

LIGAND COMPOUNDS, CONJUGATES, AND APPLICATIONS THEREOF

TECHNICAL FIELD

This disclosure relates to the technological area of nucleic acid delivery. Ligand
5 compounds, oligonucleotide conjugates, and methods of making and using the same are disclosed.

BACKGROUND

Asialoglycoprotein receptor (ASGPR) is an abundant endocytic receptor of
10 hetero-oligomers, which exists mainly on the surface of the cell membrane of liver parenchymal cells facing the side of sinusoidal space and has specificity for sugar. As the terminal sialic acid of the glycoproteins is removed through hydrolysis by enzymes or acidolysis, the exposed penultimates are galactose residues. Therefore, the sugar-binding specificity of ASGPR is actually galactosyl, and it is also called galactose-specific
15 receptor. ASGPRs are mainly distributed in the liver parenchymal cells, and low in content in other cells. As such, the ASGPRs provide possible receptors for liver targeted transport.

Glycoproteins terminated with non-reducing galactose (Gal) or *N*-acetylgalactosamine (GalNAc) residues can be recognized by ASGPRs, wherein the
20 affinity of GalNAc to ASGPR is about 50 times higher than that of Gal (Iobst ST *et al*, J Biol Chem. 1996, 271 (12) 6686-6693). *In vitro* experiments show that the affinity of clustered sugar residues is much higher than that of non-clustered sugar residues by simultaneously occupying the binding sites of the receptor with an affinity order of tetraantennary > triantennary > > biantennary > > monoantennary galactosides (Lee Y C, *et al*,
25 J Biol Chem, 1983, 258 (1): 199-202).

ASGPR receptor-mediated liver targeting oligonucleotide is a new breakthrough in the research field of nucleic acid innovative drugs. In 2012, Alnylam Pharmaceuticals Inc. covalently linked triantennary GalNAc structure previously studied with small

interfering RNA (siRNA) to achieve liver-targeted delivery of siRNA *in vivo*. Using this technology, researchers have developed drugs for amyloidosis, hemophilia, hypercholesterolemia, liver porphyrin, hepatitis B and other diseases. In 2019, the first GalNAc-siRNA drug was approved while two additional drugs are seeking approval.

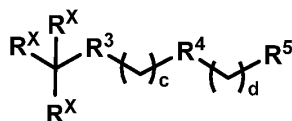
5 Over ten drug candidates have entered into clinical studies (<http://www.alnylam.com/product-pipeline/>). In 2014, ISIS Pharmaceuticals of the United States covalently linked triantennary GalNAc and antisense nucleic acid to achieve liver-targeted drug delivery in animals, with 10-fold increase in antisense nucleic acid activity after linking (Prakash, T. P. *et al*, Nucleic Acids Res. 42, 8796-807).

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SUMMARY

This disclosure features chemical entities (e.g., a compound or a pharmaceutically acceptable salt, and/or hydrate, and/or cocrystal, and/or drug combination of the compound) that comprise one or more ligand moieties for an asialoglycoprotein receptor (ASGPR). Exemplary chemical entities can further comprise an oligonucleotide. Said
 15 chemical entities are useful, e.g., in the targeted delivery of oligonucleotides to liver cells (e.g., liver parenchymal cells). The chemical entities are useful e.g., in the treatment of conditions or diseases caused by the expression (e.g., abnormal expression) of one or more genes in liver cells. This disclosure also features compositions containing the
 20 same as well as methods of using and making the same.

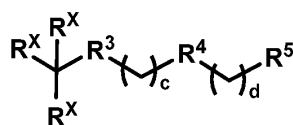
In one aspect, this disclosure features compounds of Formula (I):



Formula (I)

25 or a pharmaceutically acceptable salt thereof, wherein: R^{X} , R^3 , c , R^4 , d , and R^5 can be as defined anywhere herein.

In some embodiments, the compound of Formula (I) is a conjugate compound of Formula (II):



Formula (II)

5 or a pharmaceutically acceptable salt thereof, wherein:

R^5 is $\text{---}^L\text{---Oligo}$, wherein **Oligo** is an oligonucleotide that is attached to **L** via the 5'-end, 3'-end, or sequence middle of any strand via a phosphate group; and **-L-**, **R^x**, **R³**, **c**, **R⁴**, and **d** can be as defined for Formula (I) anywhere herein.

10 In another aspect, provided herein are pharmaceutical compositions comprising a chemical entity as described herein (e.g., a compound of Formula (II) or a pharmaceutically acceptable salt thereof) and a pharmaceutically acceptable excipient.

15 In another aspect, provided herein are methods for treating and/or preventing pathological conditions or diseases in a subject, wherein the conditions or diseases are caused by the expression of one or more genes in liver cells, the method comprising administering to the subject a chemical entity as described herein (e.g., a compound of Formula (II) or a pharmaceutically acceptable salt thereof); or a
20 pharmaceutical composition comprising a chemical entity as described herein (e.g., a compound of Formula (II) or a pharmaceutically acceptable salt thereof), and a pharmaceutically acceptable excipient.

In a further aspect, provided herein are methods for detecting or localizing RNA in the liver of a subject, comprising administering to the subject a chemical
25 entity as described herein (e.g., a compound of Formula (II) or a pharmaceutically acceptable salt thereof); or a pharmaceutical composition comprising a chemical

entity as described herein (e.g., a compound of Formula (II) or a pharmaceutically acceptable salt thereof), and a pharmaceutically acceptable excipient.

Other embodiments include those described in the Detailed Description and/or in
5 the claims.

Additional Definitions

To facilitate understanding of the disclosure set forth herein, a number of additional terms are defined below. Generally, the nomenclature used herein and the
10 laboratory procedures in organic chemistry, medicinal chemistry, and pharmacology described herein are those well-known and commonly employed in the art. Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Each of the patents, applications, published applications, and other
15 publications that are mentioned throughout the specification and the attached appendices are incorporated herein by reference in their entireties.

As used herein, the term "oligonucleotide" refers to an oligomeric compound containing a plurality of linked chemically modified or unmodified nucleotides having a length of less than about 100 nucleotides, such as, e.g., 1-20 nucleotides, 20-40
20 nucleotides, 40-60 nucleotides, 60-80 nucleotides, 80-100 nucleotides, or 1-50 nucleotides. In certain embodiments, the oligonucleotide can include a non-nucleic acid conjugate group. In certain embodiments, the oligonucleotide comprises ribonucleic acid (RNA), deoxyribonucleic acid (DNA), or peptide nucleic acid (PNA). In certain embodiments, the oligonucleotide is double-stranded or single-stranded. In certain
25 embodiments, the oligonucleotide is an siRNA, an aptamer, an antisense nucleic acid, an sgRNA, a tractRNA, or crRNA.

As used herein, the term "conjugate" or "conjugate group" means an atom or atomic group bound to an oligonucleotide. In some cases, the conjugate groups alter one or more properties of the oligonucleotide to which they are linked, including but not

limited to pharmacodynamics, pharmacokinetics, binding, absorption, cell distribution, cell uptake, charge, and/or clearance properties.

As used herein, the term "receptor" refers to a biological macromolecule composed of glycoproteins or lipoproteins, present in the cell membrane, cytoplasm, or nucleus of a cell, with different receptors having specific structures and configurations. As used herein, the term "ligand" refers to a substance that has the ability to recognize and bind to a receptor. In certain embodiments, the ligand is a ligand having affinity for an asialoglycoprotein receptor (ASGPR). In certain embodiments, the ligand is a carbohydrate, such as monosaccharides and polysaccharides, including but not limited to: galactose, *N*-acetylgalactosamine, mannose, glucose, glucosamine and fucose.

As used herein, the term "polysaccharide" refers to a polymer formed from a plurality of monosaccharide groups linked by glycosidic linkages. In the present disclosure, polysaccharides include oligoses and oligosaccharides. Generally, "oligose" refers to a polymer composed of 2-10 monosaccharide groups linked by glycosidic bonds, and "oligosaccharide" refers to a polymer composed of fewer than 20 monosaccharide groups linked by glycosidic bonds.

As used herein, the term "about" should be understood by those skilled in the art and will vary to some extent depending on the context in which it is used. If, depending on the context in which the term is used, its meaning is not clear to those skilled in the art, then the meaning of "about" is such that the deviation does not exceed plus or minus 10% of the specified value or range.

As used herein, the term "preventing" refers to preventing or delaying the onset of a disease.

As used herein, the term "treating" refers to curing or at least partially arresting the progression of a disease, or alleviating the symptoms of a disease.

As used herein, the term "effective amount" refers to an amount effective to achieve the intended purpose. For example, an amount effective to prevent a disease refers to an amount effective to prevent, arrest, or delay the onset of the disease. Determination of such effective amounts is within the ability of one skilled in the art.

The term “acceptable” with respect to a formulation, composition or ingredient, as used herein, means having no persistent detrimental effect on the general health of the subject being treated.

“API” refers to an active pharmaceutical ingredient.

5 The term “excipient” or “pharmaceutically acceptable excipient” means a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, carrier, solvent, or encapsulating material. In one embodiment, each component is “pharmaceutically acceptable” in the sense of being compatible with the other ingredients of a pharmaceutical formulation, and suitable for use in contact with the
10 tissue or organ of humans and animals without excessive toxicity, irritation, allergic response, immunogenicity, or other problems or complications, commensurate with a reasonable benefit/risk ratio. *See, e.g., Remington: The Science and Practice of Pharmacy, 21st ed.*; Lippincott Williams & Wilkins: Philadelphia, PA, 2005; *Handbook of Pharmaceutical Excipients, 6th ed.*; Rowe *et al.*, Eds.; The Pharmaceutical Press and the
15 American Pharmaceutical Association: 2009; *Handbook of Pharmaceutical Additives, 3rd ed.*; Ash and Ash Eds.; Gower Publishing Company: 2007; *Pharmaceutical Preformulation and Formulation, 2nd ed.*; Gibson Ed.; CRC Press LLC: Boca Raton, FL, 2009.

 The term “pharmaceutically acceptable salt” refers to a formulation of a
20 compound that does not cause significant irritation to an organism to which it is administered and does not abrogate the biological activity and properties of the compound. In certain instances, pharmaceutically acceptable salts are obtained by reacting a compound described herein, with acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid,
25 p-toluenesulfonic acid, salicylic acid and the like. In some instances, pharmaceutically acceptable salts are obtained by reacting a compound having acidic group described herein with a base to form a salt such as an ammonium salt, an alkali metal salt, such as a sodium or a potassium salt, an alkaline earth metal salt, such as a calcium or a magnesium salt, a salt of organic bases such as dicyclohexylamine, *N*-methyl-D-
30 glucamine, tris(hydroxymethyl)methylamine, and salts with amino acids such as arginine,

lysine, and the like, or by other methods previously determined. The pharmacologically acceptable salt is not specifically limited as far as it can be used in medicaments. Examples of a salt that the compounds described herein form with a base include the following: salts thereof with inorganic bases such as sodium, potassium, magnesium, calcium, and aluminum; salts thereof with organic bases such as methylamine, ethylamine and ethanolamine; salts thereof with basic amino acids such as lysine and ornithine; and ammonium salt. The salts may be acid addition salts, which are specifically exemplified by acid addition salts with the following: mineral acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, nitric acid, and phosphoric acid; organic acids such as formic acid, acetic acid, propionic acid, oxalic acid, malonic acid, succinic acid, fumaric acid, maleic acid, lactic acid, malic acid, tartaric acid, citric acid, methanesulfonic acid, and ethanesulfonic acid; acidic amino acids such as aspartic acid and glutamic acid.

The term “pharmaceutical composition” refers to a mixture of a compound described herein with other chemical components (referred to collectively herein as “excipients”), such as carriers, stabilizers, diluents, dispersing agents, suspending agents, and/or thickening agents. The pharmaceutical composition facilitates administration of the compound to an organism. Multiple techniques of administering a compound exist in the art including, but not limited to: rectal, oral, intravenous, aerosol, parenteral, ophthalmic, pulmonary, and topical administration.

The term “subject” refers to an animal, including, but not limited to, a primate (*e.g.*, human), monkey, cow, pig, sheep, goat, horse, dog, cat, rabbit, rat, or mouse. The terms “subject” and “patient” are used interchangeably herein in reference, for example, to a mammalian subject, such as a human.

The term “gene-related disease” refers to a disease that results from an abnormal expression of one or more genes and/or an abnormal activity of proteins expressed by these genes. Similarly, these genes are known as disease-related genes.

The term “halo” refers to fluoro (F), chloro (Cl), bromo (Br), or iodo (I).

The term "alkyl" refers to a hydrocarbon chain that may be a straight chain or branched chain, containing the indicated number of carbon atoms. For example, C₁₋₁₀ indicates that the group may have from 1 to 10 (inclusive) carbon atoms in it. Non-limiting examples include methyl, ethyl, *iso*-propyl, *tert*-butyl, *n*-hexyl.

5 The term "haloalkyl" refers to an alkyl, in which one or more hydrogen atoms is/are replaced with an independently selected halo (e.g., -CF₃).

The term "alkoxy" refers to an -O-alkyl radical (e.g., -OCH₃).

The term "alkylene" refers to a divalent alkyl (e.g., -CH₂-).

10 The term "alkenyl" refers to a hydrocarbon chain that may be a straight chain or branched chain having one or more carbon-carbon double bonds. The alkenyl moiety contains the indicated number of carbon atoms. For example, C₂₋₆ indicates that the group may have from 2 to 6 (inclusive) carbon atoms in it.

15 The term "alkynyl" refers to a hydrocarbon chain that may be a straight chain or branched chain having one or more carbon-carbon triple bonds. The alkynyl moiety contains the indicated number of carbon atoms. For example, C₂₋₆ indicates that the group may have from 2 to 6 (inclusive) carbon atoms in it.

20 The term "aryl" refers to a 6-20 carbon mono-, bi-, tri- or polycyclic group wherein at least one ring in the system is aromatic (e.g., 6-carbon monocyclic, 10-carbon bicyclic, or 14-carbon tricyclic aromatic ring system); and wherein 0, 1, 2, 3, or 4 atoms of each ring may be substituted by a substituent. Examples of aryl groups include phenyl, naphthyl, tetrahydronaphthyl, and the like.

25 The term "cycloalkyl" as used herein includes non-aromatic cyclic hydrocarbon groups having 3 to 20 ring carbons, preferably 3 to 16 ring carbons, and more preferably 3 to 12 ring carbons or 3-10 ring carbons or 3-6 ring carbons, wherein the cycloalkyl group may be optionally substituted. Cycloalkyl groups may have any degree of saturation provided that none of the rings in the ring system are aromatic. Accordingly, cycloalkyl can be fully saturated. Non-limiting examples include: cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Cycloalkyl can also include partially unsaturated cyclic hydrocarbon groups having 3 to 20 ring carbons, preferably 3 to 16 ring carbons, and more preferably 3 to 12 ring carbons or 3-10 ring carbons or 3-6

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ring carbons. Non-limiting examples can include cyclopentenyl, cyclohexenyl, cycloheptenyl, and cyclooctenyl. Cycloalkyl may include multiple fused and/or bridged rings. Non-limiting examples of fused/bridged cycloalkyl includes: bicyclo[1.1.0]butane, bicyclo[2.1.0]pentane, bicyclo[1.1.1]pentane, bicyclo[3.1.0]hexane, bicyclo[2.1.1]hexane, bicyclo[3.2.0]heptane, bicyclo[4.1.0]heptane, bicyclo[2.2.1]heptane, bicyclo[3.1.1]heptane, bicyclo[4.2.0]octane, bicyclo[3.2.1]octane, bicyclo[2.2.2]octane, and the like. Cycloalkyl also includes spirocyclic rings (e.g., spirocyclic bicycle wherein two rings are connected through just one atom). Non-limiting examples of spirocyclic cycloalkyls include spiro[2.2]pentane, spiro[2.5]octane, spiro[3.5]nonane, spiro[3.5]nonane, spiro[3.5]nonane, spiro[4.4]nonane, spiro[2.6]nonane, spiro[4.5]decane, spiro[3.6]decane, spiro[5.5]undecane, and the like.

The term “heteroaryl”, as used herein, means a mono-, bi-, tri- or polycyclic group having 5 to 20 ring atoms, alternatively 5, 6, 9, 10, or 14 ring atoms; and having 6, 10, or 14 pi electrons shared in a cyclic array; wherein at least one ring in the system is aromatic (but does not have to be a ring which contains a heteroatom, e.g. tetrahydroisoquinoliny, e.g., tetrahydroquinoliny), and at least one ring in the system contains one or more heteroatoms independently selected from the group consisting of N, O, and S. Heteroaryl groups can either be unsubstituted or substituted with one or more substituents. Examples of heteroaryl include thienyl, pyridinyl, furyl, oxazolyl, oxadiazolyl, pyrrolyl, imidazolyl, triazolyl, thiodiazolyl, pyrazolyl, isoxazolyl, thiadiazolyl, pyranlyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, thiazolyl, benzothienyl, benzoxadiazolyl, benzofuranyl, benzimidazolyl, benzotriazolyl, cinnoliny, indazolyl, indolyl, isoquinoliny, isothiazolyl, naphthyridinyl, purinyl, thienopyridinyl, pyrido[2,3-*d*]pyrimidinyl, pyrrolo[2,3-*b*]pyridinyl, quinazoliny, quinoliny, thieno[2,3-*c*]pyridinyl, pyrazolo[3,4-*b*]pyridinyl, pyrazolo[3,4-*c*]pyridinyl, pyrazolo[4,3-*c*]pyridine, pyrazolo[4,3-*b*]pyridinyl, tetrazolyl, chromane, 2,3-dihydrobenzo[*b*][1,4]dioxine, benzo[*d*][1,3]dioxole, 2,3-dihydrobenzofuran, tetrahydroquinoline, 2,3-dihydrobenzo[*b*][1,4]oxathiine, isoindoline, and others. In some embodiments, the

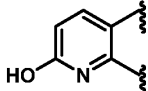
heteroaryl is selected from thienyl, pyridinyl, furyl, pyrazolyl, imidazolyl, isoindolinyl, pyranyl, pyrazinyl, and pyrimidinyl.

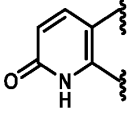
The term "heterocyclyl" refers to a mon-, bi-, tri-, or polycyclic nonaromatic ring system with 3-16 ring atoms (e.g., 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system) having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic or polycyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively), wherein 0, 1, 2 or 3 atoms of each ring may be substituted by a substituent. Examples of heterocyclyl groups include piperazinyl, pyrrolidinyl, dioxanyl, morpholinyl, tetrahydrofuranyl, and the like. Heterocyclyl may include multiple fused and bridged rings. Non-limiting examples of fused/bridged heterocyclyl includes: 2-azabicyclo[1.1.0]butane, 2-azabicyclo[2.1.0]pentane, 2-azabicyclo[1.1.1]pentane, 3-azabicyclo[3.1.0]hexane, 5-azabicyclo[2.1.1]hexane, 3-azabicyclo[3.2.0]heptane, octahydrocyclopenta[c]pyrrole, 3-azabicyclo[4.1.0]heptane, 7-azabicyclo[2.2.1]heptane, 6-azabicyclo[3.1.1]heptane, 7-azabicyclo[4.2.0]octane, 2-azabicyclo[2.2.2]octane, 3-azabicyclo[3.2.1]octane, 2-oxabicyclo[1.1.0]butane, 2-oxabicyclo[2.1.0]pentane, 2-oxabicyclo[1.1.1]pentane, 3-oxabicyclo[3.1.0]hexane, 5-oxabicyclo[2.1.1]hexane, 3-oxabicyclo[3.2.0]heptane, 3-oxabicyclo[4.1.0]heptane, 7-oxabicyclo[2.2.1]heptane, 6-oxabicyclo[3.1.1]heptane, 7-oxabicyclo[4.2.0]octane, 2-oxabicyclo[2.2.2]octane, 3-oxabicyclo[3.2.1]octane, and the like. Heterocyclyl also includes spirocyclic rings (e.g., spirocyclic bicycle wherein two rings are connected through just one atom). Non-limiting examples of spirocyclic heterocyclyls include 2-azaspiro[2.2]pentane, 4-azaspiro[2.5]octane, 1-azaspiro[3.5]nonane, 2-azaspiro[3.5]nonane, 7-azaspiro[3.5]nonane, 2-azaspiro[4.4]nonane, 6-azaspiro[2.6]nonane, 1,7-diazaspiro[4.5]decane, 7-azaspiro[4.5]decane, 2,5-diazaspiro[3.6]decane, 3-azaspiro[5.5]undecane, 2-oxaspiro[2.2]pentane, 4-oxaspiro[2.5]octane, 1-oxaspiro[3.5]nonane, 2-oxaspiro[3.5]nonane, 7-oxaspiro[3.5]nonane, 2-oxaspiro[4.4]nonane, 6-oxaspiro[2.6]nonane, 1,7-dioxaspiro[4.5]decane, 2,5-dioxaspiro[3.6]decane, 1-

oxaspiro[5.5]undecane, 3-oxaspiro[5.5]undecane, 3-oxa-9-azaspiro[5.5]undecane and the like.

In addition, atoms making up the compounds of the present embodiments are intended to include all isotopic forms of such atoms. Isotopes, as used herein, include those atoms having the same atomic number but different mass numbers. By way of
5 general example and without limitation, isotopes of hydrogen include tritium and deuterium, and isotopes of carbon include ^{13}C and ^{14}C .

In addition, the compounds generically or specifically disclosed herein are intended to include all tautomeric forms. Thus, by way of example, a compound

10 containing the moiety:  encompasses the tautomeric form containing the

moiety: . Similarly, a pyridinyl or pyrimidinyl moiety that is described to be optionally substituted with hydroxyl encompasses pyridone or pyrimidone tautomeric forms.

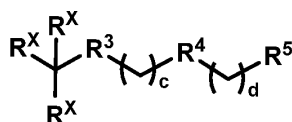
15 The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features and advantages of the invention will be apparent from the description and drawings, and from the claims.

DETAILED DESCRIPTION

This disclosure features chemical entities (e.g., a compound or a pharmaceutically
20 acceptable salt, and/or hydrate, and/or cocrystal, and/or drug combination of the compound) that comprise one or more ligand moieties for an asialoglycoprotein receptor (ASGPR). Exemplary chemical entities can further comprise an oligonucleotide. Said chemical entities are useful, e.g., in the targeted delivery of oligonucleotides to liver cells (e.g., liver parenchymal cells). The chemical entities are useful e.g., in the treatment of
25 conditions or diseases caused by the expression (e.g., abnormal expression) of one or

more genes in liver cells. This disclosure also features compositions containing the same as well as methods of using and making the same.

In one aspect, this disclosure provides compounds of Formula (I):



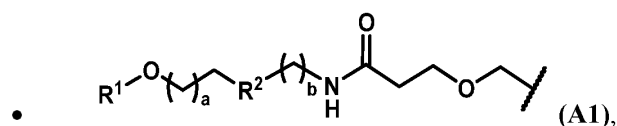
Formula (I)

or a pharmaceutically acceptable salt thereof, wherein:

each $\mathbf{R}^{\mathbf{x}}$ is independently selected from the group consisting of:

- H;
- -CH₂OR^{X2}, wherein R^{X2} is H, C₁₋₆ alkyl, or a hydroxyl protecting group;

10 and



provided that at least one $\mathbf{R}^{\mathbf{x}}$ is a group of Formula (A1);

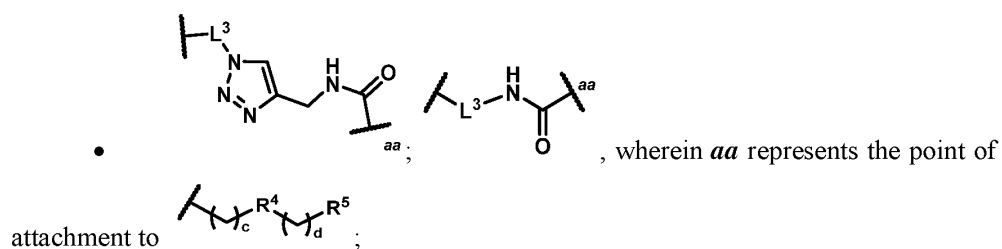
each $\mathbf{R}^1$ is an independently selected moiety capable of binding an
15 asialoglycoproteinreceptor (ASGPR);

each $\mathbf{R}^2$ is independently selected from the group consisting of: $-\text{C}(\mathbf{R}^6)_2-$; $-\text{OC}(\mathbf{R}^6)_2\text{C}(\mathbf{R}^6)_2\text{O}-$; $-\text{C}(\mathbf{R}^6)(\text{OH})-\text{C}(\mathbf{R}^6)(\text{OH})-$; $^*-\text{C}(=\text{O})\text{NR}^7-$; $^*-\text{NR}^7\text{C}(=\text{O})-$; C_{6-10} arylene; C_{2-6} alkenylene; and C_{2-6} alkynylene,

20 wherein the C₆₋₁₀ arylene, C₂₋₆ alkenylene, and C₂₋₆ alkynylene are each optionally substituted with 1-4 independently selected **R^a**, and the * represents the point of

attachment to $R^1-O-(CH_2)_a-$

$\mathbf{R}^3$ is selected from the group consisting of:

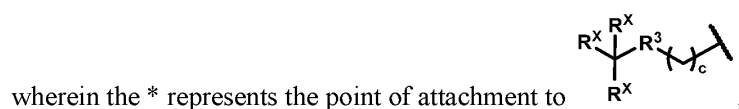


- $-(\text{CR}^6\text{R}^6)_x-\text{O}-(\text{CR}^6\text{R}^6)_y-$, wherein *x* and *y* are independently 0, 1, 2, or 3; and
- $-\text{L}^3-\text{L}^{3\text{C}}-$, wherein $\text{L}^{3\text{C}}$ is selected from the group consisting of: C_{3-10} cycloalkylene, C_{6-10} arylene, 5-10 membered heteroarylene, and 4-10 membered heterocyclylene, each of which is optionally substituted with 1-4 independently selected R^a ;

$-\text{L}^3$ is $-\text{C}(\text{R}^6)_2-$,

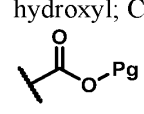
- 10 R^4 is selected from the group consisting of: $-\text{C}(\text{R}^6)_2-$; $-\text{OC}(\text{R}^6)_2\text{C}(\text{R}^6)_2\text{O}-$; $^*\text{-C(=O)NR}^7-$; $^*\text{-NR}^7\text{C(=O)-}$; C_{6-10} arylene, C_{3-10} cycloalkylene, 5-10 membered heteroarylene, and 4-10 membered heterocyclylene,

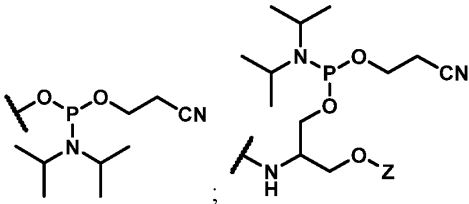
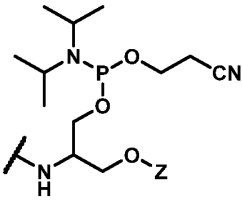
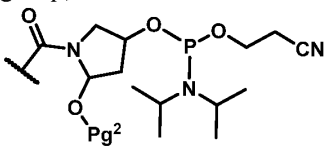
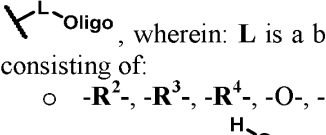
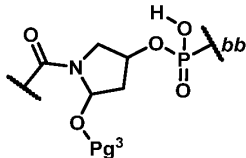
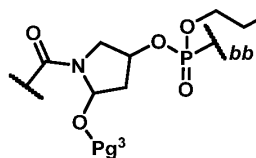
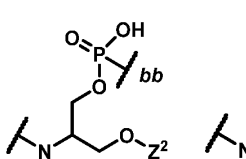
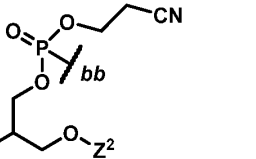
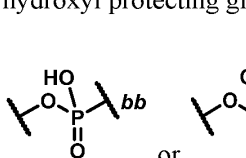
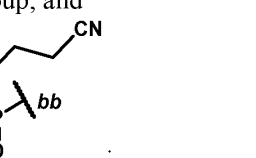
wherein the C_{6-10} arylene, C_{3-10} cycloalkylene, 5-10 membered heteroarylene, and 4-10 membered heterocyclylene are each optionally substituted with 1-4 independently selected R^a , and



wherein the * represents the point of attachment to

R^5 is selected from the group consisting of:

- 20 • hydroxyl; C(O)OH ;
-  wherein **Pg** is a carboxyl activating group or a carboxyl protecting group;

-  ;  wherein **Z** is a hydroxyl protecting group;
-  , wherein **Pg²** is a hydroxyl protecting group;
- and
-  , wherein: **L** is a bond or a divalent group selected from the group consisting of:
 - **-R²-**, **-R³-**, **-R⁴-**, **-O-**, **-C(=O)-**, **-C(=O)O-**, **-OC(=O)-**,
 -  ,  , wherein **Pg³** is H or **Pg²**;
 -  ,  wherein **Z²** is an H or a hydroxyl protecting group; and
 -  or  ;
 - wherein **bb** represents the point of attachment to **Oligo**, and
 - **Oligo** is an oligonucleotide;

each **R⁶** is independently selected from the group consisting of: H; C₁₋₃ alkyl; C₁₋₃ haloalkyl; and halo;

each **R⁷** is independently selected from the group consisting of: H; and C₁₋₃ alkyl.

a and **b** are each independently selected integers from 1 to 10;

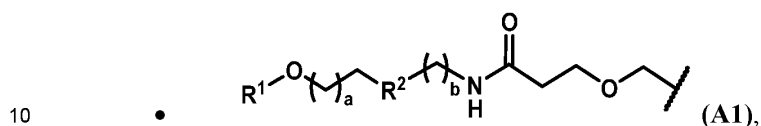
c and **d** are each independently selected integers from 0 to 10; and

each occurrence of **R^a** is independently selected from the group consisting of:
 5 halo, cyano, C₁₋₆ alkyl, C₁₋₆ haloalkyl, C₁₋₆ alkoxy, and C₁₋₆ haloalkoxy.

Variable **R^X**

In some embodiments, each **R^X** is selected from the group consisting of:

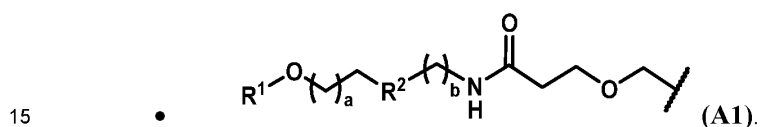
- H; and



In some embodiments, each **R^X** is selected from the group consisting of:

- -CH₂OR^{X2}, wherein **R^{X2}** is H, C₁₋₆ alkyl, or a hydroxyl protecting group;

and



In certain embodiments, the hydroxyl protecting group is selected from the group consisting of: a silyl protecting group; 4-monomethoxytrityl (MMTR); 4,4 - dimethoxytrityl (DMTR); and trityl.

20 In certain of these embodiments, the silyl protecting group is selected from the group consisting of: *tert*-butyldimethylsilyl (TBMDS); *tert*-butyldiphenylsilyl (TBDPS), and triisopropylsilyl (TIPS).

In some embodiments, at least two **R^X** are each independently a group of Formula
 25 (A1).

In certain of these embodiments, each R^X is independently a group of Formula (A1).

In some embodiments, each R^X is a group of Formula (A1); and each R^X is the same.

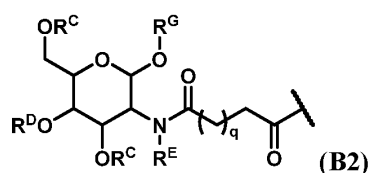
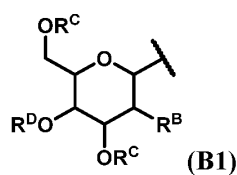
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Variable R^I

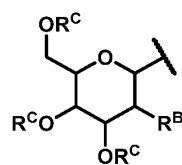
In some embodiments, each R^1 is an independently selected carbohydrate moiety. In certain of these embodiments, each R^1 is an independently monosaccharide or polysaccharide (e.g., monosaccharide or disaccharide). In certain embodiments, each R^1 is selected from the group consisting of: galactose, *N*-acetylgalactosamine, mannose,
10 glucose, glucosamine and fucose. In certain of these embodiments, each R^1 is the same.

In certain embodiments, each R^1 is independently a group of Formula (B1) or (B2):

15



wherein:



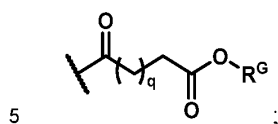
R^D is selected from the group consisting of: R^C and
each R^B is independently selected from the group consisting of: $-NR^E R^F$ and -

20 OR^C ,

each R^C is independently selected from the group consisting of: H and $C(=O)C_{1-6}$ alkyl;

each R^E is independently $C(=O)C_{1-6}$ alkyl;

each R^F is independently selected from the group consisting of: H and

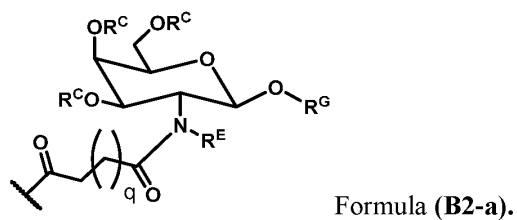
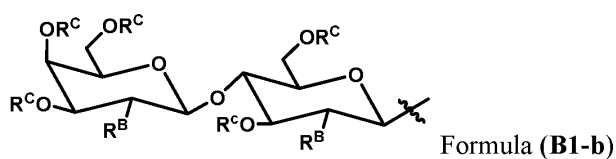
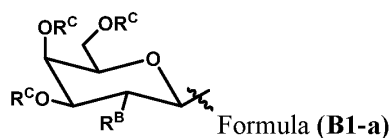


R^G is C_{1-6} alkyl; and

each q is independently an integer selected from 1 to 10.

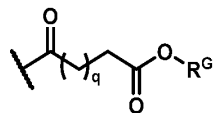
In certain of these embodiments, each R^1 is independently a group having

10 Formula (B1-a), (B1-b), or (B2-a):



15 In certain embodiments of Formula (B1), (B2), (B1-a), (B1-b), or (B2-a), each R^B is independently $NR^E R^F$. In certain of these embodiments, each R^E is independently

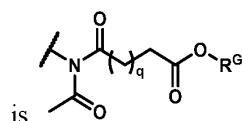
$C(=O)C_{1-6}$ alkyl. For example, each R^E can be $C(=O)CH_3$. In certain embodiments, each



R^F is H. In certain embodiments, each R^F is

In certain embodiments of Formula (B1), (B2), (B1-a), (B1-b), or (B2-a), each R^B is $NHC(=O)CH_3$.

5 In certain embodiments of Formula (B1), (B2), (B1-a), (B1-b), or (B2-a), each R^B



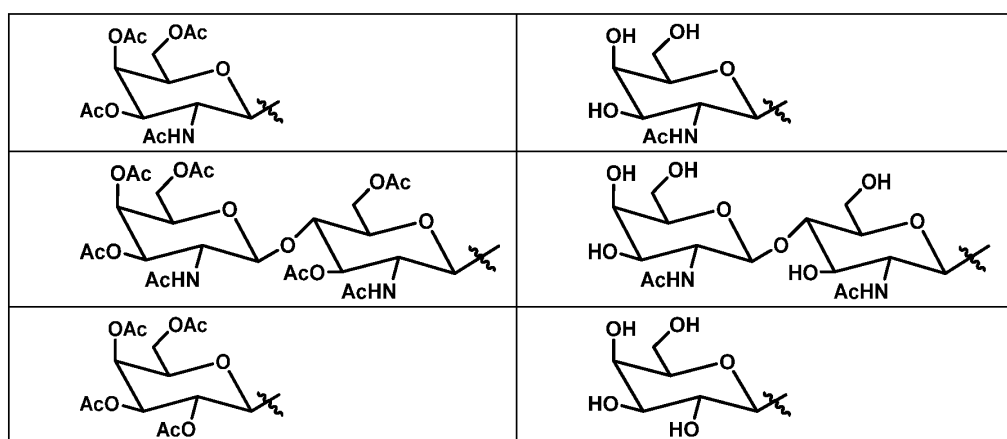
In certain embodiments of Formula (B1), (B2), (B1-a), (B1-b), or (B2-a), each R^B is independently $-OR^C$.

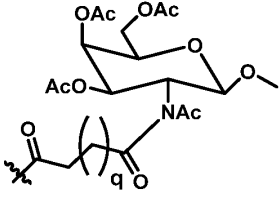
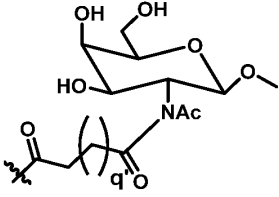
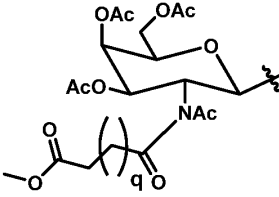
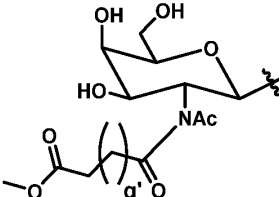
In certain embodiments of Formula (B1), (B2), (B1-a), (B1-b), or (B2-a), each
10 R^C is independently $C(=O)C_{1-6}$ alkyl. For example, each R^C can be $C(=O)CH_3$.

In certain embodiments of Formula (B1), (B2), (B1-a), (B1-b), or (B2-a), each R^C is H.

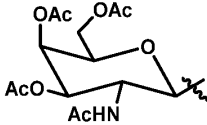
In certain embodiments of Formula (B2) or (B2-a), each R^G is CH_3 .

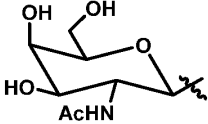
15 In certain embodiments, each R^1 is selected from the group consisting of the following:



 wherein, q is an integer selected from 1 to 10;	 wherein, q' is an integer selected from 1 to 10;
 wherein, q is an integer selected from 1 to 10;	 wherein, q' is an integer selected from 1 to 10.

In some embodiments, each R^1 is the same.

For example, each R^1 can be . As another non-limiting

5 example, each R^1 can be .

Variable R^2 , a , and b

In some embodiments, each R^2 is independently $^*-C(=O)NR^7-$ or $^*-NR^7C(=O)-$.

10 In certain embodiments, each R^2 is independently $^*-C(=O)NR^7-$. In certain of these embodiments, each R^2 is $^*-C(=O)NH-$.

In some embodiments, each R^2 is independently $-C(R^6)_2-$. In certain of these embodiments, each R^2 is $-CH_2-$. In some embodiments, each R^2 is the same.

In some embodiments, each **a** is independently 1, 2, 3, or 4. In some embodiments, each **a** is independently 5, 6, or 7. In some embodiments, each **a** is independently 8, 9, or 10.

In some embodiments, each **a** is an independently selected integer from 1 to 4. In certain embodiments, each **a** is independently 2 or 3. In some embodiments, each **a** is the same.

In some embodiments, each **b** is independently 1, 2, 3, or 4. In some embodiments, each **b** is independently 5, 6, or 7. In some embodiments, each **b** is independently 8, 9, or 10.

In some embodiments, each **b** is an independently selected integer from 1 to 4. In certain of these embodiments, each **b** is independently 2 or 3. In some embodiments, each **b** is the same.

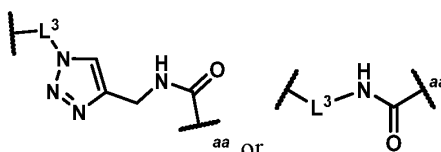
In certain embodiments, each **a** is the same; each **b** is the same; and each **R²** is the same.

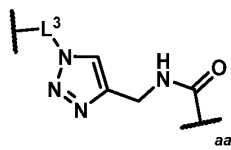
In certain of these embodiments, **a** is an integer from 1 to 4; **b** is an integer from 1 to 4; and **R²** is *-C(=O)NR⁷. For example, **a** can be 3; **b** can be 3; and **R²** can be *-C(=O)NH.

In certain embodiments (when each **a** is the same; each **b** is the same; and each **R²** is the same), **a** is an integer from 1 to 4; **b** is an integer from 1 to 4; and **R²** is -C(**R⁶**)₂. For example, **R²** can be -CH₂-; and 3 ≤ (**a**+**b**) ≤ 5.

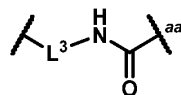
Variable **R³**

In some embodiments, **R³** is





In certain embodiments, R^3 is



In certain embodiments, R^3 is

5 In some embodiments, L^3 is $-C(R^6)_2-$. In certain embodiments, L^3 is $-CH_2-$.

Variable R^4 , c , and d

In some embodiments, c is independently 0 or 1. In some embodiments, c is independently 2, 3, or 4. In some embodiments, c is independently 5, 6, or 7. In some
10 embodiments, c is independently 8, 9, or 10.

In some embodiments, c is an integer from 1 to 2.

In some embodiments, c is an integer from 2 to 5.

In some embodiments, c is an integer from 3 to 7.

15 In some embodiments, R^4 is $^*-C(=O)NR^7-$. In certain of these embodiments, R^4 is $^*-C(=O)NH-$.

In some embodiments, R^4 is $-C(R^6)_2-$. In certain of these embodiments, R^4 is $-CH_2-$.

20 In some embodiments, d is independently 0 or 1. In some embodiments, d is independently 2, 3, or 4. In some embodiments, d is independently 5, 6, or 7. In some embodiments, d is independently 8, 9, or 10.

In some embodiments, d is an integer from 1 to 2.

In some embodiments, d is an integer from 3 to 7.

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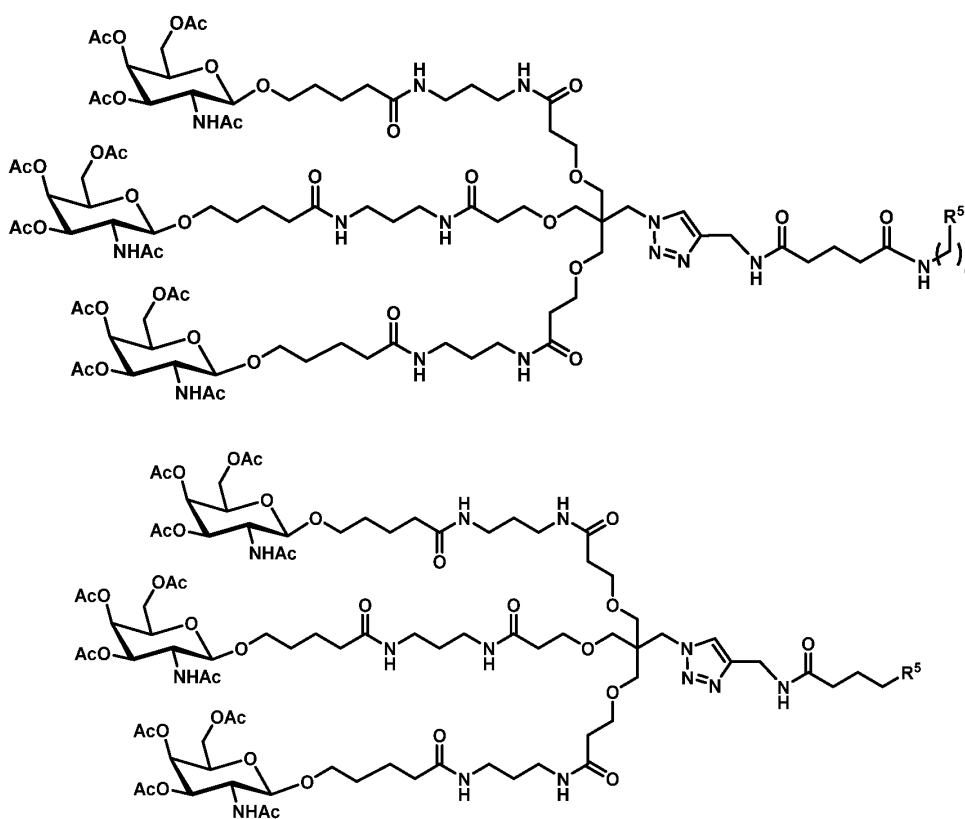
In certain embodiments, R^4 is $-C(R^6)_2-$; and each of c and d is independently 1 or 2. In certain of these embodiments, R^4 is $-CH_2-$; and each of c and d is 1.

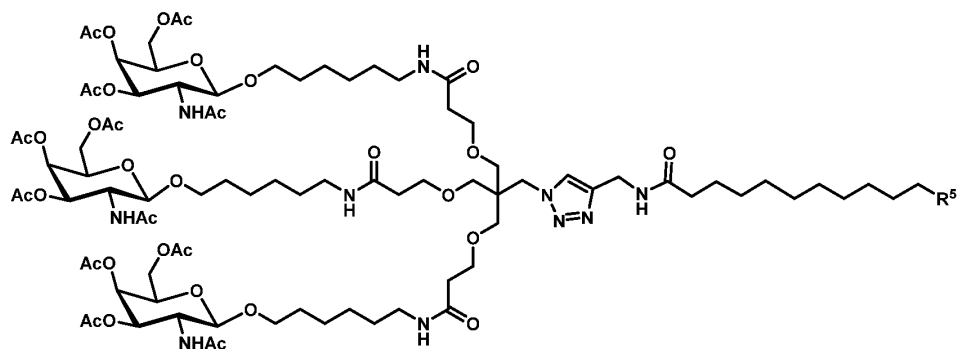
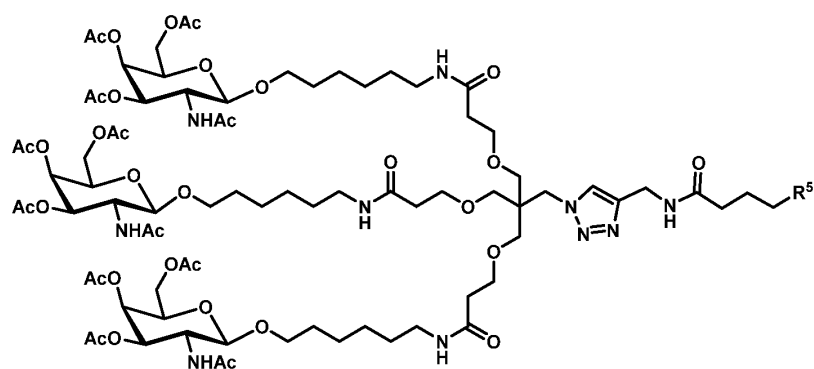
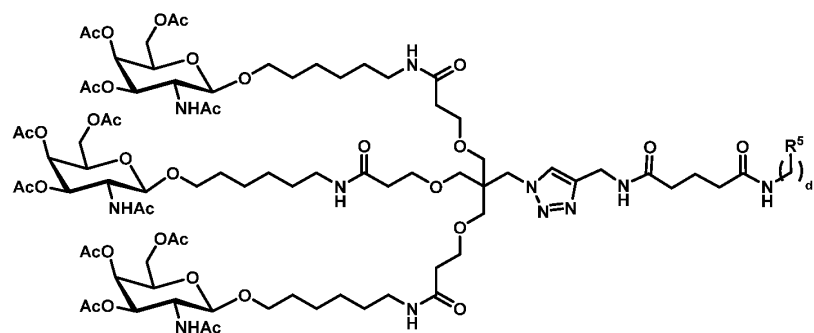
In certain embodiments, $\mathbf{R}^4$ is $-\text{C}(\mathbf{R}^6)_{2-}$; and $4 \leq (\mathbf{c} + \mathbf{d}) \leq 12$. In certain of these embodiments, $\mathbf{R}^4$ is $-\text{CH}_2-$; and $7 \leq (\mathbf{c} + \mathbf{d}) \leq 10$.

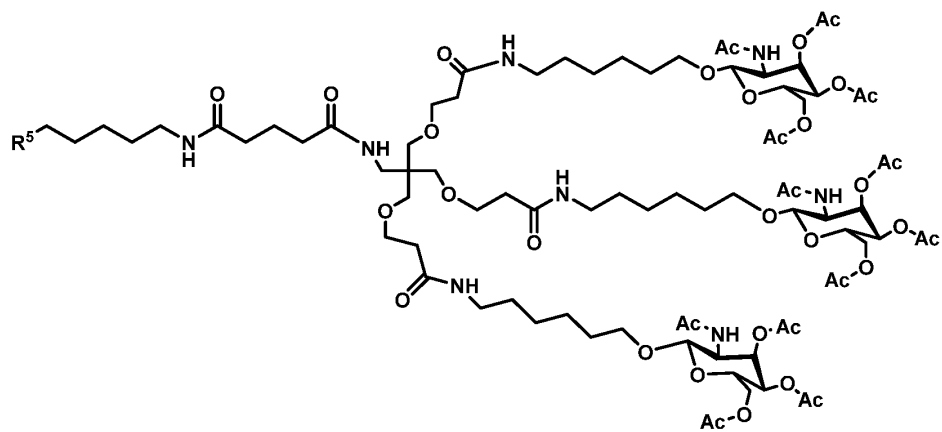
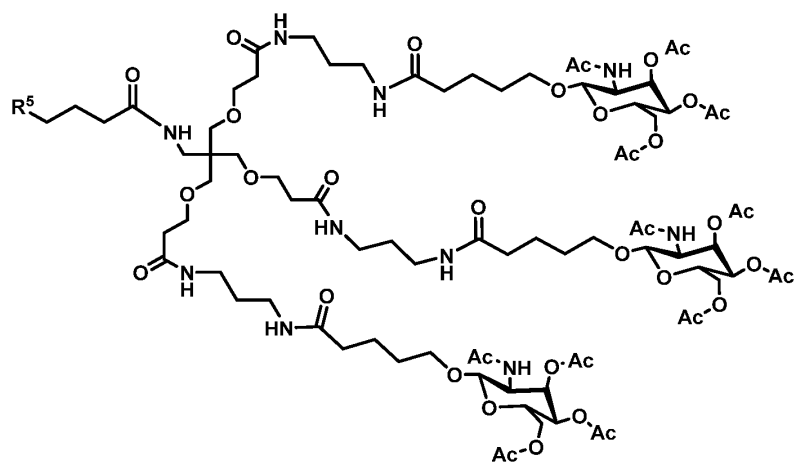
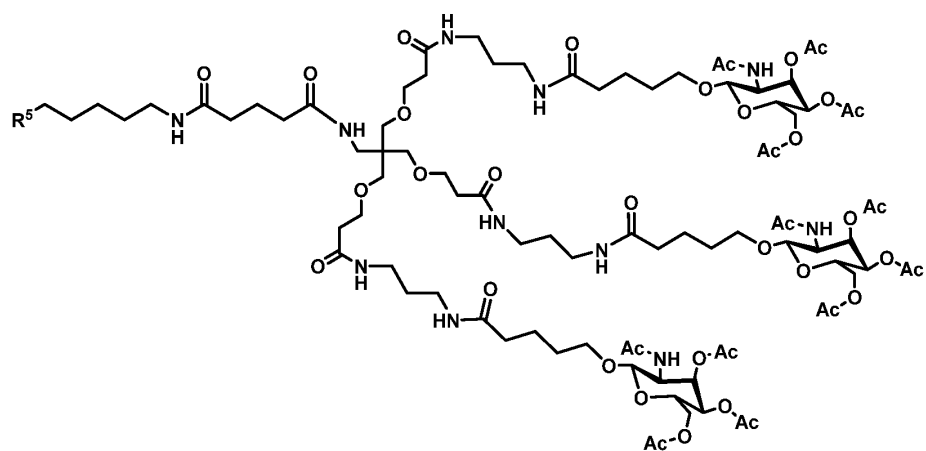
In certain embodiments, $\mathbf{R}^4$ is $^*\text{-C(=O)NR}^7\text{-}$; and $5 \leq (\mathbf{c} + \mathbf{d}) \leq 10$. In certain of these embodiments, $\mathbf{R}^4$ is $^*\text{-C(=O)NR}^7\text{-}$; and $7 \leq (\mathbf{c} + \mathbf{d}) \leq 9$. In certain of the foregoing embodiments, $\mathbf{c}$ is 3.

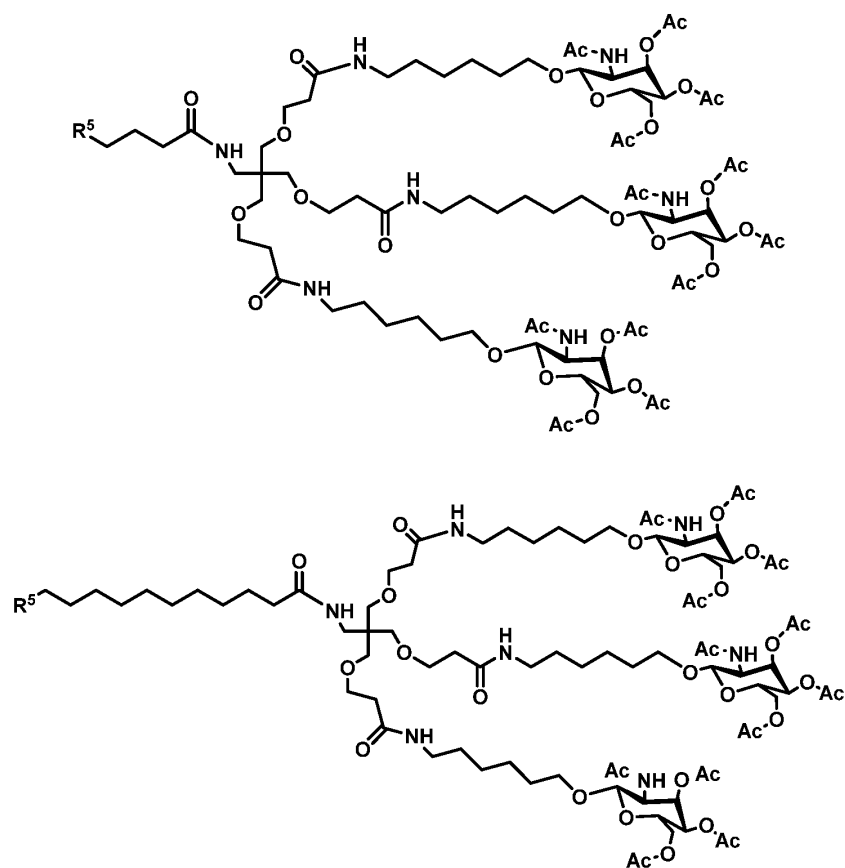
Non-Limiting Combinations

In certain embodiments, the compound of Formula (I) is selected from the group
10 consisting of the following:





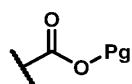




Variable R^5

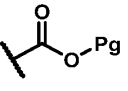
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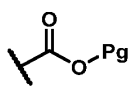
In some embodiments, R^5 is C(O)OH or

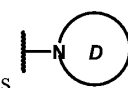


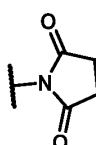
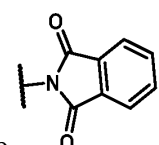
In certain embodiments, R^5 is C(O)OH.

10

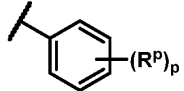
In certain embodiments, R^5 is . In certain embodiments, **Pg** is a carboxyl activating group. As used herein, a “carboxyl activating group” is a chemical moiety that, upon covalent bonding with a carboxyl oxygen atom, converts said oxygen atom into a leaving group. Accordingly, when **Pg** is a carboxyl activating group, the “-O-

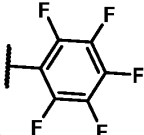
Pg group in  is a leaving group. Non-limiting examples of carboxyl activating groups include *N*-centered heterocyclyl (e.g., succinimidyl) and electron-deficient heteroaryl and aryl (e.g., pentafluorophenyl).

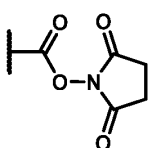
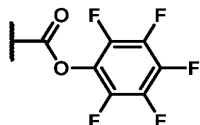
In certain embodiments, **Pg** is , wherein **Ring D** is a 5-10 membered heteroaryl or 4-10 membered heterocyclyl, each optionally substituted with 1-6 substituents each independently selected from the group consisting of: halo, oxo, NO₂, C(O)C₁₋₄ alkyl, C(O)OC₁₋₄ alkyl, S(O)C₁₋₄ alkyl, C₁₋₆ alkyl, C₁₋₆ haloalkyl, and -OH. For

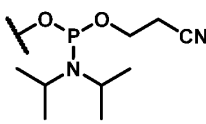
example, **Pg** can be . As another non-limiting example, **Pg** can be  which is optionally substituted with 1-6 substituents each independently selected from the group consisting of: halo, NO₂, C(O)C₁₋₄ alkyl, C(O)OC₁₋₄ alkyl, S(O)C₁₋₄ alkyl, C₁₋₆ alkyl, C₁₋₆ haloalkyl, and -OH (e.g., unsubstituted or substituted with 1-6 independently selected halo).

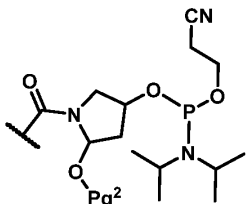
In certain embodiments, **Pg** is C₆₋₁₀ aryl or 5-10 membered heteroaryl substituted with 1-6 substituents each independently selected from the group consisting of: -F; -Cl; -NO₂; C(O)C₁₋₄ alkyl, C(O)OC₁₋₄ alkyl, and S(O)C₁₋₄ alkyl.

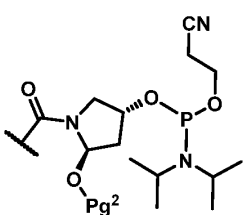
In certain of these embodiments, Pg is , wherein p is an integer from 1 to 5; and each R^p is $-\text{F}$, $-\text{Cl}$, or $-\text{NO}_2$. In certain embodiments, each R^p is $-\text{F}$. As

a non-limiting example of the foregoing embodiments, Pg can be .

5 In certain embodiments, R^5 is  or .

In some embodiments, R^5 is .

In some embodiments, R^5 is . In certain embodiments, R^5 is

10 .

In some embodiments, Pg^2 and Z are each independently selected from the group consisting of: a silyl protecting group; 4-monomethoxytrityl (MMTR); 4,4 - dimethoxytrityl (DMTR); and trityl.

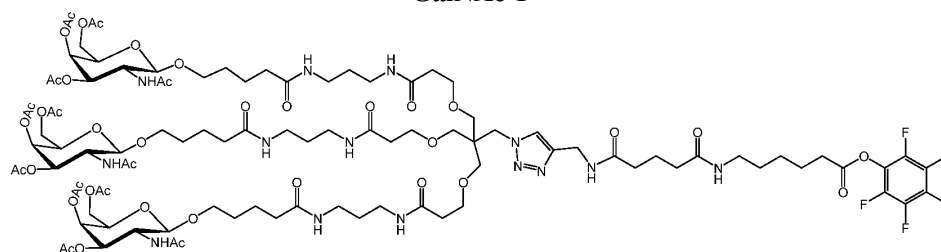
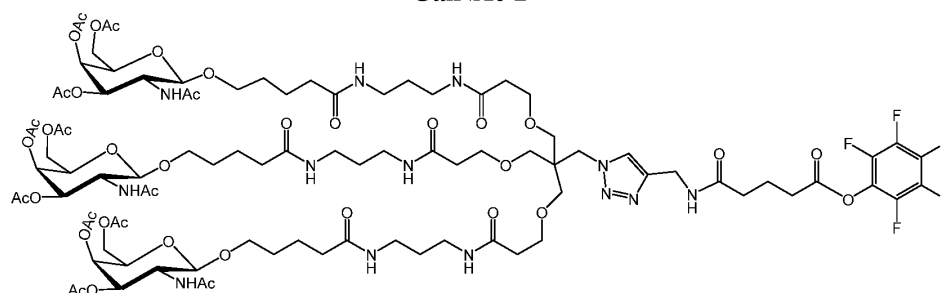
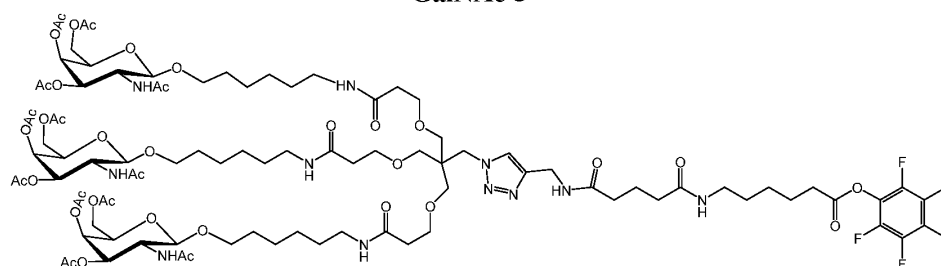
In certain of these embodiments, the silyl protecting group is selected from the group consisting of: *tert*-butyldimethylsilyl (TBMDS); *tert*-butyldiphenylsilyl (TBDPS), and triisopropylsilyl (TIPS).

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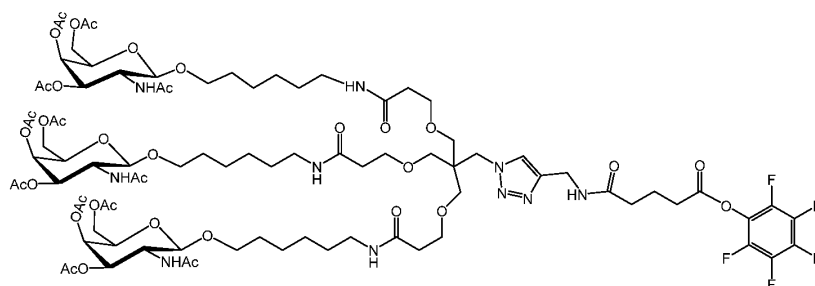
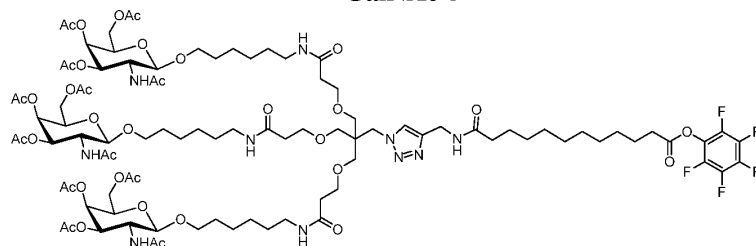
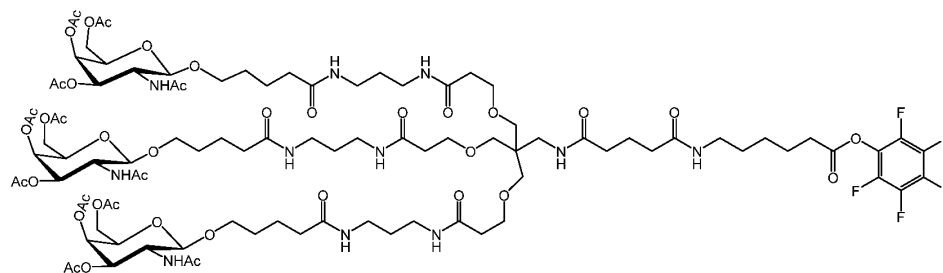
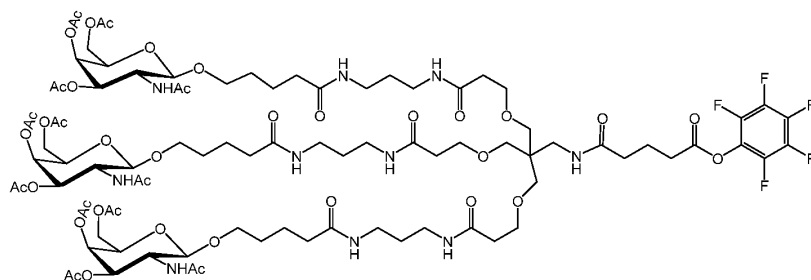
Non-Limiting Exemplary Compounds

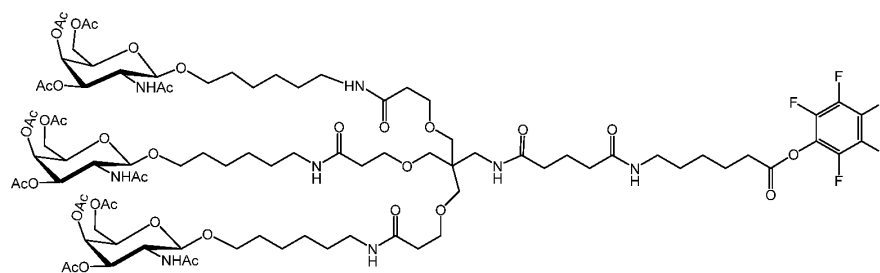
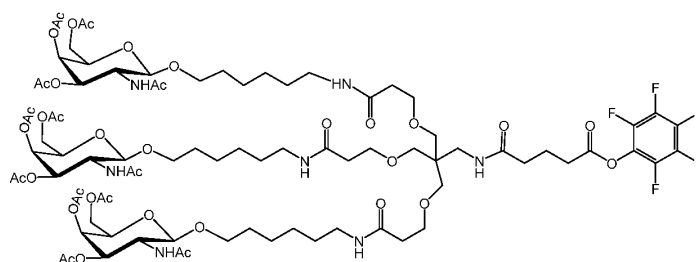
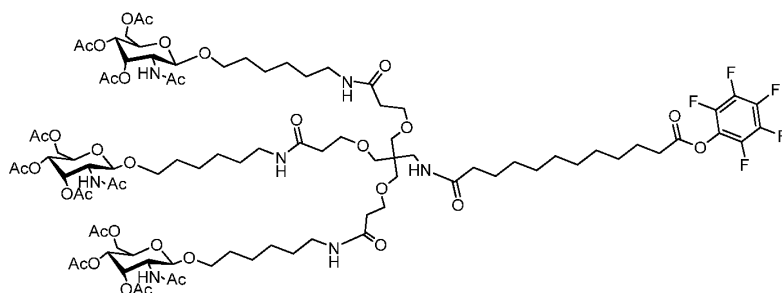
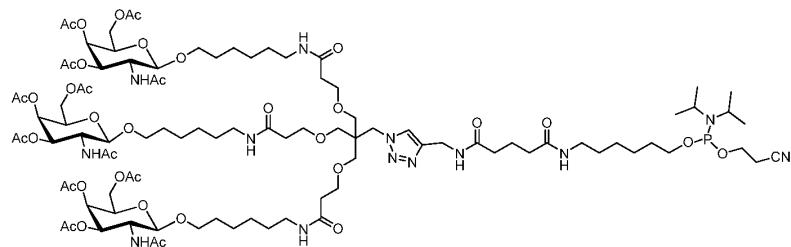
In certain embodiments, the compound is selected from the group consisting of compounds **GalNAc-1** through **GalNAc-12**. For example, the compound can be selected from the group consisting of compounds **GalNAc-1** through **GalNAc-10**.

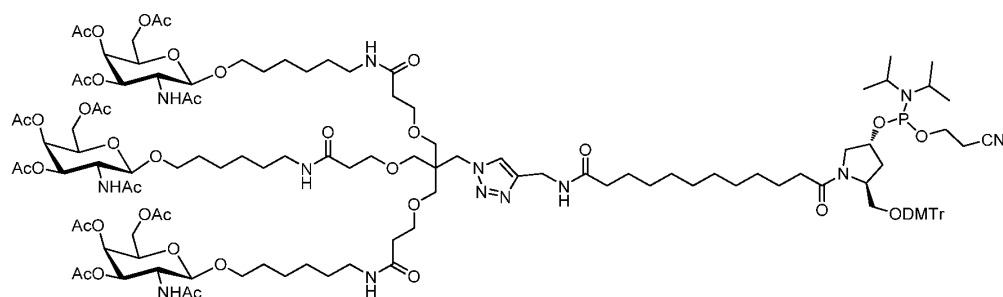
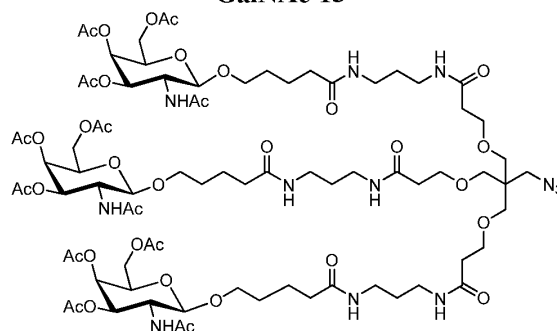
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GalNAc-1**GalNAc-2****GalNAc-3****GalNAc-4**

15

**GalNAc-5****GalNAc-6****GalNAc-7:****GalNAc-8:**

**GalNAc-9:****GalNAc-10:****GalNAc-11:****GalNAc-12:**

**GalNAc-13****GalNAc-14**

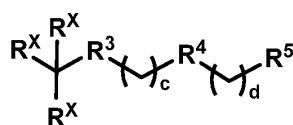
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GalNAc-13 and **GalNAc-14** are useful e.g., as intermediates in the preparation of Formula (I) compounds.

10

Conjugate Compounds

In some embodiments, the compound of Formula (I) is a conjugate compound of Formula (II):



Formula (II)

or a pharmaceutically acceptable salt thereof, wherein:

R^5 is $\text{---}L\text{---Oligo}$, wherein **Oligo** is an oligonucleotide; and
 5 $-L-$, R^X (including R^1 , R^2 , **a**, and **b**), R^3 , **c**, R^4 , and **d** can be as defined for Formula (I) anywhere herein.

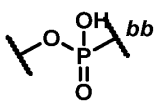
In some embodiments, **Oligo** is an oligonucleotide that is attached to **L** via the 5'-end, 3'-end, or sequence middle of any strand via a phosphate group.

10 *Variable L*

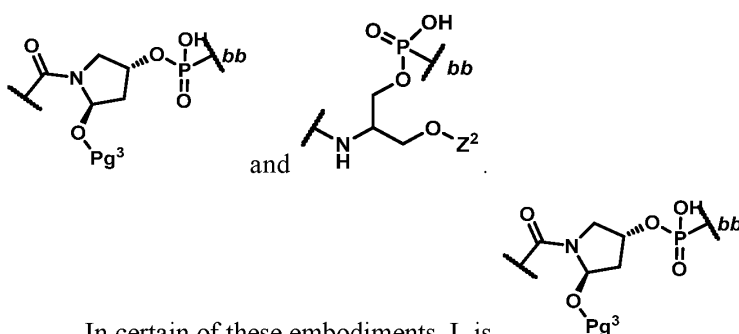
In some embodiments, **L** is a bond.

In some embodiments, **L** is $C(=O)$.

In some embodiments, **L** is $-O-$.

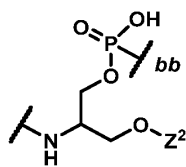
15 In some embodiments, **L** is , wherein **bb** is the point of attachment to **Oligo**.

In some embodiments, **L** is selected from the group consisting of:



In certain of these embodiments, **L** is

In certain embodiments, Pg^3 is H. In certain embodiments, Pg^3 is a hydroxyl protecting group.



In certain embodiments, L is

In certain embodiments, Z^2 is H. In certain embodiments, Z^2 is a hydroxyl protecting group.

In certain embodiments, the hydroxyl protecting group is selected from the group consisting of: a silyl protecting group; 4-monomethoxytrityl (MMTR); 4,4 - dimethoxytrityl (DMTR); and trityl.

In certain of these embodiments, the silyl protecting group is selected from the group consisting of: *tert*-butyldimethylsilyl (TBMDS); *tert*-butyldiphenylsilyl (TBDPS), and triisopropylsilyl (TIPS).

Oligonucleotide Moiety

In some embodiments, **Oligo** is an oligonucleotide that comprises a single-stranded oligonucleotide and/or a double-stranded oligonucleotide.

In certain of these embodiments, **Oligo** is a single-stranded oligonucleotide. In certain embodiments, **Oligo** is a double-stranded oligonucleotide.

In some embodiments, the oligonucleotide is selected from the group consisting of: DNA, siRNA, miRNA, pre-miRNA, antagomir, mRNA, antisense oligonucleotide (ASO), Aptamer, crRNA, tracrRNA, and sgRNA.

The oligonucleotide herein can comprise unmodified nucleotides and/or modified nucleotides. Non-limiting examples of modified nucleotides include: 2'-O-(2-methoxyethyl)-modified nucleotides; 2'-O-alkyl modified nucleotides (e.g., 2'-O-methyl modified nucleotides); 2'-O-allyl modified nucleotides; 2'-C-allyl modified nucleotides; 2'-fluoro modified nucleotides; 2'-deoxy modified nucleotides; 2'-

hydroxy modified nucleotides; locked nucleic acids (LNAs) modified nucleotides; hexitol nucleic acids (HNAs) modified nucleotides; glycol nucleic acids (GNAs) modified nucleotides, and unlocked nucleic acid (UNAs) modified nucleotides. In certain embodiments, the modified nucleotide is selected from the group consisting of: 2'-O-alkyl modified nucleotides and 2'-fluoro modified nucleotides.

In some embodiments, the oligonucleotide comprises a modifying group, wherein the modifying group is selected from the group consisting of: cholesterol, polyethylene glycol, fluorescent probes, biotin, polypeptides, vitamins, tissue targeting molecules, and a combination thereof. In certain of these embodiments, the modifying group is a terminal modifying group.

Oligo can be attached to **L** via the 5'-end, 3'-end or sequence middle of any strand via a phosphate group. In certain embodiments, the phosphate group is a phosphodiester group. In certain embodiments, the phosphate group is a modified phosphate group. In certain of these embodiments, the modified phosphate group is selected from one or more of: thio modified phosphate (e.g., phosphorothioate), and amino modified phosphate.

In some embodiments, **Oligo** comprises one or more peptide nucleic acids and/or morpholino nucleic acids (e.g., morpholino antisense oligonucleotides).

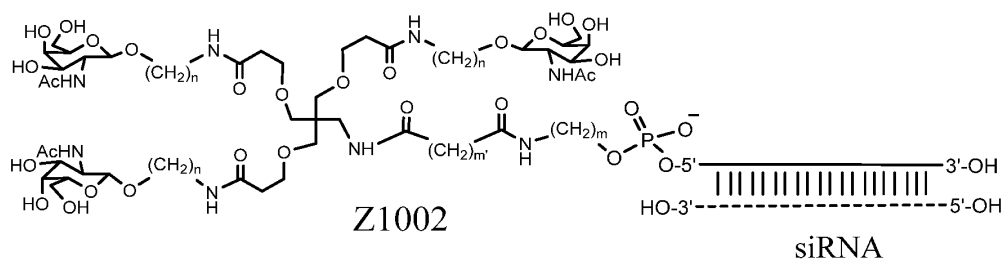
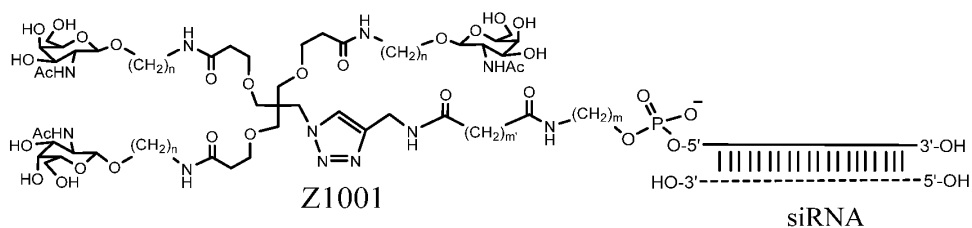
In some embodiments, the **Oligo** is an oligonucleotide of from 5 to 100 base pairs. For example, **Oligo** can be an oligonucleotide of 5 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 50, 50 to 60, 60 to 70, 70 to 80, 80 to 90, or 90 to 100 base pairs.

In some embodiments, the compound of Formula (II) is synthesized via solid-phase synthesis or liquid-phase synthesis. In certain embodiments, the compound of Formula (II) is synthesized via solid-phase synthesis. In certain embodiments, the compound of Formula (II) is synthesized via liquid-phase synthesis.

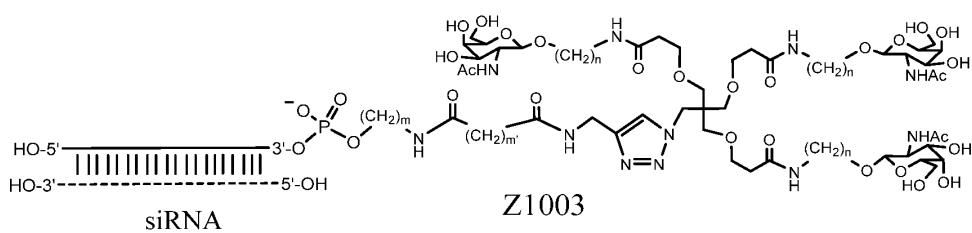
In some embodiments, the oligonucleotide can be as described anywhere in paragraphs [0335]-[410] in US 2015/0119444, or paragraphs [0341]-[416] in US 2015/0119445, and these sections are incorporated herein by reference.

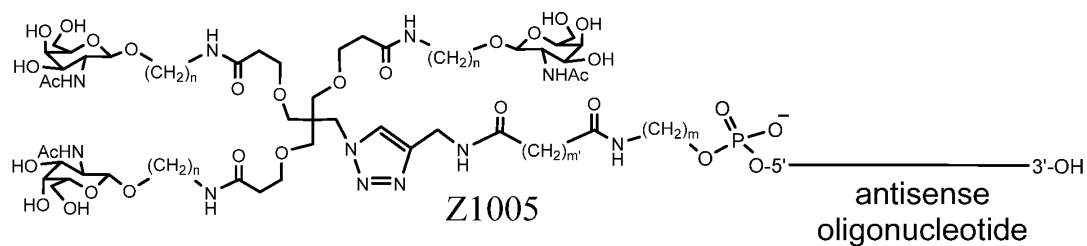
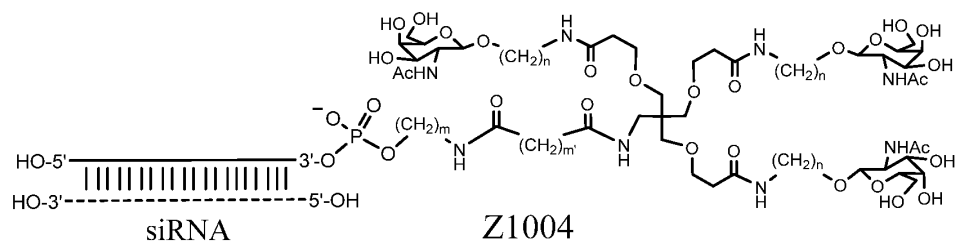
5 Non-Limiting Examples of Conjugate Compounds

Non-limiting examples of Formula (II) compounds include the following (wherein **n**, **m**, and **m'** are each independently selected integers from 1 to 10):

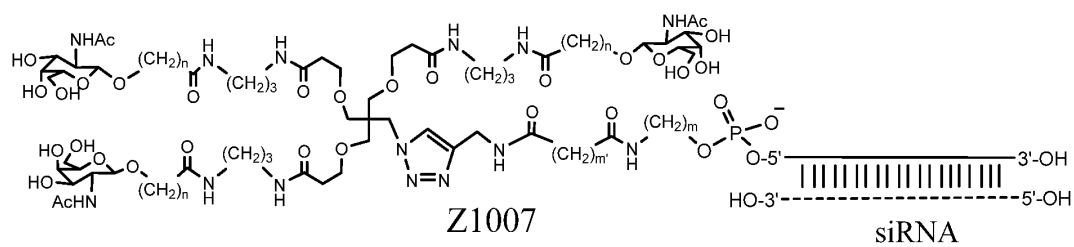
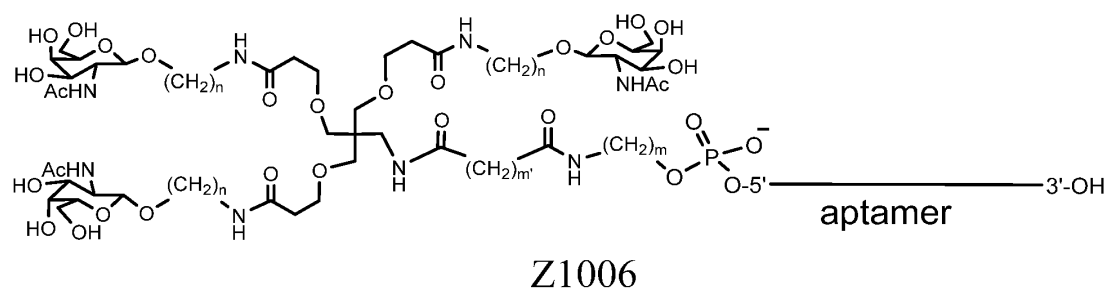


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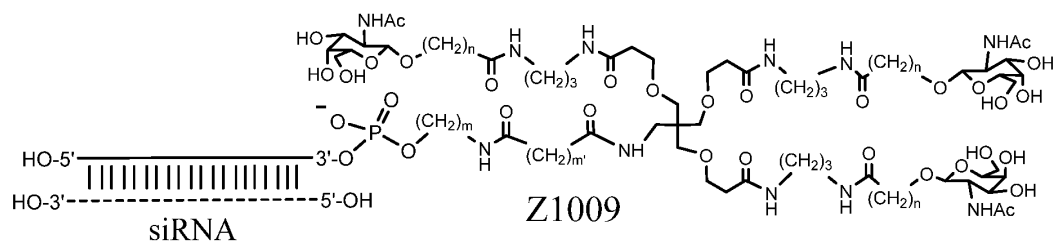
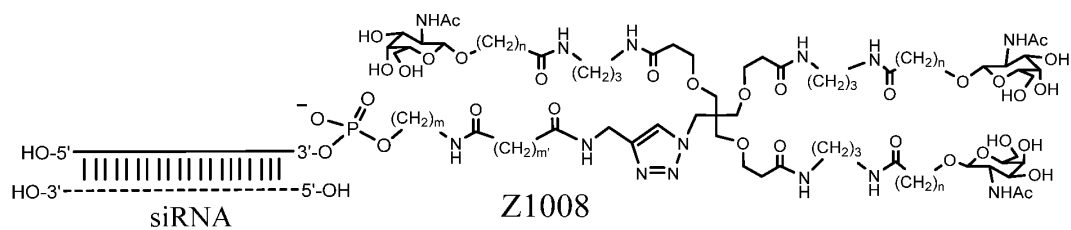




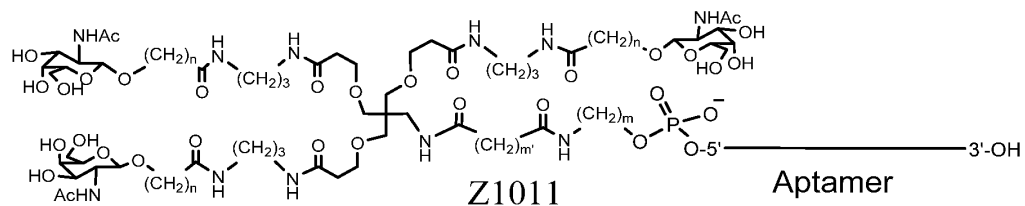
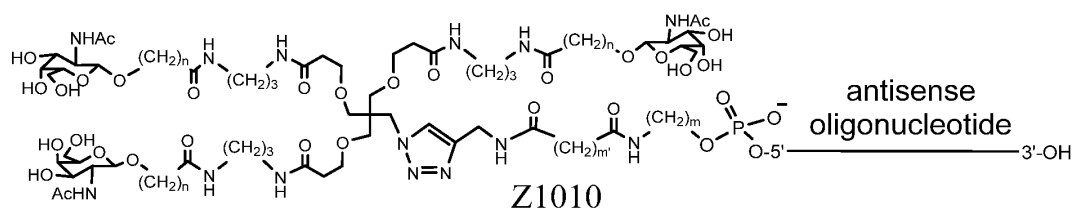
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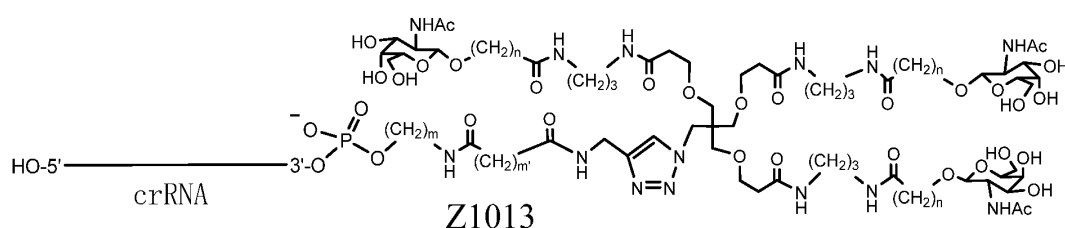
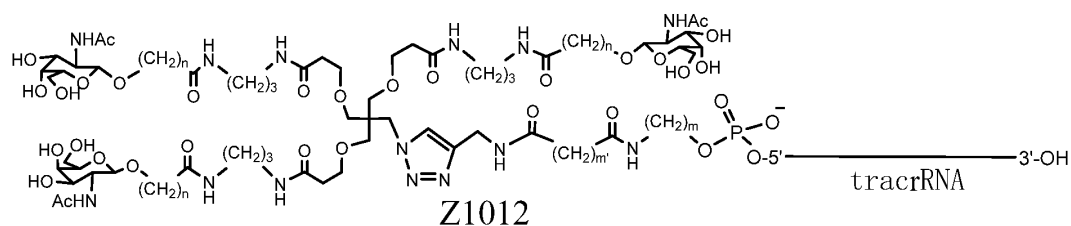
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Pharmaceutical Compositions and Administration

General

In another aspect, this disclosure features pharmaceutical compositions comprising a chemical entity as described herein (e.g., a compound of Formula (II) or a pharmaceutically acceptable salt thereof) and a pharmaceutically acceptable excipient.

In some embodiments, the chemical entities can be administered in combination with one or more conventional pharmaceutical excipients. Pharmaceutically acceptable excipients include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d- α -tocopherol polyethylene glycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens, poloxamers or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, tris, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate,

potassium hydrogen phosphate, sodium-chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethyl cellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, and wool fat. Cyclodextrins such as α -, β -, and γ -cyclodextrin, or
5 chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl- β -cyclodextrins, or other solubilized derivatives can also be used to enhance delivery of compounds described herein. Dosage forms or compositions containing a chemical entity as described herein in the range of 0.005% to 100% with the balance made up from non-toxic excipient may be prepared. The contemplated
10 compositions may contain 0.001%-100% of a chemical entity provided herein, in one embodiment 0.1-95%, in another embodiment 75-85%, in a further embodiment 20-80%. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington: The Science and Practice of Pharmacy*, 22nd Edition (Pharmaceutical Press, London, UK. 2012).

15 In some embodiments, the pharmaceutical composition is formulated in a dosage form selected from the group consisting of: powders, tablets, granules, capsules, solutions, emulsions, suspensions, injections, sprays, aerosols, dry powder inhalations, and microneedle patches.

In some embodiments, the pharmaceutical composition is suitable for
20 administration to a subject in need thereof intravenously, intramuscularly, subcutaneously, via microneedle patches, orally, via oral or nasal spray, or topically.

In some embodiments, the subject is a mammal, including bovine, equine, sheep, swine, canine, feline, rodent, and primate. For example, the subject can be human.

25

Dosages

The dosages may be varied depending on the requirement of the patient, the severity of the condition being treating and the particular compound being employed. Determination of the proper dosage for a particular situation can be determined by one

skilled in the medical arts. The total daily dosage may be divided and administered in portions throughout the day or by means providing continuous delivery.

In some embodiments, a unit dose is less than 1.4 mg per kg of bodyweight of the subject, or less than 10, 5, 2, 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00005 or 0.00001 mg per kg of bodyweight. In some embodiments, a unit dose is from 0.00001 to 10 mg per kg of bodyweight. In some embodiments, a unit dose is from 0.001 to 2 mg per kg of bodyweight.

Regimens

The foregoing dosages can be administered on a daily basis (e.g., as a single dose or as two or more divided doses) or non-daily basis (e.g., every other day, every two days, every three days, once weekly, twice weeks, once every two weeks, once a month).

Methods of Treatment

In another aspect, this disclosure features methods for treating and/or preventing pathological conditions or diseases in a subject, wherein the conditions or diseases are caused by the expression of one or more genes in liver cells, the method comprising administering to the subject a chemical entity as described herein (e.g., a compound of Formula (II) or a pharmaceutically acceptable salt thereof); or a pharmaceutical composition comprising a chemical entity as described herein (e.g., a compound of Formula (II) or a pharmaceutically acceptable salt thereof), and a pharmaceutically acceptable excipient.

In some embodiments, the one or more genes are selected from: HBV genome, HCV genome, *PCSK9*, xanthine oxidase, *URAT1*, *APOB*, liver fibrosis-related genes (*AP3S2*, *AQP2*, *AZIN1*, *DEGSI*, *STXBP5L*, *TLR4*, *TRPM5*, etc.), and genes related to non-alcoholic fatty liver disease (*PNPLA3*, *FDFTI*), primary biliary cirrhosis (*HLA-DQB1*, *IL-12*, *IL-12RB2*, etc.).

In some embodiments, the disease or condition is selected from the group consisting of: hereditary angioedema, familial tyrosinemia type I, Alagille syndrome,

α -1 -antitrypsin deficiency, bile acid synthesis and metabolic defects, biliary atresia, cystic fibrosis liver disease, idiopathic neonatal hepatitis, mitochondrial liver disease, progressive familial intrahepatic cholestasis, primary sclerosing cholangitis, transthyretin amyloidosis, hemophilia, homozygous familial hypercholesterolemia, hyperlipidemia, hepatitis B virus infection (HBV), hepatitis C virus infection (HCV),
5 steatohepatitis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), hyperglycemia and diseases involving abnormally increased hepatic glucose production similar to type II diabetes, hepatitis, hepatic porphyrins.

In some embodiments, the subject is a mammal, including bovine, equine,
10 sheep, swine, canine, feline, rodent, and primate. For example, the subject can be human.

In a further aspect, this disclosure features methods for detecting or localizing
15 RNA in the liver of a subject, comprising administering to the subject a chemical entity as described herein (e.g., a compound of Formula (II) or a pharmaceutically acceptable salt thereof); or a pharmaceutical composition comprising a chemical entity as described herein (e.g., a compound of Formula (II) or a pharmaceutically acceptable salt thereof), and a pharmaceutically acceptable excipient.

20 In some embodiments, the subject is diagnosed with one or more conditions or diseases that are caused by the expression of one or more genes in liver cells

In certain embodiments, the one or more genes are selected from: HBV genome, HCV genome, *PCSK9*, xanthine oxidase, *URAT1*, *APOB*, liver fibrosis-related genes (*AP3S2*, *AQP2*, *AZIN1*, *DEGSI*, *STXBP5L*, *TLR4*, *TRPM5*, etc.), and
25 genes related to non-alcoholic fatty liver disease (*PNPLA3*, *FDFTI*), primary biliary cirrhosis (*HLA-DQB1*, *IL-12*, *IL-12RB2*, etc.).

In certain embodiments, the disease or condition is selected from the group consisting of: hereditary angioedema, familial tyrosinemia type I, Alagille syndrome, α -1 -antitrypsin deficiency, bile acid synthesis and metabolic defects, biliary atresia,
30 cystic fibrosis liver disease, idiopathic neonatal hepatitis, mitochondrial liver disease, progressive familial intrahepatic cholestasis, primary sclerosing cholangitis,

transthyretin amyloidosis, hemophilia, homozygous familial hypercholesterolemia, hyperlipidemia, hepatitis B virus infection (HBV), hepatitis C virus infection (HCV), steatohepatitis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), hyperglycemia and diseases involving abnormally increased
5 hepatic glucose production similar to type II diabetes, hepatitis, hepatic porphyrins.

In some embodiments, the subject is a mammal, including bovine, equine, sheep, swine, canine, feline, rodent, and primate. For example, the subject can be human.

In some embodiments, the chemical entity described herein (e.g., a
10 compound of Formula (II) or a pharmaceutically acceptable salt thereof) is administered intravenously, intramuscularly, subcutaneously, via microneedle patches, orally, via oral or nasal spray, or topically.

In another aspect, provided herein is a compound (e.g., a compound of Formula
15 (II)), or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, for use in the treatment of conditions or diseases are caused by the expression of one or more genes in liver cells in a subject in need thereof. In some embodiments, the disease or condition is selected from the group consisting of: hereditary angioedema, familial tyrosinemia type I, Alagille syndrome, α -1 -
20 antitrypsin deficiency, bile acid synthesis and metabolic defects, biliary atresia, cystic fibrosis liver disease, idiopathic neonatal hepatitis, mitochondrial liver disease, progressive familial intrahepatic cholestasis, primary sclerosing cholangitis, transthyretin amyloidosis, hemophilia, homozygous familial hypercholesterolemia, hyperlipidemia, hepatitis B virus infection (HBV), hepatitis C virus infection (HCV),
25 steatohepatitis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), hyperglycemia and diseases involving abnormally increased hepatic glucose production similar to type II diabetes, hepatitis, hepatic porphyrins. The subject can be as defined anywhere herein. In some embodiments, the subject is a mammal (e.g., human).

In another aspect, provided herein is a compound (e.g., a compound of Formula (II)), or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, for use in detecting or localizing RNA in the liver of a subject. The subject can be as defined anywhere herein. In some embodiments, the subject is a mammal (e.g.,
5 human).

In another aspect, provided herein is a use for a compound (e.g., a compound of Formula (II)), or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, in the treatment of conditions or diseases are caused by the expression of one or more genes in liver cells in a subject in need thereof. In some
10 embodiments, the disease or condition is selected from the group consisting of: hereditary angioedema, familial tyrosinemia type I, Alagille syndrome, α -1 - antitrypsin deficiency, bile acid synthesis and metabolic defects, biliary atresia, cystic fibrosis liver disease, idiopathic neonatal hepatitis, mitochondrial liver disease, progressive familial intrahepatic cholestasis, primary sclerosing cholangitis,
15 transthyretin amyloidosis, hemophilia, homozygous familial hypercholesterolemia, hyperlipidemia, hepatitis B virus infection (HBV), hepatitis C virus infection (HCV), steatohepatitis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), hyperglycemia and diseases involving abnormally increased hepatic glucose production similar to type II diabetes, hepatitis, hepatic porphyrins.
20 The subject can be as defined anywhere herein. In some embodiments, the subject is a mammal (e.g., human).

In another aspect, provided herein is a use of a compound (e.g., a compound of Formula (II)), or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, in detecting or localizing RNA in the liver of a subject. The subject
25 can be as defined anywhere herein. In some embodiments, the subject is a mammal (e.g., human).

In another aspect, provided herein is a use for a compound (e.g., a compound of Formula (II)), or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, in the manufacture of a medicament for the treatment of conditions
30 or diseases are caused by the expression of one or more genes in liver cells in a

subject in need thereof. In some embodiments, the disease or condition is selected from the group consisting of: hereditary angioedema, familial tyrosinemia type I, Alagille syndrome, α -1 -antitrypsin deficiency, bile acid synthesis and metabolic defects, biliary atresia, cystic fibrosis liver disease, idiopathic neonatal hepatitis, 5 mitochondrial liver disease, progressive familial intrahepatic cholestasis, primary sclerosing cholangitis, transthyretin amyloidosis, hemophilia, homozygous familial hypercholesterolemia, hyperlipidemia, hepatitis B virus infection (HBV), hepatitis C virus infection (HCV), steatohepatitis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), hyperglycemia and diseases involving 10 abnormally increased hepatic glucose production similar to type II diabetes, hepatitis, hepatic porphyrins. The subject can be as defined anywhere herein. In some embodiments, the subject is a mammal (e.g., human).

In another aspect, provided herein is a use of a compound (e.g., a compound of Formula (II)), or a pharmaceutically acceptable salt thereof, or a pharmaceutical 15 composition thereof, in the manufacture of a medicament for detecting or localizing RNA in the liver of a subject. The subject can be as defined anywhere herein. In some embodiments, the subject is a mammal (e.g., human).

Compound Preparation

20 As can be appreciated by the skilled artisan, methods of synthesizing the compounds of the formulae herein will be evident to those of ordinary skill in the art. Synthetic chemistry transformations and protecting group methodologies (protection and deprotection) useful in synthesizing the compounds described herein are known in the art and include, for example, those such as described in R. Larock, Comprehensive Organic 25 Transformations, VCH Publishers (1989); T. W. Greene and RGM. Wuts, Protective Groups in Organic Synthesis, 2d. Ed., John Wiley and Sons (1991); L. Fieser and M. Fieser, Fieser and Fieser's Reagents for Organic Synthesis, John Wiley and Sons (1994); and L. Paquette, ed., Encyclopedia of Reagents for Organic Synthesis, John Wiley and Sons (1995), and subsequent editions thereof. The starting materials used in preparing 30 the compounds of the invention are known, made by known methods, or are

commercially available. The skilled artisan will also recognize that conditions and reagents described herein that can be interchanged with alternative art-recognized equivalents. For example, in many reactions, triethylamine can be interchanged with other bases, such as non-nucleophilic bases (e.g. diisopropylamine, 1,8-diazabicycloundec-7-ene, 2,6-di-tert-butylpyridine, or tetrabutylphosphazene).

The skilled artisan will recognize a variety of analytical methods that can be used to characterize the compounds described herein, including, for example, ¹H NMR, heteronuclear NMR, mass spectrometry, liquid chromatography, and infrared spectroscopy. The foregoing list is a subset of characterization methods available to a skilled artisan and is not intended to be limiting.

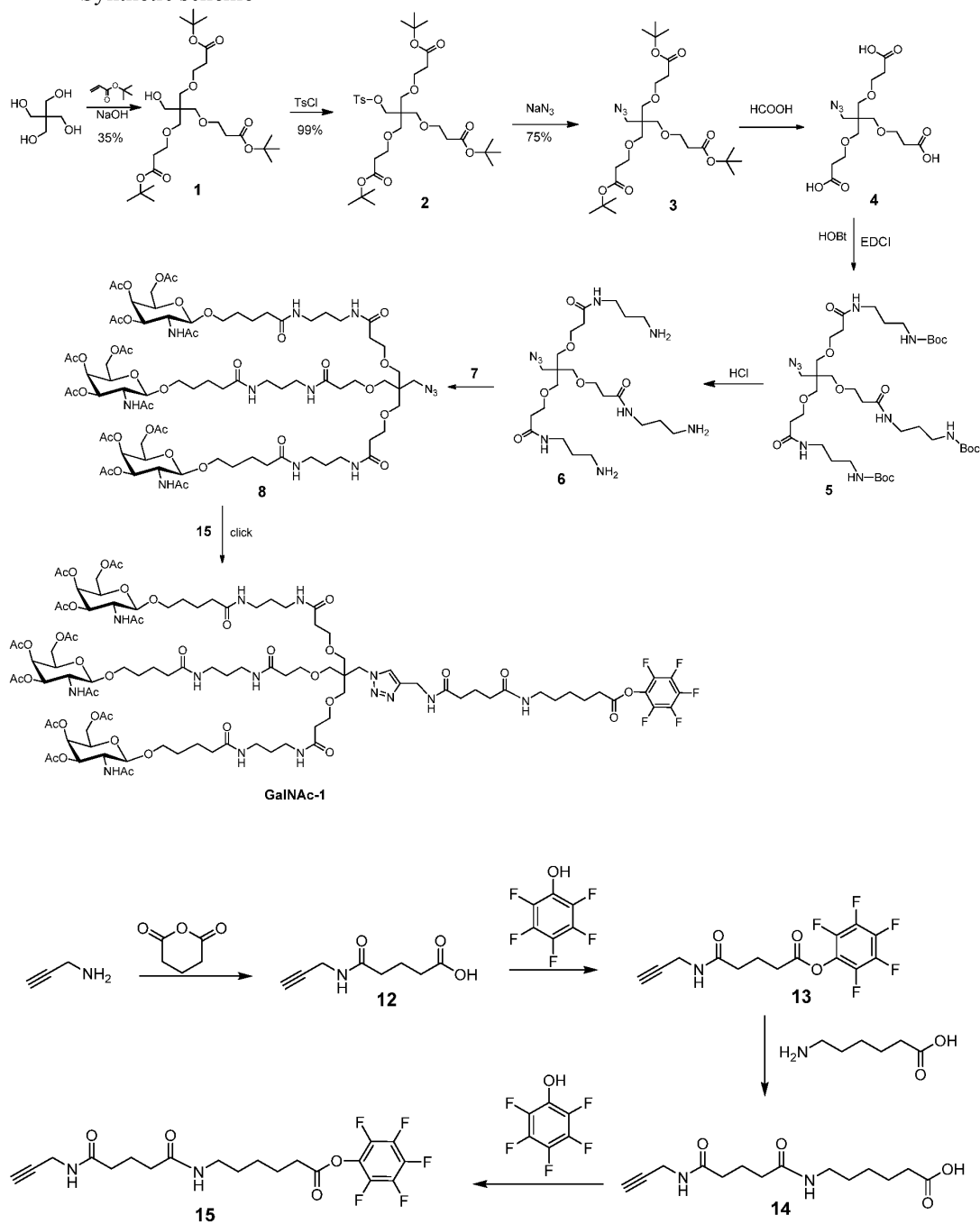
To further illustrate the foregoing, the following non-limiting, exemplary synthetic schemes are included. Variations of these examples within the scope of the claims are within the purview of one skilled in the art and are considered to fall within the scope of the invention as described, and claimed herein. The reader will recognize that the skilled artisan, provided with the present disclosure, and skill in the art is able to prepare and use the invention without exhaustive examples.

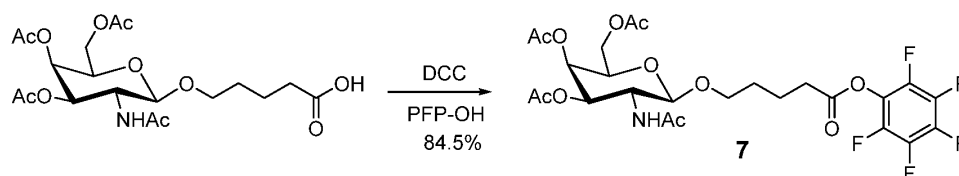
Examples

The disclosure is further described in the following examples, which do not limit the scope of the disclosure as described in the claims. Specific conditions which are not noted in the examples are carried out under conventional conditions or conditions recommended by the manufacturer. The reagents or instruments used of which the manufacturer are not noted are conventional products commercially available.

Example 1. Preparation of GalNAc-1

Synthetic scheme





Procedure

(1) Pentaerythritol (50 g), NaOH solution (6 mL, aq., 50%), and tetrabutylammonium hydroxide (9.5 g) were added to *tert*-butyl acrylate (150 g) and the reaction mixture was then stirred at room temperature until the reaction was completed. Upon completion, ethyl acetate was added to extract the organic phase, which was then concentrated to yield the crude product. After column separation and purification (PE:EA = 20:1-7:1) of the crude product, compound **1** was obtained;

(2) Compound **1** (10 g) and TsCl (6.5 g) were dissolved in pyridine (100 mL), and the resulting mixture was stirred at 70°C. Upon completion, the reaction mixture was concentrated to remove pyridine. Ethyl acetate was added to the residue, which is further concentrated and dried. After column separation and purification of the crude product (PE:EA = 20:1-10:1), compound **2** was obtained;

(3) compound **2** (2 g) was dissolved in anhydrous DMF (50 mL), and then sodium azide (0.53g) was added and the reaction mixture was heated to 100°C. Upon completion, the reaction mixture was cooled to room temperature and concentrated to remove DMF. Ethyl acetate was added to the residue, which is further concentrated and dried. After column separation and purification of the crude product (PE: EA = 20:1-10:1), compound **3** was obtained;

(4) Compound **3** (2 g) was dissolved in formic acid (10 mL) and the reaction mixture was stirred at room temperature. Upon completion, the reaction mixture was concentrated to dryness and compound **4** was obtained;

(5) Compound **4** (1.6 g) was dissolved in dichloromethane (50 ml), then HOBt (2.23 g), EDCI (3.0 g), DIEA (3.3 g), and *N*-*boc*-1,3-diaminopropane (2.7 g) were added to the mixture. The reaction was stirred at room temperature until it was completed. Upon completion, dichloromethane and water were added to the reaction for extraction, after which the organic phase was dried and concentrated to dryness and yielded a crude

product. After column separation and purification (dichloromethane: methanol = 2% - 5%) of the crude product, compound **5** was obtained;

(6) Compound **5** (2.5 g) was dissolved in dichloromethane (50 mL), then trifluoroacetic acid (20 mL, 2N) was added and the reaction was stirred at room temperature before it was completed. Upon completion, the reaction mixture was concentrated to obtain compound **6**;

(7) 5-(((2*R*,3*R*,4*R*,5*R*,6*R*)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl) tetrahydro-2*H*-pyran-2-yl)oxy)pentanoic acid (11 g) was dissolved in dichloromethane (100 mL), then DCC (6.2 g) and pentafluorophenol (5.5 g) were added, and the reaction mixture was then stirred at room temperature until the reaction was completed. Upon completion, the reaction mixture was filtered and the filtrate was washed with water, dried and concentrated to give the crude product. After column separation and purification of the crude product (PE:EA=3:1 - 1:1), compound **7** was obtained;

(8) Compound **6** (2.46 g) and compound **7** (10 g) were dissolved in dichloromethane (50 mL), then DIEA (8 mL) was added to the reaction mixture, which was then stirred at room temperature until the reaction was completed. Upon reaction, dichloromethane and water was added to the reaction mixture for extraction, after which the organic phase was dried and concentrated to yield a crude product. After column separation and purification of the crude product (dichloromethane: methanol = 10%-20%), compound **8** was obtained;

(9) Glutaric anhydride (10 g) and propargylamine (4.82 g) were dissolved in tetrahydrofuran (50 mL), and then the reaction was stirred at room temperature until it was completed. Upon completion, the reaction mixture was concentrated to dryness and compound **12** was obtained;

(10) Compound **12** (3.7 g) was dissolved in dichloromethane (50 mL), and then DCC (5.41 g), pentafluorophenol (4.83 g) were added to the reaction mixture, which was then stirred at room temperature until the reaction was completed. Upon completion, the reaction mixture was filtered and the filtrate was washed with water, dried and then concentrated to yield the crude product. After column separation and purification of the crude product (PE:EA = 3:1—1:1), compound **13** was obtained;

(11) Compound **13** (6.5 g) and aminocaproic acid (2.54 g) were dissolved in tetrahydrofuran (50 mL), then the reaction mixture was stirred at room temperature until the reaction was completed. Upon completion, the reaction mixture was concentrated to dryness. Dichloromethane and water were added to the residue for extraction. The organic phase was dried and further concentrated to yield a crude product. After column separation and purification (dichloromethane: methanol = 3%-8%) of the crude product, compound **14** was obtained;

(12) Compound **14** (15 g) was dissolved in dichloromethane (100 mL), and then DCC (3.94 g), pentafluorophenol (3.52 g) were added to the reaction mixture, which was then stirred at room temperature until the reaction was completed. Upon completion, the reaction mixture was filtered and the filtrate was washed with water, and then dried and concentrate to yield the crude product. After column separation and purification of the crude product (PE:EA=3:1 -1:1), compound **15** was obtained;

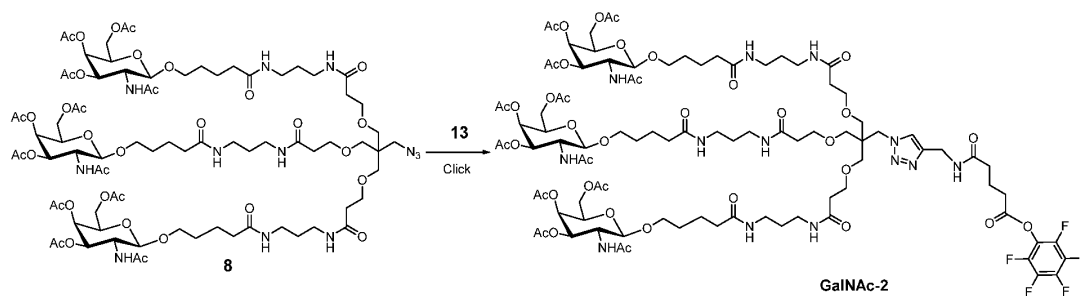
(13) Compound **8** (0.34 g) and compound **15** (0.09 g) were dissolved in THF (5 mL), then anhydrous copper sulfate (0.092 g), and sodium ascorbate 0.146g in an aqueous solution (5 mL) were added to the reaction mixture, which was then stirred at room temperature until the reaction was completed. Upon completion, THF was removed from the reaction mixture through concentration. The resulting residue was diluted with dichloromethane, decolorized by activated carbon, and dried with anhydrous sodium sulfate. The resulting solution was filtered to remove the insoluble, and then concentrated to yield a crude product. After column separation and purification of the crude product (dichloromethane: methanol =10%-15%), **GalNAc-1** was obtained.

Result

GalNAc-1 was obtained as a foam-like white solid (0.4 g). ¹H NMR (400 MHz, CDCl₃) δ: ppm 7.69 (s, 1H), 7.36 (dd, 4H), 6.97 (t, 3H), 6.81 (d, 1H), 6.74 (d, 2H), 6.40 (s, 1H), 5.35 (t, 3H), 5.19 (dd, 3H), 4.61 (d, 3H), 4.51 (d, 2H), 4.32 (s, 2H), 4.13 (m, 9H), 3.92 (dd, 6H), 3.65 (d, 6H), 3.50 (m, 3H), 3.27 (m, 18H), 2.68 (t, 2H), 2.44 (t, 6H), 2.33 (t, 2H), 2.21-1.26 (m, 61H). MS (ESI-TOF): m/z (M+H)⁺ 2283.44, (M+Na)⁺ 2305.41.

Example 2. Preparation of GalNAc-2

Synthetic scheme



5

Compound **8** and **13** were synthesized as described in **Example 1**.

Procedure

GalNAc-2 was prepared using the method described in **Example 1**.

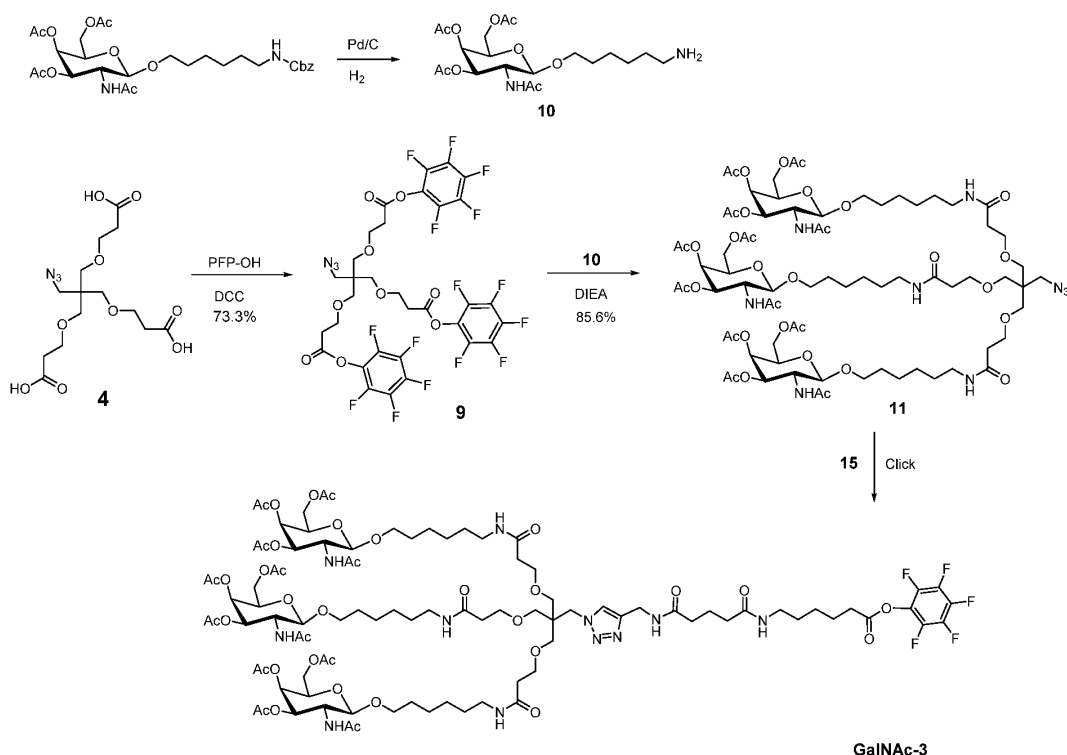
10

Result

GalNAc-2 was obtained as a white foam-like solid (0.2 g). ¹HNMR (400 MHz, CDCl₃) δ: ppm 7.73(s, 1H), 6.90-6.40(m, 10H), 5.36(d, 3H), 5.08(m, 3H), 4.60(s, 3H), 4.50(d, 2H), 4.35(s, 2H), 4.14(m, 9H), 3.92(s, 6H), 3.66(s, 6H), 3.51(m, 3H), 3.27(m, 18H), 2.79(t, 2H), 2.45(m, 6H), 2.25-1.80(m, 44H), 1.80-1.26(m, 20H). MS(ESI-TOF): m/z (M+Na)⁺ 2191.38.

Example 3. Preparation of GalNAc-3.

Synthetic scheme



Compound **4** and **15** were the same as described in example 1.

5 Procedure

Compound **9**, compound **11** and **GalNAc-3** were prepared using the methods for preparation of compound **7**, compound **8**, and **GalNAc-1** described in **Example 1**, respectively.

Cbz-hexylamine-galactose (5.5 g, purchased with Alading) was dissolved in ethyl acetate (100 mL), and then Pd/C (10%, 1g) was added to the reaction mixture, which was further stirred in a hydrogen atmosphere at room temperature before the reaction was completed. Upon completion of the hydrogenation reaction, the reaction mixture was filtered and the filtrate was dried and concentrated to yield compound **10**.

Result

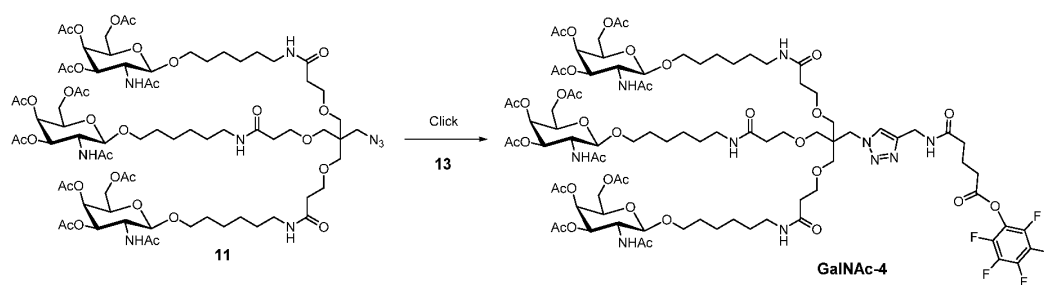
GalNAc-3 was obtained as a white foam-like solid (0.2 g). ^1H NMR (400 MHz, CDCl_3) δ : ppm. 7.47-6.32(m, 9H), 5.36(s, 3H), 5.28(m, 3H), 4.69(s, 3H), 4.54(s, 2H),

4.36(m,2H), 4.15(m,6H), 4.05(m,3H), 3.94(dd,6H), 3.66(s,6H), 3.47(m,3H),
3.25(m,12H), 2.68(t,2H), 2.44(m,6H), 2.30-1.80(m,42H), 1.80-1.26(m,32H).

MS(ESI-TOF):m/z (M+Na)⁺2133.43.

5 Example 4. Preparation of GalNAc-4

Synthetic scheme



Compounds **11** and **13** were prepared using methods described in **Examples 1** and **3**, respectively.

Procedure

GalNAc-4 was prepared from reacting compound **11** and **13** using the method described in **example 1**.

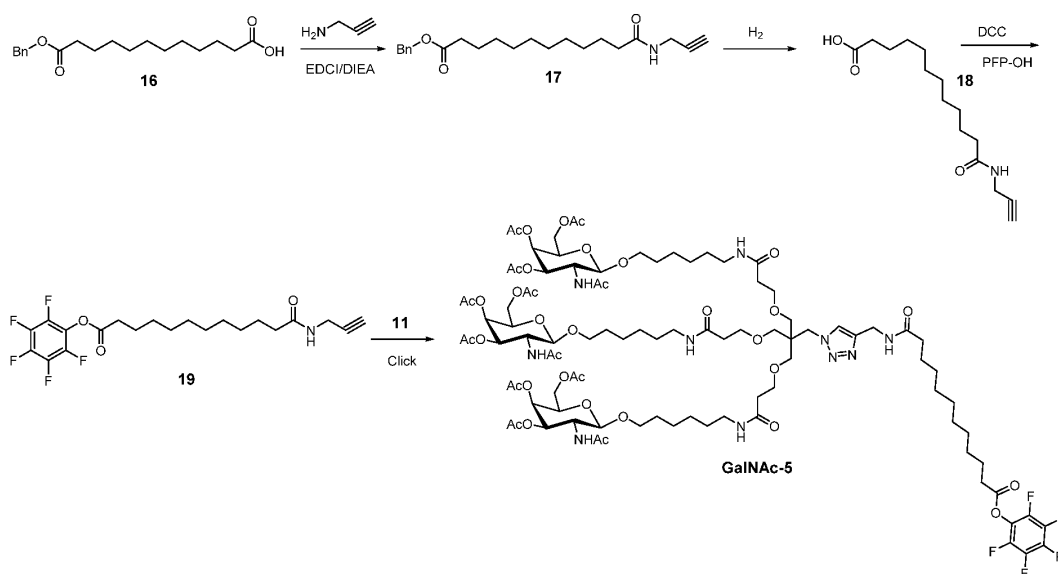
Result

GalNAc-4 was obtained as a white foam-like solid (0.2 g). ¹HNMR (400 MHz, CDCl₃) δ: ppm. 7.74-6.43(m,8H), 5.36(d,3H), 5.28(m,3H), 4.68(d,3H), 4.53(d,2H), 4.31(s,2H), 4.14(ddd,6H), 4.05(dd,3H), 3.89(ddd,6H), 3.67(m,6H), 3.46(dd, 3H), 3.24(s,12H), 2.78(t,2H), 2.43(m,6H), 2.30-1.80(m,38H), 1.80-1.26(m,26H).

MS(ESI-TOF):m/z (M+H)⁺1998.43,(M+Na)⁺2020.42.

20 Example 5. Preparation of GalNAc-5

Synthetic scheme



Compound **11** was the same as described in **Example 3**.

Procedure

(1) Compound **16** (1.6 g) was dissolved in dichloromethane (50 ml), then HOBT (2.23 g), EDCI (3.0 g), DIEA (3.3 g), and propargylamine (0.3 g) were added to the mixture. The reaction was then stirred at room temperature until the reaction was completed. Upon completion, dichloromethane and water were added to the reaction mixture for extraction, after which the organic phase was dried and concentrated to yield the crude product. After column separation and purification of the crude product (dichloromethane: methanol = 2% - 5%), compound **17** was obtained;

(2) Compound **17** (2 g) was added to methanol (10 mL), and then Pd/C (10%, 0.5 g) was added to the reaction mixture, which was further stirred in a hydrogen atmosphere at room temperature before the reaction was completed. Upon completion of the hydrogenation reaction, the reaction mixture was filtered and the filtrate was dried and concentrated to yield compound **18**;

(3) PFP-laurate (4.5 g) was dissolved in dichloromethane (100 mL), then DCC (3.94 g) and pentafluorophenol (3.53 g) were added to the reaction mixture, which was then stirred at room temperature until the reaction was completed. Upon completion, the

reaction mixture was filtered and the filtrate was washed with water, dried and concentrated to yield the crude product. After column separation and purification of the crude product (PE:EA = 3:1 - 1:1), compound **19** was obtained;

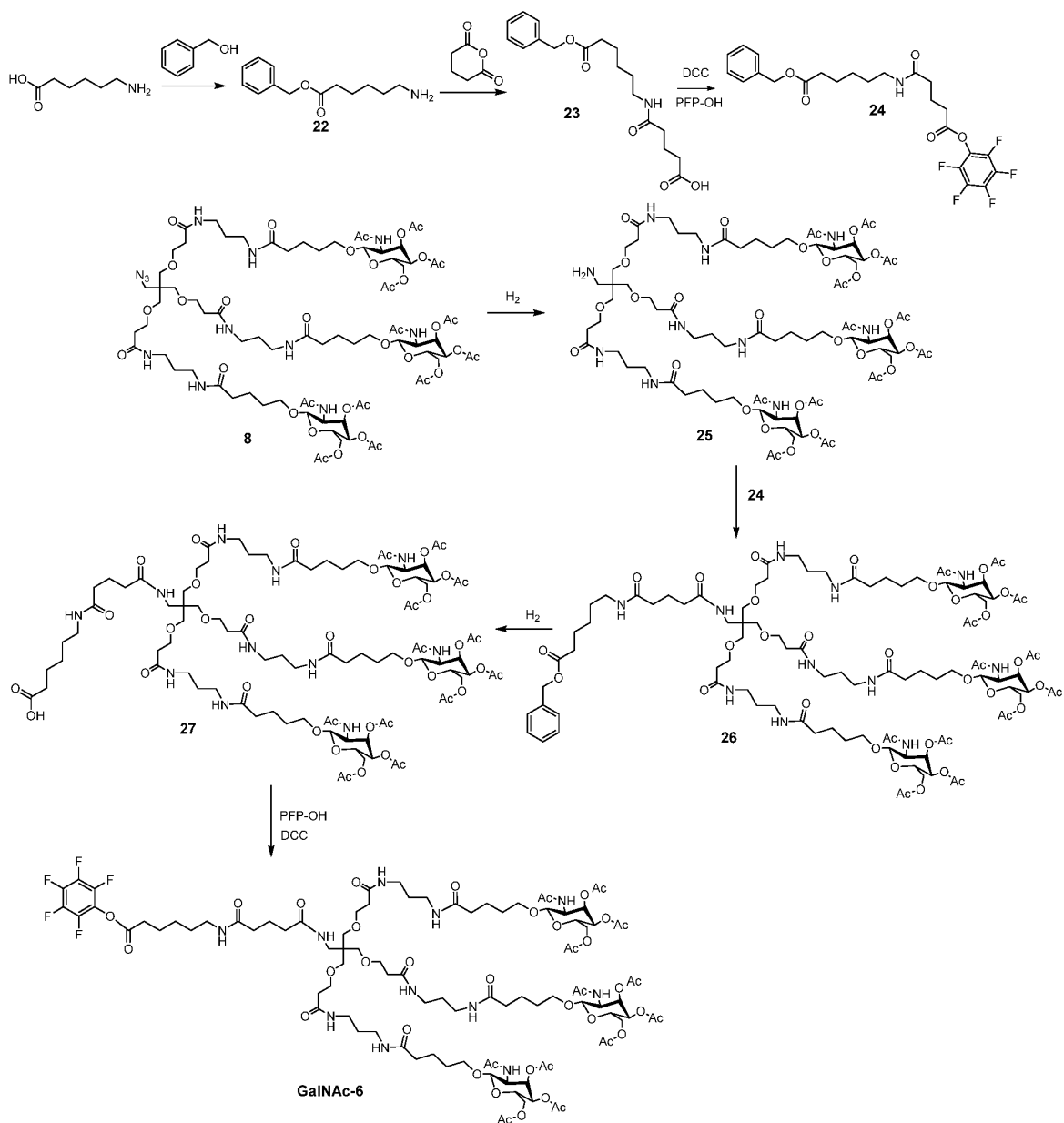
(4) **GalNAc-5** was prepared from reacting compound **11** and **19** using the method described in **Example 1**.

Result

GalNAc-5 was obtained as a white foam-like solid (0.4 g). ¹HNMR (400 MHz, CDCl₃) δ: ppm. 7.73-6.40(m, 8H), 5.35(d, 3H), 5.26(m, 3H), 4.67(d, 3H), 4.51(s, 2H), 4.33(s, 2H), 4.11(dd, 6H), 4.07(d, 3H), 3.89(dt, 6H), 3.66(m, 6H), 3.46(dd, 3H), 3.24(s, 12H), 2.75(t, 2H), 2.41(m, 6H), 2.25-1.80(m, 38H), 1.80-1.26(m, 40H). MS(ESI-TOF): m/z (M+H)⁺ 2096.19, (M+Na)⁺ 2119.20.

Example 6. Preparation of GalNAc-6

Synthetic scheme



Compound **8** was prepared using the method described in **Example 1**.

Procedure

(1) Aminocaproic acid (47.9 g) was dissolved in toluene (700 mL), then benzyl alcohol (57 mL) and 4-toluenesulfonic acid (74 g) were added to the reaction mixture, which was then heated up to 115°C while being stirred until the reaction was completed. Upon completion, the reaction mixture was cooled down to room temperature while being stirred, after which a large amount of solid precipitated. Methyl *tert*-butyl ether (500 mL) was added to the reaction mixture, which was then filtered. The solid was collected and dried to obtain compound **22**; (2) Compound **22** (19 g) was dissolved in dichloromethane (100 mL), then glutaric anhydride (11 g) in dichloromethane (100 mL) was added to the reaction mixture, which was then stirred at room temperature until the reaction was completed. Upon completion, the reaction mixture was concentrated to yield compound **23**; (3) Compound **23** (5 g) was dissolved in dichloromethane (100 mL), then DCC (3.6 g) and pentafluorophenol (3.3 g) were added to the reaction mixture, which was then stirred at room temperature before the reaction was completed. Upon completion, the reaction mixture was filtered and the filtrate was concentrated to yield the crude product. After column separation and purification of the crude product (PE:EA = 3:1-2:1), compound **24** was obtained; (4) Compound **8** (0.13 g) was dissolved in ethyl acetate (10 mL) and anhydrous methanol (10 mL), then Pd/C (0.2 g, 10%) and 3 drops of acetic acid were added to the reaction mixture, which was then stirred in a hydrogen atmosphere at room temperature until the reaction was completed. Upon completion, the reaction mixture was filtered and the filtrate was concentrated to yield compound **25**; (5) Compound **25** (2.8 g) was dissolved in dichloromethane (50 mL), then DIEA (0.5g) and compound **24** (0.78g) were added to the reaction mixture, which was then stirred at room temperature until the reaction completed. Upon reaction, the reaction mixture was concentrated to dryness. After column separation and purification of the crude product (dichloromethane: methanol 15% -25%), compound **26** was obtained; (6) Compound **26** (2.5 g) was dissolved in methanol (30 mL) and ethyl acetate (30 mL), then Pd/C (1.3 g, 10%) was added to the reaction mixture, which was then stirred in a hydrogen atmosphere at room temperature until the reaction was completed. Upon reaction completion, the reaction mixture was filtered and the filtrate was

concentrated to yield compound **27**; (7) Compound **27** (0.8 g) was dissolved in dichloromethane, then DCC (0.12g), pentafluorophenol (0.11g) were added to the reaction mixture, which was then stirred at room temperature until the reaction was completed. Upon completion, dichloromethane was added to the reaction mixture, which was then concentrated to yield the crude product. After column separation and purification of the crude product (dichloromethane: methanol = 10%-15%), **GalNAc-6** was obtained.

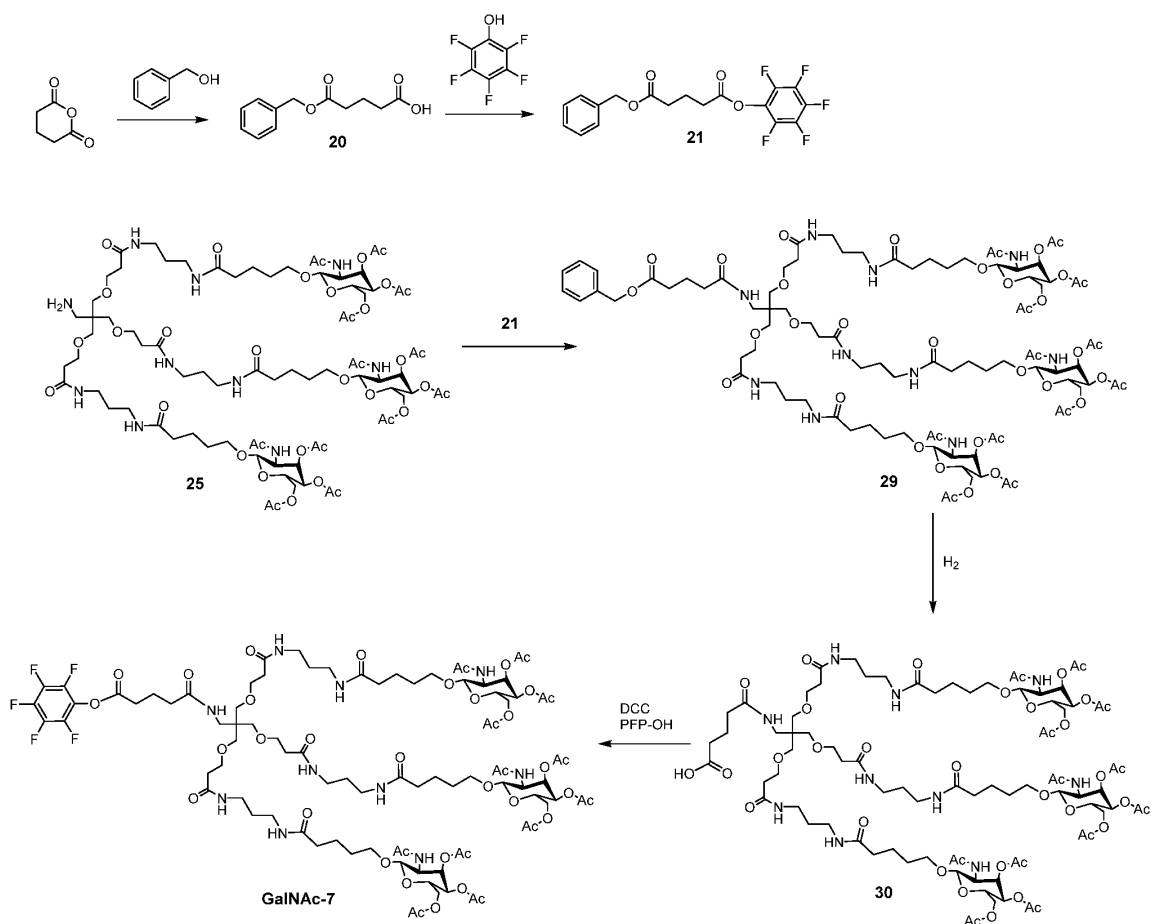
Result

GalNAc-6 was obtained as a white foam-like solid (0.7 g). ^1H NMR (400MHz, d-DMSO) δ : ppm. 7.75-7.26(m, 11H), 5.85(d, 3H), 5.59(d, 3H), 5.11(dd, 3H), 4.63(m, 6H), 4.50(d, 6H), 4.37(s, 3H), 4.17(d, 6H), 4.07(s, 3H), 3.90-3.70(m, 18H), 3.28(m, 2H), 2.92(d, 6H), 2.80-2.25(m, 52H), 2.25-1.26(m, 24H). MS(ESI-TOF): m/z (M+H) $^+$ 2201.82, (M+Na) $^+$ 2223.86.

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Example 7. Preparation of GalNAc-7

Synthetic scheme



Compound **25** was prepared as described in **Example 6**.

Procedure

- 5 Compound **21** was prepared using the method for preparation of compound **13** described in **Example 1**.

Compounds **29**, **30** and **GalNAc-7** were prepared using the methods for preparation of compounds **26**, **27** and **GalNAc-6** in **Example 6**, respectively.

Result

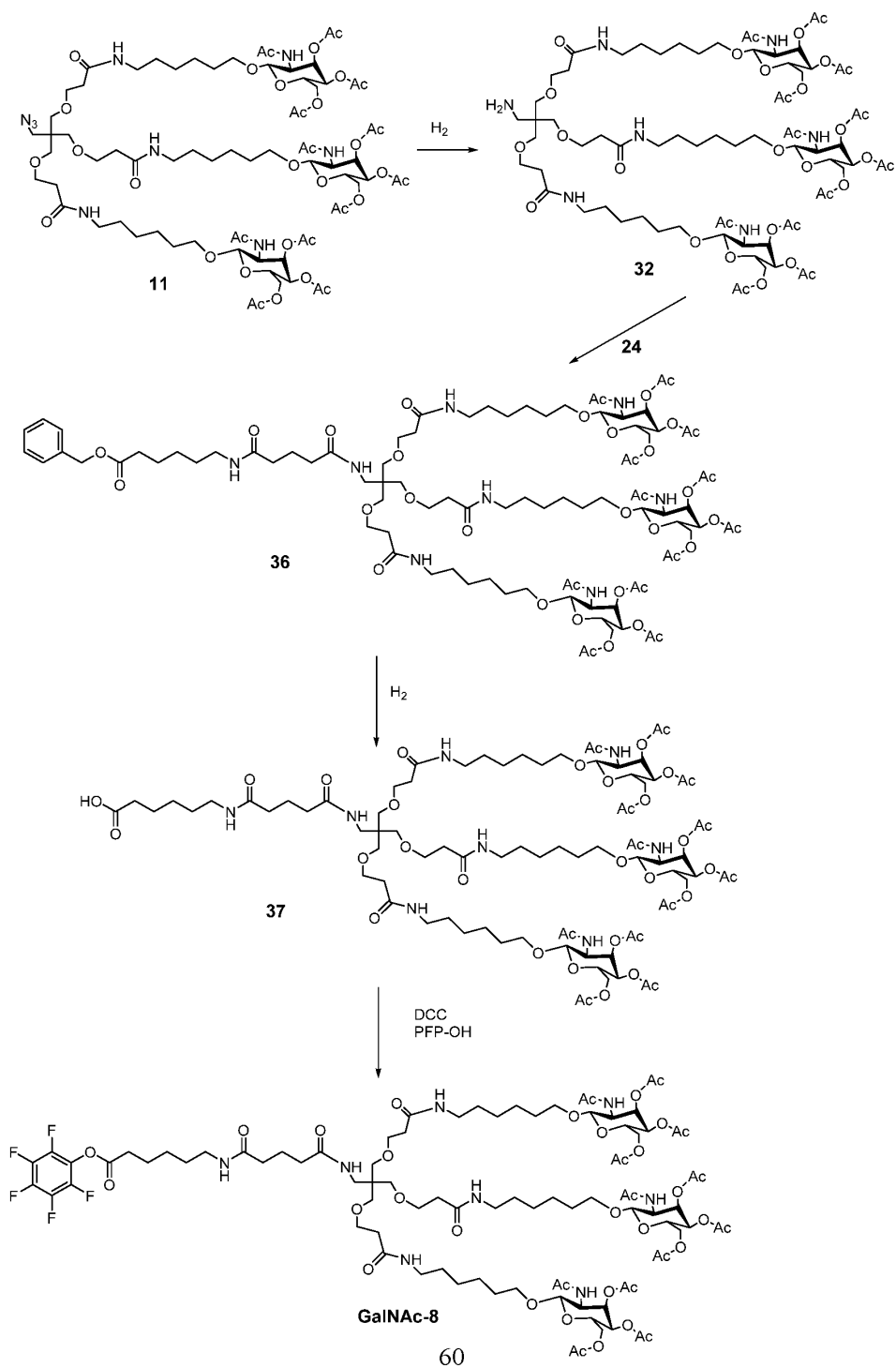
- 10 **GalNAc-7** was obtained as a white foam-like solid (0.5 g). ¹HNMR (400 MHz, CDCl₃) δ: ppm 7.32-6.63(m, 10H), 5.35(s, 3H), 5.20(s, 3H), 4.62(d, 3H),

4.13(ddd,9H), 3.92(d,6H), 3.66(dd,6H), 3.52(d,3H), 3.33(m,20H), 2.77(t, 2H), 2.44 (s, 6H), 2.25-1.80(m,44H), 1.80-1.26(m,20H). MS(ESI-TOF):m/z (M+H)⁺2088.08, (M+Na)⁺2109.36.

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Example 8. Preparation of GalNAc-8

Synthetic scheme



Compounds **11** and **24** were prepared as described in **Examples 3** and **example 6**, respectively.

Procedure

Compounds **32**, **36**, **37**, and **GalNAc-8** were prepared using the methods for preparation of compounds **25**, **26**, **27** and **GalNAc-6** in **Example 6**.

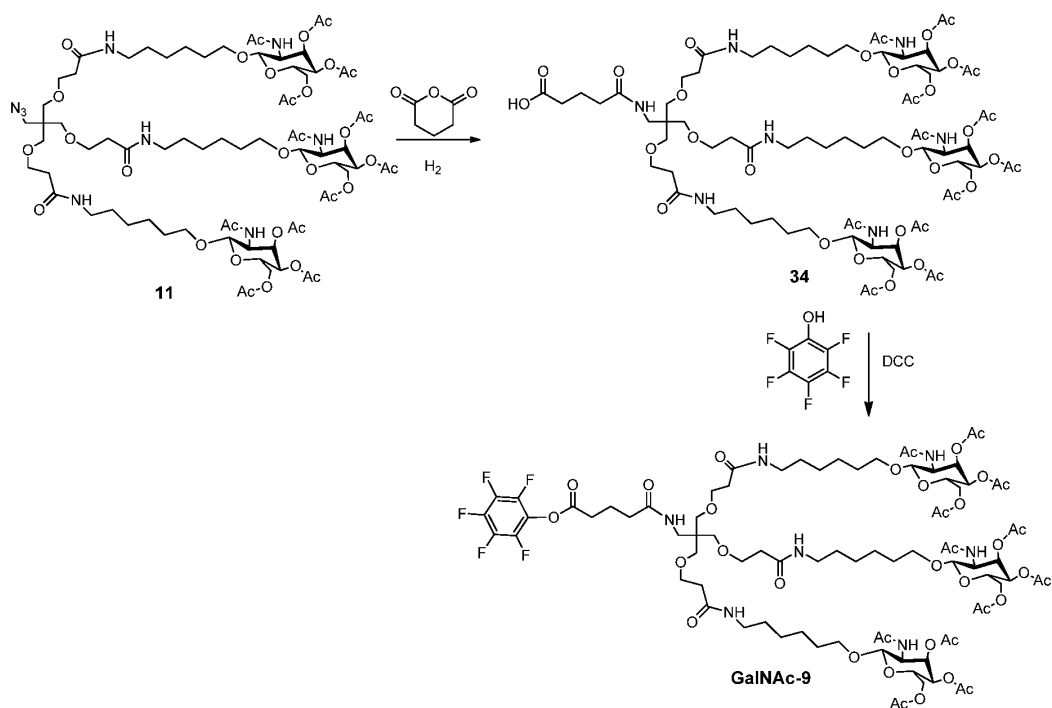
Result

^1H NMR (400MHz, CDCl_3) δ :ppm. 7.7.06-6.37(m,8H), 5.36(d,3H), 5.270(m,3H), 4.69(t,3H), 4.15(m,6H), 4.00(m,3H), 3.90(m,6H), 3.65(t,6H), 3.47(dt,3H), 3.28(m,16H), 2.68(t,2H), 2.42(m,6H), 2.29(t,4H), 2.15-1.80(s,36H), 1.80-1.26(m,32H).

MS(ESI-TOF):m/z ($\text{M}+\text{H}$) $^+$ 2030.38, ($\text{M}+\text{Na}$) $^+$ 2052.41.

Example 9. Preparation of GalNAc-9

Synthetic scheme



Compound **11** was prepared as described in **Example 3**.

Procedure

(1) Azide compound **11** (1.3 g) was dissolved in THF (15 mL), then Pd/C (0.3 g) and glutaric anhydride (0.11 g) were added to the reaction mixture, which was then stirred at room temperature in a hydrogen atmosphere until the hydrogenation reaction was completed. Upon completion, the reaction mixture was filtered and the filtrate was collected and concentrated the reaction residue. Dichloromethane and water were added to the reaction residue for extraction. The extracted organic phase was concentrated and concentrated to yield the crude product. After column separation and purification of the crude product (dichloromethane: methanol = 10%-15%), compound **34** (0.8 g) was obtained; (2) **GalNAc-9** was prepared using the method for preparation of **GalNAc-6** in example 6.

Result

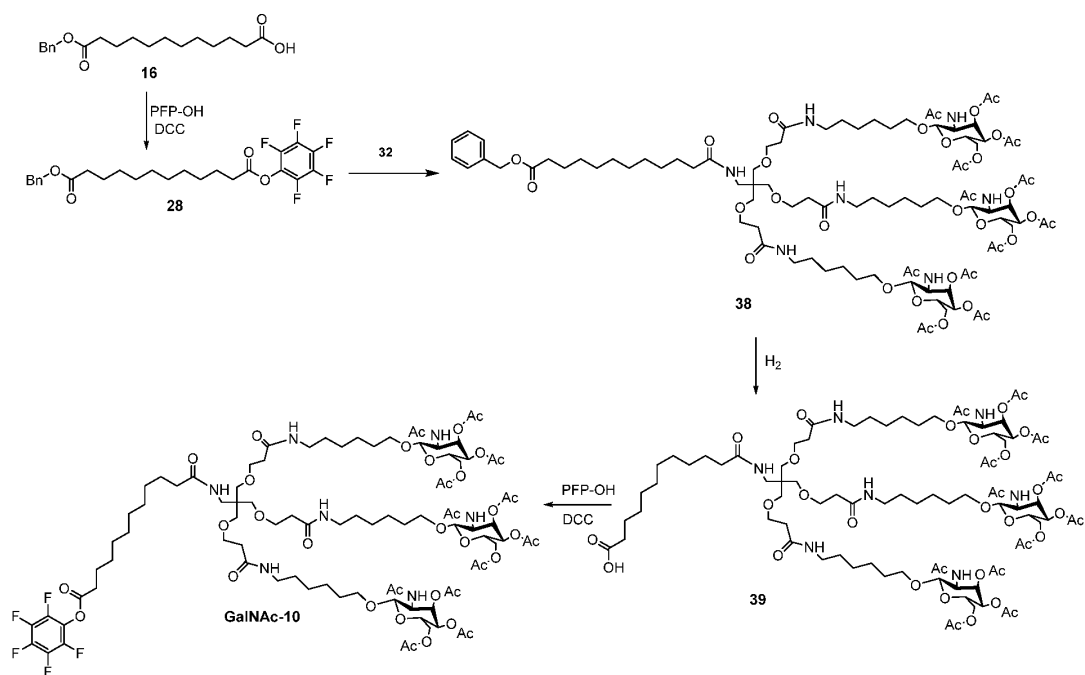
GalNAc-9 was obtained as a white foam-like solid (0.8 g).

¹HNMR(400MHz,d-DMSO) δ :ppm.7.63(s,1H), 7.42(s,3H), 7.17(s,3H), 5.85(d,3H), 5.60(t,3H), 5.10(dd,3H), 4.63(td,6H), 4.49(m,6H), 4.34(d,3H), 4.16(m,6H), 4.03(d,3H), 3.84(d,6H), 3.73(d,6H), 3.35(m,2H), 2.91(d,6H), 2.70-2.40(m,42H), 2.21-1.76(m,24H).

MS(ESI-TOF):m/z (M+H)⁺1917.93, (M+Na)⁺1938.69.

Example 10. Preparation of GalNAc-10

Synthetic scheme



Compound **28** in the present example was prepared as described for the synthesis of compound **24** in **Example 6**.

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Procedure

(1) Amino compound **32** (3 g) was dissolved in dichloromethane (30 mL), then DIEA (1ml) and compound **28** (1.31g) were added to the reaction mixture, which was then stirred at room temperature until the reaction was completed. Upon completion, dichloromethane was added to the reaction mixture and the resulting solution was concentrated to yield a crude product. After column separation and purification of the crude product (dichloromethane: methanol = 10%-15%), compound **38** was obtained; (2) Compound **39** and **GalNAc-10** were prepared using the methods for preparation of compound **27** and **GalNAc-6** in **example 6**, respectively.

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Result

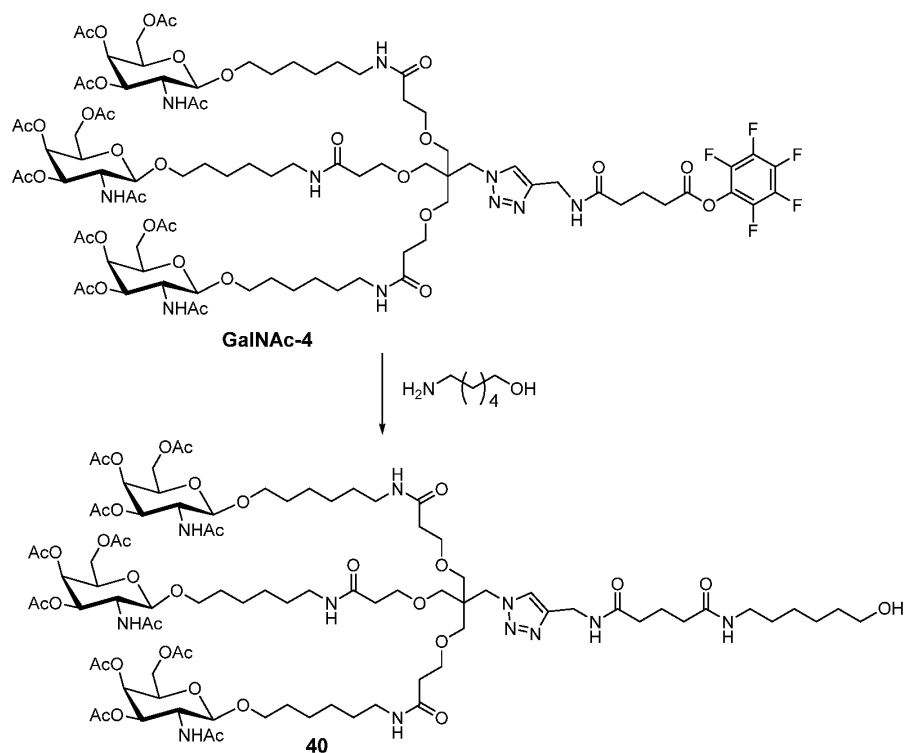
GalNAc-10 was obtained as a white foam-like solid (0.45 g). ^1H NMR (400 MHz, CDCl_3) δ : ppm 7.7.06-6.40(m,7H), 5.35(d,3H), 5.28(m,3H), 4.67(t,3H), 4.14(m,6H), 4.02(m,3H), 3.93(m,6H), 3.61(t,6H), 3.48(dt,3H), 3.29 (m,14H), 2.67(t,2H), 2.43(m,6H), 2.28(t,2H), 2.15-1.80(s,36H), 1.80-1.26(m,40H).

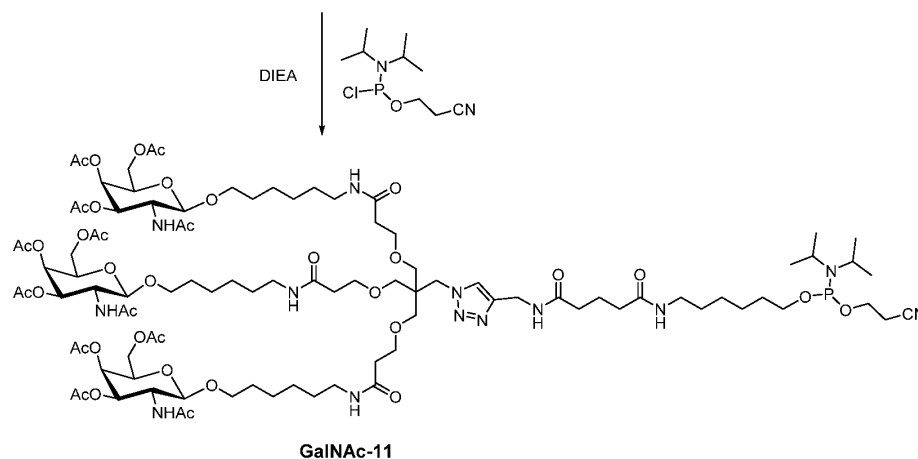
5 MS(ESI-TOF): m/z ($\text{M}+\text{H}$) $^+$ 2015.11, ($\text{M}+\text{Na}$) $^+$ 2038.47.

Example 11. Preparation of GalNAc-11

10

Synthetic scheme





Compound **GalNAc 4** is the same as described in **example 4**.

Procedure

- 5 (1) **GalNAc-4** (3 g) was dissolved in dichloromethane (30 mL), then DIEA (0.3ml), 1-hydroxyhexanoic acid (0.18g) were added to the reaction mixture, which was then stirred at room temperature until the reaction was completed. Upon completion, dichloromethane was added to the reaction mixture, which was then concentrated to yield the crude product. After column separation and purification of the crude product
- 10 (dichloromethane: methanol 10%-15%), compound **40** was obtained; (2) Compound **40** (1 g) was dissolved in anhydrous dichloromethane (10 mL), then the reaction mixture was cooled to 0 °C before DIEA (0.2ml), and phosphorous oxychloride (0.14g) was added. Then the reaction was stirred at 0 °C until it was completed. Upon completion, dichloromethane was added to the reaction mixture, which was then dried and
- 15 concentrated to yield the crude product. After column separation and purification of the crude product (dichloromethane: methanol 10%-15%), **GalNAc-11** was obtained.

Result

Compound 40: ¹HNMR (400 MHz, CDCl₃) δ: ppm. 7.71-6.38(m, 9H), 5.36(d, 3H), 5.27(m, 3H), 4.68(d, 3H), 4.53(s, 2H), 4.31(s, 2H), 4.15(m, 6H), 4.01(dd, 3H),

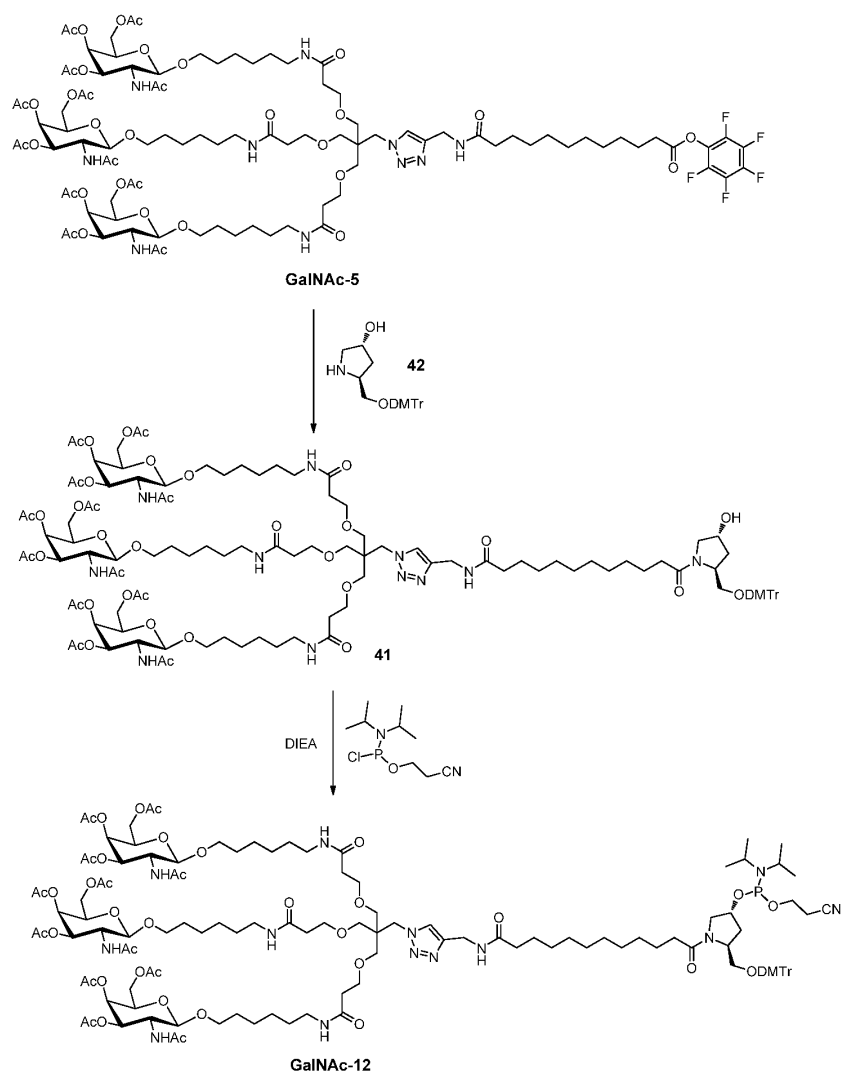
20 3.89(ddd, 6H), 3.65(dd, 8H), 3.46(m, 3H), 3.24(m, 14H), 2.46(d, 6H), 2.31(d, 2H), 2.23(s, 2H), 2.16-1.80(m, 36H), 1.80-1.26(m, 34H). MS(ESI-TOF): m/z (M-H)⁻ 1929.81.

GalNAc-11 was obtained as a white foam-like solid (0.5 g). MS(ESI-TOF):m/z (M+Na)⁺ 2153.62.

Example 12. Preparation of GalNAc-12

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Synthetic scheme



Compound **42** is purchased from Alaliding; compound **GalNAc-5** is the same as described in **Example 5**.

Procedure

(1) **GalNAc-5** (e.g., 2 g) is dissolved in dichloromethane (*ca.* 20 mL), then DIEA (*ca.* 0.3ml), compound **42** (*ca.* 0.44g) are added to the reaction, which is then stirred at room temperature before the reaction is deemed complete. Upon completion, dichloromethane is added to the reaction mixture, which is then dried and concentrated to yield the crude product. After column separation and purification of the crude product (dichloromethane: methanol using the appropriate gradient, e.g., 10%-15% methanol), compound **41** is obtained; (2) Compound **41** (e.g., 1 g) is dissolved in dry dichloromethane (*ca.* 10 mL), then the reaction mixture is cooled to 0 °C before DIEA (*ca.* 0.2ml) and phosphorous oxychloride (*ca.* 0.12g) are added. The reaction is then stirred at 0 °C until it is completed. Upon completion, dichloromethane is added to the reaction mixture, which is then dried and concentrated to yield the crude product. After column separation and purification of the crude product (dichloromethane: methanol under appropriate gradient, e.g., 10%-15% methanol), **GalNAc-12** is obtained.

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Example 13. Preparation of modified single-stranded oligonucleotide (antisense strand)

The sequence discussed in **Examples 13**, and **16-18** are as follows: 5'-Cy5-mUmGmAfCmAmAmAfCmGmGmGfCmAmAfCmAfUmAfC-3' (SEQ ID NO: 1). In this example, the modified oligonucleotide was synthesized on a 1 µmol scale. The experiment was carried out as described below:

(1) 1 µmol of standard Controlled Pore Glass (CPG) solid-phase support or 3'-cholesterol-modified CPG solid-phase support (purchased from Chemgenes), or 3'-amino-modified CPG solid-phase support (purchased from Kinovite)] was weighed. 2'-O-TBDMS-protected RNA phosphoramidite monomers, DNA monomers, 2'-methoxy monomers, 2'-fluoromonomers (purchased from Sigma Aldrich); together with amino-C16-12-phosphoramidite (or fluorescent phosphoramidite) for synthesis of 5' modification were dissolved in anhydrous acetonitrile to prepare a solution with a

30

concentration of 0.2 M. For oligonucleotides with thio-modification on the phosphate backbone, 0.2 M PADS solution was used as a thio-reagent. An acetonitrile solution with 0.25 M of 5-ethylthio-1*H*-tetrazole (purchased from Chemgenes) as the activator, a pyridine/water solution with 0.02 M of iodine as the oxidant, and a chloromethane solution with 3% of trichloroacetic acid as the deprotection reagent were prepared and placed in the designated positions of a DNA/RNA automatic synthesizer (GE AKTA™ OP100).

(2) The desired oligonucleotide base sequence was entered in the synthesis program, then the oligonucleotide synthesis is started and repeated. Each step of coupling takes 6 minutes, and each step of galactose ligand monomer coupling takes 10-20 minutes. The solid-phase oligonucleotide synthesis is completed after the automatic circulation.

(3) The CPG was dried with dry nitrogen, and then transferred to a 5 mL EP tube. Ammonia/ ethanol solution(3/1) (2 mL) was added to the EP tube, which was then heated at 55 °C for 16-18 hours. The resulting solution was centrifuged at 10,000 rpm for 10 min. The supernatant layer was collected, and its ammonia solution/ethanol was removed with reduced pressure to yield a white gel-like solid. The solid was dissolved in a TBAF solution (1M in THF, 200 μL) and shaken for 20 hours at room temperature. To the resulting mixture was added Tris-HCl buffer (ph 7.4, 1M, 0.5 mL). The mixture thus obtained as shaken at room temperature for 15 minutes and centrifuged to ½ of the original volume. The resulting mixture was extracted twice with choloroform (0.5 mL) and treated with a TEAA sampling solution (1 mL 0.1M) to provide a solution, which was then poured into a solid phase extraction column to remove excess salt in the solution.

(4) The concentration of the obtained oligonucleotide was determined by a micro-volume UV-visible spectrophotometer (KO5500). The mass spectrometry of the oligonucleotide was detected and analyzed on an Oligo HTCS LC-MS system (Novatia). After the first-level scan, the molecular weight was calculated through a normalized method on the Promass software.

Example 14. Preparation of modified single-stranded oligonucleotide (sense strand)

The sequence discussed in **Examples 14-18** are as follows: 5'-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA-3' (SEQ ID NO: 2). In this example, the modified oligonucleotide was synthesized on 1 μ mol scale. The experiment was carried out as described below.

(1) 1 μ mol of standard Controlled Pore Glass (CPG) solid-phase support or 3'-cholesterol-modified CPG solid-phase support (purchased from Chemgenes), or 3'-amino-modified CPG solid-phase support (purchased from Kinovite)], DNA monomers, 2'-methoxy monomers, 2'-fluoromonomers (purchased from Sigma Aldrich), amino-C16-12-phosphoramidite (or fluorescent phosphoramidite) for synthesis of 5'-modification were dissolved in anhydrous acetonitrile to make a solution of 0.2 M. For oligonucleotides with thio-modification on the phosphate backbone, 0.2 M PADS solution was used as a thio-reagent. An acetonitrile solution (0.25 M) of 5-ethylthio-1*H*-tetrazole (purchased from Chemgenes) as the activator, a pyridine/water solution (0.02 M) of iodine as the oxidant, and a chloromethane solution (3%) of trichloroacetic acid as the deprotection reagent were prepared and placed in the designated position in the DNA/RNA automatic synthesizer (GE AKTAOP100).

(2) The desired oligonucleotide base sequence was entered in the synthesis program, then the oligonucleotide synthesis is started and repeated. Each step of coupling takes 6 minutes, and each step of galactose ligand monomer coupling takes 6-10 minutes. The solid-phase oligonucleotide synthesis is completed after the automatic circulation.

(3) The CPG was dried with dry nitrogen, and then transferred to a 5 mL EP tube. Ammonia solution (2 mL) was added to the EP tube, which was then heated at 55 °C for 16-18 hours. The resulting solution was centrifuged at 10,000 rpm for 10 min. The supernatant layer was collected, and its ammonia solution/ethanol was removed with reduced pressure to yield a white or yellow gel-like solid. TEAA sampling solution (1 mL 0.1M) was added to the solid to create a solution, which was then poured into a solid phase extraction column to remove excess salt in the solution.

(4) The concentration of the obtained oligonucleotide was determined by a micro-volume UV-visible spectrophotometer (KO5500). The mass spectrometry of the

oligonucleotide was detected and analyzed on an Oligo HTCS LC-MS system (Novatia). After the first-level scan, the molecular weight was calculated through a normalized method on the Promass software.

5 Example 15. Preparation of GalNAc-modified oligonucleotides

The amino-modified oligonucleotide (sense strand) prepared in **Example 14** was dissolved in a buffer solution. Various GalNAc-pentafluorophenol esters (GalNAc selected from GalNAc-1 to GalNAc-10) dissolved in acetonitrile were respectively added to the amino-modified oligonucleotide solution, mixed well, and then reacted at room temperature for at least 3 hours. After the reaction is completed, the acetyl protecting group was removed, and the reaction product was purified by ion exchange chromatography (WATERS) using a linear gradient DNAPAc PA-100 ion exchange column. The mobile phase A solution was 20 mM NaOH, and the mobile phase B solution was 20 mM NaOH + 2 M NaCl mixture.

Table 1. GalNAc-modified oligonucleotides (sense strand)

Sample Number	Structure (5'-3')	Measured Molecular Weight
Sense strand -101	5'-GalNAc-1-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3'	7497.93
Sense strand -102	5'-GalNAc-2-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3'	7669.08
Sense strand -103	5'-GalNAc-3-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3'	7611.08
Sense strand -104	5'-GalNAc-4-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3'	7782.24
Sense strand -105	5'-GalNAc-5-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3'	7750.16
Sense strand -106	5'-GalNAc-6-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3'	7579.00
Sense strand -107	5'-GalNAc-7-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3'	7692.16

Sense strand -108	5'-GalNAc-8-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3'	7863.31
Sense strand -109	5'-GalNAc-9-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3'	7753.27
Sense strand -110	5'-GalNAc-10-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3'	7810.32

Note: N=RNA; dN=DNA; mN=2'OMe modification; and fN=2'F modification.

Sequence of exemplary modified oligonucleotides and corresponding molecular weight (MW) detection results are shown in Table 1.

5

Example 16. Preparation of double-stranded oligonucleotides

The experiment was carried out as follows: The 5'-Cy5-antisense strand oligonucleotides synthesized in Example 13 were respectively mixed with the sense strand-GalNAc-1-10 oligonucleotides prepared in Example 15, according to the UV 10 absorption ratio of 1:1. The mixture was incubated at 95°C for 3 minutes in a heated water bath, then cooled to room temperature to form double-stranded oligonucleotides.

Table 2. Double-stranded RNA structures

Sample number	Double-stranded structure (5'-3')
101	5'-GalNAc-1-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA-3' 3'-fCmAfUmAfCmAfCmGmGmGfCmA mAfCmA mGmU-Cy5-5'
102	5'-GalNAc-2-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3' 3'-fCmAfUmAfCmAfCmGmGmGfCmA mAfCmA mGmU-Cy5-5'
103	5'-GalNAc-3-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3' 3'-fCmAfUmAfCmAfCmGmGmGfCmA mAfCmA mGmU-Cy5-5'
104	5'-GalNAc-4-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3' 3'-fCmAfUmAfCmAfCmGmGmGfCmA mAfCmA mGmU-Cy5-5'
105	5'-GalNAc-5-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3' 3'-fCmAfUmAfCmAfCmGmGmGfCmA mAfCmA mGmU-Cy5-5'
106	5'-GalNAc-6-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3' 3'-fCmAfUmAfCmAfCmGmGmGfCmA mAfCmA mGmU-Cy5-5'
107	5'-GalNAc-7-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3' 3'-fCmAfUmAfCmAfCmGmGmGfCmA mAfCmA mGmU-Cy5-5'

108	5'-GalNAc-8-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3' 3'-fCmAfUmAfCmAfCmGmGmGfCmA mA mA fCmA mGmU-Cy5-5'
109	5'-GalNAc-9-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3' 3'-fCmAfUmAfCmAfCmGmGmGfCmA mA mA fCmA mGmU-Cy5-5'
110	5'-GalNAc-10-mGfUmAfUmGfUfUmGfCfCfCmGfUfUfUmGfUfCmA3' 3'-fCmAfUmAfCmAfCmGmGmGfCmA mA mA fCmA mGmU-Cy5-5'

Note: N=RNA; dN=DNA; mN=2'OMe modification; and fN=2'F modification.

Example 17. Detection of cell targeting capability of modified oligonucleotides

5 Modified oligonucleotides for animal experiments were filtered through a 0.22
um membrane before injection.

1. Isolation of mouse primary hepatocytes

Mice obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. were anesthetized. Mouse skin and muscle layer were cut open to expose the liver. Perfusion catheter was inserted into the portal vein, and a small opening was cut in the inferior vena cava to prepare the liver for perfusion. The perfusion Solution I (Hank's, 0.5 mM EGTA, pH 8) and perfusion Solution II (low-glucose DMEM, 100 U/mL Type IV, pH 7.4) were pre-warmed to 40°C. The perfusion solution I (at 37°C) was infused into the liver along the portal vein at a flow rate of 7 mL/min for 5 minutes until the liver turned gray. Then, the liver was perfused with 37°C perfusion Solution II at a flow rate of 7 mL/min for 7 minutes. After the perfusion is complete, the liver was isolated and placed in Solution III (10% FBS, low-glucose DMEM, 4°C) to terminate the digestion. The liver envelope was pierced with forceps, and gently shaken to release the hepatocytes. The hepatocytes were filtered with a 70 µm cell strainer, and then centrifuged at 50 g for 2 minutes. After centrifugation, supernatant was discarded. The hepatocytes were then resuspended in Solution IV (40% percoll low-glucose DMEM, 4°C), and centrifuged at 100 g for 2 minutes. Supernatant was discarded, and 2% FBS low-glucose DMEM was added to resuspend the cells for subsequent experiments. Trypan blue staining was used to measure cell viability.

2. Determination of the GalNAc binding curve and K_d value

The freshly isolated mouse primary hepatocytes were plated into a 96-well plate at 2×10^4 cells/well (100 μ L/well). Different GalNAc-siRNAs were added to each well (see Table 2). The final concentration of each GalNAc-siRNA was 0.9 nM, 2.7 nM, 8.3 nM, 25 nM, 50 nM or 100 nM. The mouse hepatocytes were incubated with GalNAc-siRNAs for 2 hours at 4°C, and then centrifuged at 50 g for 2 minutes. Supernatant was discarded. Next, the hepatocytes were resuspended in 10 μ g/ml propidium iodide (PI), stained for 10 minutes, and then centrifuged at 50 g for 2 minutes. The hepatocytes were then washed with cold PBS, followed by centrifugation at 50 g for 2 minutes. Supernatant was discarded and the hepatocytes were resuspended in PBS. The mean fluorescence intensity (MFI) of living cells were measured by a flow cytometer (Beckman). GraphPad Prism 5 software was used to perform nonlinear fitting and calculation of dissociation constant Kd.

The results are listed in Table 3 below, which showed that GalNAc-siRNA can specifically target hepatocytes (or liver cells). The Kd values of the tested GalNAc ligands binding to the cell receptor were determined between 1.5-27.6 nM.

The inhibition constant Ki was also calculated based on the function $K_i = IC_{50} / (1 + [S]/K_m)$, wherein IC50 stands for inhibitory concentration 50%, [S] is the substrate concentration, and Km is the Michaelis constant. As compared with the preferred galactose ligands disclosed in PCT/US2014/046425 (with Ki values determined between 5.2-51.3 nM, *see* Example 44), the GalNAc ligands as described herein exhibited higher binding affinities. In addition, GalNAc-siRNAs with different conjugate structures exhibited different receptor binding capabilities. For example, the structures of 101, 104, 108 and 109 exhibited relatively strong receptor binding affinities (the smaller the Kd value, the greater the affinity).

Table 3. Kd and Ki values of each experimental group (nM)

Sample number	101	102	103	104	105
Kd	1.879	3.118	4.265	1.958	19.64
Ki	1.6	2.922	1.049	1.031	12.57
Sample number	106	107	108	109	110
Kd	4.632	2.066	1.874	1.982	6.371

Ki	2.984	1.161	1.018	1.057	2.708
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Example 18. *In vivo* liver targeting test

30 male, 6-7 weeks old specific-pathogen-free Balb/c-nu mice (purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.) were used. The mice were randomly divided into 6 groups: blank control group, negative control group (or NC1, unconjugated with ligand), test group 1, test group 2, test group 3, and test group 4. The number of mice in each group was 5. The mice were administered by intravenous tail injection, and the dose was about 10 mg/kg (see Table 4 for experimental design). Live imaging of all animals, including white light imaging, was performed before administration, and 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours after administration. The mice were euthanized 6 hours after administration. The brain, salivary glands, heart, spleen, lungs, liver, kidneys and intestines were isolated for organ imaging (by Xtreme of Bruker Corporation).

Table 4. Liver targeting experiment design

Serial No.	Group	Sample No.	Administration Dose (mg/kg)	Administration Volume (mL)
1	Blank control	Saline	0	0.2
2	NC1	100	10	0.2
3	Test group 1	104	10	0.2
4	Test group 2	107	10	0.2
5	Test group 3	108	10	0.2
6	Test group 4	110	10	0.2

The *in vitro* imaging results showed that Sample Nos. 100, 104, 107, 108, and 110 were mainly distributed in the liver, kidney, and gastrointestinal tract, but less in the brain, heart, lungs, spleen and other tissues.

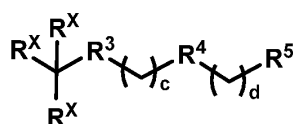
According to the statistical results of the average total photon numbers, Sample Nos. 104, 107, 108 and 110 showed some liver targeting effects, as compared with the 100 group (negative control group). Further, Sample No. 107 and 108 showed statistically significant differences ($P < 0.001$). Sample No. 104 ($P < 0.01$) and Sample No. 110 ($P < 0.05$) also showed statistically significant differences.

Table 5. Statistic results of fluorescence intensity values of isolated organs after subtracting background (average total photon number p/sec)

Group	Brain	Heart	Lungs	Liver	Left kidney	Right Kidney	Spleen	Gastrointestinal tract
Blank control	1.0E+11	3.0E+10	1.2E+11	3.9E+11	5.4E+10	6.0E+10	7.2E+10	4.5E+12
NC1	4.1E+11	4.3E+11	1.6E+12	1.4E+13	6.3E+12	6.6E+12	1.4E+12	2.5E+13
Test group 1	4.9E+11	4.7E+11	1.6E+12	3.1E+13	7.7E+12	8.2E+12	1.3E+12	2.5E+13
Test group 2	6.6E+11	5.7E+11	1.9E+12	3.2E+13	7.9E+12	8.2E+12	1.5E+12	2.8E+13
Test group 3	6.3E+11	4.6E+11	2.1E+12	3.3E+13	7.0E+12	7.2E+12	1.1E+12	2.5E+13
Test group 4	5.6E+11	4.1E+11	1.8E+12	2.9E+13	6.9E+12	7.8E+12	1.1E+12	2.3E+13

WHAT IS CLAIMED IS:

1. A compound of Formula (I):

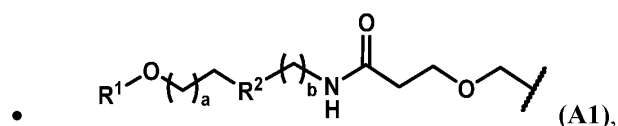


Formula (I)

or a pharmaceutically acceptable salt thereof, wherein:

each R^{X} is independently selected from the group consisting of:

- H; and
- $-\text{CH}_2\text{OR}^{\text{X}2}$, wherein $\text{R}^{\text{X}2}$ is H, C_{1-6} alkyl, or a hydroxyl protecting group;



provided that at least one R^{X} is a group of Formula (A1);

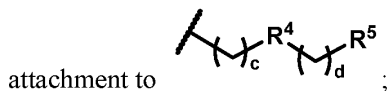
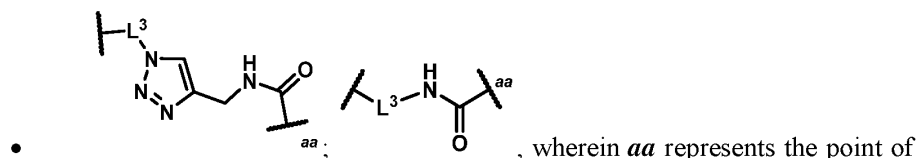
each R^1 is an independently selected moiety capable of binding an asialoglycoprotein receptor (ASGPR);

each R^2 is independently selected from the group consisting of: $-\text{C}(\text{R}^6)_2-$; $-\text{OC}(\text{R}^6)_2\text{C}(\text{R}^6)_2\text{O}-$; $-\text{C}(\text{R}^6)(\text{OH})-\text{C}(\text{R}^6)(\text{OH})-$; $^*\text{-C}(=\text{O})\text{NR}^7-$; $^*\text{-NR}^7\text{C}(=\text{O})-$; C_{6-10} arylene; C_{2-6} alkenylene; and C_{2-6} alkynylene,

wherein the C_{6-10} arylene, C_{2-6} alkenylene, and C_{2-6} alkynylene are each optionally substituted with 1-4 independently selected R^a , and the * represents the point of

attachment to $\text{R}^1 - \text{O} - \text{---} \text{---}$;

R^3 is selected from the group consisting of:

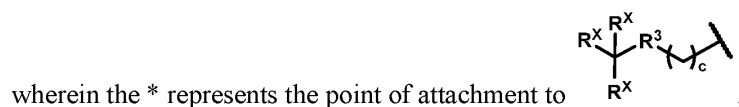


- $-(\text{CR}^6\text{R}^6)_x-\text{O}-(\text{CR}^6\text{R}^6)_y-$, wherein *x* and *y* are independently 0, 1, 2, or 3; and
- $-\text{L}^3-\text{L}^{3\text{C}}-$, wherein $\text{L}^{3\text{C}}$ is selected from the group consisting of: C_{3-10} cycloalkylene, C_{6-10} arylene, 5-10 membered heteroarylene, and 4-10 membered heterocyclylene, each of which is optionally substituted with 1-4 independently selected R^a ;

L^3 is $-\text{C}(\text{R}^6)_2-$,

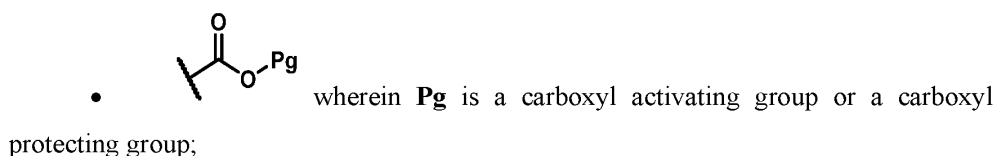
- 10 R^4 is selected from the group consisting of: $-\text{C}(\text{R}^6)_2-$; $-\text{OC}(\text{R}^6)_2\text{C}(\text{R}^6)_2\text{O}-$; $-\text{C}(\text{R}^6)(\text{OH})-\text{C}(\text{R}^6)(\text{OH})-$; $^*\text{-C}(=\text{O})\text{NR}^7-$; $^*\text{-NR}^7\text{C}(=\text{O})-$; C_{6-10} arylene, C_{3-10} cycloalkylene, 5-10 membered heteroarylene, and 4-10 membered heterocyclylene,

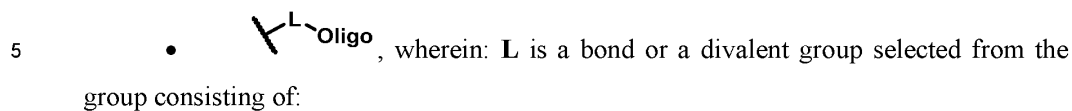
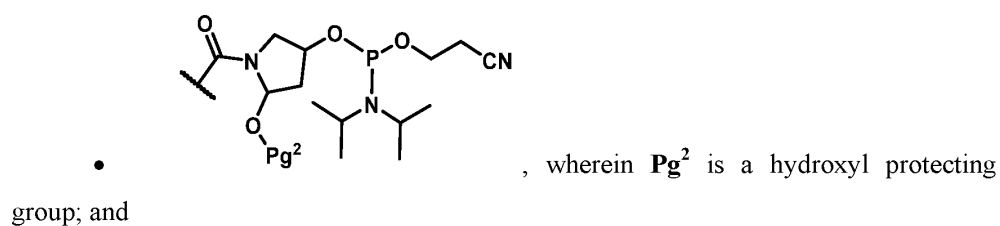
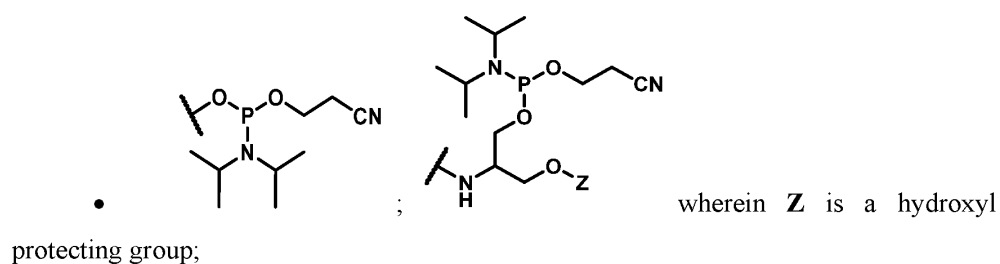
- wherein the C_{6-10} arylene, C_{3-10} cycloalkylene, 5-10 membered heteroarylene, and 4-10 membered heterocyclylene are each optionally substituted with 1-4 independently
15 selected R^a , and



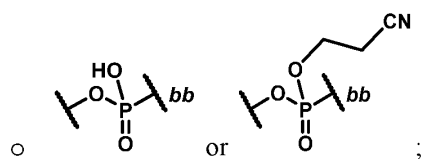
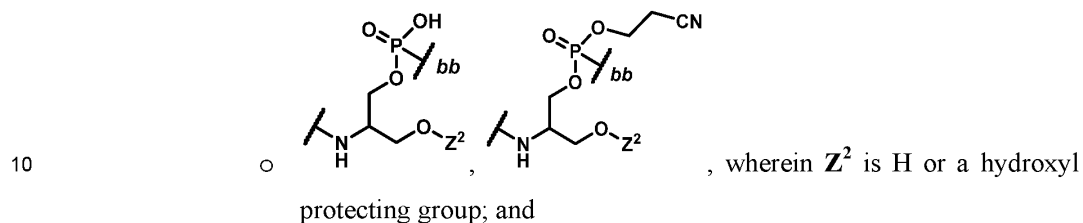
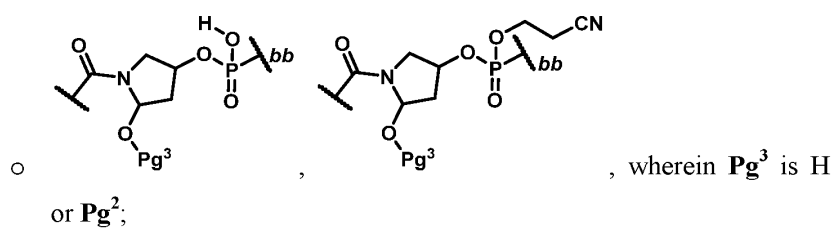
R^5 is selected from the group consisting of:

- 20 • hydroxyl; $\text{C}(\text{O})\text{OH}$;





- **-R²-, -R³-, -R⁴-, -O-, -C(=O)-, -C(=O)O-, -OC(=O)-,**



wherein **bb** represents the point of attachment to **Oligo**, and

- **Oligo** is an oligonucleotide;

each R^6 is independently selected from the group consisting of: H; C_{1-3} alkyl; C_{1-3} haloalkyl; and halo;

each R^7 is independently selected from the group consisting of: H; and C_{1-3} alkyl.

5

a and **b** are each independently selected integers from 1 to 10;

c and **d** are each independently selected integers from 0 to 10; and

each occurrence of R^a is independently selected from the group consisting of:
halo, cyano, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy.

10

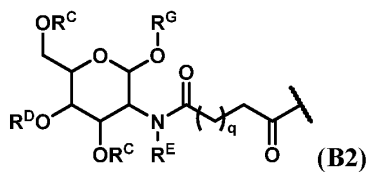
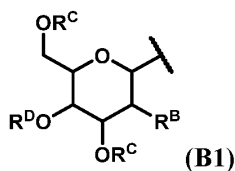
2. The compound of claim 1, wherein at least two R^x are each independently a group of Formula (A1).

15

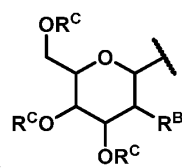
3. The compound of claims 1 or 2, wherein each R^x is independently a group of Formula (A1).

4. The compound of any one of claims 1-3, wherein each R^1 is independently a group of Formula (B1) or (B2):

20



wherein:



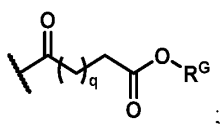
R^D is selected from the group consisting of: R^C and

each R^B is independently selected from the group consisting of: $-NR^E R^F$ and $-OR^C$;

each R^C is independently selected from the group consisting of: H and $-C(=O)C_{1-6}$ alkyl;

each R^E is independently $C(=O)C_{1-6}$ alkyl;

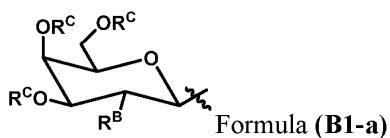
each R^F is independently selected from the group consisting of: H and



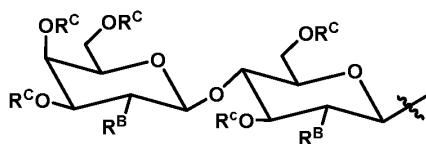
R^G is C_{1-6} alkyl; and

each q is independently an integer selected from 1 to 10.

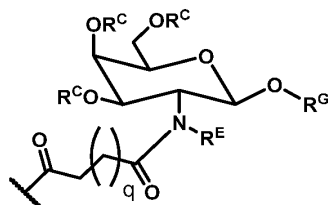
5. The compound of claim 4, wherein each R^1 is independently a group having Formula (B1-a), (B1-b), or (B2-a):



Formula (B1-a)



Formula (B1-b)



Formula (B2-a).

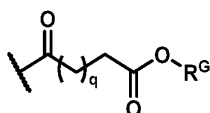
6. The compound of claims 4 or 5, wherein each R^B is independently $NR^E R^F$.

7. The compound of any one of claims 4-6, wherein each R^E is
5 independently $-C(=O)C_{1-6}$ alkyl.

8. The compound of any one of claims 4-7, wherein each R^E is $-C(=O)CH_3$.

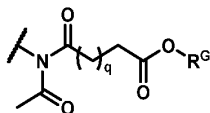
9. The compound of any one of claims 4-8, wherein each R^F is H.
10

10. The compound of any one of claims 4-8, wherein each R^F is



11. The compound of any one of claims 4-6, wherein each R^B is
15 $NHC(=O)CH_3$.

12. The compound of any one of claims 4-6, wherein each R^B is



13. The compound of claims 4 or 5, wherein each R^B is independently $-OR^C$.
20

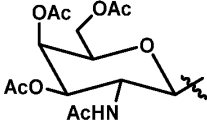
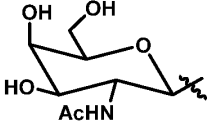
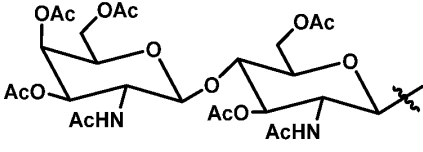
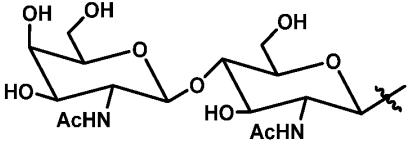
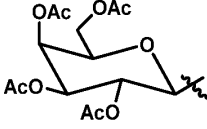
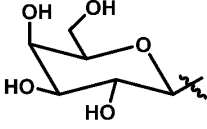
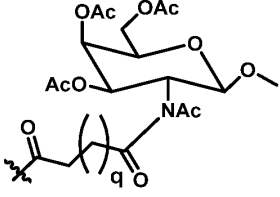
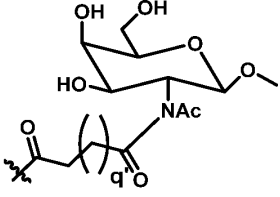
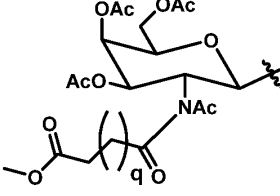
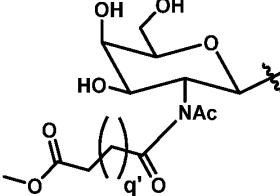
14. The compound of any one of claims 4-13, wherein each R^C is
independently $C(=O)C_{1-6}$ alkyl.

15. The compound of any one of claims 4-14, wherein each R^C is $C(=O)CH_3$.
25

16. The compound of any one of claims 4-14, wherein each R^C is H.

17. The compound of any one of claims 4-16, wherein each R^G is CH_3 .

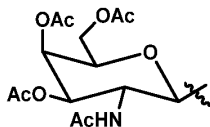
18. The compound of any one of claims 1-5, wherein each R^1 is selected from the group consisting of the following:

	
	
	
 wherein, q is an integer selected from 1 to 10;	 wherein, q' is an integer selected from 1 to 10;
 wherein, q is an integer selected from 1 to 10;	 wherein, q' is an integer selected from 1 to 10.

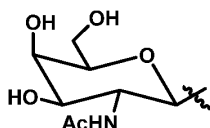
5

19. The compound of any one of claims 1-18, wherein each R^1 is the same.

20. The compound of any one of claims 1-19, wherein each R^1 is



21. The compound of any one of claims 1-19, wherein each R^1 is



5

22. The compound of any one of claims 1-21, wherein each R^2 is independently $^*-C(=O)NR^7$ - or $^*-NR^7C(=O)-$.

10 23. The compound of any one of claims 1-22, wherein each R^2 is independently $^*-C(=O)NR^7$ -.

24. The compound of any one of claims 1-23, wherein each R^2 is $^*-C(=O)NH-$.

15

25. The compound of any one of claims 1-21, wherein each R^2 is independently $-C(R^6)_2-$.

26. The compound of any one of claims 1-21 or 25, wherein each R^2 is $-CH_2-$.

20

27. The compound of any one of claims 1-26, wherein each R^2 is the same.

28. The compound of any one of claims 1-27, wherein each a is an independently selected integer from 1 to 4.

25

29. The compound of any one of claims 1-28, wherein each a is independently 2 or 3.

30. The compound of any one of claims 1-29, wherein each **a** is the same.

31. The compound of any one of claims 1-30, wherein each **b** is an
independently selected integer from 1 to 4.

32. The compound of any one of claims 1-31, wherein each **b** is independently
2 or 3.

33. The compound of any one of claims 1-32, wherein each **b** is the same.

34. The compound of any one of claim 1-21, wherein each **a** is the same; each
b is the same; and each **R**² is the same.

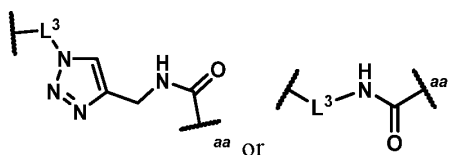
35. The compound of claim 34, wherein **a** is an integer from 1 to 4; **b** is an
integer from 1 to 4; and **R**² is *-C(=O)NR⁷.

36. The compound of claim 35, wherein **a** is 3; **b** is 3; and **R**² is *-C(=O)NH.

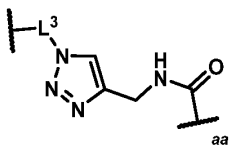
37. The compound of claim 34, wherein **a** is an integer from 1 to 4; **b** is an
integer from 1 to 4; and **R**² is -C(**R**⁶)₂-.

38. The compound of claim 37, wherein **R**² is -CH₂-; and 3≤(**a**+**b**)≤5.

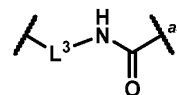
39. The compound of any one of claims 1-38, wherein **R**³ is



40. The compound of any one of claims 1-39, wherein R^3 is



41. The compound of any one of claims 1-39, wherein R^3 is



42. The compound of any one of claims 1-41, wherein L^3 is $-C(R^6)_2-$.

43. The compound of any one of claims 1-42, wherein L^3 is $-CH_2-$.

44. The compound of any one of claims 1-43, wherein c is an integer from 1 to 2.

45. The compound of any one of claims 1-43, wherein c is an integer from 2 to 5.

46. The compound of any one of claims 1-43, wherein c is an integer from 3 to 7.

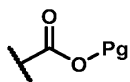
47. The compound of any one of claims 1-46, wherein R^4 is $^*-C(=O)NR^7-$.

48. The compound of any one of claims 1-47, wherein R^4 is $^*-C(=O)NH-$.

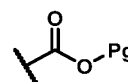
49. The compound of any one of claims 1-46, wherein R^4 is $-C(R^6)_2-$.

50. The compound of any one of claims 1-46 or 49, wherein R^4 is $-CH_2-$.
- 5 51. The compound of any one of claims 1-50, wherein d is an integer from 1 to 2.
52. The compound of any one of claims 1-50, wherein d is an integer from 3 to 7.
- 10 53. The compound of any one of claims 1-43, wherein R^4 is $-C(R^6)_2-$; and each of c and d is independently 1 or 2.
- 15 54. The compound of claim 53, wherein R^4 is $-CH_2-$; and each of c and d is 1.
- 20 55. The compound of any one of claims 1-43, wherein R^4 is $-C(R^6)_2-$; and $4 \leq (c + d) \leq 12$.
56. The compound of claim 55, wherein R^4 is $-CH_2-$; and $7 \leq (c + d) \leq 10$.
- 25 57. The compound of any one of claims 1-43, wherein R^4 is $*-C(=O)NR^7-$; and $5 \leq (c + d) \leq 10$.
58. The compound of claim 57, wherein R^4 is $*-C(=O)NR^7-$; and $7 \leq (c + d)$
- 30 ≤ 9 .
59. The compound of claims 57 or 58, wherein c is 3.

60. The compound of any one of claims 1-59, wherein R^5 is $C(O)OH$ or



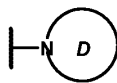
61. The compound of any one of claims 1-59, wherein R^5 is



5

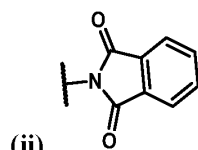
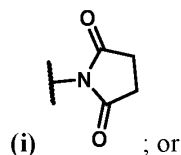
62. The compound of any one of claims 1-61, wherein **Pg** is a carboxyl activating group.

10

63. The compound of any one of claims 1-62, wherein **Pg** is , wherein **Ring D** is a 5-10 membered heteroaryl or 4-10 membered heterocyclyl, each optionally substituted with 1-6 substituents each independently selected from the group consisting of: halo, oxo, NO_2 , $C(O)C_{1-4}$ alkyl, $C(O)OC_{1-4}$ alkyl, $S(O)C_{1-4}$ alkyl, C_{1-6} alkyl, C_{1-6} haloalkyl, and $-OH$.

15

64. The compound of any one of claims 1-63, wherein **Pg** is:



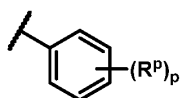
20

which is optionally substituted with 1-6 substituents each independently selected from the group consisting of: halo, NO_2 , $C(O)C_{1-4}$ alkyl, $C(O)OC_{1-4}$ alkyl, $S(O)C_{1-4}$ alkyl, C_{1-6} alkyl, C_{1-6} haloalkyl, and $-OH$.

65. The compound of any one of claims 1-62, wherein **Pg** is C₆₋₁₀ aryl or 5-10 membered heteroaryl substituted with 1-6 substituents each independently selected from the group consisting of: -F; -Cl; -NO₂; C(O)C₁₋₄ alkyl, C(O)OC₁₋₄ alkyl, and S(O)C₁₋₄ alkyl.

5

66. The compound of any one of claims 1-62 or 65, wherein **Pg** is

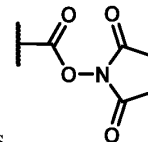
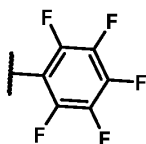


, wherein **p** is an integer from 1 to 5; and each **R^p** is -F, -Cl, or -NO₂.

10

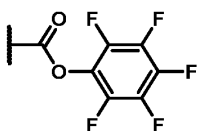
67. The compound of claim 66, wherein each **R^p** is -F.

68. The compound of any one of claims 1-62 or 65-66, wherein **Pg** is

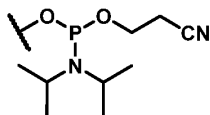


15

69. The compound of any one of claims 1-62, wherein **R⁵** is

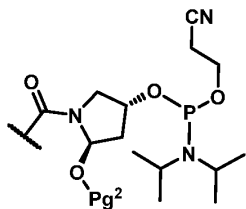


70. The compound of any one of claims 1-59, wherein **R⁵** is



20

71. The compound of any one of claims 1-59, wherein R^5 is



5 72. The compound of any one of claims 1-71, wherein each hydroxyl protecting group is independently selected from the group consisting of: a silyl protecting group; 4-monomethoxytrityl (MMTR); 4,4'-dimethoxytrityl (DMTR); and triphenylmethyl (trityl).

10 73. The compound of claim 72, wherein the silyl protecting group is selected from the group consisting of: *tert*-butyldimethylsilyl (TBMDs); *tert*-butyldiphenylsilyl (TBDPS), and triisopropylsilyl (TIPS).

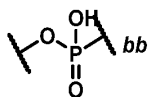
15 74. The compound of claim 1, wherein the compound is selected from the group consisting of compounds **GalNAc-1** through **GalNAc-12**.

20 75. The compound of any one of claims 1-59, wherein R^5 is $Y^L\text{-Oligo}$, wherein **Oligo** is an oligonucleotide that is attached to **L** via the 5'-end, 3'-end, or sequence middle of any strand via a phosphate group.

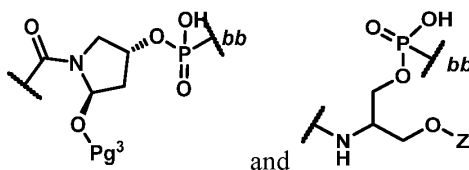
76. The compound of any one of claims 1-59 or 75, wherein **L** is a bond.

25 77. The compound of any one of claims 1-59 or 75, wherein **L** is C(=O) or –O–.

78. The compound of any one of claims 1-59 or 75, wherein **L** is



5 79. The compound of any one of claims 1-59 or 75, wherein **L** is selected



from the group consisting of:

and

80. The compound of any one of claims 1-59 or 75-79, wherein the
10 oligonucleotide comprises a single-stranded oligonucleotide and/or a double-stranded oligonucleotide.

81. The compound of any one of claims 1-59 or 75-80, wherein the
15 oligonucleotide is selected from the group consisting of: DNA, siRNA, miRNA, pre-miRNA, antagomir, mRNA, antisense oligonucleotide (ASO), aptamer, crRNA, tracrRNA, and sgRNA.

82. The compound of any one of claims 1-59 or 75-81, wherein the
20 oligonucleotide comprises unmodified nucleotides and/or modified nucleotides.

83. The conjugate compound of claim 82, wherein the modified
nucleotides are each independently selected from the group consisting of: 2'-O-(2-methoxyethyl)-modified nucleotides; 2'-O-alkyl modified nucleotides; 2'-O-allyl modified nucleotides; 2'-C-allyl modified nucleotides; 2'-fluoro modified nucleotides; 2'-deoxy modified nucleotides; 2'-hydroxy modified nucleotides; locked nucleic acids (LNAs) modified nucleotides, glycol nucleic acids (GNAs) modified nucleotides, and unlocked nucleic acids (UNAs) modified nucleotides.
25

84. The compound of claim 83, wherein the 2'-O-alkyl modified nucleotides are 2'-O-methyl nucleotides.

5 **85.** The compound of any one of claims 1-59 or 75-84, wherein the oligonucleotide comprises a modifying group, wherein the modifying group is selected from the group consisting of: cholesterol, polyethylene glycol, fluorescent probes, biotin, polypeptides, vitamins, tissue targeting molecules, and a combination thereof.

10 **86.** The compound of claim 85, wherein the modifying group is a terminal modifying group.

15 **87.** The compound of any one of claims 1-59 or 75-86, wherein the phosphate group is a phosphodiester or a modified phosphate group.

88. The conjugate compound of claim 87, wherein the modified phosphate group is selected from one or more of: thio modified phosphate, amino modified phosphate,

20 **89.** The compound of claim 88, wherein the thio modified phosphate is phosphorothioate.

90. The conjugate compound of any one of claims 1-59 or 75-89, wherein
25 the oligonucleotide comprises one or more peptide nucleic acids and/or morpholino nucleic acids.

91. The conjugate compound of any one of claims 1-59 or 75-90, wherein
30 the oligonucleotide is an oligonucleotide of from 5 to 100 base pairs.

92. The compound of any one of claims 1-59 or 75-91, wherein the oligonucleotide conjugate compound is synthesized via solid-phase synthesis or liquid-phase synthesis.

5 **93.** A method for treating and/or preventing pathological conditions or diseases in a subject, wherein the conditions or diseases are caused by the expression of one or more genes in liver cells, the method comprising administering to the subject a compound of any one of claims 75-92; or a pharmaceutical composition comprising a compound of any one of claims 75-92, and a
10 pharmaceutically acceptable excipient.

94. A method for detecting or localizing RNA in the liver of a subject, comprising administering to the subject a compound of any one of claims 75-92; or a pharmaceutical composition comprising a compound of any one of claims 75-92,
15 and a pharmaceutically acceptable excipient.

95. The method of claim 93, wherein the one or more genes are selected from: HBV genome, HCV genome, *PCSK9*, a gene expressing xanthine oxidase (e.g., *XDH*), *URAT1*, *APOB*, liver fibrosis-related genes (e.g., *AP3S2*, *AQP2*, *AZIN1*,
20 *DEGSI*, *STXBP5L*, *TLR4*, *TRPM5*), genes related to non-alcoholic fatty liver disease (e.g., *PNPLA3*, *FDFTI*), and genes related to primary biliary cirrhosis (e.g., *HLA-DQB1*, *IL-12*, *IL-12RB2*).

96. The method of any one of claims 93-95, wherein the disease or
25 condition is selected from the group consisting of: hereditary angioedema, familial tyrosinemia type I, Alagille syndrome, α -1-antitrypsin deficiency, bile acid synthesis and metabolic defects, biliary atresia, cystic fibrosis liver disease, idiopathic neonatal hepatitis, mitochondrial liver disease, progressive familial intrahepatic cholestasis, primary sclerosing cholangitis, transthyretin amyloidosis,
30 hemophilia, homozygous familial hypercholesterolemia, hyperlipidemia, hepatitis B virus infection (HBV), hepatitis C virus infection (HCV), steatohepatitis,

nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), hyperglycemia and diseases involving abnormally increased hepatic glucose production similar to type II diabetes, hepatitis, and hepatic porphyrins.

5 **97.** The method of any one of claims 93-96, wherein the compound or pharmaceutical composition is administered intravenously, intramuscularly, subcutaneously, via microneedle patches, orally, via oral or nasal spray, or topically.

10 **98.** The method of any one of claims 93-97, wherein the subject is a mammal.

99. The method of any one of claims 93-98, wherein the subject is selected from the group consisting of: bovine, equine, sheep, swine, canine, feline, rodent, and primate.

15 **100.** The method of any one of claims 93-99, wherein the subject is human.

101. A pharmaceutical composition comprising a compound of any one of claims 75-92 and a pharmaceutically acceptable excipient.

20 **102.** The pharmaceutical composition of claim 101, wherein the pharmaceutical composition is formulated in a dosage form selected from the group consisting of: powders, tablets, granules, capsules, solutions, emulsions, suspensions, injections, sprays, aerosols, dry powder inhalations, and microneedle
25 patches.

103. The pharmaceutical composition of claims 101 or 102, wherein the pharmaceutical composition is suitable for administration to a subject in need thereof intravenously, intramuscularly, subcutaneously, via microneedle patches,
30 orally, via oral or nasal spray, or topically.

104. The pharmaceutical composition of claim 103, wherein the subject is a mammal.

5 **105.** The pharmaceutical composition of claim 104, wherein the mammal is selected from the group consisting of: bovine, equine, sheep, swine, canine, feline, rodent, and primate.

10 **106.** The pharmaceutical composition of any one of claims 103-105, wherein the subject is human.