Nucleic acids for inhibiting expression of C3 in a cell

Field of the invention

The invention relates to nucleic acid products that interfere with or inhibit complement component C3 gene expression. It further relates to therapeutic uses of such inhibition such as for the treatment of diseases and disorders associated with complement pathway deregulation and/or over-activation or with ectopic expression or localisation or accumulation of the complement component C3 in the body.

Background

Double-stranded RNAs (dsRNA) able to bind through complementary base pairing to expressed mRNAs have been shown to block gene expression (Fire et al., 1998, Nature. 1998 Feb 19;391(6669):806-11 and Elbashir et al., 2001, Nature. 2001 May 24;411(6836):494-8) by a mechanism that has been termed "RNA interference (RNAi)". Short dsRNAs direct gene specific, post transcriptional silencing in many organisms, including vertebrates, and have become a useful tool for studying gene function. RNAi is mediated by the RNA induced silencing complex (RISC), a sequence specific, multi component nuclease that degrades messenger RNAs having sufficient complementary or homology to the silencing trigger loaded into the RISC complex. Interfering RNAs such as siRNAs, antisense RNAs, and micro RNAs, are oligonucleotides that prevent the formation of proteins by gene silencing, i.e., inhibiting gene translation of the protein through degradation of mRNA molecules. Gene silencing agents are becoming increasingly important for therapeutic applications in medicine.

According to Watts and Corey in the Journal of Pathology (2012; Vol 226, p 365-379), there are algorithms that can be used to design nucleic acid silencing triggers, but all of these have severe limitations. It may take various experimental methods to identify potent siRNAs, as algorithms do not take into account factors such as tertiary structure of the target mRNA or the involvement of RNA binding proteins. Therefore, the discovery of a potent nucleic acid silencing trigger with minimal off-target effects is a complex process. For the pharmaceutical development of these highly charged molecules, it is necessary that they can be synthesised economically, distributed to target tissues, enter cells and function within acceptable limits of toxicity.

The complement system or pathway is part of the innate immune system of host defence against invading pathogens. It mainly consists of a number of proteins that circulate in the

bloodstream in the form of precursors. Most of the proteins that form the complement system, including the complement component protein C3 (also referred to herein simply as C3), are largely synthesised and secreted into the bloodstream by hepatocytes in the liver. Activation of the system leads to inflammatory responses resulting in phagocyte attraction and opsonization and consequently clearance of pathogens, immune complexes and cellular debris (Janeway´s Immunobiology 9th Edition). The complement system consists of 3 pathways (Classical, Leptin and Alternative pathways), which all converge at the formation of so-called complement component 3 convertase enzyme complexes. These enzyme complexes cleave the complement component C3 protein into C3a and C3b. Once cleaved, C3b forms part of a complex that in turn cleaves C5 into C5a and C5b. After cleavage, C5b is one of the key components of the main complement pathway effectors, the membrane attack complex. C3 is therefore a key component of the complement system activation pathway.

Several diseases are associated with aberrant acquired or genetic activation of the complement pathway as well as with aberrant or over-expression of C3. Among others, these are C3 Glomerulopathy (C3G), atypical Hemolytic Uremic Syndrome (aHUS), Immune Complex-mediated Glomerulonephritis (IC-mediated GN), post-Infectious Glomerulonephritis (PIGN), Systemic Lupus Erythematosus, Lupus nephritis, Ischemia/reperfusion injury and IgA nephropathy (IgA N; reviewed in Ricklin et al., Nephrology, 2016 and others). Most of these diseases are associated with the kidney, as this organ is uniquely sensitive to complement-induced damage. However, diseases of other organs are also known to be related to complement dysfunction, such as age-related macular degeneration (AMD), Rheumatoid arthritis (RA), antineutrophil Cytoplasmic Autoantibodies-associated Vasculitis (ANCA-AV), dysbiotic periodontal Disease, Malarial Anaemia, Paroxysmal Nocturnal Hemoglobinuria (PNH) and sepsis.

In C3G, C3 accumulates in the glomeruli in the kidney and clogs them. The accumulation of C3 also leads to kidney damage. In atypical Hemolytic Uremic Syndrome (aHUS), the complement system targets red blood cells, which leads to lysis of the red blood cells.

There are currently only few treatments for complement system mediated diseases, disorders and syndromes. The monoclonal humanized antibody Eculizumab is one of them. It is known to bind complement protein C5, thereby blocking the membrane attack complex at the end of the complement cascade (Hillmen et al., 2006 NEJM). However, only a subset of patients suffering from the above listed diseases respond to Eculizumab therapy. There is thus a high unmet need for medical treatments of complement mediated or associated diseases. C3 is a

pivotal factor in the complement pathway activation. Inhibiting C3 expression therefore presents a promising therapeutic strategy for many complement-mediated diseases.

Summary of the invention

One aspect of the invention is a double-stranded nucleic acid for inhibiting expression of complement component C3, wherein the nucleic acid comprises a first strand and a second strand, wherein the first strand sequence comprises a sequence of at least 15 nucleotides differing by no more than 3 nucleotides from any one of the sequences SEQ ID NO: 370, 364, 365, 366, 368, 372, 377, 361, 95, 111, 125, 131, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 97, 99, 101, 103, 105, 107, 109, 113, 115, 117, 119, 121, 123, 127, 129, 133 or 416.

One aspect relates to a double-stranded nucleic acid that is capable of inhibiting expression of complement component C3, preferably in a cell, for use as a medicament or in associated diagnostic or therapeutic methods, wherein the nucleic acid preferably comprises or consists of a first strand and a second strand and preferably wherein the first strand comprises sequences sufficiently complementary to a complement component C3 mRNA so as to mediate RNA interference.

One aspect relates to a composition comprising a nucleic acid as disclosed herein and a solvent (preferably water) and/or a delivery vehicle and/or a physiologically acceptable excipient and/or a carrier and/or a salt and/or a diluent and/or a buffer and/or a preservative.

One aspect relates to a composition comprising a nucleic acid as disclosed herein and a further therapeutic agent selected from e.g., an oligonucleotide, a small molecule, a monoclonal antibody, a polyclonal antibody and a peptide.

One aspect relates to a nucleic acid or a composition comprising it as disclosed herein for use as a medicament or in associated methods.

One aspect relates to a nucleic acid or a composition comprising it as disclosed herein for use in the prevention, decrease of the risk of suffering from, or treatment of a disease, disorder or syndrome.

One aspect relates to the use of a nucleic acid or a composition comprising it as disclosed herein in the prevention, decrease of the risk of suffering from, or treatment of a disease, disorder or syndrome, wherein the disease, disorder or syndrome is preferably C3 Glomerulopathy (C3G).

One aspect relates to a method of preventing, decreasing the risk of suffering from, or treating a disease, disorder or syndrome comprising administering a pharmaceutically effective dose or amount of a nucleic acid or composition comprising it as disclosed herein to an individual in need of treatment, preferably wherein the nucleic acid or composition is administered to the subject subcutaneously, intravenously or by oral, rectal, pulmonary, intramuscular or intraperitoneal administration.

Detailed description of the invention

The present invention relates to a nucleic acid which is double-stranded and which comprises a sequence homologous to an expressed RNA transcript of the complement component C3, and compositions thereof. These nucleic acids, or conjugates or compositions thereof, may be used in the treatment and prevention of a variety of diseases, disorders and syndromes in which reduced expression of the C3 gene product is desirable.

A first aspect of the invention is a double-stranded nucleic acid for inhibiting expression of C3, preferably in a cell, wherein the nucleic acid comprises a first strand and a second strand, wherein the first strand sequence comprises a sequence of at least 15 nucleotides differing by no more than 3 nucleotides from any one of the sequences selected from SEQ ID NO: 370, 364, 365, 366, 368, 372, 377, 361, 95, 111, 125, 131, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 97, 99, 101, 103, 105, 107, 109, 113, 115, 117, 119, 121, 123, 127, 129, 133 or 416. These nucleic acids among others have the advantage of being active in various species that are relevant for pre-clinical and clinical development and/or of having few relevant off-target effects. Having few relevant off-target effects means that a nucleic acid specifically inhibits the intended target and does not significantly inhibit other genes or inhibits only one or few other genes at a therapeutically acceptable level.

Preferably, the first strand sequence comprises a sequence of at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all 19 nucleotides differing by no more than 3 nucleotides, preferably by no more than 2 nucleotides, more preferably by no more than 1 nucleotide, and most preferably not differing by any nucleotide from any one of

the sequences selected from SEQ ID NO: 370, 364, 365, 366, 368, 372, 377, 361, 95, 111, 125, 131, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 97, 99, 101, 103, 105, 107, 109, 113, 115, 117, 119, 121, 123, 127, 129, 133 or 416.

Preferably, the first strand sequence of the nucleic acid consists of one of the sequences selected from SEQ ID NOs: 370, 364, 365, 366, 368, 372, 377, 361, 95, 111, 125, 131, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 97, 99, 101, 103, 105, 107, 109, 113, 115, 117, 119, 121, 123, 127, 129, 133 or 416. The sequence may however be modified by a number of nucleic acid modifications that do not change the identity of the nucleotide. For example, modifications of the backbone or sugar residues of the nucleic acid do not change the identity of the nucleotide because the base itself remains the same as in the reference sequence.

A nucleic acid that comprises a sequence according to a reference sequence herein means that the nucleic acid comprises a sequence of contiguous nucleotides in the order as defined in the reference sequence.

When reference is made herein to a reference sequence comprising or consisting of nucleotides, this reference is not limited to the sequence with unmodified nucleotides. The same reference also encompasses the same nucleotide sequence in which one, several, such as two, three, four, five, six, seven or more, including all, nucleotides are modified by modifications such as 2'-OMe, 2'-F, a ligand, a linker, a 3' end or 5' end modification or any other modification. It also refers to sequences in which two or more nucleotides are linked to each other by the natural phosphodiester linkage or by any other linkage such as a phosphorothioate or a phosphorodithioate linkage.

A double-stranded nucleic acid is a nucleic acid in which the first strand and the second strand hybridise to each other over at least part of their lengths and are therefore capable of forming a duplex region under physiological conditions, such as in PBS at 37°C at a concentration of 1 μM of each strand. The first and second strand are preferably able to hybridise to each other and therefore to form a duplex region over a region of at least 15 nucleotides, preferably 16, 17, 18 or 19 nucleotides. This duplex region comprises nucleotide base parings between the two strands, preferably based on Watson-Crick base pairing and/or wobble base pairing (such as GU base pairing). All the nucleotides of the two strands within a duplex region do not have to base pair to each other to form a duplex region. A certain number of mismatches, deletions

or insertions between the nucleotide sequences of the two strands are acceptable. Overhangs on either end of the first or second strand or unpaired nucleotides at either end of the double-stranded nucleic acid are also possible. The double-stranded nucleic acid is preferably a stable double-stranded nucleic acid under physiological conditions, and preferably has a melting temperature (Tm) of 45°C or more, preferably 50°C or more, and more preferably 55°C or more for example in PBS at a concentration of 1 μM of each strand.

A stable double-stranded nucleic acid under physiological conditions is a double-stranded nucleic acid that has a Tm of 45°C or more, preferably 50°C or more, and more preferably 55°C or more, for example in PBS at a concentration of 1 μM of each strand.

The first strand and the second strand are preferably capable of forming a duplex region (i.e., are complementary to each other) over i) at least a portion of their lengths, preferably over at least 15 nucleotides of both of their lengths, ii) over the entire length of the first strand, iii) over the entire length of the second strand or iv) over the entire length of both the first and the second strand. Strands being complementary to each other over a certain length means that the strands are able to base pair to each other, either via Watson-Crick or wobble base pairing, over that length. Each nucleotide of the length does not necessarily have to be able to base pair with its counterpart in the other strand over the entire given length as long as a stable double-stranded nucleotide under physiological conditions can be formed. It is however, preferred, in certain embodiments, if each nucleotide of the length can base pair with its counterpart in the other strand over the entire given length.

A certain number of mismatches, deletions or insertions between the first strand and the target sequence, or between the first strand and the second strand can be tolerated in the context of the siRNA and even have the potential in certain cases to increase RNA interference (e.g., inhibition) activity.

The inhibition activity of the nucleic acids according to the present invention relies on the formation of a duplex region between all or a portion of the first strand and a portion of a target nucleic acid. The portion of the target nucleic acid that forms a duplex region with the first strand, defined as beginning with the first base pair formed between the first strand and the target sequence and ending with the last base pair formed between the first strand and the target sequence, inclusive, is the target nucleic acid sequence or simply, target sequence. The duplex region formed between the first strand and the second strand need not be the same as the duplex region formed between the first strand and the target sequence. That is, the second strand may have a sequence different from the target sequence; however, the first strand must

be able to form a duplex structure with both the second strand and the target sequence, at least under physiological conditions.

The complementarity between the first strand and the target sequence may be perfect (i.e., 100% identity with no nucleotide mismatches or insertions or deletions in the first strand as compared to the target sequence).

The complementarity between the first strand and the target sequence may not be perfect. The complementarity may be from about 70% to about 100%. More specifically, the complementarity may be at least 70%, 80%, 85%, 90% or 95% and intermediate values.

The identity between the first strand and the complementary sequence of the target sequence may range from about 75% to about 100%. More specifically, the complementarity may be at least 75%, 80%, 85%, 90% or 95% and intermediate values, provided a nucleic acid is capable of reducing or inhibiting the expression of the complement component C3.

A nucleic acid having less than 100% complementarity between the first strand and the target sequence may be able to reduce the expression of the complement component C3 to the same level as a nucleic acid having perfect complementarity between the first strand and target sequence. Alternatively, it may be able to reduce expression of the complement component C3 to a level that is 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or 100% of the level of reduction achieved by the nucleic acid with perfect complementarity.

In one aspect, a nucleic acid of the present disclosure is a nucleic acid wherein

(a) the first strand sequence comprises a sequence differing by no more than 3 nucleotides from any one of the first strand sequences of Table 1 and optionally wherein the second strand sequence comprises a sequence differing by no more than 3 nucleotides from the second strand sequence in the same line of the table;

(b) the first strand sequence comprises a sequence differing by no more than 2 nucleotides from any one of the first strand sequences of Table 1 and optionally wherein the second strand sequence comprises a sequence differing by no more than 2 nucleotides from the second strand sequence in the same line of the table;

(c) the first strand sequence comprises a sequence differing by no more than 1 nucleotide from any one of the first strand sequences of Table 1 and optionally wherein the second strand sequence comprises a sequence differing by no more than 1 nucleotide from the second strand sequence in the same line of the table;

(d) the first strand sequence comprises a sequence corresponding to nucleotides 2 to 17 from the 5' end of any one of the first strand sequences of Table 1 and optionally wherein the second strand sequence comprises a sequence corresponding to nucleotides 2 to 17 from the 5' end of the second strand sequence in the same line of the table;

(e) the first strand sequence comprises a sequence corresponding to nucleotides 2 to 18 from the 5' end of any one of the first strand sequences of Table 1 and optionally wherein the second strand sequence comprises a sequence corresponding to nucleotides 2 to 18 from the 5' end of the second strand sequence in the same line of the table;

(f) the first strand sequence comprises a sequence corresponding to nucleotides 2 to 19 from the 5' end of any one of the first strand sequences of Table 1 and optionally wherein the second strand sequence comprises a sequence corresponding to nucleotides 2 to 19 from the 5' end of the second strand sequence in the same line of the table;

(g) the first strand sequence comprises a sequence corresponding to nucleotides 2 to 19 from the 5' end of any one of the first strand sequences of Table 1 and optionally wherein the second strand sequence comprises a sequence corresponding to nucleotides 1 to 18 from the 5' end of the second strand sequence in the same line of the table;

(h) the first strand sequence comprises a sequence of any one of the first strand sequences of Table 1 and optionally wherein the second strand sequence comprises a sequence of the second strand sequence in the same line of the table; or

(i) the first strand sequence consists of any one of the first strand sequences of Table 1 and optionally wherein the second strand sequence consists of the sequence of the second strand sequence in the same line of the table;

wherein Table 1 is:

Table 1

First strand sequence (SEQ ID NO:)	**Second strand sequence (SEQ ID NO:)**
364	363 or 375
365	363
366	367 or 376
368	369
370	379 or 371, preferably 379
372	373
362	374
377	378
361	112
95	96
111	112

125	126
131	132
1	2
3	4
5	6
7	8
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	36
37	38
39	40
41	42
43	44
45	46
47	48
49	50
51	52
53	54
55	56
57	58
59	60
61	62
63	64
65	66
67	68

69	70
71	72
73	74
75	76
77	78
79	80
81	82
83	84
85	86
87	88
89	90
91	92
93	94
97	98
99	100
101	102
103	104
105	106
107	108
109	110
113	114
115	116
117	118
119	120
121	122
123	124
127	128
129	130
133	134
416	26

In one aspect, the nucleic acid is a nucleic acid wherein:

(a) the first strand sequence comprises the sequence of SEQ ID NO 361 and optionally wherein the second strand sequence comprises the sequence of SEQ ID NO: 112; or

(b) the first strand sequence comprises the sequence of SEQ ID NO 95 and optionally wherein the second strand sequence comprises the sequence of SEQ ID NO: 96; or

(c) the first strand sequence comprises the sequence of SEQ ID NO 111 and optionally wherein the second strand sequence comprises the sequence of SEQ ID NO: 112; or

(d) the first strand sequence comprises the sequence of SEQ ID NO 125 and optionally wherein the second strand sequence comprises the sequence of SEQ ID NO: 126; or

(e) the first strand sequence comprises the sequence of SEQ ID NO 131 and optionally wherein the second strand sequence comprises the sequence of SEQ ID NO: 132; or

(f) the first strand sequence consists of SEQ ID NO: 361 and optionally wherein the second strand sequence consists of SEQ ID NO: 112; or

(g) the first strand sequence consists of SEQ ID NO: 95 and optionally wherein the second strand sequence consists of SEQ ID NO: 96; or

(h) the first strand sequence consists of SEQ ID NO: 111 and optionally wherein the second strand sequence consists of SEQ ID NO: 112; or

(i) the first strand sequence consists of SEQ ID NO: 125 and optionally wherein the second strand sequence consists of SEQ ID NO: 126; or

(j) the first strand sequence consists of SEQ ID NO: 131 and optionally wherein the second strand sequence consists of SEQ ID NO: 132; or

(k) the first strand sequence comprises or consists of the sequence of SEQ ID NO 364 and optionally wherein the second strand sequence comprises or consists of the sequence of SEQ ID NO: 363 or 375; or

(l) the first strand sequence comprises or consists of the sequence of SEQ ID NO 365 and optionally wherein the second strand sequence comprises or consists of the sequence of SEQ ID NO: 363; or

(m) the first strand sequence comprises or consists of the sequence of SEQ ID NO 366 and optionally wherein the second strand sequence comprises or consists of the sequence of SEQ ID NO: 367 or 376; or

(n) the first strand sequence comprises or consists of the sequence of SEQ ID NO 368 and optionally wherein the second strand sequence comprises or consists of the sequence of SEQ ID NO: 369; or

(o) the first strand sequence comprises or consists of the sequence of SEQ ID NO 370 and optionally wherein the second strand sequence comprises or consists of the sequence of SEQ ID NO: 371 or 379, preferably 379; or

(p) the first strand sequence comprises or consists of the sequence of SEQ ID NO 372 and optionally wherein the second strand sequence comprises or consists of the sequence of SEQ ID NO: 373 or 380; or

(q) the first strand sequence comprises or consists of the sequence of SEQ ID NO 362 and optionally wherein the second strand sequence comprises or consists of the sequence of SEQ ID NO: 374; or

(r) the first strand sequence comprises or consists of the sequence of SEQ ID NO 377 and optionally wherein the second strand sequence comprises or consists of the sequence of SEQ ID NO: 378; or

(s) the first strand sequence comprises or consists of the sequence of SEQ ID NO 416 and optionally wherein the second strand sequence comprises or consists of the sequence of SEQ ID NO: 26.

In one aspect, if the 5'-most nucleotide of the first strand is a nucleotide other than A or U, this nucleotide is replaced by an A or U. Preferably, if the 5'-most nucleotide of the first strand is a nucleotide other than U, this nucleotide is replaced by U, and more preferably by U with a 5' vinylphosphonate.

When a nucleic acid of the invention does not comprise the entire sequence of a reference first strand and/or second strand sequence, as for example given in Table 1, or one or both strands differ from the corresponding reference sequence by one, two or three nucleotides, this nucleic acid preferably retains at least 30%, more preferably at least 50%, more preferably at least 70%, more preferably at least 80%, even more preferably at least 90%, yet more preferably at least 95% and most preferably at least 100% of the C3 inhibition activity compared to the inhibition activity of the corresponding nucleic acid that comprises the entire first strand and second strand reference sequences in a comparable experiment.

In one aspect, the nucleic acid is a nucleic acid wherein the first strand sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 361 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides of the sequence of SEQ ID NO: 112; or wherein the first strand sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 95 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides of the sequence of SEQ ID NO: 96; or wherein the first strand sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 111 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides of the sequence of SEQ ID NO: 112; or wherein the first strand sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 125 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides

of the sequence of SEQ ID NO: 126; or wherein the first strand sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 131 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides of the sequence of SEQ ID NO: 132; or wherein the first strand sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 364 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides of the sequence of SEQ ID NO: 363 or 375; or wherein the first strand sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 365 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides of the sequence of SEQ ID NO: 363; or wherein the first strand sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 366 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides of the sequence of SEQ ID NO: 367 or 376; or wherein the first strand sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 368 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides of the sequence of SEQ ID NO: 369; or wherein the first strand sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 370 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides of the sequence of SEQ ID NO: 371 or 379, preferably 379; or wherein the first strand sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 372 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides of the sequence of SEQ ID NO: 373 or 380; or wherein the first strand sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 362 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides of the sequence of SEQ ID NO: 374; or wherein the first strand sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 377 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides of the sequence of SEQ ID NO: 378; or wherein the first strand

sequence comprises, or preferably consists of, the sequence of SEQ ID NO: 416 and optionally wherein the second strand sequence comprises, or consists of, a sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides of the sequence of SEQ ID NO: 26.

In one aspect, the nucleic acid is a double-stranded nucleic acid for inhibiting expression of C3, preferably in a cell, wherein the nucleic acid comprises a first nucleic acid strand and a second nucleic acid strand, wherein the first strand is capable of hybridising under physiological conditions to a nucleic acid of sequence selected from SEQ ID NO: 379, 363, 375, 367, 376, 369, 371, 373, 380, 374, 378, 112, 96, 126, 132, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56 ,58, 60, 62, 64 ,66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 98, 100, 102, 104, 106, 108, 110, 114, 116, 118, 120, 122, 124, 128, 130 or 134; and
wherein the second strand is capable of hybridising under physiological conditions to the first strand to form a duplex region.

Nucleic acids that are capable of hybridising under physiological conditions are nucleic acids that are capable of forming base pairs, preferably Watson-Crick or wobble base-pairs, between at least a portion of the opposed nucleotides in the strands so as to form at least a duplex region. Such a double-stranded nucleic acid is preferably a stable double-stranded nucleic acid under physiological conditions (for example in PBS at 37°C at a concentration of 1 μM of each strand), meaning that under such conditions, the two strands stay hybridised to each other. The Tm of the double-stranded nucleotide is preferably 45°C or more, preferably 50°C or more and more preferably 55°C or more.

One aspect of the present invention relates to a nucleic acid for inhibiting expression of the complement component C3, wherein the nucleic acid comprises a first sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides differing by no more than 3 nucleotides, preferably no more than 2 nucleotides, more preferably no more than 1 nucleotide and most preferably not differing by any nucleotide from any of the sequences of Table 5, the first sequence being able to hybridise to a target gene transcript (such as an mRNA) under physiological conditions. Preferably, the nucleic acid further comprises a second sequence of at least 15, preferably at least 16, more preferably at least 17, yet more preferably at least 18 and most preferably all nucleotides differing by no more than 3 nucleotides, preferably no more than 2 nucleotides, more preferably no more than 1 nucleotide and most preferably not differing by any nucleotide from any of the sequences of Table 5, the second sequence being able to hybridise to the first sequence under

physiological conditions and preferably the nucleic acid being an siRNA that is capable of inhibiting C3 expression via the RNAi pathway.

One aspect relates to any double-stranded nucleic acid as disclosed in Table 3, preferably for inhibiting expression of the complement component C3, provided that the double-stranded nucleic acid is able to inhibit expression of complement component C3. These nucleic acids are all siRNAs with various nucleotide modifications. Some of them are conjugates comprising GalNAc moieties that can be specifically targeted to cells with GalNAc receptors, such as hepatocytes.

One aspect relates to a double-stranded nucleic acid that is capable of inhibiting expression of complement component C3, preferably in a cell, for use as a medicament or in associated diagnostic or therapeutic methods, wherein the nucleic acid preferably comprises or consists of a first strand and a second strand and preferably wherein the first strand comprises sequences sufficiently complementary to a complement component C3 mRNA so as to mediate RNA interference.

The nucleic acids described herein may be capable of inhibiting the expression of the complement component C3. Inhibition may be complete, i.e., 0% remaining expression. Inhibition of C3 expression may be partial, i.e., it may be 15%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more, or intermediate values of inhibition of the level of C3 expression in the absence of a nucleic acid of the invention. The level of inhibition may be measured by comparing a treated sample with an untreated sample or with a sample treated with a control such as for example a siRNA that does not target C3. Inhibition may be measured by measuring C3 mRNA and/or protein levels or levels of a biomarker or indicator that correlates with C3 presence or activity. It may be measured in cells that may have been treated *in vitro* with a nucleic acid described herein. Alternatively, or in addition, inhibition may be measured in cells, such as hepatocytes, or tissue, such as liver tissue, or an organ, such as the liver, or in a body fluid such as blood, serum, lymph or any other body part or fluid that has been taken from a subject previously treated with a nucleic acid disclosed herein. Preferably, inhibition of C3 expression is determined by comparing the C3 mRNA level measured in C3-expressing cells after 24 or 48 hours *in vitro* treatment with a double-stranded RNA disclosed herein under ideal conditions (see the examples for appropriate concentrations and conditions) to the C3 mRNA level measured in control cells that were untreated or mock treated or treated with a control double-stranded RNA under the same conditions.

One aspect of the present invention relates to a nucleic acid, wherein the first strand and the second strand are present on a single strand of a nucleic acid that loops around so that the first strand and the second strand are able to hybridise to each other and to thereby form a double-stranded nucleic acid with a duplex region.

Preferably, the first strand and the second strand of the nucleic acid are separate strands. The two separate strands are preferably each 17-25 nucleotides in length, more preferably 18-25 nucleotides in length. The two strands may be of the same or different lengths. The first strand may be 17-25 nucleotides in length, preferably it may be 18-24 nucleotides in length, it may be 18, 19, 20, 21, 22, 23 or 24 nucleotides in length. Most preferably, the first strand is 19 nucleotides in length. The second strand may independently be 17-25 nucleotides in length, preferably it may be 18-24 nucleotides in length, it may be 18, 19, 20, 21, 22, 23 or 24 nucleotides in length. More preferably, the second strand is 18 or 19 or 20 nucleotides in length, and most preferably it is 19 nucleotides in length.

Preferably, the first strand and the second strand of the nucleic acid form a duplex region of 17-25 nucleotides in length. More preferably, the duplex region is 18-24 nucleotides in length. The duplex region may be 17, 18, 19, 20, 21, 22, 23, 24 or 25 nucleotides in length. In the most preferred embodiment, the duplex region is 18 or 19 nucleotides in length. The duplex region is defined here as the region between and including the 5'-most nucleotide of the first strand that is base paired to a nucleotide of the second strand to the 3'-most nucleotide of the first strand that is base paired to a nucleotide of the second strand. The duplex region may comprise nucleotides in either or both strands that are not base-paired to a nucleotide in the other strand. It may comprise one, two, three or four such nucleotides on the first strand and/or on the second strand. However, preferably, the duplex region consists of 17-25 consecutive nucleotide base pairs. That is to say that it preferably comprises 17-25 consecutive nucleotides on both of the strands that all base pair to a nucleotide in the other strand. More preferably, the duplex region consists of 18 or 19 consecutive nucleotide base pairs, most preferably 18.

In each of the embodiments disclosed herein, the nucleic acid may be blunt ended at both ends; have an overhang at one end and a blunt end at the other end; or have an overhang at both ends.

The nucleic acid may have an overhang at one end and a blunt end at the other end. The nucleic acid may have an overhang at both ends. The nucleic acid may be blunt ended at both ends. The nucleic acid may be blunt ended at the end with the 5' end of the first strand and the

3' end of the second strand or at the 3' end of the first strand and the 5' end of the second strand.

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

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

In one embodiment, the 5' end of the first strand is a single-stranded overhang of one, two or three nucleotides, preferably of one nucleotide.

Preferably, the nucleic acid is an siRNA. siRNAs are short interfering or short silencing RNAs that are able to inhibit the expression of a target gene through the RNA interference (RNAi) pathway. Inhibition occurs through targeted degradation of mRNA transcripts of the target gene after transcription. The siRNA forms part of the RISC complex. The RISC complex specifically targets the target RNA by sequence complementarity of the first (antisense) strand with the target sequence.

Preferably, the nucleic acid mediates RNA interference (RNAi). Preferably, the nucleic acid mediates RNA interference with an efficacy of at least 50% inhibition, more preferably at least 70%, more preferably at least 80%, even more preferably at least 90%, yet more preferably at least 95% and most preferably 100% inhibition. The inhibition efficacy is preferably measured by comparing the C3 mRNA level in cells, such as hepatocytes, treated with a C3 specific siRNA to the C3 mRNA level in cells treated with a control in a comparable experiment. The control can be a treatment with a non-C3 targeting siRNA or without a siRNA. The nucleic acid, or at least the first strand of the nucleic acid, is therefore preferably able to be incorporated into the RISC complex. As a result, the nucleic acid, or at least the first strand of the nucleic

acid, is therefore able to guide the RISC complex to a specific target RNA with which the nucleic acid, or at least the first strand of the nucleic acid, is at least partially complementary. The RISC complex then specifically cleaves this target RNA and as a result leads to inhibition of the expression of the gene from which the RNA stems.

Nucleic acid modifications

Nucleic acids discussed herein include unmodified RNA as well as RNA which has been modified, e.g., to improve efficacy or stability. Unmodified RNA refers to a molecule in which the components of the nucleic acid, namely sugars, bases, and phosphate moieties, are the same or essentially the same as those which occur in nature, for example as occur naturally in the human body. The term "modified nucleotide" as used herein refers to a nucleotide in which one or more of the components of the nucleotide, namely the sugar, base, and phosphate moiety, is/are different from those which occur in nature. The term "modified nucleotide" also refers in certain cases to molecules that are not nucleotides in the strict sense of the term because they lack, or have a substitute of, an essential component of a nucleotide, such as the sugar, base or phosphate moiety. A nucleic acid comprising such modified nucleotides is still to be understood as being a nucleic acid, even if one or more of the nucleotides of the nucleic acid has been replaced by a modified nucleotide that lacks, or has a substitution of, an essential component of a nucleotide.

Modifications of the nucleic acid of the present invention generally provide a powerful tool in overcoming potential limitations including, but not limited to, *in vitro* and *in vivo* stability and bioavailability inherent to native RNA molecules. The nucleic acids according to the invention may be modified by chemical modifications. Modified nucleic acids can also minimise the possibility of inducing interferon activity in humans. Modifications can further enhance the functional delivery of a nucleic acid to a target cell. The modified nucleic acids of the present invention may comprise one or more chemically modified ribonucleotides of either or both of the first strand or the second strand. A ribonucleotide may comprise a chemical modification of the base, sugar or phosphate moieties. The ribonucleic acid may be modified by substitution with or insertion of analogues of nucleic acids or bases.

Throughout the description of the invention, "same or common modification" means the same modification to any nucleotide, be that A, G, C or U modified with a group such as a methyl group (2'-OMe) or a fluoro group (2'-F). For example, 2´-F-dU, 2´-F-dA, 2´-F-dC, 2´-F-dG are all considered to be the same or common modification, as are 2'-OMe-rU, 2'-OMe-rA; 2'-OMe-

rC; 2'-OMe-rG. In contrast, a 2'-F modification is a different modification compared to a 2'-OMe modification.

Preferably, at least one nucleotide of the first and/or second strand of the nucleic acid is a modified nucleotide, preferably a non-naturally occurring nucleotide such as preferably a 2'-F modified nucleotide.

A modified nucleotide can be a nucleotide with a modification of the sugar group. The 2′ hydroxyl group (OH) can be modified or replaced with a number of different "oxy" or "deoxy" substituents.

Examples of "oxy"-2′ hydroxyl group modifications include alkoxy or aryloxy (OR, e.g., R=H, alkyl (such as methyl), cycloalkyl, aryl, aralkyl, heteroaryl or sugar); polyethyleneglycols (PEG), $O(CH_2CH_2O)_nCH_2CH_2OR$; "locked" nucleic acids (LNA) in which the 2′ hydroxyl is connected, e.g., by a methylene bridge, to the 4′ carbon of the same ribose sugar; O-AMINE (AMINE=NH_2, alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, or diheteroaryl amino, ethylene diamine, or polyamino) and aminoalkoxy, $O(CH_2)$nAMINE, (e.g., AMINE=NH_2, alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, or diheteroaryl amino, ethylene diamine, or polyamino).

"Deoxy" modifications include hydrogen, halogen, amino (e.g., NH_2, alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, diheteroaryl amino, or amino acid); $NH(CH_2CH_2NH)_nCH_2CH_2$-AMINE (AMINE=NH_2, alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, or diheteroaryl amino), —NHC(O)R (R=alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar), cyano; mercapto; alkyl-thio-alkyl; thioalkoxy; and alkyl, cycloalkyl, aryl, alkenyl and alkynyl, which may be optionally substituted with e.g., an amino functionality. Other substituents of certain embodiments include 2'-methoxyethyl, 2'-OCH_3, 2′-O-allyl, 2′-C-allyl, and 2′-fluoro.

The sugar group can also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, a modified nucleotide may contain a sugar such as arabinose.

Modified nucleotides can also include "abasic" sugars, which lack a nucleobase at C - 1′. These abasic sugars can further contain modifications at one or more of the constituent sugar atoms.

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

One or more nucleotides of a nucleic acid of the present invention may be modified. The nucleic acid may comprise at least one modified nucleotide. The modified nucleotide may be in the first strand. The modified nucleotide may be in the second strand. The modified nucleotide may be in the duplex region. The modified nucleotide may be outside the duplex region, i.e., in a single-stranded region. The modified nucleotide may be on the first strand and may be outside the duplex region. The modified nucleotide may be on the second strand and may be outside the duplex region. The 3'-terminal nucleotide of the first strand may be a modified nucleotide. The 3'-terminal nucleotide of the second strand may be a modified nucleotide. The 5'-terminal nucleotide of the first strand may be a modified nucleotide. The 5'-terminal nucleotide of the second strand may be a modified nucleotide.

A nucleic acid of the invention may have 1 modified nucleotide or a nucleic acid of the invention may have about 2-4 modified nucleotides, or a nucleic acid may have about 4-6 modified nucleotides, about 6-8 modified nucleotides, about 8-10 modified nucleotides, about 10-12 modified nucleotides, about 12-14 modified nucleotides, about 14-16 modified nucleotides about 16-18 modified nucleotides, about 18-20 modified nucleotides, about 20-22 modified nucleotides, about 22-24 modified nucleotides, about 24-26 modified nucleotides or about 26-28 modified nucleotides. In each case the nucleic acid comprising said modified nucleotides retains at least 50% of its activity as compared to the same nucleic acid but without said modified nucleotides or vice versa. The nucleic acid may retain 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% and intermediate values of its activity as compared to the same nucleic acid but without said modified nucleotides, or may have more than 100% of the activity of the same nucleic acid without said modified nucleotides.

The modified nucleotide may be a purine or a pyrimidine. At least half of the purines may be modified. At least half of the pyrimidines may be modified. All of the purines may be modified. All of the pyrimidines may be modified. The modified nucleotides may be selected from the group consisting of a 3' terminal deoxy thymine (dT) nucleotide, a 2'-O-methyl (2'-OMe) modified nucleotide, a 2' modified nucleotide, a 2' deoxy modified nucleotide, a locked nucleotide, an abasic nucleotide, a 2' amino modified nucleotide, a 2' alkyl modified nucleotide, a 2'-deoxy-2'-fluoro (2'-F) modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a nucleotide comprising a 5' phosphate or 5' phosphate mimic and a terminal nucleotide linked to a cholesteryl derivative or a dodecanoic acid bisdecylamide group.

The nucleic acid may comprise a nucleotide comprising a modified base, wherein the base is selected from 2-aminoadenosine, 2,6-diaminopurine,inosine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidine (e.g., 5-methylcytidine), 5-alkyluridine (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine), 6-azapyrimidine, 6-alkylpyrimidine (e.g. 6-methyluridine), propyne, quesosine, 2-thiouridine, 4-thiouridine, wybutosine, wybutoxosine, 4-acetylcytidine, 5-(carboxyhydroxymethyl)uridine, 5'-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluridine, beta-D-galactosylqueosine, 1-methyladenosine, 1-methylinosine, 2,2-dimethylguanosine, 3-methylcytidine, 2-methyladenosine, 2-methylguanosine, N6-methyladenosine, 7-methylguanosine, 5-methoxyaminomethyl-2-thiouridine, 5-methylaminomethyluridine, 5-methylcarbonylmethyluridine, 5-methyloxyuridine, 5-methyl-2-thiouridine, 2-methylthio-N6-isopentenyladenosine, beta-D-mannosylqueosine, uridine-5-oxyacetic acid and 2-thiocytidine.

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

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

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

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

(i) alteration, e.g., replacement, of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens (referred to as linking even if at the 5' and 3' terminus of the nucleic acid of the invention);

(ii) alteration, e.g., replacement, of a constituent of the ribose sugar, e.g., of the 2′ hydroxyl on the ribose sugar;

(iii) replacement of the phosphate moiety with "dephospho" linkers;

(iv) modification or replacement of a naturally occurring base;

(v) replacement or modification of the ribose-phosphate backbone; and

(vi) modification of the 3′ end or 5′ end of the first strand and/or the second strand, e.g., removal, modification or replacement of a terminal phosphate group or conjugation of a moiety, e.g., a fluorescently labelled moiety, to either the 3′ or 5′ end of one or both strands.

The terms replacement, modification and alteration indicate a difference from a naturally occurring molecule.

Specific modifications are discussed in more detail below.

The nucleic acid may comprise one or more nucleotides on the second and/or first strands that are modified. Alternating nucleotides may be modified, to form modified nucleotides.

Alternating as described herein means to occur one after another in a regular way. In other words, alternating means to occur in turn repeatedly. For example, if one nucleotide is modified, the next contiguous nucleotide is not modified and the following contiguous nucleotide is modified and so on. One nucleotide may be modified with a first modification, the next contiguous nucleotide may be modified with a second modification and the following contiguous nucleotide is modified with the first modification and so on, where the first and second modifications are different.

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

In one aspect of the nucleic acid, at least nucleotides 2 and 14 of the first strand are modified, preferably by a first common modification, the nucleotides being numbered consecutively starting with nucleotide number 1 at the 5' end of the first strand. The first modification is preferably 2'-F.

In one aspect, at least one, several or preferably all the even-numbered nucleotides of the first strand are modified, preferably by a first common modification, the nucleotides being numbered consecutively starting with nucleotide number 1 at the 5' end of the first strand. The first modification is preferably 2'-F.

In one aspect, at least one, several or preferably all the odd-numbered nucleotides of the first strand are modified, the nucleotides being numbered consecutively starting with nucleotide number 1 at the 5' end of the first strand. Preferably, they are modified by a second modification. This second modification is preferably different from the first modification if the nucleic acid also comprises a first modification, for example of nucleotides 2 and 14 or of all the even-numbered nucleotides of the first strand. The first modification is preferably any 2' ribose modification that is of the same size or smaller in volume than a 2'-OH group, or a locked nucleic acid (LNA), or an unlocked nucleic acid (UNA), or a 2'-Fluoroarabino Nucleic Acid (FANA) modification. A 2' ribose modification that is of the same size or smaller in volume than a 2'-OH group can for example be a 2'-F, 2'-H, 2'-halo, or 2'-NH_2. The second modification is preferably any 2' ribose modification that is larger in volume than a 2'-OH group. A 2' ribose modification that is larger in volume than a 2'-OH group can for example be a 2'-OMe, 2'-O-MOE (2'-O-methoxyethyl), 2'-O-allyl or 2'-O-alkyl, with the proviso that the nucleic is capable of reducing the expression of the target gene to at least the same extent as the same nucleic acid without the modification(s) under comparable conditions. The first modification is preferably 2'-F and/or the second modification is preferably 2'-OMe.

In the context of this disclosure, the size or volume of a substituent, such as a 2' ribose modification, is preferably measured as the van der Waals volume.

In one aspect, at least one, several or preferably all the nucleotides of the second strand in a position corresponding to an even-numbered nucleotide of the first strand are modified, preferably by a third modification. Preferably in the same nucleic acid nucleotides 2 and 14 or all the even numbered nucleotides of the first strand are modified with a first modification. In addition, or alternatively, the odd-numbered nucleotides of the first strand are modified with a second modification. Preferably, the third modification is different from the first modification

and/or the third modification is the same as the second modification. The first modification is preferably any 2' ribose modification that is of the same size or smaller in volume than a 2'-OH group, or a locked nucleic acid (LNA), or an unlocked nucleic acid (UNA), or a 2'-Fluoroarabino Nucleic Acid (FANA) modification. A 2' ribose modification that is of the same size or smaller in volume than a 2'-OH group can for example be a 2'-F, 2'-H, 2'-halo, or 2'-NH_2. The second and/or third modification is preferably any 2' ribose modification that is larger in volume than a 2'-OH group. A 2' ribose modification that is larger in volume than a 2'-OH group can for example be a 2'-OMe, 2'-O-MOE (2'-O-methoxyethyl), 2'-O-allyl or 2'-O-alkyl, with the proviso that the nucleic is capable of reducing the expression of the target gene to at least the same extent as the same nucleic acid without the modification(s) under comparable conditions. The first modification is preferably 2'-F and/or the second and/or third modification is/are preferably 2'-OMe. The nucleotides on the first strand are numbered consecutively starting with nucleotide number 1 at the 5' end of the first strand.

A nucleotide of the second strand that is in a position corresponding, for example, to an even-numbered nucleotide of the first strand is a nucleotide of the second strand that is base-paired to an even-numbered nucleotide of the first strand.

In one aspect, at least one, several or preferably all the nucleotides of the second strand in a position corresponding to an odd-numbered nucleotide of the first strand are modified, preferably by a fourth modification. Preferably in the same nucleic acid nucleotides 2 and 14 or all the even numbered nucleotides of the first strand are modified with a first modification. In addition, or alternatively, the odd-numbered nucleotides of the first strand are modified with a second modification. In addition, or alternatively, all the nucleotides of the second strand in a position corresponding to an even-numbered nucleotide of the first strand are modified with a third modification. The fourth modification is preferably different from the second modification and preferably different from the third modification and the fourth modification is preferably the same as the first modification. The first and/or fourth modification is preferably any 2' ribose modification that is of the same size or smaller in volume than a 2'-OH group, or a locked nucleic acid (LNA), or an unlocked nucleic acid (UNA), or a 2'-Fluoroarabino Nucleic Acid (FANA) modification. A 2' ribose modification that is of the same size or smaller in volume than a 2'-OH group can for example be a 2'-F, 2'-H, 2'-halo, or 2'-NH_2. The second and/or third modification is preferably any 2' ribose modification that is larger in volume than a 2'-OH group. A 2' ribose modification that is larger in volume than a 2'-OH group can for example be a 2'-OMe, 2'-O-MOE (2'-O-methoxyethyl), 2'-O-allyl or 2'-O-alkyl, with the proviso that the nucleic is capable of reducing the expression of the target gene to at least the same extent as the same nucleic acid without the modification(s) under comparable conditions. The first and/or

the fourth modification is/are preferably a 2'-OMe modification and/or the second and/or third modification is/are preferably a 2'-F modification. The nucleotides on the first strand are numbered consecutively starting with nucleotide number 1 at the 5' end of the first strand.

In one aspect of the nucleic acid, the nucleotide/nucleotides of the second strand in a position corresponding to nucleotide 11 or nucleotide 13 or nucleotides 11 and 13 or nucleotides 11-13 of the first strand is/are modified by a fourth modification. Preferably, all the nucleotides of the second strand other than the nucleotide/nucleotides in a position corresponding to nucleotide 11 or nucleotide 13 or nucleotides 11 and 13 or nucleotides 11-13 of the first strand is/are modified by a third modification. Preferably in the same nucleic acid nucleotides 2 and 14 or all the even numbered nucleotides of the first strand are modified with a first modification. In addition, or alternatively, the odd-numbered nucleotides of the first strand are modified with a second modification. The fourth modification is preferably different from the second modification and preferably different from the third modification and the fourth modification is preferably the same as the first modification. The first and/or fourth modification is preferably any 2' ribose modification that is of the same size or smaller in volume than a 2'-OH group, or a locked nucleic acid (LNA), or an unlocked nucleic acid (UNA), or a 2'-Fluoroarabino Nucleic Acid (FANA) modification. A 2' ribose modification that is of the same size or smaller in volume than a 2'-OH group can for example be a 2'-F, 2'-H, 2'-halo, or 2'-NH_2. The second and/or third modification is preferably any 2' ribose modification that is larger in volume than a 2'-OH group. A 2' ribose modification that is larger in volume than a 2'-OH group can for example be a 2'-OMe, 2'-O-MOE (2'-O-methoxyethyl), 2'-O-allyl or 2'-O-alkyl, with the proviso that the nucleic is capable of reducing the expression of the target gene to at least the same extent as the same nucleic acid without the modification(s) under comparable conditions. The first and/or the fourth modification is/are preferably a 2'-OMe modification and/or the second and/or third modification is/are preferably a 2'-F modification. The nucleotides on the first strand are numbered consecutively starting with nucleotide number 1 at the 5' end of the first strand.

In one aspect of the nucleic acid, all the even-numbered nucleotides of the first strand are modified by a first modification, all the odd-numbered nucleotides of the first strand are modified by a second modification, all the nucleotides of the second strand in a position corresponding to an even-numbered nucleotide of the first strand are modified by a third modification, all the nucleotides of the second strand in a position corresponding to an odd-numbered nucleotide of the first strand are modified by a fourth modification, wherein the first and/or fourth modification is/are 2'-F and/or the second and/or third modification is/are 2'-OMe.

In one aspect of the nucleic acid, all the even-numbered nucleotides of the first strand are modified by a first modification, all the odd-numbered nucleotides of the first strand are modified by a second modification, all the nucleotides of the second strand in positions corresponding to nucleotides 11-13 of the first strand are modified by a fourth modification, all the nucleotides of the second strand other than the nucleotides corresponding to nucleotides 11-13 of the first strand are modified by a third modification, wherein the first and fourth modification are 2'-F and the second and third modification are 2'-OMe. In one embodiment in this aspect, the 3' terminal nucleotide of the second strand is an inverted RNA nucleotide (i.e., the nucleotide is linked to the 3' end of the strand through its 3' carbon, rather than through its 5' carbon as would normally be the case). When the 3' terminal nucleotide of the second strand is an inverted RNA nucleotide, the inverted RNA nucleotide is preferably an unmodified nucleotide in the sense that it does not comprise any modifications compared to the natural nucleotide counterpart. Specifically, the inverted RNA nucleotide is preferably a 2'-OH nucleotide. Preferably, in this aspect when the 3' terminal nucleotide of the second strand is an inverted RNA nucleotide, the nucleic acid is blunt-ended at least at the end that comprises the 5' end of the first strand.

One aspect of the present invention is a nucleic acid as disclosed herein for inhibiting expression of the C3 gene, preferably in a cell, wherein said first strand includes modified nucleotides or unmodified nucleotides at a plurality of positions in order to facilitate processing of the nucleic acid by RISC.

In one aspect, "facilitate processing by RISC" means that the nucleic acid can be processed by RISC, for example any modification present will permit the nucleic acid to be processed by RISC and preferably, will be beneficial to processing by RISC, suitably such that siRNA activity can take place.

A nucleic acid as disclosed herein, wherein the nucleotides at positions 2 and 14 from the 5' end of the first strand are not modified with a 2' OMe modification, and the nucleotide/nucleotides on the second strand which corresponds to position 11 or position 13 or positions 11 and 13 or positions 11, 12 and 13 of the first strand is/are not modified with a 2'-OMe modification (in other words, they are not modified or are modified with a modification other than 2'-OMe).

In one aspect, the nucleotide on the second strand which corresponds to position 13 of the first strand is the nucleotide that forms a base pair with position 13 (from the 5' end) of the first strand.

In one aspect, the nucleotide on the second strand which corresponds to position 11 of the first strand is the nucleotide that forms a base pair with position 11 (from the 5' end) of the first strand.

In one aspect, the nucleotide on the second strand which corresponds to position 12 of the first strand is the nucleotide that forms a base pair with position 12 (from the 5' end) of the first strand.

For example, in a 19-mer nucleic acid which is double-stranded and blunt ended, position 13 (from the 5' end) of the first strand would pair with position 7 (from the 5' end) of the second strand. Position 11 (from the 5' end) of the first strand would pair with position 9 (from the 5' end) of the second strand. This nomenclature may be applied to other positions of the second strand.

In one aspect, in the case of a partially complementary first and second strand, the nucleotide on the second strand that "corresponds to" a position on the first strand may not necessarily form a base pair if that position is the position in which there is a mismatch, but the principle of the nomenclature still applies.

One aspect is a nucleic acid as disclosed herein, wherein the nucleotides at positions 2 and 14 from the 5' end of the first strand are not modified with a 2'-OMe modification, and the nucleotides on the second strand which correspond to position 11, or 13, or 11 and 13, or 11-13 of the first strand are modified with a 2'-F modification.

One aspect is a nucleic acid as disclosed herein, wherein the nucleotides at positions 2 and 14 from the 5' end of the first strand are modified with a 2'-F modification, and the nucleotides on the second strand which correspond to position 11, or 13, or 11 and 13, or 11-13 of the first strand are not modified with a 2'-OMe modification.

One aspect is a nucleic acid as disclosed herein, wherein the nucleotides at positions 2 and 14 from the 5' end of the first strand are modified with a 2'-F modification, and the nucleotides on the second strand which correspond to position 11, or 13, or 11 and 13, or 11-13 of the first strand are modified with a 2'-F modification.

One aspect is a nucleic acid as disclosed herein wherein greater than 50% of the nucleotides of the first and/or second strand comprise a 2'-OMe modification, such as greater than 55%,

60%, 65%, 70%, 75%, 80%, or 85%, or more, of the first and/or second strand comprise a 2'-OMe modification, preferably measured as a percentage of the total nucleotides of both the first and second strands.

One aspect is a nucleic acid as disclosed herein wherein greater than 50% of the nucleotides of the first and/or second strand comprise a naturally occurring RNA modification, such as wherein greater than 55%, 60%, 65%, 70%, 75%, 80%, or 85% or more of the first and/or second strands comprise such a modification, preferably measured as a percentage of the total nucleotides of both the first and second strands. Suitable naturally occurring modifications include, as well as 2'-OMe, other 2' sugar modifications, in particular a 2'-H modification resulting in a DNA nucleotide.

One aspect is a nucleic acid as disclosed herein comprising no more than 20%, such as no more than 15% such as no more than 10%, of nucleotides which have 2' modifications that are not 2'-OMe modifications on the first and/or second strand, preferably as a percentage of the total nucleotides of both the first and second strands.

One aspect is a nucleic acid as disclosed herein, wherein the number of nucleotides in the first and/or second strand with a 2'-modification that is not a 2'-OMe modification is no more than 7, more preferably no more than 5, and most preferably no more than 3.

One aspect is a nucleic acid as disclosed herein comprising no more than 20%, (such as no more than 15% or no more than 10%) of 2'-F modifications on the first and/or second strand, preferably as a percentage of the total nucleotides of both strands.

One aspect is a nucleic acid as disclosed herein, wherein the number of nucleotides in the first and/or second strand with a 2'-F modification is no more than 7, more preferably no more than 5, and most preferably no more than 3.

One aspect is a nucleic acid as disclosed herein, wherein all nucleotides are modified with a 2'-OMe modification except positions 2 and 14 from the 5' end of the first strand and the nucleotides on the second strand which correspond to position 11, or 13, or 11 and 13, or 11-13 of the first strand. Preferably the nucleotides that are not modified with 2'-OMe are modified with fluoro at the 2' position (2'-F modification).

Preferred is a nucleic acid as disclosed herein wherein all nucleotides of the nucleic acid are modified at the 2' position of the sugar. Preferably these nucleotides are modified with a 2'-F modification where the modification is not a 2'-OMe modification.

In one aspect the nucleic acid is modified on the first strand with alternating 2'-OMe modifications and 2-F modifications, and positions 2 and 14 (starting from the 5' end) are modified with 2'-F. Preferably the second strand is modified with 2'-F modifications at nucleotides on the second strand which correspond to position 11, or 13, or 11 and 13, or 11-13 of the first strand. Preferably the second strand is modified with 2'-F modifications at positions 11-13 counting from the 3' end starting at the first position of the complementary (double-stranded) region, and the remaining modifications are naturally occurring modifications, preferably 2'-OMe. The complementary region at least in this case starts at the first position of the second strand that has a corresponding nucleotide in the first strand, regardless of whether the two nucleotides are able to base pair to each other.

In one aspect of the nucleic acid, each of the nucleotides of the first strand and of the second strand is a modified nucleotide.

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

Unless specifically stated otherwise, herein the nucleotides of the first strand are numbered contiguously starting with nucleotide number 1 at the 5' end of the first strand. Nucleotides of the second strand are numbered contiguously starting with nucleotide number 1 at the 3' end of the second strand.

One or more nucleotides on the first and/or second strand may be modified, to form modified nucleotides. One or more of the odd-numbered nucleotides of the first strand may be modified. One or more of the even-numbered nucleotides of the first strand may be modified by at least a second modification, wherein the at least second modification is different from the modification on the one or more odd nucleotides. At least one of the one or more modified even numbered-nucleotides may be adjacent to at least one of the one or more modified odd-numbered nucleotides.

A plurality of odd-numbered nucleotides in the first strand may be modified in the nucleic acid of the invention. A plurality of even-numbered nucleotides in the first strand may be modified by a second modification. The first strand may comprise adjacent nucleotides that are modified by a common modification. The first strand may also comprise adjacent nucleotides that are modified by a second different modification (i.e. the first strand may comprise nucleotides that are adjacent to each other and modified by a first modification as well as other nucleotides that are adjacent to each other and modified by a second modification that is different to the first modification).

One or more of the odd-numbered nucleotides of the second strand (wherein the nucleotides are numbered contiguously starting with nucleotide number 1 at the 3' end of the second strand) may be modified by a modification that is different to the modification of the odd-numbered nucleotides on the first strand (wherein the nucleotides are numbered contiguously starting with nucleotide number 1 at the 5' end of the first strand) and/or one or more of the even-numbered nucleotides of the second strand may be modified by the same modification of the odd-numbered nucleotides of the first strand. At least one of the one or more modified even-numbered nucleotides of the second strand may be adjacent to the one or more modified odd-numbered nucleotides. A plurality of odd-numbered nucleotides of the second strand may be modified by a common modification and/or a plurality of even-numbered nucleotides may be modified by the same modification that is present on the first stand odd-numbered nucleotides. A plurality of odd-numbered nucleotides on the second strand may be modified by a modification that is different from the modification of the first strand odd-numbered nucleotides.

The second strand may comprise adjacent nucleotides that are modified by a common modification, which may be a modification that is different from the modification of the odd-numbered nucleotides of the first strand.

In the nucleic acid of the invention, each of the odd-numbered nucleotides in the first strand and each of the even-numbered nucleotides in the second strand may be modified with a common modification and, each of the even-numbered nucleotides may be modified in the first strand with a different modification and each of the odd-numbered nucleotides may be modified in the second strand with the different modification.

The nucleic acid of the invention may have the modified nucleotides of the first strand shifted by at least one nucleotide relative to the unmodified or differently modified nucleotides of the second strand.

One or more or each of the odd numbered-nucleotides may be modified in the first strand and one or more or each of the even-numbered nucleotides may be modified in the second strand. One or more or each of the alternating nucleotides on either or both strands may be modified by a second modification. One or more or each of the even-numbered nucleotides may be modified in the first strand and one or more or each of the even-numbered nucleotides may be modified in the second strand. One or more or each of the alternating nucleotides on either or both strands may be modified by a second modification. One or more or each of the odd-numbered nucleotides may be modified in the first strand and one or more of the odd-numbered nucleotides may be modified in the second strand by a common modification. One or more or each of the alternating nucleotides on either or both strands may be modified by a second modification. One or more or each of the even-numbered nucleotides may be modified in the first strand and one or more or each of the odd-numbered nucleotides may be modified in the second strand by a common modification. One or more or each of the alternating nucleotides on either or both strands may be modified by a second modification.

The nucleic acid of the invention may comprise single- or double-stranded constructs that comprise at least two regions of alternating modifications in one or both of the strands. These alternating regions can comprise up to about 12 nucleotides but preferably comprise from about 3 to about 10 nucleotides. The regions of alternating nucleotides may be located at the termini of one or both strands of the nucleic acid of the invention. The nucleic acid may comprise from 4 to about 10 nucleotides of alternating nucleotides at each of the termini (3' and 5') and these regions may be separated by from about 5 to about 12 contiguous unmodified or differently or commonly modified nucleotides.

The odd numbered nucleotides of the first strand may be modified and the even numbered nucleotides may be modified with a second modification. The second strand may comprise adjacent nucleotides that are modified with a common modification, which may be the same as the modification of the odd-numbered nucleotides of the first strand. One or more nucleotides of the second strand may also be modified with the second modification. One or more nucleotides with the second modification may be adjacent to each other and to nucleotides having a modification that is the same as the modification of the odd-numbered nucleotides of the first strand. The first strand may also comprise phosphorothioate linkages between the two nucleotides at the 3' end and at the 5' end or a phosphorodithioate linkage between the two nucleotides at the 3' end. The second strand may comprise a phosphorothioate or phosphorodithioate linkage between the two nucleotides at the 5' end. The second strand may also be conjugated to a ligand at the 5' end.

The nucleic acid of the invention may comprise a first strand comprising adjacent nucleotides that are modified with a common modification. One or more such nucleotides may be adjacent to one or more nucleotides which may be modified with a second modification. One or more nucleotides with the second modification may be adjacent. The second strand may comprise adjacent nucleotides that are modified with a common modification, which may be the same as one of the modifications of one or more nucleotides of the first strand. One or more nucleotides of the second strand may also be modified with the second modification. One or more nucleotides with the second modification may be adjacent. The first strand may also comprise phosphorothioate linkages between the two nucleotides at the 3' end and at the 5' end or a phosphorodithioate linkage between the two nucleotides at the 3' end. The second strand may comprise a phosphorothioate or phosphorodithioate linkage between the two nucleotides at the 3' end. The second strand may also be conjugated to a ligand at the 5' end.

The nucleotides numbered from 5' to 3' on the first strand and 3' to 5' on the second strand, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25 may be modified by a modification on the first strand. The nucleotides numbered 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 may be modified by a second modification on the first strand. The nucleotides numbered 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 may be modified by a modification on the second strand. The nucleotides numbered 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 may be modified by a second modification on the second strand. Nucleotides are numbered for the sake of the nucleic acid of the present invention from 5' to 3' on the first strand and 3' to 5' on the second strand.

The nucleotides numbered 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 may be modified by a modification on the first strand. The nucleotides numbered 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 may be modified by a second modification on the first strand. The nucleotides numbered 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 may be modified by a modification on the second strand. The nucleotides numbered 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 may be modified by a second modification on the second strand.

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

One or more modified nucleotides on the first strand may be paired with modified nucleotides on the second strand having a common modification. One or more modified nucleotides on the

first strand may be paired with modified nucleotides on the second strand having a different modification. One or more modified nucleotides on the first strand may be paired with unmodified nucleotides on the second strand. One or more modified nucleotides on the second strand may be paired with unmodified nucleotides on the first strand. In other words, the alternating nucleotides can be aligned on the two strands such as, for example, all the modifications in the alternating regions of the second strand are paired with identical modifications in the first strand or alternatively the modifications can be offset by one nucleotide with the common modifications in the alternating regions of one strand pairing with dissimilar modifications (i.e. a second or further modification) in the other strand. Another option is to have dissimilar modifications in each of the strands.

The modifications on the first strand may be shifted by one nucleotide relative to the modified nucleotides on the second strand, such that common modified nucleotides are not paired with each other.

The modification and/or modifications may each and individually be selected from the group consisting of 3' terminal deoxy thymine, 2'-OMe, a 2' deoxy modification, a 2' amino modification, a 2' alkyl modification, a morpholino modification, a phosphoramidate modification, 5'-phosphorothioate group modification, a 5' phosphate or 5' phosphate mimic modification and a cholesteryl derivative or a dodecanoic acid bisdecylamide group modification and/or the modified nucleotide may be any one of a locked nucleotide, an abasic nucleotide or a non-natural base comprising nucleotide.

At least one modification may be 2'-OMe and/or at least one modification may be 2'-F. Further modifications as described herein may be present on the first and/or second strand.

The nucleic acid of the invention may comprise an inverted RNA nucleotide at one or several of the strand ends. Such inverted nucleotides provide stability to the nucleic acid. Preferably, the nucleic acid comprises at least an inverted nucleotide at the 3' end of the first and/or the second strand and/or at the 5' end of the second strand. More preferably, the nucleic acid comprises an inverted nucleotide at the 3' end of the second strand. Most preferably, the nucleic acid comprises an inverted RNA nucleotide at the 3' end of the second strand and this nucleotide is preferably an inverted A. An inverted nucleotide is a nucleotide that is linked to the 3' end of a nucleic acid through its 3' carbon, rather than its 5' carbon as would normally be the case or is linked to the 5' end of a nucleic acid through its 5' carbon, rather than its 3' carbon as would normally be the case. The inverted nucleotide is preferably present at an end of a strand not as an overhang but opposite a corresponding nucleotide in the other strand.

Accordingly, the nucleic acid is preferably blunt-ended at the end that comprises the inverted RNA nucleotide. An inverted RNA nucleotide being present at the end of a strand preferably means that the last nucleotide at this end of the strand is the inverted RNA nucleotide. A nucleic acid with such a nucleotide is stable and easy to synthesise. The inverted RNA nucleotide is preferably an unmodified nucleotide in the sense that it does not comprise any modifications compared to the natural nucleotide counterpart. Specifically, the inverted RNA nucleotide is preferably a 2'-OH nucleotide.

Nucleic acids of the invention may comprise one or more nucleotides modified at the 2' position with a 2'-H, and therefore having a DNA nucleotide within the nucleic acid. Nucleic acids of the invention may comprise DNA nucleotides at positions 2 and/or 14 of the first strand counting from the 5' end of the first strand. Nucleic acids may comprise DNA nucleotides on the second strand which correspond to position 11, or 13, or 11 and 13, or 11-13 of the first strand.

In one aspect there is no more than one DNA nucleotide per nucleic acid of the invention.

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

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

Preferably, the nucleic acid may comprise a first modification and a second or further modification which are each and individually selected from the group comprising 2'-OMe modification and 2'-F modification. The nucleic acid may comprise a modification that is 2'-OMe that may be a first modification, and a second modification that is 2'-F. The nucleic acid of the invention may also include a phosphorothioate or phosphorodithioate modification and/or a deoxy modification which may be present in or between the terminal 2 or 3 nucleotides of each or any end of each or both strands.

In one aspect of the nucleic acid, at least one nucleotide of the first and/or second strand is a modified nucleotide, wherein if the first strand comprises at least one modified nucleotide:

(i) at least one or both of the nucleotides 2 and 14 of the first strand is/are modified by a first modification; and/or

(ii) at least one, several, or all the even-numbered nucleotides of the first strand is/are modified by a first modification; and/or

(iii) at least one, several, or all the odd-numbered nucleotides of the first strand is/are modified by a second modification; and/or

wherein if the second strand comprises at least one modified nucleotide:

(iv) at least one, several, or all the nucleotides of the second strand in a position corresponding to an even-numbered nucleotide of the first strand is/are modified by a third modification; and/or

(v) at least one, several, or all the nucleotides of the second strand in a position corresponding to an odd-numbered nucleotide of the first strand is/are modified by a fourth modification; and/or

(vi) at least one, several, or all the nucleotides of the second strand in a position corresponding to nucleotide 11 or nucleotide 13 or nucleotides 11 and 13 or nucleotides 11-13 of the first strand is/are modified by a fourth modification; and/or

(vii) at least one, several, or all the nucleotides of the second strand in a position other than the position corresponding to nucleotide 11 or nucleotide 13 or nucleotides 11 and 13 or nucleotides 11-13 of the first strand is/are modified by a third modification;

wherein the nucleotides on the first strand are numbered consecutively starting with nucleotide number 1 at the 5' end of the first strand;

wherein the modifications are preferably at least one of the following:

(a) the first modification is preferably different from the second and from the third modification;

(b) the first modification is preferably the same as the fourth modification;

(c) the second and the third modification are preferably the same modification;

(d) the first modification is preferably a 2'-F modification;

(e) the second modification is preferably a 2'-OMe modification;

(f) the third modification is preferably a 2'-OMe modification; and/or

(g) the fourth modification is preferably a 2'-F modification; and

wherein optionally the nucleic acid is conjugated to a ligand.

One aspect is a double-stranded nucleic acid for inhibiting expression of C3, preferably in a cell, wherein the nucleic acid comprises a first strand and a second strand, wherein the first strand sequence comprises a sequence of at least 15 nucleotides differing by no more than 3 nucleotides from any one of the sequences SEQ ID NO: 370, 364, 365, 366, 368, 372, 377, 361, 95, 111, 125, 131, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 97, 99, 101, 103, 105, 107, 109, 113, 115, 117, 119, 121, 123, 127, 129, 133 or 416,

preferably SEQ ID NO: 370, 364, 365, 366, 368, 372, 377 or 416, wherein all the even-numbered nucleotides of the first strand are modified by a first modification, all the odd-numbered nucleotides of the first strand are modified by a second modification, all the nucleotides of the second strand in a position corresponding to an even-numbered nucleotide of the first strand are modified by a third modification, all the nucleotides of the second strand in a position corresponding to an odd-numbered nucleotide of the first strand are modified by a fourth modification, wherein the first and fourth modification are 2'-F and the second and third modification are 2'-OMe.

One aspect is a double-stranded nucleic acid for inhibiting expression of C3, preferably in a cell, wherein the nucleic acid comprises a first strand and a second strand, wherein the first strand sequence comprises a sequence of at least 15 nucleotides differing by no more than 3 nucleotides from any one of the sequences SEQ ID NO: 370, 364, 365, 366, 368, 372, 377, 361, 95, 111, 125, 131, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 97, 99, 101, 103, 105, 107, 109, 113, 115, 117, 119, 121, 123, 127, 129, 133 or 416, preferably SEQ ID NO: 370, 364, 365, 366, 368, 372, 377 or 416, wherein all the even-numbered nucleotides of the first strand are modified by a first modification, all the odd-numbered nucleotides of the first strand are modified by a second modification, all the nucleotides of the second strand in positions corresponding to nucleotides 11-13 of the first strand are modified by a fourth modification, all the nucleotides of the second strand other than the nucleotides corresponding to nucleotides 11-13 of the first strand are modified by a third modification, wherein the first and fourth modification are 2'-F and the second and third modification are 2'-OMe.

The 3′ and 5′ ends of an oligonucleotide can be modified. Such modifications can be at the 3′ end or the 5′ end or both ends of the molecule. They can include modification or replacement of an entire terminal phosphate or of one or more of the atoms of the phosphate group. For example, the 3′ and 5′ ends of an oligonucleotide can be conjugated to other functional molecular entities such as labelling moieties, e.g., fluorophores (e.g., pyrene, TAMRA, fluorescein, Cy3 or Cy5 dyes) or protecting groups (based e.g., on sulfur, silicon, boron or ester). The functional molecular entities can be attached to the sugar through a phosphate group and/or a linker. The terminal atom of the linker can connect to or replace the linking atom of the phosphate group or the C-3′ or C-5′ O, N, S or C group of the sugar. Alternatively, the linker can connect to or replace the terminal atom of a nucleotide surrogate (e.g., PNAs). These spacers or linkers can include e.g., —$(CH_2)_n$—, —$(CH_2)_nN$—, —$(CH_2)_nO$—, —$(CH_2)_nS$—, —$(CH_2CH_2O)_nCH_2CH_2O$— (e.g., n=3 or 6), abasic sugars, amide, carboxy, amine, oxyamine,

oxyimine, thioether, disulfide, thiourea, sulfonamide, or morpholino, or biotin and fluorescein reagents. The 3′ end can be an —OH group.

Other examples of terminal modifications include dyes, intercalating agents (e.g., acridines), cross-linkers (e.g., psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (e.g., phenazine, dihydrophenazine), artificial endonucleases, EDTA, lipophilic carriers (e.g., cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine) and peptide conjugates (e.g., antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (e.g., PEG-40K), MPEG, [MPEG]2, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (e.g., biotin), transport/absorption facilitators (e.g., aspirin, vitamin E, folic acid), synthetic ribonucleases (e.g., imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu3+ complexes of tetraazamacrocycles).

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

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

thiotriphosphate, etc.), 5′-phosphoramidates ($(HO)_2(O)P$—NH-5′, $(HO)(NH_2)(O)P$—O-5′), 5′-alkylphosphonates (alkyl=methyl, ethyl, isopropyl, propyl, etc., e.g. RP(OH)(O)—O-5′-(wherein R is an alkyl), $(OH)_2(O)P$-5′-CH_2-), 5′ vinylphosphonate, 5′-alkyletherphosphonates (alkylether=methoxymethyl ($MeOCH_2$-), ethoxymethyl, etc., e.g. RP(OH)(O)—O-5′- (wherein R is an alkylether)).

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

In each sequence described herein, a C-terminal "–OH" moiety may be substituted for a C-terminal "–NH_2" moiety, and vice-versa.

The invention also provides a nucleic acid according to any aspect of the invention described herein, wherein the first strand has a terminal 5' (E)-vinylphosphonate nucleotide at its 5' end. This terminal 5' (E)-vinylphosphonate nucleotide is preferably linked to the second nucleotide in the first strand by a phosphodiester linkage.

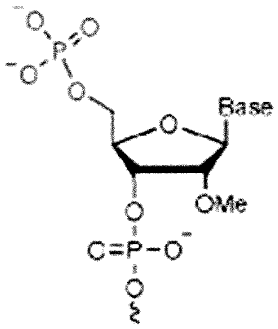
The first strand of the nucleic acid may comprise formula (I):

$$(vp)\text{-}N_{(po)}[N_{(po)}]_n\text{-} \quad (I)$$

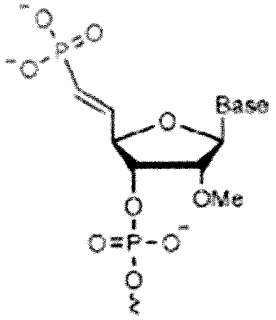
where '(vp)-' is the 5' (E)-vinylphosphonate, 'N' is a nucleotide, 'po' is a phosphodiester linkage, and n is from 1 to (the total number of nucleotides in the first strand – 2), preferably wherein n is from 1 to (the total number of nucleotides in the first strand -3), more preferably wherein n is from 1 to (the total number of nucleotides in the first strand -4).

Preferably, the terminal 5' (E)-vinylphosphonate nucleotide is an RNA nucleotide, preferably a (vp)-U.

A terminal 5' (E)-vinylphosphonate nucleotide is a nucleotide wherein the natural phosphate group at the 5'-end has been replaced with a E-vinylphosphonate, in which the bridging 5'-oxygen atom of the terminal nucleotide of the 5' phosphorylated strand is replaced with a methynyl (-CH=) group:



Nucleotides with a natural phosphate at the 5'-end



Nucleotide with a E-vinylphosphonate at the 5'-end

A 5' (E)-vinylphosphonate is a 5' phosphate mimic. A biological mimic is a molecule that is capable of carrying out the same function as and is structurally very similar to the original molecule that is being mimicked. In the context of the present invention, 5' (E)-vinylphosphonate mimics the function of a normal 5' phosphate, e.g. enabling efficient RISC loading. In addition, because of its slightly altered structure, 5' (E) vinylphosphonate is capable of stabilizing the 5'-end nucleotide by protecting it from dephosphorylation by enzymes such as phosphatases.

In one aspect, the first strand has a terminal 5' (E)-vinylphosphonate nucleotide at its 5' end, the terminal 5' (E)-vinylphosphonate nucleotide is linked to the second nucleotide in the first strand by a phosphodiester linkage and the first strand comprises a) more than 1 phosphodiester linkage; b) phosphodiester linkages between at least the terminal three 5' nucleotides and/or c) phosphodiester linkages between at least the terminal four 5' nucleotides.

In one aspect, the first strand and/or the second strand of the nucleic acid comprises at least one phosphorothioate (ps) and/or at least one phosphorodithioate (ps2) linkage between two nucleotides.

In one aspect, the first strand and/or the second strand of the nucleic acid comprises more than one phosphorothioate and/or more than one phosphorodithioate linkage.

In one aspect, the first strand and/or the second strand of the nucleic acid comprises a phosphorothioate or phosphorodithioate linkage between the terminal two 3' nucleotides or phosphorothioate or phosphorodithioate linkages between the terminal three 3' nucleotides. Preferably, the linkages between the other nucleotides in the first strand and/or the second strand are phosphodiester linkages.

In one aspect, the first strand and/or the second strand of the nucleic acid comprises a phosphorothioate linkage between the terminal two 5' nucleotides or a phosphorothioate linkages between the terminal three 5' nucleotides.

In one aspect, the nucleic acid of the present invention comprises one or more phosphorothioate or phosphorodithioate modifications on one or more of the terminal ends of the first and/or the second strand. Optionally, each or either end of the first strand may comprise one or two or three phosphorothioate or phosphorodithioate modified nucleotides (internucleoside linkage). Optionally, each or either end of the second strand may comprise one or two or three phosphorothioate or phosphorodithioate modified nucleotides (internucleoside linkage).

In one aspect, the nucleic acid comprises a phosphorothioate linkage between the terminal two or three 3' nucleotides and/or 5' nucleotides of the first and/or the second strand. Preferably, the nucleic acid comprises a phosphorothioate linkage between each of the terminal three 3' nucleotides and the terminal three 5' nucleotides of the first strand and of the second strand. Preferably, all remaining linkages between nucleotides of the first and/or of the second strand are phosphodiester linkages.

In one aspect, the nucleic acid comprises a phosphorodithioate linkage between each of the two, three or four terminal nucleotides at the 3' end of the first strand and/or comprises a phosphorodithioate linkage between each of the two, three or four terminal nucleotides at the 3' end of the second strand and/or a phosphorodithioate linkage between each of the two, three or four terminal nucleotides at the 5' end of the second strand and comprises a linkage other than a phosphorodithioate linkage between the two, three or four terminal nucleotides at the 5' end of the first strand.

In one aspect, the nucleic acid comprises a phosphorothioate linkage between the terminal three 3' nucleotides and the terminal three 5' nucleotides of the first strand and of the second strand. Preferably, all remaining linkages between nucleotides of the first and/or of the second strand are phosphodiester linkages.

In one aspect, the nucleic acid:

(i) has a phosphorothioate linkage between the terminal three 3' nucleotides and the terminal three 5' nucleotides of the first strand;

(ii) is conjugated to a triantennary ligand either on the 3' end nucleotide or on the 5' end nucleotide of the second strand;

(iii) has a phosphorothioate linkage between the terminal three nucleotides of the second strand at the end opposite to the one conjugated to the triantennary ligand; and

(iv) optionally all remaining linkages between nucleotides of the first and/or of the second strand are phosphodiester linkages.

In one aspect, the nucleic acid:

(i) has a terminal 5' (E)-vinylphosphonate nucleotide at the 5' end of the first strand;

(ii) has a phosphorothioate linkage between the terminal three 3' nucleotides on the first and second strand and between the terminal three 5' nucleotides on the second strand or it has a phosphorodithioate linkage between the terminal two 3' nucleotides on the first and second strand and between the terminal two 5' nucleotides on the second strand; and

(iii) optionally all remaining linkages between nucleotides of the first and/or of the second strand are phosphodiester linkages.

The use of a phosphorodithioate linkage in the nucleic acid of the invention reduces the variation in the stereochemistry of a population of nucleic acid molecules compared to molecules comprising a phosphorothioate in that same position. Phosphorothioate linkages introduce chiral centres and it is difficult to control which non-linking oxygen is substituted for sulphur. The use of a phosphorodithioate ensures that no chiral centre exists in that linkage and thus reduces or eliminates any variation in the population of nucleic acid molecules, depending on the number of phosphorodithioate and phosphorothioate linkages used in the nucleic acid molecule.

In one aspect, the nucleic acid comprises a phosphorodithioate linkage between the two terminal nucleotides at the 3' end of the first strand and a phosphorodithioate linkage between the two terminal nucleotides at the 3' end of the second strand and a phosphorodithioate

linkage between the two terminal nucleotides at the 5' end of the second strand and comprises a linkage other than a phosphorodithioate linkage between the two, three or four terminal nucleotides at the 5' end of the first strand. Preferably, the first strand has a terminal 5' (E)-vinylphosphonate nucleotide at its 5' end. This terminal 5' (E)-vinylphosphonate nucleotide is preferably linked to the second nucleotide in the first strand by a phosphodiester linkage. Preferably, all the linkages between the nucleotides of both strands other than the linkage between the two terminal nucleotides at the 3' end of the first strand and the linkages between the two terminal nucleotides at the 3' end and at the 5' end of the second strand are phosphodiester linkages.

In one aspect, the nucleic acid comprises a phosphorothioate linkage between each of the three terminal 3' nucleotides and/or between each of the three terminal 5' nucleotides on the first strand, and/or between each of the three terminal 3' nucleotides and/or between each of the three terminal 5' nucleotides of the second strand when there is no phosphorodithioate linkage present at that end. No phosphorodithioate linkage being present at an end means that the linkage between the two terminal nucleotides, or preferably between the three terminal nucleotides of the nucleic acid end in question are linkages other than phosphorodithioate linkages.

In one aspect, all the linkages of the nucleic acid between the nucleotides of both strands other than the linkage between the two terminal nucleotides at the 3' end of the first strand and the linkages between the two terminal nucleotides at the 3' end and at the 5' end of the second strand are phosphodiester linkages.

Other phosphate linkage modifications are possible.

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

The phosphate groups can also individually be replaced by non-phosphorus containing connectors.

Examples of moieties which can replace the phosphate group include siloxane, carbonate, carboxymethyl, carbamate, amide, thioether, ethylene oxide linker, sulfonate, sulfonamide, thioformacetal, formacetal, oxime, methyleneimino, methylenemethylimino,

methylenehydrazo, methylenedimethylhydrazo and methyleneoxymethylimino. In certain embodiments, replacements may include the methylenecarbonylamino and methylenemethylimino groups.

The phosphate linker and ribose sugar may be replaced by nuclease resistant nucleotides. Examples include the morpholino, cyclobutyl, pyrrolidine and peptide nucleic acid (PNA) nucleoside surrogates. In certain embodiments, PNA surrogates may be used.

In one aspect, the nucleic acid, which is preferably an siRNA that inhibits expression of the complement component C3, preferably via RNAi, and preferably in a cell, comprises one or more or all of:

(i) a modified nucleotide;
(ii) a modified nucleotide other than a 2'-OMe modified nucleotide at positions 2 and 14 from the 5' end of the first strand, preferably a 2'-F modified nucleotide;
(iii) each of the odd-numbered nucleotides of the first strand as numbered starting from one at the 5' end of the first strand are 2'-OMe modified nucleotides;
(iv) each of the even-numbered nucleotides of the first strand as numbered starting from one at the 5' end of the first strand are 2'-F modified nucleotides;
(v) the second strand nucleotide corresponding to position 11 and/or 13 or 11-13 of the first strand is modified by a modification other than a 2'-OMe modification, preferably wherein one or both or all of these positions comprise a 2'-F modification;
(vi) an inverted nucleotide, preferably a 3'-3' linkage at the 3' end of the second strand;
(vii) one or more phosphorothioate linkages;
(viii) one or more phosphorodithioate linkages; and/or
(ix) the first strand has a terminal 5' (E)-vinylphosphonate nucleotide at its 5' end, in which case the terminal 5' (E)-vinylphosphonate nucleotide is preferably a uridine and is preferably linked to the second nucleotide in the first strand by a phosphodiester linkage.

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

Ligands

The nucleic acids of the invention may be conjugated to a ligand. Efficient delivery of oligonucleotides, in particular double-stranded nucleic acids of the invention, to cells *in vivo* is important and requires specific targeting and substantial protection from the extracellular environment, particularly serum proteins. One method of achieving specific targeting is to

conjugate a ligand to the nucleic acid. In some embodiments, the ligand helps in targeting the nucleic acid to a target cell which has a cell surface receptor that binds to and internalizes the conjugated ligand. In such embodiments, there is a need to conjugate appropriate ligands for the desired receptor molecules in order for the conjugated molecules to be taken up by the target cells by mechanisms such as different receptor-mediated endocytosis pathways or functionally analogous processes. In other embodiments, a ligand which can mediate internalization of the nucleic acid into a target cell by mechanisms other than receptor mediated endocytosis may alternatively be conjugated to a nucleic acid of the invention for cell or tissue specific targeting.

One example of a conjugate that mediates receptor mediated endocytosis is the asialoglycoprotein receptor complex (ASGP-R) which has high affinity to the GalNAc moiety described herein. The ASGP-R complex is composed of varying ratios of multimers of membrane ASGR1 and ASGR2 receptors, which are highly abundant on hepatocytes. One of the first disclosures of the use of triantennary cluster glycosides as conjugated ligands was in US patent number US 5,885,968. Conjugates having three GalNAc ligands and comprising phosphate groups are known and are described in Dubber et al. (Bioconjug. Chem. 2003 Jan-Feb;14(1):239-46.). The ASGP-R complex shows a 50-fold higher affinity for N-Acetyl-D-Galactosamine (GalNAc) than D-Gal.

The ASGP-R complex recognizes specifically terminal β-galactosyl subunits of glycosylated proteins or other oligosaccharides (Weigel, P.H. et. al., Biochim. Biophys. Acta. 2002 Sep 19;1572(2-3):341-63) and can be used for delivering a drug to the liver's hepatocytes expressing the receptor complex by covalent coupling of galactose or galactosamine to the drug substance (Ishibashi,S.; et. al., J Biol. Chem. 1994 Nov 11;269(45):27803-6). Furthermore, the binding affinity can be significantly increased by the multi-valency effect, which is achieved by the repetition of the targeting moiety (Biessen EA, et al., J Med Chem. 1995 Apr 28;38(9):1538-46).

The ASGP-R complex is a mediator for an active uptake of terminal β-galactosyl containing glycoproteins to the cell's endosomes. Thus, the ASGPR is highly suitable for targeted delivery of drug candidates conjugated to such ligands like, e.g., nucleic acids into receptor-expressing cells (Akinc et al., Mol Ther. 2010 Jul;18(7):1357-64).

More generally the ligand can comprise a saccharide that is selected to have an affinity for at least one type of receptor on a target cell. In particular, the receptor is on the surface of a

mammalian liver cell, for example, the hepatic asialoglycoprotein receptor complex described before (ASGP-R).

The saccharide may be selected from N-acetyl galactosamine, mannose, galactose, glucose, glucosamine and fucose. The saccharide may be N-acetyl galactosamine (GalNAc).

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

The ligand may therefore comprise GalNAc.

In one aspect, the nucleic acid is conjugated to a ligand comprising a compound of formula (II):

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

wherein:

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

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

P is a phosphate or modified phosphate, preferably a thiophosphate;

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

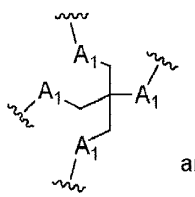
A is a branching unit;

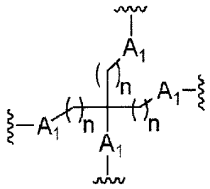
X^3 represents a bridging unit;

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

In formula (II), the branching unit "A" preferably branches into three in order to accommodate three saccharide ligands. The branching unit is preferably covalently attached to the remaining tethered portions of the ligand and the nucleic acid. The branching unit may comprise a branched aliphatic group comprising groups selected from alkyl, amide, disulphide, polyethylene glycol, ether, thioether and hydroxyamino groups. The branching unit may comprise groups selected from alkyl and ether groups.

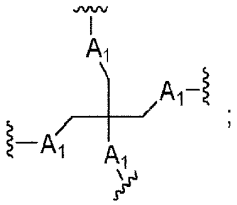
The branching unit A may have a structure selected from:

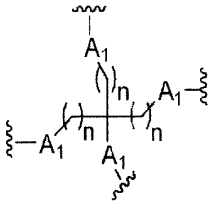


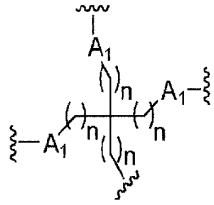
and


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

The branching unit may have a structure selected from:

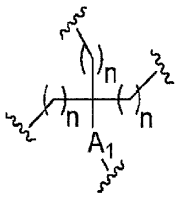


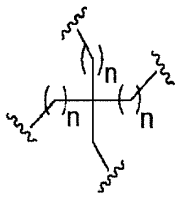
;


and


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

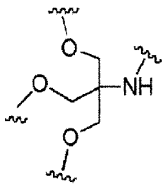
The branching unit may have a structure selected from:



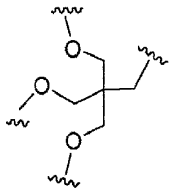
and


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

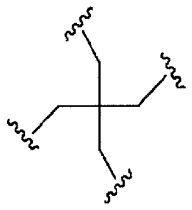
The branching unit may have the structure:



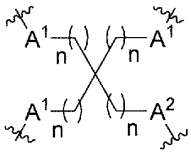
The branching unit may have the structure:



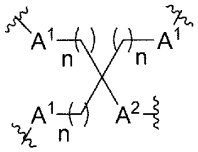
The branching unit may have the structure:



Alternatively, the branching unit A may have a structure selected from:



A^1 = O, NR^1,$C(R^1)_2$ A^2 = NR^2
n = 1 to 4



A^1 = O, NR^1, $C(R^1)_2$ A^2 = NR^2
n = 1 to 4

wherein:
R1 is hydrogen or C1-C10 alkylene;
and R2 is C1-C10 alkylene.

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

The "X^3" portion is a bridging unit. The bridging unit is linear and is covalently bound to the branching unit and the nucleic acid.

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

X^3 may be an alkylene ether of formula -(C_1-C_{20} alkylene)–O–(C_1-C_{20} alkylene)-. X^3 may be an alkylene ether of formula -(C_1-C_{20} alkylene)–O–(C_4-C_{20} alkylene)-, wherein said (C_4-C_{20} alkylene) is linked to Z. X^3 may be selected from the group consisting of -CH_2-O-C_3H_6-, -CH_2-

O-C_4H_8-, -CH_2-O-C_6H_{12}- and -CH_2-O-C_8H_{16}-, especially -CH_2-O-C_4H_8-, -CH_2-O-C_6H_{12}- and -CH_2-O-C_8H_{16}-, wherein in each case the -CH_2- group is linked to A.

In one aspect, the nucleic acid is conjugated to a ligand comprising a compound of formula (III):

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

wherein:

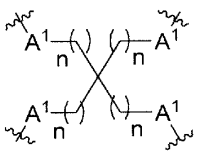
S represents a saccharide, preferably GalNAc;

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

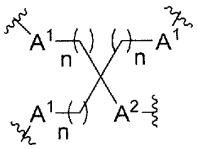
P is a phosphate or modified phosphate, preferably a thiophosphate;

X^2 is C_1-C_8 alkylene;

A is a branching unit selected from:



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

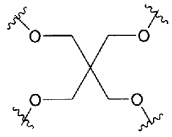


A^1 = O, NH A^2 = NH, CH_2, O
n = 1 to 4

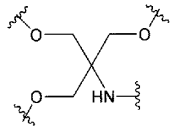
X^3 is a bridging unit;

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

The branching unit A may have the structure:



The branching unit A may have the structure:



, wherein X^3 is attached to the nitrogen atom.

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

In one aspect, the nucleic acid is conjugated to a ligand comprising a compound of formula (IV):

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

wherein:

S represents a saccharide, preferably GalNAc;

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

P is a phosphate or modified phosphate, preferably a thiophosphate;

X^2 is an alkylene ether of formula $\text{-}C_3H_6\text{-}O\text{-}CH_2\text{-}$;

A is a branching unit;

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

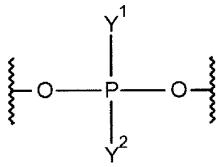
The branching unit may comprise carbon. Preferably, the branching unit is a carbon.

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

X^1 may be $(\text{-}CH_2\text{-}CH_2\text{-}O)(\text{-}CH_2)_2\text{-}$. X^1 may be $(\text{-}CH_2\text{-}CH_2\text{-}O)_2(\text{-}CH_2)_2\text{-}$. X^1 may be $(\text{-}CH_2\text{-}CH_2\text{-}O)_3(\text{-}CH_2)_2\text{-}$. Preferably, X^1 is $(\text{-}CH_2\text{-}CH_2\text{-}O)_2(\text{-}CH_2)_2\text{-}$. Alternatively, X^1 represents C_3-C_6 alkylene. X^1 may be propylene. X^1 may be butylene. X^1 may be pentylene. X^1 may be hexylene. Preferably the alkyl is a linear alkylene. In particular, X^1 may be butylene.

X^2 represents an alkylene ether of formula $\text{-}C_3H_6\text{-}O\text{-}CH_2\text{-}$ i.e. C_3 alkoxy methylene, or $\text{–}CH_2CH_2CH_2OCH_2\text{-}$.

For any of the above aspects, when P represents a modified phosphate group, P can be represented by:



wherein Y^1 and Y^2 each independently represent =O, =S, $\text{-}O^-$, -OH, -SH, $\text{-}BH_3$, $\text{-}OCH_2CO_2$, $\text{-}OCH_2CO_2R^x$, $\text{-}OCH_2C(S)OR^x$, and $\text{–}OR^x$, wherein R^x represents C_1-C_6 alkyl and wherein ⊣ indicates attachment to the remainder of the compound.

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

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

For example, Y^1 may represent -OH and Y^2 may represent =O or =S; or
Y^1 may represent $-O^-$ and Y^2 may represent =O or =S;
Y^1 may represent =O and Y^2 may represent $-CH_3$, -SH, $-OR^x$, or $-BH_3$
Y^1 may represent =S and Y^2 may represent $-CH_3$, OR^x or –SH.

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

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

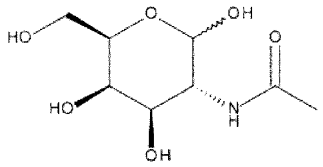
P may also be an ethylphosphate (i.e. where Y^1 represents =O and Y^2 represents OCH_2CH_3).

The saccharide may be selected to have an affinity for at least one type of receptor on a target cell. In particular, the receptor is on the surface of a mammalian liver cell, for example, the hepatic asialoglycoprotein receptor complex (ASGP-R).

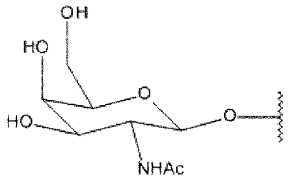
For any of the above or below aspects, the saccharide may be selected from N-acetyl with one or more of galactosamine, mannose, galactose, glucose, glucosamine and fructose. Typically a ligand to be used in the present invention may include N-acetyl galactosamine (GalNAc).

Preferably the compounds of the invention may have 3 ligands, which will each preferably include N-acetyl galactosamine.

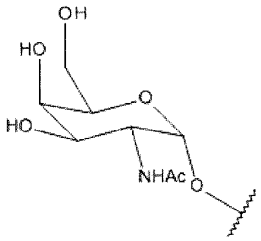
"GalNAc" refers to 2-(Acetylamino)-2-deoxy-D-galactopyranose, commonly referred to in the literature as N-acetyl galactosamine. Reference to “GalNAc” or “N-acetyl galactosamine” includes both the β- form: 2-(Acetylamino)-2-deoxy-β -D-galactopyranose and the α-form: 2-(Acetylamino)-2-deoxy-α-D- galactopyranose.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



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

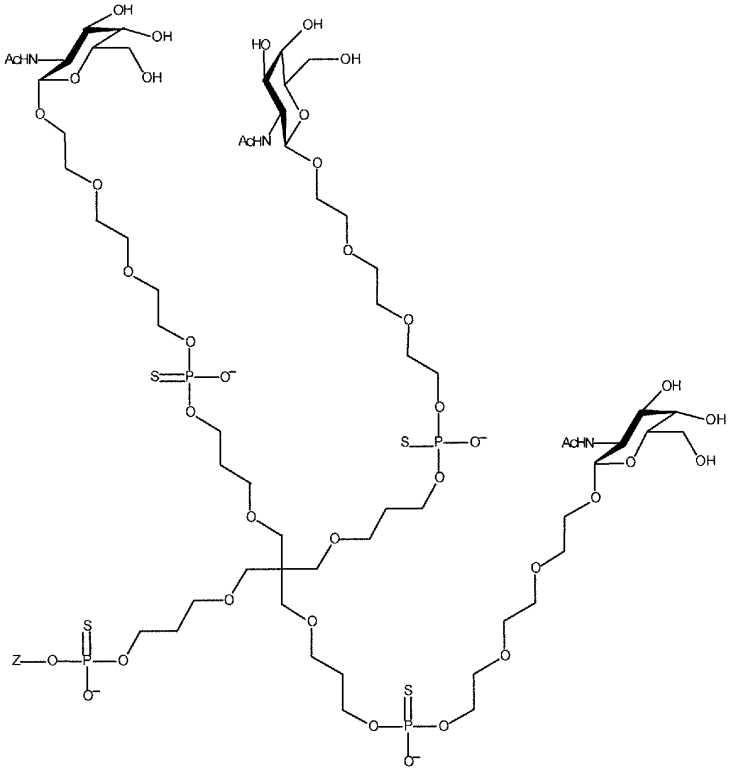


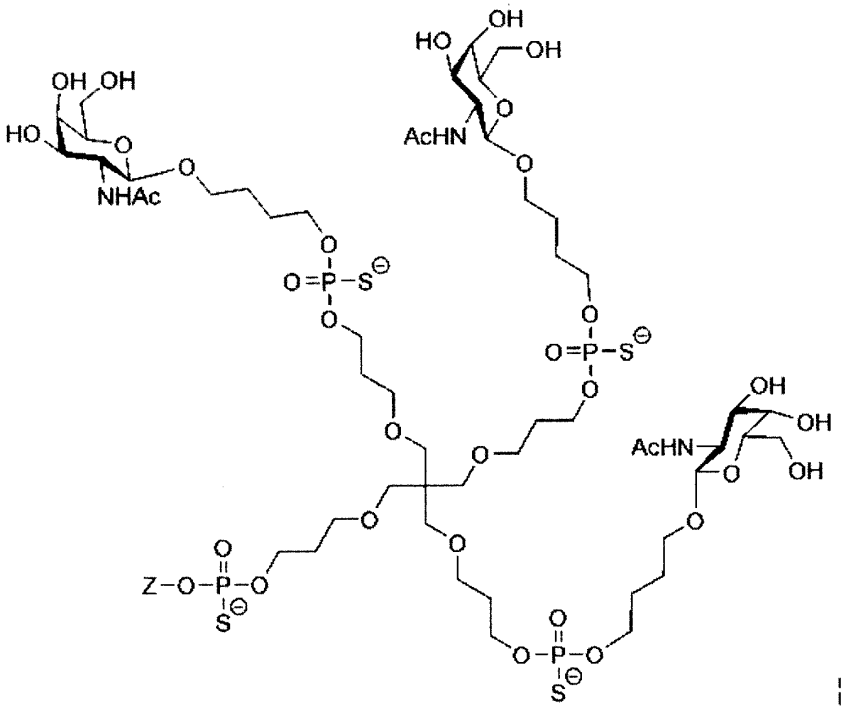
2-(Acetylamino)-2-deoxy-β-D-galactopyranose

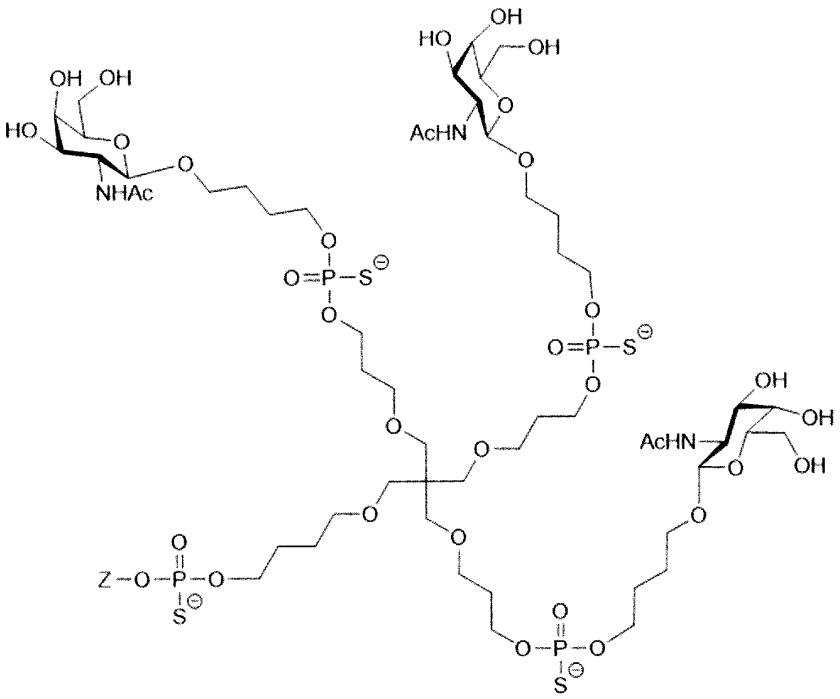


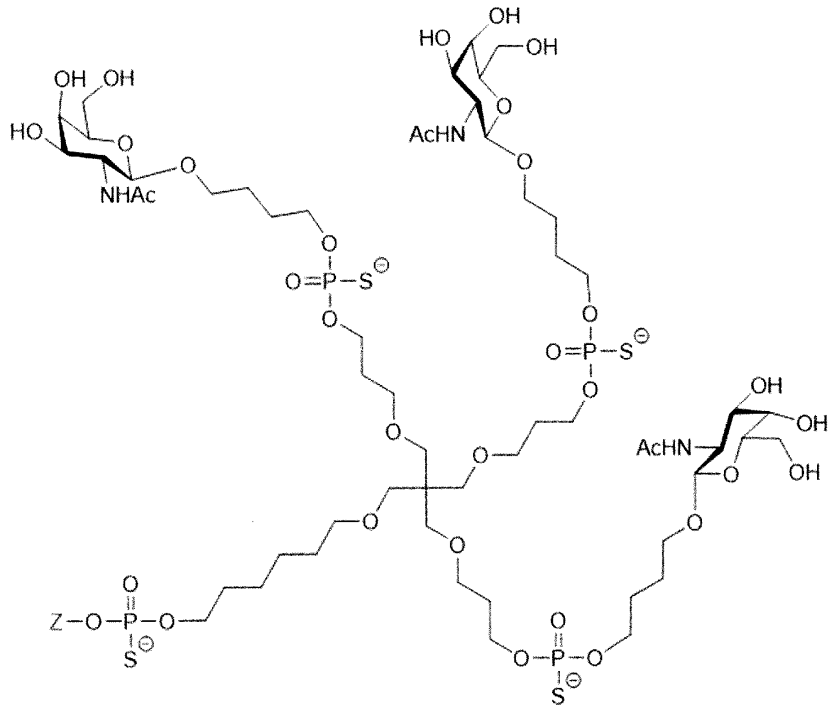
2-(Acetylamino)-2-deoxy-α-D-galactopyranose

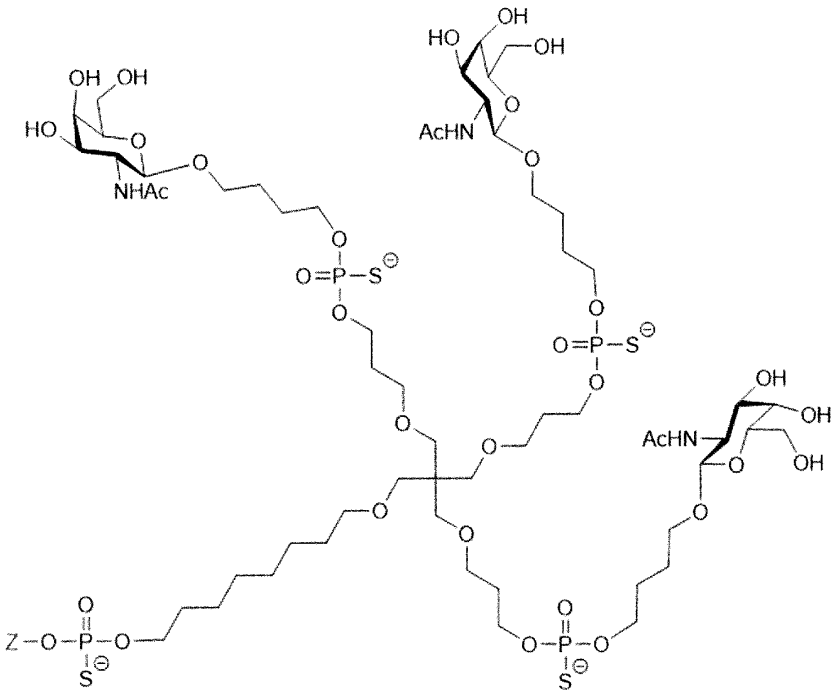
In one aspect, the nucleic acid is a conjugated nucleic acid, wherein the nucleic acid is conjugated to a triantennary ligand with one of the following structures:

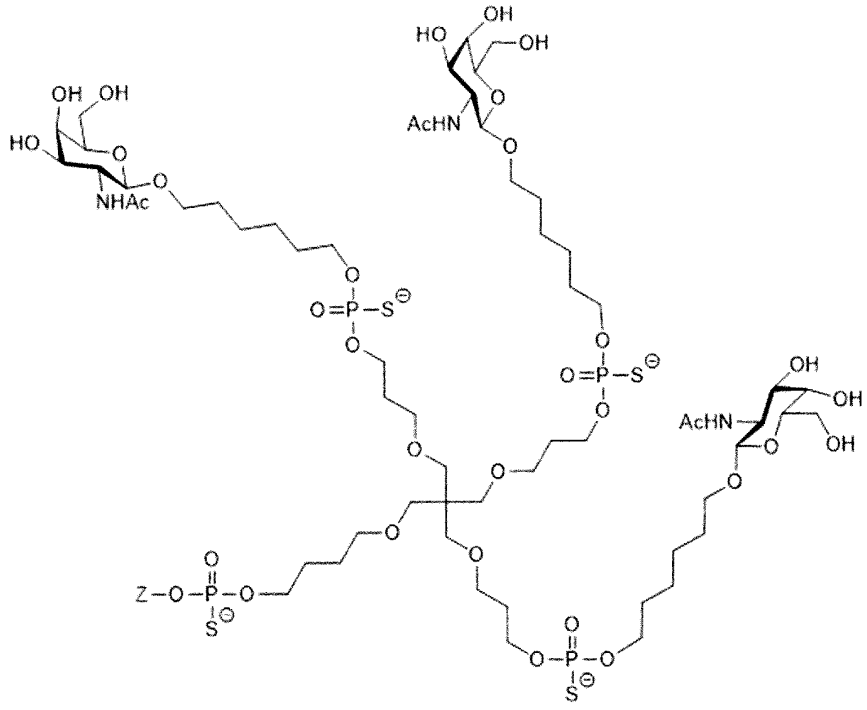


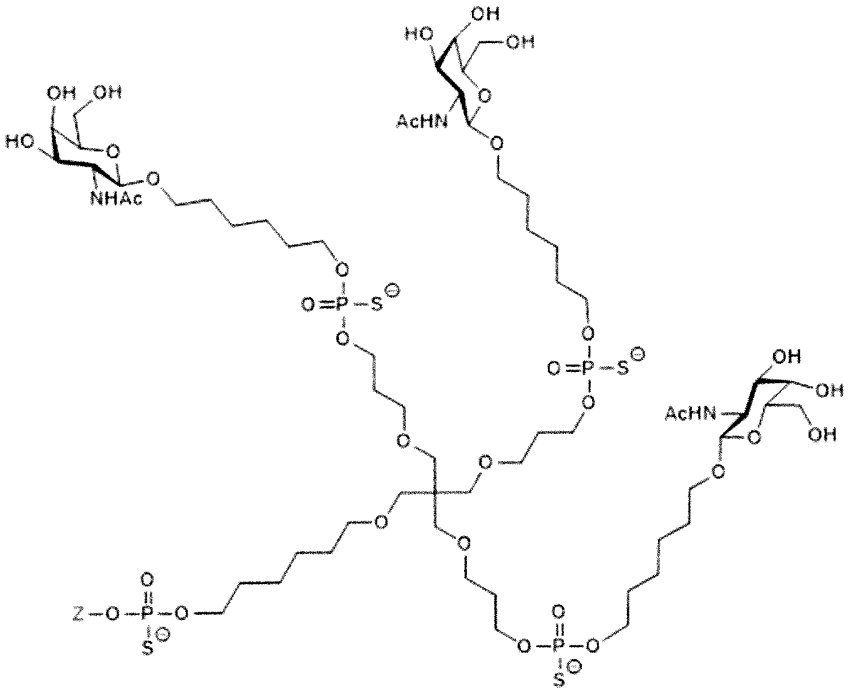


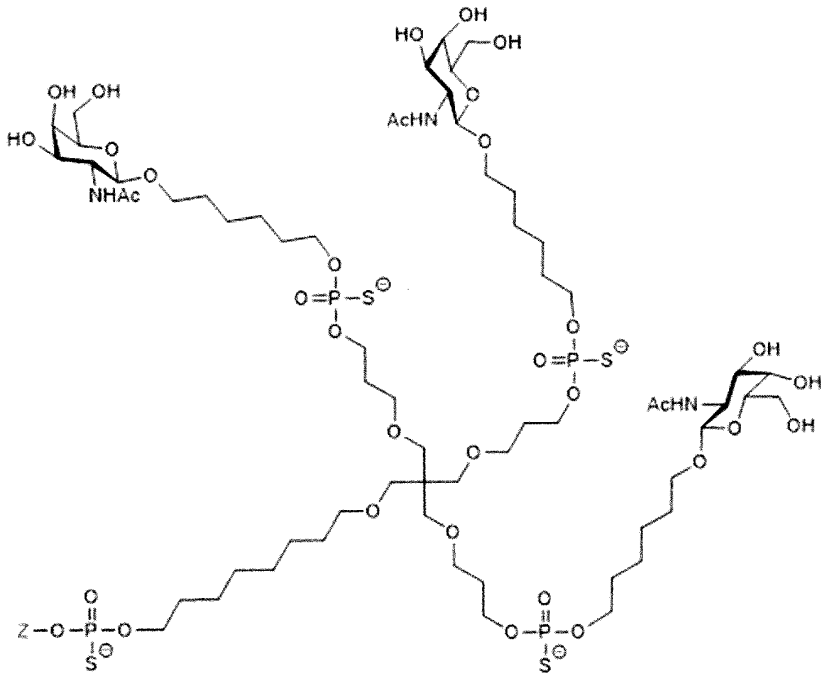


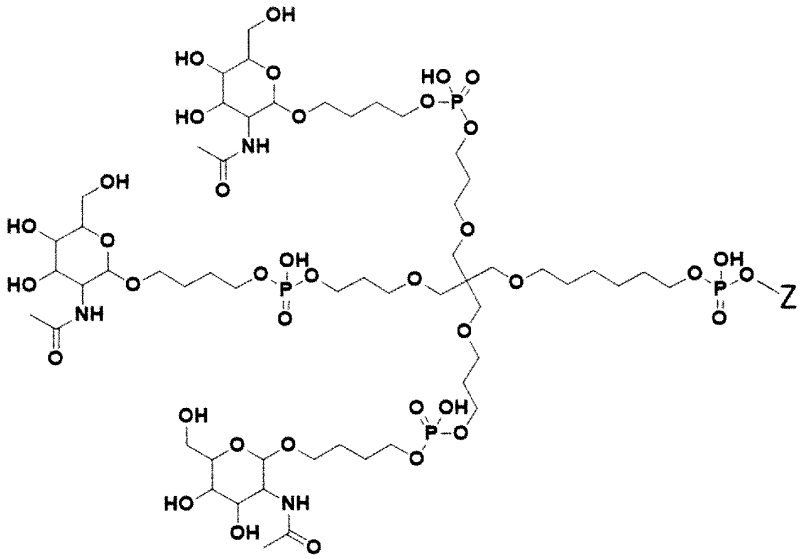






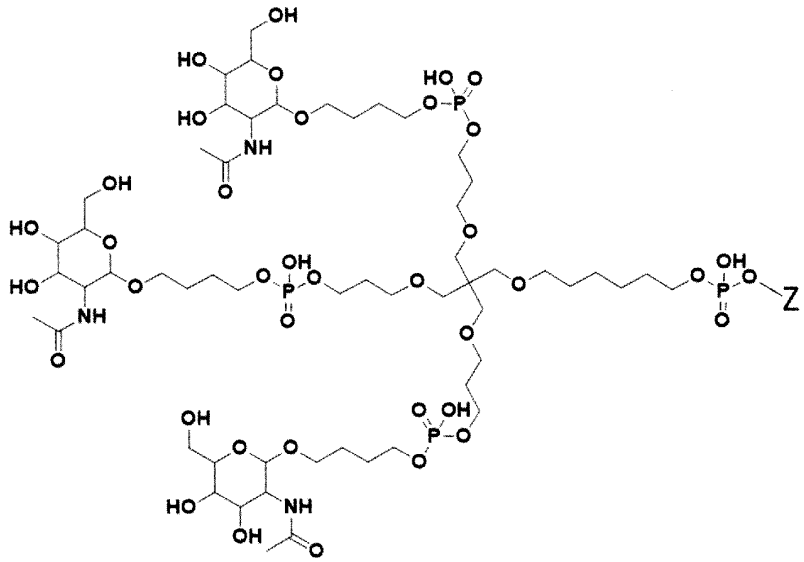






wherein Z is any nucleic acid as defined herein.

Preferably, the nucleic acid is a conjugated nucleic acid, wherein the nucleic acid is conjugated to a triantennary ligand with the following structures:



wherein Z is any nucleic acid as defined herein.

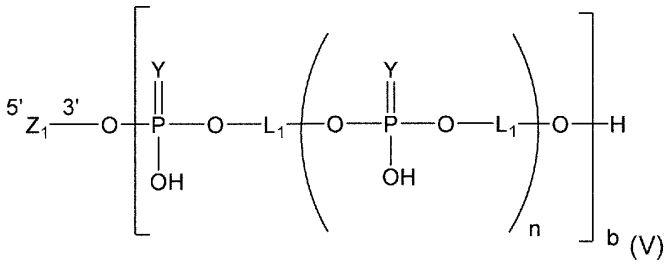
A ligand of formula (II), (III) or (IV) or any one of the triantennary ligands disclosed herein can be attached at the 3'-end of the first (antisense) strand and/or at any of the 3' and/or 5' end of the second (sense) strand. The nucleic acid can comprise more than one ligand of formula (II),

(III) or (IV) or any one of the triantennary ligands disclosed herein. However, a single ligand of formula (II), (III) or (IV) or any one of the triantennary ligands disclosed herein is preferred because a single such ligand is sufficient for efficient targeting of the nucleic acid to the target cells. Preferably in that case, at least the last two, preferably at least the last three and more preferably at least the last four nucleotides at the end of the nucleic acid to which the ligand is attached are linked by a phosphodiester linkage.

Preferably, the 5'-end of the first (antisense) strand is not attached to a ligand of formula (II), (III) or (IV) or any one of the triantennary ligands disclosed herein, since a ligand in this position can potentially interfere with the biological activity of the nucleic acid.

A nucleic acid with a single ligand of formula (II), (III) or (IV) or any one of the triantennary ligands disclosed herein at the 5' end of a strand is easier and therefore cheaper to synthesise than the same nucleic acid with the same ligand at the 3' end. Preferably therefore, a single ligand of any of formulae (II), (III) or (IV) or any one of the triantennary ligands disclosed herein is covalently attached to (conjugated with) the 5' end of the second strand of the nucleic acid.

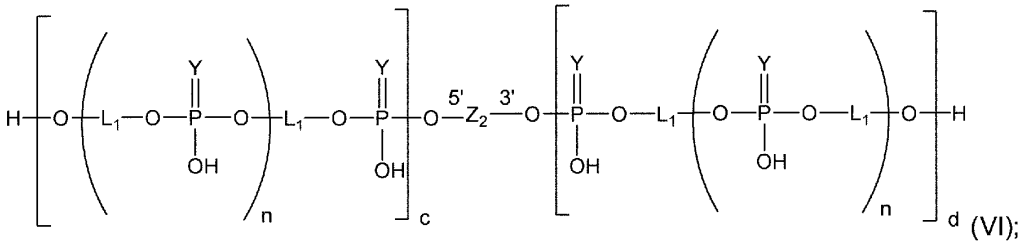
In one aspect, the first strand of the nucleic acid is a compound of formula (V):



(V)

wherein b is preferably 0 or 1; and

the second strand is a compound of formula (VI):



(VI);

wherein:

c and d are independently preferably 0 or 1;

Z_1 and Z_2 are respectively the first and second strand of the nucleic acid;

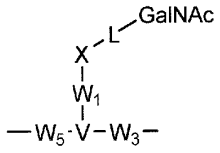
Y is independently O or S;

n is independently 0, 1, 2 or 3; and

L_1 is a linker to which a ligand is attached, wherein L_1 is the same or different in formulae (V) and (VI), and is the same or different within formulae (V) and (VI) when L_1 is present more than once within the same formula, wherein L_1 is preferably of formula (VII);

and wherein b + c + d is preferably 2 or 3.

Preferably, L_1 in formulae (V) and (VI) is of formula (VII):



(VII)

wherein:

L is selected from the group comprising, or preferably consisting of:

$-(CH_2)_r-C(O)-$, wherein r = 2-12;

$-(CH_2-CH_2-O)_s-CH_2-C(O)-$, wherein s = 1-5;

$-(CH_2)_t-CO-NH-(CH_2)_t-NH-C(O)-$, wherein t is independently 1-5;

$-(CH_2)_u-CO-NH-(CH_2)_u-C(O)-$, wherein u is independently 1-5; and

$-(CH_2)_v-NH-C(O)-$, wherein v is 2-12; and

wherein the terminal C(O), if present, is attached to X of formula (VII), or if X is absent, to W_1 of formula (VII), or if W_1 is absent, to V of formula (VII);

W_1, W_3 and W_5 are individually absent or selected from the group comprising, or preferably consisting of:

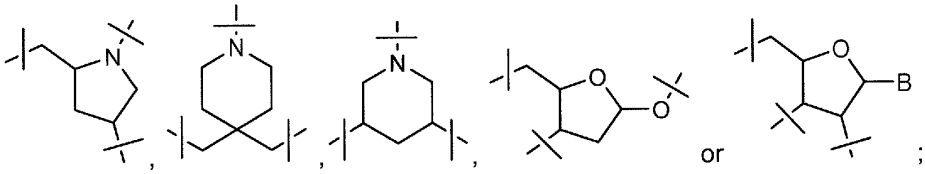
$-(CH_2)_r-$, wherein r = 1-7;

$-(CH_2)_s-O-(CH_2)_s-$, wherein s is independently 0-5;

$-(CH_2)_t-S-(CH_2)_t-$, wherein t is independently 0-5;

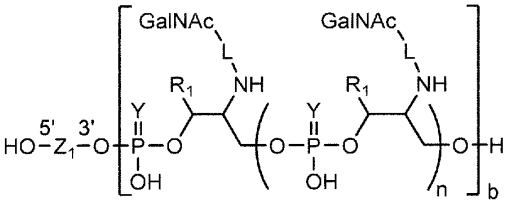
X is absent or is selected from the group comprising, or preferably consisting of: NH, NCH_3 or NC_2H_5;

V is selected from the group comprising, or preferably consisting of:

CH, N,  or ;

wherein B, if present, is a modified or natural nucleobase.

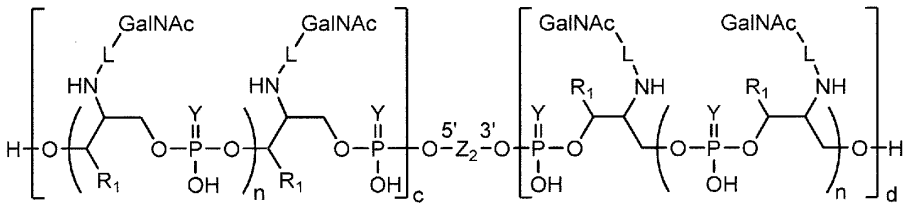
In one aspect, the first strand is a compound of formula (VIII)



(VIII)

wherein b is preferably 0 or 1; and

the second strand is a compound of formula (IX):



(IX);

wherein c and d are independently preferably 0 or 1;

wherein:

Z_1 and Z_2 are respectively the first and second strand of the nucleic acid;

Y is independently O or S;

R_1 is H or methyl;

n is independently preferably 0, 1, 2 or 3; and

L is the same or different in formulae (VIII) and (IX), and is the same or different within formulae (VIII) and (IX) when L is present more than once within the same formula, and is selected from the group comprising, or preferably consisting of:

$-(CH_2)_r-C(O)-$, wherein r = 2-12;

$-(CH_2-CH_2-O)_s-CH_2-C(O)-$, wherein s = 1-5;

$-(CH_2)_t-CO-NH-(CH_2)_t-NH-C(O)-$, wherein t is independently 1-5;

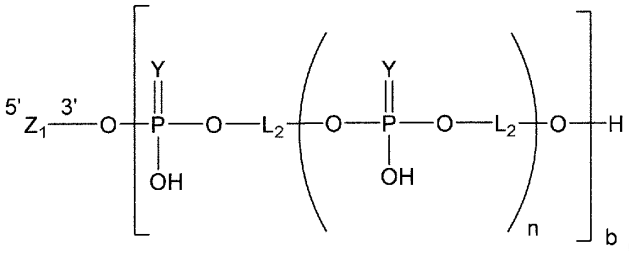
$-(CH_2)_u-CO-NH-(CH_2)_u-C(O)-$, wherein u is independently 1-5; and

$-(CH_2)_v-NH-C(O)-$, wherein v is 2-12; and

wherein the terminal C(O), if present, is attached to the NH group (of the linker, not of the targeting ligand);

and wherein b + c + d is preferably 2 or 3.

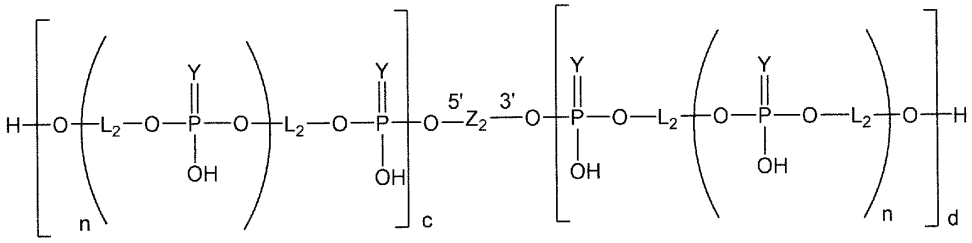
In one aspect, the first strand of the nucleic acid is a compound of formula (X):



(X)

wherein b is preferably 0 or 1; and

the second strand is a compound of formula (XI):



(XI);

wherein:

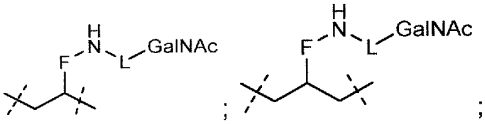
c and d are independently preferably 0 or 1;

Z_1 and Z_2 are respectively the first and second RNA strand of the nucleic;

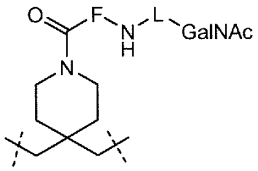
Y is independently O or S;

n is independently preferably 0, 1, 2 or 3; and

L_2 is the same or different in formulae (X) and (XI) and is the same or different in moieties bracketed by b, c and d, and is selected from the group comprising, or preferably consisting of:

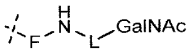


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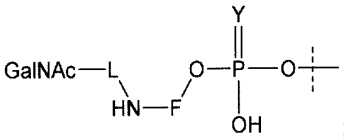
; and 

; or

n is 0 and L_2 is:



and the terminal OH group is absent such that the following moiety is formed:



;

wherein:

F is a saturated branched or unbranched (such as unbranched) C_{1-8}alkyl (e.g. C_{1-6}alkyl) chain wherein one of the carbon atoms is optionally replaced with an oxygen atom provided that said oxygen atom is separated from another heteroatom (e.g. an O or N atom) by at least 2 carbon atoms;

L is the same or different in formulae (X) and (XI) and is selected from the group comprising, or preferably consisting of:

$-(CH_2)_r-C(O)-$, wherein r = 2-12;

$-(CH_2-CH_2-O)_s-CH_2-C(O)-$, wherein s = 1-5;

$-(CH_2)_t-CO-NH-(CH_2)_t-NH-C(O)-$, wherein t is independently 1-5;

$-(CH_2)_u-CO-NH-(CH_2)_u-C(O)-$, wherein u is independently 1-5; and

$-(CH_2)_v-NH-C(O)-$, wherein v is 2-12; and

wherein the terminal C(O), if present, is attached to the NH group (of the linker, not of the targeting ligand);

and wherein b + c + d is preferably 2 or 3.

In one aspect, b is 0, c is 1 and d is 1; b is 1, c is 0 and d is 1; b is 1, c is 1 and d is 0; or b is 1, c is 1 and d is 1 in any of the nucleic acids of formulae (V) and (VI) or (VIII) and (IX) or (X) and (XI). Preferably, b is 0, c is 1 and d is 1; b is 1, c is 0 and d is 1; or b is 1, c is 1 and d is 1. Most preferably, b is 0, c is 1 and d is 1.

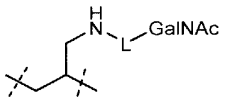
In one aspect, Y is O in any of the nucleic acids of formulae (V) and (VI) or (VIII) and (IX) or (X) and (XI). In another aspect, Y is S. In a preferred aspect, Y is independently selected from O or S in the different positions in the formulae.

In one aspect, R_1 is H or methyl in any of the nucleic acids of formulae (VIII) and (IX). In one aspect, R_1 is H. In another aspect, R_1 is methyl.

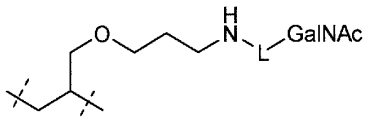
In one aspect, n is 0, 1, 2 or 3 in any of the nucleic acids of formulae (V) and (VI) or (VIII) and (IX) or (X) and (XI). Preferably, n is 0.

Examples of F moieties in any of the nucleic acids of formulae (X) and (XI) include $(CH_2)_{1-6}$ e.g. $(CH_2)_{1-4}$ e.g. CH_2, $(CH_2)_4$, $(CH_2)_5$ or $(CH_2)_6$, or $CH_2O(CH_2)_{2-3}$, e.g. $CH_2O(CH_2)CH_3$.

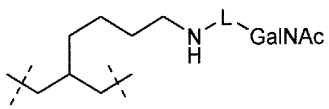
In one aspect, L_2 in formulae (X) and (XI) is:



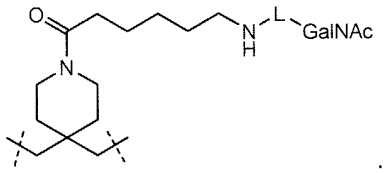
In one aspect, L_2 is:



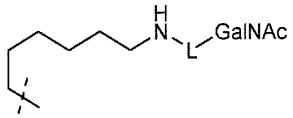
In one aspect, L_2 is:



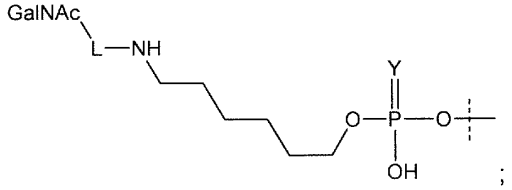
In one aspect, L_2 is:



In one aspect, n is 0 and L_2 is:



and the terminal OH group is absent such that the following moiety is formed:



;

wherein Y is O or S.

In one aspect, L in the nucleic acids of formulae (V) and (VI) or (VIII) and (IX) or (X) and (XI), is selected from the group comprising, or preferably consisting of:

$-(CH_2)_r-C(O)-$, wherein r = 2-12;

$-(CH_2-CH_2-O)_s-CH_2-C(O)-$, wherein s = 1-5;

$-(CH_2)_t-CO-NH-(CH_2)_t-NH-C(O)-$, wherein t is independently 1-5;

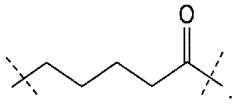
$-(CH_2)_u-CO-NH-(CH_2)_u-C(O)-$, wherein u is independently 1-5; and

$-(CH_2)_v-NH-C(O)-$, wherein v is 2-12;

wherein the terminal C(O) is attached to the NH group.

Preferably, L is $-(CH_2)_r-C(O)-$, wherein r = 2-12, more preferably r = 2-6 even more preferably, r = 4 or 6 e.g. 4.

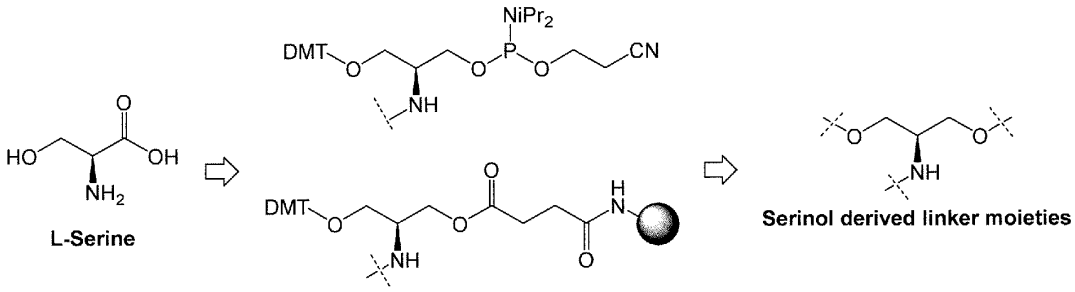
Preferably, L is:



Within the moiety bracketed by b, c and d, L_2 in the nucleic acids of formulae (X) and (XI) is typically the same. Between moieties bracketed by b, c and d, L_2 may be the same or different.

In an embodiment, L_2 in the moiety bracketed by c is the same as the L_2 in the moiety bracketed by d. In an embodiment, L_2 in the moiety bracketed by c is not the same as L_2 in the moiety bracketed by d. In an embodiment, the L_2 in the moieties bracketed by b, c and d is the same, for example when the linker moiety is a serinol-derived linker moiety.

Serinol derived linker moieties may be based on serinol in any stereochemistry i.e. derived from L-serine isomer, D-serine isomer, a racemic serine or other combination of isomers. In a preferred aspect of the invention, the serinol-GalNAc moiety (SerGN) has the following stereochemistry:



(S)-Serinol building blocks

i.e. is based on an (S)-serinol-amidite or (S)-serinol succinate solid supported building block derived from L-serine isomer.

In a preferred aspect, the first strand of the nucleic acid is a compound of formula (VIII) and the second strand of the nucleic acid is a compound of formula (IX), wherein:

b is 0;

c and d are 1,

n is 0,

Z_1 and Z_2 are respectively the first and second strand of the nucleic acid,

Y is S,

R_1 is H, and

L is $-(CH_2)_4-C(O)-$, wherein the terminal C(O) of L is attached to the N atom of the linker (ie not a possible N atom of a targeting ligand).

In another preferred aspect, the first strand of the nucleic acid is a compound of formula (V) and the second strand of the nucleic acid is a compound of formula (VI), wherein:

b is 0,

c and d are 1,

n is 0,

Z_1 and Z_2 are respectively the first and second strand of the nucleic acid,

Y is S,

L_1 is of formula (VII), wherein:

W_1 is $-CH_2-O-(CH_2)_3-$,

W_3 is $-CH_2-$,

W_5 is absent,

V is CH,

X is NH, and

L is $-(CH_2)_4-C(O)-$ wherein the terminal C(O) of L is attached to the N atom of X in formula (VII).

In another preferred aspect, the first strand of the nucleic acid is a compound of formula (V) and the second strand of the nucleic acid is a compound of formula (VI), wherein:

b is 0,

c and d are 1,

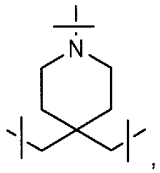
n is 0,

Z_1 and Z_2 are respectively the first and second strand of the nucleic acid,

Y is S,

L_1 is of formula (VII), wherein:

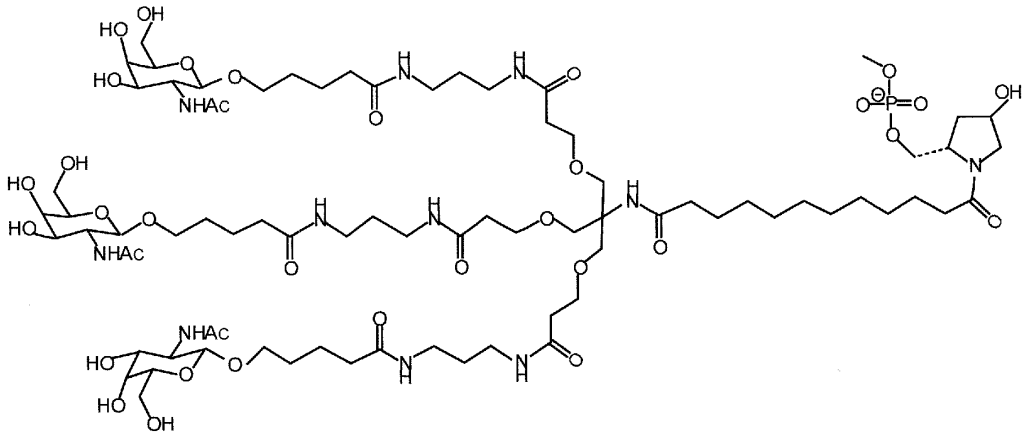
W_1, W_3 and W_5 are absent,

V is ,

X is absent, and

L is $-(CH_2)_4-C(O)-NH-(CH_2)_5-C(O)-$, wherein the terminal C(O) of L is attached to the N atom of V in formula (VII).

In one aspect, the nucleic acid is conjugated to a triantennary ligand with the following structure:



wherein the nucleic acid is conjugated to the ligand via the phosphate group of the ligand a) to the last nucleotide at the 5' end of the second strand; b) to the last nucleotide at the 3' end of the second strand; or c) to the last nucleotide at the 3' end of the first strand.

In one aspect of the nucleic acid, the cells that are targeted by the nucleic acid with a ligand are hepatocytes.

In any one of the above ligands where GalNAc is present, the GalNAc may be substituted for any other targeting ligand, such as those mentioned herein, in particular mannose, galactose, glucose, glucosamine and fucose.

A particularly preferred embodiment is a nucleic acid wherein the first strand comprises or consists of SEQ ID NO: 409 and the second strand optionally comprises or consists of SEQ ID NO: 423. This nucleic acid can be further conjugated to a ligand. Even more preferred is a nucleic acid wherein the first strand comprises or consists of SEQ ID NO: 409 and the second strand optionally comprises or consists of SEQ ID NO: 410. Most preferred is an siRNA that consists of SEQ ID NO: 409 and SEQ ID NO: 410. One aspect of the invention is EJ0020.

An alternative particularly preferred embodiment is a nucleic acid wherein the first strand comprises or consists of SEQ ID NO: 420 and the second strand optionally comprises or consists of SEQ ID NO: 424. This nucleic acid can be further conjugated to a ligand. Even more preferred is a nucleic acid wherein the first strand comprises or consists of SEQ ID NO: 420 and the second strand optionally comprises or consists of SEQ ID NO: 421. Most preferred is an siRNA that consists of SEQ ID NO: 420 and SEQ ID NO: 421. One aspect of the invention is EV0212.

An alternative particularly preferred embodiment is a nucleic acid wherein the first strand comprises or consists of SEQ ID NO: 417 and the second strand optionally comprises or consists of SEQ ID NO: 425. This nucleic acid can be further conjugated to a ligand. Even more preferred is a nucleic acid wherein the first strand comprises or consists of SEQ ID NO: 417 and the second strand optionally comprises or consists of SEQ ID NO: 418. Most preferred is an siRNA that consists of SEQ ID NO: 417 and SEQ ID NO: 418. One aspect of the invention is EV0210. Preliminary NHP data show that this siRNA is surprisingly potent *in vivo* in higher species.

In one aspect, the nucleic acid is conjugated to a ligand that comprises a lipid, and more preferably, a ligand that comprises a cholesterol.

Compositions, uses and methods

The present invention also provides compositions comprising a nucleic acid of the invention. The nucleic acids and compositions may be used as medicaments or as diagnostic agents, alone or in combination with other agents. For example, one or more nucleic acid(s) of the invention can be combined with a delivery vehicle (e.g., liposomes) and/or excipients, such as carriers, diluents. Other agents such as preservatives and stabilizers can also be added. Pharmaceutically acceptable salts or solvates of any of the nucleic acids of the invention are likewise within the scope of the present invention. Methods for the delivery of nucleic acids are known in the art and within the knowledge of the person skilled in the art.

Compositions disclosed herein are particularly pharmaceutical compositions. Such compositions are suitable for administration to a subject.

In one aspect, the composition comprises a nucleic acid disclosed herein, or a pharmaceutically acceptable salt or solvate thereof, and a solvent (preferably water) and/or a delivery vehicle and/or a physiologically acceptable excipient and/or a carrier and/or a salt and/or a diluent and/or a buffer and/or a preservative.

Pharmaceutically acceptable carriers or diluents include those used in formulations suitable for oral, rectal, nasal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, and transdermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Subcutaneous or transdermal modes of administration may be particularly suitable for the compounds described herein.

The therapeutically effective amount of a nucleic acid of the present invention will depend on the route of administration, the type of mammal being treated, and the physical characteristics of the specific mammal under consideration. These factors and their relationship to determining this amount are well known to skilled practitioners in the medical arts. This amount and the method of administration can be tailored to achieve optimal efficacy, and may depend on such factors as weight, diet, concurrent medication and other factors, well known to those skilled in the medical arts. The dosage sizes and dosing regimen most appropriate for human use may be guided by the results obtained by the present invention, and may be confirmed in properly designed clinical trials.

An effective dosage and treatment protocol may be determined by conventional means, starting with a low dose in laboratory animals and then increasing the dosage while monitoring the effects, and systematically varying the dosage regimen as well. Numerous factors may be taken into consideration by a clinician when determining an optimal dosage for a given subject. Such considerations are known to the skilled person.

Nucleic acids of the present invention, or salts thereof, may be formulated as pharmaceutical compositions prepared for storage or administration, which typically comprise a therapeutically effective amount of a nucleic acid of the invention, or a salt thereof, in a pharmaceutically acceptable carrier.

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

In one aspect, the composition comprises a nucleic acid disclosed herein and a further therapeutic agent selected from the group comprising an oligonucleotide, a small molecule, a monoclonal antibody, a polyclonal antibody and a peptide. Preferably, the further therapeutic agent is an agent that targets, preferably inhibits the expression or the activity, of the complement component C3 or of another element, such as a protein, of the immune system or more specifically of the complement pathway. Preferably, the further therapeutic agent is one of the following: a) a peptide that inhibits the expression or activity of one of the components of the complement pathway, preferably either C3 or C5 or one of their subunits;

b) an antibody that specifically binds under physiological conditions to one of the components of the complement pathway, preferably either C3 or C5 or one of their subunits; c) Eculizumab or an antigen-binding derivative thereof.

Eculizumab is a humanised monoclonal antibody that specifically binds to the complement component C5 and is commercialised under the trade name SOLIRIS®. It specifically binds the complement component C5 with high affinity and inhibits cleavage of C5 to C5a and C5b. The antibody is for example described in the patent EP 0 758 904 B1 and its family members.

In certain embodiments, two or more nucleic acids of the invention with different sequences may be administered simultaneously or sequentially.

In another aspect, the present invention provides a composition, e.g., a pharmaceutical composition, comprising one or a combination of different nucleic acids of the invention and at least one pharmaceutically acceptable carrier.

Dosage levels for the medicament and compositions of the invention can be determined by those skilled in the art by experimentation. In one aspect, a unit dose may contain between about 0.01 mg/kg and about 100 mg/kg body weight of nucleic acid or conjugated nucleic acid. Alternatively, the dose can be from 10 mg/kg to 25 mg/kg body weight, or 1 mg/kg to 10 mg/kg body weight, or 0.05 mg/kg to 5 mg/kg body weight, or 0.1 mg/kg to 5 mg/kg body weight, or 0.1 mg/kg to1 mg/kg body weight, or 0.1 mg/kg to 0.5 mg/kg body weight, or 0.5 mg/kg to 1 mg/kg body weight. Alternatively, the dose can be from about 0.5 mg/kg to about 10 mg/kg body weight, or about 0.6 mg/kg to about 8 mg/kg body weight, or about 0.7 mg/kg to about 7 mg/kg body weight, or about 0.8 mg/kg to about 6 mg/kg body weight, or about 0.9 mg/kg to about 5.5 mg/kg body weight, or about 1 mg/kg to about 5 mg/kg body weight, or about 1 mg/kg body weight, or about 3 mg/kg body weight, or about 5 mg/kg body weight, wherein "about" is a deviation of up to 30%, preferably up to 20%, more preferably up to 10%, yet more preferably up to 5% and most preferably 0% from the indicated value. Dosage levels may also be calculated via other parameters such as, e.g., body surface area.

A particularly preferred embodiment is a nucleic acid wherein the first strand comprises or consists of SEQ ID NO: 409 and the second strand optionally comprises or consists of SEQ ID NO: 423. This nucleic acid can be further conjugated to a ligand. Even more preferred is a nucleic acid wherein the first strand comprises or consists of SEQ ID NO: 409 and the second strand optionally comprises or consists of SEQ ID NO: 410. Most preferred is an siRNA that consists of SEQ ID NO: 409 and SEQ ID NO: 410. One aspect of the invention is EJ0020. A

dose unit of these nucleic acids preferably comprises about 1 mg/kg to about 5 mg/kg body weight, or about 1 mg/kg to about 3 mg/kg body weight, or about 1 mg/kg body weight, or about 3 mg/kg body weight, or about 5 mg/kg body weight. The C3 mRNA level in the liver and/or the C3 protein level in the plasma or blood of a subject treated by a dose unit of the nucleic acid is preferably decreased at the time point of maximum effect by at least 30%, at least 40%, at least 50%, at least 60% or at least 70% as compared to a control that was not treatment with the nucleic acid or treated with a control nucleic acid under comparable conditions.

The dosage and frequency of administration may vary depending on whether the treatment is therapeutic or prophylactic (e.g., preventative), and may be adjusted during the course of treatment. In certain prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a relatively long period of time. Some subjects may continue to receive treatment over their lifetime. In certain therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient may be switched to a suitable prophylactic dosing regimen.

Actual dosage levels of a nucleic acid of the invention alone or in combination with one or more other active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without causing deleterious side effects to the subject or patient. A selected dosage level will depend upon a variety of factors, such as pharmacokinetic factors, including the activity of the particular nucleic acid or composition employed, the route of administration, the time of administration, the rate of excretion of the particular nucleic acid being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the subject or patient being treated, and similar factors well known in the medical arts.

The pharmaceutical composition may be a sterile injectable aqueous suspension or solution, or in a lyophilized form.

The pharmaceutical compositions can be in unit dosage form. In such form, the composition is divided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of the

preparations, for example, packeted tablets, capsules, and powders in vials or ampoules. The unit dosage form can also be a capsule, cachet, or tablet itself, or it can be the appropriate number of any of these packaged forms. It may be provided in single dose injectable form, for example in the form of a pen. Compositions may be formulated for any suitable route and means of administration.

The pharmaceutical compositions and medicaments of the present invention may be administered to a mammalian subject in a pharmaceutically effective dose. The mammal may be selected from a human, a non-human primate, a simian or prosimian, a dog, a cat, a horse, cattle, a pig, a goat, a sheep, a mouse, a rat, a hamster, a hedgehog and a guinea pig, or other species of relevance. On this basis, "C3" as used herein denotes nucleic acid or protein in any of the above-mentioned species, if expressed therein naturally or artificially, but preferably this wording denotes human nucleic acids or proteins.

Pharmaceutical compositions of the invention may be administered alone or in combination with one or more other therapeutic or diagnostic agents. A combination therapy may include a nucleic acid of the present invention combined with at least one other therapeutic agent selected based on the particular patient, disease or condition to be treated. Examples of other such agents include, *inter alia*, a therapeutically active small molecule or polypeptide, a single chain antibody, a classical antibody or fragment thereof, or a nucleic acid molecule which modulates gene expression of one or more additional genes, and similar modulating therapeutics which may complement or otherwise be beneficial in a therapeutic or prophylactic treatment regimen.

Pharmaceutical compositions are typically sterile and stable under the conditions of manufacture and storage. The composition may be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier may be a solvent or dispersion medium containing, for example, water, alcohol such as ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), or any suitable mixtures. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by use of surfactants according to formulation chemistry well known in the art. In certain embodiments, isotonic agents, e.g., sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride may be desirable in the composition. Prolonged absorption of injectable compositions may be brought about by including in the composition an agent that delays absorption for example, monostearate salts and gelatine.

One aspect of the invention is a nucleic acid or a composition disclosed herein for use as a medicament. The nucleic acid or composition is preferably for use in the prevention, decrease of the risk of suffering from, or treatment of a disease, disorder or syndrome.

The present invention provides a nucleic acid for use, alone or in combination with one or more additional therapeutic agents in a pharmaceutical composition, for treatment or prophylaxis of conditions, diseases and disorders responsive to inhibition of C3 expression.

One aspect of the invention is the use of a nucleic acid or a composition as disclosed herein in the prevention, decrease of the risk of suffering from, or treatment of a disease, disorder or syndrome.

Nucleic acids and pharmaceutical compositions of the invention may be used in the treatment of a variety of conditions, disorders or diseases. Treatment with a nucleic acid of the invention preferably leads to *in vivo* C3 depletion, preferably in the liver and/or in blood. As such, nucleic acids of the invention, and compositions comprising them, will be useful in methods for treating a variety of pathological disorders in which inhibiting the expression of C3 may be beneficial. The present invention provides methods for treating a disease, disorder or syndrome comprising the step of administering to a subject in need thereof a therapeutically effective amount of a nucleic acid of the invention.

The invention thus provides methods of treatment or prevention of a disease, disorder or syndrome, the method comprising the step of administering to a subject (e.g., a patient) in need thereof a therapeutically effective amount of a nucleic acid or pharmaceutical composition comprising a nucleic acid of the invention.

The most desirable therapeutically effective amount is an amount that will produce a desired efficacy of a particular treatment selected by one of skill in the art for a given subject in need thereof. This amount will vary depending upon a variety of factors understood by the skilled worker, including but not limited to the characteristics of the therapeutic compound (including activity, pharmacokinetics, pharmacodynamics, and bioavailability), the physiological condition of the subject (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication), the nature of the pharmaceutically acceptable carrier or carriers in the formulation, and the route of administration. One skilled in the clinical and pharmacological arts will be able to determine a therapeutically effective amount through experimentation, namely by monitoring a subject's response to administration of a compound and adjusting the dosage accordingly. See, e.g., Remington: The Science and

Practice of Pharmacy 21st Ed., Univ. of Sciences in Philadelphia (USIP), Lippincott Williams & Wilkins, Philadelphia, PA, 2005.

In certain embodiments, nucleic acids and pharmaceutical compositions of the invention may be used to treat or prevent a disease, disorder or syndrome.

In certain embodiments, the present invention provides methods for treating a disease, disorder or syndrome in a mammalian subject, such as a human, the method comprising the step of administering to a subject in need thereof a therapeutically effective amount of a nucleic acid as disclosed herein.

Administration of a "therapeutically effective dosage" of a nucleic acid of the invention may result in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction.

Nucleic acids of the invention may be beneficial in treating or diagnosing a disease, disorder or syndrome that may be diagnosed or treated using the methods described herein. Treatment and diagnosis of other diseases, disorders or syndromes are also considered to fall within the scope of the present invention.

One aspect of the invention is a method of preventing, decreasing the risk of suffering from, or treating a disease, disorder or syndrome comprising administering a pharmaceutically effective dose or amount a nucleic acid or a composition disclosed herein to an individual in need of treatment, preferably wherein the nucleic acid or composition is administered to the subject subcutaneously, intravenously or by oral, rectal, pulmonary, intramuscular or intraperitoneal administration. Preferably, it is administered subcutaneously.

The disease, disorder or syndrome to be prevented, or treated with a nucleic acid or composition disclosed herein is preferably a complement-mediated disease, disorder or syndrome or a disease disorder or syndrome associated with the complement pathway.

The disease, disorder or syndrome to be prevented or treated with a nucleic acid or composition disclosed herein is preferably associated with aberrant activation and/or over-activation (hyper-activation) of the complement pathway and/or with over-expression or ectopic expression or localisation or accumulation of the complement component C3. One example of a disease that involves accumulation of C3 is C3 Glomerulopathy (C3G). In this disease, C3

accumulates in the kidney glomeruli. The aberrant or over activation of the complement pathway to be prevented or treated can have genetic causes or can be acquired. Preferably, the disease, disorder or syndrome to be prevented or treated is C3 Glomerulopathy (C3G).

The disease, disorder or syndrome to be prevented or treated with a nucleic acid or composition disclosed herein is preferably a) selected from the group comprising, and preferably consisting of C3 Glomerulopathy (C3G), Paroxysmal Nocturnal Hemoglobinuria (PNH), atypical Hemolytic Uremic Syndrome (aHUS), Lupus nephritis, IgA nephropathy (IgA N), Cold Agglutinin Disease (CAD), Myasthenia gravis (MG), Primary Membranous Nephropathy, Immune Complex-mediated Glomerulonephritis (IC-mediated GN), post-Infectious Glomerulonephritis (PIGN), Systemic Lupus Erythematosus (SLE), Ischemia/reperfusion injury, age-related macular degeneration (AMD), Rheumatoid arthritis (RA), antineutrophil Cytoplasmic Autoantibodies-associated Vasculitis (ANCA-AV), dysbiotic periodontal Disease, Malarial Anaemia, Neuromyelitis Optica, Post-HCT / Solid Organ Transplant (TMAs), Guillain-Barré Syndrome, Membranous Glomerulonephritis, Thrombotic Thrombocytopenic Purpura and sepsis; or b) selected from the group comprising, or preferably consisting of C3 Glomerulopathy (C3G), Paroxysmal Nocturnal Hemoglobinuria (PNH), atypical Hemolytic Uremic Syndrome (aHUS), Lupus nephritis, IgA nephropathy (IgA N) and Primary Membranous Nephropathy; or c) selected from the group comprising, or preferably consisting of C3 Glomerulopathy (C3G), antineutrophil Cytoplasmic Autoantibodies-associated Vasculitis (ANCA-AV), atypical Hemolytic Uremic Syndrome (aHUS), Cold Agglutinin Disease (CAD), Myasthenia gravis (MG), IgA nephropathy (IgA N), Paroxysmal Nocturnal Hemoglobinuria (PNH); d) selected from the group comprising, or preferably consisting of C3 Glomerulopathy (C3G), Cold Agglutinin Disease (CAD), Myasthenia gravis (MG), Neuromyelitis Optica, atypical Hemolytic Uremic Syndrome (aHUS), antineutrophil Cytoplasmic Autoantibodies-associated Vasculitis (ANCA-AV), IgA nephropathy (IgA N), Post-HCT / Solid Organ Transplant (TMAs), Guillain-Barré Syndrome, Paroxysmal Nocturnal Hemoglobinuria (PNH), Membranous Glomerulonephritis, Lupus nephritis and Thrombotic Thrombocytopenic Purpura; e) C3 Glomerulopathy (C3G), Cold Agglutinin Disease (CAD) and IgA nephropathy (IgA N) or f) it is C3 Glomerulopathy (C3G). The subjects to be treated with a nucleic acid or composition according to the invention are preferably subjects that suffer from one of these diseases, disorders or syndromes.

A nucleic acid or compositions disclosed herein may be for use in a regimen comprising treatments once or twice weekly, every week, every two weeks, every three weeks, every four weeks, every five weeks, every six weeks, every seven weeks, every eight weeks, every nine weeks, every ten weeks, every eleven weeks, every twelve weeks, every three months, every

four months, every five months, every six months or in regimens with varying dosing frequency such as combinations of the before-mentioned intervals. The nucleic acid or composition may be for use subcutaneously, intravenously or using any other application routes such as oral, rectal, pulmonary, or intraperitoneal. Preferably, it is for use subcutaneously.

In cells and/or subjects treated with or receiving a nucleic acid or composition as disclosed herein, the C3 expression may be inhibited compared to untreated cells and/or subjects by a range from 15% up to 100% but at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% or intermediate values. The level of inhibition may allow treatment of a disease associated with C3 expression or overexpression or complement over-activation, or may serve to further investigate the functions and physiological roles of the C3 gene products. The level of inhibition is preferably measured in the liver or in the blood or in the kidneys, preferably in the blood, of the subject treated with the nucleic acid or composition.

One aspect is the use of a nucleic acid or composition as disclosed herein in the manufacture of a medicament for treating a disease, disorder or syndromes, such as those as listed above or additional pathologies associated with elevated levels of C3, preferably in the blood or in the kidneys, or over activation of the complement pathway, or additional therapeutic approaches where inhibition of C3 expression is desired. A medicament is a pharmaceutical composition.

Each of the nucleic acids of the invention and pharmaceutically acceptable salts and solvates thereof constitutes an individual embodiment of the invention.

Also included in the invention is a method of treating or preventing a disease, disorder or syndrome, such as those listed above, comprising administration of a composition comprising a nucleic acid or composition as described herein, to an individual in need of treatment (to improve such pathologies). The nucleic acid or composition may be administered in a regimen comprising treatments twice every week, once every week, every two weeks, every three weeks, every four weeks, every five weeks, every six weeks, every seven weeks, or every eight to twelve or more weeks or in regimens with varying dosing frequency such as combinations of the before-mentioned intervals. The nucleic acid or conjugated nucleic acid may be for use subcutaneously or intravenously or other application routes such as oral, rectal or intraperitoneal.

A nucleic acid of the invention may be administered by any appropriate administration pathway known in the art, including but not limited to aerosol, enteral, nasal, ophthalmic, oral, parenteral, rectal, vaginal, or transdermal (e.g., topical administration of a cream, gel or ointment, or by means of a transdermal patch). "Parenteral administration" is typically associated with injection at or in communication with the intended site of action, including infraorbital, infusion, intraarterial, intracapsular, intracardiac, intradermal, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intrauterine, intravenous, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal administration.

The use of a chemical modification pattern of the nucleic acids confers nuclease stability in serum and makes for example subcutaneous application route feasible.

Solutions or suspensions used for intradermal or subcutaneous application typically include one or more of: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates; and/or tonicity adjusting agents such as, e.g., sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide, or buffers with citrate, phosphate, acetate and the like. Such preparations may be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Sterile injectable solutions may be prepared by incorporating a nucleic acid in the required amount in an appropriate solvent with one or a combination of ingredients described above, as required, followed by sterilization microfiltration. Dispersions may be prepared by incorporating the active compound into a sterile vehicle that contains a dispersion medium and optionally other ingredients, such as those described above. In the case of sterile powders for the preparation of sterile injectable solutions, the methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient in addition to any additional desired ingredient from a sterile-filtered solution thereof.

When a therapeutically effective amount of a nucleic acid of the invention is administered by, e.g., intravenous, cutaneous or subcutaneous injection, the nucleic acid will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. Methods for preparing parenterally acceptable solutions, taking into consideration appropriate pH, isotonicity, stability, and the like, are within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection will contain, in addition to a nucleic acid, an isotonic

vehicle such as sodium chloride injection, Ringer's injection, dextrose injection, dextrose and sodium chloride injection, lactated Ringer's injection, or other vehicle as known in the art. A pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives well known to those of skill in the art.

The amount of nucleic acid which can be combined with a carrier material to produce a single dosage form will vary depending on a variety of factors, including the subject being treated, and the particular mode of administration. In general, it will be an amount of the composition that produces an appropriate therapeutic effect under the particular circumstances. Generally, out of one hundred percent, this amount will range from about 0.01% to about 99% of nucleic acid, from about 0.1% to about 70%, or from about 1% to about 30% of nucleic acid in combination with a pharmaceutically acceptable carrier.

The nucleic acid may be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a dose may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by the particular circumstances of the therapeutic situation, on a case by case basis. It is especially advantageous to formulate parenteral compositions in dosage unit forms for ease of administration and uniformity of dosage when administered to the subject or patient. As used herein, a dosage unit form refers to physically discrete units suitable as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce a desired therapeutic effect. The specification for the dosage unit forms of the invention depend on the specific characteristics of the active compound and the particular therapeutic effect(s) to be achieved and the treatment and sensitivity of any individual patient.

The nucleic acid or composition of the present invention can be produced using routine methods in the art including chemical synthesis, such as solid phase chemical synthesis.

Nucleic acids or compositions of the invention may be administered with one or more of a variety of medical devices known in the art. For example, in one embodiment, a nucleic acid of the invention may be administered with a needleless hypodermic injection device. Examples of well-known implants and modules useful in the present invention are in the art, including e.g., implantable micro-infusion pumps for controlled rate delivery; devices for administering through the skin; infusion pumps for delivery at a precise infusion rate; variable flow implantable infusion devices for continuous drug delivery; and osmotic drug delivery systems. These and other such implants, delivery systems, and modules are known to those skilled in the art.

In certain embodiments, the nucleic acid or composition of the invention may be formulated to ensure a desired distribution *in vivo*. To target a therapeutic compound or composition of the invention to a particular *in vivo* location, they can be formulated, for example, in liposomes which may comprise one or more moieties that are selectively transported into specific cells or organs, thus enhancing targeted drug delivery.

The invention is characterized by high specificity at the molecular and tissue-directed delivery level. The sequences of the nucleic acids of the invention are highly specific for their target, meaning that they do not inhibit the expression of genes that they are not designed to target or only minimally inhibit the expression of genes that they are not designed to target and/or only inhibit the expression of a low number of genes that they are not designed to target. A further level of specificity is achieved when nucleic acids are linked to a ligand that is specifically recognised and internalised by a particular cell type. This is for example the case when a nucleic acid is linked to a ligand comprising GalNAc moieties, which are specifically recognised and internalised by hepatocytes. This leads to the nucleic acid inhibiting the expression of their target only in the cells that are targeted by the ligand to which they are linked. These two levels of specificity potentially confer a better safety profile than the currently available treatments. In certain embodiments, the present invention thus provides nucleic acids of the invention linked to a ligand comprising one or more GalNAc moieties, or comprising one or more other moieties that confer cell-type or tissue-specific internalisation of the nucleic acid thereby conferring additional specificity of target gene knockdown by RNA interference.

The nucleic acid as described herein may be formulated with a lipid in the form of a liposome. Such a formulation may be described in the art as a lipoplex. The composition with a lipid/liposome may be used to assist with delivery of the nucleic acid of the invention to the target cells. The lipid delivery system herein described may be used as an alternative to a

conjugated ligand. The modifications herein described may be present when using the nucleic acid of the invention with a lipid delivery system or with a ligand conjugate delivery system.

Such a lipoplex may comprise a lipid composition comprising:

i) a cationic lipid, or a pharmaceutically acceptable salt thereof;

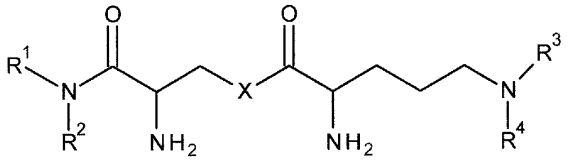
ii) a steroid;

iii) a phosphatidylethanolamine phospholipid; and/or

iv) a PEGylated lipid.

The cationic lipid may be an amino cationic lipid.

The cationic lipid may have the formula (XII):



(XII)

or a pharmaceutically acceptable salt thereof, wherein:

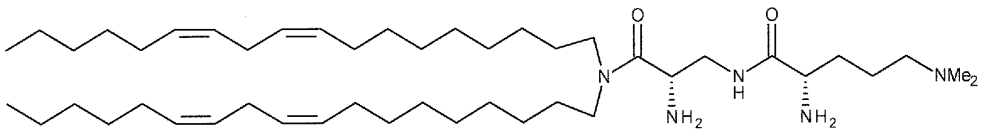
X represents O, S or NH;

R^1 and R^2 each independently represents a C_4-C_{22} linear or branched alkyl chain or a C_4-C_{22} linear or branched alkenyl chain with one or more double bonds, wherein the alkyl or alkenyl chain optionally contains an intervening ester, amide or disulfide;

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

when X represents O, R3 and R4 each independently represent hydrogen, methyl, ethyl, a mono- or polyamine moiety, or R^3 and R^4 together form a heterocyclyl ring, or R^3 represents hydrogen and R^4 represents $C(NH)(NH_2)$.

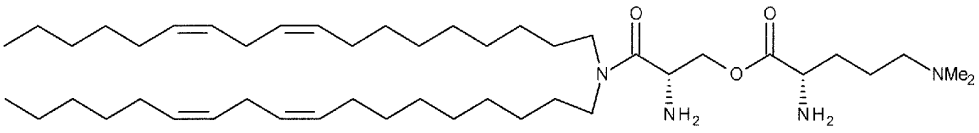
The cationic lipid may have the formula (XIII):



(XIII)

or a pharmaceutically acceptable salt thereof.

The cationic lipid may have the formula (XIV):



(XIV)

or a pharmaceutically acceptable salt thereof.

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

The compositions can further comprise a steroid. The steroid may be cholesterol. The content of the steroid may be from about 26 mol% to about 35 mol% of the overall lipid content of the lipid composition. More particularly, the content of steroid may be about 30 mol% of the overall lipid content of the lipid composition.

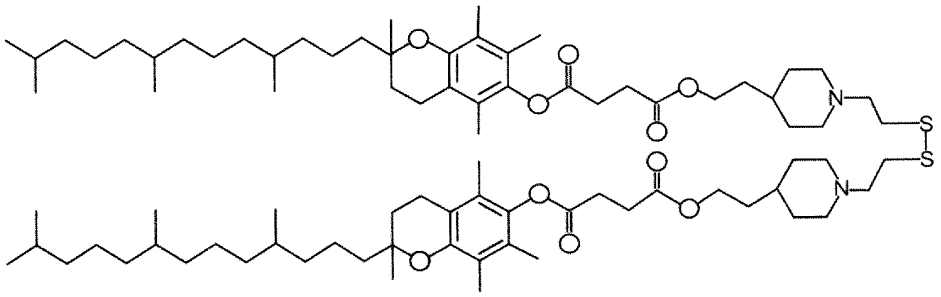
The phosphatidylethanolamine phospholipid may be selected from the group consisting of 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPhyPE), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-Dilauroyl-sn-glycero-3-phosphoethanolamine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

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

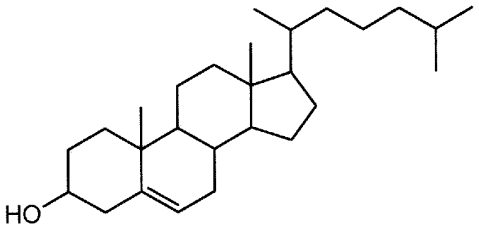
The content of the cationic lipid component in the composition may be from about 55 mol% to about 65 mol% of the overall lipid content of the lipid composition, preferably about 59 mol% of the overall lipid content of the lipid composition.

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

The composition may comprise a cationic lipid having the structure

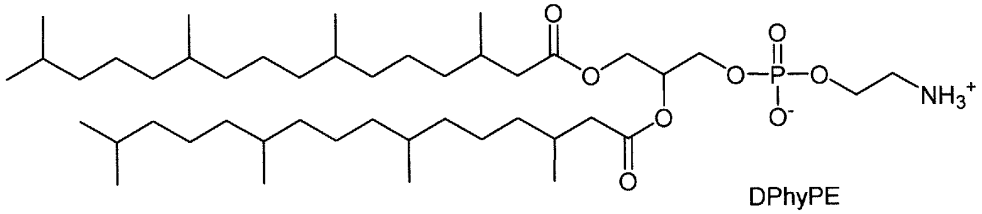


a steroid having the structure



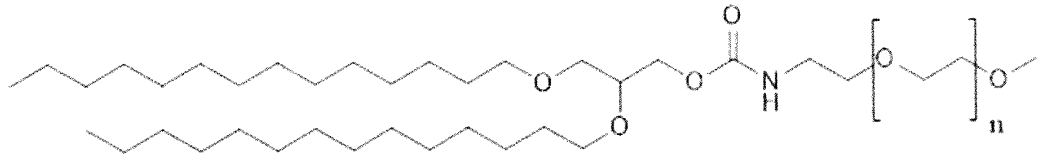
Cholesterol

a phosphatidylethanolamine phospholipid having the structure



DPhyPE

and a PEGylated lipid having the structure



mPEG 2000-DMG

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

A positively charged synthetic cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) can be used to form small liposomes that interact spontaneously with nucleic acid to form lipid-nucleic acid complexes which are capable of fusing with the negatively charged lipids of the cell membranes of tissue culture cells. DOTMA analogues can also be used to form liposomes.

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

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

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

Nucleic acid formulations of the present invention may include a surfactant. In one embodiment, the nucleic acid is formulated as an emulsion that includes a surfactant.

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

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

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

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

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

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

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

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

Definitions

As used herein, the terms "inhibit", "down-regulate", or "reduce" with respect to gene expression mean that the expression of the gene, or the level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits (e.g., mRNA), or the activity of one or more proteins or protein subunits, is reduced below that observed either in the absence of the nucleic acid or conjugated nucleic acid of the invention or as compared to that

obtained with an siRNA molecule with no known homology to the human transcript (herein termed non-silencing control). Such control may be conjugated and modified in an analogous manner to the molecule of the invention and delivered into the target cell by the same route. The expression after treatment with the nucleic acid of the invention may be reduced to 95%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 15%, 10%, 5% or 0% or to intermediate values, or less than that observed in the absence of the nucleic acid or conjugated nucleic acid. The expression may be measured in the cells to which the nucleic acid is applied. Alternatively, especially if the nucleic acid is administered to a subject, the level can be measured in a different group of cells or in a tissue or an organ or in a body fluid such as blood or plasma. The level of inhibition is preferably measured in conditions that have been selected because they show the greatest effect of the nucleic acid on the target mRNA level in cells treated with the nucleic acid *in vitro*. The level of inhibition may for example be measured after 24 hours or 48 hours of treatment with a nucleic acid at a concentration of between 0.038 nM – 10 μM, preferably 1 nM, 10 nM or 100 nM. These conditions may be different for different nucleic acid sequences or for different types of nucleic acids, such as for nucleic acids that are unmodified or modified or conjugated to a ligand or not. Examples of suitable conditions for determining levels of inhibition are described in the examples.

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

The nucleic acid may comprise ribonucleotides, modified ribonucleotides, deoxynucleotides, deoxyribonucleotides, or nucleotide analogues non-nucleotides that are able to mimic nucleotides such that they may 'pair' with the corresponding base on the target sequence or complementary strand. The nucleic acid may further comprise a double-stranded nucleic acid portion or duplex region formed by all or a portion of the first strand (also known in the art as a guide strand) and all or a portion of the second strand (also known in the art as a passenger strand). The duplex region is defined as beginning with the first base pair formed between the first strand and the second strand and ending with the last base pair formed between the first strand and the second strand, inclusive.

By duplex region it is meant the region in two complementary or substantially complementary oligonucleotides that form base pairs with one another, either by Watson-Crick base pairing or any other manner that allows for a duplex between oligonucleotide strands that are complementary or substantially complementary. For example, an oligonucleotide strand having 21 nucleotide units can base pair with another oligonucleotide of 21 nucleotide units, yet only 19 nucleotides on each strand are complementary or substantially complementary, such that the "duplex region" consists of 19 base pairs. The remaining base pairs may exist as 5′ and 3′ overhangs, or as single-stranded regions. Further, within the duplex region, 100% complementarity is not required; substantial complementarity is allowable within a duplex region. Substantial complementarity refers to complementarity between the strands such that they are capable of annealing under biological conditions. Techniques to empirically determine if two strands are capable of annealing under biological conditions are well known in the art. Alternatively, two strands can be synthesised and added together under biological conditions to determine if they anneal to one another. The portion of the first strand and second strand that forms at least one duplex region may be fully complementary and is at least partially complementary to each other. Depending on the length of a nucleic acid, a perfect match in terms of base complementarity between the first strand and the second strand is not necessarily required. However, the first and second strands must be able to hybridise under physiological conditions.

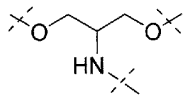
As used herein, the terms "non-pairing nucleotide analogue" means a nucleotide analogue which includes a non-base pairing moiety including but not limited to: 6 des amino adenosine (Nebularine), 4-Me-indole, 3-nitropyrrole, 5-nitroindole, Ds, Pa, N3-Me ribo U, N3-Me riboT, N3-Me dC, N3-Me-dT, N1-Me-dG,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

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

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

a first strand and a second strand at a blunt end may be base-paired. The terminal two nucleotides of a first strand and a second strand at a blunt end may not be paired.

The term "serinol-derived linker moiety" means the linker moiety comprises the following structure:



An O atom of said structure typically links to an RNA strand and the N atom typically links to the targeting ligand.

The terms "patient," "subject," and "individual" may be used interchangeably and refer to either a human or a non-human animal. These terms include mammals such as humans, primates, livestock animals (e.g., bovines, porcines), companion animals (e.g., canines, felines) and rodents (e.g., mice and rats).

As used herein, "treating" or "treatment" and grammatical variants thereof refer to an approach for obtaining beneficial or desired clinical results. The term may refer to slowing the onset or rate of development of a condition, disorder or disease, reducing or alleviating symptoms associated with it, generating a complete or partial regression of the condition, or some combination of any of the above. For the purposes of this invention, beneficial or desired clinical results include, but are not limited to, reduction or alleviation of symptoms, diminishment of extent of disease, stabilization (i.e., not worsening) of state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival relative to expected survival time if not receiving treatment. A subject (e.g., a human) in need of treatment may thus be a subject already afflicted with the disease or disorder in question. The term "treatment" includes inhibition or reduction of an increase in severity of a pathological state or symptoms relative to the absence of treatment, and is not necessarily meant to imply complete cessation of the relevant disease, disorder or condition.

As used herein, the terms "preventing" and grammatical variants thereof refer to an approach for preventing the development of, or altering the pathology of, a condition, disease or disorder. Accordingly, "prevention" may refer to prophylactic or preventive measures. For the purposes of this invention, beneficial or desired clinical results include, but are not limited to, prevention or slowing of symptoms, progression or development of a disease, whether detectable or undetectable. A subject (e.g., a human) in need of prevention may thus be a subject not yet afflicted with the disease or disorder in question. The term "prevention" includes slowing the

onset of disease relative to the absence of treatment, and is not necessarily meant to imply permanent prevention of the relevant disease, disorder or condition. Thus "preventing" or "prevention" of a condition may in certain contexts refer to reducing the risk of developing the condition, or preventing or delaying the development of symptoms associated with the condition.

As used herein, an "effective amount," "therapeutically effective amount" or "effective dose" is an amount of a composition (e.g., a therapeutic composition or agent) that produces at least one desired therapeutic effect in a subject, such as preventing or treating a target condition or beneficially alleviating a symptom associated with the condition.

As used herein, the term "pharmaceutically acceptable salt" refers to a salt that is not harmful to a patient or subject to which the salt in question is administered. It may be a salt chosen, e.g., among acid addition salts and basic salts. Examples of acid addition salts include chloride salts, citrate salts and acetate salts. Examples of basic salts include salts wherein the cation is selected from alkali metal cations, such as sodium or potassium ions, alkaline earth metal cations, such as calcium or magnesium ions, as well as substituted ammonium ions, such as ions of the type $N(R^1)(R^2)(R^3)(R^4)+$, wherein R^1, R^2, R^3 and R^4 independently will typically designate hydrogen, optionally substituted C1-6-alkyl groups or optionally substituted C2-6-alkenyl groups. Examples of relevant C1-6-alkyl groups include methyl, ethyl, 1-propyl and 2-propyl groups. Examples of C2-6-alkenyl groups of possible relevance include ethenyl, 1-propenyl and 2-propenyl. Other examples of pharmaceutically acceptable salts are described in "Remington's Pharmaceutical Sciences", 17th edition, Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, USA, 1985 (and more recent editions thereof), in the "Encyclopaedia of Pharmaceutical Technology", 3rd edition, James Swarbrick (Ed.), Informa Healthcare USA (Inc.), NY, USA, 2007, and in J. Pharm. Sci. 66: 2 (1977). A "pharmaceutically acceptable salt" retains qualitatively a desired biological activity of the parent compound without imparting any undesired effects relative to the compound. Examples of pharmaceutically acceptable salts include acid addition salts and base addition salts. Acid addition salts include salts derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphorous, phosphoric, sulfuric, hydrobromic, hydroiodic and the like, or from nontoxic organic acids such as aliphatic mono- and di-carboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include salts derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N, N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

The term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers. Pharmaceutically acceptable carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at slightly acidic or physiological pH may be used. Exemplary pH buffering agents include phosphate, citrate, acetate, tris/hydroxymethyl)aminomethane (TRIS), N-Tris(hydroxymethyl)methyl-3-aminopropanesulphonic acid (TAPS), ammonium bicarbonate, diethanolamine, histidine, which is a preferred buffer, arginine, lysine, or acetate or mixtures thereof. The term further encompasses any agents listed in the US Pharmacopeia for use in animals, including humans. A "pharmaceutically acceptable carrier" includes any and all physiologically acceptable, i.e., compatible, solvents, dispersion media, coatings, antimicrobial agents, isotonic and absorption delaying agents, and the like. In certain embodiments, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion). Depending on selected route of administration, the nucleic acid may be coated in a material or materials intended to protect the compound from the action of acids and other natural inactivating conditions to which the nucleic acid may be exposed when administered to a subject by a particular route of administration.

The term "solvate" in the context of the present invention refers to a complex of defined stoichiometry formed between a solute (*in casu*, a nucleic acid compound or pharmaceutically acceptable salt thereof according to the invention) and a solvent. The solvent in this connection may, for example, be water or another pharmaceutically acceptable, typically small-molecular organic species, such as, but not limited to, acetic acid or lactic acid. When the solvent in question is water, such a solvate is normally referred to as a hydrate.

The invention will now be described with reference to the following non-limiting Figures and Examples.

Brief description of the Figures

Figures 1A, **1B** and **1C** show concentration-response-curves of selected siRNAs.

Figures 2A and **2B** show concentration-response-curves of selected siRNA GalNAc conjugates in human primary hepatocytes.

Figures 3A and **3B** show concentration-response-curves of selected siRNA GalNAc conjugates in mouse primary hepatocytes.

Figures 4A, **4B** and **4C** show concentration-dependent C3 mRNA inhibition of selected siRNA GalNAc conjugates respectively in primary mouse, human and cynomolgus hepatocytes.

Figure 5 shows a possible synthesis route to DMT-Serinol(GalNAc)-CEP and CPG.

Figures 6A and **6B** show *in vivo* C3 mRNA levels in hepatocytes as well as C3 protein levels in serum in response to sc treatment of mice with selected siRNA GalNAc conjugates.

Figures 7A, **7B** and **7C** show relative C3 mRNA expression in primary mouse (A), cynomolgus (B) and human (C) hepatocytes after incubation with selected siRNA GalNac conjugates (1nM, 10nM and 100 nM) normalized to ACTIN mRNA.

Figures 8A and **8B** show relative C3 mRNA expression in % in murine liver 14 days (A) or 42 days (B) after a single dosing of GalNAc conjugated siRNAs EV0201, EV0203, EV0204, EV0205 and EV0207. Data is shown in bar charts as mean ± SD (n=4 per group).

Figures 9A-E show relative C3 protein serum levels in % from mouse serum samples taken before (BL), at day 4, at day 10, day 14, day 21, day 28, day 35 and day 42 of the study after dosing of 5 or 10 mg/kg siRNA. Data points depict serum C3 level of individual animals determined using a standard C3 ELISA. Data was normalized to each group's baseline mean and then to the time matched PBS control, which was set as 100%. The plotted line connects the individual group means at the respective timepoints.

A) Normalised C3 serum levels after dosing of 5 and 10 mg/kg EV0201
B) Normalised C3 serum levels after dosing of 5 and 10 mg/kg EV0203
C) Normalised C3 serum levels after dosing of 5 mg/kg EV0204
D) Normalised C3 serum levels after dosing of 5 and 10 mg/kg EV0205
E) Normalised C3 serum levels after dosing of 5 and 10 mg/kg EV0207.

Figure 10 shows C3 mRNA knockdown efficiency by various siRNAs *in vitro*.

Figure 11 shows human C3 mRNA knockdown efficiency by various siRNAs *in vitro*.

Figure 12 shows mouse C3 mRNA knockdown efficiency by various siRNAs *in vitro*.

Figure 13 shows C3 mRNA knockdown efficiency by various siRNA conjugates in mouse hepatocytes.

Figure 14 shows C3 mRNA knockdown efficiency by various siRNA conjugates in cynomolgus hepatocytes.

Figure 15 shows C3 mRNA knockdown efficiency by siRNA conjugate variants in mouse hepatocytes.

Figure 16 shows C3 mRNA knockdown efficiency by siRNA conjugate variants in cynomolgus hepatocytes.

Figure 17 shows relative C3 mRNA expression in % in murine liver 43 days after a single dosing of GalNAc conjugated C3 siRNA EJ0020.

Figure 18 shows relative C3 protein serum levels in % from mouse serum samples taken before, at day 7, day 14, day 28 and day 43 of the study after dosing of 1 or 3 mg/kg GalNAc conjugated C3 siRNA EJ0020.

Figure 19 shows expression of C3 mRNA in primary human hepatocytes after incubation with the GalNAc siRNA conjugates EV0210, EV0211, EV0212 and EV0213 at 1, 10 and 100nM.

Figure 20 shows the effect of treatment with different doses of a conjugated C3 siRNA on C3 tubular deposition in wild-type and C3G disease model mice ten days post treatment.

Figure 21 shows the effect of treatment with different doses of a conjugated C3 siRNA on Complement Factor B fragmentation in wild-type and C3G disease model mice ten days post treatment.

Figure 22A and 22B show the effect of treatment with multiple doses of a conjugated C3 siRNA on levels of C3α-chain and C3α-chain fragments in C3G disease model mice.

Figure 23 shows the effect of treatment with multiple doses of a conjugated C3 siRNA on Complement Factor B fragmentation in C3G disease model mice.

Examples

Example 1

In vitro study in HepG2 cells showing C3 mRNA knockdown efficacy of tested siRNAs after transfection of 10 nM siRNA.

C3 knockdown efficacy of siRNAs EV0001-EV0100 was determined after transfection of 10 nM siRNA in HepG2 cells. The results are shown in Table 2 below. Remaining C3 mRNA levels after knockdown were in the range of 6 to 83 %. The most potent siRNAs were EV0001, EV0007, EV0008, EV0009, EV0012, EV0013, EV0018, EV0020, EV0030, EV0033, and EV0004.

For transfection of HepG2 cells with siRNAs, cells were seeded at a density of 15,000 cells / well into collagen-coated 96-well tissue culture plates (#655150, GBO, Germany). Transfection of siRNAs was carried out with Lipofectamine RNAiMax (Invitrogen/Life Technologies, Karlsruhe, Germany) according to the manufacturer's instructions directly after seeding. The screen was performed with C3 siRNAs in quadruplicates at 10 nM, with siRNAs targeting Aha1, Firefly-Luciferase and Factor VII as unspecific controls and a mock transfection. After 24h of incubation with siRNAs, medium was removed, and cells were lysed in 150 µl Medium-Lysis Mixture (1 volume lysis mixture, 2 volumes cell culture medium) and then incubated at 53°C for 30 minutes. bDNA assay was performed according to the manufacturer's instructions.

Luminescence was read using 1420 Luminescence Counter (WALLAC VICTOR Light, Perkin Elmer, Rodgau-Jügesheim, Germany) following 30 minutes of incubation at RT in the dark. For each well, the C3 mRNA level was normalized to the respective GAPDH mRNA level. The activity of a given C3 siRNA was expressed as percent remaining C3 mRNA concentration (normalized to GAPDH mRNA) in treated cells, relative to the C3 mRNA concentration (normalized to GAPDH mRNA) averaged across control wells.

Table 2

	% remaining mRNA			% remaining mRNA	
Duplex ID	Mean	SD	Duplex ID	Mean	SD
EV0001	8.87	0.61	EV0051	11.84	0.60
EV0002	10.61	0.72	EV0052	18.23	1.26
EV0003	10.68	0.82	EV0053	15.40	0.60
EV0004	9.59	0.43	EV0054	12.80	0.69
EV0005	22.27	0.97	EV0055	44.97	4.50
EV0006	13.10	1.16	EV0056	83.23	5.03
EV0007	8.89	0.90	EV0057	55.45	3.76
EV0008	6.02	0.47	EV0058	22.12	0.84
EV0009	6.80	0.45	EV0059	12.74	0.48
EV0010	20.18	1.32	EV0060	14.38	0.79
EV0011	10.09	0.34	EV0061	12.92	0.83
EV0012	9.48	1.06	EV0062	13.26	0.83
EV0013	7.40	1.08	EV0063	21.32	1.61
EV0014	9.75	1.25	EV0064	15.14	0.86
EV0015	13.08	1.52	EV0065	12.01	0.42
EV0016	16.68	0.66	EV0066	13.14	1.32
EV0017	47.72	1.80	EV0067	13.57	0.61
EV0018	7.86	0.77	EV0068	24.89	0.87
EV0019	18.83	1.08	EV0069	15.36	2.68
EV0020	8.80	0.87	EV0070	16.50	1.29
EV0021	13.88	0.98	EV0071	9.69	0.71
EV0022	79.91	4.20	EV0072	25.55	1.40
EV0023	13.32	1.29	EV0073	12.79	1.34
EV0024	11.11	0.76	EV0074	14.63	0.51
EV0025	16.35	0.50	EV0075	13.05	0.83
EV0026	10.03	0.88	EV0076	16.29	0.87
EV0027	10.11	1.03	EV0077	18.63	0.98
EV0028	11.83	0.53	EV0078	21.78	1.18
EV0029	9.71	1.02	EV0079	20.78	1.04
EV0030	9.05	0.49	EV0080	17.59	1.31
EV0031	10.70	0.64	EV0081	15.27	0.70
EV0032	12.93	0.55	EV0082	20.75	1.03
EV0033	9.29	0.59	EV0083	11.84	0.60
EV0034	15.20	0.50	EV0084	18.23	1.26

EV0035	16.87	0.62	EV0085	15.40	0.60
EV0036	14.37	1.05	EV0086	12.80	0.69
EV0037	15.36	2.68	EV0087	44.97	4.50
EV0038	16.50	1.29	EV0088	83.23	5.03
EV0039	9.69	0.71	EV0089	55.45	3.76
EV0040	25.55	1.40	EV0090	22.12	0.84
EV0041	12.79	1.34	EV0091	12.74	0.48
EV0042	14.63	0.51	EV0092	14.38	0.79
EV0043	13.05	0.83	EV0093	12.92	0.83
EV0044	16.29	0.87	EV0094	13.26	0.83
EV0045	18.63	0.98	EV0095	21.32	1.61
EV0046	21.78	1.18	EV0096	15.14	0.86
EV0047	20.78	1.04	EV0097	12.01	0.42
EV0048	17.59	1.31	EV0098	13.14	1.32
EV0049	15.27	0.70	EV0099	13.57	0.61
EV0050	20.75	1.03	EV0100	24.89	0.87

Table 2: Results of screening of C3 siRNAs - the identity of the single strands forming each of the siRNA duplexes as well as their sequences and modifications are to be found in the tables at the end of the description.

Example 2

In vitro study in HepG2 cells showing C3 mRNA knockdown efficacy of tested siRNAs after transfection of 0.01 pM – 100 nM siRNA (concentration-response-curve experiment).

C3 knockdown efficacy of siRNAs EV0001, EV0008, EV0013, EV0030, EV0033, EV0039, EV0043, EV0053, EV0054, EV0059, EV0060, EV0061, EV0066, EV0072, EV0075, EV0081, EV0091 and EV0098 was determined after transfection of 0.01 pM -100 nM siRNA in HepG2 cells. The identity of the single strands forming each of the siRNA duplexes as well as their sequences are to be found in the tables at the end of the description. Results are presented in Figures 1A, 1B and 1C. All siRNAs showed a dose-dependent knockdown of C3 mRNA after transfection. The most potent siRNAs were EV0008, EV0033 and EV0081, with a residual C3 expression of 4.3, 5.3 and 5.3 % at 100 nM siRNA, respectively.

For transfection of HepG2 cells with siRNAs, cells were seeded at a density of 15,000 cells / well into collagen-coated 96-well tissue culture plates (#655150, GBO, Germany). Transfection of siRNA was carried out with Lipofectamine RNAiMax (Invitrogen/Life Technologies, Karlsruhe, Germany) according to the manufacturer's instructions directly after seeding. Concentration-response experiments were done with C3 siRNA in 10 concentrations transfected in quadruplicates, starting at 100 nM in 6-fold dilution steps down to 0.01 pM. Mock

transfected cells served as control in CRC experiments. After 24h of incubation with siRNAs, medium was removed and cells were lysed in 150µl Medium-Lysis Mixture (1 volume lysis mixture, 2 volumes cell culture medium) and then incubated at 53°C for 30 minutes. bDNA assay was performed according to the manufacturer's instructions. Luminescence was read using 1420 Luminescence Counter (WALLAC VICTOR Light, Perkin Elmer, Rodgau-Jügesheim, Germany) following 30 minutes of incubation at RT in the dark. For each well, the C3 mRNA level was normalized to the respective GAPDH mRNA level. The activity of a given C3 siRNA was expressed as percent remaining C3 mRNA (normalized to GAPDH mRNA) in treated cells, relative to the C3 mRNA concentration (normalized to GAPDH mRNA) averaged across control wells. Concentration-response-curves were fitted with GraphPad Prism version 7.05 using a four-parameter logistic (4PL) model without further constraints.

Example 3

In vitro study in primary human hepatocytes showing C3 mRNA knockdown efficacy of tested siRNA-GalNAc conjugates in concentration-response-curve format (0.038 nM – 10 µM siRNA conjugate).

Expression of C3 mRNA after incubation with the GalNAc siRNA conjugates EV0101, EV0102, EV0103, EV0104, EV0105, EV0106, EV0107, EV0108, EV0109, EV0110, EV0111 and EV0312 was analysed in a concentration-response format. The identity of the single strands forming each of the siRNA duplexes as well as their sequences are to be found in the tables at the end of the description. Results are shown in Figures 2A and 2B. The mRNA level of the house keeping gene GAPDH served as control for all experiments. All siRNA GalNAc conjugates were able to decrease C3 mRNA level in a concentration-dependent fashion with maximal inhibition at 10 µM between 32 and 70 %, respectively. The most potent siRNAs were EV0102 with 61 %, EV0109 with 67 %, EV0111 with 69 % and EV0312 with 70 % reduction of the C3 mRNA level at 10 µM.

Human cryopreserved primary hepatocytes were purchased from Primacyt (Schwerin, Germany, cat#GuCPI, Lot# BHum16061-P). Directly before treatment, cells were thawed, transferred to a tube with thawing medium (Primacyt, cat#HTM), centrifuged and washed with washing Medium (Primacyt, cat#HWM). Cells were seeded at a density of 90,000 cells per well in plating medium (Primacyt, cat#MPM-cryo) on collagen coated 96-well plates (Greiner-Bio-One, #655150). Directly after seeding, cells were treated with the siRNAs as they adhered as a monolayer in plating medium. Each siRNA was applied to the cells for the concentration-response-curve at concentrations starting with 10 µM, sequentially diluted in 4-fold dilution

steps down to 38 pM. Each concentration was applied as quadruplicate. After 5 hours, the medium was changed to maintenance medium (Primacyt cat#HHMM). The medium was changed every 24 hours and the cells were harvested for analysis by Quantigene bDNA assay 48 hours after seeding. The C3 mRNA concentrations were normalised to GAPDH mRNA. Concentration-response-curves were fitted with GraphPad Prism version 7.05 using a four-parameter logistic (4PL) model without further constraints.

Example 4

In vitro study in primary mouse hepatocytes showing C3 mRNA knockdown efficacy of tested siRNA-GalNAc conjugates in concentration-response-curve format (0.038 nM – 10 µM siRNA conjugate).

Expression of C3 mRNA after incubation with the GalNAc siRNA conjugates EV0104, EV0105, EV0107, EV0108, EV0109, EV0110, EV0111 and EV0312 in a concentration-response format was analysed. The identity of the single strands forming each of the siRNA duplexes as well as their sequences are to be found in the tables at the end of the description. The mRNA level of the house keeping gene GAPDH served as control. The siRNA GalNAc conjugates were able to decrease C3 mRNA levels in a concentration dependent fashion with maximal inhibition at 10 µM between 56 and 72 %. The most potent siRNAs were EV0104 with 68 %, EV0109 with 67 % and EV0111 with 72 % reduction of C3 mRNA at 10 µM, respectively.

Cryopreserved murine hepatocytes were purchased from Thermo Fisher (#MSCP10, Lot#MC817) and plated in plating medium (Thermo Fisher Sci, Cat. No. CM3000 supplement pack added to William's E Medium, no phenol red – to 500 ml total, Thermo Fisher Sci, Cat. No. A12176-01). On the day of seeding, the cells were thawed and plated at a density of 60,000 cells per well into a collagen-coated 96-well plate (Greiner-Bio-One, #655150). Directly after seeding, cells were treated with the siRNAs as they adhered as a monolayer in plating medium. Each siRNA was applied to the cells as concentration-response-curve at concentrations starting with 10 µM, sequentially diluted in 4-fold dilution steps down to 0.038 nM. Each concentration was applied as quadruplicate. After 5 hours, the medium was changed to maintenance medium (Thermo Fisher Sci, Cat. No. CM4000 supplement pack added to William's E Medium, no phenol red – to 500 ml total, Thermo Fisher Sci, Cat. No. A12176-01). The medium was changed every 24 hours and the cells were harvested for analysis by Quantigene bDNA assay 48 hours after seeding.

The results are shown in Figures 3A and 3B. They depict % remaining C3 mRNA expression in primary mouse hepatocytes after incubation with siRNA GalNAc conjugates (0.038 nM – 10 µM) normalized to GAPDH mRNA. Concentration-response curves were fitted with GraphPad Prism version 7.05 using a four-parameter logistic (4PL) model without further constraints.

Example 5

In vitro study in primary mouse, human and cynomolgus hepatocytes showing C3 mRNA knockdown efficacy of tested siRNA-GalNAc conjugates at 1, 10 and 100nM.

The expression of C3 mRNA after incubation with the GalNAc siRNA conjugates EV0312 and EV0313 at 1, 10 and 100nM was analysed. The identity of the single strands forming each of the siRNA duplexes as well as their sequences are to be found in the tables at the end of the description. The mRNA level of the house keeping gene Actin served as control.

40,000 (human), 30,000 (mouse) or 45,000 (cynomolgus) cells were seeded on collagen-coated 96-well plates. siRNAs in indicated concentrations were added immediately after seeding. 24 hours post treatment, cells were lysed using InviTrap RNA Cell HTS96 Kit/C (Stratec). qPCR was performed using mRNA-specific primers and probes against C3 and Actin.

The results are shown in Figures 4A, 4B and 4C.

Example 6 - Synthesis of building blocks

The synthesis route for DMT-Serinol(GalNAc)-CEP and CPG as described below is outlined in Figure 5. Starting material DMT-Serinol(H) (**1**) was made according to literature published methods (Hoevelmann et al. Chem. Sci., 2016,7, 128-135) from commercially available L-Serine. GalNAc(Ac_3)-C_4H_8-COOH (**2**) was prepared according to literature published methods (Nair et al. J. Am. Chem. Soc., 2014, 136 (49), pp 16958–1696), starting from commercially available per-acetylated galactose amine. Phosphitylation reagent 2-Cyanoethyl-N,N-diisopropylchlorophosphor-amidite (**4**) is commercially available. Synthesis of (vp)-mU-phos was performed as described in Prakash, Nucleic Acids Res. 2015, 43(6), 2993-3011 and Haraszti, Nucleic Acids Res. 2017, 45(13), 7581-7592. Synthesis of the phosphoramidite derivatives of ST43 (ST43-phos) as well as ST23 (ST23-phos) can be performed as described in WO2017/174657.

DMT-Serinol(GalNAc) (**3**)

HBTU (9.16 g, 24.14 mmol) was added to a stirring solution of GalNAc(Ac_3)-C_4H_8-COOH (**2**) (11.4 g, 25.4 mmol) and DIPEA (8.85 ml, 50.8 mmol). After 2 minutes activation time a solution of DMT-Serinol(H) (**1**) (10 g, 25.4 mmol) in Acetonitrile (anhydrous) (200 ml) was added to the stirring mixture. After 1h LCMS showed good conversion. The reaction mixture was concentrated in vacuo. The residue was dissolved up in EtOAc, washed subsequently with water (2x) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was further purified by column chromatography (3% MeOH in CH_2Cl_2 + 1% Et_3N, 700g silica). Product containing fractions were pooled, concentrated and stripped with CH_2Cl_2 (2x) to yield to yield 10.6g (51%) of DMT-Serinol(GalNAc) (**3**) as an off-white foam.

DMT-Serinol(GalNAc)-CEP (**5**)

2-Cyanoethyl-N,N-diisopropylchlorophosphoramidite (**4**) (5.71 ml, 25.6 mmol) was added slowly to a stirring mixture of DMT-Serinol(GalNAc) (**3**) (15.0 g, 17.0 mmol), DIPEA (14.9 ml, 85 mmol) and 4Å molecular sieves in Dichloromethane (dry) (150 ml) at 0°C under argon atmosphere. The reaction mixture was stirred at 0°C for 1h. TLC indicated complete conversion. The reaction mixture was filtered and concentrated in vacuo to give a thick oil. The residue was dissolved in Dichloromethane and was further purified by flash chromatography (0-50% acetone in toluene 1%Et3N, 220 g silica). Product containing fractions were pooled and concentrated in vacuo. The resulting oil was stripped with MeCN (2x) to yield 13.5g (77%) of the colorless DMT-Serinol(GalNAc)-CEP (**5**) foam.

DMT-Serinol(GalNAc)-succinate (**6**)

DMAP (1.11 g, 9.11 mmol) was added to a stirring solution of DMT-Serinol(GalNAc) (**3**) (7.5 g, 9.11 mmol) and succinic anhydride (4.56 g, 45.6 mmol) in a mixture of Dichloromethane (50 ml) and Pyridine (50 ml) under argon atmosphere. After 16h of stirring the reaction mixture was concentrated in vacuo and the residue was taken up in EtOAc and washed with 5% citric acid (aq). The aqueous layer was extracted with EtOAc. The combined organic layers were washed subsequently with sat $NaHCO_3$ (aq.) and brine, dried over Na_2SO_4, filtered and concentrated in vacuo. Further purification was achieved by flash chromatography (0-5% MeOH in CH_2Cl_2 +1% Et_3N, 120g silica). Product containing fractions were pooled and concentrated in vacuo. The residue was stripped with MeCN (3x) to yield 5.9g (70%) DMT-Serinol(GalNAc)-succinate (**6**).

DMT-Serinol(GalNAc)-succinyl-Icaa-CPG (**7**)

The DMT-Serinol(GalNAc)-succinate (**6**) (1 eq.) and HBTU (1.1 eq.) were dissolved in CH_3CN (10 ml). Diisopropylethylamine (2 eq.) was added to the solution, and the mixture was swirled for 2 min followed by addition native amino-Icaa-CPG (500 A, 88µmol/g, 1 eq.). The suspension was gently shaken at room temperature on a wrist-action shaker for 16h, then filtered and washed with acetonitrile. The solid support was dried under reduced pressure for 2 h. The unreacted amines on the support were capped by stirring with Ac_2O/2,6-lutidine/NMI at room temperature (2x15min). The washing of the support was repeated as above. The solid was dried under vacuum to yield DMT-Serinol(GalNAc)-succinyl-Icaa-CPG (**7**) (loading: 34 µmol/g, determined by detritylation assay).

Example 7 - Oligonucleotide Synthesis

Example compounds were synthesised according to methods described below and known to the person skilled in the art. Assembly of the oligonucleotide chain and linker building blocks was performed by solid phase synthesis applying phosphoramidite methodology.

Downstream cleavage, deprotection and purification followed standard procedures that are known in the art.

Oligonucleotide syntheses was performed on an AKTA oligopilot 10 using commercially available 2´O-Methyl RNA and 2´Fluoro-2´Deoxy RNA base loaded CPG solid support and phosphoramidites (all standard protection, ChemGenes, LinkTech) were used. Synthesis of DMT-(S)-Serinol(GalNAc)-succinyl Icaa CPG (**7**) and DMT-(S)-Serinol(GalNAc)-CEP (**5**) are described in example 6.

Ancillary reagents were purchased from EMP Biotech. Synthesis was performed using a 0.1 M solution of the phosphoramidite in dry acetonitrile (<20 ppm H_2O) and benzylthiotetrazole (BTT) was used as activator (0.3M in acetonitrile). Coupling time was 10 min. A Cap/OX/Cap or Cap/Thio/Cap cycle was applied (Cap: Ac_2O/NMI/Lutidine/Acetonitrile, Oxidizer: 0.05M I_2 in pyridine/H_2O). Phosphorothioates were introduced using commercially available thiolation reagent 50mM EDITH in acetonitrile (Link technologies). DMT cleavage was achieved by treatment with 3% dichloroacetic acid in toluene. Upon completion of the programmed synthesis cycles a diethylamine (DEA) wash was performed. All oligonucleotides were synthesized in DMT-off mode.

Attachment of the Serinol(GalNAc) moiety was achieved by use of either base-loaded (S)-DMT-Serinol(GalNAc)-succinyl-lcaa-CPG (**7**) or a (S)-DMT-Serinol(GalNAc)-CEP (**5**). Tri-antennary GalNAc clusters (ST23/ST43) were introduced by successive coupling of the branching trebler amidite derivative (C6XLT-phos) followed by the GalNAc amidite (ST23-phos). Attachment of (vp)-mU moiety was achieved by use of (vp)-mU-phos in the last synthesis cycle. The (vp)-mU-phos does not provide a hydroxy group suitable for further synthesis elongation and therefore, does not possess an DMT-group. Hence coupling of (vp)-mU-phos results in synthesis termination.

For the removal of the methyl esters masking the vinylphosphonate, the CPG carrying the fully assembled oligonucleotide was dried under reduced pressure and transferred into a 20 ml PP syringe reactor for solid phase peptide synthesis equipped with a disc frit (Carl Roth GmbH). The CPG was then brought into contact with a solution of 250 µL TMSBr and 177 µL pyridine in CH_2Cl_2 (0.5 ml/µmol solid support bound oligonucleotide) at room temperature and the reactor was sealed with a Luer cap. The reaction vessels were slightly agitated over a period of 2x15 min, the excess reagent discarded, and the residual CPG washed 2x with 10 ml acetonitrile. Further downstream processing did not alter from any other example compound.

The single strands were cleaved off the CPG by 40% aq. methylamine treatment (90 min, RT). The resulting crude oligonucleotide was purified by ion exchange chromatography (Resource Q, 6 ml, GE Healthcare) on a AKTA Pure HPLC System using a sodium chloride gradient. Product containing fractions were pooled, desalted on a size exclusion column (Zetadex, EMP Biotech) and lyophilized until further use.

All final single-stranded products were analysed by AEX-HPLC to prove their purity. Identity of the respective single-stranded products was proved by LC-MS analysis.

Example 8 – double-strand formation

Individual single strands were dissolved in a concentration of 60 OD/ml in H_2O. Both individual oligonucleotide solutions were added together in a reaction vessel. For easier reaction monitoring a titration was performed. The first strand was added in 25% excess over the second strand as determined by UV-absorption at 260 nm. The reaction mixture was heated to 80°C for 5 min and then slowly cooled to RT. Double-strand formation was monitored by ion pairing reverse phase HPLC. From the UV-area of the residual single strand the needed amount of the second strand was calculated and added to the reaction mixture. The reaction

was heated to 80°C again and slowly cooled to RT. This procedure was repeated until less than 10% of residual single strand was detected.

Example 9

In vivo study showing knockdown of C3 mRNA in murine liver tissue and serum protein after single subcutaneous dosing of 1 or 5 mg/kg GalNAc conjugated siRNAs.

Female C57BL/6N mice with an age of 8 weeks were obtained from CHARLES RIVER, Sulzfeld, Germany. Animal experiments were performed according to ethical guidelines of the German Protection of Animals Act in its version of July 2013. Mice were randomized according to weight into groups of 4 mice. On day 0 of the study animals received a single subcutaneous dose of 1 or 5 mg/kg siRNA dissolved in phosphate buffered saline (PBS) or PBS only as control. The viability, body weight and behaviour of the mice was monitored during the study without pathological findings. Serum samples were taken before the application, at day 4, day 10 and day 14. At day 14 the study was terminated, animals were euthanized, and liver samples were snap frozen and stored at – 80°C until further analysis. For analysis, RNAs were isolated using the InviTrap Spin Tissue RNA Mini Kit from Stratec according to the manufacturer´s protocol. QPCR was performed using C3 and Actin specific primer probe sets and Takyon™ One-Step Low Rox Probe 5X MasterMix dTTP on the QuantStudio6 device from Applied Biosystems in single-plex 384 well format. Expression differences were calculated using the delta delta Ct method and relative expression of C3 versus the house keeping gene actin normalized to the PBS control experiment was used for comparison of the different siRNAs. EV0107, EV0313 and EV0110 induced a dose dependent knockdown of liver C3 mRNA. The maximum achieved knockdown was observed using siRNA EV0107 (57%) and EV0110 (61%) using 5 mg/kg siRNA, respectively. Results are shown in Figure 6A. The figure shows relative C3 mRNA expression in % in murine liver 14 days after a single dosing of GalNAc conjugated siRNAs EV0107, EV0313 and EV0110. The identity of the single strands forming each of the siRNA duplexes as well as their sequences are to be found in the tables at the end of the description. Data is shown in bar charts as mean ± SD (n=4 per group).

Serum samples were analysed using commercially available C3 ELISA Kits. The analyses were carried out according to the manufacturer's protocol, and C3 serum levels were calculated relative to the respective pre dose levels. Results are shown in Figure 6B. The figure shows relative C3 protein serum levels in % from mouse serum samples taken before, at day 4, at day 10 and at day 14 of the study after dosing of 5mg/kg EV0313, EV0110 and EV0107 GalNAc conjugated siRNAs. Data is shown as means ± SD (n=3 or 4 per group).

Example 10

In vitro study in primary mouse, human and cynomolgus hepatocytes showing C3 knockdown efficacy of tested siRNA-GalNAc conjugates at 1, 10 an 100nM.

Expression of C3 mRNA after incubation with the GalNAc siRNA conjugates EV0201, EV0203, EV0204, EV0205 and EV0207 at 1, 10 and 100nM was measured (Figure 7). siRNA sequences and modifications are listed in Tables 3 and 5. The mRNA level of the house keeping gene ACTIN served as housekeeping control. Human and cynomolgus primary hepatocytes were seeded into collagen I-coated 96-well plates (Life Technologies) at a density of 40,000 cells per well. Mouse hepatocytes were seeded at a density of 25,000 cells per well. GalNAc-conjugated siRNAs were added immediately after plating in the previously defined media to final siRNA concentrations of 100, 10 and 1 nM. Plates were then incubated at 37 °C in a 5% CO2 atmosphere for 24 hours. Subsequently, cells were lysed and RNA was isolated using InviTrap RNA Cell HTS96 Kit/C (Stratec).

10 µl of RNA-solution was used for gene expression analysis by reverse transcription quantitative polymerase chain reaction (RT-qPCR) performed with amplicon sets/sequences for ACTB (Eurogentec) and C3 (BioTez GmbH, Berlin, Germany), respectively. The RT-qPCR reactions were carried out with an ABI StepOne Plus (Applied Biosystems, part of Thermo Fisher Scientific, Massachusetts, USA) using standard protocols for RT-PCR (48 °C 30 min, 95 °C 10 min, 40 cycles at 95 °C 15 s followed by 60 °C 1 min). The data were calculated by using the comparative CT method also known as the 2-deltadelta Ct method. SiRNAs EV0201, EV0203, EV0204, EV0205 and EV0207 show dose-dependent inhibition of C3 mRNA expression in primary hepatocytes.

Example 11

In vivo study showing knockdown of C3 mRNA in murine liver tissue and serum protein after a single subcutaneous dosing of 5 or 10 mg/kg GalNAc conjugated modified siRNAs.

siRNA sequences and modifications are listed in Tables 3 and 5. The mRNA level of the house keeping gene ACTIN served as housekeeping control. Male C57BL/6N mice with an age of about 8 weeks were obtained from CHARLES RIVER, Sulzfeld, Germany. Animal experiments were conducted in compliance with the principles of the Hungarian Act 1998: XXVIII regulating animal protection (latest modified by Act 2011 CLVIII) and in Government Decree 40/2013 on

animal experiments. Mice were assigned into groups of 4 mice. On day 0 of the study, the animals received a single subcutaneous dose of 5 or 10 mg/kg siRNA dissolved in phosphate buffered saline (PBS) or PBS only as control. The viability, body weight and behaviour of the mice was monitored during the study without pathological findings. Serum samples were taken before the application, at day 4, day 10, day 14, day 21, day 28, day 35 and day 42. At day 14 and at day 42 half of the groups, respectively, were terminated, the animals were euthanized, and liver samples were snap frozen and stored at – 80°C until further analysis.

For analysis, RNAs were isolated using the InviTrap Spin Tissue RNA Mini Kit from Stratec according to the manufacturer's protocol. RT-qPCR was performed using C3 and ACTIN specific primer probe sets and Takyon™ One-Step Low Rox Probe 5X MasterMix dTTP on the QuantStudio6 device from Applied Biosystems in single-plex 384 well format. Expression differences were calculated using the delta delta Ct method and relative expression of C3 versus the house keeping gene ACTIN normalized to the PBS group was used for comparison of the different siRNAs.

All tested siRNAs (EV0201, EV0203, EV0204, EV0205 and EV0207) inhibit C3 mRNA expression by more than 70 % after 14 days after a single dose of 5 or 10 mg/kg (Figure 8A). After 42 days, the inhibition of C3 expression by EV0203, EV0204, EV0205 and EV0207 was still more than 80 % knockdown with a 10 mg/kg siRNA dose (Figure 8B).

For C3 protein level analysis, serum samples were measured using commercially available C3 ELISA Kits. The analyses were carried out according the manufacturer's protocol, and % C3 serum levels were calculated relative to the group means at baseline/before the application and relative to the time matched PBS control group's means. The data for the C3 protein analyses mirror the results from the RNA analyses (Figure 9). EV0203, EV0204, EV0205 and EV0207 were able to induce a long lasting C3 serum decrease. A reduction of up to 80 % 42 days after a single application of 10 mg/kg was obtained for EV0203 (Figure 9B).

Example 12

Various siRNAs targeting C3 are active *in vitro*.

C3 knockdown efficacy of siRNAs EJ0001, EJ0002, EJ0003 and EJ0004 was determined after transfection of 0.1 - 10 nM siRNA in Hep3B cells. The results are depicted in Figure 10. After transfection with EJ0001, a dose dependent reduction of C3 mRNA levels with a maximum of ~90% knockdown is observed. EJ0002, EJ0003 and EJ0004 are in a similar activity range.

For transfection of Hep3B cells with siRNAs, cells were seeded at a density of 12,000 cells/well into 96-well tissue culture plates. Transfection of siRNA was carried out with Atufect liposomal transfection reagent 24 h after seeding. The screen was performed with siRNAs targeting C3 in triplicates at 0.1, 1 and 10 nM. An siRNA targeting Firefly Luciferase ("Luc") was used as control. After 24 h of incubation with siRNAs, medium was removed, cells were lysed, and total RNA was extracted. C3 and ApoB mRNA levels were determined by TaqMan qRT-PCR. Each bar represents mean ± SD from three technical replicates.

Example 13

Various siRNAs targeting human C3 are active *in vitro*.

C3 knockdown efficacy of siRNAs EJ0001, EJ0005, EJ0006 and EJ0007 was determined after transfection of 0.01 - 1 nM siRNA in Hep3B cells. The results are depicted in Figure 11. After transfection with 1 nM, C3 mRNA knockdown is around 90% for all tested siRNAs and at 0.1 nM, knockdown is around 50% for EJ0001, EJ0006 and EJ0007. EJ0005 performs better with 80% knockdown at 0.1 nM.

For transfection of Hep3B cells with siRNAs, cells were seeded at a density of 8,000 cells/well into 96-well tissue culture plates. Transfection of siRNA was carried out with Atufect liposomal transfection reagent 24 h after seeding. The screen was performed in triplicates at 0.01, 0.1 and 1 nM siRNA concentration. An siRNA targeting Firefly Luciferase ("Luc") was used as control. After 24 h of incubation with siRNAs, medium was removed, cells were lysed and total RNA was extracted. C3 and PTEN mRNA levels were determined by TaqMan qRT-PCR. Each bar represents mean ± SD from three technical replicates.

Example 14

Various siRNAs targeting mouse C3 are active *in vitro*.

C3 knockdown efficacy of siRNAs EJ0001, EJ005, EJ0006 and EJ0007 was determined after transfection of 0.01 - 10 nM siRNA in AML12 cells. The results are depicted in Figure 12. After siRNA transfection, a dose-dependent C3 mRNA knockdown with a maximum of around 60% is reached.

For transfection of AML12 cells, cells were seeded at a density of 6,000 cells/well into 96-well tissue culture plates. Transfection of siRNA was carried out with Atufect liposomal transfection reagent 24 h after seeding. The screen was performed with siRNAs targeting C3 in triplicates at 0.01, 0.1, 1 and 10 nM. An siRNA targeting Firefly Luciferase ("Luc") was used as control. After 24 h of incubation with siRNAs, medium was removed, cells were lysed, and total RNA was extracted. C3 and PTEN mRNA levels were determined by TaqMan qRT-PCR. Each bar represents mean ± SD from three technical replicates.

Example 15

Various GalNAc-conjugated siRNAs targeting mouse C3 are active *in vitro*.

C3 mRNA knockdown efficiency of GalNAc siRNA conjugates EV0201, EJ0010, EJ0011, EJ0012, EJ0014 and EJ0015 was determined after receptor-mediated uptake in mouse primary hepatocytes. The results are depicted in Figure 13. A dose-dependent knockdown with a maximum of around 75% was achieved.

Mouse primary hepatocytes were seeded at a density of 25,000 cells/well into 96-well tissue culture plates and treated with 100, 10, 1 and 0.1 nM GalNAc-conjugated siRNAs directly upon plating. A GalNAc-conjugated, scrambled sequence was used as non-targeting control (NTC). The cells were lysed after 24 h of incubation with GalNAc-conjugates and total RNA was extracted. C3 and APOB mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD from three technical replicates.

Example 16

Various GalNAc-conjugated siRNAs targeting cynomolgus C3 are active *in vitro*.

C3 mRNA knockdown efficiency of GalNAc siRNA conjugates EV0201, EJ0010, EJ0011, EJ0012, EJ0014 and EJ0015 was determined after receptor-mediated uptake in cynomolgus primary hepatocytes. The results are depicted in Figure 14. A dose-dependent knockdown with a maximum of around 90% was achieved.

Cynomolgus primary hepatocytes were seeded at a density of 36,500 cells/well into 96-well tissue culture plates and treated with 100 and 10 nM GalNAc-conjugated siRNAs directly upon plating. A GalNAc-conjugated, scrambled sequence was used as non-targeting control (NTC). The cells were lysed after 24 h of incubation with GalNAc-conjugates and total RNA was

extracted. C3 and APOB mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD from three technical replicates.

Example 17

Variants of GalNAc-conjugated siRNAs repress C3 in primary mouse hepatocytes.

C3 mRNA knockdown efficiency of GalNAc-siRNA conjugates EV0201 and variants thereof (EJ0009, EV0203), EJ0014 and variants thereof (EJ0019, EJ0020), EJ0015 and variants thereof (EJ0021-23) as well as EJ0012 and variants thereof (EJ0016-18) was determined after receptor-mediated uptake in mouse primary hepatocytes. The results are depicted in Figure 15. A dose-dependent knockdown with a maximum of around 85% was achieved with some of the variants.

Mouse primary hepatocytes were seeded at a density of 25,000 cells/well into 96-well tissue culture plates and treated with 100, 10 and 1 nM GalNAc-conjugated siRNAs directly upon plating. A GalNAc-conjugated, scrambled sequence was used as non-targeting control (NTC). The cells were lysed after 24 h of incubation with GalNAc conjugates and total RNA was extracted. C3 and APOB mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD from three technical replicates.

Example 18

Variants of GalNAc-conjugated siRNAs repress C3 in primary cynomolgus hepatocytes

C3 mRNA knockdown efficiency of GalNAc-siRNA conjugates EV0201 and variants thereof (EJ0009, EV0203), EJ0014 and variants thereof (EJ0019, EJ0020), EJ0015 and variants thereof (EJ0021-23) as well as EJ0012 and variants thereof (EJ0016-18) was determined after receptor-mediated uptake in cynomolgus primary hepatocytes. The results are depicted in Figure 16. A dose-dependent knockdown with a maximum of around 90% was achieved with some of the variants.

Cynomolgus primary hepatocytes were seeded at a density of 40,000 cells/well into 96-well tissue culture plates and treated with 100, 10 and 1 nM GalNAc-conjugated siRNAs directly upon plating. A GalNAc-conjugated, scrambled sequence was used as non-targeting control (NTC). The cells were lysed after 24 h of incubation with GalNAc conjugates and total RNA

was extracted. C3 and ACTB mRNA levels were determined by Taqman qRT-PCR. Each bar represents mean ± SD from three technical replicates.

Example 19

In vitro study showing knockdown of C3 mRNA in murine liver tissue and serum protein after single subcutaneous dosing of 1 or 3 mg/kg of GalNAc conjugated C3 siRNA EJ0020.

Male C57BL/6N mice aged 8 weeks were obtained from Janvier, France. Animal experiments were performed according to ethical guidelines of the German Protection of Animals Act in its version of July 2013. Mice were randomized according to weight into groups of 4 mice. On day 0 of the study, animals received a single subcutaneous dose of 1 or 3 mg/kg siRNA dissolved in phosphate buffered saline (PBS) or PBS only as control. The viability, body weight and behaviour of the mice was monitored during the study without pathological findings. Serum samples were taken before the application, at day 7, day 14, day 28 and day 43. At day 43, the study was terminated, animals were euthanized, and liver samples were snap frozen and stored at – 80°C until further analysis.

For analysis, RNAs were isolated using the InviTrap Spin Tissue RNA Mini Kit from Stratec according to the manufacturer´s protocol. qPCR was performed using C3 and Actin specific primer probe sets and Takyon™ One-Step Low Rox Probe 5X MasterMix dTTP on the QuantStudio6 device from Applied Biosystems in single-plex 384 well format. Expression was calculated using the delta delta Ct method and relative expression of C3 versus the house keeping gene normalized to PBS was used for comparisons. 38 % C3 mRNA knock down was observed using 1 mg/kg siRNA EJ0020 and 73 % C3 knock down using 3 mg/kg of the same siRNA. Results are shown in Figure 17 (data are shown in bar charts as mean ± SD (n=4 per group)).

C3 protein levels in serum were obtained using commercially available C3 ELISA Kits. The analyses were carried out according to the manufacturer´s protocol, and C3 serum levels were calculated relative to the respective pre-dose levels. Results are shown in Figure 18 (data are shown as means ± SD (n=3 or 4 per group)).

Example 20

In vitro study in primary human hepatocytes showing C3 knock down efficacy of tested siRNA-GalNAc conjugates at 1, 10 and 100nM.

Expression of C3 mRNA after incubation with the GalNAc siRNA conjugates EV0210, EV0211, EV0212 and EV0213 at 1, 10 and 100nM is shown in Figure 19. The siRNA GalNAc conjugates are listed in Table 3. mRNA level of the gene APOB served as housekeeping control.

Human primary hepatocytes were seeded into collagen I-coated 96-well plates (Life Technologies) at a density of 40,000 cells per well. GalNAc-conjugated siRNAs were added immediately after plating to final siRNA concentrations of 100, 10 and 1 nM. Plates were then incubated at 37 °C in a 5% CO_2 atmosphere for 24 hours. Subsequently, cells were lysed, and RNA was isolated using InviTrap RNA Cell HTS96 Kit/C (Stratec).

Ten µl of RNA-solution was used for gene expression analysis by reverse transcription quantitative polymerase chain reaction (RT-qPCR) performed with amplicon sets/sequences for APOB (Eurogentec) and C3 (BioTez GmbH, Berlin, Germany), respectively. The RT-qPCR reactions were carried out with an ABI StepOne Plus (Applied Biosystems, part of Thermo Fisher Scientific, Massachusetts, USA) using standard protocols for RT-PCR (48 °C 30 min, 95 °C 10 min, 40 cycles at 95 °C 15 s followed by 60 °C 1 min). The data were calculated by using the comparative CT method also known as the 2-deltadelta Ct method.

SiRNAs EV0210, EV0211, EV0212 and EV0213 were able to inhibit C3 mRNA expression in primary human hepatocytes in a dose-dependent manner.

Example 21

In vivo study of the efficacy of C3 siRNA GalNAc conjugates in a murine disease model of C3 Glomerulopathy (C3G).

EV0203 was tested in a murine disease model of C3 Glomerulopathy and in wild-type mice. Heterozygous complement factor H deficient mice (Cfh def.) were used as the C3 Glomerulopathy disease model. The animals were treated with a single dose of EV0203 or with controls (PBS or non-targeting siRNAs). The mice were sacrificed 10 days after treatment and tubular deposition of C3 was measured in the kidneys of the mice by C3 staining with an anti-C3 antibody and quantified (results are shown in Figure 20). Complement factor B (FB) fragmentation, which is determined as the ratio of Ba fragments to full length FB, was also measured in the plasma by western blot and quantified – results are shown in Figure 21. The results of the C3 staining show that tubular C3 deposits are significantly decreased in a dose-dependent manner by treatment with a conjugated C3 siRNA. The FB fragmentation data show

that Cfh def. mice have increased levels of FB fragmentation compared to wild-type mice, but that this increased fragmentation level can be reduced by treatment with a conjugated C3 siRNA. Conjugated C3 siRNAs are therefore expected to be a powerful treatment for C3-related diseases and in particular for C3 Glomerulopathy and/or at least its symptoms.

Example 22

In vivo study of the efficacy of multiple doses of a C3 siRNA GalNAc conjugate in a murine disease model of C3 Glomerulopathy (C3G).

EV0203 was tested in a murine disease model of C3 Glomerulopathy. Heterozygous complement factor H deficient mice (Cfh def.) were used as the C3 Glomerulopathy disease model. Seven- to eight-month-old mice were treated on the first day of the study and then monthly with 5 mg/kg of EV0203 or with PBS or a none-targeting siRNA as a control. Wild type mice treated with PBS were also used as a control. The mice were sacrificed three months after the start of the study (i.e., after three treatments with a conjugated C3 siRNA or a control). The levels of C3α-chain and C3α-chain fragments (activated C3 fragments) in the plasma were measured by western blot and quantified (results are shown in Figures 22A and 22B). Complement factor B (FB) fragmentation, which is determined as the ratio of Ba fragments to full length FB, was also measured in the plasma by western blot and quantified – results are shown in Figure 23. These data confirm that conjugated C3 siRNAs are effective in reducing C3 fragment levels and FB fragmentation in aged mice.

Glomerular C3d deposits were also measured, by C3d staining. Three doses of the conjugated C3 siRNA were able to reduce glomerular C3d deposits of Cfh def. mice to levels similar to those of age-matched wild-type mice. Conjugated C3 siRNAs are therefore expected to be a powerful treatment for C3-related diseases and in particular for C3 Glomerulopathy and/or at least its symptoms.

Example 23

Conjugated C3 siRNAs from the above examples, including EV0210, EV0212 and EJ0020, were tested *in vivo* in healthy cynomolgus monkeys. The animals were treated once or multiple times with different doses of conjugated C3 siRNAs. Preliminary data from monkeys treated with the tested conjugated C3 siRNAs show reduced C3 protein levels in serum. These *in vivo* experiments are currently ongoing. Additional testing of conjugated C3 siRNAs *in vivo* in healthy cynomolgus monkeys is planned. After treatment once or multiple times with different

doses of conjugated C3 siRNAs, C3 protein levels are measured in serum and C3 mRNA levels are measured in liver tissues. The C3 protein levels in serum and the C3 mRNA levels in the liver are both expected to be reduced after treatment with an effective dose of a conjugated C3 siRNA.

Statements

The following statements represent aspects of the invention.

1. A double-stranded nucleic acid for inhibiting expression of complement component C3, wherein the nucleic acid comprises a first strand and a second strand, wherein the first strand sequence comprises a sequence of at least 15 nucleotides differing by no more than 3 nucleotides from any one of the sequences SEQ ID NO: 370, 364, 365, 366, 368, 372, 377, 361, 95, 111, 125, 131, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 97, 99, 101, 103, 105, 107, 109, 113, 115, 117, 119, 121, 123, 127, 129, 133 or 416.

2. The nucleic acid of statement 1, wherein
   (a) the first strand sequence comprises a sequence of at least 18 nucleotides differing by no more than 3 nucleotides from any one of the first strand sequences of Table 1 and optionally wherein the second strand sequence comprises a sequence of at least 18 nucleotides differing by no more than 3 nucleotides from the second strand sequence in the same line of the table;
   (b) the first strand sequence comprises a sequence of at least 18 nucleotides differing by no more than 1 nucleotide from any one of the first strand sequences of Table 1 and optionally wherein the second strand sequence comprises a sequence of at least 18 nucleotides differing by no more than 1 nucleotide from the second strand sequence in the same line of the table;
   (c) the first strand sequence comprises a sequence of at least 18 nucleotides of any one of the first strand sequences of Table 1 and optionally wherein the second strand sequence comprises a sequence of at least 18 nucleotides of the second strand sequence in the same line of the table; or
   (d) the first strand sequence consists of any one of the first strand sequences of Table 1 and optionally wherein the second strand sequence consists of the sequence of the second strand sequence in the same line of the table;

   wherein Table 1 is:

First strand sequence (SEQ ID NO:)	Second strand sequence (SEQ ID NO:)
364	363 or 375
365	363
366	367 or 376
368	369
370	379 or 371, preferably 379
372	373
362	374
377	378
361	112
95	96
111	112
125	126
131	132
1	2
3	4
5	6
7	8
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	36
37	38
39	40
41	42

43	44
45	46
47	48
49	50
51	52
53	54
55	56
57	58
59	60
61	62
63	64
65	66
67	68
69	70
71	72
73	74
75	76
77	78
79	80
81	82
83	84
85	86
87	88
89	90
91	92
93	94
97	98
99	100
101	102
103	104
105	106
107	108
109	110
113	114
115	116
117	118

119	120
121	122
123	124
127	128
129	130
133	134
416	26

3. The nucleic acid of any of the preceding statements, wherein the first strand sequence comprises the sequence of SEQ ID NO: 361 and optionally wherein the second strand sequence comprises a sequence of at least 15 nucleotides of the sequence of SEQ ID NO: 112; or wherein the first strand sequence comprises the sequence of SEQ ID NO: 95 and optionally wherein the second strand sequence comprises a sequence of at least 15 nucleotides of the sequence of SEQ ID NO: 96; or wherein the first strand sequence comprises the sequence of SEQ ID NO: 125 and optionally wherein the second strand sequence comprises a sequence of at least 15 nucleotides of the sequence of SEQ ID NO: 126; or wherein the first strand sequence comprises the sequence of SEQ ID NO: 131 and optionally wherein the second strand sequence comprises a sequence of at least 15 nucleotides of the sequence of SEQ ID NO: 132; or wherein the first strand sequence comprises the sequence of SEQ ID NO: 364 and optionally wherein the second strand sequence comprises a sequence of at least 15 nucleotides of the sequence of SEQ ID NO: 363 or 375; or wherein the first strand sequence comprises the sequence of SEQ ID NO: 365 and optionally wherein the second strand sequence comprises a sequence of at least 15 nucleotides of the sequence of SEQ ID NO: 363; or wherein the first strand sequence comprises the sequence of SEQ ID NO: 366 and optionally wherein the second strand sequence comprises a sequence of at least 15 nucleotides of the sequence of SEQ ID NO:367 or 376; or wherein the first strand sequence comprises the sequence of SEQ ID NO: 368 and optionally wherein the second strand sequence comprises a sequence of at least 15 nucleotides of the sequence of SEQ ID NO: 369; or wherein the first strand sequence comprises the sequence of SEQ ID NO: 370 and optionally wherein the second strand sequence comprises a sequence of at least 15 nucleotides of the sequence of SEQ ID NO: 371 or 379, preferably 379; or wherein the first strand sequence comprises the sequence of SEQ ID NO: 372 and optionally wherein the second strand sequence comprises a sequence of at least 15 nucleotides of the sequence of SEQ ID NO: 373 or 380; or wherein the first strand sequence comprises the sequence of SEQ ID NO: 362 and optionally wherein the second strand sequence comprises a sequence of at least 15

nucleotides of the sequence of SEQ ID NO: 374; or wherein the first strand sequence comprises the sequence of SEQ ID NO: 377 and optionally wherein the second strand sequence comprises a sequence of at least 15 nucleotides of the sequence of SEQ ID NO: 378; or wherein the first strand sequence comprises the sequence of SEQ ID NO: 416 and optionally wherein the second strand sequence comprises a sequence of at least 15 nucleotides of the sequence of SEQ ID NO: 26.

4. A double-stranded nucleic acid that is capable of inhibiting expression of complement component C3 for use as a medicament, wherein the nucleic acid comprises a first strand and a second strand.

5. The nucleic acid of any of the preceding statements, wherein the first strand and the second strand are separate strands and are each 18-25 nucleotides in length.

6. The nucleic acid of any of the preceding statements, wherein the first strand and the second strand form a duplex region of from 17-25 nucleotides in length.

7. The nucleic acid of any of the preceding statements, wherein the duplex region consists of 17-25 consecutive nucleotide base pairs.

8. The nucleic acid of any of the preceding statements, wherein said nucleic acid:
   a) is blunt ended at both ends;
   b) has an overhang at one end and a blunt end at the other end; or
   c) has an overhang at both ends.

9. The nucleic acid of any of the preceding statements, wherein the nucleic acid is a siRNA.

10. The nucleic acid of any of the preceding statements, wherein the nucleic acid mediates RNA interference.

11. The nucleic acid of any of the preceding statements, wherein at least one nucleotide of the first and/or second strand is a modified nucleotide.

12. The nucleic acid of any of the preceding statements, wherein at least nucleotides 2 and 14 of the first strand are modified by a first modification, the nucleotides being numbered consecutively starting with nucleotide number 1 at the 5' end of the first strand.

13 The nucleic acid of any of the preceding statements, wherein each of the even-numbered nucleotides of the first strand are modified by a first modification, the nucleotides being numbered consecutively starting with nucleotide number 1 at the 5' end of the first strand.

14. The nucleic acid of any of statements 12-13, wherein the odd-numbered nucleotides of the first strand are modified by a second modification, wherein the second modification is different from the first modification.

15. The nucleic acid of statements 12-14, wherein the nucleotides of the second strand in a position corresponding to an even-numbered nucleotide of the first strand are modified by a third modification, wherein the third modification is different from the first modification.

16. The nucleic acid of statements 12-15, wherein the nucleotides of the second strand in a position corresponding to an odd-numbered nucleotide of the first strand are modified by a fourth modification, wherein the fourth modification is different from the second modification and different from the third modification when a second and/or a third modification are present.

17. The nucleic acid of statements 12-14, wherein the nucleotide/nucleotides of the second strand in a position corresponding to nucleotide 11 or nucleotide 13 or nucleotides 11 and 13 or nucleotides 11-13 of the first strand is/are modified by a fourth modification and preferably wherein the nucleotides of the second strand that are not modified by a fourth modification are modified by a third modification.

18. The nucleic acid of statements 12-17, wherein the first modification is the same as the fourth modification if both modifications are present in the nucleic acid and preferably wherein the second modification is the same as the third modification if both modifications are present in the nucleic acid.

19. The nucleic acid of statements 12-18, wherein the first modification is a 2'-F modification; the second modification, if present in the nucleic acid, is preferably a 2'-OMe modification; the third modification, if present in the nucleic acid, is preferably a 2'-OMe modification; and the fourth modification, if present in the nucleic acid, is preferably a 2'-F modification.

20. The nucleic acid of any of the preceding statements, wherein each of the nucleotides of the first strand and of the second strand is a modified nucleotide.

21. The nucleic acid of any of the previous statements, wherein the first strand has a terminal 5' (E)-vinylphosphonate nucleotide at its 5' end and wherein the terminal 5' (E)-vinylphosphonate nucleotide is preferably linked to the second nucleotide in the first strand by a phosphodiester linkage.

22. The nucleic acid of any of the preceding statements, wherein the nucleic acid comprises a phosphorothioate linkage between the terminal two or three 3' nucleotides and/or 5' nucleotides of the first and/or the second strand and preferably wherein the linkages between the remaining nucleotides are phosphodiester linkages.

23. The nucleic acid of any of statements 1-21, comprising a phosphorodithioate linkage between each of the two, three or four terminal nucleotides at the 3' end of the first strand and/or comprising a phosphorodithioate linkage between each of the two, three or four terminal nucleotides at the 3' end of the second strand and/or a phosphorodithioate linkage between each of the two, three or four terminal nucleotides at the 5' end of the second strand and comprising a linkage other than a phosphorodithioate linkage between the two, three or four terminal nucleotides at the 5' end of the first strand.

24. The nucleic acid of statement 23, wherein the nucleic acid comprises a phosphorothioate linkage between each of the three terminal 3' nucleotides and/or between each of the three terminal 5' nucleotides on the first strand, and/or between each of the three terminal 3' nucleotides and/or between each of the three terminal 5' nucleotides of the second strand when there is no phosphorodithioate linkage present at that end.

25 The nucleic acid of statement 23, wherein all the linkages between the nucleotides of both strands other than the linkage between the two terminal nucleotides at the 3' end of the first strand and the linkages between the two terminal nucleotides at the 3' end and at the 5' end of the second strand are phosphodiester linkages.

26. The nucleic acid of any of the preceding statements, wherein the nucleic acid is conjugated to a ligand.

27. The nucleic acid of statement 26, wherein the ligand comprises (i) one or more N-acetyl galactosamine (GalNAc) moieties or derivatives thereof, and (ii) a linker, wherein the linker conjugates the at least one GalNAc moiety or derivative thereof to the nucleic acid.

28. The nucleic acid of any of statements 1-27, wherein the nucleic acid is conjugated to a ligand comprising a compound of formula (II):

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

wherein:

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

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

P is a phosphate or modified phosphate, preferably a thiophosphate;

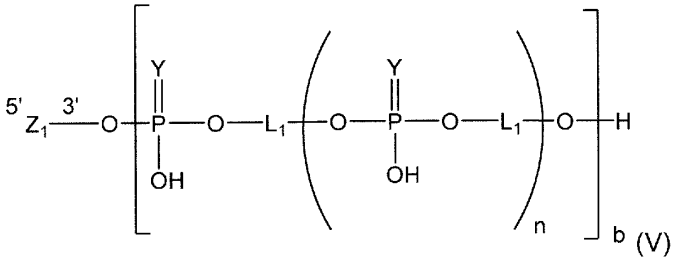
X^2 is alkylene or an alkylene ether of the formula $(\text{-}CH_2)_n\text{-}O\text{-}CH_2\text{-}$ where n = 1- 6;

A is a branching unit;

X_3 represents a bridging unit;

wherein a nucleic acid as defined in any of statements 1 to 27 is conjugated to X^3 via a phosphate or modified phosphate, preferably a thiophosphate.

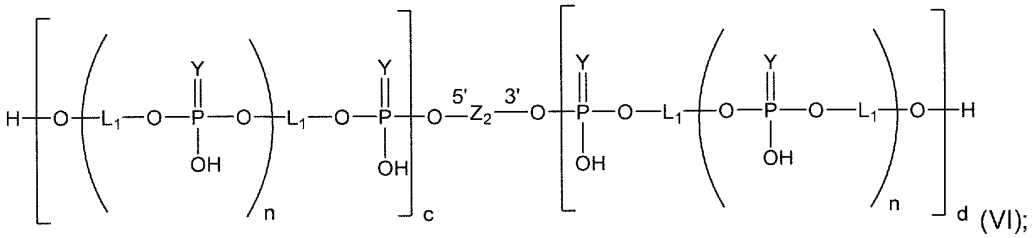
29. The nucleic acid of any of statements 1-27, wherein the first strand of the nucleic acid is a compound of formula (V):



(V)

wherein b is 0 or 1; and

wherein the second strand is a compound of formula (VI):



(VI);

wherein:

c and d are independently 0 or 1;

Z_1 and Z_2 are respectively the first and second strand of the nucleic acid;

Y is independently O or S;

n is independently 0, 1, 2 or 3; and

L_1 is a linker to which a ligand is attached, wherein L_1 is the same or different in formulae (V) and (VI), and is the same or different within formulae (V) and (VI) when L_1 is present more than once within the same formula;

and wherein b + c + d is 2 or 3.

30. A composition comprising a nucleic acid of any of the previous statements and a solvent and/or a delivery vehicle and/or a physiologically acceptable excipient and/or a carrier and/or a salt and/or a diluent and/or a buffer and/or a preservative.

31. A composition comprising a nucleic acid of any of statements 1-29 and a further therapeutic agent selected from the group comprising an oligonucleotide, a small molecule, a monoclonal antibody, a polyclonal antibody and a peptide.

32. A nucleic acid of any of statements 1-3 or 5-29 or a composition of any of statements 30-31 for use as a medicament.

33. A nucleic acid of any of statements 1-29 or a composition of any of statements 30-32 for use in the prevention, decrease of the risk of suffering from, or treatment of a disease, disorder or syndrome.

34. The nucleic acid or composition of statement 33, wherein the disease, disorder or syndrome is a complement-mediated disease, disorder or syndrome.

35. The nucleic acid or composition of any of statements 33-34, wherein the disease, disorder or syndrome is associated with aberrant activation or over-activation of the complement pathway and/or with over-expression or ectopic expression or localisation or accumulation of C3.

36. The nucleic acid or composition of any of statements 33-35, wherein the disease, disorder or syndrome is:

a) selected from the group comprising C3 Glomerulopathy (C3G), Paroxysmal Nocturnal Hemoglobinuria (PNH), atypical Hemolytic Uremic Syndrome (aHUS), Lupus nephritis, IgA nephropathy (IgA N), Cold Agglutinin Disease (CAD), Myasthenia gravis (MG), Primary Membranous Nephropathy, Immune Complex-mediated Glomerulonephritis (IC-mediated GN), post-Infectious

Glomerulonephritis (PIGN), Systemic Lupus Erythematosus (SLE), Ischemia/reperfusion injury, age-related macular degeneration (AMD), Rheumatoid arthritis (RA), antineutrophil Cytoplasmic Autoantibodies-associated Vasculitis (ANCA-AV), dysbiotic periodontal Disease, Malarial Anaemia, Neuromyelitis Optica, Post-HCT / Solid Organ Transplant (TMAs), Guillain-Barré Syndrome, Membranous Glomerulonephritis, Thrombotic Thrombocytopenic Purpura and sepsis;

b) selected from the group comprising C3 Glomerulopathy (C3G), Paroxysmal Nocturnal Hemoglobinuria (PNH), atypical Hemolytic Uremic Syndrome (aHUS), Lupus nephritis, IgA nephropathy (IgA N) and Primary Membranous Nephropathy;

c) selected from the group comprising C3 Glomerulopathy (C3G), antineutrophil Cytoplasmic Autoantibodies-associated Vasculitis (ANCA-AV), atypical Hemolytic Uremic Syndrome (aHUS), Cold Agglutinin Disease (CAD), Myasthenia gravis (MG), IgA nephropathy (IgA N), Paroxysmal Nocturnal Hemoglobinuria (PNH);

d) selected from the group comprising C3 Glomerulopathy (C3G), Cold Agglutinin Disease (CAD), Myasthenia gravis (MG), Neuromyelitis Optica, atypical Hemolytic Uremic Syndrome (aHUS), antineutrophil Cytoplasmic Autoantibodies-associated Vasculitis (ANCA-AV), IgA nephropathy (IgA N), Post-HCT / Solid Organ Transplant (TMAs), Guillain-Barré Syndrome, Paroxysmal Nocturnal Hemoglobinuria (PNH), Membranous Glomerulonephritis, Lupus nephritis and Thrombotic Thrombocytopenic Purpura

e) selected from the group comprising C3 Glomerulopathy (C3G), Cold Agglutinin Disease (CAD) and IgA nephropathy (IgA N); or

f) C3 Glomerulopathy (C3G).

37. Use of a nucleic acid of any of statements 1-29 or a composition of any of statements 30-31 in the prevention, decrease of the risk of suffering from, or treatment of a disease, disorder or syndrome, wherein the disease, disorder or syndrome is preferably C3 Glomerulopathy (C3G).

38. A method of preventing, decreasing the risk of suffering from, or treating a disease, disorder or syndrome comprising administering a pharmaceutically effective dose of a nucleic acid of any of statements 1-29 or 32-36 or a composition of any of statements 30-36 to an individual in need of treatment, preferably wherein the nucleic acid or composition is administered to the subject subcutaneously, intravenously or by oral, rectal or intraperitoneal administration.

Summary tables

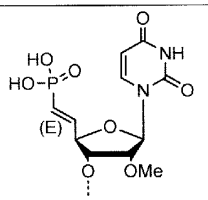
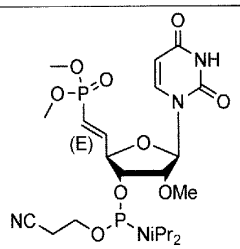
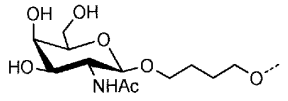
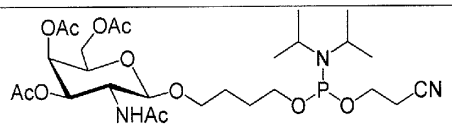
Summary duplex table – Table 3

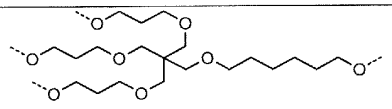
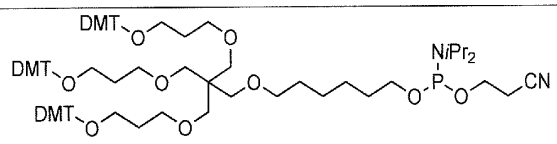
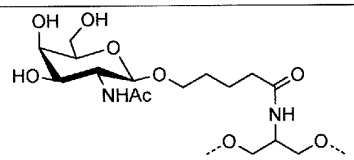
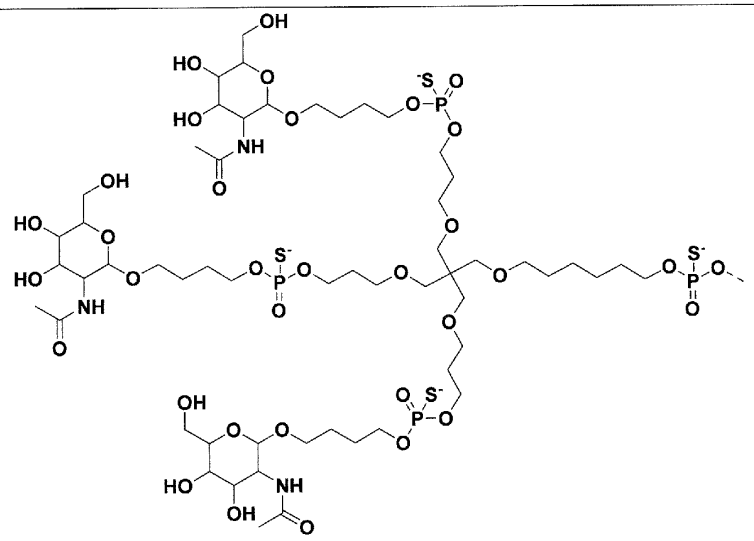
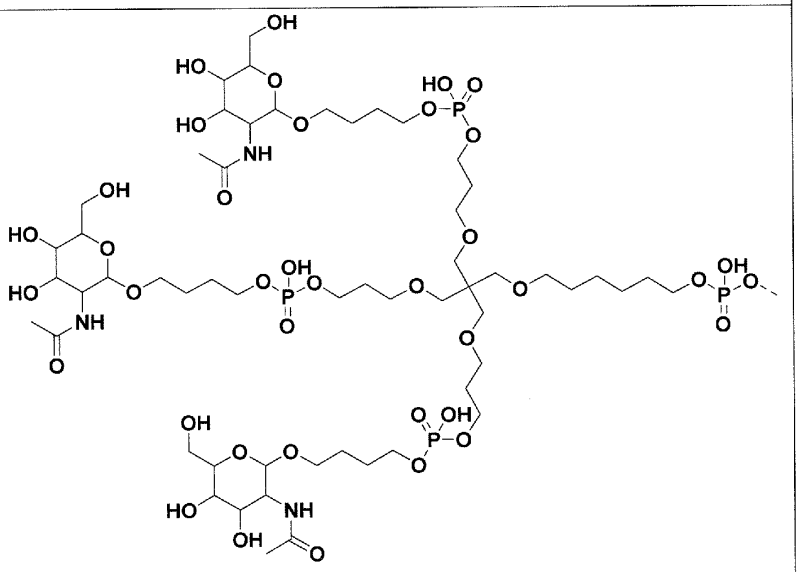
Duplex	Single Strands	Duplex	Single Strands	Duplex	Single Strands
EV0001	EV0001A EV0001B	EV0051	EV0051A EV0051B	EV0101	EV0101A EV0101B
EV0002	EV0002A EV0002B	EV0052	EV0052A EV0052B	EV0102	EV0102A EV0102B
EV0003	EV0003A EV0003B	EV0053	EV0053A EV0053B	EV0103	EV0103A EV0103B
EV0004	EV0004A EV0004B	EV0054	EV0054A EV0054B	EV0104	EV0104A EV0104B
EV0005	EV0005A EV0005B	EV0055	EV0055A EV0055B	EV0105	EV0105A EV0105B
EV0006	EV0006A EV0006B	EV0056	EV0056A EV0056B	EV0106	EV0106A EV0106B
EV0007	EV0007A EV0007B	EV0057	EV0057A EV0057B	EV0107	EV0107A EV0107B
EV0008	EV0008A EV0008B	EV0058	EV0058A EV0058B	EV0108	EV0108A EV0108B
EV0009	EV0009A EV0009B	EV0059	EV0059A EV0059B	EV0109	EV0109A EV0109B
EV0010	EV0010A EV0010B	EV0060	EV0060A EV0060B	EV0110	EV0110A EV0110B
EV0011	EV0011A EV0011B	EV0061	EV0061A EV0061B	EV0111	EV0111A EV0111B
EV0012	EV0012A EV0012B	EV0062	EV0062A EV0062B	EV0201	EV0201A EV0201B
EV0013	EV0013A EV0013B	EV0063	EV0063A EV0063B	EV0202	EV0201A EV0202B
EV0014	EV0014A EV0014B	EV0064	EV0064A EV0064B	EV0203	EV0203A EV0201B
EV0015	EV0015A EV0015B	EV0065	EV0065A EV0065B	EV0204	EV0203A EV0202B
EV0016	EV0016A EV0016B	EV0066	EV0066A EV0066B	EV0205	EV0110A EV0205B
EV0017	EV0017A EV0017B	EV0067	EV0067A EV0067B	EV0206	EV0110A EV0206B
EV0018	EV0018A EV0018B	EV0068	EV0068A EV0068B	EV0207	EV0207A EV0205B
EV0019	EV0019A EV0019B	EV0069	EV0037A EV0069B	EV0208	EV0207A EV0206B
EV0020	EV0020A EV0020B	EV0070	EV0038A EV0070B	EV0209	EV0209A EV0205B
EV0021	EV0021A EV0021B	EV0071	EV0039A EV0071B	EV0312	EV0312A EV0112B

EV0022	EV0022A EV0022B	EV0072	EV0040A EV0072B	EV0313	EV0313A EV0313B
EV0023	EV0023A EV0023B	EV0073	EV0041A EV0073B	EJ0001	EJ0001A EJ0001B
EV0024	EV0024A EV0024B	EV0074	EV0042A EV0074B	EJ0002	EJ0002A EJ0001B
EV0025	EV0025A EV0025B	EV0075	EV0043A EV0075B	EJ0003	EJ0003A EJ0001B
EV0026	EV0026A EV0026B	EV0076	EV0044A EV0076B	EJ0004	EJ0004A EJ0004B
EV0027	EV0027A EV0027B	EV0077	EV0045A EV0077B	EJ0005	EJ0005A EJ0005B
EV0028	EV0028A EV0028B	EV0078	EV0046A EV0078B	EJ0006	EJ0006A EJ0006B
EV0029	EV0029A EV0029B	EV0079	EV0047A EV0079B	EJ0007	EJ0007A EJ0007B
EV0030	EV0030A EV0030B	EV0080	EV0048A EV0080B	EJ0008	EJ0001A EJ0008B
EV0031	EV0031A EV0031B	EV0081	EV0049A EV0081B	EJ0009	EJ0001A EJ0009B
EV0032	EV0032A EV0032B	EV0082	EV0050A EV0082B	EJ0010	EJ0002A EJ0010B
EV0033	EV0033A EV0033B	EV0083	EV0051A EV0083B	EJ0011	EJ0004A EJ0011B
EV0034	EV0034A EV0034B	EV0084	EV0052A EV0084B	EJ0012	EJ0012A EJ0012B
EV0035	EV0035A EV0035B	EV0085	EV0053A EV0085B	EJ0013	EJ0005A EJ0013B
EV0036	EV0036A EV0036B	EV0086	EV0054A EV0086B	EJ0014	EJ0006A EJ0014B
EV0037	EV0037A EV0037B	EV0087	EV0055A EV0087B	EJ0015	EJ0007A EJ0015B
EV0038	EV0038A EV0038B	EV0088	EV0056A EV0088B	EJ0016	EJ0016A EJ0012B
EV0039	EV0039A EV0039B	EV0089	EV0057A EV0089B	EJ0017	EJ0017A EJ0017B
EV0040	EV0040A EV0040B	EV0090	EV0058A EV0090B	EJ0018	EJ0018A EJ0017B
EV0041	EV0041A EV0041B	EV0091	EV0059A EV0091B	EJ0019	EJ0019A EJ0014B
EV0042	EV0042A EV0042B	EV0092	EV0060A EV0092B	EJ0020	EJ0020A EJ0020B
EV0043	EV0043A EV0043B	EV0093	EV0061A EV0093B	EJ0021	EJ0021A EJ0015B
EV0044	EV0044A EV0044B	EV0094	EV0062A EV0094B	EJ0022	EJ0022A EJ0022B

EV0045	EV0045A EV0045B	EV0095	EV0063A EV0095B	EJ0023	EJ0023A EJ0022B
EV0046	EV0046A EV0046B	EV0096	EV0064A EV0096B	EV0210	EV0210A EV0210B
EV0047	EV0047A EV0047B	EV0097	EV0065A EV0097B	EV0211	EV0211A EV0210B
EV0048	EV0048A EV0048B	EV0098	EV0066A EV0098B	EV0212	EV0212A EV0211B
EV0049	EV0049A EV0049B	EV0099	EV0067A EV0099B	EV0213	EV0213A EV0211B
EV0050	EV0050A EV0050B	EV0100	EV0068A EV0100B		

Summary abbreviations table – Table 4

Abbreviation	**Meaning**
mA, mU, mC, mG	2'-O-Methyl RNA nucleotides
2'-OMe	2'-O-Methyl modification
fA, fU, fC, fG	2' deoxy-2'-F RNA nucleotides
2'-F	2'-fluoro modification
(ps)	phosphorothioate
(ps2)	phosphorodithioate
(vp)	Vinyl-(E)-phosphonate
(vp)-mU	
(vp)-mU-phos	
ivA, ivC, ivU, ivG	inverted RNA (3'-3') nucleotides
ST23	
ST23-phos	

ST43 (or C6XLT)	
ST43-phos (or C6XLT-phos)	
Ser(GN) (when at the end of a chain, one of the O--- is OH)	
[ST23 (ps)]3 ST43 (ps)	
[ST23]3 ST43	

The abbreviations as shown in the above abbreviation table may be used herein. The list of abbreviations may not be exhaustive and further abbreviations and their meaning may be found throughout this document.

Summary sequence table – Table 5

SEQ ID NO:	Name (A=1st strand; B=2nd strand)	Sequence 5´-3´	Unmodified sequence 5´-3´ counterpart
1	EV0001Aun	UUUCAUAGUAGGCUCGGAU	UUUCAUAGUAGGCUCGGAU
2	EV0001Bun	AUCCGAGCCUACUAUGAAA	AUCCGAGCCUACUAUGAAA
3	EV0002Aun	UUUCUCUGUAGGCUCCACU	UUUCUCUGUAGGCUCCACU
4	EV0002Bun	AGUGGAGCCUACAGAGAAA	AGUGGAGCCUACAGAGAAA
5	EV0003Aun	UUAUAGAUGUAGUAGAAUU	UUAUAGAUGUAGUAGAAUU
6	EV0003Bun	AAUUCUACUACAUCUAUAA	AAUUCUACUACAUCUAUAA
7	EV0004Aun	AUGACAAAGGCAGUUCCCU	AUGACAAAGGCAGUUCCCU
8	EV0004Bun	AGGGAACUGCCUUUGUCAU	AGGGAACUGCCUUUGUCAU
9	EV0005Aun	AUCUGGUAGGGAGAGGUCA	AUCUGGUAGGGAGAGGUCA
10	EV0005Bun	UGACCUCUCCCUACCAGAU	UGACCUCUCCCUACCAGAU
11	EV0006Aun	UGUGUGUUGAUGCUGAGUU	UGUGUGUUGAUGCUGAGUU
12	EV0006Bun	AACUCAGCAUCAACACACA	AACUCAGCAUCAACACACA
13	EV0007Aun	UAAUUGUUGGAGUUGCCCA	UAAUUGUUGGAGUUGCCCA
14	EV0007Bun	UGGGCAACUCCAACAAUUA	UGGGCAACUCCAACAAUUA
15	EV0008Aun	AGGAAGUUGACGUUGAGGG	AGGAAGUUGACGUUGAGGG
16	EV0008Bun	CCCUCAACGUCAACUUCCU	CCCUCAACGUCAACUUCCU
17	EV0009Aun	AUGAAGUCGGUGGUGAUGG	AUGAAGUCGGUGGUGAUGG
18	EV0009Bun	CCAUCACCACCGACUUCAU	CCAUCACCACCGACUUCAU
19	EV0010Aun	UUUACCACCAGCGAGCCCA	UUUACCACCAGCGAGCCCA
20	EV0010Bun	UGGGCUCGCUGGUGGUAAA	UGGGCUCGCUGGUGGUAAA
21	EV0011Aun	UCUAUCUUCAGGGUCAUCU	UCUAUCUUCAGGGUCAUCU
22	EV0011Bun	AGAUGACCCUGAAGAUAGA	AGAUGACCCUGAAGAUAGA
23	EV0012Aun	AUGUAGUUGCAGCAGUCCA	AUGUAGUUGCAGCAGUCCA
24	EV0012Bun	UGGACUGCUGCAACUACAU	UGGACUGCUGCAACUACAU
25	EV0013Aun	AAUAUAUUCAUGAGCUUCG	AAUAUAUUCAUGAGCUUCG

26	EV0013Bun	CGAAGCUCAUGAAUAUAUU	CGAAGCUCAUGAAUAUAUU
27	EV0014Aun	AAAUAUAUUCAUGAGCUUC	AAAUAUAUUCAUGAGCUUC
28	EV0014Bun	GAAGCUCAUGAAUAUAUUU	GAAGCUCAUGAAUAUAUUU
29	EV0015Aun	UCCUGCAUUACUGUGACCU	UCCUGCAUUACUGUGACCU
30	EV0015Bun	AGGUCACAGUAAUGCAGGA	AGGUCACAGUAAUGCAGGA
31	EV0016Aun	CAACAGAGUAGGGUAGCCG	CAACAGAGUAGGGUAGCCG
32	EV0016Bun	CGGCUACCCUACUCUGUUG	CGGCUACCCUACUCUGUUG
33	EV0017Aun	UCGAACAACAGAGUAGGGU	UCGAACAACAGAGUAGGGU
34	EV0017Bun	ACCCUACUCUGUUGUUCGA	ACCCUACUCUGUUGUUCGA
35	EV0018Aun	UUUCGAACAACAGAGUAGG	UUUCGAACAACAGAGUAGG
36	EV0018Bun	CCUACUCUGUUGUUCGAAA	CCUACUCUGUUGUUCGAAA
37	EV0019Aun	GUUUCAUCCAGGUAAUGCA	GUUUCAUCCAGGUAAUGCA
38	EV0019Bun	UGCAUUACCUGGAUGAAAC	UGCAUUACCUGGAUGAAAC
39	EV0020Aun	UUAUCUUUGGCUGUGGUCA	UUAUCUUUGGCUGUGGUCA
40	EV0020Bun	UGACCACAGCCAAAGAUAA	UGACCACAGCCAAAGAUAA
41	EV0021Aun	UGUUCAUUGAGCCAACGCA	UGUUCAUUGAGCCAACGCA
42	EV0021Bun	UGCGUUGGCUCAAUGAACA	UGCGUUGGCUCAAUGAACA
43	EV0023Aun	UUGGUAUUGAGCCAAGGCU	UUGGUAUUGAGCCAAGGCU
44	EV0023Bun	AGCCUUGGCUCAAUACCAA	AGCCUUGGCUCAAUACCAA
45	EV0024Aun	UUUAUUACAGGUGAGUUGA	UUUAUUACAGGUGAGUUGA
46	EV0024Bun	UCAACUCACCUGUAAUAAA	UCAACUCACCUGUAAUAAA
47	EV0025Aun	UUUCUGUUUCCGGUGCUGG	UUUCUGUUUCCGGUGCUGG
48	EV0025Bun	CCAGCACCGGAAACAGAAA	CCAGCACCGGAAACAGAAA
49	EV0026Aun	UCAAGGAUCAUAGUGUUCU	UCAAGGAUCAUAGUGUUCU
50	EV0026Bun	AGAACACUAUGAUCCUUGA	AGAACACUAUGAUCCUUGA
51	EV0027Aun	AUCUCAAGGAUCAUAGUGU	AUCUCAAGGAUCAUAGUGU
52	EV0027Bun	ACACUAUGAUCCUUGAGAU	ACACUAUGAUCCUUGAGAU
53	EV0028Aun	UCAUACUUGGAGAUGUAUC	UCAUACUUGGAGAUGUAUC
54	EV0028Bun	GAUACAUCUCCAAGUAUGA	GAUACAUCUCCAAGUAUGA

55	EV0029Aun	UAUCGGAGAAGGCUUUGUC	UAUCGGAGAAGGCUUUGUC
56	EV0029Bun	GACAAAGCCUUCUCCGAUA	GACAAAGCCUUCUCCGAUA
57	EV0030Aun	AUGAUGAGGGUGUUCCUAU	AUGAUGAGGGUGUUCCUAU
58	EV0030Bun	AUAGGAACACCCUCAUCAU	AUAGGAACACCCUCAUCAU
59	EV0031Aun	UAUUGGUGAACUUUGAAAG	UAUUGGUGAACUUUGAAAG
60	EV0031Bun	CUUUCAAAGUUCACCAAUA	CUUUCAAAGUUCACCAAUA
61	EV0032Aun	AGCUUGUUCAGCUUUCCAU	AGCUUGUUCAGCUUUCCAU
62	EV0032Bun	AUGGAAAGCUGAACAAGCU	AUGGAAAGCUGAACAAGCU
63	EV0033Aun	UUUGUAUGAAGCAAUUCUC	UUUGUAUGAAGCAAUUCUC
64	EV0033Bun	GAGAAUUGCUUCAUACAAA	GAGAAUUGCUUCAUACAAA
65	EV0034Aun	GUCUUGUACACAUAGUCCA	GUCUUGUACACAUAGUCCA
66	EV0034Bun	UGGACUAUGUGUACAAGAC	UGGACUAUGUGUACAAGAC
67	EV0035Aun	UGCUCAAUGGCCAUGAUGU	UGCUCAAUGGCCAUGAUGU
68	EV0035Bun	ACAUCAUGGCCAUUGAGCA	ACAUCAUGGCCAUUGAGCA
69	EV0036Aun	UUGGCAUUCGUCCUCCUCG	UUGGCAUUCGUCCUCCUCG
70	EV0036Bun	CGAGGAGGACGAAUGCCAA	CGAGGAGGACGAAUGCCAA
71	EV0037Aun	GUCUGGAUGAAGAGGUACC	GUCUGGAUGAAGAGGUACC
72	EV0037Bun	GGUACCUCUUCAUCCAGAC	GGUACCUCUUCAUCCAGAC
73	EV0038Aun	UGUCUGGAUGAAGAGGUAC	UGUCUGGAUGAAGAGGUAC
74	EV0038Bun	GUACCUCUUCAUCCAGACA	GUACCUCUUCAUCCAGACA
75	EV0039Aun	UCUGUCUGGAUGAAGAGGU	UCUGUCUGGAUGAAGAGGU
76	EV0039Bun	ACCUCUUCAUCCAGACAGA	ACCUCUUCAUCCAGACAGA
77	EV0040Aun	CUUGUCUGUCUGGAUGAAG	CUUGUCUGUCUGGAUGAAG
78	EV0040Bun	CUUCAUCCAGACAGACAAG	CUUCAUCCAGACAGACAAG
79	EV0041Aun	AUGGUCUUGUCUGUCUGGA	AUGGUCUUGUCUGUCUGGA
80	EV0041Bun	UCCAGACAGACAAGACCAU	UCCAGACAGACAAGACCAU
81	EV0042Aun	AGAUGGUCUUGUCUGUCUG	AGAUGGUCUUGUCUGUCUG
82	EV0042Bun	CAGACAGACAAGACCAUCU	CAGACAGACAAGACCAUCU
83	EV0043Aun	GUAGAUGGUCUUGUCUGUC	GUAGAUGGUCUUGUCUGUC

84	EV0043Bun	GACAGACAAGACCAUCUAC	GACAGACAAGACCAUCUAC
85	EV0044Aun	GUGUAGAUGGUCUUGUCUG	GUGUAGAUGGUCUUGUCUG
86	EV0044Bun	CAGACAAGACCAUCUACAC	CAGACAAGACCAUCUACAC
87	EV0045Aun	GGGGUGUAGAUGGUCUUGU	GGGGUGUAGAUGGUCUUGU
88	EV0045Bun	ACAAGACCAUCUACACCCC	ACAAGACCAUCUACACCCC
89	EV0046Aun	UAGGCUCGGAUCUUCCACU	UAGGCUCGGAUCUUCCACU
90	EV0046Bun	AGUGGAAGAUCCGAGCCUA	AGUGGAAGAUCCGAGCCUA
91	EV0047Aun	CUCGAAACUGGGCAGCACG	CUCGAAACUGGGCAGCACG
92	EV0047Bun	CGUGCUGCCCAGUUUCGAG	CGUGCUGCCCAGUUUCGAG
93	EV0048Aun	UUGGUGAAGUGGAUCUGGU	UUGGUGAAGUGGAUCUGGU
94	EV0048Bun	ACCAGAUCCACUUCACCAA	ACCAGAUCCACUUCACCAA
95	EV0049Aun	UCUUGGUGAAGUGGAUCUG	UCUUGGUGAAGUGGAUCUG
96	EV0049Bun	CAGAUCCACUUCACCAAGA	CAGAUCCACUUCACCAAGA
97	EV0050Aun	GUCUUGGUGAAGUGGAUCU	GUCUUGGUGAAGUGGAUCU
98	EV0050Bun	AGAUCCACUUCACCAAGAC	AGAUCCACUUCACCAAGAC
99	EV0051Aun	GGUGUCUUGGUGAAGUGGA	GGUGUCUUGGUGAAGUGGA
100	EV0051Bun	UCCACUUCACCAAGACACC	UCCACUUCACCAAGACACC
101	EV0052Aun	AACACCAUGAGGUCAAAGG	AACACCAUGAGGUCAAAGG
102	EV0052Bun	CCUUUGACCUCAUGGUGUU	CCUUUGACCUCAUGGUGUU
103	EV0053Aun	GAACACCAUGAGGUCAAAG	GAACACCAUGAGGUCAAAG
104	EV0053Bun	CUUUGACCUCAUGGUGUUC	CUUUGACCUCAUGGUGUUC
105	EV0054Aun	GUCACGAACACCAUGAGGU	GUCACGAACACCAUGAGGU
106	EV0054Bun	ACCUCAUGGUGUUCGUGAC	ACCUCAUGGUGUUCGUGAC
107	EV0055Aun	ACACAGAUCCCUUUCUUGU	ACACAGAUCCCUUUCUUGU
108	EV0055Bun	ACAAGAAAGGGAUCUGUGU	ACAAGAAAGGGAUCUGUGU
109	EV0058Aun	CCUUGCAGGAGAAUUCUGG	CCUUGCAGGAGAAUUCUGG
110	EV0058Bun	CCAGAAUUCUCCUGCAAGG	CCAGAAUUCUCCUGCAAGG
111	EV0059Aun	AGAGAGAAGACCUUGACCA	AGAGAGAAGACCUUGACCA
112	EV0059Bun	UGGUCAAGGUCUUCUCUCU	UGGUCAAGGUCUUCUCUCU

113	EV0060Aun	GAGUCGAUGGCGAUGAGGU	GAGUCGAUGGCGAUGAGGU
114	EV0060Bun	ACCUCAUCGCCAUCGACUC	ACCUCAUCGCCAUCGACUC
115	EV0061Aun	GCUUGGAACACCAUGAAGG	GCUUGGAACACCAUGAAGG
116	EV0061Bun	CCUUCAUGGUGUUCCAAGC	CCUUCAUGGUGUUCCAAGC
117	EV0062Aun	UCAUUUUCCUUGGUCUCUU	UCAUUUUCCUUGGUCUCUU
118	EV0062Bun	AAGAGACCAAGGAAAAUGA	AAGAGACCAAGGAAAAUGA
119	EV0063Aun	UGUGUCUGGAGCAAAGCCA	UGUGUCUGGAGCAAAGCCA
120	EV0063Bun	UGGCUUUGCUCCAGACACA	UGGCUUUGCUCCAGACACA
121	EV0064Aun	AGGUAGAUGAUGAGGGUGU	AGGUAGAUGAUGAGGGUGU
122	EV0064Bun	ACACCCUCAUCAUCUACCU	ACACCCUCAUCAUCUACCU
123	EV0065Aun	UUGUAAUAGGCGUAGACCU	UUGUAAUAGGCGUAGACCU
124	EV0065Bun	AGGUCUACGCCUAUUACAA	AGGUCUACGCCUAUUACAA
125	EV0066Aun	GUUGUAAUAGGCGUAGACC	GUUGUAAUAGGCGUAGACC
126	EV0066Bun	GGUCUACGCCUAUUACAAC	GGUCUACGCCUAUUACAAC
127	EV0067Aun	GGGUCUUGUACACAUAGUC	GGGUCUUGUACACAUAGUC
128	EV0067Bun	GACUAUGUGUACAAGACCC	GACUAUGUGUACAAGACCC
129	EV0068Aun	CACUUGAUGGGGCUGAUGA	CACUUGAUGGGGCUGAUGA
130	EV0068Bun	UCAUCAGCCCCAUCAAGUG	UCAUCAGCCCCAUCAAGUG
131	EV0312Aun	AUUGUAAUAGGCGUAGACC	AUUGUAAUAGGCGUAGACC
132	EV0112Bun	GGUCUACGCCUAUUACAAU	GGUCUACGCCUAUUACAAU
133	EV0313Aun	AUGUAGAUGGUCUUGUCUG	AUGUAGAUGGUCUUGUCUG
134	EV0313Bun	CAGACAAGACCAUCUACAU	CAGACAAGACCAUCUACAU
135	EV0069Bun	GGUACCUCUUCAUCCAGAA	GGUACCUCUUCAUCCAGAA
136	EV0072Bun	CUUCAUCCAGACAGACAAA	CUUCAUCCAGACAGACAAA
137	EV0073Bun	UCCAGACAGACAAGACCAA	UCCAGACAGACAAGACCAA
138	EV0074Bun	CAGACAGACAAGACCAUCA	CAGACAGACAAGACCAUCA
139	EV0075Bun	GACAGACAAGACCAUCUAA	GACAGACAAGACCAUCUAA
140	EV0076Bun	CAGACAAGACCAUCUACAA	CAGACAAGACCAUCUACAA
141	EV0077Bun	ACAAGACCAUCUACACCCA	ACAAGACCAUCUACACCCA

142	EV0079Bun	CGUGCUGCCCAGUUUCGAA	CGUGCUGCCCAGUUUCGAA
143	EV0082Bun	AGAUCCACUUCACCAAGAA	AGAUCCACUUCACCAAGAA
144	EV0083Bun	UCCACUUCACCAAGACACA	UCCACUUCACCAAGACACA
145	EV0084Bun	CCUUUGACCUCAUGGUGUA	CCUUUGACCUCAUGGUGUA
146	EV0085Bun	CUUUGACCUCAUGGUGUUA	CUUUGACCUCAUGGUGUUA
147	EV0086Bun	ACCUCAUGGUGUUCGUGAA	ACCUCAUGGUGUUCGUGAA
148	EV0087Bun	ACAAGAAAGGGAUCUGUGA	ACAAGAAAGGGAUCUGUGA
149	EV0088Bun	AGGGAUCUGUGUGGCAGAA	AGGGAUCUGUGUGGCAGAA
150	EV0089Bun	AAUGCAGGACUUCUUCAUA	AAUGCAGGACUUCUUCAUA
151	EV0090Bun	CCAGAAUUCUCCUGCAAGA	CCAGAAUUCUCCUGCAAGA
152	EV0091Bun	UGGUCAAGGUCUUCUCUCA	UGGUCAAGGUCUUCUCUCA
153	EV0092Bun	ACCUCAUCGCCAUCGACUA	ACCUCAUCGCCAUCGACUA
154	EV0093Bun	CCUUCAUGGUGUUCCAAGA	CCUUCAUGGUGUUCCAAGA
155	EV0096Bun	ACACCCUCAUCAUCUACCA	ACACCCUCAUCAUCUACCA
156	EV0098Bun	GGUCUACGCCUAUUACAAA	GGUCUACGCCUAUUACAAA
157	EV0099Bun	GACUAUGUGUACAAGACCA	GACUAUGUGUACAAGACCA
158	EV0100Bun	UCAUCAGCCCCAUCAAGUA	UCAUCAGCCCCAUCAAGUA
159	EV0001A	mU fU mU fC mA fU mA fG mU fA mG fG mC fU mC fG mG fA mU	UUUCAUAGUAGGCUCGGAU
160	EV0001B	fA mU fC mC fG mA fG mC fC mU fA mC fU mA fU mG fA mA fA	AUCCGAGCCUACUAUGAAA
161	EV0002A	mU fU mU fC mU fC mU fG mU fA mG fG mC fU mC fC mA fC mU	UUUCUCUGUAGGCUCCACU
162	EV0002B	fA mG fU mG fG mA fG mC fC mU fA mC fA mG fA mG fA mA fA	AGUGGAGCCUACAGAGAAA
163	EV0003A	mU fU mA fU mA fG mA fU mG fU mA fG mU fA mG fA mA fU mU	UUAUAGAUGUAGUAGAAUU
164	EV0003B	fA mA fU mU fC mU fA mC fU mA fC mA fU mC fU mA fU mA fA	AAUUCUACUACAUCUAUAA
165	EV0004A	mA fU mG fA mC fA mA fA mG fG mC fA mG fU mU fC mC fC mU	AUGACAAAGGCAGUUCCCU
166	EV0004B	fA mG fG mG fA mA fC mU fG mC fC mU fU mU fG mU fC mA fU	AGGGAACUGCCUUUGUCAU
167	EV0005A	mA fU mC fU mG fG mU fA mG fG mG fA mG fA mG fG mU fC mA	AUCUGGUAGGGAGAGGUCA
168	EV0005B	fU mG fA mC fC mU fC mU fC mC fC mU fA mC fC mA fG mA fU	UGACCUCUCCCUACCAGAU
169	EV0006A	mU fG mU fG mU fG mU fU mG fA mU fG mC fU mG fA mG fU mU	UGUGUGUUGAUGCUGAGUU
170	EV0006B	fA mA fC mU fC mA fG mC fA mU fC mA fA mC fA mC fA mC fA	AACUCAGCAUCAACACACA

171	EV0007A	mU fA mA fU mU fG mU fU mG fG mA fG mU fU mG fC mC fC mA	UAAUUGUUGGAGUUGCCCA
172	EV0007B	fU mG fG mG fC mA fA mC fU mC fC mA fA mC fA mA fU mU fA	UGGGCAACUCCAACAAUUA
173	EV0008A	mA fG mG fA mA fG mU fU mG fA mC fG mU fU mG fA mG fG mG	AGGAAGUUGACGUUGAGGG
174	EV0008B	fC mC fC mU fC mA fA mC fG mU fC mA fA mC fU mU fC mC fU	CCCUCAACGUCAACUUCCU
175	EV0009A	mA fU mG fA mA fG mU fC mG fG mU fG mG fU mG fA mU fG mG	AUGAAGUCGGUGGUGAUGG
176	EV0009B	fC mC fA mU fC mA fC mC fA mC fC mG fA mC fU mU fC mA fU	CCAUCACCACCGACUUCAU
177	EV0010A	mU fU mU fA mC fC mA fC mC fA mG fC mG fA mG fC mC fC mA	UUUACCACCAGCGAGCCCA
178	EV0010B	fU mG fG mG fC mU fC mG fC mU fG mG fU mG fG mU fA mA fA	UGGGCUCGCUGGUGGUAAA
179	EV0011A	mU fC mU fA mU fC mU fU mC fA mG fG mG fU mC fA mU fC mU	UCUAUCUUCAGGGUCAUCU
180	EV0011B	fA mG fA mU fG mA fC mC fC mU fG mA fA mG fA mU fA mG fA	AGAUGACCCUGAAGAUAGA
181	EV0012A	mA fU mG fU mA fG mU fU mG fC mA fG mC fA mG fU mC fC mA	AUGUAGUUGCAGCAGUCCA
182	EV0012B	fU mG fG mA fC mU fG mC fU mG fC mA fA mC fU mA fC mA fU	UGGACUGCUGCAACUACAU
183	EV0013A	mA fA mU fA mU fA mU fU mC fA mU fG mA fG mC fU mU fC mG	AAUAUAUUCAUGAGCUUCG
184	EV0013B	fC mG fA mA fG mC fU mC fA mU fG mA fA mU fA mU fA mU fU	CGAAGCUCAUGAAUAUAUU
185	EV0014A	mA fA mA fU mA fU mA fU mU fC mA fU mG fA mG fC mU fU mC	AAAUAUAUUCAUGAGCUUC
186	EV0014B	fG mA fA mG fC mU fC mA fU mG fA mA fU mA fU mA fU mU fU	GAAGCUCAUGAAUAUAUUU
187	EV0015A	mU fC mC fU mG fC mA fU mU fA mC fU mG fU mG fA mC fC mU	UCCUGCAUUACUGUGACCU
188	EV0015B	fA mG fG mU fC mA fC mA fG mU fA mA fU mG fC mA fG mG fA	AGGUCACAGUAAUGCAGGA
189	EV0016A	mC fA mA fC mA fG mA fG mU fA mG fG mG fU mA fG mC fC mG	CAACAGAGUAGGGUAGCCG
190	EV0016B	fC mG fG mC fU mA fC mC fC mU fA mC fU mC fU mG fU mU fG	CGGCUACCCUACUCUGUUG
191	EV0017A	mU fC mG fA mA fC mA fA mC fA mG fA mG fU mA fG mG fG mU	UCGAACAACAGAGUAGGGU
192	EV0017B	fA mC fC mC fU mA fC mU fC mU fG mU fU mG fU mU fC mG fA	ACCCUACUCUGUUGUUCGA
193	EV0018A	mU fU mU fC mG fA mA fC mA fA mC fA mG fA mG fU mA fG mG	UUUCGAACAACAGAGUAGG
194	EV0018B	fC mC fU mA fC mU fC mU fG mU fU mG fU mU fC mG fA mA fA	CCUACUCUGUUGUUCGAAA
195	EV0019A	mG fU mU fU mC fA mU fC mC fA mG fG mU fA mA fU mG fC mA	GUUUCAUCCAGGUAAUGCA
196	EV0019B	fU mG fC mA fU mU fA mC fC mU fG mG fA mU fG mA fA mA fC	UGCAUUACCUGGAUGAAAC
197	EV0020A	mU fU mA fU mC fU mU fU mG fG mC fU mG fU mG fG mU fC mA	UUAUCUUUGGCUGUGGUCA
198	EV0020B	fU mG fA mC fC mA fC mA fG mC fC mA fA mA fG mA fU mA fA	UGACCACAGCCAAAGAUAA
199	EV0021A	mU fG mU fU mC fA mU fU mG fA mG fC mC fA mA fC mG fC mA	UGUUCAUUGAGCCAACGCA

200	EV0021B	fU mG fC mG fU mU fG mG fC mU fC mA fA mU fG mA fA mC fA	UGCGUUGGCUCAAUGAACA
201	EV0022A	mA fA mC fA mC fC mA fU mG fA mA fG mG fU mG fG mC fC mU	AACACCAUGAAGGUGGCCU
202	EV0022B	fA mG fG mC fC mA fC mC fU mU fC mA fU mG fG mU fG mU fU	AGGCCACCUUCAUGGUGUU
203	EV0023A	mU fU mG fG mU fA mU fU mG fA mG fC mC fA mA fG mG fC mU	UUGGUAUUGAGCCAAGGCU
204	EV0023B	fA mG fC mC fU mU fG mG fC mU fC mA fA mU fA mC fC mA fA	AGCCUUGGCUCAAUACCAA
205	EV0024A	mU fU mU fA mU fU mA fC mA fG mG fU mG fA mG fU mU fG mA	UUUAUUACAGGUGAGUUGA
206	EV0024B	fU mC fA mA fC mU fC mA fC mC fU mG fU mA fA mU fA mA fA	UCAACUCACCUGUAAUAAA
207	EV0025A	mU fU mU fC mU fG mU fU mU fC mC fG mG fU mG fC mU fG mG	UUUCUGUUUCCGGUGCUGG
208	EV0025B	fC mC fA mG fC mA fC mC fG mG fA mA fA mC fA mG fA mA fA	CCAGCACCGGAAACAGAAA
209	EV0026A	mU fC mA fA mG fG mA fU mC fA mU fA mG fU mG fU mU fC mU	UCAAGGAUCAUAGUGUUCU
210	EV0026B	fA mG fA mA fC mA fC mU fA mU fG mA fU mC fC mU fU mG fA	AGAACACUAUGAUCCUUGA
211	EV0027A	mA fU mC fU mC fA mA fG mG fA mU fC mA fU mA fG mU fG mU	AUCUCAAGGAUCAUAGUGU
212	EV0027B	fA mC fA mC fU mA fU mG fA mU fC mC fU mU fG mA fG mA fU	ACACUAUGAUCCUUGAGAU
213	EV0028A	mU fC mA fU mA fC mU fU mG fG mA fG mA fU mG fU mA fU mC	UCAUACUUGGAGAUGUAUC
214	EV0028B	fG mA fU mA fC mA fU mC fU mC fC mA fA mG fU mA fU mG fA	GAUACAUCUCCAAGUAUGA
215	EV0029A	mU fA mU fC mG fG mA fG mA fA mG fG mC fU mU fU mG fU mC	UAUCGGAGAAGGCUUUGUC
216	EV0029B	fG mA fC mA fA mA fG mC fC mU fU mC fU mC fC mG fA mU fA	GACAAAGCCUUCUCCGAUA
217	EV0030A	mA fU mG fA mU fG mA fG mG fG mU fG mU fU mC fC mU fA mU	AUGAUGAGGGUGUUCCUAU
218	EV0030B	fA mU fA mG fG mA fA mC fA mC fC mC fU mC fA mU fC mA fU	AUAGGAACACCCUCAUCAU
219	EV0031A	mU fA mU fU mG fG mU fG mA fA mC fU mU fU mG fA mA fA mG	UAUUGGUGAACUUUGAAAG
220	EV0031B	fC mU fU mU fC mA fA mA fG mU fU mC fA mC fC mA fA mU fA	CUUUCAAAGUUCACCAAUA
221	EV0032A	mA fG mC fU mU fG mU fU mC fA mG fC mU fU mU fC mC fA mU	AGCUUGUUCAGCUUUCCAU
222	EV0032B	fA mU fG mG fA mA fA mG fC mU fG mA fA mC fA mA fG mC fU	AUGGAAAGCUGAACAAGCU
223	EV0033A	mU fU mU fG mU fA mU fG mA fA mG fC mA fA mU fU mC fU mC	UUUGUAUGAAGCAAUUCUC
224	EV0033B	fG mA fG mA fA mU fU mG fC mU fU mC fA mU fA mC fA mA fA	GAGAAUUGCUUCAUACAAA
225	EV0034A	mG fU mC fU mU fG mU fA mC fA mC fA mU fA mG fU mC fC mA	GUCUUGUACACAUAGUCCA
226	EV0034B	fU mG fG mA fC mU fA mU fG mU fG mU fA mC fA mA fG mA fC	UGGACUAUGUGUACAAGAC
227	EV0035A	mU fG mC fU mC fA mA fU mG fG mC fC mA fU mG fA mU fG mU	UGCUCAAUGGCCAUGAUGU
228	EV0035B	fA mC fA mU fC mA fU mG fG mC fC mA fU mU fG mA fG mC fA	ACAUCAUGGCCAUUGAGCA

229	EV0036A	mU fU mG fG mC fA mU fU mC fG mU fC mC fU mC fC mU fC mG	UUGGCAUUCGUCCUCCUCG
230	EV0036B	fC mG fA mG fG mA fG mG fA mC fG mA fA mU fG mC fC mA fA	CGAGGAGGACGAAUGCCAA
231	EV0037A	mG fU mC fU mG fG mA fU mG fA mA fG mA fG mG fU mA fC mC	GUCUGGAUGAAGAGGUACC
232	EV0037B	fG mG fU mA fC mC fU mC fU mU fC mA fU mC fC mA fG mA fC	GGUACCUCUUCAUCCAGAC
233	EV0038A	mU fG mU fC mU fG mG fA mU fG mA fA mG fA mG fG mU fA mC	UGUCUGGAUGAAGAGGUAC
234	EV0038B	fG mU fA mC fC mU fC mU fU mC fA mU fC mC fA mG fA mC fA	GUACCUCUUCAUCCAGACA
235	EV0039A	mU fC mU fG mU fC mU fG mG fA mU fG mA fA mG fA mG fG mU	UCUGUCUGGAUGAAGAGGU
236	EV0039B	fA mC fC mU fC mU fU mC fA mU fC mC fA mG fA mC fA mG fA	ACCUCUUCAUCCAGACAGA
237	EV0040A	mC fU mU fG mU fC mU fG mU fC mU fG mG fA mU fG mA fA mG	CUUGUCUGUCUGGAUGAAG
238	EV0040B	fC mU fU mC fA mU fC mC fA mG fA mC fA mG fA mC fA mA fG	CUUCAUCCAGACAGACAAG
239	EV0041A	mA fU mG fG mU fC mU fU mG fU mC fU mG fU mC fU mG fG mA	AUGGUCUUGUCUGUCUGGA
240	EV0041B	fU mC fC mA fG mA fC mA fG mA fC mA fA mG fA mC fC mA fU	UCCAGACAGACAAGACCAU
241	EV0042A	mA fG mA fU mG fG mU fC mU fU mG fU mC fU mG fU mC fU mG	AGAUGGUCUUGUCUGUCUG
242	EV0042B	fC mA fG mA fC mA fG mA fC mA fA mG fA mC fC mA fU mC fU	CAGACAGACAAGACCAUCU
243	EV0043A	mG fU mA fG mA fU mG fG mU fC mU fU mG fU mC fU mG fU mC	GUAGAUGGUCUUGUCUGUC
244	EV0043B	fG mA fC mA fG mA fC mA fA mG fA mC fC mA fU mC fU mA fC	GACAGACAAGACCAUCUAC
245	EV0044A	mG fU mG fU mA fG mA fU mG fG mU fC mU fU mG fU mC fU mG	GUGUAGAUGGUCUUGUCUG
246	EV0044B	fC mA fG mA fC mA fA mG fA mC fC mA fU mC fU mA fC mA fC	CAGACAAGACCAUCUACAC
247	EV0045A	mG fG mG fG mU fG mU fA mG fA mU fG mG fU mC fU mU fG mU	GGGGUGUAGAUGGUCUUGU
248	EV0045B	fA mC fA mA fG mA fC mC fA mU fC mU fA mC fA mC fC mC fC	ACAAGACCAUCUACACCCC
249	EV0046A	mU fA mG fG mC fU mC fG mG fA mU fC mU fU mC fC mA fC mU	UAGGCUCGGAUCUUCCACU
250	EV0046B	fA mG fU mG fG mA fA mG fA mU fC mC fG mA fG mC fC mU fA	AGUGGAAGAUCCGAGCCUA
251	EV0047A	mC fU mC fG mA fA mA fC mU fG mG fG mC fA mG fC mA fC mG	CUCGAAACUGGGCAGCACG
252	EV0047B	fC mG fU mG fC mU fG mC fC mC fA mG fU mU fU mC fG mA fG	CGUGCUGCCCAGUUUCGAG
253	EV0048A	mU fU mG fG mU fG mA fA mG fU mG fG mA fU mC fU mG fG mU	UUGGUGAAGUGGAUCUGGU
254	EV0048B	fA mC fC mA fG mA fU mC fC mA fC mU fU mC fA mC fC mA fA	ACCAGAUCCACUUCACCAA
255	EV0049A	mU fC mU fU mG fG mU fG mA fA mG fU mG fG mA fU mC fU mG	UCUUGGUGAAGUGGAUCUG
256	EV0049B	fC mA fG mA fU mC fC mA fC mU fU mC fA mC fC mA fA mG fA	CAGAUCCACUUCACCAAGA
257	EV0050A	mG fU mC fU mU fG mG fU mG fA mA fG mU fG mG fA mU fC mU	GUCUUGGUGAAGUGGAUCU

258	EV0050B	fA mG fA mU fC mC fA mC fU mU fC mA fC mC fA mA fG mA fC	AGAUCCACUUCACCAAGAC
259	EV0051A	mG fG mU fG mU fC mU fU mG fG mU fG mA fA mG fU mG fG mA	GGUGUCUUGGUGAAGUGGA
260	EV0051B	fU mC fC mA fC mU fU mC fA mC fC mA fA mG fA mC fA mC fC	UCCACUUCACCAAGACACC
261	EV0052A	mA fA mC fA mC fC mA fU mG fA mG fG mU fC mA fA mA fG mG	AACACCAUGAGGUCAAAGG
262	EV0052B	fC mC fU mU fU mG fA mC fC mU fC mA fU mG fG mU fG mU fU	CCUUUGACCUCAUGGUGUU
263	EV0053A	mG fA mA fC mA fC mC fA mU fG mA fG mG fU mC fA mA fA mG	GAACACCAUGAGGUCAAAG
264	EV0053B	fC mU fU mU fG mA fC mC fU mC fA mU fG mG fU mG fU mU fC	CUUUGACCUCAUGGUGUUC
265	EV0054A	mG fU mC fA mC fG mA fA mC fA mC fC mA fU mG fA mG fG mU	GUCACGAACACCAUGAGGU
266	EV0054B	fA mC fC mU fC mA fU mG fG mU fG mU fU mC fG mU fG mA fC	ACCUCAUGGUGUUCGUGAC
267	EV0055A	mA fC mA fC mA fG mA fU mC fC mC fU mU fU mC fU mU fG mU	ACACAGAUCCCUUUCUUGU
268	EV0055B	fA mC fA mA fG mA fA mA fG mG fG mA fU mC fU mG fU mG fU	ACAAGAAAGGGAUCUGUGU
269	EV0056A	mG fU mC fU mG fC mC fA mC fA mC fA mG fA mU fC mC fC mU	GUCUGCCACACAGAUCCCU
270	EV0056B	fA mG fG mG fA mU fC mU fG mU fG mU fG mG fC mA fG mA fC	AGGGAUCUGUGUGGCAGAC
271	EV0057A	mG fA mU fG mA fA mG fA mA fG mU fC mC fU mG fC mA fU mU	GAUGAAGAAGUCCUGCAUU
272	EV0057B	fA mA fU mG fC mA fG mG fA mC fU mU fC mU fU mC fA mU fC	AAUGCAGGACUUCUUCAUC
273	EV0058A	mC fC mU fU mG fC mA fG mG fA mG fA mA fU mU fC mU fG mG	CCUUGCAGGAGAAUUCUGG
274	EV0058B	fC mC fA mG fA mA fU mU fC mU fC mC fU mG fC mA fA mG fG	CCAGAAUUCUCCUGCAAGG
275	EV0059A	mA fG mA fG mA fG mA fA mG fA mC fC mU fU mG fA mC fC mA	AGAGAGAAGACCUUGACCA
276	EV0059B	fU mG fG mU fC mA fA mG fG mU fC mU fU mC fU mC fU mC fU	UGGUCAAGGUCUUCUCUCU
277	EV0060A	mG fA mG fU mC fG mA fU mG fG mC fG mA fU mG fA mG fG mU	GAGUCGAUGGCGAUGAGGU
278	EV0060B	fA mC fC mU fC mA fU mC fG mC fC mA fU mC fG mA fC mU fC	ACCUCAUCGCCAUCGACUC
279	EV0061A	mG fC mU fU mG fG mA fA mC fA mC fC mA fU mG fA mA fG mG	GCUUGGAACACCAUGAAGG
280	EV0061B	fC mC fU mU fC mA fU mG fG mU fG mU fU mC fC mA fA mG fC	CCUUCAUGGUGUUCCAAGC
281	EV0062A	mU fC mA fU mU fU mU fC mC fU mU fG mG fU mC fU mC fU mU	UCAUUUUCCUUGGUCUCUU
282	EV0062B	fA mA fG mA fG mA fC mC fA mA fG mG fA mA fA mA fU mG fA	AAGAGACCAAGGAAAAUGA
283	EV0063A	mU fG mU fG mU fC mU fG mG fA mG fC mA fA mA fG mC fC mA	UGUGUCUGGAGCAAAGCCA
284	EV0063B	fU mG fG mC fU mU fU mG fC mU fC mC fA mG fA mC fA mC fA	UGGCUUUGCUCCAGACACA
285	EV0064A	mA fG mG fU mA fG mA fU mG fA mU fG mA fG mG fG mU fG mU	AGGUAGAUGAUGAGGGUGU
286	EV0064B	fA mC fA mC fC mC fU mC fA mU fC mA fU mC fU mA fC mC fU	ACACCCUCAUCAUCUACCU

287	EV0065A	mU fU mG fU mA fA mU fA mG fG mC fG mU fA mG fA mC fC mU	UUGUAAUAGGCGUAGACCU
288	EV0065B	fA mG fG mU fC mU fA mC fG mC fC mU fA mU fU mA fC mA fA	AGGUCUACGCCUAUUACAA
289	EV0066A	mG fU mU fG mU fA mA fU mA fG mG fC mG fU mA fG mA fC mC	GUUGUAAUAGGCGUAGACC
290	EV0066B	fG mG fU mC fU mA fC mG fC mC fU mA fU mU fA mC fA mA fC	GGUCUACGCCUAUUACAAC
291	EV0067A	mG fG mG fU mC fU mU fG mU fA mC fA mC fA mU fA mG fU mC	GGGUCUUGUACACAUAGUC
292	EV0067B	fG mA fC mU fA mU fG mU fG mU fA mC fA mA fG mA fC mC fC	GACUAUGUGUACAAGACCC
293	EV0068A	mC fA mC fU mU fG mA fU mG fG mG fG mC fU mG fA mU fG mA	CACUUGAUGGGGCUGAUGA
294	EV0068B	fU mC fA mU fC mA fG mC fC mC fC mA fU mC fA mA fG mU fG	UCAUCAGCCCCAUCAAGUG
295	EV0069B	mG mG mU mA mC mC fU fC fU mU mC mA mU mC mC mA mG mA irA	GGUACCUCUUCAUCCAGAA
296	EV0070B	mG mU mA mC mC mU fC fU fU mC mA mU mC mC mA mG mA mC irA	GUACCUCUUCAUCCAGACA
297	EV0071B	mA mC mC mU mC mU fU fC fA mU mC mC mA mG mA mC mA mG irA	ACCUCUUCAUCCAGACAGA
298	EV0072B	mC mU mU mC mA mU fC fC fA mG mA mC mA mG mA mC mA mA irA	CUUCAUCCAGACAGACAAA
299	EV0073B	mU mC mC mA mG mA fC fA fG mA mC mA mA mG mA mC mC mA irA	UCCAGACAGACAAGACCAA
300	EV0074B	mC mA mG mA mC mA fG fA fC mA mA mG mA mC mC mA mU mC irA	CAGACAGACAAGACCAUCA
301	EV0075B	mG mA mC mA mG mA fC fA fA mG mA mC mC mA mU mC mU mA irA	GACAGACAAGACCAUCUAA
302	EV0076B	mC mA mG mA mC mA fA fG fA mC mC mA mU mC mU mA mC mA irA	CAGACAAGACCAUCUACAA
303	EV0077B	mA mC mA mA mG mA fC fC fA mU mC mU mA mC mA mC mC mC irA	ACAAGACCAUCUACACCCA
304	EV0078B	mA mG mU mG mG mA fA fG fA mU mC mC mG mA mG mC mC mU irA	AGUGGAAGAUCCGAGCCUA
305	EV0079B	mC mG mU mG mC mU fG fC fC mC mA mG mU mU mU mC mG mA irA	CGUGCUGCCCAGUUUCGAA
306	EV0080B	mA mC mC mA mG mA fU fC fC mA mC mU mU mC mA mC mC mA irA	ACCAGAUCCACUUCACCAA
307	EV0081B	mC mA mG mA mU mC fC fA fC mU mU mC mA mC mC mA mA mG irA	CAGAUCCACUUCACCAAGA
308	EV0082B	mA mG mA mU mC mC fA fC fU mU mC mA mC mC mA mA mG mA irA	AGAUCCACUUCACCAAGAA
309	EV0083B	mU mC mC mA mC mU fU fC fA mC mC mA mA mG mA mC mA mC irA	UCCACUUCACCAAGACACA
310	EV0084B	mC mC mU mU mU mG fA fC fC mU mC mA mU mG mG mU mG mU irA	CCUUUGACCUCAUGGUGUA
311	EV0085B	mC mU mU mU mG mA fC fC fU mC mA mU mG mG mU mG mU mU irA	CUUUGACCUCAUGGUGUUA
312	EV0086B	mA mC mC mU mC mA fU fG fG mU mG mU mU mC mG mU mG mA irA	ACCUCAUGGUGUUCGUGAA
313	EV0087B	mA mC mA mA mG mA fA fA fG mG mG mA mU mC mU mG mU mG irA	ACAAGAAAGGGAUCUGUGA
314	EV0088B	mA mG mG mG mA mU fC fU fG mU mG mU mG mG mC mA mG mA irA	AGGGAUCUGUGUGGCAGAA
315	EV0089B	mA mA mU mG mC mA fG fG fA mC mU mU mC mU mU mC mA mU irA	AAUGCAGGACUUCUUCAUA

316	EV0090B	mC mC mA mG mA mA fU fU fC mU mC mC mU mG mC mA mA mG irA	CCAGAAUUCUCCUGCAAGA
317	EV0091B	mU mG mG mU mC mA fA fG fG mU mC mU mU mC mU mC mU mC irA	UGGUCAAGGUCUUCUCUCA
318	EV0092B	mA mC mC mU mC mA fU fC fG mC mC mA mU mC mG mA mC mU irA	ACCUCAUCGCCAUCGACUA
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320	EV0094B	mA mA mG mA mG mA fC fC fA mA mG mG mA mA mA mA mU mG irA	AAGAGACCAAGGAAAAUGA
321	EV0095B	mU mG mG mC mU mU fU fG fC mU mC mC mA mG mA mC mA mC irA	UGGCUUUGCUCCAGACACA
322	EV0096B	mA mC mA mC mC mC fU fC fA mU mC mA mU mC mU mA mC mC irA	ACACCCUCAUCAUCUACCA
323	EV0097B	mA mG mG mU mC mU fA fC fG mC mC mU mA mU mU mA mC mA irA	AGGUCUACGCCUAUUACAA
324	EV0098B	mG mG mU mC mU mA fC fG fC mC mU mA mU mU mA mC mA mA irA	GGUCUACGCCUAUUACAAA
325	EV0099B	mG mA mC mU mA mU fG fU fG mU mA mC mA mA mG mA mC mC irA	GACUAUGUGUACAAGACCA
326	EV0100B	mU mC mA mU mC mA fG fC fC mC mC mA mU mC mA mA mG mU irA	UCAUCAGCCCCAUCAAGUA
327	EV0101A	mA (ps) fG (ps) mG fA mA fG mU fU mG fA mC fG mU fU mG fA mG (ps) fG (ps) mG	AGGAAGUUGACGUUGAGGG
328	EV0101B	[ST23 (ps)]3 ST43 (ps) fC mC fC mU fC mA fA mC fG mU fC mA fA mC fU mU fC (ps) mC (ps) fU	CCCUCAACGUCAACUUCCU
329	EV0102A	mA (ps) fA (ps) mU fA mU fA mU fU mC fA mU fG mA fG mC fU mU (ps) fC (ps) mG	AAUAUAUUCAUGAGCUUCG
330	EV0102B	[ST23 (ps)]3 ST43 (ps) fC mG fA mA fG mC fU mC fA mU fG mA fA mU fA mU fA (ps) mU (ps) fU	CGAAGCUCAUGAAUAUAUU
331	EV0103A	mA (ps) fU (ps) mG fA mU fG mA fG mG fG mU fG mU fU mC fC mU (ps) fA (ps) mU	AUGAUGAGGGUGUUCCUAU
332	EV0103B	[ST23 (ps)]3 ST43 (ps) fA mU fA mG fG mA fA mC fA mC fC mC fU mC fA mU fC (ps) mA (ps) fU	AUAGGAACACCCUCAUCAU
333	EV0104A	mU (ps) fC (ps) mU fG mU fC mU fG mG fA mU fG mA fA mG fA mG (ps) fG (ps) mU	UCUGUCUGGAUGAAGAGGU
334	EV0104B	[ST23 (ps)]3 ST43 (ps) fA mC fC mU fC mU fU mC fA mU fC mC fA mG fA mC fA (ps) mG (ps) fA	ACCUCUUCAUCCAGACAGA
335	EV0105A	mG (ps) fU (ps) mA fG mA fU mG fG mU fC mU fU mG fU mC fU mG (ps) fU (ps) mC	GUAGAUGGUCUUGUCUGUC
336	EV0105B	[ST23 (ps)]3 ST43 (ps) fG mA fC mA fG mA fC mA fA mG fA mC fC mA fU mC fU (ps) mA (ps) fC	GACAGACAAGACCAUCUAC
337	EV0106A	mG (ps) fA (ps) mA fC mA fC mC fA mU fG mA fG mG fU mC fA mA (ps) fA (ps) mG	GAACACCAUGAGGUCAAAG

338	EV0106B	[ST23 (ps)]3 ST43 (ps) fC mU fU mU fG mA fC mC fU mC fA mU fG mG fU mG fU (ps) mU (ps) fC	CUUUGACCUCAUGGUGUUC
339	EV0107A	mA (ps) fG (ps) mA fG mA fG mA fA mG fA mC fC mU fU mG fA mC (ps) fC (ps) mA	AGAGAGAAGACCUUGACCA
340	EV0107B	[ST23 (ps)]3 ST43 (ps) mU fG mG fU mC fA mA fG mG fU mC fU mU fC mU fC mU (ps) fC (ps) mU	UGGUCAAGGUCUUCUCUCU
341	EV0108A	mC (ps) fU (ps) mU fG mU fC mU fG mU fC mU fG mG fA mU fG mA (ps) fA (ps) mG	CUUGUCUGUCUGGAUGAAG
342	EV0108B	[ST23 (ps)]3 ST43 (ps) mC mU mU mC mA mU fC fC fA mG mA mC mA mG mA mC mA mA irA	CUUCAUCCAGACAGACAAA
343	EV0109A	mG (ps) fU (ps) mA fG mA fU mG fG mU fC mU fU mG fU mC fU mG (ps) fU (ps) mC	GUAGAUGGUCUUGUCUGUC
344	EV0109B	[ST23 (ps)]3 ST43 (ps) mG mA mC mA mG mA fC fA fA fA mG mA mC mC mA mU mC mU mA irA	GACAGACAAGACCAUCUAA
345	EV0110A	mU (ps) fC (ps) mU fU mG fG mU fG mA fA mG fU mG fG mA fU mC (ps) fU (ps) mG	UCUUGGUGAAGUGGAUCUG
346	EV0110B	[ST23 (ps)]3 ST43 (ps) mC mA mG mA mU mC fC fA fC mU mU mC mA mC mC mA mA mG irA	CAGAUCCACUUCACCAAGA
347	EV0111A	mG (ps) fU (ps) mU fG mU fA mA fU mA fG mG fC mG fU mA fG mA (ps) fC (ps) mC	GUUGUAAUAGGCGUAGACC
348	EV0111B	[ST23 (ps)]3 ST43 (ps) mG mG mU mC mU mA fC fG fC mC mU mA mU mU mA mC mA mA irA	GGUCUACGCCUAUUACAAA
349	EV0312A	mA (ps) fU (ps) mU fG mU fA mA fU mA fG mG fC mG fU mA fG mA (ps) fC (ps) mC	AUUGUAAUAGGCGUAGACC
350	EV0112B	[ST23 (ps)]3 ST43 (ps) fG mG fU mC fU mA fC mG fC mC fU mA fU mU fA mC fA (ps) mA (ps) fU	GGUCUACGCCUAUUACAAU
351	EV0313A	mA (ps) fU (ps) mG fU mA fG mA fU mG fG mU fC mU fU mG fU mC (ps) fU (ps) mG	AUGUAGAUGGUCUUGUCUG
352	EV0313B	[ST23 (ps)]3 ST43 (ps) fC mA fG mA fC mA fA mG fA mC fC mA fU mC fU mA fC (ps) mA (ps) fU	CAGACAAGACCAUCUACAU
353	EV0201A	mU (ps) fG (ps) mA fG mA fA mG fA mC fC mU fU mG fA mC (ps) fC (ps) mA	UGAGAGAAGACCUUGACCA
354	EV0201B	[ST23 (ps)]3 ST43 (ps) mU mG mG mU mC mA fA fG fG mU mC mU mU mC mU mC mU (ps) mC (ps) mU	UGGUCAAGGUCUUCUCUCU

355	EV0202B	Ser(GN) (ps) mU (ps) mG (ps) mG mU mC mA fA fG fG mU mC mU mU mC mU mC mU (ps) mC (ps) mU (ps) Ser(GN)	UGGUCAAGGUCUUCUCUCU
356	EV0203A	(vp)-mU fG mA fG mA fG mA fA mG fA mC fC mU fU mG fA mC (ps) fC (ps) mA	UGAGAGAAGACCUUGACCA
357	EV0205B	[ST23 (ps)]3 ST43 (ps) mC mA mG mA mU mC fC fA fC mU mU mC mA mC mC mA mA (ps) mG (ps) mA	CAGAUCCACUUCACCAAGA
358	EV0206B	Ser(GN) (ps) mC (ps) mA (ps) mG mA mU mC fC fA fC mU mU mC mA mC mC mA mA (ps) mG (ps) mA (ps) Ser(GN)	CAGAUCCACUUCACCAAGA
359	EV0207A	(vp)-mU (ps) fC (ps) mU fU mG fG mU fG mA fA mG fU mG fG mA fU mC (ps) fU (ps) mG	UCUUGGUGAAGUGGAUCUG
360	EV0209A	(vp)-mU fC mU fU mG fG mU fG mA fA mG fU mG fG mA fU mC (ps) fU (ps) mG	UCUUGGUGAAGUGGAUCUG
361	EV0201Aun	UGAGAGAAGACCUUGACCA	UGAGAGAAGACCUUGACCA
362	EJ0001Aun	UGAGAGAAGACCUUGACCA	UGAGAGAAGACCUUGACCA
363	EJ0001Bun	UGGUCAAGGUCUUCUCUCU	UGGUCAAGGUCUUCUCUCU
364	EJ0002Aun	UGAGAGACGACCUUGACCA	UGAGAGACGACCUUGACCA
365	EJ0003Aun	UGAGAGAAUACCUUGACCA	UGAGAGAAUACCUUGACCA
366	EJ0004Aun	UGAGAGAAGACCUUGACCG	UGAGAGAAGACCUUGACCG
367	EJ0004Bun	CGGUCAAGGUCUUCUCUCU	CGGUCAAGGUCUUCUCUCU
368	EJ0005Aun	AUGAGCUUCGUAGAGAUUC	AUGAGCUUCGUAGAGAUUC
369	EJ0005Bun	GAAUCUCUACGAAGCUCAU	GAAUCUCUACGAAGCUCAU
370	EJ0006Aun	UUGUAGUAGCGGAUCUUGG	UUGUAGUAGCGGAUCUUGG
371	EJ0006Bun	CCAAGAUCCGCUACUACAC	CCAAGAUCCGCUACUACAC
372	EJ0007Aun	UUCUGUCUGGAUGAAGAGG	UUCUGUCUGGAUGAAGAGG
373	EJ0007Bun	CCUCUUCAUCCAGACAGAC	CCUCUUCAUCCAGACAGAC
374	EJ0009Bun	UGGUCAAGGUCUUCUCUCA	UGGUCAAGGUCUUCUCUCA
375	EJ0010Bun	UGGUCAAGGUCGUCUCUCA	UGGUCAAGGUCGUCUCUCA
376	EJ0011Bun	CGGUCAAGGUCUUCUCUCA	CGGUCAAGGUCUUCUCUCA
377	EJ0012Aun	UGAGAGACGACCUUGACCG	UGAGAGACGACCUUGACCG
378	EJ0012Bun	CGGUCAAGGUCGUCUCUCA	CGGUCAAGGUCGUCUCUCA
379	EJ0014Bun	CCAAGAUCCGCUACUACAA	CCAAGAUCCGCUACUACAA

380	EJ0015Bun	CCUCUUCAUCCAGACAGAA	CCUCUUCAUCCAGACAGAA
381	EJ0001A	mU (ps) fG (ps) mA fG mA fG mA fA mG fA mC fC mU fU mG fA mC (ps) fC (ps) mA	UGAGAGAAGACCUUGACCA
382	EJ0001B	mU (ps) mG (ps) mG mU mC mA fA fG fG mU mC mU mU mC mU mC mU (ps) mC (ps) mU	UGGUCAAGGUCUUCUCUCU
383	EJ0002A	mU (ps) fG (ps) mA fG mA fG mA fC mG fA mC fC mU fU mG fA mC (ps) fC (ps) mA	UGAGAGACGACCUUGACCA
384	EJ0003A	mU (ps) fG (ps) mA fG mA fG mA fA mU fA mC fC mU fU mG fA mC (ps) fC (ps) mA	UGAGAGAAUACCUUGACCA
385	EJ0004A	mU (ps) fG (ps) mA fG mA fG mA fA mG fA mC fC mU fU mG fA mC (ps) fC (ps) mG	UGAGAGAAGACCUUGACCG
386	EJ0004B	mC (ps) mG (ps) mG mU mC mA fA fG fG mU mC mU mU mC mU mC mU (ps) mC (ps) mU	CGGUCAAGGUCUUCUCUCU
387	EJ0005A	mA (ps) fU (ps) mG fA mG fC mU fU mC fG mU fA mG fA mG fA mU (ps) fU (ps) mC	AUGAGCUUCGUAGAGAUUC
388	EJ0005B	mG (ps) mA (ps) mA mU mC mU fC fU fA mC mG mA mA mG mC mU mC (ps) mA (ps) mU	GAAUCUCUACGAAGCUCAU
389	EJ0006A	mU (ps) fU (ps) mG fU mA fG mU fA mG fC mG fG mA fU mC fU mU (ps) fG (ps) mG	UUGUAGUAGCGGAUCUUGG
390	EJ0006B	mC (ps) mC (ps) mA mA mG mA fU fC fC mG mC mU mA mC mU mA mC (ps) mA (ps) mC	CCAAGAUCCGCUACUACAC
391	EJ0007A	mU (ps) fU (ps) mC fU mG fU mC fU mG fG mA fU mG fA mA fG mA (ps) fG (ps) mG	UUCUGUCUGGAUGAAGAGG
392	EJ0007B	mC (ps) mC (ps) mU mC mU mU fC fA fU mC mC mA mG mA mC mA mG (ps) mA (ps) mC	CCUCUUCAUCCAGACAGAC
393	EJ0008B	[ST23 (ps)]3 ST43 (ps) mU mG mG mU mC mA fA fG fG mU mC mU mU mC mU mC mU (ps) mC (ps) mU	UGGUCAAGGUCUUCUCUCU
394	EJ0009B	[ST23 (ps)]3 ST43 (ps) mU mG mG mU mC mA fA fG fG mU mC mU mU mC mU mC mU (ps) mC (ps) mA	UGGUCAAGGUCUUCUCUCA
395	EJ0002A	mU (ps) fG (ps) mA fG mA fG mA fC mG fA mC fC mU fU mG fA mC (ps) fC (ps) mA	UGAGAGACGACCUUGACCA
396	EJ0010B	[ST23 (ps)]3 ST43 (ps) mU mG mG mU mC mA fA fG fG mU mC mG mU mC mU mC mU (ps) mC (ps) mA	UGGUCAAGGUCGUCUCUCA
397	EJ0011B	[ST23 (ps)]3 ST43 (ps) mC mG mG mU mC mA fA fG fG mU mC mU mU mC mU mC mU (ps) mC (ps) mA	CGGUCAAGGUCUUCUCUCA

398	EJ0012A	mU (ps) fG (ps) mA fG mA fG mA fC mG fA mC fC mU fU mG fA mC (ps) fC (ps) mG	UGAGAGACGACCUUGACCG
399	EJ0012B	[ST23 (ps)]3 ST43 (ps) mC mG mG mU mC mA fA fG fG mU mC mG mU mC mU mC mU (ps) mC (ps) mA	CGGUCAAGGUCGUCUCUCA
400	EJ0013B	[ST23 (ps)]3 ST43 (ps) mG mA mA mU mC mU fC fU fA mC mG mA mA mG mC mU mC (ps) mA (ps) mU	GAAUCUCUACGAAGCUCAU
401	EJ0014B	[ST23 (ps)]3 ST43 (ps) mC mC mA mA mG mA fU fC fC mG mC mU mA mC mU mA mC (ps) mA (ps) mA	CCAAGAUCCGCUACUACAA
402	EJ0015B	[ST23 (ps)]3 ST43 (ps) mC mC mU mC mU mU fC fA fU mC mC mA mG mA mC mA mG (ps) mA (ps) mA	CCUCUUCAUCCAGACAGAA
403	EJ0016A	(vp)-mU fG mA fG mA fG mA fC mG fA mC fC mU fU mG fA mC (ps) fC (ps) mG	UGAGAGACGACCUUGACCG
404	EJ0017A	(vp)-mU fG mA fG mA fG mA fC mG fA mC fC mU fU mG fA mC fC (ps2) mG	UGAGAGACGACCUUGACCG
405	EJ0017B	[ST23]3 ST43 mC (ps2) mG mG mU mC mA fA fG fG mU mC mG mU mC mU mC mU mC (ps2) mA	CGGUCAAGGUCGUCUCUCA
406	EJ0018A	mU (ps) fG (ps) mA fG mA fG mA fC mG fA mC fC mU fU mG fA mC fC (ps2) mG	UGAGAGACGACCUUGACCG
407	EJ0017B	[ST23]3 ST43 mC (ps2) mG mG mU mC mA fA fG fG mU mC mG mU mC mU mC mU mC (ps2) mA	CGGUCAAGGUCGUCUCUCA
408	EJ0019A	(vp)-mU fU mG fU mA fG mU fA mG fC mG fG mA fU mC fU mU (ps) fG (ps) mG	UUGUAGUAGCGGAUCUUGG
409	EJ0020A	(vp)-mU fU mG fU mA fG mU fA mG fC mG fG mA fU mC fU mU fG (ps2) mG	UUGUAGUAGCGGAUCUUGG
410	EJ0020B	[ST23]3 ST43 mC (ps2) mC mA mA mG mA fU fC fC mG mC mU mA mC mU mA mC mA (ps2) mA	CCAAGAUCCGCUACUACAA
411	EJ0021A	(vp)-mU fU mC fU mG fU mC fU mG fG mA fU mG fA mA fG mA (ps) fG (ps) mG	UUCUGUCUGGAUGAAGAGG
412	EJ0022A	(vp)-mU fU mC fU mG fU mC fU mG fG mA fU mG fA mA fG mA fG (ps2) mG	UUCUGUCUGGAUGAAGAGG
413	EJ0022B	[ST23]3 ST43 mC (ps2) mC mU mC mU mU fC fA fU mC mC mA mG mA mC mA mG mA (ps2) mA	CCUCUUCAUCCAGACAGAA
414	EJ0023A	mU (ps) fU (ps) mC fU mG fU mC fU mG fG mA fU mG fA mA fG mA fG (ps2) mG	UUCUGUCUGGAUGAAGAGG

415	EJ0022B	[ST23]3 ST43 mC (ps2) mC mU mC mU mU fC fA fU mC mC mA mG mA mC mA mG mA (ps2) mA	CCUCUUCAUCCAGACAGAA
416	EV0210Aun	UAUAUAUUCAUGAGCUUCG	UAUAUAUUCAUGAGCUUCG
417	EV0210A	mU (ps) fA (ps) mU fA mU fA mU fU mC fA mU fG mA fG mC fU mU (ps) fC (ps) mG	UAUAUAUUCAUGAGCUUCG
418	EV0210B	[ST23 (ps)]3 ST43 (ps) mC mG mA mA mG mC fU fC fA mU mG mA mA mU mA mU mA (ps) mU (ps) mU	CGAAGCUCAUGAAUAUAUU
419	EV0211A	(vp)-mU fA mU fA mU fA mU fU mC fA mU fG mA fG mC fU mU (ps) fC (ps) mG	UAUAUAUUCAUGAGCUUCG
420	EV0212A	(vp)-mU fA mU fA mU fA mU fU mC fA mU fG mA fG mC fU mU fC (ps2) mG	UAUAUAUUCAUGAGCUUCG
421	EV0211B	[ST23]3 ST43 mC (ps2) mG mA mA mG mC fU fC fA mU mG mA mA mU mA mU mA mU (ps2) mU	CGAAGCUCAUGAAUAUAUU
422	EV0213A	mU (ps) fA (ps) mU fA mU fA mU fU mC fA mU fG mA fG mC fU mU fC (ps2) mG	UAUAUAUUCAUGAGCUUCG
423	EJ0020B without ligand	mC (ps2) mC mA mA mG mA fU fC fC mG mC mU mA mC mU mA mC mA (ps2) mA	CCAAGAUCCGCUACUACAA
424	EV0211B without ligand	mC (ps2) mG mA mA mG mC fU fC fA mU mG mA mA mU mA mU mA mU (ps2) mU	CGAAGCUCAUGAAUAUAUU
425	EV0210B without ligand	mC mG mA mA mG mC fU fC fA mU mG mA mA mU mA mU mA (ps) mU (ps) mU	CGAAGCUCAUGAAUAUAUU