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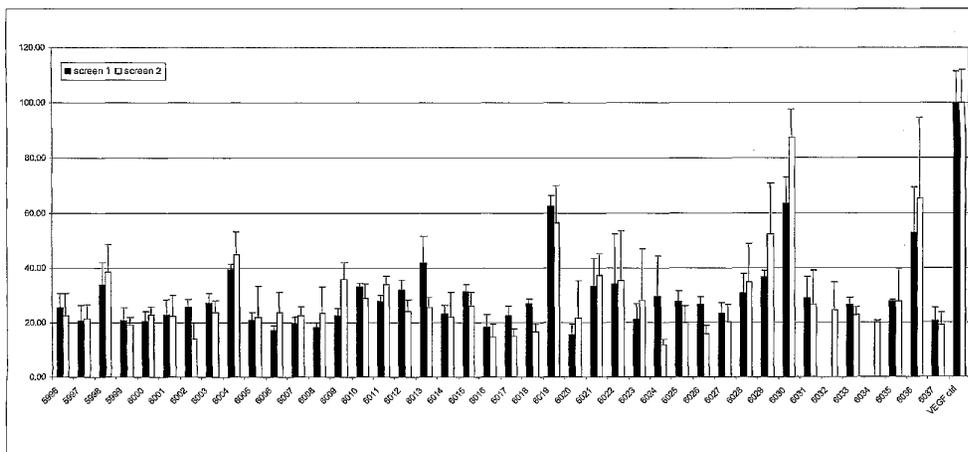
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(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF HUNTINGTIN GENE



(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 than 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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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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**COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF
HUNTINGTIN GENE**

Related Applications

5 This application corresponds to International Application No.
PCT/US2006/042420 filed October 27, 2006 in the Australian national stage, and
claims priority from 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 entire content of
10 each priority application is incorporated herein by reference.

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.
15

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
20 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
25 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
30 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
5 *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
10 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
15 (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
20 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
25 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-
5 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
10 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
15 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:

- 20 (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
25 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,
5 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
10 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
15 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
20 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
5 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)
10 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
15 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
20 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
25 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

5 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
10 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
15 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
20 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
25 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
5 *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
10 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
15 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
20 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
25 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
5 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
10 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
15 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
20 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.

25 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,
5 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.

10 "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
15 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
20 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
25 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 lilted gait, motor impersistence (inability to sustain a motor act, such as tongue protrusion), facial grimacing, ataxia, and dystonia.

5 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
10 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
15 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
20 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.
25 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.

5 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)

10 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

15 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

20 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 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.

5 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
10 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
15 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.

20 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
25 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
5 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
10 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
15 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
20 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.,
25 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-
5 (oxyphosphinicoxy-1,3-propanediol)- 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
10 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
15 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,
20 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
25 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
5 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
10 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
15 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.
20 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-
25 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-hydroxysuccinimide 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.

5 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-butyl dimethylsilyl, t-butyl diphenylsilyl, 10 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 15 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).

20 Additional amino-protecting groups include, but are not limited to, carbamate protecting groups, such as 2-trimethylsilylethoxycarbonyl (Teoc), 1-methyl-1-(4-biphenyl)ethoxycarbonyl (Bpoc), t-butoxycarbonyl (BOC), allyloxycarbonyl (Alloc), 9-fluorenylmethyloxycarbonyl (Fmoc), and benzyloxycarbonyl (Cbz); amide protecting groups, such as formyl, acetyl, trihaloacetyl, benzoyl, and 25 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.

5 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

10 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

15 bonded to the solid support via a *syn*-1,2-acetoxyposphate 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

20 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

25 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
5 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
10 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.

15 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
20 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.

25 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
5 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
10 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
15 backbones; amide backbones; and others having mixed N, O, S and CH₂ 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;
20 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
25 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
5 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.

10 Some preferred embodiments of the invention employ oligonucleotides with phosphorothioate linkages and oligonucleosides with heteroatom backbones, and in particular --CH₂--NH--O--CH₂ --, --CH₂--N(CH₃)--O--CH₂ -- [known as a methylene (methylimino) or MMI backbone], --CH₂--O--N(CH₃)--CH₂ --, --CH₂--N(CH₃)--
15 N(CH₃)--CH₂--, and --O--N(CH₃)--CH₂ --CH₂-- [wherein the native phosphodiester backbone is represented as --O--P--O--CH₂--] of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

The oligonucleotides employed in the ligand-conjugated oligonucleotides of
20 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-
25 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-
5 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
10 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-
15 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
20 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
25 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 C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Particularly preferred

5 are O[(CH₂)_nO]_mCH₃, O(CH₂)_nOCH₃, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, and O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂ CH₃, ONO₂, NO₂, N₃, NH₂,

10 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--

15 CH₂CH₂OCH₃, 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'-dimethylaminoethoxy, i.e., a O(CH₂)₂ON(CH₃)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.

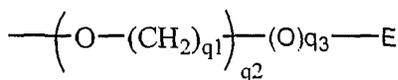
20 Other preferred modifications include 2'-methoxy (2'-O--CH₃), 2'-aminopropoxy (2'-OCH₂CH₂CH₂NH₂) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides.

As used herein, the term "sugar substituent group" or "2'-substituent group"

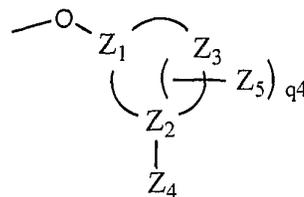
25 includes groups attached to the 2'-position of the ribofuranosyl moiety with or without an oxygen atom. Sugar substituent groups include, but are not limited to, fluoro, O-alkyl, O-alkylamino, O-alkylalkoxy, protected O-alkylamino, O-alkylaminoalkyl, O-alkyl imidazole and polyethers of the formula (O-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 Delgado et. al. (*Critical Reviews in Therapeutic Drug Carrier*
 5 *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,
 10 Substitutions," hereby incorporated by reference in its entirety.

Additional sugar substituent groups amenable to the invention include 2'-SR and 2'-NR₂ 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
 15 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

20

wherein,

E is C₁-C₁₀ alkyl, N(Q₃)(Q₄) or N=C(Q₃)(Q₄); each Q₃ and Q₄ is, independently, H, C₁-C₁₀ alkyl, dialkylaminoalkyl, a nitrogen protecting group, a

tethered or untethered conjugate group, a linker to a solid support; or Q₃ and Q₄, together, form a nitrogen protecting group or a ring structure optionally including at least one additional heteroatom selected from N and O;

q₁ is an integer from 1 to 10;

5 q₂ is an integer from 1 to 10;

q₃ is 0 or 1;

q₄ is 0, 1 or 2;

each Z₁, Z₂ and Z₃ is, independently, C₄-C₇ cycloalkyl, C₅-C₁₄ aryl or C₃-C₁₅ heterocyclyl, wherein the heteroatom in said heterocyclyl group is selected from
10 oxygen, nitrogen and sulfur;

Z₄ is OM₁, SM₁, or N(M₁)₂; each M₁ is, independently, H, C₁-C₈ alkyl, C₁-C₈ haloalkyl, C(=NH)N(H)M₂, C(=O)N(H)M₂ or OC(=O)N(H)M₂; M₂ is H or C₁-C₈ alkyl; and

Z₅ is C₁-C₁₀ alkyl, C₁-C₁₀ haloalkyl, C₂-C₁₀ alkenyl, C₂-C₁₀ alkynyl, C₆-C₁₄
15 aryl, N(Q₃)(Q₄), OQ₃, halo, SQ₃ 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
20 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, CH₂, CHF, and CF₂. See, e.g., Secrist et al., Abstract 21, *Program & Abstracts, Tenth International Roundtable, Nucleosides, Nucleotides and their Biological Applications*,
5 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;
10 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,700,920; 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.
15 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. Lett.*, 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.,
20 *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-oxysterol 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; 10 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; 15 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 20 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 25 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.,
5 *Bioorg. Med. Chem. Lett.*, 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-
10 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-
15 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
20 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
25 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 phosphorylation of the terminal alcohol functionality. Other linkers, such as
5 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
10 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,
15 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
20 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
25 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.

5 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
10 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
15 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.

20 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
25 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.

5 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
10 (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
15 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
20 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
25 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
5 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
10 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;
15 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
20 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
25 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
5 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
10 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
15 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
20 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
25 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.

5 **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
10 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

15 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
20 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
25 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
5 *Huntingtin* 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
10 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 lilted gait, motor imperistence (inability to sustain a motor act, such as tongue protrusion), facial
15 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
20 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
25 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.

5 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.

5

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.

10 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
15 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
20 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
25 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:	Remaining HD gene mRNA [% of control]
AD-10894	gaaucgagauccggauguca	1	gaaucgagauccggaugucaTT	2	ugacauccggaucucgaaucTT	3	28 ± 3
AD-10895	aaauccugcuuuagucgag	4	aaauccugcuuuagucgagTT	5	cucgacuaaagcaggaaucTT	6	45 ± 4
AD-10896	agucaguccggguagaacu	7	agucaguccggguagaacuTT	8	aguucuaaccggacugacuTT	9	38 ± 2
AD-10897	gguuuagaaucugacguua	10	gguuuagaaucugacguuaTT	11	uaacgucaguucauaaacTT	12	11 ± 2
AD-10898	guuacggguuaaauacugu	13	guuacggguuaaauacuguTT	14	acaguaaauaacccguaacTT	15	28 ± 1
AD-10899	ugcuuuagucgagaaccaa	16	ugcuuuagucgagaaccaaTT	17	uuguuucucgacuaaagaTT	18	33 ± 3
AD-10900	ucuguaccguugaguccca	19	ucuguaccguugagucccaTT	20	ugggacucaaacgguacagaTT	21	35 ± 3
AD-10901	aaauuguuagacggguac	22	aaauuguuagacggguacTT	23	guaccgucuaacacaaauuTT	24	48 ± 6
AD-10902	uggccggaaacuugcuugc	25	uggccggaaacuugcuugcTT	26	gcaagcaaguuuccggccaTT	27	46 ± 5
AD-10903	guucaguuacggguuaauu	28	guucaguuacggguuaauuTT	29	aaauaacccgguaacgaaacTT	30	32 ± 3
AD-10904	gcgggcucguuccaugauc	31	gcgggcucguuccaugaucTT	32	gaucauggaacgccccgcTT	33	31 ± 1
AD-10905	gacuccgagcacuaaacgu	34	gacuccgagcacuaaacguTT	35	acguuaagucucggagucTT	36	28 ± 3
AD-10906	cgcauggucgacaucuuug	37	cgcauggucgacaucuuugTT	38	caaggaugucgaccaauggTT	39	37 ± 2
AD-10907	aagacgagauccucgcuca	40	aagacgagauccucgcucaTT	41	ugagcggagauccucgcuuTT	42	35 ± 1
AD-10908	aagucaguccggguagaac	43	aagucaguccggguagaacTT	44	guucuaaccgggacugacuTT	45	42 ± 4
AD-10909	aaggccuucauagcgaacc	46	aaggccuucauagcgaaccTT	47	gguuucgcuagaaggccuuTT	48	65 ± 4
AD-10910	aggccuucauagcgaacc	49	aggccuucauagcgaaccTT	50	agguuucgcuagaaggccuuTT	51	23 ± 1
AD-10911	acuccgagcacuaaacgug	52	acuccgagcacuaaacgugTT	53	caaguuuagucucggagucTT	54	42 ± 4
AD-10912	uaaaggccuucauagcgaac	55	uaaaggccuucauagcgaacTT	56	uucgcuagaaggccuuuaTT	57	20 ± 1
AD-10913	ucugaauccgagauccggaug	58	ucugaauccgagauccggaugTT	59	cauccgauccggaauccagaTT	60	46 ± 4
AD-10914	ugaaaauuguuagacgggu	61	ugaaaauuguuagacggguTT	62	accgcuaacacaaauucaTT	63	35 ± 1
AD-10915	uggcucgcauggucgacau	64	uggcucgcauggucgacauTT	65	augucgaccaauggagccaTT	66	42 ± 5
AD-10916	aaagucaguccggguagaa	67	aaagucaguccggguagaaTT	68	uuuuaaccgggacugacuTT	69	42 ± 4
AD-10917	gagugcccugucgguuucu	70	gagugcccugucgguuucuTT	71	agaaaccgacacgggacucTT	72	77 ± 8
AD-10918	ggagcucgggacggauagu	73	ggagcucgggacggauaguTT	74	acuaaccgucgggacucTT	75	94 ± 9
AD-10919	agaaaacaagccuugccgc	76	agaaaacaagccuugccgcTT	77	gcggaaggcuuuuuuuuuTT	78	43 ± 4
AD-10920	auaaucacauucuuuuuuu	79	auaaucacauucuuuuuuuTT	80	aaacaagaaugugauuuTT	81	35 ± 4
AD-10921	ucugggcaucgcuauuggaa	82	ucugggcaucgcuauuggaaTT	83	uuccaugcuaugcccagaTT	84	26 ± 6
AD-10922	gcccuucauagcgaaccug	85	gcccuucauagcgaaccugTT	86	cagguuucgcuagaagcccTT	87	32 ± 12
AD-10923	cuaaaugucucuuaaggcu	88	cuaaaugucucuuaaggcuTT	89	agcuuaagagcacauuuagTT	90	24 ± 8
AD-10924	guuuuagaaucugacguuac	91	guuuuagaaucugacguuacTT	92	guaacgucaguucauaaacTT	93	18 ± 8
AD-10925	uuuuuagaaucugacguuaca	94	uuuuuagaaucugacguuacaTT	95	uguaaacgucaguucauaaaaTT	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:	Remaining HD gene mRNA [% of control]
AD-10926	augaacugacguuacauc	97	augaacugacguuacauc	98	ugauguaacgucaguucau	99	20 ± 3
AD-10927	ccacaaguuugacccgga	100	ccacaaguuugacccgga	101	ucgggucacaacaauuggg	102	20 ± 3
AD-10928	cuggggccgaagccguag	103	cuggggccgaagccguag	104	cuacggcuucggccaccag	105	38 ± 1
AD-10929	aaunuguuagacgguaacc	106	aaunuguuagacgguaacc	107	gguaaccgucuaacacaau	108	39 ± 6
AD-10930	uuuguuuagacgguaaccga	109	uuuguuuagacgguaaccga	110	ucgguaaccgucuaacaca	111	30 ± 4
AD-10931	aaaaaagccuugccgcau	112	aaaaaagccuugccgcau	113	augggcaaggcuuguuuu	114	32 ± 3
AD-10932	aagacuguaaccguuugga	115	aagacuguaaccguuugga	116	ucccaacgguaacgucuu	117	43 ± 5
AD-10933	auaccucagguccuguuac	118	auaccucagguccuguuac	119	guaacagaccugagguau	120	36 ± 4
AD-10934	uccuguuuagucgagaac	121	uccuguuuagucgagaac	122	guucugacuaaaagcagg	123	35 ± 7
AD-10935	cauaaacaauucguuuu	124	cauaaacaauucguuuu	125	acaaacgauguaauaug	126	28 ± 2
AD-10936	aagcgacugucucgacaga	127	aagcgacugucucgacaga	128	ucugucgagacagucgu	129	29 ± 3
AD-10937	ccgagcacuuacguggc	130	ccgagcacuuacguggc	131	agccacguuaagugcgg	132	38 ± 5
AD-10938	cuggcucgcauggucgaca	133	cuggcucgcauggucgaca	134	ugucgaccaugcagccag	135	35 ± 2
AD-10939	uuugcccgguuagaaug	136	uuugcccgguuagaaug	137	cauuuacacacugggau	147	37 ± 4
AD-10940	ugcaagacucacuagucc	139	ugcaagacucacuagucc	140	ggacuuaagugagucua	141	56 ± 9
AD-10941	gaaacagugaguccggaca	142	gaaacagugaguccggaca	143	ugucggacucacuguuuc	144	36 ± 4
AD-10942	aaaucccaguguggacca	145	aaaucccaguguggacca	146	ugnuccaacacugggau	147	37 ± 4
AD-10943	gcuagcuccaugcuuaagc	148	gcuagcuccaugcuuaagc	149	gcuuaagcauggagcuag	150	47 ± 4
AD-10944	uccaugcuuaagccuaagg	151	uccaugcuuaagccuaagg	152	cccuaggcuuaagcagg	153	102 ± 12
AD-10945	ccaugcuuaagccuaaggga	154	ccaugcuuaagccuaaggga	155	ucccuaggcuuaagcagg	156	34 ± 5
AD-10946	ucaacagcuacacacgugu	157	ucaacagcuacacacgugu	158	acaaguguaagcugua	159	40 ± 5
AD-10947	augugugccacugcguuuu	160	augugugccacugcguuuu	161	aaaaagcaguggcacaca	162	31 ± 3
AD-10948	ugugugccacugcguuuu	163	ugugugccacugcguuuu	164	uaaaacgacugggcacaca	165	33 ± 1
AD-10949	ucaguccggguagaacuu	166	ucaguccggguagaacuu	167	gaaguuuaccccggacuga	168	58 ± 5
AD-10950	aguccggguagaacuu	169	aguccggguagaacuu	170	cugaaguuuaccccggacu	171	34 ± 3
AD-10951	gauugugcuuagggagcgg	172	gauugugcuuagggagcgg	173	ccgucuuuaccccggacu	174	46 ± 7
AD-10952	acuuuuuacgaaauucc	175	acuuuuuacgaaauucc	176	ggacaauuuguaaacaag	177	46 ± 2
AD-10953	cuuuuuacgaaauucca	178	cuuuuuacgaaauucca	179	uggacaauuuguaaacaag	180	30 ± 1
AD-10954	gcuuccgcacaucccgcg	181	gcuuccgcacaucccgcg	182	ccggcgaugugcgggagc	183	45 ± 5
AD-10955	uaauuuuacgaaauuccu	184	uaauuuuacgaaauuccu	185	aaagcgaugugcgggagc	186	104 ± 6
AD-10956	cuuuuuuacgaaauucca	187	cuuuuuuacgaaauucca	188	uuuacagggcgaagaaag	189	59 ± 3
AD-10957	aaagggaaagcucgacag	190	aaagggaaagcucgacag	191	cucgucagucuuuccuu	192	84 ± 4
AD-10958	gcuuggcucgcauggucgac	193	gcuuggcucgcauggucgac	194	gucgacaugcagccagc	195	44 ± 4
AD-10959	ugacguuacaucaucaca	196	ugacguuacaucaucaca	197	uguguauguaacgucacu	198	19 ± 3
AD-10960	acgguaaccgacaaccagua	199	acgguaaccgacaaccagua	200	uacugguugcgguaaccg	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	gguaccgacaaccaguuuu	202	gguaccgacaaccaguuuuTT	203	aaucugguuuugcguuaccTT	204	19 ± 3
AD-10962	acgagugcucaauaauuu	205	acgagugcucaauaauuuTT	206	aaacuuuuugagcacucguTT	207	19 ± 3
AD-10963	caucggagaguuucugucc	208	caucggagaguuucuguccTT	209	ggacagaaacucuccgagTT	210	38 ± 5
AD-10964	gcgaaccuugaagucaagcu	211	gcgaaccuugaagucaagcuTT	212	agcuugacuuucagguucgTT	213	35 ± 4
AD-10965	cugaaucgagauccggaugu	214	cugaaucgagauccggauguTT	215	acaucggaucucgguuacagTT	216	31 ± 2
AD-10966	cgguaccgacaaccaguuu	217	cgguaccgacaaccaguuuTT	218	aaucugguuuugcguuaccTT	219	26 ± 2
AD-10967	acugaaccggugaucaaa	220	acugaaccggugaucaaaTT	221	cuugaucacccgguucaagTT	222	43 ± 3
AD-10968	ccuugccgcaucaaaaggug	223	ccuugccgcaucaaaaggugTT	224	caccuuugaugcggcaagTT	225	64 ± 9
AD-10969	cuuugcggauugcauucc	226	cuuugcggauugcauuccTT	227	ggaaugcaauccggccaaagTT	228	45 ± 3
AD-10970	cguuaccguugagucccaa	229	cguuaccguugagucccaaTT	230	uugggacucaaccgguaacagTT	231	33 ± 1
AD-10971	uguaccguugagucccaa	232	uguaccguugagucccaaTT	233	cuugggacucaaccgguaacagTT	234	36 ± 4
AD-10972	agucgagaaaccauagugg	235	agucgagaaaccauaguggTT	236	ccaucuuuguuucugacuuTT	237	34 ± 4
AD-10973	ccgacuaccgucgugggc	238	ccgacuaccgucgugggcTT	239	gcccaccagcgguaucgTT	240	47 ± 7
AD-10974	auaucaccggcugcugacu	241	auaucaccggcugcugacuTT	242	agucagcagccggugauuTT	243	73 ± 6
AD-10975	ugcauauccgugggcucaa	244	ugcauauccgugggcucaaTT	245	uuggcccagcgaauuacagTT	246	88 ± 1
AD-10976	uuuuuacgacgugaucua	247	uuuuuacgacgugaucuaTT	248	uagaucaucgcuuaaacaTT	249	66 ± 5
AD-10977	guguuagacgguaaccgaca	250	guguuagacgguaaccgacaTT	251	ugucgguaaccgcuuaaccTT	252	21 ± 2
AD-10978	cuugaacuaaucggaucuu	253	cuugaacuaaucggaucuuTT	254	uugcgauguaucgucagTT	255	37 ± 6
AD-10979	ggccggaaaauugcuugca	256	ggccggaaaauugcuugcaTT	257	ugcaagcaaguuuuccggccTT	258	32 ± 3
AD-10980	cugucgcgacgagauagcug	259	cugucgcgacgagauagcugTT	260	cagcuauucugcagagacagTT	261	26 ± 8
AD-10981	gcaucgcuuuggaacuuuu	262	gcaucgcuuuggaacuuuuTT	263	aaaaguucauagcgaugcTT	264	11 ± 2
AD-10982	acugacguuacaucauaca	265	acugacguuacaucauacaTT	266	uugauugauuaucgucagTT	267	13 ± 4
AD-10983	cugacguuacaucauacac	268	cugacguuacaucauacacTT	269	guguaugauuaaccgucagTT	270	31 ± 5
AD-10984	ugaucgagauccggauguc	271	ugaucgagauccggaugucTT	272	gacauccgauccgcuuacTT	273	62 ± 13
AD-10985	uagacgguaaccgacaacca	274	uagacgguaaccgacaaccaTT	275	ugguuugcguuaccgucuaTT	276	30 ± 4
AD-10986	uugccgcaucaaaaggugac	277	uugccgcaucaaaaggugacTT	278	gucacuuuugaugcggcaTT	279	68 ± 6
AD-10987	aacuaucgaucauuggag	280	aacuaucgaucauuggagTT	281	cuccaugaucgaugauuuTT	282	61 ± 5
AD-10988	uuuugcggauugcauuccu	283	uuuugcggauugcauuccuTT	284	aggaaucaauccggccaaaTT	285	48 ± 5
AD-10989	gcuuuagucgagaaccaca	286	gcuuuagucgagaaccacaTT	287	auugguuucgcgcuuaaagTT	288	29 ± 3
AD-10990	uuuagucgagaaccacauga	289	uuuagucgagaaccacaugaTT	290	ucauuuguuucgcgcuuaaTT	291	29 ± 1
AD-10991	uagucgagaaccacauga	292	uagucgagaaccacaugaTT	293	caucauuuguuucgcgcuuaTT	294	36 ± 3
AD-10992	aagugcuuaccaguuugaa	295	aagugcuuaccaguuugaaTT	296	uucaacuugguagacacuuTT	297	31 ± 3
AD-10993	ucaguuaccggguuuuuuac	298	ucaguuaccggguuuuuuacTT	299	guuuuuaccggguuuuuuacTT	300	44 ± 8
AD-10994	uuacggguuuuuuuuacuguc	301	uuacggguuuuuuuuacugucTT	302	gacaguuuuuuuuuuuuacTT	303	88 ± 17
AD-10995	uacggguuuuuuuuuuacugucu	304	uacggguuuuuuuuuuacugucuTT	305	agacaguuuuuuuuuuuuacTT	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	gucagcuauucugucgagacTT	309	32 ± 3
AD-10997	ucucgacagauagcugaca	310	ucucgacagauagcugacaTT	311	ugucagcuauucugucgagacTT	312	34 ± 2
AD-10998	ugcgggucucuccaugau	313	ugcgggucucuccaugauTT	314	aucauggaacgagcccgcaTT	315	34 ± 4
AD-10999	uucagucucguugugaaaa	316	uucagucucguugugaaaaTT	317	uuuuacacaacgagacugaaTT	318	37 ± 2
AD-11000	ugucgcccggugagaaugc	319	ugucgcccggugagaaugcTT	320	gcuuuuuaccccggcgacaTT	321	91 ± 2
AD-11001	ucggaguucaaccuaagcc	322	ucggaguucaaccuaagccTT	323	ggcuuagguuagaacucggaTT	324	70 ± 6
AD-11002	caugcuuaagccuaaggau	325	caugcuuaagccuaaggauTT	326	auccuaggcuuaagcaugTT	327	37 ± 6
AD-11003	ccgcugagucuggaucucc	328	ccgcugagucuggaucuccTT	329	ggagauccagacucagcggTT	330	70 ± 12
AD-11004	ugucaacagcuacacacgu	331	ugucaacagcuacacacguTT	332	acguguuagcuguuagacaTT	333	43 ± 4
AD-11005	guggcccggcaaccagcug	334	guggcccggcaaccagcugTT	335	cagcuggguugccggccacTT	336	40 ± 3
AD-11006	gaaagggaucccccacugc	337	gaaagggaucccccacugcTT	338	gcagugggagcuaucuuuTT	339	42 ± 2
AD-11007	aaagggaucccccacugcg	340	aaagggaucccccacugcgTT	341	cgagugggagcuaucuuuTT	342	43 ± 2
AD-11008	cgggugaacuuacagacc	343	cgggugaacuuacagaccTT	344	gggucugaaguuuaccccgTT	345	33 ± 3
AD-11009	gucgaccgagggccuuc	346	gucgaccgagggccuucTT	347	gaaagccucgucgucgagcTT	348	49 ± 4
AD-11010	agcccauacaccggcugc	349	agcccauacaccggcugcTT	350	gcagccggugauuagggcuTT	351	46 ± 1
AD-11011	uuuauagcccuguaaagu	352	uuuauagcccuguaaaguTT	353	acuuuacacgggcauagaaTT	354	100 ± 5
AD-11012	ccuuuuagucagggagagu	355	ccuuuuagucagggagaguTT	356	acucuccugacuaaaagggTT	357	94 ± 8
AD-11013	gguuaggcagucugcaugug	358	gguuaggcagucugcaugugTT	359	cacaugacagucgccaaccTT	360	156 ± 10
AD-11014	acugucucgacagauagcu	361	acugucucgacagauagcuTT	362	agcuauucugucgagacagaTT	363	39 ± 5
AD-11015	uuugucgacaauaugugaa	364	uuugucgacaauaugugaaTT	365	uuacauuuugucagacaaTT	366	21 ± 1
AD-11016	cugggcaucgcuauaggaa	367	cugggcaucgcuauaggaaTT	368	guuccauagcgaugcccagTT	369	25 ± 3
AD-11017	cucggaguuugcgugcugc	370	cucggaguuugcgugcugcTT	371	gcagcacgcaaacuccggagTT	372	29 ± 3
AD-11018	uguuuaagggccuucauagc	373	uguuuaagggccuucauagcTT	374	gcuaugaggccuuuaacaTT	375	42 ± 3
AD-11019	uuuaagggccuucauagcga	376	uuuaagggccuucauagcgaTT	377	ucgcuauagaaggccuuuaaTT	378	32 ± 4
AD-11020	gccuucauagcgaaccuga	379	gccuucauagcgaaccugaTT	380	ucaagguucgcuauagaaggcTT	381	26 ± 10
AD-11021	aaggcagcuucggagugac	382	aaggcagcuucggagugacTT	383	gucacuccgaaagcugccuuTT	384	27 ± 2
AD-11022	agguuuauagaacugacguu	385	agguuuauagaacugacguuTT	386	aaagcuguucauaaaaccuTT	387	10 ± 2
AD-11023	aacugacguuacaucauac	388	aacugacguuacaucauacTT	389	guauguauaacgucaguuTT	390	39 ± 3
AD-11024	cacaauugugaccggag	391	cacaauugugaccggagTT	392	cuccggucacaacaauugTT	393	23 ± 4
AD-11025	aaaguuugagaccggagcc	394	aaaguuugagaccggagccTT	395	gucuccggucacaacaauugTT	396	25 ± 4
AD-11026	agcagcucuucagaacgcc	397	agcagcucuucagaacgccTT	398	ggcguucugaaagcugcuTT	399	74 ± 11
AD-11027	gugfccgaagccguagugg	400	gugfccgaagccguaguggTT	401	cccauacggcnuccggccacTT	402	32 ± 4
AD-11028	cguagugggaguuuugugg	403	cguagugggaguuuuguggTT	404	ccaacaauacucccauacgTT	405	26 ± 4
AD-11029	ggaguuuugggaaucuau	406	ggaguuuugggaaucuauTT	407	auaaguuuccacaauacuccTT	408	20 ± 2
AD-11030	aguuuuugggaaucuauag	409	aguuuuugggaaucuauagTT	410	cuauaaguuuccacaauacuTT	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	gagaucggauugucagcagc	412	gagaucggauugucagcagc	413	gcugcugacauccgaucuc	414	53 ± 18
AD-11032	cagcgcgcccacucugac	415	cagcgcgcccacucugac	416	gucagaugggacggcguc	417	49 ± 4
AD-11033	ccaccgaaggccugauuc	418	ccaccgaaggccugauuc	419	gaucagggccuucgggg	420	28 ± 6
AD-11034	auuguuagacgguaaccg	421	auuguuagacgguaaccg	422	cgguaccgucuaacaca	423	111 ± 12
AD-11035	ccgacaaccaguanuuagg	424	ccgacaaccaguanuuagg	425	cccaauacuguuucgg	426	25 ± 5
AD-11036	aaacaagcucgcccgauc	427	aaacaagcucgcccgauc	428	gaucggcaaggccuuuu	429	35 ± 4
AD-11037	gccuugcccaucaaaagg	430	gccuugcccaucaaaagg	431	accuuugauguccaagg	432	36 ± 9
AD-11038	aucuugaacuaaucgauc	433	aucuugaacuaaucgauc	434	gaucgaugauucaagau	435	40 ± 5
AD-11039	aucgaucauggagaccac	436	aucgaucauggagaccac	437	guggucuccaugau	438	69 ± 5
AD-11040	uggagaccacagguucga	439	uggagaccacagguucga	440	ucgaaccugggucucc	441	39 ± 9
AD-11041	ggagaccacagguucgag	442	ggagaccacagguucgag	443	cucgaaccugggucucc	444	65 ± 14
AD-11042	ccgcuuaccgugggagau	444	ccgcuuaccgugggagau	446	aucuccacguggaagg	447	63 ± 2
AD-11043	ucuuugccggaugcauuc	448	ucuuugccggaugcauuc	449	gaucgaucucccaaga	450	60 ± 5
AD-11044	uuggcggauugcauucuu	451	uuggcggauugcauucuu	452	aaggaugcauuccgca	453	30 ± 2
AD-11045	agcagcuacagugauuag	454	agcagcuacagugauuag	455	cuacucacugucuc	456	64 ± 2
AD-11046	cgagucuaaauauguug	457	cgagucuaaauauguug	458	caacauuuuagcacuc	459	18 ± 5
AD-11047	aaauaggcucgucaccaag	460	aaauaggcucgucaccaag	461	cuuugggacaagcuauu	462	54 ± 14
AD-11048	ugggaguuaggguugcacu	463	ugggaguuaggguugcacu	464	aguccaaacuuaccucc	465	44 ± 5
AD-11049	cuugguucccauuggauc	466	cuugguucccauuggauc	467	agauccaugggaaacc	468	32 ± 4
AD-11050	uuuuggccggaacuugcu	469	uuuuggccggaacuugcu	470	agcaaguuuccggccaa	471	53 ± 12
AD-11051	ugccuucuaacaaacc	472	ugccuucuaacaaacc	473	gguuuuuuagagaagg	474	57 ± 5
AD-11052	uaagucccauccgacgaaa	475	uaagucccauccgacgaaa	476	uuucgucggauuggacu	477	43 ± 4
AD-11053	ugaucuccucagguccuguu	478	ugaucuccucagguccuguu	479	aacaggaccugagguau	480	26 ± 2
AD-11054	gaucuccucagguccuguu	481	gaucuccucagguccuguu	482	uaacaggaccugagguau	483	30 ± 5
AD-11055	uguuacaacaaguaaucc	484	uguuacaacaaguaaucc	485	ggauuuacuuuuuuaaca	486	81 ± 4
AD-11056	cuaggauaccugaaauccu	487	cuaggauaccugaaauccu	488	aggaauucaggguauccu	489	35 ± 13
AD-11057	cuuugucggaaccuauug	490	cuuugucggaaccuauug	491	cauuguuucgacuaaag	492	33 ± 6
AD-11058	acuguuuguuucaacaau	493	acuguuuguuucaacaau	494	auuuguaacacacaacag	495	39 ± 4
AD-11059	caauuguuagaacucucu	496	caauuguuagaacucucu	497	agagagucuucaacaau	498	39 ± 3
AD-11060	caagucacaaggccgagca	499	caagucacaaggccgagca	500	ugcucggccuuuagcu	501	40 ± 1
AD-11061	agucacaaggccgagcac	502	agucacaaggccgagcac	503	gucucggccuuuagcu	504	38 ± 5
AD-11062	ggcuuguaaccacucugcu	505	ggcuuguaaccacucugcu	506	agcagagugguacaagc	507	27 ± 3
AD-11063	acgacaccucggggauguu	508	acgacaccucggggauguu	509	aaccuuccgagguucgu	510	38 ± 4
AD-11064	caccucggggauguuugau	511	caccucggggauguuugau	512	aucaaaccaucccgagg	513	52 ± 11
AD-11065	cucggggauguuuugauguc	514	cucggggauguuuugauguc	515	gacaucacaaccuuccg	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	cacgguuuuuugugacacTT	519	43 ± 13
AD-11067	gugucacaaagaaccgugc	520	gugucacaaagaaccgugcTT	521	gcacgguuuuuugugacacTT	522	30 ± 6
AD-11068	aaccgugcagaagaagaug	523	aaccgugcagaagaagaugTT	524	cauuuuuauucgacgguTT	525	36 ± 7
AD-11069	accgugcagaagaagaugc	526	accgugcagaagaagaugcTT	527	gcauuuuuauucgacgguTT	528	39 ± 3
AD-11070	ccgugcagaagaagaugcu	529	ccgugcagaagaagaugcuTT	530	agcauuuuuauucgacgguTT	531	39 ± 3
AD-11071	gcagauaagaagucuauc	532	gcagauaagaagucuaucTT	533	gaauagcauuuuuucgucTT	534	37 ± 4
AD-11072	acaauucguuuuugaacc	535	acaauucguuuuugaaccTT	536	gguucaaacaagaaugTT	537	62 ± 3
AD-11073	ugaaccucuuuuuaaaa	538	ugaaccucuuuuuaaaaTT	539	uuuuuaacaagagguucaTT	540	21 ± 4
AD-11074	uuuagaauucgugcgcag	541	uuuagaauucgugcgcagTT	542	cugcgcagcaaaucuaaaTT	543	80 ± 5
AD-11075	ugguucaguuacggguuaa	544	ugguucaguuacggguuaaTT	545	uuaaaccguaacugaaccaTT	546	32 ± 13
AD-11076	gggccaguuacgggaauca	547	gggccaguuacgggaaucaTT	548	ugaauccugaaacuggccTT	549	30 ± 7
AD-11077	uggaaagcagucucgcagc	550	uggaaagcagucucgcagcTT	551	gucgagacagucguuuccTT	552	41 ± 5
AD-11078	ggaagcagucucgcagaca	553	ggaagcagucucgcagacaTT	554	ugucgagacagucguuuccTT	555	30 ± 8
AD-11079	gaagcagucucgcagcag	556	gaagcagucucgcagcagTT	557	cugucgagacagucguuuccTT	558	35 ± 8
AD-11080	gcgacugucucgcagagaa	559	gcgacugucucgcagagaaTT	560	uauucgagacagucgcTT	561	35 ± 6
AD-11081	ugucgcagacagaagcuga	562	ugucgcagacagaagcugaTT	563	ucagcuauucgucgagacaTT	564	33 ± 4
AD-11082	cucgacagaagcugacau	565	cucgacagaagcugacauTT	566	augucagcuauucgucgagTT	567	39 ± 7
AD-11083	aggggaaauagagugagca	568	aggggaaauagagugagcaTT	569	ugucacucauuuccaccuTT	570	27 ± 4
AD-11084	agugagcagcaacaucuu	571	agugagcagcaacaucuuTT	572	aaguuugugucgucacacuTT	573	23 ± 3
AD-11085	guuccgcagugauggcugu	574	guuccgcagugauggcuguTT	575	acagcacaucagucggaacTT	576	37 ± 4
AD-11086	caaccacaccgacuaccgc	577	caaccacaccgacuaccgcTT	578	gcgguagucggugugguTT	579	36 ± 5
AD-11087	aaccacaccgacuaccgc	580	aaccacaccgacuaccgcTT	581	agcgguaucggugugguTT	582	48 ± 10
AD-11088	accacaccgacuaccgcug	583	accacaccgacuaccgcugTT	584	cagcgguaucggugugguTT	585	42 ± 3
AD-11089	cccgaaaagcacacagucug	586	cccgaaaagcacacagucugTT	587	cagcgguaucggugugguTT	588	37 ± 2
AD-11090	uccagcacaagaauacuaa	589	uccagcacaagaauacuaaTT	590	uaaguuuauucgucggaTT	591	35 ± 4
AD-11091	uuggaaugugcaauagaga	592	uuggaaugugcaauagagaTT	593	ucucuauugcacaauuccaaTT	594	29 ± 6
AD-11092	agaucugaucagccuuucc	595	agaucugaucagccuuuccTT	596	ggaaggcugaucagauucTT	597	43 ± 3
AD-11093	caggcaauucagucucguu	598	caggcaauucagucucguuTT	599	aacgagacugaaugccugTT	600	31 ± 3
AD-11094	ggcaauucagucucguugu	601	ggcaauucagucucguuguTT	602	acaacgagacugaaugccTT	603	27 ± 3
AD-11095	gcaauucagucucguugug	604	gcaauucagucucguugugTT	605	caacaacgagacugaaugcTT	606	23 ± 3
AD-11096	aaucagucucguugugaa	607	aaucagucucguugugaaTT	608	uucaacaacgagacugaaTT	609	27 ± 3
AD-11097	ucagucucguugugaaaac	610	ucagucucguugugaaaacTT	611	guuuucaacacgagacugaTT	612	42 ± 8
AD-11098	aaaccuuucaaaccuacc	613	aaaccuuucaaaccuaccTT	614	gguugaguguaaagguuuTT	615	60 ± 7
AD-11099	cuuuccgugucguggcug	616	cuuuccgugucguggcugTT	617	cgagccagcacacggaaagTT	618	46 ± 4
AD-11100	ccgugucgucgucgucgcaug	619	ccgugucgucgucgucgcaugTT	620	caugcagccagcacacgTT	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	ucgacauccuugcuugcug	622	ucgacauccuugcuugcugTT	623	cgacaagaaggaugcgaTT	624	47 ± 4
AD-11102	ugcuugcgcggguagaa	625	ugcuugcgcggguagaaTT	626	uucuaaccggcgacaagcaTT	627	43 ± 8
AD-11103	gcuugcgcggguagaaa	628	gcuugcgcggguagaaaTT	629	uuuuaaccggcgacaagcTT	630	35 ± 7
AD-11104	cuugcgcggguagaaa	631	cuugcgcggguagaaaTT	632	uuuuuaccggcgacaagTT	633	37 ± 9
AD-11105	ggcccaguuuccaaugaa	634	ggcccaguuuccaaugaaTT	635	uucauuggcaaacugggccTT	636	39 ± 5
AD-11106	caguuucgucucuccacc	637	caguuucgucucuccaccTT	638	gguggagagacgaaccugTT	639	38 ± 8
AD-11107	ggcacgugucacuggaaac	640	ggcacgugucacuggaaacTT	641	uuuuccagugacacugccTT	642	39 ± 3
AD-11108	cuggaaaacagugaguccgg	643	cuggaaaacagugaguccggTT	644	cuugacucacuguuuccagTT	645	51 ± 3
AD-11109	caaauccaguuuggacc	646	caaauccaguuuggaccTT	647	gguccaacacuggganuugTT	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	agccuagggagugagaaa	655	agccuagggagugagaaaTT	656	uuuacucuaaccuccagguTT	657	34 ± 5
AD-11113	gucaacagcuacacacgug	658	gucaacagcuacacacgugTT	659	uucgugugagucuguuagcTT	660	42 ± 4
AD-11114	gaugguaccccaaacgggg	661	gaugguaccccaaacggggTT	662	cccgguuuuggugaccuacTT	663	49 ± 3
AD-11115	ugacagaaucggaaggggu	664	ugacagaaucggaagggguTT	665	accuuucgaguuucgucaTT	666	53 ± 8
AD-11116	gaagacgagauccucguc	667	gaagacgagauccucgucTT	668	gagcggagauccucguuTT	669	43 ± 7
AD-11117	acgagauccucgucagua	670	acgagauccucgucaguaTT	671	uacugagcggagauccucguTT	672	40 ± 9
AD-11118	aaccugaaaaggauccgcc	673	aaccugaaaaggauccgccTT	674	gggcgauccuuucagguTT	675	81 ± 7
AD-11119	gaucgcccacugcguagaac	676	gaucgcccacugcguagaacTT	677	uuuacgucaguuuccagguTT	678	50 ± 13
AD-11120	cacugcguagaacaucaca	679	cacugcguagaacaucacaTT	680	ugugaauuuucacgagugTT	681	40 ± 13
AD-11121	agaacuaucucuggacgu	682	agaacuaucucuggacguTT	683	acgucacagagauaguuTT	684	41 ± 8
AD-11122	gucaguccggguagaacuu	685	gucaguccggguagaacuuTT	686	aaguuuuaccggcagacTT	687	37 ± 10
AD-11123	ugaacaaagucuaucggaga	688	ugaacaaagucuaucggagaTT	689	uucucgagucuuuguucaTT	690	39 ± 6
AD-11124	aagucuaucggaguuucuu	691	aagucuaucggaguuucuuTT	692	agaaaacucuccgagacTT	693	40 ± 2
AD-11125	gucaucggagaguuucugu	694	gucaucggagaguuucuguTT	695	acagaaaacucuccgagacTT	696	37 ± 4
AD-11126	ggccaccguguguaaag	697	ggccaccguguguaaagTT	698	cuuuuacacaccgggucTT	699	48 ± 2
AD-11127	accguguguaaaggugu	700	accguguguaaagguguTT	701	acacuuuuacaccgggucTT	702	36 ± 2
AD-11128	cugacuuguuacgaaag	703	cugacuuguuacgaaagTT	704	cauuucguaaaacaagucagTT	705	33 ± 7
AD-11129	uguuuacgaaauguccaca	706	uguuuacgaaauguccacaTT	707	uguggacaauuucguaaaacTT	708	46 ± 8
AD-11130	ccaccgagccagcuugguc	709	ccaccgagccagcuuggucTT	710	gaccaagucgucggugTT	711	51 ± 12
AD-11131	caccgagccagcuuggucc	712	caccgagccagcuugguccTT	713	ggaccaagucgucggugTT	714	53 ± 15
AD-11132	caggcaaccgucguguc	715	caggcaaccgucgugucTT	716	gagacacgacgucggugTT	717	46 ± 6
AD-11133	aacgucgugucucugcca	718	aacgucgugucucugccaTT	719	uggcagagacgacgucuuTT	720	59 ± 6
AD-11134	uuuuuuuacguaacucuu	721	uuuuuuuacguaacucuuTT	722	agaguuuacguaaaaauaaTT	723	64 ± 16
AD-11135	uuuacguaacucuuuuuu	724	uuuacguaacucuuuuuuTT	725	auagaaagaguuuacguuuaTT	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	uaacguaaacucuuuuaug	727	uaacguaaacucuuuuaugTT	728	cauagaagaaguuuacguuaTT	729	72 ± 9
AD-11137	aacguaaacucuuuuaugc	730	aacguaaacucuuuuaugcTT	731	gcauagaagaaguuuacguuTT	732	68 ± 8
AD-11138	guaacucuuuuaugcccg	733	guaacucuuuuaugcccgTT	734	cgggcauagaagaaguuacTT	735	69 ± 10
AD-11139	uaugcccguaaaguaug	736	uaugcccguaaaguaugTT	737	cauacuuuacacgggcauaTT	738	102 ± 4
AD-11140	ugcccguaaaguaugug	739	ugcccguaaaguaugugTT	740	cacauacuuuacacgggcaTT	741	104 ± 9
AD-11141	ugagcaccgcugacaauu	742	ugagcaccgcugacaauuTT	743	aaaugucagcgggucucaTT	744	110 ± 25
AD-11142	caccgcugacaauuuccgu	745	caccgcugacaauuuccguTT	746	acggaaaugucagcgggugTT	747	50 ± 4
AD-11143	uuuuagucagagagugca	748	uuuuagucagagagugcaTT	749	ugcacucuccugacuaaaaTT	750	93 ± 17
AD-11144	agccaagucuuuaaaugg	751	agccaagucuuuaaauggTT	752	ccauuuuaaugacuuuggcuTT	753	62 ± 4
AD-11145	guuggcagucuaugugg	754	guuggcagucuauguggTT	755	ccacaugacagucgcccacTT	756	57 ± 4
AD-11146	gccuuuaagggaagcuacu	757	gccuuuaagggaagcuacuTT	758	aguaagcuuccuuuaagggcTT	759	74 ± 5
AD-11147	gcauaucgucgggcucaac	760	gcauaucgucgggcucaacTT	761	guugagcccagcgauaugcTT	762	61 ± 10
AD-11148	aaauagagucuaauaguaa	763	aaauagagucuaauaguaaTT	764	uuacuaaugagcucuaauuTT	765	61 ± 8
AD-11149	gugcccgugucgguuucuc	766	gugcccgugucgguuucucTT	767	gaaagaccgacacgggcacTT	768	66 ± 5
AD-11150	aaugaaaccaggguaagaa	769	aaugaaaccaggguaagaaTT	770	auucuaaccuguuuucuuTT	771	101 ± 7
AD-11151	caccagaauguaagcauc	772	caccagaauguaagcaucTT	773	agaugcuacaauucugggugTT	774	98 ± 8
AD-11152	gagcucgggacggaugua	775	gagcucgggacggauguaTT	776	uacuaucgucuccgagcucTT	777	77 ± 2
AD-11153	ugacaacugaaaggcaaccu	778	ugacaacugaaaggcaaccuTT	779	agguugccuuacaguuucaTT	780	86 ± 3
AD-11154	caacguggaccugccuaag	781	caacguggaccugccuaagTT	782	cguaggcagguccaccgguugTT	783	86 ± 4
AD-11155	gacugacgagagauuaa	784	gacugacgagagauuaaTT	785	uaaacuacucucgucagucTT	786	72 ± 2
AD-11156	acgagagauguauuuuua	787	acgagagauguauuuuuaTT	788	uuuuuuuucaucucucguTT	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	cmumummagumcmgagaaacmcmaaumgTT	790	cmuauugguucucgacumaaaagTT	791	24 ± 7
AL-DP-5997	gumcmaamaaagaaacmcmgumcmagTT	792	cugcmacggguucuuugagcTT	793	21 ± 5
AL-DP-5998	umcmggagumumcmaaacmcmaagcmcmTT	794	ggcuumaggguuagaaacucggaTT	795	36 ± 9
AL-DP-5999	gaaaumcmcmumgcmumummagumcmgagTT	796	ucgacumaaaagcmaggaauucTT	797	20 ± 4
AL-DP-6000	umcmcmumgcmumummagumcmgagaaacmTT	798	guucucgacumaaaagcmaggaTT	799	22 ± 3
AL-DP-6001	umumagumcmgagaaacmcmaaumgumTT	800	aucmauugguucucgacumaaTT	801	23 ± 7
AL-DP-6002	umagumcmgagaaacmcmaaumgumTT	802	cmuacmauugguucucgacumaaTT	803	20 ± 7
AL-DP-6003	cmumgcmumummagumcmgagaaacmcmaTT	804	ugguucucgacumaaaagcmagTT	805	26 ± 4
AL-DP-6004	cmgcmumgcmacmcmgaaacmcmaaaagaaTT	806	uucuuuggucggugcmagcTT	807	42 ± 7
AL-DP-6005	umgcmumummagumcmgagaaacmcmaaaTT	808	uugguucucgacumaaaagcmagTT	809	21 ± 8
AL-DP-6006	gaaacmumacmaumcmgumcmgagaaacmcmaTT	810	uacmaugaucgagumaguuucTT	811	21 ± 6
AL-DP-6007	umgaaacmumacmaumcmgumcmgagaaacmcmaTT	812	ccmaugaucgagumaguuucmaTT	813	21 ± 3
AL-DP-6008	cmaaaagaaacmcmgumgcmagaaacmcmaTT	814	uumaucugcmacgguuucuuugTT	815	21 ± 8
AL-DP-6009	cmcmcmacmumgcmgumgaaacmumumcmagTT	816	ugaauguuucmcmagcgggTT	817	22 ± 4
AL-DP-6010	umumumagumcmgagaaacmcmaaumgagTT	818	ucmauugguucucgacumaaaTT	819	31 ± 5
AL-DP-6011	umggaaumgumumcmcmgagaaumcmTT	820	gaauucuccggaaacmauuccmaTT	821	26 ± 4
AL-DP-6012	cmggagumumcmcaacmcmumaaagcmcmumTT	822	aggcuumaggguuagaaacucgTT	823	28 ± 6
AL-DP-6013	umggcmuuumumgaaumcmcmumgagcmTT	824	gcucmaugguucmaaaugccmaTT	825	34 ± 11
AL-DP-6014	umcmumggaaumgumcmcmgagaaacmcmaTT	826	uucuccggaaacmauucccmagTT	827	23 ± 7
AL-DP-6015	ggcmumgcmaaaumumumcmagagcmTT	828	gcucugumaaaauuugcmagcTT	829	29 ± 5
AL-DP-6016	gcmgumgaaacmumumcmcmagcmcmTT	830	uggcugugaauguuucmcmagcTT	831	17 ± 5
AL-DP-6017	umcmcmagguumumummagaaacmumgagcmTT	832	gucmaguuucmaumaaaacucggagTT	833	19 ± 5
AL-DP-6018	aggcmaaaagumcmumcmummagagTT	834	uccumaagagcmcuuugccuTT	835	22 ± 6
AL-DP-6019	aacmumacmaumcmgaaumcmumggagTT	836	cuccmaugaucgagumaguuTT	837	59 ± 10

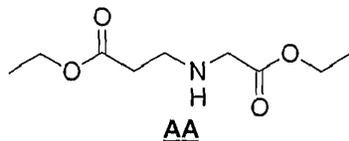
Duplex name	Sense strand sequence (5'-3')	SEQ ID NO:	Antisense strand sequence (5'-3')	SEQ ID NO:	Remaining HD gene mRNA [% of controls]
AL-DP-6020	cmuuumggaauumcmummaaaumcmTT	838	gauuumaggaauuuccmaaagTT	839	19 ± 11
AL-DP-6021	aumcmumgcmumumumagumcmgagaaTT	840	uucucgacumaaagcmaggauTT	841	35 ± 9
AL-DP-6022	acmumacmaumcmgaumcmumgagaaTT	842	ucucmaugaucgaugumaguTT	843	35 ± 18
AL-DP-6023	aaumcmumgcmumumumagumcmgagaaTT	844	ucucgacumaaagcmaggauTT	845	26 ± 16
AL-DP-6024	umgumcmcmagumumumumagumcmgTT	846	cmaguucmaumaaaccuggacmaTT	847	16 ± 5
AL-DP-6025	cmumcmggaumumcmmaacmcmumaaagcmTT	848	gcuumaggguugaacuccgagTT	849	24 ± 6
AL-DP-6026	umgaaumcmumgcmumumumagumcmgTT	850	cgacumaaagcmaggauuucmaTT	851	21 ± 6
AL-DP-6027	cmagcmumumgumcmcmagumumumumgTT	852	cmuumaaccucggacmaagcugTT	853	22 ± 6
AL-DP-6028	cmgumgaaacmaumcmcmagumcmgTT	854	cuggcugugaauugucmacgTT	855	33 ± 11
AL-DP-6029	cmumgcmumcmgumumumumagumcmgTT	856	ugucgaccmagucgagcmagTT	857	45 ± 15
AL-DP-6030	agcmumumgumcmcmagumumumumagTT	858	ucmaumaaaccucggacmaagcuTT	859	75 ± 15
AL-DP-6031	ggcmuaagumgcmumcmumumagggagTT	860	cuccumaaaggcmacuugcctTT	861	28 ± 10
AL-DP-6032	gaumcmuumumggaaumumcmummaaaTT	862	uumaggaaauuccmaauugcTT	863	25 ± 10
AL-DP-6033	cmacmumgcmgumgaaacmaumumcmacmaTT	864	ugugauguuucmacgcmagugTT	865	24 ± 3
AL-DP-6034	gumcmgagaaacmcmmaauumgumgcmTT	866	gccmaucmauugguuucucgacTT	867	20 ± 1
AL-DP-6035	cmumumgumcmcmagumumumumagaaacmTT	868	guucmaumaaaccucggacmaagTT	869	28 ± 9
AL-DP-6036	umgumgumgumgumcmumcmumgumcmgTT	870	uggccmaugaugccmaucmaacmaTT	871	50 ± 14
AL-DP-6037	cmacmaaaagaaacmcmgumgumgumgumTT	872	aucugcmacgguuucuuugugTT	873	20 ± 5

siRNA synthesis

Single-stranded RNAs were produced by solid phase synthesis on a scale of 1 μ mole 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).

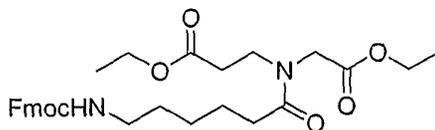
Deprotection and purification of the crude oligoribonucleotides by anion exchange HPLC were carried out according to established procedures. Yields and concentrations were determined by UV absorption of a solution of the respective RNA at a wavelength of 260 nm using a spectral photometer (DU 640B, Beckman 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 phosphorothioate 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 AA

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%).

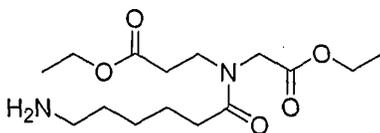
3-{Ethoxycarbonylmethyl-[6-(9H-fluoren-9-ylmethoxycarbonyl-amino)-hexanoyl]-amino}-propionic acid ethyl ester AB

AB

Fmoc-6-amino-hexanoic acid (9.12 g, 25.83 mmol) was dissolved in dichloromethane (50 mL) and cooled with ice. Diisopropylcarbodiimide (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**



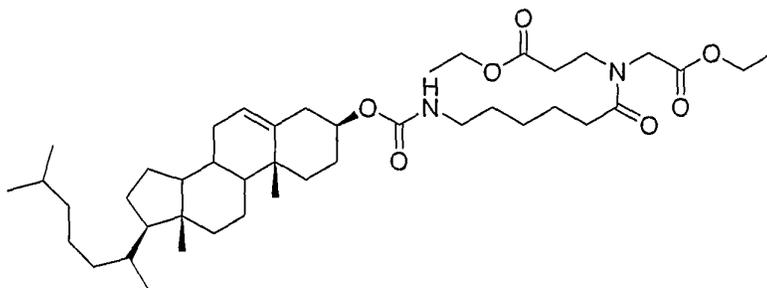
5

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.

10

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**



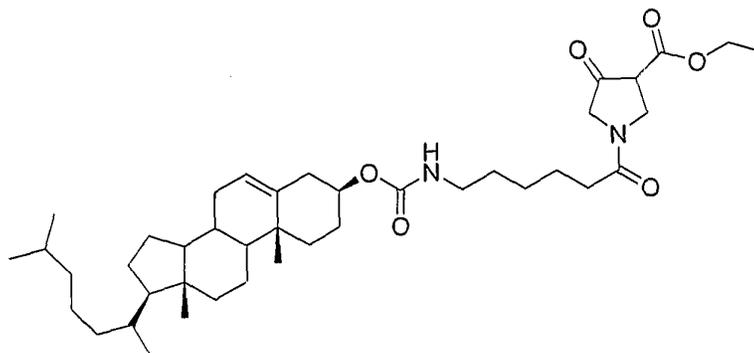
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AD

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-yloxy carbonylamino]-hexanoyl}-4-oxo-pyrrolidine-3-carboxylic acid ethyl ester **AE**



10

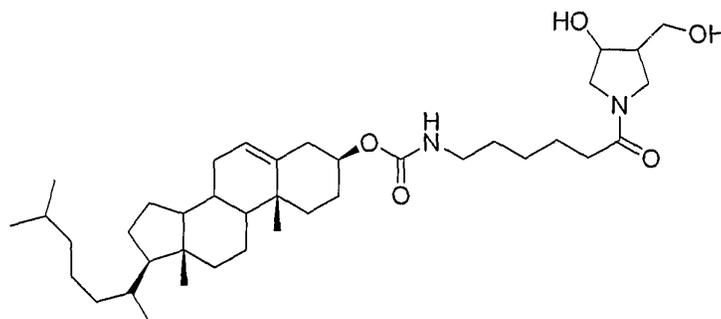
AE

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 NaH₂PO₄·H₂O 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

20

evaporated to dryness. The residue was purified by column chromatography using 25% ethylacetate/hexane to afford 1.9 g of b-ketoester (39%).

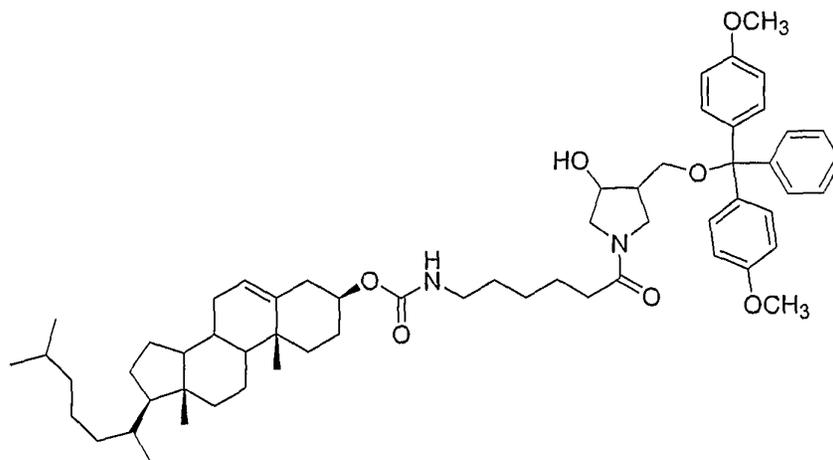
[6-(3-Hydroxy-4-hydroxymethyl-pyrrolidin-1-yl)-6-oxo-hexyl]-carbamic acid 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester **AF**



AF

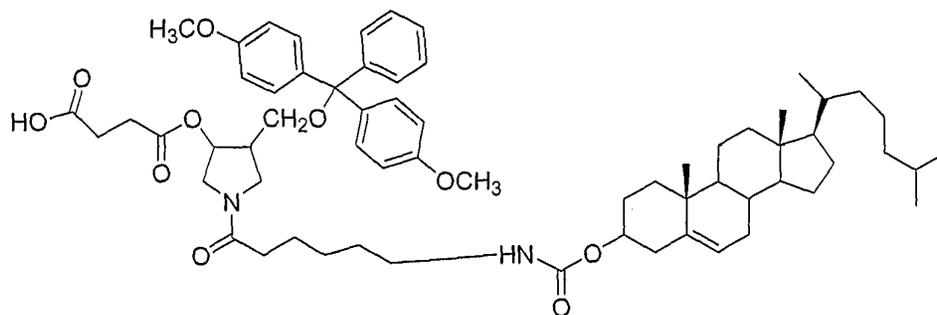
Methanol (2 mL) was added dropwise over a period of 1 h to a refluxing mixture of b-ketoester AE (1.5 g, 2.2 mmol) and sodium borohydride (0.226 g, 6 mmol) in tetrahydrofuran (10 mL). Stirring was continued at reflux temperature for 1 h. After cooling to room temperature, 1 N HCl (12.5 mL) was added, 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/CHCl₃) (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**

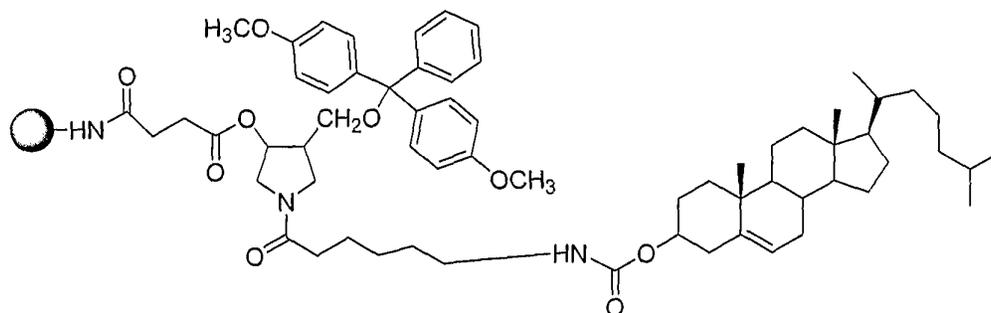
**AG**

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/CHCl₃) (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-yloxy-carbonylamino]-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
 5 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
 10 next step.

Cholesterol derivatised CPG AI**AI**

Succinate AH (0.254 g, 0.242 mmol) was dissolved in a mixture of
 15 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'- <i>O</i> -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
<u>A</u> , <u>C</u> , <u>G</u> , <u>T</u> , <u>U</u> , <u>a</u> , <u>c</u> , <u>g</u> , <u>t</u> , <u>u</u>	underlined: nucleoside-5'-phosphorothioate
<u>am</u> , <u>cm</u> , <u>gm</u> , <u>tm</u> , <u>um</u>	underlined: 2'- <i>O</i> -methyl-nucleoside-5'-phosphorothioate

^acapital 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% CO₂ in a humidified incubator (Heraeus HERAcell, Kendro Laboratory Products, Langenselbold, Germany).

For transfection with siRNA, HeLa cells were seeded at a density of 2.0×10^4 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 (Genospectra, 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, \pm 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, \pm 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] \pm SD	IC ₈₀ mean [nM] \pm SD	max. inhib. mean[%] \pm SD
AL-DP-5996	1.6 \pm 1.2	22 \pm 9	79 \pm 6
AL-DP-5997*	0.05 \pm 0.02	2 \pm 1	86 \pm 5
AL-DP-5999	0.3 \pm 0.3	8 \pm 4	82 \pm 4
AL-DP-6000*	0.1 \pm 0.1	5 \pm 3	80 \pm 2
AL-DP-6001*	0.1 \pm 0.1	3 \pm 1	83 \pm 1
AL-DP-6002	0.3 \pm 0.2	9 \pm 4	78 \pm 3
AL-DP-6003	0.3 \pm 0.2	3 \pm 2	83 \pm 3
AL-DP-6005	0.3 \pm 0.3	9 \pm 9	77 \pm 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% CO₂ 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% CO₂ in a

humidified incubator (Heraeus HERAccl, Kendro Laboratory Products, Langensfeld, 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
 5 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,
 10 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 IC ₅₀ mean [nM] +/- SD	U87MG IC ₅₀ 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

15

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').
 20 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 μ M 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.

AL-DP	% full length material after 48 hours			
	calf		swine	
	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

Table 6.

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'-gauuumaggaauuccmaau-cyclic-PO₄-3' (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	cmaumumggaauumcmcmumaaaaumcmTT	876	gauuumaggaauuccmaaumgTT	877
AL-DP-7101	gaumcmaumumggaauumcmcmumaaaTT	878	uumaggaauuccmaaugaucTT	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 μ M 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.

5 **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-
 10 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.

15 **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	gumcmacmaagaacmcmgumgcmagTT-sChol	880	cugcmacgguucuuugacTT	881
AL-DP-6983	umcmcmumgcmumumumagumcmgagaacmTT-sChol	882	guucucgacumaaagcmaggaTT	883
AL-DP-7130	cmaumumggaumumcmcmumaaaaumcmTT-sChol	884	gauuumaggaauccmaaumgTT	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
 20 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 IC₅₀ values; the mean IC₅₀ 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	gumcmacmaaagaacmcmgumgcmagTT	886	cugcmacgguucuugugacBB	887
AL-DP-1998	gumcmacmaaagaacmcmgumgcmagTT-Chol	888	cugcmacgguucuugugacBB	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 µL/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 µg 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

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0.25 μ L/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 μ g resulted in significant down-modulation of HD mRNA levels.

Additional Definition

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Prior art references

It is to be understood that any discussion of public documents, acts, materials, devices, articles or the like included herein is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters were common general knowledge in the field relevant to the present invention as it existed before the priority date of any claim of this application.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of a human *Huntingtin* (HD) gene in a cell, wherein said dsRNA comprises a sense strand and an antisense strand each comprising sequences that are complementary to each other, and wherein the sense strand comprises a first sequence and the antisense strand comprises a second sequence that is less than 30 nucleotides in length and comprises at least 15 contiguous nucleotides of SEQ ID NO: 793 and wherein said second sequence is substantially complementary to a part of an mRNA transcript of the HD gene.
2. The dsRNA according to claim 1, wherein the second sequence comprises at least 16 or 17 or 18 or 19 or 20 or 21 contiguous nucleotides of SEQ ID NO: 793.
3. The dsRNA according to claim 1 or 2, wherein the second sequence comprises the sequence set forth in SEQ ID NO: 793.
4. The dsRNA according to any one of claims 1 to 3, wherein the second sequence consists of the sequence set forth in SEQ ID NO: 793.
5. The dsRNA according to any one of claims 1 to 4, wherein the first sequence comprises at least 15 contiguous nucleotides of the sequence set forth in SEQ ID NO: 792.
6. The dsRNA according to any one of claims 1 to 5, wherein the first sequence comprises at least 16 or 17 or 18 or 19 or 20 or 21 contiguous nucleotides of SEQ ID NO: 792.
7. The dsRNA according to any one of claims 1 to 6, wherein the first sequence comprises the sequence set forth in SEQ ID NO: 792.

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8. The dsRNA according to any one of claims 1 to 7, wherein the first sequence consists of the sequence set forth in SEQ ID NO: 792.
9. The dsRNA according to any one of claims 1 to 8, wherein the first sequence comprises the sequence set forth in SEQ ID NO: 792 and the second sequence comprises the sequence set forth in SEQ ID NO: 793.
10. The dsRNA according to any one of claims 1 to 9, wherein the first sequence consists of the sequence set forth in SEQ ID NO: 792 and the second sequence consists of the sequence set forth in SEQ ID NO: 793.
11. The dsRNA according to any one of claims 1 to 10, wherein said dsRNA comprises at least one modified nucleotide.
12. The dsRNA according to claim 11, wherein at least one modified nucleotide is selected from the group consisting of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a terminal nucleotide linked to a cholesteryl derivative, a dodecanoic acid bisdecylamide group, a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural base-comprising nucleotide.
13. The dsRNA according to claim 11 or 12, wherein said dsRNA comprises at least one a 2'-O-methyl modified nucleotide and at least one nucleotide comprising a 5'-phosphorothioate group.
14. The dsRNA according to any one of claims 11 to 13, wherein the antisense strand comprises a 2'-O-methyl modified nucleotide at a position corresponding to

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- position 4 of SEQ ID NO: 793 and a phosphorothioate at a position corresponding to position 21 of SEQ ID NO: 793.
15. The dsRNA according to any one of claims 11 to 13, wherein the sense strand comprises a 2'-O-methyl modified nucleotide at each of positions corresponding to positions 2, 3, 5, 12, 13, 15, and 17 of SEQ ID NO: 792 and a 5'-phosphorothioate group at a position corresponding to position 21 of SEQ ID NO: 792.
16. The dsRNA according to any one of claims 11 to 13, wherein the sense strand comprises a 2'-O-methyl modified nucleotide at each of positions corresponding to positions 2, 3, 5, 12, 13, 15, and 17 of SEQ ID NO: 792 and a 5'-phosphorothioate group at a position corresponding to position 21 of SEQ ID NO: 792, and wherein the antisense strand comprises a 2'-O-methyl modified nucleotide at a position corresponding to position 4 of SEQ ID NO: 793 and a phosphorothioate at a position corresponding to position 21 of SEQ ID NO: 793.
17. The dsRNA according to any one of claims 1 to 16, wherein the antisense strand comprises at least one 5-bromo-2'-deoxyuridine residue at a 3'-end thereof.
18. The dsRNA according to any one of claims 1 to 17, wherein the dsRNA comprises a cholesterol moiety.
19. The dsRNA according to any one of claims 1 to 18, wherein the dsRNA is capable of inhibiting expression of a Huntingtin gene in a cell by at least 20%.
20. The dsRNA according to any one of claims 1 to 19, wherein the dsRNA is capable of reducing a level of an mRNA transcript of the HD gene in a HeLa cell by more than 70% compared to an siRNA duplex that targets expression of the HD gene in a HeLa cell.

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21. A vector comprising a regulatory sequence operably linked to at least one strand of the dsRNA according to any one of claims 1 to 20.
22. The vector according to claim 21 when used to express the dsRNA in a cell to thereby inhibit expression of a HD gene in the cell.
23. A cell comprising the dsRNA of any one of claims 1 to 20 or the vector of claim 21.
24. A pharmaceutical composition comprising the dsRNA according to any one of claims 1 to 20 or the vector according to claim 21 and a pharmaceutically acceptable carrier.
25. The pharmaceutical composition according to claim 24, wherein said composition comprises at least one additional dsRNA capable of targeting a different region of a *Huntingtin* (HD) gene to the dsRNA according to any one of claims 1 to 20, and wherein each said additional dsRNA comprises a sense strand and an antisense strand each comprising sequences that are complementary to each other, and wherein the sense strand comprises a first sequence and the antisense strand comprises a second sequence that is less than 30 nucleotides in length and comprises at least 15 contiguous nucleotides of an antisense strand sequence set forth in any one of Tables 1, 2, 7, 8 or 10 other than SEQ ID NO: 793.
26. The pharmaceutical composition according to claim 25, wherein the antisense strand of each additional dsRNA comprises at least 16 or 17 or 18 or 19 or 20 or 21 contiguous nucleotides of an antisense strand sequence set forth in any one of Tables 1, 2, 7, 8 or 10 other than SEQ ID NO: 793.

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27. The pharmaceutical composition according to claim 25 or 26, wherein the antisense strand of each additional dsRNA comprises an antisense strand sequence set forth in any one of Tables 1, 2, 7, 8 or 10 other than SEQ ID NO: 793.
28. The pharmaceutical composition according to any one of claims 25 to 27, wherein the antisense strand of each additional dsRNA consists of an antisense strand sequence set forth in any one of Tables 1, 2, 7, 8 or 10 other than SEQ ID NO: 793.
29. The pharmaceutical composition according to any one of claims 25 to 28, wherein the sense strand of each additional dsRNA comprises at least 15 contiguous nucleotides of a sense strand sequence set forth in any one of Tables 1, 2, 7, 8 or 10 other than SEQ ID NO: 792.
30. The pharmaceutical composition according to any one of claims 25 to 29, wherein the sense strand of each additional dsRNA comprises at least 16 or 17 or 18 or 19 or 20 or 21 contiguous nucleotides of a sense strand sequence set forth in any one of Tables 1, 2, 7, 8 or 10 other than SEQ ID NO: 792.
31. The pharmaceutical composition according to any one of claims 25 to 30, wherein the sense strand of each additional dsRNA comprises a sense sequence set forth in any one of Tables 1, 2, 7, 8 or 10 other than SEQ ID NO: 792.
32. The pharmaceutical composition according to any one of claims 25 to 31, wherein the sense strand of each additional dsRNA consists of a sense sequence set forth in any one of Tables 1, 2, 7, 8 or 10 other than SEQ ID NO: 792.
33. The pharmaceutical composition according to any one of claims 24 to 32 when formulated for administration to the brain of a patient.

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34. The pharmaceutical composition according to claim 34 when formulated for administration by intrastriatal infusion.
35. A method for inhibiting the expression of a *Huntingtin* (HD) gene in a cell, the method comprising:
- (a) introducing into the cell a double-stranded ribonucleic acid (dsRNA) according to any one of claims 1 to 20 or the vector according to claim 21 or the pharmaceutical composition according to any one of claims 24 to 34; and
 - (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of an mRNA transcript of the HD gene, thereby inhibiting expression of the HD gene in the cell.
36. The method according to claim 35, wherein the method comprises introducing the dsRNA, vector or pharmaceutical composition a mammalian cell *in vivo*.
37. A method of 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 a dsRNA according to any one of claims 1 to 20 or the vector according to claim 21 or the pharmaceutical composition according to any one of claims 24 to 34.
38. The method according to claim 36 or 37, wherein the method comprises introducing or administering the dsRNA, vector or pharmaceutical composition to the brain of the mammal or patient.
39. The method according to any one of claims 36 to 38, wherein the method comprises introducing or administering the dsRNA, vector or pharmaceutical composition to the brain of the mammal or patient by intrastriatal infusion.

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40. The method according to any one of claims 36 to 39, wherein the method comprises introducing or administering the dsRNA, vector or pharmaceutical composition to the brain of the mammal or patient to thereby decrease a level of an mRNA transcript of a *Huntingtin* (HD) gene in the striatum.
41. The method according to any one of claims 36 to 39, wherein the method comprises introducing or administering the dsRNA, vector or pharmaceutical composition to the brain of the mammal or patient to thereby decrease a level of an mRNA transcript of a *Huntingtin* (HD) gene in the cortex.
42. Use of a dsRNA according to any one of claims 1 to 20 or the vector according to claim 21 in the manufacture of a medicament for treating, preventing or managing Huntington's disease in a patient.
43. The use according to claim 42, wherein the medicament is a pharmaceutical composition according to any one of claims 24 to 34.
44. The dsRNA according to any one of claims 1 to 20 or the vector according to claim 21 or 22 or the cell according to claim 23 or the pharmaceutical composition according to any one of claims 24 to 34 or the method according to any one of claims 35 to 41 or the use according to claim 42 or 43 substantially as hereinbefore described with reference to the accompanying drawings and/or examples and/or Sequence Listing and comprising any one of Tables 1, 2, 7, 8 or 10.

DATED this ELEVENTH day of NOVEMBER, 2010

Alnylam Pharmaceuticals, Inc.

By patent attorneys for the applicant:

FB Rice & CO

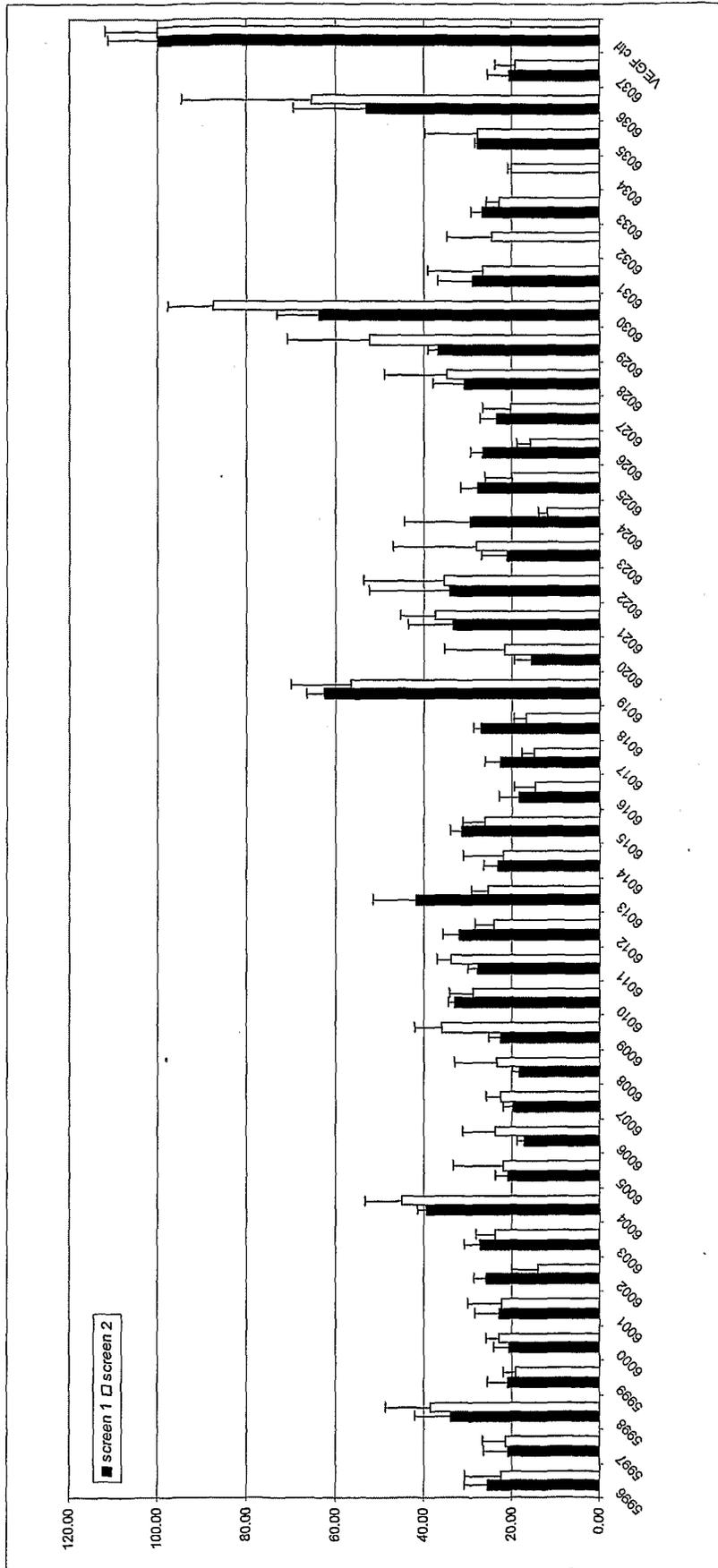


FIG. 1

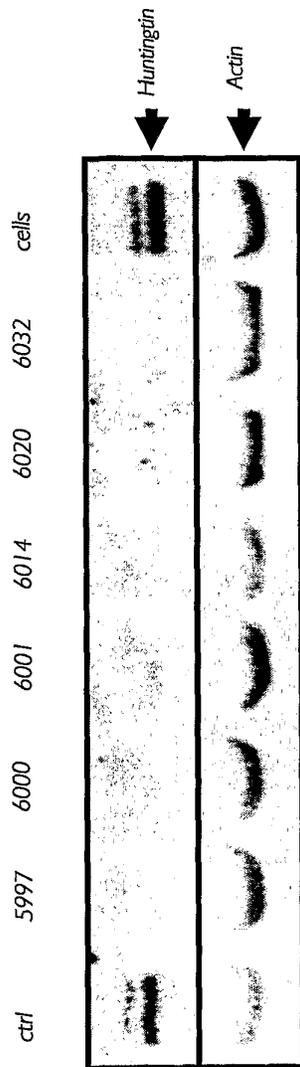
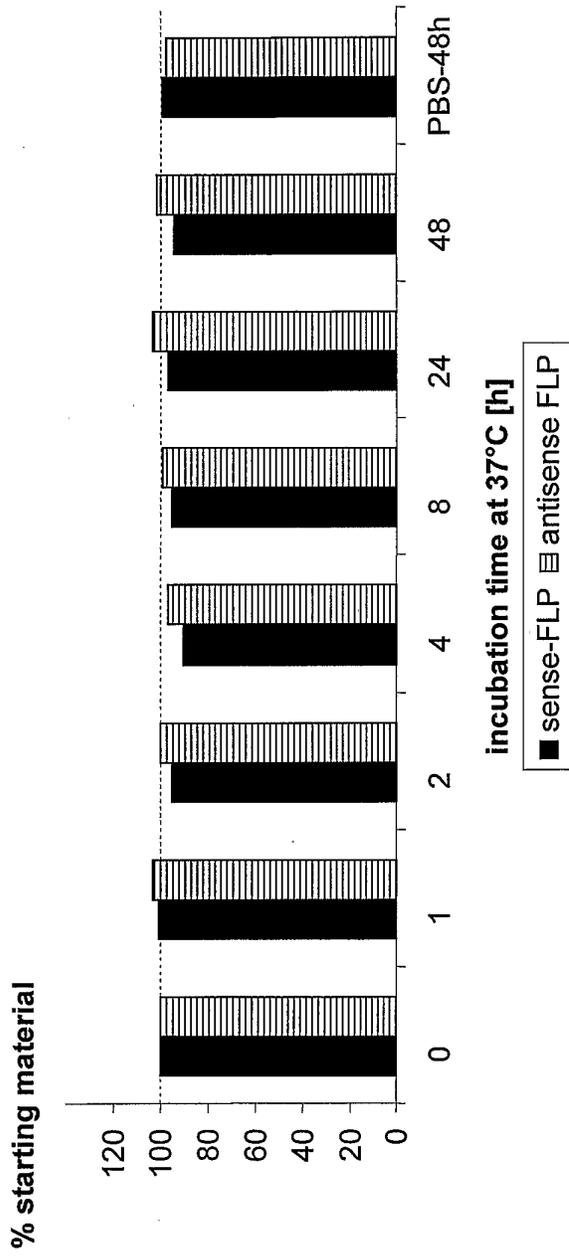


FIG. 2

AL-DP-5997



AL-DP-6020

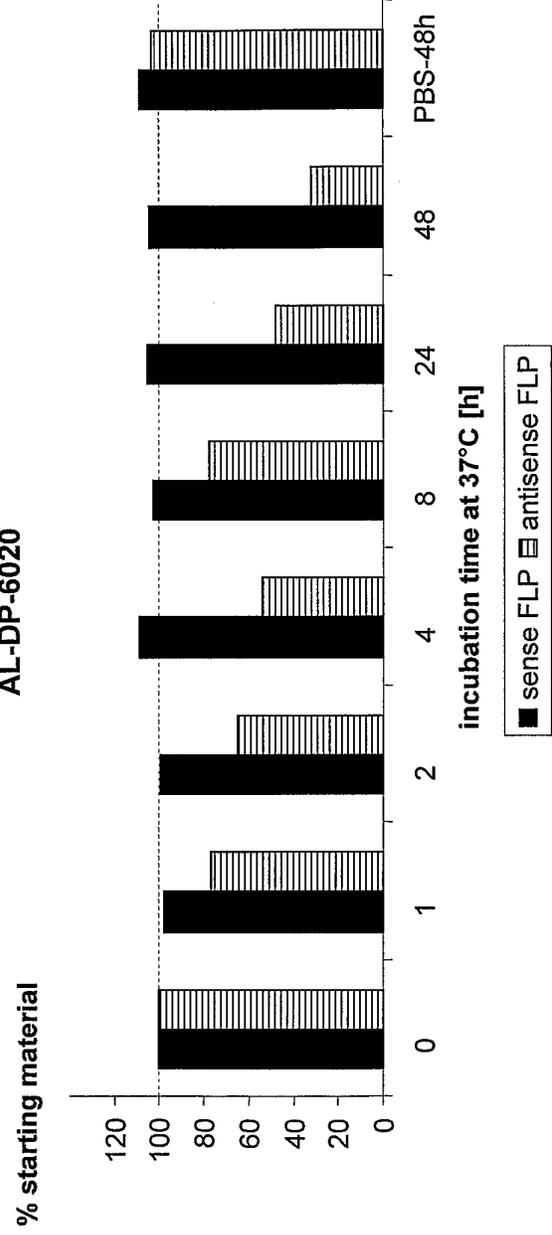


FIG. 3

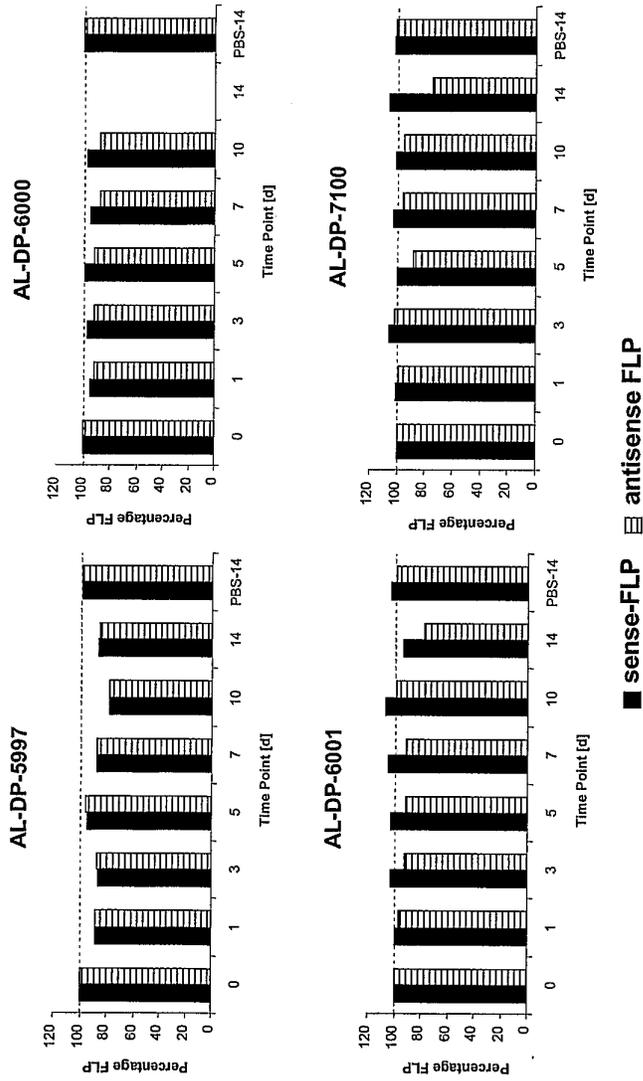


Figure 4

<110> Alnylam Pharmaceuticals, Inc.

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

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PCT/US2006/042420

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<210> 446

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FIG. 1

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FIG. 11 of WO 2007/051045, "FIG. 11"

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FIG. 11

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FIG. 10

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<210> 844

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<210> 849

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gcuuagguug aacuccgagt t 21

<210> 850

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<400> 850

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cagcuugucc agguuuaugt t 21

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<220>
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<400> 857
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<220>
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<222> 21
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<220>
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<222> 1, 2, 6, 10, 11, 12, 16, 18, 19
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<400> 858
agcuugucca gguuuau gat t 21

<210> 859
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<400> 859
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<210> 860
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<223> /mod_base = "2'-hydroxy corresponding base"

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<400> 862

gaucauugga auuccuaaat t 21

<210> 863

<211> 21

<212> DNA

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<222> 3, 12

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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

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

<400> 863

uuuaggaau ccaaugaut t 21

<210> 864

<211> 21

<212> DNA

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<222> 21

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

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ugugaauguu cacgcagugt t 21

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<211> 21
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<223> /mod_base = "5'-thio thymidine"

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gucgagaacc aaugauggct t 21

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

<220>
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<400> 867
gccaucaug guucugact t 21

<210> 868
<211> 21
<212> DNA
<213> Artificial sequence

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

<400> 868

cuuguccagg uuuugaact t 21

<210> 869

<211> 21

<212> DNA

<213> Artificial sequence

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<221> modified_base

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<400> 869

guucauaaac cuggacaagt t 21

<210> 870

<211> 21

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<213> Artificial sequence

<220>

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<221> modified_base

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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>

<221> modified_base

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<400> 870
ugugauggca ucauggccat t 21

<210> 871
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<220>
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<220>
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<220>
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uggccaugau gccaucacat t 21

<210> 872
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<220>
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<220>
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<400> 872
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<210> 873
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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
<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>
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<222> 6, 15
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<400> 874
gagggguagga aggccaag 18

<210> 875
<211> 20
<212> DNA
<213> Artificial sequence

<220>
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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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<222> 1, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18
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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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<220>
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<223> /mod_base = "5'-thio thymidine"

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

<400> 876

cauuggaauu ccuaaaau t 21

<210> 877

<211> 21

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<220>

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<220>

<221> modified_base

<222> 6, 15

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

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<220>

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<222> 21

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

<220>

<221> modified_base

<222> 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 20

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

<400> 877

gauuuuagga auuccaagt t 21

<210> 878

<211> 21

<212> DNA

<213> Artificial sequence

<220>

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<220>

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<222> 3, 4, 6, 7, 12, 13, 14, 15, 16

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

<220>

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<400> 878
gaucauugga auuccuaaat t 21

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<220>
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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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<220>
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<400> 880
gucacaaaga accgugcagt t 21

<210> 881
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<220>
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<220>
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<220>
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<400> 881
cugcacgguu cuuugugact t 21

<210> 882
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<220>
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<223> /mod_base = "2'-hydroxy corresponding base"

<400> 882
uccugcuuuu gucgagaact t 21

<210> 883
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<223> /mod_base = "5'-thio thymidine"

<220>
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<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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<222> 21
<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
caugggaauu ccuaaaauct t 21

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

<220>
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<400> 885
gauuuuagga auuccaaugt t 21

<210> 886
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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>

<221> modified_base

<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>

<221> modified_base

<222> 4

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

<400> 887

cugcacgguu cuuugugacu u 21

<210> 888

<211> 21

<212> DNA

<213> Artificial sequence

<220>

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<210> 889
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<220>
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<220>
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<220>
<221> modified_base
<222> 4
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