

DOUBLE-STRANDED RNAI AGENTS AND COMPOSITIONS FOR REDUCING
EXPRESSION OF ANGIOPOIETIN-LIKE 3 (ANGPTL3) AND METHODS OF USE
THEREOF

5 TECHNICAL FIELD

The technical field relates to certain double-stranded RNA (dsRNA) or siRNA agents for inhibition of angiopoietin-like 3 gene expression, compositions that include angiopoietin-like 3 agents and methods of use thereof.

10 SEQUENCE LISTING

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 201286-PCT01-NP_SL.xml (Homo Sapiens angiopoietin-like 3 (ANGPTL3) transcript GenBank NM_014495.4 (SEQ ID NO: 1)), created on January 31, 2025, which is 193,866 bytes in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety. Although the sequence listing accompanying this filing identifies each sequence as either “RNA” or “DNA” as required, in reality, those sequences may be modified with any combination of chemical modifications. One of skill in the art will readily appreciate that such designation as “RNA” or “DNA” to describe modified oligonucleotides is, in certain instances, arbitrary. For example, an oligonucleotide comprising a nucleoside comprising a 2'-OH sugar moiety and a thymine base could be described as a DNA having a modified sugar (2'-OH in place of one 2'-H of DNA) or as an RNA having a modified base (thymine (methylated uracil) in place of an uracil of RNA). Accordingly, nucleic acid sequences provided herein, including, but not limited to those in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, unless otherwise stated, including, but not limited to such nucleic acids having modified nucleobases.

BACKGROUND

Angiopoietin-like 3 (also called ANGPTL3, ANGPL3, ANG3, or angiopoietin-like protein 3) is an angiopoietin protein encoded by the human angiopoietin-like 3 gene that is reported to be involved in regulating lipid metabolism. ANGPTL3 is a 460-amino acid polypeptide that

consists of a signal peptide, N-terminal coiled-coil domain, and a C-terminal fibrinogen (FBN)-like domain. ANGPTL3 is known to be primarily produced in hepatocytes in humans, and after synthesis is secreted into circulation. ANGPTL3 acts as an inhibitor of lipoprotein lipase, which catalyzes hydrolysis of triglycerides, and endothelial lipase, which hydrolyzes lipoprotein phospholipids. Inhibition of these enzymes can cause increases in plasma levels of triglycerides, high-density lipoproteins (HDL), and phospholipids. Further, loss-of-function mutations in ANGPTL3 lead to familial hypobetalipoproteinemia, which is characterized by low levels of triglycerides and low-density lipoprotein (LDL-C) in plasma. In humans, loss of-function in ANGPTL3 is also correlated with a decreased risk of atherosclerotic cardiovascular disease.

An effective therapeutic that targets ANGPTL3 has been reported to potentially provide a beneficial impact in the treatment (including prophylactic treatment) of cardiometabolic diseases such as hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis type I diabetes mellitus, cardiovascular disease, coronary artery disease, non alcoholic steatohepatitis, non-alcoholic fatty liver disease, homozygous and heterozygous familial hypercholesterolemia, statin resistant hypercholesterolemia and other metabolic related disorders and diseases.

Certain double-stranded RNA (dsRNA) or siRNA agents have been identified as being capable of inhibiting the expression of an ANGPTL3 gene (see e.g. WO 2012/17784, WO 2016/168286, WO 2016/154127, WO 2019/055633 and WO 2021/188795), the specific ANGPTL3 siRNA agent disclosed herein were not previously disclosed or known, and enable highly efficient ANGPTL3-specific inhibition of expression of an ANGPTL3 gene.

That an effective therapeutic targeting ANPTL3 could provide a beneficial impact in the treatment (including prophylactic treatment) of heart failure, including HFpEF and HFrEF, or chronic kidney disease (CKD) has never been depicted.

SUMMARY

There exists a need for novel ANGPTL3-specific RNA interference (RNAi) agents, e.g. double-stranded RNA (dsRNA) or siRNA agents, that are able to inhibit the expression of an ANGPTL3 gene *in vitro* and/or *in vivo* using the ANGPTL3 siRNA agents and compositions that include ANGPTL3 siRNA agents described herein. The ANGPTL3 siRNA agents described herein can selectively and efficiently decrease or inhibit expression of an

ANGPTL3 gene, and thereby reducing triglyceride (TG) levels and/or cholesterol in a subject, e.g., a human or animal subject.

The described ANGPTL3 siRNA agents can be used in methods for therapeutic treatment (including prophylactic and preventive treatment) of symptoms and diseases associated with

5 elevated TG levels and/or elevated cholesterol levels including but not limited to hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis type I diabetes mellitus, cardiovascular disease, coronary artery disease, non-alcoholic steatohepatitis, non-alcoholic fatty liver disease, homozygous and heterozygous familial hypercholesterolemia, statin resistant hypercholesterolemia and other metabolic

10 related disorders and diseases. The ANGPTL3 siRNA agents disclosed herein can selectively reduce ANGPTL3 gene expression, which can lead to a reduction in, among other things, TG levels and or cholesterol levels, in a subject. The methods disclosed herein include the administration of one or more ANGPTL3 siRNA agents to a subject, e.g. a human or animal subject, using any suitable methods known in the art, such as subcutaneous injection or

15 intravenous administration.

An object is to provide a method of treating symptoms and diseases associated with elevated TG levels and/or elevated cholesterol levels including but not limited to hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism,

20 atherosclerosis type I diabetes mellitus, cardiovascular disease, coronary artery disease, non-alcoholic steatohepatitis, non-alcoholic fatty liver disease, homozygous and heterozygous familial hypercholesterolemia, statin resistant hypercholesterolemia and other metabolic related disorders and diseases comprising administering to a subject a therapeutically effective amount of a double-stranded siRNA comprising an antisense strand comprising a nucleobase

25 sequence that is complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, thereby treating hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis type I diabetes mellitus, cardiovascular disease, coronary artery disease, non-alcoholic steatohepatitis, non-alcoholic fatty liver disease, homozygous and heterozygous

30 familial hypercholesterolemia, statin resistant hypercholesterolemia and other metabolic related disorders and diseases.

An object is to provide a method of treating heart failure comprising administering to a subject a therapeutically effective amount of a double-stranded siRNA comprising an antisense strand comprising a nucleobase sequence that is complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, thereby treating heart failure.

A further object is to provide a method of treating chronic kidney disease comprising administering to a subject a therapeutically effective amount of a double-stranded siRNA comprising an antisense strand comprising a nucleobase sequence that is complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, thereby treating chronic kidney disease.

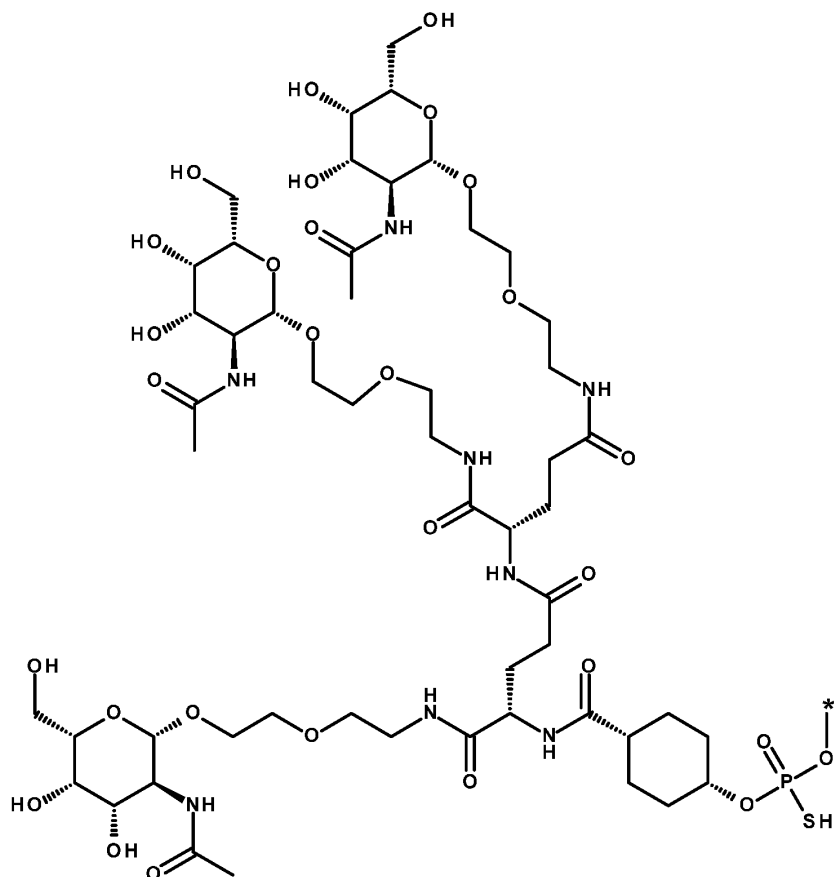
In one embodiment, the ANGPTL3 siRNA agent is Conjugate 1, a double-stranded RNA (dsRNA) or siRNA capable of inhibiting expression of the human ANGPTL3 gene comprising (i) a first (sense) strand having the sequence:

NAG-invAb (ps) mG mC mU mC mA mA mC mA fU fA fU mU mU mG mA mU mC mA mG mU mA (ps) invAb (SEQ ID NO: 10),

and (ii) a second (antisense) strand having the sequence:

mU (ps) fA (ps) mC (ps) fU mG fA mU fC mA fA mA fU mA fU mG fU mU fG mA fG (ps) mC (SEQ ID NO: 3),

where NAG is a triantennary ligand moiety linked to the 5' end of the first strand and having the structure:



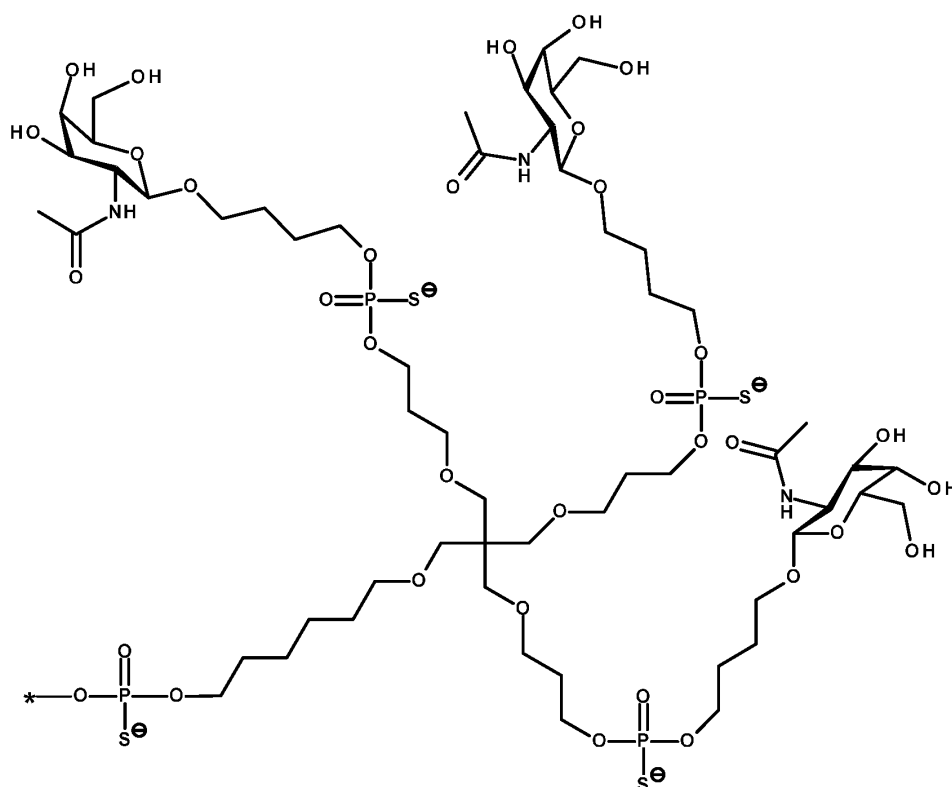
In one embodiment, the ANGPTL3 siRNA agent is Conjugate 2, a double-stranded RNA (dsRNA) or siRNA capable of inhibiting expression of the human ANGPTL3 gene comprising (i) a first (sense) strand having the sequence:

[M]-mC mU mA mC mA mU fA fU fA mA mA mC mU mA mC mA mA (ps) mG (ps) mU (SEQ ID NO: 11),

and (ii) a second (antisense) strand having the sequence:

mA (ps) fC (ps) mU fU mG fU mA fG mU fU mU fA mU fA mU fG mU (ps) fA (ps) mG
(SEQ ID NO: 5),

where [M] is a triantennary ligand moiety linked to the 5' end of the first strand and having
5 the structure:



Thus, the terminal phosphorothioate group of the ligand moiety [M] is bonded directly (via
the “free” bond indicated “*”) to the 5' position of the 5' terminal nucleotide of the first
10 strand (i.e., to the 5'-carbon of the 2'-O-methyl ribose moiety of the mC residue at the 5' end
of the first strand).

Sequences are shown in the conventional 5' to 3' direction.

In the context of the above formula, the following abbreviations are used:

Abbreviation	Meaning
mA, mU, mC, mG	2'-O-methyl ribonucleotide (adenine, uracil, cytosine, guanine)
fA, fU, fC, fG	2'-fluoro-ribonucleotide (adenine, uracil, cytosine, guanine)
(ps)	phosphorothioate linkage between adjacent nucleotides

In a further embodiment there is also provided a chirally enriched population of double-stranded siRNA, wherein the population is enriched for double-stranded siRNA comprising at least one particular phosphorothioate internucleoside linkage having a particular stereochemical configuration, or polynucleotide conjugates comprising said double-stranded siRNA.

The chirally enriched population may be enriched for double-stranded siRNA comprising at least one particular phosphorothioate internucleoside linkage having the (*Sp*) or (*Rp*) configuration, or polynucleotide conjugates comprising said double-stranded siRNA. The chirally enriched population may be enriched for double-stranded siRNA having a particular, independently selected stereochemical configuration at each phosphorothioate internucleoside linkage, or polynucleotide conjugates comprising said double-stranded siRNA. The chirally enriched population may be enriched for double-stranded siRNA having the (*Rp*) configuration at one particular phosphorothioate internucleoside linkage and the (*Sp*) configuration at each of the remaining phosphorothioate internucleoside linkages, or polynucleotide conjugates comprising said double-stranded siRNA. The chirally enriched population may be enriched for double-stranded siRNA having at least 2 or at least 3 contiguous phosphorothioate internucleoside linkages in the *Sp*, *Sp*, and *Rp* configurations, in the 5' to 3' direction, or polynucleotide conjugates comprising said double-stranded siRNA.

In a population of double-stranded siRNA comprising double-stranded or polynucleotide conjugates of the invention, all of the phosphorothioate internucleoside linkages of the double-stranded siRNA may be stereorandom.

In a further embodiment there is also provided a pharmaceutical composition comprising a double-stranded siRNA, polynucleotide conjugate, and a pharmaceutically acceptable diluent or carrier.

The pharmaceutically acceptable diluent may be water or phosphate-buffered saline. The pharmaceutical composition may consist essentially of said double-stranded siRNA, said polynucleotide conjugate and water or phosphate-buffered saline.

In a further embodiment there is also provided a method comprising administering to a subject said double-stranded siRNA, said polynucleotide conjugate, or said pharmaceutical composition.

In one embodiment, there is provided a method of treating a disease associated with ANGPTL3 comprising administering to a subject having a disease associated with ANGPTL3 a therapeutically effective amount of Conjugate 2 or said pharmaceutical composition, thereby treating the disease associated with ANGPTL3.

The disease associated with ANGPTL3 may be hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, type 2 diabetes mellitus, cardiovascular disease, coronary artery disease, non-alcoholic steatohepatitis, non-alcoholic fatty liver disease, homozygous and heterozygous familial hypercholesterolemia, statin resistant hypercholesterolemia and other metabolic-related disorders and diseases.

In a further embodiment, there is provided a method of treating heart failure comprising administering to a subject a therapeutically effective amount of a double-stranded siRNA comprising an antisense strand comprising a nucleobase sequence that is at least 80% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, thereby treating heart failure.

In still a further embodiment, there is provided a method of treating heart failure comprising administering to a subject a therapeutically effective amount of a double-stranded siRNA comprising an antisense strand comprising a nucleobase sequence that is at least 85% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, thereby treating heart failure.

In still a further embodiment, there is provided a method of treating heart failure comprising administering to a subject a therapeutically effective amount of a double-stranded siRNA comprising an antisense strand comprising a nucleobase sequence that is at least 90% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, thereby treating heart failure.

In still a further embodiment, there is provided a method of treating heart failure comprising administering to a subject a therapeutically effective amount of a double-stranded siRNA comprising an antisense strand comprising a nucleobase sequence that is at least 95%

complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, thereby treating heart failure.

In still a further embodiment, there is provided a method of treating heart failure comprising administering to a subject a therapeutically effective amount of a double-stranded
5 siRNA comprising an antisense strand comprising a nucleobase sequence that is 100% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand.; thereby treating heart failure.

In still a further embodiment, there is provided a method of treating heart failure comprising administering to a subject a therapeutically effective amount of Conjugate 2 or
10 said pharmaceutical composition.

In still a further embodiment, there is provided a method of treating heart failure comprising administering to a subject a therapeutically effective amount of Conjugate 1 or said pharmaceutical composition.

15 In a further embodiment, there is provided a method of treating chronic kidney disease (CKD) comprising administering to a subject a therapeutically effective amount of a double-stranded siRNA comprising an antisense strand comprising a nucleobase sequence that is at least 80% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, thereby treating CKD.

20 In still a further embodiment, there is provided a method of treating chronic kidney disease (CKD) comprising administering to a subject a therapeutically effective amount of a double-stranded siRNA comprising an antisense strand comprising a nucleobase sequence that is at least 85% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, thereby treating CKD.

25 In still a further embodiment, there is provided a method of treating chronic kidney disease (CKD) comprising administering to a subject a therapeutically effective amount of a double-stranded siRNA comprising an antisense strand comprising a nucleobase sequence that is at least 90% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, thereby treating CKD.

30 In still a further embodiment, there is provided a method of treating chronic kidney disease (CKD) comprising administering to a subject a therapeutically effective amount of a double-stranded siRNA comprising an antisense strand comprising a nucleobase sequence

that is at least 95% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, thereby treating CKD.

In still a further embodiment, there is provided a method of treating chronic kidney disease (CKD) comprising administering to a subject a therapeutically effective amount of a
5 double-stranded siRNA comprising an antisense strand comprising a nucleobase sequence that is 100% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, thereby treating CKD.

In still a further embodiment, there is provided a method of treating chronic kidney disease (CKD) comprising administering to a subject a therapeutically effective amount of
10 Conjugate 2 or said pharmaceutical composition.

In still a further embodiment, there is provided a method of treating chronic kidney disease (CKD) comprising administering to a subject a therapeutically effective amount of Conjugate 1 or said pharmaceutical composition.

15 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Five-concentration testing of ANGPTL3-targeting siRNAs Conjugate 2 and Conjugate 1 in primary human hepatocytes.

Figure 2 Five-concentration testing of ANGPTL3-targeting siRNAs Conjugate 2 and Conjugate 1 in primary cynomolgus monkey hepatocytes.

20 **Figure 3** Baseline-corrected circulating human ANGPTL3 protein humanized mice treated with a single dose of conjugate 2.

Figure 4 Human ANGPTL3 mRNA expression in liver on day 49 in humanized mice treated with a single dose of Conjugate 2.

25 **Figure 5** Human ANGPTL3 mRNA expression in liver on day 49 in humanized mice treated with a single dose of Conjugate 3 and 4, respectively.

Figure 6 Schematic study outline Example 5, Therapeutic effects of Conjugate 1 in an obese HFpEF ZSF1 rat model In Vivo.

Figure 7 Body weight (gr) at the end of the 12 weeks treatment period, Example 5.

30 **Figure 8** ANGPTL3 protein and TG plasma levels after administration of Conjugate 1 in ZSF-1 rats, Example 5.

Figure 9a Heart content (lipidometric analysis) after administration of Conjugate 1 in ZSF-1 rats.

Figure 9b Liver content (lipidometric analysis) after administration of Conjugate 1 in ZSF-1 rats.

Figure 10 Diastolic function (IVRT and NT-proBNP) after Conjugate 1 administration in ZSF-1 rats.

5 **Figure 11** Tissue proteomics after Conjugate 1 administration in ZSF-1 rats.

Figure 12 Renal health represented by UACR, podocyte health and glomerulosclerosis after Conjugate 1 administration in ZSF-1 Rats.

Figure 13 Baseline and Endpoint Proton-Density Fat Fraction (PDFF) results in HfpEF NHPs after treatment with ANGPTL3 inhibition.

10 **Figure 14** Baseline and Endpoint Extracellular volume (ECV) results in HfpEF NHPs after treatment with ANGPTL3 inhibition.

Figure 15 Left atria volume index (LAVi) results in HfpEF NHPs after treatment with ANGPTL3 inhibition.

15 **Figure 16** Plasma ANGPTL3 levels in HfpEF NHPs after treatment with ANGPTL3 inhibition.

Figure 17 Plasma TG levels in HFpEF NHPs after treatment with ANGPTL3 inhibition.

DETAILED DESCRIPTION

20 This detailed description and its specific examples, while indicating embodiments, are intended for purposes of illustration only. Therefore, there is no limitation to the illustrative embodiments described in this specification. In addition, it is to be appreciated that various features that are, for clarity reasons, described in the context of separate embodiments, also may be combined to form a single embodiment. Conversely, various features that are, for brevity reasons, described in the context of a single embodiment, also may be combined to

25 form sub-combinations thereof.

Listed below are definitions of various terms used in the specification and claims.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the

30 use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “including” as well as other forms, such as “includes” and “included”, is not limiting. Also, terms such as “element” or “component” encompass both elements and components

comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including, but not limited to, patents, patent applications, articles, books, and treatises, are hereby expressly incorporated-by-reference for the portions of the document discussed herein, as well as in their entirety.

Unless specific definitions are provided, the nomenclature used in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art.

The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts described herein to provide yet further embodiments of the disclosure. Moreover, due to biological functional equivalency considerations, some changes can be made in protein structure without affecting the biological or chemical action in kind or amount. These and other changes can be made to the disclosure in light of the detailed description. All such modifications are intended to be included within the scope of the appended claims.

Numeric ranges are inclusive of the numbers defining the range. Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within this disclosure. The upper and lower limits of these smaller

ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within this disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included
5 limits are also included in this disclosure.

“About” may generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are within 20 percent (%), typically, within 10%, and more typically, within 5% of a given value or range of values. Preferably, the term “about” shall be understood herein as plus or minus ($\pm$) 5%,
10 preferably $\pm 4\%$, $\pm 3\%$, $\pm 2\%$, $\pm 1\%$, $\pm 0.5\%$, $\pm 0.1\%$, of the numerical value of the number with which it is being used.

The term “consisting of” refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the invention.

15 Embodiments described herein as “comprising” one or more features may also be considered as disclosure of the corresponding embodiments “consisting of” and/or “consisting essentially of” such features.

Concentrations, amounts, volumes, percentages and other numerical values may be presented herein in a range format. It is also to be understood that such range format is used
20 merely for convenience and brevity and should be interpreted flexibly to include not only the numerical values explicitly recited as the limits of the range but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited.

The polynucleotides of the present invention may be prepared by any means known in
25 the art. For example, large amounts of the polynucleotides may be produced by replication in a suitable host cell. The polynucleotides of the present invention may preferably be produced by chemical synthesis, e.g., by the phosphoramidite method or the tri-ester method and may be performed on commercial automated oligonucleotide synthesizers. A double-stranded oligonucleotide may be obtained from the single stranded product of chemical synthesis either
30 by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Methods of determining nucleic acid percentage sequence identity are known in the art. By way of example, when assessing nucleic acid sequence identity, a sequence having a defined number of contiguous nucleotides may be aligned with a nucleic acid sequence (having the same number of contiguous nucleotides) from the corresponding portion of a nucleic acid sequence of the present invention. Tools known in the art for determining nucleic acid percentage sequence identity include Nucleotide BLAST (as described below).

The terms "decrease", "reduced", "reduction", or "inhibit" are all used herein to mean a decrease by a statistically significant amount. The terms "reduce", "reduction" or "decrease" or "inhibit" typically means a decrease by at least 10% as compared to a reference level (e.g. the absence of a given treatment) and can include, for example, a decrease by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or more. As used herein, "reduction" or "inhibition" encompasses a complete inhibition or reduction as compared to a reference level. "Complete inhibition" is a 100% inhibition (i.e., abrogation) as compared to a reference level.

The terms "increased", "increase", "enhance", or "activate" are all used herein to mean an increase by a statistically significant amount. The terms "increased", "increase", "enhance", or "activate" can mean an increase of at least 25%, at least 50% as compared to a reference level, for example an increase of at least about 50%, or at least about 75%, or at least about 80%, or at least about 90%, at least about 95%, or at least about 98%, or at least about 99%, or at least about 100%, or at least about 250% or more compared with a reference level, or at least about a 1.5-fold, or at least about a 2-fold, or at least about a 2.5-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or any increase between 1.5-fold and 10-fold or greater as compared to a reference level.

As used herein, "chirally enriched population" means a plurality of molecules of identical molecular formula, wherein the number or percentage of molecules within the population that contain a particular stereochemical configuration at a particular chiral center is greater than the number or percentage of molecules expected to contain the same particular stereochemical configuration at the same particular chiral center within the population if the particular chiral center were stereorandom. Chirally enriched populations of molecules having

multiple chiral centers within each molecule may contain one or more stereorandom chiral centers. In certain embodiments, the molecules are modified oligonucleotides. In certain embodiments, the molecules are oligomeric compounds comprising modified oligonucleotides.

5 As used herein, “complementary” in reference to an oligonucleotide means that is at least 70% of the nucleobases of the oligonucleotide and the nucleobases of another nucleic acid or one or more regions thereof are capable of hydrogen bonding with one another when the nucleobase sequence of the oligonucleotide and the other nucleic acid are aligned in opposing directions. “Complementary region” in reference to a region of an oligonucleotide
10 means that is at least 70% of the nucleobases of that region and the nucleobases of another nucleic acid or one or more regions thereof are capable of hydrogen bonding with one another when the nucleobase sequence of the oligonucleotide and the other nucleic acid are aligned in opposing directions, i.e. the nucleobase sequences are reverse complementary. For the avoidance of doubt, all references herein to an oligonucleotide (e.g. an antisense strand of a
15 double-stranded oligonucleotide of the invention) as complementary to a target sequence means that the nucleobase sequence of said oligonucleotide (e.g. the antisense strand of a double-stranded oligonucleotide of the invention) is reverse complementary to said target sequence.

 Complementary nucleobases mean nucleobases that are capable of forming hydrogen
20 bonds with one another. Complementary nucleobase pairs include adenine (A) and thymine (T), adenine (A) and uracil (U), cytosine (C) and guanine (G), 5-methyl cytosine (mC) and guanine (G). Certain modified nucleobases that pair with natural nucleobases or with other modified nucleobases are known in the art and are not considered complementary nucleobases as defined herein unless indicated otherwise. For example, inosine can pair, but is not
25 considered complementary, with adenosine, cytosine, or uracil. Complementary oligonucleotides and/or nucleic acids need not have nucleobase complementarity at each nucleoside. Rather, some mismatches are tolerated. As used herein, “fully complementary” or “100% complementary” in reference to oligonucleotides means that oligonucleotides are complementary to another oligonucleotide or nucleic acid at each nucleoside of the
30 oligonucleotide.

As used herein, “conjugate group” means a group of atoms that is directly attached to an oligonucleotide. Conjugate groups include a conjugate moiety and a conjugate linker that attaches the conjugate moiety to the oligonucleotide.

As used herein, “conjugate linker” means a single bond or a group of atoms
5 comprising at least one bond that connects a conjugate moiety to an oligonucleotide.

As used herein, “conjugate moiety” means a group of atoms that modifies one or more properties of a molecule compared to the identical molecule lacking the conjugate moiety, including but not limited to pharmacodynamics, pharmacokinetics, stability, binding, absorption, tissue distribution, cellular distribution, cellular uptake, charge and clearance.

10 As used herein, “internucleoside linkage” is the covalent linkage between adjacent nucleosides in an oligonucleotide. As used herein “modified internucleoside linkage” means any internucleoside linkage other than a phosphodiester internucleoside linkage.

As used herein, “motif” means the pattern of unmodified and/or modified sugar moieties, nucleobases, and/or internucleoside linkages, in an oligonucleotide.

15 As used herein, “modified nucleoside” means a nucleoside comprising a modified nucleobase and/or a modified sugar moiety.

As used herein, “nucleobase” means an unmodified nucleobase or a modified nucleobase. A nucleobase is a heterocyclic moiety. As used herein an “unmodified nucleobase” is adenine (A), thymine (T), cytosine (C), uracil (U), or guanine (G). As used
20 herein, a “modified nucleobase” is a group of atoms other than unmodified A, T, C, U, or G capable of pairing with at least one other nucleobase. A “5-methyl cytosine” is a modified nucleobase. A universal base is a modified nucleobase that can pair with any one of the five unmodified nucleobases.

As used herein, “nucleobase sequence” means the order of contiguous nucleobases in
25 a nucleic acid or oligonucleotide independent of any sugar or internucleoside linkage modification. By way of example and without limitation, an oligomeric compound having the nucleobase sequence “ATCGATCG” encompasses any compounds having such nucleobase sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence “AUCGAUCG” and those having
30 some DNA bases and some RNA bases such as “AUCGATCG” and compounds having other modified nucleobases, such as “AT^mCGAUCG,” wherein ^mC indicates a cytosine base comprising a methyl group at the 5-position. Finally, for clarity, unless otherwise indicated,

the phrase “nucleobase sequence of SEQ ID NO: X”, refers only to the sequence of nucleobases in that SEQ ID NO.: X, independent of any sugar or internucleoside linkage modifications also described in such SEQ ID.

As used herein, “nucleoside” means a compound or fragment of a compound
5 comprising a nucleobase and a sugar moiety. The nucleobase and sugar moiety are each, independently, unmodified or modified.

As used herein, “oligomeric agent” means a double-stranded oligonucleotide and optionally one or more additional features, such as a second oligonucleotide.

As used herein, “polynucleotide conjugate” means a double-stranded oligonucleotide
10 and optionally one or more additional features, such as a conjugate group or terminal group.

A double-stranded oligonucleotide comprises a first oligonucleotide strand that is paired with a second oligonucleotide strand that is complementary to the first oligonucleotide strand. A double-stranded oligonucleotide comprises a sense oligonucleotide or strand (also known in the art as a passenger oligonucleotide or passenger strand), and an antisense
15 oligonucleotide or strand (also known in the art as a guide oligonucleotide or guide strand).

As used herein, “antisense activity” means any detectable and/or measurable change attributable to the hybridization of an antisense strand to its target nucleic acid. In certain embodiments, antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid compared to target nucleic acid levels or
20 target protein levels in the absence of the antisense strand. In certain embodiments, antisense activity is the modulation of splicing of a target pre-mRNA.

As used herein, “antisense agent” means an antisense strand and optionally one or more additional features, such as a sense strand. Thus, a double-stranded oligonucleotide of the invention may be described as an antisense agent.

As used herein, “antisense compound” means an antisense strand and optionally one
25 or more additional features, such as a conjugate group. An antisense agent may comprise an antisense compound and a sense strand.

As used herein, “sense compound” means a sense strand and optionally one or more additional features, such as a conjugate group.

As used herein, “antisense oligonucleotide” means an oligonucleotide, including the
30 oligonucleotide portion of an antisense compound, that is capable of hybridizing to a target nucleic acid and is capable of at least one antisense activity. Antisense oligonucleotides

include but are not limited to antisense RNAi oligonucleotides and antisense RNase H oligonucleotides. The double-stranded oligonucleotides of the invention are typically siRNA and so comprise an antisense RNAi oligonucleotide.

As used herein, “sense oligonucleotide” or “sense strand” means an oligonucleotide, including the oligonucleotide portion of a sense compound, that is capable of hybridizing to an antisense oligonucleotide.

As used herein, “oligonucleotide” means a strand of linked nucleosides connected via internucleoside linkages, wherein each nucleoside and internucleoside linkage may be modified or unmodified. Unless otherwise indicated, oligonucleotides consist of 8-50 linked nucleosides. As used herein, “modified oligonucleotide” means an oligonucleotide, wherein at least one nucleoside or internucleoside linkage is modified. As used herein, “unmodified oligonucleotide” means an oligonucleotide that does not comprise any nucleoside modifications or internucleoside modifications.

As used herein, “stabilized phosphate group” means a 5'-phosphate analog that is metabolically more stable than a 5'-phosphate as naturally occurs on DNA or RNA.

As used herein, “standard cell assay” means the assays described in the Examples and reasonable variations thereof.

As used herein, “stereorandom chiral center” in the context of a population of molecules of identical molecular formula means a chiral center having a random stereochemical configuration. For example, in a population of molecules comprising a stereorandom chiral center, the number of molecules having the (S) configuration of the stereorandom chiral center may be but is not necessarily the same as the number of molecules having the (R) configuration of the stereorandom chiral center. The stereochemical configuration of a chiral center is considered random when it is the result of a synthetic method that is not designed to control the stereochemical configuration. In certain embodiments, a stereorandom chiral center is a stereorandom phosphorothioate internucleoside linkage.

As used herein, “target nucleic acid” and “target RNA” mean a nucleic acid that an oligomeric compound is designed to affect. Target RNA means an RNA transcript and includes pre-mRNA and mRNA unless otherwise specified.

As used herein, “target region” means a portion of a target nucleic acid to which an oligomeric compound is designed to hybridize.

As used herein, "terminal group" means a chemical group or group of atoms that is covalently linked to a terminus of an oligonucleotide.

As used herein, "hybridization" means the annealing of oligonucleotides and/or nucleic acids. While not limited to a particular mechanism, the most common mechanism of hybridization involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an antisense compound and a nucleic acid target. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an oligonucleotide and a nucleic acid target.

As used herein, "RNAi agent" means an antisense agent that acts, at least in part, through RISC or Ago2 to modulate a target nucleic acid and/or protein encoded by a target nucleic acid. RNAi agents include, but are not limited to double-stranded siRNA, single-stranded RNAi (ssRNAi), and microRNA, including microRNA mimics. RNAi agents may comprise conjugate groups and/or terminal groups. In certain embodiments, an RNAi agent modulates the amount and/or activity, of a target nucleic acid. The term RNAi agent excludes antisense agents that act through RNase H.

As used herein, "RNase H agent" means an antisense agent that acts through RNase H to modulate a target nucleic acid and/or protein encoded by a target nucleic acid. In certain embodiments, RNase H agents are single-stranded. In certain embodiments, RNase H agents are double-stranded. RNase H compounds may comprise conjugate groups and/or terminal groups. In certain embodiments, an RNase H agent modulates the amount and/or activity of a target nucleic acid. The term RNase H agent excludes antisense agents that act principally through RISC/Ago2.

As used herein, "reducing" or "inhibiting" ANGPTL3 means reducing expression of ANGPTL3 RNA and/or protein levels in the presence of an oligomeric compound or oligomeric agent described herein compared to expression of ANGPTL3 RNA and/or protein levels in the absence of an oligomeric compound or oligomeric agent described herein.

As used herein, "pharmaceutically acceptable carrier or diluent" means any substance suitable for use in administering to an animal. Certain such carriers enable pharmaceutical compositions to be formulated as, for example, tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspension and lozenges for the oral ingestion by a subject. In certain

embodiments, a pharmaceutically acceptable carrier or diluent is sterile water, sterile saline, sterile buffer solution or sterile artificial cerebrospinal fluid.

As used herein “pharmaceutically acceptable salts” means physiologically and pharmaceutically acceptable salts of compounds. Pharmaceutically acceptable salts retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. For example, the term “pharmaceutically acceptable” may refer to salts, excipients, carriers, diluents, etc. approved by a regulatory agency of the Federal or a state government, or listed in the U.S. Pharmacopeia, European Pharmacopeia, or other generally recognized pharmacopeia.

As used herein “pharmaceutical composition” means a mixture of substances suitable for administering to a subject. For example, a pharmaceutical composition may comprise an oligomeric compound and a sterile aqueous solution. In certain embodiments, a pharmaceutical composition shows activity in free uptake assay in certain cell lines.

As used herein “prodrug” means a therapeutic agent in a first form outside the body that is converted to a second form within an animal or cells thereof. Typically, conversion of a prodrug within the animal is facilitated by the action of an enzymes (e.g., endogenous or viral enzyme) or chemicals present in cells or tissues and/or by physiologic conditions. In certain embodiments, the first form of the prodrug is less active than the second form.

As used herein, “treating” means improving a subject’s disease or condition by administering an oligomeric agent or oligomeric compound described herein. In certain embodiments, treating a subject improves a symptom relative to the same symptom in the absence of the treatment. In certain embodiments, treatment reduces in the severity or frequency of a symptom, or delays the onset of a symptom, slows the progression of a symptom, or slows the severity or frequency of a symptom.

As used herein, “therapeutically effective amount” means an amount of a pharmaceutical agent or composition that has been observed to provide a therapeutic benefit to an animal. For example, a therapeutically effective amount may be observed to improve a symptom of a disease.

The terms “individual”, “subject”, and “patient”, are used interchangeably herein to refer to a mammalian subject for whom diagnosis, prognosis, disease monitoring, treatment, therapy, and/or therapy optimisation is desired. The mammal can be (without limitation) a human, non-human primate, mouse, rat, dog, cat, horse, or cow. In a preferred embodiment,

the individual, subject, or patient is a human. An “individual” may be an adult, juvenile or infant. An “individual” may be male or female.

A “subject in need” of treatment for a particular condition can be an individual having that condition, diagnosed as having that condition, or at risk of developing that condition.

5 A subject can be one who has been previously diagnosed with or identified as suffering from or having a condition in need of treatment or one or more complications or symptoms related to such a condition, and optionally, have already undergone treatment for a condition as defined herein or the one or more complications or symptoms related to said condition. Alternatively, a subject can also be one who has not been previously diagnosed as
10 having a condition as defined herein or one or more or symptoms or complications related to said condition. For example, a subject can be one who exhibits one or more risk factors for a condition, or one or more or symptoms or complications related to said condition or a subject who does not exhibit risk factors.

As used herein, the term “healthy individual” refers to an individual or group of
15 individuals who are in a healthy state, e.g., individuals who have not shown any symptoms of the disease, have not been diagnosed with the disease and/or are not likely to develop the disease e.g., a thrombotic event. Preferably said healthy individual(s) is not on medication affecting haemostasis and has not been diagnosed with any other disease. The one or more healthy individuals may have a similar sex, age, and/or body mass index (BMI) as compared
20 with the test individual. Application of standard statistical methods used in medicine permits determination of normal levels of expression in healthy individuals, and significant deviations from such normal levels.

Herein the terms “control” and “reference population” are used interchangeably.

25 There is provided Conjugate 2, comprising (i) a first (sense) strand having the sequence:

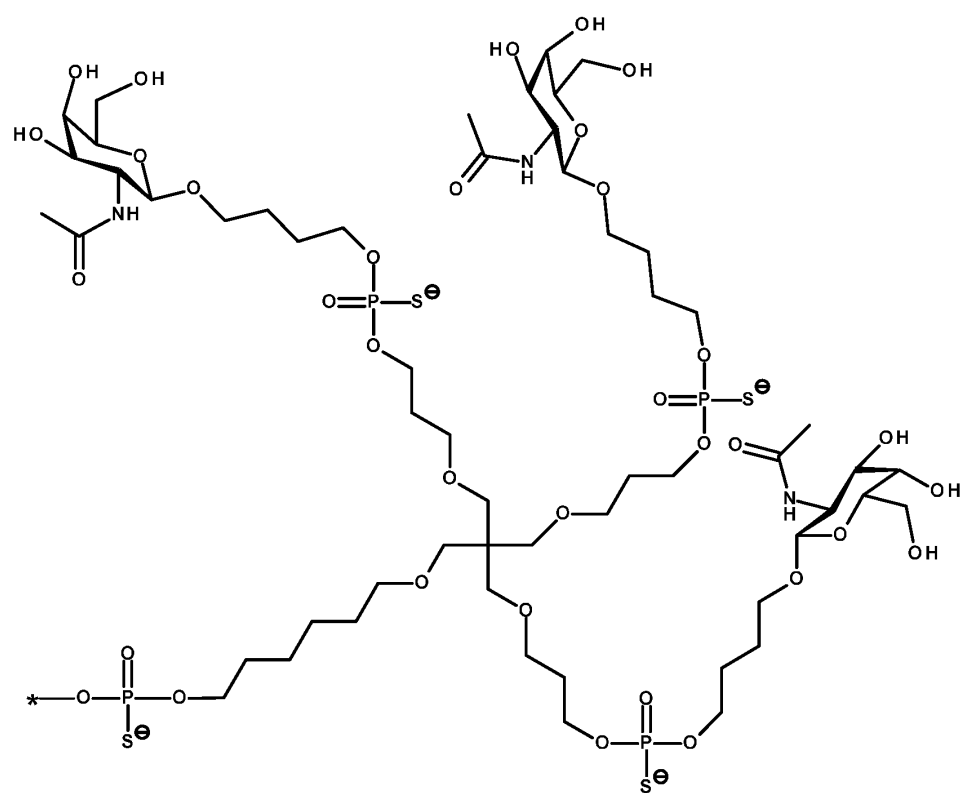
[M]-mC mU mA mC mA mU fA fU fA mA mA mC mU mA mC mA mA (ps) mG (ps) mU
(SEQ ID NO: 11),

30

and (ii) a second (antisense) strand having the sequence:

mA (ps) fC (ps) mU fU mG fU mA fG mU fU mU fA mU fA mU fG mU (ps) fA (ps) mG
(SEQ ID NO: 5),

where [M] is a triantennary ligand moiety linked to the 5' end of the second strand and having
5 the structure:



Thus, the terminal phosphorothioate group of the ligand moiety [M] is bonded directly (via
the “free” bond indicated “*”) to the 5' position of the 5' terminal nucleotide of the first
strand (i.e., to the 5'-carbon of the 2'-O-methyl ribose moiety of the mC residue at the 5' end
10 of the first strand).

Sequences are shown in the conventional 5' to 3' direction.

In the context of the above formula, the following abbreviations are used:

Abbreviation	Meaning
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mA, mU, mC, mG	2'-O-methyl ribonucleotide (adenine, uracil, cytosine, guanine)
fA, fU, fC, fG	2'-fluoro-ribonucleotide (adenine, uracil, cytosine, guanine)
(ps)	phosphorothioate linkage between adjacent nucleotides

In one embodiment there is provided a process for the preparation of conjugate (I), or pharmaceutically acceptable salts of conjugate (I), and the intermediates used in the preparation thereof.

5 Another embodiment is a product obtainable by any of the processes or examples disclosed herein.

MEDICAL AND PHARMACEUTICAL USE

10 Conjugate 2, and pharmaceutically acceptable salts thereof, is believed to be useful in the prevention or treatment of cardiometabolic diseases such as hypertriglyceridemia, obesity, hyperlipidemia. abnormal lipid and/or cholesterol metabolism, atherosclerosis type I diabetes mellitus. cardiovascular disease, coronary artery disease, non alcoholic steatohepatitis, non-alcoholic fatty liver disease, homozygous and heterozygous familial hypercholesterolemia, statin resistant hypercholesterolemia and other metabolic related disorders and diseases in a mammal, particularly a human.

15 Conjugate 2, and pharmaceutically acceptable salts thereof, is believed to be useful in the prevention or treatment of heart failure in a mammal, particularly a human.

 Conjugate 1, and pharmaceutically acceptable salts thereof, is believed to be useful in the prevention or treatment of heart failure in a mammal, particularly a human.

20 Conjugate 2, and pharmaceutically acceptable salts thereof, is believed to be useful in the prevention or treatment of chronic kidney disease in a mammal, particularly a human.

 Conjugate 1, and pharmaceutically acceptable salts thereof, is believed to be useful in the prevention or treatment of chronic kidney disease in a mammal, particularly a human.

25 For the avoidance of doubt, as used herein, the term “treatment” includes therapeutic and/or prophylactic treatment.

 When a compound or salt described herein is administered as therapy for treating a disorder, a “therapeutically effective amount” is an amount sufficient to reduce or completely alleviate symptoms or other detrimental effects of the disorder, cure the disorder, reverse,

completely stop, or slow the progress of the disorder or reduce the risk of the disorder getting worse.

The compounds described herein are thus indicated both in the therapeutic and/or prophylactic treatment of these conditions.

5 The compounds described herein have the advantage that they may be more efficacious, be less toxic, be more selective, be more potent, produce fewer side effects, be more easily absorbed, and/or have a better pharmacokinetic profile (e.g. higher oral bioavailability and/or lower clearance), than compounds known in the prior art.

For the above-mentioned therapeutic indications, the dosage administered will vary
10 with the compound employed, the mode of administration and the treatment desired. However, in general, satisfactory results are obtained when the compounds are administered at a dosage of the solid form of between 1 mg and 2000 mg per day.

Conjugate 2, and pharmaceutically acceptable salts thereof, may be used on their own, or in the form of appropriate pharmaceutical compositions in which the compound or
15 derivative is in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier. Thus, another aspect concerns a pharmaceutical composition comprising Conjugate 2, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

Conjugate 1, and pharmaceutically acceptable salts thereof, may be used on their own,
20 or in the form of appropriate pharmaceutical compositions in which the compound or derivative is in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier. Thus, another aspect concerns a pharmaceutical composition comprising Conjugate 1, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

25 In one embodiment there is provided Conjugate 2, or a pharmaceutically acceptable salt thereof for use in therapy, especially in the prevention or treatment of cardiometabolic diseases such as hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis type I diabetes mellitus, cardiovascular disease, coronary artery disease, non alcoholic steatohepatitis, non-alcoholic fatty liver disease,
30 homozygous and heterozygous familial hypercholesterolemia, statin resistant hypercholesterolemia and other metabolic related disorders and diseases in a mammal, particularly a human.

In one embodiment there is provided Conjugate 2, or a pharmaceutically acceptable salt thereof for use in therapy, especially in the prevention or treatment of heart failure in a mammal, particularly a human.

5 In one embodiment there is provided Conjugate 1, or a pharmaceutically acceptable salt thereof for use in therapy, especially in the prevention or treatment of heart failure in a mammal, particularly a human.

In one embodiment there is provided Conjugate 2, or a pharmaceutically acceptable salt thereof for use in therapy, especially in the prevention or treatment of chronic kidney disease in a mammal, particularly a human.

10 In one embodiment there is provided Conjugate 1, or a pharmaceutically acceptable salt thereof for use in therapy, especially in the prevention or treatment of chronic kidney disease in a mammal, particularly a human.

These and other embodiments are described in greater detail herein below, where
15 further aspects will be apparent to one skilled in the art from reading this specification.

COMBINATION THERAPY

Conjugate 2, or pharmaceutically acceptable salts thereof, may also be administered in conjunction with other compounds used for the treatment of the above conditions.

20 In another embodiment, there is a combination therapy wherein Conjugate 2, or a pharmaceutically acceptable salt thereof, and a second active ingredient are administered concurrently, sequentially or in admixture, for the treatment of one or more of the conditions listed above. Such a combination may be used in combination with one or more further active ingredients.

25 When used in a combination therapy, it is contemplated that Conjugate 2, or pharmaceutically acceptable salts thereof, and the other active ingredients may be administered in a single composition, completely separate compositions, or a combination thereof. It also is contemplated that the active ingredients may be administered concurrently, simultaneously, sequentially, or separately. The particular composition(s) and dosing
30 frequency(ies) of the combination therapy will depend on a variety of factors, including, for example, the route of administration, the condition being treated, the species of the patient, any potential interactions between the active ingredients when combined into a single

composition, any interactions between the active ingredients when they are administered to the animal patient, and various other factors known to physicians (in the context of human patients), veterinarians (in the context of non-human patients), and others skilled in the art.

PHARMACEUTICAL COMPOSITIONS

5 There is provided a method of treating a disease associated with ANGPTL3 comprising administering to a subject having a disease associated with ANGPTL3 a therapeutically effective amount of an ANPTL3 siRNA agent, thereby treating the disease associated with ANGPTL3.

 Typically, the pharmaceutical composition further comprises a pharmaceutically
10 acceptable diluent or carrier. The pharmaceutically acceptable diluent may be water or saline. A pharmaceutical composition may comprise or consist of a sterile saline solution and an ANPTL3 siRNA agent, which comprises an antisense strand having a nucleobase sequence complementary to a ANGPTL3 target nucleic acid. The sterile saline is preferably pharmaceutical grade saline.

15 A pharmaceutical composition may comprise or consist of an ANPTL3 siRNA agent, which comprises an antisense strand having a nucleobase sequence complementary to a ANGPTL3 target nucleic acid and sterile water. Preferably the sterile water is pharmaceutical grade water, e.g., water for injection. The saline may be phosphate-buffered saline (PBS), preferably sterile PBS.

20 A pharmaceutical composition may comprise an ANPTL3 siRNA agent, which comprises an antisense strand having a nucleobase sequence complementary to a ANGPTL3 target nucleic acid and one or more excipients. Excipients may be selected from water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylase, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose and
25 polyvinylpyrrolidone.

 In certain embodiments, an ANPTL3 siRNA agent, which comprises an antisense strand having a nucleobase sequence complementary to a ANGPTL3 target nucleic acid, may be admixed with pharmaceutically acceptable active and/or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods
30 for the formulation of pharmaceutical compositions depend on a number of criteria,

including, but not limited to, route of administration, extent of disease, or dose to be administered.

Pharmaceutical compositions comprising an ANGPTL3 siRNA agent, which comprises an antisense strand having a nucleobase sequence complementary to a
5 ANGPTL3 target nucleic acid, encompass any pharmaceutically acceptable salts of the ANPTL3 siRNA agent. Pharmaceutical compositions comprising an ANPTL3 siRNA agent, which comprises an antisense strand having a nucleobase sequence complementary to a ANGPTL3 target nucleic acid, upon administration to an animal, including a human, are typically capable of providing (directly or indirectly) the biologically active metabolite
10 or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of an ANGPTL3 siRNA agent, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

A pharmaceutical composition may comprise a co-solvent system. Certain of such
15 co-solvent systems comprise, for example, benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. In certain embodiments, such co-solvent systems are used for hydrophobic compounds. A non-limiting example of such a co-solvent system is the VPD co-solvent system, which is a solution of absolute ethanol comprising 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant Polysorbate 80™ and 65% w/v
20 polyethylene glycol 300. The proportions of such co-solvent systems may be varied considerably without significantly altering their solubility and toxicity characteristics. Furthermore, the identity of co-solvent components may be varied: for example, other surfactants may be used instead of Polysorbate 80™; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g.,
25 polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose.

A pharmaceutical composition may be prepared for administration by injection (e.g., intravenous, subcutaneous, etc.). In certain of such embodiments, a pharmaceutical composition comprises a carrier and is formulated in aqueous solution, such as water or physiologically compatible buffers such as Hanks's solution, Ringer's solution, or
30 physiological saline buffer. In certain embodiments, other ingredients are included (e.g., ingredients that aid in solubility or serve as preservatives). In certain embodiments, injectable suspensions are prepared using appropriate liquid carriers, suspending agents and

the like. Certain pharmaceutical compositions for injection are presented in unit dosage form, e.g., in ampoules or in multi-dose containers. Certain pharmaceutical compositions for injection are suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

- 5 Certain solvents suitable for use in pharmaceutical compositions for injection include, but are not limited to, lipophilic solvents and fatty oils, such as sesame oil, synthetic fatty acid esters, such as ethyl oleate or triglycerides, and liposomes.

Under certain conditions, certain compounds disclosed herein act as acids. Although such compounds may be drawn or described in protonated (free acid) form, or ionized and
10 in association with a cation (salt) form, aqueous solutions of such compounds exist in equilibrium among such forms. For example, a phosphate linkage of an oligonucleotide in aqueous solution exists in equilibrium among free acid, anion and salt forms. Unless otherwise indicated, compounds described herein are intended to include all such forms. Moreover, certain oligonucleotides have several such linkages, each of which is in
15 equilibrium. Thus, oligonucleotides in solution exist in an ensemble of forms at multiple positions all at equilibrium. The term "oligonucleotide" is intended to include all such forms. Drawn structures necessarily depict a single form. Nevertheless, unless otherwise indicated, such drawings are likewise intended to include corresponding forms. Herein, a structure depicting the free acid of a compound followed by the term "or a salt thereof"
20 expressly includes all such forms that may be fully or partially protonated/de-protonated/in association with a cation. In certain instances, one or more specific cation is identified.

An ANGPTL3 siRNA agent, which comprises an antisense strand having a nucleobase sequence complementary to a ANGPTL3 target nucleic acid, may be in aqueous solution with sodium. An ANGPTL3 siRNA agent, which comprises an antisense strand having a
25 nucleobase sequence complementary to a ANGPTL3 target nucleic acid, may be in aqueous solution with potassium. An ANGPTL3 siRNA agent, which comprises an antisense strand having a nucleobase sequence complementary to a ANGPTL3 target nucleic acid, may be in PBS. An ANGPTL3 siRNA agent, which comprises an antisense strand having a nucleobase sequence complementary to a ANGPTL3 target nucleic acid, may be in water. The pH of the
30 solution may be adjusted with NaOH and/or HCl to achieve a desired pH.

PREPARATION OF THE COMPOUNDS

Example 1

Conjugate 1 (WO2019/055633: AD05488 (guide: AM07235-AS, passenger: AM07234-SS)):

Sense (passenger): NAG-invAb (ps) mG mC mU mC mA mA mC mA fU fA fU mU mU mG
mA mU mC mA mG mU mA (ps) invAb (SEQ ID NO: 10)

- 5 Antisense (guide): mU (ps) fA (ps) mC (ps) fU mG fA mU fC mA fA mA fU mA fU mG fU
mU fG mA fG (ps) mC (SEQ ID NO: 3)

SEQ ID NO: 2 invAb (ps) mG mC mU mC mA mA mC mA fU fA fU mU mU mG mA mU
mC mA mG mU mA (ps) invAb

- 10 **SEQ ID NO: 3** mU (ps) fA (ps) mC (ps) fU mG fA mU fC mA fA mA fU mA fU mG fU mU
fG mA fG (ps) mC

Single strands synthesis, Example 1

- All synthetic reactions were performed under an inert atmosphere, unless otherwise stated. In
the following examples, when the source of the starting products is not specified, it should be
15 understood that said products are known compounds (e.g., commercially available
compounds from suppliers such as Sigma-Aldrich) and/or may be prepared according to
known methods, e.g. as described in the literature and patent publications described herein.

- Nucleotide phosphoramidites were purchased from Sigma-Aldrich or WuXi. Linker
phosphoramidites were purchased from Glen Research or WuXi. The 5'-amino-modifier C6
20 was obtained from GlenResearch. UV purities were determined using ion-pairing LCMS and
are stated at 260 nm. Yields are given based on the initial resin loading and oligonucleotide
content of the final product, as calculated from UV absorption.

General synthetic procedure, Example 1

This procedure was used unless otherwise indicated.

- 25 Oligonucleotides **SEQ ID NO: 2, 3** were synthesized on a 10 μ mol scale on a K&A system
using CUTAG CPG support (Sigma-Aldrich, 25-35 μ mol/g).

Phosphoramidites were dissolved to a final concentration to 0.1 M (3 equivalents) in DNA
grade acetonitrile (ACN) prior to use, except for the GalNAc phosphoramidite, dissolved at

0.2 M in dry DCM. Detritylation was performed using 3 vol-% dichloroacetic acid in DCM (contact time 5 x 35 s). Di- and tri-antennary linkers were deprotected using double detritylation. Activator BTT was used as activating agent (0.3 M in ACN) for the couplings.

Recirculation times of phosphoramidites were 10 min (single coupling) for all 2'-modified building blocks and 4*10min (quadruple coupling) for GalNAc phosphoramidite (SEQ ID no 1). DDTT was dissolved in pyridine (0.2 M) and used as thiolation reagent with a contact time of 5 min. Oxidizer solution was purchased from Sigma-Aldrich and used as such with a contact time of 9 s. Equal volumes of Cap A (9.1 vol-% acetic anhydride in tetrahydrofuran (THF)) and Cap B (THF/*N*-methylimidazole/ pyridine 80:10:10 vol-%) were mixed in situ for capping (contact time 50 s). Cyanoethyl backbone removal was performed with 20 vol-% diethylamine in ACN (contact time 7 x 1 min) after the synthesis complete. Oligonucleotides were cleaved from the solid support and further deprotected by treatment with ammonia (aq. 40%) solution (10 mL) at 55 °C for 18 h, and subsequently dried on a Speedvac.

15 Purification procedure, Example 1

The single strand oligonucleotides were purified using either ion-pairing HPLC or reverse phase HPLC.

Identity and purity of oligonucleotides was confirmed by liquid chromatography-mass spectrometry (LC-MS) using an Acquity I-class LC system, equipped with a PDA and coupled to an RDa via heated electrospray (BioAccord) by the Waters Corporation. Analytical runs were performed on an ACQUITY PREMIER BEH C18 1.7µm 2.1x100mm (130 Å) column at 80 °C, using a gradient of 35-50% B (100% ACN) in A (10 mM TBAA in 10% ACN/90% H₂O) over 10 min (flow rate 0.5 mL/min).

Oligonucleotide **SEQ ID NO 2** was purified using RP HPLC (XBridge C18, 5µm 19x150mm, A: 50 mM NH₄HCO₃ in water (pH8), B: ACN), gradient 5-12% B 16 min, 12-30% B 3 min, the repurified using same column and A:60 mM DBuAA in (H₂O/ACN 95/5)pH7.0, B:60 mM DBuAA in ACN, gradient 10-40% in 12min, 40-80% in 5min, UV purity obtained is 95.0% ; *m/z* (ESI-;TOF) calcd 2158.91 (4-); found: 2158.91

Oligonucleotide **SEQ ID NO 3** was purified using RP HPLC (XBridge C18, 5µm 19x150mm, A:60 mM DBuAA in (H₂O/ACN 95/5), pH 7.0, B:60 mM DbuAA in ACN, gradient 20-50%

in 12 min, 50-80% in 0.5min, UV purity obtained is 90.2% ; m/z (*ESI-;TOF*) calcd 1728.22 (4-); found: 1728.23

Double strands siRNA synthesis - annealing, Example 1

- 5 The amounts of the single strands were quantified by UV spectrophotometry at 260 nm and the solutions normalized to 2 mM using calculated extinction coefficients.

For siRNA compounds, single strands were mixed in equal molar amounts, warmed to 95°C for 5 min and then left to cool down to room temperature over 1 h.

- Double stranded siRNAs were analyzed using UPLC-MS method. Analyses of double
10 stranded siRNAs were performed at ACQUITY PREMIER BEH Amide 1.7 μ m 2.1x100mm, 300 Å (30°C, flow 0.5ml/min) using gradient: 15-60% B in 5 min, where A: 25 mM ammonium acetate in 70/30 ACN/water, B: 25 mM ammonium acetate in 30/70 ACN/water.

The final siRNAs were freeze-dried and redissolved in 1X PBS to a concentration of 10 mg/mL.

- 15 Conjugate 1: Theoretical mass: 15564.6 Deconvolved mass found: 15564.7

Example 2

Conjugate 2:

- Sense (passenger): [M]-mC mU mA mC mA mU fA fU fA mA mA mC mU mA mC mA mA
20 (ps) mG (ps) mU (SEQ ID NO: 11)

Antisense (guide): mA (ps) fC (ps) mU fU mG fU mA fG mU fU mU fA mU fA mU fG mU
(ps) fA (ps) mG (SEQ ID NO: 5)

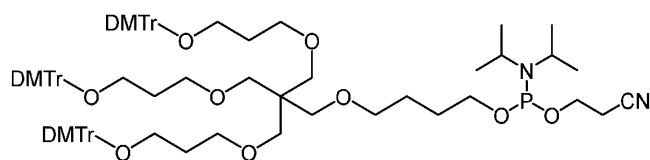
- SEQ ID NO: 4 mC mU mA mC mA mU fA fU fA mA mA mC mU mA mC mA mA (ps)
25 mG (ps) mU

SEQ ID NO: 5 mA (ps) fC (ps) mU fU mG fU mA fG mU fU mU fA mU fA mU fG mU (ps)
fA (ps) mG

Building block synthesis, Example 2

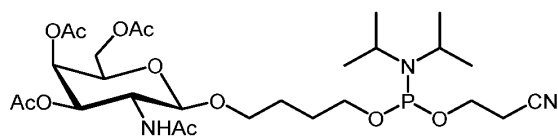
Synthesis of the phosphoramidite derivatives of [M], (ST41-phos) as well as ST23 (ST23-phos) and their precursor compounds can be performed as described in WO2017/174657.

ST41-phos:



ST41-phos

5 ST23-phos:



ST23-phos

Synthesis of Oligonucleotides, Example 2

Oligonucleotides were synthesized on an AKTA oligopilot 100 synthesizer using standard
10 phosphoramidite chemistry. Commercially available base loaded solid support (Nitto phase HL250, Kinovate, CA), 2'OMe nucleotide phosphoramidites and 2'F nucleotide phosphoramidites (all standard protection. ChemGenes) were used according to the manufacturers recommended procedures.

Ancillary reagents were purchased from EMP Biotech. Synthesis was performed using a
15 0.1 M solution of the phosphoramidite in dry acetonitrile and benzylthiotetrazole (BTT) was used as activator (0.3M in acetonitrile). Coupling time was 10 min. A Cap/OX/Cap or Cap/Thio/Cap cycle was applied (Cap: Ac₂O/NMI/Lutidine/Acetonitrile. Oxidizer: 0.1M I₂ in pyridine/H₂O). Phosphorothioates were introduced using 0.2M XH (0.2 M Xanthane hydride in pyridine). DMT cleavage was achieved by treatment with 3% dichloroacetic acid in
20 toluene. Upon completion of the programmed synthesis cycles a diethylamine (DEA) wash was performed. All other reagents and solvents were commercially available and used in standard reagent quality.

All oligonucleotides were synthesized in DMT-off mode. The single strands were cleaved off the support by 40% aq. methylamine treatment (90min, RT). The resulting crude

oligonucleotide was concentrated under reduced pressure to a concentration of ~10 mg/mL and purified by ion exchange chromatography (SourceQ, 7.5 mL, GE Healthcare) on an AKTA Pure HPLC System using a sodium chloride gradient. Product containing fractions were pooled, desalted by UFDF (Sartorius Sartoflow Smart, Sartoclon Slice 200 Hydrosart 2k, 5 10 times volume exchange against sterile water).

To prove their purity all final single stranded products were analysed by AEX-HPLC (DNA Pac PA200 4.0x250 mm & DNAPac PA200 Guard 4x50 mm at 80 °C, using a gradient of 25-70% B (10 % MeCN, 20 mM TRIS, 0.4 M LiClO₄ in water, pH =7.4) in A (10 % MeCN, 20 mM TRIS in water) over 10 min, flow rate 1 mL/min). Purity is given in %FLP (% full length product) which is the percentage of the UV-area under the assigned product signal in the UV-trace of the AEX-HPLC analysis of the final product. Identity of the respective single stranded products was confirmed by UPLC-MS analysis (Acquity UPLC BEH C18 2.1x50 mm, 1.7 µm Acquity BEH C18 1.7µm Vanguard Pre-Col at 60°C, using a gradient of 10-22% B (100 mM HFIP, 15 mM TEA in MeOH) in A (100 mM HFIP, 15 mM TEA, 5% MeOH in 15 water) over 5 min, flow rate 0.3 mL/min).

Single stranded oligonucleotides:

Name	MW. calc. (free acid)	MW (ESI-), found	%FLP (AEX-HPLC)
SEQ ID NO: 4	6247.85 Da	6248.0 Da	97.5
SEQ ID NO: 5	7834.46 Da	7835.0 Da	94.9

Double strand formation, Example 2

Individual single strands were dissolved in a concentration of 60 OD/mL in H₂O. Both 20 individual oligonucleotide solutions were added together in a reaction vessel. A titration was performed for easier reaction monitoring. The first strand was added in 25% excess over the second strand as determined by UV-absorption at 260 nm. The reaction mixture was heated to 80 °C for 5 min and then slowly cooled to RT. Double strand formation was monitored by ion pairing reverse phase HPLC (X Bridge BEH C18 2.1x50 mm 2.5µm, XP VanGuard 25 Cartridges at 15 °C, using a gradient of 5-45% B (100 mM HFIP, 15 mM TEA in MeOH) in A (100 mM HFIP, 15 mM TEA, 5% MeOH in water) over 15 min, flow rate 0.3 mL/min).

From the UV-area of the residual single strand the needed amount of the second strand was calculated and added to the reaction mixture. The reaction was heated to 80°C again and

slowly cooled to RT. This procedure was repeated until less than 10% of residual single strand was detected.

Nucleic acid conjugates:

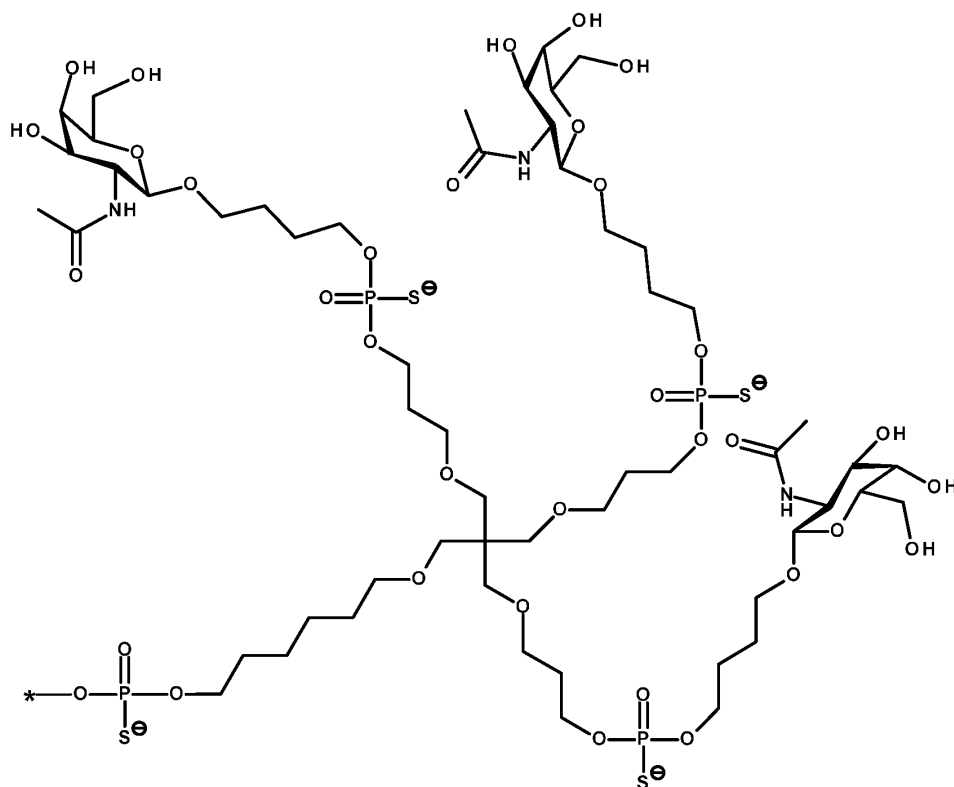
siRNA	% double strand
Conjugate 2	93.3

5

Abbreviations used in the Examples:

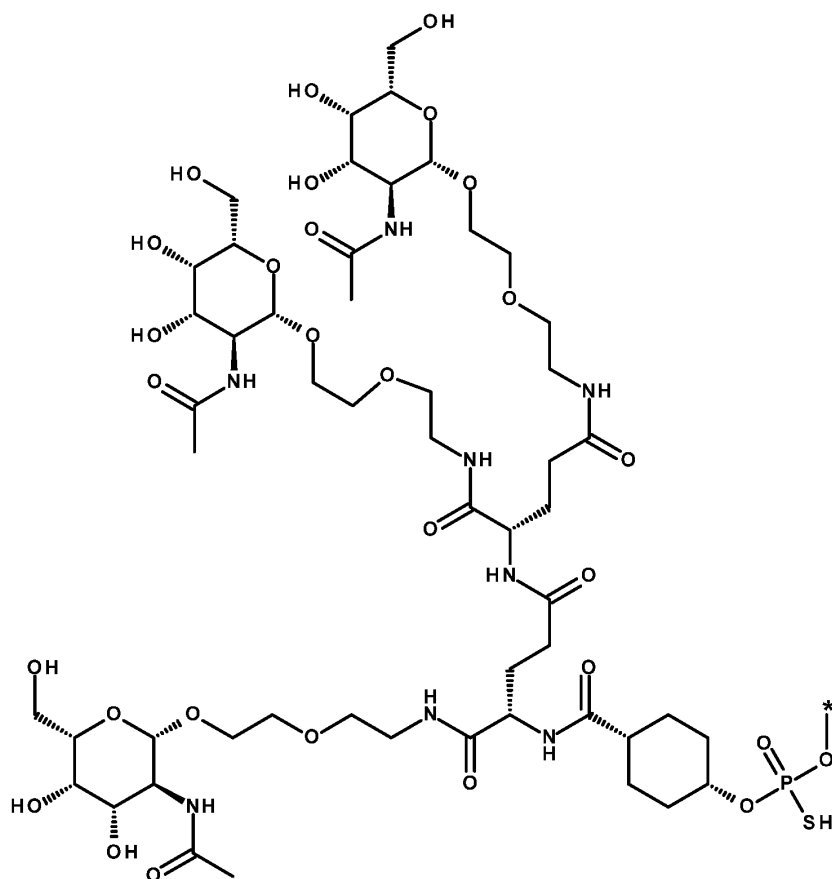
Abbreviation	Meaning
mA, mU, mC, mG	2'-O-methyl ribonucleotide (adenine, uracil, cytosine, guanine)
fA, fU, fC, fG	2'-fluoro-ribonucleotide (adenine, uracil, cytosine, guanine)
(ps)	phosphorothioate linkage between adjacent nucleotides
invAb	abasic deoxyribose
T	Thymine

[M] is:



[M] is bonded via the “free” bond indicated “*”,

NAG is:



NAG is bonded via the “free” bond indicated “*”.

5 Example 3

In vitro test of GalNac-conjugated siRNAs for inhibition of ANGPTL3 mRNA expression in primary hepatocytes

List of abbreviations for Example 3:

%	Percent
μM	Micromolar
A	Adenine
ANGPTL3	Angiopoietin-like Protein 3

C	Cytosine
c	Sequence identity: cynomolgus
Ctr	Non-targeting siRNA control
cyno	Cynomolgus monkey
DMSO	Dimethyl sulfoxide
G	Guanine
GalNAc	N-Acetylgalactosamine
h	Sequence identity: human
LPR	Lower primer for RT-PCR
mg	Milligrams
ml	Milliliter
nM	Nanomolar
nt	Nucleotides
PPIB	Peptidyl-prolyl cis-trans isomerase B
PRB	Fluorescently labelled probe for RT-PCR
RNA	Ribonucleic acid
RNAi	RNA interference
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SD	Standard deviation
siRNA	Small interfering RNA
T	Thymine
U	Uracil
UPR	Upper primer for PCR
UT	Untreated
v/v	Volume per volume

Test Items

GalNAc-conjugated siRNAs tested were:

Conjugate 1 (**Example 1**) and Conjugate 2 (**Example 2**).

5

Cell culture

Primary human hepatocytes (Lot Hu8264) and cynomolgus hepatocytes (crab-eating macaque, *Macaca fascicularis*, Lot CY411) were sourced from Life Technologies. As described by the manufacturer, primary hepatocytes were thawed and plated in Williams' E medium (Life Technologies), supplemented with 5 % fetal bovine serum, 1 µM Dexamethasone in DMSO (final concentration of DMSO = 0.01 %) and 3.6 % v/v of Thawing/Plating Cocktail-A (Thermo Fisher Scientific, CM3000) at 37 °C in a 5 % CO₂ atmosphere.

Treatment of primary hepatocytes with GalNac-conjugated siRNAs

Primary hepatocytes were seeded into collagen I-coated 96-well plates (Life Technologies) at a density of 35,000 cells and 45,000 cells per well for human and cynomolgus cells, respectively. GalNac-conjugated siRNAs Conjugate 1 and Conjugate 2 were serially diluted to achieve final concentrations of 100, 20, 4, 0.8 and 0.16 nM for human cells, and 100, 10, 1, 0.1 and 0.01 nM for cynomolgus cells in the previously defined media in triplicates. Plates were then incubated at 37 °C in a 5 % CO₂ atmosphere for 24 hours. Subsequently, cells were lysed, and RNA was isolated from the cells as described in section 4.4.

Quantitative RT-PCR

Twenty-four hours post treatment with GalNac conjugated siRNAs Conjugate 1 and Conjugate 2, total RNA was extracted using the InviTrap HTS 96-well kit (Invitex Molecular GmbH, Berlin, Germany) according to the manufacturer's instructions and eluted in 2x 30 µl of elution buffer. Eluted RNA was stored at -80 °C until analysis by qPCR. For qPCR, 10 µl of RNA were mixed with Takyon 5x Master Mix (Eurogentec, Seraing, Belgium) and primer/probe sets against either ANGPTL3 or PPIB (BioTez GmbH, Berlin, Germany or Eurogentec, Seraing, Belgium) as listed in Table 1:

Table 1: Sequences of primers and probes used for RT-qPCR.

Name	Primer/ Probe	SEQ ID NO:	Sequence 5' to 3'
hANL3	UPR	12	AGAGCACCAAGAACTACTCCCTTTC
	PRB	13	CATGATGGCATTCTGCTGAATGTACCA

	LWR	14	TGCCACTTGTATGTTACCTCTGT
cANL3	UPR	12	AGAGCACCAAGAACTACTCCCTTTC
	PRB	13	CATGATGGCATTCTGCTGAATGTACCA
	LWR	15	ACGTGCCACTTATATGTTACCTCT
hcPPIB	UPR	16	AGATGTAGGCCGGGTGATCTTT
	PRB	17	TGTTCCAAAAACAGTGGATAATTTTGTGGCC
	LWR	18	GTAGCCAAATCCTTTCTCTCCTGT

The RT-qPCR reactions were carried out at 20 ml per sample using a QuantStudio 6 Flex
cycler (Applied Biosystems, part of Thermo Fisher Scientific, Massachusetts, USA) using
standard protocols for RT-qPCR (48 °C 30 min, 95 °C 3 min, 40 cycles at 95 °C 15 s
5 followed by 60 °C 1 min).

The data were calculated by using the comparative C_T method also known as the 2^{-DDC_T}
method (Livak and Schmittgen, 2001 and Schmittgen and Livak, 2008). In brief, the amount
of ANGPTL3 mRNA was normalized to the endogenous housekeeping gene PPIB followed
by untreated control cells according to the following formula:

10
$$\text{Fold-change} = 2^{-\Delta\Delta C_T}.$$

Data is presented as means of three replicates ± SD.

Results

Unless stated otherwise, all values presented in the text refer to mean ± standard deviation
(SD). Calculations of some tabular presentations were carried out using standard spreadsheet
15 software (Graph Pad Prism 9, Microsoft® EXCEL®). A higher number of digits than tabulated
were used for the calculation of some parameters. Therefore, recalculation of parameters from
tabulated data may, in some instances, lead to minor variations. Since the original raw data is
unaltered and thus numerically correct, this occurrence is not significant for the outcome of
the study.

20 To estimate the impact of Conjugate 1 and Conjugate 2 on ANGPTL3 mRNA levels *in vitro*,
primary human and cynomolgus hepatocytes were treated at five different concentrations for
24 hours, followed by isolation of RNA and quantification of remaining ANGPTL3 transcript
levels by qPCR. Untreated cells were used for normalization. Experimental results are shown
in **Figure 1** for human primary hepatocytes and in **Figure 2** for cynomolgus hepatocytes.

As illustrated in **Figure 1**, Conjugate 2 induced a dose-dependent knockdown of ANGPTL3 in human hepatocytes, exceeding a 50% knockdown at 100 nM, whereas Conjugate 1 exceeded 50% at both 100 and 20 nM. The maximum knockdown measured in this experiment was $59 \pm 3\%$ and $60.3 \pm 2\%$ for Conjugate 2 and Conjugate 1, respectively.

5 In cynomolgus monkey hepatocytes (**Figure 2**), tested compounds appeared more potent in general, which is in line with previous observations (data not shown), resulting in a 10-fold dilution series employed in this experiment. 50% knockdown levels were exceeded at 1 nM for Conjugate 2, and 10 nM for Conjugate 1. Maximum knockdown levels were observed at
10 100 nM in both cases ($85.4 \pm 1\%$ for Conjugate 2 vs. $80.4 \pm 1\%$ for Conjugate 1). Data is presented as means of three replicates $\pm$ SD. Corresponding gene expression levels are depicted in Table 2.

Table 2: ANGPTL3 mRNA expression levels in primary hepatocytes normalized to PPIB mRNA

	Human hepatocytes			Cynomolgus hepatocytes		
siRNA	siRNA (conc.)	2 ^{-DDCt}	SD 2 ^{-DDCt}	siRNA (conc.)	2 ^{-DDCt}	SD 2 ^{-DDCt}
untreated		1.00	0.12		1.00	0.17
Conjugate 2	100nM	0.41	0.03	100nM	0.15	0.01
	20nM	0.57	0.04	10nM	0.16	0.03
	4nM	0.75	0.07	1nM	0.34	0.03
	0.8nM	1.05	0.13	0.1nM	0.66	0.06
	0.16nM	0.97	0.09	0.01nM	0.82	0.06
Conjugate 1	100nM	0.40	0.02	100nM	0.22	0.01
	20nM	0.46	0.01	10nM	0.35	0.01
	4nM	0.62	0.05	1nM	0.65	0.27
	0.8nM	0.80	0.09	0.1nM	0.99	0.09
	0.16nM	0.83	0.07	0.01nM	0.97	0.02

Reference compounds Conjugate 3 and Conjugate 4

Conjugate 3 (WO2012/177784: AD-52988.1 (guide: A-108397.1, passenger A-108396.1):

Sense (passenger): fU mA fC mA fU mA fU mA fA fA fC mU fA mC fA mA fG mU fC mA

5 fA-L96 (SEQ ID NO: 19)

Antisense (guide): mU fU mG fA mC fU mU fG mU fA mG mU mU fU mA fU mA fU mG

fU mA (ps) fG (ps) mU (SEQ ID NO: 7)

SEQ ID NO: 6 fU mA fC mA fU mA fU mA fA fA fC mU fA mC fA mA fG mU fC mA fA

10 SEQ ID NO: 7 mU fU mG fA mC fU mU fG mU fA mG mU mU fU mA fU mA fU mG fU
mA (ps) fG (ps) mU

Conjugate 4 (WO2012/177784: AD-45887.1 (guide: A-96144.1, passenger: A-96143.1):

Sense (passenger): mC mU fA mC fA mU fA mU fA fA fA mC mU fA mC fA fA fG mU dT

15 (ps) dT-L96 (SEQ ID NO: 20)

Antisense (guide): fA fC fU fU fG mU fA fG fU fU mU fA mU fA fU fG mU fA fG dT (ps)

dT (SEQ ID NO: 9)

SEQ ID NO: 8 mC mU fA mC fA mU fA mU fA fA fA mC mU fA mC fA fA fG mU dT

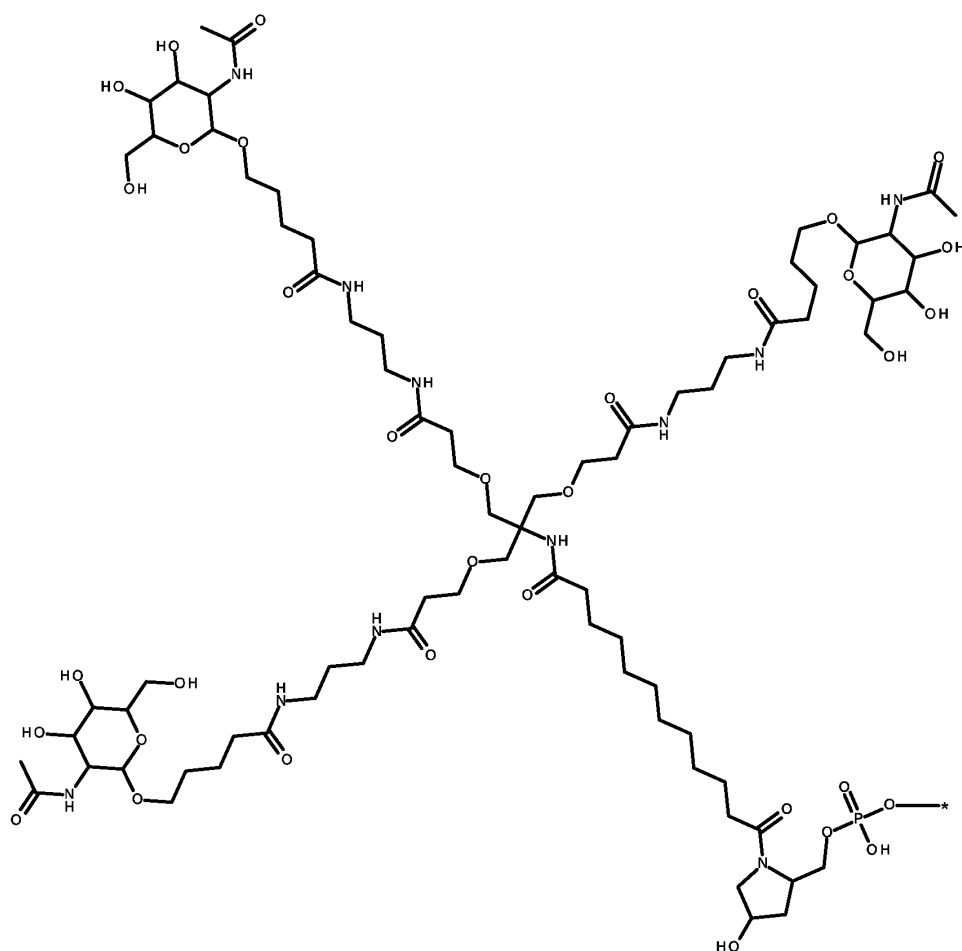
20 (ps) dT

SEQ ID NO: 9 fA fC fU fU fG mU fA fG fU fU mU fA mU fA fU fG mU fA fG dT (ps) dT

In the context of the above formula, the following abbreviations are used:

Abbreviation	Meaning
mA, mU, mC, mG	2'-O-methyl ribonucleotide (adenine, uracil, cytosine, guanine)
fA, fU, fC, fG	2'-fluoro-ribonucleotide (adenine, uracil, cytosine, guanine)
dT	2'-deoxythymidine
(ps)	phosphorothioate linkage between adjacent nucleotides

L96 is a triantennary ligand moiety having the structure:



Thus, the terminal phosphorothioate group of the ligand moiety L96 is bonded directly (via
5 the “free” bond indicated “*”) to the 3’ position of the 3’ terminal nucleotide of the first
strand.

Single strands synthesis

All synthetic reactions were performed under an inert atmosphere, unless otherwise stated. In
10 the following examples, when the source of the starting products is not specified, it should be

understood that said products are known compounds (e.g., commercially available compounds from suppliers such as Sigma-Aldrich) and/or may be prepared according to known methods, e.g. as described in the literature and patent publications described herein.

Nucleotide phosphoramidites were purchased from Sigma-Aldrich or WuXi. Linker phosphoramidites were purchased from Glen Research or WuXi. The 5'-amino-modifier C6 was obtained from GlenResearch. UV purities were determined using ion-pairing LCMS and are stated at 260 nm. Yields are given based on the initial resin loading and oligonucleotide content of the final product, as calculated from UV absorption.

General synthetic procedure

10 This procedure was used unless otherwise indicated.

Oligonucleotides **SEQ ID NO: 7, 9** were synthesised on an ÄKTA OligoPilot Plus 10 synthesizer (GE Healthcare), on a 32 µmole scale, using a standard synthesis cycle of detritylation (3% dichloroacetic acid in toluene), coupling (coupling agent: 0.25 M 5-[3,5-Bis(trifluoromethyl)phenyl]-1H-tetrazole solution in acetonitrile), capping (Cap A: 20% *N*-methylimidazole and 80% acetonitrile; Cap B: 20% pyridine, 20% acetic anhydride and 60% acetonitrile), oxidation (0.05 M iodine in pyridine and water) or thiolation (0.2 M Xanthane hydride in pyridine), and solid supports (UNY Primer Support 5G ~353 µmole/g, GE Healthcare). All fully protected β-cyanoethyl phosphoramidite monomers were dissolved in anhydrous acetonitrile (0.1 M) under argon immediately prior to use. The phosphoramidite recirculation time/coupling time for DNA monomers was 5 min and was extended to 10 min for RNA monomers. Stepwise coupling efficiencies and overall yields were determined by automated trityl cation absorption monitoring exceeding 98% for all oligonucleotides synthesized. At the end of solid phase synthesis, the solid-support bound oligonucleotides were treated with ammonia (aq. 40%) solution (10 mL) at 55 °C for 18 h, and subsequently dried on a Speedvac.

Oligonucleotides **SEQ ID NO: 6, 8** were synthesised on an ÄKTA OligoPilot Plus 10 synthesizer (GE Healthcare) on a 13 µmole scale, using a standard synthesis cycle described above and solid support Nitto Phase (loading 0.153mmol/g) preloaded with L96 GalNac.

30 Purification procedure

The single strand oligonucleotides were purified using either ion-pairing HPLC or reverse phase HPLC.

Identity and purity of oligonucleotides was confirmed by liquid chromatography-mass spectrometry (LC-MS) using an Acquity I-class LC system, equipped with a PDA and coupled to an RDa via heated electrospray (BioAccord) by the Waters Corporation. Analytical runs were performed on an ACQUITY PREMIER BEH C18 1.7 μ m 2.1x100mm (130 Å) column at 80 °C, using a gradient of 35-50% B (100% ACN) in A (10 mM TBAA in 10% ACN/90% H₂O) over 10 min (flow rate 0.5 mL/min).

Oligonucleotide **SEQ ID NO 6** was purified using RP HPLC (XBridge C18, 5 μ m 19x150mm, A: 50 mM NH₄HCO₃ in water (pH8), B: ACN), gradient 5% B for 1 min, 5-20% B in 11 min, 20-90% B in 3 min, UV purity obtained is 94% ; *m/z* (ESI-;TOF) calcd 1717.19 (5-); found: 1718.0

Oligonucleotide **SEQ ID NO 7** was purified using IP HPLC (XBridge C18, 5 μ m 19x150mm, A:60 mM DBuAA (H₂O/ACN 95/5) pH7, B:60 mM DBuAA in ACN), gradient 5% B for 1min, 20-40% B in 12min, 50-80% in 0.5 min, UV purity obtained is 95.6% ; *m/z* (ESI-;TOF) calcd 1879.0 (4-); found: 1878.98

Oligonucleotide **SEQ ID NO 8** was purified using RP HPLC (XBridge C18, 5 μ m 19x150mm, A: 50 mM NH₄HCO₃ in water (pH8), B: ACN), gradient 5% B for 1 min, 5-18% B in 11 min, 18-90% B in 3 min, UV purity obtained is 96.5% ; *m/z* (ESI-;TOF) calcd 1709.59 (5-); found: 1710.3

Oligonucleotide **SEQ ID NO 9** was purified using IP HPLC (XBridge C18, 5 μ m 19x150mm, A:60 mM DBuAA (H₂O/ACN 95/5) pH7, B:60 mM DBuAA in ACN), gradient 5% B for 1min, 20-40% B in 12min, 40-80% in 0.5 min, UV purity obtained is 96.3% ; *m/z* (ESI-;TOF) calcd 2243.27 (3-); found: 2243.26

Double strands siRNA synthesis - annealing

The amounts of the single strands were quantified by UV spectrophotometry at 260 nm and the solutions normalized to 2 mM using calculated extinction coefficients.

For siRNA compounds, single strands were mixed in equal molar amounts, warmed to 95°C for 5 min and then left to cool down to room temperature over 1 h.

Double stranded siRNAs were analyzed using UPLC-MS method. Analyses of double stranded siRNAs were performed at ACQUITY PREMIER BEH Amide 1.7µm 2.1x100mm, 300 Å (30°C, flow 0.5ml/min) using gradient: 15-60% B in 5 min, where A: 25 mM ammonium acetate in 70/30 ACN/water, B: 25 mM ammonium acetate in 30/70 ACN/water.

The final siRNAs were freeze-dried and redissolved in 1X PBS to a concentration of 10 mg/mL.

Conjugate 3: Theoretical mass: 16118.8 Deconvolved mass found: 16118.6

Conjugate 4: Theoretical mass: 15293.2 Deconvolved mass found: 15293.1

Example 4

In vivo ANGPTL3 knockdown using AAV-based humanized ANGPTL3 model.

The aim of the study was to profile the in vivo effects of Conjugate 2 in its ability to knockdown (KD) human *ANGPTL3* mRNA in the liver and reduction of circulating ANGPTL3 protein as compared to Conjugates 3 and 4.

List of abbreviations for Example 4:

AAT	Alpha1-antitrypsin
AAV	Associated Adeno Virus
ApoE	Apolipoprotein E
EDTA	Ethylendiaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
siRNA	Small interfering RNA
HDL	High-density lipoprotein
KD	Knockdown
LDL-C	Low Density Lipoprotein – Cholesterol
LPL	Lipoprotein lipase
ORF	Open reading frame

	PBS	Phosphate-buffered saline
	PCR	Polymerase chain reaction
	RNA	Ribonucleic acid
	UTR	Untranslated region
5	VLDL	Very low-density lipoprotein

Test Formulation

	Compounds:	Conjugate 2
		Conjugate 3
10		Conjugate 4
	Vehicle:	PBS
	Stock concentration:	10 mg/mL
	Dosing solution:	0.05, 0.2 and 0.6 mg/mL

15 Test System In Vivo

	Species:	Mouse
	Strain:	C57Bl6N
	Sex:	Females
	Total No of animals:	50
20	Body weight range:	21.0-26.3 grams at study start
	Supplier:	Charles River Laboratory, Germany
	Identification method:	Tail tatoo
	Acclimatisation:	At least 5 days
	Housing conditions:	Upon arrival, mice were group housed in Macrolon L3 cages.
25		Twelve-hour light/dark cycle (lights on at 6 am), 40 to 60 humidity, 20 to 22°C.
	Water:	Bottled tap water, ad lib
	Diet:	Normal chow diet ad lib (R70, Lantmännen, Kimstad, Sweden)/(A40 Safe, Augy, France)
30	Bedding:	Aspen wood chip bedding (Harlan, USA)
	Pre-treatment:	humanANGPTL3 AAV 5E+11gc
	Participation in previous studies:	No

Study Design

Dose(s): 0.25, 1 or 3 mg/kg of Conjugate 2
0.25, 1 or 3 mg/kg of Conjugate 3
5 0.25, 1 or 3 mg/kg of Conjugate 4

Volume(s) of administration: 5 mL/kg

Route(s) and frequency of

Administration: S.c.

Number/group: 5

10

In vivo design

Mice were 8 weeks of age when they arrived the test facility. All mice were put on regular chow diet from the day of arrival and throughout the study. After 6 weeks or 6 days of acclimatization, the mice were injected via the tail vein with AAV. Two weeks after the AAV
15 injection each group was then treated, receiving the assigned treatment via a single subcutaneous injection. Blood samples from the tail vein collected in EDTA glass capillaries (REF#164113 Micro Haematocrit Tubes, Vitrex Medical) were taken on day 0, 7, 22, 30, 42 and 49. Termination was carried out after the last blood sample.

At termination, all animals were anesthetized with isoflurane (Abbot, Stockholm, Sweden)
20 using a vaporizer (Univentor 400, Agnethos, Sweden) and blood was withdrawn from the orbital plexus and collected into tubes containing EDTA as anticoagulant (REF#15.1671.101, Multivette 600K3E, Sarstedt). Blood samples were centrifuged at 10.000 x g for 4 minutes at 4 °C temperature. The resulting plasma was aliquoted and stored at -20°C until further analysis was performed. Subsequently, anesthetized mice were culled by cutting out the heart.
25 The left lateral lobe from the liver was collected and snap frozen in liquid nitrogen and stored at -80°C until subsequent analysis.

AAV-ANGPTL3 generation

The liver-targeted AAV vector genome construct which carries a liver-specific hAAT-hApoE
30 promoter (a chimeric promoter with human apolipoprotein E and alpha-1-antitrypsin gene enhancer/promoter) was previously reported ((Liu, 2023 #499). Human ANGPTL3 open reading frame (ORF) sequence was synthesized by GenScripts, and the ORF was amplified by

PCR using a primer pair 5'-TTTGAATTCGCCACCATGTTTCAACAATTAAGCTCCTTCTT-3' (SEQ ID NO: 21) and 5'-TACATTGCTAGCTCATTCAAAGCTTTCTGAATCTG-3' (SEQ ID NO: 22). The PCR products were then purified using QIAquick PCR Purification Kit, digested with EcoRI and NheI and gel purified using QIAquick Gel Extraction Kit. The digested ANGPTL3 ORF was then cloned into the EcoRI and SpeI sites of AAV-hAAT-hApoE-FGF21 mutant vector plasmid, which led to the introduction of human ANGPTL3 ORF under the control of the liver specific hAAT-hApoE promoter. The resulting plasmid was named as pAAV-hAAT-hApoE-ANGPTL3_noUTRs and the insert was confirmed by sequencing using the following primers.

10	H83 ANGPTL3_L1	TCATGCCTCTTTGCACCATTTC (SEQ ID NO: 23)
	H84 ANGPTL3_L2	AATGGCCTCCTTCAGTTGGG (SEQ ID NO: 24)
	H85 ANGPTL3_L3	GCCAAGAGCACCAAGAACTAC (SEQ ID NO: 25)
	H86 ANGPTL3_L4	CACTTCAACTGTCCAGAGGGT (SEQ ID NO: 26)
	H87 ANGPTL3_R1	TGTCTTGATCAATTCTGGAGGA (SEQ ID NO: 27)
15	H88 ANGPTL3_R2	ATTGGTCTTCCACGGTCTGG (SEQ ID NO: 28)
	H89 ANGPTL3_R3	TGTTTGTTGTCTTCCAGTCTTCC (SEQ ID NO: 29)
	H90 ANGPTL3_R4	CCTTGCTCCATACCACCCC (SEQ ID NO: 30)

The full sequence of pAAV-hAAT-hApoE-ANGPTL3_noUTRs is as follows.

20	pAAV-hAAT-hApoE-ANGPTL3_noUTRs	6623 bp	DNA
	FEATURES	Location/Qualifiers	
	Annotation	143..172	
		/label="Annotation Promoter lac_promoter"	
	misc_feature	237..377	
25		/label="AAV ITR"	
	misc_feature	446..1167	
		/label="hAAT-hApoE prom"	
	misc_feature	1203..1695	
		/label="Human beta globin intron"	
30	CDS	1714..3096	
		/note="ANGPTL3 CDS" – highlighted in bold	
	Terminator	3111..3589	
		/label="Terminator human_growth_hormone_poly_A"	
	Reporter	3749..3909	
35		/label="Reporter lacZ_alpha_fragment"	
	Promoter	4753..4781	
		/label="Promoter ampicillin_resistance_gene(b-lactamase)promoter"	
	Selection	4823..5683	

Origin /label="Selection ampicillin_resistance_gene_ORF
5838..6457
/label="Origin pBR322_replication_origin

5 ORIGIN (SEQ ID NO: 31)
1 agcgcccaat acgcaaacgg cctctccccg cgcgttgccc gattcattaa tgcagctggc
61 acgacagggt tccccactgg aaagcgggca gtgagcgcaa cgcaattaat gtgagttagc
121 tcaactcatta ggcaccccag gctttacact ttatgcttcc ggctcgtagt ttgtgtggaa
181 ttgtgagcgg ataacaattt cacacaggaa acagctatga ccatgattac gaattgacctg
10 241 caggcagctg cgcgctcgct cgctcactga ggccgcccgg gcaaaagccc ggcgctgggc
301 gaccttttgt cgcggggcct cagtgagcga gcgagcgccg agagagggag tggccaactc
361 catcactagg ggttccctatc gatataaagc ttttaatagta atcaattacg gggtcattag
421 ttcatagccc atatatggag ttccgaggct cagaggcaca caggagtctc tgggtccacc
481 ctgccccctt ccaaccccctc agttcccctc ctccagcagc tgtttgtgtg ctgcctctga
15 541 agtccacact gaacaaactt cagcctactc atgtccctaa aatgggcaaa cattgcaagc
601 agcaaacagc aaacacacag cccctccctgc ctgctgacct tggagctggg gcagaggtca
661 gagacctctc tgggcccctg ccacctccaa catccactcg accccttgga atttcgggtg
721 agaggagcag aggttgtcct ggctgtgttt aggtagtgtg agaggggtac ccggggatct
781 tgctaccagt ggaacagcca ctaaggatct tgcagtgaga gcagagggcc agctaagtgg
20 841 tactctccca gagactgtct gactcacgac accccctcca ccttggaacac aggaagctgt
901 ggtttctgag ccaggtacaa tgactccttt cggtaagtgc agtggaagct gtacactgcc
961 caggcaaaagc gtccgggcag cgtaggcggg cgactcagat cccagccagt ggacttagcc
1021 cctgttttgt cctccgataa ctggggtgac cttggttaat attcaccagc agcctcccc
1081 gttgccccctc tggatccact gcttaaatac ggacgaggac agggccctgt ctccctcagt
25 1141 tcaggcacca ccactgacct gggacagtgg tttagtggat atccttaagg gcccagccgg
1201 cccgaatccc ggccgggaac ggtgcattgg aacgcggatt ccccggtcca agagtgcgt
1261 aagtaccgcc tatagagtct ataggccac aaaaaatgct ttctctttt aatatacttt
1321 tttgtttatc ttatttctaa tactttccct aatctcttct tttcagggca ataagtatac
1381 aatgtatcat gctcttttgc accattctaa agaataacag tgataatttc tgggttaagg
30 1441 caatagcaat atttctgcat ataaatattt ctgcatataa attgtaactg atgtaaggag
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1621 ttatcttctc cccacagctc ctgggcaacg tgcgtgtctg tgtgtgtggc catcactttg
1681 gcaaaagaatt gggattctgc agaattctgc accatgttca caattaagct cctcttttt
35 1741 attgttctc tagttatttc ctccagaatt gatcaagaca attcatcatt tgattctcta
1801 totccagagc caaaatcaag atttgctatg ttagacgatg taaaaatttt agccaatggc
1861 ctcccttcagt tgggacatgg tcttaaagac tttgtccata agacgaaggg ccaaaattaat
1921 gacataattc aaaaactcaa catatttgat cagtcttttt atgatctatc gctgcaaac
1981 agtgaatca aagaagaaga aaaggaactg agaagaacta catataaact acaagtcaaa
40 2041 aatgaagagg taaagaatat gtcaactgaa ctcaactcaa aacttgaaag cctcctagaa
2101 gaaaaaatc tacttcaaca aaaagtgaat tatttagaag agcaactaac taacttaatt
2161 caaatcaac ctgaaactcc agaacaccca gaagtaactt cacttaaaac tttgtagaa
2221 aaacaagata atagcatcaa agaccttctc cagaccgtgg aagaccaata taaacaatta
2281 aaccaacagc atagtcaaat aaaagaaata gaaaaatcagc tcagaaggac tagtattcaa
45 2341 gaaccacagc aaatttctct atcttccaag ccaagagcac caagaactac tccctttctt
2401 cagttgaatg aaataagaaa tgtaaaacat gatggcattc ctgctgaatg taccaccatt
2461 tataacagag gtgaacatac aagtggcatg tatgccatca gaccagcaa ctctcaagtt
2521 tttcatgtct actgtgatgt tatacaggt agtccatgga cattaattca acatogaata
2581 gatggatcac aaaacttcaa tgaaacgtgg gagaactaca aatatggttt tgggaggctt
50 2641 gatggagaat tttggttggg cctagagaag atatactcca tagtgaagca atctaattat
2701 gttttacgaa ttgagttgga agactggaaa gacaacaaac attatattga atattctttt
2761 tacttgggaa atcacgaaac caactatagc ctacatctag ttgcgattac tggcaatgtc
2821 cccaatgcaa tcccggaaaa caaagatttg gtgttttcta cttgggatac caaagcaaaa
2881 ggacacttca actgtccaga gggttattca ggaggtgggt ggtggcatga tgagtgtgga
55 2941 gaaaacaacc taaatggtaa atataacaaa ccaagagcaa aatctaagcc agagaggaga
3001 agaggattat cttggaagtc tcaaaatgga aggttatact ctataaaatc aacccaaatg
3061 ttgatccatc caacagattc agaaagcttt gaatgagcta gtgcggatcc acgggtggca

	3121	tcctctgtgac	ccctccccag	tgectctect	ggccctggaa	gttgccactc	cagtgccac
	3181	cagccttgte	ctaataaaat	taagttgcat	cattttgtct	gactagggtg	cctctataa
	3241	tattatgggg	tgagggggg	tggtatggag	caaggggcaa	gttgggaaga	caacctgtag
	3301	ggcctgcggg	gtctattggg	aaccaagctg	gagtgagtg	gcacaatctt	ggctcactgc
5	3361	aatctccgcc	tcctgggttc	aagcgattct	cctgcctcag	cctcccgagt	tgttgggatt
	3421	ccaggcatgc	atgaccaggc	tcagctaatt	tttgtttttt	tggtagagac	gggggtttcac
	3481	catattggcc	aggctgggtc	ccaactccta	atctcaggtg	atctacccac	cctggcctcc
	3541	caaattgctg	ggattacagg	cgtgaaccac	tgctcccttc	cctgtcctta	tcgatagatc
10	3601	taggaacccc	tagtgatgga	gttgccact	ccctctctgc	gcgtcgcctc	gctcaactgag
	3661	gcggggcgac	caaaggctgc	cgcagcccg	ggctttgccc	ggggcgccctc	agtgagcgag
	3721	cgagcgcgca	gctgcctgca	ggcagcttgg	cactggccgt	cgttttacaa	cgtcgtgact
	3781	gggaaaaacc	tggtgttacc	caacttaatc	gccttgagc	acatccccc	ttgcgcagct
	3841	ggcgtaatat	cgaagaggcc	cgcacccgac	gcctctccca	acagttgctc	agcctgaatg
	3901	gcgaatggcg	cctgatgcgg	tattttctcc	ttacgcctct	gtgcggtatt	tcacacccga
15	3961	tacgtcaaa	caaccatagt	acgcgcctg	tagcggcgca	ttaaagcgcg	cgggtgtggt
	4021	ggttacgcgc	agcgtgacgc	ctacacttgc	cagcgcctca	gcgcgcgctc	ccttcgcttt
	4081	cttcctctcc	tttctcgcca	cgttcgcgg	ccttccccct	caagctctaa	atcgggggct
	4141	ccctttagg	ttccgattta	gtgctttac	gcacctcgac	cccaaaaaac	ttgatttggg
20	4201	tgatggttca	cgtagtgggc	cctgcctctg	atagacggtt	tttcgcctct	tgacgttgga
	4261	gtccacgttc	tttaatatgt	gactcttgtt	ccaaactgga	acaacactca	acctatctc
	4321	gggtattctt	tttgatttat	aagggttttt	gcggtatttc	gcctatttgg	taaaaaatga
	4381	gctgatttaa	caaaaattta	acgcgaattt	taacaaaata	ttaacgttta	caattttatg
	4441	gtgcactctc	agtacaatct	gctctgatgc	cgcatagtta	agccagcccc	gacacccgc
25	4501	aacacccgct	gacgcgcctc	gacgggcttg	ctctgtcccg	gcacccgctt	acagacaaag
	4561	tgtgaccgct	tcggggagct	gcctgtgtca	gaggttttca	ccgtcatcac	cgaacgcgc
	4621	gagacgaaag	ggcctcgtga	tacgcctatt	tttatagggt	aatgtcatga	taataatggt
	4681	ttcttagacg	tcaggtggca	cctttcgggg	aaatgtgcgc	ggaaccccta	tttgtttatt
	4741	tttctaaata	cattcaaaata	tgatcccgct	catgagacaa	taacctgat	aaatgcttca
30	4801	ataatattga	aaaaggaaga	gtatgagtat	tcaacatttc	cgtgtcgcct	ttattccctt
	4861	ttttcgggca	ttttgccttc	ctgtttttgc	tcacccagaa	acgtcgttga	aagtaaaaga
	4921	tgctgaagat	cagttgggtg	cacgagtggg	ttacatcgaa	ctggatctca	acagcggtaa
	4981	gtcccttgag	agttttcgcc	ccgaagaacg	ttttccaatg	atgagcactt	ttaaagtctc
	5041	gctatgtggc	gcggtattat	ccggtattga	cgcggggcaa	gagcaactcg	gtcgcgcgat
35	5101	acactattct	cagaatgact	tggttgagta	ctcaccagtc	acagaaaagc	atcttacgga
	5161	tgccatgaca	gtaagagaat	tatgcagtgc	tgccataacc	atgagtgata	acactgcggc
	5221	caacttactt	ctgacaacga	tcggaggacc	gaaggagcta	accgcttttt	tgcaacaact
	5281	gggggatcat	gtaactcgcc	ttgatcgttg	ggaacccgag	ctgaatgaag	ccataccaaa
	5341	cgacgagcgt	gacaccacga	tgcttgtagc	aatggcaaca	acgttgcgca	aactattaac
40	5401	tgccgaacta	cttactctag	cctccgggca	acaatttaata	gactggatgg	aggcggataa
	5461	agttgcagga	ccacttctgc	gctcggccct	tcgggctggc	tggtttattg	ctgataaatc
	5521	tgagcgggtg	gagcgtgggt	ctcgcggtat	cattgcagca	ctggggccag	atggtaagcc
	5581	ctcccgatc	gtagtattct	acacgacggg	gagtcaggca	actatggatg	aacgaaatag
	5641	acagatcgct	gagatagggt	cctcactgat	taagcatttg	taactgtcag	accaagttaa
45	5701	ctcatatata	ccttagattg	atttaaaact	tcatttttaa	tttaaaagga	tctaggtgaa
	5761	gatccttttt	gataatctca	tgaccaaaat	cccttaacgt	gagttttcgt	tccactgagc
	5821	gtcagacccc	gtagaaaaga	tcaaaggatc	ttcttgagat	cctttttttc	tcgcgcgaat
	5881	ctgctgcttg	caaacaaaaa	aaccaccgct	accagcggtg	gtttgttttc	cggatcaaga
	5941	gctaccaact	ccttttccga	aggtaaactg	cctcagcaga	gcgcagatac	caaatactgt
50	6001	tcttctagt	tagccgtagt	taggccaaca	cctcaagaac	tctgtagcac	cgcctacata
	6061	cctcgcctcg	ctaactcctg	taccagtggc	tgctgccagt	ggcgataagt	cgtgtcttac
	6121	cgggttgagc	tcaagacgat	agttaccgga	taaggcgag	cggtcgggct	gaacgggggg
	6181	ttcgtgcaca	cagcccagct	tgagcggaac	gacctacacc	gaactgagat	acctacagcg
	6241	tgagctatga	gaaagcgcca	cgttcccgca	aggagagaa	ggcgacaggt	atccggtaag
	6301	cggcaggggt	ggaacaggag	agcgcacgag	ggagcttcca	gggggaaacg	cctgggtatct
55	6361	ttatagtcct	gtcgggtttc	gccacctctg	acttgagcgt	cgatttttgt	gatgctcgtc
	6421	agggggggcg	agcctatgga	aaaacggcag	caacgcggcc	tttttacggc	tcctggcctt
	6481	ttgtggcct	tttgcacaca	tggtcttttc	tgcttatctc	cctgattctg	tggtataaccg
	6541	tattacccgc	tttgagttag	ctgataccgc	tcgcccgcag	cgaacgaccg	agcgcagcga

6601 gtcagtggagc gaggaagcgg aag

Generation of recombinant AAV vectors: Ad293 cells (Agilent, Santa Clara, CA, USA) were plated in a five layered chamber in Gibco DMEM supplemented with 10% Gibco FBS and 1% Gibco penicillin-streptomycin (Thermo Fisher Scientific, Waltham, MA, USA). When these cells reached at 60–85% confluency under the microscope, they were transfected by polyethylenimine (Polyscience, Torrance, CA, USA) with triple plasmids, including pHHelper containing adenoviral E2A and E4 genes, pRep2Cap8 encoding AAV2 Rep proteins and AAV8 serotype capsid, and pAAV-hAAT-hApoE-ANGPTL3_noUTRs, in a ratio of 2:1.4:1, respectively ((Liu, 2023 #499). After 72 hr of the post-transfection, cells were harvested and lysed via three freeze-thaw cycles followed by 1 hr of benzonase treatment at 37°C. Supernatants were further purified using iodixanol gradient-based ultracentrifugation. Titers of AAV vector preps used for in vivo study were quantitated by quantitative PCR. Since the recombinant AAV8 vector was generated by a standard and helper-free 3 plasmid transfection system, this vector does not express AAV8 and adenoviral helper proteins and cannot replicate in transduced hepatocytes.

Human ANGPTL3 mRNA analysis

RNA was isolated from ~20 mg snap-frozen liver using RNeasy Mini Kit (Qiagen, cat#74104) according to manufacturer's instructions. cDNA was generated using a High-Capacity RNA-to-cDNA™ Kit from Applied Biosystems (ThermoFisher cat# 4387406) and used for real-time PCR with the QuantStudio 7 Flex (Applied Biosystems). *ANGPTL3* gene expression was measured using TaqMan Gene Expression assays from Applied Biosystems (ThermoFisher, *ANGPTL3*: FAM-MGB Hs00205581_m1 cat# 4331182, and mouse *RPLP0* primer probe set from (Sigma Aldrich) as housekeeping gene.

Forward primer: 5'-GAGGAATCAGATGAGGATATGGGA-3' (SEQ ID NO: 32),

Reverse primer 5'-AAGCAGGCTGACTTGGTTGC-3' (SEQ ID NO: 33) and

FAM-TAM labelled probe 5'-TCGGTCTCTTCGACTAATCCCGCCAA-3' (SEQ ID NO: 34)

Real-time PCR was performed on a Quantstudio 7 flex and PCR cycle numbers (Ct) determined using the Quantstudio Real-Time PCR Software (Applied Biosystems). *ANGPTL3* gene expression level was normalized against *RPLP0* the housekeeping gene.

Human ANGPTL3 mRNA analysis

Human ANGPTL3 was measured in mouse plasma using Human Angiopoietin-like 3 Quantikine ELISA Kit (DANL30, R&D Systems, Minneapolis, MN). Prior to use, the ELISA assay was shown to not cross-react with endogenous mouse ANGPTL3 present in the plasma samples (BS003151-48). The mouse plasma samples were analysed for human ANGPTL3 according to the manufacture instructions with the following modifications; the calibration curve was extended with 4 points to cover 0.02-10 ng/mL, the plasma samples were diluted 25-, 50-, or 100-fold prior to analysis. Calibration standards were analysed in duplicated and study samples as single samples, the ELISA plates were read in an absorbance microplate reader capable of measuring absorbance at 450 nm, with correction wavelength set at 540 nm (e.g. iX3, Molecular Devices). The response of the standard curve was plotted as absorbance units on the linear scale versus concentration on the log scale and the 4- parameter function was used for curve fitting (using SoftMax Pro). To control for between plate variations a human quality control samples was run, in duplicate, in the beginning and end of each analysis plate. ANGPTL3 levels was reported as ng/mL plasma.

Data Analysis

Plasma ANGPTL3 protein data were baseline-corrected, individually for each animal. The mean and standard error of each group at each day were calculated. Normality of data was investigated using the one-sample Kolmogorov-Smirnov test. Log-transformed data were suggested and used in subsequent tests. For human *ANGPTL3* mRNA expression data, mean and standard error of each group were calculated. Normality of data was investigated using the one-sample Kolmogorov-Smirnov test. Log-transformed data were suggested and used in subsequent tests. For all analyses, Tukey's post hoc test was used for multiple comparisons. Calculations were performed in MATLAB (R2022b; MathWorks). Specifically, the function `kstest` was used to test for normality, and the functions `anova1` and `multcompare` were used for multiple comparison tests.

Results

For Conjugate 2, maximum reductions of baseline-corrected ANGPTL3 protein concentrations were 37%, 58%, and 86% for single doses of 0.25, 1, and 3 mg/kg,

respectively (Table 3; Figure 3). For the treated groups, the maximum reductions were observed 22 to 30 days after dosing. The baseline-corrected ANGPTL3 protein concentration decreased over time in the placebo group with a maximum reduction of 37% at Day 42 and a reduction of 22% at the study end (Day 49). Neither the 0.25 mg/kg group nor the 1 mg/kg group showed statistically significant reductions in baseline-corrected ANGPTL3 protein concentration compared to placebo at the $P < 0.05$ level. The high-dose group of 3 mg/kg showed statistically significant reduction in baseline-corrected ANGPTL3 protein concentration compared to placebo at all measured time-points ($P < 0.05$). For the 1 mg/kg group, the baseline-corrected ANGPTL3 protein concentration started to return towards baseline, and reached the same level as the placebo group, but did not fully return to baseline, at Day 49. For the 3 mg/kg group, the baseline-corrected ANGPTL3 protein concentration did not fully return to baseline during the 7-week study. At study termination on Day 49, knockdown of human ANGPTL3 mRNA in liver was 56% compared to placebo ($P < 0.005$ versus all other groups) for the high-dose group of 3 mg/kg. The groups dosed with 0.25 and 1 mg/kg did not show knockdown of human ANGPTL3 mRNA in the liver at Day 49 (Table 4; Figure 4). For Conjugate 3, maximum reductions of baseline-corrected ANGPTL3 protein concentrations were 31%, 28%, and 43% for single doses of 0.25, 1, and 3 mg/kg, respectively (Table 3). For Conjugate 4, maximum reductions of baseline-corrected ANGPTL3 protein concentrations were 0%, 20%, and 30% for single doses of 0.25, 1, and 3 mg/kg, respectively (Table 3). None of the dose groups for Conjugates 3 or 4 did show any knockdown of human ANGPTL3 mRNA in the liver at Day 49 (Table 4; Figure 5).

Table 3 **Circulating Human ANGPTL3 Protein (% of baseline) In Humanized Mice Treated with A Single Dose Of Conjugate 2, Conjugate 3 or Conjugate 4.**

Treatment	Dose (mg/kg)		Baseline	Day 7	Day 22	Day 30	Day 42	Day 49
PBS	0		100	136	74	92	66	78
PBS	0		100	66	45	44	32	40
PBS	0		100	137	68	45	67	38
PBS	0		100	90	97	105	66	127
PBS	0		100	91	98	88	85	104
		N	5	5	5	5	5	5
		Mean	100	104	76	75	63	78

Treatment	Dose (mg/kg)		Baseline	Day 7	Day 22	Day 30	Day 42	Day 49
Conjugate 2	0.25		100	70	85	65	89	76
Conjugate 2	0.25		100	128	111	57	97	145
Conjugate 2	0.25		100	87	134	63	102	128
Conjugate 2	0.25		100	61	83	65	77	46
Conjugate 2	0.25		100	55	64	65	122	101
		N	5	5	5	5	5	5
		Mean	100	80	96	63	97	99
Conjugate 2	1		100	39	25	31	25	54
Conjugate 2	1		100	45	52	90	81	98
Conjugate 2	1		100	45	49	12	29	64
Conjugate 2	1		100	105	94	34	42	78
Conjugate 2	1		100	54	45	45	46	79
		N	5	5	5	5	5	5
		Mean	100	58	53	42	45	75
Conjugate 2	3		100	13	5	6	16	26
Conjugate 2	3		100	21	22	21	44	56
Conjugate 2	3		100	17	19	22	51	17
Conjugate 2	3		100	11	8	8	11	20
Conjugate 2	3		100	25	16	18	21	32
		N	5	5	5	5	5	5
		Mean	100	17	14	15	29	30
Conjugate 3	0.25		100	103	81	94	79	53
Conjugate 3	0.25		100	122	132	137	95	94
Conjugate 3	0.25		100	113	93	85	58	85
Conjugate 3	0.25		100	102	84	98	59	75
Conjugate 3	0.25		100	69	66	68	54	57
		N	5	5	5	5	5	5
		Mean	100	102	91	96	69	73
Conjugate 3	1		100	69	89	118	101	77
Conjugate 3	1		100	80	71	38	46	42
Conjugate 3	1		100	161	138	121	173	102
Conjugate 3	1		100	129	85	40	72	78
Conjugate 3	1		100	127	100	44	88	60
		N	5	5	5	5	5	5
		Mean	100	113	97	72	96	72

Treatment	Dose (mg/kg)		Baseline	Day 7	Day 22	Day 30	Day 42	Day 49
Conjugate 3	3		100	74	75	51	60	102
Conjugate 3	3		100	84	140	53	103	89
Conjugate 3	3		100	80	110	56	85	66
Conjugate 3	3		100	102	121	58	125	116
Conjugate 3	3		100	70	127	66	90	93
		N	5	5	5	5	5	5
		Mean	100	82	115	57	93	93
Conjugate 4	0.25		100	147	132	142	129	75
Conjugate 4	0.25		100	156	104	93	87	88
Conjugate 4	0.25		100	129	203	108	121	118
Conjugate 4	0.25		100	151	151	133	138	147
Conjugate 4	0.25		100	144	102	90	61	80
		N	5	5	5	5	5	5
		Mean	100	145	138	113	107	102
Conjugate 4	1		100	82	88	79	72	53
Conjugate 4	1		100	84	131	69	54	68
Conjugate 4	1		100	90	113	85	94	83
Conjugate 4	1		100	86	136	86	106	71
Conjugate 4	1		100	100	159	149	92	123
		N	5	5	5	5	5	5
		Mean	100	88	125	94	84	80
Conjugate 4	3		100	136	79	75	68	48
Conjugate 4	3		100	124	156	123	157	116
Conjugate 4	3		100	89	98	92	118	71
Conjugate 4	3		100	118	66	81	61	53
Conjugate 4	3		100	124	78	66	119	63
		N	5	5	5	5	5	5
		Mean	100	118	95	87	105	70

ANGPTL3, angiopoietin-like protein 3; mRNA, messenger ribonucleic acid; N, number.

5 **Table 4 Human ANGPTL3 mRNA Expression in Liver On Day 49 In Humanized Mice Treated With A Single Dose Of Conjugate 2, Conjugate 3 or Conjugate 4.**

Treatment	Dose (mg/kg)		Human ANGPTL3 mRNA (2 ^{-ΔCt} _{vs} mRPLO)
PBS	0		7.67
PBS	0		4.07
PBS	0		5.06
PBS	0		6.74
PBS	0		4.77
		N	5
		Mean	5.66
Conjugate 2	0.25		5.26
Conjugate 2	0.25		9.16
Conjugate 2	0.25		11.1
Conjugate 2	0.25		4.23
Conjugate 2	0.25		6.51
		N	5
		Mean	7.24
		Knockdown (%)	-27.9
Conjugate 2	1		4.48
Conjugate 2	1		4.87
Conjugate 2	1		5.22
Conjugate 2	1		4.73
Conjugate 2	1		9.37
		N	5
		Mean	5.73
		Knockdown (%)	-1.2
Conjugate 2	3		2.81
Conjugate 2	3		2.45
Conjugate 2	3		2.10

Treatment	Dose (mg/kg)		Human ANGPTL3 mRNA (2 ⁻ Δ Ct _{vs} mRPLO)
Conjugate 2	3		1.93
Conjugate 2	3		3.12
		N	5
		Mean	2.48
		Knockdown (%)	56.2
Conjugate 3	0.25		7.38
Conjugate 3	0.25		5.48
Conjugate 3	0.25		4.48
Conjugate 3	0.25		3.73
Conjugate 3	0.25		5.74
		N	5
		Mean	5.36
		Knockdown (%)	5.3
Conjugate 3	1		3.50
Conjugate 3	1		6.93
Conjugate 3	1		5.59
Conjugate 3	1		5.14
Conjugate 3	1		5.50
		N	5
		Mean	5.33
		Knockdown (%)	5.9
Conjugate 3	3		9.02
Conjugate 3	3		8.24
Conjugate 3	3		5.13
Conjugate 3	3		6.48
Conjugate 3	3		7.76
		N	5
		Mean	7.32
		Knockdown (%)	-29.3

Treatment	Dose (mg/kg)		Human ANGPTL3 mRNA ($2^{-\Delta Ct}$ vs mRPLO)
Conjugate 4	0.25		4.57
Conjugate 4	0.25		6.16
Conjugate 4	0.25		7.89
Conjugate 4	0.25		10.65
Conjugate 4	0.25		6.84
		N	5
		Mean	7.22
		Knockdown (%)	-27.5
Conjugate 4	1		8.64
Conjugate 4	1		5.74
Conjugate 4	1		3.23
Conjugate 4	1		5.57
Conjugate 4	1		4.68
		N	5
		Mean	5.57
		Knockdown (%)	1.6
Conjugate 4	3		4.99
Conjugate 4	3		10.68
Conjugate 4	3		6.18
Conjugate 4	3		1.40
Conjugate 4	3		4.24
		N	5
		Mean	5.50
		Knockdown (%)	2.9

ANGPTL3, angiopoietin-like protein 3; mRNA, messenger ribonucleic acid; N, number.

Example 5

Therapeutic effects of Conjugate 1 in an obese HFpEF ZSF1 rat model In Vivo.

Abbreviation or special term	Explanation
ELISA	Enzyme-linked immunosorbent assay
GalNAc	Galactose/N-acetylgalactosamine
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HS	High salt
IVRT	Isovolumic relaxation time
LPL	Lipoprotein lipase
LV	Left ventricle
NT-proBNP	N-terminal pro B-type natriuretic peptide
PBS	Phosphate buffered saline
SC	Subcutaneous
siRNA	Small interfering ribonucleic acid
T2DM	Type 2 diabetes
TG	Triglyceride
UACR	Urine albumin-creatinine ratio
Unx	Uninephrectomized - One kidney removed surgically
ZSF-1	Male obese Zucker diabetic fatty/spontaneously hypertensive heart failure F1 hybrid (ZSF1) rats with leptin mutations, when uninephrectomized and under high-salt diet they develop HFpEF

A ZSF-1 obese rat model with heart failure with preserved ejection fraction (HFpEF) was used to study the effect of ANGPTL3 protein reduction using Conjugate 1. Once secreted into the circulation, ANGPTL3 acts as an inhibitor of lipoprotein lipase (LPL), thus, play a major role in regulating total levels of circulating plasma triglycerides (Prog Lipid Res. 2022;85101140). It is known that similar to the positive metabolic profile in individuals deficient in ANGPTL3 protein (The Journal of clinical endocrinology and metabolism. 2012;97(7):E1266-75), pharmacological reduction in circulating ANGPTL3 protein has been demonstrated to cause clinically meaningful and well-tolerated reductions in cardiovascular risk factors (Evinacumab. (EVKEEZA®) Prescribing Information. Regeneron Pharmaceuticals, Inc. 2023, Circulation 2019. p. E987-8). Data from observational studies (Arterioscler Thromb Vasc Biol. 2018; 38(2): 464-72) has also indicated the potential clinical benefit of such reductions in patients with metabolically driven heart failure.

The ZSF-1 rat is a cross of two different leptin receptor mutations and the offspring can be either obese or lean. The obese ZSF-1 rats spontaneously develop obesity, hypertension, type 2 diabetes, hyperlipidemia, left ventricular (LV) dysfunction, nephropathy

and insulin resistance. The lean ZSF-1 rat is hypertensive, but shows none of the other conditions. Obese uninephrectomized ZSF-1 rats were treated for 12 weeks with Conjugate 1 or vehicle respectively, and given high salt diet (to induce HFpEF phenotype). Non-invasive imaging, echocardiography, was used in this study as the “golden standard” of evaluating heart function. It was concluded that ANGPTL3 protein reduction decreases circulating triglyceride (TG) levels, reduces TG content in heart and reduces the development of diastolic dysfunction in ZSF-1 obese rats with HFpEF (as shown by NT-proBNP and IVRT results), without significant increase of liver TGs.

Obese uninephrectomized (Unx) ZSF-1 rats were treated for 12 weeks with Conjugate 1 or vehicle, n=8 respectively, in high salt (HS) diet. Lean ZSF-1 rats (n=8) were used as a control group. Blood samples were taken before start of the treatment and after 12 weeks treatment for analysis of NT-proBNP, ANGPTL3 and circulating lipids and lipoproteins. At termination, tissue from heart, kidney and liver was collected for analysis. Non-invasive imaging, echocardiography, was used in this study as the “golden standard” for evaluating heart function. Isovolumic relaxation time (IVRT) was the primary endpoint.

The study design for each rat is outlined in Figure 6. The rats were grouped 4 and 4 in each cage and were fed pellets containing high salt diet or Purina and normal tap water ad libitum throughout the study. Once every week, the rats were weighed. The rats were randomised into groups by IVRT, glucose, UACR and body weight. Following acclimatization, baseline echocardiography and baseline blood sampling was initiated.

Study design

Dose(s):	4 mg/kg
Infusion/injection rate(s)	
and/or time:	Sub cutaneous injections
Duration of treatment:	12 weeks
Number/group:	8, Groups are outlined in Table 5
Number of groups:	7
Individual animal	Identification or reference number is the
identification No/reference No :	individual identifying number which was given to each animal in the study

Table 5 **Groups, Doses and Number of Animals**

Group number	Group	Treatment	Diet	Dose	Number of animals	Disease-population
1	ZSF1 Lean	PBS	Purina	-	8	
2	ZSF1 Obese sham	PBS	Purina	-	8	
3	ZSF1 Obese sham	Conjugate 1	Purina	4 mg/kg	8	
4	ZSF1 Obese Unx	PBS	Purina	-	8	CKD
5	ZSF1 Obese Unx	Conjugate 1	Purina	4 mg/kg	8	CKD
6	ZSF1 Obese Unx + HS	PBS	Purina + High Salt	-	8	HFpEF
7	ZSF1 Obese Unx + HS	Conjugate 1	Purina + High Salt	4 mg/kg	8	HFpEF
Total number of animals					56	

The study design for each rat is outlined in Figure 6. The rats were grouped 4 and 4 in each cage and were fed pellets containing high salt diet or Purina and normal tap water ad libitum throughout the study. Once every week, the rats were weighed. The rats were randomised into groups by IVRT, glucose, UACR and body weight. Following acclimatization, baseline echocardiography and baseline blood sampling was initiated. Obese uninephrectomized ZSF1 rats on high salt diet were treated for 12 weeks with Conjugate 1 or vehicle, n=8 respectively. Lean ZSF1 rats (n=8) were used as a control group. Treatments (either PBS or Conjugate 1) were administered subcutaneously at study week 1, 4, 7 and 10. Blood samples were taken before start of the treatment, every third week and after 12 weeks treatment for analysis of NT-proBNP, ANGPTL3 and circulating lipids. At termination, blood was collected as well as tissue from heart, kidney and liver for analysis. Non-invasive imaging, echocardiography, was used in this study as the “golden standard” of evaluating heart function. Isovolumic relaxation time (IVRT from echocardiography), was the primary endpoint. Animals N 25 (Obese Unx+ HS+AD) 30 and 32 (Obese Unx+ HS) died before endpoint.

Experimental Procedures

Blood Sampling from Tail Vein

At baseline, every third week and endpoint (as described in Figure 1), a tail vein blood sample was collected from the wake rats via a butterfly catheter (21G, BD Valu-Set™, Plymouth, UK) in EDTA coated vials (MiniCollect, K3EDTA, Kremsmünster, Austria).

Plasma Analysis

Blood was centrifuged for 10 min at 5000 x g. EDTA-plasma and was used for analysis of NT-proBNP and ANGPTL3. The assays were performed according to the manufacturer's instructions.

Metabolic cages

Animals were placed in metabolic cages urine sampling and fasting period for later blood collection. From an animal welfare perspective, a shorter time, such as 4 hours, is recommended as it is less challenging for the rats (less stressful and less impact on body weight, food, and water intake). At study weeks -1, 7, 10 and 12, animals were placed in metabolic cages for 4 hours with water ad libitum but no food. For the fasting period of the blood collection of week 4, animals were placed in a plastic box for 4 hours with water ad libitum.

Echocardiography

Animal Preparation Echocardiography

Non-invasive imaging, Echocardiography, was performed three times: at baseline, week 9 and study week 12. The animals were initially anaesthetised with isoflurane (Isoba® vet, Schering-Plough Ltd., England) gas (5%) in a gas chamber. The gas concentration was established by exerting a flow of O₂ (200 mL/min) and air (500 mL/min) to a solution of isoflurane. During the experiment the animals were kept anaesthetised by breathing isoflurane gas (approximately 2.2%) through a mask. Hair removal of the chest and left back side is done with Veet® removal cream after shaving. The skin was carefully rinsed with lukewarm tap water.

Experimental Protocol Echocardiography

Echocardiography was performed using a high-resolution ultrasound scanner (Vevo 3100, Visualsonics Inc) with MX201 solid state transducers. A parasternal long axis (LAX) view

was used to visualize a long axis image of the heart. Subsequently a short-axis (SAX) image was collected at the mid-ventricular level, with the right ventricle (RV) oriented to the left side of the field of view (FOV), and the papillary muscles on the right side. B-mode and m-mode loops were collected.

- 5 To assess diastolic function including isovolumic relaxation time (IVRT), analyses of the blood flow spectrum from a pulse wave Doppler on the mitral valve were performed. This image was acquired via a modified apical four chamber view. From the same view Tissue Doppler Imaging (TDI) was performed on the septal mitral valve annulus to assess longitudinal velocities of the myocardium.

10 **Termination and Organ Sampling**

At endpoint, the heart, lungs, spleen, liver and one kidney were harvested and weighed and the tibia was dissected and the length was measured.

Rat ANGPTL3 ELISA

- This method was set up fully automated in a Beckman Coulter Motoman robotic system (FLIPR). Including Biomek FX Span-8/96-head Biotek cellwasher, Cytomat Incubators (shake), Carousel and MD Paradigm Reader. Assay kit Mouse/Rat Angiopoietin-like protein 3 ELISA (ANGPTL3, Cat No RAG011R, Czech Republic) was used.

- Robot assay: Samples were pre-diluted in two steps from 10 μ L Sample, to in total 800 $\times$ (separate method). Standards were serial diluted (2 $\times$) and transferred to predilution plate. 100 μ L prediluted sample/standard were pipetted to ELISA plate. Incubation for 1 h at 37°C. ELISA plate was washed (ELISA3 5 $\times$ washes). 100 μ L detection antibody was added. Incubation for 1 h at 37°C. ELISA plate was washed (ELISA3 5 $\times$ washes). 100 μ L HRP Conjugate was added. Incubation for 1 h at 37°C. ELISA plate was washed (ELISA3 5 $\times$ washes). 100 μ L Substrate TMB was added. Incubation for 15 min at room temperature in the dark. 100 μ L top solution was added. Absorbance at 450 nm and reference wavelength at 540 nm (within 20 min) was read.

Rat NT-proBNP MSD ELISA Assay

- This method was set up fully automated in a Beckman Coulter SCARA robotic system (Lambda) including Biomek NX Span-8, Biomek FX 96/96-heads, biotek cellwasher, cytomat Incubators, hotels and MD Paradigm Reader and MSD S600. Rat NT-proBNP kit (Cat No K153JKD-1) was used.

Robot assay: Samples were pre-diluted (3 x) in Diluent 34 with protease inhibitor. MSD plate was washed (3 x in wash buffer). 50 µL of standards, controls and diluted sample were dispensed. Incubation for 1 h with vigorous shaking (500 rpm) at room temperature. Plate was washed (3 x in wash buffer). 25 µL detection antibody was dispensed. Incubation for 2 h with vigorous shaking (500 rpm) at room temperature. Plate was washed (3 x in wash buffer). 150µL reading buffer was dispensed. Plate was read within 2 min in the MSD Sector Imager S600.

Straight Phase HPLC for Plasma TGs

Plasma triglycerides were extracted using liquid/liquid extraction (Lofgren L, 2012). The liquid extract was evaporated under a stream of nitrogen and reconstituted in heptane:isopropanol [9:1]. The analysis was done using straight phase HPLC as described previously (J Chromatogr B Biomed Sci Appl. 1998; 708(1-2): 21-6). Quantification was made against an external calibration curve.

TG content in Liver

Pieces (app.50 mg) of liver were homogenized with isopropanol (2-isopropanol) so the triglycerides is released from the tissue. Triglycerides in sample were hydrolyzed enzymatically to glycerol and fatty acids. Glycerol generates, by two enzymatic steps, hydrogen peroxide that contributes to the formation of a red quinoneimine derivat. Colour intensity is directly proportional to the amount of triglycerides (glycerol) in the sample, and is measured colorimetrically at wave length 500 nm.

Lipidomics for TG content in the heart

Pre-weighed frozen ZSF1 heart samples were homogenized by bead beating in 10X (v/w) 3:1:6 IPA:water:EtOAc containing 1:100 EquiSplash (Avanti) internal standard mixture. A fraction of the clarified supernatant was dried via speedvac before resuspension in an equal volume of 9:1 MeOH:Toluene (10X ratio maintained). 1 µL of sample was analyzed using a C18 LCMS-based approach.

Mass Spectrometry: Untargeted (full scan) mass spectrometry analysis was performed using Thermo Tribrid ID-X with a scan range of 50 to 950 m/z for metabolomics and 50 to 1700 m/z for lipidomics. Individual samples were acquired in MS1 only mode, using a polarity switching approach.

Data analysis: Thermo raw files were analyzed in MS-DIAL ($\geq v. 4.8$) for feature detection, alignment, identification, and normalization. Peak areas were normalized to

EquiSplash standards for lipidomics and to the total ion current for metabolomics. Features were filtered in R based on QC/Blank signal >3X and a QC RSD<30%. Statistical analysis was performed using the MetaboAnalystR package (v.3.2.0) with R language (v.4.1.0).

Briefly, data was normalized by median, log-transformed and scaled with a Pareto approach.

- 5 Fold change calculations are using data before the column-wise normalization was applied (ie at the original scale).

Proteomics

- Proteomics data acquisition: Mouse heart tissues were lysed in metabolite extraction buffer (IPA:H₂O:EtOAc=6:1:3) and beads beat, protein pellets with leftover buffer will be used for
10 proteome profiling. High pH Reversed-Phase Peptide Fractionation (Thermo 84868) was performed for the peptide fractionation. LC-MS/MS analysis was conducted on a timsTOF Pro mass spectrometer (Bruker) coupled with a Evosep LC-system and nano-electrospray ion source (CaptiveSpray Source, Bruker). Data-dependent acquisition (DDA) and Data-independent acquisition (DIA)-PASEF mode were performed to obtain the peptide data. The
15 combined DDA and DIA acquisition raw files were analyzed via Spectronaut (Biognosys AG) software (Version 18) with Pulsar search engine (SN14.10.201222) to build the library, using UniProt rat proteome database (UP000002494_20231024).

- Proteomics data analysis: Protein intensities were analyzed using R (v. 4.2.3) and DEP package (v.1.20.0). Briefly, the data was normalized using svm approach, and missing values
20 were imputed using missForest approach(v.1.5). Finally, differential analysis was done using linear models and empirical Bayes statistics. PCA was calculated using the imputed values. Enrichment analysis was performed using clusterProfiler package (v.4.6.2).

Electron microscopy for Podocyte health

- A thin slice of the kidney was fixed in a mix of 2.5% glutaraldehyde + 1% paraformaldehyde
25 (both from Sigma-Aldrich) in 0.1 M phosphate buffer (pH 7.4) and stored at 4°C. Electron microscopy analysis was conducted at the electron microscopy unit at Huddinge University Hospital (Stockholm, Sweden).

Data Analysis

Holm-Sidak's multiple comparisons test or Unpaired t-test was used for statistical evaluation. Data is presented as mean $\pm$ SD. In other cases it is indicated in the footnotes of the figures.

- 5 **Histology readouts:** Glomerular score, Podocyte stress and TEM (Glomerular Basement Membrane, GBM, thickness and slit/ μ m).

Assays used for analyzing urine samples: U-Albumin and U-Creatinine are measured in Pentra 400. U-Albumin is determined colorimetric: Albumin CP ABX Pentra, ref A11A01664. U-Creatinine is determined by Jaffe's reaction (picric acid): Creatinine 120CP, 10 Horiba ABX A11A01933. U-Albumin and creatine volume required for analyses around 50 μ L.

Results

Body weight

- 15 As seen in Figure 7, changes in body weight between lean and obese rats were observed. No change in body weight was observed in obese animal treated with Conjugate 1 (C1) compared to obese control group. Filled-black circles represent individual animals. Stars indicate significant changes between the comparison groups. Not significant (ns) indicates no significant changes between the comparison groups. Holm-Sidak's multiple comparisons test was used. Lean (N=8), Obese Unx+HS (N=6) Obese, Unx+HS+C1 (N=7). Lean - control 20 animals, Obese Unx+HS - uninephrectomy + high-salt diet to induce HFpEF, Obese Unx+HS+AD, uninephrectomy + high-salt diet to induce HFpEF + treatment with Conjugate 1.

ANGPTL3 Protein and TG Plasma Levels

- 25 As seen in Figure 8, Conjugate 1 reduced more than 95% of ANGPTL3 circulating protein levels in the Obese Unx+HS+AD group compared to the control group. This changes were also observed in the reduction of circulating plasma triglyceride (TG) levels that were reduced almost to baseline levels after treatment. Filled-black circles represent individual animals. Stars indicate significant changes between the comparison groups. Holm-Sidak's multiple 30 comparisons test was used. Lean (N=8), Obese Unx+HS (N=6) Obese, Unx+HS+AD (N=7). Lean - control animals, Obese Unx+HS - uninephrectomy + high-salt diet to induce HFpEF, Obese

Unx+HS+AD, uninephrectomy + high-salt diet to induce HFpEF + treatment with Conjugate 1.

TG content (lipidomic analysis) in ZSF-1 rat hearts and liver

Figure 9a shows a significant reduction in TG content (lipidomic analysis) in heart tissue by Conjugate 1 in the Obese Unx+HS+AD group compared to control group Tissue triglyceride 51:4, 51 carbons, 4 double bonds). As seen in Figure 9b, ANGPTL3 gene silencing does not affect liver TG content in the Obese Unx+HS+AD group compared to comparison group. Filled-black circles represent individual animals. Stars indicate significant changes between the comparison groups. Not significant (ns) indicates no significant changes between the comparison groups. Holm-Sidak's multiple comparisons test was used. Lean (N=8), Obese Unx+HS (N=6) Obese, Unx+HS+C1 (N=7). Lean - control animals, Obese Unx+HS - uninephrectomy + high-salt diet to induce HFpEF, Obese Unx+HS+AD, uninephrectomy + high-salt diet to induce HFpEF + treatment with Conjugate 1.

NT-proBNP and IVRT

At a functional level, prevention of cardiac diastolic dysfunction was demonstrated by decreased isovolumic relaxation time and circulating N-terminal pro B-type natriuretic peptide levels (Figure 10). NT-proBNP is a biomarker for strain in the heart muscle and isovolumic relaxation time is an echocardiography diastolic function parameter. Filled-black circles represent individual animals. Stars indicate significant changes between the comparison groups. Not significant (ns) indicates no significant changes between the comparison groups. Holm-Sidak's multiple comparisons test was used. Lean (N=8), Obese Unx+HS (N=6) Obese, Unx+HS+C1 (N=7). Lean - control animals, Obese Unx+HS - uninephrectomy + high-salt diet to induce HFpEF, Obese Unx+HS+AD, uninephrectomy + high-salt diet to induce HFpEF + treatment with Conjugate 1.

Proteomics data (heart tissue)

Proteomics data from heart tissue showed changes in proteins involved in Fatty acid metabolism. This was confirmed in using several pathway platforms. Specifically, ANGPTL3 inhibition altered the concentration of several Apolipoproteins. This results suggest that the treatment enhances the lipid degradation in heart tissue.

A) Volcano plot showing proteins changing significantly (Adj p-value < .1 & |log2(FC)| > 0.5). B) KEGG pathways significantly enriched. C) Levels of Apolipoproteins in the different treatment groups ("***"=0.001, "**"=0.01, "*"=0.05). Lean - control animals, Obese

Unx+HS - uninephrectomy + high-salt diet to induce HFpEF, Obese Unx+HS+ARO, uninephrectomy + high-salt diet to induce HFpEF + treatment with Conjugate 1.

Renal health

As shown in Figure 8, ANGPTL3 inhibition prevents kidney damage in ZSF-1 rats with
5 CKD, represented by UACR, podocyte injury, and glomerulosclerosis. The rats used for the CKD indication were not under high salt diet. They received purina diet for the duration of the study. Filled-black circles, triangles and squares represent individual animals. Stars indicate significant changes between the comparison groups. Holm-Sidak's multiple comparisons test was used. N number varies depending on the assay performed.

10 Lean - control animals, Obese Unx - uninephrectomy to induce CKD, Obese Unx+ARO, uninephrectomy to induce CKD + treatment with Conjugate 1.

Treatment with Conjugate 1 showed that reduction in ANGPTL3 protein improves
cardiometabolic outcomes in ZSF-1 HFpEF rats by decreasing circulating TGs levels,
15 reducing TG content in heart and reducing the development of diastolic dysfunction. It is hypothesized that targeting ANGPTL3 mRNA and consequently silencing hepatic ANGPTL3 gene expression with Conjugate 2, or any other siRNA agent for inhibition of ANGPTL3, will enhance LPL enzymatic activity, promote hydrolysis of TG-rich lipoproteins, decrease circulating TG and improve cardiac and renal outcomes.

20

Example 6

Therapeutic effects of Conjugate 1 in cynomolgus macaques (*Macaca fascicularis*) with HFpEF.

The intention of this study was to investigate the chronic effects of the target inhibition of the
25 Conjugate 1 on cardiac-renal functional measures and markers in the most relevant animal model that reflects the intended human population. The cynomolgus monkey was selected as the test species of choice over other lower mammalian species for more meaningful and translatable results because it is closely related to humans, both phylogenetically and physiologically. The chronic efficacy of Conjugate 1 in the treatment of heart failure was
30 explored in the cynomolgus monkey model with heart failure with preserved ejection fraction (HFpEF) which closely approximates the adult human HFpEF phenotype.

Study Design

Group 1: Age-matched normal healthy control (4 subjects)

Group 2: HFpEF PBS control (10 subjects)

5 Group 3: HFpEF Conjugate 1 s.c. dose 3 mg/kg (10 subjects)

Group 4: HFpEF Empagliflozin p.o. dose 3 mg/kg (10 subjects)

Dosing was administered on day 1 of week 1 and day 84 of week 12. This was then followed by a 12 week post-dose observation/washout period.

10 Diet and Water

The monkeys were provided 3 meals per day consisting of 100.0 g of standard monkey formula feed (extruded pellets) in the morning 9:00-10:00 AM, one regular apple (150 g) in the afternoon 14:00-15:00 PM, and 100 g of high-fat diet (HFD) in the evening 16:00-17:00 PM. Water was provided ad libitum. All the monkeys were fasted overnight. After each
15 feeding time, all remaining food was withdrawn, and food intake determined by weighing the left-over food.

Two-Dimensional (2D) Echocardiography

Two-dimensional echocardiography (2D echo) was performed using the Mindray M9CV
20 Echocardiography System (Mindray, Shenzhen China) with SP5-1s (1.1-4.4MHz) phased array transducer under ketamine sedation administered intramuscularly (IM) at 10 mg/kg. Animals were fasted for 4-6 hours to minimize the risk of vomiting and lung aspiration during anaesthesia induction and recovery. Nevertheless, unlimited access to drinking water was provided during the fasting period. Ketamine was administered intramuscularly at 10 mg/kg
25 and the hairs on the animal's left chest was shaved thoroughly using an electric clipper and a shaving blade. The monkey were appropriately positioned on the exam table or surgical bed that has been cleaned by wiping with 70% alcohol and covered with sterile disposable drape. The animal was be kept warm on a heating pad / blanket during the entire procedure. Typically, the echocardiogram was performed with the animal in the left lateral decubitus.

30

MRI Scan Preparations

Prior to each scan, the monkey was fasted overnight or for a minimum of 6 hours prior to

imaging.

Ketamine (5-10 mg/kg, IM) was injected intramuscularly to induce sedation and atropine hydrochloride (0.02-0.04 mg/kg, IM) was administered to inhibit respiratory spasm and salivation. Endotracheal intubation was then carried out, an intravenous (IV) line was inserted into a peripheral vein, and the ECG leads were attached to the animal's chest wall. The animal was then transferred to the MRI bed for ECG and vital signs monitoring. It was connected to the mechanical ventilator and Isoflurane (1-3%) in medical air was used to induce and maintain anesthesia. The animal was positioned supine, head first on the MRI bed and an abdominal coil was used to image the heart. Each scan session did not exceed four (4) hours. The animal was kept warm during the scan. The heart rate, blood oxygenation level, noninvasive blood pressure, respiratory rate, end-tidal CO₂ level and temperature was recorded/ monitored during the entire scan session to ensure adequate level of anesthesia. ECG monitoring was continuously carried out.

MRI Procedures

All animals underwent CMR using a 3.0 Tesla magnetic resonance imaging system (Magnetom Prisma; Siemens Healthineers, Erlangen, Germany) with a 32-channel phasearray body coil. The following MRI Scan Sequences was carried out twice during the entire study (at baseline and on Week 25): Proton density fat fraction (PDFF) Imaging; Regadenoson Stress Perfusion Imaging; and Extracellular volume (ECV) Scan. The pulse sequence parameters were documented and described in the MRI report. The animals were mechanically ventilated at 20-30 breaths per min during the scan. Inspiratory breath holding was carried out to mitigate the effect of respiration on the vascular image quality. This was done only by switching the ventilator off for no more than 10 seconds while running the pulse sequence. The oxygen flow during breath holds (when the ventilator is off) was maintained.

Proton-Density Fat Fraction (PDFF) Imaging

Myocardial proton density fat fraction (mPDFF) was measured by using a 2D, ECGtriggered, multi-echo segmented gradient-echo sequence. The ECG trigger delay was to ensure data acquisition occurring at the quiescent period of cardiac cycle to minimize cardiac motion. The respiratory motion was controlled by a single breath-hold. The mPDFF was measured using

the software, VivoQuant (V2.5), by placing region-of-interest (ROI) at different myocardial locations.

Regadenoson Stress Perfusion Imaging and Extracellular volume (ECV) Scan

- 5 To perform stress imaging, a rest first-pass perfusion imaging was first be initialized with a bolus injection of MRI contrast agent (0.075 mmol/kg of Gadoteric Acid Meglumine Salt Injection; 15ml: 5.654g (a sterile solution containing 377mg/ml gadoteric acid meglumine salt; Jiangsu Hengrui Pharmaceutical Co., Ltd.). The perfusion sequence was a 2D saturation recovered single-shot gradient-echo sequence with 70 repetitions. A total of 3 slices as those
- 10 in pre-contrast T1 mapping were scanned with a temporal resolution of one RR-interval (~500 msec). Cine images along 2-, 3-, and 4-chambers were then acquired after the perfusion images. Approximately 15 min later, Regadenoson (5 µg/kg; Nanjing HaiRong Pharmaceutical LTD) was injected in a bolus to induce vasodilation and another first-pass perfusion imaging (0.075 mmol/kg of Gadoteric Acid Meglumine Salt Injection) was
- 15 performed at 1 min after the injection of Regadenoson for stress perfusion imaging. Ten minutes after the second contrast injection, post-contrast MOLLI T1 mapping was acquired with a scheme of 4(1)3(1) in the same three short-axis slices that are used for the pre-contrast T1 scans. Late gadolinium enhancement images were obtained using an electrocardiogram-gated breath-hold inversion recovery Turbo FLASH at 15 min after
- 20 contrast injection. All CMR images were prospectively analyzed. A blood sample was obtained from all monkeys immediately before each CMR study for hematocrit (Hct) measurements. Cine images were analyzed by Medis software (Medis Medical Imaging Systems BV, Leiden, Netherlands).
- Based on the pre-contrast and post-contrast T1 maps, which were automatically generated
- 25 with a prototype inline process function from the MRI system, global or regional ECV values were calculated using Hct values. If there was motion between the pre- and post-T1 series, the pre- and post-MOLLI was registered manually. The first-pass perfusion imaging was analyzed by using another custom-made software Perfusion Imaging Toolkit (PIT). Quantitative perfusion maps in 3 slices were generated for both rest and stress conditions.
- 30 Myocardial perfusion reserves were then calculated as the ratio of stress perfusion over resting perfusion.

Blood Sampling for Pharmacokinetic (PK) profiling, Target Biomarkers and Other Exploratory Pharmacodynamic (PD) Biomarkers

Approximately 6 mL of whole blood samples was collected from a peripheral vein of the non-sedated/alert monkeys directly into K2EDTA tubes for the following target biomarkers.

- 5 EDTA plasma was separated by centrifugation at 2,500 x g for 10 minutes at 4 °C. The collected plasma was aliquoted into cryotubes.

The samples were stored frozen at -80 °C. The cryotubes were marked with study number, animal number group number, experimental day number and time after dose.

- One aliquot for PK was analyzed by KBI and one was stored frozen at -80 °C as
10 backup. Samples for target engagement biomarkers, ANGPTL3, TG were stored and analyzed.

Proton-Density Fat Fraction (PDFF) Imaging

- As seen in Figure 13, animals treated with Conjugate 1(ANGPTL3 SiRNA inhibition) had
15 signific reduction in heart fat fraction. Similar results were observed with the positive control SGLT2 inhibitor (Empaglifozin) as expected.

Extracellular volume (ECV) Scan

- As seen in Figure 14, ANGPTL3 inhibition significantly reduced extracellular volume. This
20 parameter has been associated with survival rate in heart failure patients.

Left atria volume index

Left atria volume index (LAVi) is a critical parameter for the diagnosis of HfpEF and predicts poor prognosis in these patients (J Cardiovasc Med (Hagerstown). 2018 Jun;19(6):304-309).

- 25 As seen in Figure 15, ANGPTL3 inhibition, but not SGLT2 inhibition, resulted in a significant decrease of LAVi.

Plasma ANGPTL3 levels

As seen in Figure 16, ANGPTL3 circulating levels decreased significantly with treatment.

30

Plasma triglyceride (TG) levels

As a consequence of ANGPTL3 inhibition, TG circulating levels were significantly decreased as seen in Figure 17. Treatment with SGLT2 inhibitor Empaglifozin did not have any effects in reduction of TG circulating levels.

CLAIMS

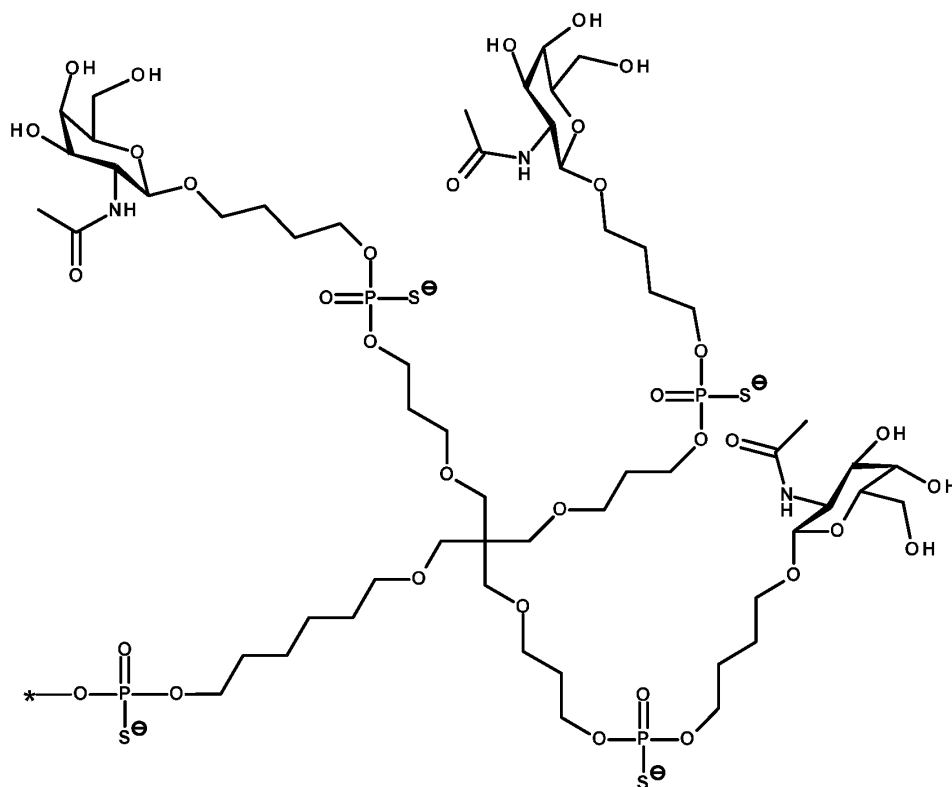
1. An ANGPTL3 siRNA agent comprising (i) a first (sense) strand having the sequence:

[M]-mC mU mA mC mA mU fA fU fA mA mA mC mU mA mC mA mA (ps) mG (ps) mU
5 (SEQ ID NO: 11),

and (ii) a second (antisense) strand having the sequence:

mA (ps) fC (ps) mU fU mG fU mA fG mU fU mU fA mU fA mU fG mU (ps) fA (ps) mG
10 (SEQ ID NO: 5),

where [M] is a triantennary ligand moiety linked to the 5' end of the first strand and having the structure:



;

or a pharmaceutically acceptable salt thereof.

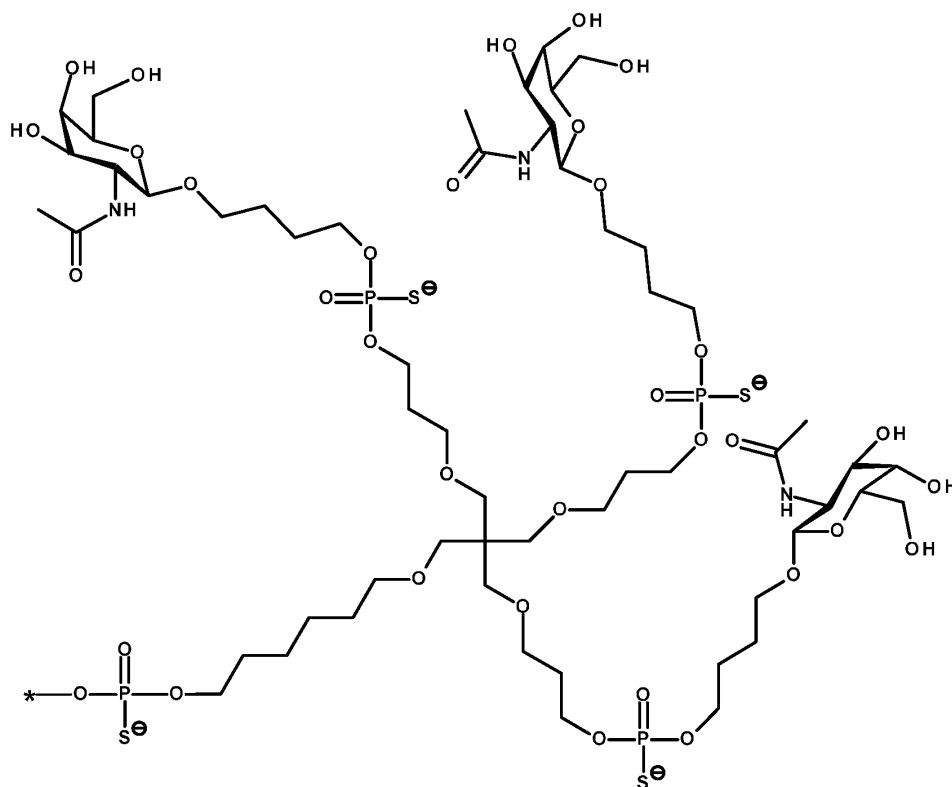
2. An ANGPTL3 siRNA agent according to claim 1, or a pharmaceutically acceptable salt thereof, for use as a medicament.
3. A pharmaceutical composition comprising an ANGPTL3 siRNA agent according to claim 1, or a pharmaceutically acceptable salts thereof, optionally in admixture with a pharmaceutically acceptable adjuvant, diluents or carrier.
4. A method of treating, or reducing the risk of, cardiovascular disease or metabolic conditions which comprises administering to a person suffering from or at risk of, said

disease or condition, a therapeutically effective amount of an ANGPTL3 siRNA agent according to Claim 1, or a pharmaceutically acceptable salt thereof.

5. The method according to claim 1, wherein said disease is hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis type I diabetes mellitus, cardiovascular disease, coronary artery disease, non alcoholic steatohepatitis, non-alcoholic fatty liver disease, homozygous and heterozygous familial hypercholesterolemia or statin resistant hypercholesterolemia.
6. A compound according to claim 1, or a pharmaceutically acceptable salt thereof, for use as a medicament.
7. A compound according to claim 1, or a pharmaceutically acceptable salt thereof, for use as in the treatment of hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis type I diabetes mellitus, cardiovascular disease, coronary artery disease, non alcoholic steatohepatitis, non-alcoholic fatty liver disease, homozygous and heterozygous familial hypercholesterolemia or statin resistant hypercholesterolemia.
8. The use of a compound according to claim 1, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament, for the treatment or prophylaxis of cardiovascular disease or metabolic conditions.
9. A method of treating, or reducing the risk of, heart failure which comprises administering to a person suffering from or at risk of heart failure a therapeutically effective amount of a double-stranded siRNA comprising an antisense strand comprising a nucleobase sequence that is at least 80% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, or a pharmaceutically acceptable salt thereof.
10. The method according to claim 9, wherein the double-stranded siRNA comprise an antisense strand comprising a nucleobase sequence that is at least 90% complementary

to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, or a pharmaceutically acceptable salt thereof.

- 5 11. The method according to claim 9, wherein the double-stranded siRNA comprise an antisense strand comprising a nucleobase sequence that is at 100% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, or a pharmaceutically acceptable salt thereof.
- 10 12. The method according to claim 9, wherein the double-stranded siRNA is an ANGPTL3 siRNA agent comprising (i) a first (sense) strand having the sequence:
[M]-mC mU mA mC mA mU fA fU fA mA mA mC mU mA mC mA mA (ps) mG (ps) mU (SEQ ID NO: 11),
- 15 and (ii) a second (antisense) strand having the sequence:
mA (ps) fC (ps) mU fU mG fU mA fG mU fU mU fA mU fA mU fG mU (ps) fA (ps) mG (SEQ ID NO: 5),
- 20 where [M] is a triantennary ligand moiety linked to the 5' end of the first strand and having the structure:



;

or a pharmaceutically acceptable salt thereof.

5

13. The method according to claim 9, wherein the double-stranded siRNA is an ANGPTL3 siRNA agent comprising (i) a first (sense) strand having the sequence:

NAG-invAb (ps) mG mC mU mC mA mA mC mA fU fA fU mU mU mG mA mU
mC mA mG mU mA (ps) invAb (SEQ ID NO: 10),

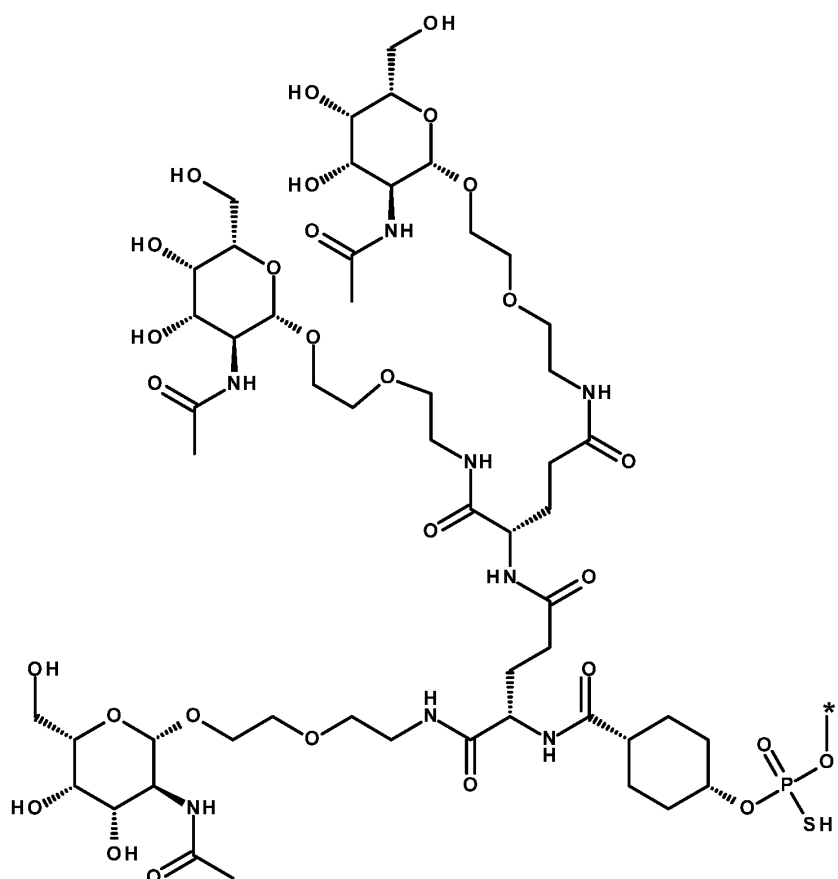
10

and (ii) a second (antisense) strand having the sequence:

mU (ps) fA (ps) mC (ps) fU mG fA mU fC mA fA mA fU mA fU mG fU mU fG mA
fG (ps) mC (SEQ ID NO: 3),

15

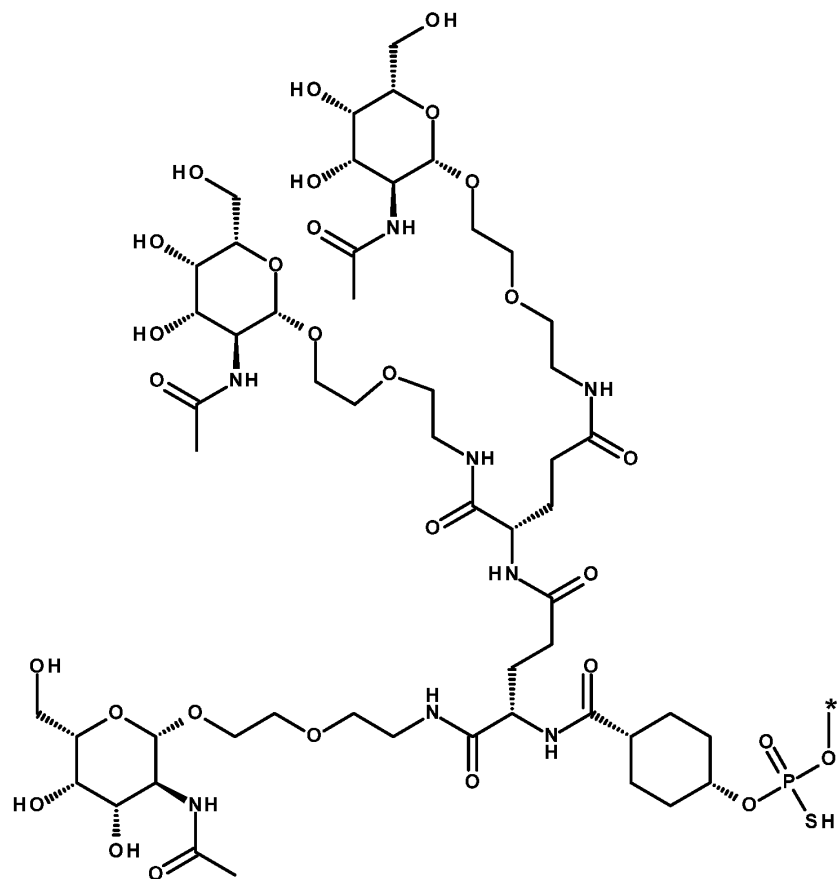
where NAG is a triantennary ligand moiety linked to the 5' end of the first strand and having the structure:



5 or a pharmaceutically acceptable salt thereof.

14. A method of treating, or reducing the risk of, chronic kidney disease which comprises administering to a person suffering from or at risk of chronic kidney disease a therapeutically effective amount of of a double-stranded siRNA comprising an antisense strand comprising a nucleobase sequence that is at least 80% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, or a pharmaceutically acceptable salt thereof.
- 10

15. The method according to claim 14, wherein the double-stranded siRNA comprise an antisense strand comprising a nucleobase sequence that is at least 90% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, or a pharmaceutically acceptable salt thereof.
16. The method according to claim 14, wherein the double-stranded siRNA comprise an antisense strand comprising a nucleobase sequence that is at 100% complementary to an equal length portion of a ANGPTL3 nucleic acid having the nucleobase sequence of SEQ ID NO: 1, and a sense strand, or a pharmaceutically acceptable salt thereof.
17. The method according to claim 14, wherein the double-stranded siRNA is an ANGPTL3 siRNA agent comprising (i) a first (sense) strand having the sequence:
- NAG-invAb (ps) mG mC mU mC mA mA mC mA fU fA fU mU mU mG mA mU mC mA mG mU mA (ps) invAb (SEQ ID NO: 10),
- and (ii) a second (antisense) strand having the sequence:
- mU (ps) fA (ps) mC (ps) fU mG fA mU fC mA fA mA fU mA fU mG fU mU fG mA fG (ps) mC (SEQ ID NO: 3),
- where NAG is a triantennary ligand moiety linked to the 5' end of the first strand and having the structure:

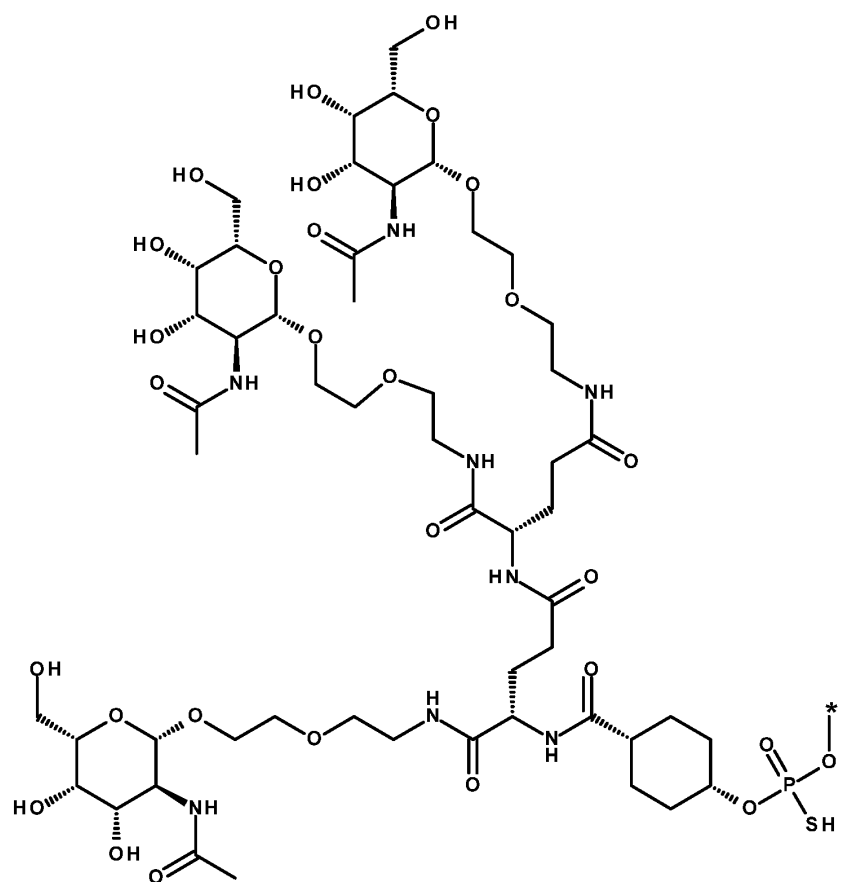


or a pharmaceutically acceptable salt thereof.

18. The method according to claim 14, wherein the double-stranded siRNA is an
 5 ANGPTL3 siRNA agent comprising (i) a first (sense) strand having the sequence:
- NAG-invAb (ps) mG mC mU mC mA mA mC mA fU fA fU mU mU mG mA mU
 mC mA mG mU mA (ps) invAb (SEQ ID NO: 10),
- 10 and (ii) a second (antisense) strand having the sequence:

mU (ps) fA (ps) mC (ps) fU mG fA mU fC mA fA mA fU mA fU mG fU mU fG mA
fG (ps) mC (SEQ ID NO: 3),

where NAG is a triantennary ligand moiety linked to the 5' end of the first strand and
having the structure:



or a pharmaceutically acceptable salt thereof.