WO 2011/126937 refers to targeted intracellular delivery of oligonucleotides via conjugation with small molecule ligands.

WO2009/025669 refers to polymeric (polyethylene glycol) linkers containing pyridyl disulphide moieties. See also Zhao et al., Bioconjugate Chem. 2005 16 758 – 766.

Chaltin *et al.,* Bioconjugate Chem. 2005 16 827 - 836 reports on cholesterol modified mono- di- and tetrameric oligonucleotides used to incorporate antisense oligonucleotides into cationic liposomes, to produce a dendrimeric delivery system. Cholesterol is conjugated to the oligonucleotides via a lysine linker.

Other non-cleavable cholesterol conjugates have been used to target siRNAs and antagomirs to the liver – see for example, Soutscheck *et al.*, Nature 2004 vol. 432 173 – 178 and Krützfeldt et al., Nature 2005 vol 438, 685 – 689. For the partially phosphorothiolated siRNAs and antagomirs, the use of cholesterol as a liver targeting entity was found to be essential for *in vivo* activity.

The present invention is based upon the discovery that highly effective targeted delivery of oligonucleotides is achieved by the use of a homing device linked to the oligonucleotide by means of a short region of nuclease labile nucleosides, such as phosphodiester linked DNA or RNA nucleosides.

SUMMARY

The disclosure provides for an oligomeric compound comprising three regions:

i) a first region (region A), which comprises 7 – 26 contiguous nucleotides;

ii) a second region (region B) which comprises between 1 – 10 nucleotides, which is covalently linked to the 5' or 3' nucleotide of the first region, such as via a internucleoside linkage group such as a phosphodiester linkage, wherein either

   a. the internucleoside linkage between the first and second region is a phosphodiester linkage and the nucleoside of the second region [such as immediately] adjacent to the first region is either DNA or RNA; and/or

   b. at least 1 nucleoside of the second region is a phosphodiester linked DNA or RNA nucleoside;

iii) a third region (C) which comprises a conjugate moiety, a targeting moiety, a reactive group, an activation group, or a blocking moiety, wherein the third region is covalently linked to the second region.

In one aspect, there is provided an oligomeric compound comprising three regions:

i) a first region (region A), which is an antisense oligomer of 10 – 22 contiguous nucleotides complementary to a nucleic acid target, wherein the first region is a gapmer;

ii) a second region (region B) which comprises between 1 – 10 nucleotides, which is covalently linked to the 5' or 3' nucleotide of the first region, such as via a internucleoside linkage group such as a phosphodiester linkage, wherein either

   a. the internucleoside linkage between the first and second region is a phosphodiester linkage and the nucleoside of the second region adjacent to the first region is either DNA or RNA; and/or

   b. at least 1 nucleoside of the second region is a phosphodiester linked DNA or RNA nucleoside;

a third region which comprises a conjugate moiety, a targeting moiety, a reactive group, or a blocking moiety, wherein the third region is covalent linked to the second region.

In some embodiments, region A and region B form a single contiguous nucleotide sequence of 8 – 35 nucleotides in length.

In some embodiments the internucleoside linkage between the first and second regions may be considered part of the second region.

In some embodiments, there is a phosphorus containing linkage group between the second and third region. The phosphorus linkage group, may, for example, be a phosphate (phosphodiester), a phosphorothioate, a phosphorodithioate or a boranophosphate group. In some embodiments, this phosphorus containing linkage group is positioned between the second region and a linker region which is attached to the third region. In some embodiments, the phosphate group is a phosphodiester.

Therefore, in some embodiments the oligomeric compound comprises at least two phosphodiester groups, wherein at least one is as according to the above statement of disclosure, and the other is positioned between the second and third regions, optionally between a linker group and the second region.

In some embodiments, the third region is an activation group, such as an activation group for use in conjugation. In this respect, the disclosure also provides activated oligomers comprising region A and B and an activation group, e.g. an intermediate which is suitable for subsequent linking to the third region, such as suitable for conjugation.

In some embodiments, the third region is a reactive group, such as a reactive group for use in conjugation. In this respect, the disclosure also provides oligomers comprising region A and B and a reactive group, e.g. an intermediate which is suitable for subsequent linking to the third region, such as suitable for conjugation. The reactive group may, in some embodiments comprise an amine of alcohol group, such as an amine group.

In some embodiments region A comprises at least one, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 internucleoside linkages other than phosphodiester, such as internucleoside linkages which are (optionally independently] selected from the group consisting of phosphorothioate, phosphorodithioate, and boranophosphate, and methylphosphonate, such as phosphorothioate. In some embodiments region A comprises at least one phosphorothioate linkage. In some embodiments at least 50%, such as at least 75%, such as at least 90% of the internucleoside linkages, such as all the internucleoside linkages within region A are other than phosphodiester, for example are phosphorothioate linkages. In some embodiments, all the internucleoside linkages in region A are other than phosphodiester.

In some embodiments, the oligomeric compound comprises an antisense oligonucleotide, such as an antisense oligonucleotide conjugate. The antisense oligonucleotide may be or may comprise the first region, and optionally the second region.

In this respect, in some embodiments, region B may form part of a contiguous nucleobase sequence which is complementary to the (nucleic acid) target. In other embodiments, region B may lack complementarity to the target.

Alternatively stated, in some embodiments, the disclosure provides a non-phosphodiester linked, such as a phosphorothioate linked, oligonucleotide (e.g. an antisense oligonucleotide) which has at least one terminal (5' and/or 3') DNA or RNA nucleoside linked to the adjacent nucleoside of the oligonucleotide via a phosphodiester linkage, wherein the terminal DNA or RNA nucleoside is further covalently linked to a conjugate moiety, a targeting moiety or a blocking moiety, optionally via a linker moiety.

In another aspect, there is provided a pharmaceutical composition comprising the oligomeric compound of the disclosure and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

In another aspect, there is provided the oligomeric compound according to the disclosure for use in the inhibition of a nucleic acid target in a cell. In some embodiments the use is in vitro. In some embodiments the use is in vivo.

The disclosure provides for the oligomeric compound of the disclosure for use in medicine, such as for use as a medicament.

In another aspect, there is provided the oligomeric compound of the disclosure for use in the treatment of a medical disease or disorder.

The disclosure provides for the use of the oligomeric compound of the disclosure for the preparation of a medicament for the treatment of a disease or disorder, such as a metabolic disease or disorder.

The disclosure provides for a method of synthesizing (or manufacture) of an oligomeric compound, such as the oligomeric compound of the disclosure, said method comprising either:

a) a step of providing a [solid phase] oligonucleotide synthesis support to which one of the following is attached [third region]:
   i) optionally a linker group (-Y-)
   ii) a group X comprising a group selected from the group consisting of a conjugate, a targeting group, a blocking group, a reactive group [e.g. an amine or an alcohol] or an activation group(X-), or an an -Y – X group

and

b) a step of [sequential] oligonucleotide synthesis of region B followed by region A, and / or:

c) a step of [sequential] oligonucleotide synthesis of a first region (A) and a second region (B), wherein the synthesis step is followed by

d) a step of adding a third region [phosphoramidite comprising]

i) optionally a linker group (-Y-)

ii) a group X comprising a group selected from the group consisting of a conjugate, a targeting group, a blocking group, a reactive group [e.g. an amine or an alcohol] or an activation group (X-) or optionally an -Y – X group

followed by

e) the cleavage of the oligomeric compound from the [solid phase] support

wherein, optionally said method further comprises a further step selected from:

f) wherein the third group is an activation group, the step of activating the activation group to produce a reactive group, followed by adding a conjugate, a blocking, or targeting group to the reactive group, optionally via a linker group (Y);

g) wherein the third region is a reactive group, the step of adding a conjugate, a blocking, or targeting group to the reactive group, optionally via a linker group (Y).

h) wherein the third region is a linker group (Y), the step of adding a conjugate, a blocking, or targeting group to the linker group (Y)

wherein steps f), g) or h) are performed either prior to or subsequent to cleavage of the oligomeric compound from the oligonucleotide synthesis support. In some embodiments, the method may be performed using standard phosphoramidite chemistry, and as such the region X and/or region X or region X and Y may be provided, prior to incorporation into the oligomer, as a phosphoramidite. Please see figures 5 – 10 which illustrate non-limiting embodiments of the method of the disclosure.

The disclosure provides for a method of synthesizing (or manufacture) of an oligomeric compound, such as the oligomeric compound of the disclosure, said method comprising a step of [sequential] oligonucleotide synthesis of a first region (A) and optionally a second region (B), wherein the synthesis step is followed by a step of adding a third region [phosphoramidite comprising] region X (also referred to as region C), or Y such as a region comprising a group selected from the group consisting of a conjugate, a targeting group, a blocking group, a functional group, a reactive group [e.g. an amine or an alcohol] or an activation group (X), or an -Y – X group followed by the cleavage of the oligomeric compound from the [solid phase] support.

It is however recognized that the region X or X-Y may be added after the cleavage from the solid support. Alternatively, the method of synthesis may comprise the steps of synthesizing a first (A), and optionally second region (B), followed by the cleavage of the oligomer from the support, with a subsequent step of adding a third region , such as X or X-Y group to the oligomer. The addition of the third region may be achieved, by example, by adding an amino phosphoramidite unit in the final step of oligomer synthesis (on the support), which can, after cleavage from the support, be used to join to the X or X-Y group, optionally via an activation group on the X or Y (when present) group. In the embodiments where the cleavable linker is not a nucleotide region, region B may be a non-nucleotide cleavable linker for example a peptide linker, which may form part of region X (also referred to as region C) or be region Y (or part thereof).

In some embodiments of the method, region X (such as C) or (X-Y), such as the conjugate (e.g. a GalNAc conjugate) comprises an activation group, (an activated functional group) and in the method of synthesis the activated conjugate (or region x, or X-Y) is added to the first and second regions, such as an amino linked oligomer. The amino group may be added to the oligomer by standard phosphoramidite chemistry, for example as the final step of oligomer synthesis (which typically will result in amino group at the 5' end of the oligomer). For example during the last step of the oligonucleotide synthesis a protected amino-alkyl phosphoramidite is used, for example a TFA-aminoC6 phosphoramidite (6-(Trifluoroacetylamino)-hexyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite).

Region X (or region C as referred to herein), such as the conjugate (e.g. a GalNac conjugate) may be activated via NHS ester method and then the aminolinked oligomer is added. For example a N-hydroxysuccinimide (NHS) may be used as activating group for region X (or region C, such as a conjugate, such as a GalNac conjugate moiety.

The disclosure provides an oligomer prepared by the method of the disclosure.

In some embodiments, region X and/or region X or region X and Y may be covalently joined (linked) to region B via a phosphate nucleoside linkage, such as those described herein, including phosphodiester or phosphorothioate, or via an alternative group, such as a triazol group.

The disclosure provides for a method of treatment of a disease or disorder in a subject in need of treatment, said method comprising the steps of administering a pharmaceutical composition comprising the oligomeric compound of the disclosure to said subject in a therapeutically effective amount.

The disclosure provides for a method of inhibiting the expression of a target gene in a cell, said method comprising administering the oligomeric compound according to the disclosure to a cell which is expressing said target gene, suitably in an amount effective to reduce the expression of the target gene in said cell. In some embodiments the method is *in vitro* (.e. not in an organism, but may be in a (e.g. ex-vivo) cell or tissue). In some embodiments the method is *in vivo*.

The disclosure also provides for an LNA oligomer, comprising a contiguous region of 8 – 24 phosphorothioate linked nucleosides, and further comprising between 1 and 6 DNA nucleosides which are contiguous with the LNA oligomer, wherein the internucleoside linkages between the DNA, and/or adjacent to the DNA nucleoside(s), is physiologically labile, such as is / are phosphodiester linkages. Such an LNA oligomer may be in the form of a conjugate, as described herein, or may, for example be an intermediate to be used in a subsequent conjugation step. When conjugated, the conjugate may, for example be or comprise a sterol, such as cholesterol or tocopherol, or may be or comprise a (non-nucleotide) carbohydrate, such as a GalNac conjugate, such as a GalNac cluster, e.g. triGalNac, or another conjugate as described herein.

The disclosure provides for an LNA antisense oligomer (which may be referred to as region A herein) comprising an antisense oligomer and an asialoglycoprotein receptor targeting moiety conjugate moiety, such as a GalNAc moiety, which may form part of a further region (referred to as region C). The LNA antisense oligomer may be 7 – 30, such as 8 – 26 nucleosides in length and it comprises at least one LNA unit (nucleoside).

The disclosure provides for an LNA antisense oligomer covalently joined to (e.g. linked to) a (non-nucleoside) carbohydrate moiety, such as a carbohydrate conjugate moiety. In some embodiments the carbohydrate moiety is not a linear carbohydrate polymer. The carbohydrate moiety may however be multi-valent, such as, for example 2, 3, 4 or 4 identical or non-identical carbohydrate moieties may be covalently joined to the oligomer, optionally via a linker or linkers.

The disclosure provides for an LNA antisense oligomer (conjugate) comprising an antisense oligomer and a conjugate moiety which comprises a carbohydrate, such as a carbohydrate conjugate moiety.

The disclosure provides for a pharmaceutical composition comprising the LNA oligomeric compound of the disclosure and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

The disclosure provides for the oligomeric compound according to the disclosure for use in the inhibition of a nucleic acid target in a cell. In some embodiments the use is in vitro. In some embodiments the use is in vivo.

The disclosure provides for the oligomeric compound of the disclosure for use in medicine, such as for use as a medicament.

The disclosure provides for the oligomeric compound of the disclosure for use in the treatment of a medical disease or disorder.

The disclosure provides for the use of the oligomeric compound of the disclosure for the preparation of a medicament for the treatment of a disease or disorder, such as a metabolic disease or disorder.

BRIEF DESCRIPTION OF FIGURES

Figure 1: Non-limiting illustration of oligomers of the invention attached to an activation group (i.e. a protected reactive group – as the third region). The internucleoside linkage L may be, for example phosphodiester, phosphorothioate, phosphorodithioate, boranophosphate or methylphosphonate, such as phosphodiester. PO is a phosphodiester linkage. Compound a) has a region B with a single DNA or RNA, the linkage between the second and the first region is PO. Compound b) has two DNA/RNA (such as DNA) nucleosides linked by a phosphodiester linkage. Compound c) has three DNA/RNA (such as DNA) nucleosides linked by a phosphodiester linkages. In some embodiments, Region B may be further extended by further phosphodiester DNA/RNA (such as DNA nucleosides). The activation group is illustrated on the left side of each compound, and may, optionally be linked to the terminal nucleoside of region B via a phosphorus nucleoside linkage group, such as phosphodiester, phosphorothioate, phosphorodithioate, boranophosphate or methylphosphonate, or in some embodiments a triazole linkage. Compounds d), e), & f) further comprise a linker (Y) between region B and the activation group, and region Y may be linked to region B via, for example, a phosphorus nucleoside linkage group, such as phosphodiester, phosphorothioate, phosphorodithioate, boranophosphate or methylphosphonate, or in some embodiments a triazole linkage.

Figure 2: Equivalent compounds as shown in figure 1; however a reactive group is used in place of the activation group. The reactive group may, in some embodiments be the result of activation of the activation group (e.g. deprotection). The reactive group may, in non-limiting examples, be an amine or alcohol.