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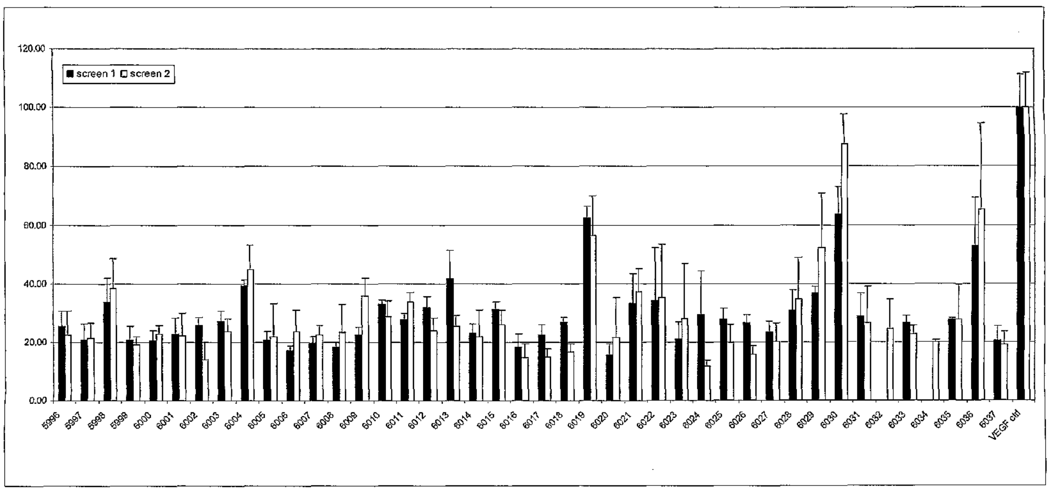
(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF HUNTINGTIN GENE



(57) Abstract: The invention relates to a double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of the *Huntingtin* gene (HD gene), comprising an antisense strand having a nucleotide sequence which is less that 25 nucleotides in length and which is substantially complementary to at least a part of the HD gene. The invention also relates to a pharmaceutical composition comprising the dsRNA together with a pharmaceutically acceptable carrier; methods for treating diseases caused by the expression of the HD gene, or a mutant form thereof, using the pharmaceutical composition; and methods for inhibiting the expression of the huntingtin gene in a cell.

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COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF HUNTINGTIN GENE

Related Applications

This application claims the benefit of U. S. Provisional Application No. 60/731,555, filed October 28, 2005, U.S. Provisional Application No. 60/819,038, filed July 7, 2006, and U.S. Provisional Application No. 60/836,040, filed August 7, 2006. The contents of each of these priority applications are incorporated herein by reference in their entirety.

Field of the Invention

This invention relates to double-stranded ribonucleic acid (dsRNA), and its use in mediating RNA interference to inhibit the expression of the *Huntingtin* gene.

Background of the Invention

Recently, double-stranded RNA molecules (dsRNA) have been shown to block gene expression in a highly conserved regulatory mechanism known as RNA interference (RNAi). WO 99/32619 (Fire et al.) discloses the use of a dsRNA of at least 25 nucleotides in length to inhibit the expression of genes in *C. elegans*. dsRNA has also been shown to degrade target RNA in other organisms, including plants (see, e.g., WO 99/53050, Waterhouse et al.; and WO 99/61631, Heifetz et al.), *Drosophila* (see, e.g., Yang, D., et al., *Curr. Biol.* (2000) 10:1191-1200), and mammals (see WO 00/44895, Limmer; and DE 101 00 586.5, Kreutzer et al.). This natural mechanism has now become the focus for the development of a new class of pharmaceutical agents for treating disorders that are caused by the aberrant regulation of genes or the expression of a mutant form of a gene.

Huntington's disease is a progressive neurodegenerative disorder characterized by motor disturbance, cognitive loss and psychiatric manifestations (Martin and

Gusella, N. Engl. J. Med. 315:1267-1276 (1986). It is inherited in an autosomal dominant fashion, and affects about 1/10,000 individuals in most populations of European origin (Harper, P. S. et al., in Huntington's disease, W. B. Saunders, Philadelphia, 1991). The hallmark of Huntington's disease is a distinctive choreic movement disorder that typically has a subtle, insidious onset in the fourth to fifth decade of life and gradually worsens over a course of 10 to 20 years until death. Occasionally, Huntington's disease is expressed in juveniles typically manifesting with more severe symptoms including rigidity and a more rapid course. Juvenile onset of Huntington's disease is associated with a preponderance of paternal transmission of the disease allele. The neuropathology of Huntington's disease also displays a distinctive pattern, with selective loss of neurons that is most severe in the caudate and putamen regions of the brain. The biochemical basis for neuronal death in Huntington's disease has not yet been explained, and there is consequently no treatment effective in delaying or preventing the onset and progression of this devastating disorder.

Although an actual mechanism for Huntington's disease remains elusive, Huntington's disease has been shown to be an autosomal dominant neurodegenerative disorder caused by an expanding glutamine repeat in a gene termed IT15 or *Huntingtin* (HD). Although this gene is widely expressed and is required for normal development, the pathology of Huntington's disease is restricted to the brain, for reasons that remain poorly understood. The *Huntingtin* gene product is expressed at similar levels in patients and controls, and the genetics of the disorder suggest that the expansion of the polyglutamine repeat induces a toxic gain of function, perhaps through interactions with other cellular proteins.

Treatment for Huntington's disease is currently not available. The choreic movements and agitated behaviors may be suppressed, usually only partially, by antipsychotics (e.g., chlorpromazine 100 to 900 mg/day po or haloperidol 10 to 90 mg/day po) or reserpine begun with 0.1 mg/day po and increased until adverse effects of lethargy, hypotension, or parkinsonism occur.

Despite significant advances in the field of RNAi and Huntington's disease treatment, there remains a need for an agent that can selectively and efficiently silence the HD gene using the cell's own RNAi machinery that has both high biological activity and in vivo stability, and that can effectively inhibit expression of a target *Huntingtin* gene.

Summary of the Invention

The invention provides double-stranded ribonucleic acid (dsRNA), as well as compositions and methods for inhibiting the expression of the HD gene in a cell or mammal using such dsRNA. The invention also provides compositions and methods for treating diseases caused by the expression of a mutant form of the HD gene. The dsRNA of the invention comprises an RNA strand (the antisense strand) having a region which is less than 30 nucleotides in length and is substantially complementary to at least part of an mRNA transcript of the HD gene.

In embodiment, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of the HD 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 comprising a second sequence. The antisense strand comprises a nucleotide sequence which is substantially complementary to at least part of an mRNA encoding the huntingtin protein, and the region of complementarity is less than 30 nucleotides in length. The dsRNA, upon contacting with a cell expressing the HD gene, inhibits the expression of the HD gene by at least 20%.

For example, the dsRNA molecules of the invention can be comprised of a first sequence of the dsRNA that is selected from the group consisting of the sense sequences of Tables 1, 2, 7, 8 or 10 and the second sequence is selected from the group consisting of the antisense sequences of Tables 1, 2, 7, 8 or 10. The dsRNA molecules of the invention can be comprised of naturally occurring nucleotides or can be comprised of at least one modified nucleotide, such as a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group. Alternatively, 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. Preferably, the first sequence of said dsRNA is selected from the group consisting of the sense sequences of Table 2 and the second sequence is selected from the group consisting of the antisense sequences of Table 2.

In another embodiment, the invention provides a cell comprising one of the dsRNAs of the invention. The cell is preferably a mammalian cell, such as a human cell.

In another embodiment, the invention provides a pharmaceutical composition for inhibiting the expression of the HD gene in an organism, comprising one or more of the dsRNA of the invention and a pharmaceutically acceptable carrier.

In another embodiment, the invention provides a method for inhibiting the expression of the HD gene in a cell, comprising the following steps:

(a) introducing into the cell a double-stranded ribonucleic acid (dsRNA), wherein 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 comprising a second sequence. The antisense strand comprises a region of complementarity which is substantially complementary to at least a part of a mRNA encoding the HD gene, and wherein the region of complementarity is less than 30 nucleotides in length and wherein the dsRNA, upon contact with a cell expressing the HD gene, inhibits expression of the HD gene by at least 20%; and

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

In another embodiment, the invention provides methods for treating, preventing or managing Huntington's disease comprising administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of one or more of the dsRNAs of the invention.

In another embodiment, the invention provides vectors for inhibiting the expression of the HD gene in a cell, comprising a regulatory sequence operably 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 cell comprising a vector for inhibiting the expression of the HD gene in a cell. The vector comprises a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of one of the dsRNA of the invention.

Brief Description of the Figures

FIG. 1. *In vitro* activity of the dsRNAs provided in Table 2 against endogenous human HD mRNA expression in HeLa cells.

FIG. 2. Activity of selected dsRNAs in reducing endogenous human HD protein formation in HeLa cells.

FIG. 3. Stability of selected dsRNAs in cerebrospinal fluid (CSF) at 37°C.

FIG. 4. Long-term stability of dsRNAs AL-DP-5997, AL-DP-6000, AL-DP-6001 and AL-DP-7100 in rat CSF

Detailed Description of the Invention

The invention provides double-stranded ribonucleic acid (dsRNA), as well as compositions and methods for inhibiting the expression of the HD gene in a cell or mammal using the dsRNA. The invention also provides compositions and methods for treating diseases in a mammal caused by the expression of the HD gene, or a mutant form thereof, using dsRNA. dsRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The process occurs in a wide variety of organisms, including mammals and other vertebrates.

The dsRNA of the invention comprises an RNA strand (the antisense strand) having a region which is less than 30 nucleotides in length and is substantially complementary to at least part of an mRNA transcript of the HD gene. The use of these dsRNAs enables the targeted degradation of mRNAs of genes that are implicated in Huntington Disease. Using cell-based and animal assays, the present inventors have demonstrated that very low dosages of these dsRNA can specifically and efficiently mediate RNAi, resulting in significant inhibition of expression of the HD gene. Thus, the methods and compositions of the invention comprising these dsRNAs are useful for treating Huntington disease.

The following detailed description discloses how to make and use the dsRNA and compositions containing dsRNA to inhibit the expression of a target HD gene, as well as compositions and methods for treating diseases and disorders caused by the expression of these genes. The pharmaceutical compositions of the invention comprise a dsRNA having an antisense strand comprising a region of complementarity which is less than 30 nucleotides in length and is substantially complementary to at least part of an RNA transcript of the HD gene, together with a pharmaceutically acceptable carrier (Human HD mRNA (NM-002111), mouse HD mRNA (NM_010414) and rat HD mRNA (U18650)).

Accordingly, certain aspects of the invention provide pharmaceutical compositions comprising the dsRNA of the invention together with a pharmaceutically acceptable carrier, methods of using the compositions to inhibit expression of the HD gene, and methods of using the pharmaceutical compositions to treat diseases caused by expression of a mutant form of the HD gene.

I. 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" and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, and uracil may be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base may base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine may be replaced in the nucleotide sequences of the invention by a nucleotide containing, for example, inosine. Sequences comprising such replacement moieties are embodiments of the invention.

The gene involved in Huntington's disease (IT-15) is located at the end of the short arm of chromosome 4. A mutation occurs in the coding region of this gene and produces an unstable expanded trinucleotide repeat (cytosine-adenosine-guanosine), resulting in a protein with an expanded glutamate sequence. The normal and abnormal functions of this protein (termed *huntingtin*) are unknown. The abnormal *huntingtin* protein appears to accumulate in neuronal nuclei of transgenic mice, but the causal relationship of this accumulation to neuronal death is uncertain.

By "*Huntingtin*" or "HD" as used herein is meant, any *Huntingtin* protein, peptide, or polypeptide associated with the development or maintenance of Huntington disease. The terms "*Huntingtin*" and "HD" also refer to nucleic acid sequences encoding any *huntingtin* protein, peptide, or polypeptide, such as *Huntingtin* RNA or *Huntingtin* DNA (see for example Van Dellen et al., Jan. 24, 2004, Neurogenetics). For the Examples, the HD mRNA sequences used were Human HD mRNA (NM-002111), mouse HD mRNA (NM_010414) and rat HD mRNA (U18650).

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of the HD 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.

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, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions may include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing. Other conditions, such as physiologically relevant conditions as may be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

This includes 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. Such sequences can be referred to as "fully complementary" with respect to each other herein. 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 4, 3 or 2 mismatched base pairs upon hybridization, while retaining the ability to hybridize under the conditions most relevant to their ultimate application. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, may yet be referred to as "fully complementary" for the purposes of the invention.

"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.

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.

As used herein, a polynucleotide which is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide which is substantially complementary to a contiguous portion of the mRNA of interest (e.g., encoding HD). For example, a polynucleotide is complementary to at least a part of a

HD mRNA if the sequence is substantially complementary to a non-interrupted portion of a mRNA encoding HD.

The term "double-stranded RNA" 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, as defined above, 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. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA. In addition to the duplex structure, a dsRNA may comprise one or more nucleotide overhangs.

As used herein, 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. "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, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches are most tolerated in the terminal regions and, if present, are preferably in a terminal region or regions, *e.g.*, within 6, 5, 4, 3, or 2 nucleotides of the 5' and/or 3' terminus.

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.

"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. In vitro introduction into a cell includes methods known in the art such as electroporation and lipofection.

The terms "silence" and "inhibit the expression of", in as far as they refer to the HD gene, herein refer to the at least partial suppression of the expression of the HD gene, as manifested by a reduction of the amount of mRNA transcribed from the HD gene which may be isolated from a first cell or group of cells in which the HD gene is transcribed and which has or have been treated such that the expression of the HD 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 HD gene transcription, e.g. the amount of protein encoded by the HD gene which is secreted by a cell, or the number of cells displaying a certain phenotype, e.g apoptosis. In principle, HD gene silencing may be determined in any cell expressing the target, either constitutively or by genomic engineering, and by any appropriate assay. However, when a reference is needed in order to determine whether a given siRNA inhibits the expression of the HD gene by a certain degree and therefore is encompassed by the instant invention, the assay provided in the Examples below shall serve as such reference.

For example, in certain instances, expression of the HD gene is suppressed by at least about 20%, 25%, 35%, or 50% by administration of the double-stranded oligonucleotide of the invention. In a preferred embodiment, the HD gene is suppressed by at least about 60%, 70%, or 80% by administration of the double-stranded oligonucleotide of the invention. In a more preferred embodiment, the HD gene is suppressed by at least about 85%, 90%, or 95% by administration of the double-stranded oligonucleotide of the invention. In a most preferred embodiment, the HD gene is suppressed by at least about 98%, 99% or more by administration of the double-stranded oligonucleotide of the invention.

As used herein, the term "treatment" refers to the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disorder, e.g., a disease or condition, a symptom of disease, or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptoms of disease, or the predisposition toward disease. A "patient" may be a human, but can also be a non-human animal. Treatment can refer to the reduction of any one of the overt symptoms of Huntington's disease, such as dementia or psychiatric disturbances, ranging from apathy and irritability to full-blown bipolar or schizophreniform disorder, motor manifestations include flicking movements of the extremities, a lilting gait, motor impersistence (inability to sustain a motor act, such as tongue protrusion), facial grimacing, ataxia, and dystonia.

As used herein, the phrases "therapeutically effective amount" and "prophylactically effective amount" refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of Huntington's disease or an overt symptom of the disease. The specific amount that is therapeutically effective can be readily determined by ordinary medical practitioner, and may vary depending on factors known in the art, such as, e.g. the type of Huntington's disease, the patient's history and age, the stage of Huntington's disease, and the administration of other anti-Huntington's disease agents.

As used herein, a "pharmaceutical composition" comprises a pharmacologically effective amount of a dsRNA and a pharmaceutically acceptable carrier. 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. For example, if a given clinical treatment is considered effective when there is at least a 25% reduction in a measurable parameter associated with a disease or disorder, a therapeutically effective amount of a drug for the treatment of that disease or disorder is the amount necessary to effect at least a 25% reduction in that parameter.

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. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

As used herein, a "transformed cell" is a cell into which a vector has been introduced from which a dsRNA molecule may be expressed.

II. Double-stranded ribonucleic acid (dsRNA)

In one embodiment, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of the HD gene in a cell or mammal, wherein the dsRNA comprises an antisense strand comprising a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of the HD gene, and wherein the region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said HD gene, inhibits the expression of said HD gene by at least 20%. The dsRNA comprises two RNA strands that are sufficiently complementary to hybridize to form a duplex structure. One strand of the dsRNA (the antisense strand) comprises a region of complementarity that is substantially complementary, and preferably fully complementary, to a target sequence, derived from the sequence of an mRNA formed during the expression of the HD gene, the other strand (the sense strand) comprises a region which is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. Preferably, the duplex structure is between 15 and 30, more preferably between 18 and 25, yet more preferably between 19 and 24, and most preferably between 21 and 23 base pairs in length. Similarly, the region of complementarity to the target sequence is between 15 and 30, more preferably between 18 and 25, yet more preferably between 19 and 24, and most preferably between 21 and 23 nucleotides in length. The dsRNA of the invention may further comprise one or more single-stranded nucleotide overhang(s). The dsRNA can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc. In a preferred embodiment, the HD gene is the human HD gene. In specific embodiments, the antisense strand of the dsRNA comprises the antisense sequences of Tables 1, 2, 7, 8 or 10 and the second sequence is selected from the group consisting of the sense sequences of Tables 1, 2, 7, 8 or 10.

In further embodiments, the dsRNA comprises at least one nucleotide sequence selected from the groups of sequences provided in Tables 1, 2, 7, 8 or 10. In other embodiments, the dsRNA comprises at least two sequences selected from this group, wherein one of the at least two sequences is complementary to another of the at least two sequences, and one of the at least two sequences is substantially complementary to a sequence of an mRNA generated in the expression of the HD gene. Preferably, the dsRNA comprises two oligonucleotides, wherein one oligonucleotide is described by Tables 1, 2, 7, 8 or 10 and the second oligonucleotide is described Tables 1, 2, 7, 8 or 10.

The skilled person is well aware that dsRNAs comprising a duplex structure of between 20 and 23, but specifically 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir et al., EMBO 2001, 20:6877-6888). However, others have found that shorter or longer dsRNAs can be effective as well. In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Tables 1, 2, 7, 8 or 10, the dsRNAs of the invention can comprise at least one strand of a length of minimally 21 nt. It can be reasonably expected that shorter dsRNAs comprising one of the sequences of Tables 1, 2, 7, 8 or 10 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs comprising a partial sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the sequences of Tables 1, 2, 7, 8 or 10, and differing in their ability to inhibit the expression of the HD gene in a FACS assay as described herein below by not more than 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated by the invention.

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 3 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 in the center of the region of complementarity. If the antisense strand of the dsRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to 5 nucleotides from either end, for example 5, 4, 3, 2, or 1 nucleotide from either the 5' or 3' end of the region of complementarity. For example, for a 23 nucleotide dsRNA strand which is complementary to a region of the HD gene, the dsRNA preferably does not contain any mismatch within the central 13 nucleotides. The methods described within the invention can be used to determine whether a dsRNA containing a mismatch to a target sequence is effective in inhibiting the expression of the HD gene. Consideration of the efficacy of dsRNAs with mismatches in inhibiting expression of the HD gene is important, especially if the particular region of complementarity in the HD gene is known to have polymorphic sequence variation within the population.

In one embodiment, at least one end of the dsRNA has a single-stranded nucleotide overhang of 1 to 4, 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. Such dsRNAs have improved stability and inhibitory activity, thus allowing administration at low dosages, i.e., less than 5 mg/kg body weight of the recipient per day. 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.

In yet another embodiment, the dsRNA is 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. Table 2 provides examples of modified RNAi agents of the invention.

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, such as, for example, without limitation, 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, 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. Oligonucleotides containing the locked nucleotide are described in (Koshkin, A.A., et al., *Tetrahedron* (1998), 54: 3607-3630 and Obika, S. et al., *Tetrahedron Lett.* (1998), 39: 5401-5404). Introduction of a locked nucleotide into an oligonucleotide improves the affinity for complementary sequences and increases the melting temperature by several degrees (Braasch, D.A. and D.R. Corey, *Chem. Biol.* (2001), 8:1-7).

Conjugating a ligand to a dsRNA can enhance its cellular absorption. 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. Li and coworkers report that attachment of folic acid to the 3'-terminus of an oligonucleotide resulted in an 8-fold increase in 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. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is 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. Nos. 5,138,045 and 5,218,105, drawn to polyamine conjugated oligonucleotides; U.S. Pat. No. 5,212,295, drawn to monomers for the preparation of oligonucleotides having chiral phosphorus linkages; U.S. Pat. Nos. 5,378,825 and 5,541,307, drawn to oligonucleotides having modified backbones; U.S. Pat. No. 5,386,023, drawn to backbone-modified oligonucleotides and the preparation thereof through reductive coupling; U.S. Pat. No. 5,457,191, drawn to modified nucleobases based on the 3-deazapurine ring system and methods of synthesis thereof; U.S. Pat. No. 5,459,255, drawn to modified nucleobases based on N-2 substituted purines; 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. Nos. 5,587,361 and 5,599,797, 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. 5,223,168, and U.S. Pat. No. 5,608,046, both drawn to conjugated 4'-desmethyl nucleoside analogs; U.S. Pat. Nos. 5,602,240, and 5,610,289, drawn to backbone-modified oligonucleotide analogs; U.S. Pat. Nos. 6,262,241, and 5,459,255, 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 the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

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, for example, are disclosed by Beaucage et al. (*Tetrahedron*, 1992, 48:2223-2311). Further 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.

Examples of hydroxyl protecting groups include, but are not limited to, t-butyl, t-butoxymethyl, methoxymethyl, tetrahydropyranyl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 2-trimethylsilylethyl, p-chlorophenyl, 2,4-dinitrophenyl, benzyl, 2,6-dichlorobenzyl, diphenylmethyl, p,p'-dinitrobenzhydryl, p-nitrobenzyl, triphenylmethyl, trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triphenylsilyl, benzoylformate, acetate, chloroacetate, trichloroacetate, trifluoroacetate, pivaloate, benzoate, p-phenylbenzoate, 9-fluorenylmethyl carbonate, mesylate and tosylate.

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; Verhart and Tesser, *Rec. Trav. Chim. Pays-Bas*, 1987, 107:621).

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. Universal Support 500 and Universal Support II are universal supports that are commercially available from Glen Research, 22825 Davis Drive, Sterling, Virginia. 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]; Azhayev, A.V. *Tetrahedron* **1999**, *55*, 787-800; and Azhayev and Antopolsky *Tetrahedron* **2001**, *57*, 4977-4986. 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.

3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; and 5,697,248, 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; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

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. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., *Science*, 1991, 254, 1497.

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. 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; and 5,808,027; all of which are hereby incorporated by reference.

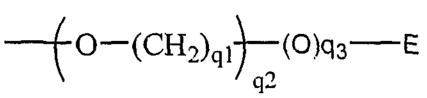
In certain embodiments, the oligonucleotides employed in the ligand-conjugated oligonucleotides of the invention may additionally or alternatively comprise one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl, O-, 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] (Martin et al., *Helv. Chim. Acta*, 1995, 78, 486), 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.

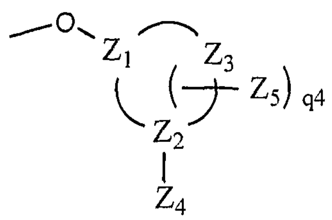
As used herein, the term "sugar substituent group" or "2'-substituent group" includes groups attached to the 2'-position of the ribofuranosyl moiety with or without an oxygen atom. Sugar substituent groups include, but are not limited to, fluoro, O-alkyl, O-alkylamino, O-alkylalkoxy, protected O-alkylamino, 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 those which are disclosed by Ouchi et al. (Drug Design and Discovery 1992, 9:93); Ravasio et al. (*J. Org. Chem.* 1991, 56:4329); and Delgardo et. al. (*Critical Reviews in Therapeutic Drug Carrier Systems* 1992, 9:249), each of which is hereby incorporated by reference in its entirety. Further sugar modifications are disclosed by Cook (*Anti-Huntingtin disease 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. See, e.g., Secrist et al., Abstract 21, *Program & Abstracts, Tenth International Roundtable, Nucleosides, Nucleotides and their Biological Applications*, Park City, Utah, Sep. 16-20, 1992.

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. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,0531 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,700,920; and 5,859,221, 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).

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

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). Representative United States patents that teach the preparation of such oligonucleotide conjugates have been listed above. 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.

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 phosphitylated.

Importantly, each of these approaches may be used for the synthesis of ligand conjugated oligonucleotides. Aminolinked 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.

III. Pharmaceutical compositions comprising dsRNA

In one embodiment, the invention provides pharmaceutical compositions comprising a dsRNA, as described in the preceding section, and a pharmaceutically acceptable carrier, as described below. The pharmaceutical composition comprising the dsRNA is useful for treating a disease or disorder associated with the expression or activity of the HD gene.

In another embodiment, the invention provides pharmaceutical compositions comprising at least two dsRNAs, designed to target different regions of the HD gene, and a pharmaceutically acceptable carrier. In this embodiment, the individual dsRNAs are prepared as described in the preceding section, which is incorporated by reference herein. One dsRNA can have a nucleotide sequence which is substantially complementary to at least one part of the HD gene; additional dsRNAs are prepared, each of which has a nucleotide sequence that is substantially complementary to different part of the HD gene. The multiple dsRNAs may be combined in the same pharmaceutical composition, or formulated separately. If formulated individually, the compositions containing the separate dsRNAs may comprise the same or different carriers, and may be administered using the same or different routes of administration. Moreover, the pharmaceutical compositions comprising the individual dsRNAs may be administered substantially simultaneously, sequentially, or at preset intervals throughout the day or treatment period.

The pharmaceutical compositions of the invention are administered in dosages sufficient to inhibit expression of the HD gene. The present inventors have found that, because of their improved efficiency, compositions comprising the dsRNA of the invention can be administered at surprisingly low dosages. A maximum dosage of 5 mg dsRNA per kilogram body weight of recipient per day is sufficient to inhibit or completely suppress expression of the HD gene.

In general, a suitable dose of dsRNA will be in the range of 0.01 to 5.0 milligrams per kilogram body weight of the recipient per day, preferably in the range of 0.1 to 200 micrograms per kilogram body weight per day, more preferably in the range of 0.1 to 100 micrograms per kilogram body weight per day, even more preferably in the range of 1.0 to 50 micrograms per kilogram body weight per day, and most preferably in the range of 1.0 to 25 micrograms per kilogram body weight per day. The pharmaceutical composition may be administered once daily, or the dsRNA may be administered as two, three, four, five, six or more sub-doses at appropriate intervals throughout the day. In that case, the dsRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, e.g., using a conventional sustained release formulation which provides sustained release of the dsRNA over a several day period. Sustained release formulations are well known in the art. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and in vivo half-lives for the individual dsRNAs encompassed by the invention can be made using conventional methodologies or on the basis of in vivo testing using an appropriate animal model, as described elsewhere herein.

Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as Huntington's disease. Such models are used for in vivo testing of dsRNA, as well as for determining a therapeutically effective dose.

The pharmaceutical compositions encompassed by the invention may be administered by any means known in the art including, but not limited to oral or parenteral routes, including intracranial (including intraparenchymal and intraventricular), intrathecal, epidural, intravenous, intramuscular, intraperitoneal, subcutaneous, transdermal, airway (aerosol), nasal, rectal, vaginal and topical (including buccal and sublingual) administration. In preferred embodiments, the pharmaceutical compositions are administered by intravenous, intrathecal or intracranial infusion or injection.

For intramuscular, intracranial, intrathecal, 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. Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride. 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 the HD gene. Such substances include, for example, micellar structures, such as liposomes or capsids, as described below. Surprisingly, the present inventors have discovered that compositions containing only naked dsRNA and a physiologically acceptable solvent are taken up by cells, where the dsRNA effectively inhibits expression of the HD gene. Although microinjection, lipofection, viruses, viroids, capsids, capsoids, or other auxiliary agents are required to introduce dsRNA into cell cultures, surprisingly these methods and agents are not necessary for uptake of dsRNA in vivo. 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. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) 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 U.S. Patent No. 4,522,811; PCT publication WO 91/06309; and European patent publication EP-A-43075, which are incorporated by reference herein.

Using the small interfering RNA vectors previously described, the invention also provides devices, systems, and methods for delivery of small interfering RNA to target locations of the brain. The envisioned route of delivery is through the use of implanted, indwelling, intraparenchymal catheters that provide a means for injecting small volumes of fluid containing the dsRNA of the invention directly into local brain tissue. Another envisioned route of delivery is through the use of implanted, indwelling, intraventricular catheters that provide a means for injecting small volumes of fluid containing the dsRNA of the invention directly into cerebrospinal fluid. The proximal end of these catheters may be connected to an implanted, intracerebral access port surgically affixed to the patient's cranium, or to an implanted drug pump located in the patient's torso.

Alternatively, implantable delivery devices, such as an implantable pump may be employed. Examples of the delivery devices within the scope of the invention include the Model 8506 investigational device (by Medtronic, Inc. of Minneapolis, Minn.), which can be implanted subcutaneously on the cranium, and provides an access port through which therapeutic agents may be delivered to the brain. Delivery occurs through a stereotactically implanted polyurethane catheter. Two models of catheters that can function with the Model 8506 access port include the Model 8770 ventricular catheter by Medtronic, Inc., for delivery to the intracerebral ventricles, which is disclosed in U.S. Pat. No. 6,093,180, incorporated herein by reference, and the IPA1 catheter by Medtronic, Inc., for delivery to the brain tissue itself (i.e., intraparenchymal delivery), disclosed in U.S. Ser. Nos. 09/540,444 and 09/625,751, which are incorporated herein by reference. The latter catheter has multiple outlets on its distal end to deliver the therapeutic agent to multiple sites along the catheter path. In addition to the aforementioned device, the delivery of the small interfering RNA vectors in accordance with the invention can be accomplished with a wide variety of devices, including but not limited to U.S. Pat. Nos. 5,735,814, 5,814,014, and 6,042,579, all of which are incorporated herein by reference. Using the teachings of the invention and those of skill in the art will recognize that these and other devices and systems may be suitable for delivery of small interfering RNA vectors for the treatment of neurodegenerative diseases in accordance with the invention.

In one such embodiment, the method further comprises the steps of implanting a pump outside the brain, the pump coupled to a proximal end of the catheter, and operating the pump to deliver the predetermined dosage of the at least one small interfering RNA or small interfering RNA vector through the discharge portion of the catheter. A further embodiment comprises the further step of periodically refreshing a supply of the at least one small interfering RNA or small interfering RNA vector to the pump outside said brain.

Thus, the invention includes the delivery of small interfering RNA vectors using an implantable pump and catheter, like that taught in U.S. Pat. No. 5,735,814 and 6,042,579, and further using a sensor as part of the infusion system to regulate the amount of small interfering RNA vectors delivered to the brain, like that taught in U.S. Pat. No. 5,814,014. Other devices and systems can be used in accordance with the method of the invention, for example, the devices and systems disclosed in U.S. Ser. Nos. 09/872,698 (filed Jun. 1, 2001) and 09/864,646 (filed May 23, 2001), which are incorporated herein by reference.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulation a range of dosage for use in humans. The dosage of compositions of the invention lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (e.g., achieving a decreased concentration of the polypeptide) that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

In addition to their administration individually or as a plurality, as discussed above, the dsRNAs of the invention can be administered in combination with other known agents effective in treatment of diseases. In any event, the administering physician can adjust the amount and timing of dsRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

Methods for treating diseases caused by expression of the HD gene

In one embodiment, the invention provides a method for treating a subject having a disease or at risk of developing a disease caused by the expression of the HD gene, or a mutant form of the HD gene. In this embodiment, the dsRNA acts as a therapeutic agent for controlling the expression of the HD protein. The method comprises administering a pharmaceutical composition of the invention to the patient (e.g., human), such that expression of the HD gene is diminished at least in part. Because of their high specificity, the dsRNAs of the invention specifically target mRNAs of the HD gene.

Neurodegenerative Diseases

Huntington's disease is also known as Huntington's Chorea, Chronic Progressive Chorea, and Hereditary Chorea. Huntington's disease is an autosomal dominant genetic disorder characterized by choreiform movements and progressive intellectual deterioration, usually beginning in middle age (35 to 50 yr). The disease affects both sexes equally. The caudate nucleus atrophies, the small-cell population degenerates, and levels of the neurotransmitters gamma-aminobutyric acid (GABA) and substance P decrease. This degeneration results in characteristic "boxcar ventricles" seen on CT scans.

The gene involved in Huntington's disease (IT-15) is located at the end of the short arm of chromosome 4. A mutation occurs in the coding region of this gene and produces an unstable expanded trinucleotide repeat (cytosine-adenosine-guanosine), resulting in a protein with an expanded glutamate sequence. The normal and abnormal functions of this protein (termed *huntingtin*) are unknown. The abnormal *huntingtin* protein appears to accumulate in neuronal nuclei of transgenic mice, but the causal relationship of this accumulation to neuronal death is uncertain.

By "*Huntingtin*" or "HD" as used herein is meant, any *Huntingtin* protein, peptide, or polypeptide associated with the development or maintenance of Huntington disease. The terms "*Huntingtin*" and "HD" also refer to nucleic acid sequences encoding any *huntingtin* protein, peptide, or polypeptide, such as *Huntingtin* RNA or *Huntingtin* DNA (see for example Van Dellen et al., Jan. 24, 2004, Neurogenetics).

Symptoms and signs develop insidiously. Dementia or psychiatric disturbances, ranging from apathy and irritability to full-blown bipolar or schizophreniform disorder, may precede the movement disorder or develop during its course. Anhedonia or asocial behavior may be the first behavioral manifestation. Motor manifestations include flicking movements of the extremities, a lilting gait, motor impersistence (inability to sustain a motor act, such as tongue protrusion), facial grimacing, ataxia, and dystonia.

Treatment for Huntington's disease is currently not available. The choreic movements and agitated behaviors may be suppressed, usually only partially, by antipsychotics (e.g., chlorpromazine 100 to 900 mg/day po or haloperidol 10 to 90 mg/day po) or reserpine begun with 0.1 mg/day po and increased until adverse effects of lethargy, hypotension, or parkinsonism occur.

Another embodiment of the present invention thus provides the use of an anti-*Huntingtin* dsRNA administered to a human, particularly the striatum of the human brain, for the treatment of Huntington's disease

The pharmaceutical compositions encompassed by the invention may be administered by any means known in the art including, but not limited to oral or parenteral routes, including intracranial (including intraparenchymal and intraventricular), intrathecal, epidural, intravenous, intramuscular, intraperitoneal, subcutaneous, transdermal, airway (aerosol), nasal, rectal, vaginal and topical (including buccal and sublingual) administration. In preferred embodiments, the pharmaceutical compositions are administered by intravenous, intrathecal or intracranial infusion or injection.

Methods for inhibiting expression of the HD gene

In yet another aspect, the invention provides a method for inhibiting the expression of the HD gene in a mammal. The method comprises administering a composition of the invention to the mammal such that expression of the target HD gene is silenced. Because of their high specificity, the dsRNAs of the invention specifically target RNAs (primary or processed) of target HD gene. Compositions and methods for inhibiting the expression of these HD genes using dsRNAs can be performed as described elsewhere herein.

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 the HD gene of the mammal to be treated. When the organism to be treated is a mammal such as a human, the composition may be administered by any means known in the art including, but not limited to oral or parenteral routes, including intracranial (including intraparenchymal and intraventricular), intrathecal, epidural, intravenous, intramuscular, intracranial, subcutaneous, transdermal, airway (aerosol), nasal, rectal, vaginal and topical (including buccal and sublingual) administration. In preferred embodiments, the compositions are administered by intravenous, intrathecal or intracranial infusion or injection.

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.

EXAMPLES

Gene Walking of the HD gene

ClustalW multiple alignment function of BioEdit Sequence Alignment Editor (version 7.0.4.1) was used to generate a global alignment of human (NM-002111), mouse (NM_010414) and rat (U18650) mRNA sequences.

Conserved regions were identified by embedded sequence analysis function of the software. Conserved regions were defined as sequence stretches with a minimum length of 19 bases for all aligned sequences containing no internal gaps. Sequence positions of conserved regions were counted according to the human sequence.

The siRNA design web interface at Whitehead Institute for Biomedical Research (http://jura.wi.mit.edu/siRNAext/) (Yuan et al., Nucl. Acids. Res. 2004 32:W130-W134) was used to identify all potential siRNAs targeting the conserved regions as well as their respective off-target hits to sequences in the human, mouse and rat RefSeq database. siRNAs satisfying the cross-reactivity criteria selected out of the candidates pool and subjected to the software embedded off-target analysis. For this, all selected siRNAs were analyzed in 3 rounds by the NCBI blast algorithm against the NCBI human, mouse and rat RefSeq database.

Blast results were downloaded and analyzed in order to extract the identity of the best off-target hit for the antisense strand as well as the positions of occurring mismatches. All siRNA candidates were ranked according to predicted properties. For this, different criteria were applied in order to identify siRNA with the following properties: targeting human, mouse and rat sequences (cross-reactivity given), absence of stretches with more than 3 Gs in a row, absence of human, mouse or rat predicted off-target hits. The siRNAs that contained the applied criteria were selected and synthesized (Tables 1 and 2).

As has been experienced by those working in the antisense field, ribonucleic acids are often quickly degraded by a range of nucleases present in virtually all biological environments, e.g. endonucleases, exonucleases etc. This vulnerability may be circumvented by chemically modifying these oligonucleotides such that nucleases may no longer attack. Consequently, siRNAs were synthesized with 2'-*O*-Methyl substitutions (Table 2) and tested for in vitro inhibitory activity on endogenous HD gene expression (HD mRNA levels).

dsRNA synthesis

Source of reagents

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.

Table 1: Sequences and activities of dsRNAs tested for HD gene expression inhibiting activity

Duplex name	sequence of total 19mer target site	SEQ ID NO:	Sense strand sequence (5'-3')	SEQ ID NO:	Antisense strand sequence (5'-3')	SEQ ID NO:	Remain-ing HD gene mRNA [% of control]
AD-10894	gaaucgagaucggauguca	1	gaaucgagaucggaugucaTT	2	ugacauccgaucucgauucTT	3	28 ± 3
AD-10895	aaauccugcuuuagucgag	4	aaauccugcuuuagucgagTT	5	cucgacuaaagcaggauuuTT	6	45 ± 4
AD-10896	agucaguccgguagaacu	7	agucaguccgguagaacuTT	8	aguucuacccggacugacuTT	9	38 ± 2
AD-10897	gguuuaugaacugacguua	10	gguuuaugaacugacguuaTT	11	uaacgucaguucauaaaccTT	12	11 ± 2
AD-10898	guuacggguuaauuacugu	13	guuacggguuaauuacuguTT	14	acaguaauuaacccguaacTT	15	28 ± 1
AD-10899	ugcuuuagucgagaaccaa	16	ugcuuuagucgagaaccaaTT	17	uugguucucgacuaaagcaTT	18	33 ± 3
AD-10900	ucuguaccguugaguccca	19	ucuguaccguugagucccaTT	20	ugggacucaacgguacagaTT	21	35 ± 3
AD-10901	aaauuguguuagacgguac	22	aaauuguguuagacgguacTT	23	guaccgucuaacacaauuuTT	24	48 ± 6
AD-10902	uggccggaaacuugcuugc	25	uggccggaaacuugcuugcTT	26	gcaagcaaguuuccggccaTT	27	46 ± 5
AD-10903	guucaguuacggguuaauu	28	guucaguuacggguuaauuTT	29	aauuaacccguaacugaacTT	30	32 ± 3
AD-10904	gcgggcucguuccaugauc	31	gcgggcucguuccaugaucTT	32	gaucauggaacgagcccgcTT	33	31 ± 1
AD-10905	gacuccgagcacuuaacgu	34	gacuccgagcacuuaacguTT	35	acguuaagugcucggagucTT	36	28 ± 3
AD-10906	cgcauggucgacauccuug	37	cgcauggucgacauccuugTT	38	caaggaugucgaccaugcgTT	39	37 ± 2
AD-10907	aagacgagauccucgcuca	40	aagacgagauccucgcucaTT	41	ugagcgaggaucucgucuuTT	42	35 ± 1
AD-10908	aagucaguccggguagaac	43	aagucaguccggguagaacTT	44	guucuacccggacugacuuTT	45	42 ± 4
AD-10909	aaggccuucauagcgaacc	46	aaggccuucauagcgaaccTT	47	gguucgcuaugaaggccuuTT	48	65 ± 4
AD-10910	aggccuucauagcgaaccu	49	aggccuucauagcgaaccuTT	50	agguucgcuaugaaggccuTT	51	23 ± 1
AD-10911	acuccgagcacuuaacgug	52	acuccgagcacuuaacgugTT	53	cacguuaagugcucggaguTT	54	42 ± 4
AD-10912	uaaaggccuucauagcgaa	55	uaaaggccuucauagcgaaTT	56	uucgcuaugaaggccuuuaTT	57	20 ± 1
AD-10913	ucugaaucgagaucggaug	58	ucugaaucgagaucggaugTT	59	cauccgaucucgauucagaTT	60	46 ± 4
AD-10914	ugaaauuguguuagacggu	61	ugaaauuguguuagacgguTT	62	accgucuaacacaauuucaTT	63	35 ± 1
AD-10915	uggcucgcauggucgacau	64	uggcucgcauggucgacauTT	65	augucgaccaugcgagccaTT	66	42 ± 5
AD-10916	aaagucaguccggguagaa	67	aaagucaguccggguagaaTT	68	uucuacccggacugacuuuTT	69	42 ± 4
AD-10917	gagugcccgugucgguucu	70	gagugcccgugucgguucuTT	71	agaaccgacacgggcacucTT	72	77 ± 8
AD-10918	ggagcucgggacggauagu	73	ggagcucgggacggauaguTT	74	acuauccgucccgagcuccTT	75	94 ± 9
AD-10919	agaaaacaagccuugccgc	76	agaaaacaagccuugccgcTT	77	gcggcaaggcuuguuuucuTT	78	43 ± 4
AD-10920	auaaucacauucguuuguu	79	auaaucacauucguuuguuTT	80	aacaaacgaaugugauuauTT	81	35 ± 4
AD-10921	ucugggcaucgcuauggaa	82	ucugggcaucgcuauggaaTT	83	uuccauagcgaugcccagaTT	84	26 ± 6
AD-10922	ggccuucauagcgaaccug	85	ggccuucauagcgaaccugTT	86	cagguucgcuaugaaggccTT	87	32 ± 12
AD-10923	cuaaaugugcucuuaggcu	88	cuaaaugugcucuuaggcuTT	89	agccuaagagcacauuuagTT	90	24 ± 8
AD-10924	guuuaugaacugacguuac	91	guuuaugaacugacguuacTT	92	guaacgucaguucauaaacTT	93	18 ± 8
AD-10925	uuuaugaacugacguuaca	94	uuuaugaacugacguuacaTT	95	uguaacgucaguucauaaaTT	96	25 ± 3

Duplex name	sequence of total 19mer target site	SEQ ID NO:	Sense strand sequence (5'-3')	SEQ ID NO:	Antisense strand sequence (5'-3')	SEQ ID NO:	Remain-ing HD gene mRNA [% of control]
AD-10926	augaacugacguuacauca	97	augaacugacguuacaucaTT	98	ugauguaacgucaguucauTT	99	20 ± 3
AD-10927	ccacaauguugugaccgga	100	ccacaauguugugaccggaTT	101	uccggucacaacauuguggTT	102	20 ± 3
AD-10928	cugguggccgaagccguag	103	cugguggccgaagccguagTT	104	cuacggcuucggccaccagTT	105	38 ± 1
AD-10929	aauuguguuagacgguacc	106	aauuguguuagacgguaccTT	107	gguaccgucuaacacaauuTT	108	39 ± 6
AD-10930	uuguguuagacgguaccga	109	uuguguuagacgguaccgaTT	110	ucgguaccgucuaacacaaTT	111	30 ± 4
AD-10931	aaaacaagccuugccgcau	112	aaaacaagccuugccgcauTT	113	augcggcaaggcuuguuuuTT	114	32 ± 3
AD-10932	aagagcuguaccguuggga	115	aagagcuguaccguugggaTT	116	ucccaacgguacagcucuuTT	117	43 ± 5
AD-10933	auaccucagguccuguuac	118	auaccucagguccuguuacTT	119	guaacaggaccugagguauTT	120	36 ± 4
AD-10934	uccugcuuuagucgagaac	121	uccugcuuuagucgagaacTT	122	guucucgacuaaagcaggaTT	123	35 ± 7
AD-10935	cauaaucacauucguuugu	124	cauaaucacauucguuuguTT	125	acaaacgaaugugauuaugTT	126	28 ± 2
AD-10936	aagcgacugucucgacaga	127	aagcgacugucucgacagaTT	128	ucugucgagacagucgcuuTT	129	29 ± 3
AD-10937	ccgagcacuuaacguggcu	130	ccgagcacuuaacguggcuTT	131	agccacguuaagugcucggTT	132	38 ± 5
AD-10938	cuggcucgcauggucgaca	133	cuggcucgcauggucgacaTT	134	ugucgaccaugcgagccagTT	135	35 ± 2
AD-10939	uugucgccggguagaaaug	136	uugucgccggguagaaaugTT	137	cauuucuacccggcgacaaTT	138	47 ± 8
AD-10940	ugcaagacucacuuagucc	139	ugcaagacucacuuaguccTT	140	ggacuaagugagucuugcaTT	141	56 ± 9
AD-10941	gaaacagugaguccggaca	142	gaaacagugaguccggacaTT	143	uguccggacucacuguuucTT	144	36 ± 4
AD-10942	aaaucccaguguuggacca	145	aaaucccaguguuggaccaTT	146	ugguccaacacugggauuuTT	147	37 ± 4
AD-10943	gcuagcuccaugcuuaagc	148	gcuagcuccaugcuuaagcTT	149	gcuuaagcauggagcuagcTT	150	47 ± 4
AD-10944	uccaugcuuaagccuaggg	151	uccaugcuuaagccuagggTT	152	cccuaggcuuaagcauggaTT	153	102 ± 12
AD-10945	ccaugcuuaagccuaggga	154	ccaugcuuaagccuagggaTT	155	ucccuaggcuuaagcauggTT	156	34 ± 5
AD-10946	ucaacagcuacacacgugu	157	ucaacagcuacacacguguTT	158	acacguguguagcuguugaTT	159	40 ± 5
AD-10947	augugugccacugcguuuu	160	augugugccacugcguuuuTT	161	aaaacgcaguggcacacauTT	162	31 ± 3
AD-10948	ugugugccacugcguuuua	163	ugugugccacugcguuuuaTT	164	uaaaacgcaguggcacacaTT	165	33 ± 1
AD-10949	ucaguccggguagaacuuc	166	ucaguccggguagaacuucTT	167	gaaguucuacccggacugaTT	168	58 ± 5
AD-10950	aguccggguagaacuucag	169	aguccggguagaacuucagTT	170	cugaaguucuacccggacuTT	171	34 ± 3
AD-10951	gauuguugcuauggagcgg	172	gauuguugcuauggagcggTT	173	ccgcuccauagcaacaaucTT	174	46 ± 7
AD-10952	acuuguuuacgaaaugucc	175	acuuguuuacgaaauguccTT	176	ggacauuucguaaacaaguTT	177	46 ± 2
AD-10953	cuuguuuacgaaaugucca	178	cuuguuuacgaaaugu ccaTT	179	uggacauuucguaaacaagTT	180	30 ± 1
AD-10954	gcuuccgcacaugccgcgg	181	gcuuccgcacaugccgcggTT	182	ccgcggcaugugcggaagcTT	183	45 ± 5
AD-10955	uaauuuuaacguaacucuu	184	uaauuuuaacguaacucuuTT	185	aagaguuacguuaaaauuaTT	186	104 ± 6
AD-10956	cuuucuaugcccguguaaa	187	cuuucuaugcccguguaaaTT	188	uuuacacgggcauagaaagTT	189	59 ± 3
AD-10957	aaagggaaggacugacgag	190	aaagggaaggacugacgagTT	191	cucgucaguccuucccuuuTT	192	84 ± 4
AD-10958	gcuggcucgcauggucgac	193	gcuggcucgcauggucgacTT	194	gucgaccaugcgagccagcTT	195	44 ± 4
AD-10959	ugacguuacaucauacaca	196	ugacguuacaucauacacaTT	197	uguguaugauguaacgucaTT	198	19 ± 3
AD-10960	acgguaccgacaaccagua	199	acgguaccgacaaccaguaTT	200	uacugguugucgguaccguTT	201	25 ± 3

Duplex name	sequence of total 19mer target site	SEQ ID NO:	Sense strand sequence (5'-3')	SEQ ID NO:	Antisense strand sequence (5'-3')	SEQ ID NO:	Remaining HD gene mRNA [% of control]
AD-10961	gguaccgacaaccaguauu	202	gguaccgacaaccaguauuTT	203	aauacugguugucgguaccTT	204	19 ± 3
AD-10962	acgagugcucaauaauguu	205	acgagugcucaauaauguuTT	206	aacauuauugagcacucguTT	207	19 ± 3
AD-10963	caucggagaguuucugucc	208	caucggagaguuucuguccTT	209	ggacagaaacucuccgaugTT	210	38 ± 5
AD-10964	gcgaaccugaagucaagcu	211	gcgaaccugaagucaagcuTT	212	agcuugacuucagguucgcTT	213	35 ± 4
AD-10965	cugaaucgagaucggaugu	214	cugaaucgagaucggauguTT	215	acauccgaucucgauucagTT	216	31 ± 2
AD-10966	cgguaccgacaaccaguau	217	cgguaccgacaaccaguauTT	218	auacugguugucgguaccgTT	219	26 ± 2
AD-10967	acugaaccgggugaucaag	220	acugaaccgggugaucaagTT	221	cuugaucacccgguucaguTT	222	43 ± 3
AD-10968	ccuugccgcaucaaaggug	223	ccuugccgcaucaaaggugTT	224	caccuuugaugcggcaaggTT	225	64 ± 9
AD-10969	cuuuggcggauugcauucc	226	cuuuggcggauugcauuccTT	227	ggaaugcaauccgccaaagTT	228	45 ± 3
AD-10970	cuguaccguugagucccaa	229	cuguaccguugagucccaaTT	230	uugggacucaacgguacagTT	231	33 ± 1
AD-10971	uguaccguugagucccaag	232	uguaccguugagucccaagTT	233	cuugggacucaacgguacaTT	234	36 ± 4
AD-10972	agucgagaaccaaugaugg	235	agucgagaaccaaugauggTT	236	ccaucauugguucucgacuTT	237	34 ± 5
AD-10973	ccgacuaccgcuggugggc	238	ccgacuaccgcuggugggcTT	239	gcccaccagcgguagucggTT	240	47 ± 7
AD-10974	auaucaccggcugcugacu	241	auaucaccggcugcugacuTT	242	agucagcagccggugauauTT	243	73 ± 6
AD-10975	ugcauaucgcugggcucaa	244	ugcauaucgcugggcucaaTT	245	uugagcccagcgauaugcaTT	246	88 ± 1
AD-10976	uuguuuacgacgugaucua	247	uuguuuacgacgugaucuaTT	248	uagaucacgucguaaacaaTT	249	66 ± 5
AD-10977	guguuagacgguaccgaca	250	guguuagacgguaccgacaTT	251	ugucgguaccgucuaacacTT	252	21 ± 2
AD-10978	cuugaacuacaucgaucau	253	cuugaacuacaucgaucauTT	254	augaucgauguaguucaagTT	255	37 ± 6
AD-10979	ggccggaaacuugcuugca	256	ggccggaaacuugcuugcaTT	257	ugcaagcaaguuuccggccTT	258	32 ± 3
AD-10980	cugucucgacagauagcug	259	cugucucgacagauagcugTT	260	cagcuaucugucgagacagTT	261	26 ± 8
AD-10981	gcaucgcuauggaacuuuu	262	gcaucgcuauggaacuuuuTT	263	aaaaguuccauagcgaugcTT	264	11 ± 2
AD-10982	acugacguuacaucauaca	265	acugacguuacaucauacaTT	266	uguaugauguaacgucaguTT	267	13 ± 4
AD-10983	cugacguuacaucauacac	268	cugacguuacaucauacacTT	269	guguaugauguaacgucagTT	270	31 ± 5
AD-10984	ugaaucgagaucggauguc	271	ugaaucgagaucggaugucTT	272	gacauccgaucucgauucaTT	273	62 ± 13
AD-10985	uagacgguaccgacaacca	274	uagacgguaccgacaaccaTT	275	ugguugucgguaccgucuaTT	276	30 ± 4
AD-10986	uugccgcaucaaaggugac	277	uugccgcaucaaaggugacTT	278	gucaccuuugaugcggcaaTT	279	68 ± 6
AD-10987	aacuacaucgaucauggag	280	aacuacaucgaucauggagTT	281	cuccaugaucgauguaguuTT	282	61 ± 5
AD-10988	uuuggcggauugcauuccu	283	uuuggcggauugcauuccuTT	284	aggaaugcaauccgccaaaTT	285	48 ± 5
AD-10989	gcuuuagucgagaaccaau	286	gcuuuagucgagaaccaauTT	287	auugguucucgacuaaagcTT	288	29 ± 3
AD-10990	uuuagucgagaaccaauga	289	uuuagucgagaaccaaugaTT	290	ucauugguucucgacuaaaTT	291	29 ± 1
AD-10991	uagucgagaaccaaugaug	292	uagucgagaaccaaugaugTT	293	caucauugguucucgacuaTT	294	36 ± 3
AD-10992	aagugucuacccaguugaa	295	aagugucuacccaguugaaTT	296	uucaacuggguagacacuuTT	297	31 ± 3
AD-10993	ucaguuacggguuaauuac	298	ucaguuacggguuaauuacTT	299	guaauuaacccguaacugaTT	300	44 ± 8
AD-10994	uuacggguuaauuacuguc	301	uuacggguuaauuacugucTT	302	gacaguaauuaacccguaaTT	303	88 ± 17
AD-10995	uacggguuaauuacugucu	304	uacggguuaauuacugucuTT	305	agacaguaauuaacccguaTT	306	65 ± 5

Duplex name	sequence of total 19mer target site	SEQ ID NO:	Sense strand sequence (5'-3')	SEQ ID NO:	Antisense strand sequence (5'-3')	SEQ ID NO:	Remaining HD gene mRNA [% of control]
AD-10996	gucucgacagauagcugac	307	gucucgacagauagcugacTT	308	gucagcuaucugucgagacTT	309	32 ± 3
AD-10997	ucucgacagauagcugaca	310	ucucgacagauagcugacaTT	311	ugucagcuaucugucgagaTT	312	34 ± 2
AD-10998	ugcgggcucguuccaugau	313	ugcgggcucguuccaugauTT	314	aucauggaacgagcccgcaTT	315	34 ± 4
AD-10999	uucagucucguugugaaaa	316	uucagucucguugugaaaaTT	317	uuuucacaacgagacugaaTT	318	37 ± 2
AD-11000	ugucgccggguagaaaugc	319	ugucgccggguagaaaugcTT	320	gcauuucuacccggcgacaTT	321	91 ± 2
AD-11001	ucggaguucaaccuaagcc	322	ucggaguucaaccuaagccTT	323	ggcuuagguugaacuccgaTT	324	70 ± 6
AD-11002	caugcuuaagccuagggau	325	caugcuuaagccuagggauTT	326	aucccuaggcuuaagcaugTT	327	37 ± 6
AD-11003	ccgcugagucuggaucucc	328	ccgcugagucuggaucuccTT	329	ggagauccagacucagcggTT	330	70 ± 12
AD-11004	ugucaacagcuacacacgu	331	ugucaacagcuacacacguTT	332	acguguguagcuguugacaTT	333	43 ± 4
AD-11005	guggccggcaacccagcug	334	guggccggcaacccagcugTT	335	cagcuggguugccggccacTT	336	40 ± 3
AD-11006	gaaagggaucgcccacugc	337	gaaagggaucgcccacugcTT	338	gcagugggcgaucccuuucTT	339	42 ± 2
AD-11007	aaagggaucgcccacugcg	340	aaagggaucgcccacugcgTT	341	cgcagugggcgaucccuuuTT	342	43 ± 2
AD-11008	cggguagaacuucagaccc	343	cggguagaacuucagacccTT	344	gggucugaaguucuacccgTT	345	33 ± 3
AD-11009	gcucgaccgcagggccuuc	346	gcucgaccgcagggccuucTT	347	gaaggcccugcggucgagcTT	348	49 ± 4
AD-11010	agcccauaucaccggcugc	349	agcccauaucaccggcugcTT	350	gcagccggugauaugggcuTT	351	46 ± 1
AD-11011	uucuaugcccguguaaagu	352	uucuaugcccguguaaaguTT	353	acuuuacacgggcauagaaTT	354	100 ± 5
AD-11012	cccuuuuagucaggagagu	355	cccuuuuagucaggagaguTT	356	acucuccugacuaaaagggTT	357	94 ± 8
AD-11013	gguuggcgacugucaugug	358	gguuggcgacugucaugugTT	359	cacaugacagucgccaaccTT	360	156 ± 10
AD-11014	acugucucgacagauagcu	361	acugucucgacagauagcuTT	362	agcuaucugucgagacaguTT	363	39 ± 5
AD-11015	uugucugacaauaugugaa	364	uugucugacaauaugugaaTT	365	uucacauauugucagacaaTT	366	21 ± 1
AD-11016	cugggcaucgcuauggaac	367	cugggcaucgcuauggaacTT	368	guuccauagcgaugcccagTT	369	25 ± 3
AD-11017	cucggaguuugcgugcugc	370	cucggaguuugcgugcugcTT	371	gcagcacgcaaacuccgagTT	372	29 ± 3
AD-11018	uguuaaaggccuucauagc	373	uguuaaaggccuucauagcTT	374	gcuaugaaggccuuuaacaTT	375	42 ± 3
AD-11019	uuaaaggccuucauagcga	376	uuaaaggccuucauagcgaTT	377	ucgcuaugaaggccuuuaaTT	378	32 ± 4
AD-11020	gccuucauagcgaaccuga	379	gccuucauagcgaaccugaTT	380	ucagguucgcuaugaaggcTT	381	26 ± 10
AD-11021	aaggcagcuucggagugac	382	aaggcagcuucggagugacTT	383	gucacuccgaagcugccuuTT	384	27 ± 2
AD-11022	agguuuaugaacugacguu	385	agguuuaugaacugacguuTT	386	aacgucaguucauaaaccuTT	387	10 ± 2
AD-11023	aacugacguuacaucauac	388	aacugacguuacaucauacTT	389	guaugauguaacgucaguuTT	390	39 ± 3
AD-11024	cacaauguugugaccggag	391	cacaauguugugaccggagTT	392	cuccggucacaacauugugTT	393	23 ± 4
AD-11025	caauguugugaccggagcc	394	caauguugugaccggagccTT	395	ggcuccggucacaacauugTT	396	25 ± 4
AD-11026	agcagcucuucagaacgcc	397	agcagcucuucagaacgccTT	398	ggcguucugaagagcugcuTT	399	74 ± 11
AD-11027	guggccgaagccguagugg	400	guggccgaagccguaguggTT	401	ccacuacggcuucggccacTT	402	32 ± 4
AD-11028	cguagugggaguauugugg	403	cguagugggaguauuguggTT	404	ccacaauacucccacuacgTT	405	26 ± 4
AD-11029	ggaguauuguggaacuuau	406	ggaguauuguggaacuuauTT	407	auaaguuccacaauacuccTT	408	20 ± 2
AD-11030	aguauuguggaacuuauag	409	aguauuguggaacuuauagTT	410	cuauaaguuccacaauacuTT	411	35 ± 3

Duplex name	sequence of total 19mer target site	SEQ ID NO:	Sense strand sequence (5'-3')	SEQ ID NO:	Antisense strand sequence (5'-3')	SEQ ID NO:	Remaining HD gene mRNA [% of control]
AD-11031	gagaucggaugucagcagc	412	gagaucggaugucagcagcTT	413	gcugcugacauccgaucucTT	414	53 ± 18
AD-11032	cagcgccgucccaucugac	415	cagcgccgucccaucugacTT	416	gucagaugggacggcgcugTT	417	49 ± 4
AD-11033	ccaccgaagggccugauuc	418	ccaccgaagggccugauucTT	419	gaaucaggcccuucgguggTT	420	28 ± 6
AD-11034	auuguguuagacgguaccg	421	auuguguuagacgguaccgTT	422	cgguaccgucuaacacaauTT	423	111 ± 12
AD-11035	ccgacaaccaguauuuggg	424	ccgacaaccaguauuugggTT	425	cccaaauacugguugucggTT	426	25 ± 5
AD-11036	aaacaagccuugccgcauc	427	aaacaagccuugccgcaucTT	428	gaugcggcaaggcuuguuuTT	429	35 ± 4
AD-11037	gccuugccgcaucaaaggu	430	gccuugccgcaucaaagguTT	431	accuuugaugcggcaaggcTT	432	36 ± 9
AD-11038	aucuugaacuacaucgauc	433	aucuugaacuacaucgaucTT	434	gaucgauguaguucaagauTT	435	40 ± 5
AD-11039	aucgaucauggagacccac	436	aucgaucauggagacccacTT	437	gugggucuccaugaucgauTT	438	69 ± 5
AD-11040	uggagacccacagguucga	439	uggagacccacagguucgaTT	440	ucgaaccugugggucuccaTT	441	39 ± 9
AD-11041	ggagacccacagguucgag	442	ggagacccacagguucgagTT	443	cucgaaccugugggucuccTT	444	65 ± 14
AD-11042	ccgcuucccacgugggagau	445	ccgcuucccacgugggagauTT	446	aucucccacguggaagcggTT	447	63 ± 2
AD-11043	ucuuuggcggauugcauuc	448	ucuuuggcggauugcauucTT	449	gaaugcaauccgccaaagaTT	450	60 ± 5
AD-11044	uuggcggauugcauuccuu	451	uuggcggauugcauuccuuTT	452	aaggaaugcaauccgccaaTT	453	30 ± 2
AD-11045	agcagcuacagugaguuag	454	agcagcuacagugaguuagTT	455	cuaacucacuguagcugcuTT	456	64 ± 2
AD-11046	cgagugcucaauaauguug	457	cgagugcucaauaauguugTT	458	caacauuauugagcacucgTT	459	18 ± 5
AD-11047	aauuaggcuugucccaaag	460	aauuaggcuugucccaaagTT	461	cuuugggacaagccuaauuTT	462	54 ± 14
AD-11048	uggaguuuagguuggcacu	463	uggaguuuagguuggcacuTT	464	agugccaaccuaaacuccaTT	465	44 ± 5
AD-11049	cuugguucccauuggaucu	466	cuugguucccauuggaucuTT	467	agauccaaugggaaccaagTT	468	32 ± 4
AD-11050	uuuuggccggaaacuugcu	469	uuuuggccggaaacuugcuTT	470	agcaaguuuccggccaaaaTT	471	53 ± 12
AD-11051	ugccuucucuaacaaaccc	472	ugccuucucuaacaaacccTT	473	ggguuuguuagagaaggcaTT	474	57 ± 5
AD-11052	uaagucccauccgacgaaa	475	uaagucccauccgacgaaaTT	476	uuucgucggaugggacuuaTT	477	43 ± 4
AD-11053	ugauaccucagguccuguu	478	ugauaccucagguccuguuTT	479	aacaggaccugagguaucaTT	480	26 ± 2
AD-11054	gauaccucagguccuguua	481	gauaccucagguccuguuaTT	482	uaacaggaccugagguaucTT	483	30 ± 5
AD-11055	uguuacaacaaguaaaucc	484	uguuacaacaaguaaauccTT	485	ggauuuacuuguuguaacaTT	486	81 ± 4
AD-11056	cuaggauaccugaaauccu	487	cuaggauaccugaaauccuTT	488	aggauuucagguauccuagTT	489	35 ± 13
AD-11057	cuuuagucgagaaccaaug	490	cuuuagucgagaaccaaugTT	491	cauugguucucgacuaaagTT	492	33 ± 6
AD-11058	acuguuuguguucaacaau	493	acuguuuguguucaacaauTT	494	auuguugaacacaaacaguTT	495	39 ± 4
AD-11059	caauuguugaagacucucu	496	caauuguugaagacucucuTT	497	agagagucuucaacaauugTT	498	39 ± 3
AD-11060	caagucacaaggccgagca	499	caagucacaaggccgagcaTT	500	ugcucggccuugugacuugTT	501	40 ± 1
AD-11061	aagucacaaggccgagcac	502	aagucacaaggccgagcacTT	503	gugcucggccuugugacuuTT	504	38 ± 5
AD-11062	ggcuuguaccacuacugcu	505	ggcuuguaccacuacugcuTT	506	agcaguagugguacaagccTT	507	27 ± 3
AD-11063	acgacaccucgggaugguu	508	acgacaccucgggaugguuTT	509	aaccaucccgaggugucguTT	510	38 ± 4
AD-11064	caccucgggaugguuugau	511	caccucgggaugguuugauTT	512	aucaaaccaucccgaggugTT	513	52 ± 11
AD-11065	cucgggaugguuugauguc	514	cucgggaugguuugaugucTT	515	gacaucaaaccaucccgagTT	516	49 ± 13

Duplex name	sequence of total 19mer target site	SEQ ID NO:	Sense strand sequence (5'-3')	SEQ ID NO:	Antisense strand sequence (5'-3')	SEQ ID NO:	Remaining HD gene mRNA [% of control]
AD-11066	agugucacaaagaaccgug	517	agugucacaaagaaccgugTT	518	cacgguucuuugugacacuTT	519	43 ± 13
AD-11067	gugucacaaagaaccgugc	520	gugucacaaagaaccgugcTT	521	gcacgguucuuugugacacTT	522	30 ± 6
AD-11068	aaccgugcagauaagaaug	523	aaccgugcagauaagaaugTT	524	cauucuuaucugcacgguuTT	525	36 ± 7
AD-11069	accgugcagauaagaaugc	526	accgugcagauaagaaugcTT	527	gcauucuuaucugcacgguTT	528	39 ± 3
AD-11070	ccgugcagauaagaaugcu	529	ccgugcagauaagaaugcuTT	530	agcauucuuaucugcacggTT	531	39 ± 3
AD-11071	gcagauaagaaugcuauuc	532	gcagauaagaaugcuauucTT	533	gaauagcauucuuaucugcTT	534	37 ± 4
AD-11072	acauucguuuguuugaacc	535	acauucguuuguuugaaccTT	536	gguucaaacaaacgaauguTT	537	62 ± 3
AD-11073	ugaaccucuuguuauaaaa	538	ugaaccucuuguuauaaaaTT	539	uuuuauaacaagagguucaTT	540	21 ± 4
AD-11074	uuuagauuugcuggcgcag	541	uuuagauuugcuggcgcagTT	542	cugcgccagcaaaucuaaaTT	543	80 ± 5
AD-11075	ugguucaguuacggguuaa	544	ugguucaguuacggguuaaTT	545	uuaacccguaacugaaccaTT	546	32 ± 13
AD-11076	gggccaguucagggaauca	547	gggccaguucagggaaucaTT	548	ugauucccugaacuggcccTT	549	30 ± 7
AD-11077	uggaagcgacugucucgac	550	uggaagcgacugucucgacTT	551	gucgagacagucgcuuccaTT	552	41 ± 5
AD-11078	ggaagcgacugucucgaca	553	ggaagcgacugucucgacaTT	554	ugucgagacagucgcuuccTT	555	30 ± 8
AD-11079	gaagcgacugucucgacag	556	gaagcgacugucucgacagTT	557	cugucgagacagucgcuucTT	558	35 ± 8
AD-11080	gcgacugucucgacagaua	559	gcgacugucucgacagauaTT	560	uaucugucgagacagucgcTT	561	35 ± 6
AD-11081	ugucucgacagauagcuga	562	ugucucgacagauagcugaTT	563	ucagcuaucugucgagacaTT	564	33 ± 4
AD-11082	cucgacagauagcugacau	565	cucgacagauagcugacauTT	566	augucagcuaucugucgagTT	567	39 ± 7
AD-11083	agguggaaaugagugagca	568	agguggaaaugagugagcaTT	569	ugcucacucauuuccaccuTT	570	27 ± 4
AD-11084	agugagcagcaacauacuu	571	agugagcagcaacauacuuTT	572	aaguauguugcugcucacuTT	573	23 ± 3
AD-11085	guuccgcagugauggcugu	574	guuccgcagugauggcuguTT	575	acagccaucacugcggaacTT	576	37 ± 4
AD-11086	caaccacaccgacuaccgc	577	caaccacaccgacuaccgcTT	578	gcgguagucggugugguugTT	579	36 ± 5
AD-11087	aaccacaccgacuaccgcu	580	aaccacaccgacuaccgcuTT	581	agcgguagucggugugguuTT	582	48 ± 10
AD-11088	accacaccgacuaccgcug	583	accacaccgacuaccgcugTT	584	cagcgguagucggugugguTT	585	42 ± 3
AD-11089	cccgaaaagacacagucug	586	cccgaaaagacacagucugTT	587	cagacugugucuuuucgggTT	588	37 ± 2
AD-11090	uccagcacaaaguuacuua	589	uccagcacaaaguuacuuaTT	590	uaaguaacuuugugcuggaTT	591	35 ± 4
AD-11091	uuggaaugugcaauagaga	592	uuggaaugugcaauagagaTT	593	ucucuauugcacauuccaaTT	594	29 ± 6
AD-11092	agaucugaucagccuuucc	595	agaucugaucagccuuuccTT	596	ggaaaggcugaucagaucuTT	597	43 ± 3
AD-11093	caggcaauucagucucguu	598	caggcaauucagucucguuTT	599	aacgagacugaauugccugTT	600	31 ± 3
AD-11094	ggcaauucagucucguugu	601	ggcaauucagucucguuguTT	602	acaacgagacugaauugccTT	603	27 ± 3
AD-11095	gcaauucagucucguugug	604	gcaauucagucucguugugTT	605	cacaacgagacugaauugcTT	606	23 ± 3
AD-11096	aauucagucucguugugaa	607	aauucagucucguugugaaTT	608	uucacaacgagacugaauuTT	609	27 ± 3
AD-11097	ucagucucguugugaaaac	610	ucagucucguugugaaaacTT	611	guuuucacaacgagacugaTT	612	42 ± 8
AD-11098	aaaccuuucaacuccaacc	613	aaaccuuucaacuccaaccTT	614	gguuggaguugaaagguuuTT	615	60 ± 7
AD-11099	cuuuccgugugcuggcucg	616	cuuuccgugugcuggcucgTT	617	cgagccagcacacggaaagTT	618	46 ± 4
AD-11100	ccgugugcuggcucgcaug	619	ccgugugcuggcucgcaugTT	620	caugcgagccagcacacggTT	621	33 ± 3

Duplex name	sequence of total 19mer target site	SEQ ID NO:	Sense strand sequence (5'-3')	SEQ ID NO:	Antisense strand sequence (5'-3')	SEQ ID NO:	Remaining HD gene mRNA [% of control]
AD-11101	ucgacauccuugcuugucg	622	ucgacauccuugcuugucgTT	623	cgacaagcaaggaugucgaTT	624	47 ± 4
AD-11102	ugcuugucgccggguagaa	625	ugcuugucgccggguagaaTT	626	uucuacccggcgacaagcaTT	627	43 ± 8
AD-11103	gcuugucgccggguagaaa	628	gcuugucgccggguagaaaTT	629	uuucuacccggcgacaagcTT	630	35 ± 7
AD-11104	cuugucgccggguagaaau	631	cuugucgccggguagaaauTT	632	auuucuacccggcgacaagTT	633	37 ± 9
AD-11105	ggcccaguugccaauggaa	634	ggcccaguugccaauggaaTT	635	uuccauuggcaacugggccTT	636	39 ± 5
AD-11106	cagguuucgucucuccacc	637	cagguuucgucucuccaccTT	638	gguggagagacgaaaccugTT	639	38 ± 8
AD-11107	ggcacgugucacuggaaac	640	ggcacgugucacuggaaacTT	641	guuuccagugacacgugccTT	642	39 ± 3
AD-11108	cuggaaacagugaguccgg	643	cuggaaacagugaguccggTT	644	ccggacucacuguuuccagTT	645	51 ± 3
AD-11109	caaaucccaguguuggacc	646	caaaucccaguguuggaccTT	647	gguccaacacugggauuugTT	648	53 ± 4
AD-11110	acucggaguucaaccuaag	649	acucggaguucaaccuaagTT	650	cuuagguugaacuccgaguTT	651	43 ± 3
AD-11111	cucggaguucaaccuaagc	652	cucggaguucaaccuaagcTT	653	gcuuagguugaacuccgagTT	654	41 ± 6
AD-11112	agccuagggaugagugaaa	655	agccuagggaugagugaaaTT	656	uuucacucaucccuaggcuTT	657	34 ± 5
AD-11113	gucaacagcuacacacgug	658	gucaacagcuacacacgugTT	659	cacguguguagcuguugacTT	660	42 ± 4
AD-11114	gauggucacccaaaccggg	661	gauggucacccaaaccgggTT	662	cccgguuugggugaccaucTT	663	49 ± 3
AD-11115	ugacagaacugcgaagggu	664	ugacagaacugcgaaggguTT	665	acccuucgcaguucugucaTT	666	53 ± 8
AD-11116	gaagacgagauccucgcuc	667	gaagacgagauccucgcucTT	668	gagcgaggaucucgucuucTT	669	43 ± 7
AD-11117	acgagauccucgcucagua	670	acgagauccucgcucaguaTT	671	uacugagcgaggaucucguTT	672	40 ± 9
AD-11118	aaccugaaagggaucgccc	673	aaccugaaagggaucgcccTT	674	gggcgaucccuuucagguuTT	675	81 ± 7
AD-11119	gaucgcccacugcgugaac	676	gaucgcccacugcgugaacTT	677	guucacgcagugggcgaucTT	678	50 ± 7
AD-11120	cacugcgugaacauucaca	679	cacugcgugaacauucacaTT	680	ugugaauguucacgcagugTT	681	40 ± 13
AD-11121	agaacuauccucuggacgu	682	agaacuauccucuggacguTT	683	acguccagaggauaguucuTT	684	41 ± 8
AD-11122	gucaguccggguagaacuu	685	gucaguccggguagaacuuTT	686	aaguucuacccggacugacTT	687	37 ± 10
AD-11123	ugaacaaagucaucggaga	688	ugaacaaagucaucggagaTT	689	ucuccgaugacuuuguucaTT	690	39 ± 6
AD-11124	aagucaucggagaguuucu	691	aagucaucggagaguuucuTT	692	agaaacucuccgaugacuuTT	693	40 ± 2
AD-11125	gucaucggagaguuucugu	694	gucaucggagaguuucuguTT	695	acagaaacucuccgaugacTT	696	37 ± 4
AD-11126	ggccaccgugguguauaag	697	ggccaccgugguguauaagTT	698	cuuauacaccacgguggccTT	699	48 ± 2
AD-11127	accgugguguauaaggugu	700	accgugguguauaagguguTT	701	acaccuuauacaccacgguTT	702	36 ± 2
AD-11128	cugacuuguuuacgaaaug	703	cugacuuguuuacgaaaugTT	704	cauuucguaaacaagucagTT	705	33 ± 7
AD-11129	uguuuacgaaauguccaca	706	uguuuacgaaauguccacaTT	707	uguggacauuucguaaacaTT	708	46 ± 8
AD-11130	ccaccgagccagcuugguc	709	ccaccgagccagcuuggucTT	710	gaccaagcuggcucgguggTT	711	51 ± 12
AD-11131	caccgagccagcuuggucc	712	caccgagccagcuugguccTT	713	ggaccaagcuggcucggugTT	714	53 ± 15
AD-11132	caggcaacgugcgugucuc	715	caggcaacgugcgugucucTT	716	gagacacgcacguugccugTT	717	46 ± 6
AD-11133	aacgugcgugucucugcca	718	aacgugcgugucucugccaTT	719	uggcagagacacgcacguuTT	720	59 ± 6
AD-11134	uuaauuuuaacguaacucu	721	uuaauuuuaacguaacucuTT	722	agaguuacguuaaaauuaaTT	723	64 ± 16
AD-11135	uuaacguaacucuuucuau	724	uuaacguaacucuuucuauTT	725	auagaaagaguuacguuaaTT	726	57 ± 6

Duplex name	sequence of total 19mer target site	SEQ ID NO:	Sense strand sequence (5'-3')	SEQ ID NO:	Antisense strand sequence (5'-3')	SEQ ID NO:	Remaining HD gene mRNA [% of control]
AD-11136	uaacguaacucuuucuaug	727	uaacguaacucuuucuaugTT	728	cauagaaagaguuacguuaTT	729	72 ± 9
AD-11137	aacguaacucuuucuaugc	730	aacguaacucuuucuaugcTT	731	gcauagaaagaguuacguuTT	732	68 ± 8
AD-11138	guaacucuuucuaugcccg	733	guaacucuuucuaugcccgTT	734	cgggcauagaaagaguuacTT	735	69 ± 10
AD-11139	uaugcccguguaaaguaug	736	uaugcccguguaaaguaugTT	737	cauacuuuacacgggcauaTT	738	102 ± 4
AD-11140	ugcccguguaaaguaugug	739	ugcccguguaaaguaugugTT	740	cacauacuuuacacgggcaTT	741	104 ± 9
AD-11141	ugagcacccgcugacauuu	742	ugagcacccgcugacauuuTT	743	aaaugucagcgggugcucaTT	744	110 ± 25
AD-11142	cacccgcugacauuuccgu	745	cacccgcugacauuuccguTT	746	acggaaaugucagcggguGTT	747	50 ± 4
AD-11143	uuuuagucaggagagugca	748	uuuuagucaggagagugcaTT	749	ugcacucuccugacuaaaaTT	750	93 ± 17
AD-11144	agccaagucauuaaaaugg	751	agccaagucauuaaaauggTT	752	ccauuuuaaugacuuggcuTT	753	62 ± 4
AD-11145	guuggcgacugucaugugg	754	guuggcgacugucauguggTT	755	ccacaugacagucgccaacTT	756	57 ± 4
AD-11146	gcccuuaagggaagcuacu	757	gcccuuaagggaagcuacuTT	758	aguagcuucccuuaagggcTT	759	74 ± 5
AD-11147	gcauaucgcugggcucaac	760	gcauaucgcugggcucaacTT	761	guugagcccagcgauaugcTT	762	61 ± 10
AD-11148	aauaugagcucauuaguaa	763	aauaugagcucauuaguaaTT	764	uuacuaaugagcucauauuTT	765	61 ± 8
AD-11149	gugcccgugucgguucuuc	766	gugcccgugucgguucuucTT	767	gaagaaccgacacgggcacTT	768	66 ± 5
AD-11150	aaugaaaccaggguagaau	769	aaugaaaccaggguagaauTT	770	auucuacccugguuucauuTT	771	101 ± 7
AD-11151	cacccagaauguagcaucu	772	cacccagaauguagcaucuTT	773	agaugcuacauucugggugTT	774	98 ± 8
AD-11152	gagcucgggacggauagua	775	gagcucgggacggauaguaTT	776	uacuauccgucccgagcucTT	777	77 ± 2
AD-11153	ugacaacugaaggcaaccu	778	ugacaacugaaggcaaccuTT	779	agguugccuucaguugucaTT	780	86 ± 3
AD-11154	caacguggaccugccuacg	781	caacguggaccugccuacgTT	782	cguaggcagguccacguugTT	783	86 ± 4
AD-11155	gacugacgagagauguaua	784	gacugacgagagauguauaTT	785	uauacaucucucgucagucTT	786	72 ± 2
AD-11156	acgagagauguauauuuaa	787	acgagagauguauauuuaaTT	788	uuaaauauacaucucucguTT	789	63 ± 3

Table 2: Sequences and activities of dsRNAs with stabilizing modifications tested for HD gene expression inhibiting activity

Duplex name	Sense strand sequence (5´-3´)	SEQ ID NO:	Antisense strand sequence (5´-3´)	SEQ ID NO:	Remaining HD gene mRNA [% of controls]
AL-DP-5996	cmumumumagumcmgagaacmcmaaumgTT	790	cmauugguucucgacumaaagTT	791	24 ± 7
AL-DP-5997	gumcmacmaaagaacmcmgumgcmagTT	792	cugcmacgguucuuugugacTT	793	21 ± 5
AL-DP-5998	umcmggagumumcmaacmcmumaagcmcmTT	794	ggcuumagguugaacuccgaTT	795	36 ± 9
AL-DP-5999	gaaaumcmcmumgcmumumumagumcmgaTT	796	ucgacumaaagcmaggauuucTT	797	20 ± 4
AL-DP-6000	umcmcmumgcmumumumagumcmgagaacmTT	798	guucucgacumaaagcmaggaTT	799	22 ± 3
AL-DP-6001	umumagumcmgagaacmcmaaumgaumTT	800	aucmauugguucucgacumaaTT	801	23 ± 7
AL-DP-6002	umagumcmgagaacmcmaaumgaumgTT	802	cmaucmauugguucucgacumaTT	803	20 ± 7
AL-DP-6003	cmumgcmumumumagumcmgagaacmcmaTT	804	ugguucucgacumaaagcmagTT	805	26 ± 4
AL-DP-6004	cmgcmumgcmacmcmgacmcmaaagaaTT	806	uucuuuggucggugcmagcgTT	807	42 ± 7
AL-DP-6005	umgcmumumumagumcmgagaacmcmaaTT	808	uugguucucgacumaaagcmaTT	809	21 ± 8
AL-DP-6006	gaacmumacmaumcmgaumcmaumggaTT	810	uccmaugaucgaugumaguucTT	811	21 ± 6
AL-DP-6007	umgaacmumacmaumcmgaumcmaumggTT	812	ccmaugaucgaugumaguucmaTT	813	21 ± 3
AL-DP-6008	cmaaagaacmcmgumgcmagaumaaTT	814	uumaucugcmacgguucuuugTT	815	21 ± 8
AL-DP-6009	cmcmcmacmumgcmgumgaacmaumumcmaTT	816	ugaauguucmacgcmagugggTT	817	22 ± 4
AL-DP-6010	umumumagumcmgagaacmcmaaumgaTT	818	ucmauugguucucgacumaaaTT	819	31 ± 5
AL-DP-6011	umggaaumgumumcmcmggagaaumcmTT	820	gauucuccggaacmauuccmaTT	821	26 ± 4
AL-DP-6012	cmggagumumcmaacmcmumaagcmcmumTT	822	aggcuumagguugaacuccgTT	823	28 ± 6
AL-DP-6013	umggcmaumumumgaumcmcmaumgagcmTT	824	gcucmauggaucmaaaugccmaTT	825	34 ± 11
AL-DP-6014	umcmumggaaumgumumcmcmggagaaTT	826	uucuccggaacmauuccmagaTT	827	23 ± 7
AL-DP-6015	ggcmumgcmaaaumumumacmagagcmTT	828	gcucugumaaauuugcmagccTT	829	29 ± 5
AL-DP-6016	gcmgumgaacmaumumcmacmagcmcmaTT	830	uggcugugaauguucmacgcTT	831	17 ± 5
AL-DP-6017	umcmcmaggumumumaumgaacmumgacmTT	832	gucmaguucmaumaaaccuggaTT	833	19 ± 5
AL-DP-6018	aggcmaaagumgcmumcmumumaggaTT	834	uccumaagagcmacuuugccuTT	835	22 ± 6
AL-DP-6019	aacmumacmaumcmgaumcmaumggagTT	836	cuccmaugaucgaugumaguuTT	837	59 ± 10

Duplex name	Sense strand sequence (5´-3´)	SEQ ID NO:	Antisense strand sequence (5´-3´)	SEQ ID NO:	Remaining HD gene mRNA [% of controls]
AL-DP-6020	cmaumumggaaumumcmcmumaaaaumcmTT	838	gauuuumaggaauuccmaaugTT	839	19 ± 11
AL-DP-6021	aumcmcmumgcmumumumagumcmgagaaTT	840	uucucgacumaaagcmaggauTT	841	35 ± 9
AL-DP-6022	acmumacmaumcmgaumcmaumggagaTT	842	ucuccmaugaucgaugumaguTT	843	35 ± 18
AL-DP-6023	aaumcmcmumgcmumumumagumcmgagaTT	844	ucucgacumaaagcmaggauuTT	845	26 ± 16
AL-DP-6024	umgumcmcmaggumumumaumgaacmumgTT	846	cmaguucmaumaaaccuggacmaTT	847	16 ± 5
AL-DP-6025	cmumcmggagumumcmaacmcmumaagcmTT	848	gcuumagguugaacuccgagTT	849	24 ± 6
AL-DP-6026	umgaaaumcmcmumgcmumumumagumcmgTT	850	cgacumaaagcmaggauuucmaTT	851	21 ± 6
AL-DP-6027	cmagcmumumgumcmcmaggumumumaumgTT	852	cmaumaaaccuggacmaagcugTT	853	22 ± 6
AL-DP-6028	cmgumgaacmaumumcmacmagcmcmagTT	854	cuggcugugaauguucmacgTT	855	33 ± 11
AL-DP-6029	cmumggcmumcmgcmaumggumcmgacmaTT	856	ugucgaccmaugcgagccmagTT	857	45 ± 15
AL-DP-6030	agcmumumgumcmcmaggumumumaumgaTT	858	ucmaumaaaccuggacmaagcuTT	859	75 ± 15
AL-DP-6031	ggcmaaagumgcmumcmumumaggagTT	860	cuccumaagagcmacuuugccTT	861	28 ± 10
AL-DP-6032	gaumcmaumumggaaumumcmcmumaaaTT	862	uuumaggaauuccmaaugaucTT	863	25 ± 10
AL-DP-6033	cmacmumgcmgumgaacmaumumcmacmaTT	864	ugugaauguucmacgcmagugTT	865	24 ± 3
AL-DP-6034	gumcmgagaacmcmaaumgaumggcmTT	866	gccmaucmauugguucucgacTT	867	20 ± 1
AL-DP-6035	cmumumgumcmcmaggumumumaumgaacmTT	868	guucmaumaaaccuggacmaagTT	869	28 ± 9
AL-DP-6036	umgumgaumggcmaumcmaumggcmcmaTT	870	uggccmaugaugccmaucmacmaTT	871	50 ± 14
AL-DP-6037	cmacmaaagaacmcmgumgcmagaumTT	872	aucugcmacgguucuuugugTT	873	20 ± 5

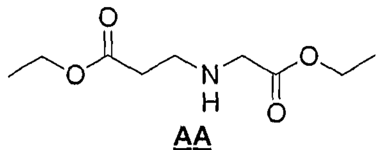
siRNA synthesis

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.

For the synthesis of 3'-cholesterol-conjugated siRNAs (herein referred to as -Chol or -sChol, depending on whether the link to the cholesteryl group is effected via a phosphodiester or a phosporothioate diester group), an appropriately modified solid support was used for RNA synthesis. The modified solid support was prepared as follows:

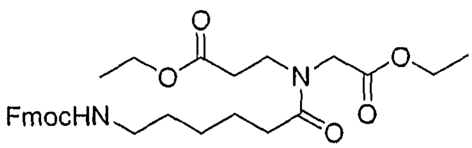
Diethyl-2-azabutane-1,4-dicarboxylate <u>AA</u>



<u>AA</u>

A 4.7 M aqueous solution of sodium hydroxide (50 mL) was added into a stirred, ice-cooled solution of ethyl glycinate hydrochloride (32.19 g, 0.23 mole) in water (50 mL). Then, ethyl acrylate (23.1 g, 0.23 mole) was added and the mixture was stirred at room temperature until completion of the reaction was ascertained by TLC. After 19 h the solution was partitioned with dichloromethane (3 x 100 mL). The organic layer was dried with anhydrous sodium sulfate, filtered and evaporated. The residue was distilled to afford AA (28.8 g, 61%).

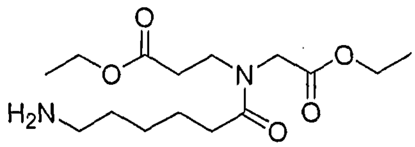
3-{Ethoxycarbonylmethyl-[6-(9H-fluoren-9-ylmethoxycarbonyl-amino)-hexanoyl]-amino}-propionic acid ethyl ester **AB**



<u>AB</u>

Fmoc-6-amino-hexanoic acid (9.12 g, 25.83 mmol) was dissolved in dichloromethane (50 mL) and cooled with ice. Diisopropylcarbodiimde (3.25 g, 3.99 mL, 25.83 mmol) was added to the solution at 0°C. It was then followed by the addition of Diethyl-azabutane-1,4-dicarboxylate (5 g, 24.6 mmol) and dimethylamino pyridine (0.305 g, 2.5 mmol). The solution was brought to room temperature and stirred further for 6 h. Completion of the reaction was ascertained by TLC. The reaction mixture was concentrated under vacuum and ethyl acetate was added to precipitate diisopropyl urea. The suspension was filtered. The filtrate was washed with 5% aqueous hydrochloric acid, 5% sodium bicarbonate and water. The combined organic layer was dried over sodium sulfate and concentrated to give the crude product which was purified by column chromatography (50 % EtOAC/Hexanes) to yield 11.87 g (88%) of AB.

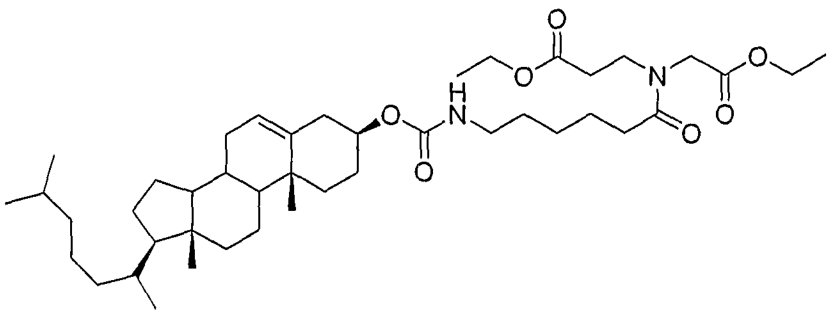
3-[(6-Amino-hexanoyl)-ethoxycarbonylmethyl-amino]-propionic acid ethyl ester **AC**



AC

3-{Ethoxycarbonylmethyl-[6-(9H-fluoren-9-ylmethoxycarbonylamino)-hexanoyl]-amino}-propionic acid ethyl ester AB (11.5 g, 21.3 mmol) was dissolved in 20% piperidine in dimethylformamide at 0°C. The solution was continued stirring for 1 h. The reaction mixture was concentrated under vacuum, water was added to the residue, and the product was extracted with ethyl acetate. The crude product was purified by conversion into its hydrochloride salt.

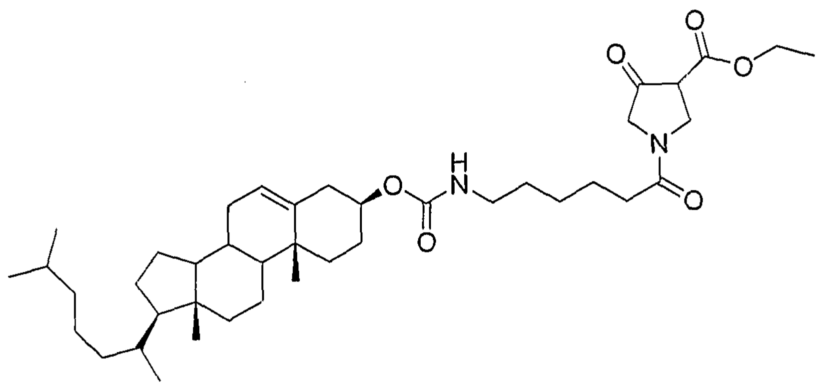
3-({6-[17-(1,5-Dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxycarbonylamino]-hexanoyl}ethoxycarbonylmethyl-amino)-propionic acid ethyl ester **AD**



AD

The hydrochloride salt of 3-[(6-Amino-hexanoyl)-ethoxycarbonylmethyl-amino]-propionic acid ethyl ester AC (4.7 g, 14.8 mmol) was taken up in dichloromethane. The suspension was cooled to 0°C on ice. To the suspension diisopropylethylamine (3.87 g, 5.2 mL, 30 mmol) was added. To the resulting solution cholesteryl chloroformate (6.675 g, 14.8 mmol) was added. The reaction mixture was stirred overnight. The reaction mixture was diluted with dichloromethane and washed with 10% hydrochloric acid. The product was purified by flash chromatography (10.3 g, 92%).

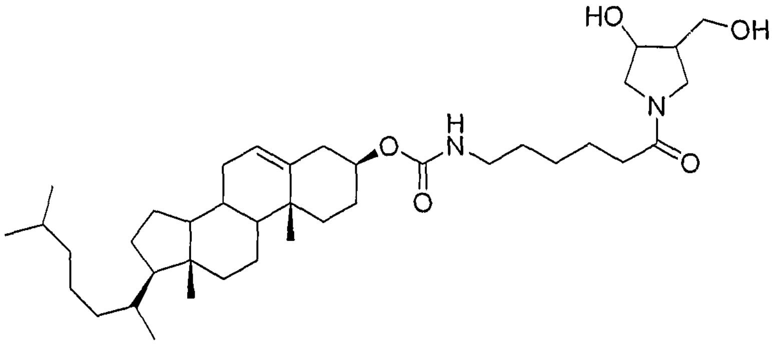
1-{6-[17-(1,5-Dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a] phenanthren-3-yloxycarbonylamino]-hexanoyl}-4-oxo-pyrrolidine-3-carboxylic acid ethyl ester **AE**



AE

Potassium t-butoxide (1.1 g, 9.8 mmol) was slurried in 30 mL of dry toluene. The mixture was cooled to 0°C on ice and 5 g (6.6 mmol) of diester AD was added slowly with stirring within 20 mins. The temperature was kept below 5°C during the addition. The stirring was continued for 30 mins at 0°C and 1 mL of glacial acetic acid was added, immediately followed by 4 g of $NaH_2PO_4 \cdot H_2O$ in 40 mL of water The resultant mixture was extracted twice with 100 mL of dichloromethane each and the combined organic extracts were washed twice with 10 mL of phosphate buffer each, dried, and evaporated to dryness. The residue was dissolved in 60 mL of toluene, cooled to 0°C and extracted with three 50 mL portions of cold pH 9.5 carbonate buffer. The aqueous extracts were adjusted to pH 3 with phosphoric acid, and extracted with five 40 mL portions of chloroform which were combined, dried and evaporated to dryness. The residue was purified by column chromatography using 25% ethylacetate/hexane to afford 1.9 g of b-ketoester (39%).

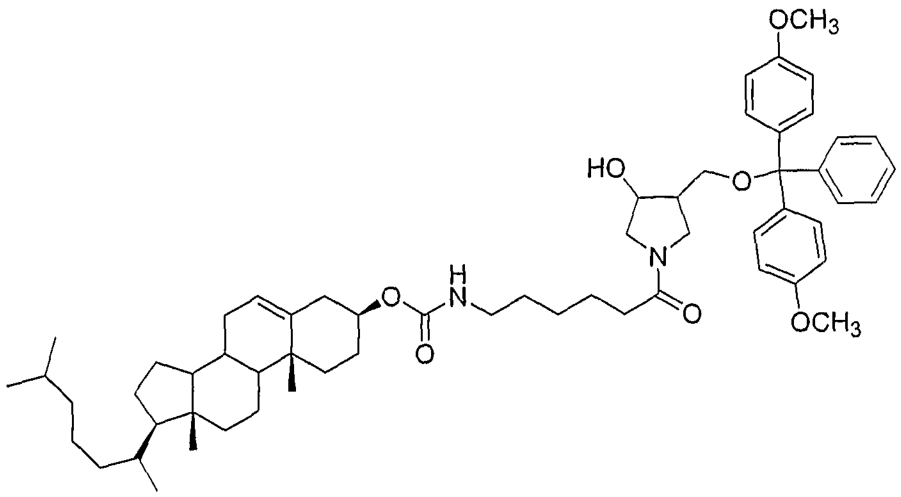
[6-(3-Hydroxy-4-hydroxymethyl-pyrrolidin-1-yl)-6-oxo-hexyl]-carbamic acid 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester **AF**



AF

Methanol (2 mL) was added dropwise over a period of 1 h to a refluxing mixture of b-ketoester AE (1.5 g, 2.2 mmol) and sodium borohydride (0.226 g, 6 mmol) in tetrahydrofuran (10 mL). Stirring was continued at reflux temperature for 1 h. After cooling to room temperature, 1 N HCl (12.5 mL) was added, the mixture was extracted with ethylacetate (3 x 40 mL). The combined ethylacetate layer was dried over anhydrous sodium sulfate and concentrated under vacuum to yield the product which was purified by column chromatography (10% MeOH/$CHCl_3$) (89%).

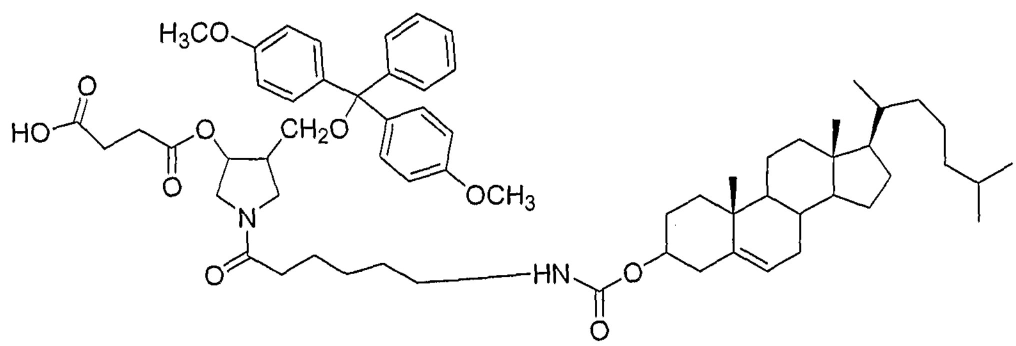
(6-{3-[Bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-4-hydroxy-pyrrolidin-1-yl}-6-oxo-hexyl)-carbamic acid 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester **AG**



AG

Diol AF (1.25 gm 1.994 mmol) was dried by evaporating with pyridine (2 x 5 mL) *in vacuo*. Anhydrous pyridine (10 mL) and 4,4'-dimethoxytritylchloride (0.724 g, 2.13 mmol) were added with stirring. The reaction was carried out at room temperature overnight. The reaction was quenched by the addition of methanol. The reaction mixture was concentrated under vacuum and to the residue dichloromethane (50 mL) was added. The organic layer was washed with 1M aqueous sodium bicarbonate. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residual pyridine was removed by evaporating with toluene. The crude product was purified by column chromatography (2% MeOH/Chloroform, Rf = 0.5 in 5% MeOH/$CHCl_3$) (1.75 g, 95%).

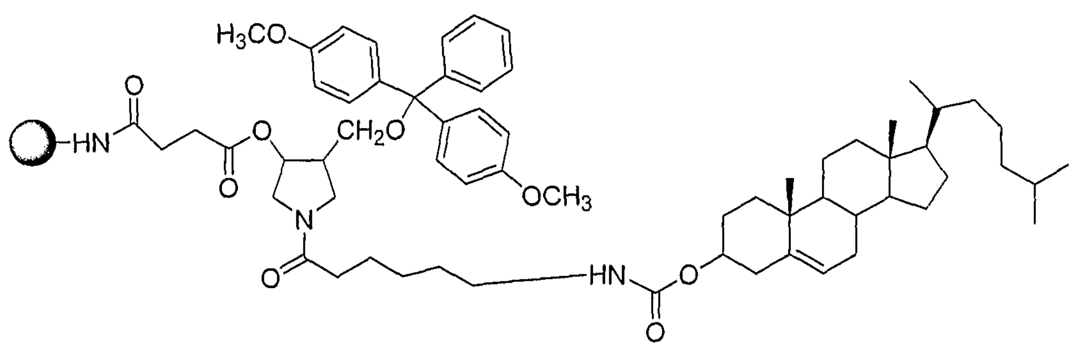
Succinic acid mono-(4-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-1-{6-[17-(1,5-dimethyl-hexyl)-10,13-dimethyl 2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H cyclopenta[a]phenanthren-3-yloxycarbonylamino]-hexanoyl}-pyrrolidin-3-yl) ester **AH**



AH

Compound AG (1.0 g, 1.05 mmol) was mixed with succinic anhydride (0.150 g, 1.5 mmol) and DMAP (0.073 g, 0.6 mmol) and dried in a vacuum at 40°C overnight. The mixture was dissolved in anhydrous dichloroethane (3 mL), triethylamine (0.318 g, 0.440 mL, 3.15 mmol) was added and the solution was stirred at room temperature under argon atmosphere for 16 h. It was then diluted with dichloromethane (40 mL) and washed with ice cold aqueous citric acid (5 wt%, 30 mL) and water (2 X 20 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated to dryness. The residue was used as such for the next step.

Cholesterol derivatised CPG **AI**



AI

Succinate AH (0.254 g, 0.242 mmol) was dissolved in a mixture of dichloromethane/acetonitrile (3:2, 3 mL). To that solution DMAP (0.0296 g, 0.242 mmol) in acetonitrile (1.25 mL), 2,2'-Dithio-bis(5-nitropyridine) (0.075 g, 0.242 mmol) in acetonitrile/dichloroethane (3:1, 1.25 mL) were added successively. To the resulting solution triphenylphosphine (0.064 g, 0.242 mmol) in acetonitrile (0.6 ml) was added. The reaction mixture turned bright orange in color. The solution was agitated briefly using a wrist-action shaker (5 mins). Long chain alkyl amine-CPG (LCAA-CPG) (1.5 g, 61 mM) was added. The suspension was agitated for 2 h. The CPG was filtered through a sintered funnel and washed with acetonitrile, dichloromethane and ether successively. Unreacted amino groups were masked using acetic anhydride/pyridine. The achieved loading of the CPG was measured by taking UV measurement (37 mM/g).

The synthesis of siRNAs bearing a 5'-12-dodecanoic acid bisdecylamide group (herein referred to as "5'-C32-") or a 5'-cholesteryl derivative group (herein referred to as "5'-Chol-") was performed as described in WO 2004/065601, except that, for the cholesteryl derivative, the oxidation step was performed using the Beaucage reagent in order to introduce a phosphorothioate linkage at the 5'-end of the nucleic acid oligomer.

Nucleic acid sequences are represented below using standard nomenclature, and specifically the abbreviations of Table 3.

Table 3: Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation[a]	Nucleotide(s)
A, a	2'-deoxy-adenosine-5'-phosphate, adenosine-5'-phosphate
C, c	2'-deoxy-cytidine-5'-phosphate, cytidine-5'-phosphate
G, g	2'-deoxy-guanosine-5'-phosphate, guanosine-5'-phosphate
T, t	2'-deoxy-thymidine-5'-phosphate, thymidine-5'-phosphate
U, u	2'-deoxy-uridine-5'-phosphate, uridine-5'-phosphate
N, n	any 2'-deoxy-nucleotide/nucleotide (G, A, C, or T, g, a, c or u)
Am	2'-*O*-methyladenosine-5'-phosphate
Cm	2'-*O*-methylcytidine-5'-phosphate
Gm	2'-*O*-methylguanosine-5'-phosphate
Tm	2'-*O*-methyl-thymidine-5'-phosphate

Abbreviation[a]	Nucleotide(s)
Um	*2'-O*-methyluridine-5'-phosphate
Af	2'-fluoro-2'-deoxy-adenosine-5'-phosphate
Cf	2'-fluoro-2'-deoxy-cytidine-5'-phosphate
Gf	2'-fluoro-2'-deoxy-guanosine-5'-phosphate
Tf	2'-fluoro-2'-deoxy-thymidine-5'-phosphate
Uf	2'-fluoro-2'-deoxy-uridine-5'-phosphate
A, C, G, T, U, a, c, g, t, u	underlined: nucleoside-5'-phosphorothioate
am, cm, gm, tm, um	underlined: 2-*O*-methyl-nucleoside-5'-phosphorothioate

[a]capital letters represent 2'-deoxyribonucleotides (DNA), lower case letters represent ribonucleotides (RNA)

Screen of HD dsRNAs against endogenous human HD mRNA expression in HeLa cells

HeLa cells were obtained from American Type Culture Collection (Rockville, MD) and cultured in Ham's F12 (Biochrom AG, Berlin, Germany) supplemented to contain 10% fetal calf serum (FCS) (Biochrom AG, Berlin, Germany), Penicillin 100 U/ml, Streptomycin 100 μg/ml (Biochrom AG, Berlin, Germany) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator (Heraeus HERAcell, Kendro Laboratory Products, Langenselbold, Germany).

For transfection with siRNA, HeLa cells were seeded at a density of 2.0 x 10^4 cells/well in 96-well plates and transfected directly. Transfection of siRNA (30nM for single dose screen) was carried out with oligofectamine (Invitrogen GmbH, Karlsruhe, Germany) as described by the manufacturer. For dose-response curves, siRNA concentrations ranged from 30 nM to 14 pM in 3-fold dilutions.

24 hours after transfection, HeLa cells were lysed and Huntingtin mRNA levels were quantified with the Quantigene Explore Kit (Genosprectra, Dumbarton Circle Fremont, USA) according to the protocol. Huntingtin mRNA levels were normalized to GAPDH mRNA. For each siRNA, four individual datapoints were collected. An siRNA duplex unrelated to the HD

gene was used as a control ('VEGF ctrl'). The activity of a given HD-specific siRNA duplex was expressed as percent HD mRNA concentration in treated cells relative to huntingtin mRNA concentration in cells treated with the control siRNA duplex.

Table 1 provides the results from four independent experiments of the *in vitro* HeLa screen where the siRNAs, the sequences of which are given in Table 1, were tested at a single dose of 30 nM. The percentage of HD mRNA remaining in treated cells compared to controls, ± standard deviation, is indicated in the rightmost column of Table 1. Figure 1 provides a graph of the results from two independent experiments of the *in vitro* HeLa screen where siRNAs, the sequences of which are given in Table 2, were tested at a single dose of 30 nM. In Table 2, duplex names are given as AL-DP-xxxx whereas the same duplex in Figure 1 is indicated by 'xxxx' only. For instance, AL-DP-5997 in Table 2 corresponds to '5997' in Figure 1. Again, the percentage of HD mRNA remaining in treated cells compared to controls, ± standard deviation, is indicated in the rightmost column of Table 2. A number of siRNAs at 30 nM were effective at reducing HD mRNA levels by more than 70% in HeLa cells.

Table 4 provides the IC50, IC80 and maximum inhibition values from two to five independent experiments for 25 selected siRNAs. Several siRNAs (AL-DP-5997, AL-DP-6000, AL-DP-6001, AL-DP-6014, AL-DP-6020 and AL-DP-6032, indicated by *) were particularly potent in this experimental paradigm, and exhibited IC50 values between 10 and 130 pM.

Table 4

Duplex name	IC-50 mean [nM] ± SD	IC-80 mean [nM] ± SD	max. inhib. mean[%] ± SD
AL-DP-5996	1.6 ± 1.2	22 ± 9	79 ± 6
AL-DP-5997*	0.05 ± 0.02	2 ± 1	86 ± 5
AL-DP-5999	0.3 ± 0.3	8 ± 4	82 ± 4
AL-DP-6000*	0.1 ± 0.1	5 ± 3	80 ± 2
AL-DP-6001*	0.1 ± 0.1	3 ± 1	83 ± 1
AL-DP-6002	0.3 ± 0.2	9 ± 4	78 ± 3
AL-DP-6003	0.3 ± 0.2	3 ± 2	83 ± 3
AL-DP-6005	0.3 ± 0.3	9 ± 9	77 ± 7

Duplex name	IC-50 mean [nM] ± SD	IC-80 mean [nM] ± SD	max. inhib. mean[%] ± SD
AL-DP-6006	0.5 ± 0.1	8 ± 5	81 ± 2
AL-DP-6007	0.2 ± 0.1	5 ± 3	77 ± 8
AL-DP-6008	0.16	13.56	75
AL-DP-6014*	0.1 ± 0.1	6 ± 3	81 ± 6
AL-DP-6016	0.2 ± 0.3	8 ± 10	81 ± 8
AL-DP-6017	0.4 ± 0.1	5 ± 4	82 ± 2
AL-DP-6018	0.2 ± 0.04	7 ± 1	81 ± 3
AL-DP-6020*	0.009 ± 0.01	1 ± 1	88 ± 5
AL-DP-6024	0.3 ± 0.1	6 ± 4	88 ± 1
AL-DP-6025	0.3 ± 0.3	11 ± 8	80 ± 1
AL-DP-6026	0.2 ± 0.2	5 ± 4	81 ± 4
AL-DP-6027	0.5 ± 0.1	8 ± 6	81 ± 2
AL-DP-6032*	0.016 ± 0.01	3 ± 5	87 ± 7
AL-DP-6033	0.3 ± 0.2	6 ± 2	78 ± 3
AL-DP-6034	0.7 ± 0.03	10 ± 3	77 ± 4
AL-DP-6035	0.8 ± 0.9	7 ± 5	80 ± 11
AL-DP-6037	0.2 ± 0.1	8 ± 7	79 ± 6

Screen of selected HD dsRNAs against endogenous HD mRNA expression in Neuroscreen and U87MG cells

Neuroscreen cells (a PC12 sub-clone) were obtained from Cellomics (Pittsburgh, PA) and cultured in RPMI 1640 (Biochrom AG, Berlin, Germany) supplemented to contain 5% fetal calf serum (FCS) (Biochrom AG, Berlin, Germany), 10% DHS (Biochrom AG, Berlin, Germany), Penicillin 100 U/ml, Streptomycin 100 μg/ml (Biochrom AG, Berlin, Germany) and 2mM L-glutamine (Biochrom AG, Berlin, Germany) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator (Heraeus HERAcell, Kendro Laboratory Products, Langenselbold, Germany).

U87MG cells were obtained from American Type Culture Collection (Rockville, MD) and cultured in Ham's F12 (Biochrom AG, Berlin, Germany) supplemented to contain 10% fetal calf serum (FCS) (Biochrom AG, Berlin, Germany), Penicillin 100 U/ml, Streptomycin 100 μg/ml (Biochrom AG, Berlin, Germany) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator (Heraeus HERAcell, Kendro Laboratory Products, Langenselbold, Germany).

Transfection of Neuroscreen and U87MG cells with six selected siRNAs (AL-DP-5997, AL-DP-6000, AL-DP-6001, AL-DP-6014, AL-DP-6020 and AL-DP-6032), and quantitation of Huntingtin and GAPDH mRNA levels with the Quantigene Explore Kit were performed in a similar manner to that described for HeLa cells.

IC50 values are provided in Table 5. In both Neuroscreen (rat) and U87MG (human) cells, IC50s were higher than in HeLa cells, in general. Of the six siRNAs tested, AL-DP-6014 was significantly less potent than the other five siRNAs (AL-DP-5997, AL-DP-6000, AL-DP-6001, AL-DP-6020 and AL-DP-6032) against HD mRNA in Neuroscreen cells, whereas AL-DP-6000 was significantly less potent than the other five siRNAs (AL-DP-5997, AL-DP-6001, AL-DP-6014, AL-DP-6020 and AL-DP-6032) against HD mRNA in U87MG cells.

Table 5.

Duplex name	Neuroscreen IC50 mean [nM] +/- SD	U87MG IC50 mean [nM]
AL-DP-5997	6 ± 2.8	2.7
AL-DP-6000	11.7 ± 10	98
AL-DP-6001	18	0.28
AL-DP-6014	264 ± 180	0.47
AL-DP-6020	1.42 ± 0.2	0.17
AL-DP-6032	4.2 ± 2.2	0.49

dsRNAs targeting HD reduce endogenous HD protein in HeLa cells

Hela cells were cultured and transfected as previously described with 100 nM of the indicated siRNAs, including six siRNAs against HD (AL-DP-5997, AL-DP-6000, AL-DP-6001, AL-DP-6014, AL-DP-6020 and AL-DP-6032) and one control unrelated siRNA ('ctrl'). 48 hours post-transfection, the cells were harvested and lysed. Proteins in the lysates were separated on an 8% denaturing PAG. Huntingtin and β-actin were detected by standard western blot protocols using antibodies that bind to the proteins. For Huntingtin detection, the membrane was probed with a mouse anti-huntingtin protein monoclonal antibody (Chemicon, U.K.) followed by a horseradish peroxidase-coupled goat anti-mouse secondary antibody (Santa Cruz Biotechnology, California). β-actin was detected by anti-actin goat polyclonal IgG (Santa Cruz, California) followed by a donkey anti-goat Ig-HRP secondary antibody (Santa Cruz, California).

Figure 2 provides the results. AL-DP-5997 ('5997'), AL-DP-6000 ('6000'), AL-DP-6001 ('6001'), AL-DP-6014 ('6014'), AL-DP-6020 ('6020') and AL-DP-6032 ('6032'), all at 100 nM, decreased the level of Huntingtin protein relative to the control protein β-actin, whereas the control unrelated siRNA ('ctrl') had no effect on the level of either protein. These results demonstrate that dsRNAs targeting HD effectively reduce not only HD mRNA levels, but also HD protein levels.

Stability in cerebrospinal fluid (CSF) of selected dsRNAs targeting HD

Six selected siRNAs (AL-DP-5997, AL-DP-6000, AL-DP-6001, AL-DP-6014, AL-DP-6020 and AL-DP-6032) were tested for stability at 5 uM over 48h at 37°C in calf and swine CSF, as well as in PBS for comparison. The incubations in CSF were stopped at 1, 2, 4, 8, 24 and 48 hours by proteinase digestion, whereas the incubation in PBS was stopped at 0 and 48 hours. Filtered samples were injected onto the IEX-HPLC under denaturing conditions, and percent recovery of each single strand was determined by measuring the area under the corresponding peak, and expressing this area relative to that obtained at 0 hours in PBS. Figure 3 and Table 6 provide the results. At least 90% of both sense and antisense strands of AL-DP-5997, AL-DP-6000 and AL-DP-6014 were recovered in both calf and swine CSF (Table 6). In contrast, although 92% of the antisense strand of AL-DP-6001 was recovered in calf CSF, only 73% of the antisense strand was recovered in swine CSF. For AL-DP-6020 and AL-DP-6032, at least 19% of the antisense strand was not recoverable in both calf and swine CSF.

Table 6.

	% full length material after 48 hours			
	calf		swine	
AL-DP	sense	antisense	sense	antisense
5997	103	99	95	101
6000	114	101	114	97
6001	100	92	100	73
6014	91	90	90	94
6020	113	68	104	32
6032	95	21	103	81

The following cleavage sites for AL-DP-6020 and AL-DP-6032 were mapped by comparing the calculated theoretical masses of all probable fragments of both strands with the experimental masses found by MALDI-TOF. For the antisense strand of AL-DP-6020, the fragment 5'-gauuuumaggaauuccmaau-cyclic-PO_4-3' (SEQ ID NO: 874) corresponds to 3'-(n-3) based on the calculated mass of 5973.5 Da, and experimental mass of 5973.0 Da. For the antisense strand of AL-DP-6032, the fragment 5'-uumaggaauuccmaaugaucTT-3' (SEQ ID NO: 875) corresponds to 5'-(n-1) based on the calculated mass of 6355.0 Da, and experimental mass of 6355.6 Da. Given these cleavage sites, 2 new duplexes were designed with additional chemical stabilization that comprises one additional 2'-OMe group (Table 7): AL-DP-7100 (parent is AL-DP-6020) and AL-DP-7101 (parent is AL-DP-6032).

Table 7: Sequences and Modifications of Further Stabilized dsRNAs AL-DP-7100 and AL-DP-7101

Duplex name	Sense strand sequence (5´-3´)	SEQ ID NO:	Antisense strand sequence (5´-3´)	SEQ ID NO:
Al-DP-7100	cmaumumggaaumumcmcmumaaaaumcmTT	876	gauuuumaggaauuccmaaumgTT	877
Al-DP-7101	gaumcmaumumggaaumumcmcmumaaaTT	878	umuumaggaauuccmaaugaucTT	879

Four selected dsRNAs (AL-DP-5997, AL-DP-6000, AL-DP-6001 and AL-DP-7100) were tested for long-term stability at 5 uM over 14 days at 37°C in rat CSF, as well as in PBS for comparison. The incubations in CSF were carried out for 0, 1, 3, 5, 7, 10, or 14 days whereas the incubation in PBS was carried out for 14 days. Samples were processed as described above. Figure 4 shows the results. For AL-DP-6000, the 14 day CSF stability timepoint is not available, for technical reasons. All four dsRNAs are highly stable for 10 to 14 days at 37°C in rat CSF, with ≤ 30% loss of antisense or sense strands.

Potency of cholesterol-conjugated dsRNAs targeting HD against endogenous human HD mRNA expression in HeLa cells

Previous studies [Soutschek et al., 2004] had demonstrated a beneficial effect of cholesterol conjugation on cellular uptake and/or efficacy of siRNA *in vivo*. We synthesized dsRNAs AL-DP-6982, AL-DP-6983 and AL-DP-7130 (Table 8) which are cholesterol-conjugated versions of AL-DP-5997, AL-DP-6000 and AL-DP-7100, respectively, in order to evaluate their biological activities *in vitro* and *in vivo*. Hela cells were cultured and transfected as previously described, with dsRNAs AL-DP-6982, AL-DP-6983, AL-DP-7130, AL-DP-5997, AL-DP-6000, and AL-DP-7100 at concentrations ranging from 30 nM to 14 pM.

Table 8: Sequences of Cholesterol-Conjugated dsRNAs AL-DP-6982, AL-DP-6983 and AL-DP-7130

Duplex name	Sense strand sequence (5´-3´)	SEQ ID NO:	Antisense strand sequence (5´-3´)	SEQ ID NO:
AL-DP-6982	gumcmacmaaagaacmcmgumgcmagTT-sChol	880	cugcmacgguucuuugugacTT	881
AL-DP-6983	umcmcmumgcmumumumagumcmgagaacmTT-sChol	882	guucucgacumaaagcmaggaTT	883
AL-DP-7130	cmaumumggaaumumcmcmumaaaaumcmTT-sChol	884	gauuuumaggaauuccmaaumgTT	885

Note: 's' represents a phosphorothioate bound inbetween T and cholesterol, Chol represents cholesterol-conjugate

24 hours after transfection, HeLa cells were lysed and Huntingtin and GAPDH mRNA levels were quantified as described above. For each siRNA, four individual datapoints were collected. An siRNA duplex unrelated to the HD gene was used as a control. The activity of a given siRNA duplex targeting HD was expressed as percent HD mRNA concentration in treated cells relative to the HD mRNA concentration in cells treated with the control siRNA

duplex. XL-fit was used to calculate IC_{50} values; the mean IC_{50} values were calculated from three independent determinations, and are shown in Table 9.

Table 9: Potency of Cholesterol-Conjugated dsRNAs AL-DP-6982, AL-DP-6983 and AL-DP-7130 Compared with Unconjugated dsRNAs AL-DP-5997, AL-DP-6000 and AL-DP-7100 against endogenous human HD mRNA expression in HeLa cells

Duplex name	IC50 (mean, nM)
AL-DP-5997	0.04
AL-DP-6982	0.73
AL-DP-6000	0.24
AL-DP-6983	14.0
AL-DP-7100	0.03
AL-DP-7130	0.38

The unconjugated dsRNAs exhibited expected (Table 4) potencies *in vitro* against HD mRNA. The cholesterol-conjugated dsRNAs retain biological activity *in vitro* against HD mRNA, although the potencies are somewhat reduced compared to the unconjugated parent molecules.

In vivo down-modulation of endogenous HD mRNA levels by CNS administration of unconjugated or cholesterol-conjugated dsRNAs targeting HD in rats and mice

To assess both the *in vivo* biological activity and distribution of unconjugated or cholesterol-conjugated dsRNAs targeting HD, dsRNAs AL-DP-1997 and AL-DP-1998 (Table 10), based on AL-DP-5997, were synthesized in which the two 2'-deoxy-thymidine-5'-phosphate nucleotides at the 3'-end of the antisense strand (outside of the dsRNA's nucleotide region that targets the HD mRNA) were replaced with 5-bromo-2'-deoxyuridine.

Table 10: Sequences of dsRNAs AL-DP-1997 and AL-DP-1998

Duplex name	Sense strand sequence (5´-3´)	SEQ ID NO:	Antisense strand sequence (5´-3´)	SEQ ID NO:
AL-DP-1997	gumcmacmaaagaacmcmgumgcmagT<u>T</u>	886	cugcmacgguucuuugugacB<u>B</u>	887
AL-DP-1998	gumcmacmaaagaacmcmgumgcmagTT-Chol	888	cugcmacgguucuuugugacB<u>B</u>	889

Note: 'B' represents 5-bromo-2'-deoxyuridine, underline designates nucleoside-5'-phosphorothioate, Chol represents cholesterol-conjugate

In rats, 1.3 mg AL-DP-1997 or AL-DP-1998, or phosphate-buffered saline (PBS, vehicle control) was administered by continuous intrastriatal infusion over 7 days. Male Sprague-Dawley rats, approximately 250-300g body weight, received stereotaxic implantation of 30-gauge infusion cannulae (Plastics One, Roanok, VA) such that unilateral injections were targeted to the center of the striatum (anteroposterior +0.7 mm, mediolateral + 3.0 mm, relative to bregma; dorsoventral 5 mm, relative to skull surface). Mini-osmotic pumps (model 1007D) were primed overnight according to the manufacturer's specifications, implanted subcutaneously, and connected via catheters, to deliver (4 rats per treatment group) PBS, 1.1 mM AL-DP-1997 or 1.1 mM AL-DP-1998 at 0.5 uL/hr over 7 days. At the end of the 7 day infusion period, animals were sacrificed, brains were removed, and ipsilateral striata encompassing the infusion site were flash frozen. Tissue samples of about 5-30 mg each were homogenized by sonication (BANDELIN electronic GmbH & Co. KG, Berlin, Germany) in Tissue and Cell Lysis solution (Epicentre, Madison, WI) containing 84 μg/ml Proteinase K (Epicentre, Madison, WI). Lysates were then stored at -80°C. For carrying out the bDNA assay, frozen lysates were thawed at room temperature, and Huntingtin and GAPDH mRNA were quantified using the Quantigene Explore Kit according to the manufacturer's instructions. For each tissue sample, the ratio of Huntingtin/GAPDH (normalized Huntingtin mRNA level) was calculated as an average of four determinations. These ratios were then averaged to obtain a group (treatment) average. The unconjugated dsRNA, AL-DP-1997, reduced the normalized Huntingtin mRNA level by 33%, relative to the PBS control group, whereas the cholesterol-conjugated dsRNA, AL-DP-1998, reduced the normalized Huntingtin mRNA level by 26%, relative to the PBS control group. Both reductions were statistically significant ($p<0.05$, ANOVA with Tukey post-hoc analysis). These results demonstrate that intrastriatal AL-DP-1997 and AL-DP-1998 are efficacious *in vivo* in down-modulating HD mRNA levels.

With an identical experimental paradigm, AL-DP-5997 and AL-DP-6000 were also found to be effective *in vivo* in down-modulating HD mRNA levels after intrastriatal infusion with 1.3 mg over 7 days (0.5 uL/hr at 1.1 mM) in rats. AL-DP-5997 and AL-DP-6000 reduced the normalized Huntingtin mRNA levels in striatal tissue by 34% and 36%, respectively, relative to the PBS control group. In addition, AL-DP-5997 and AL-DP-6000 reduced the normalized Huntingtin mRNA levels in cortical tissue by 22% and 26% respectively. These results demonstrate that these unconjugated siRNAs, after intrastriatal infusion, not only down-modulate HD mRNA levels within the striatum, but also in the cortex, another major brain region where neuronal loss occurs in Huntington's disease and which is located further from the infusion site.

In mice, 75 ug AL-DP-1998, or phosphate-buffered saline (PBS, vehicle control) was administered by a 20 minute intrastriatal infusion. Male Balb/c mice, approximately 20 – 25 g body weight, received unilateral injections of test article that were targeted to the striatum (anteroposterior +0.5 mm, mediolateral + 2.0 mm, relative to bregma; dorsoventral 3.5 mm, relative to skull surface). Test articles (1.1 mM) were injected (4 animals per test article) at

0.25 uL/min. using pre-filled, pump-regulated Hamilton micro-syringes connected to a 33 gauge needle. Approximately 72 hours following the injection, animals were sacrificed, brains were removed, and ipsilateral striata encompassing the infusion site were dissected and flash frozen. As described above for rat tissue samples, mouse tissue samples were lysed, and Huntingtin and GAPDH mRNA levels quantified. For each tissue sample, the ratio of Huntingtin/GAPDH (normalized Huntingtin mRNA level) was calculated as an average of four determinations. These ratios were then averaged to obtain a group (treatment) average. The cholesterol-conjugated dsRNA, AL-DP-1998, reduced the normalized Huntingtin mRNA level by 33%, relative to the PBS control group, which was statistically significant ($p<0.05$, ANOVA with Tukey post-hoc analysis). These results further confirm that AL-DP-1998 is efficacious *in vivo* in down-modulating HD mRNA levels. In addition, these results demonstrate that a total intrastriatal dose of AL-DP-1998 as low as 75 ug resulted in significant down-modulation of HD mRNA levels.

CLAIMS

We claim:

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of a human HD gene in a cell, wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding HD, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said HD, inhibits expression of said HD gene by at least 20%.

2. The dsRNA of claim 1, wherein said first sequence is selected from the group consisting of Tables 1, 2, 7, 8 or 10 and said second sequence is selected from the group consisting of Tables 1, 2, 7, 8 or 10.

3. The dsRNA of claim 1, wherein said dsRNA comprises at least one modified nucleotide.

4. The dsRNA of claim 2, wherein said dsRNA comprises at least one modified nucleotide.

5. The dsRNA of claims 3 or 4, wherein said modified nucleotide is chosen from the group of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group.

6. The dsRNA of claims 3 or 4, wherein said modified nucleotide is 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.

7. The dsRNA of claims 3 or 4, wherein said first sequence is selected from the group consisting of Table 2 and said second sequence is selected from the group consisting of Table 2.

8. The dsRNA of claims 3 or 4, wherein said first sequence is selected from the group consisting of Tables 1, 2, 7, 8 or 10 and said second sequence is selected from the group consisting of Tables 1, 2, 7, 8 or 10.

9. A cell comprising the dsRNA of claim 1.

10. A pharmaceutical composition for inhibiting the expression of the HD gene in an organism, comprising a dsRNA and a pharmaceutically acceptable carrier, wherein the dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding HD, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said HD, inhibits expression of said HD gene by at least 20%.

11. The pharmaceutical composition of claim 10, wherein said first sequence of said dsRNA is selected from the group consisting of Tables 1, 2, 7, 8 or 10 and said second sequence of said dsRNA is selected from the group consisting of Tables 1, 2, 7, 8 or 10.

12. The pharmaceutical composition of claim 10, wherein said first sequence of said dsRNA is selected from the group consisting of Table 2 and said second sequence of said dsRNA is selected from the group consisting of Table 2.

13. A method for inhibiting the expression of the HD gene in a cell, the method comprising:

(a) introducing into the cell a double-stranded ribonucleic acid (dsRNA), wherein the dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding HD, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said HD, inhibits expression of said HD gene by at least 20%; and

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

14. A method of treating, preventing or managing Huntingtin disease comprising administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of a dsRNA, wherein the dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding HD, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said HD, inhibits expression of said HD gene by at least 20%.

15. A vector for inhibiting the expression of the HD gene in a cell, said vector comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of a dsRNA, wherein one of the strands of said dsRNA is substantially complementary to at least a part of a mRNA encoding HD and wherein said dsRNA is less than 30 base pairs in length and wherein said dsRNA, upon contact with a cell expressing said HD, inhibits the expression of said HD gene by at least 20%.

16. A cell comprising the vector of claim 15.

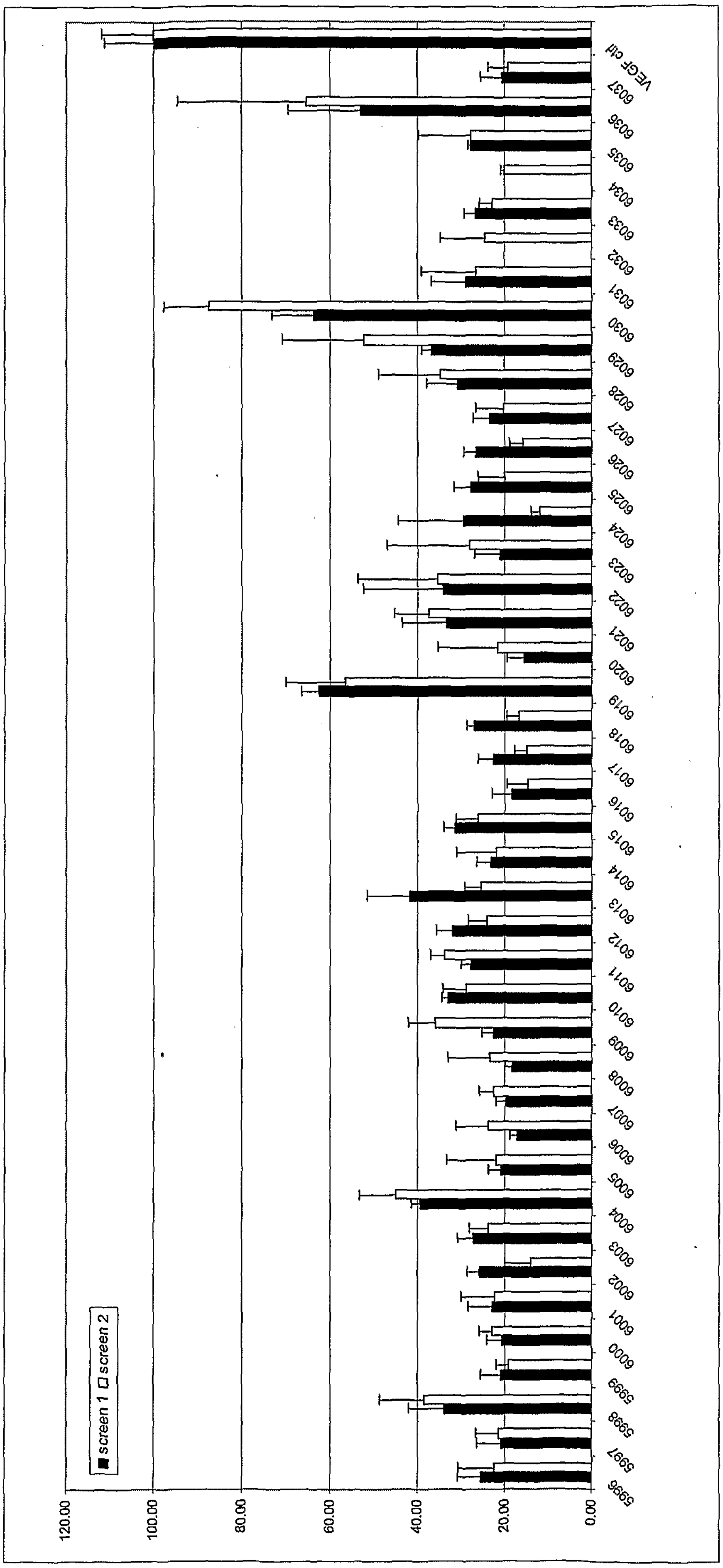


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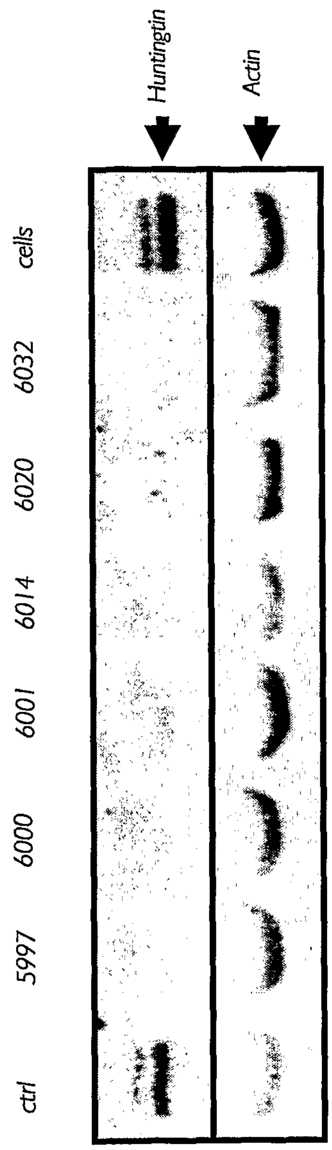
FIG. 1



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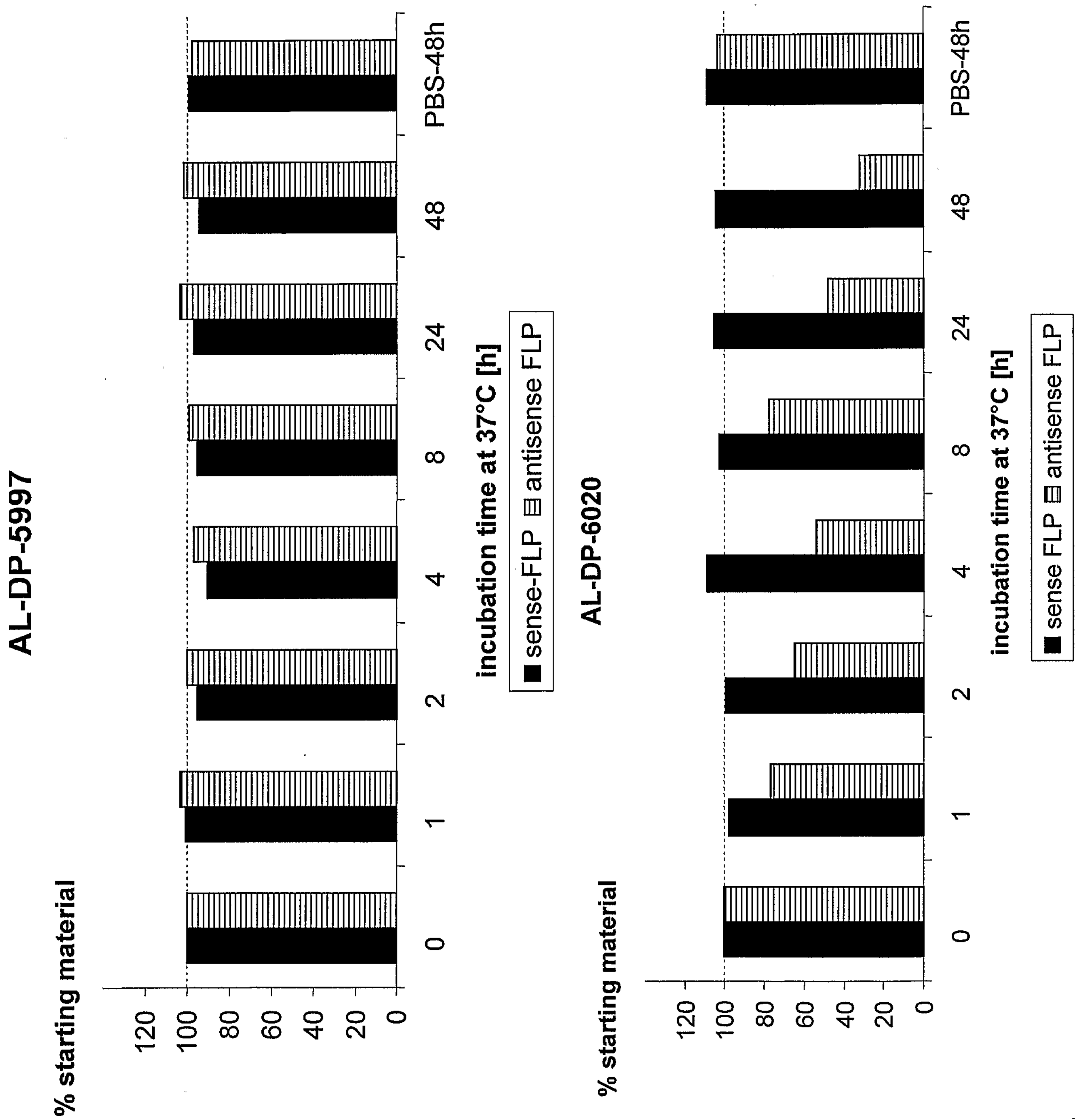
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incubation time at 37°C [h]
sense-FLP
antisense FLP
AL-DP-6020
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80
60
40
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antisense FLP

FIG. 3

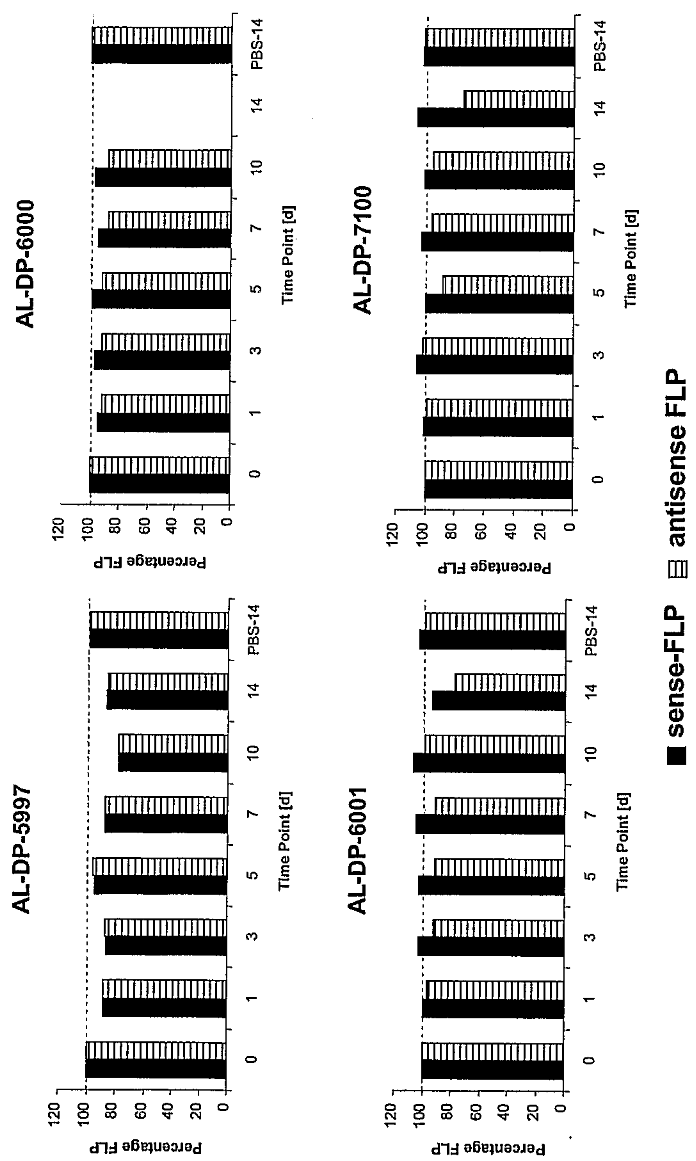
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Figure 4

<110> Alnylam Pharmaceuticals, Inc.

<120> COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF HUNTINGTIN GENE

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<400> 847
caguucauaa accuggacat t 21

<210> 848
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<223> /mod_base = "2'-hydroxy corresponding base"

<400> 848
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<220>
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<400> 850
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<220>
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<400> 852
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<220>
<221> modified_base
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<223> /mod_base = "2'-hydroxy corresponding base"

<400> 854
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<220>
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<220>
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<220>
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<222> 3, 4, 5, 7, 8, 9, 13, 14, 15, 17
<223> /mod_base = "2'-O-methyl corresponding base"

<220>
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<222> 21
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<220>
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<400> 858
agcuugucca gguuuaugat t 21

<210> 859
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<220>
<221> modified_base
<222> 21
<223> /mod_base = "5'-thio thymidine"

<220>
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<400> 859
ucauaaaccu ggacaagcut t 21

<210> 860
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<220>
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<223> /mod_base = "5'-thio thymidine"

<220>
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<400> 860
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<210> 861
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<220>
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<220>
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<222> 1, 2, 3, 4, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19
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cuccuaagag cacuuugcct t 21

<210> 862
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<220>
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<220>
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<400> 862

gaucauugga auuccuaaat t 21

<210> 863
<211> 21
<212> DNA
<213> Artificial sequence

<220>
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<220>
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<222> 21
<223> /mod_base = "5'-thio thymidine"

<220>
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<222> 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19
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<400> 863
uuuaggaauu ccaaugauct t 21

<210> 864
<211> 21
<212> DNA
<213> Artificial sequence

<220>
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<220>
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<220>
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<220>
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<223> /mod_base = "2'-hydroxy corresponding base"

<400> 864
cacugcguga acauucacat t 21

<210> 865
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<220>
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<220>
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<223> /mod_base = "2'-O-methyl corresponding base"

<220>
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<222> 21
<223> /mod_base = "5'-thio thymidine"

<220>
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<400> 865
ugugaauguu cacgcagugt t 21

<210> 866
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<212> DNA
<213> Artificial sequence

<220>
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<220>
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<223> /mod_base = "2'-O-methyl corresponding base"

<220>
<221> modified_base
<222> 21
<223> /mod_base = "5'-thio thymidine"

<220>
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<222> 1, 4, 5, 6, 7, 8, 11, 12, 14, 15, 17, 18
<223> /mod_base = "2'-hydroxy corresponding base"

<400> 866
gucgagaacc aaugauggct t 21

<210> 867
<211> 21
<212> DNA
<213> Artificial sequence

<220>
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<220>
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<220>
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<222> 21
<223> /mod_base = "5'-thio thymidine"

<220>
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<400> 867
gccaucauug guucucgact t 21

<210> 868
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<212> DNA
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<220>
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<220>
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<223> /mod_base = "2'-O-methyl corresponding base"

<220>
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<223> /mod_base = "5'-thio thymidine"

<220>
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<223> /mod_base = "2'-hydroxy corresponding base"

<400> 868
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<210> 869
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<220>
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<220>
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<220>
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<222> 21
<223> /mod_base = "5'-thio thymidine"

<220>
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<222> 1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19
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<400> 869
guucauaaac cuggacaagt t 21

<210> 870
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<220>
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<220>
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<223> /mod_base = "2'-O-methyl corresponding base"

<220>
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<222> 21
<223> /mod_base = "5'-thio thymidine"

<220>
<221> modified_base

<222> 2, 4, 5, 7, 8, 10, 13, 15, 16, 19
<223> /mod_base = "2'-hydroxy corresponding base"

<400> 870
ugugauggca ucauggccat t 21

<210> 871
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<220>
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<220>
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<223> /mod_base = "2'-O-methyl corresponding base"

<220>
<221> modified_base
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<223> /mod_base = "5'-thio thymidine"

<220>
<221> modified_base
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<400> 871
uggccaugau gccaucacat t 21

<210> 872
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<220>
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<220>
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<220>
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<220>

<221> modified_base
<222> 2, 4, 5, 6, 7, 8, 9, 12, 14, 16, 17, 18
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<400> 872
cacaaagaac cgugcagaut t 21

<210> 873
<211> 21
<212> DNA
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<220>
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<220>
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<220>
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<223> /mod_base = "2'-O-methyl corresponding base"

<220>
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<222> 21
<223> /mod_base = "5'-thio thymidine"

<220>
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<222> 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19
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<400> 873
aucugcacgg uucuuugugt t 21

<210> 874
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<220>
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<220>
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<220>
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<220>
<221> modified_base
<222> 6, 15
<223> /mod_base = "2'-O-methyl corresponding base"

<400> 874
gaggguagga aggccaag 18

<210> 875
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<220>
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<220>
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<220>
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<223> /mod_base = "2'-O-methyl corresponding base"

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<223> /mod_base = "5'-thio thymidine"

<220>
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<400> 875
guaggaaggc caaggagctt 20

<210> 876
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<212> DNA
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<220>
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<220>
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<223> /mod_base = "2'-O-methyl corresponding base"

<220>
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<223> /mod_base = "5'-thio thymidine"

<220>
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<223> /mod_base = "2'-hydroxy corresponding base"

<400> 876
cauuggaauu ccuaaaauct t 21

<210> 877
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<220>
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<223> /mod_base = "2'-O-methyl corresponding base"

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<223> /mod_base = "5'-thio thymidine"

<220>
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<400> 877
gauuuuagga auuccaaugt t 21

<210> 878
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<220>
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<220>
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<220>
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<222> 21
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<400> 878
gaucauugga auuccuaaat t 21

<210> 879
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<220>
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<220>
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<223> /mod_base = "5'-thio thymidine"

<220>
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<400> 879
uuuaggaauu ccaaugauct t 21

<210> 880
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<220>
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<223> /mod_base = "2'-O-methyl corresponding base"

<220>

<221> modified_base
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<223> /mod_base = "3'-and 5'-thio 3'-cholesterol deoxy thymidine"

<220>
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<400> 880
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<210> 881
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<220>
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<220>
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<223> /mod_base = "2'-O-methyl corresponding base"

<220>
<221> modified_base
<222> 21
<223> /mod_base = "5'-thio thymidine"

<220>
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<222> 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19
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<400> 881
cugcacgguu cuuugugact t 21

<210> 882
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<220>
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<220>
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<220>
<221> modified_base
<222> 21
<223> /mod_base = "3'-and 5'-thio 3'-cholesterol deoxy thymidine"

<220>
<221> modified_base
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<223> /mod_base = "2'-hydroxy corresponding base"

<400> 882
uccugcuuua gucgagaact t 21

<210> 883
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<220>
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<220>
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<220>
<221> modified_base
<222> 21
<223> /mod_base = "5'-thio thymidine"

<220>
<221> modified_base
<222> 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 16, 17, 18, 19
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<400> 883
guucucgacu aaagcaggat t 21

<210> 884
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<220>
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<220>
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<220>
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<223> /mod_base = "3'-and 5'-thio 3'-cholesterol deoxy thymidine"

<220>
<221> modified_base
<222> 2, 5, 6, 7, 8, 14, 15, 16, 17
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<400> 884
cauuggaauu ccuaaaauct t 21

<210> 885
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<220>
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<220>
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<220>
<221> modified_base
<222> 21
<223> /mod_base = "5'-thio thymidine"

<220>
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<222> 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 19
<223> /mod_base = "2'-hydroxy corresponding base"

<400> 885
gauuuuagga auuccaaugt t 21

<210> 886
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<212> DNA
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<220>
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<220>
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<220>
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<223> /mod_base = "2'-O-methyl corresponding base"

<220>
<221> modified_base
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<223> /mod_base = "5'-thio thymidine"

<220>
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<222> 1, 4, 6, 7, 8, 9, 10, 11, 14, 16, 18, 19
<223> /mod_base = "2'-hydroxy corresponding base"

<400> 886
gucacaaaga accgugcagt t 21

<210> 887
<211> 21
<212> RNA
<213> Artificial sequence

<220>
<223> siRNAs specific for huntingtin

<220>
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<220>
<221> modified_base
<222> 21
<223> /mod_base = "5-bromo-2'-deoxy corresponding base"

<220>
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<222> 4
<223> /mod_base = "2'-O-methyl corresponding base"

<400> 887
cugcacgguu cuuugugacu u 21

<210> 888
<211> 21
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<220>
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<220>
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<220>
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<223> /mod_base = "2'-O-methyl corresponding base"

<220>
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<222> 21
<223> /mod_base = "5'-thio 3'-cholesterol deoxy thymidine"

<220>
<221> modified_base
<222> 1, 4, 6, 7, 8, 9, 10, 11, 14, 16, 18, 19
<223> /mod_base = "2'-hydroxy corresponding base"

<400> 888
gucacaaaga accgugcagt t 21

<210> 889
<211> 21
<212> RNA
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<220>
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<220>
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<220>
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<222> 21
<223> /mod_base = "5-bromo-2'-deoxy corresponding base"

<220>
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<222> 4
<223> /mod_base = "2'-O-methyl corresponding base"

<400> 889
cugcacgguu cuuugugacu u 21