



(86) Date de dépôt PCT/PCT Filing Date: 2014/05/01
 (87) Date publication PCT/PCT Publication Date: 2014/11/06
 (45) Date de délivrance/Issue Date: 2023/10/24
 (85) Entrée phase nationale/National Entry: 2016/02/16
 (86) N° demande PCT/PCT Application No.: US 2014/036462
 (87) N° publication PCT/PCT Publication No.: 2014/179626
 (30) Priorités/Priorities: 2013/05/01 (US61/818,442);
 2013/05/15 (US61/823,826); 2013/07/08 (US61/843,887);
 2013/08/29 (US61/871,673); 2013/09/20 (US61/880,790);
 2014/04/08 (US61/976,991); 2014/04/30 (US61/986,867)

(51) Cl.Int./Int.Cl. *C12N 15/113* (2010.01),
A61K 31/7125 (2006.01), *A61K 31/713* (2006.01),
C07H 19/10 (2006.01), *C07H 19/20* (2006.01),
C07H 21/00 (2006.01), *C07K 14/775* (2006.01)
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(54) Titre : COMPOSITIONS ET PROCEDES DE MODULATION DE L'EXPRESSION DE L'APOLIPOPROTEINE C-III
 (54) Title: COMPOSITIONS AND METHODS FOR MODULATING APOLIPOPROTEIN C-III EXPRESSION

(57) Abrégé/Abstract:

Provided herein are oligomeric compounds with conjugate groups targeting apolipoprotein C-III (ApoCIII). In certain embodiments, the ApoCIII targeting oligomeric compounds are conjugated to N-Acetylgalactosamine. Also disclosed herein are conjugated oligomeric compounds targeting ApoCIII for use in decreasing ApoCIII to treat, prevent, or ameliorate diseases, disorders or conditions related to ApoCIII. Certain diseases, disorders or conditions related to ApoCIII include inflammatory, cardiovascular and/or metabolic diseases, disorders or conditions. The conjugated oligomeric compounds disclosed herein can be used to treat such diseases, disorders or conditions in an individual in need thereof.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau(10) International Publication Number
WO 2014/179626 A3(43) International Publication Date
6 November 2014 (06.11.2014)(51) International Patent Classification:
A61K 31/7125 (2006.01)(21) International Application Number:
PCT/US2014/036462(22) International Filing Date:
1 May 2014 (01.05.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/818,442	1 May 2013 (01.05.2013)	US
61/823,826	15 May 2013 (15.05.2013)	US
61/843,887	8 July 2013 (08.07.2013)	US
61/871,673	29 August 2013 (29.08.2013)	US
61/880,790	20 September 2013 (20.09.2013)	US
61/976,991	8 April 2014 (08.04.2014)	US
61/986,867	30 April 2014 (30.04.2014)	US

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Inc., 2855 Gazelle Ct., Carlsbad, CA 92010 (US).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,
OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, SA,
SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM,
ZW.(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(88) Date of publication of the international search report:
26 February 2015

(54) Title: COMPOSITIONS AND METHODS FOR MODULATING APOLIPOPROTEIN C-III EXPRESSION

(57) Abstract: Provided herein are oligomeric compounds with conjugate groups targeting apolipoprotein C-III (ApoCIII). In certain embodiments, the ApoCIII targeting oligomeric compounds are conjugated to *N*-Acetylgalactosamine. Also disclosed herein are conjugated oligomeric compounds targeting ApoCIII for use in decreasing ApoCIII to treat, prevent, or ameliorate diseases, disorders or conditions related to ApoCIII. Certain diseases, disorders or conditions related to ApoCIII include inflammatory, cardiovascular and/or metabolic diseases, disorders or conditions. The conjugated oligomeric compounds disclosed herein can be used to treat such diseases, disorders or conditions in an individual in need thereof.

WO 2014/179626 A3

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THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
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NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

COMPOSITIONS AND METHODS FOR MODULATING APOLIPOPROTEIN C-III EXPRESSION

SEQUENCE LISTING

5 The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0249WOSEQ_ST25.txt, created on 2014, which is 68 Kb in size, which forms part of the present description.

BACKGROUND OF THE INVENTION

10 The principle behind antisense technology is that an antisense compound hybridizes to a target nucleic acid and modulates the amount, activity, and/or function of the target nucleic acid. For example in certain instances, antisense compounds result in altered transcription or translation of a target. Such modulation of expression can be achieved by, for example, target mRNA degradation or occupancy-based inhibition. An example of modulation of RNA target function by degradation is RNase H-based degradation of the target RNA upon hybridization with a DNA-like antisense compound. Another example of modulation
15 of gene expression by target degradation is RNA interference (RNAi). RNAi refers to antisense-mediated gene silencing through a mechanism that utilizes the RNA-induced silencing complex (RISC). An additional example of modulation of RNA target function is by an occupancy-based mechanism such as is employed naturally by microRNA. MicroRNAs are small non-coding RNAs that regulate the expression of protein-coding RNAs. The binding of an antisense compound to a microRNA prevents that microRNA from binding
20 to its messenger RNA targets, and thus interferes with the function of the microRNA. MicroRNA mimics can enhance native microRNA function. Certain antisense compounds alter splicing of pre-mRNA. Regardless of the specific mechanism, sequence-specificity makes antisense compounds attractive as tools for target validation and gene functionalization, as well as therapeutics to selectively modulate the expression of genes involved in the pathogenesis of diseases.

25 Antisense technology is an effective means for modulating the expression of one or more specific gene products and can therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications. Chemically modified nucleosides may be incorporated into antisense compounds to enhance one or more properties, such as nuclease resistance, pharmacokinetics or affinity for a target nucleic acid. In 1998, the antisense compound, Vitravene® (fomivirsen; developed by Isis Pharmaceuticals Inc.,
30 Carlsbad, CA) was the first antisense drug to achieve marketing clearance from the U.S. Food and Drug Administration (FDA), and is currently a treatment of cytomegalovirus (CMV)-induced retinitis in AIDS patients.

 New chemical modifications have improved the potency and efficacy of antisense compounds,

uncovering the potential for oral delivery as well as enhancing subcutaneous administration, decreasing potential for side effects, and leading to improvements in patient convenience. Chemical modifications increasing potency of antisense compounds allow administration of lower doses, which reduces the potential for toxicity, as well as decreasing overall cost of therapy. Modifications increasing the resistance to degradation result in slower clearance from the body, allowing for less frequent dosing. Different types of chemical modifications can be combined in one compound to further optimize the compound's efficacy.

Apolipoprotein C-III (also called APOC3, APOC-III, ApoCIII, and APO C-III) is a constituent of HDL and of triglyceride (TG)-rich lipoproteins. Elevated ApoCIII levels are associated with elevated TG levels and diseases such as cardiovascular disease, metabolic syndrome, obesity and diabetes (Chan *et al.*, *Int J Clin Pract*, 2008, 62:799-809; Onat *et al.*, *Atherosclerosis*, 2003, 168:81-89; Mendivil *et al.*, *Circulation*, 2011, 124:2065-2072; Mauger *et al.*, *J. Lipid Res*, 2006, 47: 1212-1218; Chan *et al.*, *Clin. Chem*, 2002, 278-283; Ooi *et al.*, *Clin. Sci*, 2008, 114: 611-624; Davidsson *et al.*, *J. Lipid Res*, 2005, 46: 1999-2006; Sacks *et al.*, *Circulation*, 2000, 102: 1886-1892; Lee *et al.*, *Arterioscler Thromb Vasc Biol*, 2003, 23: 853-858). ApoCIII slows clearance of TG-rich lipoproteins by inhibiting lipolysis through inhibition of lipoprotein lipase (LPL) and through interfering with lipoprotein binding to cell-surface glycosaminoglycan matrix (Shachter, *Curr. Opin. Lipidol*, 2001, 12, 297-304).

Antisense technology is emerging as an effective means for reducing the expression of certain gene products and may prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications for the modulation of ApoCIII. Antisense compounds targeting ApoCIII and associated methods for inhibiting ApoCIII have been previously disclosed (see e.g., U.S. Patent 7,598,227, U.S. Patent 7,750,141, PCT publication WO 2004/093783, PCT publication WO 2012/149495 and PCT/US14/016546). An antisense compound targeting ApoCIII, ISIS-APOCIII_{RX}, has been tested in a Phase I and II clinical trials. However, no antisense compounds targeting ApoCIII have been approved for commercial use, accordingly, there is still a need to provide patients with additional and more potent treatment options.

SUMMARY OF THE INVENTION

In certain embodiments, the present disclosure provides conjugated antisense compounds. In certain embodiments, the present disclosure provides conjugated antisense compounds comprising an antisense oligonucleotide complementary to a nucleic acid transcript. In certain embodiments, the present disclosure provides methods comprising contacting a cell with a conjugated antisense compound comprising an antisense oligonucleotide complementary to a nucleic acid transcript. In certain embodiments, the present

disclosure provides methods comprising contacting a cell with a conjugated antisense compound comprising an antisense oligonucleotide and reducing the amount or activity of a nucleic acid transcript in a cell.

The asialoglycoprotein receptor (ASGP-R) has been described previously. See e.g., Park et al., PNAS vol. 102, No. 47, pp 17125-17129 (2005). Such receptors are expressed on liver cells, particularly hepatocytes. Further, it has been shown that compounds comprising clusters of three N-acetylgalactosamine (GalNAc) ligands are capable of binding to the ASGP-R, resulting in uptake of the compound into the cell. See e.g., Khorev et al., Bioorganic and Medicinal Chemistry, 16, 9, pp 5216-5231 (May 2008). Accordingly, conjugates comprising such GalNAc clusters have been used to facilitate uptake of certain compounds into liver cells, specifically hepatocytes. For example it has been shown that certain GalNAc-containing conjugates increase activity of duplex siRNA compounds in liver cells in vivo. In such instances, the GalNAc-containing conjugate is typically attached to the sense strand of the siRNA duplex. Since the sense strand is discarded before the antisense strand ultimately hybridizes with the target nucleic acid, there is little concern that the conjugate will interfere with activity. Typically, the conjugate is attached to the 3' end of the sense strand of the siRNA. See e.g., U.S. Patent 8,106,022. Certain conjugate groups described herein are more active and/or easier to synthesize than conjugate groups previously described.

In certain embodiments of the present invention, conjugates are attached to single-stranded antisense compounds, including, but not limited to RNase H based antisense compounds and antisense compounds that alter splicing of a pre-mRNA target nucleic acid. In such embodiments, the conjugate should remain attached to the antisense compound long enough to provide benefit (improved uptake into cells) but then should either be cleaved, or otherwise not interfere with the subsequent steps necessary for activity, such as hybridization to a target nucleic acid and interaction with RNase H or enzymes associated with splicing or splice modulation. This balance of properties is more important in the setting of single-stranded antisense compounds than in siRNA compounds, where the conjugate may simply be attached to the sense strand. Disclosed herein are conjugated single-stranded antisense compounds having improved potency in liver cells in vivo compared with the same antisense compound lacking the conjugate. Given the required balance of properties for these compounds such improved potency is surprising.

In certain embodiments, conjugate groups herein comprise a cleavable moiety. As noted, without wishing to be bound by mechanism, it is logical that the conjugate should remain on the compound long enough to provide enhancement in uptake, but after that, it is desirable for some portion or, ideally, all of the conjugate to be cleaved, releasing the parent compound (e.g., antisense compound) in its most active form. In certain embodiments, the cleavable moiety is a cleavable nucleoside. Such embodiments take advantage of endogenous nucleases in the cell by attaching the rest of the conjugate (the cluster) to the antisense oligonucleotide through a nucleoside via one or more cleavable bonds, such as those of a phosphodiester linkage. In certain embodiments, the cluster is bound to the cleavable nucleoside through a phosphodiester linkage. In certain embodiments, the cleavable nucleoside is attached to the antisense oligonucleotide (antisense compound) by a phosphodiester linkage. In certain embodiments, the conjugate group may

comprise two or three cleavable nucleosides. In such embodiments, such cleavable nucleosides are linked to one another, to the antisense compound and/or to the cluster via cleavable bonds (such as those of a phosphodiester linkage). Certain conjugates herein do not comprise a cleavable nucleoside and instead comprise a cleavable bond. It is shown that that sufficient cleavage of the conjugate from the oligonucleotide is provided by at least one bond that is vulnerable to cleavage in the cell (a cleavable bond).

In certain embodiments, conjugated antisense compounds are prodrugs. Such prodrugs are administered to an animal and are ultimately metabolized to a more active form. For example, conjugated antisense compounds are cleaved to remove all or part of the conjugate resulting in the active (or more active) form of the antisense compound lacking all or some of the conjugate.

In certain embodiments, conjugates are attached at the 5' end of an oligonucleotide. Certain such 5'-conjugates are cleaved more efficiently than counterparts having a similar conjugate group attached at the 3' end. In certain embodiments, improved activity may correlate with improved cleavage. In certain embodiments, oligonucleotides comprising a conjugate at the 5' end have greater efficacy than oligonucleotides comprising a conjugate at the 3' end (see, for example, Examples 56, 81, 83, and 84). Further, 5'-attachment allows simpler oligonucleotide synthesis. Typically, oligonucleotides are synthesized on a solid support in the 3' to 5' direction. To make a 3'-conjugated oligonucleotide, typically one attaches a pre-conjugated 3' nucleoside to the solid support and then builds the oligonucleotide as usual. However, attaching that conjugated nucleoside to the solid support adds complication to the synthesis. Further, using that approach, the conjugate is then present throughout the synthesis of the oligonucleotide and can become degraded during subsequent steps or may limit the sorts of reactions and reagents that can be used. Using the structures and techniques described herein for 5'-conjugated oligonucleotides, one can synthesize the oligonucleotide using standard automated techniques and introduce the conjugate with the final (5'-most) nucleoside or after the oligonucleotide has been cleaved from the solid support.

In view of the art and the present disclosure, one of ordinary skill can easily make any of the conjugates and conjugated oligonucleotides herein. Moreover, synthesis of certain such conjugates and conjugated oligonucleotides disclosed herein is easier and/or requires few steps, and is therefore less expensive than that of conjugates previously disclosed, providing advantages in manufacturing. For example, the synthesis of certain conjugate groups consists of fewer synthetic steps, resulting in increased yield, relative to conjugate groups previously described. Conjugate groups such as GalNAc3-10 in Example 46 and GalNAc3-7 in Example 48 are much simpler than previously described conjugates such as those described in U.S. 8,106,022 or U.S. 7,262,177 that require assembly of more chemical intermediates. Accordingly, these and other conjugates described herein have advantages over previously described compounds for use with any oligonucleotide, including single-stranded oligonucleotides and either strand of double-stranded oligonucleotides (e.g., siRNA).

Similarly, disclosed herein are conjugate groups having only one or two GalNAc ligands. As shown, such conjugates groups improve activity of antisense compounds. Such compounds are much easier to

prepare than conjugates comprising three GalNAc ligands. Conjugate groups comprising one or two GalNAc ligands may be attached to any antisense compounds, including single-stranded oligonucleotides and either strand of double-stranded oligonucleotides (e.g., siRNA).

5 In certain embodiments, the conjugates herein do not substantially alter certain measures of tolerability. For example, it is shown herein that conjugated antisense compounds are not more immunogenic than unconjugated parent compounds. Since potency is improved, embodiments in which tolerability remains the same (or indeed even if tolerability worsens only slightly compared to the gains in potency) have improved properties for therapy.

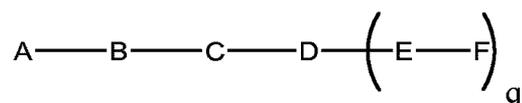
10 In certain embodiments, conjugation allows one to alter antisense compounds in ways that have less attractive consequences in the absence of conjugation. For example, in certain embodiments, replacing one or more phosphorothioate linkages of a fully phosphorothioate antisense compound with phosphodiester linkages results in improvement in some measures of tolerability. For example, in certain instances, such antisense compounds having one or more phosphodiester are less immunogenic than the same compound in which each linkage is a phosphorothioate. However, in certain instances, as shown in Example 26, that same replacement of one or more phosphorothioate linkages with phosphodiester linkages also results in reduced cellular uptake and/or loss in potency. In certain embodiments, conjugated antisense compounds described herein tolerate such change in linkages with little or no loss in uptake and potency when compared to the conjugated full-phosphorothioate counterpart. In fact, in certain embodiments, for example, in Examples 44, 15 57, 59, and 86, oligonucleotides comprising a conjugate and at least one phosphodiester internucleoside linkage actually exhibit increased potency in vivo even relative to a full phosphorothioate counterpart also comprising the same conjugate. Moreover, since conjugation results in substantial increases in uptake/potency a small loss in that substantial gain may be acceptable to achieve improved tolerability. Accordingly, in certain embodiments, conjugated antisense compounds comprise at least one phosphodiester linkage.

25 In certain embodiments, conjugation of antisense compounds herein results in increased delivery, uptake and activity in hepatocytes. Thus, more compound is delivered to liver tissue. However, in certain embodiments, that increased delivery alone does not explain the entire increase in activity. In certain such embodiments, more compound enters hepatocytes. In certain embodiments, even that increased hepatocyte uptake does not explain the entire increase in activity. In such embodiments, productive uptake of the conjugated compound is increased. For example, as shown in Example 102, certain embodiments of 30 GalNAc-containing conjugates increase enrichment of antisense oligonucleotides in hepatocytes versus non-parenchymal cells. This enrichment is beneficial for oligonucleotides that target genes that are expressed in hepatocytes.

In certain embodiments, conjugated antisense compounds herein result in reduced kidney exposure. 35 For example, as shown in Example 20, the concentrations of antisense oligonucleotides comprising certain embodiments of GalNAc-containing conjugates are lower in the kidney than that of antisense

oligonucleotides lacking a GalNAc-containing conjugate. This has several beneficial therapeutic implications. For therapeutic indications where activity in the kidney is not sought, exposure to kidney risks kidney toxicity without corresponding benefit. Moreover, high concentration in kidney typically results in loss of compound to the urine resulting in faster clearance. Accordingly for non-kidney targets, kidney accumulation is undesired.

In certain embodiments, the present disclosure provides conjugated antisense compounds represented by the formula:



10 wherein

A is the antisense oligonucleotide;

B is the cleavable moiety

C is the conjugate linker

D is the branching group

15 each E is a tether;

each F is a ligand; and

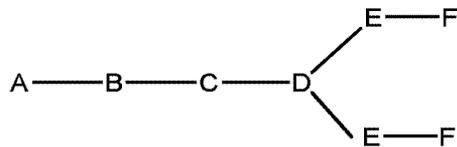
q is an integer between 1 and 5.

In the above diagram and in similar diagrams herein, the branching group “D” branches as many times as is necessary to accommodate the number of (E-F) groups as indicated by “q”. Thus, where q = 1,

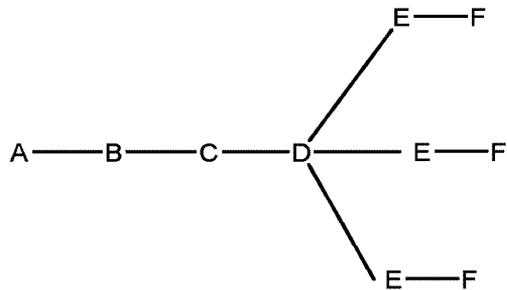
20 the formula is:



where q = 2, the formula is:

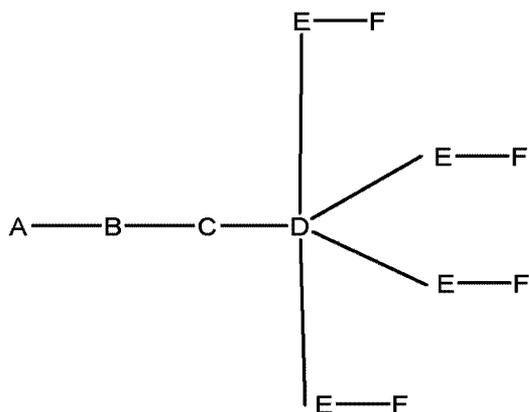


where q = 3, the formula is:

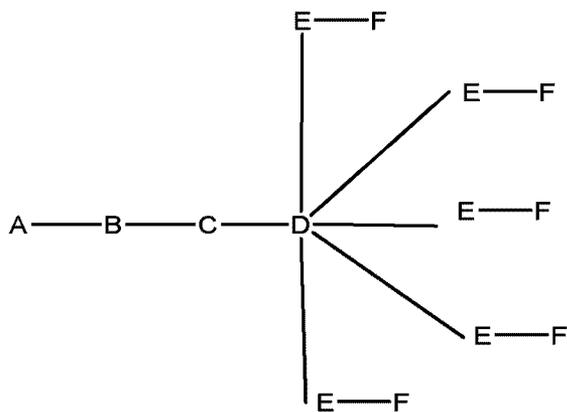


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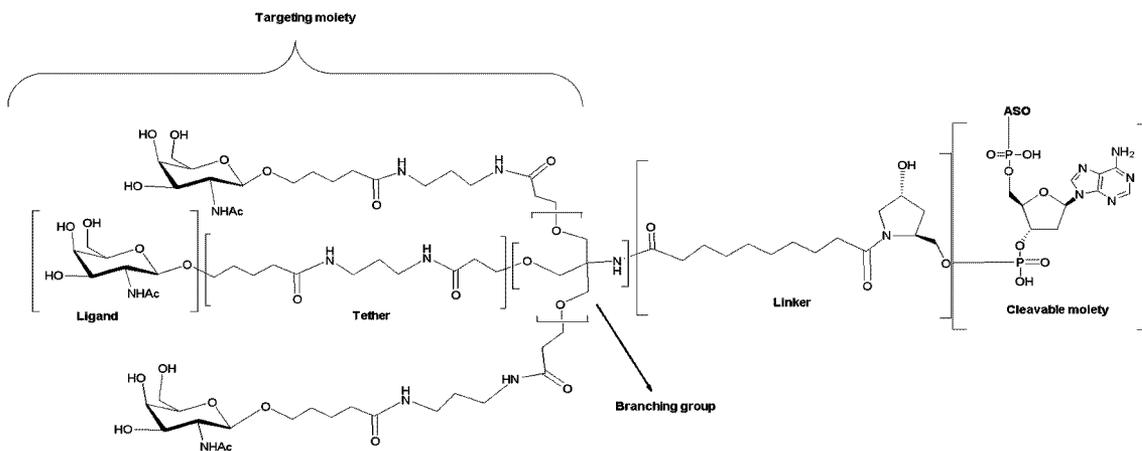
where $q = 4$, the formula is:



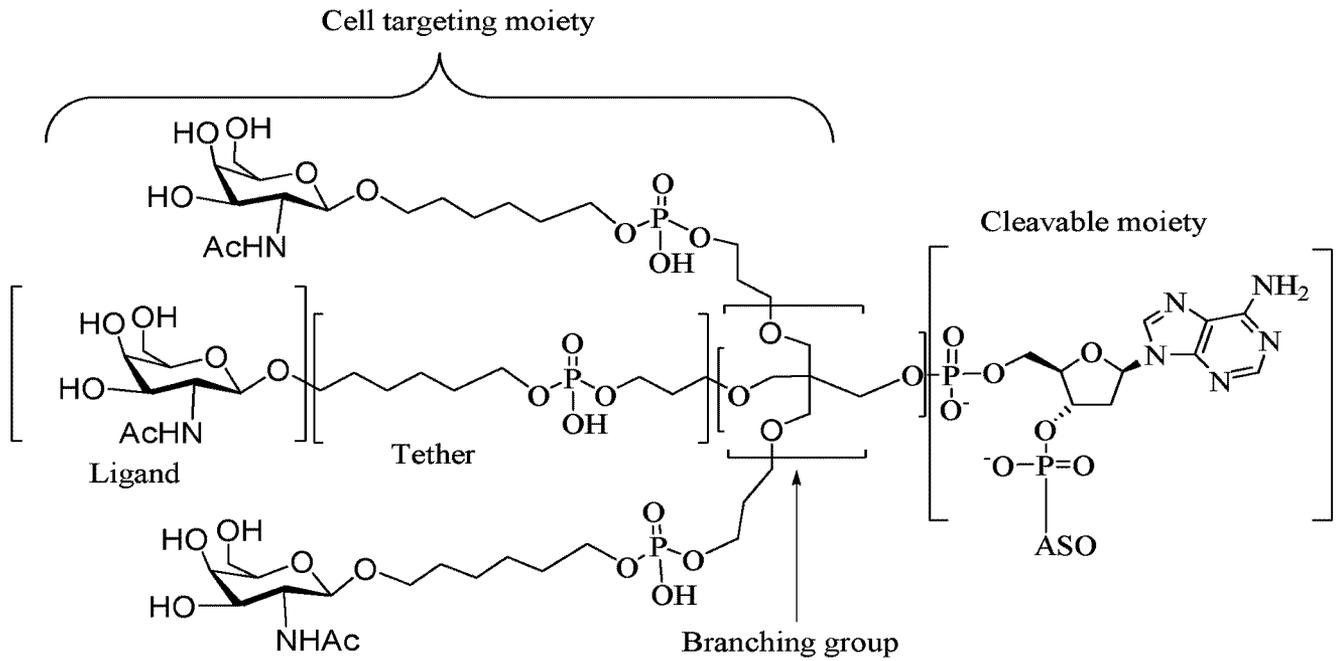
where $q = 5$, the formula is:



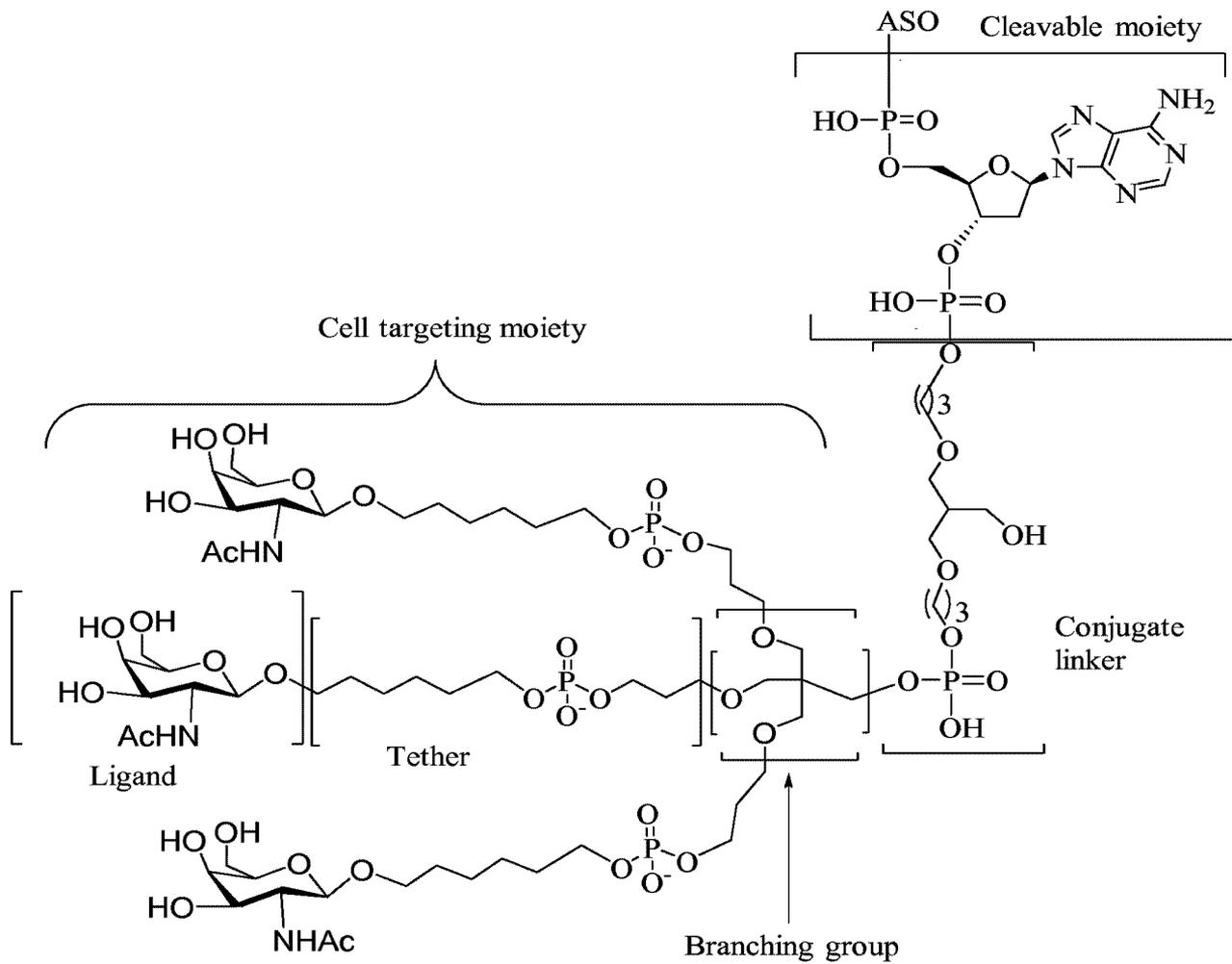
5 In certain embodiments, conjugated antisense compounds are provided having the structure:



In certain embodiments, conjugated antisense compounds are provided having the structure:

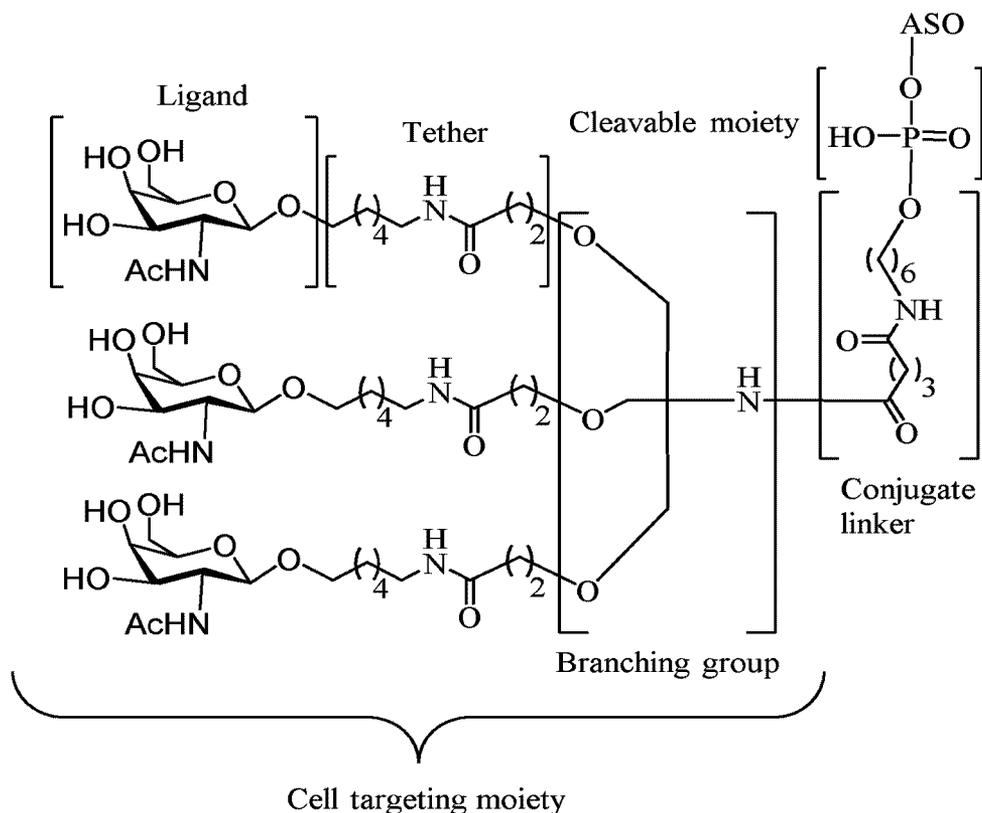


In certain embodiments, conjugated antisense compounds are provided having the structure:



5

In certain embodiments, conjugated antisense compounds are provided having the structure:



The present disclosure provides the following non-limiting embodiments:

In embodiments having more than one of a particular variable (e.g., more than one “m” or “n”),
 5 unless otherwise indicated, each such particular variable is selected independently. Thus, for a structure having more than one n, each n is selected independently, so they may or may not be the same as one another.

Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII
 and a conjugate group, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides. In
 certain embodiments, the modified oligonucleotide with the conjugate group consists of 20 linked
 10 nucleosides.

Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII
 and a conjugate group, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides and
 comprises a nucleobase sequence complementary to an equal length portion of nucleobases 3533 to 3552 of
 SEQ ID NO: 3, wherein the nucleobase sequence of the modified oligonucleotide is at least 80%
 15 complementary to SEQ ID NO: 3.

Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII
 and a conjugate group, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides and
 comprises a nucleobase sequence complementary to an equal length portion of nucleobases 3514 to 3558 of

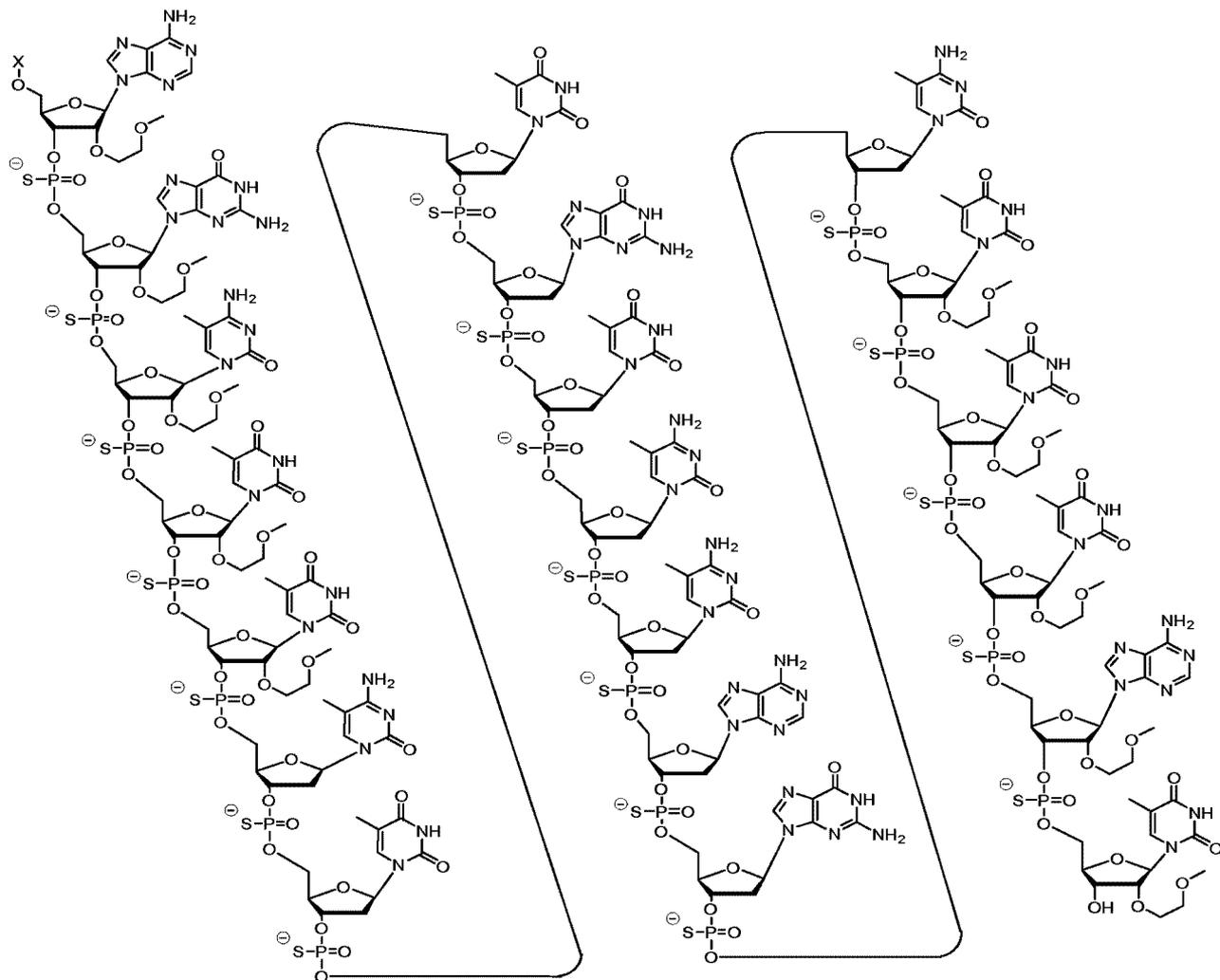
SEQ ID NO: 3, wherein the nucleobase sequence of the modified oligonucleotide is at least 80% complementary to SEQ ID NO: 3.

5 Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides and has a nucleobase sequence of any of the nucleobase sequences of SEQ ID NOs: 19-96, 209-221. In certain embodiments, the conjugated modified oligonucleotide has a nucleobase sequence comprising at least 8 contiguous nucleobases of any one of the nucleobase sequences of SEQ ID NOs: 19-96, 209-221. In certain embodiments, the compound consists of any one of SEQ ID NOs: 19-96, 209-221 and a conjugate group.

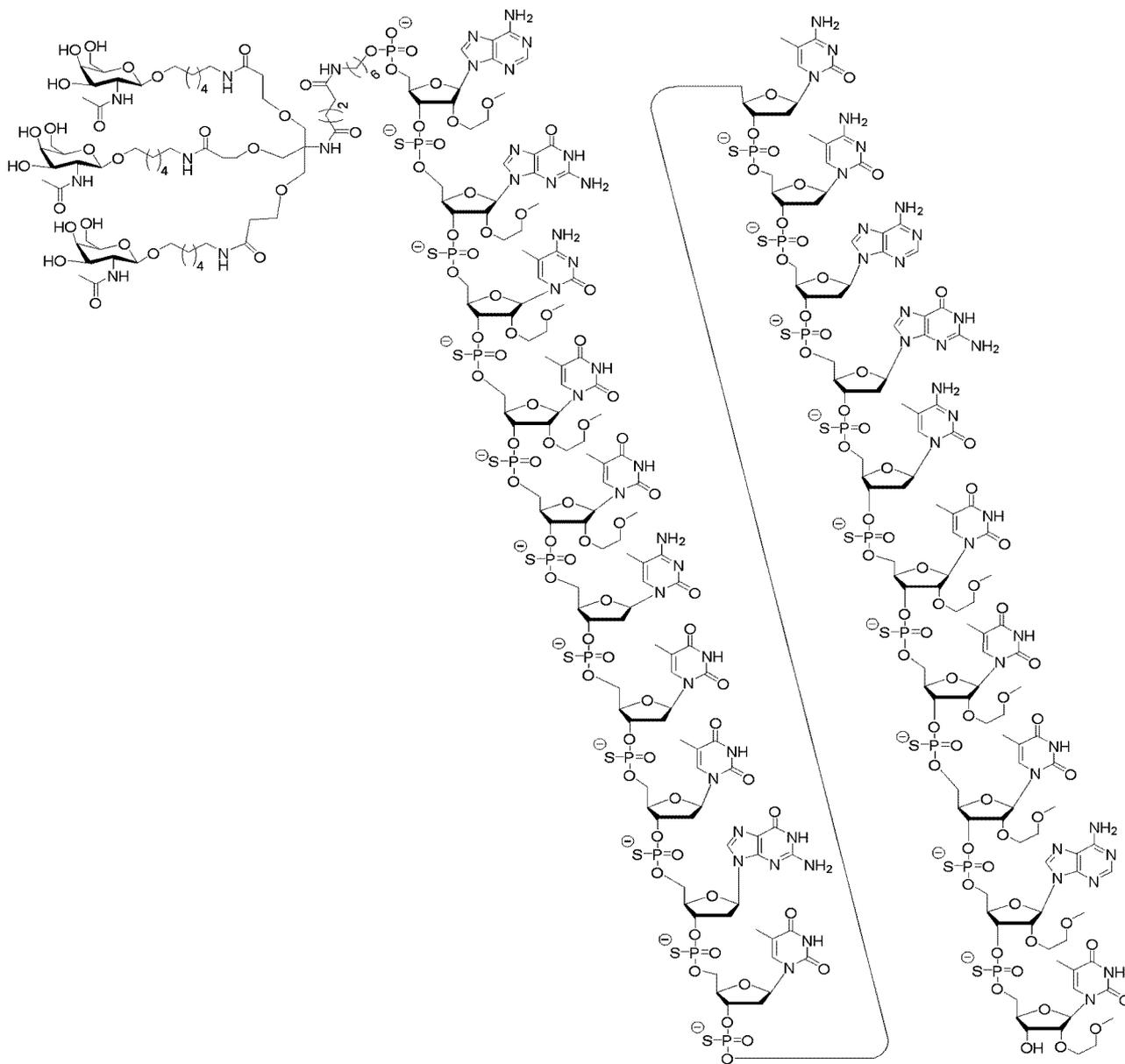
10 Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides and has a nucleobase sequence of SEQ ID NO: 87. In certain embodiments, the modified oligonucleotide with the conjugate group has a nucleobase sequence comprising at least 8 contiguous nucleobases of the nucleobase sequence of SEQ ID NO: 87. In certain embodiments, the compound consists of SEQ ID NO: 87 and a conjugate group.

15 In certain embodiments, the present disclosure provides conjugated antisense compounds represented by the following structure. In certain embodiments, the antisense compound comprises the modified oligonucleotide ISIS 304801 with a 5'-X, wherein X is a conjugate group comprising GalNAc. In certain embodiments, the antisense compound consists of the modified oligonucleotide ISIS 304801 with a 5'-X, wherein X is a conjugate group comprising GalNAc.

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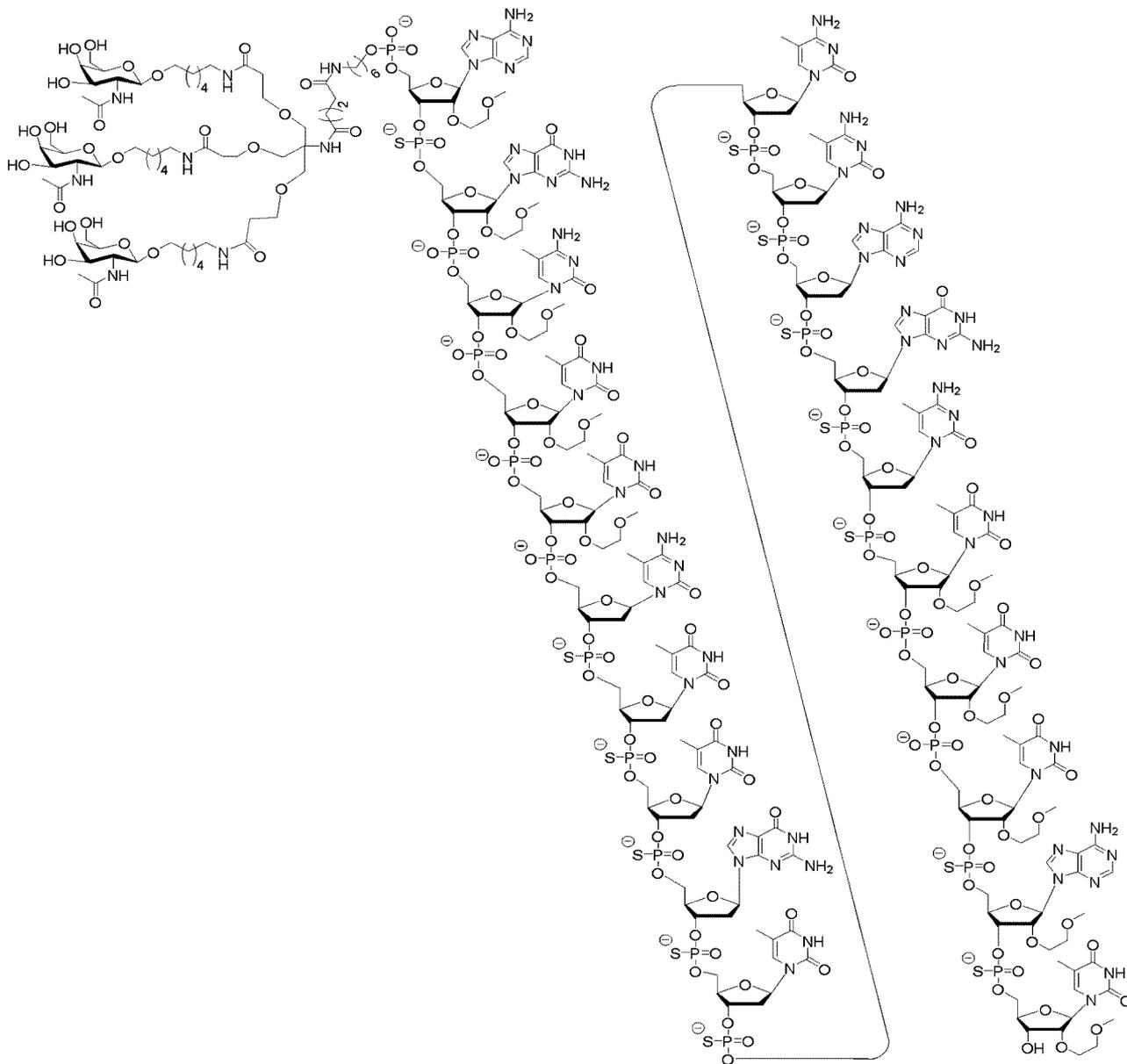


In certain embodiments, the present disclosure provides conjugated antisense compounds represented by the following structure. In certain embodiments, the antisense compound comprises the conjugated modified oligonucleotide ISIS 678354. In certain embodiments, the antisense compound consists of the conjugated modified oligonucleotide ISIS 678354.



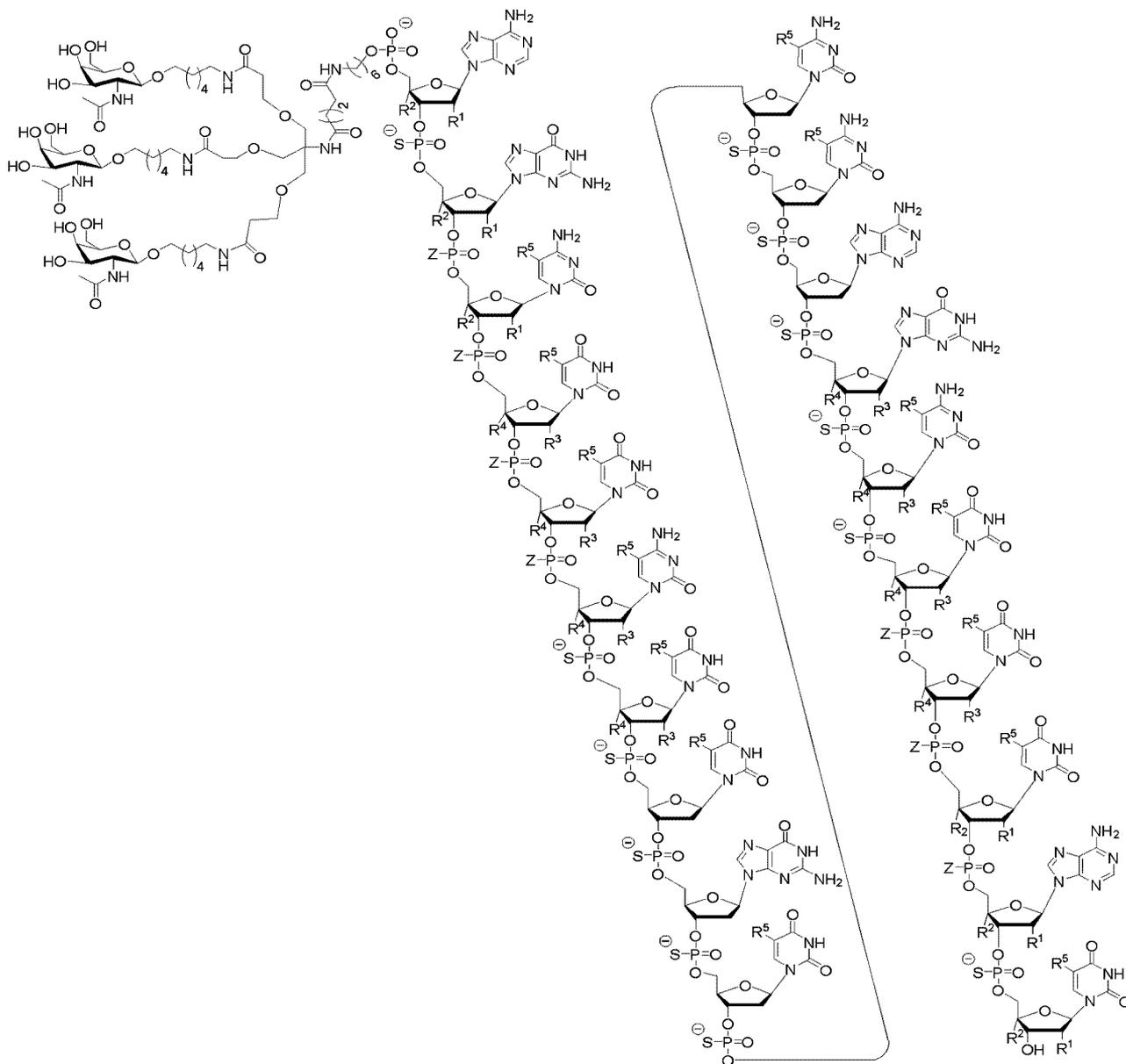
In certain embodiments, the present disclosure provides conjugated antisense compounds represented by the following structure. In certain embodiments, the antisense compound comprises the conjugated modified oligonucleotide ISIS 678357. In certain embodiments, the antisense compound consists of the

5 conjugated modified oligonucleotide ISIS 678357.



In certain embodiments, the present disclosure provides conjugated antisense compounds represented by the following structure. In certain embodiments, the antisense compound comprises a modified oligonucleotide with the nucleobase sequence of SEQ ID NO: 87 with a 5'-GalNAc with variability in the sugar mods of the wings.

5 oligonucleotide with the nucleobase sequence of SEQ ID NO: 87 with a 5'-GalNAc with variability in the sugar mods of the wings. In certain embodiments, the antisense compound consists of a modified oligonucleotide with the nucleobase sequence of SEQ ID NO: 87 with a 5'-GalNAc with variability in the sugar mods of the wings.



Wherein either R^1 is $-\text{OCH}_2\text{CH}_2\text{OCH}_3$ (MOE) and R^2 is H; or R^1 and R^2 together form a bridge, wherein R^1 is $-\text{O}-$ and R^2 is $-\text{CH}_2-$, $-\text{CH}(\text{CH}_3)-$, or $-\text{CH}_2\text{CH}_2-$, and R^1 and R^2 are directly connected such that the resulting bridge is selected from: $-\text{O}-\text{CH}_2-$, $-\text{O}-\text{CH}(\text{CH}_3)-$, and $-\text{O}-\text{CH}_2\text{CH}_2-$;

- 5 And for each pair of R^3 and R^4 on the same ring, independently for each ring: either R^3 is selected from H and $-\text{OCH}_2\text{CH}_2\text{OCH}_3$ and R^4 is H; or R^3 and R^4 together form a bridge, wherein R^3 is $-\text{O}-$, and R^4 is $-\text{CH}_2-$, $-\text{CH}(\text{CH}_3)-$, or $-\text{CH}_2\text{CH}_2-$ and R^3 and R^4 are directly connected such that the resulting bridge is selected from: $-\text{O}-\text{CH}_2-$, $-\text{O}-\text{CH}(\text{CH}_3)-$, and $-\text{O}-\text{CH}_2\text{CH}_2-$;

And R^5 is selected from H and $-\text{CH}_3$;

And Z is selected from S⁻ and O⁻.

The present disclosure provides the following non-limiting numbered embodiments:

DETAILED DESCRIPTION

5 It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the disclosure. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “including” as well as other forms, such as “includes” and “included”, is not limiting. Also, terms such as “element” or “component” encompass both
10 elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

15 A. Definitions

Unless specific definitions are provided, the nomenclature used in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical synthesis, and chemical analysis. Certain such techniques and
20 procedures may be found for example in “Carbohydrate Modifications in Antisense Research” Edited by Sangvi and Cook, American Chemical Society, Washington D.C., 1994; “Remington's Pharmaceutical Sciences,” Mack Publishing Co., Easton, Pa., 21st edition, 2005; and “Antisense Drug Technology, Principles, Strategies, and Applications” Edited by Stanley T. Crooke, CRC Press, Boca Raton, Florida; and Sambrook et al., “Molecular Cloning, A laboratory Manual,” 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

25 Unless otherwise indicated, the following terms have the following meanings:

As used herein, “nucleoside” means a compound comprising a nucleobase moiety and a sugar moiety. Nucleosides include, but are not limited to, naturally occurring nucleosides (as found in DNA and RNA) and modified nucleosides. Nucleosides may be linked to a phosphate moiety.

As used herein, “chemical modification” means a chemical difference in a compound when compared
30 to a naturally occurring counterpart. Chemical modifications of oligonucleotides include nucleoside

modifications (including sugar moiety modifications and nucleobase modifications) and internucleoside linkage modifications. In reference to an oligonucleotide, chemical modification does not include differences only in nucleobase sequence.

5 As used herein, “furanosyl” means a structure comprising a 5-membered ring comprising four carbon atoms and one oxygen atom.

As used herein, “naturally occurring sugar moiety” means a ribofuranosyl as found in naturally occurring RNA or a deoxyribofuranosyl as found in naturally occurring DNA.

As used herein, “sugar moiety” means a naturally occurring sugar moiety or a modified sugar moiety of a nucleoside.

10 As used herein, “modified sugar moiety” means a substituted sugar moiety or a sugar surrogate.

As used herein, “substituted sugar moiety” means a furanosyl that is not a naturally occurring sugar moiety. Substituted sugar moieties include, but are not limited to furanosyls comprising substituents at the 2'-position, the 3'-position, the 5'-position and/or the 4'-position. Certain substituted sugar moieties are bicyclic sugar moieties.

15 As used herein, “2'-substituted sugar moiety” means a furanosyl comprising a substituent at the 2'-position other than H or OH. Unless otherwise indicated, a 2'-substituted sugar moiety is not a bicyclic sugar moiety (i.e., the 2'-substituent of a 2'-substituted sugar moiety does not form a bridge to another atom of the furanosyl ring).

As used herein, “MOE” means $-OCH_2CH_2OCH_3$.

20 As used herein, “2'-F nucleoside” refers to a nucleoside comprising a sugar comprising fluorine at the 2' position. Unless otherwise indicated, the fluorine in a 2'-F nucleoside is in the ribo position (replacing the OH of a natural ribose).

As used herein the term "sugar surrogate" means a structure that does not comprise a furanosyl and that is capable of replacing the naturally occurring sugar moiety of a nucleoside, such that the resulting nucleoside sub-units are capable of linking together and/or linking to other nucleosides to form an oligomeric compound which is capable of hybridizing to a complementary oligomeric compound. Such structures include rings comprising a different number of atoms than furanosyl (e.g., 4, 6, or 7-membered rings); replacement of the oxygen of a furanosyl with a non-oxygen atom (e.g., carbon, sulfur, or nitrogen); or both a change in the number of atoms and a replacement of the oxygen. Such structures may also comprise substitutions corresponding to those described for substituted sugar moieties (e.g., 6-membered carbocyclic bicyclic sugar surrogates optionally comprising additional substituents). Sugar surrogates also include more complex sugar replacements (e.g., the non-ring systems of peptide nucleic acid). Sugar surrogates include without limitation morpholinos, cyclohexenyls and cyclohexitols.

30 As used herein, “bicyclic sugar moiety” means a modified sugar moiety comprising a 4 to 35 membered ring (including but not limited to a furanosyl) comprising a bridge connecting two atoms of the 4 to 7 membered ring to form a second ring, resulting in a bicyclic structure. In certain embodiments, the 4 to 7

membered ring is a sugar ring. In certain embodiments the 4 to 7 membered ring is a furanosyl. In certain such embodiments, the bridge connects the 2'-carbon and the 4'-carbon of the furanosyl.

As used herein, "nucleotide" means a nucleoside further comprising a phosphate linking group. As used herein, "linked nucleosides" may or may not be linked by phosphate linkages and thus includes, but is not limited to "linked nucleotides." As used herein, "linked nucleosides" are nucleosides that are connected
5 in a continuous sequence (i.e. no additional nucleosides are present between those that are linked).

As used herein, "nucleic acid" refers to molecules composed of monomeric nucleotides. A nucleic acid includes ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single-stranded nucleic acids (ssDNA), double-stranded nucleic acids (dsDNA), small interfering ribonucleic acids (siRNA), and
10 microRNAs (miRNA). A nucleic acid may also comprise any combination of these elements in a single molecule.

As used herein, "nucleotide" means a nucleoside further comprising a phosphate linking group. As used herein, "linked nucleosides" may or may not be linked by phosphate linkages and thus includes, but is not limited to "linked nucleotides." As used herein, "linked nucleosides" are nucleosides that are connected
15 in a continuous sequence (i.e. no additional nucleosides are present between those that are linked).

As used herein, "nucleobase" means a group of atoms that can be linked to a sugar moiety to create a nucleoside that is capable of incorporation into an oligonucleotide, and wherein the group of atoms is capable of bonding with a complementary naturally occurring nucleobase of another oligonucleotide or nucleic acid. Nucleobases may be naturally occurring or may be modified. As used herein, "nucleobase sequence" means
20 the order of contiguous nucleobases independent of any sugar, linkage, or nucleobase modification.

As used herein the terms, "unmodified nucleobase" or "naturally occurring nucleobase" means the naturally occurring heterocyclic nucleobases of RNA or DNA: the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) (including 5-methyl C), and uracil (U).

As used herein, "modified nucleobase" means any nucleobase that is not a naturally occurring
25 nucleobase.

As used herein, "modified nucleoside" means a nucleoside comprising at least one chemical modification compared to naturally occurring RNA or DNA nucleosides. Modified nucleosides comprise a modified sugar moiety and/or a modified nucleobase.

As used herein, "bicyclic nucleoside" or "BNA" means a nucleoside comprising a bicyclic sugar
30 moiety.

As used herein, "constrained ethyl nucleoside" or "cEt" means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-CH(CH₃)-O-2'bridge.

As used herein, "locked nucleic acid nucleoside" or "LNA" means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-CH₂-O-2'bridge.

As used herein, "2'-substituted nucleoside" means a nucleoside comprising a substituent at the 2'-
35 position other than H or OH. Unless otherwise indicated, a 2'-substituted nucleoside is not a bicyclic

nucleoside.

As used herein, “deoxynucleoside” means a nucleoside comprising 2'-H furanosyl sugar moiety, as found in naturally occurring deoxyribonucleosides (DNA). In certain embodiments, a 2'-deoxynucleoside may comprise a modified nucleobase or may comprise an RNA nucleobase (e.g., uracil).

5 As used herein, "oligonucleotide" means a compound comprising a plurality of linked nucleosides. In certain embodiments, an oligonucleotide comprises one or more unmodified ribonucleosides (RNA) and/or unmodified deoxyribonucleosides (DNA) and/or one or more modified nucleosides.

As used herein “oligonucleoside” means an oligonucleotide in which none of the internucleoside linkages contains a phosphorus atom. As used herein, oligonucleotides include oligonucleosides.

10 As used herein, “modified oligonucleotide” means an oligonucleotide comprising at least one modified nucleoside and/or at least one modified internucleoside linkage.

As used herein, “linkage” or “linking group” means a group of atoms that link together two or more other groups of atoms.

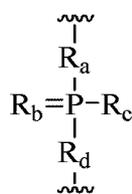
15 As used herein “internucleoside linkage” means a covalent linkage between adjacent nucleosides in an oligonucleotide.

As used herein “naturally occurring internucleoside linkage” means a 3' to 5' phosphodiester linkage.

As used herein, “modified internucleoside linkage” means any internucleoside linkage other than a naturally occurring internucleoside linkage.

20 As used herein, “terminal internucleoside linkage” means the linkage between the last two nucleosides of an oligonucleotide or defined region thereof.

As used herein, “phosphorus linking group” means a linking group comprising a phosphorus atom. Phosphorus linking groups include without limitation groups having the formula:



wherein:

25 R_a and R_d are each, independently, O, S, CH_2 , NH, or NJ_1 wherein J_1 is C_1 - C_6 alkyl or substituted C_1 - C_6 alkyl;

R_b is O or S;

R_c is OH, SH, C_1 - C_6 alkyl, substituted C_1 - C_6 alkyl, C_1 - C_6 alkoxy, substituted C_1 - C_6 alkoxy, amino or substituted amino; and

30 J_1 is R_b is O or S.

Phosphorus linking groups include without limitation, phosphodiester, phosphorothioate, phosphorodithioate, phosphonate, phosphoramidate, phosphorothioamidate, thionoalkylphosphonate, phosphotriesters,

thionoalkylphosphotriester and boranophosphate.

As used herein, "internucleoside phosphorus linking group" means a phosphorus linking group that directly links two nucleosides.

As used herein, "non-internucleoside phosphorus linking group" means a phosphorus linking group that does not directly link two nucleosides. In certain embodiments, a non-internucleoside phosphorus linking group links a nucleoside to a group other than a nucleoside. In certain embodiments, a non-internucleoside phosphorus linking group links two groups, neither of which is a nucleoside.

As used herein, "neutral linking group" means a linking group that is not charged. Neutral linking groups include without limitation phosphotriesters, methylphosphonates, MMI (-CH₂-N(CH₃)-O-), amide-3 (-CH₂-C(=O)-N(H)-), amide-4 (-CH₂-N(H)-C(=O)-), formacetal (-O-CH₂-O-), and thioformacetal (-S-CH₂-O-). Further neutral linking groups include nonionic linkages comprising siloxane (dialkylsiloxane), carboxylate ester, carboxamide, sulfide, sulfonate ester and amides (See for example: Carbohydrate Modifications in Antisense Research; Y.S. Sanghvi and P.D. Cook Eds. ACS Symposium Series 580; Chapters 3 and 4, (pp. 40-65)). Further neutral linking groups include nonionic linkages comprising mixed N, O, S and CH₂ component parts.

As used herein, "internucleoside neutral linking group" means a neutral linking group that directly links two nucleosides.

As used herein, "non-internucleoside neutral linking group" means a neutral linking group that does not directly link two nucleosides. In certain embodiments, a non-internucleoside neutral linking group links a nucleoside to a group other than a nucleoside. In certain embodiments, a non-internucleoside neutral linking group links two groups, neither of which is a nucleoside.

As used herein, "oligomeric compound" means a polymeric structure comprising two or more substructures. In certain embodiments, an oligomeric compound comprises an oligonucleotide. In certain embodiments, an oligomeric compound comprises one or more conjugate groups and/or terminal groups. In certain embodiments, an oligomeric compound consists of an oligonucleotide. Oligomeric compounds also include naturally occurring nucleic acids. In certain embodiments, an oligomeric compound comprises a backbone of one or more linked monomeric subunits where each linked monomeric subunit is directly or indirectly attached to a heterocyclic base moiety. In certain embodiments, oligomeric compounds may also include monomeric subunits that are not linked to a heterocyclic base moiety, thereby providing abasic sites. In certain embodiments, the linkages joining the monomeric subunits, the sugar moieties or surrogates and the heterocyclic base moieties can be independently modified. In certain embodiments, the linkage-sugar unit, which may or may not include a heterocyclic base, may be substituted with a mimetic such as the monomers in peptide nucleic acids.

As used herein, "terminal group" means one or more atom attached to either, or both, the 3' end or the 5' end of an oligonucleotide. In certain embodiments a terminal group is a conjugate group. In certain embodiments, a terminal group comprises one or more terminal group nucleosides.

As used herein, “conjugate” or “conjugate group” means an atom or group of atoms bound to an oligonucleotide or oligomeric compound. In general, conjugate groups modify one or more properties of the compound to which they are attached, including, but not limited to pharmacodynamic, pharmacokinetic, binding, absorption, cellular distribution, cellular uptake, charge and/or clearance properties.

5 As used herein, “conjugate linker” or “linker” in the context of a conjugate group means a portion of a conjugate group comprising any atom or group of atoms and which covalently link (1) an oligonucleotide to another portion of the conjugate group or (2) two or more portions of the conjugate group.

Conjugate groups are shown herein as radicals, providing a bond for forming covalent attachment to an oligomeric compound such as an antisense oligonucleotide. In certain embodiments, the point of
10 attachment on the oligomeric compound is the 3'-oxygen atom of the 3'-hydroxyl group of the 3' terminal nucleoside of the oligomeric compound. In certain embodiments the point of attachment on the oligomeric compound is the 5'-oxygen atom of the 5'-hydroxyl group of the 5' terminal nucleoside of the oligomeric compound. In certain embodiments, the bond for forming attachment to the oligomeric compound is a cleavable bond. In certain such embodiments, such cleavable bond constitutes all or part of a cleavable
15 moiety.

In certain embodiments, conjugate groups comprise a cleavable moiety (e.g., a cleavable bond or cleavable nucleoside) and a carbohydrate cluster portion, such as a GalNAc cluster portion. Such carbohydrate cluster portion comprises: a targeting moiety and, optionally, a conjugate linker. In certain
20 embodiments, the carbohydrate cluster portion is identified by the number and identity of the ligand. For example, in certain embodiments, the carbohydrate cluster portion comprises 3 GalNAc groups and is designated “GalNAc₃”. In certain embodiments, the carbohydrate cluster portion comprises 4 GalNAc groups and is designated “GalNAc₄”. Specific carbohydrate cluster portions (having specific tether, branching and conjugate linker groups) are described herein and designated by Roman numeral followed by subscript “a”. Accordingly “GalNAc_{3-1a}” refers to a specific carbohydrate cluster portion of a conjugate group having
25 3 GalNAc groups and specifically identified tether, branching and linking groups. Such carbohydrate cluster fragment is attached to an oligomeric compound via a cleavable moiety, such as a cleavable bond or cleavable nucleoside.

As used herein, “cleavable moiety” means a bond or group that is capable of being split under physiological conditions. In certain embodiments, a cleavable moiety is cleaved inside a cell or sub-cellular
30 compartments, such as a lysosome. In certain embodiments, a cleavable moiety is cleaved by endogenous enzymes, such as nucleases. In certain embodiments, a cleavable moiety comprises a group of atoms having one, two, three, four, or more than four cleavable bonds.

As used herein, “cleavable bond” means any chemical bond capable of being split. In certain
embodiments, a cleavable bond is selected from among: an amide, a polyamide, an ester, an ether, one or
35 both esters of a phosphodiester, a phosphate ester, a carbamate, a di-sulfide, or a peptide.

As used herein, "carbohydrate cluster" means a compound having one or more carbohydrate residues attached to a scaffold or linker group. (see, e.g., Maier et al., "Synthesis of Antisense Oligonucleotides Conjugated to a Multivalent Carbohydrate Cluster for Cellular Targeting," *Bioconjugate Chemistry*, 2003, (14): 18-29, or Rensen et al., "Design and Synthesis of Novel *N*-Acetylgalactosamine-Terminated Glycolipids for Targeting of Lipoproteins to the Hepatic Asialoglycoprotein Receptor," *J. Med. Chem.* 2004, (47): 5798-5808, for examples of carbohydrate conjugate clusters).

As used herein, "modified carbohydrate" means any carbohydrate having one or more chemical modifications relative to naturally occurring carbohydrates.

As used herein, "carbohydrate derivative" means any compound which may be synthesized using a carbohydrate as a starting material or intermediate.

As used herein, "carbohydrate" means a naturally occurring carbohydrate, a modified carbohydrate, or a carbohydrate derivative.

As used herein "protecting group" means any compound or protecting group known to those having skill in the art. Non-limiting examples of protecting groups may be found in "Protective Groups in Organic Chemistry", T. W. Greene, P. G. M. Wuts, ISBN 0-471-62301-6, John Wiley & Sons, Inc, New York.

As used herein, "single-stranded" means an oligomeric compound that is not hybridized to its complement and which lacks sufficient self-complementarity to form a stable self-duplex.

As used herein, "double stranded" means a pair of oligomeric compounds that are hybridized to one another or a single self-complementary oligomeric compound that forms a hairpin structure. In certain embodiments, a double-stranded oligomeric compound comprises a first and a second oligomeric compound.

As used herein, "antisense compound" means a compound comprising or consisting of an oligonucleotide at least a portion of which is complementary to a target nucleic acid to which it is capable of hybridizing, resulting in at least one antisense activity.

As used herein, "antisense activity" means any detectable and/or measurable change attributable to the hybridization of an antisense compound to its target nucleic acid. In certain embodiments, antisense activity includes modulation of the amount or activity of a target nucleic acid transcript (e.g. mRNA). In certain embodiments, antisense activity includes modulation of the splicing of pre-mRNA.

As used herein, "RNase H based antisense compound" means an antisense compound wherein at least some of the antisense activity of the antisense compound is attributable to hybridization of the antisense compound to a target nucleic acid and subsequent cleavage of the target nucleic acid by RNase H.

As used herein, "RISC based antisense compound" means an antisense compound wherein at least some of the antisense activity of the antisense compound is attributable to the RNA Induced Silencing Complex (RISC).

As used herein, "detecting" or "measuring" means that a test or assay for detecting or measuring is

35

performed. Such detection and/or measuring may result in a value of zero. Thus, if a test for detection or measuring results in a finding of no activity (activity of zero), the step of detecting or measuring the activity has nevertheless been performed.

As used herein, “detectable and/or measureable activity” means a statistically significant activity that
5 is not zero.

As used herein, “essentially unchanged” means little or no change in a particular parameter, particularly relative to another parameter which changes much more. In certain embodiments, a parameter is essentially unchanged when it changes less than 5%. In certain embodiments, a parameter is essentially unchanged if it changes less than two-fold while another parameter changes at least ten-fold. For example, in
10 certain embodiments, an antisense activity is a change in the amount of a target nucleic acid. In certain such embodiments, the amount of a non-target nucleic acid is essentially unchanged if it changes much less than the target nucleic acid does, but the change need not be zero.

As used herein, “expression” means the process by which a gene ultimately results in a protein. Expression includes, but is not limited to, transcription, post-transcriptional modification (e.g., splicing,
15 polyadenylation, addition of 5’-cap), and translation.

As used herein, “target nucleic acid” means a nucleic acid molecule to which an antisense compound is intended to hybridize to result in a desired antisense activity. Antisense oligonucleotides have sufficient complementarity to their target nucleic acids to allow hybridization under physiological conditions.

As used herein, “nucleobase complementarity” or “complementarity” when in reference to
20 nucleobases means a nucleobase that is capable of base pairing with another nucleobase. For example, in DNA, adenine (A) is complementary to thymine (T). For example, in RNA, adenine (A) is complementary to uracil (U). In certain embodiments, complementary nucleobase means a nucleobase of an antisense compound that is capable of base pairing with a nucleobase of its target nucleic acid. For example, if a nucleobase at a certain position of an antisense compound is capable of hydrogen bonding with a nucleobase
25 at a certain position of a target nucleic acid, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be complementary at that nucleobase pair. Nucleobases comprising certain modifications may maintain the ability to pair with a counterpart nucleobase and thus, are still capable of nucleobase complementarity.

As used herein, “non-complementary” in reference to nucleobases means a pair of nucleobases that
30 do not form hydrogen bonds with one another.

As used herein, “complementary” in reference to oligomeric compounds (e.g., linked nucleosides, oligonucleotides, or nucleic acids) means the capacity of such oligomeric compounds or regions thereof to hybridize to another oligomeric compound or region thereof through nucleobase complementarity. Complementary oligomeric compounds need not have nucleobase complementarity at each nucleoside.
35 Rather, some mismatches are tolerated. In certain embodiments, complementary oligomeric compounds or regions are complementary at 70% of the nucleobases (70% complementary). In certain embodiments,

complementary oligomeric compounds or regions are 80% complementary. In certain embodiments, complementary oligomeric compounds or regions are 90% complementary. In certain embodiments, complementary oligomeric compounds or regions are 95% complementary. In certain embodiments, complementary oligomeric compounds or regions are 100% complementary.

5 As used herein, “mismatch” means a nucleobase of a first oligomeric compound that is not capable of pairing with a nucleobase at a corresponding position of a second oligomeric compound, when the first and second oligomeric compound are aligned. Either or both of the first and second oligomeric compounds may be oligonucleotides.

10 As used herein, "hybridization" means the pairing of complementary oligomeric compounds (e.g., an antisense compound and its target nucleic acid). While not limited to a particular mechanism, the most common mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases.

As used herein, “specifically hybridizes” means the ability of an oligomeric compound to hybridize to one nucleic acid site with greater affinity than it hybridizes to another nucleic acid site.

15 As used herein, “fully complementary” in reference to an oligonucleotide or portion thereof means that each nucleobase of the oligonucleotide or portion thereof is capable of pairing with a nucleobase of a complementary nucleic acid or contiguous portion thereof. Thus, a fully complementary region comprises no mismatches or unhybridized nucleobases in either strand.

20 As used herein, “percent complementarity” means the percentage of nucleobases of an oligomeric compound that are complementary to an equal-length portion of a target nucleic acid. Percent complementarity is calculated by dividing the number of nucleobases of the oligomeric compound that are complementary to nucleobases at corresponding positions in the target nucleic acid by the total length of the oligomeric compound.

25 As used herein, “percent identity” means the number of nucleobases in a first nucleic acid that are the same type (independent of chemical modification) as nucleobases at corresponding positions in a second nucleic acid, divided by the total number of nucleobases in the first nucleic acid.

30 As used herein, "modulation" means a change of amount or quality of a molecule, function, or activity when compared to the amount or quality of a molecule, function, or activity prior to modulation. For example, modulation includes the change, either an increase (stimulation or induction) or a decrease (inhibition or reduction) in gene expression. As a further example, modulation of expression can include a change in splice site selection of pre-mRNA processing, resulting in a change in the absolute or relative amount of a particular splice-variant compared to the amount in the absence of modulation.

35 As used herein, “chemical motif” means a pattern of chemical modifications in an oligonucleotide or a region thereof. Motifs may be defined by modifications at certain nucleosides and/or at certain linking groups of an oligonucleotide.

As used herein, “nucleoside motif” means a pattern of nucleoside modifications in an oligonucleotide or a region thereof. The linkages of such an oligonucleotide may be modified or unmodified. Unless otherwise indicated, motifs herein describing only nucleosides are intended to be nucleoside motifs. Thus, in such instances, the linkages are not limited.

5 As used herein, “sugar motif” means a pattern of sugar modifications in an oligonucleotide or a region thereof.

As used herein, “linkage motif” means a pattern of linkage modifications in an oligonucleotide or region thereof. The nucleosides of such an oligonucleotide may be modified or unmodified. Unless otherwise indicated, motifs herein describing only linkages are intended to be linkage motifs. Thus, in such
10 instances, the nucleosides are not limited.

As used herein, “nucleobase modification motif” means a pattern of modifications to nucleobases along an oligonucleotide. Unless otherwise indicated, a nucleobase modification motif is independent of the nucleobase sequence.

As used herein, “sequence motif” means a pattern of nucleobases arranged along an oligonucleotide
15 or portion thereof. Unless otherwise indicated, a sequence motif is independent of chemical modifications and thus may have any combination of chemical modifications, including no chemical modifications.

As used herein, “type of modification” in reference to a nucleoside or a nucleoside of a “type” means the chemical modification of a nucleoside and includes modified and unmodified nucleosides. Accordingly, unless otherwise indicated, a “nucleoside having a modification of a first type” may be an unmodified
20 nucleoside.

As used herein, “differently modified” mean chemical modifications or chemical substituents that are different from one another, including absence of modifications. Thus, for example, a MOE nucleoside and an unmodified DNA nucleoside are “differently modified,” even though the DNA nucleoside is unmodified. Likewise, DNA and RNA are “differently modified,” even though both are naturally-occurring unmodified
25 nucleosides. Nucleosides that are the same but for comprising different nucleobases are not differently modified. For example, a nucleoside comprising a 2'-OMe modified sugar and an unmodified adenine nucleobase and a nucleoside comprising a 2'-OMe modified sugar and an unmodified thymine nucleobase are not differently modified.

As used herein, “the same type of modifications” refers to modifications that are the same as one
30 another, including absence of modifications. Thus, for example, two unmodified DNA nucleosides have “the same type of modification,” even though the DNA nucleoside is unmodified. Such nucleosides having the same type modification may comprise different nucleobases.

As used herein, “separate regions” means portions of an oligonucleotide wherein the chemical modifications or the motif of chemical modifications of any neighboring portions include at least one
35 difference to allow the separate regions to be distinguished from one another.

As used herein, “pharmaceutically acceptable carrier or diluent” means any substance suitable for use

in administering to an animal. In certain embodiments, a pharmaceutically acceptable carrier or diluent is sterile saline. In certain embodiments, such sterile saline is pharmaceutical grade saline.

As used herein the term “metabolic disorder” means a disease or condition principally characterized by dysregulation of metabolism – the complex set of chemical reactions associated with breakdown of food to produce energy.

As used herein, the term “cardiovascular disorder” means a disease or condition principally characterized by impaired function of the heart or blood vessels.

As used herein the term "mono or polycyclic ring system" is meant to include all ring systems selected from single or polycyclic radical ring systems wherein the rings are fused or linked and is meant to be inclusive of single and mixed ring systems individually selected from aliphatic, alicyclic, aryl, heteroaryl, aralkyl, arylalkyl, heterocyclic, heteroaryl, heteroaromatic and heteroarylalkyl. Such mono and poly cyclic structures can contain rings that each have the same level of saturation or each, independently, have varying degrees of saturation including fully saturated, partially saturated or fully unsaturated. Each ring can comprise ring atoms selected from C, N, O and S to give rise to heterocyclic rings as well as rings comprising only C ring atoms which can be present in a mixed motif such as for example benzimidazole wherein one ring has only carbon ring atoms and the fused ring has two nitrogen atoms. The mono or polycyclic ring system can be further substituted with substituent groups such as for example phthalimide which has two =O groups attached to one of the rings. Mono or polycyclic ring systems can be attached to parent molecules using various strategies such as directly through a ring atom, fused through multiple ring atoms, through a substituent group or through a bifunctional linking moiety.

As used herein, “prodrug” means an inactive or less active form of a compound which, when administered to a subject, is metabolized to form the active, or more active, compound (e.g., drug).

As used herein, "substituent" and "substituent group," means an atom or group that replaces the atom or group of a named parent compound. For example a substituent of a modified nucleoside is any atom or group that differs from the atom or group found in a naturally occurring nucleoside (e.g., a modified 2'-substituent is any atom or group at the 2'-position of a nucleoside other than H or OH). Substituent groups can be protected or unprotected. In certain embodiments, compounds of the present disclosure have substituents at one or at more than one position of the parent compound. Substituents may also be further substituted with other substituent groups and may be attached directly or via a linking group such as an alkyl or hydrocarbyl group to a parent compound.

Likewise, as used herein, “substituent” in reference to a chemical functional group means an atom or group of atoms that differs from the atom or a group of atoms normally present in the named functional group. In certain embodiments, a substituent replaces a hydrogen atom of the functional group (e.g., in certain embodiments, the substituent of a substituted methyl group is an atom or group other than hydrogen which replaces one of the hydrogen atoms of an unsubstituted methyl group). Unless otherwise indicated, groups amenable for use as substituents include without limitation, halogen, hydroxyl, alkyl, alkenyl, alkynyl,

acyl (-C(O)R_{aa}), carboxyl (-C(O)O-R_{aa}), aliphatic groups, alicyclic groups, alkoxy, substituted oxy (-O-R_{aa}), aryl, aralkyl, heterocyclic radical, heteroaryl, heteroarylalkyl, amino (-N(R_{bb})(R_{cc})), imino(=NR_{bb}), amido (-C(O)N(R_{bb})(R_{cc}) or -N(R_{bb})C(O)R_{aa}), azido (-N₃), nitro (-NO₂), cyano (-CN), carbamido (-OC(O)N(R_{bb})(R_{cc}) or -N(R_{bb})C(O)OR_{aa}), ureido (-N(R_{bb})C(O)N(R_{bb})(R_{cc})), thioureido (-N(R_{bb})C(S)N(R_{bb})(R_{cc})), guanidinyl (-N(R_{bb})C(=NR_{bb})N(R_{bb})(R_{cc})), amidinyl (-C(=NR_{bb})N(R_{bb})(R_{cc}) or -N(R_{bb})C(=NR_{bb})(R_{aa})), thiol (-SR_{bb}), sulfinyl (-S(O)R_{bb}), sulfonyl (-S(O)₂R_{bb}) and sulfonamidyl (-S(O)₂N(R_{bb})(R_{cc}) or -N(R_{bb})S(O)₂R_{bb}). Wherein each R_{aa}, R_{bb} and R_{cc} is, independently, H, an optionally linked chemical functional group or a further substituent group with a preferred list including without limitation, alkyl, alkenyl, alkynyl, aliphatic, alkoxy, acyl, aryl, aralkyl, heteroaryl, alicyclic, heterocyclic and heteroarylalkyl. Selected substituents within the compounds described herein are present to a recursive degree.

As used herein, "alkyl," as used herein, means a saturated straight or branched hydrocarbon radical containing up to twenty four carbon atoms. Examples of alkyl groups include without limitation, methyl, ethyl, propyl, butyl, isopropyl, n-hexyl, octyl, decyl, dodecyl and the like. Alkyl groups typically include from 1 to about 24 carbon atoms, more typically from 1 to about 12 carbon atoms (C₁-C₁₂ alkyl) with from 1 to about 6 carbon atoms being more preferred.

As used herein, "alkenyl," means a straight or branched hydrocarbon chain radical containing up to twenty four carbon atoms and having at least one carbon-carbon double bond. Examples of alkenyl groups include without limitation, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, dienes such as 1,3-butadiene and the like. Alkenyl groups typically include from 2 to about 24 carbon atoms, more typically from 2 to about 12 carbon atoms with from 2 to about 6 carbon atoms being more preferred. Alkenyl groups as used herein may optionally include one or more further substituent groups.

As used herein, "alkynyl," means a straight or branched hydrocarbon radical containing up to twenty four carbon atoms and having at least one carbon-carbon triple bond. Examples of alkynyl groups include, without limitation, ethynyl, 1-propynyl, 1-butynyl, and the like. Alkynyl groups typically include from 2 to about 24 carbon atoms, more typically from 2 to about 12 carbon atoms with from 2 to about 6 carbon atoms being more preferred. Alkynyl groups as used herein may optionally include one or more further substituent groups.

As used herein, "acyl," means a radical formed by removal of a hydroxyl group from an organic acid and has the general Formula -C(O)-X where X is typically aliphatic, alicyclic or aromatic. Examples include aliphatic carbonyls, aromatic carbonyls, aliphatic sulfonyls, aromatic sulfinyls, aliphatic sulfinyls, aromatic phosphates, aliphatic phosphates and the like. Acyl groups as used herein may optionally include further substituent groups.

As used herein, "alicyclic" means a cyclic ring system wherein the ring is aliphatic. The ring system can comprise one or more rings wherein at least one ring is aliphatic. Preferred alicyclics include rings having from about 5 to about 9 carbon atoms in the ring. Alicyclic as used herein may optionally include further substituent groups.

As used herein, "aliphatic" means a straight or branched hydrocarbon radical containing up to twenty four carbon atoms wherein the saturation between any two carbon atoms is a single, double or triple bond. An aliphatic group preferably contains from 1 to about 24 carbon atoms, more typically from 1 to about 12 carbon atoms with from 1 to about 6 carbon atoms being more preferred. The straight or branched chain of an aliphatic group may be interrupted with one or more heteroatoms that include nitrogen, oxygen, sulfur and phosphorus. Such aliphatic groups interrupted by heteroatoms include without limitation, polyalkoxys, such as polyalkylene glycols, polyamines, and polyimines. Aliphatic groups as used herein may optionally include further substituent groups.

As used herein, "alkoxy" means a radical formed between an alkyl group and an oxygen atom wherein the oxygen atom is used to attach the alkoxy group to a parent molecule. Examples of alkoxy groups include without limitation, methoxy, ethoxy, propoxy, isopropoxy, *n*-butoxy, *sec*-butoxy, *tert*-butoxy, *n*-pentoxy, neopentoxy, *n*-hexoxy and the like. Alkoxy groups as used herein may optionally include further substituent groups.

As used herein, "aminoalkyl" means an amino substituted C₁-C₁₂ alkyl radical. The alkyl portion of the radical forms a covalent bond with a parent molecule. The amino group can be located at any position and the aminoalkyl group can be substituted with a further substituent group at the alkyl and/or amino portions.

As used herein, "aralkyl" and "arylalkyl" mean an aromatic group that is covalently linked to a C₁-C₁₂ alkyl radical. The alkyl radical portion of the resulting aralkyl (or arylalkyl) group forms a covalent bond with a parent molecule. Examples include without limitation, benzyl, phenethyl and the like. Aralkyl groups as used herein may optionally include further substituent groups attached to the alkyl, the aryl or both groups that form the radical group.

As used herein, "aryl" and "aromatic" mean a mono- or polycyclic carbocyclic ring system radicals having one or more aromatic rings. Examples of aryl groups include without limitation, phenyl, naphthyl, tetrahydronaphthyl, indanyl, idenyl and the like. Preferred aryl ring systems have from about 5 to about 20 carbon atoms in one or more rings. Aryl groups as used herein may optionally include further substituent groups.

As used herein, "halo" and "halogen," mean an atom selected from fluorine, chlorine, bromine and iodine.

As used herein, "heteroaryl," and "heteroaromatic," mean a radical comprising a mono- or polycyclic aromatic ring, ring system or fused ring system wherein at least one of the rings is aromatic and includes one or more heteroatoms. Heteroaryl is also meant to include fused ring systems including systems where one or more of the fused rings contain no heteroatoms. Heteroaryl groups typically include one ring atom selected from sulfur, nitrogen or oxygen. Examples of heteroaryl groups include without limitation, pyridinyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinoliny, isoquinoliny, benzimidazolyl, benzooxazolyl,

quinoxalinyll and the like. Heteroaryl radicals can be attached to a parent molecule directly or through a linking moiety such as an aliphatic group or hetero atom. Heteroaryl groups as used herein may optionally include further substituent groups.

As used herein, “conjugate compound” means any atoms, group of atoms, or group of linked atoms suitable for use as a conjugate group. In certain embodiments, conjugate compounds may possess or impart one or more properties, including, but not limited to pharmacodynamic, pharmacokinetic, binding, absorption, cellular distribution, cellular uptake, charge and/or clearance properties.

As used herein, unless otherwise indicated or modified, the term “double-stranded” refers to two separate oligomeric compounds that are hybridized to one another. Such double stranded compounds may have one or more or non-hybridizing nucleosides at one or both ends of one or both strands (overhangs) and/or one or more internal non-hybridizing nucleosides (mismatches) provided there is sufficient complementarity to maintain hybridization under physiologically relevant conditions.

As used herein, “5’ target site” refers to the nucleotide of a target nucleic acid which is complementary to the 5’-most nucleotide of a particular antisense compound.

As used herein, “about” means within $\pm 10\%$ of a value. For example, if it is stated, “a marker may be increased by about 50%”, it is implied that the marker may be increased between 45%-55%.

As used herein, “administered concomitantly” refers to the co-administration of two agents in any manner in which the pharmacological effects of both are manifest in the patient at the same time. Concomitant administration does not require that both agents be administered in a single pharmaceutical composition, in the same dosage form, or by the same route of administration. The effects of both agents need not manifest themselves at the same time. The effects need only be overlapping for a period of time and need not be coextensive.

As used herein, “administering” or “administration” means providing a pharmaceutical agent to an individual, and includes, but is not limited to, administering by a medical professional and self-administering. Administration of a pharmaceutical agent to an individual can be continuous, chronic, short or intermittent. Administration can parenteral or non-parenteral.

As used herein, “agent” means an active substance that can provide a therapeutic benefit when administered to an animal. “First agent” means a therapeutic compound of the invention. For example, a first agent can be an antisense oligonucleotide targeting apoCIII. “Second agent” means a second therapeutic compound of the invention (e.g. a second antisense oligonucleotide targeting apoCIII) and/or a non-apoCIII therapeutic compound.

As used herein, “amelioration” or “ameliorate” or “ameliorating” refers to a lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. The severity of indicators can be determined by subjective or objective measures, which are known to those skilled in the art.

As used herein, “animal” refers to a human or non-human animal, including, but not limited to, mice, rats, rabbits, dogs, cats, pigs, and non-human primates, including, but not limited to, monkeys and chimpanzees.

As used herein, “ApoCIII”, “Apolipoprotein C-III” or “ApoC3” means any nucleic acid or protein
5 sequence encoding ApoCIII. For example, in certain embodiments, an ApoCIII includes a DNA sequence encoding ApoCIII, a RNA sequence transcribed from DNA encoding ApoCIII (including genomic DNA comprising introns and exons), a mRNA sequence encoding ApoCIII, or a peptide sequence encoding ApoCIII.

As used herein, “ApoCIII nucleic acid” means any nucleic acid encoding ApoCIII. For example, in
10 certain embodiments, an ApoCIII nucleic acid includes a DNA sequence encoding ApoCIII, a RNA sequence transcribed from DNA encoding ApoCIII (including genomic DNA comprising introns and exons), and a mRNA sequence encoding ApoCIII.

As used herein, “ApoCIII specific inhibitor” refers to any agent capable of specifically inhibiting the
expression of ApoCIII mRNA and/or the expression or activity of ApoCIII protein at the molecular level.
15 For example, ApoCIII specific inhibitors include nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of ApoCIII mRNA and/or ApoCIII protein. In certain embodiments, the nucleic acid is an antisense compound. In certain embodiments, the antisense compound is a an oligonucleotide targeting ApoCIII. In certain embodiments, the oligonucleotide targeting ApoCIII is a modified oligonucleotide targeting ApoCIII. In certain embodiments,
20 the oligonucleotide targeting ApoCIII is a modified oligonucleotide targeting ApoCIII with a conjugate group. In certain embodiments, the oligonucleotide targeting ApoCIII has a sequence as shown in SEQ ID NOs:19-96, 209-221 or another sequence (for example, such as those disclosed in PCT Publication WO 2004/093783 or PCT Publication WO 2012/149495). In certain embodiments, by specifically modulating ApoCIII mRNA level and/or ApoCIII protein expression, ApoCIII specific inhibitors may affect components
25 of the lipogenic or glucogenic pathway. Similarly, in certain embodiments, ApoCIII specific inhibitors may affect other molecular processes in an animal.

As used herein, “ApoCIII mRNA” means a mRNA encoding an ApoCIII protein.

As used herein, “ApoCIII protein” means any protein sequence encoding ApoCIII.

As used herein, “atherosclerosis” means a hardening of the arteries affecting large and medium-sized
30 arteries and is characterized by the presence of fatty deposits. The fatty deposits are called "atheromas" or “plaques,” which consist mainly of cholesterol and other fats, calcium and scar tissue, and damage the lining of arteries.

As used herein, “coronary heart disease (CHD)” means a narrowing of the small blood vessels that supply blood and oxygen to the heart, which is often a result of atherosclerosis.

As used herein, “diabetes mellitus” or “diabetes” is a syndrome characterized by disordered
35 metabolism and abnormally high blood sugar (hyperglycemia) resulting from insufficient levels of insulin or

reduced insulin sensitivity. The characteristic symptoms are excessive urine production (polyuria) due to high blood glucose levels, excessive thirst and increased fluid intake (polydipsia) attempting to compensate for increased urination, blurred vision due to high blood glucose effects on the eye's optics, unexplained weight loss, and lethargy.

5 As used herein, “diabetic dyslipidemia” or “type 2 diabetes with dyslipidemia” means a condition characterized by Type 2 diabetes, reduced HDL-C, elevated triglycerides (TG), and elevated small, dense LDL particles.

As used herein, “diluent” means an ingredient in a composition that lacks pharmacological activity, but is pharmaceutically necessary or desirable. For example, the diluent in an injected composition can be a liquid, e.g. saline solution.

10 As used herein, “dyslipidemia” refers to a disorder of lipid and/or lipoprotein metabolism, including lipid and/or lipoprotein overproduction or deficiency. Dyslipidemias can be manifested by elevation of lipids such as chylomicron, cholesterol and triglycerides as well as lipoproteins such as low-density lipoprotein (LDL) cholesterol.

15 As used herein, “dosage unit” means a form in which a pharmaceutical agent is provided, e.g. pill, tablet, or other dosage unit known in the art. In certain embodiments, a dosage unit is a vial containing lyophilized antisense oligonucleotide. In certain embodiments, a dosage unit is a vial containing reconstituted antisense oligonucleotide.

As used herein, “dose” means a specified quantity of a pharmaceutical agent provided in a single administration, or in a specified time period. In certain embodiments, a dose can be administered in one, two, or more boluses, tablets, or injections. For example, in certain embodiments where subcutaneous administration is desired, the desired dose requires a volume not easily accommodated by a single injection, therefore, two or more injections can be used to achieve the desired dose. In certain embodiments, the pharmaceutical agent is administered by infusion over an extended period of time or continuously. Doses can be stated as the amount of pharmaceutical agent per hour, day, week, or month. Doses can also be stated as mg/kg or g/kg.

20 As used herein, “effective amount” or “therapeutically effective amount” means the amount of active pharmaceutical agent sufficient to effectuate a desired physiological outcome in an individual in need of the agent. The effective amount can vary among individuals depending on the health and physical condition of the individual to be treated, the taxonomic group of the individuals to be treated, the formulation of the composition, assessment of the individual’s medical condition, and other relevant factors.

30 As used herein, “Fredrickson Type I” is also known as “Lipoprotein lipase deficiency”, “LPLD”, “Familial Chylomicronemia Syndrome” or “FCS” and exists in several forms: Type 1a (also known as Buerger-Gruestz syndrome) is a lipoprotein lipase deficiency commonly due to a deficiency of LPL or altered ApoC-II; Type 1b (also known as familial apoprotein CII deficiency) is a condition caused by lack of lipoprotein lipase activator apoprotein C-II; and Type 1c is a chylomicronemia due to circulating inhibitor of

lipoprotein lipase. Type I is a rare disorder that usually presents in childhood. It is characterized by severe elevations in chylomicrons and extremely elevated TG levels (always reaching well above 1000 mg/dL and not infrequently rising as high as 10,000 mg/dL or more) with episodes of abdominal pain, recurrent acute pancreatitis, eruptive cutaneous xanthomata, and hepatosplenomegaly. Patients rarely develop atherosclerosis, perhaps because their plasma lipoprotein particles are too large to enter into the arterial intima (Nordestgaard et al., *J Lipid Res*, 1988, 29:1491-1500; Nordestgaard et al., *Arteriosclerosis*, 1988, 8:421-428). Type I is usually caused by mutations of either the *LPL* gene, or of the gene's cofactor ApoC-II, resulting in the inability of affected individuals to produce sufficient functionally active LPL. Patients are either homozygous for such mutations or compound heterozygous. Fredrickson Type I can also be due to mutations in the *GPIHBP1*, *APOA5*, *LMF1* or other genes leading to dysfunctional LPL. Brunzell, In: Pagon RA, Adam MP, Bird TD, Dolan CR, Fong CT, Stephens K, editors. *GeneReviews™* [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2013.1999 Oct 12 [updated 2011 Dec 15]. Further, Fredrickson Type I, in some instances, can be due to the presence of LPL inhibitors (e.g., anti-LPL antibodies) in an individual causing dysfunctional LPL. The prevalence of Fredrickson Type I is approximately 1 in 1,000,000 in the general population and much higher in South Africa and Eastern Quebec as a result of a founder effect. Patients respond minimally, or not at all, to TG-lowering drugs (Tremblay et al., *J Clin Lipidol*, 2011, 5:37-44; Brisson et al., *Pharmacogenet Genom*, 2010, 20:742-747) and hence restriction of dietary fat to 20 grams/day or less is used to manage the symptoms of this rare disorder.

As used herein, “fully complementary” or “100% complementary” means each nucleobase of a nucleobase sequence of a first nucleic acid has a complementary nucleobase in a second nucleobase sequence of a second nucleic acid. In certain embodiments, a first nucleic acid is an antisense compound and a second nucleic acid is a target nucleic acid.

As used herein, “glucose” is a monosaccharide used by cells as a source of energy and inflammatory intermediate. “Plasma glucose” refers to glucose present in the plasma.

As used herein, “high density lipoprotein-C” or “HDL-C” means cholesterol associated with high density lipoprotein particles. Concentration of HDL-C in serum (or plasma) is typically quantified in mg/dL or nmol/L. “Serum HDL-C” and “plasma HDL-C” mean HDL-C in serum and plasma, respectively.

As used herein, “HMG-CoA reductase inhibitor” means an agent that acts through the inhibition of the enzyme HMG-CoA reductase, such as atorvastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, and simvastatin.

As used herein, “hypercholesterolemia” means a condition characterized by elevated cholesterol or circulating (plasma) cholesterol, LDL-cholesterol and VLDL-cholesterol, as per the guidelines of the Expert Panel Report of the National Cholesterol Educational Program (NCEP) of Detection, Evaluation of Treatment of high cholesterol in adults (see, *Arch. Int. Med.* (1988) 148, 36-39).

As used herein, “hyperlipidemia” or “hyperlipemia” is a condition characterized by elevated serum lipids or circulating (plasma) lipids. This condition manifests an abnormally high concentration of fats. The

lipid fractions in the circulating blood are cholesterol, low density lipoproteins, very low density lipoproteins, chylomicrons and triglycerides. The Fredrickson classification of hyperlipidemias is based on the pattern of TG and cholesterol-rich lipoprotein particles, as measured by electrophoresis or ultracentrifugation and is commonly used to characterize primary causes of hyperlipidemias such as hypertriglyceridemia (Fredrickson and Lee, *Circulation*, 1965, 31:321-327; Fredrickson et al., *New Eng J Med*, 1967, 276 (1): 34-42).

As used herein, “hypertriglyceridemia” means a condition characterized by elevated triglyceride levels. Hypertriglyceridemia is the consequence of increased production and/or reduced or delayed catabolism of triglyceride (TG)-rich lipoproteins: VLDL and, to a lesser extent, chylomicrons (CM). Its etiology includes primary (i.e. genetic causes) and secondary (other underlying causes such as diabetes, metabolic syndrome/insulin resistance, obesity, physical inactivity, cigarette smoking, excess alcohol and a diet very high in carbohydrates) factors or, most often, a combination of both (Yuan *et al. CMAJ*, 2007, 176:1113-1120). Hypertriglyceridemia is a common clinical trait associated with an increased risk of cardiometabolic disease (Hegele et al. 2009, *Hum Mol Genet*, 18: 4189-4194; Hegele and Pollex 2009, *Mol Cell Biochem*, 326: 35-43) as well as of occurrence of acute pancreatitis in the most severe forms (Toskes 1990, *Gastroenterol Clin North Am*, 19: 783-791; Gaudet et al. 2010, *Atherosclerosis Supplements*, 11: 55-60; Catapano et al. 2011, *Atherosclerosis*, 217S: S1-S44; Tremblay et al. 2011, *J Clin Lipidol*, 5: 37-44). Examples of cardiometabolic disease include, but are not limited to, diabetes, metabolic syndrome/insulin resistance, and genetic disorders such as familial chylomicronemia syndrome (FCS), familial combined hyperlipidemia and familial hypertriglyceridemia. Borderline high TG levels (150-199 mg/dL) are commonly found in the general population and are a common component of the metabolic syndrome/insulin resistance states. The same is true for high TG levels (200-499 mg/dL) except that as plasma TG levels increase, underlying genetic factors play an increasingly important etiologic role. Very high TG levels (≥ 500 mg/dL) are most often associated with elevated CM levels as well, and are accompanied by increasing risk for acute pancreatitis. The risk of pancreatitis is considered clinically significant if TG levels exceed 880 mg/dL (>10 mmol) and the European Atherosclerosis Society/European Society of Cardiology (EAS/ESC) 2011 guidelines state that actions to prevent acute pancreatitis are mandatory (Catapano et al. 2011, *Atherosclerosis*, 217S: S1-S44). According to the EAS/ESC 2011 guidelines, hypertriglyceridemia is the cause of approximately 10% of all cases of pancreatitis, and development of pancreatitis can occur at TG levels between 440-880 mg/dL. Based on evidence from clinical studies demonstrating that elevated TG levels are an independent risk factor for atherosclerotic CVD, the guidelines from both the National Cholesterol Education Program Adult Treatment Panel III (NCEP 2002, *Circulation*, 106: 3143-421) and the American Diabetes Association (ADA 2008, *Diabetes Care*, 31: S12-S54.) recommend a target TG level of less than 150 mg/dL to reduce cardiovascular risk.

As used herein, “identifying” or “selecting an animal with metabolic or cardiovascular disease” means identifying or selecting a subject prone to or having been diagnosed with a metabolic disease, a

cardiovascular disease, or a metabolic syndrome; or, identifying or selecting a subject having any symptom of a metabolic disease, cardiovascular disease, or metabolic syndrome including, but not limited to, hypercholesterolemia, hyperglycemia, hyperlipidemia, hypertriglyceridemia, hypertension increased insulin resistance, decreased insulin sensitivity, above normal body weight, and/or above normal body fat content or any combination thereof. Such identification can be accomplished by any method, including but not limited to, standard clinical tests or assessments, such as measuring serum or circulating (plasma) cholesterol, measuring serum or circulating (plasma) blood-glucose, measuring serum or circulating (plasma) triglycerides, measuring blood-pressure, measuring body fat content, measuring body weight, and the like.

As used herein, “improved cardiovascular outcome” means a reduction in the occurrence of adverse cardiovascular events, or the risk thereof. Examples of adverse cardiovascular events include, without limitation, death, reinfarction, stroke, cardiogenic shock, pulmonary edema, cardiac arrest, and atrial dysrhythmia.

As used herein, “immediately adjacent” means there are no intervening elements between the immediately adjacent elements, for example, between regions, segments, nucleotides and/or nucleosides.

As used herein, “increasing HDL” or “raising HDL” means increasing the level of HDL in an animal after administration of at least one compound of the invention, compared to the HDL level in an animal not administered any compound.

As used herein, “individual” or “subject” or “animal” means a human or non-human animal selected for treatment or therapy.

As used herein, “individual in need thereof” refers to a human or non-human animal selected for treatment or therapy that is in need of such treatment or therapy.

As used herein, “induce”, “inhibit”, “potentiate”, “elevate”, “increase”, “decrease”, “reduce” or the like denote quantitative differences between two states. For example, “an amount effective to inhibit the activity or expression of apoCIII” means that the level of activity or expression of apoCIII in a treated sample will differ from the level of apoCIII activity or expression in an untreated sample. Such terms are applied to, for example, levels of expression, and levels of activity.

As used herein, “inflammatory condition” refers to a disease, disease state, syndrome, or other condition resulting in inflammation. For example, rheumatoid arthritis and liver fibrosis are inflammatory conditions. Other examples of inflammatory conditions include sepsis, myocardial ischemia/reperfusion injury, adult respiratory distress syndrome, nephritis, graft rejection, inflammatory bowel disease, multiple sclerosis, arteriosclerosis, atherosclerosis and vasculitis.

As used herein, “inhibiting the expression or activity” refers to a reduction or blockade of the expression or activity of a RNA or protein and does not necessarily indicate a total elimination of expression or activity.

As used herein, “insulin resistance” is defined as the condition in which normal amounts of insulin are inadequate to produce a normal insulin response from fat, muscle and liver cells. Insulin resistance in fat cells

results in hydrolysis of stored triglycerides, which elevates free fatty acids in the blood plasma. Insulin resistance in muscle reduces glucose uptake whereas insulin resistance in liver reduces glucose storage, with both effects serving to elevate blood glucose. High plasma levels of insulin and glucose due to insulin resistance often leads to metabolic syndrome and type 2 diabetes.

5 As used herein, “insulin sensitivity” is a measure of how effectively an individual processes glucose. An individual having high insulin sensitivity effectively processes glucose whereas an individual with low insulin sensitivity does not effectively process glucose.

As used herein, “lipid-lowering” means a reduction in one or more lipids (e.g., LDL, VLDL) in a subject. “Lipid-raising” means an increase in a lipid (e.g., HDL) in a subject. Lipid-lowering or lipid-raising
10 can occur with one or more doses over time.

As used herein, “lipid-lowering therapy” or “lipid lowering agent” means a therapeutic regimen provided to a subject to reduce one or more lipids in a subject. In certain embodiments, a lipid-lowering therapy is provided to reduce one or more of apo(a), apoCIII, CETP, apoB, total cholesterol, LDL-C, VLDL-C, IDL-C, non-HDL-C, triglycerides, small dense LDL particles, and Lp(a) in a subject. Examples of lipid-
15 lowering therapy include, but are not limited to, apoB inhibitors, statins, fibrates and MTP inhibitors.

As used herein, “lipoprotein”, such as VLDL, LDL and HDL, refers to a group of proteins found in the serum, plasma and lymph and are important for lipid transport. The chemical composition of each lipoprotein differs, for example, in that the HDL has a higher proportion of protein versus lipid, whereas the VLDL has a lower proportion of protein versus lipid.

20 As used herein, “Lipoprotein Lipase” or “LPL” refers to an enzyme that hydrolyzes TGs found in lipoproteins, such as CM or VLDL, into free fatty acids and monoacylglycerols. LPL requires apo C-II as a cofactor to function in hydrolyzing TGs. LPL is mainly produced in skeletal muscle, fat tissue, and heart muscle. Hydrolysis and removal of TG from CM and VLDL normally protects against excessive postprandial rise in CM mass and TG.

25 As used herein, “Lipoprotein lipase deficient”, “lipoprotein lipase deficiency”, “LPL deficiency” or “LPLD” is also known as “Fredrickson’s Type I dyslipidemia”, “chylomicronemia”, “Familial Chylomicronemia Syndrome” or “FCS”. Although subjects with LPLD generally lack LPL or LPL activity necessary for effective breakdown of fatty acids such as TGs, these subjects may still have a minimal LPL activity or express a minimal level of LPL. In some instances, a LPLD subject may express LPL or have LPL
30 activity up to about, or no more than, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% activity. In other instances, the LPLD subject has no measurable LPL or LPL activity. One embodiment of LPLD encompasses subjects with “hyperlipoproteinemia type Ia” (also known as “Fredrickson’s Type Ia”) and refers to the inability of the subjects to produce sufficient functional lipoprotein lipase enzymes necessary for effective breakdown of fatty acids such as TGs. The inability to
35 breakdown TGs leads to hypertriglyceridemia in the subject and, often more than 12 hours after meals, hyperTG and chylomicronemia are still present and visible as lipemia. Type Ia is commonly caused by one or

more mutations in the LPL gene. As disclosed herein, LPLD also encompasses subjects that have dysfunctional lipoprotein lipase such as those subjects with "hyperlipoproteinemia type Ib" (also known as "Fredrickson's Type Ib") and "hyperlipoproteinemia type Ic" (also known as "Fredrickson's Type Ic"). Type Ib is caused by lack of lipoprotein lipase activator apoprotein C-II. Type Ic is due to a circulating inhibitor of lipoprotein lipase. As with Type 1a, Type 1b/1c subjects suffer from an inability to breakdown TGs leading to hypertriglyceridemia and hyperTG and chylomicronemia are still present and visible as lipemia often more than 12 hours after meals. In certain embodiments, LPLD is associated with at least one mutation in the LPL gene such as P207L, G188L or D9N or other mutations that affect LPL (Brunzell, In: Pagon RA, Adam MP, Bird TD, Dolan CR, Fong CT, Stephens K, editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2013.1999 Oct 12 [updated 2011 Dec 15]).

As used herein, "low density lipoprotein-cholesterol (LDL-C)" means cholesterol carried in low density lipoprotein particles. Concentration of LDL-C in serum (or plasma) is typically quantified in mg/dL or nmol/L. "Serum LDL-C" and "plasma LDL-C" mean LDL-C in the serum and plasma, respectively.

As used herein, "major risk factors" refers to factors that contribute to a high risk for a particular disease or condition. In certain embodiments, major risk factors for coronary heart disease include, without limitation, cigarette smoking, hypertension, high LDL, low HDL-C, family history of coronary heart disease, age, and other factors disclosed herein.

As used herein, "metabolic disorder" or "metabolic disease" refers to a condition characterized by an alteration or disturbance in metabolic function. "Metabolic" and "metabolism" are terms well known in the art and generally include the whole range of biochemical processes that occur within a living organism. Metabolic disorders include, but are not limited to, hyperglycemia, prediabetes, diabetes (type 1 and type 2), obesity, insulin resistance, metabolic syndrome and dyslipidemia due to type 2 diabetes.

As used herein, "metabolic syndrome" means a condition characterized by a clustering of lipid and non-lipid cardiovascular risk factors of metabolic origin. In certain embodiments, metabolic syndrome is identified by the presence of any 3 of the following factors: waist circumference of greater than 102 cm in men or greater than 88 cm in women; serum triglyceride of at least 150 mg/dL; HDL-C less than 40 mg/dL in men or less than 50 mg/dL in women; blood pressure of at least 130/85 mmHg; and fasting glucose of at least 110 mg/dL. These determinants can be readily measured in clinical practice (JAMA, 2001, 285: 2486-2497).

"Parenteral administration" means administration through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g. intrathecal or intracerebroventricular administration. Administration can be continuous, chronic, short or intermittent.

As used herein, "peptide" means a molecule formed by linking at least two amino acids by amide bonds. Peptide refers to polypeptides and proteins.

As used herein, “pharmaceutical agent” means a substance that provides a therapeutic benefit when administered to an individual. For example, in certain embodiments, an antisense oligonucleotide targeted to apoCIII is a pharmaceutical agent.

As used herein, “pharmaceutical composition” or “composition” means a mixture of substances suitable for administering to an individual. For example, a pharmaceutical composition can comprise one or more active agents and a pharmaceutical carrier e.g., a sterile aqueous solution.

As used herein, “pharmaceutically acceptable derivative” encompasses derivatives of the compounds described herein such as solvates, hydrates, esters, prodrugs, polymorphs, isomers, isotopically labelled variants, pharmaceutically acceptable salts and other derivatives known in the art.

As used herein, “pharmaceutically acceptable salts” means physiologically and pharmaceutically acceptable salts of antisense compounds, i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. The term “pharmaceutically acceptable salt” or “salt” includes a salt prepared from pharmaceutically acceptable non-toxic acids or bases, including inorganic or organic acids and bases. “Pharmaceutically acceptable salts” of the compounds described herein may be prepared by methods well-known in the art. For a review of pharmaceutically acceptable salts, see Stahl and Wermuth, Handbook of Pharmaceutical Salts: Properties, Selection and Use (Wiley-VCH, Weinheim, Germany, 2002). Sodium salts of antisense oligonucleotides are useful and are well accepted for therapeutic administration to humans. Accordingly, in one embodiment the compounds described herein are in the form of a sodium salt.

As used herein, “portion” means a defined number of contiguous (i.e. linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an antisense compound.

As used herein, “prevent” or “preventing” refers to delaying or forestalling the onset or development of a disease, disorder, or condition for a period of time from minutes to indefinitely. Prevent also means reducing risk of developing a disease, disorder, or condition.

As used herein, “raise” means to increase in amount. For example, to raise plasma HDL levels means to increase the amount of HDL in the plasma.

As used herein, “reduce” means to bring down to a smaller extent, size, amount, or number. For example, to reduce plasma triglyceride levels means to bring down the amount of triglyceride in the plasma.

As used herein, “region” or “target region” is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic. For example, a target region may encompass a 3’ UTR, a 5’ UTR, an exon, an intron, an exon/intron junction, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for apoCIII can be obtained by accession number from sequence databases such as NCBI. In certain embodiments, a target region may encompass the sequence from

a 5' target site of one target segment within the target region to a 3' target site of another target segment within the target region.

As used herein, "second agent" or "second therapeutic agent" means an agent that can be used in combination with a "first agent". A second therapeutic agent can include, but is not limited to, antisense oligonucleotides targeting apoCIII. A second agent can also include anti- apoCIII antibodies, apoCIII peptide inhibitors, cholesterol lowering agents, lipid lowering agents, glucose lowering agents and anti-inflammatory agents.

As used herein, "segments" are defined as smaller, sub-portions of regions within a nucleic acid. For example, a "target segment" means the sequence of nucleotides of a target nucleic acid to which one or more antisense compounds is targeted. "5' target site" refers to the 5'-most nucleotide of a target segment. "3' target site" refers to the 3'-most nucleotide of a target segment. Alternatively, a "start site" can refer to the 5'-most nucleotide of a target segment and a "stop site" refers to the 3'-most nucleotide of a target segment. A target segment can also begin at the "start site" of one sequence and end at the "stop site" of another sequence.

As used herein, "statin" means an agent that inhibits the activity of HMG-CoA reductase.

As used herein, "subcutaneous administration" means administration just below the skin.

As used herein, "subject" means a human or non-human animal selected for treatment or therapy.

As used herein, "symptom of cardiovascular disease or disorder" means a phenomenon that arises from and accompanies the cardiovascular disease or disorder and serves as an indication of it. For example, angina; chest pain; shortness of breath; palpitations; weakness; dizziness; nausea; sweating; tachycardia; bradycardia; arrhythmia; atrial fibrillation; swelling in the lower extremities; cyanosis; fatigue; fainting; numbness of the face; numbness of the limbs; claudication or cramping of muscles; bloating of the abdomen; or fever are symptoms of cardiovascular disease or disorder.

As used herein, "targeting" or "targeted" means the process of design and selection of an antisense compound that will specifically hybridize to a target nucleic acid and induce a desired effect.

As used herein, "therapeutically effective amount" means an amount of a pharmaceutical agent that provides a therapeutic benefit to an individual.

As used herein, "therapeutic lifestyle change" means dietary and lifestyle changes intended to lower fat/adipose tissue mass and/or cholesterol. Such change can reduce the risk of developing heart disease, and may include recommendations for dietary intake of total daily calories, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, carbohydrate, protein, cholesterol, insoluble fiber, as well as recommendations for physical activity.

As used herein, "treat" or "treating" refers to administering a compound described herein to effect an alteration or improvement of a disease, disorder, or condition.

As used herein, "triglyceride" or "TG" means a lipid or neutral fat consisting of glycerol combined with three fatty acid molecules.

As used herein, “type 2 diabetes,” (also known as “type 2 diabetes mellitus”, “diabetes mellitus, type 2”, “non-insulin-dependent diabetes”, “NIDDM”, “obesity related diabetes”, or “adult-onset diabetes”) is a metabolic disorder that is primarily characterized by insulin resistance, relative insulin deficiency, and hyperglycemia.

5

Certain Embodiments

Certain embodiments provide a compounds and methods for decreasing ApoCIII mRNA and protein expression. In certain embodiments, the compound is an ApoCIII specific inhibitor for treating, preventing, or ameliorating an ApoCIII associated disease. In certain embodiments, the compound is an antisense oligonucleotide targeting ApoCIII. In certain embodiments, the compound is an modified oligonucleotide targeting ApoCIII and a conjugate group.

In certain embodiments, a compound comprises a siRNA or antisense oligonucleotide targeted to Apolipoprotein C-III (ApoC-III) known in the art and a conjugate group described herein. Examples of antisense oligonucleotides targeted to ApoC-III suitable for conjugation include but are not limited to those disclosed in US Patent Application Publication No. US 2013/0317085. In certain embodiments, a compound comprises an antisense oligonucleotide having a nucleobase sequence of any of SEQ ID NOs 19-96 and 209-221 disclosed in US 2013/0317085 and a conjugate group described herein.

In certain embodiments, the modified oligonucleotide with the conjugate group has a nucleobase sequence comprising at least 8 contiguous nucleobases of a sequence selected from any sequence disclosed in U.S. Patent 7,598,227, U.S. Patent 7,750,141, PCT Publication WO 2004/093783 or PCT Publication WO 2012/149495. In certain embodiments, the modified oligonucleotide has a sequence selected from any sequence disclosed in U.S. Patent 7,598,227, U.S. Patent 7,750,141, PCT Publication WO 2004/093783 or PCT Publication WO 2012/149495.

Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides. In certain embodiments, the modified oligonucleotide with the conjugate group consists of 15 to 30, 18 to 24, 19 to 22, 13 to 25, 14 to 25, 15 to 25 linked nucleosides. In certain embodiments, the modified oligonucleotide with the conjugate group comprises at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29 or 30 linked nucleosides. In certain embodiments, the modified oligonucleotide with the conjugate group consists of 20 linked nucleosides.

Certain embodiments provide a compound comprising a modified oligonucleotide with a conjugate group targeting ApoCIII and has a sequence complementary to any of the sequences set forth in GENBANK

Accession No. NM_000040.1 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NT_033899.8 truncated from nucleotides 20262640 to 20266603 (incorporated herein as SEQ ID NO: 2), and/or GenBank Accession No. NT_035088.1 truncated from nucleotides 6238608 to 6242565 (incorporated herein as SEQ ID NO: 3). In certain embodiments, the modified oligonucleotide is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 100% complementary to any of SEQ ID NOs: 1-3. In certain embodiments, the compound comprises a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or 20 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3. In certain embodiments, the compound comprises a modified oligonucleotide targeting an ApoCIII segment and a conjugate group, wherein the modified oligonucleotide comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or 20 contiguous nucleobases complementary to an equal length portion of any of the target segments shown in Tables 121 and 124. In the tables, the "Start Site" refers to the 5'-most nucleotide of a target segment and "Stop Site" refers to the 3'-most nucleotide of a target segment. A target segment can range from the start site to the stop site of each sequence listed in the tables. Alternatively, the target segment can range from the start site of one sequence and end at the stop site of another sequence. For example, as shown in the tables, a target segment can range from 3533 to 3552, the start site to the stop site of SEQ ID NO: 87. In another example, as shown in the tables, a target segment can range from 3514 to 3558, the start site of SEQ ID NO: 83 to the stop site of SEQ ID NO: 88. In certain embodiments, the antisense compound comprises at least 8 nucleobases of the sequence of SEQ ID NO: 87. In certain embodiments, the antisense compound comprises the sequence of SEQ ID NO: 87. In certain embodiments, the antisense compound consists of the sequence of SEQ ID NO: 87. In certain embodiments, the antisense compound is ISIS 304801.

Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the nucleobase sequence of the modified oligonucleotide is at least 80%, at least 85%, at least 90%, at least 95%, or 100% complementary to any of SEQ ID NOs: 1-3. Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the nucleobase sequence of the modified oligonucleotide is at least 80%, at least 85%, at least 90%, at least 95%, or 100% complementary to any of the target segments disclosed herein.

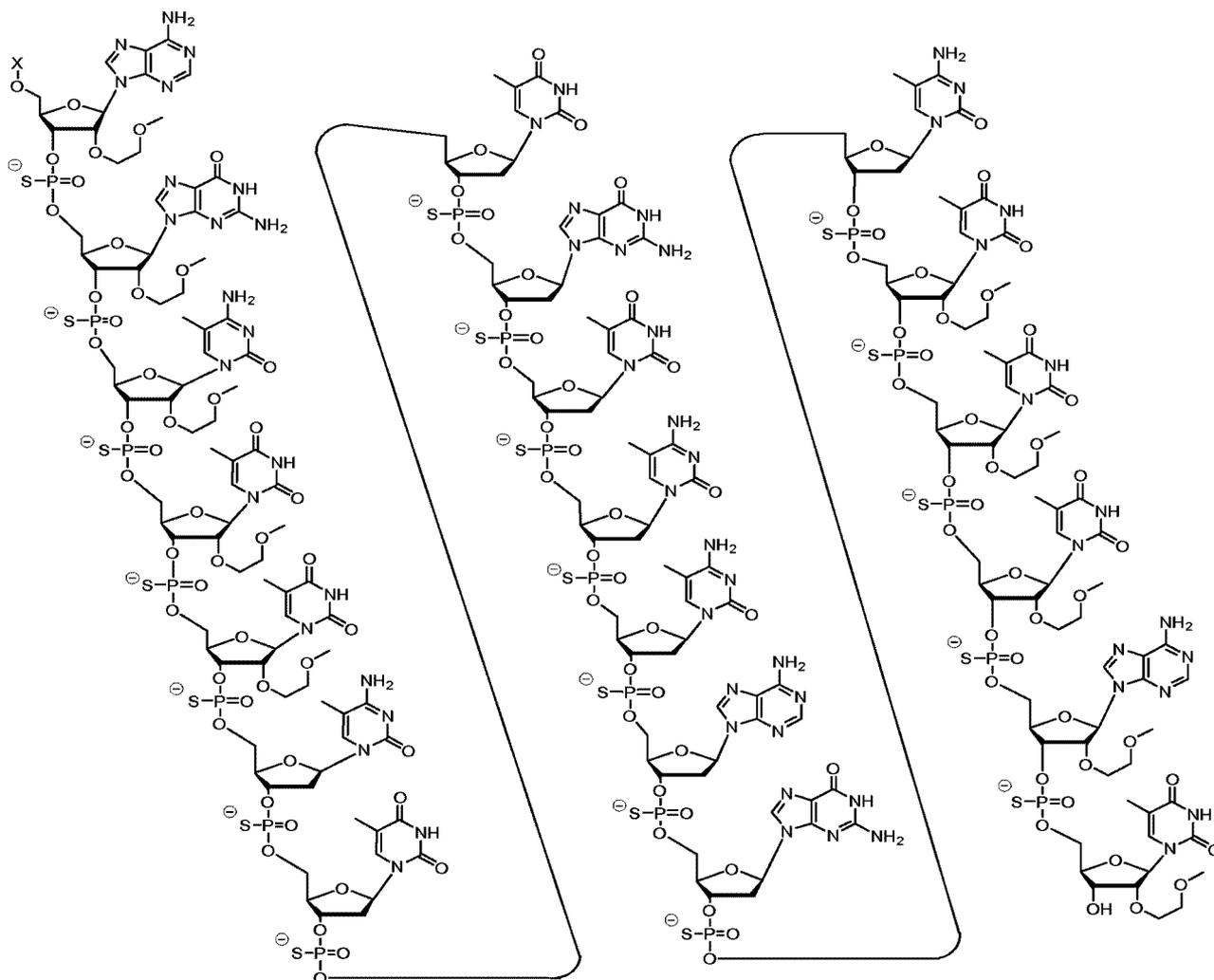
Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides and comprises a nucleobase sequence comprising a portion of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 3533 to 3552 of SEQ ID NO: 3, wherein the nucleobase sequence of the modified oligonucleotide is at least 80% complementary to SEQ ID NO: 3.

Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides and comprises a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29 or 30 contiguous nucleobases complementary to an equal length portion of nucleobases 3514 to 3558 of SEQ ID NO: 3, wherein the nucleobase sequence of the modified oligonucleotide is at least 80% complementary to SEQ ID NO: 3.

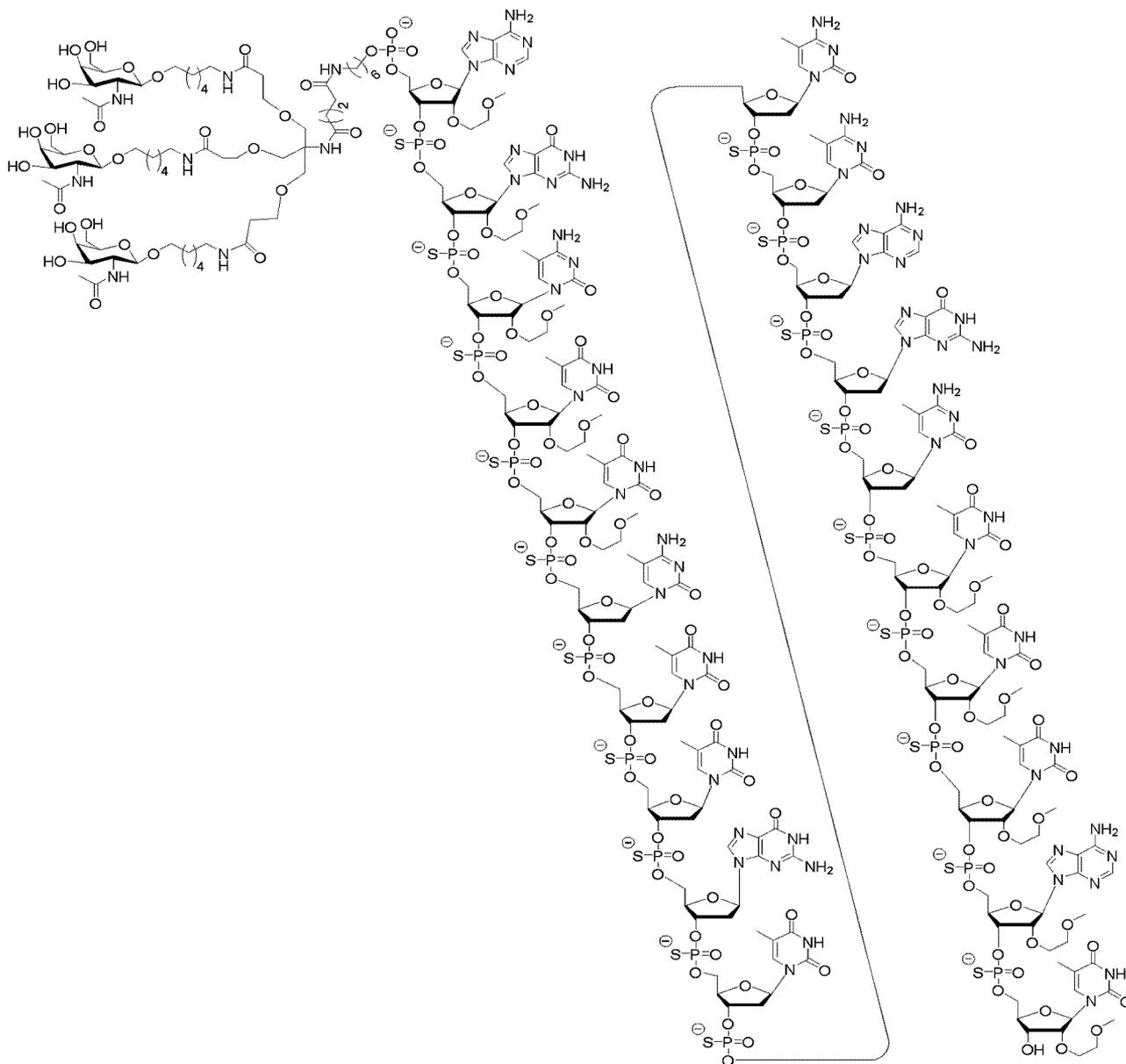
Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides and has a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or 20 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 19-96, 209-221. In certain embodiments, the conjugated modified oligonucleotide has a nucleobase sequence comprising at least 8 contiguous nucleobases of any one of the nucleobase sequences of SEQ ID NOs: 19-96, 209-221. In certain embodiments, the compound consists of any one of SEQ ID NOs: 19-96, 209-221 and a conjugate group.

Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides and has a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or 20 contiguous nucleobases of the nucleobase sequence of SEQ ID NO: 87. In certain embodiments, the modified oligonucleotide with the conjugate group has a nucleobase sequence comprising at least 8 contiguous nucleobases of the nucleobase sequence of SEQ ID NO: 87. In certain embodiments, the compound consists of SEQ ID NO: 87 and a conjugate group.

In certain embodiments, the present disclosure provides conjugated antisense compounds represented by the following structure. In certain embodiments, the antisense compound comprises a modified oligonucleotide with ISIS 304801 with a 5'-X, wherein X is a conjugate group comprising GalNAc. In certain embodiments, the antisense compound consists of a modified oligonucleotide with ISIS 304801 with a 5'-X, wherein X is a conjugate group comprising GalNAc.

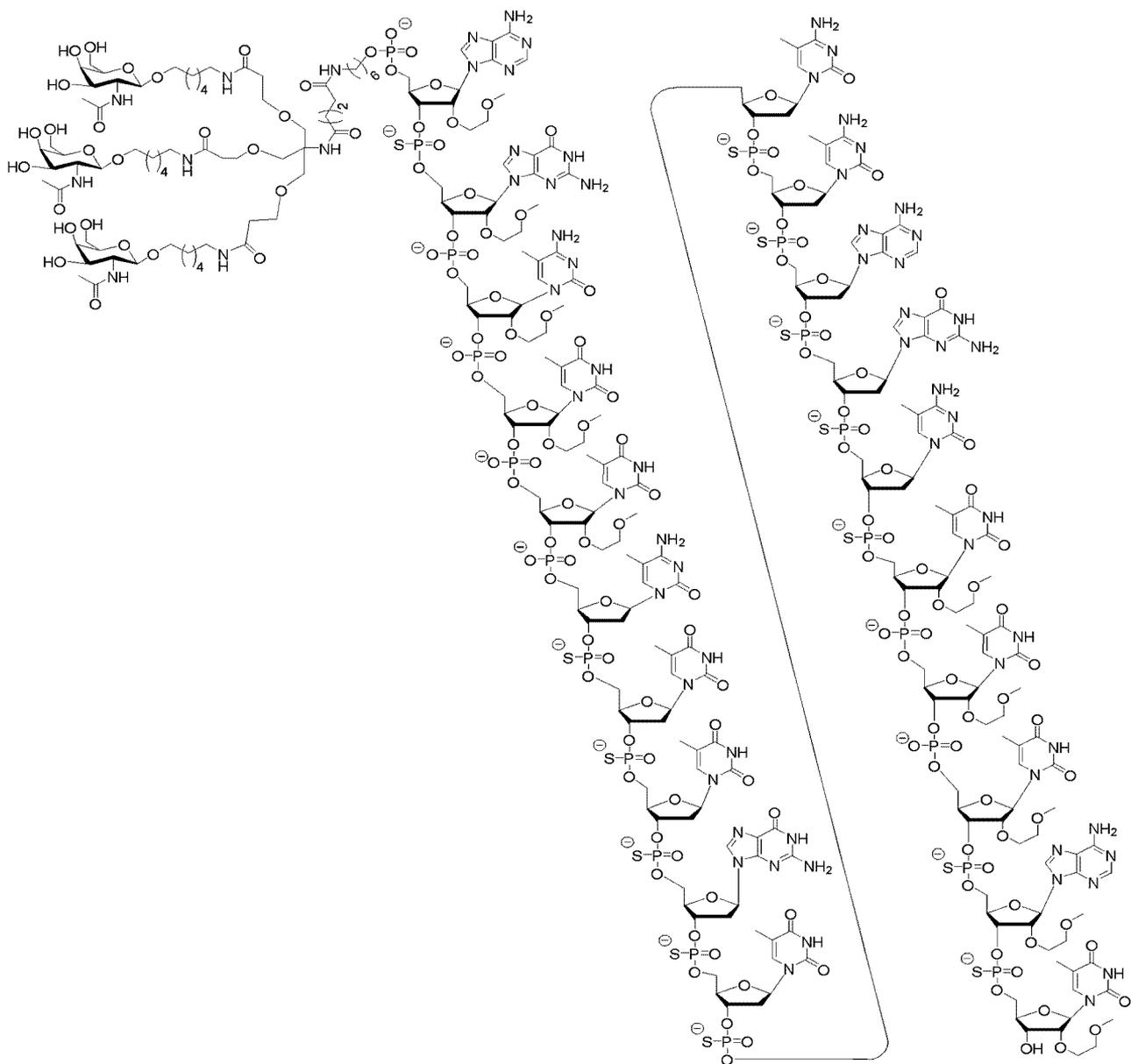


In certain embodiments, the present disclosure provides conjugated antisense compounds represented by the following structure. In certain embodiments, the antisense compound comprises the conjugated modified oligonucleotide ISIS 678354. In certain embodiments, the antisense compound consists of the conjugated modified oligonucleotide ISIS 678354.

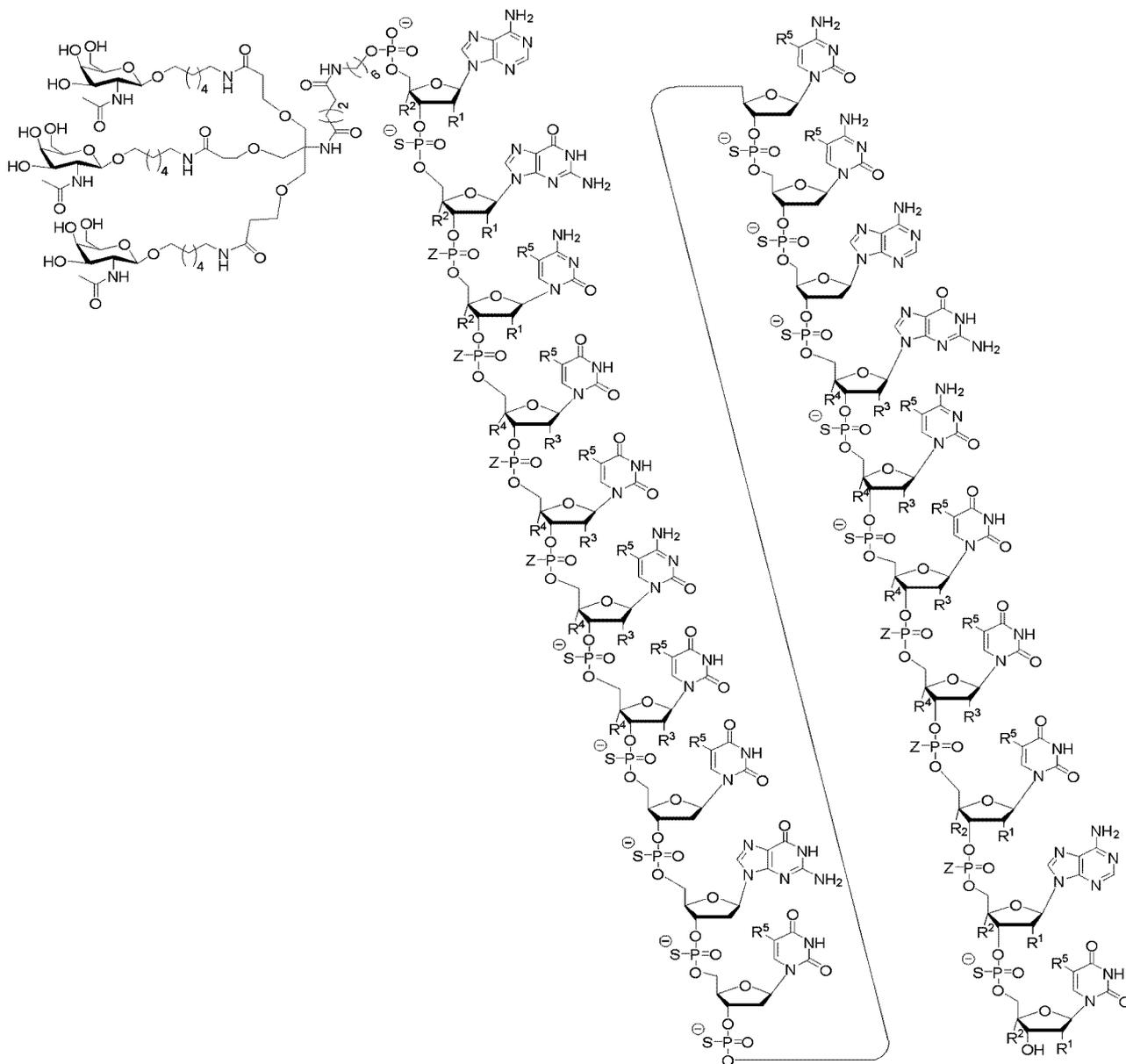


In certain embodiments, the present disclosure provides conjugated antisense compounds represented by the following structure. In certain embodiments, the antisense compound comprises the conjugated modified oligonucleotide ISIS 678357. In certain embodiments, the antisense compound consists of the

5 conjugated modified oligonucleotide ISIS 678357.



In certain embodiments, the present disclosure provides conjugated antisense compounds represented by the following structure. In certain embodiments, the antisense compound comprises a modified oligonucleotide with the nucleobase sequence of SEQ ID NO: 87 with a 5'-GalNAc with variability in the sugar mods of the wings. In certain embodiments, the antisense compound consists of a modified oligonucleotide with the nucleobase sequence of SEQ ID NO: 87 with a 5'-GalNAc with variability in the sugar mods of the wings.



Wherein either R^1 is $-\text{OCH}_2\text{CH}_2\text{OCH}_3$ (MOE) and R^2 is H; or R^1 and R^2 together form a bridge, wherein R^1 is $-\text{O}-$ and R^2 is $-\text{CH}_2-$, $-\text{CH}(\text{CH}_3)-$, or $-\text{CH}_2\text{CH}_2-$, and R^1 and R^2 are directly connected such that the resulting bridge is selected from: $-\text{O}-\text{CH}_2-$, $-\text{O}-\text{CH}(\text{CH}_3)-$, and $-\text{O}-\text{CH}_2\text{CH}_2-$;

- 5 And for each pair of R^3 and R^4 on the same ring, independently for each ring: either R^3 is selected from H and $-\text{OCH}_2\text{CH}_2\text{OCH}_3$ and R^4 is H; or R^3 and R^4 together form a bridge, wherein R^3 is $-\text{O}-$, and R^4 is $-\text{CH}_2-$, $-\text{CH}(\text{CH}_3)-$, or $-\text{CH}_2\text{CH}_2-$ and R^3 and R^4 are directly connected such that the resulting bridge is selected from: $-\text{O}-\text{CH}_2-$, $-\text{O}-\text{CH}(\text{CH}_3)-$, and $-\text{O}-\text{CH}_2\text{CH}_2-$;

And R^5 is selected from H and $-\text{CH}_3$;

And Z is selected from S⁻ and O⁻.

Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide is single-stranded.

5 Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein at least one internucleoside linkage is a modified internucleoside linkage. In certain embodiments, the modified internucleoside linkage is a phosphorothioate internucleoside linkage. In certain embodiments, at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9 or at least 10 internucleoside linkages of said modified oligonucleotide are phosphorothioate internucleoside linkages. In certain embodiments, each internucleoside linkage is a phosphorothioate
10 internucleoside linkage. In certain embodiments, the modified oligonucleotide comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9 or at least 10 phosphodiester internucleoside linkages. In certain embodiments, each internucleoside linkage of the modified oligonucleotide is selected from a phosphodiester internucleoside linkage and a phosphorothioate internucleoside linkage.

15 Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein at least one nucleoside comprises a modified nucleobase. In certain embodiments, the modified nucleobase is a 5-methylcytosine.

Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide comprises at least one modified sugar. In certain
20 embodiments, the modified sugar is a bicyclic sugar. In certain embodiments, the modified sugar comprises a 2'-O-methoxyethyl, a constrained ethyl, a 3'-fluoro-HNA or a 4'-(CH₂)_n-O-2' bridge, wherein n is 1 or 2.

Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides and comprises: (a) a gap segment consisting of linked deoxynucleosides; (b) a 5' wing segment consisting of
25 linked nucleosides; (c) a 3' wing segment consisting of linked nucleosides; and wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide consists of 20 linked nucleosides and
30 comprises: (a) a gap segment consisting of ten linked deoxynucleosides; (b) a 5' wing segment consisting of five linked nucleosides; (c) a 3' wing segment consisting of five linked nucleosides; and wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, wherein at least one internucleoside linkage is a phosphorothioate linkage and wherein each cytosine residue is a 5-methylcytosine.

Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide consists of 20 linked nucleosides and has a nucleobase sequence comprising at least 8 contiguous nucleobases of any of SEQ ID NOs: 19-96, 209-221, wherein the modified oligonucleotide comprises: (a) a gap segment consisting of ten linked
5 deoxynucleosides; (b) a 5' wing segment consisting of five linked nucleosides; (c) a 3' wing segment consisting of five linked nucleosides; and wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, wherein at least one internucleoside linkage is a phosphorothioate linkage and wherein each cytosine residue is a 5-methylcytosine.

10 Certain embodiments provide a compound comprising a modified oligonucleotide targeting ApoCIII and a conjugate group, wherein the modified oligonucleotide consists of 20 linked nucleosides and has a nucleobase sequence comprising at least 8 contiguous nucleobases of SEQ ID NO: 87, wherein the modified oligonucleotide comprises: (a) a gap segment consisting of ten linked deoxynucleosides; (b) a 5' wing segment consisting of five linked nucleosides; (c) a 3' wing segment consisting of five linked nucleosides;
15 and wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, wherein at least one internucleoside linkage is a phosphorothioate linkage and wherein each cytosine residue is a 5-methylcytosine.

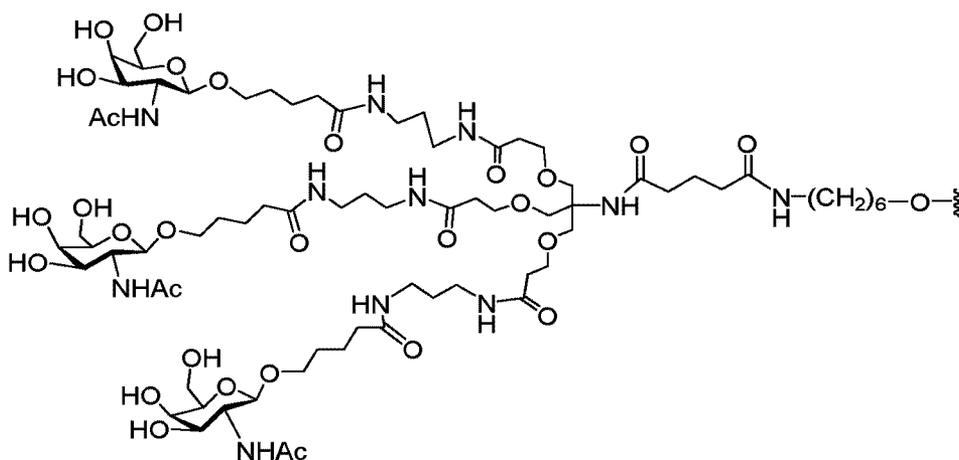
Certain embodiments provide a modified oligonucleotide targeting ApoCIII and a conjugate group,
20 wherein the modified oligonucleotide consists of 20 linked nucleosides with the nucleobase sequence of SEQ ID NO: 87, wherein the modified oligonucleotide comprises: (a) a gap segment consisting of ten linked deoxynucleosides; (b) a 5' wing segment consisting of five linked nucleosides; (c) a 3' wing segment consisting of five linked nucleosides; and wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-
25 methoxyethyl sugar, wherein at least one internucleoside linkage is a phosphorothioate linkage and wherein each cytosine residue is a 5-methylcytosine.

In certain embodiments, the conjugate group is linked to the modified oligonucleotide at the 5' end of the modified oligonucleotide. In certain embodiments, the conjugate group is linked to the modified oligonucleotide at the 3' end of the modified oligonucleotide.

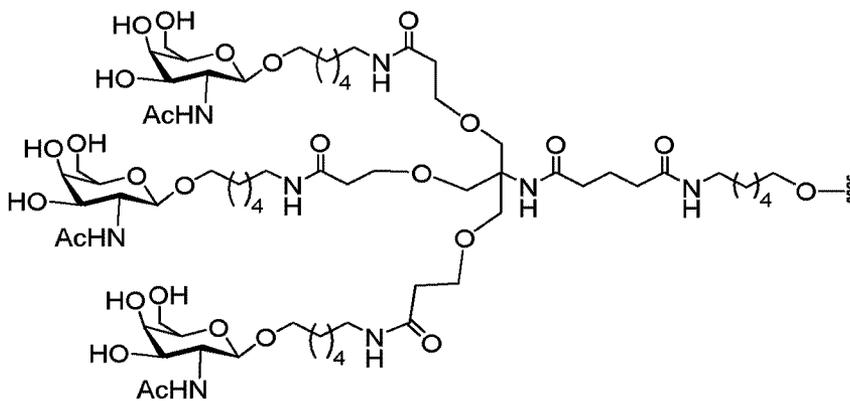
30 In certain embodiments, the conjugate group comprises exactly one ligand. In certain embodiments, the conjugate group comprises one or more ligands. In certain embodiments, the conjugate group comprises exactly two ligands. In certain embodiments, the conjugate group comprises two or more ligands. In certain embodiments, the conjugate group comprises three or more ligands. In certain embodiments, the conjugate group comprises exactly three ligands. In certain embodiments, each ligand is selected from among: a
35 polysaccharide, modified polysaccharide, mannose, galactose, a mannose derivative, a galactose derivative, D-mannopyranose, L-Mannopyranose, D-Arabinose, L-Galactose, D-xylofuranose, L-xylofuranose, D-

glucose, L-glucose, D-Galactose, L-Galactose, α -D-Mannofuranose, β -D-Mannofuranose, α -D-Mannopyranose, β -D-Mannopyranose, α -D-Glucopyranose, β -D-Glucopyranose, α -D-Glucofuranose, β -D-Glucofuranose, α -D-fructofuranose, α -D-fructopyranose, α -D-Galactopyranose, β -D-Galactopyranose, α -D-Galactofuranose, β -D-Galactofuranose, glucosamine, sialic acid, α -D-galactosamine, N-Acetylgalactosamine, 2-Amino-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy- β -D-glucopyranose, 2-Deoxy-2-methylamino-L-glucopyranose, 4,6-Dideoxy-4-formamido-2,3-di-*O*-methyl-D-mannopyranose, 2-Deoxy-2-sulfoamino-D-glucopyranose, *N*-Glycoloyl- α -neuraminic acid, 5-thio- β -D-glucopyranose, methyl 2,3,4-tri-*O*-acetyl-1-thio-6-*O*-trityl- α -D-glucopyranoside, 4-Thio- β -D-galactopyranose, ethyl 3,4,6,7-tetra-*O*-acetyl-2-deoxy-1,5-dithio- α -D-*gluco*-heptopyranoside, 2,5-Anhydro-D-allonitrile, ribose, D-ribose, D-4-thioribose, L-ribose, L-4-thioribose. In certain embodiments, each ligand is N-acetyl galactosamine.

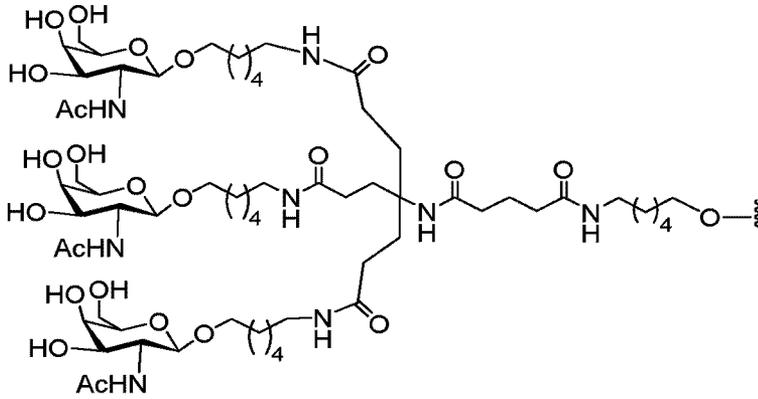
In certain embodiments, the conjugate group comprises:



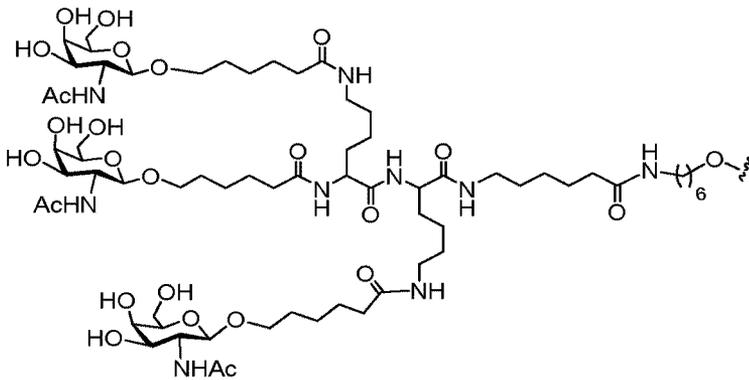
15 In certain embodiments, the conjugate group comprises:



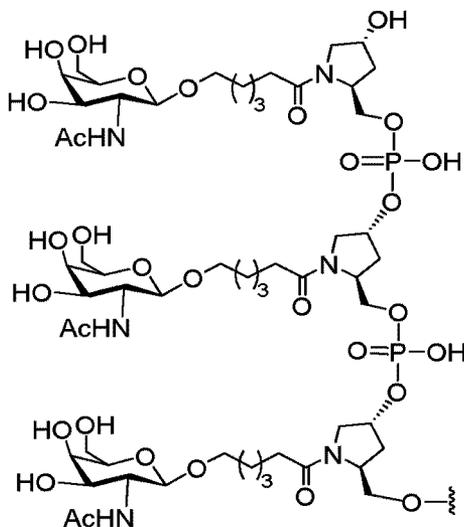
In certain embodiments, the conjugate group comprises:



5 In certain embodiments, the conjugate group comprises:



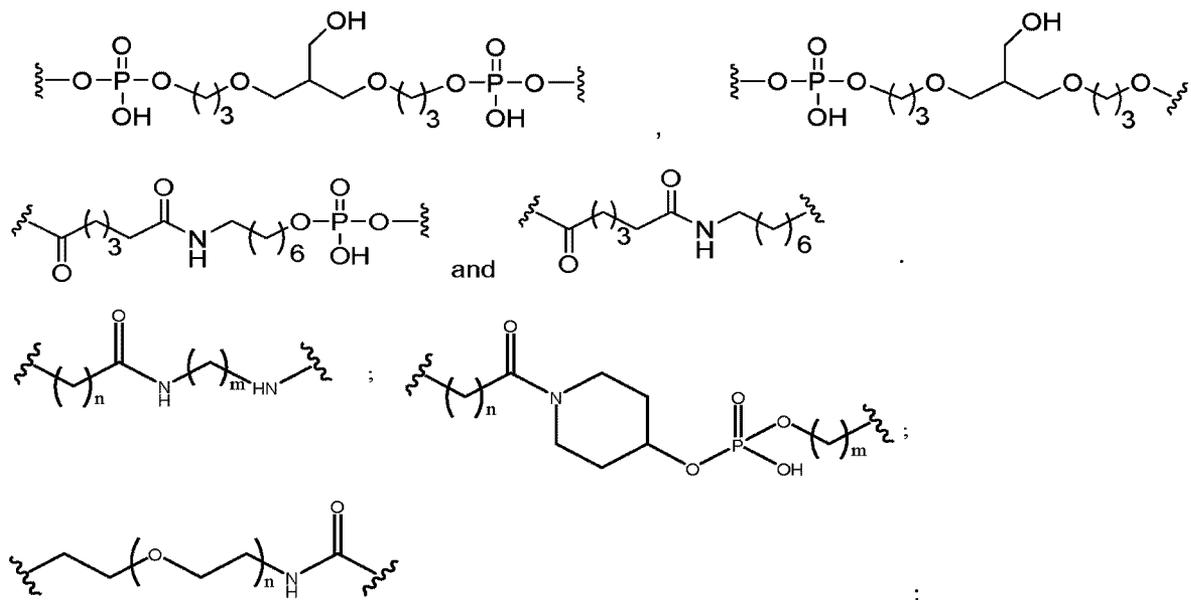
In certain embodiments, the conjugate group comprises:



In certain embodiments, the conjugate group comprises at least one phosphorus linking group or neutral linking group.

In certain embodiments, the conjugate group comprises a structure selected from among:

5

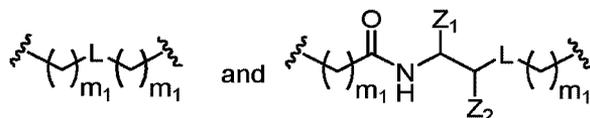


wherein n is from 1 to 12; and

wherein m is from 1 to 12.

10

In certain embodiments, the conjugate group has a tether having a structure selected from among:



wherein L is either a phosphorus linking group or a neutral linking group;

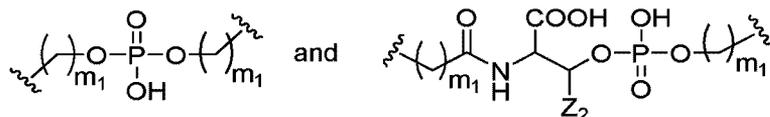
Z₁ is C(=O)O-R₂;

5 Z₂ is H, C₁-C₆ alkyl or substituted C₁-C₆ alkyl;

R₂ is H, C₁-C₆ alkyl or substituted C₁-C₆ alkyl; and

each m₁ is, independently, from 0 to 20 wherein at least one m₁ is greater than 0 for each tether.

10 In certain embodiments, the conjugate group has a tether having a structure selected from among:

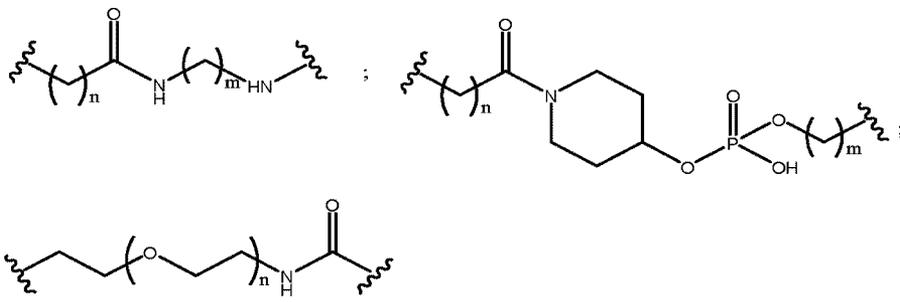


wherein Z₂ is H or CH₃; and

each m₁ is, independently, from 0 to 20 wherein at least one m₁ is greater than 0 for each

15 tether.

In certain embodiments, the conjugate group has tether having a structure selected from among:



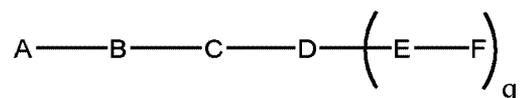
wherein n is from 1 to 12; and

20 wherein m is from 1 to 12.

In certain embodiments, the conjugate group is covalently attached to the modified oligonucleotide.

In certain embodiments, the compound has a structure represented by the formula:

25



wherein

A is the modified oligonucleotide;

5 B is the cleavable moiety

C is the conjugate linker

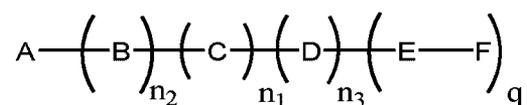
D is the branching group

each E is a tether;

each F is a ligand; and

10 q is an integer between 1 and 5.

In certain embodiments, the compound has a structure represented by the formula:



15 wherein:

A is the modified oligonucleotide;

B is the cleavable moiety

C is the conjugate linker

D is the branching group

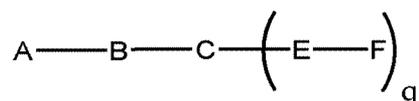
20 each E is a tether;

each F is a ligand;

each n is independently 0 or 1; and

q is an integer between 1 and 5.

25 In certain embodiments, the compound has a structure represented by the formula:



wherein

A is the modified oligonucleotide;

B is the cleavable moiety;

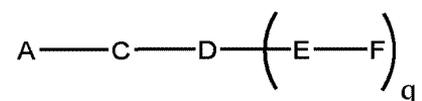
C is the conjugate linker;

each E is a tether;

each F is a ligand; and

5 q is an integer between 1 and 5.

In certain embodiments, the compound has a structure represented by the formula:



10 wherein

A is the modified oligonucleotide;

C is the conjugate linker;

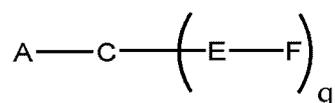
D is the branching group;

each E is a tether;

15 each F is a ligand; and

q is an integer between 1 and 5.

In certain embodiments, the compound has a structure represented by the formula:



20

wherein

A is the modified oligonucleotide;

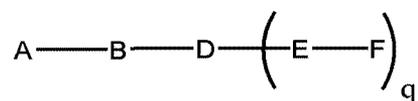
C is the conjugate linker;

each E is a tether;

25 each F is a ligand; and

q is an integer between 1 and 5.

In certain embodiments, the compound has a structure represented by the formula:



30

wherein

A is the modified oligonucleotide;

B is the cleavable moiety;

D is the branching group;

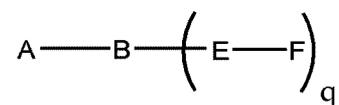
5 each E is a tether;

each F is a ligand; and

q is an integer between 1 and 5.

In certain embodiments, the compound has a structure represented by the formula:

10



wherein

A is the modified oligonucleotide;

B is the cleavable moiety;

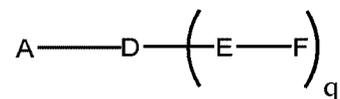
15 each E is a tether;

each F is a ligand; and

q is an integer between 1 and 5.

In certain embodiments, the compound has a structure represented by the formula:

20



wherein

A is the modified oligonucleotide;

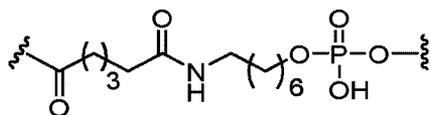
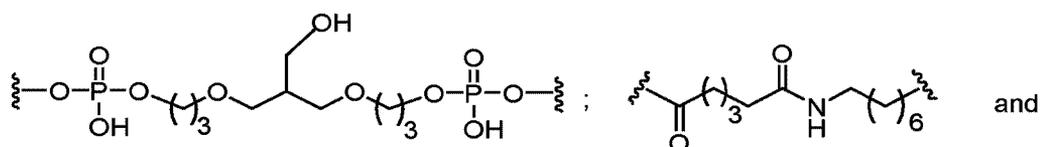
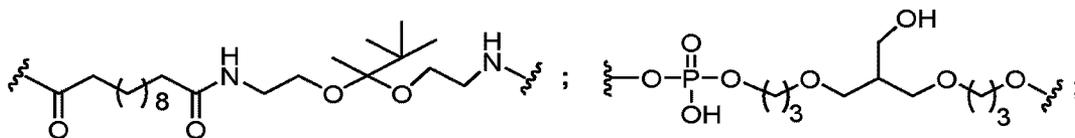
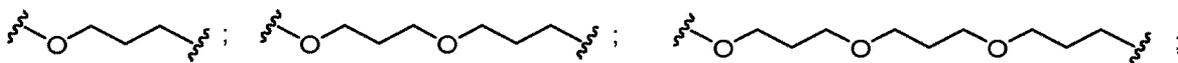
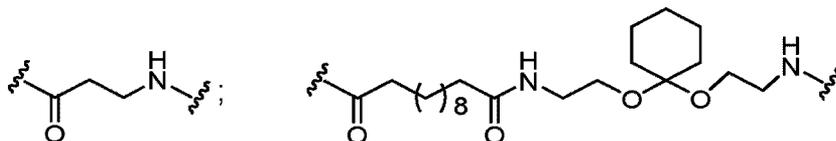
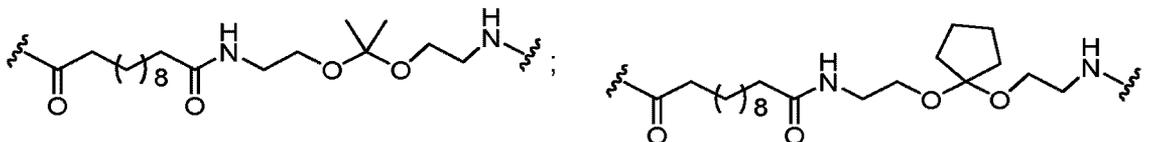
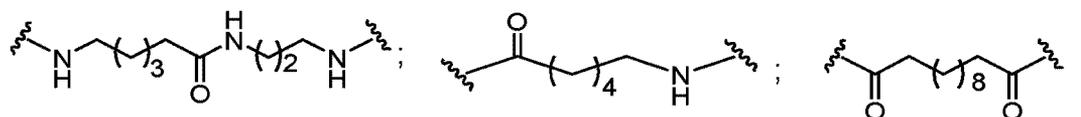
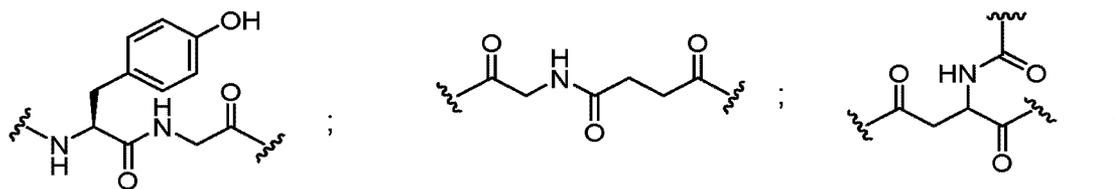
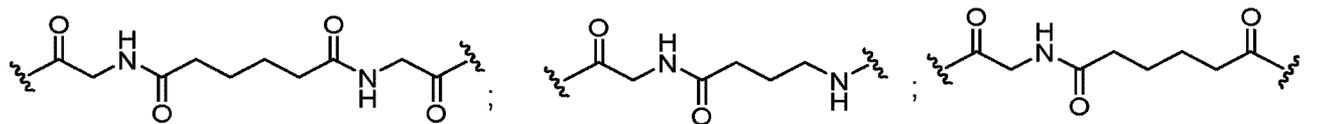
D is the branching group;

25 each E is a tether;

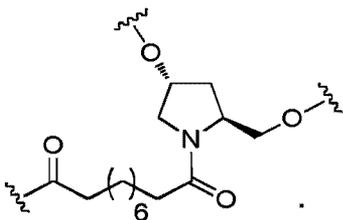
each F is a ligand; and

q is an integer between 1 and 5.

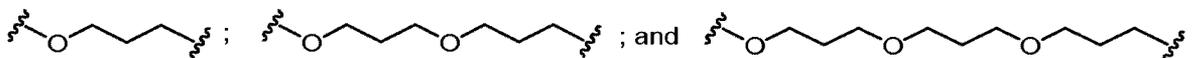
In certain embodiments, the conjugate linker has a structure selected from among:



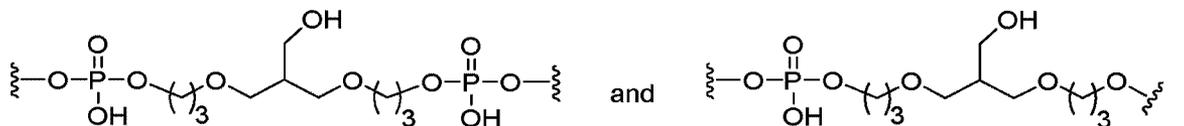
In certain embodiments, the conjugate linker has the following structure:



5 In certain embodiments, the conjugate linker has a structure selected from among:

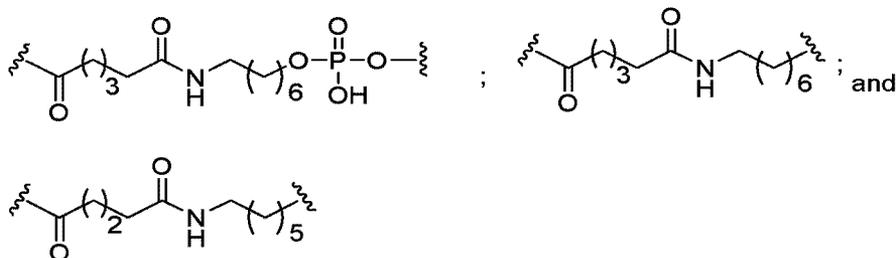


In certain embodiments, the conjugate linker has a structure selected from among:



10

In certain embodiments, the conjugate linker has a structure selected from among:



15 In certain embodiments, the conjugate linker comprises a pyrrolidine. In certain embodiments, the conjugate linker does not comprise a pyrrolidine.

In certain embodiments, the conjugate linker comprises PEG.

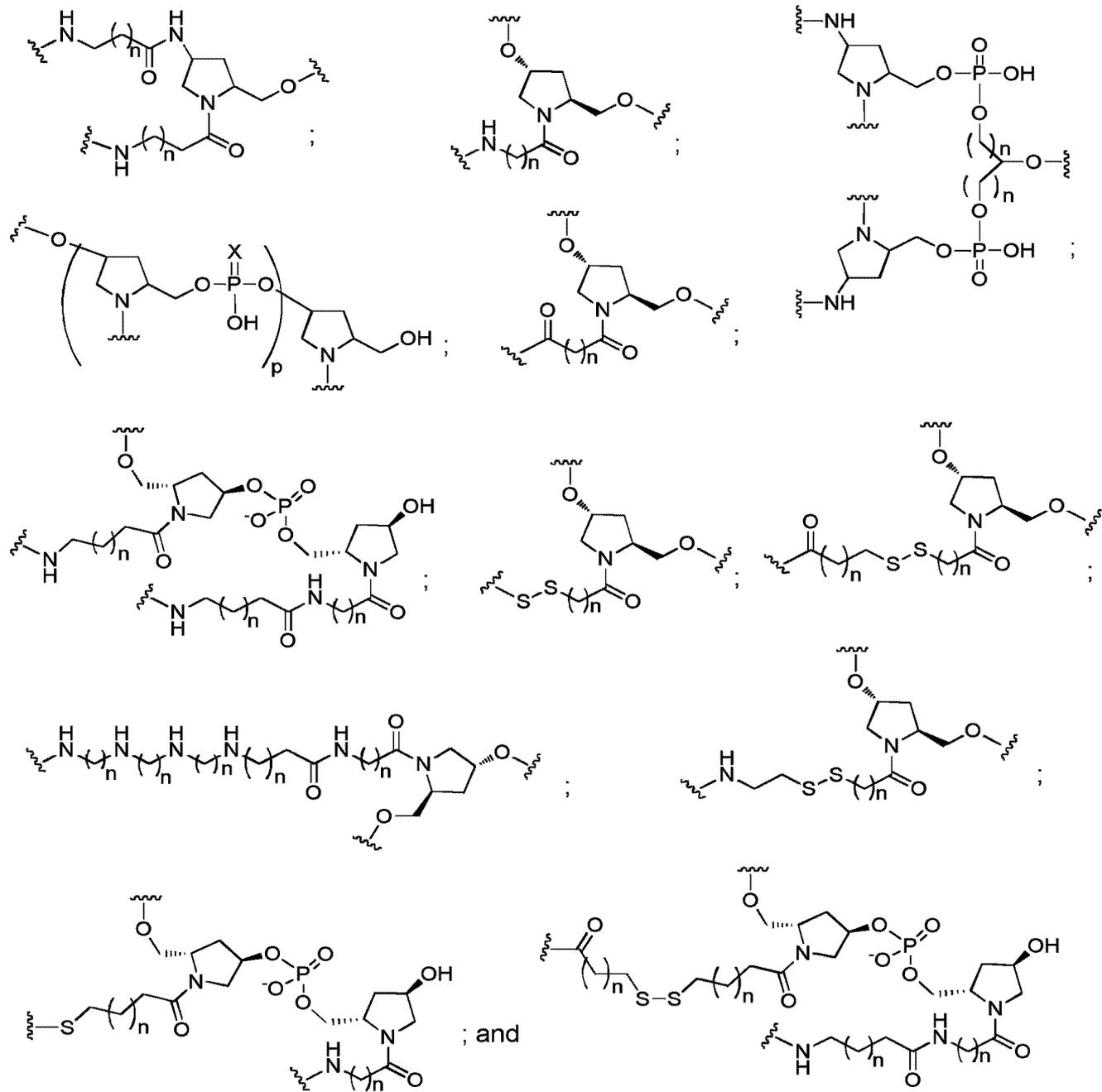
In certain embodiments, the conjugate linker comprises an amide. In certain embodiments, the conjugate linker comprises at least two amides. In certain embodiments, the conjugate linker does not comprise an amide. In certain embodiments, the conjugate linker comprises a polyamide.

- 5 In certain embodiments, the conjugate linker comprises an amine.

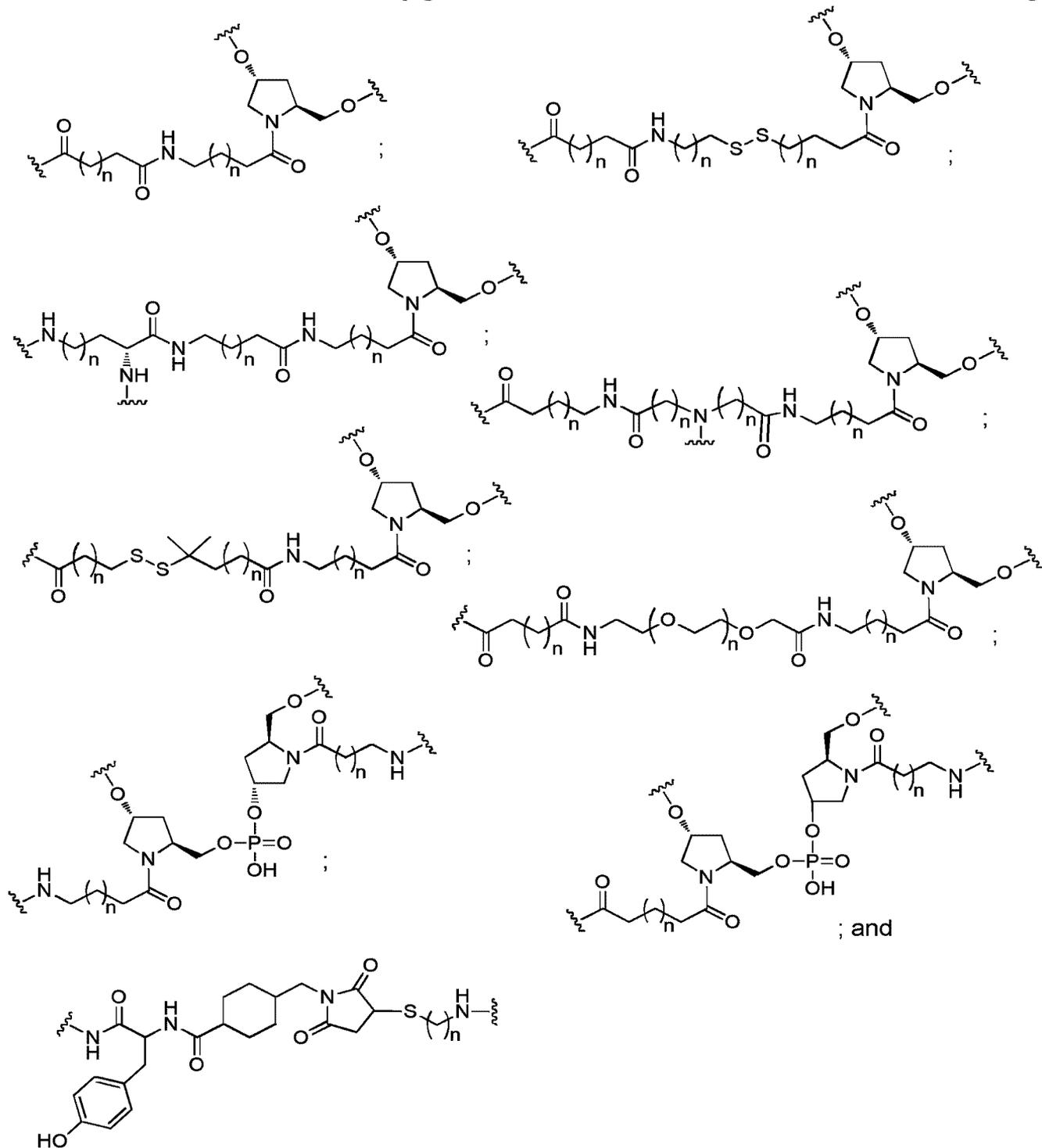
In certain embodiments, the conjugate linker comprises one or more disulfide bonds.

- 10 In certain embodiments, the conjugate linker comprises a protein binding moiety. In certain
embodiments, the protein binding moiety comprises a lipid. In certain embodiments, the protein binding
moiety is selected from among: cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid,
dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol,
menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-
15 (oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine), a vitamin (e.g., folate, vitamin A, vitamin E, biotin,
pyridoxal), a peptide, a carbohydrate (e.g., monosaccharide, disaccharide, trisaccharide, tetrasaccharide,
oligosaccharide, polysaccharide), an endosomolytic component, a steroid (e.g., uvaol, hecigenin, diosgenin),
a terpene (e.g., triterpene, e.g., sarsasapogenin, friedelin, epifriedelanol derivatized lithocholic acid), or a
cationic lipid. In certain embodiments, the protein binding moiety is selected from among: a C16 to C22 long
20 chain saturated or unsaturated fatty acid, cholesterol, cholic acid, vitamin E, adamantane or 1-
pentafluoropropyl.

In certain embodiments, the conjugate linker has a structure selected from among:



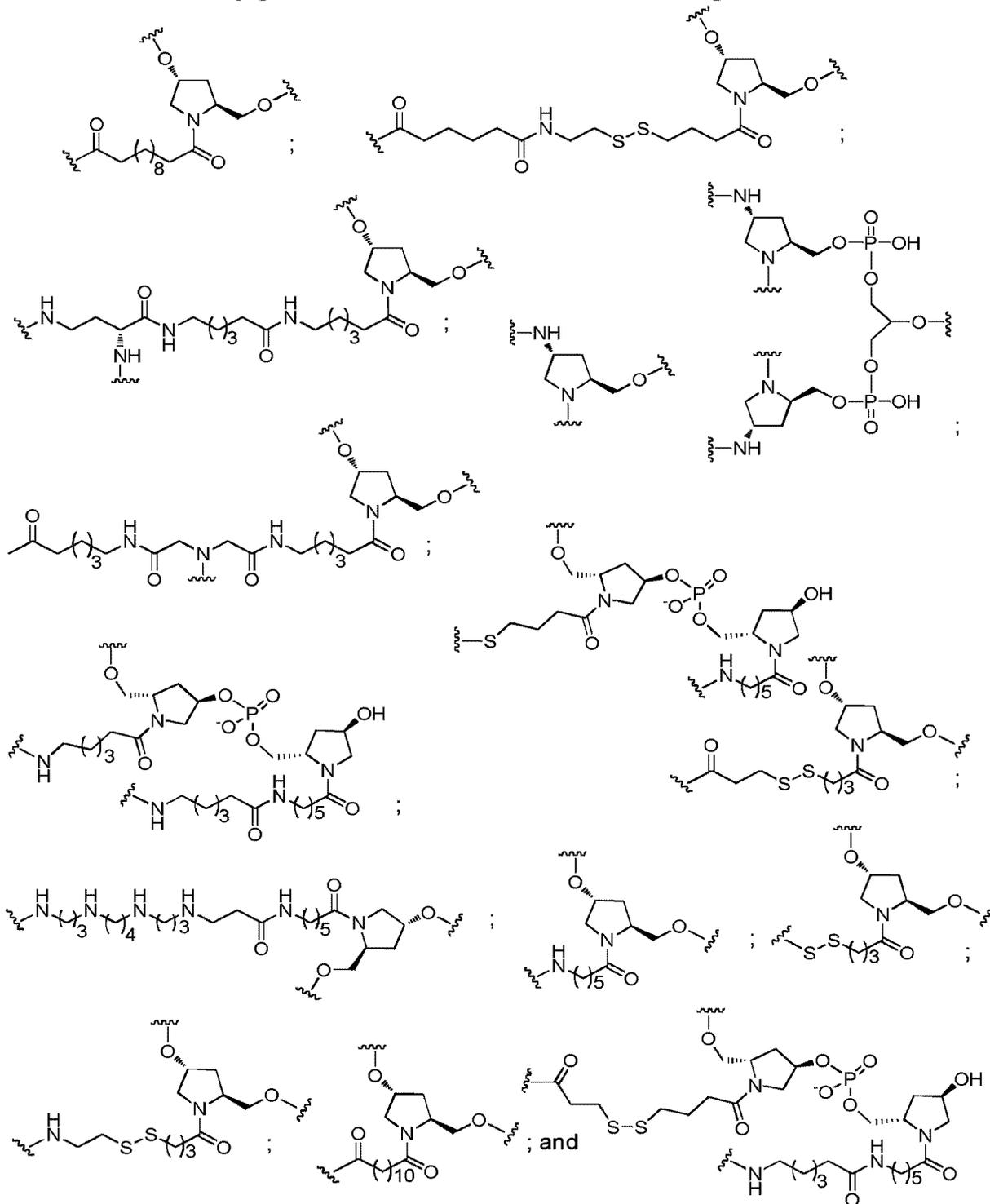
In certain embodiments, the conjugate linker has a structure selected from among:



5

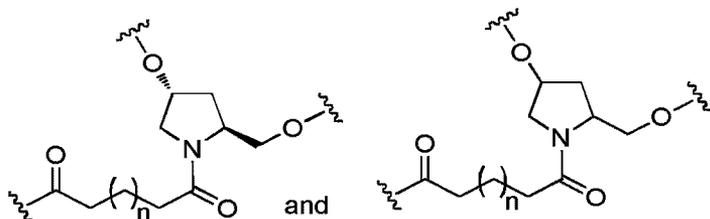
wherein each n is, independently, from 1 to 20.

In certain embodiments, the conjugate linker has a structure selected from among:



5

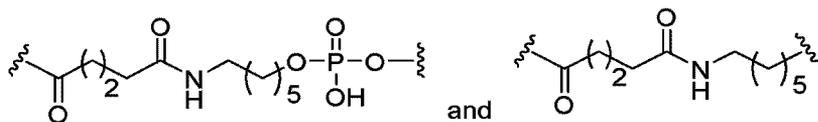
In certain embodiments, the conjugate linker has a structure selected from among:



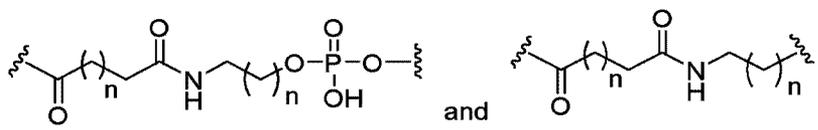
wherein n is from 1 to 20.

5

In certain embodiments, the conjugate linker has a structure selected from among:



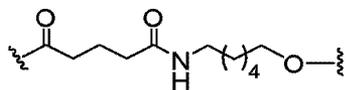
In certain embodiments, the conjugate linker has a structure selected from among:



10

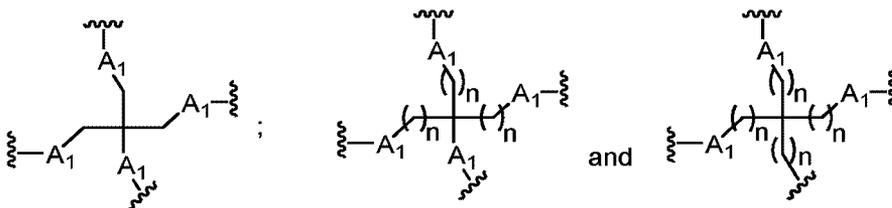
wherein each n is independently, 0, 1, 2, 3, 4, 5, 6, or 7.

In certain embodiments, the conjugate linker has the following structure:



15

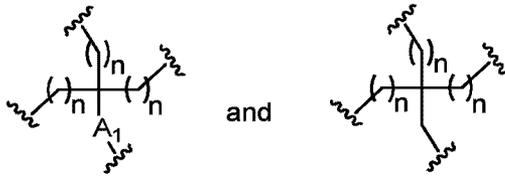
In certain embodiments, the branching group has one of the following structures:



20

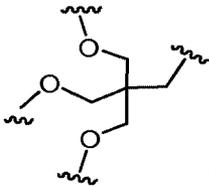
wherein each A_1 is independently, O, S, C=O or NH; and each n is, independently, from 1 to 20.

In certain embodiments, the branching group has one of the following structures:



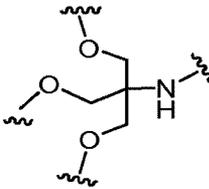
- 5 wherein each A_1 is independently, O, S, C=O or NH; and each n is, independently, from 1 to 20.

In certain embodiments, the branching group has the following structure:



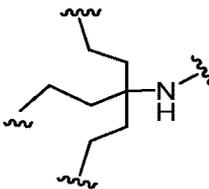
10

In certain embodiments, the branching group has the following structure:

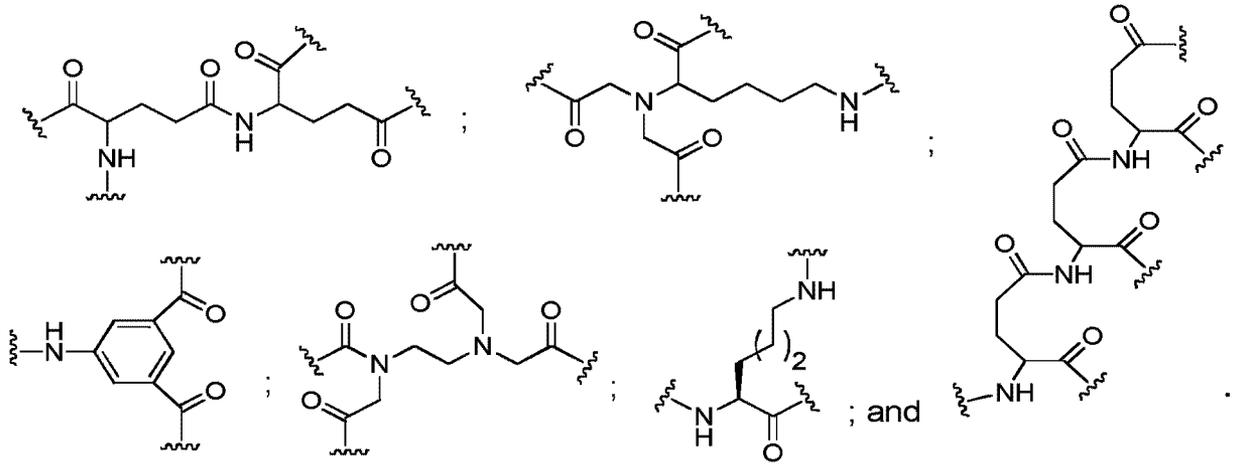


15

In certain embodiments, the branching group has the following structure:

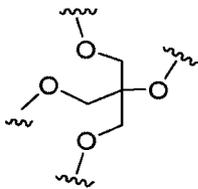


In certain embodiments, the branching group has the following structure:



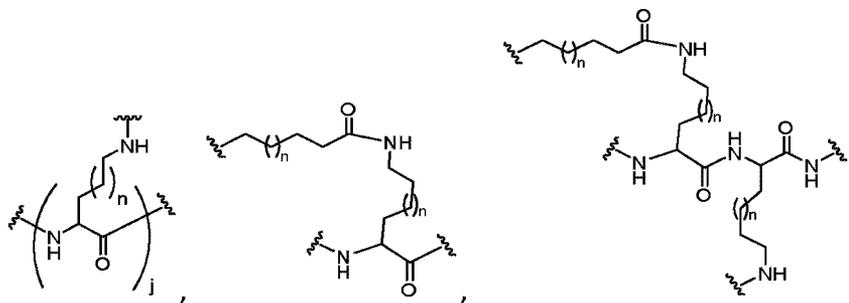
5

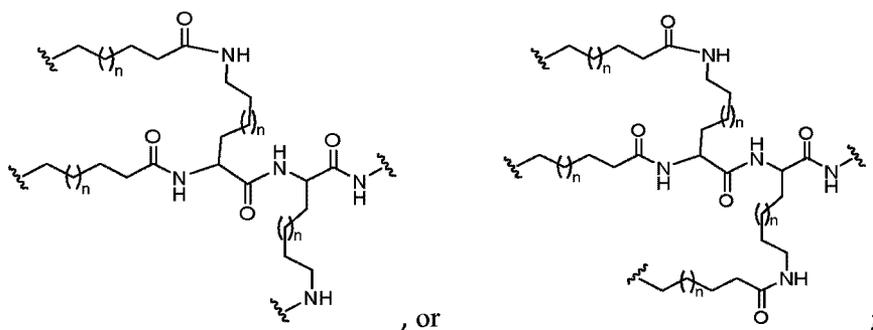
In certain embodiments, the branching group has the following structure:



10

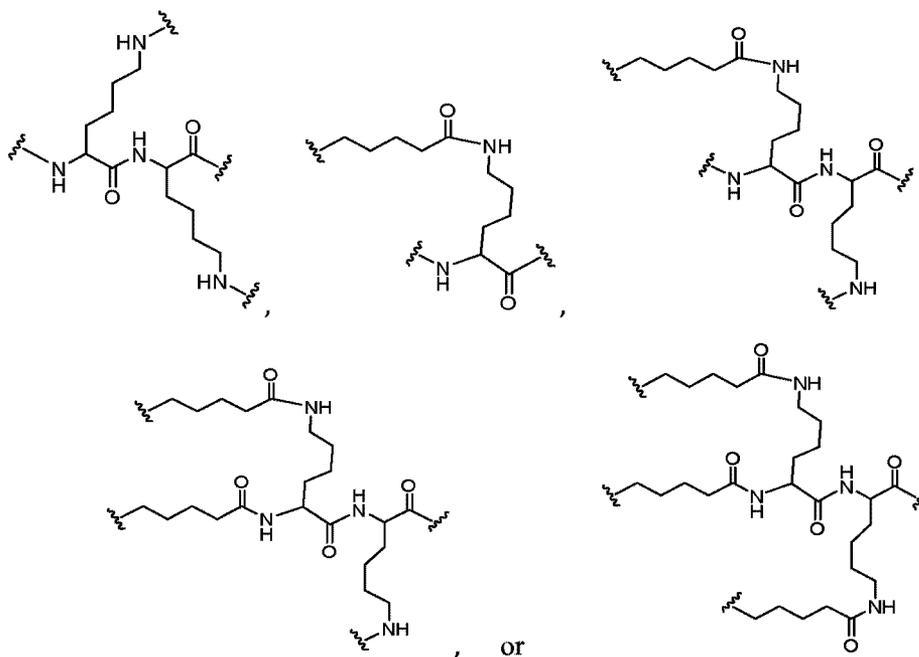
In certain embodiments, the branching group comprises:





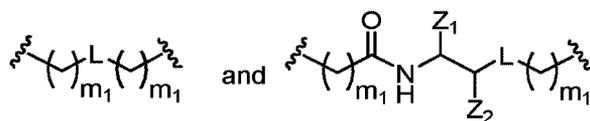
wherein each j is an integer from 1 to 3; and
 wherein each n is an integer from 1 to 20.

5 In certain embodiments, the branching group comprises:



10

In certain embodiments, each tether is selected from among:



wherein L is selected from a phosphorus linking group and a neutral linking group;

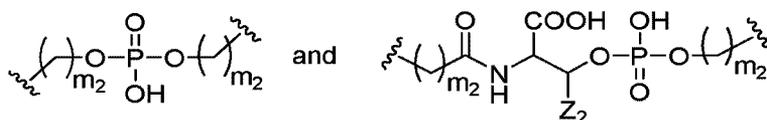
Z_1 is $C(=O)O-R_2$;

Z_2 is H, C_1-C_6 alkyl or substituted C_1-C_6 alkyl;

5 R_2 is H, C_1-C_6 alkyl or substituted C_1-C_6 alkyl; and

each m_1 is, independently, from 0 to 20 wherein at least one m_1 is greater than 0 for each tether.

In certain embodiments, each tether is selected from among:

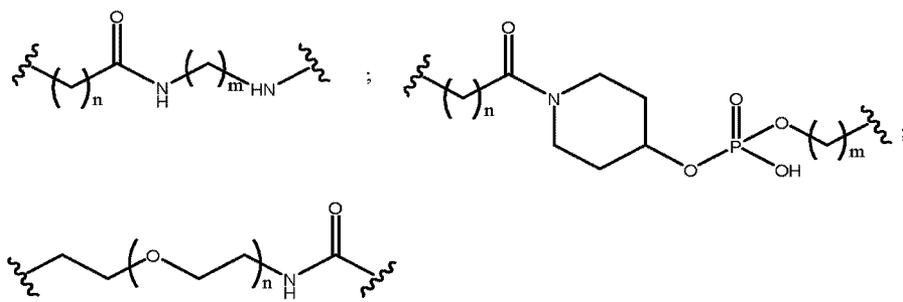


wherein Z_2 is H or CH_3 ; and

each m_2 is, independently, from 0 to 20 wherein at least one m_2 is greater than 0 for each tether.

In certain embodiments, each tether is selected from among:

15



wherein n is from 1 to 12; and

wherein m is from 1 to 12.

20

In certain embodiments, at least one tether comprises ethylene glycol.

In certain embodiments, at least one tether comprises an amide. In certain embodiments, at least one tether comprises a polyamide.

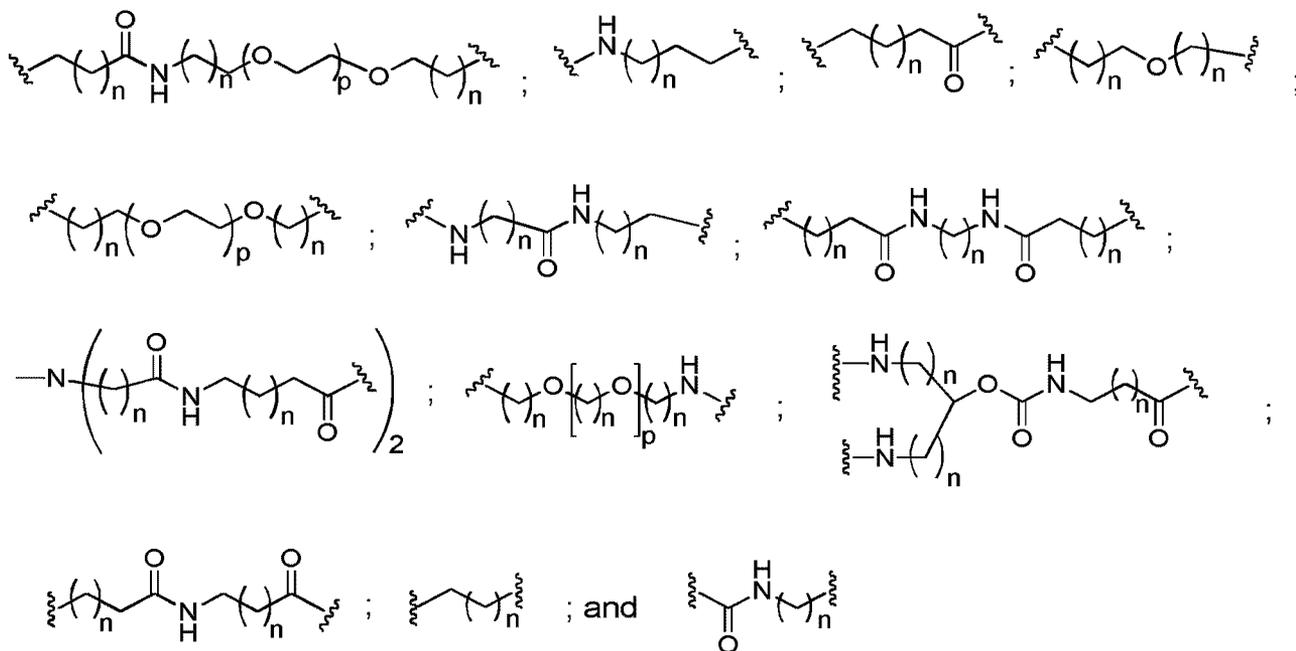
25

In certain embodiments, at least one tether comprises an amine.

In certain embodiments, at least two tethers are different from one another. In certain embodiments, all of the tethers are the same as one another.

5

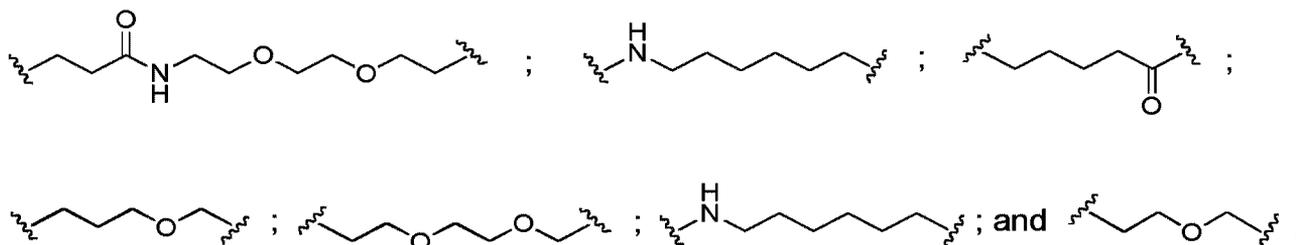
In certain embodiments, each tether is selected from among:



wherein each n is, independently, from 1 to 20; and
each p is from 1 to about 6.

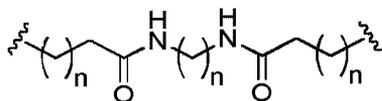
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In certain embodiments, each tether is selected from among:



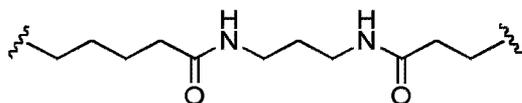
15

In certain embodiments, each tether has the following structure:



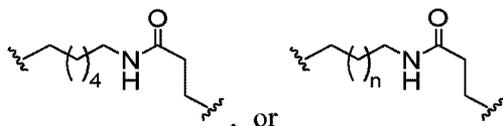
wherein each n is, independently, from 1 to 20.

In certain embodiments, each tether has the following structure:



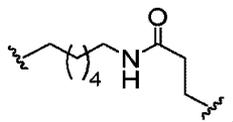
5

In certain embodiments, the tether has a structure selected from among:



10 , or ; wherein each n is independently, 0, 1, 2, 3, 4, 5, 6, or 7.

In certain embodiments, the tether has a structure selected from among:

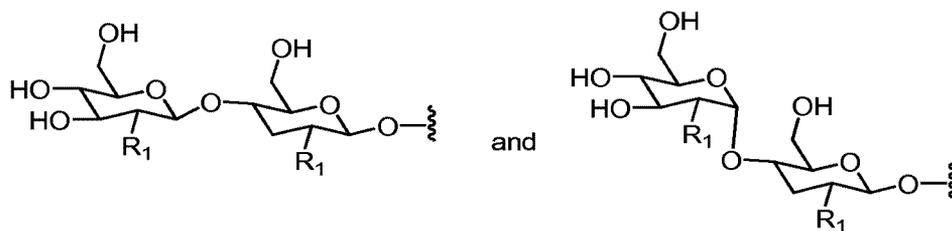


15

In certain embodiments, the ligand is galactose.

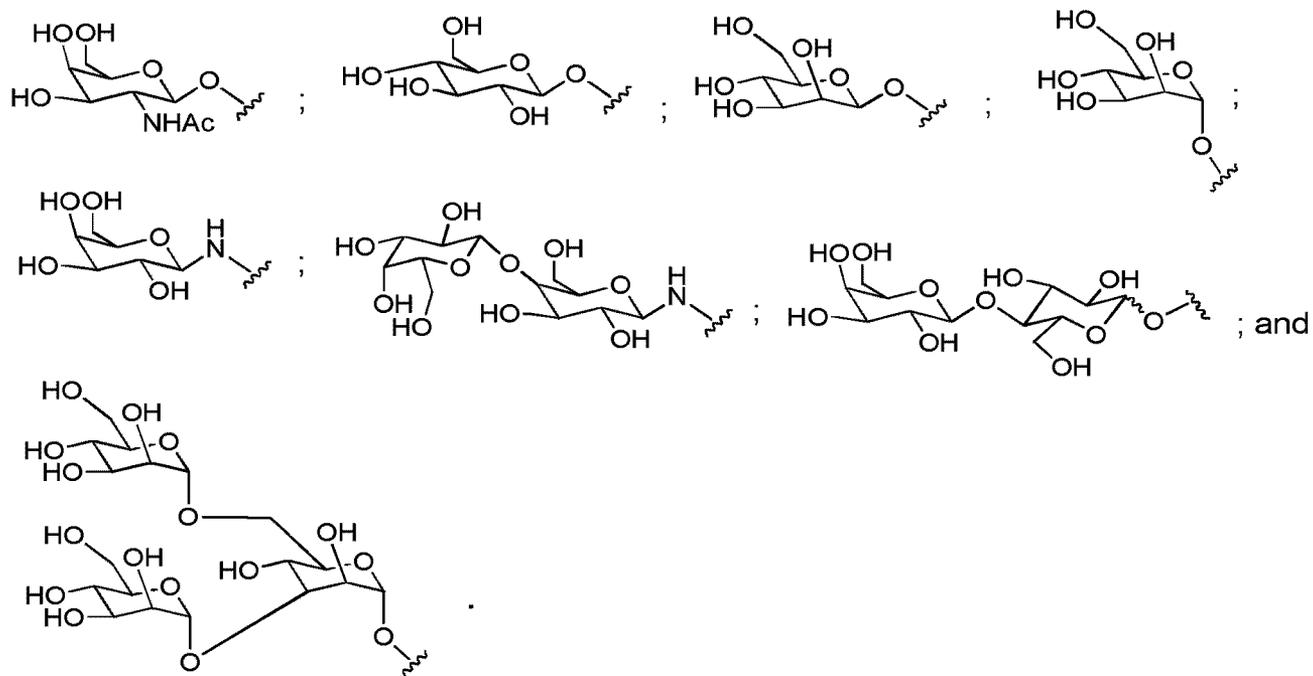
In certain embodiments, the ligand is mannose-6-phosphate.

20 In certain embodiments, each ligand is selected from among:

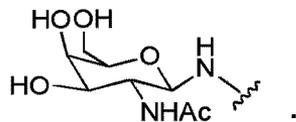


wherein each R₁ is selected from OH and NHCOOH.

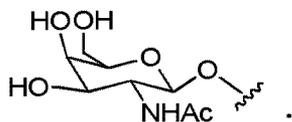
5 In certain embodiments, each ligand is selected from among:



In certain embodiments, each ligand has the following structure:



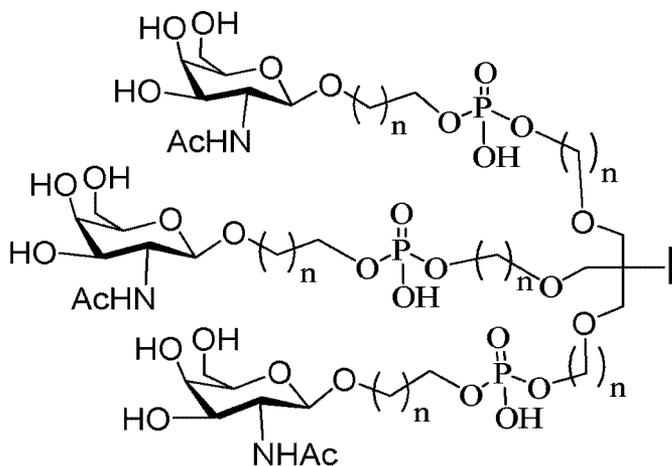
In certain embodiments, each ligand has the following structure:



In certain embodiments, the conjugate group comprises a cell-targeting moiety.

5

In certain embodiments, the conjugate group comprises a cell-targeting moiety having the following structure:

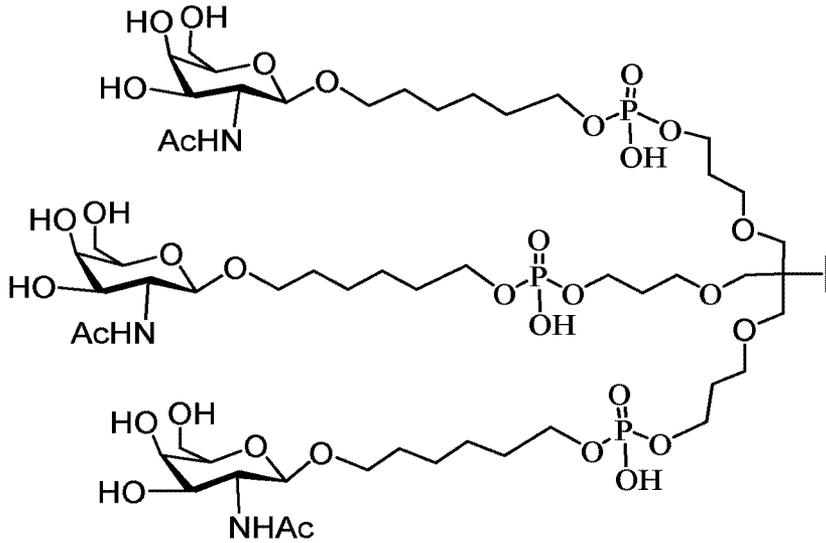


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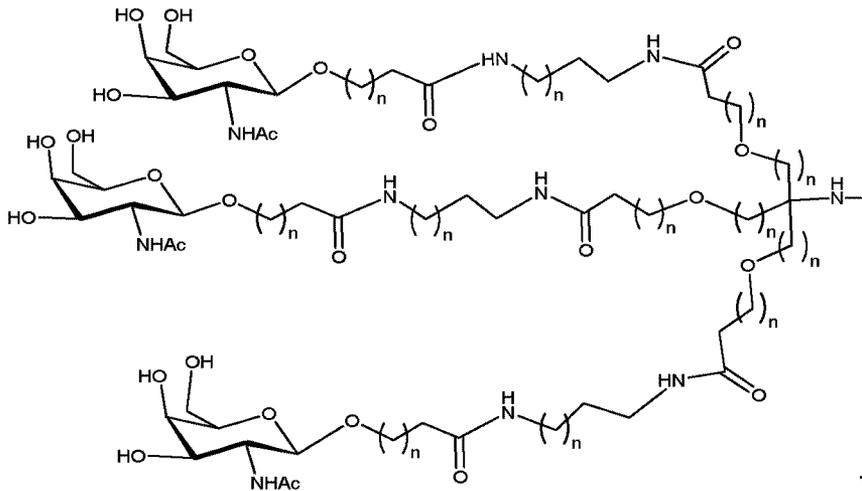
wherein each n is, independently, from 1 to 20.

In certain embodiments, the cell-targeting moiety has the following structure:

15



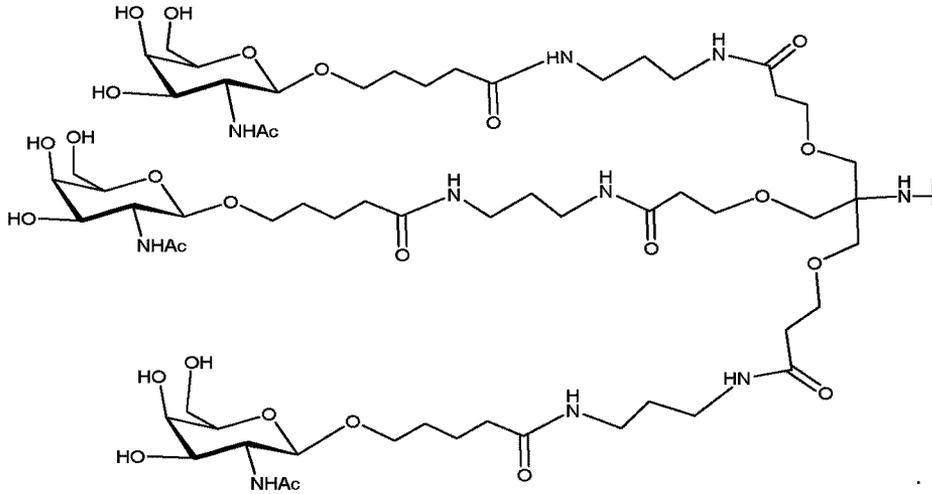
In certain embodiments, the cell-targeting moiety has the following structure:



5

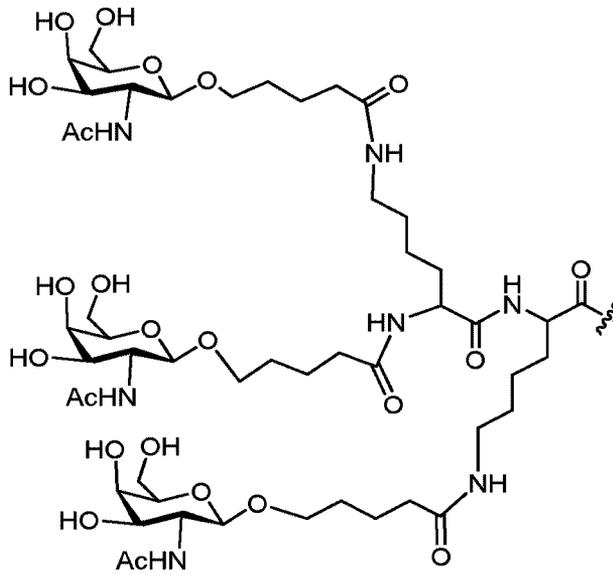
wherein each n is, independently, from 1 to 20.

10 In certain embodiments, the cell-targeting moiety has the following structure:



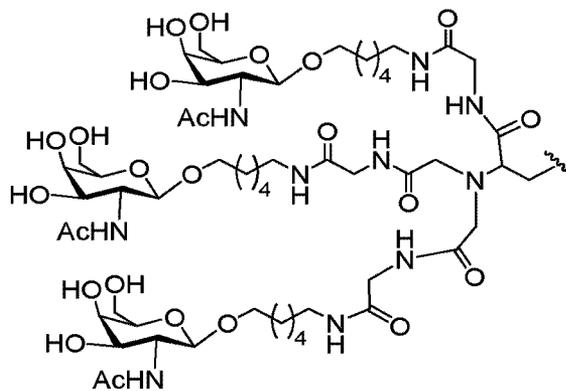
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In certain embodiments, the cell-targeting moiety comprises:

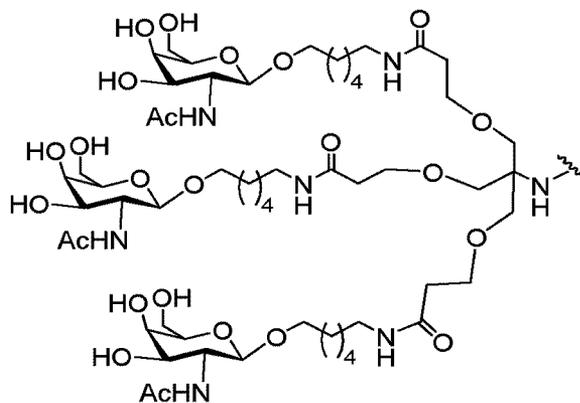


10

In certain embodiments, the cell-targeting moiety comprises:

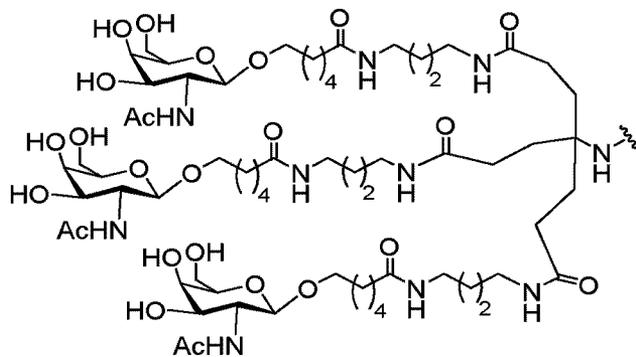


In certain embodiments, the cell-targeting moiety has the following structure:

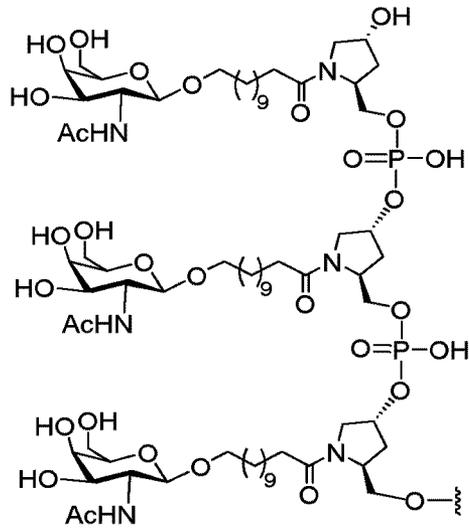


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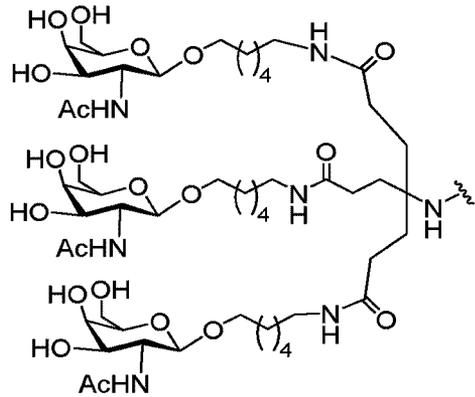
In certain embodiments, the cell-targeting moiety has the following structure:



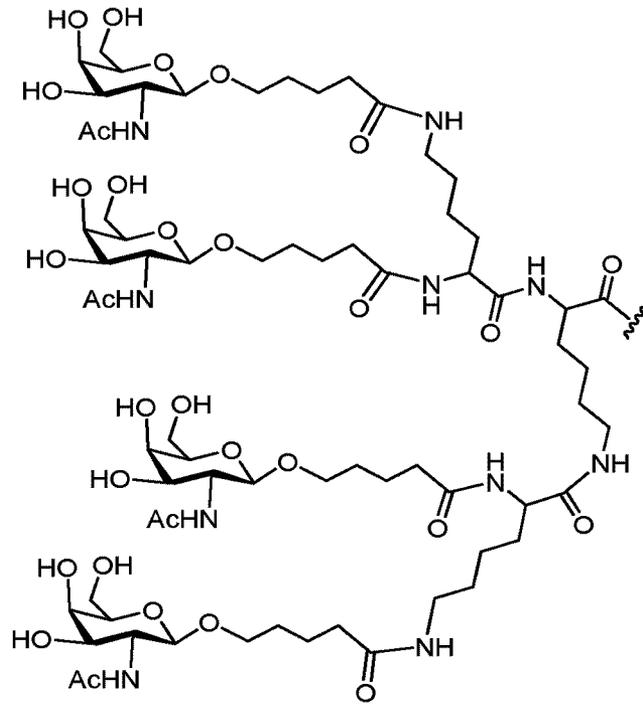
In certain embodiments, the cell-targeting moiety comprises:



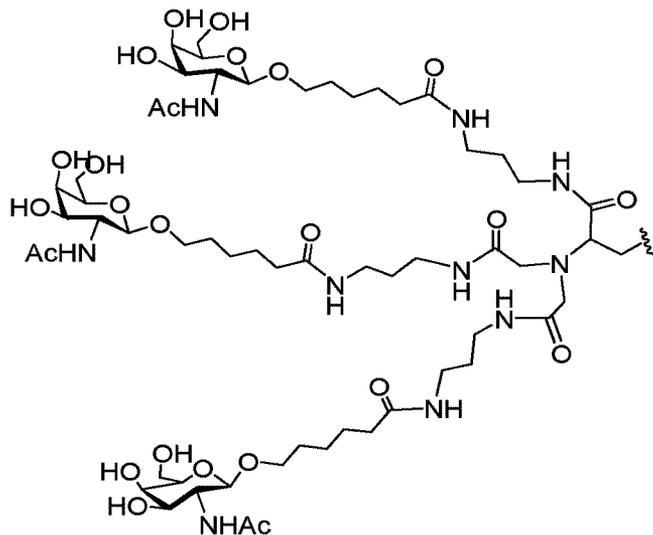
In certain embodiments, the cell-targeting moiety has the following structure:



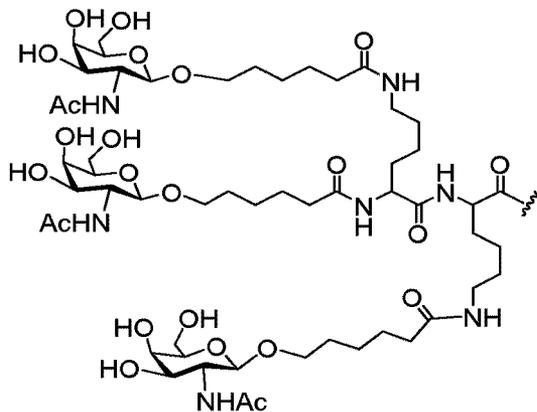
5 In certain embodiments, the cell-targeting moiety comprises:



In certain embodiments, the cell-targeting moiety comprises:

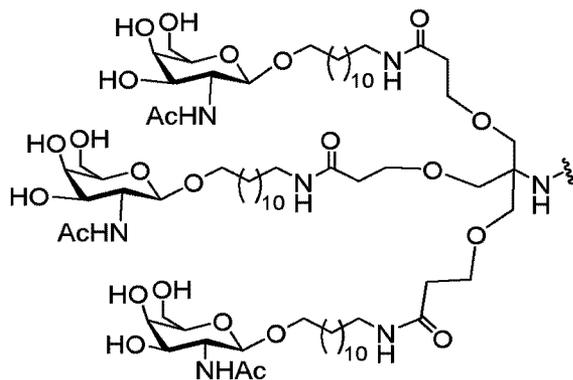


In certain embodiments, the cell-targeting moiety comprises:

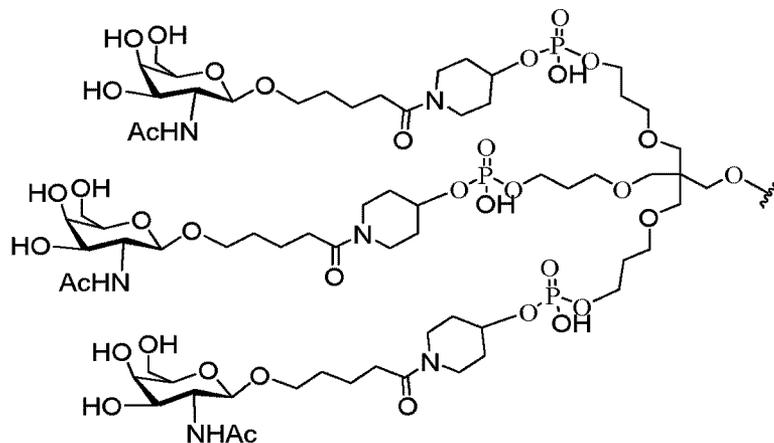


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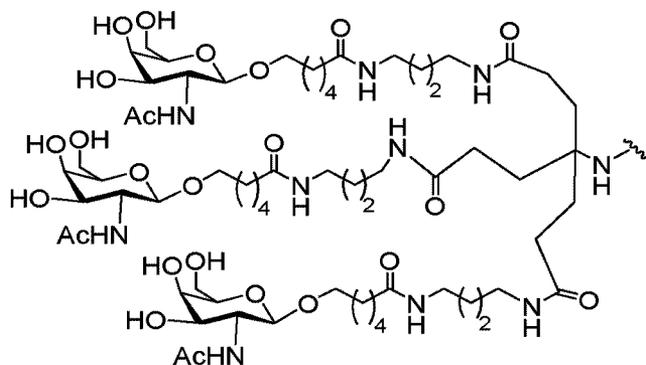
In certain embodiments, the cell-targeting moiety has the following structure:



In certain embodiments, the cell-targeting moiety has the following structure:

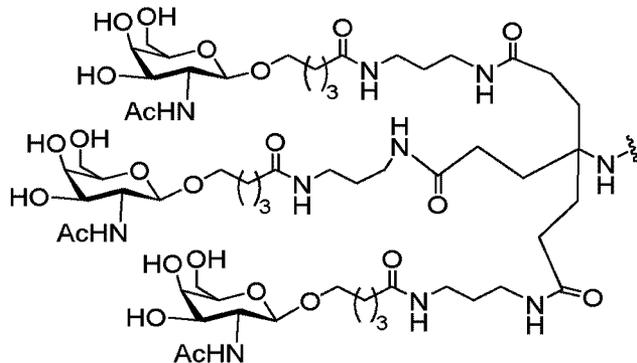


In certain embodiments, the cell-targeting moiety has the following structure:

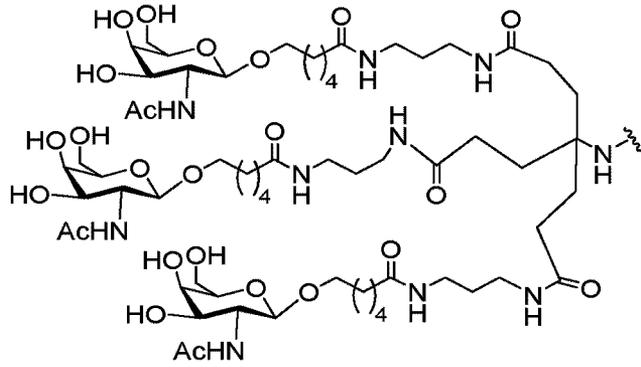


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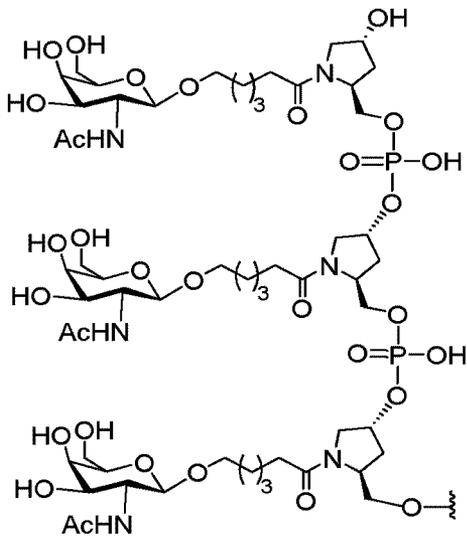
In certain embodiments, the cell-targeting moiety has the following structure:



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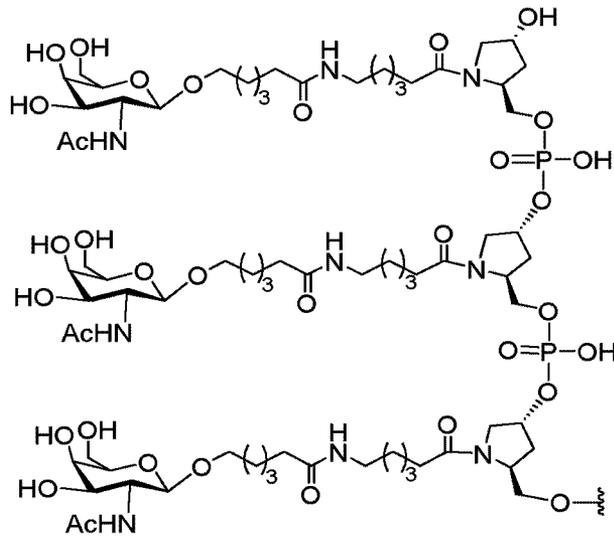


In certain embodiments, the cell-targeting moiety comprises:

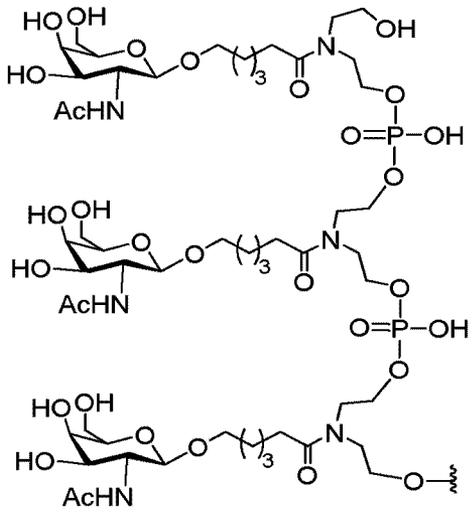


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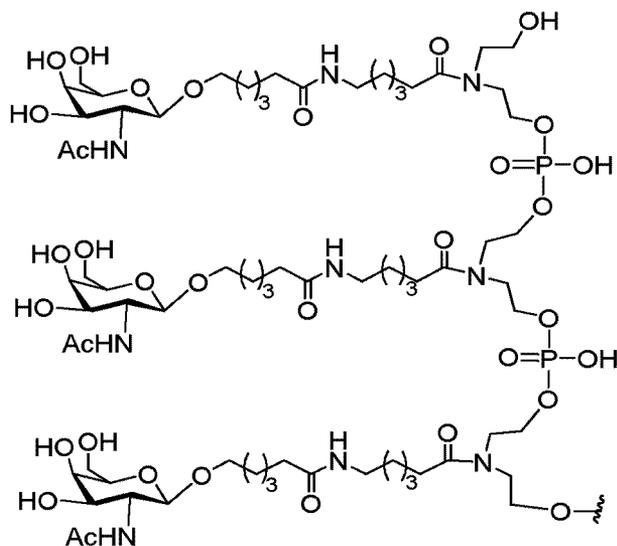
In certain embodiments, the cell-targeting moiety comprises:



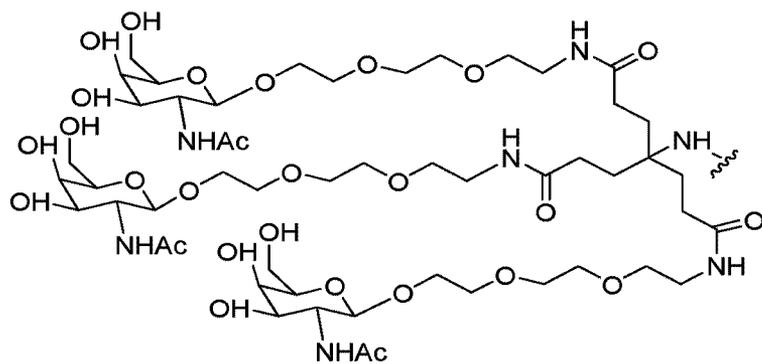
In certain embodiments, the cell-targeting moiety comprises:



5 In certain embodiments, the cell-targeting moiety comprises:

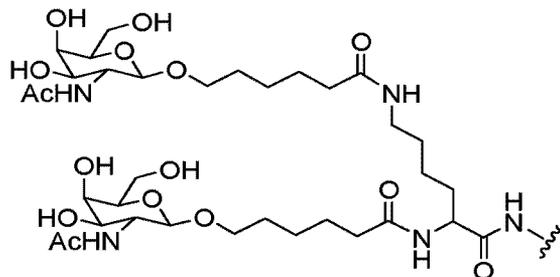


In certain embodiments, the cell-targeting moiety has the following structure:

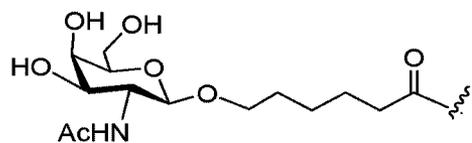


5

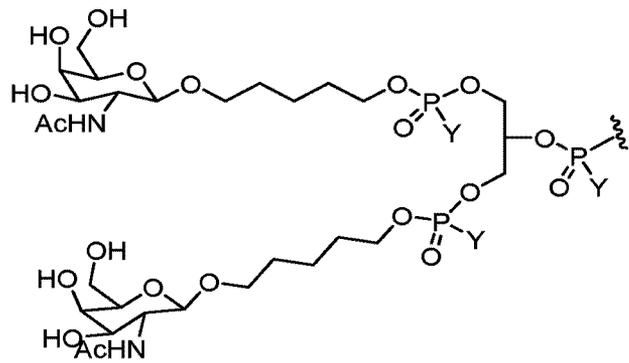
In certain embodiments, the cell-targeting moiety comprises:



In certain embodiments, the cell-targeting moiety has the following structure:

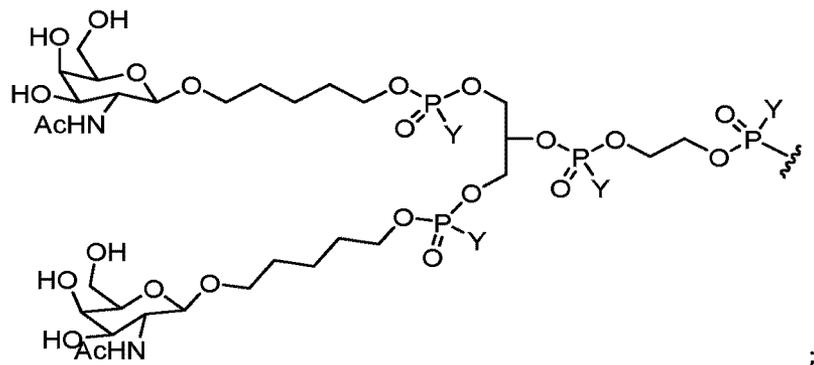


In certain embodiments, the cell-targeting moiety comprises:



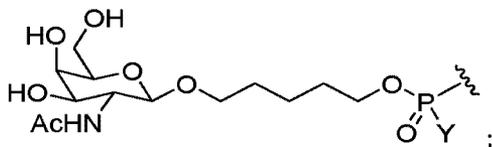
5 wherein each Y is selected from O, S, a substituted or unsubstituted C₁-C₁₀ alkyl, amino, substituted amino, azido, alkenyl or alkynyl.

In certain embodiments, the conjugate group comprises:



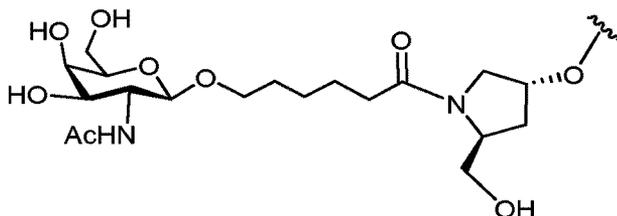
10 wherein each Y is selected from O, S, a substituted or unsubstituted C₁-C₁₀ alkyl, amino, substituted amino, azido, alkenyl or alkynyl.

In certain embodiments, the cell-targeting moiety has the following structure:

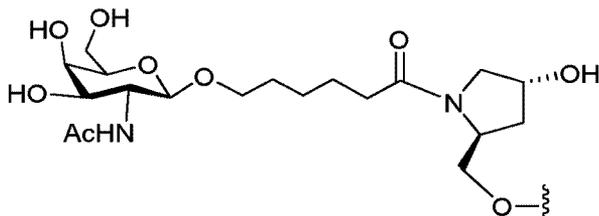


wherein each Y is selected from O, S, a substituted or unsubstituted C₁-C₁₀ alkyl, amino, substituted amino, azido, alkenyl or alkynyl.

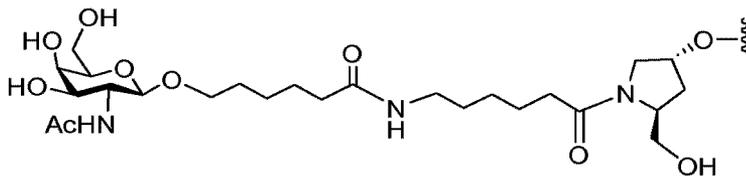
5 In certain embodiments, the conjugate group comprises:



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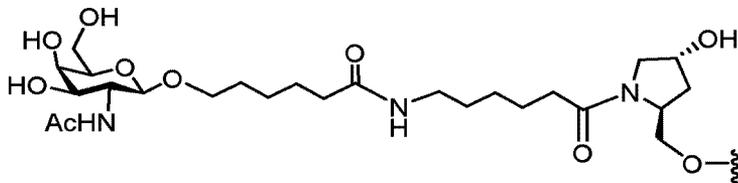


T In certain embodiments, the conjugate group comprises:



10

In certain embodiments, the conjugate group comprises:



In certain embodiments, the conjugate group comprises a cleavable moiety selected from among: a phosphodiester, an amide, or an ester.

In certain embodiments, the conjugate group comprises a phosphodiester cleavable moiety.

5

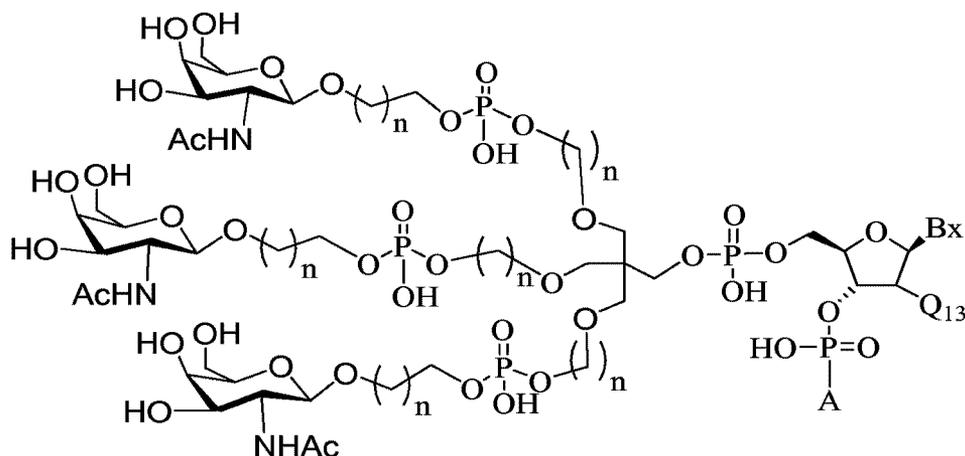
In certain embodiments, the conjugate group does not comprise a cleavable moiety, and wherein the conjugate group comprises a phosphorothioate linkage between the conjugate group and the oligonucleotide.

In certain embodiments, the conjugate group comprises an amide cleavable moiety.

10

In certain embodiments, the conjugate group comprises an ester cleavable moiety.

In certain embodiments, the compound has the following structure:



15

wherein each n is, independently, from 1 to 20;

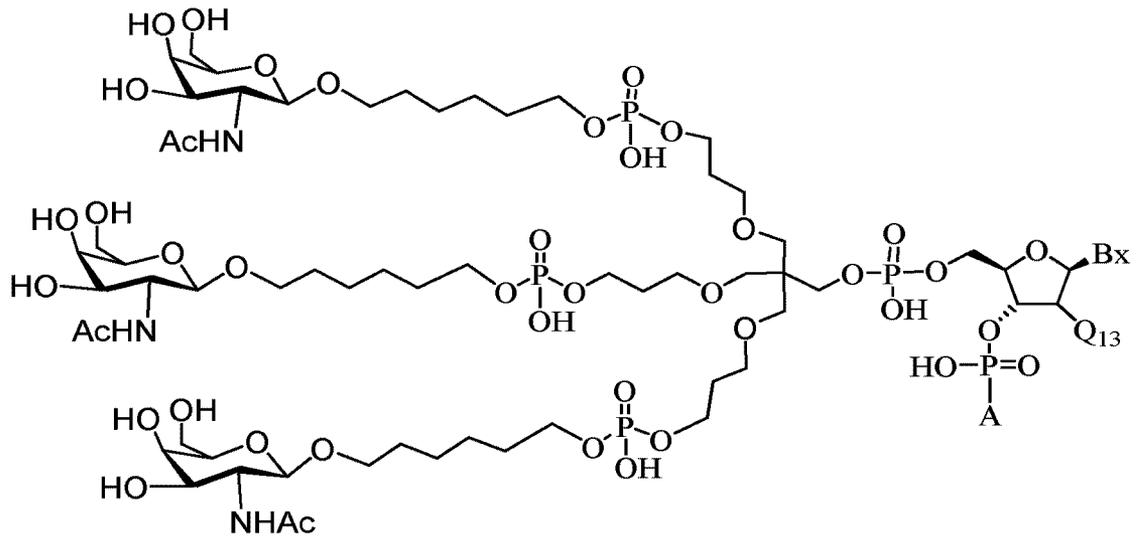
Q_{13} is H or $O(CH_2)_2-OCH_3$;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

20

In certain embodiments, the compound has the following structure:



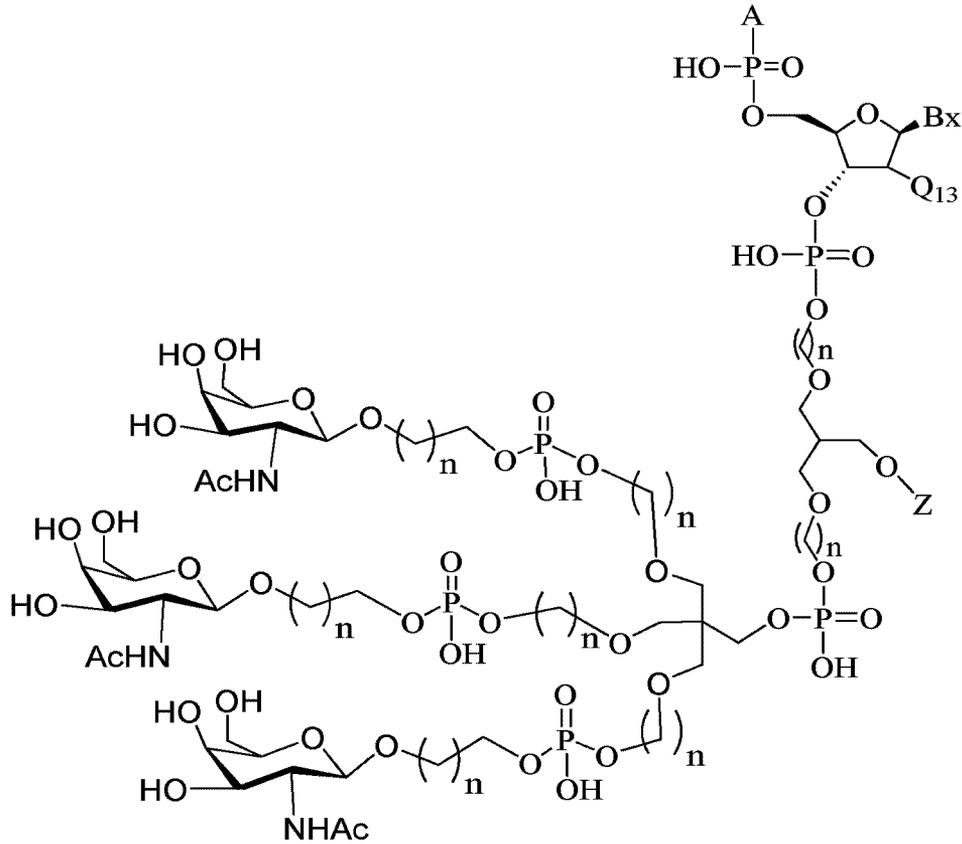
wherein each n is, independently, from 1 to 20;

5 Q_{13} is H or $O(CH_2)_2-OCH_3$;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

In certain embodiments, the compound has the following structure:



wherein each n is, independently, from 1 to 20;

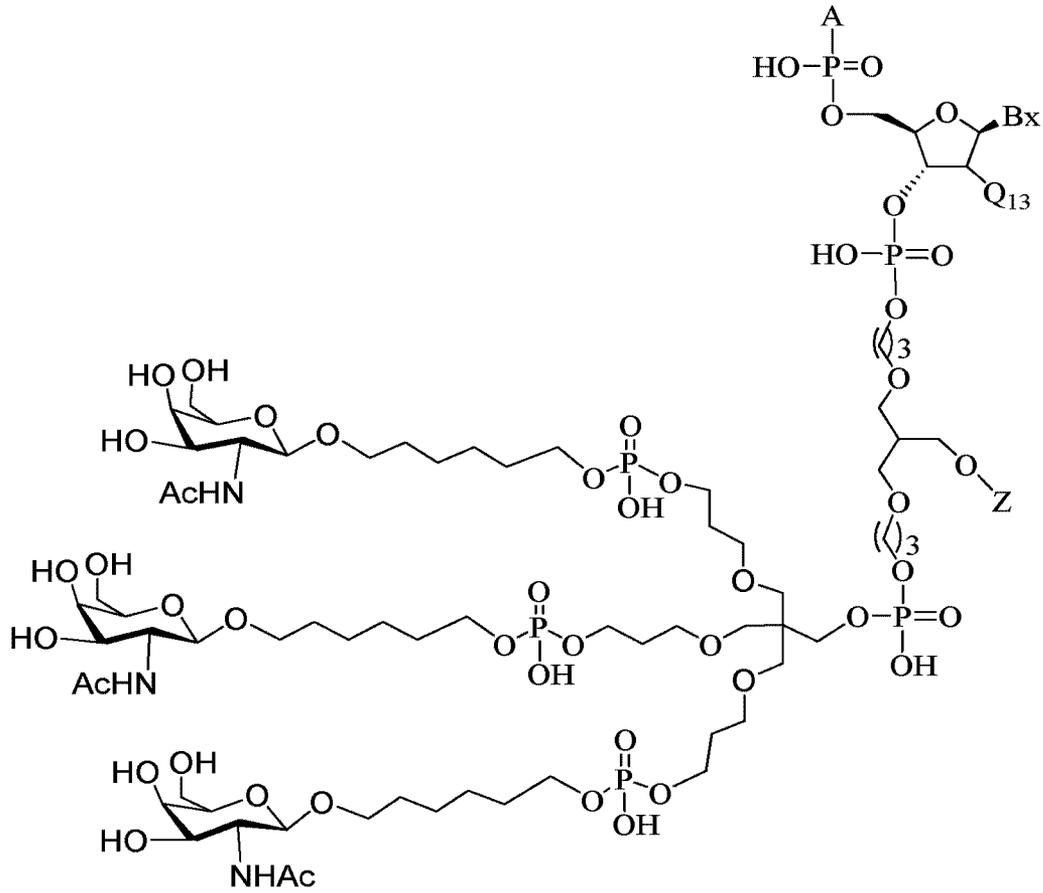
5 Q_{13} is H or $O(CH_2)_2-OCH_3$;

A is the modified oligonucleotide;

Z is H or a linked solid support; and

Bx is a heterocyclic base moiety.

In certain embodiments, the compound has the following structure:



5 wherein each n is, independently, from 1 to 20;

Q_{13} is H or $O(CH_2)_2-OCH_3$;

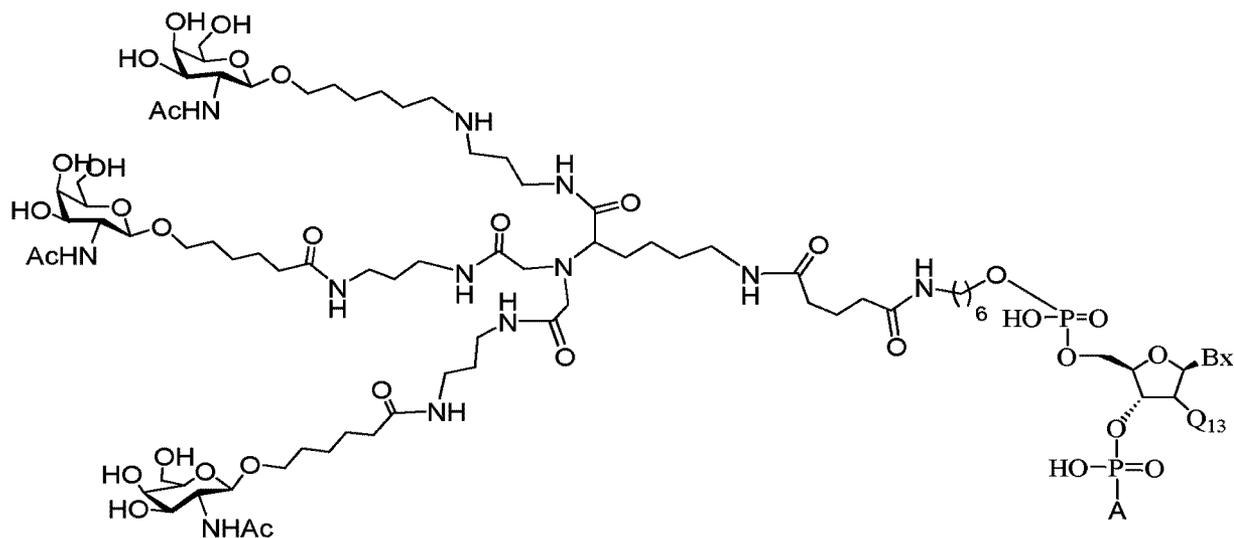
A is the modified oligonucleotide;

Z is H or a linked solid support; and

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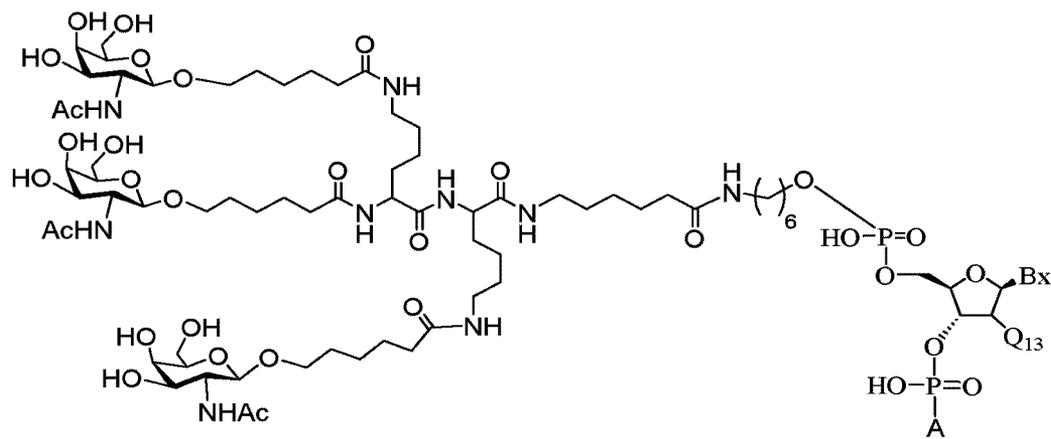


wherein Q₁₃ is H or O(CH₂)₂-OCH₃;

A is the modified oligonucleotide; and

5 Bx is a heterocyclic base moiety.

In certain embodiments, the compound has the following structure:

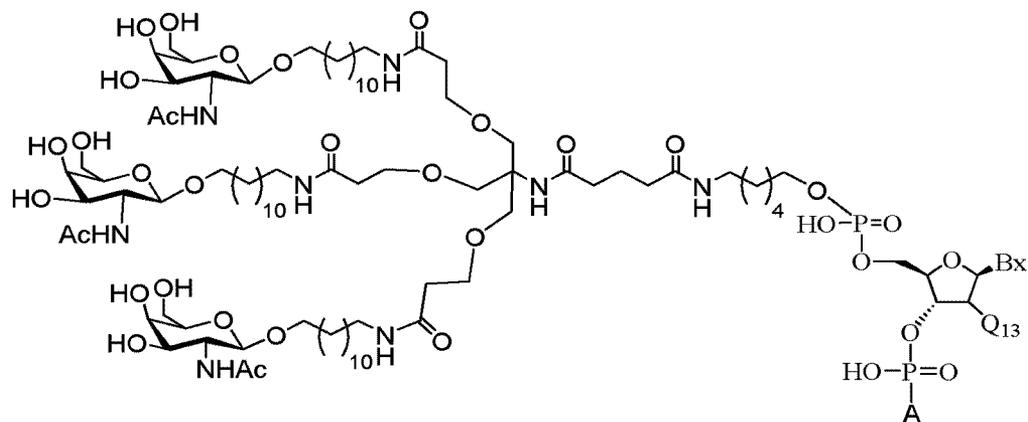


10 wherein Q₁₃ is H or O(CH₂)₂-OCH₃;

A is the modified oligonucleotide; and

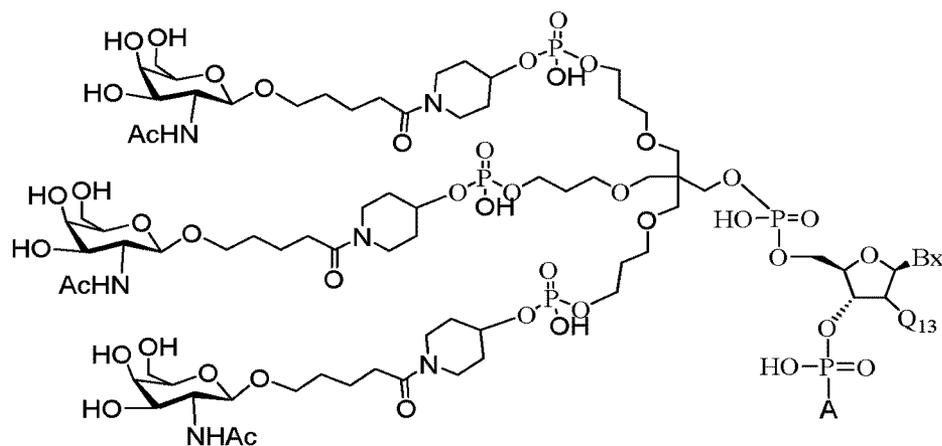
Bx is a heterocyclic base moiety.

In certain embodiments, the compound has the following structure:



- 5 wherein Q₁₃ is H or O(CH₂)₂-OCH₃;
A is the modified oligonucleotide; and
Bx is a heterocyclic base moiety.

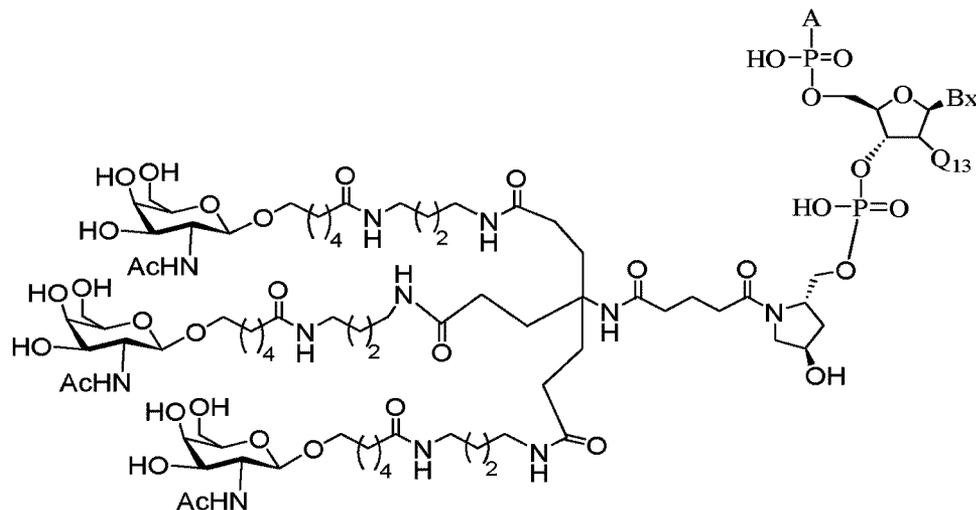
In certain embodiments, the compound has the following structure:



10

- wherein Q₁₃ is H or O(CH₂)₂-OCH₃;
A is the modified oligonucleotide; and
Bx is a heterocyclic base moiety.

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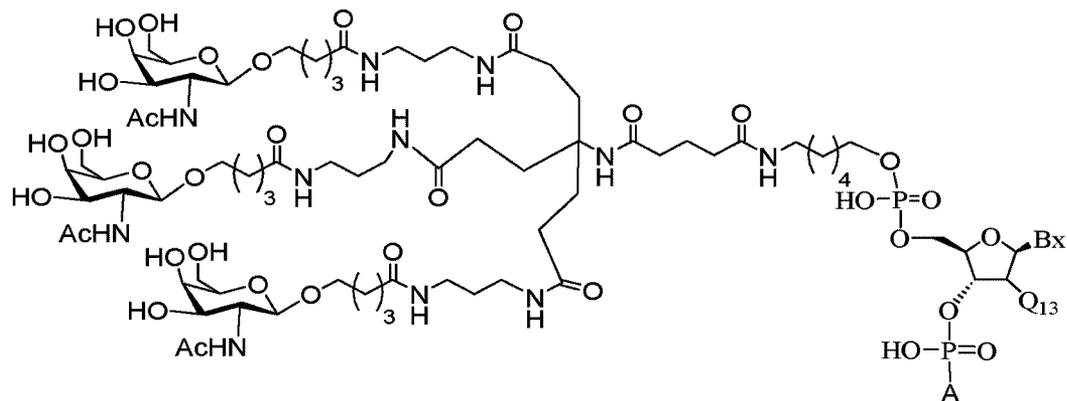


wherein Q₁₃ is H or O(CH₂)₂-OCH₃;

5 A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

In certain embodiments, the compound has the following structure:

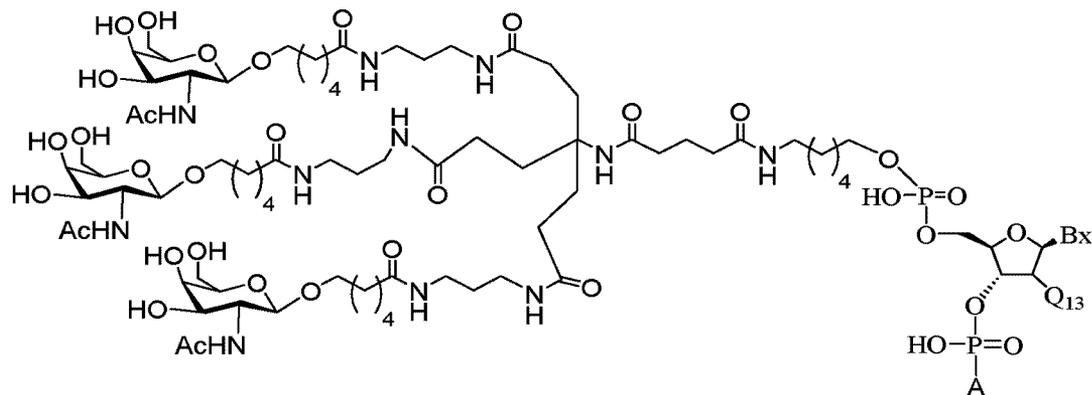


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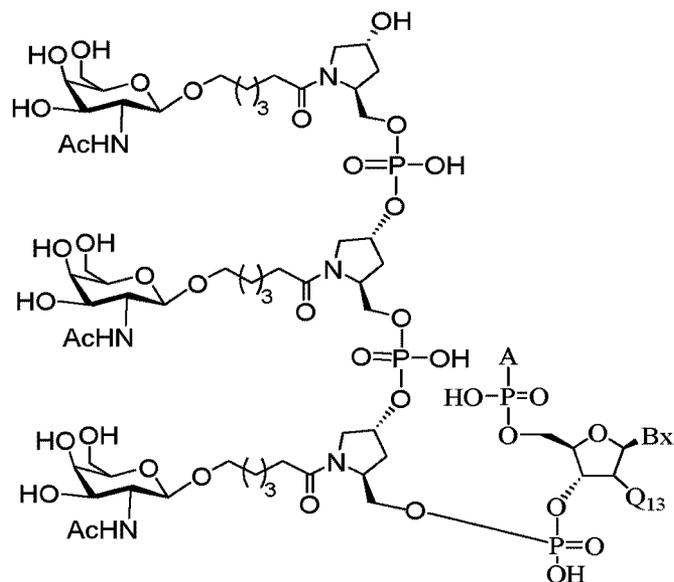


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A is the modified oligonucleotide; and

5 Bx is a heterocyclic base moiety.

In certain embodiments, the compound has the following structure:

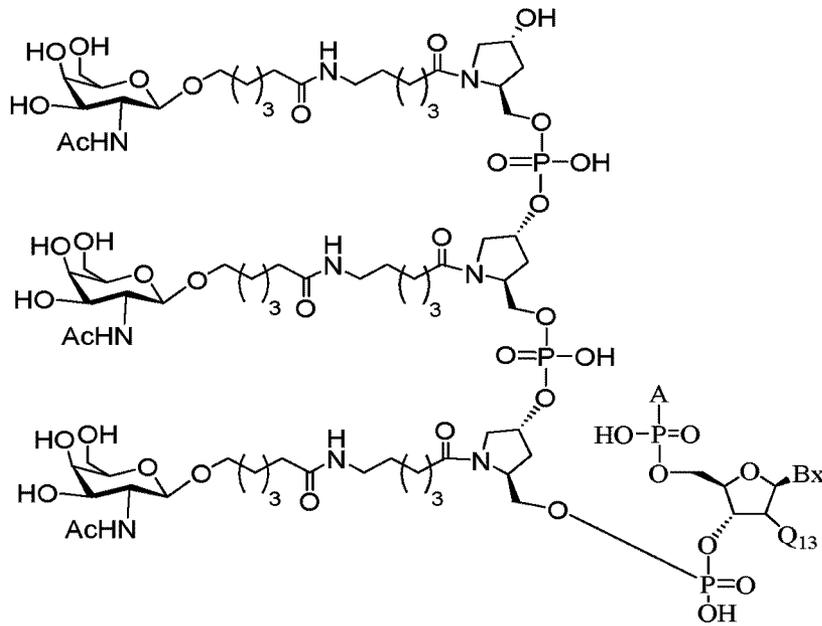


wherein Q₁₃ is H or O(CH₂)₂-OCH₃;

A is the modified oligonucleotide; and

10 Bx is a heterocyclic base moiety.

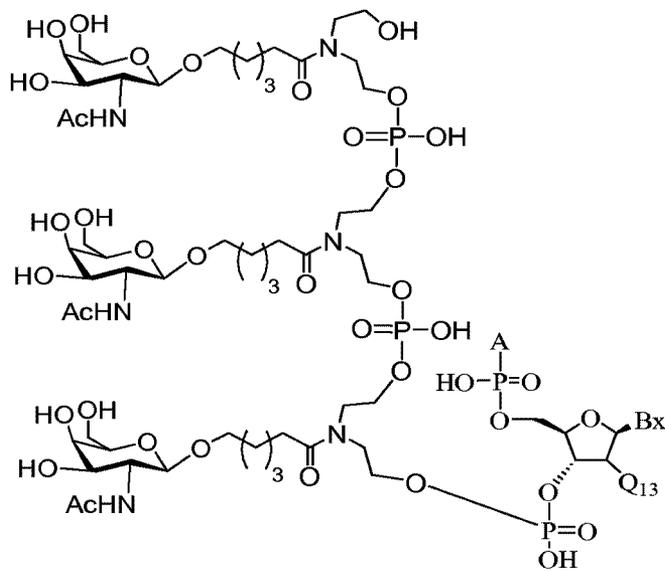
In certain embodiments, the compound has the following structure:



wherein Q_{13} is H or $O(CH_2)_2-OCH_3$;

- 5 A is the modified oligonucleotide; and
Bx is a heterocyclic base moiety.

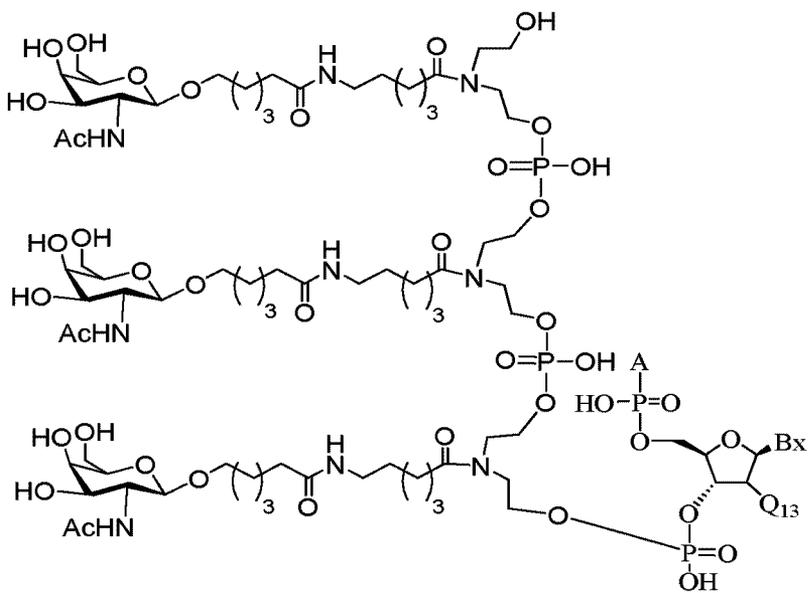
In certain embodiments, the compound has the following structure:



wherein Q_{13} is H or $O(CH_2)_2-OCH_3$;

- 5 A is the modified oligonucleotide; and
Bx is a heterocyclic base moiety.

In certain embodiments, the compound has the following structure:

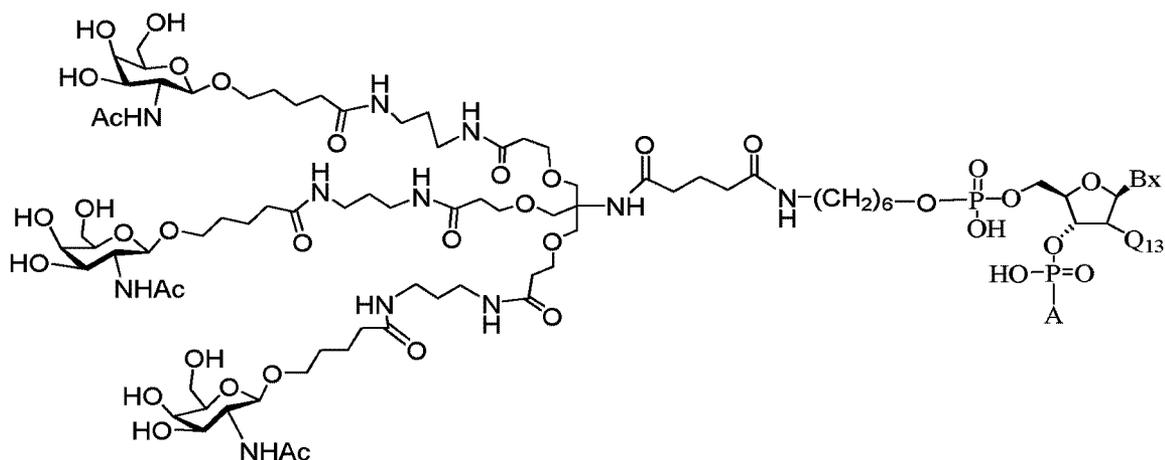


wherein Q_{13} is H or $O(CH_2)_2-OCH_3$;

- 10 A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

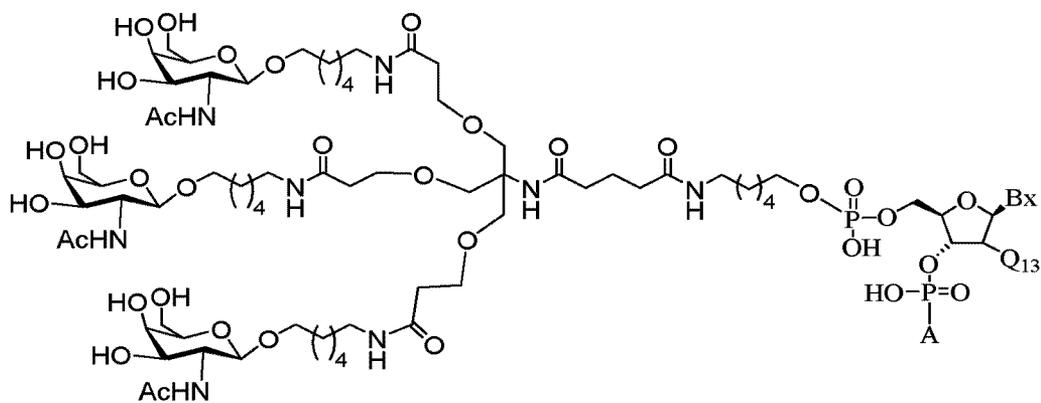
In certain embodiments, the conjugate group comprises:



- 5 wherein Q_{13} is H or $O(CH_2)_2-OCH_3$;
A is the modified oligonucleotide; and
Bx is a heterocyclic base moiety.

In certain embodiments, the conjugate group comprises:

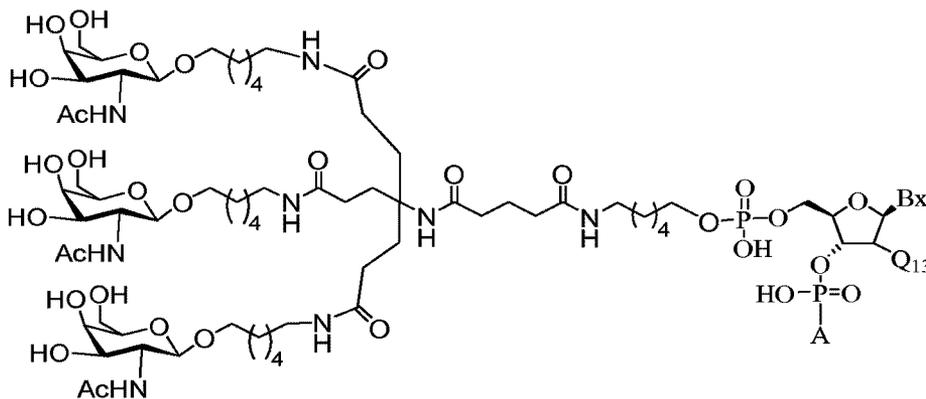
10



- wherein Q_{13} is H or $O(CH_2)_2-OCH_3$;
A is the modified oligonucleotide; and
Bx is a heterocyclic base moiety.

15

In certain embodiments, the conjugate group comprises:



wherein Q_{13} is H or $O(CH_2)_2-OCH_3$;

A is the modified oligonucleotide; and

5 Bx is a heterocyclic base moiety.

In certain embodiments, B_x is selected from among adenine, guanine, thymine, uracil, or cytosine, or 5-methyl cytosine. In certain embodiments, B_x is adenine. In certain embodiments, B_x is thymine. In certain embodiments, Q_{13} is $O(CH_2)_2-OCH_3$. In certain embodiments, Q_{13} is H.

10 Certain embodiments of the invention provide a prodrug comprising the compositions or compounds disclosed herein.

In certain embodiments, the compound is in a salt form. In further embodiments, the compound further comprises of a pharmaceutically acceptable carrier or diluent. In certain embodiments, the compound comprises a modified oligonucleotide targeting ApoCIII and a conjugate group, or a salt thereof, and a
15 pharmaceutically acceptable carrier or diluent.

Certain embodiments provide compositions and methods comprising administering to an animal a conjugated antisense compound or composition disclosed herein. In certain embodiments, administering the conjugated antisense compound prevents, treats, ameliorates, or slows progression of a cardiovascular, metabolic and/or inflammatory disease.

20 Certain embodiments provide compositions and methods for use in therapy to treat an ApoCIII related disease, disorder or condition. In certain embodiments, the ApoCIII levels are elevated in an animal. In certain embodiments, the composition is a compound comprising an ApoCIII specific inhibitor. In certain embodiments, the ApoCIII specific inhibitor is a nucleic acid. In certain embodiments, the nucleic acid is an antisense compound. In certain embodiments, the antisense compound is a modified oligonucleotide targeting
25 ApoCIII. In certain embodiments, the antisense compound is a modified oligonucleotide targeting ApoCIII and a conjugate group. In certain embodiments, the modified oligonucleotide targeting ApoCIII with the conjugate group, is used in treating, preventing, slowing progression, ameliorating an inflammatory,

cardiovascular and/or metabolic disease, disorder or condition. In certain embodiments, the compositions and methods for therapy include administering an ApoCIII specific inhibitor to an individual in need thereof.

Certain embodiments provide conjugated antisense compounds and compositions and methods for reducing ApoCIII levels. In certain embodiments, ApoCIII levels are reduced in the liver, adipose tissue, heart, skeletal muscle or small intestine.

In certain embodiments, reducing ApoCIII levels in a tissue, organ or subject increases HDL levels. In certain embodiments, the HDL levels are increased by at least 90%, by at least 80%, by at least 70%, by at least 60%, by at least 50%, by at least 45%, at least 40%, by at least 35%, by at least 30%, by at least 25%, by at least 20%, by at least 15%, by at least 10% or by at least 5% from the baseline HDL level.

In certain embodiments, reducing ApoCIII levels in a tissue, organ or subject reduces TG levels. In certain embodiments, the subject has a triglyceride level ≥ 100 mg/dL, ≥ 200 mg/dL, ≥ 300 mg/dL, ≥ 400 mg/dL, ≥ 440 mg/dL, ≥ 500 mg/dL, ≥ 600 mg/dL, ≥ 700 mg/dL, ≥ 800 mg/dL, ≥ 880 mg/dL, ≥ 900 mg/dL, ≥ 1000 mg/dL, ≥ 1100 mg/dL, ≥ 1200 mg/dL, ≥ 1300 mg/dL, ≥ 1400 mg/dL, ≥ 1500 mg/dL, ≥ 1600 mg/dL, ≥ 1700 mg/dL, ≥ 1800 mg/dL, ≥ 1900 mg/dL, ≥ 2000 mg/dL.

In certain embodiments, the TG levels (postprandial or fasting) are decreased by at least 90%, by at least 80%, by at least 70%, by at least 60%, by at least 50%, by at least 45%, at least 40%, by at least 35%, by at least 30%, by at least 25%, by at least 20%, by at least 15%, by at least 10%, by at least 5% or by at least 1% from the baseline TG level. In certain embodiments, the TG (postprandial or fasting) level is decreased to ≤ 1900 mg/dL, ≤ 1800 mg/dL, ≤ 1700 mg/dL, ≤ 1600 mg/dL, ≤ 1500 mg/dL, ≤ 1400 mg/dL, ≤ 1300 mg/dL, ≤ 1200 mg/dL, ≤ 1100 mg/dL, ≤ 1000 mg/dL, ≤ 900 mg/dL, ≤ 800 mg/dL, ≤ 750 mg/dL, ≤ 700 mg/dL, ≤ 650 mg/dL, ≤ 600 mg/dL, ≤ 550 mg/dL, ≤ 500 mg/dL, ≤ 450 mg/dL, ≤ 400 mg/dL, ≤ 350 mg/dL, ≤ 300 mg/dL, ≤ 250 mg/dL, ≤ 200 mg/dL, ≤ 150 mg/dL or ≤ 100 mg/dL.

In certain embodiments, reducing ApoCIII levels in a tissue, organ or subject improves the ratio of LDL to HDL or the ratio of TG to HDL.

In certain embodiments, reducing ApoCIII levels in a tissue, organ or subject improves insulin sensitivity.

In certain embodiments, reducing ApoCIII levels in a tissue, organ or subject increases chylomicron clearance.

Certain embodiments provide compositions and methods to reduce ApoCIII mRNA or protein expression in an animal comprising administering to the animal a conjugated antisense compound or composition disclosed herein to reduce ApoCIII mRNA or protein expression in the animal.

Certain embodiments provide conjugated antisense compounds and compositions and methods for preventing, treating, delaying, slowing the progression and/or ameliorating ApoCIII related diseases, disorders, and conditions in a subject in need thereof. In certain embodiments, such diseases, disorders, and conditions include inflammatory, cardiovascular and/or metabolic diseases, disorders, and conditions. Certain such cardiovascular diseases, disorders or conditions include, but are not limited to, chylomicronemia,

hypertriglyceridemia, aortic stenosis, aneurysm (e.g., abdominal aortic aneurysm), angina, arrhythmia, atherosclerosis, cerebrovascular disease, coronary artery disease, coronary heart disease, dyslipidemia, hypercholesterolemia, hyperlipidemia, hypertension, myocardial infarction, peripheral vascular disease (e.g., peripheral artery disease, peripheral artery occlusive disease), Fredrickson Type I dyslipidemia, FCS, LPL deficiency, retinal vascular occlusion, or stroke. Certain such metabolic diseases, disorders or conditions include, but are not limited to, hyperglycemia, prediabetes, diabetes (type I and type II), obesity, insulin resistance, metabolic syndrome and diabetic dyslipidemia. Certain such inflammatory diseases, disorders or conditions include, but are not limited to, pancreatitis, aortic stenosis, coronary artery disease (CAD), Alzheimer's Disease and thromboembolic diseases, disorder or conditions. Certain thromboembolic diseases, disorders or conditions include, but are not limited to, stroke, thrombosis (e.g., venous thromboembolism), myocardial infarction and peripheral vascular disease. Certain embodiments provide conjugated antisense compounds and compositions and methods for preventing, treating, delaying, slowing the progression and/or ameliorating hypertriglyceridemia. Certain embodiments provide conjugated antisense compounds and compositions and methods for preventing, treating, delaying, slowing the progression and/or ameliorating chylomicronemia. Certain embodiments provide conjugated antisense compounds and compositions and methods for preventing, treating, delaying, slowing the progression and/or ameliorating pancreatitis.

Certain embodiments provide a method of reducing at least one symptom of a cardiovascular disease, disorder or condition. In certain embodiments, the symptoms include, but are not limited to, angina, chest pain, shortness of breath, palpitations, weakness, dizziness, nausea, sweating, tachycardia, bradycardia, arrhythmia, atrial fibrillation, swelling in the lower extremities, cyanosis, fatigue, fainting, numbness of the face, numbness of the limbs, claudication or cramping of muscles, bloating of the abdomen, and fever. In certain embodiments, symptoms of a metabolic disease, disorder or condition include, but are not limited to, frequent urination, unusual thirst, extreme hunger, unusual weight loss, extreme fatigue, irritability, frequent infections, blurred vision, cuts/bruises that are slow to heal, tingling/numbness in the hands/feet and recurring skin, gum, or bladder infections. Certain embodiments provide a method of reducing at least one symptom of hypertriglyceridemia. Certain embodiments provide a method of reducing at least one symptom of chylomicronemia. Certain embodiments provide a method of reducing at least one symptom of pancreatitis.

In certain embodiments, the modulation of ApoCIII expression occurs in a cell, tissue or organ. In certain embodiments, the modulations occur in a cell, tissue or organ in an animal. In certain embodiments, the modulation is a reduction in ApoCIII mRNA level. In certain embodiments, the modulation is a reduction in ApoCIII protein level. In certain embodiments, both ApoCIII mRNA and protein levels are reduced. Such reduction may occur in a time-dependent or in a dose-dependent manner.

In certain embodiments, the subject or animal is human.

In certain embodiments, the compound is parenterally administered. In further embodiments, the parenteral administration is subcutaneous.

In certain embodiments, the conjugated antisense compound or composition is co-administered with a second agent or therapy. In certain embodiments, the conjugated antisense compound or composition and the second agent are administered concomitantly.

5 In certain embodiments, the second agent is a glucose-lowering agent. In certain embodiments, the second agent is a LDL, TG or cholesterol lowering agent. In certain embodiments, the second agent is an anti-inflammatory agent. In certain embodiments, the second agent is an Alzheimer Disease drug. In certain
10 embodiments, the second agent can be, but is not limited to, a non-steroidal anti-inflammatory drug (NSAID e.g., aspirin), niacin (e.g., Niaspan), nicotinic acid, an apoB inhibitor (e.g., Mipomersen), a CETP inhibitor (e.g., Anacetrapib), an apo(a) inhibitor, a thyroid hormone analog (e.g., Eprotirome), a HMG-CoA reductase inhibitor (e.g., a statin), a fibrate (e.g., Gemfibrozil) and an microsomal triglyceride transfer protein inhibitor (e.g., Lomitapide). Agents or therapies can be co-administered or administered concomitantly. Agents or therapies can be sequentially or subsequently administered.

Certain embodiments provide use of the compositions and conjugated antisense compounds described herein targeted to ApoCIII for decreasing ApoCIII levels in an animal. Certain embodiments
15 provide use of a compound targeted to ApoCIII for decreasing ApoCIII levels in an animal. Certain embodiments provide use of a compound targeted to ApoCIII for increasing HDL levels in an animal. Certain embodiments provide use of a compound targeted to ApoCIII for increasing HDL chylomicron clearance in an animal. Certain embodiments provide use of a compounds targeted to ApoCIII for the treatment, prevention, or amelioration of a disease, disorder, or condition associated with ApoCIII. Certain
20 embodiments provide use of a compound targeted to ApoCIII for the treatment, prevention, or amelioration of a hypertriglyceridemia. Certain embodiments provide use of a compound targeted to ApoCIII for the treatment, prevention, or amelioration of a chylomicronemia (e.g., FCS and/or LPLD). Certain embodiments provide use of a compound targeted to ApoCIII for the treatment, prevention, or amelioration of a pancreatitis.

25 Certain embodiments provide use of the compositions and conjugated antisense compounds described herein targeted to ApoCIII in the preparation of a medicament for decreasing ApoCIII levels in an animal. Certain embodiments provide use of the compositions and compounds for the preparation of a medicament for the treatment, prevention, or amelioration of a disease, disorder, or condition associated with ApoCIII.

30 Certain embodiments provide the use of the compositions and conjugated antisense compounds as described herein in the manufacture of a medicament for treating, ameliorating, delaying or preventing one or more of a disease related to ApoCIII.

Certain embodiments provide a kit for treating, preventing, or ameliorating a disease, disorder or condition as described herein wherein the kit comprises: (i) an ApoCIII specific inhibitor as described herein;
35 and optionally (ii) a second agent or therapy as described herein.

A kit of the present invention can further include instructions for using the kit to treat, prevent, or ameliorate a disease, disorder or condition as described herein by combination therapy as described herein.

B. Certain Compounds

5 In certain embodiments, the invention provides conjugated antisense compounds comprising antisense oligonucleotides and a conjugate.

a. Certain Antisense Oligonucleotides

10 In certain embodiments, the invention provides antisense oligonucleotides. Such antisense oligonucleotides comprise linked nucleosides, each nucleoside comprising a sugar moiety and a nucleobase. The structure of such antisense oligonucleotides may be considered in terms of chemical features (e.g., modifications and patterns of modifications) and nucleobase sequence (e.g., sequence of antisense oligonucleotide, identity and sequence of target nucleic acid).

i. Certain Chemistry Features

15 In certain embodiments, antisense oligonucleotide comprise one or more modification. In certain such embodiments, antisense oligonucleotides comprise one or more modified nucleosides and/or modified internucleoside linkages. In certain embodiments, modified nucleosides comprise a modified sugar moiety and/or modified nucleobase.

1. Certain Sugar Moieties

20 In certain embodiments, compounds of the disclosure comprise one or more modified nucleosides comprising a modified sugar moiety. Such compounds comprising one or more sugar-modified nucleosides may have desirable properties, such as enhanced nuclease stability or increased binding affinity with a target nucleic acid relative to an oligonucleotide comprising only nucleosides comprising naturally occurring sugar moieties. In certain embodiments, modified sugar moieties are substituted sugar moieties. In certain
25 embodiments, modified sugar moieties are sugar surrogates. Such sugar surrogates may comprise one or more substitutions corresponding to those of substituted sugar moieties.

In certain embodiments, modified sugar moieties are substituted sugar moieties comprising one or more non-bridging sugar substituent, including but not limited to substituents at the 2' and/or 5' positions. Examples of sugar substituents suitable for the 2'-position, include, but are not limited to: 2'-F, 2'-OCH₃ ("OMe" or "O-methyl"), and 2'-O(CH₂)₂OCH₃ ("MOE"). In certain embodiments, sugar substituents at the 2' position is selected from allyl, amino, azido, thio, O-allyl, O-C₁-C₁₀ alkyl, O-C₁-C₁₀ substituted alkyl; OCF₃, O(CH₂)₂SCH₃, O(CH₂)₂-O-N(R_m)(R_n), and O-CH₂-C(=O)-N(R_m)(R_n), where each R_m and R_n is, independently, H or substituted or unsubstituted C₁-C₁₀ alkyl. Examples of sugar substituents at the 5'
35 position, include, but are not limited to: 5'-methyl (R or S); 5'-vinyl, and 5'-methoxy. In certain embodiments, substituted sugars comprise more than one non-bridging sugar substituent, for example, 2'-F-

5'-methyl sugar moieties (*see, e.g.*, PCT International Application WO 2008/101157, for additional 5', 2'-bis substituted sugar moieties and nucleosides).

Nucleosides comprising 2'-substituted sugar moieties are referred to as 2'-substituted nucleosides. In certain embodiments, a 2'-substituted nucleoside comprises a 2'-substituent group selected from halo, allyl, amino, azido, SH, CN, OCN, CF₃, OCF₃, O, S, or N(R_m)-alkyl; O, S, or N(R_m)-alkenyl; O, S or N(R_m)-alkynyl; O-alkylenyl-O-alkyl, alkynyl, alkaryl, aralkyl, O-alkaryl, O-aralkyl, O(CH₂)₂SCH₃, O-(CH₂)₂-O-N(R_m)(R_n) or O-CH₂-C(=O)-N(R_m)(R_n), where each R_m and R_n is, independently, H, an amino protecting group or substituted or unsubstituted C₁-C₁₀ alkyl. These 2'-substituent groups can be further substituted with one or more substituent groups independently selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro (NO₂), thiol, thioalkoxy (S-alkyl), halogen, alkyl, aryl, alkenyl and alkynyl.

In certain embodiments, a 2'-substituted nucleoside comprises a 2'-substituent group selected from F, NH₂, N₃, OCF₃, O-CH₃, O(CH₂)₃NH₂, CH₂-CH=CH₂, O-CH₂-CH=CH₂, OCH₂CH₂OCH₃, O(CH₂)₂SCH₃, O-(CH₂)₂-O-N(R_m)(R_n), O(CH₂)₂O(CH₂)₂N(CH₃)₂, and N-substituted acetamide (O-CH₂-C(=O)-N(R_m)(R_n)) where each R_m and R_n is, independently, H, an amino protecting group or substituted or unsubstituted C₁-C₁₀ alkyl.

In certain embodiments, a 2'-substituted nucleoside comprises a sugar moiety comprising a 2'-substituent group selected from F, OCF₃, O-CH₃, OCH₂CH₂OCH₃, O(CH₂)₂SCH₃, O-(CH₂)₂-O-N(CH₃)₂, -O(CH₂)₂O(CH₂)₂N(CH₃)₂, and O-CH₂-C(=O)-N(H)CH₃.

In certain embodiments, a 2'-substituted nucleoside comprises a sugar moiety comprising a 2'-substituent group selected from F, O-CH₃, and OCH₂CH₂OCH₃.

Certain modified sugar moieties comprise a bridging sugar substituent that forms a second ring resulting in a bicyclic sugar moiety. In certain such embodiments, the bicyclic sugar moiety comprises a bridge between the 4' and the 2' furanose ring atoms. Examples of such 4' to 2' sugar substituents, include, but are not limited to: -[C(R_a)(R_b)]_n-, -[C(R_a)(R_b)]_n-O-, -C(R_aR_b)-N(R)-O- or, -C(R_aR_b)-O-N(R)-; 4'-CH₂-2', 4'-(CH₂)₂-2', 4'-(CH₂)₃-2', 4'-(CH₂)-O-2' (LNA); 4'-(CH₂)-S-2'; 4'-(CH₂)₂-O-2' (ENA); 4'-CH(CH₃)-O-2' (cEt) and 4'-CH(CH₂OCH₃)-O-2', and analogs thereof (*see, e.g.*, U.S. Patent 7,399,845, issued on July 15, 2008); 4'-C(CH₃)(CH₃)-O-2' and analogs thereof, (*see, e.g.*, WO2009/006478, published January 8, 2009); 4'-CH₂-N(OCH₃)-2' and analogs thereof (*see, e.g.*, WO2008/150729, published December 11, 2008); 4'-CH₂-O-N(CH₃)-2' (*see, e.g.*, US2004/0171570, published September 2, 2004); 4'-CH₂-O-N(R)-2', and 4'-CH₂-N(R)-O-2', wherein each R is, independently, H, a protecting group, or C₁-C₁₂ alkyl; 4'-CH₂-N(R)-O-2', wherein R is H, C₁-C₁₂ alkyl, or a protecting group (*see, U.S. Patent 7,427,672*, issued on September 23, 2008); 4'-CH₂-C(H)(CH₃)-2' (*see, e.g.*, Chattopadhyaya, *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂-C(=CH₂)-2' and analogs thereof (*see, published PCT International Application WO 2008/154401*, published on December 8, 2008).

In certain embodiments, such 4' to 2' bridges independently comprise from 1 to 4 linked groups independently selected from $-[C(R_a)(R_b)]_n-$, $-C(R_a)=C(R_b)-$, $-C(R_a)=N-$, $-C(=NR_a)-$, $-C(=O)-$, $-C(=S)-$, $-O-$, $-Si(R_a)_2-$, $-S(=O)_x-$, and $-N(R_a)-$;

wherein:

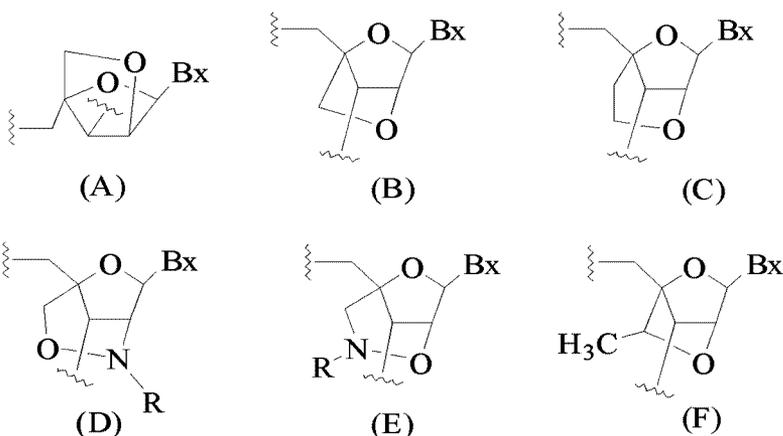
5 x is 0, 1, or 2;

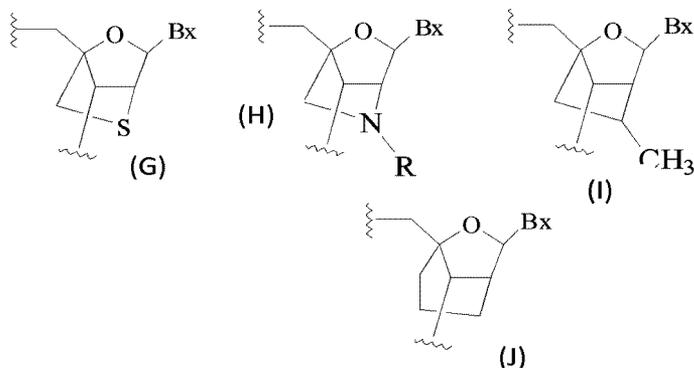
n is 1, 2, 3, or 4;

each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl,
 10 C_5 - C_7 alicyclic radical, substituted C_5 - C_7 alicyclic radical, halogen, OJ_1 , NJ_1J_2 , SJ_1 , N_3 , $COOJ_1$, acyl ($C(=O)-H$), substituted acyl, CN, sulfonyl ($S(=O)_2-J_1$), or sulfoxyl ($S(=O)-J_1$); and

each J_1 and J_2 is, independently, H, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, acyl ($C(=O)-H$), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1 - C_{12} aminoalkyl, substituted
 15 C_1 - C_{12} aminoalkyl, or a protecting group.

Nucleosides comprising bicyclic sugar moieties are referred to as bicyclic nucleosides or BNAs. Bicyclic nucleosides include, but are not limited to, (A) α -L-Methyleneoxy ($4'-CH_2-O-2'$) BNA, (B) β -D-Methyleneoxy ($4'-CH_2-O-2'$) BNA (also referred to as locked nucleic acid or LNA), (C) Ethyleneoxy ($4'-(CH_2)_2-O-2'$) BNA, (D) Aminooxy ($4'-CH_2-O-N(R)-2'$) BNA, (E) Oxyamino ($4'-CH_2-N(R)-O-2'$) BNA, (F)
 20 Methyl(methyleneoxy) ($4'-CH(CH_3)-O-2'$) BNA (also referred to as constrained ethyl or cEt), (G) methylene-thio ($4'-CH_2-S-2'$) BNA, (H) methylene-amino ($4'-CH_2-N(R)-2'$) BNA, (I) methyl carbocyclic ($4'-CH_2-CH(CH_3)-2'$) BNA, and (J) propylene carbocyclic ($4'-(CH_2)_3-2'$) BNA as depicted below.





wherein Bx is a nucleobase moiety and R is, independently, H, a protecting group, or C₁-C₁₂ alkyl.

Additional bicyclic sugar moieties are known in the art, for example: Singh et al., *Chem. Commun.*, 1998, 4, 455-456; Koshkin et al., *Tetrahedron*, 1998, 54, 3607-3630; Wahlestedt et al., *Proc. Natl. Acad. Sci. U. S. A.*, 2000, 97, 5633-5638; Kumar et al., *Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222; Singh et al., *J. Org. Chem.*, 1998, 63, 10035-10039; Srivastava et al., *J. Am. Chem. Soc.*, 129(26) 8362-8379 (Jul. 4, 2007); Elayadi et al., *Curr. Opinion Invens. Drugs*, 2001, 2, 558-561; Braasch et al., *Chem. Biol.*, 2001, 8, 1-7; Orum et al., *Curr. Opinion Mol. Ther.*, 2001, 3, 239-243; U.S. Patent Nos. 7,053,207, 6,268,490, 6,770,748, 6,794,499, 7,034,133, 6,525,191, 6,670,461, and 7,399,845; WO 2004/106356, WO 1994/14226, WO 2005/021570, and WO 2007/134181; U.S. Patent Publication Nos. US2004/0171570, US2007/0287831, and US2008/0039618; U.S. Patent Serial Nos. 12/129,154, 60/989,574, 61/026,995, 61/026,998, 61/056,564, 61/086,231, 61/097,787, and 61/099,844; and PCT International Applications Nos. PCT/US2008/064591, PCT/US2008/066154, and PCT/US2008/068922.

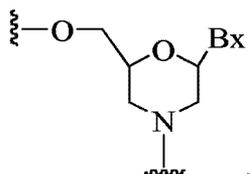
In certain embodiments, bicyclic sugar moieties and nucleosides incorporating such bicyclic sugar moieties are further defined by isomeric configuration. For example, a nucleoside comprising a 4'-2' methylene-oxy bridge, may be in the α -L configuration or in the β -D configuration. Previously, α -L-methyleneoxy (4'-CH₂-O-2') bicyclic nucleosides have been incorporated into antisense oligonucleotides that showed antisense activity (Frieden et al., *Nucleic Acids Research*, 2003, 21, 6365-6372).

In certain embodiments, substituted sugar moieties comprise one or more non-bridging sugar substituent and one or more bridging sugar substituent (e.g., 5'-substituted and 4'-2' bridged sugars). (see, PCT International Application WO 2007/134181, published on 11/22/07, wherein LNA is substituted with, for example, a 5'-methyl or a 5'-vinyl group).

In certain embodiments, modified sugar moieties are sugar surrogates. In certain such embodiments, the oxygen atom of the naturally occurring sugar is substituted, e.g., with a sulfur, carbon or nitrogen atom. In certain such embodiments, such modified sugar moiety also comprises bridging and/or non-bridging substituents as described above. For example, certain sugar surrogates comprise a 4'-sulfur atom and a substitution at the 2'-position (see, e.g., published U.S. Patent Application US2005/0130923, published on

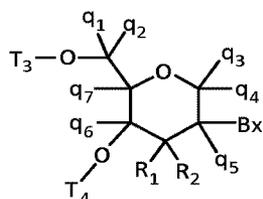
June 16, 2005) and/or the 5' position. By way of additional example, carbocyclic bicyclic nucleosides having a 4'-2' bridge have been described (*see, e.g., Freier et al., Nucleic Acids Research, 1997, 25(22), 4429-4443 and Albaek et al., J. Org. Chem., 2006, 71, 7731-7740*).

In certain embodiments, sugar surrogates comprise rings having other than 5-atoms. For example, in certain embodiments, a sugar surrogate comprises a morpholino. Morpholino compounds and their use in oligomeric compounds has been reported in numerous patents and published articles (*see for example: Braasch et al., Biochemistry, 2002, 41, 4503-4510; and U.S. Patents 5,698,685; 5,166,315; 5,185,444; and 5,034,506*). As used here, the term "morpholino" means a sugar surrogate having the following structure:



In certain embodiments, morpholinos may be modified, for example by adding or altering various substituent groups from the above morpholino structure. Such sugar surrogates are referred to herein as "modified morpholinos."

For another example, in certain embodiments, a sugar surrogate comprises a six-membered tetrahydropyran. Such tetrahydropyrans may be further modified or substituted. Nucleosides comprising such modified tetrahydropyrans include, but are not limited to, hexitol nucleic acid (HNA), antitol nucleic acid (ANA), manitol nucleic acid (MNA) (*see Leumann, CJ. Bioorg. & Med. Chem. (2002) 10:841-854*), fluoro HNA (F-HNA), and those compounds having Formula VI:



VI

wherein independently for each of said at least one tetrahydropyran nucleoside analog of Formula VI:

Bx is a nucleobase moiety;

T₃ and T₄ are each, independently, an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound or one of T₃ and T₄ is an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound and the other of T₃ and T₄ is H, a hydroxyl protecting group, a linked conjugate group, or a 5' or 3'-terminal group;

q₁, q₂, q₃, q₄, q₅, q₆ and q₇ are each, independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl, or substituted C₂-C₆ alkynyl; and

each of R₁ and R₂ is independently selected from among: hydrogen, halogen, substituted or unsubstituted alkoxy, NJ₁J₂, SJ₁, N₃, OC(=X)J₁, OC(=X)NJ₁J₂, NJ₃C(=X)NJ₁J₂, and CN, wherein X is O, S or NJ₁, and each J₁, J₂, and J₃ is, independently, H or C₁-C₆ alkyl.

In certain embodiments, the modified THP nucleosides of Formula VI are provided wherein q₁, q₂, q₃, q₄, q₅, q₆ and q₇ are each H. In certain embodiments, at least one of q₁, q₂, q₃, q₄, q₅, q₆ and q₇ is other than H. In certain embodiments, at least one of q₁, q₂, q₃, q₄, q₅, q₆ and q₇ is methyl. In certain embodiments, THP nucleosides of Formula VI are provided wherein one of R₁ and R₂ is F. In certain embodiments, R₁ is fluoro and R₂ is H, R₁ is methoxy and R₂ is H, and R₁ is methoxyethoxy and R₂ is H.

Many other bicyclo and tricyclo sugar surrogate ring systems are also known in the art that can be used to modify nucleosides for incorporation into antisense compounds (*see, e.g.*, review article: Leumann, J. C, *Bioorganic & Medicinal Chemistry*, **2002**, *10*, 841-854).

Combinations of modifications are also provided without limitation, such as 2'-F-5'-methyl substituted nucleosides (see PCT International Application WO 2008/101157 Published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides) and replacement of the ribosyl ring oxygen atom with S and further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a bicyclic nucleic acid (see PCT International Application WO 2007/134181, published on 11/22/07 wherein a 4'-CH₂-O-2' bicyclic nucleoside is further substituted at the 5' position with a 5'-methyl or a 5'-vinyl group). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (*see, e.g.*, Srivastava *et al.*, *J. Am. Chem. Soc.* 2007, *129*(26), 8362-8379).

In certain embodiments, the present disclosure provides oligonucleotides comprising modified nucleosides. Those modified nucleotides may include modified sugars, modified nucleobases, and/or modified linkages. The specific modifications are selected such that the resulting oligonucleotides possess desirable characteristics. In certain embodiments, oligonucleotides comprise one or more RNA-like nucleosides. In certain embodiments, oligonucleotides comprise one or more DNA-like nucleotides.

2. Certain Nucleobase Modifications

In certain embodiments, nucleosides of the present disclosure comprise one or more unmodified nucleobases. In certain embodiments, nucleosides of the present disclosure comprise one or more modified nucleobases.

In certain embodiments, modified nucleobases are selected from: universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases as defined herein. 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil; 5-propynylcytosine; 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C≡C-CH₃) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and

thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine, 3-deazaguanine and 3-deazaadenine, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases as defined herein. Further modified nucleobases include tricyclic pyrimidines such as phenoxazine cytidine([5,4-b][1,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido[5,4-b][1,4]benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (e.g. 9-(2-aminoethoxy)-H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5-b]indol-2-one), pyridoindole cytidine (H-pyrido[3',2':4,5]pyrrolo[2,3-d]pyrimidin-2-one). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in United States Patent No. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, Kroschwitz, J.I., Ed., John Wiley & Sons, 1990, 858-859; those disclosed by Englisch *et al.*, *Angewandte Chemie*, International Edition, 1991, 30, 613; and those disclosed by Sanghvi, Y.S., Chapter 15, *Antisense Research and Applications*, Crooke, S.T. and Lebleu, B., Eds., CRC Press, 1993, 273-288.

Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include without limitation, U.S. 3,687,808; 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121; 5,596,091; 5,614,617; 5,645,985; 5,681,941; 5,750,692; 5,763,588; 5,830,653 and 6,005,096, certain of which are commonly owned with the instant application.

3. Certain Internucleoside Linkages

In certain embodiments, the present disclosure provides oligonucleotides comprising linked nucleosides. In such embodiments, nucleosides may be linked together using any internucleoside linkage. The two main classes of internucleoside linking groups are defined by the presence or absence of a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters (PO), phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioates (PS). Representative non-phosphorus containing internucleoside linking groups include, but are not limited to, methylenemethylimino (-CH₂-N(CH₃)-O-CH₂-), thiodiester (-O-C(O)-S-), thionocarbamate (-O-C(O)(NH)-S-); siloxane (-O-Si(H)₂-O-); and N,N'-dimethylhydrazine (-CH₂-N(CH₃)-N(CH₃-). Modified linkages, compared to natural phosphodiester linkages, can be used to alter, typically increase, nuclease resistance of the oligonucleotide. In certain embodiments, internucleoside linkages having a chiral atom can be prepared as a racemic mixture, or as separate enantiomers. Representative chiral linkages include, but are not limited to, alkylphosphonates and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing internucleoside linkages are well known to those skilled in the art.

The oligonucleotides described herein contain one or more asymmetric centers and thus give rise to enantiomers, diastereomers, and other stereoisomeric configurations that may be defined, in terms of absolute stereochemistry, as (R) or (S), α or β such as for sugar anomers, or as (D) or (L) such as for amino acids etc. Included in the antisense compounds provided herein are all such possible isomers, as well as their racemic and optically pure forms.

Neutral internucleoside linkages include without limitation, phosphotriesters, methylphosphonates, MMI (3'-CH₂-N(CH₃)-O-5'), amide-3 (3'-CH₂-C(=O)-N(H)-5'), amide-4 (3'-CH₂-N(H)-C(=O)-5'), formacetal (3'-O-CH₂-O-5'), and thioformacetal (3'-S-CH₂-O-5'). Further neutral internucleoside linkages include nonionic linkages comprising siloxane (dialkylsiloxane), carboxylate ester, carboxamide, sulfide, sulfonate ester and amides (See for example: *Carbohydrate Modifications in Antisense Research*; Y.S. Sanghvi and P.D. Cook, Eds., ACS Symposium Series 580; Chapters 3 and 4, 40-65). Further neutral internucleoside linkages include nonionic linkages comprising mixed N, O, S and CH₂ component parts.

4. Certain Motifs

In certain embodiments, antisense oligonucleotides comprise one or more modified nucleoside (e.g., nucleoside comprising a modified sugar and/or modified nucleobase) and/or one or more modified internucleoside linkage. The pattern of such modifications on an oligonucleotide is referred to herein as a motif. In certain embodiments, sugar, nucleobase, and linkage motifs are independent of one another.

a. Certain sugar motifs

In certain embodiments, oligonucleotides comprise one or more type of modified sugar moieties and/or naturally occurring sugar moieties arranged along an oligonucleotide or region thereof in a defined pattern or sugar modification motif. Such motifs may include any of the sugar modifications discussed herein and/or other known sugar modifications.

In certain embodiments, the oligonucleotides comprise or consist of a region having a gapmer sugar motif, which comprises two external regions or "wings" and a central or internal region or "gap." The three regions of a gapmer sugar motif (the 5'-wing, the gap, and the 3'-wing) form a contiguous sequence of nucleosides wherein at least some of the sugar moieties of the nucleosides of each of the wings differ from at least some of the sugar moieties of the nucleosides of the gap. Specifically, at least the sugar moieties of the nucleosides of each wing that are closest to the gap (the 3'-most nucleoside of the 5'-wing and the 5'-most nucleoside of the 3'-wing) differ from the sugar moiety of the neighboring gap nucleosides, thus defining the boundary between the wings and the gap. In certain embodiments, the sugar moieties within the gap are the same as one another. In certain embodiments, the gap includes one or more nucleoside having a sugar moiety that differs from the sugar moiety of one or more other nucleosides of the gap. In certain embodiments, the sugar motifs of the two wings are the same as one another (symmetric sugar gapmer). In certain embodiments, the sugar motifs of the 5'-wing differs from the sugar motif of the 3'-wing (asymmetric sugar gapmer).

i. Certain 5'-wings

In certain embodiments, the 5'- wing of a gapmer consists of 1 to 8 linked nucleosides. In certain
embodiments, the 5'- wing of a gapmer consists of 1 to 7 linked nucleosides. In certain embodiments, the 5'-
wing of a gapmer consists of 1 to 6 linked nucleosides. In certain embodiments, the 5'- wing of a gapmer
consists of 1 to 5 linked nucleosides. In certain embodiments, the 5'- wing of a gapmer consists of 2 to 5
5 linked nucleosides. In certain embodiments, the 5'- wing of a gapmer consists of 3 to 5 linked nucleosides.
In certain embodiments, the 5'- wing of a gapmer consists of 4 or 5 linked nucleosides. In certain
embodiments, the 5'- wing of a gapmer consists of 1 to 4 linked nucleosides. In certain embodiments, the 5'-
wing of a gapmer consists of 1 to 3 linked nucleosides. In certain embodiments, the 5'- wing of a gapmer
consists of 1 or 2 linked nucleosides. In certain embodiments, the 5'- wing of a gapmer consists of 2 to 4
10 linked nucleosides. In certain embodiments, the 5'- wing of a gapmer consists of 2 or 3 linked nucleosides.
In certain embodiments, the 5'- wing of a gapmer consists of 3 or 4 linked nucleosides. In certain
embodiments, the 5'- wing of a gapmer consists of 1 nucleoside. In certain embodiments, the 5'- wing of a
gapmer consists of 2 linked nucleosides. In certain embodiments, the 5'- wing of a gapmer consists of 3
linked nucleosides. In certain embodiments, the 5'- wing of a gapmer consists of 4 linked nucleosides. In
15 certain embodiments, the 5'- wing of a gapmer consists of 5 linked nucleosides. In certain embodiments, the
5'- wing of a gapmer consists of 6 linked nucleosides.

In certain embodiments, the 5'- wing of a gapmer comprises at least one bicyclic nucleoside. In
certain embodiments, the 5'- wing of a gapmer comprises at least two bicyclic nucleosides. In certain
embodiments, the 5'- wing of a gapmer comprises at least three bicyclic nucleosides. In certain
20 embodiments, the 5'- wing of a gapmer comprises at least four bicyclic nucleosides. In certain embodiments,
the 5'- wing of a gapmer comprises at least one constrained ethyl nucleoside. In certain embodiments, the 5'-
wing of a gapmer comprises at least one LNA nucleoside. In certain embodiments, each nucleoside of the 5'-
wing of a gapmer is a bicyclic nucleoside. In certain embodiments, each nucleoside of the 5'- wing of a
gapmer is a constrained ethyl nucleoside. In certain embodiments, each nucleoside of the 5'- wing of a
25 gapmer is a LNA nucleoside.

In certain embodiments, the 5'- wing of a gapmer comprises at least one non-bicyclic modified
nucleoside. In certain embodiments, the 5'- wing of a gapmer comprises at least one 2'-substituted
nucleoside. In certain embodiments, the 5'- wing of a gapmer comprises at least one 2'-MOE nucleoside. In
certain embodiments, the 5'- wing of a gapmer comprises at least one 2'-OMe nucleoside. In certain
30 embodiments, each nucleoside of the 5'- wing of a gapmer is a non-bicyclic modified nucleoside. In certain
embodiments, each nucleoside of the 5'- wing of a gapmer is a 2'-substituted nucleoside. In certain
embodiments, each nucleoside of the 5'- wing of a gapmer is a 2'-MOE nucleoside. In certain embodiments,
each nucleoside of the 5'- wing of a gapmer is a 2'-OMe nucleoside.

In certain embodiments, the 5'- wing of a gapmer comprises at least one 2'-deoxynucleoside. In
35 certain embodiments, each nucleoside of the 5'- wing of a gapmer is a 2'-deoxynucleoside. In a certain
embodiments, the 5'- wing of a gapmer comprises at least one ribonucleoside. In certain embodiments, each

nucleoside of the 5'- wing of a gapmer is a ribonucleoside. In certain embodiments, one, more than one, or each of the nucleosides of the 5'- wing is an RNA-like nucleoside.

In certain embodiments, the 5'-wing of a gapmer comprises at least one bicyclic nucleoside and at least one non-bicyclic modified nucleoside. In certain embodiments, the 5'-wing of a gapmer comprises at least one bicyclic nucleoside and at least one 2'-substituted nucleoside. In certain embodiments, the 5'-wing of a gapmer comprises at least one bicyclic nucleoside and at least one 2'-MOE nucleoside. In certain embodiments, the 5'-wing of a gapmer comprises at least one bicyclic nucleoside and at least one 2'-OMe nucleoside. In certain embodiments, the 5'-wing of a gapmer comprises at least one bicyclic nucleoside and at least one 2'-deoxynucleoside.

In certain embodiments, the 5'-wing of a gapmer comprises at least one constrained ethyl nucleoside and at least one non-bicyclic modified nucleoside. In certain embodiments, the 5'-wing of a gapmer comprises at least one constrained ethyl nucleoside and at least one 2'-substituted nucleoside. In certain embodiments, the 5'-wing of a gapmer comprises at least one constrained ethyl nucleoside and at least one 2'-MOE nucleoside. In certain embodiments, the 5'-wing of a gapmer comprises at least one constrained ethyl nucleoside and at least one 2'-OMe nucleoside. In certain embodiments, the 5'-wing of a gapmer comprises at least one constrained ethyl nucleoside and at least one 2'-deoxynucleoside.

ii. Certain 3'-wings

In certain embodiments, the 3'- wing of a gapmer consists of 1 to 8 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 1 to 7 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 1 to 6 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 1 to 5 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 2 to 5 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 3 to 5 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 4 or 5 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 1 to 4 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 1 to 3 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 1 or 2 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 2 to 4 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 2 or 3 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 3 or 4 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 1 nucleoside. In certain embodiments, the 3'- wing of a gapmer consists of 2 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 3 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 4 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 5 linked nucleosides. In certain embodiments, the 3'- wing of a gapmer consists of 6 linked nucleosides.

In certain embodiments, the 3'- wing of a gapmer comprises at least one bicyclic nucleoside. In certain embodiments, the 3'- wing of a gapmer comprises at least one constrained ethyl nucleoside. In certain embodiments, the 3'- wing of a gapmer comprises at least one LNA nucleoside. In certain embodiments,

each nucleoside of the 3'- wing of a gapmer is a bicyclic nucleoside. In certain embodiments, each nucleoside of the 3'- wing of a gapmer is a constrained ethyl nucleoside. In certain embodiments, each nucleoside of the 3'- wing of a gapmer is a LNA nucleoside.

In certain embodiments, the 3'- wing of a gapmer comprises at least one non-bicyclic modified nucleoside. In certain embodiments, the 3'- wing of a gapmer comprises at least two non-bicyclic modified nucleosides. In certain embodiments, the 3'- wing of a gapmer comprises at least three non-bicyclic modified nucleosides. In certain embodiments, the 3'- wing of a gapmer comprises at least four non-bicyclic modified nucleosides. In certain embodiments, the 3'- wing of a gapmer comprises at least one 2'-substituted nucleoside. In certain embodiments, the 3'- wing of a gapmer comprises at least one 2'-MOE nucleoside. In certain embodiments, the 3'- wing of a gapmer comprises at least one 2'-OMe nucleoside. In certain embodiments, each nucleoside of the 3'- wing of a gapmer is a non-bicyclic modified nucleoside. In certain embodiments, each nucleoside of the 3'- wing of a gapmer is a 2'-substituted nucleoside. In certain embodiments, each nucleoside of the 3'- wing of a gapmer is a 2'-MOE nucleoside. In certain embodiments, each nucleoside of the 3'- wing of a gapmer is a 2'-OMe nucleoside.

In certain embodiments, the 3'- wing of a gapmer comprises at least one 2'-deoxynucleoside. In certain embodiments, each nucleoside of the 3'- wing of a gapmer is a 2'-deoxynucleoside. In a certain embodiments, the 3'- wing of a gapmer comprises at least one ribonucleoside. In certain embodiments, each nucleoside of the 3'- wing of a gapmer is a ribonucleoside. In certain embodiments, one, more than one, or each of the nucleosides of the 5'- wing is an RNA-like nucleoside.

In certain embodiments, the 3'-wing of a gapmer comprises at least one bicyclic nucleoside and at least one non-bicyclic modified nucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one bicyclic nucleoside and at least one 2'-substituted nucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one bicyclic nucleoside and at least one 2'-MOE nucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one bicyclic nucleoside and at least one 2'-OMe nucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one bicyclic nucleoside and at least one 2'-deoxynucleoside.

In certain embodiments, the 3'-wing of a gapmer comprises at least one constrained ethyl nucleoside and at least one non-bicyclic modified nucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one constrained ethyl nucleoside and at least one 2'-substituted nucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one constrained ethyl nucleoside and at least one 2'-MOE nucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one constrained ethyl nucleoside and at least one 2'-OMe nucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one constrained ethyl nucleoside and at least one 2'-deoxynucleoside.

In certain embodiments, the 3'-wing of a gapmer comprises at least one LNA nucleoside and at least one non-bicyclic modified nucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one LNA nucleoside and at least one 2'-substituted nucleoside. In certain embodiments, the 3'-wing of a

gapmer comprises at least one LNA nucleoside and at least one 2'-MOE nucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one LNA nucleoside and at least one 2'-OMe nucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one LNA nucleoside and at least one 2'-deoxynucleoside.

5 In certain embodiments, the 3'-wing of a gapmer comprises at least one bicyclic nucleoside, at least one non-bicyclic modified nucleoside, and at least one 2'-deoxynucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one constrained ethyl nucleoside, at least one non-bicyclic modified nucleoside, and at least one 2'-deoxynucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one LNA nucleoside, at least one non-bicyclic modified nucleoside, and at least one 2'-
10 deoxynucleoside.

In certain embodiments, the 3'-wing of a gapmer comprises at least one bicyclic nucleoside, at least one 2'-substituted nucleoside, and at least one 2'-deoxynucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one constrained ethyl nucleoside, at least one 2'-substituted nucleoside, and at least one 2'-deoxynucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one LNA
15 nucleoside, at least one 2'-substituted nucleoside, and at least one 2'-deoxynucleoside.

In certain embodiments, the 3'-wing of a gapmer comprises at least one bicyclic nucleoside, at least one 2'-MOE nucleoside, and at least one 2'-deoxynucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one constrained ethyl nucleoside, at least one 2'-MOE nucleoside, and at least one 2'-deoxynucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one LNA
20 nucleoside, at least one 2'-MOE nucleoside, and at least one 2'-deoxynucleoside.

In certain embodiments, the 3'-wing of a gapmer comprises at least one bicyclic nucleoside, at least one 2'-OMe nucleoside, and at least one 2'-deoxynucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one constrained ethyl nucleoside, at least one 2'-OMe nucleoside, and at least one 2'-deoxynucleoside. In certain embodiments, the 3'-wing of a gapmer comprises at least one LNA
25 nucleoside, at least one 2'-OMe nucleoside, and at least one 2'-deoxynucleoside.

iii. Certain Central Regions (gaps)

In certain embodiments, the gap of a gapmer consists of 6 to 20 linked nucleosides. In certain embodiments, the gap of a gapmer consists of 6 to 15 linked nucleosides. In certain embodiments, the gap of a gapmer consists of 6 to 12 linked nucleosides. In certain embodiments, the gap of a gapmer consists of 6 to
30 10 linked nucleosides. In certain embodiments, the gap of a gapmer consists of 6 to 9 linked nucleosides. In certain embodiments, the gap of a gapmer consists of 6 to 8 linked nucleosides. In certain embodiments, the gap of a gapmer consists of 6 or 7 linked nucleosides. In certain embodiments, the gap of a gapmer consists of 7 to 10 linked nucleosides. In certain embodiments, the gap of a gapmer consists of 7 to 9 linked nucleosides. In certain embodiments, the gap of a gapmer consists of 7 or 8 linked nucleosides. In certain
35 embodiments, the gap of a gapmer consists of 8 to 10 linked nucleosides. In certain embodiments, the gap of a gapmer consists of 8 or 9 linked nucleosides. In certain embodiments, the gap of a gapmer consists of 6

linked nucleosides. In certain embodiments, the gap of a gapmer consists of 7 linked nucleosides. In certain
embodiments, the gap of a gapmer consists of 8 linked nucleosides. In certain embodiments, the gap of a
gapmer consists of 9 linked nucleosides. In certain embodiments, the gap of a gapmer consists of 10 linked
nucleosides. In certain embodiments, the gap of a gapmer consists of 11 linked nucleosides. In certain
5 embodiments, the gap of a gapmer consists of 12 linked nucleosides.

In certain embodiments, each nucleoside of the gap of a gapmer is a 2'-deoxynucleoside. In certain
embodiments, the gap comprises one or more modified nucleosides. In certain embodiments, each nucleoside
of the gap of a gapmer is a 2'-deoxynucleoside or is a modified nucleoside that is "DNA-like." In such
embodiments, "DNA-like" means that the nucleoside has similar characteristics to DNA, such that a duplex
10 comprising the gapmer and an RNA molecule is capable of activating RNase H. For example, under certain
conditions, 2'-(ara)-F have been shown to support RNase H activation, and thus is DNA-like. In certain
embodiments, one or more nucleosides of the gap of a gapmer is not a 2'-deoxynucleoside and is not DNA-
like. In certain such embodiments, the gapmer nonetheless supports RNase H activation (e.g., by virtue of
the number or placement of the non-DNA nucleosides).

15 In certain embodiments, gaps comprise a stretch of unmodified 2'-deoxynucleoside interrupted by
one or more modified nucleosides, thus resulting in three sub-regions (two stretches of one or more 2'-
deoxynucleosides and a stretch of one or more interrupting modified nucleosides). In certain embodiments,
no stretch of unmodified 2'-deoxynucleosides is longer than 5, 6, or 7 nucleosides. In certain embodiments,
such short stretches is achieved by using short gap regions. In certain embodiments, short stretches are
20 achieved by interrupting a longer gap region.

In certain embodiments, the gap comprises one or more modified nucleosides. In certain
embodiments, the gap comprises one or more modified nucleosides selected from among cEt, FHNA, LNA,
and 2-thio-thymidine. In certain embodiments, the gap comprises one modified nucleoside. In certain
embodiments, the gap comprises a 5'-substituted sugar moiety selected from among 5'-Me, and 5'-(R)-Me.
25 In certain embodiments, the gap comprises two modified nucleosides. In certain embodiments, the gap
comprises three modified nucleosides. In certain embodiments, the gap comprises four modified nucleosides.
In certain embodiments, the gap comprises two or more modified nucleosides and each modified nucleoside
is the same. In certain embodiments, the gap comprises two or more modified nucleosides and each modified
nucleoside is different.

30 In certain embodiments, the gap comprises one or more modified linkages. In certain embodiments,
the gap comprises one or more methyl phosphonate linkages. In certain embodiments the gap comprises two
or more modified linkages. In certain embodiments, the gap comprises one or more modified linkages and
one or more modified nucleosides. In certain embodiments, the gap comprises one modified linkage and one
modified nucleoside. In certain embodiments, the gap comprises two modified linkages and two or more
35 modified nucleosides.

b. Certain Internucleoside Linkage Motifs

In certain embodiments, oligonucleotides comprise modified internucleoside linkages arranged along the oligonucleotide or region thereof in a defined pattern or modified internucleoside linkage motif. In certain embodiments, oligonucleotides comprise a region having an alternating internucleoside linkage motif.

5 In certain embodiments, oligonucleotides of the present disclosure comprise a region of uniformly modified internucleoside linkages. In certain such embodiments, the oligonucleotide comprises a region that is uniformly linked by phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide is uniformly linked by phosphorothioate internucleoside linkages. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate. In
10 certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate and at least one internucleoside linkage is phosphorothioate.

In certain embodiments, the oligonucleotide comprises at least 6 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 7 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 8 phosphorothioate internucleoside
15 linkages. In certain embodiments, the oligonucleotide comprises at least 9 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 10 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 11 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 12 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 13 phosphorothioate internucleoside
20 linkages. In certain embodiments, the oligonucleotide comprises at least 14 phosphorothioate internucleoside linkages.

In certain embodiments, the oligonucleotide comprises at least one block of at least 6 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 7 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the
25 oligonucleotide comprises at least one block of at least 8 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 9 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 10 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least block of at least one 12 consecutive phosphorothioate internucleoside
30 linkages. In certain such embodiments, at least one such block is located at the 3' end of the oligonucleotide. In certain such embodiments, at least one such block is located within 3 nucleosides of the 3' end of the oligonucleotide. In certain embodiments, the oligonucleotide comprises less than 15 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises less than 14 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises less than 13
35 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises less than 12 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises less

than 11 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises less than 10 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises less than 9 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises less than 8 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises less than 7 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises less than 6 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises less than 5 phosphorothioate internucleoside linkages.

c. Certain Nucleobase Modification Motifs

In certain embodiments, oligonucleotides comprise chemical modifications to nucleobases arranged along the oligonucleotide or region thereof in a defined pattern or nucleobases modification motif. In certain such embodiments, nucleobase modifications are arranged in a gapped motif. In certain embodiments, nucleobase modifications are arranged in an alternating motif. In certain embodiments, each nucleobase is modified. In certain embodiments, none of the nucleobases is chemically modified.

In certain embodiments, oligonucleotides comprise a block of modified nucleobases. In certain such embodiments, the block is at the 3'-end of the oligonucleotide. In certain embodiments the block is within 3 nucleotides of the 3'-end of the oligonucleotide. In certain such embodiments, the block is at the 5'-end of the oligonucleotide. In certain embodiments the block is within 3 nucleotides of the 5'-end of the oligonucleotide.

In certain embodiments, nucleobase modifications are a function of the natural base at a particular position of an oligonucleotide. For example, in certain embodiments each purine or each pyrimidine in an oligonucleotide is modified. In certain embodiments, each adenine is modified. In certain embodiments, each guanine is modified. In certain embodiments, each thymine is modified. In certain embodiments, each cytosine is modified. In certain embodiments, each uracil is modified.

In certain embodiments, some, all, or none of the cytosine moieties in an oligonucleotide are 5-methyl cytosine moieties. Herein, 5-methyl cytosine is not a "modified nucleobase." Accordingly, unless otherwise indicated, unmodified nucleobases include both cytosine residues having a 5-methyl and those lacking a 5 methyl. In certain embodiments, the methylation state of all or some cytosine nucleobases is specified.

In certain embodiments, chemical modifications to nucleobases comprise attachment of certain conjugate groups to nucleobases. In certain embodiments, each purine or each pyrimidine in an oligonucleotide may be optionally modified to comprise a conjugate group.

d. Certain Overall Lengths

In certain embodiments, the present disclosure provides oligonucleotides of any of a variety of ranges of lengths. In certain embodiments, oligonucleotides consist of X to Y linked nucleosides, where X represents the fewest number of nucleosides in the range and Y represents the largest number of nucleosides in the range. In certain such embodiments, X and Y are each independently selected from 8, 9, 10, 11, 12, 13,

14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50; provided that $X \leq Y$. For example, in certain embodiments, the oligonucleotide may consist of 8 to 9, 8 to 10, 8 to 11, 8 to 12, 8 to 13, 8 to 14, 8 to 15, 8 to 16, 8 to 17, 8 to 18, 8 to 19, 8 to 20, 8 to 21, 8 to 22, 8 to 23, 8 to 24, 8 to 25, 8 to 26, 8 to 27, 8 to 28, 8 to 29, 8 to 30, 9 to 10, 9 to 11, 9 to 12, 9 to 13, 9 to 14, 9 to 15, 9 to 16, 9 to 17, 9 to 18, 9 to 19, 9 to 20, 9 to 21, 9 to 22, 9 to 23, 9 to 24, 9 to 25, 9 to 26, 9 to 27, 9 to 28, 9 to 29, 9 to 30, 10 to 11, 10 to 12, 10 to 13, 10 to 14, 10 to 15, 10 to 16, 10 to 17, 10 to 18, 10 to 19, 10 to 20, 10 to 21, 10 to 22, 10 to 23, 10 to 24, 10 to 25, 10 to 26, 10 to 27, 10 to 28, 10 to 29, 10 to 30, 11 to 12, 11 to 13, 11 to 14, 11 to 15, 11 to 16, 11 to 17, 11 to 18, 11 to 19, 11 to 20, 11 to 21, 11 to 22, 11 to 23, 11 to 24, 11 to 25, 11 to 26, 11 to 27, 11 to 28, 11 to 29, 11 to 30, 12 to 13, 12 to 14, 12 to 15, 12 to 16, 12 to 17, 12 to 18, 12 to 19, 12 to 20, 12 to 21, 12 to 22, 12 to 23, 12 to 24, 12 to 25, 12 to 26, 12 to 27, 12 to 28, 12 to 29, 12 to 30, 13 to 14, 13 to 15, 13 to 16, 13 to 17, 13 to 18, 13 to 19, 13 to 20, 13 to 21, 13 to 22, 13 to 23, 13 to 24, 13 to 25, 13 to 26, 13 to 27, 13 to 28, 13 to 29, 13 to 30, 14 to 15, 14 to 16, 14 to 17, 14 to 18, 14 to 19, 14 to 20, 14 to 21, 14 to 22, 14 to 23, 14 to 24, 14 to 25, 14 to 26, 14 to 27, 14 to 28, 14 to 29, 14 to 30, 15 to 16, 15 to 17, 15 to 18, 15 to 19, 15 to 20, 15 to 21, 15 to 22, 15 to 23, 15 to 24, 15 to 25, 15 to 26, 15 to 27, 15 to 28, 15 to 29, 15 to 30, 16 to 17, 16 to 18, 16 to 19, 16 to 20, 16 to 21, 16 to 22, 16 to 23, 16 to 24, 16 to 25, 16 to 26, 16 to 27, 16 to 28, 16 to 29, 16 to 30, 17 to 18, 17 to 19, 17 to 20, 17 to 21, 17 to 22, 17 to 23, 17 to 24, 17 to 25, 17 to 26, 17 to 27, 17 to 28, 17 to 29, 17 to 30, 18 to 19, 18 to 20, 18 to 21, 18 to 22, 18 to 23, 18 to 24, 18 to 25, 18 to 26, 18 to 27, 18 to 28, 18 to 29, 18 to 30, 19 to 20, 19 to 21, 19 to 22, 19 to 23, 19 to 24, 19 to 25, 19 to 26, 19 to 27, 19 to 28, 19 to 29, 19 to 30, 20 to 21, 20 to 22, 20 to 23, 20 to 24, 20 to 25, 20 to 26, 20 to 27, 20 to 28, 20 to 29, 20 to 30, 21 to 22, 21 to 23, 21 to 24, 21 to 25, 21 to 26, 21 to 27, 21 to 28, 21 to 29, 21 to 30, 22 to 23, 22 to 24, 22 to 25, 22 to 26, 22 to 27, 22 to 28, 22 to 29, 22 to 30, 23 to 24, 23 to 25, 23 to 26, 23 to 27, 23 to 28, 23 to 29, 23 to 30, 24 to 25, 24 to 26, 24 to 27, 24 to 28, 24 to 29, 24 to 30, 25 to 26, 25 to 27, 25 to 28, 25 to 29, 25 to 30, 26 to 27, 26 to 28, 26 to 29, 26 to 30, 27 to 28, 27 to 29, 27 to 30, 28 to 29, 28 to 30, or 29 to 30 linked nucleosides. In embodiments where the number of nucleosides of an oligonucleotide of a compound is limited, whether to a range or to a specific number, the compound may, nonetheless further comprise additional other substituents. For example, an oligonucleotide comprising 8-30 nucleosides excludes oligonucleotides having 31 nucleosides, but, unless otherwise indicated, such an oligonucleotide may further comprise, for example one or more conjugate groups, terminal groups, or other substituents.

Further, where an oligonucleotide is described by an overall length range and by regions having specified lengths, and where the sum of specified lengths of the regions is less than the upper limit of the overall length range, the oligonucleotide may have additional nucleosides, beyond those of the specified regions, provided that the total number of nucleosides does not exceed the upper limit of the overall length range.

5. Certain Antisense Oligonucleotide Chemistry Motifs

In certain embodiments, the chemical structural features of antisense oligonucleotides are

characterized by their sugar motif, internucleoside linkage motif, nucleobase modification motif and overall length. In certain embodiments, such parameters are each independent of one another. Thus, each internucleoside linkage of an oligonucleotide having a gapmer sugar motif may be modified or unmodified and may or may not follow the gapmer modification pattern of the sugar modifications. Thus, the internucleoside linkages within the wing regions of a sugar-gapmer may be the same or different from one another and may be the same or different from the internucleoside linkages of the gap region. Likewise, such sugar-gapmer oligonucleotides may comprise one or more modified nucleobase independent of the gapmer pattern of the sugar modifications. One of skill in the art will appreciate that such motifs may be combined to create a variety of oligonucleotides.

In certain embodiments, the selection of internucleoside linkage and nucleoside modification are not independent of one another.

i. Certain Sequences and Targets

In certain embodiments, the invention provides antisense oligonucleotides having a sequence complementary to a target nucleic acid. Such antisense compounds are capable of hybridizing to a target nucleic acid, resulting in at least one antisense activity. In certain embodiments, antisense compounds specifically hybridize to one or more target nucleic acid. In certain embodiments, a specifically hybridizing antisense compound has a nucleobase sequence comprising a region having sufficient complementarity to a target nucleic acid to allow hybridization and result in antisense activity and insufficient complementarity to any non-target so as to avoid or reduce non-specific hybridization to non-target nucleic acid sequences under conditions in which specific hybridization is desired (e.g., under physiological conditions for *in vivo* or therapeutic uses, and under conditions in which assays are performed in the case of *in vitro* assays). In certain embodiments, oligonucleotides are selective between a target and non-target, even though both target and non-target comprise the target sequence. In such embodiments, selectivity may result from relative accessibility of the target region of one nucleic acid molecule compared to the other.

In certain embodiments, the present disclosure provides antisense compounds comprising oligonucleotides that are fully complementary to the target nucleic acid over the entire length of the oligonucleotide. In certain embodiments, oligonucleotides are 99% complementary to the target nucleic acid. In certain embodiments, oligonucleotides are 95% complementary to the target nucleic acid. In certain embodiments, such oligonucleotides are 90% complementary to the target nucleic acid.

In certain embodiments, such oligonucleotides are 85% complementary to the target nucleic acid. In certain embodiments, such oligonucleotides are 80% complementary to the target nucleic acid. In certain embodiments, an antisense compound comprises a region that is fully complementary to a target nucleic acid and is at least 80% complementary to the target nucleic acid over the entire length of the oligonucleotide. In certain such embodiments, the region of full complementarity is from 6 to 14 nucleobases in length.

In certain embodiments, oligonucleotides comprise a hybridizing region and a terminal region. In certain such embodiments, the hybridizing region consists of 12-30 linked nucleosides and is fully complementary to the target nucleic acid. In certain embodiments, the hybridizing region includes one mismatch relative to the target nucleic acid. In certain embodiments, the hybridizing region includes two mismatches relative to the target nucleic acid. In certain embodiments, the hybridizing region includes three mismatches relative to the target nucleic acid. In certain embodiments, the terminal region consists of 1-4 terminal nucleosides. In certain embodiments, the terminal nucleosides are at the 3' end. In certain embodiments, one or more of the terminal nucleosides are not complementary to the target nucleic acid.

Antisense mechanisms include any mechanism involving the hybridization of an oligonucleotide with target nucleic acid, wherein the hybridization results in a biological effect. In certain embodiments, such hybridization results in either target nucleic acid degradation or occupancy with concomitant inhibition or stimulation of the cellular machinery involving, for example, translation, transcription, or splicing of the target nucleic acid.

One type of antisense mechanism involving degradation of target RNA is RNase H mediated antisense. RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. It is known in the art that single-stranded antisense compounds which are "DNA-like" elicit RNase H activity in mammalian cells. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of DNA-like oligonucleotide-mediated inhibition of gene expression.

In certain embodiments, a conjugate group comprises a cleavable moiety. In certain embodiments, a conjugate group comprises one or more cleavable bond. In certain embodiments, a conjugate group comprises a linker. In certain embodiments, a linker comprises a protein binding moiety. In certain embodiments, a conjugate group comprises a cell-targeting moiety (also referred to as a cell-targeting group). In certain embodiments a cell-targeting moiety comprises a branching group. In certain embodiments, a cell-targeting moiety comprises one or more tethers. In certain embodiments, a cell-targeting moiety comprises a carbohydrate or carbohydrate cluster.

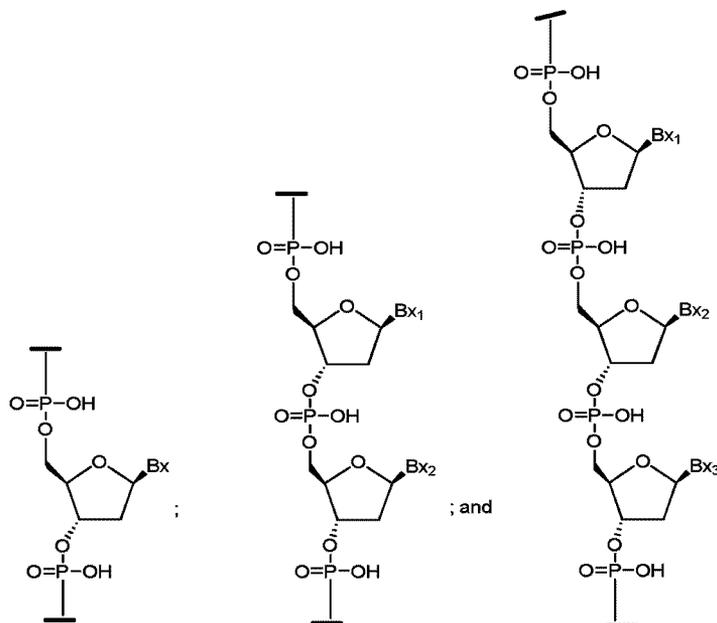
ii. Certain Cleavable Moieties

In certain embodiments, a cleavable moiety is a cleavable bond. In certain embodiments, a cleavable moiety comprises a cleavable bond. In certain embodiments, the conjugate group comprises a cleavable moiety. In certain such embodiments, the cleavable moiety attaches to the antisense oligonucleotide. In certain such embodiments, the cleavable moiety attaches directly to the cell-targeting moiety. In certain such embodiments, the cleavable moiety attaches to the conjugate linker. In certain embodiments, the cleavable moiety comprises a phosphate or phosphodiester. In certain embodiments, the cleavable moiety is a cleavable nucleoside or nucleoside analog. In certain embodiments, the nucleoside or nucleoside analog comprises an optionally protected heterocyclic base selected from a purine, substituted purine, pyrimidine or substituted pyrimidine. In certain embodiments, the cleavable moiety is a nucleoside comprising an optionally protected heterocyclic base selected from uracil, thymine, cytosine, 4-N-

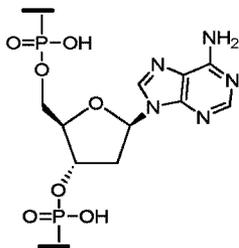
benzoylcytosine, 5-methylcytosine, 4-N-benzoyl-5-methylcytosine, adenine, 6-N-benzoyladenine, guanine and 2-N-isobutyrylguanine. In certain embodiments, the cleavable moiety is 2'-deoxy nucleoside that is attached to the 3' position of the antisense oligonucleotide by a phosphodiester linkage and is attached to the linker by a phosphodiester or phosphorothioate linkage. In certain embodiments, the cleavable moiety is 2'-deoxy adenosine that is attached to the 3' position of the antisense oligonucleotide by a phosphodiester linkage and is attached to the linker by a phosphodiester or phosphorothioate linkage. In certain embodiments, the cleavable moiety is 2'-deoxy adenosine that is attached to the 3' position of the antisense oligonucleotide by a phosphodiester linkage and is attached to the linker by a phosphodiester linkage.

In certain embodiments, the cleavable moiety is attached to the 3' position of the antisense oligonucleotide. In certain embodiments, the cleavable moiety is attached to the 5' position of the antisense oligonucleotide. In certain embodiments, the cleavable moiety is attached to a 2' position of the antisense oligonucleotide. In certain embodiments, the cleavable moiety is attached to the antisense oligonucleotide by a phosphodiester linkage. In certain embodiments, the cleavable moiety is attached to the linker by either a phosphodiester or a phosphorothioate linkage. In certain embodiments, the cleavable moiety is attached to the linker by a phosphodiester linkage. In certain embodiments, the conjugate group does not include a cleavable moiety.

In certain embodiments, the cleavable moiety is cleaved after the complex has been administered to an animal only after being internalized by a targeted cell. Inside the cell the cleavable moiety is cleaved thereby releasing the active antisense oligonucleotide. While not wanting to be bound by theory it is believed that the cleavable moiety is cleaved by one or more nucleases within the cell. In certain embodiments, the one or more nucleases cleave the phosphodiester linkage between the cleavable moiety and the linker. In certain embodiments, the cleavable moiety has a structure selected from among the following:



wherein each of Bx, Bx₁, Bx₂, and Bx₃ is independently a heterocyclic base moiety. In certain embodiments, the cleavable moiety has a structure selected from among the following:



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iii. Certain Linkers

In certain embodiments, the conjugate groups comprise a linker. In certain such embodiments, the linker is covalently bound to the cleavable moiety. In certain such embodiments, the linker is covalently bound to the antisense oligonucleotide. In certain embodiments, the linker is covalently bound to a cell-targeting moiety. In certain embodiments, the linker further comprises a covalent attachment to a solid support. In certain embodiments, the linker further comprises a covalent attachment to a protein binding moiety. In certain embodiments, the linker further comprises a covalent attachment to a solid support and further comprises a covalent attachment to a protein binding moiety. In certain embodiments, the linker includes multiple positions for attachment of tethered ligands. In certain embodiments, the linker includes multiple positions for attachment of tethered ligands and is not attached to a branching group. In certain embodiments, the linker further comprises one or more cleavable bond. In certain embodiments, the conjugate group does not include a linker.

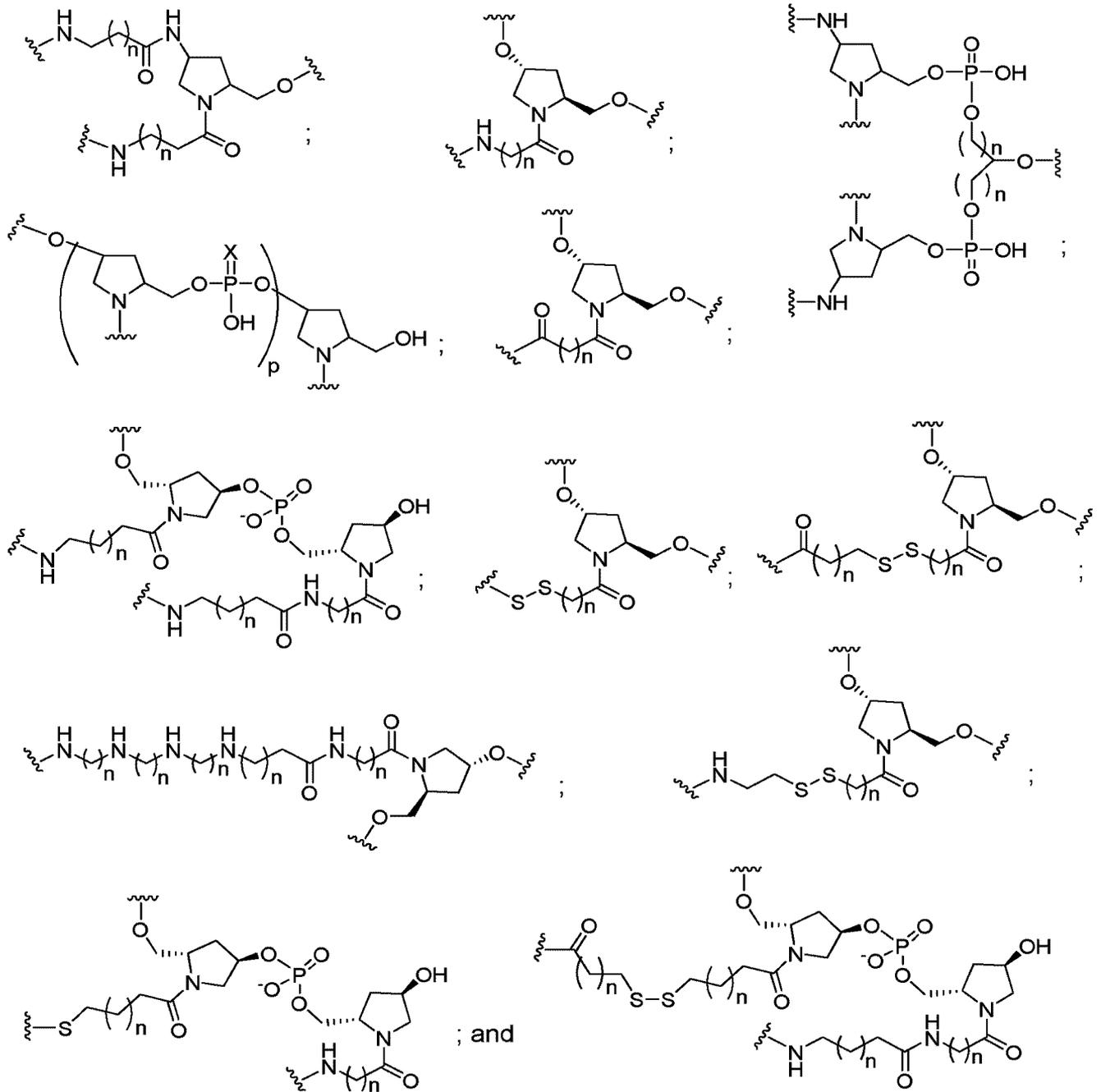
In certain embodiments, the linker includes at least a linear group comprising groups selected from alkyl, amide, disulfide, polyethylene glycol, ether, thioether (-S-) and hydroxylamino (-O-N(H)-) groups. In certain embodiments, the linear group comprises groups selected from alkyl, amide and ether groups. In certain embodiments, the linear group comprises groups selected from alkyl and ether groups. In certain embodiments, the linear group comprises at least one phosphorus linking group. In certain embodiments, the linear group comprises at least one phosphodiester group. In certain embodiments, the linear group includes at least one neutral linking group. In certain embodiments, the linear group is covalently attached to the cell-targeting moiety and the cleavable moiety. In certain embodiments, the linear group is covalently attached to the cell-targeting moiety and the antisense oligonucleotide. In certain embodiments, the linear group is covalently attached to the cell-targeting moiety, the cleavable moiety and a solid support. In certain embodiments, the linear group is covalently attached to the cell-targeting moiety, the cleavable moiety, a

solid support and a protein binding moiety. In certain embodiments, the linear group includes one or more cleavable bond.

In certain embodiments, the linker includes the linear group covalently attached to a scaffold group. In certain embodiments, the scaffold includes a branched aliphatic group comprising groups selected from alkyl, amide, disulfide, polyethylene glycol, ether, thioether and hydroxylamino groups. In certain
5 embodiments, the scaffold includes a branched aliphatic group comprising groups selected from alkyl, amide and ether groups. In certain embodiments, the scaffold includes at least one mono or polycyclic ring system. In certain embodiments, the scaffold includes at least two mono or polycyclic ring systems. In certain
10 embodiments, the linear group is covalently attached to the scaffold group and the scaffold group is covalently attached to the cleavable moiety and the linker. In certain embodiments, the linear group is covalently attached to the scaffold group and the scaffold group is covalently attached to the cleavable
15 moiety, the linker and a solid support. In certain embodiments, the linear group is covalently attached to the scaffold group and the scaffold group is covalently attached to the cleavable moiety, the linker and a protein binding moiety. In certain embodiments, the linear group is covalently attached to the scaffold group and the
20 scaffold group is covalently attached to the cleavable moiety, the linker, a protein binding moiety and a solid support. In certain embodiments, the scaffold group includes one or more cleavable bond.

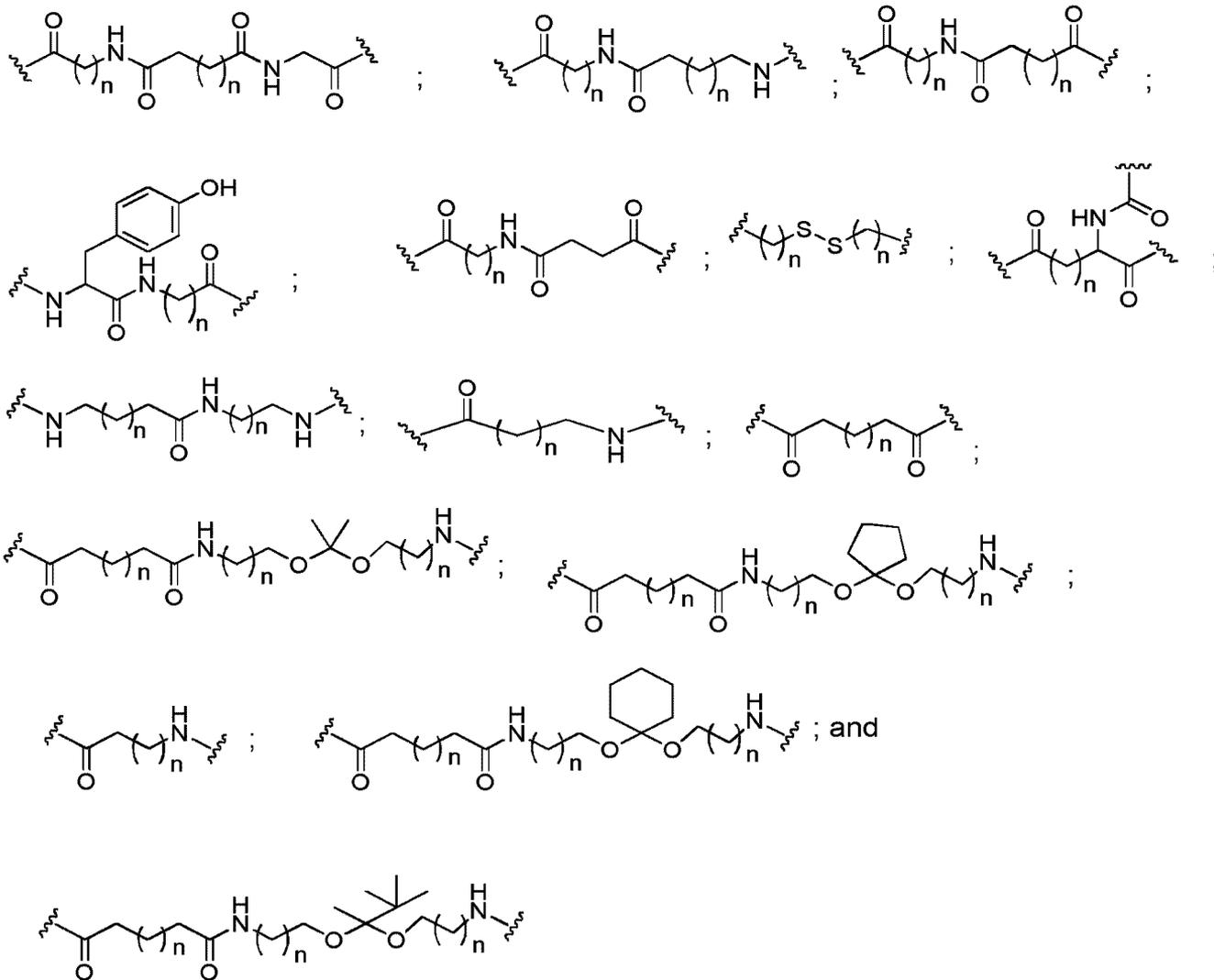
In certain embodiments, the linker includes a protein binding moiety. In certain embodiments, the protein binding moiety is a lipid such as for example including but not limited to cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol,
20 geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholonic acid, dimethoxytrityl, or phenoxazine), a vitamin (e.g., folate, vitamin A, vitamin E, biotin, pyridoxal), a peptide, a carbohydrate (e.g., monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, polysaccharide), an
25 endosomolytic component, a steroid (e.g., uvaol, hecigenin, diosgenin), a terpene (e.g., triterpene, e.g., sarsasapogenin, friedelin, epifriedelanol derivatized lithocholic acid), or a cationic lipid. In certain
embodiments, the protein binding moiety is a C16 to C22 long chain saturated or unsaturated fatty acid, cholesterol, cholic acid, vitamin E, adamantane or 1-pentafluoropropyl.

In certain embodiments, a linker has a structure selected from among:



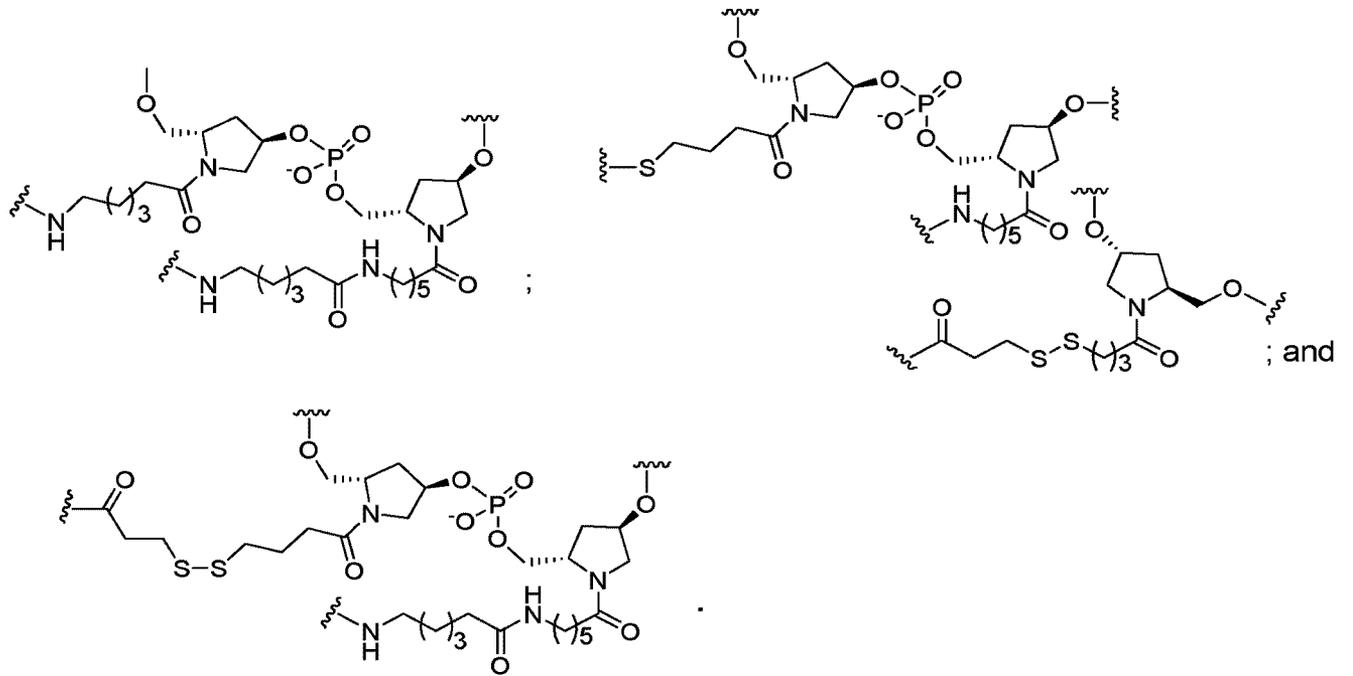
wherein each n is, independently, from 1 to 20; and p is from 1 to 6.

In certain embodiments, a linker has a structure selected from among:

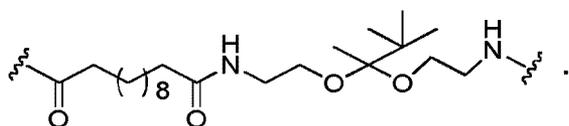
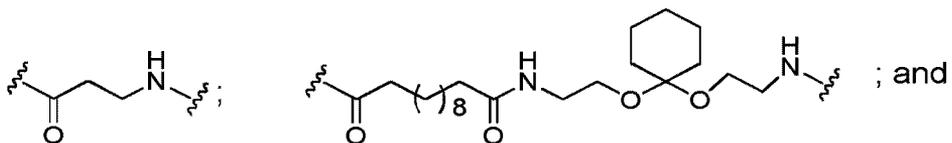
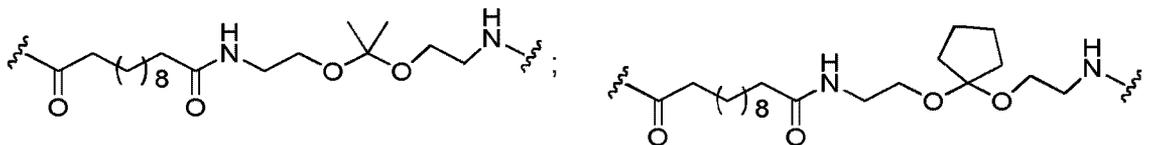
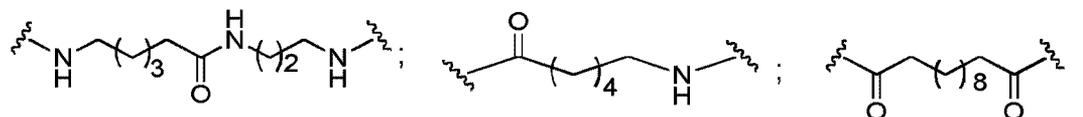
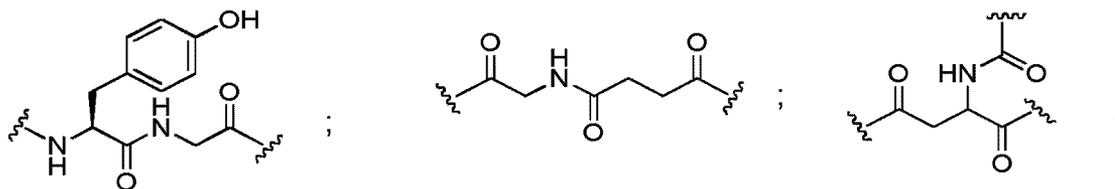
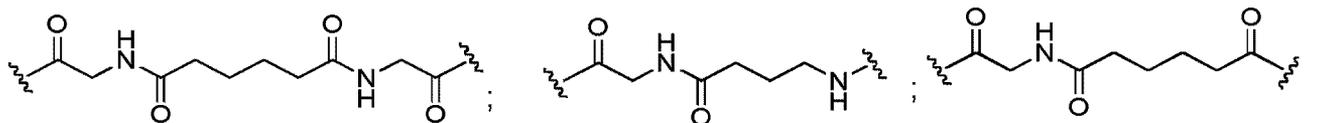


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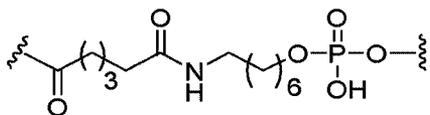
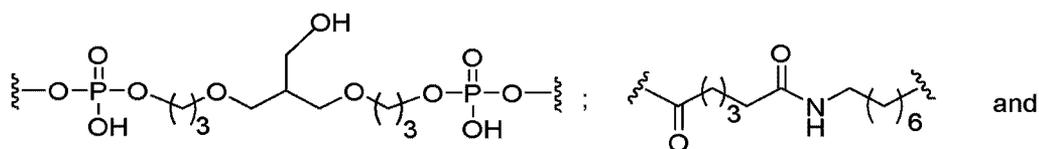
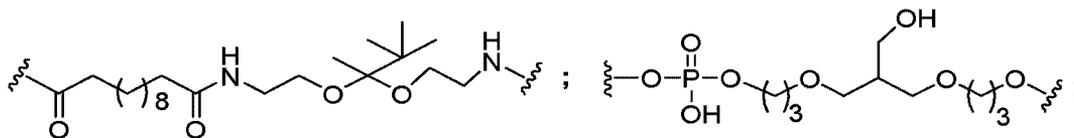
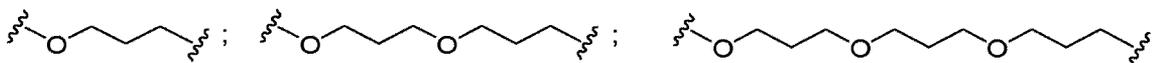
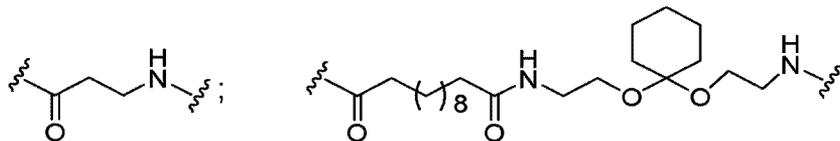
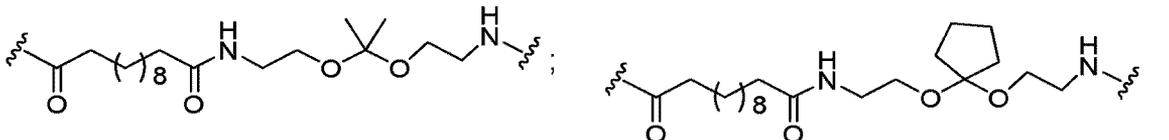
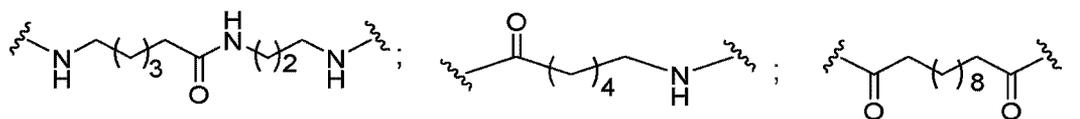
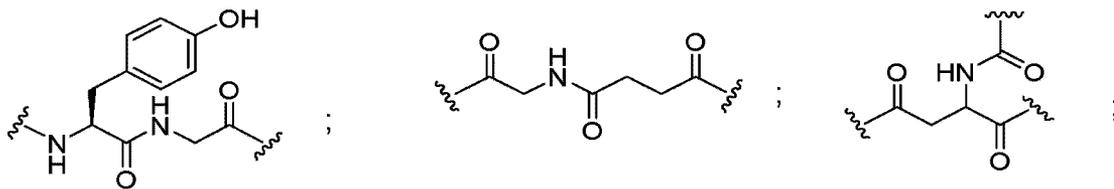
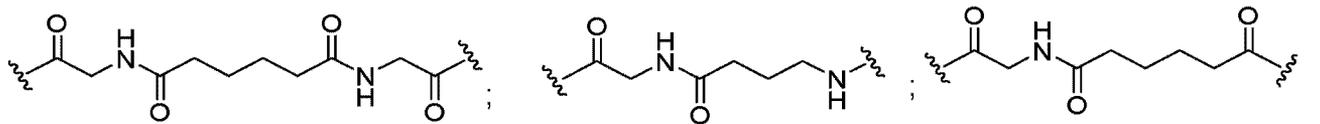
wherein n is from 1 to 20.



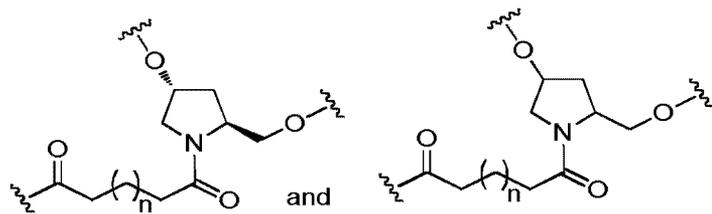
In certain embodiments, a linker has a structure selected from among:



In certain embodiments, a linker has a structure selected from among:



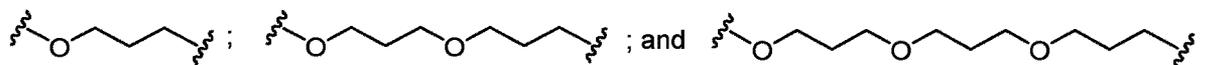
In certain embodiments, a linker has a structure selected from among:



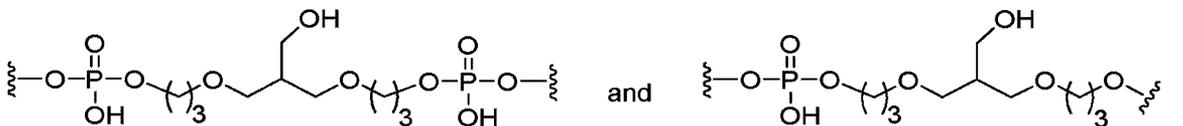
wherein n is from 1 to 20.

5

In certain embodiments, a linker has a structure selected from among:

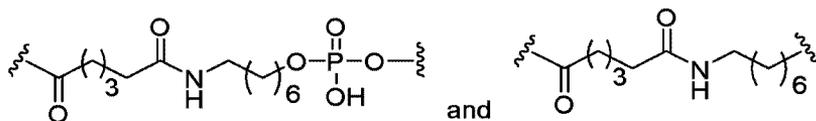


In certain embodiments, a linker has a structure selected from among:



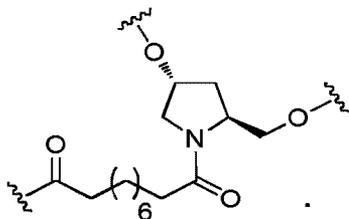
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In certain embodiments, a linker has a structure selected from among:

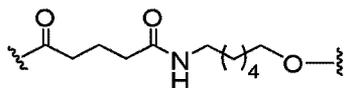


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In certain embodiments, the conjugate linker has the structure:

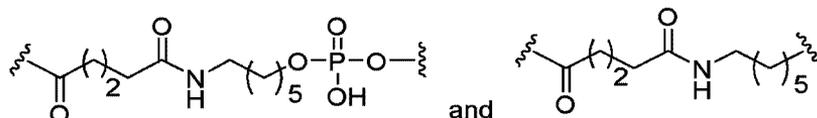


In certain embodiments, the conjugate linker has the structure:

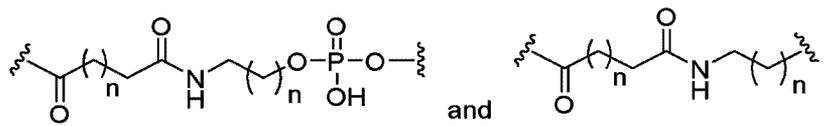


In certain embodiments, a linker has a structure selected from among:

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In certain embodiments, a linker has a structure selected from among:



5 wherein each n is independently, 0, 1, 2, 3, 4, 5, 6, or 7.

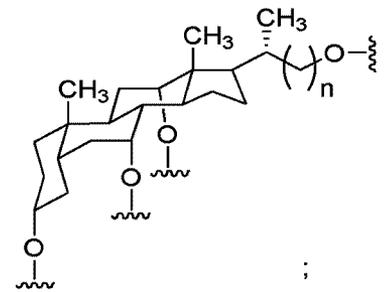
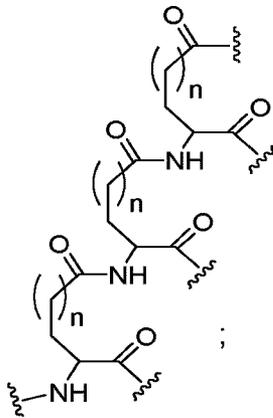
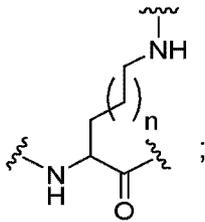
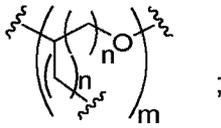
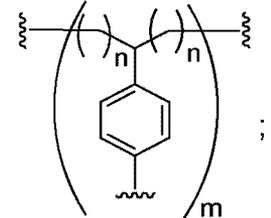
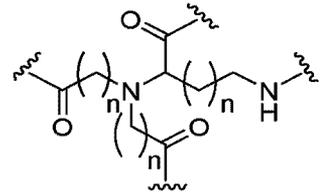
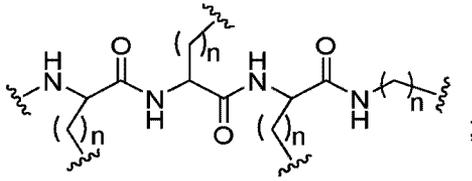
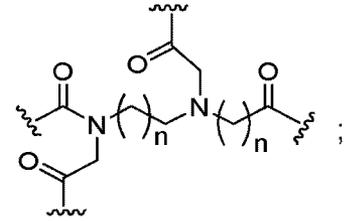
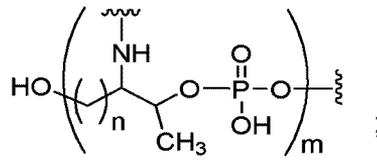
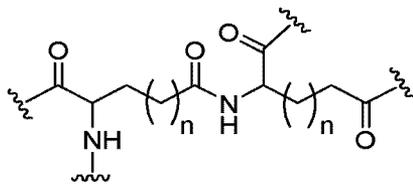
iv. Certain Cell-Targeting Moieties

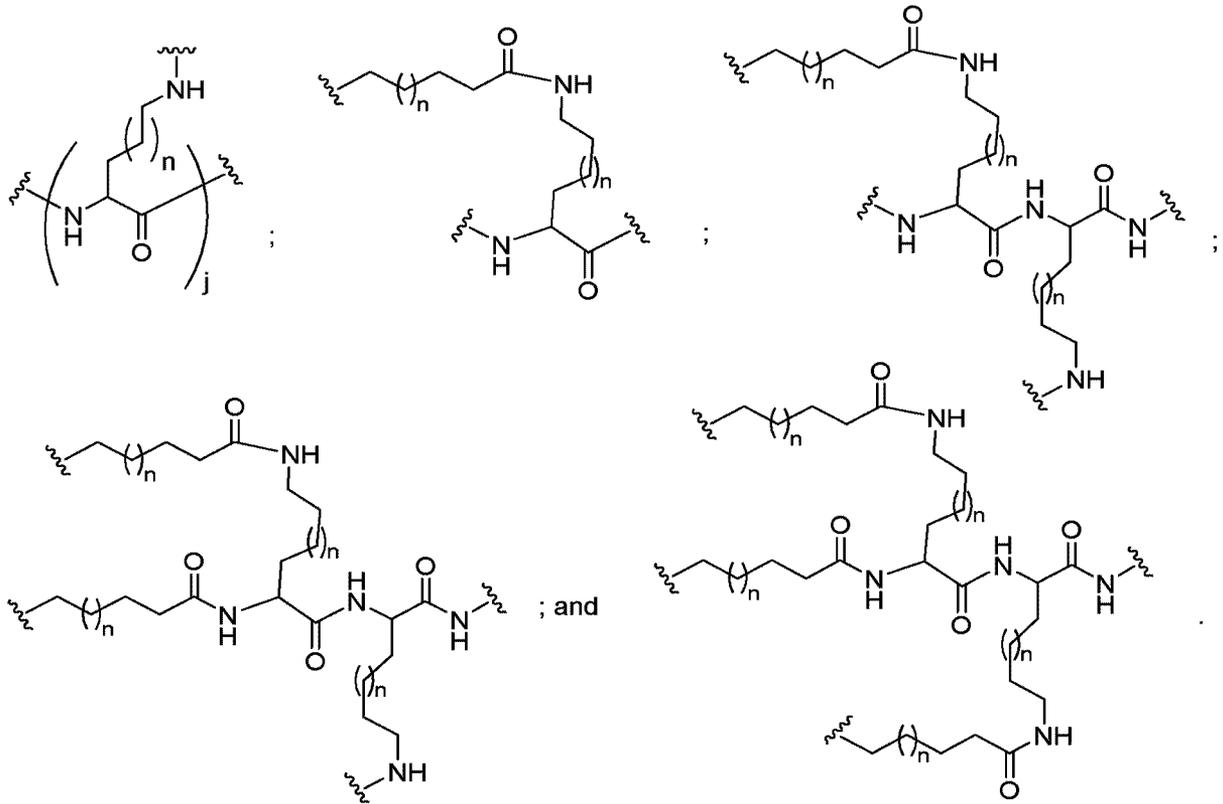
In certain embodiments, conjugate groups comprise cell-targeting moieties. Certain such cell-targeting moieties increase cellular uptake of antisense compounds. In certain embodiments, cell-targeting moieties comprise a branching group, one or more tether, and one or more ligand. In certain
10 embodiments, cell-targeting moieties comprise a branching group, one or more tether, one or more ligand and one or more cleavable bond.

1. Certain Branching Groups

In certain embodiments, the conjugate groups comprise a targeting moiety comprising a branching
15 group and at least two tethered ligands. In certain embodiments, the branching group attaches the conjugate linker. In certain embodiments, the branching group attaches the cleavable moiety. In certain embodiments, the branching group attaches the antisense oligonucleotide. In certain embodiments, the branching group is covalently attached to the linker and each of the tethered ligands. In certain embodiments, the branching group comprises a branched aliphatic group comprising groups selected from alkyl, amide, disulfide,
20 polyethylene glycol, ether, thioether and hydroxylamino groups. In certain embodiments, the branching group comprises groups selected from alkyl, amide and ether groups. In certain embodiments, the branching group comprises groups selected from alkyl and ether groups. In certain embodiments, the branching group comprises a mono or polycyclic ring system. In certain embodiments, the branching group comprises one or more cleavable bond. In certain embodiments, the conjugate group does not include a branching group.

25 In certain embodiments, a branching group has a structure selected from among:



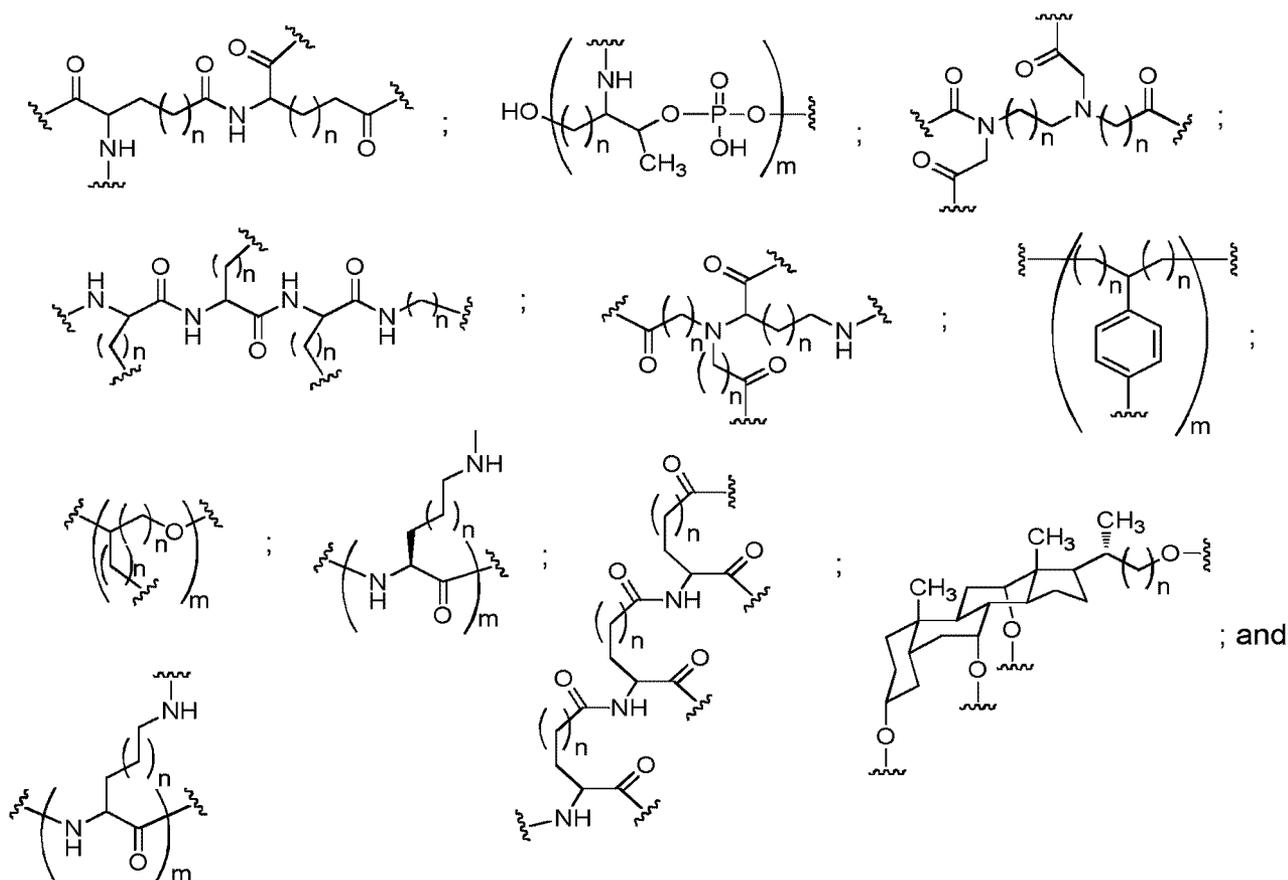


wherein each n is, independently, from 1 to 20;

j is from 1 to 3; and

m is from 2 to 6.

In certain embodiments, a branching group has a structure selected from among:



wherein each n is, independently, from 1 to 20; and

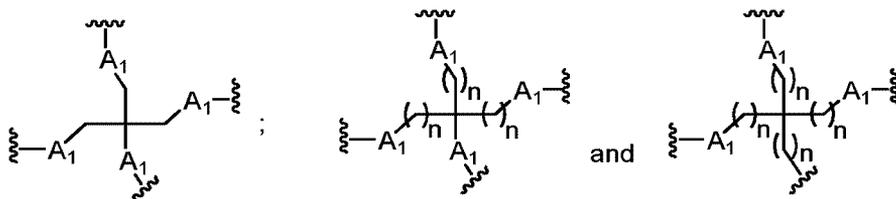
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m is from 2 to 6.

In certain embodiments, a branching group has a structure selected from among:

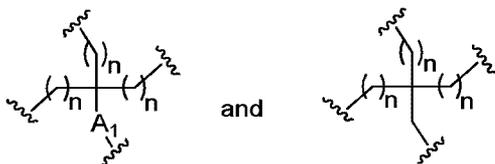
wherein each A_1 is independently, O, S, C=O or NH; and
each n is, independently, from 1 to 20.

5 In certain embodiments, a branching group has a structure selected from among:



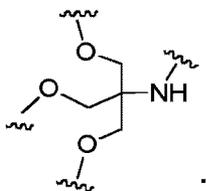
wherein each A_1 is independently, O, S, C=O or NH; and
each n is, independently, from 1 to 20.

10 In certain embodiments, a branching group has a structure selected from among:

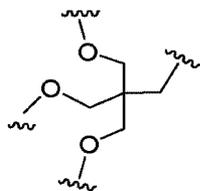


wherein A_1 is O, S, C=O or NH; and
each n is, independently, from 1 to 20.

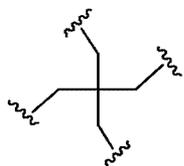
15 In certain embodiments, a branching group has a structure selected from among:



In certain embodiments, a branching group has a structure selected from among:



In certain embodiments, a branching group has a structure selected from among:



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2. Certain Tethers

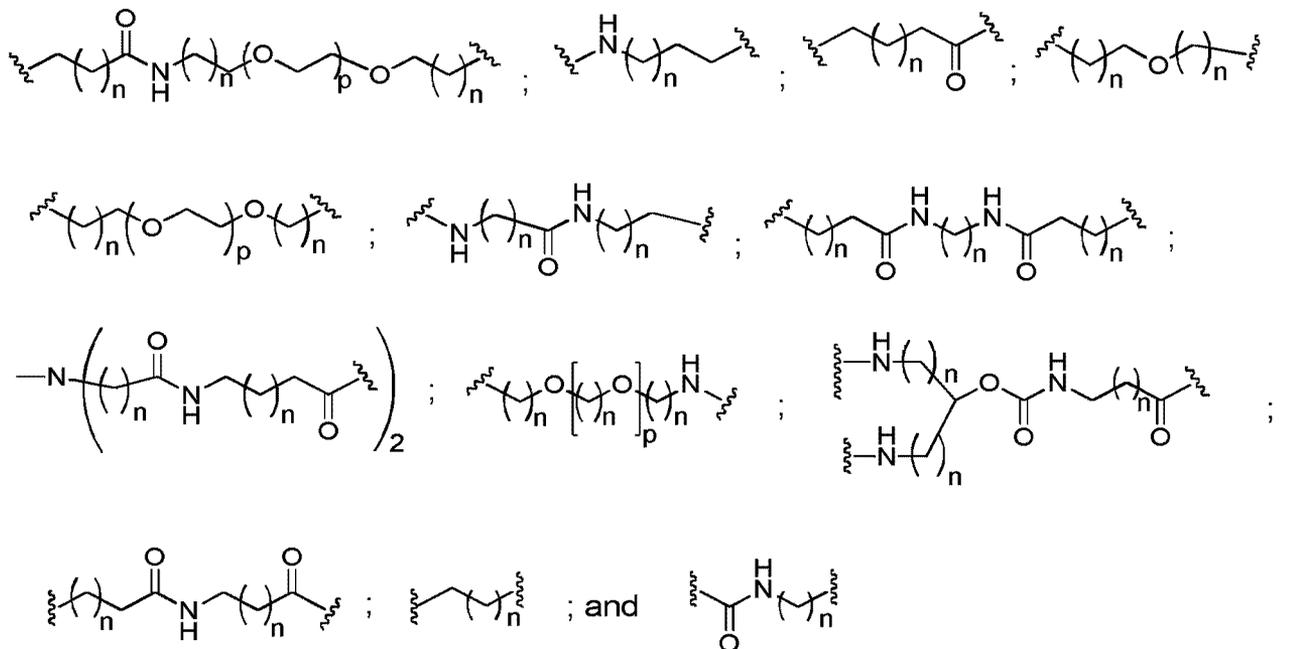
In certain embodiments, conjugate groups comprise one or more tethers covalently attached to the branching group. In certain embodiments, conjugate groups comprise one or more tethers covalently attached to the linking group. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, ether, thioether, disulfide, amide and polyethylene glycol groups in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, substituted alkyl, ether, thioether, disulfide, amide, phosphodiester and polyethylene glycol groups in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, ether and amide groups in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, substituted alkyl, phosphodiester, ether and amide groups in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl and phosphodiester in any combination. In certain embodiments, each tether comprises at least one phosphorus linking group or neutral linking group.

In certain embodiments, the tether includes one or more cleavable bond. In certain embodiments, the tether is attached to the branching group through either an amide or an ether group. In certain embodiments, the tether is attached to the branching group through a phosphodiester group. In certain embodiments, the tether is attached to the branching group through a phosphorus linking group or neutral linking group. In certain embodiments, the tether is attached to the branching group through an ether group. In certain embodiments, the tether is attached to the ligand through either an amide or an ether group. In certain embodiments, the tether is attached to the ligand through an ether group. In certain embodiments, the tether is attached to the ligand through either an amide or an ether group. In certain embodiments, the tether is attached to the ligand through an ether group.

In certain embodiments, each tether comprises from about 8 to about 20 atoms in chain length between the ligand and the branching group. In certain embodiments, each tether group comprises from

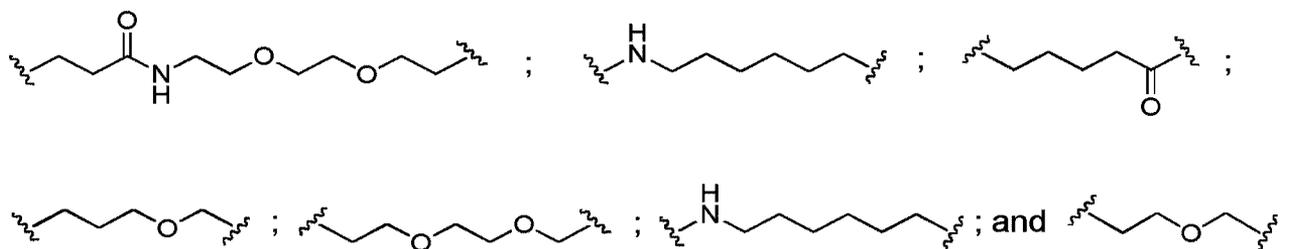
about 10 to about 18 atoms in chain length between the ligand and the branching group. In certain embodiments, each tether group comprises about 13 atoms in chain length.

In certain embodiments, a tether has a structure selected from among:

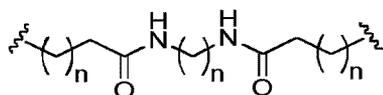


wherein each n is, independently, from 1 to 20; and each p is from 1 to about 6.

In certain embodiments, a tether has a structure selected from among:

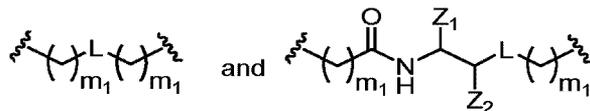


In certain embodiments, a tether has a structure selected from among:



wherein each n is, independently, from 1 to 20.

In certain embodiments, a tether has a structure selected from among:



wherein L is either a phosphorus linking group or a neutral linking group;

5

Z₁ is C(=O)O-R₂;

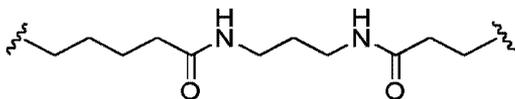
Z₂ is H, C₁-C₆ alkyl or substituted C₁-C₆ alkyl;

R₂ is H, C₁-C₆ alkyl or substituted C₁-C₆ alkyl; and

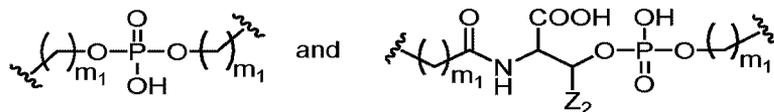
each m₁ is, independently, from 0 to 20 wherein at least one m₁ is greater than 0 for each tether.

10

In certain embodiments, a tether has a structure selected from among:



In certain embodiments, a tether has a structure selected from among:

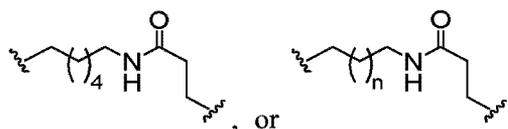


15

wherein Z₂ is H or CH₃; and

each m₁ is, independently, from 0 to 20 wherein at least one m₁ is greater than 0 for each tether.

In certain embodiments, a tether has a structure selected from among:



20

; wherein each n is independently, 0, 1, 2, 3, 4, 5, 6, or 7.

In certain embodiments, a tether comprises a phosphorus linking group. In certain embodiments, a tether does not comprise any amide bonds. In certain embodiments, a tether comprises a phosphorus linking group and does not comprise any amide bonds.

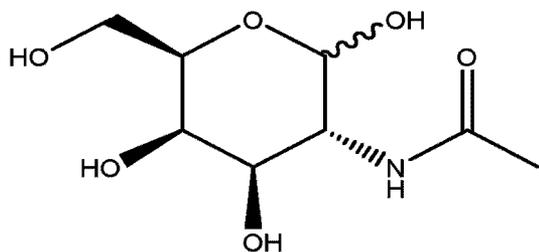
3. Certain Ligands

In certain embodiments, the present disclosure provides ligands wherein each ligand is covalently attached to a tether. In certain embodiments, each ligand is selected to have an affinity for at least one type of receptor on a target cell. In certain embodiments, ligands are selected that have an affinity for at least one type of receptor on the surface of a mammalian liver cell. In certain embodiments, ligands are selected that have an affinity for the hepatic asialoglycoprotein receptor (ASGP-R). In certain embodiments, each ligand is a carbohydrate. In certain embodiments, each ligand is, independently selected from galactose, N-acetyl galactoseamine, mannose, glucose, glucosamine and fucose. In certain embodiments, each ligand is N-acetyl galactoseamine (GalNAc). In certain embodiments, the targeting moiety comprises 2 to 6 ligands. In certain 5
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embodiments, the targeting moiety comprises 3 ligands. In certain embodiments, the targeting moiety comprises 3 N-acetyl galactoseamine ligands.

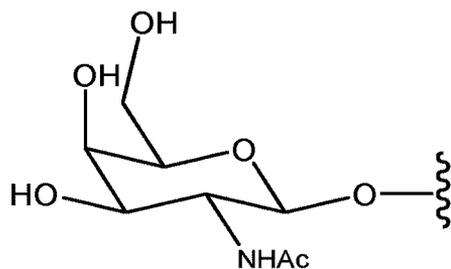
In certain embodiments, the ligand is a carbohydrate, carbohydrate derivative, modified carbohydrate, multivalent carbohydrate cluster, polysaccharide, modified polysaccharide, or polysaccharide derivative. In certain embodiments, the ligand is an amino sugar or a thio sugar. For example, amino sugars 15
may be selected from any number of compounds known in the art, for example glucosamine, sialic acid, α -D-galactosamine, N-Acetylgalactosamine, 2-acetamido-2-deoxy-D-galactopyranose (GalNAc), 2-Amino-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy- β -D-glucopyranose (β -muramic acid), 2-Deoxy-2-methylamino-L-glucopyranose, 4,6-Dideoxy-4-formamido-2,3-di-*O*-methyl-D-mannopyranose, 2-Deoxy-2-sulfoamino-D-glucopyranose and *N*-sulfo-D-glucosamine, and *N*-Glycoloyl- α -neuraminic acid. For example, thio sugars 20
may be selected from the group consisting of 5-Thio- β -D-glucopyranose, Methyl 2,3,4-tri-*O*-acetyl-1-thio-6-*O*-trityl- α -D-glucopyranoside, 4-Thio- β -D-galactopyranose, and ethyl 3,4,6,7-tetra-*O*-acetyl-2-deoxy-1,5-dithio- α -D-*gluco*-heptopyranoside.

In certain embodiments, “GalNAc” or “Gal-NAc” refers to 2-(Acetylamino)-2-deoxy-D-galactopyranose, commonly referred to in the literature as N-acetyl galactosamine. In certain embodiments, 25
“N-acetyl galactosamine” refers to 2-(Acetylamino)-2-deoxy-D-galactopyranose. In certain embodiments, “GalNAc” or “Gal-NAc” refers to 2-(Acetylamino)-2-deoxy-D-galactopyranose. In certain embodiments, “GalNAc” or “Gal-NAc” refers to 2-(Acetylamino)-2-deoxy-D-galactopyranose, which includes both the β -form: 2-(Acetylamino)-2-deoxy- β -D-galactopyranose and α -form: 2-(Acetylamino)-2-deoxy-D-galactopyranose. In certain embodiments, both the β -form: 2-(Acetylamino)-2-deoxy- β -D-galactopyranose and α -form: 2-(Acetylamino)-2-deoxy-D-galactopyranose may be used interchangeably. Accordingly, in 30
structures in which one form is depicted, these structures are intended to include the other form as well. For example, where the structure for an α -form: 2-(Acetylamino)-2-deoxy-D-galactopyranose is shown, this structure is intended to include the other form as well. In certain embodiments, In certain preferred embodiments, the β -form 2-(Acetylamino)-2-deoxy-D-galactopyranose is the preferred embodiment.

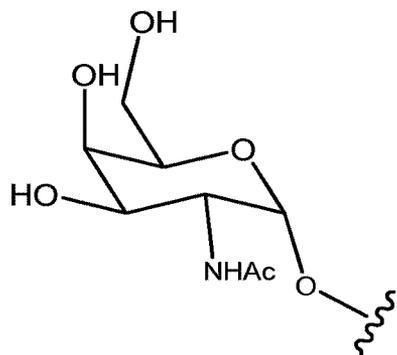
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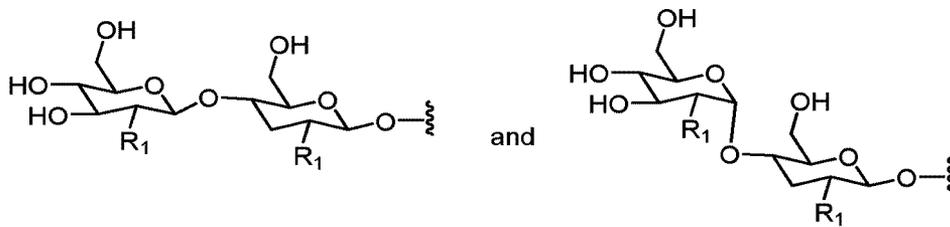
2-(Acetylamino)-2-deoxy-D-galactopyranose

2-(Acetylamino)-2-deoxy- β -D-galactopyranose

5

2-(Acetylamino)-2-deoxy- α -D-galactopyranose

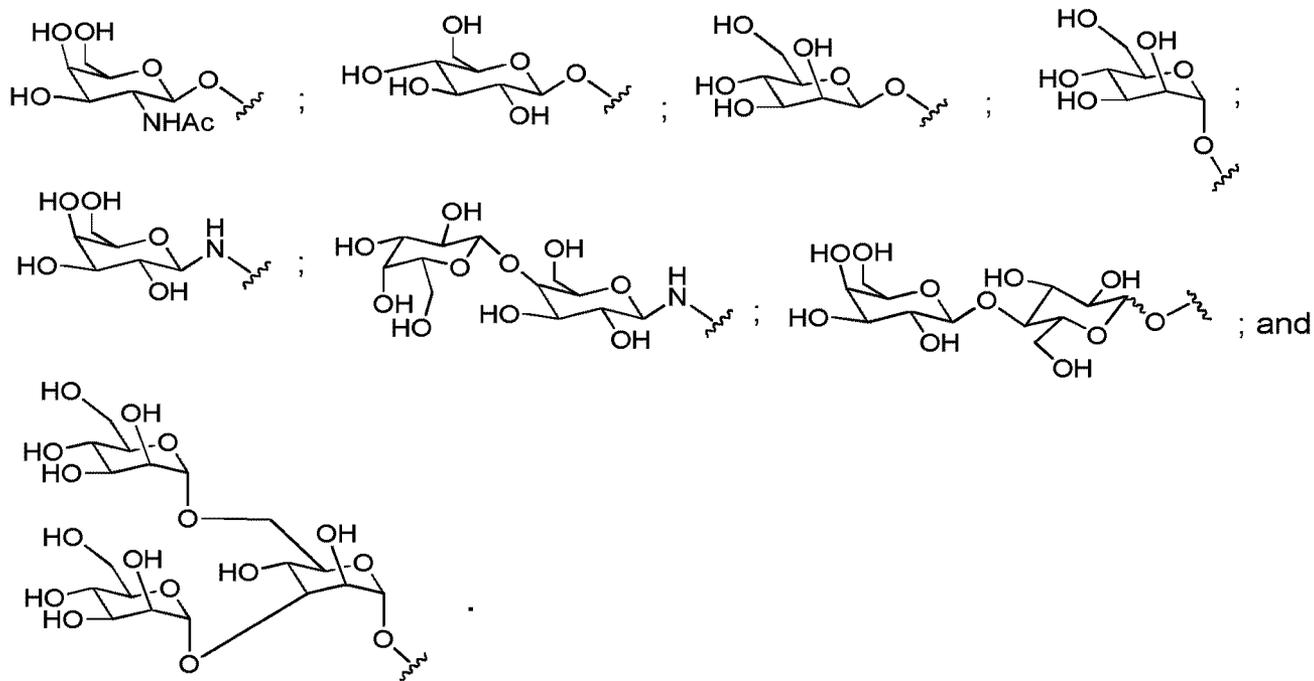
In certain embodiments one or more ligand has a structure selected from among:



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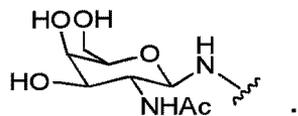
wherein each R_1 is selected from OH and NHCOOH.

In certain embodiments one or more ligand has a structure selected from among:



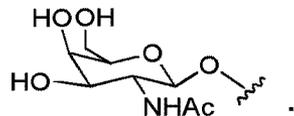
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In certain embodiments one or more ligand has a structure selected from among:



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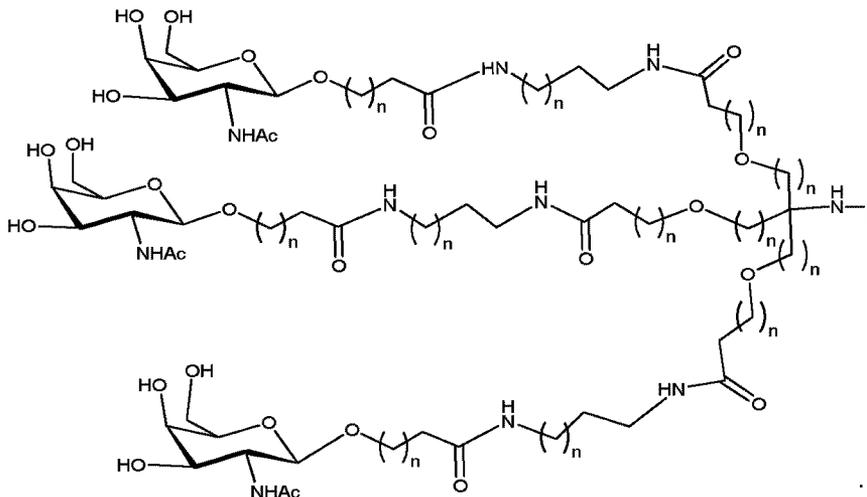
In certain embodiments one or more ligand has a structure selected from among:



i. Certain Conjugates

In certain embodiments, conjugate groups comprise the structural features above. In certain such embodiments, conjugate groups have the following structure:

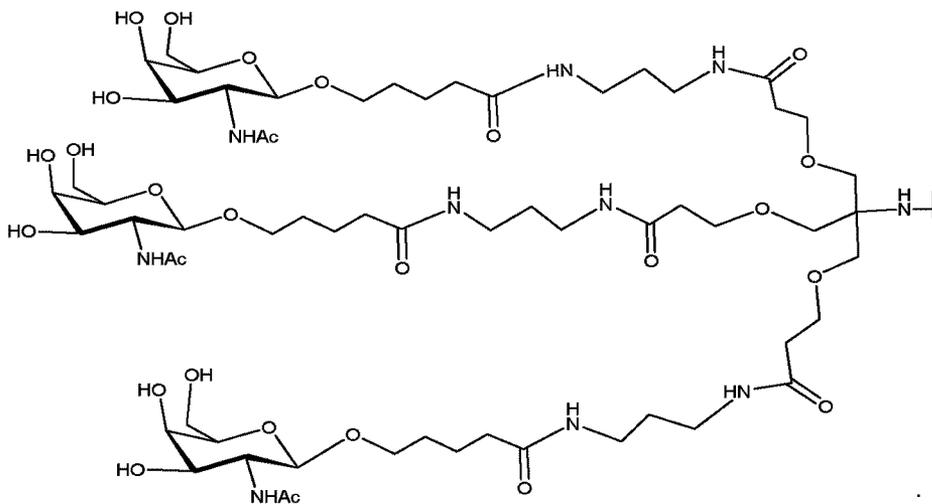
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wherein each n is, independently, from 1 to 20.

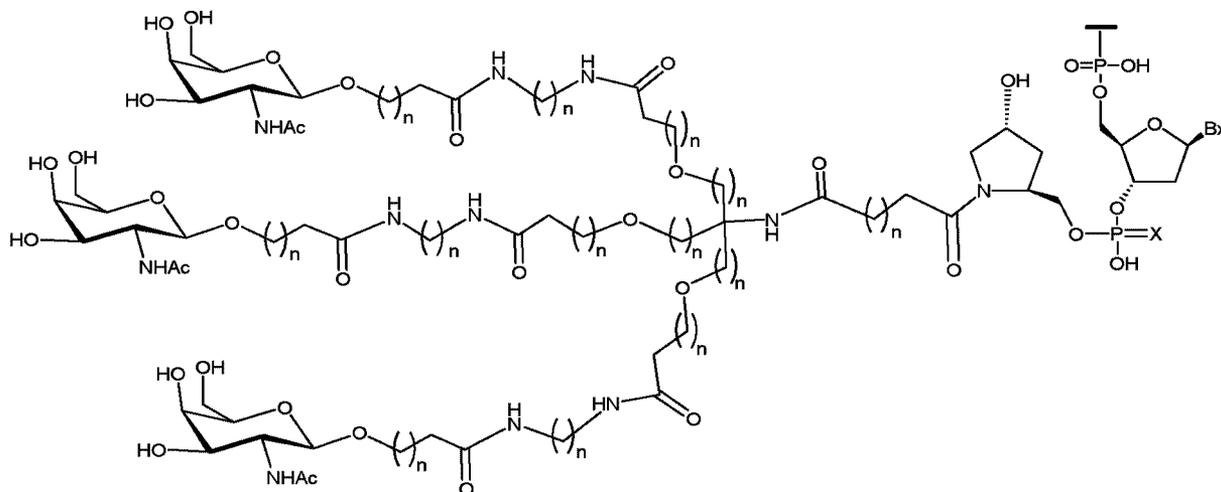
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In certain such embodiments, conjugate groups have the following structure:



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In certain such embodiments, conjugate groups have the following structure:



wherein each n is, independently, from 1 to 20;

Z is H or a linked solid support;

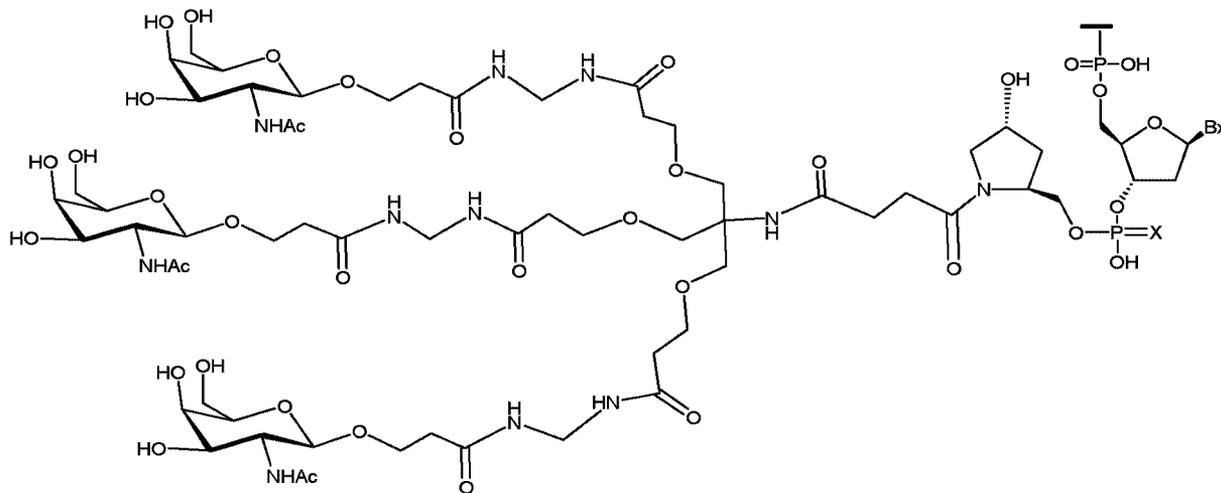
Q is an antisense compound;

5

X is O or S; and

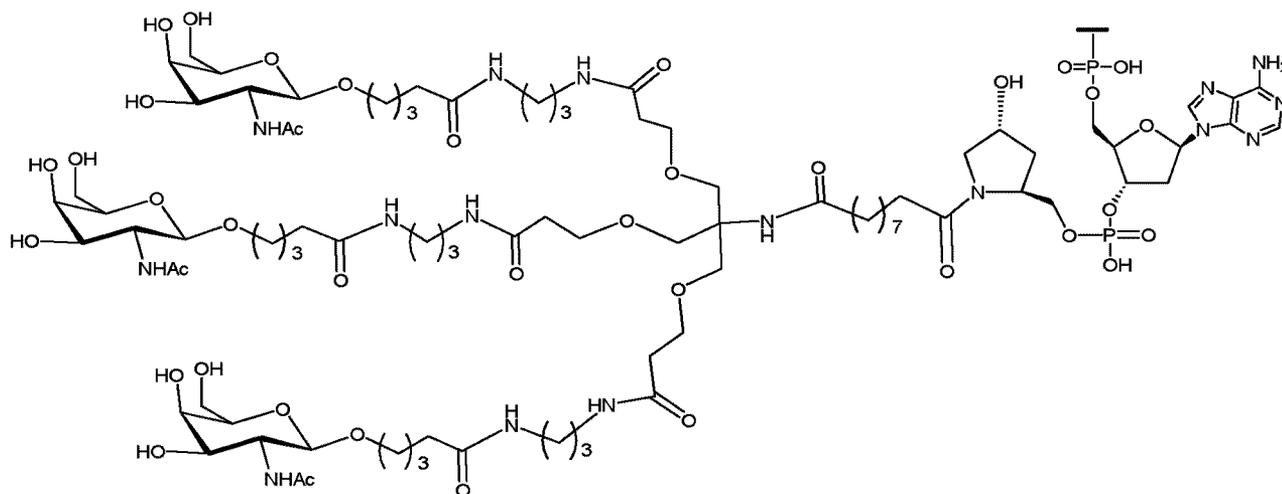
Bx is a heterocyclic base moiety.

In certain such embodiments, conjugate groups have the following structure:

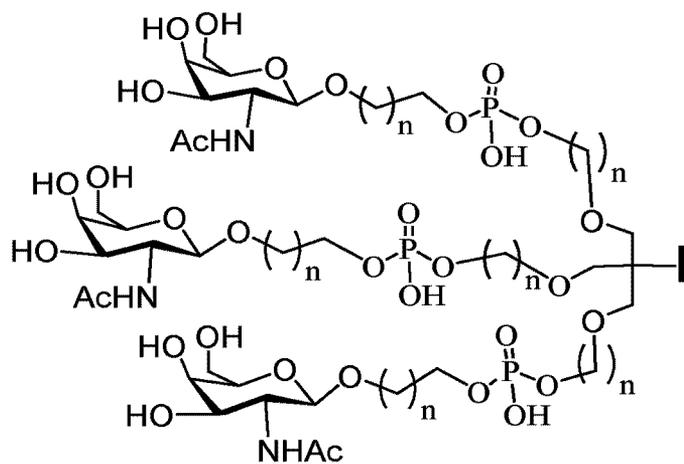


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In certain such embodiments, conjugate groups have the following structure:

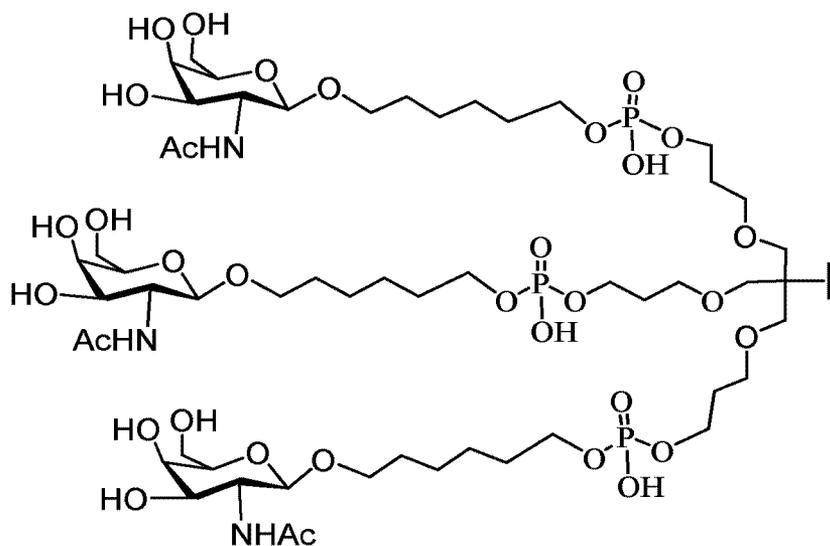


In certain such embodiments, conjugate groups have the following structure:

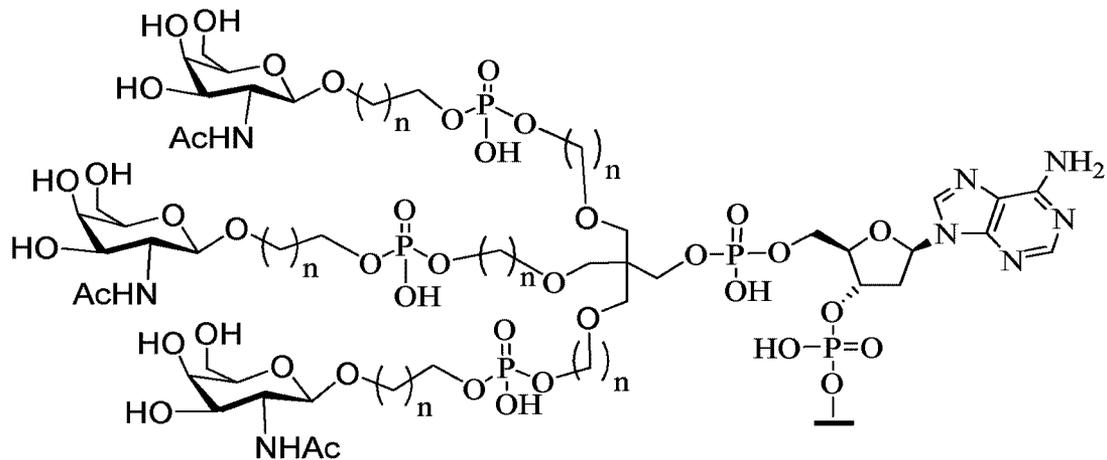


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In certain such embodiments, conjugate groups have the following structure:

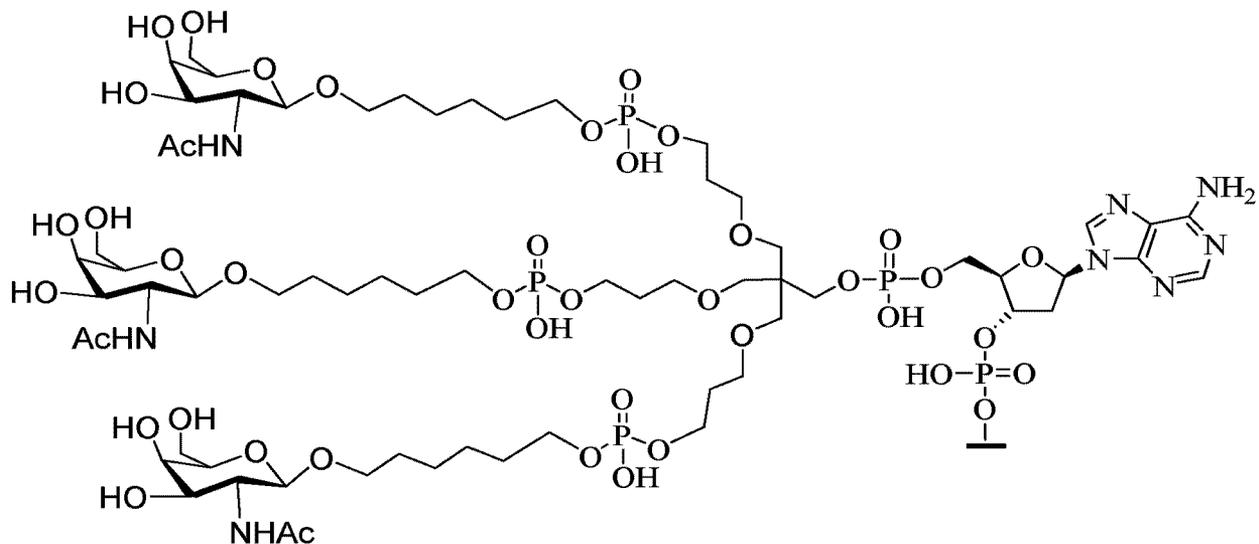


In certain such embodiments, conjugate groups have the following structure:



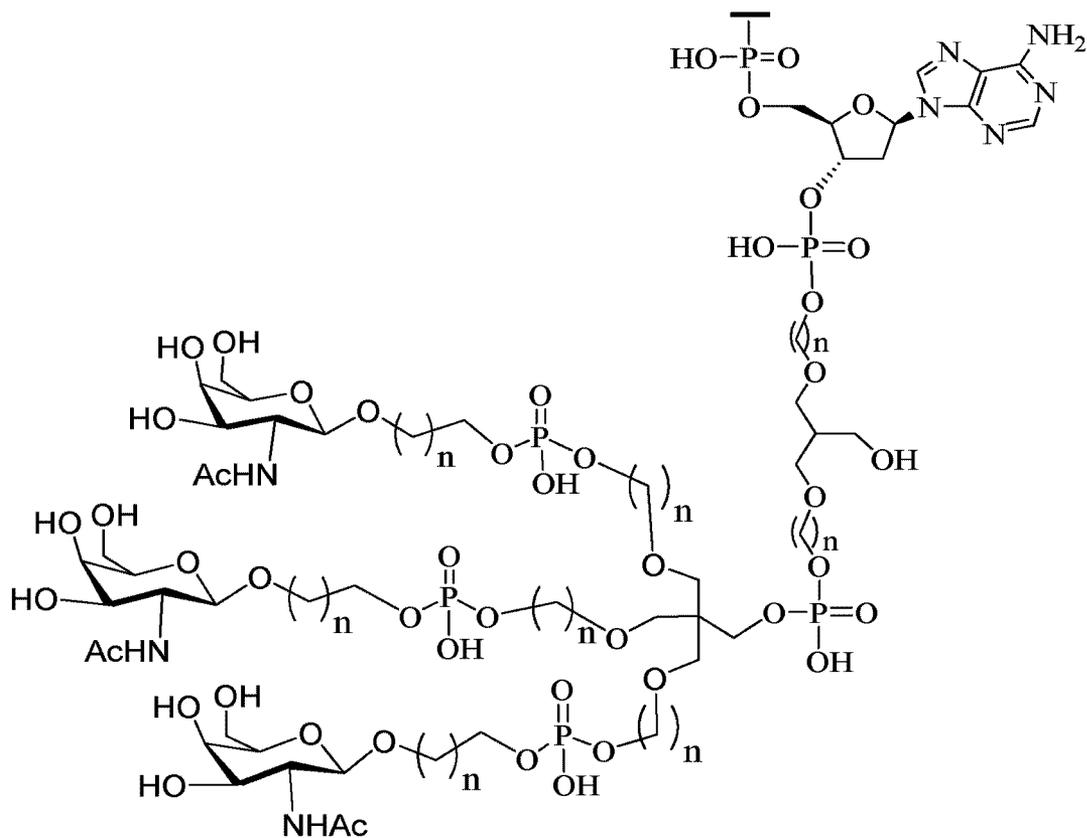
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In certain such embodiments, conjugate groups have the following structure:

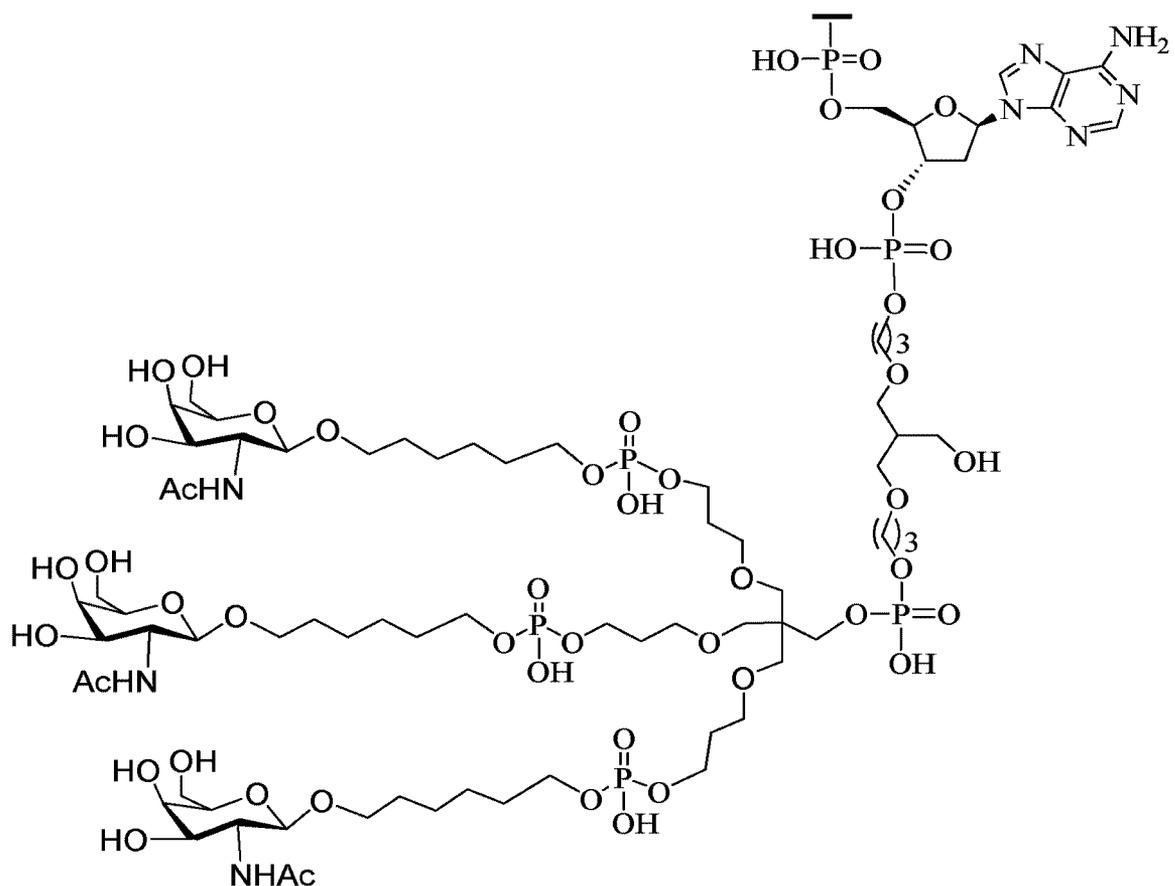


In certain such embodiments, conjugate groups have the following structure:

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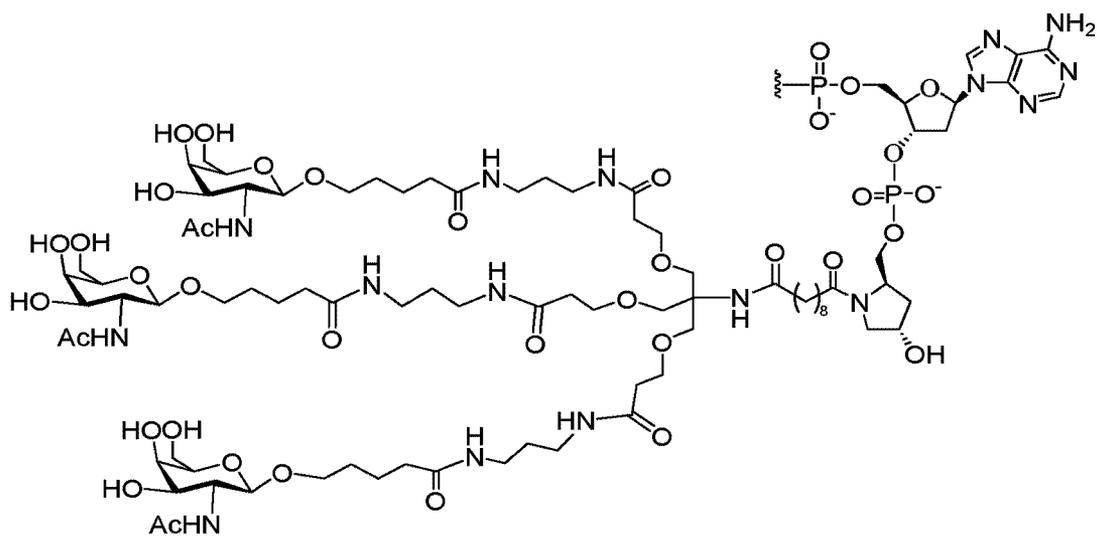


In certain such embodiments, conjugate groups have the following structure:

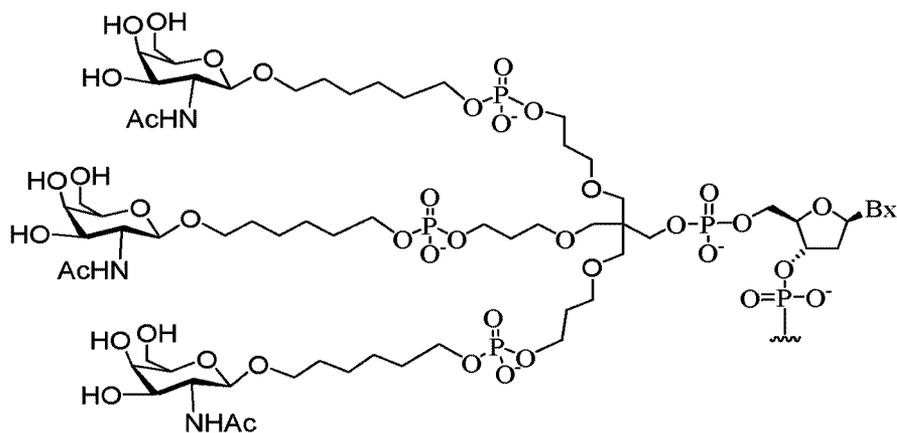


In certain embodiments, conjugates do not comprise a pyrrolidine.

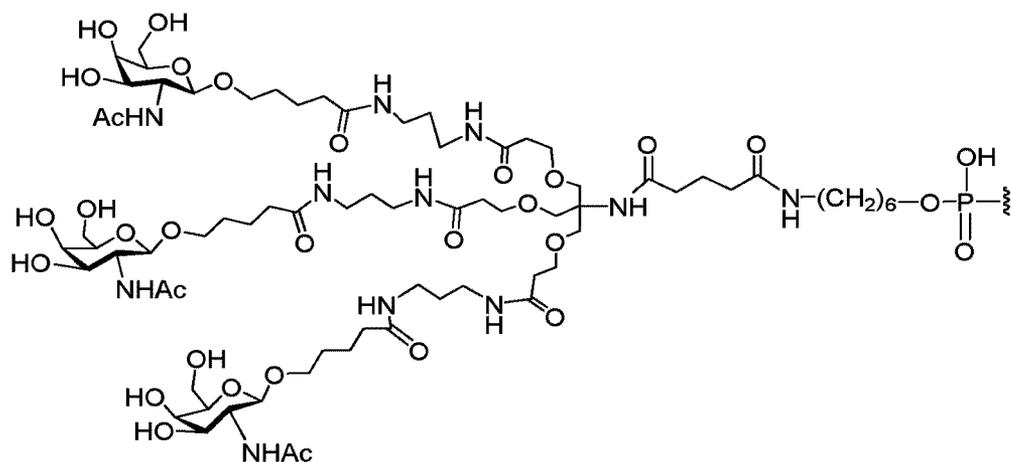
- 5 In certain such embodiments, conjugate groups have the following structure:



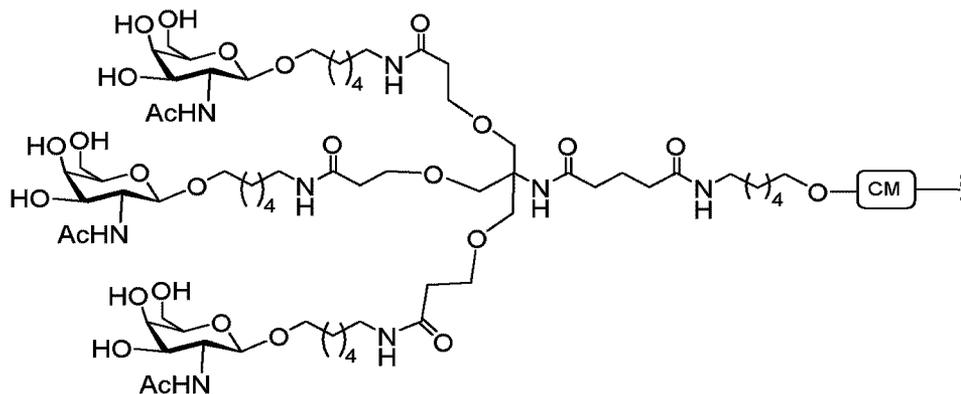
In certain such embodiments, conjugate groups have the following structure:



In certain such embodiments, conjugate groups have the following structure:

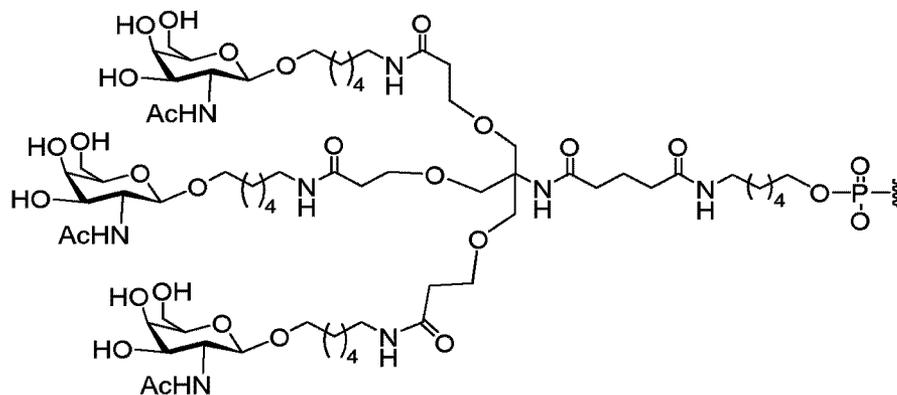


In certain such embodiments, conjugate groups have the following structure:

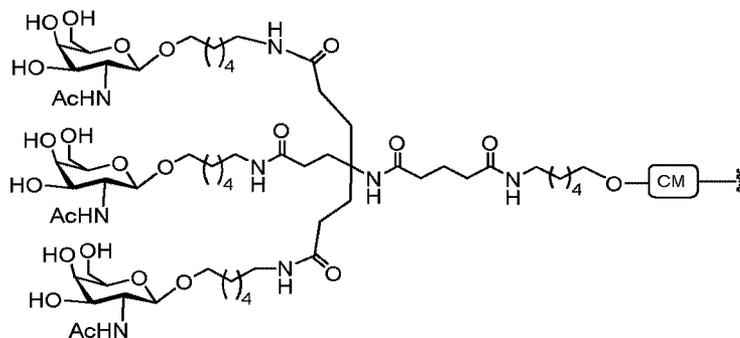


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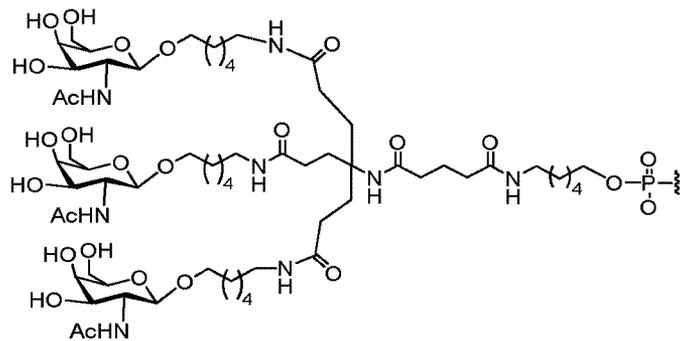
In certain such embodiments, conjugate groups have the following structure:



In certain such embodiments, conjugate groups have the following structure:

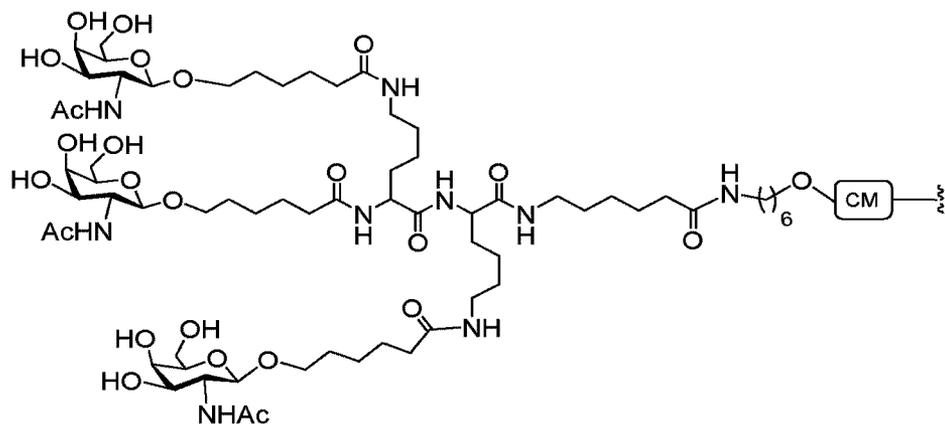


In certain such embodiments, conjugate groups have the following structure:

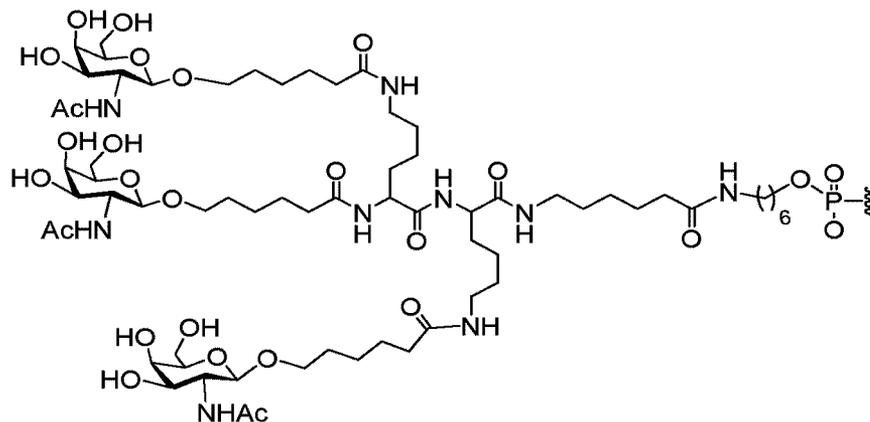


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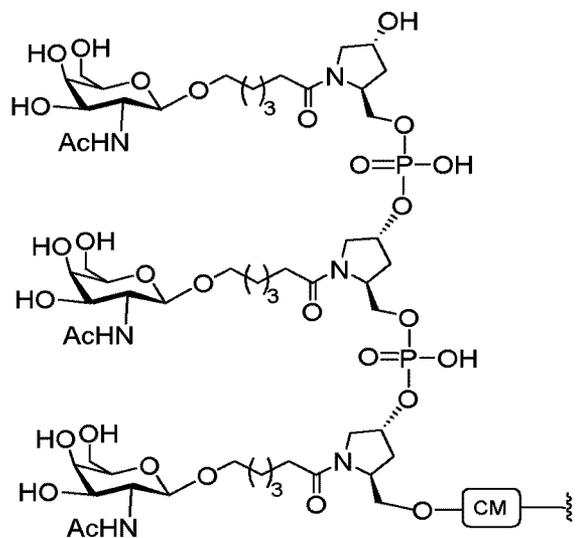
In certain such embodiments, conjugate groups have the following structure:



In certain such embodiments, conjugate groups have the following structure:

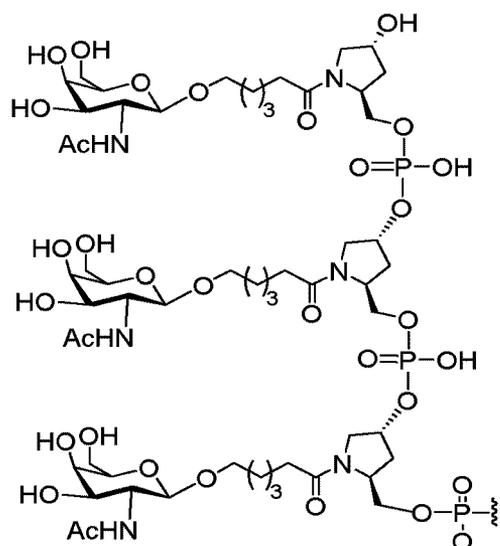


In certain such embodiments, conjugate groups have the following structure:

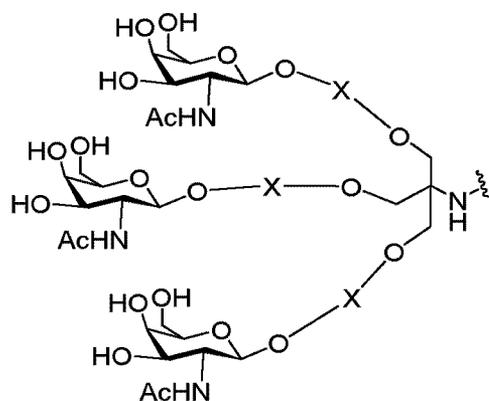


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In certain such embodiments, conjugate groups have the following structure:

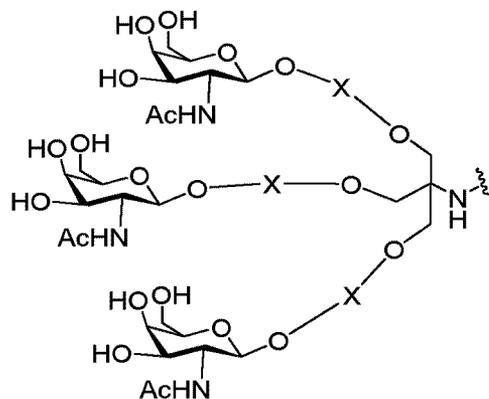


In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



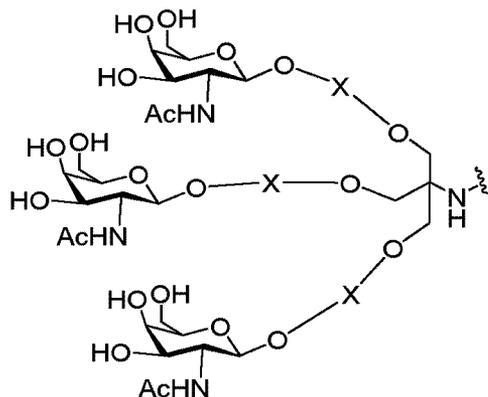
wherein X is a substituted or unsubstituted tether of six to eleven consecutively bonded atoms.

5 In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



wherein X is a substituted or unsubstituted tether of ten consecutively bonded atoms.

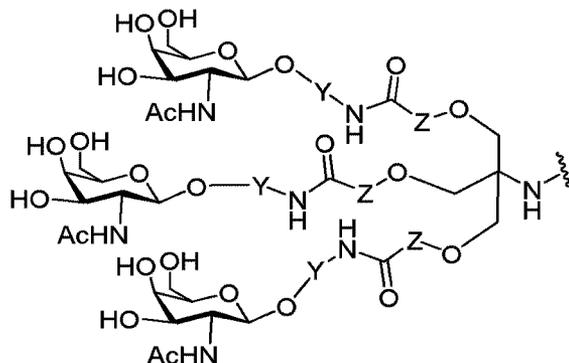
In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



wherein X is a substituted or unsubstituted tether of four to eleven consecutively bonded atoms and wherein the tether comprises exactly one amide bond.

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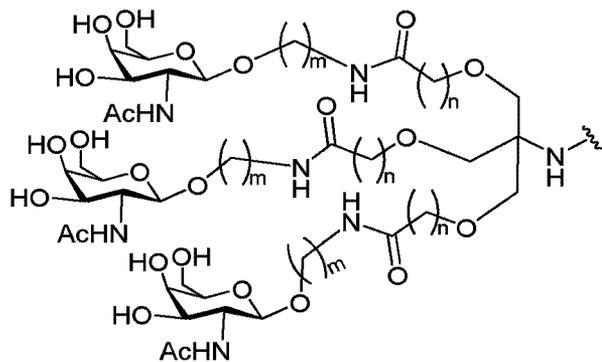
In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



wherein Y and Z are independently selected from a C₁-C₁₂ substituted or unsubstituted alkyl, alkenyl, or alkynyl group, or a group comprising an ether, a ketone, an amide, an ester, a carbamate, an amine, a piperidine, a phosphate, a phosphodiester, a phosphorothioate, a triazole, a pyrrolidine, a disulfide, or a thioether.

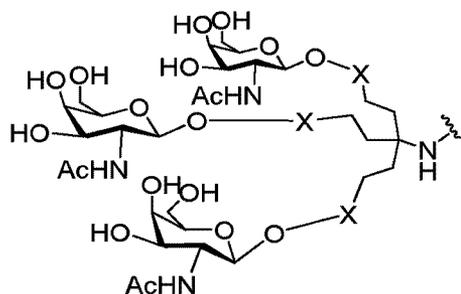
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In certain such embodiments, the cell-targeting moiety of the conjugate group has the following structure:



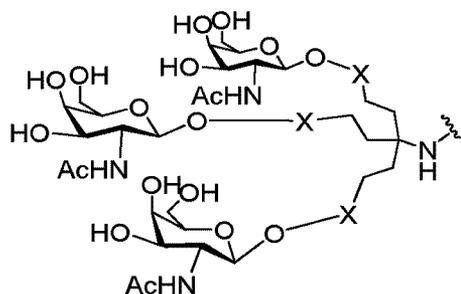
wherein m is 4, 5, 6, 7, or 8, and n is 1, 2, 3, or 4.

In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



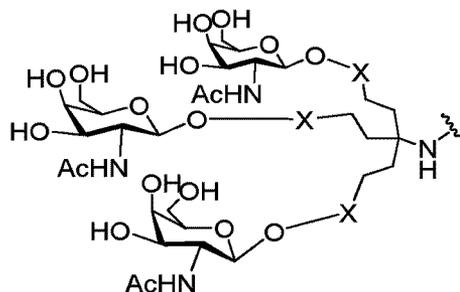
- 5 wherein X is a substituted or unsubstituted tether of four to thirteen consecutively bonded atoms, and wherein X does not comprise an ether group.

In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



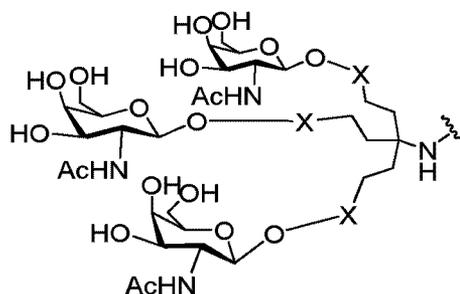
- 10 wherein X is a substituted or unsubstituted tether of eight consecutively bonded atoms, and wherein X does not comprise an ether group.

In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



wherein X is a substituted or unsubstituted tether of four to thirteen consecutively bonded atoms, and wherein the tether comprises exactly one amide bond, and wherein X does not comprise an ether group.

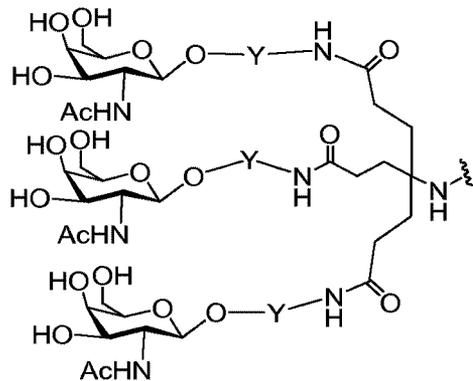
In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



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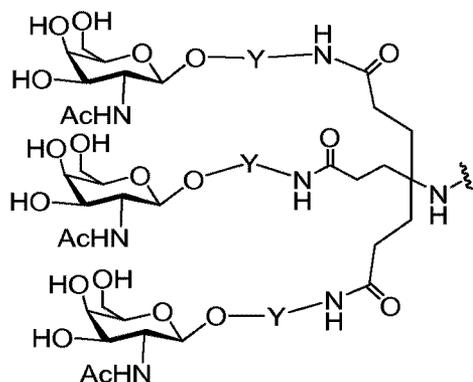
wherein X is a substituted or unsubstituted tether of four to thirteen consecutively bonded atoms and wherein the tether consists of an amide bond and a substituted or unsubstituted C₂-C₁₁ alkyl group.

In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



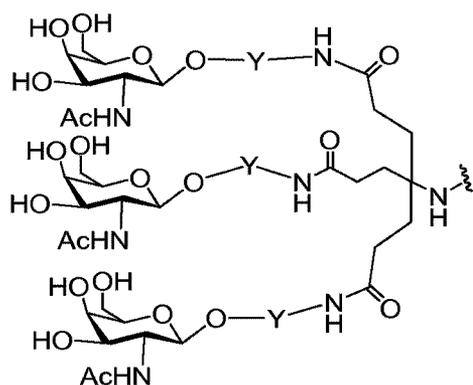
- 10 wherein Y is selected from a C₁-C₁₂ substituted or unsubstituted alkyl, alkenyl, or alkynyl group, or a group comprising an ether, a ketone, an amide, an ester, a carbamate, an amine, a piperidine, a phosphate, a phosphodiester, a phosphorothioate, a triazole, a pyrrolidine, a disulfide, or a thioether.

In certain such embodiments, the cell-targeting moiety of the conjugate group has the following structure:



wherein Y is selected from a C₁-C₁₂ substituted or unsubstituted alkyl group, or a group comprising an ether, an amine, a piperidine, a phosphate, a phosphodiester, or a phosphorothioate.

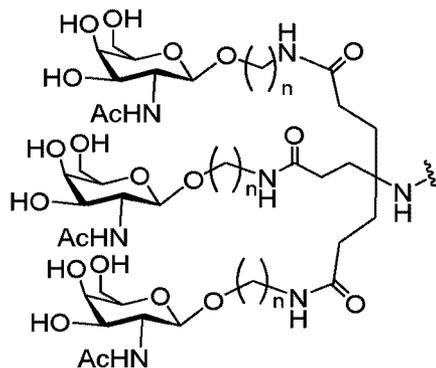
In certain such embodiments, the cell-targeting moiety of the conjugate group has the following structure:



5

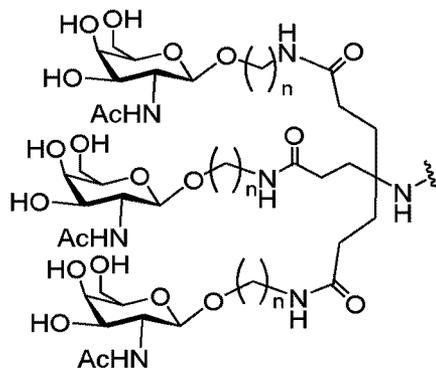
wherein Y is selected from a C₁-C₁₂ substituted or unsubstituted alkyl group.

In certain such embodiments, the cell-targeting moiety of the conjugate group has the following structure:



Wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12.

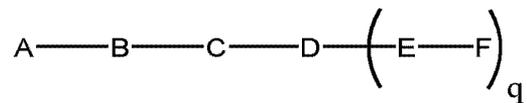
10 In certain such embodiments, the cell-targeting moiety of the conjugate group has the following structure:



wherein n is 4, 5, 6, 7, or 8.

b. Certain conjugated antisense compounds

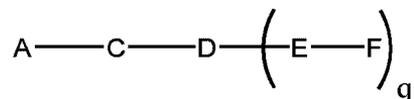
- 5 In certain embodiments, the conjugates are bound to a nucleoside of the antisense oligonucleotide at the 2', 3', or 5' position of the nucleoside. In certain embodiments, a conjugated antisense compound has the following structure:



- 10 wherein
 A is the antisense oligonucleotide;
 B is the cleavable moiety
 C is the conjugate linker
 D is the branching group
 15 each E is a tether;
 each F is a ligand; and
 q is an integer between 1 and 5.

In certain embodiments, a conjugated antisense compound has the following structure:

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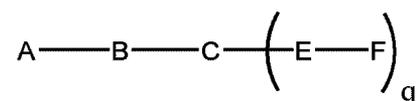
- wherein
 A is the antisense oligonucleotide;
 C is the conjugate linker

D is the branching group
 each E is a tether;
 each F is a ligand; and
 q is an integer between 1 and 5.

- 5 In certain such embodiments, the conjugate linker comprises at least one cleavable bond.
 In certain such embodiments, the branching group comprises at least one cleavable bond.
 In certain embodiments each tether comprises at least one cleavable bond.

10 In certain embodiments, the conjugates are bound to a nucleoside of the antisense oligonucleotide at the 2', 3', of 5' position of the nucleoside.

In certain embodiments, a conjugated antisense compound has the following structure:

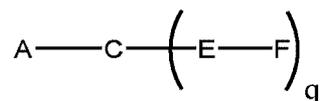


wherein

- 15 A is the antisense oligonucleotide;
 B is the cleavable moiety
 C is the conjugate linker
 each E is a tether;
 each F is a ligand; and
 20 q is an integer between 1 and 5.

In certain embodiments, the conjugates are bound to a nucleoside of the antisense oligonucleotide at the 2', 3', of 5' position of the nucleoside. In certain embodiments, a conjugated antisense compound has the following structure:

25

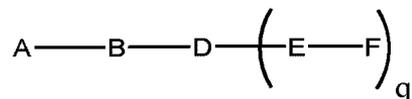


wherein

- A is the antisense oligonucleotide;
 C is the conjugate linker
 30 each E is a tether;
 each F is a ligand; and

q is an integer between 1 and 5.

In certain embodiments, a conjugated antisense compound has the following structure:



5

wherein

A is the antisense oligonucleotide;

B is the cleavable moiety

D is the branching group

10

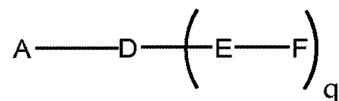
each E is a tether;

each F is a ligand; and

q is an integer between 1 and 5.

In certain embodiments, a conjugated antisense compound has the following structure:

15



wherein

A is the antisense oligonucleotide;

D is the branching group

20

each E is a tether;

each F is a ligand; and

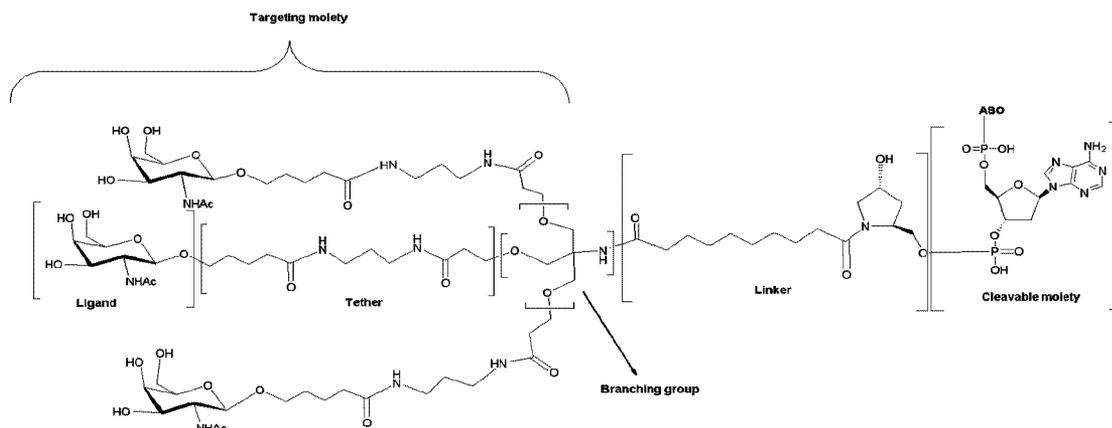
q is an integer between 1 and 5.

In certain such embodiments, the conjugate linker comprises at least one cleavable bond.

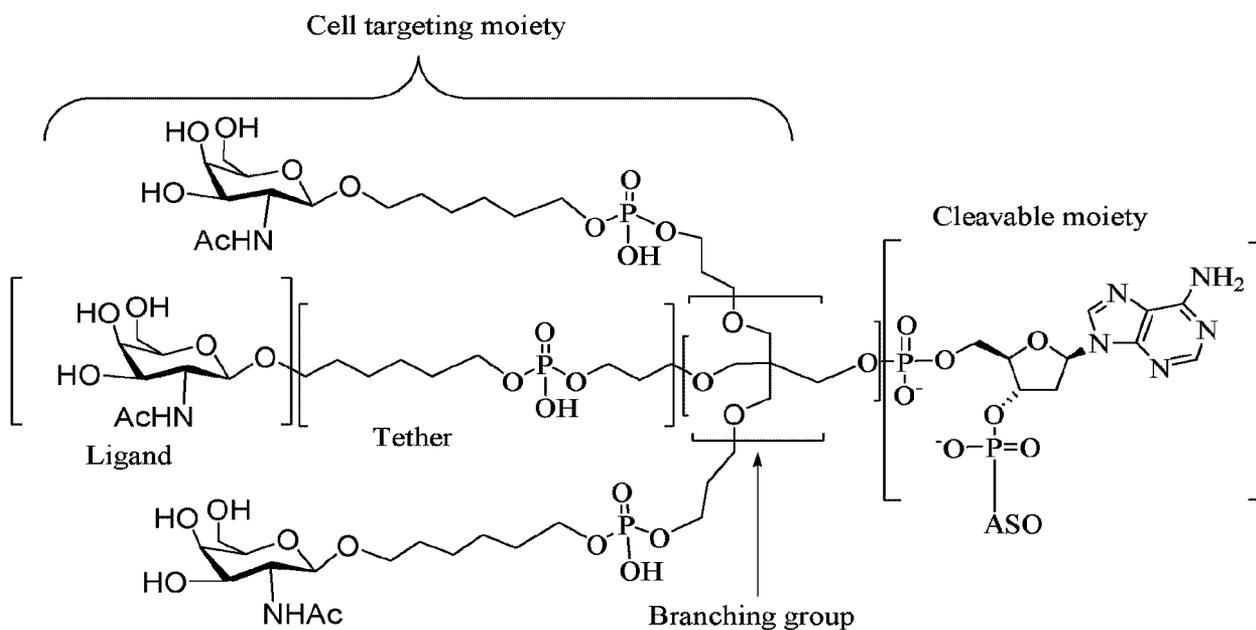
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In certain embodiments each tether comprises at least one cleavable bond.

In certain embodiments, a conjugated antisense compound has a structure selected from among the following:

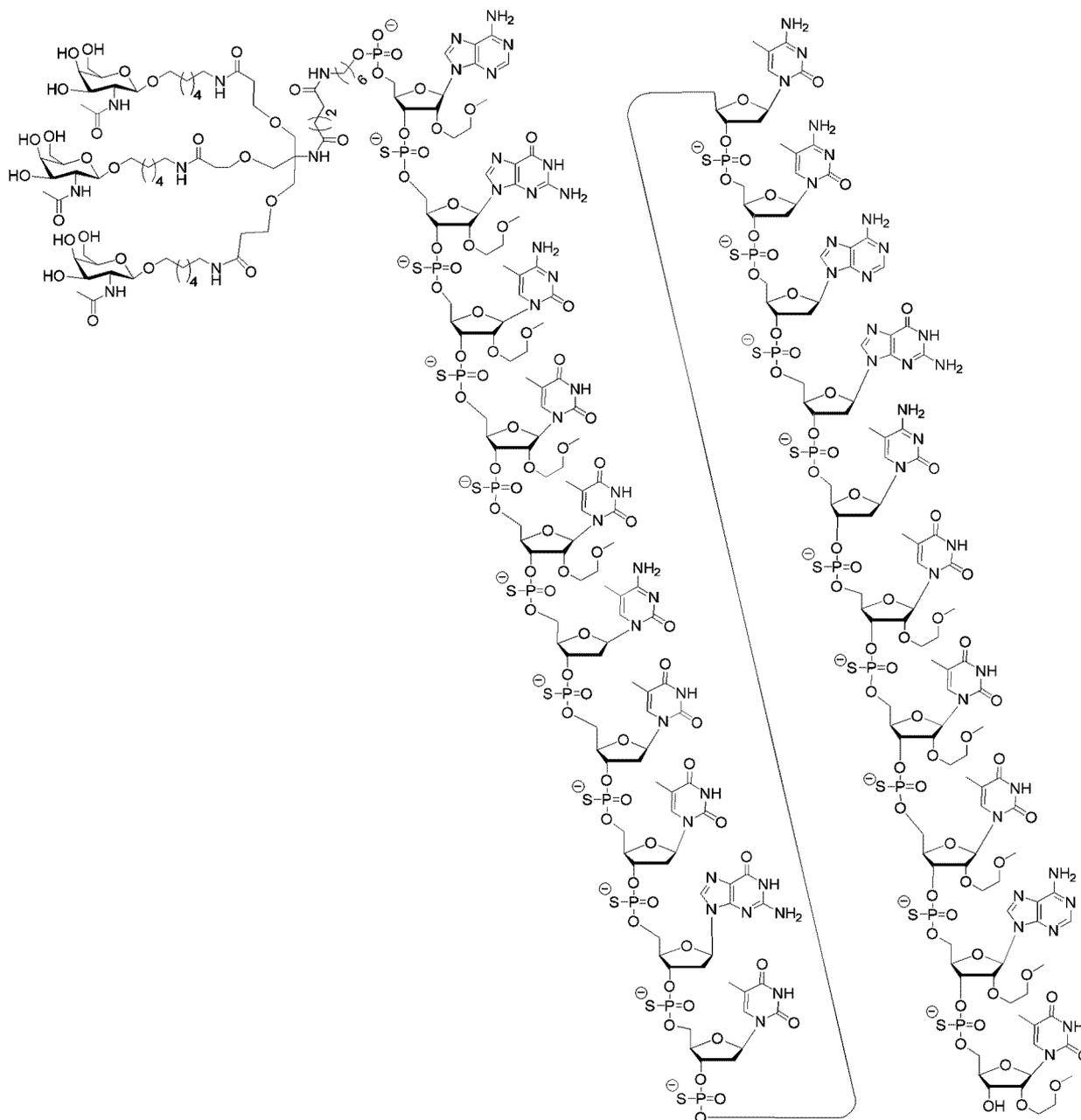


In certain embodiments, a conjugated antisense compound has a structure selected from among the following:



5

In certain embodiments, a conjugated antisense compound has a structure selected from among the following:



Representative United States patents, United States patent application publications, and international patent application publications that teach the preparation of certain of the above noted conjugates, conjugated antisense compounds, tethers, linkers, branching groups, ligands, cleavable moieties as well as other modifications include without limitation, US 5,994,517, US 6,300,319, US 6,660,720, US 6,906,182, US 7,262,177, US 7,491,805, US 8,106,022, US 7,723,509, US 2006/0148740, US 2011/0123520, WO 2013/033230 and WO 2012/037254.

Representative publications that teach the preparation of certain of the above noted conjugates, conjugated antisense compounds, tethers, linkers, branching groups, ligands, cleavable moieties as well as

other modifications include without limitation, BIESSEN et al., "The Cholesterol Derivative of a Triantennary Galactoside with High Affinity for the Hepatic Asialoglycoprotein Receptor: a Potent Cholesterol Lowering Agent" *J. Med. Chem.* (1995) 38:1846-1852, BIESSEN et al., "Synthesis of Cluster Galactosides with High Affinity for the Hepatic Asialoglycoprotein Receptor" *J. Med. Chem.* (1995) 38:1538-1546, LEE et al., "New and more efficient multivalent glyco-ligands for asialoglycoprotein receptor of mammalian hepatocytes" *Bioorganic & Medicinal Chemistry* (2011) 19:2494-2500, RENSEN et al., "Determination of the Upper Size Limit for Uptake and Processing of Ligands by the Asialoglycoprotein Receptor on Hepatocytes in Vitro and in Vivo" *J. Biol. Chem.* (2001) 276(40):37577-37584, RENSEN et al., "Design and Synthesis of Novel N-Acetylgalactosamine-Terminated Glycolipids for Targeting of Lipoproteins to the Hepatic Asialoglycoprotein Receptor" *J. Med. Chem.* (2004) 47:5798-5808, SLIEDREGT et al., "Design and Synthesis of Novel Amphiphilic Dendritic Galactosides for Selective Targeting of Liposomes to the Hepatic Asialoglycoprotein Receptor" *J. Med. Chem.* (1999) 42:609-618, and Valentijn *et al.*, "Solid-phase synthesis of lysine-based cluster galactosides with high affinity for the Asialoglycoprotein Receptor" *Tetrahedron*, 1997, 53(2), 759-770.

In certain embodiments, conjugated antisense compounds comprise an RNase H based oligonucleotide (such as a gapmer) or a splice modulating oligonucleotide (such as a fully modified oligonucleotide) and any conjugate group comprising at least one, two, or three GalNAc groups. In certain embodiments a conjugated antisense compound comprises any conjugate group found in any of the following references: Lee, *Carbohydr Res*, 1978, 67, 509-514; Connolly et al., *J Biol Chem*, 1982, 257, 939-945; Pavia et al., *Int J Pep Protein Res*, 1983, 22, 539-548; Lee et al., *Biochem*, 1984, 23, 4255-4261; Lee et al., *Glycoconjugate J*, 1987, 4, 317-328; Toyokuni et al., *Tetrahedron Lett*, 1990, 31, 2673-2676; Biessen et al., *J Med Chem*, 1995, 38, 1538-1546; Valentijn et al., *Tetrahedron*, 1997, 53, 759-770; Kim et al., *Tetrahedron Lett*, 1997, 38, 3487-3490; Lee et al., *Bioconjug Chem*, 1997, 8, 762-765; Kato et al., *Glycobiol*, 2001, 11, 821-829; Rensen et al., *J Biol Chem*, 2001, 276, 37577-37584; Lee et al., *Methods Enzymol*, 2003, 362, 38-43; Westerlind et al., *Glycoconj J*, 2004, 21, 227-241; Lee et al., *Bioorg Med Chem Lett*, 2006, 16(19), 5132-5135; Maierhofer et al., *Bioorg Med Chem*, 2007, 15, 7661-7676; Khorev et al., *Bioorg Med Chem*, 2008, 16, 5216-5231; Lee et al., *Bioorg Med Chem*, 2011, 19, 2494-2500; Kornilova et al., *Analyt Biochem*, 2012, 425, 43-46; Pujol et al., *Angew Chemie Int Ed Engl*, 2012, 51, 7445-7448; Biessen et al., *J Med Chem*, 1995, 38, 1846-1852; Sliedregt et al., *J Med Chem*, 1999, 42, 609-618; Rensen et al., *J Med Chem*, 2004, 47, 5798-5808; Rensen et al., *Arterioscler Thromb Vasc Biol*, 2006, 26, 169-175; van Rossenberg et al., *Gene Ther*, 2004, 11, 457-464; Sato et al., *J Am Chem Soc*, 2004, 126, 14013-14022; Lee et al., *J Org Chem*, 2012, 77, 7564-7571; Biessen et al., *FASEB J*, 2000, 14, 1784-1792; Rajur et al., *Bioconjug Chem*, 1997, 8, 935-940; Duff et al., *Methods Enzymol*, 2000, 313, 297-321; Maier et al., *Bioconjug Chem*, 2003, 14, 18-29; Jayaprakash et al., *Org Lett*, 2010, 12, 5410-5413; Manoharan, *Antisense Nucleic Acid Drug Dev*, 2002, 12, 103-128; Merwin et al., *Bioconjug Chem*, 1994, 5, 612-620; Tomiya et al., *Bioorg Med Chem*, 2013, 21,

5275-5281; International applications WO1998/013381; WO2011/038356; WO1997/046098; WO2008/098788; WO2004/101619; WO2012/037254; WO2011/120053; WO2011/100131; WO2011/163121; WO2012/177947; WO2013/033230; WO2013/075035; WO2012/083185; WO2012/083046; WO2009/082607; WO2009/134487; WO2010/144740; WO2010/148013; 5 WO1997/020563; WO2010/088537; WO2002/043771; WO2010/129709; WO2012/068187; WO2009/126933; WO2004/024757; WO2010/054406; WO2012/089352; WO2012/089602; WO2013/166121; WO2013/165816; U.S. Patents 4,751,219; 8,552,163; 6,908,903; 7,262,177; 5,994,517; 6,300,319; 8,106,022; 7,491,805; 7,491,805; 7,582,744; 8,137,695; 6,383,812; 6,525,031; 6,660,720; 7,723,509; 8,541,548; 8,344,125; 8,313,772; 8,349,308; 8,450,467; 8,501,930; 8,158,601; 7,262,177; 10 6,906,182; 6,620,916; 8,435,491; 8,404,862; 7,851,615; Published U.S. Patent Application Publications US2011/0097264; US2011/0097265; US2013/0004427; US2005/0164235; US2006/0148740; US2008/0281044; US2010/0240730; US2003/0119724; US2006/0183886; US2008/0206869; US2011/0269814; US2009/0286973; US2011/0207799; US2012/0136042; US2012/0165393; US2008/0281041; US2009/0203135; US2012/0035115; US2012/0095075; US2012/0101148; 15 US2012/0128760; US2012/0157509; US2012/0230938; US2013/0109817; US2013/0121954; US2013/0178512; US2013/0236968; US2011/0123520; US2003/0077829; US2008/0108801; and US2009/0203132.

C. Certain Uses and Features

20 In certain embodiments, conjugated antisense compounds exhibit potent target RNA reduction *in vivo*. In certain embodiments, unconjugated antisense compounds accumulate in the kidney. In certain embodiments, conjugated antisense compounds accumulate in the liver. In certain embodiments, conjugated antisense compounds are well tolerated. Such properties render conjugated antisense compounds particularly useful for inhibition of many target RNAs, including, but not limited to those involved in metabolic, cardiovascular and other diseases, disorders or conditions. Thus, provided herein are methods of treating 25 such diseases, disorders or conditions by contacting liver tissues with the conjugated antisense compounds targeted to RNAs associated with such diseases, disorders or conditions. Thus, also provided are methods for ameliorating any of a variety of metabolic, cardiovascular and other diseases, disorders or conditions with the conjugated antisense compounds of the present invention.

30 In certain embodiments, conjugated antisense compounds are more potent than unconjugated counterpart at a particular tissue concentration. Without wishing to be bound by any theory or mechanism, in certain embodiments, the conjugate may allow the conjugated antisense compound to enter the cell more efficiently or to enter the cell more productively. For example, in certain embodiments conjugated antisense compounds may exhibit greater target reduction as compared to its unconjugated counterpart wherein both the conjugated antisense compound and its unconjugated counterpart are present in the tissue at the same

concentrations. For example, in certain embodiments conjugated antisense compounds may exhibit greater target reduction as compared to its unconjugated counterpart wherein both the conjugated antisense compound and its unconjugated counterpart are present in the liver at the same concentrations.

Productive and non-productive uptake of oligonucleotides has been discussed previously (*See e.g.* 5 Geary, R. S., E. Wancewicz, et al. (2009). "Effect of Dose and Plasma Concentration on Liver Uptake and Pharmacologic Activity of a 2'-Methoxyethyl Modified Chimeric Antisense Oligonucleotide Targeting PTEN." *Biochem. Pharmacol.* 78(3): 284-91; & Koller, E., T. M. Vincent, et al. (2011). "Mechanisms of single-stranded phosphorothioate modified antisense oligonucleotide accumulation in hepatocytes." *Nucleic Acids Res.* 39(11): 4795-807). Conjugate groups described herein may improve productive uptake.

10 In certain embodiments, the conjugate groups described herein may further improve potency by increasing the affinity of the conjugated antisense compound for a particular type of cell or tissue. In certain embodiments, the conjugate groups described herein may further improve potency by increasing recognition of the conjugated antisense compound by one or more cell-surface receptors. . In certain embodiments, the conjugate groups described herein may further improve potency by facilitating endocytosis of the conjugated 15 antisense compound.

In certain embodiments, the cleavable moiety may further improve potency by allowing the conjugate to be cleaved from the antisense oligonucleotide after the conjugated antisense compound has entered the cell. Accordingly, in certain embodiments, conjugated antisense compounds can be administered at doses lower than would be necessary for unconjugated antisense oligonucleotides.

20 Phosphorothioate linkages have been incorporated into antisense oligonucleotides previously. Such phosphorothioate linkages are resistant to nucleases and so improve stability of the oligonucleotide. Further, phosphorothioate linkages also bind certain proteins, which results in accumulation of antisense oligonucleotide in the liver. Oligonucleotides with fewer phosphorothioate linkages accumulate less in the liver and more in the kidney (see, for example, Geary, R., "Pharmacokinetic Properties of 2'-O-(2- 25 Methoxyethyl)-Modified Oligonucleotide Analogs in Rats," *Journal of Pharmacology and Experimental Therapeutics*, Vol. 296, No. 3, 890-897; & *Pharmacological Properties of 2'-O-Methoxyethyl Modified Oligonucleotides* in *Antisense a Drug Technology*, Chapter 10, Crooke, S.T., ed., 2008) In certain 30 embodiments, oligonucleotides with fewer phosphorothioate internucleoside linkages and more phosphodiester internucleoside linkages accumulate less in the liver and more in the kidney. When treating diseases in the liver, this is undesirable for several reasons (1) less drug is getting to the site of desired action (liver); (2) drug is escaping into the urine; and (3) the kidney is exposed to relatively high concentration of drug which can result in toxicities in the kidney. Thus, for liver diseases, phosphorothioate linkages provide important benefits.

In certain embodiments, however, administration of oligonucleotides uniformly linked by phosphorothioate internucleoside linkages induces one or more proinflammatory reactions. (see for example: *J Lab Clin Med.* 1996 Sep;128(3):329-38. “Amplification of antibody production by phosphorothioate oligodeoxynucleotides”. Branda et al.; and see also for example: *Toxicologic Properties in Antisense a Drug Technology*, Chapter 12, pages 342-351, Crooke, S.T., ed., 2008). In certain embodiments, administration of oligonucleotides wherein most of the internucleoside linkages comprise phosphorothioate internucleoside linkages induces one or more proinflammatory reactions.

In certain embodiments, the degree of proinflammatory effect may depend on several variables (e.g. backbone modification, off-target effects, nucleobase modifications, and/or nucleoside modifications) see for example: *Toxicologic Properties in Antisense a Drug Technology*, Chapter 12, pages 342-351, Crooke, S.T., ed., 2008). In certain embodiments, the degree of proinflammatory effect may be mitigated by adjusting one or more variables. For example the degree of proinflammatory effect of a given oligonucleotide may be mitigated by replacing any number of phosphorothioate internucleoside linkages with phosphodiester internucleoside linkages and thereby reducing the total number of phosphorothioate internucleoside linkages.

In certain embodiments, it would be desirable to reduce the number of phosphorothioate linkages, if doing so could be done without losing stability and without shifting the distribution from liver to kidney. For example, in certain embodiments, the number of phosphorothioate linkages may be reduced by replacing phosphorothioate linkages with phosphodiester linkages. In such an embodiment, the antisense compound having fewer phosphorothioate linkages and more phosphodiester linkages may induce less proinflammatory reactions or no proinflammatory reaction. Although the the antisense compound having fewer phosphorothioate linkages and more phosphodiester linkages may induce fewer proinflammatory reactions, the antisense compound having fewer phosphorothioate linkages and more phosphodiester linkages may not accumulate in the liver and may be less efficacious at the same or similar dose as compared to an antisense compound having more phosphorothioate linkages. In certain embodiments, it is therefore desirable to design an antisense compound that has a plurality of phosphodiester bonds and a plurality of phosphorothioate bonds but which also possesses stability and good distribution to the liver.

In certain embodiments, conjugated antisense compounds accumulate more in the liver and less in the kidney than unconjugated counterparts, even when some of the phosphorothioate linkages are replaced with less proinflammatory phosphodiester internucleoside linkages. In certain embodiments, conjugated antisense compounds accumulate more in the liver and are not excreted as much in the urine compared to its unconjugated counterparts, even when some of the phosphorothioate linkages are replaced with less proinflammatory phosphodiester internucleoside linkages. In certain embodiments, the use of a conjugate allows one to design more potent and better tolerated antisense drugs. Indeed, in certain emobidments, conjugated antisense compounds have larger therapeutic indexes than unconjugated counterparts. This allows the conjugated antisense compound to be administered at a higher absolute dose, because there is less risk of proinflammatory response and less risk of kidney toxicity. This higher dose, allows one to dose less

frequently, since the clearance (metabolism) is expected to be similar. Further, because the compound is more potent, as described above, one can allow the concentration to go lower before the next dose without losing therapeutic activity, allowing for even longer periods between dosing.

5 In certain embodiments, the inclusion of some phosphorothioate linkages remains desirable. For example, the terminal linkages are vulnerable to exonucleases and so in certain embodiments, those linkages are phosphorothioate or other modified linkage. Internucleoside linkages linking two deoxynucleosides are vulnerable to endonucleases and so in certain embodiments those those linkages are phosphorothioate or other modified linkage. Internucleoside linkages between a modified nucleoside and a deoxynucleoside where the deoxynucleoside is on the 5' side of the linkage deoxynucleosides are vulnerable to endonucleases and so in certain embodiments those those linkages are phosphorothioate or other modified linkage. Internucleoside linkages between two modified nucleosides of certain types and between a deoxynucleoside and a modified nucleoside of certain typ where the modified nucleoside is at the 5' side of the linkage are sufficiently resistant to nuclease digestion, that the linkage can be phosphodiester.

15 In certain embodiments, the antisense oligonucleotide of a conjugated antisense compound comprises fewer than 16 phosphorothioate linkages. In certain embodiments, the antisense oligonucleotide of a conjugated antisense compound comprises fewer than 15 phosphorothioate linkages. In certain embodiments, the antisense oligonucleotide of a conjugated antisense compound comprises fewer than 14 phosphorothioate linkages. In certain embodiments, the antisense oligonucleotide of a conjugated antisense compound comprises fewer than 13 phosphorothioate linkages. In certain embodiments, the antisense oligonucleotide of a conjugated antisense compound comprises fewer than 12 phosphorothioate linkages. In certain embodiments, the antisense oligonucleotide of a conjugated antisense compound comprises fewer than 11 phosphorothioate linkages. In certain embodiments, the antisense oligonucleotide of a conjugated antisense compound comprises fewer than 10 phosphorothioate linkages. In certain embodiments, the antisense oligonucleotide of a conjugated antisense compound comprises fewer than 9 phosphorothioate linkages. In certain embodiments, the antisense oligonucleotide of a conjugated antisense compound comprises fewer than 8 phosphorothioate linkages.

20 In certain embodiments, antisense compounds comprising one or more conjugate group described herein has increased activity and/or potency and/or tolerability compared to a parent antisense compound lacking such one or more conjugate group. Accordingly, in certain embodiments, attachment of such conjugate groups to an oligonucleotide is desirable. Such conjugate groups may be attached at the 5'-, and/or 3'- end of an oligonucleotide. In certain instances, attachment at the 5'-end is synthetically desirable. Typically, oligonucleotides are synthesized by attachment of the 3' terminal nucleoside to a solid support and sequential coupling of nucleosides from 3' to 5' using techniques that are well known in the art. Accordingly if a conjugate group is desired at the 3'-terminus, one may (1) attach the conjugate group to the

3'-terminal nucleoside and attach that conjugated nucleoside to the solid support for subsequent preparation of the oligonucleotide or (2) attach the conjugate group to the 3'-terminal nucleoside of a completed oligonucleotide after synthesis. Neither of these approaches is very efficient and thus both are costly. In particular, attachment of the conjugated nucleoside to the solid support, while demonstrated in the Examples
5 herein, is an inefficient process. In certain embodiments, attaching a conjugate group to the 5'-terminal nucleoside is synthetically easier than attachment at the 3'-end. One may attach a non-conjugated 3' terminal nucleoside to the solid support and prepare the oligonucleotide using standard and well characterized reactions. One then needs only to attach a 5' nucleoside having a conjugate group at the final coupling step. In certain embodiments, this is more efficient than attaching a conjugated nucleoside directly to the solid
10 support as is typically done to prepare a 3'-conjugated oligonucleotide. The Examples herein demonstrate attachment at the 5'-end. In addition, certain conjugate groups have synthetic advantages. For Example, certain conjugate groups comprising phosphorus linkage groups are synthetically simpler and more efficiently prepared than other conjugate groups, including conjugate groups reported previously (e.g., WO/2012/037254).

15 In certain embodiments, conjugated antisense compounds are administered to a subject. In such embodiments, antisense compounds comprising one or more conjugate groups described herein has increased activity and/or potency and/or tolerability compared to a parent antisense compound lacking such one or more conjugate groups. Without being bound by mechanism, it is believed that the conjugate group helps with distribution, delivery, and/or uptake into a target cell or tissue. In certain embodiments, once inside the target
20 cell or tissue, it is desirable that all or part of the conjugate group to be cleaved to release the active oligonucleotide. In certain embodiments, it is not necessary that the entire conjugate group be cleaved from the oligonucleotide. For example, in Example 20 a conjugated oligonucleotide was administered to mice and a number of different chemical species, each comprising a different portion of the conjugate group remaining on the oligonucleotide, were detected (Table 23a). This conjugated antisense compound demonstrated good
25 potency (Table 23). Thus, in certain embodiments, such metabolite profile of multiple partial cleavage of the conjugate group does not interfere with activity/potency. Nevertheless, in certain embodiments it is desirable that a prodrug (conjugated oligonucleotide) yield a single active compound. In certain instances, if multiple forms of the active compound are found, it may be necessary to determine relative amounts and activities for each one. In certain embodiments where regulatory review is required (e.g., USFDA or counterpart) it is
30 desirable to have a single (or predominantly single) active species. In certain such embodiments, it is desirable that such single active species be the antisense oligonucleotide lacking any portion of the conjugate group. In certain embodiments, conjugate groups at the 5'-end are more likely to result in complete metabolism of the conjugate group. Without being bound by mechanism it may be that endogenous enzymes responsible for metabolism at the 5' end (e.g., 5' nucleases) are more active/efficient than the 3' counterparts.
35 In certain embodiments, the specific conjugate groups are more amenable to metabolism to a single active

species. In certain embodiments, certain conjugate groups are more amenable to metabolism to the oligonucleotide.

D. Antisense

In certain embodiments, oligomeric compounds of the present invention are antisense compounds.

5 In such embodiments, the oligomeric compound is complementary to a target nucleic acid. In certain embodiments, a target nucleic acid is an RNA. In certain embodiments, a target nucleic acid is a non-coding RNA. In certain embodiments, a target nucleic acid encodes a protein. In certain embodiments, a target nucleic acid is selected from a mRNA, a pre-mRNA, a microRNA, a non-coding RNA, including small non-coding RNA, and a promoter-directed RNA. In certain embodiments, oligomeric compounds are at least
10 partially complementary to more than one target nucleic acid. For example, oligomeric compounds of the present invention may be microRNA mimics, which typically bind to multiple targets.

In certain embodiments, antisense compounds comprise a portion having a nucleobase sequence at least 70% complementary to the nucleobase sequence of a target nucleic acid. In certain embodiments, antisense compounds comprise a portion having a nucleobase sequence at least 80% complementary to the
15 nucleobase sequence of a target nucleic acid. In certain embodiments, antisense compounds comprise a portion having a nucleobase sequence at least 90% complementary to the nucleobase sequence of a target nucleic acid. In certain embodiments, antisense compounds comprise a portion having a nucleobase sequence at least 95% complementary to the nucleobase sequence of a target nucleic acid. In certain
20 embodiments, antisense compounds comprise a portion having a nucleobase sequence at least 98% complementary to the nucleobase sequence of a target nucleic acid. In certain embodiments, antisense compounds comprise a portion having a nucleobase sequence that is 100% complementary to the nucleobase sequence of a target nucleic acid. In certain embodiments, antisense compounds are at least 70%, 80%, 90%, 95%, 98%, or 100% complementary to the nucleobase sequence of a target nucleic acid over the entire length of the antisense compound.

25 Antisense mechanisms include any mechanism involving the hybridization of an oligomeric compound with target nucleic acid, wherein the hybridization results in a biological effect. In certain embodiments, such hybridization results in either target nucleic acid degradation or occupancy with concomitant inhibition or stimulation of the cellular machinery involving, for example, translation, transcription, or polyadenylation of the target nucleic acid or of a nucleic acid with which the target nucleic
30 acid may otherwise interact.

One type of antisense mechanism involving degradation of target RNA is RNase H mediated antisense. RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. It is known in the art that single-stranded antisense compounds which are "DNA-like" elicit RNase H activity in

mammalian cells. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of DNA-like oligonucleotide-mediated inhibition of gene expression.

Antisense mechanisms also include, without limitation RNAi mechanisms, which utilize the RISC pathway. Such RNAi mechanisms include, without limitation siRNA, ssRNA and microRNA mechanisms.
5 Such mechanisms include creation of a microRNA mimic and/or an anti-microRNA.

Antisense mechanisms also include, without limitation, mechanisms that hybridize or mimic non-coding RNA other than microRNA or mRNA. Such non-coding RNA includes, but is not limited to promoter-directed RNA and short and long RNA that effects transcription or translation of one or more nucleic acids.

10 In certain embodiments, oligonucleotides comprising conjugates described herein are RNAi compounds. In certain embodiments, oligomeric oligonucleotides comprising conjugates described herein are ssRNA compounds. In certain embodiments, oligonucleotides comprising conjugates described herein are paired with a second oligomeric compound to form an siRNA. In certain such embodiments, the second oligomeric compound also comprises a conjugate. In certain embodiments, the second oligomeric compound
15 is any modified or unmodified nucleic acid. In certain embodiments, the oligonucleotides comprising conjugates described herein is the antisense strand in an siRNA compound. In certain embodiments, the oligonucleotides comprising conjugates described herein is the sense strand in an siRNA compound. In embodiments in which the conjugated oligomeric compound is double-stranded siRNA, the conjugate may be on the sense strand, the antisense strand or both the sense strand and the antisense strand.

20 **D. Apolipoprotein C-III (apoCIII)**

In certain embodiments, conjugated antisense compounds target any ApoCIII nucleic acid. In certain embodiments, the target nucleic acid encodes an ApoCIII target protein that is clinically relevant. In such embodiments, modulation of the target nucleic acid results in clinical benefit.

25 The targeting process usually includes determination of at least one target region, segment, or site within the target nucleic acid for the antisense interaction to occur such that the desired effect will result.

In certain embodiments, a target region is a structurally defined region of the nucleic acid. For example, in certain such embodiments, a target region may encompass a 3' UTR, a 5' UTR, an exon, an intron, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region or target segment.

30 In certain embodiments, a target segment is at least about an 8-nucleobase portion of a target region to which a conjugated antisense compound is targeted. Target segments can include DNA or RNA sequences that comprise at least 8 consecutive nucleobases from the 5'-terminus of one of the target segments (the remaining nucleobases being a consecutive stretch of the same DNA or RNA beginning immediately

upstream of the 5'-terminus of the target segment and continuing until the DNA or RNA comprises about 8 to about 30 nucleobases). Target segments are also represented by DNA or RNA sequences that comprise at least 8 consecutive nucleobases from the 3'-terminus of one of the target segments (the remaining nucleobases being a consecutive stretch of the same DNA or RNA beginning immediately downstream of the 3'-terminus of the target segment and continuing until the DNA or RNA comprises about 8 to about 30 nucleobases). Target segments can also be represented by DNA or RNA sequences that comprise at least 8 consecutive nucleobases from an internal portion of the sequence of a target segment, and may extend in either or both directions until the conjugated antisense compound comprises about 8 to about 30 nucleobases.

In certain embodiments, antisense compounds targeted to an ApoCIII nucleic acid can be modified as described herein. In certain embodiments, the antisense compounds can have a modified sugar moiety, an unmodified sugar moiety or a mixture of modified and unmodified sugar moieties as described herein. In certain embodiments, the antisense compounds can have a modified internucleoside linkage, an unmodified internucleoside linkage or a mixture of modified and unmodified internucleoside linkages as described herein. In certain embodiments, the antisense compounds can have a modified nucleobase, an unmodified nucleobase or a mixture of modified and unmodified nucleobases as described herein. In certain embodiments, the antisense compounds can have a motif as described herein.

In certain embodiments, antisense compounds targeted to ApoCIII nucleic acids can be conjugated as described herein.

ApoCIII is a constituent of HDL and of triglyceride (TG)-rich lipoproteins. Elevated ApoCIII levels are associated with elevated TG levels and diseases such as cardiovascular disease, metabolic syndrome, obesity and diabetes. Elevated TG levels are associated with pancreatitis. ApoCIII slows clearance of TG-rich lipoproteins by inhibiting lipolysis through inhibition of lipoprotein lipase (LPL) and through interfering with lipoprotein binding to cell-surface glycosaminoglycan matrix. Antisense compounds targeting ApoCIII have been previously disclosed in WO2004/093783 and WO2012/149495, each herein incorporated by reference in its entirety.

Certain Conjugated Antisense Compounds Targeted to an ApoCIII Nucleic Acid

In certain embodiments, conjugated antisense compounds are targeted to an ApoCIII nucleic acid having the sequence of any of GENBANK® Accession No. NM_000040.1 (incorporated herein as SEQ ID NO: 1); GENBANK Accession No. NT_033899.8 truncated from nucleotides 20262640 to 20266603 (incorporated herein as SEQ ID NO: 2); and GenBank Accession No. NT_035088.1 truncated from nucleotides 6238608 to 6242565 (incorporated herein as SEQ ID NO: 3). In certain such embodiments, a conjugated antisense compound is at least 90%, at least 95%, or 100% complementary to any of SEQ ID NOs: 1-3.

In certain embodiments, a conjugated antisense compound targeted to SEQ ID NO: 1 comprises an at least 8 consecutive nucleobase sequence of SEQ ID NO: 87. In certain embodiments, a conjugated antisense compound targeted to SEQ ID NO: 1 comprises a nucleobase sequence of SEQ ID NO: 87.

Table A: Antisense Compounds targeted to ApoCIII SEQ ID NO: 1

ISIS No	Target Site	Start	Sequence (5'-3')	Motif	SEQ ID NO
304801	508		AGCTTCTTGTCCAGCTTTAT	eeeeeddddddddeeeee	87
647535	508		AGCTTCTTGTCCAGCTTTAT	eeeeeddddddddeeeeeod	87
616468	508		AGCTTCTTGTCCAGCTTTAT	eeeeeddddddddeeeee	87
647536	508		AGCTTCTTGTCCAGCTTTAT	eeoeoeoddddddddeoe oeeod	87

5

ApoCIII Therapeutic Indications

In certain embodiments, the invention provides methods for using a conjugated antisense compound targeted to an ApoCIII nucleic acid for modulating the expression of ApoCIII in a subject. In certain embodiments, the expression of ApoCIII is reduced.

10 In certain embodiments, the invention provides methods for using a conjugated antisense compound targeted to an ApoCIII nucleic acid in a pharmaceutical composition for treating a subject. In certain embodiments, the subject has a cardiovascular and/or metabolic disease, disorder or condition. In certain
 15 embodiments, the subject has hypertriglyceridemia, non-familial hypertriglyceridemia, familial hypertriglyceridemia, heterozygous familial hypertriglyceridemia, homozygous familial hypertriglyceridemia, mixed dyslipidemia, atherosclerosis, a risk of developing atherosclerosis, coronary heart disease, a history of coronary heart disease, early onset coronary heart disease, one or more risk factors for coronary heart disease, type II diabetes, type II diabetes with dyslipidemia, dyslipidemia, hyperlipidemia, hypercholesterolemia, hyperfattyacidemia, hepatic steatosis, non-alcoholic steatohepatitis, pancreatitis and/or non-alcoholic fatty liver disease.

20 In certain embodiments, the invention provides methods for using a conjugated antisense compound targeted to an ApoCIII nucleic acid in the preparation of a medicament.

E. Certain Nucleic Acid GalNAc Conjugates

25 In certain embodiments, conjugated antisense compounds comprise antisense compounds having the nucleobase sequence and modifications of the antisense compounds in the Table below attached to a

GalNAc conjugate. All internucleoside linkages are phosphorothioate internucleoside linkages unless otherwise indicated. A subscript “l” indicates an LNA bicyclic nucleoside. A subscript “d” indicates a 2'-deoxy nucleoside. A subscript “e” indicates a 2'-MOE modified nucleoside. A “V” indicates a 2-amino-2'-deoxyadenosine.

5

Table B

Sequence 5' to 3'	Motif	Chemistry	Internucleoside Linkages	SEQ ID NO.
T _l G _l G _l C _d A _d A _d G _d C _d A _d T _d C _d C _d T _l G _l T _l A _d	3-9-3-1	LNA/deoxy	phosphorothioate	222
C _l T _l C _l A _l A _d T _d C _d C _d A _d T _d G _d G _d C _l A _l G _l C _d	4-8-3-1	LNA/deoxy	phosphorothioate	223
A _l C _l C _l A _d A _d G _d T _d T _d T _d C _d T _d C _d A _l G _l C _l	3-10-3	LNA/deoxy	phosphorothioate	224
G _l C _l A _d T _d T _d G _d G _d T _d A _d T _d T _l C _l A _l	2-8-3	LNA/deoxy	phosphorothioate	225
T _l T _l C _l A _l G _l C _d A _d T _d T _d G _d G _d T _d A _d T _d C _l A _l G _l T _l G _l	5-10-5	LNA/deoxy	phosphorothioate	226
C _l A _l G _l C _d A _d T _d T _d G _d G _d T _d A _d T _d T _l C _l A _l G _d	3-10-3	LNA/deoxy	phosphorothioate	227
C _l A _l G _l C _d A _d T _d T _d G _d G _d T _d A _d T _d T _l C _l A _l	3-9-3	LNA/deoxy	phosphorothioate	228
A _l G _l C _l A _d T _d T _d G _d G _d T _d A _d T _d T _l C _l A _l	3-8-3	LNA/deoxy	phosphorothioate	229
G _l C _l A _d T _d T _d G _d G _d T _d A _d T _d T _l C _l	2-8-2	LNA/deoxy	phosphorothioate	230
CGGCATGTCTATTTTGTA			phosphorothioate	231
GGCTAAATCGCTCCACCAAG			phosphorothioate	232
CTCTAGCGTCTTAAAGCCGA			phosphorothioate	233
GCTGCATGATCTCCTTGGCG			phosphorothioate	234
ACGTTGAGGGGCATCGTCGC			Morpholino	235
GGGTCTGCVGCGGGVTGGT			phosphorothioate	236
GTTVCTVCTTCCVCCTGCCTG			phosphorothioate	237
TATCCGGAGGGCTCGCCATGCTGCT			phosphorothioate	238
T _e C _e C _e C _e G _e C _e CTGTGACAT _e G _e C _e A _e T _e T _e	6-8-6	MOE/deoxy		239
C _e A _e G _e C _e AGCAGAGTCTTCAT _e C _e A _e T _e	4-13-4	MOE/deoxy		240
G _e G _e G _e A _e C _d G _d C _d G _d G _d C _d G _d C _d T _d C _d G _d G _d T _e C _e A _e T _e	4-12-4	MOE/deoxy		241
C _e C _e A _e C _e A _e A _d G _d C _d T _d G _d T _d C _d C _d A _d G _d T _e C _e T _e A _e A _e	5-10-5	MOE/deoxy		242
C _e C _e G _e C _d A _d G _d C _d A _d T _d G _d C _d G _e C _e T _e C _e T _e G _e G _e	3-9-8	MOE/deoxy		243

F. Certain Pharmaceutical Compositions

10 In certain embodiments, the present disclosure provides pharmaceutical compositions comprising one or more antisense compound. In certain embodiments, such pharmaceutical composition comprises a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutical composition comprises a sterile saline solution and one or more antisense compound. In certain embodiments, such pharmaceutical composition consists of a sterile saline solution and one or more antisense compound. In

15 certain embodiments, the sterile saline is pharmaceutical grade saline. In certain embodiments, a pharmaceutical composition comprises one or more antisense compound and sterile water. In certain embodiments, a pharmaceutical composition consists of one or more antisense compound and sterile water. In certain embodiments, the sterile saline is pharmaceutical grade water. In certain embodiments, a pharmaceutical composition comprises one or more antisense compound and phosphate-buffered saline

20 (PBS). In certain embodiments, a pharmaceutical composition consists of one or more antisense compound

and sterile phosphate-buffered saline (PBS). In certain embodiments, the sterile saline is pharmaceutical grade PBS.

In certain embodiments, antisense compounds may be admixed with pharmaceutically acceptable active and/or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions depend on a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters. In certain embodiments, pharmaceutical compositions comprising antisense compounds comprise one or more oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

A prodrug can include the incorporation of additional nucleosides at one or both ends of an oligonucleotide which are cleaved by endogenous nucleases within the body, to form the active antisense oligonucleotide.

Lipid moieties have been used in nucleic acid therapies in a variety of methods. In certain such methods, the nucleic acid is introduced into preformed liposomes or lipoplexes made of mixtures of cationic lipids and neutral lipids. In certain methods, DNA complexes with mono- or poly-cationic lipids are formed without the presence of a neutral lipid. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to a particular cell or tissue. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to fat tissue. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to muscle tissue.

In certain embodiments, pharmaceutical compositions provided herein comprise one or more modified oligonucleotides and one or more excipients. In certain such embodiments, excipients are selected from water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylase, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose and polyvinylpyrrolidone.

In certain embodiments, a pharmaceutical composition provided herein comprises a delivery system. Examples of delivery systems include, but are not limited to, liposomes and emulsions. Certain delivery systems are useful for preparing certain pharmaceutical compositions including those comprising hydrophobic compounds. In certain embodiments, certain organic solvents such as dimethylsulfoxide are used.

In certain embodiments, a pharmaceutical composition provided herein comprises one or more tissue-specific delivery molecules designed to deliver the one or more pharmaceutical agents of the present

disclosure to specific tissues or cell types. For example, in certain embodiments, pharmaceutical compositions include liposomes coated with a tissue-specific antibody.

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

In certain embodiments, a pharmaceutical composition provided herein is prepared for oral
15 administration. In certain embodiments, pharmaceutical compositions are prepared for buccal administration.

In certain embodiments, a pharmaceutical composition is prepared for administration by injection (e.g., intravenous, subcutaneous, intramuscular, etc.). In certain of such embodiments, a pharmaceutical composition comprises a carrier and is formulated in aqueous solution, such as water or physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. In certain
20 embodiments, other ingredients are included (e.g., ingredients that aid in solubility or serve as preservatives). In certain embodiments, injectable suspensions are prepared using appropriate liquid carriers, suspending agents and the like. Certain pharmaceutical compositions for injection are presented in unit dosage form, e.g., in ampoules or in multi-dose containers. Certain pharmaceutical compositions for injection are suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending,
25 stabilizing and/or dispersing agents. Certain solvents suitable for use in pharmaceutical compositions for injection include, but are not limited to, lipophilic solvents and fatty oils, such as sesame oil, synthetic fatty acid esters, such as ethyl oleate or triglycerides, and liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, such suspensions may also contain suitable stabilizers or agents that increase the
30 solubility of the pharmaceutical agents to allow for the preparation of highly concentrated solutions.

In certain embodiments, a pharmaceutical composition is prepared for transmucosal administration. In certain of such embodiments penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

In certain embodiments, a pharmaceutical composition provided herein comprises an oligonucleotide
35 in a therapeutically effective amount. In certain embodiments, the therapeutically effective amount is sufficient to prevent, alleviate or ameliorate symptoms of a disease or to prolong the survival of the subject

being treated. Determination of a therapeutically effective amount is well within the capability of those skilled in the art.

In certain embodiments, one or more modified oligonucleotide provided herein is formulated as a prodrug. In certain embodiments, upon *in vivo* administration, a prodrug is chemically converted to the biologically, pharmaceutically or therapeutically more active form of an oligonucleotide. In certain 5 embodiments, prodrugs are useful because they are easier to administer than the corresponding active form. For example, in certain instances, a prodrug may be more bioavailable (e.g., through oral administration) than is the corresponding active form. In certain instances, a prodrug may have improved solubility compared to the corresponding active form. In certain embodiments, prodrugs are less water soluble than the 10 corresponding active form. In certain instances, such prodrugs possess superior transmittal across cell membranes, where water solubility is detrimental to mobility. In certain embodiments, a prodrug is an ester. In certain such embodiments, the ester is metabolically hydrolyzed to carboxylic acid upon administration. In certain instances the carboxylic acid containing compound is the corresponding active form. In certain embodiments, a prodrug comprises a short peptide (polyaminoacid) bound to an acid group. In certain of 15 such embodiments, the peptide is cleaved upon administration to form the corresponding active form.

In certain embodiments, the present disclosure provides compositions and methods for reducing the amount or activity of a target nucleic acid in a cell. In certain embodiments, the cell is in an animal. In certain embodiments, the animal is a mammal. In certain embodiments, the animal is a rodent. In certain 20 embodiments, the animal is a primate. In certain embodiments, the animal is a non-human primate. In certain embodiments, the animal is a human.

In certain embodiments, the present disclosure provides methods of administering a pharmaceutical composition comprising an oligonucleotide of the present disclosure to an animal. Suitable administration routes include, but are not limited to, oral, rectal, transmucosal, intestinal, enteral, topical, suppository, through inhalation, intrathecal, intracerebroventricular, intraperitoneal, intranasal, intraocular, intratumoral, 25 and parenteral (e.g., intravenous, intramuscular, intramedullary, and subcutaneous). In certain embodiments, pharmaceutical intrathecal are administered to achieve local rather than systemic exposures. For example, pharmaceutical compositions may be injected directly in the area of desired effect (e.g., into the liver).

Nonlimiting disclosure

30 While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds described herein and are not intended to limit the same.

Although the sequence listing accompanying this filing identifies each sequence as either “RNA” or “DNA” as required, in reality, those sequences may be modified with any combination of chemical 35

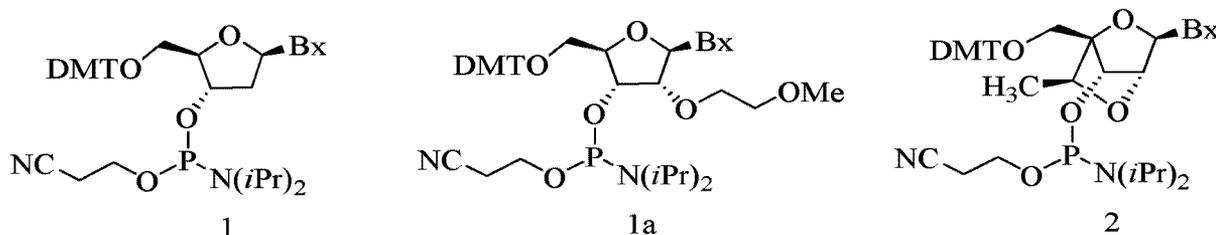
modifications. One of skill in the art will readily appreciate that such designation as “RNA” or “DNA” to describe modified oligonucleotides is, in certain instances, arbitrary. For example, an oligonucleotide comprising a nucleoside comprising a 2'-OH sugar moiety and a thymine base could be described as a DNA having a modified sugar (2'-OH for the natural 2'-H of DNA) or as an RNA having a modified base (thymine (methylated uracil) for natural uracil of RNA).

Accordingly, nucleic acid sequences provided herein, including, but not limited to those in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, including, but not limited to such nucleic acids having modified nucleobases. By way of further example and without limitation, an oligonucleotide having the nucleobase sequence “ATCGATCG” encompasses any oligonucleotides having such nucleobase sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence “AUCGAUCG” and those having some DNA bases and some RNA bases such as “AUCGATCG” and oligonucleotides having other modified bases, such as “AT^mCGAUCG,” wherein ^mC indicates a cytosine base comprising a methyl group at the 5-position.

15 EXAMPLES

The following examples illustrate certain embodiments of the present disclosure and are not limiting. Moreover, where specific embodiments are provided, the inventors have contemplated generic application of those specific embodiments. For example, disclosure of an oligonucleotide having a particular motif provides reasonable support for additional oligonucleotides having the same or similar motif. And, for example, where a particular high-affinity modification appears at a particular position, other high-affinity modifications at the same position are considered suitable, unless otherwise indicated.

Example 1: General Method for the Preparation of Phosphoramidites, Compounds 1, 1a and 2



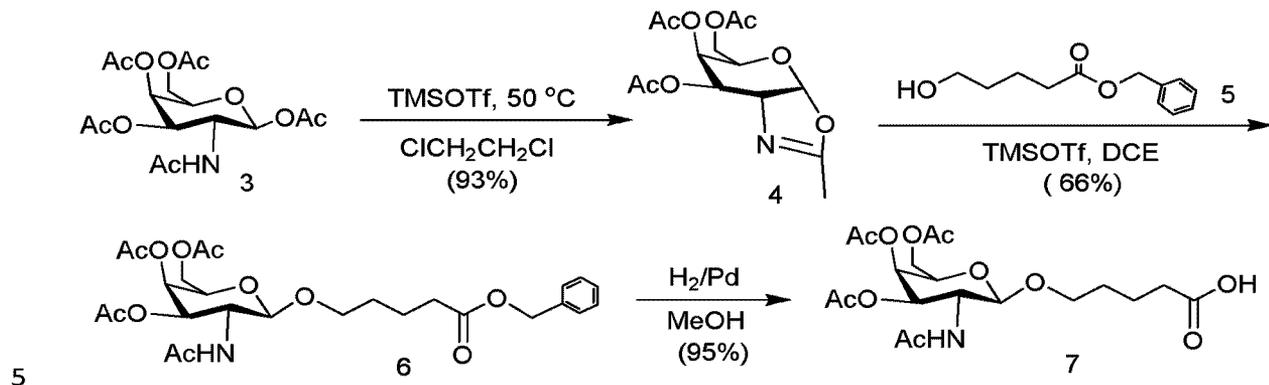
Bx is a heterocyclic base;

25

Compounds 1, 1a and 2 were prepared as per the procedures well known in the art as described in the specification herein (see Seth et al., Bioorg. Med. Chem., 2011, 21(4), 1122-1125, J. Org. Chem., 2010, 75(5), 1569-1581, Nucleic Acids Symposium Series, 2008, 52(1), 553-554); and also see published PCT

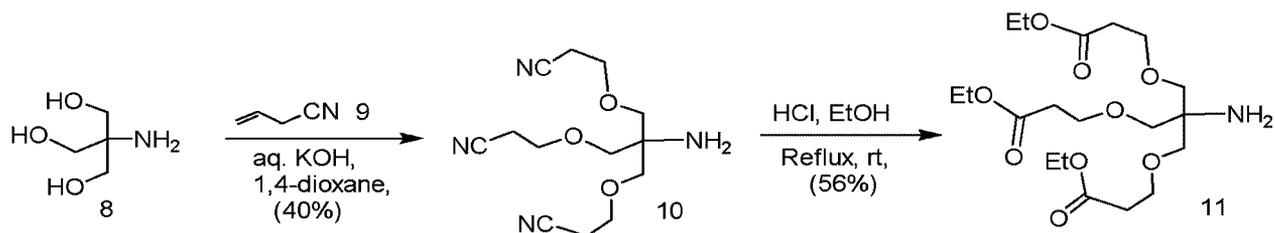
International Applications (WO 2011/115818, WO 2010/077578, WO2010/036698, WO2009/143369, WO 2009/006478, and WO 2007/090071), and US patent 7,569,686).

Example 2: Preparation of Compound 7

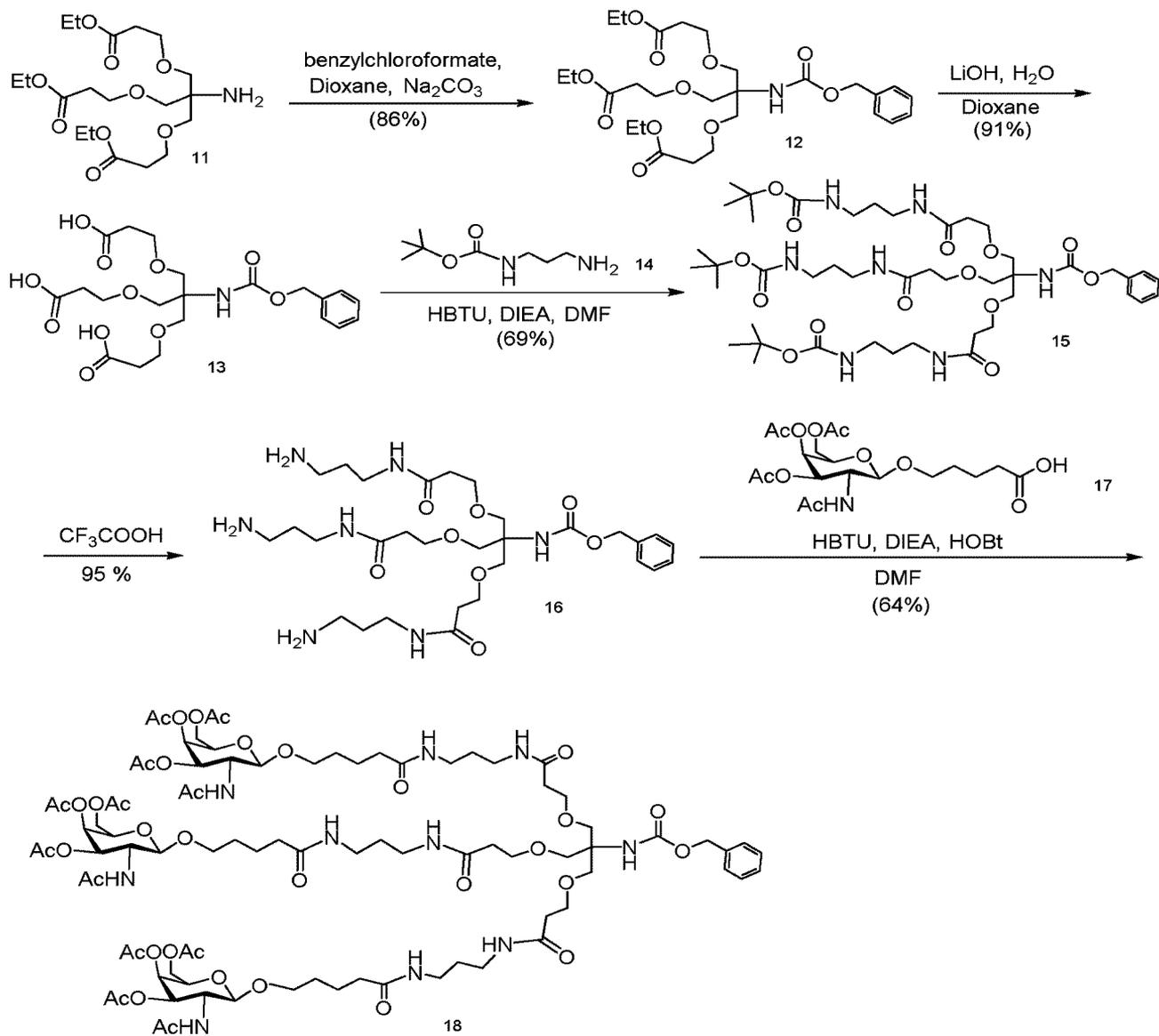


Compounds 3 (2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy- β -Dgalactopyranose or galactosamine pentaacetate) is commercially available. Compound 5 was prepared according to published procedures (Weber *et al.*, *J. Med. Chem.*, 1991, 34, 2692).

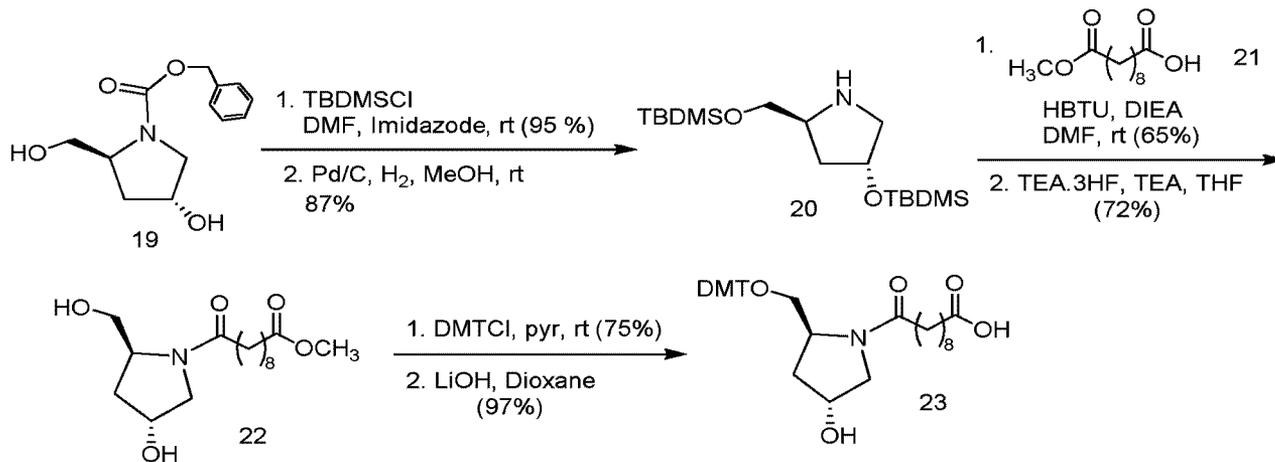
10 Example 3: Preparation of Compound 11



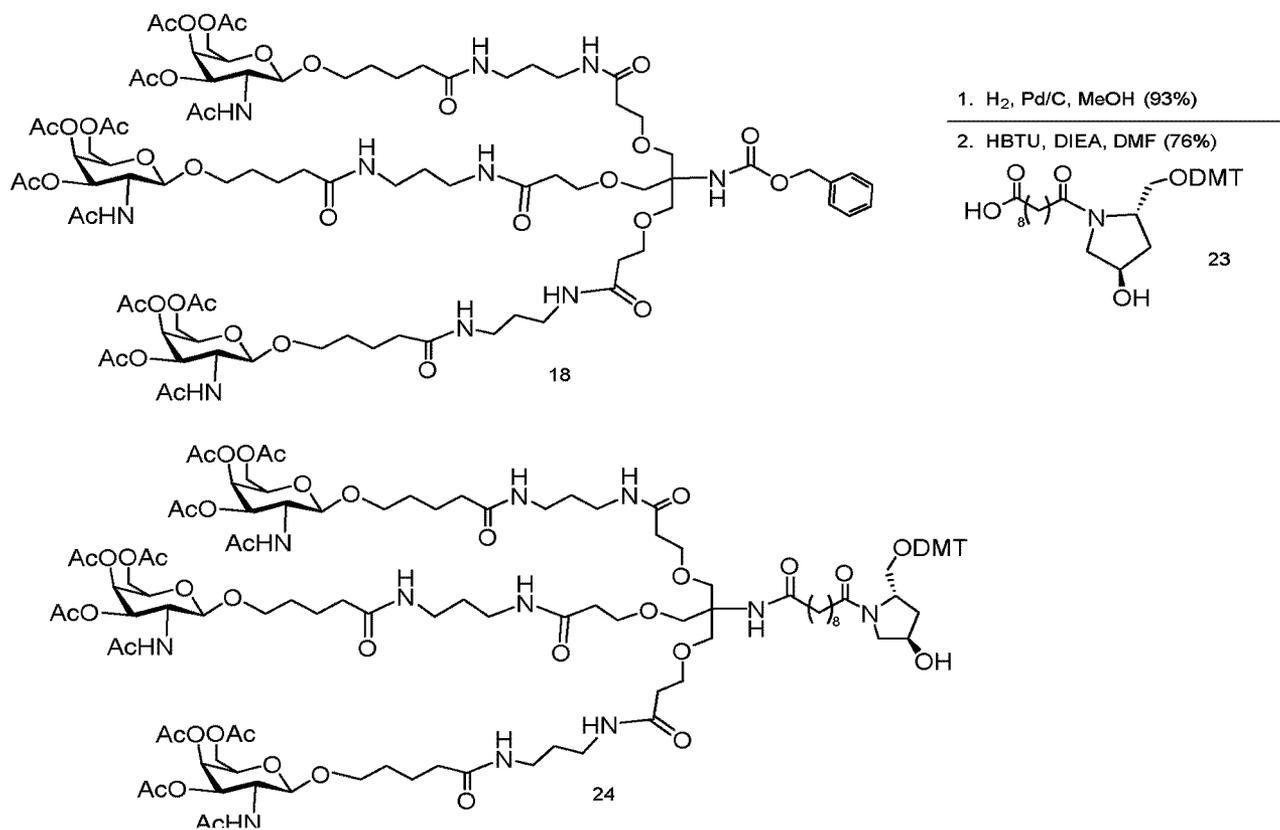
Compounds 8 and 9 are commercially available.

Example 4: Preparation of Compound 18

Compound 11 was prepared as per the procedures illustrated in Example 3. Compound 14 is commercially available. Compound 17 was prepared using similar procedures reported by Rensen *et al.*, *J. Med. Chem.*, 2004, 47, 5798-5808.

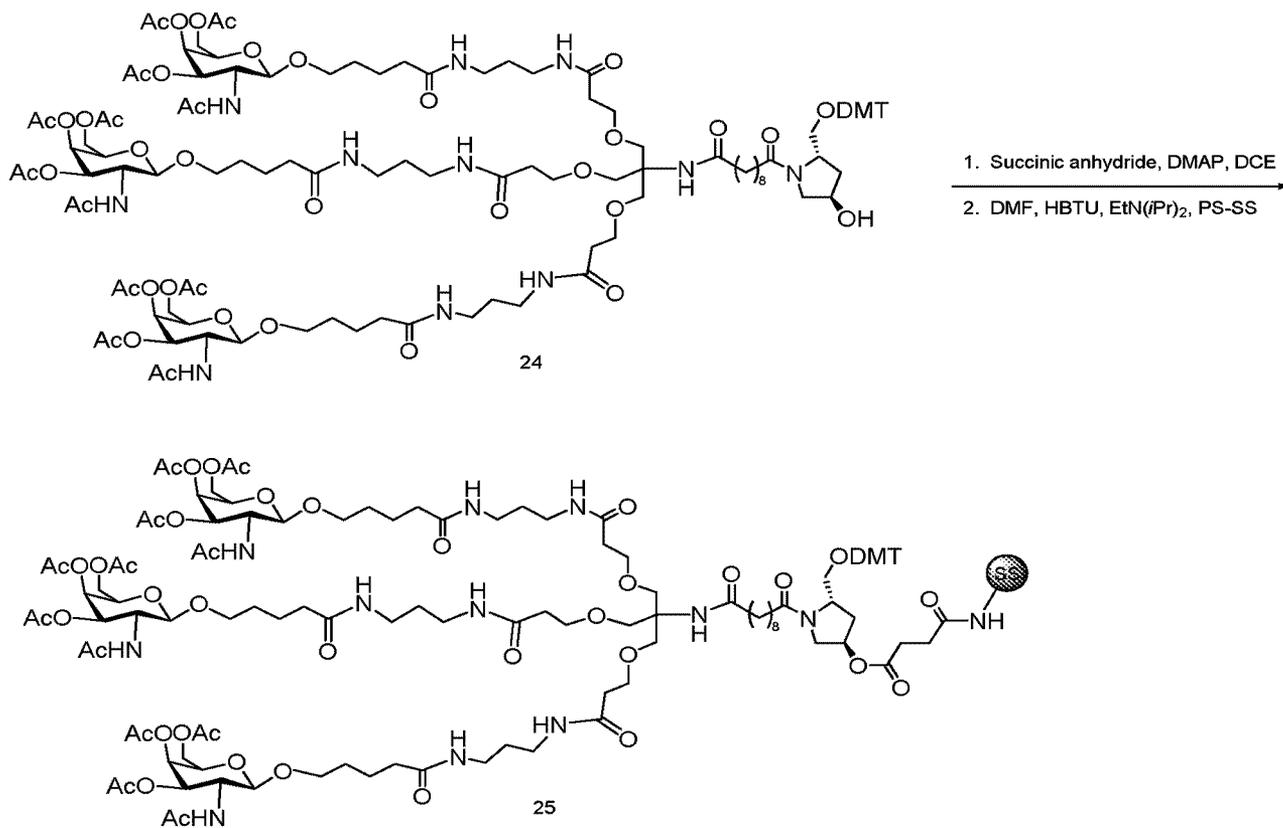
Example 5: Preparation of Compound 23

Compounds 19 and 21 are commercially available.

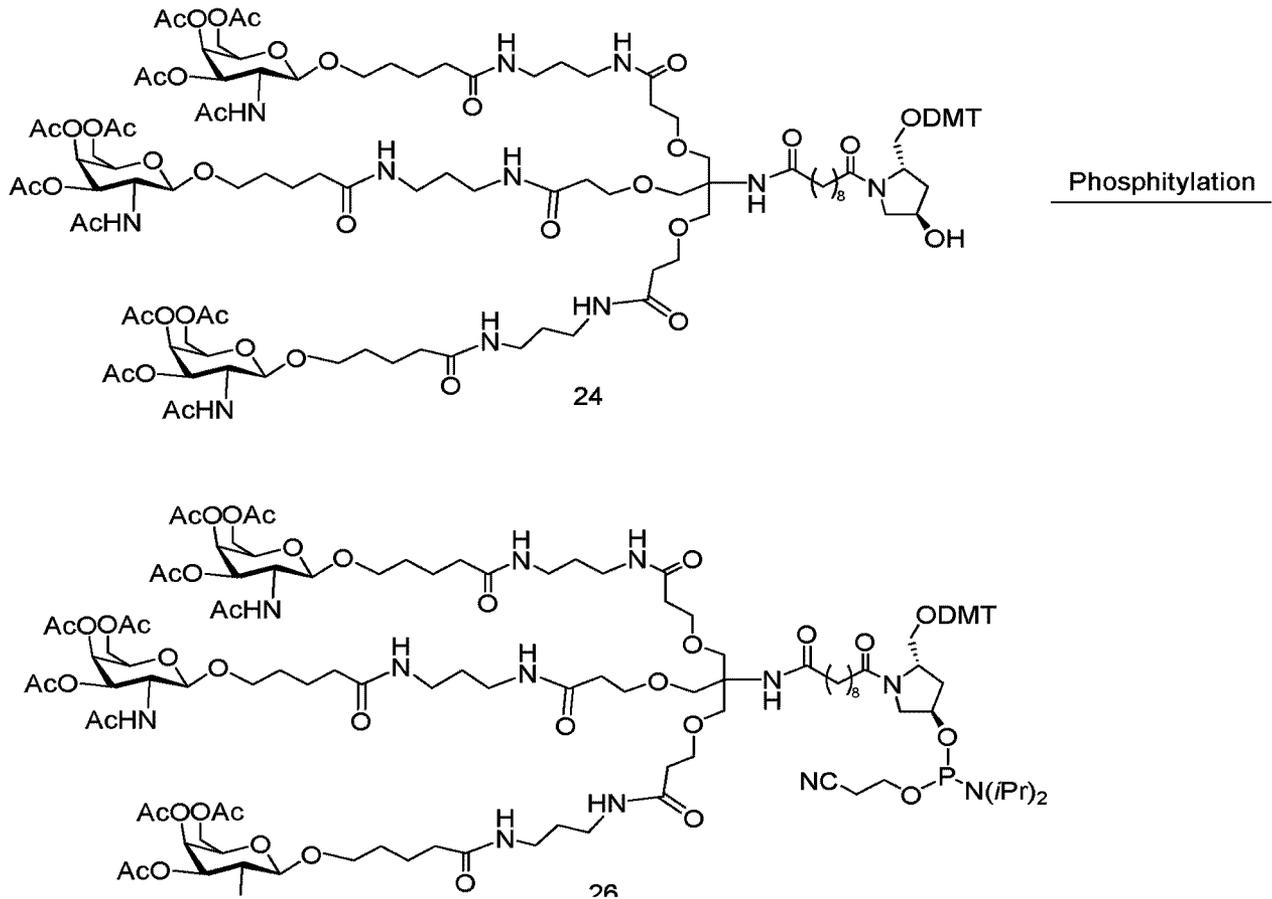
5 Example 6: Preparation of Compound 24

Compounds 18 and 23 were prepared as per the procedures illustrated in Examples 4 and 5.

Example 7: Preparation of Compound 25

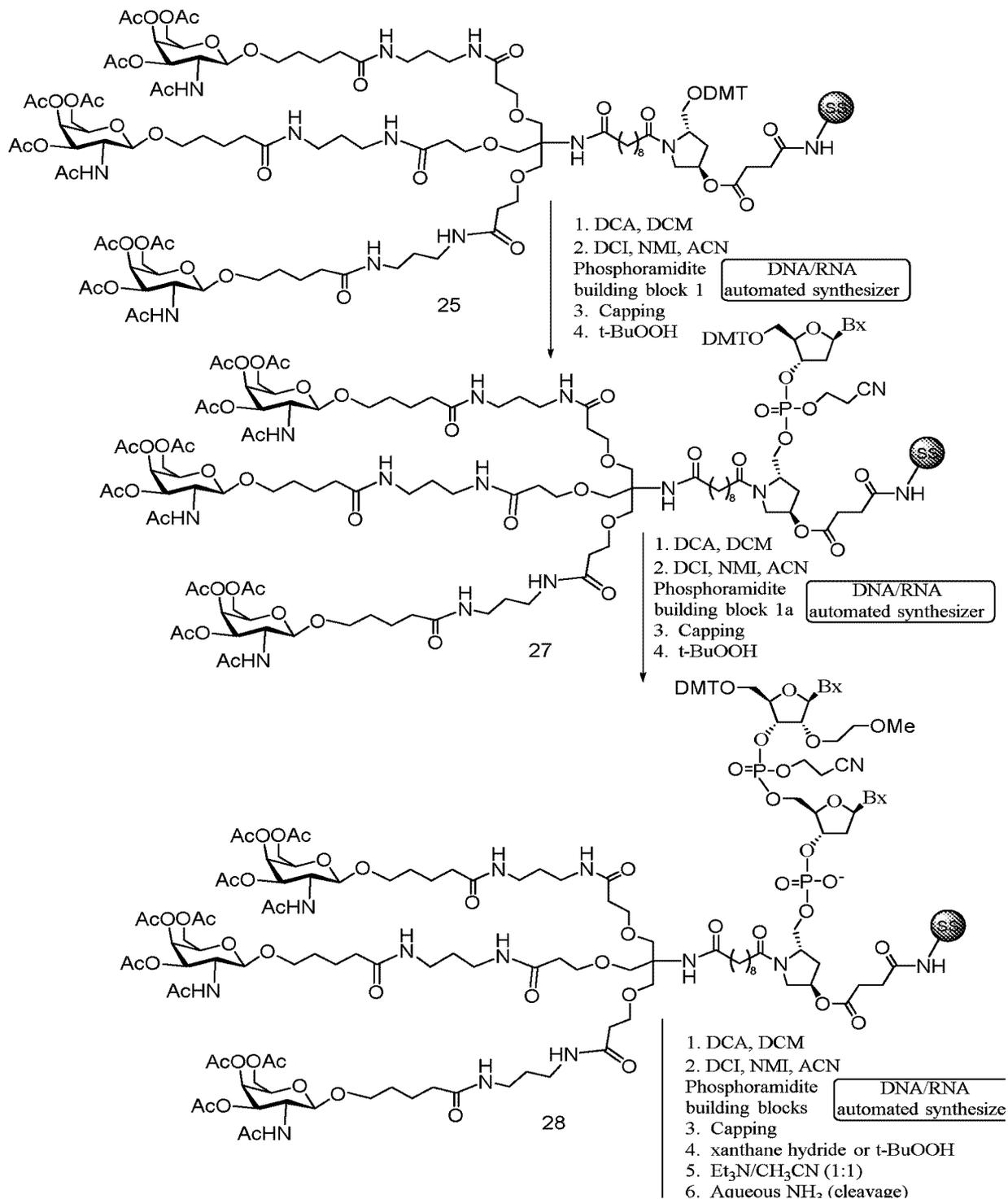


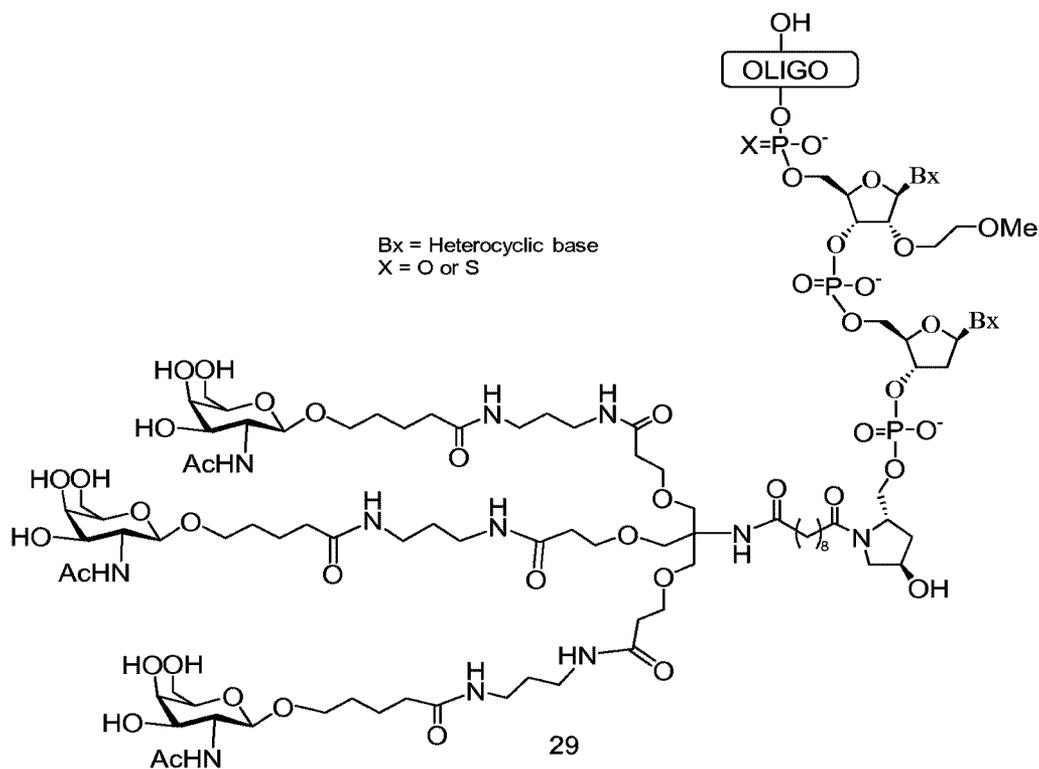
5 Compound 24 was prepared as per the procedures illustrated in Example 6.

Example 8: Preparation of Compound 26

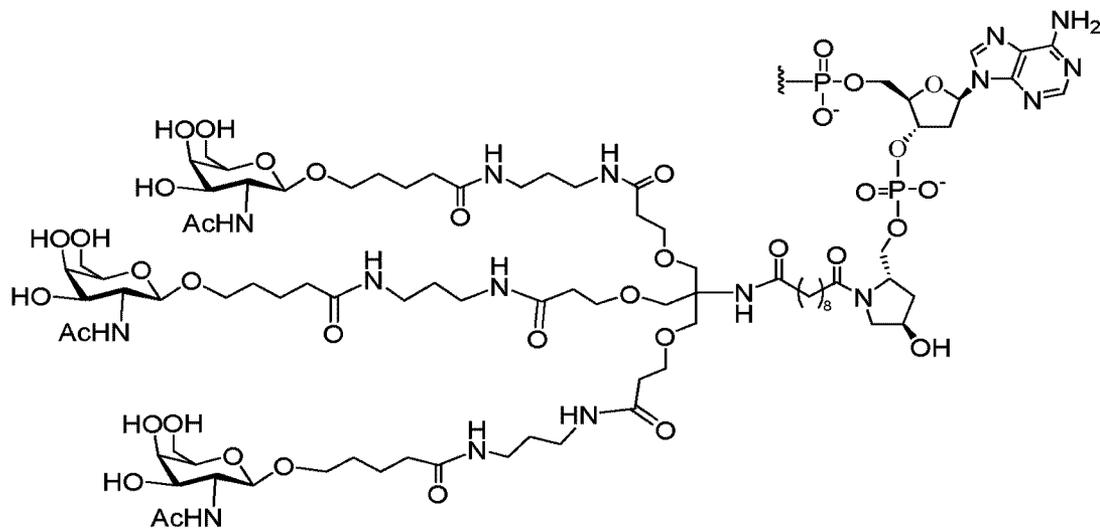
Compound 24 is prepared as per the procedures illustrated in Example 6.

**Example 9: General preparation of conjugated ASOs comprising GalNAc₃-1 at the 3' terminus,
Compound 29**



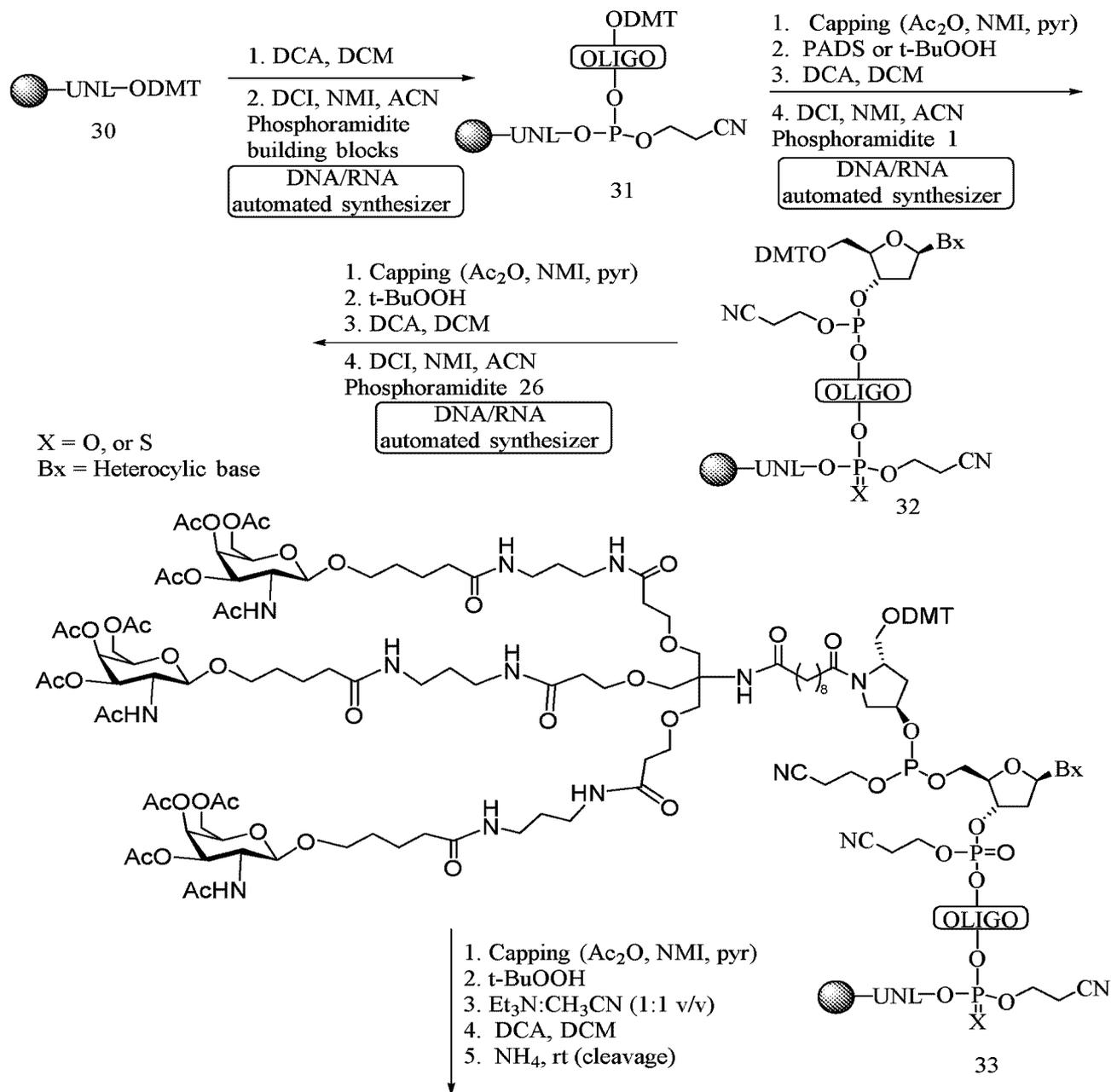


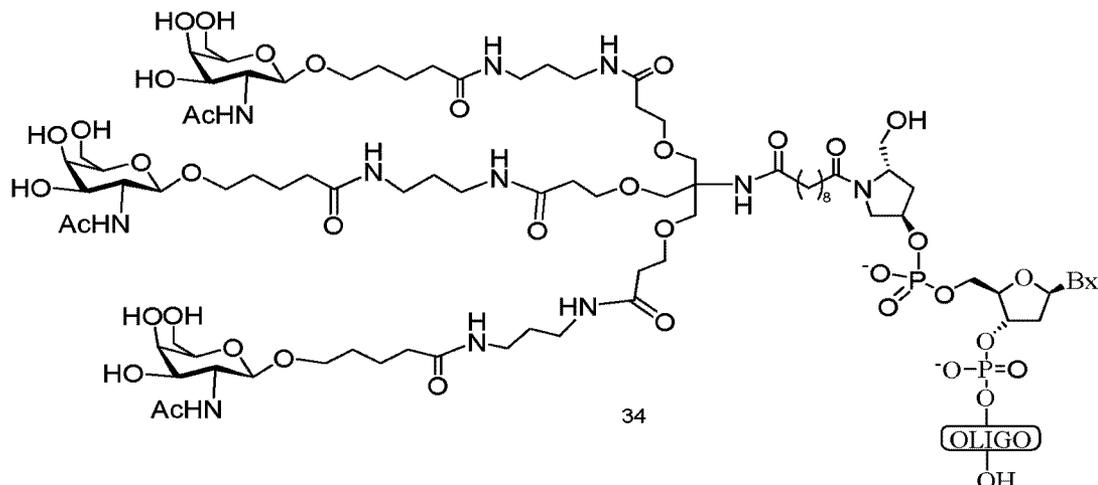
Wherein the protected GalNAc₃-1 has the structure:



The GalNAc₃ cluster portion of the conjugate group GalNAc₃-1 (GalNAc₃-1_a) can be combined with
5 any cleavable moiety to provide a variety of conjugate groups. Wherein GalNAc₃-1_a has the formula:

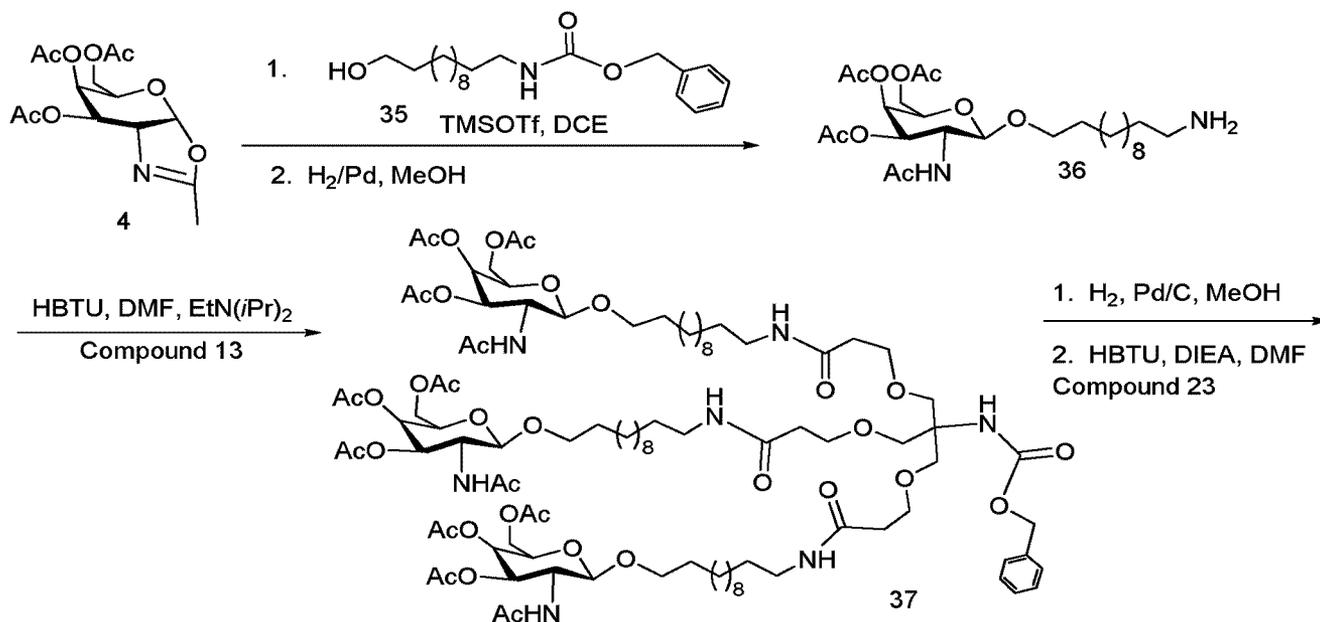
Example 10: General preparation conjugated ASOs comprising GalNAc₃-1 at the 5' terminus, Compound 34

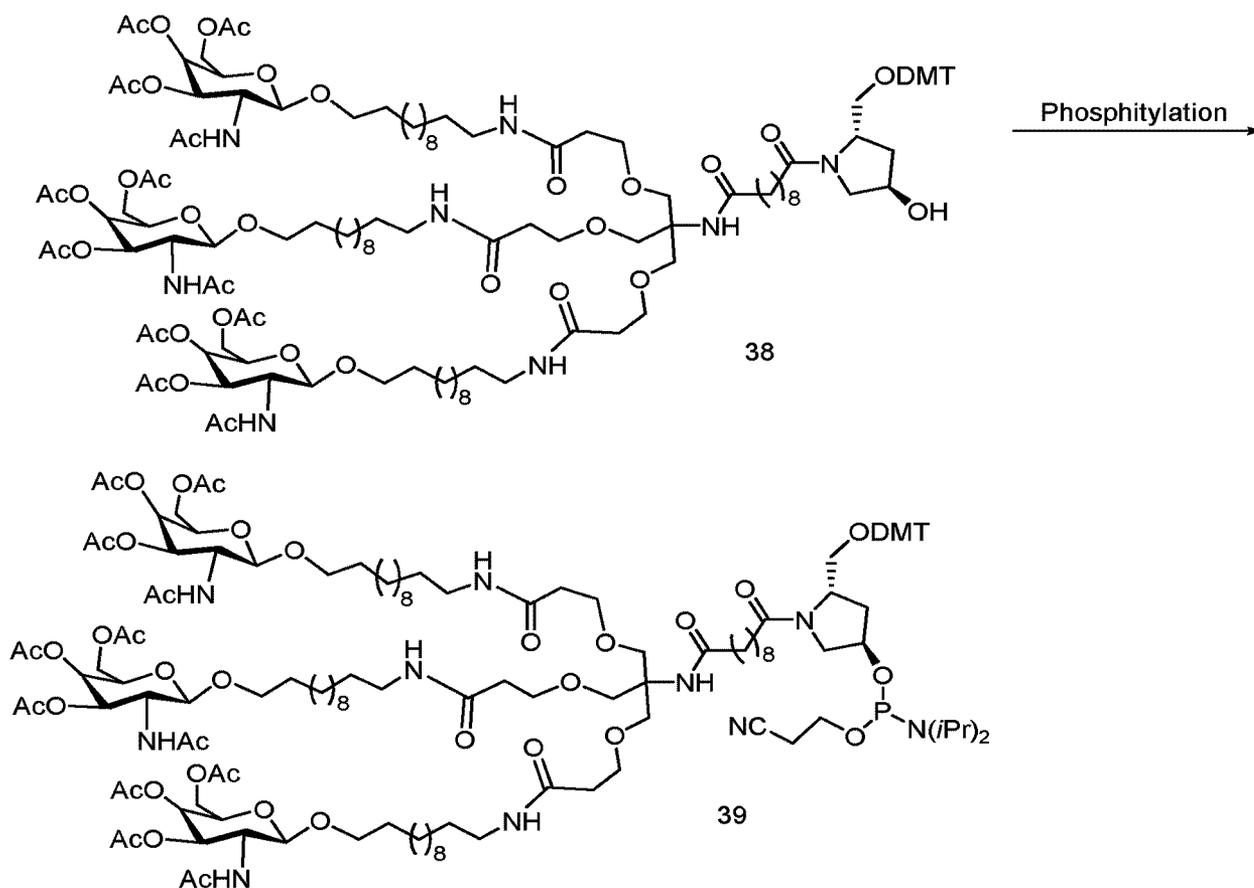




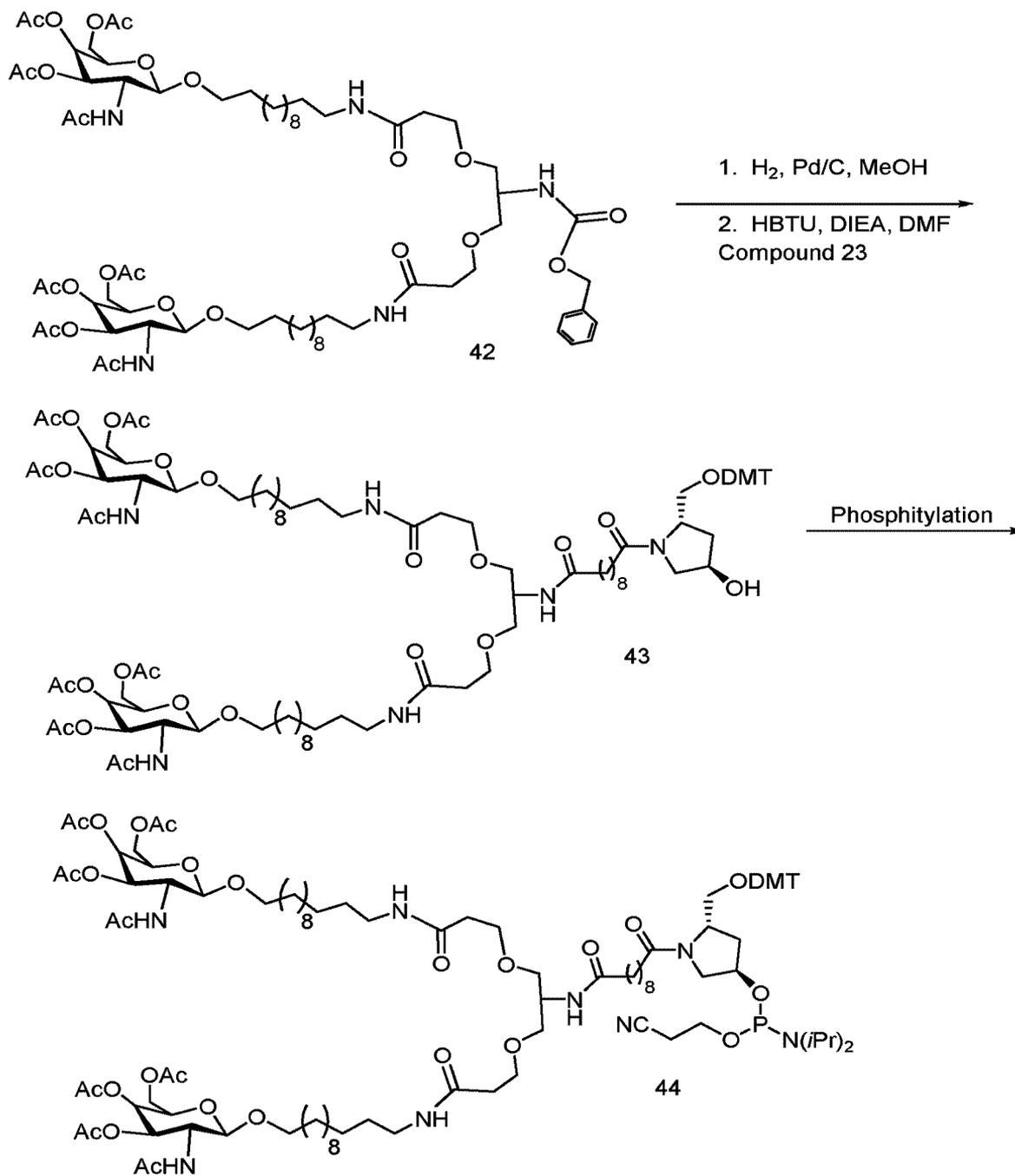
The Unylinker™ 30 is commercially available. Oligomeric Compound 34 comprising a **GalNAc₃-1** cluster at the 5' terminus is prepared using standard procedures in automated DNA/RNA synthesis (see Dupouy *et al.*, *Angew. Chem. Int. Ed.*, 2006, 45, 3623-3627). Phosphoramidite building blocks, Compounds 1 and 1a were prepared as per the procedures illustrated in Example 1. The phosphoramidites illustrated are meant to be representative and not intended to be limiting as other phosphoramidite building blocks can be used to prepare an oligomeric compound having a predetermined sequence and composition. The order and quantity of phosphoramidites added to the solid support can be adjusted to prepare gapped oligomeric compounds as described herein. Such gapped oligomeric compounds can have predetermined composition and base sequence as dictated by any given target.

Example 11: Preparation of Compound 39

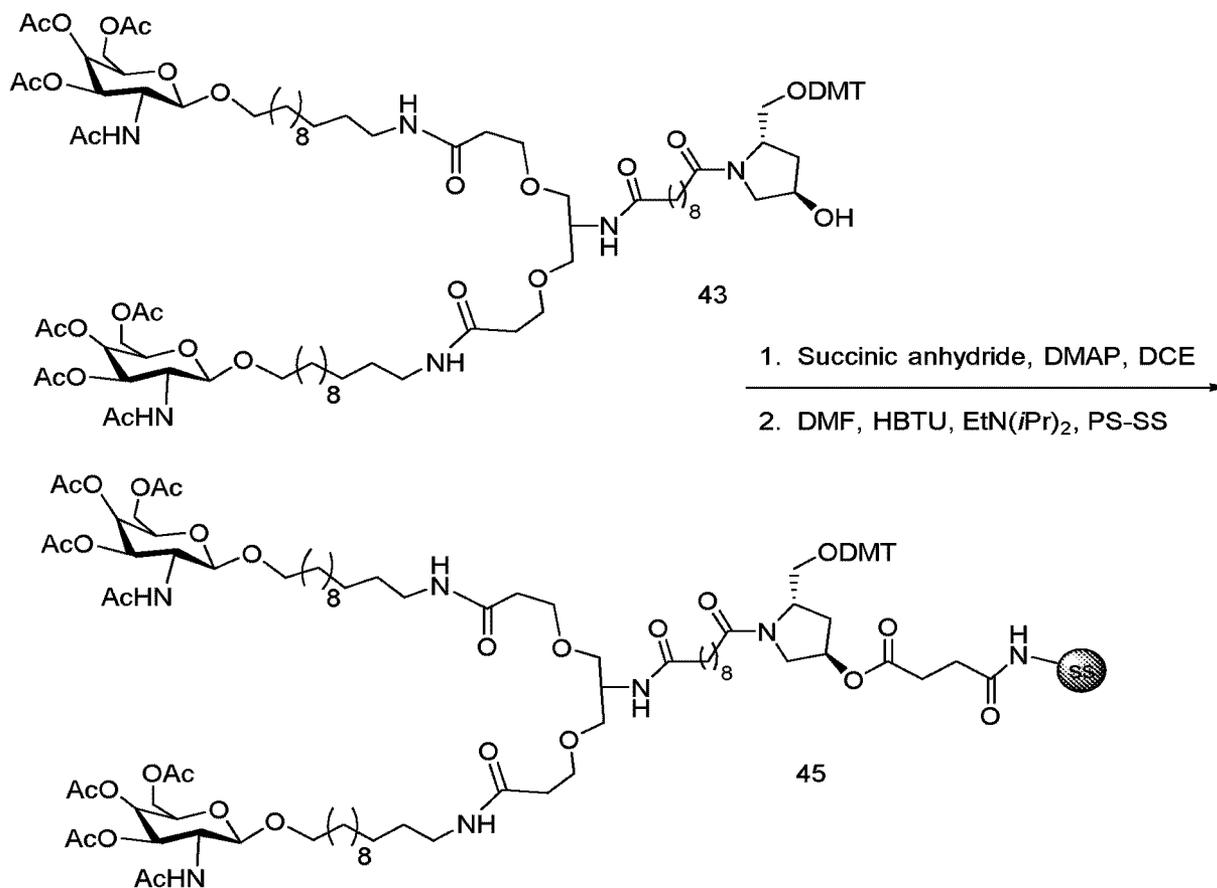




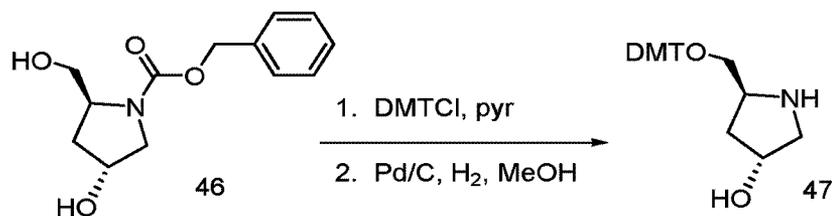
Compounds 4, 13 and 23 were prepared as per the procedures illustrated in Examples 2, 4, and 5. Compound 35 is prepared using similar procedures published in Rouchaud *et al.*, *Eur. J. Org. Chem.*, 2011, 12, 2346-2353.



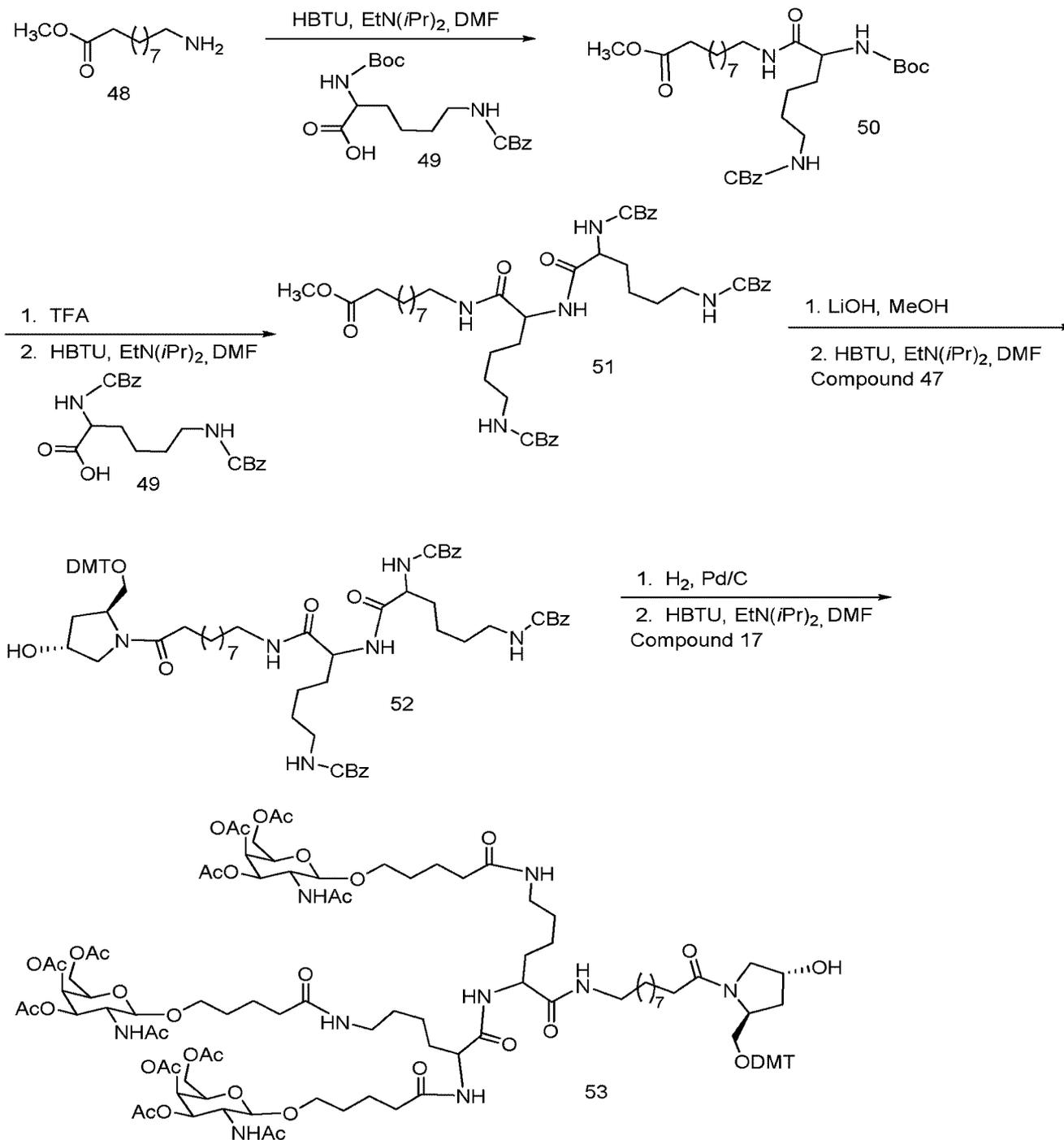
Compounds 23 and 36 are prepared as per the procedures illustrated in Examples 5 and 11. Compound 41 is prepared using similar procedures published in WO 2009082607.

Example 14: Preparation of Compound 45

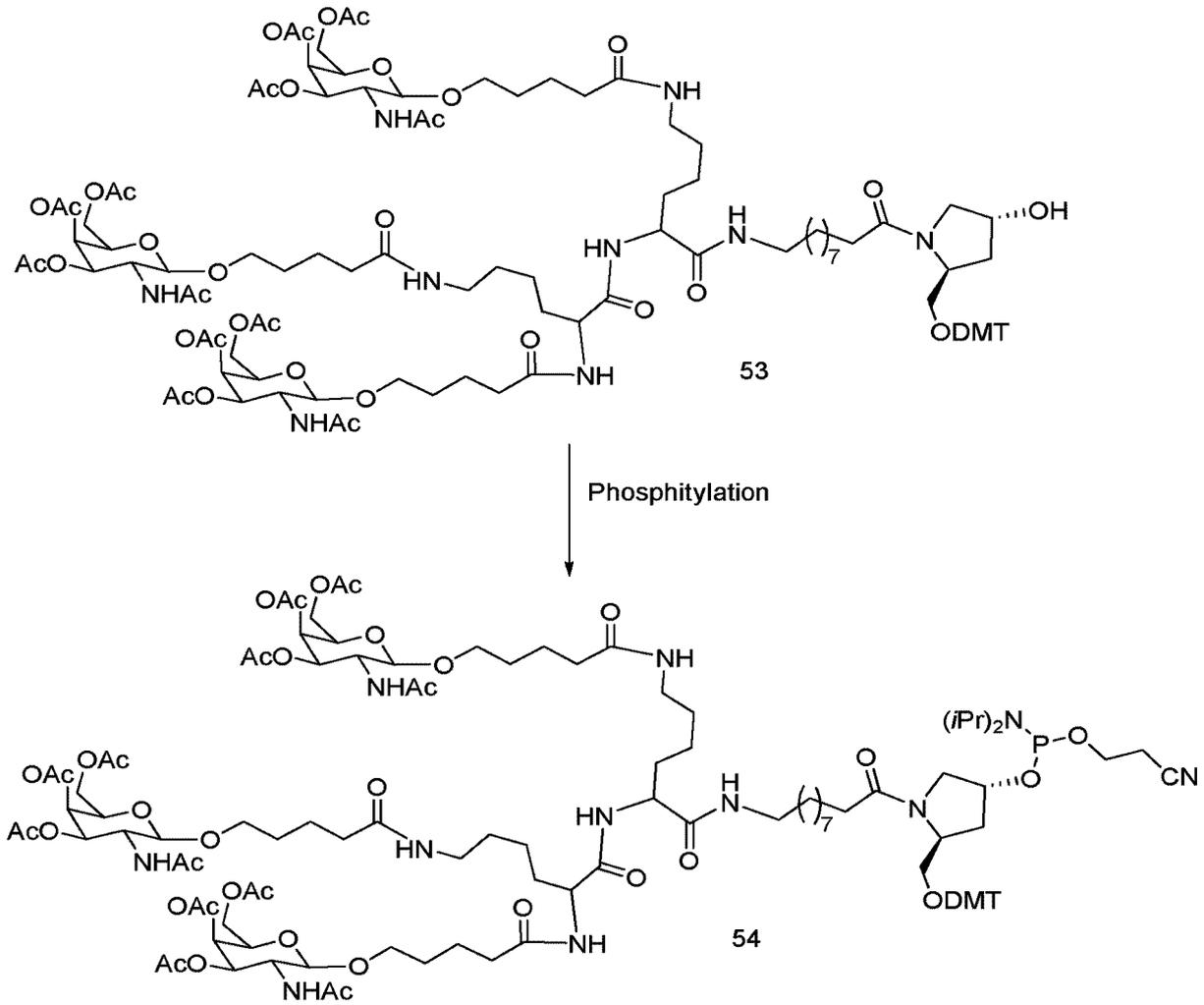
Compound 43 is prepared as per the procedures illustrated in Example 13.

5 Example 15: Preparation of Compound 47

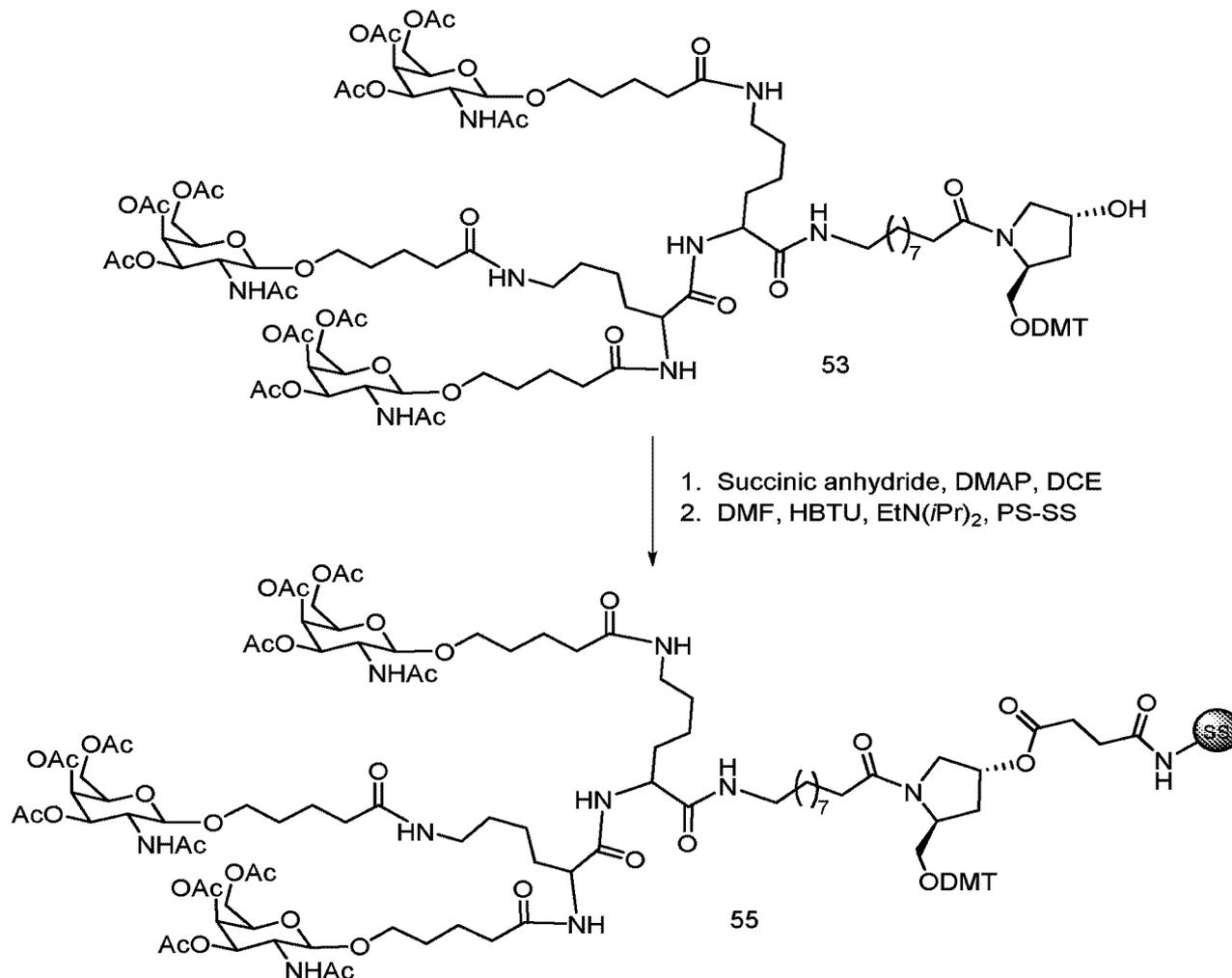
Compound 46 is commercially available.

Example 16: Preparation of Compound 53

Compounds 48 and 49 are commercially available. Compounds 17 and 47 are prepared as per the procedures illustrated in Examples 4 and 15.

Example 17: Preparation of Compound 54

Compound 53 is prepared as per the procedures illustrated in Example 16.

Example 18: Preparation of Compound 55

Compound 53 is prepared as per the procedures illustrated in Example 16.

5 **Example 19: General method for the preparation of conjugated ASOs comprising GalNAc₃-1 at the 3' position via solid phase techniques (preparation of ISIS 647535, 647536 and 651900)**

Unless otherwise stated, all reagents and solutions used for the synthesis of oligomeric compounds are purchased from commercial sources. Standard phosphoramidite building blocks and solid support are used for incorporation nucleoside residues which include for example T, A, G, and ¹³C residues. A 0.1 M solution of phosphoramidite in anhydrous acetonitrile was used for β-D-2'-deoxyribonucleoside and 2'-
10 MOE.

The ASO syntheses were performed on ABI 394 synthesizer (1-2 μmol scale) or on GE Healthcare Bioscience ÄKTA oligopilot synthesizer (40-200 μmol scale) by the phosphoramidite coupling method on an GalNAc₃-1 loaded VIMAD solid support (110 μmol/g, Guzaev *et al.*, 2003) packed in the column. For the

coupling step, the phosphoramidites were delivered 4 fold excess over the loading on the solid support and phosphoramidite condensation was carried out for 10 min. All other steps followed standard protocols supplied by the manufacturer. A solution of 6% dichloroacetic acid in toluene was used for removing dimethoxytrityl (DMT) group from 5'-hydroxyl group of the nucleotide. 4,5-Dicyanoimidazole (0.7 M) in anhydrous CH₃CN was used as activator during coupling step. Phosphorothioate linkages were introduced by sulfurization with 0.1 M solution of xanthane hydride in 1:1 pyridine/CH₃CN for a contact time of 3 minutes. A solution of 20% *tert*-butylhydroperoxide in CH₃CN containing 6% water was used as an oxidizing agent to provide phosphodiester internucleoside linkages with a contact time of 12 minutes.

After the desired sequence was assembled, the cyanoethyl phosphate protecting groups were deprotected using a 1:1 (v/v) mixture of triethylamine and acetonitrile with a contact time of 45 minutes. The solid-support bound ASOs were suspended in aqueous ammonia (28-30 wt %) and heated at 55 °C for 6 h.

The unbound ASOs were then filtered and the ammonia was boiled off. The residue was purified by high pressure liquid chromatography on a strong anion exchange column (GE Healthcare Bioscience, Source 30Q, 30 μm, 2.54 x 8 cm, A = 100 mM ammonium acetate in 30% aqueous CH₃CN, B = 1.5 M NaBr in A, 0-40% of B in 60 min, flow 14 mL min⁻¹, λ = 260 nm). The residue was desalted by HPLC on a reverse phase column to yield the desired ASOs in an isolated yield of 15-30% based on the initial loading on the solid support. The ASOs were characterized by ion-pair-HPLC coupled MS analysis with Agilent 1100 MSD system.

Antisense oligonucleotides not comprising a conjugate were synthesized using standard oligonucleotide synthesis procedures well known in the art.

Using these methods, three separate antisense compounds targeting ApoC III were prepared. As summarized in Table 17, below, each of the three antisense compounds targeting ApoC III had the same nucleobase sequence; ISIS 304801 is a 5-10-5 MOE gapmer having all phosphorothioate linkages; ISIS 647535 is the same as ISIS 304801, except that it had a **GalNAc₃-1** conjugated at its 3'-end; and ISIS 647536 is the same as ISIS 647535 except that certain internucleoside linkages of that compound are phosphodiester linkages. As further summarized in Table 17, two separate antisense compounds targeting SRB-1 were synthesized. ISIS 440762 was a 2-10-2 cEt gapmer with all phosphorothioate internucleoside linkages; ISIS 651900 is the same as ISIS 440762, except that it included a **GalNAc₃-1** at its 3'-end.

Table 17

Modified ASO targeting ApoC III and SRB-1

ASO	Sequence (5' to 3')	Target	CalCd Mass	Observed Mass	SEQ ID No.
ISIS 304801	A _{es} G _{es} ^m C _{es} T _{es} T _{es} ^m C _{ds} T _{ds} T _{ds} G _{ds} T _{ds} ^m C _{ds} ^m C _{ds} A _{ds} G _{ds} ^m C _{ds} T _{es} T _{es} T _{es} A _{es} T _e	ApoC III	7165.4	7164.4	244
ISIS 647535	A _{es} G _{es} ^m C _{es} T _{es} T _{es} ^m C _{ds} T _{ds} T _{ds} G _{ds} T _{ds} ^m C _{ds} ^m C _{ds} A _{ds} G _{ds} ^m C _{ds} T _{es} T _{es} T _{es} A _{es} T _{eo} A_{do}' - GalNAc₃-1_a	ApoC III	9239.5	9237.8	245
ISIS 647536	A _{es} G _{eo} ^m C _{eo} T _{eo} T _{eo} ^m C _{ds} T _{ds} T _{ds} G _{ds} T _{ds} ^m C _{ds} ^m C _{ds} A _{ds} G _{ds} ^m C _{ds} T _{eo} T _{eo} T _{es} A _{es} T _{eo} A_{do}' - GalNAc₃-1_a	ApoC III	9142.9	9140.8	245

ISIS 440762	$T_{ks}^m C_{ks} A_{ds} G_{ds} T_{ds}^m C_{ds} A_{ds} T_{ds} G_{ds} A_{ds}^m C_{ds} T_{ds} T_{ks}^m C_k$	SRB- 1	4647.0	4646.4	246
ISIS 651900	$T_{ks}^m C_{ks} A_{ds} G_{ds} T_{ds}^m C_{ds} A_{ds} T_{ds} G_{ds} A_{ds}^m C_{ds} T_{ds} T_{ks}^m C_{ko} A_{do} \text{-GalNAc}_3\text{-1}_a$	SRB- 1	6721.1	6719.4	247

Subscripts: “e” indicates 2’-MOE modified nucleoside; “d” indicates β -D-2’-deoxyribonucleoside; “k” indicates 6’-(S)-CH₃ bicyclic nucleoside (e.g. cEt); “s” indicates phosphorothioate internucleoside linkages (PS); “o” indicates phosphodiester internucleoside linkages (PO); and “o” indicates -O-P(=O)(OH)-. Superscript “m” indicates 5-methylcytosines. “GalNAc₃-1” indicates a conjugate group having the structure shown previously in Example 9. Note that GalNAc₃-1 comprises a cleavable adenosine which links the ASO to remainder of the conjugate, which is designated “GalNAc₃-1_a.” This nomenclature is used in the above table to show the full nucleobase sequence, including the adenosine, which is part of the conjugate. Thus, in the above table, the sequences could also be listed as ending with “GalNAc₃-1” with the “A_{do}” omitted. This convention of using the subscript “a” to indicate the portion of a conjugate group lacking a cleavable nucleoside or cleavable moiety is used throughout these Examples. This portion of a conjugate group lacking the cleavable moiety is referred to herein as a “cluster” or “conjugate cluster” or “GalNAc₃ cluster.” In certain instances it is convenient to describe a conjugate group by separately providing its cluster and its cleavable moiety.

Example 20: Dose-dependent antisense inhibition of human ApoC III in huApoC III transgenic mice

ISIS 304801 and ISIS 647535, each targeting human ApoC III and described above, were separately tested and evaluated in a dose-dependent study for their ability to inhibit human ApoC III in human ApoC III transgenic mice.

Treatment

Human ApoCIII transgenic mice were maintained on a 12-hour light/dark cycle and fed *ad libitum* Teklad lab chow. Animals were acclimated for at least 7 days in the research facility before initiation of the experiment. ASOs were prepared in PBS and sterilized by filtering through a 0.2 micron filter. ASOs were dissolved in 0.9% PBS for injection.

Human ApoC III transgenic mice were injected intraperitoneally once a week for two weeks with ISIS 304801 or 647535 at 0.08, 0.25, 0.75, 2.25 or 6.75 μ mol/kg or with PBS as a control. Each treatment group consisted of 4 animals. Forty-eight hours after the administration of the last dose, blood was drawn from each mouse and the mice were sacrificed and tissues were collected.

ApoC III mRNA Analysis

ApoC III mRNA levels in the mice’s livers were determined using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. ApoC III mRNA levels were determined relative to total RNA (using Ribogreen), prior to normalization to PBS-treated control. The results below are presented as the average percent of ApoC III

mRNA levels for each treatment group, normalized to PBS-treated control and are denoted as “% PBS”. The half maximal effective dosage (ED₅₀) of each ASO is also presented in Table 18, below.

As illustrated, both antisense compounds reduced ApoC III RNA relative to the PBS control. Further, the antisense compound conjugated to **GalNAc₃-1** (ISIS 647535) was substantially more potent than the antisense compound lacking the **GalNAc₃-1** conjugate (ISIS 304801).

Table 18

Effect of ASO treatment on ApoC III mRNA levels in human ApoC III transgenic mice

ASO	Dose (μmol/kg)	% PBS	ED ₅₀ (μmol/kg)	3' Conjugate	Internucleoside linkage/Length	SEQ ID No.
PBS	0	100	--	-	--	
ISIS 304801	0.08	95	0.77	None	PS/20	244
	0.75	42				
	2.25	32				
	6.75	19				
ISIS 647535	0.08	50	0.074	GalNAc₃-1	PS/20	245
	0.75	15				
	2.25	17				
	6.75	8				

ApoC III Protein Analysis (Turbidometric Assay)

10 Plasma ApoC III protein analysis was determined using procedures reported by Graham *et al*, *Circulation Research*, published online before print March 29, 2013.

Approximately 100 μl of plasma isolated from mice was analyzed without dilution using an Olympus Clinical Analyzer and a commercially available turbidometric ApoC III assay (Kamiya, Cat# KAI-006, Kamiya Biomedical, Seattle, WA). The assay protocol was performed as described by the vendor.

15 As shown in the Table 19 below, both antisense compounds reduced ApoC III protein relative to the PBS control. Further, the antisense compound conjugated to **GalNAc₃-1** (ISIS 647535) was substantially more potent than the antisense compound lacking the **GalNAc₃-1** conjugate (ISIS 304801).

Table 19

Effect of ASO treatment on ApoC III plasma protein levels in human ApoC III transgenic mice

ASO	Dose (μmol/kg)	% PBS	ED ₅₀ (μmol/kg)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	100	--	--	--	
ISIS 304801	0.08	86	0.73	None	PS/20	244
	0.75	51				

	2.25	23				
	6.75	13				
ISIS 647535	0.08	72	0.19	GalNAc₃-1	PS/20	245
	0.75	14				
	2.25	12				
	6.75	11				

Plasma triglycerides and cholesterol were extracted by the method of Bligh and Dyer (Bligh, E.G. and Dyer, W.J. *Can. J. Biochem. Physiol.* 37: 911-917, 1959)(Bligh, E and Dyer, W, *Can J Biochem Physiol*, 37, 911-917, 1959)(Bligh, E and Dyer, W, *Can J Biochem Physiol*, 37, 911-917, 1959) and measured by using a Beckmann Coulter clinical analyzer and commercially available reagents.

The triglyceride levels were measured relative to PBS injected mice and are denoted as “% PBS”. Results are presented in Table 20. As illustrated, both antisense compounds lowered triglyceride levels. Further, the antisense compound conjugated to **GalNAc₃-1** (ISIS 647535) was substantially more potent than the antisense compound lacking the **GalNAc₃-1** conjugate (ISIS 304801).

10

Table 20

Effect of ASO treatment on triglyceride levels in transgenic mice

ASO	Dose (μmol/kg)	% PBS	ED ₅₀ (μmol/kg)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	100	--	--	--	
ISIS 304801	0.08	87	0.63	None	PS/20	244
	0.75	46				
	2.25	21				
	6.75	12				
ISIS 647535	0.08	65	0.13	GalNAc₃-1	PS/20	245
	0.75	9				
	2.25	8				
	6.75	9				

Plasma samples were analyzed by HPLC to determine the amount of total cholesterol and of different fractions of cholesterol (HDL and LDL). Results are presented in Tables 21 and 22. As illustrated, both antisense compounds lowered total cholesterol levels; both lowered LDL; and both raised HDL. Further, the antisense compound conjugated to **GalNAc₃-1** (ISIS 647535) was substantially more potent than the antisense compound lacking the **GalNAc₃-1** conjugate (ISIS 304801). An increase in HDL and a decrease in LDL levels is a cardiovascular beneficial effect of antisense inhibition of ApoC III.

Table 21

Effect of ASO treatment on total cholesterol levels in transgenic mice

ASO	Dose ($\mu\text{mol/kg}$)	Total Cholesterol (mg/dL)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	257	--	--	
ISIS 304801	0.08	226	None	PS/20	244
	0.75	164			
	2.25	110			
	6.75	82			
ISIS 647535	0.08	230	GalNAc ₃ -1	PS/20	245
	0.75	82			
	2.25	86			
	6.75	99			

Table 22

Effect of ASO treatment on HDL and LDL cholesterol levels in transgenic mice

5

ASO	Dose ($\mu\text{mol/kg}$)	HDL (mg/dL)	LDL (mg/dL)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	17	28	--	--	
ISIS 304801	0.08	17	23	None	PS/20	244
	0.75	27	12			
	2.25	50	4			
	6.75	45	2			
ISIS 647535	0.08	21	21	GalNAc ₃ -1	PS/20	245
	0.75	44	2			
	2.25	50	2			
	6.75	58	2			

Pharmacokinetics Analysis (PK)

The PK of the ASOs was also evaluated. Liver and kidney samples were minced and extracted using standard protocols. Samples were analyzed on MSD1 utilizing IP-HPLC-MS. The tissue level ($\mu\text{g/g}$) of full-length ISIS 304801 and 647535 was measured and the results are provided in Table 23. As illustrated, liver concentrations of total full-length antisense compounds were similar for the two antisense compounds. Thus, even though the GalNAc₃-1-conjugated antisense compound is more active in the liver (as demonstrated by the RNA and protein data above), it is not present at substantially higher concentration in the liver. Indeed, the calculated EC₅₀ (provided in Table 23) confirms that the observed increase in potency of the conjugated compound cannot be entirely attributed to increased accumulation. This result suggests that

the conjugate improved potency by a mechanism other than liver accumulation alone, possibly by improving the productive uptake of the antisense compound into cells.

The results also show that the concentration of **GalNAc₃-1** conjugated antisense compound in the kidney is lower than that of antisense compound lacking the GalNAc conjugate. This has several beneficial therapeutic implications. For therapeutic indications where activity in the kidney is not sought, exposure to kidney risks kidney toxicity without corresponding benefit. Moreover, high concentration in kidney typically results in loss of compound to the urine resulting in faster clearance. Accordingly, for non-kidney targets, kidney accumulation is undesired. These data suggest that **GalNAc₃-1** conjugation reduces kidney accumulation.

10

Table 23

PK analysis of ASO treatment in transgenic mice

ASO	Dose (μmol/kg)	Liver (μg/g)	Kidney (μg/g)	Liver EC ₅₀ (μg/g)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
ISIS 304801	0.1	5.2	2.1	53	None	PS/20	244
	0.8	62.8	119.6				
	2.3	142.3	191.5				
	6.8	202.3	337.7				
ISIS 647535	0.1	3.8	0.7	3.8	GalNAc₃-1	PS/20	245
	0.8	72.7	34.3				
	2.3	106.8	111.4				
	6.8	237.2	179.3				

15

Metabolites of ISIS 647535 were also identified and their masses were confirmed by high resolution mass spectrometry analysis. The cleavage sites and structures of the observed metabolites are shown below.

The relative % of full length ASO was calculated using standard procedures and the results are presented in Table 23a. The major metabolite of ISIS 647535 was full-length ASO lacking the entire conjugate (i.e. ISIS 304801), which results from cleavage at cleavage site A, shown below. Further, additional metabolites resulting from other cleavage sites were also observed. These results suggest that introducing other cleavable bonds such as esters, peptides, disulfides, phosphoramidates or acyl-hydrazones between the **GalNAc₃-1** sugar and the ASO, which can be cleaved by enzymes inside the cell, or which may cleave in the reductive environment of the cytosol, or which are labile to the acidic pH inside endosomes and lysosomes, can also be useful.

20

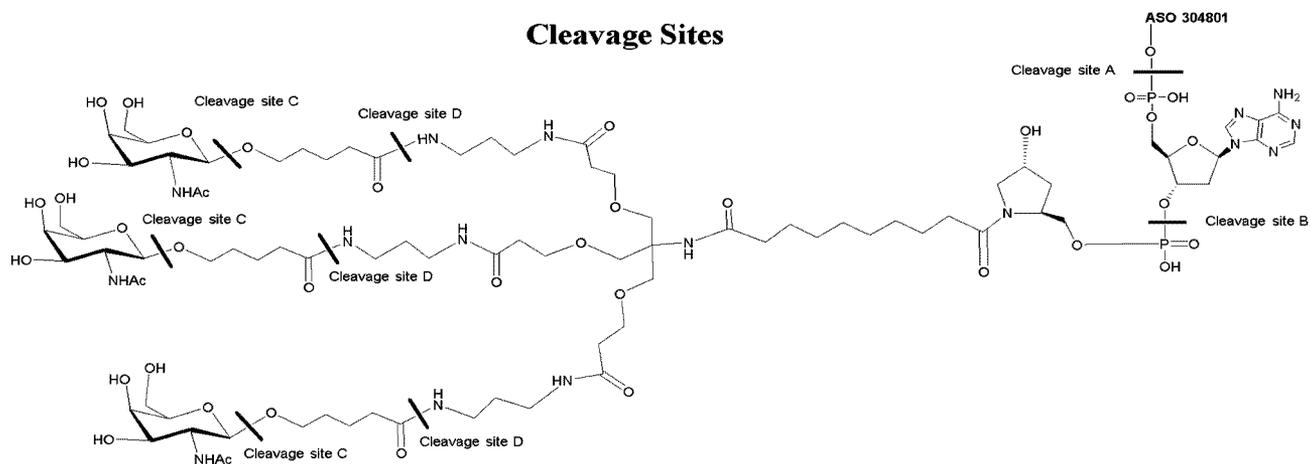
Table 23a

Observed full length metabolites of ISIS 647535

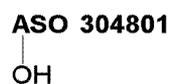
Metabolite	ASO	Cleavage site	Relative %
1	ISIS 304801	A	36.1

2	ISIS 304801 + dA	B	10.5
3	ISIS 647535 minus [3 GalNAc]	C	16.1
4	ISIS 647535 minus [3 GalNAc + 1 5-hydroxy-pentanoic acid tether]	D	17.6
5	ISIS 647535 minus [2 GalNAc + 2 5-hydroxy-pentanoic acid tether]	D	9.9
6	ISIS 647535 minus [3 GalNAc + 3 5-hydroxy-pentanoic acid tether]	D	9.8

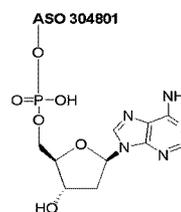
Cleavage Sites

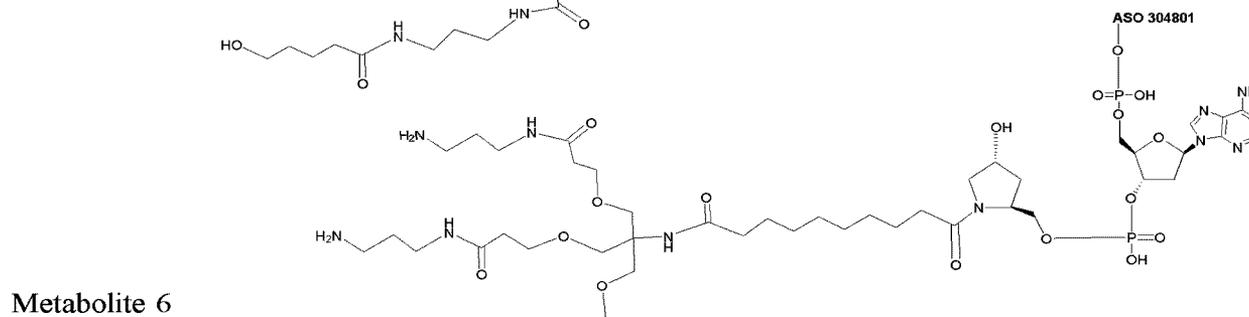
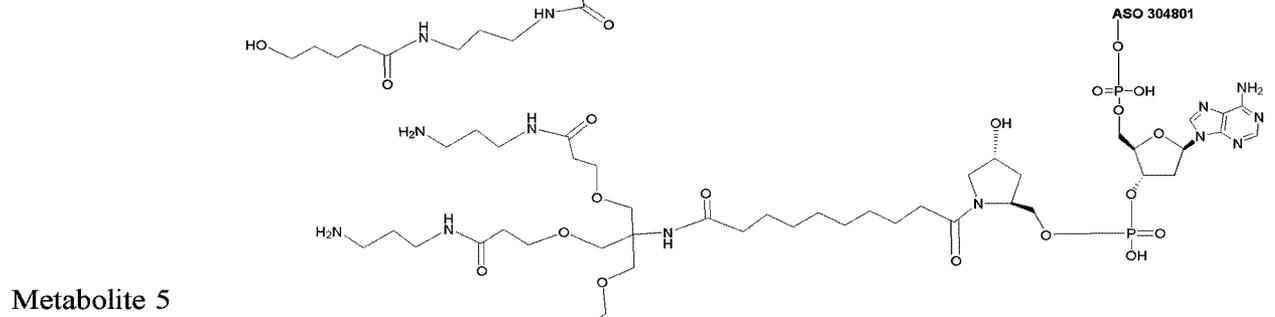
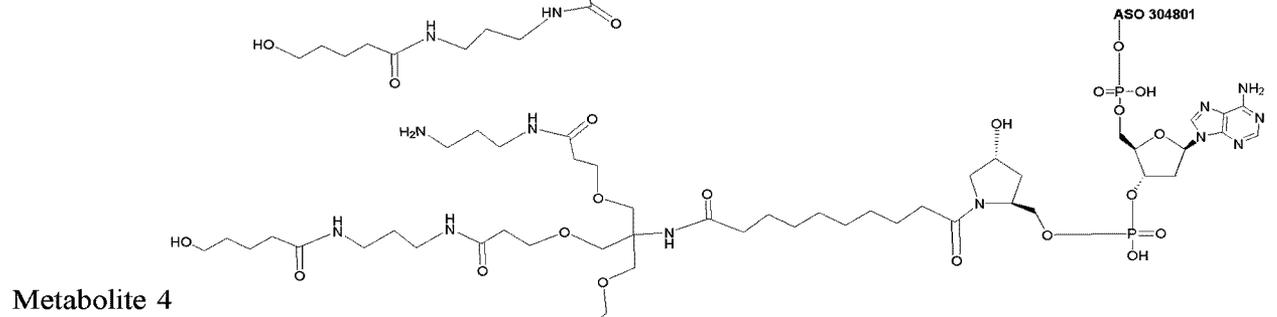
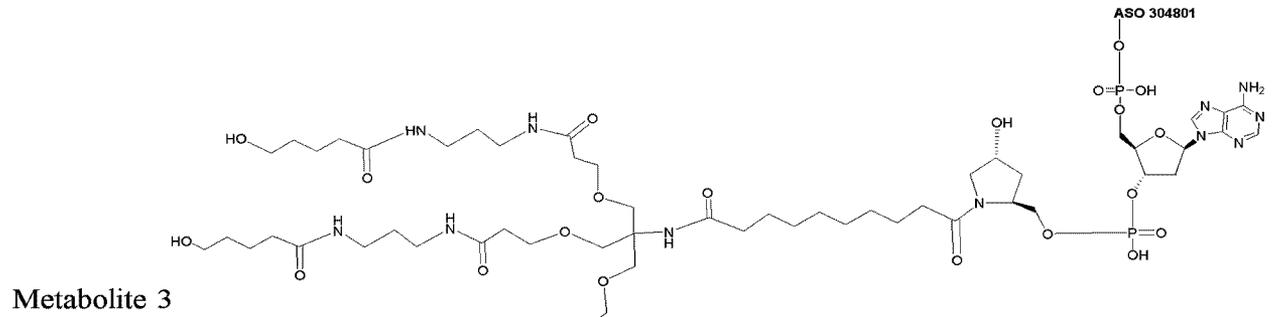


Metabolite 1



Metabolite 2





Example 21: Antisense inhibition of human ApoC III in human ApoC III transgenic mice in single administration study

ISIS 304801, 647535 and 647536 each targeting human ApoC III and described in Table 17, were further evaluated in a single administration study for their ability to inhibit human ApoC III in human ApoC
5 III transgenic mice.

Treatment

Human ApoCIII transgenic mice were maintained on a 12-hour light/dark cycle and fed *ad libitum* Teklad lab chow. Animals were acclimated for at least 7 days in the research facility before initiation of the experiment. ASOs were prepared in PBS and sterilized by filtering through a 0.2 micron filter. ASOs were
10 dissolved in 0.9% PBS for injection.

Human ApoC III transgenic mice were injected intraperitoneally once at the dosage shown below with ISIS 304801, 647535 or 647536 (described above) or with PBS treated control. The treatment group consisted of 3 animals and the control group consisted of 4 animals. Prior to the treatment as well as after the last dose, blood was drawn from each mouse and plasma samples were analyzed. The mice were sacrificed
15 72 hours following the last administration .

Samples were collected and analyzed to determine the ApoC III mRNA and protein levels in the liver; plasma triglycerides; and cholesterol, including HDL and LDL fractions were assessed as described above (Example 20). Data from those analyses are presented in Tables 24-28, below. Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured
20 relative to saline injected mice using standard protocols. The ALT and AST levels showed that the antisense compounds were well tolerated at all administered doses.

These results show improvement in potency for antisense compounds comprising a **GalNAc₃-1** conjugate at the 3' terminus (ISIS 647535 and 647536) compared to the antisense compound lacking a **GalNAc₃-1** conjugate (ISIS 304801). Further, ISIS 647536, which comprises a **GalNAc₃-1** conjugate and some phosphodiester linkages was as potent as ISIS 647535, which comprises the same conjugate and all
25 internucleoside linkages within the ASO are phosphorothioate.

Table 24

Effect of ASO treatment on ApoC III mRNA levels in human ApoC III transgenic mice

ASO	Dose (mg/kg)	% PBS	ED ₅₀ (mg/kg)	3' Conjugate	Internucleoside linkage/Length	SEQ ID No.
PBS	0	99	--	-	--	
ISIS 304801	1	104	13.2	None	PS/20	244
	3	92				
	10	71				
	30	40				
ISIS	0.3	98	1.9	GalNAc₃-1	PS/20	245

647535	1	70				
	3	33				
	10	20				
ISIS 647536	0.3	103	1.7	GalNAc₃-1	PS/PO/20	245
	1	60				
	3	31				
	10	21				

Table 25

Effect of ASO treatment on ApoC III plasma protein levels in human ApoC III transgenic mice

ASO	Dose (mg/kg)	% PBS	ED ₅₀ (mg/kg)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	99	--	--	--	
ISIS 304801	1	104	23.2	None	PS/20	244
	3	92				
	10	71				
	30	40				
ISIS 647535	0.3	98	2.1	GalNAc₃-1	PS/20	245
	1	70				
	3	33				
	10	20				
ISIS 647536	0.3	103	1.8	GalNAc₃-1	PS/PO/20	245
	1	60				
	3	31				
	10	21				

5

Table 26

Effect of ASO treatment on triglyceride levels in transgenic mice

ASO	Dose (mg/kg)	% PBS	ED ₅₀ (mg/kg)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	98	--	--	--	
ISIS 304801	1	80	29.1	None	PS/20	244
	3	92				
	10	70				
	30	47				
ISIS 647535	0.3	100	2.2	GalNAc₃-1	PS/20	245
	1	70				
	3	34				
	10	23				
ISIS 647536	0.3	95	1.9	GalNAc₃-1	PS/PO/20	245
	1	66				

	3	31				
	10	23				

Table 27

Effect of ASO treatment on total cholesterol levels in transgenic mice

ASO	Dose (mg/kg)	% PBS	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	96	--	--	
ISIS 304801	1	104	None	PS/20	244
	3	96			
	10	86			
	30	72			
ISIS 647535	0.3	93	GalNAc ₃ -1	PS/20	245
	1	85			
	3	61			
	10	53			
ISIS 647536	0.3	115	GalNAc ₃ -1	PS/PO/20	245
	1	79			
	3	51			
	10	54			

5

Table 28

Effect of ASO treatment on HDL and LDL cholesterol levels in transgenic mice

ASO	Dose (mg/kg)	HDL % PBS	LDL % PBS	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	131	90	--	--	
ISIS 304801	1	130	72	None	PS/20	244
	3	186	79			
	10	226	63			
	30	240	46			
ISIS 647535	0.3	98	86	GalNAc ₃ -1	PS/20	245
	1	214	67			
	3	212	39			
	10	218	35			
ISIS 647536	0.3	143	89	GalNAc ₃ -1	PS/PO/20	245
	1	187	56			
	3	213	33			
	10	221	34			

These results confirm that the **GalNAc₃-1** conjugate improves potency of an antisense compound. The results also show equal potency of a **GalNAc₃-1** conjugated antisense compounds where the antisense oligonucleotides have mixed linkages (ISIS 647536 which has six phosphodiester linkages) and a full phosphorothioate version of the same antisense compound (ISIS 647535).

5 Phosphorothioate linkages provide several properties to antisense compounds. For example, they resist nuclease digestion and they bind proteins resulting in accumulation of compound in the liver, rather than in the kidney/urine. These are desirable properties, particularly when treating an indication in the liver. However, phosphorothioate linkages have also been associated with an inflammatory response. Accordingly, reducing the number of phosphorothioate linkages in a compound is expected to reduce the risk of
10 inflammation, but also lower concentration of the compound in liver, increase concentration in the kidney and urine, decrease stability in the presence of nucleases, and lower overall potency. The present results show that a **GalNAc₃-1** conjugated antisense compound where certain phosphorothioate linkages have been replaced with phosphodiester linkages is as potent against a target in the liver as a counterpart having full phosphorothioate linkages. Such compounds are expected to be less proinflammatory (See Example 24
15 describing an experiment showing reduction of PS results in reduced inflammatory effect).

Example 22: Effect of GalNAc₃-1 conjugated modified ASO targeting SRB-1 *in vivo*

ISIS 440762 and 651900, each targeting SRB-1 and described in Table 17, were evaluated in a dose-dependent study for their ability to inhibit SRB-1 in Balb/c mice.

20 *Treatment*

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with ISIS 440762, 651900 or with PBS treated control. Each treatment group consisted of 4 animals. The mice were sacrificed 48 hours following the final administration to determine the SRB-1 mRNA levels in liver using real-time PCR and RIBOGREEN® RNA quantification
25 reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. SRB-1 mRNA levels were determined relative to total RNA (using Ribogreen), prior to normalization to PBS-treated control. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to PBS-treated control and is denoted as “% PBS”.

As illustrated in Table 29, both antisense compounds lowered SRB-1 mRNA levels. Further, the
30 antisense compound comprising the **GalNAc₃-1** conjugate (ISIS 651900) was substantially more potent than the antisense compound lacking the **GalNAc₃-1** conjugate (ISIS 440762). These results demonstrate that the potency benefit of **GalNAc₃-1** conjugates are observed using antisense oligonucleotides complementary to a different target and having different chemically modified nucleosides, in this instance modified nucleosides comprise constrained ethyl sugar moieties (a bicyclic sugar moiety).

Table 29

Effect of ASO treatment on SRB-1 mRNA levels in Balb/c mice

ASO	Dose (mg/kg)	Liver % PBS	ED ₅₀ (mg/kg)	3' Conjugate		Internucleosid e linkage/Lengt h	SEQ ID No.
PBS	0	100		-		--	
ISIS 440762	0.7	85	2.2	None		PS/14	246
	2	55					
	7	12					
	20	3					
ISIS 651900	0.07	98	0.3	GalNAc ₃ -1		PS/14	247
	0.2	63					
	0.7	20					
	2	6					
	7	5					

5 Example 23: Human Peripheral Blood Mononuclear Cells (hPBMC) Assay Protocol

The hPBMC assay was performed using BD Vautainer CPT tube method. A sample of whole blood from volunteered donors with informed consent at US HealthWorks clinic (Faraday & El Camino Real, Carlsbad) was obtained and collected in 4-15 BD Vacutainer CPT 8 ml tubes (VWR Cat.# BD362753). The approximate starting total whole blood volume in the CPT tubes for each donor was recorded using the
10 PBMC assay data sheet.

The blood sample was remixed immediately prior to centrifugation by gently inverting tubes 8-10 times. CPT tubes were centrifuged at rt (18-25 °C) in a horizontal (swing-out) rotor for 30 min. at 1500-1800 RCF with brake off (2700 RPM Beckman Allegra 6R). The cells were retrieved from the buffy coat interface (between Ficoll and polymer gel layers); transferred to a sterile 50 ml conical tube and pooled up to 5 CPT
15 tubes/50 ml conical tube/donor. The cells were then washed twice with PBS (Ca⁺⁺, Mg⁺⁺ free; GIBCO). The tubes were topped up to 50 ml and mixed by inverting several times. The sample was then centrifuged at 330 x g for 15 minutes at rt (1215 RPM in Beckman Allegra 6R) and aspirated as much supernatant as possible without disturbing pellet. The cell pellet was dislodged by gently swirling tube and resuspended cells in
20 RPMI+10% FBS+pen/strep (~1 ml / 10 ml starting whole blood volume). A 60 µl sample was pipette into a sample vial (Beckman Coulter) with 600 µl VersaLyse reagent (Beckman Coulter Cat# A09777) and was gently vortexed for 10-15 sec. The sample was allowed to incubate for 10 min. at rt and being mixed again before counting. The cell suspension was counted on Vicell XR cell viability analyzer (Beckman Coulter) using PBMC cell type (dilution factor of 1:11 was stored with other parameters). The live cell/ml and viability were recorded. The cell suspension was diluted to 1 x 10⁷ live PBMC/ml in RPMI+ 10%
25 FBS+pen/strep.

The cells were plated at 5×10^5 in 50 μ l/well of 96-well tissue culture plate (Falcon Microtest). 50 μ l/well of 2x concentration oligos/controls diluted in RPMI+10% FBS+pen/strep. was added according to experiment template (100 μ l/well total). Plates were placed on the shaker and allowed to mix for approx. 1 min. After being incubated for 24 hrs at 37 °C; 5% CO₂, the plates were centrifuged at 400 x g for 10 minutes before removing the supernatant for MSD cytokine assay (i.e. human IL-6, IL-10, IL-8 and MCP-1).

Example 24: Evaluation of Proinflammatory Effects in hPBMC Assay for GalNAc₃-1 conjugated ASOs

The antisense oligonucleotides (ASOs) listed in Table 30 were evaluated for proinflammatory effect in hPBMC assay using the protocol described in Example 23. ISIS 353512 is an internal standard known to be a high responder for IL-6 release in the assay. The hPBMCs were isolated from fresh, volunteered donors and were treated with ASOs at 0, 0.0128, 0.064, 0.32, 1.6, 8, 40 and 200 μ M concentrations. After a 24 hr treatment, the cytokine levels were measured.

The levels of IL-6 were used as the primary readout. The EC₅₀ and E_{max} was calculated using standard procedures. Results are expressed as the average ratio of E_{max}/EC₅₀ from two donors and is denoted as "E_{max}/EC₅₀." The lower ratio indicates a relative decrease in the proinflammatory response and the higher ratio indicates a relative increase in the proinflammatory response.

With regard to the test compounds, the least proinflammatory compound was the PS/PO linked ASO (ISIS 616468). The GalNAc₃-1 conjugated ASO, ISIS 647535 was slightly less proinflammatory than its non-conjugated counterpart ISIS 304801. These results indicate that incorporation of some PO linkages reduces proinflammatory reaction and addition of a GalNAc₃-1 conjugate does not make a compound more proinflammatory and may reduce proinflammatory response. Accordingly, one would expect that an antisense compound comprising both mixed PS/PO linkages and a GalNAc₃-1 conjugate would produce lower proinflammatory responses relative to full PS linked antisense compound with or without a GalNAc₃-1 conjugate. These results show that GalNAc₃-1 conjugated antisense compounds, particularly those having reduced PS content are less proinflammatory.

Together, these results suggest that a GalNAc₃-1 conjugated compound, particularly one with reduced PS content, can be administered at a higher dose than a counterpart full PS antisense compound lacking a GalNAc₃-1 conjugate. Since half-life is not expected to be substantially different for these compounds, such higher administration would result in less frequent dosing. Indeed such administration could be even less frequent, because the GalNAc₃-1 conjugated compounds are more potent (See Examples 20-22) and re-dosing is necessary once the concentration of a compound has dropped below a desired level, where such desired level is based on potency.

Table 30

Modified ASOs

ASO	Sequence (5' to 3')	Target	SEQ ID No.
ISIS 104838	G ^{es} C ^{es} T ^{es} G ^{es} A ^{es} T ^{ds} T ^{ds} A ^{ds} G ^{ds} A ^{ds} G ^{ds} A ^{ds} G ^{ds} A ^{ds} G ^{ds} G ^{es} T ^{es} ^m C ^{es} ^m C ^{es} ^m C ^e	TNF α	248
ISIS 353512	T ^{es} ^m C ^{es} ^m C ^{es} ^m C ^{ds} A ^{ds} T ^{ds} T ^{ds} T ^{ds} ^m C ^{ds} A ^{ds} G ^{ds} G ^{ds} A ^{ds} G ^{ds} A ^{ds} ^m C ^{ds} ^m C ^{ds} T ^{es} G ^{es} G ^e	CRP	249
ISIS 304801	A ^{es} G ^{es} ^m C ^{es} T ^{es} T ^{es} ^m C ^{ds} T ^{ds} T ^{ds} G ^{ds} T ^{ds} ^m C ^{ds} ^m C ^{ds} A ^{ds} G ^{ds} ^m C ^{ds} T ^{es} T ^{es} T ^{es} A ^{es} T ^e	ApoC III	244
ISIS 647535	A ^{es} G ^{es} ^m C ^{es} T ^{es} T ^{es} ^m C ^{ds} T ^{ds} T ^{ds} G ^{ds} T ^{ds} ^m C ^{ds} ^m C ^{ds} A ^{ds} G ^{ds} ^m C ^{ds} T ^{es} T ^{es} T ^{es} A ^{es} T ^{eo} A_{do}'-GalNAc₃-1_a	ApoC III	245
ISIS 616468	A ^{es} G ^{eo} ^m C ^{eo} T ^{eo} T ^{eo} ^m C ^{ds} T ^{ds} T ^{ds} G ^{ds} T ^{ds} ^m C ^{ds} ^m C ^{ds} A ^{ds} G ^{ds} ^m C ^{ds} T ^{eo} T ^{eo} T ^{es} A ^{es} T ^e	ApoC III	244

Subscripts: “e” indicates 2'-MOE modified nucleoside; “d” indicates β -D-2'-
5 deoxyribonucleoside; “k” indicates 6'-(S)-CH₃ bicyclic nucleoside (e.g. cEt); “s” indicates phosphorothioate internucleoside linkages (PS); “o” indicates phosphodiester internucleoside linkages (PO); and “o” indicates -O-P(=O)(OH)-. Superscript “m” indicates 5-methylcytosines. “A_{do}'-GalNAc₃-1_a” indicates a conjugate having the structure GalNAc₃-1 shown in Example 9 attached to the 3'-end of the antisense oligonucleotide, as indicated.

10

Table 31

Proinflammatory Effect of ASOs targeting ApoC III in hPBMC assay

ASO	EC ₅₀ (μ M)	E _{max} (μ M)	E _{max} /EC ₅₀	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
ISIS 353512 (high responder)	0.01	265.9	26,590	None	PS/20	249
ISIS 304801	0.07	106.55	1,522	None	PS/20	244
ISIS 647535	0.12	138	1,150	GalNAc₃-1	PS/20	245
ISIS 616468	0.32	71.52	224	None	PS/PO/20	244

Example 25: Effect of GalNAc₃-1 conjugated modified ASO targeting human ApoC III *in vitro*

ISIS 304801 and 647535 described above were tested *in vitro*. Primary hepatocyte cells from
15 transgenic mice at a density of 25,000 cells per well were treated with 0.03, 0.08, 0.24, 0.74, 2.22, 6.67 and 20 μ M concentrations of modified oligonucleotides. After a treatment period of approximately 16 hours, RNA

was isolated from the cells and mRNA levels were measured by quantitative real-time PCR and the hApoC III mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN.

The IC₅₀ was calculated using the standard methods and the results are presented in Table 32. As illustrated, comparable potency was observed in cells treated with ISIS 647535 as compared to the control, 5 ISIS 304801.

Table 32

Modified ASO targeting human ApoC III in primary hepatocytes

ASO	IC ₅₀ (μM)	3' Conjugate	Internucleoside linkage/Length	SEQ ID No.
ISIS 304801	0.44	None	PS/20	244
ISIS 647535	0.31	GalNAc₃-1	PS/20	245

In this experiment, the large potency benefits of **GalNAc₃-1** conjugation that are observed *in vivo* 10 were not observed *in vitro*. Subsequent free uptake experiments in primary hepatocytes *in vitro* did show increased potency of oligonucleotides comprising various GalNAc conjugates relative to oligonucleotides that lacking the GalNAc conjugate.(see Examples 60, 82, and 92)

Example 26: Effect of PO/PS linkages on ApoC III ASO Activity

Human ApoC III transgenic mice were injected intraperitoneally once at 25 mg/kg of ISIS 304801, 15 or ISIS 616468 (both described above) or with PBS treated control once per week for two weeks. The treatment group consisted of 3 animals and the control group consisted of 4 animals. Prior to the treatment as well as after the last dose, blood was drawn from each mouse and plasma samples were analyzed. The mice were sacrificed 72 hours following the last administration.

Samples were collected and analyzed to determine the ApoC III protein levels in the liver as 20 described above (Example 20). Data from those analyses are presented in Table 33, below.

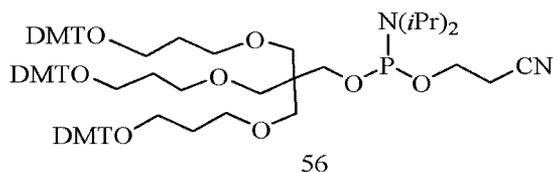
These results show reduction in potency for antisense compounds with PO/PS (ISIS 616468) in the wings relative to full PS (ISIS 304801).

Table 33

Effect of ASO treatment on ApoC III protein levels in human ApoC III transgenic mice

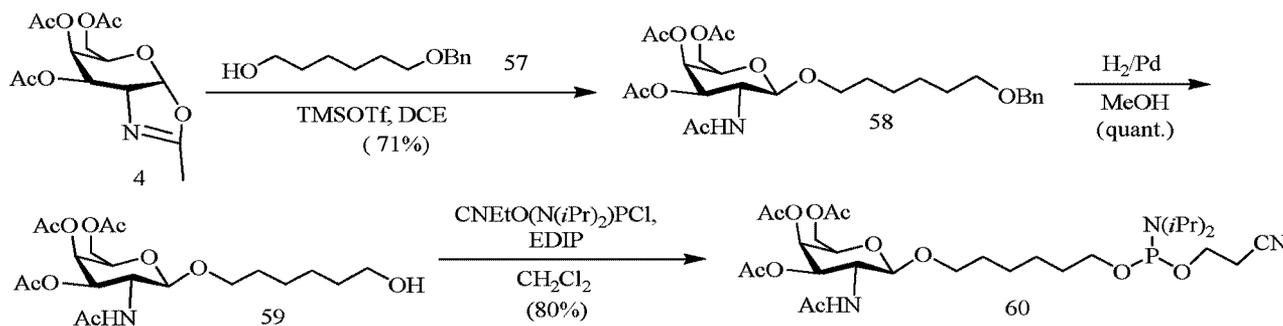
ASO	Dose (mg/kg)	% PBS	3' Conjugate	Internucleoside linkage/Length	SEQ ID No.
PBS	0	99	-	--	

ISIS 304801	25 mg/kg/wk for 2 wks	24	None	Full PS	244
ISIS 616468	25 mg/kg/wk for 2 wks	40	None	14 PS/6 PO	244

Example 27: Compound 56

5

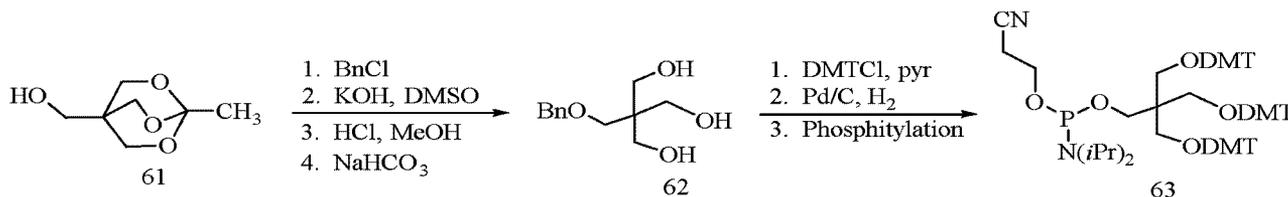
Compound 56 is commercially available from Glen Research or may be prepared according to published procedures reported by Shchepinov *et al.*, *Nucleic Acids Research*, 1997, 25(22), 4447-4454.

Example 28: Preparation of Compound 60

Compound 4 was prepared as per the procedures illustrated in Example 2. Compound 57 is commercially available. Compound 60 was confirmed by structural analysis.

Compound 57 is meant to be representative and not intended to be limiting as other monoprotected substituted or unsubstituted alkyl diols including but not limited to those presented in the specification herein can be used to prepare phosphoramidites having a predetermined composition.

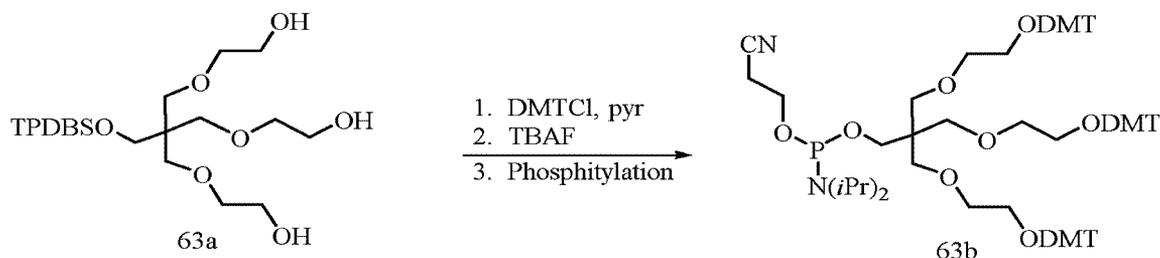
15

Example 29: Preparation of Compound 63

Compounds 61 and 62 are prepared using procedures similar to those reported by Tober *et al.*, *Eur. J. Org. Chem.*, 2013, 3, 566-577; and Jiang *et al.*, *Tetrahedron*, 2007, 63(19), 3982-3988.

Alternatively, Compound 63 is prepared using procedures similar to those reported in scientific and patent literature by Kim *et al.*, *Synlett*, 2003, 12, 1838-1840; and Kim *et al.*, published PCT International Application, WO 2004063208.

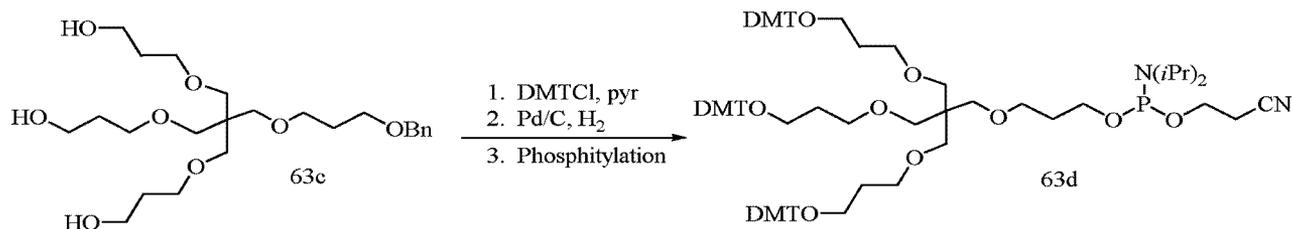
Example 30: Preparation of Compound 63b



Compound 63a is prepared using procedures similar to those reported by Hanessian *et al.*, *Canadian Journal of Chemistry*, 1996, 74(9), 1731-1737.

10

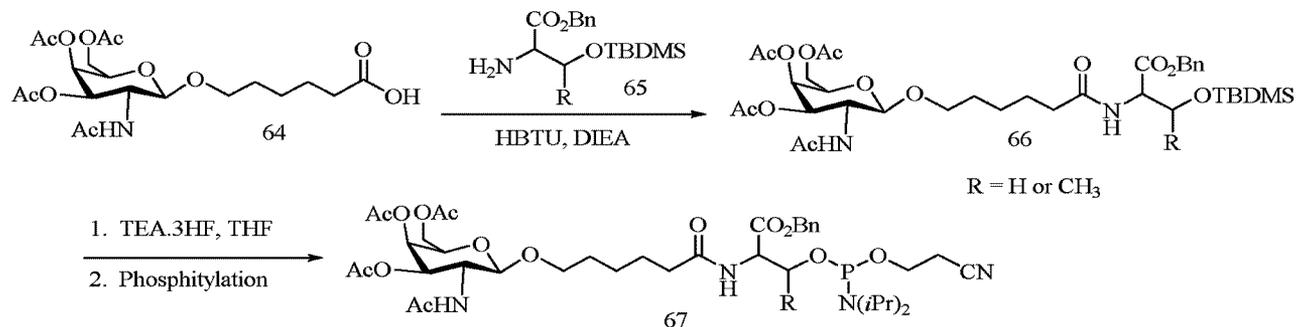
Example 31: Preparation of Compound 63d



Compound 63c is prepared using procedures similar to those reported by Chen *et al.*, *Chinese Chemical Letters*, 1998, 9(5), 451-453.

15

Example 32: Preparation of Compound 67

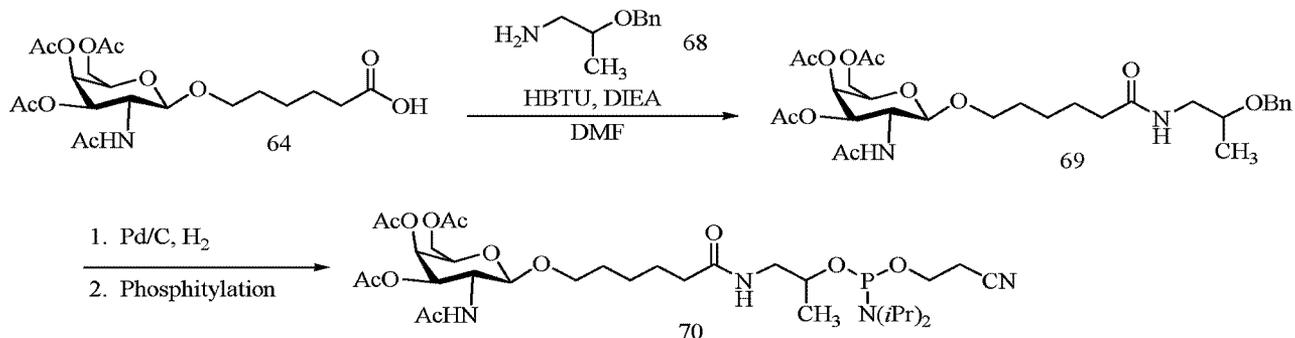


Compound 64 was prepared as per the procedures illustrated in Example 2. Compound 65 is prepared using procedures similar to those reported by Or *et al.*, published PCT International Application, WO 2009003009. The protecting groups used for Compound 65 are meant to be representative and not

20

intended to be limiting as other protecting groups including but not limited to those presented in the specification herein can be used.

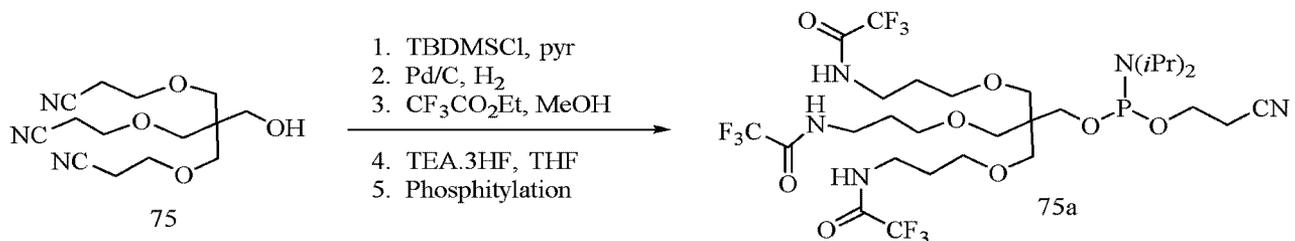
Example 33: Preparation of Compound 70



Compound 64 was prepared as per the procedures illustrated in Example 2. Compound 68 is commercially available. The protecting group used for Compound 68 is meant to be representative and not intended to be limiting as other protecting groups including but not limited to those presented in the specification herein can be used.

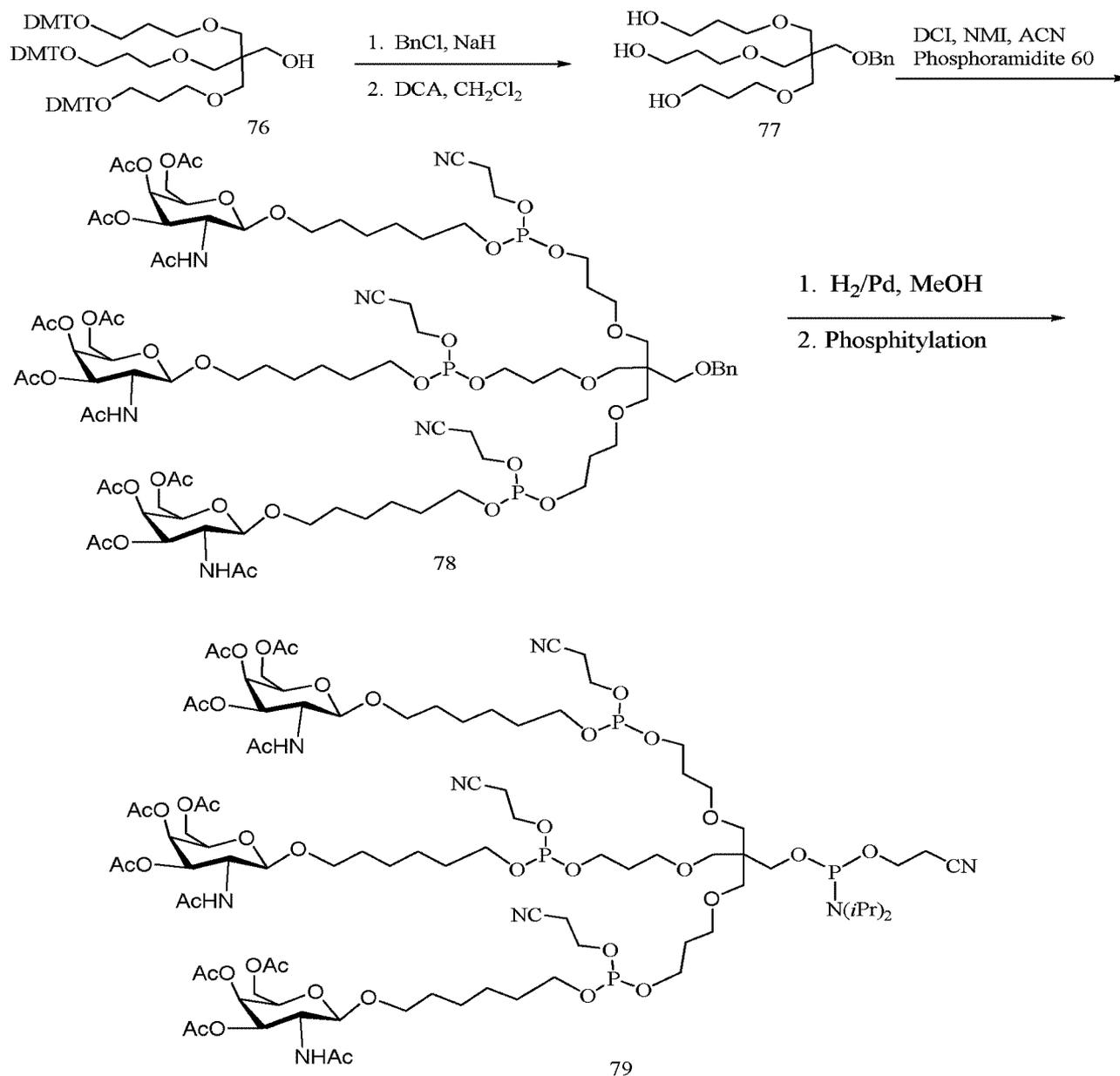
10

Example 34: Preparation of Compound 75a



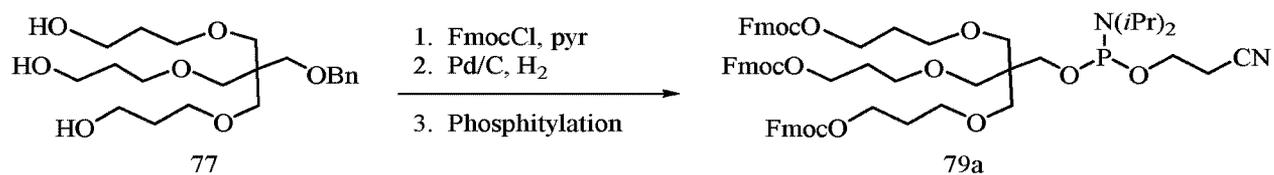
Compound 75 is prepared according to published procedures reported by Shchepinov *et al.*, *Nucleic Acids Research*, 1997, 25(22), 4447-4454.

15

Example 35: Preparation of Compound 79

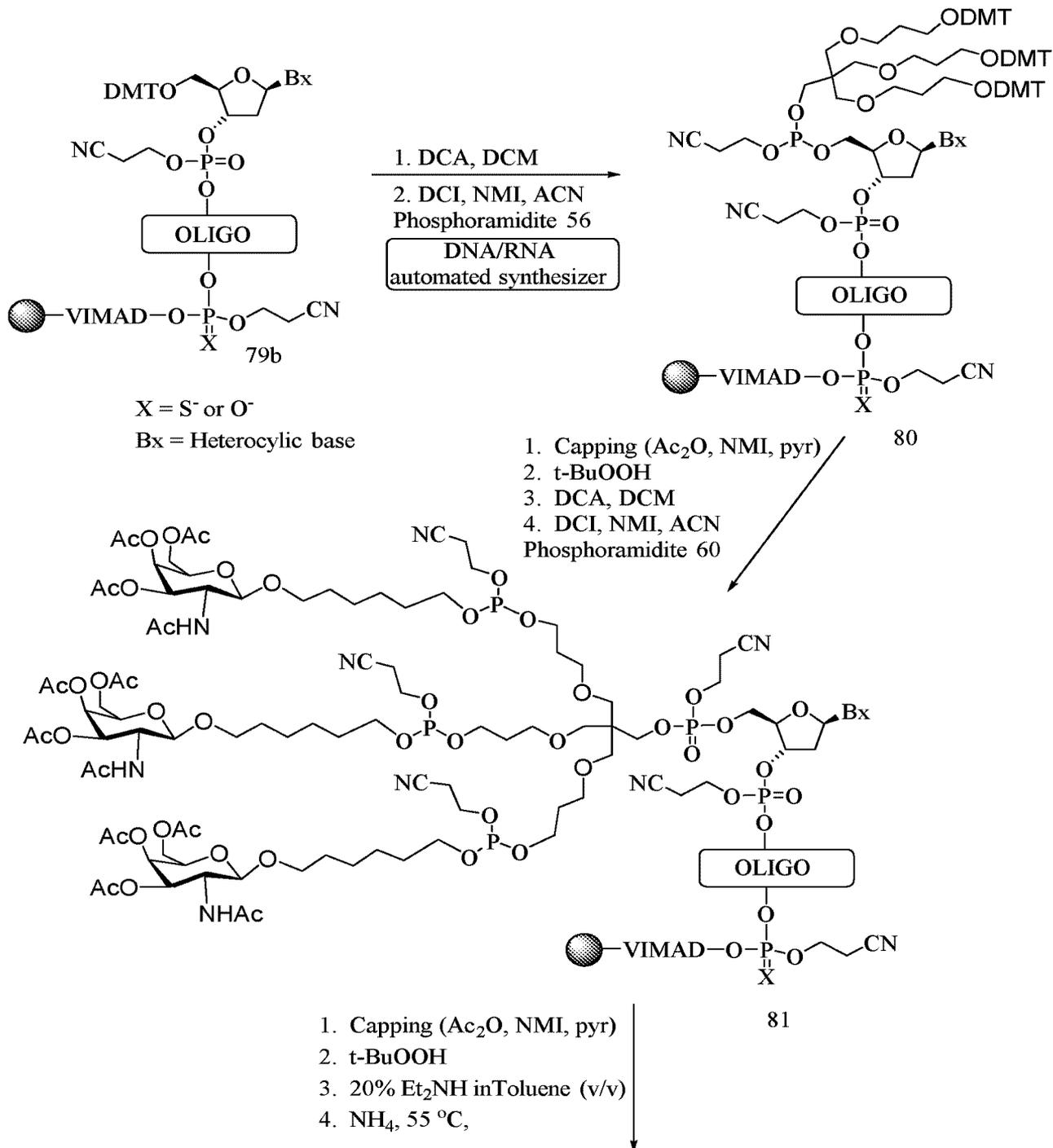
Compound 76 was prepared according to published procedures reported by Shchepinov *et al.*, *Nucleic Acids Research*, 1997, 25(22), 4447-4454.

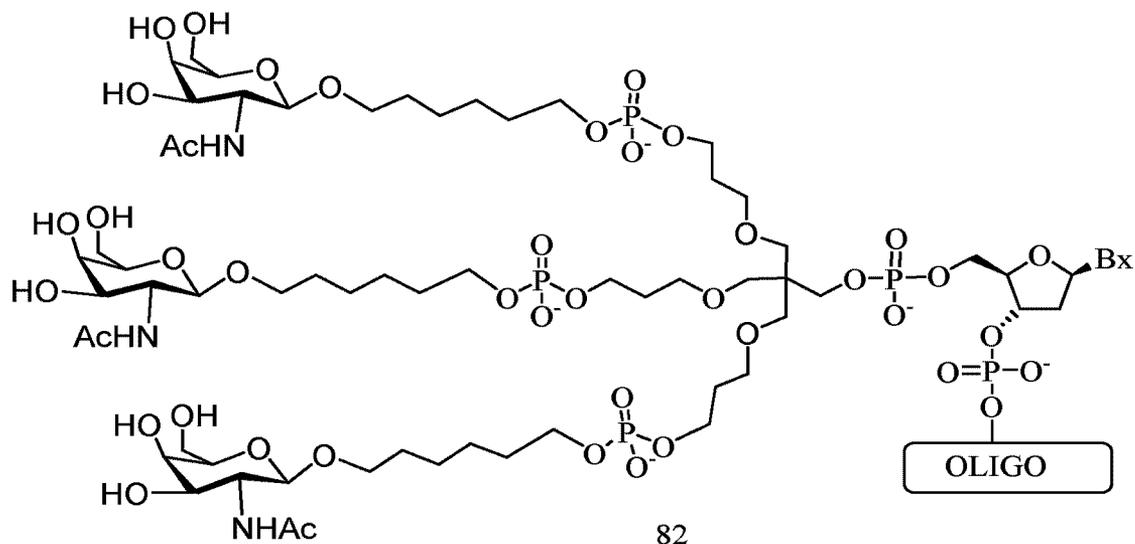
5

Example 36: Preparation of Compound 79a

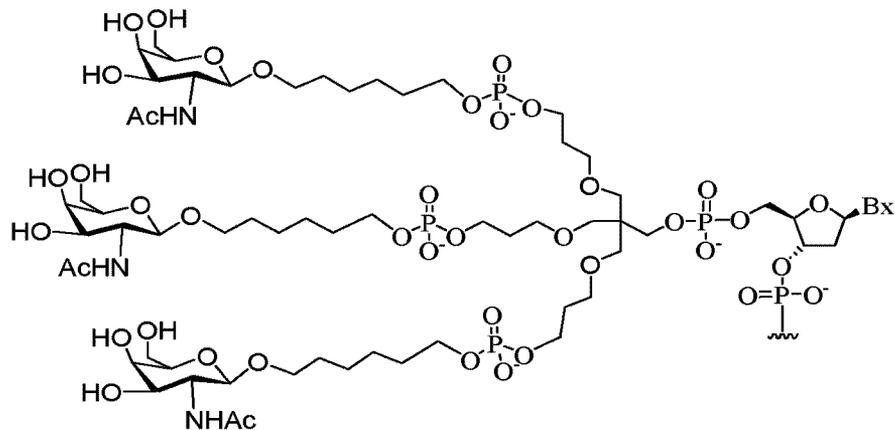
Compound 77 is prepared as per the procedures illustrated in Example 35.

Example 37: General method for the preparation of conjugated oligomeric compound 82 comprising a phosphodiester linked GalNAc₃-2 conjugate at 5' terminus *via* solid support (Method I)

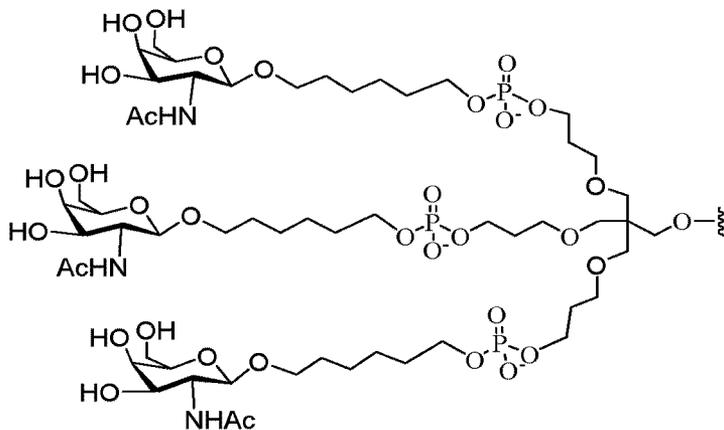




wherein GalNAc₃-2 has the structure:

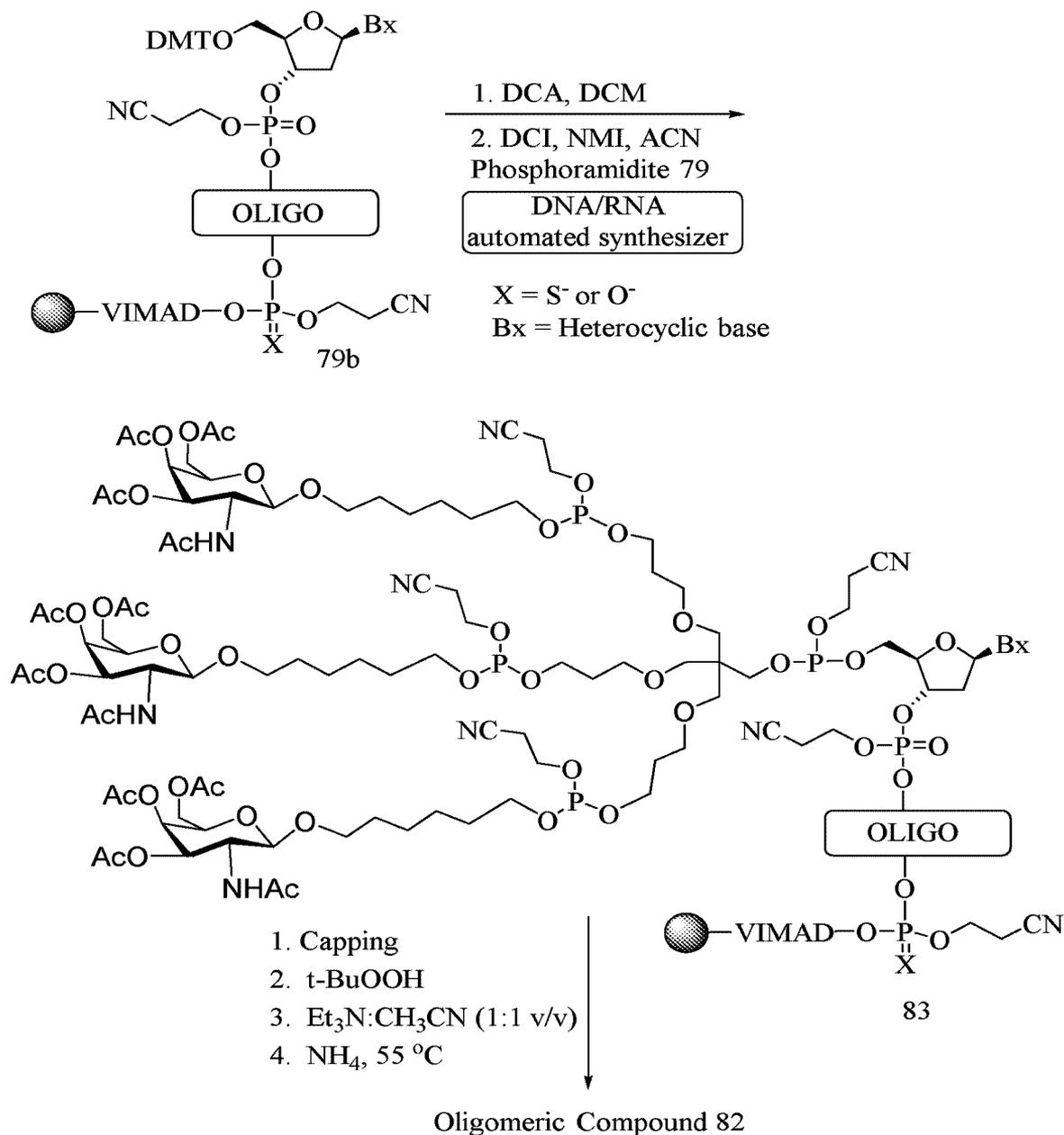


The GalNAc₃ cluster portion of the conjugate group GalNAc₃-2 (GalNAc₃-2_a) can be combined with
 5 any cleavable moiety to provide a variety of conjugate groups. Wherein GalNAc₃-2_a has the formula:



The VIMAD-bound oligomeric compound 79b was prepared using standard procedures for automated DNA/RNA synthesis (see Dupouy *et al.*, *Angew. Chem. Int. Ed.*, 2006, 45, 3623-3627). The phosphoramidite Compounds 56 and 60 were prepared as per the procedures illustrated in Examples 27 and 28, respectively. The phosphoramidites illustrated are meant to be representative and not intended to be limiting as other phosphoramidite building blocks including but not limited those presented in the specification herein can be used to prepare an oligomeric compound having a phosphodiester linked conjugate group at the 5' terminus. The order and quantity of phosphoramidites added to the solid support can be adjusted to prepare the oligomeric compounds as described herein having any predetermined sequence and composition.

Example 38: Alternative method for the preparation of oligomeric compound 82 comprising a phosphodiester linked GalNAc₃-2 conjugate at 5' terminus (Method II)

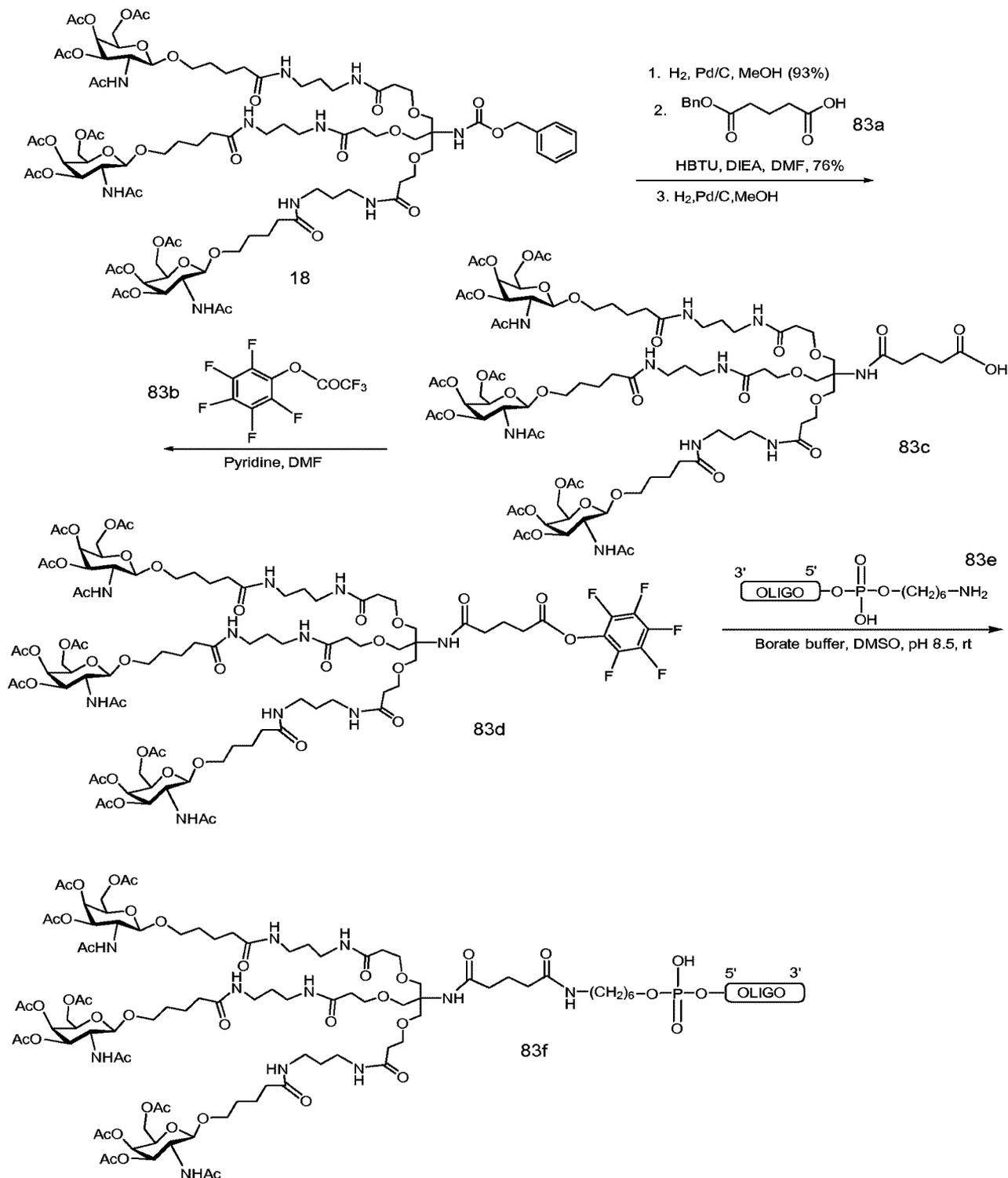


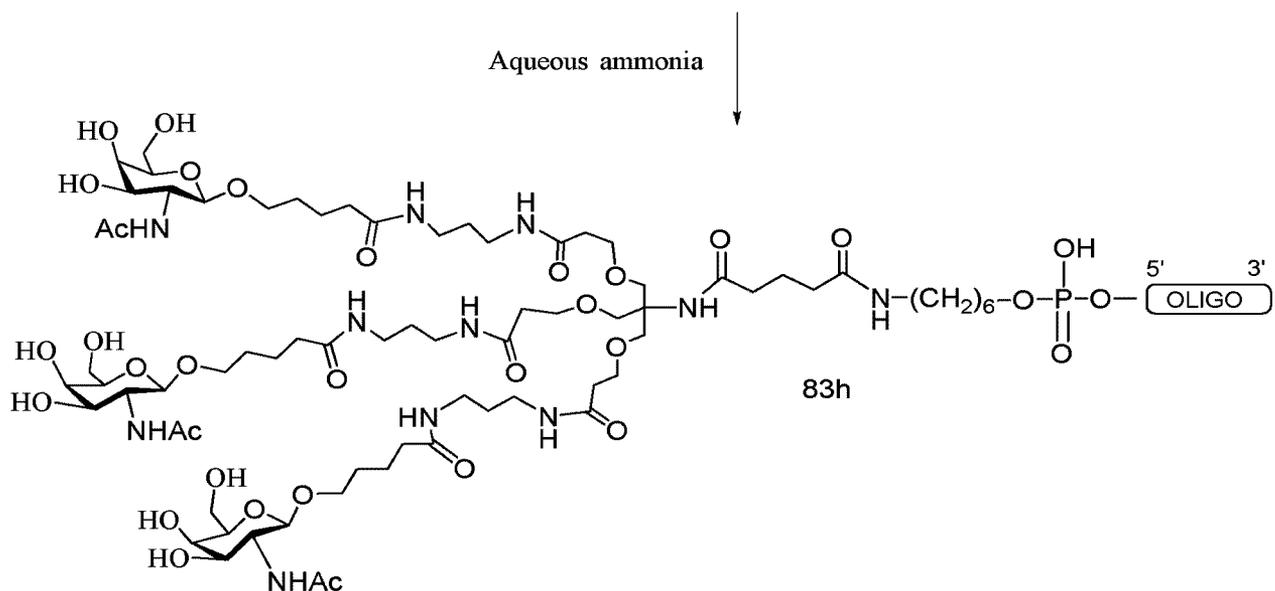
- 5 The VIMAD-bound oligomeric compound 79b was prepared using standard procedures for automated DNA/RNA synthesis (see Dupouy *et al.*, *Angew. Chem. Int. Ed.*, 2006, 45, 3623-3627). The GalNAc₃-2 cluster phosphoramidite, Compound 79 was prepared as per the procedures illustrated in Example 35. This alternative method allows a one-step installation of the phosphodiester linked GalNAc₃-2 conjugate to the oligomeric compound at the final step of the synthesis. The phosphoramidites illustrated are meant to

be representative and not intended to be limiting, as other phosphoramidite building blocks including but not limited to those presented in the specification herein can be used to prepare oligomeric compounds having a phosphodiester conjugate at the 5' terminus. The order and quantity of phosphoramidites added to the solid support can be adjusted to prepare the oligomeric compounds as described herein having any predetermined sequence and composition.

5

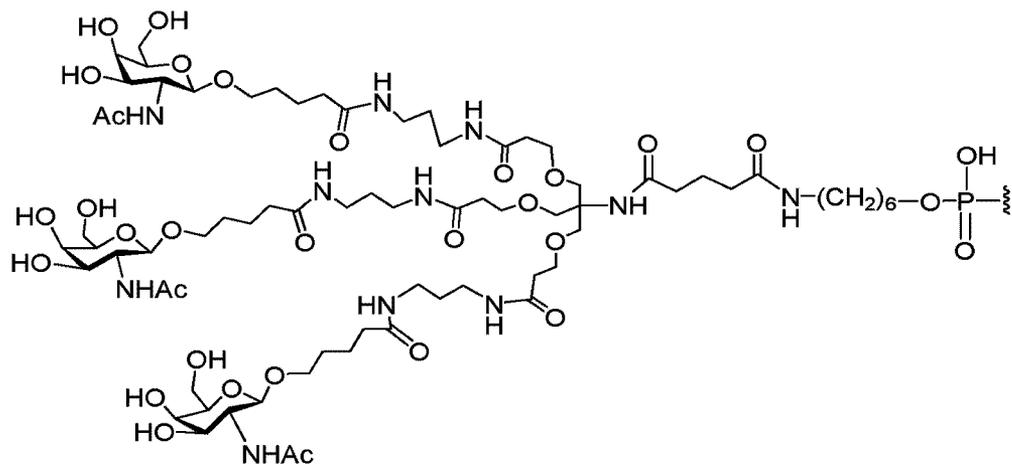
Example 39: General method for the preparation of oligomeric compound 83h comprising a GalNAc₃-3 Conjugate at the 5' Terminus (GalNAc₃-1 modified for 5' end attachment) *via* Solid Support



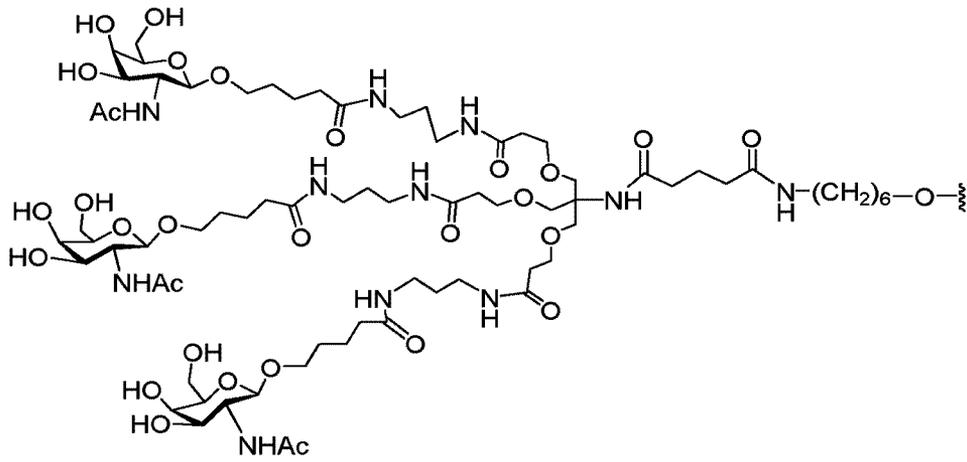


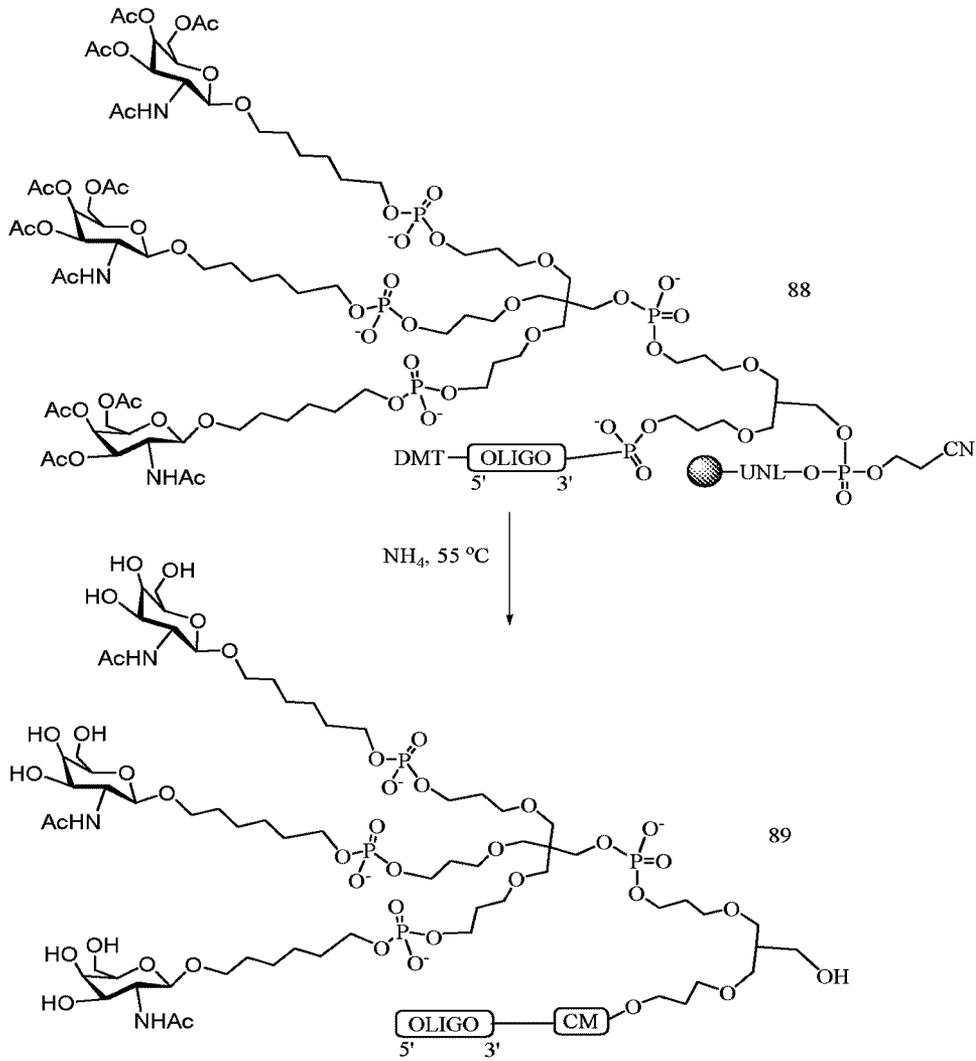
Compound 18 was prepared as per the procedures illustrated in Example 4. Compounds 83a and 83b are commercially available. Oligomeric Compound 83e comprising a phosphodiester linked hexylamine was prepared using standard oligonucleotide synthesis procedures. Treatment of the protected oligomeric compound with aqueous ammonia provided the 5'-GalNAc₃-3 conjugated oligomeric compound (83h).

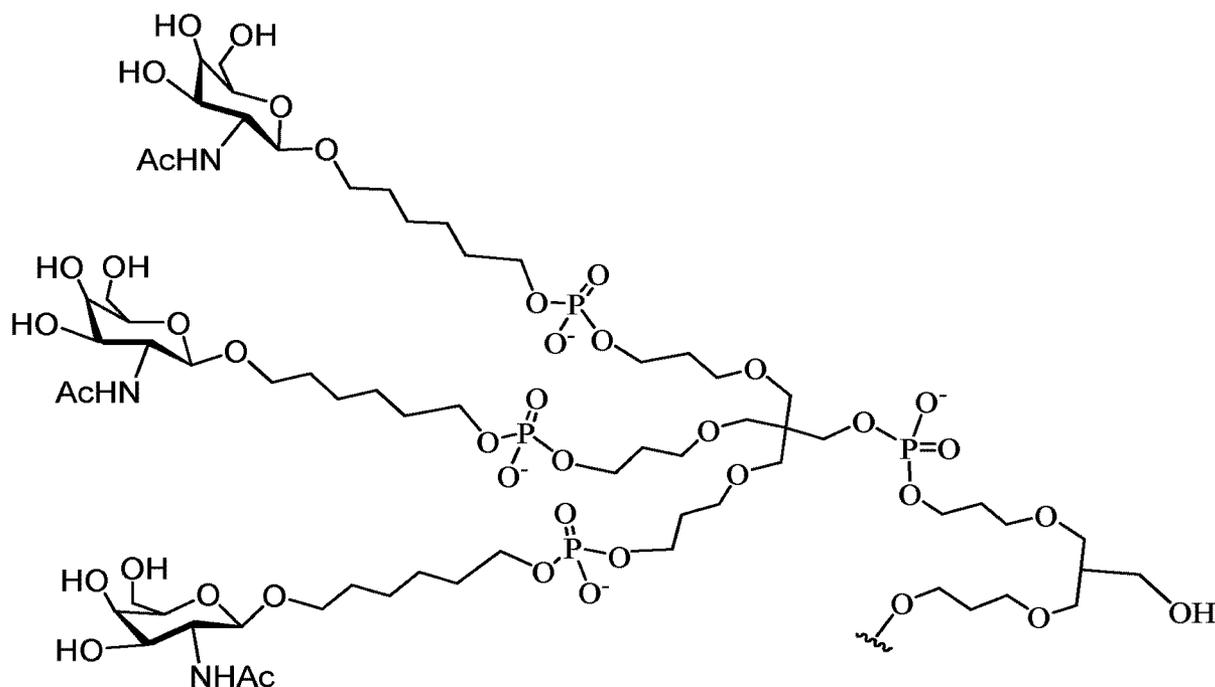
Wherein GalNAc₃-3 has the structure:



The GalNAc₃ cluster portion of the conjugate group GalNAc₃-3 (GalNAc₃-3_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. Wherein GalNAc₃-3_a has the formula:







The protected Unylinker functionalized solid support Compound 30 is commercially available.

5 Compound 84 is prepared using procedures similar to those reported in the literature (*see* Shchepinov *et al.*, *Nucleic Acids Research*, 1997, 25(22), 4447-4454; Shchepinov *et al.*, *Nucleic Acids Research*, 1999, 27, 3035-3041; and Hornet *et al.*, *Nucleic Acids Research*, 1997, 25, 4842-4849).

10 The phosphoramidite building blocks, Compounds 60 and 79a are prepared as per the procedures illustrated in Examples 28 and 36. The phosphoramidites illustrated are meant to be representative and not intended to be limiting as other phosphoramidite building blocks can be used to prepare an oligomeric compound having a phosphodiester linked conjugate at the 3' terminus with a predetermined sequence and composition. The order and quantity of phosphoramidites added to the solid support can be adjusted to prepare the oligomeric compounds as described herein having any predetermined sequence and composition.

15 **Example 41: General method for the preparation of ASOs comprising a phosphodiester linked GalNAc₃-2 (see Example 37, Bx is adenine) conjugate at the 5' position *via* solid phase techniques (preparation of ISIS 661134)**

20 Unless otherwise stated, all reagents and solutions used for the synthesis of oligomeric compounds are purchased from commercial sources. Standard phosphoramidite building blocks and solid support are used for incorporation nucleoside residues which include for example T, A, G, and ^mC residues. Phosphoramidite compounds 56 and 60 were used to synthesize the phosphodiester linked GalNAc₃-2

conjugate at the 5' terminus. A 0.1 M solution of phosphoramidite in anhydrous acetonitrile was used for β -D-2'-deoxyribonucleoside and 2'-MOE.

The ASO syntheses were performed on ABI 394 synthesizer (1-2 μ mol scale) or on GE Healthcare Bioscience \ddot{A} KTA oligopilot synthesizer (40-200 μ mol scale) by the phosphoramidite coupling method on VIMAD solid support (110 μ mol/g, Guzaev *et al.*, 2003) packed in the column. For the coupling step, the phosphoramidites were delivered at a 4 fold excess over the initial loading of the solid support and phosphoramidite coupling was carried out for 10 min. All other steps followed standard protocols supplied by the manufacturer. A solution of 6% dichloroacetic acid in toluene was used for removing the dimethoxytrityl (DMT) groups from 5'-hydroxyl groups of the nucleotide. 4,5-Dicyanoimidazole (0.7 M) in anhydrous CH₃CN was used as activator during the coupling step. Phosphorothioate linkages were introduced by sulfurization with 0.1 M solution of xanthane hydride in 1:1 pyridine/CH₃CN for a contact time of 3 minutes. A solution of 20% *tert*-butylhydroperoxide in CH₃CN containing 6% water was used as an oxidizing agent to provide phosphodiester internucleoside linkages with a contact time of 12 minutes.

After the desired sequence was assembled, the cyanoethyl phosphate protecting groups were deprotected using a 20% diethylamine in toluene (v/v) with a contact time of 45 minutes. The solid-support bound ASOs were suspended in aqueous ammonia (28-30 wt %) and heated at 55 °C for 6 h.

The unbound ASOs were then filtered and the ammonia was boiled off. The residue was purified by high pressure liquid chromatography on a strong anion exchange column (GE Healthcare Bioscience, Source 30Q, 30 μ m, 2.54 x 8 cm, A = 100 mM ammonium acetate in 30% aqueous CH₃CN, B = 1.5 M NaBr in A, 0-40% of B in 60 min, flow 14 mL min⁻¹, λ = 260 nm). The residue was desalted by HPLC on a reverse phase column to yield the desired ASOs in an isolated yield of 15-30% based on the initial loading on the solid support. The ASOs were characterized by ion-pair-HPLC coupled MS analysis with Agilent 1100 MSD system.

Table 34

ASO comprising a phosphodiester linked GalNAc₃-2 conjugate at the 5' position targeting SRB-1

ISIS No.	Sequence (5' to 3')	CalCd Mass	Observed Mass	SEQ ID No.
661134	GalNAc ₃ -2 _a -o'A _{do} T _{ks} ^m C _{ks} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{ks} ^m C _k	6482.2	6481.6	250

Subscripts: "e" indicates 2'-MOE modified nucleoside; "d" indicates β -D-2'-deoxyribonucleoside; "k" indicates 6'-(*S*)-CH₃ bicyclic nucleoside (e.g. cEt); "s" indicates phosphorothioate internucleoside linkages (PS); "o" indicates phosphodiester internucleoside linkages (PO); and "o" indicates -O-P(=O)(OH)-. Superscript "m" indicates 5-methylcytosines. The structure of GalNAc₃-2_a is shown in Example 37.

Example 42: General method for the preparation of ASOs comprising a GalNAc₃-3 conjugate at the 5' position *via* solid phase techniques (preparation of ISIS 661166)

The synthesis for ISIS 661166 was performed using similar procedures as illustrated in Examples 39 and 41.

ISIS 661166 is a 5-10-5 MOE gapmer, wherein the 5' position comprises a GalNAc₃-3 conjugate. The ASO was characterized by ion-pair-HPLC coupled MS analysis with Agilent 1100 MSD system.

Table 34a

ASO comprising a GalNAc₃-3 conjugate at the 5' position via a hexylamino phosphodiester linkage targeting Malat-1

ISIS No.	Sequence (5' to 3')	Conjugate	Calcd Mass	Observed Mass	SEQ ID No.
661166	5'-GalNAc ₃ -3 _{a-o} ^m C _{es} G _{es} G _{es} T _{es} G _{es} ^m C _{ds} A _{ds} A _{ds} G _{ds} G _{ds} ^m C _{ds} T _{ds} T _{ds} A _{ds} G _{ds} G _{es} A _{es} A _{es} T _{es} T _e	5'-GalNAc ₃ -3	8992.16	8990.51	251

Subscripts: "e" indicates 2'-MOE modified nucleoside; "d" indicates β-D-2'-deoxyribonucleoside; "s" indicates phosphorothioate internucleoside linkages (PS); "o" indicates phosphodiester internucleoside linkages (PO); and "o" indicates -O-P(=O)(OH)-. Superscript "m" indicates 5-methylcytosines. The structure of "5'-GalNAc₃-3a" is shown in Example 39.

Example 43: Dose-dependent study of phosphodiester linked GalNAc₃-2 (see examples 37 and 41, Bx is adenine) at the 5' terminus targeting SRB-1 *in vivo*

ISIS 661134 (see Example 41) comprising a phosphodiester linked GalNAc₃-2 conjugate at the 5' terminus was tested in a dose-dependent study for antisense inhibition of SRB-1 in mice. Unconjugated ISIS 440762 and 651900 (GalNAc₃-1 conjugate at 3' terminus, see Example 9) were included in the study for comparison and are described previously in Table 17.

Treatment

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with ISIS 440762, 651900, 661134 or with PBS treated control. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the liver SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. SRB-1 mRNA levels were determined relative to total RNA (using Ribogreen), prior to normalization to PBS-treated control. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group,

normalized to PBS-treated control and is denoted as “% PBS”. The ED₅₀s were measured using similar methods as described previously and are presented below.

As illustrated in Table 35, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner. Indeed, the antisense oligonucleotides comprising the phosphodiester linked GalNAc₃-2 conjugate at the 5' terminus (ISIS 661134) or the GalNAc₃-1 conjugate linked at the 3' terminus (ISIS 651900) showed substantial improvement in potency compared to the unconjugated antisense oligonucleotide (ISIS 440762). Further, ISIS 661134, which comprises the phosphodiester linked GalNAc₃-2 conjugate at the 5' terminus was equipotent compared to ISIS 651900, which comprises the GalNAc₃-1 conjugate at the 3' terminus.

10

Table 35

ASOs containing GalNAc₃-1 or GalNAc₃-2 targeting SRB-1

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA levels (% PBS)	ED ₅₀ (mg/kg)	Conjugate	SEQ ID No.
PBS	0	100	--	--	
440762	0.2	116	2.58	No conjugate	250
	0.7	91			
	2	69			
	7	22			
	20	5			
651900	0.07	95	0.26	3' GalNAc ₃ -1	247
	0.2	77			
	0.7	28			
	2	11			
	7	8			
661134	0.07	107	0.25	5' GalNAc ₃ -2	250
	0.2	86			
	0.7	28			
	2	10			
	7	6			

Structures for 3' GalNAc₃-1 and 5' GalNAc₃-2 were described previously in Examples 9 and 37.

15 *Pharmacokinetics Analysis (PK)*

The PK of the ASOs from the high dose group (7 mg/kg) was examined and evaluated in the same manner as illustrated in Example 20. Liver sample was minced and extracted using standard protocols. The full length metabolites of 661134 (5' GalNAc₃-2) and ISIS 651900 (3' GalNAc₃-1) were identified and their masses were confirmed by high resolution mass spectrometry analysis. The results showed that the major metabolite detected for the ASO comprising a phosphodiester linked GalNAc₃-2 conjugate at the 5' terminus (ISIS 661134) was ISIS 440762 (data not shown). No additional metabolites, at a detectable level, were observed. Unlike its counterpart, additional metabolites similar to those reported previously in Table 23a were observed for the ASO having the GalNAc₃-1 conjugate at the 3' terminus (ISIS 651900). These results

20

suggest that having the phosphodiester linked GalNAc₃-1 or GalNAc₃-2 conjugate may improve the PK profile of ASOs without compromising their potency.

Example 44: Effect of PO/PS linkages on antisense inhibition of ASOs comprising GalNAc₃-1 conjugate (see Example 9) at the 3' terminus targeting SRB-1

ISIS 655861 and 655862 comprising a GalNAc₃-1 conjugate at the 3' terminus each targeting SRB-1 were tested in a single administration study for their ability to inhibit SRB-1 in mice. The parent unconjugated compound, ISIS 353382 was included in the study for comparison.

The ASOs are 5-10-5 MOE gapmers, wherein the gap region comprises ten 2'-deoxyribonucleosides and each wing region comprises five 2'-MOE modified nucleosides. The ASOs were prepared using similar methods as illustrated previously in Example 19 and are described Table 36, below.

Table 36

Modified ASOs comprising GalNAc₃-1 conjugate at the 3' terminus targeting SRB-1

ISIS No.	Sequence (5' to 3')	Chemistry	SEQ ID No.
353382 (parent)	G ^{es} _{es} C ^m _{es} T ^{es} _{es} T ^{es} _{es} C ^m _{es} A ^{ds} _{ds} G ^{ds} _{ds} T ^{ds} _{ds} ^m C ^{ds} _{ds} A ^{ds} _{ds} T ^{ds} _{ds} G ^{ds} _{ds} A ^{ds} _{ds} ^m C ^{ds} _{ds} T ^{ds} _{ds} T ^{es} _{es} ^m C ^{es} _{es} ^m C ^{es} _{es} T ^{es} _{es} T ^e	Full PS no conjugate	252
655861	G ^{es} _{es} C ^m _{es} T ^{es} _{es} T ^{es} _{es} C ^m _{es} A ^{ds} _{ds} G ^{ds} _{ds} T ^{ds} _{ds} ^m C ^{ds} _{ds} A ^{ds} _{ds} T ^{ds} _{ds} G ^{ds} _{ds} A ^{ds} _{ds} ^m C ^{ds} _{ds} T ^{ds} _{ds} T ^{es} _{es} ^m C ^{es} _{es} ^m C ^{es} _{es} T ^{es} _{es} T ^{eo} _{eo} A_{do}-GalNAc₃-1_a	Full PS with GalNAc ₃ -1 conjugate	253
655862	G ^{es} _{es} C ^{eo} _{eo} T ^{eo} _{eo} T ^{eo} _{eo} ^m C ^{eo} _{eo} A ^{ds} _{ds} G ^{ds} _{ds} T ^{ds} _{ds} ^m C ^{ds} _{ds} A ^{ds} _{ds} T ^{ds} _{ds} G ^{ds} _{ds} A ^{ds} _{ds} ^m C ^{ds} _{ds} T ^{ds} _{ds} T ^{eo} _{eo} ^m C ^{eo} _{eo} ^m C ^{es} _{es} T ^{es} _{es} T ^{eo} _{eo} A_{do}-GalNAc₃-1_a	Mixed PS/PO with GalNAc ₃ -1 conjugate	253

Subscripts: "e" indicates 2'-MOE modified nucleoside; "d" indicates β-D-2'-deoxyribonucleoside; "s" indicates phosphorothioate internucleoside linkages (PS); "o" indicates phosphodiester internucleoside linkages (PO); and "o" indicates -O-P(=O)(OH)-. Superscript "m" indicates 5-methylcytosines. The structure of "GalNAc₃-1" is shown in Example 9.

Treatment

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with ISIS 353382, 655861, 655862 or with PBS treated control. Each treatment group consisted of 4 animals. Prior to the treatment as well as after the last dose, blood was drawn from each mouse and plasma samples were analyzed. The mice were sacrificed 72 hours following the final administration to determine the liver SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. SRB-1 mRNA levels were determined relative to total RNA (using Ribogreen), prior to normalization to PBS-treated control. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to PBS-treated control and is denoted as "% PBS". The ED₅₀s were measured using similar methods as described previously and are reported below.

As illustrated in Table 37, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner compared to PBS treated control. Indeed, the antisense oligonucleotides comprising the GalNAc₃-1 conjugate at the 3' terminus (ISIS 655861 and 655862) showed substantial improvement in potency comparing to the unconjugated antisense oligonucleotide (ISIS 353382). Further, 5 ISIS 655862 with mixed PS/PO linkages showed an improvement in potency relative to full PS (ISIS 655861).

Table 37

**Effect of PO/PS linkages on antisense inhibition of ASOs
comprising GalNAc₃-1 conjugate at 3' terminus targeting SRB-1**

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA levels (% PBS)	ED ₅₀ (mg/kg)	Chemistry	SEQ ID No.
PBS	0	100	--	--	
353382 (parent)	3	76.65	10.4	Full PS without conjugate	252
	10	52.40			
	30	24.95			
655861	0.5	81.22	2.2	Full PS with GalNAc ₃ -1 conjugate	253
	1.5	63.51			
	5	24.61			
	15	14.80			
655862	0.5	69.57	1.3	Mixed PS/PO with GalNAc ₃ -1 conjugate	253
	1.5	45.78			
	5	19.70			
	15	12.90			

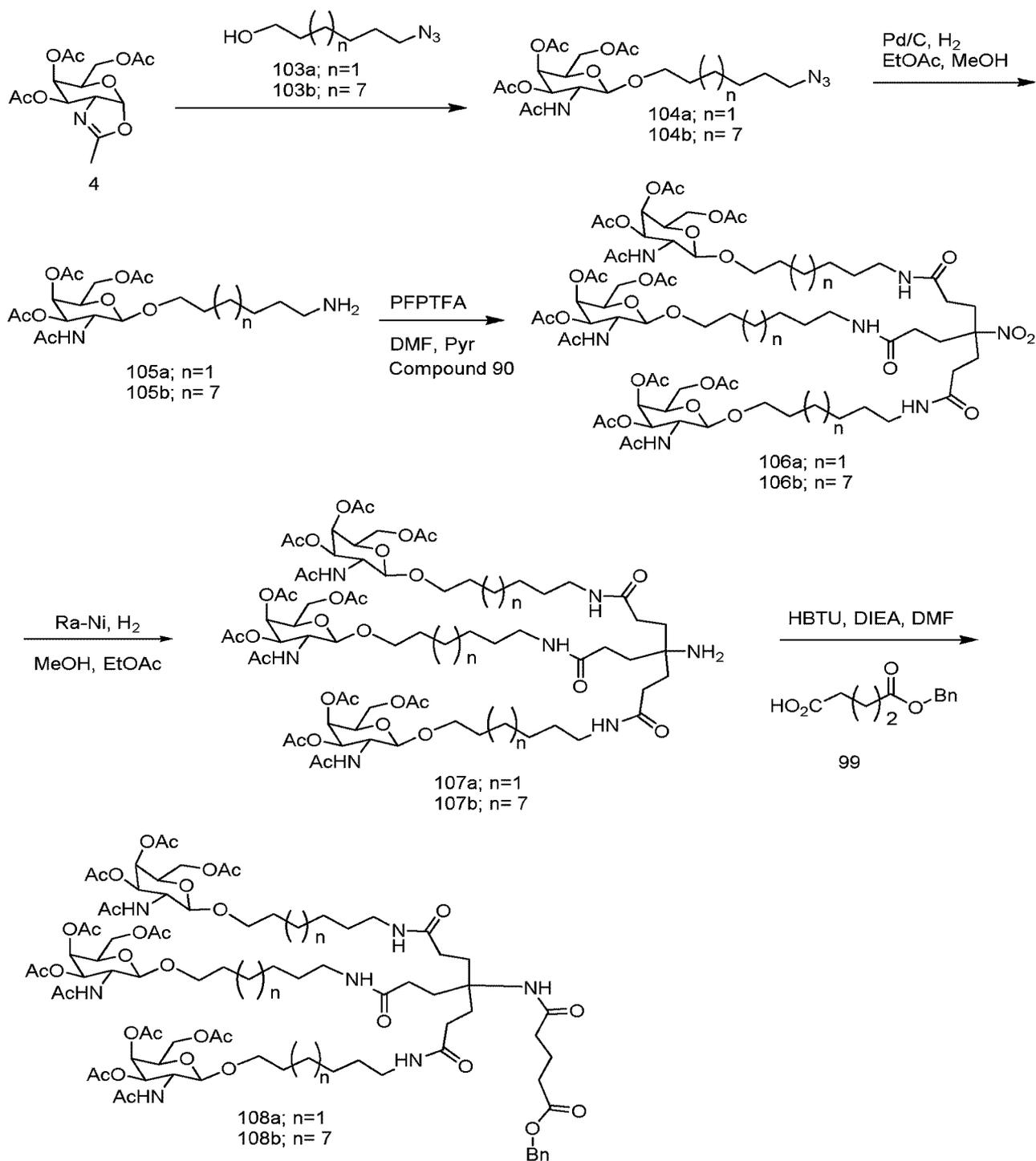
Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Organ weights were also 15 evaluated. The results demonstrated that no elevation in transaminase levels (Table 38) or organ weights (data not shown) were observed in mice treated with ASOs compared to PBS control. Further, the ASO with mixed PS/PO linkages (ISIS 655862) showed similar transaminase levels compared to full PS (ISIS 655861).

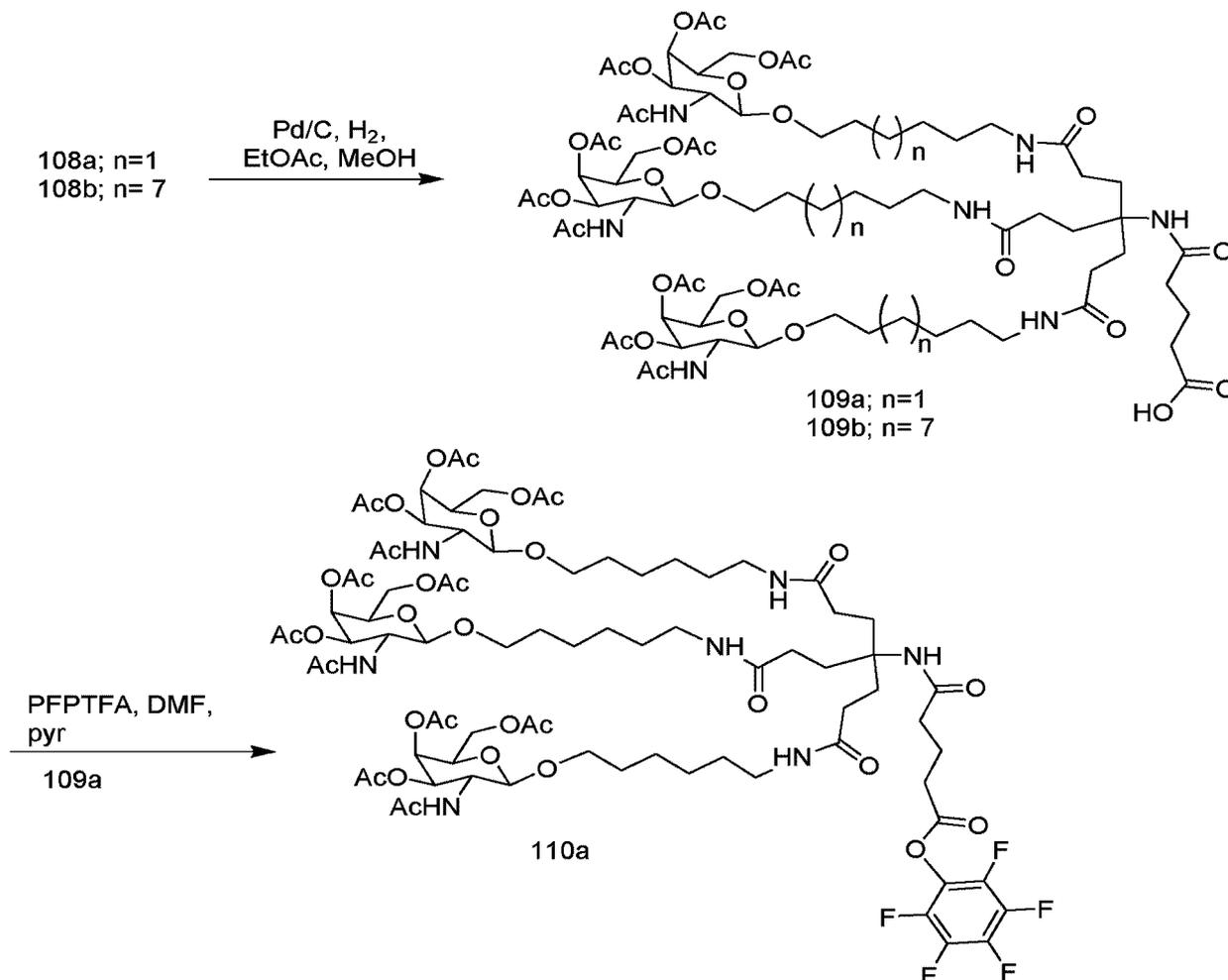
Table 38

**Effect of PO/PS linkages on transaminase levels of ASOs
comprising GalNAc₃-1 conjugate at 3' terminus targeting SRB-1**

ISIS No.	Dosage (mg/kg)	ALT (U/L)	AST (U/L)	Chemistry	SEQ ID No.
PBS	0	28.5	65	--	
353382 (parent)	3	50.25	89	Full PS without conjugate	252
	10	27.5	79.3		
	30	27.3	97		
655861	0.5	28	55.7	Full PS with	253

	1.5	30	78	GalNAc₃-1	
	5	29	63.5		
	15	28.8	67.8		
655862	0.5	50	75.5	Mixed PS/PO with GalNAc₃-1	253
	1.5	21.7	58.5		
	5	29.3	69		
	15	22	61		

Example 45: Preparation of PFP Ester, Compound 110a



Compound 4 (9.5g, 28.8 mmoles) was treated with compound 103a or 103b (38 mmoles), individually, and TMSOTf (0.5 eq.) and molecular sieves in dichloromethane (200 mL), and stirred for 16 hours at room temperature. At that time, the organic layer was filtered thru celite, then washed with sodium bicarbonate, water and brine. The organic layer was then separated and dried over sodium sulfate, filtered and reduced under reduced pressure. The resultant oil was purified by silica gel chromatography (2%-->10% methanol/dichloromethane) to give compounds 104a and 104b in >80% yield. LCMS and proton NMR was consistent with the structure.

10 Compounds 104a and 104b were treated to the same conditions as for compounds 100a-d (Example 47), to give compounds 105a and 105b in >90% yield. LCMS and proton NMR was consistent with the structure.

15 Compounds 105a and 105b were treated, individually, with compound 90 under the same conditions as for compounds 901a-d, to give compounds 106a (80%) and 106b (20%). LCMS and proton NMR was consistent with the structure.

Compounds 106a and 106b were treated to the same conditions as for compounds 96a-d (Example 47), to give 107a (60%) and 107b (20%). LCMS and proton NMR was consistent with the structure.

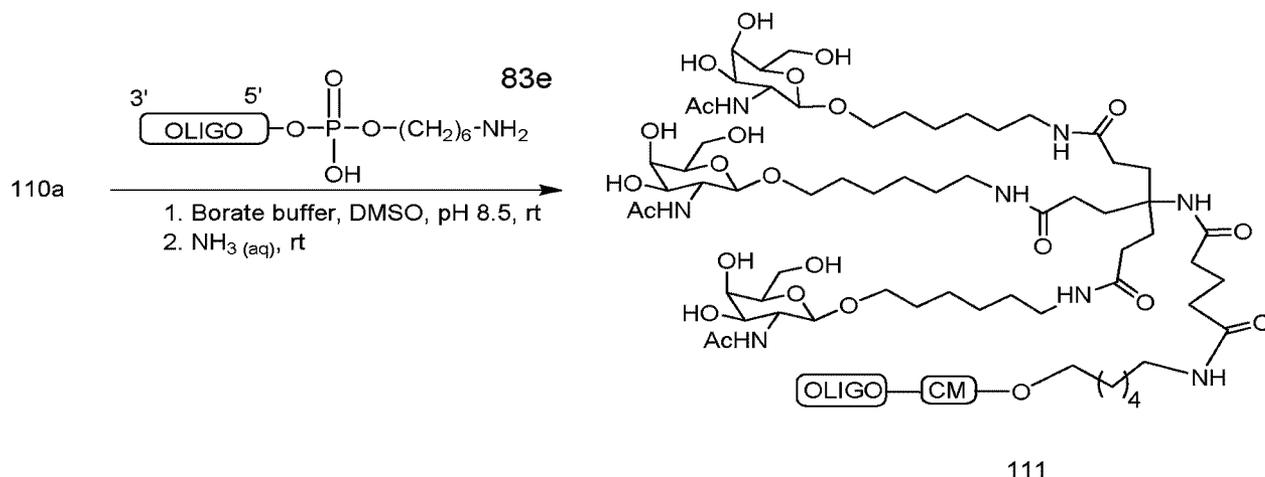
Compounds 107a and 107b were treated to the same conditions as for compounds 97a-d (Example 47), to give compounds 108a and 108b in 40-60% yield. LCMS and proton NMR was consistent with the structure.

Compounds 108a (60%) and 108b (40%) were treated to the same conditions as for compounds 100a-d (Example 47), to give compounds 109a and 109b in >80% yields. LCMS and proton NMR was consistent with the structure.

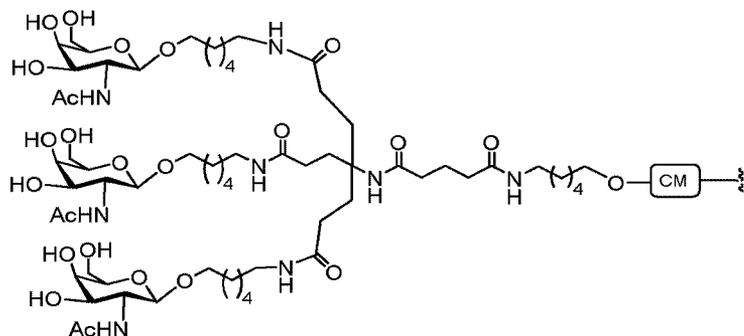
Compound 109a was treated to the same conditions as for compounds 101a-d (Example 47), to give Compound 110a in 30-60% yield. LCMS and proton NMR was consistent with the structure. Alternatively, Compound 110b can be prepared in a similar manner starting with Compound 109b.

Example 46: General Procedure for Conjugation with PFP Esters (Oligonucleotide 111); Preparation of ISIS 666881 (GalNAc₃-10)

A 5'-hexylamino modified oligonucleotide was synthesized and purified using standard solid-phase oligonucleotide procedures. The 5'-hexylamino modified oligonucleotide was dissolved in 0.1 M sodium tetraborate, pH 8.5 (200 μ L) and 3 equivalents of a selected PFP esterified GalNAc₃ cluster dissolved in DMSO (50 μ L) was added. If the PFP ester precipitated upon addition to the ASO solution DMSO was added until all PFP ester was in solution. The reaction was complete after about 16 h of mixing at room temperature. The resulting solution was diluted with water to 12 mL and then spun down at 3000 rpm in a spin filter with a mass cut off of 3000 Da. This process was repeated twice to remove small molecule impurities. The solution was then lyophilized to dryness and redissolved in concentrated aqueous ammonia and mixed at room temperature for 2.5 h followed by concentration *in vacuo* to remove most of the ammonia. The conjugated oligonucleotide was purified and desalted by RP-HPLC and lyophilized to provide the GalNAc₃ conjugated oligonucleotide.



Oligonucleotide 111 is conjugated with GalNAc₃-10. The GalNAc₃ cluster portion of the conjugate group GalNAc₃-10 (GalNAc₃-10_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)- as shown in the oligonucleotide (ISIS 666881) synthesized with GalNAc₃-10 below. The structure of GalNAc₃-10 (GalNAc₃-10_a-CM-) is shown below:



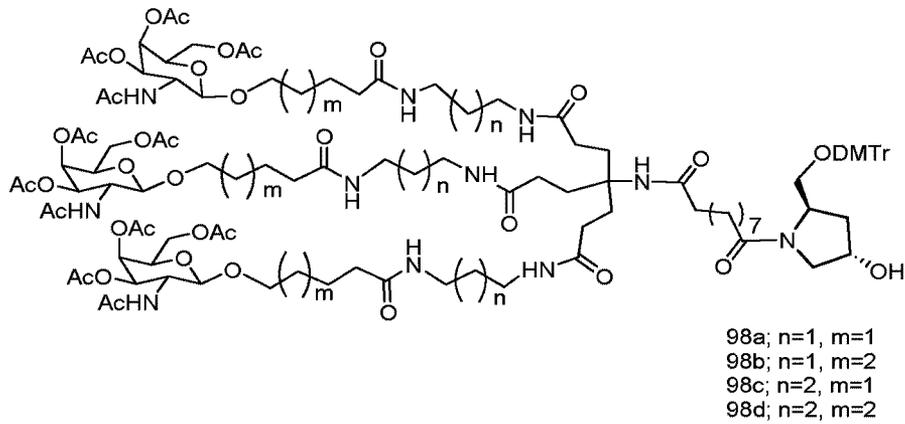
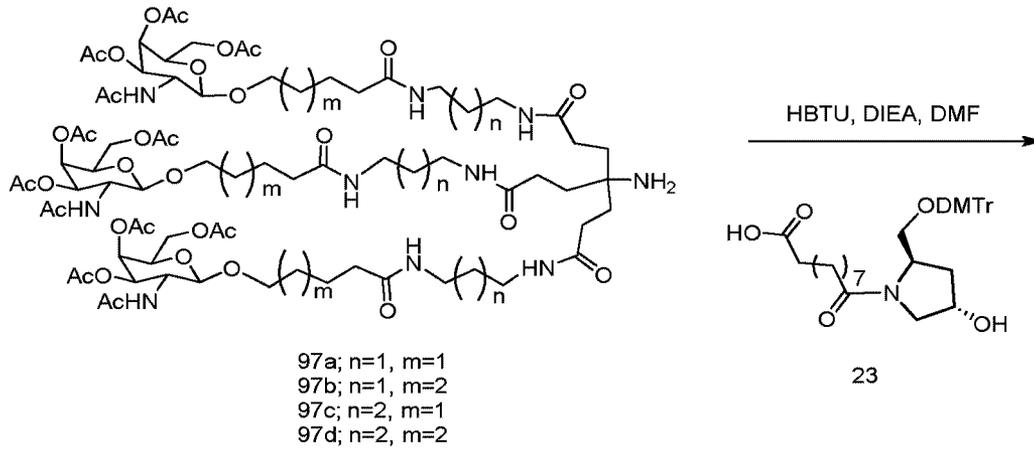
Following this general procedure ISIS 666881 was prepared. 5'-hexylamino modified oligonucleotide, ISIS 660254, was synthesized and purified using standard solid-phase oligonucleotide procedures. ISIS 660254 (40 mg, 5.2 μmol) was dissolved in 0.1 M sodium tetraborate, pH 8.5 (200 μL) and 3 equivalents PFP ester (Compound 110a) dissolved in DMSO (50 μL) was added. The PFP ester precipitated upon addition to the ASO solution requiring additional DMSO (600 μL) to fully dissolve the PFP ester. The reaction was complete after 16 h of mixing at room temperature. The solution was diluted with water to 12 mL total volume and spun down at 3000 rpm in a spin filter with a mass cut off of 3000 Da. This process was repeated twice to remove small molecule impurities. The solution was lyophilized to dryness and redissolved in concentrated aqueous ammonia with mixing at room temperature for 2.5 h followed by

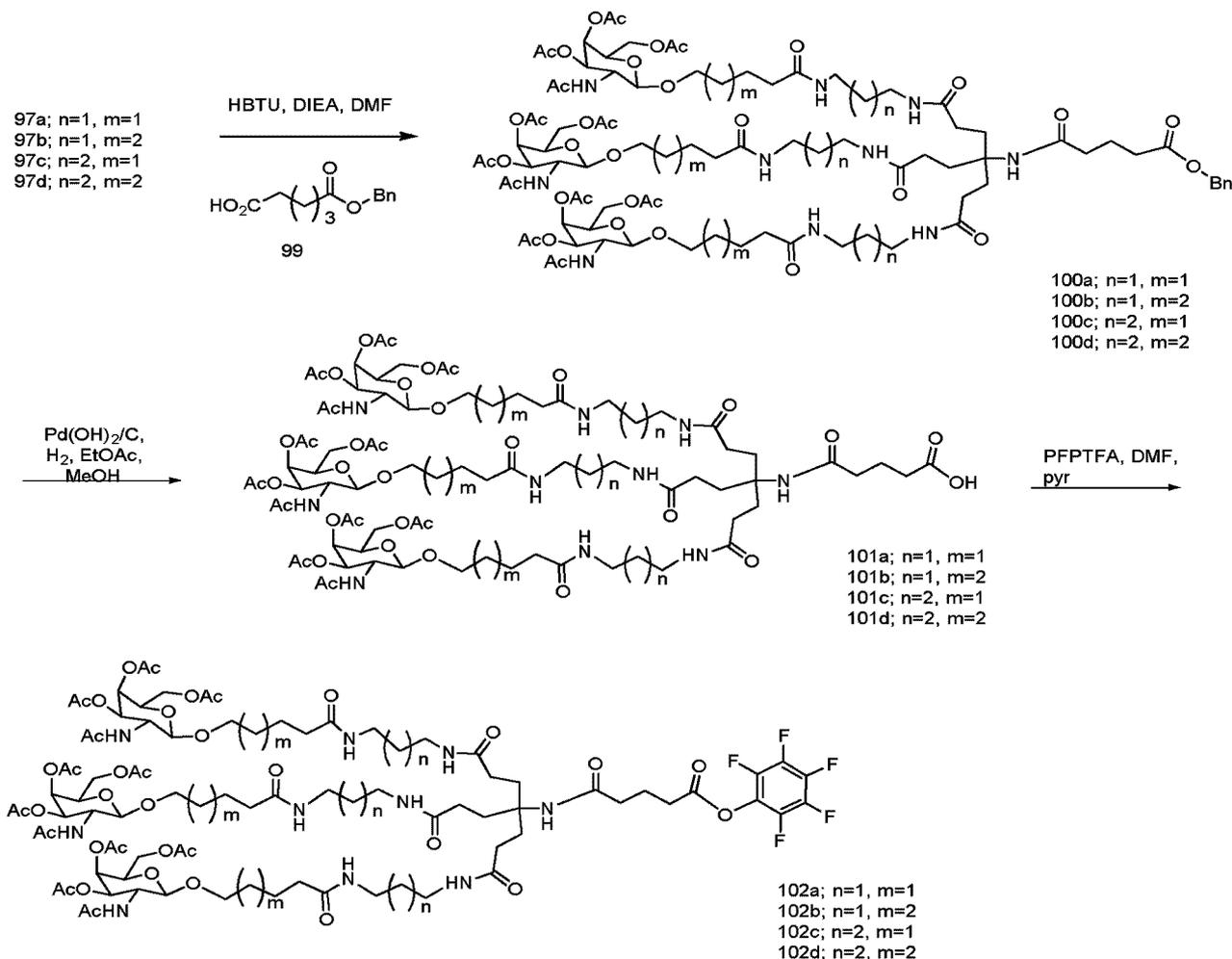
concentration *in vacuo* to remove most of the ammonia. The conjugated oligonucleotide was purified and desalted by RP-HPLC and lyophilized to give ISIS 666881 in 90% yield by weight (42 mg, 4.7 μ mol).

GalNAc₃-10 conjugated oligonucleotide

ASO	Sequence (5' to 3')	5' group	SEQ ID No.
ISIS 660254	NH ₂ (CH ₂) ₆ -o-A _{do} G _{es} ^m C _{es} T _{es} T _{es} ^m C _{es} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{es} ^m C _{es} ^m C _{es} T _{es} T _e	Hexylamine	254
ISIS 666881	GalNAc₃-10 -a-o-A _{do} G _{es} ^m C _{es} T _{es} T _{es} ^m C _{es} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{es} ^m C _{es} ^m C _{es} T _{es} T _e	GalNAc₃-10	254

- 5 Capital letters indicate the nucleobase for each nucleoside and ^mC indicates a 5-methyl cytosine. Subscripts: “e” indicates a 2'-MOE modified nucleoside; “d” indicates a β -D-2'-deoxyribonucleoside; “s” indicates a phosphorothioate internucleoside linkage (PS); “o” indicates a phosphodiester internucleoside linkage (PO); and “a” indicates -O-P(=O)(OH)-. Conjugate groups are in bold.





The triacid 90 (4 g, 14.43 mmol) was dissolved in DMF (120 mL) and *N,N*-Diisopropylethylamine (12.35 mL, 72 mmoles). Pentafluorophenyl trifluoroacetate (8.9 mL, 52 mmoles) was added dropwise, under argon, and the reaction was allowed to stir at room temperature for 30 minutes. Boc-diamine 91a or 91b (68.87 mmol) was added, along with *N,N*-Diisopropylethylamine (12.35 mL, 72 mmoles), and the reaction was allowed to stir at room temperature for 16 hours. At that time, the DMF was reduced by >75% under reduced pressure, and then the mixture was dissolved in dichloromethane. The organic layer was washed with sodium bicarbonate, water and brine. The organic layer was then separated and dried over sodium sulfate, filtered and reduced to an oil under reduced pressure. The resultant oil was purified by silica gel chromatography (2%-->10% methanol/dichloromethane) to give compounds 92a and 92b in an approximate 80% yield. LCMS and proton NMR were consistent with the structure.

Compound 92a or 92b (6.7 mmoles) was treated with 20 mL of dichloromethane and 20 mL of trifluoroacetic acid at room temperature for 16 hours. The resultant solution was evaporated and then

dissolved in methanol and treated with DOWEX-OH resin for 30 minutes. The resultant solution was filtered and reduced to an oil under reduced pressure to give 85-90% yield of compounds 93a and 93b.

Compounds 7 or 64 (9.6 mmoles) were treated with HBTU (3.7g, 9.6 mmoles) and *N,N*-Diisopropylethylamine (5 mL) in DMF (20 mL) for 15 minutes. To this was added either compounds 93a or 93b (3 mmoles), and allowed to stir at room temperature for 16 hours. At that time, the DMF was reduced by >75% under reduced pressure, and then the mixture was dissolved in dichloromethane. The organic layer was washed with sodium bicarbonate, water and brine. The organic layer was then separated and dried over sodium sulfate, filtered and reduced to an oil under reduced pressure. The resultant oil was purified by silica gel chromatography (5%-->20% methanol/dichloromethane) to give compounds 96a-d in 20-40% yield. LCMS and proton NMR was consistent with the structure.

Compounds 96a-d (0.75 mmoles), individually, were hydrogenated over Raney Nickel for 3 hours in Ethanol (75 mL). At that time, the catalyst was removed by filtration thru celite, and the ethanol removed under reduced pressure to give compounds 97a-d in 80-90% yield. LCMS and proton NMR were consistent with the structure.

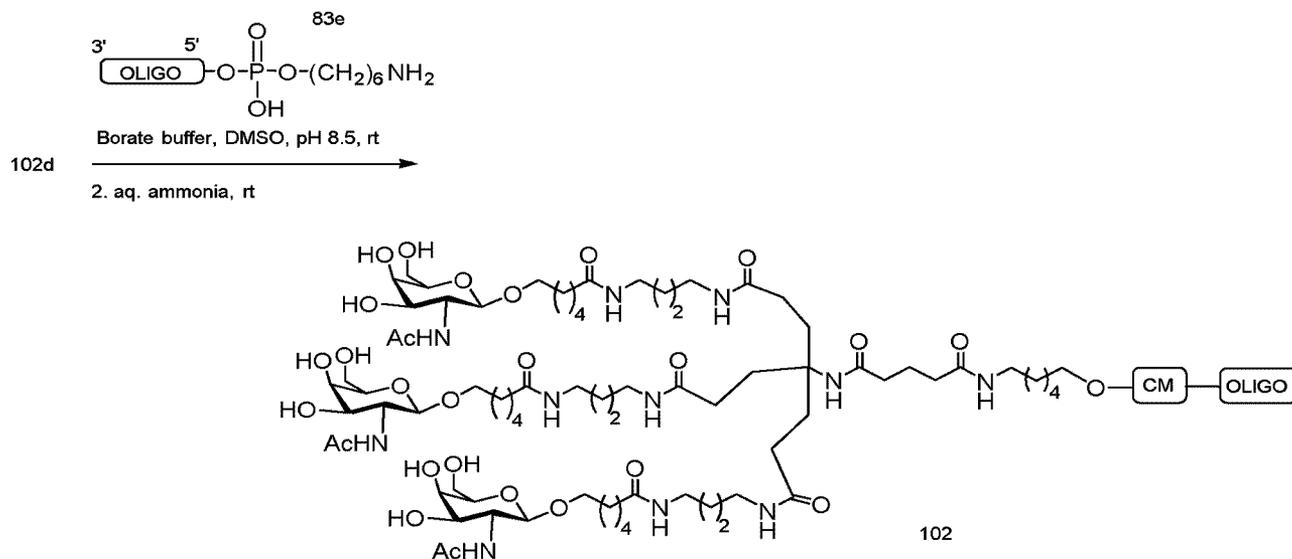
Compound 23 (0.32g, 0.53 mmoles) was treated with HBTU (0.2g, 0.53 mmoles) and *N,N*-Diisopropylethylamine (0.19 mL, 1.14 mmoles) in DMF (30mL) for 15 minutes. To this was added compounds 97a-d (0.38 mmoles), individually, and allowed to stir at room temperature for 16 hours. At that time, the DMF was reduced by >75% under reduced pressure, and then the mixture was dissolved in dichloromethane. The organic layer was washed with sodium bicarbonate, water and brine. The organic layer was then separated and dried over sodium sulfate, filtered and reduced to an oil under reduced pressure. The resultant oil was purified by silica gel chromatography (2%-->20% methanol/dichloromethane) to give compounds 98a-d in 30-40% yield. LCMS and proton NMR was consistent with the structure.

Compound 99 (0.17g, 0.76 mmoles) was treated with HBTU (0.29 g, 0.76 mmoles) and *N,N*-Diisopropylethylamine (0.35 mL, 2.0 mmoles) in DMF (50mL) for 15 minutes. To this was added compounds 97a-d (0.51 mmoles), individually, and allowed to stir at room temperature for 16 hours. At that time, the DMF was reduced by >75% under reduced pressure, and then the mixture was dissolved in dichloromethane. The organic layer was washed with sodium bicarbonate, water and brine. The organic layer was then separated and dried over sodium sulfate, filtered and reduced to an oil under reduced pressure. The resultant oil was purified by silica gel chromatography (5%-->20% methanol/ dichloromethane) to give compounds 100a-d in 40-60% yield. LCMS and proton NMR was consistent with the structure.

Compounds 100a-d (0.16 mmoles), individually, were hydrogenated over 10% Pd(OH)₂/C for 3 hours in methanol/ethyl acetate (1:1, 50 mL). At that time, the catalyst was removed by filtration thru celite, and the organics removed under reduced pressure to give compounds 101a-d in 80-90% yield. LCMS and proton NMR was consistent with the structure.

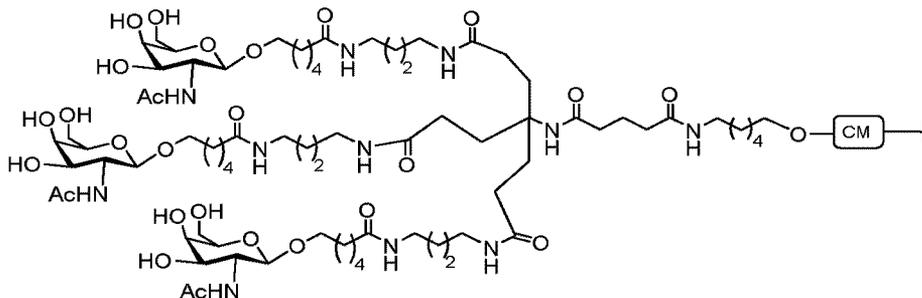
Compounds 101a-d (0.15 mmoles), individually, were dissolved in DMF (15 mL) and pyridine (0.016 mL, 0.2 mmoles). Pentafluorophenyl trifluoroacetate (0.034 mL, 0.2 mmoles) was added dropwise,

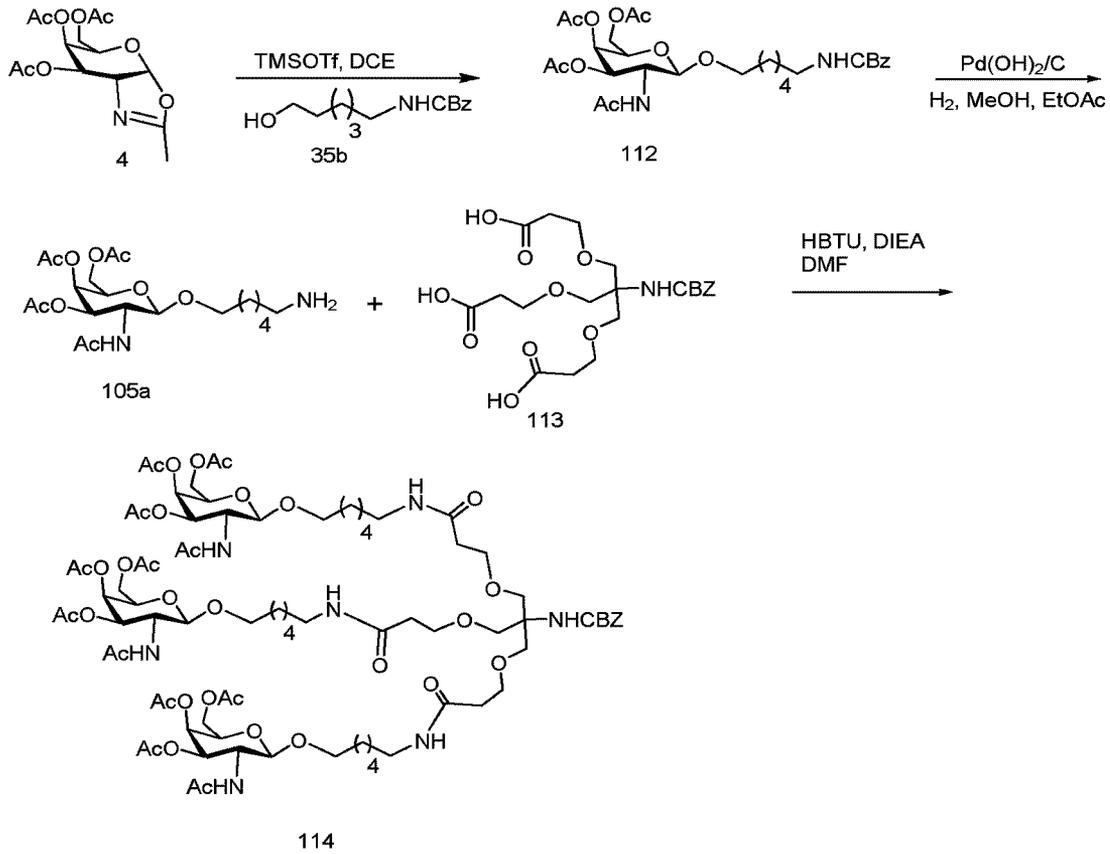
under argon, and the reaction was allowed to stir at room temperature for 30 minutes. At that time, the DMF was reduced by >75% under reduced pressure, and then the mixture was dissolved in dichloromethane. The organic layer was washed with sodium bicarbonate, water and brine. The organic layer was then separated and dried over sodium sulfate, filtered and reduced to an oil under reduced pressure. The resultant oil was purified by silica gel chromatography (2%-->5% methanol/dichloromethane) to give compounds 102a-d in an approximate 80% yield. LCMS and proton NMR were consistent with the structure.

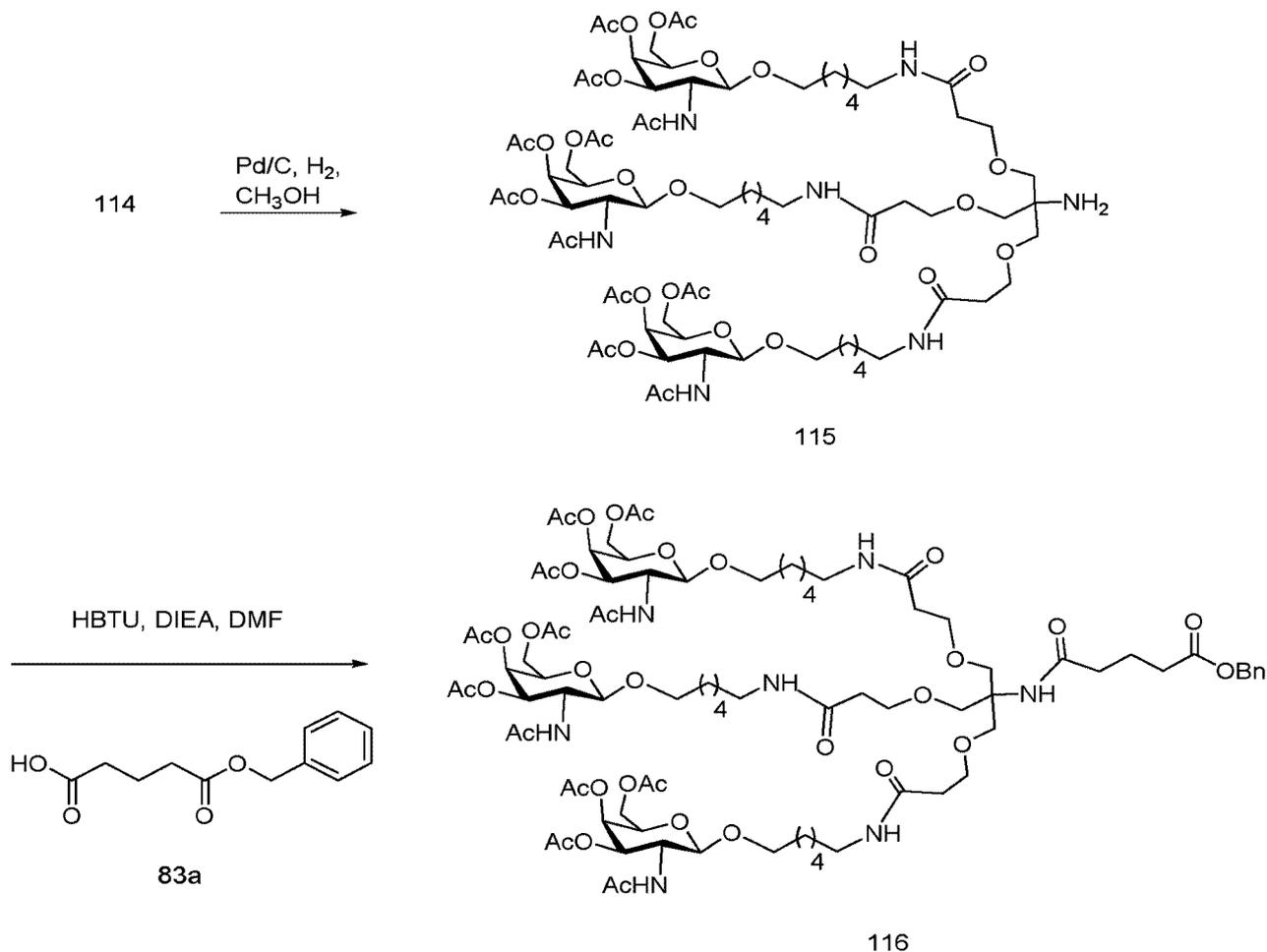


Oligomeric Compound 102, comprising a GalNAc₃-8 conjugate group, was prepared using the general procedures illustrated in Example 46. The GalNAc₃ cluster portion of the conjugate group GalNAc₃-8 (GalNAc₃-8_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In a preferred embodiment, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-.

The structure of GalNAc₃-8 (GalNAc₃-8_a-CM-) is shown below:



Example 48: Preparation of Oligonucleotide 119 Comprising GalNAc₃-7



Compound 112 was synthesized following the procedure described in the literature (*J. Med. Chem.* 2004, 47, 5798-5808).

5 Compound 112 (5 g, 8.6 mmol) was dissolved in 1:1 methanol/ethyl acetate (22 mL/22 mL). Palladium hydroxide on carbon (0.5 g) was added. The reaction mixture was stirred at room temperature under hydrogen for 12 h. The reaction mixture was filtered through a pad of celite and washed the pad with 1:1 methanol/ethyl acetate. The filtrate and the washings were combined and concentrated to dryness to yield Compound 105a (quantitative). The structure was confirmed by LCMS.

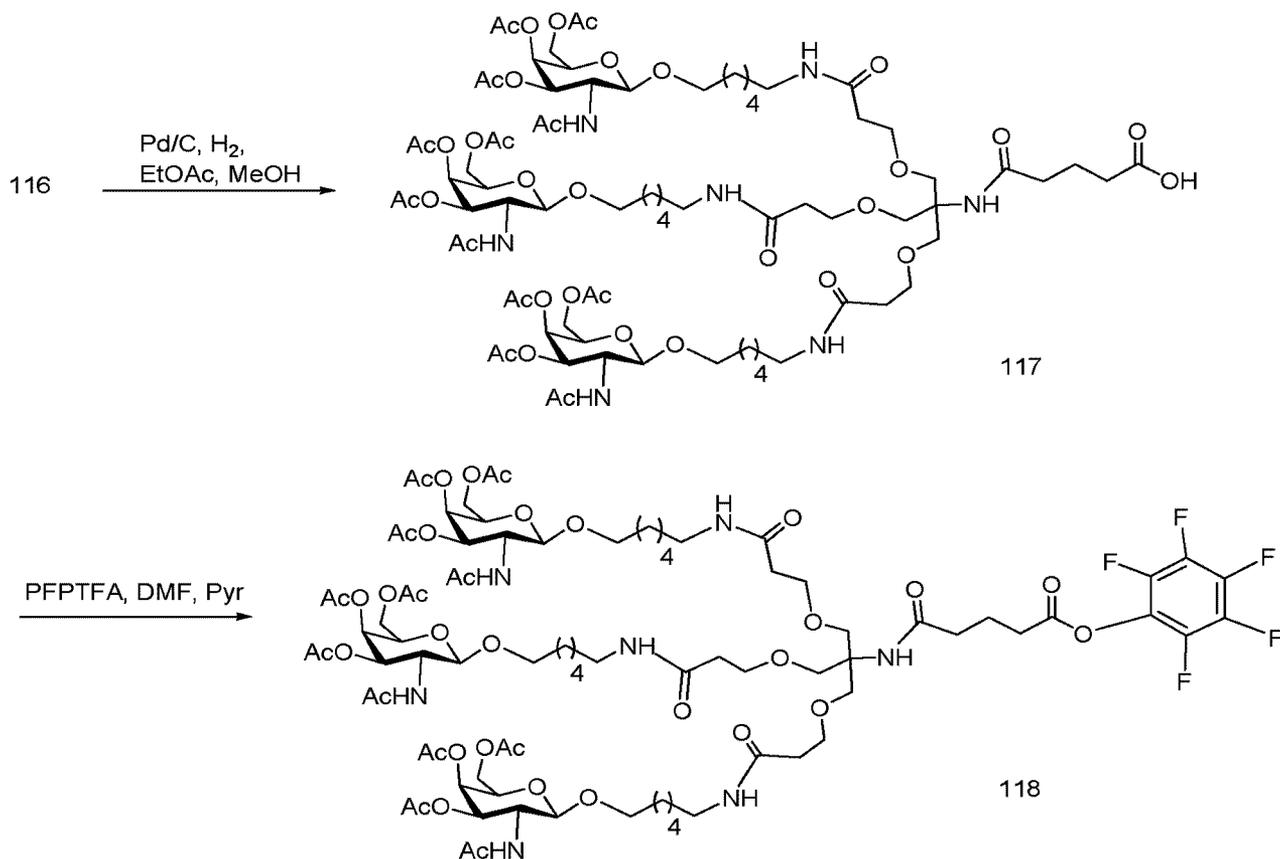
10 Compound 113 (1.25 g, 2.7 mmol), HBTU (3.2 g, 8.4 mmol) and DIEA (2.8 mL, 16.2 mmol) were dissolved in anhydrous DMF (17 mL) and the reaction mixture was stirred at room temperature for 5 min. To this a solution of Compound 105a (3.77 g, 8.4 mmol) in anhydrous DMF (20 mL) was added. The reaction was stirred at room temperature for 6 h. Solvent was removed under reduced pressure to get an oil. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with aqueous saturated NaHCO_3 solution (100 mL) and brine (100 mL). The organic phase was separated, dried (Na_2SO_4), filtered and evaporated. The residue

15

was purified by silica gel column chromatography and eluted with 10 to 20 % MeOH in dichloromethane to yield Compound 114 (1.45 g, 30%). The structure was confirmed by LCMS and ^1H NMR analysis.

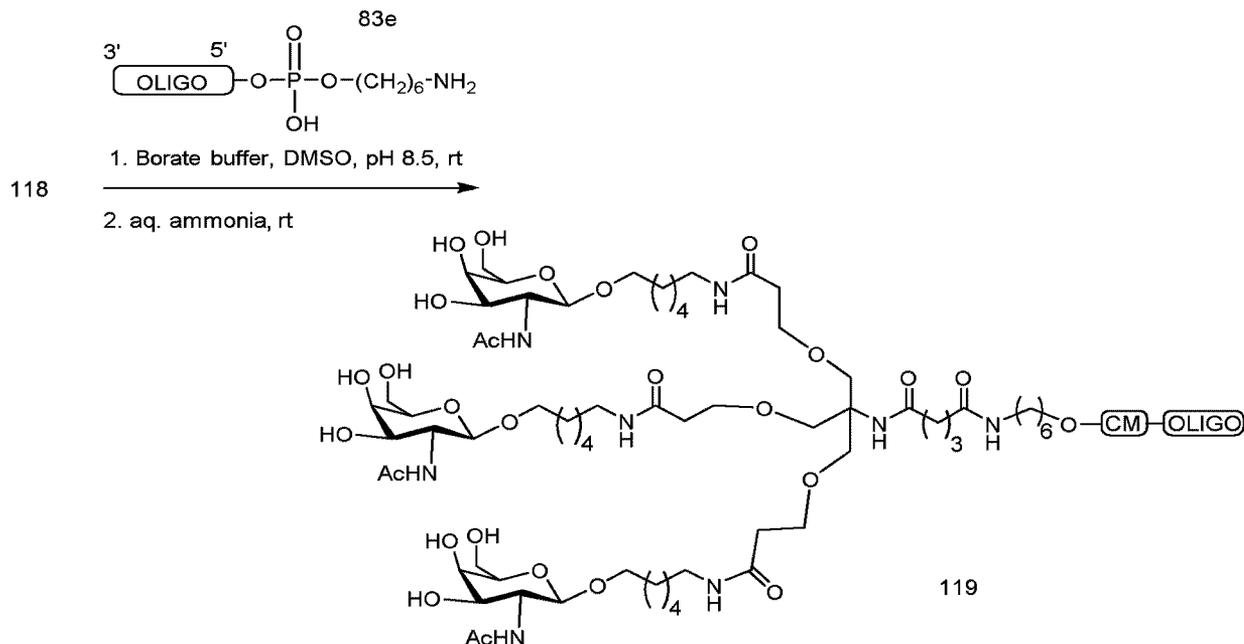
Compound 114 (1.43 g, 0.8 mmol) was dissolved in 1:1 methanol/ethyl acetate (4 mL/4 mL). Palladium on carbon (wet, 0.14 g) was added. The reaction mixture was flushed with hydrogen and stirred at room temperature under hydrogen for 12 h. The reaction mixture was filtered through a pad of celite. The celite pad was washed with methanol/ethyl acetate (1:1). The filtrate and the washings were combined together and evaporated under reduced pressure to yield Compound 115 (quantitative). The structure was confirmed by LCMS and ^1H NMR analysis.

Compound 83a (0.17 g, 0.75 mmol), HBTU (0.31 g, 0.83 mmol) and DIEA (0.26 mL, 1.5 mmol) were dissolved in anhydrous DMF (5 mL) and the reaction mixture was stirred at room temperature for 5 min. To this a solution of Compound 115 (1.22 g, 0.75 mmol) in anhydrous DMF was added and the reaction was stirred at room temperature for 6 h. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 . The organic layer was washed aqueous saturated NaHCO_3 solution and brine and dried over anhydrous Na_2SO_4 and filtered. The organic layer was concentrated to dryness and the residue obtained was purified by silica gel column chromatography and eluted with 3 to 15 % MeOH in dichloromethane to yield Compound 116 (0.84 g, 61%). The structure was confirmed by LC MS and ^1H NMR analysis.



Compound 116 (0.74 g, 0.4 mmol) was dissolved in 1:1 methanol/ethyl acetate (5 mL/5 mL). Palladium on carbon (wet, 0.074 g) was added. The reaction mixture was flushed with hydrogen and stirred at room temperature under hydrogen for 12 h. The reaction mixture was filtered through a pad of celite. The celite pad was washed with methanol/ethyl acetate (1:1). The filtrate and the washings were combined together and evaporated under reduced pressure to yield compound 117 (0.73 g, 98%). The structure was confirmed by LCMS and ^1H NMR analysis.

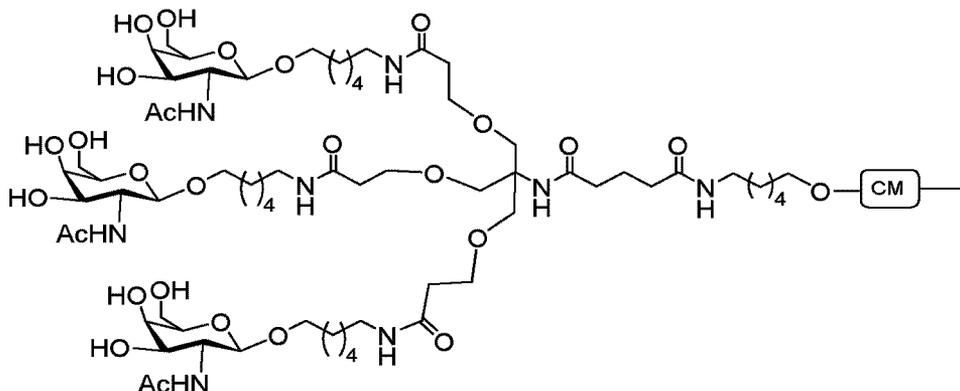
Compound 117 (0.63 g, 0.36 mmol) was dissolved in anhydrous DMF (3 mL). To this solution *N,N*-Diisopropylethylamine (70 μL , 0.4 mmol) and pentafluorophenyl trifluoroacetate (72 μL , 0.42 mmol) were added. The reaction mixture was stirred at room temperature for 12 h and poured into a aqueous saturated NaHCO_3 solution. The mixture was extracted with dichloromethane, washed with brine and dried over anhydrous Na_2SO_4 . The dichloromethane solution was concentrated to dryness and purified with silica gel column chromatography and eluted with 5 to 10 % MeOH in dichloromethane to yield compound 118 (0.51 g, 79%). The structure was confirmed by LCMS and ^1H and ^{19}F NMR.



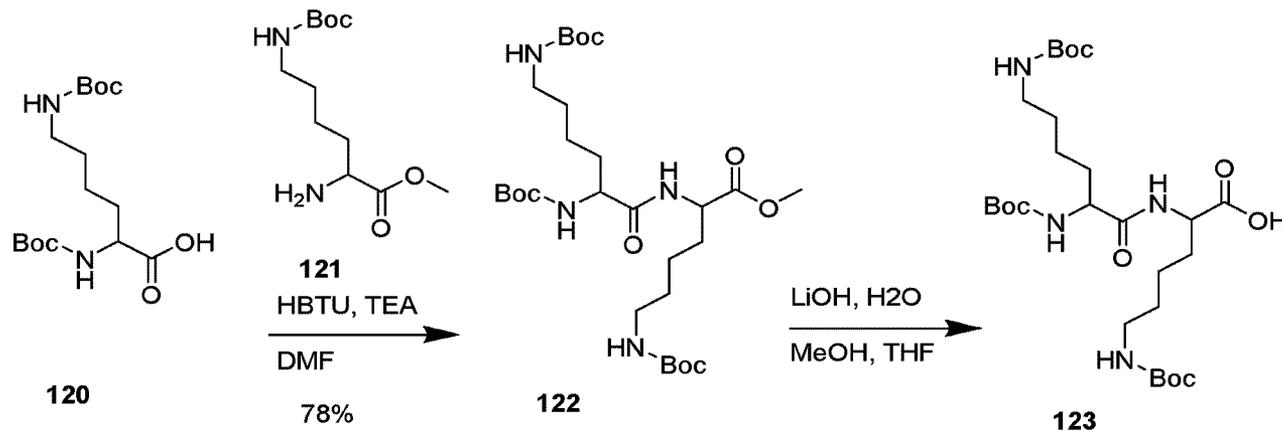
Oligomeric Compound 119, comprising a $\text{GalNAc}_3\text{-7}$ conjugate group, was prepared using the general procedures illustrated in Example 46. The GalNAc_3 cluster portion of the conjugate group $\text{GalNAc}_3\text{-7}$ ($\text{GalNAc}_3\text{-7}_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is $-\text{P}(=\text{O})(\text{OH})-\text{A}_d-\text{P}(=\text{O})(\text{OH})-$.

The structure of $\text{GalNAc}_3\text{-7}$ ($\text{GalNAc}_3\text{-7}_a\text{-CM-}$) is shown below:

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Example 49: Preparation of Oligonucleotide 132 Comprising GalNAc₃-5

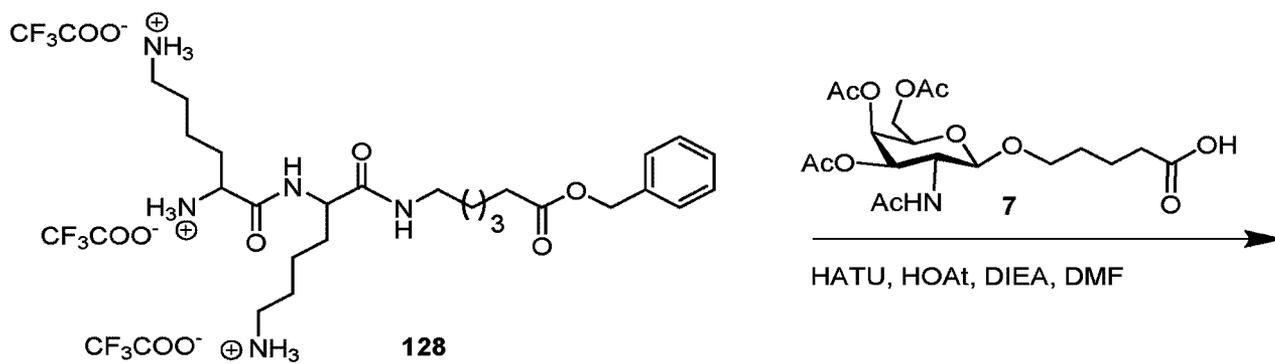
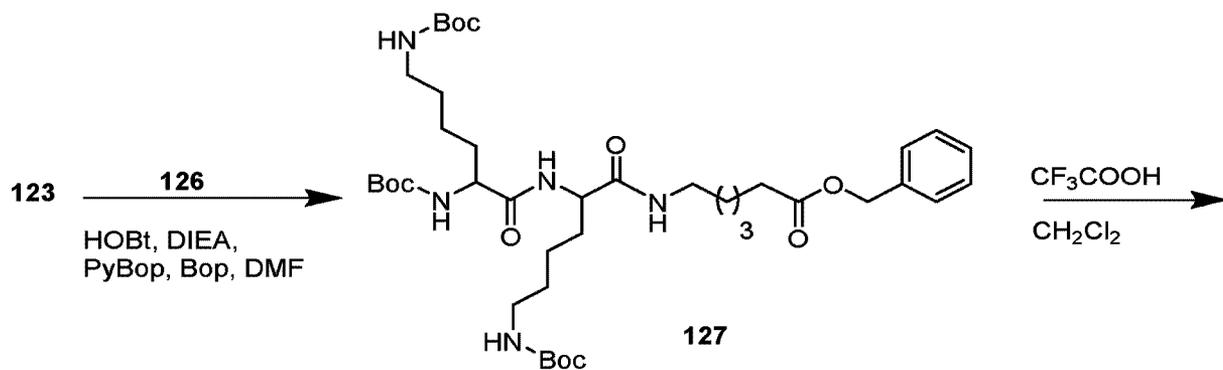


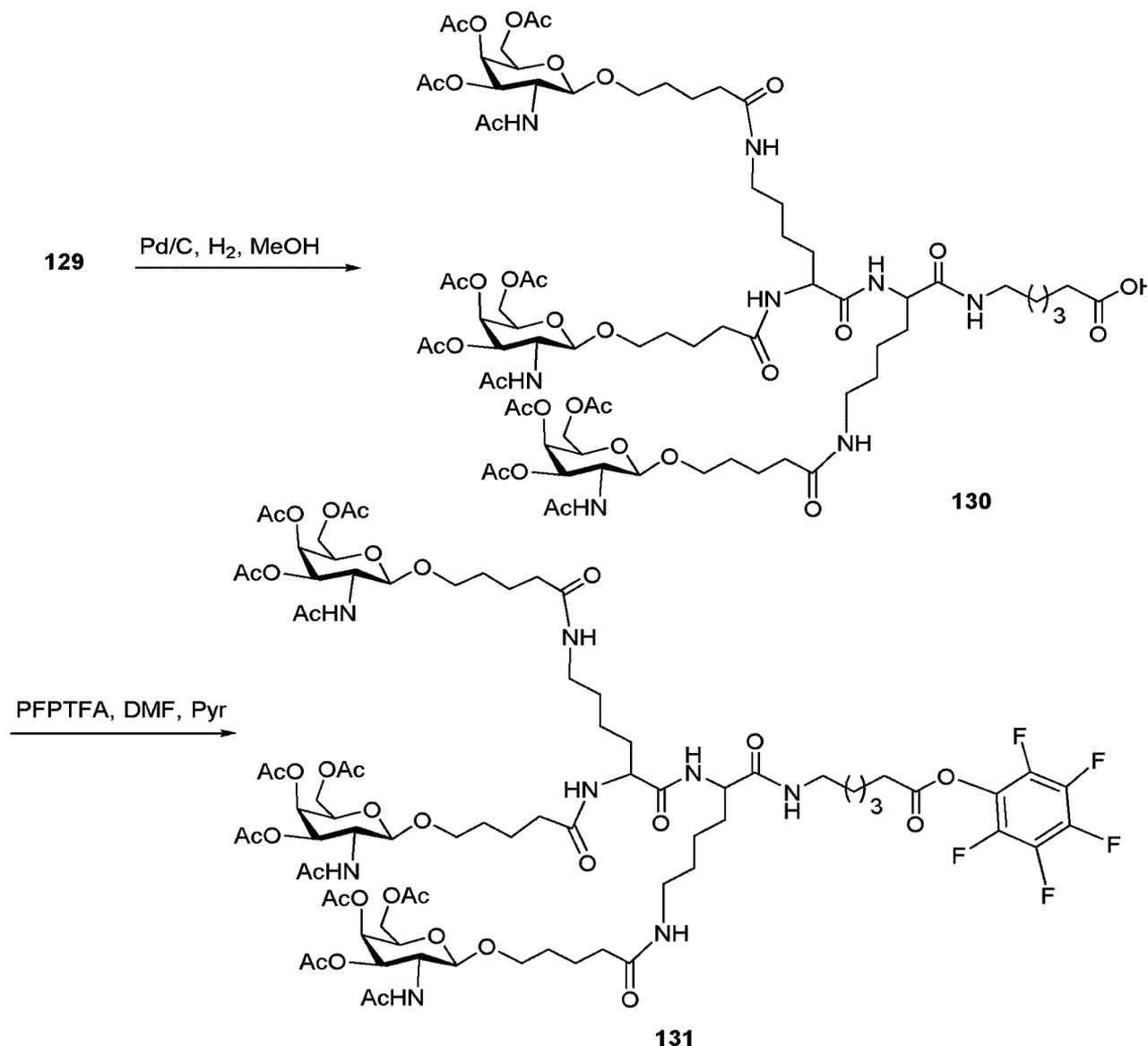
5

Compound 120 (14.01 g, 40 mmol) and HBTU (14.06 g, 37 mmol) were dissolved in anhydrous DMF (80 mL). Triethylamine (11.2 mL, 80.35 mmol) was added and stirred for 5 min. The reaction mixture was cooled in an ice bath and a solution of compound 121 (10 g, mmol) in anhydrous DMF (20 mL) was added. Additional triethylamine (4.5 mL, 32.28 mmol) was added and the reaction mixture was stirred for 18 h under an argon atmosphere. The reaction was monitored by TLC (ethyl acetate:hexane; 1:1; $R_f = 0.47$). The solvent was removed under reduced pressure. The residue was taken up in EtOAc (300 mL) and washed with 1M NaHSO₄ (3 x 150 mL), aqueous saturated NaHCO₃ solution (3 x 150 mL) and brine (2 x 100 mL). Organic layer was dried with Na₂SO₄. Drying agent was removed by filtration and organic layer was concentrated by rotary evaporation. Crude mixture was purified by silica gel column chromatography and eluted by using 35 – 50% EtOAc in hexane to yield a compound 122 (15.50 g, 78.13%). The structure was confirmed by LCMS and ¹H NMR analysis. Mass m/z 589.3 [M + H]⁺.

15

A solution of LiOH (92.15 mmol) in water (20 mL) and THF (10 mL) was added to a cooled solution of Compound 122 (7.75 g, 13.16 mmol) dissolved in methanol (15 mL). The reaction mixture was stirred at





Compound 123 (7.419 g, 12.91 mmol), HOBt (3.49 g, 25.82 mmol) and compound 126 (6.33 g, 16.14 mmol) were dissolved in and DMF (40 mL) and the resulting reaction mixture was cooled in an ice bath. To this *N,N*-Diisopropylethylamine (4.42 mL, 25.82 mmol), PyBop (8.7 g, 16.7 mmol) followed by Bop coupling reagent (1.17 g, 2.66 mmol) were added under an argon atmosphere. The ice bath was removed and the solution was allowed to warm to room temperature. The reaction was completed after 1 h as determined by TLC (DCM:MeOH:AA; 89:10:1). The reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (200 mL) and washed with 1 M NaHSO₄ (3x100 mL), aqueous saturated NaHCO₃ (3x100 mL) and brine (2x100 mL). The organic phase separated dried (Na₂SO₄), filtered and concentrated. The residue was purified by silica gel column chromatography with a gradient of 50% hexanes/EtOAc to 100% EtOAc to yield Compound 127 (9.4 g) as a white foam. LCMS and ¹H NMR

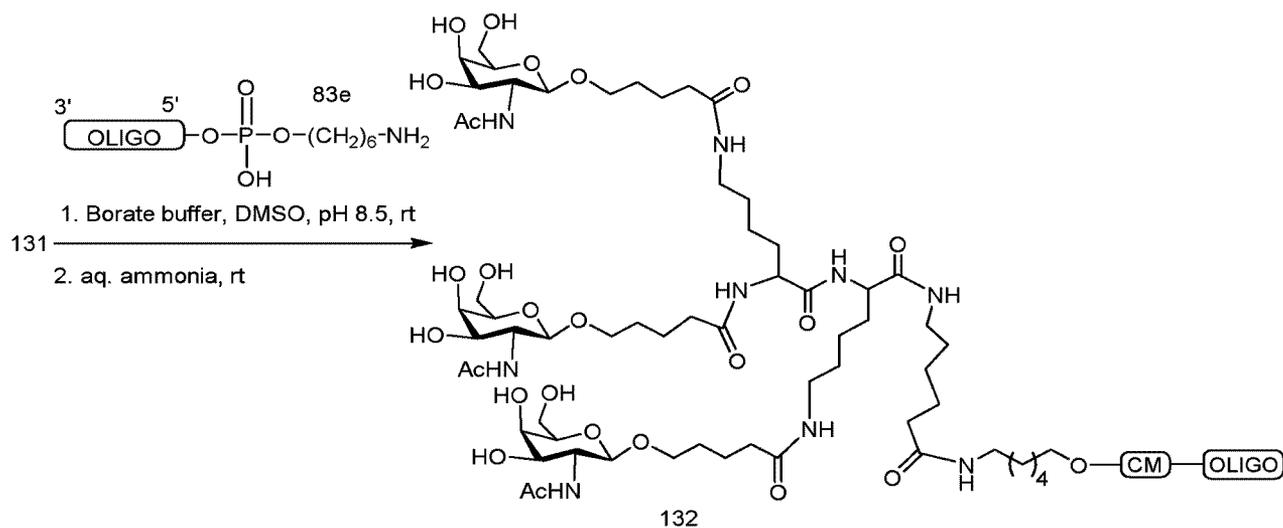
were consistent with structure. Mass m/z 778.4 $[M + H]^+$.

Trifluoroacetic acid (12 mL) was added to a solution of compound 127 (1.57 g, 2.02 mmol) in dichloromethane (12 mL) and stirred at room temperature for 1 h. The reaction mixture was co-evaporated with toluene (30 mL) under reduced pressure to dryness. The residue obtained was co-evaporated twice with acetonitrile (30 mL) and toluene (40 mL) to yield Compound 128 (1.67 g) as trifluoro acetate salt and used for next step without further purification. LCMS and 1H NMR were consistent with structure. Mass m/z 478.2 $[M + H]^+$.

Compound 7 (0.43 g, 0.963 mmol), HATU (0.35 g, 0.91 mmol), and HOAt (0.035 g, 0.26 mmol) were combined together and dried for 4 h over P_2O_5 under reduced pressure in a round bottom flask and then dissolved in anhydrous DMF (1 mL) and stirred for 5 min. To this a solution of compound 128 (0.20 g, 0.26 mmol) in anhydrous DMF (0.2 mL) and *N,N*-Diisopropylethylamine (0.2 mL) was added. The reaction mixture was stirred at room temperature under an argon atmosphere. The reaction was complete after 30 min as determined by LCMS and TLC (7% MeOH/DCM). The reaction mixture was concentrated under reduced pressure. The residue was dissolved in DCM (30 mL) and washed with 1 M $NaHSO_4$ (3x20 mL), aqueous saturated $NaHCO_3$ (3 x 20 mL) and brine (3x20 mL). The organic phase was separated, dried over Na_2SO_4 , filtered and concentrated. The residue was purified by silica gel column chromatography using 5-15% MeOH in dichloromethane to yield Compound 129 (96.6 mg). LC MS and 1H NMR are consistent with structure. Mass m/z 883.4 $[M + 2H]^+$.

Compound 129 (0.09 g, 0.051 mmol) was dissolved in methanol (5 mL) in 20 mL scintillation vial. To this was added a small amount of 10% Pd/C (0.015 mg) and the reaction vessel was flushed with H_2 gas. The reaction mixture was stirred at room temperature under H_2 atmosphere for 18 h. The reaction mixture was filtered through a pad of Celite and the Celite pad was washed with methanol. The filtrate washings were pooled together and concentrated under reduced pressure to yield Compound 130 (0.08 g). LCMS and 1H NMR were consistent with structure. The product was used without further purification. Mass m/z 838.3 $[M + 2H]^+$.

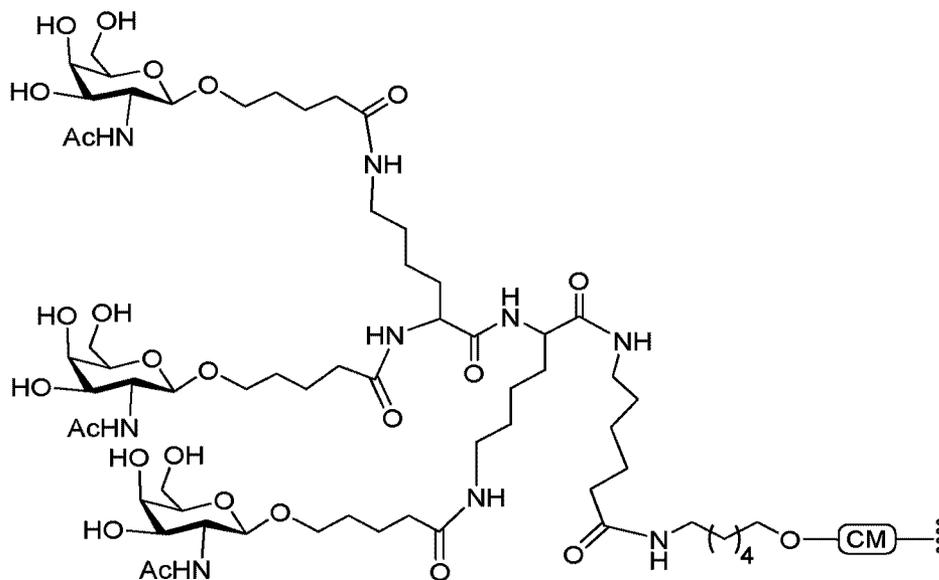
To a 10 mL pointed round bottom flask were added compound 130 (75.8 mg, 0.046 mmol), 0.37 M pyridine/DMF (200 μ L) and a stir bar. To this solution was added 0.7 M pentafluorophenyl trifluoroacetate/DMF (100 μ L) drop wise with stirring. The reaction was completed after 1 h as determined by LC MS. The solvent was removed under reduced pressure and the residue was dissolved in $CHCl_3$ (~ 10 mL). The organic layer was partitioned against $NaHSO_4$ (1 M, 10 mL), aqueous saturated $NaHCO_3$ (10 mL) and brine (10 mL) three times each. The organic phase separated and dried over Na_2SO_4 , filtered and concentrated to yield Compound 131 (77.7 mg). LCMS is consistent with structure. Used without further purification. Mass m/z 921.3 $[M + 2H]^+$.

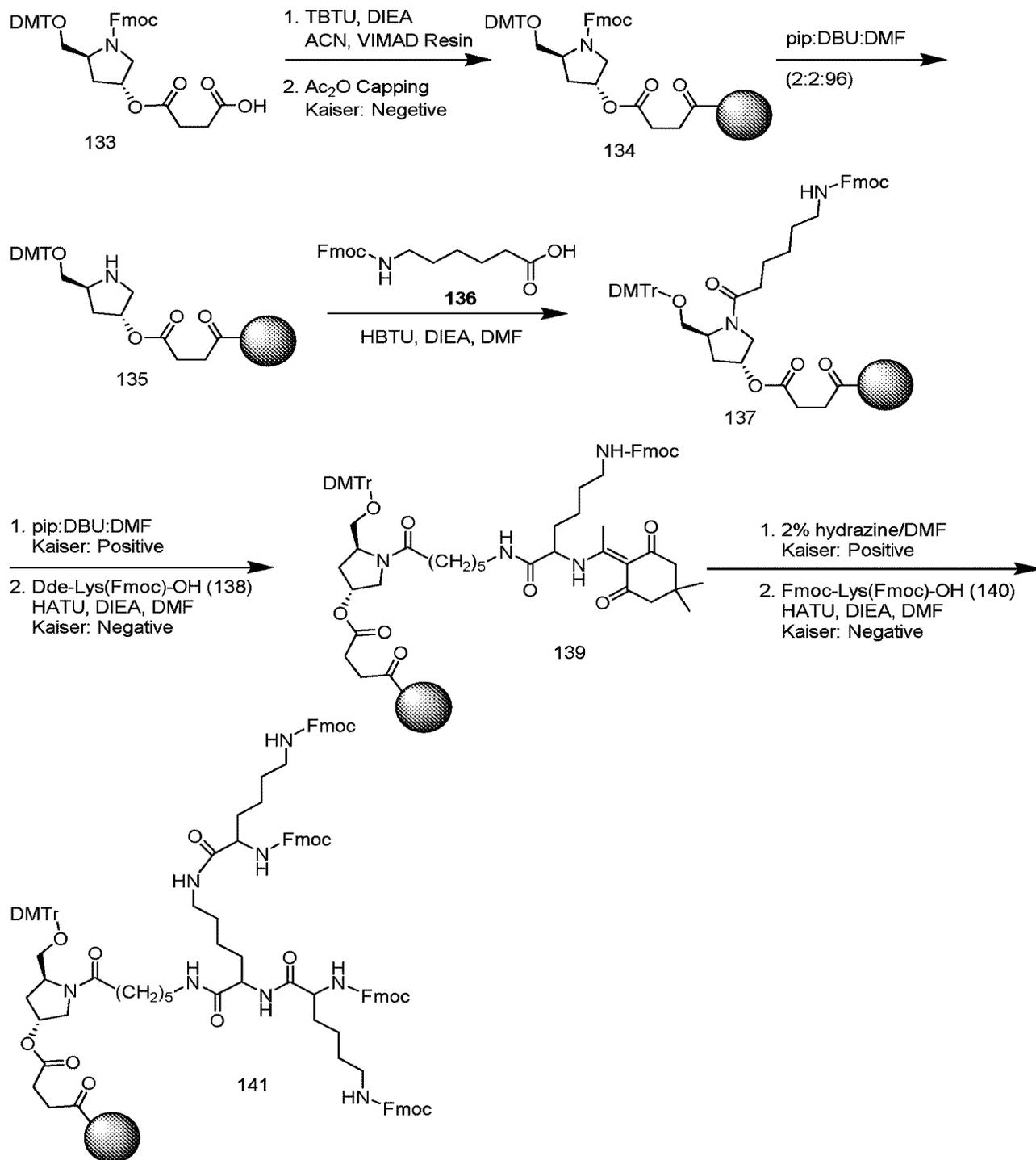


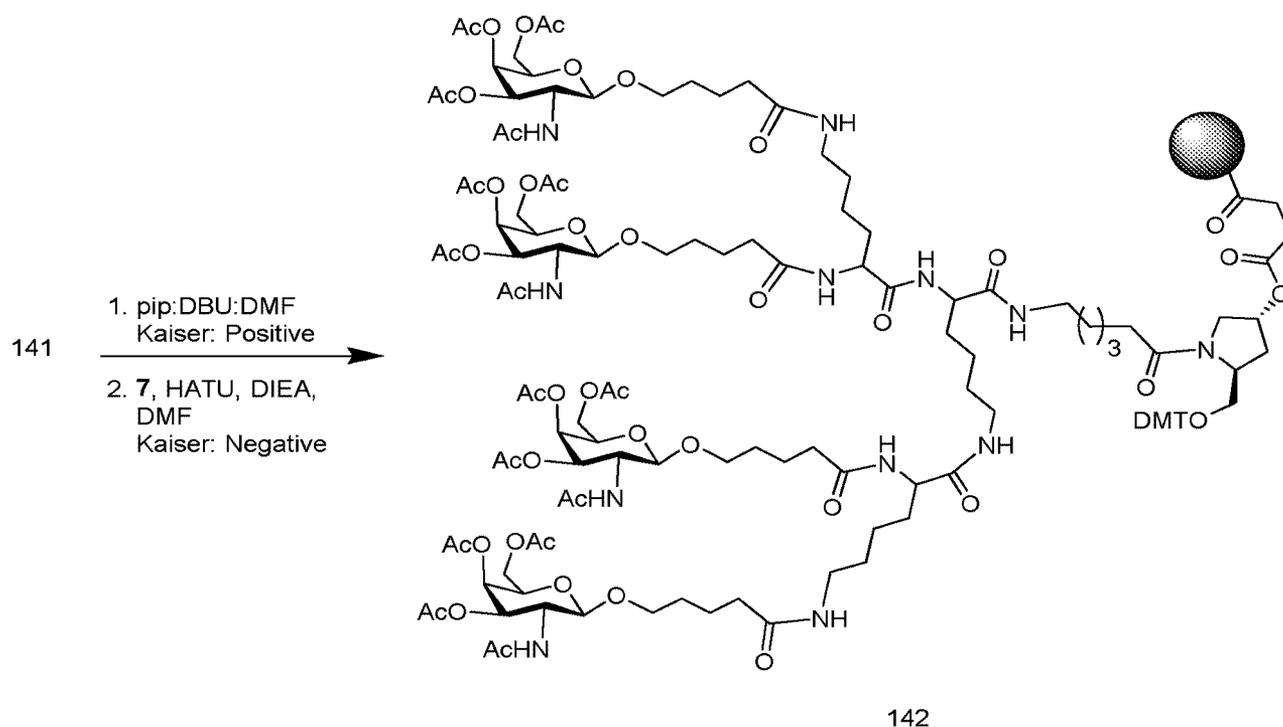
- 5 Oligomeric Compound 132, comprising a GalNAc₃-5 conjugate group, was prepared using the general procedures illustrated in Example 46. The GalNAc₃ cluster portion of the conjugate group GalNAc₃-5 (GalNAc₃-5_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-.

The structure of GalNAc₃-5 (GalNAc₃-5_a-CM-) is shown below:

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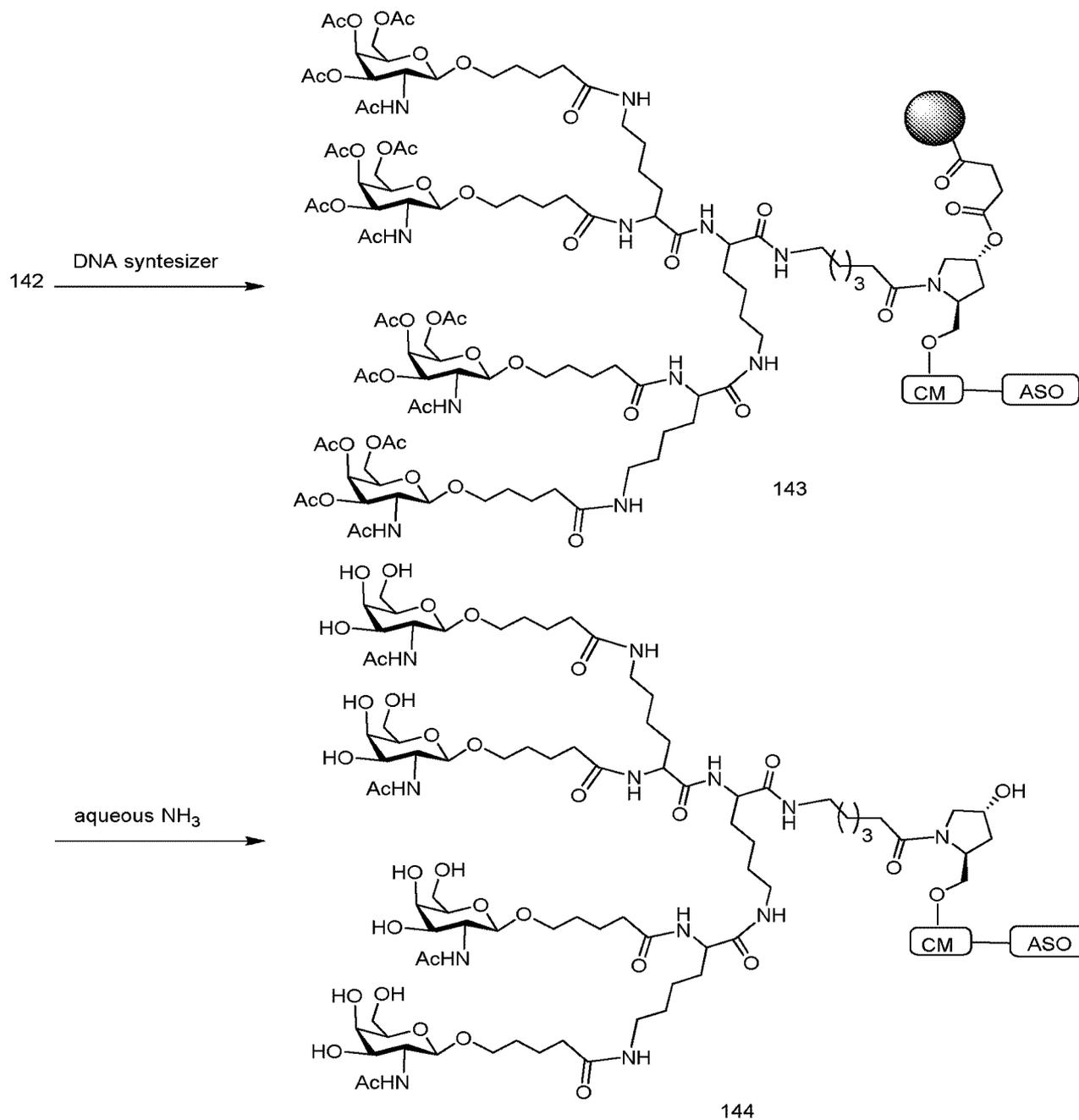
Example 50: Preparation of Oligonucleotide 144 Comprising GalNAc₄-11



Synthesis of Compound 134. To a Merrifield flask was added aminomethyl VIMAD resin (2.5 g, 450 $\mu\text{mol/g}$) that was washed with acetonitrile, dimethylformamide, dichloromethane and acetonitrile. The resin was swelled in acetonitrile (4 mL). Compound 133 was pre-activated in a 100 mL round bottom flask by adding 20 (1.0 mmol, 0.747 g), TBTU (1.0 mmol, 0.321 g), acetonitrile (5 mL) and DIEA (3.0 mmol, 0.5 mL). This solution was allowed to stir for 5 min and was then added to the Merrifield flask with shaking. The suspension was allowed to shake for 3 h. The reaction mixture was drained and the resin was washed with acetonitrile, DMF and DCM. New resin loading was quantitated by measuring the absorbance of the DMT cation at 500 nm (extinction coefficient = 76000) in DCM and determined to be 238 $\mu\text{mol/g}$. The resin was capped by suspending in an acetic anhydride solution for ten minutes three times.

The solid support bound compound 141 was synthesized using iterative Fmoc-based solid phase peptide synthesis methods. A small amount of solid support was withdrawn and suspended in aqueous ammonia (28-30 wt%) for 6 h. The cleaved compound was analyzed by LC-MS and the observed mass was consistent with structure. Mass m/z 1063.8 $[\text{M} + 2\text{H}]^+$.

The solid support bound compound 142 was synthesized using solid phase peptide synthesis methods.



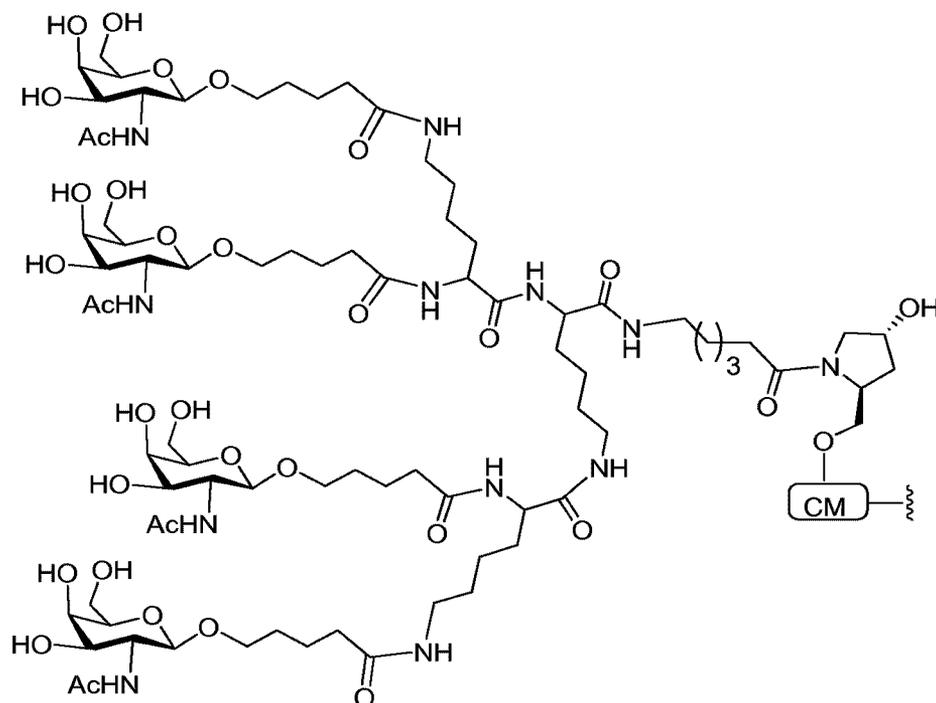
The solid support bound compound 143 was synthesized using standard solid phase synthesis on a DNA synthesizer.

5 The solid support bound compound 143 was suspended in aqueous ammonia (28-30 wt%) and heated at 55 °C for 16 h. The solution was cooled and the solid support was filtered. The filtrate was concentrated and the residue dissolved in water and purified by HPLC on a strong anion exchange column. The fractions containing full length compound 144 were pooled together and desalted. The resulting GalNAc₄-11

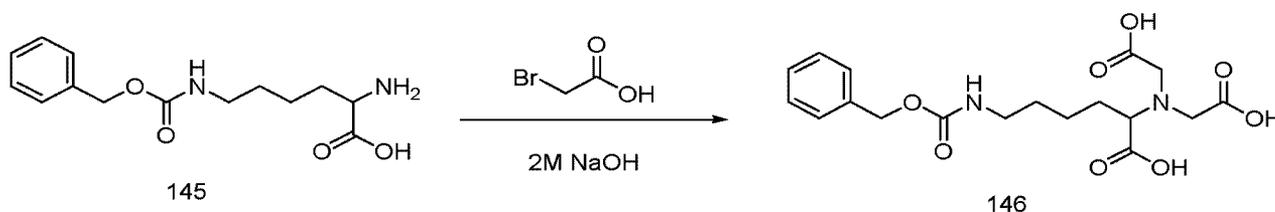
conjugated oligomeric compound was analyzed by LC-MS and the observed mass was consistent with structure.

The GalNAc₄ cluster portion of the conjugate group GalNAc₄-11 (GalNAc₄-11_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-.

The structure of GalNAc₄-11 (GalNAc₄-11_a-CM) is shown below:

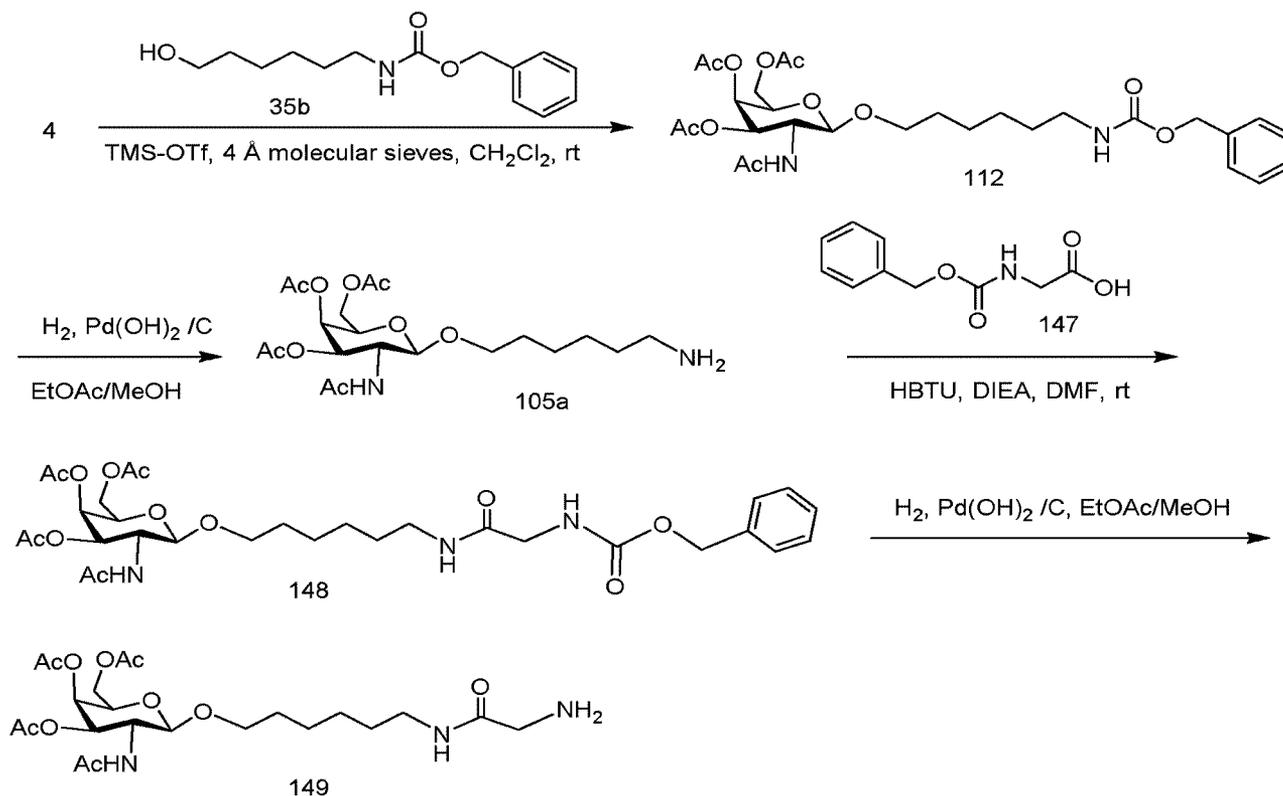


Example 51: Preparation of Oligonucleotide 155 Comprising GalNAc₃-6



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Compound 146 was synthesized as described in the literature (*Analytical Biochemistry* 1995, 229, 54-60).



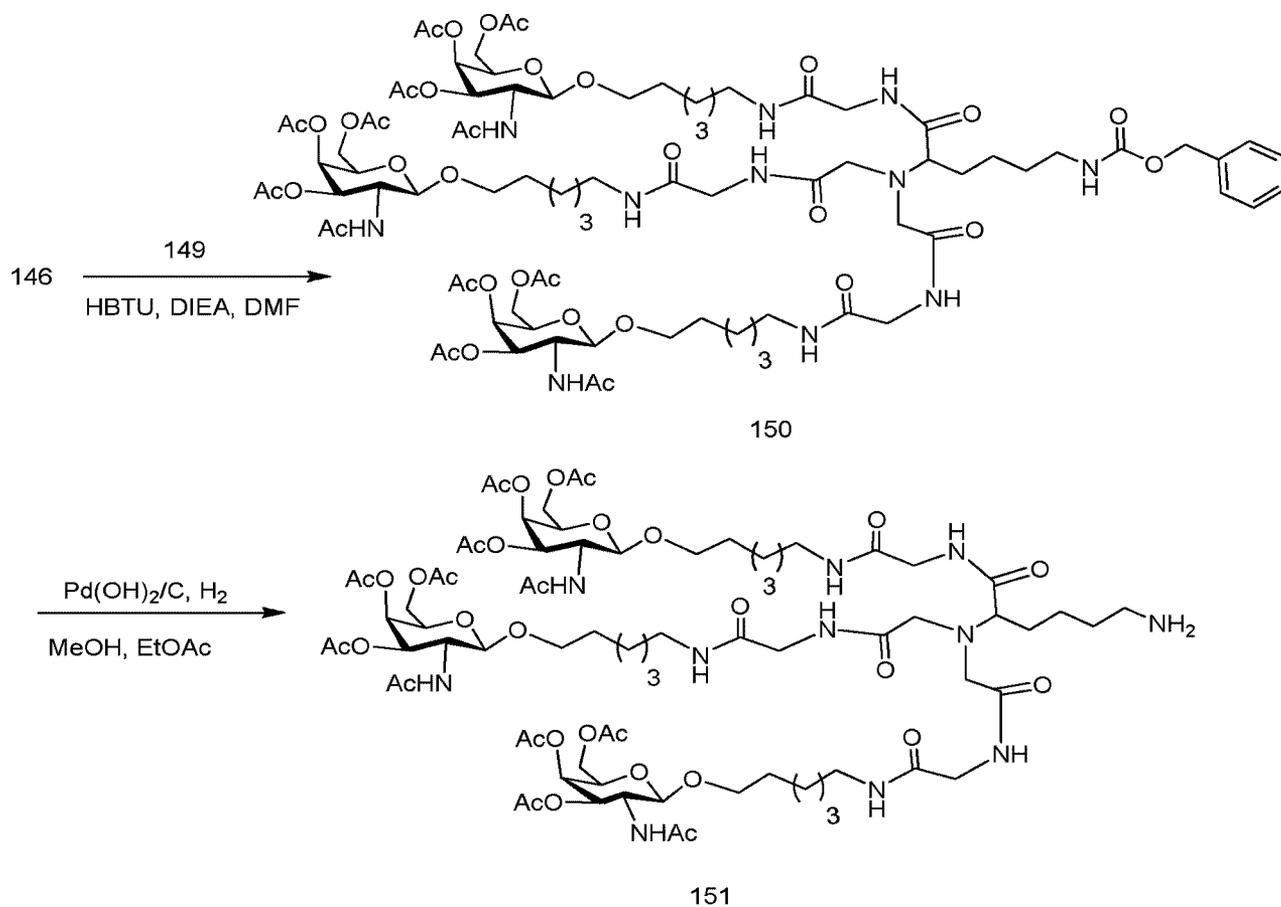
Compound 4 (15 g, 45.55 mmol) and compound 35b (14.3 grams, 57 mmol) were dissolved in CH₂Cl₂ (200 ml). Activated molecular sieves (4 Å, 2 g, powdered) were added, and the reaction was allowed to stir for 30 minutes under nitrogen atmosphere. TMS-OTf was added (4.1 ml, 22.77 mmol) and the reaction was allowed to stir at room temp overnight. Upon completion, the reaction was quenched by pouring into solution of saturated aqueous NaHCO₃ (500 ml) and crushed ice (~ 150 g). The organic layer was separated, washed with brine, dried over MgSO₄, filtered, and was concentrated to an orange oil under reduced pressure. The crude material was purified by silica gel column chromatography and eluted with 2-10 % MeOH in CH₂Cl₂ to yield Compound 112 (16.53 g, 63 %). LCMS and ¹H NMR were consistent with the expected compound.

Compound 112 (4.27 g, 7.35 mmol) was dissolved in 1:1 MeOH/EtOAc (40 ml). The reaction mixture was purged by bubbling a stream of argon through the solution for 15 minutes. Pearlman's catalyst (palladium hydroxide on carbon, 400 mg) was added, and hydrogen gas was bubbled through the solution for 30 minutes. Upon completion (TLC 10% MeOH in CH₂Cl₂, and LCMS), the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated by rotary evaporation, and was dried briefly under high vacuum to yield Compound 105a (3.28 g). LCMS and ¹H NMR were consistent with desired product.

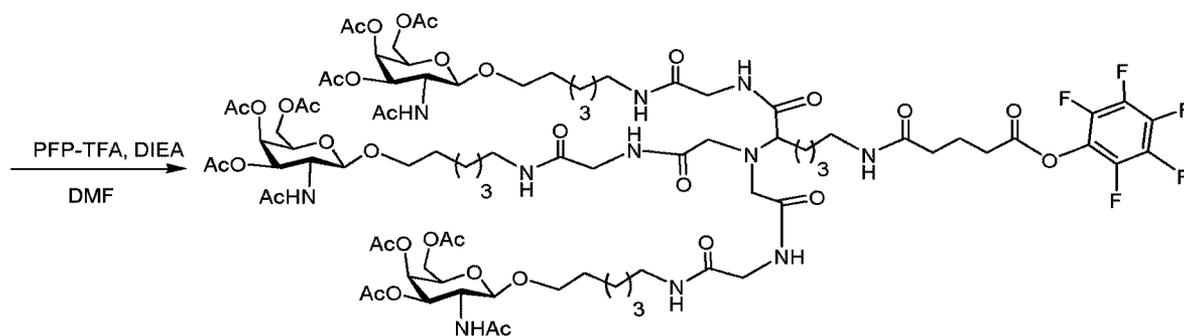
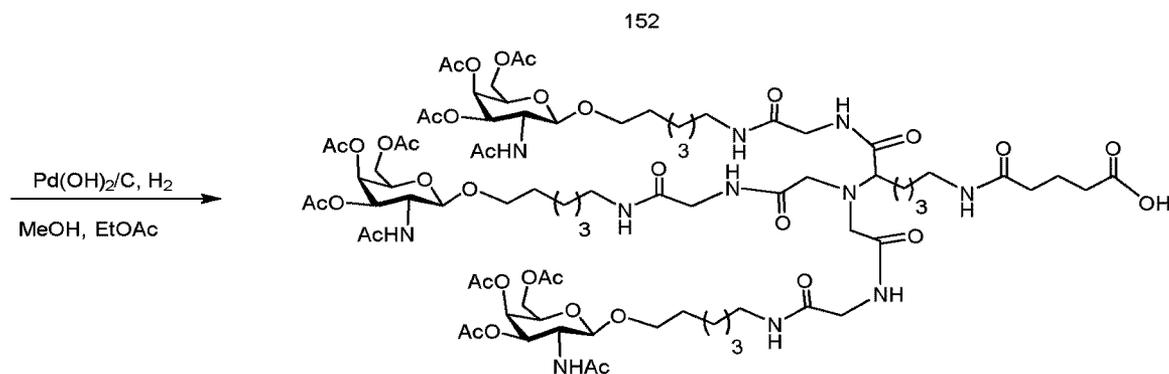
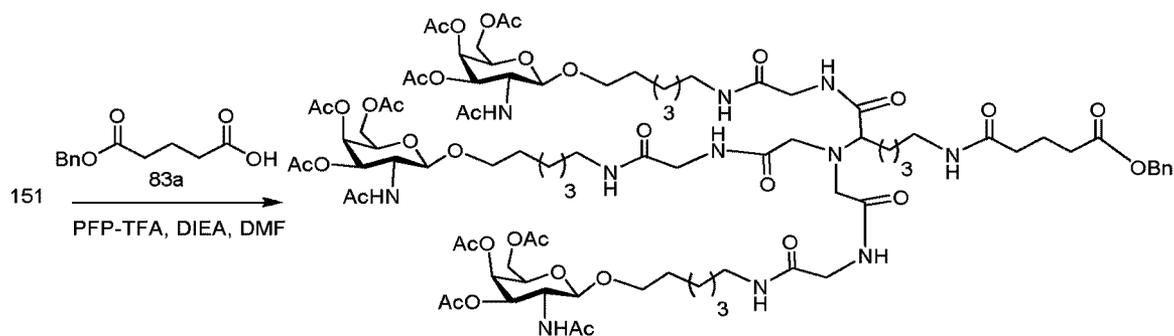
Compound 147 (2.31 g, 11 mmol) was dissolved in anhydrous DMF (100 mL). *N,N*-Diisopropylethylamine (DIEA, 3.9 mL, 22 mmol) was added, followed by HBTU (4 g, 10.5 mmol). The

reaction mixture was allowed to stir for ~ 15 minutes under nitrogen. To this a solution of compound 105a (3.3 g, 7.4 mmol) in dry DMF was added and stirred for 2 h under nitrogen atmosphere. The reaction was diluted with EtOAc and washed with saturated aqueous NaHCO₃ and brine. The organics phase was separated, dried (MgSO₄), filtered, and concentrated to an orange syrup. The crude material was purified by column chromatography 2-5 % MeOH in CH₂Cl₂ to yield Compound 148 (3.44 g, 73 %). LCMS and ¹H NMR were consistent with the expected product.

Compound 148 (3.3 g, 5.2 mmol) was dissolved in 1:1 MeOH/EtOAc (75 ml). The reaction mixture was purged by bubbling a stream of argon through the solution for 15 minutes. Pearlman's catalyst (palladium hydroxide on carbon) was added (350 mg). Hydrogen gas was bubbled through the solution for 30 minutes. Upon completion (TLC 10% MeOH in DCM, and LCMS), the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated by rotary evaporation, and was dried briefly under high vacuum to yield Compound 149 (2.6 g). LCMS was consistent with desired product. The residue was dissolved in dry DMF (10 ml) was used immediately in the next step.



- Compound 146 (0.68 g, 1.73 mmol) was dissolved in dry DMF (20 ml). To this DIEA (450 μ L, 2.6 mmol, 1.5 eq.) and HBTU (1.96 g, 0.5.2 mmol) were added. The reaction mixture was allowed to stir for 15 minutes at room temperature under nitrogen. A solution of compound 149 (2.6 g) in anhydrous DMF (10 mL) was added. The pH of the reaction was adjusted to pH = 9-10 by addition of DIEA (if necessary). The reaction was allowed to stir at room temperature under nitrogen for 2 h. Upon completion the reaction was diluted with EtOAc (100 mL), and washed with aqueous saturated aqueous NaHCO₃, followed by brine. The organic phase was separated, dried over MgSO₄, filtered, and concentrated. The residue was purified by silica gel column chromatography and eluted with 2-10 % MeOH in CH₂Cl₂ to yield Compound 150 (0.62 g, 20 %). LCMS and ¹H NMR were consistent with the desired product.
- Compound 150 (0.62 g) was dissolved in 1:1 MeOH/ EtOAc (5 L). The reaction mixture was purged by bubbling a stream of argon through the solution for 15 minutes. Pearlman's catalyst (palladium hydroxide on carbon) was added (60 mg). Hydrogen gas was bubbled through the solution for 30 minutes. Upon completion (TLC 10% MeOH in DCM, and LCMS), the catalyst was removed by filtration (syringe-tip Teflon filter, 0.45 μ m). The filtrate was concentrated by rotary evaporation, and was dried briefly under high vacuum to yield Compound 151 (0.57 g). The LCMS was consistent with the desired product. The product was dissolved in 4 mL dry DMF and was used immediately in the next step.

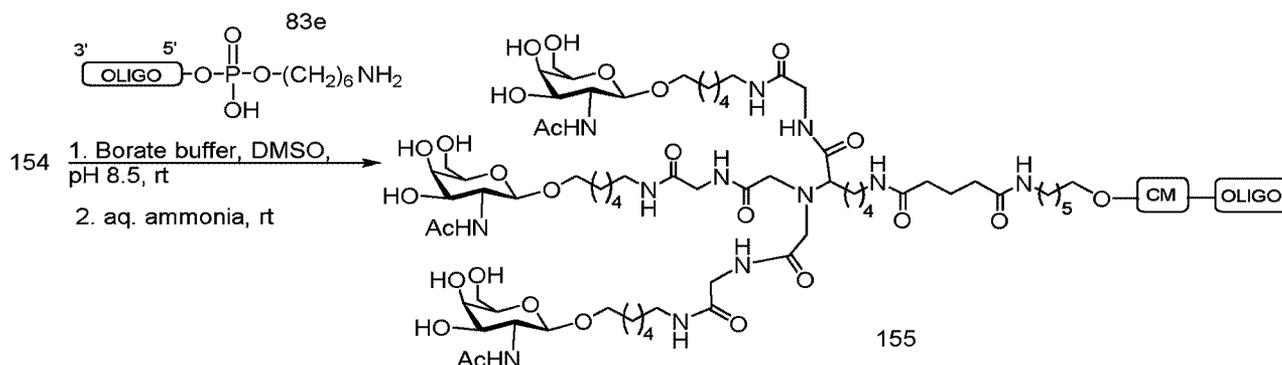


Compound 83a (0.11 g, 0.33 mmol) was dissolved in anhydrous DMF (5 mL) and *N,N*-Diisopropylethylamine (75 μL , 1 mmol) and PFP-TFA (90 μL , 0.76 mmol) were added. The reaction mixture turned magenta upon contact, and gradually turned orange over the next 30 minutes. Progress of reaction was monitored by TLC and LCMS. Upon completion (formation of the PFP ester), a solution of compound 151 (0.57 g, 0.33 mmol) in DMF was added. The pH of the reaction was adjusted to pH = 9-10 by addition of *N,N*-Diisopropylethylamine (if necessary). The reaction mixture was stirred under nitrogen for ~30 min. Upon completion, the majority of the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and washed with aqueous saturated NaHCO_3 , followed by brine. The organic phase separated, dried over MgSO_4 , filtered, and concentrated to an orange syrup. The residue was purified by

silica gel column chromatography (2-10 % MeOH in CH₂Cl₂) to yield Compound 152 (0.35 g, 55 %). LCMS and ¹H NMR were consistent with the desired product.

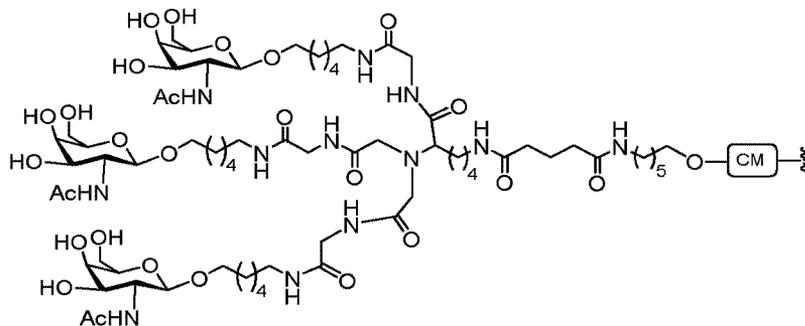
Compound 152 (0.35 g, 0.182 mmol) was dissolved in 1:1 MeOH/EtOAc (10 mL). The reaction mixture was purged by bubbling a stream of argon thru the solution for 15 minutes. Pearlman's catalyst (palladium hydroxide on carbon) was added (35 mg). Hydrogen gas was bubbled thru the solution for 30 minutes. Upon completion (TLC 10% MeOH in DCM, and LCMS), the catalyst was removed by filtration (syringe-tip Teflon filter, 0.45 μm). The filtrate was concentrated by rotary evaporation, and was dried briefly under high vacuum to yield Compound 153 (0.33 g, quantitative). The LCMS was consistent with desired product.

Compound 153 (0.33 g, 0.18 mmol) was dissolved in anhydrous DMF (5 mL) with stirring under nitrogen. To this *N,N*-Diisopropylethylamine (65 μL, 0.37 mmol) and PFP-TFA (35 μL, 0.28 mmol) were added. The reaction mixture was stirred under nitrogen for ~ 30 min. The reaction mixture turned magenta upon contact, and gradually turned orange. The pH of the reaction mixture was maintained at pH = 9-10 by adding more *N,N*-Diisopropylethylamine. The progress of the reaction was monitored by TLC and LCMS. Upon completion, the majority of the solvent was removed under reduced pressure. The residue was diluted with CH₂Cl₂ (50 mL), and washed with saturated aqueous NaHCO₃, followed by brine. The organic layer was dried over MgSO₄, filtered, and concentrated to an orange syrup. The residue was purified by column chromatography and eluted with 2-10 % MeOH in CH₂Cl₂ to yield Compound 154 (0.29 g, 79 %). LCMS and ¹H NMR were consistent with the desired product.

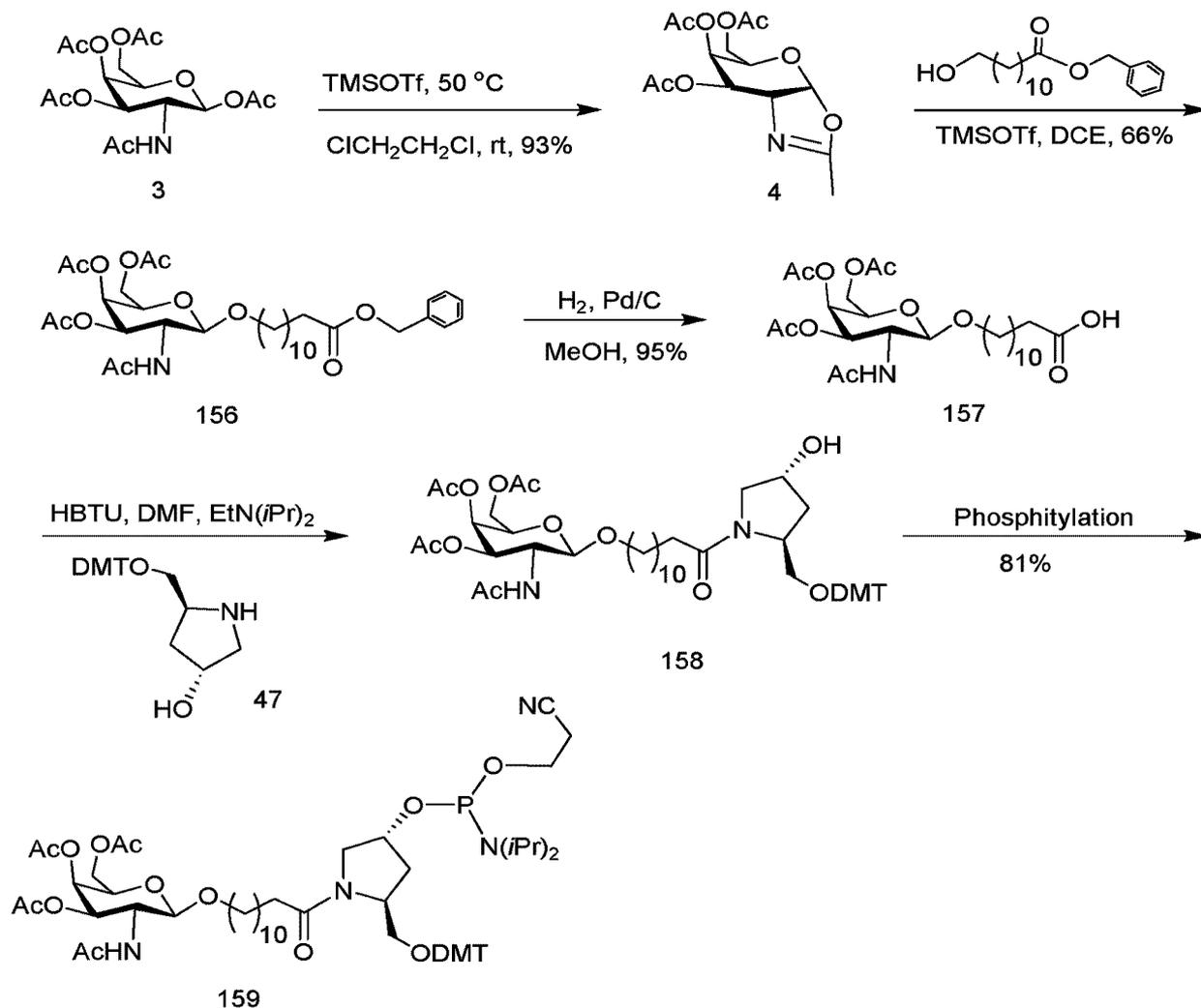


Oligomeric Compound 155, comprising a GalNAc₃-6 conjugate group, was prepared using the general procedures illustrated in Example 46. The GalNAc₃ cluster portion of the conjugate group GalNAc₃-6 (GalNAc₃-6_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-.

The structure of GalNAc₃-6 (GalNAc₃-6_a-CM-) is shown below:



Example 52: Preparation of Oligonucleotide 160 Comprising GalNAc₃-9



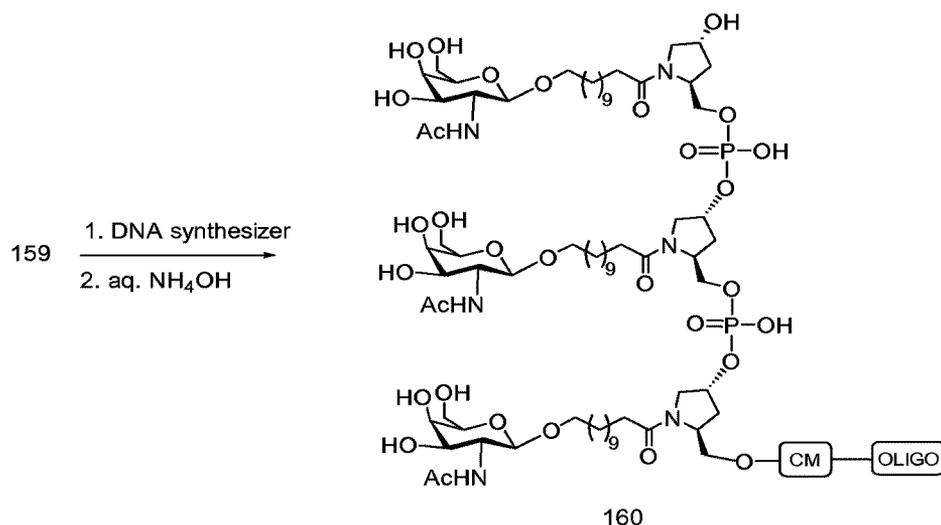
Compound 156 was synthesized following the procedure described in the literature (*J. Med. Chem.* 2004, 47, 5798-5808).

Compound 156, (18.60 g, 29.28 mmol) was dissolved in methanol (200 mL). Palladium on carbon (6.15 g, 10 wt%, loading (dry basis), matrix carbon powder, wet) was added. The reaction mixture was stirred at room temperature under hydrogen for 18 h. The reaction mixture was filtered through a pad of

celite and the celite pad was washed thoroughly with methanol. The combined filtrate was washed and concentrated to dryness. The residue was purified by silica gel column chromatography and eluted with 5-10 % methanol in dichloromethane to yield Compound 157 (14.26 g, 89%). Mass m/z 544.1 [M-H].

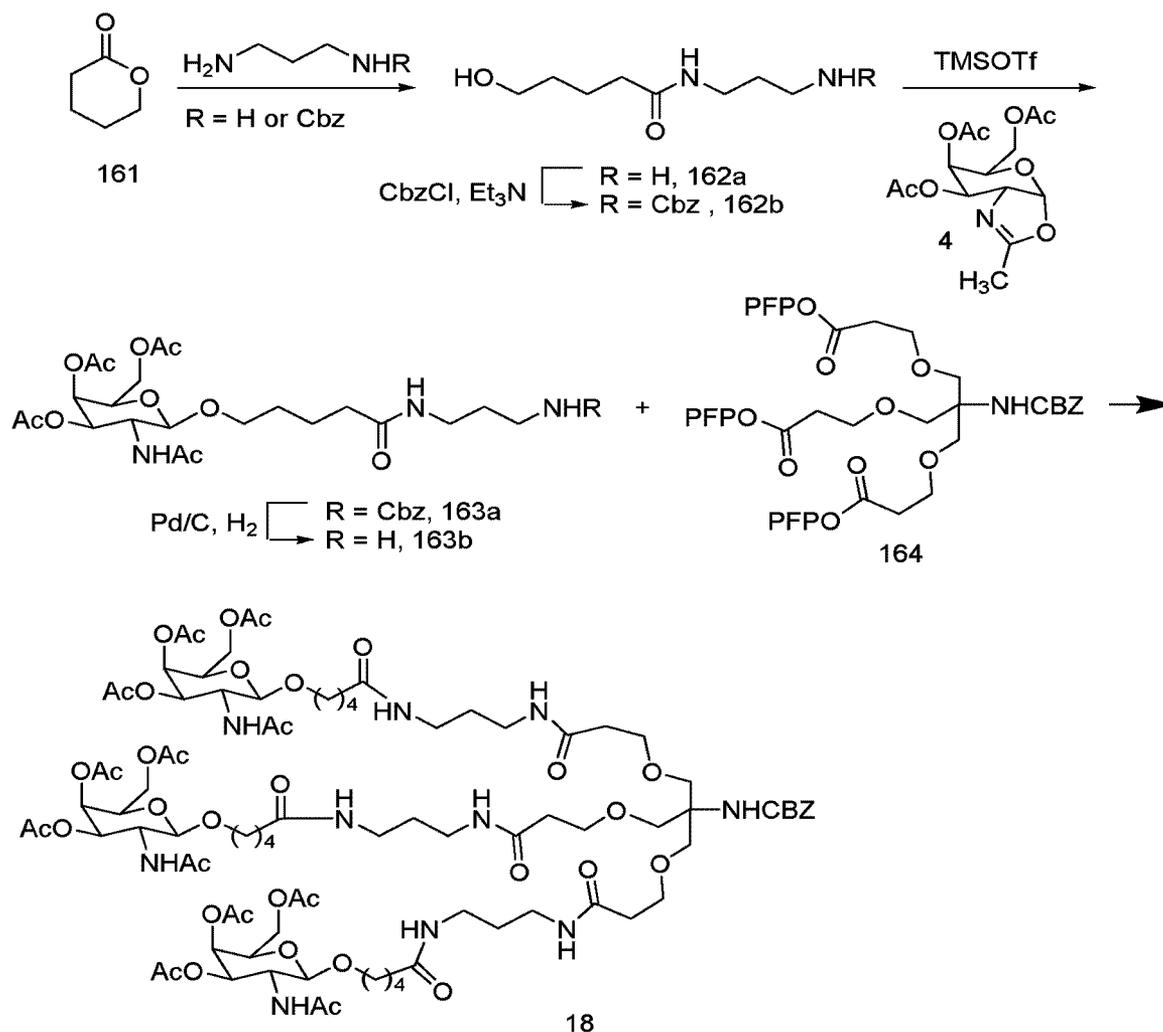
Compound 157 (5 g, 9.17 mmol) was dissolved in anhydrous DMF (30 mL). HBTU (3.65 g, 9.61 mmol) and *N,N*-Diisopropylethylamine (13.73 mL, 78.81 mmol) were added and the reaction mixture was stirred at room temperature for 5 minutes. To this a solution of compound 47 (2.96 g, 7.04 mmol) was added. The reaction was stirred at room temperature for 8 h. The reaction mixture was poured into a saturated NaHCO₃ aqueous solution. The mixture was extracted with ethyl acetate and the organic layer was washed with brine and dried (Na₂SO₄), filtered and evaporated. The residue obtained was purified by silica gel column chromatography and eluted with 50% ethyl acetate in hexane to yield compound 158 (8.25g, 73.3%). The structure was confirmed by MS and ¹H NMR analysis.

Compound 158 (7.2 g, 7.61 mmol) was dried over P₂O₅ under reduced pressure. The dried compound was dissolved in anhydrous DMF (50 mL). To this 1H-tetrazole (0.43 g, 6.09 mmol) and *N*-methylimidazole (0.3 mL, 3.81 mmol) and 2-cyanoethyl-*N,N,N',N'*-tetraisopropyl phosphorodiamidite (3.65 mL, 11.50 mmol) were added. The reaction mixture was stirred under an argon atmosphere for 4 h. The reaction mixture was diluted with ethyl acetate (200 mL). The reaction mixture was washed with saturated NaHCO₃ and brine. The organic phase was separated, dried (Na₂SO₄), filtered and evaporated. The residue was purified by silica gel column chromatography and eluted with 50-90 % ethyl acetate in hexane to yield Compound 159 (7.82 g, 80.5%). The structure was confirmed by LCMS and ³¹P NMR analysis.

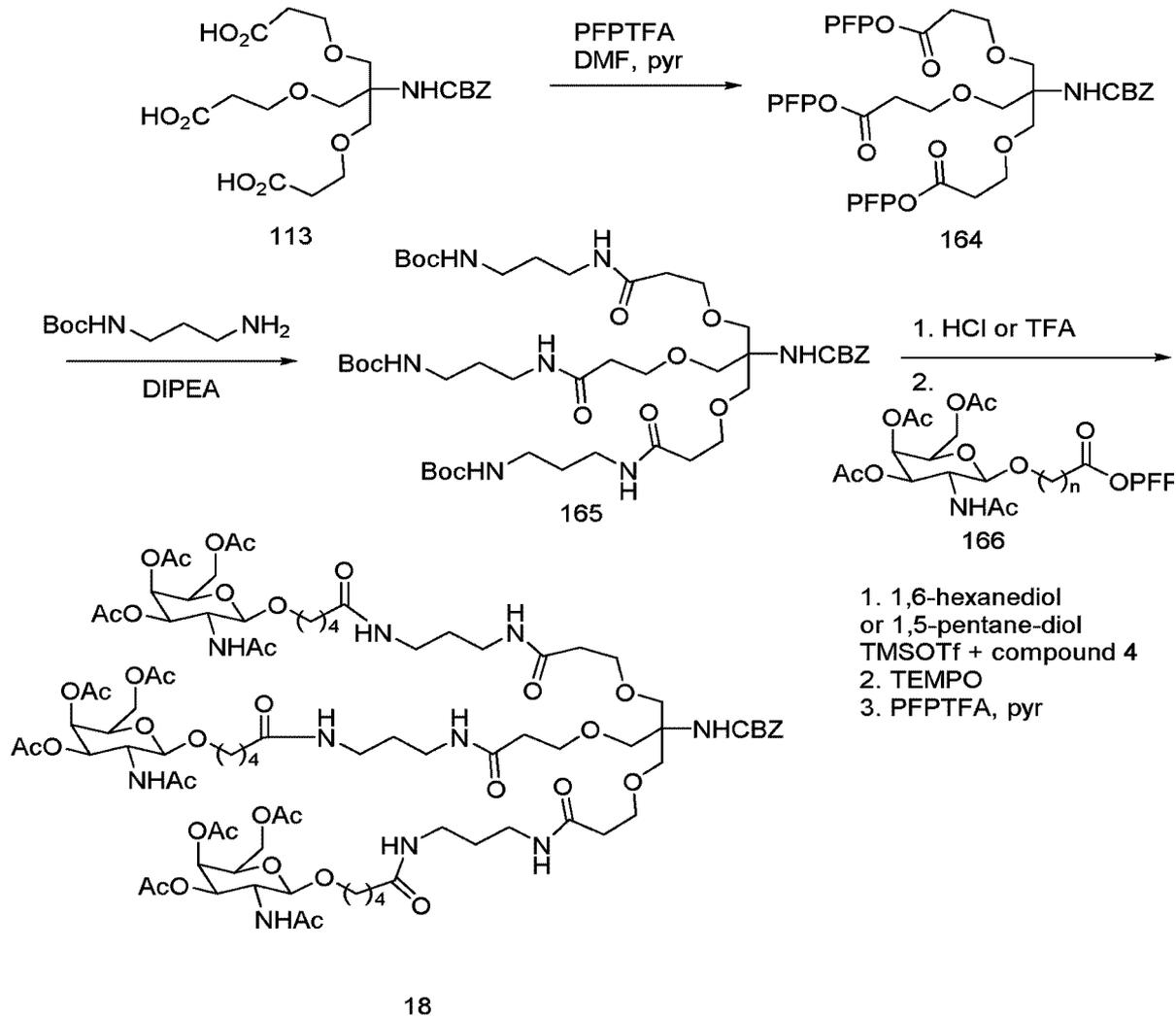


Oligomeric Compound 160, comprising a GalNAc₃₋₉ conjugate group, was prepared using standard oligonucleotide synthesis procedures. Three units of compound 159 were coupled to the solid support, followed by nucleotide phosphoramidites. Treatment of the protected oligomeric compound with aqueous ammonia yielded compound 160. The GalNAc₃ cluster portion of the conjugate group GalNAc₃₋₉ (GalNAc₃₋

25

Example 53: Alternate procedure for preparation of Compound 18 (GalNAc₃-1a and GalNAc₃-3a)

Lactone 161 was reacted with diamino propane (3-5 eq) or Mono-Boc protected diamino propane (1
 5 eq) to provide alcohol 162a or 162b. When unprotected propanediamine was used for the above reaction, the
 excess diamine was removed by evaporation under high vacuum and the free amino group in 162a was
 protected using CbzCl to provide 162b as a white solid after purification by column chromatography.
 Alcohol 162b was further reacted with compound 4 in the presence of TMSOTf to provide 163a which was
 converted to 163b by removal of the Cbz group using catalytic hydrogenation. The pentafluorophenyl (PFP)
 10 ester 164 was prepared by reacting triacid 113 (see Example 48) with PFPTFA (3.5 eq) and pyridine (3.5 eq)
 in DMF (0.1 to 0.5 M). The triester 164 was directly reacted with the amine 163b (3–4 eq) and DIPEA (3–4
 eq) to provide Compound 18. The above method greatly facilitates purification of intermediates and
 minimizes the formation of byproducts which are formed using the procedure described in Example 4.

Example 54: Alternate procedure for preparation of Compound 18 (GalNAc₃-1a and GalNAc₃-3a)

The triPFP ester 164 was prepared from acid 113 using the procedure outlined in example 53 above and reacted with mono-Boc protected diamine to provide 165 in essentially quantitative yield. The Boc groups were removed with hydrochloric acid or trifluoroacetic acid to provide the triamine which was reacted with the PFP activated acid 166 in the presence of a suitable base such as DIPEA to provide Compound 18.

The PFP protected Gal-NAc acid 166 was prepared from the corresponding acid by treatment with PFPTFA (1-1.2 eq) and pyridine (1-1.2 eq) in DMF. The precursor acid in turn was prepared from the corresponding alcohol by oxidation using TEMPO (0.2 eq) and BAIB in acetonitrile and water. The precursor alcohol was prepared from sugar intermediate 4 by reaction with 1,6-hexanediol (or 1,5-pentane-diol or other diol for other n values) (2-4 eq) and TMSOTf using conditions described previously in example 47.

Example 55: Dose-dependent study of oligonucleotides comprising either a 3' or 5'-conjugate group (comparison of GalNAc₃-1, 3, 8 and 9) targeting SRB-1 *in vivo*

The oligonucleotides listed below were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice. Unconjugated ISIS 353382 was included as a standard. Each of the various GalNAc₃ conjugate groups was attached at either the 3' or 5' terminus of the respective oligonucleotide by a phosphodiester linked 2'-deoxyadenosine nucleoside (cleavable moiety).

Table 39
Modified ASO targeting SRB-1

ASO	Sequence (5' to 3')	Motif	Conjugate	SEQ ID No.
ISIS 353382 (parent)	G _{es} ^m C _{es} T _{es} T _{es} ^m C _{es} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{es} ^m C _{es} ^m C _{es} T _{es} T _e	5/10/5	none	252
ISIS 655861	G _{es} ^m C _{es} T _{es} T _{es} ^m C _{es} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{es} ^m C _{es} ^m C _{es} T _{es} T _{eo} A_{do}-GalNAc₃-1_a	5/10/5	GalNAc₃-1	253
ISIS 664078	G _{es} ^m C _{es} T _{es} T _{es} ^m C _{es} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{es} ^m C _{es} ^m C _{es} T _{es} T _{eo} A_{do}-GalNAc₃-9_a	5/10/5	GalNAc₃-9	253
ISIS 661161	GalNAc₃-3_a-o'-A_{do} G _{es} ^m C _{es} T _{es} T _{es} ^m C _{es} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{es} ^m C _{es} ^m C _{es} T _{es} T _e	5/10/5	GalNAc₃-3	254
ISIS 665001	GalNAc₃-8_a-o'-A_{do} G _{es} ^m C _{es} T _{es} T _{es} ^m C _{es} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{es} ^m C _{es} ^m C _{es} T _{es} T _e	5/10/5	GalNAc₃-8	254

Capital letters indicate the nucleobase for each nucleoside and ^mC indicates a 5-methyl cytosine. Subscripts: "e" indicates a 2'-MOE modified nucleoside; "d" indicates a β-D-2'-deoxyribonucleoside; "s" indicates a phosphorothioate internucleoside linkage (PS); "o" indicates a phosphodiester internucleoside linkage (PO); and "o'" indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

The structure of GalNAc₃-1_a was shown previously in Example 9. The structure of GalNAc₃-9 was shown previously in Example 52. The structure of GalNAc₃-3 was shown previously in Example 39. The structure of GalNAc₃-8 was shown previously in Example 47.

Treatment

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with ISIS 353382, 655861, 664078, 661161, 665001 or with saline. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the liver SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to the saline control.

As illustrated in Table 40, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner. Indeed, the antisense oligonucleotides comprising the phosphodiester linked GalNAc₃-1 and GalNAc₃-9 conjugates at the 3' terminus (ISIS 655861 and ISIS 664078) and the GalNAc₃-3 and GalNAc₃-8 conjugates linked at the 5' terminus (ISIS 661161 and ISIS 665001) showed substantial improvement in potency compared to the unconjugated antisense oligonucleotide (ISIS 353382). Furthermore, ISIS 664078, comprising a GalNAc₃-9 conjugate at the 3' terminus was essentially equipotent compared to ISIS 655861, which comprises a GalNAc₃-1 conjugate at the 3' terminus. The 5' conjugated antisense oligonucleotides, ISIS 661161 and ISIS 665001, comprising a GalNAc₃-3 or GalNAc₃-9, respectively, had increased potency compared to the 3' conjugated antisense oligonucleotides (ISIS 655861 and ISIS 664078).

Table 40
ASOs containing GalNAc₃-1, 3, 8 or 9 targeting SRB-1

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA (% Saline)	Conjugate
Saline	n/a	100	
353382	3	88	none
	10	68	
	30	36	
655861	0.5	98	GalNAc ₃ -1 (3')
	1.5	76	
	5	31	
	15	20	
664078	0.5	88	GalNAc ₃ -9 (3')
	1.5	85	
	5	46	
	15	20	
661161	0.5	92	GalNAc ₃ -3 (5')
	1.5	59	
	5	19	
	15	11	
665001	0.5	100	GalNAc ₃ -8 (5')
	1.5	73	
	5	29	
	15	13	

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Total bilirubin and BUN were also evaluated. The change in body weights was evaluated with no significant change from the saline group. ALTs, ASTs, total bilirubin and BUN values are shown in the table below.

Table 41

ISIS No.	Dosage mg/kg	ALT	AST	Total Bilirubin	BUN	Conjugate
Saline		24	59	0.1	37.52	
353382	3	21	66	0.2	34.65	none
	10	22	54	0.2	34.2	
	30	22	49	0.2	33.72	
655861	0.5	25	62	0.2	30.65	GalNac ₃ -1 (3')
	1.5	23	48	0.2	30.97	
	5	28	49	0.1	32.92	
	15	40	97	0.1	31.62	
664078	0.5	40	74	0.1	35.3	GalNac ₃ -9 (3')
	1.5	47	104	0.1	32.75	
	5	20	43	0.1	30.62	
	15	38	92	0.1	26.2	
661161	0.5	101	162	0.1	34.17	GalNac ₃ -3 (5')
	1.5 g	42	100	0.1	33.37	
	5 g	23	99	0.1	34.97	
	15	53	83	0.1	34.8	
665001	0.5	28	54	0.1	31.32	GalNac ₃ -8 (5')
	1.5	42	75	0.1	32.32	
	5	24	42	0.1	31.85	
	15	32	67	0.1	31.	

Example 56: Dose-dependent study of oligonucleotides comprising either a 3' or 5'-conjugate group (comparison of GalNac₃-1, 2, 3, 5, 6, 7 and 10) targeting SRB-1 *in vivo*

The oligonucleotides listed below were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice. Unconjugated ISIS 353382 was included as a standard. Each of the various GalNac₃ conjugate groups was attached at the 5' terminus of the respective oligonucleotide by a phosphodiester linked 2'-deoxyadenosine nucleoside (cleavable moiety) except for ISIS 655861 which had the GalNac₃ conjugate group attached at the 3' terminus.

Table 42

Modified ASO targeting SRB-1

ASO	Sequence (5' to 3')	Motif	Conjugate	SEQ ID No.
ISIS 353382 (parent)	G ^{es} C ^{es} T ^{es} T ^{es} C ^{es} A ^{ds} G ^{ds} T ^{ds} C ^{ds} A ^{ds} T ^{ds} G ^{ds} A ^{ds} C ^{ds} T ^{ds} T ^{es} C ^{es} C ^{es} T ^{es} T ^e	5/10/5	no conjugate	252
ISIS 655861	G ^{es} C ^{es} T ^{es} T ^{es} C ^{es} A ^{ds} G ^{ds} T ^{ds} C ^{ds} A ^{ds} T ^{ds} G ^{ds} A ^{ds} C ^{ds} T ^{ds} T ^{es} C ^{es} C ^{es} T ^{es} T ^{eo} A ^{do} GalNac ₃ -1 _a	5/10/5	GalNac ₃ -1	253
ISIS 664507	GalNac ₃ -2 _a -o'-A ^{do} G ^{es} C ^{es} T ^{es} T ^{es} C ^{es} A ^{ds} G ^{ds} T ^{ds} C ^{ds} A ^{ds} T ^{ds} G ^{ds} A ^{ds} C ^{ds} T ^{ds} T ^{es} C ^{es} C ^{es} T ^{es} T ^e	5/10/5	GalNac ₃ -2	254
ISIS 661161	GalNac ₃ -3 _a -o'-A ^{do} G ^{es} C ^{es} T ^{es} T ^{es} C ^{es} A ^{ds} G ^{ds} T ^{ds} C ^{ds} A ^{ds} T ^{ds} G ^{ds} A ^{ds} C ^{ds} T ^{ds} T ^{es} C ^{es} C ^{es} T ^{es} T ^e	5/10/5	GalNac ₃ -3	254
ISIS 666224	GalNac ₃ -5 _a -o'-A ^{do} G ^{es} C ^{es} T ^{es} T ^{es} C ^{es} A ^{ds} G ^{ds} T ^{ds} C ^{ds} A ^{ds} T ^{ds} G ^{ds} A ^{ds} C ^{ds} T ^{ds} T ^{es} C ^{es} C ^{es} T ^{es} T ^e	5/10/5	GalNac ₃ -5	254

	^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{es} ^m C _{es} ^m C _{es} T _{es} T _e			
ISIS 666961	GalNAc₃-6_a-o'-A_{do} G _{es} ^m C _{es} T _{es} T _{es} ^m C _{es} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{es} ^m C _{es} ^m C _{es} T _{es} T _e	5/10/5	GalNAc₃-6	254
ISIS 666981	GalNAc₃-7_a-o'-A_{do} G _{es} ^m C _{es} T _{es} T _{es} ^m C _{es} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{es} ^m C _{es} ^m C _{es} T _{es} T _e	5/10/5	GalNAc₃-7	254
ISIS 666881	GalNAc₃-10_a-o'-A_{do} G _{es} ^m C _{es} T _{es} T _{es} ^m C _{es} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{es} ^m C _{es} ^m C _{es} T _{es} T _e	5/10/5	GalNAc₃-10	254

Capital letters indicate the nucleobase for each nucleoside and ^mC indicates a 5-methyl cytosine. Subscripts: “e” indicates a 2'-MOE modified nucleoside; “d” indicates a β-D-2'-deoxyribonucleoside; “s” indicates a phosphorothioate internucleoside linkage (PS); “o” indicates a phosphodiester internucleoside linkage (PO); and “o'” indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

The structure of GalNAc₃-1_a was shown previously in Example 9. The structure of GalNAc₃-2_a was shown previously in Example 37. The structure of GalNAc₃-3_a was shown previously in Example 39. The structure of GalNAc₃-5_a was shown previously in Example 49. The structure of GalNAc₃-6_a was shown previously in Example 51. The structure of GalNAc₃-7_a was shown previously in Example 48. The structure of GalNAc₃-10_a was shown previously in Example 46.

Treatment

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with ISIS 353382, 655861, 664507, 661161, 666224, 666961, 666981, 666881 or with saline. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the liver SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to the saline control.

As illustrated in Table 43, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner. Indeed, the conjugated antisense oligonucleotides showed substantial improvement in potency compared to the unconjugated antisense oligonucleotide (ISIS 353382). The 5' conjugated antisense oligonucleotides showed a slight increase in potency compared to the 3' conjugated antisense oligonucleotide.

Table 43

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA (% Saline)	Conjugate
Saline	n/a	100.0	
353382	3	96.0	none
	10	73.1	
	30	36.1	
655861	0.5	99.4	GalNAc₃-1 (3')

	1.5	81.2	
	5	33.9	
	15	15.2	
664507	0.5	102.0	GalNac₃-2 (5')
	1.5	73.2	
	5	31.3	
	15	10.8	
661161	0.5	90.7	GalNac₃-3 (5')
	1.5	67.6	
	5	24.3	
	15	11.5	
666224	0.5	96.1	GalNac₃-5 (5')
	1.5	61.6	
	5	25.6	
	15	11.7	
666961	0.5	85.5	GalNac₃-6 (5')
	1.5	56.3	
	5	34.2	
	15	13.1	
666981	0.5	84.7	GalNac₃-7 (5')
	1.5	59.9	
	5	24.9	
	15	8.5	
666881	0.5	100.0	GalNac₃-10 (5')
	1.5	65.8	
	5	26.0	
	15	13.0	

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Total bilirubin and BUN were also evaluated. The change in body weights was evaluated with no significant change from the saline group.

5 ALTs, ASTs, total bilirubin and BUN values are shown in Table 44 below.

Table 44

ISIS No.	Dosage mg/kg	ALT	AST	Total Bilirubin	BUN	Conjugate
Saline		26	57	0.2	27	
353382	3	25	92	0.2	27	none
	10	23	40	0.2	25	
	30	29	54	0.1	28	
655861	0.5	25	71	0.2	34	GalNac₃-1 (3')
	1.5	28	60	0.2	26	
	5	26	63	0.2	28	
	15	25	61	0.2	28	
664507	0.5	25	62	0.2	25	GalNac₃-2 (5')
	1.5	24	49	0.2	26	
	5	21	50	0.2	26	
	15	59	84	0.1	22	

661161	0.5	20	42	0.2	29	GalNac ₃ -3 (5')
	1.5 g	37	74	0.2	25	
	5 g	28	61	0.2	29	
	15	21	41	0.2	25	
666224	0.5	34	48	0.2	21	GalNac ₃ -5 (5')
	1.5	23	46	0.2	26	
	5	24	47	0.2	23	
	15	32	49	0.1	26	
666961	0.5	17	63	0.2	26	GalNac ₃ -6 (5')
	1.5	23	68	0.2	26	
	5	25	66	0.2	26	
	15	29	107	0.2	28	
666981	0.5	24	48	0.2	26	GalNac ₃ -7 (5')
	1.5	30	55	0.2	24	
	5	46	74	0.1	24	
	15	29	58	0.1	26	
666881	0.5	20	65	0.2	27	GalNac ₃ -10 (5')
	1.5	23	59	0.2	24	
	5	45	70	0.2	26	
	15	21	57	0.2	24	

Example 57: Duration of action study of oligonucleotides comprising a 3'-conjugate group targeting ApoC III *in vivo*

Mice were injected once with the doses indicated below and monitored over the course of 42 days for ApoC-III and plasma triglycerides (Plasma TG) levels. The study was performed using 3 transgenic mice that express human APOC-III in each group.

Table 45
Modified ASO targeting ApoC III

ASO	Sequence (5' to 3')	Linkages	SEQ ID No.
ISIS 304801	A _{es} G _{es} ^m C _{es} T _{es} T _{es} ^m C _{ds} T _{ds} T _{ds} G _{ds} T _{ds} ^m C _{ds} ^m C _{ds} A _{ds} G _{ds} ^m C _{ds} T _{es} T _{es} T _{es} A _{es} T _e	PS	244
ISIS 647535	A _{es} G _{es} ^m C _{es} T _{es} T _{es} ^m C _{ds} T _{ds} T _{ds} G _{ds} T _{ds} ^m C _{ds} ^m C _{ds} A _{ds} G _{ds} ^m C _{ds} T _{es} T _{es} T _{es} A _{es} T _{eo} A_{do}'-GalNac₃-1_a	PS	245
ISIS 647536	A _{es} G _{eo} ^m C _{eo} T _{eo} T _{eo} ^m C _{ds} T _{ds} T _{ds} G _{ds} T _{ds} ^m C _{ds} ^m C _{ds} A _{ds} G _{ds} ^m C _{ds} T _{eo} T _{eo} T _{es} A _{es} T _{eo} A_{do}'-GalNac₃-1_a	PO/PS	245

Capital letters indicate the nucleobase for each nucleoside and ^mC indicates a 5-methyl cytosine. Subscripts: "e" indicates a 2'-MOE modified nucleoside; "d" indicates a β-D-2'-deoxyribonucleoside; "s" indicates a phosphorothioate internucleoside linkage (PS); "o" indicates a phosphodiester internucleoside linkage (PO); and "o" indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

The structure of GalNac₃-1_a was shown previously in Example 9.

Table 46

ApoC III mRNA (% Saline on Day 1) and Plasma TG Levels (% Saline on Day 1)

ASO	Dose	Target	Day 3	Day 7	Day 14	Day 35	Day 42
Saline	0 mg/kg	ApoC-III	98	100	100	95	116
ISIS 304801	30 mg/kg	ApoC-III	28	30	41	65	74
ISIS 647535	10 mg/kg	ApoC-III	16	19	25	74	94
ISIS 647536	10 mg/kg	ApoC-III	18	16	17	35	51
Saline	0 mg/kg	Plasma TG	121	130	123	105	109
ISIS 304801	30 mg/kg	Plasma TG	34	37	50	69	69
ISIS 647535	10 mg/kg	Plasma TG	18	14	24	18	71
ISIS 647536	10 mg/kg	Plasma TG	21	19	15	32	35

As can be seen in the table above the duration of action increased with addition of the 3'-conjugate group compared to the unconjugated oligonucleotide. There was a further increase in the duration of action for the conjugated mixed PO/PS oligonucleotide 647536 as compared to the conjugated full PS oligonucleotide 647535.

Example 58: Dose-dependent study of oligonucleotides comprising a 3'-conjugate group (comparison of GalNAc₃-1 and GalNAc₄-11) targeting SRB-1 *in vivo*

The oligonucleotides listed below were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice. Unconjugated ISIS 440762 was included as an unconjugated standard. Each of the conjugate groups were attached at the 3' terminus of the respective oligonucleotide by a phosphodiester linked 2'-deoxyadenosine nucleoside cleavable moiety.

The structure of GalNAc₃-1_a was shown previously in Example 9. The structure of GalNAc₃-11_a was shown previously in Example 50.

Treatment

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with ISIS 440762, 651900, 663748 or with saline. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the liver SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to the saline control.

As illustrated in Table 47, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner. The antisense oligonucleotides comprising the phosphodiester linked GalNAc₃-1 and GalNAc₄-11 conjugates at the 3' terminus (ISIS 651900 and ISIS 663748) showed substantial

improvement in potency compared to the unconjugated antisense oligonucleotide (ISIS 440762). The two conjugated oligonucleotides, GalNac₃-1 and GalNac₄-11, were equipotent.

Table 47
Modified ASO targeting SRB-1

ASO	Sequence (5' to 3')	Dose mg/kg	% Saline control	SEQ ID No.
Saline			100	
ISIS 440762	T _{ks} ^m C _{ks} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{ks} ^m C _k	0.6	73.45	246
		2	59.66	
		6	23.50	
ISIS 651900	T _{ks} ^m C _{ks} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{ks} ^m C _{ko} A_{do}'-GalNac₃-1_a	0.2	62.75	247
		0.6	29.14	
		2	8.61	
		6	5.62	
ISIS 663748	T _{ks} ^m C _{ks} A _{ds} G _{ds} T _{ds} ^m C _{ds} A _{ds} T _{ds} G _{ds} A _{ds} ^m C _{ds} T _{ds} T _{ks} ^m C _{ko} A_{do}'-GalNac₄-11_a	0.2	63.99	247
		0.6	33.53	
		2	7.58	
		6	5.52	

5

Capital letters indicate the nucleobase for each nucleoside and ^mC indicates a 5-methyl cytosine. Subscripts: "e" indicates a 2'-MOE modified nucleoside; "k" indicates 6'-(S)-CH₃ bicyclic nucleoside; "d" indicates a β-D-2'-deoxyribonucleoside; "s" indicates a phosphorothioate internucleoside linkage (PS); "o" indicates a phosphodiester internucleoside linkage (PO); and "o'" indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

10

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Total bilirubin and BUN were also evaluated. The change in body weights was evaluated with no significant change from the saline group. ALTs, ASTs, total bilirubin and BUN values are shown in Table 48 below.

15

Table 48

ISIS No.	Dosage mg/kg	ALT	AST	Total Bilirubin	BUN	Conjugate
Saline		30	76	0.2	40	
440762	0.60	32	70	0.1	35	none
	2	26	57	0.1	35	
	6	31	48	0.1	39	
651900	0.2	32	115	0.2	39	GalNac ₃ -1 (3')
	0.6	33	61	0.1	35	
	2	30	50	0.1	37	
	6	34	52	0.1	36	
663748	0.2	28	56	0.2	36	GalNac ₄ -11 (3')
	0.6	34	60	0.1	35	
	2	44	62	0.1	36	

	6	38	71	0.1	33	
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Example 59: Effects of GalNAc₃-1 conjugated ASOs targeting FXI *in vivo*

The oligonucleotides listed below were tested in a multiple dose study for antisense inhibition of FXI in mice. ISIS 404071 was included as an unconjugated standard. Each of the conjugate groups was attached at the 3' terminus of the respective oligonucleotide by a phosphodiester linked 2'-deoxyadenosine nucleoside cleavable moiety.

Table 49
Modified ASOs targeting FXI

ASO	Sequence (5' to 3')	Linkages	SEQ ID No.
ISIS 404071	T _{es} G _{es} G _{es} T _{es} A _{es} A _{ds} T _{ds} ^m C _{ds} ^m C _{ds} A _{ds} ^m C _{ds} T _{ds} T _{ds} T _{ds} ^m C _{ds} A _{es} G _{es} A _{es} G _{es} G _e	PS	255
ISIS 656172	T _{es} G _{es} G _{es} T _{es} A _{es} A _{ds} T _{ds} ^m C _{ds} ^m C _{ds} A _{ds} ^m C _{ds} T _{ds} T _{ds} T _{ds} ^m C _{ds} A _{es} G _{es} A _{es} G _{es} G _{eo} A_{do}'-GalNAc₃-1_a	PS	256
ISIS 656173	T _{es} G _{eo} G _{eo} T _{eo} A _{eo} A _{ds} T _{ds} ^m C _{ds} ^m C _{ds} A _{ds} ^m C _{ds} T _{ds} T _{ds} T _{ds} ^m C _{ds} A _{eo} G _{eo} A _{es} G _{es} G _{eo} A_{do}'-GalNAc₃-1_a	PO/PS	256

Capital letters indicate the nucleobase for each nucleoside and ^mC indicates a 5-methyl cytosine. Subscripts: “e” indicates a 2'-MOE modified nucleoside; “d” indicates a β-D-2'-deoxyribonucleoside; “s” indicates a phosphorothioate internucleoside linkage (PS); “o” indicates a phosphodiester internucleoside linkage (PO); and “o'” indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

The structure of GalNAc₃-1_a was shown previously in Example 9.

Treatment

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously twice a week for 3 weeks at the dosage shown below with ISIS 404071, 656172, 656173 or with PBS treated control. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the liver FXI mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. Plasma FXI protein levels were also measured using ELISA. FXI mRNA levels were determined relative to total RNA (using RIBOGREEN®), prior to normalization to PBS-treated control. The results below are presented as the average percent of FXI mRNA levels for each treatment group. The data was normalized to PBS-treated control and is denoted as “% PBS”. The ED₅₀s were measured using similar methods as described previously and are presented below.

Table 50
Factor XI mRNA (% Saline)

ASO	Dose mg/kg	% Control	Conjugate	Linkages
-----	------------	-----------	-----------	----------

Saline		100	none	
ISIS 404071	3	92	none	PS
	10	40		
	30	15		
ISIS 656172	0.7	74	GalNAc ₃ -1	PS
	2	33		
	6	9		
ISIS 656173	0.7	49	GalNAc ₃ -1	PO/PS
	2	22		
	6	1		

As illustrated in Table 50, treatment with antisense oligonucleotides lowered FXI mRNA levels in a dose-dependent manner. The oligonucleotides comprising a 3'-GalNAc₃-1 conjugate group showed substantial improvement in potency compared to the unconjugated antisense oligonucleotide (ISIS 404071).
 5 Between the two conjugated oligonucleotides an improvement in potency was further provided by substituting some of the PS linkages with PO (ISIS 656173).

As illustrated in Table 50a, treatment with antisense oligonucleotides lowered FXI protein levels in a dose-dependent manner. The oligonucleotides comprising a 3'-GalNAc₃-1 conjugate group showed substantial improvement in potency compared to the unconjugated antisense oligonucleotide (ISIS 404071).
 10 Between the two conjugated oligonucleotides an improvement in potency was further provided by substituting some of the PS linkages with PO (ISIS 656173).

Table 50a
Factor XI protein (% Saline)

ASO	Dose mg/kg	Protein Control	(%)	Conjugate	Linkages
Saline		100		none	
ISIS 404071	3	127		none	PS
	10	32			
	30	3			
ISIS 656172	0.7	70		GalNAc ₃ -1	PS
	2	23			
	6	1			
ISIS 656173	0.7	45		GalNAc ₃ -1	PO/PS
	2	6			
	6	0			

15 Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Total bilirubin, total albumin, CRE and BUN were also evaluated. The change in body weights was evaluated with no significant change from the saline group. ALTs, ASTs, total bilirubin and BUN values are shown in the table below.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 275

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JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 275

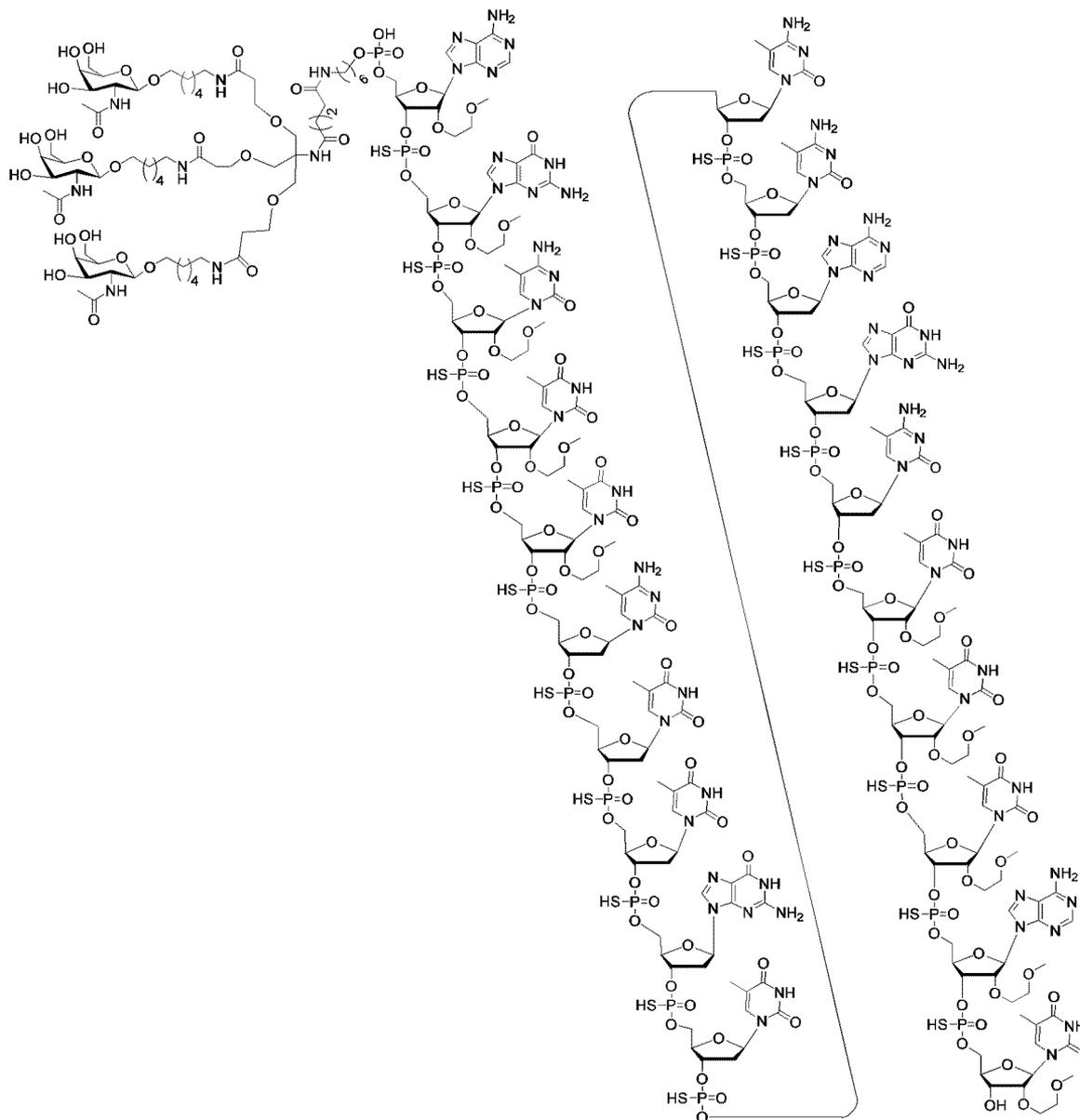
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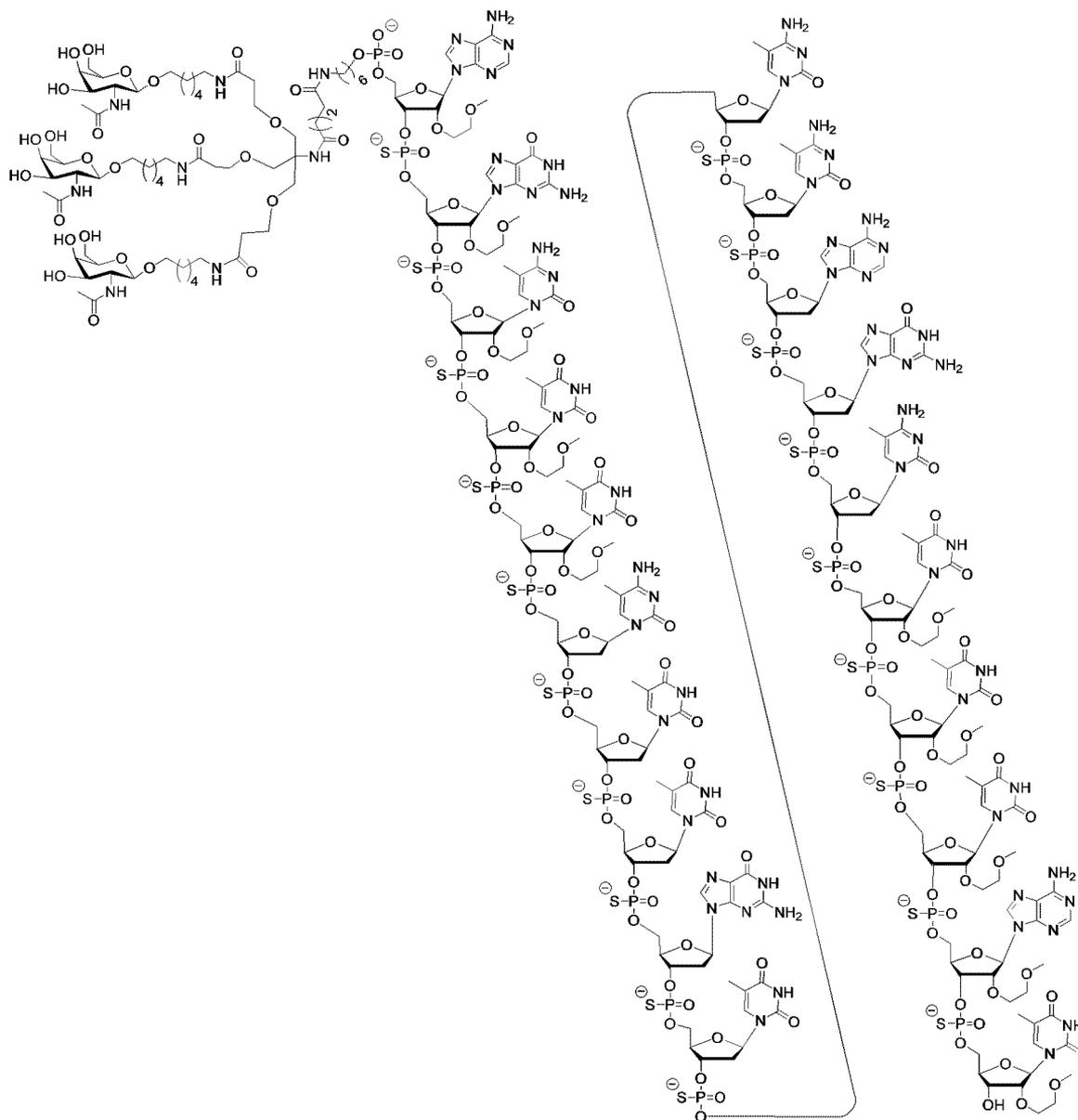
1. An oligomeric compound according to the following chemical structure:



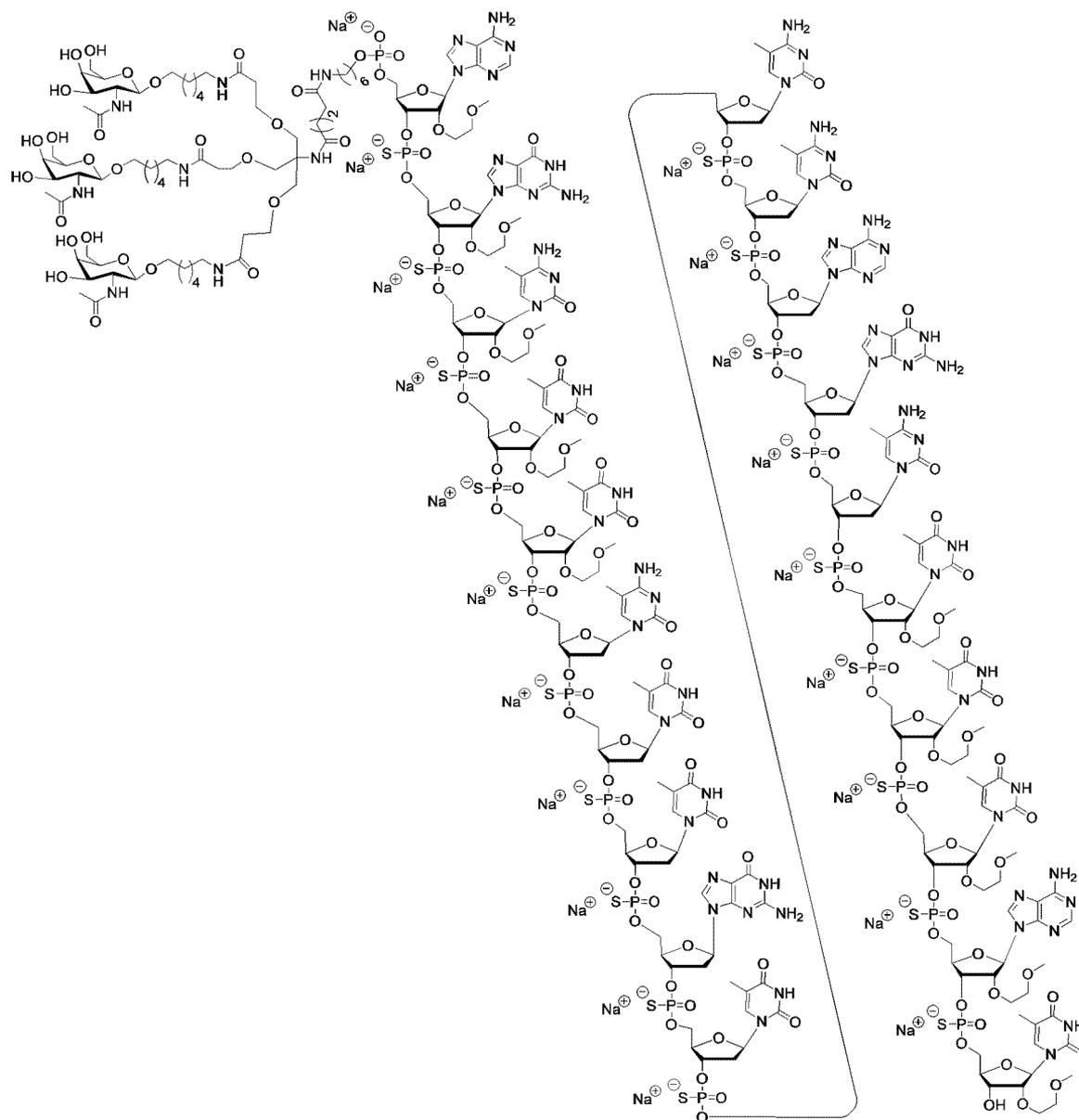
(SEQ ID NO: 87), or a salt thereof.

2. The oligomeric compound of claim 2, which is the sodium salt or the potassium salt.

3. An oligomeric compound, wherein the anion form of the oligomeric compound has the following chemical structure:



4. An oligomeric compound according to the following chemical structure:



(SEQ ID NO: 87).

5. An oligomeric compound comprising a modified oligonucleotide according to the following formula: $A_{es}G_{es}{}^mC_{es}T_{es}T_{es}{}^mC_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^mC_{ds}{}^mC_{ds}A_{ds}G_{ds}{}^mC_{ds}T_{es}T_{es}T_{es}A_{es}T_e$

(SEQ ID NO: 87); wherein,

A = an adenine nucleobase,

mC = a 5-methylcytosine nucleobase,

G = a guanine nucleobase,

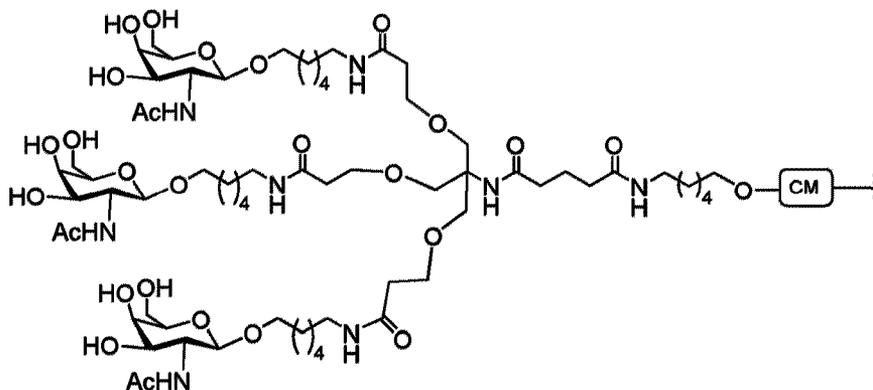
T = a thymine nucleobase,

$_e$ = a 2'-O-methoxyethyl modified sugar moiety,

$_d$ = a 2'-deoxy sugar moiety, and

$_s$ = a phosphorothioate internucleoside linkage,

wherein the compound further comprises a conjugate attached at the 5' terminal nucleoside of the oligonucleotide, wherein the conjugate has the formula:



wherein the modified oligonucleotide is linked to the conjugate via a cleavable moiety (CM), and wherein the cleavable moiety is 5'-P(OH)(=O)-O-3'.

6. A pharmaceutical composition comprising the oligomeric compound of any one of claims 1-5 and a pharmaceutically acceptable diluent.

7. The pharmaceutical composition of claim 6, wherein the pharmaceutically acceptable diluent is PBS.

8. The pharmaceutical composition of claim 7, wherein the pharmaceutical composition consists essentially of the oligomeric compound and PBS.