COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF GLUCOCORTICOID RECEPTOR (GCR) GENES

This invention relates to double-stranded ribonucleic acids (dsRNAs), and their use in mediating RNA interference to inhibit the expression of the GCR gene. Furthermore, the use of said dsRNAs to treat/prevent a wide range of diseases/disorders which are associated with the expression of the GCR gene, like diabetes, dyslipidemia, obesity, hypertension, cardiovascular diseases or depression is part of the invention.

Glucocorticoids are responsible for several physiological functions including answer to stress, immune and inflammatory responses as well as stimulation of hepatic gluconeogenesis and glucose utilization at the periphery. Glucocorticoids act via an intracellular glucocorticoid receptor (GCR) belonging to the family of the nuclear steroidal receptors. The non-activated GCR is located in the cellular cytoplasm and is associated with several chaperone proteins. When a ligand activates the receptor, the complex is translocated in the cell nucleus and interacts with the glucocorticoid response element which is located in several gene promoters. The receptor could act in the cell nucleus as an homodimer or an heterodimer. Moreover several associated co-activators or co-repressors could also interact with the complex. This large range of possible combinations leads to several GCR conformations and several possible physiological answers making difficult to identify a small chemical entity which can act as a full and specific GCR inhibitor.

Pathologies like diabetes, Cushing's syndrome or depression have been associated with moderate to severe hypercortisolism (Chiodini et al, Eur. J. Endocrinol. 2005, Vol. 153, pp 837-844; Young, Stress 2004, Vol. 7 (4), pp 205-208). GCR antagonist administration has been proven to be clinically active in depression (Flores et al, Neuropsychopharmacology 2006, Vol. 31, pp 628-636) or in Cushing's syndrome (Chu et al, J. Clin. Endocrinol. Metab. 2001, Vol. 86, pp 3568-3573). These clinical evidences illustrate the potential clinical value of a potent and selective GCR antagonist in many indications like diabetes, dyslipidemia, obesity, hypertension, cardiovascular diseases or depression (Von Geldern et al, J. Med. Chem. 2004, Vol 47 (17), pp 4213-4230; Hu et al, Drug Develop. Res. 2006, Vol. 67, pp 871-883; Andrews, Handbook of the

stress and the brain 2005, Vol. 15, pp 437-450). This approach might also improve peripheral insulin sensitivity (Zinker et al, Meta. Clin. Exp. 2007, Vol. 57, pp 380-387) and protect pancreatic beta cells (Delauney et al, J. Clin. Invest. 1997, Vol. (100, pp 2094-2098).

Diabetic patients have an increased level of fasting blood glucose which has been correlated in clinic with an impaired control of gluconeogenesis (DeFronzo, Med. Clin. N. Am. 2004, Vol. 88 pp 787-835). The hepatic gluconeogenesis process is under the control of glucocorticoids. Clinical administration of a non-specific GCR antagonist (RU486 / mifepristone) leads acutely to a decrease of fasting plasma glucose in normal volunteers (Garrel et al, J. Clin. Endocrinol. Metab. 1995, Vol. 80 (2), pp 379-385) and chronically to a decrease of plasmatic HbA1c in Cushing's patients (Nieman et al, J. Clin. Endocrinol. Metab. 1985, Vol. 61 (3), pp 536-540). Moreover, this drug given to leptin deficient animals normalizes fasting plasma glucose (ob/ob mice, Gettys et al, Int. J. Obes. 1997, Vol. 21, pp 865-873) as well as the activity of gluconeogenic enzymes (db/db mice, Friedman et al, J. Biol. Chem. 1997, Vol. 272 (50) pp 31475-31481). Liver-specific knockout mice have been produced and these animals display a moderate hypoglycemia when they are fasted for 48h minimizing the risk of severe hypoglycemia (Opherk et al, Mol. Endocrinol. 2004, Vol. 18 (6), pp 1346-1353). Moreover, hepatic and adipose tissue GCR silencing in diabetic mice (db/db mice) with an antisense approach leads to significant reduction of blood glucose (Watts et al, Diabetes, 2005, Vol 54,pp 1846-1853).

Endogenous corticosteroid secretion at the level of the adrenal gland can be modulated by the Hypothalamus-Pituitary gland- Adrenal gland axis (HPA). Low plasma level of endogenous corticosteroids can activate this axis via a feed-back mechanism which leads to an increase of endogenous corticosteroids circulating in the blood. Mifepristone which crosses the blood brain barrier is known to stimulate the HPA axis which ultimately leads to an increase of endogenous corticosteroids circulating in the blood (Gaillard et al, Pro. Natl. Acad. Sci. 1984, Vol. 81, pp 3879-3882). Mifepristone also induces some adrenal insufficiency symptoms after long term treatment (up to 1 year, for review see: Sitruk-Ware et al, 2003, Contraception, Vol. 68, pp 409-420). Moreover because of its lack of tissue selectivity Mifepristone inhibits the effect of glucocorticoids at the periphery in preclinical models as well as in human (Jacobson et al, 2005 J. Pharm. Exp. Ther. Vol 314 (1) pp 191-200; Gaillard et al, 1985 J. Clin. Endo. Met., Vol. 61 (6), pp 1009-1011)

For GCR modulator to be used in indications such as diabetes, dyslipidemia, obesity, hypertension and cardiovascular diseases it is necessary to limit the risk to activate or inhibit the HPA axis and to inhibit GCR at the periphery in other organs than liver. Silencing directly GCR in hepatocytes can be an approach to modulate/normalize hepatic gluconeogenesis as demonstrated recently. However this effect has been seen only at rather high concentrations (in vitro IC50 in the range of 25nM / Watts et al, Diabetes, 2005, Vol 54,pp 1846-1853). To minimize the risk of off target effect as well as to limit pharmacological activity at the periphery in other organs than liver it would be necessary to get more potent GCR silencing agent.

Double-stranded ribonucleic acid (dsRNA) molecules have been shown to block gene expression in a highly conserved regulatory mechanism known as RNA interference (RNAi). The invention provides double-stranded ribonucleic acid (dsRNA) molecules able to selectively and efficiently decrease the expression of GCR. The use of GCR RNAi provides a method for the therapeutic and/or prophylactic treatment of diseases/disorders which are associated with any dysregulation of the glucocorticoid pathway. These diseases/disorders can occur due to systemic or local overproduction of endogenous glucocorticoids or due to treatment with synthetic glucocorticoids (e.g. diabetic-like syndrome in patients treated with high doses of glucocorticoids). Particular disease/disorder states include the therapeutic and/or prophylactic treatment of type 2 diabetes, obesity, dislipidemia, diabetic atherosclerosis, hypertension and depression, which method comprises administration of dsRNA targeting GCR to a human being or animal. Further, the invention provides a method for the therapeutic and/or prophylactic treatment of Metabolic Syndrome X, Cushing's Syndrome, Addison's disease, inflammatory diseases such as asthma, rhinitis, and arthritis, allergy, autoimmune disease, immunodeficiency, anorexia, cachexia, bone loss or bone frailty, and wound healing. Metabolic Syndrome X refers to a cluster of risk factors that include obesity, dyslipidimia, particularly high triglycerides, glucose intolerance, high blood sugar and high blood pressure.

In one preferred embodiment the described dsRNA molecule is capable of inhibiting the expression of a GCR gene by at least 70 %, preferably by at least 80%, most preferably by at least 90%. The invention also provides compositions and methods for specifically targeting the liver with GCR dsRNA, for treating pathological conditions and diseases caused by the expression of the GCR gene including those described above. In other embodiments the invention provides compositions and methods for specifically targeting other tissues or organs affected, including, but not limited to adipose tissue, the hypothalamus, kidneys or the pancreas.

In one embodiment, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a GCR gene, in particular the expression of the mammalian or human GCR gene. The dsRNA comprises at least two sequences that are complementary to each other. The dsRNA comprises a sense strand comprising a first sequence and an antisense strand may comprise a second sequence, see sequences provided in the sequence listing and also provision of specific dsRNA pairs in the appended tables 1 and 4. In one embodiment the sense strand comprises a sequence which has an identity of at least 90% to at least a portion of an mRNA encoding GCR. Said sequence is located in a region of complementarity of the sense strand to the antisense strand, preferably within nucleotides 2-7 of the 5' terminus of the antisense strand. In one preferred embodiment the dsRNA targets particularly the human GCR gene, in yet another preferred embodiment the dsRNA targets the mouse (Mus musculus) and rat (Rattus norvegicus) GCR gene.

In one embodiment, the antisense strand comprises a nucleotide sequence which is substantially complementary to at least part of an mRNA encoding said GCR gene, and the region of complementarity is most preferably less than 30 nucleotides in length. Furthermore, it is preferred that the length of the herein described inventive dsRNA molecules (duplex length) is in the range of about 16 to 30 nucleotides, in particular in the range of about 18 to 28 nucleotides. Particularly useful in context of this invention are duplex lengths of about 19, 20, 21, 22, 23 or 24 nucleotides. Most preferred are duplex stretches of 19, 21 or 23 nucleotides. The dsRNA, upon contacting with a cell expressing a GCR gene, inhibits the expression of a GCR gene in vitro by at least 70%, preferably by at least 80%, most preferred by 90%.

Appended Table 13 relates to preferred molecules to be used as dsRNA in accordance with this invention. Also modified dsRNA molecules are provided herein and are in particular disclosed in appended tables 1 and 4, providing illustrative examples of modified dsRNA molecules of the present invention. As pointed out herein above, Table 1 provides for illustrative examples of modified dsRNAs of this invention (whereby the corresponding sense strand and antisense strand is provided in this table). The relation of the unmodified preferred molecules shown in Table 13 to the modified dsRNAs of Table 1 is illustrated in Table 14. Yet, the illustrative modifications of these constituents of the inventive dsRNAs are provided herein as examples of modifications.

Tables 2 and 3 provide for selective biological, clinically and pharmaceutical relevant parameters of certain dsRNA molecules of this invention.

Most preferred dsRNA molecules are provided in the appended table 13 and, inter alia and preferably, wherein the sense strand is selected from the group consisting of the nucleic acid sequences depicted in SEQ ID Nos 873, 929, 1021, 1023, 967 and 905 and the antisense strand is selected from the from the group consting of the nucleic acid sequences depicted in SEQ ID Nos 874, 930, 1022, 1024, 968 and 906. Accordingly, the inventive dsRNA molecule may, inter alia, comprise the sequence pairs selected from the group consisting of SEQ ID NOs: 873/874, 929/930, 1021/1022, 1023/1024, 967/968 and 905/906. In context of specific dsRNA molecules provided herein, pairs of SEQ ID NOs relate to corresponding sense and antisense strands sequences (5' to 3') as also shown in appended and included tables.

In one embodiment said dsRNA molecules comprise an antisense strand with a 3' overhang of 1-5 nucleotides length, preferably of 1-2 nucleotides length. Preferably said overhang of the antisense strand comprises uracil or nucleotides which are complementary to the mRNA encoding GCR.

In another preferred embodiment, said dsRNA molecules comprise a sense strand with a 3' overhang of 1-5 nucleotides length, preferably of 1-2 nucleotides length. Preferably said overhang of the sense strand comprises uracil or nucleotides which are identical to the mRNA encoding GCR.

In another preferred embodiment, said dsRNA molecules comprise a sense strand with a 3' overhang of 1-5 nucleotides length, preferably of 1-2 nucleotides length, and an antisense strand with a 3' overhang of 1-5 nucleotides length, preferably of 1-2 nucleotides length. Preferably said overhang of the sense strand comprises uracil or nucleotides which are at least 90% identical to the mRNA encoding GCR and said overhang of the antisense strand comprises uracil or nucleotides which are at least 90% complementary to the mRNA encoding GCR

Most preferred dsRNA molecules are provided in the tables 1 and 4 below and, inter alia and preferably, wherein the sense strand is selected from the group consisting of the nucleic acid sequences depicted in SEQ ID NOs: 7, 31, 3, 25, 33, 55, 83, 747 and 764 the antisense strand is selected from the group consisting of the nucleic acid sequences depicted in SEQ ID NOs: 8, 32, 4, 26, 34, 56, 84, 753 and 772. Accordingly, the inventive dsRNA molecule may, inter alia, comprise the sequence pairs selected from the group consisting of SEQ ID NOs: 7/8, 31/32, 3/4,

25/26, 33/34, 55/56, 83/84, 747/753 and 764/772. In context of specific dsRNA molecules provided herein, pairs of SEQ ID NOs relate to corresponding sense and antisense strands sequences (5' to 3') as also shown in appended and included tables.

The dsRNA molecules of the invention may be comprised of naturally occurring nucleotides or may be comprised of at least one modified nucleotide, such as a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, inverted deoxythymidine and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group. 2' modified nucleotides may have the additional advantage that certain immunostimulatory factors or cytokines are suppressed when the inventive dsRNA molecules are employed in vivo, for example in a medical setting. Alternatively and non-limiting, the modified nucleotide may be chosen from the group of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide. In one preferred embodiment the dsRNA molecules comprises at least one of the following modified nucleotides: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group and a deoxythymidine. In another preferred embodiment all pyrimidines of the sense strand are 2'-O-methyl modified nucleotides, and all pyrimidines of the antisense strand are 2'-deoxy-2'-fluoro modified nucleotides. In one preferred embodiment two deoxythymidine nucleotides are found at the 3' of both strands of the dsRNA molecule. In another embodiment at least one of these deoxythymidine nucleotides at the 3' end of both strands of the dsRNA molecule comprises a 5'-phosphorothioate group. In another embodiment all cytosines followed by adenine, and all uracils followed by either adenine, guanine or uracil in the sense strand are 2'-O-methyl modified nucleotides, and all cytosines and uracils followed by adenine of the antisense strand are 2'-O-methyl modified nucleotides. Preferred dsRNA molecules comprising modified nucleotides are given in tables 1 and 4.

In a preferred embodiment the inventive dsRNA molecules comprise modified nucleotides as detailed in the sequences given in tables 1 and 4. In one preferred embodiment the inventive dsRNA molecule comprises sequence pairs selected from the group consisting of SEQ ID NOs: 7/8, 31/32, 3/4, 25/26, 33/34, 55/56 and 83/84, and comprise modifications as detailed in table 1.

In another embodiment the inventive dsRNAs comprise modified nucleotides on positions different from those disclosed in tables 1 and 4. In one preferred embodiment two

deoxythymidine nucleotides are found at the 3' of both strands of the dsRNA molecule. In another preferred embodiment one of those deoxythymidine nucleotides at the 3' of both strand is a inverted deoxythymidine.

In one embodiment the dsRNA molecules of the invention comprise of a sense and an antisense strand wherein both strands have a half-life of at least 9 hours. In one preferred embodiment the dsRNA molecules of the invention comprise of a sense and an antisense strand wherein both strands have a half-life of at least 9 hours in human serum. In another embodiment the dsRNA molecules of the invention comprise of a sense and an antisense strand wherein both strands have a half-life of at least 24 hours in human serum.

In another embodiment the dsRNA molecules of the invention are non-immunostimulatory, e.g. do not stimulate INF-alpha and TNF-alpha in vitro.

The invention also provides for cells comprising at least one of the dsRNAs of the invention. The cell is preferably a mammalian cell, such as a human cell. Furthermore, also tissues and/or non-human organisms comprising the herein defined dsRNA molecules are comprised in this invention, whereby said non-human organism is particularly useful for research purposes or as research tool, for example also in drug testing.

Furthermore, the invention relates to a method for inhibiting the expression of a GCR gene, in particular a mammalian or human GCR gene, in a cell, tissue or organism comprising the following steps:

(a) introducing into the cell, tissue or organism a double-stranded ribonucleic acid (dsRNA) as defined herein;

(b) maintaining said cell, tissue or organism produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a GCR gene, thereby inhibiting expression of a GCR gene in a given cell.

The invention also relates to pharmaceutical compositions comprising the inventive dsRNAs of this invention. These pharmaceutical compositions are particularly useful in the inhibition of the expression of a GCR gene in a cell, a tissue or an organism. The pharmaceutical composition comprising one or more of the dsRNA of the invention may also comprise (a) pharmaceutically acceptable carrier(s), diluent(s) and/or excipient(s).

In another embodiment, the invention provides methods for treating, preventing or managing disorders which are associated type 2 diabetes, obesity, dislipidemia, diabetic atherosclerosis, hypertension and depression, said method comprising administering to a subject in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of one or more of the dsRNAs of the invention. Preferably, said subject is a mammal, most preferably a human patient.

In one embodiment, the invention provides a method for treating a subject having a pathological condition mediated by the expression of a GCR gene. Such conditions comprise disorders associated with diabetes and obesity, as described above. In this embodiment, the dsRNA acts as a therapeutic agent for controlling the expression of a GCR gene. The method comprises administering a pharmaceutical composition of the invention to the patient (e.g., human), such that expression of a GCR gene is silenced. Because of their high specificity, the dsRNAs of the invention specifically target mRNAs of a GCR gene. In one preferred embodiment the described dsRNAs specifically decrease GCR mRNA levels and do not directly affect the expression and / or mRNA levels of off-target genes in the cell. In another preferred embodiment the described dsRNAs specifically decrease GCR mRNA levels as well as mRNA levels of genes that are normally activated by GCR In another embodiment the inventive dsRNAs decrease glucose levels in vivo.

In one preferred embodiment the described dsRNA decrease GCR mRNA levels in the liver by at least 70%, preferably by at least 80%, most preferably by at least 90% in vivo. Preferably the dsRNAs of the invention decrease glycemia without change in liver transaminases. In another embodiment the described dsRNAs decrease GCR mRNA levels in vivo for at least 4 days. In another embodiment the described dsRNAs decrease GCR mRNA levels in vivo by at least 60% for at least 4 days.

Particularly useful with respect to therapeutic dsRNAs is the set of dsRNAs targeting mouse and rat GCR which can be used to estimate toxicity, therapeutic efficacy and effective dosages and in vivo half-lives for the individual dsRNAs in an animal or cell culture model.

In another embodiment, the invention provides vectors for inhibiting the expression of a GCR gene in a cell, in particular GCR gene comprising a regulatory sequence operable linked to a nucleotide sequence that encodes at least one strand of one of the dsRNA of the invention.

In another embodiment, the invention provides a cell comprising a vector for inhibiting the expression of a GCR gene in a cell. Said vector comprises a regulatory sequence operable linked to a nucleotide sequence that encodes at least one strand of one of the dsRNA of the invention. Yet, it is preferred that said vector comprises, besides said regulatory sequence a sequence that encodes at least one "sense strand" of the inventive dsRNA and at least one "anti sense strand" of said dsRNA. It is also envisaged that the claimed cell comprises two or more vectors comprising, besides said regulatory sequences, the herein defined sequence(s) that encode(s) at least one strand of one of the dsRNA of the invention.

In one embodiment, the method comprises administering a composition comprising a dsRNA, wherein the dsRNA comprises a nucleotide sequence which is complementary to at least a part of an RNA transcript of a GCR gene of the mammal to be treated. As pointed out above, also vectors and cells comprising nucleic acid molecules that encode for at least one strand of the herein defined dsRNA molecules can be used as pharmaceutical compositions and may, therefore, also be employed in the herein disclosed methods of treating a subject in need of medical intervention. It is also of note that these embodiments relating to pharmaceutical compositions and to corresponding methods of treating a (human) subject also relate to approaches like gene therapy approaches. GCR specific dsRNA molecules as provided herein or nucleic acid molecules encoding individual strands of these inventive dsRNA molecules may also be inserted into vectors and used as gene therapy vectors for human patients. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

In another aspect of the invention, GCR specific dsRNA molecules that modulate GCR gene expression activity are expressed from transcription units inserted into DNA or RNA vectors (see, e.g., Skillern, A., et al., International PCT Publication No. WO 00/22113). These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be incorporated and inherited as a transgene integrated into the host genome. The transgene can

also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, et al., Proc. Natl. Acad. Sci. USA (1995) 92:1292).

The individual strands of a dsRNA can be transcribed by promoters on two separate expression vectors and co-transfected into a target cell. Alternatively each individual strand of the dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In a preferred embodiment, a dsRNA is expressed as an inverted repeat joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

The recombinant dsRNA expression vectors are preferably DNA plasmids or viral vectors. dsRNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus (for a review, see Muzyczka, et al., *Curr. Topics Micro. Immunol.* (1992) 158:97-129)); adenovirus (see, for example, Berkner, et al., BioTechniques (1998) 6:616), Rosenfeld et al. (1991, Science 252:431-434), and Rosenfeld et al. (1992), *Cell* 68:143-155)); or alphavirus as well as others known in the art. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, in vitro and/or in vivo (see, e.g., Danos and Mulligan, *Proc. NatI. Acad. Sci. USA* (1998) 85:6460-6464). Recombinant retroviral vectors capable of transducing and expressing genes inserted into the genome of a cell can be produced by transfecting the recombinant retroviral genome into suitable packaging cell lines such as PA317 and Psi-CRIP (Comette et al., 1991, Human Gene Therapy 2:5-10; Cone et al., 1984, Proc. Natl. Acad. Sci. USA 81:6349). Recombinant adenoviral vectors can be used to infect a wide variety of cells and tissues in susceptible hosts (e.g., rat, hamster, dog, and chimpanzee) (Hsu et al., 1992, J. Infectious Disease, 166:769), and also have the advantage of not requiring mitotically active cells for infection.

The promoter driving dsRNA expression in either a DNA plasmid or viral vector of the invention may be a eukaryotic RNA polymerase I (e.g. ribosomal RNA promoter), RNA polymerase II (e.g. CMV early promoter or actin promoter or U1 snRNA promoter) or preferably RNA polymerase III promoter (e.g. U6 snRNA or 7SK RNA promoter) or a prokaryotic promoter, for example the T7 promoter, provided the expression plasmid also encodes T7 RNA polymerase required for transcription from a T7 promoter. The promoter can also direct transgene expression to the pancreas (see, e.g. the insulin regulatory sequence for pancreas (Bucchini et al., 1986, Proc. Natl. Acad. Sci. USA 83:2511-2515)).

In addition, expression of the transgene can be precisely regulated, for example, by using an inducible regulatory sequence and expression systems such as a regulatory sequence that is sensitive to certain physiological regulators, e.g., circulating glucose levels, or hormones (Docherty et al., 1994, FASEB J. 8:20-24). Such inducible expression systems, suitable for the control of transgene expression in cells or in mammals include regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D1 - thiogalactopyranoside (EPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the dsRNA transgene.

Preferably, recombinant vectors capable of expressing dsRNA molecules are delivered as described below, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of dsRNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the dsRNAs bind to target RNA and modulate its function or expression. Delivery of dsRNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

dsRNA expression DNA plasmids are typically transfected into target cells as a complex with cationic lipid carriers (e.g. Oligofectamine) or non-cationic lipid-based carriers (e.g. Transit-TKO^{TM}). Multiple lipid transfections for dsRNA-mediated knockdowns targeting different regions of a single GCR gene or multiple GCR genes over a period of a week or more are also contemplated by the invention. Successful introduction of the vectors of the invention into host cells can be monitored using various known methods. For example, transient transfection can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection of ex vivo cells can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (e.g., antibiotics and drugs), such as hygromycin B resistance.

The following detailed description discloses how to make and use the dsRNA and compositions containing dsRNA to inhibit the expression of a target GCR gene, as well as compositions and methods for treating diseases and disorders caused by the expression of said GCR gene.

DEFINITIONS

For convenience, the meaning of certain terms and phrases used in the specification, examples, and appended claims, are provided below. If there is an apparent discrepancy between the usage of a term in other parts of this specification and its definition provided in this section, the definition in this section shall prevail.

"G," "C," "A", "U" and "T" or "dT" respectively, each generally stand for a nucleotide that contains guanine, cytosine, adenine, uracil and deoxythymidine as a base, respectively. However, the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. Sequences comprising such replacement moieties are embodiments of the invention. As detailed below, the herein described dsRNA molecules may also comprise "overhangs", i.e. unpaired, overhanging nucleotides which are not directly involved in the RNA double helical structure normally formed by the herein defined pair of "sense strand" and "anti sense strand". Often, such an overhanging stretch comprises the deoxythymidine nucleotide, in most embodiments, 2 deoxythymidines in the 3' end. Such overhangs will be described and illustrated below.

The term „GCR" as used herein relates in particular to the intracellular glucocorticoid receptor (GCR) and said term relates to the corresponding gene, also known as NR3C1 gene, encoded mRNA, encoded protein/polypeptide as well as functional fragments of the same. Preferred is the human GCR gene. In other preferred embodiments the dsRNAs of the invention target the GCR gene of rat (Rattus norvegicus) and mouse (Mus musculus), in yet another preferred embodiment the dsRNAs of the invention target the human (H.sapiens) and cynomolgous monkey (Macaca fascicularis) GCR gene. The term "GCR gene/sequence" does not only relate to (the) wild-type sequence(s) but also to mutations and alterations which may be comprised in said gene/sequence. Accordingly, the present invention is not limited to the specific dsRNA molecules provided herein. The invention also relates to dsRNA molecules that comprise an antisense strand that is at least 85% complementary to the corresponding nucleotide stretch of an RNA transcript of a GCR gene that comprises such mutations/alterations.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a GCR gene, including mRNA that is a product of RNA processing of a primary transcription product.

As used herein, the term “strand comprising a sequence” refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature. However, as detailed herein, such a “strand comprising a sequence” may also comprise modifications, like modified nucleotides.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence. “Complementary” sequences, as used herein, may also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in as far as the above requirements with respect to their ability to hybridize are fulfilled.

Sequences referred to as “fully complementary” comprise base-pairing of the oligonucleotide or polynucleotide comprising the first nucleotide sequence to the oligonucleotide or polynucleotide comprising the second nucleotide sequence over the entire length of the first and second nucleotide sequence.

However, where a first sequence is referred to as “substantially complementary” with respect to a second sequence herein, the two sequences can be fully complementary, or they may form one or more, but preferably not more than 13 mismatched base pairs upon hybridization.

The terms “complementary”, “fully complementary” and “substantially complementary” herein may be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a dsRNA and a target sequence, as will be understood from the context of their use.

The term “double-stranded RNA”, “dsRNA molecule”, or “dsRNA”, as used herein, refers to a ribonucleic acid molecule, or complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands. The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3’-end of one strand and the 5’end of the respective other strand forming the duplex structure, the connecting

RNA chain is referred to as a "hairpin loop". Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker". The RNA strands may have the same or a different number of nucleotides. In addition to the duplex structure, a dsRNA may comprise one or more nucleotide overhangs. The nucleotides in said "overhangs" may comprise between 0 and 5 nucleotides, whereby "0" means no additional nucleotide(s) that form(s) an "overhang" and whereas "5" means five additional nucleotides on the individual strands of the dsRNA duplex. These optional "overhangs" are located in the 3' end of the individual strands. As will be detailed below, also dsRNA molecules which comprise only an "overhang" in one the two strands may be useful and even advantageous in context of this invention. The "overhang" comprises preferably between 0 and 2 nucleotides. Most preferably 2 "dT" (deoxythymidine) nucleotides are found at the 3' end of both strands of the dsRNA. Also 2 "U"(uracil) nucleotides can be used as overhangs at the 3' end of both strands of the dsRNA. Accordingly, a "nucleotide overhang" refers to the unpaired nucleotide or nucleotides that protrude from the duplex structure of a dsRNA when a 3'-end of one strand of the dsRNA extends beyond the 5'-end of the other strand, or vice versa. For example the antisense strand comprises 23 nucleotides and the sense strand comprises 21 nucleotides, forming a 2 nucleotide overhang at the 3' end of the antisense strand. Preferably, the 2 nucleotide overhang is fully complementary to the mRNA of the target gene. "Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the dsRNA, i.e., no nucleotide overhang. A "blunt ended" dsRNA is a dsRNA that is double-stranded over its entire length, i.e., no nucleotide overhang at either end of the molecule.

The term "antisense strand" refers to the strand of a dsRNA which includes a region that is substantially complementary to a target sequence. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence. Where the region of complementarity is not fully complementary to the target sequence, the mismatches are most tolerated outside nucleotides 2-7 of the 5' terminus of the antisense strand

The term "sense strand," as used herein, refers to the strand of a dsRNA that includes a region that is substantially complementary to a region of the antisense strand. "Substantially complementary" means preferably at least 85% of the overlapping nucleotides in sense and antisense strand are complementary.

"Introducing into a cell", when referring to a dsRNA, means facilitating uptake or absorption into the cell, as is understood by those skilled in the art. Absorption or uptake of dsRNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. The meaning of this term is not limited to cells in vitro; a dsRNA may also be "introduced into a cell", wherein the cell is part of a living organism. In such instance, introduction into the cell will include the delivery to the organism. For example, for in vivo delivery, dsRNA can be injected into a tissue site or administered systemically. It is, for example envisaged that the dsRNA molecules of this invention be administered to a subject in need of medical intervention. Such an administration may comprise the injection of the dsRNA, the vector or an cell of this invention into a diseased side in said subject, for example into liver tissue/cells or into cancerous tissues/cells, like liver cancer tissue. However, also the injection in close proximity of the diseased tissue is envisaged. In vitro introduction into a cell includes methods known in the art such as electroporation and lipofection.

The terms "silence", "inhibit the expression of" and "knock down", in as far as they refer to a GCR gene, herein refer to the at least partial suppression of the expression of a GCR gene, as manifested by a reduction of the amount of mRNA transcribed from a GCR gene which may be isolated from a first cell or group of cells in which a GCR gene is transcribed and which has or have been treated such that the expression of a GCR gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). The degree of inhibition is usually expressed in terms of

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \bullet 100\%$$

Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to the GCR gene transcription, e.g. the amount of protein encoded by a GCR gene which is secreted by a cell, or the number of cells displaying a certain phenotype.

As illustrated in the appended examples and in the appended tables provided herein, the inventive dsRNA molecules are capable of inhibiting the expression of a human GCR by at least about 70%, preferably by at least 80%, most preferably by at least 90% in vitro assays, i.e in vitro. The term "in vitro" as used herein includes but is not limited to cell culture assays. In

another embodiment the inventive dsRNA molecules are capable of inhibiting the expression of a mouse or rat GCR by at least 70 %.preferably by at least 80%, most preferably by at least 90%. The person skilled in the art can readily determine such an inhibition rate and related effects, in particular in light of the assays provided herein.

The term "off target" as used herein refers to all non-target mRNAs of the transcriptome that are predicted by in silico methods to hybridize to the described dsRNAs based on sequence complementarity. The dsRNAs of the present invention preferably do specifically inhibit the expression of GCR, i.e. do not inhibit the expression of any off-target.

Particular preferred dsRNAs are provided, for example in appended Table 1 and 2 (sense strand and antisense strand sequences provided therein in 5' to 3' orientation), with the most preferred dsRNAs in table 2.

The term "half-life" as used herein is a measure of stability of a compound or molecule and can be assessed by methods known to a person skilled in the art, especially in light of the assays provided herein.

The term "non-immunostimulatory" as used herein refers to the absence of any induction of a immune response by the invented dsRNA molecules. Methods to determine immune responses are well know to a person skilled in the art, for example by assessing the release of cytokines, as described in the examples section.

The terms "treat", "treatment", and the like, mean in context of this invention to relief from or alleviation of a disorder related to GCR expression, like diabetes, dyslipidemia, obesity, hypertension, cardiovascular diseases or depression.

As used herein, a "pharmaceutical composition" comprises a pharmacologically effective amount of a dsRNA and a pharmaceutically acceptable carrier. However, such a "pharmaceutical composition" may also comprise individual strands of such a dsRNA molecule or the herein described vector(s) comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of a sense or an antisense strand comprised in the dsRNAs of this invention. It is also envisaged that cells, tissues or isolated organs that express or comprise the herein defined dsRNAs may be used as "pharmaceutical compositions". As used herein, "pharmacologically effective amount," "therapeutically effective amount" or

simply "effective amount" refers to that amount of an RNA effective to produce the intended pharmacological, therapeutic or preventive result.

The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The term specifically excludes cell culture medium. For drugs administered orally, pharmaceutically acceptable carriers include, but are not limited to pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives as known to persons skilled in the art.

It is in particular envisaged that the pharmaceutically acceptable carrier allows for the systemic adminstration of the dsRNAs, vectors or cells of this invention. Whereas also the enteric administration is envisaged the parentral administration and also transdermal or transmucosal (e.g. insufflation, buccal, vaginal, anal) administration as well was inhalation of the drug are feasible ways of administering to a patient in need of medical intervention the compounds of this invention. When parenteral administration is employed, this can comprise the direct injection of the compounds of this invention into the diseased tissue or at least in close proximity. However, also intravenous, intraarterial, subcutaneous, intramuscular, intraperitoneal, intradermal, intrathecal and other administrations of the compounds of this invention are within the skill of the artisan, for example the attending physician.

For intramuscular, subcutaneous and intravenous use, the pharmaceutical compositions of the invention will generally be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity. In a preferred embodiment, the carrier consists exclusively of an aqueous buffer. In this context, "exclusively" means no auxiliary agents or encapsulating substances are present which might affect or mediate uptake of dsRNA in the cells that express a GCR gene. Aqueous suspensions according to the invention may include suspending agents such as cellulose derivatives, sodium alginate, polyvinyl-pyrrolidone and gum tragacanth, and a wetting agent such as lecithin. Suitable preservatives for aqueous suspensions include ethyl and n-propyl p-hydroxybenzoate. The pharmaceutical compositions useful according to the invention also include encapsulated formulations to protect the dsRNA against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid.

Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in PCT publication WO 91/06309 which is incorporated by reference herein.

As used herein, a "transformed cell" is a cell into which at least one vector has been introduced from which a dsRNA molecule or at least one strand of such a dsRNA molecule may be expressed. Such a vector is preferably a vector comprising a regulatory sequence operably linked to nucleotide sequence that encodes at least one of a sense strand or an antisense strand comprised in the dsRNAs of this invention.

It can be reasonably expected that shorter dsRNAs comprising one of the sequences of Table 1 and 4 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. As pointed out above, in most embodiments of this invention, the dsRNA molecules provided herein comprise a duplex length (i.e. without "overhangs") of about 16 to about 30 nucleotides. Particular useful dsRNA duplex lengths are about 19 to about 25 nucleotides. Most preferred are duplex structures with a length of 19 nucleotides. In the inventive dsRNA molecules, the antisense strand is at least partially complementary to the sense strand.

In one preferred embodiment the inventive dsRNA molecules comprise nucleotides 1-19 of the sequences given in Table 13.

The dsRNA of the invention can contain one or more mismatches to the target sequence. In a preferred embodiment, the dsRNA of the invention contains no more than 13 mismatches. If the antisense strand of the dsRNA contains mismatches to a target sequence, it is preferable that the area of mismatch not be located within nucleotides 2-7 of the 5' terminus of the antisense strand. In another embodiment it is preferable that the area of mismatch not to be located within nucleotides 2-9 of the 5' terminus of the antisense strand. .

As mentioned above, at least one end/strand of the dsRNA may have a single-stranded nucleotide overhang of 1 to 5, preferably 1 or 2 nucleotides. dsRNAs having at least one nucleotide overhang have unexpectedly superior inhibitory properties than their blunt-ended counterparts. Moreover, the present inventors have discovered that the presence of only one nucleotide overhang strengthens the interference activity of the dsRNA, without affecting its

overall stability. dsRNA having only one overhang has proven particularly stable and effective in vivo, as well as in a variety of cells, cell culture mediums, blood, and serum. Preferably, the single-stranded overhang is located at the 3'-terminal end of the antisense strand or, alternatively, at the 3‘-terminal end of the sense strand. The dsRNA may also have a blunt end, preferably located at the 5’-end of the antisense strand. Preferably, the antisense strand of the dsRNA has a nucleotide overhang at the 3’-end, and the 5’-end is blunt. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

The dsRNA of the present invention may also be chemically modified to enhance stability. The nucleic acids of the invention may be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry", Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Chemical modifications may include, but are not limited to 2’ modifications, introduction of non-natural bases, covalent attachment to a ligand, and replacement of phosphate linkages with thiophosphate linkages. In this embodiment, the integrity of the duplex structure is strengthened by at least one, and preferably two, chemical linkages. Chemical linking may be achieved by any of a variety of well-known techniques, for example by introducing covalent, ionic or hydrogen bonds; hydrophobic interactions, van der Waals or stacking interactions; by means of metal-ion coordination, or through use of purine analogues. Preferably, the chemical groups that can be used to modify the dsRNA include, without limitation, methylene blue; bifunctional groups, preferably bis-(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)cystamine; 4-thiouracil; and psoralen. In one preferred embodiment, the linker is a hexa-ethylene glycol linker. In this case, the dsRNA are produced by solid phase synthesis and the hexa-ethylene glycol linker is incorporated according to standard methods (e.g., Williams, D.J., and K.B. Hall, *Biochem.* (1996) 35:14665-14670). In a particular embodiment, the 5'-end of the antisense strand and the 3'-end of the sense strand are chemically linked via a hexaethylene glycol linker. In another embodiment, at least one nucleotide of the dsRNA comprises a phosphorothioate or phosphorodithioate groups. The chemical bond at the ends of the dsRNA is preferably formed by triple-helix bonds.

In certain embodiments, a chemical bond may be formed by means of one or several bonding groups, wherein such bonding groups are preferably poly-(oxyphosphinicooxy-1,3-propandiol)- and/or polyethylene glycol chains. In other embodiments, a chemical bond may also be formed by means of purine analogs introduced into the double-stranded structure instead of

purines. In further embodiments, a chemical bond may be formed by azabenzene units introduced into the double-stranded structure. In still further embodiments, a chemical bond may be formed by branched nucleotide analogs instead of nucleotides introduced into the double-stranded structure. In certain embodiments, a chemical bond may be induced by ultraviolet light.

In yet another embodiment, the nucleotides at one or both of the two single strands may be modified to prevent or inhibit the activation of cellular enzymes, for example certain nucleases. Techniques for inhibiting the activation of cellular enzymes are known in the art including, but not limited to, 2'-amino modifications, 2'-amino sugar modifications, 2'-F sugar modifications, 2'-F modifications, 2'-alkyl sugar modifications, uncharged backbone modifications, morpholino modifications, 2'-O-methyl modifications, inverted thymidine and phosphoramidate (see, e.g., Wagner, *Nat. Med.* (1995) 1:1116-8). Thus, at least one 2'-hydroxyl group of the nucleotides on a dsRNA is replaced by a chemical group, preferably by a 2'-amino or a 2'-methyl group. Also, at least one nucleotide may be modified to form a locked nucleotide. Such locked nucleotide contains a methylene bridge that connects the 2'-oxygen of ribose with the 4'-carbon of ribose. Introduction of a locked nucleotide into an oligonucleotide improves the affinity for complementary sequences and increases the melting temperature by several degrees.

The compounds of the present invention can be synthesized using one or more inverted nucleotides, for example inverted thymidine or inverted adenine (see, for example, Takei, et al., 2002, JBC 277(26):23800-06).

Modifications of dsRNA molecules provided herein may positively influence their stability in vivo as well as in vitro and also improve their delivery to the (diseased) target side. Furthermore, such structural and chemical modifications may positively influence physiological reactions towards the dsRNA molecules upon administration, e.g. the cytokine release which is preferably suppressed. Such chemical and structural modifications are known in the art and are, inter alia, illustrated in Nawrot (2006) Current Topics in Med Chem, 6, 913-925.

Conjugating a ligand to a dsRNA can enhance its cellular absorption as well as targeting to a particular tissue. In certain instances, a hydrophobic ligand is conjugated to the dsRNA to facilitate direct permeation of the cellular membrane. Alternatively, the ligand conjugated to the dsRNA is a substrate for receptor-mediated endocytosis. These approaches have been used to facilitate cell permeation of antisense oligonucleotides. For example, cholesterol has been conjugated to various antisense oligonucleotides resulting in compounds that are substantially

more active compared to their non-conjugated analogs. See M. Manoharan *Antisense & Nucleic Acid Drug Development* 2002, *12*, 103. Other lipophilic compounds that have been conjugated to oligonucleotides include 1-pyrene butyric acid, 1,3-bis-O-(hexadecyl)glycerol, and menthol. One example of a ligand for receptor-mediated endocytosis is folic acid. Folic acid enters the cell by folate-receptor-mediated endocytosis. dsRNA compounds bearing folic acid would be efficiently transported into the cell via the folate-receptor-mediated endocytosis. Attachment of folic acid to the 3'-terminus of an oligonucleotide results in increased cellular uptake of the oligonucleotide (Li, S.; Deshmukh, H. M.; Huang, L. *Pharm. Res.* 1998, *15*, 1540). Other ligands that have been conjugated to oligonucleotides include polyethylene glycols, carbohydrate clusters, cross-linking agents, porphyrin conjugates, and delivery peptides.

In certain instances, conjugation of a cationic ligand to oligonucleotides often results in improved resistance to nucleases. Representative examples of cationic ligands are propylammonium and dimethylpropylammonium. Interestingly, antisense oligonucleotides were reported to retain their high binding affinity to mRNA when the cationic ligand was dispersed throughout the oligonucleotide. See M. Manoharan *Antisense & Nucleic Acid Drug Development* 2002, *12*, 103 and references therein.

The ligand-conjugated dsRNA of the invention may be synthesized by the use of a dsRNA that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the dsRNA. This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto. The methods of the invention facilitate the synthesis of ligand-conjugated dsRNA by the use of, in some preferred embodiments, nucleoside monomers that have been appropriately conjugated with ligands and that may further be attached to a solid-support material. Such ligand-nucleoside conjugates, optionally attached to a solid-support material, are prepared according to some preferred embodiments of the methods of the invention via reaction of a selected serum-binding ligand with a linking moiety located on the 5' position of a nucleoside or oligonucleotide. In certain instances, an dsRNA bearing an aralkyl ligand attached to the 3'-terminus of the dsRNA is prepared by first covalently attaching a monomer building block to a controlled-pore-glass support via a long-chain aminoalkyl group. Then, nucleotides are bonded via standard solid-phase synthesis techniques to the monomer building-block bound to the solid support. The

monomer building block may be a nucleoside or other organic compound that is compatible with solid-phase synthesis.

The dsRNA used in the conjugates of the invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

Teachings regarding the synthesis of particular modified oligonucleotides may be found in the following U.S. patents: U.S. Pat. No. 5,218,105, drawn to polyamine conjugated oligonucleotides; U.S. Pat. Nos. 5,541,307, drawn to oligonucleotides having modified backbones; U.S. Pat. No. 5,521,302, drawn to processes for preparing oligonucleotides having chiral phosphorus linkages; U.S. Pat. No. 5,539,082, drawn to peptide nucleic acids; U.S. Pat. No. 5,554,746, drawn to oligonucleotides having β-lactam backbones; U.S. Pat. No. 5,571,902, drawn to methods and materials for the synthesis of oligonucleotides; U.S. Pat. No. 5,578,718, drawn to nucleosides having alkylthio groups, wherein such groups may be used as linkers to other moieties attached at any of a variety of positions of the nucleoside; U.S. Pat. No 5,587,361 drawn to oligonucleotides having phosphorothioate linkages of high chiral purity; U.S. Pat. No. 5,506,351, drawn to processes for the preparation of 2'-O-alkyl guanosine and related compounds, including 2,6-diaminopurine compounds; U.S. Pat. No. 5,587,469, drawn to oligonucleotides having N-2 substituted purines; U.S. Pat. No. 5,587,470, drawn to oligonucleotides having 3-deazapurines; U.S. Pat. No. 5,608,046, both drawn to conjugated 4'-desmethyl nucleoside analogs; U.S. Pat. No. 5,610,289, drawn to backbone-modified oligonucleotide analogs; U.S. Pat. No 6,262,241 drawn to, inter alia, methods of synthesizing 2'-fluoro-oligonucleotides.

In the ligand-conjugated dsRNA and ligand-molecule bearing sequence-specific linked nucleosides of the invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand

molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. Oligonucleotide conjugates bearing a variety of molecules such as steroids, vitamins, lipids and reporter molecules, has previously been described (see Manoharan et al., PCT Application WO 93/07883). In a preferred embodiment, the oligonucleotides or linked nucleosides of the invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to commercially available phosphoramidites.

The incorporation of a 2'-O-methyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-allyl, 2'-O-aminoalkyl or 2'-deoxy-2'-fluoro group in nucleosides of an oligonucleotide confers enhanced hybridization properties to the oligonucleotide. Further, oligonucleotides containing phosphorothioate backbones have enhanced nuclease stability. Thus, functionalized, linked nucleosides of the invention can be augmented to include either or both a phosphorothioate backbone or a 2'-O-methyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-aminoalkyl, 2'-O-allyl or 2'-deoxy-2'-fluoro group.

In some preferred embodiments, functionalized nucleoside sequences of the invention possessing an amino group at the 5'-terminus are prepared using a DNA synthesizer, and then reacted with an active ester derivative of a selected ligand. Active ester derivatives are well known to those skilled in the art. Representative active esters include N-hydrosuccinimide esters, tetrafluorophenolic esters, pentafluorophenolic esters and pentachlorophenolic esters. The reaction of the amino group and the active ester produces an oligonucleotide in which the selected ligand is attached to the 5'-position through a linking group. The amino group at the 5'-terminus can be prepared utilizing a 5'-Amino-Modifier C6 reagent. In a preferred embodiment, ligand molecules may be conjugated to oligonucleotides at the 5'-position by the use of a ligand-nucleoside phosphoramidite wherein the ligand is linked to the 5'-hydroxy group directly or indirectly via a linker. Such ligand-nucleoside phosphoramidites are typically used at the end of an automated synthesis procedure to provide a ligand-conjugated oligonucleotide bearing the ligand at the 5'-terminus.

In one preferred embodiment of the methods of the invention, the preparation of ligand conjugated oligonucleotides commences with the selection of appropriate precursor molecules upon which to construct the ligand molecule. Typically, the precursor is an appropriately-protected derivative of the commonly-used nucleosides. For example, the synthetic precursors for the synthesis of the ligand-conjugated oligonucleotides of the invention include, but are not limited to, 2'-aminoalkoxy-5'-ODMT-nucleosides, 2'-6-aminoalkylamino-5'-ODMT-nucleosides, 5'-6-aminoalkoxy-2'-deoxy-nucleosides, 5'-6-aminoalkoxy-2-protected-nucleosides, 3'-6-

aminoalkoxy-5'-ODMT-nucleosides, and 3'-aminoalkylamino-5'-ODMT-nucleosides that may be protected in the nucleobase portion of the molecule. Methods for the synthesis of such amino-linked protected nucleoside precursors are known to those of ordinary skill in the art.

In many cases, protecting groups are used during the preparation of the compounds of the invention. As used herein, the term "protected" means that the indicated moiety has a protecting group appended thereon. In some preferred embodiments of the invention, compounds contain one or more protecting groups. A wide variety of protecting groups can be employed in the methods of the invention. In general, protecting groups render chemical functionalities inert to specific reaction conditions, and can be appended to and removed from such functionalities in a molecule without substantially damaging the remainder of the molecule.

Representative hydroxyl protecting groups, as well as other representative protecting groups, are disclosed in Greene and Wuts, *Protective Groups in Organic Synthesis*, Chapter 2, 2d ed., John Wiley & Sons, New York, 1991, and *Oligonucleotides And Analogues A Practical Approach*, Ekstein, F. Ed., IRL Press, N.Y, 1991.

Amino-protecting groups stable to acid treatment are selectively removed with base treatment, and are used to make reactive amino groups selectively available for substitution. Examples of such groups are the Fmoc (E. Atherton and R. C. Sheppard in *The Peptides*, S. Udenfriend, J. Meienhofer, Eds., Academic Press, Orlando, 1987, volume 9, p.1) and various substituted sulfonylethyl carbamates exemplified by the Nsc group (Samukov et al., *Tetrahedron Lett.*, 1994, 35:7821.

Additional amino-protecting groups include, but are not limited to, carbamate protecting groups, such as 2-trimethylsilylethoxycarbonyl (Teoc), 1-methyl-1-(4-biphenylyl)ethoxycarbonyl (Bpoc), t-butoxycarbonyl (BOC), allyloxycarbonyl (Alloc), 9-fluorenylmethyloxycarbonyl (Fmoc), and benzyloxycarbonyl (Cbz); amide protecting groups, such as formyl, acetyl, trihaloacetyl, benzoyl, and nitrophenylacetyl; sulfonamide protecting groups, such as 2-nitrobenzenesulfonyl; and imine and cyclic imide protecting groups, such as phthalimido and dithiasuccinoyl. Equivalents of these amino-protecting groups are also encompassed by the compounds and methods of the invention.

Many solid supports are commercially available and one of ordinary skill in the art can readily select a solid support to be used in the solid-phase synthesis steps. In certain

embodiments, a universal support is used. A universal support allows for preparation of oligonucleotides having unusual or modified nucleotides located at the 3'-terminus of the oligonucleotide. For further details about universal supports see Scott et al., *Innovations and Perspectives in solid-phase Synthesis*, *3rd International Symposium*, 1994, Ed. Roger Epton, Mayflower Worldwide, 115-124]. In addition, it has been reported that the oligonucleotide can be cleaved from the universal support under milder reaction conditions when oligonucleotide is bonded to the solid support via a *syn*-1,2-acetoxyphosphate group which more readily undergoes basic hydrolysis. See Guzaev, A. I.; Manoharan, M. *J. Am. Chem. Soc.* 2003, *125*, 2380.

The nucleosides are linked by phosphorus-containing or non-phosphorus-containing covalent internucleoside linkages. For the purposes of identification, such conjugated nucleosides can be characterized as ligand-bearing nucleosides or ligand-nucleoside conjugates. The linked nucleosides having an aralkyl ligand conjugated to a nucleoside within their sequence will demonstrate enhanced dsRNA activity when compared to like dsRNA compounds that are not conjugated.

The aralkyl-ligand-conjugated oligonucleotides of the invention also include conjugates of oligonucleotides and linked nucleosides wherein the ligand is attached directly to the nucleoside or nucleotide without the intermediacy of a linker group. The ligand may preferably be attached, via linking groups, at a carboxyl, amino or oxo group of the ligand. Typical linking groups may be ester, amide or carbamate groups.

Specific examples of preferred modified oligonucleotides envisioned for use in the ligand-conjugated oligonucleotides of the invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined here, oligonucleotides having modified backbones or internucleoside linkages 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 the invention, modified oligonucleotides that do not have a phosphorus atom in their intersugar backbone can also be considered to be oligonucleosides.

Specific oligonucleotide chemical modifications are described below. It is not necessary for all positions in a given compound to be uniformly modified. Conversely, more than one modifications may be incorporated in a single dsRNA compound or even in a single nucleotide thereof.

Preferred modified internucleoside linkages or 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.

Representative United States Patents relating to the preparation of the above phosphorus-atom-containing linkages include, but are not limited to, U.S. Pat. Nos. 4,469,863; 5,023,243; 5,264,423; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233 and 5,466,677, each of which is herein incorporated by reference.

Preferred modified internucleoside linkages or backbones that do not include a phosphorus atom therein (i.e., oligonucleosides) have backbones that are formed by short chain alkyl or cycloalkyl intersugar linkages, mixed heteroatom and alkyl or cycloalkyl intersugar linkages, or one or more short chain heteroatomic or heterocyclic intersugar 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.

Representative United States patents relating to the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,214,134; 5,216,141; 5,264,562; 5,466,677; 5,470,967; 5,489,677; 5,602,240 and 5,663,312, each of which is herein incorporated by reference.

In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleoside units are replaced with novel groups. The nucleobase units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligonucleotide, 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 atoms of the amide portion of the backbone. Teaching of PNA compounds can be found for example in U.S. Pat. No. 5,539,082.

Some preferred embodiments of the invention employ oligonucleotides with phosphorothioate linkages 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_2--N(CH_3)--N(CH_3)--CH_2--, and --O--N(CH_3)--CH_2 --CH_2-- [wherein the native phosphodiester backbone is represented as --O--P--O--CH_2--] of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

The oligonucleotides employed in the ligand-conjugated oligonucleotides of the invention may additionally or alternatively comprise nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases include other synthetic and natural nucleobases, such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in the *Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J. I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., *Angewandte Chemie, International Edition*, 1991, 30, 613, and those disclosed by Sanghvi, Y. S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Crooke, S. T. and Lebleu, B., ed., CRC

Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligonucleotides 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. (Id., pages 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-methoxyethyl sugar modifications.

Representative United States patents relating to the preparation of certain of the above-noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos 5,134,066; 5,459,255; 5,552,540; 5,594,121 and 5,596,091 all of which are hereby incorporated by reference.

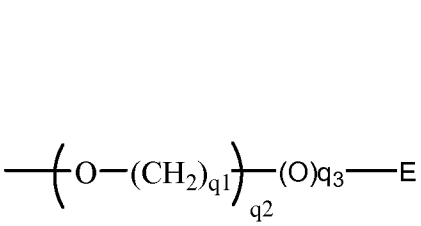
In certain embodiments, the oligonucleotides employed in the ligand-conjugated oligonucleotides of the invention may additionally or alternatively comprise one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl, O-, S-, or N-alkenyl, or O, S- or N-alkynyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2 CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. a preferred modification includes 2'-methoxyethoxy [2'-O--$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE], 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 U.S. Pat. No. 6,127,533, filed on Jan. 30, 1998, the contents of which are incorporated by reference.

Other preferred modifications include 2'-methoxy (2'-O--CH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other

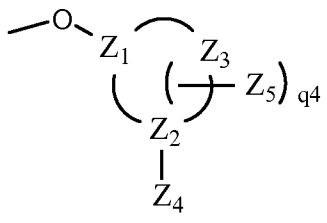
positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides.

As used herein, the term "sugar substituent group" or "2'-substituent group" includes groups attached to the 2'-position of the ribofuranosyl moiety with or without an oxygen atom. Sugar substituent groups include, but are not limited to, fluoro, O-alkyl, O-alkylamino, O-alkylalkoxy, protected O-alkylamino, O-alkylaminoalkyl, O-alkyl imidazole and polyethers of the formula $(O\text{-alkyl})_m$, wherein m is 1 to about 10. Preferred among these polyethers are linear and cyclic polyethylene glycols (PEGs), and (PEG)-containing groups, such as crown ethers and, inter alia, those which are disclosed by Delgardo et. al. (*Critical Reviews in Therapeutic Drug Carrier Systems* 1992, 9:249), which is hereby incorporated by reference in its entirety. Further sugar modifications are disclosed by Cook (*Anti-fibrosis Drug Design*, 1991, 6:585-607). Fluoro, O-alkyl, O-alkylamino, O-alkyl imidazole, O-alkylaminoalkyl, and alkyl amino substitution is described in U.S. Patent 6,166,197, entitled "Oligomeric Compounds having Pyrimidine Nucleotide(s) with 2' and 5' Substitutions," hereby incorporated by reference in its entirety.

Additional sugar substituent groups amenable to the invention include 2'-SR and 2'-NR_2 groups, wherein each R is, independently, hydrogen, a protecting group or substituted or unsubstituted alkyl, alkenyl, or alkynyl. 2'-SR Nucleosides are disclosed in U.S. Pat. No. 5,670,633, hereby incorporated by reference in its entirety. The incorporation of 2'-SR monomer synthons is disclosed by Hamm et al. (*J. Org. Chem.*, 1997, 62:3415-3420). 2'-NR nucleosides are disclosed by Goettingen, M., *J. Org. Chem.*, 1996, 61, 6273-6281; and Polushin et al., *Tetrahedron Lett.*, 1996, 37, 3227-3230. Further representative 2'-substituent groups amenable to the invention include those having one of formula I or II:



I



II

wherein,

E is C_1 -C_{10} alkyl, $N(Q_3)(Q_4)$ or $N{=}C(Q_3)(Q_4)$; each Q_3 and Q_4 is, independently, H, C_1-C_{10} alkyl, dialkylaminoalkyl, a nitrogen protecting group, a tethered or untethered conjugate

group, a linker to a solid support; or Q_3 and Q_4, together, form a nitrogen protecting group or a ring structure optionally including at least one additional heteroatom selected from N and O;

q_1 is an integer from 1 to 10;

q_2 is an integer from 1 to 10;

q_3 is 0 or 1;

q_4 is 0, 1 or 2;

each Z_1, Z_2 and Z_3 is, independently, C_4-C_7 cycloalkyl, C_5-C_{14} aryl or C_3-C_{15} heterocyclyl, wherein the heteroatom in said heterocyclyl group is selected from oxygen, nitrogen and sulfur;

Z_4 is OM_1, SM_1, or $N(M_1)_2$; each M_1 is, independently, H, C_1-C_8 alkyl, C_1-C_8 haloalkyl, $C(=NH)N(H)M_2$, $C(=O)N(H)M_2$ or $OC(=O)N(H)M_2$; M_2 is H or C_1-C_8 alkyl; and

Z_5 is C_1-C_{10} alkyl, C_1 -C_{10} haloalkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_6-C_{14} aryl, $N(Q_3)(Q_4)$, OQ_3, halo, SQ_3 or CN.

Representative 2'-O-sugar substituent groups of formula I are disclosed in U.S. Pat. No. 6,172,209, entitled "Capped 2'-Oxyethoxy Oligonucleotides," hereby incorporated by reference in its entirety. Representative cyclic 2'-O-sugar substituent groups of formula II are disclosed in U.S. Patent 6,271,358, entitled "RNA Targeted 2'-Modified Oligonucleotides that are Conformationally Preorganized," hereby incorporated by reference in its entirety.

Sugars having O-substitutions on the ribosyl ring are also amenable to the invention. Representative substitutions for ring O include, but are not limited to, S, CH_2, CHF, and CF_2.

Oligonucleotides may also have sugar mimetics, such as cyclobutyl moieties, in place of the pentofuranosyl sugar. Representative United States patents relating to the preparation of such modified sugars include, but are not limited to, U.S. Pat. Nos. 5,359,044; 5,466,786; 5,519,134; 5,591,722; 5,597,909; 5,646,265 and 5,700,920, all of which are hereby incorporated by reference.

Additional modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide. For example, one additional

modification of the ligand-conjugated oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more additional non-ligand 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), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4, 1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660, 306; Manoharan et al., *Bioorg. Med. Chem. Let.*, 1993, 3, 2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20, 533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10, 111; Kabanov et al., *FEBS Lett.*, 1990, 259, 327; Svinarchuk et al., *Biochimie*, 1993, 75, 49), 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, 3651; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14, 969), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264, 229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277, 923).

The invention also includes compositions employing oligonucleotides that are substantially chirally pure with regard to particular positions within the oligonucleotides. Examples of substantially chirally pure oligonucleotides include, but are not limited to, those having phosphorothioate linkages that are at least 75% Sp or Rp (Cook et al., U.S. Pat. No. 5,587,361) and those having substantially chirally pure (Sp or Rp) alkylphosphonate, phosphoramidate or phosphotriester linkages (Cook, U.S. Pat. Nos. 5,212,295 and 5,521,302).

In certain instances, the oligonucleotide may be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to oligonucleotides in order to enhance the activity, cellular distribution or cellular uptake of the oligonucleotide, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86:6553), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4:1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660:306; Manoharan et al., *Bioorg. Med. Chem. Let.*, 1993, 3:2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues

(Saison-Behmoaras et al., *EMBO J.*, 1991, 10:111; Kabanov et al., *FEBS Lett.*, 1990, 259:327; Svinarchuk et al., *Biochimie*, 1993, 75:49), 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, 36:3651; Shea et al., *Nucl. Acids Res.*, 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14:969), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36:3651), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277:923). Typical conjugation protocols involve the synthesis of oligonucleotides bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction may be performed either with the oligonucleotide still bound to the solid support or following cleavage of the oligonucleotide in solution phase. Purification of the oligonucleotide conjugate by HPLC typically affords the pure conjugate. The use of a cholesterol conjugate is particularly preferred since such a moiety can increase targeting to tissues in the liver, a site of GCR protein production.

Alternatively, the molecule being conjugated may be converted into a building block, such as a phosphoramidite, via an alcohol group present in the molecule or by attachment of a linker bearing an alcohol group that may be phosphorylated.

Importantly, each of these approaches may be used for the synthesis of ligand conjugated oligonucleotides. Amino linked oligonucleotides may be coupled directly with ligand via the use of coupling reagents or following activation of the ligand as an NHS or pentfluorophenolate ester. Ligand phosphoramidites may be synthesized via the attachment of an aminohexanol linker to one of the carboxyl groups followed by phosphitylation of the terminal alcohol functionality. Other linkers, such as cysteamine, may also be utilized for conjugation to a chloroacetyl linker present on a synthesized oligonucleotide.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including

definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The above provided embodiments and items of the present invention are now illustrated with the following, non-limiting examples.

Description of figures and appended tables:

Figure 1- Effect of GCR dsRNA comprising SEQ ID pair 55/56 on silencing off-target sequences. Expression of renilla luciferase protein after transfection of COS7 cells expressing dual-luciferase constructs, representative for either 19 mer target site of GCR mRNA ("on") or *in silico* predicted off-target sequences ("off 1" to "off 15"; with "off 1" – "off 12" being antisense strand off- targets and "off 13" to "off 15" being sense strand off -targets), with 50 nM GCR dsRNA. Perfect matching off-target dsRNAs are controls.

Figure 2 – Effect of GCR dsRNA comprising SEQ ID pair 83/84 on silencing off-target sequences. Expression of renilla luciferase protein after transfection of COS7 cells expressing dual-luciferase constructs, representative for either 19 mer target site of GCR mRNA ("on") or *in silico* predicted off-target sequences ("off 1" to "off 14"; with "off 1" – "off 11" being antisense strand off- targets and "off 12" and "off 14" being sense strand off -targets), with 50 nM GCR dsRNA. Perfect matching off-target dsRNAs are controls.

Figure 3– Effect of GCR dsRNA comprising SEQ ID pair 7/8 on silencing off-target sequences. Expression of renilla luciferase protein after transfection of COS7 cells expressing dual-luciferase constructs, representative for either 19 mer target site of GCR mRNA ("on") or *in silico* predicted off-target sequences ("off 1" to "off 14"; with "off 1" – "off 11" being antisense strand off- targets and "off 12" to "off 14" being sense strand off -targets), with 50 nM GCR dsRNA. Perfect matching off-target dsRNAs are controls.

Figure 4– mRNA levels, expressed in Quantigene 2.0 units / cell, for GCR (NR3C1) gene, or for housekeeping gene GUSB , in human primary hepatocytes 96h post-transfection with GCR dsRNAs or Luciferase dsRNA control, in comparison to control cells exposed to DharmaFECT-1 transfection reagent alone.

Figure 5– mRNA levels, expressed in Quantigene 2.0 units / cell, for GCR (NR3C1) gene (a), GUSB housekeeping gene (b) and GCR-target genes PCK1 (c) , G6Pc (d) and TAT (e), in human primary hepatocytes exposed for 48h to LNP01-formulated dsRNAs

Figure 6– Glucose output measured in primary human hepatocytes exposed for 48h to LNP01-dsRNAs (a) Luciferase dsRNA control b) GCR dsRNA comprising SEQ ID pair 55/56 c) GCR dsRNA comprising SEQ ID pair 83/84, and starved for 96h before incubation for 5h in the presence of gluconeogenic precursors (lactate and pyruvate).

Figure 7– Cell ATP content measured in primary human hepatocytes exposed for 48h to LNP01-dsRNAs (a) Luciferase dsRNA control b) GCR dsRNA comprising SEQ ID pair 55/56 c) GCR dsRNA comprising SEQ ID pair 83/84, and starved for 96h before incubation for 5h in the presence of gluconeogenic precursors (lactate and pyruvate) .

Figure 8– Liver mRNA levels, relative to GUSB housekeeping mRNA level, obtained for GCR (NR3C1 gene, Figure 8 a) and GCR-upregulated genes TAT (Figure 8a), PCK1 (Figure 8b), G6Pc (Figure 8b), and HES1 (down-regulated by GCR, Figure 8c), 103 h after single iv administration of LPNO1-formulated dsRNAs for GCR comprising SEQ ID pair 517/518 or Luciferase control SEQ ID pair 681/682 in hyperglycemic and diabetic 14 wks-old male db/db mice.

Figure 9– Time-course efficacy on blood glucose levels after single iv administration of LPNO1-dsRNAs in hyperglycemic and diabetic 14 wks-old male db/db mice. (*: $p<0.05$ versus vehicle). Efficacy of LPNO1-dsRNA for GCR comprising SEQ ID pair 517/518 in decreasing glucose level observed at +55-, +79-, +103h was of -13%, at -31% and -29%, respectively, when compared to the placebo (LNP01-Luciferase dsRNA SEQ ID pair 681/682). n = 4, mean values +/- SEM, t-test assuming equal variance for each day.

Figure 10– Time-course plasma levels in ALT and AST in hyperglycemic and diabetic 14 wks-old male db/db mice, 55, 79 and 103h after single iv administration of of LPNO1-dsRNAs for GCR comprising SEQ ID pair 517/518 or Luciferase control dsRNA (SEQ ID pair 681/682).

Figure 11 – GCR mRNA levels in liver biopsy of cynomolgus monkeys measured by bDNA assay 3 days post single i.v. bolus injection of Luciferase dsRNA (Seq. ID pair 681/682) or GCR dsRNAs (Seq. ID pair 747/753 or Seq. ID pair 764/772). Dose with respect to dsRNA

given for each group as mg/kg. N=2 female and male cynomolgus monkeys. Values are normalized to mean of GAPDH values of each individual monkey (a), or relative to Luciferase dsRNA (Seq. ID pair 681/682) with error bars indicating variations between monkeys (b).

Table 1 – dsRNA targeting human GCR gene. Letters in capitals represent RNA nucleotides, lower case letters "c", "g", "a" and "u" represent 2' O-methyl-modified nucleotides, "s" represents phosphorothioate and "dT" deoxythymidine, "invdT" inverted deoxythymidine, "f" represents 2' fluoro modification of the preceding nucleotide.

Table 2 - Characterization of dsRNAs targeting human GCR: Activity testing for dose response in HepG2 and HeLaS3 cells. IC 50: 50 % inhibitory concentration.

Table 3 - Characterization of dsRNAs targeting human GCR: Stability and Cytokine Induction. t ½ : half-life of a strand as defined in examples, PBMC: Human peripheral blood mononuclear cells.

Table 4 - dsRNAs targeting mouse and rat GCR genes. Letters in capitals represent RNA nucleotides, lower case letters "c", "g", "a" and "u" represent 2' O-methyl-modified nucleotides, "s" represents phosphorothioate and "dT" deoxythymidine. "f" represents 2' fluoro modification of the preceding nucleotide.

Table 5 - Characterization of dsRNA targeting mouse and rat GCR genes: Stability and Cytokine Induction. t ½ : half-life of a strand as defined in examples, PBMC: Human peripheral blood mononuclear cells.

Table 6 - Selected off-targets of dsRNAs targeting human GCR comprising sequence ID pair 55/56.

Table 7 - Selected off-targets of dsRNAs targeting human GCR comprising sequence ID pair 83/84.

Table 8 - Selected off-targets of dsRNAs targeting human GCR comprising sequence ID pair 7/8.

Table 9 - Sequences of bDNA probes for determination of human GAPDH; LE= label extender, CE= capture extender, BL= blocking probe.

Table 10 - Sequences of bDNA probes for determination of human GCR; LE= label extender, CE= capture extender, BL= blocking probe.

Table 11 - Sequences of bDNA probes for determination of mouse GCR; LE= label extender, CE= capture extender, BL= blocking probe.

Table 12- Sequences of bDNA probes for determination of mouse GAPDH; LE= label extender, CE= capture extender, BL= blocking probe.

Table 13 – dsRNA targeting human GCR gene. Letters in capitals represent RNA nucleotides.

Table 14 - dsRNA targeting human GCR gene without modifications and their modified counterparts. Letters in capitals represent RNA nucleotides, lower case letters "c", "g", "a" and "u" represent 2' O-methyl-modified nucleotides, "s" represents phosphorothioate and "dT" deoxythymidine, "invdT" inverted deoxythymidine.

EXAMPLES

Identification of dsRNAs for therapeutic use

dsRNA design was carried out to identify dsRNAs specifically targeting human GCR for therapeutic use. First, the known mRNA sequences of human (Homo sapiens) GCR (NM_000176.2, NM_001018074.1, NM_001018075.1, NM_001018076.1, NM_001018077.1, NM_001020825.1, NM_001024094.1 listed as SEQ ID NO. 659, SEQ ID NO. 660, SEQ ID NO. 661, SEQ ID NO. 662, SEQ ID NO. 663, SEQ ID NO. 664, and SEQ ID NO. 665) were downloaded from NCBI Genbank.

mRNAs of rhesus monkey (Macaca mulatta) GCR (XM_001097015.1, XM_001097126.1, XM_001097238.1, XM_001097341.1, XM_001097444.1, XM_001097542.1, XM_001097640.1, XM_001097749.1, XM_001097846.1 and XM_001097942.1) were downloaded from NCBI Genbank (SEQ ID NO. 666, SEQ ID NO. 667, SEQ ID NO. 668, SEQ

ID NO. 669, SEQ ID NO. 670, SEQ ID NO. 671, SEQ ID NO. 672, SEQ ID NO. 673, SEQ ID NO. 674, and SEQ ID NO. 675).

An EST of cynomolgus monkey (Macaca fascicularis) GCR (BB878843.1) was downloaded from NCBI Genbank (SEQ ID NO. 676).

The monkey sequences were examined together with the human GCR mRNA sequences (SEQ ID NO. 677) by computer analysis to identify homologous sequences of 19 nucleotides that yield RNA interference (RNAi) agents cross-reactive to human and rhesus monkey or human and cynomolgus monkey sequences.

In identifying RNAi agents, the selection was limited to 19mer sequences having at least 2 mismatches in the antisense strand to any other sequence in the human RefSeq database (release 27), which we assumed to represent the comprehensive human transcriptome, by using a proprietary algorithm.

The cynomolgous monkey GCR gene was sequenced (see SEQ ID NO. 678) and examined for target regions of RNAi agents.

dsRNAs cross-reactive to human as well as cynomolgous monkey GCR were defined as most preferable for therapeutic use. All sequences containing 4 or more consecutive G's (poly-G sequences) were excluded from the synthesis.

The sequences thus identified formed the basis for the synthesis of the RNAi agents in appended Tables 1, and 14 .

Identification of dsRNAs for *in vivo* proof of concept studies

dsRNA design was carried out to identify dsRNAs targeting mouse (*Mus musculus*) and rat (*Rattus norvegicus*) for *in vivo* proof-of-concept experiments. First, the transcripts for mouse GCR (NM_008173.3, SEQ ID NO. 679) and rat GCR (NM_012576.2, SEQ ID NO. 680) were examined by computer analysis to identify homologous sequences of 19 nucleotides that yield RNAi agents cross-reactive between these sequences.

In identifying RNAi agents, the selection was limited to 19mer sequences having at least 2 mismatches in the antisense strand to any other sequence in the mouse and rat RefSeq database

(release 27), which we assumed to represent the comprehensive mouse and rat transcriptome, by using a proprietary algorithm.

All sequences containing 4 or more consecutive G's (poly-G sequences) were excluded from the synthesis. The sequences thus identified formed the basis for the synthesis of the RNAi agents in appended Table 4.

dsRNA synthesis

Where the source of a reagent is not specifically given herein, such reagent may be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

Single-stranded RNAs were produced by solid phase synthesis on a scale of 1 µmole using an Expedite 8909 synthesizer (Applied Biosystems, Applera Deutschland GmbH, Darmstadt, Germany) and controlled pore glass (CPG, 500Å, Proligo Biochemie GmbH, Hamburg, Germany) as solid support. RNA and RNA containing *2'-O*-methyl nucleotides were generated by solid phase synthesis employing the corresponding phosphoramidites and *2'-O*-methyl phosphoramidites, respectively (Proligo Biochemie GmbH, Hamburg, Germany). These building blocks were incorporated at selected sites within the sequence of the oligoribonucleotide chain using standard nucleoside phosphoramidite chemistry such as described in Current protocols in nucleic acid chemistry, Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA. Phosphorothioate linkages were introduced by replacement of the iodine oxidizer solution with a solution of the Beaucage reagent (Chruachem Ltd, Glasgow, UK) in acetonitrile (1%). Further ancillary reagents were obtained from Mallinckrodt Baker (Griesheim, Germany).

Deprotection and purification of the crude oligoribonucleotides by anion exchange HPLC were carried out according to established procedures. Yields and concentrations were determined by UV absorption of a solution of the respective RNA at a wavelength of 260 nm using a spectral photometer (DU 640B, Beckman Coulter GmbH, Unterschleißheim, Germany). Double stranded RNA was generated by mixing an equimolar solution of complementary strands in annealing buffer (20 mM sodium phosphate, pH 6.8; 100 mM sodium chloride), heated in a water bath at 85 - 90°C for 3 minutes and cooled to room temperature over a period of 3 - 4 hours. The annealed RNA solution was stored at –20 °C until use.

Activity testing

Activity of dsRNAs targeting human GCR

The activity of the GCR-dsRNAs for therapeutic use described above was tested in HeLaS3 cells. Cells in culture were used for quantitation of GCR mRNA by branched DNA in total mRNA derived from cells incubated with GCR-specific dsRNAs.

HeLaS3 cells were obtained from *American Type Culture Collection* (Rockville, Md., cat. No. CCL-2.2) and cultured in Ham's F12 (Biochrom AG, Berlin, Germany, cat. No. FG 0815) supplemented to contain 10% fetal calf serum (FCS) (Biochrom AG, Berlin, Germany, cat. No. S0115), Penicillin 100 U/ml, Streptomycin 100 mg/ml (Biochrom AG, Berlin, Germany, cat. No. A2213) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator (Heraeus HERAAcell, Kendro Laboratory Products, Langenselbold, Germany).

Cell seeding and transfection of dsRNA were performed at the same time. For transfection with dsRNA, HeLaS3 cells were seeded at a density of 2.0.times.10.sup.4 cells/well in 96-well plates. Transfection of dsRNA was carried out with lipofectamine 2000 (Invitrogen GmbH, Karlsruhe, Germany, cat.No. 11668-019) as described by the manufacturer. In a first single dose experiment dsRNAs were transfected at a concentration of 30 nM. Two independent experiments were performed. Most effective dsRNAs showing a mRNA knockdown of more than 80% from the first single dose screen at 30nM were further characterized by dose response curves. For dose response curves, transfections were performed in HeLaS3 cells as described for the single dose screen above, but with the following concentrations of dsRNA (nM): 24, 6, 1.5, 0.375, 0.0938, 0.0234, 0.0059, 0.0015, 0.0004 and 0.0001 nM . After transfection cells were incubated for 24 h at 37°C and 5 % CO_2 in a humidified incubator (Heraeus GmbH, Hanau, Germany). For measurement of GCR mRNA cells were harvested and lysed at 53°C following procedures recommended by the manufacturer of the Quantigene 1.0 Assay Kit (Panomics, Fremont, Calif., USA, cat. No. QG-0004) for bDNA quantitation of mRNA. Afterwards, 50 µl of the lysates were incubated with probesets specific to human GCR and human GAPDH (sequence of probesets see table 9 and 10) and processed according to the manufacturer's protocol for QuantiGene. Chemoluminescence was measured in a Victor2-Light (Perkin Elmer, Wiesbaden, Germany) as RLUs (relative light units) and values obtained with the human GCR probeset were normalized to the respective human GAPDH values for each well. Unrelated control dsRNAs were used as a negative control.

Inhibition data are given in appended tables 1 and 2.

Activity of dsRNAs targeting rodent GCR

The activity of the GCR-siRNAs for use in rodent models was tested in Hepa1-6 cells. Hepa1-6 cells in culture were used for quantitation of GCR mRNA by branched DNA assay from whole cell lysates derived from cells transfected with GCR-specific siRNAs.

Hepa1-6 cells were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig Germany, cat. No. ACC 175) and cultured in DMEM (Biochrom AG, Berlin, Germany, cat. No. FG 0815) supplemented to contain 10% fetal calf serum (FCS) (Biochrom AG, Berlin, Germany, cat. No. S0115), Penicillin 100 U/ml, Streptomycin 100 mg/ml (Biochrom AG, Berlin, Germany, cat. No. A2213), L-Glutamine 4 mM (Biochrom AG, Berlin, Germany, cat. No. K0283) at 37°C in an atmosphere with 5% CO2 in a humidified incubator (Heraeus HERAcell, Kendro Laboratory Products, Langenselbold, Germany).

Cell seeding and transfection of siRNA were performed at the same time. For transfection with siRNA, Hepa1-6 cells were seeded at a density of 15000 cells/well in 96-well plates. Transfection of siRNA was carried out with lipofectamine 2000 (Invitrogen GmbH, Karlsruhe, Germany, cat.No. 11668-019) as described by the manufacturer. The two chemically different screening sets of siRNAs were transfected at a concentration of 50 nM. For measurement of GCR mRNA cells were harvested 24 h after transfection and lysed at 53°C following procedures recommended by the manufacturer of the Quantigene 1.0 Assay Kit (Panomics, Fremont, Calif., USA, cat. No. QG-0004) for bDNA quantitation of mRNA. Afterwards, 50 µl of the lysates were incubated with probesets specific to mouse GCR and mouse GAPDH (sequence of probesets see below) and processed according to the manufacturer's protocol for QuantiGene. Chemiluminescence was measured in a Victor2-Light (Perkin Elmer, Wiesbaden, Germany) as RLUs (relative light units) and values obtained with the mouse GCR probeset were normalized to the respective mouse GAPDH values for each well. Unrelated control siRNAs were used as a negative control.

Most efficacious three siRNAs were used for pharmacological proove of concept studies in rodent in vivo experiments.

Inhibition data are given in appended table 4.

Stability of dsRNAs

Stability of dsRNAs was determined in in vitro assays with either human serum or plasma from cynomolgous monkey for dsRNAs targeting human GCR and with mouse serum for dsRNAs targeting mouse/rat PTB1B by measuring the half-life of each single strand.

Measurements were carried out in triplicates for each time point, using 3µl 50µM dsRNA sample mixed with 30µl human serum or cynomolgous plasma (Sigma Aldrich). Mixtures were incubated for either 0min, 30min, 1h, 3h, 6h, 24h, or 48h at 37°C. As control for unspecific degradation dsRNA was incubated with 30µl 1x PBS pH 6.8 for 48h. Reactions were stopped by the addition of 4µl proteinase K (20mg/ml), 25µl of "Tissue and Cell Lysis Solution" (Epicentre) and 38µl Millipore water for 30 min at 65°C. Samples were afterwards spin filtered through a 0.2 µm 96 well filter plate at 1400 rpm for 8 min, washed with 55µl Millipore water twice and spin filtered again.

For separation of single strands and analysis of remaining full length product (FLP), samples were run through an ion exchange Dionex Summit HPLC under denaturing conditions using as eluent A 20mM Na3PO4 in 10% ACN pH=11 and for eluent B 1 M NaBr in eluent A.

The following gradient was applied:

Time	%A	%B
-1.0 min	75	25
1.00 min	75	25
19.0 min	38	62
19.5 min	0	100
21.5 min	0	100
22.0 min	75	25
24.0 min	75	25

For every injection, the chromatograms were integrated automatically by the Dionex Chromeleon 6.60 HPLC software, and were adjusted manually if necessary. All peak areas were corrected to the internal standard (IS) peak and normalized to the incubation at t=0 min. The area under the peak and resulting remaining FLP was calculated for each single strand and triplicate separately. Half-life (t1/2) of a strand was defined by the average time point [h] for triplicates at which half of the FLP was degraded.

Results are given in appended tables 3 and 5.

Cytokine induction

Potential cytokine induction of dsRNAs was determined by measuring the release of INF-a and TNF-a in an *in vitro* PBMC assay.

Human peripheral blood mononuclear cells (PBMC) were isolated from buffy coat blood of two donors by Ficoll centrifugation at the day of transfection. Cells were transfected in quadruplicates with dsRNA and cultured for 24h at 37°C at a final concentration of 130nM in Opti-MEM, using either Gene Porter 2 (GP2) or DOTAP. dsRNA sequences that were known to induce INF-a and TNF-a in this assay, as well as a CpG oligo, were used as positive controls. Chemical conjugated dsRNA or CpG oligonucleotides that did not need a transfection reagent for cytokine induction, were incubated at a concentration of 500nM in culture medium. At the end of incubation, the quadruplicate culture supernatant were pooled.

INF-a and TNF-a was then measured in these pooled supernatants by standard sandwich ELISA with two data points per pool. The degree of cytokine induction was expressed relative to positive controls using a score from 0 to 5, with 5 indicating maximum induction.

Results are given in appended tables 3 and 5.

In vitro off-target analysis of dsRNA targeting human GCR

The psiCHECK™- vector (Promega) contains two reporter genes for monitoring RNAi activity: a synthetic version of the Renilla luciferase (hRluc) gene and a synthetic firefly luciferase gene (hluc+). The firefly luciferase gene permits normalization of changes in Renilla luciferase expression to firefly luciferase expression. Renilla and firefly luciferase activities were measured using the Dual-Glo® Luciferase Assay System (Promega). To use the psiCHECK™ vectors for analyzing off-target effects of the inventive dsRNAs, the predicted off-target sequence was cloned into the multiple cloning region located 3´ to the synthetic Renilla luciferase gene and its translational stop codon. After cloning, the vector is transfected into a mammalian cell line, and subsequently cotransfected with dsRNAs targeting GCR. If the dsRNA effectively initiates the RNAi process on the target RNA of the predicted off-target, the fused Renilla target gene mRNA sequence will be degraded, resulting in reduced Renilla luciferase activity.

In silico off-target prediction

The human genome was searched by computer analysis for sequences homologous to the inventive dsRNAs. Homologous sequences that displayed less than 6 mismatches with the inventive dsRNAs were defined as a possible off-targets. Off-targets selected for *in vitro* off-target analysis are given in appended tables 6, 7 and 8.

Generation of psiCHECK vectors containing predicted off-target sequences

The strategy for analyzing off target effects for an dsRNA lead candidate includes the cloning of the predicted off target sites into the psiCHECK2 Vector system (Dual Glo®-system, Promega, Braunschweig, Germany cat. No C8021) via XhoI and NotI restriction sites. Therefore, the off target site is extended with 10 nucleotides upstream and downstream of the dsRNA target site. Additionally, a NheI restriction site is integrated to prove insertion of the fragment by restriction analysis. The single-stranded oligonucleotides were annealed according to a standard protocol (e.g. protocol by Metabion) in a Mastercycler (Eppendorf) and then cloned into psiCHECK (Promega) previously digested with XhoI and NotI. Successful insertion was verified by restriction analysis with NheI and subsequent sequencing of the positive clones. The selected primer (Seq ID No. 677) for sequencing binds at position 1401 of vector psiCHECK. After clonal production the plasmids were analyzed by sequencing and than used in cell culture experiments.

Analysis of dsRNA off-target effects

Cell culture:

Cos7 cells were obtained from Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany, cat. No. ACC-60) and cultured in DMEM (Biochrom AG, Berlin, Germany, cat. No. F0435) supplemented to contain 10% fetal calf serum (FCS) (Biochrom AG, Berlin, Germany, cat. No. S0115), Penicillin 100 U/ml, and Streptomycin 100 µg/ml (Biochrom AG, Berlin, Germany, cat. No. A2213) and 2 mM L-Glutamine (Biochrom AG, Berlin, Germany, cat. No. K0283) as well as 12 µg/ml Natrium-bicarbonate at 37°C in an atmosphere with 5% CO2 in a humidified incubator (Heraeus HERAcell, Kendro Laboratory Products, Langenselbold, Germany).

Transfection and Luciferase quantification:

For transfection with plasmids, Cos-7 cells were seeded at a density of 2.25 x 104 cells/well in 96-well plates and transfected directly. Transfection of plasmids was carried out with lipofectamine 2000 (Invitrogen GmbH, Karlsruhe, Germany, cat. No. 11668-019) as described by the manufacturer at a concentration of 50 ng/well. 4 hours after transfection, the medium was discarded and fresh medium was added. Now the dsRNAs were transfected in a concentration at 50 nM using lipofectamine 2000 as described above. 24h after dsRNA transfection the cells were lysed using Luciferase reagent described by the manufacturer (Dual-GloTM Luciferase Assay system, Promega, Mannheim, Germany, cat. No. E2980) and Firefly and Renilla Luciferase were quantified according to the manufacturer´s protocol. Renilla Luciferase protein levels were normalized to Firefly Luciferase levels. For each dsRNA eight individual data points were collected in two independent experiments. A dsRNA unrelated to all target sites was used as a control to determine the relative Renilla Luciferase protein levels in dsRNA treated cells.

Results are given in figures 1,2 and 3.

Efficacy of dsRNAs targeting GCR in human primary hepatocytes

GCR target gene knockdown after transfection of dsRNAs

Fresh suspensions of human primary hepatocytes, isolated from surgery resections, were purchased from HepaCult GmbH and were plated in 12 well collagen coated plates, at a density of 325 000 cells / well in William's E media (Sigma-Aldrich Inc, cat. No W1878.) supplemented with 10% Fetal Calf Serum (FCS), 1% GlutaMAX 200 mM (Invitrogen GmbH, cat. No 35050-038.) and antibiotics (penicillin, streptomycin and gentamycin) . After overnight culture (at 37°C in an atmosphere with 5% CO2 in a humidified incubator), medium was replaced with DMEM medium (Invitrogen GmbH, cat. No 21885) similarly supplemented, and dsRNAs transfections were performed at a final concentration of 15 nM, using DharmaFECT-1 transfection reagent (ThermoFisher Scientific Inc, cat. No T2001). 72h later, medium was replaced with fresh medium supplemented with 2 uM cAMP (Sigma-Aldrich Inc, cat. No S3912) and cells were further cultured overnight to allow for induction of gene expression. Cells were then exposed to Dexamethasone 500nM (Sigma-Aldrich Inc, cat. No D4902) for 6h to trigger activation and translocation of GCR to the nuclei and were recovered for gene expression analysis by branched-DNA technology, according to Panomics/Affymetrix Inc protocols for Quantigene 2.0

technology (http://www.panomics.com/index.php?id=product_1). In these conditions, exposure of human primary hepatocytes to dsRNA for GCR led to up to 90 % KD of GCR gene expression

Results are shown in Figure 4.

Effect of LNP01-formulated dsRNAs for GCR on GCR and GCR-regulated genes expression

Human primary hepatocytes were plated and cultivated as described above, except that 450 000 cells were seeded per well. After overnight culture, cells were exposed for 48h to dsRNAs packaged into cationic liposomal formulation LNP01 at doses ranging from 1 to 100 nM. After 32h exposure to dsRNAs, cAMP was added at 2 uM final concentration. Medium was further supplemented with Dexamethasone at 500 nM final concentration 6h before cell recovery for gene expression analysis. In these conditions, cell exposure to LNP01-formulated dsRNA for GCR led to dose response inhibition of GCR gene expression, with 80% KD of GCR gene expression reached at 100 nM exposure without change in the expression of GUSB housekeeping gene. GCR KD translated into strong inhibition of expression of TAT and PCK1 genes, and to a lesser extend, to G6Pc gene inhibition, which expressions are induced by GCR receptor upon activation.

Results are shown in Figure 5.

Effect of LNP01-formulated dsRNAs for GCR on glucose output

Glucose output assays were performed on primary human hepatocytes seeded and exposed to LNP01-formulated dsRNAs as described above, except that 96 well plates format were used with 35 000 cells seeded / well, and that after 48h exposure to LNP01-formulated dsRNAs, cells were cultivated in starvation conditions for 72h in glucose-free RPMI 1640 media (Invitrogen GmbH, cat. No 11879) supplemented with 1% FCS and antibiotics, before medium was refreshed and supplemented with 2 uM cAMP and with 30 nM Dexamethasone for overnight incubation. Control cells treated with cAMP alone, or with cAMP, Dexamethasone and Mifepristone 1 uM (a GCR antagonist), were also performed. Cells were then further incubated in the presence of gluconeogenic precursors (lactate and pyruvate) to induce glucose production for 5h in DPBS (Invitrogen GmbH, cat. No 1404) containing 0.1% free-fatty acid

BSA, 20 mM sodium pyruvate and 2mM lactate. Glucose produced was evaluated with Amplex-Red Glucose/Glucose oxydase assay kit (Invitrogen GmbH, cat. No A22189) in culture supernatants. As an indicator of cell viability, cellular ATP content was also measured using Cell-titer Glo luminescent cell viability assay (Promega Corporation, cat. No G7571). Cell exposure to LNP01-formulated dsRNA for GCR led to dose-response inhibition of glucose production up to the maximum level expected from full antagonism of GCR activity achieved by Mifepristone.

Results are shown in Figure 6 and 7.

In vivo effects of dsRNA targeting mice and rat GCR

RNAi-mediated GCR KD in liver, and efficacy on blood glucose in db/db mice after single i.v. injection.

A group of 30 males db/db mice (Jackson laboratories) were fed a regular chow diet (Kliba 3436). Homogenous groups of 4 mice each were organized according to their BW and blood glucose measured under fed conditions the day of the experiment and 2h after was food removed.

Mice were treated with single iv injection of either LNP01-formulated ds RNA for Luciferase control (SEQ ID pair 681/682) or LNP01-formulated dsRNA for GCR (SEQ ID pair 517/518) at 5.76 mg/kg for up to 103h.

Blood glucose levels were measured with Accu-Chek (Aviva) 2 days, 3 days and 4 days after iv injection (+55h, +79h and +103h post treatment) in the afternoon corresponding to10h after food was removed. Mice were then sacrificed. Plasma ALT and AST were analyzed by Hitachi. Liver was harvested and snap frozen in liquid nitrogen for mRNA expression analysis of GCR and GCR-regulated genes (TAT, PCK1, G6Pc and HES1 genes) by branched-DNA, processing the largest lobe (left lateral lobe) according to Panomics/Quantigene 2.0 sample processing protocol for animal tissues (Panomics-Affymetrix Inc, cat. No QS0106). Db/db mice treatment with GCR dsRNA.resulted in significant KD of GCR gene expression in mice liver and in decreased glycemia without change in liver transaminases.

Results are shown in Figures 8, 9 and 10.

In vivo effects of dsRNA targeting GCR (Macaca fascicularis)

For the following studies a sterile formulation of dsRNA lipid particles in isotonic buffer (e.g. Semple SC et al.,Nat Biotechnol. 2010 Feb;28(2):172-6. Epub 2010 Jan 17. Rational design of cationic lipids for siRNA delivery.) were used.

Single Dose Titration Study in Monkeys (Macaca fascicularis)

Monkeys received single i.v. bolus injections of GCR dsRNA (Seq. ID pair 747/753) of either 0.5, 1.5 or 3 mg/kg, or dsRNA (Seq. ID pair 764/772) in a dose of 1.5 mg/kg. Control groups received a 1.5 mg/kg of Luciferase dsRNA (Seq. ID pair 681/682) in order to discriminate between effects caused by the lipid particle and RNAi-mediated effects. All treatment groups were run with one male and one female monkey. Liver biopsy samples were taken on day 3 after injection.

GCR mRNA levels were measured from liver biopsy samples by bDNA assay as described above.

GCR dsRNA treated groups showed a dose-dependent decrease in GCR mRNA levels starting with 1.5 mg/kg of GCR dsRNA resulting in a decrease of about 24% by GCR dsRNA (Seq. ID pair 747/753) and 29% decrease by GCR dsRNA (Seq. ID pair 764/772), and reaching a 45% decrease in GCR mRNA with 3 mg/kg of GCR dsRNA (Seq. ID pair 747/753) (Figure 11).

Table 1

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
757	ugGucGAAcAGuuuuuuccdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)	**93**	0
756	uGGucGAAcAGuuuuuuccdT(abasic)	760	pAGAAAAAACUGUUCGACcAdT(abasic)	**93**	1
756	uGGucGAAcAGuuuuuuccdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)	**93**	1
755	ugGucGAAcAGuuuuuucudT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)	**93**	1
748	uGGucGAAcAGuuuuuuccdT(invdT)	753	AGAAAAAACUGUUCGACcAdT(invdT)	**93**	2
749	ugGucGAAcAGuuuuuuccdT(invdT)	753	AGAAAAAACUGUUCGACcAdT(invdT)	**93**	2
749	ugGucGAAcAGuuuuuuccdT(invdT)	752	pAGAAAAAACUGUUCGACcAdT(invdT)	**93**	0
758	ugGucGAAcAGuuuuuucGdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)	**92**	2
754	uGGucGAAcAGuuuuuucudT(abasic)	760	pAGAAAAAACUGUUCGACcAdT(abasic)	**92**	1
755	ugGucGAAcAGuuuuuucudT(abasic)	760	pAGAAAAAACUGUUCGACcAdT(abasic)	**92**	0
754	uGGucGAAcAGuuuuuucudT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)	**92**	0
758	ugGucGAAcAGuuuuuucGdT(abasic)	760	pAGAAAAAACUGUUCGACcAdT(abasic)	**92**	1
748	uGGucGAAcAGuuuuuuccdT(invdT)	752	pAGAAAAAACUGUUCGACcAdT(invdT)	**92**	0
757	ugGucGAAcAGuuuuuuccdT(abasic)	760	pAGAAAAAACUGUUCGACcAdT(abasic)	**92**	2
750	uGGucGAAcAGuuuuuucGdT(invdT)	753	AGAAAAAACUGUUCGACcAdT(invdT)	**92**	0
750	uGGucGAAcAGuuuuuucGdT(invdT)	752	pAGAAAAAACUGUUCGACcAdT(invdT)	**92**	1
751	ugGucGAAcAGuuuuuucGdT(invdT)	752	pAGAAAAAACUGUUCGACcAdT(invdT)	**92**	1
759	uGGucGAAcAGuuuuuucGdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)	**92**	1
751	ugGucGAAcAGuuuuuucGdT(invdT)	753	AGAAAAAACUGUUCGACcAdT(invdT)	**92**	1
746	uGGucGAAcAGuuuuuucudT(invdT)	753	AGAAAAAACUGUUCGACcAdT(invdT)	**92**	0

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
747	ugGucGAAcAGuuuuuucudT(invdT)	753	AGAAAAAACUGUUCGACcAdT(invdT)	**92**	1
740	uGGucGAAcAGuuuuuuccdTsdT	744	pAGAAAAAACUGUUCGACcAdTsdT	**91**	1
742	uGGucGAAcAGuuuuuucGdTsdT	745	pAGAAAAAACUGUUCGACcAdTsdT	**91**	1
740	uGGucGAAcAGuuuuuuccdTsdT	56	AGAAAAAACUGUUCGACcAdTsdT	**91**	1
743	ugGucGAAcAGuuuuuucGdTsdT	745	pAGAAAAAACUGUUCGACcAdTsdT	**91**	2
743	ugGucGAAcAGuuuuuucGdTsdT	56	AGAAAAAACUGUUCGACcAdTsdT	**91**	1
741	ugGucGAAcAGuuuuuuccdTsdT	56	AGAAAAAACUGUUCGACcAdTsdT	**91**	1
742	uGGucGAAcAGuuuuuucGdTsdT	56	AGAAAAAACUGUUCGACcAdTsdT	**91**	2
1	cAuGuAcGAccAAuGuAAAdTsdT	2	UfUfUfACfAUfUfGGUfCfGUfACfAUfGdTsdT	**91**	2
3	uuGcuuAAcuAcAuAuAGAdTsdT	4	UfCfUfAUfAUfGUfAGUfUfAAGCfAAdTsdT	**90**	1
5	AAAuAAcuuGcuuAAcuAcdTsdT	6	GUfAGUfUfAAGCfAAGUfUfAUfUfUfdTsdT	**90**	2
741	ugGucGAAcAGuuuuuuccdTsdT	744	pAGAAAAAACUGUUCGACcAdTsdT	**90**	2
739	ugGucGAAcAGuuuuuucudTsdT	744	pAGAAAAAACUGUUCGACcAdTsdT	**90**	2
739	ugGucGAAcAGuuuuuucudTsdT	56	AGAAAAAACUGUUCGACcAdTsdT	**90**	1
747	ugGucGAAcAGuuuuuucudT(invdT)	752	pAGAAAAAACUGUUCGACcAdT(invdT)	**90**	1
746	uGGucGAAcAGuuuuuucudT(invdT)	752	pAGAAAAAACUGUUCGACcAdT(invdT)	**90**	2
7	uGcuuAAcuAcAuAuAGAudTsdT	8	AUfCfUfAUfAUfGUfAGUfUfAAGCfAdTsdT	**89**	2
9	GuAuGAAAAccuuAcuGcudTsdT	10	AGCfAGUfAAGGUfUfUfUfCfAUfACfdTsdT	**89**	1
11	cAGuGAGAGuuGGuuAcucdTsdT	12	GAGUfAACfCfAACfUfCfUfCfACfUfGdTsdT	**89**	2
13	GGGuGGAGAucAuAuAGAcdTsdT	14	GUCuAuAUGAUCUCcACCCdTsdT	**89**	2
762	GuuccAGAcucAAcuuGGcdTsdT	770	pUCcAAGUUGAGUCUGGAACdTsdT	**89**	2
762	GuuccAGAcucAAcuuGGcdTsdT	84	UCcAAGUUGAGUCUGGAACdTsdT	**89**	2

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
55	uGGucGAAcAGuuuuuucudTsdT	744	pAGAAAAAACUGUUCGACcAdTsdT	**89**	3
763	GuuccAGAcucAAcuuGGudTsdT	84	UCcAAGUUGAGUCUGGAACdTsdT	**89**	1
763	GuuccAGAcucAAcuuGGudTsdT	770	pUCcAAGUUGAGUCUGGAACdTsdT	**88**	0
765	GuuccAGAcucAAcuuGGcdT(invdT)	771	pUCcAAGUUGAGUCUGGAACdT(invdT)	**88**	1
768	GuuccAGAcucAAcuuGGcdT(abasic)	774	UCcAAGUUGAGUCUGGAACdT(abasic)	**88**	1
769	GuuccAGAcucAAcuuGGudT(abasic)	774	UCcAAGUUGAGUCUGGAACdT(abasic)	**88**	1
769	GuuccAGAcucAAcuuGGudT(abasic)	773	pUCcAAGUUGAGUCUGGAACdT(abasic)	**87**	1
765	GuuccAGAcucAAcuuGGcdT(invdT)	772	UCcAAGUUGAGUCUGGAACdT(invdT)	**87**	1
766	GuuccAGAcucAAcuuGGudT(invdT)	771	pUCcAAGUUGAGUCUGGAACdT(invdT)	**87**	1
766	GuuccAGAcucAAcuuGGudT(invdT)	772	UCcAAGUUGAGUCUGGAACdT(invdT)	**87**	1
764	GuuccAGAcucAAcuuGGAdT(invdT)	772	UCcAAGUUGAGUCUGGAACdT(invdT)	**87**	2
767	GuuccAGAcucAAcuuGGAdT(abasic)	774	UCcAAGUUGAGUCUGGAACdT(abasic)	**87**	2
15	GGGuGGAGAucAuAuAGAcdTsdT	16	GUfCfUfAUfAUfGAUfCfUfCfCfACfCfCfdTsdT	**87**	2
17	cAGuGAGAGuuGGuuAcucdTsdT	18	GAGuAACcAACUCUcACUGdTsdT	**87**	2
19	cAuAuAGAcAAucAAGuGcdTsdT	20	GCfACfUfUfUfGAUfUfGUfCfUfAUfAUfGdTsdT	**87**	2
21	ccuAuGuAuGuGuuAucuGdTsdT	22	CfAGAUfAACfACfAUfACfAUfAGGdTsdT	**87**	1
23	uuAAuGucAuuccAccAAudTsdT	24	AUfUfGGUfGGAAUfGACfAUfUfAAdTsdT	**87**	2
25	uuGcuuAAcuAcAuAuAGAdTsdT	26	UCuAuAUGuAGUuAAGcAAdTsdT	**86**	3
27	uGGucGAAcAGuuuuuucudTsdT	28	AGAAAAAACfUfGUfUfCfGACfCfAdTsdT	**86**	1
29	cAcAcAuuAAucuGAuuuudTsdT	30	AAAAUfCfAGAUfUfAAUfGUfGUfGdTsdT	**86**	2
83	GuuccAGAcucAAcuuGGAdTsdT	770	pUCcAAGUUGAGUCUGGAACdTsdT	**86**	5
768	GuuccAGAcucAAcuuGGcdT(abasic)	773	pUCcAAGUUGAGUCUGGAACdT(abasic)	**86**	2

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
764	GuuccAGAcucAAcuuGGAdT(invdT)	771	pUCcAAGUUGAGUCUGGAACdT(invdT)	**86**	3
767	GuuccAGAcucAAcuuGGAdT(abasic)	773	pUCcAAGUUGAGUCUGGAACdT(abasic)	**83**	8
83	GuuccAGAcucAAcuuGGAdTsdT	770	pUCcAAGUUGAGUCUGGAACdTsdT	**86**	5
31	GuAuGAAAAccuuAcuGcudTsdT	32	AGcAGuAAGGUUUUcAuACdTsdT	**85**	3
33	cuAcAGGAGucucAcAAGAdTsdT	34	UCUUGUGAGACUCCUGuAGdTsdT	**84**	2
35	cuGuAuGAAAAuAcccuccdTsdT	36	GGAGGGuAUUUUcAuAcAGdTsdT	**85**	3
37	uccuAuGuAuGuGuuAucudTsdT	38	AGAuAAcAcAuAcAuAGGAdTsdT	**85**	5
39	GGuGGAGAucAuAuAGAcAdTsdT	40	UfGUfCfUfAUfAUfGAUfCfUfCfCfACfCfdTsdT	**84**	1
41	AuGuAcGAccAAuGuAAAcdTsdT	42	GUfUfUfACfAUfUfGGUfCfGUfACfAUfdTsdT	**84**	2
43	AcuGGcAGcGGuuuuAucAdTsdT	44	UfGAUfAAAACfCfGCfUfGCfCfAGUfdTsdT	**84**	2
45	AGuGAGAGuuGGuuAcucAdTsdT	46	UfGAGUfAACfCfAACfUfCfUfCfACfUfdTsdT	**84**	2
47	AAuAAcuuGcuuAAcuAcAdTsdT	48	UfGUfAGUfUfAAGCfAAGUfUfAUfUfdTsdT	**84**	1
49	GuGAGAGuuGGuuAcucAcdTsdT	50	GUfGAGUfAACfCfAACfUfCfUfCfACfdTsdT	**83**	3
51	cAucAucGAuAAAAuucGAdTsdT	52	UfCfGAAUfUfUfUfAUfCfGAUfGAUfGdTsdT	**83**	2
53	cuGuAuGAAAAuAcccuccdTsdT	54	GGAGGGUfAUfUfUfUfCfAUfACfAGdTsdT	**83**	4
55	uGGucGAAcAGuuuuuucudTsdT	56	AGAAAAAACUGUUCGACcAdTsdT	**82**	4
57	AcGAuucAuuccuuuuGGAdTsdT	58	UfCfCfAAAAGGAAUfGAAUfCfGUfdTsdT	**82**	2
59	cuGuAuGAAAAccuuAcuGdTsdT	60	cAGuAAGGUUUUcAuAcAGdTsdT	**82**	3
61	GuGAGAGuuGGuuAcucAcdTsdT	62	GUGAGuAACcAACUCUcACdTsdT	**82**	4
63	uGuAcGAccAAuGuAAAcAdTsdT	64	UfGUfUfUfACfAUfUfGGUfCfGUfACfAdTsdT	**82**	3
65	uAccGGAcAcuAAAcccAAdTsdT	66	UfUfGGGUfUfUfAGUfGUfCfCfGGUfAdTsdT	**82**	2
67	ccGcuAucGAAAAuGucuudTsdT	68	AAGACfAUfUfUfUfCfGAUfAGCfGGdTsdT	**81**	1
69	AGAucAGAccuGuuGAuAGdTsdT	70	CfUfAUfCfAACfAGGUfCfUfGAUfCfUfdTsdT	**81**	4

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
71	uccuAuGuAuGuGuuAucudTsdT	72	AGAUfAACfACfAUfACfAUfAGGAdTsdT	**81**	2
73	ucuGuAuGAAAAccuuAcudTsdT	74	AGUfAAGGUfUfUfUfCfAUfACfAGAdTsdT	**81**	1
75	AAAAcAAuAGuuccuGcAAdTsdT	76	UfUfGCfAGGAACfUfAUfUfGUfUfUfUfdTsdT	**80**	3
77	GucuuAAcuuGuGGAAGcudTsdT	78	AGCfUfUfCfCfACfAAGUfUfAAGACfdTsdT	**80**	1
79	AcAAuAGuuccuGcAAcGudTsdT	80	ACfGUfUfGCfAGGAACfUfAUfUfGUfdTsdT	**80**	3
81	AGGcuuuucAuuAAAuGGGdTsdT	82	CfCfCfAUfUfUfAAUfGAAAAGCfCfUfdTsdT	**80**	3
83	GuuccAGAcucAAcuuGGAdTsdT	84	UCcAAGUUGAGUCUGGAACdTsdT	**80**	7
85	AuGuAcGAccAAuGuAAAcdTsdT	86	GUUuAcAUUGGUCGuAcAUdTsdT	**80**	4
87	cuAcAGGAGucucAcAAGAdTsdT	88	UfCfUfUfGUfGAGACfUfCfCfUfGUfAGdTsdT	**80**	2
89	uGuAcGAccAAuGuAAAcAdTsdT	90	UGUUuAcAUUGGUCGuAcAdTsdT	**79**	3
91	AGGAucAGAAGccuAuuuudTsdT	92	AAAAUfAGGCfUfUfCfUfGAUfCfCfUfdTsdT	**79**	3
93	GAAAuuAGAAuGAccuAcAdTsdT	94	UGuAGGUcAUUCuAAUUUCdTsdT	**79**	2
95	uucuGuucAuGGuGuGAGudTsdT	96	ACfUfCfACfACfCfAUfGAACfAGAAdTsdT	**79**	2
97	GuuccAGAcucAAcuuGGAdTsdT	98	UfCfCfAAGUfUfGAGUfCfUfGGAACfdTsdT	**79**	2
99	ccAGAuGuAAGcucuccucdTsdT	100	GAGGAGAGCUuAcAUCUGGdTsdT	**79**	4
101	uuucuAAuGGcuAuucAAGdTsdT	102	CfUfUfGAAUfAGCfCfAUfUfAGAAAdTsdT	**79**	2
103	AuGccGcuAucGAAAAuGudTsdT	104	ACfAUfUfUfUfCfGAUfAGCfGGCfAUfdTsdT	**79**	2
105	ccAGcAuGccGcuAucGAAdTsdT	106	UfUfCfGAUfAGCfGGCfAUfGCfUfGGdTsdT	**79**	2
107	uuGGcGcucAAAAAAuAGAdTsdT	108	UCuAUUUUUUGAGCGCcAAdTsdT	**78**	4
109	uccAccAAuucccGuuGGudTsdT	110	ACfCfAACfGGGAAUfUfGGUfGGAdTsdT	**78**	2
111	AAAcAAuAGuuccuGcAAcdTsdT	112	GUfUfGCfAGGAACfUfAUfUfGUfUfUfdTsdT	**78**	2
113	uucuGuucAuGGuGuGAGudTsdT	114	ACUcAcACcAUGAAcAGAAdTsdT	**78**	5
115	AGcAuuGcAAAccucAAuAdTsdT	116	uAUUGAGGUUUGcAAUGCUdTsdT	**78**	5

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
117	GccucucAuuuuAccGGAcdTsdT	118	GUfCfCfGGUfAAAAUfGAGAGGCfdTsdT	**78**	2
119	cAGcAucccuuucucAAcAdTsdT	120	UGUUGAGAAAGGGAUGCUGdTsdT	**77**	5
121	GAGAucAuAuAGAcAAucAdTsdT	122	UGAUUGUCuAuAUGAUCUCdTsdT	**77**	2
123	GGcuGuAuGAAAAuAcccudTsdT	124	AGGGuAUUUUcAuAcAGCCdTsdT	**77**	2
125	AcGAuucAuuccuuuuGGAdTsdT	126	UCcAAAAGGAAUGAAUCGUdTsdT	**77**	3
127	uGGGAAAuGAccuGGGAuudTsdT	128	AAUCCcAGGUcAUUUCCcAdTsdT	**77**	4
129	cccAGGuAAAGAGAcGAAudTsdT	130	AUfUfCfGUfCfUfCfUfUfUfACfCfUfGGGdTsdT	**77**	5
131	cAGcAucccuuucucAAcAdTsdT	132	UfGUfUfGAGAAAGGGAUfGCfUfGdTsdT	**77**	3
133	cAGGuAAAGAGAcGAAuGAdTsdT	134	UfCfAUfUfCfGUfCfUfCfUfUfUfACfCfUfGdTsdT	**77**	4
135	AAuAAcuuGcuuAAcuAcAdTsdT	136	UGuAGUuAAGcAAGUuAUUdTsdT	**77**	4
137	cuGuAuGAAAAccuuAcuGdTsdT	138	CfAGUfAAGGUfUfUfUfCfAUfACfAGdTsdT	**76**	4
139	GcucuGuuccAGAcucAAcdTsdT	140	GUfUfGAGUfCfUfGGAACfAGAGCfdTsdT	**76**	3
141	GGcucAGuAAGcAAuGcGcdTsdT	142	GCfGCfAUfUfGCfUfUfACfUfGAGCfCfdTsdT	**76**	5
143	GAGAucAuAuAGAcAAucAdTsdT	144	UfGAUfUfGUfCfUfAUfAUfGAUfCfUfCfdTsdT	**76**	2
145	AGGAucAGAAGccuAuuuudTsdT	146	AAAAuAGGCUUCUGAUCCUdTsdT	**76**	4
147	cAGcAuGccGcuAucGAAAdTsdT	148	UUUCGAuAGCGGcAUGCUGdTsdT	**76**	4
149	uGuuAuAuGcAGGAuAuGAdTsdT	150	UfCfAUfAUfCfCfUfGCfAUfAUfAACfAdTsdT	**76**	1
151	cGcuAucGAAAAuGucuucdTsdT	152	GAAGACfAUfUfUfUfCfGAUfAGCfGdTsdT	**76**	1
153	GGuGGAGAucAuAuAGAcAdTsdT	154	UGUCuAuAUGAUCUCcACCdTsdT	**76**	2
155	uuGGcGcucAAAAAAuAGAdTsdT	156	UfCfUfAUfUfUfUfUfUfGAGCfGCfCfAAdTsdT	**76**	2
157	ucAuuuuAccGGAcAcuAAdTsdT	158	UuAGUGUCCGGuAAAAUGAdTsdT	**75**	3
159	cAucAucGAuAAAAuucGAdTsdT	160	UCGAAUUUuAUCGAUGAUGdTsdT	**75**	8
161	ccAGGuAAAGAGAcGAAuGdTsdT	162	cAUUCGUCUCUUuACCUGGdTsdT	**75**	5

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
163	cAGGcuucAGGuAucuuAudTsdT	164	AuAAGAuACCUGAAGCCUGdTsdT	**75**	3
165	uuuccAAAAGGcucAGuAAdTsdT	166	UuACUGAGCCUUUUGGAAAdTsdT	**75**	2
167	cAcAcAuuAAucuGAuuuudTsdT	168	AAAAUcAGAUuAAUGUGUGdTsdT	**75**	6
169	GGcuGuAuGAAAAuAcccudTsdT	170	AGGGUfAUfUfUfUfCfAUfACfAGCfCfdTsdT	**75**	3
171	cAGGuuucAGGAAcuuAcAdTsdT	172	UGuAAGUUCCUGAAACCUGdTsdT	**75**	3
173	GAAAuuAGAAuGAccuAcAdTsdT	174	UfGUfAGGUfCfAUfUfCfUfAAUfUfUfCfdTsdT	**75**	2
175	ccAAGcAGcGAAGAcuuuudTsdT	176	AAAAGUfCfUfUfCfGCfUfGCfUfUfGGdTsdT	**74**	4
177	uccAccAAuucccGuuGGudTsdT	178	ACcAACGGGAAUUGGUGGAdTsdT	**74**	8
179	ccAAcAAucuuGGcGcucAdTsdT	180	UGAGCGCcAAGAUUGUUGGdTsdT	**74**	7
181	cucAGuAAGcAAuGcGcAGdTsdT	182	CUGCGcAUUGCUuACUGAGdTsdT	**74**	4
183	ucucAAuGGGAcuGuAuAudTsdT	184	AUfAUfACfAGUfCfCfCfAUfUfGAGAdTsdT	**74**	3
185	AAAAAGAAGAuuucAucGAdTsdT	186	UfCfGAUfGAAAUfCfUfUfCfUfUfUfUfUfdTsdT	**73**	3
187	GAAcuGGcAGcGGuuuuAudTsdT	188	AUfAAAACfCfGCfUfGCfCfAGUfUfCfdTsdT	**73**	2
189	GcucuGuuccAGAcucAAcdTsdT	190	GUUGAGUCUGGAAcAGAGCdTsdT	**73**	1
191	cAccAAuucccGuuGGuucdTsdT	192	GAACfCfAACfGGGAAUfUfGGUfGdTsdT	**73**	3
193	cGcuAucGAAAAuGucuucdTsdT	194	GAAGAcAUUUUCGAuAGCGdTsdT	**73**	6
195	AGcAuGccGcuAucGAAAAdTsdT	196	UfUfUfUfCfGAUfAGCfGGCfAUfGCfUfdTsdT	**73**	2
197	cucAAcuuGGAGGAucAuGdTsdT	198	cAUGAUCCUCcAAGUUGAGdTsdT	**73**	7
199	ccAGAuGuAAGcucuccucdTsdT	200	GAGGAGAGCfUfUfACfAUfCfUfGGdTsdT	**73**	2
201	AGuGAGAGuuGGuuAcucAdTsdT	202	UGAGuAACcAACUCUcACUdTsdT	**73**	5
203	GGGcGGcAAGuGAuuGcAGdTsdT	204	CUGcAAUcACUUGCCGCCCdTsdT	**72**	4
205	uGuGAuGGAcuucuAuAAAdTsdT	206	UfUfUfAUfAGAAGUfCfCfAUfCfACfAdTsdT	**72**	5
207	ccAAGcAGcGAAGAcuuuudTsdT	208	AAAAGUCUUCGCUGCUUGGdTsdT	**72**	4

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
209	AAAAcAAuAGuuccuGcAAdTsdT	210	UUGcAGGAACuAUUGUUUUdTsdT	**72**	3
211	ccGcuAucGAAAAuGucuudTsdT	212	AAGAcAUUUUCGAuAGCGGdTsdT	**71**	5
213	cAGcAuGccGcuAucGAAAdTsdT	214	UfUfUfCfGAUfAGCfGGCfAUfGCfUfGdTsdT	**71**	3
215	cuGGuGuGcucuGAuGAAGdTsdT	216	CfUfUfCfAUfCfAGAGCfACfACfCfAGdTsdT	**71**	3
217	AcGcucAAcAuGuuAGGAGdTsdT	218	CUCCuAAcAUGUUGAGCGUdTsdT	**71**	4
219	ucccAAcAAucuuGGcGcudTsdT	220	AGCfGCfCfAAGAUfUfGUfUfGGGAdTsdT	**71**	4
221	AGAcGAAuGAGAGuccuuGdTsdT	222	CfAAGGACfUfCfUfCfAUfUfCfGUfCfUfdTsdT	**71**	6
223	uAccGGAcAcuAAAcccAAdTsdT	224	UUGGGUUuAGUGUCCGGuAdTsdT	**70**	9
225	cuGcAAcGuuAccAcAAcudTsdT	226	AGUUGUGGuAACGUUGcAGdTsdT	**70**	4
227	ccAGcAuGccGcuAucGAAdTsdT	228	UUCGAuAGCGGcAUGCUGGdTsdT	**70**	4
229	AGcAuuGcAAAccucAAuAdTsdT	230	UfAUfUfGAGGUfUfUfGCfAAUfGCfUfdTsdT	**70**	5
231	ucccAAcAAucuuGGcGcudTsdT	232	AGCGCcAAGAUUGUUGGGAdTsdT	**70**	6
233	ccAccAAuucccGuuGGuudTsdT	234	AACcAACGGGAAUUGGUGGdTsdT	**70**	5
235	ucAGAccuGuuGAuAGAuGdTsdT	236	CfAUfCfUfAUfCfAACfAGGUfCfUfGAdTsdT	**70**	4
237	uuAccGGAcAcuAAAcccAdTsdT	238	UGGGUUuAGUGUCCGGuAAdTsdT	**70**	8
239	cccAAcAAucuuGGcGcucdTsdT	240	GAGCfGCfCfAAGAUfUfGUfUfGGGdTsdT	**70**	4
241	uuucuAAuGGcuAuucAAGdTsdT	242	CUUGAAuAGCcAUuAGAAAdTsdT	**70**	8
243	uuAAuGucAuuccAccAAudTsdT	244	AUUGGUGGAAUGAcAUuAAdTsdT	**70**	5
245	GGcucAGuAAGcAAuGcGcdTsdT	246	GCGcAUUGCUuACUGAGCCdTsdT	**69**	6
247	GucuuAAcuuGuGGAAGcudTsdT	248	AGCUUCcAcAAGUuAAGACdTsdT	**69**	5
249	ucAuuuuAccGGAcAcuAAdTsdT	250	UfUfAGUfGUfCfCfGGUfAAAAUfGAdTsdT	**69**	5
251	AGAcGAAuGAGAGuccuuGdTsdT	252	cAAGGACUCUcAUUCGUCUdTsdT	**69**	6
253	AcuGuAAAAccuuGuGuGGdTsdT	254	CfCfACfACfAAGGUfUfUfUfACfAGUfdTsdT	**68**	3

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
255	AAccucAAuAGGucGAccAdTsdT	256	UGGUCGACCuAUUGAGGUUdTsdT	**68**	4
257	cAuGcuGAAuAAuAAucuGdTsdT	258	CfAGAUfUfAUfUfAUfUfCfAGCfAUfGdTsdT	**68**	3
259	uGcAAAccucAAuAGGucGdTsdT	260	CGACCuAUUGAGGUUUGcAdTsdT	**68**	4
261	ccAAcAAucuuGGcGcucAdTsdT	262	UfGAGCfGCfCfAAGAUfUfGUfUfGGdTsdT	**68**	4
263	GGuuucAGGAAcuuAcAccdTsdT	264	GGUGuAAGUUCCUGAAACCdTsdT	**68**	2
265	GGuuucAGGAAcuuAcAccdTsdT	266	GGUfGUfAAGUfUfCfCfUfGAAACfCfdTsdT	**68**	2
267	uAGuGAccAGGuuuucAGGdTsdT	268	CCUGAAAACCUGGUcACuAdTsdT	**68**	3
269	cuGcAAcGuuAccAcAAcudTsdT	270	AGUfUfGUfGGUfAACfGUfUfGCfAGdTsdT	**68**	3
271	AGcAuGccGcuAucGAAAAdTsdT	272	UUUUCGAuAGCGGcAUGCUdTsdT	**67**	4
273	uGcAAcGuuAccAcAAcucdTsdT	274	GAGUUGUGGuAACGUUGcAdTsdT	**67**	3
275	uGAAccuGAAGuGuuAuAudTsdT	276	AUfAUfAACfACfUfUfCfAGGUfUfCfAdTsdT	**66**	3
277	cAccAAuucccGuuGGuucdTsdT	278	GAACcAACGGGAAUUGGUGdTsdT	**66**	7
279	ccAGGuAAAGAGAcGAAuGdTsdT	280	CfAUfUfCfGUfCfUfCfUfUfUfACfCfUfGGdTsdT	**66**	4
281	cucucAAuGGGAcuGuAuAdTsdT	282	UfAUfACfAGUfCfCfCfAUfUfGAGAGdTsdT	**66**	6
283	uGGcGcucAAAAAAuAGAAdTsdT	284	UfUfCfUfAUfUfUfUfUfUfGAGCfGCfCfAdTsdT	**66**	3
285	AuAcccuccucAAAuAAcudTsdT	286	AGUfUfAUfUfUfGAGGAGGGUfAUfdTsdT	**65**	1
287	GGGcGGcAAGuGAuuGcAGdTsdT	288	CfUfGCfAAUfCfACfUfUfGCfCfGCfCfCfdTsdT	**65**	2
289	uGcuuAAcuAcAuAuAGAudTsdT	290	AUCuAuAUGuAGUuAAGcAdTsdT	**65**	4
291	AuuccAccAAuucccGuuGdTsdT	292	CfAACfGGGAAUfUfGGUfGGAAUfdTsdT	**64**	5
293	AccucAAuAGGucGAccAGdTsdT	294	CUGGUCGACCuAUUGAGGUdTsdT	**64**	4
295	GuucAuGGuGuGAGuAccudTsdT	296	AGGUfACfUfCfACfACfCfAUfGAACfdTsdT	**63**	4
297	ccucucAuuuuAccGGAcAdTsdT	298	UGUCCGGuAAAAUGAGAGGdTsdT	**63**	5
299	AGccucucAuuuuAccGGAdTsdT	300	UfCfCfGGUfAAAAUfGAGAGGCfUfdTsdT	**63**	5

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
301	ucAAuGGGAcuGuAuAuGGdTsdT	302	CfCfAUfAUfACfAGUfCfCfCfAUfUfGAdTsdT	**63**	6
303	cAGGcuucAGGuAucuuAudTsdT	304	AUfAAGAUfACfCfUfGAAGCfCfUfGdTsdT	**63**	4
305	AuucAGcAGGccAcuAcAGdTsdT	306	CfUfGUfAGUfGGCfCfUfGCfUfGAAUfdTsdT	**63**	2
307	cccAAcAAucuuGGcGcucdTsdT	308	GAGCGCcAAGAUUGUUGGGdTsdT	**62**	4
309	AuGAGAccAGAuGuAAGcudTsdT	310	AGCUuAcAUCUGGUCUcAUdTsdT	**62**	5
311	cAuGcuGAAuAAuAAucuGdTsdT	312	cAGAUuAUuAUUcAGcAUGdTsdT	**62**	5
313	AcuGGcAGcGGuuuuAucAdTsdT	314	UGAuAAAACCGCUGCcAGUdTsdT	**62**	5
315	AucuGGuuuuGucAAGcccdTsdT	316	GGGCfUfUfGACfAAAACfCfAGAUfdTsdT	**62**	6
317	uGAGAGuuGGuuAcucAcAdTsdT	318	UGUGAGuAACcAACUCUcAdTsdT	**61**	4
319	ccAccAAuucccGuuGGuudTsdT	320	AACfCfAACfGGGAAUfUfGGUfGGdTsdT	**61**	4
321	AAAcuGGGcAcAGuuuAcudTsdT	322	AGUfAAACfUfGUfGCfCfCfAGUfUfUfdTsdT	**61**	6
323	GuucAuGGuGuGAGuAccudTsdT	324	AGGuACUcAcACcAUGAACdTsdT	**60**	8
325	AuAcccuccucAAAuAAcudTsdT	326	AGUuAUUUGAGGAGGGuAUdTsdT	**59**	5
327	uuAccGGAcAcuAAAcccAdTsdT	328	UfGGGUfUfUfAGUfGUfCfCfGGUfAAdTsdT	**59**	5
329	AcuuAcAccuGGAuGAccAdTsdT	330	UfGGUfCfAUfCfCfAGGUfGUfAAGUfdTsdT	**59**	3
331	cucAGuAAGcAAuGcGcAGdTsdT	332	CfUfGCfGCfAUfUfGCfUfUfACfUfGAGdTsdT	**59**	7
333	uuuGAcAuuuuGcAGGAuudTsdT	334	AAUfCfCfUfGCfAAAAUfGUfCfAAAdTsdT	**59**	9
335	ucAGAccuGuuGAuAGAuGdTsdT	336	cAUCuAUcAAcAGGUCUGAdTsdT	**58**	6
337	AuucAGcAGGccAcuAcAGdTsdT	338	CUGuAGUGGCCUGCUGAAUdTsdT	**57**	5
339	AuAGuuccuGcAAcGuuAcdTsdT	340	GUfAACfGUfUfGCfAGGAACfUfAUfdTsdT	**56**	3
341	uGcAAcGuuAccAcAAcucdTsdT	342	GAGUfUfGUfGGUfAACfGUfUfGCfAdTsdT	**56**	5
343	uAGuuuuuuAuucAuGcuGdTsdT	344	CfAGCfAUfGAAUfAAAAAACfUfAdTsdT	**56**	6
345	uGGGAAAuGAccuGGGAuudTsdT	346	AAUfCfCfCfAGGUfCfAUfUfUfCfCfCfAdTsdT	**56**	7

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
347	uuuGAcAuuuuGcAGGAuudTsdT	348	AAUCCUGcAAAAUGUcAAAdTsdT	**56**	5
349	AcGcucAAcAuGuuAGGAGdTsdT	350	CfUfCfCfUfAACfAUfGUfUfGAGCfGUfdTsdT	**54**	6
351	uGcuGuucuGGuAuuAccAdTsdT	352	UfGGUfAAUfACfCfAGAACfAGCfAdTsdT	**54**	3
353	cccAGGuAAAGAGAcGAAudTsdT	354	AUUCGUCUCUUuACCUGGGdTsdT	**53**	11
355	uGcAAAccucAAuAGGucGdTsdT	356	CfGACfCfUfAUfUfGAGGUfUfUfGCfAdTsdT	**53**	5
357	GccucucAuuuuAccGGAcdTsdT	358	GUCCGGuAAAAUGAGAGGCdTsdT	**52**	6
359	uGcuGuucuGGuAuuAccAdTsdT	360	UGGuAAuACcAGAAcAGcAdTsdT	**52**	4
361	GAAcuGGcAGcGGuuuuAudTsdT	362	AuAAAACCGCUGCcAGUUCdTsdT	**52**	4
363	ccuAuGuAuGuGuuAucuGdTsdT	364	cAGAuAAcAcAuAcAuAGGdTsdT	**51**	5
365	AGAAGAuuucAucGAAcucdTsdT	366	GAGUfUfCfGAUfGAAAUfCfUfUfCfUfdTsdT	**51**	6
367	cucuGAAcuucccuGGucGdTsdT	368	CfGACfCfAGGGAAGUfUfCfAGAGdTsdT	**51**	3
369	cuGGuGuGcucuGAuGAAGdTsdT	370	CUUcAUcAGAGcAcACcAGdTsdT	**51**	6
371	cucAAcuuGGAGGAucAuGdTsdT	372	CfAUfGAUfCfCfUfCfCfAAGUfUfGAGdTsdT	**50**	5
373	AGccucucAuuuuAccGGAdTsdT	374	UCCGGuAAAAUGAGAGGCUdTsdT	**50**	7
375	AuAGuuccuGcAAcGuuAcdTsdT	376	GuAACGUUGcAGGAACuAUdTsdT	**50**	6
377	AAcAAuAGuuccuGcAAcGdTsdT	378	CfGUfUfGCfAGGAACfUfAUfUfGUfUfdTsdT	**50**	3
379	AucuGGuuuuGucAAGcccdTsdT	380	GGGCUUGAcAAAACcAGAUdTsdT	**49**	6
381	AcuGuAAAAccuuGuGuGGdTsdT	382	CcAcAcAAGGUUUuAcAGUdTsdT	**49**	6
383	AAcucuuGGAuucuAuGcAdTsdT	384	UGcAuAGAAUCcAAGAGUUdTsdT	**49**	7
385	uAGuGAccAGGuuuucAGGdTsdT	386	CfCfUfGAAAACfCfUfGGUfCfACfUfAdTsdT	**49**	6
387	AAccucAAuAGGucGAccAdTsdT	388	UfGGUfCfGACfCfUfAUfUfGAGGUfUfdTsdT	**49**	5
389	ccucucAuuuuAccGGAcAdTsdT	390	UfGUfCfCfGGUfAAAAUfGAGAGGdTsdT	**48**	4
391	uGAccAAAuGAcccuAcuGdTsdT	392	CfAGUfAGGGUfCfAUfUfUfGGUfCfAdTsdT	**48**	6

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
393	AGAucAGAccuGuuGAuAGdTsdT	394	CuAUcAAcAGGUCUGAUCUdTsdT	**48**	10
395	cAGGuuucAGGAAcuuAcAdTsdT	396	UfGUfAAGUfUfCfCfUfGAAACfCfUfGdTsdT	**47**	5
397	uAGuuuuuuAuucAuGcuGdTsdT	398	cAGcAUGAAuAAAAAACuAdTsdT	**47**	7
399	uGuGAuGGAcuucuAuAAAdTsdT	400	UUuAuAGAAGUCcAUcAcAdTsdT	**45**	4
401	uGGcGcucAAAAAAuAGAAdTsdT	402	UUCuAUUUUUUGAGCGCcAdTsdT	**45**	10
403	AAcAAuAGuuccuGcAAcGdTsdT	404	CGUUGcAGGAACuAUUGUUdTsdT	**44**	6
405	uGAAccuGAAGuGuuAuAudTsdT	406	AuAuAAcACUUcAGGUUcAdTsdT	**44**	5
407	cucucAAuGGGAcuGuAuAdTsdT	408	uAuAcAGUCCcAUUGAGAGdTsdT	**42**	6
409	ucuGuAuGAAAAccuuAcudTsdT	410	AGuAAGGUUUUcAuAcAGAdTsdT	**41**	2
411	AuGccGcuAucGAAAAuGudTsdT	412	AcAUUUUCGAuAGCGGcAUdTsdT	**41**	7
413	uAGuuccuGcAAcGuuAccdTsdT	414	GGuAACGUUGcAGGAACuAdTsdT	**40**	7
415	AAcAAucuuGGcGcucAAAdTsdT	416	UfUfUfGAGCfGCfCfAAGAUfUfGUfUfdTsdT	**40**	8
417	AAAccucAAuAGGucGAccdTsdT	418	GGUfCfGACfCfUfAUfUfGAGGUfUfUfdTsdT	**40**	4
419	uuuccAAAAGGcucAGuAAdTsdT	420	UfUfACfUfGAGCfCfUfUfUfUfGGAAAdTsdT	**38**	7
421	ucucAAuGGGAcuGuAuAudTsdT	422	AuAuAcAGUCCcAUUGAGAdTsdT	**38**	8
423	AAcAAucuuGGcGcucAAAdTsdT	424	UUUGAGCGCcAAGAUUGUUdTsdT	**38**	7
425	AAcucuuGGAuucuAuGcAdTsdT	426	UfGCfAUfAGAAUfCfCfAAGAGUfUfdTsdT	**38**	8
427	AAAAAGAAGAuuucAucGAdTsdT	428	UCGAUGAAAUCUUCUUUUUdTsdT	**37**	9
429	cAuAuAGAcAAucAAGuGcdTsdT	430	GcACUUGAUUGUCuAuAUGdTsdT	**37**	3
431	AcuuAcAccuGGAuGAccAdTsdT	432	UGGUcAUCcAGGUGuAAGUdTsdT	**34**	14
433	uuuAccGGAcAcuAAAcccdTsdT	434	GGGUfUfUfAGUfGUfCfCfGGUfAAAdTsdT	**33**	8
435	cAGGuAAAGAGAcGAAuGAdTsdT	436	UcAUUCGUCUCUUuACCUGdTsdT	**32**	11
437	cccAGcAuGccGcuAucGAdTsdT	438	UfCfGAUfAGCfGGCfAUfGCfUfGGGdTsdT	**31**	8

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
439	cccAGcAuGccGcuAucGAdTsdT	440	UCGAuAGCGGcAUGCUGGGdTsdT	**31**	8
441	GGAGGAcAGAuGuAccAcudTsdT	442	AGUfGGUfACfAUfCfUfGUfCfCfUfCfCfdTsdT	**30**	5
443	cAuGuAcGAccAAuGuAAAdTsdT	444	UUuAcAUUGGUCGuAcAUGdTsdT	**30**	4
445	uAGuuccuGcAAcGuuAccdTsdT	446	GGUfAACfGUfUfGCfAGGAACfUfAdTsdT	**30**	7
447	AAcuuAcAccuGGAuGAccdTsdT	448	GGUfCfAUfCfCfAGGUfGUfAAGUfUfdTsdT	**29**	5
449	AAAcAAuAGuuccuGcAAcdTsdT	450	GUUGcAGGAACuAUUGUUUdTsdT	**29**	11
451	uuuuAccGGAcAcuAAAccdTsdT	452	GGUfUfUfAGUfGUfCfCfGGUfAAAAdTsdT	**28**	7
453	uGAGAGuuGGuuAcucAcAdTsdT	454	UfGUfGAGUfAACfCfAACfUfCfUfCfAdTsdT	**28**	7
455	AcAAuAGuuccuGcAAcGudTsdT	456	ACGUUGcAGGAACuAUUGUdTsdT	**27**	8
457	GGuccAcccAGGAuuAGuGdTsdT	458	CfACfUfAAUfCfCfUfGGGUfGGACfCfdTsdT	**27**	7
459	uGuuAuAuGcAGGAuAuGAdTsdT	460	UcAuAUCCUGcAuAuAAcAdTsdT	**27**	6
461	AuGAGAccAGAuGuAAGcudTsdT	462	AGCfUfUfACfAUfCfUfGGUfCfUfCfAUfdTsdT	**26**	5
463	AccucAAuAGGucGAccAGdTsdT	464	CfUfGGUfCfGACfCfUfAUfUfGAGGUfdTsdT	**26**	2
465	AGAAGAuuucAucGAAcucdTsdT	466	GAGUUCGAUGAAAUCUUCUdTsdT	**26**	7
467	AAAccucAAuAGGucGAccdTsdT	468	GGUCGACCuAUUGAGGUUUdTsdT	**25**	7
469	uuccAccAAuucccGuuGGdTsdT	470	CfCfAACfGGGAAUfUfGGUfGGAAdTsdT	**24**	10
471	uGAccAAAuGAcccuAcuGdTsdT	472	cAGuAGGGUcAUUUGGUcAdTsdT	**23**	6
473	AuuccAccAAuucccGuuGdTsdT	474	cAACGGGAAUUGGUGGAAUdTsdT	**23**	12
475	uGGuccAcccAGGAuuAGudTsdT	476	ACfUfAAUfCfCfUfGGGUfGGACfCfAdTsdT	**22**	6
477	AGGAAuucAGcAGGccAcudTsdT	478	AGUGGCCUGCUGAAUUCCUdTsdT	**22**	8
479	AcuucccuGGucGAAcAGudTsdT	480	ACfUfGUfUfCfGACfCfAGGGAAGUfdTsdT	**22**	8
481	uuuuAccGGAcAcuAAAccdTsdT	482	GGUUuAGUGUCCGGuAAAAdTsdT	**20**	12
483	AAAuAAcuuGcuuAAcuAcdTsdT	484	GuAGUuAAGcAAGUuAUUUdTsdT	**20**	10

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
485	AAGGcucAGuAAGcAAuGcdTsdT	486	GCfAUfUfGCfUfUfACfUfGAGCfCfUfUfdTsdT	**16**	9
487	AGGAAuucAGcAGGccAcudTsdT	488	AGUfGGCfCfUfGCfUfGAAUfUfCfCfUfdTsdT	**16**	8
489	cucuGAAcuucccuGGucGdTsdT	490	CGACcAGGGAAGUUcAGAGdTsdT	**15**	9
491	ucAAuGGGAcuGuAuAuGGdTsdT	492	CcAuAuAcAGUCCcAUUGAdTsdT	**14**	8
493	AAAcuGGGcAcAGuuuAcudTsdT	494	AGuAAACUGUGCCcAGUUUdTsdT	**13**	12
495	AAGccucucAuuuuAccGGdTsdT	496	CfCfGGUfAAAAUfGAGAGGCfUfUfdTsdT	**9**	6
497	AcuucccuGGucGAAcAGudTsdT	498	ACUGUUCGACcAGGGAAGUdTsdT	**8**	13
499	AAcuuAcAccuGGAuGAccdTsdT	500	GGUcAUCcAGGUGuAAGUUdTsdT	**8**	6
501	GGAGGAcAGAuGuAccAcudTsdT	502	AGUGGuAcAUCUGUCCUCCdTsdT	**8**	8
503	GGuccAcccAGGAuuAGuGdTsdT	504	cACuAAUCCUGGGUGGACCdTsdT	**8**	7
505	AAGGcucAGuAAGcAAuGcdTsdT	506	GcAUUGCUuACUGAGCCUUdTsdT	**7**	7
507	uuccAccAAuucccGuuGGdTsdT	508	CcAACGGGAAUUGGUGGAAdTsdT	**7**	8
509	uuuAccGGAcAcuAAAcccdTsdT	510	GGGUUuAGUGUCCGGuAAAdTsdT	**1**	13
511	uGGuccAcccAGGAuuAGudTsdT	512	ACuAAUCCUGGGUGGACcAdTsdT	**0**	15
513	AAGccucucAuuuuAccGGdTsdT	514	CCGGuAAAAUGAGAGGCUUdTsdT	**-1**	12
515	AGGcuuuucAuuAAAuGGGdTsdT	516	CCcAUUuAAUGAAAAGCCUdTsdT	**-14**	16

Table 2

SEQ ID NO pair	Activity testing for dose response in HeLaS3 cells - transfection 1				Activity testing for dose response in HeLaS3 cells - transfection 2			
	mean IC50 [nM]	mean IC80 [nM]	mean IC20 [nM]	mean maximal inhibition [%]	mean IC50 [nM]	mean IC80 [nM]	mean IC20 [nM]	mean maximal inhibition [%]
7/8	0.003	0.047	0	87	n.d.	n.d.	n.d.	n.d.
31/32	0.004	0.09	0	89	n.d.	n.d.	n.d.	n.d.
3/4	0.005	0.072	0.001	88	n.d.	n.d.	n.d.	n.d.
25/26	0.006	0.139	0.001	91	n.d.	n.d.	n.d.	n.d.
33/34	0.008	0.114	0.001	86	n.d.	n.d.	n.d.	n.d.
83/84	0.009	0.201	0.002	84	0.0033	0.0739	0.0005	84
55/56	0.009	0.105	0.002	84	0.0055	0.0844	0.001	81
27/28	0.011	0.221	0.001	83	n.d.	n.d.	n.d.	n.d.
9/10	0.012	0.238	0.001	87	n.d.	n.d.	n.d.	n.d.
15/16	0.015	0.131	0.003	86	n.d.	n.d.	n.d.	n.d.
35/36	0.016	0.358	0.002	89	n.d.	n.d.	n.d.	n.d.
17/18	0.025	0.179	0.005	92	n.d.	n.d.	n.d.	n.d.
37/38	0.025	0.563	0.003	82	n.d.	n.d.	n.d.	n.d.
11/12	0.031	0.35	0.005	88	n.d.	n.d.	n.d.	n.d.
13/14	0.036	0.304	0.007	87	n.d.	n.d.	n.d.	n.d.
19/20	0.04	0.446	0.009	86	n.d.	n.d.	n.d.	n.d.
57/58	0.041	1'717	0.006	83	n.d.	n.d.	n.d.	n.d.
59/60	0.044	0.488	0.008	87	n.d.	n.d.	n.d.	n.d.
1/2	0.052	0.397	0.011	90	n.d.	n.d.	n.d.	n.d.
21/22	0.055	0.627	0.009	86	n.d.	n.d.	n.d.	n.d.
5/6	0.056	0.565	0.01	89	n.d.	n.d.	n.d.	n.d.
29/30	0.058	0.824	0.011	85	n.d.	n.d.	n.d.	n.d.

SEQ ID NO pair	Activity testing for dose response in HeLaS3 cells - transfection 1				Activity testing for dose response in HeLaS3 cells - transfection 2			
	mean IC50 [nM]	mean IC80 [nM]	mean IC20 [nM]	mean maximal inhibition [%]	mean IC50 [nM]	mean IC80 [nM]	mean IC20 [nM]	mean maximal inhibition [%]
23/24	0.06	0.798	0.011	85	n.d.	n.d.	n.d.	n.d.
61/62	0.082	0.827	0.016	87	n.d.	n.d.	n.d.	n.d.
85/86	0.083	2'072	0.017	84	n.d.	n.d.	n.d.	n.d.
83/770	n.d.	n.d.	n.d.	n.d.	0.0041	0.0889	0.0006	84
739/744	n.d.	n.d.	n.d.	n.d.	0.0047	0.0549	0.0008	85
755/760	n.d.	n.d.	n.d.	n.d.	0.0051	0.0864	0.0006	87
55/744	n.d.	n.d.	n.d.	n.d.	0.0064	0.1011	0.0009	86
747/753	n.d.	n.d.	n.d.	n.d.	0.0083	0.0895	0.0013	89
764/771	n.d.	n.d.	n.d.	n.d.	0.0087	0.2156	0.0014	83
747/752	n.d.	n.d.	n.d.	n.d.	0.0095	0.1057	0.0016	88
764/772	n.d.	n.d.	n.d.	n.d.	0.0096	0.2988	0.0015	83
767/773	n.d.	n.d.	n.d.	n.d.	0.0105	0.2057	0.0017	85
755/761	n.d.	n.d.	n.d.	n.d.	0.015	0.1494	0.0024	90
767/774	n.d.	n.d.	n.d.	n.d.	0.0268	17'741	0.0033	82

Table 3

	Stability Human Serum		Stability Cynomolgous Serum		Human PBMC assay	
SEQ ID NO pair	**Sense strand**	**Antisense strand**	**Sense strand**	**Antisense strand**	**IFN-a**	**TNF-a**
	t1/2 [hr]	**t1/2 [hr]**	**t1/2 [hr]**	**t1/2 [hr]**		
747/753	>48hrs	>48hrs	>48hrs	>48hrs	0	0
764/772	>48hrs	27.3	>48hrs	24.1	0	0
3/4	>24	>24	5.5	5.3	0	0
7/8	>24	>24	9.3	6.0	0	0
55/56	>24	>24	21.9	8.2	0	0
25/26	>24	13.2	5.3	4.4	0	0
83/84	>24	11.0	4.5	6.4	0	0
31/32	>24	10.7	15.0	10.0	0	0
33/34	>24	9.1	6.7	3.9	0	0

Table 4

Rank	SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 50nM dsRNA in Hepa1-6 cells	
					mean % knock-down	standard deviation
1	517	uGAAcuAuGcuuGcucGuudTsdT	518	AACGAGcAAGcAuAGUUcAdTsdT	59	7
2	519	AuGAAuAcAGcAucccuuudTsdT	520	AAAGGGAUGCUGuAUUcAUdTsdT	58	6
3	521	uucucAGGcAGAuuccAAGdTsdT	522	CUUGGAAUCUGCCUGAGAAdTsdT	52	6
4	523	AAcAuuAAuuuccGuGuGAdTsdT	524	UcAcACGGAAAUuAAUGUUdTsdT	52	9
5	525	GAAcuAuGcuuGcucGuuudTsdT	526	AAACGAGcAAGcAuAGUUCdTsdT	51	9
6	527	uccuAGAcGcuAAcAuuAAdTsdT	528	UuAAUGUuAGCGUCuAGGAdTsdT	51	6
7	529	uAAuGucAuuccAccAAuudTsdT	530	AAUUGGUGGAAUGAcAUuAdTsdT	50	6
8	531	uuAuuuuAccGGAcAcuAAdTsdT	532	UuAGUGUCCGGuAAAAuAAdTsdT	50	9
9	533	AAcAuuAAuuuccGuGuGAdTsdT	534	UfCfACfACfGGAAAUfUfAAUfGUfUfdTsdT	50	5
10	535	AuAucAAAGAGcuAGGAAAdTsdT	536	UUUCCuAGCUCUUUGAuAUdTsdT	50	14
11	537	GAcGcuAAcAuuAAuuuccdTsdT	538	GGAAAUuAAUGUuAGCGUCdTsdT	50	3
12	539	uuccGuGuGAAAAuGGGucdTsdT	540	GACCcAUUUUcAcACGGAAdTsdT	49	7
13	541	GuGAAcuAuGcuuGcucGudTsdT	542	ACGAGcAAGcAuAGUUcACdTsdT	47	17
14	543	AuAucAAAGAGcuAGGAAAdTsdT	544	UfUfUfCfCfUfAGCfUfCfUfUfUfGAUfAUfdTsdT	46	10
15	545	uccuAGAcGcuAAcAuuAAdTsdT	546	UfUfAAUfGUfUfAGCfGUfCfUfAGGAdTsdT	46	11
16	547	uGcAuGuAuGAccAAuGuAdTsdT	548	UfACfAUfUfGGUfCfAUfACfAUfGCfAdTsdT	45	1
17	549	cccccuGGuAGAGAcGAAGdTsdT	550	CfUfUfGGAAUfCfUfGCfCfUfGAGAAdTsdT	45	5
18	551	uuuAucAuGAcAuGuuAuAdTsdT	552	uAuAAcAUGUcAUGAuAAAdTsdT	45	19
19	553	AAccucAAuAGGucGAccAdTsdT	554	UGGUCGACCuAUUGAGGUUdTsdT	44	6
20	555	uuAuccAAAGccGuuucAcdTsdT	556	GUGAAACGGCUUUGGAuAAdTsdT	43	21

Rank	SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 50nM dsRNA in Hepa1-6 cells	
					mean % knock-down	standard deviation
21	557	uuccGuGuGAAAAuGGGucdTsdT	558	GACfCfCfAUfUfUfUfCfACfACfGGAAdTsdT	43	9
22	559	AccucAAuAGGucGAccAGdTsdT	560	CUGGUCGACCuAUUGAGGUdTsdT	43	9
23	561	GAAcuAuGcuuGcucGuuudTsdT	562	AAACfGAGCfAAGCfAUfAGUfUfCfdTsdT	43	6
24	563	AGAcGcuAAcAuuAAuuucdTsdT	564	GAAAUfUfAAUfGUfUfAGCfGUfCfUfdTsdT	43	8
25	565	uuuAucAuGAcAuGuuAuAdTsdT	566	UfAUfAACfAUfGUfCfAUfGAUfAAAdTsdT	42	18
26	567	GuGAAcuAuGcuuGcucGudTsdT	568	ACfGAGCfAAGCfAUfAGUfUfCfACfdTsdT	42	19
27	569	AGAcGcuAAcAuuAAuuucdTsdT	570	GAAAUuAAUGUuAGCGUCUdTsdT	42	11
28	571	ccGGAcAcuAAAccuAAAAdTsdT	572	UfUfUfUfAGGUfUfUfAGUfGUfCfCfGGdTsdT	41	8
29	573	uGcAAAccucAAuAGGucGdTsdT	574	CGACCuAUUGAGGUUUGcAdTsdT	41	16
30	575	cuGAAAAcuGGAAuAGGuGdTsdT	576	CfACfCfUfAUfUfCfCfAGUfUfUfUfCfAGdTsdT	40	3
31	577	uGuuAuAuGGuuAAAcccAdTsdT	578	UGGGUUuAACcAuAuAAcAdTsdT	38	13
32	579	uGuuAuAuGGuuAAAcccAdTsdT	580	UfGGGUfUfUfAACfCfAUfAUfAACfAdTsdT	36	2
33	581	uGGuuuAAAuuGGucucAAdTsdT	582	UfUfGAGACfCfAAUfUfUfAAACfCfAdTsdT	35	6
34	583	ccGGAcAcuAAAccuAAAAdTsdT	584	UUUuAGGUUuAGUGUCCGGdTsdT	35	6
35	585	uuAAuGucAuuccAccAAudTsdT	586	AUfUfGGUfGGAAUfGACfAUfUfAAdTsdT	34	12
36	587	uGuAAuGGuuuAAAuuGGudTsdT	588	ACfCfAAUfUfUfAAACfCfAUfUfACfAdTsdT	33	1
37	589	uGGuuuAAAuuGGucucAAdTsdT	590	UUGAGACcAAUUuAAACcAdTsdT	33	7
38	591	uuuAAuuAcuGGuAGGAcAdTsdT	592	UGUCCuACcAGuAAUuAAAdTsdT	33	6
39	593	GAcGcuAAcAuuAAuuuccdTsdT	594	GGAAAUfUfAAUfGUfUfAGCfGUfCfdTsdT	32	6
40	595	uuAuuuuAccGGAcAcuAAdTsdT	596	UfUfAGUfGUfCfCfGGUfAAAAUfAAdTsdT	32	5
41	597	uuAuccAAAGccGuuucAcdTsdT	598	GUfGAAACfGGCfUfUfUfGGAUfAAdTsdT	32	24
42	599	uuuAccGGAcAcuAAAccudTsdT	600	AGGUUuAGUGUCCGGuAAAdTsdT	31	4
43	601	uuuAAuuAcuGGuAGGAcAdTsdT	602	UfGUfCfCfUfACfCfAGUfAAUfUfAAAdTsdT	30	5

Rank	SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 50nM dsRNA in Hepa1-6 cells	
					mean % knock-down	standard deviation
44	603	uGAAcuAuGcuuGcucGuudTsdT	604	AACfGAGCfAAGCfAUfAGUfUfCfAdTsdT	29	10
45	605	GGuuuAAAuuGGucucAAAdTsdT	606	UUUGAGACcAAUUuAAACCdTsdT	27	4
46	607	GGuuuAAAuuGGucucAAAdTsdT	608	UfUfUfGAGACfCfAAUfUfUfAAACfCfdTsdT	26	1
47	609	uGcuGAAuAAccuGuAGuudTsdT	610	AACuAcAGGUuAUUcAGcAdTsdT	26	10
48	611	AAAuGGGcAAAGGcGAuAcdTsdT	612	GUfAUfCfGCfCfUfUfUfGCfCfCfAUfUfUfdTsdT	26	8
49	613	uGuAAuGGuuuAAAuuGGudTsdT	614	ACcAAUUuAAACcAUuAcAdTsdT	25	6
50	615	AuGAAuAcAGcAucccuuudTsdT	616	AAAGGGAUfGCfUfGUfAUfUfCfAUfdTsdT	23	4
51	617	uGuuAGucAGccAuuuAcAdTsdT	618	UfGUfAAAUfGGCfUfGACfUfAACfAdTsdT	21	8
52	619	uuAAuGucAuuccAccAAudTsdT	620	AUUGGUGGAAUGAcAUuAAdTsdT	21	26
53	621	GuGuGGcuucAuAccGuucdTsdT	622	GAACfGGUfAUfGAAGCfCfACfACfdTsdT	20	4
54	623	GuGuGGcuucAuAccGuucdTsdT	624	GAACGGuAUGAAGCcAcACdTsdT	18	5
55	625	uGuuAGucAGccAuuuAcAdTsdT	626	UGuAAAUGGCUGACuAAcAdTsdT	17	10
56	627	uGuGGcuucAuAccGuuccdTsdT	628	GGAACGGuAUGAAGCcAcAdTsdT	16	5
57	629	uGcuGAAuAAccuGuAGuudTsdT	630	AACfUfACfAGGUfUfAUfUfCfAGCfAdTsdT	14	20
58	631	uuuAccGGAcAcuAAAccudTsdT	632	AGGUfUfUfAGUfGUfCfCfGGUfAAAdTsdT	14	13
59	633	cuGAAAAcuGGAAuAGGuGdTsdT	634	cACCuAUUCcAGUUUUcAGdTsdT	13	21
60	635	AAAccucAAuAGGucGAccdTsdT	636	GGUfCfGACfCfUfAUfUfGAGGUfUfUfdTsdT	12	8
61	637	AAccucAAuAGGucGAccAdTsdT	638	UfGGUfCfGACfCfUfAUfUfGAGGUfUfdTsdT	10	1
62	639	AGuAAAuGuuAGucAGccAdTsdT	640	UfGGCfUfGACfUfAACfAUfUfUfACfUfdTsdT	10	3
63	641	uGcAuGuAuGAccAAuGuAdTsdT	642	uAcAUUGGUcAuAcAUGcAdTsdT	10	26
64	643	uGcAAAccucAAuAGGucGdTsdT	644	CfGACfCfUfAUfUfGAGGUfUfUfGCfAdTsdT	2	8
65	645	AAAccucAAuAGGucGAccdTsdT	646	GGUCGACCuAUUGAGGUUUdTsdT	1	4
66	647	AGuAAAuGuuAGucAGccAdTsdT	648	UGGCUGACuAAcAUUuACUdTsdT	-2	11

Rank	SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)	Activity testing with 50nM dsRNA in Hepa1-6 cells	
					mean % knock-down	standard deviation
67	649	ucuuAuuuuAccGGAcAcudTsdT	650	AGUGUCCGGuAAAAuAAGAdTsdT	-5	5
68	651	AccucAAuAGGucGAccAGdTsdT	652	CfUfGGUfCfGACfCfUfAUfUfGAGGUfdTsdT	-6	12
69	653	AAAuGGGcAAAGGcGAuAcdTsdT	654	GuAUCGCCUUUGCCcAUUUdTsdT	-7	11
70	655	uGuGGcuucAuAccGuuccdTsdT	656	GGAACfGGUfAUfGAAGCfCfACfAdTsdT	-14	3
71	657	ucuuAuuuuAccGGAcAcudTsdT	658	AGUfGUfCfCfGGUfAAAAUfAAGAdTsdT	-19	2

Table 5

Rank	SEQ ID NO pair	Stability Mouse Serum		Stability Rat Serum		Human PBMC assay	
		Sense strand t1/2 [hr]	Antisense strand t1/2 [hr]	Sense strand t1/2 [hr]	Antisense strand t1/2 [hr]	IFN-a	TNF-a
1	517/518	>24	6.3	>24	15.5	0	0
9	533/534	5.1	6	16.4	16.7	0	0
2	519/520	17.5	1.7	23.7	8	0	0

Table 6

		accession	description	spec. Score	num. mm	mismatch pos. from 5' end of as	region
antisense	ON	NM_001018077.1	Homo sapiens nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) (NR3C1), transcript variant 1, mRNA	0.00	0		CDS
	OFF-1	NM_002649.2	Homo sapiens phosphoinositide-3-kinase, catalytic, gamma polypeptide (PIK3CG), mRNA	3.00	4	15 16 17 19	3UTR
	OFF-2	NM_017506.1	Homo sapiens olfactory receptor, family 7, subfamily A, member 5 (OR7A5), mRNA	3.00	4	14 17 18 19	3UTR
	OFF-3	NM_003343.4	Homo sapiens ubiquitin-conjugating enzyme E2G 2 (UBC7 homolog, yeast) (UBE2G2), transcript variant 1, mRNA	3.00	5	1 13 14 16 19	3UTR
	OFF-4	NM_014872.1	Homo sapiens zinc finger and BTB domain containing 5 (ZBTB5), mRNA	3.00	3	14 15 17	3UTR
	OFF-5	NM_003112.3	Homo sapiens Sp4 transcription factor (SP4), mRNA	3.20	3	11 15 17	3UTR
	OFF-6	NM_001125.2	Homo sapiens ADP-ribosylarginine hydrolase (ADPRH), mRNA	3.20	3	11 14 17	3UTR
	OFF-7	NM_024770.3	Homo sapiens methyltransferase like 8 (METTL8), mRNA	3.20	4	10 14 17 19	3UTR
	OFF-8	NM_018424.2	Homo sapiens erythrocyte membrane protein band 4.1 like 4B (EPB41L4B), transcript variant 1, mRNA	3.25	4	9 14 18 19	3UTR
	OFF-9	NM_207303.2	Homo sapiens attractin-like 1 (ATRNL1), mRNA	3.50	5	1 8 12 14 19	3UTR
	OFF-10	NM_032811.2	Homo sapiens transforming growth factor beta regulator 1 (TBRG1), transcript variant 1, mRNA	3.70	5	1 8 10 15 19	3UTR
	OFF-11	NM_032714.1	Homo sapiens chromosome 14 open reading frame 151 (C14orf151), mRNA	11.00	4	1 4 15 19	3UTR
	OFF-12	NM_018230.2	Homo sapiens nucleoporin 133kDa (NUP133), mRNA	11.20	2	5 11	CDS
sense	OFF-13	NM_001013579.1	Homo sapiens diacylglycerol O-acyltransferase 2-like 3 (DGAT2L3), mRNA	2	3	15 17 19	CDS
	OFF-14	NM_032973.1	Homo sapiens protocadherin 11 Y-linked (PCDH11Y), transcript variant c, mRNA	11	2	5 14	CDS

		accession	description	spec. Score	num. mm	mismatch pos. from 5' end of as	region
	OFF-15	NM_130797.2	Homo sapiens dipeptidyl-peptidase 6 (DPP6), transcript variant 1, mRNA	11.2	3	1 6 11	CDS

Table 7

antisense	accession	description	spec. Score	num. mm	mismatch pos. from 5' end of as	region
ON	NM_001018077.1	Homo sapiens nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) (NR3C1), transcript variant 1, mRNA	0.00	0		CDS
OFF-1	NM_213607.1	Homo sapiens coiled-coil domain containing 103 (CCDC103), mRNA	3.00	3	12 13 14	3UTR
OFF-2	NM_001080485.1	Homo sapiens zinc finger protein 275 (ZNF275), mRNA	3.20	4	10 16 17 19	3UTR
OFF-3	NM_002205.2	Homo sapiens integrin, alpha 5 (fibronectin receptor, alpha polypeptide) (ITGA5), mRNA	3.40	4	10 11 14 19	3UTR
OFF-4	XM_001716748.1	PREDICTED: Homo sapiens hypothetical LOC731508 (LOC731508), mRNA	3.45	4	9 10 12 19	3UTR
OFF-5	NM_020476.2	Homo sapiens ankyrin 1, erythrocytic (ANK1), transcript variant 1, mRNA	3.45	5	1 9 11 17 19	3UTR
OFF-6	NM_001025247.1	Homo sapiens TAF5-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor, 65kDa (TAF5L), transcript variant 2, mRNA	3.70	4	8 10 17 19	3UTR
OFF-7	NM_001101396.1	Homo sapiens similar to cAMP-regulated phosphoprotein (LOC646227), mRNA	11.00	4	1 3 13 19	3UTR
OFF-8	NM_018667.2	Homo sapiens sphingomyelin phosphodiesterase 3, neutral membrane	11.00	3	1 2 15	3UTR

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antisense	accession	description	spec. Score	num. mm	mismatch pos. from 5' end of as	region
		(neutral sphingomyelinase II) (SMPD3), mRNA				
OFF-9	NM_001080449.1	Homo sapiens DNA replication helicase 2 homolog (yeast) (DNA2), mRNA	11.20	4	1 3 11 19	CDS
OFF-10	NM_015039.2	Homo sapiens nicotinamide nucleotide adenylyltransferase 2 (NMNAT2), transcript variant 1, mRNA	12.00	5	1 7 12 14 19	3UTR
OFF-11	NM_000520.4	Homo sapiens hexosaminidase A (alpha polypeptide) (HEXA), mRNA	12.20	4	1 5 10 18	3UTR
sense						
OFF-12	NM_133432.2	Homo sapiens titin (TTN), transcript variant novex-1, mRNA	2	4	1 13 14 19	CDS
OFF-13	NM_033210.3	Homo sapiens zinc finger protein 502 (ZNF502), mRNA	2.25	4	1 9 17 19	3UTR
OFF-14	NM_005076.2	Homo sapiens contactin 2 (axonal) (CNTN2), mRNA	2.5	3	1 8 13	CDS

Table 8

OFF-13	NM_003211.3	Homo sapiens thymine-DNA glycosylase (TDG), mRNA	2.2	4	1 10 17 19	3UTR
OFF-14	NM_002645.2	Homo sapiens phosphoinositide-3-kinase, class 2, alpha polypeptide (PIK3C2A), mRNA	11	4	1 3 16 19	3UTR
			spec.	**num.**	**mismatch pos.**	**region**
antisense	**accession**	**description**	**Score**	**mm**	**from 5' end of as**	
ON	NM_001018077.1	Homo sapiens nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) (NR3C1), transcript variant 1, mRNA	0.00	0		3UTR
OFF-1	NM_006710.4	Homo sapiens COP9 constitutive photomorphogenic homolog subunit 8 (Arabidopsis) (COPS8), transcript variant 1, mRNA	3.00	4	1 12 15 17	3UTR
OFF-2	NM_194285.2	Homo sapiens SPT2, Suppressor of Ty, domain containing 1 (S. cerevisiae) (SPTY2D1), mRNA	3.00	4	1 16 17 18	3UTR
OFF-3	NM_004929.2	Homo sapiens calbindin 1, 28kDa (CALB1), mRNA	3.20	4	10 17 18 19	3UTR
OFF-4	NM_021101.3	Homo sapiens claudin 1 (CLDN1), mRNA	3.25	3	9 12 18	3UTR
OFF-5	NM_058191.3	Homo sapiens chromosome 21 open reading frame 66 (C21orf66), transcript variant 4, mRNA	3.50	4	1 8 13 18	3UTR
OFF-6	NM_130446.2	Homo sapiens kelch-like 6 (Drosophila) (KLHL6), mRNA	3.75	5	1 8 9 13 19	3UTR
OFF-7	NM_015525.2	Homo sapiens inhibitor of Bruton agammaglobulinemia tyrosine kinase (IBTK), mRNA	11.00	3	1 3 12	3UTR
OFF-8	NM_001080.3	Homo sapiens aldehyde dehydrogenase 5 family, member A1 (succinate-semialdehyde dehydrogenase) (ALDH5A1), nuclear gene encoding mitochondrial protein, transcript variant 2, mRNA	12.00	5	1 3 13 18 19	3UTR
OFF-9	NM_018003.2	Homo sapiens uveal autoantigen with coiled-coil domains and ankyrin repeats (UACA), transcript variant 1, mRNA	12.00	3	6 12 15	3UTR
OFF-10	NM_020346.1	Homo sapiens solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6 (SLC17A6), mRNA	12.20	4	5 10 17 19	3UTR
OFF-11	NM_004969.2	Homo sapiens insulin-degrading enzyme (IDE), mRNA	12.20	5	1 6 11 14 19	3UTR
sense						
OFF-12	NM_024422.3	Homo sapiens desmocollin 2 (DSC2), transcript variant Dsc2a, mRNA	2	4	1 13 15 19	CDS

Table 9

FPL Name	Function	Sequence	SEQ ID No.
hGAP001	CE	GAATTTGCCATGGGTGGAATTTTTTCTCTTGGAAAGAAAGT	683
hGAP002	CE	GGAGGGATCTCGCTCCTGGATTTTTCTCTTGGAAAGAAAGT	684
hGAP003	CE	CCCCAGCCTTCTCCATGGTTTTTTCTCTTGGAAAGAAAGT	685
hGAP004	CE	GCTCCCCCCTGCAAATGAGTTTTTCTCTTGGAAAGAAAGT	686
hGAP005	LE	AGCCTTGACGGTGCCATGTTTTTAGGCATAGGACCCGTGTCT	687
hGAP006	LE	GATGACAAGCTTCCCGTTCTCTTTTTAGGCATAGGACCCGTGTCT	688
hGAP007	LE	AGATGGTGATGGGATTTCCATTTTTTTAGGCATAGGACCCGTGTCT	689
hGAP008	LE	GCATCGCCCCACTTGATTTTTTTTTAGGCATAGGACCCGTGTCT	690
hGAP009	LE	CACGACGTACTCAGCGCCATTTTTAGGCATAGGACCCGTGTCT	691
hGAP010	LE	GGCAGAGATGATGACCCTTTTGTTTTTAGGCATAGGACCCGTGTCT	692
hGAP011	BL	GGTGAAGACGCCAGTGGACTC	693

Table 10

FPL Name	Function	Sequence	SEQ ID No.
hGcR3001	CE	TCCCATGCTAATTATCCAGCACTTTTTCTCTTGGAAAGAAAGT	694
hGcR3002	CE	TGGCATGCCCAGAGCTCATTTTTCTCTTGGAAAGAAAGT	695
hGcR3003	CE	GGAGCGTGGCTTTCCTTCATTTTTCTCTTGGAAAGAAAGT	696
hGcR3004	CE	CCCTGCCTCTGAATTCTGAAGTTTTTCTCTTGGAAAGAAAGT	697
hGcR3005	CE	CCTCCTTACACTTTTATTTCCCTTCTTTTTCTCTTGGAAAGAAAGT	698
hGcR3006	CE	TTTTCTAGAGAGAAGCAAATCCTTTTTTTTCTCTTGGAAAGAAAGT	699
hGcR3007	CE	GAGGGTATTTTCATACAGCCTTTCTTTTTCTCTTGGAAAGAAAGT	700
hGcR3008	LE	TTCATAGACACAAATCATGTTAGTTTTCTTTTTAGGCATAGGACCCGTGTCT	701
hGcR3009	LE	TCCATGGTGATGTAGTTTTCAGGTTTTTAGGCATAGGACCCGTGTCT	702
hGcR3010	LE	ACAAAAACACATTCACCTACAGCTACTTTTTAGGCATAGGACCCGTGTCT	703

FPL Name	Function	Sequence	SEQ ID No.
hGcR3011	LE	TGACACTAAAACCAGACACACACACTTTTTAGGCATAGGACCCGTGTCT	704
hGcR3012	LE	AATCTATATGTAGTTAAGCAAGTTATTTGAGTTTTTAGGCATAGGACCCGTGTCT	705
hGcR3013	BL	GACTTAGGTGAAACTGGAATTGCT	706
hGcR3014	BL	GTTTTTAAAAGGGAACTAAAATTATGA	707
hGcR3015	BL	GATCAATGTATTGTATAACAATATTTTTCAT	708

Table 11

FPL Name	Function	Sequence	SEQ ID No.
mmNR3C1 001	CE	ATCTGGTCTCATTCCAGGGCTTTTTTCTCTTGGAAAGAAAGT	709
mmNR3C1 002	CE	CAGGCAGAGTTTGGGAGGTGGTTTTTCTCTTGGAAAGAAAGT	710
mmNR3C1 003	CE	TTCCAGGTTCATTCCAGCTTGTTTTTCTCTTGGAAAGAAAGT	711
mmNR3C1 004	CE	TTTTTTTCTTCGTTTTTCGAGCTTTTTCTCTTGGAAAGAAAGT	712
mmNR3C1 005	CE	AGTGGCTTGCTGAATTCCTTTAATTTTTCTCTTGGAAAGAAAGT	713
mmNR3C1 006	CE	GGAACTATTGTTTTGTTAGCGTTTTCTTTTTCTCTTGGAAAGAAAGT	714
mmNR3C1 007	LE	TCCCGTTGCTGTGGAGGATTTTTAGGCATAGGACCCGTGTCT	715
mmNR3C1 008	LE	CCGAAGCTTCATCGGAGCACACTTTTTAGGCATAGGACCCGTGTCT	716
mmNR3C1 009	LE	CAGCACCCCATAATGGCATCTTTTTAGGCATAGGACCCGTGTCT	717
mmNR3C1 010	LE	TCCAGCACAAAGGTAATTGTGCTTTTTAGGCATAGGACCCGTGTCT	718
mmNR3C1 011	LE	TTTTATCAATGATGCAATCATTTCTTTTTTAGGCATAGGACCCGTGTCT	719
mmNR3C1 012	LE	AAGACATTTTCGATAGCGGCATTTTTAGGCATAGGACCCGTGTCT	720
mmNR3C1 013	BL	GCTGGACGGAGGAGAACTCAC	721
mmNR3C1 014	BL	GAAGACTTTACAGCTTCCACACGT	722
mmNR3C1 015	BL	TGTCCTTCCACTGCTCTTTTAAA	723
mmNR3C1 016	BL	TGCTGGACAGTTTTTTCTTCGAA	724
mmNR3C1 017	BL	AGAAGTGTCTTGTGAGACTCCTGC	725

Table 12

FPL Name	Function	Sequence	SEQ ID No.
mGAP001	CE	CAAATGGCAGCCCTGGTGATTTTTCTCTTGGAAAGAAAGT	726
mGAP002	CE	CCTTGACTGTGCCGTTGAATTTTTTTTCTCTTGGAAAGAAAGT	727
mGAP003	CE	GTCTCGCTCCTGGAAGATGGTTTTTCTCTTGGAAAGAAAGT	728
mGAP004	CE	CCCGGCCTTCTCCATGGTTTTTTCTCTTGGAAAGAAAGT	729
mGAP005	LE	AACAATCTCCACTTTGCCACTGTTTTTAGGCATAGGACCCGTGTCT	730
mGAP006	LE	CATGTAGACCATGTAGTTGAGGTCAATTTTTAGGCATAGGACCCGTGTCT	731
mGAP007	LE	GACAAGCTTCCCATTCTCGGTTTTTAGGCATAGGACCCGTGTCT	732
mGAP008	LE	TGATGGGCTTCCCGTTGATTTTTTAGGCATAGGACCCGTGTCT	733
mGAP009	LE	GACATACTCAGCACCGGCCTTTTTTAGGCATAGGACCCGTGTCT	734
mGAP010	BL	TGAAGGGGTCGTTGATGGC	735
mGAP011	BL	CCGTGAGTGGAGTCATACTGGAA	736
mGAP012	BL	CACCCCATTTGATGTTAGTGGG	737
mGAP013	BL	GGTGAAGACACCAGTAGACTCCAC	738

Table 13

SEQ ID NO	Sense strand sequence (5´-3´)	SEQ ID NO	Antisense strand sequence (5´-3´)
775	UGCAAACCUCAAUAGGUCG	776	CGACCUAUUGAGGUUUGCA
777	AAACCUCAAUAGGUCGACC	778	GGUCGACCUAUUGAGGUUU
779	AACCUCAAUAGGUCGACCA	780	UGGUCGACCUAUUGAGGUU
781	ACCUCAAUAGGUCGACCAG	782	CUGGUCGACCUAUUGAGGU
783	UUAAUGUCAUUCCACCAAU	784	AUUGGUGGAAUGACAUUAA
785	UGUGAUGGACUUCUAUAAA	786	UUUAUAGAAGUCCAUCACA
787	CCAAGCAGCGAAGACUUUU	788	AAAAGUCUUCGCUGCUUGG
789	UUUCCAAAAGGCUCAGUAA	790	UUACUGAGCCUUUUGGAAA
791	AAGGCUCAGUAAGCAAUGC	792	GCAUUGCUUACUGAGCCUU
793	GGCUCAGUAAGCAAUGCGC	794	GCGCAUUGCUUACUGAGCC
795	CUCAGUAAGCAAUGCGCAG	796	CUGCGCAUUGCUUACUGAG
797	CUCUCAAUGGGACUGUAUA	798	UAUACAGUCCCAUUGAGAG
799	UCUCAAUGGGACUGUAUAU	800	AUAUACAGUCCCAUUGAGA
801	UCAAUGGGACUGUAUAUGG	802	CCAUAUACAGUCCCAUUGA
803	UGGGAAAUGACCUGGGAUU	804	AAUCCCAGGUCAUUUCCCA
805	AGCAUUGCAAACCUCAAUA	806	UAUUGAGGUUUGCAAUGCU
807	UUUGACAUUUUGCAGGAUU	808	AAUCCUGCAAAAUGUCAAA
809	CCCAGGUAAAGAGACGAAU	810	AUUCGUCUCUUUACCUGGG
811	CCAGGUAAAGAGACGAAUG	812	CAUUCGUCUCUUUACCUGG
813	CAGGUAAAGAGACGAAUGA	814	UCAUUCGUCUCUUUACCUG
815	AGACGAAUGAGAGUCCUUG	816	CAAGGACUCUCAUUCGUCU

817	AGAUCAGACCUGUUGAUAG	818	CUAUCAACAGGUCUGAUCU
819	UCAGACCUGUUGAUAGAUG	820	CAUCUAUCAACAGGUCUGA
821	ACGAUUCAUUCCUUUUGGA	822	UCCAAAAGGAAUGAAUCGU
823	AAGCCUCUCAUUUUACCGG	824	CCGGUAAAAUGAGAGGCUU
825	AGCCUCUCAUUUUACCGGA	826	UCCGGUAAAAUGAGAGGCU
827	GCCUCUCAUUUUACCGGAC	828	GUCCGGUAAAAUGAGAGGC
829	CCUCUCAUUUUACCGGACA	830	UGUCCGGUAAAAUGAGAGG
831	UCAUUUUACCGGACACUAA	832	UUAGUGUCCGGUAAAAUGA
833	UUUUACCGGACACUAAACC	834	GGUUUAGUGUCCGGUAAAA
835	UUUACCGGACACUAAACCC	836	GGGUUUAGUGUCCGGUAAA
837	UUACCGGACACUAAACCCA	838	UGGGUUUAGUGUCCGGUAA
839	UACCGGACACUAAACCCAA	840	UUGGGUUUAGUGUCCGGUA
841	AUCUGGUUUUGUCAAGCCC	842	GGGCUUGACAAAACCAGAU
843	AAAAAGAAGAUUUCAUCGA	844	UCGAUGAAAUCUUCUUUUU
845	AGAAGAUUUCAUCGAACUC	846	GAGUUCGAUGAAAUCUUCU
847	AAACUGGGCACAGUUUACU	848	AGUAAACUGUGCCCAGUUU
849	UUCUGUUCAUGGUGUGAGU	850	ACUCACACCAUGAACAGAA
851	GUUCAUGGUGUGAGUACCU	852	AGGUACUCACACCAUGAAC
853	GGAGGACAGAUGUACCACU	854	AGUGGUACAUCUGUCCUCC
855	CAGCAUCCCUUUCUCAACA	856	UGUUGAGAAAGGGAUGCUG
857	AGGAUCAGAAGCCUAUUUU	858	AAAAUAGGCUUCUGAUCCU
859	AUUCCACCAAUUCCCGUUG	860	CAACGGGAAUUGGUGGAAU
861	UUCCACCAAUUCCCGUUGG	862	CCAACGGGAAUUGGUGGAA
863	UCCACCAAUUCCCGUUGGU	864	ACCAACGGGAAUUGGUGGA
865	CCACCAAUUCCCGUUGGUU	866	AACCAACGGGAAUUGGUGG
867	CACCAAUUCCCGUUGGUUC	868	GAACCAACGGGAAUUGGUG
869	CUCUGAACUUCCCUGGUCG	870	CGACCAGGGAAGUUCAGAG

871	ACUUCCCUGGUCGAACAGU	872	ACUGUUCGACCAGGGAAGU
873	UGGUCGAACAGUUUUUUCU	874	AGAAAAAACUGUUCGACCA
875	UUUCUAAUGGCUAUUCAAG	876	CUUGAAUAGCCAUUAGAAA
877	AUGAGACCAGAUGUAAGCU	878	AGCUUACAUCUGGUCUCAU
879	CCAGAUGUAAGCUCUCCUC	880	GAGGAGAGCUUACAUCUGG
881	CUGGUGUGCUCUGAUGAAG	882	CUUCAUCAGAGCACACCAG
883	GUCUUAACUUGUGGAAGCU	884	AGCUUCCACAAGUUAAGAC
885	CAUCAUCGAUAAAAUUCGA	886	UCGAAUUUUAUCGAUGAUG
887	CCCAGCAUGCCGCUAUCGA	888	UCGAUAGCGGCAUGCUGGG
889	CCAGCAUGCCGCUAUCGAA	890	UUCGAUAGCGGCAUGCUGG
891	CAGCAUGCCGCUAUCGAAA	892	UUUCGAUAGCGGCAUGCUG
893	AGCAUGCCGCUAUCGAAAA	894	UUUUCGAUAGCGGCAUGCU
895	AUGCCGCUAUCGAAAAUGU	896	ACAUUUUCGAUAGCGGCAU
897	CCGCUAUCGAAAAUGUCUU	898	AAGACAUUUUCGAUAGCGG
899	CGCUAUCGAAAAUGUCUUC	900	GAAGACAUUUUCGAUAGCG
901	AGGAAUUCAGCAGGCCACU	902	AGUGGCCUGCUGAAUUCCU
903	AUUCAGCAGGCCACUACAG	904	CUGUAGUGGCCUGCUGAAU
905	CUACAGGAGUCUCACAAGA	906	UCUUGUGAGACUCCUGUAG
907	AAAACAAUAGUUCCUGCAA	908	UUGCAGGAACUAUUGUUUU
909	AAACAAUAGUUCCUGCAAC	910	GUUGCAGGAACUAUUGUUU
911	AACAAUAGUUCCUGCAACG	912	CGUUGCAGGAACUAUUGUU
913	ACAAUAGUUCCUGCAACGU	914	ACGUUGCAGGAACUAUUGU
915	AUAGUUCCUGCAACGUUAC	916	GUAACGUUGCAGGAACUAU
917	UAGUUCCUGCAACGUUACC	918	GGUAACGUUGCAGGAACUA
919	CUGCAACGUUACCACAACU	920	AGUUGUGGUAACGUUGCAG
921	UGCAACGUUACCACAACUC	922	GAGUUGUGGUAACGUUGCA
923	UGAACCUGAAGUGUUAUAU	924	AUAUAACACUUCAGGUUCA

925	UGUUAUAUGCAGGAUAUGA	926	UCAUAUCCUGCAUAUAACA
927	GCUCUGUUCCAGACUCAAC	928	GUUGAGUCUGGAACAGAGC
929	GUUCCAGACUCAACUUGGA	930	UCCAAGUUGAGUCUGGAAC
931	CUCAACUUGGAGGAUCAUG	932	CAUGAUCCUCCAAGUUGAG
933	ACGCUCAACAUGUUAGGAG	934	CUCCUAACAUGUUGAGCGU
935	GGGCGGCAAGUGAUUGCAG	936	CUGCAAUCACUUGCCGCCC
937	CAGGUUUCAGGAACUUACA	938	UGUAAGUUCCUGAAACCUG
939	GGUUUCAGGAACUUACACC	940	GGUGUAAGUUCCUGAAACC
941	AACUUACACCUGGAUGACC	942	GGUCAUCCAGGUGUAAGUU
943	ACUUACACCUGGAUGACCA	944	UGGUCAUCCAGGUGUAAGU
945	UGACCAAAUGACCCUACUG	946	CAGUAGGGUCAUUUGGUCA
947	GGGUGGAGAUCAUAUAGAC	948	GUCUAUAUGAUCUCCACCC
949	GGUGGAGAUCAUAUAGACA	950	UGUCUAUAUGAUCUCCACC
951	GAGAUCAUAUAGACAAUCA	952	UGAUUGUCUAUAUGAUCUC
953	CAUAUAGACAAUCAAGUGC	954	GCACUUGAUUGUCUAUAUG
955	CAUGUACGACCAAUGUAAA	956	UUUACAUUGGUCGUACAUG
957	AUGUACGACCAAUGUAAAC	958	GUUUACAUUGGUCGUACAU
959	UGUACGACCAAUGUAAACA	960	UGUUUACAUUGGUCGUACA
961	CAGGCUUCAGGUAUCUUAU	962	AUAAGAUACCUGAAGCCUG
963	UCUGUAUGAAAACCUUACU	964	AGUAAGGUUUUCAUACAGA
965	CUGUAUGAAAACCUUACUG	966	CAGUAAGGUUUUCAUACAG
967	GUAUGAAAACCUUACUGCU	968	AGCAGUAAGGUUUUCAUAC
969	GAAAUUAGAAUGACCUACA	970	UGUAGGUCAUUCUAAUUUC
971	GAACUGGCAGCGGUUUUAU	972	AUAAAACCGCUGCCAGUUC
973	ACUGGCAGCGGUUUUAUCA	974	UGAUAAAACCGCUGCCAGU
975	AACUCUUGGAUUCUAUGCA	976	UGCAUAGAAUCCAAGAGUU
977	CACACAUUAAUCUGAUUUU	978	AAAAUCAGAUUAAUGUGUG

979	UCCCAACAAUCUUGGCGCU	980	AGCGCCAAGAUUGUUGGGA
981	CCCAACAAUCUUGGCGCUC	982	GAGCGCCAAGAUUGUUGGG
983	CCAACAAUCUUGGCGCUCA	984	UGAGCGCCAAGAUUGUUGG
985	AACAAUCUUGGCGCUCAAA	986	UUUGAGCGCCAAGAUUGUU
987	UUGGCGCUCAAAAAAUAGA	988	UCUAUUUUUUGAGCGCCAA
989	UGGCGCUCAAAAAAUAGAA	990	UUCUAUUUUUUGAGCGCCA
991	AGGCUUUUCAUUAAAUGGG	992	CCCAUUUAAUGAAAAGCCU
993	UCCUAUGUAUGUGUUAUCU	994	AGAUAACACAUACAUAGGA
995	CCUAUGUAUGUGUUAUCUG	996	CAGAUAACACAUACAUAGG
997	CAGUGAGAGUUGGUUACUC	998	GAGUAACCAACUCUCACUG
999	AGUGAGAGUUGGUUACUCA	1000	UGAGUAACCAACUCUCACU
1001	GUGAGAGUUGGUUACUCAC	1002	GUGAGUAACCAACUCUCAC
1003	UGAGAGUUGGUUACUCACA	1004	UGUGAGUAACCAACUCUCA
1005	UGGUCCACCCAGGAUUAGU	1006	ACUAAUCCUGGGUGGACCA
1007	GGUCCACCCAGGAUUAGUG	1008	CACUAAUCCUGGGUGGACC
1009	UAGUGACCAGGUUUUCAGG	1010	CCUGAAAACCUGGUCACUA
1011	GGCUGUAUGAAAAUACCCU	1012	AGGGUAUUUUCAUACAGCC
1013	CUGUAUGAAAAUACCCUCC	1014	GGAGGGUAUUUUCAUACAG
1015	AUACCCUCCUCAAAUAACU	1016	AGUUAUUUGAGGAGGGUAU
1017	AAAUAACUUGCUUAACUAC	1018	GUAGUUAAGCAAGUUAUUU
1019	AAUAACUUGCUUAACUACA	1020	UGUAGUUAAGCAAGUUAUU
1021	UUGCUUAACUACAUAUAGA	1022	UCUAUAUGUAGUUAAGCAA
1023	UGCUUAACUACAUAUAGAU	1024	AUCUAUAUGUAGUUAAGCA
1025	UAGUUUUUUAUUCAUGCUG	1026	CAGCAUGAAUAAAAAACUA
1027	CAUGCUGAAUAAUAAUCUG	1028	CAGAUUAUUAUUCAGCAUG
1029	ACUGUAAAACCUUGUGUGG	1030	CCACACAAGGUUUUACAGU
1031	UGCUGUUCUGGUAUUACCA	1032	UGGUAAUACCAGAACAGCA

1033	UGGUCGAACAGUUUUUUCC	874	AGAAAAAACUGUUCGACCA
1034	UGGUCGAACAGUUUUUUCG	874	AGAAAAAACUGUUCGACCA
1035	GUUCCAGACUCAACUUGGC	930	UCCAAGUUGAGUCUGGAAC
1036	GUUCCAGACUCAACUUGGU	930	UCCAAGUUGAGUCUGGAAC

Table 14

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
955	CAUGUACGACCAAUGUAAA	956	UUUACAUUGGUCGUACAUG	1	cAuGuAcGAccAAuGuAAAdTsdT	2	UfUfUfACfAUfUfGGUfCfGUfACfAUfGdTsdT
1021	UUGCUUAACUACAUAUAGA	1022	UCUAUAUGUAGUUAAGCAA	3	uuGcuuAAcuAcAuAuAGAdTsdT	4	UfCfUfAUfAUfGUfAGUfUfAAGCfAAdTsdT
1017	AAAUAACUUGCUUAACUAC	1018	GUAGUUAAGCAAGUUAUUU	5	AAAuAAcuuGcuuAAcuAcdTsdT	6	GUfAGUfUfAAGCfAAGUfUfAUfUfUfdTsdT
1023	UGCUUAACUACAUAUAGAU	1024	AUCUAUAUGUAGUUAAGCA	7	uGcuuAAcuAcAuAuAGAudTsdT	8	AUfCfUfAUfAUfGUfAGUfUfAAGCfAdTsdT
967	GUAUGAAAACCUUACUGCU	968	AGCAGUAAGGUUUUCAUAC	9	GuAuGAAAAccuuAcuGcudTsdT	10	AGCfAGUfAAGGUfUfUfUfCfAUfACfdTsdT
997	CAGUGAGAGUUGGUUACUC	998	GAGUAACCAACUCUCACUG	11	cAGuGAGAGuuGGuuAcucdTsdT	12	GAGUfAACfCfAACfUfCfUfCfACfUfGdTsdT
947	GGGUGGAGAUCAUAUAGAC	948	GUCUAUAUGAUCUCCACCC	13	GGGuGGAGAucAuAuAGAcdTsdT	14	GUCuAuAUGAUCUCcACCCdTsdT
947	GGGUGGAGAUCAUAUAGAC	948	GUCUAUAUGAUCUCCACCC	15	GGGuGGAGAucAuAuAGAcdTsdT	16	GUfCfUfAUfAUfGAUfCfUfCfCfACfCfCfdTsdT
997	CAGUGAGAGUUGGUUACUC	998	GAGUAACCAACUCUCACUG	17	cAGuGAGAGuuGGuuAcucdTsdT	18	GAGuAACcAACUCUcACUGdTsdT
953	CAUAUAGACAAUCAAGUGC	954	GCACUUGAUUGUCUAUAUG	19	cAuAuAGAcAAucAAGuGcdTsdT	20	GCfACfUfUfGAUfUfGUfCfUfAUfAUfGdTsdT
995	CCUAUGUAUGUGUUAUCUG	996	CAGAUAACACAUACAUAGG	21	ccuAuGuAuGuGuuAucuGdTsdT	22	CfAGAUfAACfACfAUfACfAUfAGGdTsdT
783	UUAAUGUCAUUCCAC	784	AUUGGUGGAAU	23	uuAAuGucAuuccAccAAu	24	AUfUfGGUfGGAAUfGACf

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
	CAAU		GACAUUAA		dTsdT		AUfUfAAdTsdT
1021	UUGCUUAACUACAUAUAGA	1022	UCUAUAUGUAGUUAAGCAA	25	uuGcuuAAcuAcAuAuAGAdTsdT	26	UCuAuAUGuAGUuAAGcAAdTsdT
873	UGGUCGAACAGUUUUUUCU	874	AGAAAAAACUGUUCGACCA	27	uGGucGAAcAGuuuuuucudTsdT	28	AGAAAAAACfUfGUfUfCfGACfCfAdTsdT
977	CACACAUUAAUCUGAUUUU	978	AAAAUCAGAUUAAUGUGUG	29	cAcAcAuuAAucuGAuuuudTsdT	30	AAAAUfCfAGAUfUfAAUfGUfGUfGdTsdT
967	GUAUGAAAACCUUACUGCU	968	AGCAGUAAGGUUUUCAUAC	31	GuAuGAAAAccuuAcuGcudTsdT	32	AGcAGuAAGGUUUUcAuACdTsdT
905	CUACAGGAGUCUCACAAGA	906	UCUUGUGAGACUCCUGUAG	33	cuAcAGGAGucucAcAAGAdTsdT	34	UCUUGUGAGACUCCUGuAGdTsdT
1013	CUGUAUGAAAAUACCCUCC	1014	GGAGGGUAUUUUCAUACAG	35	cuGuAuGAAAAuAcccuccdTsdT	36	GGAGGGuAUUUUcAuAcAGdTsdT
993	UCCUAUGUAUGUGUUAUCU	994	AGAUAACACAUACAUAGGA	37	uccuAuGuAuGuGuuAucudTsdT	38	AGAuAAcAcAuAcAuAGGAdTsdT
949	GGUGGAGAUCAUAUAGACA	950	UGUCUAUAUGAUCUCCACC	39	GGuGGAGAucAuAuAGAcAdTsdT	40	UfGUfCfUfAUfAUfGAUfCfUfCfCfACfCfdTsdT
957	AUGUACGACCAAUGUAAAC	958	GUUUACAUUGGUCGUACAU	41	AuGuAcGAccAAuGuAAAcdTsdT	42	GUfUfUfACfAUfUfGGUfCfGUfACfAUfdTsdT
973	ACUGGCAGCGGUUUUAUCA	974	UGAUAAAACCGCUGCCAGU	43	AcuGGcAGcGGuuuuAucAdTsdT	44	UfGAUfAAAACfCfGCfUfGCfCfAGUfdTsdT
999	AGUGAGAGUUGGUUACUCA	1000	UGAGUAACCAACUCUCACU	45	AGuGAGAGuuGGuuAcucAdTsdT	46	UfGAGUfAACfCfAACfUfCfUfCfACfUfdTsdT
1019	AAUAACUUGCUUAACUACA	1020	UGUAGUUAAGCAAGUUAUU	47	AAuAAcuuGcuuAAcuAcAdTsdT	48	UfGUfAGUfUfAAGCfAAGUfUfAUfUfdTsdT

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
1001	GUGAGAGUUGGUUACUCAC	1002	GUGAGUAACCAACUCUCAC	49	GuGAGAGuuGGuuAcucAcdTsdT	50	GUfGAGUfAACfCfAACfUfCfUfCfACfdTsdT
885	CAUCAUCGAUAAAAUUCGA	886	UCGAAUUUUAUCGAUGAUG	51	cAucAucGAuAAAAuucGAdTsdT	52	UfCfGAAUfUfUfUfAUfCfGAUfGAUfGdTsdT
1013	CUGUAUGAAAAUACCCUCC	1014	GGAGGGUAUUUUCAUACAG	53	cuGuAuGAAAAuAcccuccdTsdT	54	GGAGGGUfAUfUfUfUfCfAUfACfAGdTsdT
873	UGGUCGAACAGUUUUUUCU	874	AGAAAAAACUGUUCGACCA	55	uGGucGAAcAGuuuuuucudTsdT	56	AGAAAAAACUGUUCGACcAdTsdT
821	ACGAUUCAUUCCUUUUGGA	822	UCCAAAAGGAAUGAAUCGU	57	AcGAuucAuuccuuuuGGAdTsdT	58	UfCfCfAAAAGGAAUfGAAUfCfGUfdTsdT
965	CUGUAUGAAAACCUUACUG	966	CAGUAAGGUUUUCAUACAG	59	cuGuAuGAAAAccuuAcuGdTsdT	60	cAGuAAGGUUUUcAuAcAGdTsdT
1001	GUGAGAGUUGGUUACUCAC	1002	GUGAGUAACCAACUCUCAC	61	GuGAGAGuuGGuuAcucAcdTsdT	62	GUGAGuAACcAACUCUcACdTsdT
959	UGUACGACCAAUGUAAACA	960	UGUUUACAUUGGUCGUACA	63	uGuAcGAccAAuGuAAAcAdTsdT	64	UfGUfUfUfACfAUfUfGGUfCfGUfACfAdTsdT
839	UACCGGACACUAAACCCAA	840	UUGGGUUUAGUGUCCGGUA	65	uAccGGAcAcuAAAcccAAdTsdT	66	UfUfGGGUfUfUfAGUfGUfCfCfGGUfAdTsdT
897	CCGCUAUCGAAAAUGUCUU	898	AAGACAUUUUCGAUAGCGG	67	ccGcuAucGAAAAuGucuudTsdT	68	AAGACfAUfUfUfUfCfGAUfAGCfGGdTsdT
817	AGAUCAGACCUGUUGAUAG	818	CUAUCAACAGGUCUGAUCU	69	AGAucAGAccuGuuGAuAGdTsdT	70	CfUfAUfCfAACfAGGUfCfUfGAUfCfUfdTsdT
993	UCCUAUGUAUGUGUUAUCU	994	AGAUAACACAUACAUAGGA	71	uccuAuGuAuGuGuuAucudTsdT	72	AGAUfAACfACfAUfACfAUfAGGAdTsdT
963	UCUGUAUGAAAACCU	964	AGUAAGGUUUUC	73	ucuGuAuGAAAAccuuAcu	74	AGUfAAGGUfUfUfUfCfA

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
	UACU		AUACAGA		dTsdT		UfACfAGAdTsdT
907	AAAACAAUAGUUCCUGCAA	908	UUGCAGGAACUAUUGUUUU	75	AAAAcAAuAGuuccuGcAAdTsdT	76	UfUfGCfAGGAACfUfAUfUfGUfUfUfUfdTsdT
883	GUCUUAACUUGUGGAAGCU	884	AGCUUCCACAAGUUAAGAC	77	GucuuAAcuuGuGGAAGcudTsdT	78	AGCfUfUfCfCfACfAAGUfUfAAGACfdTsdT
913	ACAAUAGUUCCUGCAACGU	914	ACGUUGCAGGAACUAUUGU	79	AcAAuAGuuccuGcAAcGudTsdT	80	ACfGUfUfGCfAGGAACfUfAUfUfGUfdTsdT
991	AGGCUUUUCAUUAAAUGGG	992	CCCAUUUAAUGAAAAGCCU	81	AGGcuuuucAuuAAAuGGGdTsdT	82	CfCfCfAUfUfUfAAUfGAAAAGCfCfUfdTsdT
929	GUUCCAGACUCAACUUGGA	930	UCCAAGUUGAGUCUGGAAC	83	GuuccAGAcucAAcuuGGAdTsdT	84	UCcAAGUUGAGUCUGGAACdTsdT
957	AUGUACGACCAAUGUAAAC	958	GUUUACAUUGGUCGUACAU	85	AuGuAcGAccAAuGuAAAcdTsdT	86	GUUuAcAUUGGUCGuAcAUdTsdT
905	CUACAGGAGUCUCACAAGA	906	UCUUGUGAGACUCCUGUAG	87	cuAcAGGAGucucAcAAGAdTsdT	88	UfCfUfUfGUfGAGACfUfCfCfUfGUfAGdTsdT
959	UGUACGACCAAUGUAAACA	960	UGUUUACAUUGGUCGUACA	89	uGuAcGAccAAuGuAAAcAdTsdT	90	UGUUuAcAUUGGUCGuAcAdTsdT
857	AGGAUCAGAAGCCUAUUUU	858	AAAAUAGGCUUCUGAUCCU	91	AGGAucAGAAGccuAuuuudTsdT	92	AAAAUfAGGCfUfUfCfUfGAUfCfCfUfdTsdT
969	GAAAUUAGAAUGACCUACA	970	UGUAGGUCAUUCUAAUUUC	93	GAAAuuAGAAuGAccuAcAdTsdT	94	UGuAGGUcAUUCuAAUUUCdTsdT
849	UUCUGUUCAUGGUGUGAGU	850	ACUCACACCAUGAACAGAA	95	uucuGuucAuGGuGuGAGudTsdT	96	ACfUfCfACfACfCfAUfGAACfAGAAdTsdT
929	GUUCCAGACUCAACUUGGA	930	UCCAAGUUGAGUCUGGAAC	97	GuuccAGAcucAAcuuGGAdTsdT	98	UfCfCfAAGUfUfGAGUfCfUfGGAACfdTsdT

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
879	CCAGAUGUAAGCUCUCCUC	880	GAGGAGAGCUUACAUCUGG	99	ccAGAuGuAAGcucuccucdTsdT	100	GAGGAGAGCUuAcAUCUGGdTsdT
875	UUUCUAAUGGCUAUUCAAG	876	CUUGAAUAGCCAUUAGAAA	101	uuucuAAuGGcuAuucAAGdTsdT	102	CfUfUfGAAUfAGCfCfAUfUfAGAAAdTsdT
895	AUGCCGCUAUCGAAAAUGU	896	ACAUUUUCGAUAGCGGCAU	103	AuGccGcuAucGAAAAuGudTsdT	104	ACfAUfUfUfUfCfGAUfAGCfGGCfAUfdTsdT
889	CCAGCAUGCCGCUAUCGAA	890	UUCGAUAGCGGCAUGCUGG	105	ccAGcAuGccGcuAucGAAdTsdT	106	UfUfCfGAUfAGCfGGCfAUfGCfUfGGdTsdT
987	UUGGCGCUCAAAAAAUAGA	988	UCUAUUUUUUGAGCGCCAA	107	uuGGcGcucAAAAAAuAGAdTsdT	108	UCuAUUUUUUGAGCGCcAAdTsdT
863	UCCACCAAUUCCCGUUGGU	864	ACCAACGGGAAUUGGUGGA	109	uccAccAAuucccGuuGGudTsdT	110	ACfCfAACfGGGAAUfUfGGUfGGAdTsdT
909	AAACAAUAGUUCCUGCAAC	910	GUUGCAGGAACUAUUGUUU	111	AAAcAAuAGuuccuGcAAcdTsdT	112	GUfUfGCfAGGAACfUfAUfUfGUfUfUfdTsdT
849	UUCUGUUCAUGGUGUGAGU	850	ACUCACACCAUGAACAGAA	113	uucuGuucAuGGuGuGAGudTsdT	114	ACUcAcACcAUGAAcAGAAdTsdT
805	AGCAUUGCAAACCUCAAUA	806	UAUUGAGGUUUGCAAUGCU	115	AGcAuuGcAAAccucAAuAdTsdT	116	uAUUGAGGUUUGcAAUGCUdTsdT
827	GCCUCUCAUUUUACCGGAC	828	GUCCGGUAAAAUGAGAGGC	117	GccucucAuuuuAccGGAcdTsdT	118	GUfCfCfGGUfAAAAUfGAGAGGCfdTsdT
855	CAGCAUCCCUUUCUCAACA	856	UGUUGAGAAAGGGAUGCUG	119	cAGcAucccuuucucAAcAdTsdT	120	UGUUGAGAAAGGGAUGCUGdTsdT
951	GAGAUCAUAUAGACAAUCA	952	UGAUUGUCUAUAUGAUCUC	121	GAGAucAuAuAGAcAAucAdTsdT	122	UGAUUGUCuAuAUGAUCUCdTsdT
1011	GGCUGUAUGAAAAUA	1012	AGGGUAUUUUCA	123	GGcuGuAuGAAAAuAccc	124	AGGGuAUUUUcAuAcAG

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
	CCCU		UACAGCC		udTsdT		CCdTsdT
821	ACGAUUCAUUCCUUUUGGA	822	UCCAAAAGGAAUGAAUCGU	125	AcGAuucAuuccuuuuGGAdTsdT	126	UCcAAAAGGAAUGAAUCGUdTsdT
803	UGGGAAAUGACCUGGGAUU	804	AAUCCCAGGUCAUUUCCCA	127	uGGGAAAuGAccuGGGAuudTsdT	128	AAUCCcAGGUcAUUUCCcAdTsdT
809	CCCAGGUAAAGAGACGAAU	810	AUUCGUCUCUUUACCUGGG	129	cccAGGuAAAGAGAcGAAudTsdT	130	AUfUfCfGUfCfUfCfUfUfUfACfCfUfGGGdTsdT
855	CAGCAUCCCUUUCUCAACA	856	UGUUGAGAAAGGGAUGCUG	131	cAGcAucccuuucucAAcAdTsdT	132	UfGUfUfGAGAAAGGGAUfGCfUfGdTsdT
813	CAGGUAAAGAGACGAAUGA	814	UCAUUCGUCUCUUUACCUG	133	cAGGuAAAGAGAcGAAuGAdTsdT	134	UfCfAUfUfCfGUfCfUfCfUfUfUfACfCfUfGdTsdT
1019	AAUAACUUGCUUAACUACA	1020	UGUAGUUAAGCAAGUUAUU	135	AAuAAcuuGcuuAAcuAcAdTsdT	136	UGuAGUuAAGcAAGUuAUUdTsdT
965	CUGUAUGAAAACCUUACUG	966	CAGUAAGGUUUUCAUACAG	137	cuGuAuGAAAAccuuAcuGdTsdT	138	CfAGUfAAGGUfUfUfUfCfAUfACfAGdTsdT
927	GCUCUGUUCCAGACUCAAC	928	GUUGAGUCUGGAACAGAGC	139	GcucuGuuccAGAcucAAcdTsdT	140	GUfUfGAGUfCfUfGGAACfAGAGCfdTsdT
793	GGCUCAGUAAGCAAUGCGC	794	GCGCAUUGCUUACUGAGCC	141	GGcucAGuAAGcAAuGcGcdTsdT	142	GCfGCfAUfUfGCfUfUfACfUfGAGCfCfdTsdT
951	GAGAUCAUAUAGACAAUCA	952	UGAUUGUCUAUAUGAUCUC	143	GAGAucAuAuAGAcAAucAdTsdT	144	UfGAUfUfGUfCfUfAUfAUfGAUfCfUfCfdTsdT
857	AGGAUCAGAAGCCUAUUUU	858	AAAAUAGGCUUCUGAUCCU	145	AGGAucAGAAGccuAuuuudTsdT	146	AAAAuAGGCUUCUGAUCCUdTsdT
891	CAGCAUGCCGCUAUCGAAA	892	UUUCGAUAGCGGCAUGCUG	147	cAGcAuGccGcuAucGAAAdTsdT	148	UUUCGAuAGCGGcAUGCUGdTsdT

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5′-3′)**	**SEQ ID NO**	**Antisense strand sequence (5′-3′)**	**SEQ ID NO**	**Sense strand sequence (5′-3′)**	**SEQ ID NO**	**Antisense strand sequence (5′-3′)**
925	UGUUAUAUGCAGGAUAUGA	926	UCAUAUCCUGCAUAUAACA	149	uGuuAuAuGcAGGAuAuGAdTsdT	150	UfCfAUfAUfCfCfUfGCfAUfAUfAACfAdTsdT
899	CGCUAUCGAAAAUGUCUUC	900	GAAGACAUUUUCGAUAGCG	151	cGcuAucGAAAAuGucuucdTsdT	152	GAAGACfAUfUfUfUfCfGAUfAGCfGdTsdT
949	GGUGGAGAUCAUAUAGACA	950	UGUCUAUAUGAUCUCCACC	153	GGuGGAGAucAuAuAGAcAdTsdT	154	UGUCuAuAUGAUCUCcACCdTsdT
987	UUGGCGCUCAAAAAAUAGA	988	UCUAUUUUUUGAGCGCCAA	155	uuGGcGcucAAAAAAuAGAdTsdT	156	UfCfUfAUfUfUfUfUfUfGAGCfGCfCfAAdTsdT
831	UCAUUUUACCGGACACUAA	832	UUAGUGUCCGGUAAAAUGA	157	ucAuuuuAccGGAcAcuAAdTsdT	158	UuAGUGUCCGGuAAAAUGAdTsdT
885	CAUCAUCGAUAAAAUUCGA	886	UCGAAUUUUAUCGAUGAUG	159	cAucAucGAuAAAAuucGAdTsdT	160	UCGAAUUUuAUCGAUGAUGdTsdT
811	CCAGGUAAAGAGACGAAUG	812	CAUUCGUCUCUUUACCUGG	161	ccAGGuAAAGAGAcGAAuGdTsdT	162	cAUUCGUCUCUUuACCUGGdTsdT
961	CAGGCUUCAGGUAUCUUAU	962	AUAAGAUACCUGAAGCCUG	163	cAGGcuucAGGuAucuuAudTsdT	164	AuAAGAuACCUGAAGCCUGdTsdT
789	UUUCCAAAAGGCUCAGUAA	790	UUACUGAGCCUUUUGGAAA	165	uuuccAAAAGGcucAGuAAdTsdT	166	UuACUGAGCCUUUUGGAAAdTsdT
977	CACACAUUAAUCUGAUUUU	978	AAAAUCAGAUUAAUGUGUG	167	cAcAcAuuAAucuGAuuuudTsdT	168	AAAAUcAGAUuAAUGUGUGdTsdT
1011	GGCUGUAUGAAAAUACCCU	1012	AGGGUAUUUUCAUACAGCC	169	GGcuGuAuGAAAAuAcccudTsdT	170	AGGGUfAUfUfUfUfCfAUfACfAGCfCfdTsdT
937	CAGGUUUCAGGAACUUACA	938	UGUAAGUUCCUGAAACCUG	171	cAGGuuucAGGAAcuuAcAdTsdT	172	UGuAAGUUCCUGAAACCUGdTsdT
969	GAAAUUAGAAUGACC	970	UGUAGGUCAUUC	173	GAAAuuAGAAuGAccuA	174	UfGUfAGGUfCfAUfUfCfU

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
	UACA		UAAUUUC		cAdTsdT		fAAUfUfUfCfdTsdT
787	CCAAGCAGCGAAGACUUUU	788	AAAAGUCUUCGCUGCUUGG	175	ccAAGcAGcGAAGAcuuuudTsdT	176	AAAAGUfCfUfUfCfGCfUfGCfUfUfGGdTsdT
863	UCCACCAAUUCCCGUUGGU	864	ACCAACGGGAAUUGGUGGA	177	uccAccAAuucccGuuGGudTsdT	178	ACcAACGGGAAUUGGUGGAdTsdT
983	CCAACAAUCUUGGCGCUCA	984	UGAGCGCCAAGAUUGUUGG	179	ccAAcAAucuuGGcGcucAdTsdT	180	UGAGCGCcAAGAUUGUUGGdTsdT
795	CUCAGUAAGCAAUGCGCAG	796	CUGCGCAUUGCUUACUGAG	181	cucAGuAAGcAAuGcGcAGdTsdT	182	CUGCGcAUUGCUuACUGAGdTsdT
799	UCUCAAUGGGACUGUAUAU	800	AUAUACAGUCCCAUUGAGA	183	ucucAAuGGGAcuGuAuAudTsdT	184	AUfAUfACfAGUfCfCfCfAUfUfGAGAdTsdT
843	AAAAAGAAGAUUUCAUCGA	844	UCGAUGAAAUCUUCUUUUU	185	AAAAAGAAGAuuucAucGAdTsdT	186	UfCfGAUfGAAAUfCfUfUfCfUfUfUfUfUfdTsdT
971	GAACUGGCAGCGGUUUUAU	972	AUAAAACCGCUGCCAGUUC	187	GAAcuGGcAGcGGuuuuAudTsdT	188	AUfAAAACfCfGCfUfGCfCfAGUfUfCfdTsdT
927	GCUCUGUUCCAGACUCAAC	928	GUUGAGUCUGGAACAGAGC	189	GcucuGuuccAGAcucAAcdTsdT	190	GUUGAGUCUGGAAcAGAGCdTsdT
867	CACCAAUUCCCGUUGGUUC	868	GAACCAACGGGAAUUGGUG	191	cAccAAuucccGuuGGuucdTsdT	192	GAACfCfAACfGGGAAUfUfGGUfGdTsdT
899	CGCUAUCGAAAAUGUCUUC	900	GAAGACAUUUUCGAUAGCG	193	cGcuAucGAAAAuGucuucdTsdT	194	GAAGAcAUUUUCGAuAGCGdTsdT
893	AGCAUGCCGCUAUCGAAAA	894	UUUUCGAUAGCGGCAUGCU	195	AGcAuGccGcuAucGAAAAdTsdT	196	UfUfUfUfCfGAUfAGCfGGCfAUfGCfUfdTsdT
931	CUCAACUUGGAGGAUCAUG	932	CAUGAUCCUCCAAGUUGAG	197	cucAAcuuGGAGGAucAuGdTsdT	198	cAUGAUCCUCcAAGUUGAGdTsdT

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
879	CCAGAUGUAAGCUCUCCUC	880	GAGGAGAGCUUACAUCUGG	199	ccAGAuGuAAGcucuccucdTsdT	200	GAGGAGAGCfUfUfACfAUfCfUfGGdTsdT
999	AGUGAGAGUUGGUUACUCA	1000	UGAGUAACCAACUCUCACU	201	AGuGAGAGuuGGuuAcucAdTsdT	202	UGAGuAACcAACUCUcACUdTsdT
935	GGGCGGCAAGUGAUUGCAG	936	CUGCAAUCACUUGCCGCCC	203	GGGcGGcAAGuGAuuGcAGdTsdT	204	CUGcAAUcACUUGCCGCCCdTsdT
785	UGUGAUGGACUUCUAUAAA	786	UUUAUAGAAGUCCAUCACA	205	uGuGAuGGAcuucuAuAAAdTsdT	206	UfUfUfAUfAGAAGUfCfCfAUfCfACfAdTsdT
787	CCAAGCAGCGAAGACUUUU	788	AAAAGUCUUCGCUGCUUGG	207	ccAAGcAGcGAAGAcuuuudTsdT	208	AAAAGUCUUCGCUGCUUGGdTsdT
907	AAAACAAUAGUUCCUGCAA	908	UUGCAGGAACUAUUGUUUU	209	AAAAcAAuAGuuccuGcAAdTsdT	210	UUGcAGGAACuAUUGUUUUdTsdT
897	CCGCUAUCGAAAAUGUCUU	898	AAGACAUUUUCGAUAGCGG	211	ccGcuAucGAAAAuGucuudTsdT	212	AAGAcAUUUUCGAuAGCGGdTsdT
891	CAGCAUGCCGCUAUCGAAA	892	UUUCGAUAGCGGCAUGCUG	213	cAGcAuGccGcuAucGAAAdTsdT	214	UfUfUfCfGAUfAGCfGGCfAUfGCfUfGdTsdT
881	CUGGUGUGCUCUGAUGAAG	882	CUUCAUCAGAGCACACCAG	215	cuGGuGuGcucuGAuGAAGdTsdT	216	CfUfUfCfAUfCfAGAGCfACfACfCfAGdTsdT
933	ACGCUCAACAUGUUAGGAG	934	CUCCUAACAUGUUGAGCGU	217	AcGcucAAcAuGuuAGGAGdTsdT	218	CUCCuAAcAUGUUGAGCGUdTsdT
979	UCCCAACAAUCUUGGCGCU	980	AGCGCCAAGAUUGUUGGGA	219	ucccAAcAAucuuGGcGcudTsdT	220	AGCfGCfCfAAGAUfUfGUfUfGGGAdTsdT
815	AGACGAAUGAGAGUCCUUG	816	CAAGGACUCUCAUUCGUCU	221	AGAcGAAuGAGAGuccuuGdTsdT	222	CfAAGGACfUfCfUfCfAUfUfCfGUfCfUfdTsdT
839	UACCGGACACUAAAC	840	UUGGGUUUAGU	223	uAccGGAcAcuAAAcccA	224	UUGGGUUuAGUGUCCG

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
	CCAA		GUCCGGUA		AdTsdT		GuAdTsdT
919	CUGCAACGUUACCAC AACU	920	AGUUGUGGUAAC GUUGCAG	225	cuGcAAcGuuAccAcAAcu dTsdT	226	AGUUGUGGuAACGUUGc AGdTsdT
889	CCAGCAUGCCGCUAU CGAA	890	UUCGAUAGCGGC AUGCUGG	227	ccAGcAuGccGcuAucGA AdTsdT	228	UUCGAuAGCGGcAUGCU GGdTsdT
805	AGCAUUGCAAACCUC AAUA	806	UAUUGAGGUUU GCAAUGCU	229	AGcAuuGcAAAccucAAu AdTsdT	230	UfAUfUfGAGGUfUfUfGCf AAUfGCfUfdTsdT
979	UCCCAACAAUCUUGG CGCU	980	AGCGCCAAGAUU GUUGGGA	231	ucccAAcAAucuuGGcGcu dTsdT	232	AGCGCcAAGAUUGUUG GGAdTsdT
865	CCACCAAUUCCCGUU GGUU	866	AACCAACGGGAA UUGGUGG	233	ccAccAAuucccGuuGGuud TsdT	234	AACcAACGGGAAUUGG UGGdTsdT
819	UCAGACCUGUUGAUA GAUG	820	CAUCUAUCAACA GGUCUGA	235	ucAGAccuGuuGAuAGAu GdTsdT	236	CfAUfCfUfAUfCfAACfAG GUfCfUfGAdTsdT
837	UUACCGGACACUAAA CCCA	838	UGGGUUUAGUG UCCGGUAA	237	uuAccGGAcAcuAAAccc AdTsdT	238	UGGGUUuAGUGUCCGGu AAdTsdT
981	CCCAACAAUCUUGGC GCUC	982	GAGCGCCAAGAU UGUUGGG	239	cccAAcAAucuuGGcGcuc dTsdT	240	GAGCfGCfCfAAGAUfUfG UfUfGGGdTsdT
875	UUUCUAAUGGCUAUU CAAG	876	CUUGAAUAGCCA UUAGAAA	241	uuucuAAuGGcuAuucAA GdTsdT	242	CUUGAAuAGCcAUuAGA AAdTsdT
783	UUAAUGUCAUUCCAC CAAU	784	AUUGGUGGAAU GACAUUAA	243	uuAAuGucAuuccAccAAu dTsdT	244	AUUGGUGGAAUGAcAUu AAdTsdT
793	GGCUCAGUAAGCAAU GCGC	794	GCGCAUUGCUUA CUGAGCC	245	GGcucAGuAAGcAAuGc GcdTsdT	246	GCGcAUUGCUuACUGAG CCdTsdT
883	GUCUUAACUUGUGGA AGCU	884	AGCUUCCACAAG UUAAGAC	247	GucuuAAcuuGuGGAAGc udTsdT	248	AGCUUCcAcAAGUuAAG ACdTsdT

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5′-3′)**	**SEQ ID NO**	**Antisense strand sequence (5′-3′)**	**SEQ ID NO**	**Sense strand sequence (5′-3′)**	**SEQ ID NO**	**Antisense strand sequence (5′-3′)**
831	UCAUUUUACCGGACACUAA	832	UUAGUGUCCGGUAAAAUGA	249	ucAuuuuAccGGAcAcuAAdTsdT	250	UfUfAGUfGUfCfCfGGUfAAAAUfGAdTsdT
815	AGACGAAUGAGAGUCCUUG	816	CAAGGACUCUCAUUCGUCU	251	AGAcGAAuGAGAGuccuuGdTsdT	252	cAAGGACUCUcAUUCGUCUdTsdT
1029	ACUGUAAAACCUUGUGUGG	1030	CCACACAAGGUUUUACAGU	253	AcuGuAAAAccuuGuGuGGdTsdT	254	CfCfACfACfAAGGUfUfUfUfACfAGUfdTsdT
779	AACCUCAAUAGGUCGACCA	780	UGGUCGACCUAUUGAGGUU	255	AAccucAAuAGGucGAccAdTsdT	256	UGGUCGACCuAUUGAGGUUdTsdT
1027	CAUGCUGAAUAAUAAUCUG	1028	CAGAUUAUUAUUCAGCAUG	257	cAuGcuGAAuAAuAAucuGdTsdT	258	CfAGAUfUfAUfUfAUfUfCfAGCfAUfGdTsdT
775	UGCAAACCUCAAUAGGUCG	776	CGACCUAUUGAGGUUUGCA	259	uGcAAAccucAAuAGGucGdTsdT	260	CGACCuAUUGAGGUUUGcAdTsdT
983	CCAACAAUCUUGGCGCUCA	984	UGAGCGCCAAGAUUGUUGG	261	ccAAcAAucuuGGcGcucAdTsdT	262	UfGAGCfGCfCfAAGAUfUfGUfUfGGdTsdT
939	GGUUUCAGGAACUUACACC	940	GGUGUAAGUUCCUGAAACC	263	GGuuucAGGAAcuuAcAccdTsdT	264	GGUGuAAGUUCCUGAAACCdTsdT
939	GGUUUCAGGAACUUACACC	940	GGUGUAAGUUCCUGAAACC	265	GGuuucAGGAAcuuAcAccdTsdT	266	GGUfGUfAAGUfUfCfCfUfGAAACfCfdTsdT
1009	UAGUGACCAGGUUUUCAGG	1010	CCUGAAAACCUGGUCACUA	267	uAGuGAccAGGuuuucAGGdTsdT	268	CCUGAAAACCUGGUcACuAdTsdT
919	CUGCAACGUUACCACAACU	920	AGUUGUGGUAACGUUGCAG	269	cuGcAAcGuuAccAcAAcudTsdT	270	AGUfUfGUfGGUfAACfGUfUfGCfAGdTsdT
893	AGCAUGCCGCUAUCGAAAA	894	UUUUCGAUAGCGGCAUGCU	271	AGcAuGccGcuAucGAAAAdTsdT	272	UUUUCGAuAGCGGcAUGCUdTsdT
921	UGCAACGUUACCACA	922	GAGUUGUGGUA	273	uGcAAcGuuAccAcAAcuc	274	GAGUUGUGGuAACGUU

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
	ACUC		ACGUUGCA		dTsdT		GcAdTsdT
923	UGAACCUGAAGUGUUAUAU	924	AUAUAACACUUCAGGUUCA	275	uGAAccuGAAGuGuuAuAudTsdT	276	AUfAUfAACfACfUfUfCfAGGUfUfCfAdTsdT
867	CACCAAUUCCCGUUGGUUC	868	GAACCAACGGGAAUUGGUG	277	cAccAAuucccGuuGGuucdTsdT	278	GAACcAACGGGAAUUGGUGdTsdT
811	CCAGGUAAAGAGACGAAUG	812	CAUUCGUCUCUUUACCUGG	279	ccAGGuAAAGAGAcGAAuGdTsdT	280	CfAUfUfCfGUfCfUfCfUfUfUfACfCfUfGGdTsdT
797	CUCUCAAUGGGACUGUAUA	798	UAUACAGUCCCAUUGAGAG	281	cucucAAuGGGAcuGuAuAdTsdT	282	UfAUfACfAGUfCfCfCfAUfUfGAGAGdTsdT
989	UGGCGCUCAAAAAAUAGAA	990	UUCUAUUUUUUGAGCGCCA	283	uGGcGcucAAAAAAuAGAAdTsdT	284	UfUfCfUfAUfUfUfUfUfUfGAGCfGCfCfAdTsdT
1015	AUACCCUCCUCAAAUAACU	1016	AGUUAUUUGAGGAGGGUAU	285	AuAcccuccucAAAuAAcudTsdT	286	AGUfUfAUfUfUfGAGGAGGGUfAUfdTsdT
935	GGGCGGCAAGUGAUUGCAG	936	CUGCAAUCACUUGCCGCCC	287	GGGcGGcAAGuGAuuGcAGdTsdT	288	CfUfGCfAAUfCfACfUfUfGCfCfGCfCfCfdTsdT
1023	UGCUUAACUACAUAUAGAU	1024	AUCUAUAUGUAGUUAAGCA	289	uGcuuAAcuAcAuAuAGAudTsdT	290	AUCuAuAUGuAGUuAAGcAdTsdT
859	AUUCCACCAAUUCCCGUUG	860	CAACGGGAAUUGGUGGAAU	291	AuuccAccAAuucccGuuGdTsdT	292	CfAACfGGGAAUfUfGGUfGGAAUfdTsdT
781	ACCUCAAUAGGUCGACCAG	782	CUGGUCGACCUAUUGAGGU	293	AccucAAuAGGucGAccAGdTsdT	294	CUGGUCGACCuAUUGAGGUdTsdT
851	GUUCAUGGUGUGAGUACCU	852	AGGUACUCACACCAUGAAC	295	GuucAuGGuGuGAGuAccudTsdT	296	AGGUfACfUfCfACfACfCfAUfGAACfdTsdT
829	CCUCUCAUUUUACCGGACA	830	UGUCCGGUAAAAUGAGAGG	297	ccucucAuuuuAccGGAcAdTsdT	298	UGUCCGGuAAAAUGAGAGGdTsdT

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
825	AGCCUCUCAUUUUACCGGA	826	UCCGGUAAAAUGAGAGGCU	299	AGccucucAuuuuAccGGAdTsdT	300	UfCfCfGGUfAAAAUfGAGAGGCfUfdTsdT
801	UCAAUGGGACUGUAUAUGG	802	CCAUAUACAGUCCCAUUGA	301	ucAAuGGGAcuGuAuAuGGdTsdT	302	CfCfAUfAUfACfAGUfCfCfCfAUfUfGAdTsdT
961	CAGGCUUCAGGUAUCUUAU	962	AUAAGAUACCUGAAGCCUG	303	cAGGcuucAGGuAucuuAudTsdT	304	AUfAAGAUfACfCfUfGAAGCfCfUfGdTsdT
903	AUUCAGCAGGCCACUACAG	904	CUGUAGUGGCCUGCUGAAU	305	AuucAGcAGGccAcuAcAGdTsdT	306	CfUfGUfAGUfGGCfCfUfGCfUfGAAUfdTsdT
981	CCCAACAAUCUUGGCGCUC	982	GAGCGCCAAGAUUGUUGGG	307	cccAAcAAucuuGGcGcucdTsdT	308	GAGCGCcAAGAUUGUUGGGdTsdT
877	AUGAGACCAGAUGUAAGCU	878	AGCUUACAUCUGGUCUCAU	309	AuGAGAccAGAuGuAAGcudTsdT	310	AGCUuAcAUCUGGUCUcAUdTsdT
1027	CAUGCUGAAUAAUAAUCUG	1028	CAGAUUAUUAUUCAGCAUG	311	cAuGcuGAAuAAuAAucuGdTsdT	312	cAGAUuAUuAUUcAGcAUGdTsdT
973	ACUGGCAGCGGUUUUAUCA	974	UGAUAAAACCGCUGCCAGU	313	AcuGGcAGcGGuuuuAucAdTsdT	314	UGAuAAAACCGCUGCcAGUdTsdT
841	AUCUGGUUUUGUCAAGCCC	842	GGGCUUGACAAAACCAGAU	315	AucuGGuuuuGucAAGcccdTsdT	316	GGGCfUfUfGACfAAAACfCfAGAUfdTsdT
1003	UGAGAGUUGGUUACUCACA	1004	UGUGAGUAACCAACUCUCA	317	uGAGAGuuGGuuAcucAcAdTsdT	318	UGUGAGuAACcAACUCUcAdTsdT
865	CCACCAAUUCCCGUUGGUU	866	AACCAACGGGAAUUGGUGG	319	ccAccAAuucccGuuGGuudTsdT	320	AACfCfAACfGGGAAUfUfGGUfGGdTsdT
847	AAACUGGGCACAGUUUACU	848	AGUAAACUGUGCCCAGUUU	321	AAAcuGGGcAcAGuuuAcudTsdT	322	AGUfAAACfUfGUfGCfCfCfAGUfUfUfdTsdT
851	GUUCAUGGUGUGAGU	852	AGGUACUCACAC	323	GuucAuGGuGuGAGuAcc	324	AGGuACUcAcACcAUGAA

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5′-3′)**	**SEQ ID NO**	**Antisense strand sequence (5′-3′)**	**SEQ ID NO**	**Sense strand sequence (5′-3′)**	**SEQ ID NO**	**Antisense strand sequence (5′-3′)**
	ACCU		CAUGAAC		udTsdT		CdTsdT
1015	AUACCCUCCUCAAAUAACU	1016	AGUUAUUUGAGGAGGGUAU	325	AuAcccuccucAAAuAAcudTsdT	326	AGUuAUUUGAGGAGGGuAUdTsdT
837	UUACCGGACACUAAACCCA	838	UGGGUUUAGUGUCCGGUAA	327	uuAccGGAcAcuAAAcccAdTsdT	328	UfGGGUfUfUfAGUfGUfCfCfGGUfAAdTsdT
943	ACUUACACCUGGAUGACCA	944	UGGUCAUCCAGGUGUAAGU	329	AcuuAcAccuGGAuGAccAdTsdT	330	UfGGUfCfAUfCfCfAGGUfGUfAAGUfdTsdT
795	CUCAGUAAGCAAUGCGCAG	796	CUGCGCAUUGCUUACUGAG	331	cucAGuAAGcAAuGcGcAGdTsdT	332	CfUfGCfGCfAUfUfGCfUfUfACfUfGAGdTsdT
807	UUUGACAUUUUGCAGGAUU	808	AAUCCUGCAAAAUGUCAAA	333	uuuGAcAuuuuGcAGGAudTsdT	334	AAUfCfCfUfGCfAAAAUfGUfCfAAAdTsdT
819	UCAGACCUGUUGAUAGAUG	820	CAUCUAUCAACAGGUCUGA	335	ucAGAccuGuuGAuAGAuGdTsdT	336	cAUCuAUcAAcAGGUCUGAdTsdT
903	AUUCAGCAGGCCACUACAG	904	CUGUAGUGGCCUGCUGAAU	337	AuucAGcAGGccAcuAcAGdTsdT	338	CUGuAGUGGCCUGCUGAAUdTsdT
915	AUAGUUCCUGCAACGUUAC	916	GUAACGUUGCAGGAACUAU	339	AuAGuuccuGcAAcGuuAcdTsdT	340	GUfAACfGUfUfGCfAGGAACfUfAUfdTsdT
921	UGCAACGUUACCACAACUC	922	GAGUUGUGGUAACGUUGCA	341	uGcAAcGuuAccAcAAcucdTsdT	342	GAGUfUfGUfGGUfAACfGUfUfGCfAdTsdT
1025	UAGUUUUUUAUUCAUGCUG	1026	CAGCAUGAAUAAAAAACUA	343	uAGuuuuuuAuucAuGcuGdTsdT	344	CfAGCfAUfGAAUfAAAAAACfUfAdTsdT
803	UGGGAAAUGACCUGGGAUU	804	AAUCCCAGGUCAUUUCCCA	345	uGGGAAAuGAccuGGGAuudTsdT	346	AAUfCfCfCfAGGUfCfAUfUfUfCfCfCfAdTsdT
807	UUUGACAUUUUGCAGGAUU	808	AAUCCUGCAAAAUGUCAAA	347	uuuGAcAuuuuGcAGGAudTsdT	348	AAUCCUGcAAAAUGUcAAAdTsdT

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5′-3′)**	**SEQ ID NO**	**Antisense strand sequence (5′-3′)**	**SEQ ID NO**	**Sense strand sequence (5′-3′)**	**SEQ ID NO**	**Antisense strand sequence (5′-3′)**
933	ACGCUCAACAUGUUAGGAG	934	CUCCUAACAUGUUGAGCGU	349	AcGcucAAcAuGuuAGGAGdTsdT	350	CfUfCfCfUfAACfAUfGUfUfGAGCfGUfdTsdT
1031	UGCUGUUCUGGUAUUACCA	1032	UGGUAAUACCAGAACAGCA	351	uGcuGuucuGGuAuuAccAdTsdT	352	UfGGUfAAUfACfCfAGAACfAGCfAdTsdT
809	CCCAGGUAAAGAGACGAAU	810	AUUCGUCUCUUUACCUGGG	353	cccAGGuAAAGAGAcGAAudTsdT	354	AUUCGUCUCUUuACCUGGGdTsdT
775	UGCAAACCUCAAUAGGUCG	776	CGACCUAUUGAGGUUUGCA	355	uGcAAAccucAAuAGGucGdTsdT	356	CfGACfCfUfAUfUfGAGGUfUfUfGCfAdTsdT
827	GCCUCUCAUUUUACCGGAC	828	GUCCGGUAAAAUGAGAGGC	357	GccucucAuuuuAccGGAcdTsdT	358	GUCCGGuAAAAUGAGAGGCdTsdT
1031	UGCUGUUCUGGUAUUACCA	1032	UGGUAAUACCAGAACAGCA	359	uGcuGuucuGGuAuuAccAdTsdT	360	UGGuAAuACcAGAAcAGcAdTsdT
971	GAACUGGCAGCGGUUUUAU	972	AUAAAACCGCUGCCAGUUC	361	GAAcuGGcAGcGGuuuuAudTsdT	362	AuAAAACCGCUGCcAGUUCdTsdT
995	CCUAUGUAUGUGUUAUCUG	996	CAGAUAACACAUACAUAGG	363	ccuAuGuAuGuGuuAucuGdTsdT	364	cAGAuAAcAcAuAcAuAGGdTsdT
845	AGAAGAUUUCAUCGAACUC	846	GAGUUCGAUGAAAUCUUCU	365	AGAAGAuuucAucGAAcucdTsdT	366	GAGUfUfCfGAUfGAAAUfCfUfUfCfUfdTsdT
869	CUCUGAACUUCCCUGGUCG	870	CGACCAGGGAAGUUCAGAG	367	cucuGAAcuucccuGGucGdTsdT	368	CfGACfCfAGGGAAGUfUfCfAGAGdTsdT
881	CUGGUGUGCUCUGAUGAAG	882	CUUCAUCAGAGCACACCAG	369	cuGGuGuGcucuGAuGAAGdTsdT	370	CUUcAUcAGAGcAcACcAGdTsdT
931	CUCAACUUGGAGGAUCAUG	932	CAUGAUCCUCCAAGUUGAG	371	cucAAcuuGGAGGAucAuGdTsdT	372	CfAUfGAUfCfCfUfCfCfAAGUfUfGAGdTsdT
825	AGCCUCUCAUUUUAC	826	UCCGGUAAAAUG	373	AGccucucAuuuuAccGGA	374	UCCGGuAAAAUGAGAG

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5′-3′)**	**SEQ ID NO**	**Antisense strand sequence (5′-3′)**	**SEQ ID NO**	**Sense strand sequence (5′-3′)**	**SEQ ID NO**	**Antisense strand sequence (5′-3′)**
	CGGA		AGAGGCU		dTsdT		GCUdTsdT
915	AUAGUUCCUGCAACG UUAC	916	GUAACGUUGCAG GAACUAU	375	AuAGuuccuGcAAcGuuAc dTsdT	376	GuAACGUUGcAGGAACu AUdTsdT
911	AACAAUAGUUCCUGC AACG	912	CGUUGCAGGAAC UAUUGUU	377	AAcAAuAGuuccuGcAAc GdTsdT	378	CfGUfUfGCfAGGAACfUf AUfUfGUfUfdTsdT
841	AUCUGGUUUUGUCAA GCCC	842	GGGCUUGACAAA ACCAGAU	379	AucuGGuuuuGucAAGccc dTsdT	380	GGGCUUGAcAAAACcAG AUdTsdT
1029	ACUGUAAAACCUUGU GUGG	1030	CCACACAAGGUU UUACAGU	381	AcuGuAAAAccuuGuGuG GdTsdT	382	CcAcAcAAGGUUUuAcAG UdTsdT
975	AACUCUUGGAUUCUA UGCA	976	UGCAUAGAAUCC AAGAGUU	383	AAcucuuGGAuucuAuGcA dTsdT	384	UGcAuAGAAUCcAAGAG UUdTsdT
1009	UAGUGACCAGGUUUU CAGG	1010	CCUGAAAACCUG GUCACUA	385	uAGuGAccAGGuuuucAG GdTsdT	386	CfCfUfGAAAACfCfUfGG UfCfACfUfAdTsdT
779	AACCUCAAUAGGUCG ACCA	780	UGGUCGACCUAU UGAGGUU	387	AAccucAAuAGGucGAcc AdTsdT	388	UfGGUfCfGACfCfUfAUfU fGAGGUfUfdTsdT
829	CCUCUCAUUUUACCG GACA	830	UGUCCGGUAAAA UGAGAGG	389	ccucucAuuuuAccGGAcAd TsdT	390	UfGUfCfCfGGUfAAAAUf GAGAGGdTsdT
945	UGACCAAAUGACCCU ACUG	946	CAGUAGGGUCAU UUGGUCA	391	uGAccAAAuGAcccuAcu GdTsdT	392	CfAGUfAGGGUfCfAUfUf UfGGUfCfAdTsdT
817	AGAUCAGACCUGUUG AUAG	818	CUAUCAACAGGU CUGAUCU	393	AGAucAGAccuGuuGAuA GdTsdT	394	CuAUcAAcAGGUCUGAU CUdTsdT
937	CAGGUUUCAGGAACU UACA	938	UGUAAGUUCCUG AAACCUG	395	cAGGuuucAGGAAcuuAc AdTsdT	396	UfGUfAAGUfUfCfCfUfGA AACfCfUfGdTsdT
1025	UAGUUUUUUAUUCAU GCUG	1026	CAGCAUGAAUAA AAAACUA	397	uAGuuuuuuAuucAuGcuG dTsdT	398	cAGcAUGAAuAAAAAAC uAdTsdT

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
785	UGUGAUGGACUUCUAUAAA	786	UUUAUAGAAGUCCAUCACA	399	uGuGAuGGAcuucuAuAAAdTsdT	400	UUuAuAGAAGUCcAUcAcAdTsdT
989	UGGCGCUCAAAAAAUAGAA	990	UUCUAUUUUUUGAGCGCCA	401	uGGcGcucAAAAAAuAGAAdTsdT	402	UUCuAUUUUUUGAGCGCcAdTsdT
911	AACAAUAGUUCCUGCAACG	912	CGUUGCAGGAACUAUUGUU	403	AAcAAuAGuuccuGcAAcGdTsdT	404	CGUUGcAGGAACuAUUGUUdTsdT
923	UGAACCUGAAGUGUUAUAU	924	AUAUAACACUUCAGGUUCA	405	uGAAccuGAAGuGuuAuAudTsdT	406	AuAuAAcACUUcAGGUUcAdTsdT
797	CUCUCAAUGGGACUGUAUA	798	UAUACAGUCCCAUUGAGAG	407	cucucAAuGGGAcuGuAuAdTsdT	408	uAuAcAGUCCcAUUGAGAGdTsdT
963	UCUGUAUGAAAACCUUACU	964	AGUAAGGUUUUCAUACAGA	409	ucuGuAuGAAAAccuuAcudTsdT	410	AGuAAGGUUUUcAuAcAGAdTsdT
895	AUGCCGCUAUCGAAAAUGU	896	ACAUUUUCGAUAGCGGCAU	411	AuGccGcuAucGAAAAuGudTsdT	412	AcAUUUUCGAuAGCGGcAUdTsdT
917	UAGUUCCUGCAACGUUACC	918	GGUAACGUUGCAGGAACUA	413	uAGuuccuGcAAcGuuAccdTsdT	414	GGuAACGUUGcAGGAACuAdTsdT
985	AACAAUCUUGGCGCUCAAA	986	UUUGAGCGCCAAGAUUGUU	415	AAcAAucuuGGcGcucAAAdTsdT	416	UfUfUfGAGCfGCfCfAAGAUfUfGUfUfdTsdT
777	AAACCUCAAUAGGUCGACC	778	GGUCGACCUAUUGAGGUUU	417	AAAccucAAuAGGucGAccdTsdT	418	GGUfCfGACfCfUfAUfUfGAGGUfUfUfdTsdT
789	UUUCCAAAAGGCUCAGUAA	790	UUACUGAGCCUUUUGGAAA	419	uuuccAAAAGGcucAGuAAdTsdT	420	UfUfACfUfGAGCfCfUfUfUfUfGGAAAdTsdT
799	UCUCAAUGGGACUGUAUAU	800	AUAUACAGUCCCAUUGAGA	421	ucucAAuGGGAcuGuAuAudTsdT	422	AuAuAcAGUCCcAUUGAGAdTsdT
985	AACAAUCUUGGCGCU	986	UUUGAGCGCCAA	423	AAcAAucuuGGcGcucAA	424	UUUGAGCGCcAAGAUU

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
	CAAA		GAUUGUU		AdTsdT		GUUdTsdT
975	AACUCUUGGAUUCUAUGCA	976	UGCAUAGAAUCCAAGAGUU	425	AAcucuuGGAuucuAuGcAdTsdT	426	UfGCfAUfAGAAUfCfCfAAGAGUfUfdTsdT
843	AAAAAGAAGAUUUCAUCGA	844	UCGAUGAAAUCUUCUUUUU	427	AAAAAGAAGAuuucAucGAdTsdT	428	UCGAUGAAAUCUUCUUUUUdTsdT
953	CAUAUAGACAAUCAAGUGC	954	GCACUUGAUUGUCUAUAUG	429	cAuAuAGAcAAucAAGuGcdTsdT	430	GcACUUGAUUGUCuAuAUGdTsdT
943	ACUUACACCUGGAUGACCA	944	UGGUCAUCCAGGUGUAAGU	431	AcuuAcAccuGGAuGAccAdTsdT	432	UGGUcAUCcAGGUGuAAGUdTsdT
835	UUUACCGGACACUAAACCC	836	GGGUUUAGUGUCCGGUAAA	433	uuuAccGGAcAcuAAAcccdTsdT	434	GGGUfUfUfAGUfGUfCfCfGGUfAAAdTsdT
813	CAGGUAAAGAGACGAAUGA	814	UCAUUCGUCUCUUUACCUG	435	cAGGuAAAGAGAcGAAuGAdTsdT	436	UcAUUCGUCUCUUuACCUGdTsdT
887	CCCAGCAUGCCGCUAUCGA	888	UCGAUAGCGGCAUGCUGGG	437	cccAGcAuGccGcuAucGAdTsdT	438	UfCfGAUfAGCfGGCfAUfGCfUfGGGdTsdT
887	CCCAGCAUGCCGCUAUCGA	888	UCGAUAGCGGCAUGCUGGG	439	cccAGcAuGccGcuAucGAdTsdT	440	UCGAuAGCGGcAUGCUGGGdTsdT
853	GGAGGACAGAUGUACCACU	854	AGUGGUACAUCUGUCCUCC	441	GGAGGAcAGAuGuAccAcudTsdT	442	AGUfGGUfACfAUfCfUfGUfCfCfUfCfCfdTsdT
955	CAUGUACGACCAAUGUAAA	956	UUUACAUUGGUCGUACAUG	443	cAuGuAcGAccAAuGuAAAdTsdT	444	UUuAcAUUGGUCGuAcAUGdTsdT
917	UAGUUCCUGCAACGUUACC	918	GGUAACGUUGCAGGAACUA	445	uAGuuccuGcAAcGuuAccdTsdT	446	GGUfAACfGUfUfGCfAGGAACfUfAdTsdT
941	AACUUACACCUGGAUGACC	942	GGUCAUCCAGGUGUAAGUU	447	AAcuuAcAccuGGAuGAccdTsdT	448	GGUfCfAUfCfCfAGGUfGUfAAGUfUfdTsdT

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
909	AAACAAUAGUUCCUGCAAC	910	GUUGCAGGAACUAUUGUUU	449	AAAcAAuAGuuccuGcAAcdTsdT	450	GUUGcAGGAACuAUUGUUUdTsdT
833	UUUUACCGGACACUAAACC	834	GGUUUAGUGUCCGGUAAAA	451	uuuuAccGGAcAcuAAAccdTsdT	452	GGUfUfUfAGUfGUfCfCfGGUfAAAAdTsdT
1003	UGAGAGUUGGUUACUCACA	1004	UGUGAGUAACCAACUCUCA	453	uGAGAGuuGGuuAcucAcAdTsdT	454	UfGUfGAGUfAACfCfAACfUfCfUfCfAdTsdT
913	ACAAUAGUUCCUGCAACGU	914	ACGUUGCAGGAACUAUUGU	455	AcAAuAGuuccuGcAAcGudTsdT	456	ACGUUGcAGGAACuAUUGUdTsdT
1007	GGUCCACCCAGGAUUAGUG	1008	CACUAAUCCUGGGUGGACC	457	GGuccAcccAGGAuuAGuGdTsdT	458	CfACfUfAAUfCfCfUfGGGUfGGACfCfdTsdT
925	UGUUAUAUGCAGGAUAUGA	926	UCAUAUCCUGCAUAUAACA	459	uGuuAuAuGcAGGAuAuGAdTsdT	460	UcAuAUCCUGcAuAuAAcAdTsdT
877	AUGAGACCAGAUGUAAGCU	878	AGCUUACAUCUGGUCUCAU	461	AuGAGAccAGAuGuAAGcudTsdT	462	AGCfUfUfACfAUfCfUfGGUfCfUfCfAUfdTsdT
781	ACCUCAAUAGGUCGACCAG	782	CUGGUCGACCUAUUGAGGU	463	AccucAAuAGGucGAccAGdTsdT	464	CfUfGGUfCfGACfCfUfAUfUfGAGGUfdTsdT
845	AGAAGAUUUCAUCGAACUC	846	GAGUUCGAUGAAAUCUUCU	465	AGAAGAuuucAucGAAcucdTsdT	466	GAGUUCGAUGAAAUCUUCUdTsdT
777	AAACCUCAAUAGGUCGACC	778	GGUCGACCUAUUGAGGUUU	467	AAAccucAAuAGGucGAccdTsdT	468	GGUCGACCuAUUGAGGUUUdTsdT
861	UUCCACCAAUUCCCGUUGG	862	CCAACGGGAAUUGGUGGAA	469	uuccAccAAuucccGuuGGdTsdT	470	CfCfAACfGGGAAUfUfGGUfGGAAdTsdT
945	UGACCAAAUGACCCUACUG	946	CAGUAGGGUCAUUUGGUCA	471	uGAccAAAuGAcccuAcuGdTsdT	472	cAGuAGGGUcAUUUGGUcAdTsdT
859	AUUCCACCAAUUCCC	860	CAACGGGAAUUG	473	AuuccAccAAuucccGuuGd	474	cAACGGGAAUUGGUGG

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
	GUUG		GUGGAAU		TsdT		AAUdTsdT
1005	UGGUCCACCCAGGAU UAGU	1006	ACUAAUCCUGGG UGGACCA	475	uGGuccAcccAGGAuuAG udTsdT	476	ACfUfAAUfCfCfUfGGGUf GGACfCfAdTsdT
901	AGGAAUUCAGCAGGC CACU	902	AGUGGCCUGCUG AAUUCCU	477	AGGAAuucAGcAGGccA cudTsdT	478	AGUGGCCUGCUGAAUU CCUdTsdT
871	ACUUCCCUGGUCGAA CAGU	872	ACUGUUCGACCA GGGAAGU	479	AcuucccuGGucGAAcAGu dTsdT	480	ACfUfGUfUfCfGACfCfAG GGAAGUfdTsdT
833	UUUUACCGGACACUA AACC	834	GGUUUAGUGUCC GGUAAAA	481	uuuuAccGGAcAcuAAAcc dTsdT	482	GGUUuAGUGUCCGGuAA AAdTsdT
1017	AAAUAACUUGCUUAA CUAC	1018	GUAGUUAAGCAA GUUAUUU	483	AAAuAAcuuGcuuAAcuA cdTsdT	484	GuAGUuAAGcAAGUuAU UUdTsdT
791	AAGGCUCAGUAAGCA AUGC	792	GCAUUGCUUACU GAGCCUU	485	AAGGcucAGuAAGcAAu GcdTsdT	486	GCfAUfUfGCfUfUfACfUf GAGCfCfUfUfdTsdT
901	AGGAAUUCAGCAGGC CACU	902	AGUGGCCUGCUG AAUUCCU	487	AGGAAuucAGcAGGccA cudTsdT	488	AGUfGGCfCfUfGCfUfGA AUfUfCfCfUfdTsdT
869	CUCUGAACUUCCCUG GUCG	870	CGACCAGGGAAG UUCAGAG	489	cucuGAAcuucccuGGucGd TsdT	490	CGACcAGGGAAGUUcAG AGdTsdT
801	UCAAUGGGACUGUAU AUGG	802	CCAUAUACAGUC CCAUUGA	491	ucAAuGGGAcuGuAuAu GGdTsdT	492	CcAuAuAcAGUCCcAUUG AdTsdT
847	AAACUGGGCACAGUU UACU	848	AGUAAACUGUGC CCAGUUU	493	AAAcuGGGcAcAGuuuAc udTsdT	494	AGuAAACUGUGCCcAGU UUdTsdT
823	AAGCCUCUCAUUUUA CCGG	824	CCGGUAAAAUGA GAGGCUU	495	AAGccucucAuuuuAccGG dTsdT	496	CfCfGGUfAAAAUfGAGA GGCfUfUfdTsdT
871	ACUUCCCUGGUCGAA CAGU	872	ACUGUUCGACCA GGGAAGU	497	AcuucccuGGucGAAcAGu dTsdT	498	ACUGUUCGACcAGGGAA GUdTsdT

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5′-3′)**	**SEQ ID NO**	**Antisense strand sequence (5′-3′)**	**SEQ ID NO**	**Sense strand sequence (5′-3′)**	**SEQ ID NO**	**Antisense strand sequence (5′-3′)**
941	AACUUACACCUGGAUGACC	942	GGUCAUCCAGGUGUAAGUU	499	AAcuuAcAccuGGAuGAccdTsdT	500	GGUcAUCcAGGUGuAAGUUdTsdT
853	GGAGGACAGAUGUACCACU	854	AGUGGUACAUCUGUCCUCC	501	GGAGGAcAGAuGuAccAcudTsdT	502	AGUGGuAcAUCUGUCCUCCdTsdT
1007	GGUCCACCCAGGAUUAGUG	1008	CACUAAUCCUGGGUGGACC	503	GGuccAcccAGGAuuAGuGdTsdT	504	cACuAAUCCUGGGUGGACCdTsdT
791	AAGGCUCAGUAAGCAAUGC	792	GCAUUGCUUACUGAGCCUU	505	AAGGcucAGuAAGcAAuGcdTsdT	506	GcAUUGCUuACUGAGCCUUdTsdT
861	UUCCACCAAUUCCCGUUGG	862	CCAACGGGAAUUGGUGGAA	507	uuccAccAAuucccGuuGGdTsdT	508	CcAACGGGAAUUGGUGGAAdTsdT
835	UUUACCGGACACUAAACCC	836	GGGUUUAGUGUCCGGUAAA	509	uuuAccGGAcAcuAAAcccdTsdT	510	GGGUUuAGUGUCCGGuAAAdTsdT
1005	UGGUCCACCCAGGAUUAGU	1006	ACUAAUCCUGGGUGGACCA	511	uGGuccAcccAGGAuuAGudTsdT	512	ACuAAUCCUGGGUGGACcAdTsdT
823	AAGCCUCUCAUUUUACCGG	824	CCGGUAAAAUGAGAGGCUU	513	AAGccucucAuuuuAccGGdTsdT	514	CCGGuAAAAUGAGAGGCUUdTsdT
991	AGGCUUUUCAUUAAAUGGG	992	CCCAUUUAAUGAAAAGCCU	515	AGGcuuuucAuuAAAuGGGdTsdT	516	CCcAUUuAAUGAAAAGCCUdTsdT
873	UGGUCGAACAGUUUUUUCU	874	AGAAAAAACUGUUCGACCA	55	uGGucGAAcAGuuuuuucudTsdT	744	pAGAAAAAACUGUUCGACcAdTsdT
873	UGGUCGAACAGUUUUUUCU	874	AGAAAAAACUGUUCGACCA	739	ugGucGAAcAGuuuuuucudTsdT	744	pAGAAAAAACUGUUCGACcAdTsdT
873	UGGUCGAACAGUUUUUUCU	874	AGAAAAAACUGUUCGACCA	739	ugGucGAAcAGuuuuuucudTsdT	56	AGAAAAAACUGUUCGACcAdTsdT
1033	UGGUCGAACAGUUUU	874	AGAAAAAACUGU	740	uGGucGAAcAGuuuuuucc	744	pAGAAAAAACUGUUCG

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
	UUCC		UCGACCA		dTsdT		ACcAdTsdT
1033	UGGUCGAACAGUUUUUCC	874	AGAAAAAACUGUUCGACCA	741	ugGucGAAcAGuuuuuccdTsdT	744	pAGAAAAAACUGUUCGACcAdTsdT
1033	UGGUCGAACAGUUUUUCC	874	AGAAAAAACUGUUCGACCA	740	uGGucGAAcAGuuuuuccdTsdT	56	AGAAAAAACUGUUCGACcAdTsdT
1033	UGGUCGAACAGUUUUUCC	874	AGAAAAAACUGUUCGACCA	741	ugGucGAAcAGuuuuuccdTsdT	56	AGAAAAAACUGUUCGACcAdTsdT
1034	UGGUCGAACAGUUUUUCG	874	AGAAAAAACUGUUCGACCA	742	uGGucGAAcAGuuuuuucGdTsdT	745	pAGAAAAAACUGUUCGACcAdTsdT
1034	UGGUCGAACAGUUUUUCG	874	AGAAAAAACUGUUCGACCA	743	ugGucGAAcAGuuuuuucGdTsdT	745	pAGAAAAAACUGUUCGACcAdTsdT
1034	UGGUCGAACAGUUUUUCG	874	AGAAAAAACUGUUCGACCA	742	uGGucGAAcAGuuuuuucGdTsdT	56	AGAAAAAACUGUUCGACcAdTsdT
1034	UGGUCGAACAGUUUUUCG	874	AGAAAAAACUGUUCGACCA	743	ugGucGAAcAGuuuuuucGdTsdT	56	AGAAAAAACUGUUCGACcAdTsdT
873	UGGUCGAACAGUUUUUCU	874	AGAAAAAACUGUUCGACCA	746	uGGucGAAcAGuuuuuucudT(invdT)	752	pAGAAAAAACUGUUCGACcAdT(invdT)
873	UGGUCGAACAGUUUUUCU	874	AGAAAAAACUGUUCGACCA	747	ugGucGAAcAGuuuuuucudT(invdT)	752	pAGAAAAAACUGUUCGACcAdT(invdT)
873	UGGUCGAACAGUUUUUCU	874	AGAAAAAACUGUUCGACCA	746	uGGucGAAcAGuuuuuucudT(invdT)	753	AGAAAAAACUGUUCGACcAdT(invdT)
873	UGGUCGAACAGUUUUUCU	874	AGAAAAAACUGUUCGACCA	747	ugGucGAAcAGuuuuuucudT(invdT)	753	AGAAAAAACUGUUCGACcAdT(invdT)
1033	UGGUCGAACAGUUUUUCC	874	AGAAAAAACUGUUCGACCA	748	uGGucGAAcAGuuuuuuccdT(invdT)	752	pAGAAAAAACUGUUCGACcAdT(invdT)

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
1033	UGGUCGAACAGUUUUUUCC	874	AGAAAAAACUGUUCGACCA	749	ugGucGAAcAGuuuuuucc dT(invdT)	752	pAGAAAAAACUGUUCGACCcAdT(invdT)
1033	UGGUCGAACAGUUUUUUCC	874	AGAAAAAACUGUUCGACCA	748	uGGucGAAcAGuuuuuucc dT(invdT)	753	AGAAAAAACUGUUCGACCcAdT(invdT)
1033	UGGUCGAACAGUUUUUUCC	874	AGAAAAAACUGUUCGACCA	749	ugGucGAAcAGuuuuuucc dT(invdT)	753	AGAAAAAACUGUUCGACCcAdT(invdT)
1034	UGGUCGAACAGUUUUUUCG	874	AGAAAAAACUGUUCGACCA	750	uGGucGAAcAGuuuuuuc GdT(invdT)	752	pAGAAAAAACUGUUCGACCcAdT(invdT)
1034	UGGUCGAACAGUUUUUUCG	874	AGAAAAAACUGUUCGACCA	751	ugGucGAAcAGuuuuuucG dT(invdT)	752	pAGAAAAAACUGUUCGACCcAdT(invdT)
1034	UGGUCGAACAGUUUUUUCG	874	AGAAAAAACUGUUCGACCA	750	uGGucGAAcAGuuuuuuc GdT(invdT)	753	AGAAAAAACUGUUCGACCcAdT(invdT)
1034	UGGUCGAACAGUUUUUUCG	874	AGAAAAAACUGUUCGACCA	751	ugGucGAAcAGuuuuuucG dT(invdT)	753	AGAAAAAACUGUUCGACCcAdT(invdT)
873	UGGUCGAACAGUUUUUUCU	874	AGAAAAAACUGUUCGACCA	754	uGGucGAAcAGuuuuuucu dT(abasic)	760	pAGAAAAAACUGUUCGACCcAdT(abasic)
873	UGGUCGAACAGUUUUUUCU	874	AGAAAAAACUGUUCGACCA	755	ugGucGAAcAGuuuuuucu dT(abasic)	760	pAGAAAAAACUGUUCGACCcAdT(abasic)
873	UGGUCGAACAGUUUUUUCU	874	AGAAAAAACUGUUCGACCA	754	uGGucGAAcAGuuuuuucu dT(abasic)	761	AGAAAAAACUGUUCGACCcAdT(abasic)
873	UGGUCGAACAGUUUUUUCU	874	AGAAAAAACUGUUCGACCA	755	ugGucGAAcAGuuuuuucu dT(abasic)	761	AGAAAAAACUGUUCGACCcAdT(abasic)
1033	UGGUCGAACAGUUUUUUCC	874	AGAAAAAACUGUUCGACCA	756	uGGucGAAcAGuuuuuucc dT(abasic)	760	pAGAAAAAACUGUUCGACCcAdT(abasic)
1033	UGGUCGAACAGUUUUUUCC	874	AGAAAAAACUGUUCGACCA	757	ugGucGAAcAGuuuuuucc	760	pAGAAAAAACUGUUCG

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
	UUCC		UCGACCA		dT(abasic)		ACcAdT(abasic)
1033	UGGUCGAACAGUUUUUUCC	874	AGAAAAAACUGUUCGACCA	756	uGGucGAAcAGuuuuuuccdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)
1033	UGGUCGAACAGUUUUUUCC	874	AGAAAAAACUGUUCGACCA	757	ugGucGAAcAGuuuuuuccdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)
1034	UGGUCGAACAGUUUUUUCG	874	AGAAAAAACUGUUCGACCA	758	ugGucGAAcAGuuuuuucGdT(abasic)	760	pAGAAAAAACUGUUCGACcAdT(abasic)
1034	UGGUCGAACAGUUUUUUCG	874	AGAAAAAACUGUUCGACCA	759	uGGucGAAcAGuuuuuucGdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)
1034	UGGUCGAACAGUUUUUUCG	874	AGAAAAAACUGUUCGACCA	758	ugGucGAAcAGuuuuuucGdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)
929	GUUCCAGACUCAACUUGGA	930	UCCAAGUUGAGUCUGGAAC	83	GuuccAGAcucAAcuuGGAdTsdT	770	pUCcAAGUUGAGUCUGGAACdTsdT
1035	GUUCCAGACUCAACUUGGC	930	UCCAAGUUGAGUCUGGAAC	762	GuuccAGAcucAAcuuGGcdTsdT	770	pUCcAAGUUGAGUCUGGAACdTsdT
1035	GUUCCAGACUCAACUUGGC	930	UCCAAGUUGAGUCUGGAAC	762	GuuccAGAcucAAcuuGGcdTsdT	84	UCcAAGUUGAGUCUGGAACdTsdT
1036	GUUCCAGACUCAACUUGGU	930	UCCAAGUUGAGUCUGGAAC	763	GuuccAGAcucAAcuuGGudTsdT	770	pUCcAAGUUGAGUCUGGAACdTsdT
1036	GUUCCAGACUCAACUUGGU	930	UCCAAGUUGAGUCUGGAAC	763	GuuccAGAcucAAcuuGGudTsdT	84	UCcAAGUUGAGUCUGGAACdTsdT
929	GUUCCAGACUCAACUUGGA	930	UCCAAGUUGAGUCUGGAAC	764	GuuccAGAcucAAcuuGGAdT(invdT)	771	pUCcAAGUUGAGUCUGGAACdT(invdT)
929	GUUCCAGACUCAACUUGGA	930	UCCAAGUUGAGUCUGGAAC	764	GuuccAGAcucAAcuuGGAdT(invdT)	772	UCcAAGUUGAGUCUGGAACdT(invdT)

unmodified sequence				modified sequence			
SEQ ID NO	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**	**SEQ ID NO**	**Sense strand sequence (5´-3´)**	**SEQ ID NO**	**Antisense strand sequence (5´-3´)**
1035	GUUCCAGACUCAACU UGGC	930	UCCAAGUUGAGU CUGGAAC	765	GuuccAGAcucAAcuuGGc dT(invdT)	771	pUCcAAGUUGAGUCUGG AACdT(invdT)
1035	GUUCCAGACUCAACU UGGC	930	UCCAAGUUGAGU CUGGAAC	765	GuuccAGAcucAAcuuGGc dT(invdT)	772	UCcAAGUUGAGUCUGG AACdT(invdT)
1036	GUUCCAGACUCAACU UGGU	930	UCCAAGUUGAGU CUGGAAC	766	GuuccAGAcucAAcuuGGu dT(invdT)	771	pUCcAAGUUGAGUCUGG AACdT(invdT)
1036	GUUCCAGACUCAACU UGGU	930	UCCAAGUUGAGU CUGGAAC	766	GuuccAGAcucAAcuuGGu dT(invdT)	772	UCcAAGUUGAGUCUGG AACdT(invdT)
929	GUUCCAGACUCAACU UGGA	930	UCCAAGUUGAGU CUGGAAC	767	GuuccAGAcucAAcuuGG AdT(abasic)	773	pUCcAAGUUGAGUCUGG AACdT(abasic)
929	GUUCCAGACUCAACU UGGA	930	UCCAAGUUGAGU CUGGAAC	767	GuuccAGAcucAAcuuGG AdT(abasic)	774	UCcAAGUUGAGUCUGG AACdT(abasic)
1035	GUUCCAGACUCAACU UGGC	930	UCCAAGUUGAGU CUGGAAC	768	GuuccAGAcucAAcuuGGc dT(abasic)	773	pUCcAAGUUGAGUCUGG AACdT(abasic)
1035	GUUCCAGACUCAACU UGGC	930	UCCAAGUUGAGU CUGGAAC	768	GuuccAGAcucAAcuuGGc dT(abasic)	774	UCcAAGUUGAGUCUGG AACdT(abasic)
1036	GUUCCAGACUCAACU UGGU	930	UCCAAGUUGAGU CUGGAAC	769	GuuccAGAcucAAcuuGGu dT(abasic)	773	pUCcAAGUUGAGUCUGG AACdT(abasic)
1036	GUUCCAGACUCAACU UGGU	930	UCCAAGUUGAGU CUGGAAC	769	GuuccAGAcucAAcuuGGu dT(abasic)	774	UCcAAGUUGAGUCUGG AACdT(abasic)

Claims

1. A double-stranded ribonucleic acid molecule capable of inhibiting the expression of GCR gene in vitro by at least 70 %, preferably by at least 80% and most preferably by at least 90%.

2. The double-stranded ribonucleic acid molecule of claim 1, wherein said double-stranded ribonucleic acid molecule comprises a sense strand and an antisense strand, the antisense strand being at least partially complementary to the sense strand, whereby the sense strand comprises a sequence, which has an identity of at least 90 % to at least a portion of an mRNA encoding GCR, wherein said sequence is (i) located in the region of complementarity of said sense strand to said antisense strand; and (ii) wherein said sequence is less than 30 nucleotides in length.

3. The double-stranded ribonucleic acid molecule of claims 1 to 2, wherein said sense strand comprises nucleotide acid sequences depicted in SEQ ID Nos: 873, 929, 1021, 1023, 967 and 905, and said antisense strand comprises nucleic acid sequences depicted in SEQ ID Nos: 874, 930, 1022, 1024, 968 and 906, wherein said double-stranded ribonucleic acid molecule comprises the sequence pairs selected from the group consisting of SEQ ID NOs: 873/874, 929/930, 1021/1022, 1023/1024, 967/968 and 905/906.

4. The double-stranded ribonucleic acid molecule of claim 3, wherein the antisense strand further comprises a 3' overhang of 1-5 nucleotides in length, preferably of 1-2 nucleotides in length.

5. The double-stranded ribonucleic acid molecule of claim 4, wherein the overhang of the antisense strand comprises uracil or nucleotides which are complementary to the mRNA encoding GCR.

6. The double-stranded ribonucleic acid molecule of any of claims 3 to 5, wherein the sense strand further comprises a 3' overhang of 1-5 nucleotides in length, preferably of 1-2 nucleotides in length.

7. The double-stranded ribonucleic acid molecule of claim 6 wherein the overhang of the sense strand comprises uracil or nucleotides which are identical to the mRNA encoding GCR.

8. The double-stranded ribonucleic acid molecule of any one of claims 1 to 7, wherein said double-stranded ribonucleic acid molecule comprises at least one modified nucleotide.

9. The double-stranded ribonucleic acid molecule of claim 8, wherein said modified nucleotide is selected from the group consisting of a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or a dodecanoic acid bisdecylamide group, a 2'-deoxy-2'-fluoro modified nucleotide, an inverted deoxythymidine, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

10. The double-stranded ribonucleic acid molecule of any one of claims 8 and 9, wherein said modified nucleotide is a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, an inverted deoxythymidine and a deoxythymidine.

11. The double-stranded ribonucleic acid molecule of claims 3 to 10, wherein said sense strand and / or said antisense strand comprise an overhang of 1-2 deoxythymidines and/or inverted deoxythymidine.

12. The double-stranded ribonucleic acid molecule of any one of claims 1 to 11, wherein said sense strand is selected from the group consisting of the nucleic acid sequences depicted in SEQ ID Nos: 3, 7, 55, 25, 83, 31, 33, 747 and 764 and said antisense strand is selected from the group consisting of the nucleic acid sequences depicted in SEQ ID Nos: 4, 8, 56, 26, 84, 32, 34, 753 and 772 wherein said double-stranded ribonucleic acid molecule comprises the sequence pairs selected from the group consisting of SEQ ID NOs: 3/4, 7/8, 55/56, 25/26, 83/84, 31/32, 33/34, 747/753 and 764/772.

13. A nucleic acid sequence encoding a sense strand and/or an antisense strand comprised in the double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12.

14. A vector comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one of a sense strand or an antisense strand comprised in the double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12 or comprising the nucleic acid sequence of claim 13.

15. A cell, tissue or non-human organism comprising the double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12, the nucleic acid molecule of claim 13 or the vector of claim 14.

16. A pharmaceutical composition comprising the double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12, the nucleic acid molecule of claim 13, the vector of claim 14 or the cell or tissue of claim15.

17. The pharmaceutical composition of claim 16, further comprising a pharmaceutically acceptable carrier, stablilizer and/or diluent.

18. A method for inhibiting the expression of GCR gene in a cell, a tissue or an organism comprising the following steps:

(a) introducing into the cell, tissue or organism the double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12, the nucleic acid molecule of claim 13, the vector of claim 14; and

(b) maintaining the cell, tissue or organism produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a GCR gene, thereby inhibiting expression of a GCR gene in the cell.

19. A method of treating, preventing or managing pathological conditions and diseases caused by the expression of the GCR gene disease comprising administering to a subject in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of a the double-stranded ribonucleic acid molecule as

defined in any one of claims 1 to 12, a nucleic acid molecule of claim 13, a vector of claim 14 and/or a pharmaceutical composition as defined in claims 16 or 17.

20. The method of claim 19, wherein said subject is a human.

21. A double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12, a nucleic acid molecule of claim 13, a vector of claim 14 and/or a pharmaceutical composition as defined in claims 16 or 17 for use in treating type 2 diabetes, obesity, dislipidemia, diabetic artherosclerosis, hypertension or depression.

22. Use of a the double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12, a nucleic acid molecule of claim 13, a vector of claim 14 and/or a cell or tissue of claim 15 for the preparation of a pharmaceutical composition for the treatment of type 2 diabetes, obesity, dislipidemia, diabetic artherosclerosis, hypertension or depression.