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WO-A1-2009/002944
WO-A1-2010/078536
WO-A1-2013/075035
WO-A1-2016/085852
WO-A1-2016/161388
WO-A1-2017/030973
WO-A2-2007/112414
WO-A2-2013/074974
US-A1- 2005 080 246
ALLERSON C R ET AL: "Fully 2'-modified oligonucleotide duplexes with improved in vitro potency and stability compared to unmodified small interfering RNA", JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, vol. 48, no. 4, 20 January 2005 (2005-01-20), pages 901-904, XP002404763, ISSN: 0022-2623, DOI: 10.1021/JM049167J
J. B. BRAMSEN ET AL: "A large-scale chemical modification screen identifies design rules to generate siRNAs with high activity, high stability and low toxicity", NUCLEIC ACIDS RESEARCH, vol. 37, no. 9, 12 March 2009

Fortsættes ...

(2009-03-12) , pages 2867-2881, XP055000747, ISSN: 0305-1048, DOI: 10.1093/nar/gkp106
SHEN WEN ET AL: "2'-Fluoro-modified phosphorothioate oligonucleotide can cause rapid degradation of P54nrb and PSF", NUCLEIC ACIDS RESEARCH, vol. 43, no. 9, 19 May 2015 (2015-05-19), pages 4569-4578, XP55863433, GB ISSN: 0305-1048, DOI: 10.1093/nar/gkv298 Retrieved from the Internet: URL:https://academic.oup.com/nar/article-p df/43/9/4569/5153015/gkv298.pdf>

DESCRIPTION

[0001] The present invention relates to products and compositions and their uses. In particular the invention relates to nucleic acid products that interfere with gene expression or inhibits its expression and therapeutic uses such as for the treatment of diseases and disorders.

Background

[0002] Double-stranded RNA (dsRNA) has been shown to block gene expression (Fire *et al,* 1998 and Elbashir *et al,* 2001) and this has been termed RNA interference (RNAi). Short dsRNAs direct gene-specific, post-transcriptional silencing in many organisms, including vertebrates, and has provided a new tool for studying gene function. RNAi is mediated by RNA-induced silencing complex (RISC), a sequence-specific, multi-component nuclease that destroys messenger RNAs homologous to the silencing trigger. Interfering RNA (iRNA) such as nucleic acid, antisense RNA, and micro-RNA are oligonucleotides that prevent the formation of proteins by gene-silencing i.e. inhibiting gene translation of the protein. Gene-silencing agents are becoming increasingly important for therapeutic applications in medicine.

[0003] However, delivery of nucleic acids, such as RNA, to cells avoiding degradation by cellular nucleases, whilst maintaining efficacy and target specificity has proved challenging to those in the field of developing nucleic acid molecules for therapeutic use.

[0004] According to Watts and Corey in the Journal of Pathology (2012; Vol 226, p 365-379) there are algorithms that can be used to design nucleic acid but none is perfect. It may take various experimental methods to identify potent nucleic acid, as algorithms do not take into account factors such as tertiary structure or the involvement of RNA binding proteins. Therefore the discovery of a potent nucleic acid with minimal off-target effects is a complex process but necessary for the pharmaceutical development of these highly charged molecules to be synthesised economically, distributed to target tissues, enter cells and function within acceptable limits of toxicity. Thus, means for efficient delivery of oligonucleotides, in particular double stranded siRNAs, to cells in vivo is becoming increasingly important and requires specific targeting and substantial protection from the extracellular environment, particularly serum proteins. One method of achieving specific targeting is to conjugate a targeting moiety to the iRNA duplex agent. The targeting moiety helps in targeting the iRNA duplex agent to the required target site and there is a need to design appropriate targeting moieties for the desired receptor sites for the conjugated molecules to be taken up by the cells such as by endocytosis.

[0005] However, targeting ligands developed so far do not always translate to in vivo setting and there is a clear need for more efficacous receptor specific ligand conjugated iRNA duplex agents and methods for their preparation for the in vivo delivery of oligonucleotide therapeutics, nucleic acids and double stranded siRNAs.

[0006] Rather than a lipid delivery system alone, the present invention addresses the structure of the nucleic acid itself.

[0007] Accordingly, the present invention provides a nucleic acid for inhibiting expression of a target gene in a cell, comprising at least one duplex region that comprises at least a portion of a first strand and at least a portion of a second strand that is at least partially complementary to the first strand, wherein said first strand is at least partially complementary to at least a portion of a RNA transcribed from said target gene,

wherein all nucleotides of the nucleic acid are modified at the 2' position of the sugar, wherein positions 2 and 14 on the first strand starting from the 5' end are modified with 2' fluoro,

wherein the nucleic acid is modified on the first strand with alternating 2' O-methyl modifications and 2' fluoro modifications, and

wherein the second strand is modified with 2' fluoro modifications at positions 11-13 counting from the 3' end starting at the first position of the double strand region and the remaining modifications are 2' O-methyl modifications.

[0008] The first strand and the second strand may be separate strands.

[0009] The nucleic acid may comprise a single strand that comprises the first strand and the second strand.

[0010] The first strand and/or said second strand may each be from 17-35 nucleotides in length and the at least one duplex region may be from 10-25 nucleotides in length. The duplex may comprise two separate strands or it may comprise a single strand which comprises the first strand and the second strand.

[0011] In one aspect the second strand may be as short as 11 nucleotides in length such as 11, 12, 13, 14, 15, 16, 17, 18 , 19 nucleotides or more.

[0012] The nucleic acid may: a) be blunt ended at both ends; b) have an overhang at one end and a blunt end at the other; or c) have an overhang at both ends.

[0013] A nucleic acid of the invention may comprise a phosphorothioate linkage between the terminal one, two or three 3' nucleotides and/or one two or three 5' nucleotides of the first and/or the second strand. It may comprise two phosphorothioate linkages between each of the three terminal 3' and between each of the three terminal 5' nucleotides on the first strand, and two phosphorothioate linkages between the three terminal nucleotides of the 3' end of the second strand.

[0014] Such a nucleic acid may be conjugated to a ligand.

[0015] The invention further provides a nucleic acid for inhibiting expression of a target gene in a cell, comprising at least one duplex region that comprises at least a portion of a first strand and at least a portion of a second strand that is at least partially complementary to the first strand, wherein said first strand is at least partially complementary to at least a portion of a RNA transcribed from said target gene wherein said first strand includes modified nucleotides or unmodified nucleotides at a plurality of positions in order to facilitate processing of the nucleic acid by RISC, and wherein the nucleotide sequence is conjugated to a ligand.

[0016] The ligand may comprise (i) one or more N-acetyl galactosamine (GalNac) moieties and derivatives thereof, and (ii) a linker, wherein the linker conjugates the GalNac moieties to a sequence as defined in any preceding aspects. The linker may be a bivalent or trivalent or tetravalent branched structure. The nucleotides may be modified as defined herein.

[0017] The ligand may comprise the formula I:

$$[S\text{-}X^1\text{-}P\text{-}X^2]_3\text{-}A\text{-}X^3\text{-} \qquad (I)$$

wherein:

S represents a saccharide, wherein the saccharide is N-acetyl galactosamine;

X^1 represents C_3-C_6 alkylene or $(\text{-}CH_2\text{-}CH_2\text{-}O)_m(\text{-}CH_2)_2$- wherein m is 1, 2, or 3;

P is a phosphate or modified phosphate (preferably a thiophosphate);

X^2 is alkylene or an alkylene ether of the formula $(\text{-}CH_2)_n\text{-}O\text{-}CH_2$- where n = 1- 6;

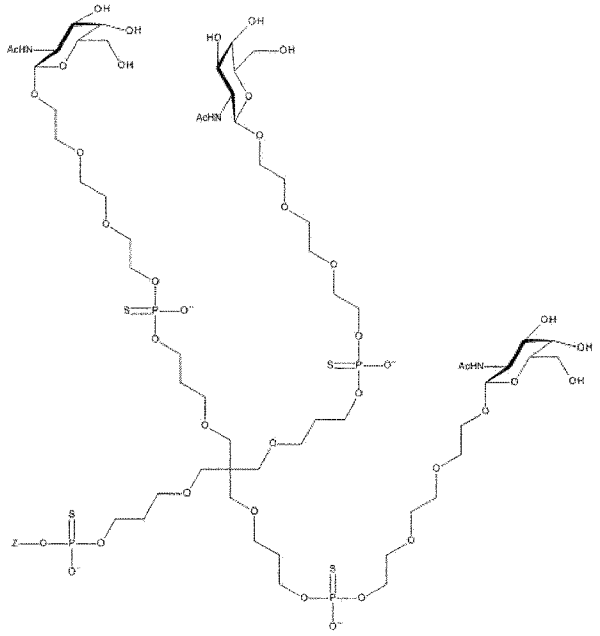
A is a branching unit;

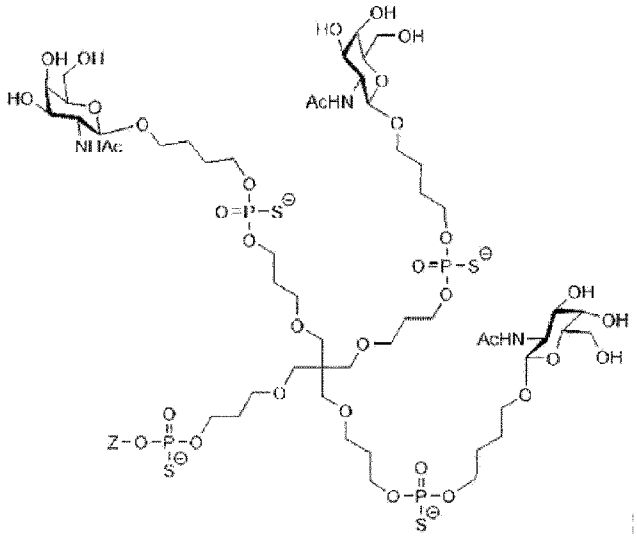
X^3 represents a bridging unit;

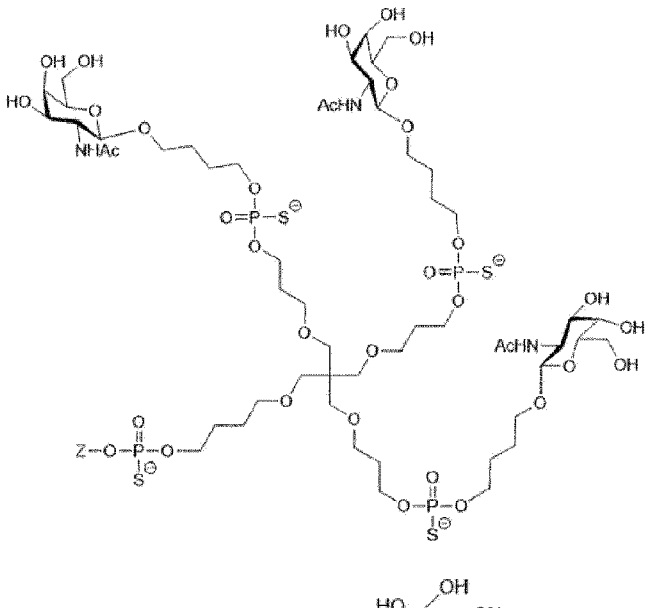
wherein a nucleic acid according to the present invention is conjugated to X^3 via a phosphate or modified phosphate (preferably a thiophosphate).

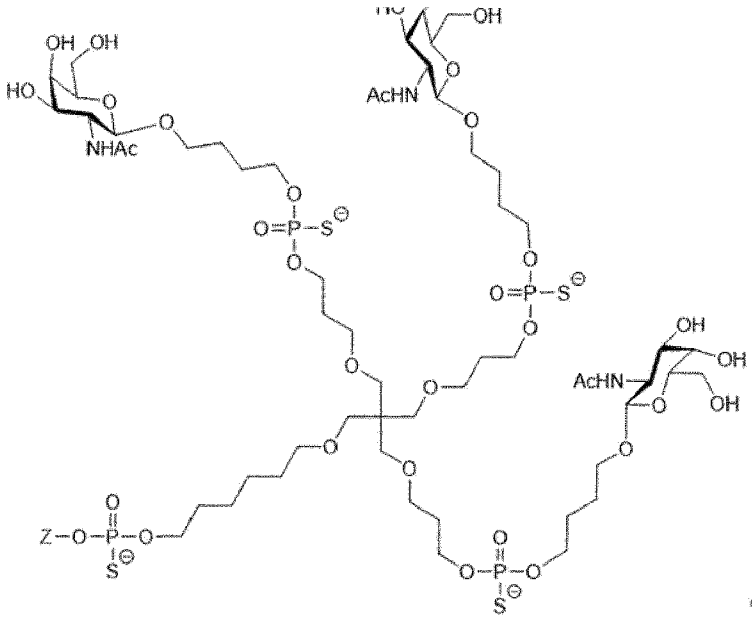
[0018] The present invention therefore additionally provides a conjugated nucleic acid having one of the following

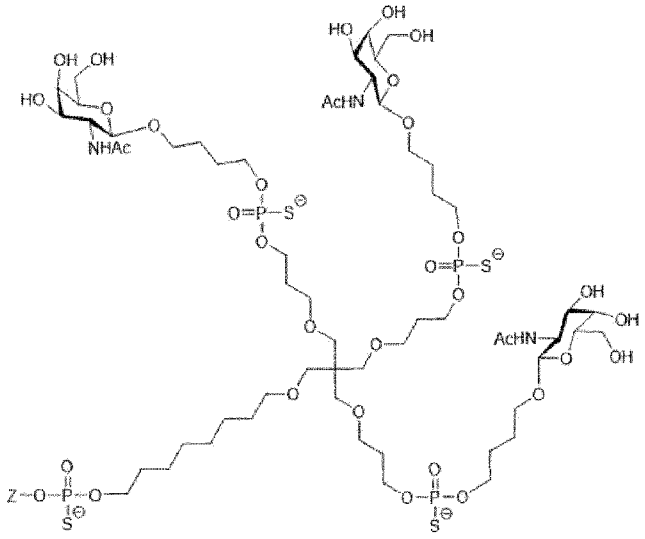
structures

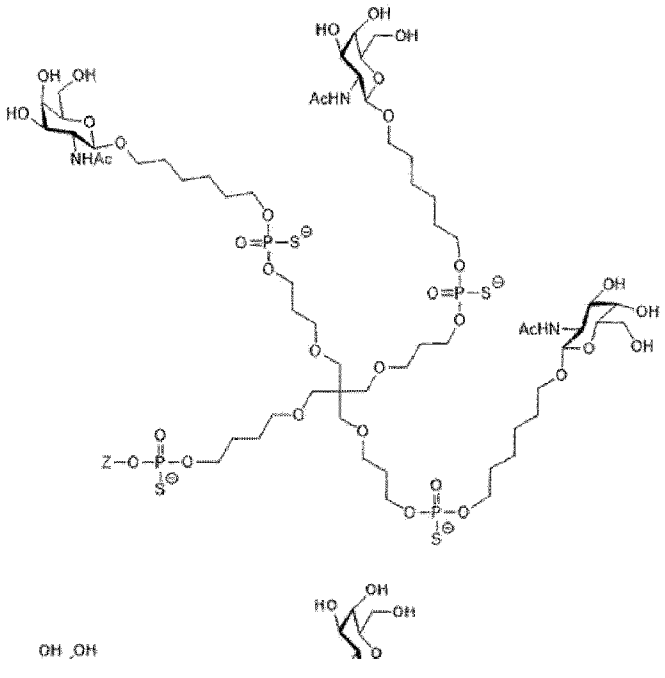


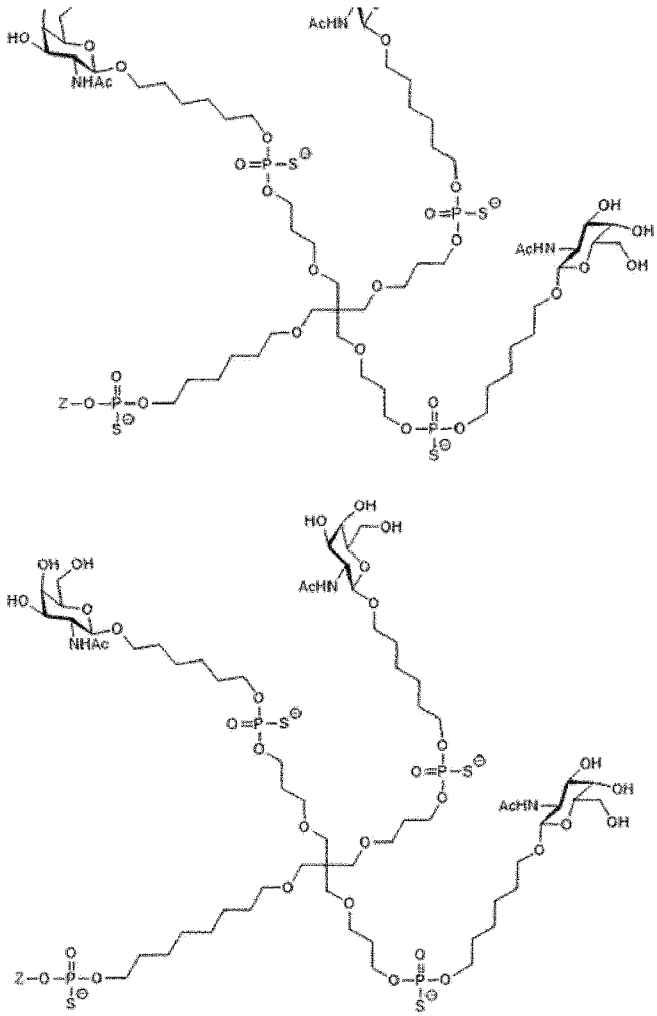






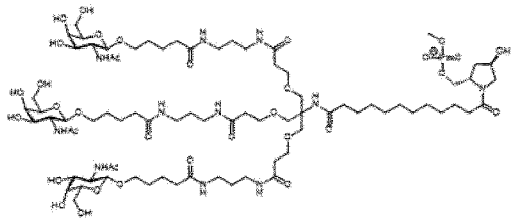






wherein Z represents a nucleic acid as defined herein before.

[0019] The ligand may comprise

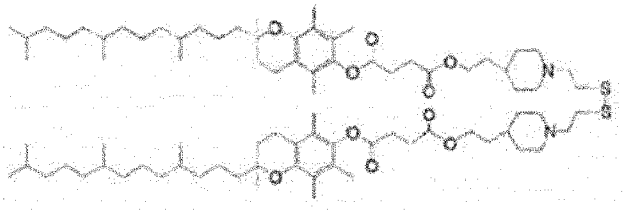


[0020] The invention also provides a composition comprising a nucleic acid or conjugated nucleic acid as defined herein and a physiologically acceptable excipient. The composition can comprise the following excipients:

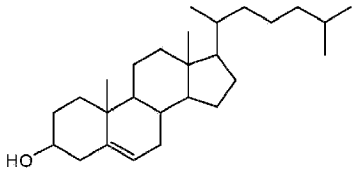
1. i) a cationic lipid, or a pharmaceutically acceptable salt thereof;
2. ii) a steroid;
3. iii) a phosphatidylethanolamine phospholipid;
4. iv) a PEGylated lipid.

[0021] The content of the cationic lipid component in the composition may be from about 55 mol% to about 65 mol% of the overall lipid content of the lipid formulation, preferably about 59 mol% of the overall lipid content of the lipid composition.

[0022] The composition may comprise a cationic lipid having the structure

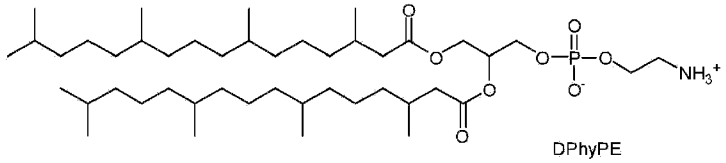


a steroid having the structure



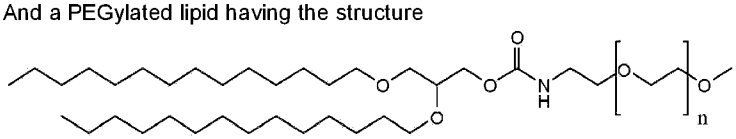
Cholesterol

a phosphatidylethanolamine phospholipid having the structure



DPhyPE

And a PEGylated lipid having the structure



mPEG-2000-DMG

[0023] A method of making a nucleic acid or conjugated nucleic acid according to the invention is also disclosed.

Detailed Description

[0024] The present invention relates to a nucleic acid which is double stranded and directed to an expressed RNA transcript of a target gene and compositions thereof. These nucleic acids can be used in the treatment of a variety of diseases and disorders where reduced expression of the target gene product is desirable.

[0025] The invention relates to a nucleic acid for inhibiting expression of a target gene in a cell, as described in the claims.

[0026] By nucleic acid it is meant a nucleic acid comprising two strands comprising nucleotides, that is able to interfere with gene expression. Inhibition may be complete or partial and results in down regulation of gene expression in a targeted manner. The nucleic acid comprises two separate polynucleotide strands; the first strand, which may also be a guide strand; and a second strand, which may also be a passenger strand. The first strand and the second strand may be part of the same polynucleotide strand that is self complementary which 'folds' to form a double stranded molecule. The nucleic acid may be an siRNA molecule.

[0027] The nucleic acid may comprise ribonucleotides, modified ribonucleotides, deoxynucleotides, deoxyribonucleotides, or nucleotide analogues. The nucleic acid may further comprise a double-stranded nucleic acid portion or duplex region formed by all or a portion of the first strand (also known in the art as a guide strand) and all or a portion of the second strand (also known in the art as a passenger strand). The duplex region is defined as beginning with the first base pair formed between the first strand and the second strand and ending with the last base pair formed between the first strand and the second strand, inclusive.

[0028] By duplex region is meant the region in two complementary or substantially complementary oligonucleotides that form base pairs with one another, either by Watson-Crick base pairing or any other manner that allows for a duplex between oligonucleotide strands that are complementary or substantially complementary. For example, an

oligonucleotide strand having 21 nucleotide units can base pair with another oligonucleotide of 21 nucleotide units, yet only 19 nucleotides on each strand are complementary or substantially complementary, such that the "duplex region" consists of 19 base pairs. The remaining base pairs may exist as 5' and 3' overhangs, or as single stranded regions. Further, within the duplex region, 100% complementarity is not required; substantial complementarity is allowable within a duplex region. Substantial complementarity refers to complementarity between the strands such that they are capable of annealing under biological conditions. Techniques to empirically determine if two strands are capable of annealing under biological conditions are well known in the art. Alternatively, two strands can be synthesised and added together under biological conditions to determine if they anneal to one another.

[0029] The portion of the first strand and second strand that form at least one duplex region may be fully complementary and are at least partially complementary to each other.

[0030] Depending on the length of an nucleic acid, a perfect match in terms of base complementarity between the first strand and second strand is not necessarily required. However, the first and second strands must be able to hybridise under physiological conditions.

[0031] The complementarity between the first strand and second strand in the at least one duplex region may be perfect in that there are no nucleotide mismatches or additional/deleted nucleotides in either strand. Alternatively, the complementarity may not be perfect. The complementarity may be at least 70%, 75%, 80%, 85%, 90% or 95%.

[0032] The first strand and the second strand may each comprise a region of complementarity which comprises at least 15 contiguous nucleotides.

[0033] The nucleic acid involves the formation of a duplex region between all or a portion of the first strand and a portion of the target nucleic acid. The portion of the target nucleic acid that forms a duplex region with the first strand, defined as beginning with the first base pair formed between the first strand and the target sequence and ending with the last base pair formed between the first strand and the target sequence, inclusive, is the target nucleic acid sequence or simply, target sequence. The duplex region formed between the first strand and the second strand need not be the same as the duplex region formed between the first strand and the target sequence. That is, the second strand may have a sequence different from the target sequence however, the first strand must be able to form a duplex structure with both the second strand and the target sequence.

[0034] The complementarity between the first strand and the target sequence may be perfect (no nucleotide mismatches or additional/deleted nucleotides in either nucleic acid).

[0035] The complementarity between the first strand and the target sequence may not be perfect. The complementarity may be at least 70%, 80%, 85%, 90% or 95%.

[0036] The identity between the first strand and the complementary sequence of the target sequence may be at least 75%, 80%, 85%, 90% or 95%, provided an nucleic acid is capable of reducing or inhibiting the expression of the target gene.

[0037] The nucleic acid may be able to reduce expression of the target gene by at least 25%, 50% or 75% of a comparative nucleic acid with perfect identity to the first strand and target sequence.

[0038] The nucleic acid may comprise a first strand and a second strand that are each from 17-35 or 19-25 nucleotides in length. The first strand and the second strand may be of different lengths.

[0039] The nucleic acid may be 15-25 nucleotide pairs in length. The nucleic acid may be 17-23 nucleotide pairs in length. The nucleic acid may be 17-25 nucleotide pairs in length. The nucleic acid may be 23-24 nucleotide pairs in length. The nucleic acid may be 19-21 nucleotide pairs in length. The nucleic acid may be 21-23 nucleotide pairs in length.

[0040] The nucleic acid may comprise a duplex region that consists of 19-25 nucleotide base pairs. The duplex region may consist of 17, 18, 19, 20, 21, 22, 23, 24 or 25 base pairs which may be contiguous.

[0041] The nucleic acid may be blunt ended at both ends; have an overhang at one end and a blunt end at the other end; or have an overhang at both ends.

[0042] An "overhang" as used herein has its normal and customary meaning in the art, i.e. a single stranded portion of a nucleic acid that extends beyond the terminal nucleotide of a complementary strand in a double strand nucleic acid. The term "blunt end" includes double stranded nucleic acid whereby both strands terminate at the same position, regardless of whether the terminal nucleotide(s) are base paired. The terminal nucleotide of a first strand and a second strand at a blunt end may be base paired. The terminal nucleotide of a first strand and a second strand at a blunt end may not be paired. The terminal two nucleotides of an first strand and a second strand at a blunt end may be base paired. The terminal two nucleotides of an first strand and a second strand at a blunt end may not be paired.

[0043] The nucleic acid may have an overhang at one end and a blunt end at the other. The nucleic acid may have an overhang at both ends. The nucleic acid may be blunt ended at both ends. The nucleic acid may be blunt ended at the end with the 5'-end of the first strand and the 3'-end of the second strand or at the 3'-end of the first strand and the 5'-end of the second strand.

[0044] The nucleic acid may comprise an overhang at a 3'- or 5'-end. The nucleic acid may have a 3'-overhang on the first strand. The nucleic acid may have a 3'-overhang on the second strand. The nucleic acid may have a 5'-overhang on the first strand. The nucleic acid may have a 5'-overhang on the second strand. The nucleic acid may have an overhang at both the 5'-end and 3'-end of the first strand. The nucleic acid may have an overhang at both the 5'-end and 3'-end of the second strand. The nucleic acid may have a 5' overhang on the first strand and a 3' overhang on the second strand. The nucleic acid may have a 3' overhang on the first strand and a 5' overhang on the second strand. The nucleic acid may have a 3' overhang on the first strand and a 3' overhang on the second strand. The nucleic acid may have a 5' overhang on the first strand and a 5' overhang on the second strand.

[0045] An overhang at the 3'-end or 5' end of the second strand or the first strand may be selected from consisting of 1, 2, 3, 4 and 5 nucleotides in length. Optionally, an overhang may consist of 1 or 2 nucleotides, which may or may not be modified.

[0046] Unmodified polynucleotides, particularly ribonucleotides, may be prone to degradation by cellular nucleases, and, as such, modified nucleotides are included in the nucleic acid of the invention.

[0047] Nucleotides of the nucleic acid of the invention are modified at least as set out in the claims.

[0048] Modifications of the nucleic acid of the present invention generally provide a powerful tool in overcoming potential limitations including, but not limited to, in vitro and in vivo stability and bioavailability inherent to native RNA molecules. Modified nucleic acid can also minimise the possibility of inducing interferon activity in humans. Modification can further enhance the functional delivery of a nucleic acid to a target cell.

[0049] The nucleic acid may comprise a nucleotide comprising a modified nucleotide, wherein the base is selected from 2-aminoadenosine, 2,6-diaminopurine,inosine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidine (e.g., 5-methylcytidine), 5-alkyluridine (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine), 6-azapyrimidine, 6-alkylpyrimidine (e.g. 6-methyluridine), propyne, quesosine, 2-thiouridine, 4-thiouridine, wybutosine, wybutoxosine, 4-acetylcytidine, 5-(carboxyhydroxymethyl)uridine, 5'-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluridine, beta-D-galactosylqueosine, 1-methyladenosine, 1-methylinosine, 2,2-dimethylguanosine, 3-methylcytidine, 2-methyladenosine, 2-methylguanosine, N6-methyladenosine, 7-methylguanosine, 5-methoxyaminomethyl-2-thiouridine, 5-methylaminomethyluridine, 5-methylcarbonylmethyluridine, 5-methyloxyuridine, 5-methyl-2-thiouridine, 2-methylthio-N6-isopentenyladenosine, beta-D-mannosylqueosine, uridine-5-oxyacetic acid and 2-thiocytidine. Unmodified RNA refers to a molecule in which the components of the nucleic acid, namely sugars, bases, and phosphate moieties, are the same or essentially the same as that which occur in nature, for example as occur naturally in the human body. Modified nucleotide as used herein refers to a nucleotide in which one or more of the components of the nucleic acid, namely sugars, bases, and phosphate moieties, are different from that which occur in nature. While they are referred to as modified nucleotides they will of course, because of the modification, include molecules which are not nucleotides, for example a polynucleotide molecules in which the ribophosphate backbone is replaced with a non-ribophosphate construct that allows hybridisation between strands i.e. the modified nucleotides mimic the ribophosphate backbone.

[0050] Many of the modifications described below that occur within a nucleic acid will be repeated within a polynucleotide molecule, such as a modification of a base, or a phosphate moiety, or the a non-linking O of a phosphate moiety. In some cases the modification will occur at all of the possible positions/nucleotides in the polynucleotide but in many cases it will not. A modification may only occur at a 3' or 5' terminal position, may only occur in a terminal regions, such as at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand. A modification may occur in a double strand region, a single strand region, or in both. A modification may occur only in the double strand region of an nucleic acid of the invention or may only occur in a single strand region of an nucleic acid of the invention. A phosphorothioate modification at a non-linking O position may only occur at one or both termini, may only occur in a terminal region, e.g., at a position on a terminal nucleotide or in the last 2, 3, 4 or 5 nucleotides of a strand, or may occur in duplex and/or in single strand regions, particularly at termini. The 5' end or 3' ends may be phosphorylated.

[0051] Stability of an nucleic acid of the invention may be increased by including particular bases in overhangs, or to include modified nucleotides, in single strand overhangs, e.g., in a 5' or 3' overhang, or in both. Purine nucleotides may be included in overhangs. All or some of the bases in a 3' or 5' overhang may be modified. Modifications can include the use of modifications at the 2' OH group of the ribose sugar, the use of deoxyribonucleotides, instead of ribonucleotides, and modifications in the phosphate group, such as phosphothioate modifications. Overhangs need not be homologous with the target sequence.

[0052] The 5'- or 3'- overhangs at the first strand, second strand or both strands of the dsRNA agent of the invention may be phosphorylated. In some embodiments, the overhang region contains two nucleotides having a phosphorothioate between the two nucleotides, where the two nucleotides can be the same or different. In one embodiment, the overhang is present at the 3 '-end of the first strand, second strand or both strands. In one embodiment, this 3 '-overhang is present in the first strand. In one embodiment, this 3 '-overhang is present in the second strand.

[0053] Nucleases can hydrolyze nucleic acid phosphodiester bonds. However, chemical modifications to nucleic acids can confer improved properties, and, can render oligoribonucleotides more stable to nucleases.

[0054] Modified nucleic acids, as used herein, can include one or more of:

1. (i) alteration, e.g., replacement, of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens (referred to as linking even if at the 5' and 3' terminus of the nucleic acid of the invention);
2. (ii) alteration, e.g., replacement, of a constituent of the ribose sugar, e.g., of the 2' hydroxyl on the ribose sugar;
3. (iii) replacement of the phosphate moiety with "dephospho" linkers;
4. (iv) modification or replacement of a naturally occurring base;
5. (v) replacement or modification of the ribose-phosphate backbone;
6. (vi) modification of the 3' end or 5' end of the RNA, e.g., removal, modification or replacement of a terminal phosphate group or conjugation of a moiety, e.g., a fluorescently labeled moiety, to either the 3' or 5' end of RNA.

[0055] The terms replacement, modification, alteration, indicates a difference from a naturally occurring molecule.

[0056] Specific modifications are discussed in more detail below.

[0057] Examples of modified phosphate groups include phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen phosphonates, phosphoroamidates, alkyl or aryl phosphonates and phosphotriesters. Phosphorodithioates have both non-linking oxygens replaced by sulphur. One, each or both non-linking oxygens in the phosphate group can be independently any one of S, Se, B, C, H, N, or OR (R is alkyl or aryl).

[0058] The phosphate linker can also be modified by replacement of a linking oxygen with nitrogen (bridged phosphoroamidates), sulfur (bridged phosphorothioates) and carbon (bridged methylenephosphonates). The replacement can occur at a terminal oxygen. Replacement of the non-linking oxygens with nitrogen is possible.

[0059] The sugar group can also contain one or more carbons that possess the opposite stereochemical configuration

than that of the corresponding carbon in ribose. Thus, a modified nucleotides may contain a sugar such as arabinose.

[0060] Modified nucleotides can also include "abasic" sugars, which lack a nucleobase at C-l'. These abasic sugars can further contain modifications at one or more of the constituent sugar atoms.

[0061] The 2' modifications may be used in combination with one or more phosphate linker modifications (e.g., phosphorothioate).

[0062] The phosphate group can be replaced by non-phosphorus containing connectors.

[0063] Examples of moieties which can replace the phosphate group include siloxane, carbonate, carboxymethyl, carbamate, amide, thioether, ethylene oxide linker, sulfonate, sulfonamide, thioformacetal, formacetal, oxime, methyleneimino, methylenemethylimino, methylenehydrazo, methylenedimethylhydrazo and methyleneoxymethylimino. In certain embodiments, replacements may include the methylenecarbonylamino and methylenemethylimino groups.

[0064] The phosphate linker and ribose sugar may be replaced by nuclease resistant nucleotides.

[0065] Examples include the mophilino, cyclobutyl, pyrrolidine and peptide nucleic acid (PNA) nucleoside surrogates. In certain embodiments, PNA surrogates may be used.

[0066] The 3' and 5' ends of an oligonucleotide can be modified. Such modifications can be at the 3' end or the 5' end or both ends of the molecule. They can include modification or replacement of an entire terminal phosphate or of one or more of the atoms of the phosphate group. For example, the 3' and 5' ends of an oligonucleotide can be conjugated to other functional molecular entities such as labeling moieties, e.g., fluorophores (e.g., pyrene, TAMRA, fluorescein, Cy3 or Cy5 dyes) or protecting groups (based e.g., on sulfur, silicon, boron or ester). The functional molecular entities can be attached to the sugar through a phosphate group and/or a linker. The terminal atom of the linker can connect to or replace the linking atom of the phosphate group or the C-3' or C-5' O, N, S or C group of the sugar. Alternatively, the linker can connect to or replace the terminal atom of a nucleotide surrogate (e.g., PNAs). These spacers or linkers can include e.g., $-(CH_2)_n-$, $-(CH_2)_nN-$, $-(CH_2)_nO-$, $-(CH_2)_nS-$, $O(CH_2CH_2O)nCH_2CH_2OH$ (e.g., n=3 or 6), abasic sugars, amide, carboxy, amine, oxyamine, oxyimine, thioether, disulfide, thiourea, sulfonamide, or morpholino, or biotin and fluorescein reagents. The 3' end can be an -OH group.

[0067] Other examples of terminal modifications include dyes, intercalating agents (e.g., acridines), cross-linkers (e.g., psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (e.g., phenazine, dihydrophenazine), artificial endonucleases (e.g., EDTA), lipophilic carriers (e.g., cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine) and peptide conjugates (e.g., antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (e.g., PEG-40K), MPEG, [MPEG]2, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (e.g., biotin), transport/absorption facilitators (e.g., aspirin, vitamin E, folic acid), synthetic ribonucleases (e.g., imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu^{3+} complexes of tetraazamacrocycles).

[0068] Terminal modifications can be added for a number of reasons, including to modulate activity or to modulate resistance to degradation. Terminal modifications useful for modulating activity include modification of the 5' end with phosphate or phosphate analogs. Nucleic acids of the invention, on the first or second strand, may be 5' phosphorylated or include a phosphoryl analog at the 5' prime terminus. 5'-phosphate modifications include those which are compatible with RISC mediated gene silencing. Suitable modifications include: 5'-monophosphate ($(HO)_2(O)P-O-5'$); 5'-diphosphate ($(HO)_2(O)P-O-P(HO)(O)-O-5'$); 5'-triphosphate ($(HO)_2(O)P-O-(HO)(O)P-O-P(HO)(O)-O-5'$); 5'-guanosine cap (7-methylated or non-methylated) (7m-G-O-5'-(HO)(O)P-O-(HO)(O)P-O-P(HO)(O)-O-5'); 5'-adenosine cap (Appp), and any modified or unmodified nucleotide cap structure (N-O-5'-(HO)(O)P-O-(HO)(O)P-O-P(HO)(O)-O-5'); 5'-monothiophosphate (phosphorothioate; $(HO)_2(S)P-O-5'$); 5'-monodithiophosphate (phosphorodithioate; (HO)(HS)(S)P-O-5'), 5'-phosphorothiolate ($(HO)_2(O)P-S-5'$); any additional combination of oxygen/sulfur replaced monophosphate, diphosphate and triphosphates (e.g., 5'-alpha-thiotriphosphate, 5'-gamma-thiotriphosphate, etc.), 5'-phosphoramidates ($(HO)_2(O)P-NH-5'$, $(HO)(NH_2)(O)P-O-5'$), 5'-alkylphosphonates (R=alkyl=methyl, ethyl, isopropyl, propyl, etc., e.g.,

RP(OH)(O)-O-5'-,

[0069] $(OH)_2(O)P$-5'-CH_2-), 5'vinylphosphonate, 5'-alkyletherphosphonates (R=alkylether=methoxymethyl ($MeOCH_2$-), ethoxymethyl, etc., e.g., RP(OH)(O)-O-5'-).

[0070] The nucleic acid of the present invention may include one or more phosphorothioate modifications on one or more of the terminal ends of the first and/or the second strand. Optionally, each or either end of the first strand may comprise one or two or three phosphorothioate modified nucleotides. Optionally, each or either end of the second strand may comprise one or two or three phosphorothioate modified nucleotides. Optionally, both ends of the first strand and the 5' end of the second strand may comprise two phosphorothioate modified nucleotides. By phosphorothioate modified nucleotide it is meant that the linkage between the nucleotide and the adjacent nucleotide comprises a phosphorothioate group instead of a standard phosphate group.

[0071] Terminal modifications can also be useful for monitoring distribution, and in such cases the groups to be added may include fluorophores, e.g., fluorscein or an Alexa dye. Terminal modifications can also be useful for enhancing uptake, useful modifications for this include cholesterol. Terminal modifications can also be useful for cross-linking an RNA agent to another moiety.

[0072] Adenine, guanine, cytosine and uracil are the most common bases found in RNA. These bases can be modified or replaced to provide RNA's having improved properties. E.g., nuclease resistant oligoribonucleotides can be prepared with these bases or with synthetic and natural nucleobases (e.g., inosine, thymine, xanthine, hypoxanthine, nubularine, isoguanisine, or tubercidine) and any one of the above modifications. Alternatively, substituted or modified analogs of any of the above bases and "universal bases" can be employed. Examples include 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 5-halouracil, 5-(2-aminopropyl)uracil, 5-amino allyl uracil, 8-halo, amino, thiol, thioalkyl, hydroxyl and other 8-substituted adenines and guanines, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine, 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine, dihydrouracil, 3-deaza-5-azacytosine, 2-aminopurine, 5-alkyluracil, 7-alkylguanine, 5-alkyl cytosine, 7-deazaadenine, N6,N6-dimethyladenine, 2,6-diaminopurine, 5-amino-allyl-uracil, N3-methyluracil, substituted 1,2,4-triazoles, 2-pyridinone, 5-nitroindole, 3-nitropyrrole, 5-methoxyuracil, uracil-5-oxyacetic acid, 5-methoxycarbonylmethyluracil, 5-methyl-2-thiouracil, 5-methoxycarbonylmethyl-2-thiouracil, 5-methylaminomethyl-2-thiouracil, 3-(3-amino-3-carboxypropyl)uracil, 3-methylcytosine, 5-methylcytosine, N<4>-acetyl cytosine, 2-thiocytosine, N6-methyladenine, N6-isopentyladenine, 2-methylthio-N6-isopentenyladenine, N-methylguanines, or O-alkylated bases.

[0073] As used herein, the terms "non-pairing nucleotide analog" means a nucleotide analog which includes a non-base pairing moiety including but not limited to: 6 des amino adenosine (Nebularine), 4-Me-indole, 3-nitropyrrole, 5-nitroindole, Ds, Pa, N3-Me ribo U, N3-Me riboT, N3-Me dC, N3-Me-dT, N1-Me-dG, N1-Me-dA, N3-ethyl-dC, N3-Me dC. In some embodiments the non-base pairing nucleotide analog is a ribonucleotide. In other embodiments it is a deoxyribonucleotide.

[0074] As used herein, the term, "terminal functional group" includes without limitation a halogen, alcohol, amine, carboxylic, ester, amide, aldehyde, ketone, ether groups.

[0075] Certain moieties may be linked to the 5' terminus of the first strand or the second strand and includes abasic ribose moiety, abasic deoxyribose moiety, modifications abasic ribose and abasic deoxyribose moieties including 2' O alkyl modifications; inverted abasic ribose and abasic deoxyribose moieties and modifications thereof, C6-imino-Pi; a mirror nucleotide including L-DNA and L-RNA; 5'OMe nucleotide; and nucleotide analogs including 4',5'-methylene nucleotide; 1-(β-D-erythrofuranosyl)nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate, 3-aminopropyl phosphate; 6-aminohexyl phosphate; 12-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; alpha-nucleotide; threo-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted abasic moiety; 1,4-butanediol phosphate; 5'-amino; and bridging or non bridging methylphosphonate and 5'-mercapto moieties.

[0076] The nucleic acids of the invention may be included one or more inverted nucleotides, for example inverted

thymidine or inverted adenine (for example see Takei, et al., 2002. JBC 277 (26):23800-06).

[0077] As used herein, the term "inhibit", "down-regulate", or "reduce" with respect to gene expression means the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits (e.g., mRNA), or activity of one or more proteins or protein subunits, is reduced below that observed in the absence of a nucleic acid of the invention; for example the expression may be reduced to 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 15% or less than that observed in the absence of an inhibitor.

[0078] The nucleic acid of the present invention may comprise an abasic nucleotide. The term "abasic" as used herein, refers to moieties lacking a base or having other chemical groups in place of a base at the 1' position, for example a 3',3'-linked or 5',5'-linked deoxyabasic ribose derivative.

[0079] "Alternating" as described herein means to occur one after another in a regular way. In other words, alternating means to occur in turn repeatedly.

[0080] The term "odd numbered" as described herein means a number not divisible by two. Examples of odd numbers are 1, 3, 5, 7, 9, 11 and so on. The term "even numbered" as described herein means a number which is evenly divisible by two. Examples of even numbers are 2, 4, 6, 8, 10, 12, 14 and so on.

[0081] The nucleotides for the purposes of modification as described herein (unless otherwise indicated) are numbered from 5' to 3' on the first strand and 3' and 5' on the second strand.

[0082] Clearly, if the first and/or the second strand are shorter or longer than 25 nucleotides in length, such as 19 nucleotides in length, there are no nucleotides numbered 20, 21, 22, 23, 24 and 25 to be modified. The skilled person understands the description to apply to shorter or longer strands, accordingly.

[0083] Throughout the description of the invention, "same or common modification" means the same modification to any nucleotide, be that A, G, C or U modified with a group such as such as a methyl group or a fluoro group. Is it not taken to mean the same addition on the same nucleotide. For example, 2'F-dU, 2'F-dA, 2'F-dC, 2'F-dG are all considered to be the same or common modification, as are 2'-OMe-rU, 2'-OMe-rA; 2'-OMe-rC; 2'-OMe-rG. A 2'F modification is a different modification to a 2'OMe modification.

[0084] Some representative modified nucleic acid sequences of the present invention are shown in the examples. These examples are meant to be representative and not limiting.

[0085] The nucleic acid of the invention may be conjugated to a ligand.

[0086] Some ligands can have endosomolytic properties. The endosomolytic ligands promote the lysis of the endosome and/or transport of the composition of the invention, or its components, from the endosome to the cytoplasm of the cell. The endosomolytic ligand may be a polyanionic peptide or peptidomimetic which shows pH-dependent membrane activity and fusogenicity. The endosomolytic component may contain a chemical group which undergoes a change in charge or protonation in response to a change in pH. The endosomolytic component may be linear or branched.

[0087] Ligands can include therapeutic modifiers, e.g., for enhancing uptake; diagnostic compounds or reporter groups e.g., for monitoring distribution; cross-linking agents; and nuclease-resistance conferring moieties. General examples include lipids, steroids, vitamins, sugars, proteins, peptides, polyamines, and peptide mimics. Ligands can include a naturally occurring substance, such as a protein, carbohydrate, or lipid. The ligand may be a recombinant or synthetic molecule.

[0088] Ligands can also include targeting groups, e.g. a cell or tissue targeting agent. The targeting ligand may be a lectin, glycoprotein, lipid or protein.

[0089] Other examples of ligands include dyes, intercalating agents, cross-linkers, porphyrins, polycyclic aromatic hydrocarbons, artificial endonucleases or a chelator, lipophilic molecules, alkylating agents, phosphate, amino, mercapto, PEG, MPEG, alkyl, substituted alkyl, radiolabelled markers, enzymes, haptens, transport/absorption facilitators, synthetic ribonucelases, or imidazole clusters.

[0090] Ligands can be proteins, e.g. glycoproteins or peptides. Ligands may also be hormones or hormone receptors. They may also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, or cofactors.

[0091] The ligand may be a substance such as a drug which can increase the uptake of the nucleic acid into a cell, for example, by disrupting the cell's cytoskeleton.

[0092] The ligand may increase uptake of the nucleic acid into the cell by activating an inflammatory response. Such ligands include tumour necrosis factor alpha (TNF-alpha), interleukin-1 beta, or gamma interferon.

[0093] The ligand may be a lipid or lipid-based molecule. The lipid or lipid-based molecule preferably binds a serum protein. Preferably, the lipid-based ligand binds human serum albumin (HSA). A lipid or lipid-based molecule can increase resistance to degradation of the conjugate, increase targeting or transport into target cell, and/or can adjust binding to a serum protein. A lipid-based ligand can be used to modulate binding of the conjugate to a target tissue.

[0094] The ligand may be a steroid. Preferably, the ligand is cholesterol or a cholesterol derivative.

[0095] The ligand may be a moiety e.g. a vitamin, which is taken up by a target cell. Exemplary vitamins include vitamin A, E, K, and the B vitamins. Vitamins may be taken up by a proliferating cell, which may be useful for delivering the nucleic acid to cells such as malignant or non-malignant tumour cells.

[0096] The ligand may be a cell-permeation agent, such as a helical cell-permeation agent. Preferably such an agent is amphipathic.

[0097] The ligand may be a peptide or peptidomimetic. A peptidomimetic is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The peptide or peptidomimetic ligand may include naturally occurring or modified peptides, or both. A peptide or peptidomimetic can be a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide. The peptide moiety can be a dendrimer peptide, constrained peptide, or crosslinked peptide. The peptide moiety can include a hydrophobic membrane translocation sequence. The peptide moiety can be a peptide capable of carrying large polar molecules such as peptides, oligonucleotides, and proteins across cell membranes, e.g. sequences from the HIV Tat protein (GRKKRRQRRRPPQ) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK). Preferably the peptide or peptidomimetic is a cell targeting peptide, e.g. arginine-glycine-aspartic acid (RGD)-peptide.

[0098] The ligand may be a cell permeation peptide that is capable of permeating, for example, a microbial cell or a mammalian cell.

[0099] The ligand may be a pharmacokinetic modulator. The pharmacokinetic modulator may be lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins, etc.

[0100] When two or more ligands are present, the ligands can all have the same properties, all have different properties, or some ligands have the same properties while others have different properties. For example, a ligand can have targeting properties, have endosomolytic activity or have PK modulating properties. In a preferred embodiment, all the ligands have different properties.

[0101] Ligands can be coupled to the nucleic acid at the 3'-end, 5'-end, and/or at an internal position. Preferably the ligand is coupled to the nucleic acid via an intervening tether or linker.

[0102] In some embodiments the nucleic acid is a double-stranded nucleic acid. In a double-stranded nucleic acid the ligand may be attached to one or both strands. In some embodiments, a double-stranded nucleic acid contains a ligand conjugated to the second strand. In other embodiments, a double-stranded nucleic acid contains a ligand conjugated to the first strand.

[0103] Ligands can be conjugated to nucleobases, sugar moieties, or internucleosidic linkages of nucleic acid molecules. Conjugation to purine nucleobases or derivatives thereof can occur at any position including endocyclic and exocyclic atoms. Conjugation to pyrimidine nucleotides or derivatives thereof can also occur at any position. Conjugation to sugar

moieties of nucleosides can occur at any carbon atom. Conjugation to internucleosidic linkages may occur at the phosphorus atom of a phosphorus-containing linkage or at an oxygen, nitrogen, or sulphur atom bonded to the phosphorus atom. For amine- or amide-containing internucleosidic linkages, conjugation may occur at the nitrogen atom of the amine or amide or to an adjacent carbon atom.

[0104] The ligand is typically a carbohydrate, e.g. a monosaccharide, disaccharide, trisaccharide, tetrasaccharide or polysaccharide. The ligand may be conjugated to the nucleic acid by a linker. The saccharide may be selected from N-acetyl galactoseamine, mannose, galactose, glucose, glucosamine and fucose. The saccharide may be N-acetyl galactoseamine (GalNAc).

[0105] A ligand for use in the present invention may therefore comprise (i) one or more N-acetyl galactosamine (GalNac) moieties and derivatives thereof, and (ii) a linker, wherein the linker conjugates the GalNac moieties to a sequence as defined in any preceding aspects. The linker may be a bivalent or trivalent or tetravalent branched structure. The nucleotides may be modified as defined herein.

[0106] Means for efficient delivery of oligonucleotides, in particular double stranded nucleic acids of the invention, to cells *in vivo* is important and requires specific targeting and substantial protection from the extracellular environment, particularly serum proteins. One method of achieving specific targeting is to conjugate a ligand to the nucleic acid. The targeting moiety helps in targeting the nucleic acid to the required target site and there is a need to conjugate appropriate ligands for the desired receptor sites for the conjugated molecules to be taken up by the cells such as by endocytosis. The ligand can be any moiety or ligand that is capable of targeting a specific receptor.

[0107] For example, the Asialoglycoprotein receptor (ASGP-R) is a high capacity receptor, which is highly abundant on hepatocytes. One of the first disclosures of triantennary cluster glycosides was in US patent number US 5,885,968. Conjugates having three GalNAc ligands and comprising phosphate groups are known and are described in Dubber et al. (2003). The ASGP-R shows a 50-fold higher affinity for N-Acetyl-D-Galactosylamine (GalNAc) than D-Gal.

[0108] Hepatocytes expressing the lectin (asialoglycoprotein receptor; ASGPR), which recognizes specifically terminal β-galactosyl subunits of glycosylated proteins or other oligosaccharides (P. H. Weigel et. al., 2002,) can be used for targeting a drug to the liver by covalent coupling of galactose or galactoseamine to the drug substance (S.Ishibashi, et. al. 1994). Furthermore the binding affinity can be significantly increased by the multi-valency effect, which is achieved by the repetition of the targeting unit (E. A. L. Biessen et. al., 1995).

[0109] The ASGPR is a mediator for an active endosomal transport of terminal β-galactosyl containing glycoproteins, thus ASGPR is highly suitable for targeted delivery of drug candidates like nucleic acid, which have to be delivered into a cell (Akinc et al.).

[0110] The saccharide, which can also be referred to as the ligand, may be selected to have an affinity for at least one type of receptor on a target cell. In particular, the receptor is on the surface of a mammalian liver cell, for example, the hepatic asialoglycoprotein receptor (ASGP-R).

[0111] The saccharide may be selected from N-acetyl galactoseamine, mannose, galactose, glucose, glucosamone and fucose. The saccharide may be N-acetyl galactoseamine (GalNAc).

[0112] "GalNAc" refers to 2-(Acetylamino)-2-deoxy-D- galactopyranose, commonly referred to in the literature as N-acetyl galactosamine. Reference to "GalNAc" or "N-acetyl galactoseamine" includes both the β- form: 2-(Acetylamino)-2-deoxy-β -D-galactopyranose and the α-form: 2-(Acetylamino)-2-deoxy-α-D- galactopyranose. Both the β-form: 2-(Acetylarnino)-2-deoxy-β-D-galactopyranose and α-form: 2-(Acetylamino)-2-deoxy-α-D-galactopyranose may be used interchangeably. Preferably, the compounds of the invention comprise the β-form, 2-(Acetylarnino)-2-deoxy-β-D-galactopyranose.

[0113] The ligand may comprise GalNAc..

[0114] The ligand may comprise a compound of formula I:

$$[S\text{-}X^1\text{-}P\text{-}X^2]_3\text{-}A\text{-}X^3\text{-} \qquad (I)$$

wherein:

S represents a saccharide, wherein the saccharide is N-acetyl galactosamine;

X^1 represents C_3-C_6 alkylene or $(-CH_2-CH_2-O)_m(-CH_2)_2-$ wherein m is 1, 2, or 3;

P is a phosphate or modified phosphate (preferably a thiophosphate);

X^2 is alkylene or an alkylene ether of the formula $(-CH_2)_n-O-CH_2-$ where n = 1- 6;

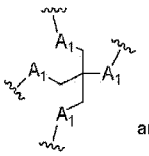
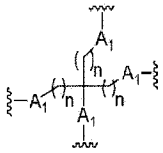
A is a branching unit;

X^3 represents a bridging unit;

wherein a nucleic acid according to the present invention is conjugated to X^3 via a phosphate or modified phosphate (preferably a thiophosphate).

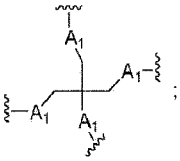
[0115] In formula I, branching unit "A" branches into three in order to accommodate the three saccharide ligands. The branching unit is covalently attached to the ligands and the nucleic acid. The branching unit may comprise a branched aliphatic group comprising groups selected from alkyl, amide, disulphide, polyethylene glycol, ether, thioether and hydroxyamino groups. The branching unit may comprise groups selected from alkyl and ether groups.

[0116] The branching unit A may have a structure selected from:


and


wherein each A_1 independently represents O, S, C=O or NH; and
each n independently represents an integer from 1 to 20.

[0117] The branching unit may have a structure selected from: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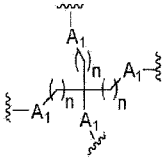
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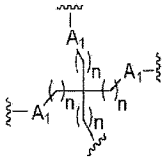
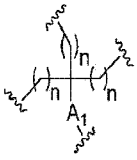
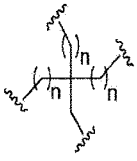


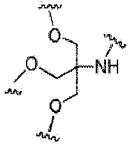
wherein each A_1 independently represents O, S, C=O or NH; and
each n independently represents an integer from 1 to 20.

[0118] The branching unit may have a structure selected from:

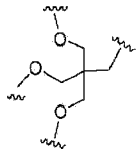

and


wherein A_1 is O, S, C=O or NH; and
each n independently represents an integer from 1 to 20.

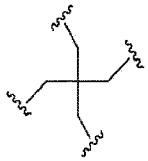
[0119] The branching unit may have the structure:



[0120] The branching unit may have the structure:



[0121] The branching unit may have the structure:



[0122] Optionally, the branching unit consists of only a carbon atom.

[0123] X^3 may be selected from -C_1-C_{20} alkylene-, -C_2-C_{20} alkenylene-, an alkylene ether of formula - (C_1-C_{20} alkylene)-O-(C_1-C_{20} alkylene)-, -C(O)-C_1-C_{20} alkylene-, -C_0-C_4 alkylene(Cy)C_0-C_4 alkylene- wherein Cy represents a substituted or unsubstituted 5 or 6 membered cycloalkylene, arylene, heterocyclylene or heteroarylene ring, -C_1-C_4 alkylene-NHC(O)-C_1-C_4 alkylene-, -C_1-C_4 alkylene-C(O)NH-C_1-C_4 alkylene-, -C_1-C_4 alkylene-SC(O)-C_1-C_4 alkylene-, - C_1-C_4 alkylene-C(O)S-C_1-C_4 alkylene-, -C_1-C_4 alkylene-OC(O)-C_1-C_4 alkylene-, -C_1-C_4 alkylene-C(O)O-C_1-C_4 alkylene-, and -C_1-C_6 alkylene-S-S-C_1-C_6 alkylene-.

[0124] X^3 may be an alkylene ether of formula -(C_1-C_{20} alkylene)-O-(C_1-C_{20} alkylene)-. X^3 may be an alkylene ether of formula -(C_1-C_{20} alkylene)-O-(C_4-C_{20} alkylene)-, wherein said (C_4-C_{20} alkylene) is linked to Z. X^3 may be selected from the group consisting of -CH_2-O-C_3H_6-, -CH_2-O-C4H_8-, -CH_2-O-C6H_{12}- and -CH_2-O-C8H_{16}-, especially -CH_2-O-C4H_8-, -CH_2-O-C6H_{12}- and - CH_2-O-C_8H_{16}-, wherein in each case the -CH_2- group is linked to A.

[0125] The ligand may comprise a compound of formula (II):

$$[S\text{-}X^1\text{-}P\text{-}X^2]_3\text{-}A\text{-}X^3\text{-} \qquad \text{(II)}$$

wherein:

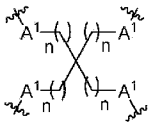
S represents a saccharide;

X^1 represents C_3-C_6 alkylene or (-CH_2-CH_2-O)$_m$(-CH_2)$_2$- wherein m is 1, 2, or 3;

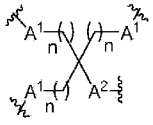
P is a phosphate or modified phosphate (preferably a thiophosphate);

X^2 is C_1-C_8 alkylene;

A is a branching unit selected from:



A^1 = O, NH
n = 1 to 4

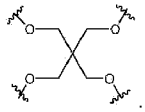


A^1 = O, NH A^2 = NH, CH_2, O
n = 1 to 4

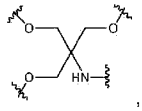
X^3 is a bridging unit;

wherein a nucleic acid according to the present invention is conjugated to X^3 via a phosphate or modified phosphate (preferably a thiophosphate).

[0126] Branching unit A may have the structure:



[0127] Branching unit A may have the structure:



wherein X^3 is attached to the nitrogen atom.

[0128] X^3 may be C_1-C_{20} alkylene. Preferably, X^3 is selected from the group consisting of -C_3H_6-, -C_4H_8-,-C_6H_{12}- and -C_8H_{16}-, especially -C_4H_8-,-C_6H_{12}- and -C_8H_{16}-.

[0129] The ligand may comprise a compound of formula (III):

$$[S\text{-}X^1\text{-}P\text{-}X^2]_3\text{-}A\text{-}X^3\text{-} \qquad \text{(III)}$$

wherein:

S represents a saccharide;

X^1 represents C_3-C_6 alkylene or $(\text{-}CH_2\text{-}CH_2\text{-}O)_m(\text{-}CH_2)_2$- wherein m is 1, 2, or 3;

P is a phosphate or modified phosphate (preferably a thiophosphate);

X^2 is an alkylene ether of formula -C_3H_6-O-CH_2-;

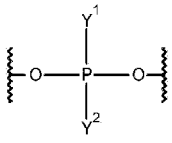
A is a branching unit;

X^3 is an alkylene ether of formula selected from the group consisting of -CH_2-O-CH_2-, - CH_2-O-C_2H_4-, -CH_2-O-C_3H_6-, -CH_2-O-C_4H_8-, -CH_2-O-C_5H_{10}-, -CH_2-O-C_6H_{12}-, -CH_2-O-C_7H_{14}-, and -CH_2-O-C_8H_{16}-, wherein in each case the -CH_2- group is linked to A, wherein a nucleic acid according to the present invention is conjugated to X^3 via a phosphate or modified phosphate (preferably a thiophosphate).

[0130] The branching unit may comprise carbon. Preferably, the carbon unit is carbon.

[0131] X^3 may be selected from the group consisting of -CH_2-O-C_4H_8-, -CH_2-O-C_5H_{10}-, -CH_2-O-C_6H_{12}-, -CH_2-O-C_7H_{14}-, and -CH_2-O-C_8H_{16}-. Preferably, X^3 is selected from the group consisting of-CH_2-O-C_4H_8-, -CH_2-O-C_6H_{12}- and -CH_2-O-C_8H_{16}.

[0132] For any of the above aspects, P represents a modified phosphate group. P can be represented by:



wherein Y^1 and Y^2 each independently represent =O, =S, $-O^-$, -OH, -SH, $-BH_3$, $-OCH_2CO_2$, -

[0133] $OCH_2CO_2R^X$, $-OCH_2C(S)OR^X$, and $-OR^X$, wherein R^X represents C_1-C_6 alkyl and wherein indicates attachment to the remainder of the compound.

[0134] For example, Y^1 may represent -OH and Y^2 may represent =O or =S; or

Y^1 may represent $-O^-$ and Y^2 may represent =O or =S;

Y^1 may represent =O and Y^2 may represent $-CH_3$, -SH, $-OR^X$, or $-BH_3$

Y^1 may represent =S and Y^2 may represent $-CH_3$, OR^X or -SH.

[0135] It will be understood by the skilled person that in certain instances there will be delocalisation between Y^1 and Y^2.

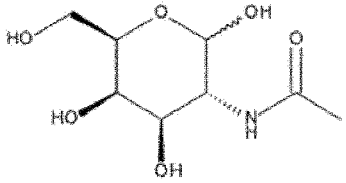
[0136] Preferably, the modified phosphate group is a thiophosphate group. Thiophosphate groups include bithiophosphate (i.e. where Y^1 represents =S and Y^2 represents $-S^-$) and monothiophosphate (i.e. where Y^1 represents $-O^-$ and Y^2 represents =S, or where Y^1 represents =O and Y^2 represents $-S^-$). Preferably, P is a monothiophosphate. The inventors have found that conjugates having thiophosphate groups in replacement of phosphate groups have improved potency and duration of action *in vivo*.

[0137] P may also be an ethylphosphate (i.e. where Y^1 represents =O and Y^2 represents OCH_2CH_3).

[0138] The saccharide, which can also be referred to as the ligand, may be selected to have an affinity for at least one type of receptor on a target cell. In particular, the receptor is on the surface of a mammalian liver cell, for example, the hepatic asialoglycoprotein receptor (ASGP-R).

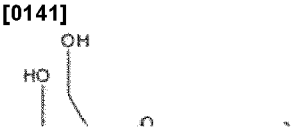
[0139] For any of the above aspects, the saccharide may be selected from N-acetyl with one or more of galactosamine, mannose, galactose, glucose, glucosamine and fructose. Preferably, the saccharide is two molecules of N-acetyl galactosamine (GalNAc). The compounds of the invention may have 3 ligands which are each preferably N-acetyl galactosamine.

[0140] "GalNAc" refers to 2-(Acetylamino)-2-deoxy-D- galactopyranose, commonly referred to in the literature as N-acetyl galactosamine. Reference to "GalNAc" or "N-acetyl galactosamine" includes both the β- form: 2-(Acetylamino)-2-deoxy-β -D-galactopyranose and the α-form: 2-(Acetylamino)-2-deoxy-α-D- galactopyranose. In certain embodiments, both the β-form: 2-(Acetylarnino)-2-deoxy-β-D-galactopyranose and α-form: 2-(Acetylamino)-2-deoxy-α-D-galactopyranose may be used interchangeably. Preferably, the compounds of the invention comprise the β-form, 2-(Acetylarnino)-2-deoxy-β-D-galactopyranose.



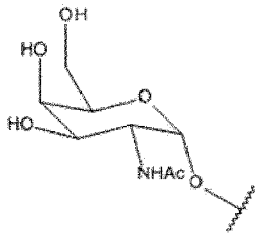
2-(Acetylamino)-2-deoxy-D-galactopyranose

[0141]



2-(Acetylamino)-2-deoxy-β-D-galactopyranose

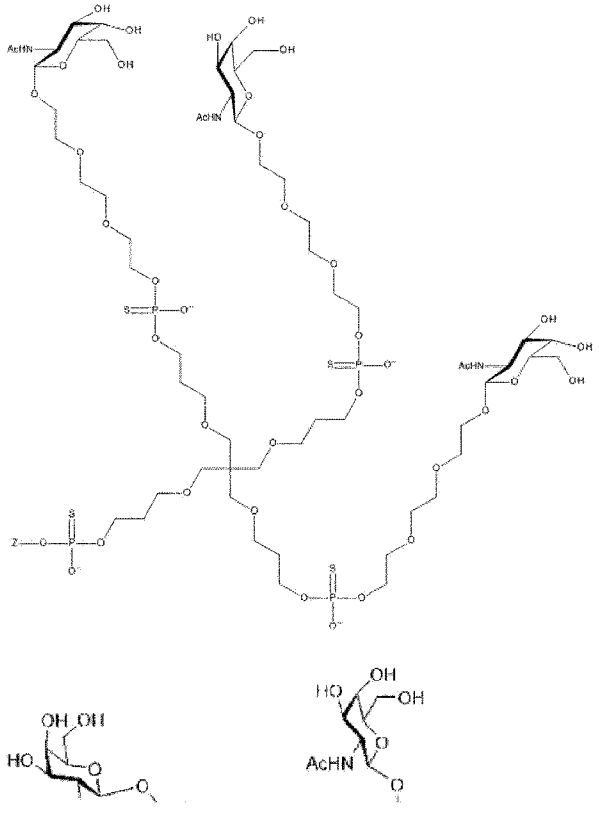
[0142]

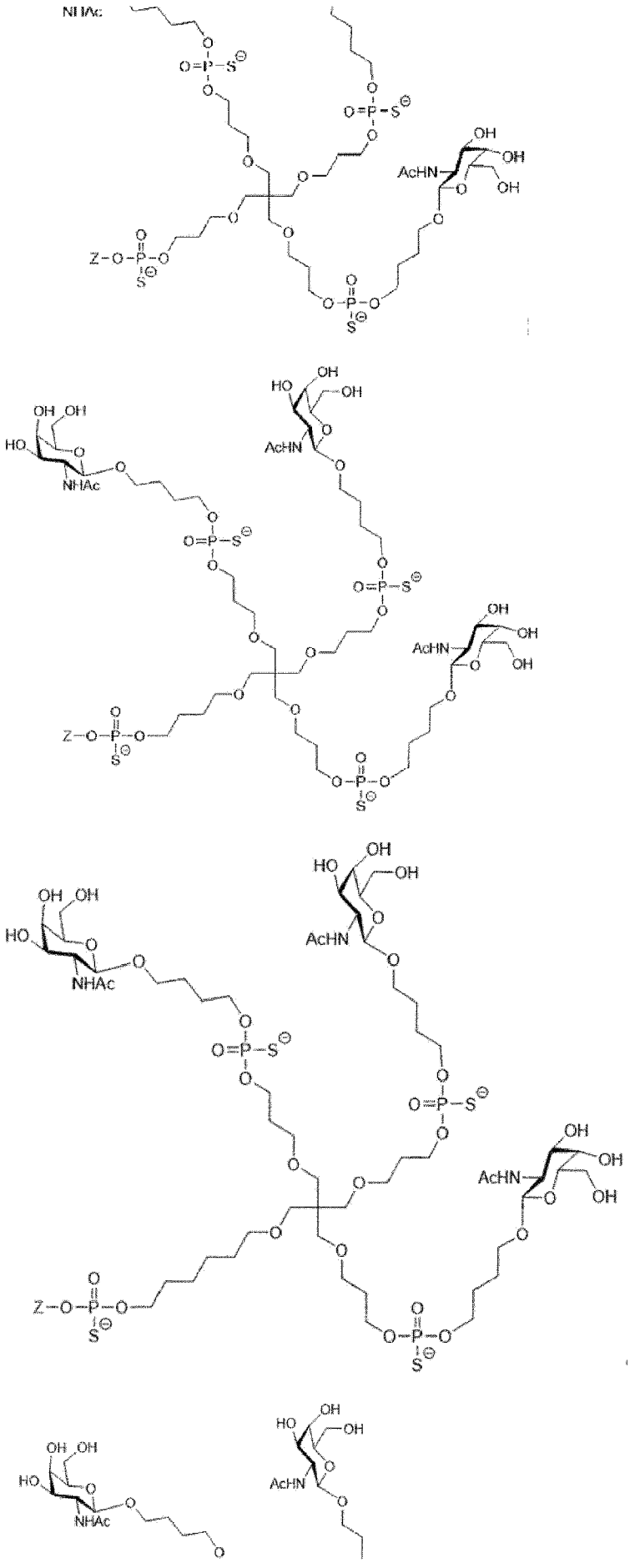


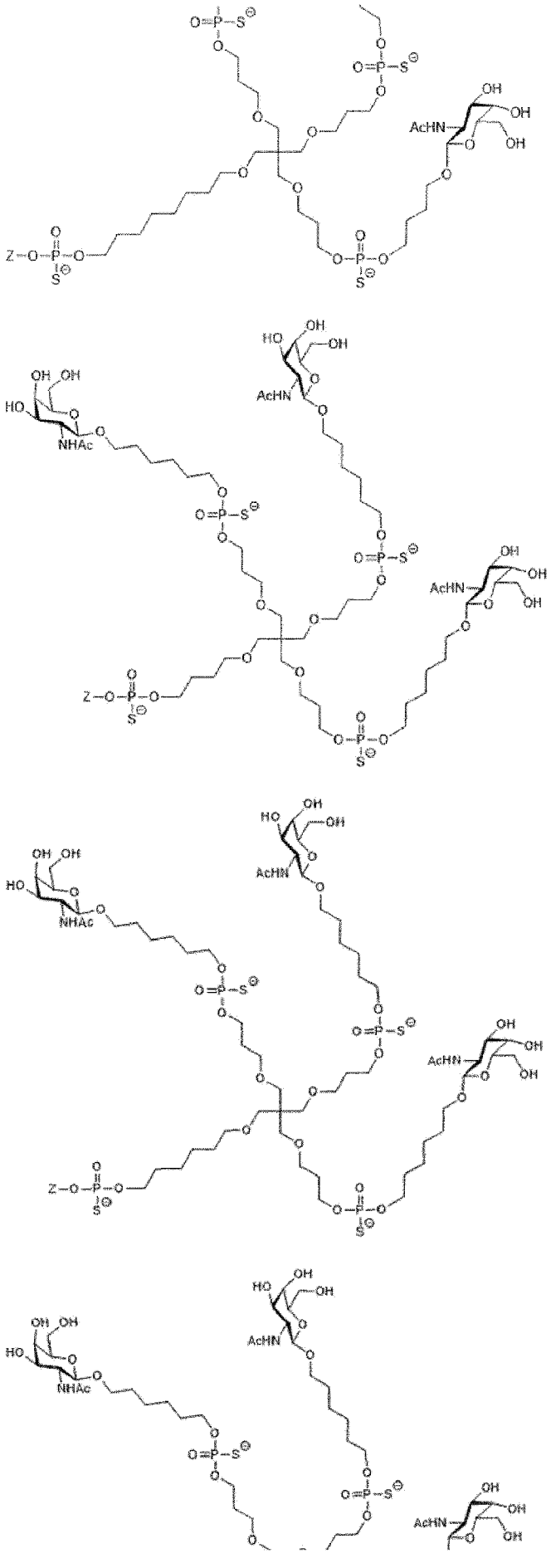
2-(Acetylamino)-2-deoxy-α-D-galactopyranose

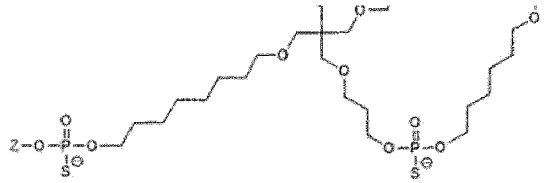
[0143] For any of the above compounds of formula (III), X^1 may be $(-CH_2-CH_2-O)_m(-CH_2)_2-$ wherein m is 1, 2, or 3. X^1 may be $(-CH_2-CH_2-O)(-CH_2)_2-$. X^1 may be $(-CH_2-CH_2-O)_2(-CH_2)_2-$. X^1 may be $(-CH_2-CH_2-O)_3(-CH_2)_2-$. Preferably, X^1 is $(-CH_2-CH_2-O)_2(-CH_2)_2-$. Alternatively, X^1 represents C_3-C_6 alkylene. X^1 may be propylene. X^1 may be butylene. X^1 may be pentylene. X^1 may be hexylene. Preferably the alkyl is a linear alkylene. In particular, X^1 may be butylene. For compounds of formula (III), X^2 represents an alkylene ether of formula $-C_3H_6-O-CH_2-$ i.e. C_3 alkoxy methylene, or $-CH_2CH_2CH_2OCH_2-$.

[0144] The present invention therefore additionally provides a conjugated nucleic acid having one of the following structures









wherein Z represents a nucleic acid as defined herein before.

[0145] Described herein is a nucleic acid or conjugated nucleic acid for inhibiting expression of a target gene in a cell, comprising at least one duplex region that comprises at least a portion of a first strand and at least a portion of a second strand that is at least partially complementary to the first strand, wherein said first strand is at least partially complementary to at least a portion of a RNA transcribed from the target gene, wherein said first strand comprises a modified nucleotide at selected position in order to facilitate processing of the nucleic acid by RISC, wherein the nucleic acid is conjugated indirectly or directly to a ligand via a linker. The nucleic acid may be conjugated to a ligand as herein described. The nucleotides of the first and/or second strand may be modified, as herein described.

[0146] The ligand may be conjugated to the nucleic acid via a linker as set out in formula I and wherein the first strand is modified with a 2'OMe modification on the odd numbered nucleotides, and modified with a 2'F on the even numbered nucleotides, and the second strand is modified with a 2'OMe on the even numbered nucleotides and modified with a 2'F on the odd numbered nucleotides.

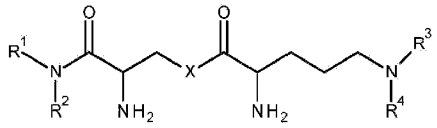
[0147] The ligand may be GalNac and be attached via a linker.

[0148] The nucleic acid as described herein may be formulated with a lipid in the form of a liposome. Such a formulation may be described in the art as a lipoplex. The formulation with a lipid/liposome may be used to assist with delivery of the nucleic acid of the invention to the target cells. The lipid delivery system herein described may be used as an alternative to a conjugated ligand. The modifications herein described may be present when using a nucleic acid of the invention with a lipid delivery system or with a ligand conjugate delivery system. Such a lipoplex may comprise a lipid formulation comprising:

1. i) a cationic lipid, or a pharmaceutically acceptable salt thereof;
2. ii) a steroid;
3. iii) a phosphatidylethanolamine phospholipid;
4. iv) a PEGylated lipid.

[0149] The cationic lipid may be an amino cationic lipid.

[0150] The cationic lipid may have the formula (I):



(I)

or a pharmaceutically acceptable salt thereof, wherein:

X represents O, S or NH;

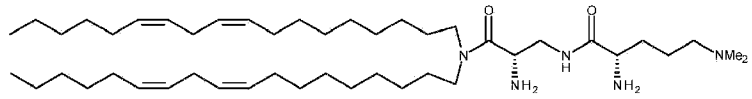
R^1 and R^2 each independently represents a C4-C22 linear or branched alkyl chain or a C4-C22 linear or branched alkenyl chain with one or more double bonds, wherein the alkyl or alkenyl chain optionally contains an intervening ester, amide or disulfide;

when X represents S or NH, R^3 and R^4 each independently represent hydrogen, methyl, ethyl, a mono- or polyamine moiety, or R^3 and R^4 together form a heterocyclyl ring;

when X represents O, R^3 and R^4 each independently represent hydrogen, methyl, ethyl, a mono- or polyamine moiety,

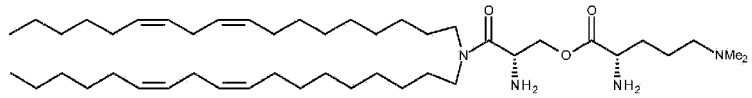
or R^3 and R^4 together form a heterocyclyl ring, or R^3 represents hydrogen and R^4 represents $C(NH)(NH_2)$.

[0151] The cationic lipid may have the formula (IA):



(IA)
or a pharmaceutically acceptable salt thereof.

[0152] The cationic lipid may have the formula (IB):



(IB)
or a pharmaceutically acceptable salt thereof.

[0153] The content of the cationic lipid component may be from about 55 mol% to about 65 mol% of the overall lipid content of the formulation. In particular, the cationic lipid component is about 59 mol% of the overall lipid content of the formulation.

[0154] The formulations further comprise a steroid. the steroid may be cholesterol. The content of the steroid may be from about 26 mol% to about 35 mol% of the overall lipid content of the lipid formulation. More particularly, the content of steroid may be about 30 mol% of the overall lipid content of the lipid formulation.

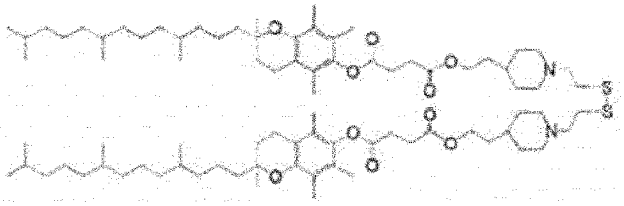
[0155] The phosphatidylethanolamine phospholipid may be selected from group consisting of 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPhyPE), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-Dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE), 1,2-Dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE), 1,2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE), 1,2-Dilinoleoyl-sn-glycero-3-phosphoethanolamine (DLoPE), 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), 1,2-Dierucoyl-sn-glycero-3-phosphoethanolamine (DEPE), 1,2-Disqualeoyl-sn-glycero-3-phosphoethanolamine (DSQPE) and 1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine (SLPE). The content of the phospholipid may be about 10 mol% of the overall lipid content of the formulation.

[0156] The PEGylated lipid may be selected from the group consisting of 1,2-dimyristoyl-sn-glycerol, methoxypolyethylene glycol (DMG-PEG) and C16-Ceramide-PEG. The content of the PEGylated lipid may be about 1 to 5 mol% of the overall lipid content of the formulation.

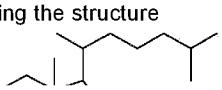
[0157] The content of the cationic lipid component in the formulation may be from about 55 mol% to about 65 mol% of the overall lipid content of the lipid formulation, preferably about 59 mol% of the overall lipid content of the lipid formulation.

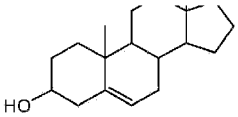
[0158] The formulation may have a molar ratio of the components of i):ii): iii): iv) selected from 55:34:10:1; 56:33:10:1; 57:32:10:1; 58:31:10:1; 59:30:10:1; 60:29:10:1; 61:28:10:1; 62:27:10:1; 63:26:10:1; 64:25:10:1; and 65:24:10:1.

[0159] The formulation may comprise a cationic lipid having the structure



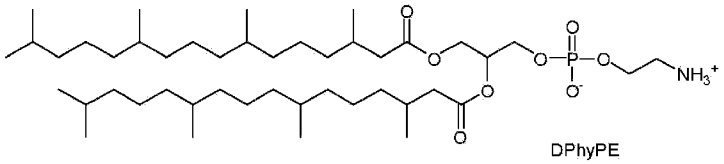
a steroid having the structure





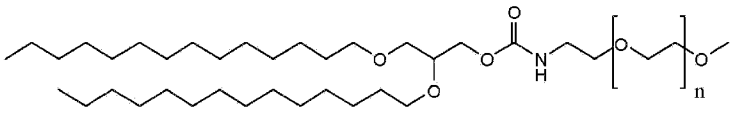
Cholesterol

a phosphatidylethanolamine phospholipid having the structure



DPhyPE

And a PEGylated lipid having the structure



mPEG-2000-DMG

[0160] Neutral liposome compositions may be formed from, for example, dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions may be formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes may be formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition may be formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

[0161] A positively charged synthetic cationic lipid, N-[l-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) can be used to form small liposomes that interact spontaneously with nucleic acid to form lipid-nucleic acid complexes which are capable of fusing with the negatively charged lipids of the cell membranes of tissue culture cells. DOTMA analogues can also be used to form liposomes.

[0162] Derivatives and analogues of lipids described herein may also be used to form liposomes.

[0163] A liposome containing a nucleic acid can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or lipid conjugate. The detergent can have a high critical micelle concentration and may be nonionic. Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The nucleic acid preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the nucleic acid and condense around the nucleic acid to form a liposome. After condensation, the detergent is removed, e.g., by dialysis, to yield a liposomal preparation of nucleic acid.

[0164] If necessary a carrier compound that assists in condensation can be added during the condensation reaction, e.g., by controlled addition. For example, the carrier compound can be a polymer other than a nucleic acid (e.g., spermine or spermidine). pH can also be adjusted to favour condensation.

[0165] Nucleic acid formulations may include a surfactant. In one embodiment, the nucleic acid is formulated as an emulsion that includes a surfactant.

[0166] A surfactant that is not ionized is a non-ionic surfactant. Examples include non-ionic esters, such as ethylene glycol esters, propylene glycol esters, glyceryl esters etc., nonionic alkanolamides, and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers.

[0167] A surfactant that carries a negative charge when dissolved or dispersed in water is an anionic surfactant. Examples include carboxylates, such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates.

[0168] A surfactant that carries a positive charge when dissolved or dispersed in water is a cationic surfactant. Examples include quaternary ammonium salts and ethoxylated amines.

[0169] A surfactant that has the ability to carry either a positive or negative charge is an amphoteric surfactant. Examples include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

[0170] "Micelles" are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic. A micelle may be formed by mixing an aqueous solution of the nucleic acid, an alkali metal alkyl sulphate, and at least one micelle forming compound.

[0171] Exemplary micelle forming compounds include lecithin, hyaluronic acid, pharmaceutically acceptable salts of hyaluronic acid, glycolic acid, lactic acid, chamomile extract, cucumber extract, oleic acid, linoleic acid, linolenic acid, monoolein, monooleates, monolaurates, borage oil, evening of primrose oil, menthol, trihydroxy oxo cholanyl glycine and pharmaceutically acceptable salts thereof, glycerin, polyglycerin, lysine, polylysine, triolein, polyoxyethylene ethers and analogues thereof, polidocanol alkyl ethers and analogues thereof, chenodeoxycholate, deoxycholate, and mixtures thereof.

[0172] Phenol and/or m-cresol may be added to the mixed micellar composition to act as a stabiliser and preservative. An isotonic agent such as glycerine may as be added.

[0173] A nucleic acid preparation may be incorporated into a particle such as a microparticle. Microparticles can be produced by spray-drying, lyophilisation, evaporation, fluid bed drying, vacuum drying, or a combination of these methods.

[0174] The present invention also provides pharmaceutical compositions comprising a nucleic acid or conjugated nucleic acid of the invention. The pharmaceutical compositions may be used as medicaments or as diagnostic agents, alone or in combination with other agents. For example, a nucleic acid or conjugated nucleic acid of the invention can be combined with a delivery vehicle (e.g., liposomes) and excipients, such as carriers, diluents. Other agents such as preservatives and stabilizers can also be added. Methods for the delivery of nucleic acids are known in the art and within the knowledge of the person skilled in the art.

[0175] A nucleic acid or conjugated nucleic acid of the present invention can also be administered in combination with other therapeutic compounds, either administrated separately or simultaneously, e.g., as a combined unit dose. The invention also includes a pharmaceutical composition comprising a nucleic acid or conjugated nucleic acid according to the present invention in a physiologically/pharmaceutically acceptable excipient, such as a stabilizer, preservative, diluent, buffer, and the like.

[0176] The pharmaceutical composition may be specially formulated for administration in solid or liquid form. The composition may be formulated for oral administration, parenteral administration (including, for example, subcutaneous, intramuscular, intravenous, or epidural injection), topical application, intravaginal or intrarectal administration, sublingual administration, ocular administration, transdermal administration, or nasal administration. Delivery using subcutaneous or intravenous methods are preferred.

[0177] Dosage levels for the medicament and pharmaceutical compositions of the invention can be determined by those skilled in the art by routine experimentation. In one embodiment, a unit dose may contain between about 0.01 mg/kg and about 100 mg/kg body weight of nucleic acid. Alternatively, the dose can be from 10 mg/kg to 25 mg/kg body weight, or 1 mg/kg to 10 mg/kg body weight, or 0.05 mg/kg to 5 mg/kg body weight, or 0.1 mg/kg to 5 mg/kg body weight, or 0.1 mg/kg to1 mg/kg body weight, or 0.1 mg/kg to 0.5 mg/kg body weight, or 0.5 mg/kg to 1 mg/kg body weight. Dosage levels may also be calculated via other parameters such as, e.g., body surface area.

[0178] The pharmaceutical composition may be a sterile injectable aqueous suspension or solution, or in a lyophilized form. In one embodiment, the pharmaceutical composition may comprise lyophilized lipoplexes or an aqueous suspension of lipoplexes. The lipoplexes preferably comprises a nucleic acid of the present invention. Such lipoplexes may be used to deliver the nucleic acid of the invention to a target cell either in vitro or in vivo.

[0179] The pharmaceutical compositions and medicaments of the present invention may be administered to a mammalian subject in a pharmaceutically effective dose. The mammal may be selected from humans, dogs, cats, horses, cattle, pig, goat, sheep, mouse, rat, hamster and guinea pig.

[0180] Pharmaceutically acceptable compositions may comprise a therapeutically-effective amount of a nucleic acid or conjugated nucleic acid in any embodiment according to the invention, taken alone or formulated with one or more pharmaceutically acceptable carriers, excipient and/or diluents.

[0181] Examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium state, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen- free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

[0182] Stabilisers may be agents that stabilise a nucleic acid or conjugated nucleic acid, for example a protein that can complex with the nucleic acid, chelators (e.g. EDTA), salts, RNAse inhibitors, and DNAse inhibitors.

[0183] In some cases it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection in order to prolong the effect of a drug. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

[0184] The nucleic acid described herein may be capable of inhibiting the expression of the target gene in a cell. The nucleic acid described herein may be capable of partially inhibiting the expression of the target gene in a cell. Inhibition may be complete, i.e. 0% of the expression level of target gene expression in the absence of the nucleic acid of the invention. Inhibition of target gene expression may be partial, i.e. it may be 15%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% of target gene expression in the absence of a nucleic acid of the invention. Inhibition may last 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks or up to 3 months, when used in a subject, such as a human subject. The nucleic acid or conjugated nucleic acid or composition comprising the same may be for use once, every week, every two weeks, every three weeks, every four weeks, every five weeks, every six weeks, every seven weeks, or every eight weeks. The nucleic acid or conjugated nucleic acid may be for use subcutaneously or intravenously.

[0185] In cells and/or subjects treated with or receiving a nucleic acid or conjugated nucleic acid of the present invention, the target gene expression may be inhibited compared to untreated cells and/or subjects by at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100%. The level of inhibition may allow treatment of a disease associated with target gene expression or overexpression, or may allow further investigation into the functions of the target gene product.

[0186] The target gene may be Factor VII, Eg5, PCSK9, TPX2, apoB, SAA, TTR, RSV, PDGF beta gene, Erb-B gene, Src gene, CRK gene, GRB2 gene, RAS gene, MEKK gene, JNK gene, RAF gene, Erkl/2 gene, PCNA(p21) gene, MYB gene, JU gene, FOS gene, BCL-2 gene, hepcidin, Activated Protein C, Cyclin D gene, VEGF gene, EGFR gene, Cyclin A gene, Cyclin E gene, WNT-1 gene, beta-catenin gene, c-MET gene, PKC gene, NFKB gene, STAT3 gene, survivin gene, Her2/Neu gene, topoisomerase I gene, topoisomerase II alpha gene, mutations in the p73 gene, mutations in the p21(WAF I/CIPI) gene, mutations in the p27(KIPI) gene, mutations in the PPM ID gene, mutations in the RAS gene, mutations in the caveolin I gene, mutations in the MIB I gene, mutations in the MTAI gene, mutations in the M68 gene, mutations in tumor suppressor genes, and mutations in the p53 tumor suppressor gene. In particular, the target gene may be TMPRSS6 or ALDH2.

[0187] A method of treating a disease or disorder (not according to the claimed invention) may comprise administration of a pharmaceutica composition comprising an nucleic acid as described herein, to an individual in need of treatment. The nucleic acid composition may be administered twice every week, once every week, every two weeks, every three weeks, every four weeks, every five weeks, every six weeks, every seven weeks, or every eight weeks. The nucleic acid may be administered to the subject subcutaneously or intravenously.

[0188] A subject may be administered an initial dose and one or more maintenance doses of a a nucleic acid or conjugated nucleic acid. The maintenance dose or doses can be the same or lower than the initial dose, e.g., one-half less of the initial dose. The maintenance doses are, for example, administered no more than once every 2, 5, 10, or 30 days. The treatment regimen may last for a period of time which will vary depending upon the nature of the particular disease, its severity and the overall condition of the patient.

[0189] The composition may include a plurality of nucleic acid agent species. In another embodiment, the nucleic acid agent species has sequences that are non-overlapping and non-adjacent to another species with respect to a naturally occurring target sequence. In another embodiment, the plurality of nucleic acid agent species is specific for different naturally occurring target genes. In another embodiment, the nucleic acid agent is allele specific.

[0190] The nucleic acid or conjugated nucleic acid of the present invention can also be administered or for use in combination with other therapeutic compounds, either administered separately or simultaneously, e.g. as a combined unit dose.

[0191] The nucleic acid or conjugated nucleic acid of the present invention can be produced using routine methods in the art including chemically synthesis or expressing the nucleic acid either in vitro (e.g., run off transcription) or in vivo. For example, using solid phase chemical synthesis or using an expression vector. In one embodiment, the expression vector can produce the nucleic acid of the invention in a target cell. Methods for the synthesis of the nucleic acid described herein are known to persons skilled in the art.

[0192] The modifications present in the nucleic acids of the invention may facilitate processing of the nucleic acid by RISC.

[0193] In one aspect "facilitate processing by RISC" means that the nucleic acid can be processed by RISC, for example any modification present will permit the nucleic acid to be processed by RISC, suitably such that SiRNA activity can take place.

[0194] Nucleic acids of the invention may comprise one or more LNA nucleotides. Nucleic acids of the invention may comprise LNA nucleotides at positions 2 and/or 14 of the first strand counting from the 5' end of the first strand. Nucleic acids may comprise LNA on the second strand which correspond to position 11, or 13, or 11 and 13, or 11-13 of the first strand.

[0195] Preferably the nucleic acid as disclosed herein is an SiRNA.

[0196] The nucleic acid is modified on the first strand with alternating 2-O methyl modifications and 2 fluoro modifications, and positions 2 and 14 (starting from the 5' end) are modified with 2' fluoro. The second strand is modified with 2' fluoro modifications at positions 11-13 counting from the 3' end starting at the first position of the complementary (double stranded) region, and the remaining modifications are 2' O-methyl.

[0197] In one aspect the nucleic acid of the invention comprise one or more inverted ribonucleotides, preferably an inverted adenine, using a 5'-5' linkage or a 3'-3' linkage, preferably a 3'-3' linkage at the 3' end of the second strand.

[0198] In one aspect the nucleic acid comprises one or more phosphorodithioate linkages, such as 1, 2, 3 or 4 phosphorodithioate linkages. Preferably there are up to 4 phosphorodithioate linkages, one each at the 5' and 3' ends of the first and second strands.

[0199] All the features of the nucleic acids can be combined with all other aspects of the invention disclosed herein.

[0200] In one aspect the nucleic acid is not any one or more or all of Patisiran, Revusiran, Fitusiran, Cemdisiran, Givosiran, Inclisiran, lumasiran, Votrisiran, Cosdosiran and Teprasiran.

[0201] These have the sequences below.

Patisiran	3'CAUUGGUUCUCAUAAGGUA 5' 5'GUAACCAAGAGUAUUCCAU 3'
Revusiran	3'-CUACCCUAAAGUACAUUGGUUCU- 5' 5'-UGGGAUUUCAUGUAACCAAGA 3'
Fitusiran	3'-GACCAAUUGUGGUAAAUGAAGUU- 5' 5'-GGUUAACACCAUUUACUUCAA 3'
Cemdisiran	3'-TTUUUUCGUUCUAUAAAAAUAUUAU- 5' 5'-AAGCAAGAUAUUUUUAUAAUA 3'
Givosiran	3'-UGGUCUUUCUCACAGAGUAGAAU 5' 5'-CAGAAAGAGUGUCUCAUCUUA 3'
Inclisiran	3'-AAGAUCUG G AC AAA ACG AAA AC A 5' 5'-CUAGACCUGUTUUGCUUUUGU 3'

Sequences of these molecules are also available on the WHO website http://www.who.int/medicines/services/inn/en/

[0202] For example,

Cemdisian is
duplex of [(2S,4R)-1-{1-[(2-acetamido-2-deoxy-β-Dgalactopyranosyl) oxy]-16,16-bis({3-[(3-{5-[(2-acetamido-2-deoxy-β-D-galactopyranosyl)oxy] pentanamido} propyl)amino]-3-oxopropoxy}methyl)-5,11,18-trioxo-14-oxa-6, 10, 17 - triazanonacosan-29-oyl}-4-hydroxypyrrolidin-2-yl]methyl hydrogen all-P-ambo-2'-O-methyl-Pthioadenylyl-(3'→5')-2'-O-methyl-P-thioadenylyl-(3'→5')-2'-deoxy-2'-fluoroguanylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-Omethyladenylyl-(3'→5')-2'-deoxy-2'-fluoroguanylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-O-methyl-3'-adenylate and all-P-ambo-thymidylyl-(5'→3')-thymidylyl-(5'→3')-2'-O-methyl-P-thiouridylyl-(5'→3')-2'-Omethyl-P-thiouridylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-O-methylcytidylyl-(5'→3')-2'-deoxy-2'-fluoroguanylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-deoxy-2'-fluorouridylyl-(5'→3')-2'-Omethylcytidylyl-(5'→3')-2'-deoxy-2'-fluorouridylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-Omethyluridylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-deoxy-2'-fluoro-P-thiouridylyl-(5'→3')-2'-deoxy-2'-fluoro-P-thioadenylyl-(5'→3')-2'-Omethyluridine

Patisiran is
RNA duplex of guanylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-adenylyl-(3'→5')-adenylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-2'-Omethylcytidylyl-(3'→5')-adenylyl-(3'→5')-adenylyl-(3'→5')-guanylyl-(3'→5')-adenylyl-(3'→5')-guanylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-adenylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-2'-Omethylcytidylyl-(3'→5')-adenylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-thymidylyl-(3'→5')-thymidine with thymidylyl-(5'→3')-thymidylyl-(5'→3')-cytidylyl-(5'→3')-adenylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-uridylyl-(5'→3')-guanylyl-(5'→3')-guanylyl-(5'→3')-uridylyl-(5'→3')-uridylyl-(5'→3')-cytidylyl-(5'→3')-uridylyl-(5'→3')-cytidylyl-(5'→3')-adenylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-adenylyl-(5'→3')-adenylyl-(5'→3')-guanylyl-(5'→3')-guanylyl-(5'→3')-uridylyl-(5'→3')-adenosine

Inclisiran is
duplex of [(2S,4R)-1-{1-[(2-acetamido-2-deoxy-β-Dgalactopyranosyl) oxy]-16,16-bis({3-[(3-{5-[(2-acetamido-2-deoxy-β-D-galactopyranosyl)oxy] pentanamido} propyl)amino]-3-oxopropoxy}methyl)-5,11,18-trioxo-14-oxa-6, 10, 17 - triazanonacosan-29-oyl}-4-hydroxypyrrolidin-2-yl]methyl hydrogen all-P-ambo-2'-O-methyl-Pthiocytidylyl-(3'→5')-2'-O-methyl-P-thiouridylyl-(3'→5')-2'-Omethyladenylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-Omethyladenylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluoroguanylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-thymidylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-Omethylcytidylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-Omethyl-3'-uridylate and all-P-ambo-2'-O-methyl-P-thioadenylyl-(5'→3')-2'-O-methyl-P-thioadenylyl-(5'→3')-2'-O-methylguanylyl-(5'→3')-2'-O-methyladenylyl-

(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-deoxy-2'-fluorocytidylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-deoxy-2'-fluoroguanylyl-(5'→3')-2'-O-methylguanylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-O-methylcytidylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-Omethyladenylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-deoxy-2'-fluorocytidylyl-(5'→3')-2'-O-methylguanylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-O-methyl-P-thioadenylyl-(5'→3')-2'-deoxy-2'-fluoro-P-thiocytidylyl-(5'→3')-2'-O-methyladenosine

Givosiran is
duplex of [(2S,4R)-1-{1-[(2-acetamido-2-deoxy-β-Dgalactopyranosyl) oxy]-16,16-bis({3-[(3-{5-[(2-acetamido-2-deoxy-β-D-galactopyranosyl)oxy] pentanamido}propyl) amino]-3-oxopropoxy}methyl)-5,11,18-trioxo-14-oxa-6,10,17 -triazanonacosan-29-oyl}-4-hydroxypyrrolidin-2-yl]methyl hydrogen all-P-ambo-2'-O-methyl-P-thiocytidylyl-(3'→5')-2'-O-methyl-P-thioadenylyl-(3'→5')-2'-Omethylguanylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-Omethyladenylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-deoxy-2'-fluoroguanylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-deoxy-2'-fluoroguanylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-2'-deoxy-2'-fluoroguanylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-O-methyl-3'-adenylate and all-P-ambo-2'-O-methyl-P-thiouridylyl-(5'→3')-2'-O-methyl-P-thioguanylyl-(5'→3')-2'-Omethylguanylyl-(5'→3')-2'-deoxy-2'-fluorouridylyl-(5'→3')-2'-O-methylcytidylyl-(5'→3')-2'-deoxy-2'-fluorouridylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-deoxy-2'-fluorouridylyl-(5'→3')-2'-O-methylcytidylyl-(5'→3')-2'-deoxy-2'-fluorouridylyl-(5'→3')-2'-O-methylcytidylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-Omethylcytidylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-O-methylguanylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-O-methylguanylyl-(5'→3')-2'-deoxy-2'-fluorouridylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-deoxy-2'-fluoroguanylyl-(5'→3')-2'-deoxy-2'-fluoro-P-thioadenylyl-(5'→3')-2'-deoxy-2'-fluoro-P-thioadenylyl-(5'→3')-2'-O-methyluridine

Revusiran is
[(2S,4R)-1-{30-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-14,14-bis[16-(2-acetamido-2-deoxy-β-Dgalactopyranosyl)-5, 11-dioxo-2, 16-dioxa-6, 10-diazahexadecyl]-12, 19,25-trioxo-16,30-dioxa-13,20,24-triazatriacontanoyl}-4-hydroxypyrrolidin-2-yl]methyl hydrogen 2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-deoxy-2'-fluoroguanylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-deoxy-2'-fluoroadenylate duplex with 2'-O-methyl-Pthiocytidylyl-(5'→3')-2'-deoxy-2'-fluoro-P-thiouridylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-deoxy-2'-fluorocytidylyl-(5'→3')-2'-O-methylcytidylyl-(5'→3')-2'-deoxy-2'-fluorocytidylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-O-methylguanylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-Omethyladenylyl-(5'→3')-2'-deoxy-2'-fluorocytidylyl-(5'→3')-2'-Omethyladenylyl-(5'→3')-2'-deoxy-2'-fluorouridylyl-(5'→3')-2'-deoxy-2'-fluorouridylyl-(5'→3')-2'-deoxy-2'-fluoroguanylyl-(5'→3')-2'-O-methylguanylyl-(5'→3')-2'-deoxy-2'-fluorouridylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-deoxy-2'-fluorocytidylyl-(5'→3')-2'-O-methyluridine

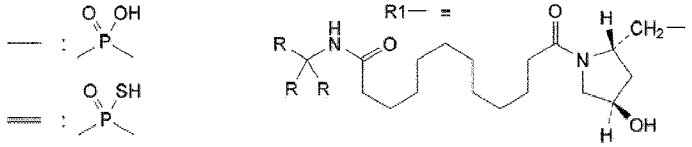
Fitusiran is
duplex of [(2S,4R)-1-{30-(2-acetamido-2-deoxy-β-Dgalactopyranosyl)-14, 14-bis[16-(2-acetamido-2-deoxy-β-Dgalactopyranosyl)-5,11-dioxo-2,16-dioxa-6,10-diazahexadecyl]-12,19,25-trioxo-16,30-dioxa-13,20,24-triazatriacontanoyl}-4-hydroxypyrrolidin-2-yl]methyl hydrogen (P-RS)-2'-deoxy-2'-fluoro-P-thioguanylyl-(3'→5')-(P-RS)-2'-O-methyl-P-thioguanylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-deoxy-2'-fluoroadenylate and and (P-RS)-2'-O-methyl-P-thiouridylyl-(3'→5')-(P-RS)-2'-deoxy-2'-fluoro-P-thiouridylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-Omethyladenylyl-(3'→5')-2'-deoxy-2'-fluoroguanylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-Omethylguanylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-Omethyladenylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-(P-RS)-2'-O-methyl-P-thiocytidylyl-(3'→5')-(P-RS)-2'-Omethyl-P-thioadenylyl-(3'→5')-2'-

O-methylguanosine

Lumasiran is
{(2S,4R)-1-{1-[(2-acetamido-2-deoxy-β-Dgalactopyranosyl) oxy]-16,16-bis-({3-[(3-{5-[(2-acetamido-2-deoxy-β-Dgalactopyranosyl) oxy]pentanamido}propyl)amino]-3-oxopropoxy}methyl)-5,11,18-trioxo-14-oxa-6,10,17-triazanonacosan-29-oyl}-4-hydroxypyrrolidin-2-yl}methyl hydrogen all-P-ambo-2'-O-methyl-P-thioguanylyl-(3'→5')-2'-O-methyl-P-thioadenylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-Omethylguanylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-Omethyladenylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-2'-O-methyl-3'-adenylate duplex with all-P-ambo-2'-O-methyl-P-thioadenylyl-(5'→3')-2'-O-methyl-P-thiocytidylyl-(5'→3')-2'-O-methylcytidylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-O-methylguanylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-Omethylguanylyl-(5'→3')-2'-deoxy-2'-fluorouridylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-O-methylguanylyl-(5'→3')-2'-O-methylguanylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-deoxy-2'-fluorocytidylyl-(5'→3')-2'-deoxy-2'-fluorocytidylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-deoxy-2'-fluorouridylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-Omethyladenylyl-(5'→3')-2'-O-methyl-P-thiouridylyl-(5'→3')-2'-deoxy-2'-fluoro-P-thioadenylyl-(5'→3')-2'-Omethyluridine
(3'-5')G = A = C - U - U - U - C - A - U - C - C - U - G - G - A - A - A - U - A - U - A - (R1)
(5'-3')A = C = C - U - G - A - A - A - G - U - A - G - G - A - C - C - U - U - U - A - U = A = U

X: 2'-deoxy-2'-fluoro-X / X : 2'-désoxy-2'-fluoro-X

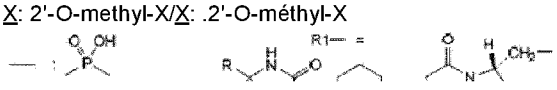
X : 2'-O-methyl-X/X : 2'-O-méthyl-X

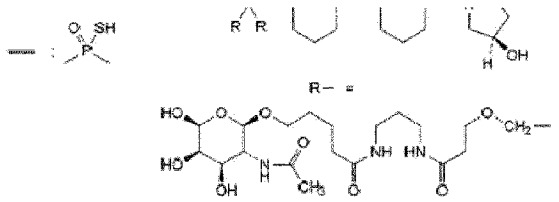


Votrisiran is:
{(2S,4R)-1-{1-[(2-acetamido-2-deoxy-β-Dgalactopyranosyl) oxy]-16,16-bis-({3-[(3-{5-[(2-acetamido-2-deoxy-β-Dgalactopyranosyl) oxy]pentanamido}propyl)amino]-3-oxopropoxy}methyl)-5,11,18-trioxo-14-oxa-6,10,17-triazanonacosan-29-oyl}-4-hydroxypyrrolidin-2-yl}methyl hydrogen all-P-ambo-2'-O-methyl-P-thiouridylyl-(3'→5')-2'-O-methyl-P-thioguanylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-deoxy-2'-fluorocytidylyl-(3'→5')-2'-deoxy-2'-fluoroadenylyl-(3'→5')-2'-deoxy-2'-fluorouridylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-2'-O-methyl-3'-adenylate duplex with all-P-ambo-2'-O-methyl-P-thiocytidylyl-(5'→3')-2'-O-methyl-P-thiouridylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-O-methylcytidylyl-(5'→3')-2'-O-methylcytidylyl-(5'→3')-2'-O-methylcytidylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-O-methylguanylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-O-methyladenylyl-(5'→3')-2'-O-methylcytidylyl-(5'→3')-2'-deoxy-2'-fluoroadenylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-Omethyluridylyl-(5'→3')-2'-deoxy-2'-fluoroguanylyl-(5'→3')-2'-O-methylguanylyl-(5'→3')-2'-O-methyluridylyl-(5'→3')-2'-Omethyl-P-thiouridylyl-(5'→3')-2'-deoxy-2'-fluoro-Pthiocytidylyl-(5'→3')-2'-O-methyluridine
(3'-5')U = G = G - G - A - U - U - U - C - A - U - G - U - A - A - C - C - A - A - G - A - (R1)
(5'-3')C = U = A - C - C - C - U - A - A - A - G - U - A - C - A - U - U - G - G - U - U = C = U

X : 2'-decxy-2'-fluoro-X/X: 2'-désoxy-2'-fluoro-x

X: 2'-O-methyl-X/X: .2'-O-méthyl-X





Cosdosiran is:
adenylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-guanylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-guanylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-uridylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-cytidylyl-(3'→5')-adenylyl-(3'→5')-2'-Omethylcytidylyl-(3'→5')-adenylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-uridylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-uridylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-guanylyl-(3'→5')-2'-O-methylcytidine duplex with [(2R,3S)-3-hydroxyoxolan-2-yl]methyl hydrogen uridylyl-(5'→3')-2'-deoxycytidylyl-(5'→3')-cytidylyl-(5'→3')-uridylyl-(5'→3')-cytidylyl-(5'→3')-adenylyl-(5'→3')-adenylyl-(5'→3')-guanylyl-(5'→3')-guanylyl-(5'→3')-uridylyl-(5'→3')-guanylyl-(5'→3')-uridylyl-(5'→3')-adenylyl-(5'→3')-adenylyl-(5'→3')-guanylyl-(5'→3')-adenylyl-(5'→3')-cytidylyl-(5'→3')-cytidylyl-(5'→3')-5 '-guanylate

Teprasiran is:
guanylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-guanylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-adenylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-adenylyl-(3'→5')-2'-O-m ethyluridylyl-(3'→5')-uridylyl-(3'→5')-2'-O-methyluridylyl-(3'→5')-cytidylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-cytidylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-cytidylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-uridylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-adenosine duplex with 2'-O-methyluridylyl-(3'→5')-guanylyl-(3'→5')-2'-O-methyladenylyl-(3'→5')-adenylyl-(3'→5')-2'-O-methylguanylyl-(3'→5')-guanylyl-(3'→5')-2'-Omethylguanylyl-(3'→5')-uridylyl-(3'→5')-2'-Omethylguanylyl-(3'→5')-adenylyl-(3'→5')-2'-Omethyladenylyl-(3'→5')-adenylyl-(3'→5')-2'-Omethyluridylyl-(3'→5')-adenylyl-(3'→5')-2'-O-m ethyluridylyl-(3'→5')-uridylyl-(3'→5')-2'-O-methylcytidylyl-(3'→5')-uridylyl-(3'→5')-2'-O-methylcytidine

[0203] In further embodiments of the invention, the terminal nucleotide at the 3' end of at least one of the first strand and the second strand is an inverted nucleotide and is attached to the adjacent nucleotide via the 3' carbon of the terminal nucleotide and the 3' carbon of the adjacent nucleotide and/ or the terminal nucleotide at the 5' end of at least one of the first strand and the second strand is an inverted nucleotide and is attached to the adjacent nucleotide via the 5' carbon of the terminal nucleotide and the 5' carbon of the adjacent nucleotide,
optionally wherein

1. a. the 3' and/or 5' inverted nucleotide of the first and/or second strand is attached to the adjacent nucleotide via a phosphate group by way of a phosphodiester linkage; or
2. b. the 3' and/or 5' inverted nucleotide of the first and/or second strand is attached to the adjacent nucleotide via a phosphorothioate group or
3. c. the 3' and/or 5' inverted nucleotide of the first and/or second strand is attached to the adjacent nucleotide via a phosphorodithioate group.

[0204] In further embodiments of the invention, the nucleic acid comprises a phosphorodithioate linkage, optionally wherein the linkage is between the 2 most 5' nucleosides and/or the 2 most 3' nucleosides of the second strand, and/or optionally wherein the nucleic acid additionally does not comprise any internal phosphorothioate linkages.

[0205] The disclosure also relates to any first strand or any second strand of nucleic acid as disclosed herein, which comprises no more than 2 base changes when compared to the specific sequence ID provided. For example, one base may be changed within any sequence.

[0206] In one instance, the change may be made to the 5' most nucleotide of the antisense (first) strand. In one instance, the change may be made to the 3' most nucleotide of the antisense (first) strand. In one example, the change may be made to the 5' most nucleotide of the sense (second) strand. In one example, the change may be made to the 3' most nucleotide of the sense (second) strand.

[0207] In one instance, the change is made to the 5' most nucleotide of the antisense (first) strand. The base of the 5'

nucleotide may be changed to any other nucleotide. An A or a U at the 5' end are preferred, and an A or a U are taught herein as the potential 5' terminal base for all of the antisense sequences disclosed herein

[0208] The invention will now be described with reference to the following non-limiting figures and examples in which:

Figure 1a and 1b show in vitro knockdown activity of siRNAs that are modified with 2'-OMe or 2'-OH at position 14 of the first strand;

Figure 2a and 2b show in vitro knockdown activity of siRNAs with 2'-OMe or 2'-OH at position 14 of the first strand;

Figure 3a and 3b show in vitro knockdown activity of siRNAs with 2'-OMe or 2'-OH at positions 2, 3 and 4 of the first strand;

Figure 4a and 4b show in vitro knockdown activity of siRNAs with 2'-OMe and 2'-OH at positions 2, 3 and 4 of the first strand;

Figure 5a and 5b show in vitro knockdown activity of siRNAs with 2'-OMe and 2'-F at position 2 of the first strand;

Figure 6 a-c show knockdown activity of differently modified ALDH2 variants derived from one sequence;

Figure 7a and b show knockdown activity of differently modified ALDH2 sequences;

Figure 8a and b show knockdown activity of differently modified ALDH2 sequences;

Figure 9a and b show knockdown activity of differently modified DGAT2 sequences;

Figure 10a and b show the effect of DNA modifications at certain positions of a TMPRSS6 siRNA sequence;

Figure 11a and b show the effect of LNA modifications at certain positions of a TMPRSS6 siRNA sequence;

Figure 12a - d show knockdown activity of GalNAc conjugates with different modification patterns both in liposomal transfections and receptor-mediated uptake;

Figure 13a and b show tolerance for DNA modification at more than one position in a TMPRSS6 siRNA sequence;

Figure 14a and b disclose tolerance for DNA in an siRNA targeting ALDH2 ;

Figure 15 and b disclose tolerance for DNA in a second siRNA targeting ALDH2 ;

Figure 16a and b disclose tolerance for DNA in an siRNA targeting DGAT2 ;

Figure 17a and b disclose the effect of 2-O-MOE at certain positions; and

Figure 18a and b disclose tolerance for 2'-OMe in an siRNA targeting GHR.

Examples

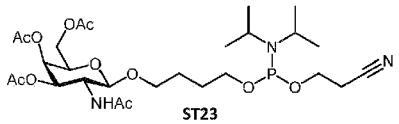
[0209] In the following, only those nucleic acids which meet the requirements of claim 1 are nucleic acids of the invention. The others provide useful context.

Example 1

[0210] All Oligonucleotides were either obtained from a commercial oligonucleotide manufacturer (Eurogentec, Belgium) or synthesized on an AKTA oligopilot 10 synthesiser (GE Healthcare) in a 10µmol scale using phosphoramidite chemistry. Commercially available base loaded CPG solid support (500A, 50µmol/g), 2'O-Methyl RNA phosphoramidites and 2'Fluoro DNA phosphoramidites (ChemGenes and LinkTech) were used according to the manufacturers recommended procedures. Amidite coupling was performed using 0.1 M solutions of the phosphoramidite in acetonitrile in presence of 0.3 M benzylthiotetrazole (BTT) activator. As ancillary reagents, 0.05 M I_2 in pyridine/H_2O (9/1, v/v) as

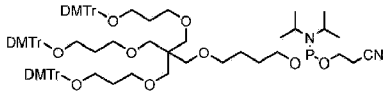
oxidizer, 40% Ac2O in acetonitrile as CapA, 20% N-methylimidazole in acetonitrile as CapB, 3% dichloroacetic acid in toluene as DMT removal and 20% diethylamine in acetonitrile as final wash were used (EMP Biotech). EDITH (LinkTech) was used as thiolation reagent. Acetonitrile (<20 ppm H_2O) was purchased from EMP Biotech. All other reagents and solvents were commercially available and used in standard reagent quality.

[0211] ST23 is a GalNac C4 phosphoramidite (structure components as below, described in WO2017/174657):



ST23

[0212] ST41 is as follows (and as described in WO2017/174657):



ST41

[0213] Phosphorothioates were introduced using 50 mM EDITH in acetonitrile. All oligonucleotides were synthesised in DMT-off mode. Diethylamine wash was performed upon completion of the assembly of the oligonucleotide chain on the solid support.

[0214] The single strands were cleaved off the CPG and all remaining protective groups were removed by using in 40% aq. methylamine solution (90 min, RT). The crude product was concentrated and purified by Ion exchange chromatography (Resource Q, 6mL, GE Healthcare) on a AKTA Pure HPLC System (GE Healthcare) using a Sodium chloride gradient (10 mM Tris buffer pH = 7.5, 10% acetonitril). Product containing fractions were analysed and pooled and concentrated. Salt removal was achieved by size exclusion chromatography (Zetadex, EMP Biotech). Finally, the individual single strands were lyophilised.

[0215] For duplex formation, single strands were reconstituted in ~2mg/mL concentration in water. Equimolar amounts of the respective single strands were added, mixed and heated to 80°C for 5min. After cooling the resulting siRNA was analyzed for full double strand formation by native IP-RP HPLC. Product solutions were stored at -20°C until further use.

[0216] The present examples utilise 19mer SiRNAs, unless otherwise apparent from the description and figures.

Example 2

[0217] The influence of 2'-OMe at position 14 of the first strand on siRNA activity was tested using a sequence targeting mouse CLIC4. CLC01 is modified with alternating 2'-OMe/2'-OH. CLC15 is modified with 2'-OMe at position 14 of the first strand, whereas CLC16 is not modified with 2'-OMe at this position. All other positions in CLC15 and CLC16 are modified similarly. "UT" indicates an untreated sample the siRNA-treated samples were normalized to. "Luc" was used as non-targeting control.

[0218] The experiment was conducted in MS1. Cells were seeded at a density of 40,000 cells per 6-well 24 h before transfection, transfected with 5 nM siRNA and 1 µg/ml Atufect and lysed after 48 h. Total RNA was extracted and CLIC4 and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three technical replicates.

[0219] Data are shown in Figures 1a and 1b.

Example 3

[0220] The influence of 2'-OMe at position 14 of the first strand on siRNA activity was tested using a sequence targeting

mouse CLIC4. CLC01 is modified with alternating 2'-OMe/2'-OH. CLC22 is modified with 2'-OMe at position 4, 9 and 14 of the first strand, whereas CLC28 is modified with 2'-OMe at positions 4, 9 and 15 of the first strand. The second strands of CLC22 and CLC28 are modified similarly. "UT"indicates an untreated sample the siRNA-treated samples were normalized to. "Luc" was used as non-targeting control.

[0221] The experiment was conducted in MS1. Cells were seeded at a density of 40,000 cells per 6-well 24 h before transfection, transfected with 5 nM siRNA and 1 µg/ml Atufect and lysed after 48 h. Total RNA was extracted and CLIC4 and Actin mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three technical replicates.

[0222] Data are shown in Figure 2a and 2b.

Example 4

[0223] The influence of 2'-OMe at position 2 of the first strand on siRNA activity was tested using a sequence targeting mouse CLIC4. CLC56 is modified with 2'-OMe at position 2 and 4 of the first strand, and 2'-OH at position 3. In contrast, CLC57 has 2'-OH at positions 2 and 4, and 2'-OMe at position 3. All other positions of the first and second strand are modified similarly. "UT"indicates an untreated sample the siRNA-treated samples were normalized to. "Luc" was used as non-targeting control.

[0224] The experiment was conducted in MS1. Cells were seeded at a density of 40,000 cells per 6-well 24 h before transfection, transfected with 5 and 1 nM siRNA and 1 µg/ml Atufect and lysed after 48 h. Total RNA was extracted and CLIC4 and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three technical replicates.

[0225] Data are shown in Figures 3a and 3b (A).

Example 5

[0226] The influence of 2'-OMe at position 2 of the first strand on siRNA activity was tested using a sequence targeting mouse CLIC4. CLC56 is modified with 2'-OMe at position 2 and 4 of the first strand, and 2'-OH at position 3. In contrast, CLC57 has 2'-OH at positions 2 and 4, and 2'-OMe at position 3. All other positions of the first and second strand are modified similarly. "UT" indicates an untreated sample the siRNA-treated samples were normalized to. "Luc" was used as non-targeting control.

[0227] The experiment was conducted in MS1. Cells were seeded at a density of 40,000 cells per 6-well 24 h before transfection, transfected with 1 to 0.008 nM siRNA and 1 µg/ml Atufect and lysed after 48 h. Total RNA was extracted and CLIC4 and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three technical replicates.

[0228] Data are shown in Figures 3a and 3b (B).

Example 6

[0229] The influence of 2'-OMe at position 2 of the first strand on siRNA activity was tested using a sequence targeting mouse CLIC4. CLC01 is modified with alternating 2'-OMe/2'-OH. CLC28 has 2'-OMe at position 4 of the first strand, whereas CLC59 has 2'-OMe at position 2 and CLC60 has 2'-OMe at position 3 of the first strand. All other positions of the first and second strand are modified similarly. "UT" indicates an untreated sample the siRNA-treated samples were normalized to. "Luc" was used as non-targeting control.

[0230] The experiment was conducted in MS1. Cells were seeded at a density of 40,000 cells per 6-well 24 h before transfection, transfected with 5 and 1 nM siRNA (A) or 1 to 0.008 nM siRNA (B) and 1 µg/ml Atufect and lysed after 48 h.

Total RNA was extracted and CLIC4 and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three technical replicates.

[0231] Data are shown in Figures 4a and 4b.

Example 7

[0232] The influence of 2'-OMe at position 2 of the first strand on siRNA activity was tested using a sequence targeting human HFE2. In the first strand, HFE04 is modified with 2'-F at position 2 and 2'-OMe at position 3, whereas HFE06 is modified with 2'-OMe at position 2 and 2'-F at position 3. All other positions of the first and second strand are modified similarly. "UT" indicates an untreated sample the siRNA-treated samples were normalized to. "Luc" was used as non-targeting control.

[0233] The experiment was conducted in Hep3B. Cells were seeded at a density of 120,000 cells per 6-well 24 h before transfection, transfected with 1 nM siRNA and 1 µg/ml Atufect and lysed after 72 h. Total RNA was extracted and HFE2 and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three technical replicates.

[0234] Data are shown in Figures 5a and 5b.

Example 8

[0235] Examples 8a and 8b represent biological replicates of the same experiment.

[0236] Example 8a The tolerance for 2'-OMe was investigated by addressing one position at a time in the context of an alternating pattern (change 2'-OMe into 2'-F and vice versa). ALD01 is completely alternating, ALD13 - ALD21 contains 2'-F into 2'-Me changes at all even positions of the first strand, ALD22 - ALD31 contains 2'-OMe into 2'-F changes at all odd positions of the first strand, ALD32 - ALD41 contains 2'-F into 2'-OMe changes at all odd positions of the second strand, ALD42 - ALD50 contains 2'-OMe into 2'-F changes at all even positions of the second strand. ALD13 contains 2'-OMe at first strand position 2, ALD19 contains 2'-OMe at first strand position 14, ALD35 contains 2'-OMe at second position 7, ALD36 contains 2'-OMe at second strand position 9.

[0237] The experiment was conducted in Hep3B. Cells were seeded at a density of 150,000 cells per 6-well 24 h before transfection, transfected with 0.1 nM siRNA and 1 µg/ml Atufect and lysed after 48 h. Total RNA was extracted and ALDH2 and actin mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three technical replicates.

[0238] Sequences are listed in Figure 6a and results are shown in figure 6b.

Example 8b:

[0239] The tolerance for 2'-OMe was investigated by addressing one position at a time in the context of an alternating pattern (change 2'-OMe into 2'-F and vice versa). ALD01 is completely alternating, ALD13 - ALD21 contains 2'-F into 2'-Me changes at all even positions of the first strand, ALD22 - ALD31 contains 2'-OMe into 2'-F changes at all odd positions of the first strand, ALD32 - ALD41 contains 2'-F into 2'-OMe changes at all odd positions of the second strand, ALD42 - ALD50 contains 2'-OMe into 2'-F changes at all even positions of the second strand. ALD13 contains 2'-OMe at first strand position 2, ALD19 contains 2'-OMe at first strand position 14, ALD35 contains 2'-OMe at second strand position 7, ALD36 contains 2'-OMe at second strand position 9.

[0240] The experiment was conducted in Hep3B. Cells were seeded at a density of 150,000 cells per 6-well 24 h before transfection, transfected with 0.1 nM siRNA and 1 µg/ml Atufect and lysed after 48 h. Total RNA was extracted and ALDH2 and actin mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three

technical replicates.

[0241] Sequences are listed in Figure 6a and results are shown in Figure 6c.

Example 9

[0242] Influence of modifications on the activity of two different ALDH2 siRNA sequences

[0243] Experiment 9-1 Tolerance of positions 2 and 14 for 2'-OMe in the first strand and tolerance of positions 7 and 9 for 2'-OMe in the second strand of an siRNA against ALDH2 was analysed. ALD58 contains alternating 2'-OMe/2'-F in both strands. ALD59 - ALD61 contain 2'-F at position 2 and/or 14 of the first strand with an all alternating second strand, whereas ALD62 - ALD64 contain 2'-F at position 7 and/or 9 of the second strand with an all alternating first strand. Positions 2 (ALD60) and 14 (ALD59) loose activity upon modification with 2'-OMe, whereas no 2'-OMe at position 2 and 14 restores activity (ALD61). Of the second strand, position 7 (ALD63) and position 9 (ALD62) loose activity upon modification with 2'OMe, whereas no 2'-OMe at positions 7 and 9 restores activity (ALD64).

[0244] The experiment was conducted in Hep3B. Cells were seeded at a density of 150,000 cells per 6-well, transfected with 0.1 nM siRNA and 1 µg/ml Atufect after 24 h and lysed after 48 h. Total RNA was extracted and ALDH2 and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD from three technical replicates.

[0245] Results are shown in Figure 7a and b.

Experiment 9-2

[0246] Tolerance of positions 2 and 14 for 2'-OMe in the first strand and tolerance of positions 7 and 9 for 2'-OMe in the second strand of a different siRNA against ALDH2 was analyzed. ALD72 contains alternating 2'-OMe/2'-F in both strands. ALD73 - ALD75 contain 2'-F at position 2 and/or 14 of the first strand with an all alternating second strand, whereas ALD76 - ALD78 contain 2'-F at position 7 and/or 9 of the second strand with an all alternating first strand. Positions 2 (ALD74) and 14 (ALD73) loose activity upon modification with 2'-OMe, whereas no 2'-OMe at position 2 and 14 restores activity (ALD75). Of the second strand, position 7 (ALD77) and position 9 (ALD76) loose activity upon modification with 2'OMe, whereas no 2'-OMe at positions 7 and 9 restores activity (ALD78).

[0247] The experiment was conducted in Hep3B. Cells were seeded at a density of 150,000 cells per 6-well, transfected with 0.1 nM siRNA and 1µg/ml Atufect after 24 h and lysed after 48 h. Total RNA was extracted and ALDH2 and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD from three technical replicates.

[0248] Results are shown in Figure 8a and b.

Example 10

[0249] Influence of modifications on the activity of an siRNA targeting DGAT2

[0250] Tolerance of positions 2 and 14 for 2'-OMe in the first strand and tolerance of positions 7 and 9 for 2'-OMe in the second strand of an siRNA against DGAT2 was analyzed. DGT01 contains alternating 2'-OMe/2'-F in both strands. DGT02 - DGT04 contain 2'-F at position 2 and/or 14 of the first strand with an all alternating second strand, whereas DGT05 - DGT07 contain 2'-F at position 7 and/or 9 of the second strand with an all alternating first strand. Positions 2 (DGT03) and 14 (DGT02) loose activity upon modification with 2'-OMe, whereas no 2'-OMe at position 2 and 14 restores activity at least partially (DGT04). Of the second strand, Position 7 (DGT06) and position 9 (DGT05) loose activity upon modification with 2'OMe, whereas no 2'-OMe at positions 7 and 9 restores activity (DGT07).

[0251] The experiment was conducted in Huh-7. Cells were seeded at a density of 80,000 cells per 6-well, transfected with 1 nM siRNA and 1 µg/ml Atufect after 24 h and lysed after 48 h. Total RNA was extracted and ALDH2 and PTEN

mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three technical replicates.

[0252] Results are shown in Figure 9a and b.

Example 11

[0253] Influence of DNA modifications on siRNA activity

[0254] Tolerance of positions 2 and 14 for DNA modification in the first strand and tolerance of positions 7 and 9 for DNA modification in the second strand of an siRNA against TMPRSS6 was analszed. TMP01 contains alternating 2'-OMe/2'-F in both strands. TMP93 contains 2'-OMe at position 14 of the first strand, whereas TMP113 contains 2'-H at the same position. TMP94 contains 2'-OMe at position 2 of the first strand, whereas TMP112 contains 2'-H at the same position. TMP97 contains 2'-OMe at position 9 of the second strand, whereas TMP117 contains 2'-H at the same position. TMP98 contains 2'-OMe at position 7 of the second strand, whereas TMP116 contains 2'-H at the same position.

[0255] The experiment was conducted in Hep3B. Cells were seeded at a density of 150,000 cells per 6-well, transfected with 0.1 nM siRNA and 1µg/ml Atufect after 24 h and lysed after 48 h. Total RNA was extracted and ALDH2 and Actin mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD from three technical replicates.

[0256] Results are shown in Figure 10 a and b.

Example 12

[0257] Influence of LNA modifications on siRNA activity

[0258] Tolerance of positions 2 and 14 for LNA modification in the first strand and tolerance of positions 7 and 9 for LNA modification in the second strand of an siRNA against TMPRSS6 was analysed. TMP01 contains alternating 2'-OMe/2'-F in both strands. TMP93 contains 2'-OMe at position 14 of the first strand, whereas TMP111 contains LNA at the same position. TMP94 contains 2'-OMe at position 2 of the first strand, whereas TMP110 contains LNA at the same position. TMP97 contains 2'-OMe at position 9 of the second strand, whereas TMP115 contains LNA at the same position. TMP98 contains 2'-OMe at position 7 of the second strand, whereas TMP114 contains LNA at the same position.

[0259] The experiment was conducted in Hep3B. Cells were seeded at a density of 150,000 cells per 6-well, transfected with 0.1 nM siRNA and 1 µg/ml Atufect after 24 h and lysed after 48 h. Total RNA was extracted and ALDH2 and Actin mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD from three technical replicates.

[0260] Results are shown in Figure 11a and b.

Example 13

[0261] Knockdown activity of different GalNAc-siRNA conjugates targeting TMPRSS6

13A

[0262] The influence of 2-O-methylation at certain second strand strand positions was investigated in the context of GalNAc-siRNA conjugates. All conjugates contain the same first strand. STS12009V23 contains an all-2'-O-methylated second strand, STS12009V25 has one 2'-F modification at second strand position 9, STS12009V26 has one 2'-F modification at second strand position 7, and STS12009V27 has three 2'-F modifications at second strand positions 7-9.

[0263] The experiment was conducted in Hep3B. Cells were seeded at a density of 150,000 cells per 6-well, transfected with 5 to 0.005 nM siRNA and 1 µg/ml Atufect after 24 h and lysed after 72 h. Total RNA was extracted and TMPRSS6

and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD from three technical replicates.

13B

[0264] The influence of 2-O-methylation at certain second strand positions was investigated in the context of GalNAc-siRNA conjugates. All conjugates contain the same first strand. STS12009V41L4 contains a second strand with alternating 2'-F/2'-OMe, STS12009V23 contains an all-2'-O-methylated second strand, STS12009V25 has one 2'-F modification at second strand position 9, STS12009V26 has one 2'-F modification at second strand position 7, and STS12009V27 has three 2'-F modifications at second strand positions 7-9.

[0265] The experiment was conducted in Hep3B. Cells were seeded at a density of 150,000 cells per 6-well, transfected with 10 to 0.001 nM siRNA and 1 µg/ml Atufect after 24 h and lysed after 72 h. Total RNA was extracted and TMPRSS6 and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD from three technical replicates.

13C

[0266] The influence of 2-O-methylation at certain second strand positions was investigated in the context of GalNAc-siRNA conjugates. All conjugates contain the same first strand. STS12009V23 contains an all-2'-O-methylated second strand, STS12009V25 has one 2'-F modification at second strand position 9, STS12009V26 has one 2'-F modification at second strand position 7, and STS12009V27 has three 2'-F modifications at second strand positions 7-9.

[0267] The experiment was conducted in mouse primary hepatocytes. Cells were seeded at a density of 250,000 cells per 6-well and treated with 100 to 0.25 nM GalNAc-siRNA. Transfections with 10 nM GalNAc-siRNA and 1 µg/ml Atufect served as control.

[0268] Cells were lysed after 24 h, total RNA was extracted and TMPRSS6 and Actin mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD from three technical replicates.

[0269] Results are shown in Figure 12a - d.

Example 14

[0270] Influence of DNA modification at multiple positions

[0271] Tolerance of positions 2 and 14 for DNA in the first strand and tolerance of positions 7-9 for DNA in the second strand of an siRNA against TMPRSS6 was analyzed. TMP70 contains alternating 2'-OMe/2'-F in both strands, whereas TMP119 contains 2'-OMe at all positions except of first strand positions 2 and 14 and second strand positions 7-9. TMP120-TMP126 contain a different number of DNA substitutions at 2'-F positions.

[0272] The experiment was conducted in Hep3B. Cells were seeded at a density of 150,000 cells per 6-well, transfected with 1 nM and 0.1 nM siRNA and 1µg/ml Atufect after 24 h and lysed after 48 h. Total RNA was extracted and TMPRSS6 and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD from three technical replicates.

[0273] Results are shown in Figure 13a and b.

Example 15

[0274] Incorporation of DNA at key positions.

[0275] Tolerance of first strand positions 2 and 14 for DNA and tolerance of second strand positions 7-9 for DNA was analyzed with an siRNA targeting human ALDH2. ALD58 contains alternating 2'-OMe/2'-F in both strands, whereas ALD61 and ALD90-ALD92 contain a reduced 2'-F pattern in the first strand with DNA at position 2 (ALD90), DNA at position 14 (ALD91) and DNA at position 2 and 14 (ALD92), ALD93-ALD96 contain a reduced 2'-F pattern in the second strand with DNA at position 7 (ALD94), DNA at position 9 (ALD95) and DNA at position 7 and 9 (ALD96). ALD97 contains 2'-F at positions 7, 8 and 9 of the second strand, whereas ALD98 contains DNA at these positions.

[0276] The experiment was conducted in Hep3B. Cells were seeded at a density of 150,000 cells per 6-well, transfected with 0.1 nM and 0.01 nM siRNA and 1 µg/ml Atufect after 24 h and lysed after 48 h. Total RNA was extracted and ALDH2 and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three technical replicates.

[0277] Data is shown in Figure 14a and b.

Example 16

[0278] Incorporation of DNA at key positions.

[0279] Tolerance of first strand positions 2 and 14 for DNA and tolerance of second strand positions 7-9 for DNA was analyzed with a second siRNA targeting human ALDH2. ALD72 contains alternating 2'-OMe/2'-F in both strands, whereas ALD75 and ALD99-ALD101 contain a reduced 2'-F pattern in the first strand with DNA at position 2 (ALD99), DNA at position 14 (ALD100) and DNA at position 2 and 14 (ALD101). ALD102-ALD105 contain a reduced 2'-F pattern in the second strand with DNA at position 7 (ALD103), DNA at position 9 (ALD104) and DNA at position 7 and 9 (ALD105). ALD106 contains 2'-F at positions 7, 8 and 9 of the second strand, whereas ALD107 contains DNA at these positions.

[0280] The experiment was conducted in Hep3B. Cells were seeded at a density of 150,000 cells per 6-well, transfected with 0.1 nM and 0.01 nM siRNA and 1 µg/ml Atufect after 24 h and lysed after 48 h. Total RNA was extracted and ALDH2 and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three technical replicates.

[0281] Data is shown in Figure 15a and b.

Example 17

[0282] Incorporation of DNA at key positions.

[0283] Tolerance of first strand positions 2 and 14 for DNA and tolerance of second strand positions 7-9 for DNA was analyzed with an siRNA targeting human DGAT2. DGT01 contains alternating 2'-OMe/2'-F in both strands, whereas DGT04 and DGT11-DGT13 contain a reduced 2'-F pattern in the first strand with DNA at position 2 (DGT11), DNA at position 14 (DGT12) and DNA at position 2 and 14 (DGT13). DGT14-DGT17 contain a reduced 2'-F pattern in the second strand with DNA at position 7 (DGT15), DNA at position 9 (DGT16) and DNA at position 7 and 9 (DGT17). DGT18 contains 2'-F at positions 7, 8 and 9 of the second strand, whereas DGT19 contains DNA at these positions.

[0284] The experiment was conducted in Huh7. Cells were seeded at a density of 80,000 cells per 6-well, transfected with 10 nM and 1 nM siRNA and 1 µg/ml Atufect after 24 h and lysed after 72 h. Total RNA was extracted and DGAT2 and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three technical replicates.

[0285] Data is shown in Figure 16a and b.

Example 18

[0286] Incorporation of 2'-O-methoxyethyl (MOE) at key positions.

[0287] Tolerance of first strand positions 2 and 14 for 2'-O-MOE and tolerance of second strand positions 7 and 9 for 2'-O-MOE was analyzed with an siRNA targeting ALDH2. ALD108 contains a reduced number of 2'-F in both strands. In this context, 2'-O-MOE is placed at position 2 (ALD115), at position 14 (ALD116) or at both positions 2 and 14 of the first strand (ALD117). Similarly, 2'-O-MOE is placed at position 7 (ALD118), position 9 (ALD119) or at both positions 7 and 9 of the second strand (ALD120). An siRNA against Luciferase was used as non-targeting control ("Luc").

[0288] The experiment was conducted in Hep3B. Cells were seeded at a density of 150,000 cells per 6-well, transfected with 0.1 nM siRNA and 1 µg/ml Atufect after 24 h and lysed after 48 h. Total RNA was extracted and ALDH2 and Actin mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three technical replicates.

[0289] Data is shown in Figure 17a and b.

Example 19

[0290] Identification of key positions in the first strand.

[0291] The tolerance for 2'-OMe was investigated by addressing one position at a time in the context of an alternating pattern (change 2'-OMe into 2'-F and vice versa) in an siRNA targeting GHR. GHR03 contains completely alternating 2'-OMe/2'-F, GHR07 - GHR15 contain 2'-F into 2'-OMe changes at all even positions of the first strand, GHR16 - GHR25 contain 2'-OMe into 2'-F changes at all odd positions of the first strand. GHR07 contains 2'-OMe at first strand position 2, GHR13 contains 2'-OMe at first strand position 14. An siRNA targeting Luciferase ("Luc") was used as control.

[0292] The experiment was conducted in MCF-7 cells. The cells were seeded at a density of 120,000 cells per 6-well 24 h before transfection, transfected with 1 nM siRNA and 1 µg/ml Atufect and lysed after 48 h. Total RNA was extracted and GHR and PTEN mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD of three technical replicates.

[0293] Data is shown in Figures 18a and b.

SEQ ID	Name	Sequence (5'-3')
1	CLC28-a	AUGmCAAAAmUACACUmUCUAC
2	CLC28-b	GmUAGAAGmUGmUAmUmUmUmUGmCAmU
3	CLC59-a	AmUGCAAAAmUACACUmUCUAC
4	CLC59-b	GmUAGAAGmUGmUAmUmUmUmUGmCAmU
5	CLC60-a	AUmGCAAAAmUACACUmUCUAC
6	CLC60-b	GmUAGAAGmUGmUAmUmUmUmUGmCAmU
7	CLC56-a	AmUGmCAmAAmAUmACmACUUmCUmAC
8	CLC56-b	mGUmAGmAAmGUGUmAUmUUmUGmCAmU
9	CLC57-a	AUmGCAmAAmAUmACmACUUmCUmAC
10	CLC57-b	mGUmAGmAAmGUGUmAUmUUmUGmCAmU
11	CLC01-a	mAUmGCmAAmAAmUAmCAmCUmUCmUAmC
12	CLC01-b	GmUAmGAmAGmUGmUAmUUmUUmGCmAU
13	CLC22-a	AUGmCAAAAmUACACmUUCUAC
14	CLC22-b	GmUAGAAGmUGmUAmUmUmUmUGmCAmU
15	CLC28-a	AUGmCAAAAmUACACUmUCUAC
16	CLC28-b	GmUAGAAGmUGmUAmUmUmUmUGmCAmU
17	CLC16-a	AmUGmCAmAAmAUmACmACUUmCUmAC

SEQ ID	Name	Sequence (5'-3')
18	CLC16-b	mGUmAGmAAmGUmGUmAUmUUmUGmCAmU
19	CLC15-a	AmUGmCAmAAmAUmACmACmUUmCUmAC
20	CLC15-b	mGUmAGmAAmGUmGUmAUmUUmUGmCAmU
21	HFE04-a	fAfUmUfGfAmUfAfGfAfAmCfCfAfUmCfUfUmCfA
22	HFE04-b	mUfGfAfAfGfAmUfGfGmUmUmCmUfAmUmCfAfAmU
23	HFE06-a	fAmUfUfGfAmUfAfGfAfAmCfCfAfUmCfUfUmCfA
24	HFE06-b	mUfGfAfAfGfAmUfGfGmUmUmCmUfAmUmCfAfAmU
25	ALD01-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
26	ALD01-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
27	ALD13-a	mA(ps)mA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
28	ALD13-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
29	ALD14-a	mA(ps)fA(ps)mUmGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
30	ALD14-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
31	ALD15-a	mA(ps)fA(ps)mUfGmUmUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
32	ALD15-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
33	ALD16-a	mA(ps)fA(ps)mUfGmUfUmUmUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
34	ALD16-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
35	ALD17-a	mA(ps)fA(ps)mUfGmUfUmUfUmCmCmUfGmCfUmGfAmC(ps)fG(ps)mG
36	ALD17-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
37	ALD18-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUmGmCfUmGfAmC(ps)fG(ps)mG
38	ALD18-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
39	ALD19-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCmUmGfAmC(ps)fG(ps)mG
40	ALD19-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
41	ALD20-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGmAmC(ps)fG(ps)mG
42	ALD20-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
43	ALD21-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)mG(ps)mG
44	ALD21-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
45	ALD22-a	fA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
46	ALD22-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
47	ALD23-a	mA(ps)fA(ps)fUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
48	ALD23-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
49	ALD24-a	mA(ps)fA(ps)mUfGfUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
50	ALD24-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
51	ALD25-a	mA(ps)fA(ps)mUfGmUfUfUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
52	ALD25-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
53	ALD26-a	mA(ps)fA(ps)mUfGmUfUmUfUfCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
54	ALD26-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
55	ALD27-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCfUfGmCfUmGfAmC(ps)fG(ps)mG
56	ALD27-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
57	ALD28-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGfCfUmGfAmC(ps)fG(ps)mG
58	ALD28-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
59	ALD29-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUfGfAmC(ps)fG(ps)mG
60	ALD29-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU

SEQ ID	Name	Sequence (5'-3')
61	ALD30-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAfC(ps)fG(ps)mG
62	ALD30-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
63	ALD31-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)fG
64	ALD31-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
65	ALD32-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
66	ALD32-b	mC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)m U(ps)f U
67	ALD33-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
68	ALD33-b	fC(ps)mC(ps)mGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
69	ALD34-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
70	ALD34-b	fC(ps)mC(ps)fGmUmCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
71	ALD35-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
72	ALD35-b	fC(ps)mC(ps)fGmUfCmAmGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
73	ALD36-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
74	ALD36-b	fC(ps)mC(ps)fGmUfCmAfGmCmAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
75	ALD37-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
76	ALD37-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGmGmAfAmAfAmCfA(ps)mU(ps)fU
77	ALD38-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
78	ALD38-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAmAmAfAmCfA(ps)mU(ps)fU
79	ALD39-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
80	ALD39-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAmAmCfA(ps)mU(ps)fU
81	ALD40-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
82	ALD40-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCmA(ps)mU(ps)fU
83	ALD41-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
84	ALD41-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)mU
85	ALD42-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
86	ALD42-b	fC(ps)fC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
87	ALD43-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
88	ALD43-b	fC(ps)mC(ps)fGfUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
89	ALD44-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
90	ALD44-b	fC(ps)mC(ps)fGmUfCfAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
91	ALD45-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
92	ALD45-b	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
93	ALD46-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
94	ALD46-b	fC(ps)mC(ps)fGmUfCmAfGmCfAfGfGmAfAmAfAmCfA(ps)mU(ps)fU
95	ALD47-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
96	ALD47-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGfAfAmAfAmCfA(ps)mU(ps)fU
97	ALD48-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
98	ALD48-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAfAfAmCfA(ps)mU(ps)fU
99	ALD49-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
100	ALD49-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAfCfA(ps)mU(ps)fU
101	ALD50-a	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG
102	ALD50-b	fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)fU(ps)fU
103	ALD58-a	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
104	ALD58-b	fCmCfGm UfCmAfGmCfAmGfGmAfAmAfAmCfAm UfU

SEQ ID	Name	Sequence (5'-3')
105	ALD59-a	mAfAmUmGmUmUmUmUmCmCmUmGmCmUmGmAmCmGmG
106	ALD59-b	fCmCfGm UfCmAfGmCfAmGfGmAfAmAfAmCfAm UfU
107	ALD60-a	mAmAmUmGmUmUmUmUmCmCmUmGmCfUmGmAmCmGmG
108	ALD60-b	fCmCfGm UfCmAfGmCfAmGfGmAfAmAfAmCfAm UfU
109	ALD61-a	mAfAmUmGmUmUmUmUmCmCmUmGmCfUmGmAmCmGmG
110	ALD61-b	fCmCfGm UfCmAfGmCfAmGfGmAfAmAfAmCfAm UfU
111	ALD62-a	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
112	ALD62-b	mCmCmGmUmCmAfGmCmAmGmGmAmAmAmAmCmAmUmU
113	ALD63-a	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
114	ALD63-b	mCmCmGmUmCmAmGmCfAmGmGmAmAmAmAmCmAmUmU
115	ALD64-a	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
116	ALD64-b	mCmCmGmUmCmAfGmCfAmGmGmAmAmAmAmCmAmUmU
117	ALD72-a	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
118	ALD72-b	fAmGfAmAfGmAfU mCfCm UfCmGfGmCfUmAfCmAfU
119	ALD73-a	mAfUmGmUmAmGmCmCmGmAmGmGmAmUmCmUmUmCmU
120	ALD73-b	fAmGfAmAfGmAfU mCfCm UfCmGfGmCfUmAfCmAfU
121	ALD74-a	mAmUmGmUmAmGmCmCmGmAmGmGmAfUmCmUmUmCmU
122	ALD74-b	fAmGfAmAfGmAfU mCfCm UfCmGfGmCfUmAfCmAfU
123	ALD75-a	mAfUmGmUmAmGmCmCmGmAmGmGmAfUmCmUmUmCmU
124	ALD75-b	fAmGfAmAfGmAfU mCfCm UfCmGfGmCfUmAfCmAfU
125	ALD76-a	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
126	ALD76-b	mAmGmAmAmGmAfUmCmCmUmCmGmGmCmUmAmCmAmU
127	ALD77-a	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
128	ALD77-b	mAmGmAmAmGmAmUmCfCmUmCmGmGmCmUmAmCmAmU
129	ALD78-a	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
130	ALD78-b	mAmGmAmAmGmAfUmCfCmUmCmGmGmCmUmAmCmAmU
131	DGT01-a	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
132	DGT01-b	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
133	DGT02-a	mUfUmAmAmAmUmAmAmCmCmCmAmCmAmGmAmCmAmC
134	DGT02-b	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
135	DGT03-a	mUmUmAmAmAmUmAmAmCmCmCmAmCfAmGmAmCmAmC
136	DGT03-b	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
137	DGT04-a	mUfUmAmAmAmUmAmAmCmCmCmAmCfAmGmAmCmAmC
138	DGT04-b	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
139	DGT05-a	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
140	DGT05-b	mGmUmGmUmCmUfGmUmGmGmGmUmUmAmUmUmUmAmA
141	DGT06-a	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
142	DGT06-b	mGmUmGmUmCmUmGmUfGmGmGmUmUmAmUmUmUmAmA
143	DGT07-a	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
144	DGT07-b	mGmUmGmUmCmUfGmUfGmGmGmUmUmAmUmUmUmAmA
145	TMP01-a	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
146	TMP01-b	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
147	TMP93-a	mAfAmCmCmAmGmAmAmGmAmAmGmCmAmGmGmUmGmA

SEQ ID	Name	Sequence (5'-3')
148	TMP93-b	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
149	TMP94-a	mAmAmCmCmAmGmAmAmGmAmAmGmCfAmGmGmUmGmA
150	TMP94-b	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
151	TMP97-a	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
152	TMP97-b	mUmCmAmCmCmUfGmCmUmUmCmUmUmCmUmGmGmUmU
153	TMP98-a	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
154	TMP98-b	mUmCmAmCmCmUmGmCfUmUmCmUmUmCmUmGmGmUmU
155	TMP112-a	mA[A]mCmCmAmGmAmAmGmAmAmGmCfAmGmGmUmGmA
156	TMP112-b	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
157	TMP113-a	mAfAmCmCmAmGmAmAmGmAmAmGmC[A]mGmGmUmGmA
158	TMP113-b	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
159	TMP116-a	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
160	TMP116-b	mUmCmAmCmCmU[G]mCfUmUmCmUmUmCmUmGmGmUmU
161	TMP117-a	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
162	TMP117-b	mUmCmAmCmCmUfGmC[U]mUmCmUmUmCmUmGmGmUmU
163	TMP110-a	mA{A}mCmCmAmGmAmAmGmAmAmGmCfAmGmGmUmGmA
164	TMP110-b	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
165	TMP111-a	mAfAmCmCmAmGmAmAmGmAmAmGmC{A}mGmGmUmGmA
166	TMP111-b	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
167	TMP114-a	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
168	TMP114-b	mUmCmAmCmCmU{G}mCfUmUmCmUmUmCmUmGmGmUmU
169	TMP115-a	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
170	TMP115-a	mUmCmAmCmCmUfGmC{U}mUmCmUmUmCmUmGmGmUmU
171	STS12009V23L4-a	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAfGmCfAmGmGmU(ps)mG(ps)mA
172	STS12009V23L4-b	GalNAc-mUmCmAmCmCmUmGmCmUmUmCmUmUmCmUmGmG(ps)mU(ps)mU
173	STS12009V25L4-a	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAfGmCfAmGmGmU(ps)mG(ps)mA
174	STS12009V25L4-b	GalNAc-mUmCmAmCmCmUmGmCfUmUmCmUmUmCmUmGmG(ps)mU(ps)mU
175	STS12009V26L4-a	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAfGmCfAmGmGmU(ps)mG(ps)mA
176	STS12009V26L4-b	GalNAc-mUmCmAmCmCmUfGmCmUmUmCmUmUmCmUmGmG(ps)mU(ps)mU
177	STS12009V27L4-a	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAfGmCfAmGmGmU(ps)mG(ps)mA
178	STS12009V27L4-b	GalNAc-mUmCmAmCmCmUfGfCfUmUmCmUmUmCmUmGmG(ps)mU(ps)mU
179	STS12009V41 L4-a	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAfGmCfAmGmGmU(ps)mG(ps)mA
180	STS12009V41 L4-b	GalNAc-fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfG(ps)mU(ps)fU
181	TMP70-a	mA(ps)fA(ps)mCfCmAfGmAfAmGfAmAfGmCfAmGfGmU(ps)fG(ps)mA
182	TMP70-b	fU(ps)mC(ps)fAmCfCmUfGmCfUmUfCmUfUmCfUmGfG(ps)mU(ps)fU
183	TMP119-A	mA(ps)fA(ps)mCfCmAfGmAfAmGfAmAfGmCfAmGfGmU(ps)fG(ps)mA
184	TMP119-B	mU(ps)mC(ps)mAmCmCmUfGfCfUmUmCmUmUmCmUmGmG(ps)mU(ps)mU

SEQ ID	Name	Sequence (5'-3')
185	TMP120-A	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAmGmCfAmGmGmU(ps)mG(ps)mA
186	TMP120-B	mU(ps)mC(ps)mAmCmCmUfGfCfUmUmCmUmUmCmUmGmG(ps)mU(ps)mU
187	TMP121-A	mA(ps)fA(ps)mCfCmAfGmAfAmGfAmAfGmCfAmGfGmU(ps)fG(ps)mA
188	TMP121-B	mU(ps)mC(ps)mAmCmCmU[G][C][T]mUmCmUmUmCmUmGmG(ps)mU(ps)mU
189	TMP122-A	mA(ps)fA(ps)mCfCmAfGmAfAmGfAmAfGmCfAmGfGmU(ps)fG(ps)mA
190	TMP122-B	mU(ps)mC(ps)mAmCmCmU[G]mC[T]mUmCmUmUmCmUmGmG(ps)mU(ps)mU
191	TMP123-A	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAmGmCfAmGmGmU(ps)mG(ps)mA
192	TMP123-B	mU(ps)mC(ps)mAmCmCmU[G][C][T]mUmCmUmUmCmUmGmG(ps)mU(ps)mU
193	TMP124-A	mA(ps)[A](ps)mCmCmAmGmAmAmGmAmAmGmC[A]mGmGmU(ps)mG(ps)mA
194	TMP124-B	mU(ps)mC(ps)mAmCmCmU[G][C][T]mUmCmUmUmCmUmGmG(ps)mU(ps)mU
195	TMP125-A	mA(ps)[A](ps)mCmCmAmGmAmAmGmAmAmGmCfAmGmGmU(ps)mG(ps)mA
196	TMP125-B	mU(ps)mC(ps)mAmCmCmU[G][C][T]mUmCmUmUmCmUmGmG(ps)mU(ps)mU
197	TMP126-A	mA(ps)[A](ps)mCmCmAmGmAmAmGmAmAmGmCAmGmGmU(ps)mG(ps)mA
198	TMP126-B	mU(ps)mC(ps)mAmCmCmU[G][C][T]mUmCmUmUmCmUmGmG(ps)mU(ps)mU
199	ALD91-A	mAfAmUmGmUmUmUmUmCmCmUmGmC [T] mGmAmCmGmG
200	ALD91-B	fCmCfGm UfCmAfGmCfAmGfGmAfAmAfAmCfAm UfU
201	ALD92-A	mA [A] mUmGmUmUmUmUmCmCmUmGmC [T] mGmAmCmGmG
202	ALD92-B	fCmCfGm UfCmAfGmCfAmGfGmAfAmAfAmCfAm UfU
203	ALD93-A	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
204	ALD93-B	mCmCmGmUmCmAfGmCfAmGmGmAmAmAmAmCmAmUmU
205	ALD94-A	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
206	ALD94-B	mCmCmGmUmCmA [G] mCfAmGmGmAmAmAmAmCmAmUmU
207	ALD95-A	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
208	ALD95-B	mCmCmGmUmCmAfGmC [A] mGmGmAmAmAmAmCmAmUmU
209	ALD96-A	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
210	ALD96-B	mCmCmGmUmCmA [G] mC [A] mGmGmAmAmAmAmCmAmUmU
211	ALD97-A	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
212	ALD97-B	mCmCmGmUmCmAfGfCfAmmGmGmAmAmAmAmCmAmUmU
213	ALD98-A	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
214	ALD98-B	mCmCmGmUmCmA [G] [C] [A] mGmGmAmAmAmAmCmAmUmU
215	ALD99-A	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
216	ALD99-B	fAmGfAmAfGmAfU mCfCm UfCmGfGmCfUmAfCmAfU
217	ALD100-A	mAfUmGmUmAmGmCmCmGmAmGmGmA [T] mCmUmUmCmU
218	ALD100-B	fAmGfAmAfGmAfU mCfCm UfCmGfGmCfUmAfCmAfU
219	ALD101-A	mA [T] mGmUmAmGmCmCmGmAmGmGmA [T] mCmUmUmCmU
220	ALD101-B	fAmGfAmAfGmAfU mCfCm UfCmGfGmCfUmAfCmAfU
221	ALD102-A	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
222	ALD102-B	mAmGmAmAmGmAfUmCfCmUmCmGmGmCmUmAmCmAmU
223	ALD103-A	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
224	ALD103-B	mAmGmAmAmGmA [T] mCfCmUmCmGmGmCmUmAmCmAmU
225	ALD104-A	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
226	ALD104-B	mAmGmAmAmGmAmUmC [C] mUmCmGmGmCmUmAmCmAmU
227	ALD105-A	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU

SEQ ID	Name	Sequence (5'-3')
228	ALD105-B	mAmGmAmAmGmA [U] mC [C] mUmCmGmGmCmUmAmCmAmU
229	ALD106-A	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
230	ALD106-B	mAmGmAmAmGmAfUfCfCmUmCmGmGmCmUmAmCmAmU
231	ALD107-A	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
232	ALD107-B	mAmGmAmAmGmA [T] [C] [C] mUmCmGmGmCmUmmAmCmAmU
233	DGT11-A	mU [T] mAmAmAmUmAmAmmCmCmCmAmCfAmGmAmCmAmC
234	DGT11-B	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
235	DGT12-A	mUfUmAmAmAmUmAmAmCmCmCmAmC [A] mGmAmCmAmC
236	DGT12-B	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
237	DGT13-A	mUfUmAmAmAmUmAmAmCmCmCmAmC [A] mGmAmCmAmC
238	DGT13-B	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
239	DGT14-A	mU [T] mAmAmAmUmAmAmCmCmCmAmC [A] mGmAmCmAmC
240	DGT14-B	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
241	DGT15-A	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
242	DGT15-B	mGmUmGmUmCmUfGmUfGmGmGmUmUmAmUmUmUmAmA
243	DGT16-A	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
244	DGT16-B	mGmUmGmUmCmUfGmU [G] mGmGmUmUmAmUmUmUmAmA
245	DGT17-A	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
246	DGT17-B	mGmUmGmUmCmU [G] mU [G] mGmGmUmUmAmUmUmUmAmA
247	DGT18-A	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
248	DGT18-B	mGmUmGmUmCmUfGfUfGmGmGmUmUmAmUmUmUmAmA
249	DGT19-A	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
250	DGT19-B	mGmUmGmUmCmU [G] [T] [G] mGmGmUmUmAmUmUmUmAmA
251	ALD108-A	mA (ps) fU (ps) mGmUmAmGmCmCmGmAmGmGmAfUmCmUmU (ps) mC (ps) mU
252	ALD108-B	mA (ps) mG (ps) mAmAmGmAfUmCfCmUmCmGmGmCmUmAmC (ps) mA (ps) mU
253	ALD115-A	mA (ps) (MOE-U) (ps) mGmUmAmGmCmCmGmAmGmGmAfUmCmUmU (ps) mC (ps) mU
254	ALD115-B	mA (ps) mG (ps) mAmAmGmAfUmCfCmUmCmGmGmCmUmAmC (ps) mA (ps) mU
255	ALD116-A	mA (ps) fU (ps) mGmUmAmGmCmCmGmAmGmGmA (MOE-U) mCmUmU (ps) mC (ps) mU
256	ALD116-B	mA (ps) mG (ps) mAmAmGmAfUmCfCmUmCmGmGmCmUmAmC (ps) mA (ps) mU
257	ALD117-A	mA (ps) (MOE-U) (ps) mGmUmAmGmCmCmGmAmGmGmA (MOE-U) mCmUmU (ps) mC (ps) mU
258	ALD117-B	mA (ps) mG (ps) mAmAmGmAfUmCfCmUmCmGmGmCmUmAmC (ps) mA (ps) mU
259	ALD118-A	mA (ps) fU (ps) mGmUmAmGmCmCmGmAmGmGmAfUmCmUmU (ps) mC (ps) mU
260	ALD118-B	mA (ps) mG (ps) mAmAmGmA (MOE-U) mCfCmUmCmGmGmCmUmAmC (ps) mA (ps) mU
261	ALD119-A	mA (ps) fU (ps) mGmUmAmGmCmCmGmAmGmGmAfUmCmUmU (ps) mC (ps) mU
262	ALD119-B	mA (ps) mG (ps) mAmAmGmAfUmC (MOE-C) mUmCmGmGmCmUmAmC (ps) mA (ps) mU

SEQ ID	Name	Sequence (5'-3')
263	ALD120-A	mA (ps) fU (ps) mGmUmAmGmCmCmGmAmGmGmAfUmCmUmU (ps) mC (ps) mU
264	ALD120-B	mA (ps) mG (ps) mAmAmGmA (MOE-U) mC (MOE-C) mUmCmGmGmCmUmAmC (ps) mA (ps) mU
265	CLC28-a	AUGCAAAAUACACUUCUAC
266	CLC28-b	GUAGAAGUGUAUUUUGCAU
267	HFE04-a	AUUGAUAGAACCAUCUUCA
268	HFE04-b	UGAAGAUGGUUCUAUCAAU
269	ALD01-a	AAUGUUUUCCUGCUGACGG
270	ALD01-b	CCGUCAGCAGGAAAACAUU
271	ALD72-a	AUGUAGCCGAGGAUCUUCU
272	ALD72-b	AGAAGAUCCUCGGCUACAU
273	DGT01-a	UUAAAUAACCCACAGACAC
274	DGT01-b	GUGUCUGUGGGUUAUUUAA
275	TMP01-a	AACCAGAAGAAGCAGGUGA
276	TMP01-b	UCACCUGCUUCUUCUGGUU
277	STS12009V23L4-a	AACCAGAAGAAGCAGGUGA
278	STS12009V23L4-b	UCACCUGCUUCUUCUGGUU

Key

[0294]

A, U, C, G - RNA

mA, mU, mC, mG - 2'-OMe RNA

fA, fU, fC, fG - 2'-F RNA

(ps) - phosphorothioate

[A], [U], [C], [G] - 2'-H (DNA)

{A}, {U}, {C}, {G} - LNA

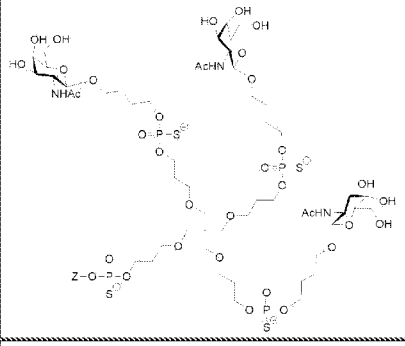
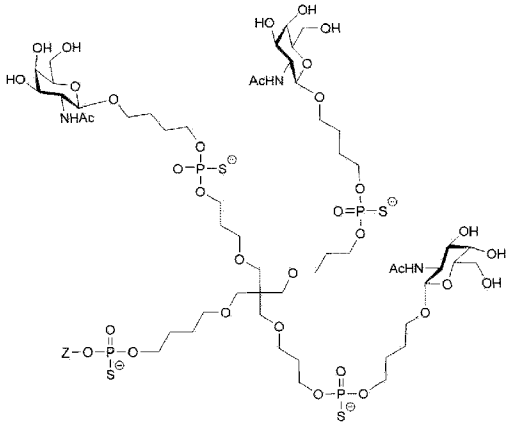
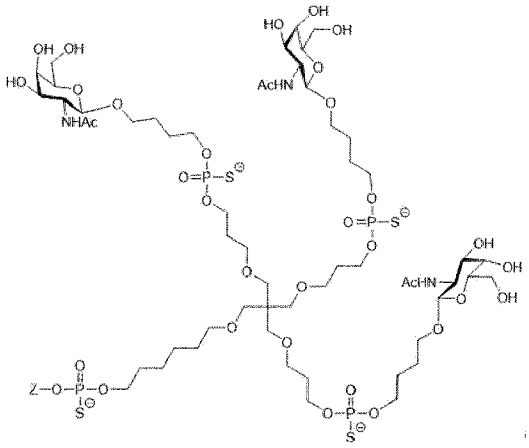
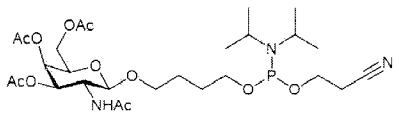
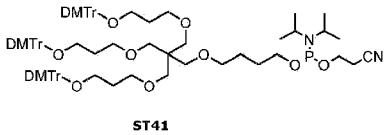
GalNAc - [ST23 (ps)]3 ST41 (ps)

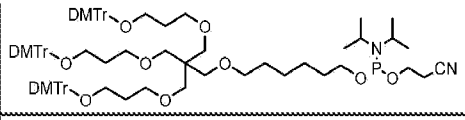
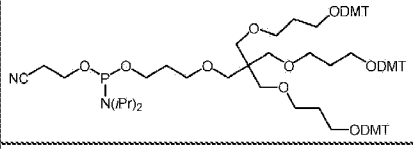
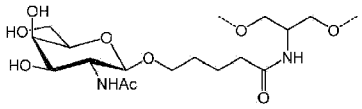
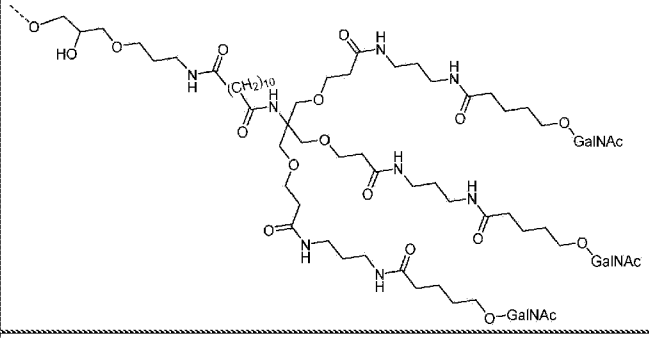
(MOE-U), (MOE-C) - 2'methoxyethyl RNA

[0295] The sequences listed above may be disclosed with a linker or ligand, such as GalNAC or (ps) or (ps2) linkages for example. These form an optional, but preferred, part of the sequence of the sequence listing.

[0296] The following abbreviations may be used:

ivN	Inverted nucleotide, either 3'-3' or 5'-5'
(ps2)	Phosphorodithioate
vinylphosphonate	Vinyl-(E)-phosphonate

FAM	6-Carboxyfluorescein
TAMRA	5-Carboxytetramethylrhodamine
BHQ1	Black Hole Quencher 1
(ps)	Phosphorothioate
GN	
GN2	
GN3	
GNo	Same as GN2 but with phosphodiesters instead of phosphorothioates
ST23	
ST41/C4XLT	 ST41
ST43/C6XLT	

	
Long trebler/ltrb/STKS	
Ser(GN)	
GlyC3Am(GalNAc)	
GalNAc (only in when used in sequences)	GN2 (see above)
(MOE-U), (MOE-C)	2'methoxyethyl RNA
{A}, {U}, {C}, {G}	LNA
[ST23 (ps)]3 ST41 (ps)	GN2 (see above)
[ST23 (ps)]3 ST43 (ps)	GN3 (see above)
ST23(ps) long trebler(ps)	GN (see above)

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Patent documents cited in the description

- US5885968A [0107]
- WO2017174657A [0211] [0212]

Non-patent literature cited in the description

- **WATTSCOREY**Journal of Pathology, 2012, vol. 226, 365-379 [0004]
- **TAKEI et al.**JBC, 2002, vol. 277, 2623800-06 [0076]

Patentkrav

1. Nukleinsyre til inhibering af ekspression af et målgen i en celle, omfattende mindst én duplex-region, der omfatter mindst en del af en første streng og mindst en del af en anden streng, der er mindst delvist komplementær til den første streng, hvor den første streng er i det mindste delvist komplementær til i det mindste en del af RNA, der er transskriberet fra målgenet,
hvor alle nukleotider i nukleinsyren er modificeret i 2'-positionen af sukkeret,
hvor position 2 og 14 på den første streng startende fra 5'-enden er modificeret med 2'-fluor,
hvor nukleinsyren er modificeret på den første streng med skiftende 2' O-methyl-modifikationer og 2'-fluormodifikationer, og
hvor den anden streng er modificeret med 2'-fluormodifikationer i positionerne 11-13, der tæller fra 3'-enden startende ved den første position af dobbeltstrengsområdet, og de resterende modifikationer er 2'-O-methylmodifikationer.

2. Nukleinsyre ifølge krav 1, hvor det terminale nukleotid ved 3'-enden af mindst én af den første streng, og den anden streng er et omvendt nukleotid og er bundet til det tilstødende nukleotid via 3'-carbonatomet i det terminale nukleotid og 3'-carbonatomet i det tilstødende nukleotid, og/eller det terminale nukleotid ved 5'-enden af mindst én af den første streng og den anden streng er et omvendt nukleotid og er knyttet til det tilstødende nukleotid via 5'-carbonatomet i den terminale nukleotid og 5'-carbonatomet af det tilstødende nukleotid, eller hvor nukleinsyren omfatter en phosphordithioat-binding.

3. Nukleinsyre ifølge et hvilket som helst af de foregående krav, hvor nukleinsyren er stump i begge ender.

4. Nukleinsyre ifølge et hvilket som helst af de foregående krav, hvor den første streng og den anden streng hver er 19 nukleotider lange.

5. Nukleinsyre ifølge et hvilket som helst af de foregående krav, hvor nukleinsyren er stump i begge ender, og den første og den anden streng hver er 19 nukleotider lange.

6. Konjugeret nukleinsyre, omfattende en nukleinsyre ifølge et hvilket som helst af de foregående krav, konjugeret med en ligand.

7. Konjugeret nukleinsyre ifølge krav 6, hvor nukleinsyren er konjugeret til en ligand med formlen (III):

$$[S\text{-}X^1\text{-}P\text{-}X2]3\text{-}A\text{-}X3\text{-} \qquad (III)$$

hvor:
S repræsenterer et saccharid;
X^1 repræsenterer C_3-C_6-alkylen eller $(-CH_2-CH_2-O)_m(-CH_2)_2-$ hvor m er 1, 2 eller 3;
P er et phosphat eller modificeret phosphat;
X^2 er en alkylenether med formel $-C_3H_6-O-CH_2-$;
A er en forgreningsenhed;
X^3 er en alkylenether med formel udvalgt fra gruppen bestående af $-CH_2-O-C_4H_8-$, $-CH_2-O-C_5H_{10}-$, $-CH_2-O-C_6H_{12}-$, $-CH_2-O-C_7H_{14}-$ og $-CH_2-O-C_8H_{16}-$, hvor $-CH_2-$gruppen i hvert tilfælde er bundet til A,
hvor en nukleinsyre ifølge et hvilket som helst af kravene 1 til 5 er konjugeret til X^3 via et fosfat eller modificeret fosfat.

8. Konjugeret nukleinsyre ifølge krav 7, hvor P i formlen (III) er et thiophosphat.

9. Konjugeret nukleinsyre ifølge krav 7 eller 8, hvor nukleinsyren ifølge et hvilket som helst af kravene 1 til 5 er konjugeret til X^3 via et thiophosphat.

10. Sammensætning omfattende en nukleinsyre ifølge et hvilket som helst af kravene 1 til 5 eller en konjugeret nukleinsyre ifølge et hvilket som helst af kravene 6 til 9 og en fysiologisk acceptabel excipiens.

11. Nukleinsyre ifølge et hvilket som helst af kravene 1 til 5 eller en konjugeret nukleinsyre ifølge et hvilket som helst af kravene 6 til 9 eller en sammensætning ifølge krav 10 til anvendelse i behandlingen af en sygdom eller lidelse.

DRAWINGS

Figure 1a

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
CLC01	mAUmGCmAAmAAmUAmCAmCUmUCmUAmC
	GmUAmGAmAGmUGmUAmUUmUUmGCmAU
CLC15	AmUGmCAmAAmAUmACmACmUUmCUmAC
	mGUmAGmAAmGUmGUmAUmUUmUGmCAmU
CLC16	AmUGmCAmAAmAUmACmACUUmCUmAC
	mGUmAGmAAmGUmGUmAUmUUmUGmCAmU

A, U, C, G - RNA
mA, mU, mC, mG – 2'-OMe RNA

Figure 1b

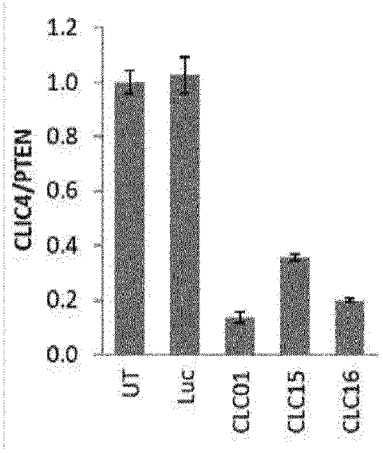


Figure 2a

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
CLC01	mAUmGCmAAmAAmUAmCAmCUmUCmUAmC
	GmUAmGAmAGmUGmUAmUUmUUmGCmAU
CLC22	AUGmCAAAAmUACACmUUCUAC
	GmUAGAAGmUGmUAmUmUmUmUGmCAmU
CLC28	AUGmCAAAAmUACACUmUCUAC
	GmUAGAAGmUGmUAmUmUmUmUGmCAmU

A, U, C, G - RNA

mA, mU, mC, mG – 2'-OMe RNA

Figure 2b

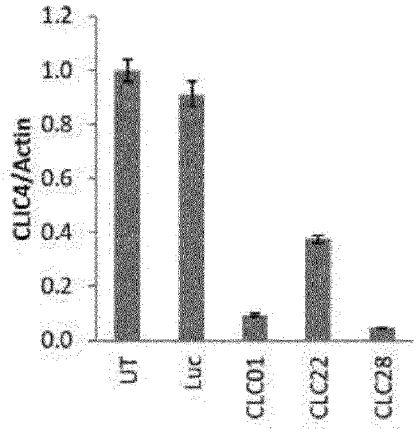


Figure 3a

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
CLC56	AmUGmCAmAAmAUmACmACUUmCUmAC
	mGUmAGmAAmGUGUmAUmUUmUGmCAmU
CLC57	AUmGCAmAAmAUmACmACUUmCUmAC
	mGUmAGmAAmGUGUmAUmUUmUGmCAmU

A, U, C, G - RNA

mA, mU, mC, mG – 2'-OMe RNA

Figure 3b

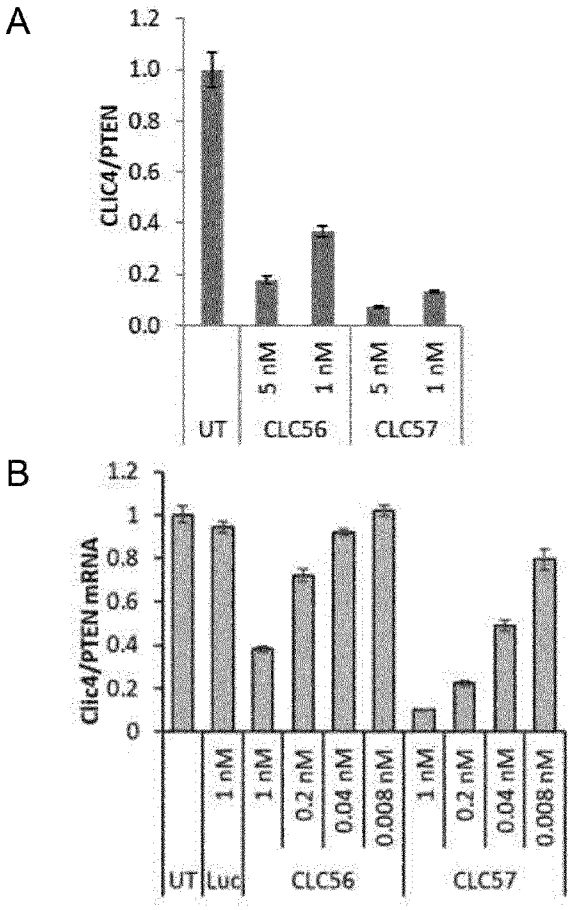


Figure 4a

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
CLC01	mAUmGCmAAmAAmUAmCAmCUmUCmUAmC
	GmUAmGAmAGmUGmUAmUUmUUmGCmAU
CLC28	AUGmCAAAAmUACACUmUCUAC
	GmUAGAAGmUGmUAmUmUmUmUGmCAmU
CLC59	AmUGCAAAAmUACACUmUCUAC
	GmUAGAAGmUGmUAmUmUmUmUGmCAmU
CLC60	AUmGCAAAAmUACACUmUCUAC
	GmUAGAAGmUGmUAmUmUmUmUGmCAmU

A, U, C, G - RNA

mA, mU, mC, mG – 2'-OMe RNA

Figure 4b

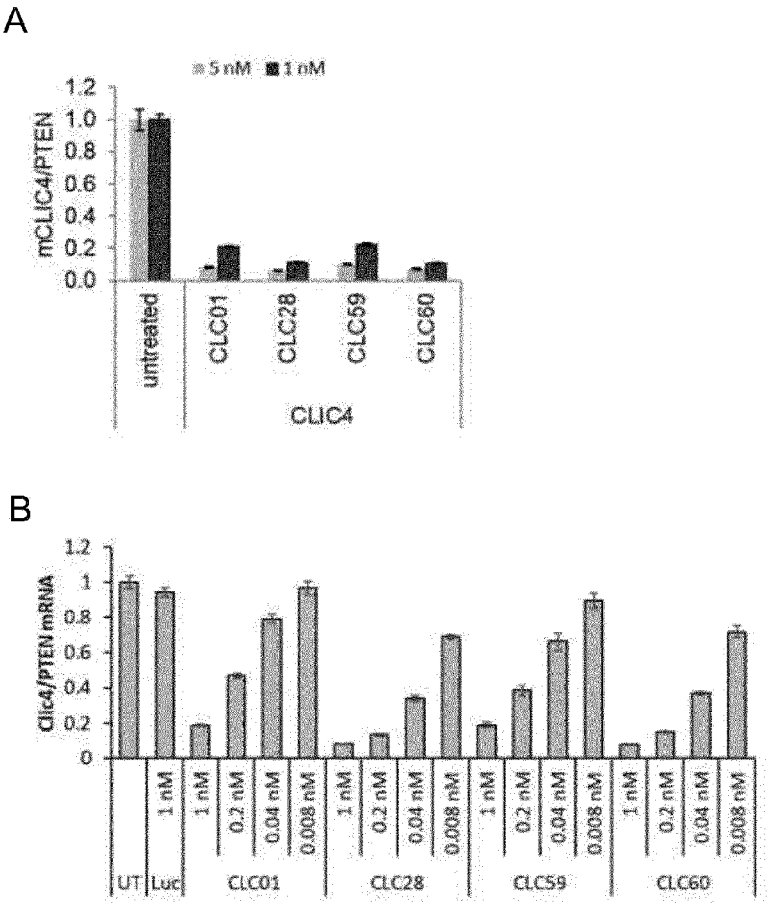


Figure 5a

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
HFE04	fAfUmUfGfAmUfAfGfAfAmCfCfAfUmCfUfUmCfA
	mUfGfAfAfGfAmUfGfGmUmUmCmUfAmUmCfAfAmU
HFE06	fAmUfUfGfAmUfAfGfAfAmCfCfAfUmCfUfUmCfA
	mUfGfAfAfGfAmUfGfGmUmUmCmUfAmUmCfAfAmU

A, U, C, G - RNA
mA, mU, mC, mG – 2'-OMe RNA
fA, fU, fC, fG – 2'-F RNA

Figure 5b

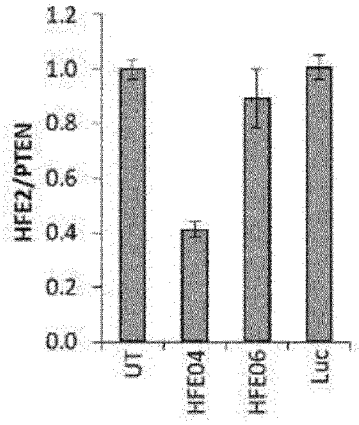


Figure 6a: Sequences used in Figures 6b and 6c

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
ALD01	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD13	mA(ps)mA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD14	mA(ps)fA(ps)mUmGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD15	mA(ps)fA(ps)mUfGmUmUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD16	mA(ps)fA(ps)mUfGmUfUmUmUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD17	mA(ps)fA(ps)mUfGmUfUmUfUmCmCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD18	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUmGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD19	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCmUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD20	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGmAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD21	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)mG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD22	fA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD23	mA(ps)fA(ps)fUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD24	mA(ps)fA(ps)mUfGfUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD25	mA(ps)fA(ps)mUfGmUfUfUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD26	mA(ps)fA(ps)mUfGmUfUmUfUfCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD27	mA(ps)fA(ps)mUfGmUfUmUfUmCfCfUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD28	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGfCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD29	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUfGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD30	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAfC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD31	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)fG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU

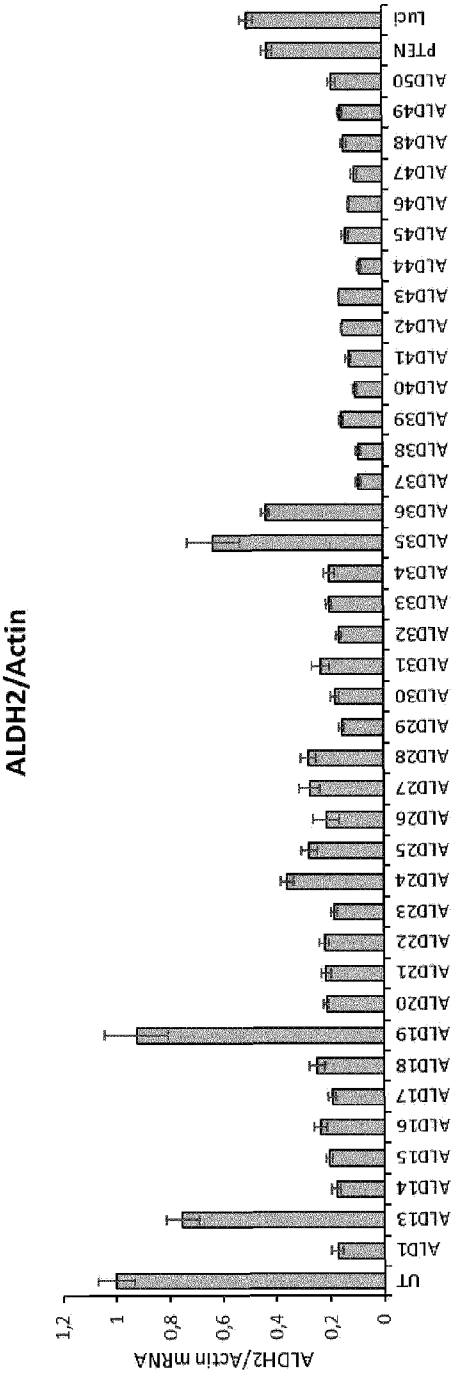
mA, mU, mC, mG – 2'-OMe RNA
fA, fU, fC, fG – 2'-F RNA
(ps) – phosphorothioate

Figure 6a (continued):

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
ALD01	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD32	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG mC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD33	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)mGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD34	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUmCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD35	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAmGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD36	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCmAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD37	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGmGmAfAmAfAmCfA(ps)mU(ps)fU
ALD38	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAmAmAfAmCfA(ps)mU(ps)fU
ALD39	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAmAmCfA(ps)mU(ps)fU
ALD40	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCmA(ps)mU(ps)fU
ALD41	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)mU
ALD42	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)fC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD43	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGfUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD44	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCfAfGmCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD45	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGfCfAmGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD46	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAfGfGmAfAmAfAmCfA(ps)mU(ps)fU
ALD47	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGfAfAmAfAmCfA(ps)mU(ps)fU
ALD48	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAfAfAmCfA(ps)mU(ps)fU
ALD49	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAfCfA(ps)mU(ps)fU
ALD50	mA(ps)fA(ps)mUfGmUfUmUfUmCfCmUfGmCfUmGfAmC(ps)fG(ps)mG fC(ps)mC(ps)fGmUfCmAfGmCfAmGfGmAfAmAfAmCfA(ps)fU(ps)fU

mA, mU, mC, mG – 2'-OMe RNA
fA, fU, fC, fG – 2'-F RNA
(ps) – phosphorothioate

Fig 6b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ALDH2/Actin mRNA
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0,4
0,6
0,8
1
1,2
UT
ALD1
ALD13
ALD14
ALD15
ALD16
ALD17
ALD18
ALD19
ALD20
ALD21
ALD22
ALD23
ALD24
ALD25
ALD26
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ALD28
ALD29
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ALD31
ALD32
ALD33
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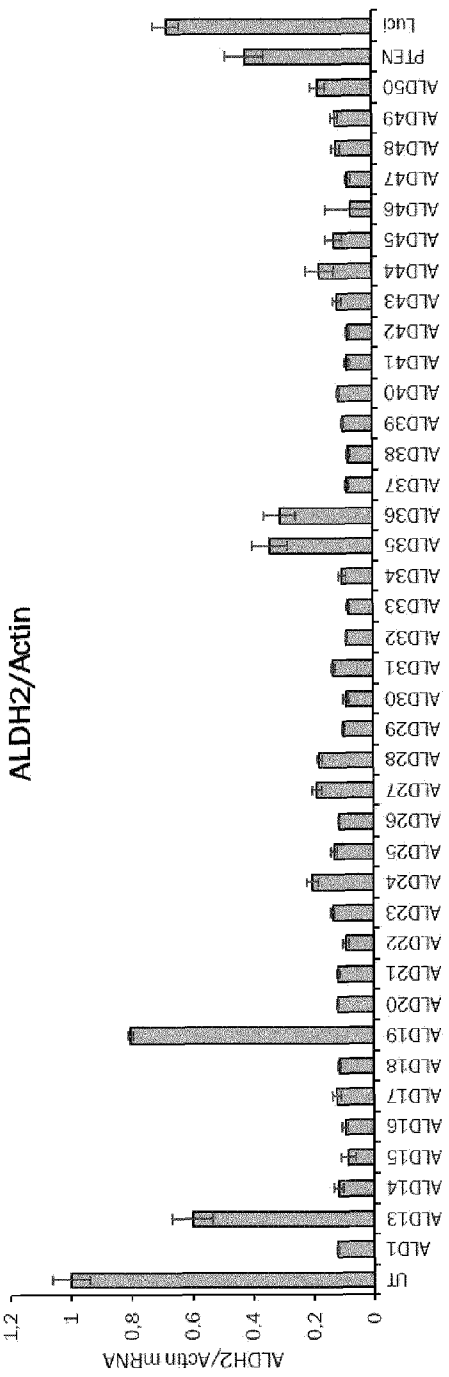
ALD38
ALD39
ALD40
ALD41
ALD42
ALD43
ALD44
ALD45
ALD46
ALD47
ALD48
ALD49
ALD50
PTEN
Luci

Fig6c


ALDH2/Actin
ALDH2/Actin mRNA
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0.2
0.4
0.6
0.8
1
1.2
UT
ALD1
ALD13
ALD14
ALD15
ALD16
ALD17
ALD18
ALD19
ALD20
ALD21
ALD22
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ALD39
ALD40
ALD41
ALD42
ALD43
ALD44
ALD45
ALD46
ALD47
ALD48
ALD49
ALD50
PTEN
Luci

Fig 7a

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
ALD58	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
	fCmCfGmUfCmAfGmCfAmGfGmAfAmAfAmCfAmUfU
ALD59	mAfAmUmGmUmUmUmUmCmCmUmGmCmUmGmAmCmGmG
	fCmCfGmUfCmAfGmCfAmGfGmAfAmAfAmCfAmUfU
ALD60	mAmAmUmGmUmUmUmUmCmCmUmGmCfUmGmAmCmGmG
	fCmCfGmUfCmAfGmCfAmGfGmAfAmAfAmCfAmUfU
ALD61	mAfAmUmGmUmUmUmUmCmCmUmGmCfUmGmAmCmGmG
	fCmCfGmUfCmAfGmCfAmGfGmAfAmAfAmCfAmUfU
ALD62	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
	mCmCmGmUmCmAfGmCmAmGmGmAmAmAmAmCmAmUmU
ALD63	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
	mCmCmGmUmCmAmGmCfAmGmGmAmAmAmAmCmAmUmU
ALD64	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
	mCmCmGmUmCmAfGmCfAmGmGmAmAmAmAmCmAmUmU

mA, mU, mC, mG – 2'-OMe RNA
fA, fU, fC, fG – 2'-F RNA
(ps) – phosphorothioate

Fig 7b

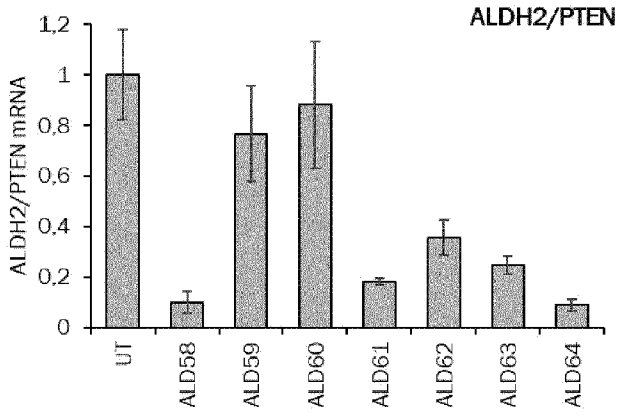


Figure 8a

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
ALD72	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
	fAmGfAmAfGmAfUmCfCmUfCmGfGmCfUmAfCmAfU
ALD73	mAfUmGmUmAmGmCmCmGmAmGmGmAmUmCmUmUmCmU
	fAmGfAmAfGmAfUmCfCmUfCmGfGmCfUmAfCmAfU
ALD74	mAmUmGmUmAmGmCmCmGmAmGmGmAfUmCmUmUmCmU
	fAmGfAmAfGmAfUmCfCmUfCmGfGmCfUmAfCmAfU
ALD75	mAfUmGmUmAmGmCmCmGmAmGmGmAfUmCmUmUmCmU
	fAmGfAmAfGmAfUmCfCmUfCmGfGmCfUmAfCmAfU
ALD76	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
	mAmGmAmAmGmAfUmCmCmUmCmGmGmCmUmAmCmAmU
ALD77	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
	mAmGmAmAmGmAmUmCfCmUmCmGmGmCmUmAmCmAmU
ALD78	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
	mAmGmAmAmGmAfUmCfCmUmCmGmGmCmUmAmCmAmU

mA, mU, mC, mG – 2'-OMe RNA
fA, fU, fC, fG – 2'-F RNA
(ps) – phosphorothioate

Figure 8b

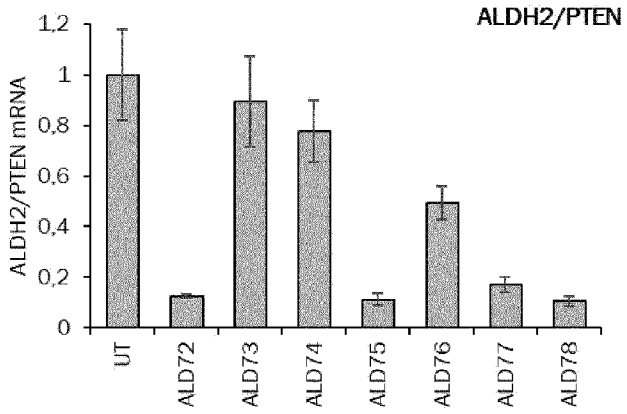


Figure 9a

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
DGT01	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
DGT02	mUfUmAmAmAmUmAmAmCmCmCmAmCmAmGmAmCmAmC
	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
DGT03	mUmUmAmAmAmUmAmAmCmCmCmAmCfAmGmAmCmAmC
	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
DGT04	mUfUmAmAmAmUmAmAmCmCmCmAmCfAmGmAmCmAmC
	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
DGT05	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
	mGmUmGmUmCmUfGmUmGmGmGmUmUmAmUmUmUmAmA
DGT06	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
	mGmUmGmUmCmUmGmUfGmGmGmUmUmAmUmUmUmAmA
DGT07	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
	mGmUmGmUmCmUfGmUfGmGmGmUmUmAmUmUmUmAmA

mA, mU, mC, mG – 2'-OMe RNA
fA, fU, fC, fG – 2'-F RNA
(ps) – phosphorothioate

Figure 9b

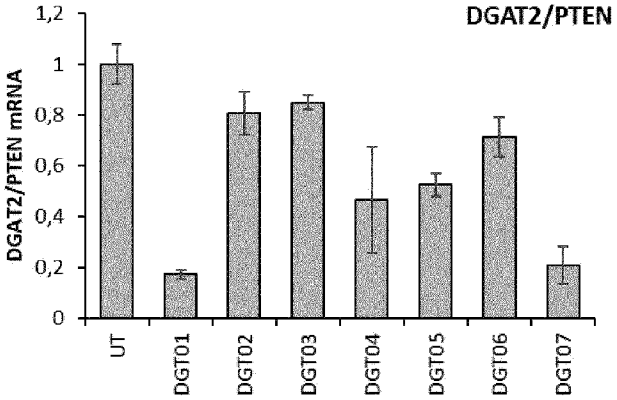


Figure 10a Modification with DNA: sequences

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
TMP01	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
TMP93	mAfAmCmCmAmGmAmAmGmAmAmGmCmAmGmGmUmGmA
	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
TMP94	mAmAmCmCmAmGmAmAmGmAmAmGmCfAmGmGmUmGmA
	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
TMP97	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
	mUmCmAmCmCmUfGmCmUmUmCmUmUmCmUmGmGmUmU
TMP98	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
	mUmCmAmCmCmUmGmCfUmUmCmUmUmCmUmGmGmUmU
TMP112	mA[A]mCmCmAmGmAmAmGmAmAmGmCfAmGmGmUmGmA
	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
TMP113	mAfAmCmCmAmGmAmAmGmAmAmGmC[A]mGmGmUmGmA
	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
TMP116	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
	mUmCmAmCmCmU[G]mCfUmUmCmUmUmCmUmGmGmUmU
TMP117	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
	mUmCmAmCmCmUfGmC[U]mUmCmUmUmCmUmGmGmUmU

mA, mU, mC, mG – 2'-OMe RNA
fA, fU, fC, fG – 2'-F RNA
[A], [U], [C], [G] - 2'-H (DNA)

Figure 10b

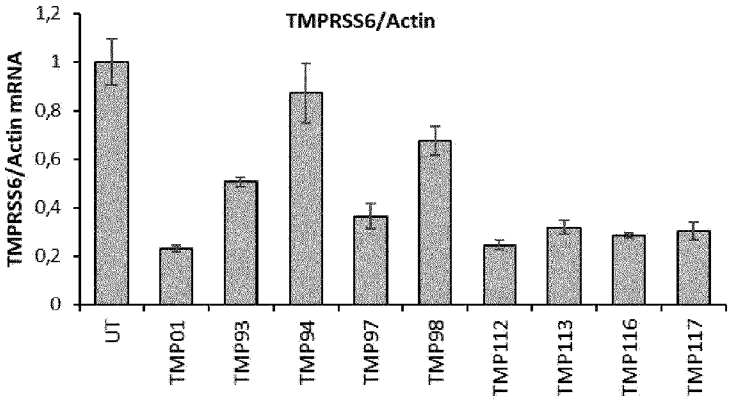


Figure 11a Modification with LNA: sequences

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
TMP01	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
TMP93	mAfAmCmCmAmGmAmAmGmAmAmGmCmAmGmGmUmGmA
	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
TMP94	mAmAmCmCmAmGmAmAmGmAmAmGmCfAmGmGmUmGmA
	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
TMP97	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
	mUmCmAmCmCmUfGmCmUmUmCmUmUmCmUmGmGmUmU
TMP98	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
	mUmCmAmCmCmUmGmCfUmUmCmUmUmCmUmGmGmUmU
TMP110	mA{A}mCmCmAmGmAmAmGmAmAmGmCfAmGmGmUmGmA
	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
TMP111	mAfAmCmCmAmGmAmAmGmAmAmGmC{A}mGmGmUmGmA
	fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfGmUfU
TMP114	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
	mUmCmAmCmCmU{G}mCfUmUmCmUmUmCmUmGmGmUmU
TMP115	mAfAmCfCmAfGmAfAmGfAmAfGmCfAmGfGmUfGmA
	mUmCmAmCmCmUfGmC{U}mUmCmUmUmCmUmGmGmUmU

mA, mU, mC, mG – 2'-OMe RNA
fA, fU, fC, fG – 2'-F RNA
{A}, {U}, {C}, {G} - LNA

Fig 11b

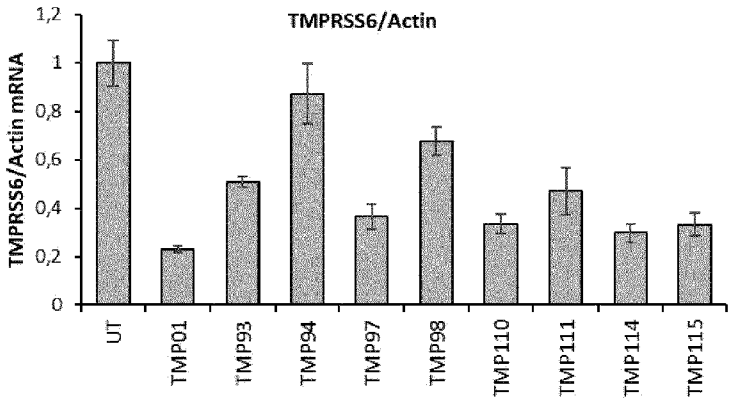


Fig 12a GalNAc-siRNA conjugates with 2'-OMe at B7 and B9

Duplex ID	sequence and chemistry (top: first strand, bottom: second strand, both 5'-3')
STS12009V23L4	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAfGmCfAmGmGmU(ps)mG(ps)mA GalNAc- mUmCmAmCmCmUmGmCmUmUmCmUmUmCmUmGmG(ps)mU(ps)mU
STS12009V25L4	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAfGmCfAmGmGmU(ps)mG(ps)mA GalNAc- mUmCmAmCmCmUmGmCfUmUmCmUmUmCmUmGmG(ps)mU(ps)mU
STS12009V26L4	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAfGmCfAmGmGmU(ps)mG(ps)mA GalNAc- mUmCmAmCmCmUfGmCmUmUmCmUmUmCmUmGmG(ps)mU(ps)mU
STS12009V27L4	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAfGmCfAmGmGmU(ps)mG(ps)mA GalNAc- mUmCmAmCmCmUfGfCfUmUmCmUmUmCmUmGmG(ps)mU(ps)mU
STS12009V41L4	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAfGmCfAmGmGmU(ps)mG(ps)mA GalNAc-fUmCfAmCfCmUfGmCfUmUfCmUfUmCfUmGfG(ps)mU(ps)fU

mA, mU, mC, mG – 2'-OMe RNA
fA, fU, fC, fG – 2'-F RNA
(ps) – phosphorothioate
GalNAc - [ST23 (ps)]3 ST41 (ps)

Figure 12b

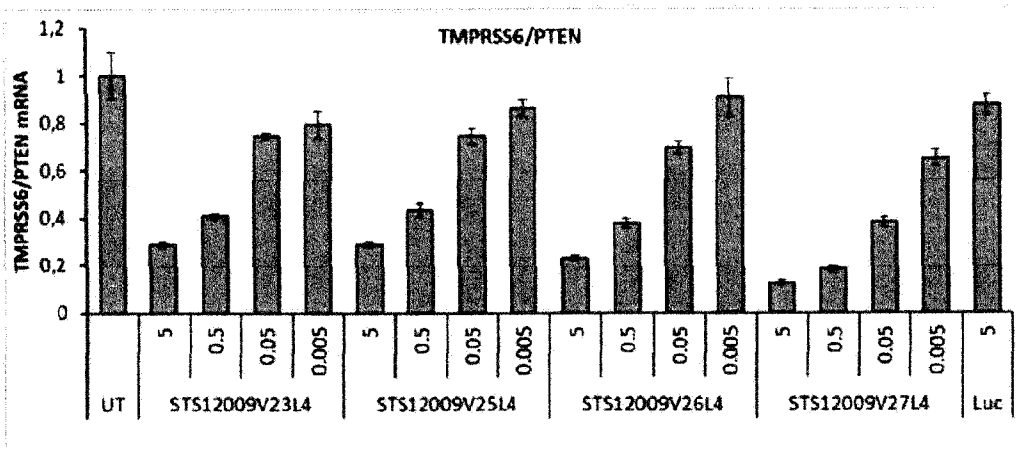

TMPRSS6/PTEN
TMPRSS6/PTEN mRNA
1,2
1
0,8
0,6
0,4
0,2
0
5
0.5
0.05
0.005
UT
STS12009V23L4
STS12009V25L4
STS12009V26L4
STS12009V27L4
Luc

Fig 12c

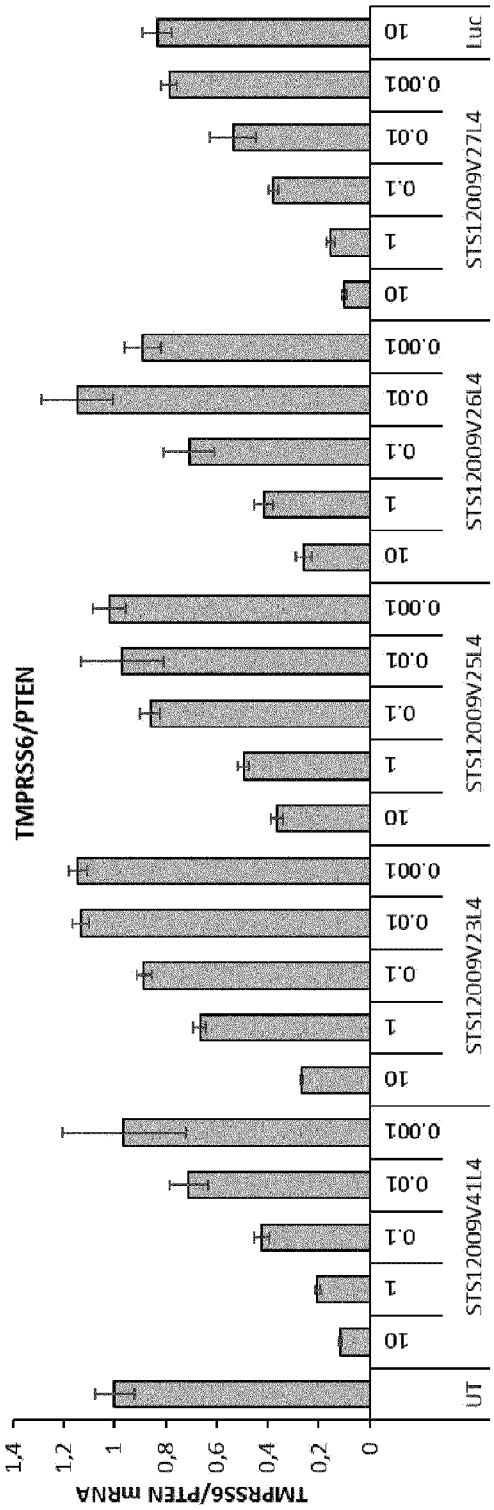

TMPRSS6/PTEN
TMPRSS6/PTEN mRNA
1,4
1,2
1
0,8
0,6
0,4
0,2
0
UT
STS12009V41L4
STS12009V23L4
STS12009V25L4
STS12009V26L4
STS12009V27L4
Luc
10
1
0.1
0.01
0.001

Figure 12d

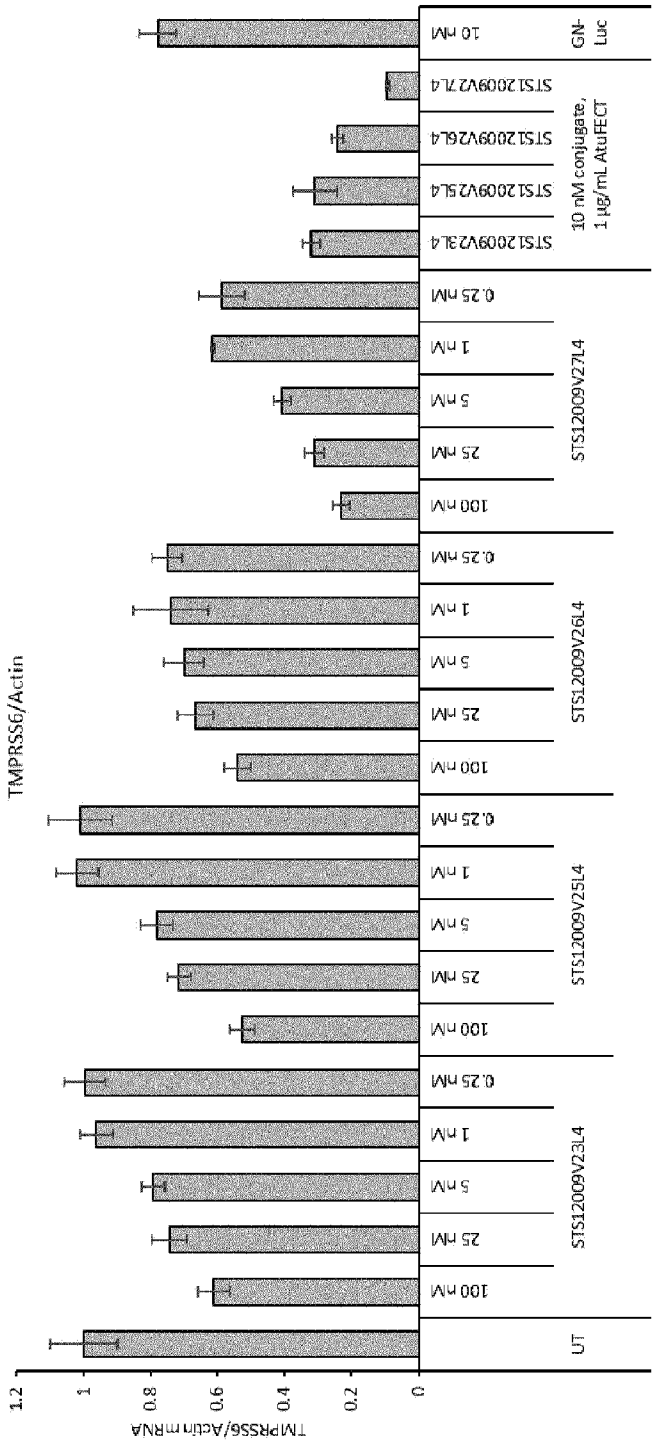

TMPRSS6/Actin
TMPRSS6/Actin mRNA
1.2
1
0.8
0.6
0.4
0.2
0
UT
100 nM
25 nM
5 nM
1 nM
0.25 nM
STS12009V23L4
STS12009V25L4
STS12009V26L4
STS12009V27L4
10 nM conjugate, 1 µg/mL AtuFECT
STSI2009V23L4
STSI2009V25L4
STSI2009V26L4
STSI2009V27L4
GN-Luc
10 nM

Figure 13a Tolerance for DNA at more than one position

Duplex ID	sequence and chemistry (top: first strand, bottom: second strand, both 5'-3')
TMP70	mA(ps)fA(ps)mCfCmAfGmAfAmGfAmAfGmCfAmGfGmU(ps)fG(ps)mA fU(ps)mC(ps)fAmCfCmUfGmCfUmUfCmUfUmCfUmGfG(ps)mU(ps)fU
TMP119	mA(ps)fA(ps)mCfCmAfGmAfAmGfAmAfGmCfAmGfGmU(ps)fG(ps)mA mU(ps)mC(ps)mAmCmCmUfGfCfUmUmCmUmUmCmUmGmG(ps)mU(ps)mU
TMP120	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAmGmCfAmGmGmU(ps)mG(ps)mA mU(ps)mC(ps)mAmCmCmUfGfCfUmUmCmUmUmCmUmGmG(ps)mU(ps)mU
TMP121	mA(ps)fA(ps)mCfCmAfGmAfAmGfAmAfGmCfAmGfGmU(ps)fG(ps)mA mU(ps)mC(ps)mAmCmCmU[G][C][T]mUmCmUmUmCmUmGmG(ps)mU(ps)mU
TMP122	mA(ps)fA(ps)mCfCmAfGmAfAmGfAmAfGmCfAmGfGmU(ps)fG(ps)mA mU(ps)mC(ps)mAmCmCmU[G]mC[T]mUmCmUmUmCmUmGmG(ps)mU(ps)mU
TMP123	mA(ps)fA(ps)mCmCmAmGmAmAmGmAmAmGmCfAmGmGmU(ps)mG(ps)mA mU(ps)mC(ps)mAmCmCmU[G][C][T]mUmCmUmUmCmUmGmG(ps)mU(ps)mU
TMP124	mA(ps)[A](ps)mCmCmAmGmAmAmGmAmAmGmC[A]mGmGmU(ps)mG(ps)mA mU(ps)mC(ps)mAmCmCmU[G][C][T]mUmCmUmUmCmUmGmG(ps)mU(ps)mU
TMP125	mA(ps)[A](ps)mCmCmAmGmAmAmGmAmAmGmCfAmGmGmU(ps)mG(ps)mA mU(ps)mC(ps)mAmCmCmU[G][C][T]mUmCmUmUmCmUmGmG(ps)mU(ps)mU
TMP126	mA(ps)[A](ps)mCmCmAmGmAmAmGmAmAmGmCAmGmGmU(ps)mG(ps)mA mU(ps)mC(ps)mAmCmCmU[G][C][T]mUmCmUmUmCmUmGmG(ps)mU(ps)mU

A, U, C, G - RNA
mA, mU, mC, mG – 2'-OMe RNA
fA, fU, fC, fG – 2'-F RNA
[A], [T], [C], [G] - 2'-H (DNA)
(ps) – phosphorothioate

Fig 13b

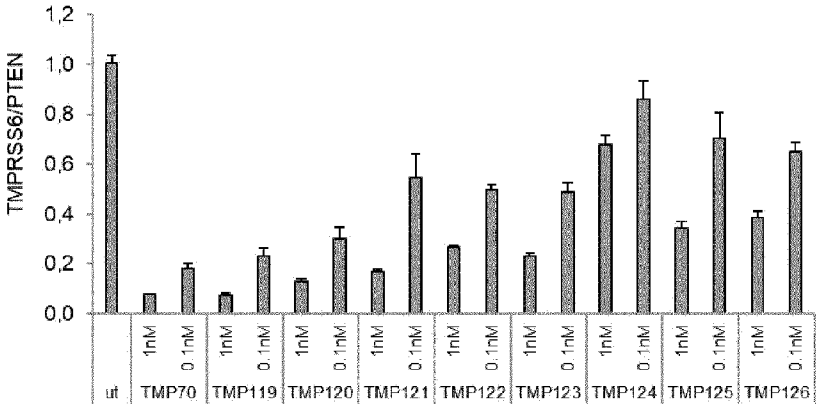


Figure 14a

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
ALD58	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
	fCmCfGmUfCmAfGmCfAmGfGmAfAmAfAmCfAmUfU
ALD61	mAfAmUmGmUmUmUmUmCmCmUmGmCfUmGmAmCmGmG
	fCmCfGmUfCmAfGmCfAmGfGmAfAmAfAmCfAmUfU
ALD90	mA[A]mUmGmUmUmUmUmCmCmUmGmCfUmGmAmCmGmG
	fCmCfGmUfCmAfGmCfAmGfGmAfAmAfAmCfAmUfU
ALD91	mAfAmUmGmUmUmUmUmCmCmUmGmC[T]mGmAmCmGmG
	fCmCfGmUfCmAfGmCfAmGfGmAfAmAfAmCfAmUfU
ALD92	mA[A]mUmGmUmUmUmUmCmCmUmGmC[T]mGmAmCmGmG
	fCmCfGmUfCmAfGmCfAmGfGmAfAmAfAmCfAmUfU
ALD93	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
	mCmCmGmUmCmAfGmCfAmGmGmAmAmAmAmCmAmUmU
ALD94	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
	mCmCmGmUmCmA[G]mCfAmGmGmAmAmAmAmCmAmUmU
ALD95	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
	mCmCmGmUmCmAfGmC[A]mGmGmAmAmAmAmCmAmUmU
ALD96	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
	mCmCmGmUmCmA[G]mC[A]mGmGmAmAmAmAmCmAmUmU
ALD97	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
	mCmCmGmUmCmAfGfCfAmGmGmAmAmAmAmCmAmUmU
ALD98	mAfAmUfGmUfUmUfUmCfCmUfGmCfUmGfAmCfGmG
	mCmCmGmUmCmA[G][C][A]mGmGmAmAmAmAmCmAmUmU

mA, mU, mC, mG – 2'-OMe RNA
fA, fU, fC, fG – 2'-F RNA
[A], [T], [C], [G] - DNA

Figure 14b

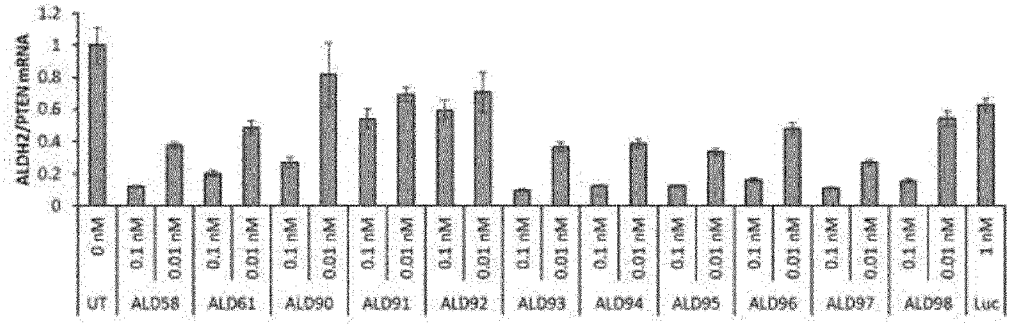


Figure 15a

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
ALD72	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
	fAmGfAmAfGmAfUmCfCmUfCmGfGmCfUmAfCmAfU
ALD75	mAfUmGmUmAmGmCmCmGmAmGmGmAfUmCmUmUmCmU
	fAmGfAmAfGmAfUmCfCmUfCmGfGmCfUmAfCmAfU
ALD99	mA[T]mGmUmAmGmCmCmGmAmGmGmAfUmCmUmUmCmU
	fAmGfAmAfGmAfUmCfCmUfCmGfGmCfUmAfCmAfU
ALD100	mAfUmGmUmAmGmCmCmGmAmGmGmA[T]mCmUmUmCmU
	fAmGfAmAfGmAfUmCfCmUfCmGfGmCfUmAfCmAfU
ALD101	mA[T]mGmUmAmGmCmCmGmAmGmGmA[T]mCmUmUmCmU
	fAmGfAmAfGmAfUmCfCmUfCmGfGmCfUmAfCmAfU
ALD102	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
	mAmGmAmAmGmAfUmCfCmUmCmGmGmCmUmAmCmAmU
ALD103	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
	mAmGmAmAmGmA[T]mCfCmUmCmGmGmCmUmAmCmAmU
ALD104	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
	mAmGmAmAmGmAfUmC[C]mUmCmGmGmCmUmAmCmAmU
ALD105	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
	mAmGmAmAmGmA[T]mC[C]mUmCmGmGmCmUmAmCmAmU
ALD106	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
	mAmGmAmAmGmAfUfCfCmUmCmGmGmCmUmAmCmAmU
ALD107	mAfUmGfUmAfGmCfCmGfAmGfGmAfUmCfUmUfCmU
	mAmGmAmAmGmA[T][C][C]mUmCmGmGmCmUmAmCmAmU

mA, mU, mC, mG – 2'-OMe RNA
fA, fU, fC, fG – 2'-F RNA
[A], [T], [C], [G] - DNA

Figure 15b

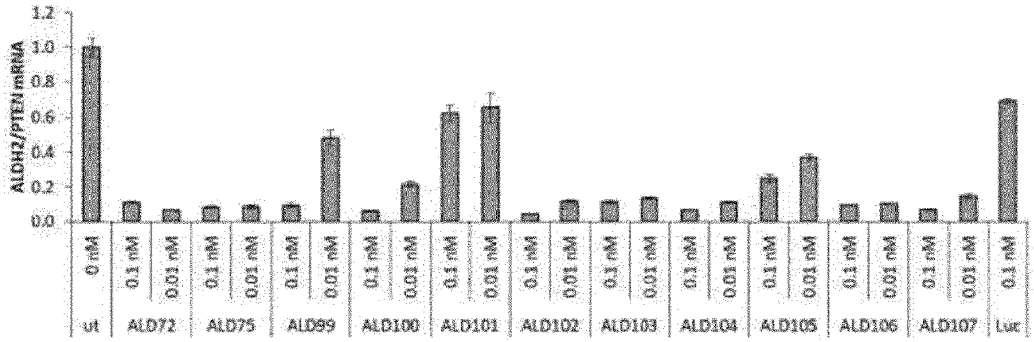


Figure 16a

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
DGT01	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
DGT04	mUfUmAmAmAmUmAmAmCmCmCmAmCfAmGmAmCmAmC
	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
DGT11	mU[T]mAmAmAmUmAmAmCmCmCmAmCfAmGmAmCmAmC
	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
DGT12	mUfUmAmAmAmUmAmAmCmCmCmAmC[A]mGmAmCmAmC
	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
DGT13	mU[T]mAmAmAmUmAmAmCmCmCmAmC[A]mGmAmCmAmC
	fGmUfGmUfCmUfGmUfGmGfGmUfUmAfUmUfUmAfA
DGT14	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
	mGmUmGmUmCmUfGmUfGmGmGmUmUmAmUmUmUmAmA
DGT15	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
	mGmUmGmUmCmU[G]mUfGmGmGmUmUmAmUmUmUmAmA
DGT16	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
	mGmUmGmUmCmUfGmU[G]mGmGmUmUmAmUmUmUmAmA
DGT17	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
	mGmUmGmUmCmU[G]mU[G]mGmGmUmUmAmUmUmUmAmA
DGT18	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
	mGmUmGmUmCmUfGfUfGmGmGmUmUmAmUmUmUmAmA
DGT19	mUfUmAfAmAfUmAfAmCfCmCfAmCfAmGfAmCfAmC
	mGmUmGmUmCmU[G][T][G]mGmGmUmUmAmUmUmUmAmA

mA, mU, mC, mG – 2'-OMe RNA
fA, fU, fC, fG – 2'-F RNA
[A], [T], [C], [G] - DNA

Figure 16b

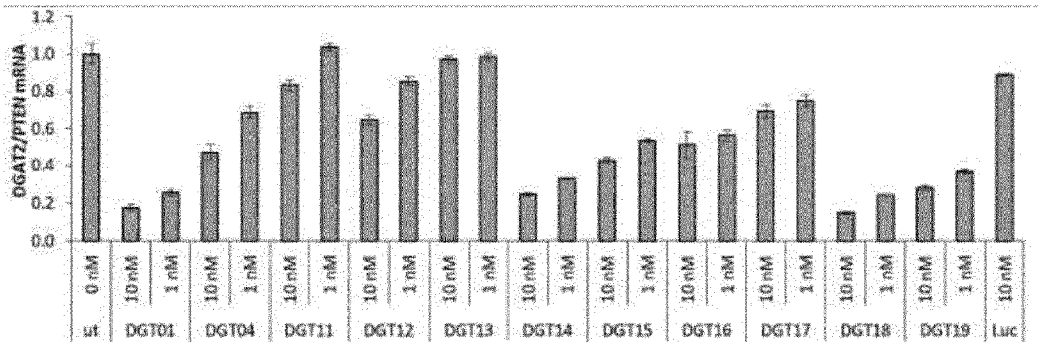


Figure 17a

Duplex ID	sequence and chemistry top: first strand, bottom: second strand, both 5'-3'
ALD108	mA(ps)fU(ps)mGmUmAmGmCmCmGmAmGmGmAfUmCmUmU(ps)mC(ps)mU
	mA(ps)mG(ps)mAmAmGmAfUmCfCmUmCmGmGmCmUmAmC(ps)mA(ps)mU
ALD115	mA(ps)(MOE-U)(ps)mGmUmAmGmCmCmGmAmGmGmAfUmCmUmU(ps)mC(ps)mU
	mA(ps)mG(ps)mAmAmGmAfUmCfCmUmCmGmGmCmUmAmC(ps)mA(ps)mU
ALD116	mA(ps)fU(ps)mGmUmAmGmCmCmGmAmGmGmA(MOE-U)mCmUmU(ps)mC(ps)mU
	mA(ps)mG(ps)mAmAmGmAfUmCfCmUmCmGmGmCmUmAmC(ps)mA(ps)mU
ALD117	mA(ps)(MOE-U)(ps)mGmUmAmGmCmCmGmAmGmGmA(MOE-U)mCmUmU(ps)mC(ps)mU
	mA(ps)mG(ps)mAmAmGmAfUmCfCmUmCmGmGmCmUmAmC(ps)mA(ps)mU
ALD118	mA(ps)fU(ps)mGmUmAmGmCmCmGmAmGmGmAfUmCmUmU(ps)mC(ps)mU
	mA(ps)mG(ps)mAmAmGmA(MOE-U)mCfCmUmCmGmGmCmUmAmC(ps)mA(ps)mU
ALD119	mA(ps)fU(ps)mGmUmAmGmCmCmGmAmGmGmAfUmCmUmU(ps)mC(ps)mU
	mA(ps)mG(ps)mAmAmGmAfUmC(MOE-C)mUmCmGmGmCmUmAmC(ps)mA(ps)mU
ALD120	mA(ps)fU(ps)mGmUmAmGmCmCmGmAmGmGmAfUmCmUmU(ps)mC(ps)mU
	mA(ps)mG(ps)mAmAmGmA(MOE-U)mC(MOE-C)mUmCmGmGmCmUmAmC(ps)mA(ps)mU

mA, mU, mC, mG – 2′-OMe RNA
fA, fU, fC, fG – 2′-F RNA
(ps) - phosphorothioate
(MOE-U), (MOE-C) - 2'-methoxyethyl RNA

Figure 17b

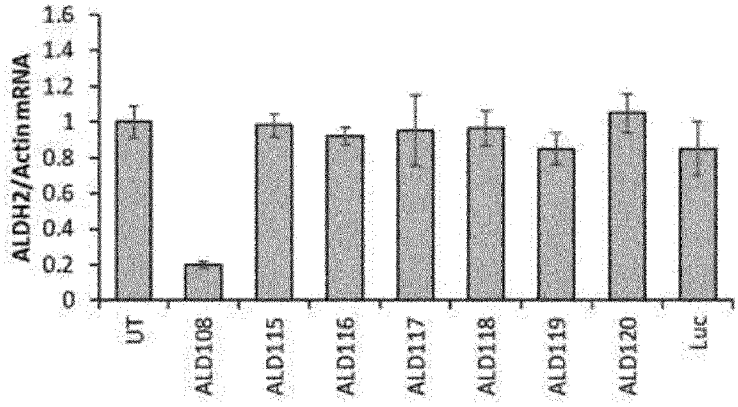


Figure 18a

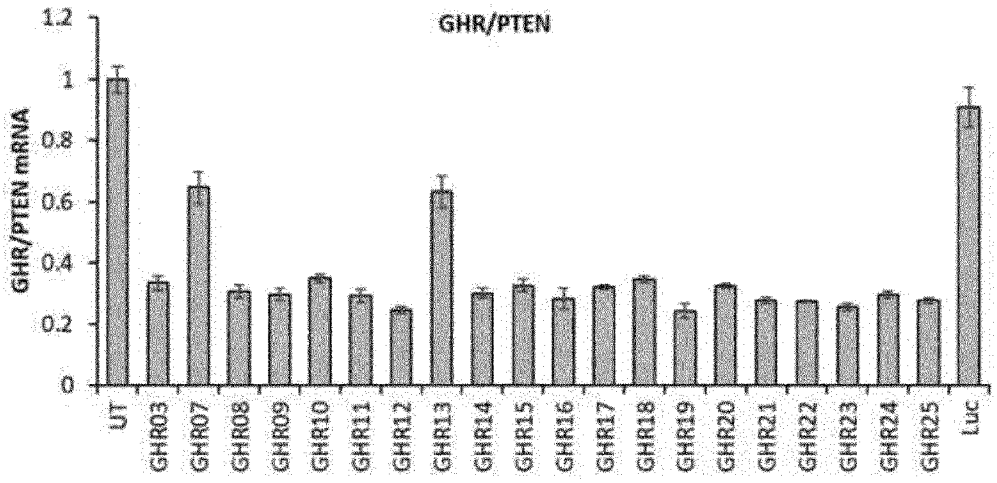
Duplex ID		sequence and chemistry (top: first strand, bottom: second strand, both 5'-3')
GHR03	GHR00A	mA(ps)fA(ps)mUfCmAfGmGfGmCfAmUfUmCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR07	GHR02A	mA(ps)mA(ps)mUfCmAfGmGfGmCfAmUfUmCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR08	GHR04A	mA(ps)fA(ps)mUmCmAfGmGfGmCfAmUfUmCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR09	GHR06A	mA(ps)fA(ps)mUfCmAmGmGfGmCfAmUfUmCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR10	GHR08A	mA(ps)fA(ps)mUfCmAfGmGmGmCfAmUfUmCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR11	GHR10A	mA(ps)fA(ps)mUfCmAfGmGfGmCmAmUfUmCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR12	GHR12A	mA(ps)fA(ps)mUfCmAfGmGfGmCfAmUmUmCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR13	GHR14A	mA(ps)fA(ps)mUfCmAfGmGfGmCfAmUfUmCrUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR14	GHR16A	mA(ps)fA(ps)mUfCmAfGmGfGmCfAmUfUmCfUmUmUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR15	GHR18A	mA(ps)fA(ps)mUfCmAfGmGfGmCfAmUfUmCfUmUfUmC(ps)mC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR16	GHR01A	fA(ps)fA(ps)mUfCmAfGmGfGmCfAmUfUmCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR17	GHR03A	mA(ps)fA(ps)fUfCmAfGmGfGmCfAmUfUmCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR18	GHR05A	mA(ps)fA(ps)mUfCfAfGmGfGmCfAmUfUmCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR19	GHR07A	mA(ps)fA(ps)mUfCmAfGmGfGmCfAmUfUmCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR20	GHR09A	mA(ps)fA(ps)mUfCmAfGmGfGfCfAmUfUmCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR21	GHR11A	mA(ps)fA(ps)mUfCmAfGmGfGmCfAfUfUmCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR22	GHR13A	mA(ps)fA(ps)mUfCmAfGmGfGmCfAmUfUfCfUmUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR23	GHR15A	mA(ps)fA(ps)mUfCmAfGmGfGmCfAmUfUmCfUfUfUmC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR24	GHR17A	mA(ps)fA(ps)mUfCmAfGmGfGmCfAmUfUmCfUmUfUfC(ps)fC(ps)mA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU
GHR25	GHR19A	mA(ps)fA(ps)mUfCmAfGmGfGmCfAmUfUmCfUmUfUmC(ps)fC(ps)fA
	GHR00B	fU(ps)mG(ps)fGmAfAmAfGmAfAmUfGmCfCmCfUmGfA(ps)mU(ps)fU

mA, mU, mC, mG – 2'-OMe RNA

fA, fU, fC, fG – 2'-F RNA

(ps) - phosphorothioate

Figure 18b


GHR/PTEN
GHR/PTEN mRNA
1.2
1
0.8
0.6
0.4
0.2
0
UT
GHR03
GHR07
GHR08
GHR09
GHR10
GHR11
GHR12
GHR13
GHR14
GHR15
GHR16
GHR17
GHR18
GHR19
GHR20
GHR21
GHR22
GHR23
GHR24
GHR25
Luc