In one aspect, the disclosure provides for an oligomeric compound of 8 – 35 nucleotides in length, comprising three regions:

i) a first region (region A), which is an antisense oligomer ~~comprising~~ consisting of 7 – ~~26~~ 25 contiguous nucleotides complementary to a nucleic acid target, wherein the first region comprises at least one nucleoside analog and wherein the internucleoside linkages of the first region comprise at least 50% internucleoside linkage other than phosphodiester;

ii) a second region (region B) which ~~comprises~~ consists of between 1 – ~~10~~ 4 DNA or RNA 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 2 nucleosides of the second region are phosphodiester linked DNA or RNA nucleosides;

iii) a third region which comprises a conjugate moiety, a targeting moiety, or a blocking moiety, wherein the third region is covalently 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 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, the disclosure provides for a pharmaceutical composition comprising the oligomeric compound of the disclosure and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

In another aspect, 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.

In another aspect, the disclosure provides for the oligomeric compound of the disclosure for use in medicine. The oligomeric compound of the disclosure may be for use as a medicament.

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

In another aspect, 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~~selected from the group consisting of alpha-1 antitrypsin liver disease (AAT-LivD), alcohol dependence, anemia or inflammation/chronic kidney disease (CKD), atherosclerosis, atherosclerosis/high lipoprotein (a) (Lp(a)), cancer, liver cancer, cardiometabolic diseases, viral disease, hepatitis C virus (HCV), polycythemia, hepatitis B, BKV, human immunodeficiency virus (HIV), rare diseases (or genetic disorder), hemochromatosis, hemophilia A, B, hypertriglyceridemia, hyperlipidemia, high/low density lipoprotein (HDL/LDL) cholesterol imbalance, dyslipidemias, nonalcoholic steatohepatitis (NASH), porphyria, rare bleeding disorders, serum amyloid A (SAA)-amyloidosis, thrombosis, acromegaly, hypercholesterolemia, inflammatory disorders, obesity, transthyretin (TTR) amyloidosis, diabetes (type I and/or II), cushing's syndrome, glucose homeostasis, chronic heart failure, cardiovascular disease or disorder, myocardial infarction (MI), blood clotting disorder, fibrosis, liver fibrosis, cardiac fibrosis, local fibrosis, disease or disorder that

results (or is associated with) bone-lose, liver disease or disorder, metabolic disorder, metabolic syndrome, familial combined hyperlipidemia (FCHL), acquired hyperlipidemia, statin-resistant, vascular disease, peripheral arterial disease, heart disease, ischemia, acute coronary syndrome (ACS), coronary artery disease (CAD), coronary heart disease (CHD).

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 another aspect, the disclosure provdes for a method of synthesizing an oligomeric compound comprising a first region, a second region and a third region~~, and optionally a linker region (Y),~~ as defined herein, said method comprising

either:

a) a step of providing an oligonucleotide synthesis support to which one of the following is attached:

~~i) a linker group (-Y-)~~

~~ii)~~i) a group (X-) selected from the group consisting of a conjugate, a targeting group or a blocking group,

~~iii)~~ii) an -Y – X group, where Y is a linker group,

and

b) a step of oligonucleotide synthesis of region B followed by region A,

or:

c) a step of 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

~~i) a linker group (-Y-)~~

~~ii)~~i) a group (X-) selected from the group consisting of a conjugate, a targeting group or a blocking group

~~iii)~~ii) an -Y – X group, where Y is a linker group,

followed by

e) the cleavage of the oligomeric compound from the support.

~~wherein, optionally said method further comprises a further step 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) 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 aspects 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 disclosureprovides 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 disclosureto said subject in a therapeutically effective amount.

~~In another aspect, the~~ The disclosure provides ~~for~~ an oligomeric compound as defined herein for use in the treatment of a disease or disorder in a subject.

CLAIMS

1. An oligomeric compound of 8 – 35 nucleotides in length, comprising three regions:

i) a first region (region A), which is an antisense oligomer ~~comprising~~ consisting of 7 – ~~26~~ 25 contiguous nucleotides complementary to a nucleic acid target, wherein the first region comprises at least one nucleoside analog and wherein the internucleoside linkages of the first region comprise at least 50% internucleoside linkage other than phosphodiester;

ii) a second region (region B) which ~~comprises~~ consists of between 1 – ~~10~~ 4 DNA or RNA 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 2 nucleosides of the second region are phosphodiester linked DNA or RNA nucleosides;

iii) a third region which comprises a conjugate moiety, a targeting moiety, or a blocking moiety, wherein the third region is covalently linked to the second region.

2. The oligomeric compound according to claim 1, wherein the internucleoside linkages of the first region comprise at least 75%, such as at least 90%, such as 100% internucleoside linkages other than phosphodiester.

3. The oligomeric compound according to claim 1 or 2, wherein the internucleoside linkages other than phosphodiester are selected from the group consisting of phosphorothioate, phosphorodithioate and boranophosphate.

4. The oligomeric compound according to any one of claims 1 – 3, wherein the nucleic acid target is selected from the group consisting of a microRNA, a mRNA, a lncRNA (long non-coding RNA), a snRNA, snoRNA, and a viral RNA.

5. The oligomeric compound according to any one of claims 1 - 4, wherein the first region of the oligomeric compound~~, such as the first region, comprises~~ is a gapmer, a mixmer, or a totalmer.

6. The oligomeric compound according to any one of claims 1 - 5, wherein the first region of the oligomeric compound~~, such as the first region,~~ comprises at least one bicyclic nucleotide analogue (LNA).

7. The oligomeric compound according to any one of claims 1 – 6, wherein the first region and the second region form a contiguous nucleotide sequence.

8. The oligomeric compound according to any one of claims 1 – 7, wherein the second region is 5' to the first region.

9. The oligomeric compound according to any one of claims 1 – 7, wherein the second region is 3' to the first region.

10. The oligomeric compound according to any one of claims 1 – 9, wherein the second region comprises at least two~~, such as at least three~~ consecutive ~~nucleoside units, selected from the group of~~ DNA ~~and~~ or RNA units, which ~~may be~~are linked by phosphodiester linkages.

11. The oligomeric compound according to any one of claims 1 – 10, wherein the second region is covalently linked to the third region at the terminal nucleoside of the second region (i.e. the 3' or 5' terminus, depending on the position of the first region).

12. The oligomeric compound according to any one of claims 1 – 11, wherein the third region ~~comprises~~ is a conjugate group comprising a non-nucleotide moiety~~, such as a conjugate group, such as a sterol, for example cholesterol, or a carbohydrate, such as GalNac/GalNac cluster.~~

~~13. The oligomeric compound according to claim 0, wherein the third region comprises a moiety~~ selected from the group consisting of: a carbohydrate, a lipophilic group ~~(e.g. a lipid, a fatty acid, a sterol)~~, a protein, a peptide, an antibody or fragment thereof, a polymer, a reporter group, a dye, a receptor ligand, a small molecule drug, a prodrug, and a vitamin.

13. The oligomeric compound according to claim 3, wherein the conjugate group is selected from sterol, fatty acid, cholesterol, GalNAc or a GalNAc cluster.

14. The oligomeric compound according to any one of claims 1 – 11, wherein the third region ~~comprises a nucleic acid or polymer thereof, such as an aptamer, or a blocking nucleotide sequence~~is a blocker group.

15. The oligomeric compound according to any one of claims 1 – 14, wherein the third region comprises a targeting group.

~~16. The oligomeric compound according to any one of claims 1 – 11, wherein the third region comprises an activated group.~~

~~17~~16. The oligomeric compound according to any one of claims 1 – ~~16~~15, wherein the second and third regions are covalently joined by a linker group.

~~18~~17. The oligomeric compound according to any one of claims 1 – ~~17~~16, wherein the linker group between the second and third regions comprises a group selected from phosphodiester, a phosphorothioate, and phosphorodithioate or a boranophosphate group, such as a phosphodiester group.

~~19~~18. A pharmaceutical composition comprising the oligomeric compound of any one of claims 1-~~18~~17, and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

~~20~~19. The oligomeric compound according to any one of claims 1-~~18~~ 17 for use in the inhibition of a nucleic acid target in a cell.

~~21~~20. The oligomeric compound according to any one of claims 1-~~18~~ 17 for use in medicine.

~~22~~21. The oligomeric compound according to any one of claims 1-~~18~~ 17 for use in the treatment of a medical disease or disorder.

~~23~~22. The use of the oligomeric compound according to any one of claims 1-~~18~~ 17 for the preparation of a medicament for the treatment of a disease or disorder~~,~~ selected from the group consisting of alpha-1 antitrypsin liver disease (AAT-LivD), alcohol dependence, anemia or inflammation/chronic kidney disease (CKD), atherosclerosis, atherosclerosis/high lipoprotein (a) (Lp(a)), cancer, liver cancer, cardiometabolic diseases, viral disease, hepatitis C virus (HCV), polycythemia, hepatitis B, BKV, human immunodeficiency virus (HIV), rare diseases (or genetic disorder), hemochromatosis, hemophilia A, B, hypertriglyceridemia, hyperlipidemia, high/low density lipoprotein (HDL/LDL) cholesterol imbalance, dyslipidemias, nonalcoholic steatohepatitis (NASH), porphyria, rare bleeding disorders, serum amyloid A (SAA)-amyloidosis, thrombosis, acromegaly, hypercholesterolemia, inflammatory disorders, obesity, transthyretin (TTR) amyloidosis, diabetes (type I and/or II), cushing's syndrome, glucose homeostasis, chronic heart failure, cardiovascular disease or disorder, myocardial

infarction (MI), blood clotting disorder, fibrosis, liver fibrosis, cardiac fibrosis, local fibrosis, disease or disorder that results (or is associated with) bone-lose, liver disease or disorder, metabolic disorder, metabolic syndrome, familial combined hyperlipidemia (FCHL), acquired hyperlipidemia, statin-resistant, vascular disease, peripheral arterial disease, heart disease, ischemia, acute coronary syndrome (ACS), coronary artery disease (CAD), coronary heart disease (CHD).. ~~such as a metabolic disease or disorder.~~

~~24~~23. A method of synthesizing an oligomeric compound comprising a first region, a second region and a third region~~, and optionally a linker region (Y),~~ as defined in claims 1-~~18~~17, said method comprising

either:

a) a step of providing an oligonucleotide synthesis support to which one of the following is attached:

~~i) a linker group (-Y-)~~

~~ii)~~i) a group (X-) selected from the group consisting of a conjugate, a targeting group or a blocking group,

~~iii)~~ii) an -Y – X group, where Y is a linker group,

and

b) a step of oligonucleotide synthesis of region B followed by region A,

or:

c) a step of 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

~~i) a linker group (-Y-)~~

~~ii)~~i) a group (X-) selected from the group consisting of a conjugate, a targeting group or a blocking group

~~iii)~~ii) an -Y – X group, where Y is a linker group,

followed by

e) the cleavage of the oligomeric compound from the support.

~~wherein, optionally said method further comprises a further step~~

~~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) are performed either prior to or subsequent to cleavage of the oligomeric compound from the oligonucleotide synthesis support.~~

~~25. An oligomeric compound according to any one of claims 1-18 for use in the treatment of a disease or disorder in a subject.~~

~~26~~24. An oligomeric compound according to any one of claims 1-~~18~~ 17 for use in inhibiting the expression of a target gene in a cell, wherein said cell expresses said target gene, and wherein said oligomeric compound is to be administered in an amount effective to reduce the expression of the target gene in said cell.