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(54) **Title:** ANTI HCV OLIGOMERS

(57) **Abstract:** The present invention relates to oligomer compounds (oligomers), which target HCV mRNA in a cell, leading to reduced expression of HCV. Reduction of HCV expression is beneficial for the treatment of certain medical disorders, such as Hepatitis C virus infection.

ANTI HCV OLIGOMERS

FIELD OF INVENTION

The present invention relates to oligomeric compounds (oligomers) that target HCV RNA in a cell, leading to reduced expression of HCV RNA. Reduction of HCV expression is beneficial for a range of medical disorders, such as Hepatitis C Virus infection.

BACKGROUND

Hepatitis C virus (HCV) is a member of the Flaviviridae family of viruses, which also includes the viruses that cause yellow fever and dengue. There are six major genotypes of HCV, which differ slightly in their genetic constitution and vary in their response to treatment. HCV has a single strand of ribonucleic acid (RNA) as its genetic material and the virus replicates by copying this RNA. Previous research has shown that the three-dimensional structure of HCV RNA appears to be crucial for initiating the viral replication process. HCV infections affect approximately 3 percent of the worldwide population and often lead to cirrhosis and hepatocellular carcinoma. The current therapy of pegylated-interferon and ribavirin induces serious side effects and provides viral eradication in less than 50% of patients.

Numerous attempts have been made to develop antisense oligonucleotides that will be effective in treating HCV infection in patients, however, so far no such compound has reached the market. One such oligonucleotide, ISIS 14803, which is a 20 nucleotide long phosphorothioate antisense oligodeoxynucleotide targeting the HCV IRES region (Soler et al 2004, Antiviral Therapy, 9, 953-968). This oligomer was tested in clinical trials, but its development was abandoned due to its low efficacy in reducing virus titres and possibly also due to ALT flares.

The present invention provides novel effective compounds for use in the treatment of HCV infections, both as a treatment to be combined with existing treatments, or as a single treatment.

SUMMARY OF INVENTION

The invention provides an oligomer from 10 - 50, such as 10 - 30 nucleotides in length which comprises a contiguous nucleotide sequence (a first region) of a total of from 10 - 30 nucleotides, wherein said contiguous nucleotide sequence (a first region) is at least 80% (e.g., 85%, 90%, 95%, 98%, or 99%) homologous to a region corresponding to the reverse

complement of a mammalian HCV gene or mRNA, such as SEQ ID NO: 1 or 2 or naturally occurring variant thereof. Thus, for example, the oligomer hybridizes to a single stranded nucleic acid molecule having the sequence of a portion of SEQ ID NO: 1 or 2. In one preferred embodiment, the oligomer target a sequence within the 5'untranslated region, such as within the region that correspond to nucleotides 20-42 of genotype 1a (SEQ ID NO 1) or nucleotides 20 - 44 in genotype 1b (SEQ ID NO 2), or such as in the region in any other HCV genotype that correspond to any one of SEQ ID NO's 1 or 2. HCV genotypes that may be targeted by oligomers of the present invention, may in non-limiting example be any one of genotypes 1a, b, c, d, e, f, g, h, i, j, k, or 11, 2a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p or 2q, 3a, b, c, d, e, f, g, h, i, or 3k, 4a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r or 4t, 5a, and 6a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, or 6q, and 7a(geno-/subtypes according to Simmonds et al. 2005, Hepatology vol 42, No. 4, p. 962-973, and Murphy D, Chamberland J, Dandavino R, Sablon E. A new genotype of hepatitis C virus orginating from central Africa [Abstract]. HEPATOLOGY 2007; 46: 623A.).

The invention provides for a conjugate comprising the oligomer according to the invention, and at least one non-nucleotide or non-polynucleotide moiety covalently attached to said oligomer.

The invention provides for a pharmaceutical composition comprising the oligomer or the conjugate according to the invention, and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

The invention provides for the oligomer or the conjugate according to invention, for use as a medicament, such as for the treatment of Hepatitis C Virus infection.

The invention provides for the use of an oligomer or the conjugate according to the invention, for the manufacture of a medicament for the treatment of Hepatitis C virus infection.

The invention provides for a method of treating Hepatitis C virus infection, said method comprising administering an, e.g. effective dose of, an oligomer, a conjugate or a pharmaceutical composition according to the invention, to a patient suffering from, or likely to suffer from Hepatitis C virus infection(such as a patient suffering from or susceptible to the disease or disorder).

In one embodiment, the disease or disorder or condition is associated with overexpression of HCV.

The invention provides for a method for the inhibition of HCV in a cell which is expressing HCV, said method comprising administering an oligomer, or a conjugate according to the invention to said cell so as to affect the inhibition of HCV in said cell.

The invention provides an oligomer of from 10-50 monomers, which comprises a first region of 10-50 contiguous monomers, wherein the sequence of the first region is at least

80% identical to a region corresponding to a mammalian HCV gene or to the reverse complement of a target region of a nucleic acid which encodes a mammalian HCV.

The invention further provides a conjugate comprising the oligomer according to the invention, which comprises at least one non-nucleotide or non-polynucleotide moiety ("conjugated moiety") covalently attached to the oligomer of the invention.

The invention provides for pharmaceutical compositions comprising an oligomer or conjugate of the invention, and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

The invention further provides for an oligomer according to the invention, for use in medicine.

The invention further provides for the use of the oligomer of the invention for the manufacture of a medicament for the treatment of one or more of the diseases referred to herein, such as a disease selected from the group consisting of Hepatitis C virus infection.

The invention further provides for an oligomer according to the invention, for use for the treatment of one or more of the diseases referred to herein, such as a disease selected from the group consisting of Hepatitis C virus infection.

Pharmaceutical and other compositions comprising an oligomer of the invention are also provided. Further provided are methods of down-regulating the expression of HCV in cells or tissues comprising contacting said cells or tissues, *in vitro* or *in vivo,* with an effective amount of one or more of the oligomers, conjugates or compositions of the invention.

Also disclosed are methods of treating an animal (a non-human animal or a human) suspected of having, or susceptible to, a disease or condition, associated with expression, or over-expression of HCV by administering to the non-human animal or human a therapeutically or prophylactically effective amount of one or more of the oligomers, conjugates or pharmaceutical compositions of the invention. Further, methods of using oligomers for the inhibition of expression of HCV, and for treatment of diseases associated with activity of HCV are provided.

The invention provides for a method for treating a disease selected from the group consisting of: Hepatitis C virus infection, the method comprising administering an effective amount of one or more oligomers, conjugates, or pharmaceutical compositions thereof to an animal in need thereof (such as a patient in need thereof).

The invention provides for methods of inhibiting (e.g., by down-regulating) the expression of HCV in a cell or a tissue, the method comprising the step of contacting the cell or tissue, *in vitro* or *in vivo,* with an effective amount of one or more oligomers, conjugates, or pharmaceutical compositions thereof, to affect down-regulation of expression of HCV.

FIGURES

Figure 1. Representation of how the TT and UU oligos will target both HCV genotype 1a and 1b by having one mismatch against each genotype.

Figure 2. Structure of the P-base and schematic representation of the base-pairing between the P-base and the G and A nucleotide, respectively.

Figure 3. Dose dependent activity/toxicity plots after screening of the TT and UU oligonucleotides. The genotype 1b specific pre-lead is shown in the left column. Y-axis: 1.0 = 100% activity - black line, 1.0 = 100% toxicity - red line. Data are average values ± stdev from five independent experiments. Data originate from vir-08-076.

DETAILED DESCRIPTION OF INVENTION

The Oligomer

The present invention employs oligomeric compounds (referred herein as oligomers), for use in modulating the function of nucleic acid molecules encoding mammalian HCV, and naturally occurring variants of such nucleic acid molecules encoding mammalian HCV. The present invention relates to oligomers targeting the Genomic RNA sequences of SEQ ID NOS 1, 2, 3, 4, 5, 6, or 7, or naturally occurring variants of such nucleic acid sequences within HCV Genomic RNA. The term "oligomer" in the context of the present invention, refers to a molecule formed by covalent linkage of two or more nucleotides *(i.e.* an oligonucleotide). Herein, a single nucleotide (unit) may also be referred to as a monomer or unit. In some embodiments, the terms "nucleoside", "nucleotide", "unit" and "monomer" are used interchangeably. It will be recognised that when referring to a sequence of nucleotides or monomers, what is referred to is the sequence of bases, such as A, T, G, C or U.

The oligomer consists or comprises of a contiguous nucleotide sequence of from 10 - 50, such as 10 - 30 nucleotides in length.

In various embodiments, the compound of the invention does not comprise RNA (units). It is preferred that the compound according to the invention is a linear molecule or is synthesised as a linear molecule. The oligomer is a single stranded molecule, and preferably does not comprise short regions of, for example, at least 3, 4 or 5 contiguous nucleotides, which are complementary to equivalent regions within the same oligomer *(i.e.* duplexes) - in this regards, the oligomer is not (essentially) double stranded. In some embodiments, the oligomer is essentially not double stranded, such as is not a siRNA. In various embodiments, the oligomer of the invention may consist entirely of the contiguous nucleotide region. Thus, the oligomer is not substantially self-complementary.

The Target

Suitably the oligomer of the invention is capable of down-regulating (e.g. reducing or removing) expression of the HCV gene. In this regards, the oligomer of the invention can affect the inhibition of HCV, typically in a mammalian such as a human cell, such as a liver cell. In one preferred embodiment, the oligomer target a sequence within the 5' untranslated region, such as within the region that correspond to nucleotides 20-44 of genotype 1a (SEQ ID NO 1) or nucleotides 20 - 44 in genotype 1b (SEQ ID NO 2), or within any one of SEQ ID NOS 3, 4, 5, 6 or 7 or such as in the region in any other HCV genotype that correspond to any one of SEQ ID NO's 1, 2, 3, 4, 5, 6 or 7. HCV genotypes that may be targeted by oligomers of the present invention, may in non-limiting example be any one of genotypes 1a, b, c, d, e, f, g, h, i, j, k, or 11, 2a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p or 2q, 3a, b, c, d, e, f, g, h, i, or 3k, 4a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r or 4t, 5a, and 6a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, or 6q, and 7a. In some embodiments, the oligomers of the invention bind to the target nucleic acid and affect inhibition of expression of at least 10% or 20% compared to the normal expression level, more preferably at least a 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% inhibition compared to the normal expression level (such as the expression level in the absence of the oligomer(s) or conjugate(s)). In some embodiments, such modulation is seen when using from 0.04 and 25nM, such as from 0.8 and 20nM concentration of the compound of the invention. In the same or a different embodiment, the inhibition of expression is less than 100%, such as less than 98% inhibition, less than 95% inhibition, less than 90% inhibition, less than 80% inhibition, such as less than 70% inhibition. Modulation of expression level may be determined by measuring protein levels, *e.g.* by the methods such as SDS-PAGE followed by western blotting using suitable antibodies raised against the target protein, or by measuring luciferase activity, if expression of the target is linked to the production of luciferase protein - as in the HUH7 replicon system used in this invention. Alternatively, modulation of expression levels can be determined by measuring levels of mRNA, e.g. by northern blotting or quantitative RT-PCR. When measuring via mRNA levels, the level of down-regulation when using an appropriate dosage, such as from 0.04 and 25nM, such as from 0.8 and 20nM concentration, is, In some embodiments, typically to a level of from 10-20% the normal levels in the absence of the compound, conjugate or composition of the invention.

As illustrated herein the cell type may, in some embodiments, be HUH7 replicon cells (e.g. *in vitro* - transfected cells). The oligomer concentration used (e.g. in HUH7 replicon cells) may, in some embodiments, be 5nM. The oligomer concentration used may, in some embodiments be 25nM (e.g. in HUH7 replicon cells). The oligomer concentration used may, in some embodiments be 1nM (e.g. in HUH7 replicon cells). It should be noted that the concentration of oligomer used to treat the cell is typically performed in an *in vitro* cell assay, using transfection (Lipofecton), as illustrated in the examples. In the absence of a

transfection agent, the oligo concentration required to obtain the down-regulation of the target is typically between 1 and 25μM, such as 5μM.

As used herein, the phrase "potent inhibitor" refers to an oligomer with an IC50 of less than 5nM as determined by the lipofectamin transfection assay of the examples. In some embodiments, the IC50 is less than 4nM, such as less than 2nM.

The invention therefore provides a method of down-regulating or inhibiting the expression of HCV protein and/or mRNA in a cell which is expressing HCV protein and/or mRNA, said method comprising administering the oligomer or conjugate according to the invention to said cell to down-regulating or inhibiting the expression of HCV protein and/or mRNA in said cell. Suitably the cell is a mammalian cell such as a human cell. The administration may occur, in some embodiments, *in vitro.* The administration may occur, in some embodiments, *in vivo.*

The term "target nucleic acid", as used herein refers to the DNA or RNA encoding mammalian HCV polypeptide, such as human HCV, such as SEQ ID NO: 1 or 2 or 3, 4, 5, 6 or 7. HCV encoding nucleic acids or naturally occurring variants thereof, and RNA nucleic acids derived therefrom, preferably mRNA, such as pre-mRNA, although preferably mature mRNA. In some embodiments, for example when used in research or diagnostics the "target nucleic acid" may be a cDNA or a synthetic oligonucleotide derived from the above DNA or RNA nucleic acid targets. The oligomer according to the invention is preferably capable of hybridising to the target nucleic acid. It will be recognised that SEQ ID NO: 1 or 2 is a cDNA sequences, and as such, corresponds to the genomic RNA target sequence, although uracil is replaced with thymidine in the cDNA sequences.

The term "naturally occurring variant thereof" refers to variants of the HCV polypeptide of nucleic acid sequence which exist naturally within the defined taxonomic group.

Sequences

The oligomers comprise or consist of a contiguous nucleotide sequence which corresponds to the reverse complement of a nucleotide sequence present in any one of SEQ ID NOs: 1-2, or 3-7 or in the corresponding sequences in any of the 7 known genotypes of HCV, such as in any another HCV genotype, such as in genotype 1a, b, c, d, e, f, g, h, i, j, k, or 11, 2a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p or 2q, 3a, b, c, d, e, f, g, h, i, or 3k, 4a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r or 4t, 5a, and 6a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, or 6q, and 7a. In a preferred embodiment, the oligomer can comprise or consist of a sequence selected from the group consisting of SEQ ID NOS: 23-31 and SEQ ID NOS: 78-86, wherein said oligomer (or contiguous nucleotide portion thereof) may optionally have one, two, or three mismatches against said selected sequence. In one preferred embodiment, the oligomer can comprise or consist of a sequence 10-16 nucleotides long,

which is present in any one of the sequences consisting of SEQ ID NOS: 23-31 and SEQ ID NOS: 78-86, or a sequence from another HCV genotype that corresponds to the sequence of any one of SEQ ID NOS: 23-31 and SEQ ID NOS: 78-86. In one embodiment, the oligomer is targeted to one or more of genotypes 1, 2, 3, 4, 5, 6 or 7. In one embodiment, the oligomer of the invention is targeted to a sequence in one or more genotypes/subtypes selected from the list of 1a, b, c, d, e, f, g, h, i, j, k, or 1l, 2a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p or 2q, 3a, b, c, d, e, f, g, h, i, or 3k, 4a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r or 4t, 5a, and 6a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, or 6q, and 7a, that corresponds to or is at least 70% homologous, such as 75%, such as 80%, such as 90% homologous to any one of SEQ ID NOS 1, 2, 3, 4, 5, 6 or 7. In one embodiment, the oligomer of the invention is targeted to a sequence in any one of subtypes 1a, 1b, 2a, 2b, 2c, 3a, 4a, 5a, or 6f that corresponds to or is at least 80% homologous to a sequence in any one of SEQ ID NOS 1-7.

TABLE1

SEQ ID	MOTIF SEQUENCE
1	GACACTCCACCATGAATCACTCC
2	GACACTCCACCATAGATCACTCC
3	ACACUCCGCCAUGAAUCACUCC
4	ACACUCCACCAUGGAUCACUCC
5	ACACUCCACCAUGAACCGCUCC
6	ACACUCCACCAUGAUCACUCC
7	ACACUCCACCAUGAUCACUCC
23	TCTATGGTGGAGTGTC
24	ATCTATGGTGGAGTGT
25	GATCTATGGTGGAGTG
26	TGATCTATGGTGGAGT
27	GTGATCTATGGTGGAG
28	AGTGATCTATGGTGGA
29	GAGTGATCTATGGTGG
30	GGAGTGATCTATGGTG
31	TGATTCATGGTGGAGT
78	TTCATGGTGGAGTGTC
79	ATTCATGGTGGAGTGT
80	GATTCATGGTGGAGTG
81	TGATTCATGGTGGAGT
82	GTGATTCATGGTGGAG
83	AGTGATTCATGGTGGA
84	GAGTGATTCATGGTGG
85	GGAGTGATTCATGGTG
86	TGATTCATGGTGGAGT

The oligomer may comprise or consist of a contiguous nucleotide sequence which is fully complementary (perfectly complementary) to the equivalent region of a nucleic acid which encodes a mammalian HCV (e.g., any one of SEQ ID NO: 1-2 or 3-7). Thus, the oligomer can comprise or consist of an antisense nucleotide sequence.

However, in some embodiments, the oligomer may tolerate 1, 2, 3, or 4 (or more) mismatches, when hybridising to the target sequence and still sufficiently bind to the target to show the desired effect, *i.e.* down-regulation of the target. Mismatches may, for example, be compensated by increased length of the oligomer nucleotide sequence and/or an increased number of nucleotide analogues, such as LNA, present within the nucleotide sequence.

In some embodiments, the oligomer contains has a T/G mismatch, to allow the oligomer having equal activity towards two genotypes of HCV. In some embodiments, universal bases may be introduced to compensate for natural sequence variability between genotypes or species of the virus.

In some embodiments, the contiguous nucleotide sequence comprises no more than 3, such as no more than 2 mismatches when hybridizing to the target sequence, such as to the corresponding region of a nucleic acid which encodes a mammalian HCV.

In some embodiments, the contiguous nucleotide sequence comprises no more than a single mismatch when hybridizing to the target sequence, such as the corresponding region of a nucleic acid which encodes a mammalian HCV.

The nucleotide sequence of the oligomers of the invention or the contiguous nucleotide sequence is preferably at least 80% homologous to a corresponding sequence selected from the group consisting of SEQ ID NOS: 1-7, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% homologous, at least 97% homologous, at least 98% homologous, at least 99% homologous, such as 100% homologous (identical).

The nucleotide sequence of the oligomers of the invention or the contiguous nucleotide sequence is preferably at least 80% homologous to the reverse complement of a corresponding sequence present in SEQ ID NO: 1-7, such as at least 85%, at least 90%, at least 91%, at least 92%at least 93%, at least 94%, at least 95%, at least 96% homologous, at least 97% homologous, at least 98% homologous, at least 99% homologous, such as 100% homologous (identical).

The nucleotide sequence of the oligomers of the invention or the contiguous nucleotide sequence is preferably at least 80% complementary to a sub-sequence present in SEQ ID NO: 1-2 or 3-7, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% complementary, at least 97%

complementary, at least 98% complementary, at least 99% complementary, such as 100% complementary (perfectly complementary).

In some embodiments, the term "first region" as used herein refers to a portion (sub-sequence) of an oligomer. For example, the 16 monomer sequence SEQ ID No 23 is a subsequence of the 23 monomer sequence SEQ ID No 2 and comprises 16 contiguous monomers of SEQ ID No 2.

In some embodiments the oligomer (or contiguous nucleotide portion thereof) is selected from, or comprises, one of the sequences selected from the group consisting of SEQ ID NOS: 23-31 and SEQ ID NOS: 78-86, or a sub-sequence of at least 10 contiguous nucleotides thereof, wherein said oligomer (or contiguous nucleotide portion thereof) may optionally comprise one, two, or three mismatches when compared to the sequence.

In some embodiments the sub-sequence may consist of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23contiguous nucleotides, such as from 12 -22, such as from 12-18 nucleotides. Suitably, in some embodiments, the sub-sequence is of the same length as the contiguous nucleotide sequence of the oligomer of the invention.

However, it is recognised that, in some embodiments the nucleotide sequence of the oligomer may comprise additional 5' or 3' nucleotides, such as, independently, 1, 2, 3, 4 or 5 additional nucleotides 5' and/or 3', which are non-complementary to the target sequence. In this respect the oligomer of the invention, may, in some embodiments, comprise a contiguous nucleotide sequence which is flanked 5' and or 3' by additional nucleotides. In some embodiments the additional 5' or 3' nucleotides are naturally occurring nucleotides, such as DNA or RNA. In some embodiments, the additional 5' or 3' nucleotides may represent region D as referred to in the context of gapmer oligomers herein.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:23, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:24, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:25, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:26, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:27, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:28, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:29, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:30, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:31 , or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:78, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:79, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:80, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:81 , or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:82, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:83, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:84, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:85, or a sub-sequence of thereof.

In some embodiments the oligomer according to the invention consists or comprises of a nucleotide sequence according to SEQ ID NO:86, or a sub-sequence of thereof.

In determining the degree of "complementarity" between oligomers of the invention (or regions thereof) and the target region of the nucleic acid which encodes mammalian HCV, such as those disclosed herein, the degree of "complementarity" (also, "homology" or "identity") is expressed as the percentage identity (or percentage homology) between the sequence of the oligomer (or region thereof) and the sequence of the target region (or the reverse complement of the target region) that best aligns therewith. The percentage is calculated by counting the number of aligned bases that are identical between the 2 sequences, dividing by the total number of contiguous monomers in the oligomer, and multiplying by 100. In such a comparison, if gaps exist, it is preferable that such gaps are merely mismatches rather than areas where the number of monomers within the gap differs between the oligomer of the invention and the target region.

HCV. As used herein, the terms "homologous" and "homology" are interchangeable with the terms "identity" and "identical".

The terms "corresponding to" and "corresponds to" refer to the comparison between the nucleotide sequence of the oligomer *(i.e.* the nucleobase or base sequence) or

contiguous nucleotide sequence (a first region) and the equivalent contiguous nucleotide sequence of a further sequence selected from either i) a sub-sequence of the reverse complement of the nucleic acid target, such as the mRNA which encodes the HCV protein. Nucleotide analogues are compared directly to their equivalent or corresponding nucleotides. A first sequence which corresponds to a further sequence under i) or ii) typically is identicial to that sequence over the length of the first sequence (such as the contiguous nucleotide sequence) or, as described herein may, in some embodiments, is at least 80% homologous to a corresponding sequence, such as at least 85%, at least 90%, at least 91%, at least 92%at least 93%, at least 94%, at least 95%, at least 96% homologous, such as 100% homologous (identical).

The terms "corresponding nucleotide analogue" and "corresponding nucleotide" are intended to indicate that the nucleotide in the nucleotide analogue and the naturally occurring nucleotide are identical. For example, when the 2-deoxyribose unit of the nucleotide is linked to an adenine, the "corresponding nucleotide analogue" contains a pentose unit (different from 2-deoxyribose) linked to an adenine.

The terms "reverse complement", "reverse complementary" and "reverse complementarity" as used herein are interchangeable with the terms "complement", "complementary" and "complementarity".

Length

The oligomers may comprise or consist of a contiguous nucleotide sequence of a total of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous nucleotides in length.

In some embodiments, the oligomers comprise or consist of a contiguous nucleotide sequence of a total of from 10 - 22, such as 12 - 18, such as 13 - 17 or 12 - 16, such as 10, 11, 12, 13, 14, 15, or 16 contiguous nucleotides in length.

In some embodiments, the oligomers comprise or consist of a contiguous nucleotide sequence of a total of 10, 11, 12, 13, or 14 contiguous nucleotides in length.

In some embodiments, the oligomer according to the invention consists of no more than 22 nucleotides, such as no more than 20 nucleotides, such as no more than 18 nucleotides, such as 15, 16 or 17 nucleotides. In some embodiments the oligomer of the invention comprises less than 20 nucleotides. It should be understood that when a range is given for an oligomer, or contiguous nucleotide sequence length it includes the lower an upper lengths provided in the range, for example from (or between) 10 - 30, includes both 10 and 30.

Nucleosides and Nucleoside analogues

In some embodiments, the terms "nucleoside analogue" and "nucleotide analogue" are used interchangeably.

The term "nucleotide" as used herein, refers to a glycoside comprising a sugar moiety, a base moiety and a covalently linked group (linkage group), such as a phosphate or phosphorothioate internucleotide linkage group, and covers both naturally occurring nucleotides, such as DNA or RNA, and non-naturally occurring nucleotides comprising modified sugar and/or base moieties, which are also referred to as "nucleotide analogues" herein. Herein, a single nucleotide (unit) may also be referred to as a monomer or nucleic acid unit.

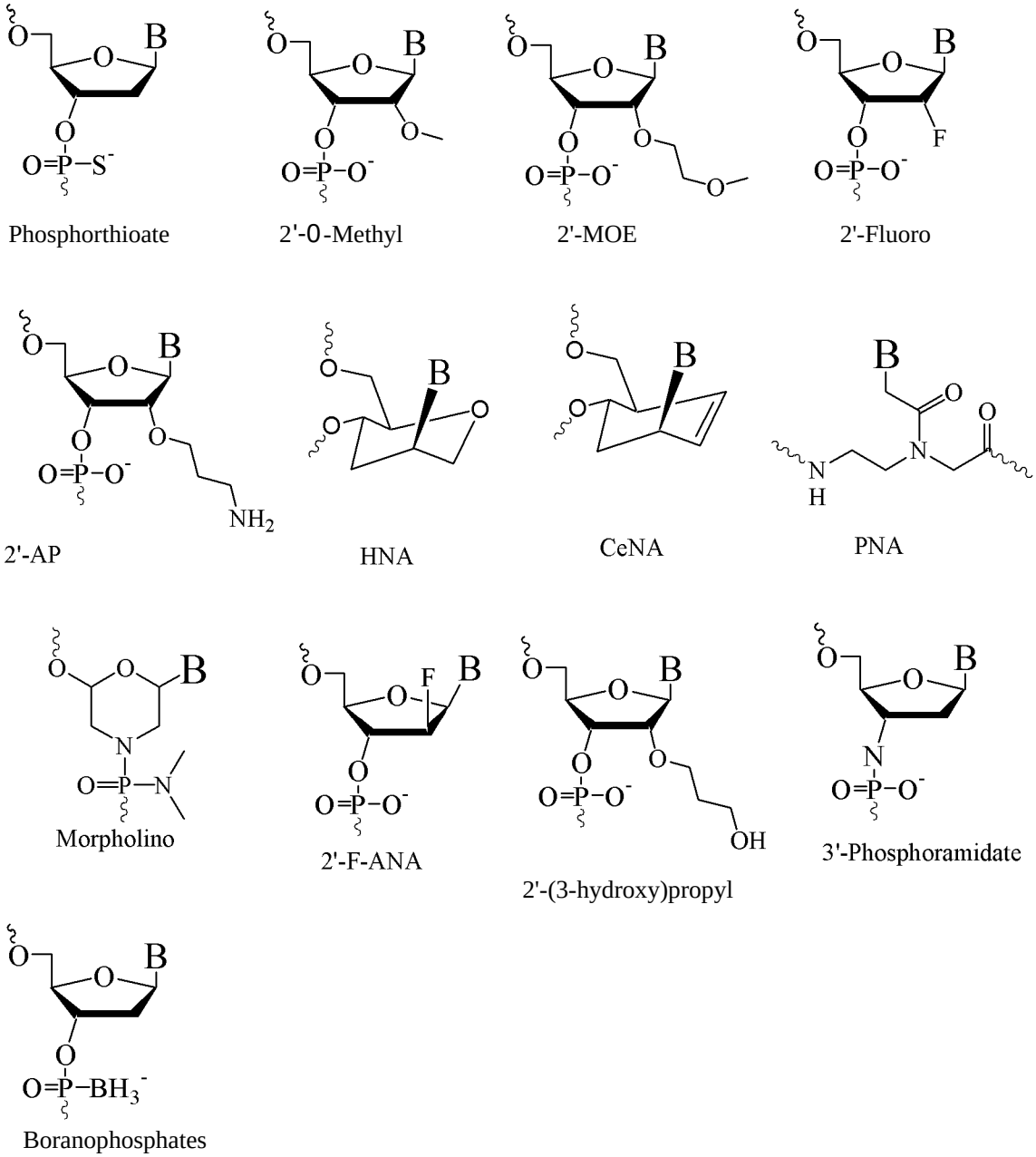
In field of biochemistry, the term "nucleoside" is commonly used to refer to a glycoside comprising a sugar moiety and a base moiety, and may therefore be used when referring to the nucleotide units, which are covalently linked by the internucleotide linkages between the nucleotides of the oligomer. In the field of biotechnology, the term "nucleotide" is often used to refer to a nucleic acid monomer or unit, and as such in the context of an oligonucleotide may refer to the base - such as the "nucleotide sequence", typically refer to the nucleobase sequence *(i.e.* the presence of the sugar backbone and internucleoside linkages are implicit). Likewise, particularly in the case of oligonucleotides where one or more of the internucleoside linkage groups are modified, the term "nucleotide" may refer to a "nucleoside" for example the term "nucleotide" may be used, even when specifiying the presence or nature of the linkages between the nucleosides.

As one of ordinary skill in the art would recognise, the 5' terminal nucleotide of an oligonucleotide does not comprise a 5' internucleotide linkage group, although may or may not comprise a 5' terminal group.

Non-naturally occurring nucleotides include nucleotides which have modified sugar moieties, such as bicyclic nucleotides or 2' modified nucleotides, such as 2' substituted nucleotides.

"Nucleotide analogues" are variants of natural nucleotides, such as DNA or RNA nucleotides, by virtue of modifications in the sugar and/or base moieties. Analogues could in principle be merely "silent" or "equivalent" to the natural nucleotides in the context of the oligonucleotide, *i.e.* have no functional effect on the way the oligonucleotide works to inhibit target gene expression. Such "equivalent" analogues may nevertheless be useful if, for example, they are easier or cheaper to manufacture, or are more stable to storage or manufacturing conditions, or represent a tag or label. Preferably, however, the analogues will have a functional effect on the way in which the oligomer works to inhibit expression; for example by producing increased binding affinity to the target and/or increased resistance to intracellular nucleases and/or increased ease of transport into the cell. Specific examples of nucleoside analogues are described by *e.g.* Freier & Altmann; *Nucl. Acid Res.,* 1997, 25,

4429-4443 and Uhlmann; *Curr. Opinion in Drug Development,* 2000, 3(2), 293-213, and in Scheme 1:



Scheme 1

The oligomer may thus comprise or consist of a simple sequence of natural occurring nucleotides - preferably 2'-deoxynucleotides (referred to here generally as "DNA"), but also possibly ribonucleotides (referred to here generally as "RNA"), or a combination of such naturally occurring nucleotides and one or more non-naturally occurring nucleotides, *i.e.* nucleotide analogues. Such nucleotide analogues may suitably enhance the affinity of the oligomer for the target sequence.

Examples of suitable and preferred nucleotide analogues are provided by WO2007/031091or are referenced therein.

Incorporation of affinity-enhancing nucleotide analogues in the oligomer, such as LNA or 2'-substituted sugars, can allow the size of the specifically binding oligomer to be reduced, and may also reduce the upper limit to the size of the oligomer before non-specific or aberrant binding takes place.

In some embodiments, the oligomer comprises at least 1 nucleoside analogue. In some embodiments the oligomer comprises at least 2 nucleotide analogues. In some embodiments, the oligomer comprises from 3-8 nucleotide analogues, *e.g.* 6 or 7 nucleotide analogues. In the by far most preferred embodiments, at least one of said nucleotide analogues is a locked nucleic acid (LNA); for example at least 3 or at least 4, or at least 5, or at least 6, or at least 7, or 8, of the nucleotide analogues may be LNA. In some embodiments all the nucleotides analogues may be LNA.

It will be recognised that when referring to a preferred nucleotide sequence motif or nucleotide sequence, which consists of only nucleotides, the oligomers of the invention which are defined by that sequence may comprise a corresponding nucleotide analogue in place of one or more of the nucleotides present in said sequence, such as LNA units or other nucleotide analogues, which raise the duplex stability/T_m of the oligomer/target duplex *(i.e.* affinity enhancing nucleotide analogues).

In some embodiments, any mismatches between the nucleotide sequence of the oligomer and the target sequence are preferably found in regions outside the affinity enhancing nucleotide analogues, such as region B as referred to herein, and/or region D as referred to herein, and/or at the site of non modified such as DNA nucleotides in the oligonucleotide, and/or in regions which are 5' or 3' to the contiguous nucleotide sequence.

Examples of such modification of the nucleotide include modifying the sugar moiety to provide a 2'-substituent group or to produce a bridged (locked nucleic acid) structure which enhances binding affinity and may also provide increased nuclease resistance.

A preferred nucleotide analogue is LNA, such as oxy-LNA (such as beta-D-oxy-LNA, and alpha-L-oxy-LNA), and/or amino-LNA (such as beta-D-amino-LNA and alpha-L-amino-LNA) and/or thio-LNA (such as beta-D-thio-LNA and alpha-L-thio-LNA) and/or ENA (such as beta-D-ENA and alpha-L-ENA). Most preferred is beta-D-oxy-LNA.

In some embodiments the nucleotide analogues present within the oligomer of the invention (such as in regions A and C mentioned herein) are independently selected from, for example: 2'-0-alkyl-RNA units, 2'-amino-DNA units, 2'-fluoro-DNA units, LNA units, arabino nucleic acid (ANA) units, 2'-fluoro-ANA units, HNA units, INA (intercalating nucleic acid -Christensen, 2002. Nucl. Acids. Res. 2002 30: 4918-4925, hereby incorporated by reference) units and 2'MOE units. In some embodiments there is only one of the above types of nucleotide analogues present in the oligomer of the invention, or contiguous nucleotide sequence thereof.

In some embodiments the nucleotide analogues are 2'-0-methoxyethyl-RNA (2'MOE), 2'-fluoro-DNA monomers or LNA nucleotide analogues, and as such the oligonucleotide of the invention may comprise nucleotide analogues which are independently selected from these three types of analogue, or may comprise only one type of analogue selected from the three types. In some embodiments at least one of said nucleotide analogues is 2'-MOE-RNA, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 2'-MOE-RNA nucleotide units. In some embodiments at least one of said nucleotide analogues is 2'-fluoro DNA, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 2'-fluoro-DNA nucleotide units.

In some embodiments, the oligomer according to the invention comprises at least one Locked Nucleic Acid (LNA) unit, such as 1, 2, 3, 4, 5, 6, 7, or 8 LNA units, such as from 3 - 7 or 4 to 8 LNA units, or 3, 4, 5, 6 or 7 LNA units. In some embodiments, all the nucleotide analogues are LNA. In some embodiments, the oligomer may comprise both beta-D-oxy-LNA, and one or more of the following LNA units: thio-LNA, amino-LNA, oxy-LNA, and/or ENA in either the beta-D or alpha-L configurations or combinations thereof. In some embodiments all LNA cytosine units are 5'methyl-Cytosine. In some embodiments of the invention, the oligomer may comprise both LNA and DNA units. Preferably the combined total of LNA and DNA units is 10-25, such as 10 - 24, preferably 10-20, such as 10 - 18, even more preferably 12-16. In some embodiments of the invention, the nucleotide sequence of the oligomer, such as the contiguous nucleotide sequence consists of at least one LNA and the remaining nucleotide units are DNA units. In some embodiments the oligomer comprises only LNA nucleotide analogues and naturally occurring nucleotides (such as RNA or DNA, most preferably DNA nucleotides), optionally with modified internucleotide linkages such as phosphorothioate.

The term "nucleobase" refers to the base moiety of a nucleotide and covers both naturally occuring a well as non-naturally occurring variants. Thus, "nucleobase" covers not only the known purine and pyrimidine heterocycles but also heterocyclic analogues and tautomeres thereof.

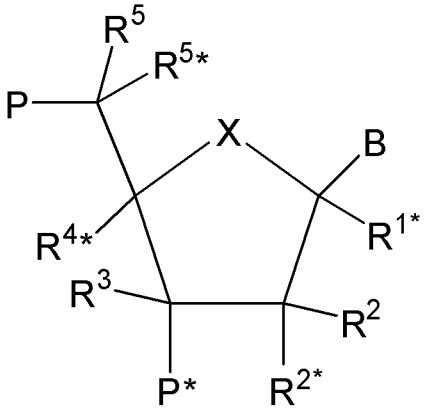
Examples of nucleobases include, but are not limited to adenine, guanine, cytosine, thymidine, uracil, xanthine, hypoxanthine, 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine.

In some embodiments, at least one of the nucleobases present in the oligomer is a modified nucleobase selected from the group consisting of 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine.

LNA

The term "LNA" refers to a bicyclic nucleoside analogue, known as "Locked Nucleic Acid". It may refer to an LNA monomer, or, when used in the context of an "LNA oligonucleotide", LNA refers to an oligonucleotide containing one or more such bicyclic nucleotide analogues. LNA nucleotides are characterised by the presence of a linker group (such as a bridge) between C2' and C4' of the ribose sugar ring - for example as shown as the biradical R^{4*} - R^{2*} as described below.

The LNA used in the oligonucleotide compounds of the invention preferably has the structure of the general formula I



Formula 1

wherein for all chiral centers, asymmetric groups may be found in either R or S orientation;

wherein X is selected from -0-, -S-, -N(R^{N*})-, -C(R^6R^{6*})-, such as, in some embodiments -0-;

B is selected from hydrogen, optionally substituted C_{1-4}-alkoxy, optionally substituted C_{1-4}-alkyl, optionally substituted C_{1-4}-acyloxy, nucleobases including naturally occurring and nucleobase analogues, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands; preferably, B is a nucleobase or nucleobase analogue;

P designates an internucleotide linkage to an adjacent monomer, or a 5'-terminal group, such internucleotide linkage or 5'-terminal group optionally including the substituent R^5 or equally applicable the substituent R^{5*};

P* designates an internucleotide linkage to an adjacent monomer, or a 3'-terminal group;

R^{4*} and R^{2*} together designate a bivalent linker group consisting of 1 - 4 groups/atoms selected from -C(R^aR^b)-, -C(R^a)=C(R^b)-, -C(R^a)=N-, -0-, -Si(R^a)$_2$-, -S-, -S0 $_2$-, -N(R^a)-, and >C=Z, wherein Z is selected from -0-, -S-, and -N(R^a)-, and R^a and R^b each is independently selected from hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, optionally substituted Ci-$_{12}$-alkoxy, C_{2-12}-alkoxyalkyl, C_{2-12}-alkenyloxy, carboxy, Ci-i$_2$-alkoxycarbonyl, Ci-i$_2$-alkylcarbonyl, formyl, aryl,

aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(Ci-6-alkyl)amino, carbamoyl, mono- and di(Ci-6-alkyl)-amino-carbonyl, amino-Ci-6-alkyl-aminocarbonyl, mono- and di(Ci-6-alkyl)amino-Ci-6-alkyl-aminocarbonyl, C1-6-alkyl-carbonylamino, carbamido, C1-6-alkanoyloxy, sulphono, C1-6-alkylsulphonyloxy, nitro, azido, sulphanyl, C1-6-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH_2), wherein for all chiral centers, asymmetric groups may be found in either *R* or S orientation, and;

each of the substituents R^{1*}, R^2, R^3, R^5, R^{5*}, R^6 and R^{6*}, which are present is independently selected from hydrogen, optionally substituted Ci_i2-alkyl, optionally substituted C2-i2-alkenyl, optionally substituted C2-i2-alkynyl, hydroxy, Ci-12-alkoxy, C2-12-alkoxyalkyl, C2-i2-alkenyloxy, carboxy, Ci-12-alkoxycarbonyl, Ci-12-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(Ci-6-alkyl)amino, carbamoyl, mono- and di(Ci-6-alkyl)-amino-carbonyl, amino-Ci-6-alkyl-aminocarbonyl, mono- and di(Ci-6-alkyl)amino-Ci-6-alkyl-aminocarbonyl, C1-6-alkyl-carbonylamino, carbamido, C1-6-alkanoyloxy, sulphono, C1-6-alkylsulphonyloxy, nitro, azido, sulphanyl, C1-6-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene; ; wherein R^N is selected from hydrogen and C1-4-alkyl, and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond; and R^{N*}, when present and not involved in a biradical, is selected from hydrogen and C1-4-alkyl; and basic salts and acid addition salts thereof. For all chiral centers, asymmetric groups may be found in either *R* or S orientation.

In some embodiments, R^{4*} and R^{2*} together designate a biradical consisting of a groups selected from the group consisting of $C(R^aR^b)$-$C(R^aR^b)$-, $C(R^aR^b)$-0-, $C(R^aR^b)$-NR^a-, $C(R^aR^b)$-S-, and $C(R^aR^b)$-$C(R^aR^b)$-0-, wherein each R^a and R^b may optionally be independently selected. In some embodiments, R^a and R^b may be, optionally independently selected from the group consisting of hydrogen and Ci-6alkyl, such as methyl, such as hydrogen.

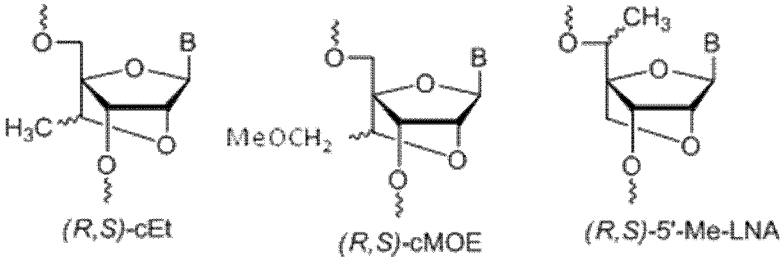
In some embodiments, R^{4*} and R^{2*} together designate the biradical -0-$CH(CH_2OCH_3)$- (2'O-methoxyethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem) - in either the R- or S- configuration.

In some embodiments, R^{4*} and R^{2*} together designate the biradical -0-CH(CH $_2$CH$_3$)- (2'0-ethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem). - in either the R- or S-configuration.

In some embodiments, R^{4*} and R^{2*} together designate the biradical -0-CH(CH $_3$)-. - in either the R- or S- configuration. In some embodiments, R^{4*} and R^{2*} together designate the biradical -0-CH $_2$-0-CH $_2$- - (Seth at al., 2010, J. Org. Chem).

In some embodiments, R^{4*} and R^{2*} together designate the biradical -0-NR-CH $_3$- - (Seth at al., 2010, J. Org. Chem) .

In some embodiments, the LNA units have a structure selected from the following group:



In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. For all chiral centers, asymmetric groups may be found in either *R* or S orientation.

In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen.

In some embodiments, R^{1*}, R^2, R^3 are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. For all chiral centers, asymmetric groups may be found in either *R* or S orientation.

In some embodiments, R^{1*}, R^2, R^3 are hydrogen.

In some embodiments, R^5 and R^{5*} are each independently selected from the group consisting of H, -CH $_3$, -CH$_2$-CH$_3$,- CH$_2$-0-CH $_3$, and -CH=CH$_2$. Suitably in some embodiments, either R^5 or R^{5*} are hydrogen, where as the other group (R^5 or R^{5*} respectively) is selected from the group consisting of C_{1-5} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{1-6} alkyl, substituted C_{2-6} alkenyl, substituted C_{2-6} alkynyl or substituted acyl (-C(=0)-); wherein each substituted group is mono or poly substituted with substituent groups independently selected from halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl,

substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, OJ1 , SJi , NJ_1J_2, N_3, COOJ1 , CN, 0-C(=0)NJ $_1J_2$, N(H)C(=N H)NJ ,J_2 or N(H)C(=X)N(H)J $_2$ wherein X is O or S ; and each J, and J_2 is, independently, H, Ci_6 alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, Ci-$_6$ aminoalkyl, substituted Ci-$_6$ aminoalkyl or a protecting group. In some embodiments either R^5 or R^{5*} is substituted C_{1-6} alkyl. In some embodiments either R^5 or R^{5*} is substituted methylene wherein preferred substituent groups include one or more groups independently selected from F, NJ_1J_2, N_3, CN, OJ1 , SJi , O-C(=0)NJ $_1J_2$, N(H)C(=N H)NJ , J_2 or N(H)C(0)N(H)J $_2$. In some embodiments each J, and J_2 is, independently H or C_{1-6} alkyl. In some embodiments either R^5 or R^{5*} is methyl, ethyl or methoxymethyl. In some embodiments either R^5 or R^{5*} is methyl. In a further embodiment either R^5 or R^{5*} is ethylenyl. In some embodiments either R^5 or R^{5*} is substituted acyl. In some embodiments either R^5 or R^{5*} is C(=0)NJ $_1J_2$. For all chiral centers, asymmetric groups may be found in either *R* or S orientation. Such 5' modified bicyclic nucleotides are disclosed in WO 2007/134181, which is hereby incorporated by reference in its entirety.

In some embodiments B is a nucleobase, including nucleobase analogues and naturally occurring nucleobases, such as a purine or pyrimidine, or a substituted purine or substituted pyrimidine, such as a nucleobase referred to herein, such as a nucleobase selected from the group consisting of adenine, cytosine, thymine, adenine, uracil, and/or a modified or substituted nucleobase, such as 5-thiazolo-uracil, 2-thio-uracil, 5-propynyl-uracil, 2'thio-thymine, 5-methyl cytosine, 5-thiozolo-cytosine, 5-propynyl-cytosine, and 2,6-diaminopurine.

In some embodiments, R^{4*} and R^{2*} together designate a biradical selected from -C(R^aR^b)-0-, -C(R^aR^b)-C(R^cR^d)-0-, -C(R^aR^b)-C(R^cR^d)-C(R^eR^f)-0-, -C(R^aR^b)-0-C(R^cR^d)-, -C(R^aR^b)-0-C(R^cR^d)-0-, -C(R^aR^b)-C(R^cR^d)-, -C(R^aR^b)-C(R^cR^d)-C(R^eR^f)-, -C(R^a)=C(R^b)-C(R^cR^d)-, -C(R^aR^b)-N(R^c)-, -C(R^aR^b)-C(R^cR^d)- N(R^e)-, -C(R^aR^b)-N(R^c)-0-, and -C(R^aR^b)-S-, -C(R^aR^b)-C(R^cR^d)-S-, wherein R^a, R^b, R^c, R^d, R^e, and R^f each is independently selected from hydrogen, optionally substituted Ci-i $_2$-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, Ci-i $_2$-alkoxy, C_{2-12}-alkoxyalkyl, C_{2-12}-alkenyloxy, carboxy, Ci-i $_2$-alkoxycarbonyl, Ci-i $_2$-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(Ci $_6$-alkyl)amino, carbamoyl, mono- and di(Ci $_6$-alkyl)-amino-carbonyl, amino-Ci-6-alkyl-aminocarbonyl, mono- and di(Ci-6-alkyl)amino-Ci-$_6$-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b

together may designate optionally substituted methylene (=CH_2). For all chiral centers, asymmetric groups may be found in either *R* or S orientation.

In a further embodiment R^{4*} and R^{2*} together designate a biradical (bivalent group) selected from -CH_2-0-, -CH_2-S-, -CH_2-NH-, -CH_2-N(CH_3)-, -CH_2-CH_2-0-, -CH_2-CH(CH_3)-, -CH_2-CH_2-S-, -CH_2-CH_2-NH-, -CH_2-CH_2-CH_2-, -CH_2-CH_2-CH_2-0-, -CH_2-CH_2-CH(CH_3)-, -CH=CH-CH_2-, -CH_2-0-CH_2-0-, -CH_2-NH-0-, -CH_2-N(CH_3)-0-, -CH_2-0-CH_2-, -CH(CH_3)-0-, and -CH(CH_2-0-CH_3)-0-, and/or, -CH_2-CH_2-, and -CH=CH- For all chiral centers, asymmetric groups may be found in either *R* or S orientation.

In some embodiments, R^{4*} and R^{2*} together designate the biradical C(R^aR^b)-N(R^c)-0-, wherein R^a and R^b are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{i-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen, and; wherein R^c is selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen.

In some embodiments, R^{4*} and R^{2*} together designate the biradical C(R^aR^b)-0-C(R^cR^d) -0-, wherein R^a, R^b, R^c, and R^d are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, Ci-6 aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen.

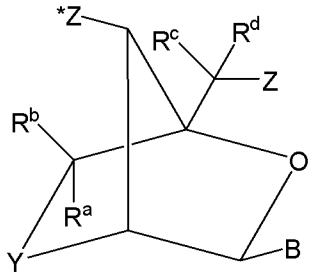
In some embodiments, R^{4*} and R^{2*} form the biradical -CH(Z)-0-, wherein Z is selected from the group consisting of C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{1-6} alkyl, substituted C_{2-6} alkenyl, substituted C_{2-6} alkynyl, acyl, substituted acyl, substituted amide, thiol or substituted thio; and wherein each of the substituted groups, is, independently, mono or poly substituted with optionally protected substituent groups independently selected from halogen, oxo, hydroxyl, OJi, NJiJ$_2$, SJi, N_3, OC(=X)Ji, OC(=X)NJ$_1$J$_2$, NJ^{3}C(=X)NJ$_1$J$_2$ and CN, wherein each J_1, J_2 and J_3 is, independently, H or C_{1-6} alkyl, and X is O, S or N^. In some embodiments Z is C_{1-6} alkyl or substituted C_{1-6} alkyl. In some embodiments Z is methyl. In some embodiments Z is substituted C_{1-6} alkyl. In some embodiments said substituent group is C_{1-6} alkoxy. In some embodiments Z is CH_3OCH_2-. For all chiral centers, asymmetric groups may be found in either *R* or S orientation. Such bicyclic nucleotides are disclosed in US 7,399,845 which is hereby incorporated by reference in its entirety. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. In some some embodiments, R^{1*}, R^2, R^{3*}

are hydrogen, and one or both of R^5, R^{5*} may be other than hydrogen as referred to above and in WO 2007/134181 .

In some embodiments, R^{4*} and R^{2*} together designate a biradical which comprise a substituted amino group in the bridge such as consist or comprise of the biradical -CH_2-N(R^c)-, wherein R^c is C_{1-12} alkyloxy. In some embodiments R^{4*} and R^{2*} together designate a biradical -Cq_3q_4-NOR -, wherein q_3 and q_4 are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl; wherein each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, OJi, SJi, $NJiJ_2$, COOJi, CN, 0-C(=0)NJ_1J_2, N(H)C(=NH)NJ_1J_2 or N(H)C(=X=N(H)J_2 wherein X is O or S; and each of J_1 and J_2 is, independently, H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} aminoalkyl or a protecting group. For all chiral centers, asymmetric groups may be found in either *R* or S orientation. Such bicyclic nucleotides are disclosed in WO2008/150729 which is hereby incorporated by reference in its entirity. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. In some embodiments, R^{1*}, R^2, R^3 are hydrogen and one or both of R^5, R^{5*} may be other than hydrogen as referred to above and in WO 2007/134181 . In some embodiments R^{4*} and R^{2*} together designate a biradical (bivalent group) C(R^aR^b)-0-, wherein R^a and R^b are each independently halogen, Ci-Ci_2 alkyl, substituted C_1-C_{12} alkyl, C_2-Ci_2 alkenyl, substituted C_2-Ci_2 alkenyl, C_2-Ci_2 alkynyl, substituted C_2-Ci_2 alkynyl, C_1-C_{12} alkoxy, substituted C_1-C_{12} alkoxy, OJi SJi, SOJi, $S0_2Ji$, $NJiJ_2$, N_3, CN, C(=O)OJ_1, C(=0)NJ_1J_2, C(=O)J_1, 0-C(=0)NJ_1J_2, N(H)C(=NH)NJ_1J_2, N(H)C(=0)NJ_1J_2 or N(H)C(=S)NJ_1J_2; or R^a and R^b together are =C(q3)(q4); q_3 and q_4 are each, independently, H, halogen, Ci-Ci_2alkyl or substituted Ci-Ci_2 alkyl; each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, C_1-C_6 alkyl, substituted Ci-C_6 alkyl, C_2- C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_2-C_6 alkynyl, OJi, SJi, $NJiJ_2$, N_3, CN, C(=0)OJi, C(=0)NJ_1J_2, C(=0)Ji, O-C(=0)NJ_1J_2, N(H)C(=0)NJ_1J_2 or N(H)C(=S)NJ_1J_2. and; each J, and J_2 is, independently, H, C1-C_6 alkyl, substituted C1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_2-C_6 alkynyl, C1-C_6 aminoalkyl, substituted C1-C_6 aminoalkyl or a protecting group. Such compounds are disclosed in WO2009006478A, hereby incorporated in its entirety by reference.

In some embodiments, R^{4*} and R^{2*} form the biradical -Q-, wherein Q is $C(qi)(q_2)C(q_3)(q4)$, $C(qi)=C(q_3)$, $C[=C(qi)(q_2)]-C(q_3)(q4)$ or $C(qi)(q_2)-C[=C(q_3)(q_4)]$; qi, q_2, q_3, q_4 are each independently. H, halogen, C_{1-12} alkyl, substituted Ci_{-i2} alkyl, C_{2-12} alkenyl, substituted $Ci-i_2$ alkoxy, OJi, SJi, SOJi, $S0_2Ji$, $NJiJ_2$, N_3, CN, $C(=O)OJ_1$, $C(=0)-NJ_1J_2$, $C(=0)$ Ji, $-C(=0)NJ_1J_2$, $N(H)C(=NH)NJ_1J_2$, $N(H)C(=0)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$; each J_1 and J_2 is, independently, H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} aminoalkyl or a protecting group; and, optionally wherein when Q is $C(qi)(q_2)(q_3)(q_4)$ and one of q_3 or q_4 is CH_3 then at least one of the other of q_3 or q_4 or one of q_1 and q_2 is other than H. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. For all chiral centers, asymmetric groups may be found in either *R* or S orientation. Such bicyclic nucleotides are disclosed in WO2008/154401 which is hereby incorporated by reference in its entirity. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. In some embodiments, R^{1*}, R^2, R^3 are hydrogen and one or both of R^5, R^{5*} may be other than hydrogen as referred to above and in WO 2007/134181 or WO2009/067647 (alpha-L-bicyclic nucleic acids analogs).

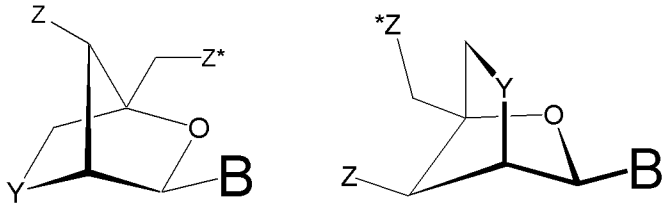
In some embodiments the LNA used in the oligonucleotide compounds of the invention preferably has the structure of the general formula II:



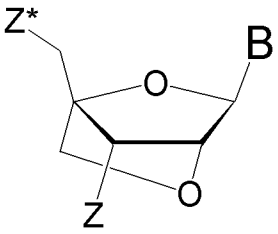
Formula II

wherein Y is selected from the group consisting of -0-, $-CH_20-$, -S-, -NH-, $N(R^e)$ and/or $-CH_2-$; Z and Z* are independently selected among an internucleotide linkage, R^H, a terminal group or a protecting group; B constitutes a natural or non-natural nucleotide base moiety (nucleobase), and R^H is selected from hydrogen and C_{1-4}-alkyl; R^a, R^b R^c, R^d and R^e are, optionally independently, selected from the group consisting of hydrogen, optionally substituted $Ci-i_2$-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, $Ci-i_2$-alkoxy, C_{2-12}-alkoxyalkyl, C_{2-12}-alkenyloxy, carboxy, $Ci-i_2$-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and $di(Ci_{-6}$-alkyl)amino, carbamoyl, mono- and $di(Ci_{-6}$-alkyl)-amino-carbonyl, amino-Ci_{-6}-alkyl-aminocarbonyl, mono-

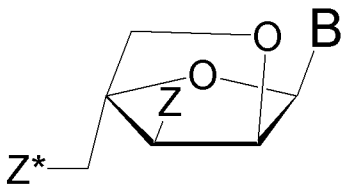
and di(Ci-6-alkyl)amino-Ci-6-alkyl-aminocarbonyl, Ci-$_6$-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH_2); and R^H is selected from hydrogen and C_{1-4}-alkyl. In some embodiments R^a, R^b R^c, R^d and R^e are, optionally independently, selected from the group consisting of hydrogen and Ci$_{-6}$ alkyl, such as methyl. For all chiral centers, asymmetric groups may be found in either *R* or S orientation, for example, two exemplary stereochemical isomers include the beta-D and alpha-L isoforms, which may be illustrated as follows:



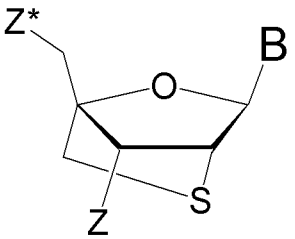
Specific exemplary LNA units are shown below:



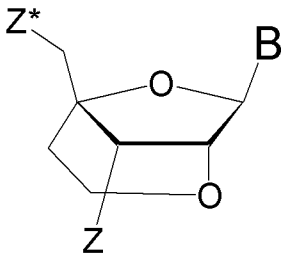
β-D-oxy-LNA



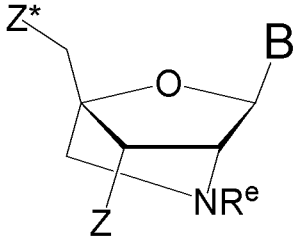
α-L-Oxy-LNA



β-D-thio-LNA



β-D-ENA



β-D-amino-LNA

The term "thio-LNA" comprises a locked nucleotide in which Y in the general formula above is selected from S or -CH_2-S-. Thio-LNA can be in both beta-D and alpha-L-configuration.

The term "amino-LNA" comprises a locked nucleotide in which Y in the general formula above is selected from -N(H)-, N(R)-, CH_2-N(H)-, and -CH_2-N(R)- where R is selected from hydrogen and C_{1-4}-alkyl. Amino-LNA can be in both beta-D and alpha-L-configuration.

The term "oxy-LNA" comprises a locked nucleotide in which Y in the general formula above represents -0-. Oxy-LNA can be in both beta-D and alpha-L-configuration.

The term "ENA" comprises a locked nucleotide in which Y in the general formula above is -CH_2-0- (where the oxygen atom of -CH_2-0- is attached to the 2'-position relative to the base B). R^e is hydrogen or methyl.

In some exemplary embodiments LNA is selected from beta-D-oxy-LNA, alpha-L-oxy-LNA, beta-D-amino-LNA and beta-D-thio-LNA, in particular beta-D-oxy-LNA.

RNAse recruitment

It is recognised that an oligomeric compound may function via non RNase mediated degradation of target mRNA, such as by steric hindrance of translation, or other methods, however, the preferred oligomers of the invention are capable of recruiting an endoribonuclease (RNase), such as RNase H.

It is preferable that the oligomer, or contiguous nucleotide sequence, comprises of a region of at least 6, such as at least 7 consecutive nucleotide units, such as at least 8 or at least 9 consecutive nucleotide units (residues), including 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 consecutive nucleotides, which, when formed in a duplex with the complementary target RNA is capable of recruiting RNase. The contiguous sequence which is capable of recruiting RNAse may be region B as referred to in the context of a gapmer as described herein. In some embodiments the size of the contiguous sequence which is capable of recruiting RNAse, such as region B, may be higher, such as 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleotide units.

EP 1 222 309 provides *in vitro* methods for determining RNaseH activity, which may be used to determine the ability to recruit RNaseH. A oligomer is deemed capable of recruiting RNase H if, when provided with the complementary RNA target, it has an initial rate, as measured in pmol/l/min, of at least 1 %, such as at least 5%, such as at least 10% or ,more than 20% of the of the initial rate determined using DNA only oligonucleotide, having the same base sequence but containing only DNA monomers, with no 2'

substitutions, with phosphorothioate linkage groups between all monomers in the oligonucleotide,, using the methodology provided by Example 91 - 95 of EP 1 222 309.

In some embodiments, an oligomer is deemed essentially incapable of recruiting RNaseH if, when provided with the complementary RNA target, and RNaseH, the RNaseH initial rate, as measured in pmol/l/min, is less than 1%, such as less than 5%, such as less than 10% or less than 20% of the initial rate determined using the equivalent DNA only oligonucleotide, with no 2' substitutions, with phosphorothioate linkage groups between all nucleotides in the oligonucleotide, using the methodology provided by Example 91 - 95 of EP 1 222 309.

In other embodiments, an oligomer is deemed capable of recruiting RNaseH if, when provided with the complementary RNA target, and RNaseH, the RNaseH initial rate, as measured in pmol/l/min, is at least 20%, such as at least 40 %, such as at least 60 %, such as at least 80 % of the initial rate determined using the equivalent DNA only oligonucleotide, with no 2' substitutions, with phosphorothioate linkage groups between all nucleotides in the oligonucleotide, using the methodology provided by Example 91 - 95 of EP 1 222 309.

Typically the region of the oligomer which forms the consecutive nucleotide units which, when formed in a duplex with the complementary target RNA is capable of recruiting RNase consists of nucleotide units which form a DNA/RNA like duplex with the RNA target - and include both DNA units and LNA units which are in the alpha-L configuration, particularly preferred being alpha-L-oxy LNA.

The oligomer of the invention may comprise a nucleotide sequence which comprises both nucleotides and nucleotide analogues, and may be in the form of a gapmer, a headmer or a mixmer.

A "headmer" is defined as an oligomer that comprises a region X and a region Y that is contiguous thereto, with the 5'-most monomer of region Y linked to the 3'-most monomer of region X. Region X comprises a contiguous stretch of non-RNase recruiting nucleoside analogues and region Y comprises a contiguous stretch (such as at least 7 contiguous monomers) of DNA monomers or nucleoside analogue monomers recognizable and cleavable by the RNase.

A "tailmer" is defined as an oligomer that comprises a region X and a region Y that is contiguous thereto, with the 5'-most monomer of region Y linked to the 3'-most monomer of the region X. Region X comprises a contiguous stretch (such as at least 7 contiguous monomers) of DNA monomers or nucleoside analogue monomers recognizable and cleavable by the RNase, and region X comprises a contiguous stretch of non-RNase recruiting nucleoside analogues.

Other "chimeric" oligomers, called "mixmers", consist of an alternating composition of (i) DNA monomers or nucleoside analogue monomers recognizable and cleavable by RNase, and (ii) non-RNase recruiting nucleoside analogue monomers.

In some embodiments, in addition to enhancing affinity of the oligomer for the target region, some nucleoside analogues also mediate RNase (e.g., RNaseH) binding and cleavage. Since a-L-LNA monomers recruit RNaseH activity to a certain extent, in some embodiments, gap regions (e.g., region B as referred to herein) of oligomers containing a-L-LNA monomers consist of fewer monomers recognizable and cleavable by the RNaseH, and more flexibility in the mixmer construction is introduced.

Gapmer Design

Preferably, the oligomer of the invention is a gapmer. A gapmer oligomer is an oligomer which comprises a contiguous stretch of nucleotides which is capable of recruiting an RNAse, such as RNAseH, such as a region of at least 6 or 7 DNA nucleotides, referred to herein in as region B (B), wherein region B is flanked both 5' and 3' by regions of affinity enhancing nucleotide analogues, such as from 1 - 6 nucleotide analogues 5' and 3' to the contiguous stretch of nucleotides which is capable of recruiting RNAse - these regions are referred to as regions A (A) and C (C) respectively.

In some embodiments, the monomers which are capable of recruiting RNAse are selected from the group consisting of DNA monomers, alpha-L-LNA monomers, C4' alkylayted DNA monomers (see PCT/EP2009/050349 and Vester ***et at.,*** Bioorg. Med. Chem. Lett. 18 (2008) 2296 - 2300, hereby incorporated by reference), and UNA (unlinked nucleic acid) nucleotides (see Flutter e*t* a/., Mol. Biosyst., 2009, 10, 1039 hereby incorporated by reference). UNA is unlocked nucleic acid, typically where the C2 - C3 C-C bond of the ribose has been removed, forming an unlocked "sugar" residue. Preferably the gapmer comprises a (poly)nucleotide sequence of formula (5' to 3'), A-B-C, or optionally A-B-C-D or D-A-B-C, wherein; region A (A) (5' region) consists or comprises of at least one nucleotide analogue, such as at least one LNA unit, such as from 1-6 nucleotide analogues, such as LNA units, and; region B (B) consists or comprises of at least five consecutive nucleotides which are capable of recruiting RNAse (when formed in a duplex with a complementary RNA molecule, such as the mRNA target), such as DNA nucleotides, and; region C (C) (3'region) consists or comprises of at least one nucleotide analogue, such as at least one LNA unit, such as from 1-6 nucleotide analogues, such as LNA units, and; region D (D), when present consists or comprises of 1, 2 or 3 nucleotide units, such as DNA nucleotides.

In some embodiments, region A consists of 1, 2, 3, 4, 5 or 6 nucleotide analogues, such as LNA units, such as from 2-5 nucleotide analogues, such as 2-5 LNA units, such as 3 or 4 nucleotide analogues, such as 3 or 4 LNA units; and/or region C consists of 1, 2, 3, 4,

5 or 6 nucleotide analogues, such as LNA units, such as from 2-5 nucleotide analogues, such as 2-5 LNA units, such as 3 or 4 nucleotide analogues, such as 3 or 4 LNA units.

In some embodiments B consists or comprises of 5, 6, 7, 8, 9, 10, 11 or 12 consecutive nucleotides which are capable of recruiting RNAse, or from 6-10, or from 7-9, such as 8 consecutive nucleotides which are capable of recruiting RNAse. In some embodiments region B consists or comprises at least one DNA nucleotide unit, such as 1-12 DNA units, preferably from 4-12 DNA units, more preferably from 6-10 DNA units, such as from 7-10 DNA units, most preferably 8, 9 or 10 DNA units.

In some embodiments region A consist of 3 or 4 nucleotide analogues, such as LNA, region B consists of 7, 8, 9 or 10 DNA units, and region C consists of 3 or 4 nucleotide analogues, such as LNA. Such designs include (A-B-C) 3-10-3, 3-10-4, 4-10-3, 3-9-3, 3-9-4, 4-9-3, 3-8-3, 3-8-4, 4-8-3, 4-8-4, 3-7-3, 3-7-4, 4-7-3, and may further include region D, which may have one or 2 nucleotide units, such as DNA units.

Further gapmer designs are disclosed in WO2004/046160, which is hereby incorporated by reference. WO2008/1 13832, which claims priority from US provisional application 60/977,409 hereby incorporated by reference, refers to 'shortmer' gapmer oligomers. In some embodiments, oligomers presented here may be such shortmer gapmers.

In some embodiments the oligomer is consisting of a contiguous nucleotide sequence of a total of 10, 11, 12, 13 or 14 nucleotide units, wherein the contiguous nucleotide sequence is of formula (5' - 3'), A-B-C, or optionally A-B-C-D or D-A-B-C, wherein; A consists of 1, 2 or 3 nucleotide analogue units, such as LNA units; B consists of 7, 8 or 9 contiguous nucleotide units which are capable of recruiting RNAse when formed in a duplex with a complementary RNA molecule (such as a mRNA target); and C consists of 1, 2 or 3 nucleotide analogue units, such as LNA units. When present, D consists of a single DNA unit.

In some embodiments A consists of 1 LNA unit. In some embodiments A consists of 2 LNA units. In some embodiments A consists of 3 LNA units. In some embodiments A consists of 4 LNA units. In some embodiments C consists of 1 LNA unit. In some embodiments C consists of 2 LNA units. In some embodiments C consists of 3 LNA units. In some embodiments C consists of 4 LNA units. In some embodiments B consists of 7 nucleotide units. In some embodiments B consists of 8 nucleotide units. In some embodiments B consists of 9 nucleotide units. . In certain embodiments, region B consists of 10 nucleoside monomers. In certain embodiments, region B comprises 1 - 10 DNA monomers. In some embodiments B comprises of from 1 - 9 DNA units, such as 2, 3, 4, 5, 6, 7 , 8 or 9 DNA units. In some embodiments B consists of DNA units. In some embodiments B comprises of at least one LNA unit which is in the alpha-L configuration,

such as 2, 3, 4, 5, 6, 7, 8 or 9 LNA units in the alpha-L-configuration. In some embodiments B comprises of at least one alpha-L-oxy LNA unit or wherein all the LNA units in the alpha-L-configuration are alpha-L-oxy LNA units. In some embodiments the number of nucleotides present in A-B-C are selected from the group consisting of (nucleotide analogue units - region B - nucleotide analogue units): 1-8-1, 1-8-2, 2-8-1, 2-8-2, 3-8-3, 2-8-3, 3-8-2, 4-8-1, 4-8-2, 4-8-3, 4-8-4, 1-8-4, 2-8-4, or; 1-9-1, 1-9-2, 2-9-1, 2-9-2, 2-9-3, 3-9-2, 1-9-3, 3-9-1, 4-9-1, 1-9-4, or; 1-10-1, 1-10-2, 2-10-1, 2-10-2, 1-10-3, 3-10-1. In some embodiments the number of nucleotides in A-B-C are selected from the group consisting of: 2-7-1, 1-7-2, 2-7-2, 3-7-3, 2-7-3, 3-7-2, 3-7-4, and 4-7-3. In certain embodiments, each of regions A and C consists of three LNA monomers, and region B consists of 8 or 9 or 10 nucleoside monomers, preferably DNA monomers. In some embodiments both A and C consists of two LNA units each, and B consists of 8 or 9 nucleotide units, preferably DNA units. In various embodiments, other gapmer designs include those where regions A and/or C consists of 3, 4, 5 or 6 nucleoside analogues, such as monomers containing a 2'-0-methoxyethyl-ribose sugar (2'-MOE) or monomers containing a 2'-fluoro-deoxyribose sugar, and region B consists of 8, 9, 10, 11 or 12 nucleosides, such as DNA monomers, where regions A-B-C have 3-9-3, 3-10-3, 5-10-5 or 4-12-4 monomers. Further gapmer designs are disclosed in WO 2007/14651 1A2, hereby incorporated by reference. In a preferred embodiment, regions A and C each consist of 3 or 4 LNA units, and B consist of 7, 8 or 9 DNA or other RNaseH recruiting nucleotides.

Internucleotide Linkages

The monomers of the oligomers described herein are coupled together via linkage groups. Suitably, each monomer is linked to the 3' adjacent monomer via a linkage group.

The person having ordinary skill in the art would understand that, in the context of the present invention, the 5' monomer at the end of an oligomer does not comprise a 5' linkage group, although it may or may not comprise a 5' terminal group.

The terms "linkage group" or "internucleotide linkage" are intended to mean a group capable of covalently coupling together two nucleotides. Specific and preferred examples include phosphate groups and phosphorothioate groups.

The nucleotides of the oligomer of the invention or contiguous nucleotides sequence thereof are coupled together via linkage groups. Suitably each nucleotide is linked to the 3' adjacent nucleotide via a linkage group.

Suitable internucleotide linkages include those listed within WO2007/031091, for example the internucleotide linkages listed on the first paragraph of page 34 of WO2007/031091 (hereby incorporated by reference).

It is, in some embodiments, preferred to modify the internucleotide linkage from its normal phosphodiester to one that is more resistant to nuclease attack, such as phosphorothioate or boranophosphate - these two, being cleavable by RNase H, also allow that route of antisense inhibition in reducing the expression of the target gene.

Suitable sulphur (S) containing internucleotide linkages as provided herein may be preferred. Phosphorothioate internucleotide linkages are also preferred, particularly for the gap region (B) of gapmers. Phosphorothioate linkages may also be used for the flanking regions (A and C, and for linking A or C to D, and within region D, as appropriate).

Regions A, B and C, may however comprise internucleotide linkages other than phosphorothioate, such as phosphodiester linkages, particularly, for instance when the use of nucleotide analogues protects the internucleotide linkages within regions A and C from endo-nuclease degradation - such as when regions A and C comprise LNA nucleotides.

The internucleotide linkages in the oligomer may be phosphodiester, phosphorothioate or boranophosphate so as to allow RNase H cleavage of targeted RNA. Phosphorothioate is preferred, for improved nuclease resistance and other reasons, such as ease of manufacture.

In one aspect of the oligomer of the invention, the nucleotides and/or nucleotide analogues are linked to each other by means of phosphorothioate groups.

It is recognised that the inclusion of phosphodiester linkages, such as one or two linkages, into an otherwise phosphorothioate oligomer, particularly between or adjacent to nucleotide analogue units (typically in region A and or C) can modify the bioavailability and/or bio-distribution of an oligomer - see WO2008/053314, hereby incorporated by reference.

In some embodiments, such as the embodiments referred to above, where suitable and not specifically indicated, all remaining linkage groups are either phosphodiester or phosphorothioate, or a mixture thereof.

In some embodiments all the internucleotide linkage groups are phosphorothioate.

When referring to specific gapmer oligonucleotide sequences, such as those provided herein it will be understood that, in various embodiments, when the linkages are phosphorothioate linkages, alternative linkages, such as those disclosed herein may be used, for example phosphate (phosphodiester) linkages may be used, particularly for linkages between nucleotide analogues, such as LNA, units. Likewise, when referring to specific gapmer oligonucleotide sequences, such as those provided herein, when the C residues are annotated as 5'methyl modified cytosine, in various embodiments, one or more of the Cs present in the oligomer may be unmodified C residues.

Oligomehc Compounds

The oligomers of the invention may, for example, be selected from the group consisting of: SEQ ID NOs: 32-48, or SEQ ID NOs: 49 - 71, or SEQ ID NOS: 72-77

In a preferred embodiment, the compound of the invention is anyone of SEQ ID NO: 52, 56, 57, 60, 63, 64, 65, 66, 67, 72, 73, 74, 75, 76, or 77.

In an especially preferred embodiment, the compound of the invention is anyone of SEQ ID NOs: 52, 56, 65, 66, 67, 72, 73, 74, 75, 76, or 77.

Conjugates

In the context the term "conjugate" is intended to indicate a heterogenous molecule formed by the covalent attachment ("conjugation") of the oligomer as described herein to one or more non-nucleotide, or non-polynucleotide moieties. Examples of non-nucleotide or non- polynucleotide moieties include macromolecular agents such as proteins, fatty acid chains, sugar residues, glycoproteins, polymers, or combinations thereof. Typically proteins may be antibodies for a target protein. Typical polymers may be polyethylene glycol.

Therefore, in various embodiments, the oligomer of the invention may comprise both a polynucleotide region which typically consists of a contiguous sequence of nucleotides, and a further non-nucleotide region. When referring to the oligomer of the invention consisting of a contiguous nucleotide sequence, the compound may comprise non-nucleotide components, such as a conjugate component.

In various embodiments of the invention the oligomeric compound is linked to ligands/conjugates, which may be used, e.g. to increase the cellular uptake of oligomeric compounds. WO2007/031091 provides suitable ligands and conjugates, which are hereby incorporated by reference.

The invention also provides for a conjugate comprising the compound according to the invention as herein described, and at least one non-nucleotide or non-polynucleotide moiety covalently attached to said compound. Therefore, in various embodiments where the compound of the invention consists of a specified nucleic acid or nucleotide sequence, as herein disclosed, the compound may also comprise at least one non-nucleotide or non-polynucleotide moiety (e.g. not comprising one or more nucleotides or nucleotide analogues) covalently attached to said compound.

Conjugattion (to a conjugate moiety) may enhance the activity, cellular distribution or cellular uptake of the oligomer of the invention. Such moieties include, but are not limited to, antibodies, polypeptides, lipid moieties such as a cholesterol moiety, cholic acid, a thioether, e.g. Hexyl-s-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipids, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-o-hexadecyl-rac-glycero-3-h-phosphonate, a polyamine or a polyethylene glycol chain, an

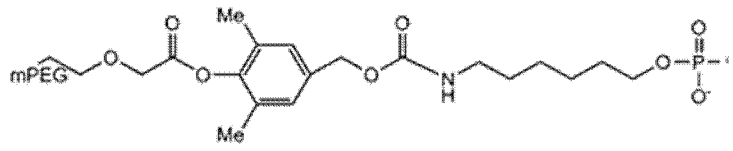
adamantane acetic acid, a palmityl moiety, an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety.

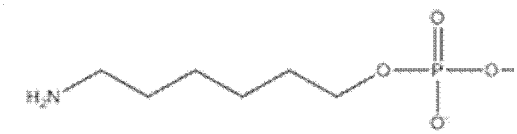
The oligomers of the invention may also be conjugated to active drug substances, for example, aspirin, ibuprofen, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

In certain embodiments the conjugated moiety is a sterol, such as cholesterol.

In various embodiments, the conjugated moiety comprises or consists of a positively charged polymer, such as a positively charged peptides of, for example from 1 -50, such as 2 - 20 such as 3 - 10 amino acid residues in length, and/or polyalkylene oxide such as polyethylglycol(PEG) or polypropylene glycol - see WO 2008/034123, hereby incorporated by reference. Suitably the positively charged polymer, such as a polyalkylene oxide may be attached to the oligomer of the invention via a linker such as the releasable inker described in WO 2008/034123.

By way of example, the following conjugate moieties may be used in the conjugates of the invention:





Activated oligomers

The term "activated oligomer," as used herein, refers to an oligomer of the invention that is covalently linked (i.e., functionalized) to at least one functional moiety that permits covalent linkage of the oligomer to one or more conjugated moieties, i.e., moieties that are not themselves nucleic acids or monomers, to form the conjugates herein described. Typically, a functional moiety will comprise a chemical group that is capable of covalently bonding to the oligomer via, e.g., a 3'-hydroxyl group or the exocyclic NH_2 group of the adenine base, a spacer that is preferably hydrophilic and a terminal group that is capable of binding to a conjugated moiety (e.g., an amino, sulfhydryl or hydroxyl group). In some embodiments, this terminal group is not protected, e.g., is an NH_2 group. In other embodiments, the terminal group is protected, for example, by any suitable protecting group such as those described in "Protective Groups in Organic Synthesis" by Theodora W Greene and Peter G M Wuts, 3rd edition (John Wiley & Sons, 1999). Examples of suitable hydroxyl protecting groups include esters such as acetate ester, aralkyl groups such as

benzyl, diphenylmethyl, or triphenylmethyl, and tetrahydropyranyl. Examples of suitable amino protecting groups include benzyl, alpha-methylbenzyl, diphenylmethyl, triphenylmethyl, benzyloxycarbonyl, tert-butoxycarbonyl, and acyl groups such as trichloroacetyl or trifluoroacetyl. In some embodiments, the functional moiety is self-cleaving. In other embodiments, the functional moiety is biodegradable. See e.g., U.S. Patent No. 7,087,229, which is incorporated by reference herein in its entirety.

In some embodiments, oligomers of the invention are functionalized at the 5' end in order to allow covalent attachment of the conjugated moiety to the 5' end of the oligomer. In other embodiments, oligomers of the invention can be functionalized at the 3' end. In still other embodiments, oligomers of the invention can be functionalized along the backbone or on the heterocyclic base moiety. In yet other embodiments, oligomers of the invention can be functionalized at more than one position independently selected from the 5' end, the 3' end, the backbone and the base.

In some embodiments, activated oligomers of the invention are synthesized by incorporating during the synthesis one or more monomers that is covalently attached to a functional moiety. In other embodiments, activated oligomers of the invention are synthesized with monomers that have not been functionalized, and the oligomer is functionalized upon completion of synthesis. In some embodiments, the oligomers are functionalized with a hindered ester containing an aminoalkyi linker, wherein the alkyl portion has the formula $(CH_2)_W$, wherein w is an integer ranging from 1 to 10, preferably about 6, wherein the alkyl portion of the alkylamino group can be straight chain or branched chain, and wherein the functional group is attached to the oligomer via an ester group (-O-C(O)-$(CH_2)_w$NH).

In other embodiments, the oligomers are functionalized with a hindered ester containing a $(CH_2)_w$-sulfhydryl (SH) linker, wherein w is an integer ranging from 1 to 10, preferably about 6, wherein the alkyl portion of the alkylamino group can be straight chain or branched chain, and wherein the functional group attached to the oligomer via an ester group (-0-C(0)-$(CH_2)_w$SH)

In some embodiments, sulfhydryl-activated oligonucleotides are conjugated with polymer moieties such as polyethylene glycol or peptides (via formation of a disulfide bond).

Activated oligomers containing hindered esters as described above can be synthesized by any method known in the art, and in particular by methods disclosed in PCT Publication No. WO 2008/034122 and the examples therein, which is incorporated herein by reference in its entirety.

In still other embodiments, the oligomers of the invention are functionalized by introducing sulfhydryl, amino or hydroxyl groups into the oligomer by means of a functionalizing reagent substantially as described in U.S. Patent Nos. 4,962,029 and

4,914,210, i.e., a substantially linear reagent having a phosphoramidite at one end linked through a hydrophilic spacer chain to the opposing end which comprises a protected or unprotected sulfhydryl, amino or hydroxyl group. Such reagents primarily react with hydroxyl groups of the oligomer. In some embodiments, such activated oligomers have a functionalizing reagent coupled to a 5'-hydroxyl group of the oligomer. In other embodiments, the activated oligomers have a functionalizing reagent coupled to a 3'-hydroxyl group. In still other embodiments, the activated oligomers of the invention have a functionalizing reagent coupled to a hydroxyl group on the backbone of the oligomer. In yet further embodiments, the oligomer of the invention is functionalized with more than one of the functionalizing reagents as described in U.S. Patent Nos. 4,962,029 and 4,914,210, incorporated herein by reference in their entirety. Methods of synthesizing such functionalizing reagents and incorporating them into monomers or oligomers are disclosed in U.S. Patent Nos. 4,962,029 and 4,914,210.

In some embodiments, the 5'-terminus of a solid-phase bound oligomer is functionalized with a dienyl phosphoramidite derivative, followed by conjugation of the deprotected oligomer with, e.g., an amino acid or peptide via a Diels-Alder cycloaddition reaction.

In various embodiments, the incorporation of monomers containing 2'-sugar modifications, such as a 2'-carbamate substituted sugar or a 2'-(0-pentyl-N-phthalimido)-deoxyribose sugar into the oligomer facilitates covalent attachment of conjugated moieties to the sugars of the oligomer. In other embodiments, an oligomer with an amino-containing linker at the 2'-position of one or more monomers is prepared using a reagent such as, for example, 5'-dimethoxytrityl-2'-0-(e-phthalimidylaminopentyl)-2'-deoxyadenosine-3'- N,N-diisopropyl-cyanoethoxy phosphoramidite. See, e.g., Manoharan, et al., Tetrahedron Letters, 1991 , 34, 7171 .

In still further embodiments, the oligomers of the invention may have amine-containing functional moieties on the nucleobase, including on the N6 purine amino groups, on the exocyclic N2 of guanine, or on the N4 or 5 positions of cytosine. In various embodiments, such functionalization may be achieved by using a commercial reagent that is already functionalized in the oligomer synthesis.

Some functional moieties are commercially available, for example, heterobifunctional and homobifunctional linking moieties are available from the Pierce Co. (Rockford, III.). Other commercially available linking groups are 5'-Amino-Modifier C6 and 3'-Amino-Modifier reagents, both available from Glen Research Corporation (Sterling, Va.). 5'-Amino-Modifier C6 is also available from ABI (Applied Biosystems Inc., Foster City, Calif.)

as Aminolink-2, and 3'-Amino-Modifier is also available from Clontech Laboratories Inc. (Palo Alto, Calif.).

Compositions

The oligomer of the invention may be used in pharmaceutical formulations and compositions. Suitably, such compositions comprise a pharmaceutically acceptable diluent, carrier, salt or adjuvant. PCT/DK2006/000512 provides suitable and preferred pharmaceutically acceptable diluent, carrier and adjuvants - which are hereby incorporated by reference. Suitable dosages, formulations, administration routes, compositions, dosage forms, combinations with other therapeutic agents, pro-drug formulations are also provided in PCT/DK2006/000512 - which are also hereby incorporated by reference. The invention provides for pharmaceutical compositions comprising an oligomer or conjugate of the invention, and a pharmaceutically acceptable solvent, such as water or saline water, diluent, carrier, salt or adjuvant.

Applications

The oligomers of the invention may be utilized as research reagents for, for example, diagnostics, therapeutics and prophylaxis.

In research, such oligomers may be used to specifically inhibit the synthesis of HCV protein (typically by degrading or inhibiting the mRNA and thereby prevent protein formation) in cells and experimental animals thereby facilitating functional analysis of the target or an appraisal of its usefulness as a target for therapeutic intervention.

In diagnostics the oligomers may be used to detect and quantitate HCV expression in cell and tissues by northern blotting, *in-situ* hybridisation or similar techniques.

For therapeutics, an animal or a human, suspected of having a disease or disorder, which can be treated by modulating the expression of HCV is treated by administering oligomeric compounds in accordance with this invention. Further provided are methods of treating a mammal, such as treating a human, suspected of having or being prone to a disease or condition, associated with expression of HCV by administering a therapeutically or prophylactically effective amount of one or more of the oligomers or compositions of the invention. The oligomer, a conjugate or a pharmaceutical composition according to the invention is typically administered in an effective amount.

The invention also provides for the use of the compound or conjugate of the invention as described for the manufacture of a medicament for the treatment of a disorder as referred to herein, or for a method of the treatment of as a disorder as referred to herein.

The invention also provides for a method for treating a disorder as referred to herein said method comprising administering a compound according to the invention as herein

described, and/or a conjugate according to the invention, and/or a pharmaceutical composition according to the invention to a patient in need thereof.

Medical Indications

The oligomers and other compositions according to the invention can be used for the treatment of conditions associated with over expression or expression of mutated version of the HCV.

The invention further provides use of a compound of the invention in the manufacture of a medicament for the treatment of a disease, disorder or condition as referred to herein.

Generally stated, one aspect of the invention is directed to a method of treating a mammal suffering from or susceptible to conditions associated with abnormal levels of HCV, comprising administering to the mammal and therapeutically effective amount of an oligomer targeted to HCV that comprises one or more LNA units. The oligomer, a conjugate or a pharmaceutical composition according to the invention is typically administered in an effective amount.

The disease or disorder, as referred to herein, may, in some embodiments be associated with a mutation in the HCV gene or a gene whose protein product is associated with or interacts with HCV. Therefore, in some embodiments, the target RNA is a mutated form of the HCV sequence.

An interesting aspect of the invention is directed to the use of an oligomer (compound) as defined herein or a conjugate as defined herein for the preparation of a medicament for the treatment of a disease, disorder or condition as referred to herein.

The methods of the invention are preferably employed for treatment or prophylaxis against diseases caused by abnormal levels of HCV.

Alternatively stated, In some embodiments, the invention is furthermore directed to a method for treating abnormal levels of HCV, said method comprising administering a oligomer of the invention, or a conjugate of the invention or a pharmaceutical composition of the invention to a patient in need thereof.

The invention also relates to an oligomer, a composition or a conjugate as defined herein for use as a medicament.

Moreover, the invention relates to a method of treating a subject suffering from a disease or condition such as those referred to herein.

A patient who is in need of treatment is a patient suffering from or likely to suffer from the disease or disorder.

In some embodiments, the term 'treatment' as used herein refers to both treatment of an existing disease (e.g. a disease or disorder as herein referred to), or prevention of a

disease, *i.e.* prophylaxis. It will therefore be recognised that treatment as referred to herein may, in some embodiments, be prophylactic.

In some embodiments, the invention relates to an oligomer, a composition or a conjugate as defined herein for use as a medicament, wherein the medicament is for use in combination with another active ingredient. Another active ingredient means an ingredient which in combination with the oligomer of the invention will provide an added or a synergistic effect on a disease parameter of HCV, such as in non limiting example on virus titer, liver fibrosis, liver cancer risk, or another pathologic parameter characterising HCV infection.

EMBODIMENTS

The following embodiments of the present invention may be used in combination with the other embodiments described herein.

In one embodiment, an oligomer is designed to target a sequence in a particular genotype of HCV, that correspond to that targeted in genotype 1b by any one of SEQ ID NOs: 23-31 . In another preferred embodiment, an anti HCV oligomer is designed to target the region of non 1b genotype which corresponds to any one of SEQ ID NOs: 32-48, and which are designed according to that corresponding SEQ ID NO. In one preferred embodiment, an anti HCV oligomer is designed which target the same region in a HCV genotype other than 1b and which has a similar design as any one of SEQ ID NO: 49-71.

In a preferred embodiment, all internucleoside bonds are phosphorothioate bonds. In a further preferred embodiment, all LNAs are oxy-LNA such as beta-D-oxy-LNA.

In one highly preferred embodiment, the design of the oligonucleotides targeting genotype 1a and 1b by having a G/T mismatch against both genotypes. "TT-oligos designed corresponding to all preferred oligonucleotides in Table 2 (preferred compounds based on in vitro activity). Preferred "TT" oligos which will target both genotype 1a and 1b, and their corresponding genotype 1b specific versions can be seen in Table 2. In another preferred embodiment, G/T mismatches may be overcome between any genotype by designing the antisense oligonucleotide as a "TT" oligo. In one embodiment, G/A mismatches between genotypes can be overcome by inserting a P base at the site of the mismatch (in Figure 2, P base pairing with G and A is shown).

Other types of mismatches may be overcome by in one embodiment to insert a universal base instead of the normal base. Universal bases are well known by the skilled person, examples of universal bases may be 2,4-difluorotoluyl or 5-nitroindole as exemplified in WO201 001 1895. Other examples of universal bases may be found in Loakes, 2001 , Nucl. Acids Research, vol 29, no 12, p 2437-2447, and Herdewijn, Nucleosides (2008), 277-303. And Burgess and Welch, 1995, Organic Chemistry, 8(3), 187-190. In one

embodiment, the antisense oligonucleotides have no more than 1 "TT", such as no more than 2 "TT"s, such as no more than 3 "TT"s.

In one embodiment, the antisense oligonucleotides have no more than 1 universal base, such as no more than 2 universal bases, such as no more than 3 universal bases.

In one embodiment, the antisense oligonucleotide of the invention is for use in a combination treatment with one or more of the following types of compounds: a miR-122 inhibitor, such as an antisense oligomer, an NS3a protease inhibitor or an HCV NS3/4A protease inhibitor. Ribavirin, or a virally active derivative thereof and/or Interferon.

EXAMPLES

LNA monomer and oligonucleotide synthesis were performed using the methodology referred to in Examples 1 and 2 of PCT/EP2007/060703.

The stability of LNA oligonucleotides in human or rat plasma is performed using the methodology referred to in Example 4 of PCT/EP2007/060703

The treatment of in vitro cells with LNA anti-HCV antisense oligonucleotides is performed using the methodology referred to in Examples 5 and 6 of PCT/EP2007/060703

The above mentioned examples of PCT/EP2007/060703 are hereby specifically incorporated by reference.

The efficacy of anti-HCV antisense oligonucleotides in inhibiting HCV RNA was measured using the HCV 1b replicon assay of Friebe et al. ((Friebe et al. Journal of Virology 2001),).

Example 1: Design of the oligonucleotide

In a specific preferred design of the oligonucleotides of the invention, oligomers comprising 16 nucleotide sequences of Table 2 are designed as 3-10-3 or 4-8-4 (LNA-DNA-LNA), or oligomers, wherein the LNAs are independently selected from oxy-LNA, thio-LNA, and amino-LNA, in either of the D-β and L-a configurations or combinations thereof.

Table 2 (Oligomers designed to target HCV 1b)

SEQ ID	MOTIF SEQUENCE	SEQ ID	GAPMER DESIGN	SEQ ID	LNA GAPMERS
23	TCTATGGTGGAGTGTC	32	5'- TCTatggtggagtGTC -3'	49	5'- $T_s^{om}C_s^{o}T_s^{o}a_s^{'}t_s^{'}g_s^{'}g_s^{'}t_s^{'}g_s^{'}g_s^{'}a_s^{'}g_s^{'}t_s^{'}G_s^{o}T_s^{om}C^{o}$-3'
		33	5'- TCTAtggtggagTGTC -3'	50	5'- $T_s^{om}C_s^{o}T_s^{o}A_s^{o}t_s^{'}g_s^{'}g_s^{'}t_s^{'}g_s^{'}g_s^{'}a_s^{'}g_s^{'}T_s^{o}G_s^{o}T_s^{om}C^{o}$-3'

24	ATCTATGGTGGAGTGT	34	5'- ATCtatggtggagTGT - 3'	51	5'- $A_s{}^oT_s{}^{om}C_s{}^ot_sa_st_sg_sg_st_sg_sg_sa_sg_sT_s{}^oG_s{}^oT^o$ -3'
		35	5'- ATCTatggtggaGTGT -3'	52	5'- $A_s{}^oT_s{}^{om}C_s{}^oT_s{}^oa_st_sg_sg_st_sg_sg_sa_sG_s{}^oT_s{}^oG_s{}^oT^o$ -3'
25	GATCTATGGTGGAGTG	36	5'- GATctatggtggaGTG -3'	53	5'- $G_s{}^oA_s{}^oT_s{}^oc_st_sa_st_sg_sg_st_sg_sg_sa_sG_s{}^oT_s{}^oG^o$-3'
		37	5'- GATCtatggtggAGTG -3'	54	5'- $G_s{}^oA_s{}^oT_s{}^{om}C_s{}^ot_sa_st_sg_sg_st_sg_sg_sA_s{}^oG_s{}^oT_s{}^oG^o$-3'
26	TGATCTATGGTGGAGT	38	5'-TGAtctatggtggAGT-3'	55	5'- $T_s{}^oG_s{}^oA_s{}^ot_sc_st_sa_st_sg_sg_st_sg_sg_sA_s{}^oG_s{}^oT^o$-3'
		39	5'-TGATctatggtgGAGT-3'	56	5'- $T_s{}^oG_s{}^oA_s{}^oT_s{}^oc_st_sa_st_sg_sg_st_sg_sG_s{}^oA_s{}^oG_s{}^oT^o$-3'
27	GTGATCTATGGTGGAG	40	5'-GTGatctatggtgGAG-3'	57	5'- $G_s{}^oT_s{}^oG_s{}^oa_st_sc_st_sa_st_sg_sg_st_sg_sG_s{}^oA_s{}^oG^o$-3'
		41	5'-GTGAtctatggtGGAG-3'	58	5'- $G_s{}^oT_s{}^oG_s{}^oA_s{}^ot_sc_st_sa_st_sg_sg_st_sG_s{}^oG_s{}^oA_s{}^oG^o$-3'
28	AGTGATCTATGGTGGA	42	5'-AGTgatctatggtGGA-3'	59	5'- $A_s{}^oG_s{}^oT_s{}^og_sa_st_sc_st_sa_st_sg_sg_st_sG_s{}^oG_s{}^oA^o$-3'
		43	5'- AGTGatctatggTGGA-3'	60	5'- $A_s{}^oG_s{}^oT_s{}^oG_s{}^oa_st_sc_st_sa_st_sg_sg_sT_s{}^oG_s{}^oG_s{}^oA^o$-3'

29	GAGTGATCTATG GTGG	44	5'-GAGtgatctatggTGG-3'	61	5'- $G_s^oA_s^oG_s^ot_sg_sa_st_sc_st_sa_st_sg_sg_sT_s^oG_s^oG^o$-3'
		45	5'-GAGTgatctatgGTGG-3'	62	5'- $G_s^oA_s^oG_s^oT_s^og_sa_st_sc_st_sa_st_sg_sG_s^oT_s^oG_s^oG^o$-3'
30	GGAGTGATCTAT GGTG	46	5'-GGAgtgatctatgGTG-3'	63	5'- $G_s^oG_s^oA_s^og_st_sg_sa_st_sc_st_sa_st_sg_sG_s^oT_s^oG^o$-3'
		47	5'-GGAGtgatctatGGTG-3'	64	5'- $G_s^oG_s^oA_s^oG_s^ot_sg_sa_st_sc_st_sa_st_sG_s^oG_s^oT_s^oG^o$-3'
31	TGATTCATGGTGG AGT	48	5'-TGATtcatggtgGAGT-3'	65	5'- $T_s^oG_s^oA_s^oT_s^ot_sc_sa_st_sg_sg_st_sg_sG_s^oA_s^oG_s^oT^o$-3'

Motif sequence represents the sequences of bases.

Gapmer design sequences - capital letters are nucleotide analogue nucleotides, such as those described herein, small letters (not subscript or superscript) are DNA nucleotides. Nucleotide analogue cytosines may optionally be 5-methyl cytosine, internucleoside linkages may be as disclosed herein, preferably phosphorothioate.

LNA gapmers - capital letters are LNA units, such as beta-D-oxy-LNA, small letters (not subscript or superscript) are DNA units. ° indicate that the LNA unit is oxy LNA. LNA cytosines may optionally be 5-methyl cytosine, internucleoside linkages may be as disclosed herein, preferably phosphorothioate.

In a preferred embodiment, internucleoside bonds are fully thiolated. In a further preferred embodiment, all LNA are oxy-LNA such as beta-D-oxy-LNA, ^{m} indicate (optional) 5'methylation (in connection with cytosines)

Table 3. IC50s and tolerability of selected genotype 1b targeting oligomers in HCV genotype 1b replicon system.

SEQ ID NO	Position	Design	IC50 - activity	Tolerability 50% (*)	Genotype specificity (1a and 1b)
52	22+	4-8-4	~ 2nM	>50nM	1b
56	24+	4-8-4	~ 1nM	>50nM	1b
57	25+	3-10-3	~ 1nM	>50nM	1b
60	26+	4-8-4	~ 1nM	>50nM	1b
63	28+	3-10-3	<1nM	>50nM	1b
64	28+	4-8-4	~ 1.5nM	>50nM	1b

Table 4. Design of oligonucleotides targeting genotype 1a and 1b by having a G/T mismatch against both genotypes.

Table 4		
SEQ ID NO	Genotype	Sequence
52	1b	5'-$A_s^oT_s^o{}^{m}\underline{C}_s^o\underline{T}_s^o a_s t_s g_s g_s t_s g_s g_s a_s G_s^o T_s^o G_s^o T^o$-3'
66	1a/1b	5'-$A_s^oT_s^o\underline{T}_s^o\underline{T}_s^o a_s t_s g_s g_s t_s g_s g_s a_s G_s^o T_s^o G_s^o T^o$-3'
72	1a/1b	5'-$A_s^oT_s^o\underline{U}_s^o\underline{U}_s^o a_s t_s g_s g_s t_s g_s g_s a_s G_s^o T_s^o G_s^o T^o$-3'
56	1b	5'-$T_s^oG_s^oA_s^oT_s^o\underline{c}_s\underline{t}_s a_s t_s g_s g_s t_s g_s G_s^o A_s^o G_s^o T^o$-3'
67	1a/1b	5'-$T_s^oG_s^oA_s^oT_s^o\underline{t}_s\underline{t}_s a_s t_s g_s g_s t_s g_s G_s^o A_s^o G_s^o T^o$-3'
73	1a/1b	5'-$T_s^oG_s^oA_s^oT_s^o\underline{u}_s\underline{u}_s a_s t_s g_s g_s t_s g_s G_s^o A_s^o G_s^o T^o$-3'
57	1b	5'-$G_s^oT_s^oG_s^o a_s t_s\underline{c}_s\underline{t}_s a_s t_s g_s g_s t_s g_s G_s^o A_s^o G^o$-3'
68	1a/1b	5'-$G_s^oT_s^oG_s^o a_s t_s\underline{t}_s\underline{t}_s a_s t_s g_s g_s t_s g_s G_s^o A_s^o G^o$-3'
74	1a/1b	5'-$G_s^oT_s^oG_s^o a_s t_s\underline{u}_s\underline{u}_s a_s t_s g_s g_s t_s g_s G_s^o A_s^o G^o$-3'
60	1b	5'-$A_s^oG_s^oT_s^oG_s^o a_s t_s\underline{c}_s\underline{t}_s a_s t_s g_s g_s T_s^o G_s^o G_s^o A^o$-3'
69	1a/1b	5'-$A_s^oG_s^oT_s^oG_s^o a_s t_s\underline{t}_s\underline{t}_s a_s t_s g_s g_s T_s^o G_s^o G_s^o A^o$-3'

75	1a/1b	5'-A$_s^o$-G$_s^o$-T$_s^o$-G$_s^o$-a$_s$t$_s$U$_s$U$_s$a$_s$t$_s$g$_s$g$_s$T$_s^o$-G$_s^o$-G$_s^o$-A^o-3'
63	1b	5'-G$_s^o$-G$_s^o$-A$_s^o$-g$_s$t$_s$g$_s$a$_s$t$_s$c$_s$t$_s$a$_s$t$_s$g$_s$G$_s^o$-T$_s^o$-G^o-3'
70	1a/1b	5'-G$_s^0$G$_s^0$A$_s^0$g$_s$t$_s$g$_s$a$_s$t$_s$t$_s$t$_s$a$_s$t$_s$g$_s$G$_s^0$T$_s^0$G^o-3'
76	1a/1b	5'-G$_s^o$-G$_s^o$-A$_s^o$-g$_s$t$_s$g$_s$a$_s$t$_s$U$_s$U$_s$a$_s$t$_s$g$_s$G$_s^o$-T$_s^o$-G^o-3'
64	1b	5'-G$_s^0$G$_s^0$A$_s^0$G$_s^0$t$_s$g$_s$a$_s$t$_s$c$_s$t$_s$a$_s$t$_s$G$_s^0$G$_s^0$T$_s^0$G^o-3'
71	1a/1b	5'-G$_s^0$G$_s^0$A$_s^0$G$_s^0$t$_s$g$_s$a$_s$t$_s$t$_s$t$_s$a$_s$t$_s$G$_s^0$G$_s^0$T$_s^0$G^o-3'
77	1a/1b	5'-G$_s^0$G$_s^0$A$_s^0$G$_s^o$t$_s$g$_s$a$_s$t$_s$U$_s$U$_s$a$_s$t$_s$G$_s^0$G$_s^0$T$_s^0$G^0-3'

Design of oligomers that can target both HCV genotypes 1a and 1b

The difference in sequence between genotypes 1b and 1a is a change from "AG" to "GA" in positions 34 and 35 of the HCV genome.

An oligo which can hybridize to both A and G in the given positions would be able to efficiently target both genotype 1a and 1b.

One option for designing oligos that could target both genotypes would be to design oligos that have TT or UU in the given positions. These oligos would have a G/T or G/U mismatch against both genotype 1a and 1b.

As the G/T and G/U mismatches are the least discriminating mismatches, these so called TT or UU oligos should be able to efficiently target both genotypes (see Figure 1).

The complete list of "TT" and "UU" oligos was screened for activity and toxicity in the HCV 1b replicon system (Bartenschlager and Pletschmann, 2005, PNAS, vol 102, no. 28, p. 9739-9740). The genotype 1b specific "parent compound" was also included in the screen for comparison with the TT and UU versions. The LNA antimiR-122 (SPC3649) used as positive control, the ISIS14803 reference compound (SPC4475), and the 16-mer LNA gapmer targeting luciferase (SPC2570) were included as controls in the screen.

The oligos were screened at 1, 2.5, 5, 10, 25 and 50 nM concentrations and activity/toxicity was analysed 48h after transfection in the HCV replicon system. Five independent experiments were performed.

Dose-dependent inhibition of luciferase activity for each oligonucleotide plotted together with in-assay resazurin toxicity is presented in (Figure 3).

The results show that in general, the TT oligos show similar activity to the HCV genotype 1b specific pre-leads, while the UU oligos show lower activity.

The most potent TT oligos are SEQ ID NO: 67 which corresponds to SEQ ID NO: 56; SEQ ID NO: 70 which corresponds to SEQ ID NO: 63; and SEQ ID NO: 71 which corresponds to SEQ ID NO: 64.

These results show that an HCV oligo with a G/T mismatch to HCV genotype 1b still shows potent anti-viral activity in the HCV replicon system.

Another option would be to design oligos that contain two P-bases at the given positions. The P-base is a pyrimidine derivative which when introduced into oligonucleotides can base pair with either A or G (see Fig 2).

Screening of TT and UU oligos

The six selected preferred oligonucleotides targeting the miR-122 binding site region in the 5'UTR of HCV genotype 1b were designed as "TT" and "UU"- versions, meaning that they have a base composition of TT or UU in the position of the oligo that hybridizes to the region having two mismatches between genotype 1a and 1b.

Example 2: Assays

HCV replicon system

The *in vitro* screens were performed using the bicistronic luciferase HCV NS genes replicon system in HUH7 cells (Friebe et al. Journal of Virology 2001), which is considered an appropriate surrogate assay for evaluating the inhibitory potential of LNA oligos on HCV replication and will be referred to as "HCV replicon system".

The replicon contains the 5'UTR of HCV genotype 1b and the non-structural genes NS3, NS4A, NS4B, NS5A and NS5B, which are required for RNA replication, fused to the gene encoding the firefly luciferase. The luciferase activity in the HCV replicon system is a direct measure of the amount of HCV replicon present.

Transfection

For the transfection experiments which were performed in 96-well plates, luciferase activity was measured 48h after transfection with oligonucleotides in the HCV replicon system, to evaluate the inhibitory potential of the LNA oligonucleotides on HCV replication.

In addition, a resazurin assay was used for evaluation of cell viability, since a decrease in cell viability can lead to decreased HCV replication, leading to false positive results. For this reason it is important to evaluate the therapeutic window between activity (luciferase activity) and toxicity (resazurin assay), when evaluating the potency of the different compounds.

Measurement of resazurin and luciferase activity

Resazurin

The resazurin assay was performed by adding 85 µl of non-fluorescent resazurin (VWR, catalogue no. 330884Y) to the cell culture media (85 µl) 45h after transfection and incubating for 3 hours at 37°C/5%$C0_2$. Measurement of fluorescent resofurin, which is directly correlated to cell viability, was thereafter performed at 460-490 nm using BMG Fluorostar (BMG Labtechnologies Ltd).

Luciferase

After the resazurin measurements, all media was removed and 50µl of Bright-Glow (Promega, catalogue no. E2620) was added into each well and left in the dark for 3 minutes to allow cell lysis before measurement of luminescence. Luminescent measurements were also performed with the BMG Fluorostar (BMG Labtechnologies Ltd). Reference:

Friebe P, Lohmann V, Krieger N, Bartenschlager R., 2001. Sequences in the 5' nontranslated region of hepatitis C virus required for RNA replication. ***J Virol*** 75(24): 12047-57.

Example 3: In vitro model: Cell culture

The effect of antisense compounds on target nucleic acid expression can be tested in any of a variety of cell types provided that the target nucleic acid is present at measurable levels. Target can be expressed endogenously or by transient or stable transfection of a nucleic acid encoding said nucleic acid.

The expression level of target nucleic acid can be routinely determined using, for example, Northern blot analysis, Quantitative PCR, Ribonuclease protection assays. The following cell types are provided for illustrative purposes, but other cell types can be routinely used, provided that the target is expressed in the cell type chosen.

Cells were cultured in the appropriate medium as described below and maintained at 37°C at 95-98% humidity and 5% $C0_2$. Cells were routinely passaged 2-3 times weekly.

HuH-7: Human liver cell line HuH-7 was purchased from ATCC and cultured in Eagle MEM (Sigma) with 10% FBS + Glutamax I + non-essential amino acids + gentamicin.

Example 4: In vitro model: Treatment with antisense oligonucleotide

Cell culturing and transfections: Huh-7 cells were seeded in 6-well plates at 37°C (5% $C0_2$) in growth media supplemented with 10% FBS, Glutamax I and Gentamicin. When the cells were 60-70% confluent, they were transfected in duplicates with different concentrations of oligonucleotides (0.04 - 25 nM) using Lipofectamine 2000 (5 µg/mL). Transfections were carried out essentially as described by Dean et al. (1994, JBC 269:16416-16424). In short, cells were preincubated for 7 min. with Lipofectamine in OptiMEM followed by addition of oligonucleotide to a total volume of 1.5 mL transfection mix per well. After 4 hours, the transfection mix was removed; cells were washed and grown at

37°C for approximately 20 hours (mRNA analysis and protein analysis) in the appropriate growth medium. Cells were then harvested for protein and RNA analysis.

In the Gymnosis experiments HepG2 cells were used in 6 well plates without transfection agent, the oligonucleotides were dissolved in the media.

Example 5: in vitro model: Extraction of RNA and cDNA synthesis

Total RNA Isolation

Total RNA was isolated using RNeasy mini kit (Qiagen). Cells were washed with PBS, and Cell Lysis Buffer (RTL, Qiagen) supplemented with 1% mercaptoethanol was added directly to the wells. After a few minutes, the samples were processed according to manufacturer's instructions.

First strand synthesis

First strand synthesis was performed using either OmniScript Reverse Transcriptase kit or M-MLV Reverse transcriptase (essentially as described by manufacturer (Ambion)) according to the manufacturer's instructions (Qiagen). When using OmniScript Reverse Transcriptase 0.5 µg total RNA each sample, was adjusted to 12 µl and mixed with 0.2 µl poly (**dT**)**i2-i8** (0.5 µg/µl) (Life Technologies), 2 µl dNTP mix (5 mM each), 2 µl 10x RT buffer, 0.5 µl RNAguard™ RNase Inhibitor (33 units/mL, Amersham) and 1 µl OmniScript Reverse Transcriptase followed by incubation at 37°C for 60 min. and heat inactivation at 93°C for 5 min.

When first strand synthesis was performed using random decamers and M-MLV-Reverse Transcriptase (essentially as described by manufacturer (Ambion)) 0.25 µg total RNA of each sample was adjusted to 10.8 µl in H_20 . 2 µl decamers and 2 µl dNTP mix (2.5 mM each) was added. Samples were heated to 70°C for 3 min. and cooled immediately in ice water and added 3.25 µl of a mix containing (2 µl 10x RT buffer; 1 µl M-MLV Reverse Transcriptase; 0.25 µl RNAase inhibitor). cDNA is synthesized at 42°C for 60 min followed by heating inactivation step at 95 °C for 10 min and finally cooled to 4 °C.

ALT and AST levels were determined in the blood serum, free from red blood cells, obtained from the mice at the time of sacrifice (48 hours after last administration). The activity of alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) in mouse serum was determined using an enzymatic assay (ABX Pentra A 11A01627 (ALT) or A 11A01629 (AST), Horiba ABX Diagnostics, France) according to the manufacturer's instruction but adjusted to 96-well format. In short, serum samples were diluted 2.5 fold with H_20 and assayed in duplicate. After addition of 50 µl diluted sample or standard (multical from ABX Pentra, A 11A01652) to each well, 200 µl of 37 °C ALT reagent mix was added to each well. Kinetic measurements were performed at 340nm and 37 °C for 5 min with an

interval of 30s. Data were correlated to the 2-fold diluted standard curve and results were presented as ALT or AST activity in U/L.

Example methods

Tissue and plasma oligonucleotide analysis (drug content)

Sample and standard preparation

Tissue samples (100 mg) were collected in 2 ml Eppendorf tubes and kept on dry ice. Extraction buffer 500 µl (0.5% Igepal CA-630 (Sigma-Aldrich), 25 mM Tris pH 8.0, 25 mM EDTA, 100 mM NaCl, pH 8.0) containing proteinase K (1 mg/ml) (Sigma-Aldrich P4850) and two tungsten carbide beads (3 mm) were added. The samples were homogenized mechanically by a Retsch MM300 (8 min. at 25 revolutions per seconds) and homogenates were incubated overnight at 37°C. Control tissue from untreated animals were spiked with the relevant oligonucleotides at 5 -250 µg/g tissue and treated as described for the samples above.

Extraction of samples, standard - and QC-samples

One ml phenol-chloroform-isoamyl-alcohol (25:24: 1(v/v/v)), saturated with 10 M Tris, pH 8.0, 1 mM EDTA (Sigma P2069) was added to each tissue samples and vortexed for 5 min. Phase separation was achieved by centrifugation at 4000g for 15 min. The aqueous phase (upper-phase) was diluted 100 times. These dilutions were kept at 4°C and were stable for up to two weeks.

Oligonucleotide content determination by ELISA.

Streptavidin-coated strips (Immobilizer Streptavidin LockWell module plate, Nunc) were washed three times in300 µl 5x SSCT buffer (750 mM NaCl, 75 mM sodium citrate, 0.05% Tween-20, pH 7.0). Each well was incubated for 30 min. at room temperature under gentle agitation with 100 µl of a 0.02 µM solution of biotinylated capture probe (7-mer fully LNA-modified phosphodiester oligonucleotide complementary to the 5'-end oligonucleotide) in 5x SSCT buffer . The wells were aspirated and washed three times with 300 µl of 2x SSCT buffer (300 mM NaCl, 30 mM sodium citrate, 0.05% Tween-20, pH 7.0). One hundred microliters of the extracted and diluted oligonucleotide samples (pmol range) were added to the wells, which were agitated at room temperature for 0.5 hours. The wells were aspirated and washed three times with 300 µl of 2x SSCT buffer. One hundred microliters of a 0.025 µM solution of a 5'-digitoxinated conjugated (Dig) detection probe (5x SSCT buffer with 7-mer fully LNA modified phosphorodiester oligonucleotide, complementary to the 3'-end of the oligonucleotide) was added to each well and incubated for 1 hour at room temperature under gentle agitation. The wells were aspirated and washed three times with 300 µl of 2x

SSCT buffer. One hundred microliters of anti-Dig-POD Fab fragments (Roche Applied Science) diluted 1:4000 in PBS containing 0.05% Tween-20 (pH 7.2) were added to each well and incubated for 1 hour at room temperature under gentle agitation. The wells were aspirated and washed three times with 300 µl of 2x SSCT buffer. One hundred microliters of substrate solution (TMB+Substrate-Chromogen, Dako) was added to each well and incubated for 3-5 min. at room temperature under gentle agitation, after which the incubation was stopped by addition of sulphuric acid (100 µl 0.5 M). The intensity of the color development was measured spectrophotometncally at 450 nm, and the test samples were referenced against the standard samples.

CLAIMS

1. An oligomer of from 10 - 30 nucleotides in length which comprises a contiguous nucleotide sequence of a total of from 10 - 23 nucleotides, wherein said contiguous nucleotide sequence is at least 80% homologous to any one of SEQ ID NOS: 1 - 7 or a region corresponding to any one of these in a HCV genome or to the reverse complement of any one of SEQ ID NOS: 1-7 or naturally occurring variants thereof.
2. The oligomer according to claim 1, wherein the contiguous nucleotide sequence is at least 80% homologous to a region corresponding to any of SEQ ID NO: 23-31 .
3. The oligomer according to claim 1 or 2, wherein the contiguous nucleotide sequence comprises no mismatches or no more than one or two mismatches with the reverse complement of the corresponding region of SEQ ID NO: 1 or 2 .
4. The oligomer according to any one of claims 1 - 3, wherein the nucleotide sequence of the oligomer consists of the contiguous nucleotide sequence.
5. The oligomer according to any one of claims 1 - 4, wherein the contiguous nucleotide sequence is from 10 - 18 nucleotides in length.
6. The oligomer according to any one of claims 1-5, wherein the contiguous nucleotide sequence is 16 nucleotides in length.
7. The oligomer according to any one of claims 1 - 6, wherein the contiguous nucleotide sequence comprises nucleotide analogues.
8. The oligomer according to claim 7, wherein the nucleotide analogues are sugar modified nucleotides, such as sugar modified nucleotides selected from the group consisting of: Locked Nucleic Acid (LNA) units; 2'-0-alkyl-RNA units, 2'-OMe-RNA units, 2'-amino-DNA units, and 2'-fluoro-DNA units.
9. The oligomer according to claim 7, wherein the nucleotide analogues are LNA.
10. The oligomer according to any one of claims 7 - 9 which is a gapmer.
11. The oligomer according to any one of claims 1-10 wherein the oligonucleotide comprises or consists of any one of SEQ ID NO: 32-71 or SEQ ID NO: 72-78.
12. The oligomer according to any one of claims 1 - 11, which inhibits the expression of HCV gene or mRNA in a cell which is expressing HCV gene or mRNA.
13. A conjugate comprising the oligomer according to any one of claims 1 - 12, and at least one non-nucleotide or non-polynucleotide moiety covalently attached to said oligomer.
14. A pharmaceutical composition comprising the oligomer according to any one of claims 1 - 12, or the conjugate according to claim 13, and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.
15. The oligomer according to any one of claims 1 - 12, or the conjugate according to claim 13, for use as a medicament, such as for the treatment of Hepatitis C virus infection.

16. The use of an oligomer according to any one of the claims 1-12, or a conjugate as defined in claim 13, for the manufacture of a medicament for the treatment of Hepatitis C virus infection.

17. The use of an oligomer according to any one of the claims 1-12, or a conjugate as defined in claim 13, for the manufacture of a medicament for the treatment of Hepatitis C virus infection, wherein the medicament is for use in combination with another active ingredient.

18. A method of treating Hepatitis C virus infection, said method comprising administering an effective amount of an oligomer according to any one of the claims 1-12, or a conjugate according to claim 13, or a pharmaceutical composition according to claim 14, to a patient suffering from, or likely to suffer from Hepatitis C virus infection.

19. A method for the inhibition of HCV in a cell which is expressing HCV, said method comprising administering an oligomer according to any one of the claims 1-12, or a conjugate according to claim 13 to said cell so as to inhibit HCV in said cell.

FIGURES

Figure 1

The region with difference in sequence between genotypes (nt 34 and 35) contains one of the following sequence motifs:

AG	GA	AA

By designing oligonucleotides that have TT or UU in the given position, oligonucleotides will have a G/T or G/U mismatch against the genotypes containing either the AG or GA motif and a perfect match to the AA motif.

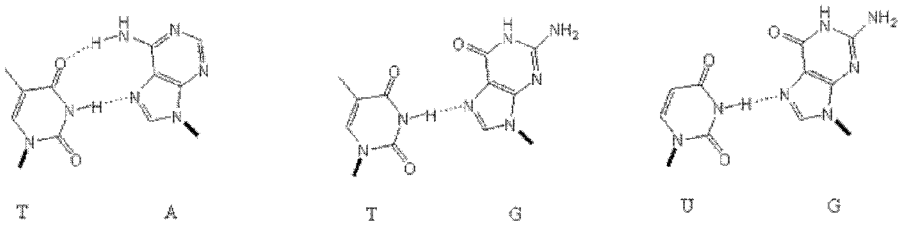


Figure 2

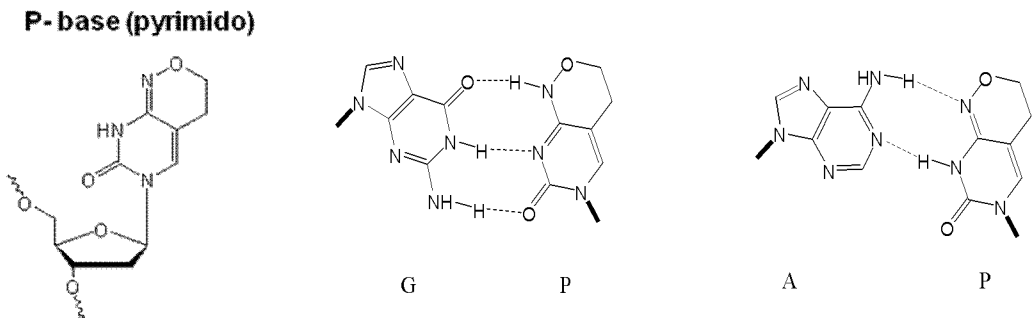


Figure 3

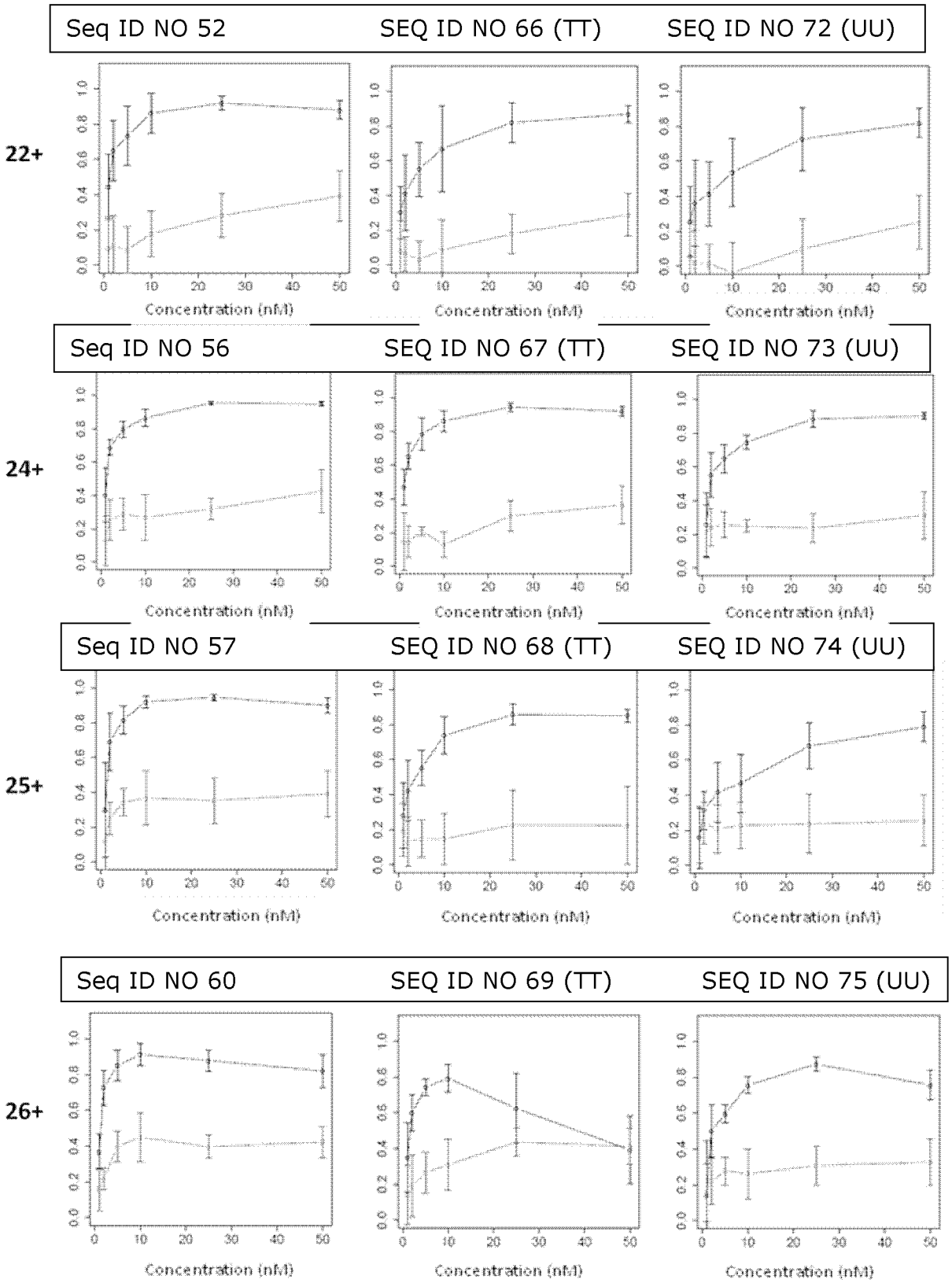
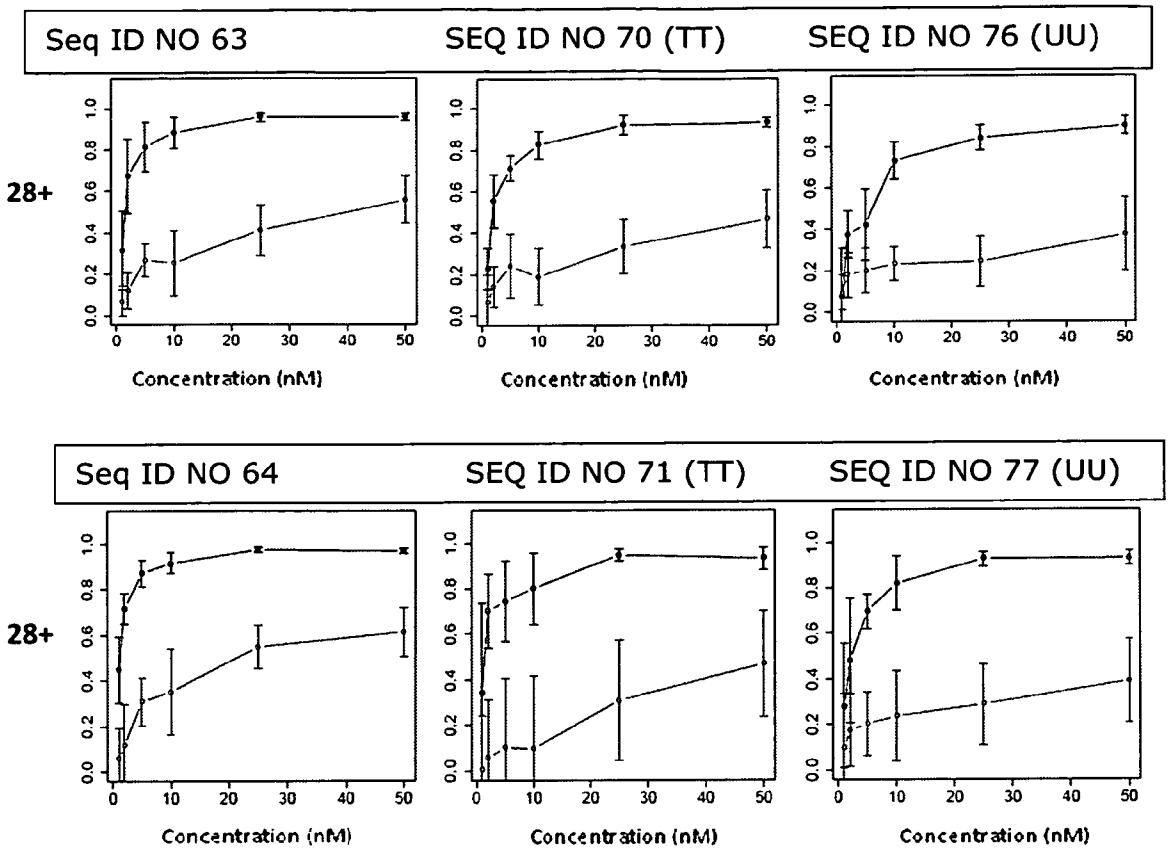

Seq ID NO 52
SEQ ID NO 66 (TT)
SEQ ID NO 72 (UU)
22+
Seq ID NO 56
SEQ ID NO 67 (TT)
SEQ ID NO 73 (UU)
24+
Seq ID NO 57
SEQ ID NO 68 (TT)
SEQ ID NO 74 (UU)
25+
Seq ID NO 60
SEQ ID NO 69 (TT)
SEQ ID NO 75 (UU)
26+
Concentration (nM)

Figure 3 continued



INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2 011/061884

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purpose of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/061884

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/712 A61K31/7125
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal , WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIMA W F ET AL: "Combinatorial screening and rational optimization for hybridization to folded hepatitis C virus RNA of oligonucleotides with biological antisense activity" , JOURNAL OF BIOLOGICAL CHEMISTRY, THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INC, US, vol . 272 , no. 1, 3 January 1997 (1997-01-03) , pages 626-638, XP002959559 , ISSN: 0021-9258, DOI: 10.1074/JBC.272.1.626	1-4
Y	figure 2	1-19

-/- ·

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" documentwhich may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 November 2011	09/11/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Romano, Alper

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/061884

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94/05813 Al (MOCHIDA PHARM CO LTD [JP] ; CHEMO SERO THERAPEUT RES INST [JP] ; ISIS PH) 17 March 1994 (1994-03-17)	1-4,7 ,8
Y	tabl es 2,4; sequence 2	1-19

X	CN 1 253 138 A (INST OF RADIOACTIVE MEDICAL SC [CN]) 17 May 2000 (2000-05-17)	1-4, 15 , 16, 19
Y	page 2; claim 1; tabl e 1	1-19

Y	MCHUTCHISON J G ET AL: "A phase I tri al of an anti sense inhi bitor of hepati tis C virus (ISIS 14803) , admi ni stered to chroni c hepati tis C pati ents" , JOURNAL OF HEPAT0L0GY, MUNKSGAARD INTERNATIONAL PUBLISHERS, COPENHAGEN , DK, vol . 44, no. 1, 1 January 2006 (2006-01-01) , pages 88-96, XP025053323 , ISSN : 0168-8278, D0I : 10. 1016/J .JHEP.2005 .09 .009 [retri eved on 2006-01-01] the whol e document	1-19

X, P	LAXT0N CARL ET AL: "Sel ecti on, optimi zati on, and pharmacoki neti c properti es of a novel , potent anti viral l ocked nucl ei c aci d-based anti sense oligomer targeti ng hepati tis C virus i nternal ri bosome entry si te. ", ANTIMICROBIAL AGENTS AND CHEMOTHERAPY JUL 2011 LNKD- PUBMED:21502629 , vol . 55, no. 7, 18 Apri l 2011 (2011-04-18) , pages 3105-3114, XP008144780, ISSN : 1098-6596 the whol e document	1-19

A	JURA INGA HENKE ET AL: "mi croRNA-122 stimul ates transl ati on of hepati tis C virus RNA" , THE EMB0 JOURNAL, vol . 27, no. 24, 17 December 2008 (2008-12-17) , pages 3300-3310, XP55010930, ISSN : 0261-4189 , D0I : 10. 1038/emboj .2008.244 the whol e document	1-19

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/061884

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9405813 A1	17-03-1994	AT 202383 T	15-07-2001
		AU 680435 B2	31-07-1997
		AU 4983793 A	29-03-1994
		CA 2143678 A1	17-03-1994
		DE 69330372 D1	26-07-2001
		DE 69330372 T2	14-03-2002
		DK 662157 T3	27-08-2001
		EP 0662157 A1	12-07-1995
		JP H08506479 A	16-07-1996
		NZ 255578 A	26-11-1996
		NZ 286209 A	29-09-2000
		US 6284458 B1	04-09-2001
		US 2004033978 A1	19-02-2004
CN 1253138 A	17-05-2000	NONE	