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(71) Applicant *(for all designated States except US)*: **PHARMACIA CORPORATION** [US/US]; Corporate Patent Department, P.O. Box 1027, St. Louis, MO 63006 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **WEINSTEIN, Edward, J.** [US/US]; 15449 Highcroft Drive, Chesterfield, MO 63017 (US).

(74) Agents: **BAUER, S. Christopher** et al.; Pharmacia Corporation, Corporate Patent Department, P.O. Box 1027, St. Louis, MO 63006 (US).

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(54) Title: ANTISENSE MODULATION OF VEGF CO-REGULATED CHEMOKINE-1 EXPRESSION

(57) Abstract: Antisense compounds, compositions, and methods are provided for modulating the expression of VEGF Co-regulated chemokine-1 (VCC-1). The compositions comprise antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding VCC-1. Methods of using these compounds for modulation of VCC-1 expression and for treatment of diseases associated with expression of VCC-1 are provided.

ANTISENSE MODULATION OF VEGF CO-REGULATED CHEMOKINE-1 EXPRESSION

The present application claims priority under Title 35, United States Code, §119 to United States Provisional application Serial No. 60/404,484, filed August 19, 2002, which is incorporated by reference in its entirety as if written herein.

FIELD OF THE INVENTION

[001] The present invention provides compositions and methods for modulating the expression of VEGF Co-regulated chemokine-1 (VCC-1). In particular, this invention relates to antisense compounds, particularly oligonucleotides, specifically hybridizable with nucleic acids encoding VEGF Co-regulated chemokine-1. Such oligonucleotides have been shown to modulate the expression of VEGF Co-regulated chemokine-1.

BACKGROUND OF THE INVENTION

[002] Angiogenesis is the growth of new capillary blood vessels from pre-existing vessels and capillaries and is crucial in a large number of processes, such as wound repair, embryonic development, and the growth of solid tumors. In neovascularization, endothelial cells will undergo migration, elongation, proliferation, and orientation leading to lumen formation, re-establishment of a basement membrane and eventual anastomosis with other vessels (Patan, S., 2000 *J. Neurooncol.* **50**(1-2): 1-15).

[003] Cytokines are small proteins that bind to cell surface receptors in order to modulate activity of a variety of cells. VCC-1 appears to be a CXC chemokine, which is a sub-family of the cytokines, named due to their conserved Cys-Xaa-Cys sequence near the N-terminus of the protein. Family members also contain two additional conserved cysteine residues and are roughly 70 – 130 amino acids in size. They are secreted proteins with a leader sequence of 20 – 25 amino acids, which is cleaved off before release. A characteristic three-dimensional folding of

the chemokines is stabilized by the disulfide bonds that form between the conserved cysteine 1 and cysteine 2 and between cysteine 3 and cysteine 4 (reviewed in Baggiolini, M., 2001 *J. Int. Med.* **250**: 91-104).

[004] Among the known CXC chemokines are interleukin-8 (IL-8), γ-interferon-inducible protein 10 (IP-10), platelet factor 4 (PF4), monokine induced by γ-interferon (MIG), epithelial neutrophil activating protein-78 (ENA-78), the growth related oncogene peptides (GRO) GRO-α, GRO-β and GRO-γ, and others. These proteins mediate a diverse number of activities including activation of neutrophils, induction of chemotaxis, induction of angiogenesis and tumorigenesis, as well as inhibition of angiogenesis and tumorigenesis (Belperio, J.A., *et al.*, 2000 *J. Leuk. Bio.* **68:** 1-8).

[005] All of the biological effects of chemokines are exerted through their interaction with a cell surface receptor. There are six CXC chemokine receptors (CXCRs) identified to date (reviewed by Horuk et al., 2001 *Cytokine Growth Factor Rev.* **12**: 313-335). The CXCRs are members of the superfamily of serpentine proteins that signal through heterotrimeric G-proteins. These proteins have been shown to possess the ability to bind multiple chemokines with high affinity.

[006] The regulation of angiogenesis is controlled at least in part by angiostatic and angiogenic cytokines. IL-8 has been shown to mediate endothelial cell chemotactic and proliferative activity *in vitro* (Strieter R.M., *et al.*, 1992, *Am. J. Pathol.* **141:** 1279-1284 and Koch, A.E., *et al.*, 1992 *Science* **258:**1798-1801). In contrast, IP-10, MIG, and PF4 have been found to have angiostatic properties both *in vitro* and *in vivo* (Maione, T.E., *et al.*, 1990, *Science* **247:** 77-79; Strieter, R.M., *et al.*, 1995, *Biochem. Biophys. Res. Commun.* **210**(1): 51-57; and Arenberg, DA, *et al.*, 1997 *Methods Enzymol* **283:** 190-220).

[007] Since tumor growth is dependent upon angiogenesis, it follows that CXC chemokines play a role in growth and metastasis of tumors. The clearest example of angiogenic chemokines modulating tumorigenesis and growth was shown by over-expression of GRO α, β and γ in human melanocytes, which lead to an anchorage-independent growth phenotype *in vitro* and the ability to form tumors *in vivo* in nude mice (Luan, J., *et al.*, 1997, *J. Leukoc. Bio.* **62:** 588-597 and Owen,

J.D., *et al.*, 1997 *Int. J. Cancer* **73:** 94-103). Furthermore, both IL-8 and ENA-78 expression in non-small cell lung carcinoma (NSCLC) has been correlated with tumor angiogenesis (Yatsunami, J., *et al.*, 1997, *Cancer Lett.* **120:** 101-108, and Arenberg, DA, *et al.*, 1998 *J. Clin. Invest.* **102:** 465-472).

[008] Other CXC chemokines appear to either inhibit tumor cell growth or induce necrosis of tumor cells. Nude mice with Burkitt's tumor subcutaneously implanted were inoculated daily with recombinant MIG. This consistently caused tumor necrosis with vascular damage (Sgadari, C., *et al.*, 1997 *Blood* **89**(8): 2635-). The same was seen in Burkitt's tumor bearing nude mice treated with IP-10 (Sgadari, C., *et al.*, 1996 *Proc. Natl. Acad. Sci. U.S.A.* **93:**13791-13796). SCID mice bearing NSCLC tumors and treated with MIG also show growth inhibition, decreased numbers of metastasis, and a decrease in tumor-derived vessel density (Addison, C.L., *et al.*, 2000 *Hum. Gene Ther.* **11:** 247-261).

[009] 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 VCC-1 expression.

SUMMARY OF THE INVENTION

[0010] The present invention is directed to antisense compounds, particularly oligonucleotides, which are targeted to a nucleic acid encoding VCC-1, and which modulate the expression of VCC-1. Pharmaceutical and other compositions comprising the antisense compounds of the invention are also provided. Further provided are methods of modulating the expression of VCC-1 in cells or tissues comprising contacting said cells or tissues with one or more of the antisense compounds or compositions of the invention. Further provided are methods of treating an animal, particularly a human, suspected of having or being prone to a disease or condition associated with expression of VCC-1 by administering a therapeutically or prophylactically effective amount of one or more of the antisense compounds or compositions of the invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the cDNA sequence and the VCC-1 protein sequence encoded therefrom.

DETAILED DESCRIPTION OF THE INVENTION

[0011] The present invention employs oligomeric antisense compounds, particularly oligonucleotides, for use in modulating the function of nucleic acid molecules encoding VCC-1, ultimately modulating the amount of VCC-1 produced. This is accomplished by providing antisense compounds, which specifically hybridize with one or more nucleic acids encoding VCC-1. As used herein, the terms "target nucleic acid" and "nucleic acid encoding VCC-1" encompass DNA encoding VCC-1, RNA (including pre-mRNA and mRNA) transcribed from such DNA, and also cDNA derived from such RNA. The specific hybridization of an oligomeric compound with its target nucleic acid interferes with the normal function of the nucleic acid. This modulation of function of a target nucleic acid by compounds, which specifically hybridize to it, is generally referred to as "antisense". The functions of DNA to be interfered with include replication and transcription. The functions of RNA to be interfered with include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of VCC-1. In the context of the present invention, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene. In the context of the present invention, inhibition is the preferred form of modulation, of gene expression and mRNA is a preferred target.

[0012] It is preferred to target specific nucleic acids for antisense. "Targeting" an antisense compound to a particular nucleic acid, in the context of this invention, is a multistep process. The process usually begins

with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target is a nucleic acid molecule encoding VCC-1. The targeting process also includes determination of a site or sites within this gene for the antisense interaction to occur such that the desired effect, e.g., detection or modulation of expression of the protein, will result. Within the context of the present invention, a preferred intragenic site is the region encompassing the translation initiation or termination codon of the open reading frame (ORF) of the gene. Since, as is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in vivo. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used in vivo to initiate translation of an mRNA molecule transcribed from a gene encoding VCC-1, regardless of the sequence(s) of such codons.

[0013] It is also known in the art that a translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e. 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively). The terms "start codon region" and "translation initiation codon region "refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either

direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region "refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon.

[0014] The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Other target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA or corresponding nucleotides on the gene, and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA or corresponding nucleotides on the gene. The 5' cap of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap. The 5' cap region may also be a preferred target region.

[0015] Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. mRNA splice sites, i.e., intron-exon junctions, may also be preferred target regions, and are particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular mRNA splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also preferred targets. It has also been found that introns can also be

effective, and therefore preferred, target regions for antisense compounds targeted, for example, to DNA or pre-mRNA.

[0016] Once one or more target sites have been identified, oligonucleotides are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect.

[0017] In the context of this invention, "hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases. For example, adenine and thymine are complementary nucleobases, which pair through the formation of hydrogen bonds. "Complementary," as used herein, refers to the capacity for precise pairing between two nucleotides. For example, if a nucleotide at a certain position of an oligonucleotide is capable of hydrogen bonding with a nucleotide at the same position of a DNA or RNA molecule, then the oligonucleotide and the DNA or RNA are considered to be complementary to each other at that position. The oligonucleotide and the DNA or RNA are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the oligonucleotide and the DNA or RNA target. It is understood in the art that the sequence of an antisense compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. An antisense compound is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are performed.

[0018] Antisense compounds are commonly used as research reagents and diagnostics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes. Antisense compounds are also used, for example, to distinguish between functions of various members of a biological pathway. Antisense modulation has, therefore, been harnessed for research use.

[0019] The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotides have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans. In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides composed of naturally occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

[0020] VCC-1 antisense oligonucleotides that have activity in the cardiovascular, angiogenic, and endothelial assays described herein, and/or whose gene product has been found to be localized to the cardiovascular system, is likely to have therapeutic uses in a variety of cardiovascular, endothelial, and angiogenic disorders, including systemic disorders that affect vessels, such as diabetes mellitus. Its therapeutic utility could include diseases of the arteries, capillaries, veins, and/or lymphatics. Examples of treatments hereunder include treating muscle wasting disease, treating osteoporosis, aiding in implant fixation to stimulate the growth of cells around the implant and therefore facilitate its attachment to its intended site,

increasing IGF stability in tissues or in serum, if applicable, and increasing binding to the IGF receptor (since IGF has been shown in vitro to enhance human marrow erythroid and granulocytic progenitor cell growth).

[0021] VCC-1 antisense oligonucleotides can be used to inhibit the production of excess connective tissue during wound healing or pulmonary fibrosis if VCC-1 promotes such production. This would include treatment of acute myocardial infarction and heart failure.

[0022] Moreover, the present invention provides the treatment of cardiac hypertrophy, regardless of the underlying cause, by administering a therapeutically effective dose of VCC-1 antisense oligonucleotides.

[0023] The treatment for cardiac hypertrophy can be performed at any of its various stages, which may result from a variety of diverse pathologic conditions, including myocardial infarction, hypertension, hypertrophic cardiomyopathy, and valvular regurgitation. The treatment extends to all stages of the progression of cardiac hypertrophy, with or without structural damage of the heart muscle, regardless of the underlying cardiac disorder.

[0024] VCC-1 antisense oligonucleotides would be useful for treatment of disorders where it is desired to limit or prevent angiogenesis. Examples of such disorders include vascular tumors such as hemangioma, tumor angiogenesis, neovascularization in the retina, choroid, or cornea, associated with diabetic retinopathy or premature infant retinopathy or macular degeneration and proliferative vitreoretinopathy, rheumatoid arthritis, Crohn's disease, atherosclerosis, ovarian hyperstimulation, psoriasis, endometriosis associated with neovascularization, restenosis subsequent to balloon angioplasty, sear tissue overproduction, for example, that seen in a keloid that forms after surgery, fibrosis after myocardial infarction, or fibrotic lesions associated with pulmonary fibrosis.

[0025] Specific types of diseases are described below, where VCC-1 antisense oligonucleotides may serve as useful for vascular- related drug targeting or as therapeutic targets for the treatment or prevention of the disorders.

[0026] Atherosclerosis is a disease characterized by accumulation of plaques of intimal thickening in arteries, due to accumulation of lipids, proliferation of smooth muscle cells, and formation of fibrous tissue within the arterial wall. The disease can affect large, medium, and small arteries in any organ. Changes in endothelial

and vascular smooth muscle cell function are known to play an important role in modulating the accumulation and regression of these plaques.

[0027] Hypertension is characterized by raised vascular pressure in the systemic arterial, pulmonary arterial, or portal venous systems. Elevated pressure may result from or result in impaired endothelial function and/or vascular disease.

[0028] Inflammatory vasculitides include giant cell arteritis, Takayasu's arteritis, polyarteritis nodosa (including the microangiopathic form), Kawasaki's disease, microscopic polyarightis, Wegener's granulomatosis, and a variety 101 of infectious-related vascular disorders (including Henoch-Schonlein Prupura). Altered endothelial cell function has been shown to be important in these diseases. Reynaud's disease and Reynaud's phenomenon are characterized by intermittent abnormal impairment of the circulation through the extremities on exposure to cold. Altered endothelial cell function has been shown to be important in this disease.

[0029] Aneurysms are saccular or fusiform dilatations of the arterial or venous tree that are associated with altered endothelial cell and/or vascular smooth muscle cells.

[0030] Arterial restenosis (restenosis of the arterial wall) may occur following angioplasty as a result of alteration in the function and proliferation of endothelial and vascular smooth muscle cells.

[0031] Thrombophlebitis and lymphangitis are inflammatory disorders of veins and lymphatics, respectively, that may result from, and/or in, altered endothelial cell function. Similarly, lymphedema is a condition involving impaired lymphatic vessels resulting from endothelial cell function.

[0032] The family of benign and malignant vascular tumors is characterized by abnormal proliferation and growth of cellular elements of the vascular system. For example, lymphangiomas are benign tumors of the lymphatic system that are congenital, often cystic, malformations of the lymphatics that usually occur in newborns.

[0033] Cystic tumors tend to grow into the adjacent tissue. Cystic tumors usually occur in the cervical and axillary region. They can also occur in the soft tissue of the extremities. The main symptoms are dilated, sometimes reticular, structured lymphatics and lymphocysts surrounded by connective tissue.

[0034] Lymphangiomas are assumed to be caused by improperly connected embryonic lymphatics or their deficiency. The result is impaired local lymph drainage.

[0035] Another use for VCC-1 antisense antagonists is in the prevention of tumor angiogenesis, which involves vascularization of a tumor to enable it to growth and/or metastasize. This process is dependent on the growth of new blood vessels. Examples of neoplasms and related conditions that involve tumor angiogenesis include breast carcinomas, lung carcinomas, gastric carcinomas, esophageal carcinomas, colorectal carcinomas, liver carcinomas, ovarian carcinomas, thecomas, arrhenoblastomas, cervical carcinomas, endometrial carcinoma, endometrial hyperplasia, endometriosis, fibrosarcomas, choriocarcinoma, head and neck cancer, nasopharyngeal carcinoma, laryngeal carcinomas, hepatoblastoma, Kaposi's sarcoma, melanoma, skin carcinomas, hemangioma, cavernous hemangioma, hemangioblastoma, pancreas carcinomas, retinoblastoma, astrocytoma, glioblastoma, Schwannoma, oligodendroglioma, medulloblastoma, neuroblastomas, rhabdomyosarcoma, osteogenic sarcoma, leiomyosarcomas, urinary tract carcinomas, thyroid carcinomas, Wilm's tumor, renal cell carcinoma, prostate carcinoma, abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

[0036] Healing of trauma such as wound healing and tissue repair is also a targeted use for VCC-1 antisense oligonucleotides. Formation and regression of new blood vessels is essential for tissue healing and repair. This category includes bone, cartilage, tendon, ligament, and/or nerve tissue growth or regeneration, as well as wound healing and tissue repair and replacement, and in the treatment of bums, incisions, and ulcers.

[0037] VCC-1 antisense oligonucleotides that induce cartilage and/or bone growth in circumstances where bone is not normally formed have application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing VCC-1 antisense oligonucleotides may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or

oncologic, resection-induced craniofacial defects, and also is useful in cosmetic plastic surgery.

[0038] It is expected that VCC-1 antisense oligonucleotides may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, or endothelium), muscle (smooth, skeletal, or cardiac), and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate.

[0039] VCC-1 antisense oligonucleotides may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage. Also, VCC-1 antisense oligonucleotides may be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells, or for inhibiting the growth of tissues described above.

[0040] VCC-1 antisense oligonucleotides may also be used in the treatment of periodontal diseases and in other tooth-repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells, or induce differentiation of progenitors of bone-forming cells VCC-1 antisense oligonucleotides may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes, since blood vessels play an important role in the regulation of bone turnover and growth.

[0041] Another category of tissue regeneration activity that may be attributable to VCC-1 antisense oligonucleotides is tendon/ligament formation. A protein that induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed has application in the healing of tendon or ligament tears, deformities, and other tendon or ligament defects in humans and other animals. Such a preparation may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of

VCC-1 antisense oligonucleotides contributes to the repair of congenital, trauma-induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions herein may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament forming cells, or induce growth of tendon/ligament cells or progenitors ex vivo for return in vivo to effect tissue repair. The compositions herein may also be useful in the treatment of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

[0042] VCC-1 antisense oligonucleotides may also be administered prophylactically to patients with cardiac hypertrophy, to prevent the progression of the condition, and avoid sudden death, including death of asymptomatic patients. Such preventative therapy is particularly warranted in the case of patients diagnosed with massive left ventricular cardiac hypertrophy (a maximal wall thickness of 35 mm. or more in adults, or a comparable value in children), or in instances when the hemodynamic burden on the heart is particularly strong.

[0043] VCC-1 antisense oligonucleotides may also be useful in the management of atrial fibrillation, which develops in a substantial portion of patients diagnosed with hypertrophic cardiomyopathy. Further indications include angina, myocardial infarctions such as acute myocardial infarctions, and heart failure such as congestive heart failure. Additional non-neoplastic conditions include psoriasis, diabetic and other proliferative retinopathies including retinopathy of prematurity, retrolental fibroplasia, neovascular glaucoma, thyroid hyperplasias (including Grave's disease), corneal and other tissue transplantation, chronic inflammation, lung inflammation, nephrotic syndrome, preeclampsia, ascites, pericardial effusion (such as that associated with pericarditis), and pleural effusion.

[0044] In view of the above, VCC-1 antisense oligonucleotides, which are shown to alter or impact endothelial cell function, proliferation, and/or form, are likely to play an important role in the etiology and pathogenesis of many or all of the disorders noted above, and as such can serve as

therapeutic targets to augment or inhibit these processes or for vascular-related drug targeting in these disorders.

Combination Therapies

[0045] The effectiveness of VCC-1 antisense oligonucleotides in preventing or treating the disorder in question may be improved by administering the active agent serially or in combination with another agent that is effective for those purposes, either in the same composition or as separate compositions. For example, for treatment of cardiac hypertrophy, VCC-1 antisense therapy can be combined with the administration of inhibitors of known cardiac myocyte hypertrophy factors, e.g., inhibitors of cc-adrenergic agonists such as phenylephrine; endothelin-1 inhibitors such as BOSENTAN™ and MOXONODIN™; inhibitors to CT- I (US Pat. No. 5,679,545); inhibitors to LIF; ACE inhibitors; des- aspartate-angiotensin I inhibitors (U.S. Pat. No. 5,773,415), and angiotensin II inhibitors.

[0046] For treatment of cardiac hypertrophy associated with hypertension, VCC-1 antisense oligonucleotides can be administered in combination with P-adrenergic receptor blocking agents, e.g., propranolol, timolol, tertalolol, carteolol, nadolol, betaxolol, penbutolol, acetobutolol, atenolol, metoprolol, or carvedilol; ACE inhibitors, e.g., quinapril, captopril, enalapril, ramipril, benazepril, fosinopril, or lisinopril; diuretics, e.g., chlorothiazide, hydrochlorothiazide, hydroflumethiazide, methylchlothiazide, benzthiazide, dichlorphenamide, acetazolamide, or indapamide; and/or calcium channel blockers, e.g., diltiazem, nifedipine, verapamil, or nicardipine. Pharmaceutical compositions comprising the therapeutic agents identified herein by their generic names are commercially available, and are to be administered following the manufacturers' instructions for dosage, administration, adverse effects, contraindications, etc. 119 See, e.z., *Physicians' Desk Reference* (Medical Economics Data Production Co.: Montvale, N.J., 1997), 51 st Edition. Preferred candidates for combination therapy in the treatment of hypertrophic cardiormyopathy are P-adrenergic-blocking drugs (e.g., propranolol, timolol, tertalolol, carteolol, nadolol, betaxolol, penbutolol, acetobutolol, atenolol, metoprolol, or carvedilol), verapamil, difedipine, or diltiazem. Treatment of hypertrophy associated with high blood pressure may require the use of antihypertensive drug therapy, using calcium channel blockers,

e.g., diltiazem, nifedipine, verapamil, or nicardipine; P-adrenergic blocking agents; diuretics, e.g., chlorothiazide, hydrochlorothiazide, hydroflumethiazide, methylchlothiazide, benzthiazide, dichlorphenamide, acetazolamide, or indapamide; and/or ACE-inhibitors, e. g., quinapril, captopril, enalapril, ramipril, benazepril, fosinopril, or lisinopril.

[0047] For other indications, VCC-1 antisense oligonucleotides may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as EGF, PDGF, TGF- or TGF-, IGF, FGF, and CTGF.

[0048] In addition, VCC-1 antisense oligonucleotides used to treat cancer may be combined with cytotoxic, chemotherapeutic, or growth-inhibitory agents as identified above. Also, for cancer treatment, VCC-1 antisense oligonucleotides are suitably administered serially or in combination with radiological treatments, whether involving irradiation or administration of radioactive substances.

[0049] The effective amounts of the therapeutic agents administered in combination with VCC-1 antisense oligonucleotides thereof will be at the physician's, or veterinarian's discretion. Dosage administration and adjustment is done to achieve maximal management of the conditions to be treated. For example, for treating hypertension, these amounts ideally take into account use of diuretics or digitalis, and conditions such as hyper- or hypotension, renal impairment, etc. The dose will additionally depend on such factors as the type of the therapeutic agent to be used and the specific patient being treated. Typically, the amount employed will be the same dose as that used, if the given therapeutic agent is administered without VCC-1 antisense oligonucleotides.

[0050] For treatment of breast carcinoma, VCC-1 antisense oligonucleotides can be administered in combination with, but not limited to, Trastuzumab (Herceptin) with chemotherapy, paclitaxel, docetaxel, epirubicin, mitoxantrone, topotecan, capecitabine, vinorelbine, thiotepa, vincristine, vinblastine, carboplatin or cisplatin, plicamycin, anastrozole, letrozole, exemestane, toremifine, or progestins.

[0051] For treatment of acute lymphocytic leukemia, VCC-1 antisense oligonucleotides can be administered in combination with, but not limited to,

doxorubicin, cytarabine, cyclophosphamide, etoposide, teniposide, allopurinol, or autologous bone marrow transplantation.

[0052] For treatment of acute myelocytic and myelomonocytic leukemia, VCC-1, antisense oligonucleotides can be administered in combination with, but not limited to, gemtuzumab ozogamicin (Mylotarg), mitoxantrone, idarubicin, etoposide, mercaptopurine, thioguanine, azacitidine, amsacrine, methotrexate, doxorubicin, tretinoin, allopurinol, leukapheresis, prednisone, or arsenic trioxide for acute promyelocytic leukemia.

[0053] For treatment of chronic myelocytic leukemia, VCC-1 antisense oligonucleotides can be administered in combination with, but not limited to, busulfan, mercaptopurine, thioguanine, cytarabine, plicamycin, melphalan, autologous bone marrow transplantation, or allopurinol.

[0054] For treatment of chronic lymphocytic leukemia, VCC-1 antisense oligonucleotides can be administered in combination with, but not limited to, vincristine, cyclophosphamide, doxorubicin, cladribine (2-chlorodeoxyadenosine; CdA), allogeneic bone marrow transplant, androgens, or allopurinol.

[0055] For treatment of multiple myeloma, VCC-1 antisense oligonucleotides can be administered in combination with, but not limited to, etoposide, cytarabine, alpha interferon, dexamethasone, or autologous bone marrow transplantation.

[0056] For treatment of carcinoma of the lung (small cell and non-small cell), VCC-1 antisense oligonucleotides can be administered in combination with, but not limited to, cyclophosphamide, doxorubicin, vincristine, etoposide, mitomycin, ifosfamide, paclitaxel, irinotecan, or radiation therapy.

[0057] For treatment of carcinoma of the colon and rectum, VCC-1 antisense oligonucleotides can be administered in combination with, but not limited to, capecitabine, methotrexate, mitomycin, carmustine, cisplatin, irinotecan, or floxuridine.

[0058] For treatment of carcinoma of the kidney, VCC-1 antisense oligonucleotides can be administered in combination with, but not limited to, alpha interferon, progestins, infusional FUDR, or fluorouracil.

[0059] For treatment of carcinoma of the prostate, VCC-1 antisense oligonucleotides can be administered in combination with, but not limited to,

ketoconazole, doxorubicin, aminoglutethimide, progestins, cyclophosphamide, cisplatin, vinblastine, etoposide, suramin, PC-SPES, or estramustine phosphate.

[0060] For treatment of melanoma, VCC-1 antisense oligonucleotides can be administered in combination with, but not limited to, carmustine, lomustine, melphalan, thiotepa, cisplatin, paclitaxel, tamoxifen, or vincristine.

[0061] For treatment of carcinoma of the ovary, VCC-1 antisense oligonucleotides can be administered in combination with, but not limited to, docetaxel, doxorubicin, topotecan, cyclophosphamide, doxorubicin, etoposide, or liposomal doxorubicin.

[0062] While antisense oligonucleotides are a preferred form of antisense compound, the present invention comprehends other oligomeric antisense compounds, including but not limited to oligonucleotide mimetics such as are described below. The antisense compounds in accordance with this invention preferably comprise from about 8 to about 30 nucleobases (i.e. from about 8 to about 30 linked nucleo sides). Particularly preferred antisense compounds are antisense oligonucleotides, even more preferably those comprising from about 12 to about 25 nucleobases. As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn the respective ends of this linear polymeric structure can be further joined to form a circular structure, however, open linear structures are generally preferred. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal I linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

[0063] 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.

[0064] Preferred modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

[0065] Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference.

[0066] Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and

thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

[0067] Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

[0068] In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. 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.

[0069] Most preferred embodiments of the invention are oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular $-CH_2-NH-O-CH_2-$, $-CH_2-N(CH_3)-O-CH_2-$ [known as a methylene (methylimino) or MMI backbone], $-CH_2-O-N(CH_3)-CH_2-$, $-CH_2N(CH_3)-N(CH_3)-CH_2-$ and $-O-N(CH_3)-CH_2-CH_2-$ [wherein the native phosphodiester backbone is represented as -O-P-

O-CH_2-] of the above referenced U.S. patent 5,489,677, and the amide backbones of the above referenced U.S. patent 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. patent 5,034,506.

[0070] Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_n,OCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_{2n}ON[(CH_2)_nCH_3)]_2$ where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C_1 to C_{10}, (lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, $ON0_2$, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2' -methoxyethoxy (2' -O-$CH_2CH_2OCH_3$, also known as 2'-O- (2-methoxyethyl) or 2'-MOE) (Martin et al., *Helv. Chim. Acta*, 1995, 78, 486-504) i.e., an alkoxyalkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O-CH_2-O-CH_2-N $(CH_2)_2$, also described in examples herein below.

[0071] Other preferred modifications include 2'-methoxy (2'-O CH_3) , 2'-aminopropoxy (2'-O CH_2 CH_2 CH_2NH_2) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in

2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference in its entirety.

[0072] Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylquanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in United States Patent No. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J.I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., *Angewandte Chemie*, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y.S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Crooke, S.T. and Lebleu, B. ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These

include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds, *Antisense Research and Applications*, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

[0073] 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. 3,687,808, as well as U.S.: 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 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; 5,750,692, and 5,681,941, each of which is herein incorporated by reference.

[0074] Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates, which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Let.*, 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660, 306-309; Manoharan et al., *Bioorg. Med. Chem. Let.*, 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10, 1111-1118; Kabanov et al., *FEBS Lett.*, 1990, 259, 327-330; Svinarchuk et al., *Biochimie*, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 365'-3654; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Mancharan et al., *Nucleosides & Nucleotides*, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et

al., *Tetrahedron Lett.*, 1995, 36, 365'-3654), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277, 923-937).

[0075] Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference.

[0076] 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. The present invention also includes antisense compounds, which are chimeric compounds. "Chimeric" antisense compounds or "chimeras," in the context of this invention, are antisense compounds, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease, which cleaves the RNA strand of RNA:DNA

duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

[0077] 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. 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, each of which is herein incorporated by reference in its entirety.

[0078] 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, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is well known to use similar techniques to prepare oligonucleotides such as the phosphorothioates and alkylated derivatives.

[0079] The antisense compounds of the invention are synthesized in vitro and do not include antisense compositions of biological origin, or genetic vector constructs designed to direct the in vivo synthesis of antisense molecules. The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative United

States patents that teach the preparation of such uptake, distribution and/or absorption assisting formulations include, but are not limited to, U.S. 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.

[0080] 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.

[0081] 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 December 9, 1993 or in WO 94/26764 to Imbach et al.

[0082] 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.

[0083] Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N, N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine,

dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine (see, for example, Berge et al., "Pharmaceutical Salts," *J. of Pharma Sci.*, 1977, 66, 119). The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention. As used herein, a "pharmaceutical addition salt" includes a pharmaceutically acceptable salt of an acid form of one of the components of the compositions of the invention. These include organic or inorganic acid salts of the amines. Preferred acid salts are the hydrochlorides, acetates, salicylates, nitrates and phosphates. Other suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of a variety of inorganic and organic acids, such as, for example, with inorganic acids, such as for example hydrochloric acid, hydrobromic acid, sulfuric acid or phosphoric acid; with organic carboxylic, sulfonic, sulfo or phospho acids or N-substituted sulfamic acids, for example acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, fumaric acid, malic acid, tartaric acid, lactic acid, oxalic acid, gluconic acid, glucaric acid, glucuronic acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, salicylic acid, 4-aminosalicylic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, embonic acid, nicotinic acid or isonicotinic acid; and with amino acids, such as the 20 alpha-amino acids involved in the synthesis of proteins in nature, for example glutamic acid or aspartic acid, and also with phenylacetic acid, methanesulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, benzenesulfonic acid, 4-methylbenzenesulfoic acid, naphthalene-2-sulfonic acid, naphthalene-1,5-disulfonic acid, 2- or 3-phosphoglycerate, glucose-6-phosphate, N-cyclohexylsulfamic acid (with the formation of cyclamates), or with other acid organic compounds, such as ascorbic acid.

Pharmaceutically acceptable salts of compounds may also be prepared with a pharmaceutically acceptable cation. Suitable pharmaceutically acceptable cations are well known to those skilled in the art and include alkaline, alkaline earth, ammonium and quaternary ammonium cations. Carbonates or hydrogen carbonates are also possible.

[0084] For oligonucleotides, preferred examples of pharmaceutically acceptable salts include but are not limited to (a) salts formed with cations such as sodium, potassium, ammonium, magnesium, calcium, polyamines such as spermine and spermidine, etc.; (b) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; (c) salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (d) salts formed from elemental anions such as chlorine, bromine, and iodine.

[0085] The antisense compounds of the present invention can be utilized for diagnostics, therapeutics, and prophylaxis and as research reagents and kits. For therapeutics, an animal, preferably a human, suspected of having a disease or disorder, which can be treated by modulating the expression of VCC-1, is treated by administering antisense compounds in accordance with this invention. The compounds of the invention can be utilized in pharmaceutical compositions by adding an effective amount of an antisense compound to a suitable pharmaceutically acceptable diluent or carrier. Use of the antisense compounds and methods of the invention may also be useful prophylactically, e.g., to prevent or delay infection, inflammation or tumor formation, for example.

[0086] The antisense compounds of the invention are useful for research and diagnostics, because these compounds hybridize to nucleic acids encoding VCC-1, enabling sandwich and other assays to easily be constructed to exploit this fact. Hybridization of the antisense

oligonucleotides of the invention with a nucleic acid encoding VCC-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 VCC-1 in a sample may also be prepared.

[0087] 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.

[0088] 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.

[0089] Compositions and formulations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

[0090] 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.

[0091] Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

[0092] 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.

[0093] The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances, which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0094] In one embodiment of the present invention the pharmaceutical compositions may be formulated and used as foams. Pharmaceutical foams include formulations such as, but not limited to, emulsions, microemulsions, creams, jellies and liposomes. While basically similar in nature these formulations vary in the components and the consistency of the final product. The preparation of such compositions and formulations is generally known to those skilled in the pharmaceutical and formulation arts and may be applied to the formulation of the compositions of the present invention.

Emulsions

[0095] The compositions of the present invention may be prepared and formulated as emulsions. Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. (Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi et al., in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA, 1985, p. 301). Emulsions are often biphasic systems comprising of two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions may be either water-in-oil (w/o) or of the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions may contain additional components in addition to the dispersed phases and the active drug, which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants may also be present in emulsions as needed. Pharmaceutical emulsions may also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous provides an o/w/o emulsion.

[0096] Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well

dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion may be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that may be incorporated into either phase of the emulsion. Emulsifiers may broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (Idson, in *Pharmaceutical Dosaqe Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

[0097] Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (Rieger, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants may be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric (Rieger, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

[0098] Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, non-swelling clays such as

bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

[0099] A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives, and antioxidants (Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

[00100] Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed phase droplets and by increasing the viscosity of the external phase.

[00101] Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that may readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used may be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

[00102] The application of emulsion formulations via dermatological, oral, and parenteral routes and methods for their manufacture has been reviewed in the literature (Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of reasons of ease of formulation, efficacy from an absorption and bioavailability standpoint. (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

[00103] In one embodiment of the present invention, the compositions of oligonucleotides and nucleic acids are formulated as microemulsions. A microemulsion may be defined as a system of water, oil and amphiphile, which is a single optically isotropic, and thermodynamically stable liquid solution (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: *Controlled Release of Drugs: Polymers and Aggregate Systems*, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 1852-5). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric

packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA, 1985, p. 271).

[00104] The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

[00105] Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (S0750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions may, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase may typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase may include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and triglycerides, polyoxyethylated glyceryl fatty acid

esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

[00106] Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (Constantinides et al., *Pharmaceutical Research*, 1994, 11, 1385-1390; Ritschel, *Meth. Find. Exp. Clin. Pharmacol.*, 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (Constantinides et al., *Pharmaceutical Research*, 1994, 11, 1385; Ho et al., *J. Pharm. Sci.*, 1996, 85, 138-143). Often microemulsions may form spontaneously when their components are brought together at ambient temperature. This may be particularly advantageous when formulating thermolabile drugs, peptides or oligonucleotides. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of oligonucleotides and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of oligonucleotides and nucleic acids within the gastrointestinal tract, vagina, buccal cavity and other areas of administration.

[00107] Microemulsions of the present invention may also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the oligonucleotides and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention may be classified as belonging to one of five broad categories - surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee et al., *Critical Reviews in*

Therapeutic Drug Carrier Systems, 1991, p. 92). Each of these classes has been discussed above.

Liposomes

[00108] There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

[00109] Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Noncationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages in vivo.

[00110] In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. Therefore, it is desirable to use a liposome, which is highly deformable and able to pass through such fine pores.

[00111] Further advantages of liposomes include; liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, P. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

[00112] Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is

structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes. As the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent may act.

[00113] Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side-effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

[00114] Several reports have detailed the ability of liposomes to deliver agents including high-molecular weight DNA into the skin. Compounds including analgesics, antibodies, hormones and high-molecular weight DNAs have been administered to the skin. The majority of applications resulted in the targeting of the upper epidermis.

[00115] Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes, which interact with the negatively charged DNA molecules to form a stable complex. The positively charged DNA/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang et al., *Biochem. Biophys. Res. Commun.*, 1987, 147, 980 - 985)

[00116] Liposomes, which are pH-sensitive or negatively charged, entrap DNA rather than complex with it. Since both the DNA and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some DNA is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver DNA encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou et al., *Journal of Controlled Release*, 1992, 19, 269-274).

[00117] One major type of liposomal composition includes phospholipids other than naturally derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

[00118] Several studies have assessed the topical delivery of liposomal drug formulations to the skin. Application of liposomes containing interferon to guinea pig skin resulted in a reduction of skin herpes sores while delivery of interferon via other means (e.g. as a solution or as an emulsion) was ineffective (Weiner et al., *Journal of Drug Targeting*, 1992, 2, 405-410). Further, an additional study tested the efficacy of interferon administered as part of a liposomal formulation to the administration of interferon using an aqueous system, and concluded that the liposomal formulation was superior to aqueous administration (du Plessis et al., *Antiviral Research*, 1992, 18, 259-265).

[00119] Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome ™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/ cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporin-A into different layers of the skin (Hu et al. *S.T.P.Pharma. Sci.*, 1994, 4, 6, 466).

[00120] Liposomes also include "sterically stabilized" liposomes, a term, which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced

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

[00121] Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos et al. (*Ann. N.Y. Acad. Sci.*, 1987, 507, 64) reported the ability of monosialoganglioside G_{M1}, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon et al. (*Proc. Natl. Acad. Sci. U.S.A.*, 1988, 85, 6949). U.S. Patent No. 4,837,028 and WO 88/04924, both to Allen et al., disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside Gjor a galactocerebroside sulfate ester. U.S. Patent No. 5,543,152 (Webb et al.) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim et al.).

[00122] Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto et al. (*Bull. Chem. Soc. Jpn.*, 1980, 53, 2778) described liposomes comprising a nonionic detergent, $2C_{12}15G$ that contains a PEG moiety. Illum et al. (*FEBS Lett.*, 1984, 167, 79) noted that hydrophilic coating of polystyrene particles with polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols (e.g., PEG) are described by Sears (U.S. Patent Nos. 4,426,330 and 4,534,899). Klibanov et al. (*FEBS Lett.*, 1990, 268, 235) described experiments

demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume et al. (*Biochimica et Biophysica Acta*, 1990, 1029, 91) extended such observations to other PEG derivatized phospholipids, e.g., DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle et al. (U.S. Patent Nos. 5,013,556 and 5,356,633) and Martin et al. (U.S. Patent No. 5,213,804 and European Patent No. EP 0 496 813 Bl). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Patent No. 5,225,212 (both to Martin et al.) and in WO 94/20073 (Zalipsky et al.) Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi et al.). U.S. Patent Nos. 5,540,935 (Miyazaki et al.) and 5,556,948 (Tagawa et al.) describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces.

[00123] A limited number of liposomes comprising nucleic acids are known in the art. WO 96/40062 to Thierry et al. discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Patent No. 5,264,221 to Tagawa et al. discloses protein-bonded liposomes and asserts that the contents of such liposomes may include an antisense RNA. U.S. Patent No. 5,665,710 to Rahman et al. describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 to Love et al. discloses liposomes comprising antisense oligonucleotides targeted to the raf gene.

[00124] Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes may be described as lipid droplets, which are so highly deformable that they are easily able to penetrate through pores that are smaller than the droplet. Transfersomes are adaptable to the

environment in which they are used, e.g. they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

[00125] Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, NY, 1988, p. 285)

[00126] If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

[00127] If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most

important members of the anionic surfactant class are the alkyl sulfates and the soaps.

[00128] If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

[00129] If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

[00130] The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, NY, 1988, p. 285). Penetration Enhancers

[00131] In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids particularly oligonucleotides, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs may cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

[00132] 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 nonsurfactants (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p.92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

[00133] Surfactants: In connection with the present invention, surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the

result that absorption of oligonucleotides through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p.92); and perfluorochemical emulsions, such as FC-43. Takahashi et al., *J. Pharm. Pharmacol.*, 1988, 40, 252).

[00134] Fatty acids: Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-.rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C_{1-10} alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p.92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7, 1-33; El Hariri et al., *J. Pharm. Pharmacol.*, 1992, 44, 651-654).

[00135] Bile salts: The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 in: Goodman & Gilman's *The Pharmacological Basis of Therapeutics*, 9th Ed., Hardman et al. Eds. McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. The bile salts of the invention include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate

(STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92; Swinyard, Chapter 39 In: *Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990, pages 782-783; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7, 1-33; Yamamoto et al., *J. Pharm. Exp. Ther.*, 1992, 263, 25; Yamashita et al., *J. Pharm. Sci.*, 1990, 79, 579-583).

[00136] Chelating Agents: Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of oligonucleotides through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, *J. Chromatogr.*, 1993, 618, 315-339). Chelating agents of the invention include but are not limited to disodium. ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9, and N-amino acyl derivatives of beta-diketones (enamines)(Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7, 1-33; Buur et al., *J. Control Rel.*, 1990, 14, 43-51).

[00137] Non-chelating non-surfactants: As used herein, nonchelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of oligonucleotides through the alimentary mucosa (Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7, 1-33). This class of penetration enhancers includes, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin, and

phenylbutazone (Yamashita et al., *J. Pharm. Pharmacol.*, 1987, 39, 621-626).

[00138] Agents that enhance uptake of oligonucleotides at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi et al, U.S. Patent No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo et al., PCT Application WO 97/30731), are also known to enhance the cellular uptake of oligonucleotides.

[00139] Other agents may be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

Carriers

[00140] Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (i.e., does not possess biological activity per se) but is recognized as a nucleic acid by in vivo processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate oligonucleotide in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'disulfonic acid (Miyao et al., *Antisense Res. Dev.,* 1995, 5, 115-121; Takakura et al., *Antisense & Nucl. Acid Drug Dev.*, 1996, 6, 177-183).

Excipients

[00141] In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient may be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc.).

[00142] Pharmaceutically acceptable organic or inorganic excipient suitable for non-parenteral administration, which does not deleteriously react with nucleic acids, can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

[00143] Formulations for topical administration of nucleic acids may include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions may also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration, which do not deleteriously react with nucleic acids, can be used.

[00144] Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin,

lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Other Components

[00145] The compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention.' The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

[00146] Aqueous suspensions may contain substances, which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol, and/or dextran. The suspension may also contain stabilizers.

[00147] Certain embodiments of the invention provide pharmaceutical compositions containing (a) one or more antisense compounds and (b) one or more other chemotherapeutic agents which function by a non-antisense mechanism. Examples of such chemotherapeutic agents include, but are not limited to, anticancer drugs such as daunorubicin, dactinomycin, doxorubicin, bleomycin, mitomycin, nitrogen mustard, chlorambucil, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5-fluorouracil (5-FU), floxuridine (5-FUdR), methotrexate (MTX), colchicine, vincristine, vinblastine, etoposide, teniposide, cisplatin and

diethylstilbestrol (DES). See, generally, *The Merck Manual of Diagnosis and Therapy*, 15th Ed., Berkow et al., eds., 1987, Rahway, N.J., pages 1206-1228). Anti-inflammatory drugs, including but not limited to 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. See, generally, *The Merck Manual of Diagnosis and Therapy*, 15th Ed., Berkow et al., eds., 1987, Rahway, N.J., pages 2499-2506 and 46-49, respectively). other non-antisense chemotherapeutic agents are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

[00148] In another related embodiment, compositions of the invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. Numerous examples of antisense compounds are known in the art. Two or more combined compounds may be used together or sequentially.

[00149] The formulation of therapeutic compositions and their subsequent administration is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50}s found to be effective in in vitro and in vivo animal models. In general, dosage is from 0.01 μg to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be

desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 μg to 100 g per kg of body weight, once or more daily, to once every 20 years.

[00150] While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same.

EXAMPLES

Example 1

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

[00151] 2'-Deoxy and 2'-methoxy beta-cyanoethyldiisopropyl phosphoramidites are available from commercial sources (e.g. Chemgenes, Needham MA or Glen Research, Inc. Sterling VA). Other 2'-O-alkoxy substituted nucleoside amidites are prepared as described in U.S. Patent 5,506,351, herein incorporated by reference. For oligonucleotides synthesized using 2'-alkoxy amidites, the standard cycle for unmodified oligonucleotides is utilized, except the wait step after pulse delivery of tetrazole and base is increased to 360 seconds.

[00152] Oligonucleotides containing 5-methyl-2'-deoxycytidine (5-Me-C) nucleotides are synthesized according to published methods [Sanghvi, et. al., *Nucleic Acids Research*, 1993, 21, 3197-3203] using commercially available phosphoramidites (Glen Research, Sterling VA or ChemGenes, Needham MA).

2'-Fluoro amidites

2'-Fluorodeoxyadenosine amidites

[00153] 2'-fluoro oligonucleotides are synthesized as described previously [Kawasaki, et. al., *J. Med. Chem.*, 1993, 36, 831-841] and United States patent 5,670,633, herein incorporated by reference. Briefly, the protected nucleoside N6-benzoyl-2'-deoxy-2'-fluoroadenosine is

synthesized utilizing commercially available 9-beta-D-arabinofuranosyladenine as starting material and by modifying literature procedures whereby the 2'-alpha-fluoro atom is introduced by a S_N2-displacement of a 2'-beta-trityl group. Thus N6-benzoyl-9-beta-D-arabinofuranosyladenine is selectively protected in moderate yield as the 3',5'-ditetrahydropyranyl (THP) intermediate. Deprotection of the THP and N6-benzoyl groups is accomplished using standard methodologies and standard methods are used to obtain the 5'-dimethoxytrityl-(DMT) and 5'-DMT-3'-phosphoramidite intermediates.

2'-Fluorodeoxyguanosine

[00154] The synthesis of 2'-deoxy-2'-fluoroguanosine is accomplished using tetraisopropyldisiloxanyl (TPDS) protected 9-beta-D-arabinofuranosylguanine as starting material, and conversion to the intermediate diisobutyrylarabinofuranosylguanosine. Deprotection of the TPDS group is followed by protection of the hydroxyl group with THP to give diisobutyryl di-THP protected arabinofuranosylguanine. Selective O-deacylation and triflation is followed by treatment of the crude product with fluoride, then deprotection of the THP groups. Standard methodologies are used to obtain the 5'-DMT- and 5'-DMT-3'-phosphoramidites.

2'-Fluorouridine

[00155] Synthesis of 2'-deoxy-2'-fluorouridine is accomplished by the modification of a literature procedure in which 2,2'anhydro-l-beta-D-arabinofuranosyluracil is treated with 70% hydrogen fluoride-pyridine. Standard procedures are used to obtain the 5'-DMT and 5'-DMT-3'-phosphoramidites.

2'-Fluorodeoxycytidine

[00156] 2'-deoxy-2'-fluorocytidine is synthesized via amination of 2'-deoxy-2'-fluorouridine, followed by selective protection to give N4-benzoyl-2'-deoxy-2'-fluorocytidine. Standard procedures are used to obtain the 5'-DMT and 5'-DMT-3'phosphoramidites.

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

[00157] 2'-O-Methoxyethyl-substituted nucleoside amidites are prepared as follows, or alternatively, as per the methods of Martin, P., *Helvetica Chimica Acta*, 1995, 78, 486-504.

2,2'-Anhydro[l-(beta-D-arabinofuranosyl)-5-methyluridinel

[00158] 5-Methyluridine (ribosylthymine, commercially available through Yamasa, Choshi, Japan) (72.0 g, 0.279 M), diphenylcarbonate (90.0 g, 0.420 M) and sodium bicarbonate (2.0 g, 0.024 M) are added to DMF (300 mL). The mixture is heated to reflux, with stirring, allowing the evolved carbon dioxide gas to be released in a controlled manner. After 1 hour, the slightly darkened solution is concentrated under reduced pressure. The resulting syrup is poured into diethylether (2.5 L), with stirring. The product formed a gum. The ether is decanted and the residue is dissolved in a minimum amount of methanol (ca. 400 mL). The solution is poured into fresh ether (2.5 L) to yield a stiff gum. The ether is decanted and the gum is dried in a vacuum oven (60°C at 1 mm Hg for 24 h) to give a solid that is crushed to a light tan powder. The material is used as is for further reactions (or it can be purified further by column chromatography using a gradient of methanol in ethyl acetate (10-25%) to give a white solid.

2'-O-Methoxyethyl-5-methyluridine

[00159] 2,2'-Anhydro-5-methyluridine (195 g, 0.81 M), tris(2-methoxyethyl)borate (231 g, 0.98 M) and 2-methoxyethanol (1.2 L) are added to a 2 L stainless steel pressure vessel and placed in a pre-heated oil bath at 160°C. After heating for 48 hours at 155-160°C, the vessel is opened and the solution evaporated to dryness and triturated with MeOH (200 mL). The residue is suspended in hot acetone (1 L). The insoluble salts are filtered, washed with acetone (150 mL) and the filtrate evaporated. The residue (280 g) is dissolved in CH_3CN (600 mL) and evaporated. A silica gel column (3 kg) is packed in CH_2Cl_2 /acetone /MeOH (20:5:3) containing 0.5% Et_3NH. The residue is dissolved in CH_2C1_2 (250 mL) and adsorbed onto silica (150 g) prior to loading onto the column. The product is eluted with the packing solvent to give the title product. Additional material can be obtained by reworking impure fractions.

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

[00160] 2'-O-Methoxyethyl-5-methyluridine (160 g, 0.506 M) is co-evaporated with pyridine (250 mL) and the dried residue dissolved in pyridine (1.3 L). A first aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) is added and the mixture stirred at room temperature for one hour. A second aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) is added and the reaction stirred for an additional one hour. Methanol (170 mL) is then added to stop the reaction. The solvent is evaporated and triturated with CH_3CN (200 mL) The residue is dissolved in CHCl (1.5 L) and extracted with 2x500 mL of saturated $NaHCO_3$ and 2x500 mL of saturated NaCl. The organic phase is dried over Na_2SO_4, filtered, and evaporated. The residue is purified on a 3.5 kg silica gel column, packed and eluted with EtOAc/hexane/ acetone (5:5:1) containing 0-5% Et_3NH. The pure fractions are evaporated to give the title product.

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

[00161] 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (106 g, 0.167 M), DMF/pyridine (750 mL of a 3:1 mixture prepared from 562 mL of DMF and 188 mL of pyridine) and acetic anhydride (24.38 mL, 0.258 M) are combined and stirred at room temperature for 24 hours. The reaction is monitored by TLC by first quenching the TLC sample with the addition of MeOH. Upon completion of the reaction, as judged by TLC, MeOH (50 mL) is added and the mixture evaporated at 35°C. The residue is dissolved in $CHCl_3$ (800 mL) and extracted with 2x200 mL of saturated sodium bicarbonate and 2x200 mL of saturated NaCl. The water layers are back extracted with 200 mL of $CHCl_3$. The combined organics are dried with sodium sulfate and evaporated to a residue. The residue is purified on a 3.5 kg silica gel column and eluted using EtOAc/hexane(4:1). Pure product fractions are evaporated to yield the title compounds.

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

[00162] A first solution is prepared by dissolving 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (96 g, 0.144 M) in CH_3CN (700 mL) and set aside. Triethylamine (189 mL, 1.44 M) is added to a solution of triazole (90 g, 1.3 M) in CH_3CN (1 L), cooled to –5°C and

stirred for 0.5 h using an overhead stirrer. $POCl_3$ is added dropwise, over a 30 minute period, to the stirred solution maintained at 0-10°C, and the resulting mixture stirred for an additional 2 hours. The first solution is added dropwise, over a 45 minute period, to the latter solution. The resulting reaction mixture is stored overnight in a cold room. Salts are filtered from the reaction mixture and the solution is evaporated. The residue is dissolved in EtOAc (1 L) and the insoluble solids are removed by filtration. The filtrate is washed with 1x300 mL of $NaHCO_3$ and 2x300 mL of saturated NaCl, dried over sodium sulfate and evaporated. The residue is triturated with EtOAc to give the title compound.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine

[00163] A solution of 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine (103 g, 0.141 M) in dioxane (500 mL) and NH_4OH (30 mL) is stirred at room temperature for 2 hours. The dioxane solution is evaporated and the residue azeotroped with MeOH (2x200 mL). The residue is dissolved in MeOH (300 mL) and transferred to a 2-liter stainless steel pressure vessel. MeOH (400 mL) saturated with NH_3 gas is added and the vessel heated to 100°C for 2 hours (TLC showed complete conversion). The vessel contents are evaporated to dryness and the residue is dissolved in EtOAc (500 mL) and washed once with saturated NaCl (200 mL). The organics are dried over sodium sulfate and the solvent is evaporated to give the title compound.

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

[00164] 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (85 g, 0.134 M) is dissolved in DMF (800 mL) and benzoic anhydride (37.2 g, 0.165 M) is added with stirring. After stirring for 3 hours, TLC showed the reaction to be approximately 95% complete. The solvent is evaporated and the residue azeotroped with MeOH (200 mL). The residue is dissolved in $CHCl_3$ (700 mL) and extracted with saturated NaHCO, (2x300 mL) and saturated NaCl (2x300 mL) , dried over $MgSO_4$ and evaporated to give a residue. The residue is chromatographed on a 1.5 kg silica column using

EtOAc/hexane (1:1) containing 0-5% Et_3NH as the eluting solvent. The pure product fractions are evaporated to give the title compound.

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

[00165] N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (74 g, 0.10 M) is dissolved in CH_2Cl_2 (1 L) Tetrazole diisopropylamine (7.1 g) and 2-cyanoethoxy-tetra(isopropyl)phosphite (40.5 mL, 0.123 M) are added with stirring, under a nitrogen atmosphere. The resulting mixture is stirred for 20 hours at room temperature (TLC showed the reaction to be 95% complete). The reaction mixture is extracted with saturated $NaHCO_3$ (1x300 mL) and saturated NaCl (3x300 mL). The aqueous washes are back-extracted with CH_2Cl_2 (300 mL), and the extracts are combined, dried over $MgSO_4$ and concentrated. The residue obtained is chromatographed on a 1.5 kg silica column using EtOAc/hexane (3:1) as the eluting solvent. The pure fractions were combined to give the title compound.

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

2'-(Dimethylaminooxyethoxy) nucleoside amidites

[00166] 2'-(Dimethylaminooxyethoxy) nucleoside amidites [also known in the art as 2'-O-(dimethylaminooxyethyl) nucleoside amidites] are prepared as described in the following paragraphs. Adenosine, cytidine and guanosine nucleoside amidites are prepared similarly to the thymidine (5-methyluridine) except the exocyclic amines are protected with a benzoyl moiety in the case of adenosine and cytidine and with isobutyryl in the case of guanosine.

5'-O-tert-Butyldiphenylsilyl -O^2 -2'-anhydro-5-methyluridine

[00167] O^2 -2'-anhydro-5-methyluridine (Pro. Bio. Sint., Varese, Italy, 100.0g, 0.4'6 mmol), dimethylaminopyridine (0.66g, 0.013eq, 0.0054mmol) are dissolved in dry pyridine (500 ml) at ambient temperature under an argon atmosphere and with mechanical stirring. tert-Butyldiphenylchlorosilane (125.8g, 119.0mL, 1.1eq, 0.458mmol) is added in one portion. The reaction is stirred for 16 h at ambient temperature. TLC

(Rf 0.22, ethyl acetate) indicated a complete reaction. The solution is concentrated under reduced pressure to a thick oil. This is partitioned between dichloromethane (1 L) and saturated sodium bicarbonate (2xl L) and brine (1 L). The organic layer is dried over sodium sulfate and concentrated under reduced pressure to a thick oil. The oil is dissolved in a 1:1 mixture of ethyl acetate and ethyl ether (600mL) and the solution is cooled to –10°C. The resulting crystalline product is collected by filtration, washed with ethyl ether (3x200 mL), and dried (40°C, 1mm Hg, 24 h) to a white solid

5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine

[00168] In a 2 L stainless steel, unstirred pressure reactor is added borane in tetrahydrofuran (1.0 M, 2.0 eq, 622 mL). In the fume hood and with manual stirring, ethylene glycol (350 mL, excess) is added cautiously at first until the evolution of hydrogen gas subsides. 5'-O-tert-Butyldiphenylsilyl-O^2-2'anhydro-5-methyluridine (149 g, 0.3'1 mol) and sodium bicarbonate (0.074 g, 0.003 eq) are added with manual stirring. The reactor is sealed and heated in an oil bath until an internal temperature of 160°C is reached and then maintained for 16 h (pressure < 100 psig). The reaction vessel is cooled to ambient and opened. TLC (Rf 0.67 for desired product and Rf 0.82 for ara-T side product, ethyl acetate) indicated about 70% conversion to the product. In order to avoid additional side product formation, the reaction is stopped, concentrated under reduced pressure (10 to 1mm, Hg) in a warm water bath (40-100°C) with the more extreme conditions used to remove the ethylene glycol. [Alternatively, once the low boiling solvent is gone, the remaining solution can be partitioned between ethyl acetate and water. The product will be in the organic phase.] The residue is purified by column chromatography (2kg silica gel, ethyl acetate-hexanes gradient 1:1 to 4:1). The appropriate fractions are combined, stripped and dried to product as a white crisp foam, contaminated starting material, and pure reusable starting material.

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

[00169] 5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine (20g, 36.98mmol) is mixed with triphenylphosphine (11.63g,

44.36mmol) and N-hydroxyphthalimide (7.24g, 44.36mmol). It is then dried over P_2O_5 under high vacuum for two days at 40°C. The reaction mixture is flushed with argon and dry THF (369.8mL, Aldrich, sure seal bottle) is added to get a clear solution. Diethyl-azodicarboxylate (6.98mL, 44.36mmol) is added dropwise to the reaction mixture. The rate of addition is maintained such that resulting deep red coloration is just discharged before adding the next drop. After the addition is complete, the reaction is stirred for 4 hrs. By that time TLC showed the completion of the reaction (ethylacetate:hexane, 60:40). The solvent is evaporated in vacuum. Residue obtained is placed on a flash column and eluted with ethyl acetate:hexane (60:40), to get 2'-O-([2-phthalimidoxy)ethyl]-5'-t-butyldiphenylsilyl-5-methyluridine as white foam.

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

[00170] 2'-O-([2-phthalimidoxy)ethyl]-5'-t-butyldiphenylsilyl-5-methyluridine (3.1g, 4.5mmol) is dissolved in dry CH_2C1_2 (4.5mL) and methylhydrazine (300mL, 4.64mmol) is added dropwise at –10°C to 0°C. After 1 h the mixture is filtered, the filtrate is washed with ice cold CH_2Cl_2 and the combined organic phase is washed with water, brine and dried over anhydrous Na_2SO_4. The solution is concentrated to get 2'-O(aminooxyethyl) thymidine, which is then dissolved in MeOH (67.5mL). To this formaldehyde (20% aqueous solution, w/w, 1.1 eq.) is added and the resulting mixture is stirred for 1 h. Solvent is removed under vacuum; residue chromatographed to get 5'-O-tert-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy) ethyl]-5-methyluridine as white foam.

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

[00171] 5'-O-tert-butyldiphenylsilyl-2'-O-[(2- formadoximinooxy)ethyl]-5-methyluridine (1.77g, 3.12mmol) is dissolved in a solution of 1M pyridinium p-toluenesulfonate (PPTS) in dry MeOH (30.6mL). Sodium cyanoborohydride (0.39g, 6.13mmol) is added to this solution at 10°C under inert atmosphere. The reaction mixture is stirred for 10 minutes at 10°C. After that the reaction vessel is removed from the ice bath and stirred at

room temperature for 2 h, the reaction monitored by TLC (5% MeOH in CH_2Cl_2). Aqueous $NaHCO_3$ solution (5%, 10mL) is added and extracted with ethyl acetate (2x20mL). Ethyl acetate phase is dried over anhydrous Na_2SO_4, evaporated to dryness. Residue is dissolved in a solution of 1M PPTS in MeOH (30.6mL). Formaldehyde (20% w/w, 30mL, 3.37mmol) is added and the reaction mixture is stirred at room temperature for 10 minutes. Reaction mixture cooled to 10°C in an ice bath, sodium cyanoborohydride (0.39g, 6.13mmol) is added, and reaction mixture stirred at 10°C for 10 minutes. After 10 minutes, the reaction mixture is removed from the ice bath and stirred at room temperature for 2 hrs. To the reaction mixture 5% $NaHCO_3$ (25mL) solution is added and extracted with ethyl acetate (2x25mL). Ethyl acetate layer is dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue obtained is purified by flash column chromatography and eluted with 5% MeOH in CH_2Cl_2 to get 5'-O-tertbutyldiphenylsilyl-2'-O-[N,N-dimethylaminooxyethyl]-5- methyluridine as a white foam.

2'-O-(dimethylaminooxyethyl)-5-methyluridine

[00172] Triethylamine trihydrofluoride (3.91mL, 24.0mmol) is dissolved in dry THF and triethylamine (1.67mL, 12mmol, dry, kept over KOH). This mixture of triethylamine-2HF is then added to 5'-O-tert-butyldiphenylsilyl-2'-O-[N,N-dimethylaminooxyethyl]-5-methyluridine (1.40g, 2.4mmol) and stirred at room temperature for 24 hrs. Reaction is monitored by TLC (5% MeOH in CH_2Cl_2). Solvent is removed under vacuum and the residue placed on a flash column and eluted with 10% MeOH in CH_2Cl_2 to get 2'-O-(dimethylaminooxyethyl)-5-methyluridine.

5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine

[00173] 2'-O-(dimethylaminooxyethyl)-5-methyluridine (750mg, 2.17mmol) is dried over P_2O_5 under high vacuum overnight at 40°C. It is then co-evaporated with anhydrous pyridine (20mL). The residue obtained is dissolved in pyridine (11mL) under argon atmosphere. 4-dimethylaminopyridine (26.5mg, 2.60mmol), 4,4'-dimethoxytrityl chloride (880mg, 2.60mmol) is added to the mixture and the reaction mixture is stirred at room temperature until all of the starting material disappeared.

Pyridine is removed under vacuum and the residue chromatographed and eluted with 10% MeOH in CH_2Cl_2 (containing a few drops of pyridine) to get 5'-O-DMT-2'-0(dimethylamino-oxyethyl)-5-methyluridine.

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

[00174] 5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine (1.08g, 1.67mmol) is co-evaporated with toluene (20mL). To the residue N,N-diisopropylamine tetrazonide (0.29g, 1.67mmol) is added and dried over P20, under high vacuum overnight at 40°C. Then the reaction mixture is dissolved in anhydrous acetonitrile (8.4mL) and 2-cyanoethyl-N,N,N^1,N^1-tetraisopropylphosphoramidite (2.12mL, 6.08mmol) is added. The reaction mixture is stirred at ambient temperature for 4 hrs under inert atmosphere. The progress of the reaction is monitored by TLC (hexane:ethyl acetate 1:1). The solvent is evaporated, then the residue is dissolved in ethyl acetate (70mL) and washed with 5% aqueous $NaHCO_3$ (40mL). Ethyl acetate layer is dried over anhydrous Na_2SO_4 and concentrated. Residue obtained is chromatographed (ethyl acetate as eluent) to get 5'-O-DMT-2'-O-(2-N,N-dimethylaminooxyethyl)-5-methyluridine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] as a foam.

2'-(Aminooxyethoxy) nucleoside amidites

[00175] 2'-(Aminooxyethoxy) nucleoside amidites [also known in the art as 2'-O-(aminooxyethyl) nucleoside amidites] are prepared as described in the following paragraphs. Adenosine, cytidine and thymidine nucleoside amidites are prepared similarly.

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

[00176] The 2'-O-aminooxyethyl guanosine analog may be obtained by selective 2'-O-alkylation of diaminopurine riboside. Multigram quantities of diaminopurine riboside may be purchased from Schering AG (Berlin) to provide 2'-O-(2-ethylacetyl) diaminopurine riboside along with a minor amount of the 3'-O-isomer. 2'-O-(2-ethylacetyl) diaminopurine riboside may be resolved and converted to 2'-O-(2ethylacetyl)guanosine by

treatment with adenosine deaminase. (McGee, D. P. C., Cook, P. D., Guinosso, C. J., WO 94/02501 Al 940203.) Standard protection procedures should afford 2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine and 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine which may be reduced to provide 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine. As before the hydroxyl group may be displaced by N-hydroxyphthalimide via a Mitsunobu reaction, and the protected nucleoside may phosphitylated as usual to yield 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramiditel.

2'-dimethylaminoethoxyethoxy (2'-DMAEOE) nucleoside amidites

[00177] 2'-dimethylaminoethoxyethoxy nucleoside amidites (also known in the art as 2'-O-dimethylaminoethoxyethyl, i.e., 2'O-CH_2-O-CH_2-$N(CH_2)_2$, or 2'-DMAEOE nucleoside amidites) are prepared as follows. Other nucleoside amidites are prepared similarly.

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

[00178] 2[2-(Dimethylamino)ethoxylethanol (Aldrich, 6.66 g, 50 mmol) is slowly added to a solution of borane in tetrahydrofuran (1 M, 10 mL, 10 mmol) with stirring in a 100 mL bomb. Hydrogen gas evolves as the solid dissolves. O^2-, 2' - anhydro-5-methyluridine (1.2 g, 5 mmol), and sodium bicarbonate (2.5 mg) are added and the bomb is sealed, placed in an oil bath, and heated to 155°C for 26 hours. The bomb is cooled to room temperature and opened. The crude solution is concentrated and the residue partitioned between water (200 mL) and hexanes (200 mL). The excess phenol is extracted into the hexane layer. The aqueous layer is extracted with ethyl acetate (3x200 mL) and the combined organic layers are washed once with water, dried over anhydrous sodium sulfate and concentrated. The residue is columned on silica gel using methanol/methylene chloride 1:20 (which has 2% triethylamine) as the eluent. As the column fractions are concentrated a colorless solid forms which is collected to give the title compound as a white solid.

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

[00179] To 0.5 g (1.3 mmol) of 2'-O-[2(2-N,N-dimethylaminoethoxy)ethyl)1-5-methyl uridine in anhydrous pyridine (8 mL), triethylamine (0.36 mL) and dimethoxytrityl chloride (DMT-Cl, 0.87 g, 2 eq.) are added and stirred for 1 hour. The reaction mixture is poured into water (200 mL) and extracted with CH_2Cl_2 (2x200 mL). The combined CH_2Cl_2 layers are washed with saturated $NaHCO_3$ solution, followed by saturated NaCl solution and dried over anhydrous sodium sulfate. Evaporation of the solvent followed by silica gel chromatography using MeOH: CH_2Cl_2:Et_3N (20:1, v/v, with 1% triethylamine) gives the title compound.

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

[00180] Diisopropylaminotetrazolide (0.6 g) and 2-cyanoethoxyN,N-diisopropyl phosphoramidite (1.1 mL, 2 eq.) are added to a solution of 5'-O-dimethoxytrityl-2'-O-[2(2-N,N-dimethylaminoethoxy)ethyl)]-5-methyluridine (2.17 g, 3 mmol) dissolved in CH_2Cl_2 (20 mL) under an atmosphere of argon. The reaction mixture is stirred overnight and the solvent evaporated. The resulting residue is purified by silica gel flash column chromatography with ethyl acetate as the eluent to give the title compound.

Example 2

Oligonucleotide synthesis

[00181] Unsubstituted and substituted phosphodiester (P=O) oligonucleotides are synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine.

[00182] Phosphorothioates (P=S) are synthesized as for the phosphodiester oligonucleotides except the standard oxidation bottle is replaced by 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation

wait step is increased to 68 sec and is followed by the capping step. After cleavage from the CPG column and deblocking in concentrated ammonium hydroxide at 55°C (18 h), the oligonucleotides are purified by precipitating twice with 2.5 volumes of ethanol from a 0.5 M NaCl solution. Phosphinate oligonucleotides are prepared as described in U.S. Patent 5,508,270, herein incorporated by reference.

[00183] Alkyl phosphonate oligonucleotides are prepared as described in U.S. Patent 4,469,863, herein incorporated by reference.

[00184] 3'-Deoxy-3'-methylene phosphonate oligonucleotides are prepared as described in U.S. Patents 5,610,289 or 5,625,050, herein incorporated by reference.

[00185] Phosphoramidite oligonucleotides are prepared as described in U.S. Patent, 5,256,775 or U.S. Patent 5,366,878, herein incorporated by reference.

[00186] Alkylphosphonothioate oligonucleotides are prepared as described in WO 94/17093 and WO 94/02499 herein incorporated by reference.

[00187] 3'-Deoxy-3'-amino phosphoramidate oligonucleotides are prepared as described in U.S. Patent 5,476,925, herein incorporated by reference.

[00188] Phosphotriester oligonucleotides are prepared as described in U.S. Patent 5,023,243, herein incorporated by reference.

[00189] Borano phosphate oligonucleotides are prepared as described in U.S. Patents 5,130,302 and 5,177,198, both herein incorporated by reference.

Example 3

Oligonucleoside Synthesis

[00190] 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. Patents 5,378,825; 5,386,023; 5,489,677; 5,602,240; and 5,610,289, all of which are herein incorporated by reference.

[00191] Formacetal and thioformacetal linked oligonucleosides are prepared as described in U.S. Patents 5,264,562 and 5,264,564, herein incorporated by reference.

[00192] Ethylene oxide linked oligonucleosides are prepared as described in U.S. Patent 5,223,618, herein incorporated by reference.

Example 4

PNA Synthesis

[00193] Peptide nucleic acids (PNAs) are prepared in accordance with any of the various procedures referred to in Peptide Nucleic Acids (PNA): Synthesis, Properties and Potential Applications, *Bioorganic & Medicinal Chemistry*, 1996, 4, 523. They may also be prepared in accordance with U.S. Patents 5,539,082; 5,700,922; and 5,719,262, herein incorporated by reference.

Example 5

Synthesis of Chimeric Oligonucleotides

[00194] 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".

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

[00195] Chimeric oligonucleotides having 2'-O-alkyl phosphorothioate and 2'-deoxy phosphorothioate oligonucleotide segments are synthesized using an Applied Biosystems automated DNA synthesizer Model 380B, as above. Oligonucleotides are synthesized using the automated synthesizer and 2'-deoxy-5'-dimethoxytrityl-3'-O-phosphoramidite for the DNA portion and 5'-dimethoxytrityl-2'-O-methyl-3'-O-phosphoramidite for 5' and 3' wings. The standard synthesis cycle is modified by increasing the wait step after the delivery of tetrazole and base to 600 s repeated four times for RNA and twice for 2'-O-methyl. The fully protected oligonucleotide is cleaved from the support and the phosphate group is deprotected in 3:1 ammonia/ethanol at room temperature overnight then lyophilized to dryness. Treatment in methanolic ammonia for 24 hrs at room temperature is then done to deprotect all bases and sample is again lyophilized to dryness. The pellet is resuspended in 1M TBAF in THF for 24 hrs at room temperature to deprotect the 2' positions. The reaction is then quenched with 1M TEAA and the sample is then reduced to 1/2 volume by rotovac before being desalted on a G25 size exclusion column. The oligo recovered is then analyzed spectrophotometrically for yield and for purity by capillary electrophoresis and by mass spectrometry.

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

[00196] [2'-O-(2-methoxyethyl)]--[2'-deoxy]—[-2'-O-(methoxyethyl)] chimeric phosphorothioate oligonucleotides are prepared as per the procedure above for the 2'-O-methyl chimeric oligonucleotide, with the substitution of phorothioate oligonucleotides are prepared as per the procedure above for 2'-O-(methoxyethyl) amidites for the 2'-O-methyl amidites.

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

[00197] [2'-O-(2-methoxyethyl phosphodiester]--[2'-deoxy phosphorothioate]--[2'-O-(methcixyethyl) phosphodiester] chimeric oligonucleotides are prepared as per the above procedure for the 2'-O-methyl chimeric oligonucleotide with the substitution of 2'-O-

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

[00198] Other chimeric oligonucleotides, chimeric oligonucleosides and mixed chimeric oligonucleotides/oligonucleosides are synthesized according to United States patent 5,623,065, herein incorporated by reference.

Example 6

Oligonucleotide Isolation

[00199] After cleavage from the controlled pore glass column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide at 55°C for 18 hours, the oligonucleotides or oligonucleosides are purified by precipitation twice out of 0.5 M NaCl with 2.5 volumes ethanol. Synthesized oligonucleotides are analyzed by polyacrylamide gel electrophoresis on denaturing gels and judged to be at least 85% full-length material. The relative amounts of phosphorothioate and phosphodiester linkages obtained in synthesis are periodically checked by "P nuclear magnetic resonance spectroscopy, and for some studies oligonucleotides are purified by HPLC, as described by Chiang et al., *J. Biol. Chem.* 1991, 266, 18162-18171.

Example 7

Oligonucleotide Synthesis - 96 Well Plate Format

[00200] Oligonucleotides are synthesized via solid phase P(III) phosphoramidite chemistry on an automated synthesizer capable of assembling 96 sequences simultaneously in a standard 96 well format. Phosphodiester internucleotide linkages are afforded by oxidation with aqueous iodine. Phosphorothioate internucleotide linkages are generated by sulfurization utilizing 3,H-1,2 benzodithiole-3-one 1,1 dioxide (Beaucage Reagent) in anhydrous acetonitrile. Standard base-protected beta-cyanoethyldiisopropyl phosphoramidites can be purchased from commercial

vendors (e.g. PE-Applied Biosystems, Foster City, CA, or Pharmacia, Piscataway, NJ). Non-standard nucleosides are synthesized as per known literature or patented methods. They are utilized as base protected betacyanoethyldiisopropyl phosphoramidites.

[00201] Oligonucleotides are cleaved from support and deprotected with concentrated NH_4OH at elevated temperature (55-60°C) for 12-16 hours and the released product then dried in vacuo. The dried product is 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

[00202] The concentration of oligonucleotide in each well is assessed by dilution of samples and UV absorption spectroscopy. The full-length integrity of the individual products is 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 is confirmed by mass analysis of the compounds utilizing electrospray-mass spectroscopy. All assay test plates are diluted from the master plate using single and multi-channel robotic pipettors. Plates are judged to be acceptable if at least 85% of the compounds on the plate are at least 85% full length.

Example 9

Cell culture and oligonucleotide treatment

[00203] The effect of antisense compounds on target nucleic acid expression can be tested in any of a variety of cell types provided that the target nucleic acid is present at measurable levels. This can be routinely determined using, for example, PCR or Northern blot analysis. The following 6 cell types are provided for illustrative purposes, but other cell types can be routinely used, provided that the target is expressed in the cell type chosen. This can be readily determined by methods routine in the art,

for example Northern blot analysis, Ribonuclease protection assays, or RT-PCR.

T-24 cells:

[00204] The human transitional cell bladder carcinoma cell line T-24 is obtained from the American Type Culture Collection (ATCC) (Manassas, VA). T-24 cells are routinely cultured in complete McCoy's 5A basal media (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Gaithersburg, MD), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Gibco/Life Technologies, Gaithersburg, MD). Cells are routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells are seeded into 96-well plates (Falcon-Primaria #3872) at a density of 7000 cells/well for use in RT-PCR analysis.

[00205] 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.

A549 cells:

[00206] The human lung carcinoma cell line A549 can be obtained from the American Type Culture Collection (ATCC) (Manassas, VA). A549 cells are routinely cultured in DMEM basal media (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Gaithersburg, MD), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Gibco/Life Technologies, Gaithersburg, MD). Cells are routinely passaged by trypsinization and dilution when they reached 90% confluence.

NHDF cells:

[00207] Human neonatal dermal fibroblast (NHDF) can be obtained from the Clonetics Corporation (Walkersville MD). NHDFs are routinely maintained in Fibroblast Growth Medium (Clonetics Corporation, Walkersville MD) supplemented as recommended by the supplier. Cells are maintained for up to 10 passages as recommended by the supplier.

HEK cells:

[00208] Human embryonic keratinocytes (HEK) can be obtained from the Clonetics Corporation (Walkersville MD). HEKs are routinely maintained in Keratinocyte Growth Medium (Clonetics Corporation, Walkersville MD) formulated as recommended by the supplier. Cells are routinely maintained for up to 10 passages as recommended by the supplier.

MCF-7 cells:

[00209] The human breast carcinoma cell line MCF-7 is obtained from the American Type Culture Collection (Manassas, VA). MCF-7 cells are routinely cultured in DMEM low glucose (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Gaithersburg, MD). Cells are routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells are seeded into 96-well plates (Falcon-Primaria #3872) at a density of 7000 cells/well for use in RT-PCR analysis.

[00210] 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.

LA4 cells:

[00211] The mouse lung epithelial cell line LA4 is obtained from the American Type Culture Collection (Manassas, VA). LA4 cells are routinely cultured in F12K medium (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 15% fetal calf serum (Gibco/Life Technologies, Gaithersburg, MD). Cells are routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells are seeded into 96-well plates (Falcon-Primaria #3872) at a density of 3000-6000 cells/ well for use in RT-PCR analysis.

[00212] 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.

Treatment with antisense compounds:

[00213] When cells reached 80% confluence, they are treated with oligonucleotide. For cells grown in 96-well plates, wells are washed once with 200 µL OPTI-MEM™-1 reduced-serum medium (Gibco BRL) and

then treated with 130 µL of OPTI-MEM™-1 containing 3.75 µg/mL LIPOFECTIN™ (Gibco BRL) and the desired concentration of oligonucleotide. After 4-7 hours of treatment, the medium is replaced with fresh medium. Cells are harvested 16-24 hours after oligonucleotide treatment.

[00214] 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.

Example 10

Analysis of oligonucleotide inhibition of VCC-1 expression

[00215] Antisense modulation of VCC-1 expression can be assayed in a variety of ways known in the art. For example, VCC-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. Methods of RNA isolation are taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 1, pp. 4.1.1-4.2.9 and 4.5.1-4.5.3, John Wiley & Sons, Inc., 1993. Northern blot analysis is routine in the art and is taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 1, pp. 4.2.1-4.2.9, John Wiley & Sons, Inc., 1996. Real-time quantitative (PCR) can be conveniently accomplished using the commercially available ABI PRISM™ 7700 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions. Prior to quantitative PCR analysis, primer-probe sets specific to the target gene being measured are evaluated for their ability to be "multiplexed" with a GAPDH amplification reaction. In multiplexing, both the target gene and the internal standard gene GAPDH are amplified concurrently in a single sample. In this analysis, mRNA isolated from untreated cells is serially diluted. Each dilution is amplified in the presence of primer-probe sets specific for GAPDH only, target gene only ("single-plexing"), or both

(multiplexing). Following PCR amplification, standard curves of GAPDH and target mRNA signal as a function of dilution are generated from both the single-plexed and multiplexed samples. If both the slope and correlation coefficient of the GAPDH and target signals generated from the multiplexed samples fall within 10% of their corresponding values generated from the single-plexed samples, the primer-probe set specific for that target is deemed as multiplexable. Other methods of PCR are also known in the art.

[00216] Protein levels of VCC-1 can be quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), ELISA or fluorescence-activated cell sorting (FACS). Antibodies directed to VCC-1 can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional antibody generation methods. Methods for preparation of polyclonal antisera are taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.12.1-11.12.9, John Wiley & Sons, Inc., 1997. Preparation of monoclonal antibodies is taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.4.1-11.11.5, John Wiley Sons, Inc., 1997.

[00217] Immunoprecipitation methods are standard in the art and can be found at, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 10.16.110.16.11, John Wiley & Sons, Inc., 1998. Western blot (immunoblot) analysis is standard in the art and can be found at, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 10.8.1-10.8.21, John Wiley Sons, Inc., 1997. Enzyme-linked immunosorbent assays (ELISA) are standard in the art and can be found at, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.2.1-11.2.22, John Wiley & Sons, Inc., 1991.

Example 11

Poly(A)+ mRNA isolation

[00218] Poly(A)+ mRNA is isolated according to Miura et al., *Clin. Chem.*, 1996, 42, 1758-1764. Other methods for poly(A)+ mRNA isolation are taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 1, pp. 4.5.1-4.5.3, John Wiley & Sons, Inc., 1993. Briefly, for cells grown on 96-well plates, growth medium is removed from the cells and each well is washed with 200 μL cold PBS. 60μL 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) is added to each well, the plate is gently agitated and then incubated at room temperature for five minutes. 55 μL of lysate is transferred to Oligo d(T) coated 96-well plates (AGCT Inc., Irvine CA). Plates are incubated for 60 minutes at room temperature, washed 3 times with 200 μL of wash buffer (10 mM Tris-HC1 pH 7.6, 1 mM EDTA, 0.3 M NaCl). After the final wash, the plate is blotted on paper towels to remove excess wash buffer and then air-dried for 5 minutes. 60 pL of elution buffer (5 mM Tris-HCl pH 7.6), preheated to 70•C is added to each well, the plate is incubated on a 90•C hot plate for 5 minutes, and the eluate is then transferred to a fresh 96-well plate.

[00219] Cells grown on 100 mm or other standard plates may be treated similarly, using appropriate volumes of all solutions.

Example 12

Total RNA Isolation

[00220] Total mRNA is isolated using an RNEASY 96™ kit and buffers purchased from Qiagen Inc. (Valencia CA) following the manufacturer's recommended procedures. Briefly, for cells grown on 96-well plates, growth medium is removed from the cells and each well is washed with 200 μL cold PBS. 100 μL Buffer RLT is added to each well and the plate vigorously agitated for 20 seconds. 100 μL of 70% ethanol is then added to each well and the contents mixed by pipetting three times up and down. The samples are 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 is applied for 15 seconds. 1 mL of Buffer RW1 is added to each well of the RNEASY 96™ plate and the vacuum again applied for 15 seconds. 1 mL of Buffer RPE is then added to each well of the RNEASY 96™ plate and the vacuum applied for a period of 15 seconds. The Buffer RPE wash is then repeated and the vacuum is applied for an additional 10 minutes. The plate is then removed from the QIAVAC™ manifold and blotted dry on paper towels. The plate is then re-attached to the QIAVAC™ manifold fitted with a collection tube rack containing 1.2 mL collection tubes. RNA is then eluted by pipetting 60μL water into each well, incubating 1 minute, and then applying the vacuum for 30 seconds. The elution step is repeated with an additional 60μL water.

[00221] The repetitive pipetting and elution steps may be automated using a QIAGEN Bio-Robot 9604 (Qiagen, Inc., Valencia CA). 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 VCC-1 mRNA Levels

[00222] Quantitation of VCC-1 mRNA levels is determined by real-time quantitative PCR using the ABI PRISM™ 7700 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to manufacturer's instructions. This is a closed-tube, non-gel-based, fluorescence detection system which allows high-throughput quantitation of polymerase chain reaction (PCR) products in real-time. As opposed to standard PCR, in which amplification products are quantitated after the PCR is completed, products in real-time quantitative PCR are quantitated as they accumulate. This is accomplished by including in the PCR reaction an oligonucleotide probe that anneals specifically between the forward and reverse PCR primers, and contains two fluorescent dyes. A reporter dye (e.g., JOE, FAM™, or VIC, obtained from either Operon Technologies Inc., Alameda, CA or PE-Applied Biosystems, Foster City, CA) is attached to the 5' end of the probe and a quencher dye (e.g., TAMRA, obtained from either Operon

Technologies Inc., Alameda, CA or PE-Applied Biosystems, Foster City, CA) is attached to the 3' end of the probe. When the probe and dyes are intact, reporter dye emission is quenched by the proximity of the 3' quencher dye. During amplification, annealing of the probe to the target sequence creates a substrate that can be cleaved by the 5'-exonuclease activity of Taq polymerase. During the extension phase of the PCR amplification cycle, cleavage of the probe by Taq polymerase releases the reporter dye from the remainder of the probe (and hence from the quencher moiety) and a sequence-specific fluorescent signal is generated. With each cycle, additional reporter dye molecules are cleaved from their respective probes, and the fluorescence intensity is monitored at regular intervals by laser optics built into the ABI PRISM™ 7700 Sequence Detection System. In each assay, a series of parallel reactions containing serial dilutions of mRNA from untreated control samples generates a standard curve that is used to quantitate the percent inhibition after antisense oligonucleotide treatment of test samples.

[00223] PCR reagents can be obtained from PE-Applied Biosystems, Foster City, CA. RT-PCR reactions are carried out by adding 25μL PCR cocktail (1x TAQMAN™ buffer A, 5.5 MM $MgCl_2$, 300 μM each of dATP, dCTP and dGTP, 600 μM of dUTP, 100 nM each of forward primer, reverse primer, and probe, 20 Units RNAse inhibitor, 1.25 Units AMPLITAQ GOLD™, and 12.5 Units MuLV reverse transcriptase) to 96 well plates containing 25 μL poly(A) mRNA solution. The RT reaction is carried out by incubation for 30 minutes at 48°C. Following a 10 minute incubation at 95°C to activate the AMPLITAQ GOLD™, 40 cycles of a two-step PCR protocol are carried out: 95°C for 15 seconds (denaturation) followed by 60°C for 1.5 minutes (annealing/extension).

[00224] Probes and primers to human VCC-1 were designed to hybridize to a human VCC-1 sequence, using published sequence, information (GenBank accession number XM_058945, incorporated herein as Figure 1. For human VCC-1 the PCR primers were:

forward primer: CGACAGTTGCGATGAAAGTTCT SEQ ID NO:1100

reverse primer: AGAGACCATGGACATCAGCATTAG SEQ ID NO:1101 and the PCR probe is: FAM™- TCTCTTCCCTCCTCCTGTTGCTGCC SEQ ID NO:1102 -TAMRA where FAM™ (PE-Applied Biosystems, Foster City, CA) is the fluorescent reporter dye) and TAMRA (PE-Applied Biosystems, Foster City, CA) is the quencher dye. For human cyclophilin the PCR primers were:

forward primer: CCCACCGTGTTCTTCGACAT SEQ ID NO:1103 reverse primer: TTTCTGCTGTCTTTGGGACCTT SEQ ID NO 1104 and the PCR probe is: 5' JOE- CGCGTCTCCTTTGAGCTGTTTGCA SEQ ID NO:1105 - TAMRA 3' where JOE (PE-Applied Biosystems, Foster City, CA) is the fluorescent reporter dye) and TAMRA (PE-Applied Biosystems, Foster City, CA) is the quencher dye.

Example 14

Antisense inhibition of human VCC-1 expression by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

[00225] In accordance with the present invention, a series of oligonucleotides are designed to target different regions of the human VCC-1 RNA, using published sequences (XM_058945, incorporated herein as Figure 1. The oligonucleotides are shown in Table 1. "Position" indicates the first (5'-most) nucleotide number on the particular target sequence to which the oligonucleotide binds. The indicated parameters for each oligo were predicted using RNAstructure 3.7 by David H. Mathews, Michael Zuker, and Douglas H. Turner. The parameters are described either as free energy (The energy that is released when a reaction occurs. The more negative the number, the more likely the reaction will occur. All free energy units are in kcal/mol.) or melting temperature (The temperature at which two anneal strands of polynucleic acid separate. The higher the temperature, greater the affinity between the 2 strands.) When designing an antisense oligonucleotide that will bind with high affinity, it is desirable to consider the structure of the target RNA strand and the antisense oligomer.

Specifically, for an oligomer to bind tightly (in the table described as 'duplex formation'), it should be complementary to a stretch of target RNA that has little self-structure (in the table the free energy of which is described as 'target structure'). Also, the oligomer should have little self-structure, either intramolecular (in the table the free energy of which is described as 'intramolecular oligo') or bimolecular (in the table the free energy of which is described as 'intermolecular oligo'). Breaking up any self-structure amounts to a binding penalty. 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 four-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE) nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. Cytidine residues in the 2'-MOE wings are 5-methylcytidines. All cytidine residues are 5-methylcytidines.

TABLE 1

position	oligo	total binding	duplex formation	Tm of Duplex	target structure	Intra-molecular oligo	Inter-molecular oligo
414	CTGTGGTGCCTTTGGTGTCT SEQ ID NO:1	-26.2	-28.3	82.5	-2.1	0	-5.7
419	GCTTTCTGTGGTGCCTTTGG SEQ ID NO;2	-25.8	-27.9	80.7	-2.1	0	-5.7
415	TCTGTGGTGCCTTTGGTGTC SEQ ID NO:3	-25.7	-27.8	82.4	-2.1	0	-5
410	GGTGCCTTTGGTGTCTTGTT SEQ ID NO:4	-25.5	-27.6	81.5	-2.1	0	-4.9
411	TGGTGCCTTTGGTGTCTTGT SEQ ID NO:5	-25.4	-27.5	80.8	-2.1	0	-5.7
412	GTGGTGCCTTTGGTGTCTTG SEQ ID NO:6	-25.4	-27.5	80.8	-2.1	0	-5.7
413	TGTGGTGCCTTTGGTGTCTT SEQ ID NO:7	-25.4	-27.5	80.8	-2.1	0	-5.7
416	TTCTGTGGTGCCTTTGGTGT SEQ ID NO:8	-25.4	-27.5	80.8	-2.1	0	-5.7
418	CTTTCTGTGGTGCCTTTGGT SEQ ID NO:9	-25.2	-27.3	79.8	-2.1	0	-5.7
424	GTTTGGCTTTCTGTGGTGCC SEQ ID NO:10	-24.8	-28.2	82.4	-2.1	-1.2	-5.2
956	GTGAGGGTCTTGGTGGGGAT SEQ ID NO:11	-24.7	-27.4	80.4	-2.7	0	-2.4
409	GTGCCTTTGGTGTCTTGTTT SEQ ID NO:12	-24.4	-26.5	79.1	-2.1	0	-3.4
420	GGCTTTCTGTGGTGCCTTTG SEQ ID NO:13	-24.4	-27.9	80.7	-2.1	-1.3	-5.7
417	TTTCTGTGGTGCCTTTGGTG SEQ ID NO:14	-24.3	-26.4	77.5	-2.1	0	-5.7

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
425	TGTTTGGCTTTCTGTGGTGC SEQ ID NO:15	-24.1	-26.2	78.4	-2.1	0	-3.7
421	TGGCTTTCTGTGGTGCCTTT SEQ ID NO:16	-23.8	-27.9	80.7	-2.1	-2	-6
422	TTGGCTTTCTGTGGTGCCTT SEQ ID NO:17	-23.8	-27.9	80.7	-2.1	-2	-6
423	TTTGGCTTTCTGTGGTGCCT SEQ ID NO:18	-23.8	-27.9	80.7	-2.1	-2	-6
407	GCCTTTGGTGTCTTGTTTTC SEQ ID NO:19	-23.7	-25.8	77.8	-2.1	0	-3.2
957	AGTGAGGGTCTTGGTGGGGA SEQ ID NO:20	-23.4	-27.4	80.8	-4	0	-2.4
408	TGCCTTTGGTGTCTTGTTTT SEQ ID NO:21	-23.3	-25.4	75.7	-2.1	0	-3.4
955	TGAGGGTCTTGGTGGGGATA SEQ ID NO:22	-23.2	-25.9	76	-2.7	0	-2.4
952	GGGTCTTGGTGGGGATAAGT SEQ ID NO:23	-23.1	-25.8	75.8	-2.7	0	-3.2
171	GGCAGCAACAGGAGGAGGGA SEQ ID NO:24	-22.6	-27	75.9	-4.4	0	-5.3
566	GAGTGTCTGGTAGGTGTGCT SEQ ID NO:25	-22.5	-26.7	81.5	-4.2	0	-3.6
954	GAGGGTCTTGGTGGGGATAA SEQ ID NO:26	-22.5	-25.2	73.6	-2.7	0	-2.4
426	TTGTTTGGCTTTCTGTGGTG SEQ ID NO:27	-22.4	-24.5	74	-2.1	0	-3.7
565	AGTGTCTGGTAGGTGTGCTC SEQ ID NO:28	-22.3	-26.5	82.1	-4.2	0	-3.6
403	TTGGTGTCTTGTTTTCTTCA SEQ ID NO:29	-22.2	-23.1	72	-0.7	0	-1.9
404	TTTGGTGTCTTGTTTTCTTC SEQ ID NO:30	-22.1	-22.5	71.2	0	0	-1.5
613	GAATGATTTAGGGGTGGGTA SEQ ID NO:31	-22.1	-22.5	67	0	0	-2.1
172	TGGCAGCAACAGGAGGAGGG SEQ ID NO:32	-22	-26.4	74.4	-4.4	0	-5.3
614	GGAATGATTTAGGGGTGGGT SEQ ID NO:33	-22	-24	70.2	-2	0	-2.3
889	GGGTCATCTGGTTGTGAATT SEQ ID NO:34	-21.9	-23.7	71	-1.8	0	-3.3
953	AGGGTCTTGGTGGGGATAAG SEQ ID NO:35	-21.9	-24.6	72.5	-2.7	0	-2.4
1	CGTTCCCATTTGAGGGCGAG SEQ ID NO:36	-21.8	-27.6	74.4	-4.5	-1.2	-6.4
890	TGGGTCATCTGGTTGTGAAT SEQ ID NO:37	-21.8	-23.6	70.4	-1.8	0	-3.3
891	ATGGGTCATCTGGTTGTGAA SEQ ID NO:38	-21.8	-23.6	70.4	-1.8	0	-3.3
892	AATGGGTCATCTGGTTGTGA SEQ ID NO:39	-21.8	-23.6	70.4	-1.8	0	-3.3
567	AGAGTGTCTGGTAGGTGTGC SEQ ID NO:40	-21.6	-25.8	79.6	-4.2	0	-2.6
951	GGTCTTGGTGGGGATAAGTA SEQ ID NO:41	-21.6	-24.3	72.4	-2.7	0	-3.2
715	CTGGGTAAGGGGAGGGCACA SEQ ID NO:42	-21.5	-27.5	77	-6	0	-4
958	GAGTGAGGGTCTTGGTGGGG SEQ ID NO:43	-21.4	-27.4	80.8	-6	0	-2.2
405	CTTTGGTGTCTTGTTTTCTT SEQ ID NO:44	-21.3	-23	71.5	-1.7	0	-1.3
174	AGTGGCAGCAACAGGAGGAG SEQ ID NO:45	-21	-25.2	72.9	-4.2	0	-2.4
562	GTCTGGTAGGTGTGCTCACT SEQ ID NO:46	-20.9	-27.1	81.9	-4.2	-2	-4.2

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
173	GTGGCAGCAACAGGAGGAGG SEQ ID NO:47	-20.8	-26.4	75.3	-5.6	0	-6.1
161	GGAGGAGGGAAGAGATTAGA SEQ ID NO:48	-20.7	-21.5	64.7	-0.6	0	-1.5
170	GCAGCAACAGGAGGAGGGAA SEQ ID NO:49	-20.7	-25.1	71	-4.4	0	-4.7
175	TAGTGGCAGCAACAGGAGGA SEQ ID NO:50	-20.7	-24.9	72	-4.2	0	-2.4
888	GGTCATCTGGTTGTGAATTG SEQ ID NO:51	-20.7	-22.5	68.1	-1.8	0	-3.1
714	TGGGTAAGGGGAGGGCACAG SEQ ID NO:52	-20.6	-26.6	75.4	-6	0	-4
897	GGTAAAATGGGTCATCTGGT SEQ ID NO:53	-20.6	-22.4	66.3	-1.8	0	-2.9
898	GGGTAAAATGGGTCATCTGG SEQ ID NO:54	-20.6	-22.4	65.7	-1.8	0	-2.9
227	GGCCTCTGGCGACCCCTGGA SEQ ID NO:55	-20.5	-34.5	87.6	-11.5	-2.5	-8.4
564	GTGTCTGGTAGGTGTGCTCA SEQ ID NO:56	-20.5	-27.2	82.9	-6.7	0	-0.6
893	AAATGGGTCATCTGGTTGTG SEQ ID NO:57	-20.5	-22.3	66.7	-1.8	0	-2.9
950	GTCTTGGTGGGGATAAGTAT SEQ ID NO:58	-20.4	-23.1	69.6	-2.7	0	-3.2
946	TGGTGGGGATAAGTATGTGT SEQ ID NO:59	-20.2	-22.9	68.7	-2.7	0	-1.8
162	AGGAGGAGGGAAGAGATTAG SEQ ID NO:60	-20.1	-20.9	63.6	-0.6	0	-1.5
226	GCCTCTGGCGACCCCTGGAT SEQ ID NO:61	-20.1	-33.3	85.2	-11.5	-1.7	-7.8
612	AATGATTTAGGGGTGGGTAC SEQ ID NO:62	-20.1	-22.1	66.2	-2	0	-4
948	CTTGGTGGGGATAAGTATGT SEQ ID NO:63	-20	-22.7	67.8	-2.7	0	-2.1
228	TGGCCTCTGGCGACCCCTGG SEQ ID NO:64	-19.9	-33.9	86.2	-11.5	-2.5	-8.1
229	GTGGCCTCTGGCGACCCCTG SEQ ID NO:65	-19.9	-33.9	87.2	-11.5	-2.5	-8.3
402	TGGTGTCTTGTTTTCTTCAC SEQ ID NO:66	-19.9	-23.2	72.3	-3.3	0	-3.6
427	CTTGTTTGGCTTTCTGTGGT SEQ ID NO:67	-19.9	-25.4	76.3	-5.5	0	-3.7
560	CTGGTAGGTGTGCTCACTGT SEQ ID NO:68	-19.9	-26.7	79.6	-4.8	-2	-4.2
945	GGTGGGGATAAGTATGTGTA SEQ ID NO:69	-19.9	-22.6	68.2	-2.7	0	-1.8
135	ATCGCAACTGTCGGTGCAGC SEQ ID NO:70	-19.8	-27.2	75.3	-5.8	-1.6	-6.8
406	CCTTTGGTGTCTTGTTTTCT SEQ ID NO:71	-19.8	-24.9	75.1	-5.1	0	-2
606	TTAGGGGTGGGTACAGTGGG SEQ ID NO:72	-19.8	-26.4	77.4	-5.9	-0.4	-5.2
894	AAAATGGGTCATCTGGTTGT SEQ ID NO:73	-19.8	-21.6	64.5	-1.8	0	-2.9
2	GCGTTCCCATTTGAGGGCGA SEQ ID NO:74	-19.7	-29.4	78.2	-8.2	-1.4	-7.1
401	GGTGTCTTGTTTTCTTCACA SEQ ID NO:75	-19.7	-23.9	73.7	-3	-1.1	-4.7
561	TCTGGTAGGTGTGCTCACTG SEQ ID NO:76	-19.7	-25.9	77.7	-4.2	-2	-4.2
225	CCTCTGGCGACCCCTGGATT SEQ ID NO:77	-19.6	-31.6	81.5	-11.5	-0.1	-4.5
137	TCATCGCAACTGTCGGTGCA SEQ ID NO:78	-19.5	-26.5	73.5	-5.8	-1.1	-7

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
605	TAGGGGTGGGTACAGTGGGA SEQ ID NO:79	-19.5	-26.9	78.5	-7.4	0.2	-5.2
896	GTAAAATGGGTCATCTGGTT SEQ ID NO:80	-19.5	-21.3	64.1	-1.8	0	-2.9
1048	GTATGCTTTTTTTTTTTTGT SEQ ID NO:81	-19.5	-19.9	63.1	0	0	-3.6
1049	GGTATGCTTTTTTTTTTTTG SEQ ID NO:82	-19.5	-19.9	62.5	0	0	-2.9
1050	TGGTATGCTTTTTTTTTTTT SEQ ID NO:83	-19.5	-19.9	62.5	0	0	-3.6
1051	TTGGTATGCTTTTTTTTTTT SEQ ID NO:84	-19.5	-19.9	62.5	0	0	-3.6
132	GCAACTGTCGGTGCAGCTGT SEQ ID NO:85	-19.4	-28.1	79.1	-7.3	-1.3	-9.7
899	AGGGTAAAATGGGTCATCTG SEQ ID NO:86	-19.4	-21.2	63.4	-1.8	0	-2.9
140	CTTTCATCGCAACTGTCGGT SEQ ID NO:87	-19.3	-25.1	71	-5.8	0	-4.7
158	GGAGGGAAGAGATTAGAACT SEQ ID NO:88	-19.3	-20.1	60.9	-0.6	0	-2.3
965	GGAGACAGAGTGAGGGTCTT SEQ ID NO:89	-19.3	-24.7	74.4	-3.9	-1.4	-5.5
138	TTCATCGCAACTGTCGGTGC SEQ ID NO:90	-19.2	-25.9	72.8	-5.8	-0.8	-7
176	TTAGTGGCAGCAACAGGAGG SEQ ID NO:91	-19.2	-24.4	71	-5.2	0	-2.4
949	TCTTGGTGGGGATAAGTATG SEQ ID NO:92	-19.2	-21.9	66.1	-2.7	0	-2.7
963	AGACAGAGTGAGGGTCTTGG SEQ ID NO:93	-19.2	-24.1	72.7	-3.9	-0.9	-5.1
400	GTGTCTTGTTTTCTTCACAT SEQ ID NO:94	-19.1	-22.7	70.8	-3	-0.3	-3.9
611	ATGATTTAGGGGTGGGTACA SEQ ID NO:95	-19.1	-23.5	69.8	-3.7	-0.4	-5.2
615	TGGAATGATTTAGGGGTGGG SEQ ID NO:96	-19.1	-22.8	66.8	-3.7	0	-2.3
900	TAGGGTAAAATGGGTCATCT SEQ ID NO:97	-19.1	-20.9	62.9	-1.8	0	-2.9
947	TTGGTGGGGATAAGTATGTG SEQ ID NO:98	-19.1	-21.8	65.7	-2.7	0	-1.8
962	GACAGAGTGAGGGTCTTGGT SEQ ID NO:99	-19	-25.3	76.1	-5.8	-0.1	-4.4
169	CAGCAACAGGAGGAGGGAAG SEQ ID NO:100	-18.9	-23.3	67.1	-4.4	0	-4.1
160	GAGGAGGGAAGAGATTAGAA SEQ ID NO:101	-18.8	-19.6	60	-0.6	0	-1.5
168	AGCAACAGGAGGAGGGAAGA SEQ ID NO:102	-18.8	-23.2	67.2	-4.4	0	-4.1
887	GTCATCTGGTTGTGAATTGG SEQ ID NO:103	-18.8	-22.5	68.1	-3.7	0	-3.1
1065	CCGTGTCTGGTTCATTGGTA SEQ ID NO:104	-18.8	-26.3	76	-7.5	0	-2.9
64	TCCCTGGGGATGACTCAGGT SEQ ID NO:105	-18.7	-28.7	80.3	-6.9	-3.1	-9.3
136	CATCGCAACTGTCGGTGCAG SEQ ID NO:106	-18.7	-26.1	72.2	-5.8	-1.6	-8.4
607	TTTAGGGGTGGGTACAGTGG SEQ ID NO:107	-18.7	-25.3	75.1	-5.9	-0.4	-5.2
1061	GTCTGGTTCATTGGTATGCT SEQ ID NO:108	-18.7	-25	75.5	-5.8	-0.1	-3.6
568	AAGAGTGTCTGGTAGGTGTG SEQ ID NO:109	-18.5	-23.3	71.8	-4.8	0	-2.9
685	GACGAGAGAAGAAGACACTA SEQ ID NO:110	-18.5	-18.9	57.3	0	0	-3.5

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
966	TGGAGACAGAGTGAGGGTCT SEQ ID NO:111	-18.5	-24.6	73.8	-4.8	-1.2	-5.9
1052	ATTGGTATGCTTTTTTTTTT SEQ ID NO:112	-18.5	-19.8	62.1	-1.2	0	-3.6
1064	CGTGTCTGGTTCATTGGTAT SEQ ID NO:113	-18.5	-24.3	72.2	-5.8	0	-2.7
159	AGGAGGGAAGAGATTAGAAC SEQ ID NO:114	-18.4	-19.2	59.2	-0.6	0	-1.4
686	TGACGAGAGAAGAAGACACT SEQ ID NO:115	-18.4	-19.2	57.8	-0.6	0	-3.5
1047	TATGCTTTTTTTTTTTTGTC SEQ ID NO:116	-18.4	-19.1	61.3	-0.4	0	-3.6
141	ACTTTCATCGCAACTGTCGG SEQ ID NO:117	-18.3	-24.1	68.4	-5.8	0	-4.7
683	CGAGAGAAGAAGACACTAGA SEQ ID NO:118	-18.3	-18.7	56.9	0	0	-4.5
895	TAAAATGGGTCATCTGGTTG SEQ ID NO:119	-18.3	-20.1	60.9	-1.8	0	-2.9
3	AGCGTTCCCATTTGAGGGCG SEQ ID NO:120	-18.2	-28.8	77.2	-9	-1.5	-9.2
157	GAGGGAAGAGATTAGAACTT SEQ ID NO:121	-18.2	-19	58.7	-0.6	0	-2.6
563	TGTCTGGTAGGTGTGCTCAC SEQ ID NO:122	-18.2	-26.2	79.5	-6.7	-1.2	-3.3
901	ATAGGGTAAAATGGGTCATC SEQ ID NO:123	-18.2	-20	61	-1.8	0	-2.9
155	GGGAAGAGATTAGAACTTTC SEQ ID NO:124	-18.1	-18.9	58.9	-0.6	0	-3.2
964	GAGACAGAGTGAGGGTCTTG SEQ ID NO:125	-18.1	-23.5	71.3	-3.9	-1.4	-5.5
716	CCTGGGTAAGGGGAGGGCAC SEQ ID NO:126	-18	-28.8	79.5	-10	-0.6	-5.2
934	GTATGTGTAGAATCTGGATT SEQ ID NO:127	-18	-20.1	62.6	-2.1	0	-6.7
233	CCCTGTGGCCTCTGGCGACC SEQ ID NO:128	-17.9	-33.9	87.2	-16	1.9	-7.2
684	ACGAGAGAAGAAGACACTAG SEQ ID NO:129	-17.9	-18.3	56.2	0	0	-4
935	AGTATGTGTAGAATCTGGAT SEQ ID NO:130	-17.9	-20	62.5	-2.1	0	-4.5
65	ATCCCTGGGGATGACTCAGG SEQ ID NO:131	-17.8	-27.5	76.7	-6.9	-2.8	-11.1
224	CTCTGGCGACCCCTGGATTC SEQ ID NO:132	-17.8	-30	80	-11.5	-0.4	-5.2
271	GCCTTCCTGGAGCCATCTCC SEQ ID NO:133	-17.8	-32.1	87.2	-11.9	-2.4	-6.8
399	TGTCTTGTTTTCTTCACATT SEQ ID NO:134	-17.8	-21.6	67.5	-3.8	0	-2.7
485	GCAGAGCAAAGCTTCTTAGC SEQ ID NO:135	-17.8	-23.9	70.4	-4.8	-1.2	-7.7
713	GGGTAAGGGGAGGGCACAGG SEQ ID NO:136	-17.8	-27.8	78.2	-10	0	-4
905	GTGAATAGGGTAAAATGGGT SEQ ID NO:137	-17.8	-19.6	59.2	-1.8	0	-1.2
1062	TGTCTGGTTCATTGGTATGC SEQ ID NO:138	-17.8	-24.1	73.1	-5.8	-0.1	-2.6
151	AGAGATTAGAACTTTCATCG SEQ ID NO:139	-17.7	-18.5	57.7	-0.6	0	-4.2
156	AGGGAAGAGATTAGAACTTT SEQ ID NO:140	-17.7	-18.5	57.7	-0.6	0	-3.2
232	CCTGTGGCCTCTGGCGACCC SEQ ID NO:141	-17.7	-33.9	87.2	-16.2	1.9	-6.5
903	GAATAGGGTAAAATGGGTCA SEQ ID NO:142	-17.7	-19.5	58.9	-1.8	0	-2.1

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
959	AGAGTGAGGGTCTTGGTGGG SEQ ID NO:143	-17.7	-26.2	78.3	-8.5	0	-2.5
1063	GTGTCTGGTTCATTGGTATG SEQ ID NO:144	-17.7	-23.5	72.1	-5.8	0	-2.7
139	TTTCATCGCAACTGTCGGTG SEQ ID NO:145	-17.6	-24.2	69	-5.8	-0.6	-6.7
223	TCTGGCGACCCCTGGATTCA SEQ ID NO:146	-17.6	-29.8	79.1	-11.5	-0.4	-5.2
428	GCTTGTTTGGCTTTCTGTGG SEQ ID NO:147	-17.6	-26	77.3	-8.4	0	-3.7
486	GGCAGAGCAAAGCTTCTTAG SEQ ID NO:148	-17.6	-23.3	68.7	-4.8	-0.7	-7.7
1060	TCTGGTTCATTGGTATGCTT SEQ ID NO:149	-17.6	-23.9	72.2	-5.8	-0.1	-3.6
487	AGGCAGAGCAAAGCTTCTTA SEQ ID NO:150	-17.5	-23.3	68.7	-4.8	-0.9	-7.7
608	ATTTAGGGGTGGGTACAGTG SEQ ID NO:151	-17.5	-24.1	72.2	-5.9	-0.4	-5.2
680	GAGAAGAAGACACTAGAGAG SEQ ID NO:152	-17.5	-17.9	56.4	0	0	-4.5
681	AGAGAAGAAGACACTAGAGA SEQ ID NO:153	-17.5	-17.9	56.4	0	0	-4.5
682	GAGAGAAGAAGACACTAGAG SEQ ID NO:154	-17.5	-17.9	56.4	0	0	-4.5
981	GAACAAGTAGGCCAATGGAG SEQ ID NO:155	-17.5	-21.8	63.2	-3.8	0	-7.7
982	TGAACAAGTAGGCCAATGGA SEQ ID NO:156	-17.5	-21.8	62.9	-3.8	0	-7.7
1053	CATTGGTATGCTTTTTTTTT SEQ ID NO:157	-17.5	-20.4	63	-2.9	0	-3.6
163	CAGGAGGAGGGAAGAGATTA SEQ ID NO:158	-17.4	-21.6	64.6	-4.2	0	-1.5
220	GGCGACCCCTGGATTCAGGC SEQ ID NO:159	-17.3	-31.5	82.7	-11.5	-2.7	-11
862	CCCATTTGAAGGAAACAATT SEQ ID NO:160	-17.3	-19.5	57	-2.2	0	-3.4
1059	CTGGTTCATTGGTATGCTTT SEQ ID NO:161	-17.3	-23.6	70.8	-5.8	-0.1	-3.6
131	CAACTGTCGGTGCAGCTGTA SEQ ID NO:162	-17.2	-26	74.1	-7.3	-1.3	-9.9
936	AAGTATGTGTAGAATCTGGA SEQ ID NO:163	-17.2	-19.3	60.3	-2.1	0	-4
961	ACAGAGTGAGGGTCTTGGTG SEQ ID NO:164	-17.2	-24.7	74.5	-7.5	0	-2.8
230	TGTGGCCTCTGGCGACCCCT SEQ ID NO:165	-17.1	-33.9	87.2	-16.8	1.9	-7.6
902	AATAGGGTAAAATGGGTCAT SEQ ID NO:166	-17.1	-18.9	57.6	-1.8	0	-2.9
972	GGCCAATGGAGACAGAGTGA SEQ ID NO:167	-17.1	-24.7	70.4	-6.7	-0.8	-8.5
219	GCGACCCCTGGATTCAGGCT SEQ ID NO:168	-17	-31.2	82.1	-11.5	-2.7	-9.6
222	CTGGCGACCCCTGGATTCAG SEQ ID NO:169	-17	-29.4	77.8	-11.5	-0.7	-6.6
554	GGTGTGCTCACTGTCTTCTT SEQ ID NO:170	-17	-26.5	80.4	-7.5	-2	-4.2
904	TGAATAGGGTAAAATGGGTC SEQ ID NO:171	-17	-18.8	57.6	-1.8	0	-1.7
1058	TGGTTCATTGGTATGCTTTT SEQ ID NO:172	-17	-22.8	69.1	-5.8	0.5	-3.6
150	GAGATTAGAACTTTCATCGC SEQ ID NO:173	-16.9	-20.3	61.6	-3.4	0	-4.2
154	GGAAGAGATTAGAACTTTCA SEQ ID NO:174	-16.9	-18.4	57.6	-0.6	-0.4	-4.6

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
164	ACAGGAGGAGGGAAGAGATT SEQ ID NO:175	-16.9	-22.1	65.7	-5.2	0	-1.3
555	AGGTGTGCTCACTGTCTTCT SEQ ID NO:176	-16.9	-26.4	80.3	-7.5	-2	-4.2
619	GCACTGGAATGATTTAGGGG SEQ ID NO:177	-16.9	-22.8	66.5	-5.9	0	-3.4
967	ATGGAGACAGAGTGAGGGTC SEQ ID NO:178	-16.9	-23.7	71.6	-5.9	-0.8	-5.2
983	ATGAACAAGTAGGCCAATGG SEQ ID NO:179	-16.9	-21.2	61.6	-3.8	0	-7.7
1066	ACCGTGTCTGGTTCATTGGT SEQ ID NO:180	-16.9	-26.8	77.3	-9	-0.7	-4.7
610	TGATTTAGGGGTGGGTACAG SEQ ID NO:181	-16.6	-23.5	70.1	-6.2	-0.4	-5.2
679	AGAAGAAGACACTAGAGAGA SEQ ID NO:182	-16.6	-17.9	56.4	-1.2	0	-4.5
906	AGTGAATAGGGTAAAATGGG SEQ ID NO:183	-16.6	-18.4	56.5	-1.8	0	-1.2
1057	GGTTCATTGGTATGCTTTTT SEQ ID NO:184	-16.6	-22.9	69.7	-5.8	-0.1	-3.6
142	AACTTTCATCGCAACTGTCG SEQ ID NO:185	-16.4	-22.2	63.8	-5.8	0	-4.1
153	GAAGAGATTAGAACTTTCAT SEQ ID NO:186	-16.4	-17.2	55	-0.6	0	-4.6
177	ATTAGTGGCAGCAACAGGAG SEQ ID NO:187	-16.4	-23.2	68.4	-6.8	0	-2.4
687	CTGACGAGAGAAGAAGACAC SEQ ID NO:188	-16.4	-19.2	57.8	-2.8	0	-3.5
973	AGGCCAATGGAGACAGAGTG SEQ ID NO:189	-16.4	-24.1	69.4	-6.7	-0.8	-9.2
149	AGATTAGAACTTTCATCGCA SEQ ID NO:190	-16.3	-20.4	61.5	-4.1	0	-4.2
231	CTGTGGCCTCTGGCGACCCC SEQ ID NO:191	-16.3	-33.9	87.2	-17.6	1.9	-7.3
237	CGGTCCCTGTGGCCTCTGGC SEQ ID NO:192	-16.3	-33.9	90.1	-16	-1.5	-7.2
559	TGGTAGGTGTGCTCACTGTC SEQ ID NO:193	-16.3	-26.2	79.5	-7.9	-2	-4.2
616	CTGGAATGATTTAGGGGTGG SEQ ID NO:194	-16.3	-22.5	66.2	-6.2	0	-2.3
618	CACTGGAATGATTTAGGGGT SEQ ID NO:195	-16.3	-22.2	65.5	-5.9	0	-2.3
932	ATGTGTAGAATCTGGATTCA SEQ ID NO:196	-16.3	-20.3	62.8	-2.1	-1.7	-11
937	TAAGTATGTGTAGAATCTGG SEQ ID NO:197	-16.3	-18.4	58.4	-2.1	0	-4
984	GATGAACAAGTAGGCCAATG SEQ ID NO:198	-16.3	-20.6	60.4	-3.8	0	-7.7
985	AGATGAACAAGTAGGCCAAT SEQ ID NO:199	-16.3	-20.6	60.7	-3.8	0	-7.7
1054	TCATTGGTATGCTTTTTTTT SEQ ID NO:200	-16.3	-20.7	64.2	-3.9	-0.1	-3.6
99	AATATAATGGAAGGTTCCCT SEQ ID NO:201	-16.2	-20.9	61.3	-3.7	-0.8	-7.1
143	GAACTTTCATCGCAACTGTC SEQ ID NO:202	-16.2	-22	64.8	-5.8	0	-3.6
152	AAGAGATTAGAACTTTCATC SEQ ID NO:203	-16.2	-17	55	-0.6	0	-4.6
217	GACCCCTGGATTCAGGCTGC SEQ ID NO:204	-16.2	-30.4	82.4	-11.5	-2.7	-9.6
429	TGCTTGTTTGGCTTTCTGTG SEQ ID NO:205	-16.2	-24.8	74.3	-7.7	-0.7	-3.7
430	ATGCTTGTTTGGCTTTCTGT SEQ ID NO:206	-16.2	-24.8	74.4	-7.7	-0.7	-3.7

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
718	AGCCTGGGTAAGGGGAGGGC SEQ ID NO:207	-16.2	-29.7	82.6	-12.1	-1.3	-6.7
933	TATGTGTAGAATCTGGATTC SEQ ID NO:208	-16.2	-19.3	60.9	-2.1	-0.6	-9.7
971	GCCAATGGAGACAGAGTGAG SEQ ID NO:209	-16.2	-23.5	68.1	-6.7	-0.3	-6.3
270	CCTTCCTGGAGCCATCTCCT SEQ ID NO:210	-16.1	-31.2	84.7	-11.9	-3.2	-7.4
398	GTCTTGTTTTCTTCACATTG SEQ ID NO:211	-16.1	-21.6	67.5	-5.5	0	-2.7
558	GGTAGGTGTGCTCACTGTCT SEQ ID NO:212	-16.1	-27.1	81.9	-9.7	-1.2	-3.4
886	TCATCTGGTTGTGAATTGGC SEQ ID NO:213	-16.1	-23.1	69.1	-7	0	-3.1
974	TAGGCCAATGGAGACAGAGT SEQ ID NO:214	-16.1	-23.8	69	-6.7	-0.8	-9.2
480	GCAAAGCTTCTTAGCTGACA SEQ ID NO:215	-16	-23.2	68	-4.8	-2.4	-8.1
569	GAAGAGTGTCTGGTAGGTGT SEQ ID NO:216	-16	-23.9	73.5	-7.9	0	-2.9
604	AGGGGTGGGTACAGTGGGAG SEQ ID NO:217	-16	-27.2	79.4	-10.5	-0.4	-5.2
100	GAATATAATGGAAGGTTCCC SEQ ID NO:218	-15.9	-20.6	60.7	-3.7	-0.8	-7.1
609	GATTTAGGGGTGGGTACAGT SEQ ID NO:219	-15.9	-24.7	73.9	-8.1	-0.4	-5.2
130	AACTGTCGGTGCAGCTGTAA SEQ ID NO:220	-15.8	-24.6	70.6	-7.3	-1.3	-9.9
144	AGAACTTTCATCGCAACTGT SEQ ID NO:221	-15.8	-21.6	63.6	-5.8	0	-4.2
481	AGCAAAGCTTCTTAGCTGAC SEQ ID NO:222	-15.8	-22.5	67.1	-4.8	-1.9	-8.8
863	CCCCATTTGAAGGAAACAAT SEQ ID NO:223	-15.8	-21.4	60.1	-5.6	0	-3.4
103	GAAGAATATAATGGAAGGTT SEQ ID NO:224	-15.7	-16.1	51.7	0	0	-2.5
218	CGACCCCTGGATTCAGGCTG SEQ ID NO:225	-15.7	-29.4	77.8	-11.5	-2.2	-9.1
221	TGGCGACCCCTGGATTCAGG SEQ ID NO:226	-15.7	-29.7	78.4	-11.5	-2.5	-11
939	GATAAGTATGTGTAGAATCT SEQ ID NO:227	-15.7	-17.8	57.1	-2.1	0	-3.6
944	GTGGGGATAAGTATGTGTAG SEQ ID NO:228	-15.7	-21.4	65.7	-5.7	0	-1.8
993	TGAGTGAAAGATGAACAAGT SEQ ID NO:229	-15.7	-16.9	53.4	-1.1	0	-2.9
1002	TTTGTCGAATGAGTGAAAGA SEQ ID NO:230	-15.7	-18.1	55.9	-2.4	0	-5
63	CCCTGGGGATGACTCAGGTC SEQ ID NO:231	-15.6	-28.7	80.3	-10	-3.1	-9
104	TGAAGAATATAATGGAAGGT SEQ ID NO:232	-15.6	-16	51.4	0	0	-2.7
133	CGCAACTGTCGGTGCAGCTG SEQ ID NO:233	-15.6	-27.7	75.4	-10.5	-1.6	-8.3
1001	TTGTCGAATGAGTGAAAGAT SEQ ID NO:234	-15.6	-18	55.6	-2.4	0	-5
717	GCCTGGGTAAGGGGAGGGCA SEQ ID NO:235	-15.5	-30.4	83.3	-13.4	-1.4	-7
990	GTGAAAGATGAACAAGTAGG SEQ ID NO:236	-15.5	-17.2	54.1	-1.7	0	-2.9
1000	TGTCGAATGAGTGAAAGATG SEQ ID NO:237	-15.5	-17.9	55.3	-2.4	0	-5
178	CATTAGTGGCAGCAACAGGA SEQ ID NO:238	-15.4	-23.9	69.3	-8.5	0	-1.6

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
236	GGTCCCTGTGGCCTCTGGCG SEQ ID NO:239	-15.4	-33.9	90.1	-16	-2.5	-7.7
475	GCTTCTTAGCTGACATTGTT SEQ ID NO:240	-15.4	-23.5	70.9	-6.8	-1.2	-7.2
980	AACAAGTAGGCCAATGGAGA SEQ ID NO:241	-15.4	-21.8	63.2	-5.9	0	-7.7
992	GAGTGAAAGATGAACAAGTA SEQ ID NO:242	-15.4	-16.6	52.9	-1.1	0	-2.9
94	AATGGAAGGTTCCCTGCTGG SEQ ID NO:243	-15.3	-26.1	72.6	-9.9	-0.8	-7.1
488	AAGGCAGAGCAAAGCTTCTT SEQ ID NO:244	-15.3	-22.9	67	-6.6	-0.9	-7.7
1055	TTCATTGGTATGCTTTTTTT SEQ ID NO:245	-15.3	-20.7	64.2	-4.9	-0.1	-3.6
90	GAAGGTTCCCTGCTGGAGGC SEQ ID NO:246	-15.2	-29.2	81.2	-13.1	-0.8	-7.8
98	ATATAATGGAAGGTTCCCTG SEQ ID NO:247	-15.2	-21.6	63.2	-5.5	-0.8	-7.1
484	CAGAGCAAAGCTTCTTAGCT SEQ ID NO:248	-15.2	-23	68.1	-5.6	-2.2	-8.5
603	GGGGTGGGTACAGTGGGAGA SEQ ID NO:249	-15.1	-27.8	80.5	-12	-0.4	-5.2
938	ATAAGTATGTGTAGAATCTG SEQ ID NO:250	-15.1	-17.2	55.7	-2.1	0	-4
1003	ATTTGTCGAATGAGTGAAAG SEQ ID NO:251	-15.1	-17.5	54.7	-2.4	0	-4.5
474	CTTCTTAGCTGACATTGTTT SEQ ID NO:252	-15	-21.8	66.8	-6.8	0	-5.3
678	GAAGAAGACACTAGAGAGAG SEQ ID NO:253	-15	-17.9	56.4	-2.9	0	-4.5
975	GTAGGCCAATGGAGACAGAG SEQ ID NO:254	-15	-23.8	69	-7.8	-0.8	-9.2
28	GTGGTCTATGCTTTAGTCCC SEQ ID NO:255	-14.9	-26.8	79.2	-11.9	0	-4
66	GATCCCTGGGGATGACTCAG SEQ ID NO:256	-14.9	-26.9	75.5	-10	-1.4	-11.9
482	GAGCAAAGCTTCTTAGCTGA SEQ ID NO:257	-14.9	-22.9	67.8	-5.6	-2.4	-8.8
847	CAATTTTGATCTGTGACATT SEQ ID NO:258	-14.9	-19	58.8	-4.1	0	-4.9
134	TCGCAACTGTCGGTGCAGCT SEQ ID NO:259	-14.8	-28.1	77.2	-11.7	-1.6	-8.4
620	AGCACTGGAATGATTTAGGG SEQ ID NO:260	-14.8	-21.6	64.1	-6.8	0	-4.1
858	TTTGAAGGAAACAATTTTGA SEQ ID NO:261	-14.8	-15.6	50.5	-0.6	0	-4.4
991	AGTGAAAGATGAACAAGTAG SEQ ID NO:262	-14.8	-16	51.8	-1.1	0	-2.9
1046	ATGCTTTTTTTTTTTTGTCC SEQ ID NO:263	-14.8	-21.4	65.9	-6.6	0	-3.6
1069	AAGACCGTGTCTGGTTCATT SEQ ID NO:264	-14.8	-24.3	70.5	-8.1	-1.3	-8.3
1077	TCTTTAATAAGACCGTGTCT SEQ ID NO:265	-14.8	-20.8	62.2	-4.8	-1.1	-8
483	AGAGCAAAGCTTCTTAGCTG SEQ ID NO:266	-14.7	-22.3	66.7	-5.2	-2.4	-8.8
885	CATCTGGTTGTGAATTGGCA SEQ ID NO:267	-14.7	-23.4	68.7	-8.7	0	-4
91	GGAAGGTTCCCTGCTGGAGG SEQ ID NO:268	-14.6	-28.6	79.4	-13.1	-0.8	-6.8
102	AAGAATATAATGGAAGGTTC SEQ ID NO:269	-14.6	-15.9	51.7	-1.2	0	-3.3
165	AACAGGAGGAGGGAAGAGAT SEQ ID NO:270	-14.6	-21.3	63.2	-6.7	0	-1.1

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
476	AGCTTCTTAGCTGACATTGT SEQ ID NO:271	-14.6	-23.4	70.8	-6.8	-2	-7.7
711	GTAAGGGGAGGGCACAGGCT SEQ ID NO:272	-14.6	-28.1	79.4	-12.1	-1.3	-4
994	ATGAGTGAAAGATGAACAAG SEQ ID NO:273	-14.5	-15.7	50.7	-1.1	0	-2.9
968	AATGGAGACAGAGTGAGGGT SEQ ID NO:274	-14.4	-22.6	67.5	-7.3	-0.8	-3.7
1070	TAAGACCGTGTCTGGTTCAT SEQ ID NO:275	-14.4	-23.9	69.5	-8.1	-1.3	-8.3
1071	ATAAGACCGTGTCTGGTTCA SEQ ID NO:276	-14.4	-23.9	69.5	-8.1	-1.3	-8.3
145	TAGAACTTTCATCGCAACTG SEQ ID NO:277	-14.3	-20.1	60	-5.8	0	-4.2
431	AATGCTTGTTTGGCTTTCTG SEQ ID NO:278	-14.3	-22.9	68.4	-7.7	-0.7	-3.7
712	GGTAAGGGGAGGGCACAGGC SEQ ID NO:279	-14.3	-28.4	80	-13.4	-0.5	-4
4	CAGCGTTCCCATTTGAGGGC SEQ ID NO:280	-14.2	-28.7	78.6	-13.2	-1.2	-9.2
101	AGAATATAATGGAAGGTTCC SEQ ID NO:281	-14.2	-18.6	57.2	-3.7	-0.4	-6.7
844	TTTTGATCTGTGACATTTAA SEQ ID NO:282	-14.2	-18.1	57.3	-3.9	0	-4.9
907	CAGTGAATAGGGTAAAATGG SEQ ID NO:283	-14.2	-17.9	55.3	-3.7	0	-3.1
89	AAGGTTCCCTGCTGGAGGCT SEQ ID NO:284	-14.1	-29.5	81.8	-14	-1.3	-8
93	ATGGAAGGTTCCCTGCTGGA SEQ ID NO:285	-14.1	-27.4	76.3	-12.4	-0.8	-7.1
688	ACTGACGAGAGAAGAAGACA SEQ ID NO:286	-14.1	-19.2	57.8	-5.1	0	-3.4
869	GGCAGACCCCATTTGAAGGA SEQ ID NO:287	-14.1	-27.1	73.5	-13	0	-4
979	ACAAGTAGGCCAATGGAGAC SEQ ID NO:288	-14.1	-22.7	65.8	-8.1	0	-7.7
491	ACAAAGGCAGAGCAAAGCTT SEQ ID NO:289	-13.9	-21.7	62.9	-6.8	-0.9	-7.5
676	AGAAGACACTAGAGAGAGCA SEQ ID NO:290	-13.9	-20.5	62.6	-6.6	0	-4.5
95	TAATGGAAGGTTCCCTGCTG SEQ ID NO:291	-13.8	-24.6	69.6	-9.9	-0.8	-7.1
269	CTTCCTGGAGCCATCTCCTA SEQ ID NO:292	-13.8	-28.9	80.6	-11.9	-3.2	-7.4
489	AAAGGCAGAGCAAAGCTTCT SEQ ID NO:293	-13.8	-22.1	64.5	-7.3	-0.9	-7.7
864	ACCCCATTTGAAGGAAACAA SEQ ID NO:294	-13.8	-21.6	60.5	-7.8	0	-3.4
1078	ATCTTTAATAAGACCGTGTC SEQ ID NO:295	-13.8	-19.9	60.3	-4.8	-1.2	-6.8
148	GATTAGAACTTTCATCGCAA SEQ ID NO:296	-13.7	-19.7	59.3	-6	0	-3.6
394	TGTTTTCTTCACATTGCCCT SEQ ID NO:297	-13.7	-25.7	74.2	-12	0	-3
719	AAGCCTGGGTAAGGGGAGGG SEQ ID NO:298	-13.7	-27.2	75.7	-12.1	-1.3	-5.2
913	AGTCTGCAGTGAATAGGGTA SEQ ID NO:299	-13.7	-23.1	70.1	-8.8	0	-8.6
105	TTGAAGAATATAATGGAAGG SEQ ID NO:300	-13.6	-14.9	49.1	-1.2	0	-2.7
213	CCTGGATTCAGGCTGCTAGA SEQ ID NO:301	-13.6	-26.8	76.5	-11	-2.2	-9.4
216	ACCCCTGGATTCAGGCTGCT SEQ ID NO:302	-13.6	-30.7	83	-14.4	-2.7	-9.6

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
272	CGCCTTCCTGGAGCCATCTC SEQ ID NO:303	-13.6	-30.9	83.1	-16.4	-0.7	-6.7
363	CAGGGGCACTGCTTCTTTGG SEQ ID NO:304	-13.6	-27.4	78.2	-13.1	-0.5	-6
368	GATCACAGGGGCACTGCTTC SEQ ID NO:305	-13.6	-27	77.8	-12.7	-0.5	-7.7
492	TACAAAGGCAGAGCAAAGCT SEQ ID NO:306	-13.6	-21.3	62.1	-6.8	-0.7	-5.7
557	GTAGGTGTGCTCACTGTCTT SEQ ID NO:307	-13.6	-26	79.4	-10.4	-2	-4.2
677	AAGAAGACACTAGAGAGAGC SEQ ID NO:308	-13.6	-19.1	59.2	-5.5	0	-4.5
998	TCGAATGAGTGAAAGATGAA SEQ ID NO:309	-13.6	-16.6	52.1	-3	0	-4.2
1045	TGCTTTTTTTTTTTTGTCCC SEQ ID NO:310	-13.6	-23.4	69.9	-9.8	0	-3.6
1056	GTTCATTGGTATGCTTTTTT SEQ ID NO:311	-13.6	-21.8	67.3	-7.7	-0.1	-3.6
88	AGGTTCCCTGCTGGAGGCTC SEQ ID NO:312	-13.5	-30.6	86.6	-15.9	-1.1	-8
128	CTGTCGGTGCAGCTGTAAGT SEQ ID NO:313	-13.5	-26.3	76.2	-12	-0.4	-8.9
188	TGGACATCAGCATTAGTGGC SEQ ID NO:314	-13.5	-24.3	71.7	-10.8	0	-4.1
274	GCCGCCTTCCTGGAGCCATC SEQ ID NO:315	-13.5	-33.4	87	-19.2	-0.4	-6.7
289	GCACTCACATTCTTGGCCGC SEQ ID NO:316	-13.5	-28.7	78.9	-14.7	0	-7.6
92	TGGAAGGTTCCCTGCTGGAG SEQ ID NO:317	-13.4	-27.4	76.6	-13.1	-0.8	-7.1
601	GGTGGGTACAGTGGGAGAGT SEQ ID NO:318	-13.4	-26.6	79.1	-12.5	-0.4	-4.6
602	GGGTGGGTACAGTGGGAGAG SEQ ID NO:319	-13.4	-26.6	78.1	-12.5	-0.4	-5.2
617	ACTGGAATGATTTAGGGGTG SEQ ID NO:320	-13.4	-21.5	64.2	-8.1	0	-2.3
843	TTTGATCTGTGACATTTAAA SEQ ID NO:321	-13.4	-17.3	55	-3.9	0	-4.9
853	AGGAAACAATTTTGATCTGT SEQ ID NO:322	-13.3	-18	56.1	-4.7	0	-5.8
67	TGATCCCTGGGGATGACTCA SEQ ID NO:323	-13.2	-26.9	75	-11.7	-1.4	-11.9
179	GCATTAGTGGCAGCAACAGG SEQ ID NO:324	-13.2	-25.1	72.2	-11.9	0	-2.4
366	TCACAGGGGCACTGCTTCTT SEQ ID NO:325	-13.2	-27.4	78.9	-12.7	-1.4	-6.5
397	TCTTGTTTTCTTCACATTGC SEQ ID NO:326	-13.2	-22.2	68.6	-9	0	-2.7
857	TTGAAGGAAACAATTTTGAT SEQ ID NO:327	-13.2	-15.5	50.2	-2.3	0	-4.4
62	CCTGGGGATGACTCAGGTCA SEQ ID NO:328	-13.1	-27.4	77.8	-11.7	-2.6	-8
97	TATAATGGAAGGTTCCCTGC SEQ ID NO:329	-13.1	-23.4	67.2	-9.4	-0.8	-7.1
367	ATCACAGGGGCACTGCTTCT SEQ ID NO:330	-13.1	-27.3	78.5	-12.7	-1.4	-6.5
710	TAAGGGGAGGGCACAGGCTA SEQ ID NO:331	-13.1	-26.6	75.2	-12.1	-1.3	-4
882	CTGGTTGTGAATTGGCAGAC SEQ ID NO:332	-13.1	-23.1	68.1	-10	0	-4
1079	TATCTTTAATAAGACCGTGT SEQ ID NO:333	-13.1	-19.2	58.4	-4.8	-1.2	-6
393	GTTTTCTTCACATTGCCCTT SEQ ID NO:334	-13	-25.8	74.7	-12.8	0	-3

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
570	AGAAGAGTGTCTGGTAGGTG SEQ ID NO:335	-13	-22.7	70	-9.7	0	-2.9
859	ATTTGAAGGAAACAATTTTG SEQ ID NO:336	-13	-15	49.3	-2	0	-3.9
914	CAGTCTGCAGTGAATAGGGT SEQ ID NO:337	-13	-24.1	71.9	-10.5	0	-8.6
395	TTGTTTTCTTCACATTGCCC SEQ ID NO:338	-12.9	-24.9	72.6	-12	0	-3
931	TGTGTAGAATCTGGATTCAG SEQ ID NO:339	-12.9	-20.3	63	-5.6	-1.7	-11
976	AGTAGGCCAATGGAGACAGA SEQ ID NO:340	-12.9	-23.8	69	-9.9	-0.8	-9.2
1004	GATTTGTCGAATGAGTGAAA SEQ ID NO:341	-12.9	-18.1	55.8	-5.2	0	-5
1067	GACCGTGTCTGGTTCATTGG SEQ ID NO:342	-12.9	-26.2	75.1	-11.9	-1.3	-7.8
129	ACTGTCGGTGCAGCTGTAAG SEQ ID NO:343	-12.8	-25.3	73.3	-11	-1.3	-9.9
845	ATTTTGATCTGTGACATTTA SEQ ID NO:344	-12.8	-18.8	59.3	-6	0	-4.2
852	GGAAACAATTTTGATCTGTG SEQ ID NO:345	-12.8	-18	55.9	-4.7	-0.2	-5.8
870	TGGCAGACCCCATTTGAAGG SEQ ID NO:346	-12.8	-26.5	72.1	-13	-0.5	-4.4
988	GAAAGATGAACAAGTAGGCC SEQ ID NO:347	-12.8	-19.8	58.9	-7	0	-6.4
573	AGAAGAAGAGTGTCTGGTAG SEQ ID NO:348	-12.7	-20.2	63.1	-7.5	0	-2.9
930	GTGTAGAATCTGGATTCAGT SEQ ID NO:349	-12.7	-21.5	66.5	-7.4	-1.1	-10.2
1044	GCTTTTTTTTTTTTTGTCCCA SEQ ID NO:350	-12.7	-24.1	71.2	-11.4	0	-2.8
75	GAGGCTCCTGATCCCTGGGG SEQ ID NO:351	-12.6	-31.3	84.9	-18.1	-0.2	-8.2
238	TCGGTCCCTGTGGC2CTGG SEQ ID NO:352	-12.6	-32.5	87.6	-18.3	-1.5	-7.2
795	TCCTGATTGCATTT3AGGTT SEQ ID NO:353	-12.6	-22.2	66	-9.1	-0.1	-5.4
796	TTCCTGATTGCATT4AAGGT SEQ ID NO:354	-12.6	-22.2	66	-9.1	-0.1	-5.4
842	TTGATCTGTGACAT5TAAAA SEQ ID NO:355	-12.6	-16.5	52.9	-3.9	0	-5
865	GACCCCATTTGAAG6AAACA SEQ ID NO:356	-12.6	-22.9	63.5	-10.3	0	-3.4
943	TGGGGATAAGTATGTGTAGA SEQ ID NO:357	-12.6	-20.8	63.8	-8.2	0	-1.6
989	TGAAAGATGAACAAGTAGGC SEQ ID NO:358	-12.6	-17.8	55.2	-5.2	0	-2.9
999	GTCGAATGAGTGAAAGATGA SEQ ID NO:359	-12.6	-18.5	56.6	-5.9	0	-5
9	CAGGCCAGCGTTCCCATTTG SEQ ID NO:360	-12.5	-29.6	79.2	-16.6	0	-7.7
215	CCCCTGGATTCAGGCTGCTA SEQ ID NO:361	-12.5	-30.2	81.8	-15	-2.7	-9.6
8	AGGCCAGCGTTCCCATTTGA SEQ ID NO:362	-12.4	-29.5	79.5	-16.6	0	-7.7
96	ATAATGGAAGGTTCCCTGCT SEQ ID NO:363	-12.4	-24.6	69.7	-11.5	-0.4	-6.4
369	TGATCACAGGGGCACTGCTT SEQ ID NO:364	-12.4	-26.6	75.8	-12.7	-1.4	-7.5
391	TTTCTTCACATTGCCCTTGA SEQ ID NO:365	-12.4	-25.1	72.1	-12.7	0	-3
479	CAAAGCTTCTTAGCTGACAT SEQ ID NO:366	-12.4	-21.4	63.8	-6.6	-2.4	-7

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
522	TTAATTGGAAGAGTGGGCGC SEQ ID NO:367	-12.4	-22.9	65.9	-10.5	0	-7.2
794	CCTGATTGCATTTAAGGTTA SEQ ID NO:368	-12.4	-21.5	63.9	-9.1	0	-5.1
27	TGGTCTATGCTTTAGTCCCA SEQ ID NO:369	-12.3	-26.3	76.6	-13	-0.9	-5.7
370	ATGATCACAGGGGCACTGCT SEQ ID NO:370	-12.3	-26.5	75.4	-12.7	-1.4	-8.7
551	GTGCTCACTGTCTTCTTGGC SEQ ID NO:371	-12.3	-27.1	81.3	-14.8	0	-4.7
912	GTCTGCAGTGAATAGGGTAA SEQ ID NO:372	-12.3	-22.4	67.4	-9.5	0	-8.6
74	AGGCTCCTGATCCCTGGGGA SEQ ID NO:373	-12.2	-31.3	84.9	-18.1	-0.2	-9.9
110	GTTGCTTGAAGAATATAATG SEQ ID NO:374	-12.2	-16.6	53.1	-4.4	0	-3.6
111	AGTTGCTTGAAGAATATAAT SEQ ID NO:375	-12.2	-16.6	53.3	-4.4	0	-3.6
187	GGACATCAGCATTAGTGGCA SEQ ID NO:376	-12.2	-25	73	-11.9	-0.8	-4.1
234	TCCCTGTGGCCTCTGGCGAC SEQ ID NO:377	-12.2	-32.3	85.8	-17.6	-2.5	-8.6
521	TAATTGGAAGAGTGGGCGCT SEQ ID NO:378	-12.2	-23.7	67.4	-11	-0.1	-8.1
689	GACTGACGAGAGAAGAAGAC SEQ ID NO:379	-12.2	-19.1	57.8	-6.9	0	-3.5
868	GCAGACCCCATTTGAAGGAA SEQ ID NO:380	-12.2	-25.2	69	-13	0	-3.4
878	TTGTGAATTGGCAGACCCCA SEQ ID NO:381	-12.2	-26.5	72.4	-13.6	-0.5	-4
969	CAATGGAGACAGAGTGAGGG SEQ ID NO:382	-12.2	-22.1	65.4	-9	-0.8	-4.5
1076	CTTTAATAAGACCGTGTCTG SEQ ID NO:383	-12.2	-20.4	60.8	-6.8	-1.3	-8.3
275	GGCCGCCTTCCTGGAGCCAT SEQ ID NO:384	-12.1	-34.2	87.6	-19.2	-2.9	-9.6
364	ACAGGGGCACTGCTTCTTTG SEQ ID NO:385	-12.1	-26.4	76.2	-12.8	-1.4	-6.5
675	GAAGACACTAGAGAGAGCAA SEQ ID NO:386	-12.1	-19.8	60.3	-7.7	0	-4.5
690	AGACTGACGAGAGAAGAAGA SEQ ID NO:387	-12.1	-18.9	57.5	-6.8	0	-3.5
877	TGTGAATTGGCAGACCCCAT SEQ ID NO:388	-12.1	-26.4	72.1	-13.6	-0.5	-4
940	GGATAAGTATGTGTAGAATC SEQ ID NO:389	-12.1	-18.1	57.8	-6	0	-2.7
549	GCTCACTGTCTTCTTGGCTG SEQ ID NO:390	-12	-26.8	79.5	-14.8	0	-3.7
553	GTGTGCTCACTGTCTTCTTG SEQ ID NO:391	-12	-25.3	77.2	-12	-1.2	-3.3
978	CAAGTAGGCCAATGGAGACA SEQ ID NO:392	-12	-23.2	66.4	-10.3	-0.6	-8.9
1080	TTATCTTTAATAAGACCGTG SEQ ID NO:393	-12	-18.1	55.9	-4.8	-1.2	-6
1081	ATTATCTTTAATAAGACCGT SEQ ID NO:394	-12	-18.1	55.9	-4.8	-1.2	-6
113	TAAGTTGCTTGAAGAATATA SEQ ID NO:395	-11.9	-16.3	52.7	-4.4	0	-4.3
273	CCGCCTTCCTGGAGCCATCT SEQ ID NO:396	-11.9	-32.5	84.6	-20	-0.3	-6.7
874	GAATTGGCAGACCCCATTTG SEQ ID NO:397	-11.9	-25.4	69.8	-13	-0.2	-4
520	AATTGGAAGAGTGGGCGCTC SEQ ID NO:398	-11.8	-24.4	69.5	-11	-1.6	-8.3

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
840	GATCTGTGACATTTAAAAAT SEQ ID NO:399	-11.8	-15.7	51	-3.9	0	-5
841	TGATCTGTGACATTTAAAAA SEQ ID NO:400	-11.8	-15.7	50.9	-3.9	0	-5
29	GGTGGTCTATGCTTTAGTCC SEQ ID NO:401	-11.7	-26	78.2	-14.3	0	-3.9
87	GGTTCCCTGCTGGAGGCTCC SEQ ID NO:402	-11.7	-32.6	89.7	-19.7	-1.1	-8
106	CTTGAAGAATATAATGGAAG SEQ ID NO:403	-11.7	-14.6	48.5	-2.9	0	-2.7
181	CAGCATTAGTGGCAGCAACA SEQ ID NO:404	-11.7	-24.6	70.8	-12	-0.8	-2.4
189	ATGGACATCAGCATTAGTGG SEQ ID NO:405	-11.7	-22.5	67.2	-10.8	0	-4.1
290	TGCACTCACATTCTTGGCCG SEQ ID NO:406	-11.7	-26.9	74.5	-14.7	0	-7.6
750	GTTTCCTGGAATCTTTCAGG SEQ ID NO:407	-11.7	-23.6	70.2	-10.1	-1.8	-8.8
871	TTGGCAGACCCCATTTGAAG SEQ ID NO:408	-11.7	-25.4	70.1	-13	-0.5	-4
872	ATTGGCAGACCCCATTTGAA SEQ ID NO:409	-11.7	-25.4	69.8	-13	-0.5	-4
873	AATTGGCAGACCCCATTTGA SEQ ID NO:410	-11.7	-25.4	69.8	-13	-0.5	-4
996	GAATGAGTGAAAGATGAACA SEQ ID NO:411	-11.7	-16.3	51.8	-4.6	0	-2.9
1005	AGATTTGTCGAATGAGTGAA SEQ ID NO:412	-11.7	-18.8	57.8	-6.2	-0.7	-5
304	CAGGAACCAATCTTTGCACT SEQ ID NO:413	-11.6	-23.1	66	-11	-0.1	-7.8
390	TTCTTCACATTGCCCTTGAA SEQ ID NO:414	-11.6	-24.3	69.4	-12.7	0	-3.5
571	AAGAAGAGTGTCTGGTAGGT SEQ ID NO:415	-11.6	-22	67.7	-10.4	0	-2.9
645	GATCTTGAAAAACATGCTTT SEQ ID NO:416	-11.6	-17.6	54.6	-6	0	-5
724	AGCCTAAGCCTGGGTAAGGG SEQ ID NO:417	-11.6	-27.4	75.8	-14.4	-1.3	-8.2
846	AATTTTGATCTGTGACATTT SEQ ID NO:418	-11.6	-18.4	57.9	-6.8	0	-4.9
1008	GAAAGATTTGTCGAATGAGT SEQ ID NO:419	-11.6	-18.1	56	-5.6	-0.7	-5
112	AAGTTGCTTGAAGAATATAA SEQ ID NO:420	-11.5	-15.9	51.5	-4.4	0	-2.9
214	CCCTGGATTCAGGCTGCTAG SEQ ID NO:421	-11.5	-28.2	78.7	-14	-2.7	-9.6
396	CTTGTTTTCTTCACATTGCC SEQ ID NO:422	-11.5	-23.8	70.8	-12.3	0	-3
550	TGCTCACTGTCTTCTTGGCT SEQ ID NO:423	-11.5	-26.8	79.5	-14.8	-0.1	-3.7
908	GCAGTGAATAGGGTAAAATG SEQ ID NO:424	-11.5	-18.5	56.7	-7	0	-4.2
127	TGTCGGTGCAGCTGTAAGTT SEQ ID NO:425	-11.4	-25.5	74.6	-13.4	0	-8.9
182	TCAGCATTAGTGGCAGCAAC SEQ ID NO:426	-11.4	-24.3	71.3	-12	-0.8	-5.8
276	TGGCCGCCTTCCTGGAGCCA SEQ ID NO:427	-11.4	-34.2	87.4	-19.2	-3.6	-10.7
621	GAGCACTGGAATGATTTAGG SEQ ID NO:428	-11.4	-21	62.9	-9.6	0	-4.1
709	AAGGGGAGGGCACAGGCTAA SEQ ID NO:429	-11.4	-26.2	73.4	-13.4	-1.3	-4
749	TTTCCTGGAATCTTTCAGGT SEQ ID NO:430	-11.4	-23.6	70.2	-10.1	-2.1	-8.9

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
851	GAAACAATTTTGATCTGTGA SEQ ID NO:431	-11.4	-17.4	54.7	-5.5	-0.2	-5.8
921	CTGGATTCAGTCTGCAGTGA SEQ ID NO:432	-11.4	-24.7	73.9	-11.8	-0.5	-10.9
997	CGAATGAGTGAAAGATGAAC SEQ ID NO:433	-11.4	-16.4	51.5	-5	0	-2
68	CTGATCCCTGGGGATGACTC SEQ ID NO:434	-11.3	-27.1	75.9	-13.8	-1.4	-11.9
277	TTGGCCGCCTTCCTGGAGCC SEQ ID NO:435	-11.3	-33.6	86.9	-19.8	-2.5	-10
303	AGGAACCAATCTTTGCACTC SEQ ID NO:436	-11.3	-22.8	66.3	-11	-0.1	-7.8
352	CTTCTTTGGCAGCCCAGACA SEQ ID NO:437	-11.3	-28.2	78.5	-15.8	-1	-8.1
362	AGGGGCACTGCTTCTTTGGC SEQ ID NO:438	-11.3	-28.5	81.7	-16.5	-0.4	-6.3
876	GTGAATTGGCAGACCCCATT SEQ ID NO:439	-11.3	-26.5	72.6	-14.5	-0.5	-4
26	GGTCTATGCTTTAGTCCCAG SEQ ID NO:440	-11.2	-26.3	77.2	-14.6	-0.2	-4.6
264	TGGAGCCATCTCCTAGAAGC SEQ ID NO:441	-11.2	-26.3	74.8	-11.9	-3.2	-8.6
262	GAGCCATCTCCTAGAAGCCT SEQ ID NO:442	-11.1	-28	77.9	-15.9	-0.9	-5.6
456	TTGAGAAATTGCTGGCAGGC SEQ ID NO:443	-11.1	-23.4	67.6	-11.5	-0.3	-9
478	AAAGCTTCTTAGCTGACATT SEQ ID NO:444	-11.1	-20.8	62.9	-7.3	-2.4	-7
705	GGAGGGCACAGGCTAAGACT SEQ ID NO:445	-11.1	-26.2	74.5	-14.4	-0.5	-4.3
5	CCAGCGTTCCCATTTGAGGG SEQ ID NO:446	-11	-28.9	77.8	-16.8	-1	-9.2
40	ATACTCAGCCTGGTGGTCTA SEQ ID NO:447	-11	-26.4	77.5	-14.8	-0.3	-4.8
41	GATACTCAGCCTGGTGGTCT SEQ ID NO:448	-11	-27.3	79.6	-15.7	-0.3	-4.9
180	AGCATTAGTGGCAGCAACAG SEQ ID NO:449	-11	-23.9	69.9	-12	-0.8	-2.4
345	GGCAGCCCAGACACTGTCAT SEQ ID NO:450	-11	-29.1	80.5	-16.6	-1.4	-8.9
357	CACTGCTTCTTTGGCAGCCC SEQ ID NO:451	-11	-29.6	81.9	-15.5	-3.1	-8.1
446	GCTGGCAGGCTCTGGAATGC SEQ ID NO:452	-11	-28.5	80.1	-16.6	-0.7	-6
490	CAAAGGCAGAGCAAAGCTTC SEQ ID NO:453	-11	-21.9	63.8	-9.9	-0.9	-7.7
748	TTCCTGGAATCTTTCAGGTA SEQ ID NO:454	-11	-23.2	69.3	-10.1	-2.1	-8.9
1007	AAAGATTTGTCGAATGAGTG SEQ ID NO:455	-11	-17.5	54.7	-5.6	-0.7	-5
473	TTCTTAGCTGACATTGTTTG SEQ ID NO:456	-10.9	-20.9	64.6	-10	0	-5.1
523	TTTAATTGGAAGAGTGGGCG SEQ ID NO:457	-10.9	-21.2	62.2	-10.3	0	-4
720	TAAGCCTGGGTAAGGGGAGG SEQ ID NO:458	-10.9	-25.7	72.5	-13.4	-1.3	-4.9
838	TCTGTGACATTTAAAAATAT SEQ ID NO:459	-10.9	-14.8	49.2	-3.9	0	-5
839	ATCTGTGACATTTAAAAATA SEQ ID NO:460	-10.9	-14.8	49.2	-3.9	0	-5
922	TCTGGATTCAGTCTGCAGTG SEQ ID NO:461	-10.9	-24.5	74.3	-11.8	-1.1	-11.7
923	ATCTGGATTCAGTCTGCAGT SEQ ID NO:462	-10.9	-24.5	74.5	-11.8	-1.1	-11.7

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
960	CAGAGTGAGGGTCTTGGTGG SEQ ID NO:463	-10.9	-25.7	76.6	-14.8	0	-2.6
970	CCAATGGAGACAGAGTGAGG SEQ ID NO:464	-10.9	-22.9	66.5	-11.1	-0.8	-5.2
1068	AGACCGTGTCTGGTTCATTG SEQ ID NO:465	-10.9	-25	72.7	-12.7	-1.3	-7.5
1082	TATTATCTTTAATAAGACCG SEQ ID NO:466	-10.9	-16.6	52.7	-4.8	-0.7	-4.7
32	CCTGGTGGTCTATGCTTTAG SEQ ID NO:467	-10.8	-25.3	74.5	-14.5	0	-3.6
330	GTCATGAATTTTCTTCTCGG SEQ ID NO:468	-10.8	-21.6	65.2	-10.8	0.1	-6.7
432	GAATGCTTGTTTGGCTTTCT SEQ ID NO:469	-10.8	-23.5	69.9	-11	-1.7	-5.4
494	CCTACAAAGGCAGAGCAAAG SEQ ID NO:470	-10.8	-21.5	61.8	-9.8	-0.7	-4.6
691	AAGACTGACGAGAGAAGAAG SEQ ID NO:471	-10.8	-17.6	54.4	-6.8	0	-3.5
114	GTAAGTTGCTTGAAGAATAT SEQ ID NO:472	-10.7	-17.8	56.2	-7.1	0	-4.3
263	GGAGCCATCTCCTAGAAGCC SEQ ID NO:473	-10.7	-28.3	78.6	-15.1	-2.5	-8.2
358	GCACTGCTTCTTTGGCAGCC SEQ ID NO:474	-10.7	-29.4	82.9	-15.6	-3.1	-9.8
371	AATGATCACAGGGGCACTGC SEQ ID NO:475	-10.7	-24.9	71	-12.7	-1.4	-8.4
455	TGAGAAATTGCTGGCAGGCT SEQ ID NO:476	-10.7	-24.2	69.2	-12.3	-1.1	-7.5
647	ATGATCTTGAAAAACATGCT SEQ ID NO:477	-10.7	-17.4	54	-6.7	0	-5
755	CTACAGTTTCCTGGAATCTT SEQ ID NO:478	-10.7	-22.7	67.6	-10.6	-1.3	-4.6
797	TTTCCTGATTGCATTTAAGG SEQ ID NO:479	-10.7	-21.1	63.2	-10.4	0	-5.1
1006	AAGATTTGTCGAATGAGTGA SEQ ID NO:480	-10.7	-18.8	57.8	-7.2	-0.7	-5
239	CTCGGTCCCTGTGGCCTCTG SEQ ID NO:481	-10.6	-32.2	86.9	-20	-1.5	-7.2
267	TCCTGGAGCCATCTCCTAGA SEQ ID NO:482	-10.6	-28.5	80	-14.7	-3.2	-7.9
291	TTGCACTCACATTCTTGGCC SEQ ID NO:483	-10.6	-26.2	75	-15.6	0	-6.2
361	GGGGCACTGCTTCTTTGGCA SEQ ID NO:484	-10.6	-29.2	82.4	-17.3	-1.2	-7.2
365	CACAGGGGCACTGCTTCTTT SEQ ID NO:485	-10.6	-27.1	77.5	-15	-1.4	-6.5
519	ATTGGAAGAGTGGGCGCTCA SEQ ID NO:486	-10.6	-25.8	72.9	-12.5	-2.7	-10
644	ATCTTGAAAAACATGCTTTT SEQ ID NO:487	-10.6	-17.1	53.7	-6	-0.2	-7.1
856	TGAAGGAAACAATTTTGATC SEQ ID NO:488	-10.6	-15.8	51	-5.2	0	-5.8
881	TGGTTGTGAATTGGCAGACC SEQ ID NO:489	-10.6	-24.2	69.9	-12.9	-0.4	-4.7
147	ATTAGAACTTTCATCGCAAC SEQ ID NO:490	-10.5	-19.3	58.6	-8.8	0	-4.2
346	TGGCAGCCCAGACACTGTCA SEQ ID NO:491	-10.5	-29.1	80.3	-16.6	-2	-9.6
351	TTCTTTGGCAGCCCAGACAC SEQ ID NO:492	-10.5	-27.5	77.1	-16.1	-0.7	-8.1
708	AGGGGAGGGCACAGGCTAAG SEQ ID NO:493	-10.5	-26.9	76.1	-15	-1.3	-4
743	GGAATCTTTCAGGTAATTAA SEQ ID NO:494	-10.5	-18.2	57.1	-6.8	-0.8	-5.8

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
760	GGAAGCTACAGTTTCCTGGA SEQ ID NO:495	-10.5	-24.9	72.3	-12.9	-1.4	-9.1
1014	ACCTCAGAAAGATTTGTCGA SEQ ID NO:496	-10.5	-21.2	62.4	-9.8	-0.7	-4.8
6	GCCAGCGTTCCCATTTGAGG SEQ ID NO:497	-10.4	-29.5	79.5	-19.1	0	-4.1
39	TACTCAGCCTGGTGGTCTAT SEQ ID NO:498	-10.4	-26.4	77.5	-15.5	-0.2	-4.9
72	GCTCCTGATCCCTGGGGATG SEQ ID NO:499	-10.4	-30.1	81.7	-17.7	-1.4	-11.9
124	CGGTGCAGCTGTAAGTTGCT SEQ ID NO:500	-10.4	-26.6	75.8	-12.2	-4	-9.4
574	GAGAAGAAGAGTGTCTGGTA SEQ ID NO:501	-10.4	-20.8	64.3	-10.4	0	-2.9
728	ATTAAGCCTAAGCCTGGGTA SEQ ID NO:502	-10.4	-24.8	70.3	-14.4	0	-5.4
10	CCAGGCCAGCGTTCCCATTT SEQ ID NO:503	-10.3	-31.6	82.7	-20.8	0	-7.7
265	CTGGAGCCATCTCCTAGAAG SEQ ID NO:504	-10.3	-25.4	72.4	-11.9	-3.2	-7.4
389	TCTTCACATTGCCCTTGAAA SEQ ID NO:505	-10.3	-23.5	66.9	-12.7	-0.2	-3.6
746	CCTGGAATCTTTCAGGTAAT SEQ ID NO:506	-10.3	-22	65	-10.1	-1.5	-7.7
860	CATTTGAAGGAAACAATTTT SEQ ID NO:507	-10.3	-15.7	50.6	-5.4	0	-3.2
493	CTACAAAGGCAGAGCAAAGC SEQ ID NO:508	-10.2	-21.3	62.1	-10.2	-0.7	-4.6
548	CTCACTGTCTTCTTGGCTGA SEQ ID NO:509	-10.2	-25.6	76.2	-15.4	0	-3.7
747	TCCTGGAATCTTTCAGGTAA SEQ ID NO:510	-10.2	-22.4	66.6	-10.1	-2.1	-8.9
987	AAAGATGAACAAGTAGGCCA SEQ ID NO:511	-10.2	-19.9	58.8	-9.2	0	-7.7
209	GATTCAGGCTGCTAGAGACC SEQ ID NO:512	-10.1	-25.5	74.3	-14.9	-0.1	-6.1
356	ACTGCTTCTTTGGCAGCCCA SEQ ID NO:513	-10.1	-29.6	81.9	-16.4	-3.1	-8.1
725	AAGCCTAAGCCTGGGTAAGG SEQ ID NO:514	-10.1	-25.5	71	-14.4	-0.9	-7.5
764	GCTAGGAAGCTACAGTTTCC SEQ ID NO:515	-10.1	-24.6	72.5	-12.9	-1.5	-9.1
855	GAAGGAAACAATTTTGATCT SEQ ID NO:516	-10.1	-16.7	52.9	-6.6	0	-5.8
76	GGAGGCTCCTGATCCCTGGG SEQ ID NO:517	-10	-31.3	84.9	-20.5	-0.5	-8.6
208	ATTCAGGCTGCTAGAGACCA SEQ ID NO:518	-10	-25.6	74	-14.9	-0.4	-6.1
268	TTCCTGGAGCCATCTCCTAG SEQ ID NO:519	-10	-28	79	-15.5	-2.5	-7.3
288	CACTCACATTCTTGGCCGCC SEQ ID NO:520	-10	-28.9	78.1	-18.4	0	-7.6
344	GCAGCCCAGACACTGTCATG SEQ ID NO:521	-10	-27.9	77.7	-16.6	-1.2	-8.9
354	TGCTTCTTTGGCAGCCCAGA SEQ ID NO:522	-10	-29.1	81	-18	-1	-8.1
472	TCTTAGCTGACATTGTTTGA SEQ ID NO:523	-10	-21.4	65.6	-11.4	0	-5.4
848	ACAATTTTGATCTGTGACAT SEQ ID NO:524	-10	-19.1	59	-9.1	0	-4.9
880	GGTTGTGAATTGGCAGACCC SEQ ID NO:525	-10	-26.2	73.6	-15.5	-0.5	-4.1
925	GAATCTGGATTCAGTCTGCA SEQ ID NO:526	-10	-23.2	69.4	-11.8	-1.1	-10.3

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
146	TTAGAACTTTCATCGCAACT SEQ ID NO:527	-9.9	-20.2	60.4	-10.3	0	-4.2
167	GCAACAGGAGGAGGGAAGAG SEQ ID NO:528	-9.9	-23.2	67.2	-13.3	0	-3.4
355	CTGCTTCTTTGGCAGCCCAG SEQ ID NO:529	-9.9	-29.4	81.6	-17.3	-2.2	-7.9
388	CTTCACATTGCCCTTGAAAT SEQ ID NO:530	-9.9	-23.1	65.4	-12.7	-0.2	-3.6
692	TAAGACTGACGAGAGAAGAA SEQ ID NO:531	-9.9	-17.3	53.8	-7.4	0	-3.5
693	CTAAGACTGACGAGAGAAGA SEQ ID NO:532	-9.9	-18.9	57.4	-9	0	-3.5
757	AGCTACAGTTTCCTGGAATC SEQ ID NO:533	-9.9	-23.5	69.8	-12.9	-0.4	-8.3
849	AACAATTTTGATCTGTGACA SEQ ID NO:534	-9.9	-18.4	57.1	-8	-0.2	-4.9
866	AGACCCCATTTGAAGGAAAC SEQ ID NO:535	-9.9	-22.2	62.6	-12.3	0	-3.4
1009	AGAAAGATTTGTCGAATGAG SEQ ID NO:536	-9.9	-16.9	53.4	-7	0.1	-5
1098	TTTTTTTTTAAACCTATATT SEQ ID NO:537	-9.9	-15.7	51.6	-5.8	0	-4.4
1099	TTTTTTTTTTAAACCTATAT SEQ ID NO:538	-9.9	-15.7	51.6	-5.8	0	-4.4
212	CTGGATTCAGGCTGCTAGAG SEQ ID NO:539	-9.8	-24.8	73.1	-14.2	-0.6	-7.6
235	GTCCCTGTGGCCTCTGGCGA SEQ ID NO:540	-9.8	-33.3	88.8	-21	-2.5	-7.7
302	GGAACCAATCTTTGCACTCA SEQ ID NO:541	-9.8	-23.5	67.2	-13.2	-0.1	-5.1
353	GCTTCTTTGGCAGCCCAGAC SEQ ID NO:542	-9.8	-29.3	81.9	-18.4	-1	-8.1
556	TAGGTGTGCTCACTGTCTTC SEQ ID NO:543	-9.8	-25.2	77.4	-13.4	-2	-4.2
600	GTGGGTACAGTGGGAGAGTG SEQ ID NO:544	-9.8	-25.4	76	-15.6	0	-5.2
646	TGATCTTGAAAAACATGCTT SEQ ID NO:545	-9.8	-17.5	54.3	-7.7	0	-5
785	ATTTAAGGTTAAATGACACT SEQ ID NO:546	-9.8	-16.6	53.1	-6.2	-0.3	-6.5
920	TGGATTCAGTCTGCAGTGAA SEQ ID NO:547	-9.8	-23.1	69.3	-11.8	-0.5	-10.8
13	GTCCCAGGCCAGCGTTCCCA SEQ ID NO:548	-9.7	-35	90.5	-24.8	0	-7.7
35	CAGCCTGGTGGTCTATGCTT SEQ ID NO:549	-9.7	-28	80.4	-17.7	-0.3	-4.9
73	GGCTCCTGATCCCTGGGGAT SEQ ID NO:550	-9.7	-31.3	84.5	-19.7	-1.2	-11.9
123	GGTGCAGCTGTAAGTTGCTT SEQ ID NO:551	-9.7	-25.9	76.4	-12.2	-4	-11.4
166	CAACAGGAGGAGGGAAGAGA SEQ ID NO:552	-9.7	-22	64.4	-12.3	0	0
329	TCATGAATTTTCTTCTCGGG SEQ ID NO:553	-9.7	-21.6	64.6	-11.1	-0.6	-5.9
552	TGTGCTCACTGTCTTCTTGG SEQ ID NO:554	-9.7	-25.3	76.2	-15.6	0	-5.5
674	AAGACACTAGAGAGAGCAAC SEQ ID NO:555	-9.7	-19.4	59.5	-9.7	0	-4.5
744	TGGAATCTTTCAGGTAATTA SEQ ID NO:556	-9.7	-18.9	59.1	-8.3	-0.8	-5.6
915	TCAGTCTGCAGTGAATAGGG SEQ ID NO:557	-9.7	-23.3	70.1	-13	0	-8.4
1083	ATATTATCTTTAATAAGACC SEQ ID NO:558	-9.7	-15.8	51.8	-4.8	-1.2	-5.2

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
107	GCTTGAAGAATATAATGGAA SEQ ID NO:559	-9.6	-16.4	52.1	-6.8	0	-2.8
305	TCAGGAACCAATCTTTGCAC SEQ ID NO:560	-9.6	-22.6	65.6	-12.5	-0.1	-7.8
392	TTTTCTTCACATTGCCCTTG SEQ ID NO:561	-9.6	-24.6	71.1	-15	0	-3
721	CTAAGCCTGGGTAAGGGGAG SEQ ID NO:562	-9.6	-25.4	71.9	-15	-0.6	-4.7
850	AAACAATTTTGATCTGTGAC SEQ ID NO:563	-9.6	-17	54	-6.9	-0.2	-4.9
1013	CCTCAGAAAGATTTGTCGAA SEQ ID NO:564	-9.6	-20.3	59.9	-9.8	-0.7	-5
1015	TACCTCAGAAAGATTTGTCG SEQ ID NO:565	-9.6	-20.3	60.6	-9.8	-0.7	-3.2
328	CATGAATTTTCTTCTCGGGG SEQ ID NO:566	-9.5	-22.4	65.7	-12.1	-0.6	-4.4
752	CAGTTTCCTGGAATCTTTCA SEQ ID NO:567	-9.5	-23.1	68.7	-12.7	-0.8	-4.6
924	AATCTGGATTCAGTCTGCAG SEQ ID NO:568	-9.5	-22.6	68.3	-11.8	-1.1	-9.9
941	GGGATAAGTATGTGTAGAAT SEQ ID NO:569	-9.5	-18.9	59	-9.4	0	-1.8
207	TTCAGGCTGCTAGAGACCAT SEQ ID NO:570	-9.4	-25.6	74	-14.9	-1.2	-6.7
445	CTGGCAGGCTCTGGAATGCT SEQ ID NO:571	-9.4	-27.6	77.7	-16.6	-1.5	-6.7
702	GGGCACAGGCTAAGACTGAC SEQ ID NO:572	-9.4	-25.2	72.1	-14.4	-1.3	-5.6
875	TGAATTGGCAGACCCCATTT SEQ ID NO:573	-9.4	-25.4	69.8	-15.3	-0.5	-4
33	GCCTGGTGGTCTATGCTTTA SEQ ID NO:574	-9.3	-27.1	78.8	-17.2	-0.3	-4.7
240	CCTCGGTCCCTGTGGCCTCT SEQ ID NO:575	-9.3	-34.2	90.5	-23.3	-1.5	-7.2
247	AGCCTGGCCTCGGTCCCTGT SEQ ID NO:576	-9.3	-34.7	91.5	-24.6	0	-9.2
301	GAACCAATCTTTGCACTCAC SEQ ID NO:577	-9.3	-22.5	65.3	-13.2	0	-5
377	CCTTGAAATGATCACAGGGG SEQ ID NO:578	-9.3	-22.4	64.2	-11.5	-1.6	-7.1
787	GCATTTAAGGTTAAATGACA SEQ ID NO:579	-9.3	-18	55.8	-6	-2.7	-11
986	AAGATGAACAAGTAGGCCAA SEQ ID NO:580	-9.3	-19.9	58.8	-10.1	0	-7.7
61	CTGGGGATGACTCAGGTCAG SEQ ID NO:581	-9.2	-25.4	74.4	-13.8	-2.4	-6.6
71	CTCCTGATCCCTGGGGATGA SEQ ID NO:582	-9.2	-28.9	78.7	-17.7	-1.4	-11.9
84	TCCCTGCTGGAGGCTCCTGA SEQ ID NO:583	-9.2	-31.6	85.9	-21.1	-1.2	-7.1
86	GTTCCCTGCTGGAGGCTCCT SEQ ID NO:584	-9.2	-32.3	89	-21.8	-1.2	-8
116	CTGTAAGTTGCTTGAAGAAT SEQ ID NO:585	-9.2	-19	58.6	-9.8	0	-4.3
477	AAGCTTCTTAGCTGACATTG SEQ ID NO:586	-9.2	-21.5	65	-9.9	-2.4	-7.1
703	AGGGCACAGGCTAAGACTGA SEQ ID NO:587	-9.2	-25	71.7	-14.4	-1.3	-5.6
704	GAGGGCACAGGCTAAGACTG SEQ ID NO:588	-9.2	-25	71.7	-14.4	-1.3	-5.3
739	TCTTTCAGGTAATTAAGCCT SEQ ID NO:589	-9.2	-21.8	65.5	-12	-0.3	-5.4
761	AGGAAGCTACAGTTTCCTGG SEQ ID NO:590	-9.2	-24.3	71.2	-12.9	-2.2	-10.6

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
246	GCCTGGCCTCGGTCCCTGTG SEQ ID NO:591	-9.1	-34.7	90.8	-25.1	0	-8
648	AATGATCTTGAAAAACATGC SEQ ID NO:592	-9.1	-15.8	50.6	-6.7	0	-5
707	GGGGAGGGCACAGGCTAAGA SEQ ID NO:593	-9.1	-27.5	77.2	-17	-1.3	-4
729	AATTAAGCCTAAGCCTGGGT SEQ ID NO:594	-9.1	-24.4	68.6	-14.4	-0.8	-5.4
745	CTGGAATCTTTCAGGTAATT SEQ ID NO:595	-9.1	-20.1	61.6	-10.1	-0.8	-4.3
11	CCCAGGCCAGCGTTCCCATT SEQ ID NO:596	-9	-33.5	85.5	-24	0	-7.7
14	AGTCCCAGGCCAGCGTTCCC SEQ ID NO:597	-9	-34.3	90	-24.8	0	-7.7
31	CTGGTGGTCTATGCTTTAGT SEQ ID NO:598	-9	-24.5	74.3	-15.5	0	-3.9
190	CATGGACATCAGCATTAGTG SEQ ID NO:599	-9	-22	65.8	-13	0	-4.1
701	GGCACAGGCTAAGACTGACG SEQ ID NO:600	-9	-24.8	69.5	-14.4	-1.3	-5.4
722	CCTAAGCCTGGGTAAGGGGA SEQ ID NO:601	-9	-27.4	75.1	-17	-1.3	-6.9
753	ACAGTTTCCTGGAATCTTTC SEQ ID NO:602	-9	-22.6	68.1	-12.2	-1.3	-4.6
38	ACTCAGCCTGGTGGTCTATG SEQ ID NO:603	-8.9	-26.7	77.9	-17.2	-0.3	-4.9
70	TCCTGATCCCTGGGGATGAC SEQ ID NO:604	-8.9	-28.2	77.4	-17.7	-0.8	-11.3
464	GACATTGTTTGAGAAATTGC SEQ ID NO:605	-8.9	-18.7	57.8	-9.8	0	-5.5
673	AGACACTAGAGAGAGCAACA SEQ ID NO:606	-8.9	-20.8	62.8	-11.9	0	-4.1
742	GAATCTTTCAGGTAATTAAG SEQ ID NO:607	-8.9	-17	54.7	-8.1	0	-5
754	TACAGTTTCCTGGAATCTTT SEQ ID NO:608	-8.9	-21.9	66	-11.6	-1.3	-4.6
861	CCATTTGAAGGAAACAATTT SEQ ID NO:609	-8.9	-17.6	53.8	-8.7	0	-3.2
919	GGATTCAGTCTGCAGTGAAT SEQ ID NO:610	-8.9	-23.1	69.4	-11.8	-1.5	-12.8
926	AGAATCTGGATTCAGTCTGC SEQ ID NO:611	-8.9	-22.5	68.5	-11.8	-1.7	-11
995	AATGAGTGAAAGATGAACAA SEQ ID NO:612	-8.9	-15	49	-6.1	0	-2.5
83	CCCTGCTGGAGGCTCCTGAT SEQ ID NO:613	-8.8	-31.2	84	-21.1	-1.2	-7.1
211	TGGATTCAGGCTGCTAGAGA SEQ ID NO:614	-8.8	-24.5	72.4	-15.7	0	-6.6
331	TGTCATGAATTTTCTTCTCG SEQ ID NO:615	-8.8	-20.4	62.5	-10.8	-0.6	-6.7
386	TCACATTGCCCTTGAAATGA SEQ ID NO:616	-8.8	-22.7	64.4	-12.7	-1.1	-4.3
643	TCTTGAAAAACATGCTTTTT SEQ ID NO:617	-8.8	-17.2	54	-7.5	-0.7	-8.5
700	GCACAGGCTAAGACTGACGA SEQ ID NO:618	-8.8	-24.2	68.3	-14.4	-0.9	-5.4
727	TTAAGCCTAAGCCTGGGTAA SEQ ID NO:619	-8.8	-24.1	68.1	-14.4	-0.8	-4.9
740	ATCTTTCAGGTAATTAAGCC SEQ ID NO:620	-8.8	-20.9	63.5	-12.1	0	-5
798	CTTTCCTGATTGCATTTAAG SEQ ID NO:621	-8.8	-20.8	62.5	-12	0	-5.1
1075	TTTAATAAGACCGTGTCTGG SEQ ID NO:622	-8.8	-20.7	61.4	-10.5	-1.3	-8.3

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
12	TCCCAGGCCAGCGTTCCCAT SEQ ID NO:623	-8.7	-33.8	86.9	-25.1	0	-6.9
69	CCTGATCCCTGGGGATGACT SEQ ID NO:624	-8.7	-28.7	77.6	-18	-1.4	-11.9
266	CCTGGAGCCATCTCCTAGAA SEQ ID NO:625	-8.7	-27.4	75.7	-15.5	-3.2	-7.7
360	GGGCACTGCTTCTTTGGCAG SEQ ID NO:626	-8.7	-28	80.1	-17.3	-2	-9.7
378	CCCTTGAAATGATCACAGGG SEQ ID NO:627	-8.7	-23.2	65.3	-11.5	-3	-7.9
726	TAAGCCTAAGCCTGGGTAAG SEQ ID NO:628	-8.7	-24	68	-14.4	-0.8	-4.9
759	GAAGCTACAGTTTCCTGGAA SEQ ID NO:629	-8.7	-23	67.3	-12.9	-1.3	-8.6
867	CAGACCCCATTTGAAGGAAA SEQ ID NO:630	-8.7	-22.7	63.2	-14	0	-3.4
1034	TTTTGTCCCACCTCGCTCTT SEQ ID NO:631	-8.7	-29	79.9	-20.3	0	-3.1
1035	TTTTTGTCCCACCTCGCTCT SEQ ID NO:632	-8.7	-29	79.9	-20.3	0	-3.1
348	TTTGGCAGCCCAGACACTGT SEQ ID NO:633	-8.6	-28.2	78.3	-18.5	-1	-9.1
381	TTGCCCTTGAAATGATCACA SEQ ID NO:634	-8.6	-22.7	64.4	-13.4	-0.5	-6.8
387	TTCACATTGCCCTTGAAATG SEQ ID NO:635	-8.6	-22.2	63.5	-12.7	-0.7	-4
444	TGGCAGGCTCTGGAATGCTT SEQ ID NO:636	-8.6	-26.8	76.1	-16.6	-1.5	-6.7
454	GAGAAATTGCTGGCAGGCTC SEQ ID NO:637	-8.6	-24.6	70.9	-14.8	-1.1	-7.5
496	CTCCTACAAAGGCAGAGCAA SEQ ID NO:638	-8.6	-23.5	66.7	-13.7	-1.1	-6.3
575	GGAGAAGAAGAGTGTCTGGT SEQ ID NO:639	-8.6	-22.3	67.6	-13.7	0	-2.9
738	CTTTCAGGTAATTAAGCCTA SEQ ID NO:640	-8.6	-21.1	63.4	-12	-0.2	-5.3
763	CTAGGAAGCTACAGTTTCCT SEQ ID NO:641	-8.6	-23.7	70.1	-12.9	-2.2	-10.7
788	TGCATTTAAGGTTAAATGAC SEQ ID NO:642	-8.6	-17.3	54.6	-6	-2.7	-11
929	TGTAGAATCTGGATTCAGTC SEQ ID NO:643	-8.6	-20.7	64.7	-10.3	-1.7	-11
115	TGTAAGTTGCTTGAAGAATA SEQ ID NO:644	-8.5	-17.8	56.1	-9.3	0	-4.3
119	CAGCTGTAAGTTGCTTGAAG SEQ ID NO:645	-8.5	-21.6	65	-12.2	-0.6	-8.8
122	GTGCAGCTGTAAGTTGCTTG SEQ ID NO:646	-8.5	-24.7	73.5	-12.2	-4	-11.4
350	TCTTTGGCAGCCCAGACACT SEQ ID NO:647	-8.5	-28.3	78.7	-18.7	-1	-7.7
380	TGCCCTTGAAATGATCACAG SEQ ID NO:648	-8.5	-22.6	64.3	-13.4	-0.5	-6.8
789	TTGCATTTAAGGTTAAATGA SEQ ID NO:649	-8.5	-17.2	54.4	-6	-2.7	-11
1016	TTACCTCAGAAAGATTTGTC SEQ ID NO:650	-8.5	-19.6	60.4	-11.1	0	-2.5
117	GCTGTAAGTTGCTTGAAGAA SEQ ID NO:651	-8.4	-20.8	62.7	-12.4	0	-4.3
385	CACATTGCCCTTGAAATGAT SEQ ID NO:652	-8.4	-22.3	63.1	-12.7	-1.1	-4.3
463	ACATTGTTTGAGAAATTGCT SEQ ID NO:653	-8.4	-19	58.5	-10.6	0	-4
524	GTTTAATTGGAAGAGTGGGC SEQ ID NO:654	-8.4	-21.6	65	-13.2	0	-2.9

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
622	AGAGCACTGGAATGATTTAG SEQ ID NO:655	-8.4	-19.8	60.5	-11.4	0	-4.1
756	GCTACAGTTTCCTGGAATCT SEQ ID NO:656	-8.4	-24.4	71.6	-14.6	-1.3	-8.3
786	CATTTAAGGTTAAATGACAC SEQ ID NO:657	-8.4	-16.4	52.5	-6	-2	-9.9
25	GTCTATGCTTTAGTCCCAGG SEQ ID NO:658	-8.3	-26.3	77.2	-18	0	-3.9
283	ACATTCTTGGCCGCCTTCCT SEQ ID NO:659	-8.3	-30.3	81.1	-21.5	0	-8
300	AACCAATCTTTGCACTCACA SEQ ID NO:660	-8.3	-22.6	65.2	-14.3	0	-5
349	CTTTGGCAGCCCAGACACTG SEQ ID NO:661	-8.3	-27.9	76.8	-18.5	-1	-8.5
1033	TTTGTCCCACCTCGCTCTTA SEQ ID NO:662	-8.3	-28.6	79	-20.3	0	-3.1
1043	CTTTTTTTTTTTTGTCCCAC SEQ ID NO:663	-8.3	-22.5	67.4	-14.2	0	-1.6
287	ACTCACATTCTTGGCCGCCT SEQ ID NO:664	-8.2	-29.1	78.9	-20.4	0	-8
332	CTGTCATGAATTTTCTTCTC SEQ ID NO:665	-8.2	-20.5	64.1	-11.5	-0.6	-6.7
433	GGAATGCTTGTTTGGCTTTC SEQ ID NO:666	-8.2	-23.8	70.6	-13.9	-1.7	-5.4
460	TTGTTTGAGAAATTGCTGGC SEQ ID NO:667	-8.2	-21.1	63.2	-12.9	0	-5.5
510	GTGGGCGCTCAGAGCTCCTA SEQ ID NO:668	-8.2	-30.3	84.5	-20.8	1.5	-10.6
511	AGTGGGCGCTCAGAGCTCCT SEQ ID NO:669	-8.2	-30.6	85.5	-21.1	1.5	-10.6
1092	TTTAAACCTATATTATCTTT SEQ ID NO:670	-8.2	-16.3	52.9	-8.1	0	-4
1093	TTTTAAACCTATATTATCTT SEQ ID NO:671	-8.2	-16.3	52.9	-8.1	0	-4.4
108	TGCTTGAAGAATATAATGGA SEQ ID NO:672	-8.1	-17.1	53.7	-9	0	-3.6
284	CACATTCTTGGCCGCCTTCC SEQ ID NO:673	-8.1	-30.1	80.2	-21.5	0	-8
374	TGAAATGATCACAGGGGCAC SEQ ID NO:674	-8.1	-22.1	64.1	-13.4	-0.3	-6.3
384	ACATTGCCCTTGAAATGATC SEQ ID NO:675	-8.1	-22	63.3	-12.7	-1.1	-4.3
461	ATTGTTTGAGAAATTGCTGG SEQ ID NO:676	-8.1	-19.3	59.2	-11.2	0	-4
624	TGAGAGCACTGGAATGATTT SEQ ID NO:677	-8.1	-20.7	62.1	-12.6	0	-3.4
625	TTGAGAGCACTGGAATGATT SEQ ID NO:678	-8.1	-20.7	62.1	-12.6	0	-4.2
758	AAGCTACAGTTTCCTGGAAT SEQ ID NO:679	-8.1	-22.4	66	-12.9	-1.3	-8.6
928	GTAGAATCTGGATTCAGTCT SEQ ID NO:680	-8.1	-21.6	66.9	-11.7	-1.7	-11
299	ACCAATCTTTGCACTCACAT SEQ ID NO:681	-8	-23.3	67.3	-15.3	0	-5
572	GAAGAAGAGTGTCTGGTAGG SEQ ID NO:682	-8	-21.4	65.6	-13.4	0	-2.9
120	GCAGCTGTAAGTTGCTTGAA SEQ ID NO:683	-7.9	-23.4	69	-12.2	-3.3	-11.4
121	TGCAGCTGTAAGTTGCTTGA SEQ ID NO:684	-7.9	-24.1	71.3	-12.2	-4	-11.4
359	GGCACTGCTTCTTTGGCAGC SEQ ID NO:685	-7.9	-28.6	82	-17.6	-3.1	-10.1
375	TTGAAATGATCACAGGGGCA SEQ ID NO:686	-7.9	-22	63.9	-13.4	-0.5	-6.8

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
631	TGCTTTTTGAGAGCACTGGA SEQ ID NO:687	-7.9	-23.7	70	-13.8	-2	-5.9
741	AATCTTTCAGGTAATTAAGC SEQ ID NO:688	-7.9	-18.2	57.5	-10.3	0	-5
17	TTTAGTCCCAGGCCAGCGTT SEQ ID NO:689	-7.8	-29.8	81.7	-21.5	0	-7.7
294	TCTTTGCACTCACATTCTTG SEQ ID NO:690	-7.8	-22.6	68.2	-14.8	0	-5
295	ATCTTTGCACTCACATTCTT SEQ ID NO:691	-7.8	-22.6	68.4	-14.8	0	-4.7
630	GCTTTTTGAGAGCACTGGAA SEQ ID NO:692	-7.8	-23	67.8	-13.8	-1.3	-4.6
771	GACACTAGCTAGGAAGCTAC SEQ ID NO:693	-7.8	-22.4	66.9	-11.8	-2.8	-9.9
780	AGGTTAAATGACACTAGCTA SEQ ID NO:694	-7.8	-19.5	59.8	-11.2	-0.1	-5.6
1091	TTAAACCTATATTATCTTTA SEQ ID NO:695	-7.8	-15.9	52	-8.1	0	-2.3
1097	TTTTTTTTAAACCTATATTA SEQ ID NO:696	-7.8	-15.3	50.7	-7.5	0	-4.1
278	CTTGGCCGCCTTCCTGGAGC SEQ ID NO:697	-7.7	-32.5	85.5	-23.6	-0.8	-10
306	CTCAGGAACCAATCTTTGCA SEQ ID NO:698	-7.7	-23.3	66.9	-15.6	0.2	-6.3
335	ACACTGTCATGAATTTTCTT SEQ ID NO:699	-7.7	-19.9	61.5	-12.2	0	-6.7
507	GGCGCTCAGAGCTCCTACAA SEQ ID NO:700	-7.7	-28.1	77.5	-19.8	2.3	-9.1
599	TGGGTACAGTGGGAGAGTGA SEQ ID NO:701	-7.7	-24.8	73.7	-17.1	0	-5.2
697	CAGGCTAAGACTGACGAGAG SEQ ID NO:702	-7.7	-22.1	64.4	-14.4	0	-4.9
1074	TTAATAAGACCGTGTCTGGT SEQ ID NO:703	-7.7	-21.8	64.1	-12.7	-1.3	-8.3
34	AGCCTGGTGGTCTATGCTTT SEQ ID NO:704	-7.6	-27.4	79.8	-19.2	-0.3	-4.9
36	TCAGCCTGGTGGTCTATGCT SEQ ID NO:705	-7.6	-28.3	82	-20.1	-0.3	-4.9
373	GAAATGATCACAGGGGCACT SEQ ID NO:706	-7.6	-23	66.1	-14.9	-0.2	-7
449	ATTGCTGGCAGGCTCTGGAA SEQ ID NO:707	-7.6	-26.8	76.1	-18	-1.1	-7.5
694	GCTAAGACTGACGAGAGAAG SEQ ID NO:708	-7.6	-20.1	60	-12.5	0	-3.5
730	TAATTAAGCCTAAGCCTGGG SEQ ID NO:709	-7.6	-22.9	65.1	-14.4	-0.8	-5.8
126	GTCGGTGCAGCTGTAAGTTG SEQ ID NO:710	-7.5	-25.5	74.6	-16.9	-1	-8.9
184	CATCAGCATTAGTGGCAGCA SEQ ID NO:711	-7.5	-25.5	74.3	-18	0	-5.3
437	CTCTGGAATGCTTGTTTGGC SEQ ID NO:712	-7.5	-24.5	71.7	-17	0	-3.6
518	TTGGAAGAGTGGGCGCTCAG SEQ ID NO:713	-7.5	-25.8	73.2	-15.6	-2.7	-10.1
762	TAGGAAGCTACAGTTTCCTG SEQ ID NO:714	-7.5	-22.8	67.9	-12.9	-2.4	-11.1
879	GTTGTGAATTGGCAGACCCC SEQ ID NO:715	-7.5	-27	74.6	-18.8	-0.5	-4
319	TCTTCTCGGGGCTCTCAGGA SEQ ID NO:716	-7.4	-28.4	82	-21	0	-4.1
327	ATGAATTTTCTTCTCGGGGC SEQ ID NO:717	-7.4	-23.5	68.7	-15.3	-0.6	-4.1
457	TTTGAGAAATTGCTGGCAGG SEQ ID NO:718	-7.4	-21.7	63.9	-13.6	0	-9

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
629	CTTTTTGAGAGCACTGGAAT SEQ ID NO:719	-7.4	-21.2	63.5	-13.8	0	-4.2
765	AGCTAGGAAGCTACAGTTTC SEQ ID NO:720	-7.4	-22.6	68.9	-12.9	-2.3	-7.8
779	GGTTAAATGACACTAGCTAG SEQ ID NO:721	-7.4	-19.5	59.8	-11.2	-0.1	-9.5
781	AAGGTTAAATGACACTAGCT SEQ ID NO:722	-7.4	-19.1	58.4	-11.2	-0.1	-5.1
1084	TATATTATCTTTAATAAGAC SEQ ID NO:723	-7.4	-13.5	47.3	-4.8	-1.2	-5.2
286	CTCACATTCTTGGCCGCCTT SEQ ID NO:724	-7.3	-29	78.7	-21.2	0	-8
341	GCCCAGACACTGTCATGAAT SEQ ID NO:725	-7.3	-25.3	71	-17.3	-0.4	-7.1
517	TGGAAGAGTGGGCGCTCAGA SEQ ID NO:726	-7.3	-26.3	74.2	-17.1	-1.9	-10.1
672	GACACTAGAGAGAGCAACAA SEQ ID NO:727	-7.3	-20.1	60.5	-12.8	0	-4.5
778	GTTAAATGACACTAGCTAGG SEQ ID NO:728	-7.3	-19.5	59.8	-11.2	0	-9.9
791	GATTGCATTTAAGGTTAAAT SEQ ID NO:729	-7.3	-17.2	54.4	-9.3	-0.3	-6.5
918	GATTCAGTCTGCAGTGAATA SEQ ID NO:730	-7.3	-21.6	66.1	-11.8	-1.7	-12.9
191	CCATGGACATCAGCATTAGT SEQ ID NO:731	-7.2	-24	69.7	-16.8	0	-7.3
347	TTGGCAGCCCAGACACTGTC SEQ ID NO:732	-7.2	-28.5	79.7	-20.1	-1.1	-8.7
379	GCCCTTGAAATGATCACAGG SEQ ID NO:733	-7.2	-23.8	66.8	-14.9	-1.7	-6.8
434	TGGAATGCTTGTTTGGCTTT SEQ ID NO:734	-7.2	-23.4	68.8	-15.3	-0.7	-4
442	GCAGGCTCTGGAATGCTTGT SEQ ID NO:735	-7.2	-26.8	77	-18.5	-1	-6.7
784	TTTAAGGTTAAATGACACTA SEQ ID NO:736	-7.2	-16.3	52.6	-8.6	-0.1	-4.7
916	TTCAGTCTGCAGTGAATAGG SEQ ID NO:737	-7.2	-22.2	67.7	-13.9	-0.2	-10.2
917	ATTCAGTCTGCAGTGAATAG SEQ ID NO:738	-7.2	-21	64.9	-11.8	-1.1	-12
7	GGCCAGCGTTCCCATTTGAG SEQ ID NO:739	-7.1	-29.5	79.5	-22.4	0	-7
495	TCCTACAAAGGCAGAGCAAA SEQ ID NO:740	-7.1	-21.9	62.9	-13.6	-1.1	-6.2
626	TTTGAGAGCACTGGAATGAT SEQ ID NO:741	-7.1	-20.7	62.1	-13.6	0	-4.2
751	AGTTTCCTGGAATCTTTCAG SEQ ID NO:742	-7.1	-22.4	67.8	-14.4	-0.8	-8.3
884	ATCTGGTTGTGAATTGGCAG SEQ ID NO:743	-7.1	-22.7	67.7	-15.6	0	-4
37	CTCAGCCTGGTGGTCTATGC SEQ ID NO:744	-7	-28.3	82	-20.7	-0.3	-4.9
497	GCTCCTACAAAGGCAGAGCA SEQ ID NO:745	-7	-26	73.1	-16.6	-2.4	-7.9
699	CACAGGCTAAGACTGACGAG SEQ ID NO:746	-7	-22.4	64.6	-14.4	-0.9	-5.4
723	GCCTAAGCCTGGGTAAGGGG SEQ ID NO:747	-7	-28.6	78	-20.2	-1.3	-8.2
772	TGACACTAGCTAGGAAGCTA SEQ ID NO:748	-7	-22.2	66.2	-12.4	-2.8	-9
790	ATTGCATTTAAGGTTAAATG SEQ ID NO:749	-7	-16.6	53.1	-7.3	-2.3	-10.5
1090	TAAACCTATATTATCTTTAA SEQ ID NO:750	-7	-15.1	50	-8.1	0	-2.2

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
206	TCAGGCTGCTAGAGACCATG SEQ ID NO:751	-6.9	-25.5	73.5	-17.3	-1.2	-6.7
320	TTCTTCTCGGGGCTCTCAGG SEQ ID NO:752	-6.9	-27.9	81	-21	0	-4.1
698	ACAGGCTAAGACTGACGAGA SEQ ID NO:753	-6.9	-22.3	64.7	-14.4	-0.9	-5.4
883	TCTGGTTGTGAATTGGCAGA SEQ ID NO:754	-6.9	-23.3	69.1	-15.7	-0.5	-4.2
334	CACTGTCATGAATTTTCTTC SEQ ID NO:755	-6.8	-20.1	62.4	-13.3	0	-6.2
448	TTGCTGGCAGGCTCTGGAAT SEQ ID NO:756	-6.8	-26.8	76.1	-18.8	-1.1	-7.5
637	AAAACATGCTTTTTGAGAGC SEQ ID NO:757	-6.8	-18.9	57.8	-11.1	-0.9	-6.3
767	CTAGCTAGGAAGCTACAGTT SEQ ID NO:758	-6.8	-22.7	68.3	-12.9	-3	-8.5
59	GGGGATGACTCAGGTCAGGA SEQ ID NO:759	-6.7	-26.3	76.7	-17.7	-1.9	-6.1
450	AATTGCTGGCAGGCTCTGGA SEQ ID NO:760	-6.7	-26.8	76.1	-18.9	-1.1	-7.5
777	TTAAATGACACTAGCTAGGA SEQ ID NO:761	-6.7	-18.9	58.1	-11.2	0	-9.9
30	TGGTGGTCTATGCTTTAGTC SEQ ID NO:762	-6.6	-24	74	-17.4	0	-3.9
77	TGGAGGCTCCTGATCCCTGG SEQ ID NO:763	-6.6	-30.1	82.1	-22.2	-1.2	-7
109	TTGCTTGAAGAATATAATGG SEQ ID NO:764	-6.6	-16.6	52.8	-10	0	-3.6
376	CTTGAAATGATCACAGGGGC SEQ ID NO:765	-6.6	-22.2	64.6	-14.9	-0.5	-6.8
436	TCTGGAATGCTTGTTTGGCT SEQ ID NO:766	-6.6	-24.5	71.7	-17	-0.7	-4
770	ACACTAGCTAGGAAGCTACA SEQ ID NO:767	-6.6	-22.5	66.7	-12.9	-3	-9.9
773	ATGACACTAGCTAGGAAGCT SEQ ID NO:768	-6.6	-22.5	66.7	-13.6	-2.3	-9.9
1032	TTGTCCCACCTCGCTCTTAC SEQ ID NO:769	-6.6	-28.7	79.2	-22.1	0	-3.1
799	ACTTTCCTGATTGCATTTAA SEQ ID NO:770	-6.5	-21	62.9	-14.5	0	-5.1
854	AAGGAAACAATTTTGATCTG SEQ ID NO:771	-6.5	-16.1	51.6	-9.6	0	-5.8
1010	CAGAAAGATTTGTCGAATGA SEQ ID NO:772	-6.5	-17.6	54.4	-10.2	-0.7	-5
118	AGCTGTAAGTTGCTTGAAGA SEQ ID NO:773	-6.4	-21.5	65.1	-14.4	-0.5	-6.2
326	TGAATTTTCTTCTCGGGGCT SEQ ID NO:774	-6.4	-24.4	70.7	-17.2	-0.6	-4.3
336	GACACTGTCATGAATTTTCT SEQ ID NO:775	-6.4	-20.4	62.5	-13.3	-0.4	-6.9
382	ATTGCCCTTGAAATGATCAC SEQ ID NO:776	-6.4	-22	63.3	-14.9	-0.5	-6.8
465	TGACATTGTTTGAGAAATTG SEQ ID NO:777	-6.4	-16.9	53.8	-10.5	0	-5.5
471	CTTAGCTGACATTGTTTGAG SEQ ID NO:778	-6.4	-21	64.3	-14.6	0	-5.4
1073	TAATAAGACCGTGTCTGGTT SEQ ID NO:779	-6.4	-21.8	64.1	-14	-1.3	-7.8
186	GACATCAGCATTAGTGGCAG SEQ ID NO:780	-6.3	-23.8	70.6	-16.6	-0.8	-4.1
241	GCCTCGGTCCCTGTGGCCTC SEQ ID NO:781	-6.3	-35.1	93.1	-26.8	-2	-7.2
261	AGCCATCTCCTAGAAGCCTG SEQ ID NO:782	-6.3	-27.4	76.4	-20.1	-0.9	-4.3

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
318	CTTCTCGGGGCTCTCAGGAA SEQ ID NO:783	-6.3	-27.3	77.4	-21	0	-4.1
627	TTTTGAGAGCACTGGAATGA SEQ ID NO:784	-6.3	-20.8	62.5	-14.5	0	-4.2
737	TTTCAGGTAATTAAGCCTAA SEQ ID NO:785	-6.3	-19.5	59.4	-12.5	-0.4	-5.5
1085	CTATATTATCTTTAATAAGA SEQ ID NO:786	-6.3	-14.2	48.7	-6.8	-1	-5.2
298	CCAATCTTTGCACTCACATT SEQ ID NO:787	-6.2	-23.2	67.1	-17	0	-5
462	CATTGTTTGAGAAATTGCTG SEQ ID NO:788	-6.2	-18.8	57.9	-12.6	0	-4
623	GAGAGCACTGGAATGATTTA SEQ ID NO:789	-6.2	-20.4	61.6	-14.2	0	-4.2
766	TAGCTAGGAAGCTACAGTTT SEQ ID NO:790	-6.2	-21.9	66.6	-12.9	-2.8	-8.3
833	GACATTTAAAAATATTTATT SEQ ID NO:791	-6.2	-12.3	44.2	-5.4	-0.4	-6.7
1096	TTTTTTTAAACCTATATTAT SEQ ID NO:792	-6.2	-15.2	50.4	-9	0	-4.4
42	GGATACTCAGCCTGGTGGTC SEQ ID NO:793	-6.1	-27.6	80.2	-20.9	-0.3	-4.9
245	CCTGGCCTCGGTCCCTGTGG SEQ ID NO:794	-6.1	-34.1	88.9	-28	0.3	-7.2
909	TGCAGTGAATAGGGTAAAAT SEQ ID NO:795	-6.1	-18.5	56.7	-12.4	0	-4.7
942	GGGGATAAGTATGTGTAGAA SEQ ID NO:796	-6.1	-20.1	61.7	-14	0	-1.8
1042	TTTTTTTTTTTTGTCCCACC SEQ ID NO:797	-6.1	-23.6	69.2	-17.5	0	-1.7
16	TTAGTCCCAGGCCAGCGTTC SEQ ID NO:798	-6	-30.1	83.1	-23.6	0	-7.7
506	GCGCTCAGAGCTCCTACAAA SEQ ID NO:799	-6	-26.2	72.6	-18.7	-1.4	-9.6
642	CTTGAAAAACATGCTTTTTG SEQ ID NO:800	-6	-16.8	52.8	-9.2	-1.5	-9.1
649	AAATGATCTTGAAAAACATG SEQ ID NO:801	-6	-13.3	45.6	-7.3	0	-4.9
816	ATTGACTTCTGTTTGCTACT SEQ ID NO:802	-6	-22.1	67.4	-16.1	0	-3.6
834	TGACATTTAAAAATATTTAT SEQ ID NO:803	-6	-12.2	43.9	-5.5	-0.4	-6.7
836	TGTGACATTTAAAAATATTT SEQ ID NO:804	-6	-13.7	46.9	-7.7	0	-6.4
439	GGCTCTGGAATGCTTGTTTG SEQ ID NO:805	-5.9	-24.5	71.7	-17.9	-0.5	-4
441	CAGGCTCTGGAATGCTTGTT SEQ ID NO:806	-5.9	-25.1	72.9	-18.5	-0.5	-5.4
776	TAAATGACACTAGCTAGGAA SEQ ID NO:807	-5.9	-18.1	55.9	-11.2	0	-9.9
783	TTAAGGTTAAATGACACTAG SEQ ID NO:808	-5.9	-16.2	52.4	-10.3	0.7	-4
1072	AATAAGACCGTGTCTGGTTC SEQ ID NO:809	-5.9	-22.5	66.1	-15.2	-1.3	-8.3
85	TTCCCTGCTGGAGGCTCCTG SEQ ID NO:810	-5.8	-31.1	85	-24	-1.2	-8
321	TTTCTTCTCGGGGCTCTCAG SEQ ID NO:811	-5.8	-26.8	78.7	-21	0	-4.1
829	TTTAAAAATATTTATTGACT SEQ ID NO:812	-5.8	-12.5	44.7	-6	-0.4	-6.2
248	AAGCCTGGCCTCGGTCCCTG SEQ ID NO:813	-5.7	-32.8	85.1	-26.3	0	-9.2
323	ATTTTCTTCTCGGGGCTCTC SEQ ID NO:814	-5.7	-26.2	77.5	-20.5	0	-4.1

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
325	GAATTTTCTTCTCGGGGCTC SEQ ID NO:815	-5.7	-24.8	72.5	-19.1	0	-3.9
466	CTGACATTGTTTGAGAAATT SEQ ID NO:816	-5.7	-17.8	55.8	-12.1	0	-5.5
800	TACTTTCCTGATTGCATTTA SEQ ID NO:817	-5.7	-21.4	64.4	-15.7	0	-5.1
830	ATTTAAAAATATTTATTGAC SEQ ID NO:818	-5.7	-11.6	42.9	-5.2	-0.4	-6.7
210	GGATTCAGGCTGCTAGAGAC SEQ ID NO:819	-5.6	-24.7	73.2	-19.1	0	-6.1
638	AAAAACATGCTTTTTGAGAG SEQ ID NO:820	-5.6	-16.4	52.2	-9.8	-0.9	-8.3
1039	TTTTTTTTTGTCCCACCTCG SEQ ID NO:821	-5.6	-25.4	71.7	-19.8	0	-2.4
24	TCTATGCTTTAGTCCCAGGC SEQ ID NO:822	-5.5	-26.9	78.1	-21.4	0	-3.6
183	ATCAGCATTAGTGGCAGCAA SEQ ID NO:823	-5.5	-24.1	70.6	-17.7	-0.8	-5.3
185	ACATCAGCATTAGTGGCAGC SEQ ID NO:824	-5.5	-25	73.8	-18.6	-0.8	-4.7
202	GCTGCTAGAGACCATGGACA SEQ ID NO:825	-5.5	-25.9	73.4	-19.7	0	-8.8
296	AATCTTTGCACTCACATTCT SEQ ID NO:826	-5.5	-21.8	65.6	-16.3	0	-5
525	TGTTTAATTGGAAGAGTGGG SEQ ID NO:827	-5.5	-19.8	60.7	-14.3	0	-2.6
547	TCACTGTCTTCTTGGCTGAG SEQ ID NO:828	-5.5	-24.7	74.4	-19.2	0	-4.2
632	ATGCTTTTTGAGAGCACTGG SEQ ID NO:829	-5.5	-23.1	68.6	-15.2	-2.4	-6.7
768	ACTAGCTAGGAAGCTACAGT SEQ ID NO:830	-5.5	-22.8	68.5	-14.3	-3	-9.9
835	GTGACATTTAAAAATATTTA SEQ ID NO:831	-5.5	-13.4	46.4	-7.4	-0.2	-6.7
279	TCTTGGCCGCCTTCCTGGAG SEQ ID NO:832	-5.4	-31.1	83.1	-24.6	-0.3	-10
534	GGCTGAGAATGTTTAATTGG SEQ ID NO:833	-5.4	-20.1	60.9	-14.7	0	-3.7
576	GGGAGAAGAAGAGTGTCTGG SEQ ID NO:834	-5.4	-22.3	67	-16.9	0	-2.9
636	AAACATGCTTTTTGAGAGCA SEQ ID NO:835	-5.4	-20.3	61	-13.2	-1.7	-5.9
911	TCTGCAGTGAATAGGGTAAA SEQ ID NO:836	-5.4	-20.5	61.9	-14.5	0	-8.6
1031	TGTCCCACCTCGCTCTTACC SEQ ID NO:837	-5.4	-30.6	82.2	-25.2	0	-3.1
60	TGGGGATGACTCAGGTCAGG SEQ ID NO:838	-5.3	-25.7	75.1	-18	-2.4	-6.6
769	CACTAGCTAGGAAGCTACAG SEQ ID NO:839	-5.3	-22.3	66.4	-14	-3	-9.9
910	CTGCAGTGAATAGGGTAAAA SEQ ID NO:840	-5.3	-19.4	58.6	-14.1	0	-7.4
1041	TTTTTTTTTTTTGTCCCACCT SEQ ID NO:841	-5.3	-24.4	70.8	-19.1	0	-1.7
342	AGCCCAGACACTGTCATGAA SEQ ID NO:842	-5.2	-25.3	71.3	-18.8	-1.2	-7.6
503	CTCAGAGCTCCTACAAAGGC SEQ ID NO:843	-5.2	-24.8	71.3	-18.4	-1.1	-8.4
792	TGATTGCATTTAAGGTTAAA SEQ ID NO:844	-5.2	-17.2	54.4	-12	0	-5.3
793	CTGATTGCATTTAAGGTTAA SEQ ID NO:845	-5.2	-18.8	58.1	-13.6	0	-4.8
440	AGGCTCTGGAATGCTTGTTT SEQ ID NO:846	-5.1	-24.5	72.1	-18.7	-0.5	-4

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
443	GGCAGGCTCTGGAATGCTTG SEQ ID NO:847	-5.1	-26.8	76.1	-20.1	-1.5	-6.7
501	CAGAGCTCCTACAAAGGCAG SEQ ID NO:848	-5.1	-24.2	69.2	-17.9	-1.1	-8.4
826	AAAAATATTTATTGACTTCT SEQ ID NO:849	-5.1	-14	47.7	-8.9	0	-6.7
58	GGGATGACTCAGGTCAGGAT SEQ ID NO:850	-5	-25.1	73.9	-17.7	-2.4	-6.6
201	CTGCTAGAGACCATGGACAT SEQ ID NO:851	-5	-24.1	69.2	-18.4	0	-8.8
340	CCCAGACACTGTCATGAATT SEQ ID NO:852	-5	-23.6	67.2	-17.3	-1.2	-7.6
467	GCTGACATTGTTTGAGAAAT SEQ ID NO:853	-5	-19.5	59.4	-14.5	0	-5.5
468	AGCTGACATTGTTTGAGAAA SEQ ID NO:854	-5	-19.5	59.6	-14.5	0	-4.9
695	GGCTAAGACTGACGAGAGAA SEQ ID NO:855	-5	-21.3	62.2	-16.3	0	-3.7
15	TAGTCCCAGGCCAGCGTTCC SEQ ID NO:856	-4.9	-32	86.2	-26.6	0	-7.7
435	CTGGAATGCTTGTTTGGCTT SEQ ID NO:857	-4.9	-24.2	70.4	-18.4	-0.7	-4
509	TGGGCGCTCAGAGCTCCTAC SEQ ID NO:858	-4.9	-29.3	81.5	-23.1	1.5	-10.6
512	GAGTGGGCGCTCAGAGCTCC SEQ ID NO:859	-4.9	-30.3	84.9	-23.1	-1.9	-12.4
706	GGGAGGGCACAGGCTAAGAC SEQ ID NO:860	-4.9	-26.5	75.2	-20.2	-1.3	-4
1011	TCAGAAAGATTTGTCGAATG SEQ ID NO:861	-4.9	-17.4	54.4	-11.6	-0.7	-5
1040	TTTTTTTTTTGTCCCACCTC SEQ ID NO:862	-4.9	-24.7	72.1	-19.8	0	-1.7
828	TTAAAAATATTTATTGACTT SEQ ID NO:863	-4.8	-12.5	44.7	-7	-0.4	-6.7
458	GTTTGAGAAATTGCTGGCAG SEQ ID NO:864	-4.7	-21.7	64.4	-15.9	0	-10.1
546	CACTGTCTTCTTGGCTGAGA SEQ ID NO:865	-4.7	-24.9	74.1	-20.2	0	-6
774	AATGACACTAGCTAGGAAGC SEQ ID NO:866	-4.7	-20.9	62.6	-14.6	-1.5	-9.9
1020	GCTCTTACCTCAGAAAGATT SEQ ID NO:867	-4.7	-21.9	65.1	-16.5	-0.4	-3.6
1030	GTCCCACCTCGCTCTTACCT SEQ ID NO:868	-4.7	-31.5	84.3	-26.8	0	-3.1
1038	TTTTTTTTGTCCCACCTCGC SEQ ID NO:869	-4.7	-27.1	75.5	-22.4	0	-2.7
256	TCTCCTAGAAGCCTGGCCTC SEQ ID NO:870	-4.6	-29.2	81.4	-23.5	0	-10.1
322	TTTTCTTCTCGGGGCTCTCA SEQ ID NO:871	-4.6	-26.9	78.7	-22.3	0	-4.1
324	AATTTTCTTCTCGGGGCTCT SEQ ID NO:872	-4.6	-25.1	73.1	-20.5	0	-4.1
200	TGCTAGAGACCATGGACATC SEQ ID NO:873	-4.5	-23.6	68.8	-18.4	0	-8.8
650	AAAATGATCTTGAAAAACAT SEQ ID NO:874	-4.5	-12.6	44.2	-8.1	0	-4.2
671	ACACTAGAGAGAGCAACAAA SEQ ID NO:875	-4.5	-18.8	57.3	-14.3	0	-4.5
736	TTCAGGTAATTAAGCCTAAG SEQ ID NO:876	-4.5	-19.4	59.3	-14.2	-0.4	-5.5
977	AAGTAGGCCAATGGAGACAG SEQ ID NO:877	-4.5	-22.5	65.4	-17.1	-0.8	-8.4
18	CTTTAGTCCCAGGCCAGCGT SEQ ID NO:878	-4.4	-30.6	83.2	-25.7	0	-7.7

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
333	ACTGTCATGAATTTTCTTCT SEQ ID NO:879	-4.4	-20.3	63.1	-15.1	-0.6	-6.7
337	AGACACTGTCATGAATTTTC SEQ ID NO:880	-4.4	-19.5	60.7	-13.8	-1.2	-7.6
500	AGAGCTCCTACAAAGGCAGA SEQ ID NO:881	-4.4	-24.1	69.3	-18.5	-1.1	-8.4
514	AAGAGTGGGCGCTCAGAGCT SEQ ID NO:882	-4.4	-27.2	77.1	-20.1	-2.7	-9.6
598	GGGTACAGTGGGAGAGTGAG SEQ ID NO:883	-4.4	-24.8	74.3	-20.4	0	-5.2
43	AGGATACTCAGCCTGGTGGT SEQ ID NO:884	-4.3	-27.2	78.7	-21.8	-1	-6.7
438	GCTCTGGAATGCTTGTTTGG SEQ ID NO:885	-4.3	-24.5	71.7	-20.2	0	-3.6
628	TTTTTGAGAGCACTGGAATG SEQ ID NO:886	-4.3	-20.3	61.5	-16	0	-4.2
639	GAAAAACATGCTTTTTGAGA SEQ ID NO:887	-4.3	-17	53.3	-11.1	-1.5	-9.1
731	GTAATTAAGCCTAAGCCTGG SEQ ID NO:888	-4.3	-22.9	65.6	-17.7	-0.8	-6.5
257	ATCTCCTAGAAGCCTGGCCT SEQ ID NO:889	-4.2	-28.8	79.5	-23.5	0	-10.1
260	GCCATCTCCTAGAAGCCTGG SEQ ID NO:890	-4.2	-28.6	78.6	-23.7	-0.5	-4.2
292	TTTGCACTCACATTCTTGGC SEQ ID NO:891	-4.2	-24.3	71.7	-20.1	0	-5
505	CGCTCAGAGCTCCTACAAAG SEQ ID NO:892	-4.2	-24.4	68.8	-18.7	-1.4	-9.6
535	TGGCTGAGAATGTTTAATTG SEQ ID NO:893	-4.2	-18.9	58.3	-14.7	0	-3.7
827	TAAAAATATTTATTGACTTC SEQ ID NO:894	-4.2	-12.8	45.4	-8.1	-0.1	-6.7
1086	CCTATATTATCTTTAATAAG SEQ ID NO:895	-4.2	-15.6	51.3	-10.5	-0.8	-3.3
199	GCTAGAGACCATGGACATCA SEQ ID NO:896	-4.1	-24.3	70.1	-19.5	0	-8.8
383	CATTGCCCTTGAAATGATCA SEQ ID NO:897	-4.1	-22.5	63.9	-17.8	-0.3	-6.5
451	AAATTGCTGGCAGGCTCTGG SEQ ID NO:898	-4.1	-25.5	72.3	-20.7	-0.5	-7.5
499	GAGCTCCTACAAAGGCAGAG SEQ ID NO:899	-4.1	-24.1	69.3	-18.8	-1.1	-7.2
515	GAAGAGTGGGCGCTCAGAGC SEQ ID NO:900	-4.1	-26.9	76.4	-20.1	-2.7	-10.1
498	AGCTCCTACAAAGGCAGAGC SEQ ID NO:901	-4	-25.3	72.3	-19.2	-2.1	-7.1
125	TCGGTGCAGCTGTAAGTTGC SEQ ID NO:902	-3.9	-26.1	75.5	-19.7	-2.5	-9.4
205	CAGGCTGCTAGAGACCATGG SEQ ID NO:903	-3.9	-26.3	74.4	-21.1	-1.2	-8.3
285	TCACATTCTTGGCCGCCTTC SEQ ID NO:904	-3.9	-28.5	78.5	-24.1	0	-8
1037	TTTTTTTGTCCCACCTCGCT SEQ ID NO:905	-3.9	-27.9	77	-24	0	-3.1
23	CTATGCTTTAGTCCCAGGCC SEQ ID NO:906	-3.8	-28.5	79.9	-24.7	0	-6.4
502	TCAGAGCTCCTACAAAGGCA SEQ ID NO:907	-3.8	-24.6	70.5	-19.6	-1.1	-8.4
459	TGTTTGAGAAATTGCTGGCA SEQ ID NO:908	-3.7	-21.7	64.1	-17.4	0	-8.4
696	AGGCTAAGACTGACGAGAGA SEQ ID NO:909	-3.7	-22	64.5	-18.3	0	-3.7
837	CTGTGACATTTAAAAATATT SEQ ID NO:910	-3.7	-14.5	48.4	-10.8	0	-5

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
1021	CGCTCTTACCTCAGAAAGAT SEQ ID NO:911	-3.7	-22.6	65	-18.2	-0.4	-3.6
78	CTGGAGGCTCCTGATCCCTG SEQ ID NO:912	-3.6	-29.8	81.5	-24.9	-1.2	-7
508	GGGCGCTCAGAGCTCCTACA SEQ ID NO:913	-3.6	-30	82.7	-25.5	1.5	-9.9
825	AAAATATTTATTGACTTCTG SEQ ID NO:914	-3.6	-14.7	49.3	-11.1	0	-6.7
1012	CTCAGAAAGATTTGTCGAAT SEQ ID NO:915	-3.6	-18.3	56.3	-13.8	-0.7	-5
1094	TTTTTAAACCTATATTATCT SEQ ID NO:916	-3.6	-16.3	52.9	-12.7	0	-4.4
1095	TTTTTTAAACCTATATTATC SEQ ID NO:917	-3.6	-15.5	51.3	-11.9	0	-4.4
57	GGATGACTCAGGTCAGGATA SEQ ID NO:918	-3.5	-23.6	70.5	-17.7	-2.4	-6.6
81	CTGCTGGAGGCTCCTGATCC SEQ ID NO:919	-3.5	-29.6	82.4	-24.9	-1.1	-6.3
293	CTTTGCACTCACATTCTTGG SEQ ID NO:920	-3.5	-23.4	69.3	-19.9	0	-5
536	TTGGCTGAGAATGTTTAATT SEQ ID NO:921	-3.5	-19	58.7	-15.5	0	-3.7
82	CCTGCTGGAGGCTCCTGATC SEQ ID NO:922	-3.4	-29.6	82.4	-24.9	-1.2	-7.1
249	GAAGCCTGGCCTCGGTCCCT SEQ ID NO:923	-3.4	-33.4	86.6	-29.2	0	-9.2
635	AACATGCTTTTTGAGAGCAC SEQ ID NO:924	-3.4	-21.2	63.6	-15.4	-2.4	-6.7
832	ACATTTAAAAATATTTATTG SEQ ID NO:925	-3.4	-11.7	43	-7.6	-0.4	-6.7
927	TAGAATCTGGATTCAGTCTG SEQ ID NO:926	-3.4	-20.4	63.4	-15.2	-1.7	-11
309	GCTCTCAGGAACCAATCTTT SEQ ID NO:927	-3.3	-23.9	69.4	-20.1	-0.1	-4.6
372	AAATGATCACAGGGGCACTG SEQ ID NO:928	-3.3	-22.4	64.7	-17.8	-1.2	-8.5
447	TGCTGGCAGGCTCTGGAATG SEQ ID NO:929	-3.3	-26.7	75.5	-22.2	-1.1	-7
526	ATGTTTAATTGGAAGAGTGG SEQ ID NO:930	-3.3	-18.6	58.1	-15.3	0	-2.9
192	ACCATGGACATCAGCATTAG SEQ ID NO:931	-3.2	-23	67	-19.1	0	-8.8
244	CTGGCCTCGGTCCCTGTGGC SEQ ID NO:932	-3.2	-33.9	90.1	-28.3	-2.4	-7.2
343	CAGCCCAGACACTGTCATGA SEQ ID NO:933	-3.2	-26.7	74.7	-22.2	-1.2	-7.6
782	TAAGGTTAAATGACACTAGC SEQ ID NO:934	-3.2	-17.9	56	-14.2	-0.1	-4.5
824	AAATATTTATTGACTTCTGT SEQ ID NO:935	-3.2	-16.6	53.9	-13.4	0	-5.8
339	CCAGACACTGTCATGAATTT SEQ ID NO:936	-3.1	-21.7	64	-17.3	-1.2	-7.6
823	AATATTTATTGACTTCTGTT SEQ ID NO:937	-3.1	-17.4	56.1	-14.3	0	-3.8
651	CAAAATGATCTTGAAAAACA SEQ ID NO:938	-3	-13.3	45.4	-10.3	0	-4.9
504	GCTCAGAGCTCCTACAAAGG SEQ ID NO:939	-2.9	-24.8	71.3	-20.1	-1.8	-10.2
19	GCTTTAGTCCCAGGCCAGCG SEQ ID NO:940	-2.8	-31.2	84	-27.9	-0.2	-7.7
670	CACTAGAGAGAGCAACAAAC SEQ ID NO:941	-2.8	-18.8	57.3	-16	0	-4.5
735	TCAGGTAATTAAGCCTAAGC SEQ ID NO:942	-2.8	-21.1	63	-17.6	-0.4	-5.5

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
45	TCAGGATACTCAGCCTGGTG SEQ ID NO:943	-2.6	-25.9	75.3	-21.1	-2.2	-6.6
577	TGGGAGAAGAAGAGTGTCTG SEQ ID NO:944	-2.6	-21.1	64.2	-18.5	0	-2.9
453	AGAAATTGCTGGCAGGCTCT SEQ ID NO:945	-2.5	-24.9	71.5	-21.2	-1.1	-7.5
1028	CCCACCTCGCTCTTACCTCA SEQ ID NO:946	-2.5	-31	81.8	-28.5	0	-3.1
1087	ACCTATATTATCTTTAATAA SEQ ID NO:947	-2.5	-15.8	51.7	-12.7	-0.3	-3.3
313	CGGGGCTCTCAGGAACCAAT SEQ ID NO:948	-2.4	-26.8	72.7	-23.4	-0.9	-4.6
802	GCTACTTTCCTGATTGCATT SEQ ID NO:949	-2.4	-24.3	70.9	-21.9	0	-5.1
312	GGGGCTCTCAGGAACCAATC SEQ ID NO:950	-2.3	-26.4	74.4	-23.1	-0.9	-4.6
811	CTTCTGTTTGCTACTTTCCT SEQ ID NO:951	-2.3	-24.7	73.6	-22.4	0	-3.6
1019	CTCTTACCTCAGAAAGATTT SEQ ID NO:952	-2.3	-20.2	61.3	-17.2	-0.4	-3.6
46	GTCAGGATACTCAGCCTGGT SEQ ID NO:953	-2.2	-27.1	79.2	-22.7	-2.2	-6.6
307	TCTCAGGAACCAATCTTTGC SEQ ID NO:954	-2.1	-23	67.3	-20.4	-0.1	-4.1
280	TTCTTGGCCGCCTTCCTGGA SEQ ID NO:955	-2	-31.2	83.1	-28.1	-0.3	-10
338	CAGACACTGTCATGAATTTT SEQ ID NO:956	-2	-19.8	60.5	-16.5	-1.2	-7.6
633	CATGCTTTTTGAGAGCACTG SEQ ID NO:957	-2	-22.6	67.1	-18.2	-2.4	-6.7
663	GAGAGCAACAAACAAAATGA SEQ ID NO:958	-2	-15.9	50.2	-13.9	0	-4.1
665	GAGAGAGCAACAAACAAAAT SEQ ID NO:959	-2	-15.9	50.4	-13.9	0	-4.1
666	AGAGAGAGCAACAAACAAAA SEQ ID NO:960	-2	-15.9	50.5	-13.9	0	-4.1
813	GACTTCTGTTTGCTACTTTC SEQ ID NO:961	-2	-22.6	69.7	-20.6	0	-3.6
55	ATGACTCAGGTCAGGATACT SEQ ID NO:962	-1.9	-22.9	69.1	-18.1	-2.9	-7.2
259	CCATCTCCTAGAAGCCTGGC SEQ ID NO:963	-1.9	-28.6	78.6	-26	0	-8.8
530	GAGAATGTTTAATTGGAAGA SEQ ID NO:964	-1.9	-16.7	53.4	-14.8	0	-2.9
775	AAATGACACTAGCTAGGAAG SEQ ID NO:965	-1.9	-18.4	56.7	-15.5	0	-9.9
831	CATTTAAAAATATTTATTGA SEQ ID NO:966	-1.9	-12.1	43.7	-9.5	-0.4	-6.7
801	CTACTTTCCTGATTGCATTT SEQ ID NO:967	-1.8	-22.6	67	-20.8	0	-5.1
80	TGCTGGAGGCTCCTGATCCC SEQ ID NO:968	-1.7	-30.7	83.9	-27.7	-1.2	-7
203	GGCTGCTAGAGACCATGGAC SEQ ID NO:969	-1.7	-26.4	74.9	-23.9	-0.4	-8.8
314	TCGGGGCTCTCAGGAACCAA SEQ ID NO:970	-1.7	-27.2	74.3	-24.5	-0.9	-4.6
1017	CTTACCTCAGAAAGATTTGT SEQ ID NO:971	-1.7	-20.1	60.9	-18.4	0	-2.5
242	GGCCTCGGTCCCTGTGGCCT SEQ ID NO:972	-1.6	-35.9	93.6	-30.1	-4.2	-10.8
21	ATGCTTTAGTCCCAGGCCAG SEQ ID NO:973	-1.5	-28.6	79.9	-26.6	0	-7.7
805	TTTGCTACTTTCCTGATTGC SEQ ID NO:974	-1.5	-23.7	70	-22.2	0	-3.6

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
281	ATTCTTGGCCGCCTTCCTGG SEQ ID NO:975	-1.4	-30.6	81.8	-28.2	0	-10
597	GGTACAGTGGGAGAGTGAGG SEQ ID NO:976	-1.4	-24.8	74.3	-23.4	0	-5.2
662	AGAGCAACAAACAAAATGAT SEQ ID NO:977	-1.4	-15.3	49.1	-13.9	0	-4.1
664	AGAGAGCAACAAACAAAATG SEQ ID NO:978	-1.4	-15.3	49.2	-13.9	0	-3.3
732	GGTAATTAAGCCTAAGCCTG SEQ ID NO:979	-1.4	-22.9	65.6	-20.6	-0.8	-6.5
812	ACTTCTGTTTGCTACTTTCC SEQ ID NO:980	-1.4	-24	72.2	-22.6	0	-3.6
529	AGAATGTTTAATTGGAAGAG SEQ ID NO:981	-1.3	-16.1	52.3	-14.8	0	-2.9
593	CAGTGGGAGAGTGAGGTGGG SEQ ID NO:982	-1.3	-26.1	76.8	-24.8	0	-3.1
1022	TCGCTCTTACCTCAGAAAGA SEQ ID NO:983	-1.3	-23	66.5	-21.2	-0.2	-3.5
1036	TTTTTTGTCCCACCTCGCTC SEQ ID NO:984	-1.3	-28.2	78.4	-26.9	0	-3.1
315	CTCGGGGCTCTCAGGAACCA SEQ ID NO:985	-1.2	-28.8	78.5	-26.6	-0.9	-4.6
44	CAGGATACTCAGCCTGGTGG SEQ ID NO:986	-1.1	-26.7	76.2	-24	-1.6	-6.7
79	GCTGGAGGCTCCTGATCCCT SEQ ID NO:987	-1.1	-31.6	86.1	-29.2	-1.2	-7
1029	TCCCACCTCGCTCTTACCTC SEQ ID NO:988	-1.1	-30.7	82.6	-29.6	0	-3.1
255	CTCCTAGAAGCCTGGCCTCG SEQ ID NO:989	-1	-29.6	79.2	-27.7	-0.3	-9.5
310	GGCTCTCAGGAACCAATCTT SEQ ID NO:990	-1	-25	71.6	-23.5	-0.1	-4.6
578	GTGGGAGAAGAAGAGTGTCT SEQ ID NO:991	-1	-22.3	67.6	-21.3	0	-2.9
1088	AACCTATATTATCTTTAATA SEQ ID NO:992	-1	-15.8	51.7	-14	-0.6	-3.1
282	CATTCTTGGCCGCCTTCCTG SEQ ID NO:993	-0.9	-30.1	80.3	-28.7	0	-8
452	GAAATTGCTGGCAGGCTCTG SEQ ID NO:994	-0.9	-24.9	71.1	-22.8	-1.1	-7.2
533	GCTGAGAATGTTTAATTGGA SEQ ID NO:995	-0.9	-19.5	59.6	-18.6	0	-2.9
804	TTGCTACTTTCCTGATTGCA SEQ ID NO:996	-0.9	-24.3	70.8	-22.9	-0.2	-4.8
20	TGCTTTAGTCCCAGGCCAGC SEQ ID NO:997	-0.8	-30.4	84.5	-29.1	0	-7.7
470	TTAGCTGACATTGTTTGAGA SEQ ID NO:998	-0.8	-20.7	63.6	-19.9	0	-5.4
542	GTCTTCTTGGCTGAGAATGT SEQ ID NO:999	-0.7	-23.6	71.1	-22	-0.8	-8.1
661	GAGCAACAAACAAAATGATC SEQ ID NO:1000	-0.7	-15.7	50	-15	0	-4.1
810	TTCTGTTTGCTACTTTCCTG SEQ ID NO:1001	-0.7	-23.8	71.4	-23.1	0	-3.6
198	CTAGAGACCATGGACATCAG SEQ ID NO:1002	-0.6	-22.5	66.1	-21.2	0	-8.8
308	CTCTCAGGAACCAATCTTTG SEQ ID NO:1003	-0.6	-22.1	65.1	-21	-0.1	-4.6
669	ACTAGAGAGAGCAACAAACA SEQ ID NO:1004	-0.6	-18.8	57.3	-18.2	0	-4.5
803	TGCTACTTTCCTGATTGCAT SEQ ID NO:1005	-0.6	-24.2	70.4	-23.1	-0.2	-5.1
814	TGACTTCTGTTTGCTACTTT SEQ ID NO:1006	-0.6	-22.2	67.8	-21.6	0	-3.6

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
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818	TTATTGACTTCTGTTTGCTA SEQ ID NO:1008	-0.5	-20.8	64.5	-20.3	0	-3.6
1023	CTCGCTCTTACCTCAGAAAG SEQ ID NO:1009	-0.5	-23.3	67.1	-22.8	0	-3.1
311	GGGCTCTCAGGAACCAATCT SEQ ID NO:1010	-0.4	-26.1	73.8	-25.2	-0.1	-4.6
532	CTGAGAATGTTTAATTGGAA SEQ ID NO:1011	-0.3	-17	53.8	-16.7	0	-2.9
806	GTTTGCTACTTTCCTGATTG SEQ ID NO:1012	-0.2	-23.1	69	-22.9	0	-3.6
1089	AAACCTATATTATCTTTAAT SEQ ID NO:1013	-0.2	-15.4	50.5	-15.2	0	-2.5
54	TGACTCAGGTCAGGATACTC SEQ ID NO:1014	-0.1	-23.3	70.8	-20.5	-2.7	-6.8
808	CTGTTTGCTACTTTCCTGAT SEQ ID NO:1015	-0.1	-23.9	70.7	-23.8	0	-3.6
596	GTACAGTGGGAGAGTGAGGT SEQ ID NO:1016	0	-24.8	75.2	-24.8	0	-4.6
654	AAACAAAATGATCTTGAAAA SEQ ID NO:1017	0	-11.9	42.9	-11.9	0	-5
297	CAATCTTTGCACTCACATTC SEQ ID NO:1018	0.1	-21.6	64.9	-21.7	0	-5
469	TAGCTGACATTGTTTGAGAA SEQ ID NO:1019	0.1	-19.9	61.1	-20	0	-5.3
819	TTTATTGACTTCTGTTTGCT SEQ ID NO:1020	0.2	-21.2	65.5	-21.4	0	-3.6
53	GACTCAGGTCAGGATACTCA SEQ ID NO:1021	0.3	-24	72.2	-22.2	-2.1	-5.1
516	GGAAGAGTGGGCGCTCAGAG SEQ ID NO:1022	0.3	-26.3	74.7	-23.9	-2.7	-10.1
531	TGAGAATGTTTAATTGGAAG SEQ ID NO:1023	0.3	-16.1	52.1	-16.4	0	-2.9
655	CAAACAAAATGATCTTGAAA SEQ ID NO:1024	0.3	-13.3	45.4	-13.6	0	-5
815	TTGACTTCTGTTTGCTACTT SEQ ID NO:1025	0.3	-22.2	67.8	-22.5	0	-3.6
1018	TCTTACCTCAGAAAGATTTG SEQ ID NO:1026	0.3	-19.3	59.3	-19.1	-0.2	-3.5
537	CTTGGCTGAGAATGTTTAAT SEQ ID NO:1027	0.4	-19.8	60.3	-20.2	0	-4
541	TCTTCTTGGCTGAGAATGTT SEQ ID NO:1028	0.4	-22.5	68	-22	-0.8	-8.1
317	TTCTCGGGGCTCTCAGGAAC SEQ ID NO:1029	0.5	-26.6	76.1	-27.1	0	-4.1
204	AGGCTGCTAGAGACCATGGA SEQ ID NO:1030	0.6	-26.2	74.6	-25.5	-1.2	-8.8
251	TAGAAGCCTGGCCTCGGTCC SEQ ID NO:1031	0.6	-30.2	81.3	-29.9	-0.3	-9.5
668	CTAGAGAGAGCAACAAACAA SEQ ID NO:1032	0.8	-17.9	55	-18.7	0	-4.1
316	TCTCGGGGCTCTCAGGAACC SEQ ID NO:1033	0.9	-28.5	79.3	-29.4	0	-3.3
809	TCTGTTTGCTACTTTCCTGA SEQ ID NO:1034	0.9	-24.3	72.4	-25.2	0	-3.6
528	GAATGTTTAATTGGAAGAGT SEQ ID NO:1035	1	-17.3	55	-18.3	0	-2.9
538	TCTTGGCTGAGAATGTTTAA SEQ ID NO:1036	1	-20.2	61.7	-21.2	0	-5.8
652	ACAAAATGATCTTGAAAAAC SEQ ID NO:1037	1	-12.8	44.6	-13.8	0	-5
653	AACAAAATGATCTTGAAAAA SEQ ID NO:1038	1.1	-11.9	42.9	-13	0	-5

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
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807	TGTTTGCTACTTTCCTGATT SEQ ID NO:1040	1.2	-23.1	69	-24.3	0	-3.4
250	AGAAGCCTGGCCTCGGTCCC SEQ ID NO:1041	1.4	-32.5	85.2	-33	-0.3	-9.5
822	ATATTTATTGACTTCTGTTT SEQ ID NO:1042	1.4	-18.2	58.5	-19.6	0	-2.5
47	GGTCAGGATACTCAGCCTGG SEQ ID NO:1043	1.6	-27.1	78.2	-26.5	-2.2	-7
539	TTCTTGGCTGAGAATGTTTA SEQ ID NO:1044	1.6	-21	64.2	-21.8	-0.6	-7.8
50	TCAGGTCAGGATACTCAGCC SEQ ID NO:1045	1.7	-26.1	76.9	-26.7	-1	-4.6
820	ATTTATTGACTTCTGTTTGC SEQ ID NO:1046	1.7	-20.3	63.4	-22	0	-2.6
258	CATCTCCTAGAAGCCTGGCC SEQ ID NO:1047	1.8	-28.6	78.6	-29.3	0	-10.1
656	ACAAACAAAATGATCTTGAA SEQ ID NO:1048	1.8	-14.2	47.2	-16	0	-5
49	CAGGTCAGGATACTCAGCCT SEQ ID NO:1049	1.9	-26.6	77.1	-26.7	-1.8	-4.9
243	TGGCCTCGGTCCCTGTGGCC SEQ ID NO:1050	1.9	-35	91.4	-32.8	-4.1	-10.6
513	AGAGTGGGCGCTCAGAGCTC SEQ ID NO:1051	1.9	-28.3	81.6	-27.5	-2.7	-12.3
579	GGTGGGAGAAGAAGAGTGTC SEQ ID NO:1052	2	-22.6	68.3	-24.6	0	-1.8
817	TATTGACTTCTGTTTGCTAC SEQ ID NO:1053	2	-20.9	64.7	-22.9	0	-3.6
527	AATGTTTAATTGGAAGAGTG SEQ ID NO:1054	2.1	-16.7	53.6	-18.8	0	-2.9
545	ACTGTCTTCTTGGCTGAGAA SEQ ID NO:1055	2.2	-23.5	70.3	-24.9	-0.6	-7.8
52	ACTCAGGTCAGGATACTCAG SEQ ID NO:1056	2.4	-23.4	71.1	-24.9	-0.8	-3.8
197	TAGAGACCATGGACATCAGC SEQ ID NO:1057	2.4	-23.4	68.4	-25.1	0	-8.8
1024	CCTCGCTCTTACCTCAGAAA SEQ ID NO:1058	2.4	-25.3	70.4	-27.7	0	-3.1
821	TATTTATTGACTTCTGTTTG SEQ ID NO:1059	2.5	-18.2	58.4	-20.7	0	-2.5
51	CTCAGGTCAGGATACTCAGC SEQ ID NO:1060	2.6	-25	75.1	-26.7	-0.8	-4.2
544	CTGTCTTCTTGGCTGAGAAT SEQ ID NO:1061	2.6	-23.3	69.7	-25.1	-0.6	-7.9
641	TTGAAAAACATGCTTTTTGA SEQ ID NO:1062	2.6	-16.5	52.2	-17.5	-1.5	-9.1
657	AACAAACAAAATGATCTTGA SEQ ID NO:1063	2.6	-14.2	47.2	-16.8	0	-5
1026	CACCTCGCTCTTACCTCAGA SEQ ID NO:1064	2.6	-27.6	76.7	-30.2	0	-3.1
634	ACATGCTTTTTGAGAGCACT SEQ ID NO:1065	2.8	-22.8	67.8	-23.2	-2.4	-6.7
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733	AGGTAATTAAGCCTAAGCCT SEQ ID NO:1067	3.4	-22.9	66	-25.4	-0.8	-6.6
196	AGAGACCATGGACATCAGCA SEQ ID NO:1068	3.5	-24.4	70.1	-27.3	0	-8.5
640	TGAAAAACATGCTTTTTGAG SEQ ID NO:1069	3.5	-16.4	52.1	-18.3	-1.5	-9.1
658	CAACAAACAAAATGATCTTG SEQ ID NO:1070	3.5	-14.3	47.3	-17.8	0	-4.9

position	oligo	total binding	duplex form-ation	Tm of Duplex	target struc-ture	Intra-molecular oligo	Inter-molecular oligo
667	TAGAGAGAGCAACAAACAAA SEQ ID NO:1071	3.8	-16.3	51.6	-20.1	0	-4.1
734	CAGGTAATTAAGCCTAAGCC SEQ ID NO:1072	3.8	-22.7	65.2	-25.6	-0.8	-6.8
1027	CCACCTCGCTCTTACCTCAG SEQ ID NO:1073	3.8	-29	78.8	-32.8	0	-3.1
543	TGTCTTCTTGGCTGAGAATG SEQ ID NO:1074	3.9	-22.4	67.5	-25.4	-0.8	-8.1
580	AGGTGGGAGAAGAAGAGTGT SEQ ID NO:1075	4	-22.2	67	-26.2	0	0
587	GAGAGTGAGGTGGGAGAAGA SEQ ID NO:1076	4	-22.9	68.7	-26.9	0	0
254	TCCTAGAAGCCTGGCCTCGG SEQ ID NO:1077	4.1	-29.9	79.8	-33.4	0.2	-8.7
253	CCTAGAAGCCTGGCCTCGGT SEQ ID NO:1078	4.3	-30.7	81.4	-34.1	-0.3	-9.5
540	CTTCTTGGCTGAGAATGTTT SEQ ID NO:1079	4.3	-22.2	66.8	-25.6	-0.8	-8.1
592	AGTGGGAGAGTGAGGTGGGA SEQ ID NO:1080	4.3	-26	77.1	-30.3	0	0
595	TACAGTGGGAGAGTGAGGTG SEQ ID NO:1081	4.5	-23.6	71.3	-28.1	0	-4.6
193	GACCATGGACATCAGCATTA SEQ ID NO:1082	4.7	-23.6	68.1	-27.6	0	-8.8
194	AGACCATGGACATCAGCATT SEQ ID NO:1083	4.9	-23.9	68.9	-28.1	0	-8.8
581	GAGGTGGGAGAAGAAGAGTG SEQ ID NO:1084	5.3	-21.6	65	-26.9	0	0
586	AGAGTGAGGTGGGAGAAGAA SEQ ID NO:1085	5.3	-21.6	65	-26.9	0	0
252	CTAGAAGCCTGGCCTCGGTC SEQ ID NO:1086	5.6	-29.1	79.8	-33.8	-0.3	-9.5
22	TATGCTTTAGTCCCAGGCCA SEQ ID NO:1087	5.7	-28.3	79	-33.5	0	-7.7
589	GGGAGAGTGAGGTGGGAGAA SEQ ID NO:1088	5.8	-24.7	72.5	-30.5	0	0
590	TGGGAGAGTGAGGTGGGAGA SEQ ID NO:1089	6.1	-25.4	74.8	-31.5	0	0
195	GAGACCATGGACATCAGCAT SEQ ID NO:1090	6.2	-24.4	69.8	-29.9	0	-8.8
594	ACAGTGGGAGAGTGAGGTGG SEQ ID NO:1091	6.4	-25.1	74.7	-31.5	0	-4.6
588	GGAGAGTGAGGTGGGAGAAG SEQ ID NO:1092	7	-23.5	70	-30.5	0	0
591	GTGGGAGAGTGAGGTGGGAG SEQ ID NO:1093	7.3	-26	77.1	-33.3	0	0
659	GCAACAAACAAAATGATCTT SEQ ID NO:1094	9	-16.1	50.7	-25.1	0	-4.9
582	TGAGGTGGGAGAAGAAGAGT SEQ ID NO:1095	9.4	-21.6	65	-31	0	-0.1
48	AGGTCAGGATACTCAGCCTG SEQ ID NO:1096	9.5	-25.9	75.8	-33.6	-1.8	-7
584	AGTGAGGTGGGAGAAGAAGA SEQ ID NO:1097	9.6	-21.6	65	-31.2	0	0
583	GTGAGGTGGGAGAAGAAGAG SEQ ID NO:1098	11.4	-21.6	65	-33	0	0
585	GAGTGAGGTGGGAGAAGAAG SEQ ID NO:1099	11.9	-21.6	65	-33.5	0	0

Example 15

Western blot analysis of VCC-1 protein levels

[00226] Western blot analysis (immunoblot analysis) is carried out using standard methods. Cells are harvested 16-20 h after oligonucleotide treatment, washed once with PBS, suspended in Laemmli buffer (100 ul/well), boiled for 5 minutes and loaded on a 16% SDS-PAGE gel. Gels are run for 1.5 hours at 150 V, and transferred to membrane for western blotting. Appropriate primary antibody directed to VCC-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 CA).

WHAT IS CLAIMED IS:

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

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

3. The antisense oligonucleotide of claim 2 comprising a nucleic acid sequence selected from the group consisting of at least eight contiguous bases of SEQ ID NO:1 – SEQ ID NO:1099.

4. The antisense oligonucleotide of claim 2 comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 – SEQ ID NO:1099.

5. The antisense compound of claim 2, 3, or 4 wherein the antisense oligonucleotide comprises at least one modified internucleoside linkage.

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

7. The antisense compound of claim 2, 3, or 4 wherein the antisense oligonucleotide comprises at least one modified sugar moiety.

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

9. The antisense compound of claim 2, 3, or 4 wherein the antisense oligonucleotide comprises at least one modified nucleobase.

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

11. The antisense compound of claim 2, 3, or 4 wherein the antisense oligonucleotide is a chimeric oligonucleotide.

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

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

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

15. A method of inhibiting the expression of VCC-1 in cells or tissues comprising contacting said cells or tissues with the antisense compound of claim 1 so that expression of VCC-1 is inhibited.

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

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

18. The method of claim 16 wherein the disease or condition is an immunological disorder.

19. The method of claim 16 wherein the disease or condition is a cardiovascular disorder.

20. The method of claim 16 wherein the disease or condition is a neurologic disorder.

21. The method of claim 16 wherein the disease or condition is ischemia/reperfusion injury.

22. The method of claim 16 wherein the disease or condition is any form of cancer.

23. The method of claim 16 wherein the disease or condition is an angiogenic disorder.

Figure 1

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