# INHIBITORS OF EXPRESSION AND/OR FUNCTION

## FIELD

The present invention provides inhibitors, such as nucleic acid compounds, such as siRNA, suitable for therapeutic use. Additionally, the present invention provides methods of making these compounds, as well as methods of using such compounds for the treatment of various diseases and condition

## BACKGROUND OF THE INVENTION

Inhibitors, such as oligonucleoside/ oligonucleotide compounds which are inhibitors of gene expression and/or expression or function of other targets such as LNCRNAs, can have important therapeutic applications in medicine. Oligonucleotides/ oligonucleosides can be used to silence genes that are responsible for a particular disease. Gene-silencing prevents formation of a protein by inhibiting translation. Importantly, gene-silencing agents are a promising alternative to traditional small, organic compounds that inhibit the function of the protein linked to the disease. siRNA, antisense RNA, and micro-RNA are oligonucleoside /oligonucleotides that prevent the formation of proteins by gene-silencing.

A number of modified siRNA compounds in particular have been developed in the last two decades for diagnostic and therapeutic purposes, including siRNA / RNAi therapeutic agents for the treatment of various diseases including central-nervous-system diseases, inflammatory diseases, metabolic disorders, oncology, infectious diseases, and ocular diseases.

The present invention relates to inhibitors, such oligomers e.g. nucleic acids, e.g. oligonucleoside /oligonucleotide compounds, and their use in the treatment and / or prevention of disease.

CFHR4, a gene linked to the complement system, plays a role in the immune response. The complement system is a complex network of proteins in the blood that enhances the body's ability to fight off infections and remove damaged cells. It acts as a rapid and powerful defence mechanism by marking pathogens for destruction, promoting inflammation, and aiding in the removal of cellular debris. CFHR4 encodes the protein FHR-4 that regulates complement

activation, a key defence mechanism against infections. CFHR4 has been associated with various diseases, including kidney disorders and age-related macular degeneration. This gene is part of a family of complement factor H-related genes, contributing to the intricate balance between immune defence and self-tolerance.

## STATEMENTS OF INVENTION

The invention is defined as in the claims and relates to, *inter alia*:

In one aspect, the invention relates to an inhibitor of expression and / or function of CFHR4/FHR-4, wherein said inhibitor is conjugated to one or more ligand moieties, preferably wherein the ligand moiety allows targeting of hepatocytes.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein said inhibitor is an siRNA oligomer.

In another aspect, the invention relates to an inhibitor of expression and / or function of CFHR4/FHR-4, wherein said inhibitor is an siRNA oligomer.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein said inhibitor comprises an siRNA oligomer conjugated to one or more ligand moieties, preferably wherein the ligand moiety allows targeting of hepatocytes.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein said one or more ligand moieties comprise one or more GalNAc ligands or comprise one or more GalNAc ligand derivatives.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein said one or more ligand moieties comprise one or more GalNAc ligand derivatives.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the target of the inhibitor is CFHR4/FHR-4.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the inhibitor is a nucleic acid for inhibiting expression of CFHR4 comprising a duplex region that comprises a first strand and a second strand that is at least partially complementary to the first strand, wherein

(i) at least partially complementary to a portion of RNA transcribed from the CFHR4 gene, and

(ii) comprises at least 17 contiguous nucleosides differing by 0 or 1 nucleosides from any one of the first strand sequences as listed in Table 2.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the inhibitor is a nucleic acid for inhibiting expression of CFHR4 comprising a duplex region that comprises a first strand and a second strand that is at least partially complementary to the first strand, wherein said first strand is:

(i) at least partially complementary to a portion of RNA transcribed from the CFHR4 gene, and

(ii) comprises at least 17 contiguous nucleosides differing by 0 or 1 nucleosides from any one of the first strand modified sequences as listed in Table 3.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the first strand comprises nucleosides 2-18 of any one of the sequences defined in claim 8 or 9, in particular wherein the first strand comprises nucleosides 2-18 of any one of the sequences defined in Tables 2 or 3.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the second strand comprises a nucleoside sequence of at least 17 contiguous nucleosides differing by 0 or 1 nucleosides from any one of the second strand sequences as listed in Table 2, and wherein the second strand has a region of at least 85% complementarity over the 17 contiguous nucleosides to the first strand.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the second strand comprises a nucleoside sequence of at least 17 contiguous nucleosides differing by 0 or 1 nucleosides from any one of the second strand modified sequences as listed in Table 4, and wherein the second strand has a region of at least 85% complementarity over the 17 contiguous nucleosides to the first strand.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the first strand comprises any one of the first strand sequences as listed in Table 2.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the first strand comprises any one of the first strand modified sequences as listed in Table 3.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the second strand comprises any one of the second strand sequences as listed in Table 2.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the second strand comprises any one of the first strand modified sequences as listed in Table 4.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the first strand comprises any one of the following sequences: SEQ ID NO:149, SEQ ID NO:188, SEQ ID NO:198, and SEQ ID NO:221.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the first strand comprises any one of the following sequences: SEQ ID NO:415, SEQ ID NO:454, SEQ ID NO:464, and SEQ ID NO:487.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the second strand comprises any one of the following sequences: SEQ ID NO:282, SEQ ID NO:321, SEQ ID NO:331, and SEQ ID NO:354.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the second strand comprises any one of the following sequences: SEQ ID NO:548, SEQ ID NO:587, SEQ ID NO:597, and SEQ ID NO:620.

In a further aspect, the invention relates to an inhibitor according to the invention, comprising first and second strands that comprise, consist of, or consist essentially of a nucleoside sequence differing by 0 or 1 nucleosides from any one of the following combinations of first and second sequences:

| Unmodified first strand | Unmodified second strand |
|---|---|
| SEQ ID NO:149 | SEQ ID NO:282 |
| SEQ ID NO:188 | SEQ ID NO:321 |
| SEQ ID NO:198 | SEQ ID NO:331 |
| SEQ ID NO:221 | SEQ ID NO:354 |

In a further aspect, the invention relates to an inhibitor according to the invention, comprising first and second strands that comprise, consist of, or consist essentially of a nucleoside sequence differing by 0 or 1 nucleosides from any one of the following combinations of first and second sequences:

| Unmodified first strand | Unmodified second strand |
|---|---|
| SEQ ID NO:149 | SEQ ID NO:282 |

In a further aspect, the invention relates to an inhibitor according to the invention, comprising first and second strands that comprise, consist of, or consist essentially of a nucleoside sequence differing by 0 or 1 nucleosides from any one of the following combinations of first and second sequences:

| Unmodified first strand | Unmodified second strand |
|---|---|
| SEQ ID NO:415 | SEQ ID NO:548 |
| SEQ ID NO:454 | SEQ ID NO:587 |
| SEQ ID NO:464 | SEQ ID NO:597 |
| SEQ ID NO:487 | SEQ ID NO:620 |

In a further aspect, the invention relates to an inhibitor according to the invention, comprising first and second strands that comprise, consist of, or consist essentially of a nucleoside sequence differing by 0 or 1 nucleosides from any one of the following combinations of first and second sequences:

| Unmodified first strand | Unmodified second strand |
|---|---|
| SEQ ID NO:415 | SEQ ID NO:548 |

In a further aspect, the invention relates to an inhibitor according to the invention, which is an siRNA oligomer having a first and a second strand wherein:

i) the first strand of the siRNA has a length in the range of 15 to 30 nucleosides, preferably 19 to 25 nucleosides, more preferably 23 or 25; even more preferably 23; and / or

ii) the second strand of the siRNA has a length in the range of 15 to 30 nucleosides, preferably 19 to 25 nucleosides, more preferably 21 nucleosides.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the first strand has a length in the range of 17 to 30 nucleosides, preferably 19 to 25 nucleosides, more preferably 19 or 23 nucleosides.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the second strand has a length in the range of 17 to 30 nucleosides, preferably 19 to 25 nucleosides, more preferably 19 or 21 or 23 nucleosides.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the duplex region of the nucleic acid is between 17 and 30 nucleosides in length, more preferably is 19 or 21 or 23 nucleosides in length.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the region of complementarity between the first strand and the portion of RNA transcribed from the CFHR4 gene is between 17 and 30 nucleosides in length.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the nucleic acid further comprises one or more single-stranded nucleoside overhangs, optionally wherein the overhang is present on the first or second strand, preferably at the 3' terminus of the first or second strand, and/or wherein the overhang comprises 1 to 4 nucleosides, more preferably 2 nucleosides.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the nucleic acid is an siRNA oligonucleoside.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the second sense strand further comprises one or more abasic nucleosides in a terminal region of the second strand, and wherein said abasic nucleoside(s) is / are connected to an adjacent nucleoside through a reversed internucleoside linkage.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the second strand comprises:

i) 2, or more than 2, abasic nucleosides in a terminal region of the second strand; and / or

ii) 2, or more than 2, abasic nucleosides in either the 5' or 3' terminal region of the second strand; and / or

iii) 2, or more than 2, abasic nucleosides in either the 5' or 3' terminal region of the second strand, wherein the abasic nucleosides are present in an overhang as herein described; and / or

iv) 2, or more than 2, consecutive abasic nucleosides in a terminal region of the second strand, wherein preferably one such abasic nucleoside is a terminal nucleoside; and / or

v) 2, or more than 2, consecutive abasic nucleosides in either the 5' or 3' terminal region of the second strand, wherein preferably one such abasic nucleoside is a terminal nucleoside in either the 5' or 3' terminal region of the second strand; and / or

vi) a reversed internucleoside linkage connects at least one abasic nucleoside to an adjacent basic nucleoside in a terminal region of the second strand; and / or

vii) a reversed internucleoside linkage connects at least one abasic nucleoside to an adjacent basic nucleoside in either the 5' or 3' terminal region of the second strand; and / or

viii) an abasic nucleoside as the penultimate nucleoside which is connected via the reversed linkage to the nucleoside which is not the terminal nucleoside (called the antepenultimate nucleoside herein); and / or

ix) abasic nucleosides as the 2 terminal nucleosides connected via a 5'-3' linkage when reading the strand in the direction towards that terminus;

x) abasic nucleosides as the 2 terminal nucleosides connected via a 3'-5' linkage when reading the strand in the direction towards the terminus comprising the terminal nucleosides;

xi) abasic nucleosides as the terminal 2 positions, wherein the penultimate nucleoside is connected via the reversed linkage to the antepenultimate nucleoside, and wherein the reversed linkage is a 5-5' reversed linkage or a 3'-3' reversed linkage;

xii) abasic nucleosides as the terminal 2 positions, wherein the penultimate nucleoside is connected via the reversed linkage to the antepenultimate nucleoside, and wherein either

(1) the reversed linkage is a 5-5' reversed linkage and the linkage between the terminal and penultimate abasic nucleosides is 3'5' when reading towards the terminus comprising the terminal and penultimate abasic nucleosides; or

(2) the reversed linkage is a 3-3' reversed linkage and the linkage between the terminal and penultimate abasic nucleosides is 5'3' when reading towards the terminus comprising the terminal and penultimate abasic nucleosides.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the reversed internucleoside linkage is at a terminal region which is distal to the 5' terminal region

of the second strand, or at a terminal region which is distal to the 3' terminal region of the second strand.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the reversed internucleoside linkage is a 3'3 reversed linkage.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the reversed internucleoside linkage is a 5'5 reversed linkage.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the second sense strand further comprises one or more abasic nucleosides in a terminal region of the second strand, and wherein said abasic nucleoside(s) is / are connected to an adjacent nucleoside through a reversed internucleoside linkage.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the second strand comprises 2 consecutive abasic nucleosides in the 5' terminal region of the second strand, wherein one such abasic nucleoside is a terminal nucleoside at the 5' terminal region of the second strand and the other abasic nucleoside is a penultimate nucleoside at the 5' terminal region of the second strand, wherein:

(a) said penultimate abasic nucleoside is connected to an adjacent first basic nucleoside in an adjacent 5' near terminal region through a reversed internucleoside linkage; and

(b) the reversed linkage is a 5-5' reversed linkage; and

(c) the linkage between the terminal and penultimate abasic nucleosides is 3'5' when reading towards the terminus comprising the terminal and penultimate abasic nucleosides.12. An inhibitor, or inhibitor for use, according claim 10 or 11, wherein the reversed internucleoside linkage is at a terminal region which is distal to the 5' terminal region of the second strand, or at a terminal region which is distal to the 3' terminal region of the second strand.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein

(i) the first strand and the second strand each has a length of 23 nucleosides;

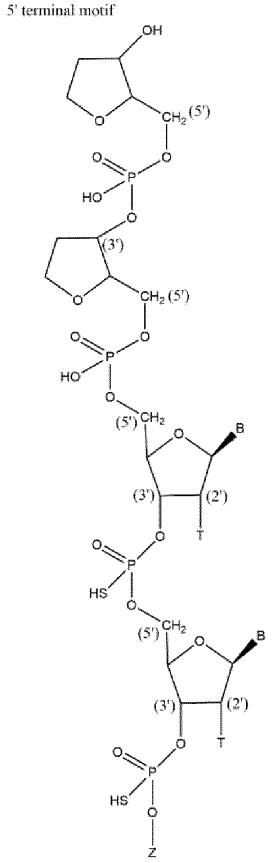
(ii) two phosphorothioate internucleoside linkages are respectively between three consecutive positions in said 5' near terminal region of the second strand, wherein a first phosphorothioate internucleoside linkage is present between said adjacent first basic

nucleoside of (a) and an adjacent second basic nucleoside in said 5' near terminal region of the second strand, and a second phosphorothioate internucleoside linkage is present between said adjacent second basic nucleoside and an adjacent third basic nucleoside in said 5' near terminal region of the second strand;

(iii) two phosphorothioate internucleoside linkages are respectively between three consecutive positions in both 5' and 3' terminal regions of the first strand, whereby a terminal nucleoside respectively at each of the 5' and 3' terminal regions of said first strand is each attached to a respective 5' and 3' adjacent penultimate nucleoside by a phosphorothioate internucleoside linkage, and each first 5' and 3' penultimate nucleoside is attached to a respective 5' and 3' adjacent antepenultimate nucleoside by a phosphorothioate internucleoside linkage; and

(iv) the second strand of the nucleic acid is conjugated directly or indirectly to one or more ligand moieties at the 3' terminal region of the second strand.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein, wherein the 2 consecutive inverted abasic nucleosides in the 5' terminal region of the second strand present as the following 5' terminal motif




wherein:

T represents a 2'Me ribose modification,

B represents the nucleoside bases of the first two basic nucleosides in the 5' terminal region of the second strand, and

Z represents the remaining 19 contiguous basic nucleosides of said second strand.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein one or more nucleosides on the first strand and / or the second strand is / are modified, to form modified nucleosides.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the modification is a modification at the 2'-OH group of the ribose sugar, optionally selected from 2'-Me or 2'-F modifications.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the first strand comprises a 2'-F at any of position 14, position 2, position 6, or any combination thereof, counting from position 1 of said first strand.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the second strand comprises a 2'-F modification at position 7 and / or 9, and / or 11 and / or 13, counting from position 1 of said second strand.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the first and second strand each comprise 2'-Me and 2'-F modifications.

In a further aspect, the invention relates to an inhibitor according to the invention, which is an siRNA, wherein the siRNA comprises at least one thermally destabilizing modification, suitably at one or more of positions 1 to 9 of the first strand counting from position 1 of the first strand, and / or at one or more of positions on the second strand aligned with positions 1 to 9 of the first strand, wherein the destabilizing modification is selected from a modified unlocked nucleic acid (UNA) and a glycol nucleic acid (GNA), preferably a glycol nucleic acid.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the siRNA comprises at least one thermally destabilizing modification at position 7 of the first strand, counting from position 1 of the first strand.

In a further aspect, the invention relates to an inhibitor according to the invention, which is an siRNA, wherein the siRNA comprises 3 or more 2'-F modifications at positions 7 to 13 of the second strand, such as 4, 5, 6 or 7 2'-F modifications at positions 7 to 13 of the second strand, counting from position 1 of said second strand

In a further aspect, the invention relates to an inhibitor according to the invention, which is an siRNA, wherein said second strand comprises at least 3, such as 4, 5 or 6, 2'-Me modifications at positions 1 to 6 of the second strand, counting from position 1 of said second strand.

In a further aspect, the invention relates to an inhibitor according to the invention, which is an siRNA, wherein said first strand comprises at least 5 2'-Me consecutive modifications at the 3' terminal region, preferably including the terminal nucleoside at the 3' terminal region, or at least within 1 or 2 nucleosides from the terminal nucleoside at the 3' terminal region.

In a further aspect, the invention relates to an inhibitor according to the invention, which is an siRNA wherein said first strand comprises 7 2'-Me consecutive modifications at the 3' terminal region, preferably including the terminal nucleoside at the 3' terminal region.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the siRNA oligomer further comprises one or more phosphorothioate internucleoside linkages.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein said one or more phosphorothioate internucleoside linkages are respectively between at least three consecutive positions in a 5' or 3' near terminal region of the second strand, whereby said near terminal region is preferably adjacent said terminal region wherein said one or more abasic nucleosides of said second strand is / are located as defined herein.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein said one or more phosphorothioate internucleoside linkages are respectively between at least three consecutive positions in a 5' and / or 3' terminal region of the first strand, whereby preferably a terminal position at the 5' and / or 3' terminal region of said first strand is attached to its adjacent position by a phosphorothioate internucleoside linkage.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the oligomer is an siRNA and the second strand of the siRNA is conjugated directly or indirectly to one or more ligand moiety(s), wherein said ligand moiety is typically present at a terminal region of the second strand, preferably at the 3' terminal region thereof.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the ligand moiety comprises

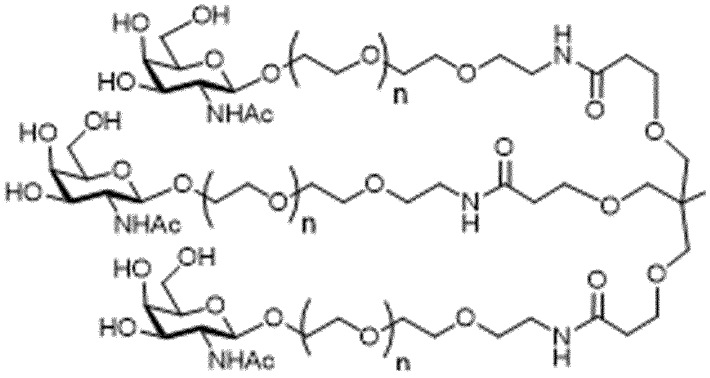
i) one or more GalNAc ligands; and / or

ii) one or more GalNAc ligand derivatives; and / or

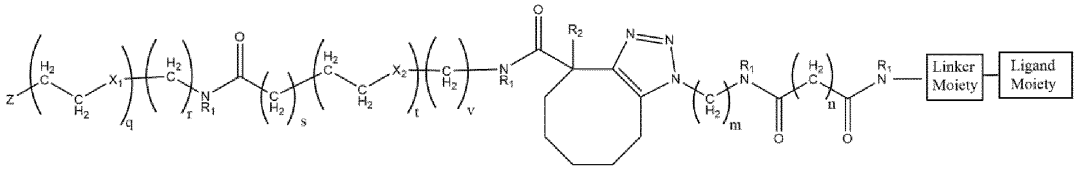
iii) one or more GalNAc ligands and / or GalNAc ligand derivatives conjugated to said siRNA through a linker.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein said one or more GalNAc ligands and / or GalNAc ligand derivatives are conjugated directly or indirectly to the 5' or 3' terminal region of the second strand of the siRNA oligomer, preferably at the 3' terminal region thereof.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the ligand moiety comprises



In a further aspect, the invention relates to an inhibitor according to the invention, comprising the structure:




wherein:

$R_1$ at each occurrence is independently selected from the group consisting of hydrogen, methyl and ethyl;

$R_2$ is selected from the group consisting of hydrogen, hydroxy, -$OC_{1-3}$alkyl, -$C(=O)OC_{1-3}$alkyl, halo and nitro;

$X_1$ and $X_2$ at each occurrence are independently selected from the group consisting of methylene, oxygen and sulfur;

m is an integer of from 1 to 6;

n is an integer of from 1 to 10;

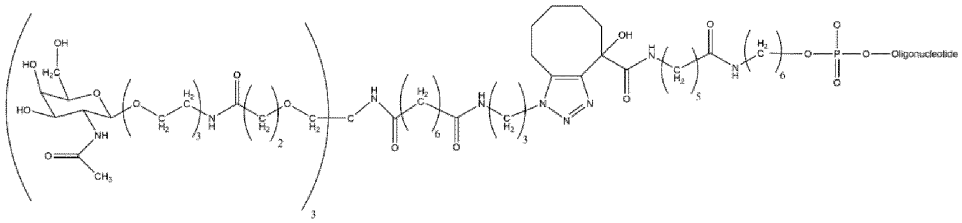
q, r, s, t, v are independently integers from 0 to 4, with the proviso that:

(i) q and r cannot both be 0 at the same time; and

(ii) s, t and v cannot all be 0 at the same time;

Z is an oligonucleoside moiety.

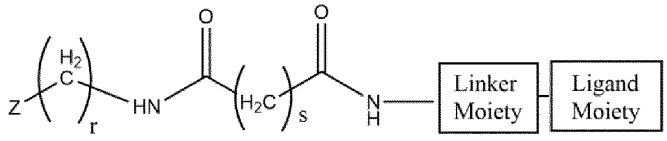
In a further aspect, the invention relates to an inhibitor according to the invention, comprising the structure




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wherein oligonucleotide represents the contiguous nucleosides of the second strand.

In a further aspect, the invention relates to an inhibitor according to the invention, comprising the structure

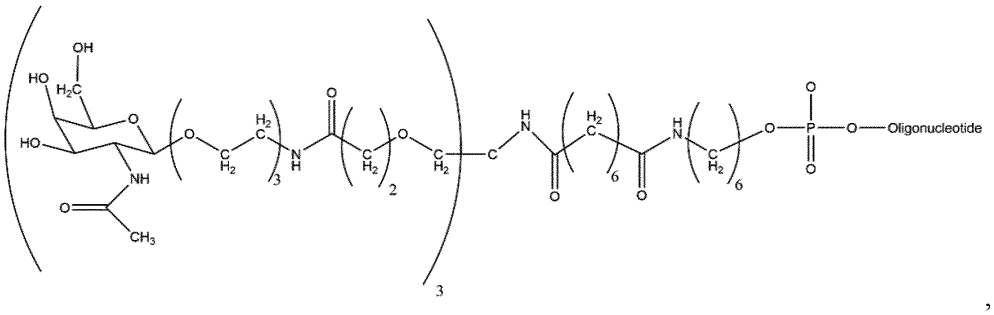



wherein:

r and s are independently an integer selected from 1 to 16; and

Z is an oligonucleoside moiety.

In a further aspect, the invention relates to an inhibitor according to the invention, comprising the structure




,

wherein oligonucleotide represents the contiguous nucleosides of the second strand.

In a further aspect, the invention relates to an inhibitor according to the invention, wherein the structure is conjugated to the 3' terminal region of the second strand.

In a further aspect, the invention relates to an inhibitor according to the invention, formulated as a pharmaceutical composition with an excipient and / or carrier.

In another aspect, the invention relates to a pharmaceutical composition comprising an inhibitor according to one or more preceding aspects, in combination with a pharmaceutically acceptable excipient or carrier.

In another aspect, the invention relates to an inhibitor according to the invention or a pharmaceutical composition according to the invention for use in therapy.

In another aspect, the invention relates to an inhibitor according to the invention or a pharmaceutical composition according to the invention for use in treating a disease or disorder associated with alterations in complement pathway protein levels and/or function in a patient, in particular wherein the disease or disorder is associated with elevated levels of complement factor H-related protein 4 (FHR-4).

In a further aspect, the invention relates to an inhibitor or a pharmaceutical composition for use according to the invention, wherein the disease or disorder associated with alterations in complement pathway protein levels and/or function is age-related macular degeneration (AMD), preferably dry age-related macular degeneration.

In a further aspect, the invention relates to an inhibitor or a pharmaceutical composition for use according to the invention, wherein the disease or disorder associated with alterations in complement pathway protein levels and/or function is a kidney disease, such as atypical haemolytic uraemic syndrome (aHUS), C3 glomerulonephropathy (C3G), IgA nephropathy (IgAN), or membranous (glomerulo) nephropathy (MN).

In a further aspect, the invention relates to an inhibitor or a pharmaceutical composition for use according to the invention, wherein the inhibitor of expression and/or function of CFHR4/FHR-4 slows the progression of the disease or disorder associated with alterations in complement pathway protein levels and/or function.

In another aspect, the invention relates to an inhibitor according to the invention or a pharmaceutical composition according to the invention for use in the treatment of age-related macular degeneration (AMD), preferably dry age-related macular degeneration.

In a further aspect, the invention relates to an inhibitor or a pharmaceutical composition for use according to the invention, wherein the patient has elevated levels of FHR-4.

In a further aspect, the invention relates to an inhibitor or a pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles rs61818956TT and/or rs10494745GG.

In a further aspect, the invention relates to an inhibitor or a pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT and rs10494745GG; (ii) rs61818956TT and rs10494745GA; (iii) rs61818956TT and rs10494745AA; (iv) rs61818956CT and rs10494745GG; (v) rs61818956CT and rs10494745GA; (vi) rs61818956CT and rs10494745AA; (vii) rs61818956CC and rs10494745GG; or (viii) rs61818956CC or rs10494745GA.

In a further aspect, the invention relates to an inhibitor or a pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT and rs10494745GG; (ii) rs61818956TT and rs10494745GA; (iii) rs61818956TT and rs10494745AA; (iv) rs61818956CT and rs10494745GG; or (v) rs61818956CT and rs10494745GA.

In a further aspect, the invention relates to an inhibitor or a pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT and rs10494745GG or (ii) rs61818956TT and rs10494745GA; or (iii) rs61818956CT and rs10494745GG.

In a further aspect, the invention relates to an inhibitor or a pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT and rs10494745GG or (ii) rs61818956TT and rs10494745GA.

In a further aspect, the invention relates to an inhibitor or a pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; (ii) rs61818956CT, rs10494745GG, and rs7531555CC; (iii) rs61818956TT, rs10494745GA, and rs7531555CT; (iv) rs61818956TT, rs10494745GA, and rs7531555CC; (v) rs61818956CC, rs10494745GG, and rs7531555CC; (vi) rs61818956TC, rs10494745GA, and rs7531555CC; (vii) rs61818956TC, rs10494745GG, and rs7531555CT; (viii) rs61818956CT, rs10494745GA, and rs7531555CT; (ix) rs61818956CT, rs10494745GA, and rs7531555CC; (x) rs61818956TT, rs10494745GG, and rs7531555CT; (xi) rs61818956TT, rs10494745AA, and rs7531555TT; (xii) rs61818956TT, rs10494745AA, and rs7531555TC; or (xiii) rs61818956TT, rs10494745AA, and rs7531555CC.

In a further aspect, the invention relates to an inhibitor or a pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; (ii) rs61818956CT, rs10494745GG, and rs7531555CC; (iii) rs61818956TT, rs10494745GA, and rs7531555CT; or (iv) rs61818956TT, rs10494745GA, and rs7531555CC.

In a further aspect, the invention relates to an inhibitor or a pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; (ii) rs61818956CT, rs10494745GG, and rs7531555CC; (iii) rs61818956CC, rs10494745GG, and rs7531555CC; (iv) rs61818956TC, rs10494745GA, and rs7531555CC; (v) rs61818956TC, rs10494745GG, and rs7531555CT.

In a further aspect, the invention relates to an inhibitor or a pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; or (ii) rs61818956CT, rs10494745GG, and rs7531555CC.

In another aspect, the invention relates to the use of CFHR4/FHR-4 as a target for identifying one or more therapeutic agents for the treatment of age-related macular degeneration (AMD).

In another aspect, the invention relates to a method of treating or preventing age-related macular degeneration (AMD), which comprises administering to a patient an inhibitor of CFHR4/FHR-4, such as an inhibitor as defined according to one or more preceding aspects.

In another aspect, the invention relates to the use of an inhibitor according to the invention or a pharmaceutical composition according to the invention, in the preparation of a medicament for the treatment of age-related macular degeneration (AMD).

In another aspect, the invention relates to CFHR4/FHR-4 for use as a biomarker of age-related macular degeneration (AMD).

In another aspect, the invention relates to CFHR4/FHR-4 for use in an *in vivo* method of predicting susceptibility to age-related macular degeneration (AMD), typically by monitoring the sequence and/ or level of expression and / or function of CFHR4/FHR-4 in a sample obtained from a patient.

In another aspect, the invention relates to a method of predicting susceptibility to age-related macular degeneration (AMD), and optionally treating age-related macular degeneration (AMD), in a patient, said method comprising:

(a) obtaining a sample from the patient,

(b) detecting the sequence and / or expression and / or function of CFHR4/FHR-4 in said sample obtained from the patient,

(c) predicting susceptibility to age-related macular degeneration (AMD), based on the sequence and / or expression and / or function of CFHR4/FHR-4 in said sample obtained from the patient,

(d) preferably administering to the diagnosed patient an effective amount of an inhibitor of CFHR4/FHR-4.

In another aspect, the invention relates to a method of selecting a patient having a disease or disorder associated with alterations in complement pathway protein levels, in particular age-related macular degeneration (AMD), for a treatment with the inhibitor according to the invention, and optionally treating the disease or disorder associated with alterations in complement pathway protein levels, in particular age-related macular degeneration (AMD), in said patient, said method comprising:

(a) providing a sample from the patient,

(b) detecting the sequence and / or expression and / or function of CFHR4/FHR-4 in said sample obtained from the patient,

(c) selecting the patient having the disease or disorder associated with alterations in complement pathway protein levels, in particular age-related macular degeneration (AMD), for a treatment with the inhibitor according to the invention based on the sequence and / or expression and / or function of CFHR4/FHR-4 in said sample obtained from the patient,

(d) preferably administering to the diagnosed patient an effective amount of an inhibitor of CFHR4/FHR-4.

In a further aspect, the invention relates to the method according to the invention, wherein the patient is selected for a treatment with the inhibitor according to the invention, if the protein

levels of FHR-4 and/or the activity of FHR-4 in the sample obtained from said patient are elevated compared to a suitable control, preferably wherein the suitable control is a comparable sample from a healthy donor.

In a further aspect, the invention relates to the method according to the invention, wherein the method comprises a step of determining the presence of a single nucleotide polymorphism (SNP) at positions rs61818956, rs10494745, and/or rs7531555 in the sample obtained from said patient.

In a further aspect , the invention relates to the method according to the invention, wherein the patient is determined to have elevated levels of FHR-4, if the patient is a carrier of diplotype alleles rs61818956TT and/or rs10494745GG and/or rs7531555CC;

preferably of diplotype alleles (i) rs61818956TT and rs10494745GG; (ii) rs61818956TT and rs10494745GA; (iii) rs61818956TT and rs10494745AA; (iv) rs61818956CT and rs10494745GG; (v) rs61818956CT and rs10494745GA; (vi) rs61818956CT and rs10494745AA; (vii) rs61818956CC and rs10494745GG; or (viii) rs61818956CC or rs10494745GA;

more preferably of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; (ii) rs61818956CT, rs10494745GG, and rs7531555CC; (iii) rs61818956TT, rs10494745GA, and rs7531555CT; (iv) rs61818956TT, rs10494745GA, and rs7531555CC; (v) rs61818956CC, rs10494745GG, and rs7531555CC; (vi) rs61818956TC, rs10494745GA, and rs7531555CC; (vii) rs61818956TC, rs10494745GG, and rs7531555CT; (viii) rs61818956CT, rs10494745GA, and rs7531555CT; (ix) rs61818956CT, rs10494745GA, and rs7531555CC; (x) rs61818956TT, rs10494745GG, and rs7531555CT; (xi) rs61818956TT, rs10494745AA, and rs7531555TT; (xii) rs61818956TT, rs10494745AA, and rs7531555TC; or (xiii) rs61818956TT, rs10494745AA, and rs7531555CC;

even more preferably of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; (ii) rs61818956CT, rs10494745GG, and rs7531555CC; (iii) rs61818956TT, rs10494745GA, and rs7531555CT; or (iv) rs61818956TT, rs10494745GA, and rs7531555CC or

of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; (ii) rs61818956CT, rs10494745GG, and rs7531555CC; (iii) rs61818956CC, rs10494745GG, and rs7531555CC; (iv) rs61818956TC, rs10494745GA, and rs7531555CC; (v) rs61818956TC, rs10494745GG, and rs7531555CT;

most preferably of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; or (ii) rs61818956CT, rs10494745GG, and rs7531555CC.

## FIGURES

**Figure 1a:** An exemplary linear configuration for a conjugate.

**Figure 1b**: An exemplary branched configuration for a conjugate.

**Figure 2:** Linker and ligand portions of constructs suitable for use according to the present invention including tether 1a. While Figure 2 depicts the linker to be conjugated to an oligonucleotide, it is to be understood that the present invention also encompasses conjugates of the same linker with an oligonucleoside as disclosed herein.

It should also be understood that while Figure 2 depicts as a product molecules based on the linker and ligand portions as specifically depicted in Figure 2 attached to an oligonucleoside moiety as also depicted herein, this product may alternatively further comprise, or consist essentially of, molecules wherein the linker and ligand portions are essentially as depicted in Figure 2 attached to an oligonucleoside moiety but having the F substituent as shown in Figure 2 on the cyclo-octyl ring replaced by a substituent, which could occur as a result of hydrolytic displacement, such as an OH substituent, or the OH substituent could be synthesized as a linker in its own right. In this way, (a) tether 1a constructs can consist essentially of molecules having linker and ligand portions specifically as depicted in Figure 2, with a F substituent on the cyclo-octyl ring; or (b) tether 1a constructs can consist essentially of molecules having linker and ligand portions essentially as depicted in Figure 2 but having the F substituent as shown in Figure 2 on the cyclo-octyl ring replaced by an OH substituent, or (c) tether 1a constructs can comprise a mixture of molecules as defined in (a) and/or (b).

**Figure 3:** Linker and ligand portions of constructs suitable for use according to the present invention including tether 1b. While Figure 3 depicts the linker to be conjugated to an oligonucleotide, it is to be understood that the present invention also encompasses conjugates of the same linker with an oligonucleoside as disclosed herein.

The comments made in relation to Figure 2 and the possible replacement of the F substituent as shown in Figure 2 on the cyclo-octyl ring replaced by a substituent, which could occur as a result of hydrolytic displacement, such as an OH substituent, or the OH substituent could be synthesized as a linker in its own right, apply equally to tether 1b constructs. In this way, (a) tether 1b constructs can consist essentially of molecules having linker and ligand portions specifically as depicted in Figure 3, with a F substituent on the cyclo-octyl ring; or (b) tether 1b

constructs can consist essentially of molecules having linker and ligand portions essentially as depicted in Figure 3 but having the F substituent as shown in Figure 3 on the cyclo-octyl ring replaced by an OH substituent, or (c) tether 1b constructs can comprise a mixture of molecules as defined in (a) and/or (b).

**Figure 4:** Linker and ligand portions of constructs suitable for use according to the present invention including tether 2a. While Figure 4 depicts the linker to be conjugated to an oligonucleotide, it is to be understood that the present invention also encompasses conjugates of the same linker with an oligonucleoside as disclosed herein.

**Figure 5:** Linker and ligand portions of constructs suitable for use according to the present invention including tether 2b. While Figure 5 depicts the linker to be conjugated to an oligonucleotide, it is to be understood that the present invention also encompasses conjugates of the same linker with an oligonucleoside as disclosed herein.

**Figure 6:** Formulae described in Sentences 1-101 disclosed herein.

**Figure 7:** Formulae described in Clauses 1-56 disclosed herein.

**Figures 8a and 8b:** Inverted abasic constructs that can be used with nucleic acid sequences according to the present invention as described herein. For Figure 8a, a GalNAc linker is attached to the 5' end region of the sense strand in use (not depicted in Figure 8a). For Figure 8b, a GalNAc linker is attached to the 3' end region of the sense strand in use (not depicted in Figure 8b).

iaia as shown at the 3' end region of the sense strand in Figure 8a represents (i) two abasic nucleosides provided as the penultimate and terminal nucleosides at the 3' end region of the sense strand, (ii) wherein a 3'-3' reversed linkage is provided between the antepenultimate nucleoside (namely at position 21 of the sense strand, wherein position 1 is the terminal 5' nucleoside of the sense strand) and the adjacent penultimate abasic residue of the sense strand, and (iii) the linkage between the terminal and penultimate abasic nucleosides is 5'-3' when reading towards the 3' end region comprising the terminal and penultimate abasic nucleosides.

iaia as shown at the 5' end region of the sense strand in Figure 8b represents (i) two abasic nucleosides provided as the penultimate and terminal nucleosides at the 5' end region of the sense strand, (ii) wherein a 5'-5' reversed linkage is provided between the antepenultimate nucleoside (namely at position 1 of the sense strand, not including the iaia motif at the 5' end region of the sense strand in the nucleoside position numbering on the sense strand) and the

adjacent penultimate abasic residue of the sense strand, and (iii) the linkage between the terminal and penultimate abasic nucleosides is 3'-5' when reading towards the 5' end region comprising the terminal and penultimate abasic nucleosides.

**Figures 9a and 9b:** Duplex constructs according to Table 5.

**Figure 10:** Overview of multiple-dose, 56-day study in non-human primates (NHPs).

**Figure 11:** Results of hepatic CFHR4 mRNA knockdown (top) and hepatic FHR-4 protein knockdown (bottom) in NHPs with siRNA candidates ETX-M00001347, ETX-M00001697, ETX-M00001674 and ETX-M00001664.

**Figure 12:** Results of plasma FHR-4 protein knockdown in NHPs with siRNA candidates ETX-M00001347, ETX-M00001697, ETX-M00001674 and ETX-M00001664.

**Figure 13:** Results of choroid FHR-4 protein knockdown in NHPs with siRNA candidates ETX-M00001347, ETX-M00001697, ETX-M00001674 and ETX-M00001664..

**Figure 14:** Tolerability assessmentin NHPs following treatment with siRNA candidates ETX-M00001347, ETX-M00001697, ETX-M00001674 and ETX-M00001664. ALT: Alanine Transaminase; AST: Aspartate Transaminase; GGT: Gamma-glutamyltransferase.

**Figure 15:** Summary of hepatic mRNA, hepatic protein, plasma protein and eye protein levels after treatment with siRNA candidate ETX-M00001347 (ETX-407).

**Figure 16:** Overview of single-dose human mRNA expression study in mice.

**Figure 17:** Results of human hepatic CFHR4 mRNA knockdown in mice with siRNA candidates ETX-M00001347, ETX-M00001697, ETX-M00001674 and ETX-M00001664.

**Figure 18:** Summary of human hepatic CFHR4 mRNA knockdown in mice with siRNA candidate ETX-M00001347.

## DETAILED DESCRIPTION

The present invention, inter alia, provides inhibitors, for example oligomers such as nucleic acids, such as inhibitory RNA molecules (which may be referred to as iRNA or siRNA ), and compositions containing the same which can affect expression of a target, for example by

binding to mRNA transcribed from a gene. The target may be within a cell, e.g. a cell within a subject, such as a human. The inhibitors can be used to prevent and/or treat medical conditions associated with the e.g. the expression of a target gene.

In particular the present invention identifies inhibitors of CFHR4/FHR-4 as useful in the prevention and/or treatment of age-related macular degeneration (AMD).

Complement Factor H Related 4 (CFHR4) is a protein circulating in the blood and was found to be upregulated in patients suffering from age-related macular degeneration. It is a member of the CFH-related (CFHR) family of genes. In humans, CFHR4 is encoded by the CFHR4 gene (SEQ ID NO:666).

SEQ ID NO:666 (CFHR4)

AAGTGACCTTAAAGCCCTAGCTTTGTGGTAGTGCACTTAAATTCAGAATCACACTTGGTAACTAATAA
TGAAAGATTTCAAACCCCAAACAGTGCAACTGAAACTTTTGCATTACTATACTACTGAGAATATCTAA
CATGTTGTTACTAATCAATGTCATTCTGACCTTGTGGGTTTCCTGTGCTAATGGACAAGGTAAGTTGAA
AGAGATCTAAACACTCAGCTTCCCTCTTAAATGTAACTTCGTGTAATATCTAACTTCATATGTCTATAA
TTTTTTTATAAATCTGATAGGATATATTCACTATGCTAGCAGAAGTAGCATATTTTGTGAGAGTATAAC
AGAAATTAATTTTATGAAAAAATATCTCATAATTTAAAGAAAAAATGAATAATATTTTCTTTGTTTTAC
AAATTCTACAGTGTAAAAAGCATCTAATATTCATTATGGAGATATATGAATATACTCATAAAACTTTA
AGTAGGAAACATGTCATTAGATAGATTCTAACCTTAAAATGTTCAGAATTTTCTTATTCATAATACAAG
GGAAACTTTGGGAGTGATTGATACTTTCATAATCTTACATACAGTGATTTTTTATTGATTCATATATCA
AAACAGCAAATAAAATATTTTAAATTATGCCATTTCTTATATTTCTATTATACCTTAAGAAAGCTGCTA
CATCTAATTAACATTAATATGAATTTAATATTTCAACAAGATTAGCAATATGTAAATCACAACATATAT
GTAATCAAAAAGCAGAATATATACTTAACTTACATTGGAATGACAGTAAATTTTAATTTGCATTTCTGA
GTTCACTAGCAAATGTTCAATAAATAAATACATAAATAATTTTCATAGCTTTATGTCATTGTTCTCTGA
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CACAATTTATTAAAAGAATATTAACTTAAAATAATTTTTTACTCTTTACTCATGGATACGTATATACCC
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CTGCCTGCCATGTGGCTAAGGAGCGTGGTATGAACATATGACACTGTGCTAGGAGCTAAAATTGACTG
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GTACAATCAGACAAATTCTGGCCATGCAACTGTAAAGGTGAGGAGTCAGATTCCAGGTGTTTCCTCCT
CCGCCATCTTCCCAGATTTCTTTTTCTATATTTCTTAGATTGCTTTCAGTAAATAGAATAACAAGCTAAT
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GTTACATATGTATAAATACGCTGTGTTGGTTTGCTGCACTCATCATTTACATTAGGTATCAAACATAAA
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GGTATACTTCTAGAATTTTGTCCCCCAAGTACTATGAACTTGAAGCAACACAATTCAAAAAAATATAA
GCCACAATAATTATGGCAACAACAAGAAAACCTAAAAGAAAACTTGCCACAAGTTTCTACTAGCCTAT
GAGGTAGAGCCCTGGGATACCCATCAGTCCTTGCATGTCAGATAACCTATTCCTATCACAGCTCTTAA
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GATATGTACTATGTTAAGAAATTTATTTAAGTAAGTACTTGAAAGATGTATTAAAATATGATTGGGGA
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TGTAAGAATTAGGCTTAATACCTAGGGTATGGGTTGATAGGTACAGCAAACCACCATGGCACACATTT
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AGGCATAGTGAGATAGAGTAATTTGTCCATAATCATGTAGGGATTCACATTAACTCCTGTGTGCTTATC
CTTGCCTCTTTAAAATAGAAAATGAAGATGACAGGGATAGATGATGGTGATAGAGAGAGAGATAATT
GACAGATATTGAGGTGTATGTATGTATGTATGTATGTATATATAGAGAGTTTTATTTTAATAGAT
GACTATTAGAAAGATTATACCTTTCTACAGTTCTTTTTCTACACTGTGGGCATAAGAGAAAAATATAAA
CATAGAATTAAATTAGCAAAATGTGAGTACATTTGGAGTGCTTGTAGTCACGGCTTGTCAAATGGGGG
AAAGGAGGATAAGTAACCATGACTCCAGTGGAACATGTTAGCATAATCCTTTTTGATATGAAGTCACT
AGGGTGGACACAACATGTTTTAGGGAAGAAGAGTTCAGGCTCTAGAGAAAGACTGCCAGGGTTCACG
TTTATCACCTCACCTGATGATCTCTGCTGTGACGTTGGGGAAGTGCAACTGAACTTATTATATTTTTGA
AATGCTAAAGTCAGTATGTAGCACAAATTAATAACTATTAACTATTTGGATTATTTTATAATTTTATTTT
ATCCTAAACTACTCATTAGGATGCATTTTATTTGCTCATGAAAGGCAAGATTATGATTGTTAATTGTTT
CTTTTTCTGCTTTCAGATCCATGTATAATAACTGAAGAAAACATGAATAAAAATAACATACAGTTAAA
AGGAAAAAGTGACATAAAATATTATGCAAAAACAGGGGATACCATTGAATTTATGTGTAAATTGGGA
TATAATGCGAATACATCAGTTCTATCATTTCAAGCAGTGTGTAGGGAAGGCATAGTGGAATACCCCAG
ATGCGAATAAGGCAGCATTGTTACCCTAAATGTATGTCCAACTTCCACTTCTCACTCTTATGGTCTCAA
AGCTTGCAAAGATAGCTTCTGATATTGTTGTAATTTCTACTTTATTTCAAAGAAAATTAATATAATAGT
TTCAATTTGCAACTTAATATGTTCTCAAAAATATGTTAAAACAAACTAAATTATTGCTTATGCTTGTAC
TAAAATAATAAAAACTACCCTTATATTGGACTTCCTATCAATGAATTAGTAAGTATAGAAACAGATAA
CTGAATGGCTTTCTGCATATTGTACAGTATACCTAGACATAGAAACAAAATGACTTTAGACTTTATTTG
GGGAAGTAATAATAACATAAAATTAGATGTTAAAATTGTAAGTGAAAATAAACAC

The inhibition disclosed herein may be of the gene or protein resulting from expression of the gene and reference to CFHR4 hereby explicitly incorporates a reference to inhibition of the expression or function of the gene and, separately, of the protein product FHR-4.

## DEFINITIONS

The “first strand”, also called the antisense strand or guide strand herein and which can be used interchangeably herein, refers to the nucleic acid strand, e.g. the strand of an siRNA, e.g. a dsiRNA, which includes a region that is substantially complementary to a target sequence, e.g. to an mRNA. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. In some embodiments, a double stranded nucleic acid e.g. an siRNA agent of the invention includes a nucleotide mismatch in the antisense strand.

The “second strand” (also called the sense strand or passenger strand herein, and which can be used interchangeably herein), refers to the strand of a nucleic acid e.g. siRNA that includes a

region that is substantially complementary to a region of the antisense strand as that term is defined herein.

In the context of molecule comprising a nucleic acid provided with a ligand moiety, optionally also with a linker moiety, the nucleic acid of the invention may be referred to as an oligonucleotide moiety or oligonucleoside moiety

Oligonucleotides are short nucleic acid polymers. Whilst oligonucleotides contain phosphodiester bonds between the nucleoside component thereof (base plus sugar), the present invention is not limited to oligonucleotides always joined by such a phosphodiester bond between adjacent nucleosides, and other oligomers of nucleosides joined by bonds which are bonds other than a phosphate bond are contemplated. For example, a bond between nucleotides may be a phosphorothioate bond. Therefore, the term “oligonucleoside” herein covers both oligonucleotides and other oligomers of nucleosides. An oligonucleoside which is a nucleic acid having at least a portion which is an oligonucleotide is preferred according to the present invention. An oligonucleoside having one or more, or a majority of, phosphodiester backbone bonds between nucleosides is also preferred according to the present invention. An oligonucleoside having one or more, or a majority of, phosphodiester backbone bonds between nucleosides, and also having one or more phosphorothioate backbone bonds between nucleosides (typically in a terminal region of the first and / or second strands) is also preferred according to the present invention.

It is preferred herein that the nucleic acid according to the invention is a double stranded oligonucleoside comprising one or more phosphorothioate backbone bonds between nucleosides. Accordingly, in all instances in which the present application refers to an oligonucleotide, particularly in the chemical structures disclosed herein, the oligonucleotide may equally be an oligonucleoside as defined herein.

In some embodiments, a double stranded nucleic acid e.g. siRNA agent of the invention includes a nucleoside mismatch in the sense strand. In some embodiments, the nucleoside mismatch is, for example, within 5, 4, 3, 2, or 1 nucleosides from the 3 '-end of the nucleic acid e.g. siRNA.

In another embodiment, the nucleoside mismatch is, for example, in the 3'- terminal nucleoside of the nucleic acid e.g. siRNA.

A "target sequence" (which may be called a target RNA or a target mRNA) refers to a contiguous portion of the nucleoside sequence of an mRNA molecule formed during the transcription of a gene, including mRNA that is a product of RNA processing of a primary

transcription product, or can be a contiguous portion of the nucleotide sequence of any RNA molecule such as a LNCRNA which it is desired to inhibit.

The target sequence may be from about 10-35 nucleosides in length, e.g., about 15-30 nucleosides in length. For example, the target sequence can be from about 15-30 nucleosides, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18- 28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20- 21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleosides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

The term “ribonucleoside” or “nucleoside” can also refer to a modified nucleoside as further detailed below.

A nucleic acid can be a DNA or an RNA, and can comprise modified nucleosides. RNA is a preferred nucleic acid.

The terms "iRNA", “siRNA”, "RNAi agent," and "iRNA agent," "RNA interference agent" as used interchangeably herein, refer to an agent that contains RNA, and which mediates the targeted cleavage of an RNA transcript via an RNA-induced silencing complex (RISC) pathway. siRNA directs the sequence-specific degradation of mRNA through RNA interference (RNAi).

A double stranded RNA is referred to herein as a "double stranded siRNA (dsiRNA) agent", "double stranded siRNA (dsiRNA) molecule", "double stranded RNA (dsRNA) agent", "double stranded RNA (dsRNA) molecule", "dsiRNA agent", "dsiRNA molecule", or "dsiRNA", which refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target RNA. The majority of nucleosides of each strand of the nucleic acid, e.g. a dsRNA molecule, are preferably ribonucleosides, but in that case each or both strands can also include one or more non-ribonucleosides, e.g., a deoxyribonucleoside or a modified ribonucleoside. In addition, as used in this specification, an "siRNA" may include ribonucleosides with chemical modifications.

The term "modified nucleoside" refers to a nucleoside having, independently, a modified sugar moiety, a modified internucleoside linkage, or modified nucleobase, or any combination thereof. Thus, the term modified nucleoside encompasses substitutions, additions or removal of, e.g., a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. Any such

modifications, as used in a siRNA type molecule, are encompassed by "iRNA" or "RNAi agent" or “siRNA” or “siRNA agent” for the purposes of this specification and claims.

The duplex region of a nucleic acid of the invention e.g. a dsRNA may range from about 9 to 40 base pairs in length such as 9 to 36 base pairs in length, e.g., about 15- 30 base pairs in length, for example, about 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs in length, such as about 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18- 27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21- 30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length.

The two strands forming the duplex structure may be different portions of one larger molecule, or they may be separate molecules e.g. RNA molecules.

The term "nucleoside overhang" refers to at least one unpaired nucleoside that extends from the duplex structure of a double stranded nucleic acid. A ds nucleic acid can comprise an overhang of at least one nucleoside; alternatively, the overhang can comprise at least two nucleosides, at least three nucleosides, at least four nucleosides, at least five nucleosides, or more. A nucleoside overhang can comprise or consist of a nucleoside analog, including a deoxynucleoside. The overhang(s) can be on the sense strand, the antisense strand, or any combination thereof. Furthermore, the /nucleoside(s) of an overhang can be present on the 5'-end, 3'-end, or both ends of either an antisense or sense strand.

In certain embodiments, the antisense strand has a 1-10 nucleoside, e.g., 0-3, 1-3, 2-4, 2-5, 4-10, 5-10, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleoside overhang at the 3'-end or the 5'-end.

"Blunt" or "blunt end" means that there are no unpaired nucleoside at that end of the double stranded nucleic acid, i.e., no nucleoside overhang. The nucleic acids of the invention include those with no nucleoside overhang at one end or with no nucleoside overhangs at either end.

Unless otherwise indicated, the term "complementary," when used to describe a first nucleoside sequence in relation to a second nucleoside sequence, refers to the ability of an oligonucleoside comprising the first nucleoside sequence to hybridize and form a duplex structure under certain conditions with an oligonucleoside or polynucleoside comprising the second nucleoside sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH

6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing (see, e.g., "Molecular Cloning: A Laboratory Manual, Sambrook, et al. (1989) Cold Spring Harbor Laboratory Press).

Complementary sequences within nucleic acid e.g. a dsiRNA, as described herein, include base-pairing of the oligonucleoside or polynucleoside comprising a first nucleoside sequence to an oligonucleoside or polynucleoside comprising a second nucleoside sequence over the entire length of one or both nucleoside sequences. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" or “partially complementary”with respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more mismatched base pairs, such as 2, 4, or 5 mismatched base pairs, but preferably not more than 5, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, e.g., inhibition of gene expression via a RISC pathway. Overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a nucleic acid e.g. dsRNA comprising one oligonucleoside 17 nucleosides in length and another oligonucleoside 19 nucleosides in length, wherein the longer oligonucleoside comprises a sequence of 17 nucleosides that is fully complementary to the shorter oligonucleoside, can yet be referred to as "fully complementary".

"Complementary" sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs or base pairs formed from non-natural and modified nucleosides, in so far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogstein base pairing.

The terms "complementary," "fully complementary" and "substantially/partially complementary" herein can be used with respect to the base matching between the sense strand and the antisense strand of a nucleic acid e.g. dsiRNA, or between the antisense strand of a double stranded nucleic acid e.g. siRNA agent and a target sequence.

Within the present invention, the second strand of the nucleic acid according to the invention, in particular a dsiRNA for inhibiting CFHR4, is at least partially complementary to the first strand of said nucleic acid. In certain embodiments, a first and second strand of a nucleic acid according to the invention are partially complementary if they form a duplex region having a length of at least 17 base pairs and comprising not more than 1, 2, 3, 4, or 5 mismatched base pairs.

In certain embodiments, a first and second strand of the nucleic acid according to the invention are partially complementary if they form a duplex region having a length of 19 base pairs and

comprising not more than 1, 2, 3, 4, or 5 mismatched base pairs. In certain embodiments, a first and second strand of the nucleic acid according to the invention are partially complementary if they form a duplex region having a length of 21 base pairs comprising not more than 1, 2, 3, 4, or 5 mismatched base pairs.

Alternatively, a first and second strand of the nucleic acid according to the invention are partially complementary if they form a duplex region having a length of at least 17 base pairs, wherein at least 14, 15, 16 or 17 of said base pairs are complementary base pairs, in particular Watson-Crick base pairs.

In certain embodiments, a first and second strand of the nucleic acid according to the invention are partially complementary if they form a duplex region having a length of 19 base pairs, wherein at least 14, 15, 16, 17, 18 or all 19 base pairs are complementary base pairs, in particular Watson-Crick base pairs. In certain embodiments, a first and second strand of the nucleic acid according to the invention are partially complementary if they form a duplex region having a length of 21 base pairs, wherein at least 16, 17, 18, 19, 20 or all 21 base pairs are complementary base pairs, in particular Watson-Crick base pairs.

As used herein, a nucleic acid that is "substantially complementary" or "partially complementary" to at least part of a messenger RNA (mRNA) refers to a nucleic acid that is substantially or partially complementary to a contiguous portion of the mRNA of interest (e.g., an mRNA encoding a gene). In certain embodiments, the contiguous portion of the mRNA is a sequence as listed in Table 1, i.e., any one of SEQ ID NOs:1-133. For example, a nucleic acid is complementary to at least a part of an mRNA of a gene of interest if the sequence is substantially or partially complementary to a non-interrupted portion of an mRNA encoding that gene.

Accordingly, in some preferred embodiments, the antisense oligonucleosides as disclosed herein are fully complementary to the target gene sequence.

In other embodiments, the antisense oligonucleosides disclosed herein are substantially or partially complementary to a target RNA sequence and comprise a contiguous nucleoside sequence which is at least about 80% complementary over its entire length to the equivalent region of the target RNA sequence, such as at least about 85%, 86%, 87%, 88%, 89%, about 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% complementary or 100% complementary.

In certain embodiments, the first (antisense) strand of a nucleic acid according to the invention is partially or fully complementary to a contiguous portion of RNA transcribed from the CFHR4

gene. In certain embodiments, the first strand of the nucleic acid according to the invention is partially or fully complementary to a contiguous portion of at least 17 nucleosides of the CFHR4 mRNA. In certain embodiments, the first strand of the nucleic acid according to the invention is partially or fully complementary to a contiguous portion of 17, 18, 19, 20, 21, 22 or 23 nucleosides of the CFHR4 mRNA. In certain embodiments, the first strand of the nucleic acid according to the invention is partially or fully complementary to a contiguous portion of 17, 18, 19, 20, 21, 22 or 23 nucleosides of any one of the sequences as listed in Table 1, i.e., any one of SEQ ID NOs:1-133.

In certain embodiments, the first (antisense) strand of the nucleic acid according to the invention is partially complementary to a contiguous portion of the CFHR4 mRNA if it comprises a contiguous nucleoside sequence of at least 17 nucleosides, wherein at least 14, 15, 16 or 17 nucleosides of said contiguous nucleoside sequence are complementary to a contiguous portion of the CFHR4 mRNA. In certain embodiments, the first strand of the nucleic acid according to the invention comprises a contiguous nucleoside sequence of at least 17 nucleosides, wherein at least 14, 15, 16 or 17 nucleosides of said contiguous nucleoside sequence are complementary to a contiguous portion of any one of the sequences listed in Table 1, i.e., any one of SEQ ID NOs: 1-133. In certain embodiments, the first strand of the nucleic acid according to the invention comprises a contiguous nucleoside sequence of 19 nucleosides, wherein at least 14, 15, 16, 17, 18 or all 19 nucleosides of said contiguous nucleoside sequence are complementary to a contiguous portion of any one of the sequences listed in Table 1, i.e., any one of SEQ ID NOs: 1-133. In certain embodiments, the first strand of the nucleic acid according to the invention comprises a contiguous nucleoside sequence of 23 nucleosides, wherein at least 18, 19, 20, 21, 22 or all 23 nucleosides of said contiguous nucleoside sequence are complementary to a contiguous portion of any one of the sequences listed in Table 1, i.e., any one of SEQ ID NOs: 1-133.

In some embodiments, a nucleic acid e.g. an siRNA of the invention includes a sense strand that is substantially or partially complementary to an antisense oligonucleoside which, in turn, is complementary to a target gene sequence and comprises a contiguous nucleoside sequence. The nucleoside sequence of the sense strand is typically at least about 80% complementary over its entire length to the equivalent region of the nucleoside sequence of the antisense strand, such as about 85%, 86%, 87%, 88%, 89%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% complementary, or 100% complementary.

In some embodiments, a nucleic acid e.g. an siRNA of the invention includes an antisense strand that is substantially or partially complementary to the target sequence and comprises a contiguous nucleoside sequence which is at least 80% complementary over its entire length to the target sequence such as about 85%, 86%, 87%, 88%, 89%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% complementary, or 100% complementary.

As used herein, a "subject" is an animal, such as a mammal, including a primate (such as a human, a non-human primate, e.g., a monkey, and a chimpanzee), or a non-primate or a bird that expresses the target gene, either endogenously or heterologously, when the target gene sequence has sufficient complementarity to the nucleic acid e.g. iRNA agent to promote target knockdown. In certain preferred embodiments, the subject is a human.

The terms "treating" or "treatment" refer to a beneficial or desired result including, but not limited to, alleviation or amelioration of one or more symptoms associated with gene expression. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

The terms “prevent” or “prevention” as used herein are defined as eliminating or reducing the likelihood of occurrence of one or more symptoms of a disease or disorder. For example, the inhibitor disclosed herein can be used to prevent the occurrence of age-related macular degeneration.

"Therapeutically effective amount," as used herein, is intended to include the amount of a nucleic acid e.g. an iRNA that, when administered to a patient for treating a subject having disease, is sufficient to effect treatment of the disease (e.g., by diminishing, ameliorating or maintaining the existing disease or one or more symptoms of disease or its related comorbidities).

The phrase "pharmaceutically acceptable" is employed herein to refer to compounds, materials, compositions, or dosage forms which are suitable for use in contact with the tissues of human subjects and animal subjects without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject being treated.

Where a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to".

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise. For example, "sense strand or antisense strand" is understood as "sense strand or antisense strand or sense strand and antisense strand."

The term "about" is used herein to mean within the typical ranges of tolerances in the art. For example, "about" can be understood as about 2 standard deviations from the mean. In certain embodiments, about means +10%. In certain embodiments, about means +5%. When about is present before a series of numbers or a range, it is understood that "about" can modify each of the numbers in the series or range.

The term "at least" prior to a number or series of numbers is understood to include the number adjacent to the term "at least", and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, "at least 18 nucleosides of a 21 nucleoside nucleic acid molecule" means that 18, 19, 20, or 21 nucleosides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range.

As used herein, "no more than" or "less than" is understood as the value adjacent to the phrase and logical lower values or integers, as logical from context, to zero. For example, a duplex with an overhang of "no more than 2 nucleosides" has a 2, 1, or 0 nucleoside overhang. When "no more than" is present before a series of numbers or a range, it is understood that "no more than" can modify each of the numbers in the series or range.

The terminal region of a strand is the last 5 nucleotides from the 5' or the 3' end.

A nucleobase sequence is the sequence of the bases of the nucleic acid in an oligomer.

Various embodiments of the invention can be combined as determined appropriate by one of skill in the art.

## TARGET

A target for inhibition disclosed herein may be, without limitation, an mRNA, polypeptide, protein, or gene.

These targets are a target the inhibition of which helps in the prevention or treatment of age-related macular degeneration (AMD).

The target for inhibition is the gene CFHR4 or a gene product thereof, such as an mRNA transcribed from the CFHR4 gene or the FHR-4 protein, and inhibition may be effected by inhibition of expression or function of the CFHR4/FHR-4 gene or protein or both.

In a preferred embodiment, the target is an mRNA expressed from the CFHR4 gene. Exemplary target sequences on the CFHR4 mRNA are listed below in Table 1.

Following Table 1 provides oligonucleoside mRNA target sequences of CFHR4, together with the corresponding positions in transcript ENST00000608469.6. It is to be understood that SEQ ID NO: 1 to 133 refer to human (Homo sapiens) mRNA sequences.

**Table 1**

| SEQ ID NO | Oligonucleotide mRNA target sequence 5' → 3' | Starting position on ENST00000608469.6 |
|---|---|---|
| SEQ ID NO: 1 | CCUCAGAACAUGCUCAAAAUCAG | 1092,351 |
| SEQ ID NO: 2 | UUUCCAGAAAUUCAACAUGGAGG | 175 |
| SEQ ID NO: 3 | AUUUUCCAGAAAUUCAACAUGGA | 173 |
| SEQ ID NO: 4 | AAUAACAUACAGUUAAAAGGAAA | 1675 |
| SEQ ID NO: 5 | AUGCCUCAGAACAUGCUCAAAAU | 348,1089 |
| SEQ ID NO: 6 | UGCCUCAGAACAUGCUCAAAAUC | 349,1090 |
| SEQ ID NO: 7 | UUCCAGAAAUUCAACAUGGAGGU | 176 |
| SEQ ID NO: 8 | GAUUUUCCAGAAAUUCAACAUGG | 172 |
| SEQ ID NO: 9 | UUUUCCAGAAAUUCAACAUGGAG | 174 |
| SEQ ID NO: 10 | UCCAGAAAUUCAACAUGGAGGUC | 177 |
| SEQ ID NO: 11 | UCCCAUGCCUCAGAACAUGCUCA | 344,1085 |
| SEQ ID NO: 12 | GCCUCAGAACAUGCUCAAAAUCA | 350,1091 |
| SEQ ID NO: 13 | CCAGAAAUUCAACAUGGAGGUCU | 178 |
| SEQ ID NO: 14 | UAUAAUUCUUCAGAAAAGUGUGG | 1453 |
| SEQ ID NO: 15 | CAUGCCUCAGAACAUGCUCAAAA | 347,1088 |
| SEQ ID NO: 16 | CCAUGCCUCAGAACAUGCUCAAA | 346,1087 |
| SEQ ID NO: 17 | CCCAUGCCUCAGAACAUGCUCAA | 345,1086 |

| | | |
|---|---|---|
| SEQ ID NO: 18 | GUGGGUUUCCUGUGCUAAUGGAC | 132 |
| SEQ ID NO: 19 | UGUGGGUUUCCUGUGCUAAUGGA | 131 |
| SEQ ID NO: 20 | UUGUGGGUUUCCUGUGCUAAUGG | 130 |
| SEQ ID NO: 21 | CUUGUGGGUUUCCUGUGCUAAUG | 129 |
| SEQ ID NO: 22 | CCUUGUGGGUUUCCUGUGCUAAU | 128 |
| SEQ ID NO: 23 | ACCUUGUGGGUUUCCUGUGCUAA | 127 |
| SEQ ID NO: 24 | UUGUUACUAAUCAAUGUCAUUCU | 103 |
| SEQ ID NO: 25 | GUUGUUACUAAUCAAUGUCAUUC | 102 |
| SEQ ID NO: 26 | UGUUGUUACUAAUCAAUGUCAUU | 101 |
| SEQ ID NO: 27 | AUGUUGUUACUAAUCAAUGUCAU | 100 |
| SEQ ID NO: 28 | CAUGUUGUUACUAAUCAAUGUCA | 99 |
| SEQ ID NO: 29 | ACAUGUUGUUACUAAUCAAUGUC | 98 |
| SEQ ID NO: 30 | AACAUGUUGUUACUAAUCAAUGU | 97 |
| SEQ ID NO: 31 | UAACAUGUUGUUACUAAUCAAUG | 96 |
| SEQ ID NO: 32 | CUAACAUGUUGUUACUAAUCAAU | 95 |
| SEQ ID NO: 33 | UCUAACAUGUUGUUACUAAUCAA | 94 |
| SEQ ID NO: 34 | AUCUAACAUGUUGUUACUAAUCA | 93 |
| SEQ ID NO: 35 | UAUCUAACAUGUUGUUACUAAUC | 92 |
| SEQ ID NO: 36 | AUAUCUAACAUGUUGUUACUAAU | 91 |
| SEQ ID NO: 37 | AAUAUCUAACAUGUUGUUACUAA | 90 |
| SEQ ID NO: 38 | GAAUAUCUAACAUGUUGUUACUA | 89 |
| SEQ ID NO: 39 | AGAAUAUCUAACAUGUUGUUACU | 88 |
| SEQ ID NO: 40 | GAGAAUAUCUAACAUGUUGUUAC | 87 |
| SEQ ID NO: 41 | UGAGAAUAUCUAACAUGUUGUUA | 86 |
| SEQ ID NO: 42 | CUGAGAAUAUCUAACAUGUUGUU | 85 |
| SEQ ID NO: 43 | ACUGAGAAUAUCUAACAUGUUGU | 84 |
| SEQ ID NO: 44 | UACUGAGAAUAUCUAACAUGUUG | 83 |
| SEQ ID NO: 45 | UUCAGGAAGUUACUGGGAUUACA | 288 |
| SEQ ID NO: 46 | CUUCAGGAAGUUACUGGGAUUAC | 287 |
| SEQ ID NO: 47 | CCUUCAGGAAGUUACUGGGAUUA | 286 |
| SEQ ID NO: 48 | UCCUUCAGGAAGUUACUGGGAUU | 285 |
| SEQ ID NO: 49 | AGUUUCAAUUUGCAACUUAAUAU | 1962 |
| SEQ ID NO: 50 | UAGUUUCAAUUUGCAACUUAAUA | 1961 |
| SEQ ID NO: 51 | AUAGUUUCAAUUUGCAACUUAAU | 1960 |
| SEQ ID NO: 52 | AAUAGUUUCAAUUUGCAACUUAA | 1959 |
| SEQ ID NO: 53 | UAAUAGUUUCAAUUUGCAACUUA | 1958 |
| SEQ ID NO: 54 | AUAAUAGUUUCAAUUUGCAACUU | 1957 |
| SEQ ID NO: 55 | GAUAGCUUCUGAUAUUGUUGUAA | 1906 |
| SEQ ID NO: 56 | AGAUAGCUUCUGAUAUUGUUGUA | 1905 |
| SEQ ID NO: 57 | AAGAUAGCUUCUGAUAUUGUUGU | 1904 |
| SEQ ID NO: 58 | AAAGAUAGCUUCUGAUAUUGUUG | 1903 |

| | | |
|---|---|---|
| SEQ ID NO: 59 | CAAAGAUAGCUUCUGAUAUUGUU | 1902 |
| SEQ ID NO: 60 | GCAAAGAUAGCUUCUGAUAUUGU | 1901 |
| SEQ ID NO: 61 | UGCAAAGAUAGCUUCUGAUAUUG | 1900 |
| SEQ ID NO: 62 | UUGCAAAGAUAGCUUCUGAUAUU | 1899 |
| SEQ ID NO: 63 | CUUGCAAAGAUAGCUUCUGAUAU | 1898 |
| SEQ ID NO: 64 | GCUUGCAAAGAUAGCUUCUGAUA | 1897 |
| SEQ ID NO: 65 | AGCUUGCAAAGAUAGCUUCUGAU | 1896 |
| SEQ ID NO: 66 | AAGCUUGCAAAGAUAGCUUCUGA | 1895 |
| SEQ ID NO: 67 | AAAGCUUGCAAAGAUAGCUUCUG | 1894 |
| SEQ ID NO: 68 | CAAAGCUUGCAAAGAUAGCUUCU | 1893 |
| SEQ ID NO: 69 | UCAAAGCUUGCAAAGAUAGCUUC | 1892 |
| SEQ ID NO: 70 | CUCAAAGCUUGCAAAGAUAGCUU | 1891 |
| SEQ ID NO: 71 | UCUCAAAGCUUGCAAAGAUAGCU | 1890 |
| SEQ ID NO: 72 | GUCUCAAAGCUUGCAAAGAUAGC | 1889 |
| SEQ ID NO: 73 | UUGUUACCCUAAAUGUAUGUCCA | 1844 |
| SEQ ID NO: 74 | AUUGUUACCCUAAAUGUAUGUCC | 1843 |
| SEQ ID NO: 75 | CAUUGUUACCCUAAAUGUAUGUC | 1842 |
| SEQ ID NO: 76 | GGGGAUACCAUUGAAUUUAUGUG | 1726 |
| SEQ ID NO: 77 | AGGGGAUACCAUUGAAUUUAUGU | 1725 |
| SEQ ID NO: 78 | CAGGGGAUACCAUUGAAUUUAUG | 1724 |
| SEQ ID NO: 79 | ACAGGGGAUACCAUUGAAUUUAU | 1723 |
| SEQ ID NO: 80 | AACAGGGGAUACCAUUGAAUUUA | 1722 |
| SEQ ID NO: 81 | AAACAGGGGAUACCAUUGAAUUU | 1721 |
| SEQ ID NO: 82 | AAAACAGGGGAUACCAUUGAAUU | 1720 |
| SEQ ID NO: 83 | AAAUAACAUACAGUUAAAAGGAA | 1674 |
| SEQ ID NO: 84 | AAAAUAACAUACAGUUAAAAGGA | 1673 |
| SEQ ID NO: 85 | AACUGAAGAAAACAUGAAUAAAA | 1653 |
| SEQ ID NO: 86 | UAACUGAAGAAAACAUGAAUAAA | 1652 |
| SEQ ID NO: 87 | AUAACUGAAGAAAACAUGAAUAA | 1651 |
| SEQ ID NO: 88 | AAUAACUGAAGAAAACAUGAAUA | 1650 |
| SEQ ID NO: 89 | UAAUAACUGAAGAAAACAUGAAU | 1649 |
| SEQ ID NO: 90 | AUAAUAACUGAAGAAAACAUGAA | 1648 |
| SEQ ID NO: 91 | UAUAAUAACUGAAGAAAACAUGA | 1647 |
| SEQ ID NO: 92 | GUAUAAUAACUGAAGAAAACAUG | 1646 |
| SEQ ID NO: 93 | UGUAUAAUAACUGAAGAAAACAU | 1645 |
| SEQ ID NO: 94 | AUGUAUAAUAACUGAAGAAAACA | 1644 |
| SEQ ID NO: 95 | CAUGUAUAAUAACUGAAGAAAAC | 1643 |
| SEQ ID NO: 96 | CCAUGUAUAAUAACUGAAGAAAA | 1642 |
| SEQ ID NO: 97 | UCCAUGUAUAAUAACUGAAGAAA | 1641 |
| SEQ ID NO: 98 | AUCCAUGUAUAAUAACUGAAGAA | 1640 |
| SEQ ID NO: 99 | ACAUGUAGUAAUGGAGAGUGGUC | 1597 |

| | | |
|---|---|---|
| SEQ ID NO: 100 | AACAUGUAGUAAUGGAGAGUGGU | 1596 |
| SEQ ID NO: 101 | UAACAUGUAGUAAUGGAGAGUGG | 1595 |
| SEQ ID NO: 102 | GUAACAUGUAGUAAUGGAGAGUG | 1594 |
| SEQ ID NO: 103 | UGUAACAUGUAGUAAUGGAGAGU | 1593 |
| SEQ ID NO: 104 | AUGUAACAUGUAGUAAUGGAGAG | 1592 |
| SEQ ID NO: 105 | GUCAAGAGUCGAGUACCAAUGCC | 1539 |
| SEQ ID NO: 106 | AGUCAAGAGUCGAGUACCAAUGC | 1538 |
| SEQ ID NO: 107 | CAGUCAAGAGUCGAGUACCAAUG | 1537 |
| SEQ ID NO: 108 | ACAGUCAAGAGUCGAGUACCAAU | 1536 |
| SEQ ID NO: 109 | CACAGUCAAGAGUCGAGUACCAA | 1535 |
| SEQ ID NO: 110 | CCACAGUCAAGAGUCGAGUACCA | 1534 |
| SEQ ID NO: 111 | CCUCCUCCACCUAUUAGCAAUGG | 736 |
| SEQ ID NO: 112 | GCCUCCUCCACCUAUUAGCAAUG | 735 |
| SEQ ID NO: 113 | GGCCUCCUCCACCUAUUAGCAAU | 734 |
| SEQ ID NO: 114 | GGGCCUCCUCCACCUAUUAGCAA | 733 |
| SEQ ID NO: 115 | UGGGCCUCCUCCACCUAUUAGCA | 732 |
| SEQ ID NO: 116 | GUGGGCCUCCUCCACCUAUUAGC | 731 |
| SEQ ID NO: 117 | UGUGGGCCUCCUCCACCUAUUAG | 730 |
| SEQ ID NO: 118 | GUGUGGGCCUCCUCCACCUAUUA | 1470 |
| SEQ ID NO: 119 | AGUGUGGGCCUCCUCCACCUAUU | 1469 |
| SEQ ID NO: 120 | AAGUGUGGGCCUCCUCCACCUAU | 1468 |
| SEQ ID NO: 121 | AAAGUGUGGGCCUCCUCCACCUA | 1467 |
| SEQ ID NO: 122 | AAAAGUGUGGGCCUCCUCCACCU | 1466 |
| SEQ ID NO: 123 | GAAAAGUGUGGGCCUCCUCCACC | 1465 |
| SEQ ID NO: 124 | AGAAAAGUGUGGGCCUCCUCCAC | 1464 |
| SEQ ID NO: 125 | CAGAAAAGUGUGGGCCUCCUCCA | 1463 |
| SEQ ID NO: 126 | UCAGAAAAGUGUGGGCCUCCUCC | 1462 |
| SEQ ID NO: 127 | UUCAGAAAAGUGUGGGCCUCCUC | 1461 |
| SEQ ID NO: 128 | CUUCAGAAAAGUGUGGGCCUCCU | 1460 |
| SEQ ID NO: 129 | UCUUCAGAAAAGUGUGGGCCUCC | 1459 |
| SEQ ID NO: 130 | UUCUUCAGAAAAGUGUGGGCCUC | 1458 |
| SEQ ID NO: 131 | AUUCUUCAGAAAAGUGUGGGCCU | 1457 |
| SEQ ID NO: 132 | AAUUCUUCAGAAAAGUGUGGGCC | 1456 |
| SEQ ID NO: 133 | UAAUUCUUCAGAAAAGUGUGGGC | 1455 |

It is to be understood that SEQ ID NOs: 1 to 133 relate to human (Homo sapiens) mRNA sequences.

## DISEASE/CONDITIONS

The invention relates to an inhibitor suitable for use, or for use, in treatment of age-related macular degeneration (AMD), as defined in more detail elsewhere herein.

## INHIBITORS

Inhibitors of the invention include nucleic acids such as siRNAs, antibodies and antigen binding fragments thereof, e.g., monoclonal antibodies, polypeptides, antibody–drug conjugates, and small molecules. Preferred are nucleic acids such as siRNA.

Certain preferred features of inhibitors of the invention, where these are oligonucelosides such as siRNA, are given below.

In certain embodiments, the nucleic acid comprises a first strand comprising a sequence that is at least partially complementary to a portion of RNA transcribed from the CFHR4 gene (SEQ ID NO:666). In a preferred embodiment, the nucleic acid comprises a first strand comprising a sequence that is at least partially complementary to a CFHR4 mRNA (ENST00000608469.6).

In certain embodiments, the nucleic acid for inhibiting expression of CFHR4 comprises a duplex region that comprises a first strand and a second strand that is at least partially complementary to the first strand, wherein said first strand is:

(i) at least partially complementary to a portion of RNA transcribed from the CFHR4 gene, and

(ii) comprises at least 17 contiguous nucleosides differing by 0 or 1 nucleosides from any one of SEQ ID NO:134-266.In certain embodiments, the first strand comprises nucleosides 2-18 of any one of the sequences set forth in SEQ ID NO:134-266.

In certain embodiments, the first strand comprises any one of SEQ ID NO:134-266.

In certain embodiments, the second strand comprises a nucleoside sequence of at least 17 contiguous nucleosides differing by 0 or 1 nucleosides from any one of SEQ ID NO:267-399; wherein the second strand has a region of at least 85% complementarity over the 17 contiguous nucleosides to the first strand.

In certain embodiments, the second strand comprises any one of SEQ ID NO: 267-399.

In certain embodiments, the nucleic acid comprises a first strand that comprises, consists of, or consists essentially of a nucleoside sequence differing by 0 or 1 nucleosides from any one of SEQ ID NO: 134-266;

and a second strand that comprises, consists of, or consists essentially of a nucleoside sequence differing by 0 or 1 nucleosides from any one of SEQ ID NO: 267-399.

It is preferred herein that the duplex region is formed between a first (antisense) strand and a complementary second (sense) strand. Exemplary pairs of complementary antisense and sense strands are listed in Table 2 below.

Table 2 provides the unmodified first (antisense) and corresponding unmodified second (sense) strand sequences for siRNA oligonucleosides according to the present invention, together with the corresponding positions in the overall gene sequence of SEQ ID NO:666 as follows.

**Table 2:**

| SEQ ID NO (AS) | Antisense Strand Base Sequence 5' → 3' (Shown as an Unmodified Nucleotide Sequence) | SEQ ID NO (SS) | Sense Strand Base Sequence 5' → 3' (Shown as an Unmodified Nucleotide Sequence) | Corresponding positions on ENST00000608469.6 |
|---|---|---|---|---|
| SEQ ID NO: 149 | UUUGAGCAUGUUCUGAGGCAUGG | SEQ ID NO: 282 | AUGCCUCAGAACAUGCUCAAA | 346-369,1087-1110 |
| SEQ ID NO: 221 | UAUUCAUGUUUUCUUCAGUUAUU | SEQ ID NO: 354 | UAACUGAAGAAAACAUGAAUA | 1650-1673 |
| SEQ ID NO: 198 | AUCAGAAGCUAUCUUUGCAAGCU | SEQ ID NO: 331 | CUUGCAAAGAUAGCUUCUGAU | 1896-1919 |
| SEQ ID NO: 188 | UUACAACAAUAUCAGAAGCUAUC | SEQ ID NO: 321 | UAGCUUCUGAUAUUGUUGUAA | 1906-1929 |
| SEQ ID NO: 134 | CUGAUUUUGAGCAUGUUCUGAGG | SEQ ID NO: 267 | UCAGAACAUGCUCAAAAUCAG | 1092-1115,351-374 |
| SEQ ID NO: 135 | CCUCCAUGUUGAAUUUCUGGAAA | SEQ ID NO: 268 | UCCAGAAAUUCAACAUGGAGG | 175-198 |
| SEQ ID NO: 136 | UCCAUGUUGAAUUUCUGGAAAAU | SEQ ID NO: 269 | UUUCCAGAAAUUCAACAUGGA | 173-196 |
| SEQ ID NO: 137 | UUUCCUUUUAACUGUAUGUUAUU | SEQ ID NO: 270 | UAACAUACAGUUAAAAGGAAA | 1675-1698 |
| SEQ ID NO: 138 | AUUUUGAGCAUGUUCUGAGGCAU | SEQ ID NO: 271 | GCCUCAGAACAUGCUCAAAAU | 348-371,1089-1112 |

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|---|---|---|---|---|
| SEQ ID NO: 139 | GAUUUUGAGCAUGUUCUGAGGCA | SEQ ID NO: 272 | CCUCAGAACAUGCUCAAAAUC | 349-372,1090-1113 |
| SEQ ID NO: 140 | ACCUCCAUGUUGAAUUUCUGGAA | SEQ ID NO: 273 | CCAGAAAUUCAACAUGGAGGU | 176-199 |
| SEQ ID NO: 141 | CCAUGUUGAAUUUCUGGAAAAUC | SEQ ID NO: 274 | UUUUCCAGAAAUUCAACAUGG | 172-195 |
| SEQ ID NO: 142 | CUCCAUGUUGAAUUUCUGGAAAA | SEQ ID NO: 275 | UUCCAGAAAUUCAACAUGGAG | 174-197 |
| SEQ ID NO: 143 | GACCUCCAUGUUGAAUUUCUGGA | SEQ ID NO: 276 | CAGAAAUUCAACAUGGAGGUC | 177-200 |
| SEQ ID NO: 144 | UGAGCAUGUUCUGAGGCAUGGGA | SEQ ID NO: 277 | CCAUGCCUCAGAACAUGCUCA | 344-367,1085-1108 |
| SEQ ID NO: 145 | UGAUUUUGAGCAUGUUCUGAGGC | SEQ ID NO: 278 | CUCAGAACAUGCUCAAAAUCA | 350-373,1091-1114 |
| SEQ ID NO: 146 | AGACCUCCAUGUUGAAUUUCUGG | SEQ ID NO: 279 | AGAAAUUCAACAUGGAGGUCU | 178-201 |
| SEQ ID NO: 147 | CCACACUUUUCUGAAGAAUUAUA | SEQ ID NO: 280 | UAAUUCUUCAGAAAAGUGUGG | 1453-1476 |
| SEQ ID NO: 148 | UUUUGAGCAUGUUCUGAGGCAUG | SEQ ID NO: 281 | UGCCUCAGAACAUGCUCAAAA | 347-370,1088-1111 |
| SEQ ID NO: 150 | UUGAGCAUGUUCUGAGGCAUGGG | SEQ ID NO: 283 | CAUGCCUCAGAACAUGCUCAA | 345-368,1086-1109 |
| SEQ ID NO: 151 | GUCCAUUAGCACAGGAAACCCAC | SEQ ID NO: 284 | GGGUUUCCUGUGCUAAUGGAC | 132-155 |
| SEQ ID NO: 152 | UCCAUUAGCACAGGAAACCCACA | SEQ ID NO: 285 | UGGGUUUCCUGUGCUAAUGGA | 131-154 |
| SEQ ID NO: 153 | CCAUUAGCACAGGAAACCCACAA | SEQ ID NO: 286 | GUGGGUUUCCUGUGCUAAUGG | 130-153 |
| SEQ ID NO: 154 | CAUUAGCACAGGAAACCCACAAG | SEQ ID NO: 287 | UGUGGGUUUCCUGUGCUAAUG | 129-152 |
| SEQ ID NO: 155 | AUUAGCACAGGAAACCCACAAGG | SEQ ID NO: 288 | UUGUGGGUUUCCUGUGCUAAU | 128-151 |
| SEQ ID NO: 156 | UUAGCACAGGAAACCCACAAGGU | SEQ ID NO: 289 | CUUGUGGGUUUCCUGUGCUAA | 127-150 |
| SEQ ID NO: 157 | AGAAUGACAUUGAUUAGUAACAA | SEQ ID NO: 290 | GUUACUAAUCAAUGUCAUUCU | 103-126 |
| SEQ ID NO: 158 | GAAUGACAUUGAUUAGUAACAAC | SEQ ID NO: 291 | UGUUACUAAUCAAUGUCAUUC | 102-125 |
| SEQ ID NO: 159 | AAUGACAUUGAUUAGUAACAACA | SEQ ID NO: 292 | UUGUUACUAAUCAAUGUCAUU | 101-124 |

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|---|---|---|---|---|
| SEQ ID NO: 160 | AUGACAUUGAUUAGUAACA ACAU | SEQ ID NO: 293 | GUUGUUACUAAUCAAUG UCAU | 100-123 |
| SEQ ID NO: 161 | UGACAUUGAUUAGUAACAA CAUG | SEQ ID NO: 294 | UGUUGUUACUAAUCAAU GUCA | 99-122 |
| SEQ ID NO: 162 | GACAUUGAUUAGUAACAAC AUGU | SEQ ID NO: 295 | AUGUUGUUACUAAUCAA UGUC | 98-121 |
| SEQ ID NO: 163 | ACAUUGAUUAGUAACAACA UGUU | SEQ ID NO: 296 | CAUGUUGUUACUAAUCA AUGU | 97-120 |
| SEQ ID NO: 164 | CAUUGAUUAGUAACAACAU GUUA | SEQ ID NO: 297 | ACAUGUUGUUACUAAUC AAUG | 96-119 |
| SEQ ID NO: 165 | AUUGAUUAGUAACAACAUG UUAG | SEQ ID NO: 298 | AACAUGUUGUUACUAAU CAAU | 95-118 |
| SEQ ID NO: 166 | UUGAUUAGUAACAACAUGU UAGA | SEQ ID NO: 299 | UAACAUGUUGUUACUAA UCAA | 94-117 |
| SEQ ID NO: 167 | UGAUUAGUAACAACAUGUU AGAU | SEQ ID NO: 300 | CUAACAUGUUGUUACUA AUCA | 93-116 |
| SEQ ID NO: 168 | GAUUAGUAACAACAUGUUA GAUA | SEQ ID NO: 301 | UCUAACAUGUUGUUACU AAUC | 92-115 |
| SEQ ID NO: 169 | AUUAGUAACAACAUGUUAG AUAU | SEQ ID NO: 302 | AUCUAACAUGUUGUUAC UAAU | 91-114 |
| SEQ ID NO: 170 | UUAGUAACAACAUGUUAGA UAUU | SEQ ID NO: 303 | UAUCUAACAUGUUGUUA CUAA | 90-113 |
| SEQ ID NO: 171 | UAGUAACAACAUGUUAGAU AUUC | SEQ ID NO: 304 | AUAUCUAACAUGUUGUU ACUA | 89-112 |
| SEQ ID NO: 172 | AGUAACAACAUGUUAGAUA UUCU | SEQ ID NO: 305 | AAUAUCUAACAUGUUGU UACU | 88-111 |
| SEQ ID NO: 173 | GUAACAACAUGUUAGAUAU UCUC | SEQ ID NO: 306 | GAAUAUCUAACAUGUUG UUAC | 87-110 |
| SEQ ID NO: 174 | UAACAACAUGUUAGAUAUU CUCA | SEQ ID NO: 307 | AGAAUAUCUAACAUGUU GUUA | 86-109 |
| SEQ ID NO: 175 | AACAACAUGUUAGAUAUUC UCAG | SEQ ID NO: 308 | GAGAAUAUCUAACAUGU UGUU | 85-108 |
| SEQ ID NO: 176 | ACAACAUGUUAGAUAUUCU CAGU | SEQ ID NO: 309 | UGAGAAUAUCUAACAUG UUGU | 84-107 |
| SEQ ID NO: 177 | CAACAUGUUAGAUAUUCUC AGUA | SEQ ID NO: 310 | CUGAGAAUAUCUAACAU GUUG | 83-106 |
| SEQ ID NO: 178 | UGUAAUCCCAGUAACUUCC UGAA | SEQ ID NO: 311 | CAGGAAGUUACUGGGAU UACA | 288-311 |
| SEQ ID NO: 179 | GUAAUCCCAGUAACUUCCU GAAG | SEQ ID NO: 312 | UCAGGAAGUUACUGGGA UUAC | 287-310 |

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|---|---|---|---|---|
| SEQ ID NO: 180 | UAAUCCCAGUAACUUCCUGAAGG | SEQ ID NO: 313 | UUCAGGAAGUUACUGGGAUUA | 286-309 |
| SEQ ID NO: 181 | AAUCCCAGUAACUUCCUGAAGGA | SEQ ID NO: 314 | CUUCAGGAAGUUACUGGGAUU | 285-308 |
| SEQ ID NO: 182 | AUAUUAAGUUGCAAAUUGAAACU | SEQ ID NO: 315 | UUUCAAUUUGCAACUUAAUAU | 1962-1985 |
| SEQ ID NO: 183 | UAUUAAGUUGCAAAUUGAAACUA | SEQ ID NO: 316 | GUUUCAAUUUGCAACUUAAUA | 1961-1984 |
| SEQ ID NO: 184 | AUUAAGUUGCAAAUUGAAACUAU | SEQ ID NO: 317 | AGUUUCAAUUUGCAACUUAAU | 1960-1983 |
| SEQ ID NO: 185 | UUAAGUUGCAAAUUGAAACUAUU | SEQ ID NO: 318 | UAGUUUCAAUUUGCAACUUAA | 1959-1982 |
| SEQ ID NO: 186 | UAAGUUGCAAAUUGAAACUAUUA | SEQ ID NO: 319 | AUAGUUUCAAUUUGCAACUUA | 1958-1981 |
| SEQ ID NO: 187 | AAGUUGCAAAUUGAAACUAUUAU | SEQ ID NO: 320 | AAUAGUUUCAAUUUGCAACUU | 1957-1980 |
| SEQ ID NO: 189 | UACAACAAUAUCAGAAGCUAUCU | SEQ ID NO: 322 | AUAGCUUCUGAUAUUGUUGUA | 1905-1928 |
| SEQ ID NO: 190 | ACAACAAUAUCAGAAGCUAUCUU | SEQ ID NO: 323 | GAUAGCUUCUGAUAUUGUUGU | 1904-1927 |
| SEQ ID NO: 191 | CAACAAUAUCAGAAGCUAUCUUU | SEQ ID NO: 324 | AGAUAGCUUCUGAUAUUGUUG | 1903-1926 |
| SEQ ID NO: 192 | AACAAUAUCAGAAGCUAUCUUUG | SEQ ID NO: 325 | AAGAUAGCUUCUGAUAUUGUU | 1902-1925 |
| SEQ ID NO: 193 | ACAAUAUCAGAAGCUAUCUUUGC | SEQ ID NO: 326 | AAAGAUAGCUUCUGAUAUUGU | 1901-1924 |
| SEQ ID NO: 194 | CAAUAUCAGAAGCUAUCUUUGCA | SEQ ID NO: 327 | CAAAGAUAGCUUCUGAUAUUG | 1900-1923 |
| SEQ ID NO: 195 | AAUAUCAGAAGCUAUCUUUGCAA | SEQ ID NO: 328 | GCAAAGAUAGCUUCUGAUAUU | 1899-1922 |
| SEQ ID NO: 196 | AUAUCAGAAGCUAUCUUUGCAAG | SEQ ID NO: 329 | UGCAAAGAUAGCUUCUGAUAU | 1898-1921 |
| SEQ ID NO: 197 | UAUCAGAAGCUAUCUUUGCAAGC | SEQ ID NO: 330 | UUGCAAAGAUAGCUUCUGAUA | 1897-1920 |
| SEQ ID NO: 199 | UCAGAAGCUAUCUUUGCAAGCUU | SEQ ID NO: 332 | GCUUGCAAAGAUAGCUUCUGA | 1895-1918 |
| SEQ ID NO: 200 | CAGAAGCUAUCUUUGCAAGCUUU | SEQ ID NO: 333 | AGCUUGCAAAGAUAGCUUCUG | 1894-1917 |
| SEQ ID NO: 201 | AGAAGCUAUCUUUGCAAGCUUUG | SEQ ID NO: 334 | AAGCUUGCAAAGAUAGCUUCU | 1893-1916 |

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|---|---|---|---|---|
| SEQ ID NO: 202 | GAAGCUAUCUUUGCAAGCUUUGA | SEQ ID NO: 335 | AAAGCUUGCAAAGAUAGCUUC | 1892-1915 |
| SEQ ID NO: 203 | AAGCUAUCUUUGCAAGCUUUGAG | SEQ ID NO: 336 | CAAAGCUUGCAAAGAUAGCUU | 1891-1914 |
| SEQ ID NO: 204 | AGCUAUCUUUGCAAGCUUUGAGA | SEQ ID NO: 337 | UCAAAGCUUGCAAAGAUAGCU | 1890-1913 |
| SEQ ID NO: 205 | GCUAUCUUUGCAAGCUUUGAGAC | SEQ ID NO: 338 | CUCAAAGCUUGCAAAGAUAGC | 1889-1912 |
| SEQ ID NO: 206 | UGGACAUACAUUUAGGGUAACAA | SEQ ID NO: 339 | GUUACCCUAAAUGUAUGUCCA | 1844-1867 |
| SEQ ID NO: 207 | GGACAUACAUUUAGGGUAACAAU | SEQ ID NO: 340 | UGUUACCCUAAAUGUAUGUCC | 1843-1866 |
| SEQ ID NO: 208 | GACAUACAUUUAGGGUAACAAUG | SEQ ID NO: 341 | UUGUUACCCUAAAUGUAUGUC | 1842-1865 |
| SEQ ID NO: 209 | CACAUAAAUUCAAUGGUAUCCCC | SEQ ID NO: 342 | GGAUACCAUUGAAUUUAUGUG | 1726-1749 |
| SEQ ID NO: 210 | ACAUAAAUUCAAUGGUAUCCCCU | SEQ ID NO: 343 | GGGAUACCAUUGAAUUUAUGU | 1725-1748 |
| SEQ ID NO: 211 | CAUAAAUUCAAUGGUAUCCCCUG | SEQ ID NO: 344 | GGGGAUACCAUUGAAUUUAUG | 1724-1747 |
| SEQ ID NO: 212 | AUAAAUUCAAUGGUAUCCCCUGU | SEQ ID NO: 345 | AGGGGAUACCAUUGAAUUUAU | 1723-1746 |
| SEQ ID NO: 213 | UAAAUUCAAUGGUAUCCCCUGUU | SEQ ID NO: 346 | CAGGGGAUACCAUUGAAUUUA | 1722-1745 |
| SEQ ID NO: 214 | AAAUUCAAUGGUAUCCCCUGUUU | SEQ ID NO: 347 | ACAGGGGAUACCAUUGAAUUU | 1721-1744 |
| SEQ ID NO: 215 | AAUUCAAUGGUAUCCCCUGUUUU | SEQ ID NO: 348 | AACAGGGGAUACCAUUGAAUU | 1720-1743 |
| SEQ ID NO: 216 | UUCCUUUUAACUGUAUGUUAUUU | SEQ ID NO: 349 | AUAACAUACAGUUAAAAGGAA | 1674-1697 |
| SEQ ID NO: 217 | UCCUUUUAACUGUAUGUUAUUUU | SEQ ID NO: 350 | AAUAACAUACAGUUAAAAGGA | 1673-1696 |
| SEQ ID NO: 218 | UUUUAUUCAUGUUUUCUUCAGUU | SEQ ID NO: 351 | CUGAAGAAAACAUGAAUAAAA | 1653-1676 |
| SEQ ID NO: 219 | UUUAUUCAUGUUUUCUUCAGUUA | SEQ ID NO: 352 | ACUGAAGAAAACAUGAAUAAA | 1652-1675 |
| SEQ ID NO: 220 | UUAUUCAUGUUUUCUUCAGUUAU | SEQ ID NO: 353 | AACUGAAGAAAACAUGAAUAA | 1651-1674 |
| SEQ ID NO: 222 | AUUCAUGUUUUCUUCAGUUAUUA | SEQ ID NO: 355 | AUAACUGAAGAAAACAUGAAU | 1649-1672 |

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|---|---|---|---|---|
| SEQ ID NO: 223 | UUCAUGUUUUCUUCAGUUAUUAU | SEQ ID NO: 356 | AAUAACUGAAGAAAACAUGAA | 1648-1671 |
| SEQ ID NO: 224 | UCAUGUUUUCUUCAGUUAUUAUA | SEQ ID NO: 357 | UAAUAACUGAAGAAAACAUGA | 1647-1670 |
| SEQ ID NO: 225 | CAUGUUUUCUUCAGUUAUUAUAC | SEQ ID NO: 358 | AUAAUAACUGAAGAAAACAUG | 1646-1669 |
| SEQ ID NO: 226 | AUGUUUUCUUCAGUUAUUAUACA | SEQ ID NO: 359 | UAUAAUAACUGAAGAAAACAU | 1645-1668 |
| SEQ ID NO: 227 | UGUUUUCUUCAGUUAUUAUACAU | SEQ ID NO: 360 | GUAUAAUAACUGAAGAAAACA | 1644-1667 |
| SEQ ID NO: 228 | GUUUUCUUCAGUUAUUAUACAUG | SEQ ID NO: 361 | UGUAUAAUAACUGAAGAAAAC | 1643-1666 |
| SEQ ID NO: 229 | UUUUCUUCAGUUAUUAUACAUGG | SEQ ID NO: 362 | AUGUAUAAUAACUGAAGAAAA | 1642-1665 |
| SEQ ID NO: 230 | UUUCUUCAGUUAUUAUACAUGGA | SEQ ID NO: 363 | CAUGUAUAAUAACUGAAGAAA | 1641-1664 |
| SEQ ID NO: 231 | UUCUUCAGUUAUUAUACAUGGAU | SEQ ID NO: 364 | CCAUGUAUAAUAACUGAAGAA | 1640-1663 |
| SEQ ID NO: 232 | GACCACUCUCCAUUACUACAUGU | SEQ ID NO: 365 | AUGUAGUAAUGGAGAGUGGUC | 1597-1620 |
| SEQ ID NO: 233 | ACCACUCUCCAUUACUACAUGUU | SEQ ID NO: 366 | CAUGUAGUAAUGGAGAGUGGU | 1596-1619 |
| SEQ ID NO: 234 | CCACUCUCCAUUACUACAUGUUA | SEQ ID NO: 367 | ACAUGUAGUAAUGGAGAGUGG | 1595-1618 |
| SEQ ID NO: 235 | CACUCUCCAUUACUACAUGUUAC | SEQ ID NO: 368 | AACAUGUAGUAAUGGAGAGUG | 1594-1617 |
| SEQ ID NO: 236 | ACUCUCCAUUACUACAUGUUACA | SEQ ID NO: 369 | UAACAUGUAGUAAUGGAGAGU | 1593-1616 |
| SEQ ID NO: 237 | CUCUCCAUUACUACAUGUUACAU | SEQ ID NO: 370 | GUAACAUGUAGUAAUGGAGAG | 1592-1615 |
| SEQ ID NO: 238 | GGCAUUGGUACUCGACUCUUGAC | SEQ ID NO: 371 | CAAGAGUCGAGUACCAAUGCC | 1539-1562 |
| SEQ ID NO: 239 | GCAUUGGUACUCGACUCUUGACU | SEQ ID NO: 372 | UCAAGAGUCGAGUACCAAUGC | 1538-1561 |
| SEQ ID NO: 240 | CAUUGGUACUCGACUCUUGACUG | SEQ ID NO: 373 | GUCAAGAGUCGAGUACCAAUG | 1537-1560 |
| SEQ ID NO: 241 | AUUGGUACUCGACUCUUGACUGU | SEQ ID NO: 374 | AGUCAAGAGUCGAGUACCAAU | 1536-1559 |
| SEQ ID NO: 242 | UUGGUACUCGACUCUUGACUGUG | SEQ ID NO: 375 | CAGUCAAGAGUCGAGUACCAA | 1535-1558 |

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|---|---|---|---|---|
| SEQ ID NO: 243 | UGGUACUCGACUCUUGACU GUGG | SEQ ID NO: 376 | ACAGUCAAGAGUCGAGU ACCA | 1534-1557 |
| SEQ ID NO: 244 | CCAUUGCUAAUAGGUGGAG GAGG | SEQ ID NO: 377 | UCCUCCACCUAUUAGCA AUGG | 736-759 |
| SEQ ID NO: 245 | CAUUGCUAAUAGGUGGAGG AGGC | SEQ ID NO: 378 | CUCCUCCACCUAUUAGC AAUG | 735-758 |
| SEQ ID NO: 246 | AUUGCUAAUAGGUGGAGGA GGCC | SEQ ID NO: 379 | CCUCCUCCACCUAUUAG CAAU | 734-757 |
| SEQ ID NO: 247 | UUGCUAAUAGGUGGAGGAG GCCC | SEQ ID NO: 380 | GCCUCCUCCACCUAUUA GCAA | 733-756 |
| SEQ ID NO: 248 | UGCUAAUAGGUGGAGGAGG CCCA | SEQ ID NO: 381 | GGCCUCCUCCACCUAUU AGCA | 732-755 |
| SEQ ID NO: 249 | GCUAAUAGGUGGAGGAGGC CCAC | SEQ ID NO: 382 | GGGCCUCCUCCACCUAU UAGC | 731-754 |
| SEQ ID NO: 250 | CUAAUAGGUGGAGGAGGCC CACA | SEQ ID NO: 383 | UGGGCCUCCUCCACCUA UUAG | 730-753 |
| SEQ ID NO: 251 | UAAUAGGUGGAGGAGGCCC ACAC | SEQ ID NO: 384 | GUGGGCCUCCUCCACCU AUUA | 1470-1493 |
| SEQ ID NO: 252 | AAUAGGUGGAGGAGGCCCA CACU | SEQ ID NO: 385 | UGUGGGCCUCCUCCACC UAUU | 1469-1492 |
| SEQ ID NO: 253 | AUAGGUGGAGGAGGCCCAC ACUU | SEQ ID NO: 386 | GUGUGGGCCUCCUCCAC CUAU | 1468-1491 |
| SEQ ID NO: 254 | UAGGUGGAGGAGGCCCACA CUUU | SEQ ID NO: 387 | AGUGUGGGCCUCCUCCA CCUA | 1467-1490 |
| SEQ ID NO: 255 | AGGUGGAGGAGGCCCACAC UUUU | SEQ ID NO: 388 | AAGUGUGGGCCUCCUCC ACCU | 1466-1489 |
| SEQ ID NO: 256 | GGUGGAGGAGGCCCACACU UUUC | SEQ ID NO: 389 | AAAGUGUGGGCCUCCUC CACC | 1465-1488 |
| SEQ ID NO: 257 | GUGGAGGAGGCCCACACUU UUCU | SEQ ID NO: 390 | AAAAGUGUGGGCCUCCU CCAC | 1464-1487 |
| SEQ ID NO: 258 | UGGAGGAGGCCCACACUUU UCUG | SEQ ID NO: 391 | GAAAAGUGUGGGCCUCC UCCA | 1463-1486 |
| SEQ ID NO: 259 | GGAGGAGGCCCACACUUUU CUGA | SEQ ID NO: 392 | AGAAAAGUGUGGGCCUC CUCC | 1462-1485 |
| SEQ ID NO: 260 | GAGGAGGCCCACACUUUUC UGAA | SEQ ID NO: 393 | CAGAAAAGUGUGGGCCU CCUC | 1461-1484 |
| SEQ ID NO: 261 | AGGAGGCCCACACUUUUCU GAAG | SEQ ID NO: 394 | UCAGAAAAGUGUGGGCC UCCU | 1460-1483 |
| SEQ ID NO: 262 | GGAGGCCCACACUUUUCUG AAGA | SEQ ID NO: 395 | UUCAGAAAAGUGUGGGC CUCC | 1459-1482 |

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|---|---|---|---|---|
| SEQ ID NO: 263 | GAGGCCCACACUUUUCUGAAGAA | SEQ ID NO: 396 | CUUCAGAAAAGUGUGGGCCUC | 1458-1481 |
| SEQ ID NO: 264 | AGGCCCACACUUUUCUGAAGAAU | SEQ ID NO: 397 | UCUUCAGAAAAGUGUGGGCCU | 1457-1480 |
| SEQ ID NO: 265 | GGCCCACACUUUUCUGAAGAAUU | SEQ ID NO: 398 | UUCUUCAGAAAAGUGUGGGCC | 1456-1479 |
| SEQ ID NO: 266 | GCCCACACUUUUCUGAAGAAUUA | SEQ ID NO: 399 | AUUCUUCAGAAAAGUGUGGGC | 1455-1478 |

In certain embodiments, the nucleic acid for inhibiting expression of CFHR4 comprises a duplex region that comprises a first strand and a second strand that is at least partially complementary to the first strand, wherein said first strand is:

(i) at least partially complementary to a portion of RNA transcribed from the CFHR4 gene, and

(ii) comprises at least 17 contiguous nucleosides differing by 0 or 1 nucleosides from any one of SEQ ID NO:400-532.

In certain embodiments, the first strand comprises nucleosides 2-18 of any one of the sequences set forth in SEQ ID NO: 400-532.

In certain embodiments, the first strand comprises any one of SEQ ID NO: 400-532.

The modification pattern of the nucleic acids as set forth in SEQ ID NO: 400-532 is summarized in Table 3 below.

Table 3 provides the modified first (antisense) sequences, together with the corresponding unmodified first (antisense) sequences for siRNA oligonucleosides according to the present invention as follows.

**Table 3**

| Antisense strand ID | Modified Antisense Strand 5' → 3' | SEQ ID NO (AS - mod) | Underlying Base Sequence 5' → 3' (Shown as an Unmodified Nucleotide Sequence) | SEQ ID NO (AS - unmod) |
|---|---|---|---|---|
| ETX-S00005642 | UmsUfsUmGmAmGfCmAmUmGmUmUmCmUfGmAfGmGfCmAmUmsGmsGm | SEQ ID NO: 415 | UUUGAGCAUGUUCUGAGGCAUGG | SEQ ID NO: 149 |

| | | | | |
|---|---|---|---|---|
| ETX-S00004474 | UmsAfsUmUmCmAfUmGmUmUmUmUm CmUfUmCfAmGfUmUmAmsUmsUm | SEQ ID NO: 487 | UAUUCAUGUUUUC UUCAGUUAUU | SEQ ID NO: 221 |
| ETX-S00004382 | AmsUfsCmAmGmAfAmGmCmUmAmUm CmUfUmUfGmCfAmAmGmsCmsUm | SEQ ID NO: 464 | AUCAGAAGCUAUC UUUGCAAGCU | SEQ ID NO: 198 |
| ETX-S00004342 | UmsUfsAmCmAmAfCmAmAmUmAmUm CmAfGmAfAmGfCmUmAmsUmsCm | SEQ ID NO: 454 | UUACAACAAUAUC AGAAGCUAUC | SEQ ID NO: 188 |
| ETX-S00005582 | CmsUfsGmAmUmUfUmUmGmAmGmCm AmUfGmUfUmCfUmGmAmsGmsGm | SEQ ID NO: 400 | CUGAUUUUGAGCA UGUUCUGAGG | SEQ ID NO: 134 |
| ETX-S00005586 | CmsCfsUmCmCmAfUmGmUmUmGmAm AmUfUmUfCmUfGmGmAmsAmsAm | SEQ ID NO: 401 | CCUCCAUGUUGAA UUUCUGGAAA | SEQ ID NO: 135 |
| ETX-S00005590 | UmsCfsCmAmUmGfUmUmGmAmAmUm UmUfCmUfGmGfAmAmAmsAmsUm | SEQ ID NO: 402 | UCCAUGUUGAAUU UCUGGAAAAU | SEQ ID NO: 136 |
| ETX-S00005594 | UmsUfsUmCmCmUfUmUmUmAmAmCm UmGfUmAfUmGfUmUmAmsUmsUm | SEQ ID NO: 403 | UUUCCUUUUAACU GUAUGUUAUU | SEQ ID NO: 137 |
| ETX-S00005598 | AmsUfsUmUmUmGfAmGmCmAmUmGm UmUfCmUfGmAfGmGmCmsAmsUm | SEQ ID NO: 404 | AUUUUGAGCAUGU UCUGAGGCAU | SEQ ID NO: 138 |
| ETX-S00005602 | GmsAfsUmUmUmUfGmAmGmCmAmUm GmUfUmCfUmGfAmGmGmsCmsAm | SEQ ID NO: 405 | GAUUUUGAGCAUG UUCUGAGGCA | SEQ ID NO: 139 |
| ETX-S00005606 | AmsCfsCmUmCmCfAmUmGmUmUmGm AmAfUmUfUmCfUmGmGmsAmsAm | SEQ ID NO: 406 | ACCUCCAUGUUGA AUUUCUGGAA | SEQ ID NO: 140 |
| ETX-S00005610 | CmsCfsAmUmGmUfUmGmAmAmUmUm UmCfUmGfGmAfAmAmAmsUmsCm | SEQ ID NO: 407 | CCAUGUUGAAUUU CUGGAAAAUC | SEQ ID NO: 141 |
| ETX-S00005614 | CmsUfsCmCmAmUfGmUmUmGmAmAm UmUfUmCfUmGfGmAmAmsAmsAm | SEQ ID NO: 408 | CUCCAUGUUGAAU UUCUGGAAAA | SEQ ID NO: 142 |
| ETX-S00005618 | GmsAfsCmCmUmCfCmAmUmGmUmUm GmAfAmUfUmUfCmUmGmsGmsAm | SEQ ID NO: 409 | GACCUCCAUGUUG AAUUUCUGGA | SEQ ID NO: 143 |
| ETX-S00005622 | UmsGfsAmGmCmAfUmGmUmUmCmUm GmAfGmGfCmAfUmGmGmsGmsAm | SEQ ID NO: 410 | UGAGCAUGUUCUG AGGCAUGGGA | SEQ ID NO: 144 |
| ETX-S00005626 | UmsGfsAmUmUmUfUmGmAmGmCmAm UmGfUmUfCmUfGmAmGmsGmsCm | SEQ ID NO: 411 | UGAUUUUGAGCAU GUUCUGAGGC | SEQ ID NO: 145 |
| ETX-S00005630 | AmsGfsAmCmCmUfCmCmAmUmGmUm UmGfAmAfUmUfUmCmUmsGmsGm | SEQ ID NO: 412 | AGACCUCCAUGUU GAAUUUCUGG | SEQ ID NO: 146 |
| ETX-S00005634 | CmsCfsAmCmAmCfUmUmUmUmCmUm GmAfAmGfAmAfUmUmAmsUmsAm | SEQ ID NO: 413 | CCACACUUUUCUG AAGAAUUAUA | SEQ ID NO: 147 |
| ETX-S00005638 | UmsUfsUmUmGmAfGmCmAmUmGmUm UmCfUmGfAmGfGmCmAmsUmsGm | SEQ ID NO: 414 | UUUUGAGCAUGUU CUGAGGCAUG | SEQ ID NO: 148 |
| ETX-S00005646 | UmsUfsGmAmGmCfAmUmGmUmUmCm UmGfAmGfGmCfAmUmGmsGmsGm | SEQ ID NO: 416 | UUGAGCAUGUUCU GAGGCAUGGG | SEQ ID NO: 150 |
| ETX-S00004194 | GmsUfsCmCmAmUfUmAmGmCmAmCm AmGfGmAfAmAfCmCmCmsAmsCm | SEQ ID NO: 417 | GUCCAUUAGCACA GGAAACCCAC | SEQ ID NO: 151 |

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|---|---|---|---|---|
| ETX-S00004198 | UmsCfsCmAmUmUfAmGmCmAmCmAmGmGfAmAfAmCfCmCmAmsCmsAm | SEQ ID NO: 418 | UCCAUUAGCACAGGAAACCCACA | SEQ ID NO: 152 |
| ETX-S00004202 | CmsCfsAmUmUmAfGmCmAmCmAmGmGmAfAmAfCmCfCmAmCmsAmsAm | SEQ ID NO: 419 | CCAUUAGCACAGGAAACCCACAA | SEQ ID NO: 153 |
| ETX-S00004206 | CmsAfsUmUmAmGfCmAmCmAmGmGmAmAfAmCfCmCfAmCmAmsAmsGm | SEQ ID NO: 420 | CAUUAGCACAGGAAACCCACAAG | SEQ ID NO: 154 |
| ETX-S00004210 | AmsUfsUmAmGmCfAmCmAmGmGmAmAmAfCmCfCmAfCmAmAmsGmsGm | SEQ ID NO: 421 | AUUAGCACAGGAAACCCACAAGG | SEQ ID NO: 155 |
| ETX-S00004214 | UmsUfsAmGmCmAfCmAmGmGmAmAmAmCfCmCfAmCfAmAmGmsGmsUm | SEQ ID NO: 422 | UUAGCACAGGAAACCCACAAGGU | SEQ ID NO: 156 |
| ETX-S00004218 | AmsGfsAmAmUmGfAmCmAmUmUmGmAmUfUmAfGmUfAmAmCmsAmsAm | SEQ ID NO: 423 | AGAAUGACAUUGAUUAGUAACAA | SEQ ID NO: 157 |
| ETX-S00004222 | GmsAfsAmUmGmAfCmAmUmUmGmAmUmUfAmGfUmAfAmCmAmsAmsCm | SEQ ID NO: 424 | GAAUGACAUUGAUUAGUAACAAC | SEQ ID NO: 158 |
| ETX-S00004226 | AmsAfsUmGmAmCfAmUmUmGmAmUmUmAfGmUfAmAfCmAmAmsCmsAm | SEQ ID NO: 425 | AAUGACAUUGAUUAGUAACAACA | SEQ ID NO: 159 |
| ETX-S00004230 | AmsUfsGmAmCmAfUmUmGmAmUmUmAmGfUmAfAmCfAmAmCmsAmsUm | SEQ ID NO: 426 | AUGACAUUGAUUAGUAACAACAU | SEQ ID NO: 160 |
| ETX-S00004234 | UmsGfsAmCmAmUfUmGmAmUmUmAmGmUfAmAfCmAfAmCmAmsUmsGm | SEQ ID NO: 427 | UGACAUUGAUUAGUAACAACAUG | SEQ ID NO: 161 |
| ETX-S00004238 | GmsAfsCmAmUmUfGmAmUmUmAmGmUmAfAmCfAmAfCmAmUmsGmsUm | SEQ ID NO: 428 | GACAUUGAUUAGUAACAACAUGU | SEQ ID NO: 162 |
| ETX-S00004242 | AmsCfsAmUmUmGfAmUmUmAmGmUmAmAfCmAfAmCfAmUmGmsUmsUm | SEQ ID NO: 429 | ACAUUGAUUAGUAACAACAUGUU | SEQ ID NO: 163 |
| ETX-S00004246 | CmsAfsUmUmGmAfUmUmAmGmUmAmAmCfAmAfCmAfUmGmUmsUmsAm | SEQ ID NO: 430 | CAUUGAUUAGUAACAACAUGUUA | SEQ ID NO: 164 |
| ETX-S00004250 | AmsUfsUmGmAmUfUmAmGmUmAmAmCmAfAmCfAmUfGmUmUmsAmsGm | SEQ ID NO: 431 | AUUGAUUAGUAACAACAUGUUAG | SEQ ID NO: 165 |
| ETX-S00004254 | UmsUfsGmAmUmUfAmGmUmAmAmCmAmAfCmAfUmGfUmUmAmsGmsAm | SEQ ID NO: 432 | UUGAUUAGUAACAACAUGUUAGA | SEQ ID NO: 166 |
| ETX-S00004258 | UmsGfsAmUmUmAfGmUmAmAmCmAmAmCfAmUfGmUfUmAmGmsAmsUm | SEQ ID NO: 433 | UGAUUAGUAACAACAUGUUAGAU | SEQ ID NO: 167 |
| ETX-S00004262 | GmsAfsUmUmAmGfUmAmAmCmAmAmCmAfUmGfUmUfAmGmAmsUmsAm | SEQ ID NO: 434 | GAUUAGUAACAACAUGUUAGAUA | SEQ ID NO: 168 |
| ETX-S00004266 | AmsUfsUmAmGmUfAmAmCmAmAmCmAmUfGmUfUmAfGmAmUmsAmsUm | SEQ ID NO: 435 | AUUAGUAACAACAUGUUAGAUAU | SEQ ID NO: 169 |
| ETX-S00004270 | UmsUfsAmGmUmAfAmCmAmAmCmAmUmGfUmUfAmGfAmUmAmsUmsUm | SEQ ID NO: 436 | UUAGUAACAACAUGUUAGAUAUU | SEQ ID NO: 170 |
| ETX-S00004274 | UmsAfsGmUmAmAfCmAmAmCmAmUmGmUfUmAfGmAfUmAmUmsUmsCm | SEQ ID NO: 437 | UAGUAACAACAUGUUAGAUAUUC | SEQ ID NO: 171 |

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|---|---|---|---|---|
| ETX-S00004278 | AmsGfsUmAmAmCfAmAmCmAmUmGmUmUfAmGfAmUfAmUmUmsCmsUm | SEQ ID NO: 438 | AGUAACAACAUGUUAGAUAUUCU | SEQ ID NO: 172 |
| ETX-S00004282 | GmsUfsAmAmCmAfAmCmAmUmGmUmUmAfGmAfUmAfUmUmCmsUmsCm | SEQ ID NO: 439 | GUAACAACAUGUUAGAUAUUCUC | SEQ ID NO: 173 |
| ETX-S00004286 | UmsAfsAmCmAmAfCmAmUmGmUmUmAmGfAmUfAmUfUmCmUmsCmsAm | SEQ ID NO: 440 | UAACAACAUGUUAGAUAUUCUCA | SEQ ID NO: 174 |
| ETX-S00004290 | AmsAfsCmAmAmCfAmUmGmUmUmAmGmAfUmAfUmUfCmUmCmsAmsGm | SEQ ID NO: 441 | AACAACAUGUUAGAUAUUCUCAG | SEQ ID NO: 175 |
| ETX-S00004294 | AmsCfsAmAmCmAfUmGmUmUmAmGmAmUfAmUfUmCfUmCmAmsGmsUm | SEQ ID NO: 442 | ACAACAUGUUAGAUAUUCUCAGU | SEQ ID NO: 176 |
| ETX-S00004298 | CmsAfsAmCmAmUfGmUmUmAmGmAmUmAfUmUfCmUfCmAmGmsUmsAm | SEQ ID NO: 443 | CAACAUGUUAGAUAUUCUCAGUA | SEQ ID NO: 177 |
| ETX-S00004302 | UmsGfsUmAmAmUfCmCmCmAmGmUmAmAfCmUfUmCfCmUmGmsAmsAm | SEQ ID NO: 444 | UGUAAUCCCAGUAACUUCCUGAA | SEQ ID NO: 178 |
| ETX-S00004306 | GmsUfsAmAmUmCfCmCmAmGmUmAmAmCfUmUfCmCfUmGmAmsAmsGm | SEQ ID NO: 445 | GUAAUCCCAGUAACUUCCUGAAG | SEQ ID NO: 179 |
| ETX-S00004310 | UmsAfsAmUmCmCfCmAmGmUmAmAmCmUfUmCfCmUfGmAmAmsGmsGm | SEQ ID NO: 446 | UAAUCCCAGUAACUUCCUGAAGG | SEQ ID NO: 180 |
| ETX-S00004314 | AmsAfsUmCmCmCfAmGmUmAmAmCmUmUfCmCfUmGfAmAmGmsGmsAm | SEQ ID NO: 447 | AAUCCCAGUAACUUCCUGAAGGA | SEQ ID NO: 181 |
| ETX-S00004318 | AmsUfsAmUmUmAfAmGmUmUmGmCmAmAfAmUfUmGfAmAmAmsCmsUm | SEQ ID NO: 448 | AUAUUAAGUUGCAAAUUGAAACU | SEQ ID NO: 182 |
| ETX-S00004322 | UmsAfsUmUmAmAfGmUmUmGmCmAmAmAfUmUfGmAfAmAmCmsUmsAm | SEQ ID NO: 449 | UAUUAAGUUGCAAAUUGAAACUA | SEQ ID NO: 183 |
| ETX-S00004326 | AmsUfsUmAmAmGfUmUmGmCmAmAmAmUfUmGfAmAfAmCmUmsAmsUm | SEQ ID NO: 450 | AUUAAGUUGCAAAUUGAAACUAU | SEQ ID NO: 184 |
| ETX-S00004330 | UmsUfsAmAmGmUfUmGmCmAmAmAmUmUfGmAfAmAfCmUmAmsUmsUm | SEQ ID NO: 451 | UUAAGUUGCAAAUUGAAACUAUU | SEQ ID NO: 185 |
| ETX-S00004334 | UmsAfsAmGmUmUfGmCmAmAmAmUmUmGfAmAfAmCfUmAmUmsUmsAm | SEQ ID NO: 452 | UAAGUUGCAAAUUGAAACUAUUA | SEQ ID NO: 186 |
| ETX-S00004338 | AmsAfsGmUmUmGfCmAmAmAmUmUmGmAfAmAfCmUfAmUmUmsAmsUm | SEQ ID NO: 453 | AAGUUGCAAAUUGAAACUAUUAU | SEQ ID NO: 187 |
| ETX-S00004346 | UmsAfsCmAmAmCfAmAmUmAmUmCmAmGfAmAfGmCfUmAmUmsCmsUm | SEQ ID NO: 455 | UACAACAAUAUCAGAAGCUAUCU | SEQ ID NO: 189 |
| ETX-S00004350 | AmsCfsAmAmCmAfAmUmAmUmCmAmGmAfAmGfCmUfAmUmCmsUmsUm | SEQ ID NO: 456 | ACAACAAUAUCAGAAGCUAUCUU | SEQ ID NO: 190 |
| ETX-S00004354 | CmsAfsAmCmAmAfUmAmUmCmAmGmAmAfGmCfUmAfUmCmUmsUmsUm | SEQ ID NO: 457 | CAACAAUAUCAGAAGCUAUCUUU | SEQ ID NO: 191 |
| ETX-S00004358 | AmsAfsCmAmAmUfAmUmCmAmGmAmAmGfCmUfAmUfCmUmUmsUmsGm | SEQ ID NO: 458 | AACAAUAUCAGAAGCUAUCUUUG | SEQ ID NO: 192 |

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|---|---|---|---|---|
| ETX-S00004362 | AmsCfsAmAmUmAfUmCmAmGmAmAmGmCfUmAfUmCfUmUmUmsGmsCm | SEQ ID NO: 459 | ACAAUAUCAGAAGCUAUCUUUGC | SEQ ID NO: 193 |
| ETX-S00004366 | CmsAfsAmUmAmUfCmAmGmAmAmGmCmUfAmUfCmUfUmUmGmsCmsAm | SEQ ID NO: 460 | CAAUAUCAGAAGCUAUCUUUGCA | SEQ ID NO: 194 |
| ETX-S00004370 | AmsAfsUmAmUmCfAmGmAmAmGmCmUmAfUmCfUmUfUmGmCmsAmsAm | SEQ ID NO: 461 | AAUAUCAGAAGCUAUCUUUGCAA | SEQ ID NO: 195 |
| ETX-S00004374 | AmsUfsAmUmCmAfGmAmAmGmCmUmAmUfCmUfUmUfGmCmAmsAmsGm | SEQ ID NO: 462 | AUAUCAGAAGCUAUCUUUGCAAG | SEQ ID NO: 196 |
| ETX-S00004378 | UmsAfsUmCmAmGfAmAmGmCmUmAmUmCfUmUfUmGfCmAmAmsGmsCm | SEQ ID NO: 463 | UAUCAGAAGCUAUCUUUGCAAGC | SEQ ID NO: 197 |
| ETX-S00004386 | UmsCfsAmGmAmAfGmCmUmAmUmCmUmUfUmGfCmAfAmGmCmsUmsUm | SEQ ID NO: 465 | UCAGAAGCUAUCUUUGCAAGCUU | SEQ ID NO: 199 |
| ETX-S00004390 | CmsAfsGmAmAmGfCmUmAmUmCmUmUmUfGmCfAmAfGmCmUmsUmsUm | SEQ ID NO: 466 | CAGAAGCUAUCUUUGCAAGCUUU | SEQ ID NO: 200 |
| ETX-S00004394 | AmsGfsAmAmGmCfUmAmUmCmUmUmUmGfCmAfAmGfCmUmUmsUmsGm | SEQ ID NO: 467 | AGAAGCUAUCUUUGCAAGCUUUG | SEQ ID NO: 201 |
| ETX-S00004398 | GmsAfsAmGmCmUfAmUmCmUmUmUmGmCfAmAfGmCfUmUmUmsGmsAm | SEQ ID NO: 468 | GAAGCUAUCUUUGCAAGCUUUGA | SEQ ID NO: 202 |
| ETX-S00004402 | AmsAfsGmCmUmAfUmCmUmUmUmGmCmAfAmGfCmUfUmUmGmsAmsGm | SEQ ID NO: 469 | AAGCUAUCUUUGCAAGCUUUGAG | SEQ ID NO: 203 |
| ETX-S00004406 | AmsGfsCmUmAmUfCmUmUmUmGmCmAmAfGmCfUmUfUmGmAmsGmsAm | SEQ ID NO: 470 | AGCUAUCUUUGCAAGCUUUGAGA | SEQ ID NO: 204 |
| ETX-S00004410 | GmsCfsUmAmUmCfUmUmUmGmCmAmAmGfCmUfUmUfGmAmGmsAmsCm | SEQ ID NO: 471 | GCUAUCUUUGCAAGCUUUGAGAC | SEQ ID NO: 205 |
| ETX-S00004414 | UmsGfsGmAmCmAfUmAmCmAmUmUmUmAfGmGfGmUfAmAmCmsAmsAm | SEQ ID NO: 472 | UGGACAUACAUUUAGGGUAACAA | SEQ ID NO: 206 |
| ETX-S00004418 | GmsGfsAmCmAmUfAmCmAmUmUmUmAmGfGmGfUmAfAmCmAmsAmsUm | SEQ ID NO: 473 | GGACAUACAUUUAGGGUAACAAU | SEQ ID NO: 207 |
| ETX-S00004422 | GmsAfsCmAmUmAfCmAmUmUmUmAmGmGfGmGfUmUfAmAfCmAmAmsUmsGm | SEQ ID NO: 474 | GACAUACAUUUAGGGUAACAAUG | SEQ ID NO: 208 |
| ETX-S00004426 | CmsAfsCmAmUmAfAmAmUmUmCmAmAmUfGmGfUmAfUmCmCmsCmsCm | SEQ ID NO: 475 | CACAUAAAUUCAAUGGUAUCCCC | SEQ ID NO: 209 |
| ETX-S00004430 | AmsCfsAmUmAmAfAmUmUmCmAmAmUmGfGmGfUmUfAmUfCmCmCmsCmsUm | SEQ ID NO: 476 | ACAUAAAUUCAAUGGUAUCCCCU | SEQ ID NO: 210 |
| ETX-S00004434 | CmsAfsUmAmAmAfUmUmCmAmAmUmGmGfUmAfUmCfCmCmCmsUmsGm | SEQ ID NO: 477 | CAUAAAUUCAAUGGUAUCCCCUG | SEQ ID NO: 211 |
| ETX-S00004438 | AmsUfsAmAmAmUfUmCmAmAmUmGmGmUfAmUfCmCfCmCmUmsGmsUm | SEQ ID NO: 478 | AUAAAUUCAAUGGUAUCCCCUGU | SEQ ID NO: 212 |
| ETX-S00004442 | UmsAfsAmAmUmUfCmAmAmUmGmGmUmAfUmCfCmCfCmUmGmsUmsUm | SEQ ID NO: 479 | UAAAUUCAAUGGUAUCCCCUGUU | SEQ ID NO: 213 |

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|---|---|---|---|---|
| ETX-S00004446 | AmsAfsAmUmUmCfAmAmUmGmGmUmAmUfCmCfCmCfUmGmUmsUmsUm | SEQ ID NO: 480 | AAAUUCAAUGGUAUCCCCUGUUU | SEQ ID NO: 214 |
| ETX-S00004450 | AmsAfsUmUmCmAfAmUmGmGmUmAmUmCfCmCfCmUfGmUmUmsUmsUm | SEQ ID NO: 481 | AAUUCAAUGGUAUCCCCUGUUUU | SEQ ID NO: 215 |
| ETX-S00004454 | UmsUfsCmCmUmUfUmUmAmAmCmUmGmUfAmUfGmUfUmAmUmsUmsUm | SEQ ID NO: 482 | UUCCUUUUAACUGUAUGUUAUUU | SEQ ID NO: 216 |
| ETX-S00004458 | UmsCfsCmUmUmUfUmAmAmCmUmGmUmAfUmGfUmUfAmUmUmsUmsUm | SEQ ID NO: 483 | UCCUUUUAACUGUAUGUUAUUUU | SEQ ID NO: 217 |
| ETX-S00004462 | UmsUfsUmUmAmUfUmCmAmUmGmUmUmUfCmCfUmUfCmAmGmsUmsUm | SEQ ID NO: 484 | UUUUAUUCAUGUUUUCUUCAGUU | SEQ ID NO: 218 |
| ETX-S00004466 | UmsUfsUmAmUmUfCmAmUmGmUmUmUmUfCmUfUmCfAmGmUmsUmsAm | SEQ ID NO: 485 | UUUAUUCAUGUUUUCUUCAGUUA | SEQ ID NO: 219 |
| ETX-S00004470 | UmsUfsAmUmUmCfAmUmGmUmUmUmUmCfUmUfCmAfGmUmUmsAmsUm | SEQ ID NO: 486 | UUAUUCAUGUUUUCUUCAGUUAU | SEQ ID NO: 220 |
| ETX-S00004478 | AmsUfsUmCmAmUfGmUmUmUmUmCmUmUfCmAfGmUfUmAmUmsUmsAm | SEQ ID NO: 488 | AUUCAUGUUUUCUUCAGUUAUUA | SEQ ID NO: 222 |
| ETX-S00004482 | UmsUfsCmAmUmGfUmUmUmUmCmUmUmCfAmGfUmUfAmUmUmsAmsUm | SEQ ID NO: 489 | UUCAUGUUUUCUUCAGUUAUUAU | SEQ ID NO: 223 |
| ETX-S00004486 | UmsCfsAmUmGmUfUmUmUmCmUmUmCmAfGmUfUmAfUmUmAmsUmsAm | SEQ ID NO: 490 | UCAUGUUUUCUUCAGUUAUUAUA | SEQ ID NO: 224 |
| ETX-S00004490 | CmsAfsUmGmUmUfUmUmCmUmUmCmAmGfUmUfAmUfUmAmUmsAmsCm | SEQ ID NO: 491 | CAUGUUUUCUUCAGUUAUUAUAC | SEQ ID NO: 225 |
| ETX-S00004494 | AmsUfsGmUmUmUfUmCmUmUmCmAmGmUfUmAfUmAfUmUfAmUmAmsCmsAm | SEQ ID NO: 492 | AUGUUUUCUUCAGUUAUUAUACA | SEQ ID NO: 226 |
| ETX-S00004498 | UmsGfsUmUmUmUfCmUmUmCmAmGmUmUfAmUfAmUfUmAfUmAmCmsAmsUm | SEQ ID NO: 493 | UGUUUUCUUCAGUUAUUAUACAU | SEQ ID NO: 227 |
| ETX-S00004502 | GmsUfsUmUmUmCfUmUmCmAmGmUmUmAfUmUfAmUfAmCmAmsUmsGm | SEQ ID NO: 494 | GUUUUCUUCAGUUAUUAUACAUG | SEQ ID NO: 228 |
| ETX-S00004506 | UmsUfsUmUmCmUfUmCmAmGmUmUmAmUfUmAfUmAfUmAfCmAmUmsGmsGm | SEQ ID NO: 495 | UUUUCUUCAGUUAUUAUACAUGG | SEQ ID NO: 229 |
| ETX-S00004510 | UmsUfsUmCmUmUfCmAmGmUmUmAmUmUfAmUfAmUfCmAfAmUmGmsGmsAm | SEQ ID NO: 496 | UUUCUUCAGUUAUUAUACAUGGA | SEQ ID NO: 230 |
| ETX-S00004514 | UmsUfsCmUmUmCfAmGmUmUmAmUmAfUmAfUmAfCmAfUmGmGmsAmsUm | SEQ ID NO: 497 | UUCUUCAGUUAUUAUACAUGGAU | SEQ ID NO: 231 |
| ETX-S00004518 | GmsAfsCmCmAmCfUmCmUmCmCmAmUmUfAmCfUmAfCmAmUmsGmsUm | SEQ ID NO: 498 | GACCACUCUCCAUUACUACAUGU | SEQ ID NO: 232 |
| ETX-S00004522 | AmsCfsCmAmCmUfCmUmCmCmAmUmUmAfCmUfAmCfAmUmGmsUmsUm | SEQ ID NO: 499 | ACCACUCUCCAUUACUACAUGUU | SEQ ID NO: 233 |
| ETX-S00004526 | CmsCfsAmCmUmCfUmCmCmAmUmUmAmCfUmAfCmAfUmGmUmsUmsAm | SEQ ID NO: 500 | CCACUCUCCAUUACUACAUGUUA | SEQ ID NO: 234 |

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|---|---|---|---|---|
| ETX-S00004530 | CmsAfsCmUmCmUfCmCmAmUmUmAmCmUfAmCfAmUfGmUmUmsAmsCm | SEQ ID NO: 501 | CACUCUCCAUUACUACAUGUUAC | SEQ ID NO: 235 |
| ETX-S00004534 | AmsCfsUmCmUmCfCmAmUmUmAmCmUmAfCmAfUmGfUmUmAmsCmsAm | SEQ ID NO: 502 | ACUCUCCAUUACUACAUGUUACA | SEQ ID NO: 236 |
| ETX-S00004538 | CmsUfsCmUmCmCfAmUmUmAmCmUmAmCfAmUfGmUfUmAmCmsAmsUm | SEQ ID NO: 503 | CUCUCCAUUACUACAUGUUACAU | SEQ ID NO: 237 |
| ETX-S00004542 | GmsGfsCmAmUmUfGmGmUmAmCmUmCmGfAmCfUmCfUmUmGmsAmsCm | SEQ ID NO: 504 | GGCAUUGGUACUCGACUCUUGAC | SEQ ID NO: 238 |
| ETX-S00004546 | GmsCfsAmUmUmGfGmUmAmCmUmCmGmAfCmUfCmUfUmGmAmsCmsUm | SEQ ID NO: 505 | GCAUUGGUACUCGACUCUUGACU | SEQ ID NO: 239 |
| ETX-S00004550 | CmsAfsUmUmGmGfUmAmCmUmCmGmAmCfUmCfUmUfGmAmCmsUmsGm | SEQ ID NO: 506 | CAUUGGUACUCGACUCUUGACUG | SEQ ID NO: 240 |
| ETX-S00004554 | AmsUfsUmGmGmUfAmCmUmCmGmAmCmUfCmUfUmGfAmCmUmsGmsUm | SEQ ID NO: 507 | AUUGGUACUCGACUCUUGACUGU | SEQ ID NO: 241 |
| ETX-S00004558 | UmsUfsGmGmUmAfCmUmCmGmAmCmUmCfUmUfGmAfCmUmGmsUmsGm | SEQ ID NO: 508 | UUGGUACUCGACUCUUGACUGUG | SEQ ID NO: 242 |
| ETX-S00004562 | UmsGfsGmUmAmCfUmCmGmAmCmUmCmUfUmGfAmCfUmGmUmsGmsGm | SEQ ID NO: 509 | UGGUACUCGACUCUUGACUGUGG | SEQ ID NO: 243 |
| ETX-S00004566 | CmsCfsAmUmUmGfCmUmAmAmUmAmGmGfUmGfGmAfGmGmAmsGmsGm | SEQ ID NO: 510 | CCAUUGCUAAUAGGUGGAGGAGG | SEQ ID NO: 244 |
| ETX-S00004570 | CmsAfsUmUmGmCfUmAmAmUmAmGmGmUfGmGfAmGfGmAmGmsGmsCm | SEQ ID NO: 511 | CAUUGCUAAUAGGUGGAGGAGGC | SEQ ID NO: 245 |
| ETX-S00004574 | AmsUfsUmGmCmUfAmAmUmAmGmGmUmGfGmAfGmGfAmGmGmsCmsCm | SEQ ID NO: 512 | AUUGCUAAUAGGUGGAGGAGGCC | SEQ ID NO: 246 |
| ETX-S00004578 | UmsUfsGmCmUmAfAmUmAmGmGmUmGmGfAmGfGmAfGmGmCmsCmsCm | SEQ ID NO: 513 | UUGCUAAUAGGUGGAGGAGGCCC | SEQ ID NO: 247 |
| ETX-S00004582 | UmsGfsCmUmAmAfUmAmGmGmUmGmGmAfGmGfAmGfGmCmCmsCmsAm | SEQ ID NO: 514 | UGCUAAUAGGUGGAGGAGGCCCA | SEQ ID NO: 248 |
| ETX-S00004586 | GmsCfsUmAmAmUfAmGmGmUmGmGmAmGfGmAfGmGfCmCmCmsAmsCm | SEQ ID NO: 515 | GCUAAUAGGUGGAGGAGGCCCAC | SEQ ID NO: 249 |
| ETX-S00004590 | CmsUfsAmAmUmAfGmGmUmGmGmAmGmGfAmGfGmCfCmCmAmsCmsAm | SEQ ID NO: 516 | CUAAUAGGUGGAGGAGGCCCACA | SEQ ID NO: 250 |
| ETX-S00004594 | UmsAfsAmUmAmGfGmUmGmGmAmGmGmAfGmGfCmCfCmAmCmsAmsCm | SEQ ID NO: 517 | UAAUAGGUGGAGGAGGCCCACAC | SEQ ID NO: 251 |
| ETX-S00004598 | AmsAfsUmAmGmGfUmGmGmAmGmGmAmGfGmCfCmCfAmCmAmsCmsUm | SEQ ID NO: 518 | AAUAGGUGGAGGAGGCCCACACU | SEQ ID NO: 252 |
| ETX-S00004602 | AmsUfsAmGmGmUfGmGmAmGmGmAmGmGfCmCfCmAfCmAmCmsUmsUm | SEQ ID NO: 519 | AUAGGUGGAGGAGGCCCACACUU | SEQ ID NO: 253 |
| ETX-S00004606 | UmsAfsGmGmUmGfGmAmGmGmAmGmGmCfCmCfAmCfAmCmUmsUmsUm | SEQ ID NO: 520 | UAGGUGGAGGAGGCCCACACUUU | SEQ ID NO: 254 |

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|---|---|---|---|---|
| ETX-S00004610 | AmsGfsGmUmGmGfAmGmGmAmGmGm CmCfCmAfCmAfCmUmUmsUmsUm | SEQ ID NO: 521 | AGGUGGAGGAGGC CCACACUUUU | SEQ ID NO: 255 |
| ETX-S00004614 | GmsGfsUmGmGmAfGmGmAmGmGmCm CmCfAmCfAmCfUmUmUmsUmsCm | SEQ ID NO: 522 | GGUGGAGGAGGCC CACACUUUUC | SEQ ID NO: 256 |
| ETX-S00004618 | GmsUfsGmGmAmGfGmAmGmGmCmCm CmAfCmAfCmUfUmUmUmsCmsUm | SEQ ID NO: 523 | GUGGAGGAGGCCC ACACUUUUCU | SEQ ID NO: 257 |
| ETX-S00004622 | UmsGfsGmAmGmGfAmGmGmCmCmCm AmCfAmCfUmUfUmUmCmsUmsGm | SEQ ID NO: 524 | UGGAGGAGGCCCA CACUUUUCUG | SEQ ID NO: 258 |
| ETX-S00004626 | GmsGfsAmGmGmAfGmGmCmCmCmAm CmAfCmUfUmUfUmCmUmsGmsAm | SEQ ID NO: 525 | GGAGGAGGCCCAC ACUUUUCUGA | SEQ ID NO: 259 |
| ETX-S00004630 | GmsAfsGmGmAmGfGmCmCmCmAmCm AmCfUmUfUmUfCmUmGmsAmsAm | SEQ ID NO: 526 | GAGGAGGCCCACA CUUUUCUGAA | SEQ ID NO: 260 |
| ETX-S00004634 | AmsGfsGmAmGmGfCmCmCmAmCmAm CmUfUmUfUmCfUmGmAmsAmsGm | SEQ ID NO: 527 | AGGAGGCCCACAC UUUUCUGAAG | SEQ ID NO: 261 |
| ETX-S00004638 | GmsGfsAmGmGmCfCmCmAmCmAmCm UmUfUmUfCmUfGmAmAmsGmsAm | SEQ ID NO: 528 | GGAGGCCCACACU UUUCUGAAGA | SEQ ID NO: 262 |
| ETX-S00004642 | GmsAfsGmGmCmCfCmAmCmAmCmUm UmUfUmCfUmGfAmAmGmsAmsAm | SEQ ID NO: 529 | GAGGCCCACACUU UUCUGAAGAA | SEQ ID NO: 263 |
| ETX-S00004646 | AmsGfsGmCmCmCfAmCmAmCmUmUm UmUfCmUfGmAfAmGmAmsAmsUm | SEQ ID NO: 530 | AGGCCCACACUUU UCUGAAGAAU | SEQ ID NO: 264 |
| ETX-S00004650 | GmsGfsCmCmCmAfCmAmCmUmUmUm UmCfUmGfAmAfGmAmAmsUmsUm | SEQ ID NO: 531 | GGCCCACACUUUU CUGAAGAAUU | SEQ ID NO: 265 |
| ETX-S00004654 | GmsCfsCmCmAmCfAmCmUmUmUmUm CmUfGmAfAmGfAmAmUmsUmsAm | SEQ ID NO: 532 | GCCCACACUUUUC UGAAGAAUUA | SEQ ID NO: 266 |

In certain embodiments, the second strand comprises a nucleoside sequence of at least 17 contiguous nucleosides differing by 0 or 1 nucleosides from any one of SEQ ID NO:533-665; wherein the second strand has a region of at least 85% complementarity over the 17 contiguous nucleosides to the first strand.

In certain embodiments, the second strand comprises any one of SEQ ID NO: 533-665.

The modification pattern of the nucleic acids as set forth in SEQ ID NO: 533-665 is summarized in Table 4 below.

Table 4 provides the modified second (sense) sequences, together with the corresponding unmodified second (sense) sequences for siRNA oligonucleosides according to the present invention as follows.

**Table 4**

| Sense strand ID | Modified Sense Strand 5' → 3' | SEQ ID NO (SS - mod) | Underlying Base Sequence 5' → 3' (Shown as an Unmodified Nucleotide Sequence) | SEQ ID NO (SS - unmod) |
|---|---|---|---|---|
| ETX-S00005641 | iaiaAmsUmsGmCmCmUmCmAmGfAfAfCmAmUmGmCmUmCmAmAmAm | SEQ ID NO: 548 | AUGCCUCAGAACAUGCUCAAA | SEQ ID NO: 282 |
| ETX-S00004473 | iaiaUmsAmsAmCmUmGmAmAmGfAfAfAmAmCmAmUmGmAmAmUmAm | SEQ ID NO: 620 | UAACUGAAGAAAACAUGAAUA | SEQ ID NO: 354 |
| ETX-S00004381 | iaiaCmsUmsUmGmCmAmAmAmGfAfUfAmGmCmUmUmCmUmGmAmUm | SEQ ID NO: 597 | CUUGCAAAGAUAGCUUCUGAU | SEQ ID NO: 331 |
| ETX-S00004341 | iaiaUmsAmsGmCmUmUmCmUmGfAfUfAmUmUmGmUmUmGmUmAmAm | SEQ ID NO: 587 | UAGCUUCUGAUAUUGUUGUAA | SEQ ID NO: 321 |
| ETX-S00005581 | iaiaUmsCmsAmGmAmAmCmAmUfGfCfUmCmAmAmAmAmUmCmAmGm | SEQ ID NO: 533 | UCAGAACAUGCUCAAAAUCAG | SEQ ID NO: 267 |
| ETX-S00005585 | iaiaUmsCmsCmAmGmAmAmAmUfUfCfAmAmCmAmUmGmGmAmGmGm | SEQ ID NO: 534 | UCCAGAAAUUCAACAUGGAGG | SEQ ID NO: 268 |
| ETX-S00005589 | iaiaUmsUmsUmCmCmAmGmAmAfAfUfUmCmAmAmCmAmUmGmGmAm | SEQ ID NO: 535 | UUUCCAGAAAUUCAACAUGGA | SEQ ID NO: 269 |
| ETX-S00005593 | iaiaUmsAmsAmCmAmUmAmCmAfGfUfUmAmAmAmAmGmGmAmAmAm | SEQ ID NO: 536 | UAACAUACAGUUAAAGGAAA | SEQ ID NO: 270 |
| ETX-S00005597 | iaiaGmsCmsCmUmCmAmGmAmAfCfAfUmGmCmUmCmAmAmAmAmUm | SEQ ID NO: 537 | GCCUCAGAACAUGCUCAAAAU | SEQ ID NO: 271 |
| ETX-S00005601 | iaiaCmsCmsUmCmAmGmAmAmCfAfUfGmCmUmCmAmAmAmAmUmCm | SEQ ID NO: 538 | CCUCAGAACAUGCUCAAAAUC | SEQ ID NO: 272 |
| ETX-S00005605 | iaiaCmsCmsAmGmAmAmAmUmUfCfAfAmCmAmUmGmGmAmGmGmUm | SEQ ID NO: 539 | CCAGAAAUUCAACAUGGAGGU | SEQ ID NO: 273 |
| ETX-S00005609 | iaiaUmsUmsUmUmCmCmAmGmAfAfAfUmUmCmAmAmCmAmUmGmGm | SEQ ID NO: 540 | UUUUCCAGAAAUUCAACAUGG | SEQ ID NO: 274 |
| ETX-S00005613 | iaiaUmsUmsCmCmAmGmAmAmAfUfUfCmAmAmCmAmUmGmGmAmGm | SEQ ID NO: 541 | UUCCAGAAAUUCAACAUGGAG | SEQ ID NO: 275 |
| ETX-S00005617 | iaiaCmsAmsGmAmAmAmUmUmCfAfAfCmAmUmGmGmAmGmGmUmCm | SEQ ID NO: 542 | CAGAAAUUCAACAUGGAGGUC | SEQ ID NO: 276 |
| ETX-S00005621 | iaiaCmsCmsAmUmGmCmCmUmCfAfGfAmAmCmAmUmGmCmUmCmAm | SEQ ID NO: 543 | CCAUGCCUCAGAACAUGCUCA | SEQ ID NO: 277 |
| ETX-S00005625 | iaiaCmsUmsCmAmGmAmAmCmAfUfGfCmUmCmAmAmAmAmUmCmAm | SEQ ID NO: 544 | CUCAGAACAUGCUCAAAAUCA | SEQ ID NO: 278 |

| | | | | |
|---|---|---|---|---|
| ETX-S00005629 | iaiaAmsGmsAmAmAmUmUmCmAfAfCfAmUmGmGmAmGmGmUmCmUm | SEQ ID NO: 545 | AGAAAUUCAACAUGGAGGUCU | SEQ ID NO: 279 |
| ETX-S00005633 | iaiaUmsAmsAmUmUmCmUmUmCfAfGfAmAmAmAmGmUmGmUmGmGm | SEQ ID NO: 546 | UAAUUCUUCAGAAAAGUGUGG | SEQ ID NO: 280 |
| ETX-S00005637 | iaiaUmsGmsCmCmUmCmAmGmAfAfCfAmUmGmCmUmCmAmAmAmAm | SEQ ID NO: 547 | UGCCUCAGAACAUGCUCAAAA | SEQ ID NO: 281 |
| ETX-S00005645 | iaiaCmsAmsUmGmCmCmUmCmAfGfAfAmCmAmUmGmCmUmCmAmAm | SEQ ID NO: 549 | CAUGCCUCAGAACAUGCUCAA | SEQ ID NO: 283 |
| ETX-S00004193 | iaiaGmsGmsGmUmUmUmCmCmUfGfUfGmCmUmAmAmUmGmGmAmCm | SEQ ID NO: 550 | GGGUUUCCUGUGCUAAUGGAC | SEQ ID NO: 284 |
| ETX-S00004197 | iaiaUmsGmsGmGmUmUmUmCmCfUfGfUmGmCmUmAmAmUmGmGmAm | SEQ ID NO: 551 | UGGGUUUCCUGUGCUAAUGGA | SEQ ID NO: 285 |
| ETX-S00004201 | iaiaGmsUmsGmGmGmUmUmUmCfCfUfGmUmGmCmUmAmAmUmGmGm | SEQ ID NO: 552 | GUGGGUUUCCUGUGCUAAUGG | SEQ ID NO: 286 |
| ETX-S00004205 | iaiaUmsGmsUmGmGmGmUmUmUfCfCfUmGmUmGmCmUmAmAmUmGm | SEQ ID NO: 553 | UGUGGGUUUCCUGUGCUAAUG | SEQ ID NO: 287 |
| ETX-S00004209 | iaiaUmsUmsGmUmGmGmGmUmUfUfCfCmUmGmUmGmCmUmAmAmUm | SEQ ID NO: 554 | UUGUGGGUUUCCUGUGCUAAU | SEQ ID NO: 288 |
| ETX-S00004213 | iaiaCmsUmsUmGmUmGmGmGmUfUfUfCmCmUmGmUmGmCmUmAmAm | SEQ ID NO: 555 | CUUGUGGGUUUCCUGUGCUAA | SEQ ID NO: 289 |
| ETX-S00004217 | iaiaGmsUmsUmAmCmUmAmAmUfCfAfAmUmGmUmCmAmUmUmCmUm | SEQ ID NO: 556 | GUUACUAAUCAAUGUCAUUCU | SEQ ID NO: 290 |
| ETX-S00004221 | iaiaUmsGmsUmUmAmCmUmAmAfUfCfAmAmUmGmUmCmAmUmUmCm | SEQ ID NO: 557 | UGUUACUAAUCAAUGUCAUUC | SEQ ID NO: 291 |
| ETX-S00004225 | iaiaUmsUmsGmUmUmAmCmUmAfAfUfCmAmAmUmGmUmCmAmUmUm | SEQ ID NO: 558 | UUGUUACUAAUCAAUGUCAUU | SEQ ID NO: 292 |
| ETX-S00004229 | iaiaGmsUmsUmGmUmUmAmCmUfAfAfUmCmAmAmUmGmUmCmAmUm | SEQ ID NO: 559 | GUUGUUACUAAUCAAUGUCAU | SEQ ID NO: 293 |
| ETX-S00004233 | iaiaUmsGmsUmUmGmUmUmAmCfUfAfAmUmCmAmAmUmGmUmCmAm | SEQ ID NO: 560 | UGUUGUUACUAAUCAAUGUCA | SEQ ID NO: 294 |
| ETX-S00004237 | iaiaAmsUmsGmUmUmGmUmUmAfCfUfAmAmUmCmAmAmUmGmUmCm | SEQ ID NO: 561 | AUGUUGUUACUAAUCAAUGUC | SEQ ID NO: 295 |
| ETX-S00004241 | iaiaCmsAmsUmGmUmUmGmUmUfAfCfUmAmAmUmCmAmAmUmGmUm | SEQ ID NO: 562 | CAUGUUGUUACUAAUCAAUGU | SEQ ID NO: 296 |
| ETX-S00004245 | iaiaAmsCmsAmUmGmUmUmGmUfUfAfCmUmAmAmUmCmAmAmUmGm | SEQ ID NO: 563 | ACAUGUUGUUACUAAUCAAUG | SEQ ID NO: 297 |
| ETX-S00004249 | iaiaAmsAmsCmAmUmGmUmUmGfUfUfAmCmUmAmAmUmCmAmAmUm | SEQ ID NO: 564 | AACAUGUUGUUACUAAUCAAU | SEQ ID NO: 298 |
| ETX-S00004253 | iaiaUmsAmsAmCmAmUmGmUmUfGfUfUmAmCmUmAmAmUmCmAmAm | SEQ ID NO: 565 | UAACAUGUUGUUACUAAUCAA | SEQ ID NO: 299 |

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|---|---|---|---|---|
| ETX-S00004257 | iaiaCmsUmsAmAmCmAmUmGmUfUfGfUmUmAmCmUmAmAmUmCmAm | SEQ ID NO: 566 | CUAACAUGUUGUUACUAAUCA | SEQ ID NO: 300 |
| ETX-S00004261 | iaiaUmsCmsUmAmAmCmAmUmGfUfUfGmUmUmAmCmUmAmAmUmCm | SEQ ID NO: 567 | UCUAACAUGUUGUUACUAAUC | SEQ ID NO: 301 |
| ETX-S00004265 | iaiaAmsUmsCmUmAmAmCmAmUfGfUfUmGmUmUmAmCmUmAmAmUm | SEQ ID NO: 568 | AUCUAACAUGUUGUUACUAAU | SEQ ID NO: 302 |
| ETX-S00004269 | iaiaUmsAmsUmCmUmAmAmCmAfUfGfUmUmGmUmUmAmCmUmAmAm | SEQ ID NO: 569 | UAUCUAACAUGUUGUUACUAA | SEQ ID NO: 303 |
| ETX-S00004273 | iaiaAmsUmsAmUmCmUmAmAmCfAfUfGmUmUmGmUmUmAmCmUmAm | SEQ ID NO: 570 | AUAUCUAACAUGUUGUUACUA | SEQ ID NO: 304 |
| ETX-S00004277 | iaiaAmsAmsUmAmUmCmUmAmAfCfAfUmGmUmUmGmUmUmAmCmUm | SEQ ID NO: 571 | AAUAUCUAACAUGUUGUUACU | SEQ ID NO: 305 |
| ETX-S00004281 | iaiaGmsAmsAmUmAmUmCmUmAfAfCfAmUmGmUmUmGmUmUmAmCm | SEQ ID NO: 572 | GAAUAUCUAACAUGUUGUUAC | SEQ ID NO: 306 |
| ETX-S00004285 | iaiaAmsGmsAmAmUmAmUmCmUfAfAfCmAmUmGmUmUmGmUmUmAm | SEQ ID NO: 573 | AGAAUAUCUAACAUGUUGUUA | SEQ ID NO: 307 |
| ETX-S00004289 | iaiaGmsAmsGmAmAmUmAmUmCfUfAfAmCmAmUmGmUmUmGmUmUm | SEQ ID NO: 574 | GAGAAUAUCUAACAUGUUGUU | SEQ ID NO: 308 |
| ETX-S00004293 | iaiaUmsGmsAmGmAmAmUmAmUfCfUfAmAmCmAmUmGmUmUmGmUm | SEQ ID NO: 575 | UGAGAAUAUCUAACAUGUUGU | SEQ ID NO: 309 |
| ETX-S00004297 | iaiaCmsUmsGmAmGmAmAmUmAfUfCfUmAmAmCmAmUmGmUmUmGm | SEQ ID NO: 576 | CUGAGAAUAUCUAACAUGUUG | SEQ ID NO: 310 |
| ETX-S00004301 | iaiaCmsAmsGmGmAmAmGmUmUfAfCfUmGmGmGmAmUmUmAmCmAm | SEQ ID NO: 577 | CAGGAAGUUACUGGGAUUACA | SEQ ID NO: 311 |
| ETX-S00004305 | iaiaUmsCmsAmGmGmAmAmGmUfUfAfCmUmGmGmGmAmUmUmAmCm | SEQ ID NO: 578 | UCAGGAAGUUACUGGGAUUAC | SEQ ID NO: 312 |
| ETX-S00004309 | iaiaUmsUmsCmAmGmGmAmAmGfUfUfAmCmUmGmGmGmAmUmUmAm | SEQ ID NO: 579 | UUCAGGAAGUUACUGGGAUUA | SEQ ID NO: 313 |
| ETX-S00004313 | iaiaCmsUmsUmCmAmGmGmAmAfGfUfUmAmCmUmGmGmGmAmUmUm | SEQ ID NO: 580 | CUUCAGGAAGUUACUGGGAUU | SEQ ID NO: 314 |
| ETX-S00004317 | iaiaUmsUmsUmCmAmAmUmUmUfGfCfAmAmCmUmUmAmAmUmAmUm | SEQ ID NO: 581 | UUUCAAUUUGCAACUUAAUAU | SEQ ID NO: 315 |
| ETX-S00004321 | iaiaGmsUmsUmUmCmAmAmUmUfUfGfCmAmAmCmUmUmAmAmUmAm | SEQ ID NO: 582 | GUUUCAAUUUGCAACUUAAUA | SEQ ID NO: 316 |
| ETX-S00004325 | iaiaAmsGmsUmUmUmCmAmAmUfUfUfGmCmAmAmCmUmUmAmAmUm | SEQ ID NO: 583 | AGUUUCAAUUUGCAACUUAAU | SEQ ID NO: 317 |
| ETX-S00004329 | iaiaUmsAmsGmUmUmUmCmAmAfUfUfUmGmCmAmAmCmUmUmAmAm | SEQ ID NO: 584 | UAGUUUCAAUUUGCAACUUAA | SEQ ID NO: 318 |
| ETX-S00004333 | iaiaAmsUmsAmGmUmUmUmCmAfAfUfUmUmGmCmAmAmCmUmUmAm | SEQ ID NO: 585 | AUAGUUUCAAUUUGCAACUUA | SEQ ID NO: 319 |

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|---|---|---|---|---|
| ETX-S00004337 | iaiaAmsAmsUmAmGmUmUmUmCfAfAfUmUmUmGmCmAmAmCmUmUm | SEQ ID NO: 586 | AAUAGUUUCAAUUUGCAACUU | SEQ ID NO: 320 |
| ETX-S00004345 | iaiaAmsUmsAmGmCmUmUmCmUfGfAfUmAmUmUmGmUmUmGmUmAm | SEQ ID NO: 588 | AUAGCUUCUGAUAUUGUUGUA | SEQ ID NO: 322 |
| ETX-S00004349 | iaiaGmsAmsUmAmGmCmUmUmCfUfGfAmUmAmUmUmGmUmUmGmUm | SEQ ID NO: 589 | GAUAGCUUCUGAUAUUGUUGU | SEQ ID NO: 323 |
| ETX-S00004353 | iaiaAmsGmsAmUmAmGmCmUmUfCfUfGmAmUmAmUmUmGmUmUmGm | SEQ ID NO: 590 | AGAUAGCUUCUGAUAUUGUUG | SEQ ID NO: 324 |
| ETX-S00004357 | iaiaAmsAmsGmAmUmAmGmCmUfUfCfUmGmAmUmAmUmUmGmUmUm | SEQ ID NO: 591 | AAGAUAGCUUCUGAUAUUGUU | SEQ ID NO: 325 |
| ETX-S00004361 | iaiaAmsAmsAmGmAmUmAmGmCfUfUfCmUmGmAmUmAmUmUmGmUm | SEQ ID NO: 592 | AAAGAUAGCUUCUGAUAUUGU | SEQ ID NO: 326 |
| ETX-S00004365 | iaiaCmsAmsAmAmGmAmUmAmGfCfUfUmCmUmGmAmUmAmUmUmGm | SEQ ID NO: 593 | CAAAGAUAGCUUCUGAUAUUG | SEQ ID NO: 327 |
| ETX-S00004369 | iaiaGmsCmsAmAmAmGmAmUmAfGfCfUmUmCmUmGmAmUmAmUmUm | SEQ ID NO: 594 | GCAAAGAUAGCUUCUGAUAUU | SEQ ID NO: 328 |
| ETX-S00004373 | iaiaUmsGmsCmAmAmAmGmAmUfAfGfCmUmUmCmUmGmAmUmAmUm | SEQ ID NO: 595 | UGCAAAGAUAGCUUCUGAUAU | SEQ ID NO: 329 |
| ETX-S00004377 | iaiaUmsUmsGmCmAmAmAmGmAfUfAfGmCmUmUmCmUmGmAmUmAm | SEQ ID NO: 596 | UUGCAAAGAUAGCUUCUGAUA | SEQ ID NO: 330 |
| ETX-S00004385 | iaiaGmsCmsUmUmGmCmAmAmAfGfAfUmAmGmCmUmUmCmUmGmAm | SEQ ID NO: 598 | GCUUGCAAAGAUAGCUUCUGA | SEQ ID NO: 332 |
| ETX-S00004389 | iaiaAmsGmsCmUmUmGmCmAmAfAfGfAmUmAmGmCmUmUmCmUmGm | SEQ ID NO: 599 | AGCUUGCAAAGAUAGCUUCUG | SEQ ID NO: 333 |
| ETX-S00004393 | iaiaAmsAmsGmCmUmUmGmCmAfAfAfGmAmUmAmGmCmUmUmCmUm | SEQ ID NO: 600 | AAGCUUGCAAAGAUAGCUUCU | SEQ ID NO: 334 |
| ETX-S00004397 | iaiaAmsAmsAmGmCmUmUmGmCfAfAfAmGmAmUmAmGmCmUmUmCm | SEQ ID NO: 601 | AAAGCUUGCAAAGAUAGCUUC | SEQ ID NO: 335 |
| ETX-S00004401 | iaiaCmsAmsAmAmGmCmUmUmGfCfAfAmAmGmAmUmAmGmCmUmUm | SEQ ID NO: 602 | CAAAGCUUGCAAAGAUAGCUU | SEQ ID NO: 336 |
| ETX-S00004405 | iaiaUmsCmsAmAmAmGmCmUmUfGfCfAmAmAmGmAmUmAmGmCmUm | SEQ ID NO: 603 | UCAAAGCUUGCAAAGAUAGCU | SEQ ID NO: 337 |
| ETX-S00004409 | iaiaCmsUmsCmAmAmAmGmCmUfUfGfCmAmAmAmGmAmUmAmGmCm | SEQ ID NO: 604 | CUCAAAGCUUGCAAAGAUAGC | SEQ ID NO: 338 |
| ETX-S00004413 | iaiaGmsUmsUmAmCmCmCmUmAfAfAfUmGmUmAmUmGmUmCmCmAm | SEQ ID NO: 605 | GUUACCCUAAAUGUAUGUCCA | SEQ ID NO: 339 |
| ETX-S00004417 | iaiaUmsGmsUmUmAmCmCmCmUfAfAfAmUmGmUmAmUmGmUmCmCm | SEQ ID NO: 606 | UGUUACCCUAAAUGUAUGUCC | SEQ ID NO: 340 |
| ETX-S00004421 | iaiaUmsUmsGmUmUmAmCmCmCfUfAfAmAmUmGmUmAmUmGmUmCm | SEQ ID NO: 607 | UUGUUACCCUAAAUGUAUGUC | SEQ ID NO: 341 |

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|---|---|---|---|---|
| ETX-S00004425 | iaiaGmsGmsAmUmAmCmCmAmUfUfGfAmAmUmUmUmAmUmGmUmGm | SEQ ID NO: 608 | GGAUACCAUUGAAUUUAUGUG | SEQ ID NO: 342 |
| ETX-S00004429 | iaiaGmsGmsGmAmUmAmCmCmAfUfUfGmAmAmUmUmUmAmUmGmUm | SEQ ID NO: 609 | GGGAUACCAUUGAAUUUAUGU | SEQ ID NO: 343 |
| ETX-S00004433 | iaiaGmsGmsGmGmAmUmAmCmCfAfUfUmGmAmAmUmUmUmAmUmGm | SEQ ID NO: 610 | GGGGAUACCAUUGAAUUUAUG | SEQ ID NO: 344 |
| ETX-S00004437 | iaiaAmsGmsGmGmGmAmUmAmCfCfAfUmUmGmAmAmUmUmUmAmUm | SEQ ID NO: 611 | AGGGGAUACCAUUGAAUUUAU | SEQ ID NO: 345 |
| ETX-S00004441 | iaiaCmsAmsGmGmGmGmAmUmAfCfCfAmUmUmGmAmAmUmUmUmAm | SEQ ID NO: 612 | CAGGGGAUACCAUUGAAUUUA | SEQ ID NO: 346 |
| ETX-S00004445 | iaiaAmsCmsAmGmGmGmGmAmUfAfCfCmAmUmUmGmAmAmUmUmUm | SEQ ID NO: 613 | ACAGGGGAUACCAUUGAAUUU | SEQ ID NO: 347 |
| ETX-S00004449 | iaiaAmsAmsCmAmGmGmGmGmAfUfAfCmCmAmUmUmGmAmAmUmUm | SEQ ID NO: 614 | AACAGGGGAUACCAUUGAAUU | SEQ ID NO: 348 |
| ETX-S00004453 | iaiaAmsUmsAmAmCmAmUmAmCfAfGfUmUmAmAmAmAmGmGmAmAm | SEQ ID NO: 615 | AUAACAUACAGUUAAAAGGAA | SEQ ID NO: 349 |
| ETX-S00004457 | iaiaAmsAmsUmAmAmCmAmUmAfCfAfGmUmUmAmAmAmAmGmGmAm | SEQ ID NO: 616 | AAUAACAUACAGUUAAAAGGA | SEQ ID NO: 350 |
| ETX-S00004461 | iaiaCmsUmsGmAmAmGmAmAmAfAfCfAmUmGmAmAmUmAmAmAmAm | SEQ ID NO: 617 | CUGAAGAAAACAUGAAUAAAA | SEQ ID NO: 351 |
| ETX-S00004465 | iaiaAmsCmsUmGmAmAmGmAmAfAfAfCmAmUmGmAmAmUmAmAmAm | SEQ ID NO: 618 | ACUGAAGAAAACAUGAAUAAA | SEQ ID NO: 352 |
| ETX-S00004469 | iaiaAmsAmsCmUmGmAmAmGmAfAfAfAmCmAmUmGmAmAmUmAmAm | SEQ ID NO: 619 | AACUGAAGAAAACAUGAAUAA | SEQ ID NO: 353 |
| ETX-S00004477 | iaiaAmsUmsAmAmCmUmGmAmAfGfAfAmAmAmCmAmUmGmAmAmUm | SEQ ID NO: 621 | AUAACUGAAGAAAACAUGAAU | SEQ ID NO: 355 |
| ETX-S00004481 | iaiaAmsAmsUmAmAmCmUmGmAfAfGfAmAmAmAmCmAmUmGmAmAm | SEQ ID NO: 622 | AAUAACUGAAGAAAACAUGAA | SEQ ID NO: 356 |
| ETX-S00004485 | iaiaUmsAmsAmUmAmAmCmUmGfAfAfGmAmAmAmAmCmAmUmGmAm | SEQ ID NO: 623 | UAAUAACUGAAGAAAACAUGA | SEQ ID NO: 357 |
| ETX-S00004489 | iaiaAmsUmsAmAmUmAmAmCmUfGfAfAmGmAmAmAmAmCmAmUmGm | SEQ ID NO: 624 | AUAAUAACUGAAGAAAACAUG | SEQ ID NO: 358 |
| ETX-S00004493 | iaiaUmsAmsUmAmAmUmAmAmCfUfGfAmAmGmAmAmAmCmAmUm | SEQ ID NO: 625 | UAUAAUAACUGAAGAAAACAU | SEQ ID NO: 359 |
| ETX-S00004497 | iaiaGmsUmsAmUmAmAmUmAmAfCfUfGmAmAmGmAmAmAmCmAm | SEQ ID NO: 626 | GUAUAAUAACUGAAGAAAACA | SEQ ID NO: 360 |
| ETX-S00004501 | iaiaUmsGmsUmAmUmAmAmUmAfAfCfUmGmAmAmGmAmAmAmCm | SEQ ID NO: 627 | UGUAUAAUAACUGAAGAAAAC | SEQ ID NO: 361 |
| ETX-S00004505 | iaiaAmsUmsGmUmAmUmAmAmUfAfAfCmUmGmAmAmGmAmAmAm | SEQ ID NO: 628 | AUGUAUAAUAACUGAAGAAAA | SEQ ID NO: 362 |

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|---|---|---|---|---|
| ETX-S00004509 | iaiaCmsAmsUmGmUmAmUmAmAfUfAfAmCmUmGmAmAmGmAmAmAm | SEQ ID NO: 629 | CAUGUAUAAUAACUGAAGAAA | SEQ ID NO: 363 |
| ETX-S00004513 | iaiaCmsCmsAmUmGmUmAmUmAfAfUfAmAmCmUmGmAmAmGmAmAm | SEQ ID NO: 630 | CCAUGUAUAAUAACUGAAGAA | SEQ ID NO: 364 |
| ETX-S00004517 | iaiaAmsUmsGmUmAmGmUmAmAfUfGfGmAmGmAmGmUmGmGmUmCm | SEQ ID NO: 631 | AUGUAGUAAUGGAGAGUGGUC | SEQ ID NO: 365 |
| ETX-S00004521 | iaiaCmsAmsUmGmUmAmGmUmAfAfUfGmGmAmGmAmGmUmGmGmUm | SEQ ID NO: 632 | CAUGUAGUAAUGGAGAGUGGU | SEQ ID NO: 366 |
| ETX-S00004525 | iaiaAmsCmsAmUmGmUmAmGmUfAfAfUmGmGmAmGmAmGmUmGmGm | SEQ ID NO: 633 | ACAUGUAGUAAUGGAGAGUGG | SEQ ID NO: 367 |
| ETX-S00004529 | iaiaAmsAmsCmAmUmGmUmAmGfUfAfAmUmGmGmAmGmAmGmUmGm | SEQ ID NO: 634 | AACAUGUAGUAAUGGAGAGUG | SEQ ID NO: 368 |
| ETX-S00004533 | iaiaUmsAmsAmCmAmUmGmUmAfGfUfAmAmUmGmGmAmGmAmGmUm | SEQ ID NO: 635 | UAACAUGUAGUAAUGGAGAGU | SEQ ID NO: 369 |
| ETX-S00004537 | iaiaGmsUmsAmAmCmAmUmGmUfAfGfUmAmAmUmGmGmAmGmAmGm | SEQ ID NO: 636 | GUAACAUGUAGUAAUGGAGAG | SEQ ID NO: 370 |
| ETX-S00004541 | iaiaCmsAmsAmGmAmGmUmCmGfAfGfUmAmCmCmAmAmUmGmCmCm | SEQ ID NO: 637 | CAAGAGUCGAGUACCAAUGCC | SEQ ID NO: 371 |
| ETX-S00004545 | iaiaUmsCmsAmAmGmAmGmUmCfGfAfGmUmAmCmCmAmAmUmGmCm | SEQ ID NO: 638 | UCAAGAGUCGAGUACCAAUGC | SEQ ID NO: 372 |
| ETX-S00004549 | iaiaGmsUmsCmAmAmGmAmGmUfCfGfAmGmUmAmCmCmAmAmUmGm | SEQ ID NO: 639 | GUCAAGAGUCGAGUACCAAUG | SEQ ID NO: 373 |
| ETX-S00004553 | iaiaAmsGmsUmCmAmAmGmAmGfUfCfGmAmGmUmAmCmCmAmAmUm | SEQ ID NO: 640 | AGUCAAGAGUCGAGUACCAAU | SEQ ID NO: 374 |
| ETX-S00004557 | iaiaCmsAmsGmUmCmAmAmGmAfGfUfCmGmAmGmUmAmCmCmAmAm | SEQ ID NO: 641 | CAGUCAAGAGUCGAGUACCAA | SEQ ID NO: 375 |
| ETX-S00004561 | iaiaAmsCmsAmGmUmCmAmAmGfAfGfUmCmGmAmGmUmAmCmCmAm | SEQ ID NO: 642 | ACAGUCAAGAGUCGAGUACCA | SEQ ID NO: 376 |
| ETX-S00004565 | iaiaUmsCmsCmUmCmCmAmAmCfUfAfUmUmAmGmCmAmAmUmGmGm | SEQ ID NO: 643 | UCCUCCACCUAUUAGCAAUGG | SEQ ID NO: 377 |
| ETX-S00004569 | iaiaCmsUmsCmCmUmCmCmAmAmCfCfUfAmUmUmAmGmCmAmAmUmGm | SEQ ID NO: 644 | CUCCUCCACCUAUUAGCAAUG | SEQ ID NO: 378 |
| ETX-S00004573 | iaiaCmsCmsUmCmCmUmCmCmAmAfCfCfUmAmUmUmAmGmCmAmAmUm | SEQ ID NO: 645 | CCUCCUCCACCUAUUAGCAAU | SEQ ID NO: 379 |
| ETX-S00004577 | iaiaGmsCmsCmUmCmCmUmCmCfAfCfCmUmAmUmUmAmGmCmAmAm | SEQ ID NO: 646 | GCCUCCUCCACCUAUUAGCAA | SEQ ID NO: 380 |
| ETX-S00004581 | iaiaGmsGmsCmCmUmCmCmUmCfCfAfCmCmUmAmUmUmAmGmCmAm | SEQ ID NO: 647 | GGCCUCCUCCACCUAUUAGCA | SEQ ID NO: 381 |
| ETX-S00004585 | iaiaGmsGmsGmCmCmUmCmCmUfCfCfAmCmCmUmAmUmUmAmGmCm | SEQ ID NO: 648 | GGGCCUCCUCCACCUAUUAGC | SEQ ID NO: 382 |

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|---|---|---|---|---|
| ETX-S00004589 | iaiaUmsGmsGmGmCmCmUmCmCfUfCfCmAmCmCmUmAmUmUmAmGm | SEQ ID NO: 649 | UGGGCCUCCUCCACCUAUUAG | SEQ ID NO: 383 |
| ETX-S00004593 | iaiaGmsUmsGmGmGmCmCmUmCfCfUfCmCmAmCmCmUmAmUmUmAm | SEQ ID NO: 650 | GUGGGCCUCCUCCACCUAUUA | SEQ ID NO: 384 |
| ETX-S00004597 | iaiaUmsGmsUmGmGmGmCmCmUfCfCfUmCmCmAmCmCmUmAmUmUm | SEQ ID NO: 651 | UGUGGGCCUCCUCCACCUAUU | SEQ ID NO: 385 |
| ETX-S00004601 | iaiaGmsUmsGmUmGmGmGmCmCfUfCfCmUmCmCmAmCmCmUmAmUm | SEQ ID NO: 652 | GUGUGGGCCUCCUCCACCUAU | SEQ ID NO: 386 |
| ETX-S00004605 | iaiaAmsGmsUmGmUmGmGmGmCfCfUfCmCmUmCmCmAmCmCmUmAm | SEQ ID NO: 653 | AGUGUGGGCCUCCUCCACCUA | SEQ ID NO: 387 |
| ETX-S00004609 | iaiaAmsAmsGmUmGmUmGmGmGfCfCfUmCmCmUmCmCmAmCmCmUm | SEQ ID NO: 654 | AAGUGUGGGCCUCCUCCACCU | SEQ ID NO: 388 |
| ETX-S00004613 | iaiaAmsAmsAmGmUmGmUmGmGfGfCfCmUmCmCmUmCmCmAmCmCm | SEQ ID NO: 655 | AAAGUGUGGGCCUCCUCCACC | SEQ ID NO: 389 |
| ETX-S00004617 | iaiaAmsAmsAmAmGmUmGmUmGfGfGfCmCmUmCmCmUmCmCmAmCm | SEQ ID NO: 656 | AAAAGUGUGGGCCUCCUCCAC | SEQ ID NO: 390 |
| ETX-S00004621 | iaiaGmsAmsAmAmAmGmUmGmUfGfGfGmCmCmUmCmCmUmCmCmAm | SEQ ID NO: 657 | GAAAAGUGUGGGCCUCCUCCA | SEQ ID NO: 391 |
| ETX-S00004625 | iaiaAmsGmsAmAmAmAmGmUmGfUfGfGmGmCmCmUmCmCmUmCmCm | SEQ ID NO: 658 | AGAAAAGUGUGGGCCUCCUCC | SEQ ID NO: 392 |
| ETX-S00004629 | iaiaCmsAmsGmAmAmAmAmGmUfGfUfGmGmGmCmCmUmCmCmUmCm | SEQ ID NO: 659 | CAGAAAAGUGUGGGCCUCCUC | SEQ ID NO: 393 |
| ETX-S00004633 | iaiaUmsCmsAmGmAmAmAmAmGfUfGfUmGmGmGmCmCmUmCmCmUm | SEQ ID NO: 660 | UCAGAAAAGUGUGGGCCUCCU | SEQ ID NO: 394 |
| ETX-S00004637 | iaiaUmsUmsCmAmGmAmAmAmAfGfUfGmUmGmGmGmCmCmUmCmCm | SEQ ID NO: 661 | UUCAGAAAAGUGUGGGCCUCC | SEQ ID NO: 395 |
| ETX-S00004641 | iaiaCmsUmsUmCmAmGmAmAmAfAfGfUmGmUmGmGmGmCmCmUmCm | SEQ ID NO: 662 | CUUCAGAAAAGUGUGGGCCUC | SEQ ID NO: 396 |
| ETX-S00004645 | iaiaUmsCmsUmUmCmAmGmAmAfAfAfGmUmGmUmGmGmGmCmCmUm | SEQ ID NO: 663 | UCUUCAGAAAAGUGUGGGCCU | SEQ ID NO: 397 |
| ETX-S00004649 | iaiaUmsUmsCmUmUmCmAmGmAfAfAfAmGmUmGmUmGmGmGmCmCm | SEQ ID NO: 664 | UUCUUCAGAAAAGUGUGGGCC | SEQ ID NO: 398 |
| ETX-S00004653 | iaiaAmsUmsUmCmUmUmCmAmGfAfAfAmAmGmUmGmUmGmGmGmCm | SEQ ID NO: 665 | AUUCUUCAGAAAAGUGUGGGC | SEQ ID NO: 399 |

As used herein, and in particular in Tables 3 and 4, the following abbreviations are used for modified nucleosides:

A – adenosine

C – cytidine

G – guanosine

T – thymidine

m – 2’-O-methyl

f – 2’fluro

s – phosphorothioate bond

o - thermally destabilised nucleoside

ia - inverted abasic nucleoside

Am stands for 2'-O-methyl-adenosine, Cm stands for 2'-O-methyl-cytidine, Gm stands for 2'-O-methyl-guanosine, Um stands for 2'-O-methyl-uridine, Af stands for 2'-Fluoro-adenosine, Cf stands for 2'-Fluoro-cytidine, Gf stands for 2'-Fluoro-guanosine and Uf stands for 2'-Fluoro-uridine.

Furthermore, the letter “s” is used as abbreviation for a phosphorothioate linkage between two consecutive (modified) nucleosides. For example, the abbreviation “AmsAm” is used for two consecutive 2'-O-methyl-adenosine nucleosides that are linked via a 3’5’ phosphorothioate linkage. No abbreviation is used for nucleosides that are linked via a standard 3’5’ phosphodiester linkage. For example, the abbreviation “AmAm” is used for two consecutive 2'-O-methyl-adenosine nucleosides that are linked via a 3’5’ phosphodiester linkage.

Some of the modified second strand sequences as illustrated above in Table 4 include the preferred 5’ iaia motif. However, it should also be understood that the scope of these modified second strand sequences additionally includes the Me / F modified second strand in the absence of the 5’iaia motif.

In certain embodiments, the nucleic acid comprises a first strand that comprises, consists of, or consists essentially of a (modified) nucleoside sequence differing by 0 or 1 nucleosides from any one of SEQ ID NO:400-532;

and a second strand that comprises, consists of, or consists essentially of a (modified) nucleoside sequence differing by 0 or 1 nucleosides from any one of SEQ ID NO:533-665.

Preferred combinations of complementary modified antisense (first) and sense (second) strands are listed below in Table 5.

Table 5 identifies duplexes with Duplex IDs referencing the modified antisense and sense IDs from previous Tables 3 and 4.

**Table 5**

| Duplex ID | First (Antisense) strand ID | Second (Sense) strand ID |
|---|---|---|
| ETX-M00001347 | ETX-S00005642 | ETX-S00005641 |
| ETX-M00001697 | ETX-S00004474 | ETX-S00004473 |
| ETX-M00001674 | ETX-S00004382 | ETX-S00004381 |
| ETX-M00001664 | ETX-S00004342 | ETX-S00004341 |
| ETX-M00001327 | ETX-S00005582 | ETX-S00005581 |
| ETX-M00001328 | ETX-S00005586 | ETX-S00005585 |
| ETX-M00001329 | ETX-S00005590 | ETX-S00005589 |
| ETX-M00001330 | ETX-S00005594 | ETX-S00005593 |
| ETX-M00001331 | ETX-S00005598 | ETX-S00005597 |
| ETX-M00001333 | ETX-S00005602 | ETX-S00005601 |
| ETX-M00001335 | ETX-S00005606 | ETX-S00005605 |
| ETX-M00001336 | ETX-S00005610 | ETX-S00005609 |
| ETX-M00001337 | ETX-S00005614 | ETX-S00005613 |
| ETX-M00001338 | ETX-S00005618 | ETX-S00005617 |
| ETX-M00001339 | ETX-S00005622 | ETX-S00005621 |
| ETX-M00001341 | ETX-S00005626 | ETX-S00005625 |
| ETX-M00001343 | ETX-S00005630 | ETX-S00005629 |
| ETX-M00001344 | ETX-S00005634 | ETX-S00005633 |
| ETX-M00001345 | ETX-S00005638 | ETX-S00005637 |
| ETX-M00001349 | ETX-S00005646 | ETX-S00005645 |
| ETX-M00001627 | ETX-S00004194 | ETX-S00004193 |
| ETX-M00001628 | ETX-S00004198 | ETX-S00004197 |
| ETX-M00001629 | ETX-S00004202 | ETX-S00004201 |
| ETX-M00001630 | ETX-S00004206 | ETX-S00004205 |
| ETX-M00001631 | ETX-S00004210 | ETX-S00004209 |
| ETX-M00001632 | ETX-S00004214 | ETX-S00004213 |
| ETX-M00001633 | ETX-S00004218 | ETX-S00004217 |
| ETX-M00001634 | ETX-S00004222 | ETX-S00004221 |
| ETX-M00001635 | ETX-S00004226 | ETX-S00004225 |
| ETX-M00001636 | ETX-S00004230 | ETX-S00004229 |
| ETX-M00001637 | ETX-S00004234 | ETX-S00004233 |
| ETX-M00001638 | ETX-S00004238 | ETX-S00004237 |
| ETX-M00001639 | ETX-S00004242 | ETX-S00004241 |
| ETX-M00001640 | ETX-S00004246 | ETX-S00004245 |
| ETX-M00001641 | ETX-S00004250 | ETX-S00004249 |
| ETX-M00001642 | ETX-S00004254 | ETX-S00004253 |
| ETX-M00001643 | ETX-S00004258 | ETX-S00004257 |

| | | |
|---|---|---|
| ETX-M00001644 | ETX-S00004262 | ETX-S00004261 |
| ETX-M00001645 | ETX-S00004266 | ETX-S00004265 |
| ETX-M00001646 | ETX-S00004270 | ETX-S00004269 |
| ETX-M00001647 | ETX-S00004274 | ETX-S00004273 |
| ETX-M00001648 | ETX-S00004278 | ETX-S00004277 |
| ETX-M00001649 | ETX-S00004282 | ETX-S00004281 |
| ETX-M00001650 | ETX-S00004286 | ETX-S00004285 |
| ETX-M00001651 | ETX-S00004290 | ETX-S00004289 |
| ETX-M00001652 | ETX-S00004294 | ETX-S00004293 |
| ETX-M00001653 | ETX-S00004298 | ETX-S00004297 |
| ETX-M00001654 | ETX-S00004302 | ETX-S00004301 |
| ETX-M00001655 | ETX-S00004306 | ETX-S00004305 |
| ETX-M00001656 | ETX-S00004310 | ETX-S00004309 |
| ETX-M00001657 | ETX-S00004314 | ETX-S00004313 |
| ETX-M00001658 | ETX-S00004318 | ETX-S00004317 |
| ETX-M00001659 | ETX-S00004322 | ETX-S00004321 |
| ETX-M00001660 | ETX-S00004326 | ETX-S00004325 |
| ETX-M00001661 | ETX-S00004330 | ETX-S00004329 |
| ETX-M00001662 | ETX-S00004334 | ETX-S00004333 |
| ETX-M00001663 | ETX-S00004338 | ETX-S00004337 |
| ETX-M00001665 | ETX-S00004346 | ETX-S00004345 |
| ETX-M00001666 | ETX-S00004350 | ETX-S00004349 |
| ETX-M00001667 | ETX-S00004354 | ETX-S00004353 |
| ETX-M00001668 | ETX-S00004358 | ETX-S00004357 |
| ETX-M00001669 | ETX-S00004362 | ETX-S00004361 |
| ETX-M00001670 | ETX-S00004366 | ETX-S00004365 |
| ETX-M00001671 | ETX-S00004370 | ETX-S00004369 |
| ETX-M00001672 | ETX-S00004374 | ETX-S00004373 |
| ETX-M00001673 | ETX-S00004378 | ETX-S00004377 |
| ETX-M00001675 | ETX-S00004386 | ETX-S00004385 |
| ETX-M00001676 | ETX-S00004390 | ETX-S00004389 |
| ETX-M00001677 | ETX-S00004394 | ETX-S00004393 |
| ETX-M00001678 | ETX-S00004398 | ETX-S00004397 |
| ETX-M00001679 | ETX-S00004402 | ETX-S00004401 |
| ETX-M00001680 | ETX-S00004406 | ETX-S00004405 |
| ETX-M00001681 | ETX-S00004410 | ETX-S00004409 |
| ETX-M00001682 | ETX-S00004414 | ETX-S00004413 |
| ETX-M00001683 | ETX-S00004418 | ETX-S00004417 |
| ETX-M00001684 | ETX-S00004422 | ETX-S00004421 |
| ETX-M00001685 | ETX-S00004426 | ETX-S00004425 |
| ETX-M00001686 | ETX-S00004430 | ETX-S00004429 |

| | | |
|---|---|---|
| ETX-M00001687 | ETX-S00004434 | ETX-S00004433 |
| ETX-M00001688 | ETX-S00004438 | ETX-S00004437 |
| ETX-M00001689 | ETX-S00004442 | ETX-S00004441 |
| ETX-M00001690 | ETX-S00004446 | ETX-S00004445 |
| ETX-M00001691 | ETX-S00004450 | ETX-S00004449 |
| ETX-M00001692 | ETX-S00004454 | ETX-S00004453 |
| ETX-M00001693 | ETX-S00004458 | ETX-S00004457 |
| ETX-M00001694 | ETX-S00004462 | ETX-S00004461 |
| ETX-M00001695 | ETX-S00004466 | ETX-S00004465 |
| ETX-M00001696 | ETX-S00004470 | ETX-S00004469 |
| ETX-M00001698 | ETX-S00004478 | ETX-S00004477 |
| ETX-M00001699 | ETX-S00004482 | ETX-S00004481 |
| ETX-M00001700 | ETX-S00004486 | ETX-S00004485 |
| ETX-M00001701 | ETX-S00004490 | ETX-S00004489 |
| ETX-M00001702 | ETX-S00004494 | ETX-S00004493 |
| ETX-M00001703 | ETX-S00004498 | ETX-S00004497 |
| ETX-M00001704 | ETX-S00004502 | ETX-S00004501 |
| ETX-M00001705 | ETX-S00004506 | ETX-S00004505 |
| ETX-M00001706 | ETX-S00004510 | ETX-S00004509 |
| ETX-M00001707 | ETX-S00004514 | ETX-S00004513 |
| ETX-M00001708 | ETX-S00004518 | ETX-S00004517 |
| ETX-M00001709 | ETX-S00004522 | ETX-S00004521 |
| ETX-M00001710 | ETX-S00004526 | ETX-S00004525 |
| ETX-M00001711 | ETX-S00004530 | ETX-S00004529 |
| ETX-M00001712 | ETX-S00004534 | ETX-S00004533 |
| ETX-M00001713 | ETX-S00004538 | ETX-S00004537 |
| ETX-M00001714 | ETX-S00004542 | ETX-S00004541 |
| ETX-M00001715 | ETX-S00004546 | ETX-S00004545 |
| ETX-M00001716 | ETX-S00004550 | ETX-S00004549 |
| ETX-M00001717 | ETX-S00004554 | ETX-S00004553 |
| ETX-M00001718 | ETX-S00004558 | ETX-S00004557 |
| ETX-M00001719 | ETX-S00004562 | ETX-S00004561 |
| ETX-M00001720 | ETX-S00004566 | ETX-S00004565 |
| ETX-M00001721 | ETX-S00004570 | ETX-S00004569 |
| ETX-M00001722 | ETX-S00004574 | ETX-S00004573 |
| ETX-M00001723 | ETX-S00004578 | ETX-S00004577 |
| ETX-M00001724 | ETX-S00004582 | ETX-S00004581 |
| ETX-M00001725 | ETX-S00004586 | ETX-S00004585 |
| ETX-M00001726 | ETX-S00004590 | ETX-S00004589 |
| ETX-M00001727 | ETX-S00004594 | ETX-S00004593 |
| ETX-M00001728 | ETX-S00004598 | ETX-S00004597 |

| | | |
|---|---|---|
| ETX-M00001729 | ETX-S00004602 | ETX-S00004601 |
| ETX-M00001730 | ETX-S00004606 | ETX-S00004605 |
| ETX-M00001731 | ETX-S00004610 | ETX-S00004609 |
| ETX-M00001732 | ETX-S00004614 | ETX-S00004613 |
| ETX-M00001733 | ETX-S00004618 | ETX-S00004617 |
| ETX-M00001734 | ETX-S00004622 | ETX-S00004621 |
| ETX-M00001735 | ETX-S00004626 | ETX-S00004625 |
| ETX-M00001736 | ETX-S00004630 | ETX-S00004629 |
| ETX-M00001737 | ETX-S00004634 | ETX-S00004633 |
| ETX-M00001738 | ETX-S00004638 | ETX-S00004637 |
| ETX-M00001739 | ETX-S00004642 | ETX-S00004641 |
| ETX-M00001740 | ETX-S00004646 | ETX-S00004645 |
| ETX-M00001741 | ETX-S00004650 | ETX-S00004649 |
| ETX-M00001742 | ETX-S00004654 | ETX-S00004653 |

For duplexes of Table 5:

ETX-M00001327 – ETX-M00001349 and ETX-M00001627 - ETX-M00001742 preferably have a duplex structure according to Figure 8b.

In a particularly preferred embodiment, the invention relates to a nucleic acid comprising first and second strands that comprise, consist of, or consist essentially of a nucleoside sequence differing by 0 or 1 nucleosides from any one of the following first and second sequences:

| Duplex ID | Modified first strand | Modified second strand |
|---|---|---|
| ETX-M00001347 | ETX-S00005642 (SEQ ID NO:415) | ETX-S00005641 (SEQ ID NO:548) |
| ETX-M00001697 | ETX-S00004474 (SEQ ID NO:487) | ETX-S00004473 (SEQ ID NO:620) |
| ETX-M00001674 | ETX-S00004382 (SEQ ID NO:464) | ETX-S00004381 (SEQ ID NO:597) |
| ETX-M00001664 | ETX-S00004342 (SEQ ID NO:454) | ETX-S00004341 (SEQ ID NO:587) |

In an even more preferred embodiment, the invention relates to a nucleic acid comprising first and second strands that comprise, consist of, or consist essentially of a nucleoside sequence differing by 0 or 1 nucleosides from any one of the following first and second sequences:

| Duplex ID | Modified first strand | Modified second strand |
|---|---|---|
| ETX-M00001347 | ETX-S00005642 (SEQ ID NO:415) | ETX-S00005641 (SEQ ID NO:548) |

In case of ambiguity between the sequences in this specification and the sequences in the attached sequence listing, the sequences provided herein are considered to be the correct sequences.

**ABASIC NUCLEOTIDES**

In certain embodiments, there are 1, e.g. 2, e.g. 3, e.g. 4 or more abasic nucleosides present in nucleic acids according to the invention. Abasic nucleosides are modified nucleosides because they lack the base normally seen at position 1 of the sugar moiety. Typically, there will be a hydrogen at position 1 of the sugar moiety of the abasic nucleosides present in a nucleic acid according to the present invention.

The abasic nucleosides are in the terminal region of the second strand, preferably located within the terminal 5 nucleosides of the end of the strand. The terminal region may be the terminal 5 nucleosides, which includes abasic nucleosides.

The second strand may comprise, as preferred features (which are all specifically contemplated in combination unless mutually exclusive):

2, or more than 2, abasic nucleosides in a terminal region of the second strand; and/or

2, or more than 2, abasic nucleosides in either the 5' or 3' terminal region of the second strand; and/or

2, or more than 2, abasic nucleosides in either the 5' or 3' terminal region of the second strand, wherein the abasic nucleosides are present in an overhang as herein described; and/or

2, or more than 2, consecutive abasic nucleosides in a terminal region of the second strand, wherein preferably one such abasic nucleosides is a terminal nucleosides; and/or

2, or more than 2, consecutive abasic nucleosides in either the 5' or 3' terminal region of the second strand, wherein preferably one such abasic nucleosides is a terminal nucleosides in either the 5' or 3' terminal region of the second strand; and/or

a reversed internucleoside linkage connects at least one abasic nucleoside to an adjacent basic nucleoside in a terminal region of the second strand; and/or

a reversed internucleoside linkage connects at least one abasic nucleoside to an adjacent basic nucleoside in either the 5' or 3' terminal region of the second strand; and/or

an abasic nucleoside as the penultimate nucleoside which is connected via the reversed linkage to the nucleoside which is not the terminal nucleoside (called the antepenultimate nucleoside herein); and/or

abasic nucleosides as the 2 terminal nucleosides connected via a 5'-3' linkage when reading the strand in the direction towards the terminus comprising the terminal nucleosides;

abasic nucleosides as the 2 terminal nucleosides connected via a 3'-5' linkage when reading the strand in the direction towards the terminus comprising the terminal nucleosides;

abasic nucleosides as the terminal 2 positions, wherein the penultimate nucleoside is connected via the reversed linkage to the antepenultimate nucleoside, and wherein the reversed linkage is a 5-5' reversed linkage or a 3'-3' reversed linkage;

abasic nucleosides as the terminal 2 positions, wherein the penultimate nucleoside is connected via the reversed linkage to the antepenultimate nucleoside, and wherein either

(1) the reversed linkage is a 5-5' reversed linkage and the linkage between the terminal and penultimate abasic nucleosides is 3'5' when reading towards the terminus comprising the terminal and penultimate abasic nucleosides; or

(2) the reversed linkage is a 3-3' reversed linkage and the linkage between the terminal and penultimate abasic nucleosides is 5'3' when reading towards the terminus comprising the terminal and penultimate abasic nucleosides.

Preferably there is an abasic nucleoside at the terminus of the second strand.

Preferably there are 2 or at least 2 abasic nucleosides in the terminal region of the second strand, preferably at the terminal and penultimate positions.

Preferably 2 or more abasic nucleosides are consecutive, for example all abasic nucleosides may be consecutive. For example, the terminal 1 or terminal 2 or terminal 3 or terminal 4 nucelotides may be abasic nucleosides.

An abasic nucleoside may also be linked to an adjacent nucleoside through a 5'-3' phosphodiester linkage or reversed linkage unless there is only 1 abasic nucleoside at the terminus, in which case it will have a reversed linkage to the adjacent nucleoside.

A reversed linkage (which may also be referred to as an inverted linkage, which is also seen in the art), comprises either a 5'-5', a 3-'3', a 3'-2' or a 2'-3' phosphodiester linkage between the adjacent sugar moieties of the nucleosides.

Abasic nucleosides which are not terminal will have 2 phosphodiester bonds, one with each adjacent nucleoside, and these may be a reversed linkage or may be a 5'-3 phosphodiester bond or may be one of each.

A preferred embodiment comprises 2 abasic nucleosides at the terminal and penultimate positions of the second strand, and wherein the reversed internucleoside linkage is located between the penultimate (abasic) nucleoside and the antepenultimate nucleoside.

Preferably there are 2 abasic nucleosides at the terminal and penultimate positions of the second strand and the penultimate nucleoside is linked to the antepenultimate nucleoside through a reversed internucleoside linkage and is linked to the terminal nucleoside through a 5'-3' or 3'-5' phosphodiester linkage (reading in the direction of the terminus of the molecule).

Preferably a nucleic acid according to the present invention comprises one or more abasic nucleosides, optionally wherein the one or more abasic nucleosides are in a terminal region of the second strand, and/or wherein at least one abasic nucleoside is linked to an adjacent basic nucleoside through a reversed internucleoside linkage.

Different preferred features are as follows:

The reversed internucleoside linkage is a 3'-3' reversed linkage. The reversed internucleoside linkage is at a terminal region which is distal to the 5' terminal phosphate of the second strand.

The reversed internucleoside linkage is a 5'-5' reversed linkage. The reversed internucleoside linkage is at a terminal region which is distal to the 3' terminal hydroxide of the second strand.

Typically, the second strand comprises 2 consecutive abasic nucleosides in the 5' terminal region of the second strand, wherein one such abasic nucleoside is a terminal nucleoside at the 5' terminal region of the second strand and the other abasic nucleoside is a penultimate nucleoside at the 5' terminal region of the second strand, wherein: (a) said penultimate abasic nucleoside is connected to an adjacent first basic nucleoside in an adjacent 5' near terminal region through a reversed internucleoside linkage; and (b) the reversed linkage is a 5-5' reversed linkage; and (c) the linkage between the terminal and penultimate abasic nucleosides is 3'5' when reading towards the terminus comprising the terminal and penultimate abasic nucleosides. More

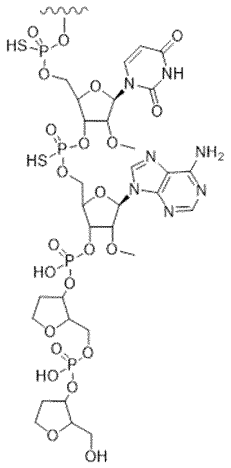
typically, (i) the first strand and the second strand each has a length of 23 nucleosides; (ii) two phosphorothioate internucleoside linkages are respectively between three consecutive positions in said 5' near terminal region of the second strand, wherein a first phosphorothioate internucleoside linkage is present between said adjacent first basic nucleoside of (a) and an adjacent second basic nucleoside in said 5' near terminal region of the second strand, and a second phosphorothioate internucleoside linkage is present between said adjacent second basic nucleoside and an adjacent third basic nucleoside in said 5' near terminal region of the second strand; (iii) two phosphorothioate internucleoside linkages are respectively between three consecutive positions in both 5' and 3' terminal regions of the first strand, whereby a terminal nucleoside respectively at each of the 5' and 3' terminal regions of said first strand is each attached to a respective 5' and 3' adjacent penultimate nucleoside by a phosphorothioate internucleoside linkage, and each first 5' and 3' penultimate nucleoside is attached to a respective 5' and 3' adjacent antepenultimate nucleoside by a phosphorothioate internucleoside linkage; and (iv) the second strand of the nucleic acid is conjugated directly or indirectly to one or more ligand moieties at the 3' terminal region of the second strand.

Alternatively the second strand comprises 2 consecutive abasic nucleosides preferably in an overhang in the 3' terminal region of the second strand, wherein one such abasic nucleoside is a terminal nucleoside at the 3' terminal region of the second strand and the other abasic nucleoside is a penultimate nucleoside at the 3' terminal region of the second strand, wherein: (a) said penultimate abasic nucleoside is connected to an adjacent first basic nucleoside in an adjacent 3' near terminal region through a reversed internucleoside linkage; and (b) the reversed linkage is a 3-3' reversed linkage; and (c) the linkage between the terminal and penultimate abasic nucleosides is 5'-3' when reading towards the terminus comprising the terminal and penultimate abasic nucleosides. More typically, (i) the first strand and the second strand each has a length of 23 nucleosides; (ii) two phosphorothioate internucleoside linkages are respectively between three consecutive positions in said 3' near terminal region of the second strand, wherein a first phosphorothioate internucleoside linkage is present between said adjacent first basic nucleoside of (a) and an adjacent second basic nucleoside in said 3' near terminal region of the second strand, and a second phosphorothioate internucleoside linkage is present between said adjacent second basic nucleoside and an adjacent third basic nucleoside in said 3' near terminal region of the second strand; (iii) two phosphorothioate internucleoside linkages are respectively between three consecutive positions in both 5' and 3' terminal regions of the first strand, whereby a terminal nucleoside respectively at each of the 5' and 3' terminal regions of said first strand is each attached to a respective 5' and 3' adjacent penultimate nucleoside by a phosphorothioate

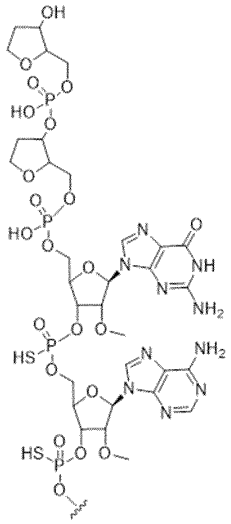
internucleoside linkage, and each first 5' and 3' penultimate nucleoside is attached to a respective 5' and 3' adjacent antepenultimate nucleoside by a phosphorothioate internucleoside linkage; and (iv) the second strand of the nucleic acid is conjugated directly or indirectly to one or more ligand moieties at the 5' terminal region of the second strand.

Examples of the structures are as follows (where the specific RNA nucleosides shown are not limiting and could be any RNA nucleoside):

A A 3'-3' reversed bond (and also showing the 5'-3 direction of the last phosphodiester bond between the two abasic molecules reading towards the terminus of the molecule)



B Illustrating a 5'-5' reversed bond (and also showing the 3'-5' direction of the last phosphodiester bond between the two abasic molecules reading towards the terminus of the molecule)



The abasic nucleoside or abasic nucleosides present in the nucleic acid are provided in the presence of a reversed internucleoside linkage or linkages, namely a 5'-5' or a 3'-3' reversed internucleoside linkage. A reversed linkage occurs as a result of a change of orientation of an adjacent nucleoside sugar, such that the sugar will have a 3' – 5' orientation as opposed to the conventional 5' – 3' orientation (with reference to the numbering of ring atoms on the nucleoside sugars). The abasic nucleoside or nucleosides as present in the nucleic acids of the invention preferably include such inverted nucleoside sugars.

In the case of a terminal nucleoside having an inverted orientation, then this will result in an "inverted" end configuration for the overall nucleic acid. Whilst certain structures drawn and referenced herein are represented using conventional 5' - 3' direction (with reference to the numbering of ring atoms on the nucleoside sugars), it will be appreciated that the presence of a terminal nucleoside having a change of orientation and a proximal 3'-3' reversed linkage, will result in a nucleic acid having an overall 5'- 5' end structure (i.e. the conventional 3' end nucleoside becomes a 5' end nucleoside). Alternatively, it will be appreciated that the presence of a terminal nucleoside having a change of orientation and a proximal 5'-5' reversed linkage will result in a nucleic acid with an overall 3'- 3' end structure.

The proximal 3'-3' or 5'-5' reversed linkage as herein described, may comprise the reversed linkage being directly adjacent / attached to a terminal nucleoside having an inverted orientation, such as a single terminal nucleoside having an inverted orientation. Alternatively, the proximal 3'-3' or 5'-5' reversed linkage as herein described, may comprise the reversed linkage being adjacent 2, or more than 2, nucleosides having an inverted orientation, such as 2, or more than 2, terminal region nucleosides having an inverted orientation, such as the terminal and penultimate nucleosides. In this way, the reversed linkage may be attached to a penultimate nucleoside having an inverted orientation. While a skilled addressee will appreciate that inverted orientations as described above can result in nucleic acid molecules having overall 3' - 3' or 5'-5' end structures as described herein, it will also be appreciated that with the presence of one or more additional reversed linkages and / or nucleosides having an inverted orientation, then the overall nucleic acid may have 3' - 5' end structures corresponding to the conventionally positioned 5' / 3' ends.

In one aspect the nucleic acid may have a 3'-3' reversed linkage, and the terminal sugar moiety may comprise a 5' OH rather than a 5' phosphate group at the 5' position of that terminal sugar.

A skilled person would therefore clearly understand that 5'-5', 3'-3' and 3'-5' (reading in the direction of that terminus) end variants of the more conventional 5'-3' structures (with reference

to the numbering of ring atoms on the end nucleoside sugars) drawn herein are included in the scope of the disclosure, where a reversed linkage or linkages is / are present.

In the situation of e.g. a reversed internucleoside linkage and / or one or more nucleosides having an inverted orientation creating an inverted end, and where the relative position of a linkage (e.g. to a linker) or the location of an internal feature (such as a modified nucleoside) is defined relative to the 5' or 3' end of the nucleic acid, then the 5' or 3' end is the conventional 5' or 3' end which would have existed had a reversed linkage not been in place, and wherein the conventional 5' or 3' end is determined by consideration of the directionality of the majority of the internal nucleoside linkages and / or nucleoside orientation within the nucleic acid. It is possible to tell from these internal bonds and / or nucleoside orientation which ends of the nucleic acid would constitute the conventional 5' and 3' ends (with reference to the numbering of ring atoms on the end nucleoside sugars) of the molecule absent the reversed linkage.

For example, in the structure shown below there are abasic residues in the first 2 positions located at the "5'" end. Where the terminal nucleoside has an inverted orientation then the "5'" end indicated in the diagram below, which is the conventional 5' end, can in fact comprise a 3' OH in view of the inverted nucleoside at the terminal position. Nevertheless the majority of the molecule will comprise conventional internucleoside linkages that run from the 3' OH of the sugar to the 5' phosphate of the next sugar, when reading in the standard 5' [PO4] to 3' [OH] direction of a nucleic acid molecule (with reference to the numbering of ring atoms on the nucleoside sugars), which can be used to determine the conventional 5' and 3' ends that would be found absent the inverted end configuration.

A 5' A-A-Me-Me-Me-Me-Me-Me-F-Me-F-F-F-Me-Me-Me-Me-Me-Me-Me-Me-Me 3'

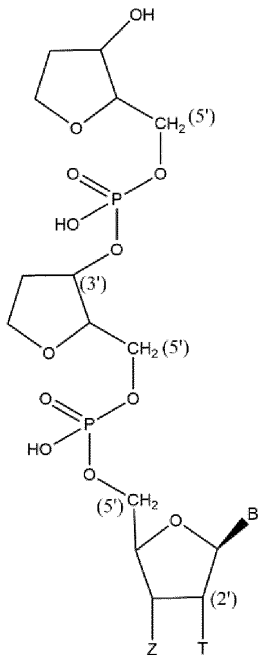
The reversed bond is preferably located at the end of the nucleic acid e.g. RNA which is distal to a ligand moiety, such as a GalNAc containing portion, of the molecule.

GalNAc-siRNA constructs with a 5'-GalNAc on the sense strand can have a reversed linkage on the opposite end of the sense strand.

GalNAc-siRNA constructs with a 3'-GalNAc on the sense strand can have a reversed linkage on the opposite end of the sense strand.

In some embodiments, the second (sense) strand of the nucleic acid according to the invention comprises 2 consecutive abasic nucleosides in the 5' terminal region as shown in the following 5' terminal motif

5' terminal motif




wherein:

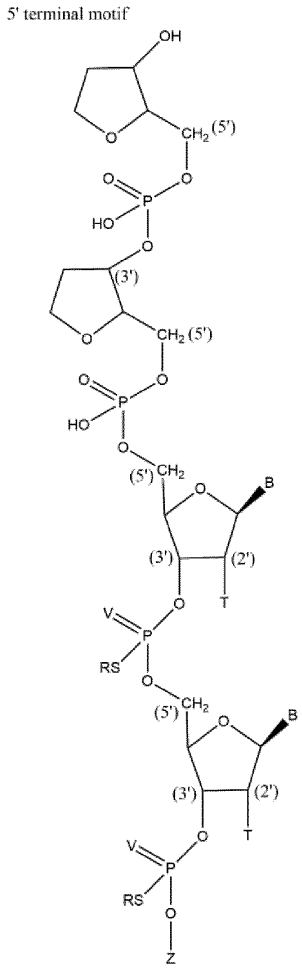
B represents a nucleoside base,

T represent H, OH or a 2' ribose modification,

Z represents the remaining nucleosides of said second strand.

In some embodiments, the second (sense) strand of the nucleic acid according to the invention comprises 2 consecutive abasic nucleosides in the 5' terminal region as shown in the following 5' terminal motif

5' terminal motif




wherein:

B represents a nucleoside base,

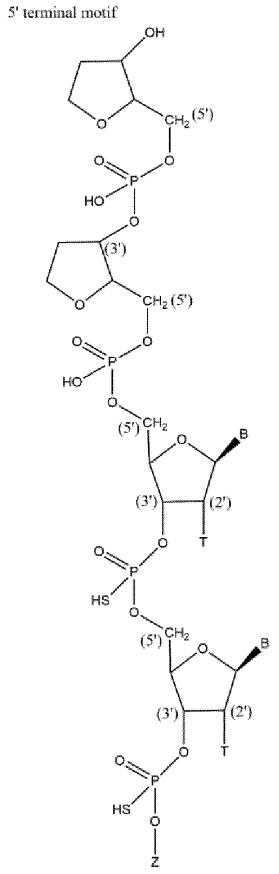
T represents H, OH or a 2' ribose modification (preferably a 2' ribose modification, more preferably a 2'Me or 2'F ribose modification),

V represents O or S (preferably O),

R represents H or $C_{1-4}$ alkyl (preferably H),

Z represents the remaining nucleosides of said second strand,

more preferably the following 5' terminal motif




wherein:

B represents a nucleoside base,

T represents a 2' ribose modification (preferably a 2'Me or 2'F ribose modification),

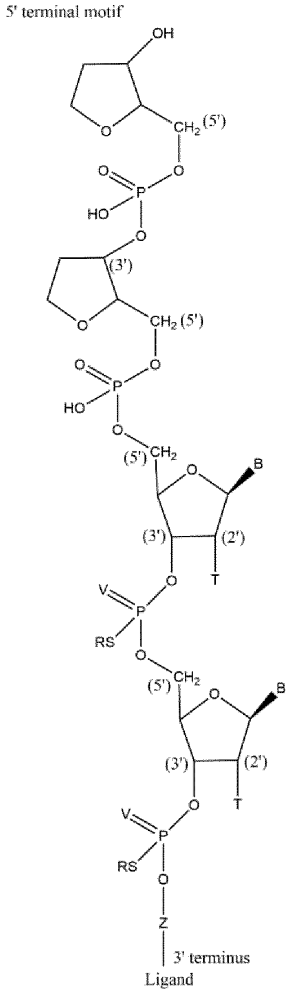
Z represents the remaining nucleosides of said second strand.

The reversed bond is preferably located at the end of the nucleic acid eg RNA which is distal to a ligand moiety, such as a GalNAc containing portion, of the molecule.

GalNAc-siRNA constructs with a 5'-GalNAc on the sense strand can have a reversed linkage on the opposite end of the sense strand.

GalNAc-siRNA constructs with a 3'-GalNAc on the sense strand can have a reversed linkage on the opposite end of the sense strand.

In a preferred embodiment, the second (sense) strand of the nucleic acid according to the invention comprises 2 consecutive abasic nucleosides in the 5' terminal region as shown in the following 5' terminal motif




wherein:

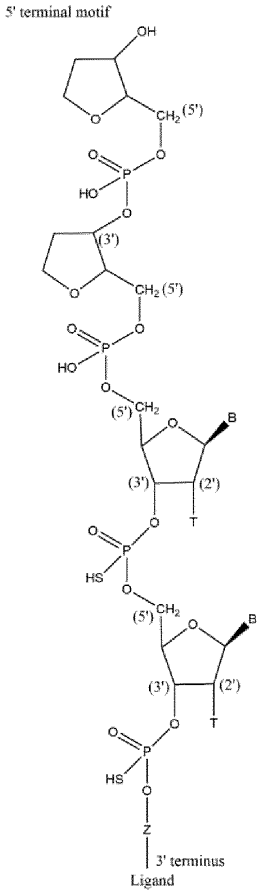
B represents a nucleoside base,

T represent H, OH or a 2' ribose modification (preferably a 2' ribose modification, more preferably a 2'Me or 2'F ribose modification),

V represent O or S (preferably O),

R represent H or $C_{1-4}$ alkyl (preferably H),

Z comprises 11 to 26 contiguous nucleosides, preferably 15 to 21 contiguous nucleosides, and more preferably 19 contiguous nucleosides, more preferably the following 5' terminal motif




wherein:

B represents a nucleoside base,

T represents a 2' ribose modification (preferably a 2'Me or 2'F ribose modification),

Z comprises 19 contiguous nucleosides.

## NUCLEIC ACID LENGTHS

In one aspect the i) the first strand of the nucleic acid has a length in the range of 17 to 30 nucleosides, preferably 19 to 25 nucleosides, more preferably 19 or 23 nucleosides; and / or ii) the second strand of the nucleic acid has a length in the range of 17 to 30 nucleosides, preferably 19 to 25 nucleosides, more preferably 19 or 21 nucleosides.

Typically, the duplex region of the nucleic acid is between 17 and 30 nucleosides in length, more preferably is 19 or 21 nucleosides in length. Similarly, the region of complementarity between the first strand and the portion of RNA transcribed from the CFHR4 gene is between 17 and 30 nucleosides in length.

Generally, the duplex structure of the nucleic acid e.g. an iRNA is about 15 to 30 base pairs in length, e.g., 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19- 23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

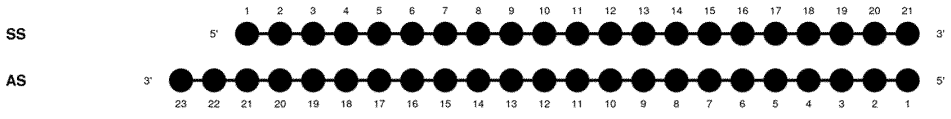
Similarly, the region of complementarity of an antisense sequence to a target sequence and/or the region of complementarity of an antisense sequence to a sense sequence is about 15 to 30 nucleosides in length, e.g., 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20- 24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleosides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

In certain preferred embodiments, the region of complementarity of an antisense sequence to a target sequence and/or the region of complementarity of an antisense sequence to a sense sequence is at least 17 nucleosides in length. For example, the region of complementarity between the antisense strand and the target is 19 to 21 nucleosides in length, for example, the region of complementarity is 21 nucleosides in length.

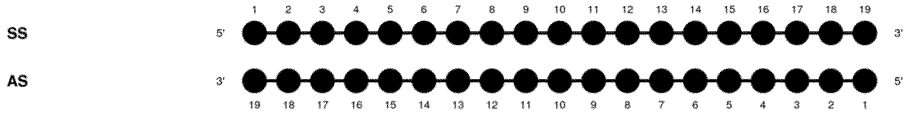
In preferred embodiments, each strand is no more than 30 nucleosides in length.

In certain preferred embodiments, the duplex structure of the nucleic acid e.g. an siRNA is 19 or 21 base pairs in length. In particularly preferred embodiment, the duplex may have one of the following structures:

e.g., ETX-M00001327 – ETX-M00001349 and ETX-M00001627 ETX-M00001742




or




A nucleic acid e.g. a dsRNA as described herein can further include one or more single-stranded nucleoside overhangs e.g., 1-4, 2-4, 1-3, 2-3, 1, 2, 3, or 4 nucleosides. A nucleoside overhang can comprise or consist of a nucleoside/nucleoside analog, including a deoxynucleoside/nucleoside. The overhang(s) can be on the sense strand, the antisense strand, or any combination thereof. Furthermore, the nucleoside(s) of an overhang can be present on the 5'-end, 3'- end, or both ends of an antisense or sense strand of a nucleic acid e.g. a dsRNA.

In certain preferred embodiments, at least one strand comprises a 3' overhang of at least 1 nucleoside, e.g. , at least one strand comprises a 3' overhang of at least 2 nucleosides. The overhang is suitably on the antisense/ guide strand and/or the sense / passenger strand.

**NUCLEIC ACID MODIFICATIONS**

In certain embodiments, the nucleic acid e.g. an RNA of the invention e.g., a dsiRNA, does not comprise further modifications, e.g., chemical modifications or conjugations known in the art and described herein.

In other preferred embodiments, the nucleic acid e.g. RNA of the invention, e.g., a dsiRNA, is further chemically modified to enhance stability or other beneficial characteristics.

In certain embodiments of the invention, substantially all of the nucleosides are modified.

The nucleic acids featured in the invention can be synthesized or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference.

Modifications include, for example, end modifications, e.g., 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3 '-end modifications (conjugation, DNA nucleosides within an RNA, or RNA nucleosides within a DNA, inverted linkages, etc.); base modifications, e.g., replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, conjugated bases; sugar modifications (e.g. , at the 2'-position or 4'- position) or replacement of the sugar; or backbone modifications, including modification or replacement of the phosphodiester linkages.

Specific examples of nucleic acids such as siRNA compounds useful in the embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. Nucleic acids such as RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified nucleic acids e.g. RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified nucleic acid e.g. an siRNA will have a phosphorus atom in its internucleoside backbone.

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

Modified nucleic acids e.g. RNAs can also contain one or more substituted sugar moieties. The nucleic acids e.g. siRNAs, e.g., dsiRNAs, featured herein can include one of the following at the 2'-position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted. 2' O- methyl and 2'-F are preferred modifications.

In certain preferred embodiments, the nucleic acid comprises at least one modified nucleoside.

The nucleic acid of the invention may comprise one or more modified nucleosides on the first strand and/or the second strand.

In some embodiments, substantially all of the nucleosides of the sense strand and all of the nucleosides of the antisense strand comprise a modification.

In some embodiments, all of the nucleosides of the sense strand and substantially all of the nucleosides of the antisense strand comprise a modification.

In some embodiments, all of the nucleosides of the sense strand and all of the nucleosides of the antisense strand comprise a modification.

In one embodiment, at least one of the modified nucleosides is selected from the group consisting of a deoxy- nucleoside, a 3 '-terminal deoxy-thymine (dT) nucleoside, a 2'-O-methyl modified nucleoside (also called herein 2'-Me, where Me is a methoxy) , a 2'-fluoro modified nucleoside, a 2'-deoxy- modified nucleoside, a locked nucleoside, an unlocked nucleoside, a conformationally restricted nucleoside, a constrained ethyl nucleoside, an abasic nucleoside, a 2' -amino- modified nucleoside, a 2'-O-allyl- modified nucleoside, 2' -C-alkyl- modified nucleoside, 2'-hydroxly-modified nucleoside, a 2'- methoxyethyl modified nucleoside, a 2'-O-alkyl-modified nucleoside, a morpholino nucleoside, a phosphoramidate, a non-natural base comprising nucleoside, a tetrahydropyran modified nucleoside, a 1 ,5-anhydrohexitol modified nucleoside, a cyclohexenyl modified nucleoside, a nucleoside comprising a phosphorothioate group, a nucleoside comprising a methylphosphonate group, a nucleoside comprising a 5 '-phosphate, and a nucleoside comprising a 5 '-phosphate mimic. In another embodiment, the modified nucleosides comprise a short sequence of 3 '-terminal deoxy-thymine nucleosides (dT).

Modifications on the nucleosides may preferably be selected from the group including, but not limited to, LNA, HNA, CeNA, 2 -methoxyethyl, 2'-O-alkyl, 2-O-allyl, 2'-C-allyl, 2'-fluoro, 2'-deoxy, 2'-hydroxyl, and combinations thereof. In another embodiment, the modifications on the nucleosides are 2 -O-methyl ("2-Me") or 2'-fluoro modifications.

One preferred modification is a modification at the 2'-OH group of the ribose sugar, optionally selected from 2'-Me or 2'-F modifications.

In certain embodiments, the nucleic acid e.g. RNAi agent further comprises at least one phosphorothioate or methylphosphonate internucleoside linkage. For example the phosphorothioate or methylphosphonate internucleoside linkage can be at the 3 '-terminus or in the terminal region of one strand, i.e. , the sense strand or the antisense strand; or at the ends of both strands, the sense strand and the antisense strand.

In certain embodiments, the phosphorothioate or methylphosphonate internucleoside linkage is at the 5 'terminus or in the terminal region of one strand, i.e. , the sense strand or the antisense strand; or at the ends of both strands, the sense strand and the antisense strand.

In certain embodiments, a phosphorothioate or a methylphosphonate internucleoside linkage is at both the 5'- and 3 '-terminus or in the terminal region of one strand, i.e. , the sense strand or the antisense strand; or at the ends of both strands, the sense strand and the antisense strand.

Any nucleic acid may comprise one or more phosphorothioate (PS) modifications within the nucleic acid, such as at least two PS internucleoside bonds at the ends of a strand.

At least one of the oligoribonucleoside strands preferably comprises at least two consecutive phosphorothioate modifications in the last 3 nucleosides of the oligonucleoside.

The invention therefore also relates to: A nucleic acid disclosed herein which comprises phosphorothioate internucleoside linkages respectively between at least two or three consecutive positions, such as in a 5' and/or 3' terminal region and/or near terminal region of the second strand, whereby said near terminal region is preferably adjacent said terminal region wherein said one or more abasic nucleosides of said second strand is / are located.

A nucleic acid disclosed herein which comprises phosphorothioate internucleoside linkages respectively between at least two or three consecutive positions in a 5' and / or 3' terminal region of the first strand, whereby preferably the terminal position at the 5' and / or 3' terminal region of said first strand is attached to its adjacent position by a phosphorothioate internucleoside linkage.

The nucleic acid strand may be an RNA comprising a phosphorothioate internucleoside linkage between the three nucleosides contiguous with 2 terminally located abasic nucleosides.

A preferred nucleic acid is a double stranded RNA comprising 2 adjacent abasic nucleosides at the 5' terminus of the second strand and a ligand moiety comprising one or more GalNAc ligand moieties at the opposite 3' end of the second strand. Further preferred, the same nucleic acid may also comprise a phosphorothioate bond between nucelotides at positions 3-4 and 4-5 of the second strand, reading from the position 1 of the second strand.

Position 1 of the first or the second strand is the nucleoside which is the closest to the end of the nucleic acid (ignoring any abasic nucleosides) and that is joined to an adjacent nucleoside (at Position 2) via a 3' to 5' internal bond, with reference to the bonds between the sugar moieties of the backbone, and reading in a direction away from that end of the molecule.

It can therefore be seen that "position 1 of the sense strand" is the 5' most nucleoside (not including abasic nucleosides) at the conventional 5' end of the sense strand. Typically, the nucleoside at this position 1 of the sense strand will be equivalent to the 5' nucleoside of the selected target nucleic acid sequence, and more generally the sense strand will have equivalent nucleosides to those of the target nucleic acid sequence starting from this position 1 of the sense strand, whilst also allowing for acceptable mismatches between the sequences.

As used herein, "position 1 of the antisense strand" is the 5' most nucleoside (not including abasic nucleosides) at the conventional 5' end of the antisense strand. As hereinbefore

described, there will be a region of complementarity between the sense and antisense strands, and in this way the antisense strand will also have a region of complementarity to the target nucleic acid sequence as referred to above.

Preferred modifications that can be used with sequences according to the present invention can be as follows:

Modification 1:

First strand modification:
NmsNfsNmNfNmNfNmNfNfNmNmNmNmNfNmNfNmNmNmNmNmsNmsNm (5' to 3')

Second strand modification:
iaiaNmsNmsNmNmNmNmNfNfNfNfNfNmNmNmNmNmNmNmNfNmNm (5' to 3')

Modification 2:

First strand modification:
NmsNfsNmNfNmNfNmNfNfNmNmNmNmNfNmNfNmNmNmNmNmsNmsNm (5' to 3')

Second strand modification:
iaiaNmsNmsNmNmNmNfNfNmNfNfNfNfNmNmNmNmNmNmNmNmNm (5' to 3')

Modification 3:

First strand modification:
NmsNfsNmNfNmNfNmNfNfNmNmNmNmNfNmNfNmNmNmNmNmsNmsNm (5' to 3')

Second strand modification:
iaiaNmsNmsNmNmNmNmNfNmNfNfNfNfNmNmNmNmNmNmNmNmNm (5' to 3')

Modification 4:

First strand modification:
NmsNfsNmNfNmNfNmNmNmNmNmNmNmNfNmNfNmNmNmNmNmsNmsNm (5' to 3')

Second strand modification:
iaiaNmsNmsNmNmNmNmNfNmNfNfNfNfNmNmNmNmNmNmNmNmNm (5' to 3')

Modification 5:

First strand modification:

NmsNfsNmNmNmNfNmNmNfNmNmNmNmNfNmNfNmNmNmNmNmsNmsNm (5’ to 3’)

Second strand modification:

iaiaNmsNmsNmNmNmNmNmNmNfNfNfNmNmNmNmNmNmNmNmNmNm (5’ to 3’)

Modification 6:

First strand modification:

NmsNfsNmNmNmNfNmNmNmNmNmNmNmNfNmNfNmNfNmNmNmsNmsNm (5’ to 3’)

Second strand modification:

iaiaNmsNmsNmNmNmNmNmNmNfNfNfNmNmNmNmNmNmNmNmNmNm (5’ to 3’)

Modification 7:

First strand modification:

NmsNfsNmNmNmNyNmNmNmNmNmNmNmNfNmNfNmNmNmNmNmsNmsNm (5’ to 3’)

Second strand modification:

iaiaNmsNmsNmNmNmNmNmNmNfNfNfNmNmNmNmNmNmNmNmNmNm (5’ to 3’)

Modification 8:

First strand modification:

NmsNfsNmNmNmNyNmNfNfNmNmNmNmNfNmNfNmNmNmNmNmsNmsNm (5’ to 3’)

Second strand modification:

iaiaNmsNmsNmNmNmNmNmNmNfNfNfNmNmNmNmNmNmNmNmNmNm (5’ to 3’)

Modification 9:

First strand modification:

NmsNfsNmNmNmNfNmNmNmNmNmNmNmNfNmNfNmNmNmNfNmsNmsNm (5’ to 3’)

Second strand modification:

iaiaNmsNmsNmNmNmNmNmNmNfNfNfNmNmNmNmNmNmNmNmNmNm (5’ to 3’)

Modification 10:

First strand modification:

NmsNfsNmNfNmNfNmNmNmNmNmNmNfNmNfNmNmNmNmNmsNmsNm (5' to 3')

Second strand modification:

iaiaNmsNmsNmNmNmNmNmNmNfNfNfNmNmNmNmNmNmNmNmNmNm (5' to 3')

Modification 11:

First strand modification:

NmsNfsNmNfNmNfNmNfNfNmNmNmNmNfNmNfNmNmNmNmNmsNmsNm (5' to 3')

Second strand modification:

iaiaNmsNmsNmNmNmNmNmNmNfNfNfNmNmNmNmNmNmNmNmNmNm (5' to 3')

Modification 12:

First strand modification:

NmsNfsNmNmNmNfNmNfNfNmNmNmNmNfNmNfNmNfNmNmNmsNmsNm (5' to 3')

Second strand modification:

iaiaNmsNmsNmNmNmNmNmNmNfNfNfNmNmNmNmNmNmNmNmNmNm (5' to 3')

Modification 13:

First strand modification:

NmsNfsNmNmNmNfNmNfNfNmNmNmNmNfNmNfNmNmNmNfNmsNmsNm (5' to 3')

Second strand modification:

iaiaNmsNmsNmNmNmNmNmNmNfNfNfNmNmNmNmNmNmNmNmNmNm (5' to 3')

wherein in each of the above modifications:

ia represents an inverted abasic nucleoside;

Nm represents a 2'Me ribose modified nucleoside;

Nf represents a 2'F ribose modified nucleoside;

Ny represents a nucleoside with a thermally destabilizing modification, preferably wherein the destabilizing modification is selected from a modified unlocked nucleic acid (UNA) and a glycol

nucleic acid (GNA), more preferably a glycol nucleic acid, most preferably an (S)-glycol nucleic acid;

s represents a phosphorothioate internucleoside bond.

In a preferred embodiment, the nucleic acid according to the invention has the following modification pattern:

Modification 6:

First strand modification:
NmsNfsNmNmNmNfNmNmNmNmNmNmNmNfNmNfNmNfNmNmNmsNmsNm (5' to 3')

Second strand modification:
iaiaNmsNmsNmNmNmNmNmNmNfNfNfNmNmNmNmNmNmNmNmNmNm (5' to 3')

**CONJUGATION OF NUCLEIC ACID TO LIGAND**

Another modification of a nucleic acid e.g. RNA e.g. an siRNA of the invention involves linking the nucleic acid e.g. the siRNA to one or more ligand moieties e.g. to enhance the activity, cellular distribution, or cellular uptake of the nucleic acid e.g. siRNA e.g., into a cell.

In certain embodiments, the inhibitor according to the invention is conjugated to a ligand moiety that enables and/or facilitates targeting of hepatocytes. In certain embodiments, targeting of hepatocytes is achieved using N-acetylgalactosamine (GalNAc) conjugates as described in more detail herein below. That is, in certain embodiments, the inhibitor according to the invention is an siRNA-GalNAc conjugate.

In some embodiments, the ligand moiety described can be attached to a nucleic acid e.g. an siRNA oligonucleoside, via a linker that can be cleavable or non-cleavable. The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, e.g., covalently attaches two parts of a compound.

The ligand can be attached to the 3' or 5' end of the sense strand.

The ligand is preferably conjugated to 3' end of the sense strand of the nucleic acid e.g. an siRNA agent.

The invention therefore relates in a further aspect to a conjugate for inhibiting expression of a target e.g. a target gene, in a cell, said conjugate comprising a nucleic acid portion and one or more ligand moieties, said nucleic acid portion comprising a nucleic acid as disclosed herein.

In one aspect the second strand of the nucleic acid is conjugated directly or indirectly (e.g. via a linker) to the one or more ligand moiety(s), wherein said ligand moiety is typically present at a terminal region of the second strand, preferably at the 3' terminal region thereof.

In certain embodiments, the ligand moiety comprises a GalNAc or GalNAc derivative attached to the nucleic acid e.g. dsiRNA through a linker.

Therefore, the invention relates to a conjugate wherein the ligand moiety comprises

i) one or more GalNAc ligands; and/or

ii) one or more GalNAc ligand derivatives; and/or

iii) one or more GalNAc ligands conjugated to said nucleic acid through a linker.

Said GalNAc ligand may be conjugated directly or indirectly to the 5' or 3' terminal region of the second strand of the nucleic acid, preferably at the 3' terminal region thereof.

GalNAc ligands are well known in the art and described in, inter alia, EP3775207A1.

In some embodiments, the ligand moiety comprises one or more ligands.

In some embodiments, the ligand moiety comprises one or more carbohydrate ligands.

In some embodiments, the one or more carbohydrates can be a monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide and / or polysaccharide.

In some embodiments, the one or more carbohydrates comprise one or more galactose moieties, one or more lactose moieties, one or more N-AcetylGalactosamine moieties, and / or one or more mannose moieties.

In some embodiments, the one or more carbohydrates comprise one or more N-Acetyl-Galactosamine moieties.

In some embodiments, the compounds as described anywhere herein comprise two or three N-AcetylGalactosamine moieties.

In some embodiments, the one or more ligands are attached in a linear configuration, or in a branched configuration, for example each configuration being respectively attached to a branch point in an overall linker.

Exemplary linear configurations and Exemplary branched configurations are shown in Figures 1a and 1b:

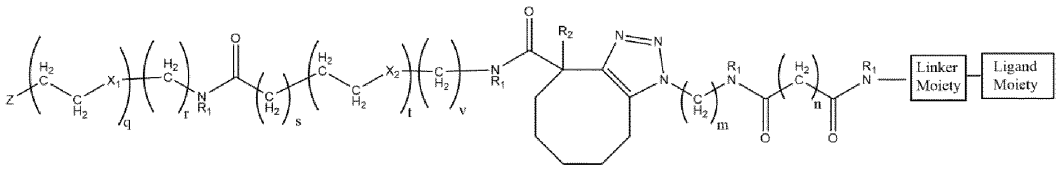
In Fig 1a, (linear), (a) and / or (b) can typically represent connecting bonds or groups, such as phosphate or phosphorothioate groups.

In Fig 1b, (branched), in some embodiments, the one or more ligands are attached as a biantennary or triantennary branched configuration. Typically, a triantennary branched configuration can be preferred, such as an N-AcetylGalactosamine triantennary branched configuration.

Linker

Exemplary compounds of the invention comprise a 'linker moiety', such as that as depicted in Formula (I), that is part of an overall 'linker'.

Formula I




wherein:

$R_1$ at each occurrence is independently selected from the group consisting of hydrogen, methyl and ethyl;

$R_2$ is selected from the group consisting of hydrogen, hydroxy, -$OC_{1-3}$alkyl, -$C(=O)OC_{1-3}$alkyl, halo and nitro;

$X_1$ and $X_2$ at each occurrence are independently selected from the group consisting of methylene, oxygen and sulfur;

m is an integer of from 1 to 6;

n is an integer of from 1 to 10;

q, r, s, t, v are independently integers from 0 to 4, with the proviso that:

(i) q and r cannot both be 0 at the same time; and

(ii) s, t and v cannot all be 0 at the same time;

Z is an oligonucleoside moiety.

As will be further understood in the art, exemplary compounds of the invention comprise an overall linker that is located between the oligonucleoside moiety and the ligand moiety of these compounds. The overall linker, thereby 'links' the oligonucleoside moiety and the ligand moiety to each other.

The overall linker is often notionally envisaged as comprising one or more linker building blocks. For example, there is a linker portion that is depicted as the 'linker moiety' as represented in Formula (I) positioned adjacent the ligand moiety and attaching the ligand moiety, typically via a branch point, directly or indirectly to the oligonucleoside moiety. The linker moiety as depicted in Formula (I) can also often be referred to as the 'ligand arm or arms' of the overall linker. There can also, but not always, be a further linker portion between the oligonucleoside moiety and the branch point, that is often referred to as the 'tether moiety' of the overall linker, 'tethering' the oligonucleoside moiety to the remainder of the conjugated compound. Such 'ligand arms' and / or 'linker moieties' and / or 'tether moieties' can be envisaged by reference to the linear and / or branched configurations as set out above.

As can be seen from the claims, and the reminder of the patent specification, the scope of the present invention extends to linear or branched configurations, and with no limitation as to the number of individual ligands that might be present. Furthermore, the addressee will also be aware that there are many structures that could be used as the linker moiety, based on the state of the art and the expertise of an oligonucleoside chemist.

The remainder of the overall linker (other than the linker moiety) as set out in the claims, and the remainder of the patent specification, is shown by its chemical constituents in Formula (I), which the inventors consider to be particularly unique to the current invention. In more general terms, however, these chemical constituents could be described as a 'tether moiety' as hereinbefore described, wherein the 'tether moiety' is that portion of the overall linker which comprises the group of atoms between Z, namely the oligonucleoside moiety, and the linker moiety as depicted in Formula (I).

*Tether moiety of Formula I*

In relation to Formula (I), the 'tether moiety' comprises the group of atoms between Z, namely the oligonucleoside moiety, and the linker moiety.

In some embodiments, $R_1$ is hydrogen at each occurrence. In some embodiments, $R_1$ is methyl. In some embodiments, $R_1$ is ethyl.

In some embodiments, $R_2$ is hydroxy. In some embodiments, $R_2$ is halo. In some embodiments, $R_2$ is fluoro. In some embodiments, $R_2$ is chloro. In some embodiments, $R_2$ is bromo. In some embodiments, $R_2$ is iodo. In some embodiments, $R_2$ is nitro.

In some embodiments, $X_1$ is methylene. In some embodiments, $X_1$ is oxygen. In some embodiments, $X_1$ is sulfur.

In some embodiments, $X_2$ is methylene. In some embodiments, $X_2$ is oxygen. In some embodiments, $X_2$ is sulfur.

In some embodiments, m = 3.

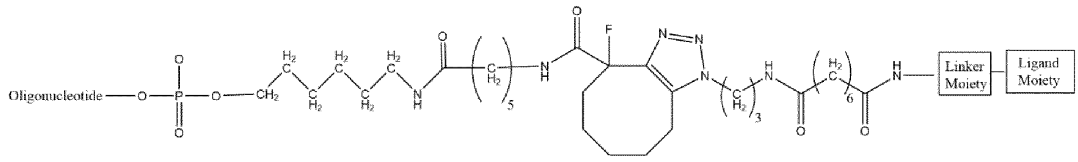
In some embodiments, n = 6.

In some embodiments, $X_1$ is oxygen and $X_2$ is methylene. In some embodiments, both $X_1$ and $X_2$ are methylene.

In some embodiments, q = 1, r = 2, s = 1, t = 1, v = 1. In some embodiments, q = 1, r = 3, s = 1, t = 1, v = 1.

In some embodiments, $R_1$ is hydrogen at each occurrence, n = 6, m = 3, $R_2$ is fluoro, $X_2$ is methylene, v = 1, t = 1, s = 1, $X_1$ is methylene, q = 1 and r = 2.

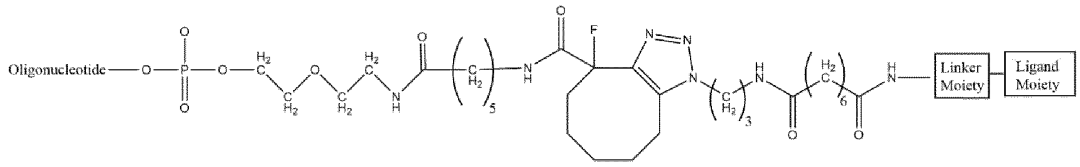
Thus, in some embodiments, exemplary compounds of the invention comprise the following structure:




Formula (IV)

In some embodiments, $R_1$ is hydrogen at each occurrence, n = 6, m = 3, $R_2$ is fluoro, $X_2$ is methylene, v = 1, t = 1, s = 1, $X_1$ is oxygen, q = 1 and r = 2.

Thus, in some embodiments, exemplary compounds of the invention comprise the following structure:




Formula (II)

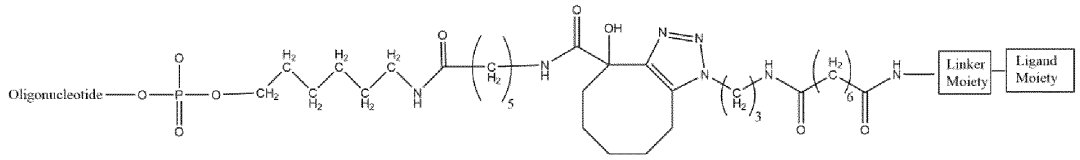
*Alternative tether moieties*

During the synthesis of compounds of the present invention, alternative tether moiety structures may arise. In some embodiments, alternative tether moieties have a change of one or more atoms in the tether moiety of the overall linker compared to tether moieties described anywhere herein.

In some embodiments, the alternative tether moiety is a compound of Formula (I) as described anywhere herein, wherein $R_2$ is hydroxy.

In some embodiments, $R_1$ is hydrogen at each occurrence, n = 6, m = 3, $R_2$ is hydroxy, $X_2$ is methylene, v = 1, t = 1, s = 1, $X_1$ is methylene, q = 1 and r = 2.

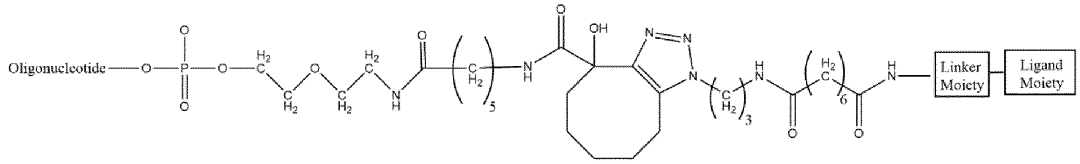
Thus, in some embodiments, compounds of the invention comprise the following structure:




Formula (V)

In some embodiments, $R_1$ is hydrogen at each occurrence, n = 6, m = 3, $R_2$ is hydroxy, $X_2$ is methylene, v = 1, t = 1, s = 1, $X_1$ is oxygen, q = 1 and r = 2.

Thus, in some embodiments, compounds of the invention comprise the following structure:

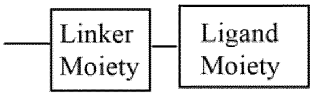



Formula (III)

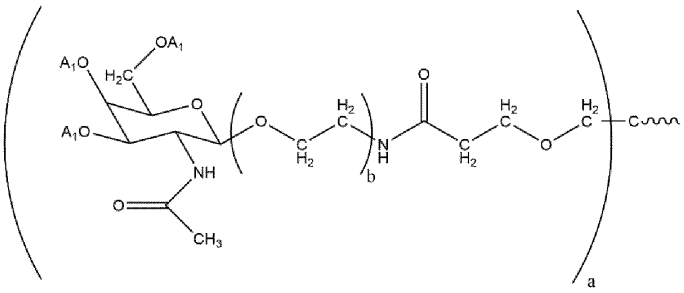
*Linker moiety*

In relation to Formula (I), the 'linker moiety' as depicted in Formula (I) comprises the group of atoms located between the tether moiety as described anywhere herein, and the ligand moiety as described anywhere herein.

In some embodiments:




as depicted in Formula (I) as described anywhere herein is any of Formulae (VIa), (VIb) or (VIc), preferably Formula (VIa):



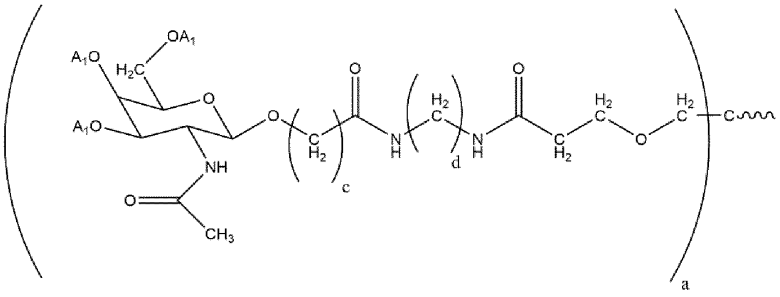
Formula (VIa)

wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

a is an integer of 2 or 3; and

b is an integer of 2 to 5; or



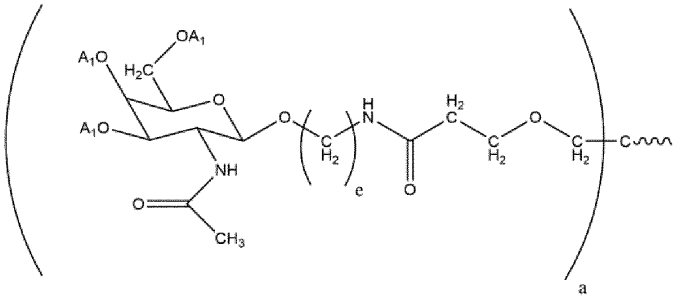
Formula (VIb)

wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

a is an integer of 2 or 3; and

c and d are independently integers of 1 to 6; or



Formula (VIc)

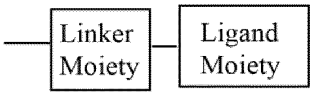
wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

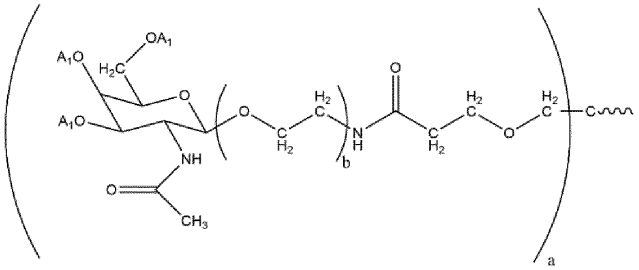
a is an integer of 2 or 3; and

e is an integer of 2 to 10.

In some embodiments, the moiety:




as depicted in Formula (I) is Formula (VIa):



Formula (VIa)

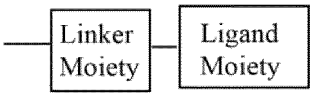
wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

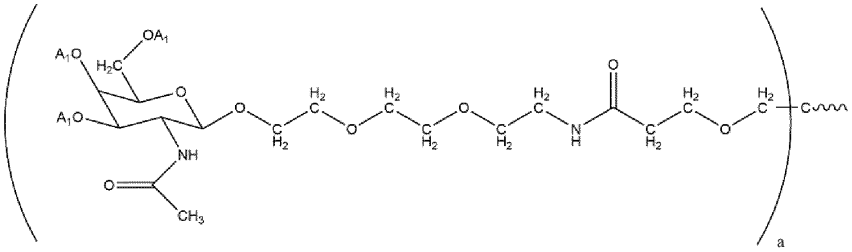
a is 3; and

b is an integer of 3.

In some embodiments, the moiety:




as depicted in Formula (I) as described anywhere herein is Formula (VII):



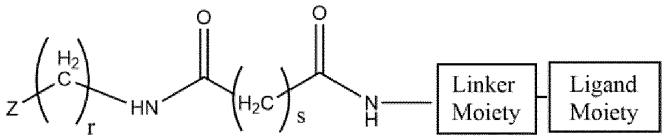
Formula (VII)

wherein:

$A_I$ is hydrogen;

a is an integer of 2 or 3, preferably 3.

Other exemplary compounds of the invention comprise a 'linker moiety', as depicted in Formula (I*), that is part of an overall 'linker'.




Formula I*

Where:

r and s are independently an integer selected from 1 to 16; and

Z is an oligonucleoside moiety.

As will be further understood in the art, exemplary compounds of the invention comprise an overall linker that is located between the oligonucleoside moiety and the ligand moiety of these compounds. The overall linker, thereby 'links' the oligonucleoside moiety and the ligand moiety to each other.

The overall linker is often notionally envisaged as comprising one or more linker building blocks. For example, there is a linker portion that is depicted as the 'linker moiety' as represented in Formula (I*) positioned adjacent the ligand moiety and attaching the ligand moiety, typically via a branch point, directly or indirectly to the oligonucleoside moiety. The linker moiety as depicted in Formula (I*) can also often be referred to as the 'ligand arm or arms' of the overall linker. There can also, but not always, be a further linker portion between the oligonucleoside moiety and the branch point, that is often referred to as the 'tether moiety' of the overall linker, 'tethering' the oligonucleoside moiety to the remainder of the conjugated compound. Such 'ligand arms' and / or 'linker moieties' and / or 'tether moieties' can be envisaged by reference to the linear and / or branched configurations as set out above.

As can be seen from the claims, and the reminder of the patent specification, the scope of the present invention extends to linear or branched configurations, and with no limitation as to the number of individual ligands that might be present. Furthermore, the addressee will also be aware that there are many structures that could be used as the linker moiety, based on the state of the art and the expertise of an oligonucleoside chemist.

The remainder of the overall linker (other than the linker moiety) as set out in the claims, and the remainder of the patent specification, is shown by its chemical constituents in Formula (I), which the inventors consider to be particularly unique to the current invention. In more general terms, however, these chemical constituents could be described as a 'tether moiety' as hereinbefore described, wherein the 'tether moiety' is that portion of the overall linker which comprises the

group of atoms between Z, namely the oligonucleoside moiety, and the linker moiety as depicted in Formula (I).

*Tether moiety*

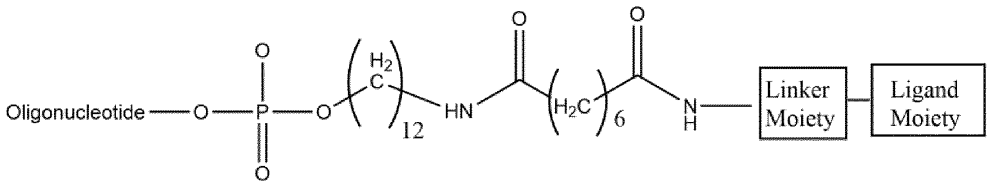
In relation to Formula (I*), the 'tether moiety' comprises the group of atoms between Z, namely the oligonucleoside moiety, and the linker moiety.

In some embodiments, s is an integer selected from 4 to 12. In some embodiments, s is 6.

In some embodiments, r is an integer selected from 4 to 14. In some embodiments, r is 6. In some embodiments, r is 12.

In some embodiments, r is 12 and s is 6.

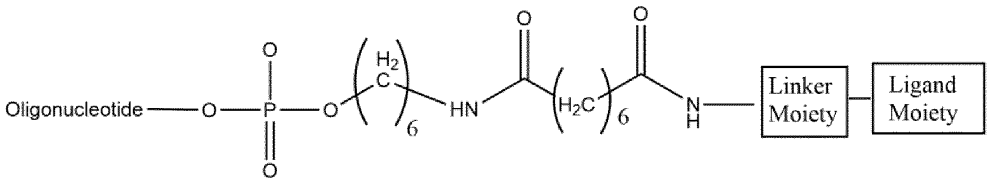
Thus, in some embodiments, exemplary compounds of the invention comprise the following structure:




Formula (II*)

In some embodiments, r is 6 and s is 6.

Thus, in some embodiments, exemplary compounds of the invention comprise the following structure:

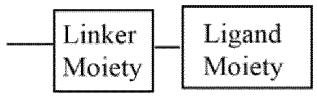



Formula (III*)

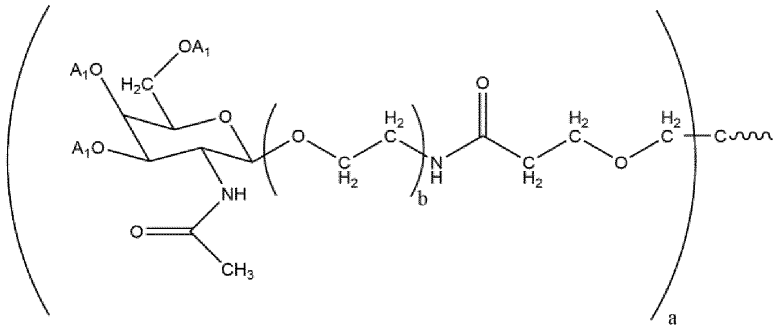
*Linker moiety*

In relation to Formula (I*), the 'linker moiety' as depicted in Formula (I) comprises the group of atoms located between the tether moiety as described anywhere herein, and the ligand moiety as described anywhere herein.

In some embodiments, the moiety:




as depicted in Formula (I*) as described anywhere herein is any of Formulae (IV*), (V*) or (VI*), preferably Formula (IV*):



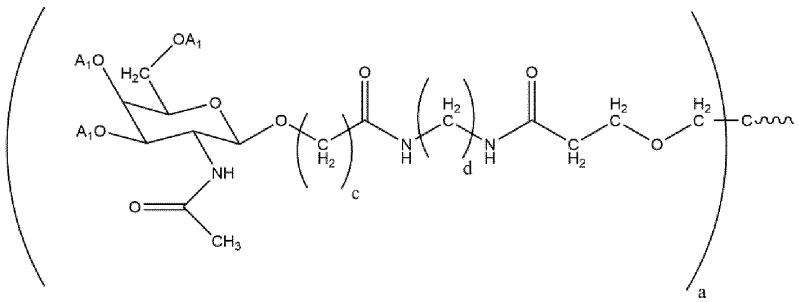
Formula (IV*)

wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

a is an integer of 2 or 3; and

b is an integer of 2 to 5; or



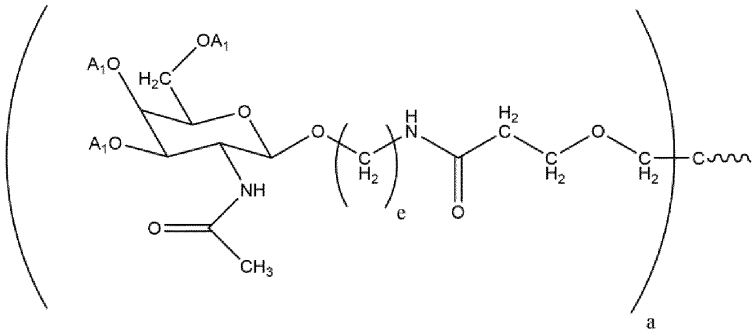
Formula (V*)

wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

a is an integer of 2 or 3; and

c and d are independently integers of 1 to 6; or



Formula (VI*)

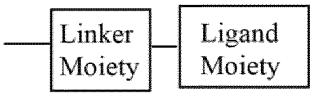
wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

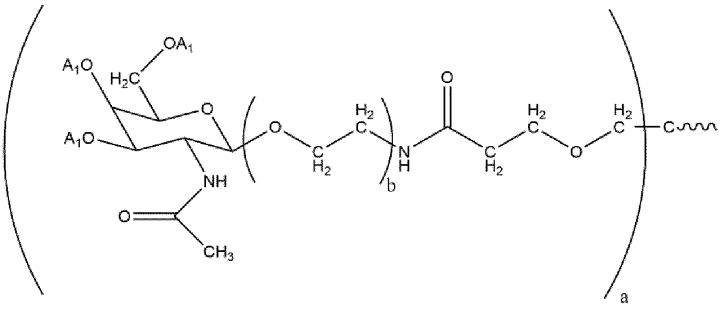
a is an integer of 2 or 3; and

e is an integer of 2 to 10.

In some embodiments, the moiety:




as depicted in Formula (I) is Formula (VIa*):



Formula (VIa*)

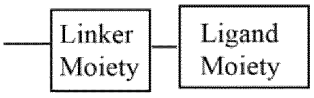
wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

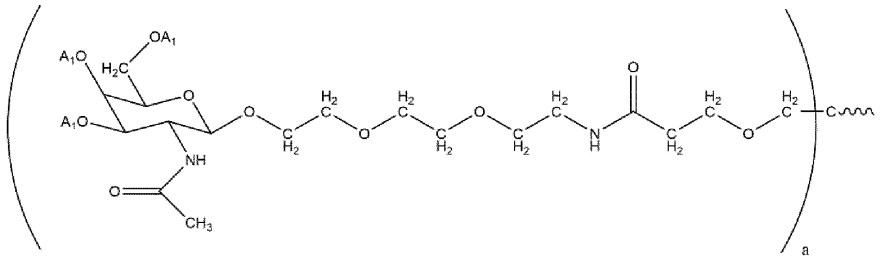
a is 3; and

b is an integer of 3.

In some embodiments, the moiety:




as depicted in Formula (I) as described anywhere herein is Formula (VII*):




Formula (VII*)

wherein:

$A_I$ is hydrogen;

a is an integer of 2 or 3.

In some embodiments, a = 2. In some embodiments, a = 3. In some embodiments, b = 3.

In some embodiments, the GalNAc ligand is comprised in any one of the linkers shown in Figures 2 to 5 or Figure 6 (Formula XI), wherein the "oligonucleotide" may be any nucleic acid disclosed herein. Accordingly, the "oligonucleotide" may comprise other bonds than a phosphodiester bond, such as one or more phosphorothioate bonds. Preferably, the nucleic acid according to the invention is a double stranded oligonucleoside as defined herein and the linker is conjugated to the second strand, more preferably to the 3' terminal region of the second strand, via a phosphodiester bond.

In some embodiments, the GalNAc ligand is comprised in the linker shown in Figure 4, wherein the "oligonucleotide" may be any nucleic acid disclosed herein. Accordingly, the "oligonucleotide" may comprise other bonds than a phosphodiester bond, such as one or more phosphorothioate bonds. Preferably, the nucleic acid according to the invention is a double stranded oligonucleoside as defined herein and the linker is conjugated to the second strand, more preferably to the 3' terminal region of the second strand, via a phosphodiester bond.

In some embodiments, the GalNAc ligand is comprised in the linker shown in Figure 6 (Formula XI), wherein the "oligonucleotide" may be any nucleic acid disclosed herein. Accordingly, the "oligonucleotide" may comprise other bonds than a phosphodiester bond, such as one or more phosphorothioate bonds. Preferably, the nucleic acid according to the invention is a double stranded oligonucleoside as defined herein and the linker is conjugated to the second strand, more preferably to the 3' terminal region of the second strand, via a phosphodiester bond.

In some embodiments, the GalNAc ligand is comprised in any one of the linkers shown in Figures 2 to 5 or Figure 6 (Formula XI), wherein the "oligonucleotide" represents a nucleic acid according to the invention, wherein the nucleic acid according to the invention comprises a modified or unmodified second strand comprising or consisting of any one of SEQ ID NO:267 to SEQ ID NO:399, preferably wherein the linker is conjugated to the 3' terminal region of the second strand, i.e., to the 3' terminal region of any one of SEQ ID NO:267 to SEQ ID NO:399, via a phosphodiester bond.

In some embodiments, the GalNAc ligand is comprised in the linker shown in Figure 4, wherein the "oligonucleotide" represents a nucleic acid according to the invention, wherein the nucleic acid according to the invention comprises a modified or unmodified second strand comprising or consisting of any one of SEQ ID NO:267 to SEQ ID NO:399, preferably wherein the linker is conjugated to the 3' terminal region of the second strand, i.e., to the 3' terminal region of any one of SEQ ID NO:267 to SEQ ID NO:399, via a phosphodiester bond.

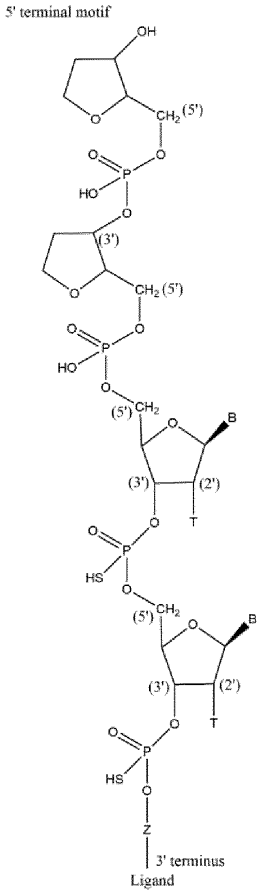
In some embodiments, the GalNAc ligand is comprised in the linker shown in Figure 6 (Formula XI), wherein the "oligonucleotide" represents a nucleic acid according to the invention, wherein the nucleic acid according to the invention comprises a modified or unmodified second strand comprising or consisting of any one of SEQ ID NO:267 to SEQ ID NO:399, preferably wherein the linker is conjugated to the 3' terminal region of the second strand, i.e., to the 3' terminal region of any one SEQ ID NO:267 to SEQ ID NO:399, via a phosphodiester bond.

In some embodiments, the GalNAc ligand is comprised in any one of the linkers shown in Figures 2 to 5 or Figure 6 (Formula XI), wherein the "oligonucleotide" represents a nucleic acid according to the invention, wherein the nucleic acid according to the invention comprises a modified second strand comprising or consisting of any one of SEQ ID NO:533 to SEQ ID NO:665, preferably wherein the linker is conjugated to the 3' terminal region of the second strand, i.e., to the 3' terminal region of any one of SEQ ID NO:533 to SEQ ID NO:665, via a phosphodiester bond.

In some embodiments, the GalNAc ligand is comprised in the linker shown in Figure 4, wherein the "oligonucleotide" represents a nucleic acid according to the invention, wherein the nucleic acid according to the invention comprises a modified second strand comprising or consisting of any one of SEQ ID NO:533 to SEQ ID NO:665, preferably wherein the linker is conjugated to the 3' terminal region of the second strand, i.e., to the 3' terminal region of any one of SEQ ID NO:533 to SEQ ID NO:665, via a phosphodiester bond.

In some embodiments, the GalNAc ligand is comprised in the linker shown in Figure 6 (Formula XI), wherein the "oligonucleotide" represents a nucleic acid according to the invention, wherein the nucleic acid according to the invention comprises a modified second strand comprising or consisting of any one of SEQ ID NO:533 to SEQ ID NO:665, preferably wherein the linker is conjugated to the 3' terminal region of the second strand, i.e., to the 3' terminal region of any one of SEQ ID NO:533 to SEQ ID NO:665, via a phosphodiester bond.

In some embodiments, the GalNAc ligand is comprised in the linker shown in Figures 2 to 5 or Figure 6 (Formula XI), wherein the "oligonucleotide" represents a nucleic acid according to the invention, wherein the nucleic acid according to the invention comprises a modified second strand comprising or consisting of any one of SEQ ID NO:533 to SEQ ID NO:665, preferably of any one of SEQ ID NO:548, SEQ ID NO:620, SEQ ID NO:597 and SEQ ID NO:587, wherein the second strand has the following structure



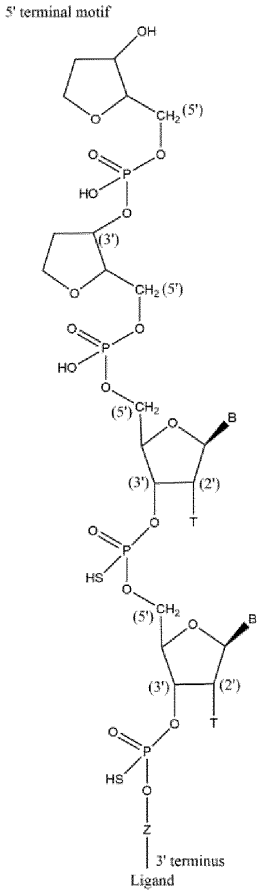

wherein:

T represents a 2'Me ribose modification,

B represents the nucleoside bases of the first two basic nucleosides in the 5' terminal region of any one of SEQ ID NO:533 to SEQ ID NO:665 preferably of any one of SEQ ID NO:548, SEQ ID NO:620, SEQ ID NO:597 and SEQ ID NO:587, and

Z represents the remaining 19 contiguous basic nucleosides of any one of SEQ ID NO:533 to SEQ ID NO:665 preferably of any one of SEQ ID NO:548, SEQ ID NO:620, SEQ ID NO:597 and SEQ ID NO:587, respectively.

In some embodiments, the GalNAc ligand is comprised in the linker shown in Figure 6 (Formula XI), wherein the "oligonucleotide" represents a nucleic acid according to the invention, wherein the nucleic acid according to the invention comprises a modified second strand comprising or consisting of any one of SEQ ID NO:533 to SEQ ID NO:665 preferably of any one of SEQ ID NO:548, SEQ ID NO:620, SEQ ID NO:597 and SEQ ID NO:587, wherein the second strand has the following structure



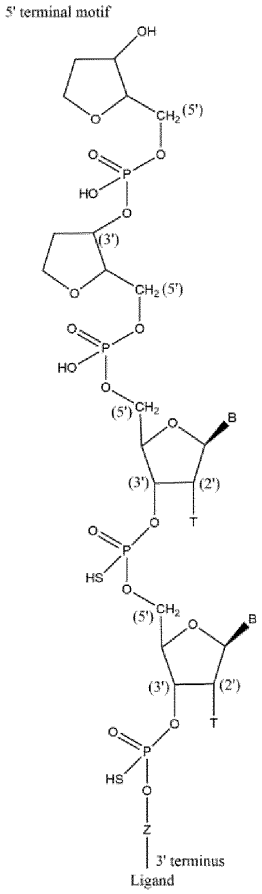

wherein:

T represents a 2'Me ribose modification,

B represents the nucleoside bases of the first two basic nucleosides in the 5' terminal region of any one of SEQ ID NO:533 to SEQ ID NO:665 preferably of any one of SEQ ID NO:548, SEQ ID NO:620, SEQ ID NO:597 and SEQ ID NO:587, and

Z represents the remaining 19 contiguous basic nucleosides of any one of SEQ ID NO:533 to SEQ ID NO:665 preferably of any one of SEQ ID NO:548, SEQ ID NO:620, SEQ ID NO:597 and SEQ ID NO:587, respectively.

In some embodiments, the GalNAc ligand is comprised in the linker shown in Figure 4, wherein the "oligonucleotide" represents a nucleic acid according to the invention, wherein the nucleic acid according to the invention comprises a modified second strand comprising or consisting of any one of SEQ ID NO:533 to SEQ ID NO:665 preferably of any one of SEQ ID NO:548, SEQ ID NO:620, SEQ ID NO:597 and SEQ ID NO:587, wherein the second strand has the following structure




wherein:

T represents a 2'Me ribose modification,

B represents the nucleoside bases of the first two basic nucleosides in the 5' terminal region of any one of SEQ ID NO:533 to SEQ ID NO:665 preferably of any one of SEQ ID NO:548, SEQ ID NO:620, SEQ ID NO:597 and SEQ ID NO:587, and

Z represents the remaining 19 contiguous basic nucleosides of any one of SEQ ID NO:533 to SEQ ID NO:665 preferably of any one of SEQ ID NO:548, SEQ ID NO:620, SEQ ID NO:597 and SEQ ID NO:587, respectively.

**VECTOR AND CELL**

In one aspect, the invention provides a cell containing a nucleic acid, such as inhibitory RNA [RNAi] as described herein.

In one aspect, the invention provides a cell comprising a vector as described herein.

In one aspect the invention provides a vector comprising an oligonucleotide inhibitor, e.g.an iRNA e.g. siRNA.

## PHARMACEUTICALLY ACCEPTABLE COMPOSITIONS

In one aspect, the invention provides a pharmaceutical composition for inhibiting expression of a target gene, the composition comprising an inhibitor such as an oligomer such as a nucleic acid as disclosed herein.

The pharmaceutically acceptable composition may comprise an excipient and or carrier.

Some 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 stearate, 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 poly anhydrides; (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.

Typical pharmaceutical carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g. , magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g. , starch, sodium starch glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc).

Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable excipients

include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone, and the like.

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

In one embodiment, the nucleic acid or composition is administered in an unbuffered solution. In certain embodiments, the unbuffered solution is saline or water. In other embodiments, the nucleic acid e.g. RNAi agent is administered in a buffered solution. In such embodiments, the buffer solution can comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. For example, the buffer solution can be phosphate buffered saline (PBS).

**DOSAGES**

The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of a gene or modify the expression or function of a target. In general, where the composition comprising a nucleic acid, a suitable dose of a nucleic acid e.g. an siRNA of the invention will be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. Typically, a suitable dose of a nucleic acid e.g. an siRNA of the invention will be in the range of about 0.1 mg/kg to about 5.0 mg/kg, e.g., about 0.3 mg/kg and about 3.0 mg/kg.

A repeat-dose regimen may include administration of a therapeutic amount of a nucleic acid e.g. siRNA on a regular basis, such as every other day or once a year. In certain embodiments, the nucleic acid e.g. siRNA is administered about once per month to about once per quarter (i.e., about once every three months).

In various embodiments, the nucleic acid e.g. siRNA agent is administered at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 50 mg/kg. In some embodiments, the nucleic acid e.g. siRNA agent is administered at a dose of about 10 mg/kg to about 30 mg/kg. In certain embodiments, the nucleic acid e.g. siRNA agent is administered at a dose selected from about 0.5 mg/kg 1 mg/kg, 1.5 mg/kg, 3 mg/kg, 5 mg/kg, 10 mg/kg, and 30 mg/kg. In certain embodiments, the nucleic acid e.g. siRNA agent is administered about once per week, once per

month, once every other two months, or once a quarter (i.e., once every three months) at a dose of about 0.1 mg/kg to about 5.0 mg/kg. In certain embodiments, the nucleic acid e.g. siRNA agent is administered to the subject once a week. In certain embodiments, the nucleic acid e.g. siRNA agent is administered to the subject once a month. In certain embodiments, the nucleic acid e.g. siRNA agent is administered once per quarter (i.e., every three months).

After an initial treatment regimen, the treatments can be administered on a less frequent basis. For example, after administration weekly or biweekly for three months, administration can be repeated once per month, for six months, or a year; or longer.

The pharmaceutical composition can be administered once daily, or administered as two, three, or more sub-doses at appropriate intervals throughout the day or even using continuous infusion or delivery through a controlled release formulation. In that case, the nucleic acid e.g. siRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, e.g., using a conventional sustained release formulation which provides sustained release of the nucleic acid e.g. siRNA over a several day period. Sustained release formulations are well known in the art and are particularly useful for delivery of agents at a particular site, such as could be used with the agents of the present invention. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

In other embodiments, a single dose of the pharmaceutical compositions can be long lasting, such that subsequent doses are administered at not more than 3, 4, or 5 day intervals, or at not more than 1, 2, 3, or 4 week intervals. In some embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered once per week. In other embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered bimonthly. In certain embodiments, the siRNA is administered about once per month to about once per quarter (i.e., about once every three months), or even every 6 months or 12 months.

Estimates of effective dosages and *in vivo* half-lives for the individual nucleic acid e.g. siRNAs encompassed by the invention can be made using conventional methodologies or on the basis of *in vivo* testing using an appropriate animal model, as known in the art.

The pharmaceutical compositions of the present invention can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be topical (e.g., by a transdermal patch), pulmonary, e.g., by

inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal, or intramuscular injection or infusion; subdermal, e.g., via an implanted device; or intracranial, e.g., by intraparenchymal, intrathecal or intraventricular administration. In certain preferred embodiments, the compositions are administered by intravenous infusion or injection. In certain embodiments, the compositions are administered by subcutaneous injection.

In one embodiment, the nucleic acid e.g. siRNA agent is administered to the subject subcutaneously.

The inhibitor e.g. nucleic acid e.g. siRNA can be delivered in a manner to target a particular tissue (e.g. in particular liver cells).

**METHODS FOR INHIBITING GENE EXPRESSION OR INHIBITION OF TARGET EXPRESSION OR FUNCTION**

The present invention also provides methods of inhibiting expression of a gene in a cell and methods for inhibiting expression and/or function of other target molecules. The methods include contacting a cell with a nucleic acid of the invention e.g. siRNA agent, such as double stranded siRNA in an amount effective to inhibit expression of the gene in the cell, thereby inhibiting expression of the gene in the cell. In a preferred embodiment, the gene is CFHR4. It is to be noted that a nucleic acid "for inhibiting the expression of CFHR4" is a nucleic acid that is capable of inhibiting CFHR4 expression, preferably as described herein below.

Contacting of a cell with the inhibitor e.g. the nucleic acid e.g. an siRNA, such as a double stranded siRNA agent, may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the inhibitor nucleic acid e.g. siRNA includes contacting a cell or group of cells within a subject, e.g., a human subject, with the nucleic acid e.g. siRNA. Combinations of *in vitro* and *in vivo* methods of contacting a cell are also possible. Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand moiety, including any ligand moiety described herein or known in the art. In preferred embodiments, the targeting ligand moiety is a carbohydrate moiety, e.g. a GalNAc3 ligand, or any other ligand moiety that directs the siRNA agent to a site of interest.

The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating", "suppressing", and other similar terms, and includes any level of inhibition.

In some embodiments of the methods of the invention, expression or activity of a gene or an inhibition target is inhibited by at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay, preferably when determined by qPCR as described herein and/or when the siRNA is introduced into the target cell by transfection. In certain embodiments, the methods include a clinically relevant inhibition of expression of a target gene e.g. as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of the gene and/ or activity of the target.

In some embodiments, when transfected into the cells, the nucleic acid of the invention inhibits expression of the CFHR4 gene with an IC50 value below a defined threshold value. In some embodiments, the threshold value may be 2500 pM, 2000 pM, 1900 pM, 1800 pM, 1700 pM, 1600 pM, 1500 pM, 1400 pM, 1300 pM, 1200 pM, 1100 pM, 1000 pM, 900 pM, 800 pM, 700 pM, 600 pM, 500 pM, 400 pM, 300 pM, 200 pM or 100 pM, preferably when determined by qPCR, more preferably by reverse transcriptase (RT)-qPCR, as described herein.

In a preferred embodiment, when transfected into the cells, the nucleic acid of the invention inhibits expression of the CFHR4 gene with an IC50 value lower than 2500 pM. In a more preferred embodiment, when transfected into the cells, the nucleic acid of the invention inhibits expression of the CFHR4 gene with an IC50 value lower than 1000 pM. In an even more preferred embodiment, when transfected into the cells, the nucleic acid of the invention inhibits expression of the CFHR4 gene with an IC50 value lower than 500 pM. In a most preferred embodiment, when transfected into the cells, the nucleic acid of the invention inhibits expression of the CFHR4 gene with an IC50 value lower than 100 pM.

Inhibition of the CFHR4 gene may be quantified by the following method:

Huh7 cells (human hepatocyte-derived cell line, obtained from JCRB Cell Bank) may be maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS at 37°C in an atmosphere of 5% $CO_2$. Cells may then be transfected with siRNA duplexes targeting CFHR4 mRNA or a negative control siRNA (siRNA-control; sense strand 5'-UUCUCCGAACGUGUCACGUTT-3' (SEQ ID NO:668), antisense strand 5'-ACGUGACACGUUCGGAGAATT-3' (SEQ ID NO:667)) using 10x3-fold serial dilutions over a final duplex concentration range of 20 nM to 1 pM. Transfection may be carried out by adding 9.7 µL Opti-MEM (ThermoFisher) plus 0.3 µL Lipofectamine RNAiMAX (ThermoFisher) to 10 µL of each siRNA duplex. The mixture may be incubated at room temperature for 15 minutes before being added to 100 µL of complete growth medium containing 20,000 Huh7 cells. Cells may be incubated for 24 hours at 37°C/5% $CO_2$ prior to total RNA purification using a RNeasy

96 Kit (Qiagen). Each duplex may be tested by transfection in duplicate wells in a single experiment.

cDNA synthesis may be performed using FastQuant RT (with gDNase) Kit (Tiangen). Real-time quantitative PCR (qPCR) may be performed on an ABI Prism 7900HT or ABI QuantStudio 7 with primers specific for human CFHR4 (forward: TGTGTAAATTGGGATATAATGCGA (SEQ ID NO:671), reverse: CCTTATTCGCATCTGGGGTA (SEQ ID NO:672)) and human GAPDH (forward: GAAGGTGAAGGTCGGAGTC (SEQ ID NO:673), reverse: GAAGATGGTGATGGGATTTC (SEQ ID NO:674)) using FastStart Universal Probe Master Kit (Roche).

qPCR may be performed in duplicate on cDNA derived from each well and the mean cycle threshold (Ct) calculated. Relative CFHR4 expression may be calculated from mean Ct values using the comparative Ct ($\Delta\Delta$Ct) method, normalised to GAPDH and relative to untreated cells. Maximum percent inhibition of CFHR4 expression and IC50 values may be calculated using a four parameter (variable slope) model using GraphPad Prism 9.

Alternatively or in addition, a pEC50 value may be calculated to quantify and or compare the inhibitory potential of the siRNAs according to the invention.

In some embodiments, when transfected into the cells, the nucleic acid of the invention inhibits expression of the CFHR4 gene with an pEC50 value lower than 5, 6, 7, 8, 9 or 10, preferably when determined by qPCR, more preferably by reverse transcriptase (RT)-qPCR, as described herein.

For that, Huh7 cells (human hepatocyte-derived cell line, obtained from JCRB Cell Bank) may be maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% non-essential amino acids at 37°C, 5% $CO_2$, 95% humidity. Cells may be transfected with siRNA duplexes targeting either CFHR4 mRNA or a negative control siRNA (siRNA-control; sense strand 5'-GCGAAUACAUCAGUUCUAUTT-3' (SEQ ID NO:669), antisense strand 5'-AUAGAACUGAUGUAUUCGCTT-3' (SEQ ID NO:670)) in a 6-point, log dose response curve to give final in assay concentrations of 3nM to 0.03pM. Transfection may be carried out by diluting Lipofectamine RNAiMAX (ThermoFisher) in Opti-MEM (ThermoFisher) medium at a ratio of 48.5:1.5. This solution may be added to an equal volume of siRNA, diluted to the required concentration in phosphate buffered saline. The lipofectamine RNAiMAX and siRNA mixture may be incubated at room temperature for 15 minutes before 20 µL may be added to wells of a 96 well plate. Huh7 cells may bemay dissociated from flasks using trypsin

and resuspended at a density of 300,000 cells/mL. 100 µL of Huh7 cell suspension may be added to each well of the siRNA-containing 96-well plates. Cells may be incubated for 48 hours at 37°C, 5% $CO_2$,95% humidity. Each siRNA may be tested in triplicate wells and on two separate days for a total of six replicates.

Intracellular RNA may be isolated using an RNeasy kit (Qiagen) according to the manufacturer's instructions. cDNA synthesis may be performed using a FastKing RT kit, with gDNase (Tiangen). Target cDNA may be the quantified by qPCR on an ABI Prism 7900HT or ABI QuantStudio 7 with primers specific for human CFHR4 (forward: TGTGTAAATTGGGATATAATGCGA (SEQ ID NO:671), reverse: CCTTATTCGCATCTGGGGTA (SEQ ID NO:672)) and human GAPDH (forward: GAAGGTGAAGGTCGGAGTC (SEQ ID NO:673), reverse: GAAGATGGTGATGGGATTTC (SEQ ID NO:674)) using a SensiFAST SYBR Hi-ROX kit (Meridian).

qPCR may be performed in duplicate on cDNA derived from each well and the mean Ct calculated. Relative CFHR4 expression may be calculated from mean Ct values using the comparative Ct (ΔΔCt) method, normalised to GAPDH and relative to untreated cells. Maximum percent inhibition of CFHR4 expression and pEC50 values (-$\log_{10}$ of the EC50) may be calculated using a four parameter (variable slope) model using NumPy (Python).

Alternatively or in addition, inhibition of expression of the CFHR4 gene may be characterized by a reduction of mean relative expression of the CFHR4 gene.

In some embodiments, when cells are transfected with 0.1 nM of the nucleic acid of the invention, the mean relative expression of CFHR4 is below 1, 0.9, 0.8, 0.7, 0.6, 0.5, or 0.4, preferably when determined by qPCR, more preferably by reverse transcriptase (RT)-qPCR, as described herein.

In some embodiments, when cells are transfected with 5 nM of the nucleic acid of the invention, the mean relative expression of CFHR4 is below 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4 or 0.3, preferably when determined by qPCR, more preferably by reverse transcriptase (RT)-qPCR, as described herein.

Mean relative expression of the CFHR4 gene may be quantified by the following method:

Huh7 cells (human hepatocyte-derived cell line, obtained from JCRB Cell Bank) may be maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS at 37°C in at atmosphere of 5% $CO_2$. Cells may be transfected with siRNA duplexes targeting

CFHR4 mRNA or a negative control siRNA (siRNA-control; sense strand 5'-UUCUCCGAACGUGUCACGUTT-3' (SEQ ID NO:668), antisense strand 5'-ACGUGACACGUUCGGAGAATT-3' (SEQ ID NO:667)) at a final duplex concentration of 5 nM and 0.1 nM. Transfection may be carried out by adding 9.7 µL Opti-MEM (ThermoFisher) plus 0.3 µL Lipofectamine RNAiMAX (ThermoFisher) to 10 µL of each siRNA duplex. The mixture may be incubated at room temperature for 15 minutes before being added to 100 µL of complete growth medium containing 20,000 Huh7 cells. Cells may be incubated for 24 hours at 37°C/5% $CO_2$ prior to total RNA purification using a RNeasy 96 Kit (Qiagen). Each duplex may be tested by transfection in duplicate wells in two independent experiments.

cDNA synthesis may be performed using FastQuant RT (with gDNase) Kit (Tiangen). Real-time quantitative PCR (qPCR) may be performed on an ABI Prism 7900HT or ABI QuantStudio 7 with primers specific for human CFHR4 (forward: TGTGTAAATTGGGATATAATGCGA (SEQ ID NO:671), reverse: CCTTATTCGCATCTGGGGTA (SEQ ID NO:672)) and human GAPDH (forward: GAAGGTGAAGGTCGGAGTC (SEQ ID NO:673), reverse: GAAGATGGTGATGGGATTTC (SEQ ID NO:674)) using FastStart Universal Probe Master Kit (Roche).

qPCR may be performed in duplicate on cDNA derived from each well and the mean Ct calculated. Relative CFHR4 expression may be calculated from mean Ct values using the comparative Ct ($\Delta\Delta$Ct) method, normalised to GAPDH and relative to untreated cells.

Inhibition of the expression of CFHR4 gene may be manifested by a reduction of the amount of mRNA of the target CFHR4 gene in comparison to a suitable control.

In other embodiments, inhibition of the expression of CFHR4 gene may be assessed in terms of a reduction of a parameter that is functionally linked to gene expression, e.g , protein expression or signaling pathways.

## METHODS OF TREATING OR PREVENTING DISEASES ASSOCIATED WITH GENE EXPRESSION/ EXPRESSION OF FUNCTION OF A TARGET

The present invention also provides methods of using nucleic acid e.g. an siRNA of the invention or a composition containing nucleic acid e.g. an siRNA of the invention to reduce or inhibit gene expression in a cell or reduce expression or function of a target. The methods include contacting the cell with a nucleic acid e.g. dsiRNA of the invention and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of a gene, thereby inhibiting expression

of the gene in the cell. Reduction in gene expression or function of a target can be assessed by any methods known in the art. In a preferred embodiment, the gene is CFHR4.

In the methods of the invention the cell may be contacted *in vitro* or *in vivo*, i.e., the cell may be within a subject.

A cell suitable for treatment using the methods of the invention may be any cell that expresses a gene of interest or target of interest associated with disease.

The *in vivo* methods of the invention may include administering to a subject a composition containing a nucleic acid of the invention e.g. an iRNA, where the nucleic acid e.g. siRNA includes a nucleoside sequence that is complementary to at least a part of an RNA transcript of the gene of the mammal to be treated, or complementary to another nucleic acid the expression and /or function of which is associated with diseases.

The present invention further provides methods of treatment of a subject in need thereof. The treatment methods of the invention include administering a nucleic acid such as an siRNA of the invention to a subject, e.g., a subject that would benefit from a reduction or inhibition of the expression of a gene and/or expression and/or function of a target, in a therapeutically effective amount e.g. a nucleic acid such as an siRNA targeting a gene or a pharmaceutical composition comprising the nucleic acid targeting a gene.

The inhibitor, such as the nucleic acid, according to the invention may be used in the prevention and/or treatment of a disease or disorder associated with alterations in complement pathway protein levels and/or function in a patient.

A disease or disorder associated with alterations in complement pathway protein levels and/or function may be any disease that is characterized by abnormal levels and or functions of one or more proteins involved in the complement pathway.

The complement system is a complex network of proteins in the blood and tissues that enhances the body's ability to fight off infections and remove damaged cells. It acts as a rapid and powerful defence mechanism by marking pathogens for destruction, promoting inflammation, and aiding in the removal of cellular debris. Three biochemical pathways activate the complement system: the classical complement pathway, the alternative complement pathway, and the lectin pathway. The alternative pathway accounts for the majority of terminal pathway activation and so therapeutic efforts in disease have revolved around its inhibition.

CFHR4 encodes a protein that regulates complement activation as part of the alternative complement pathway. CFHR4 has been associated with various diseases, including kidney disorders and age-related macular degeneration. This gene is part of a family of complement factor H-related genes, contributing to the intricate balance between immune defence and self-tolerance.

In certain embodiments, the disease or disorder to be treated may be a disease or disorder associated or caused by an alteration of normal complement factor H (CFH/FH) and/or Factor H-like-1 (FHL-1) function. In certain embodiments, the disease or disorder to be treated may be a disease or disorder associated with or caused by reduced levels and/or function of complement factor H (CFH/FH) and/or Factor H-like-1 (FHL-1).

Abnormal function of complement factor H and/or Factor H-like-1 (FHL-1) often results in overactivation of the alternative complement system. Overactivation of the alternative complement system has been described as the cause for various diseases, including AMD and several kidney diseases disclosed herein. Accordingly, in certain embodiments, the inhibitor according to the invention may be used in the prevention and/or treatment of a disease or disorder associated with alterations in complement pathway protein levels and/or function in a patient, wherein the alterations in complement pathway protein levels and/or function result in overactivation of the alternative complement system.

In certain embodiments, the inhibitor according to the invention may be used in the prevention and/or treatment of a disease or disorder associated with an overactivation of the alternative complement system. Preferably, the disease or disorder associated with alterations in complement pathway protein levels and/or function is a disease or disorder associated with elevated levels and/or functions of complement factor H-related protein 4 (FHR-4).

The inhibitor according to the invention may be used in the treatment of any disease or disorder that is associated with alterations in complement pathway protein levels and/or function. In particular, the inhibitor according to the invention may result in normalization of the complement pathway protein levels and/or function. Normalization of the complement pathway protein levels and/or function may preferably slow down progression of the disease or disorder that is associated with alterations in complement pathway protein levels and/or function.

In a preferred embodiment, the inhibitor according to the invention is used for the treatment of a disease or disorder associated with elevated levels and/or function of complement factor H-related protein 4 (FHR-4) in a patient. In particular, the inhibitor according to the invention may

reduce the levels and/or the function of FHR-4 in said patient and thereby slow down the progression of the disease or disorder associated with elevated levels of complement factor H-related protein 4 (FHR-4).

In certain embodiments, the disease or disorder associated with alterations in complement pathway protein levels and/or function is age-related macular degeneration (AMD), preferably dry age-related macular degeneration, as described in more detail herein below.

In certain embodiments, the disease or disorder associated with alterations in complement pathway protein levels and/or function is a kidney disease, such as atypical haemolytic uraemic syndrome (aHUS), C3 glomeruloneoropathy (C3G), IgA nephropathy (IgAN), or membranous (glomerulo) nephropathy (MN).

The inhibitor, such as the nucleic acid, according to the invention may be used in the prevention and/or treatment of age-related macular degeneration (AMD).

More specifically, the present invention relates to inhibitors of expression and/or function of the gene CFHR4 and the use of such inhibitors in the treatment of age-related macular degeneration (AMD).

CFHR4, a gene linked to the complement system, plays a role in the immune response. The complement system is a complex network of proteins in the blood that enhances the body's ability to fight off infections and remove damaged cells. It acts as a rapid and powerful defence mechanism by marking pathogens for destruction, promoting inflammation, and aiding in the removal of cellular debris. CFHR4 encodes a protein that regulates complement activation, a key defence mechanism against infections. CFHR4 has been associated with various diseases, including kidney disorders and age-related macular degeneration. This gene is part of a family of complement factor H-related genes, contributing to the intricate balance between immune defence and self-tolerance.

Age-related macular degeneration (AMD) is a progressive chronic disease of the central retina and a leading cause of vision loss worldwide. Most visual loss occurs in the late stages of the disease due to one of two processes: neovascular AMD (known as "wet AMD") and geographic atrophy (GA, known as "dry AMD").

In both cases, AMD affects the complex of photoreceptors, retinal pigment epithelium (RPE), Bruch’s membrane (BrM) and the choroid with the most pronounced pathological alterations occurring in the macula.

AMD is characterized by the accumulation of drusen (extracellular deposits of lipids, proteins, and cellular debris) that lead to progressive degeneration of photoreceptors and RPE, resulting in loss of central vision. Size of the drusen is used to define different stages of AMD and assess severity of the disease, including non-sight-threatening earlier stages. For instances, medium-sized drusen are classified as "early" AMD while larger drusen are classified as "intermediate" AMD.

Over the years classification systems have been refined as methods used (e.g., retinal imaging, molecular, genetics) become more precise. Classification started from epidemiological studies that have traditionally classified AMD into early and late stages, with late AMD including dry or wet AMD. More recently, clinically based studies often use the AREDS classification system; the Age-Related Eye Disease Study (AREDS) classification which contains 4 categories with the severity of the disease increasing from category 1 (no AMD) to category 4 (advanced AMD). In 2013, a modified classification system based on AREDS was proposed by the Beckman Initiative for Macular Research Classification Committee. This classification system groups the disease into 5 stages including early, intermediate and late AMD; note that the two first stages correspond to no AMD (no apparent ageing changes and normal ageing changes). In the Beckman classification, the term dry AMD is used only for GA and not for early stages of AMD (Ferris F.L. *et al.* Ophthalmology, 2013, 120(4):844-51).

GA usually leads to progressive loss of central visual field. GA is characterized by confluent atrophy of photoreceptors, RPE and choriocapillaris. Areas of GA enlarge over time, leading to a sharply demarcated area of depigmentation and resulting in a dark patch in AMD patient central vision (scotoma) (Fleckenstein, M. *et al.* Ophthalmology, 2018, 125(3):369-390).

At any stage of AMD, new vessels may invade the outer retina, subretinal space or subRPE space, resulting in macular neovascularization (MNV), which is the hallmark lesion of neovascular AMD (Spaide, R. F. *et al.* Ophthalmology, 2020, 127(5):616-636). The exudative stage of neovascular AMD becomes apparent when these new vessels leak or rupture, resulting in fluid accumulation and/or haemorrhages, and distortion and deterioration in vision (metamorphopsia). Without treatment, exudative MNV typically results in extensive fibrosis with severe central vision loss. Note that VEGF, a key regulator of angiogenesis, play an important role in the growth of choroidal neovascularisation. In summary GA and MNV involve different mechanisms both resulting in central vision loss.

Thus, AMD is regarded as a multifactorial and complex disease, representing a spectrum of phenotypes which reflect the intricate combination of different mechanisms. These mechanisms

include genetic susceptibility, ageing-associated dysfunction of normal retinal homeostasis, impaired lipid metabolism, immune activation and progression to chronic inflammation, oxidative stress, and ECM dysfunction, all of which seem to contribute to the disease.

AMD pathogenesis is characterised by disruption in the normal homeostatic mechanisms of the retina, where ageing changes coupled with chronic inflammation, increased lipid and lipoprotein deposition, oxidative stress and impaired extracellular matrix (ECM) maintenance lead to an imbalance, which manifests as this disease (Miller, J. W. Am J Ophthalmol, 2013, 155(1):1-35.e13).

The patient to be treated may be a patient that already has age-related macular degeneration (AMD) or that is at risk of developing age-related macular degeneration (AMD). That is, in certain embodiments, the inhibitor of the present invention may be used in the treatment of age-related macular degeneration (AMD). Treatment of age-related macular degeneration (AMD) with the nucleic acid of the present invention may slow the progression of age-related macular degeneration (AMD).

In certain embodiments, the patient to be treated may have neovascular AMD (known as "wet AMD") and geographic atrophy (GA, known as "dry AMD"). In a preferred embodiment, the patient to be treated has dry AMD. In a more preferred embodiment, the patient to be treated has intermediate dry AMD as defined herein.

In certain embodiments, the nucleic acid of the present invention may be used to prevent manifestation of age-related macular degeneration (AMD) in a patient that is at risk of developing age-related macular degeneration (AMD).

Thus, in a particular embodiment, the invention relates to a nucleic acid suitable for use, or for use, in prevention and/or treatment of age-related macular degeneration (AMD).

The inhibitor of the invention may be used in the treatment of any type of AMD, as defined elsewhere herein. That is, the inhibitor of the invention may be used in the treatment of dry AMD (GA) or wet/neovascular AMD of any grade or severity.

In certain embodiments, the inhibitor of the invention is used in the treatment of dry AMD or geographic atrophy AMD. In certain embodiments, the inhibitor of the invention is used in the treatment of intermediate dry AMD as classified by the Beckman Initiative for Macular Research Classification Committee (Ophthalmology, 2013, 120(4):844-51), which is fully incorporated herein by reference. In certain embodiments, dry AMD is characterized by drusen larger than

125 μm and/or the presence of any AMD pigmentary abnormalities, wherein AMD pigmentary abnormalities are characterized as any definite hyper- or hypopigmentary abnormalities associated with medium (63-125 μm) or large (above 125 μm) drusen, but not associated with known disease entities.

The patient to be treated with the inhibitor according to the invention, such as a patient having AMD or any other disease or disorder associated with alterations in complement pathway protein levels and/or function, is preferably a patient having elevated levels of FHR-4. As described elsewhere herein, FHR-4 is a protein that is synthesized in the liver and then circulates in the bloodstream to reach other tissues/organs. The skilled person is able to determine whether a patient has elevated levels of FHR-4. For example, elevated levels of FHR-4 may be determined by quantifying the levels of FHR-4 in a sample derived from the patient and in a corresponding sample from a healthy individual and by comparing the levels of FHR-4 in the two samples, wherein higher levels in the sample obtained from the patient are indicative of elevated levels of FHR-4. The sample may be a blood sample or a tissue sample that has been obtained by biopsy.

However, it is preferred herein that the patient having elevated levels of FHR-4 has a genetic profile that is indicative of elevated levels of FHR-4. It has been shown in Example 11 and by Zouache et al. (Nat Commun, 2024, 15(1):443), which is fully incorporated herein by reference, that the genetic markers rs61818956, rs10494745 and rs7531555 independently influence FHR-4 levels in the blood, as well as in different organs.

The SNP rs61818956 is located at genomic position chr 1:196917093. The following alleles have been described: C (major allele) and T (minor allele).

The SNP rs10494745 is located at genomic position chr 1:196918327. The following alleles have been described: G (major allele) and C (minor allele).

The SNP rs7531555 is located at genomic position chr 1:196960180. The following alleles have been described: C (major allele) and T (minor allele).

Surprisingly, in Example 11 it was shown that taken into account all three loci of interest renders the involvement of FHR-4 as a driver of complement-mediated disease progression plausible. Accordingly, the inventors have identified genetic profiles that correlate with elevated levels of FHR-4. Patients with these genetic profiles, in particular patients having AMD or any other disease or disorder associated with alterations in complement pathway protein levels and/or function, are thus particularly likely to respond to treatment with an inhibitor of CFHR4/FHR-4 expression and/or function, such as any of the inhibitors disclosed herein.

In certain embodiments, the invention relates to an inhibitor or pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles rs61818956TT and/or rs10494745GG and/or rs7531555CC.

In certain embodiments, the invention relates to an inhibitor or pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT and rs10494745GG; (ii) rs61818956TT and rs10494745GA; (iii) rs61818956TT and rs10494745AA; (iv) rs61818956CT and rs10494745GG; (v) rs61818956CT and rs10494745GA; (vi) rs61818956CT and rs10494745AA; (vii) rs61818956CC and rs10494745GG; or (viii) rs61818956CC or rs10494745GA.

In certain embodiments, the invention relates to an inhibitor or pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT and rs10494745GG; (ii) rs61818956TT and rs10494745GA; (iii) rs61818956TT and rs10494745AA; (iv) rs61818956CT and rs10494745GG; or (v) rs61818956CT and rs10494745GA.

In certain embodiments, the invention relates to an inhibitor or pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT and rs10494745GG or (ii) rs61818956TT and rs10494745GA; or (iii) rs61818956CT and rs10494745GG.

In certain embodiments, the invention relates to an inhibitor or pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT and rs10494745GG or (ii) rs61818956TT and rs10494745GA.

In certain embodiments, the invention relates to an inhibitor or pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT, rs10494745GG, rs7531555CC; (ii) rs61818956CT, rs10494745GG, rs7531555CC; (iii) rs61818956TT, rs10494745GA, rs7531555CT; (iv) rs61818956TT, rs10494745GA, rs7531555CC; (v) rs61818956CC, rs10494745GG, rs7531555CC; (vi) rs61818956TC, rs10494745GA, rs7531555CC; (vii) rs61818956TC, rs10494745GG, rs7531555CT; (viii) rs61818956CT, rs10494745GA, rs7531555CT; (ix) rs61818956CT, rs10494745GA, rs7531555CC; (x) rs61818956TT, rs10494745GG, rs7531555CT; (xi) rs61818956TT, rs10494745AA, rs7531555TT; (xii) rs61818956TT, rs10494745AA, rs7531555TC; or (xiii) rs61818956TT, rs10494745AA, rs7531555CC.

In certain embodiments, the invention relates to an inhibitor or pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT, rs10494745GG, rs7531555CC; (ii) rs61818956CT, rs10494745GG, rs7531555CC; (iii) rs61818956TT, rs10494745GA, rs7531555CT; or (iv) rs61818956TT, rs10494745GA, rs7531555CC.

In certain embodiments, the invention relates to an inhibitor or pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT, rs10494745GG, rs7531555CC; (ii) rs61818956CT, rs10494745GG, rs7531555CC; (iii) rs61818956CC, rs10494745GG, rs7531555CC; (iv) rs61818956TC, rs10494745GA, rs7531555CC; (v) rs61818956TC, rs10494745GG, rs7531555CT.

In certain embodiments, the invention relates to an inhibitor or pharmaceutical composition for use according to the invention, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT, rs10494745GG, rs7531555CC; or (ii) rs61818956CT, rs10494745GG, rs7531555CC.The genotype of a SNP can be determined in a biological sample by any suitable method. Many methods are available for detection of one or more alleles of a SNP, including sequencing methods, re-sequencing methods, amplification methods, and hybridization methods. Analysis of nucleic acids in a biological sample from an individual, whether amplified or not, may be performed using any of these methods. Exemplary methods include but are not limited to polymerase chain reaction (PCR), restriction fragment length polymorphism analysis (RFLP), reverse-transcription PCR (RT-PCR), isothermal amplification, 5' fluorescence nuclease assay (e.g. TAQMAN assay), molecular beacon assays, heteroduplex mobility assays (HMA), single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), planar microarrays, bead arrays, sequencing, chemical cleavage of mismatch (CCM), and denaturing high performance liquid chromatography (DHPLC). One of ordinary skill in the art would understand that any known method of amplification of a nucleotide could be incorporated into a method to detect one or more alleles of a SNP. One of ordinary skill in the art would further understand that these methods of amplification of a nucleotide could use DNA, RNA, or a combination of the two.

These assays may be multiplexed, meaning two or more reactions may be conducted simultaneously in the same physical location, such as in the same tube or on the same substrate, such as a biochip, ensuring that the reaction products of the multiplexed reactions can be distinguished. For example, TAQMAN or molecular beacon assays can be multiplexed by use of

any by monitoring of accumulation or depletion of two different fluorochromes corresponding to different sequence specific probes.

As used herein, "PCR" is any method involving the amplification of a nucleotide sequence based upon complementary primer binding to a target sequence. One of ordinary skill in the art will understand that PCR may be employed as part of many techniques for identifying a SNP, including but not limited to Tetra-primer amplification refractory mutation system PCR (ARMS-PCR). In ARMS-PCR, primers are employed whose 3' ends encompass the SNP location, with each primer encoding a different allele at the SNP location. The primers are also designed to produce different length amplification fragments, thus allowing discrimination of the SNP genotype based upon the length of the amplified fragments.

As used herein, "RFLP" is any method for distinguishing genetic polymorphisms using a restriction enzyme, which is an endonuclease that catalyses the degradation of nucleic acid and recognizes a specific base sequence, generally a palindrome or inverted repeat. One of ordinary skill in the art would understand that the use of RFLP analysis depends upon an enzyme that can differentiate two alleles at a polymorphic site.

As used herein, "RT-PCR" is any method involving the amplification of a RNA sequence using a reverse transcriptase to produce a cDNA sequence, followed by amplification of a nucleotide sequence based upon complementary primer binding to a target sequence. One of ordinary skill in the art will understand that RT-PCR may be employed as part of many techniques for identifying a SNP.

As used herein, "isothermal amplification" is any method involving amplification of a nucleotide sequence based upon complementary primer binding to a target sequence performed at a constant temperature. One example of an isothermal amplification method is loop-mediated isothermal amplification (LAMP). Generally, LAMP is used to amplify from a DNA sequence and is performed using multiple primer sets and a polymerase with a high strand displacement activity. Another example of an isothermal amplification method is nucleic acid sequence based amplification (NASBA). Generally, NASBA is used to amplify from a RNA sequence and is performed using a reverse transcriptase, an RNAse, and a RNA polymerase.

As used herein, a "5' fluorescence nuclease assay" is any method using a target allele specific probe bearing a 5' fluorescent dye label. In general, when the allele specific probe is used to amplify the target sequence, the 5'-nuclease activity of the polymerase cleaves the 5' fluorescent dye label off of the probe, changing the molecular weight of the fluorescent dye molecule and

therefore changing the fluorescence polarization. This change in fluorescence polarization may be detected, thereby confirming the presence of the target allele.

As used herein, "hybridization methods" mean methods relying on the use of a labelled oligonucleotide probe having a sequence complementary, for example, to the sequence encompassing a disease-predisposing allele. Under appropriate conditions, the allele-specific probe hybridizes to a nucleic acid containing the disease -predisposing allele but does not hybridize to the one or more other alleles, which have one or more nucleotide mismatches as compared to the probe. If desired, a second allele- specific oligonucleotide probe that matches an alternate allele also can be used to selectively amplify, for example, a non-disease- predisposing allele by using an allele-specific oligonucleotide primer that is complementary to the nucleotide sequence of the non-disease-predisposing allele but which has one or more mismatches as compared to other alleles. One of ordinary skill in the art will understand that the one or more nucleotide mismatches that distinguish between the disease-predisposing allele (or the non-disease promoting allele) and one or more other alleles are preferably located in the center of an allele- specific oligonucleotide primer to be used in allele-specific oligonucleotide hybridization. In contrast, an allele-specific oligonucleotide primer to be used in PCR amplification preferably contains the one or more nucleotide mismatches that distinguish between the disease-associated and other alleles at the 3' end of the primer. Nonlimiting examples of hybridization methods useful herein include molecular beacon assays.

As used herein, a "HMA assay" is useful for detecting the presence of a polymorphic sequence since a DNA duplex carrying a mismatch has reduced mobility in a polyacrylamide gel compared to the mobility of a perfectly base-paired duplex.

As used herein, "SSCP" can be used to detect mutations based on differences in the secondary structure of single-strand DNA that produce an altered electrophoretic mobility upon non-denaturing gel electrophoresis. Polymorphic fragments are detected by comparison of the electrophoretic pattern of the test fragment to corresponding standard fragments containing known alleles.

As used herein, "DGGE" can be used to detect SNPs by electrophoresis of double-stranded DNA in a gel containing an increasing concentration of denaturant. The double-stranded DNA fragments containing mismatched alleles will have segments that will likely melt more rapidly, causing such fragments to migrate at a different rate compared to perfectly complementary sequences.

When implementing methods for detection of one or more SNPs, an array may be used to perform a high-throughput assay. The array generally comprises one or more reagents, such as nucleic acid primers and/or probes, for identifying in a nucleic acid sample from a subject the occurrence of an allelic variation corresponding to one or more SNPs. These reagents may be immobilized onto a substrate in a spatially addressable manner, such that each reagent is located at a different, identifiable, position on the array. The substrate may include multi-welled plates, ceramic chips, or beads. In a non-limiting example, the substrate may be a 96 well dish, with each well constituting a reaction chamber within which separate reactions comprising identified constituents may be performed. The reaction constituents may include primers for amplifying DNA or probes for binding specific sequences and reaction reagents. The reagents may be in any suitable form, including in solution, dried, lyophilized, or glassified. In a further non-limiting example, the array may include two or more sets of beads, with each bead having an identifiable marker, such as a quantum dot or fluorescent tag, so that the beads may be individually identified using, for example, a flow cytometer. Various array technologies are commercially available, for example from Applied Biosystems. Informatics and/or statistical software or other computer-implemented processes for analysing array data and/or identifying genetic risk factors from data obtained from a patient sample are well known in the art and would be readily understood by the ordinarily skilled artisan.

Other molecular methods useful for determining genotype of a SNP known in the art may also be used when performing the methods disclosed herein.

In certain embodiments, the patient that is to be treated with the inhibitor of function and/or expression of CFHR4/FHR-4 may be characterized based on a combination of different types of biomarkers. For example, the patient group may be characterized based on a combination of genomic biomarkers, i.e., the SNPs defined herein above, and blood biomarkers. Preferably, a FHR protein is used as the blood biomarker. More preferably, FHR-4 is used as the blood biomarker.

Methods for detecting, measuring or quantifying the amount of FHR-4 in a blood sample using an antibody are known in the art, and include e.g. ELISA, see e.g. Crowther JR, Methods in Molecular Biology, The ELISA Guidebook. Second Edition. Humana Press, a part of Springer Science + Business Media, LLC 2009; Butler J.E. The Behaviour of Antigens and Antibodies Immobilized on a Solid Phase. In: M.H.V. Van Regenmortel, ed. Structure of Antigens. Boca Raton, FL: CRC Press, 1992: 209-259. Vol.1 , 209; CRC Press, Inc.; Lequin RM. Clinical chemistry 51.12 (2005): 2415-2418; and Engvall and Perlmann. Immunochemistry 8.9 (1971 ):

871-874, which are hereby incorporated by reference in their entirety. Other methods may include mass spectrometry, Western blotting, protein immunostaining, Immunoelectrophoresis, and protein immunoprecipitation, which are described hereinbelow and/or will be apparent to one skilled in the art.

In certain embodiments, the invention relates to a method of selecting a patient having a disease or disorder associated with alterations in complement pathway protein levels, in particular a patient having age-related macular degeneration (AMD), for a treatment with the inhibitor according to the invention, and optionally treating the disease or disorder associated with alterations in complement pathway protein levels, in particular age-related macular degeneration (AMD), in said patient, said method comprising:

(a) providing a sample from the patient,

(b) detecting the sequence and / or expression and / or function of CFHR4/FHR-4 in said sample obtained from the patient,

(c) selecting the patient having the disease or disorder associated with alterations in complement pathway protein levels, in particular age-related macular degeneration (AMD), for a treatment with the inhibitor according to the invention based on the sequence and / or expression and / or function of CFHR4/FHR-4 in said sample obtained from the patient,

(d) preferably administering to the diagnosed patient an effective amount of an inhibitor of expression and/or function of CFHR4/FHR-4.

As explained in Example 11, elevated protein levels of FHR-4 are drivers of AMD and other disease or disorder associated with alterations in complement pathway protein levels. Thus, patients having elevated levels and/or function of FHR-4 can be expected to respond particularly well to a treatment with the inhibitor of the present invention.

To identify a patient that would benefit from the treatment with an inhibitor according to the invention, first a sample from said patient is to be provided. The sample is preferably a blood sample or a tissue sample that has been obtained by biopsy. In a next step, the sequence and/or expression and/or function of CFHR4/FHR-4 in that sample is determined and compared to a suitable control. A suitable control may be a sample from a healthy donor, preferably wherein the samples that are to be compared have been obtained from a similar source.

A patient is to be selected for a treatment with the inhibitor according to the invention based on the sequence and / or expression and / or function of CFHR4/FHR-4 in the sample obtained from said patient. Preferably, a patient is selected for a treatment with the inhibitor according to the invention if the protein levels of FHR-4 and/or the activity of FHR-4 in the sample are elevated.

Thus, in certain embodiments, the invention relates to the method according to the invention, wherein the patient is selected for a treatment with the inhibitor according to the invention, if the protein levels of FHR-4 and/or the activity of FHR-4 in the sample obtained from said patient are elevated compared to a suitable control, preferably wherein the suitable control is a comparable sample from a healthy donor.

Elevated levels of FHR-4 may be determined biochemically as described herein above. However, elevated levels of FHR-4 may also be determined indirectly by analysing specific SNPs that have been reported to correlate with FHR-4 protein levels, in particular the SNPs rs61818956, rs10494745, and rs7531555.

Accordingly, in certain embodiments, the invention relates to the method according to the invention, wherein the method comprises a step of determining the presence of a single nucleotide polymorphism (SNP) at positions rs61818956, rs10494745, and/or rs7531555 in the sample obtained from said patient.

In particular, the patient may be determined to have elevated levels of FHR-4 if the patient is a carrier of any of the diplotypes disclosed herein. That is, in certain embodiments, the invention relates to the method according to the invention, wherein the patient is determined to have elevated levels of FHR-4, if the patient is a carrier of diplotype alleles rs61818956TT and/or rs10494745GG and/or rs7531555CC;

preferably of diplotype alleles (i) rs61818956TT and rs10494745GG; (ii) rs61818956TT and rs10494745GA; (iii) rs61818956TT and rs10494745AA; (iv) rs61818956CT and rs10494745GG; (v) rs61818956CT and rs10494745GA; (vi) rs61818956CT and rs10494745AA; (vii) rs61818956CC and rs10494745GG; or (viii) rs61818956CC or rs10494745GA;

more preferably of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; (ii) rs61818956CT, rs10494745GG, and rs7531555CC; (iii) rs61818956TT, rs10494745GA, and rs7531555CT; (iv) rs61818956TT, rs10494745GA, and rs7531555CC; (v) rs61818956CC, rs10494745GG, and rs7531555CC; (vi) rs61818956TC, rs10494745GA, and rs7531555CC; (vii) rs61818956TC, rs10494745GG, and rs7531555CT; (viii) rs61818956CT, rs10494745GA, and

rs7531555CT; (ix) rs61818956CT, rs10494745GA, and rs7531555CC; (x) rs61818956TT, rs10494745GG, and rs7531555CT; (xi) rs61818956TT, rs10494745AA, and rs7531555TT; (xii) rs61818956TT, rs10494745AA, and rs7531555TC; or (xiii) rs61818956TT, rs10494745AA, and rs7531555CC;

even more preferably of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; (ii) rs61818956CT, rs10494745GG, and rs7531555CC; (iii) rs61818956TT, rs10494745GA, and rs7531555CT; or (iv) rs61818956TT, rs10494745GA, and rs7531555CC or

of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; (ii) rs61818956CT, rs10494745GG, and rs7531555CC; (iii) rs61818956CC, rs10494745GG, and rs7531555CC; (iv) rs61818956TC, rs10494745GA, and rs7531555CC; (v) rs61818956TC, rs10494745GG, and rs7531555CT;

most preferably of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; or (ii) rs61818956CT, rs10494745GG, and rs7531555CC.

If a patient has been diagnosed as being susceptible to a treatment of a disease or disorder associated with alterations in complement pathway protein levels, in particular AMD, with an effective amount of an inhibitor of expression and/or function of CFHR4/FHR-4, said patient is preferably administered with an effective amount of an inhibitor of expression and/or function of CFHR4/FHR-4, such as any one of the inhibitors disclosed herein.

A nucleic acid e.g. siRNA of the invention may be administered as a "free" nucleic acid or “free” siRNA, administered in the absence of a pharmaceutical composition. The naked nucleic acid may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution can be adjusted such that it is suitable for administering to a subject.

Alternatively, a nucleic acid e.g. siRNA of the invention may be administered as a pharmaceutical composition, such as a dsiRNA liposomal formulation.

In one embodiment, the method includes administering a composition featured herein such that expression of the target gene is decreased, such as for about 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 18, 24 hours, 28, 32, or about 36 hours. In one embodiment, expression of the target gene is decreased for an extended duration, e.g., at least about two, three, four days or more, e.g., about one week, two weeks, three weeks, or four weeks or longer, e.g., about 1 month, 2 months, or 3 months.

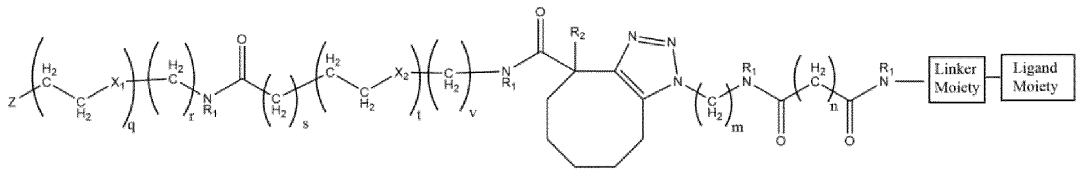
Subjects can be administered a therapeutic amount of nucleic acid e.g. siRNA, such as about 0.01 mg/kg to about 200 mg/kg, so as to prevent and/or treat age-related macular degeneration (AMD).

The nucleic acid e.g. siRNA can be administered by intravenous infusion over a period of time, on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. Administration of the siRNA can reduce gene product levels of a target gene , e.g., in a cell or tissue of the patient by at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or below the level of detection of the assay method used. In certain embodiments, administration results in clinical stabilization or preferably clinically relevant reduction of at least one sign or symptom of a gene-associated disorder.

Alternatively, the nucleic acid e.g. siRNA can be administered subcutaneously, i.e., by subcutaneous injection. One or more injections may be used to deliver the desired daily dose of nucleic acid e.g. s iRNA to a subject. The injections may be repeated over a period of time. The administration may be repeated on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. A repeat-dose regimen may include administration of a therapeutic amount of nucleic acid on a regular basis, such as every other day or to once a year. In certain embodiments, the nucleic acid is administered about once per month to about once per quarter (i.e., about once every three months).

In one aspect the present invention may be applied in the compounds, processes, compositions or uses of the following Sentences numbered 1-101 wherein reference to any Formula in the Sentences 1-101 refers only to those Formulas that are defined within Sentences 1-101. These formulae are reproduced in Figure 6. Specifically, an oligonucleoside moiety as represented by Z in any of the following sentences can comprise a nucleic acid for inhibiting expression of CFHR4 as defined in any of the claims hereinafter.

1. A compound comprising the following structure:




Formula (I)

wherein:

$R_1$ at each occurrence is independently selected from the group consisting of hydrogen, methyl and ethyl;

$R_2$ is selected from the group consisting of hydrogen, hydroxy, -$OC_{1-3}$alkyl, -$C(=O)OC_{1-3}$alkyl, halo and nitro;

$X_1$ and $X_2$ at each occurrence are independently selected from the group consisting of methylene, oxygen and sulfur;

m is an integer of from 1 to 6;

n is an integer of from 1 to 10;

q, r, s, t, v are independently integers from 0 to 4, with the proviso that:

(i) q and r cannot both be 0 at the same time; and

(ii) s, t and v cannot all be 0 at the same time;

Z is an oligonucleoside moiety.

2. A compound according to Sentence 1, wherein $R_1$ is hydrogen at each occurrence.

3. A compound according to Sentence 1, wherein $R_1$ is methyl.

4. A compound according to Sentence 1, wherein $R_1$ is ethyl.

5. A compound according to any of Sentences 1 to 4, wherein $R_2$ is hydroxy.

6. A compound according to any of Sentences 1 to 4, wherein $R_2$ is halo.

7. A compound according to Sentence 6, wherein $R_2$ is fluoro.

8. A compound according to Sentence 6, wherein $R_2$ is chloro.

9. A compound according to Sentence 6, wherein $R_2$ is bromo.

10. A compound according to Sentence 6, wherein $R_2$ is iodo.

11. A compound according to Sentence 6, wherein $R_2$ is nitro.

12. A compound according to any of Sentences 1 to 11, wherein $X_1$ is methylene.

13. A compound according to any of Sentences 1 to 11, wherein $X_1$ is oxygen.

14. A compound according to any of Sentences 1 to 11, wherein $X_1$ is sulfur.

15. A compound according to any of Sentences 1 to 14, wherein $X_2$ is methylene.

16. A compound according to any of Sentences 1 to 15, wherein $X_2$ is oxygen.

17. A compound according to any of Sentences 1 to 16, wherein $X_2$ is sulfur.

18. A compound according to any of Sentences 1 to 17, wherein m = 3.

19. A compound according to any of Sentences 1 to 18, wherein n = 6.

20. A compound according to Sentences 13 and 15, wherein $X_1$ is oxygen and $X_2$ is methylene, and preferably wherein:

q = 1,

r = 2,

s = 1,

t = 1,

v = 1.

21. A compound according to Sentences 12 and 15, wherein both $X_1$ and $X_2$ are methylene, and preferably wherein:

q = 1,

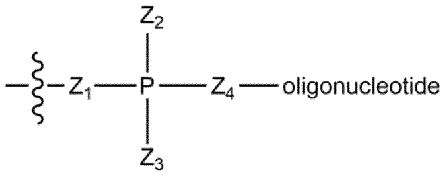
r = 3,

s = 1,

t = 1,

v = 1.

22. A compound according to any of Sentences 1 to 21, wherein Z is:




wherein:

$Z_1$, $Z_2$, $Z_3$, $Z_4$ are independently at each occurrence oxygen or sulfur; and

one the bonds between P and $Z_2$, and P and $Z_3$ is a single bond and the other bond is a double bond.

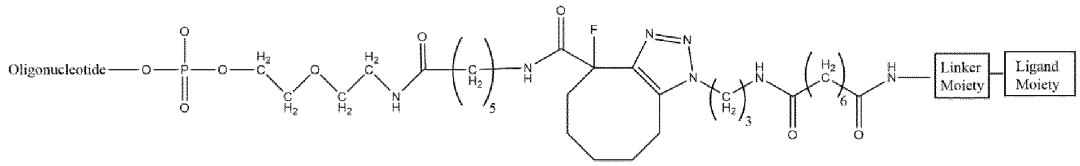
23. A compound according to Sentence 22, wherein said oligonucleoside is an RNA compound capable of modulating, preferably inhibiting, expression of a target gene.

24. A compound according to Sentence 23, wherein said RNA compound comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends.

25. A compound according to Sentence 24, wherein the RNA compound is attached at the 5' end of its second strand to the adjacent phosphate.

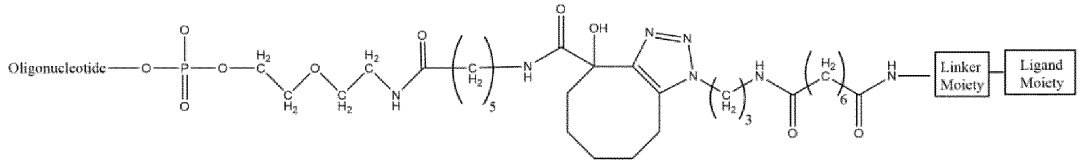
26. A compound according to Sentence 24, wherein the RNA compound is attached at the 3' end of its second strand to the adjacent phosphate.

27. A compound of Formula (II):




Formula (II)

28. A compound of Formula (III):



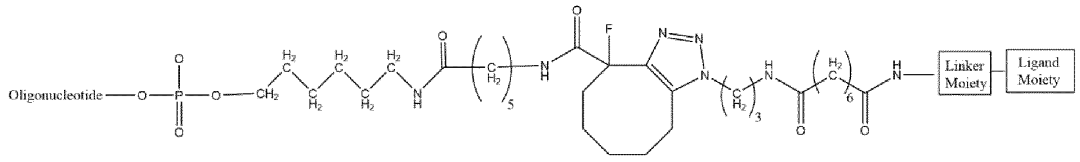

Formula (III)

29. A compound according to Sentence 27 or 28, wherein the oligonucleoside comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends, and wherein said RNA duplex is attached at the 5' end of its second strand to the adjacent phosphate.

30. A composition comprising a compound of Formula (II) as defined in Sentence 27, and a compound of Formula (III) as defined in Sentence 28, optionally dependent on Sentence 29.

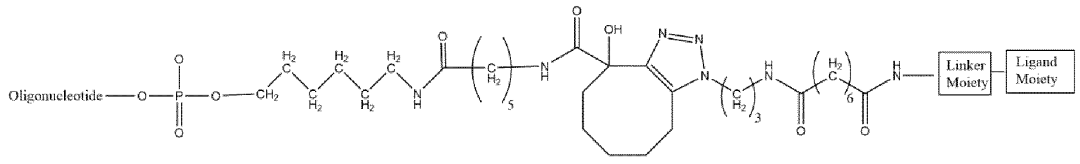
31. A composition according to Sentence 30, wherein said compound of Formula (III) as defined in Sentence 28 is present in an amount in the range of 10 to 15% by weight of said composition.

32. A compound of Formula (IV):




Formula (IV)

33. A compound of Formula (V):




Formula (V)

34. A compound according to Sentence 32 or 33, wherein the oligonucleoside comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends, and wherein said RNA duplex is attached at the 3' end of its second strand to the adjacent phosphate.

35. A composition comprising a compound of Formula (IV) as defined in Sentence 32, and a compound of Formula (V) as defined in Sentence 33, optionally dependent on Sentence 34.

36. A composition according to Sentence 35, wherein said compound of Formula (V) as defined in Sentence 33 is present in an amount in the range of 10 to 15% by weight of said composition.

37. A compound as defined in any of Sentences 1 to 29, or 32 to 34, wherein the oligonucleoside comprises an RNA duplex which further comprises one or more riboses modified at the 2' position, preferably a plurality of riboses modified at the 2' position.

38. A compound according to Sentence 37, wherein the modifications are chosen from 2'-O-methyl, 2'-deoxy-fluoro, and 2'-deoxy.

39. A compound according to any of Sentences 1 to 29, or 32 to 34, or 37 to 38, wherein the oligonucleoside further comprises one or more degradation protective moieties at one or more ends.

40. A compound according to Sentence 39, wherein said one or more degradation protective moieties are not present at the end of the oligonucleoside strand that carries the ligand moieties, and / or wherein said one or more degradation protective moieties is selected from phosphorothioate internucleoside linkages, phosphorodithioate internucleoside linkages and inverted abasic nucleosides, wherein said inverted abasic nucleosides are present at the distal end of the strand that carries the ligand moieties.

41. A compound according to any of Sentences 1 to 29, or 32 to 34, or 37 to 40, wherein said ligand moiety as depicted in Formula (I) in Sentence 1 comprises one or more ligands.

42. A compound according to Sentence 41, wherein said ligand moiety as depicted in Formula (I) in Sentence 1 comprises one or more carbohydrate ligands.

43. A compound according to Sentence 42, wherein said one or more carbohydrates can be a monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide or polysaccharide.

44. A compound according to Sentence 43, wherein said one or more carbohydrates comprise one or more galactose moieties, one or more lactose moieties, one or more N-AcetylGalactosamine moieties, and / or one or more mannose moieties.

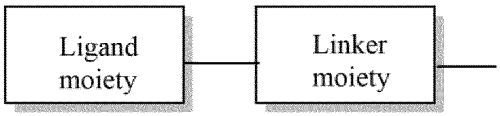
45. A compound according to Sentence 44, wherein said one or more carbohydrates comprise one or more N-Acetyl-Galactosamine moieties.

46. A compound according to Sentence 45, which comprises two or three N-AcetylGalactosamine moieties.

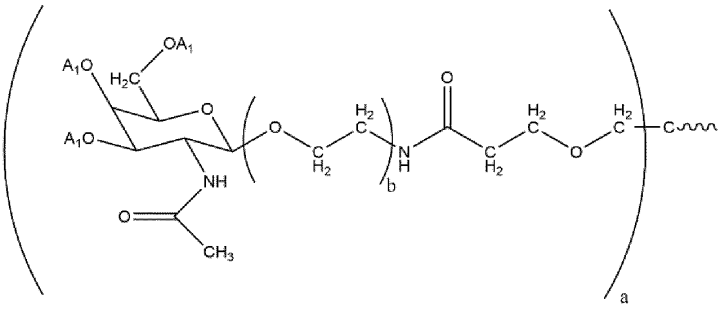
47. A compound according to any of Sentences 41 to 46, wherein said one or more ligands are attached in a linear configuration, or in a branched configuration.

48. A compound according to Sentence 47, wherein said one or more ligands are attached as a biantennary or triantennary branched configuration.

49. A compound according to Sentences 46 to 48, wherein said moiety:




as depicted in Formula (I) in Sentence 1 is any of Formulae (VIa), (VIb) or (VIc), preferably Formula (VIa):



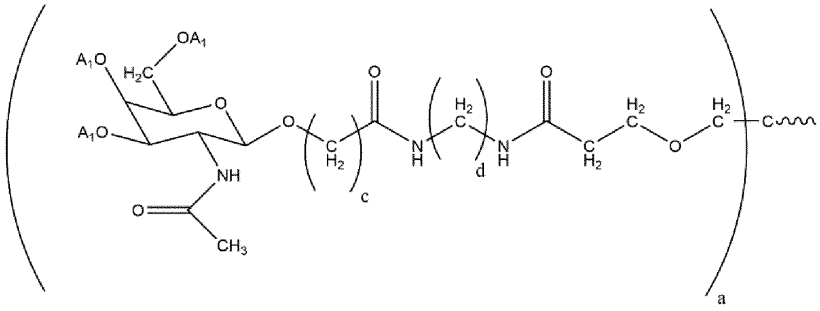

Formula (VIa)

wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

a is an integer of 2 or 3; and

b is an integer of 2 to 5; or



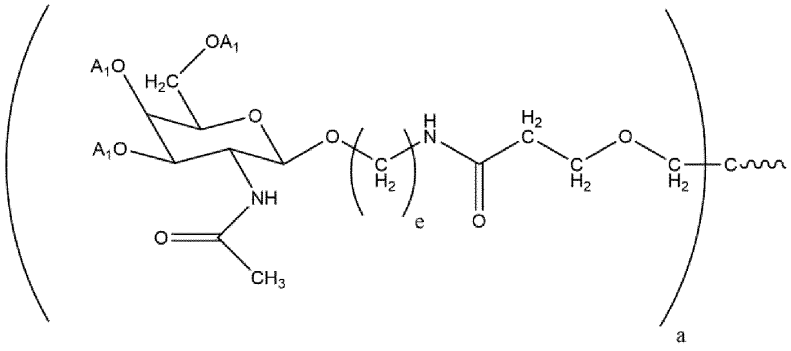
Formula (VIb)

wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

a is an integer of 2 or 3; and

c and d are independently integers of 1 to 6; or



Formula (VIc)

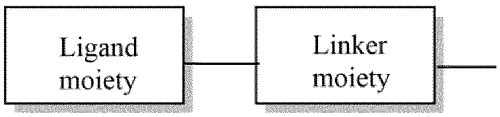
wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

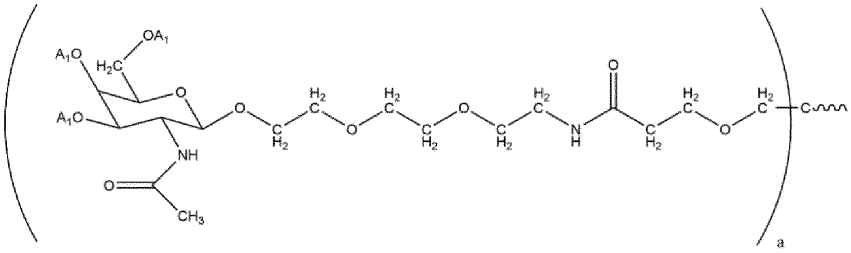
a is an integer of 2 or 3; and

e is an integer of 2 to 10.

50. A compound according to Sentences 46 to 48, wherein said moiety:




as depicted in Formula (I) in Sentence 1 is Formula (VII):



Formula (VII)

wherein:

$A_I$ is hydrogen;

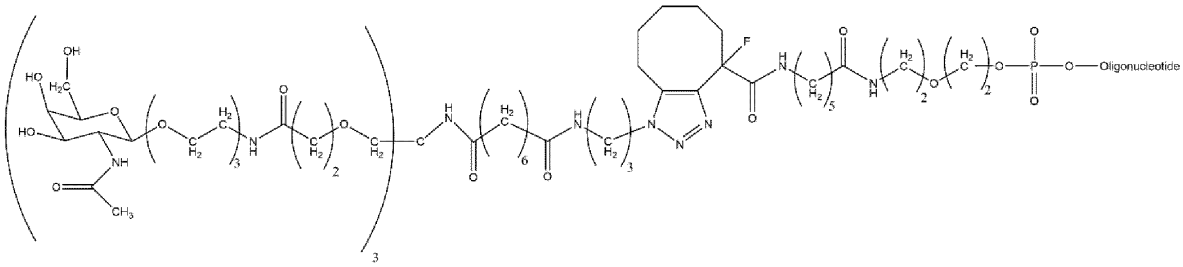
a is an integer of 2 or 3.

51. A compound according to Sentence 49 or 50, wherein a = 2.

52. A compound according to Sentence 49 or 50, wherein a = 3.

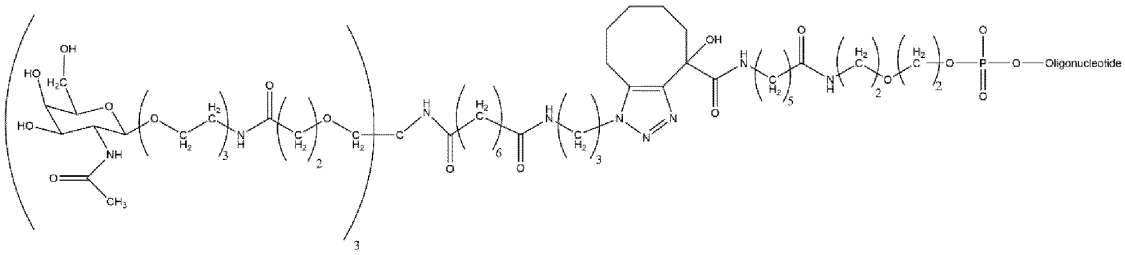
53. A compound according to Sentence 49, wherein b = 3.

54. A compound of Formula (VIII):




Formula (VIII)

55. A compound of Formula (IX):



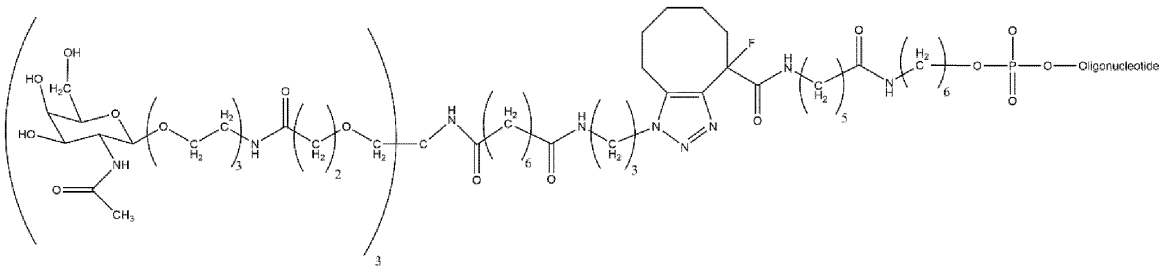

Formula (IX)

56. A compound according to Sentence 54 or 55, wherein the oligonucleoside comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends, and wherein said RNA duplex is attached at the 5' end of its second strand to the adjacent phosphate.

57. A composition comprising a compound of Formula (VIII) as defined in Sentence 54, and a compound of Formula (IX) as defined in Sentence 55, optionally dependent on Sentence 56.

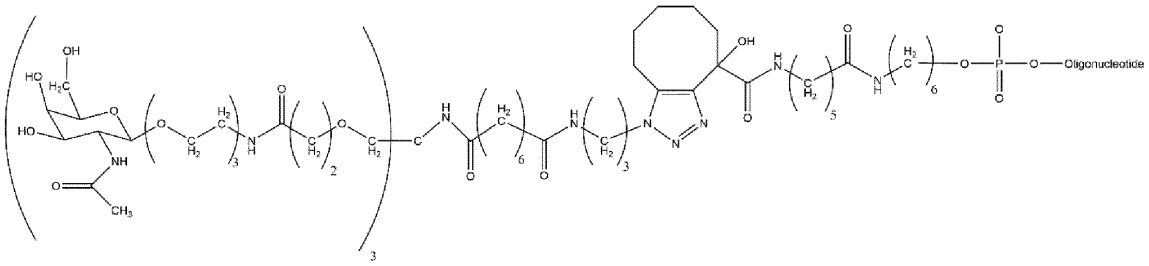
58. A composition according to Sentence 57, wherein said compound of Formula (IX) as defined in Sentence 55 is present in an amount in the range of 10 to 15% by weight of said composition.

59. A compound of Formula (X):




Formula (X)

60. A compound of Formula (XI):




Formula (XI)

61. A compound according to Sentence 59 or 60, wherein the oligonucleoside comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends, and wherein said RNA duplex is attached at the 3' end of its second strand to the adjacent phosphate.

62. A composition comprising a compound of Formula (X) as defined in Sentence 59, and a compound of Formula (XI) as defined in Sentence 60, optionally dependent on Sentence 61.

63. A composition according to Sentence 62, wherein said compound of Formula (XI) as defined in Sentence 60 is present in an amount in the range of 10 to 15% by weight of said composition.

64. A compound as defined in any of Sentences 54 to 63, wherein the oligonucleoside comprises an RNA duplex which further comprises one or more riboses modified at the 2' position, preferably a plurality of riboses modified at the 2' position.

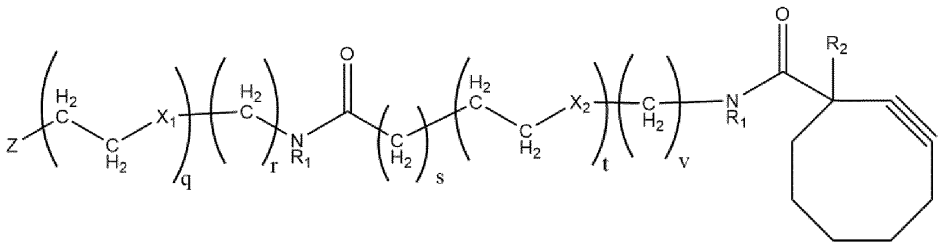
65. A compound according to Sentence 64, wherein the modifications are chosen from 2'-O-methyl, 2'-deoxy-fluoro, and 2'-deoxy.

66. A compound according to any of Sentences 54 to 65, wherein the oligonucleoside further comprises one or more degradation protective moieties at one or more ends.

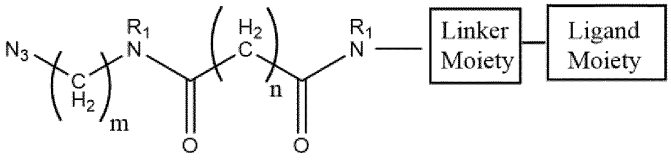
67. A compound according to Sentence 66, wherein said one or more degradation protective moieties are not present at the end of the oligonucleoside strand that carries the ligand moieties, and / or wherein said one or more degradation protective moieties is selected from phosphorothioate internucleoside linkages, phosphorodithioate internucleoside linkages and inverted abasic nucleosides, wherein said inverted abasic nucleosides are

present at the distal end of the strand that carries the ligand moieties, as shown in any of Formulae (VIII), (IX), (X) or (XI) in any of Sentences 54, 55, 59 or 60.

68. A process of preparing a compound according to any of Sentences 1 to 29, 32 to 34, 37 to 56, 59 to 61, and 64 to 67, and / or a composition according to any of Sentences 30, 31, 35, 36, 57, 58, 62, 63, which comprises reacting compounds of Formulae (XII) and (XIII):



Formula (XII)




Formula (XIII)

herein:

$R_1$ at each occurrence is independently selected from the group consisting of hydrogen, methyl and ethyl;

$R_2$ is selected from the group consisting of hydrogen, hydroxy, -$OC_{1-3}$alkyl, -$C(=O)OC_{1-3}$alkyl, halo and nitro;

$X_1$ and $X_2$ at each occurrence are independently selected from the group consisting of methylene, oxygen and sulfur;

m is an integer of from 1 to 6;

n is an integer of from 1 to 10;

q, r, s, t, v are independently integers from 0 to 4, with the proviso that:

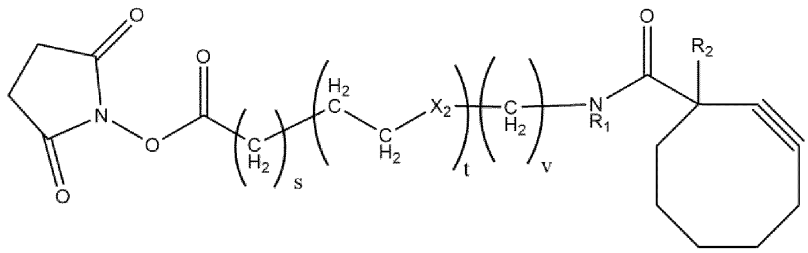
(i) q and r cannot both be 0 at the same time; and

(ii) s, t and v cannot all be 0 at the same time;

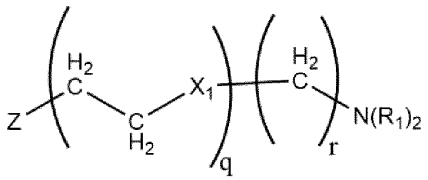
Z is an oligonucleoside moiety;

and where appropriate carrying out deprotection of the ligand and / or annealing of a second strand for the oligonucleoside moiety.

69. A process according to Sentence 68, wherein a compound of Formula (XII) is prepared by reacting compounds of Formulae (XIV) and (XV):



Formula (XIV)



Formula (XV)

$R_1$ at each occurrence is independently selected from the group consisting of hydrogen, methyl and ethyl;

$R_2$ is selected from the group consisting of hydrogen, hydroxy, -$OC_{1-3}$alkyl, -$C(=O)OC_{1-3}$alkyl, halo and nitro;

$X_1$ and $X_2$ at each occurrence are independently selected from the group consisting of methylene, oxygen and sulfur;

q, r, s, t, v are independently integers from 0 to 4, with the proviso that:

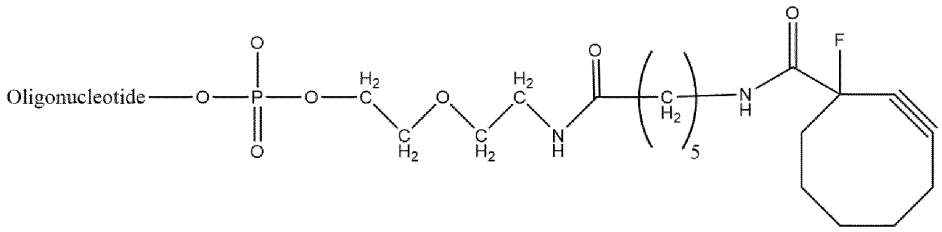
(i) q and r cannot both be 0 at the same time; and

(ii) s, t and v cannot all be 0 at the same time;

Z is an oligonucleoside moiety.

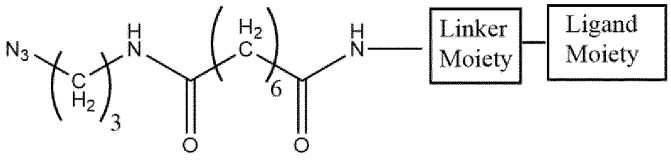
70. A process according to Sentence 68, to prepare a compound according to any of Sentences 20, 25, 27, 29, 54, 56, and / or a composition according to any of Sentences 30, 31, 57, 58, wherein:

compound of Formula (XII) is Formula (XIIa):




Formula (XIIa)

and compound of Formula (XIII) is Formula (XIIIa):

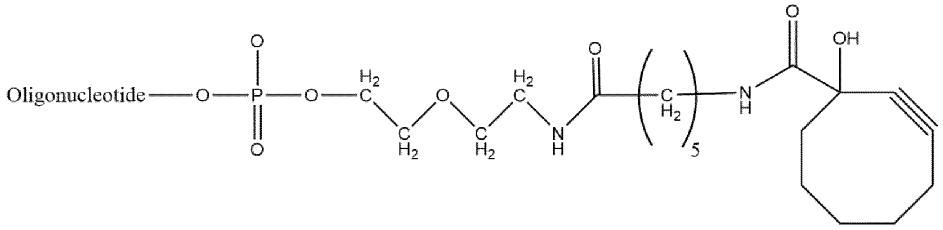



Formula (XIIIa)

wherein the oligonucleoside comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends, and wherein said RNA duplex is attached at the 5' end of its second strand to the adjacent phosphate.

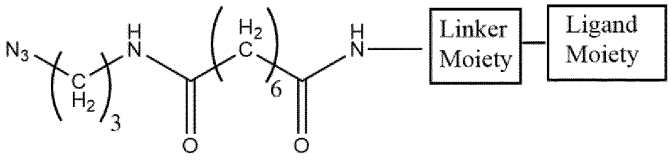
71. A process according to Sentence 68, to prepare a compound according to any of Sentences 20, 25, 28, 29, 55, 56, and / or a composition according to any of Sentences 30, 31, 57, 58, wherein:

compound of Formula (XII) is Formula (XIIb):




Formula (XIIb)

and compound of Formula (XIII) is Formula (XIIIa):

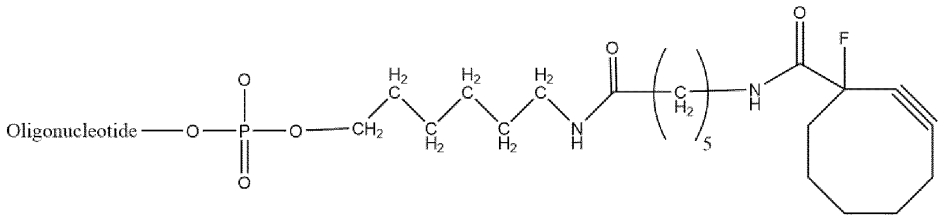



Formula (XIIIa)

wherein the oligonucleoside comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends, and wherein said RNA duplex is attached at the 5' end of its second strand to the adjacent phosphate.

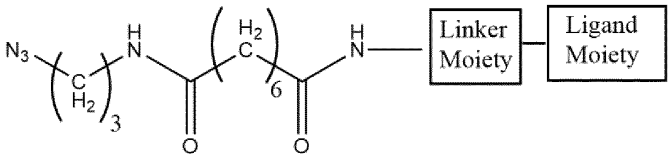
72. A process according to Sentence 68, to prepare a compound according to any of Sentences 21, 26, 32, 34, 59, 61, and / or a composition according to any of Sentences 35, 36, 62, 63, wherein:

compound of Formula (XII) is Formula (XIIc):




Formula (XIIc)

and compound of Formula (XIII) is Formula (XIIIa):

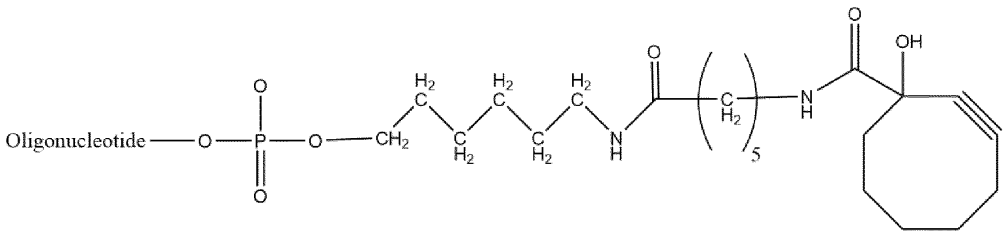



Formula (XIIIa)

wherein the oligonucleoside comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends, and wherein said RNA duplex is attached at the 3' end of its second strand to the adjacent phosphate.

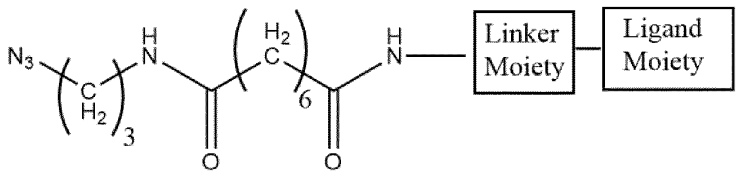
73. A process according to Sentence 68, to prepare a compound according to any of Sentences 21, 26, 33, 34, 60, 61, and / or a composition according to any of Sentences 35, 36, 62, 63, wherein:

compound of Formula (XII) is Formula (XIId):




Formula (XIId)

and compound of Formula (XIII) is Formula (XIIIa):



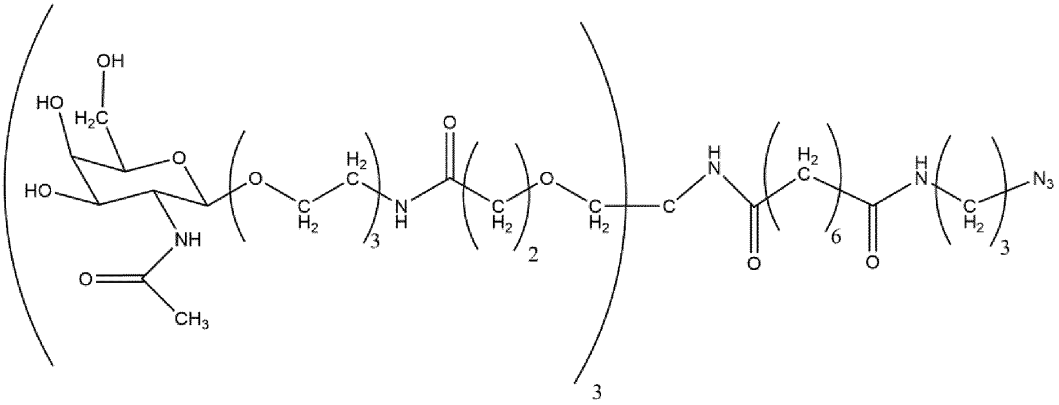

Formula (XIIIa)

wherein the oligonucleoside comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of

the first and second strands have 5' and 3' ends, and wherein said RNA duplex is attached at the 3' end of its second strand to the adjacent phosphate.

74. A process according to any of Sentences 70 to 73, wherein:

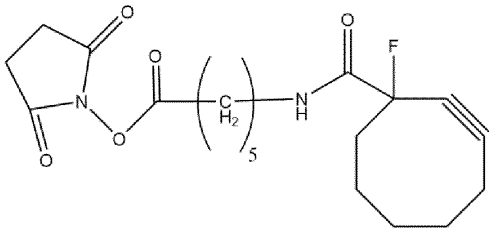
compound of Formula (XIIIa) is Formula (XIIIb):



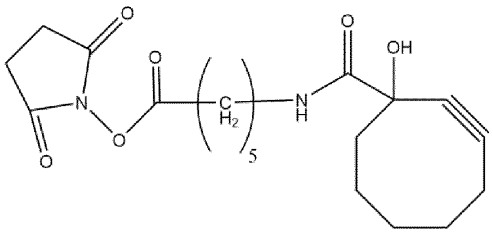
Formula (XIIIb)

75. A process according to Sentences 69, as dependent on Sentences 70 to 73, wherein:

compound of Formula (XIV) is either Formula (XIVa) or Formula (XIVb):

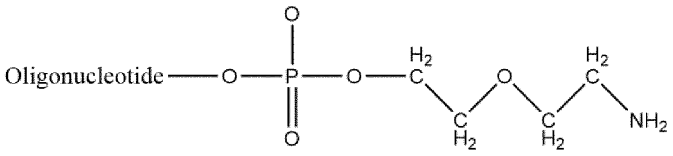


Formula (XIVa)

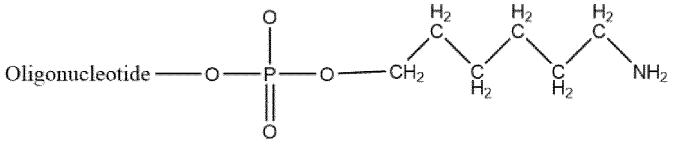


Formula (XIVb)

and compound of Formula (XV) is either Formula (XVa) or Formula (XIVb):



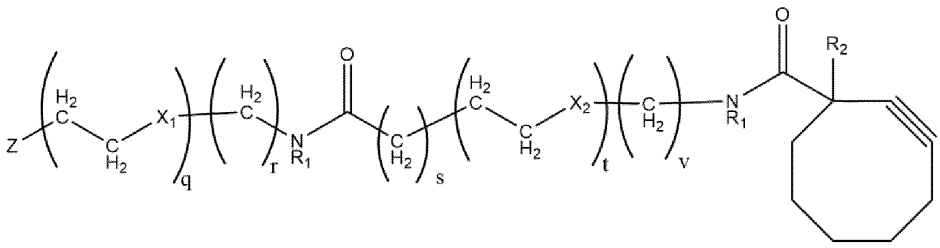
Formula (XVa)



Formula (XVb)

wherein the oligonucleoside comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends, and wherein (i) said RNA duplex is attached at the 5' end of its second strand to the adjacent phosphate in Formula (XVa), or (ii) said RNA duplex is attached at the 3' end of its second strand to the adjacent phosphate in Formula (XVb).

76. A compound of Formula (XII):



Formula (XII)

wherein:

$R_1$ at each occurrence is independently selected from the group consisting of hydrogen, methyl and ethyl;

$R_2$ is selected from the group consisting of hydrogen, hydroxy, -$OC_{1-3}$alkyl, -$C(=O)OC_{1-3}$alkyl, halo and nitro;

$X_1$ and $X_2$ at each occurrence are independently selected from the group consisting of methylene, oxygen and sulfur;

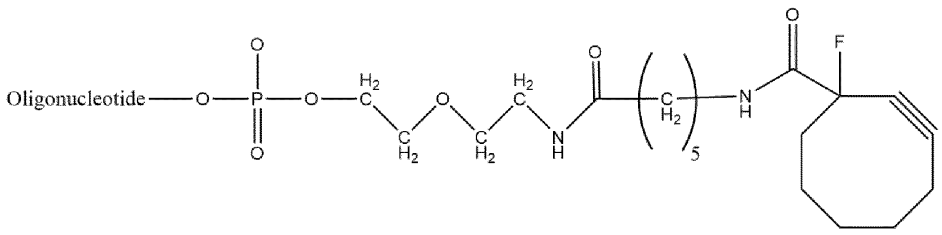
q, r, s, t, v are independently integers from 0 to 4, with the proviso that:

(i) q and r cannot both be 0 at the same time; and

(ii) s, t and v cannot all be 0 at the same time;

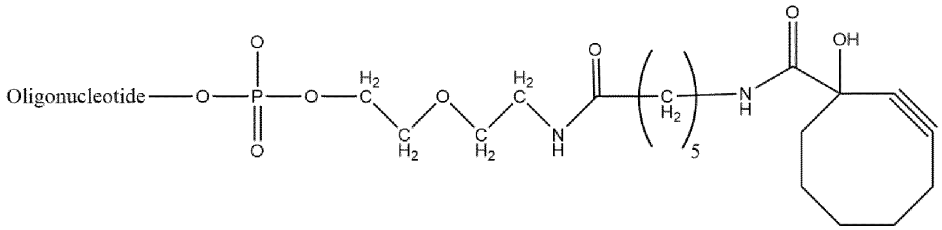
Z is an oligonucleoside moiety.

77. A compound of Formula (XIIa):



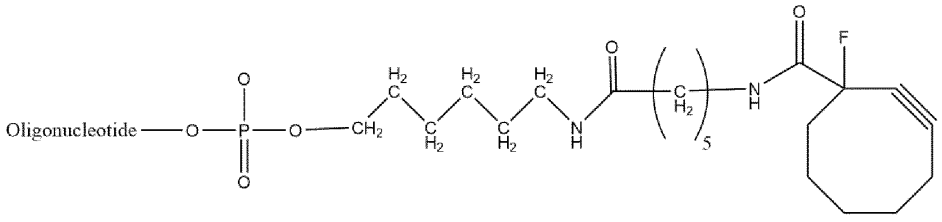

Formula (XIIa)

78. A compound of Formula (XIIb):



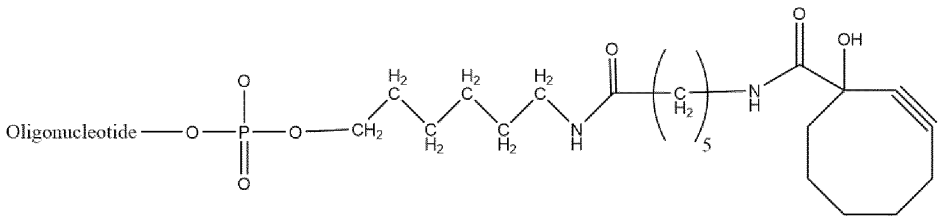

Formula (XIIb)

79. A compound of Formula (XIIc):



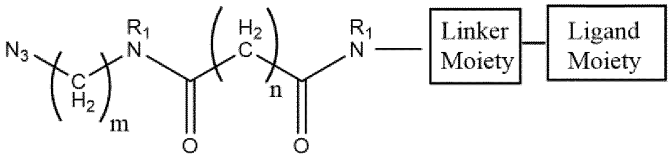

Formula (XIIc)

80. A compound of Formula (XIId):




Formula (XIId)

81. A compound of Formula (XIII):




Formula (XIII)

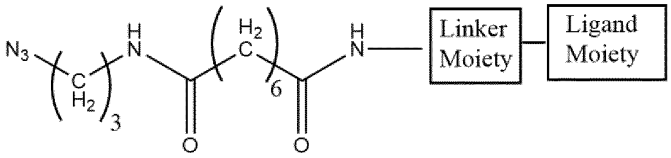
wherein:

$R_1$ at each occurrence is independently selected from the group consisting of hydrogen, methyl and ethyl;

m is an integer of from 1 to 6;

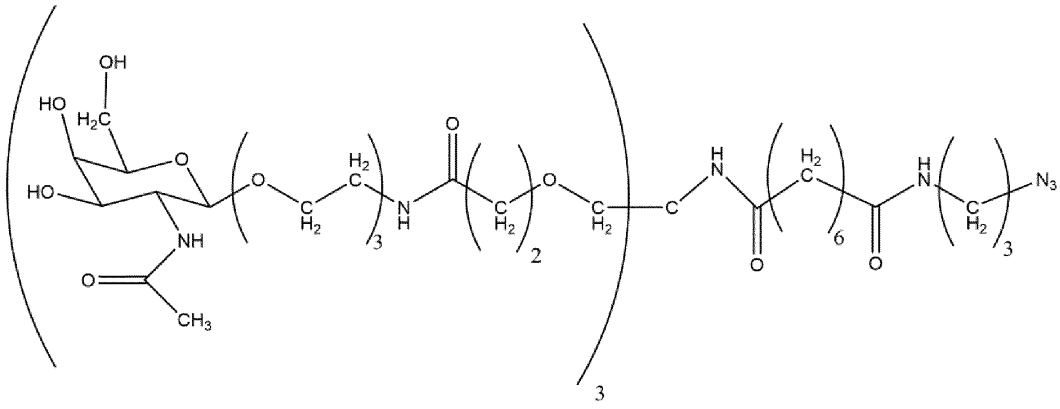
n is an integer of from 1 to 10.

82. A compound of Formula (XIIIa):



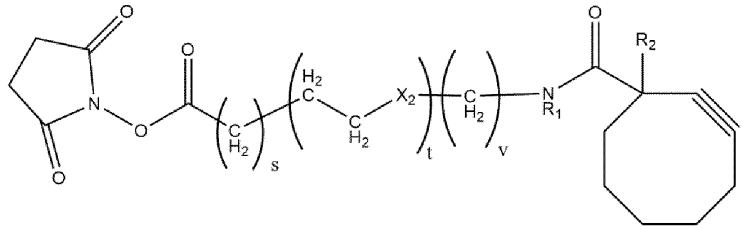

Formula (XIIIa)

83. A compound of Formula (XIIIb):



Formula (XIIIb)

84. A compound of Formula (XIV):



Formula (XIV)

wherein:

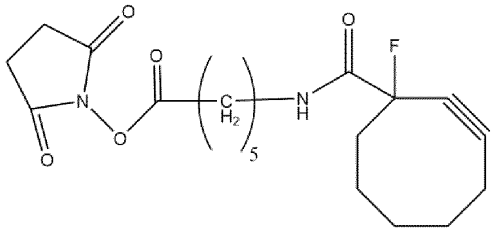
$R_1$ is selected from the group consisting of hydrogen, methyl and ethyl;

$R_2$ is selected from the group consisting of hydrogen, hydroxy, -$OC_{1-3}$alkyl, -$C(=O)OC_{1-3}$alkyl, halo and nitro;

$X_2$ is selected from the group consisting of methylene, oxygen and sulfur;

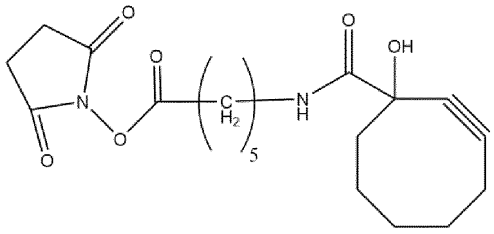
s, t, v are independently integers from 0 to 4, with the proviso that s, t and v cannot all be 0 at the same time.

85. A compound of Formula (XIVa):



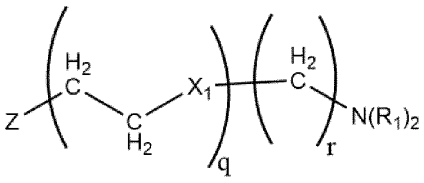
Formula (XIVa)

86. A compound of Formula (XIVb):



Formula (XIVb)

87. A compound of Formula (XV):



Formula (XV)

wherein:

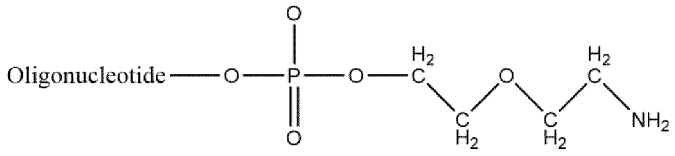
$R_1$ at each occurrence is independently selected from the group consisting of hydrogen, methyl and ethyl;

$X_1$ is selected from the group consisting of methylene, oxygen and sulfur;

q and r are independently integers from 0 to 4, with the proviso that q and r cannot both be 0 at the same time;

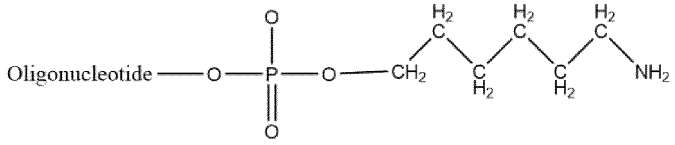
Z is an oligonucleoside moiety.

88. A compound of Formula (XVa):




Formula (XVa)

89. A compound of Formula (XVb):




Formula (XVb)

90. Use of a compound according to any of Sentences 76, 81 to 84, 87, for the preparation of a compound according to any of Sentences 1 to 29, 32 to 34, 37 to 56, 59 to 61, and 64 to 67, and / or a composition according to any of Sentences 30, 31, 35, 36, 57, 58, 62 and 63.

91. Use of a compound according to Sentence 85, for the preparation of a compound according to any of Sentences 1 to 29, 32 to 34, 37 to 56, 59 to 61, and 64 to 67, and / or a composition according to any of Sentences 30, 31, 35, 36, 57, 58, 62 and 63, wherein $R_2$ = F.

92. Use of a compound according to Sentence 86, for the preparation of a compound according to any of Sentences 1 to 29, 32 to 34, 37 to 56, 59 to 61, and 64 to 67, and / or a composition according to any of Sentences 30, 31, 35, 36, 57, 58, 62 and 63, wherein $R_2$ = OH.

93. Use of a compound according to Sentence 77, for the preparation of a compound according to any of Sentences 20, 25, 27, 29, 54, 56, and / or a composition according to any of Sentences 30, 31, 57, 58.

94. Use of a compound according to Sentence 78, for the preparation of a compound according to any of Sentences 20, 25, 28, 29, 55, 56, and / or a composition according to any of Sentences 30, 31, 57, 58.

95. Use of a compound according to Sentence 79, for the preparation of a compound according to any of Sentences 21, 26, 32, 34, 59, 61, and / or a composition according to any of Sentences 35, 36, 62, 63.

96. Use of a compound according to Sentence 80, for the preparation of a compound according to any of Sentences 21, 26, 33, 34, 60, 61, and / or a composition according to any of Sentences 35, 36, 62, 63.

97. Use of a compound according to Sentence 88, for the preparation of a compound according to any of Sentences 20, 25, 27 to 29, 54 to 56, and / or a composition according to any of Sentences 30, 31, 57, 58.

98. Use of a compound according to Sentence 89, for the preparation of a compound according to any of Sentences 21, 26, 32 to 34, 59 to 61, and / or a composition according to any of Sentences 35, 36, 62, 63.

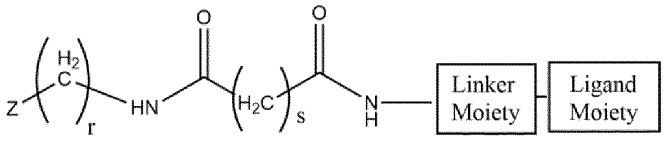
99. A compound or composition obtained, or obtainable by a process according to any of Sentences 68 to 75.

100. A pharmaceutical composition comprising of a compound according to any of Sentences 1 to 29, 32 to 34, 37 to 56, 59 to 61, and 64 to 67, and / or a composition according to any of Sentences 30, 31, 35, 36, 57, 58, 62 and 63, together with a pharmaceutically acceptable carrier, diluent or excipient.

101. A compound according to any of Sentences 1 to 29, 32 to 34, 37 to 56, 59 to 61, and 64 to 67, and / or a composition according to any of Sentences 30, 31, 35, 36, 57, 58, 62 and 63, for use in therapy.

In another aspect the present invention may be applied in the compounds, processes, compositions or uses of the following Clauses numbered 1-56 wherein reference to any Formula in the Clauses refers only to those Formulas that are defined within Clause 1-56. These formulae are reproduced in Figure 7. Specifically, an oligonucleoside moiety as represented by Z in any of the following clauses can comprise a nucleic acid for inhibiting expression of CFHR4 as defined in any of the claims hereinafter.

1. A compound comprising the following structure:




Formula (I*)

wherein:

r and s are independently an integer selected from 1 to 16; and

Z is an oligonucleoside moiety.

2. A compound according to Clause 1, wherein s is an integer selected from 4 to 12.

3. A compound according to Clause 2, wherein s is 6.

4. A compound according to any of Clauses 1 to 3, wherein r is an integer selected from 4 to 14.

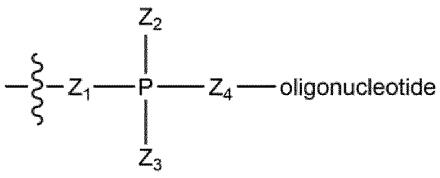
5. A compound according to Clause 4, wherein r is 6.

6. A compound according to Clause 4, wherein r is 12.

7. A compound according to Clause 5, which is dependent on Clause 3.

8. A compound according to Clause 6, which is dependent on Clause 3.

9. A compound according to any of Clauses 1 to 8, wherein Z is:




wherein:

$Z_1$, $Z_2$, $Z_3$, $Z_4$ are independently at each occurrence oxygen or sulfur; and

one the bonds between P and $Z_2$, and P and $Z_3$ is a single bond and the other bond is a double bond.

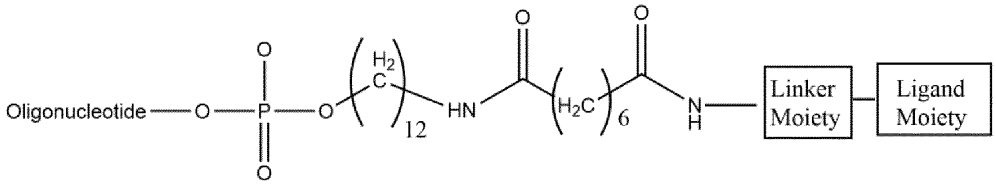
10. A compound according to any of Clauses 1 to 9, wherein said oligonucleoside is an RNA compound capable of modulating, preferably inhibiting, expression of a target gene.

11. A compound according to any of Clause 10, wherein said RNA compound comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends.

12. A compound according to Clause 11, preferably also dependent on Clauses 3 and 6, wherein the RNA compound is attached at the 5' end of its second strand to the adjacent phosphate.

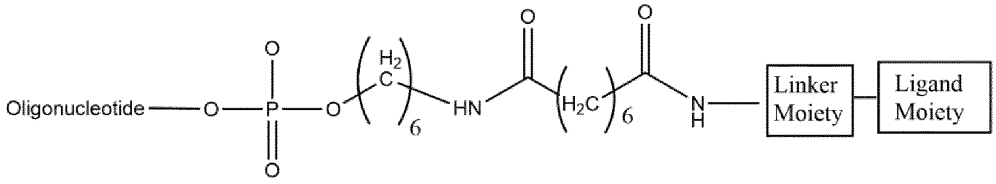
13. A compound according to Clause 11, preferably also dependent on Clauses 3 and 5, wherein the RNA compound is attached at the 3' end of its second strand to the adjacent phosphate.

14. A compound of Formula (II*), preferably dependent on Clause 12:




Formula (II*)

15. A compound of Formula (III*), preferably dependent on Clause 13:




Formula (III*)

16. A compound as defined in any of Clauses 1 to 15, wherein the oligonucleoside comprises an RNA duplex which further comprises one or more riboses modified at the 2' position, preferably a plurality of riboses modified at the 2' position.

17. A compound according to Clause 16, wherein the modifications are chosen from 2'-O-methyl, 2'-deoxy-fluoro, and 2'-deoxy.

18. A compound according to any of Clauses 1 to 17, wherein the oligonucleoside further comprises one or more degradation protective moieties at one or more ends.

19. A compound according to Clause 18, wherein said one or more degradation protective moieties are not present at the end of the oligonucleoside strand that carries the linker / ligand moieties, and / or wherein said one or more degradation protective moieties is selected from phosphorothioate internucleoside linkages, phosphorodithioate internucleoside linkages and inverted abasic nucleosides, wherein said inverted abasic nucleosides are present at the distal end of the same strand to the end that carries the linker / ligand moieties.

20. A compound according to any of Clauses 1 to 19, wherein said ligand moiety as depicted in Formula (I*) in Clause 1 comprises one or more ligands.

21. A compound according to Clause 20, wherein said ligand moiety as depicted in Formula (I*) in Clause 1 comprises one or more carbohydrate ligands.

22. A compound according to Clause 21, wherein said one or more carbohydrates can be a monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide or polysaccharide.

23. A compound according to Clause 22, wherein said one or more carbohydrates comprise one or more galactose moieties, one or more lactose moieties, one or more N-AcetylGalactosamine moieties, and / or one or more mannose moieties.

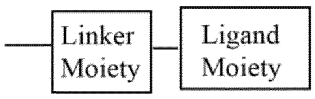
24. A compound according to Clause 23, wherein said one or more carbohydrates comprise one or more N-Acetyl-Galactosamine moieties.

25. A compound according to Clause 24, which comprises two or three N-AcetylGalactosamine moieties.

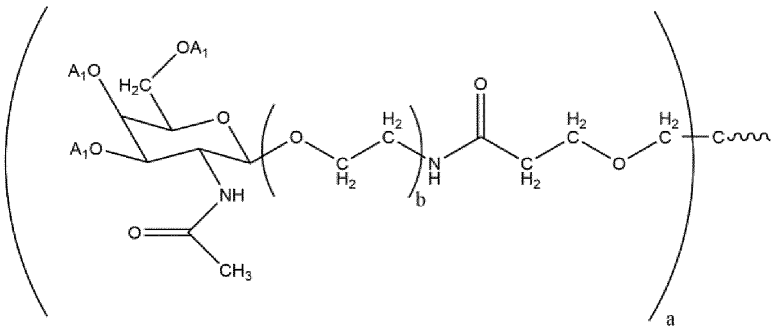
26. A compound according to any of the preceding Clauses, wherein said one or more ligands are attached in a linear configuration, or in a branched configuration.

27. A compound according to Clause 26, wherein said one or more ligands are attached as a biantennary or triantennary branched configuration.

28. A compound according to Clauses 20 to 27, wherein said moiety:




as depicted in Formula (I*) in Clause 1 is any of Formulae (IV*), (V*) or (VI*), preferably Formula (IV*):



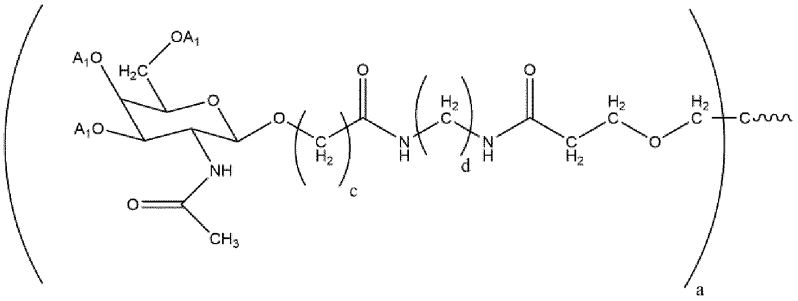

Formula (IV*)

wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

a is an integer of 2 or 3; and

b is an integer of 2 to 5; or



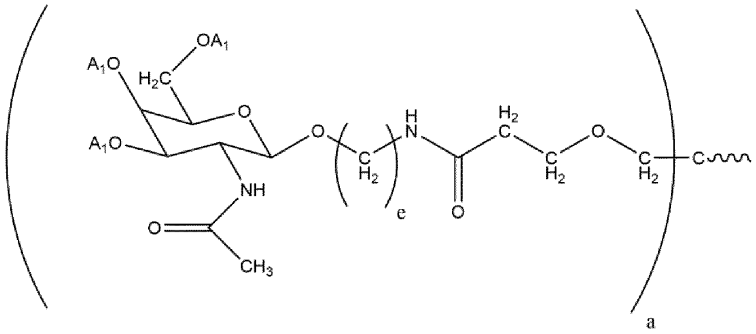

Formula (V*)

wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

a is an integer of 2 or 3; and

c and d are independently integers of 1 to 6; or



Formula (VI*)

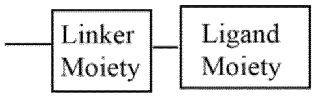
wherein:

$A_I$ is hydrogen, or a suitable hydroxy protecting group;

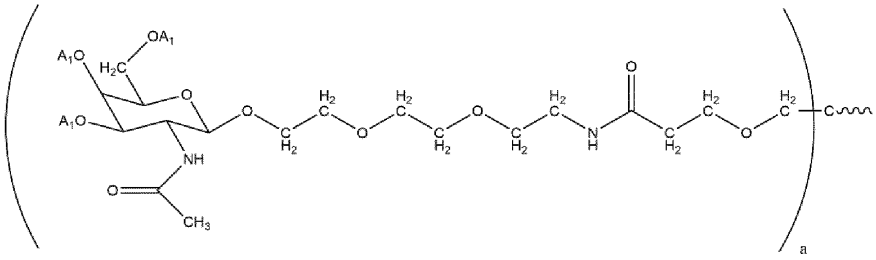
a is an integer of 2 or 3; and

e is an integer of 2 to 10.

29. A compound according to any of Clauses 1 to 28, wherein said moiety:




as depicted in Formula (I*) in Clause 1 is Formula (VII*):



Formula (VII*)

wherein:

$A_I$ is hydrogen;

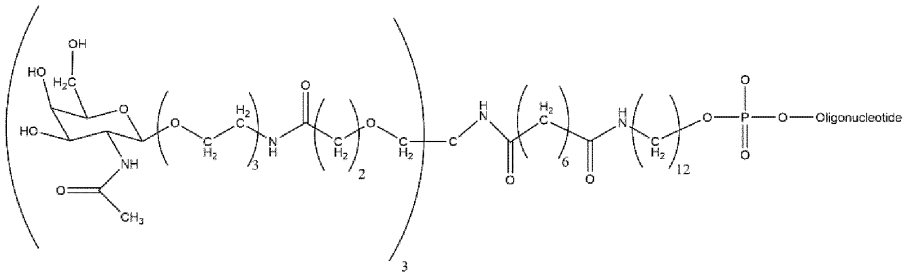
a is an integer of 2 or 3.

30. A compound according to Clause 28 or 29, wherein a = 2.

31. A compound according to Clause 28 or 29, wherein a = 3.

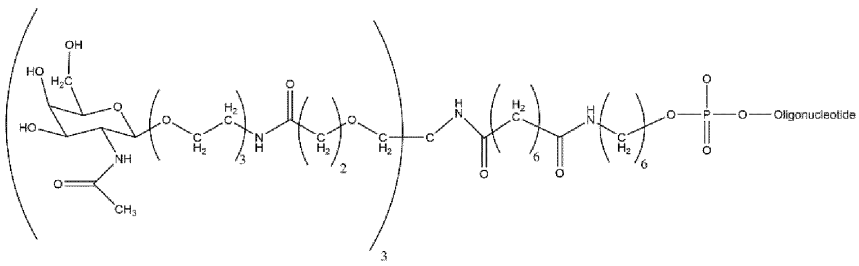
32. A compound according to Clause 28, wherein b = 3.

33. A compound of Formula (VIII*):




Formula (VIII*)

34. A compound of Formula (IX*):




Formula (IX*)

35. A compound according to Clause 33 or 34, wherein the oligonucleoside comprises an RNA duplex which further comprises one or more riboses modified at the 2' position, preferably a plurality of riboses modified at the 2' position.

36. A compound according to Clause 35, wherein the modifications are chosen from 2'-O-methyl, 2'-deoxy-fluoro, and 2'-deoxy.

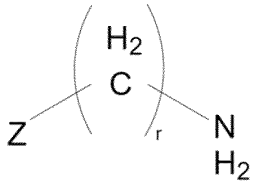
37. A compound according to any of Clauses 33 to 36, wherein the oligonucleoside further comprises one or more degradation protective moieties at one or more ends.

38. A compound according to Clause 37, wherein said one or more degradation protective moieties are not present at the end of the oligonucleoside strand that carries the linker / ligand moieties, and / or wherein said one or more degradation protective moieties is selected from phosphorothioate internucleoside linkages, phosphorodithioate internucleoside linkages and inverted abasic nucleosides, wherein said inverted abasic nucleosides are present at the distal end of the same strand to the end that carries the linker / ligand moieties.

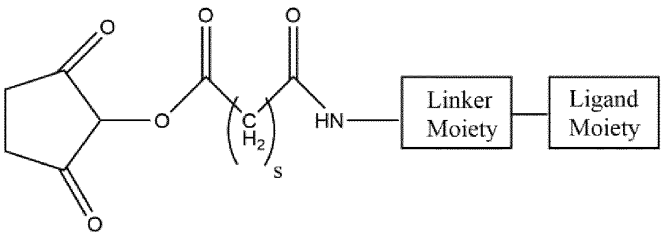
39. A compound according to Clause 33, wherein the oligonucleoside comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends, and wherein said RNA duplex is attached at the 5' end of its second strand to the adjacent phosphate.

40. A compound according to Clause 34, wherein the oligonucleoside comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends, and wherein said RNA duplex is attached at the 3' end of its second strand to the adjacent phosphate.

41. A process of preparing a compound according to any of Clauses 1 to 40, which comprises reacting compounds of Formulae (X*) and (XI*):




Formula (X*)



Formula (XI*)

wherein:

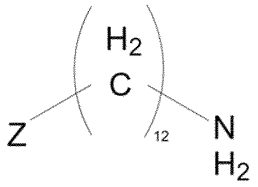
r and s are independently an integer selected from 1 to 16; and

Z is an oligonucleoside moiety;

and where appropriate carrying out deprotection of the ligand and / or annealing of a second strand for the oligonucleoside.

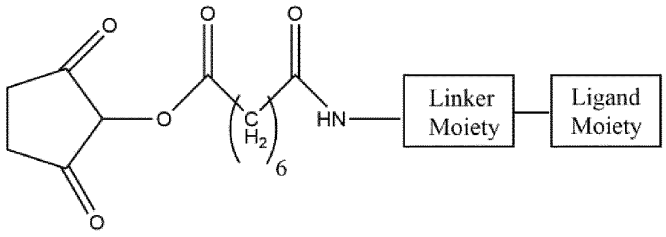
42. A process according to Clause 41, to prepare a compound according to any of Clauses 6, 8 to 14, 16 to 33, and 35 to 40, wherein:

compound of Formula (X*) is Formula (Xa*):




Formula (Xa*)

and compound of Formula (XI*) is Formula (XIa*):

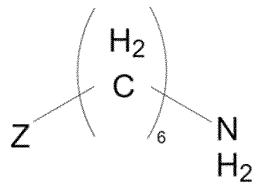



Formula (XIa*)

wherein the oligonucleoside comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends, and wherein said RNA duplex is attached at the 5' end of its second strand to the adjacent phosphate.

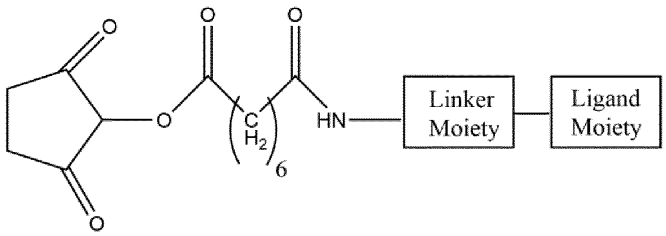
43. A process according to Clause 41, to prepare a compound according to any of Clauses 5, 7, 9 to 13, 15 to 32, and 34 to 40, wherein:

compound of Formula (X*) is Formula (Xb*):




Formula (Xb*)

and compound of Formula (XI*) is Formula (XIa*):

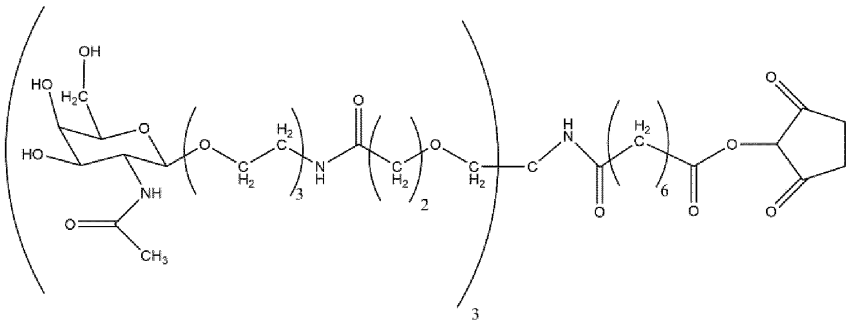



Formula (XIa*)

wherein the oligonucleoside comprises an RNA duplex comprising first and second strands, wherein the first strand is at least partially complementary to an RNA sequence of a target gene, and the second strand is at least partially complementary to said first strand, and wherein each of the first and second strands have 5' and 3' ends, and wherein said RNA duplex is attached at the 3' end of its second strand to the adjacent phosphate.

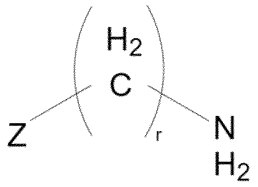
44. A process according to Clauses 42 or 43, wherein:

compound of Formula (XIa*) is Formula (XIb*):



Formula (XIb*)

45. A compound of Formula (X*):



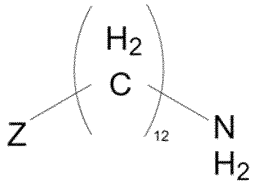

Formula (X*)

wherein:

r is independently an integer selected from 1 to 16; and

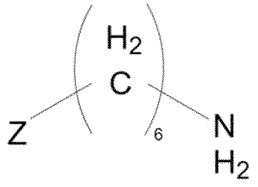
Z is an oligonucleoside moiety.

46. A compound of Formula (Xa*):



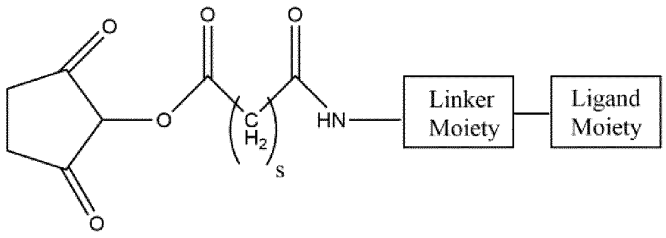

Formula (Xa*)

47. A compound of Formula (Xb*):




Formula (Xb*)

48. A compound of Formula (XI*):



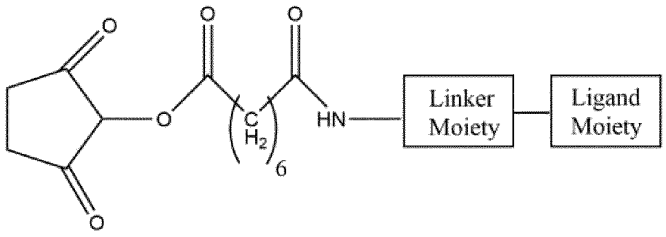

Formula (XI*)

wherein:

s is independently an integer selected from 1 to 16; and

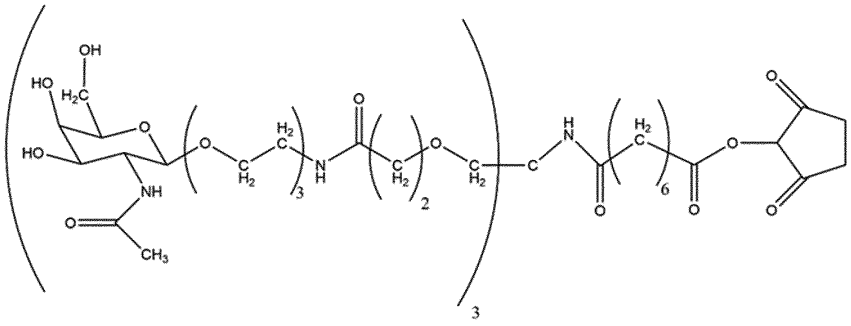
Z is an oligonucleoside moiety.

49. A compound of Formula (XIa*):




Formula (XIa*)

50. A compound of Formula (XIb*):




Formula (XIb*)

51. Use of a compound according to any of Clauses 45 and 48 to 50, for the preparation of a compound according to any of Clauses 1 to 40.

52. Use of a compound according to Clause 46, for the preparation of a compound according to any of Clauses 6, 8 to 14, 16 to 33, and 35 to 40.

53. Use of a compound according to Clause 47, for the preparation of a compound according to any of Clauses 5, 7, 9 to 13, 15 to 32, and 34 to 40.

54. A compound or composition obtained, or obtainable by a process according to any of Clauses 41 to 44.

55. A pharmaceutical composition comprising of a compound according to any of Clauses 1 to 40, together with a pharmaceutically acceptable carrier, diluent or excipient.

A compound according to any of Clauses 1 to 40, for use in therapy.

## EXAMPLES

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

**Example 1: Synthesis of tether 1**

General Experimental conditions:

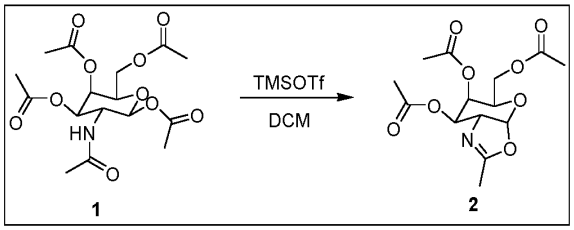
Thin layer chromatography (TLC) was performed on silica-coated aluminium plates with fluorescence indicator 254 nm from Macherey-Nagel. Compounds were visualized under UV light (254 nm), or after spraying with the 5% $H_2SO_4$ in methanol (MeOH) or ninhydrin reagent according to Stahl (from Sigma-Aldrich), followed by heating. Flash chromatography was performed with a Biotage Isolera One flash chromatography instrument equipped with a dual variable UV wavelength detector (200-400 nm) using Biotage Sfär Silica 10, 25, 50 or 100 g columns (Uppsala, Sweden).

All moisture-sensitive reactions were carried out under anhydrous conditions using dry glassware, anhydrous solvents, and argon atmosphere. All commercially available reagents were purchased from Sigma-Aldrich and solvents from Carl Roth GmbH + Co. KG. D-Galactosamine pentaacetate was purchased from AK scientific.

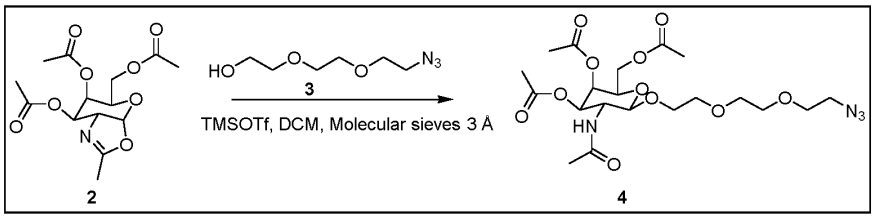
HPLC/ESI-MS was performed on a Dionex UltiMate 3000 RS UHPLC system and Thermo Scientific MSQ Plus Mass spectrometer using an Acquity UPLC Protein BEH C4 column from Waters (300Å, 1.7 µm, 2.1 x 100 mm) at 60 °C. The solvent system consisted of solvent A with $H_2O$ containing 0.1% formic acid and solvent B with acetonitrile (ACN) containing 0.1% formic acid. A gradient from 5-100% of B over 15 min with a flow rate of 0.4 mL/min was employed. Detector and conditions: Corona ultra-charged aerosol detection (from esa). Nebulizer Temp.: 25 °C. $N_2$ pressure: 35.1 psi. Filter: Corona.

$_1H$ and $_{13}C$ NMR spectra were recorded at room temperature on a Varian spectrometer at 500 MHz ($_1H$ NMR) and 125 MHz ($_{13}C$ NMR). Chemical shifts are given in ppm referenced to the solvent residual peak ($CDCl_3$ – $_1H$ NMR: δ at 7.26 ppm and $_{13}C$ NMR δ at 77.2 ppm; DMSO-d6 – $_1H$ NMR: δ at 2.50 ppm and $_{13}C$ NMR δ at 39.5 ppm). Coupling constants are given in Hertz. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t) or multiplet (m).

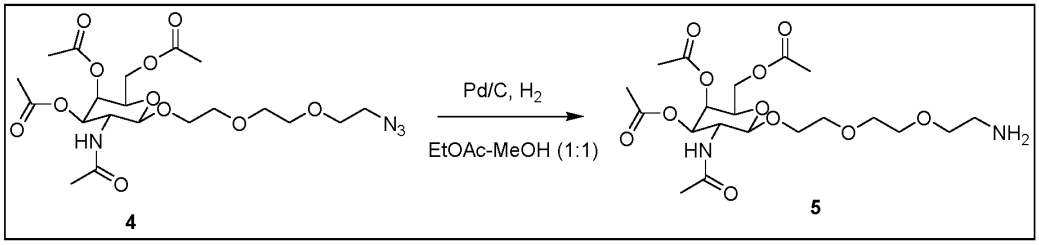
Synthesis route for the conjugate building block TriGalNAc _Tether1:



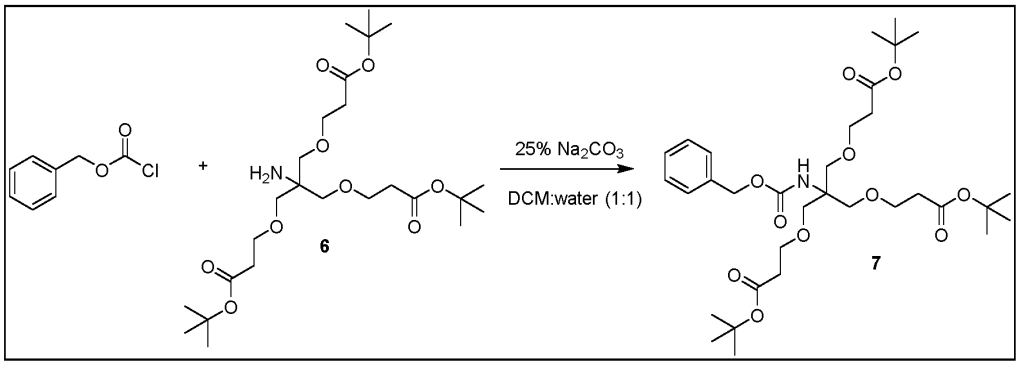

Preparation of compound 2: D-Galactosamine pentaacetate (3.00 g, 7.71 mmol, 1.0 eq.) was dissolved in anhydrous dichloromethane (DCM) (30 mL) under argon and trimethylsilyl trifluoromethanesulfonate (TMSOTf, 4.28 g, 19.27 mmol, 2.5 eq.) was added. The reaction was stirred at room temperature for 3 h. The reaction mixture was diluted with DCM (50 mL) and washed with cold saturated aq. $NaHCO_3$ (100 mL) and water (100 mL). The organic layer was separated, dried over $Na_2SO_4$ and concentrated to afford the title compound as yellow oil, which was purified by flash chromatography (gradient elution: 0-10% MeOH in DCM in 10 CV). The product was obtained as colourless oil (2.5 g, 98%, rf= 0.45 (2% MeOH in DCM)).



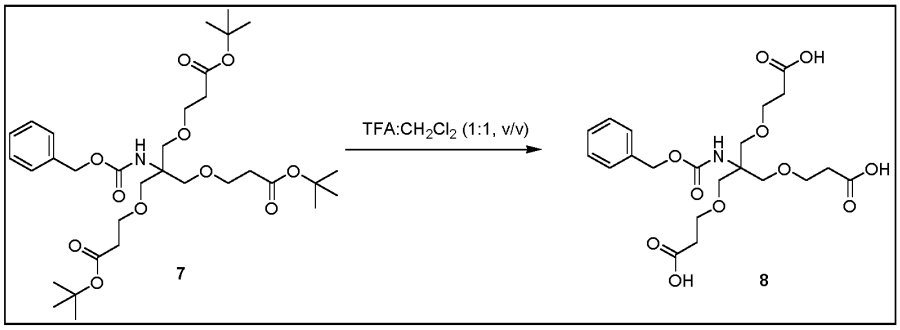
Preparation of compound 4: Compound 2 (2.30 g, 6.98 mmol, 1.0 eq.) and azido-PEG3-OH (1.83 g, 10.5 mmol, 1.5 eq.) were dissolved in anhydrous DCM (40 mL) under argon and molecular sieves 3 Å (5 g) were added to the solution. The mixture was stirred at room temperature for 1 h. TMSOTf (0.77 g, 3.49 mmol, 0.5 eq.) was then added to the mixture and the reaction was stirred overnight. The molecular sieves were filtered, the filtrate was diluted with DCM (100 mL) and washed with cold saturated aq. $NaHCO_3$ (100 mL) and water (100 mL). The organic layer was separated, dried over $Na_2SO_4$ and the solvent was removed under reduced pressure. The crude material was purified by flash chromatography (gradient elution: 0-3% MeOH in DCM in 10 CV) to afford the title product as light yellow oil (3.10 g, 88%, rf = 0.25 (2% MeOH in DCM)). MS: calculated for $C_{20}H_{32}N_4O_{11}$, 504.21. Found 505.4. 1H NMR (500 MHz, CDCl3) δ 6.21-6.14 (m, 1H), 5.30 (dd, J = 3.4, 1.1 Hz, 1H), 5.04 (dd, J = 11.2, 3.4 Hz,1H), 4.76 (d, J = 8.6 Hz, 1H), 4.23-4.08 (m, 3H), 3.91-3.80 (m, 3H), 3.74-3.59 (m, 9H), 3.49-3.41 (m, 2H), 2.14 (s, 3H), 2.02 (s, 3H), 1.97 (d, J = 4.2 Hz, 6H). 13C NMR (125 MHz, CDCl3) δ 170.6 (C), 170.5 (C), 170.4 (C), 170.3 (C), 102.1 (CH), 71.6 (CH), 70.8 (CH), 70.6 (CH), 70.5 (CH), 70.3 (CH2), 69.7 (CH2), 68.5 (CH2), 66.6 (CH2), 61.5 (CH2), 23.1 (CH3), 20.7 (3xCH3).




Preparation of compound 5: Compound 4 (1.00 g, 1.98 mmol, 1.0 eq.) was dissolved in a mixture of ethyl acetate (EtOAc) and MeOH (30 mL 1:1 v/v) and Pd/C (100 mg) was added. The reaction mixture was degassed using vacuum/argon cycles (3x) and hydrogenated under balloon pressure overnight. The reaction mixture was filtered through celite and washed with EtOAc (30 mL). The solvent was removed under reduced pressure to afford the title compound as colourless oil (0.95 g, quantitative yield, rf = 0.25 (10% MeOH in DCM)). The compound was used without further purification. MS: calculated for $C_{20}H_{34}N_2O_{11}$, 478.2. Found 479.4.

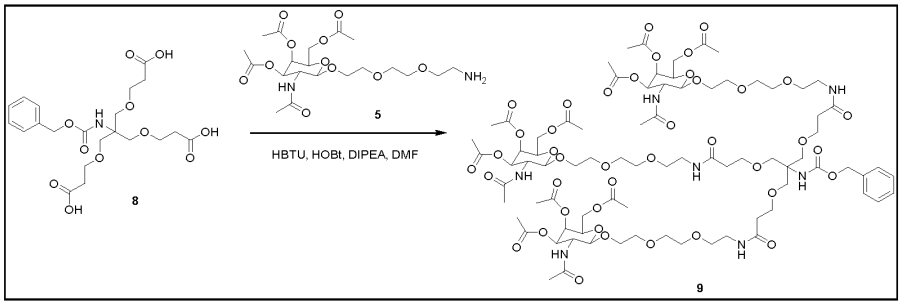



Preparation of compound 7: Tris{[2-(tert-butoxycarbonyl)ethoxy]methyl}-methylamine 6 (3.37 g, 6.67 mmol, 1.0 eq.) was dissolved in a mixture of DCM/water (40 mL 1:1 v/v) and $Na_2CO_3$ (0.18 g, 1.7 mmol, 0.25 eq.) was added while stirring vigorously. Benzyl chloroformate (2.94 mL, 20.7 mmol, 3.10 eq.) was added dropwise to the previous mixture and the reaction was stirred at room temperature for 24 h. The reaction mixture was diluted with $CH_2Cl_2$ (100 mL) and washed with water (100 mL). The organic layer was separated and dried over Na2SO4. The solvent was removed under reduced pressure and the resulting crude material was purified by flash chromatography (gradient elution: 0-10% EtOAc in cyclohexane in 12 CV) to afford the title compound as pale yellowish oil (3.9 g, 91%, rf = 0.56 (10% EtOAc in cyclohexane)). MS: calculated for $C_{33}H_{53}NO_{11}$, 639.3. Found 640.9. 1H NMR (500 MHz, DMSO-d6) δ 7.38-7.26 (m, 5H), 4.97 (s, 2H), 3.54 (t, 6H), 3.50 (s, 6H), 2.38 (t, 6H), 1.39 (s, 27H). 13C NMR (125 MHz, DMSO-d6) δ 170.3 (3xC), 154.5 (C), 137.1 (C), 128.2 (2xCH), 127.7 (CH), 127.6 (2xCH), 79.7 (3xC), 68.4 (3xCH2), 66.8 (3xCH2), 64.9 (C), 58.7 (CH2), 35.8 (3xCH2), 27.7 (9xCH3).

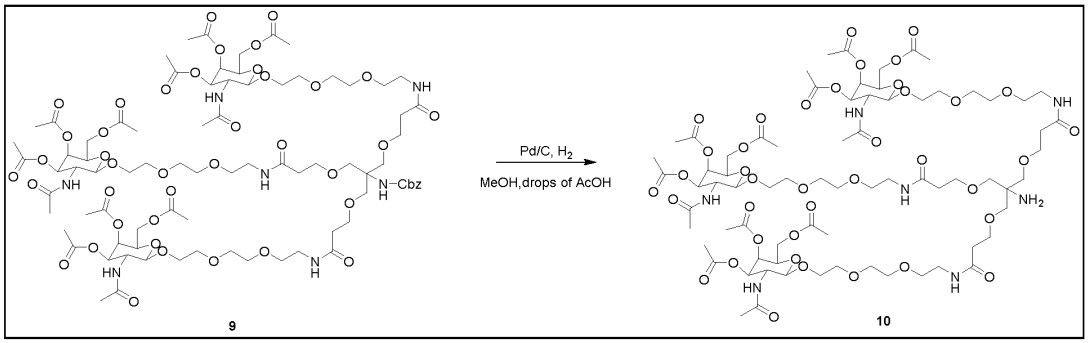



Preparation of compound 8: Cbz-NH-tris-Boc-ester 7 (0.20 g, 0.39 mmol, 1.0 eq.) was dissolved in $CH_2Cl_2$ (1 mL) under argon, trifluoroacetic acid (TFA, 1 mL) was added and the reaction was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, the residue was co-evaporated 3 times with toluene (5 mL) and dried under high vacuum to get the

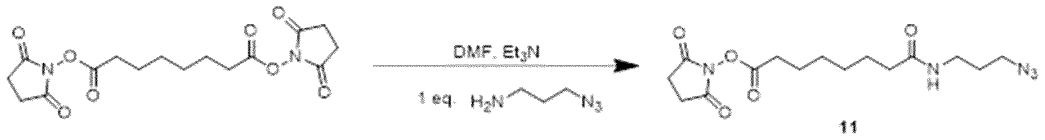
compound as its TFA salt (0.183 g, 98%). The compound was used without further purification. MS: calculated for $C_{21}H_{29}NO_{11}$, 471.6. Found 472.4.



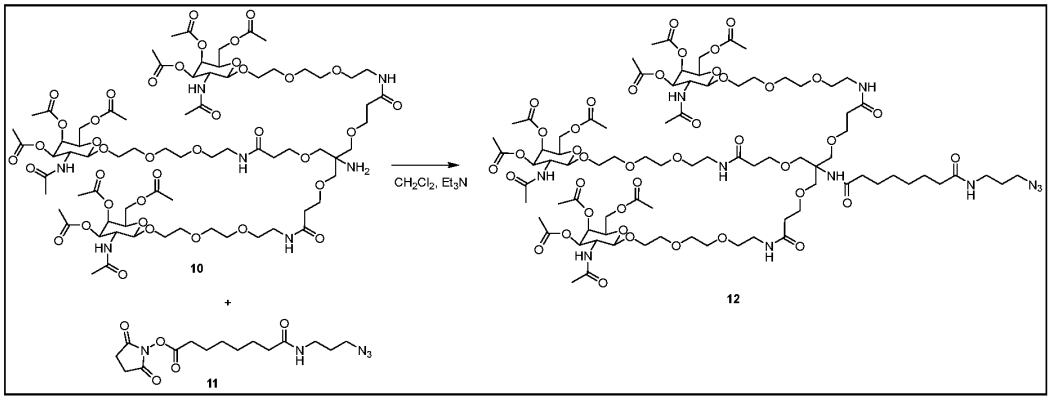

Preparation of compound 9: CbzNH-tris-COOH 8 (0.72 g, 1.49 mmol, 1.0 eq.) and GalNAc-PEG3-$NH_2$ 5 (3.56 g, 7.44 mmol, 5.0 eq.) were dissolved in N,N-dimethylformamide (DMF) (25 mL). Then N,N,N′,N′-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (2.78 g, 7.44 mmol, 5.0 eq.), 1-hydroxybenzotriazole hydrate (HOBt) (1.05 g, 7.44 mmol, 5.0 eq.) and N,N-diisopropylethylamine (DIPEA) (2.07 mL, 11.9 mmol, 8.0 eq.) were added to the solution and the reaction was stirred for 72 h. The solvent was removed under reduced pressure, the residue was dissolved in DCM (100 mL) and washed with saturated aq. $NaHCO_3$ (100 mL). The organic layer was dried over $Na_2SO_4$, the solvent evaporated and the crude material was purified by flash chromatography (gradient elution: 0-5% MeOH in DCM in 14 CV). The product was obtained as pale yellowish oil (1.2 g, 43%, rf = 0.20 (5% MeOH in DCM)). MS: calculated for $C_{81}H_{125}N_7O_{41}$, 1852.9. Found 1854.7. 1H NMR (500 MHz, DMSO-d6) δ 7.90-7.80 (m, 10H), 7.65-7.62 (m, 4H), 7.47-7.43 (m, 3H), 7.38-7.32 (m, 8H), 5.24-5.22 (m, 3H), 5.02-4.97 (m, 4H), 4.60-4.57 (m, 3 H), 4.07-3.90 (m 10H), 3.67-3.36 (m, 70H), 3.23-3.07 (m, 25H), 2.18 (s, 10H), 2.00 (s, 13H), 1.89 (s, 11H), 1.80-1.78 (m, 17H). 13C NMR (125 MHz, DMSO-d6) δ 170.1 (C), 169.8 (C), 169.7 (C), 169.4 (C), 169.2 (C), 169.1 (C), 142.7 (C), 126.3 (CH), 123.9 (CH), 118.7 (CH), 109.7 (CH), 100.8 (CH), 70.5 (CH), 69.8 (CH), 69.6 (CH), 69.5 (CH), 69.3 (CH2), 69.0 (CH2), 68.2 (CH2), 67.2 (CH2), 66.7 (CH2), 61.4 (CH2), 22.6 (CH2), 22.4 (3xCH3), 20.7 (9xCH3).



Preparation of compound 10: Triantennary GalNAc compound 9 (0.27 g, 0.14 mmol, 1.0 eq.) was dissolved in MeOH (15 mL), 3 drops of acetic acid (AcOH) and Pd/C (30 mg) was added. The reaction mixture was degassed using vacuum/argon cycles (3x) and hydrogenated under balloon pressure overnight. The completion of the reaction was followed by mass spectrometry and the resulting mixture was filtered through a thin pad of celite. The solvent was evaporated and the residue obtained was dried under high vacuum and used for the next step without further purification. The product was obtained as pale yellowish oil (0.24 g, quantitative yield). MS: calculated for $C_{73}H_{119}N_7O_{39}$, 1718.8. Found 1719.3.




Preparation of compound 11: Commercially available suberic acid bis(N-hydroxysuccinimide ester) (3.67 g, 9.9 mmol, 1.0 eq.) was dissolved in DMF (5 mL) and triethylamine (1.2 mL) was added. To this solution was added dropwise a solution of 3-azido-1-propylamine (1.0 g, 9.9 mmol, 1.0 eq.) in DMF (5 mL). The reaction was stirred at room temperature for 3 h. The reaction mixture was diluted with EtOAc (100 mL) and washed with water (50 mL). The organic layer was separated, dried over $Na_2SO_4$ and the solvent was removed under reduced pressure. The crude material was purified by flash chromatography (gradient elution: 0-5% MeOH in DCM in 16 CV). The product was obtained as white solid (1.54 g, 43%, rf = 0.71 (5% MeOH in DCM)). MS: calculated for $C_{15}H_{23}N_5O_5$, 353.4. Found 354.3.




Preparation of TriGalNAc (12): Triantennary GalNAc compound 10 (0.35 g, 0.24 mmol, 1.0 eq.) and compound 11 (0.11 g, 0.31 mmol, 1.5 eq.) were dissolved in DCM (5 mL) under argon and triethylamine (0.1 mL, 0.61 mmol, 3.0 eq.) was added. The reaction was stirred at room temperature overnight. The solvent was removed under reduced pressure, the residue was

dissolved in EtOAc (100 mL) and washed with water (100 mL). The organic layer was separated and dried over $Na_2SO_4$. The solvent was evaporated and the resulting crude material was purified by flash chromatography (elution gradient: 0-10% MeOH in DCM in 20 CV) to afford the title compound as white fluffy solid (0.27 g, 67%, rf = 0.5 (10% MeOH in DCM)). MS: calculated for $C_{84}H_{137}N_{11}O_{41}$, 1957.1. Found 1959.6.

Conjugation of Tether 1 to a siRNA strand: Monofluoro cyclooctyne (MFCO) conjugation at 5'- or 3'-end

5'-end MFCO conjugation



3'-end MFCO conjugation



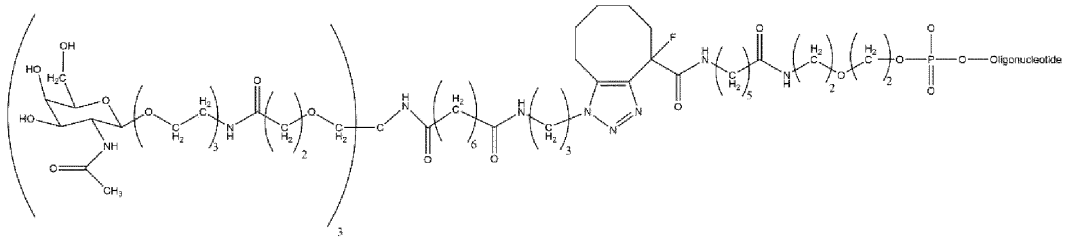
General conditions for MFCO conjugation: Amine-modified single strand was dissolved at 700 OD/mL in 50 mM carbonate/bicarbonate buffer pH 9.6/dimethyl sulfoxide (DMSO) 4:6 (v/v) and to this solution was added one molar equivalent of a 35 mM solution of MFCO-C6-NHS ester (Berry&Associates, Cat. # LK 4300) in DMF. The reaction was carried out at room temperature and after 1 h another molar equivalent of the MFCO solution was added. The reaction was allowed to proceed for an additional hour and was monitored by LC/MS. At least two molar equivalent excess of the MFCO NHS ester reagent relative to the amino modified oligonucleotide were needed to achieve quantitative consumption of the starting material. The reaction mixture was diluted 15-fold with water, filtered through a 1.2 µm filter from Sartorius and then purified by reserve phase (RP HPLC) on an Äkta Pure instrument (GE Healthcare).

Purification was performed using a XBridge C18 Prep 19 x 50 mm column from Waters. Buffer A was 100 mM TEAAc pH 7 and buffer B contained 95% acetonitrile in buffer A. A flow rate of 10 mL/min and a temperature of 60°C were employed. UV traces at 280 nm were recorded. A gradient of 0-100% B within 60 column volumes was employed.

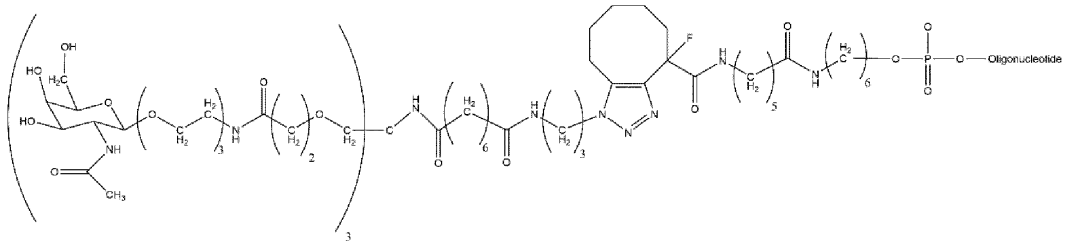
Fractions containing full length conjugated oligonucleotide were pooled, precipitated in the freezer with 3 M NaOAc, pH 5.2 and 85% ethanol and the collected pellet was dissolved in

water. Samples were desalted by size exclusion chromatography and concentrated using a speed-vac concentrator to yield the conjugated oligonucleotide in an isolated yield of 40–80%.

**5'-GalNAc-T1 conjugates**




**3'-GalNAc-T1 conjugates**




General procedure for TriGalNAc conjugation: MFCO-modified single strand was dissolved at 2000 OD/mL in water and to this solution was added one equivalent solution of compound 12 (10 mM) in DMF. The reaction was carried out at room temperature and after 3 h 0.7 molar equivalent of the compound 12 solution was added. The reaction was allowed to proceed overnight and completion was monitored by LCMS. The conjugate was diluted 15-fold in water, filtered through a 1.2 µm filter from Sartorius and then purified by RP HPLC on an Äkta Pure instrument (GE Healthcare).

RP HPLC purification was performed using a XBridge C18 Prep 19 x 50 mm column from Waters. Buffer A was 100 mM triethylammonium acetate pH 7 and buffer B contained 95% acetonitrile in buffer A. A flow rate of 10 mL/min and a temperature of 60°C were employed. UV traces at 280 nm were recorded. A gradient of 0-100% B within 60 column volumes was employed.

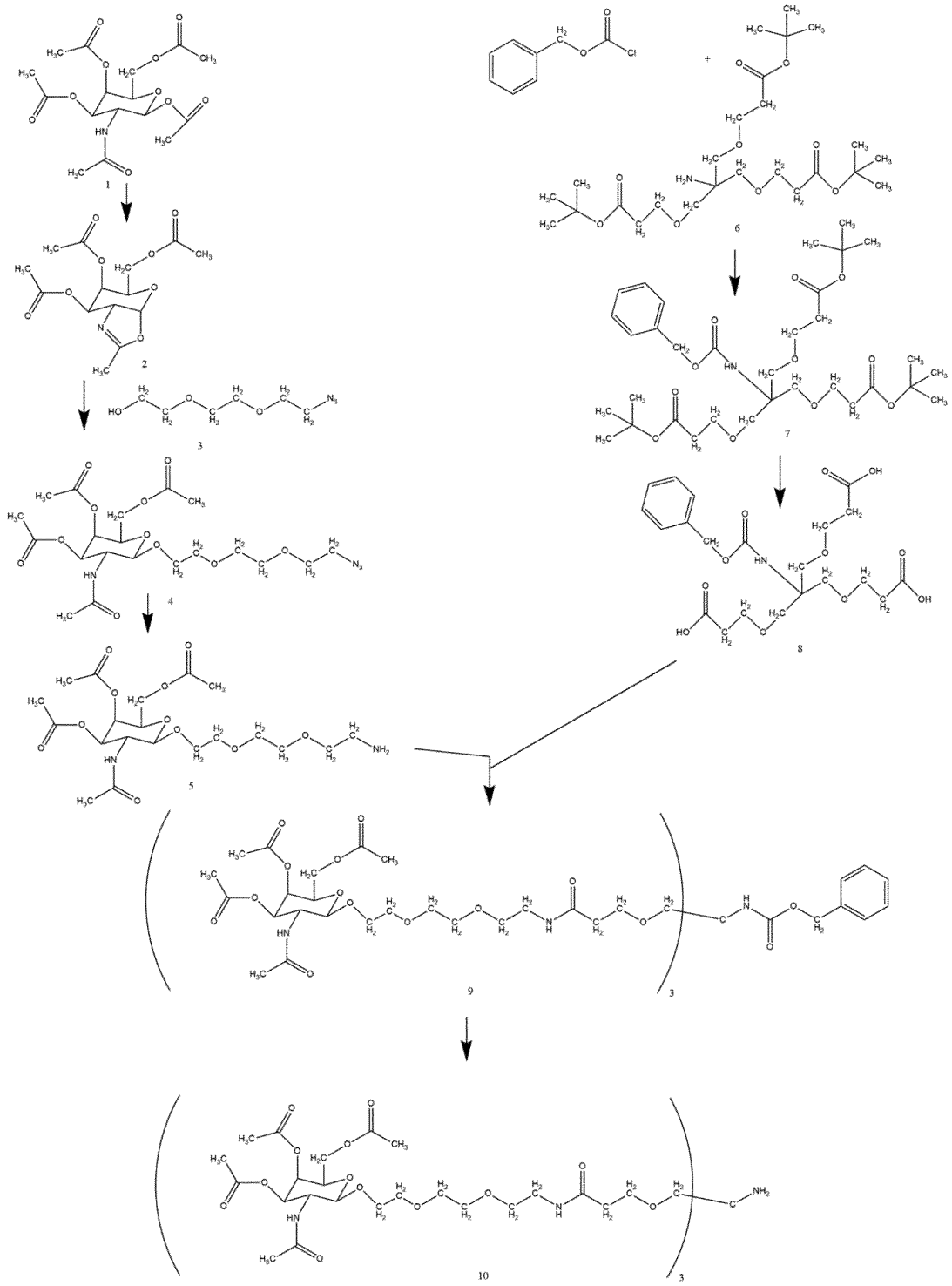
Fractions containing full-length conjugated oligonucleotide were pooled, precipitated in the freezer with 3 M NaOAc, pH 5.2 and 85% ethanol and the collected pellet was dissolved in water to give an oligonucleotide solution of about 1000 OD/mL. The O-acetates were removed

by adding 20% aqueous ammonia. Quantitative removal of these protecting groups was verified by LC-MS.

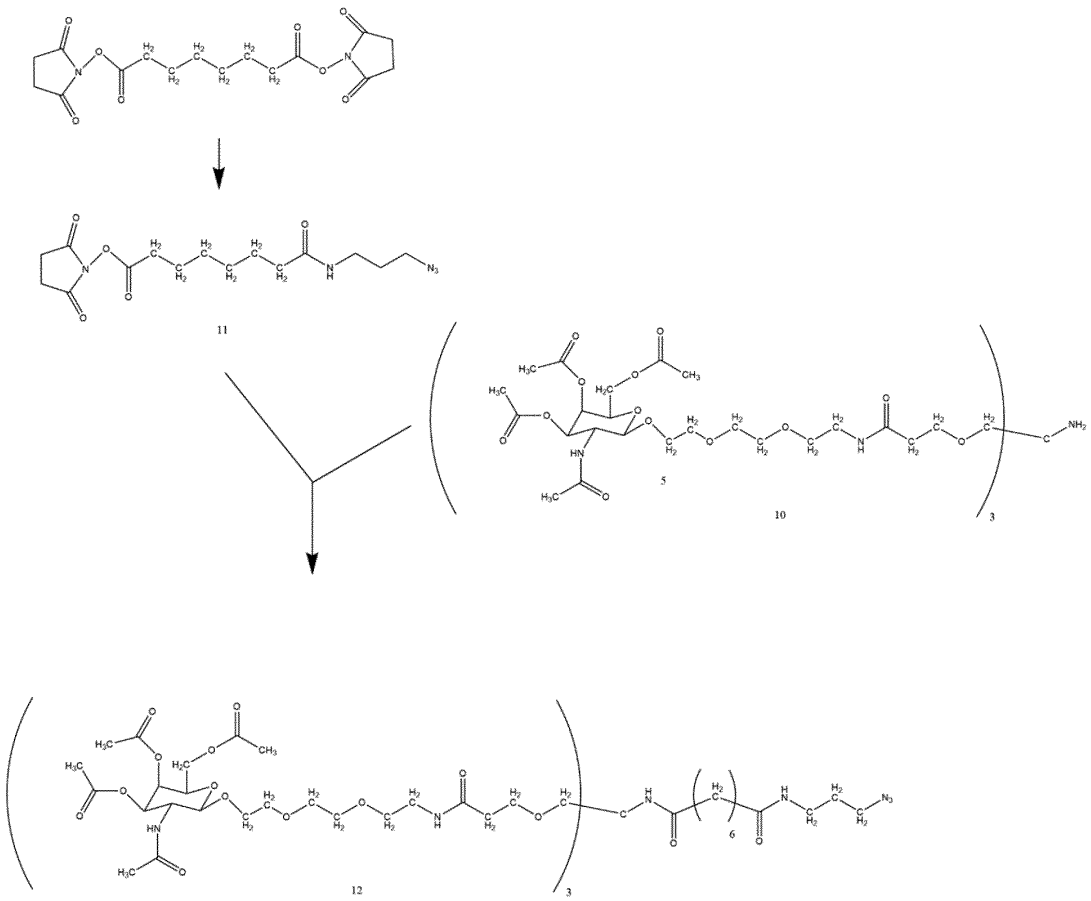
The conjugates were desalted by size exclusion chromatography using Sephadex G25 Fine resin (GE Healthcare) on an Äkta Pure (GE Healthcare) instrument to yield the conjugated oligonucleotides in an isolated yield of 50–70%.

The following schemes further set out the routes of synthesis:

**Scheme 1:**

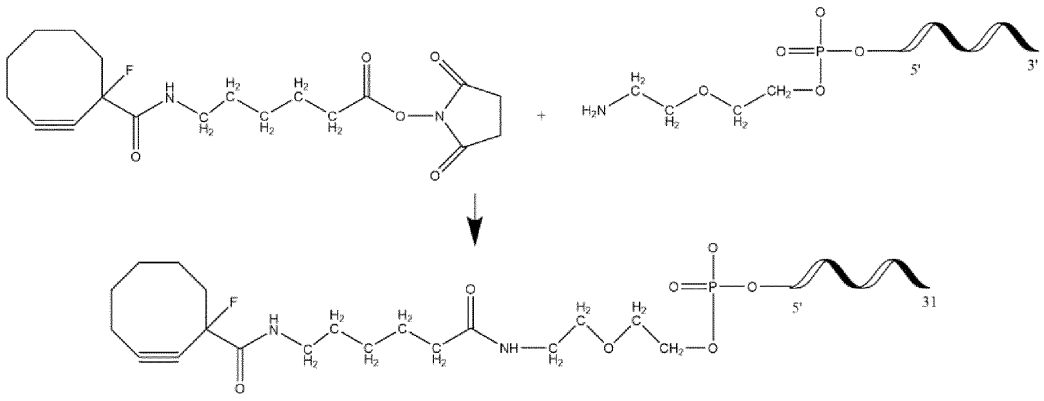


**Scheme 2:**

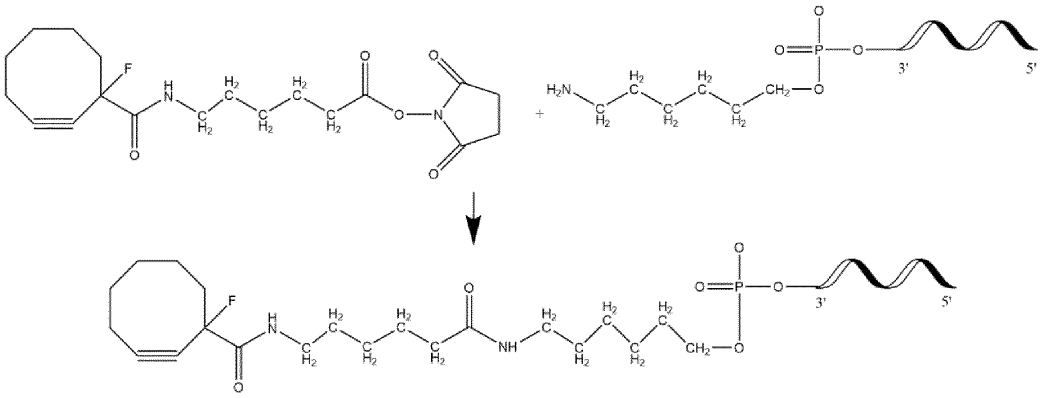


**Scheme 3:**

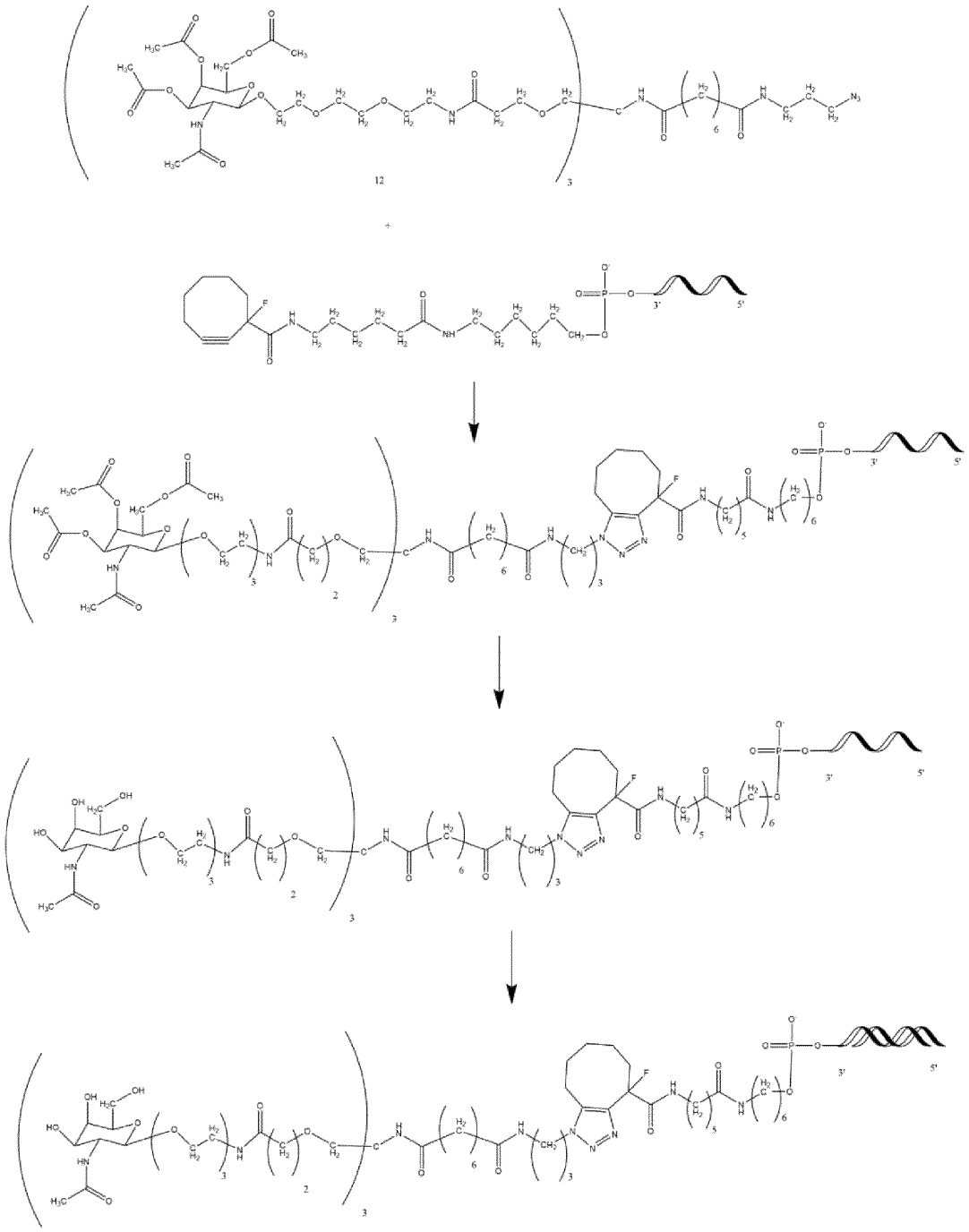
5' end MFCO conjugation



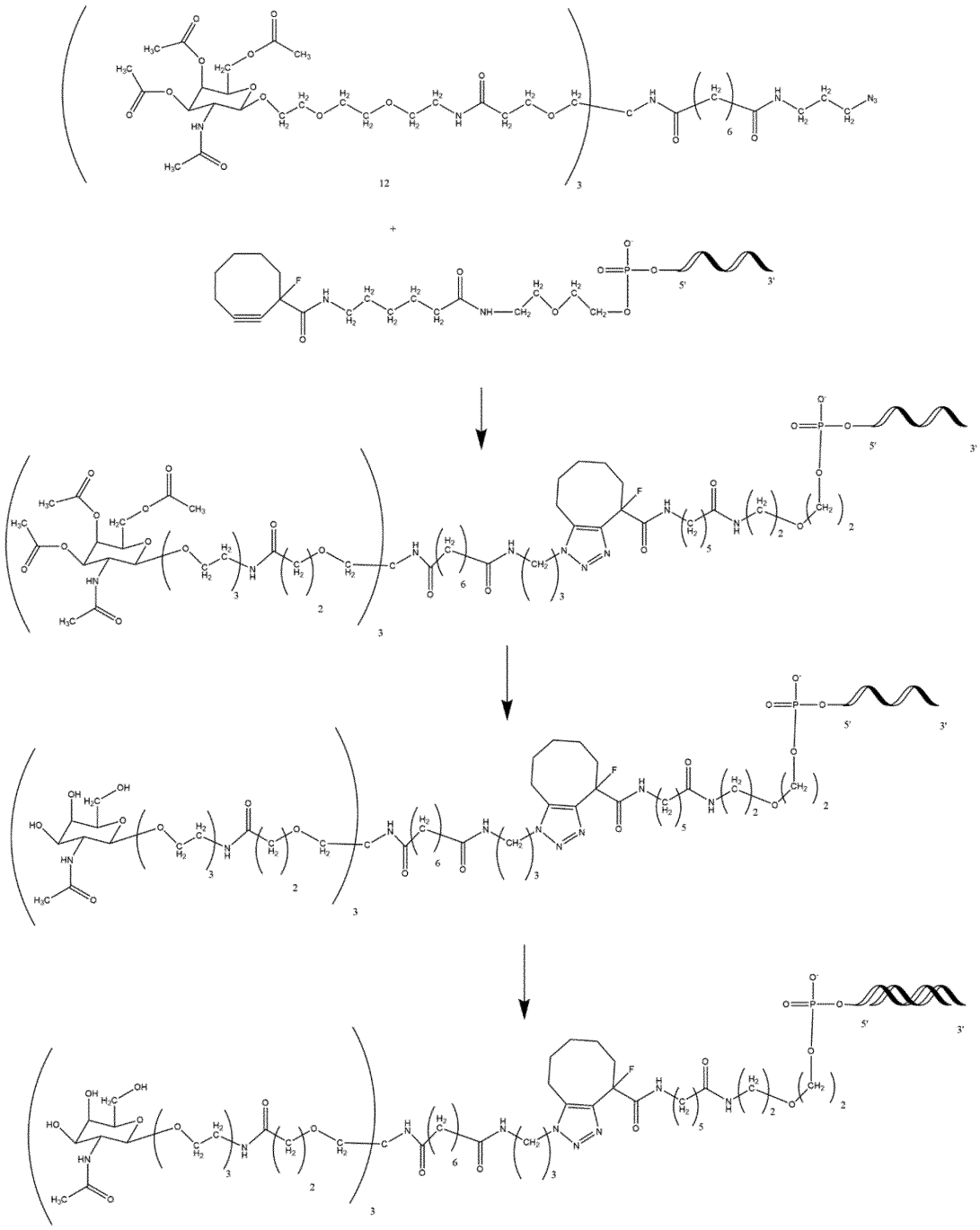
3' end MFCO conjugation



**Scheme 4:**



**Scheme 5:**



**Example 2: Duplex Annealing**

To generate the desired siRNA duplex, the two complementary strands were annealed by combining equimolar aqueous solutions of both strands. The mixtures were placed into a water bath at 70°C for 5 minutes and subsequently allowed to cool to ambient temperature within 2 h. The duplexes were lyophilized for 2 days and stored at -20°C.

The duplexes were analyzed by analytical SEC HPLC on Superdex™ 75 Increase 5/150 GL column 5 x 153-158 mm (Cytiva) on a Dionex Ultimate 3000 (Thermo Fisher Scientific) HPLC system. Mobile phase consisted of 1x PBS containing 10% acetonitrile. An isocratic gradient was run in 10 min at a flow rate of 1.5 mL/min at room temperature. UV traces at 260 and 280 nm were recorded. Water (LC-MS grade) was purchased from Sigma-Aldrich and Phosphate-buffered saline (PBS; 10x, pH 7.4) was purchased from GIBCO (Thermo Fisher Scientific).

**Example 3: Synthesis of tether 2**

General Experimental conditions:

Thin layer chromatography (TLC) was performed on silica-coated aluminium plates with fluorescence indicator 254 nm from Macherey-Nagel. Compounds were visualized under UV light (254 nm), or after spraying with the 5% $H_2SO_4$ in methanol (MeOH) or ninhydrin reagent according to Stahl (from Sigma-Aldrich), followed by heating. Flash chromatography was performed with a Biotage Isolera One flash chromatography instrument equipped with a dual variable UV wavelength detector (200-400 nm) using Biotage Sfär Silica 10, 25, 50 or 100 g columns (Uppsala, Sweden).

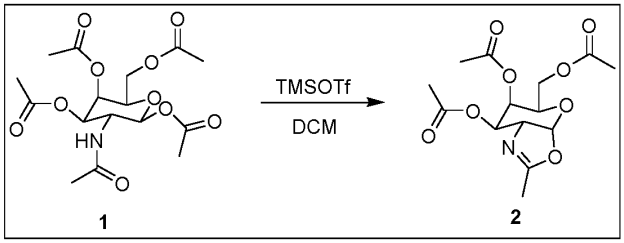
All moisture-sensitive reactions were carried out under anhydrous conditions using dry glassware, anhydrous solvents, and argon atmosphere. All commercially available reagents were purchased from Sigma-Aldrich and solvents from Carl Roth GmbH + Co. KG. D-Galactosamine pentaacetate was purchased from AK scientific.

HPLC/ESI-MS was performed on a Dionex UltiMate 3000 RS UHPLC system and Thermo Scientific MSQ Plus Mass spectrometer using an Acquity UPLC Protein BEH C4 column from Waters (300Å, 1.7 µm, 2.1 x 100 mm) at 60 °C. The solvent system consisted of solvent A with H2O containing 0.1% formic acid and solvent B with acetonitrile (ACN) containing 0.1% formic acid. A gradient from 5-100% of B over 15 min with a flow rate of 0.4 mL/min was employed.

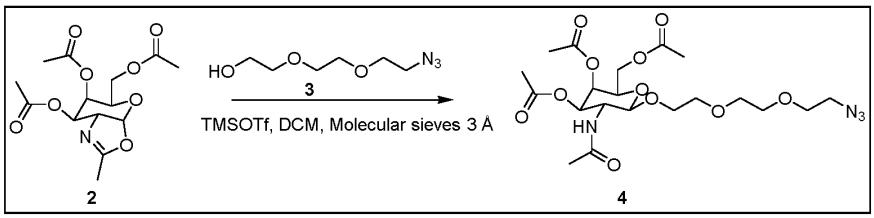
Detector and conditions: Corona ultra-charged aerosol detection (from esa). Nebulizer Temp.: 25 °C. $N_2$ pressure: 35.1 psi. Filter: Corona.

$_1H$ and $_{13}C$ NMR spectra were recorded at room temperature on a Varian spectrometer at 500 MHz ($_1H$ NMR) and 125 MHz ($_{13}C$ NMR). Chemical shifts are given in ppm referenced to the solvent residual peak ($CDCl_3$ – $_1H$ NMR: δ at 7.26 ppm and $_{13}C$ NMR δ at 77.2 ppm; DMSO-d6 – $_1H$ NMR: δ at 2.50 ppm and $_{13}C$ NMR δ at 39.5 ppm). Coupling constants are given in Hertz. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t) or multiplet (m).

Synthesis route for the conjugate building block TriGalNAc _Tether2:

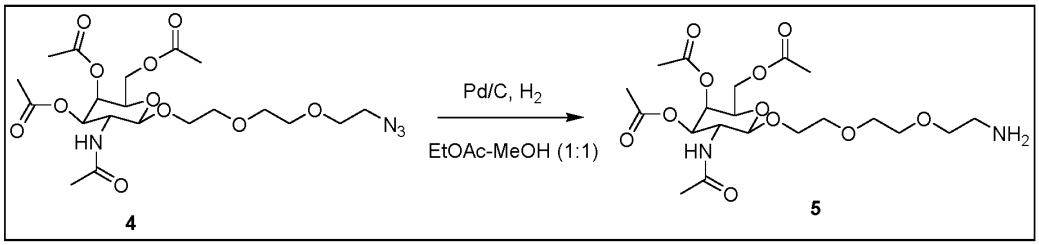



Preparation of compound 2: D-Galactosamine pentaacetate (3.00 g, 7.71 mmol, 1.0 eq.) was dissolved in anhydrous dichloromethane (DCM) (30 mL) under argon and trimethylsilyl trifluoromethanesulfonate (TMSOTf, 4.28 g, 19.27 mmol, 2.5 eq.) was added. The reaction was stirred at room temperature for 3 h. The reaction mixture was diluted with DCM (50 mL) and washed with cold saturated aq. $NaHCO_3$ (100 mL) and water (100 mL). The organic layer was separated, dried over $Na_2SO_4$, and concentrated to afford the title compound as yellow oil, which was purified by flash chromatography (gradient elution: 0-10% MeOH in DCM in 10 CV). The product was obtained as colourless oil (2.5 g, 98%, rf= 0.45 (2% MeOH in DCM)).

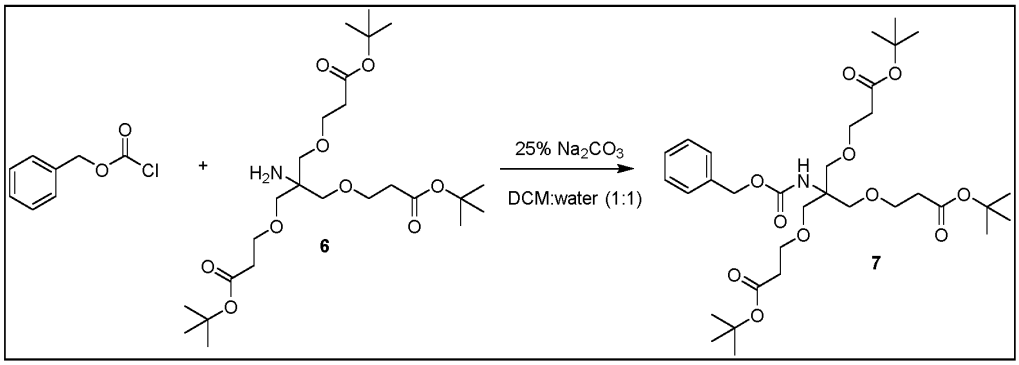



Preparation of compound 4: Compound 2 (2.30 g, 6.98 mmol, 1.0 eq.) and azido-PEG3-OH (1.83 g, 10.5 mmol, 1.5 eq.) were dissolved in anhydrous DCM (40 mL) under argon and molecular sieves 3 Å (5 g) were added to the solution. The mixture was stirred at room temperature for 1 h. TMSOTf (0.77 g, 3.49 mmol, 0.5 eq.) was then added to the mixture and the reaction was stirred overnight. The molecular sieves were filtered, the filtrate was diluted with DCM (100 mL) and washed with cold saturated aq. $NaHCO_3$ (100 mL) and water (100 mL). The

organic layer was separated, dried over Na2SO4 and the solvent was removed under reduced pressure. The crude material was purified by flash chromatography (gradient elution: 0-3% MeOH in DCM in 10 CV) to afford the title product as light-yellow oil (3.10 g, 88%, rf = 0.25 (2% MeOH in DCM)). MS: calculated for $C_{20}H_{32}N_4O_{11}$, 504.21. Found 505.4. 1H NMR (500 MHz, CDCl3) δ 6.21-6.14 (m, 1H), 5.30 (dd, J = 3.4, 1.1 Hz, 1H), 5.04 (dd, J = 11.2, 3.4 Hz,1H), 4.76 (d, J = 8.6 Hz, 1H), 4.23-4.08 (m, 3H), 3.91-3.80 (m, 3H), 3.74-3.59 (m, 9H), 3.49-3.41 (m, 2H), 2.14 (s, 3H), 2.02 (s, 3H), 1.97 (d, J = 4.2 Hz, 6H). 13C NMR (125 MHz, CDCl3) δ 170.6 (C), 170.5 (C), 170.4 (C), 170.3 (C), 102.1 (CH), 71.6 (CH), 70.8 (CH), 70.6 (CH), 70.5 (CH), 70.3 (CH2), 69.7 (CH2), 68.5 (CH2), 66.6 (CH2), 61.5 (CH2), 23.1 (CH3), 20.7 (3xCH3).

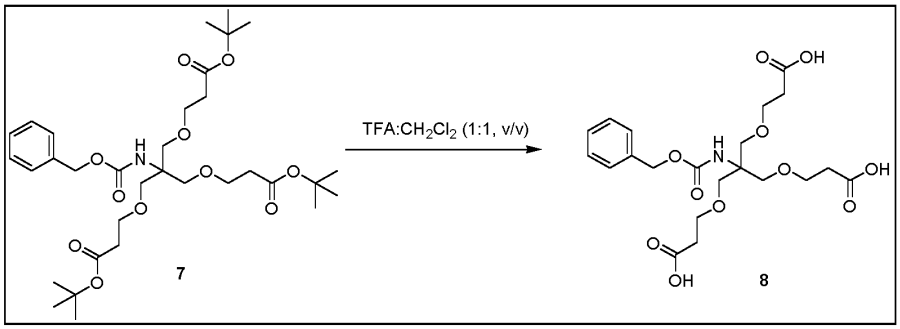



Preparation of compound 5: Compound 4 (1.00 g, 1.98 mmol, 1.0 eq.) was dissolved in a mixture of ethyl acetate (EtOAc) and MeOH (30 mL 1:1 v/v) and Pd/C (100 mg) was added. The reaction mixture was degassed using vacuum/argon cycles (3x) and hydrogenated under balloon pressure overnight. The reaction mixture was filtered through celite and washed with EtOAc (30 mL). The solvent was removed under reduced pressure to afford the title compound as colourless oil (0.95 g, quantitative yield, rf = 0.25 (10% MeOH in DCM)). The compound was used without further purification. MS: calculated for $C_{20}H_{34}N_2O_{11}$, 478.2. Found 479.4.

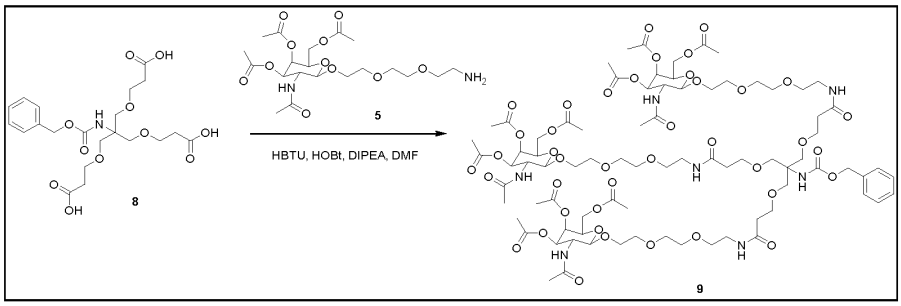



Preparation of compound 7: Tris{[2-(tert-butoxycarbonyl)ethoxy]methyl}-methylamine 6 (3.37 g, 6.67 mmol, 1.0 eq.) was dissolved in a mixture of DCM/water (40 mL 1:1 v/v) and $Na_2CO_3$ (0.18 g, 1.7 mmol, 0.25 eq.) was added while stirring vigorously. Benzyl chloroformate (2.94 mL, 20.7 mmol, 3.10 eq.) was added dropwise to the previous mixture and the reaction was

stirred at room temperature for 24 h. The reaction mixture was diluted with $CH_2Cl_2$ (100 mL) and washed with water (100 mL). The organic layer was separated and dried over $Na_2SO_4$. The solvent was removed under reduced pressure and the resulting crude material was purified by flash chromatography (gradient elution: 0-10% EtOAc in cyclohexane in 12 CV) to afford the title compound as pale yellowish oil (3.9 g, 91%, rf = 0.56 (10% EtOAc in cyclohexane)). MS: calculated for $C_{33}H_{53}NO_{11}$, 639.3. Found 640.9. 1H NMR (500 MHz, DMSO-d6) δ 7.38-7.26 (m, 5H), 4.97 (s, 2H), 3.54 (t, 6H), 3.50 (s, 6H), 2.38 (t, 6H), 1.39 (s, 27H). 13C NMR (125 MHz, DMSO-d6) δ 170.3 (3xC), 154.5 (C), 137.1 (C), 128.2 (2xCH), 127.7 (CH), 127.6 (2xCH), 79.7 (3xC), 68.4 (3xCH2), 66.8 (3xCH2), 64.9 (C), 58.7 (CH2), 35.8 (3xCH2), 27.7 (9xCH3).

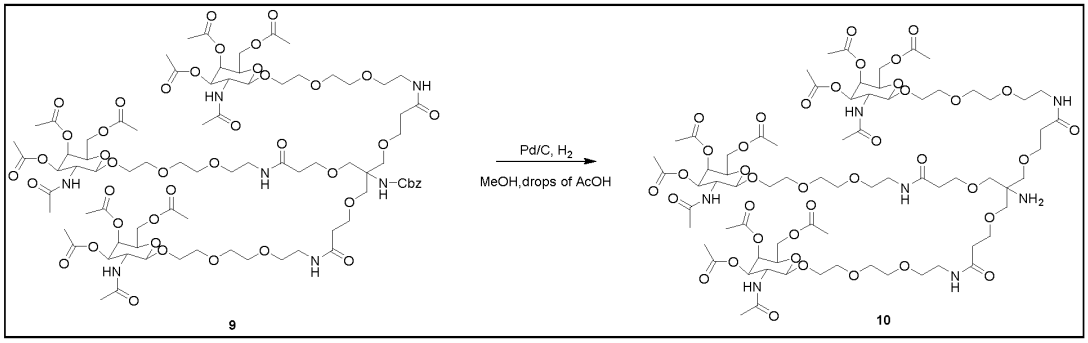



Preparation of compound 8: Cbz-NH-tris-Boc-ester 7 (0.20 g, 0.39 mmol, 1.0 eq.) was dissolved in $CH_2Cl_2$ (1 mL) under argon, trifluoroacetic acid (TFA, 1 mL) was added and the reaction was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, the residue was co-evaporated 3 times with toluene (5 mL) and dried under high vacuum to get the compound as its TFA salt (0.183 g, 98%). The compound was used without further purification. MS: calculated for $C_{21}H_{29}NO_{11}$, 471.6. Found 472.4.

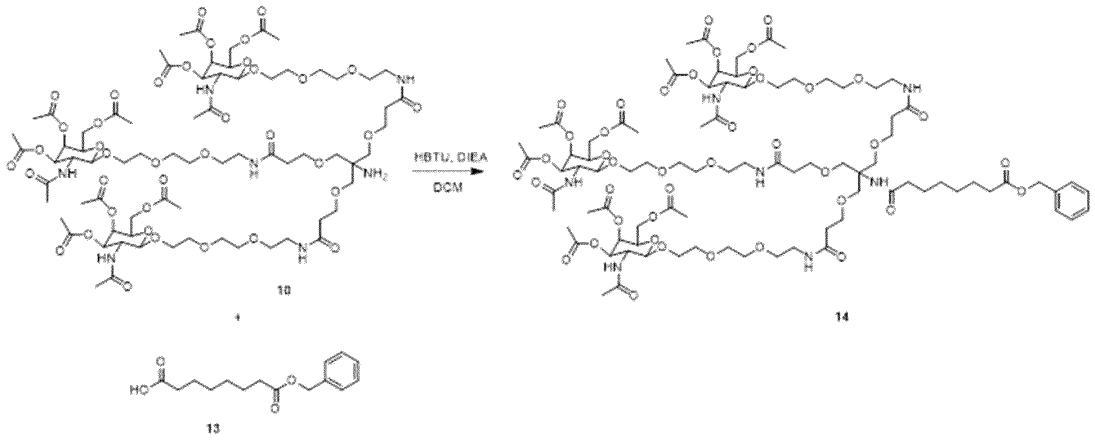



Preparation of compound 9: CbzNH-tris-COOH 8 (0.72 g, 1.49 mmol, 1.0 eq.) and GalNAc-PEG3-NH2 5 (3.56 g, 7.44 mmol, 5.0 eq.) were dissolved in N,N-dimethylformamide (DMF) (25 mL). Then N,N,N′,N′-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate

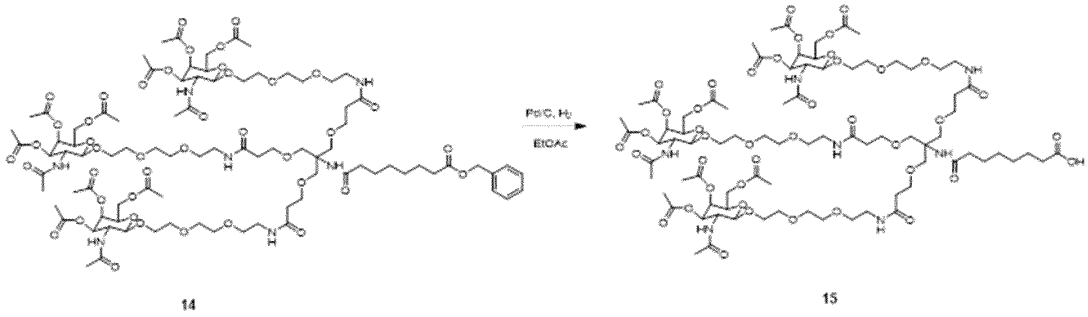
(HBTU) (2.78 g, 7.44 mmol, 5.0 eq.), 1-hydroxybenzotriazole hydrate (HOBt) (1.05 g, 7.44 mmol, 5.0 eq.) and N,N-diisopropylethylamine (DIPEA) (2.07 mL, 11.9 mmol, 8.0 eq.) were added to the solution and the reaction was stirred for 72 h. The solvent was removed under reduced pressure, the residue was dissolved in DCM (100 mL) and washed with saturated aq. NaHCO3 (100 mL). The organic layer was dried over $Na_2SO_4$, the solvent evaporated and the crude material was purified by flash chromatography (gradient elution: 0-5% MeOH in DCM in 14 CV). The product was obtained as pale yellowish oil (1.2 g, 43%, rf = 0.20 (5% MeOH in DCM)). MS: calculated for $C_{81}H_{125}N_7O_{41}$, 1852.9. Found 1854.7. 1H NMR (500 MHz, DMSO-d6) δ 7.90-7.80 (m, 10H), 7.65-7.62 (m, 4H), 7.47-7.43 (m, 3H), 7.38-7.32 (m, 8H), 5.24-5.22 (m, 3H), 5.02-4.97 (m, 4H), 4.60-4.57 (m, 3 H), 4.07-3.90 (m 10H), 3.67-3.36 (m, 70H), 3.23-3.07 (m, 25H), 2.18 (s, 10H), 2.00 (s, 13H), 1.89 (s, 11H), 1.80-1.78 (m, 17H). 13C NMR (125 MHz, DMSO-d6) δ 170.1 (C), 169.8 (C), 169.7 (C), 169.4 (C), 169.2 (C), 169.1 (C), 142.7 (C), 126.3 (CH), 123.9 (CH), 118.7 (CH), 109.7 (CH), 100.8 (CH), 70.5 (CH), 69.8 (CH), 69.6 (CH), 69.5 (CH), 69.3 (CH2), 69.0 (CH2), 68.2 (CH2), 67.2 (CH2), 66.7 (CH2), 61.4 (CH2), 22.6 (CH2), 22.4 (3xCH3), 20.7 (9xCH3).




Preparation of compound 10: Triantennary GalNAc compound 9 (0.27 g, 0.14 mmol, 1.0 eq.) was dissolved in MeOH (15 mL), 3 drops of acetic acid (AcOH) and Pd/C (30 mg) was added. The reaction mixture was degassed using vacuum/argon cycles (3x) and hydrogenated under balloon pressure overnight. The completion of the reaction was followed by mass spectrometry and the resulting mixture was filtered through a thin pad of celite. The solvent was evaporated, and the residue obtained was dried under high vacuum and used for the next step without further purification. The product was obtained as pale yellowish oil (0.24 g, quantitative yield). MS: calculated for $C_{73}H_{119}N_7O_{39}$, 1718.8. Found 1719.3.



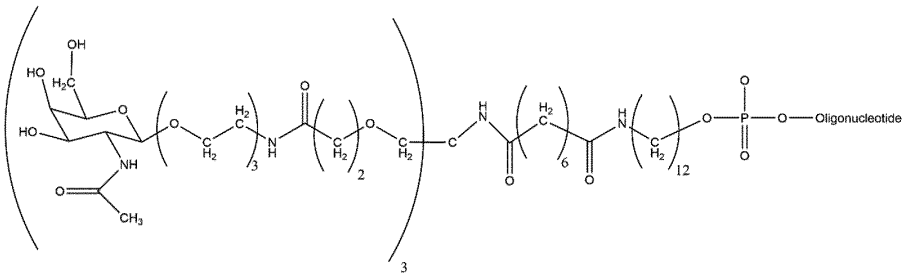

Preparation of compound 14: Triantennary GalNAc compound 10 (0.45 g, 0.26 mmol, 1.0 eq.), HBTU (0.19 g, 0.53 mmol, 2.0 eq.) and DIPEA (0.23 mL, 1.3 mmol, 5.0 eq.) were dissolved in DCM (10 mL) under argon. To this mixture, it was added dropwise a solution of compound 13 (0.14 g, 0.53 mmol, 2.0 eq.) in DCM (5 mL). The reaction was stirred at room temperature overnight. The solvent was removed, and the residue was dissolved in EtOAc (50 mL), washed with water (50 mL) and dried over $Na_2SO_4$. The solvent was evaporated, and the crude material was purified by flash chromatography (gradient elution: 0-5% MeOH in DCM in 20 CV). The product was obtained as white fluffy solid (0.25 g, 48%, rf = 0.4 (10% MeOH in DCM)). MS: calculated for $C_{88}H_{137}N_7O_{42}$, 1965.1. Found 1965.6.



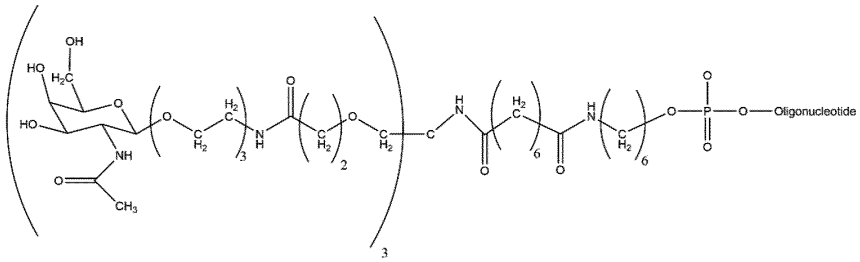

Preparation of TriGalNAc (15): Triantennary GalNAc compound 14 (0.31 g, 0.15 mmol, 1.0 eq.) was dissolved in EtOAc (15 mL) and Pd/C (40 mg) was added. The reaction mixture was degassed by using vacuum/argon cycles (3x) and hydrogenated under balloon pressure overnight. The completion of the reaction was monitored by mass spectrometry and the resulting mixture was filtered through a thin pad of celite. The solvent was removed under reduced pressure and the resulting residue was dried under high vacuum overnight. The residue was used for conjugations to oligonucleosides without further purification (0.28 g, quantitative yield). MS: calculated for $C_{81}H_{131}N_7O_{42}$, 1874.9. Found 1875.3.

Conjugation of Tether 2 to a siRNA strand: TriGalNAc tether 2 (GalNAc-T2) conjugation at 5'-end or 3'-end

**5'-GalNAc-T2 conjugates**




**3'-GalNAc-T2 conjugates**




Preparation of TriGalNAc tether 2 NHS ester: To a solution of carboxylic acid tether 2 (compound 15, 227 mg, 121 µmol) in DMF (2.1 mL), N-hydroxysuccinimide (NHS) (15.3 mg, 133 µmol) and N,N′-diisopropylcarbodiimide (DIC) (19.7 µL, 127 µmol) were added. The solution was stirred at room temperature for 18 h and used without purification for the subsequent conjugation reactions.

General procedure for triGalNAc tether 2 conjugation: Amine-modified single strand was dissolved at 700 OD/mL in 50 mM carbonate/bicarbonate buffer pH 9.6/DMSO 4:6 (v/v) and to this solution was added one molar equivalent of Tether 2 NHS ester (57 mM) solution in DMF. The reaction was carried out at room temperature and after 1 h another molar equivalent of the NHS ester solution was added. The reaction was allowed to proceed for one more hour and reaction progress was monitored by LCMS. At least two molar equivalent excess of the NHS ester reagent relative to the amino modified oligonucleoside were needed to achieve quantitative consumption of the starting material. The reaction mixture was diluted 15-fold with water, filtered once through 1.2 µm filter from Sartorius and then purified by reserve phase (RP HPLC) on an Äkta Pure (GE Healthcare) instrument.

The purification was performed using a XBridge C18 Prep 19 x 50 mm column from Waters. Buffer A was 100 mM TEAA pH 7 and buffer B contained 95% acetonitrile in buffer A. A flow rate of 10 mL/min and a temperature of 60°C were employed. UV traces at 280 nm were recorded. A gradient of 0–100% B within 60 column volumes was employed.

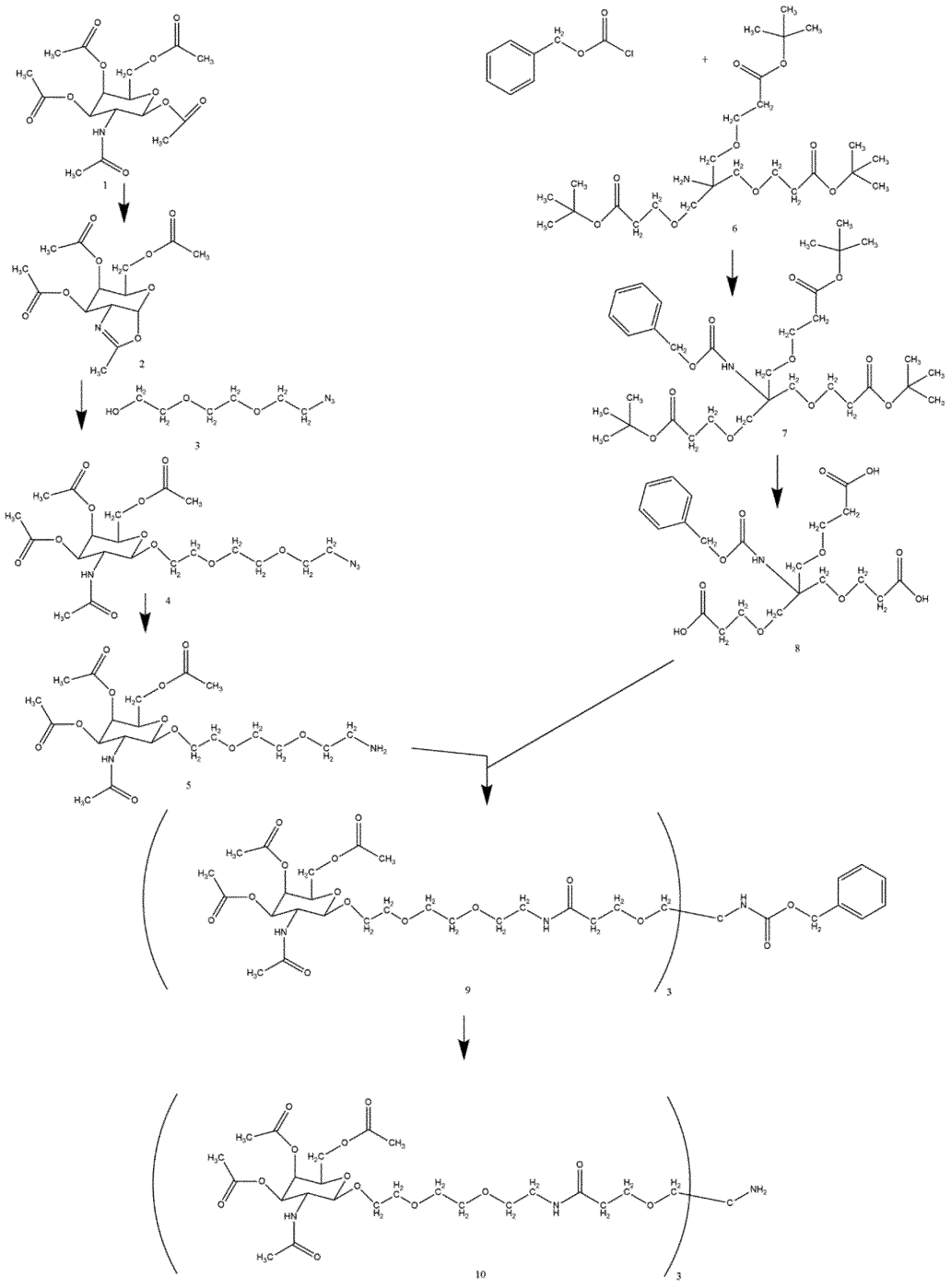
Fractions containing full-length conjugated oligonucleosides were pooled together, precipitated in the freezer with 3 M NaOAc, pH 5.2 and 85% ethanol and then dissolved at 1000 OD/mL in water. The O-acetates were removed with 20% ammonium hydroxide in water until completion (monitored by LC-MS).

The conjugates were desalted by size exclusion chromatography using Sephadex G25 Fine resin (GE Healthcare) on an Äkta Pure (GE Healthcare) instrument to yield the conjugated oligonucleotides in an isolated yield of 60–80%.

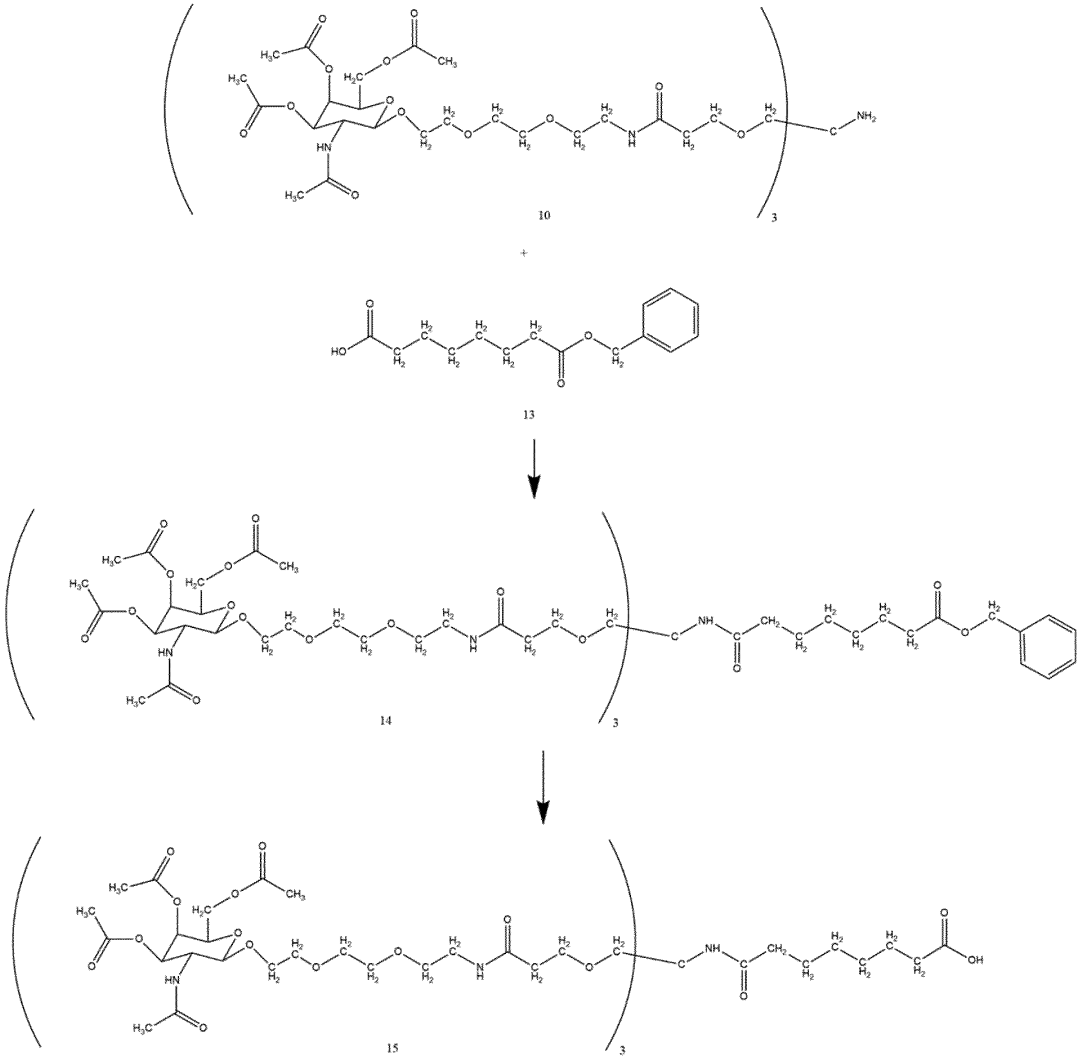
The conjugates were characterized by HPLC–MS analysis with a 2.1 x 50 mm XBridge C18 column (Waters) on a Dionex Ultimate 3000 (Thermo Fisher Scientific) HPLC system equipped with a Compact ESI-Qq-TOF mass spectrometer (Bruker Daltonics). Buffer A was 16.3 mM triethylamine, 100 mM HFIP in 1% MeOH in H2O and buffer B contained 95% MeOH in buffer A. A flow rate of 250 µL/min and a temperature of 60°C were employed. UV traces at 260 and 280 nm were recorded. A gradient of 1-100% B within 31 min was employed.

The following schemes further set out the routes of synthesis:

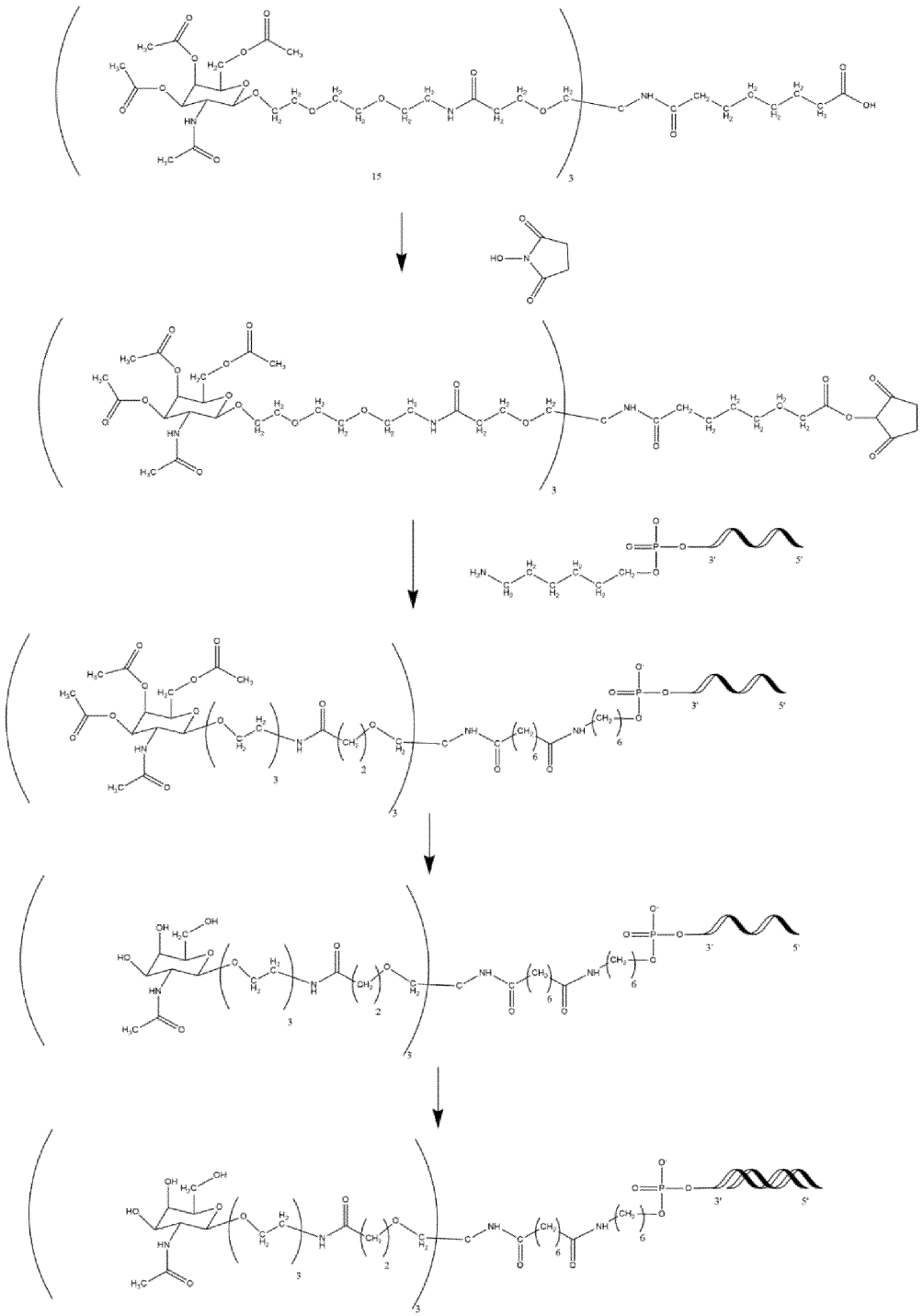
**Scheme 6:**



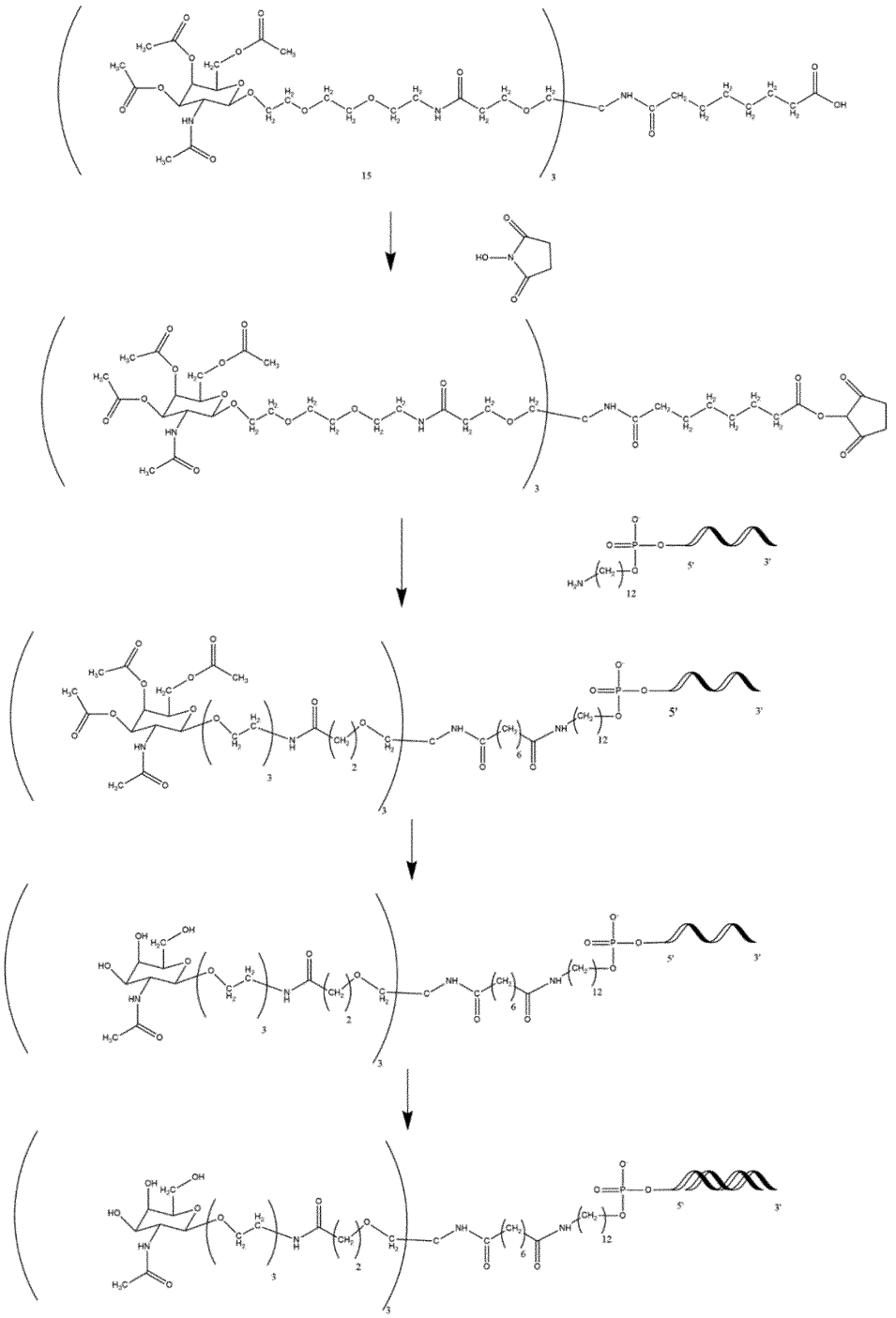
**Scheme 7:**



**Scheme 8:**



**Scheme 9:**

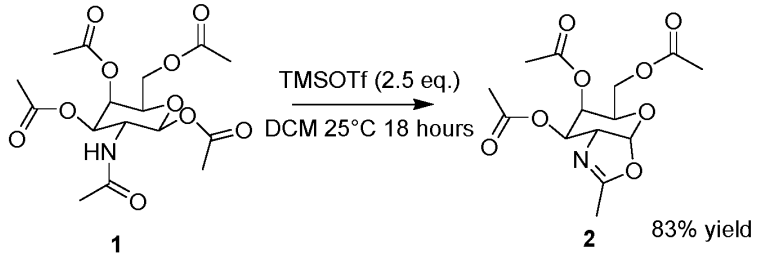


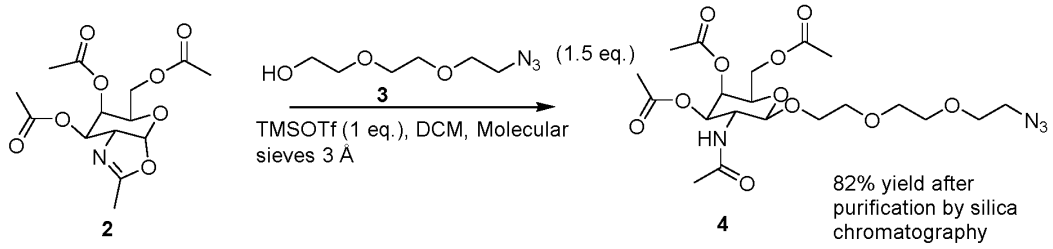
**Example 4: Duplex Annealing**

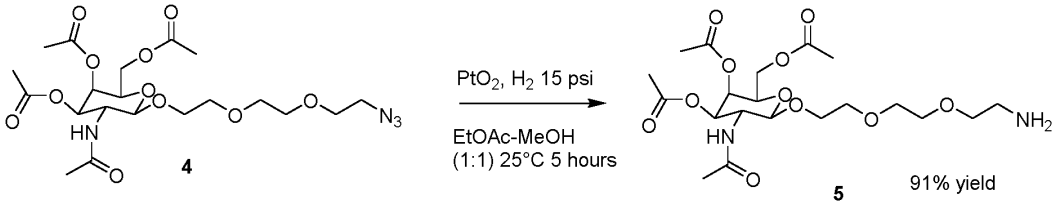
To generate the desired siRNA duplex, the two complementary strands were annealed by combining equimolar aqueous solutions of both strands. The mixtures were placed into a water bath at 70°C for 5 minutes and subsequently allowed to cool to ambient temperature within 2 h. The duplexes were lyophilized for 2 days and stored at -20°C.

The duplexes were analyzed by analytical SEC HPLC on Superdex™ 75 Increase 5/150 GL column 5 x 153-158 mm (Cytiva) on a Dionex Ultimate 3000 (Thermo Fisher Scientific) HPLC system. Mobile phase consisted of 1x PBS containing 10% acetonitrile. An isocratic gradient was run in 10 min at a flow rate of 1.5 mL/min at room temperature. UV traces at 260 and 280 nm were recorded. Water (LC-MS grade) was purchased from Sigma-Aldrich and Phosphate-buffered saline (PBS; 10x, pH 7.4) was purchased from GIBCO (Thermo Fisher Scientific).

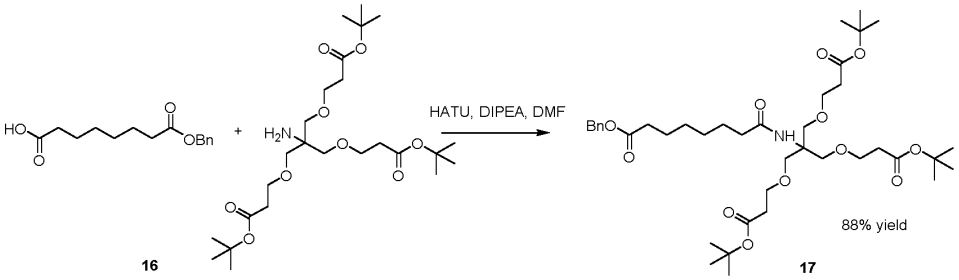
**Example 5: Alternative synthesis route for the conjugate building block TriGalNAc _Tether2**



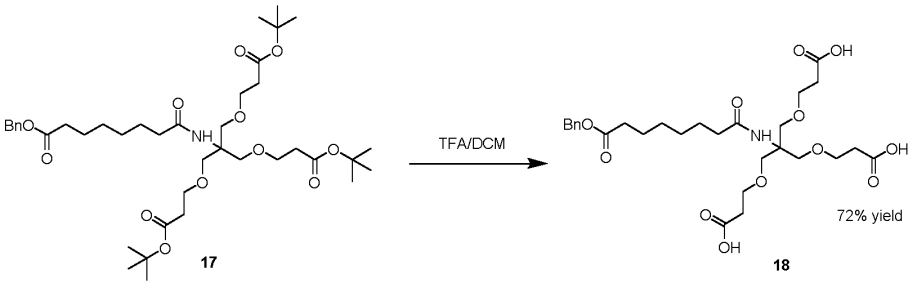





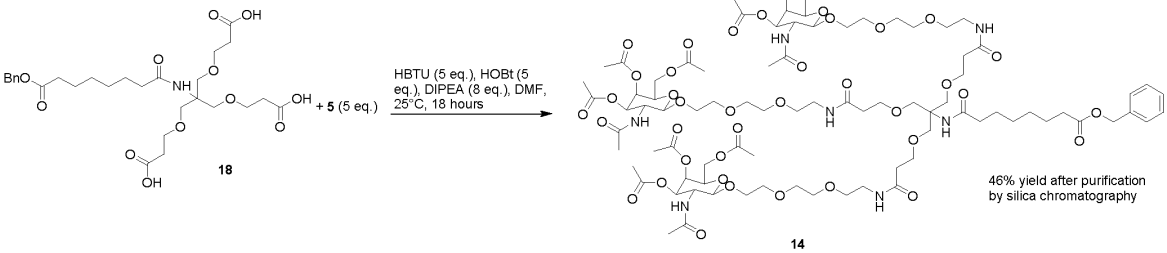

4 5



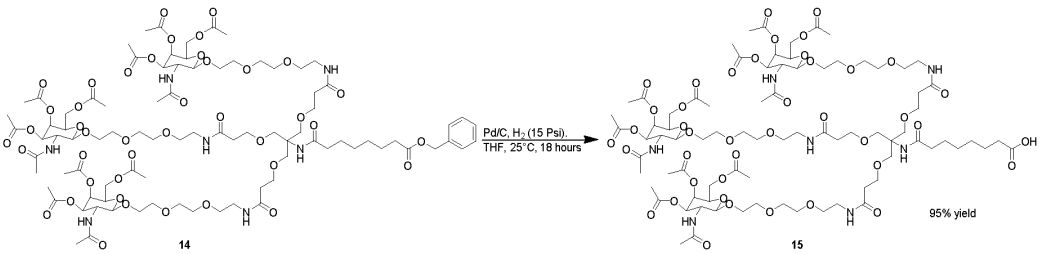

16 17




17 18



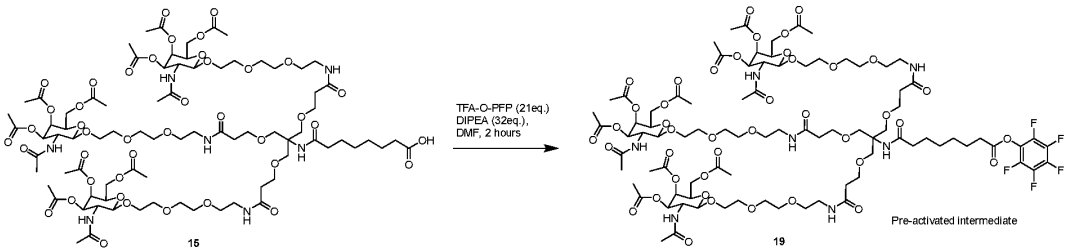

18 14




14 15

Conjugation of Tether 2 to a siRNA strand: TriGalNAc tether 2 (GalNAc-T2) conjugation at 5'-end or 3'-end

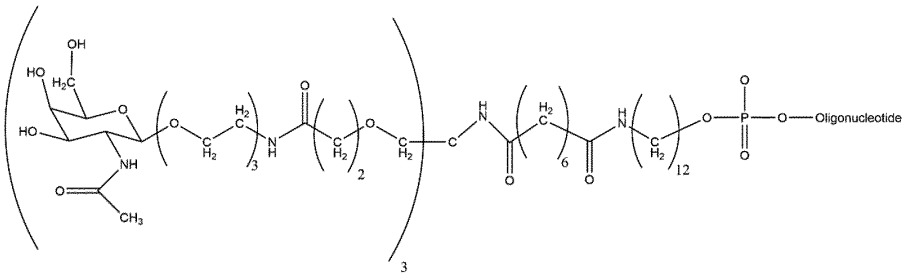
Conjugation conditions



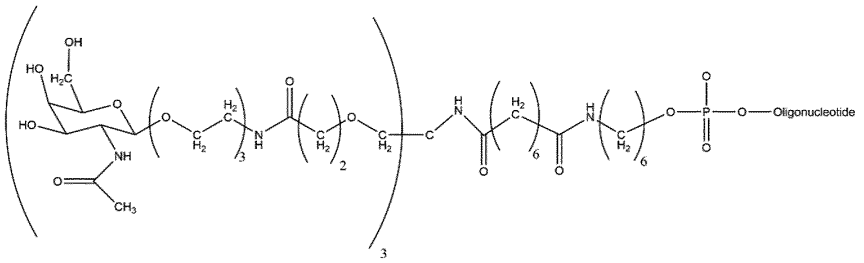

Pre-activation: To a solution of compound 15 (16 umol, 4 eq.) in DMF (160 µL) was added TFA-O-PFP (15 µl, 21 eq.) followed by DIPEA (23 µl, 32 eq.) at 25°C. The tube was shaken for 2 h at 25°C. The reaction was quenched with H2O (10 µL).

Coupling: The resulting mixture was diluted with DMF (400 µl), followed by addition of oligo-amine solution (4.0 µmol in 10 x PBS, pH 7.4, 500 µL; final oligo concentration in organic and aqueous solution: 4 µmol/ml = 4 mM). The tube was shaken at 25°C for 16 h and the reaction was analysed by LCMS. The resulting mixture was treated with 28% NH4OH (4.5 ml) and shaken for 2 h at 25°C. The mixture was analysed by LCMS, concentrated, and purified by IP-RP HPLC to produce the oligonucleotides conjugated to tether 2 GalNAc.

**5'-GalNAc-T2 conjugates**



**3'-GalNAc-T2 conjugates**




**Example 6: Solid phase synthesis method: scale ≤1µmol**

Syntheses of siRNA sense and antisense strands were performed on a MerMade192X synthesiser with commercially available solid supports made of controlled pore glass with universal linker (Universal CPG, with a loading of 40 µmol/g; LGC Biosearch or Glen Research).

RNA phosphoramidites were purchased from ChemGenes or Hongene.

The 2'-O-Methyl phosphoramidites used were the following: 5'-(4,4'-dimethoxytrityl)-N-benzoyl-adenosine 2'-O-methyl-3'- [(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5'-(4,4'-dimethoxytrityl)-N-acetyl-cytidine 2'-O-methyl-3'- [(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5'-(4,4'-dimethoxytrityl)-N-isobutyryl-guanosine 2'-O-methyl-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5'-(4,4'-dimethoxytrityl)-uridine 2'-O-methyl-3'-[(2-cyanoethyl)- (N,N-diisopropyl)]-phosphoramidite.

The 2'-F phosphoramidites used were the following: 5'-dimethoxytrityl-N-benzoyl-deoxyadenosine 2'-fluoro-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5'-dimethoxytrityl-N-acetyl-deoxycytidine 2'-fluoro-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5'-dimethoxytrityl-N-isobutyryl-deoxyguanosine 2'-fluoro-3'- [(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite and 5'-dimethoxytrityl-deoxyuridine 2'-fluoro-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite.

All phosphoramidites were dissolved in anhydrous acetonitrile (Honeywell Research Chemicals) at a concentration of 0.05M, except 2'-O-methyl-uridine phosphoramidite which was dissolved in DMF/MeCN (1:4, v/v). Iodine at 0.02M in acetonitrile/Pyridine/$H_2O$ (DNAchem) was used as oxidizing reagent. Thiolation for phosphorothioate linkages was performed with 0.2 M PADS

(TCI) in acetonitrile/pyridine 1:1 v/v. 5-Ethyl thiotetrazole (ETT), 0.25M mM in acetonitrile was used as activator solution.

Inverted abasic phosphoramidite, 3-O-Dimethoxytrityl-2-deoxyribose-5-[(2-cyanoethyl)-(N, N-diisopropyl)]-phosphoramidite were purchased from Chemgenes (ANP-1422) or Hongene (OP-040).

At each cycle, the DMT was removed by deblock solution, 3% TCA in DCM (DNAchem).

The coupling time was 180 seconds. The oxidizer contact time was set to 80 seconds and thiolation time was 2*100 seconds.

At the end of the synthesis, the oligonucleotides were cleaved from the solid support using a $NH_4OH$:EtOH solution 4:1 (v/v) for 20 hours at 45°C (TCI). The solid support was then filtered off, the filter was thoroughly washed with $H_2O$ and the volume of the combined solution was reduced by evaporation under reduced pressure.

Oligonucleotide were treated to form the sodium salt by ultracentrifugation using Amicon Ultra-2 Centrifugal Filter Unit; PBS buffer (10x, Teknova, pH 7.4, Sterile) or by EtOH precipitation from 1M sodium acetate.

The single strands identity were assessed by MS ESI- and then, were annealed in water to form the final duplex siRNA and duplex purity were assessed by size exclusion chromatography.

**Example 7: Solid phase synthesis method: scale ≥5 µmol**

Syntheses of siRNA sense and antisense strands were performed on a MerMade12 synthesiser with commercially available solid supports made of controlled pore glass with universal linker (Universal CPG, with a loading of 40 µmol/g; LGC Biosearch or Glen Research) at 5 µmol scale. Sense strand destined to 3' conjugation were sytnthesised at 12 µmol on 3'-PT-Amino-Modifier C6 CPG 500 Å solid support with a loading of 86 µmol/g (LGC).

RNA phosphoramidites were purchased from ChemGenes or Hongene.

The 2'-O-Methyl phosphoramidites used were the following: 5'-(4,4'-dimethoxytrityl)-N-benzoyl-adenosine 2'-O-methyl-3'- [(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5'-(4,4'-dimethoxytrityl)-N-acetyl-cytidine 2'-O-methyl-3'- [(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5'-(4,4'-dimethoxytrityl)-N-isobutyryl-guanosine 2'-O-methyl-3'-[(2-

cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5'-(4,4'-dimethoxytrityl)-uridine 2'-O-methyl-3'-[(2-cyanoethyl)- (N,N-diisopropyl)]-phosphoramidite.

The 2'-F phosphoramidites used were the following: 5'-dimethoxytrityl-N-benzoyl-deoxyadenosine 2'-fluoro-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5'-dimethoxytrityl-N-acetyl-deoxycytidine 2'-fluoro-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5'-dimethoxytrityl-N-isobutyryl-deoxyguanosine 2'-fluoro-3'- [(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite and 5'-dimethoxytrityl-deoxyuridine 2'-fluoro-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite.

Inverted abasic phosphoramidite, 3-O-Dimethoxytrityl-2-deoxyribose-5-[(2-cyanoethyl)-(N, N-diisopropyl)]-phosphoramidite were purchased from Chemgenes (ANP-1422) or Hongene (OP-040).

All phosphoramidites were dissolved in anhydrous acetonitrile (Honeywell Research Chemicals) at a concentration of 0.05M, except 2'-O-methyl-uridine phosphoramidite which was dissolved in DMF/MeCN (1:4, v/v). Iodine at 0.02M in acetonitrile/Pyridine/$H_2O$ (DNAchem) was used as oxidizing reagent. Thiolation for phosphorothioate linkages was performed with 0.2 M PADS (TCI) in acetonitrile/pyridine 1:1 v/v. 5-Ethyl thiotetrazole (ETT), 0.25M mM in acetonitrile was used as activator solution.

At each cycle, the DMT was removed by deblock solution, 3% TCA in DCM (DNAchem).

For strands synthesised on universal CPG the coupling was performed with 8 eq. of amidite for 130 seconds. The oxidation time was 47 seconds, the thiolation time was 210 seconds.

For strands synthesised on 3'-PT-Amino-Modifier C6 CPG the coupling was performed with 8 eq. of amidite for 2*150 seconds. The oxidation time was 47 seconds, the thiolation time was 250 seconds

At the end of the synthesis, the oligonucleotides were cleaved from the solid support using a NH4OH:EtOH solution 4:1 (v/v) for 20 hours at 45°C (TCI). The solid support was then filtered off, the filter was thoroughly washed with $H_2O$ and the volume of the combined solution was reduced by evaporation under reduced pressure.

Oligonucleotide were treated to form the sodium salt by EtOH precipitation from 1M sodium acetate.

The single strand oligonucleotides were purified by IP-RP HPLC on Xbridge BEH C18 5 µm, 130 Å, 19x150 mm (Waters) column with an increasing gradient of B in A. Mobile phase A: 240 mM HFIP, 7 mM TEA and 5% methanol in water; mobile phase B: 240 mM HFIP, 7 mM TEA in methanol.

The single strands purity and identity were assessed by UPLC/MS ESI- on Xbridge BEH C18 2.5 µm, 3x50 mm (Waters) column with an increasing gradient of B in A. Mobile phase A: 100 mM HFIP, 5 mM TEA in water; mobile phase B: 20% mobile phase A: 80% Acetonitrile (v/v).

Sense strands were conjugated as per protocol provided in any of Examples 2, 4, 6.

Sense and Antisense strands were then annealed in water to form the final duplex siRNA and duplex purity were assessed by size exclusion chromatography.

**Example 8: Dose-response for Inhibition of CFHR4 Expression in Human Huh7 Cells**

Huh7 cells (human hepatocyte-derived cell line, obtained from JCRB Cell Bank) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% non-essential amino acids at 37°C, 5% $CO_2$, 95% humidity. Cells were transfected with siRNA duplexes targeting either CFHR4 mRNA or a negative control siRNA (siRNA-control; sense strand 5'-GCGAAUACAUCAGUUCUAUTT-3' (SEQ ID NO:669), antisense strand 5'-AUAGAACUGAUGUAUUCGCTT-3' (SEQ ID NO:670)) in a 6-point, log dose response curve to give final in assay concentrations of 3nM to 0.03pM. Transfection was carried out by diluting Lipofectamine RNAiMAX (ThermoFisher) in Opti-MEM (ThermoFisher) medium at a ratio of 48.5:1.5. This solution was added to an equal volume of siRNA, diluted to the required concentration in phosphate buffered saline. The lipofectamine RNAiMAX and siRNA mixture was incubated at room temperature for 15 minutes before 20 µL was added to wells of a 96 well plate. Huh7 cells were dissociated from flasks using trypsin and resuspended at a density of 300,000 cells/mL. 100 µL of Huh7 cell suspension was added to each well of the siRNA-containing 96-well plates. Cells were incubated for 48 hours at 37°C, 5% $CO_2$,95% humidity. Each siRNA was tested in triplicate wells and on two separate days for a total of six replicates.

Intracellular RNA was isolated using an RNeasy kit (Qiagen) according to the manufacturer's instructions. cDNA synthesis was performed using a FastKing RT kit, with gDNase (Tiangen). Target cDNA was the quantified by qPCR on an ABI Prism 7900HT or ABI QuantStudio 7 with primers specific for human CFHR4 (forward: TGTGTAAATTGGGATATAATGCGA (SEQ ID NO:671), reverse: CCTTATTCGCATCTGGGGTA (SEQ ID NO:672)) and human GAPDH

(forward: GAAGGTGAAGGTCGGAGTC (SEQ ID NO:673), reverse: GAAGATGGTGATGGGATTTC (SEQ ID NO:674)) using a SensiFAST SYBR Hi-ROX kit (Meridian).

qPCR was performed in duplicate on cDNA derived from each well and the mean Ct calculated. Relative CFHR4 expression was calculated from mean Ct values using the comparative Ct (ΔΔCt) method, normalised to GAPDH and relative to untreated cells. Maximum percent inhibition of CFHR4 expression and pEC50 values (-$\log_{10}$ of the EC50) were calculated using a four parameter (variable slope) model using NumPy (Python). Results are shown in Table 6. Sequences of RNAi molecules are depicted in the relevant Tables herein.

**Table 6 -** Results of dose-response experiments for inhibition of CFHR4 mRNA expression in human Huh7 cells. Observed and fitted pEC50s are reported with observed minimum and maximum relative expression values (relative to medium control) for two experimental replicates (repeated on separate days with three repeats per day).

| ETXM ID | Minimum r1 | Maximum r1 | pEC50 r1 | Minimum r2 | Maximum r2 | pEC50 r2 |
|---|---|---|---|---|---|---|
| ETX-M00001327 | 0.45 | 0.91 | 7.32 | 0.46 | 0.8 | 10.97 |
| ETX-M00001328 | 0.5 | 0.82 | 6.42 | 0.48 | 0.92 | 8.0 |
| ETX-M00001329 | 0.57 | 0.86 | 12.67 | 0.53 | 1.01 | 12.38 |
| ETX-M00001330 | 0.38 | 0.9 | 8.62 | 0.35 | 1.08 | 9.65 |
| ETX-M00001331 | 0.36 | 1.06 | 11.24 | 0.59 | 1.06 | 10.53 |
| ETX-M00001333 | 0.35 | 1.04 | 9.24 | 0.53 | 1.1 | 10.28 |
| ETX-M00001335 | 0.54 | 0.87 | 7.31 | 0.34 | 0.93 | 7.83 |
| ETX-M00001336 | 0.51 | 0.89 | 10.92 | 0.56 | 1.05 | 0.0 |
| ETX-M00001337 | 0.4 | 0.93 | 10.14 | 0.46 | 1.05 | 8.16 |
| ETX-M00001338 | 0.53 | 0.84 | 17.9 | 0.62 | 0.96 | 5.12 |
| ETX-M00001339 | 0.46 | 0.85 | 31.19 | 0.59 | 1.36 | 10.44 |
| ETX-M00001341 | 0.52 | 0.88 | 5.25 | 0.51 | 1.04 | 5.99 |
| ETX-M00001343 | 0.54 | 0.79 | 12.69 | 0.71 | 0.98 | 16.94 |
| ETX-M00001344 | 0.55 | 0.76 | 4.15 | 0.61 | 1.01 | 7.62 |
| ETX-M00001345 | 0.26 | 0.82 | 10.95 | 0.3 | 0.87 | 9.18 |
| ETX-M00001347 | 0.16 | 0.89 | 10.21 | 0.21 | 1.15 | 9.57 |
| ETX-M00001349 | 0.39 | 0.92 | 10.79 | 0.41 | 1.23 | 9.61 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ETX-M00001627 | 0.49 | 1.11 | 13.21 | 0.63 | 1.01 | 31.54 |
| ETX-M00001628 | 0.61 | 0.86 | 0.51 | 0.75 | 1.22 | 13.76 |
| ETX-M00001629 | 0.58 | 0.99 | 14.41 | 0.59 | 1.19 | 12.01 |
| ETX-M00001630 | 0.72 | 0.98 | 23.04 | ? | ? | ? |
| ETX-M00001631 | 0.65 | 1.0 | 6.59 | 0.74 | 1.04 | 12.57 |
| ETX-M00001632 | 0.6 | 1.02 | 9.52 | 0.75 | 1.14 | 13.55 |
| ETX-M00001633 | 0.57 | 1.02 | 14.69 | 0.72 | 1.2 | 0.0 |
| ETX-M00001634 | 0.44 | 0.83 | 16.36 | 0.64 | 0.99 | 5.37 |
| ETX-M00001635 | 0.63 | 0.98 | 6.4 | 0.57 | 0.94 | 7.59 |
| ETX-M00001636 | 0.59 | 1.11 | 7.09 | 0.55 | 1.66 | 11.56 |
| ETX-M00001637 | 0.66 | 1.25 | 18.57 | 0.67 | 1.66 | 11.3 |
| ETX-M00001638 | 0.63 | 1.04 | 7.12 | 0.65 | 1.13 | 8.05 |
| ETX-M00001639 | 0.71 | 1.25 | 9.09 | 0.68 | 1.5 | 12.82 |
| ETX-M00001640 | 0.72 | 1.23 | 0.0 | 0.75 | 1.28 | 10.02 |
| ETX-M00001641 | 0.61 | 0.93 | 10.25 | 0.68 | 1.12 | 11.63 |
| ETX-M00001642 | 0.57 | 0.96 | 6.2 | 0.62 | 0.97 | 2.19 |
| ETX-M00001643 | 0.68 | 1.14 | 11.58 | 0.64 | 1.38 | 22.24 |
| ETX-M00001644 | 0.66 | 0.95 | 11.1 | 0.65 | 1.05 | 13.19 |
| ETX-M00001645 | 0.44 | 0.96 | 8.42 | 0.5 | 1.09 | 9.59 |
| ETX-M00001646 | 0.53 | 0.94 | 9.26 | 0.6 | 1.14 | 8.42 |
| ETX-M00001647 | 0.51 | 1.14 | 7.08 | 0.75 | 1.12 | 12.82 |
| ETX-M00001648 | 0.42 | 0.96 | 8.49 | 0.64 | 0.94 | 13.63 |
| ETX-M00001649 | 0.52 | 1.02 | 10.65 | 0.54 | 0.91 | 1.64 |
| ETX-M00001650 | 0.54 | 0.9 | 3.98 | 0.71 | 0.95 | 12.56 |
| ETX-M00001651 | 0.49 | 0.93 | 13.61 | 0.63 | 0.9 | 40.37 |
| ETX-M00001652 | 0.55 | 0.91 | 13.62 | 0.58 | 1.03 | 13.05 |
| ETX-M00001653 | 0.48 | 0.72 | 20.93 | 0.7 | 0.89 | 13.56 |
| ETX-M00001654 | 0.31 | 0.77 | 10.84 | 0.46 | 1.04 | 10.82 |
| ETX-M00001655 | 0.45 | 0.89 | 7.12 | 0.53 | 1.07 | 10.49 |
| ETX-M00001656 | 0.26 | 0.83 | 9.68 | 0.45 | 0.92 | 10.35 |
| ETX-M00001657 | 0.34 | 0.67 | 10.78 | 0.5 | 1.01 | 11.59 |
| ETX-M00001658 | 0.28 | 0.7 | 10.71 | 0.41 | 1.0 | 10.8 |
| ETX-M00001659 | 0.23 | 0.81 | 9.92 | 0.28 | 0.95 | 9.69 |
| ETX-M00001660 | 0.24 | 0.66 | 10.15 | 0.33 | 0.87 | 11.04 |
| ETX-M00001661 | 0.17 | 0.68 | 10.24 | 0.23 | 0.9 | 9.61 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ETX-M00001662 | 0.22 | 0.79 | 13.26 | 0.37 | 0.88 | 10.45 |
| ETX-M00001663 | 0.18 | 0.81 | 9.84 | 0.28 | 0.83 | 10.22 |
| ETX-M00001664 | 0.11 | 0.83 | 10.13 | 0.17 | 0.79 | 10.31 |
| ETX-M00001665 | 0.19 | 0.84 | 10.35 | 0.16 | 0.93 | 10.9 |
| ETX-M00001666 | 0.27 | 0.84 | 10.83 | 0.32 | 1.19 | 11.0 |
| ETX-M00001667 | 0.37 | 0.93 | 10.5 | 0.19 | 0.8 | 8.98 |
| ETX-M00001668 | 0.3 | 0.87 | 10.79 | 0.27 | 0.81 | 9.3 |
| ETX-M00001669 | 0.23 | 0.89 | 9.59 | 0.21 | 0.96 | 10.18 |
| ETX-M00001670 | 0.23 | 0.78 | 9.67 | 0.32 | 0.85 | 9.63 |
| ETX-M00001671 | 0.2 | 0.85 | 10.81 | 0.34 | 0.85 | 10.11 |
| ETX-M00001672 | 0.28 | 0.8 | 13.28 | 0.38 | 0.86 | 10.05 |
| ETX-M00001673 | 0.22 | 0.92 | 10.49 | 0.31 | 0.9 | 8.85 |
| ETX-M00001674 | 0.19 | 0.92 | 11.71 | 0.26 | 0.93 | 10.63 |
| ETX-M00001675 | 0.22 | 0.84 | 9.91 | 0.4 | 0.91 | 11.06 |
| ETX-M00001676 | 0.25 | 0.75 | 9.54 | 0.26 | 1.06 | 9.52 |
| ETX-M00001677 | 0.2 | 0.81 | 9.96 | 0.33 | 1.05 | 10.24 |
| ETX-M00001678 | 0.34 | 0.94 | 10.52 | 0.31 | 0.89 | 10.18 |
| ETX-M00001679 | 0.32 | 0.95 | 9.87 | 0.32 | 1.0 | 8.48 |
| ETX-M00001680 | 0.31 | 0.87 | 11.7 | 0.39 | 0.95 | 8.89 |
| ETX-M00001681 | 0.46 | 0.82 | 10.83 | 0.47 | 1.0 | 6.98 |
| ETX-M00001682 | 0.4 | 0.81 | 8.62 | 0.4 | 0.93 | 10.58 |
| ETX-M00001683 | 0.31 | 0.86 | 9.1 | 0.29 | 1.01 | 8.98 |
| ETX-M00001684 | 0.37 | 0.95 | 8.28 | 0.46 | 0.86 | 5.54 |
| ETX-M00001685 | 0.31 | 0.81 | 9.46 | 0.4 | 1.0 | 9.36 |
| ETX-M00001686 | 0.45 | 0.9 | 8.41 | 0.33 | 0.89 | 8.67 |
| ETX-M00001687 | 0.29 | 0.99 | 10.05 | 0.44 | 0.88 | 10.64 |
| ETX-M00001688 | 0.27 | 1.31 | 9.19 | 0.31 | 0.96 | 9.18 |
| ETX-M00001689 | 0.39 | 0.82 | 0.29 | 0.43 | 0.92 | 8.54 |
| ETX-M00001690 | 0.26 | 0.8 | 12.1 | 0.36 | 0.81 | 8.85 |
| ETX-M00001691 | 0.35 | 0.91 | 8.54 | 0.37 | 0.88 | 10.87 |
| ETX-M00001692 | 0.26 | 0.8 | 9.56 | 0.27 | 0.9 | 9.59 |
| ETX-M00001693 | 0.25 | 0.66 | 11.37 | 0.35 | 0.88 | 10.17 |
| ETX-M00001694 | 0.32 | 0.77 | 10.63 | 0.21 | 0.9 | 11.13 |
| ETX-M00001695 | 0.26 | 1.0 | 17.19 | 0.2 | 0.93 | 9.78 |
| ETX-M00001696 | 0.34 | 0.85 | 9.1 | 0.28 | 1.1 | 10.77 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ETX-M00001697 | 0.11 | 0.89 | 10.42 | 0.2 | 0.98 | 10.61 |
| ETX-M00001698 | 0.23 | 1.08 | 9.58 | 0.26 | 0.84 | 10.53 |
| ETX-M00001699 | 0.38 | 1.14 | 10.13 | 0.39 | 0.94 | 12.05 |
| ETX-M00001700 | 0.47 | 1.16 | 10.53 | 0.32 | 0.93 | 11.86 |
| ETX-M00001701 | 0.47 | 1.12 | 10.49 | 0.3 | 0.97 | 11.23 |
| ETX-M00001702 | 0.43 | 1.08 | 9.42 | 0.3 | 0.9 | 8.87 |
| ETX-M00001703 | 0.46 | 1.24 | 9.78 | 0.4 | 0.8 | 12.64 |
| ETX-M00001704 | 0.63 | 1.19 | 9.58 | 0.33 | 0.96 | 9.39 |
| ETX-M00001705 | ? | ? | ? | 0.3 | 0.88 | 8.77 |
| ETX-M00001706 | 0.31 | 1.1 | 8.64 | 0.14 | 0.82 | 10.06 |
| ETX-M00001707 | 0.45 | 1.04 | 10.48 | 0.31 | 0.77 | 8.85 |
| ETX-M00001708 | 0.71 | 1.42 | 0.0 | 0.23 | 0.94 | 9.32 |
| ETX-M00001709 | 0.46 | 1.01 | 9.7 | 0.31 | 0.84 | 8.31 |
| ETX-M00001710 | 0.5 | 1.04 | 10.91 | 0.32 | 81.43 | 8.16 |
| ETX-M00001711 | 0.53 | 1.07 | 8.6 | 0.45 | 0.87 | 13.06 |
| ETX-M00001712 | 0.34 | 1.09 | 8.12 | 0.26 | 1.01 | 12.24 |
| ETX-M00001713 | 0.59 | 0.98 | 11.54 | 0.3 | 0.9 | 12.26 |
| ETX-M00001714 | 0.49 | 0.95 | 7.39 | 0.25 | 0.73 | 10.18 |
| ETX-M00001715 | 0.34 | 0.99 | 9.84 | 0.23 | 0.82 | 9.1 |
| ETX-M00001716 | 0.41 | 1.12 | 9.96 | 0.22 | 0.84 | 10.05 |
| ETX-M00001717 | 0.41 | 1.12 | 15.84 | 0.08 | 0.68 | 13.81 |
| ETX-M00001718 | 0.42 | 1.18 | 0.62 | 0.34 | 0.88 | 12.02 |
| ETX-M00001719 | 0.5 | 1.03 | 10.46 | 0.44 | 0.93 | 9.55 |
| ETX-M00001720 | 0.61 | 1.08 | 9.74 | 0.36 | 0.78 | 8.33 |
| ETX-M00001721 | 0.56 | 1.04 | 9.47 | 0.39 | 0.84 | 8.61 |
| ETX-M00001722 | 0.48 | 1.27 | 9.51 | 0.51 | 1.09 | 9.83 |
| ETX-M00001723 | 0.53 | 1.21 | 9.7 | 0.48 | 1.06 | 10.49 |
| ETX-M00001724 | 0.58 | 1.26 | 9.48 | 0.65 | 1.15 | 10.44 |
| ETX-M00001725 | 0.58 | 1.33 | 9.34 | 0.71 | 1.27 | 0.0 |
| ETX-M00001726 | 0.63 | 1.33 | 9.52 | 0.55 | 1.11 | 10.21 |
| ETX-M00001727 | 0.65 | 1.29 | 9.46 | 0.62 | 1.1 | 9.45 |
| ETX-M00001728 | 0.65 | 1.53 | 9.51 | 0.6 | 1.16 | 117.87 |
| ETX-M00001729 | 0.46 | 1.19 | 9.49 | 0.57 | 1.02 | 8.52 |
| ETX-M00001730 | 0.75 | 1.22 | 0.0 | 0.63 | 1.03 | 12.19 |
| ETX-M00001731 | ? | ? | ? | 0.71 | 1.11 | 12.12 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ETX-M00001732 | 0.71 | 1.12 | 10.3 | 0.57 | 1.31 | 0.0 |
| ETX-M00001733 | 0.58 | 1.17 | 9.56 | ? | ? | ? |
| ETX-M00001734 | 0.93 | 1.3 | 0.06 | 0.62 | 1.0 | 3.27 |
| ETX-M00001735 | 0.83 | 1.13 | 9.43 | 0.75 | 1.02 | 8.28 |
| ETX-M00001736 | 0.69 | 1.21 | 9.39 | 0.63 | 1.1 | 9.49 |
| ETX-M00001737 | 0.61 | 1.25 | 9.79 | 0.33 | 1.08 | 8.07 |
| ETX-M00001738 | 0.4 | 1.27 | 9.43 | 0.52 | 0.82 | 2.12 |
| ETX-M00001739 | 0.75 | 1.16 | 9.53 | 0.77 | 1.03 | 0.0 |
| ETX-M00001740 | 0.57 | 1.1 | 8.02 | 0.83 | 1.15 | 8.18 |
| ETX-M00001741 | 0.46 | 1.3 | 9.09 | 0.58 | 1.32 | 13.58 |
| ETX-M00001742 | 0.41 | 0.94 | 4.31 | 0.48 | 0.94 | 7.8 |

**Example 9: Pharmacodynamic study of ETX-407 in non-human primates (NHPs)**

This study was designed to evaluate the activity of 4 lead siRNAs and demonstrate proof of concept. Specifically, a 56-day, multiple dose study was designed to effectively demonstrate that reduction of target mRNA in the liver results in reduced levels of target protein in the eye.

To this end, 20 non-naïve male cynomolgus macaques aged 2-6 years received 3 mg/kg ETX siRNA subcutaneously on days 0 and 28. Liver biopsies were taken on study days -7, 28 and 56 for measurement of target mRNA and protein levels. Interim blood samples for measurement of circulating target protein levels were collected on study days -7, -3, 0, 3, 7, 14, 21, 28, 42 and 56 post-dose. A terminal choroidal sample to measure target protein levels in the eye was collected on day 56. A summary of the study design is shown in Figure 10.

The following siRNAs were tested in this study:

- ETX-M00001347 (SEQ ID NO:415 and SEQ ID NO:548)
- ETX-M00001697 (SEQ ID NO:487 and SEQ ID NO:620)
- ETX-M00001674 (SEQ ID NO:464 and SEQ ID NO:597)
- ETX-M00001664 (SEQ ID NO:454 and SEQ ID NO:587)

The following endpoints were assessed in this study:

- Clinical observations
- qPCR, liver tissue, *CFHR4* mRNA knockdown

- LC-MS for FHR-4 protein in plasma, liver, and choroid
- Hepatic function measurements (ALT, AST, ALP, GGT)

Hepatic CFHR4 mRNA and FHR-4 protein levels in NHPs

Hepatic CFHR4 mRNA and FHR-4 protein levels were determined after 1 or 2 doses of 3 mg/kg siRNA. Treatment of NHPs with ETX-M00001347 resulted in 70-75% knockdown of FHR-4 in liver tissue after 1 or 2 doses (Figure 11).

Plasma FHR-4 protein levels in NHPs

FHR-4 plasma protein levels were determined after 1 or 2 doses of 3 mg/kg siRNA. After 2 doses (Day 56), ETX-M00001347 reached a maximum of more than 75% knockdown of FHR-4 protein expression. ETX-M00001347 and ETX-M00001697 show statistically significant additivity (** $p < 0.01$) from single-dose nadir at Day 14 to 2-dose nadir at Day 42 (Figure 12).

Choroid FHR-4 protein levels in NHPs

FHR-4 choroid protein levels were determined after 2 doses of 3 mg/kg siRNA. After 2 doses (Day 56), ETX-M00001347 shows approximately 75% knockdown of FHR4 protein expression. Accordingly, knockdown of hepatic FHR4 successfully reduced FHR4 protein levels in the eye (Figure 13).

Tolerability of siRNAs

Hepatic function measurements were performed after 1 or 2 doses of 3 mg/kg siRNA. All measurements for hepatic function test (ALT, AST, alkaline phosphatase, and GGT) were within the normal range for NHPs (Figure 14).

Conclusion

ETX-M00001347 effectively reduced target mRNA and protein levels in the liver resulting in significantly reduced plasma protein levels and reduction of target protein levels in the eye (Figure 15).

**Example 10: Confirmation of Lead siRNA Activity in a Human hydrodynamic injection (HDI) Mouse Model**

The transient expression of human mRNA in mice was evaluated to assess the activity of siRNAs against human mRNA sequences. A single subcutaneous dose of siRNAs (3 mg/kg) was administered on Day -6. Plasmid hydrodynamic injection (HDI) of a plasmid expressing human CFHR4 mRNA was performed on Day 0. The study was terminated and mRNA expression was analysed on Day 1. As a negative control, a non-target specific siRNA was tested in parallel. An overview of the study is shown in Figure 16.

The following siRNAs were tested in this study:

- ETX-M00001347 (SEQ ID NO:415 and SEQ ID NO:548)
- ETX-M00001697 (SEQ ID NO:487 and SEQ ID NO:620)
- ETX-M00001674 (SEQ ID NO:464 and SEQ ID NO:597)
- ETX-M00001664 (SEQ ID NO:454 and SEQ ID NO:587)

Activity of all tested siRNAs was confirmed in the human HDI mouse model (Figure 17). Activity of ETX-M00001347 is shown in Figure 18.

**Example 11: Analysis of FHR-4 expression in AMD patients**

It has been established by Zouache et al. (Nat Commun, 2024, 15(1):443) that FHR-4 protein levels in the eye, blood and other organs are influenced independently by 3 protein quantitative trait loci (pQTLs), i.e., the loci rs61818956, rs10494745, rs7531555 on chromosome 1. However, it has been concluded by Zouache et al. that these pQTLs are distinct from loci that are associated with AMD and that genetically driven variation in FHR-4 levels do not influence AMD susceptibility or its course of progression.

Herein, the inventors have re-analysed the patient progression data in Figure 4 of Zouache et al. and surprisingly shown the plausibility of FHR-4 as a relevant driver of complement-mediated disease progression (as opposed to risk of developing the disease de novo) in AMD.

For that, the following workflow was used:

At each location described by the ID numbers rs61818956, rs10494745 and rs7531555, the genetic marker or variant or SNP may have one of two forms dependent on the DNA base at that

location (designated C, A, T or G for cytosine, adenine, thymine or guanine): the major allele or base; or the minor or effect allele.

| pQTL Variant Identifier | Major Allele | Minor Allele |
|---|---|---|
| rs61818956 | C | T |
| rs10494745 | G | A |
| rs7531555 | C | T |

These FHR-4 influencing SNPs have the potential to interact with each other to modulate the levels and/or activity of FHR-4 in either the same or opposing directions; furthermore, since humans are 'diploid', inheriting two copies of each chromosome, there is the possibility of interaction between as well as within chromosomes making the genotype-phenotype relationship complex and non-obvious.

The inventors have assigned a score to each SNP to encode the direction of the effect and have treated the effect sizes as equal in order to illustrate the basis for a predictor. For rs61818956, a minor allele score of 1 was assigned. For rs10494745 and rs7531555 a minor allele score of -1 was assigned.

Predicted effects for the haplotypes observed in Zouache et al. are summarised in the table below.

| **pQTL Variant Identifier** | **Observed Haplotypes** | | | | **Predicted Haplotype Effect** | | | |
|---|---|---|---|---|---|---|---|---|
| | **H1** | **H2** | **H3** | **H4** | **H1** | **H2** | **H3** | **H4** |
| rs61818956 | C | T | C | C | 0,0 | 1,0 | 0,0 | 0,0 |
| rs10494745 | G | G | A | G | 0,0 | 0,0 | -1,0 | 0,0 |
| rs7531555 | C | C | C | T | 0,0 | 0,0 | 0,0 | -1,0 |
| | | | | | **0,0** | **1,0** | **-1,0** | **-1,0** |

Furthermore, there are other possible haplotypes that were not observed and are likely rarer - possibly due to linkage disequilibrium. These are also summarised separately in the table along with their predicted effects on FHR-4 protein levels in the body.

| **pQTL Variant Identifier** | **Unobserved Haplotypes** | | | | **Predicted Haplotype Effect** | | | |
|---|---|---|---|---|---|---|---|---|
| | **H5** | **H6** | **H7** | **H8** | **H5** | **H6** | **H7** | **H8** |
| rs61818956 | C | T | T | T | 0,0 | 1,0 | 1,0 | 1,0 |
| rs10494745 | A | A | G | A | -1,0 | 0,0 | -1,0 | -1,0 |
| rs7531555 | T | T | T | C | -1,0 | -1,0 | -1,0 | 0,0 |
| | | | | | **-2,0** | **0,0** | **-1,0** | **0,0** |

The potential diplotypes that could result from combinations of these 3 QTL loci within and between chromosome 1 pairs is shown in the table below (column 1). The predicted aggregate effect per diplotype is shown in column 2 and results from a (weighted - here the weighting is 1) summation of the magnitude and direction of each individual independent effect. Shaded in grey are diplotypes consisting of haplotypes that were observed by Zouache et al.

| **Potential Diplotypes** | **Predicted Diplotype Effect** | **Responder Rank** |
|---|---|---|
| H2:H2 | 2 | 1 |
| H1:H2 | 1 | 2 |
| H2:H6 | 1 | 2 |
| H2:H8 | 1 | 2 |
| H1:H1 | 0 | 3 |
| H2:H3 | 0 | 3 |
| H2:H4 | 0 | 3 |
| H1:H6 | 0 | 3 |
| H1:H8 | 0 | 3 |
| H2:H7 | 0 | 3 |
| H6:H6 | 0 | 3 |
| H6:H8 | 0 | 3 |

| | | |
|---|---|---|
| H8:H8 | 0 | 3 |
| H1:H3 | -1 | 4 |
| H1:H4 | -1 | 4 |
| H1:H7 | -1 | 4 |
| H2:H5 | -1 | 4 |
| H3:H6 | -1 | 4 |
| H3:H8 | -1 | 4 |
| H4:H6 | -1 | 4 |
| H4:H8 | -1 | 4 |
| H6:H7 | -1 | 4 |
| H7:H8 | -1 | 4 |
| H3:H3 | -2 | 5 |
| H3:H4 | -2 | 5 |
| H4:H4 | -2 | 5 |
| H1:H5 | -2 | 5 |
| H3:H7 | -2 | 5 |
| H4:H7 | -2 | 5 |
| H5:H6 | -2 | 5 |
| H5:H8 | -2 | 5 |
| H7:H7 | -2 | 5 |
| H3:H5 | -3 | 6 |
| H4:H5 | -3 | 6 |
| H5:H7 | -3 | 6 |
| H5:H5 | -4 | 7 |

This allows potential responders to be ranked based on their genetic profile (column 3) according to the likely magnitude of the influence on mean circulating FHR-4 protein levels over time and is preferable to measuring serum FHR-4 at arbitrary time points and setting arbitrary thresholds.

The ranking was generated by summing all of the allele scores in the table columns for all of the alleles in a haplotype & then adding the 2 haplotype scores for the diplotype. Each score is an integer (including zero to cover the effect of the major allele) with a sign +/- to indicate the direction of the effect. Each individual score can be multiplied by a weighing factor between 0 & 1 to allow for the fact that the effects may not be of equal magnitude.

When considering the potential diplotypes arising from combinations of the observed haplotypes in Zouache et al., it becomes apparent that their choice of the 'Baseline' group in Figure 4 is suboptimal because their focus on just 2 out of the 3 possible diplotypes that satisfy their conditions results in 3 different predicted FHR-4 levels for this group and would be expected to inject noise and error into their calculations.

| **Fig.4 of Zouchane et al.** | **rs61818956** | **rs10494745** | **Haplotypes satisfying condition** | **Diplotypes satisfying condition** | **Score** |
|---|---|---|---|---|---|
| Chr1_low | C:C | A:A | H3 | H3:H3 | -2 |
| Chr1_medium | C:C | G:G | H1, H4 | H1:H1, H1:H4, H4:H4 | 0, -1, -2 |
| Chr1_high | T:T | G:G | H2 | H2:H2 | 2 |

The conclusion of Zouache et al. that FHR-4 protein levels do not correlate with AMD progression is based largely on Figure 4c, which shows the median age of first recorded conversion to late AMD. The statistical test used in this analysis ($x^2$) did not reach significance ($p = 0.36$), leading the authors to conclude that there is no statistically significant correlation between FHR-4 levels and AMD progression. However, Figure 4c of Zouache et al. shows that there is a clear dose-dependent trend between patient groups from Chr1_low to Chr_1_high. This led the inventors to re-analyse the data in Zouache et al. When applying a more robust statistical test, including a linear model fit with a one-tailed t-test of the co-efficients, the inventors obtained a p-value of 0.07, indicating that there is only a 7% chance of seeing these observations if the null hypothesis, i.e. that there is no correlation between FHR-4 protein levels and AMD progression, is true (see table below). It is therefore plausible that FHR-4 protein levels are correlated with AMD progression. Importantly, these results were obtained even with the more 'noisy' data used by Zouache et al. It is therefore expected that the correlation between FHR-4 protein levels and AMD progression will become even more apparent when patients are correctly grouped based on all possible diplotypes, as described herein above.

| | **Estimate** | **Std Error** | **t value** | **p** |
|---|---|---|---|---|
| Intercept | 84.4 | 3.61 | 23.37 | <2e16 |

| | | | | |
|---|---|---|---|---|
| Group | -2.61 | 1.73 | -1.51 | 0.072 |

The present invention is not intended to be limited in scope to the particular disclosed embodiments, which are provided, for example, to illustrate various aspects of the invention. Various modifications to the compositions and methods described will become apparent from the description and teachings herein. Such variations may be practiced without departing from the true scope and spirit of the disclosure and are intended to fall within the scope of the present disclosure.

New PCT Patent Application
Based on EP 23 155 091.4
e-therapeutics plc
Voss.-Ref.: AF3460 PCT BS

## CLAIMS

1. An inhibitor of expression and / or function of CFHR4/FHR-4, wherein said inhibitor is conjugated to one or more ligand moieties, preferably wherein the ligand moiety allows targeting of hepatocytes.

2. An inhibitor according to claim 1, wherein said inhibitor is an siRNA oligomer.

3. An inhibitor of expression and / or function of CFHR4/FHR-4, wherein said inhibitor is an siRNA oligomer.

4. An inhibitor according to claim 3, wherein said inhibitor comprises an siRNA oligomer conjugated to one or more ligand moieties, preferably wherein the ligand moiety allows targeting of hepatocytes.

5. An inhibitor according to claim 1, 2 or 4, wherein said one or more ligand moieties comprise one or more GalNAc ligands or comprise one more GalNAc ligand derivatives.

6. An inhibitor according to claim 1, 2 or 4 wherein said one or more ligand moieties comprise one or more GalNAc ligand derivatives.

7. An inhibitor according to one or more preceding claims, wherein the target of the inhibitor is CFHR4/FHR-4.

8. An inhibitor according to one or more preceding claims, wherein the inhibitor is a nucleic acid for inhibiting expression of CFHR4 comprising a duplex region that comprises a first strand and a second strand that is at least partially complementary to the first strand, wherein

(i) at least partially complementary to a portion of RNA transcribed from the CFHR4 gene, and

(ii) comprises at least 17 contiguous nucleosides differing by 0 or 1 nucleosides from any one of the first strand sequences as listed in Table 2.

9. An inhibitor according to one or more preceding claims, wherein the inhibitor is a nucleic acid for inhibiting expression of CFHR4 comprising a duplex region that comprises a first strand and a second strand that is at least partially complementary to the first strand, wherein said first strand is:

(i) at least partially complementary to a portion of RNA transcribed from the CFHR4 gene, and

(ii) comprises at least 17 contiguous nucleosides differing by 0 or 1 nucleosides from any one of the first strand modified sequences as listed in Table 3.

10. An inhibitor according claim 8 or 9, wherein the first strand comprises nucleosides 2-18 of any one of the sequences defined in claim 8 or 9, in particular wherein the first strand comprises nucleosides 2-18 of any one of the sequences defined in Tables 2 or 3.

11. An inhibitor according to claim 8, wherein the second strand comprises a nucleoside sequence of at least 17 contiguous nucleosides differing by 0 or 1 nucleosides from any one of the second strand sequences as listed in Table 2, and wherein the second strand has a region of at least 85% complementarity over the 17 contiguous nucleosides to the first strand.

12. An inhibitor according to claim 9, wherein the second strand comprises a nucleoside sequence of at least 17 contiguous nucleosides differing by 0 or 1 nucleosides from any one of the second strand modified sequences as listed in Table 4, and wherein the second strand has a region of at least 85% complementarity over the 17 contiguous nucleosides to the first strand.

13. An inhibitor according to claim 8, wherein the first strand comprises any one of the first strand sequences as listed in Table 2.

14. An inhibitor according to claim 9, wherein the first strand comprises any one of the first strand modified sequences as listed in Table 3.

15. An inhibitor according to claim 11, wherein the second strand comprises any one of the second strand sequences as listed in Table 2.

16. An inhibitor according to claim 12, wherein the second strand comprises any one of the second strand modified sequences as listed in Table 4.

17. An inhibitor according to claim 13, wherein the first strand comprises any one of the following sequences: SEQ ID NO:149, SEQ ID NO:221, SEQ ID NO:198, and SEQ ID NO:188.

18. An inhibitor according to claim 14, wherein the first strand comprises any one of the following sequences: SEQ ID NO:415, SEQ ID NO:487, SEQ ID NO:464, and SEQ ID NO:454.

19. An inhibitor according to claim 15, wherein the second strand comprises any one of the following sequences: SEQ ID NO:282, SEQ ID NO:354, SEQ ID NO:331, and SEQ ID NO:321.

20. An inhibitor according to claim 16, wherein the second strand comprises any one of the following sequences: SEQ ID NO:548, SEQ ID NO:620, SEQ ID NO:597, and SEQ ID NO:587.

21. An inhibitor according any one of claims 8 and 11, comprising first and second strands that comprise, consist of, or consist essentially of a nucleoside sequence differing by 0 or 1 nucleosides from any one of the following combinations of first and second sequences:

| Unmodified first strand | Unmodified second strand |
|---|---|
| SEQ ID NO:149 | SEQ ID NO:282 |
| SEQ ID NO:221 | SEQ ID NO:354 |
| SEQ ID NO:198 | SEQ ID NO:331 |
| SEQ ID NO:188 | SEQ ID NO:321 |

22. An inhibitor according any one of claims 9 and 12, comprising first and second strands that comprise, consist of, or consist essentially of a nucleoside sequence differing by 0 or 1 nucleosides from any one of the following combinations of first and second sequences:

| Unmodified first strand | Unmodified second strand |
|---|---|
| SEQ ID NO:415 | SEQ ID NO:548 |
| SEQ ID NO:487 | SEQ ID NO:620 |
| SEQ ID NO:464 | SEQ ID NO:597 |
| SEQ ID NO:454 | SEQ ID NO:587 |

23. An inhibitor according any one of claims 8 to 22, wherein the first strand has a length in the range of 17 to 30 nucleosides, preferably 19 to 25 nucleosides, more preferably 19 or 23 nucleosides.

24. An inhibitor according any one of claims 8 to 23, wherein the second strand has a length in the range of 17 to 30 nucleosides, preferably 19 to 25 nucleosides, more preferably 19 or 21 or 23 nucleosides.

25. An inhibitor according any one of claims 8 to 24, wherein the duplex region of the nucleic acid is between 17 and 30 nucleosides in length, more preferably is 19 or 21 or 23 nucleosides in length.

26. An inhibitor according any one of claims 8 to 25, wherein the region of complementarity between the first strand and the portion of RNA transcribed from the CFHR4 gene is between 17 and 30 nucleosides in length.

27. An inhibitor according any one of claims 8 to 26, wherein the nucleic acid further comprises one or more single-stranded nucleoside overhangs, optionally wherein the overhang is present on the first or second strand, preferably at the 3' terminus of the first or second strand, and/or wherein the overhang comprises 1 to 4 nucleosides, more preferably 2 nucleosides.

28. An inhibitor according any one of claims 8 to 27, wherein the nucleic acid is an siRNA oligonucleoside.

29. An inhibitor according to any one of claims 8 to 28, wherein the second sense strand further comprises one or more abasic nucleosides in a terminal region of the second strand, and wherein said abasic nucleoside(s) is / are connected to an adjacent nucleoside through a reversed internucleoside linkage.

30. An inhibitor according to any one of claims 8 to 29, wherein the second strand comprises 2 consecutive abasic nucleosides in the 5' terminal region of the second strand, wherein one such abasic nucleoside is a terminal nucleoside at the 5' terminal region of the second strand and the other abasic nucleoside is a penultimate nucleoside at the 5' terminal region of the second strand, wherein:

(a) said penultimate abasic nucleoside is connected to an adjacent first basic nucleoside in an adjacent 5' near terminal region through a reversed internucleoside linkage; and

(b) the reversed linkage is a 5-5' reversed linkage; and

(c) the linkage between the terminal and penultimate abasic nucleosides is 3'5' when reading towards the terminus comprising the terminal and penultimate abasic nucleosides.12. An inhibitor, or inhibitor for use, according claim 10 or 11, wherein the reversed internucleoside linkage is at a terminal region which is distal to the 5' terminal region of

the second strand, or at a terminal region which is distal to the 3' terminal region of the second strand.

31. An inhibitor according to claim 30, wherein

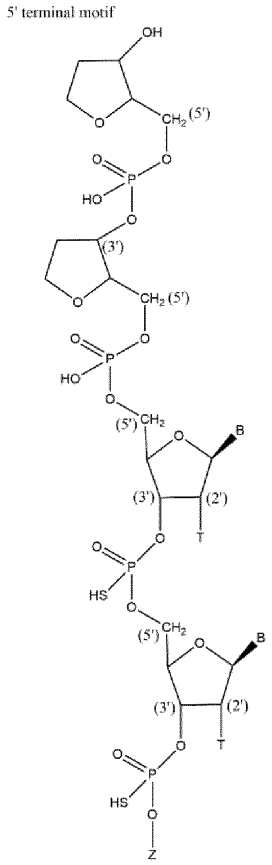
(i) the first strand and the second strand each has a length of 23 nucleosides;

(ii) two phosphorothioate internucleoside linkages are respectively between three consecutive positions in said 5' near terminal region of the second strand, wherein a first phosphorothioate internucleoside linkage is present between said adjacent first basic nucleoside of (a) and an adjacent second basic nucleoside in said 5' near terminal region of the second strand, and a second phosphorothioate internucleoside linkage is present between said adjacent second basic nucleoside and an adjacent third basic nucleoside in said 5' near terminal region of the second strand;

(iii) two phosphorothioate internucleoside linkages are respectively between three consecutive positions in both 5' and 3' terminal regions of the first strand, whereby a terminal nucleoside respectively at each of the 5' and 3' terminal regions of said first strand is each attached to a respective 5' and 3' adjacent penultimate nucleoside by a phosphorothioate internucleoside linkage, and each first 5' and 3' penultimate nucleoside is attached to a respective 5' and 3' adjacent antepenultimate nucleoside by a phosphorothioate internucleoside linkage; and

(iv) the second strand of the nucleic acid is conjugated directly or indirectly to one or more ligand moieties at the 3' terminal region of the second strand.

32. An inhibitor according to claim 30 or 31, wherein, wherein the 2 consecutive inverted abasic nucleosides in the 5' terminal region of the second strand present as the following 5' terminal motif




wherein:

T represents a 2'Me ribose modification,

B represents the nucleoside bases of the first two basic nucleosides in the 5' terminal region of the second strand, and

Z represents the remaining 19 contiguous basic nucleosides of said second strand.

33. An inhibitor according to any one of claims 8 to 32, wherein the nucleic acid is conjugated directly or indirectly to one or more ligand moieties, optionally wherein said ligand moiety is present at a terminal region of the second strand, preferably at the 3' terminal region thereof.

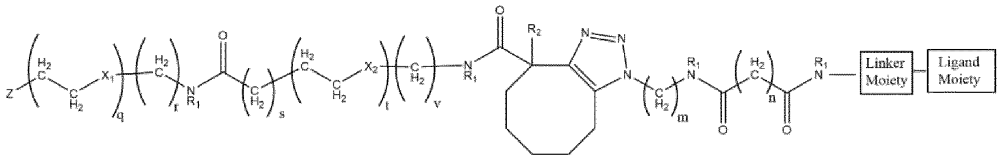
34. An inhibitor according to claim 33, wherein the ligand moiety comprises:

(i) one or more N-acetyl galactosamine (GalNAc) ligands, and / or

(ii) one or more N-acetyl galactosamine (GalNAc) ligand derivatives.

35. An inhibitor according to claim 34, wherein said one or more GalNAc ligands and / or GalNAc ligand derivatives are conjugated directly or indirectly to the 5' or 3' terminal region of the second strand of the nucleic acid, preferably at the 3' terminal region thereof.

36. An inhibitor according to any one of claims 33 to 35, comprising the structure:




wherein:

$R_1$ at each occurrence is independently selected from the group consisting of hydrogen, methyl and ethyl;

$R_2$ is selected from the group consisting of hydrogen, hydroxy, -$OC_{1-3}$alkyl, -$C(=O)OC_{1-3}$alkyl, halo and nitro;

$X_1$ and $X_2$ at each occurrence are independently selected from the group consisting of methylene, oxygen and sulfur;

m is an integer of from 1 to 6;

n is an integer of from 1 to 10;

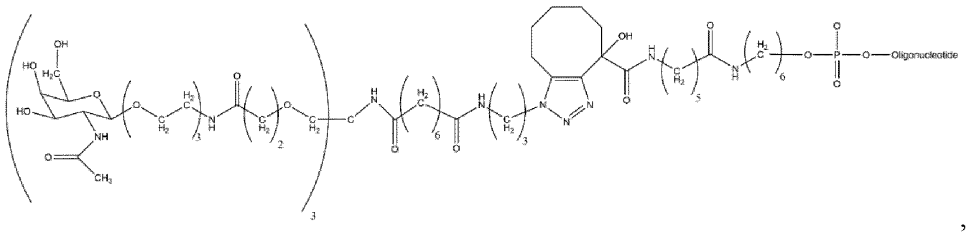
q, r, s, t, v are independently integers from 0 to 4, with the proviso that:

(i) q and r cannot both be 0 at the same time; and

(ii) s, t and v cannot all be 0 at the same time;

Z is an oligonucleoside moiety.

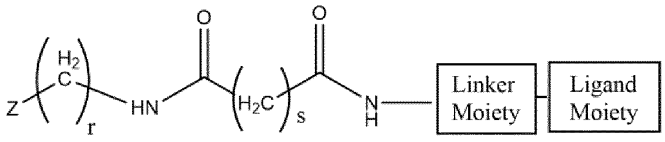
37. An inhibitor according to claim 36, comprising the structure




,

wherein oligonucleotide represents the contiguous nucleosides of the second strand.

38. An inhibitor according to any one of claims 33 to 35, comprising the structure:

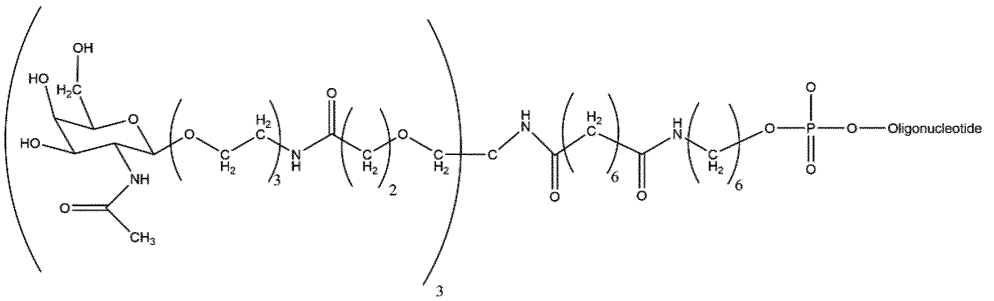



wherein:

r and s are independently an integer selected from 1 to 16; and

Z is an oligonucleoside moiety.

39. An inhibitor according to claim 38, comprising the structure




,

wherein oligonucleotide represents the contiguous nucleosides of the second strand.

40. An inhibitor according to any one of claims 37 or 39, wherein the structure is conjugated to the 3' terminal region of the second strand.

41. An inhibitor according to any one of claims 22, 32, 37 and 40.

42. An inhibitor according to any one of claims 22, 32, 39 and 40.

43. An inhibitor according to one or more preceding claims, formulated as a pharmaceutical composition with an excipient and / or carrier.

44. A pharmaceutical composition comprising an inhibitor according to one or more preceding claims, in combination with a pharmaceutically acceptable excipient or carrier.

45. An inhibitor according to any one of claims 1 to 43 or a pharmaceutical composition according to claim 44, for use in therapy.

46. An inhibitor according to any one of claims 1 to 43 or a pharmaceutical composition according to claim 44 for use in treating a disease or disorder associated with alterations in complement pathway protein levels and/or function in a patient, in particular wherein the disease

or disorder is associated with elevated levels of complement factor H-related protein 4 (FHR-4).47. An inhibitor or a pharmaceutical composition for use according to claim 46, wherein the disease or disorder associated with alterations in complement pathway protein levels and/or function is age-related macular degeneration (AMD), preferably dry age-related macular degeneration.

48. An inhibitor or a pharmaceutical composition for use according to claim 46, wherein the disease or disorder associated with alterations in complement pathway protein levels and/or function is a kidney disease, such as atypical haemolytic uraemic syndrome (aHUS), C3 glomeruloneoropathy (C3G), IgA nephropathy (IgAN), or mebranous (glomerulo) nephropathy (MN).

49. An inhibitor or a pharmaceutical composition for use according to any one of claims 46 to 48, wherein the inhibitor of expression and/or function of CFHR4/FHR-4 slows the progression of the disease or disorder associated with alterations in complement pathway protein levels and/or function.

50. An inhibitor according to any one of claims 1 to 43 or a pharmaceutical composition according to claim 44, for use in the treatment of age-related macular degeneration (AMD) in a patient, preferably dry age-related macular degeneration.

51. An inhibitor or pharmaceutical composition for use according to any one of claims 46 to 50, wherein the patient has elevated levels of FHR-4.

52. An inhibitor or pharmaceutical composition for use according to claim 51, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles rs61818956TT and/or rs10494745GG and/or rs7531555CC.

53. An inhibitor or pharmaceutical composition for use according to claim 51, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT and rs10494745GG; (ii) rs61818956TT and rs10494745GA; (iii) rs61818956TT and rs10494745AA; (iv) rs61818956CT and rs10494745GG; (v) rs61818956CT and rs10494745GA; (vi) rs61818956CT and rs10494745AA; (vii) rs61818956CC and rs10494745GG; or (viii) rs61818956CC or rs10494745GA.

54. An inhibitor or pharmaceutical composition for use according to claim 51, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT and

rs10494745GG; (ii) rs61818956TT and rs10494745GA; (iii) rs61818956TT and rs10494745AA; (iv) rs61818956CT and rs10494745GG; or (v) rs61818956CT and rs10494745GA.

55. An inhibitor or pharmaceutical composition for use according to claim 51, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT and rs10494745GG or (ii) rs61818956TT and rs10494745GA; or (iii) rs61818956CT and rs10494745GG.

56. An inhibitor or pharmaceutical composition for use according to claim 51, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT and rs10494745GG or (ii) rs61818956TT and rs10494745GA.

57. An inhibitor or pharmaceutical composition for use according to claim 51, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT, rs10494745GG, rs7531555CC; (ii) rs61818956CT, rs10494745GG, rs7531555CC; (iii) rs61818956TT, rs10494745GA, rs7531555CT; (iv) rs61818956TT, rs10494745GA, rs7531555CC; (v) rs61818956CC, rs10494745GG, rs7531555CC; (vi) rs61818956TC, rs10494745GA, rs7531555CC; (vii) rs61818956TC, rs10494745GG, rs7531555CT; (viii) rs61818956CT, rs10494745GA, rs7531555CT; (ix) rs61818956CT, rs10494745GA, rs7531555CC; (x) rs61818956TT, rs10494745GG, rs7531555CT; (xi) rs61818956TT, rs10494745AA, rs7531555TT; (xii) rs61818956TT, rs10494745AA, rs7531555TC; or (xiii) rs61818956TT, rs10494745AA, rs7531555CC.

58. An inhibitor or pharmaceutical composition for use according to claim 51, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT, rs10494745GG, rs7531555CC; (ii) rs61818956CT, rs10494745GG, rs7531555CC; (iii) rs61818956TT, rs10494745GA, rs7531555CT; or (iv) rs61818956TT, rs10494745GA, rs7531555CC.

59. An inhibitor or pharmaceutical composition for use according to claim 51, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT, rs10494745GG, rs7531555CC; (ii) rs61818956CT, rs10494745GG, rs7531555CC; (iii) rs61818956CC, rs10494745GG, rs7531555CC; (iv) rs61818956TC, rs10494745GA, rs7531555CC; (v) rs61818956TC, rs10494745GG, rs7531555CT.

60. An inhibitor or pharmaceutical composition for use according to claim 51, wherein the patient having elevated levels of FHR-4 is a carrier of diplotype alleles (i) rs61818956TT, rs10494745GG, rs7531555CC; or (ii) rs61818956CT, rs10494745GG, rs7531555CC.

61. Use of CFHR4/FHR-4 as a target for identifying one or more therapeutic agents for the treatment of age-related macular degeneration (AMD).

62. A method of treating or preventing age-related macular degeneration (AMD), which comprises administering to a patient an inhibitor of CFHR4/FHR-4, such as an inhibitor as defined according to any one of claims 1 to 43, or a pharmaceutical composition comprising an inhibitor of CFHR4/FHR-4, such as a composition as defined according to claim 44.

63. Use of an inhibitor according to any one of claims 1 to 43 or a pharmaceutical composition according to claim 44, in the preparation of a medicament for the treatment of age-related macular degeneration (AMD).

64. CFHR4/FHR-4 for use as a biomarker of age-related macular degeneration (AMD).

65. CFHR4/FHR-4 for use in an *in vivo* method of predicting susceptibility to age-related macular degeneration (AMD), typically by monitoring the sequence and/ or level of expression and / or function of CFHR4 in a sample obtained from a patient.

66. A method of predicting susceptibility to age-related macular degeneration (AMD), and optionally treating age-related macular degeneration (AMD), in a patient, said method comprising:

(a) obtaining a sample from the patient,

(b) detecting the sequence and / or expression and / or function of CFHR4/FHR-4 in said sample obtained from the patient,

(c) predicting susceptibility to age-related macular degeneration (AMD), based on the sequence and / or expression and / or function of CFHR4/FHR-4 in said sample obtained from the patient,

(d) preferably administering to the diagnosed patient an effective amount of an inhibitor of CFHR4/FHR-4, preferably an inhibitor of CFHR4/FHR-4 according to any one of

claims 1 to 43, or of a pharmaceutical composition comprising an inhibitor of CFHR4/FHR-4, such as a composition as defined according to claim 44.

67. A method of selecting a patient having a disease or disorder associated with alterations in complement pathway protein levels, in particular age-related macular degeneration (AMD), for a treatment with the inhibitor according to the invention, and optionally treating the disease or disorder associated with alterations in complement pathway protein levels, in particular age-related macular degeneration (AMD), in said patient, said method comprising:

(a) providing a sample from the patient,

(b) detecting the sequence and / or expression and / or function of CFHR4/FHR-4 in said sample obtained from the patient,

(c) selecting the patient having the disease or disorder associated with alterations in complement pathway protein levels, in particular age-related macular degeneration (AMD), for a treatment with the inhibitor according to the invention based on the sequence and / or expression and / or function of CFHR4/FHR-4 in said sample obtained from the patient,

(d) preferably administering to the diagnosed patient an effective amount of an inhibitor of CFHR4/FHR-4.

68. A method according to claim 67, wherein the patient is selected for a treatment with the inhibitor according to the invention, if the protein levels of FHR-4 and/or the activity of FHR-4 in the sample obtained from said patient are elevated compared to a suitable control, preferably wherein the suitable control is a comparable sample from a healthy donor.

69. A method according to claim 67 or 68, wherein the method comprises a step of determining the presence of a single nucleotide polymorphism (SNP) at positions rs61818956, rs10494745, and/or rs7531555 in the sample obtained from said patient.

70. A method according to claim 68 or 69, wherein the patient is determined to have elevated levels of FHR-4, if the patient is a carrier of diplotype alleles rs61818956TT and/or rs10494745GG and/or rs7531555CC; preferably of diplotype alleles (i) rs61818956TT and rs10494745GG; (ii) rs61818956TT and rs10494745GA; (iii) rs61818956TT and rs10494745AA; (iv) rs61818956CT and rs10494745GG; (v) rs61818956CT and rs10494745GA; (vi) rs61818956CT and rs10494745AA; (vii) rs61818956CC and rs10494745GG; or (viii)

rs61818956CC or rs10494745GA; more preferably of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; (ii) rs61818956CT, rs10494745GG, and rs7531555CC; (iii) rs61818956TT, rs10494745GA, and rs7531555CT; (iv) rs61818956TT, rs10494745GA, and rs7531555CC; (v) rs61818956CC, rs10494745GG, and rs7531555CC; (vi) rs61818956TC, rs10494745GA, and rs7531555CC; (vii) rs61818956TC, rs10494745GG, and rs7531555CT; (viii) rs61818956CT, rs10494745GA, and rs7531555CT; (ix) rs61818956CT, rs10494745GA, and rs7531555CC; (x) rs61818956TT, rs10494745GG, and rs7531555CT; (xi) rs61818956TT, rs10494745AA, and rs7531555TT; (xii) rs61818956TT, rs10494745AA, and rs7531555TC; or (xiii) rs61818956TT, rs10494745AA, and rs7531555CC; even more preferably of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; (ii) rs61818956CT, rs10494745GG, and rs7531555CC; (iii) rs61818956TT, rs10494745GA, and rs7531555CT; or (iv) rs61818956TT, rs10494745GA, and rs7531555CC or of alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; (ii) rs61818956CT, rs10494745GG, and rs7531555CC; (iii) rs61818956CC, rs10494745GG, and rs7531555CC; (iv) rs61818956TC, rs10494745GA, and rs7531555CC; (v) rs61818956TC, rs10494745GG, and rs7531555CT; most preferably of diplotype alleles (i) rs61818956TT, rs10494745GG, and rs7531555CC; or (ii) rs61818956CT, rs10494745GG, and rs7531555CC.