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(54) Title: OLIGONUCLEOTIDES CONJUGATED TO FATTY ACIDS

(57) Abstract: Provided are lipid conjugated oligonucleotides that allow for delivery of the oligonucleotides to specific tissues in the body. The lipid on the conjugated oligonucleotide comprises an acyl group having a free terminal carboxylic acid group. Also provided are methods for synthesizing lipid conjugated oligonucleotides having with a lipid comprising an acyl group having a free terminal carboxylic acid group, along with methods for delivering the lipid conjugated oligonucleotides and methods for using the lipid conjugated oligonucleotides for treating disease.

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# OLIGONUCLEOTIDES CONJUGATED TO FATTY ACIDS

## SUMMARY

**[0001]** The present disclosure relates to oligonucleotides conjugated to acyl chains having a terminal carboxyl group. In some embodiments, the disclosure provides methods of making oligonucleotides conjugated to acyl chains having a terminal carboxyl group.

**[0002]** Oligonucleotide based therapies – such as antisense therapies, gene therapies and CRISPR gene editing therapies – are thought to hold promise for treatment of various conditions. However, delivery of oligonucleotides to specific tissues in the body has been a challenge for oligonucleotide-based therapies.

**[0003]** One strategy for delivering oligonucleotides to specific tissues is to deliver the oligonucleotides packaged into lipid nanoparticles or polymer nanoparticles. Because oligonucleotides, unless specially modified, have a polyanionic backbone, cationic lipids or polymers are used in forming nanoparticles. The electrostatic interaction between the anionic oligonucleotides and cationic lipids or polymers causes the nanoparticles to form.

**[0004]** While a wide variety of lipid nanoparticles has been developed, almost all of these nanoparticles have limitations for use in therapeutics. One limitation is the amount of carrier, i.e., lipid, material that must be delivered to support the administration of oligonucleotides. For example, for small interfering RNAs (siRNAs) packaged in lipid nanoparticles, the siRNA makes up only a few percent of the total mass of the deliverable, with the remaining mass being lipids. Juliano, *Nucleic Acids Research* 2016, 44(14):6518-6548. The delivery of large amount of cationic lipids can be problematic as many biological macromolecules are anionic and thus electrostatically associate with the cationic lipids leading to toxicity issues.

**[0005]** Further, lipid nanoparticles typically only accumulate in tissues having a fenestrated (e.g., porous) endothelium: liver, spleen and some tumor tissues. Osborn *et al.*, *Nucleic Acid Therapeutics* 2018, 28(3):128-136. This makes the use of lipid nanoparticles impractical for therapies where the oligonucleotide needs to be delivered to other tissues.

**[0006]** Lipid conjugated oligonucleotides are being developed to improve the delivery of oligonucleotides. As the oligonucleotide is chemically linked to the lipid, an electrostatic interaction between the two is not needed and potentially toxic cationic lipids are not required.

Further, as a single oligonucleotide molecule is usually conjugated to only one or two lipid chains, the amount of lipid carrier material is substantially reduced. Thus, side effects with lipid conjugated oligonucleotides should be reduced.

**[0007]** As the lipids used for conjugation do not have to be suitable for lipid nanoparticle formation, a larger variety of lipids can be used. By varying the lipid portion of the lipid conjugated oligonucleotide, it is possible to target a wider range of tissues for uptake of the conjugates.

**[0008]** The present disclosure is directed to acid acyl conjugated oligonucleotides comprising:

a) a carboxy acyl group;

b) an oligonucleotide; and

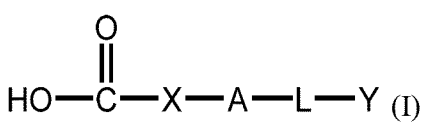
c) a linker connecting the carboxy acyl group to the oligonucleotide.

**[0009]** In some embodiments, the carboxy acyl group is $C_4$ to $C_{32}$ and may be saturated, for example, monounsaturated or unsaturated, for instance polyunsaturated.

**[0010]** In some embodiments, the acid acyl conjugated oligonucleotides comprise a linker bound to the 5' end of the oligonucleotide. In some embodiments, the acid acyl conjugated oligonucleotides comprise a linker comprising an amino terminus connected to the carboxy acyl group. In some embodiments, the acid acyl conjugated oligonucleotides comprise a linker comprising a phosphate terminus connected to the oligonucleotide.

**[0011]** In some embodiments, the acid acyl conjugated oligonucleotides comprise a DNA oligonucleotide. In some embodiments, the acid acyl conjugated oligonucleotides comprise a RNA oligonucleotide. In some embodiments, the acid acyl conjugated oligonucleotides comprise an antisense oligonucleotide. In some embodiments, the oligonucleotide is a phosphorothioate oligonucleotide.

**[0012]** According to the present disclosure, the acid acyl conjugated oligonucleotides of the present disclosure may be chosen from compounds of formula (I):



(I)

wherein:

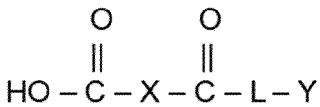
X is $C_4$ to $C_{32}$ alkyl or alkenyl;

A is a conjugation group;

L is a linker; and

Y is an oligonucleotide.

**[0013]** Also disclosed herein are acid acyl conjugated oligonucleotides of formula (Ia):



(Ia)

wherein:

X is $C_{10}$ to $C_{26}$ alkyl;

L is a linker -NH-$C_6H_{12}$-O-$PO_2$-; and

Y is DNA,

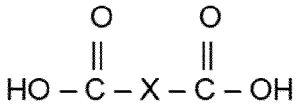
wherein the linker is attached to the DNA via a phosphate linkage at the 5' end of the DNA.

**[0014]** The present disclosure further comprises methods to improve the reduction in cardiac cell expression compared comprising administering an acid acyl conjugated oligonucleotide disclosed herein to a mammal, wherein the acid acyl conjugated oligonucleotide improves reduction in cardiac cell expression compared to a non-acyl conjugated oligonucleotide.

**[0015]** Also disclosed are methods of reducing expression of a gene of interest in the cardiac cells of a mammal, comprising administering an acid acyl conjugated oligonucleotide disclosed herein to the mammal, wherein the acid acyl conjugated oligonucleotide reduces expression of a gene of interest in cardiac cells compared to a non-acyl conjugated oligonucleotide

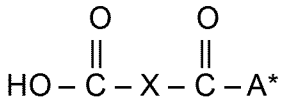
**[0016]** Further disclosed herein are methods of making an acid acyl oligonucleotide of formula (Ia), the method comprising:

A) providing a fatty diacid of formula (II)



(II);

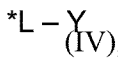
B) reacting the fatty diacid with an activating group, A, to form an acid acyl compound of formula (III):



(III);

wherein A* is the activating group attached to the fatty acyl compound;

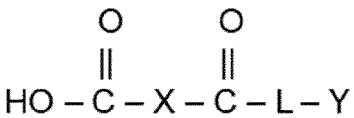
C) reacting the compound of formula (III) with a compound of formula (IV):



(IV),

wherein *L is a linker with a reactive group;

to form the compound of formula (Ia):



(Ia)

**[0017]** The present disclosure further provides methods of delivering an oligonucleotide to cardiac tissue in a subject comprising:

a) providing acid acyl conjugated oligonucleotide according to the present disclosure, and

b) administering the acid acyl conjugated oligonucleotide to the subject.

**[0018]** In the following description, certain details are set forth in order to provide a thorough understanding of various embodiments. However, one skilled in the art will understand that the disclosed embodiments may be practiced without these details. These and other embodiments will become apparent upon reference to the following detailed description and attached drawings.

## BRIEF DESCRIPTION OF DRAWINGS

**[0019] FIG. 1** shows the dissociation constants of 5' lipid conjugated Malat-1 ASOs from Human (HSA) and rat (RSA) serum albumin measured by surface plasmon resonance (SPR) as exemplified in Example 25.

**[0020] FIG. 2** shows the concentration dependent knock down of Malat-1 gene expression in human THP-1 monocytes after treatment with FA-ASO conjugates as exemplified in Example 26.

**[0021] FIGS. 3A-D** shows the results of Example 27, that the lipidated CamK2D ASOs of Examples 12 (**FIG. 3A**), Example 13 (**FIG. 3B**), Example 14 (**FIG. 3C**), and Example 15 (**FIG. 3D**) maintained functional activity in vitro.

**[0022] FIGS. 4A-B** shows that conjugation to a saturated $C_{22}$ acid or $C_{18}$ (9Z) monounsaturated fatty diacid led to similar or increased knock down in the heart but also to an attenuation of the knock down measured in the liver and kidney compared to the parent ASO with **FIG. 4A** exemplifying Examples 12 and 15 MALAT-1 gene expression in the heart and **FIG. 4B** exemplifying Examples 7 and 10 MALAT-1 gene expression in the liver and kidney. ns: Analysis was carried out via two-way ANOVA followed by Bonferroni multiple comparisons. ***: $p<0.001$, one way ANOVA followed by Dunnett multiple comparisons.

**[0023] FIG. 5** shows CamK2D gene expression level in the heart, kidney, and liver and shows that the saturated $C_{22}$ acid chain conjugation improved knock down in the heart and tend to attenuate the knock-down in sink organs like kidney and liver in comparison to the naked parent ASOs as exemplified in Example 29. ** $p<0.01$. *** $p<0.001$, two way ANOVA followed by Bonferroni multiple comparisons.

## DETAILED DESCRIPTION

**[0024]** The present disclosure provides lipid conjugated oligonucleotides where the lipid comprises an acyl group and a free terminal carboxylic acid group. The present disclosure includes methods of making lipid conjugated oligonucleotides where the lipid comprises and acyl group and a free terminal carboxylic acid group. The present disclosure includes methods of delivering the lipid conjugate oligonucleotides disclosed herein to a subject. The present

disclosure also includes methods of administering the lipid conjugate oligonucleotides disclosed herein to treat disease in a subject.

**[0025]** It should be appreciated that the particular implementations shown and described herein are examples and are not intended to otherwise limit the scope of the application in any way.

**[0026]** As used interchangeably herein, a "nucleic acid," "nucleic acid molecule," "nucleotide," "nucleotide sequence," "oligonucleotide," or "polynucleotide" means a polymeric compound including covalently linked nucleotides. A nucleotide includes a nucleoside linked to a phosphate group. A nucleoside includes a nucleobase and sugar moiety. The nucleobase may be naturally occurring or synthetic. The nucleobase and sugar base may each, independently, be modified or unmodified. "Modified nucleoside" means a nucleoside comprising a modified nucleobase and/or a modified sugar moiety. Modified nucleosides can include abasic nucleosides, which lack a nucleobase. Polynucleotides or oligonucleotides may be modified or unmodified and may contain one or more modified nucleosides. "Modified polynucleotide" or "modified oligonucleotide" means a polynucleotide or oligonucleotide, wherein at least one sugar, nucleobase, or internucleoside linkage is modified. In some embodiments, the modified polynucleotide or modified oligonucleotide is oligonucleotide is a phosphorothioate polynucleotide. "Unmodified polynucleotide" means a polynucleotide that does not comprise any sugar, nucleobase, or internucleoside modification. The term "nucleic acid" includes ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), both of which may be single- or double-stranded. DNA includes, but is not limited to, complementary DNA (cDNA), genomic DNA, plasmid or vector DNA, and synthetic DNA. In some embodiments the polynucleotide or oligonucleotide is double stranded DNA. In some embodiments the polynucleotide or oligonucleotide is single stranded DNA. In some embodiments the polynucleotide or oligonucleotide is double stranded RNA. In some embodiments the polynucleotide or oligonucleotide is single stranded RNA. In some embodiments the polynucleotide or oligonucleotide is an antisense RNA. Nucleic acids of the present disclosure may be any length. In some embodiments, a nucleic acid provided herein is 8 to 80 nucleotides in length. In some embodiments, a nucleic acid provided herein is 10 to 70 nucleotides in length, 12 to 60 nucleotides in length, 15 to 50 nucleotides in length, 15 to 45 nucleotides in length, 16 to 40 nucleotides in length, 17 to 35 nucleotides in length, 18 to 30 nucleotides in length, 19 to 29 nucleotides in length or 20 to 28 nucleotides in length.

**[0027]** As used herein, the term "antisense molecule" means an oligomeric molecule that is capable of undergoing hybridization to a target nucleic acid, e.g., through hydrogen bonding. Examples of antisense molecules include single-stranded and double-stranded nucleic acids, such as, e.g., antisense oligonucleotides (ASO), small interfering RNAs (siRNA), short hairpin RNAs (shRNA), small nucleolar RNAs (snoRNA), microRNAs (miRNA), and meroduplexes (mdRNA), and satellite repeat sequences.

**[0028]** An "antisense oligonucleotide" or "ASO" refers to a polynucleotide comprising a sequence that is complementary to a target nucleic acid or region or segment thereof. In some embodiments, an ASO is specifically hybridizable to a target nucleic acid or region or segment thereof. In some embodiments, ASOs are capable of influencing RNA processing and/or modulating protein expression. In general, an ASO is a single-stranded oligonucleotide that binds to single-stranded RNA to inactivate the RNA. In some embodiments, an ASO binds to messenger RNA (mRNA) for a gene, thereby inactivating the gene. In some embodiments, an ASO binds to a non-coding mRNA, thereby disrupting the function of the non-coding mRNA. In some embodiments, an ASO binds to a transcription initiation site, a translation initiation site, 5'-untranslated sequence, 3'-untranslated sequence, coding sequence, a pre-mRNA sequence, an mRNA splice site, and/or an intron/exon junction of an mRNA encoding a gene, thereby inactivating the gene. In some embodiments, the ASO includes DNA, RNA, or combination thereof. ASOs are further described in, e.g., Goodchild, Methods Mol Biol 764:1-15, 2011; Smith et al., Ann Rev Pharmacol Toxicol 59:605-630, 2019; and Stein et al., Mol Ther 25(5):1069-1075, 2017.

**[0029]** In embodiments of any of the above, the oligonucleotide can be unmodified DNA, RNA or may be modified. Modified oligonucleotides comprise at least one modification relative to unmodified RNA or DNA (i.e., comprise at least one modified nucleoside (comprising a modified sugar moiety and/or a modified nucleobase and/or at least one modified internucleoside linkage). In embodiments, the oligonucleotide can be selected from any of the oligonucleotides described herein. In embodiments, the oligonucleotide is an antisense oligonucleotide. In embodiments, the antisense oligonucleotide contains at least one phosphorothioate internucleoside linkage. In embodiments, the antisense oligonucleotide contains at least one modified sugar moiety, for example, a bicyclic sugar moiety (e.g., comprising two rings, wherein the second ring is formed via a bridge connecting two of the

atoms in the first ring thereby forming a bicyclic structure), such as a furanosyl moiety. In embodiments, the antisense oligonucleotide contains at least one modified nucleobase.

**Carboxy acyl groups and linkers**

**[0030]** In embodiments, the present disclosure provides an acyl acid conjugated oligonucleotide comprising a carboxy acyl group connected to an oligonucleotide. In embodiments, the carboxy acyl group is connected to the oligonucleotide by a linker.

**[0031]** In embodiments, the carboxy acyl group of the conjugate is a fatty acid chain having a free terminal carboxylic acid group. In embodiments, the acyl group has a length of from $C_4$ to $C_{32}$. In embodiments, the acyl group has a length of from $C_6$ to $C_{30}$. In embodiments, the acyl group has a length of from $C_8$ to $C_{28}$. In embodiments, the acyl group has a length of from $C_{10}$ to $C_{26}$. In embodiments, the acyl group has a length of from $C_{12}$ to $C_{26}$. In embodiments, the acyl group has a length of from $C_{14}$ to $C_{24}$. In embodiments, the acyl group has a length of from $C_{16}$ to $C_{22}$. In embodiments, the acyl group has a length of $C_{16}$. In embodiments, the acyl group has a length of $C_{18}$. In embodiments, the acyl group has a length of $C_{22}$.

**[0032]** In embodiments, the carboxy terminal on the acyl group provides improved uptake of the conjugated oligonucleotide in specific tissues and/or organs upon administration. In embodiments, the carboxy terminal on the acyl group provides improved uptake in cardiac tissue or liver tissue. In embodiments, the carboxyl terminal on the acyl group provides improved uptake in the heart or liver. In embodiments, the carboxyl terminal on the acyl group provides improved uptake in cardiac tissue. In embodiments, the carboxyl terminal on the acyl group provides improved uptake in the heart.

**[0033]** In embodiments, the acyl group is saturated, i.e., having only single carbon-carbon bonds. In embodiments, the acyl group is unsaturated, i.e., having one or more double carbon-carbon bonds. In embodiments, the acyl group is monounsaturated. In embodiments, the acyl group is polyunsaturated, e.g., having 2-15 double bond, e.g., having 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 double bonds.

**[0034]** In embodiments, the lipid conjugated oligonucleotides can include a single carboxy acyl group attached to the oligonucleotide. In embodiments, the lipid conjugated oligonucleotides can include a two carboxy acyl groups attached to the oligonucleotide. In embodiments, the lipid conjugated oligonucleotides can include a three or more carboxy acyl

groups attached to the oligonucleotide. In embodiments, the lipid conjugated oligonucleotides can include a single carboxy acyl group attached to the oligonucleotide along with one, two or more other acyl groups that do not have a terminal carboxy. In embodiments, the lipid conjugated oligonucleotides can include two carboxy acyl groups attached to the oligonucleotide along with one, two or more other acyl groups that do not have a terminal carboxy.

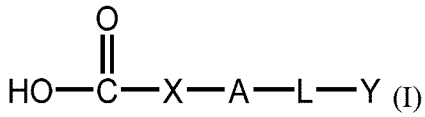
**[0035]** In embodiments, the carboxyl acyl group is attached to the 5' end of the oligonucleotide. In embodiments, the carboxyl acyl group is attached to the 3' end of the oligonucleotide. In embodiments, the carboxy acyl group is attached to the oligonucleotide at a modified base in the oligonucleotide instead of the 5′ or 3′ ends of the oligonucleotide.

**[0036]** In embodiments, the carboxyl acyl group is connected to the oligonucleotide by a linker. In embodiments, the linker is bound to the 5' end of the oligonucleotide. In embodiments, the linker forms an amide bond with the carboxy acyl group. In embodiments, the linker comprises a phosphate linkage connected to the oligonucleotide. In embodiments, the linker is attached to the 3' end of the oligonucleotide. In embodiments, the linker is attached to the oligonucleotide at a modified base in the oligonucleotide.

**[0037]** The linker may be any chemical moiety that allows for chemical attachment of the carboxy acyl group at one end of the linker and the oligonucleotide at another end of the linker. In embodiments, the linker forms an amide bond with the carboxy acyl group. In embodiments, the linker comprises a phosphate linkage connected to the oligonucleotide. In embodiments, the phosphate linkage of the linker can be a 5' phosphate on the oligonucleotide.

**[0038]** In embodiments, the linker comprises an acyl chain between termini. In embodiments, the linker comprises an acyl chain of $C_1$ to $C_{10}$ in length. In embodiments, the acyl chain can be saturated. In embodiments, the acyl chain can be monounsaturated or polyunsaturated. In embodiments, the linker forms an amide bond with the carboxy acyl group, a phosphate linkage with the oligonucleotide, and an acyl chain of $C_1$ to $C_{10}$ in length connecting the amide bond with the phosphate terminal.

**[0039]** In embodiments, the present disclosure provides an acid acyl conjugated oligonucleotide of formula (I)



(I)

wherein: X is $C_4$ to $C_{32}$ alkyl or alkenyl; A is a conjugation group; L is a linker; and Y is an oligonucleotide.

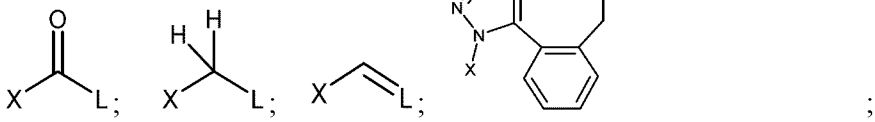
**[0040]** In embodiments of formula (I), X is $C_6$ to $C_{30}$ alkyl or alkenyl. In embodiments, X is $C_8$ to $C_{28}$ alkyl or alkenyl. In embodiments, X is $C_{10}$ to $C_{26}$ alkyl or alkenyl. In embodiments, X is $C_{12}$ to $C_{26}$ alkyl or alkenyl. In embodiments, X is $C_{14}$ to $C_{24}$ alkyl or alkenyl. In embodiments, X is $C_{14}$ alkyl or alkenyl. In embodiments, X is $C_{16}$ alkyl or alkenyl. In embodiments, X is $C_{20}$ alkyl or alkenyl.

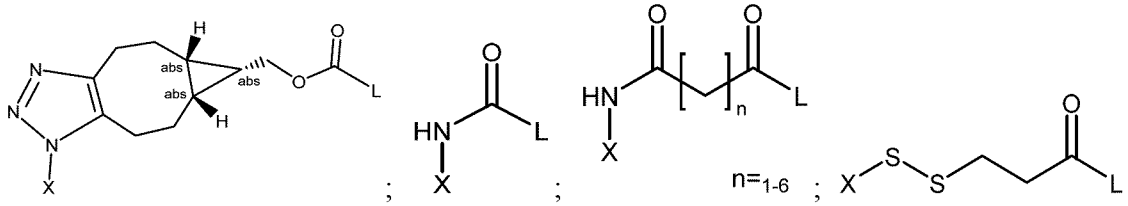
**[0041]** In embodiments of formula (I), X is $C_6$ to $C_{30}$ alkyl. In embodiments, X is $C_8$ to $C_{28}$ alkyl. In embodiments, X is $C_{10}$ to $C_{26}$ alkyl. In embodiments, X is $C_{12}$ to $C_{26}$ alkyl. In embodiments, X is $C_{14}$ to $C_{24}$ alkyl. In embodiments, X is $C_{14}$ alkyl. In embodiments, X is $C_{16}$ alkyl. In embodiments, X is $C_{20}$ alkyl.

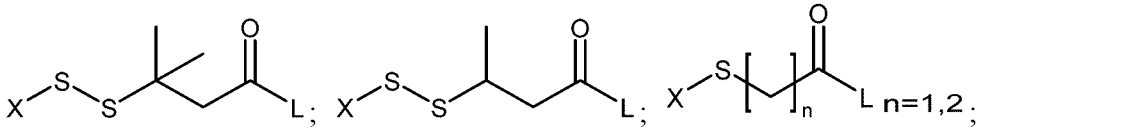
**[0042]** In embodiments of formula (I), X is $C_6$ to $C_{30}$ monounsaturated alkenyl. In embodiments, X is $C_8$ to $C_{28}$ monounsaturated alkenyl. In embodiments, X is $C_{10}$ to $C_{26}$ monounsaturated alkenyl. In embodiments, X is $C_{12}$ to $C_{26}$ monounsaturated alkenyl. In embodiments, X is $C_{14}$ to $C_{24}$ monounsaturated alkenyl. In embodiments, X is $C_{14}$ monounsaturated alkenyl. In embodiments, X is $C_{16}$ monounsaturated alkenyl. In embodiments, X is $C_{20}$ monounsaturated alkenyl.

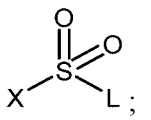
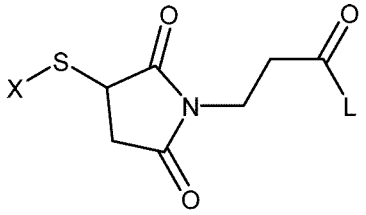
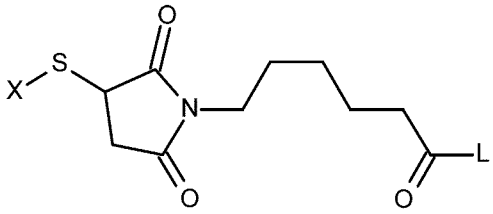
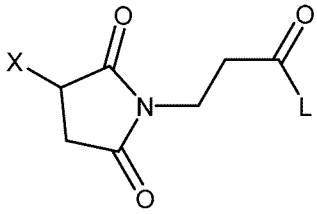
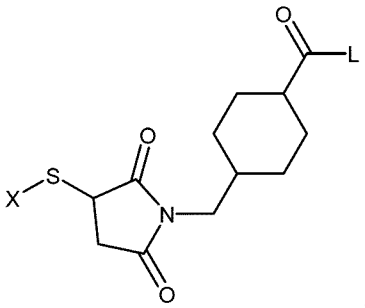
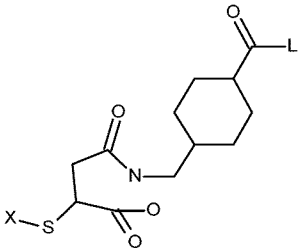
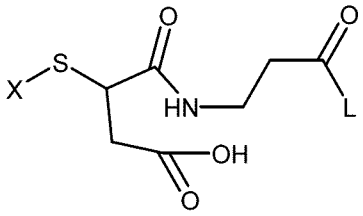
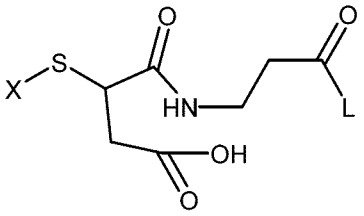
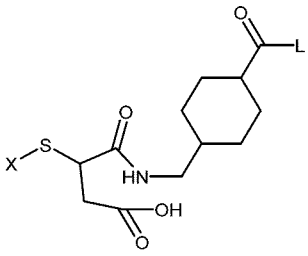
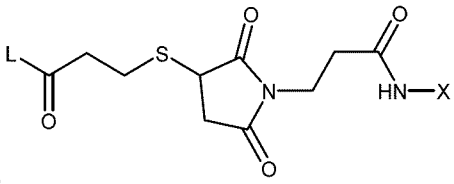
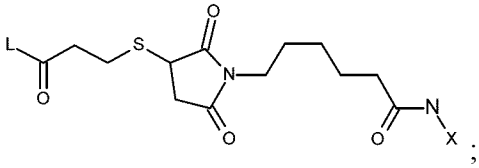
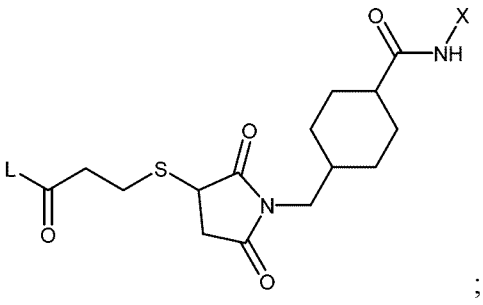
**[0043]** In embodiments of formula (I), X is $C_6$ to $C_{30}$ polyunsaturated alkenyl. In embodiments, X is $C_8$ to $C_{28}$ polyunsaturated alkenyl. In embodiments, X is $C_{10}$ to $C_{26}$ polyunsaturated alkenyl. In embodiments, X is $C_{12}$ to $C_{26}$ polyunsaturated alkenyl. In embodiments, X is $C_{14}$ to $C_{24}$ polyunsaturated alkenyl. In embodiments, X is $C_{14}$ polyunsaturated alkenyl. In embodiments, X is $C_{16}$ polyunsaturated alkenyl. In embodiments, X is $C_{20}$ polyunsaturated alkenyl.

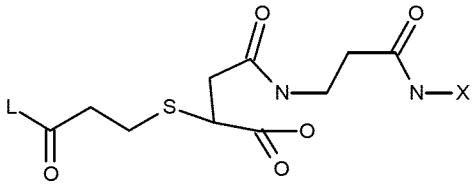
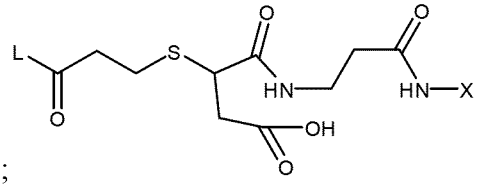
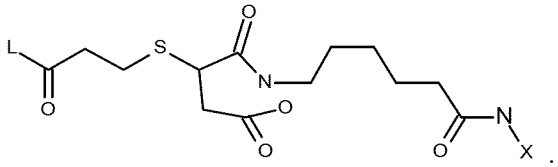
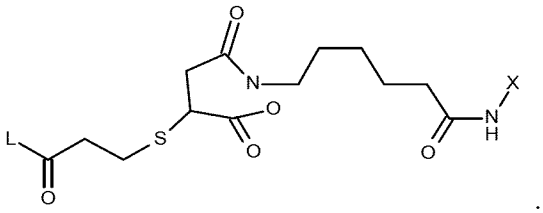
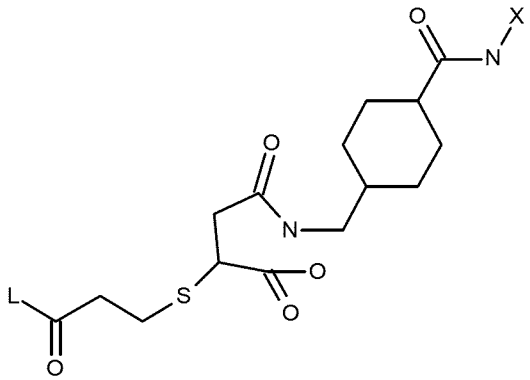
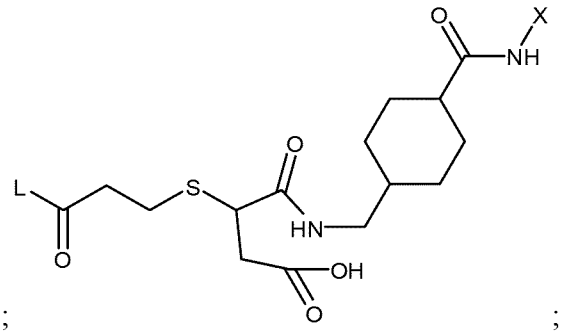
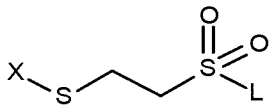
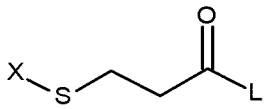
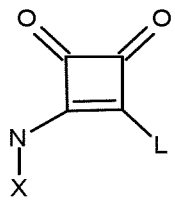
**[0044]** In embodiments of formula (I), A is a conjugation group where A is C=O. In certain embodiments, A is a conjugation group chosen from the following:



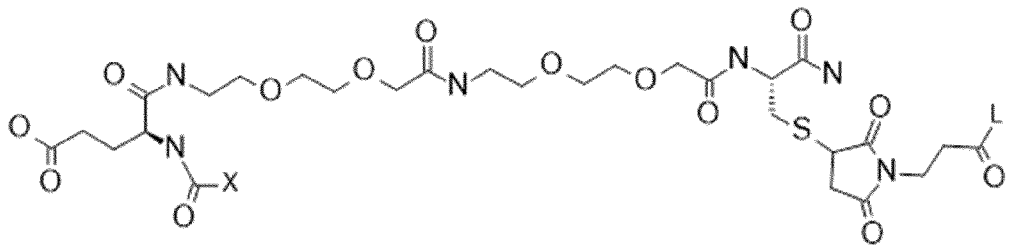


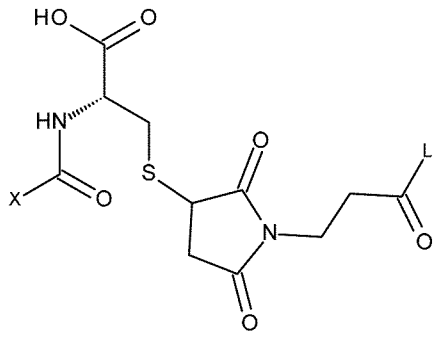



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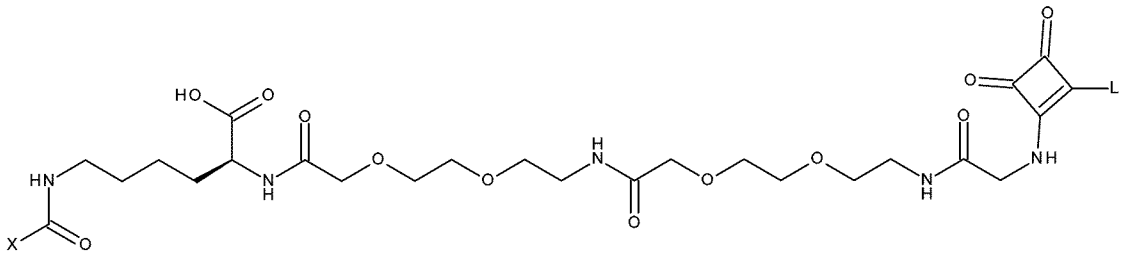

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**[0045]** In some embodiments, the conjugation group A contains at least one spacer between the conjugation group A and X in the formula (I) above (e.g., a polyethylene glycol (PEG) chain (e.g., molecular weight ranging from 100 to 2000 Da) and/or at least one amino acid (e.g., cysteine, glutamic acid, lysine, glycine, etc.). Non-limiting examples include:

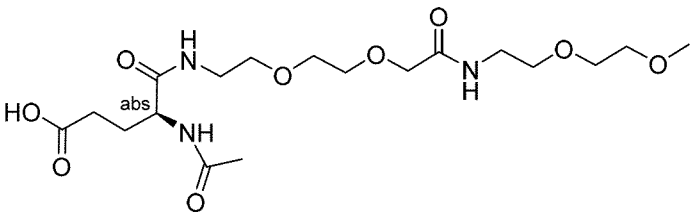

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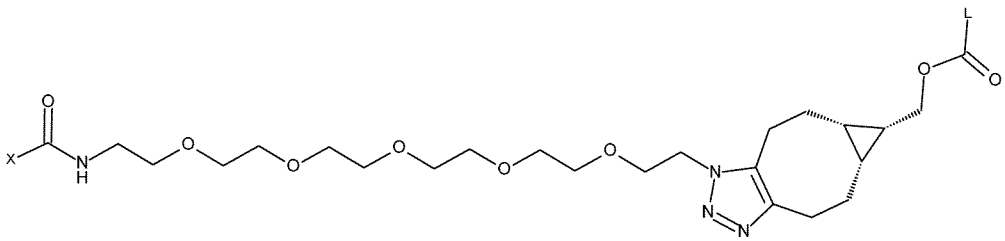
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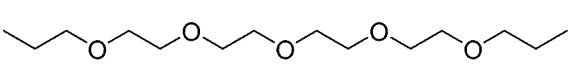
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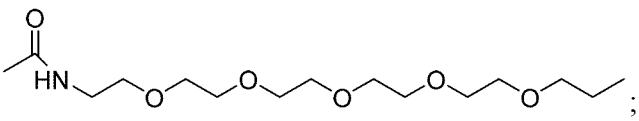
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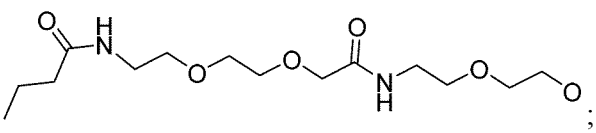
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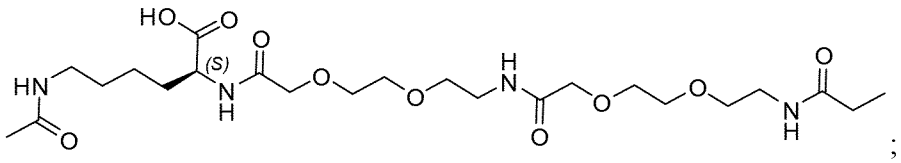
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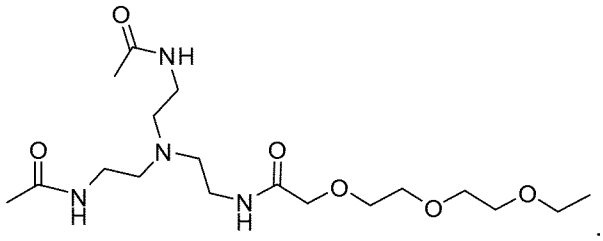
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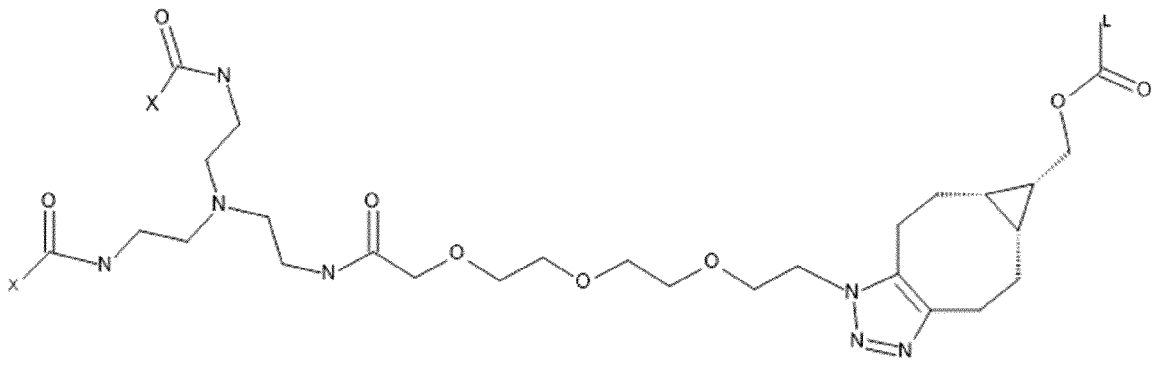


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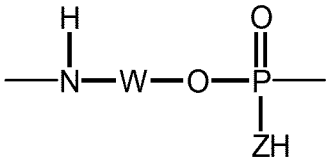
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**[0046]** In some embodiments, the conjugation group A contains at least one other fatty acid chain other than the fatty acid chain of formula (I). For example, the conjugation group A contains two fatty acid chains such as illustrated below:



wherein in each instance, X is $C_4$ to $C_{32}$ alkyl or alkenyl.

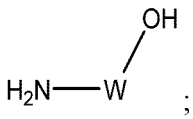
**[0047]** In embodiments of formula (I), L is a linker:

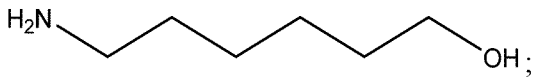


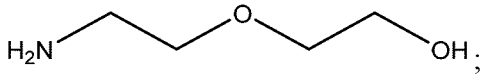
where Z and W are defined below.

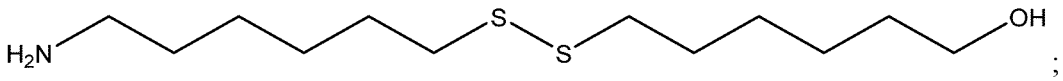
**[0048]** In embodiments of formula (I), Z is O or S.

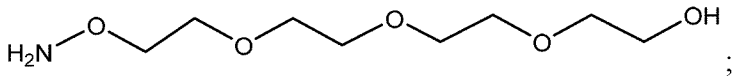
**[0049]** In embodiments of formula (I), W is $C_1$ to $C_{10}$ alkyl or alkenyl, or in some embodiments, W is

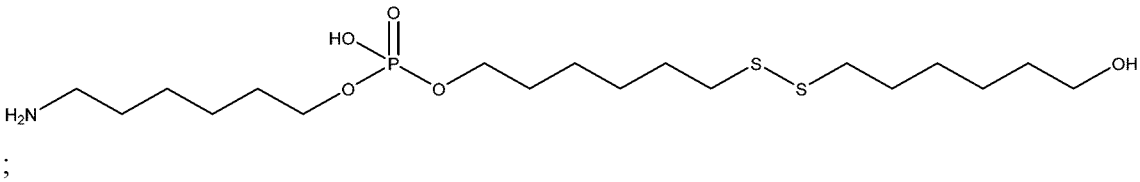


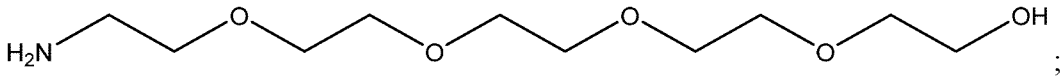


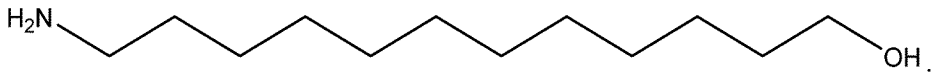












**[0050]** In embodiments, W is $C_2$ to $C_9$ alkyl, such as $C_3$ to $C_8$ alkyl, for example, W is $C_4$ to $C_8$ alkyl. In some embodiments, W is $C_5$ to $C_7$ alkyl.

**[0051]** In embodiments, W is $C_2$ to $C_9$ monounsaturated alkenyl, for instance W is $C_3$ to $C_8$ monounsaturated alkenyl. In some embodiments, W is $C_4$ to $C_8$ monounsaturated alkenyl, such as W is $C_5$ to $C_7$ monounsaturated alkenyl.

**[0052]** In embodiments, W is $C_2$ to $C_9$ polyunsaturated alkenyl, for example W is $C_3$ to $C_8$ polyunsaturated alkenyl. In some embodiments, W is $C_4$ to $C_8$ polyunsaturated alkenyl, such as W is $C_5$ to $C_7$ polyunsaturated alkenyl.

**[0053]** In embodiments, W is $C_1$ alkyl. In embodiments, W is $C_2$ alkyl. In embodiments, W is $C_3$ alkyl. In embodiments, W is $C_4$ alkyl. In embodiments, W is $C_5$ alkyl. In embodiments, W is $C_6$ alkyl. In embodiments, W is $C_7$ alkyl. In embodiments, W is $C_8$ alkyl. In embodiments, W is $C_9$ alkyl. In embodiments, W is $C_{10}$ alkyl.

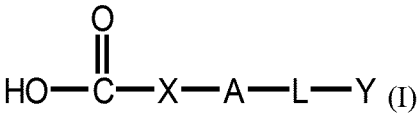
**[0054]** In embodiments, W is $C_2$ monounsaturated alkenyl. In embodiments, W is $C_3$ monounsaturated alkenyl. In embodiments, W is $C_4$ monounsaturated alkenyl. In embodiments, W is $C_5$ monounsaturated alkenyl. In embodiments, W is $C_6$ monounsaturated alkenyl. In embodiments, W is $C_7$ monounsaturated alkenyl. In embodiments, W is $C_8$ monounsaturated alkenyl. In embodiments, W is $C_9$ monounsaturated alkenyl. In embodiments, W is $C_{10}$ monounsaturated alkenyl.

**[0055]** In embodiments, W is $C_2$ polyunsaturated alkenyl. In embodiments, W is $C_3$ polyunsaturated alkenyl. In embodiments, W is $C_4$ polyunsaturated alkenyl. In embodiments, W is $C_5$ polyunsaturated alkenyl. In embodiments, W is $C_6$ polyunsaturated alkenyl. In embodiments, W is $C_7$ polyunsaturated alkenyl. In embodiments, W is $C_8$ polyunsaturated alkenyl. In embodiments, W is $C_9$ polyunsaturated alkenyl. In embodiments, W is $C_{10}$ polyunsaturated alkenyl.

**[0056]** In embodiments, L is -NH-$CH_2$-O-$PO_2$-. In embodiments, L is -NH-$C_2H_4$-O-$PO_2$-. In embodiments, L is -NH-$C_3H_6$-O-$PO_2$-. In embodiments, L is -NH-$C_4H_8$-O-$PO_2$-. In embodiments, L is -NH-$C_5H_{10}$-O-$PO_2$-. In embodiments, L is -NH-$C_6H_{12}$-O-$PO_2$-. In embodiments, L is -NH-$C_7H_{14}$-O-$PO_2$-. In embodiments, L is -NH-$C_8H_{16}$-O-$PO_2$-. In embodiments, L is -NH-$C_9H_{18}$-O-$PO_2$-. In embodiments, L is -NH-$C_{10}H_{20}$-O-$PO_2$-.

**[0057]** In embodiments, L is attached to the 5' end of the oligonucleotide Y. In embodiments, the phosphate group in L is a 5' phosphate group on the oligonucleotide. In embodiments, L is attached to the 3' end of the oligonucleotide Y. In embodiments, L is attached to the oligonucleotide at a modified base in the oligonucleotide Y.

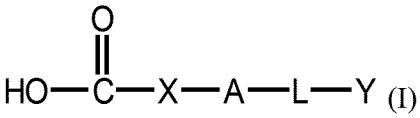
**[0058]** In embodiments, the present disclosure provides an acid acyl conjugated oligonucleotide of formula (I)



(I)

wherein: X is $C_{10}$ to $C_{26}$ alkyl; A is a conjugation group chosen from the groups described above; L is -NH-$C_6H_{12}$-O-$PO_2$-; and Y is an oligonucleotide as described herein attached to L at the 5' end of Y.

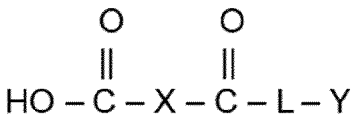
**[0059]** In embodiments, the present disclosure provides an acid acyl conjugated oligonucleotide of formula (I)



(I)

wherein: X is chosen from $C_{14}$ alkyl, $C_{16}$ alkyl, and $C_{20}$ alkyl; A is a conjugation group chosen from the groups described above; L is -NH-$C_6H_{12}$-O-$PO_2$-; and Y is an oligonucleotide as described herein attached to L at the 5' end of Y.

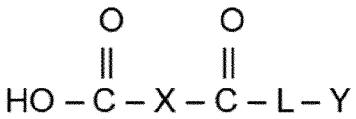
**[0060]** In embodiments, the present disclosure provides an acid acyl conjugated oligonucleotide of formula (Ia)



(Ia)

wherein: X is $C_{14}$ alkyl; L is -NH-$C_6H_{12}$-O-$PO_2$-; and Y is an oligonucleotide as described herein attached to L at the 5' end of Y.

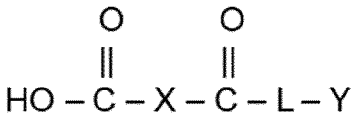
**[0061]** In embodiments, the present disclosure provides an acid acyl conjugated oligonucleotide of formula (Ia)



(Ia)

wherein: X is $C_{16}$ alkyl; L is -NH-$C_6H_{12}$-O-$PO_2$-; and Y is an oligonucleotide as described herein attached to L at the 5' end of Y.

**[0062]** In embodiments, the present disclosure provides an acid acyl conjugated oligonucleotide of formula (Ia)



(Ia)

wherein: X is $C_{20}$ alkyl; L is -NH-$C_6H_{12}$-O-$PO_2$-; and Y is an oligonucleotide as described herein attached to L at the 5' end of Y.

**[0063]** In any of the above embodiments of formula (I), the oligonucleotide Y can be any oligonucleotide described herein.

**Oligonucleotides for conjugation and methods of treatment**

**[0064]** In any embodiment of the present disclosure, the oligonucleotide (Y) of the compounds described herein can comprise DNA, RNA or nucleic acids having unnatural backbones. The oligonucleotide can comprise the natural DNA and RNA nucleobases: adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U), and may also comprise non-natural and modified nucleobases. The oligonucleotide can have a non-natural backbone such as a phosphorothioate backbone. In some embodiments, the oligonucleotide is single stranded. In some embodiments, the oligonucleotide is double stranded.

**[0065]** In embodiments, the oligonucleotide comprises an antisense oligonucleotide. In embodiments, the oligonucleotide comprises a sequence that can be expressed in a cell, e.g., a coding sequence such as a gene or a messenger RNA (mRNA). In embodiments, the oligonucleotide comprises a sequence that does not encode a protein but has another function in the cell, e.g., a non-coding RNA, a transfer RNA (tRNA), a ribosomal RNA (rRNA) or a small-nucleolar RNA (snRNA). In embodiments, the oligonucleotide is a CRISPR guide RNA (gRNA).

**[0066]** In embodiments, the oligonucleotide is an antisense oligonucleotide targeting MALAT1, apolipoprotein B, apolipoprotein C III, endothelial lipase, p53, clusterin, signal transducer and activator of transcription 3 (STAT3), Mothers against decapentaplegic homolog 7 (SMAD7), intercellular adhesion molecule 1 (CD54), dystrophin (for example, myotonic dystrophy protein kinase (DMPK), transthyretin (TTR), huntingtin (HTT), microRNA-122 (miR-122). In embodiments, the oligonucleotide is an antisense oligonucleotide that interferes

with splicing of dystrophin mRNA such as eteplirsen, golodirsen, casimersen, drisapersen and viltolarsen). In embodiments, the oligonucleotide is an antisense oligonucleotide that reduces expression of any of the above targets. In embodiments, the oligonucleotide is an antisense oligonucleotide that reduces mRNA splicing of any of the above targets. In embodiments, the oligonucleotide is an antisense oligonucleotide that targets a non-coding sequence in order to reduce expression of any of the above targets, e.g., an exon, a 5' non-coding sequence or a 3' non-coding sequence.

**[0067]** In some embodiments the oligonucleotide comprises a sequence that can be expressed in a cell, such as a protein expressed by a gene, the lipid conjugated oligonucleotide can be used in methods of treating subjects that lack sufficient expression of the protein, e.g., in gene therapy type methods of treatment. In embodiments where the oligonucleotide comprises a sequence that does not encode a protein but has another function, the lipid conjugated oligonucleotide can be used in methods of treating subjects that lack the function provided by the oligonucleotide.

**[0068]** In embodiments where the oligonucleotide is antisense to a target, the lipid conjugated oligonucleotide can be used in methods of treating subjects having disorders relating to increased levels of expression of the target. In embodiments where the oligonucleotide is antisense to a target, the oligonucleotide reduces expression of the target in cells by about 1% to about 100%. In embodiments, the oligonucleotide reduces expression of the target in cells by about 1%, by about 10%, by about 20%, by about 30%, by about 40%, by about 50%, by about 60%, by about 70%, by about 80%, by about 90%, by about 95% or by about 100%. In embodiments, the oligonucleotide reduces expression of the target in cells by about 1% to about 100%, by about 5% to about 50%, by about 10% to about 50%, by about 30%, by about 10% to about 30%, or by about 15% to about 25%.

**[0069]** In some embodiments, the oligonucleotide is an antisense oligonucleotide targeting a condition effecting the heart, as the carboxy acyl conjugate can be used to preferentially target to the oligonucleotide to cardiac tissue. In embodiments where the oligonucleotide is antisense to a target, the oligonucleotide reduces expression of the target in cardiac cells by about 1% to about 100%. In embodiments, the oligonucleotide reduces expression of the target in cardiac cells by about 1%, by about 10%, by about 20%, by about 30%, by about 40%, by about 50%, by about 60%, by about 70%, by about 80%, by about 90%, by about 95% or by about 100%. In embodiments, the oligonucleotide reduces expression of the target in cardiac cells by about

1% to about 100%, by about 5% to about 50%, by about 10% to about 50%, by about 30%, by about 10% to about 30%, or by about 15% to about 25%.

**[0070]** In embodiments, the oligonucleotide is an antisense oligonucleotide targeting metastasis-associated lung adenocarcinoma transcript 1 (MALAT1). In embodiments, the oligonucleotide is an antisense oligonucleotide targeting MALAT1, variant 1 (SEQ ID NO:1). In embodiments, the oligonucleotide is an antisense oligonucleotide targeting MALAT1, variant 2 (SEQ ID NO:2). In embodiments, the oligonucleotide is an antisense oligonucleotide targeting MALAT1, variant 3 (SEQ ID NO:3). In embodiments, the antisense oligonucleotide targets MALAT1 and has the sequence: tcagcattctaatagcagc (SEQ ID NO:4). In embodiments, the antisense oligonucleotide targets MALAT1 and has the sequence: tm5cagm5cattm5ctaatagm5cagm5c, where m5c is 5-methylcytidine (SEQ ID NO:5). In embodiments, the antisense oligonucleotide targets MALAT1 and has the sequence: gcattctaatagcagc (SEQ ID NO:6). In embodiments, the antisense oligonucleotide targets MALAT1 and has the sequence: gm5cattm5ctaatagm5cagm5c, where m5c is 5-methylcytidine (SEQ ID NO:7). In embodiments, the oligonucleotide is an antisense oligonucleotide that targets MALAT1 and reduces expression of MALAT1 in cells by about 1% to about 100%. In embodiments, the oligonucleotide is an antisense oligonucleotide that targets MALAT1 and reduces expression of MALAT1 in cells by about 1%, by about 10%, by about 20%, by about 30%, by about 40%, by about 50%, by about 60%, by about 70%, by about 80%, by about 90%, by about 95% or by about 100%.

**[0071]** In embodiments, the oligonucleotide is an antisense oligonucleotide targeting metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) comprising at least one nucleic acid with a locked sugar modified moiety ("LNA"). In such embodiments, the antisense oligonucleotide targets MALAT1 and has the sequence: GM5CAttm5ctaatagm5cAGM5C, where m5c is 5-methylcytidine and capital letters are LNA nucleosides (SEQ ID NO:8). In embodiments, the oligonucleotide is an antisense oligonucleotide that targets MALAT1 and reduces expression of MALAT1 in cells by about 1% to about 100%. In embodiments, the oligonucleotide is an antisense oligonucleotide that targets MALAT1 and reduces expression of MALAT1 in cells by about 1%, by about 10%, by about 20%, by about 30%, by about 40%, by about 50%, by about 60%, by about 70%, by about 80%, by about 90%, by about 95% or by about 100%.

**[0072]** In embodiments, the oligonucleotide is an antisense oligonucleotide targeting Calcium/Calmodulin Dependent Protein Kinase II Delta (CAMK2D). In embodiments, the antisense oligonucleotide targeting CAMK2D comprises at least one nucleic acid with a locked sugar modified moiety ("LNA"). In some embodiments, the antisense oligonucleotide targets CAMK2D and has the sequence: GTTtggtattm5cttTAG, where m5c is 5-methylcytidine and capital letters are LNA nucleosides (SEQ ID NO:9). In some embodiments, the antisense oligonucleotide targets CAMK2D and has the sequence: GTGtm5caam5caam5cm5caTTT, where m5c is 5-methylcytidine and capital letters are LNA nucleosides (SEQ ID NO:10). In some embodiments, the antisense oligonucleotide targets CAMK2D and has the sequence: M5CAM5CAaatttattaaM5CTM5CT, where m5c is 5-methylcytidine and capital letters are LNA nucleosides (SEQ ID NO:11). In some embodiments, the antisense oligonucleotide targets CAMK2D and has the sequence: M5CTGttm5cttm5caAtaATG, where m5c is 5-methylcytidine and capital letters are LNA nucleosides (SEQ ID NO:12). In some embodiments, the antisense oligonucleotide targets CAMK2D and has the sequence: AM5CM5Catgagm5ctataM5CTT, where m5c is 5-methylcytidine and capital letters are LNA nucleosides (SEQ ID NO:13). In embodiments, the oligonucleotide is an antisense oligonucleotide that targets CAMK2D and reduces expression of CAMK2D in cells by about 1% to about 100%. In embodiments, the oligonucleotide is an antisense oligonucleotide that targets CAMK2D and reduces expression of CAMK2D in cells by about 1%, by about 10%, by about 20%, by about 30%, by about 40%, by about 50%, by about 60%, by about 70%, by about 80%, by about 90%, by about 95% or by about 100%.

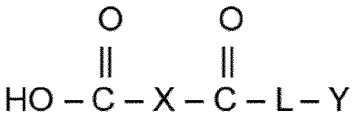
**[0073]** In embodiments, the oligonucleotide is an antisense oligonucleotide that targets MALAT1 and reduces expression of MALAT1 in cardiac cells by about 1% to about 100%. In embodiments, the oligonucleotide is an antisense oligonucleotide that targets MALAT1 and reduces expression of MALAT1 in cardiac cells by about 1%, by about 10%, by about 20%, by about 30%, by about 40%, by about 50%, by about 60%, by about 70%, by about 80%, by about 90%, by about 95% or by about 100%.

**[0074]** In embodiments where the oligonucleotide is an antisense oligonucleotide that targets MALAT1, the lipid conjugated oligonucleotide can be used in a method of treatment of a cardiac disease in a subject. In embodiments where the oligonucleotide is an antisense oligonucleotide that targets MALAT1, the lipid conjugated oligonucleotide can be used in a method of treatment of a myocardial infarction in a subject. In embodiments where the

oligonucleotide is an antisense oligonucleotide that targets MALAT1, the lipid conjugated oligonucleotide can be used in a method of preventing a myocardial infarction in a subject. In embodiments where the oligonucleotide is an antisense oligonucleotide that targets MALAT1, the oligonucleotide is administered to a subject at risk for a myocardial infarction.

**Methods of making lipid conjugated oligonucleotides**

**[0075]** In embodiments, the present disclosure provides methods of making the lipid conjugated oligonucleotides described herein. In embodiments, the present disclosure provides a method of making an acid acyl oligonucleotide of formula (Ia):

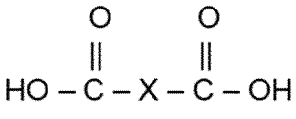


(Ia)

wherein: X is $C_4$ to $C_{32}$ alkyl or alkenyl; L is a linker; and Y is an oligonucleotide.

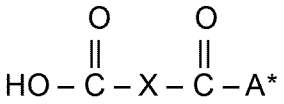
**[0076]** In embodiments, the method of making formula (I) comprises the following steps:

A) providing a fatty diacid of formula (II)



(II);

B) reacting the fatty diacid with an activating group, A, to form an acid acyl compound of formula (III):



(III);

wherein A* is the activating group attached to the fatty acyl compound; and

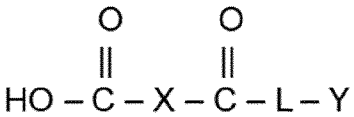
C) reacting the compound of formula (III) with a compound of formula (IV):



(IV),

wherein *L is a linker with a reactive group;

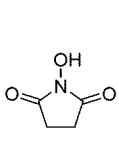
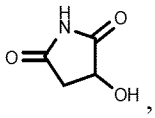
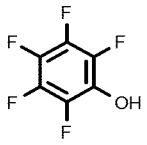
to form the compound of formula (Ia):



(Ia)

wherein: X is $C_4$ to $C_{32}$ alkyl or alkenyl; L is a linker; and Y is an oligonucleotide.

**[0077]** In embodiments of the method of making formula (I), the activating group A* is an activated ester amine. In embodiments, the activating group A* in step B) may be chosen from:


,

,

, TBTU, and HATU.

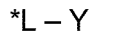
**[0078]** In embodiments of the method of making formula (I), the reaction in step B) requires reacting a carboxylic acid with an amine to form an activated ester amine. In embodiments, the reaction in step B) of the method of making requires reacting a carboxylic acid with N-hydroxysuccinimide (NHS) to form an NHS ester as an activated ester amine.

**[0079]** In embodiments, the reaction in step B) of the method of making is performed in an organic solvent. In embodiments, the reaction in step B) of the method of making is performed in a solvent comprising one or more of ethyl acetate, dioxane, tetrahydrofuran, dimethylfuran and dichloromethane and mixtures thereof.

**[0080]** In embodiments, the reaction in step B) of the method of making is performed in the presence of a coupling reagent. In embodiments, the coupling reagent is a diimine. In embodiments, the coupling reagent is dicyclohexylmethanediimine.

**[0081]** In embodiments of the method of making formula (I), the reaction in step C) requires coupling between the activating group A* attached to the fatty acyl compound and a primary amine on the linker (L).

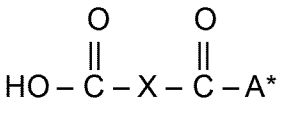
**[0082]** In embodiments of the method of making formula (I), the compound of formula (IV)



(IV)

is dissolved in an aqueous buffer. In embodiments, the compoud of formula (IV) is dissolved in a phosphate buffer, a borate buffer, a carbonate buffer, an acetate buffer, a Tris buffer, a HEPES buffer, a MOPS buffer or a PIPES buffer, or is dissolved in pure water with a base such as triethylamine or DIPEA.

**[0083]** In embodiments of the method of making formula (I), the compound of formula (III)

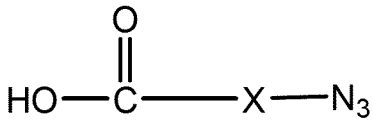


(III);

is dissolved in a polar aprotic solvent. In embodiments of the method of making formula (I), the compound of formula (III) is dissolved in acetonitrile. In other embodiments of the method of making formula (I), the compound of formula (III) is dissolved in dimethyl sulfoxide. In other embodiments of the method of making formula (I), the compound of formula (III) is dissolved in a mixture of acetonitrile and dimethyl sulfoxide.

**[0084]** In other embodiments, the method of making formula (I) comprises the following steps:

A) providing a fatty acid of formula (IIa)



(IIa);

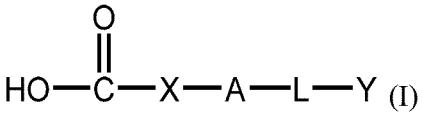
B) reacting the compound of formula (IIa) with a compound of formula (IV):



(IV),

wherein *L is a linker with a reactive group;

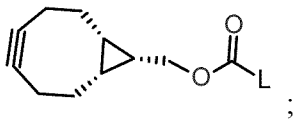
to form the compound of formula (I):



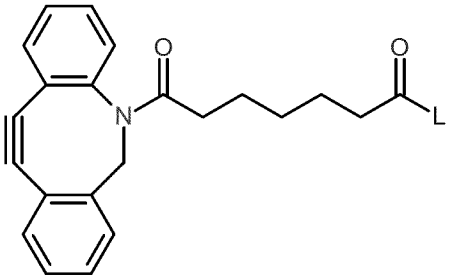
(I)

wherein: X is $C_4$ to $C_{32}$ alkyl or alkenyl; A is a conjugation group; L is a linker; and Y is an oligonucleotide as described above.

**[0085]** In embodiments of the method of making formula (I), the linker with a reactive group L* is



;



**[0086]** In embodiments of the method of making formula (I), the resultant product of either step B) and /or step C) can be purified. In embodiments, the resultant product of either step B) and/or step C) is purified by chromatography. In embodiments, the resultant product of either step B) and/or step C) is purified by flash chromatography.

**[0087]** In embodiments of any of the above methods for making formula (I), the length of the acyl group, X, is $C_6$ to $C_{30}$ alkyl or alkenyl. In embodiments, X is $C_8$ to $C_{28}$ alkyl or alkenyl. In embodiments, X is $C_{10}$ to $C_{26}$ alkyl or alkenyl. In embodiments, X is $C_{12}$ to $C_{26}$ alkyl or alkenyl. In embodiments, X is $C_{14}$ to $C_{24}$ alkyl or alkenyl. In embodiments, X is $C_{14}$ alkyl or alkenyl. In embodiments, X is $C_{16}$ alkyl or alkenyl. In embodiments, X is $C_{20}$ alkyl or alkenyl.

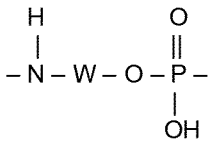
**[0088]** In embodiments of any of the above methods for making formula (I), the length of the acyl group, X, is $C_6$ to $C_{30}$ alkyl. In embodiments, X is $C_8$ to $C_{28}$ alkyl. In embodiments, X is $C_{10}$ to $C_{26}$ alkyl. In embodiments, X is $C_{12}$ to $C_{26}$ alkyl. In embodiments, X is $C_{14}$ to $C_{24}$ alkyl. In embodiments, X is $C_{14}$ alkyl. In embodiments, X is $C_{16}$ alkyl. In embodiments, X is $C_{20}$ alkyl.

**[0089]** In embodiments of any of the above methods for making formula (I), X is $C_6$ to $C_{30}$ monounsaturated alkenyl. In embodiments, X is $C_8$ to $C_{28}$ monounsaturated alkenyl. In embodiments, X is $C_{10}$ to $C_{26}$ monounsaturated alkenyl. In embodiments, X is $C_{12}$ to $C_{26}$ monounsaturated alkenyl. In embodiments, X is $C_{14}$ to $C_{24}$ monounsaturated alkenyl. In embodiments, X is $C_{14}$ monounsaturated alkenyl. In embodiments, X is $C_{16}$ monounsaturated alkenyl. In embodiments, X is $C_{20}$ monounsaturated alkenyl.

**[0090]** In any of the above methods for making formula (I), X is $C_6$ to $C_{30}$ polyunsaturated alkenyl. In embodiments, X is $C_8$ to $C_{28}$ polyunsaturated alkenyl. In embodiments, X is $C_{10}$ to $C_{26}$ polyunsaturated alkenyl. In embodiments, X is $C_{12}$ to $C_{26}$ polyunsaturated alkenyl. In embodiments, X is $C_{14}$ to $C_{24}$ polyunsaturated alkenyl. In embodiments, X is $C_{14}$ polyunsaturated alkenyl. In embodiments, X is $C_{16}$ polyunsaturated alkenyl. In embodiments, X is $C_{20}$ polyunsaturated alkenyl.

**[0091]** In embodiments of any of the above methods for making formula (I), the linker (L) comprises a $C_3$-$C_{10}$ amine. In embodiments of the method of making formula (I), the linker (L) comprises a $C_4$-$C_9$ amine. In embodiments of the method of making formula (I), the linker (L) comprises a $C_5$-$C_8$ amine. In embodiments of the method of making formula (I), the linker (L) comprises a $C_6$ or $C_7$ amine. In embodiments of the method of making formula (I), the linker (L) comprises a $C_6$ amine (hexylamine).

**[0092]** In embodiments, the linker (L) forms an amide bond with the carboxy acyl group. In embodiments, the linker (L) comprises a phosphate terminal connected to the oligonucleotide. In some embodiments, the linker (L) is



wherein W is $C_1$ to $C_{10}$ alkyl or alkenyl.

**[0093]** In embodiments, W is $C_2$ to $C_9$ alkyl. In embodiments, W is $C_3$ to $C_8$ alkyl. In embodiments, W is $C_4$ to $C_8$ alkyl. In embodiments, W is $C_5$ to $C_7$ alkyl.

**[0094]** In embodiments, W is $C_2$ to $C_9$ monounsaturated alkenyl. In embodiments, W is $C_3$ to $C_8$ monounsaturated alkenyl. In embodiments, W is $C_4$ to $C_8$ monounsaturated alkenyl. In embodiments, W is $C_5$ to $C_7$ monounsaturated alkenyl.

**[0095]** In embodiments, W is $C_2$ to $C_9$ polyunsaturated alkenyl. In embodiments, W is $C_3$ to $C_8$ polyunsaturated alkenyl. In embodiments, W is $C_4$ to $C_8$ polyunsaturated alkenyl. In embodiments, W is $C_5$ to $C_7$ polyunsaturated alkenyl.

**[0096]** In embodiments, W is $C_1$ alkyl. In embodiments, W is $C_2$ alkyl. In embodiments, W is $C_3$ alkyl. In embodiments, W is $C_4$ alkyl. In embodiments, W is $C_5$ alkyl. In embodiments, W is $C_6$ alkyl. In embodiments, W is $C_7$ alkyl. In embodiments, W is $C_8$ alkyl. In embodiments, W is $C_9$ alkyl. In embodiments, W is $C_{10}$ alkyl.

**[0097]** In embodiments, W is $C_2$ monounsaturated alkenyl. In embodiments, W is $C_3$ monounsaturated alkenyl. In embodiments, W is $C_4$ monounsaturated alkenyl. In embodiments, W is $C_5$ monounsaturated alkenyl. In embodiments, W is $C_6$ monounsaturated alkenyl. In embodiments, W is $C_7$ monounsaturated alkenyl. In embodiments, W is $C_8$ monounsaturated alkenyl. In embodiments, W is $C_9$ monounsaturated alkenyl. In embodiments, W is $C_{10}$ monounsaturated alkenyl.

**[0098]** In embodiments, W is $C_2$ polyunsaturated alkenyl. In embodiments, W is $C_3$ polyunsaturated alkenyl. In embodiments, W is $C_4$ polyunsaturated alkenyl. In embodiments, W is $C_5$ polyunsaturated alkenyl. In embodiments, W is $C_6$ polyunsaturated alkenyl. In embodiments, W is $C_7$ polyunsaturated alkenyl. In embodiments, W is $C_8$ polyunsaturated alkenyl. In embodiments, W is $C_9$ polyunsaturated alkenyl. In embodiments, W is $C_{10}$ polyunsaturated alkenyl.

**[0099]** In embodiments, the linker (L) is -NH-$CH_2$-O-$PO_2$-. In embodiments, L is -NH-$C_3H_6$-O-$PO_2$-. In embodiments, L is -NH-$C_4H_8$-O-$PO_2$-. In embodiments, L is -NH-$C_5H_{10}$-O-$PO_2$-. In embodiments, L is -NH-$C_6H_{12}$-O-$PO_2$-. In embodiments, L is -NH-$C_7H_{14}$-O-$PO_2$-. In embodiments, L is -NH-$C_8H_{16}$-O-$PO_2$-. In embodiments, L is -NH-$C_9H_{18}$-O-$PO_2$-. In embodiments, L is -NH-$C_{10}H_{20}$-O-$PO_2$-.

**[00100]** In embodiments of any of the above methods for making formula (I), L is attached to the 5' end of the oligonucleotide Y. In embodiments, L is attached to the 3' end of the oligonucleotide Y. In embodiments, L is attached to the oligonucleotide at a modified base in the oligonucleotide Y.

**[00101]** In embodiments of any of the above methods for making formula (I), the oligonucleotide can be unmodified DNA, RNA or may be modified. Modified oligonucleotides comprise at least one modification relative to unmodified RNA or DNA (i.e., comprise at least one modified nucleoside (comprising a modified sugar moiety and/or a modified nucleobase and/or at least one modified internucleoside linkage). In embodiments, the oligonucleotide can be selected from any of the oligonucleotides described herein. In embodiments, the

oligonucleotide is an antisense oligonucleotide. In embodiments, the antisense oligonucleotide contains at least one phosphorothioate internucleoside linkage. In embodiments, the antisense oligonucleotide contains at least one modified sugar moiety, for example, a bicyclic sugar moiety (e.g., comprising two rings, wherein the second ring is formed via a bridge connecting two of the atoms in the first ring thereby forming a bicyclic structure). In embodiments, the antisense oligonucleotide contains at least one modified nucleobase.

**Methods of delivery and use of lipid conjugated oligonucleotides**

**[00102]** In embodiments, the lipid conjugated oligonucleotides are preferentially delivered to a specific tissue in the body upon administration. In embodiments, the lipid conjugated oligonucleotides are, for instance, delivered to cardiac tissue upon administration. In embodiments, the lipid conjugated oligonucleotides are, for example, delivered to liver tissue upon administration. In embodiments, the lipid conjugated oligonucleotides are, for instance, delivered to spleen tissue upon administration. In embodiments, the lipid conjugated oligonucleotides are, for example, delivered to kidney tissue upon administration. In embodiments, the lipid conjugated oligonucleotides are, for example, delivered to skin tissue upon administration. In embodiments, the lipid conjugated oligonucleotides are, for example, delivered to muscle tissue upon administration. In embodiments, the lipid conjugated oligonucleotides are, for example, delivered to lung tissue upon administration. In embodiments, the lipid conjugated oligonucleotides are, for example, delivered to adipose tissue upon administration.

**[00103]** In embodiments, the present disclosure provides a method of delivering an oligonucleotide to cardiac tissue in a subject comprising: a) providing an acid acyl conjugated oligonucleotide as described herein, and b) administering the acid acyl conjugated oligonucleotide to the subject.

**[00104]** In embodiments, the present disclosure provides a method of reducing expression of a gene of interest in the cardiac cells of a subject, comprising a) providing an acid acyl conjugated oligonucleotide as described herein, and b) administering the acid acyl conjugated oligonucleotide to the subject.

**[00105]** In embodiments of the method of reducing expression of a gene of interest in the cardiac cells of a subject, the gene of interest is any antisense target described herein. In

embodiments of the method of reducing expression of a gene of interest in the cardiac cells of a subject, the gene of interest is MALAT1.

**[00106]** In embodiments of the methods herein, the oligonucleotide can be DNA, RNA or a modified oligonucleotide. In embodiments, the oligonucleotide can be selected from any of the oligonucleotides described herein. In embodiments, the oligonucleotide is an antisense oligonucleotide. In embodiments, the oligonucleotide has a phosphorothioate backbone.

**[00107]** In embodiments of the methods herein, the subject is a mammal. In embodiments of the methods herein, the mammalian subject is an animal such as an agricultural animal (e.g., cattle, sheep, swine), research animal (e.g., mice, rats, monkeys, chimpanzees) or companion animal (e.g., dogs, cats and rabbits). In embodiments of the methods herein, the mammalian subject is a human.

**[00108]** In embodiments of the methods herein, the oligonucleotide is administered to treat a cardiac disease. In embodiments of the methods herein, the oligonucleotide is administered to treat a myocardial infarction. In embodiments of the methods herein, the oligonucleotide is administered to prevent a myocardial infarction. In embodiments of the method of delivering an oligonucleotide to cardiac tissue in a subject, the oligonucleotide is administered to a subject at risk for a myocardial infarction.

**Pharmaceutical formulations**

**[00109]** In embodiments, the lipid conjugated oligonucleotides described herein are formulated into a pharmaceutically acceptable formulation. In some embodiments, the pharmaceutical formulation further comprises a pharmaceutically acceptable excipient, e.g., tonicity adjusting agent, preservative, solubilizing agent, complexing agent, dispersing agent, buffering agent, or combination thereof. In some embodiments, the pharmaceutical formulation is suitable for administration to a patient. In some embodiments, the pharmaceutical formulation is suitable for intramuscular, subcutaneous, intravenous, intraperitoneal or oral administration to a patient.

## EXAMPLES

**[00110] INTERMEDIATES**

**[00111] Activated C22 Acid (Intermediate 1) – 22-((2,5-dioxopyrrolidin-1-yl)oxy)-22-oxodocosanoic acid**

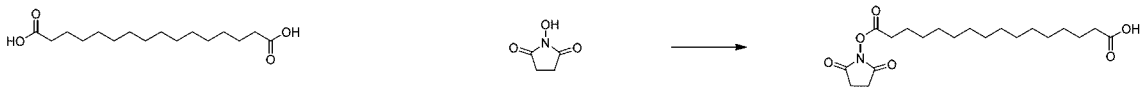


Docosanedioic acid (0.5 g, 1.35 mmol), 1-hydroxypyrrolidine-2,5-dione (0.155 g, 1.35 mmol) and N,N-dimethylpyridin-4-amine (cat. amount) were added to tetrahydrofuran (THF) (17 mL) and stirred at room temperature for 10 min. Dicyclohexylmethanediimine (0.278 g, 1.35 mmol) solvated in THF (6.00 mL) was added dropwise for 30 min. and the mixture was then stirred for 24 h. at room temperature. After filtration, the mixture was evaporated. Methanol (MeOH) (5 ml) was added to the residue, heated to 45°C and was then stirred for 1 h. at room temperature. The product (22-((2,5-dioxopyrrolidin-1-yl)oxy)-22-oxodocosanoic acid) was crystalized and was separated by filtration, washed with a small amount of MeOH and dried under reduced pressure to give the desired product: 22-((2,5-dioxopyrrolidin-1-yl)oxy)-22-oxodocosanoic acid. Yield was 353 mg (56%). LC/MS and H, NMR are in agreement with the expected product.

MS (ESI) m/z 466.6 $[M - H]^-$

$^1H$ NMR (500 MHz, $CDCl_3$) 1.1 – 1.46 (33H, m), 1.63 (2H, t), 1.68 – 1.79 (2H, m), 2.35 (2H, td), 2.60 (2H, t) 2.84 (4H, d),

**[00112] NHS Activated C16 Acid (Intermediate 2) – 16-((2,5-dioxopyrrolidin-1-yl)oxy)-16-oxohexadecanoic acid**



The compound was made according to **Intermediate 1** starting from hexadecanedioic acid (0.3g, 1.05 mmol). After crystallization, the residue was purified with flash chromatography on silica using ethyl acetate/ Heptane 1/1 as eluent. The pure fractions was evaporated to give the desired product (16-((2,5-dioxopyrrolidin-1-yl)oxy)-16-oxohexadecanoic acid) Yield: 120 mg (30%).

MS (ESI) m/z 382.0 $[M - H]^-$

$^1$H NMR (500 MHz, CDCl3) 1.28 (21H, d), 1.64 (2H, td), 1.69 – 1.79 (2H, m), 2.35 (2H, td), 2.60 (2H, t), 2.84 (4H, d).

**[00113] NHS Activated C17 Acid (Intermediate 3) - 17-((2,5-dioxopyrrolidin-1-yl)oxy)-17-oxoheptadecanoic acid**

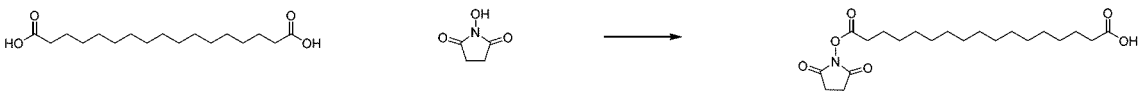


The compound was made according to **Intermediate 1** starting from heptadecanedioic acid (0.3g, 1.0 mmol). After crystallization, the residue was purified with flash chromatography on silica using ethyl acetate/ Heptane 1/1 as eluent. The pure fraction was evaporated to give of the desired product (17-((2,5-dioxopyrrolidin-1-yl)oxy)-17-oxoheptadecanoic acid) Yield: 99 mg, 25%.

MS (ESI) m/z 396.2 $[M - H]^-$

$^1$H NMR (500 MHz, $CDCl_3$) 1.27 (23H, d), 1.55 – 1.69 (2H, m), 1.74 (2H, p), 2.35 (2H, td), 2.60 (2H, t), 2.76 – 2.91 (4H, m).

**[00114] NHS Activated C18 Acid (Intermediate 4) - 18-((2,5-dioxopyrrolidin-1-yl)oxy)-18-oxooctadecanoic acid**



The compound was made according to **Intermediate 1** starting from octadecanedioic acid (0.3g, 0.95 mmol). After crystallization, the residue was purified with flash chromatography on silica using ethyl acetate/ Heptane 2/1 as eluent. The pure fraction was evaporated to give of the desired product (18-((2,5-dioxopyrrolidin-1-yl)oxy)-18-oxooctadecanoic acid) Yield: 93 mg, 24%.

MS (ESI) m/z 410.3 $[M - H]^-$

$^1$H NMR (500 MHz, $CDCl_3$) 1.25 (25H, s), 1.63 (2H, q), 1.74 (2H, p), 2.35 (2H, t), 2.60 (2H, t), 2.83 (4H, s).

**[00115] NHS Activated C20 Acid (Intermediate 5) - 20-((2,5-dioxopyrrolidin-1-yl)oxy)-20-oxoicosanoic acid**

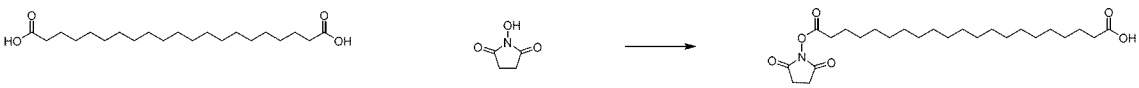


The compound (20-((2,5-dioxopyrrolidin-1-yl)oxy)-20-oxoicosanoic acid) was made according to **Intermediate 1** starting from icosanedioic acid (0.3g, 0.88 mmol). Yield: 229 mg, 59%.

MS (ESI) m/z 438.2 $[M - H]^-$

$^1H$ NMR (500 MHz, $CDCl_3$) 1.2 – 1.45 (29H, m), 1.63 (2H, qd), 1.74 (2H, p), 2.35 (2H, td), 2.60 (2H, t), 2.83 (4H, d).

**[00116] NHS Activated C21 Acid (Intermediate 6) - 21-((2,5-dioxopyrrolidin-1-yl)oxy)-21-oxohenicosanoic acid**

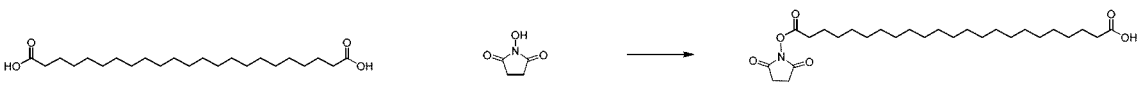


The compound was made according to **Intermediate 1** starting from henicosanedioic acid (0.3g, 1.05 mmol). After crystallization, the residue was purified with flash chromatography on silica using ethyl acetate/ Heptane 1/1 as eluent. The pure fraction was evaporated to give of the desired product (21-((2,5-dioxopyrrolidin-1-yl)oxy)-21-oxohenicosanoic acid) Yield: 21 mg, (6%).

MS (ESI) m/z 452.0 $[M - H]^-$

1H NMR (500 MHz, CDCl3) 1.27 (31H, d), 1.58 – 1.69 (2H, m), 1.69 – 1.81 (2H, m), 2.35 (2H, t), 2.60 (2H, t), 2.77 – 2.91 (4H, m).

**[00117] NHS Activated C23 Acid (Intermediate 7) - 23-((2,5-dioxopyrrolidin-1-yl)oxy)-23-oxotricosanoic acid**

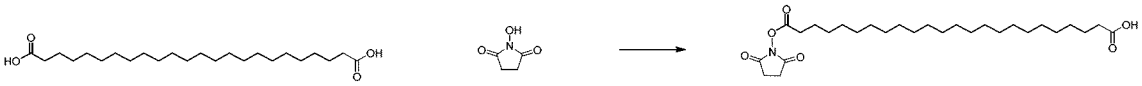


The compound (23-((2,5-dioxopyrrolidin-1-yl)oxy)-23-oxotricosanoic acid) was made according to **Intermediate 1** starting from tricosanedioic acid (66 mg, 0.17 mmol). The solids were isolated by centrifugation. Yield: 60 mg, (73%).

MS (ESI) m/z 480.1 $[M - H]^-$

$^1H$ NMR (500 MHz, $CDCl_3$) 1.26 (35H, d), 1.64 (2H, td), 1.74 (2H, p), 2.3 – 2.38 (2H, m), 2.60 (2H, t), 2.77 – 2.9 (4H, m).

**[00118] NHS Activated C24 Acid (Intermediate 8) - 24-((2,5-dioxopyrrolidin-1-yl)oxy)-24-oxotetracosanoic acid**



The compound was made according to **Intermediate 1** starting from tetracosanedioic acid (0.3g, 0.75 mmol). After crystallization, the residue was purified with flash chromatography on silica using ethyl acetate/ Heptane 1/1 as eluent. The pure fraction was evaporated to give of the desired product (24-((2,5-dioxopyrrolidin-1-yl)oxy)-24-oxotetracosanoic acid) Yield: 44 mg, 12%.

MS (ESI) m/z 494.1 $[M - H]^-$

$^1H$ NMR (500 MHz, $CDCl_3$) 1.25 (37H, s), 1.6 – 1.67 (2H, m), 1.69 – 1.79 (2H, m), 2.35 (2H, t), 2.60 (2H, t), 2.84 (4H, d).

**[00119] NHS Activated C19 Acid (Intermediate 9) - 1-(tert-butyl) 19-(2,5-dioxopyrrolidin-1-yl) nonadecanedioate**

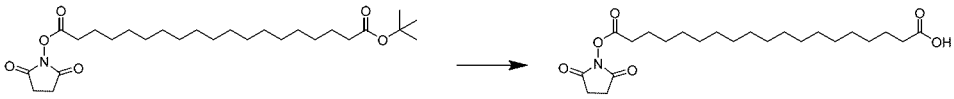


19-(tert-butoxy)-19-oxononadecanoic acid (0.4 g, 1.04 mmol), 1-hydroxypyrrolidine-2,5-dione (0.120 g, 1.04 mmol) and N,N-dimethylpyridin-4-amine (0.635 mg, 5.20 µmol) was added to THF (7 mL) and was stirred at r.t. for 10 min. dicyclohexylmethanediimine (0.215 g, 1.04 mmol) solved in THF (3 mL) was added dropwise during 30 min. and the mixture was then stirred for 24 h. at r.t.. After filtration the mixture was evaporated. MeOH (5 ml) was added to the residue, heated to 60°C and was then stirred for 1 h. at r.t. The product (1-(tert-

butyl) 19-(2,5-dioxopyrrolidin-1-yl) nonadecanedioate) was crystalized and was separated by filtration, washed with a small amount of MeOH and dried under reduced pressure. Yield: 361 mg, 72%

$^1$H NMR (500 MHz, $CDCl_3$) 1.27 (24H, d), 1.40 (2H, s), 1.44 (9H, s), 1.51 – 1.63 (2H, m), 1.69 – 1.8 (2H, m), 2.19 (2H, t), 2.60 (2H, t), 2.83 (4H, d).

**[00120] Intermediate 10 - 19-((2,5-dioxopyrrolidin-1-yl)oxy)-19-oxononadecanoic acid**



**Intermediate 9** - 1-(tert-butyl) 19-(2,5-dioxopyrrolidin-1-yl) nonadecanedioate (150 mg, 0.31 mmol) was added to 2,2,2-trifluoroacetic acid (3 mL, 0.31 mmol) and the reaction mixture was stirred 4h. at r.t. The TFA was evaporated and co-evaporated 3 times with toluene and one time with DCM. The residue was solved in MeOH (4ml) at 60 °C and the solution was stirred at r.t for 15 min. The product was precipitated, filtered and dried under vacuum to give 76 mg (57%) of the desired compound (19-((2,5-dioxopyrrolidin-1-yl)oxy)-19-oxononadecanoic acid).

MS (ESI) m/z 424.0 $[M - H]^-$

$^1$H NMR (500 MHz, $CDCl_3$) 1.26 (27H, d), 1.63 (2H, q), 1.74 (2H, p), 2.35 (2H, t), 2.60 (2H, t), 2.76 – 2.9 (4H, m).

**[00121] NHS Activated C18 (9Z) Acid (Intermediate 11) - (Z)-18-((2,5-dioxopyrrolidin-1-yl)oxy)-18-oxooctadec-9-enoic acid**



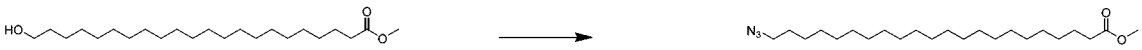
(Z)-octadec-9-enedioic acid (88 mg, 0.28 mmol), 1-hydroxypyrrolidine-2,5-dione (32.4 mg, 0.28 mmol) and N,N-dimethylpyridin-4-amine (0.344 mg, 2.82 µmol) was added to THF (2 mL) and was stirred at r.t. for 10 min. The solution was cooled on an icebath to 0 gr and dicyclohexylmethanediimine (58.1 mg, 0.28 mmol) solved in THF (1 mL) was added dropwise during 15 min. and the mixture was then stirred for 24 h. at r.t. After filtration the mixture was evaporated. MeOH (0.5 ml) was added to the residue. The solids were removed

by filtration (impurities).and the filtrate was evaporated to give the desired product (Z)-18-((2,5-dioxopyrrolidin-1-yl)oxy)-18-oxooctadec-9-enoic acid). Yield: 90 mg (78 %)

MS (ESI) m/z 408.2 $[M - H]^-$

$^1H$ NMR (500 MHz, $CDCl_3$) 1.22 – 1.45 (16H, m), 1.62 (3H, qd), 1.74 (2H, p), 2.00 (4H, q), 2.3 – 2.39 (2H, m), 2.59 (2H, t), 2.83 (4H, d), 5.3 – 5.42 (2H, m).

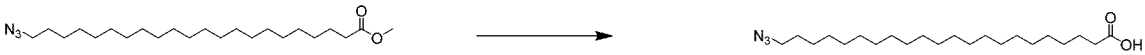
**[00122] Azid C22 Acid (Intermediate 12) - methyl 22-azidodocosanoate**



Diphenyl phosphorazidate (46.4 µl, 0.22 mmol) solved in THF (1 mL) was added to methyl 22-hydroxydocosanoate (40 mg, 0.11 mmol), diisopropyl (E)-diazene-1,2-dicarboxylate (42.5 µl, 0.22 mmol) and triphenylphosphane (56.6 mg, 0.22 mmol) solved in THF (2 mL) at r.t. The reaction mixture was stirred for 3 h. The THF was evaporated and the residue was purified with flash chromatography on silica using heptane/ethylacetate10/1 as eluent. The product was purified a second time with flash. heptane/ethyl acetate 10/1. The pure fraction was evaporated to give 31mg, 91% of the desired product (methyl 22-azidodocosanoate).

$^1H$ NMR (500 MHz, $CDCl_3$) 1.25 (34H, s), 1.54 – 1.66 (4H, m), 2.29 (2H, t), 3.25 (2H, t), 3.66 (3H, s).

**[00123] Intermediate 13 – 22-azidodocosanoic acid**



**Intermediate 12 -** Methyl 22-azidodocosanoate (35 mg, 0.09 mmol) was added to a solution of lithium hydroxide (10.59 mg, 0.44 mmol) in MeOH (0.5 mL) and water (0.1 mL). The reaction mixture was stirred over night at r.t. Ethyl acetate (1 ml), water (1ml) and hydrogen chloride (36.9 µl, 0.44 mmol) were added. The organic phase was separated, dried with Na2SO4, filtered and evaporated to give the desired compound (22-azidodocosanoic acid). Yield: 28 mg, 83%

MS (ESI) m/z 380.3 $[M - H]^-$

$^1H$ NMR (500 MHz, $CDCl_3$) 1.25 (35H, s), 1.55 – 1.67 (4H, m), 2.34 (2H, t), 3.25 (2H, t).

**[00124] Intermediate 14 - [(1R,8S)-9-bicyclo[6.1.0]non-4-ynyl]methyl activated MALAT1-LNA**

MALAT1-LNA-hexylamine was dissolved in water (2400 µl) and triethylamine (28.2 µl, 0.20 mmol) was added. ((1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-yl)methyl (2,5-dioxopyrrolidin-1-yl) carbonate (9.89 mg, 0.03 mmol) predissolved in acetonitrile (650 µl) was added and the reaction mixture was stirred for 5 min. LCMS showed full conversion. sodium acetate 3M, pH 5.2 (300 µl) was added and the oligo was precipitated by addition of ethanol (24 ml), vortexed briefly and left standing at -20C overnight. It was centrifuged at 0 C for 10 mins at 3800 rpm, and the clear supernatant was removed and the pellets was dried on vacum. Yield: 101 mg.

MS ($ESI^-$) m/z 1424.8 (z=4)

**[00125] Intermediate 15 - *tert*-butyl (1-azido-15-{2-[(*tert*-butoxycarbonyl)amino]ethyl}-11-oxo-3,6,9-trioxa-12,15-diazaheptadecan-17-yl)carbamate**

A solution of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetic acid (94 mg, 0.40 mmol) in dichloromethane (1mL) and 0.2mL of dimethylformamide (0.2mL) was cooled in ice and N-ethyl-N-isopropylpropan-2-amine (0.140 mL, 0.80 mmol) and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (153 mg, 0.40 mmol) was added. The mixture was stirred for 5min and then a solution of di-tert-butyl (((2-aminoethyl)azanediyl)bis(ethane-2,1-diyl))dicarbamate (CAS 161038-11-5) (139 mg, 0.40 mmol) in dichloromethane and dimethylformamide (0.2mL) was added. The yellow reaction mixture was stirred for 1h at rt. The reaction mixture was diluted with ethyl acetate and washed with water and brine. The organic phase was dried over magnesium sulphate, filtered and evaporated to dryness. The compound was purified by preparative HPLC on a XBridge C18 column (10 µm 250x50 ID mm) using a gradient of 20-75% acetonitrile in ammonia(0.2%) buffer over 20minutes with a flow of 100 mL/min. The product was detected by LS-MS analyses. Product fractions were concentrated to give the desired compound (*tert*-butyl (1-azido-15-{2-[(*tert*-butoxycarbonyl)amino]ethyl}-11-oxo-3,6,9-trioxa-12,15-diazaheptadecan-17-yl)carbamate). Yield 131mg (58.1%)

MS ($ESI^+$) m/z 562.5 (z=1)

1H NMR (500 MHz, CDCl3) 1.46 (18H, s), 3.30 (4H, s), 3.4 – 3.47 (3H, m), 3.48 – 3.63 (4H, m), 3.72 (13H, dq), 4.05 (2H, s), 5.84 (2H, s), 7.87 (1H, s).

**[00126] Intermediate 16 - 1-azido-15-(2-(21-carboxyhenicosanamido)ethyl)-11,19-dioxo-3,6,9-trioxa-12,15,18-triazatetracontan-40-oic acid**

To a solution of **Intermediate 15** (60 mg, 110 µmol) and triisopropylsilane (5 µl, 110 µmol) in dichloromethane (500 µl) was added 2,2,2-trifluoroacetic acid (500 µl) at rt. The mixture was stirred at rt for 5h and evaporated to dryness (MS ($ESI^+$) m/z 362.4 (z=1). To the crude TFA-salt (15mg, 30 µmol) was added acetonitrile (100 µl), water (100 µl), triethylamine (28.3 µl, 0.21 mmol) and N,N-dimethylpyridin-4-amine (0.156 mg, 1.28 µmol) to give a clear solution. Intermediate 1 (40mg, 90 µmol) dissolved in tetrahydrofuran (300 µl) was added and the mixture was stirred at at 45 °C for 4h. The reaction was evaporated, diluted with DMSO and purified by preparative HPLC on a XBridge C18 column (10 µm 250x50 ID mm) using a gradient of 20-75% acetonitrile in ammonia(0.2%) buffer over 20minutes with a flow of 100 mL/min. The product was detected by LS-MS analyses. Product fractions were concentrated to give the desired compound (1-azido-15-(2-(21-carboxyhenicosanamido)ethyl)-11,19-dioxo-3,6,9-trioxa-12,15,18-triazatetracontan-40-oic acid). Yield 4.4mg (16.2%)

MS ($ESI^+$) m/z 1066.6 (z=1)

**[00127] Intermediate 17 – Maleimide activated MALAT1-LNA**



MALAT1-LNA-hexylamine was dissolved in Phosphate buffer (0.1M pH 7.34, 660 µl) and 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate (9.76 mg, 0.04 mmol) dissolved in acetonitrile (330 µL) was added. The clear solution was stirred for 1.5h at rt. Sodium acetate (3M, pH 5.2, 100 µl) was added and then the oligo was precipitated by addition of ethanol (4mL), vortexed briefly and left standing at -20C for 30 min. The mixture was centrifuged at 0 °C for 10 mins at 3500 rpm and the clear supernatant was removed. The oligo was washed once more by dissolving the pellet in 1mL of water and Sodium acetate 3M, pH 5.2 (100 µl) (shaking necessary for full dissolution) followed by addition of 4 mL of

ethanol, cooled for 30 mins at -20, then centrifuged at 0 °C for 10 mins at 3500 rpm. The supernatant was discarded, and the pellet was dried under a nitrogen flow. Yield 27mg (97 %)

MS ($ESI^-$) m/z 1418.1 (z=4)

**[00128] Intermediate 18 - di-tert-butyl 22,22'-(((2R,2'R)-disulfanediylbis(3-(tert-butoxy)-3-oxopropane-1,2-diyl))bis(azanediyl))bis(22-oxodocosanoate)**

To 22-(tert-butoxy)-22-oxodocosanoic acid (87mg, 0.20 mmol) in N-methyl-pyrrolidinone ( 1 ml) was added DIPEA (31.7 µl, 0.18 mmol) and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (69 mg, 0.18 mmol). di-tert-butyl 3,3'-disulfanediyl(2R,2'R)-bis(2-aminopropanoate) (32mg, 0.09 mmol) in dimethylformamide (1 ml) was then added. The resulting opaque reaction mixture was stirred for 24h at rt. The mixture was diluted with diethyl ether and washed with water, sodium bicarbonate (10%), potassium hydrogen sulphate (0.5M), water and brine. The organic phase was evaporated, and the residue was passed through a 2g silica plug with heptane and increasing diethyl ether. The product was detected by TLC with cerium ammonium molybdate stain. Pure product fractions were evaporated to give the desired product (di-tert-butyl 22,22'-(((2R,2'R)-disulfanediylbis(3-(tert-butoxy)-3-oxopropane-1,2-diyl))bis(azanediyl))bis(22-oxodocosanoate). Yield: 85 mg, (80 %).

1H NMR (500 MHz, CDCl3) 1.27 (64H, s), 1.47 (19H, s), 1.50 (17H, s), 1.55 – 1.62 (4H, m), 1.66 (4H, t), 2.22 (4H, t), 2.27 (4H, td), 3.22 (4H, dd), 4.77 (2H, dt), 6.46 (2H, d).

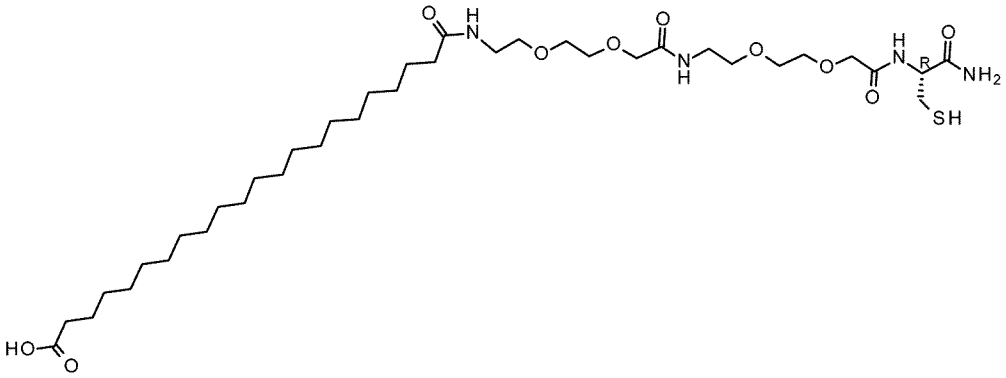
**[00129] Intermediate 19 – 22,22'-(((1R,1'R)-disulfanediylbis(1-carboxyethane-2,1-diyl))bis(azanediyl))bis(22-oxodocosanoic acid)**

To a solution of Intermediate 18 (15 mg, 10 µmol) in dichloromethane (100 µl) was added 2,2,2-trifluoroacetic acid (500 µl) at rt. The mixture was stirred at rt for 15h and evaporated to dryness and then co-evaporated with toluene to give the desired compound (22,22'-(((1R,1'R)-disulfanediylbis(1-carboxyethane-2,1-diyl))bis(azanediyl))bis(22-oxodocosanoic acid)).

Yield 12mg (99%)

MS ($ESI^+$) m/z 944.1 (z=1)

**[00130] Intermediate 21 - (1-{[(2*R*)-1-amino-1-oxo-3-sulfanylpropan-2-yl]amino}-1,10,19-trioxo-3,6,12,15-tetraoxa-9,18-diazatetracontan-40-oic acid)**



**[00131]** The synthesis was performed by Automated Solid Phase Synthesis in a Biotage Alstra peptide synthesizer equipped with a microwave heater. Rink amide Chem Matrix resin (0.4g, 0.16mmol, loading 0.4mmol/g) was weighed into a 10mL reaction vial and swelled twice in DMF for 10 min at 55°C under agitation. Solutions of N-(((9H-fluoren-9-yl)methoxy)carbonyl)-S-trityl-L-cysteine (0.387g, 0.2M, 3.3mL, 0.66mmol), Oxyma (0.5M, 4mL, 1.3mL, 0.66mmol) and diisopropylcarbodiimide (2M, 0.265mL, 0.53mmol) in NMP was added and the reaction was agitated at 40°C for 20min. The resin was washed with DMF (4x) and treated twice with piperidine (20% in DMF) for 3+10min. The same coupling procedure was repeated twice for 1-(9H-fluoren-9-yl)-3-oxo-2,7,10-trioxa-4-azadodecan-12-oic acid.

**[00132]** Solutions of 22-(tert-butoxy)-22-oxodocosanoic acid (137 mg, 0.32 mmol) (4 eq, in DMF/NMP 1:1, 4mL), 1-(bis(dimethylamino)methylene)-1H-[1,2,3]triazolo[4,5-b]pyridine-1-ium 3-oxide hexafluorophosphate(V) (HATU) (122 mg, 0.32 mmol) and N-ethyl-N-isopropylpropan-2-amine (62.9 µl, 0.36 mmol) were then added to the resin and the reaction was agitated at rt for 1h45 min. The resin was finally washed with DMF (4 x), methanol and DCM. A mixture of TFA/H2O/DODT//TIPS (94/2.5/2.5/1) (3 mL) was added to the resin and the reaction mixture was agitated at room temperature for 2h. The resin was filtered off and washed with TFA (~2 mL) which was combined with the filtrate. The product was precipitated in cold Et2O. The precipitated product was centrifugated and the supernatant discarded. The solid material was washed three times by addition of cold Et2O and centrifugation. The resulting solid material was suspended in MeCH/H2O/TFA (50/50/0.1) and freeze dried.

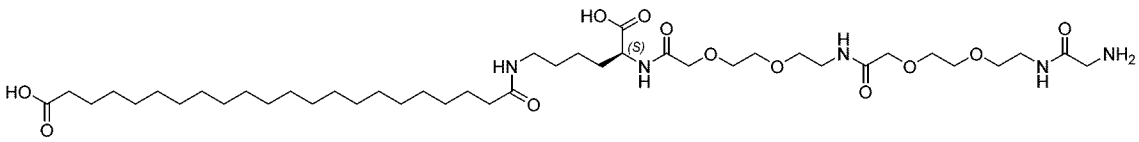
**[00133]** The compound was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x50 ID mm) using a gradient of 35-80% acetonitrile in H2O/ACN/FA 95/5/0.2 buffer over 20 minutes with a flow of 100 mL/min. The compound was detected by UV at 220 nm. The product fractions were freeze dried to give the desired compound.

Yield 27mg (44%)

1H NMR (500 MHz, $CDCl_3$) 1.27 (32H, s), 1.61 – 1.67 (4H, m), 2.23 (2H, t), 2.34 (2H, t), 2.65 (3H, s), 2.85 (1H, ddd), 3.09 (1H, ddd), 3.45 – 3.54 (3H, m), 3.59 (2H, t), 3.61 – 3.73 (10H, m), 3.78 (1H, qd), 4.05 (2H, s), 4.78 (1H, ddd), 6.52 (2H, d), 6.75 (1H, s), 7.32 (1H, t), 7.78 (1H, d). Carboxylic acid proton not integrated.

MS ($ESI^+$) m/z 763.9 (z=1)

**[00134] Intermediate 22 - (S)-1-amino-22-carboxy-2,11,20,28-tetraoxo-6,9,15,18-tetraoxa-3,12,21,27-tetraazanonatetracontan-49-oic acid**

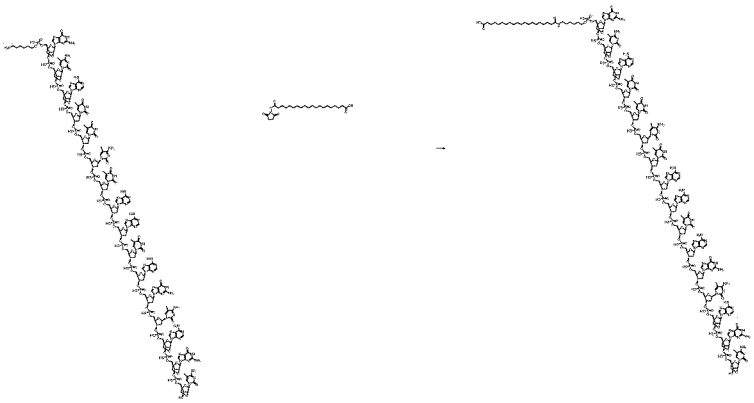


**[00135]** The resin bound precursor (S)-23-(4-((1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl)amino)butyl)-1-(9H-fluoren-9-yl)-3,12,21-trioxo-2,7,10,16,19-pentaoxa-4,13-diazatetracosan-24-oic acid was synthesized according to the methods described for coupling and deprotection steps for the synthesis of intermediate 21 staring from Wang resin bound (S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-6-((1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl)amino)hexanoic acid (Fmoc-Lys-IvDde-Wang resin, loading 0.6mmol/g, 0.3g, ), 1-(9H-fluoren-9-yl)-3-oxo-2,7,10-trioxa-4-azadodecan-12-oic acid and (tert-butoxycarbonyl)glycine. The IvDde-group was then removed by treating the resin with 5%Hydrazine/DMF six times at rt for 2+5+5+5+5+5min. The same procedures used in the synthesis of intermediate 21 was then performed for the coupling of 22-(tert-butoxy)-22-oxodocosanoic acid (137 mg, 0.32 mmol) and for the cleavage and purification of the final product.

MS ($ESI^+$) m/z 846.7 (z=1)

1H NMR (500 MHz, DMSO) 1.23 (34H, s), 1.33 (2H, p), 1.46 (4H, dt), 1.56 (1H, d), 1.68 (1H, d), 2.00 (2H, t), 2.17 (2H, t), 2.95 (2H, q), 3.28 (3H, p), 3.32 (1H, d), 3.42 (2H, s), 3.47 (4H, q), 3.51 – 3.63 (8H, m), 3.84 (2H, s), 3.88 – 3.96 (3H, m), 7.58 (1H, d), 7.71 (1H, d), 7.87 (1H, t), 8.45(1H,s). NH2 and 2xCO2H not integrated

**[00136] EXAMPLE 1 – SYNTHESIS OF $C_{20}$ SATURATED ACID CONJUGATE OF MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**



**[00137]** MALAT1-LNA-hexylamine (34 mg, 6.15 µmol) was dissolved in water (800 µl) and triethylamine (9.53 µl, 0.07 mmol) was added. 20-((2,5-dioxopyrrolidin-1-yl)oxy)-20-oxoicosanoic acid (4.06 mg, 9.23 µmol) **(Intermediate 5)** solved in a mixture of warm (60 °C) acetonitrile (200 µl) and DMSO (120 µl) was added to the solved oligonucleotide and the reaction mixture was stirred at RT for 60 min. Sodium acetate 3M, pH 5.2 (90 µl) was added and then the oligo-conjugate was precipitated by addition of ethanol (8000 µl), The mixture was centrifuged at 0 °C for 10 mins at 3500 rpm and the clear supernatant was removed. The pellet was dried under vacuum and the residue was purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 5-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. The pure fractions was freeze-dried twice to give the desired compound as ammonium salt. The yield was 15 mg (40%).

MS ($ESI^-$) m/z 1461.4 (z=4)

**[00138] EXAMPLE 2 – SYNTHESIS OF $C_{16}$ SATURATED ACID CONJUGATE OF MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**

**[00139]** The Example 2 compound was made according to **Example 1** (C20-acid-MALAT1-LNA) starting from MALAT1-LNA-hexylamine (36 mg, 6.52 µmol) and 16-((2,5-

dioxopyrrolidin-1-yl)oxy)-16-oxohexadecanoic acid (5.00 mg, 13.0 µmol **(Intermediate 2)**. The yield was 20 mg (50%).

MS ($ESI^-$) m/z 1447.5 (z=4)

**[00140] EXAMPLE 3 – SYNTHESIS OF $C_{17}$ SATURATED ACID CONJUGATE OF MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**

**[00141]** The Example 3 compound was made according to **Example 1** (C20-acid-MALAT1-LNA) starting from MALAT1-LNA-hexylamine (34 mg, 6.15 µmol) and 17-((2,5-dioxopyrrolidin-1-yl)oxy)-17-oxoheptadecanoic acid (4.89 mg, 12.3 µmol) **(Intermediate 3)** Reaction time 10 min. The yield was 18 mg (48%).

MS ($ESI^-$) m/z 1450.9 (z=4)

**[00142] EXAMPLE 4 – SYNTHESIS OF $C_{18}$ SATURATED ACID CONJUGATE OF MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**

**[00143]** The Example 4 compound was made according to **Example 1** (C20-acid-MALAT1-LNA) starting from MALAT1-LNA-hexylamine (38 mg, 6.88 µmol) and triethylamine (9.53 µl, 0.07 mmol) was added. 18-((2,5-dioxopyrrolidin-1-yl)oxy)-18-oxooctadecanoic acid (5.66 mg, 13.8 µmol) **(Intermediate 4)**. Reaction time: 90 min. Purification on a XBridge C18, 5µm 19x150 mm column by using a gradient from 15-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. Yield: 24 mg (57%).

MS ($ESI^-$) m/z 1454.4 (z=4)

**[00144] EXAMPLE 5 – SYNTHESIS OF $C_{19}$ SATURATED ACID CONJUGATE OF MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**

**[00145]** The compound was made according to **Example 1** (C20-acid-MALAT1-LNA) starting from MALAT1-LNA-hexylamine (34 mg, 6.15 µmol) and 19-((2,5-dioxopyrrolidin-1-yl)oxy)-19-oxononadecanoic acid (5.24 mg, 12.3 mmol) **(Intermediate 10)**.Reaction time: 30 min. The yield was 21 mg (56%).

MS ($ESI^-$) m/z 1457.7 (z=4)

**[00146] EXAMPLE 6 – SYNTHESIS OF $C_{21}$ SATURATED ACID CONJUGATE OF MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**

**[00147]** The compound was made according to **Example 1** (C20-acid-MALAT1-LNA) starting from MALAT1-LNA-hexylamine (34 mg, 6.15 µmol) and 21-((2,5-dioxopyrrolidin-1-yl)oxy)-21-oxohenicosanoic acid (5.58 mg, 12.3 µmol l) **(Intermediate 6)**. Yield 20 mg (53%).

MS ($ESI^-$) m/z 1465.1 (z=4)

**[00148] EXAMPLE 7 – SYNTHESIS OF $C_{22}$ SATURATED ACID CONJUGATE OF MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**

**[00149]** MALAT1-LNA-hexylamine (100 mg, 0.02 mmol) and triethylamine (10.03 µl, 0.07 mmol) dissolved in water (2.4ml) and 22-((2,5-dioxopyrrolidin-1-yl)oxy)-22-oxodocosanoic acid (21.16 mg, 0.05 mmol) (**Intermediate 1**) dissolved in warm acetonitrile (400 µl) and DMSO (200 µl) was added. Stirred at 40 °C for 24 h. Additional 2.5 eqv of the activated lipid was added and the mixture stirred additional 24 h. at 40 °C. Sodium acetate 3M, pH 5.2 (270 µl) was added and then the oligo was precipitated by addition of ethanol (24 ml). The mixture was centrifuged at 0 °C for 10 mins at 3500 rpm and the clear supernatant was removed. The residue was washed with EtOH (5 ml.) and the pellet was dried under vacuum. The residue was purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 15-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. The pure fractions was freeze-dried twice. Yield: 28 mg (25%).

MS ($ESI^-$) m/z 1468.6 (z=4)

**[00150] EXAMPLE 8 – SYNTHESIS OF $C_{23}$ SATURATED ACID CONJUGATE OF MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**

**[00151]** The compound was made according to **Example 1** (C20-acid-MALAT1-LNA) starting from MALAT1-LNA-hexylamine (39 mg, 7.06 µmol) and 23-((2,5-dioxopyrrolidin-1-yl)oxy)-23-oxotricosanoic acid (8.50 mg, 15.2 µmo) **(Intermediate 7)**. The yield was 17 mg (39%).

MS (ESI-) m/z 1472.5 (z=4)

**[00152] EXAMPLE 9 – SYNTHESIS OF $C_{24}$ SATURATED ACID CONJUGATE OF MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**

**[00153]** The compound was made according to **Example 1** C20-acid-MALAT1-LNA starting from MALAT1-LNA-hexylamine (41 mg, 7.42 μmol) and 24-((2,5-dioxopyrrolidin-1-yl)oxy)-24-oxotetracosanoic acid (7.36 mg, 0.01 mmol) **(Intermediate 8)**. Reaction time: over night. The yield was 23 mg (50%).

MS ($ESI^-$) m/z 1476.0 (z=4)

**[00154] EXAMPLE 10 – SYNTHESIS OF $C_{18}$ (9Z) SATURATED ACID CONJUGATE OF MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**

**[00155]** MALAT1-LNA-hexylamine (180 mg, 0.03 mmol) was dissolved in Borate buffer 0.1M pH 9.5 (4.8 ml). (Z)-18-((2,5-dioxopyrrolidin-1-yl)oxy)-18-oxooctadec-9-enoic acid **(Intermediate 11)** (26.7 mg, 0.07 mmol) dissolved in acetonitrile (1.2 mL) was added and the mixture was stirred at RT for 45 m. Additional 1 eqv of **(Intermediate 11)** was added and the mixture was stirred for additional 2 h. 1M NaOH solution (1.4 ml) was added during 1 h. Sodium acetate 3M, pH 5.2 (540 μL) was added and then the oligo was precipitated by addition of ethanol (48 ml). The mixture was centrifuged at 0 °C for 10 mins at 3500 rpm, the clear supernatant was removed. The pellet was dried under vacuum. The residue was purified on a XBridge C18, 5μm 19x150 mm column by using a gradient from 5-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. The pure fractions was freeze-dried twice. Yield: 79 mg (40%).

MS ($ESI^-$) m/z 1454.1(z=4)

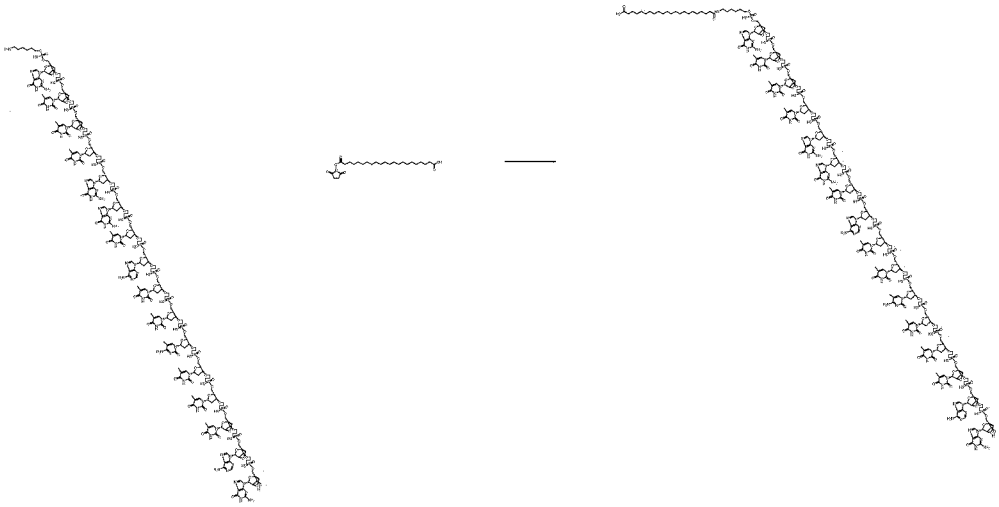
**[00156] EXAMPLE 11 – SYNTHESIS OF $C_{22}$ CLICK SATURATED ACID CONJUGATE OF MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**

**[00157]** [(1R,8S)-9-bicyclo[6.1.0]non-4-ynyl]methyl activated MALAT1- **(Intermediate 14)** (50 mg, 8.77 μmol) was added to a mixture of 22-azidodocosanoic acid (5.02 mg, 0.01 mmol) **(Intermediate 13)** in DMSO (0.2 mL) and acetonitrile (0.200 mL). water (0.5 mL) was added and the mixture was stirred for 3h. Sodium acetate 3M, pH 5.2 (120 μL) and ethanol (10

mL) was added. The mixture was centrifuged at 0 °C for 10 mins at 3500 rpm, the clear supernatant was removed. The pellet was dried under vacuum. The residue was purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 10-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. The pure fractions was freeze-dried twice. Yield: 5.0 mg.

MS ($ESI^-$) m/z 1520.1 (z=4)

**[00158] EXAMPLE 12 – SYNTHESIS OF $C_{22}$ SATURATED ACID CONJUGATE OF CAMK2D ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 9)**



**[00159]** The compound was made according to **Example 1** (C20-acid-MALAT1-LNA) starting from CamK2D ASO -hexylamine (40 mg, 7.24 µmol) and 22-((2,5-dioxopyrrolidin-1-yl)oxy)-22-oxodocosanoic acid (**Intermediate 1)** (6.77 mg, 14.5 µmol). Reaction time: 90 min. Purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 15-90% methanol in $NH_4HCO_3$ (50 mM, pH8) at R.T. The yield was 8 mg (18%).

MS ($ESI^-$) m/z 1469.2 (z=4)

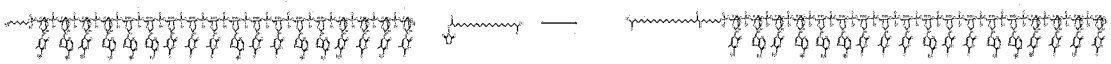
**[00160] EXAMPLE 13 – SYNTHESIS OF $C_{22}$ SATURATED ACID CONJUGATE OF CAMK2D ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 10)**



**[00161]** The compound was made according to **Example 1** (C20-acid-MALAT1-LNA) starting from CamK2D ASO -hexylamine (40 mg, 7.24 µmol) and 22-((2,5-dioxopyrrolidin-1-yl)oxy)-22-oxodocosanoic acid (**Intermediate 1)** (6.77 mg, 14.5 µmol). Reaction time: 120 min. Purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 10-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. The yield was 16 mg (36%).

MS ($ESI^-$) m/z 1462.9 (z=4)

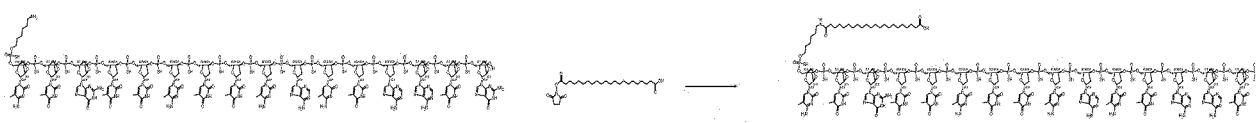
**[00162] EXAMPLE 14 – SYNTHESIS OF $C_{22}$ SATURATED ACID CONJUGATE OF CAMK2D ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 11)**



**[00163]** The compound was made according to **Example 2** C22-acid-MALAT1-LNA starting from CamK2D ASO -hexylamine (35 mg, 5.68 µmol) and 22-((2,5-dioxopyrrolidin-1-yl)oxy)-22-oxodocosanoic acid (**Intermediate 1)** (5.31 mg, 11.4 µmol). Reaction time: 40 min. Purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 10-50% acetonitrile 11min, 50-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. The yield was 20.4 mg (52%).

MS ($ESI^-$) m/z 1628.5 (z=4)

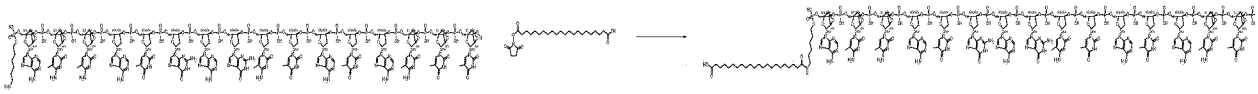
**[00164] EXAMPLE 15 – SYNTHESIS OF $C_{22}$ SATURATED ACID CONJUGATE OF CAMK2D ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 12)**



**[00165]** The compound was made according to **Example 2** C22-acid-MALAT1-LNA starting from CamK2D ASO -hexylamine (35 mg, 5.68 µmol) and 22-((2,5-dioxopyrrolidin-1-yl)oxy)-22-oxodocosanoic acid (**Intermediate 1)** (5.31 mg, 11.4 µmol). Reaction time: 40 min. Purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 10-50% AC 11min, 50-90% ACN in $NH_4HCO_3$ (50 mM, pH8) at R.T. The yield was 20.4 mg (56%).

MS ($ESI^-$) m/z 1458.4 (z=4)

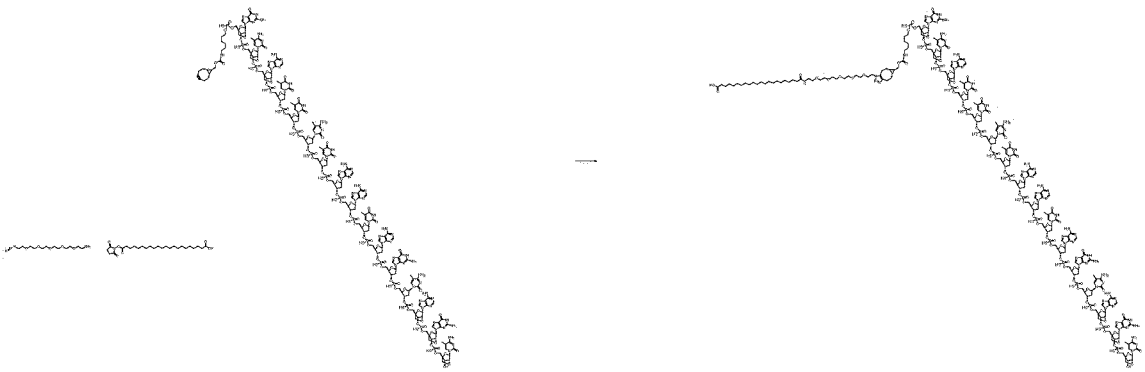
**[00166] EXAMPLE 16 – SYNTHESIS OF $C_{22}$ SATURATED ACID CONJUGATE OF CAMK2D ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 13)**



**[00167]** The compound was made according to **Example 2** C22-acid-MALAT1-LNA starting from CamK2D ASO -hexylamine (35 mg, 5.68 µmol) and 22-((2,5-dioxopyrrolidin-1-yl)oxy)-22-oxodocosanoic acid (**Intermediate 1)** (5.31 mg, 11.4 µmol). Reaction time: 40 min. Purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 10-50% acetonitrile 11min, 50-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. The yield was 18.5 mg (53%).

MS ($ESI^-$) m/z 1462.6 (z=4)

**[00168] EXAMPLE 17 – SYNTHESIS OF $C_{22}$ SATURATED ACID CONJUGATE OF PEG-4 MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**

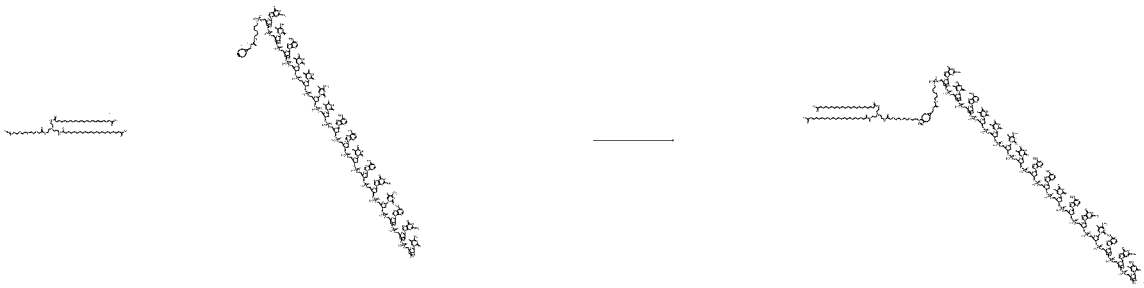


**[00169]** To **Intermediate 14** (20 mg, 3.5 µmol) dissolved in water (600 µl) was added a solution of 17-azido-3,6,9,12,15-pentaoxaheptadecan-1-amine in acetonitrile (150 µl) and

DMSO (90 µl). Stirred at rt for 30min. To the reaction mixture was added triethylamine (5.83 µl ). The mixture was heated to 45 °C and a warm solution of 22-((2,5-dioxopyrrolidin-1-yl)oxy)-22-oxodocosanoic acid (**Intermediate 1)** (5 mg, 10.5 µmol) was added. The mixture was stirred at 45 °C for 30min. Sodium acetate 3M, pH 5.2 (500 µl) was added and then the oligo was precipitated by addition of ethanol (24 ml). The mixture was centrifuged at 0 °C for 10 mins at 3500 rpm and the clear supernatant was removed. The residue was washed with EtOH (5 ml.) and the pellet was dried under vacuum. The residue was purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 15-60% acetonitrile 11min, 60-90% acetonitrile 3min in $NH_4HCO_3$ (50 mM, pH8) at R.T. The pure fractions were freeze-dried twice. Yield: 9.8 mg (42%).

MS ($ESI^-$) m/z 1589.9 (z=4)

**[00170] EXAMPLE 18 – SYNTHESIS OF BILIPID $C_{22}$ SATURATED ACID CONJUGATE OF PEG-4 MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**



**[00171]** To a warm solution of **Intermediate 16** (4.3 mg, 4.05 µmol) in water (200 µl) and THF (100 µl) was added a warm solution of **Intermediate 14** (21 mg, 3.68 µmol) in water (300 µl) and tetrahydrofuran (200 µl). The mixture was stirred at 45 °C for 3h. Sodium acetate 3M, pH 5.2 (500 µl) was added and then the oligo was precipitated by addition of ethanol (24 ml). The mixture was centrifuged at 0 °C for 10 mins at 3500 rpm and the clear supernatant was removed. The residue was washed with EtOH (5 ml.) and the pellet was dried under vacuum. The residue was purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 20-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. The pure fractions were evaporated to 2/3 of the volume and freeze-dried twice. Yield: 3 mg (11%).

MS ($ESI^-$) m/z 1691.3 (z=4)

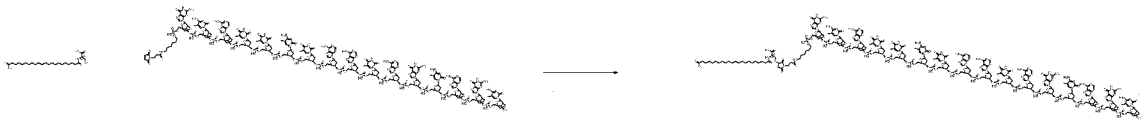
**[00172] EXAMPLE 19 – SYNTHESIS OF $C_{22}$ SATURATED ACID CONJUGATE OF OPEN MALEIMIDE MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**



**[00173]** To C22-acid Maleimide-MALAT1-LNA (40 mg, 6.31 µmol) was added a saturated water solution of sodium hydrogen carbonate (2ml), water (0.6mL) and acetonitrile (0.600 ml). The mixture was stirred at rt for 3h. Sodium hydroxide (1M, 150 µl, 150 µmol). The reaction was stirred at 30 °C for 17h then at 40 °C for 10h. The mixture was cooled in ice and pH was adjusted to pH5 with acetic acid. The milky solution was filtered and freeze dried. The residue was purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 10-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. The pure fractions were freeze-dried twice. Yield: 13 mg (32.2%).

MS ($ESI^-$) m/z 1516.1 (z=4)

**[00174] EXAMPLE 20 – SYNTHESIS OF $C_{22}$ SATURATED ACID CONJUGATE OF CYSTEINE MALEIMIDE MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**



**[00175] Intermediate 19** (6.8 mg, 7.2 µmol) was treated with 3,3',3''-phosphanetriyltripropionic acid hydrochloride (4.12 mg, 10 µmol) in a mixture of tetrahydrofuran (300 µl), sodium acetate buffer (pH 5.3, 3M, 50.0 µl) and water (100 µl) at 40 °C for 10 mins. The mixture was diluted with water and the sulphide was extracted with dichloromethane. The organic phase was evaporated and the desired sulphide (3 mg, 6.33 µmol) was dissolved in dimethyl formamide (0.200 mL) and added to **Intermediate 17** (Maleimide-MALAT1-LNA (25 mg, 4.40 µmol) in sodium acetate buffer (3M, pH5.2, 100 µl), water (500 µl) and dimethyl formamide (200 µl). The mixture was heated at 45 °C for 2h. Acetonitrile (100 µl) and another portion of sulphide (3 mg, 6.33 µmol) dissolved in dimethyl formamide (0.200 mL) was added and heating was continued for another 1h. The oligo was

precipitated by addition of ethanol (14mL), vortexed briefly and left standing at -20C for 30 min. The mixture was centrifuged at 0 °C for 10 mins at 3500 rpm and the clear supernatant was removed. The oligo was washed once more by dissolving the pellet in 1mL of water and Sodium acetate 3M, pH 5.2 (100 µl) followed by addition of 14 mL of ethanol, cooled for 30 mins at -20, then centrifuged at 0 °C for 10 mins at 3500 rpm. The supernatant was discarded, and the product was dried in vacuum. The residue was purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 5-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. The pure fractions were freeze-dried twice. Yield: 7.5 mg (26.4%).

MS ($ESI^-$) m/z 1536.7 (z=4)

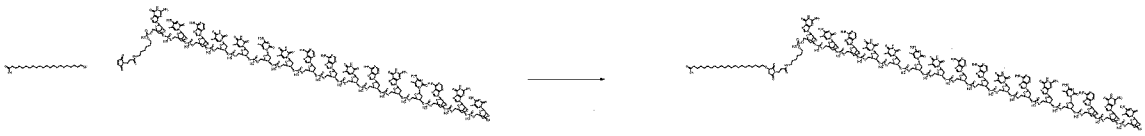
**[00176] EXAMPLE 21 – SYNTHESIS OF $C_{22}$ SATURATED ACID CONJUGATE OF PEG CYSTEINE MALEIMIDE MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**



**[00177]** To a solution of **Intermediate 17** (Maleimide-MALAT1-LNA (30 mg, 5.28 µmol) in water (1 ml), was added **Intermediate 21** (1-{[(2*R*)-1-amino-1-oxo-3-sulfanylpropan-2-yl]amino}-1,10,19-trioxo-3,6,12,15-tetraoxa-9,18-diazatetracontan-40-oic acid) (12.1 mg, 20 µmol) as a solution in dimethylformamide (1.5ml). The mixture was heated at 45 °C for 30min. Water (1ml) was added, resulting in some precipitation. The mixture was extracted with diethylether/tetrahydofuran (0.5ml) to remove excess of **Intermediate 21**. To the water phase was then added ethanol (14mL) to precipitate the oligo. The mixture was vortexed briefly and left standing at -20C for 30 min, centrifuged at 0 °C for 10 mins at 3500 rpm and the clear supernatant was removed. The oligo was washed once more by dissolving the pellet in 1mL of water and adding sodium acetate 3M, pH 5.2 (100 µl) followed by addition of 14 mL of ethanol, cooled for 30 mins at -20, then centrifuged at 0 °C for 10 mins at 3500 rpm. The supernatant was discarded and the product was dried in vacuum. The residue was purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 10-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. The pure fractions were freeze-dried twice. Yield: 11.5 mg (32.3%).

MS ($ESI^-$) m/z 1609.0 (z=4)

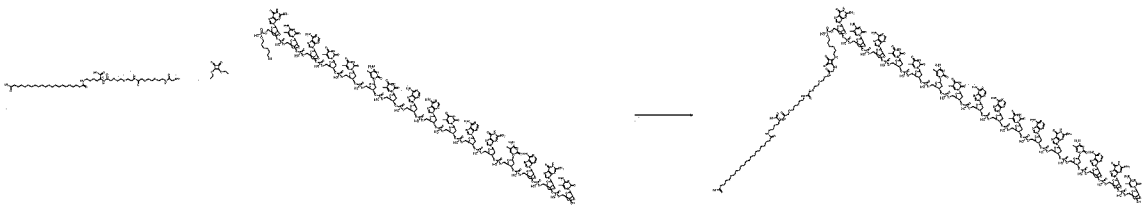
**[00178] EXAMPLE 22 – SYNTHESIS OF $C_{22}$ SATURATED ACID CONJUGATE OF MALEIMIDE MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**



**[00179]** The compound was made according to C22-acid Cystein Maleimide-MALAT1-LNA starting from **Intermediate 17** (Maleimide-MALAT1-LNA (10 mg, 1.76 µmol) and 22-mercaptodocosanoic acid (2.00 mg, 5.28 µmol) published compound JACS 2003 (125)p7704-7714 Khoshtariya, Dimitri et al) The yield was 2.7 mg (24.2%).

MS ($ESI^-$) m/z 1511.3 (z=4)

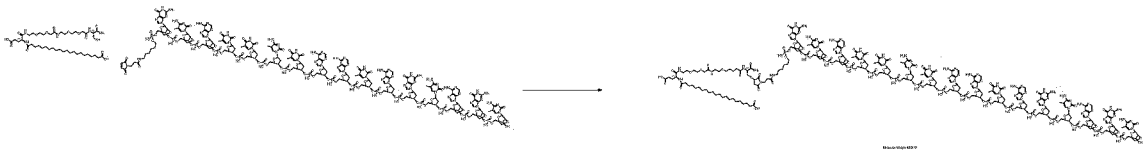
**[00180] EXAMPLE 23 – SYNTHESIS OF $C_{22}$ SATURATED ACID CONJUGATE OF LYS-PEG SQUARIC AMIDE MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**



**[00181]** To MALAT1-LNA-hexylamine (27 mg, 4.89 µmol) in sodium hydrogen carbonate (pH 10.0, 0.025M, 600 µL) and acetonitrile (200 µL) was added a solution of 3,4-diethoxycyclobut-3-ene-1,2-dione (1.1 mg, 6.35 µmol) in acetonitrile (127 µl). The mixture was stirred at rt for 30min. Another portion of 3,4-diethoxycyclobut-3-ene-1,2-dione (1.1 mg, 6.35 µmol) in acetonitrile (127 µl) was then added for complete reaction. After stirring for 30 min, a solution of **Intermediate 22** (S)-1-amino-22-carboxy-2,11,20,28-tetraoxo-6,9,15,18-tetraoxa-3,12,21,27-tetraazanonatetracontan-49-oic acid (20.67 mg, 0.02 mmol) in DMSO (300 µL) and sodium bicarbonate, 0.15M (489 µl, 0.07 mmol) was then added and the reaction was heated at 40 °C for 18h. The product was purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 10-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. The pure fractions were freeze-dried twice. Yield: 8.5 mg (25.7%).

MS ($ESI^-$) m/z 1611.4 (z=4)

**[00182] EXAMPLE 24 – SYNTHESIS OF $C_{22}$ SATURATED ACID CONJUGATE OF gamma GLU-PEG MALEIMIDE MALAT-1 LNA ANTISENSE OLIGONUCLEOTIDE (SEQ ID NO: 8)**



**[00183]** **Intermediate 17** (Maleimide-MALAT1-LNA) (32mg, 5.64 µmol) was dissolved in water (1 mL) and a warm solution of (2R,23S)-1-amino-23-(2-carboxyethyl)-2-(mercaptomethyl)-1,4,13,22,25-pentaoxo-6,9,15,18-tetraoxa-3,12,21,24-tetraazahexatetracontan-46-oic acid (7.54mg, 8.46 µmol) in DMF/ ACN / Water (0.2ml/0.2ml/0.05m) was added. DMF (0.5mL) was added to get a clear solution. The mixture was stirred for 30 min at 45 °C. The product was purified on a XBridge C18, 5µm 19x150 mm column by using a gradient from 10-90% acetonitrile in $NH_4HCO_3$ (50 mM, pH8) at R.T. The pure fractions were freeze-dried twice. Yield 12.5mg (32.3%)

MS ($ESI^-$) m/z 1641.2 (z=4)

**[00184] EXAMPLE 25 - DETERMINATION OF ALBUMIN BINDING AFFINITY AND ESTIMATION OF FREE FRACTION OF 5' LIPID-CONJUGATED MALAT-1 ASOS USING SURFACE PLASMON RESONANCE (SPR) BIOSENSOR**

**[00185]** Affinity determination of serum albumin from different species (human, bovine, rat) with Malat1 ASO gapmer carrying different ligands was achieved using a Biacore S200 (Cytiva). All experiments were performed at 20°C on a HLC30M chip (Xantec) using running buffer: 10 mM HEPES, pH 7.4, 150mM NaCl at 30µl/min. Albumin was covalently immobilized on the chip by EDC-NHS-activated coupling by injecting 100nM albumin in 10mM acetate buffer, pH 5.0. Reference flow cell lacked immobilized protein. Analyte solutions were injected as three-fold serial dilutions up to 150µM in running buffer, 11 concentrations per analyte, in triplicates. Data were evaluated with Biacore S200 evaluation software (Cytiva), and the obtained sensorgrams were fitted into a 1:1 steady-state affinity (fixed Rmax). The resulting apparent affinity ($K_{d,\,app}$) is used to estimate the free fraction (*fu*) according to

**[00186]** $fu = 1 - \frac{[L]_{bound}}{[L]_{tot}} = 1 - \frac{[R]}{\alpha K_{d,app} + [R]}$

**[00187]** where [R] is the plasma concentration of albumin and α is an assay calibration factor. Estimation assumes that the albumin interaction is the dominant contributor to plasma protein binding and that [R] = $[R]_{tot}$ (i.e. [R] >> [L]).

**[00188]** The data in **FIG. 1** indicated that the increased affinity of lipid-ASO conjugates for albumin correlates well with the increased lipophilicity/chain length of the fatty acid part. This can be further modulated by the introduction of unsaturation on the fatty acid chain but also by modifying linker and spacer properties (lipophilicity and introduction of additional polar groups) between the oligonucleotide and the fatty acid chain. The observed modulation of albumin affinity based on differences in lipids and linker composition translated well across the species studied.

**[00189] EXAMPLE 26 - DETERMINATION OF KNOCK DOWN EFFICIENCY OF SELECTED 5' LIPID-CONJUGATED MALAT-1 ASOS IN THP-1 CELLS**

**[00190]** THP-1 cells (ATCC® TIB-202™) were cultured according to standard procedures in RPMI 1640 with GlutaMax, 2g/L glucose, HEPES, MEM Non-Essential Amino Acids, 1mM sodium pyruvate, 10% FBS and 50μM β-mercaptoethanol. Cells were collected by centrifugation, resuspended in serum free medium and plated at 70.000 cells per well in 96 well culture plates. FA-ASO conjugates were dosed into the medium at final concentrations 1, 0.3, 0.1, and 0.03 μM.

**[00191]** Cells were incubated at 37°C, 5% $CO_2$ for 24 hours.

**[00192]** After incubation, cells were transferred to 96-well V-bottom polypropylene microplates and pelleted at 500g for 3 minutes. Medium was removed and cells lysed in 20μL lysis buffer (Qiagen RNeasy RLN lysis buffer with 4% RNAsecure™ RNase Inactivation Reagent from Invitrogen) for 5 minutes at room temperature. 2μL of lysates were used as templates in 20μL-reverse transcription (RT) reactions (50% RT buffer and 5% enzyme mix from Invitrogen's Cells-to-CT Bulk RT Reagents), and RT was performed at 37°C for 60 min, then 95°C for 5 min.

**[00193]** The cDNA samples were diluted 1:4 and Real-Time PCR reactions were set up using 3μL cDNA, TaqMan™ Fast Advanced Master Mix, and Malat-1 or GAPDH TaqMan™ Gene

Expression Assays (Hs00273907_s1 and Hs99999905_m1, all Applied Biosystems) in a total volume of 10µL. Amplifications were performed on a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems) and were conducted at 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Quantification cycle (Cq) values were determined by the software using the Auto Baseline and Auto Threshold options and were then used to calculate relative Malat-1 expression (2^-dCq) normalized against the reference gene GAPDH.s

**[00194]** The data in **FIG. 2** indicated that the introduction in the 5' position of an ASO of a saturated fatty acid chain bearing a carboxylic acid group improved in vitro knock down of Malat-1 in THP-1 cells. The increased functional activity and correlated with increasing lipophilicty of the lipid component. Variation of the linker and spacer chemistries can modulate the in vitro activity.

**[00195] EXAMPLE 27 - DETERMINATION OF KNOCK DOWN EFFICIENCY OF SELECTED 5' LIPID-CONJUGATED CAMK2D ASOS IN LA-4 CELLS**

**[00196]** LA-4 cells (ATCC CCL- 196 ® ™) were cultured according to standard procedures in Ham's F12 nutrient mix media with GlutaMax, 1.8g/L glucose, 1% MEM Non-Essential Amino Acids and 15% FBS. Cells were trypsinised, resuspended in standard culture medium and plated at 6000 cells per well in 384 well culture plates. 16h later the media was removed from the cells and replaced with serum free media. 12-point dilution series (concentration range 0.000085-15µM) were prepared for both naked and C22 lipid conjugated ASOs. These were dosed into the medium and cells were incubated at 37°C, 5% $CO_2$ for 24 hours.

**[00197]** After incubation, medium was removed, cells were washed in PBS and lysed in 10µL Cells to CT lysis buffer (Life Technologies) + 1% DNAse per well for 5 minutes at room temperature with shaking. 2ul of Cells to CT stop solution (Life Technologies) was then added per well. 4µL of each cell lysate was used a template in a 9µL reverse transcription (RT) reactions (50% RT buffer and 5% enzyme mix from Invitrogen's Cells-to-CT Bulk RT Reagents), and RT was performed at 37°C for 30 min, followed by RT inactivation at 95°C for 5 min.

**[00198]** Real-Time PCR reactions were set up using 3µL cDNA, TaqMan™ Fast Advanced Master Mix, and Malat-1 or Rplp0 TaqMan™ Gene Expression Assays (Mm00499266_m1

and Mm00725448_s1, all Applied Biosystems) in a total volume of 10µL. Amplifications were performed on a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems) and were conducted at 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Quantification cycle (Cq) values were determined by the software using the Auto Baseline and Auto Threshold options and were then used to calculate relative CamK2D expression (2^-dCq) normalized against the reference gene Rplp0. 2^-dCq values for CamK2D were then normalized to values obtained from $H_2O$ treated samples. The data in **FIGS. 3A-D** indicates that the lipidated CamK2D ASOs maintained functional activity in vitro.

**[00199] EXAMPLE 28 - KNOCKDOWN EFFECT OF FATTY-ACID-CONJUGATED MALAT-1 ASOS ON THE TARGET GENE IN B6NTAC MICE**

**[00200]** Male *B6NTac* mice arrived at 8-10 weeks of age, 2/cage-housed and placed on Chow Diet. Mice were allowed to acclimate for at least one week before subjected to the study. On day -1, mice were weighed and randomized to appropriate drug treatment groups based on the body weight. Compounds were dosed by subcutaneous injection through tail vein at 5mg/Kg in PBS at pH7.4 at days 0, 2, 4, 7 and 21. Mice were euthanized via $CO_2$ inhalation at day 28. The hearts were removed and approximately 20 mg of apex were weighed out for RNA extraction. One piece of the liver left lobe was snap frozen in liquid nitrogen for RNA extraction. The right kidney were removed and snap frozen for RNA extraction. Knock-down effect was evaluated by RT-qPCR as described in example 26.

**[00201]** The data indicated in **FIGS. 4A-B** show that conjugation to a saturated $C_{22}$ acid or $C_{18}$ (9Z) monounsaturated fatty diacid led to similar or increased knock down in the heart but also to an attenuation of the knock down measured in the liver and kidney compared to the parent ASO.

**[00202] EXAMPLE 29 - KNOCKDOWN EFFECT OF FATTY-ACID-CONJUGATED CAMK2D ASOS ON THE TARGET GENE IN B6NTAC MICE**

**[00203]** Male *B6NTac* mice arrived at 8-10 weeks of age, 2/cage-housed and placed on Chow Diet. Mice were allowed to acclimate for at least one week before subjected to the study. On day 0, mice were weighed and randomized to appropriate drug treatment groups based on the body weight. On day1 compounds were dosed by subcutaneous injection through tail vein at 5mg/Kg in PBS at pH7.4. Mice were euthanized via $CO_2$ inhalation at day 4. The hearts were removed and approximately 20 mg of apex were weighed out for RNA extraction. One piece

of the liver left lobe was snap frozen in liquid nitrogen for RNA extraction. The right kidney was removed and snap frozen for RNA extraction. Knock-down effect was evaluated as described in example 26.

**[00204]** The data indicated in **FIG. 5** shows that the saturated $C_{22}$ acid chain conjugation improved knock down in the heart and tend to attenuate the knock-down in sink organs like kidney and liver in comparison to the naked parent ASOs.

**[00205]** It is to be understood that while certain embodiments have been illustrated and described herein, the claims are not to be limited to the specific forms or arrangement of parts described and shown. In the specification, there have been disclosed illustrative embodiments and, although specific terms are employed, they are used in a generic and descriptive sense only and not for purposes of limitation. Modifications and variations of the embodiments are possible in light of the above teachings. It is therefore to be understood that the embodiments may be practiced otherwise than as specifically described.

**SEQUENCES**

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| 6 | gcattctaatagcagc |
| 7 | gm5cattm5ctaatagm5cagm5c<br><br>m5c – 5-methylcytidine |
| 8 | GM5CAttm5ctaatagm5cAGM5C<br><br>m5c– 5-methylcytidine and capital letters are LNA nucleosides |
| 9 | GTTtggtattm5cttTAG<br><br>m5c– 5-methylcytidine and capital letters are LNA nucleosides |
| 10 | GTGtm5caam5caam5cm5caTTT<br><br>m5c – 5-methylcytidine and capital letters are LNA nucleosides |
| 11 | M5CAM5CAaatttattaaM5CTM5CT<br><br>m5c – 5-methylcytidine and capital letters are LNA nucleosides |
| 12 | M5CTGttm5cttm5caAtaATG<br><br>m5c – 5-methylcytidine and capital letters are LNA nucleosides |
| 13 | AM5CM5Catgagm5ctataM5CTT<br><br>m5c – 5-methylcytidine and capital letters are LNA nucleosides |

## CLAIMS

1. An acid acyl conjugated oligonucleotide comprising:
   a) a carboxy acyl group;
   b) an oligonucleotide; and
   c) a linker connecting the carboxy acyl group to the oligonucleotide.

2. The acid acyl conjugated oligonucleotide of claim 1, wherein the carboxy acyl group is $C_4$ to $C_{32}$.

3. The acid acyl conjugated oligonucleotide of claim 1 or 2, wherein the carboxy acyl group is saturated.

4. The acid acyl conjugated oligonucleotide of claim 1 or 2, wherein the carboxy acyl group is monounsaturated or polyunsaturated.

5. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the linker is bound to the 5' end of the oligonucleotide.

6. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the linker comprises an amino terminus connected to the carboxy acyl group.

7. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the linker comprises a phosphate terminus connected to the oligonucleotide.

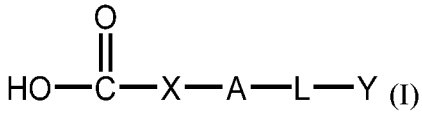
8. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the oligonucleotide is DNA.

9. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the oligonucleotide is RNA.

10. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the oligonucleotide is an antisense oligonucleotide.

11. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the oligonucleotide is a phosphorothioate oligonucleotide.

12. An acid acyl conjugated oligonucleotide of formula (I):



(I)

wherein:

X is $C_4$ to $C_{32}$ alkyl or alkenyl;

A is a conjugation group;

L is a linker; and

Y is an oligonucleotide.

13. The acid acyl conjugated oligonucleotide of claim 12, wherein X is $C_{10}$ to $C_{26}$ alkyl or alkenyl.

14. The acid acyl conjugated oligonucleotide of claim 12, wherein X is $C_{14}$, $C_{16}$ or $C_{22}$ alkyl or alkenyl.

15. The acid acyl conjugated oligonucleotide of claim 12, wherein X is $C_{10}$ to $C_{26}$ alkyl.

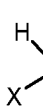
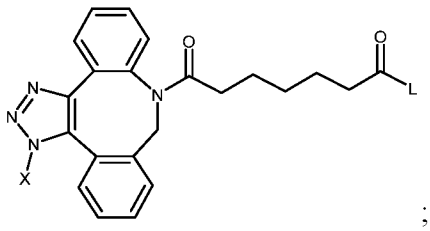
16. The acid acyl conjugated oligonucleotide of claim 15, wherein X is $C_{10}$ to $C_{22}$ alkyl

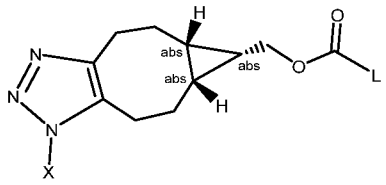
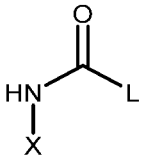
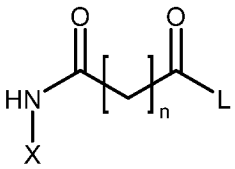
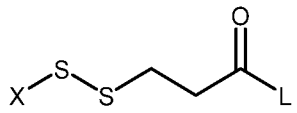
17. The acid acyl conjugated oligonucleotide of claim 15, wherein X is $C_{14}$ alkyl, $C_{16}$ alkyl or $C_{22}$ alkyl.

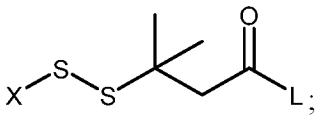
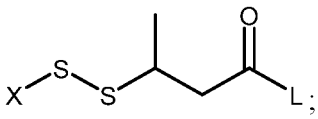
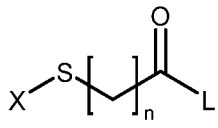
18. The acid acyl conjugated oligonucleotide of claim 15, wherein X is alkenyl.

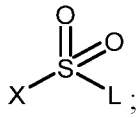
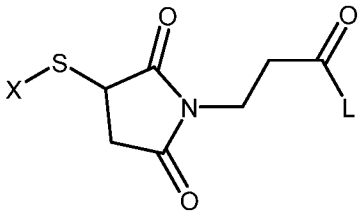
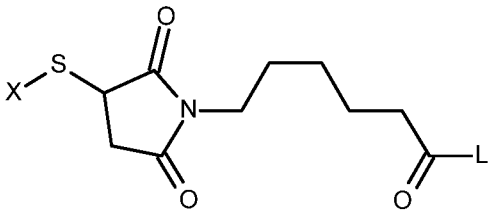
19. The acid acyl conjugated oligonucleotide of claim 18, wherein X is $C_{10}$ to $C_{26}$ alkenyl.

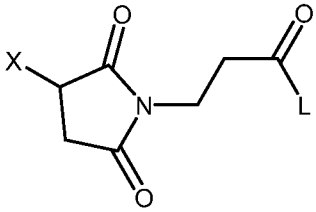
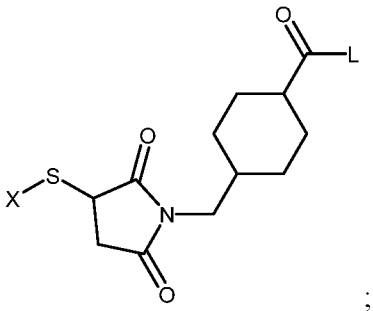
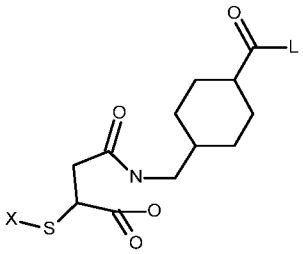
20. The acid acyl conjugated oligonucleotide of claim 12, wherein A is chosen from:

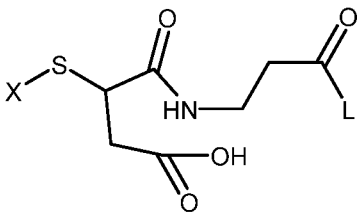
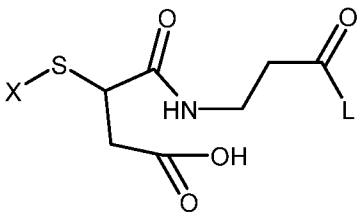
C=O;  ;  ;  ;  ;

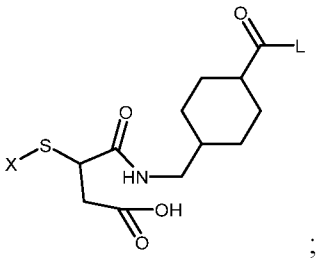
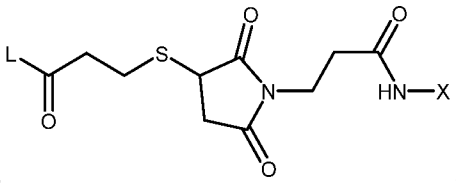
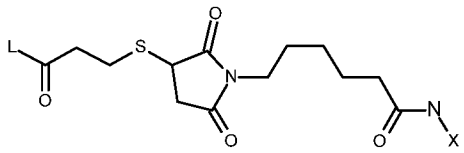
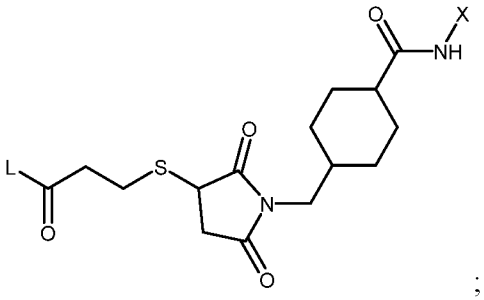
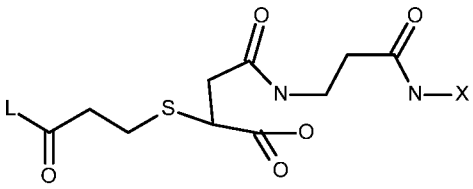
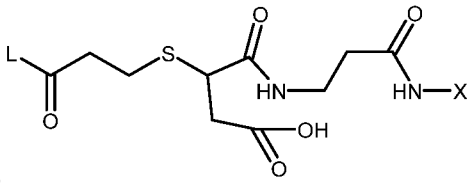
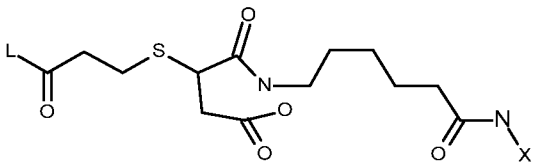
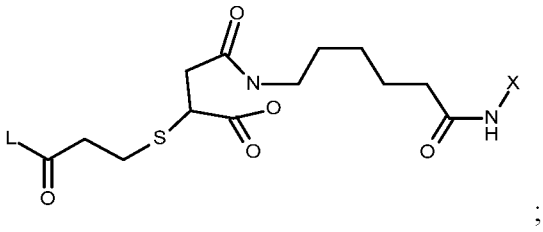

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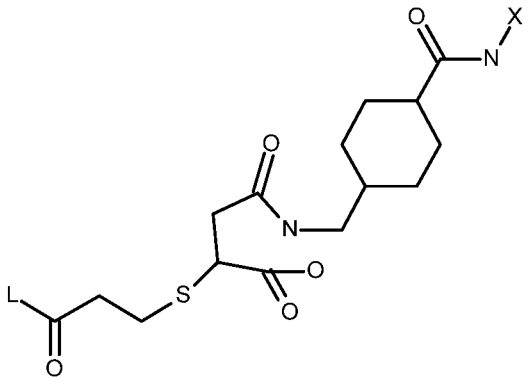
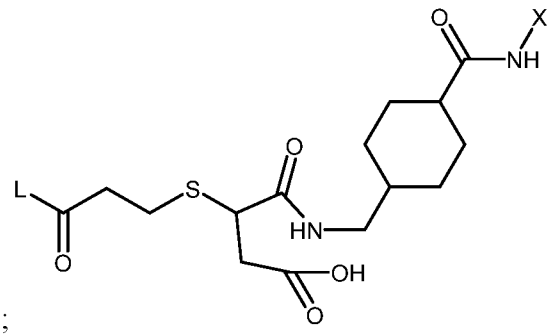
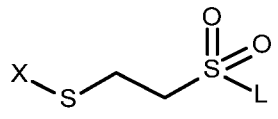
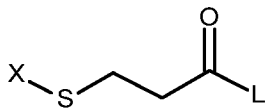
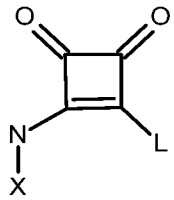

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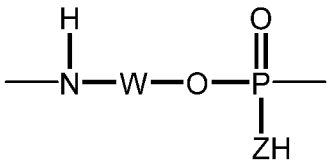
21. The acid acyl conjugated oligonucleotide of claim 20, wherein A is C=O.

22. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the linker (L) is bound to the 5' end of the oligonucleotide.

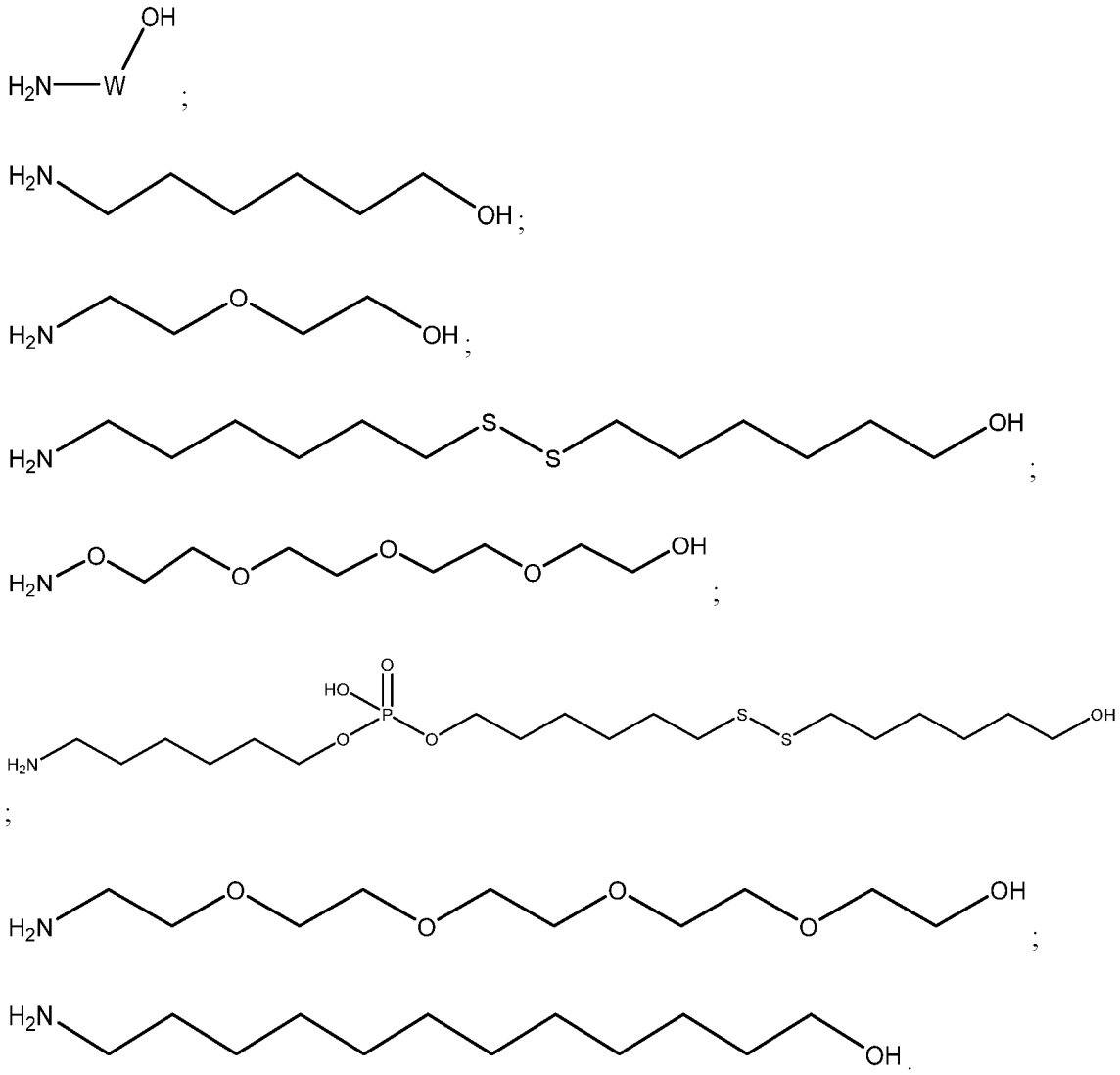
23. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the linker (L) comprises an amino terminus connected to the carboxy acyl group.

24. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the linker (L) comprises a phosphate terminus connected to the oligonucleotide.

25. The acid acyl conjugated oligonucleotide of any one of the proceeding claims, wherein the linker (L) is



wherein Z is O or S and W is chosen from $C_1$ to $C_{10}$ alkyl or alkenyl or one of the following:



26. The acid acyl conjugated oligonucleotide of claim 25, wherein W is $C_4$ to $C_8$ alkyl.

27. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the linker (L) is -NH-$C_6H_{12}$-O-$PO_2$-.

28. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the linker (L) is attached to the oligonucleotide via a phosphate linkage.

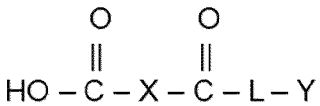
29. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the oligonucleotide is DNA.

30. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the oligonucleotide is RNA.

31. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the oligonucleotide is an antisense oligonucleotide.

32. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the oligonucleotide is a phosphorothioate oligonucleotide.

33. An acid acyl conjugated oligonucleotide of formula (Ia):



(Ia)

wherein:

X is $C_{10}$ to $C_{26}$ alkyl;

L is a linker -NH-$C_6H_{12}$-O-$PO_2$-; and

Y is DNA,

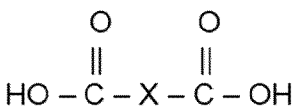
wherein the linker is attached to the DNA via a phosphate linkage at the 5' end of the DNA.

34. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the oligonucleotide improves reduction in cardiac cell expression compared to a non-acyl conjugated oligonucleotide.

35. The acid acyl conjugated oligonucleotide of any one of the preceding claims, wherein the oligonucleotide is an antisense oligonucleotide that specifically binds to MALAT1.

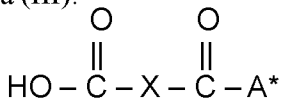
36. A method of making an acid acyl oligonucleotide of formula (Ia), the method comprising:

A) providing a fatty diacid of formula (II)



(II);

B) reacting the fatty diacid with an activating group, A, to form an acid acyl compound of formula (III):



(III);

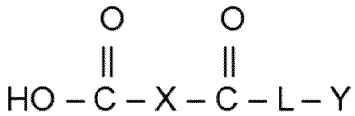
wherein A* is the activating group attached to the fatty acyl compound;

C) reacting the compound of formula (III) with a compound of formula (IV):

$$*L-Y \quad (IV),$$

wherein *L is a linker with a reactive group;

to form the compound of formula (Ia):



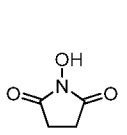
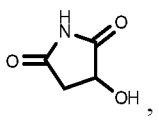
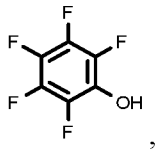
(Ia)

wherein: X is $C_4$ to $C_{32}$ alkyl or alkenyl; L is a linker; and Y is an oligonucleotide.

37. The method of claim 36, wherein the reaction in B) is reacting a carboxylic acid with an amine to form an activated ester amine.

38. The method of claim 37, wherein the amine is N-hydroxysuccinimide (NHS) and the activated ester amine is an NHS ester.

39. The method of making of claim 36, wherein the activating group A* is chosen from


,

,

, TBTU, and HATU.

40. The method of making of any one of claims 36 to 39, wherein the reaction in c) is a coupling between the activating group A* attached to the fatty acyl compound and a primary amine on the linker (L).

41. The method of making of any one of claims 36 to 40, wherein the activating group A* attached to the fatty acyl compound is an activated ester amine.

42. The method of making of any one of claims 36 to 41, wherein the L* is a $C_3$-$C_{10}$ amine.

43. The method of making of any one of claims 36 to 41, wherein the L* is a hexylamine.

44. The method of any one of claims 36 to 43, wherein X is $C_{10}$ to $C_{26}$ alkyl or alkenyl.

45. The method of any one of claims 36 to 44, wherein X is $C_{16}$ or $C_{22}$ alkyl or alkenyl.

46. The method of any one of claims 36 to 45, wherein X is alkyl.

47. The method of claim 46, wherein X is $C_{10}$ to $C_{26}$ alkyl

48. The method of claim 46, wherein X is $C_{16}$ alkyl or $C_{22}$ alkyl.

49. The method of any one of claims 36 to 45, wherein X is alkenyl.

50. The method of claim 49, wherein X is $C_{10}$ to $C_{26}$ alkenyl.

51. The method of claim 49 or claim 50, wherein the alkenyl is monounsaturated.

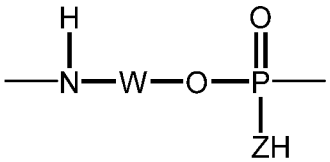
52. The method of claim 49 or claim 50, wherein the alkenyl is polyunsaturated.

53. The method of any one of claims 36 to 52, wherein the linker (L) is bound to the 5' end of the oligonucleotide.

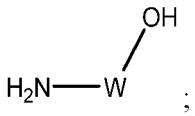
54. The method of any one of claims 36 to 53, wherein the linker (L) comprises an amino terminus connected to the carboxy acyl group.

55. The method of any one of claims 36 to 54, wherein the linker (L) comprises a phosphate terminus connected to the oligonucleotide.

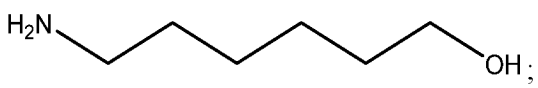
56. The method of any one of claims 36 to 55, wherein the linker (L) is;



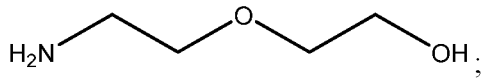
wherein Z is O or S and W is chosen from $C_1$ to $C_{10}$ alkyl or alkenyl or one of the following:



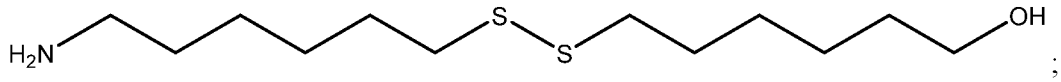
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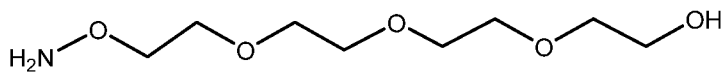
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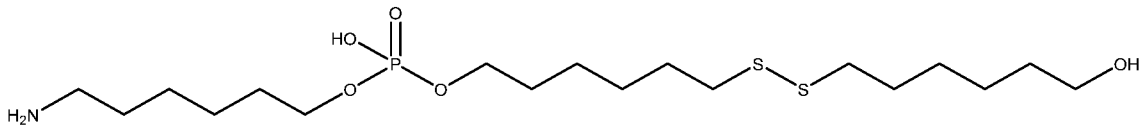
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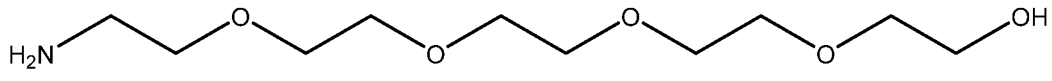
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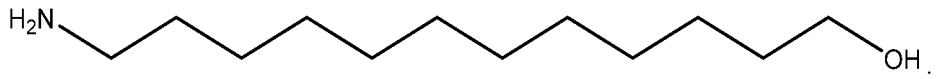
;



;



; and



.

57. The method of claim 56, wherein W is $C_4$ to $C_8$ alkyl.

58. The method of any one of claims 36 to 57, wherein the linker (L) is -NH-$C_6H_{12}$-O-$PO_2$-.

59. The method of any one of claims 36 to 58, wherein the linker (L) is attached to the oligonucleotide via a phosphate linkage.

60. The method of any one of claims 36 to 59, wherein the oligonucleotide is DNA.

61. The method of any one of claims 36 to 59, wherein the oligonucleotide is RNA.

62. The method of any one of claims 36 to 59, wherein the oligonucleotide is an antisense oligonucleotide.

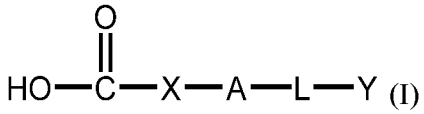
63. The method of any one of claims 36 to 62, wherein the oligonucleotide is a phosphorothioate oligonucleotide.

64. A method of delivering an oligonucleotide to cardiac tissue in a subject comprising:

a) providing acid acyl conjugated oligonucleotide of any one of claim 1 to 35, and

b) administering the acid acyl conjugated oligonucleotide to the subject.

65. The method of claim 64, wherein the acid acyl conjugated oligonucleotide is a compound of formula (I):



(I)

wherein:

X is $C_{10}$ to $C_{26}$ alkyl;

A is C=O;

L is a linker -NH-$C_6H_{12}$-O-$PO_2$-; and

Y is DNA,

wherein the linker is attached to the DNA via a phosphate linkage at the 5' end of the DNA.

66. The method of claim 64 or 65, wherein the oligonucleotide is an antisense oligonucleotide.

67. The method of any one of claims 64-66, wherein the oligonucleotide is an antisense oligonucleotide that specifically binds to a target selected from MALAT1.

68. The method of any one of claims 64 to 67, wherein the subject is a mammal.

69. The method of claim 68, wherein the mammal is a human.

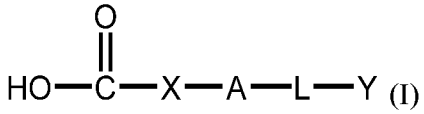
70. The method of any one of claims 64 to 69, wherein the oligonucleotide is administered to treat a cardiac disease.

71. A method of reducing expression of a gene of interest in the cardiac cells of a subject, the method comprising:

a) providing acid acyl conjugated oligonucleotide of any one of claim 1 to 35, and

b) administering the acid acyl conjugated oligonucleotide to the subject.

72. The method of claim 71, wherein the acid acyl conjugated oligonucleotide is a compound of formula (I):



(I)

wherein:

X is $C_{10}$ to $C_{26}$ alkyl;

A is C=O;

L is a linker -NH-$C_6H_{12}$-O-$PO_2$-; and

Y is DNA,

wherein the linker is attached to the DNA via a phosphate linkage at the 5' end of the DNA.

73. The method of claims 71 or 72, wherein the oligonucleotide is an antisense oligonucleotide.

74. The method of any one of claims 71 to 73, wherein the oligonucleotide is an antisense oligonucleotide that specifically binds to a target selected from MALAT1.

75. The method of any one of claims 72 to 74, wherein the subject is a mammal.

76. The method of claim 75, wherein the mammal is a human.

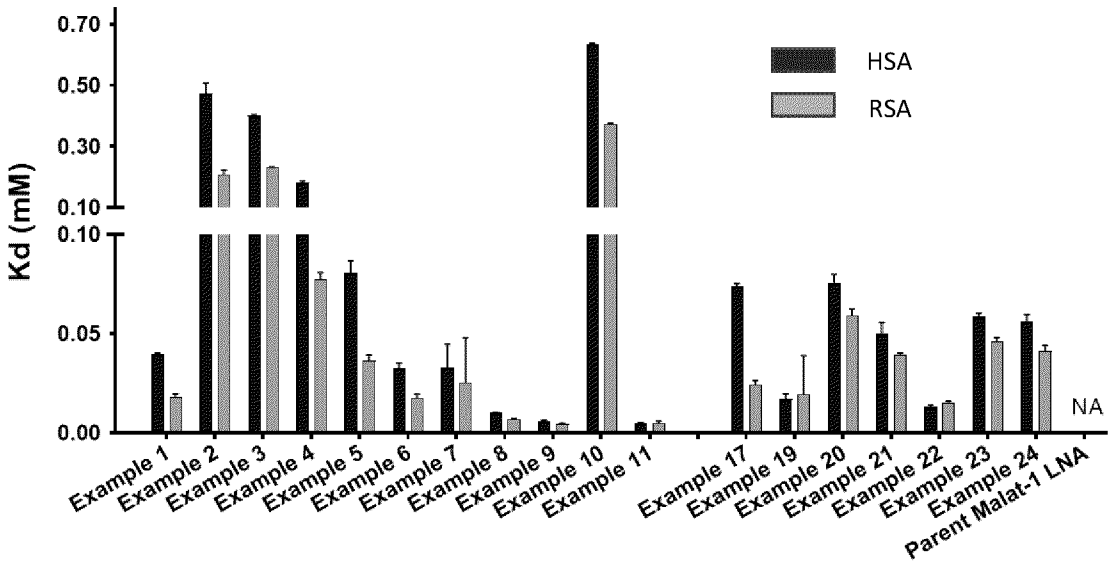

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Example 1
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Example 3
Example 4
Example 5
Example 6
Example 7
Example 8
Example 9
Example 10
Example 11
Example 17
Example 19
Example 20
Example 21
Example 22
Example 23
Example 24
Parent Malat-1 LNA

FIG. 1

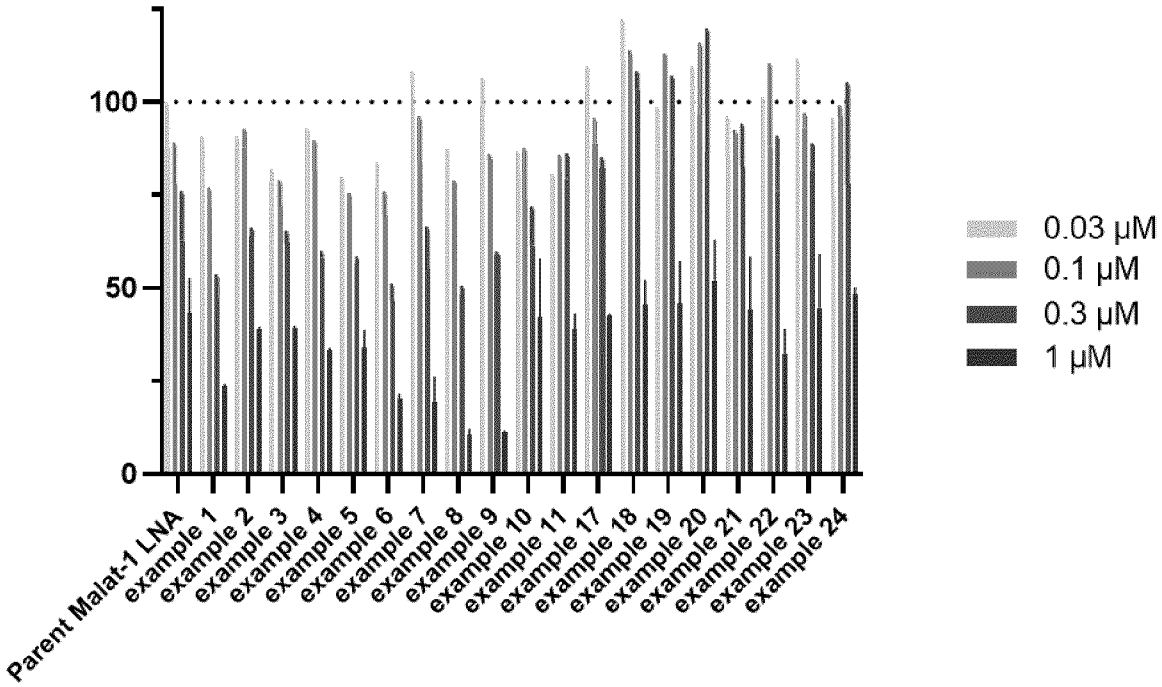

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0.03 µM
0.1 µM
0.3 µM
1 µM
Parent Malat-1 LNA
example 1
example 2
example 3
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example 5
example 6
example 7
example 8
example 9
example 10
example 11
example 17
example 18
example 19
example 20
example 21
example 22
example 23
example 24

FIG. 2

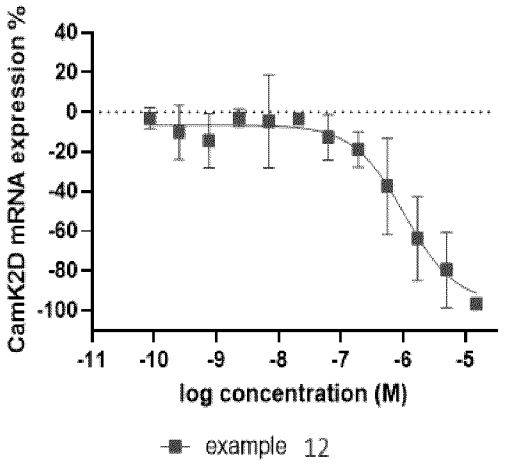



FIG. 3A

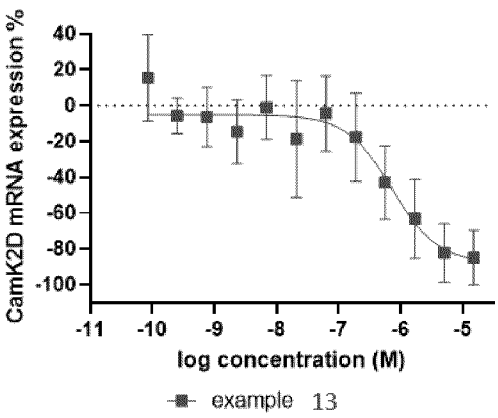



FIG. 3B

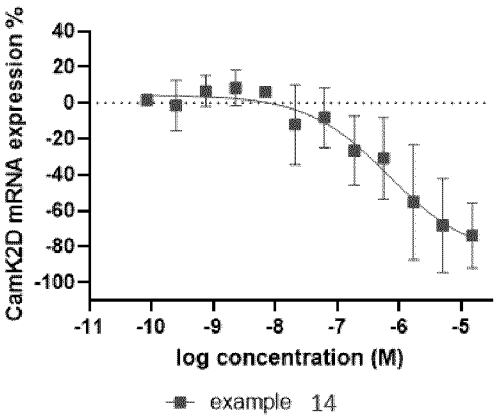



FIG. 3C

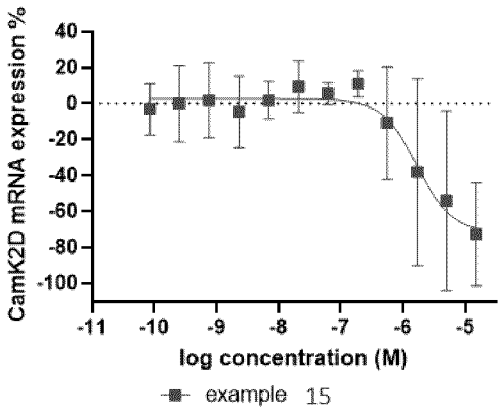



FIG. 3D

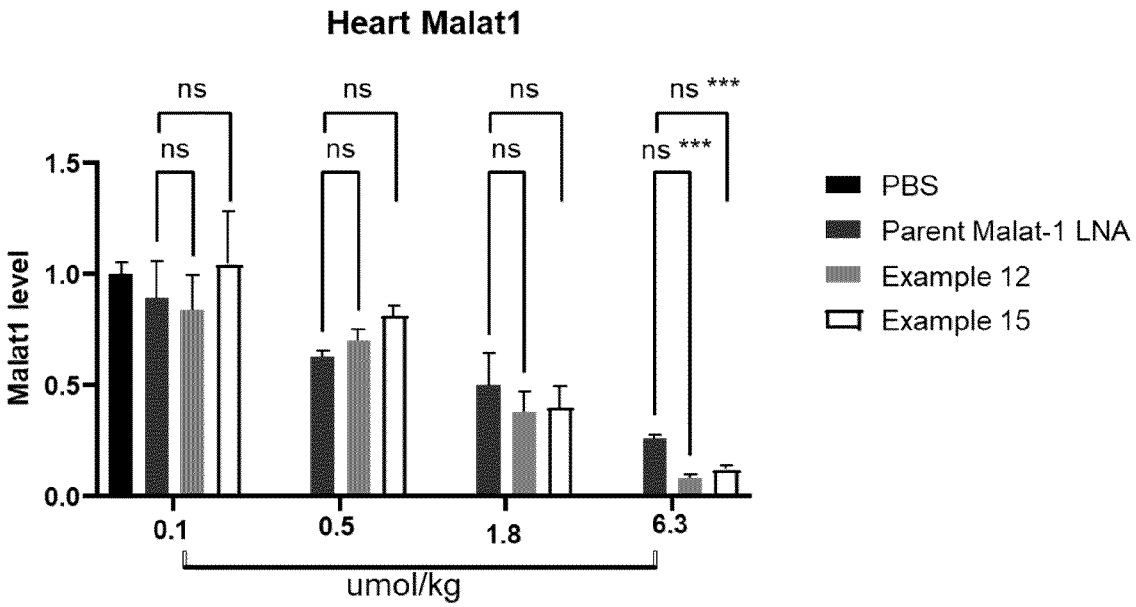

Heart Malat1
Malat1 level
1.5
1.0
0.5
0.0
ns
ns
ns
ns
ns
ns
ns
ns
ns ***
ns ***
0.1
0.5
1.8
6.3
umol/kg
PBS
Parent Malat-1 LNA
Example 12
Example 15

FIG. 4A

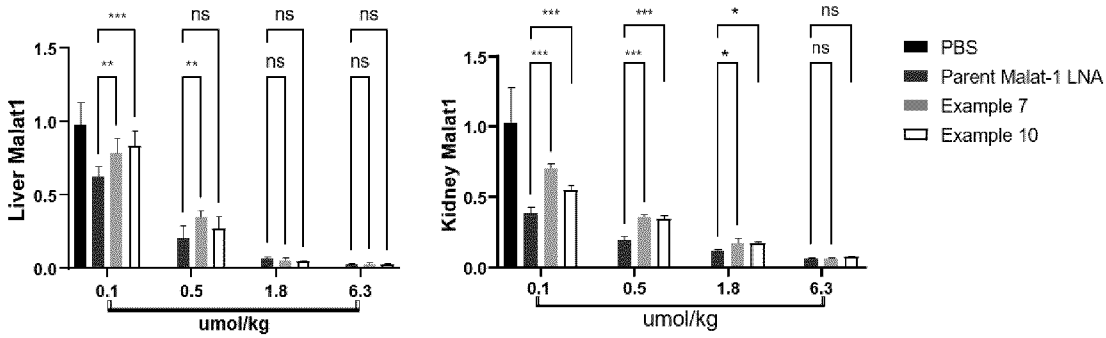

Liver Malat1
Kidney Malat1
1.5
1.0
0.5
0.0
***
**
ns
0.1
0.5
1.8
6.3
umol/kg
*
PBS
Parent Malat-1 LNA
Example 7
Example 10

FIG. 4B

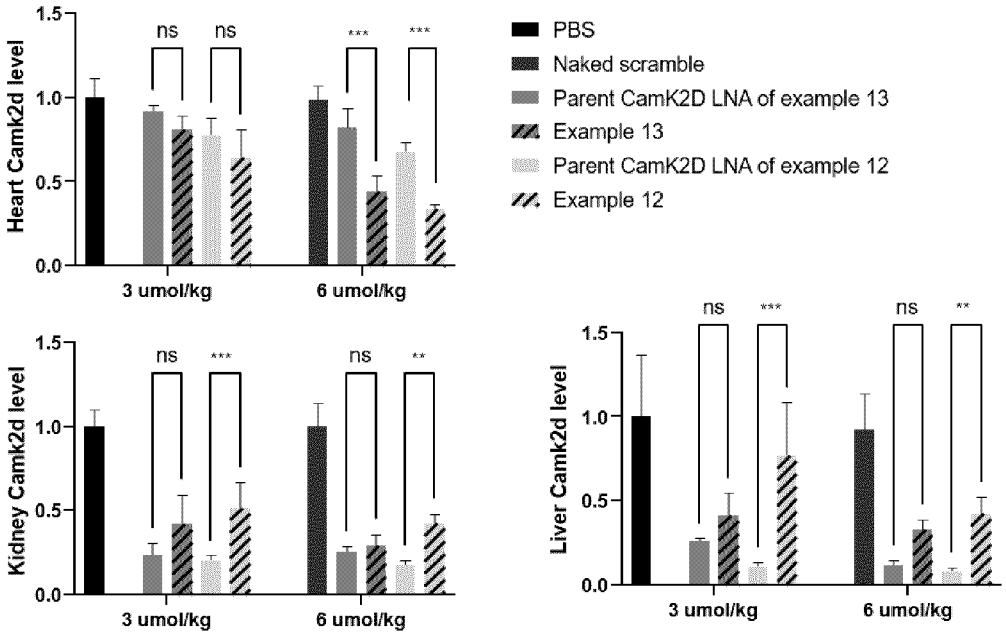

Heart Camk2d level
1.5
1.0
0.5
0.0
ns
ns
***
***
3 umol/kg
6 umol/kg
PBS
Naked scramble
Parent CamK2D LNA of example 13
Example 13
Parent CamK2D LNA of example 12
Example 12
Kidney Camk2d level
ns
***
ns
**
Liver Camk2d level
ns
***
ns
**

FIG. 5

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2021/075386

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

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

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

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      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

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

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2021/075386

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/54
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 2020/046846 A1 (ROLOFF ALEXANDER [US] ET AL) 13 February 2020 (2020-02-13)<br>paragraphs [0002], [0006], [0007], [0020], [0077], [0084]<br>tables 1-3<br>claims 1-40<br>example 1<br>reaction scheme on page 12 | 1-76 |
| X,P | WO 2021/046497 A2 (SHEN HAIFA [US])<br>11 March 2021 (2021-03-11)<br>paragraphs [0036], [0044], [0074] - [0079], [0109]<br>examples 8, 9<br>claims 1, 2, 9-16, 23-29<br>figures 2, 5A-5H, 17A, 17B | 1-76 |

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 6 December 2021 | 22/12/2021 |

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

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2021/075386

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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| | | CN 110636865 A | 31-12-2019 |
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| | | US 2020046846 A1 | 13-02-2020 |
| | | WO 2018175592 A1 | 27-09-2018 |
| WO 2021046497 A2 | 11-03-2021 | NONE | |