

Multivalent Ligand Clusters with Diamine Scaffold for Targeted Delivery of Therapeutic Agents

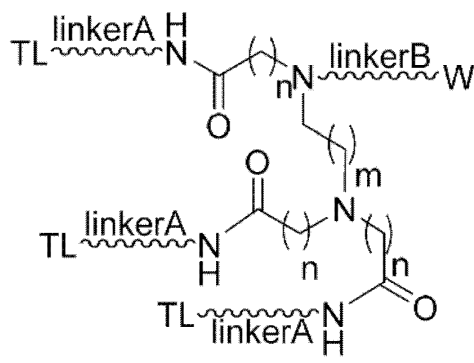
BACKGROUND

Due to their high molecular weight and polyanionic nature, oligonucleotides generally have low cell membrane permeability. Thus, target ligands are often conjugated to oligonucleotide compounds to enhance cell uptake and improve tissue specificity of in vivo delivery, through a well-known mechanism of receptor-mediated endocytosis. In some cases, multivalent ligand clusters have an advantage over single ligands in enhancing delivery to targeted tissues via specific receptors. Asialoglycoprotein receptor (ASGPR) is one of such receptors.

It has been demonstrated that N-acetylgalactosamine (GalNAc), a ligand for ASGPR, can facilitate delivery of oligonucleotide drugs into hepatocytes. It has also been demonstrated that multivalent GalNAc ligand clusters have higher binding affinity to ASGPR than individual GalNAc ligands, and thus higher efficiency in delivering therapeutic oligonucleotides into liver hepatocytes.

SUMMARY

One aspect of the present disclosure relates to a compound for targeted delivery of one or more pharmaceutical agents, where the compound has the formula:



where each TL is an independently selected targeting ligand, m is an integral number between 1 and 10, each n is an independently selected integral number between 1 and 10, each linkerA is an independently selected spacer, linkerB is a spacer, and W is either the one or more pharmaceutical agents or a functional group capable of linking to the one or more pharmaceutical agents. In some embodiments, m is 1. In some embodiments, m is 2.

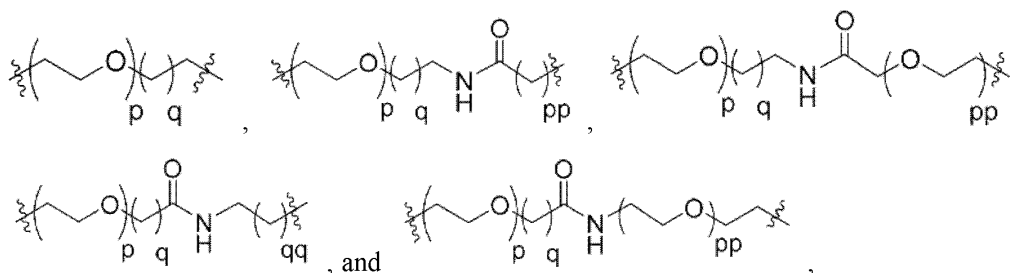
In some embodiments, n is 1. In some embodiments, n is 2.

In some embodiments, at least one of the independently selected TLs is capable of binding to one or more cell receptors, cell channels, and cell transporters capable of facilitating endocytosis. In some embodiments, at least one of the independently selected TLs comprises at least one small molecule ligand. In some embodiments, at least one small molecule comprises at least one of N-acetylgalactosamine, galactose, galactosamine, N-formyl-galactosamine, N-propionylgalactosamine, N-butanoylgalactosamine, and N-iso-butanoylgalactosamine, a macrocycle, a folate molecule, a fatty acid, a bile acid, and a cholesterol. In some embodiments, at least one of the independently selected TLs comprises at least one peptide. In some embodiments, at least one of the independently selected TLs comprises at least one cyclic peptide. In some embodiments, at least one of the independently selected TLs comprises at least one aptamer. In some embodiments, at least one of the independently selected TLs is capable of binding to at least one Asialoglycoprotein receptor (ASGPR). In some embodiments, at least one of the independently selected TLs is capable of binding to at least one transferrin receptor. In some embodiments, at least one of the independently selected TLs is capable of binding to at least one integrin receptor. In some embodiments, at least one of the independently selected TLs is capable of binding to at least one folate receptor. In some embodiments, at least one of the independently selected TLs is capable of binding to at least one G-protein-coupled receptor (GPCR).

In some embodiments, at least one of the independently selected linkerAs comprises at least one of polyethylene glycol, an alkyl group, a cycloalkyl group, an alkenyl group, a cycloalkenyl group, an alkynyl group, an aryl group, an aralkyl group, an aralkenyl group, and an aralkynyl group. In some embodiments, at least one of the independently selected linkerAs comprises at least one heteroatom. In some embodiments, the at least one heteroatom comprises at least one of oxygen, nitrogen, sulfur, or phosphorous. In some embodiments, at least one of the independently selected linkerAs comprises at least one aliphatic heterocycle. In some embodiments, the at least one aliphatic heterocycle comprises at least one of tetrahydrofuran, tetrahydropyran, morpholine, piperidine, piperazine, pyrrolidine, and azetidine. In some embodiments, at least one of the independently selected linkerAs comprises at least one heteroaryl group. In some embodiments, the at least one heteroaryl group comprises at least one of imidazole, pyrazole, pyridine, pyrimidine, triazole, and 1,2,3-triazole. In some embodiments, at least one of the independently selected linkerAs comprises at least one amino acid. In some embodiments, at least one of the independently

selected linkerAs comprises at least one nucleotide. In some embodiments, at least one of the independently selected linkerAs comprises at least one saccharide. In some embodiments, the at least one saccharide comprises at least one of glucose, fructose, mannose, galactose, ribose, and glucosamine. In some embodiments, at least one of the independently selected linkerAs

5 comprises one or more of:



where p is an integral number between 0 and 12, pp is an integral number between 0 and 12, q is an integral number between 1 and 12, and qq is an integral number between 1 and 12.

10 In some embodiments, linkerB comprises at least one of a polyethylene glycol, an alkyl group, a cycloalkyl group, an alkenyl group, a cycloalkenyl group, an alkynyl group, an aryl group, an aralkyl group, an aralkenyl group, and an aralkynyl group. In some

embodiments, linkerB comprises at least one heteroatom. In some embodiments, the at least one heteroatom comprises at least one of oxygen, nitrogen, sulfur, and phosphorous. In some

15 embodiments, linkerB comprises at least one aliphatic heterocycle. In some embodiments, the at least one aliphatic heterocycle comprises at least one of tetrahydrofuran, tetrahydropyran, morpholine, piperidine, piperazine, pyrrolidine, and azetidine. In some embodiments, linkerB comprises at least one heteroaryl group. In some embodiments, the at least one heteroaryl

group comprises at least one of imidazole, pyrazole, pyridine, pyrimidine, triazole, and 1,2,3-

20 triazole. In some embodiments, linkerB comprises at least one amino acid. In some

embodiments, linkerB comprises at least one nucleotide. In some embodiments, the at least one nucleotide comprises at least one of an abasic nucleotide and an inverted abasic

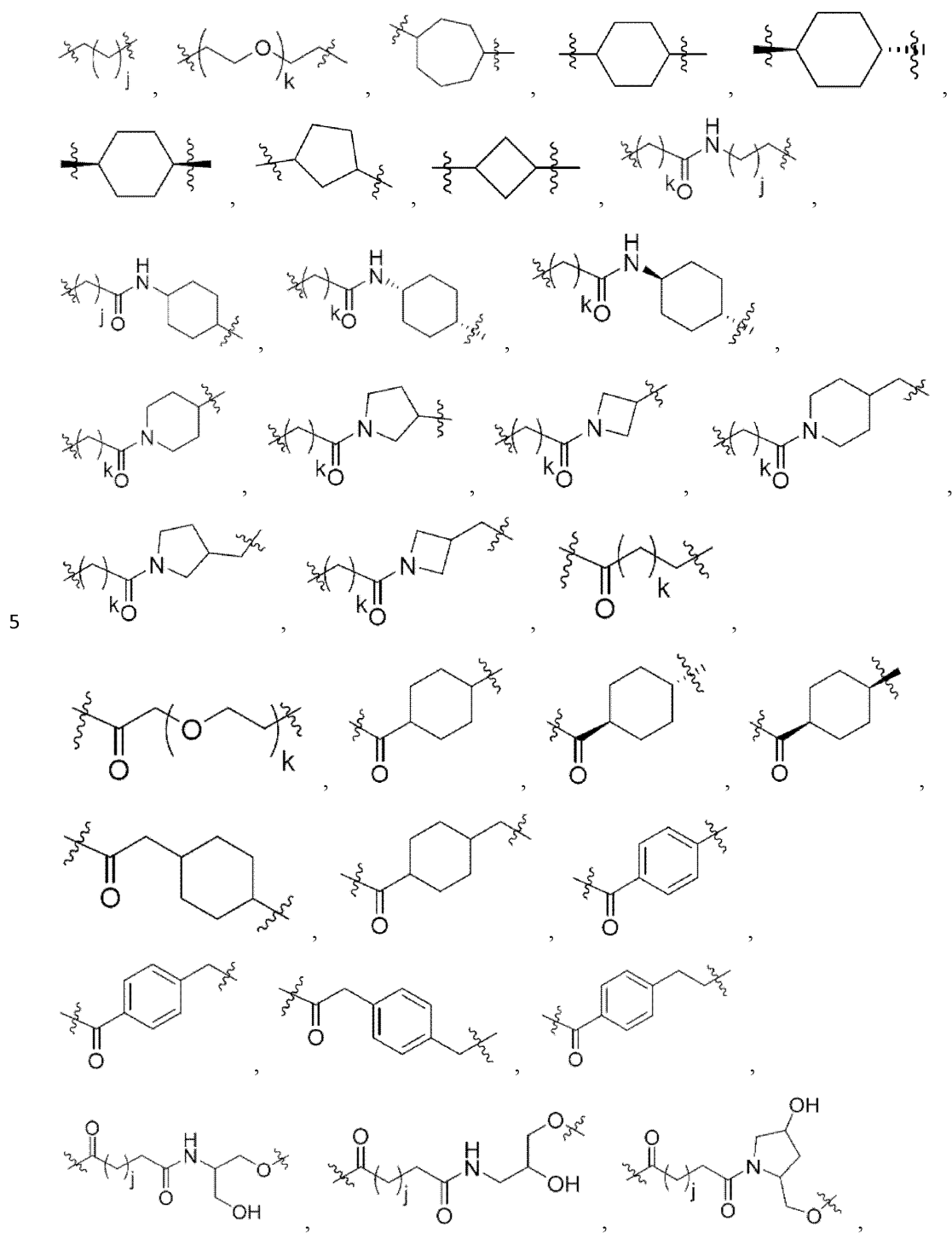
nucleotide. In some embodiments, the abasic nucleotide is an abasic deoxyribonucleic acid. In some embodiments, the inverted abasic nucleotide is an inverted abasic deoxyribonucleic

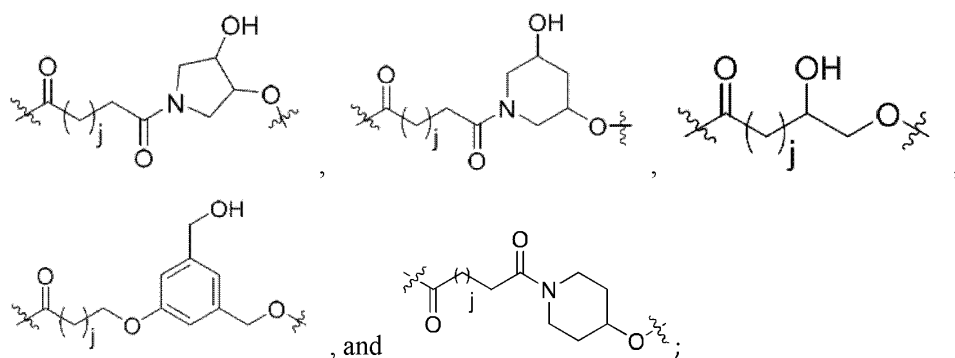
25 acid. In some embodiments, the abasic nucleotide is an abasic ribonucleic acid. In some

embodiments, the inverted abasic nucleotide is an inverted abasic ribonucleic acid. In some

embodiments, linkerB comprises at least one saccharide. In some embodiments, the at least one saccharide comprises at least one of glucose, fructose, mannose, galactose, ribose, and

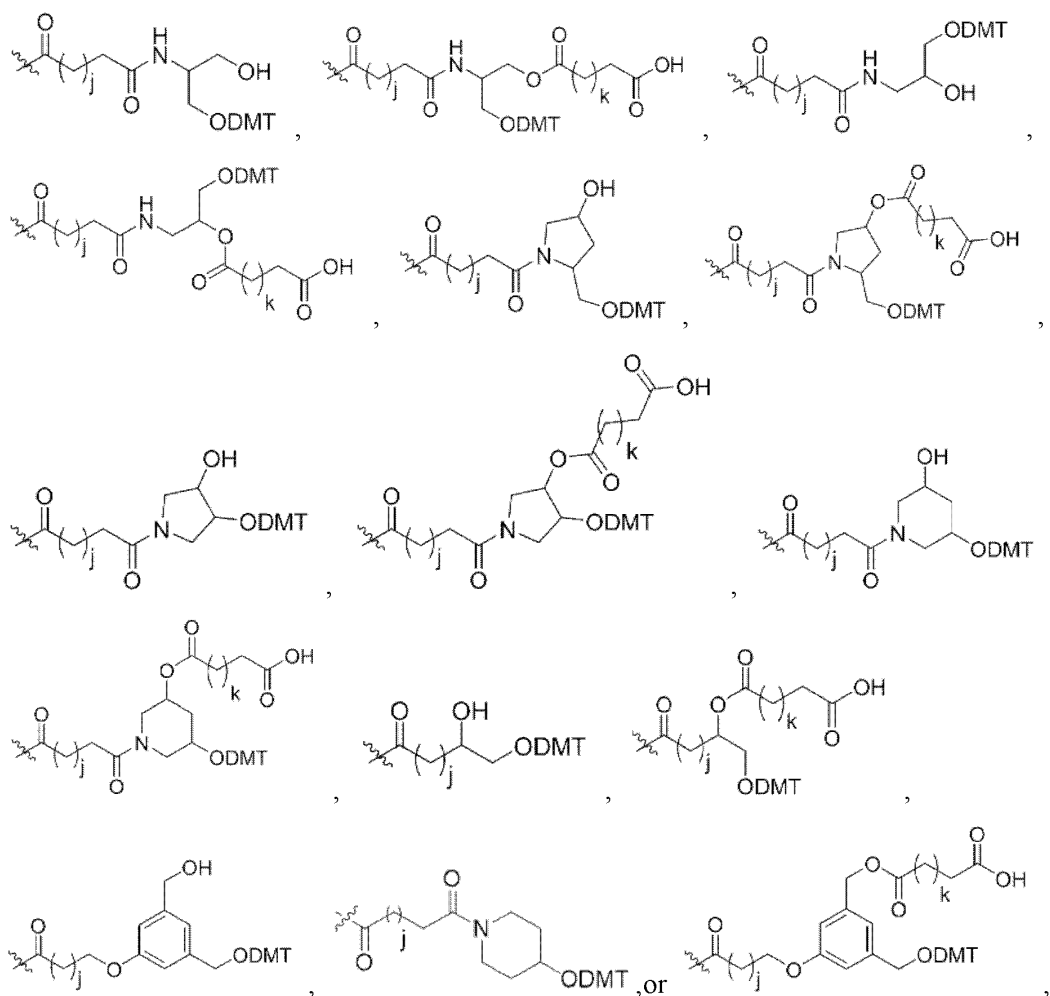
glucosamine. In some embodiments, linkerB comprises at least one of:





where j is an integral number between 1 and 12, and k is an integral number between 0 and 12.

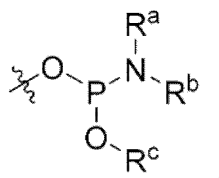
5 In some embodiments, linkerB-W is:



where j is an integral number between 0 and 12, and k is an integral number between 0 and 12.

In some embodiments, W is a hydroxy group. In some embodiments, W is a protected hydroxy group. In some embodiments, the protected hydroxy group is protected using at least one of 4,4'-dimethoxytrityl (DMT), monomethoxytrityl (MMT), 9-(*p*-methoxyphenyl)xanthen-9-yl (Mox), and 9-phenylxanthen-9-yl (Px). In some embodiments,

- 5 W is a phosphoramidite group having the formula:



where:

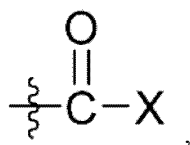
R^a is a C1 to C6 alkyl, C3 to C6 cycloalkyl, an isopropyl group, or R^a joins with R^b through a nitrogen atom to form a cycle,

- 10 R^b is a C1 to C6 alkyl, C3 to C6 cycloalkyl, an isopropyl group, or R^b joins with R^a through a nitrogen atom to form a cycle, and

R^c is a phosphite protecting group, phosphate protecting group, or a 2-cyanoethyl group.

- In some embodiments, the phosphite protecting group comprises at least one of
- 15 methyl, allyl, 2-cyanoethyl, 4-cyano-2-butenyl, 2-cyano-1,1-dimethylethyl, 2-(trimethylsilyl)ethyl, 2-(S-acetylthio)ethyl, 2-(S-pivaloylthio)ethyl, 2-(4-nitrophenyl)ethyl, 2,2,2-trichloroethyl, 2,2,2-trichloro-1, 1-dimethylethyl, 1,1,1,3,3,3-hexafluoro-2-propyl, fluorenyl-9-methyl, 2-chlorophenyl, 4-chlorophenyl, and 2,4-dichlorophenyl. In some embodiments, the phosphate protecting group comprises at least one of methyl, allyl, 2-
- 20 cyanoethyl, 4-cyano-2-butenyl, 2-cyano-1,1-dimethylethyl, 2-(trimethylsilyl)ethyl, 2-(S-acetylthio)ethyl, 2-(S-pivaloylthio)ethyl, 2-(4-nitrophenyl)ethyl, 2,2,2-trichloroethyl, 2,2,2-trichloro-1,1-dimethylethyl, 1,1,1,3,3,3-hexafluoro-2-propyl, fluorenyl-9-methyl, 2-chlorophenyl, 4-chlorophenyl, and 2,4-dichlorophenyl.

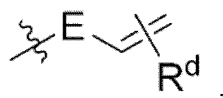
- In some embodiments, W is a carboxyl group. In some embodiments, W is an
- 25 activated carboxyl group having the formula:



where X is a leaving group. In some embodiments, the leaving group is selected from the group consisting of carboxylate, sulfonate, chloride, phosphate, imidazole,

hydroxybenzotriazole (HOBt), N-hydroxysuccinimide (NHS), tetrafluorophenol, pentafluorophenol, and *para*-nitrophenol.

In some embodiments, W is a Michael acceptor. In some embodiments, the Michael acceptor has the formula:

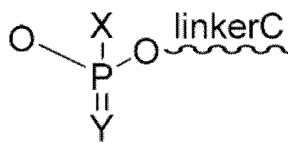


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where E is an electron withdrawing group, and R^d is hydrogen or a C1-C6 alkyl substitution group on olefin. In some embodiments, the electron withdrawing group is carboxamide or an ester. In some embodiments, E and the carbon-carbon double bond are part of maleimide.

In some embodiments, W is an oligonucleotide. In some embodiments, the oligonucleotide is a single-stranded oligonucleotide. In some embodiments, the oligonucleotide is a double-stranded oligonucleotide. In some embodiments, the oligonucleotide comprises at least 3 independently selected nucleotides. In some embodiments, the oligonucleotide comprises between 16 and 23 independently selected nucleotides. In some embodiments, the oligonucleotide comprises about 100 independently selected nucleotides. In some embodiments, the oligonucleotide comprises up to fourteen thousand independently selected nucleotides.

In some embodiments, W is:

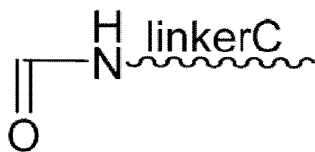


where:

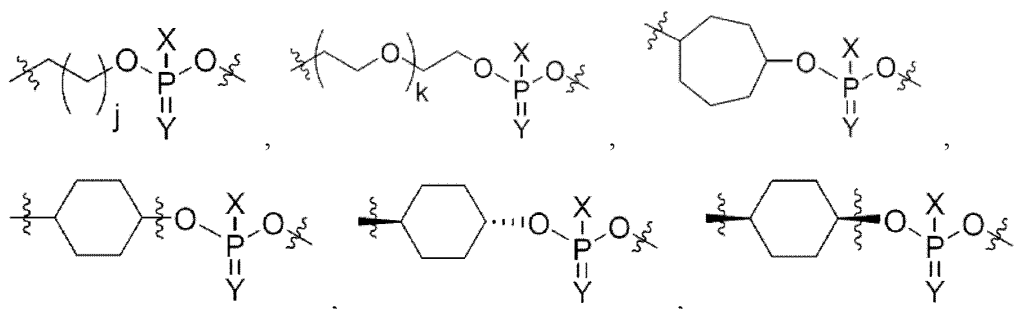
linkerC is absent or a spacer attached to a 3' or 5' end of an oligonucleotide, X is a methyl group, oxygen, sulfur, or an amino group, and Y is oxygen, sulfur, or an amino group.

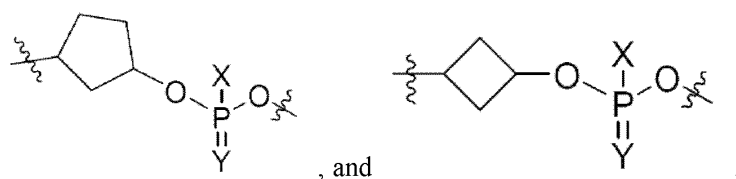
In some embodiments, linkerC comprises at least a heterocyclic compound. In some embodiments, the heterocyclic compound is an abasic nucleotide or an inverted abasic nucleotide.

In some embodiments, W is:



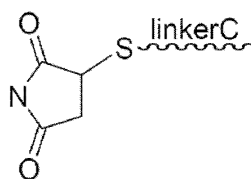
where linkerC is a spacer attached to a 3' or 5' end of an oligonucleotide. In some embodiments, linkerC comprises at least one of polyethylene glycol (PEG), an alkyl group, and a cycloalkyl group. In some embodiments, linkerC comprises at least one heteroatom. In some embodiments, the at least one heteroatom comprises at least one of oxygen, nitrogen, sulfur, and phosphorous. In some embodiments, linkerC comprises at least one aliphatic heterocycle. In some embodiments, the at least one aliphatic heterocycle comprises at least one of tetrahydrofuran, tetrahydropyran, morpholine, piperidine, piperazine, pyrrolidine, and azetidine. In some embodiments, linkerC comprises at least one heteroaryl group. In some embodiments, the at least one heteroaryl group comprises at least one of imidazole, pyrazole, pyridine, pyrimidine, triazole, and 1,2,3-triazole. In some embodiments, linkerC comprises at least one amino acid. In some embodiments, linkerC comprises at least one nucleotide. In some embodiments, the at least one nucleotide comprises at least one of an abasic nucleotide and an inverted abasic nucleotide. In some embodiments, the abasic nucleotide is an abasic deoxyribonucleic acid (DNA). In some embodiments, the inverted abasic nucleotide is an inverted abasic deoxyribonucleic acid (DNA). In some embodiments, the abasic nucleotide is an abasic ribonucleic acid (RNA). In some embodiments, the inverted abasic nucleotide is an inverted abasic ribonucleic acid (RNA). In some embodiments, linkerC comprises at least one saccharide. In some embodiments, the at least one saccharide comprises at least one of glucose, fructose, mannose, galactose, ribose, and glucosamine. In some embodiments, linkerC comprises one or more of:





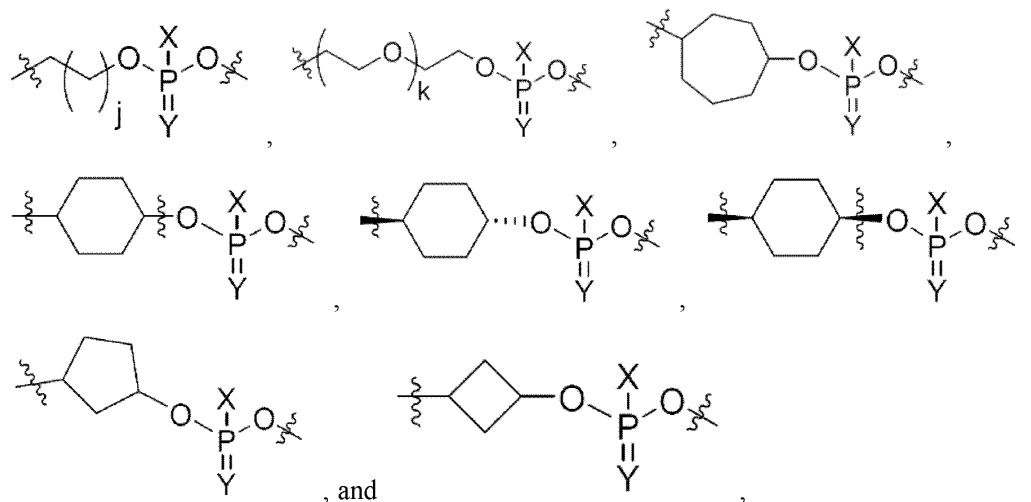
where j is an integral number between 1 and 12, and k is an integral number between 0 and 12.

In some embodiments, W is:



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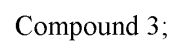
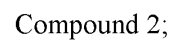
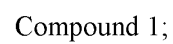
where linkerC is a spacer attached to a 3' or 5' end of an oligonucleotide. In some embodiments, linkerC comprises at least one of polyethylene glycol (PEG), an alkyl group, and a cycloalkyl group. In some embodiments, linkerC comprises one or more of:

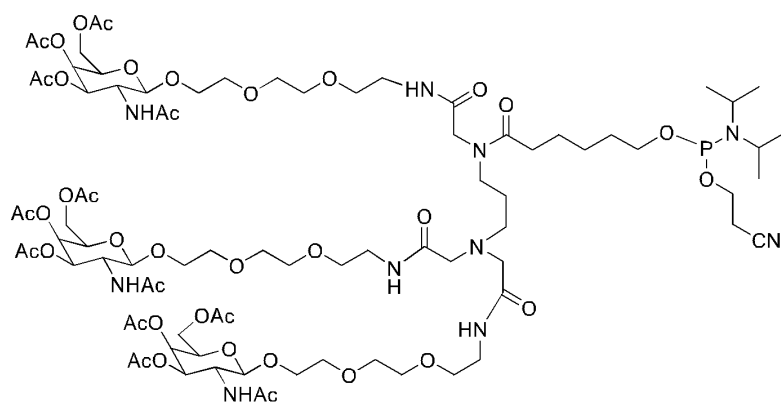


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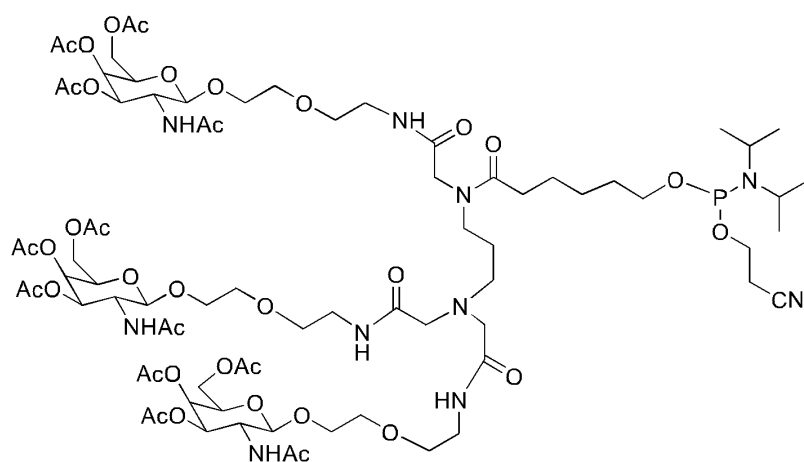
where j is an integral number between 1 and 12, and k is an integral number between 0 and 12.

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

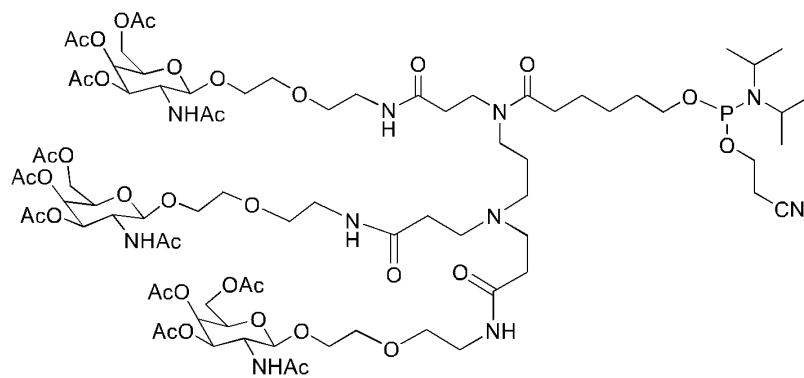




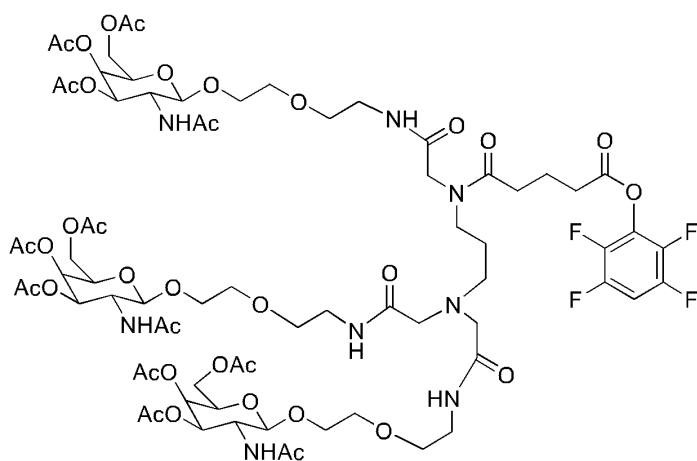
Compound 4;



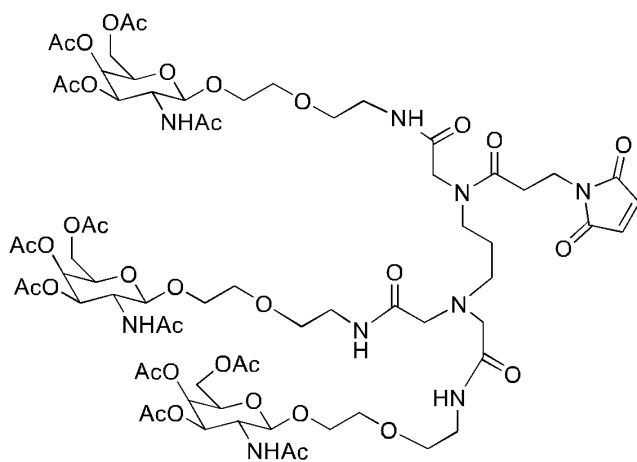
Compound 5;



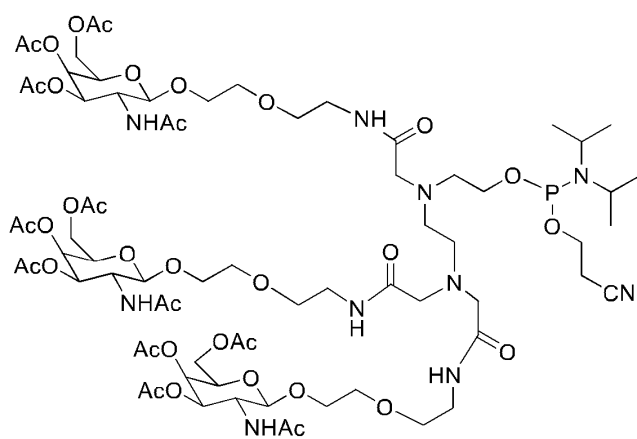
Compound 6;



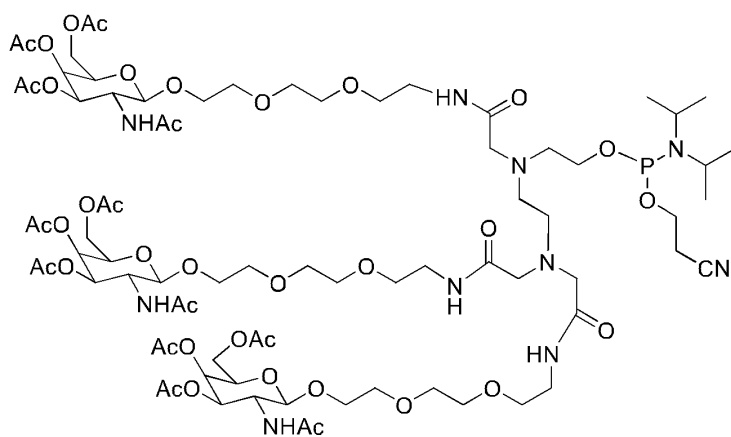
Compound 7;



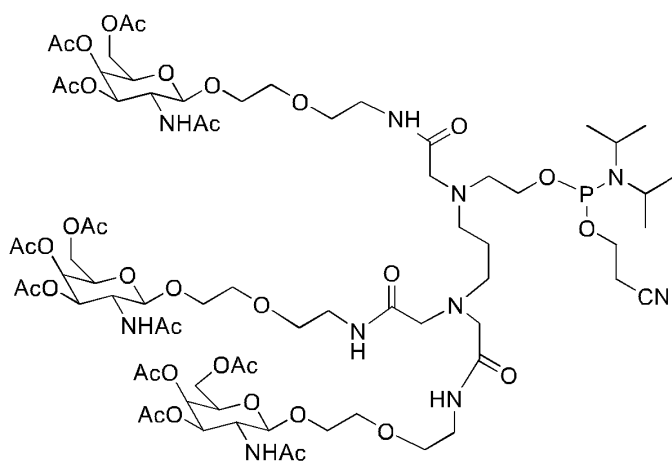
Compound 8;



Compound 9;



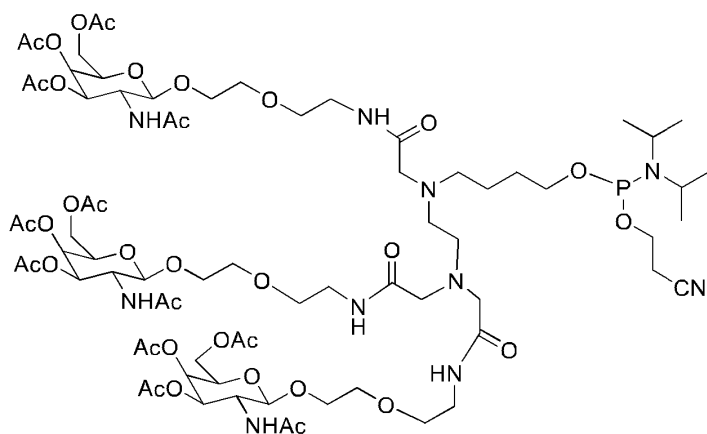
Compound 10;



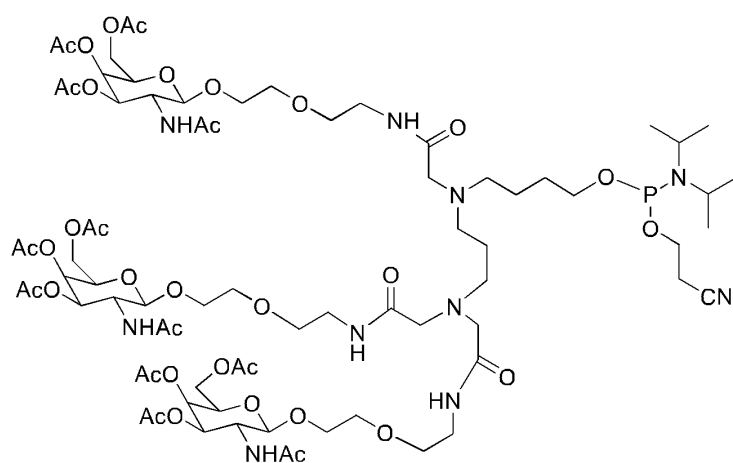
Compound

11;

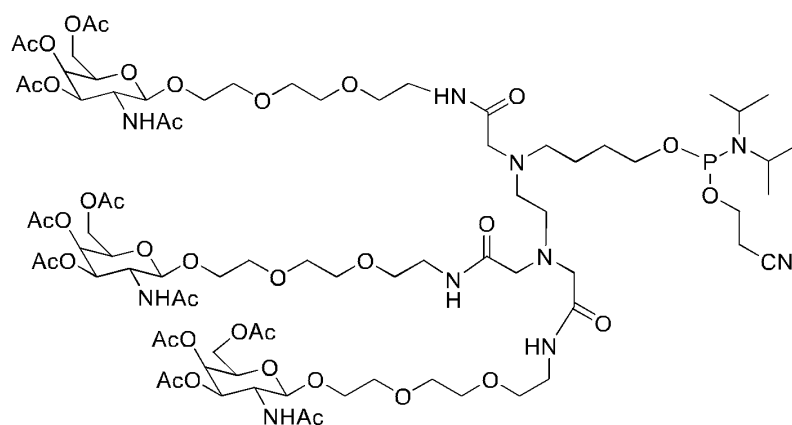
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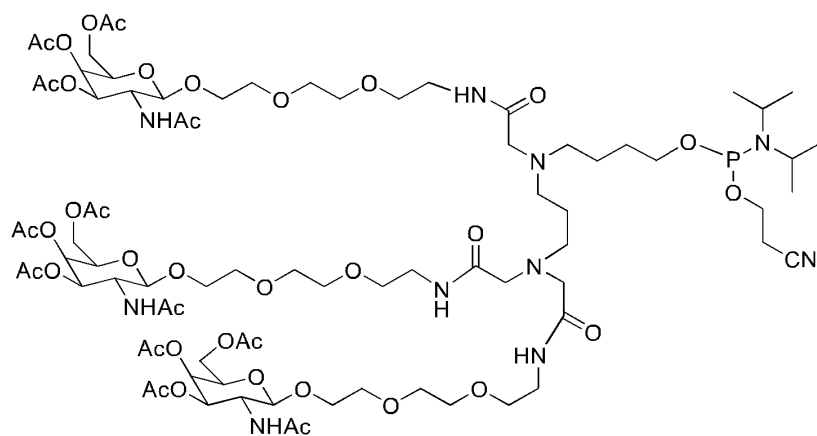
Compound 12;



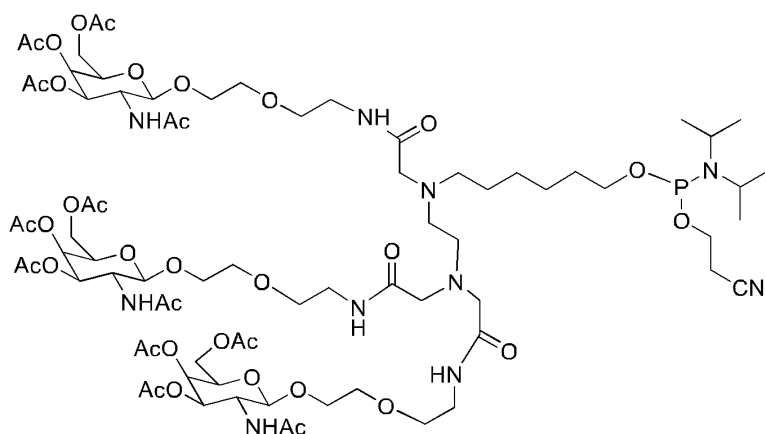
Compound 13;



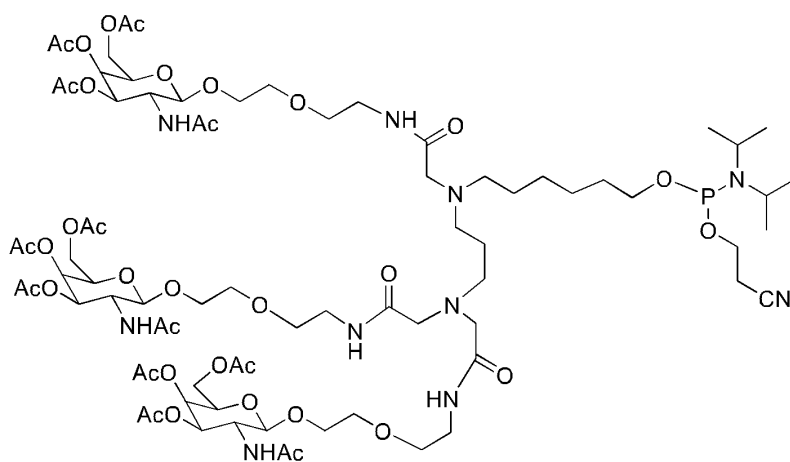
Compound 14;



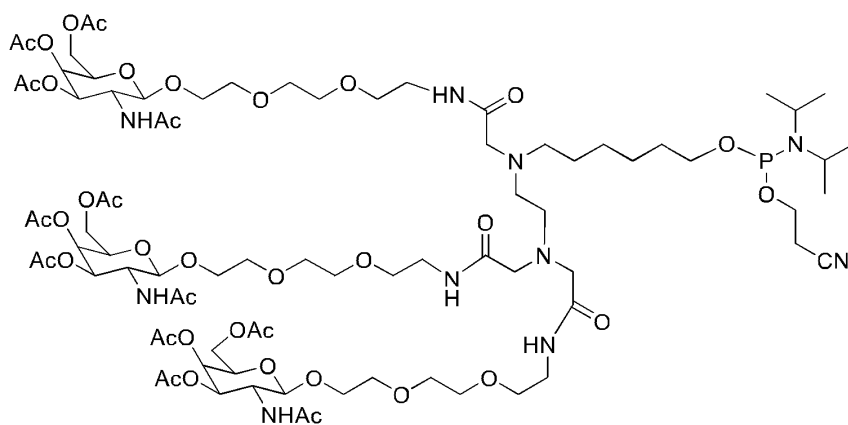
Compound 15;



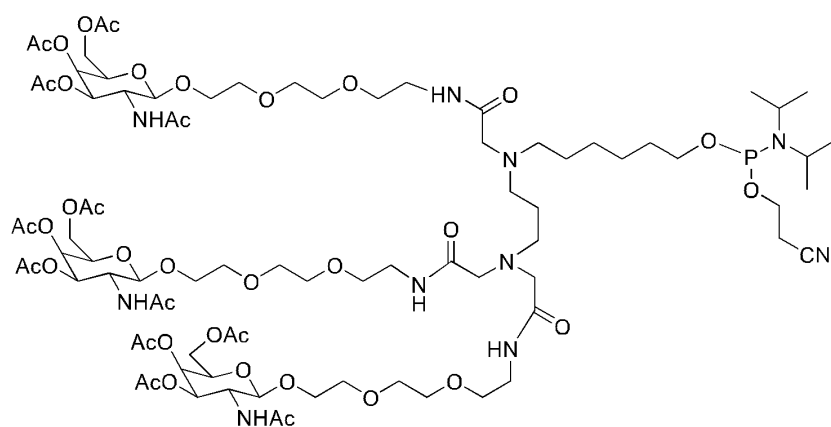
Compound 16;



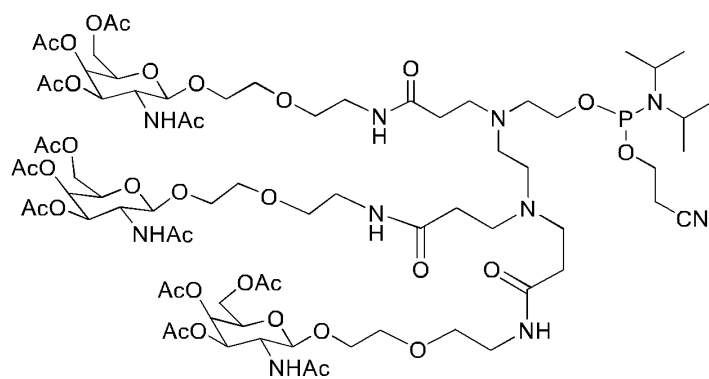
Compound 17;



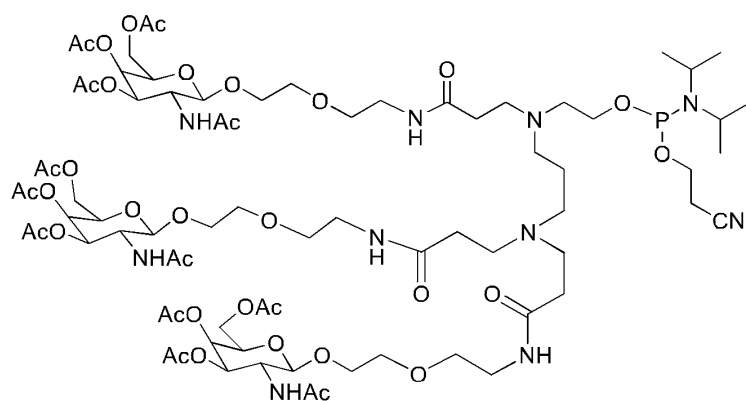
Compound 18;



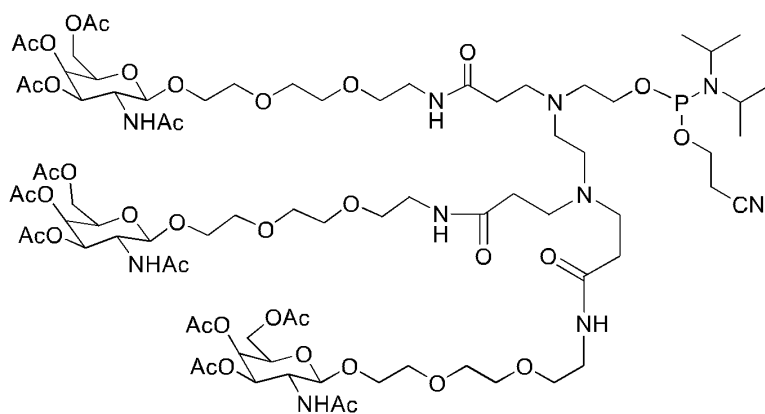
Compound 19;



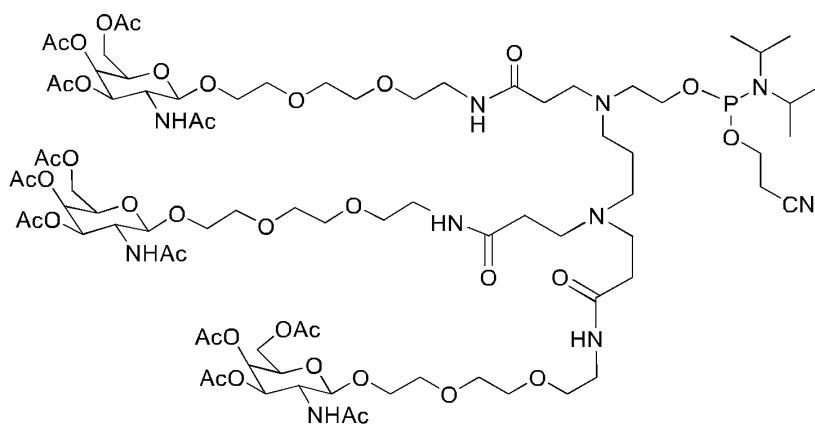
Compound 20;



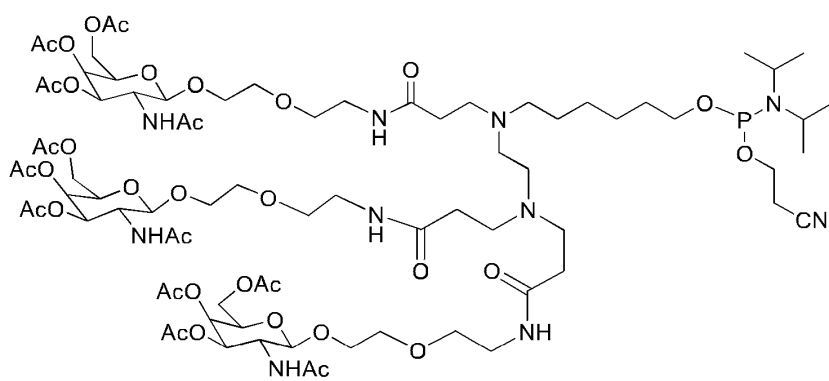
Compound 21;



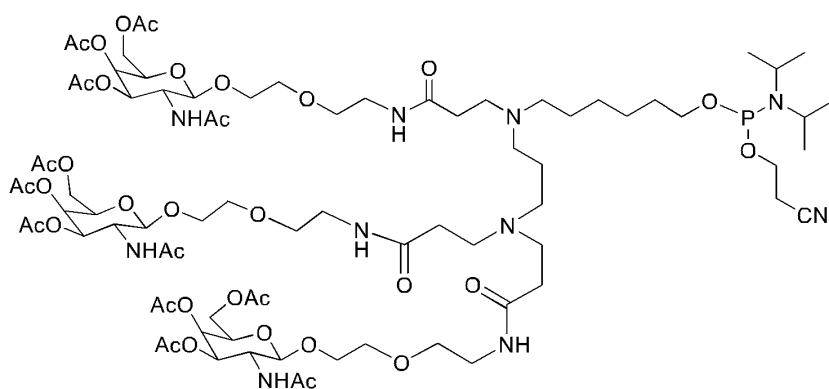
Compound 22;



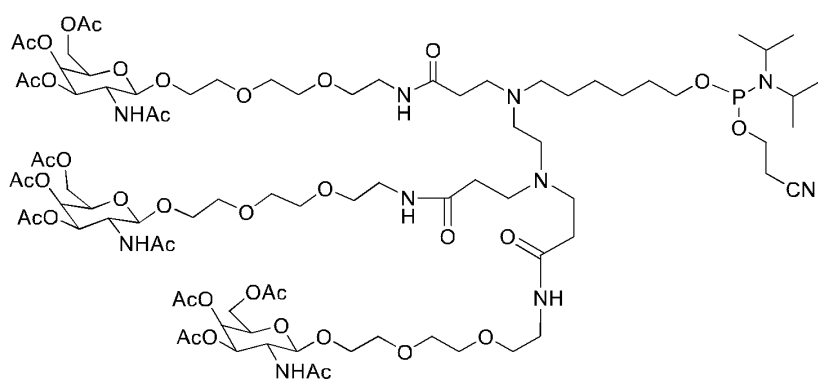
Compound 23;



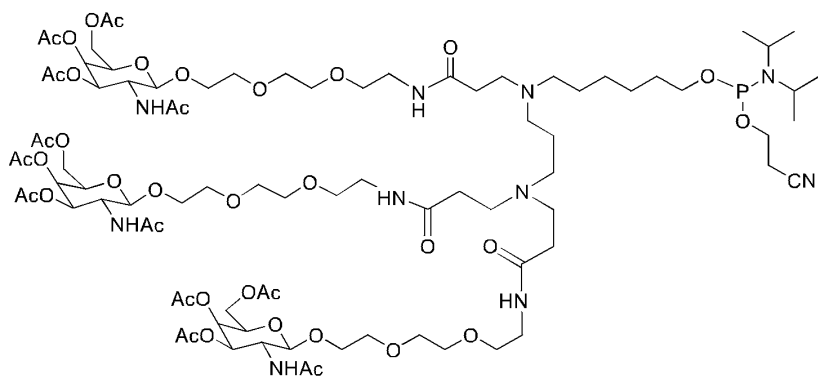
Compound 24;



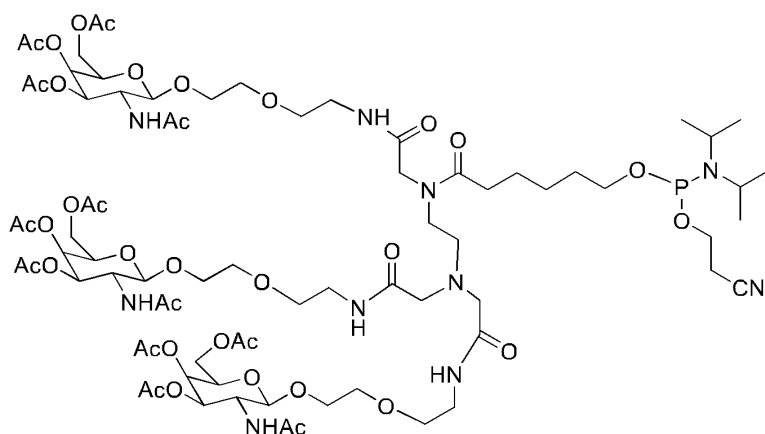
Compound 25;



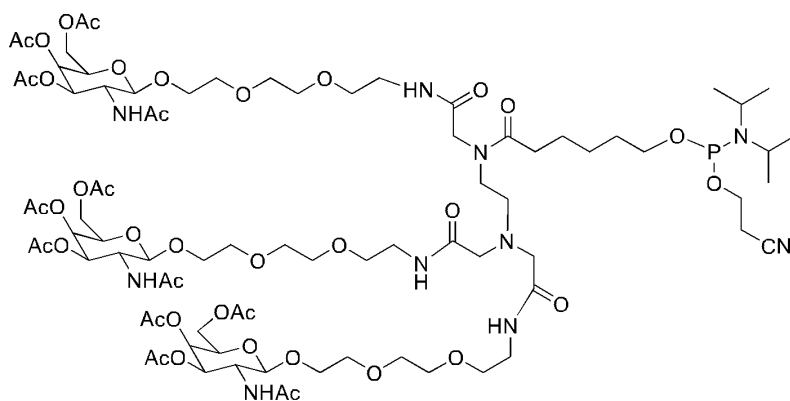
Compound 26;



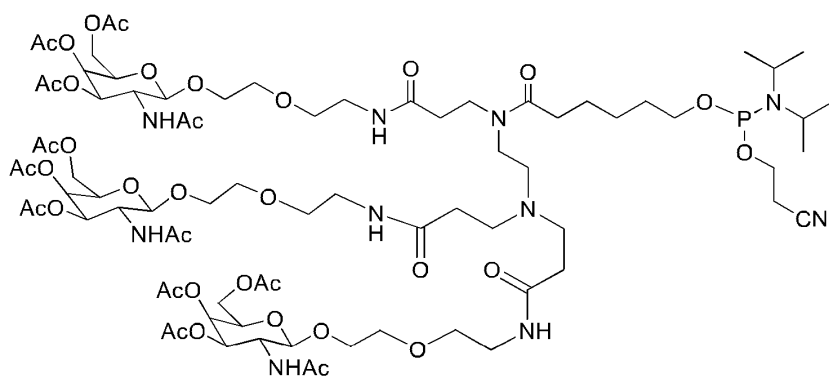
Compound 27;



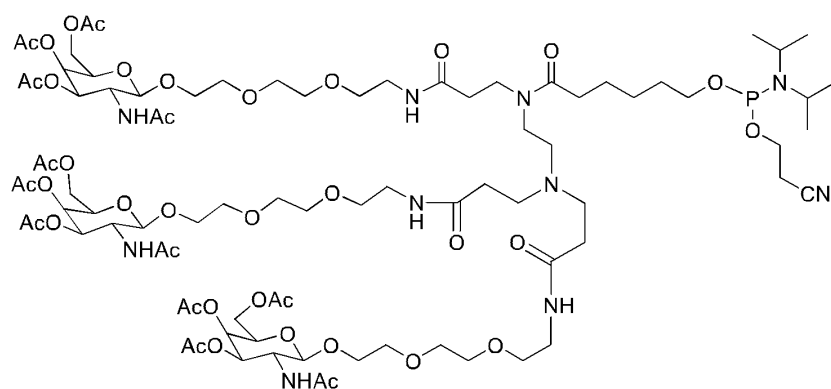
Compound 28;



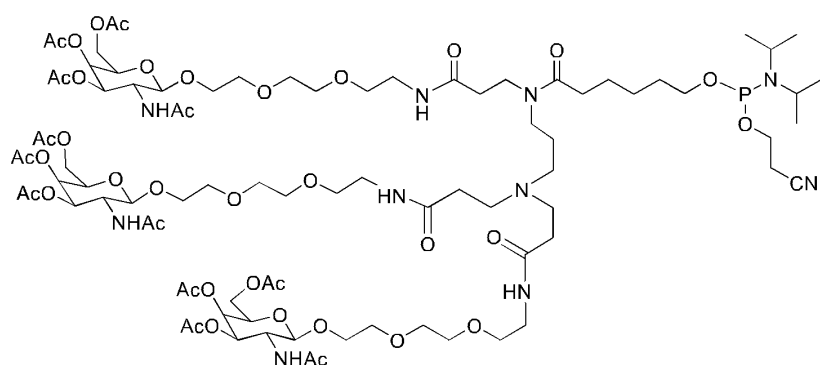
Compound 29;



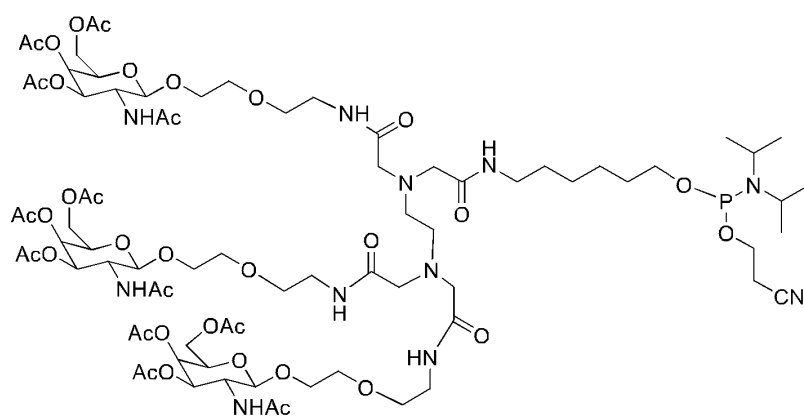
Compound 30;



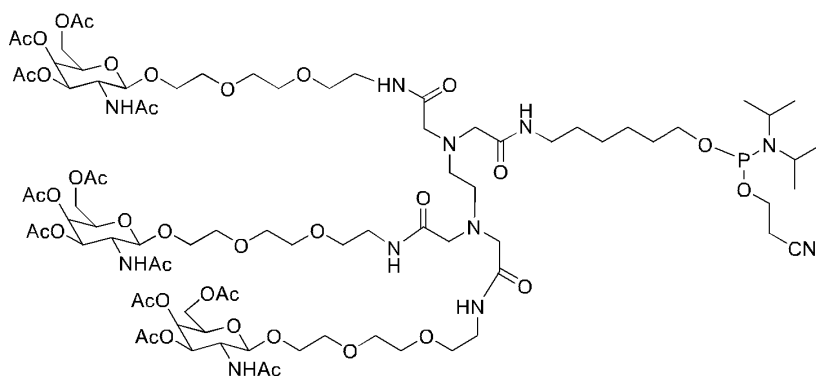
Compound 31;



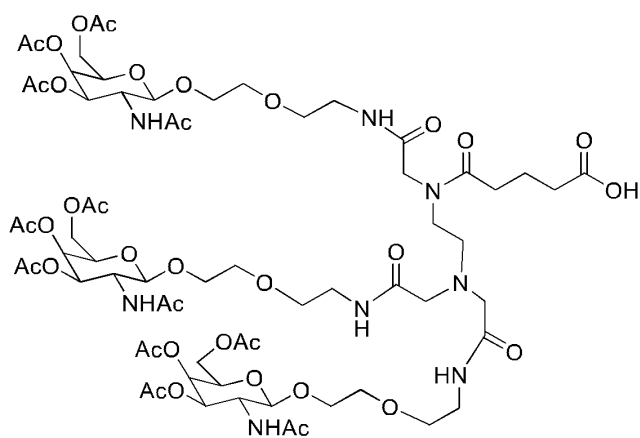
Compound 32;



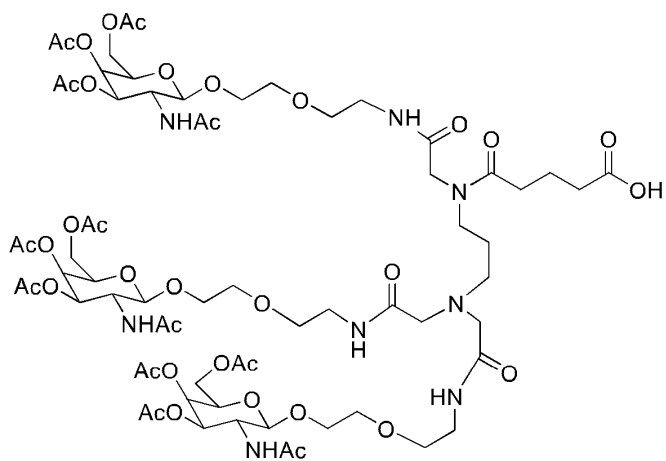
Compound 33;



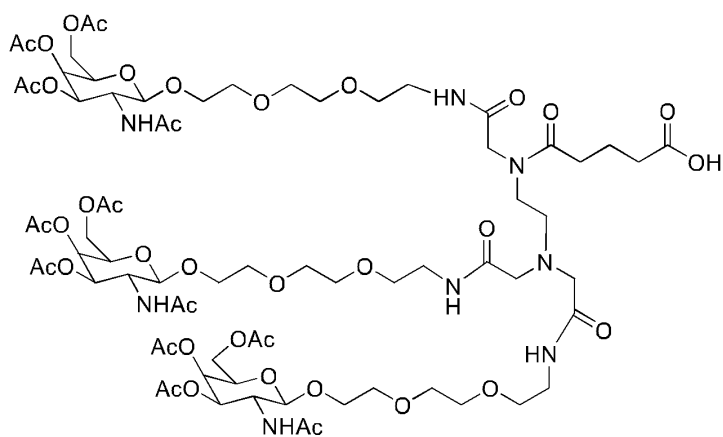
Compound 34;



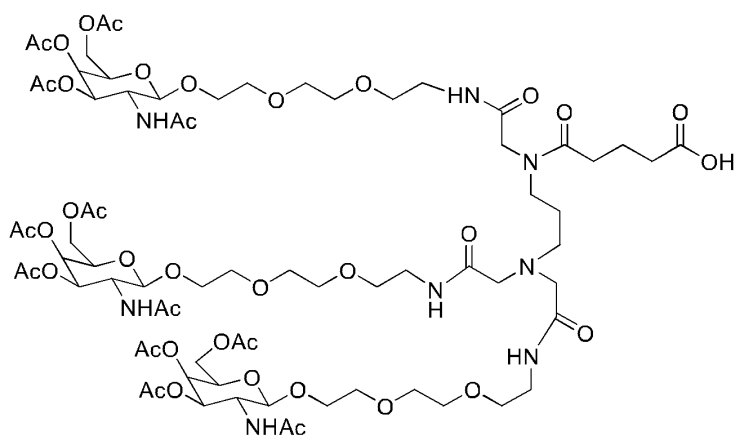
Compound 35;



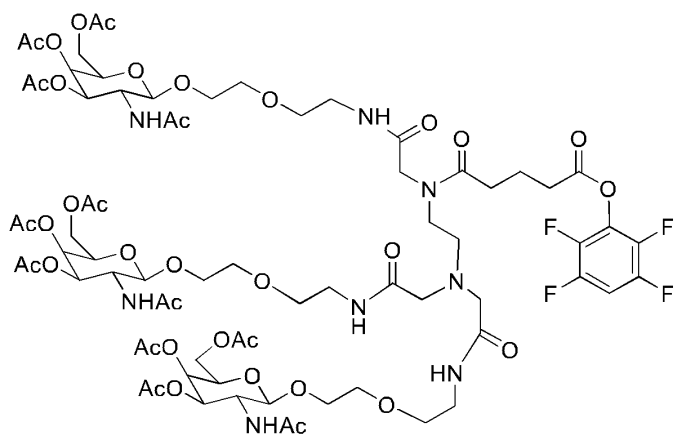
Compound 36;



Compound 37;



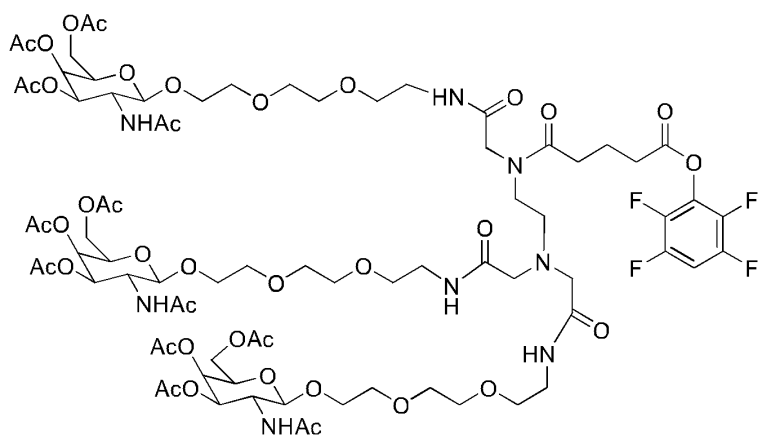
Compound 38;



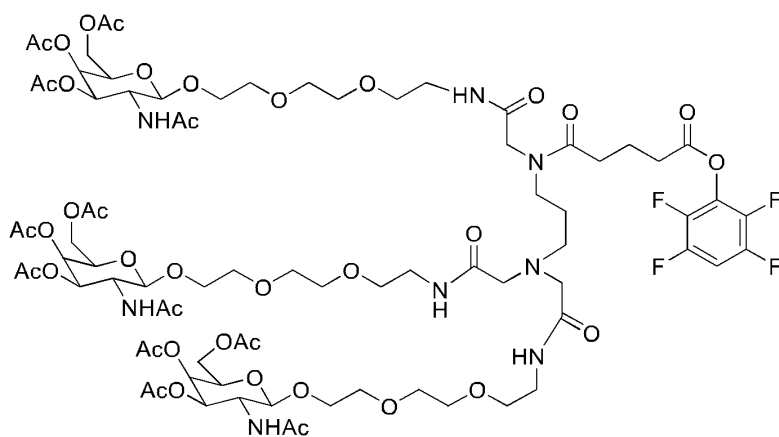
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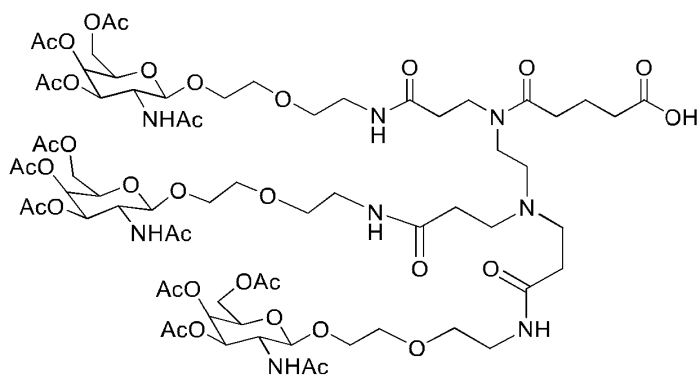
Compound



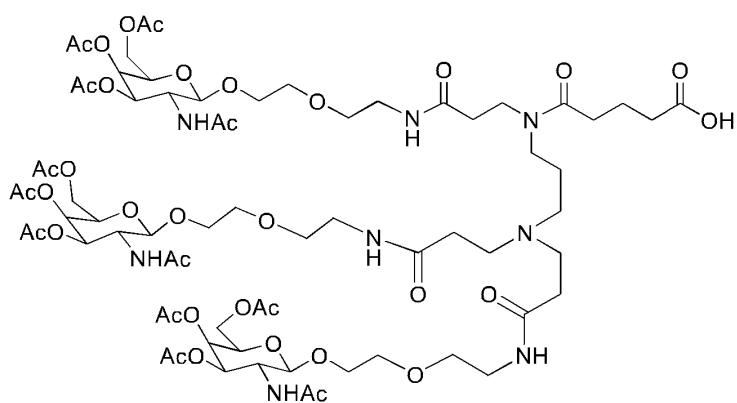
Compound 40;



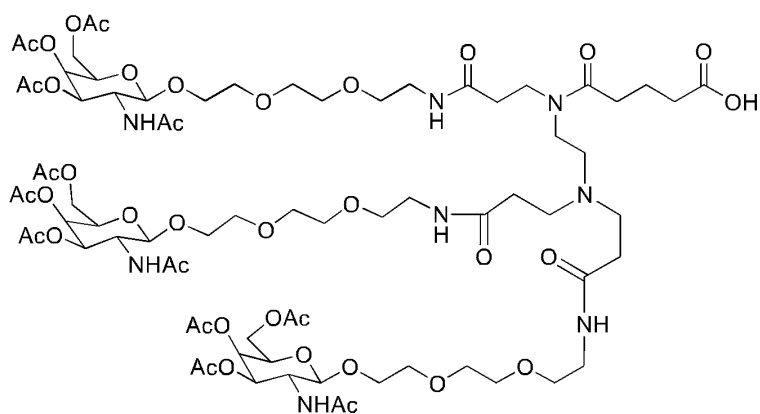
Compound 41;



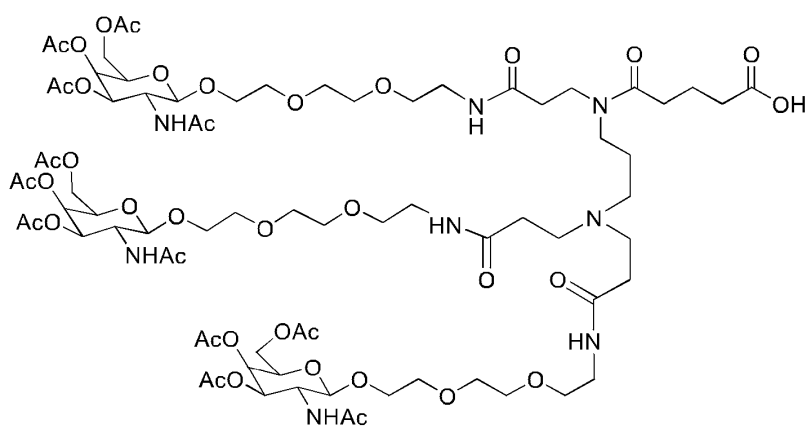
Compound 42;



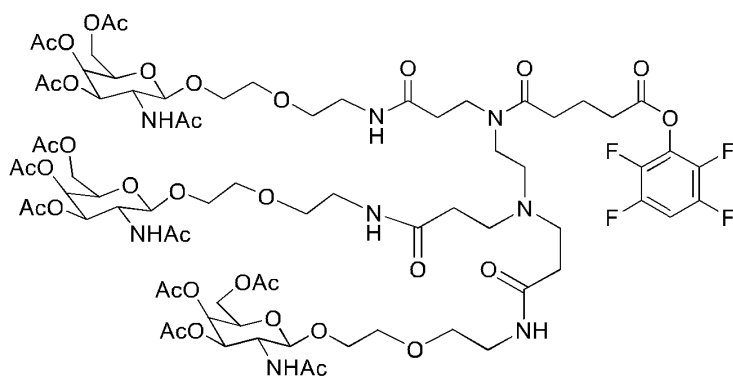
Compound 43;



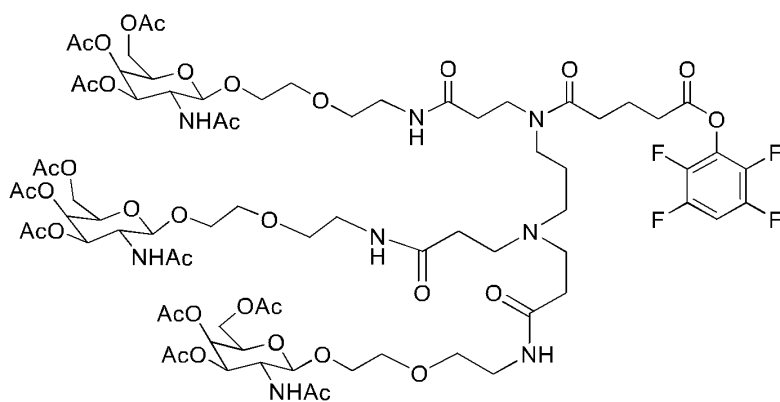
Compound 44;



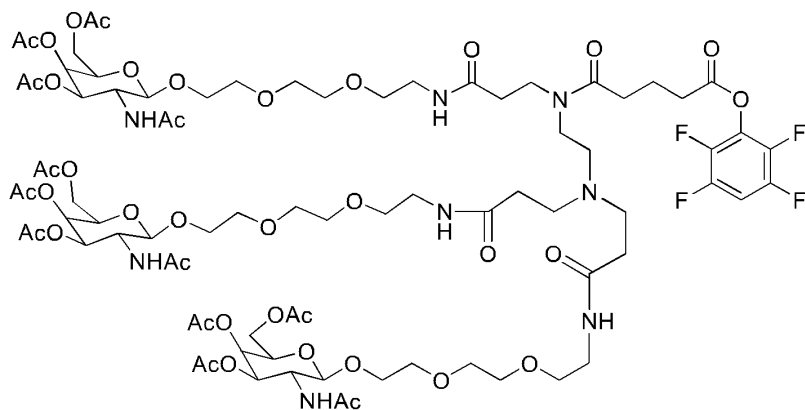
Compound 45;



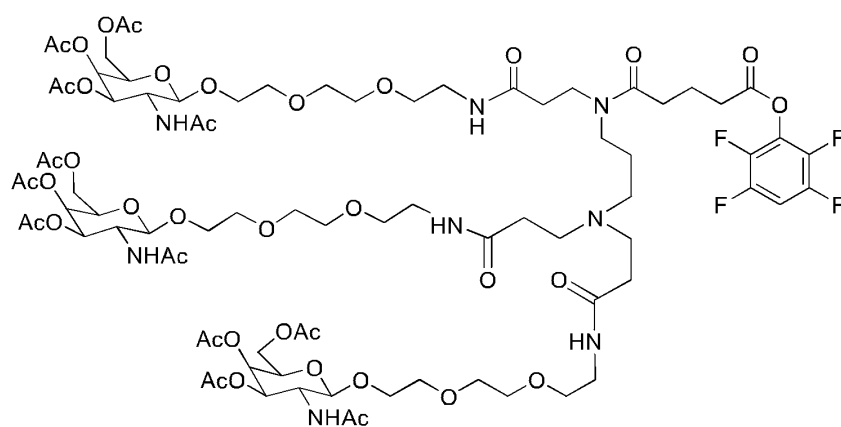
Compound 46;



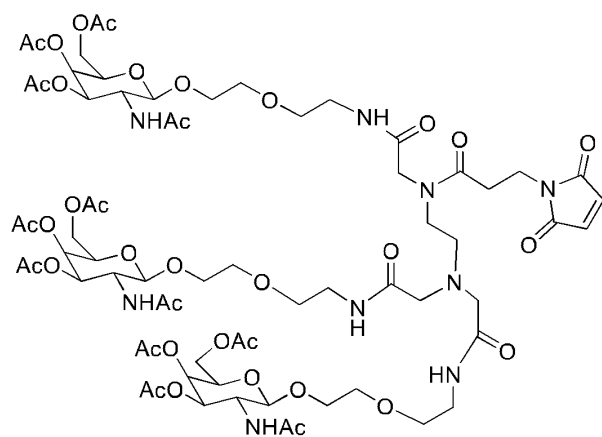
Compound 47;



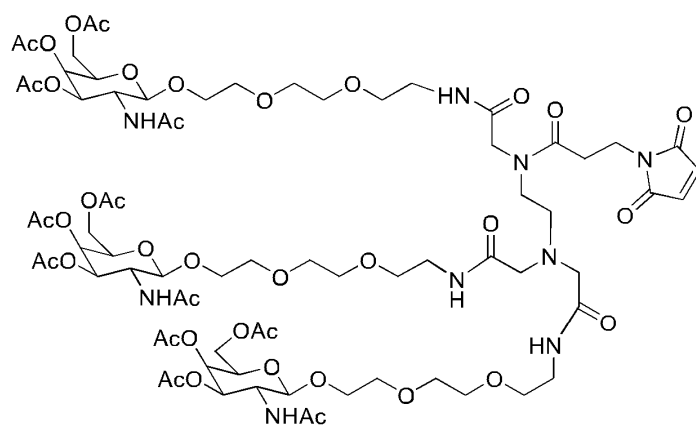
Compound 48;



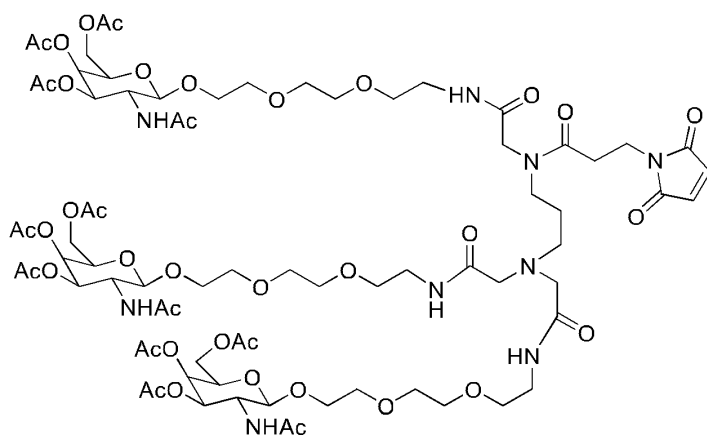
Compound 49;



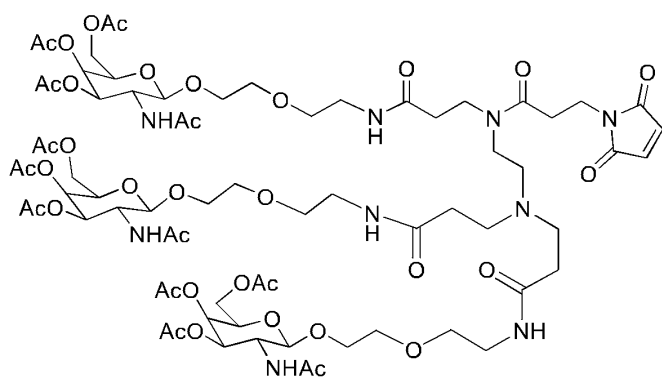
Compound 50;



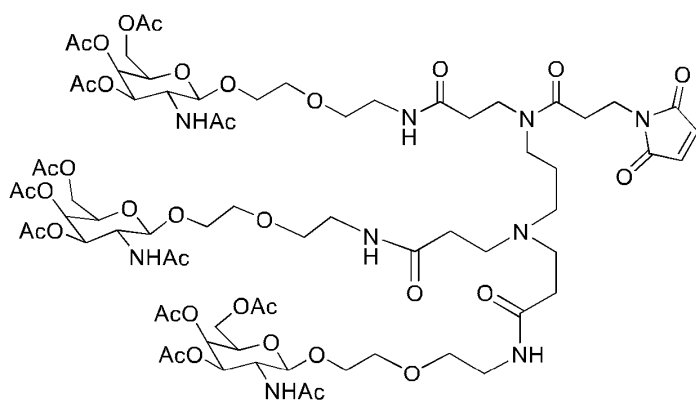
Compound 51;



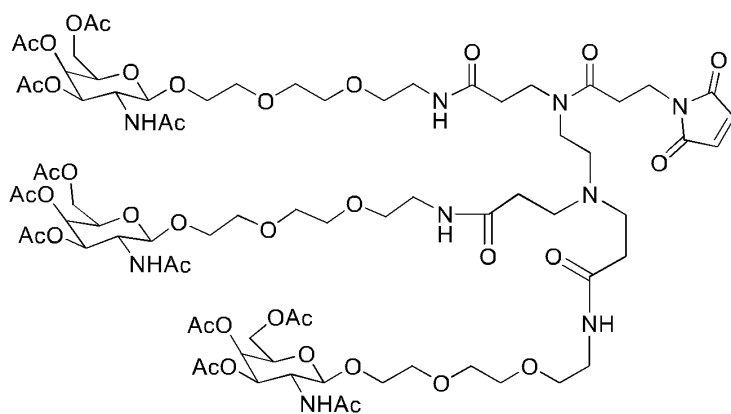
Compound 52;



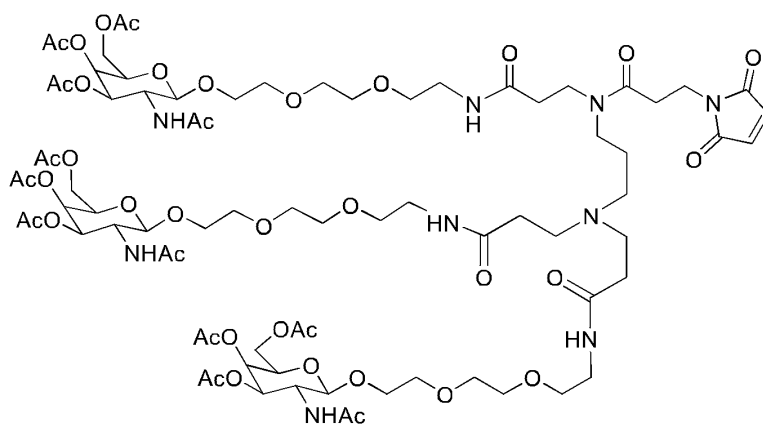
Compound 53;



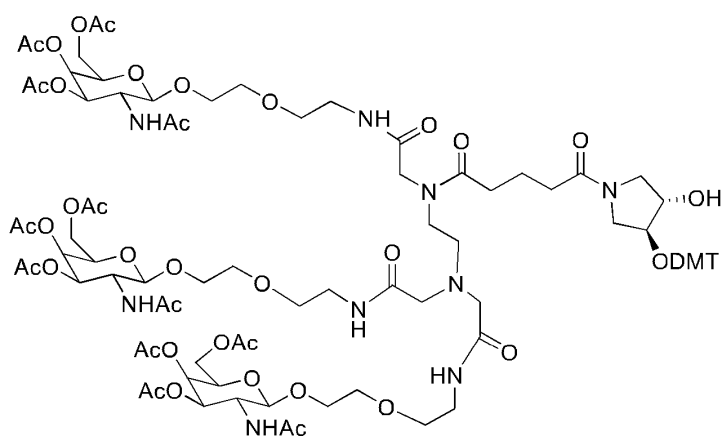
Compound 54;



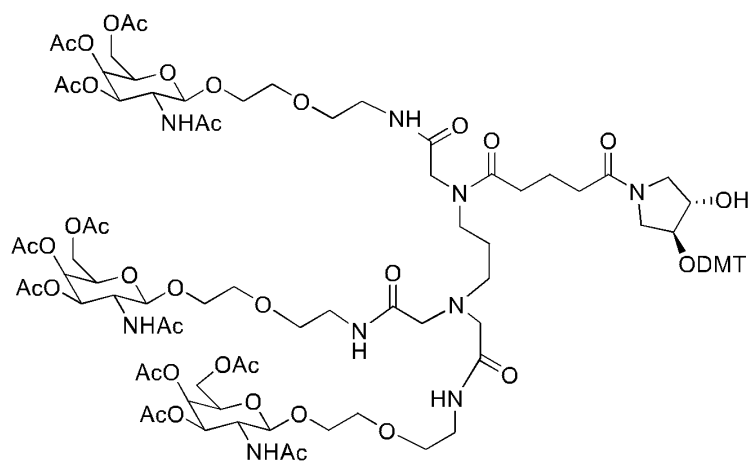
Compound 55;



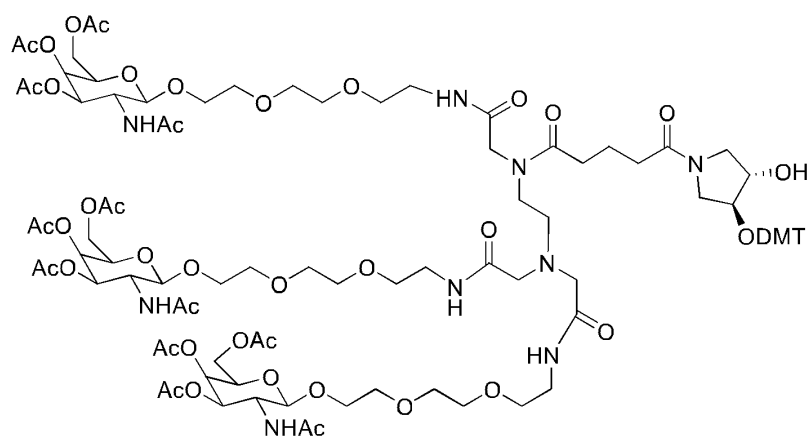
Compound 56;



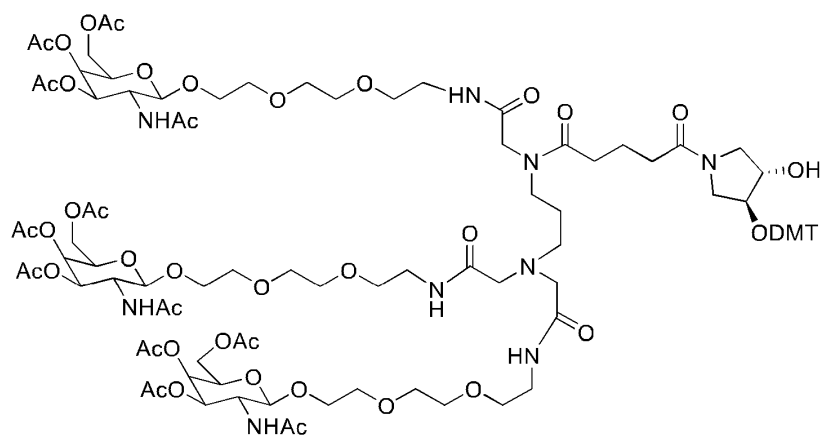
Compound 57;



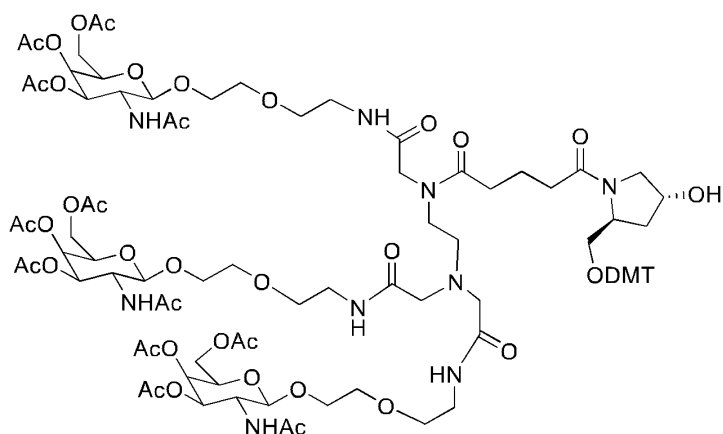
Compound 58;



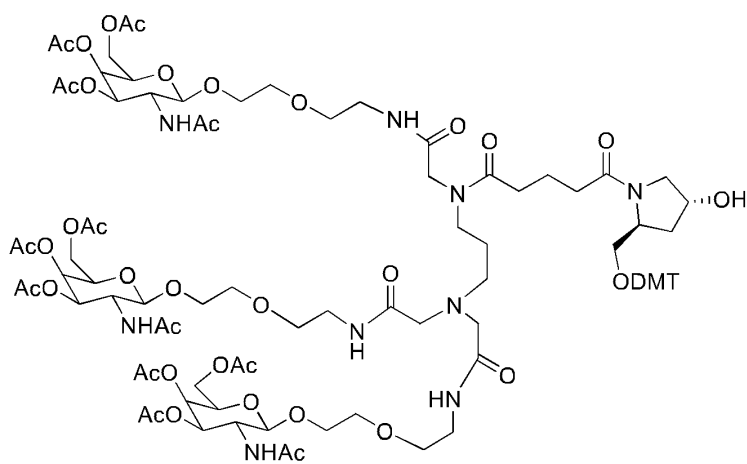
Compound 59;



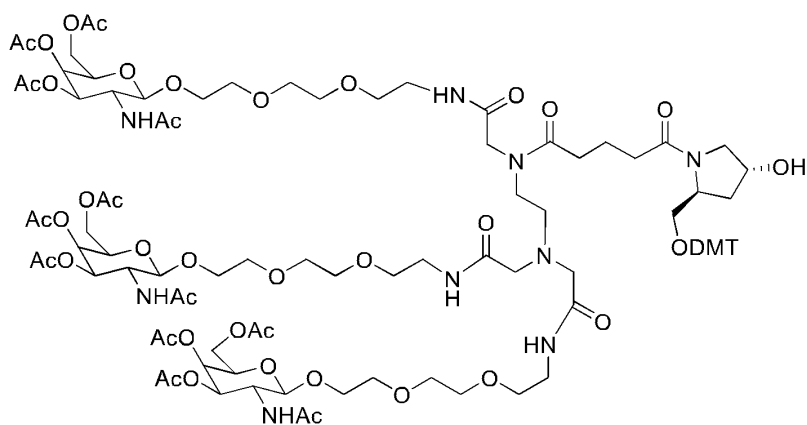
Compound 60;



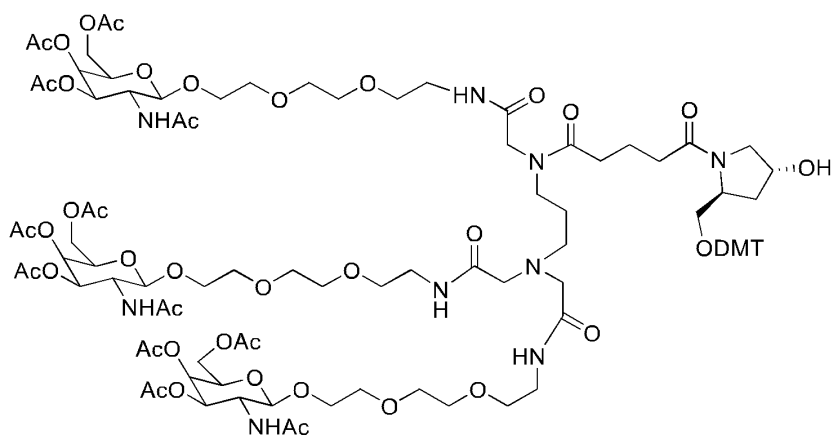
Compound 61;



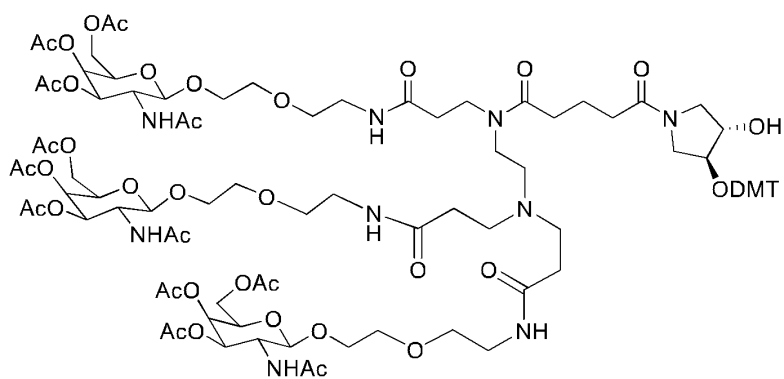
Compound 62;



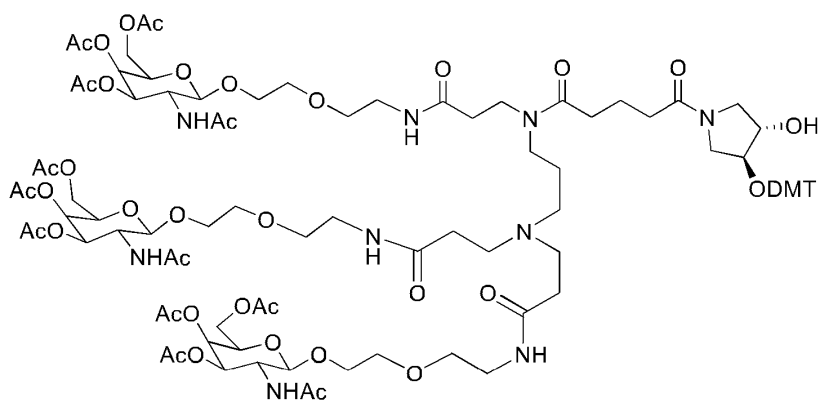
Compound 63;



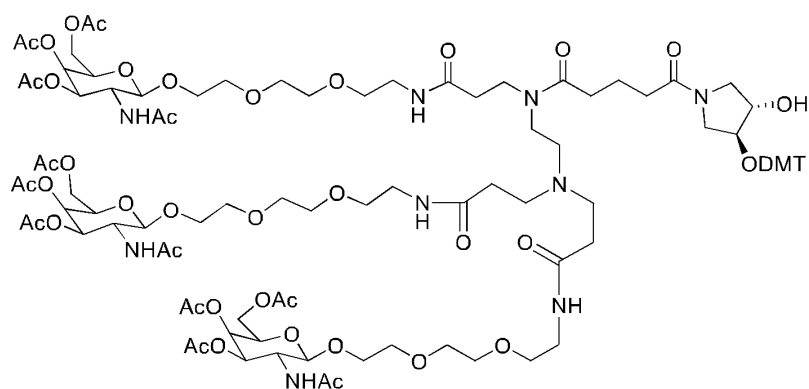
Compound 64;



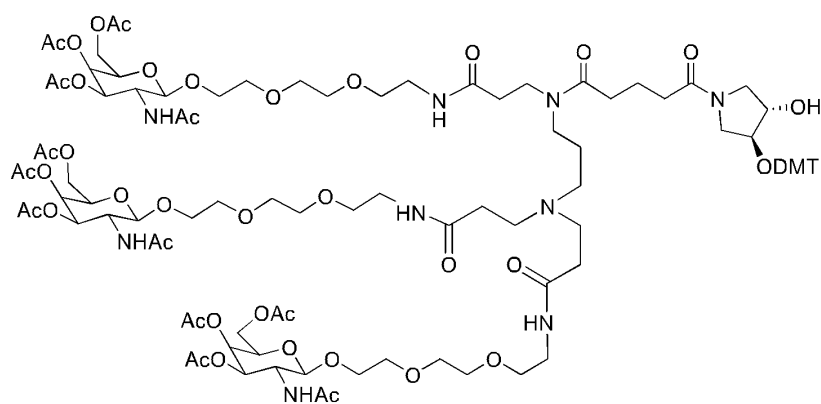
Compound 65;



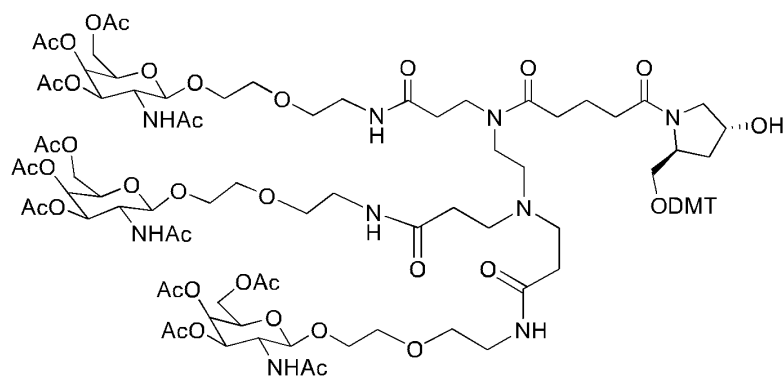
Compound 66;



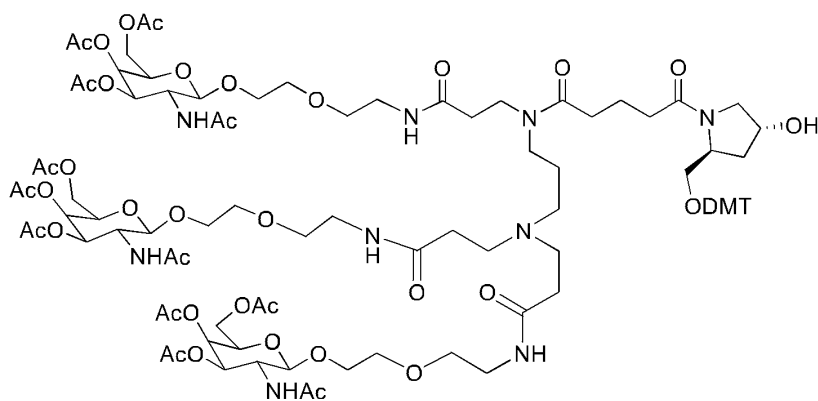
Compound 67;



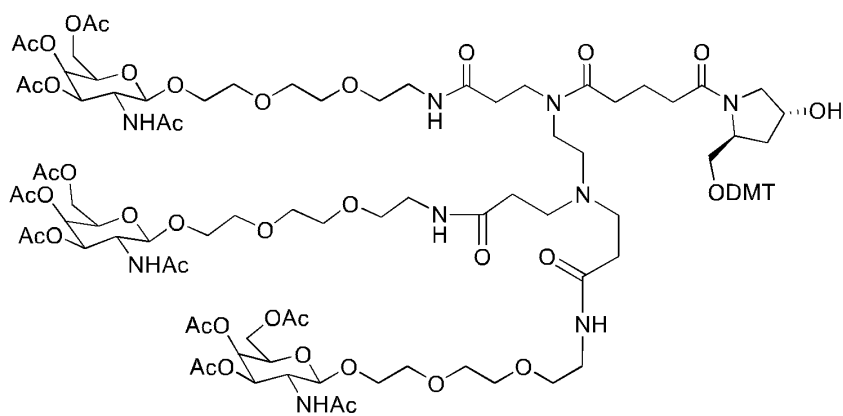
Compound 68;



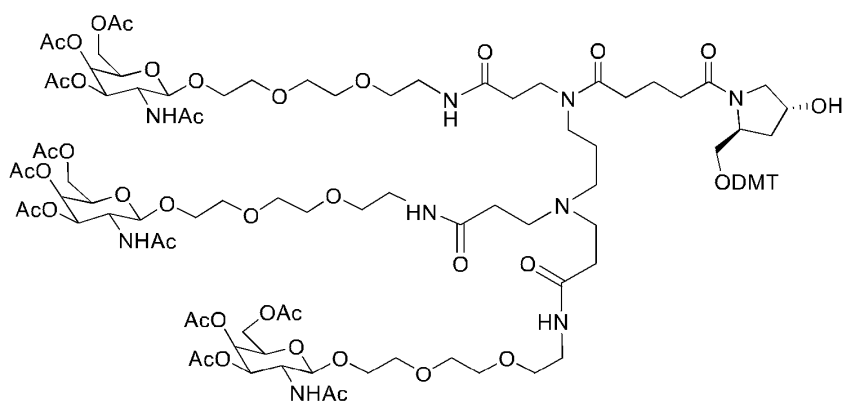
Compound 69;



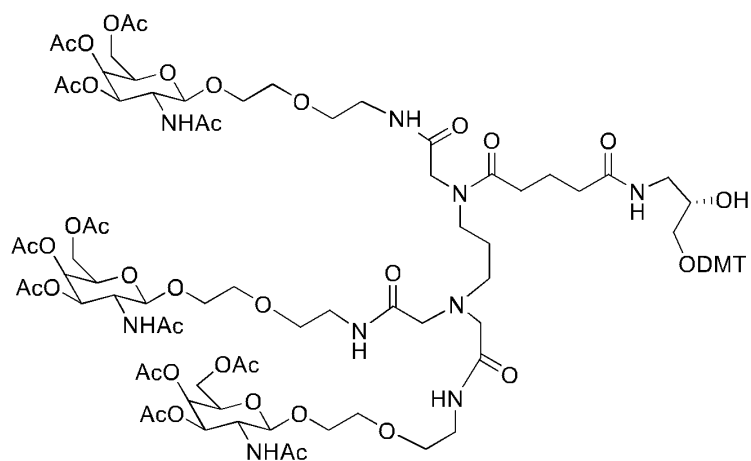
Compound 70;



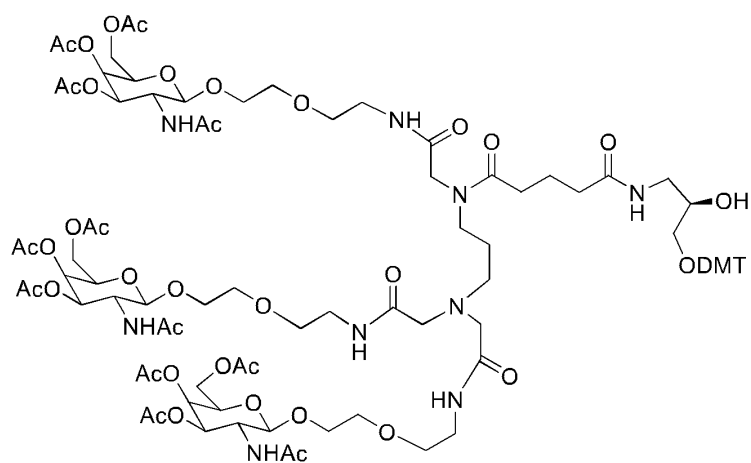
Compound 71;



Compound 72;

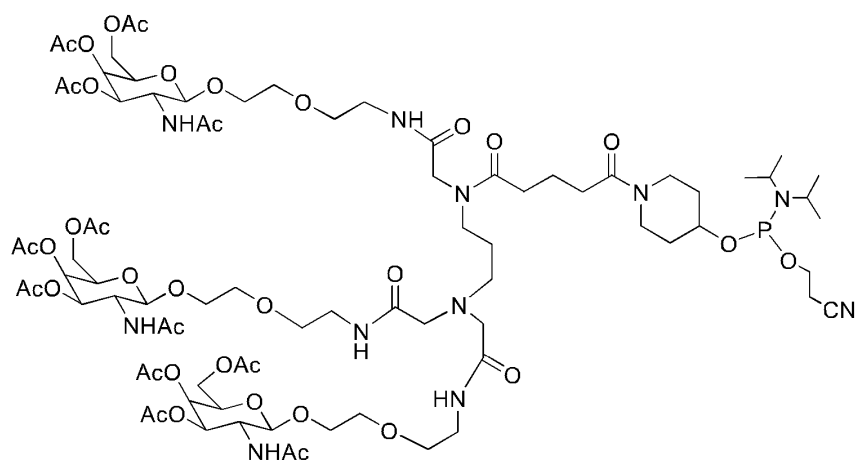


Compound 73;



Compound 74;

and



Compound 75.

5 In some embodiments, the compound is a stereoisomer of one of Compound 1-75.

In some embodiments, W is the one or more pharmaceutical agents. In some embodiments, the one or more pharmaceutical agents comprises at least one of a small

interfering RNA (siRNA), a single strand siRNA, a double stranded siRNA, a small activating RNA, an RNAi, a microRNA (miRNA), an antisense oligonucleotide, a short guide RNA (gRNA), a single guide RNA (sgRNA), a messenger RNA (mRNA), a ribozyme, a plasmid, an immune-stimulating nucleic acid, an antagomir, and an aptamer. In some
5 embodiments, the double stranded siRNA comprises at least one modified ribonucleotide. In some embodiments, the double stranded siRNA each strand is 19-23 nucleotides in length. In some embodiments, substantially all ribonucleotides of the double stranded siRNA are modified. In some embodiments, all ribonucleotides of the double stranded siRNA are modified. In some embodiments, the modified ribonucleotide comprises a 2'-O-methyl
10 nucleotide, 2'-Fluoro nucleotide, 2'-deoxy nucleotide, 2'3'-seco nucleotide mimic, locked nucleotide, 2'-F-Arabino nucleotide, 2'-methoxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted abasic nucleotide, inverted 2'-OMe nucleotide, inverted 2'deoxy nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, and 3'-OMe nucleotide, a nucleotide comprising a 5'-phosphorothioate group, or a
15 5'-(E)-vinyl phosphonate nucleotide (antisense strand only), or a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, a phosphoramidate, or a non-natural base comprising nucleotide. In some embodiments, at least one strand of the double-stranded siRNA comprises at least one phosphorothioate linkage. In some embodiments, at least one
20 strand of the double-stranded siRNA comprises up to 6 phosphorothioate linkages. In some embodiments, the double-stranded siRNA comprises at least one locked nucleic acid. In some embodiments, the double-stranded siRNA comprises at least one unlocked nucleic acid. In some embodiments, the double-stranded siRNA comprises at least one glycerol nucleic acid.

Another aspect of the present disclosure relates to a pharmaceutical composition
25 comprising the any one of the compounds detailed above. In some embodiments, the pharmaceutical composition comprises one or more pharmaceutical agents. In some embodiments, the pharmaceutical composition comprises one or more therapeutic agents. In some embodiments, the pharmaceutical composition comprises a pharmaceutically acceptable carrier.

30 A further aspect of the present disclosure relates to a composition for targeted delivery of one or more pharmaceutical agents, where the composition comprises any one of the compounds described above, and where W is the one or more pharmaceutical agents. In some embodiments, the one or more pharmaceutical agents comprises at least one of a small

interfering RNA (siRNA), a single strand siRNA, a double stranded siRNA, a small activating RNA, a microRNA (miRNA), an antisense oligonucleotide, a short guide RNA (gRNA), a single guide RNA (sgRNA), a messenger RNA (mRNA), a ribozyme, a plasmid, an immune stimulating nucleic acid, an antagomir, and an aptamer. In some embodiments, the double-stranded siRNA comprises at least one modified ribonucleotide in one or both strands of the siRNA. In some embodiments, the double stranded siRNA each strand is 19-23 nucleotides in length. In some embodiments, substantially all ribonucleotides of the double-stranded siRNA are modified. In some embodiments, all ribonucleotides of the double-stranded siRNA are modified. In some embodiments, the modified ribonucleotide comprises: a 2'-O-methyl nucleotide, 2'-Fluoro nucleotide, 2'-deoxy nucleotide, 2'3'-seco nucleotide mimic, locked nucleotide, 2'-F-Arabino nucleotide, 2'-methoxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted abasic nucleotide, inverted 2'-OMe nucleotide, inverted 2'-deoxy nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, mopholino nucleotide, and 3'-OMe nucleotide, a nucleotide comprising a 5'-phosphorothioate group, or a 5'-(E)-vinyl phosphonate nucleotide (antisense strand only), or a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, a phosphoramidate, or a non-natural base comprising nucleotide. In some embodiments, at least one strand of the double stranded siRNA comprises at least one phosphorothioate linkage. In some embodiments, at least one strand of the double-stranded siRNA comprises up to 6 phosphorothioate linkages. In some embodiments, the double-stranded siRNA comprises at least one locked nucleic acid. In some embodiments, the double-stranded siRNA comprises at least one unlocked nucleic acid. In some embodiments, the double-stranded siRNA comprises at least one glycerol nucleic acid.

Another aspect of the present disclosure relates to a pharmaceutical composition comprising any one of the compositions described above. In some embodiments, the pharmaceutical composition comprises one or more therapeutic agents. In some embodiments, the pharmaceutical composition comprises a pharmaceutically acceptable carrier.

A further aspect of the present disclosure relates to a method for making a compound for targeted delivery of one or more pharmaceutical agents, where the method comprises: receiving a first compound comprising a diamine, the diamine comprises a first nitrogen and a second nitrogen, the first nitrogen being a primary amine, the second nitrogen being a secondary amine comprising a protecting group; producing a second compound by coupling a

plurality of protected carboxylic acids to the first compound, the first nitrogen in the second compound being a tertiary amine comprising a first protected carboxylic acid and a second protected carboxylic acid, the second nitrogen of the second compound being a tertiary amine comprising the protecting group and a third protected carboxylic acid; producing a third
5 compound by deprotecting the second nitrogen of the second compound, resulting in the second nitrogen becoming a secondary amine comprising the third protected carboxylic acid; producing a fourth compound by attached a moiety comprising a hydroxy group to the second nitrogen of the third compound, resulting in the second nitrogen becoming a tertiary amine or an amide comprising the third protected carboxylic acid and the moiety comprising the
10 hydroxy group; producing a fifth compound by converting the protected carboxylic acids of the fourth compound into carboxylic acids; and producing a sixth compound by performing an amide coupling reaction using the fifth compound, the first nitrogen in the sixth compound being a tertiary amine comprising a first amide and a second amide, the second nitrogen in the sixth compound being a tertiary amine comprising the moiety comprising the hydroxy
15 group and a third amide, wherein the first amide, the second amide, and the third amide are each coupled to an independently selected targeting ligand.

In some embodiments, the protecting group is selected from the group consisting of a benzyl group and a triphenylmethyl group. In some embodiments, producing the second compound comprises performing a S_N2 substitution reaction using the first compound. In
20 some embodiments, producing the second compound comprises performing a reductive amination reaction using the first compound. In some embodiments, producing the second compound comprises performing a Michael addition reaction using the first compound. In some embodiments, the protecting group is a benzyl group, and producing the third compound comprises performing a hydrogenation reaction using the second compound. In
25 some embodiments, the protecting group is a triphenylmethyl group, and producing the third compound comprises reacting the second component with at least one acid. In some embodiments, producing the fourth compound comprises performing a S_N2 substitution reaction using the third compound. In some embodiments, producing the fourth compound comprises performing a reductive amination reaction using the third compound. In some
30 embodiments, producing the fourth compound comprises performing a Michael addition reaction using the third compound. In some embodiments, producing the fourth compound comprises performing an amide coupling reaction using the third compound. In some embodiments, producing the fourth compound comprises performing a nucleophilic addition

reaction using the third compound. In some embodiments, the moiety comprising the hydroxy group is attached to the second nitrogen using any linkerB described above. In some embodiments, producing the fifth compound comprises reacting the fourth compound with at least one acid. In some embodiments, the at least one acid comprises at least one of

5 hydrochloric acid, hydrobromic acid, trifluoroacetic acid, and formic acid. In some embodiments, producing the fifth compound comprises performing a hydrogenation reaction using the fourth compound. In some embodiments, producing the fifth compound comprises performing a hydrolysis reaction using the fourth compound. In some embodiments, the first amide, the second amide, and the third amide are each coupled to an independently selected

10 targeting ligand using any independently selected linkerA described above. In some embodiments, the independently selected targeting ligand is an independently selected targeting ligand described above. In some embodiments, the method further comprises converting the hydroxy group to a phosphoramidite group using a phosphitylation reaction. In some embodiments, converting the hydroxy group to the phosphoramidite group is performed

15 after performing the amide coupling reaction to produce the sixth compound.

Another aspect of the present disclosure relates to a method for making a compound for targeted delivery of one or more pharmaceutical agents, where the method comprises: receiving a first compound comprising a diamine, the diamine comprising a first nitrogen and a second nitrogen, the first nitrogen being a secondary amine comprising a first protecting

20 group, the second nitrogen being an amine comprising a second protecting group; producing a second compound by coupling a first protected carboxylic acid to the first nitrogen of the first compound, resulting in the first nitrogen becoming a tertiary amine; removing the first protecting group from the first nitrogen of the second compound to produce a third compound comprising the first nitrogen and the second nitrogen, the first nitrogen being a secondary

25 amine comprising the first protected carboxylic acid, the second nitrogen being an amine comprising the second protecting group; producing a fourth compound by coupling a second protected carboxylic acid to the first nitrogen of the third compound, resulting in the first nitrogen becoming a tertiary amine; removing the second protecting group from the fourth compound to produce a fifth compound comprising the first nitrogen and the second nitrogen,

30 the first nitrogen being a tertiary amine comprising the first protected carboxylic acid and the second protected carboxylic acid, the second nitrogen being a primary amine; producing a sixth compound by coupling a third protected carboxylic acid to the second nitrogen of the fifth compound, resulting in the second nitrogen becoming a secondary amine; producing a

seventh compound by attaching a moiety comprising a hydroxy group to the second nitrogen of sixth compound, resulting in the second nitrogen becoming a tertiary amine; producing an eighth compound by converting the third protected carboxylic acid of the seventh compound into a first carboxylic acid; producing a ninth compound by performing an amide coupling reaction using the eighth compound, the first nitrogen of the ninth compound comprising the first protected carboxylic acid and the second protected carboxylic acid, the second nitrogen of the ninth compound comprising the a first amide having a first targeting ligand coupled thereto and the moiety comprising the hydroxy group; producing a tenth compound by converting the second protected carboxylic acid of the ninth compound into a second carboxylic acid; producing an eleventh compound by performing an amide coupling reaction using the tenth compound, the first nitrogen of the eleventh compound comprising the first protected carboxylic acid and a second amide having a second targeting ligand coupled thereto, the second nitrogen of the eleventh compound comprising the first amide having the first targeting ligand coupled thereto and the moiety comprising the hydroxy group; producing a twelfth compound by converting the first protected carboxylic acid of the eleventh compound into a third carboxylic acid; and producing a thirteenth compound by performing an amide coupling reaction using the twelfth compound, the first nitrogen of the thirteenth compound comprising the second amide having the second targeting ligand coupled thereto and a third amide having a third targeting ligand coupled thereto, the second nitrogen of the thirteenth compound comprising the first amide having the first targeting ligand coupled thereto and the moiety comprising the hydroxy group.

In some embodiments, the first protecting group is a benzyl group and the second protecting group is a *tert*-butoxycarbonyl (Boc) group. In some embodiments, producing the second compound comprises performing a S_N2 substitution reaction using the first compound. In some embodiments, producing the second compound comprises performing a reductive amination reaction using the first compound. In some embodiments, producing the second compound comprises performing a Michael addition reaction using the first compound. In some embodiments, producing the third compound comprises performing a hydrogenation reaction using the second compound. In some embodiments, producing the fourth compound comprises performing a S_N2 substitution reaction using the third compound. In some embodiments, producing the fourth compound comprises performing a reductive amination reaction using the third compound. In some embodiments, producing the fourth compound comprises performing a Michael addition reaction using the third compound. In

some embodiments, producing the fourth compound comprises performing an amide coupling reaction using the third compound. In some embodiments, producing the fourth compound comprises performing a nucleophilic addition reaction using the third compound. In some embodiments, producing the fifth compound comprises reacting the fourth

5 compound with at least one acid. In some embodiments, the at least one acid comprises at least one of hydrochloric acid and trifluoroacetic acid. In some embodiments, producing the sixth compound comprises performing a S_N2 substitution reaction using the fifth compound. In some embodiments, producing the sixth compound comprises performing a reductive amination reaction using the fifth compound. In some embodiments, producing the sixth

10 compound comprises performing a Michael addition reaction using the fifth compound. In some embodiments, producing the seventh compound comprises performing a S_N2 substitution reaction using the sixth compound. In some embodiments, producing the seventh compound comprises performing a reductive amination reaction using the sixth compound. In some embodiments, producing the seventh compound comprises performing a Michael

15 addition reaction using the sixth compound. In some embodiments, producing the seventh compound comprises performing an amide coupling reaction using the sixth compound. In some embodiments, producing the seventh compound comprises performing a nucleophilic addition reaction using the sixth compound. In some embodiments, the first amide is coupled to the first targeting ligand using an independently selected linkerA described above. In some

20 embodiments, the second amide is coupled to the second targeting ligand using an independently selected linkerA described above. In some embodiments, the third amide is coupled to the third targeting ligand using an independently selected linkerA described above. In some embodiments, the first targeting ligand, the second targeting ligand, and the third targeting ligand are independently selected to be one or more of the targeting ligands

25 described above. In some embodiments, the hydroxy group is coupled to the second nitrogen using a linkerB described above. In some embodiments, the method further comprises converting the hydroxy group to a phosphoramidite group using a phosphitylation reaction. In some embodiments, converting the hydroxy group to the phosphoramidite group is performed after producing the thirteenth compound.

30 A further aspect of the present disclosure relates to a method for delivering a pharmaceutical agent to a subject, the method comprising administering, to the subject, (a) a compound described above, where W is the one or more pharmaceutical agents, or (b) a composition described above. In some embodiments, the subject is a vertebrate. In some

embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In some embodiments, the compound is administered in a pharmaceutically acceptable carrier.

Another aspect of the present disclosure relates to a method for delivering a pharmaceutical agent to a subject, the method comprising administering, to the subject, a pharmaceutical composition described above. In some embodiments, the subject is a vertebrate. In some embodiments, the subject is a mammal, optionally the mammal is a human. In some embodiments, the one or more pharmaceutical agents comprises at least one of a small interfering RNA (siRNA), a single strand siRNA, a double stranded siRNA, a small activating RNA, a microRNA (miRNA), an antisense oligonucleotide, a short guide RNA (gRNA), a single guide RNA (sgRNA), a messenger RNA (mRNA), a ribozyme, a plasmid, an immune stimulating nucleic acid, an antagomir, and an aptamer. In some embodiments, the double-stranded siRNA comprises at least one modified ribonucleotide in one or both strands of the siRNA. In some embodiments, substantially all ribonucleotides of the double-stranded siRNA are modified. In some embodiments, all ribonucleotides of the double-stranded siRNA are modified. In some embodiments, the modified ribonucleotide comprises: a 2'-O-methyl nucleotide, 2'-Fluoro nucleotide, 2'-deoxy nucleotide, 2'3'-seco nucleotide mimic, locked nucleotide, 2'-F-Arabino nucleotide, 2'-methoxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted abasic nucleotide, inverted 2'-OMe nucleotide, inverted 2'deoxy nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, mopholino nucleotide, and 3'-OMe nucleotide, a nucleotide comprising a 5'-phosphorothioate group, or a 5'-(E)-vinyl phosphonate nucleotide (antisense strand only), or a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, a phosphoramidate, or a non-natural base comprising nucleotide. In some embodiments, at least one strand of the double stranded siRNA comprises at least one phosphorothioate linkage. In some embodiments, at least one strand of the double-stranded siRNA comprises up to 6 phosphorothioate linkages. In some embodiments, the double-stranded siRNA comprises at least one locked nucleic acid. In some embodiments, the double-stranded siRNA comprises at least one unlocked nucleic acid. In some embodiments, the double-stranded siRNA comprises at least one glycerol nucleic acid. In some embodiments, the pharmaceutical composition further comprises one or more therapeutic agents.

Another aspect of the present disclosure the compounds for use to deliver a pharmaceutical agent to a subject. In some embodiments, the subject is a vertebrate. In some

embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In some embodiments, the compound is administered in a pharmaceutically acceptable carrier.

Another aspect of the present disclosure, the dsRNA agent comprises 2'-fluoro modified nucleotides at positions 2, 7, 12, 14 and 16 of the antisense strand (counting from the first paired nucleotide from the 5' end of the antisense strand), and/or 2'-fluorine-
5 modified nucleotides at positions 9, 11 and 13 of the sense strand (counting from the first paired nucleotide from the 3' end of the sense strand).

DETAILED DESCRIPTION

10 Overview

The present disclosure provides multivalent ligand clusters, having a diamine scaffold, for targeted delivery of pharmaceutical agents conjugated thereto. In some embodiments, the multivalent ligand cluster may comprise one or more N-acetylgalactosamine (GalNAc) targeting ligands. In some embodiments, the multivalent ligand cluster may be conjugated to
15 one or more small interfering ribonucleic acids (siRNAs), with siRNA being an example of a pharmaceutical agent. The present disclosure also provides compositions comprising the multivalent ligand clusters of the present disclosure, and methods of making and using the multivalent ligand clusters of the present disclosure.

Definitions

20 Before further description of the invention, and in order that the invention may be more readily understood, certain terms are first defined and collected herein for convenience.

As used herein, the term “treat,” “treating,” or “treatment” may include prophylaxis and means to ameliorate, alleviate symptoms, eliminate the causation of the symptoms either on a temporary or permanent basis, or to prevent or slow the appearance of symptoms of the
25 named disorder or condition.

As used herein, the term “about,” in connection with a measured quantity, refers to the normal variations in that measured quantity, as expected by the skilled artisan making the measurement and exercising a level of care commensurate with the objective of measurement and the precision of the measuring equipment.

30 As used herein, the term “conjugate” or “conjugate group” means an atom or group of atoms bound to an oligonucleotide or other oligomer. In general, conjugate groups modify one or more properties of the compound to which they are attached, including, but not limited

to pharmacodynamics, pharmacokinetic, binding, absorption, cellular distribution, cellular uptake, charge, and/or clearance properties.

As used herein, the term “linked,” when referring to the connection between two molecules, means the two molecules are joined, directly or indirectly, by a covalent bond or that the two molecules are associated via noncovalent bonds (e.g., hydrogen bonds or ionic bonds). An example of a Compound A being directly joined to a Compound B may be represented as A-B. An example of a Compound A being indirectly joined to a Compound B may be represented as A-C-B, where Compound A is indirectly joined to Compound B through Compound C. It will be appreciated that more than one intermediary compound may be present in situations of indirect joining of compounds.

As used herein, the term “nucleic acid” refers to molecules composed of monomeric nucleotides. A nucleic acid includes ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs), single-stranded nucleic acids (ssDNAs), double-stranded nucleic acids (dsDNAs), small interfering ribonucleic acids (siRNAs) and microRNAs (miRNAs). A nucleic acid may also comprise any combination of these elements in a single molecule. A nucleic acid may include natural nucleic acids, non-natural nucleic acids, or a combination of natural and non-natural nucleic acids. A nucleic acid may also be referred to herein as a nucleotide sequence, or as a polynucleotide.

As used herein, the term “oligomer” refers to nucleotide sequence containing up to 5, up to 10, up to 15, up to 20, or more than 20 nucleotides or nucleotide base pairs. In some embodiments, an oligomer has a nucleobase sequence that is at least partially complementary to a coding sequence in an expressed target nucleic acid or target gene within a cell. In some embodiments, the oligomers, upon delivery to a cell expressing a gene, are able to inhibit the expression of the underlying gene. The gene expression can be inhibited *in vitro* or *in vivo*. Non-limiting examples of oligomers that may be included in methods and complexes of the invention are oligonucleotides, single-stranded oligonucleotides, single-stranded antisense oligonucleotides, short interfering RNAs (siRNAs), single-stranded siRNA, double-strand RNAs (dsRNA), micro RNAs (miRNAs), short hairpin RNAs (shRNA), ribozymes, interfering RNA molecules, and dicer substrates.

As used herein, the term “oligonucleotide” refers to a polymer of linked nucleotides each of which can be independently modified or unmodified.

As used herein, the term “single-stranded oligonucleotide” refers to a single-stranded oligomer and in certain embodiments a single-stranded oligonucleotide may comprise a

sequence at least partially complementary to a target mRNA, that is capable of hybridizing to a target mRNA through hydrogen bonding under mammalian physiological conditions (or comparable conditions *in vitro*). In some embodiments, a single-stranded oligonucleotide is a single stranded antisense oligonucleotide.

- 5 As used herein, the term “siRNA” refers to a short interfering RNA or silencing RNA. siRNAs are a class of double-stranded RNA molecules, that may be 20-25 (or shorter) base pairs in length, similar to microRNA (miRNA) that operate within the RNA interference (RNAi) pathway. siRNAs interfere with the expression of specific genes with complementary nucleotide sequences to the siRNA by degrading mRNA after transcription, preventing
10 translation. siRNAs act in cells to silence gene expression by inducing the RNA-induced silencing complex (RISC) to cleave messenger RNA (mRNA).

As used herein, the term “effective amount,” “therapeutically effective amount,” or “effective dose” refers to an amount sufficient to elicit the desired pharmacological or therapeutic effects, thus resulting in effective prevention or treatment of the disorder.

- 15 Prevention of the disorder is manifested by delaying the onset of the symptoms of the disorder to a medically significant extent. Treatment of the disorder is manifested by a decrease in the symptoms associated with the disorder or an amelioration of the reoccurrence of the symptoms of the disorder.

- As used herein, the term “pharmaceutical composition” or “composition” refers to a
20 mixture of substances suitable for administering to an individual. For example, though not intended to be limiting, a pharmaceutical composition can comprise one or more active agents and a pharmaceutical carrier, also referred to herein as a “pharmaceutically acceptable carrier” (e.g. a sterile aqueous solution). In some embodiments, a pharmaceutical composition is sterile.

- 25 As used herein, the term “alkyl” as used by itself or as part of another group refers to a straight- or branched-chain aliphatic hydrocarbon containing one to twelve carbon atoms (i.e., C₁₋₁₂ alkyl) or the number of carbon atoms designated (i.e., a C₁ alkyl such as methyl, a C₂ alkyl such as ethyl, a C₃ alkyl such as propyl or isopropyl, etc.). Non-limiting illustrative C₁₋₁₀ alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl, iso-
30 butyl, 3-pentyl, hexyl, heptyl, octyl, nonyl, decyl, and the like.

As used herein, the term “substituted alkyl” by itself or as part of another group means that the alkyl as defined herein is substituted with one or more (e.g., one, two, or three) independently selected substituents. A non-limiting list of independently selected substituents

includes amino, (alkyl)amino, (alkyl)carbonyl, (aryl)carbonyl, (alkoxy)carbonyl, [(alkoxy)carbonyl]amino, carboxy, aryl, heteroaryl, ureido, guanidino, halogen, sulfonamido, hydroxyl, (alkyl)sulfanyl, nitro, haloalkoxy, aryloxy, aralkyloxy, (alkyl)sulfonyl, (cycloalkyl)sulfonyl, (aryl)sulfonyl, cycloalkyl, sulfanyl, caboxamido, heterocyclyl, and
 5 (heterocyclyl)sulfonyl.

As used herein, the term “cycloalkyl” by itself or as part of another group refers to saturated and partially unsaturated (containing one or two double bonds) cyclic aliphatic hydrocarbons containing one to three rings having from three to twelve carbon atoms (i.e., C₃₋₁₂ cycloalkyl) or the number of carbons designated. Non-limiting illustrative cycloalkyl
 10 groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, norbornyl, decalin, adamantyl, cyclohexenyl, cyclopentenyl, cyclohexenyl, and the like.

As used herein, the term “substituted cycloalkyl” by itself or as part of another group means that the cycloalkyl as defined herein is substituted with one, two, or three independently selected substituents. A non-limiting list of independently selected substituents
 15 includes halo, nitro, cyano, hydroxyl, amino, (alkyl)amino, (dialkyl)amino, haloalkyl, (hydroxyl)alkyl, (dihydroxy)alkyl, alkoxy, haloalkoxy, aryloxy, aralkyloxy, alkylthio, carboxamido, sulfonamido, (alkyl)carbonyl, (aryl)carbonyl, (alkyl)sulfonyl, arylsulfonyl, ureido, guanidino, carboxy, (carboxy)alkyl, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocyclyl, (alkoxy)alkyl, (amino)alkyl, (hydroxyl)alkylamino,
 20 (alkylamino)alkyl, (dialkylamino)alkyl, (cyano)alkyl, (carboxamido)alkyl, (alkyl)sulfanyl, (heterocyclo)alkyl, (heteroaryl)alkyl, (alkoxy)carbonyl, and mercaptoalkyl.

As used herein, the term “alkenyl” by itself or as part of another group refers to an alkyl group as defined herein containing one, two or three carbon-to-carbon double bonds. Non-limiting illustrative alkenyl groups include ethenyl, propenyl, isopropenyl, butenyl, sec-
 25 butenyl, pentenyl, and hexenyl.

As used herein, the term “substituted alkenyl” by itself or as part of another group means the alkenyl as defined herein is substituted with one, two, or three independently selected substituents. A non-limiting list of independently selected substituents includes halo, nitro, cyano, hydroxyl, amino, (alkyl)amino, (dialkyl)amino, haloalkyl, (hydroxy)alkyl,
 30 (dihydroxy)alkyl, alkoxy, haloalkoxy, aryloxy, aralkyloxy, (alkyl)sulfanyl, carboxamido, sulfonamido, (alkyl)carbonyl, (aryl)carbonyl, (alkyl)sulfonyl, (aryl)sulfonyl, ureido, guanidino, carboxy, (carboxy)alkyl, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, and heterocyclyl.

As used herein, the term “cycloalkenyl” by itself or as part of another group refers to non-aromatic cyclic alkyl groups of from 4 to 10 carbon atoms having single or multiple cyclic rings and having at least one $>C=C<$ ring unsaturation and preferably from 1 to 2 sites of $>C=C<$ ring unsaturation.

- 5 As used herein, the term “substituted cycloalkenyl” by itself or as part of another group refers to a cycloalkenyl as defined herein having from 1 to 5 independently selected substituents. A non-limiting list of independently selected substituents includes oxo, thione, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, substituted amino, aminocarbonyl, aminothiocarbonyl, aminocarbonylamino, aminothiocarbonylamino, aminocarbonyloxy, 10 aminosulfonyl, aminosulfonyloxy, aminosulfonylamino, amidino, aryl, substituted aryl, aryloxy, substituted aryloxy, arylthio, substituted arylthio, azido, carboxyl, carboxyl ester, (carboxyl ester)amino, (carboxyl ester)oxy, cyano, cyanate, cycloalkyl, substituted cycloalkyl, cycloalkyloxy, substituted cycloalkyloxy, cycloalkylthio, substituted cycloalkylthio, 15 cycloalkenyl, substituted cycloalkenyl, cycloalkenyloxy, substituted cycloalkenyloxy, cycloalkenylthio, substituted cycloalkenylthio, guanidino, substituted guanidino, halo, hydroxy, hydroxyamino, alkoxyamino, hydrazino, substituted hydrazino, heteroaryl, substituted heteroaryl, heteroaryloxy, substituted heteroaryloxy, heteroarylthio, substituted heteroarylthio, heterocyclic, substituted heterocyclic, heterocyclyloxy, substituted heterocyclyloxy, heterocyclylthio, substituted heterocyclylthio, nitro, SO_3H , substituted sulfonyl, sulfonyloxy, thioacyl, thiocyanate, thiol, alkylthio, and substituted alkylthio. 20

- As used herein, the term “alkynyl” by itself or as part of another group refers to an alkyl group as defined herein containing one to three carbon-to-carbon triple bonds. Non-limiting illustrative alkynyl groups include ethynyl, propynyl, butynyl, 2-butynyl, pentynyl, 25 and hexynyl groups.

- As used herein, the term “substituted alkynyl” by itself or as part of another group means the alkynyl as defined herein is substituted with one, two, or three independently selected substituents. A non-limiting list of independently selected substituents includes halo, nitro, cyano, hydroxyl, amino, alkylamino, dialkylamino, haloalkyl, (hydroxy)alkyl, 30 (dihydroxy)alkyl, alkoxy, haloalkoxy, aryloxy, aralkyloxy, (alkyl)sulfanyl, carboxamido, sulfonamido, alkylcarbonyl, arylcarbonyl, alkylsulfonyl, arylsulfonyl, ureido, guanidino, carboxy, (carboxy)alkyl, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, and heterocyclyl.

As used herein, the term “haloalkyl” by itself or as part of another group refers to an alkyl group substituted by one or more fluorine, chlorine, bromine and/or iodine atoms. Non-limiting illustrative haloalkyl groups include fluoromethyl, difluoromethyl, trifluoromethyl, pentafluoroethyl, 1,1-difluoroethyl, 2,2-difluoroethyl, 2,2,2-trifluoroethyl, 3,3,3-trifluoropropyl, 4,4,4-trifluorobutyl, and trichloromethyl groups.

As used herein, the term “alkoxy” by itself or as part of another group refers to an alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, or substituted alkynyl attached to a terminal oxygen atom.

As used herein, the term “haloalkoxy” by itself or as part of another group refers to a haloalkyl attached to a terminal oxygen atom. Non-limiting illustrative haloalkoxy groups include fluoromethoxy, difluoromethoxy, trifluoromethoxy, and 2,2,2-trifluoroethoxy.

As used herein, the term “aryl” by itself or as part of another group refers to a monocyclic or bicyclic aromatic ring system having from six to fourteen carbon atoms (i.e., C₆-C₁₄ aryl). Non-limiting illustrative aryl groups include phenyl, naphthyl, phenanthryl, anthracyl, indenyl, azulenyl, biphenyl, biphenylenyl, and fluorenyl groups.

As used herein, the term “substituted aryl” by itself or as part of another group means that the aryl as defined herein is substituted with one to five independently selected substituents. A non-limiting list of independently selected substituents includes halo, nitro, cyano, hydroxyl, amino, alkylamino, dialkylamino, haloalkyl, (hydroxy)alkyl, (dihydroxy)alkyl, alkoxy, haloalkoxy, aryloxy, heteroaryloxy, aralkyloxy, alkylthio, carboxamido, sulfonamido, (alkyl)carbonyl, (aryl)carbonyl, (alkyl)sulfonyl, (aryl)sulfonyl, ureido, guanidino, carboxy, carboxyalkyl, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocyclo, (alkoxy)alkyl, (amino)alkyl, [(hydroxy)alkyl]amino, [(alkyl)amino]alkyl, [(dialkyl)amino]alkyl, (cyano)alkyl, (carboxamido)alkyl, mercaptoalkyl, (heterocyclo)alkyl, (cycloalkylamino)alkyl, (halo(C₁-C₄))alkoxyalkyl, (heteroaryl)alkyl, and the like. Non-limiting illustrative substituted aryl groups include 2-methylphenyl, 2-methoxyphenyl, 2-fluorophenyl, 2-chlorophenyl, 2-bromophenyl, 3-methylphenyl, 3-methoxyphenyl, 3-fluorophenyl, 3-chlorophenyl, 4-methylphenyl, 4-ethylphenyl, 4-methoxyphenyl, 4-fluorophenyl, 4-chlorophenyl, 2,6-di-fluorophenyl, 2,6-di-chlorophenyl, 2-methyl, 3-methoxyphenyl, 2-ethyl, 3-methoxyphenyl, 3,4-di-methoxyphenyl, 3,5-di-fluorophenyl, 3,5-di-methylphenyl, 3,5-dimethoxy, 4-methylphenyl, 2-fluoro-3-chlorophenyl, and 3-chloro-4-fluorophenyl. The term substituted aryl is meant to include groups having fused substituted cycloalkyl and fused substituted heterocyclo rings.

As used herein, the term “aryloxy” by itself or as part of another group refers to an aryl or substituted aryl attached to a terminal oxygen atom. A non-limiting illustrative aryloxy group is PhO—.

As used herein, the term “heteroaryloxy” by itself or as part of another group refers to
5 a heteroaryl or substituted heteroaryl attached to a terminal oxygen atom.

As used herein, the term “aralkyloxy” by itself or as part of another group refers to an aralkyl group attached to a terminal oxygen atom. A non-limiting illustrative aralkyloxy group is PhCH₂O—.

As used herein, the term “heteroaryl” refers to monocyclic and bicyclic aromatic ring
10 systems having 5 to 14 ring atoms (i.e., C₅-C₁₄ heteroaryl) and 1, 2, 3, or 4 heteroatoms independently chosen from oxygen (O), nitrogen (N), and sulfur (S). Non-limiting illustrative heteroaryl groups include thienyl, benzo[b]thienyl, naphtho[2,3-b]thienyl, thianthrenyl, furyl, benzofuryl, pyranyl, isobenzofuranyl, benzooxazolyl, chromenyl, xanthenyl, 2H-pyrrolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, isoindolyl, 3H-
15 indolyl, indolyl, indazolyl, purinyl, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, cinnolinyl, quinazolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, β-carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, thiazolyl, isothiazolyl, phenothiazolyl, isoxazolyl, furazanyl, and phenoxazinyl. The term “heteroaryl” is also meant to include possible N-oxides. Illustrative N-oxides include pyridyl N-oxide and the like.

As used herein, the term “substituted heteroaryl” by itself or as part of another group
20 means that the heteroaryl as defined herein is substituted with one to four independently selected substituents. A non-limiting list of independently selected substituents includes halo, nitro, cyano, hydroxy, amino, (alkyl)amino, (dialkyl)amino, haloalkyl, (hydroxy)alkyl, (dihydroxy)alkyl, alkoxy, haloalkoxy, aryloxy, aralkyloxy, alkylthio, carboxamido, sulfonamido, (alkyl)carbonyl, (aryl)carbonyl, (alkyl) sulfonyl, (aryl)sulfonyl, ureido,
25 guanidino, carboxy, (carboxy)alkyl, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocyclo, (alkoxy)alkyl, (amino)alkyl, [(hydroxy)alkyl]amino, [(alkyl)amino]alkyl, [(dialkyl)amino]alkyl, (cyano)alkyl, (carboxamido)alkyl, mereaptoalkyl, (heterocyclo)alkyl, and (heteroaryl)alkyl. Any available carbon or nitrogen atom can be substituted.

As used herein, the term “heterocyclo” or “heterocyclyl” by itself or as part of another
30 group refers to saturated and partially unsaturated (e.g., containing one or two double bonds) cyclic groups containing one, two, or three rings having from three to fourteen ring members (i.e., a 3- to 14-membered heterocyclo) and at least one heteroatom. Each heteroatom is

independently selected. The term “heterocyclo” or “heterocyclyl” is meant to include cyclic ureido groups, such as, 2-imidazolidinone, and cyclic amide groups, such as, β -lactam, γ -lactam, δ -lactam and ϵ -lactam. The term “heterocyclo” or “heterocyclyl” is also meant to include groups having fused aryl or substituted aryl groups, e.g., indolinyl. The heterocyclo or heterocyclyl can be linked to the rest of the molecule through a carbon or nitrogen atom. Non-limiting illustrative heterocyclo (or heterocyclyl) groups include 2-oxopyrrolidin-3-yl, 2-imidazolidinone, piperidinyl, morpholinyl, piperazinyl, pyrrolidinyl, and indolinyl.

As used herein, the term “substituted heterocyclo” or “substituted heterocyclyl” by itself or part of another group means the heterocyclo or heterocyclyl group as defined above is substituted with one to four independently selected substituents. A non-limiting list of independently selected substituents includes halo, nitro, cyano, hydroxyl, amino, (alkyl)amino, (dialkyl)amino, haloalkyl, (hydroxy)alkyl, (dihydroxy)alkyl, alkoxy, haloalkoxy, aryloxy, aralkyloxy, alkylthio, carboxamido, sulfonamido, (alkyl)carbonyl, (aryl)carbonyl, (alkyl)sulfonyl, (aryl)sulfonyl, ureido, guanidino, carboxy, carboxyalkyl, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocyclyl, alkoxyalkyl, (amino)alkyl, [(hydroxy)alkyl]amino, [(alkyl)amino]alkyl, [(dialkyl)amino]alkyl, (cyano)alkyl, (carboxamido)alkyl, mercaptoalkyl, (heterocyclyl)alkyl, and (heteroaryl)alkyl. Substitution may occur on any available carbon or nitrogen atom, and may form a spirocycle.

As used herein, the term “amino” by itself or as part of another group refers to —NH_2 .

As used herein, the term “alkylamino” or “(alkyl)amino” by itself or as part of another group refers to —NHR , wherein R is alkyl.

As used herein, the term “dialkylamino” or “(dialkyl)amino” by itself or as part of another group refers to $\text{—NR}^{\prime}\text{R}^{\prime\prime}$, wherein $\text{R}^{\prime}$ and $\text{R}^{\prime\prime}$ are each independently alkyl or $\text{R}^{\prime}$ and $\text{R}^{\prime\prime}$ are taken together to form a 3- to 8-membered heterocyclo or substituted heterocyclo.

As used herein, the term “cycloalkylamino” by itself or as part of another group refers to $\text{—NR}^{\prime}\text{R}^{\prime\prime}$, wherein $\text{R}^{\prime}$ is cycloalkyl or substituted cycloalkyl, and $\text{R}^{\prime\prime}$ is hydrogen or alkyl.

As used herein, the term “(amino)alkyl” by itself or as part of another group refers to an alkyl group substituted with an amino group. Non-limiting illustrative (amino)alkyl groups include $\text{—CH}_2\text{CH}_2\text{NH}_2$, $\text{—CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$, and $\text{—CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$.

As used herein, the term “(alkylamino)alkyl” or “[(alkyl)amino]alkyl” by itself or as part of another group refers to an alkyl group substituted with an alkylamino group. A non-limiting illustrative (alkylamino)alkyl group is $\text{—CH}_2\text{CH}_2\text{N(H)CH}_3$.

As used herein, the term “(dialkylamino)alkyl” by itself or as part of another group refers to an alkyl group substituted by a dialkylamino group. Non-limiting illustrative (dialkylamino)alkyl groups include $\text{—CH}_2\text{N}(\text{CH}_3)_2$ and $\text{—CH}_2\text{CH}_2\text{N}(\text{CH}_3)_2$.

As used herein, the term “(cycloalkylamino)alkyl” by itself or as part of another group refers to an alkyl group substituted by a cycloalkylamino group. Non-limiting illustrative (cycloalkylamino)alkyl groups include $\text{—CH}_2\text{N}(\text{H})\text{cyclopropyl}$, $\text{—CH}_2\text{N}(\text{H})\text{cyclobutyl}$, and $\text{—CH}_2\text{N}(\text{H})\text{cyclohexyl}$.

As used herein, the term “carboxamido” by itself or as part of another group refers to a radical of formula —C(=O)NR'R'' , where R' and R'' are each independently hydrogen, alkyl, substituted alkyl, aralkyl, substituted aralkyl, aryl, substituted aryl, heteroaryl, or substituted heteroaryl, or R' and R'' taken together with the nitrogen to which they are attached form a 3- to 8-membered heterocyclo group. Non-limiting illustrative carboxamido groups include —CONH_2 , $\text{—CON}(\text{H})\text{CH}_3$, $\text{CON}(\text{CH}_3)_2$, and $\text{CON}(\text{H})\text{Ph}$.

As used herein, the term “sulfonamido” by itself or as part of another group refers to a radical of the formula $\text{—SO}_2\text{NR'R''}$, where R' and R'' are each independently hydrogen, alkyl, substituted alkyl, aryl, or substituted aryl, or R' and R'' taken together with the nitrogen to which they are attached form a 3- to 8-membered heterocyclo group. Non-limiting illustrative sulfonamido groups include $\text{—SO}_2\text{NH}_2$, $\text{—SO}_2\text{N}(\text{H})\text{CH}_3$, and $\text{—SO}_2\text{N}(\text{H})\text{Ph}$.

As used herein, the term “(alkyl)carbonyl” by itself or as part of another group refers to a carbonyl group, i.e., —C(=O)— , substituted by an alkyl group. A non-limiting illustrative alkylcarbonyl group is —COCH_3 .

As used herein, the term “(alkoxy)carbonyl” (or “ester”) by itself or as part of another group refers to a carbonyl group, i.e., —C(=O)— , substituted by an alkoxy group. A non-limiting illustrative (alkoxy)carbonyl group is —C(O)OCH_3 .

As used herein, the term “(aryl)carbonyl” by itself or as part of another group refers to a carbonyl group, i.e., —C(=O)— , substituted by an aryl or substituted aryl group. A non-limiting illustrative arylcarbonyl group is —COPh .

As used herein, the term “sulfanyl” by itself or as part of another group refers to a —SH group.

As used herein, the term “(alkyl)sulfanyl” or “alkylthio” by itself or as part of another group refers to a sulfur atom substituted by an alkyl or substituted alkyl group. Non-limiting illustrative alkylthio groups include —SCH_3 , and $\text{—SCH}_2\text{CH}_3$.

As used herein, the term “mercaptoalkyl” by itself or as part of another group refers to an alkyl group substituted by a —SH group.

As used herein, the term “alkylsulfonyl” or “(alkyl)sulfonyl” by itself or as part of another group refers to a sulfonyl group, i.e., —SO₂—, substituted by an alkyl or substituted alkyl group. A non-limiting illustrative alkylsulfonyl group is —SO₂CH₃.

As used herein, the term “arylsulfonyl” or “(aryl)sulfonyl” by itself or as part of another group refers to a sulfonyl group, i.e., —SO₂—, substituted by an aryl or substituted aryl group. A non-limiting illustrative arylsulfonyl group is —SO₂Ph.

As used herein, the term “carboxy” by itself or as part of another group refers to a radical of the formula —COOH.

As used herein, the term “(carboxy)alkyl” by itself or as part of another group refers to an alkyl group substituted with a —COOH. A non-limiting illustrative carboxyalkyl group is —CH₂CO₂H.

As used herein, the term “aralkyl” by itself or as part of another group refers to a residue in which an aryl moiety is attached to an alkyl residue. The aralkyl group may be attached to the parent structure at either the aryl or the alkyl residue.

As used herein, the term “substituted aralkyl” by itself or as part of another group refers to a residue in which an aryl moiety is attached to a substituted alkyl residue.

As used herein, the term “aralkenyl” by itself or as part of another group refers to a radical of the formula —R_d—R_e where R_d is an alkenylene chain and R_e is one or more aryl radicals.

As used herein, the term “substituted aralkenyl” by itself or as part of another group refers to an aralkenyl radical where the alkenylene chain of the aralkenyl radical is an optionally substituted alkenylene chain, and each aryl radical of the aralkenyl radical is an optionally substituted aryl radical.

As used herein, the term “aralkynyl” by itself or as part of another group refer to a radical of the formula —R_eR_c, where R_e is an alkynylene chain and R_c is one or more aryl radicals.

As used herein, the term “substituted aralkynyl” by itself or as part of another group refers to an aralkynyl radical where the alkynylene chain of the aralkynyl radical is an optionally substituted alkynylene chain, and each aryl radical of the aralkynyl radical is an optionally substituted aryl radical.

As used herein, the term “aliphatic heterocycle” by itself or as part of another group refers to a non-aromatic ring in which one or more of the ring-forming atoms is a heteroatom.

As used herein, the term “heteroatom” refers to an atom inserted between a carbon atom and its parent molecule (i.e., between the points of attachment). Non-limiting
 5 illustrative heteroatoms include oxygen, nitrogen, sulfur (including sulfoxide and sulfone), and phosphorous (P).

As used herein, the term “saccharide” refers to a single sugar moiety or monosaccharide unit as well as combinations of two or more single sugar moieties or monosaccharide units covalently linked to form disaccharides, oligosaccharides, and
 10 polysaccharides. The polysaccharide may be linear or branched.

As used herein, the term “monosaccharide” refers to a single sugar residue in an oligosaccharide.

As used herein, the term “disaccharide” refers to a polysaccharide composed of two monosaccharide units or moieties linked together by a glycosidic bond.

As used herein, an “oligosaccharide” refers to a compound containing two or more
 15 monosaccharide units or moieties. Within the context of an oligosaccharide, an individual monomer unit or moiety is a monosaccharide which is, or can be, bound through a hydroxy group to another monosaccharide unit or moiety. Oligosaccharides can be prepared by either chemical synthesis from protected single residue sugars or by chemical degradation of
 20 biologically produced polysaccharides. Alternatively, oligosaccharides may be prepared by in vitro enzymatic methods.

As used herein, the term “ureido” by itself or as part of another group refers to a radical of the formula $\text{—NR}^{\text{'}}\text{—C(=O)—NR}^{\text{'}}\text{R}^{\text{'}}$, wherein $\text{R}^{\text{'}}$ is hydrogen, alkyl, aryl, or substituted aryl, and $\text{R}^{\text{'}}$ and $\text{R}^{\text{'}}$ are each independently hydrogen, alkyl, aryl or substituted
 25 aryl, or $\text{R}^{\text{'}}$ and $\text{R}^{\text{'}}$ taken together with the nitrogen to which they are attached form a 4- to 8-membered heterocyclo group. Non-limiting illustrative ureido groups include —NH—C(=O)—NH_2 and —NH—C(=O)—NHCH_3 .

As used herein, the term “guanidino” by itself or as part of another group refers to a radical of the formula $\text{—NR}^{\text{'}}\text{—C(=NR}^{\text{'}}\text{)—NR}^{\text{'}}\text{R}^{\text{'}}$, wherein $\text{R}^{\text{'}}$, $\text{R}^{\text{'}}$, and $\text{R}^{\text{'}}$ are each
 30 independently hydrogen, alkyl, aryl, or substituted aryl, and $\text{R}^{\text{'}}$ is hydrogen, alkyl, cyano, alkylsulfonyl, alkylcarbonyl, carboxamido, or sulfonamido. Non-limiting illustrative guanidino groups include —NH—C(=NH)—NH_2 , —NH—C(=NCN)—NH_2 , and —NH—C(=NH)—NHCH_3 .

As used herein, the term “(heteroaryl)alkyl” by itself or as part of another group refers to an alkyl group substituted with one, two, or three heteroaryl or substituted heteroaryl groups.

As used herein, the term “heteroalkyl” by itself or part of another group refers to a stable straight or branched chain hydrocarbon radical containing at least one heteroatom, which can be the same or different. The heteroatom(s) can be placed at any interior position or terminal position of the heteroalkyl group, or at a position at which the heteroalkyl group is attached to the remainder of the molecule. Non-limiting illustrative heteroalkyl groups include $\text{—CH}_2\text{N(H)CH}_2\text{CH}_2\text{N(CH}_3)_2$, $\text{—CH}_2\text{N(CH}_3)\text{CH}_2\text{CH}_2\text{N(CH}_3)_2$, $\text{—CH}_2\text{N(H)CH}_2\text{CH}_2\text{CH}_2\text{N(CH}_3)_2$, $\text{—CH}_2\text{N(H)CH}_2\text{CH}_2\text{OH}$, $\text{—CH}_2\text{N(CH}_3)\text{CH}_2\text{CH}_2\text{OH}$, $\text{—CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_3$, $\text{—OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_3$, $\text{—CH}_2\text{NHCH}_2\text{CH}_2\text{OCH}_2\text{—}$, $\text{—OCH}_2\text{CH}_2\text{NH}_2$, and $\text{—NHCH}_2\text{CH}_2\text{N(H)CH}_3$.

As used herein, the term “(heterocyclo)alkyl” or “(heterocyclyl)alkyl” by itself or as part of another group refers to an alkyl group substituted with one heterocyclyl or substituted heterocyclyl group, and optionally one hydroxy group.

As used herein, the term “(carboxamido)alkyl” by itself or as part of another group refers to an alkyl group substituted with one carboxamido group, and optionally one heterocyclo, amino, alkylamino, or dialkylamino group.

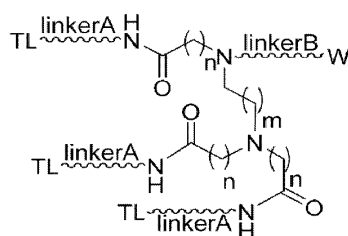
As used herein, the term “N-oxide” refers to a compound that contains a functional group, wherein N^+ is further connected to H and/or the rest of the compound structure.

As used herein, the term “integral number” refers to an integer including, but not limited to 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, etc.

Compound

General Structure

A multivalent ligand cluster, having a diamine scaffold, may have the general structure of Formula 1:



Formula 1

where:

each TL is an independently selected targeting ligand,

m is an integral number between 1 and 10,

each n is an independently selected integral number between 1 and 10,

5 each linkerA is an independently selected spacer, with one end attached to a TL and the other end attached to the nitrogen of an alkylcarboxamide,

linkerB is a spacer, with one end attached to a pharmaceutical agent or a functional group capable of linking to one or more pharmaceutical agents, and the other end attached to a diamine nitrogen, and

10 W is either one or more pharmaceutical agents, or a functional group capable of linking to one or more pharmaceutical agents.

In some embodiments, m may be configured based on a starting material used to synthesize the multivalent ligand cluster. For example, m may be 1 due to ethylenediamine being used as a starting material, m may be 2 due to 1,3-propanediamine being used as a starting material, m may be 3 due to 1,4-butanediamine being used as a starting material, etc.

15 As used herein, a “spacer” refers to a compound or molecule that links other groups together. Example linkerA and linkerB spacers are described in detail herein below.

As used herein in reference to elements of multivalent ligand clusters of the invention, the term “independently selected” means that each of a given type of element may differ from one or more others of the same type of element in a multivalent ligand cluster. For example, a multivalent ligand cluster may include more than one TLs, where each may be selected to be different from or the same as one or more other TLs in that multivalent ligand cluster. For further example, a multivalent ligand cluster may include more than one “n,” where each may be selected to be different from or the same as one or more other “n” in that multivalent ligand cluster. In another example, a multivalent ligand cluster may include more than one linkerA, where each may be selected to be different from or the same as one or more other linkerAs in that multivalent ligand cluster.

30 *Targeting Ligands*

As mentioned above with respect to Formula 1, a multivalent ligand cluster of the present disclosure may include multiple (e.g., three) independently selected targeting ligands. In this context, the term “independently selected” means that each targeting ligand may be

selected to be different from or the same as one or more other targeting ligands in the same multivalent ligand cluster.

At least one of the independently selected targeting ligands of Formula 1 may be capable of binding to one or more cell receptors, cell channels, and/or cell transporters
5 capable of facilitating endocytosis.

In some embodiments, at least one of the independently selected targeting ligands of Formula 1 may comprise at least one small molecule ligand. As used herein, a “small molecule ligand” refers to a ligand smaller than a protein. In some embodiments, at least one small molecular ligand may comprise at least one of N-acetylgalactosamine (GalNAc),
10 galactose, galactosamine, N-formyl-galactosamine, N-propionylgalactosamine, N-butanoylgalactosamine, and N-iso-butanoylgalactosamine, a macrocycle, a folate molecule, a fatty acid, a bile acid, a cholesterol, and derivatives thereof.

A macrocycle is a molecule or ion containing a twelve or more membered ring. The present disclosure is not limited to any particular macrocycle. A non-limiting list of
15 macrocycles within the scope of the present disclosure include terpenoid macrocycles, porphyrins, and cyclodextrins.

Folate, also known as vitamin B9 and folacin, is used by the human body to make DNA and RNA, and metabolize amino acids necessary for cell division. Folate receptors bind folate, and reduced folic acid derivatives. Thus, in some embodiments, at least one of the
20 independently selected targeting ligands may comprise a reduced folic acid derivative.

A fatty acid is a carboxylic acid with a long aliphatic chain. In some embodiments, the fatty acid may be saturated, meaning the aliphatic chain has all single carbon-to-carbon bonds. In some embodiments, the fatty acid may be unsaturated, meaning the aliphatic chain includes at least one double or triple carbon-to-carbon bond. In some embodiments, the fatty
25 acid may include a branched chain. In some embodiments, the fatty acid may include a ring structure. Fatty acids are known to assist in the uptake of pharmaceutical agents into cells. *See Prakash et al.* “Fatty acid conjugation enhances potency of antisense oligonucleotides in muscle.” *Nucleic Acids Res.* 2019;47(12):6029-6044. doi:10.1093/nar/gkz354; Raouane *et al.* “Lipid Conjugated Oligonucleotides: A Useful Strategy for Delivery.” *Bioconjugate Chemistry* 2012 23 (6), 1091-1104, DOI: 10.1021/bc200422w; and Osborn *et al.* “Improving siRNA Delivery In Vivo Through Lipid Conjugation.” *Nucleic Acid Ther.* 2018;28(3):128-136. doi:10.1089/nat.2018.0725.
30

A bile acid is a steroidal acid found in bile. Bile acid is a known ligand for the farnesoid X receptor (FXR) and G protein-coupled bile acid receptor 1 (GPBAR1) (TGR5). In some embodiments, the bile acid may be a primary bile acid synthesized in the liver. In some embodiments, the bile acid may be a secondary bile acid resulting from bacterial actions in the colon. Bile acids are known to be beneficial in inhibiting RNA translation. *See* González-Carmona *et al.* “Inhibition of hepatitis C virus RNA translation by antisense bile acid conjugated phosphorothioate modified oligodeoxynucleotides (ODN).” *Antiviral Res.* 2013, 97, 49–59. doi:10.1089/nat.2018.0725.

10 In some embodiments, at least one of the independently selected targeting ligands of Formula 1 may comprise at least one peptide. Various peptides and corresponding peptide receptors are known to those skilled in the art. The present disclosure is not limited to any particular peptide. Peptides known and not yet discovered are within the scope of the present disclosure.

15 In some embodiments, at least one of the independently selected targeting ligands of Formula 1 may comprise at least one cyclic peptide. As known to those skilled in the art, a cyclic peptide is a polypeptide chain having a cyclic ring structure. In some embodiments, the cyclic ring structure may be formed by linking one end of the peptide to the other with an amide bond, or other chemically stable bond such as lactone, ether, thioether, disulfide, etc. In some embodiments, a cyclic peptide of the present disclosure may be a biologically active cyclic peptide where a head-to-tail (or N-to-C) cyclization is formed by an amide bond between amino and carboxyl termini. In some embodiments, a cyclic peptide of the present disclosure may be a biologically active cyclic peptide where cyclization is formed by “click chemistry.” *See* Rashad A.A. (2019) Click Chemistry for Cyclic Peptide Drug Design. In: Goetz G. (eds) Cyclic Peptide Design. Methods in Molecular Biology, vol 2001. Humana, New York, NY. https://doi.org/10.1007/978-1-4939-9504-2_8. The present disclosure is not limited to any particular cyclic peptide. A cyclic peptide of the present disclosure may be naturally occurring or synthetically produced.

30 In some embodiments, at least one of the independently selected targeting ligands of Formula 1 may comprise at least one aptamer. An aptamer is a short, single-stranded DNA or RNA molecule that can selectively bind to a specific target, such as a protein, peptide, carbohydrate, small molecule, toxin, or live cell. Aptamers assume a variety of shapes, as they tend to form helices and single-stranded loops. The present disclosure is not limited to

any particular aptamer. Aptamers known and not yet discovered are within the scope of the present disclosure.

In some embodiments, at least one of the independently selected targeting ligands of Formula 1 may be capable of binding to at least one Asialoglycoprotein receptor (ASGPR).

5 ASGPRs are lectins, located on liver cells, that bind galactose residues. ASGPR has been demonstrated to have high expression on the surface of hepatocytes, human carcinoma cell lines, and liver cancers. ASGPR is also weakly expressed by glandular cells of the gallbladder and stomach.

In some embodiments, at least one of the independently selected targeting ligands of
10 Formula 1 may be capable of binding to at least one transferrin receptor. A transferrin receptor is a membrane glycoprotein that mediates cellular uptake of transferrin, a protein in the blood that binds to iron and transports it through the body. The transferrin receptor-mediated endocytosis pathway is known to those skilled in the art. *See Qian et al.* "Targeted drug delivery via the transferrin receptor-mediated endocytosis pathway." *Pharmacol Rev.*
15 2002 Dec; 54(4):561-87. doi: 10.1124/pr.54.4.561. PMID: 12429868. The present disclosure is not limited to any particular ligand capable of binding to at least one transferring receptor. Transferrin receptor ligands known and not yet developed are within the scope of the present disclosure.

In some embodiments, at least one of the independently selected targeting ligands of
20 Formula 1 may be capable of binding to at least one integrin receptor. An integrin receptor is a transmembrane receptor that facilitates cell-cell and cell-extracellular matrix (ECM) adhesion. Upon ligand binding, integrin receptors activate signal transduction pathways that mediate cellular signals such as regulation of the cell cycle, organization of the intracellular cytoskeleton, and movement of new receptors to the cell membrane. Integrin targeted
25 delivery of gene therapeutics is known to those skilled in the art. *See Juliano et al.* "Integrin targeted delivery of gene therapeutics." *Theranostics* vol. 1 211-9. 2 Mar. 2011, doi:10.7150/thno/v01p0211. The present disclosure is not limited to any particular ligand capable of binding to at least one integrin receptor. Integrin receptor ligands known and not yet developed are within the scope of the present disclosure.

30 In some embodiments, at least one of the independently selected targeting ligands of Formula 1 may be capable of binding to at least one folate receptor. A folate receptor binds folate and reduced folic acid derivatives, and mediates delivery of tetrahydrofolate to the interior of cells. Targeted drug delivery via folate receptors is known to those skilled in the

art. *See Zhao et al.* "Targeted drug delivery via folate receptors." *Expert Opin Drug Deliv.* 2008 Mar; 5(3):309-19. doi: 10.1517/17425247.5.3.309. PMID: 18318652. The present disclosure is not limited to any particular ligand capable of binding to at least one folate receptor. Folate receptor ligands known and not yet developed are within the scope of the present disclosure.

In some embodiments, at least one of the independently selected targeting ligands of Formula 1 may be capable of binding to at least one G-protein-coupled receptor (GPCR). GPCRs are cell surface receptors that bind, among other things, peptides, lipids, sugars, and proteins. GPCRs interact with G proteins in the plasma membrane. When an external signaling molecule binds to a GPCR, it causes a conformational change in the GPCR, which triggers the interaction between the GPCR and a nearby G protein. GPCRs consist of a single polypeptide that is folded into a globular shape and embedded in a cell's plasma membrane. GPCR targeted delivery of oligonucleotide therapeutics is known to those skilled in the art. *See Knerr et al.* "Glucagon Like Peptide 1 Receptor Agonists for Targeted Delivery of Antisense Oligonucleotides to Pancreatic Beta Cell" *J. Am. Chem. Soc.*, 2021 143 (9), 3416-3429. DOI: 10.1021/jacs.0c12043.

LinkerAs

As mentioned above with respect to Formula 1, a multivalent ligand cluster of the present disclosure may include multiple (e.g., three) independently selected linkerAs. In this context, the term "independently selected" means that each linkerA may be selected to be different from or the same as one or more other linkerAs in the same multivalent ligand cluster.

Each linkerA is an independently selected spacer, with one end attached to a targeting ligand (TL in Formula 1) and the other end attached to the nitrogen of an alkylcarboxamide of the multivalent ligand cluster.

In some embodiments, at least one of the independently selected linkerAs may comprise polythethylene glycol (PEG). The PEG may have any number of repeating O-CH₂-CH₂ units. For example, the PEG may be PEG1, PEG2, PEG3, PEG4, PEG5, PEG6, PEG7, PEG8, PEG9, PEG10, PEG11, PEG12, PEG13, PEG14, PEG15, PEG16, PEG17, PEG18, PEG19, PEG20, PEG21, PEG22, PEG23, PEG24, PEG25, PEG26, PEG27, PEG28, PEG29, PEG30, PEG31, PEG32, PEG33, PEG34, PEG35, PEG36, PEG37, PEG38, PEG39, PEG40, PEG41, PEG42, PEG43, PEG44, PEG45, PEG46, PEG47, PEG48, PEG49, PEG50, PEG51,

PEG52, PEG53, PEG54, PEG55, PEG56, PEG57, PEG58, PEG59, PEG60, PEG61, PEG62, PEG63, PEG64, PEG65, PEG66, PEG67, PEG68, PEG69, PEG70, PEG71, PEG72, PEG73, PEG74, PEG75, PEG76, PEG77, PEG78, PEG79, PEG80, PEG81, PEG82, PEG83, PEG84, PEG85, PEG86, PEG87, PEG88, PEG89, PEG90, PEG91, PEG92, PEG93, PEG94, PEG95, 5 PEG96, PEG97, PEG98, PEG99, PEG100, or larger.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one alkyl group. In some embodiments, an alkyl group may have 2 carbons, 3 carbons, 4 carbons, 5 carbons, 6 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 17 carbons, 18 carbons, 10 19 carbons, or 20 carbons.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one substituted alkyl group. In some embodiments, a substituted alkyl group may have 2 carbons, 3 carbons, 4 carbons, 5 carbons, 6 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 15 17 carbons, 18 carbons, 19 carbons, or 20 carbons. In some embodiments, a substituted alkyl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one cycloalkyl group. In some embodiments, a cycloalkyl group may be a 20 C3 cycloalkyl (i.e., cyclopropane), C4 cycloalkyl (i.e., cyclobutene), C5 cycloalkyl (i.e., cyclopentane), C6 cycloalkyl (i.e., cyclohexane), C7 cycloalkyl (i.e., cycloheptane), C8 cycloalkyl (i.e., cyclooctane), C9 cycloalkyl (i.e., cyclononane), or C10 cycloalkyl (i.e., cyclodecane).

In some embodiments, at least one of the independently selected linkerAs may 25 comprise at least one substituted cycloalkyl group. In some embodiments, a substituted cycloalkyl group may be a C3 substituted cycloalkyl, C4 substituted cycloalkyl, C5 substituted cycloalkyl, C6 substituted cycloalkyl, C7 substituted cycloalkyl, C8 substituted cycloalkyl, C9 substituted cycloalkyl, or C10 substituted cycloalkyl. In some embodiments, a substituted cycloalkyl group may include one or more of the following substitution groups: 30 alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one alkenyl group. In some embodiments, an alkenyl group may have 4

carbons, 5 carbons, 6 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 17 carbons, 18 carbons, 19 carbons, or 20 carbons.

In some embodiments, at least one of the independently selected linkerAs may
5 comprise at least one substituted alkenyl group. In some embodiments, an alkenyl group may have 4 carbons, 5 carbons, 6 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 17 carbons, 18 carbons, 19 carbons, or 20 carbons. In some embodiments, a substituted alkenyl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl,
10 amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one cycloalkenyl group. In some embodiments, a cycloalkenyl group may be a C5 cycloalkenyl, C6 cycloalkenyl, C7 cycloalkenyl, C8 cycloalkenyl, C9 cycloalkenyl, or C10 cycloalkenyl.

15 In some embodiments, at least one of the independently selected linkerAs may comprise at least one substituted cycloalkenyl group. In some embodiments, a cycloalkenyl group may be a C5 cycloalkenyl, C6 cycloalkenyl, C7 cycloalkenyl, C8 cycloalkenyl, C9 cycloalkenyl, or C10 cycloalkenyl. In some embodiments, a substituted cycloalkenyl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy,
20 alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one alkynyl group. In some embodiments, an alkynyl group may have 4 carbons, 5 carbons, 6 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 17 carbons, 18 carbons, 19 carbons,
25 or 20 carbons.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one substituted alkynyl group. In some embodiments, a substituted alkynyl group may have 4 carbons, 5 carbons, 6 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 17 carbons, 18
30 carbons, 19 carbons, or 20 carbons. In some embodiments, a substituted alkynyl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one aryl or heteroaryl group. Examples include phenyl, naphthyl, and pyridinyl, although it is noted that other aryl and heteroaryl groups, that fall within the definitions provided herein, may be used.

5 In some embodiments, at least one of the independently selected linkerAs may comprise at least one substituted aryl group. In some embodiments, a substituted aryl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

10 In some embodiments, at least one of the independently selected linkerAs may comprise at least one aralkyl group. Example aralkyl groups include, but are not limited to, phenylmethyl, phenylethyl, and phenylpropyl.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one substituted aralkyl group. In some embodiments, a substituted aralkyl group may include one or more of the following substitution groups: alkyl, cycloalkyl,
15 hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one aralkenyl group. Example aralkenyl groups include, but are not limited to ethenylbenzene and propenylbenzene.

20 In some embodiments, at least one of the independently selected linkerAs may comprise at least one substituted aralkenyl group. In some embodiments, a substituted aralkenyl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one aralkynyl group. Example aralkynyl groups include, but are not limited
25 to ethynylbenzene and propynylbenzene.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one substituted aralkynyl group. In some embodiments, a substituted aralkynyl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

30 In some embodiments, at least one of the independently selected linkerAs may comprise at least one heteroatom. In some embodiments, at least one of the independently selected linkerAs may comprise one or more oxygen (O) heteroatoms, one or more nitrogen (N) heteroatoms, one or more sulfur (S) heteroatoms, and/or one or more phosphorous (P)

heteroatoms. In some embodiments, at least one of the independently selected linkerAs may comprise at least one heteroalkyl group.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one aliphatic heterocycle. In some embodiments, at least one of the independently selected linkerAs may comprise at least one of tetrahydrofuran (THF), tetrahydropyran (THP), morpholine, piperidine, piperazine, pyrrolidine, and/or azetidine.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one heteroaryl group. In some embodiments, at least one of the independently selected linkerAs may comprise one or more of imidazole, pyrazole, pyridine, pyrimidine, triazole, and/or 1,2,3-triazole.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one substituted heteroaryl group. In some embodiments, a substituted heteroaryl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one amino acid. Various amino acids are known to those skilled in the art. An independently selected linkerA is not limited to including one or more specific amino acids. For example, an independently selected linkerA may comprise one or more arginine (Arg) amino acids, one or more histidine (His) amino acids, one or more lysine (Lys) amino acids, one or more aspartic acid (Asp) amino acids, one or more glutamic acid (Glu) amino acids, one or more serine (Ser) amino acids, one or more threonine (Thr) amino acids, one or more asparagine (Asn) amino acids, one or more glutamine (Gln) amino acids, one or more cysteine (Cys) amino acids, one or more selenocysteine (Sec) amino acids, one or more glycine (Gly) amino acids, one or more proline (Pro) amino acids, one or more alanine (Ala) amino acids, one or more valine (Val) amino acids, one or more isoleucine (Ile) amino acids, one or more leucine (Leu) amino acids, one or more methionine (Met) amino acids, one or more phenylalanine (Phe) amino acids, one or more tyrosine (Tyr) amino acids, and/or one or more tryptophan (Trp) amino acids.

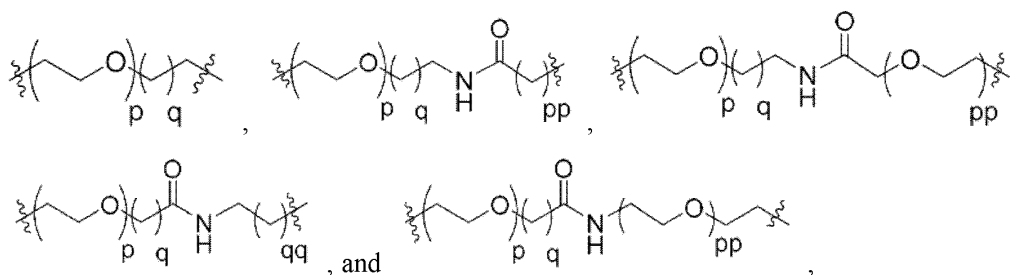
In some embodiments, at least one of the independently selected linkerAs may comprise at least one nucleotide. Various nucleotides are known to those skilled in the art. An independently selected linkerA is not limited to including one or more specific nucleotides. For example, an independently selected linkerA may comprise one or more nucleotides comprising a guanine nucleobase, one or more nucleotides comprising an adenine nucleobase,

one or more nucleotides comprising a cytosine nucleobase, one or more nucleotides comprising a thymine nucleobase, and/or one or more nucleotides comprising a uracil nucleobase.

In some embodiments, at least one independently selected linkerA may comprise at least one abasic nucleotide. As known in the art, an abasic nucleotide is a nucleotide having an abasic site, which is a location that has neither a purine nor a pyrimidine base. For example, at least one independently selected linkerA may comprise one or more abasic DNAs and/or one or more abasic RNAs. In some embodiments, at least one independently selected linkerA may comprise at least one inverted abasic nucleotide. As known in the art, an inverted abasic nucleotide is an abasic nucleotide whose 5' end connects to a 5' end of a next nucleotide, and whose 3' end connects to a 3' end of a next nucleotide. For example, at least one independently selected linkerA may comprise one or more inverted abasic DNAs and/or one or more inverted abasic RNAs.

In some embodiments, at least one of the independently selected linkerAs may comprise at least one saccharide. In some embodiments, at least one of the independently selected linkerAs may comprise at least one glucose monosaccharide unit, at least one fructose monosaccharide unit, at least one mannose monosaccharide unit, at least one galactose monosaccharide unit, at least one ribose monosaccharide unit, and/or at least one glucosamine monosaccharide unit.

In some embodiments, at least one of the independently selected linkerAs may comprise one or more of:



where:

- p is an integral number between 0 and 12,
- pp is an integral number between 0 and 12,
- q is an integral number between 1 and 12, and
- qq is an integral number between 1 and 12.

In some embodiments, p is an integral number independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12. In some embodiments, pp is an integral number independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12. In some embodiments, q is an integral number independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12. In some
5 embodiments, qq is an integral number independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12.

LinkerB

LinkerB is a spacer, with one end attached to a pharmaceutical agent or a functional group capable of linking to one or more pharmaceutical agents, and the other end attached to
10 a diamine nitrogen of the multivalent ligand cluster.

In some embodiments, linkerB may comprise polythethylene glycol (PEG). The PEG may have any number of repeating O-CH₂-CH₂ units. For example, the PEG may be PEG1, PEG2, PEG3, PEG4, PEG5, PEG6, PEG7, PEG8, PEG9, PEG10, PEG11, PEG12, PEG13, PEG14, PEG15, PEG16, PEG17, PEG18, PEG19, PEG20, PEG21, PEG22, PEG23, PEG24,
15 PEG25, PEG26, PEG27, PEG28, PEG29, PEG30, PEG31, PEG32, PEG33, PEG34, PEG35, PEG36, PEG37, PEG38, PEG39, PEG40, PEG41, PEG42, PEG43, PEG44, PEG45, PEG46, PEG47, PEG48, PEG49, PEG50, PEG51, PEG52, PEG53, PEG54, PEG55, PEG56, PEG57, PEG58, PEG59, PEG60, PEG61, PEG62, PEG63, PEG64, PEG65, PEG66, PEG67, PEG68, PEG69, PEG70, PEG71, PEG72, PEG73, PEG74, PEG75, PEG76, PEG77, PEG78, PEG79,
20 PEG80, PEG81, PEG82, PEG83, PEG84, PEG85, PEG86, PEG87, PEG88, PEG89, PEG90, PEG91, PEG92, PEG93, PEG94, PEG95, PEG96, PEG97, PEG98, PEG99, PEG100, or larger. In some embodiments, it may be beneficial for the PEG to be PEG10 or less.

In some embodiments, linkerB may comprise at least one alkyl group. In some embodiments, linkerB may comprise at least one substituted alkyl group. In some
25 embodiments, an alkyl group may have 2 carbons, 3 carbons, 4 carbons, 5 carbons, 6 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 17 carbons, 18 carbons, 19 carbons, or 20 carbons.

In some embodiments, linkerB may comprise at least one substituted alkyl group. In some embodiments, a substituted alkyl group may have 2 carbons, 3 carbons, 4 carbons, 5
30 carbons, 6 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 17 carbons, 18 carbons, 19 carbons, or 20 carbons. In some embodiments, a substituted alkyl group may include one or more of the

following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, linkerB may comprise at least one cycloalkyl group. In some embodiments, a cycloalkyl group may be a C3 cycloalkyl (i.e., cyclopropane), C4 cycloalkyl (i.e., cyclobutene), C5 cycloalkyl (i.e., cyclopentane), C6 cycloalkyl (i.e., cyclohexane), C7 cycloalkyl (i.e., cycloheptane), C8 cycloalkyl (i.e., cyclooctane), C9 cycloalkyl (i.e., cyclononane), or C10 cycloalkyl (i.e., cyclodecane).

In some embodiments, linkerB may comprise at least one at least one substituted cycloalkyl group. In some embodiments, a substituted cycloalkyl group may be a C3 substituted cycloalkyl, C4 substituted cycloalkyl, C5 substituted cycloalkyl, C6 substituted cycloalkyl, C7 substituted cycloalkyl, C8 substituted cycloalkyl, C9 substituted cycloalkyl, or C10 substituted cycloalkyl. In some embodiments, a substituted cycloalkyl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, linkerB may comprise at least one alkenyl group. In some embodiments, an alkenyl group may have 4 carbons, 5 carbons, 6 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 17 carbons, 18 carbons, 19 carbons, or 20 carbons.

In some embodiments, linkerB may comprise at least one substituted alkenyl group. In some embodiments, an alkenyl group may have 4 carbons, 5 carbons, 6 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 17 carbons, 18 carbons, 19 carbons, or 20 carbons. In some embodiments, a substituted alkenyl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, linkerB may comprise at least one cycloalkenyl group. In some embodiments, a cycloalkenyl group may be a C5 cycloalkenyl, C6 cycloalkenyl, C7 cycloalkenyl, C8 cycloalkenyl, C9 cycloalkenyl, or C10 cycloalkenyl.

In some embodiments, linkerB may comprise at least one substituted cycloalkenyl group. In some embodiments, a cycloalkenyl group may be a C5 cycloalkenyl, C6 cycloalkenyl, C7 cycloalkenyl, C8 cycloalkenyl, C9 cycloalkenyl, or C10 cycloalkenyl. In some embodiments, a substituted cycloalkenyl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, linkerB may comprise at least one alkynyl group. In some embodiments, an alkynyl group may have 4 carbons, 5 carbons, 6 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 17 carbons, 18 carbons, 19 carbons, or 20 carbons.

5 In some embodiments, linkerB may comprise at least one substituted alkynyl group. In some embodiments, a substituted alkynyl group may have 4 carbons, 5 carbons, 6 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 17 carbons, 18 carbons, 19 carbons, or 20 carbons. In some embodiments, a substituted alkynyl group may include one or more of the following
10 substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, linkerB may comprise at least one aryl group or heteroaryl group. Examples include phenyl, naphthyl, and pyridinyl, although it is noted that other aryl and heteroaryl groups, that fall within the definitions provided herein, may be used.

15 In some embodiments, linkerB may comprise at least one substituted aryl group. In some embodiments, a substituted aryl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

In some embodiments, linkerB may comprise at least one aralkyl group. Example
20 aralkyl groups include, but are not limited to, phenylmethyl, phenylethyl, and phenylpropyl.

In some embodiments, linkerB may comprise at least one substituted aralkyl group. In some embodiments, a substituted aralkyl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

25 In some embodiments, linkerB may comprise at least one aralkenyl group. Example aralkenyl groups include, but are not limited to ethenylbenzene and propenylbenzene.

In some embodiments, linkerB may comprise at least one substituted aralkenyl group. In some embodiments, a substituted aralkenyl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide,
30 halide, sulfonyl, and sulfonamide.

In some embodiments, linkerB may comprise at least one aralkynyl group. Example aralkynyl groups include, but are not limited to ethynylbenzene and propynylbenzene.

In some embodiments, linkerB may comprise at least one substituted aralkynyl group. In some embodiments, a substituted aralkynyl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

5 In some embodiments, linkerB may comprise at least one heteroatom. In some embodiments, linkerB may comprise one or more oxygen (O) heteroatoms, one or more nitrogen (N) heteroatoms, one or more sulfur (S) heteroatoms, and/or one or more phosphorous (P) heteroatoms. In some embodiments, at least one of the independently selected linkerAs may comprise at least one heteroalkyl group.

10 In some embodiments, linkerB may comprise at least one aliphatic heterocycle. In some embodiments, linkerB may comprise at least one of tetrahydrofuran (THF), tetrahydropyran (THP), morpholine, piperidine, piperazine, pyrrolidine, and/or azetidine.

In some embodiments, linkerB may comprise at least one heteroaryl group. In some embodiments, linkerB may comprise one or more of imidazole, pyrazole, pyridine,
15 pyrimidine, triazole, and/or 1,2,3-triazole.

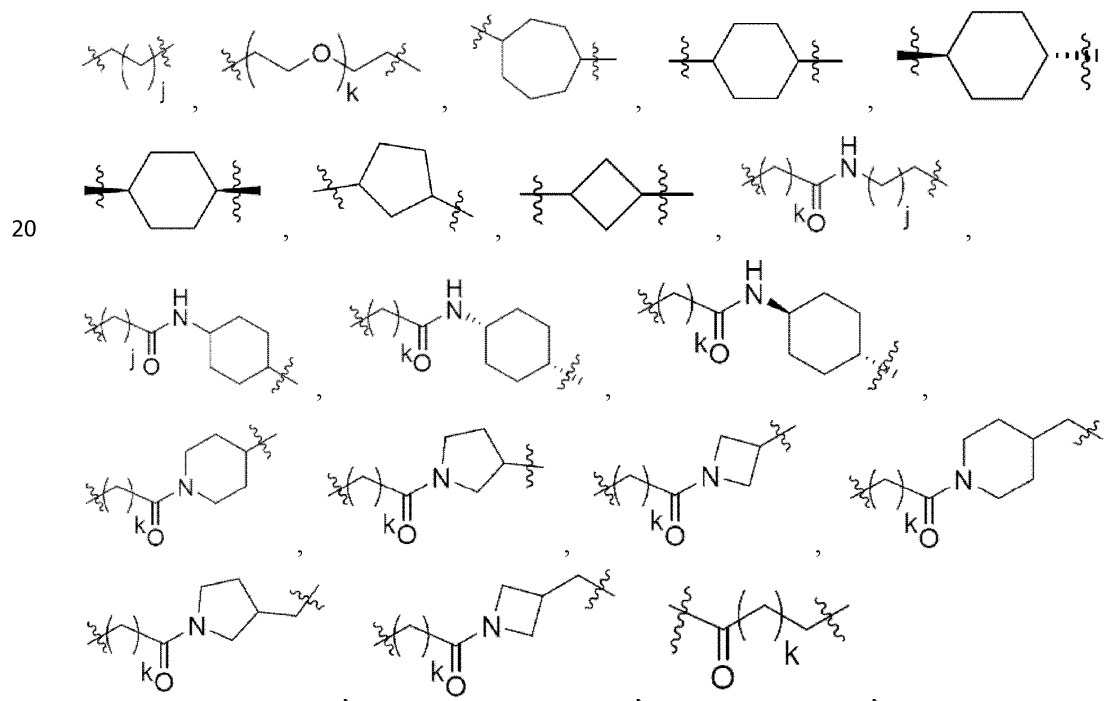
In some embodiments, linkerB may comprise at least one substituted heteroaryl group. In some embodiments, a substituted heteroaryl group may include one or more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

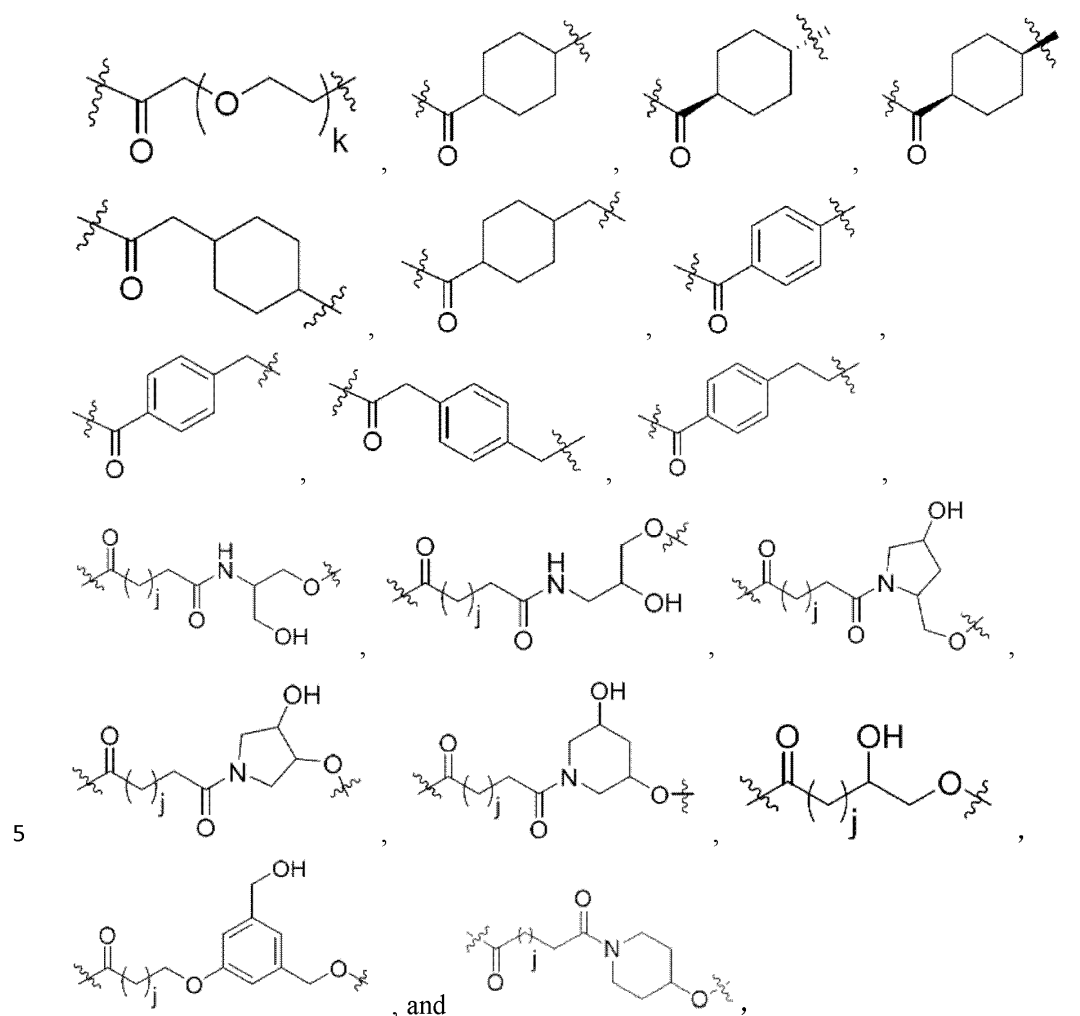
20 In some embodiments, linkerB may comprise at least one amino acid. Various amino acids are known to those skilled in the art. LinkerB is not limited to including one or more specific amino acids. For example, linkerB may comprise one or more arginine (Arg) amino acids, one or more histidine (His) amino acids, one or more lysine (Lys) amino acids, one or more aspartic acid (Asp) amino acids, one or more glutamic acid (Glu) amino acids, one or
25 more serine (Ser) amino acids, one or more threonine (Thr) amino acids, one or more asparagine (Asn) amino acids, one or more glutamine (Gln) amino acids, one or more cysteine (Cys) amino acids, one or more selenocysteine (Sec) amino acids, one or more glycine (Gly) amino acids, one or more proline (Pro) amino acids, one or more alanine (Ala) amino acids, one or more valine (Val) amino acids, one or more isoleucine (Ile) amino acids,
30 one or more leucine (Leu) amino acids, one or more methionine (Met) amino acids, one or more phenylalanine (Phe) amino acids, one or more tyrosine (Tyr) amino acids, and/or one or more tryptophan (Trp) amino acids.

In some embodiments, linkerB may comprise at least one nucleotide. Various nucleotides are known to those skilled in the art. LinkerB is not limited to including one or more specific nucleotides. For example, linkerB may comprise one or more nucleotides comprising a guanine nucleobase, one or more nucleotides comprising an adenine nucleobase, one or more nucleotides comprising a cytosine nucleobase, one or more nucleotides comprising a thymine nucleobase, and/or one or more nucleotides comprising a uracil nucleobase. In some embodiments, linkerB may comprise at least one abasic nucleotide. As known in the art, an abasic nucleotide is a nucleotide having an abasic site, which is a location that has neither a purine nor a pyrimidine base. For example, linkerB may comprise one or more abasic DNAs and/or one or more abasic RNAs. In some embodiments, linkerB may comprise at least one inverted abasic nucleotide. For example, linkerB may comprise one or more inverted abasic DNAs and/or one or more inverted abasic RNAs.

In some embodiments, linkerB may comprise at least one saccharide. In some embodiments, linkerB may comprise at least one glucose monosaccharide unit, at least one fructose monosaccharide unit, at least one mannose monosaccharide unit, at least one galactose monosaccharide unit, at least one ribose monosaccharide unit, and/or at least one glucosamine monosaccharide unit.

In some embodiments, linkerB may comprise one or more of:





where:

j is an integral number between 1 and 12, and

10 k is an integral number between 0 and 12.

In some embodiments, j is an integral number independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12. In some embodiments, k is an integral number independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12. In some cases, the present invention also found that when linkerB contains a six-membered ring fragment, especially a 4-Hydroxypiperidinyl group, when it is used as targeted delivery of pharmaceutical agents, compared with a five-membered ring, it shows better in vivo stability and activity, such as the compound 75 of the present invention.

Pharmaceutical Agents

In some embodiments a pharmaceutical agent is a diagnostic or therapeutic drug, molecule, compound, or combination of drugs, molecules, or compounds that have a property of assisting in diagnosis, prevention, treatment, and/or mitigation of a disease or condition, for example in a cell or subject. In certain embodiments, a pharmaceutical agent is a drug, molecule, compound or combination of drugs, molecules, or compounds that have a property of assisting in the enhancement of a desirable condition, for example in a cell or subject.

In some embodiments, a pharmaceutical agent may be an oligonucleotide. In some embodiments, the oligonucleotide may comprise a siRNA. In some embodiments, the oligonucleotide may comprise a double stranded siRNA. In some embodiments, the double stranded siRNA may comprise at least one modified ribonucleotide. In some embodiments of compositions and methods of the invention, the at least one modified nucleotide comprises: a 2'-O-methyl nucleotide, 2'-Fluoro nucleotide, 2'-deoxy nucleotide, 2'3'-seco nucleotide mimic, locked nucleotide, unlocked nucleic acid nucleotide (UNA), glycol nucleic acid nucleotide (GNA), 2'-F-Arabino nucleotide, 2'-methoxyxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted abasic nucleotide, inverted 2'-Ome nucleotide, inverted 2'-deoxy nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a 3'-Ome nucleotide, a nucleotide comprising a 5'-phosphorothioate group, or a 5'-(E)-vinyl phosphonate nucleotide (antisense strand only), or a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a 2'-amino-modified nucleotide, a phosphoramidate, or a non-natural base comprising nucleotide. In some embodiments, substantially all (i.e., greater than 85 %) ribonucleotides of the double stranded siRNA may be modified.

In some embodiments, all ribonucleotides of the double stranded siRNA may be modified. In some embodiments, at least one strand of the double stranded siRNA may comprise at least one phosphorothioate linkage. In some embodiments, at least one strand of the double stranded siRNA may comprise up to 6 phosphorothioate linkages. In some embodiments, a double stranded siRNA may comprise at least one locked nucleic acid (LNA). As known in the art, a LNA (sometimes referred to as a bridged nucleic acid (BNA) or an inaccessible RNA) is a modified RNA molecule where the ribose moiety is modified with an extra bridge connecting the 2' oxygen and the 4' carbon, thereby locking the ribose in the 3'-endo conformation. LNA is known to increase stability against enzymatic degradation, and improve specificity and affinity. In some embodiments, a double stranded siRNA may

comprise at least one unlocked nucleic acid (UNA). As known in the art, a UNA is an acyclic derivative of RNA lacking a C2'-C3'-bond of the ribose ring of RNA. It is known to those skilled in the art that including a UNA in certain positions of an antisense strand of a siRNA is tolerated for activity, and may promote reduced off target activity.

5 In some embodiments, a double stranded siRNA may comprise at least one glycerol nucleic acid (GNA). As known in the art, a GNA (sometimes referred to as a glycol nucleic acid) is a nucleic acid similar to RNA but differing in the composition of its sugar-phosphodiester backbone. It is known to those skilled in the art that including a GNA in certain positions of an antisense strand of a siRNA is tolerated for activity, and may promote
10 reduced off target activity.

 In some embodiments, the oligonucleotide may comprise a siRNA including one or more modified nucleotides, including but not limited to a 2'-modified nucleotide (e.g. F and MeO), an abasic nucleotide, an inverted abasic nucleotide, a locked nucleotide, UNA an unlocked nucleic acid (UNA), and a glycerol nucleic acid (GNA).

15 In some embodiments, the oligonucleotide may comprise a siRNA including one or more phosphorothioate backbone linkages.

 In some embodiments, the oligonucleotide may comprise a single strand siRNA. In some embodiments, the oligonucleotide may comprise a small activating RNA. In some
20 embodiments, the oligonucleotide may comprise a microRNA (miRNA). In some embodiments, the oligonucleotide may comprise an antisense oligonucleotide. In some embodiments, the oligonucleotide may comprise a short guide RNA (gRNA). In some embodiments, the oligonucleotide may comprise a single guide RNA (sgRNA). In some embodiments, the oligonucleotide may comprise a messenger RNA (mRNA). In some
25 embodiments, the oligonucleotide may comprise a ribozyme. In some embodiments, the oligonucleotide may comprise a plasmid. In some embodiments, the oligonucleotide may comprise an immune stimulating nucleic acid. In some embodiments, the oligonucleotide may comprise an antagomir. In some embodiments, the oligonucleotide may comprise an aptamer. An aptamer is a short, single-stranded DNA or RNA molecule that can selectively
30 bind to a specific target, such as a protein, peptide, carbohydrate, small molecule, toxin, or live cell. Aptamers assume a variety of shapes, as they tend to form helices and single-stranded loops. The present disclosure is not limited to any particular aptamer. Aptamers known and not yet discovered are within the scope of the present disclosure.

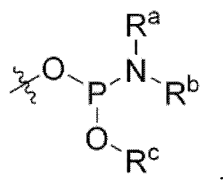
In some embodiments, the oligonucleotide may comprise at least 3 independently selected nucleotides. In some embodiments, the oligonucleotide may comprise between 16 and 23 independently selected nucleotides, for example when the oligonucleotide is a siRNA. In some embodiments, the oligonucleotide may comprise about 100 independently selected nucleotides, for example when the oligonucleotide is a sgRNA. In some embodiments, the oligonucleotide may comprise up to fourteen thousand independently selected nucleotides, for example when the oligonucleotide is an mRNA.

Functional Groups Capable of Linking to One or More Pharmaceutical Agents

As indicated above, "W" in formula 1 may be a functional group capable of linking to one or more pharmaceutical agents.

In some embodiments, the functional group may be a hydroxy group (OH). In some embodiments, the functional group may be a protected hydroxy group. One skilled in the art will appreciate that various protecting groups may be used to protect a hydroxy group. Each of the various processing groups are within the scope of the present disclosure. For example, and not limitation, the hydroxy group may be protected using at least one of 4,4'-dimethoxytrityl (DMT), monomethoxytrityl (MMT), 9-(*p*-methoxyphenyl)xanthen-9-yl (Mox), and 9-phenylxanthen-9-yl (Px).

In some embodiments, the functional group may be a phosphoramidite group having the formula:



Formula 2

where:

R^a is a C1 to C6 alkyl, C3 to C6 cycloalkyl, an isopropyl group, or R^a joins with R^b through a nitrogen atom to form a cycle,

R^b is a C1 to C6 alkyl, C3 to C6 cycloalkyl, an isopropyl group, or R^b joins with R^a through a nitrogen atom to form a cycle, and

R^c is a phosphite protecting group, phosphate protecting group, or a 2-cyanoethyl group.

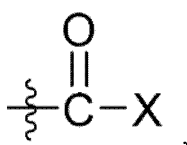
R^c may be one of various phosphite protecting groups known to those skilled in the art.

In some embodiments, the phosphite protecting group may comprise at least one of methyl,

allyl, 2-cyanoethyl, 4-cyano-2-butenyl, 2-cyano-1,1-dimethylethyl, 2-(trimethylsilyl)ethyl, 2-(S-acetylthio)ethyl, 2-(S-pivaloylthio)ethyl, 2-(4-nitrophenyl)ethyl, 2,2,2-trichloroethyl, 2,2,2-trichloro-1, 1-dimethylethyl, 1,1,1,3,3,3-hexafluoro-2-propyl, fluorenyl-9-methyl, 2-chlorophenyl, 4-chlorophenyl, and 2,4-dichlorophenyl.

- 5 R^c may be one of various phosphate protecting groups known to those skilled in the art. In some embodiments, the phosphate protecting group may comprise at least one of methyl, allyl, 2-cyanoethyl, 4-cyano-2-butenyl, 2-cyano-1,1-dimethylethyl, 2-(trimethylsilyl)ethyl, 2-(S-acetylthio)ethyl, 2-(S-pivaloylthio)ethyl, 2-(4-nitrophenyl)ethyl, 2,2,2-trichloroethyl, 2,2,2-trichloro-1, 1-dimethylethyl, 1,1,1,3,3,3-hexafluoro-2-propyl, fluorenyl-9-methyl, 2-chlorophenyl, 4-chlorophenyl, and 2,4-dichlorophenyl.

In some embodiments, the functional group may be a carboxyl group (CO_2H). In some embodiments, the functional group may be an activated carboxyl group having the formula:

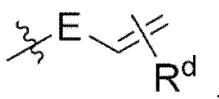


Formula 3

- 15 where X is a leaving group.

Various activated carboxyl groups are known to those skilled in the art, all of which are within the scope of the present disclosure. In some embodiments, the leaving group (X) may be one of carboxylate, sulfonate, chloride, phosphate, imidazole, hydroxybenzotriazole (HOBt), N-hydroxysuccinimide (NHS), tetrafluorophenol, pentafluorophenol, of *para*-nitrophenol.

In some embodiments, the functional group may be a Michael acceptor. In some embodiments, the Michael acceptor may have the formula:



Formula 4

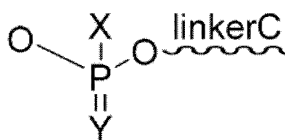
where:

- 25 E is an electron withdrawing group; and
 R^d is hydrogen or a C1-C6 alkyl substitution group on olefin (meaning E and R^d may be *cis*, *trans*, or *iso* with respect to the carbon-carbon double bond).

Various electron withdrawing groups are known to those skilled in the art, all of which are within the scope of the present disclosure. In some embodiments, the electron withdrawing group (E) may be carboxamide or an ester. In some embodiments, the electron

withdrawing group (E) and the carbon-carbon double bond of the Michael acceptor may be part of maleimide, a cyclic dicarboximide in which the two carboacyl groups on nitrogen together with the nitrogen itself form a 1H-pyrrole-2,5-dione structure.

In some embodiments, the functional group may have the formula:



Formula 5

where:

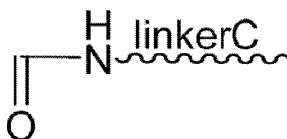
linkerC is absent or a spacer attached to a 3' or 5' end of an oligonucleotide,

X is a methyl group, oxygen, sulfur, or an amino group, and

Y is oxygen, sulfur, or an amino group.

In some embodiments, linkerC of Formula 5 may comprise at least a heterocyclic compound. In some embodiments, the heterocyclic compound may be an abasic nucleotide or an inverted abasic nucleotide.

In some embodiments, the functional group may have the formula:



Formula 6

where linkerC is a spacer having one end attached to a nitrogen of a carboxamide and the other end attached to a 3' or 5' end of an oligonucleotide.

In some embodiments, linkerB is attached to the carbonyl of the carboxyamide of Formula 6.

In some embodiments, linkerC in Formula 6 may comprise at least one PEG. The PEG may have any number of repeating O-CH₂-CH₂ units. For example, the PEG may be PEG1, PEG2, PEG3, PEG4, PEG5, PEG6, PEG7, PEG8, PEG9, PEG10, PEG11, PEG12, PEG13, PEG14, PEG15, PEG16, PEG17, PEG18, PEG19, PEG20, PEG21, PEG22, PEG23, PEG24, PEG25, PEG26, PEG27, PEG28, PEG29, PEG30, PEG31, PEG32, PEG33, PEG34, PEG35, PEG36, PEG37, PEG38, PEG39, PEG40, PEG41, PEG42, PEG43, PEG44, PEG45, PEG46, PEG47, PEG48, PEG49, PEG50, PEG51, PEG52, PEG53, PEG54, PEG55, PEG56, PEG57, PEG58, PEG59, PEG60, PEG61, PEG62, PEG63, PEG64, PEG65, PEG66, PEG67, PEG68, PEG69, PEG70, PEG71, PEG72, PEG73, PEG74, PEG75, PEG76, PEG77, PEG78, PEG79, PEG80, PEG81, PEG82, PEG83, PEG84, PEG85, PEG86, PEG87, PEG88, PEG89,

PEG90, PEG91, PEG92, PEG93, PEG94, PEG95, PEG96, PEG97, PEG98, PEG99, PEG100, or larger.

In some embodiments, linkerC in Formula 6 may comprise at least one alkyl group. In some embodiments, an alkyl group may have 2 carbons, 3 carbons, 4 carbons, 5 carbons, 6
5 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 17 carbons, 18 carbons, 19 carbons, or 20 carbons.

In some embodiments, linkerC in Formula 6 may comprise at least one cycloalkyl group. In some embodiments, a cycloalkyl group may be a C3 cycloalkyl (i.e., cyclopropane), C4 cycloalkyl (i.e., cyclobutene), C5 cycloalkyl (i.e., cyclopentane), C6 cycloalkyl (i.e.,
10 cyclohexane), C7 cycloalkyl (i.e., cycloheptane), C8 cycloalkyl (i.e., cyclooctane), C9 cycloalkyl (i.e., cyclononane), or C10 cycloalkyl (i.e., cyclodecane).

In some embodiments, linkerC in Formula 6 may comprise at least one heteroatom. In some embodiments, linkerC may comprise one or more oxygen (O) heteroatoms, one or more nitrogen (N) heteroatoms, one or more sulfur (S) heteroatoms, and/or one or more
15 phosphorous (P) heteroatoms.

In some embodiments, linkerC in Formula 6 may comprise at least one aliphatic heterocycle. In some embodiments, linkerC may comprise at least one of tetrahydrofuran (THF), tetrahydropyran (THP), morpholine, piperidine, piperazine, pyrrolidine, and/or azetidine.

20 In some embodiments, linkerC in Formula 6 may comprise at least one heteroaryl group. In some embodiments, linkerC may comprise one or more of imidazole, pyrazole, pyridine, pyrimidine, triazole, and/or 1,2,3-triazole.

In some embodiments, linkerC in Formula 6 may comprise at least one substituted heteroaryl group. In some embodiments, a substituted heteroaryl group may include one or
25 more of the following substitution groups: alkyl, cycloalkyl, hydroxy, alkoxide, carboxyl, amine, amide, halide, sulfonyl, and sulfonamide.

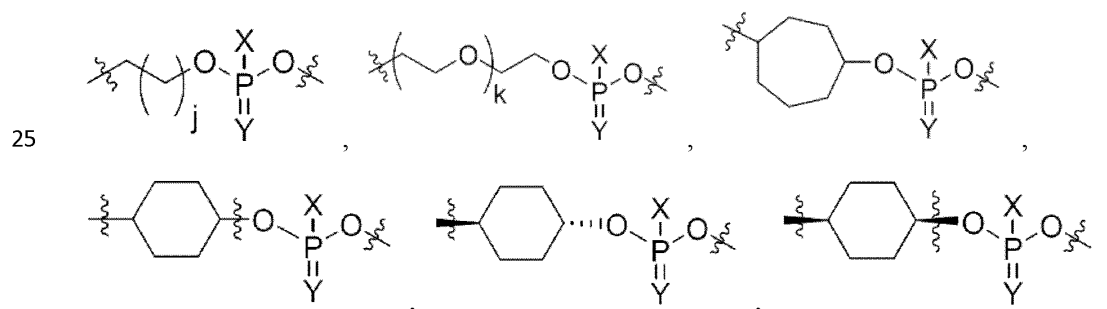
In some embodiments, linkerC in Formula 6 may comprise at least one amino acid. Various amino acids are known to those skilled in the art. LinkerC is not limited to including one or more specific amino acids. For example, linkerC may comprise one or more arginine
30 (Arg) amino acids, one or more histidine (His) amino acids, one or more lysine (Lys) amino acids, one or more aspartic acid (Asp) amino acids, one or more glutamic acid (Glu) amino acids, one or more serine (Ser) amino acids, one or more threonine (Thr) amino acids, one or more asparagine (Asn) amino acids, one or more glutamine (Gln) amino acids, one or more

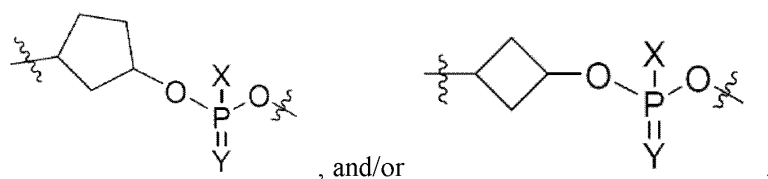
cysteine (Cys) amino acids, one or more selenocysteine (Sec) amino acids, one or more glycine (Gly) amino acids, one or more proline (Pro) amino acids, one or more alanine (Ala) amino acids, one or more valine (Val) amino acids, one or more isoleucine (Ile) amino acids, one or more leucine (Leu) amino acids, one or more methionine (Met) amino acids, one or more phenylalanine (Phe) amino acids, one or more tyrosine (Tyr) amino acids, and/or one or more tryptophan (Trp) amino acids.

In some embodiments, linkerC in Formula 6 may comprise at least one nucleotide. Various nucleotides are known to those skilled in the art. LinkerC is not limited to including one or more specific nucleotides. For example, linkerC may comprise one or more nucleotides comprising a guanine nucleobase, one or more nucleotides comprising an adenine nucleobase, one or more nucleotides comprising a cytosine nucleobase, one or more nucleotides comprising a thymine nucleobase, and/or one or more nucleotides comprising a uracil nucleobase. In some embodiments, linkerC may comprise at least one abasic nucleotide. As known in the art, an abasic nucleotide is a nucleotide having an abasic site, which is a location that has neither a purine nor a pyrimidine base. For example, linkerC may comprise one or more abasic DNAs and/or one or more abasic RNAs. In some embodiments, linkerC may comprise at least one inverted abasic nucleotide. For example, linkerC may comprise one or more inverted abasic DNAs and/or one or more inverted abasic RNAs.

In some embodiments, linkerC in Formula 6 may comprise at least one saccharide. In some embodiments, linkerC may comprise at least one glucose monosaccharide unit, at least one fructose monosaccharide unit, at least one mannose monosaccharide unit, at least one galactose monosaccharide unit, at least one ribose monosaccharide unit, and/or at least one glucosamine monosaccharide unit.

In some embodiments, linkerC in Formula 6 may comprise one or more of:



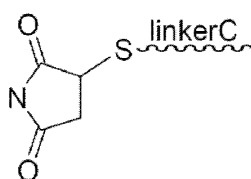


where:

j is an integral number between 1 and 12, and

k is an integral number between 0 and 12.

- 5 In some embodiments, the functional group may have the formula:



Formula 7

where linkerC is a spacer with one end attached to one of the succinimide ring carbons through a thioether bond and the other end attached to a 3' or 5' end of an oligonucleotide.

In some embodiments, linkerB is attached to the succinimide nitrogen of Formula 7.

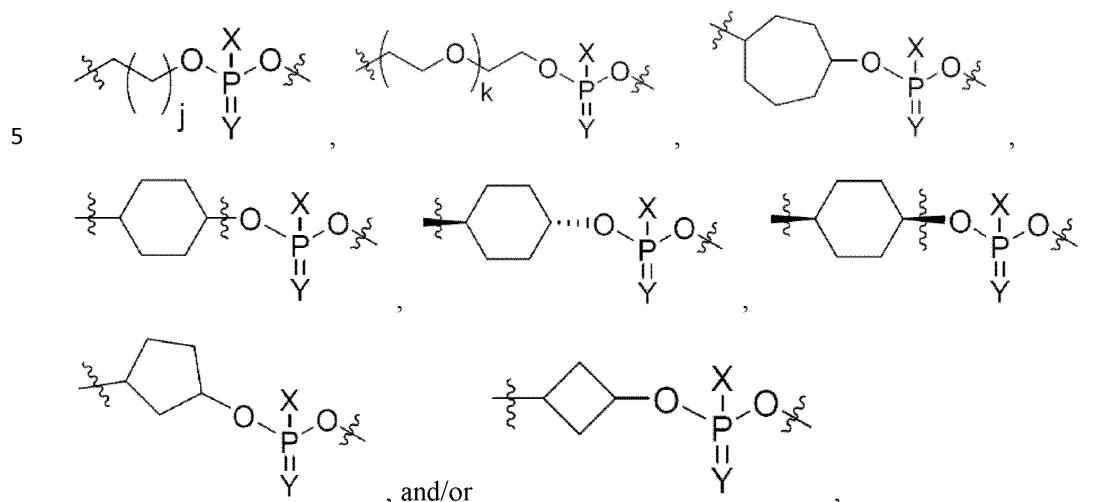
- 10 In some embodiments, linkerC in Formula 7 may comprise at least one PEG. The PEG may have any number of repeating O-CH₂-CH₂ units. For example, the PEG may be PEG1, PEG2, PEG3, PEG4, PEG5, PEG6, PEG7, PEG8, PEG9, PEG10, PEG11, PEG12, PEG13, PEG14, PEG15, PEG16, PEG17, PEG18, PEG19, PEG20, PEG21, PEG22, PEG23, PEG24, PEG25, PEG26, PEG27, PEG28, PEG29, PEG30, PEG31, PEG32, PEG33, PEG34, PEG35, PEG36, PEG37, PEG38, PEG39, PEG40, PEG41, PEG42, PEG43, PEG44, PEG45, PEG46, PEG47, PEG48, PEG49, PEG50, PEG51, PEG52, PEG53, PEG54, PEG55, PEG56, PEG57, PEG58, PEG59, PEG60, PEG61, PEG62, PEG63, PEG64, PEG65, PEG66, PEG67, PEG68, PEG69, PEG70, PEG71, PEG72, PEG73, PEG74, PEG75, PEG76, PEG77, PEG78, PEG79, PEG80, PEG81, PEG82, PEG83, PEG84, PEG85, PEG86, PEG87, PEG88, PEG89, PEG90, PEG91, PEG92, PEG93, PEG94, PEG95, PEG96, PEG97, PEG98, PEG99, PEG100, or larger.

- 25 In some embodiments, linkerC in Formula 7 may comprise at least one alkyl group. In some embodiments, an alkyl group may have 2 carbons, 3 carbons, 4 carbons, 5 carbons, 6 carbons, 7 carbons, 8 carbons, 9 carbons, 10 carbons, 11 carbons, 12 carbons, 13 carbons, 14 carbons, 15 carbons, 16 carbons, 17 carbons, 18 carbons, 19 carbons, or 20 carbons.

In some embodiments, linkerC in Formula 7 may comprise at least one cycloalkyl group. In some embodiments, a cycloalkyl group may be a C3 cycloalkyl (i.e., cyclopropane),

C4 cycloalkyl (i.e., cyclobutene), C5 cycloalkyl (i.e., cyclopentane), C6 cycloalkyl (i.e., cyclohexane), C7 cycloalkyl (i.e., cycloheptane), C8 cycloalkyl (i.e., cyclooctane), C9 cycloalkyl (i.e., cyclononane), or C10 cycloalkyl (i.e., cyclodecane).

In some embodiments, linkerC in Formula 7 may comprise one or more of:



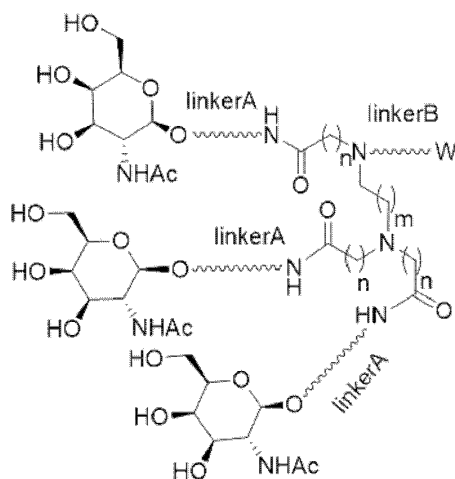
where:

j is an integral number between 1 and 12, and

10 k is an integral number between 0 and 12.

Multivalent Ligand Clusters Comprising GalNAc Targeting Ligands

In some embodiments, a multivalent ligand cluster of the present disclosure may have the general structure of Formula 8:



Formula 8

where:

m is an integral number between 1 and 10,

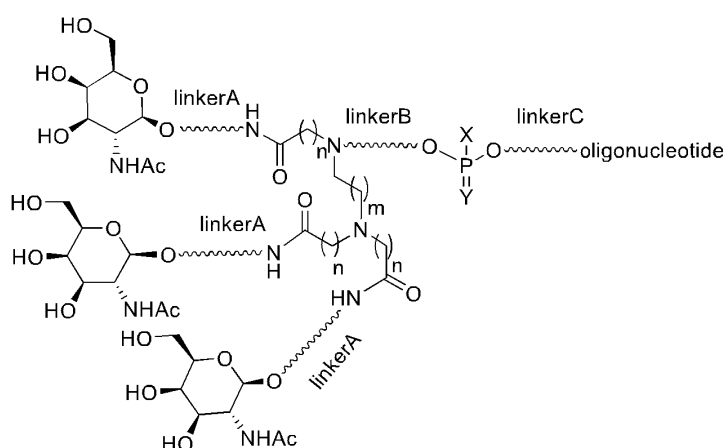
each n is an independently selected integral number between 1 and 10,

5 each linkerA is an independently selected spacer, with one end attached to a TL and the other end attached to the nitrogen of an alkylcarboxamide,

linkerB is a spacer, with one end attached to a pharmaceutical agent or a functional group capable of linking to one or more pharmaceutical agents, and the other end attached to a diamine nitrogen, and

10 W is either one or more pharmaceutical agents, or a functional group capable of linking to one or more pharmaceutical agents.

In some embodiments, a multivalent ligand cluster of the present disclosure may have the general structure of Formula 9:



15

Formula 9

where:

m is an integral number between 1 and 10,

each n is an independently selected integral number between 1 and 10,

each linkerA is an independently selected spacer,

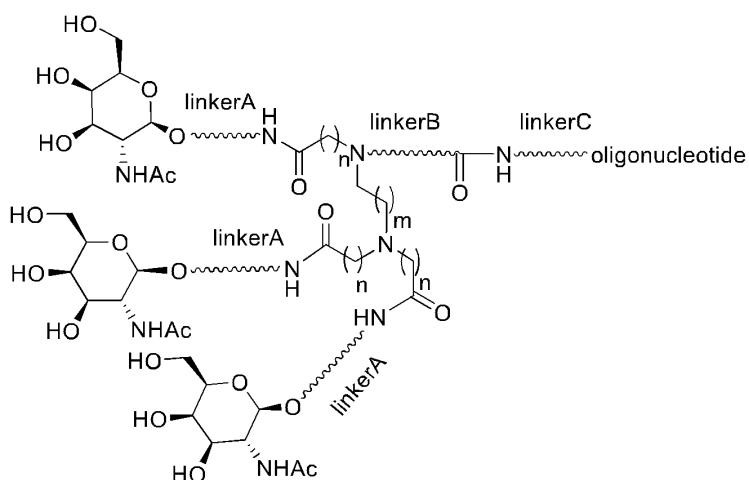
20 linkerB is a spacer,

linkerC is a spacer or absent,

X is a methyl group, oxygen, sulfur, or an amino group, and

Y is oxygen, sulfur, or an amino group.

In some embodiments, a multivalent ligand cluster of the present disclosure may have
25 the general structure of Formula 10:



Formula 10

where:

m is an integral number between 1 and 10,

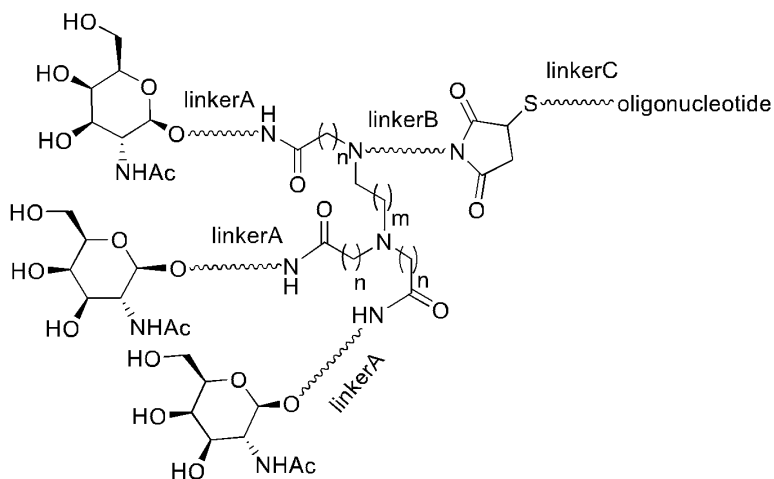
5 each n is an independently selected integral number between 1 and 10,

each linkerA is an independently selected spacer,

linkerB is a spacer, and

linkerC is a spacer.

10 In some embodiments, a multivalent ligand cluster of the present disclosure may have the general structure of Formula 11:



Formula 11

where:

m is an integral number between 1 and 10,

15 each n is an independently selected integral number between 1 and 10,

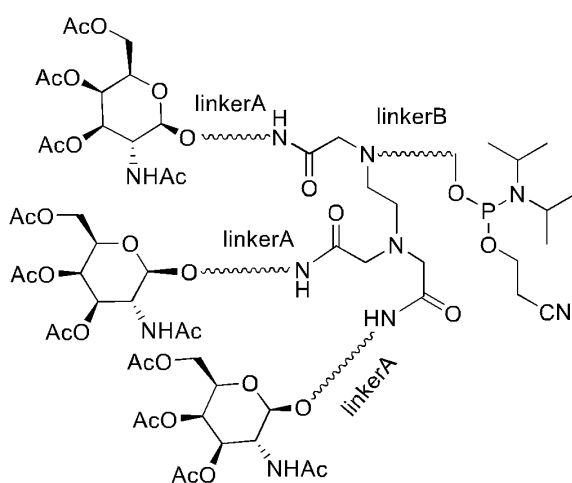
each linkerA is an independently selected spacer,

linkerB is a spacer, and

linkerC is a spacer.

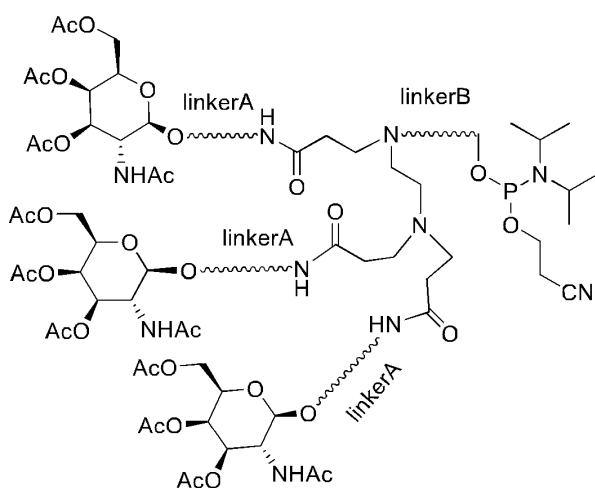
The following are example formulas for multivalent ligand clusters comprising

- 5 GalNAc or protected GalNAc targeting ligands, various “m” from Formula 1, various “n” from Formula 1, and various functional groups capable of linking to one or more pharmaceutical agents:

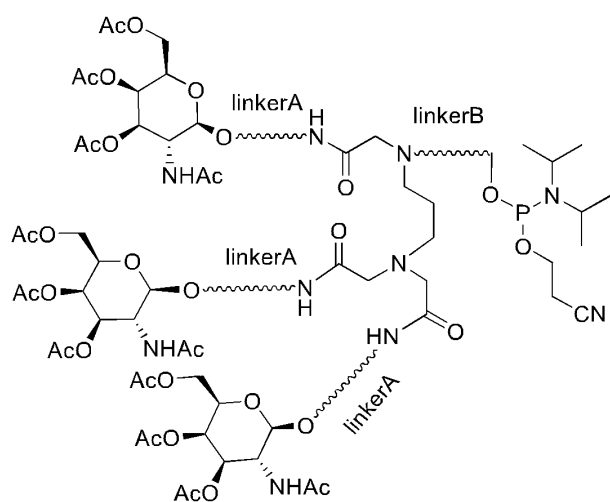


Formula 12

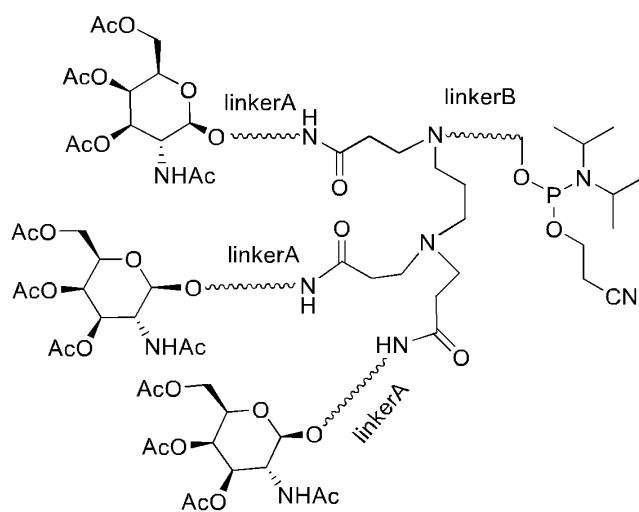
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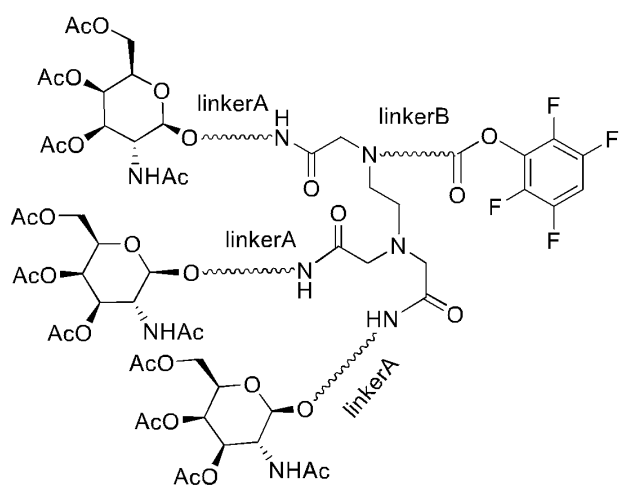
Formula 13



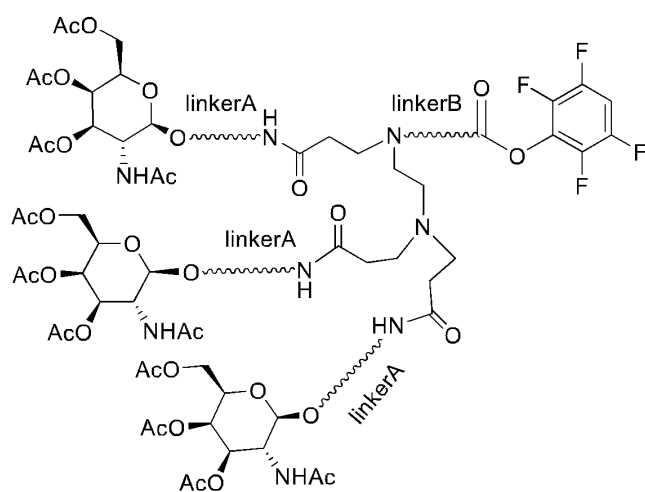
Formula 14



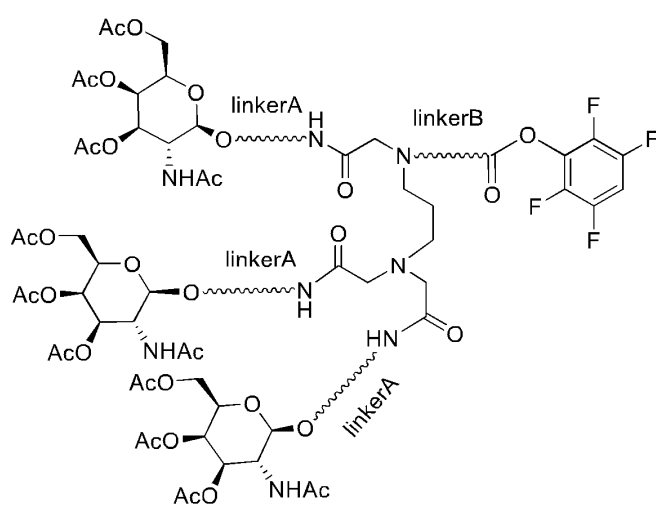
Formula 15



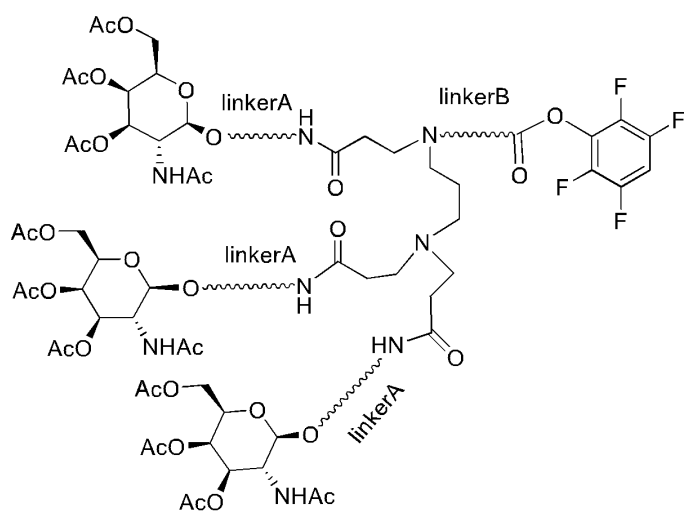
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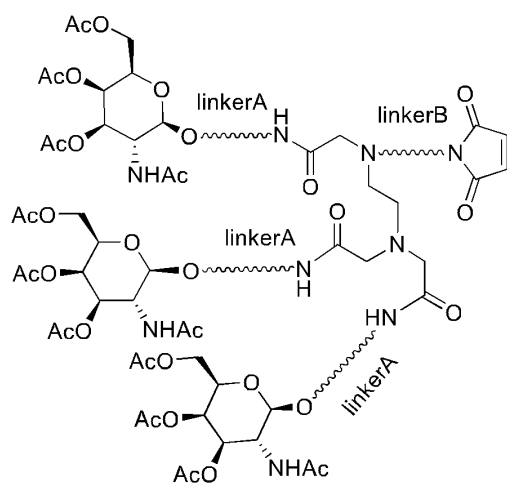
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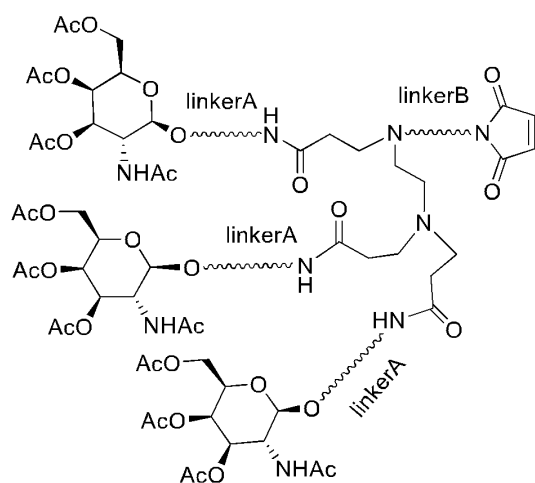
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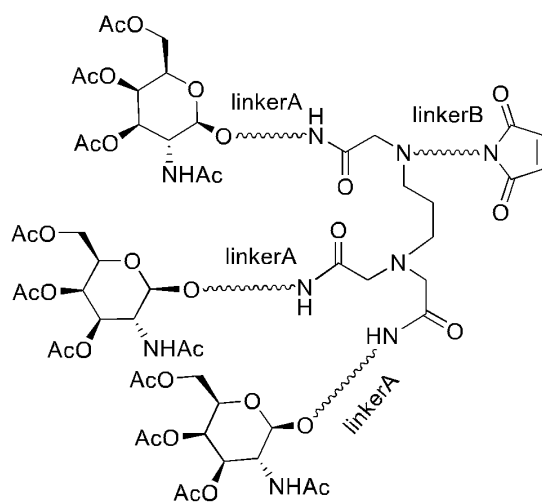
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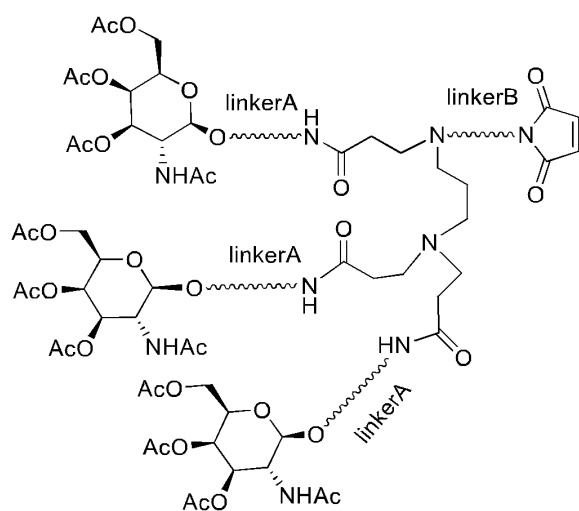
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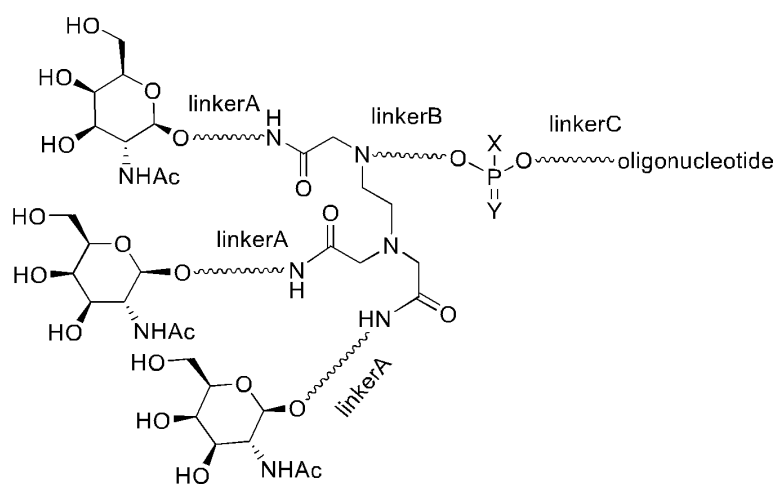
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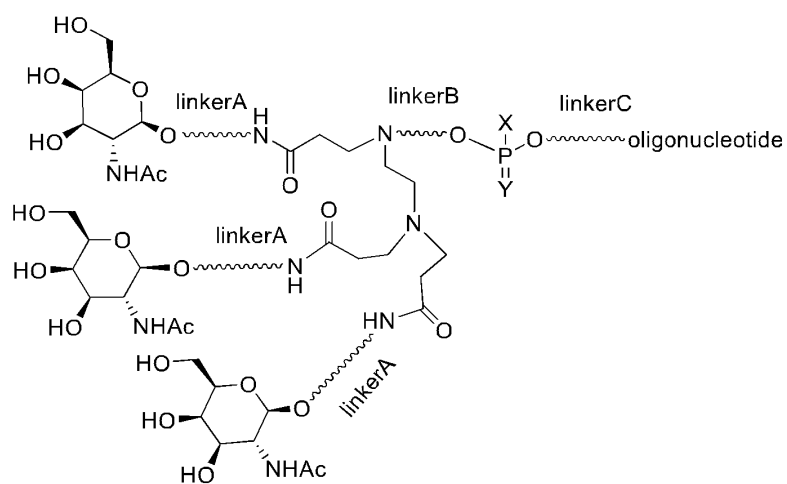
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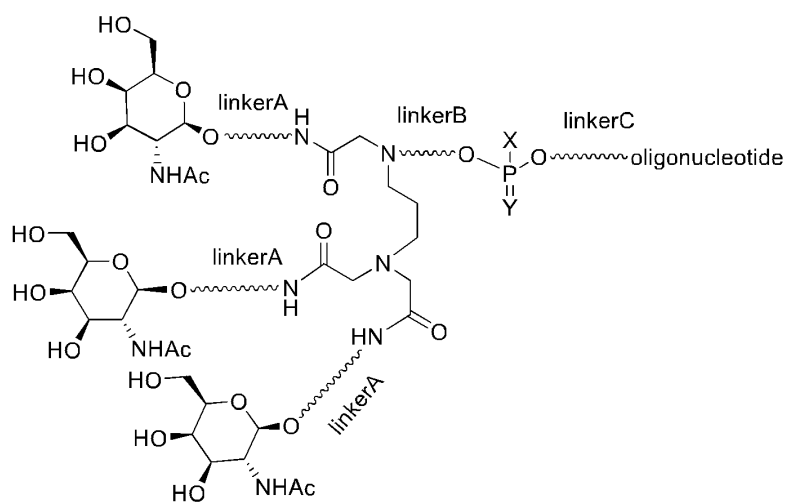
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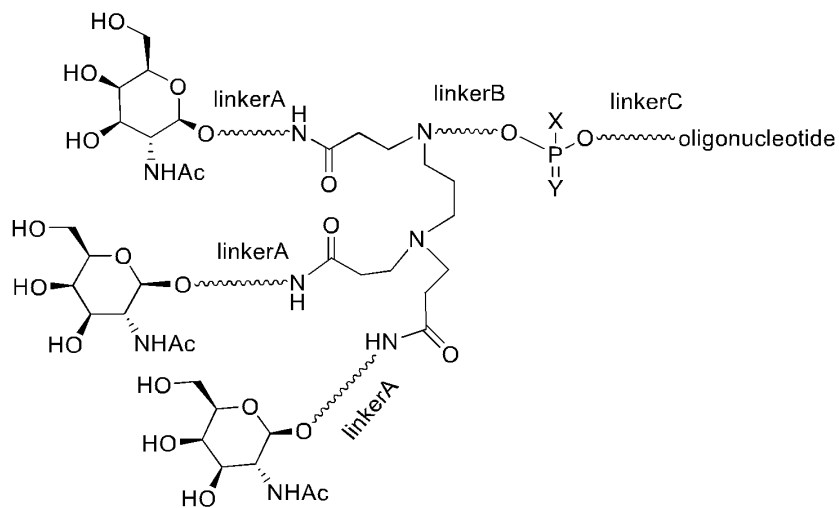
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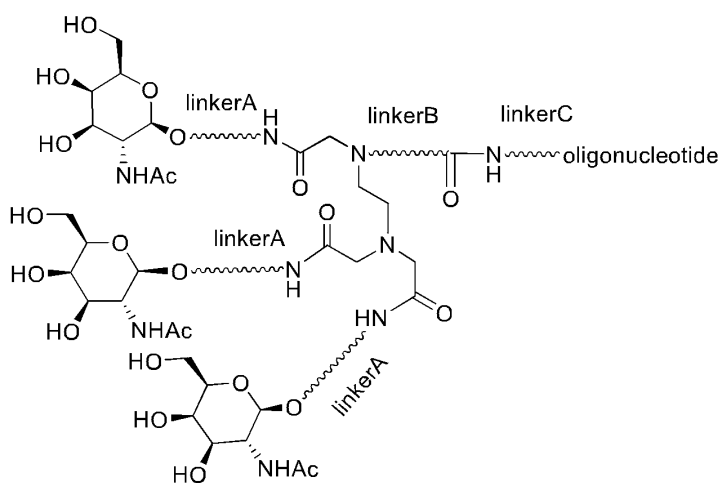
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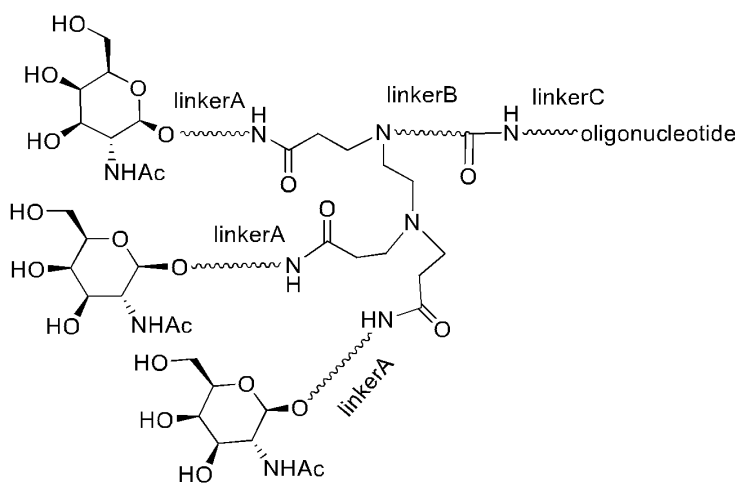
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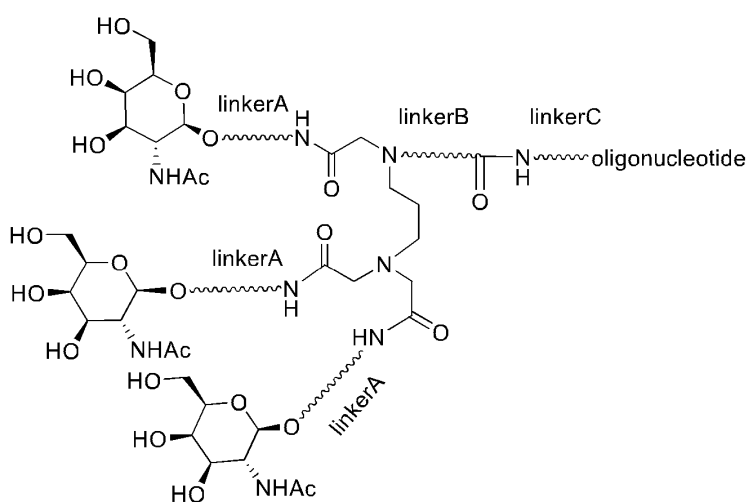
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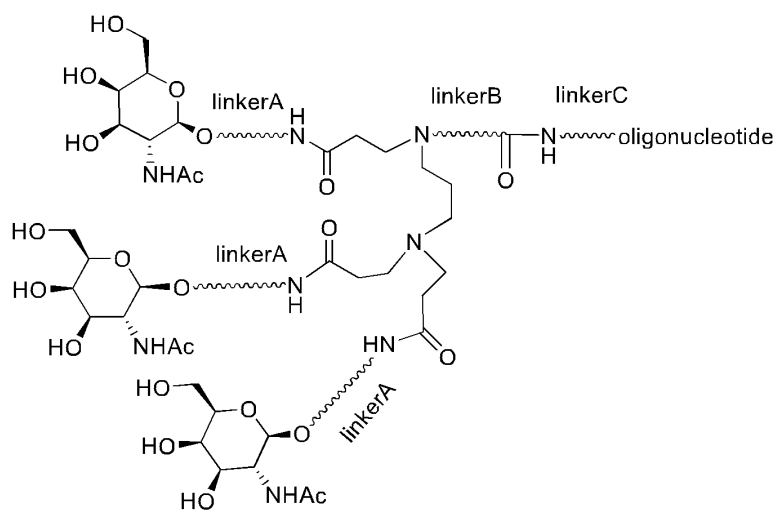
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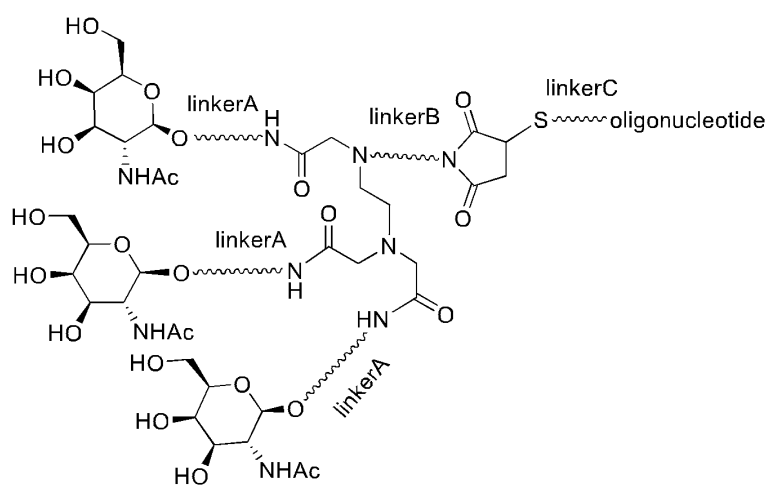
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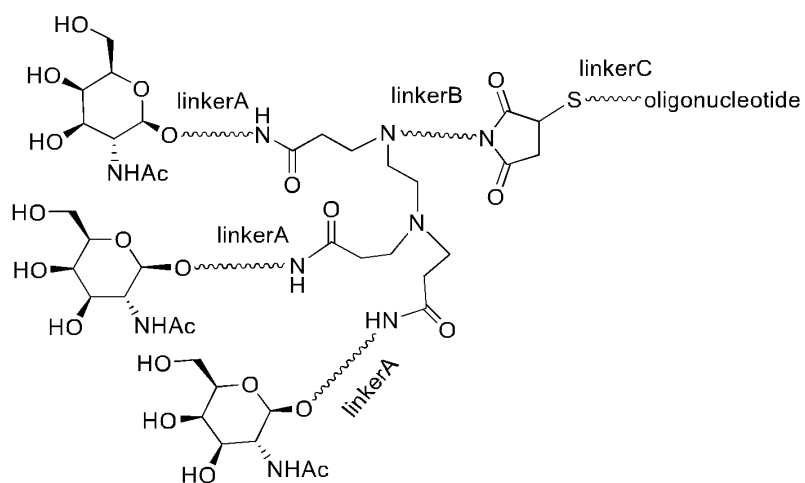
Formula 30



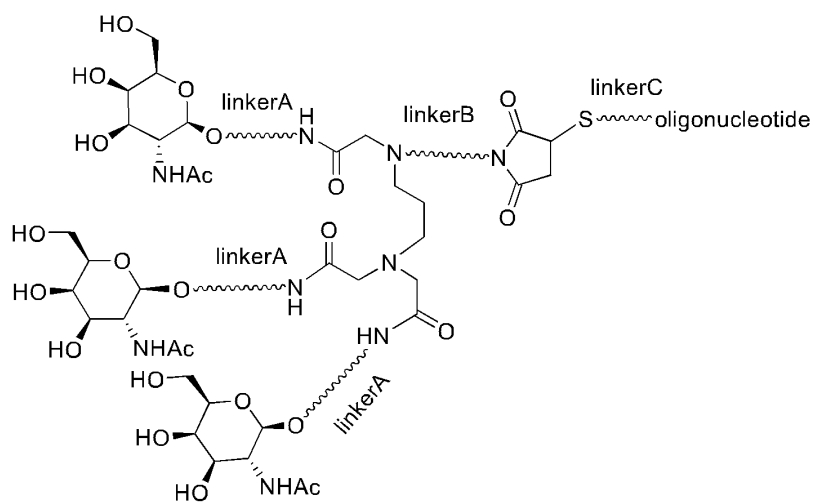
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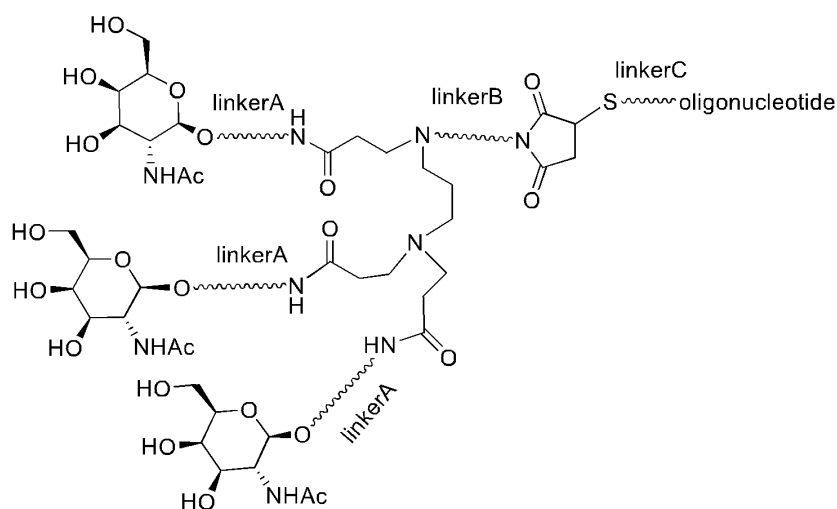
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Formula 33



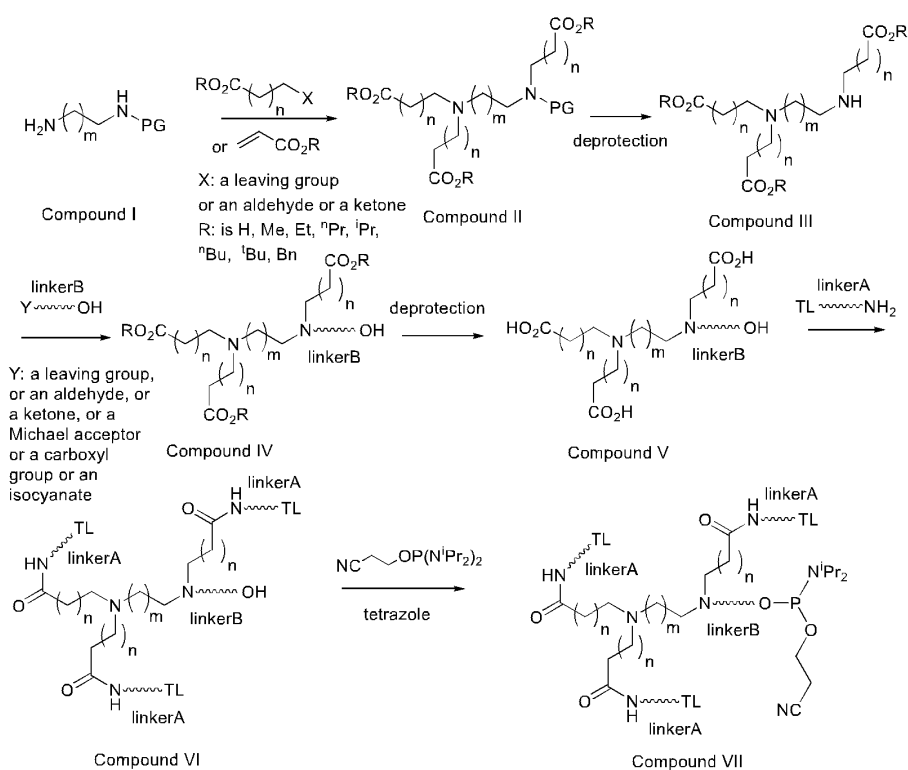
Formula 34



Formula 35

First Example Method of Preparation

One method for the preparation of examples of compounds with general Formula 1 is depicted in Scheme 1 below. Starting materials and intermediates may be purchased from commercial sources, made from known procedures, or are otherwise illustrated. The order of carrying out the steps of the reaction scheme may be varied.



Scheme 1

Scheme 1 starts with a mono-protected diamine (Compound I). The mono-protected diamine comprises a first nitrogen and a second nitrogen, where the first nitrogen is a primary amine, and the second nitrogen is a secondary amine comprising a protecting group (PG) in Scheme 1).

Various protecting groups are known to those skilled in the art, and may be used. In some embodiments, the protecting group may be a benzyl group. In some embodiments, the protecting group may be a triphenylmethyl group.

“m” in Scheme 1 may be any integral number. In some embodiments, m in Scheme 1 may be an integral number between 1 and 10.

Starting with Compound I, a triester Compound II can be synthesized in one step. In some embodiments, Compound II may be synthesized via a S_N2 substitution reaction using Compound I and one or more appropriate substrates. In some embodiments, Compound II may be synthesized via a reductive amination reaction using Compound I and one or more appropriate substrates. In some embodiments, Compound II may be synthesized via a Michael addition reaction using Compound I and one or more appropriate substrates.

As illustrated in Scheme 1, Compound II results from various protected carboxylic acids being coupled to Compound I. In Compound II, the first nitrogen is a tertiary amine comprise a first protected carboxylic acid and a second protected carboxylic acid, and the second nitrogen is a tertiary amine comprising the protecting group and a third protected carboxylic acid.

Each “n” in Scheme 1 is an independently selected integral number. In some embodiments, each n in Scheme 1 is an independently selected integral number between 0 and 10.

Compound III is produced by deprotecting the second nitrogen of Compound II, resulting in the second nitrogen of Compound III being a secondary amine comprising the third protected carboxylic acid. In embodiments where the protecting group is a benzyl group, Compound III may be produced by performing a hydrogenation reaction using Compound II. In embodiments where the protecting group is a triphenylmethyl group, Compound III may be produced by reacting the second compound with at least one acid. Example acids include, but are not limited to, hydrochloric acid (HCl) and trifluoroacetic acid (TFA).

Compound IV is produced by attaching a moiety comprising a hydroxy group to the second nitrogen of Compound III, resulting in the second nitrogen of Compound IV being a

tertiary amine or an amide comprising the third protected carboxylic acid and the moiety comprising the hydroxy group. The moiety comprising the hydroxy group may be attached to the second nitrogen using any linkerB described herein above.

In some embodiments, producing triester Compound IV comprises performing a S_N2 reaction using Compound III and one or more appropriate substrates. In some embodiments, producing Compound IV comprises performing a reductive amination reaction using Compound III and one or more appropriate substrates. In some embodiments, producing Compound IV comprises performing a Michael addition reaction using Compound III and one or more appropriate substrates. In some embodiments, producing Compound IV comprises performing an amide coupling reaction using Compound III and one or more appropriate substrates. In some embodiments, producing Compound IV comprises performing a nucleophilic addition reaction using Compound III and one or more appropriate substrates. Examples substrates include, but are not limited to, isocyanate and isothiocyanate.

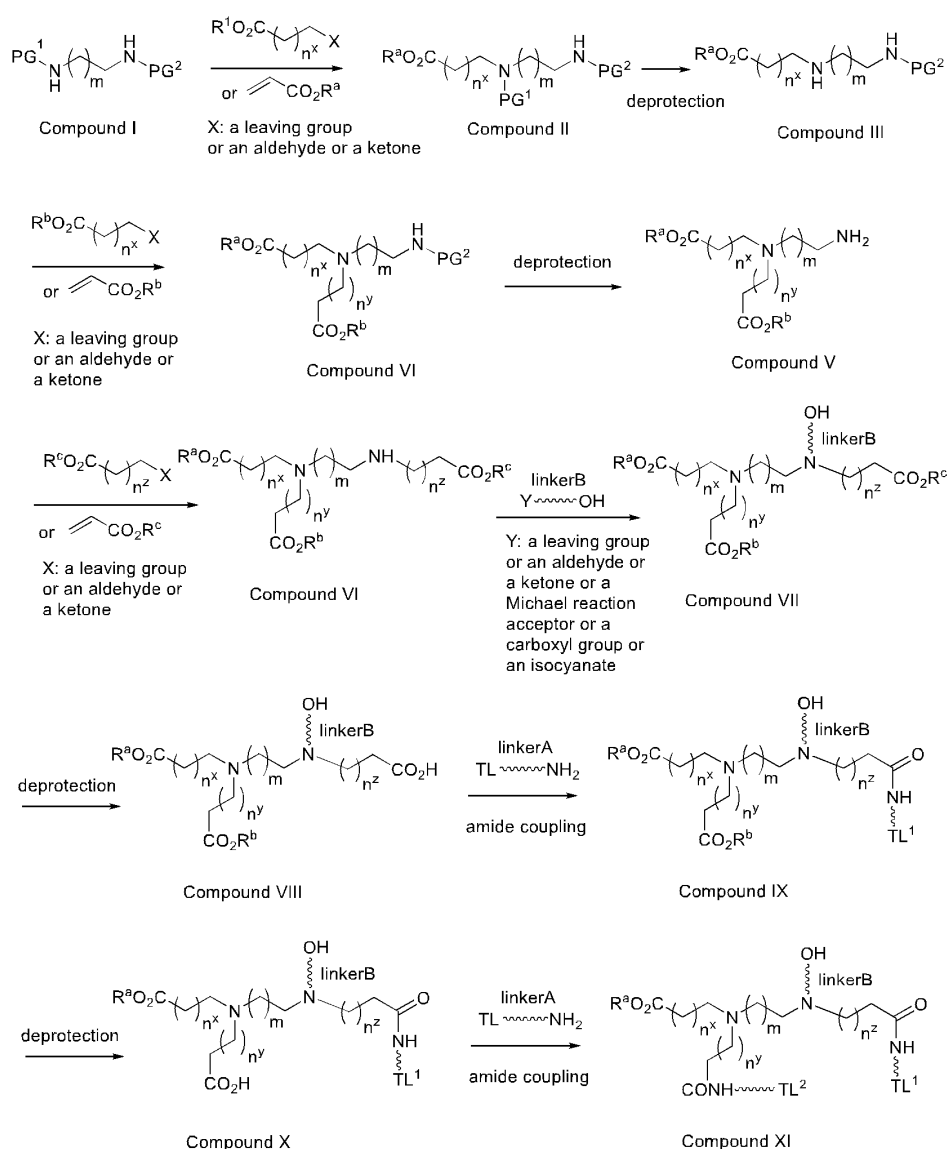
Triacid Compound V is produced by converting the protected carboxylic acids of Compound IV into carboxylic acids. In some embodiments, Compound V may be produced by reacting Compound IV with one or more acids (e.g., when R in Scheme 1 is an acid sensitive group, such as a *tert*-butyl group). In some embodiments, the one or more acids may comprise hydrochloric acid (HCl), hydrobromic acid (HBr), trifluoroacetic acid (TFA), and formic acid. In some embodiments, producing Compound V may comprise performing a hydrogenation reaction using Compound IV (e.g., when R in Scheme 1 is a benzyl group). In some embodiments, producing Compound V may comprise performing a hydrolysis reaction using Compound IV.

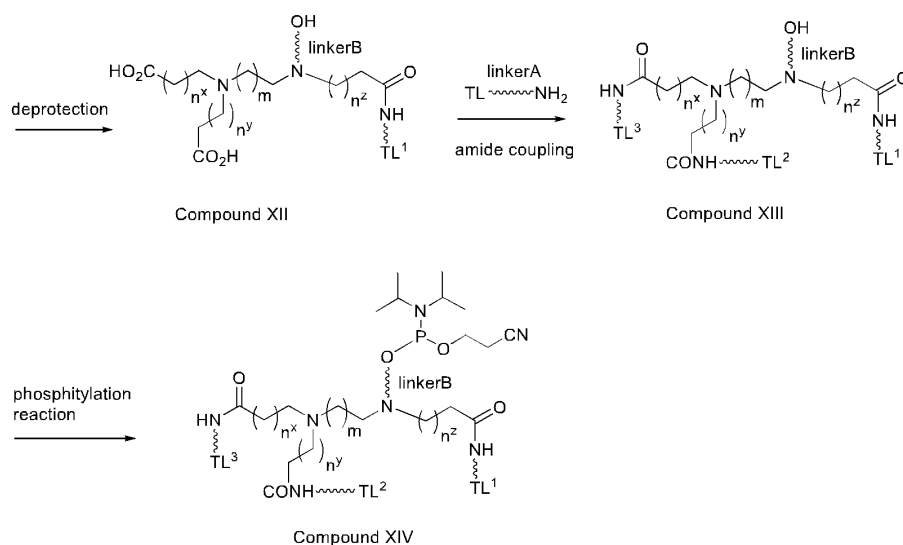
Compound VI may be produced by perform an amide coupling reaction using the Compound V. In Compound VI the first nitrogen is a tertiary amine comprising a first amide and a second amide, and the second nitrogen is a tertiary amine comprising the moiety comprising the hydroxy group and a third amide. The first amide, the second amide, and the third amide may each be coupled to an independently selected targeting ligand. linkerA in Scheme 1 may be any linkerA described herein. TL in Scheme 1 may be any TL described herein.

Compound VII is produced by converting the hydroxy group (attached to linkerB) of Compound VI to a phosphoramidite group using a phosphitylation reaction. As illustrated in Scheme 1, in some embodiments converting the hydroxy group to the phosphoramidite group may be performed after performing the amide coupling reaction to produce Compound VI.

Second Example Method of Preparation

Another method for the preparation of examples of compounds with general Formula 1 is depicted in Scheme 2 below. Scheme 2 allows for a compound with general Formula 1 to have one or more different targeting ligands. Scheme 2 allows for stepwise introduction of targeting ligands. Starting materials and intermediates may be purchased from commercial sources, made from known procedures, or are otherwise illustrated. The order of carrying out the steps of the reaction scheme may be varied.





Scheme 2

Scheme 2 starts with a double-protected diamine (Compound I). The double-protected diamine comprises a first nitrogen and a second nitrogen, where the first nitrogen is a secondary amine comprising a first protecting group (PG¹), and the second nitrogen is a primary or a secondary amine comprising a second protecting group (PG²).

The first protecting group and the second protecting group may be different, thereby allowing different linkerAs and targeting ligands to be attached to the diamine scaffold. Various protecting groups are known to those skilled in the art, and may be used. In some embodiments, the first protecting group may be a benzyl group, and the second protecting group may be a *tert*-butoxycarbonyl (Boc) group.

“m” in Scheme 2 may be any integral number. In some embodiments, m in Scheme 2 may be an integral number between 1 and 10.

Compound II may be produced by coupling a first protected carboxylic acid to the first nitrogen of Compound I, resulting in the first nitrogen of Compound II being a tertiary amine. One skilled in the art will appreciate that, by differentiating the protecting groups in Compound I, the protecting groups may be strategically removed and replaced. For example, in embodiments where the first protecting group is a benzyl group and the second protecting group is a Boc group, the amine with the benzyl group (and not the amine with the Boc group) of Compound I may undergo a S_N2 substitution reaction, a reductive amination reaction, or a Michael addition reaction with one or more appropriate reagents to form Compound II.

Compound III may be produced by removing the first protecting group from Compound II. In Compound III, the first nitrogen is a secondary amine having the first

protected carboxylic acid, and the second nitrogen is a primary or a secondary amine having the second protecting group. In some embodiments, producing Compound III may comprise performing a hydrogenation reaction (e.g., when the first protecting group is a benzyl group).

Compound IV may be produced by coupling a second protected carboxylic acid to the first nitrogen of Compound III, resulting in the first nitrogen of Compound IV being a tertiary amine. In some embodiments, producing Compound IV may comprise performing a S_N2 substitution reaction using Compound III and one or more other appropriate reagents. In some embodiments, producing Compound IV may comprise performing a reductive amination reaction using Compound III and one or more other appropriate reagents. In some
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embodiments, producing Compound IV may comprise performing a Michael addition reaction using Compound III and one or more other appropriate reagents. In some embodiments, producing Compound IV may comprise performing an amide coupling reaction using Compound III and one or more other appropriate reagents. In some embodiments, producing Compound IV may comprise performing a nucleophilic addition reaction using Compound III and one or more other appropriate reagents.

Compound V may be produced by removing the second protecting group from Compound IV, resulting in the first nitrogen of Compound V being a tertiary amine comprising the first protected carboxylic acid and the second protected carboxylic acid, and the second nitrogen of Compound V being a primary amine. In some embodiments (e.g.,
20
when the second protecting group is a Boc group), Compound V may be produced by reacting Compound IV with at least one acid. Example acids include, but are not limited to, hydrochloric acid (HCl) and trifluoroacetic acid (TFA). Compound VI may be produced by coupling a third protected carboxylic acid to the second nitrogen of Compound V, resulting in the second nitrogen of Compound VI being a secondary amine. In some embodiments,
25
Compound VI may be produced by performing a S_N2 substitution reaction using Compound V and one or more other appropriate reagents. In some embodiments, Compound VI may be produced by performing a reductive amination reaction using Compound V and one or more other appropriate reagents. In some embodiments, Compound VI may be produced by performing a Michael addition reaction using Compound V and one or more other
30
appropriate reagents.

Compound VII may be produced by attaching a moiety comprising a hydroxy group to the second nitrogen of Compound VI, resulting in the second nitrogen of Compound VII being a tertiary amine or an amide or a urea. The moiety comprising the hydroxy group may

be attached to the second nitrogen using any linkerB described herein above. In some embodiments, Compound VII may be produced by performing a S_N2 substitution reaction using Compound VI and one or more other appropriate reagents. In some embodiments, Compound VII may be produced by performing a reductive amination reaction using
5 Compound VI and one or more other appropriate reagents. In some embodiments, Compound VII may be produced by performing a Michael addition reaction using Compound VI and one or more other appropriate reagents. In some embodiments, Compound VII may be produced by performing an amide coupling reaction using Compound VI and one or more other appropriate reagents. In some embodiments, Compound VII may be produced by performing
10 a nucleophilic addition reaction using Compound VI and one or more other appropriate reagents.

In the example of Scheme 2, R^a, R^b, and R^c may be sufficiently different such that targeting ligands may be selectively attached, for example in one scenario R^a, R^b, and R^c may be methyl, benzyl and *tert*-butyl groups, respectively. The following describes such selective
15 attachment of targeting ligands.

Compound VIII is produced by converting the third protected carboxylic acid of Compound VII into a first carboxylic acid. In some embodiments, Compound VIII may be produced by reacting Compound VII with one or more acids (e.g., when R^c in Scheme 2 is an acid sensitive group, for example a *tert*-butyl group).

20 Compound IX may be produced by performing an amide coupling reaction using Compound VIII. In Compound IX, the first nitrogen comprises the first protected carboxylic acid and the second protected carboxylic acid, and the second nitrogen of Compound IX comprises a first amide having a first targeting ligand coupled thereto and the moiety comprising the hydroxy group.

25 Compound X is produced by converting the second protected carboxylic acid of Compound IX into a second carboxylic acid. In some embodiments, producing Compound X may comprise performing a hydrogenation reaction using Compound IX (e.g., when R^b in Scheme 2 is a benzyl group).

30 Compound XI may be produced by performing an amide coupling reaction using Compound X. In Compound XI, the first nitrogen comprises the first protected carboxylic acid and a second amide having a second targeting ligand coupled thereto, and the second nitrogen of Compound XI comprises the first amide having the first targeting ligand coupled thereto and the moiety comprising the hydroxy group.

Compound XII is produced by converting the first protected carboxylic acid of Compound XI into a third carboxylic acid. In some embodiments, producing Compound XII may comprise performing a hydrolysis reaction using Compound XI (e.g., when R^a in Scheme 2 is a methyl group).

5 Compound XIII may be produced by performing an amide coupling reaction using Compound XII. In Compound XIII, the first nitrogen comprises the second amide having the second targeting ligand coupled thereto and a third amide having a third targeting ligand coupled thereto, and the second nitrogen of Compound XI comprises a first amide having a first targeting ligand coupled thereto and the moiety comprising the hydroxy group.

10 The first amide may be coupled to the first targeting ligand using any independently selected linkerA described herein. The second amide may be coupled to the second targeting ligand using any independently selected linkerA described herein. The third amide may be coupled to the third targeting ligand using any independently selected linkerA described herein.

15 One or more of the first targeting ligand, the second targeting ligand, and the third targeting ligand may be independently selected to be one or more of the targeting ligands described herein.

 The hydroxy group may be coupled to the second nitrogen using any linkerB described herein.

20 In some embodiments, the hydroxy group may be converted to a phosphoramidite group using a phosphorylation reaction. In some embodiments, the hydroxy group may be converted to the phosphoramidite group producing Compound XIV.

 Each “n^x”, “n^y” or “n^z” in Scheme 2 is an independently selected integral number. In some embodiments, each “n^x”, “n^y” or “n^z” in Scheme 2 is an independently selected
25 integral number between 0 and 10.

Certain Elements of Preparation and Use

Embodiments of multivalent ligand clusters of the invention can be prepared and used to deliver oligonucleotide agents to cells, tissues, and organs. Non-limiting examples of
30 agents that can be delivered include therapeutic agents such as siRNA. Delivery methods using multivalent ligand clusters of the invention can be used to deliver siRNAs and other agents conjugated to a target ligand cluster of the invention to *in vitro* and *in vivo* cells. Multivalent ligand clusters of the invention can be used as a delivery vehicle with which to

deliver agents, such as but not limited to agents comprising nucleic acids, to a cell. As used herein, the term “multivalent ligand cluster/pharmaceutical agent complex” means a multivalent ligand cluster as described herein that is linked to a pharmaceutical agent as described herein. In some embodiments of the invention, the pharmaceutical agent is an siRNA.

Another aspect of the present disclosure, the dsRNA agent comprises 2'-fluoro modified nucleotides at position 2, 7, 12, 14 and 16 of the antisense strand (counting from the first paired nucleotide from the 5' end of the antisense strand), and/or 2'-fluorine-modified nucleotides at position 9, 11 and 13 of the sense strand (counting from the first paired nucleotide from the 3' end of the sense strand). In some embodiments, no other positions of the dsRNA agent contain 2' fluorine-modified nucleotides. In some embodiments, all nucleotides of the antisense strand and /or sense strand of the dsRNA agent are modified nucleotides. In some embodiments, the dsRNA agent has 2'-fluoro modified nucleotides at position 2, 7, 12, 14 and 16 of the antisense strand and/or 2' fluorine-modified nucleotide at positions 9, 11 and 13 of the sense strand, with other positions containing modified nucleotides selected from: 2'-O-methyl nucleotide, 2'-deoxy nucleotide, 2'3'-seco nucleotide mimic, locked nucleotide, unlocked nucleic acid nucleotide (UNA), glycol nucleic acid nucleotide (GNA), 2'-F-Arabino nucleotide, 2'-methoxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted abasic nucleotide, inverted 2'-Ome nucleotide, inverted 2'-deoxy nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, and 3'-OMe nucleotide, a nucleotide including a 5'-phosphorothioate group, or a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a 2'-amino-modified nucleotide, a phosphoramidate, or a non-natural base containing nucleotide. In some embodiments, the dsRNA agent includes an E-vinylphosphonate nucleotide at the 5' end of the guide strand. In certain embodiments, the dsRNA agent includes at least one phosphorothioate internucleoside linkage. In certain embodiments, the sense strand includes at least one phosphorothioate internucleoside linkage. In some embodiments, the antisense strand includes at least one phosphorothioate internucleoside linkage. In some embodiments, the sense strand includes 1, 2, 3, 4, 5, or 6, phosphorothioate internucleoside linkages. In some embodiments, the antisense strand includes 1, 2, 3, 4, 5, or 6, phosphorothioate internucleoside linkages. In certain embodiments, the sense strand is complementary or substantially complementary to the antisense strand, and the region of complementarity is between 16 and 23 nucleotides in length. In some embodiments, the

region of complementarity is 19-21 nucleotides in length. In certain embodiments, the region of complementarity is 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. In some embodiments, each strand is no more than 30 nucleotides in length. In some embodiments, each strand is no more than 25 nucleotides in length. In some
5 embodiments, each strand is no more than 23 nucleotides in length. In certain embodiments, the dsRNA agent includes at least one modified nucleotide and further includes one or more targeting groups or linking groups. In some embodiments, the one or more targeting groups or linking groups are conjugated to the sense strand. In some embodiments, the targeting group comprises N-acetyl-galactosamine (GalNAc). In some embodiments, the targeting
10 group contains a structure of GalNAc described above.

In some aspects of the invention, a multivalent ligand cluster may be used to deliver a pharmaceutical agent to a cell in a subject. Means of administering a multivalent ligand cluster/pharmaceutical agent complex to a subject may include art-known methods. As a non-
15 limiting example, a multivalent ligand cluster/pharmaceutical agent complex may be locally delivered *in vivo* by direct injection or by use of an infusion pump. In some aspects of the invention, a multivalent ligand cluster/pharmaceutical agent complex is in a pharmaceutical composition and may be referred to as a pharmaceutical agent. In some embodiments, a pharmaceutical agent of the invention is administered to a subject in an amount effective to
20 prevent, modulate the occurrence, treat, or alleviate a symptom of a disease state in the subject.

Cells and Subjects

As used herein, a subject shall mean a human or vertebrate mammal including but not
25 limited to a dog, cat, horse, goat, cow, sheep, rodent, and primate, e.g., monkey. Thus, the invention can be used to treat diseases or conditions in human and non-human subjects. For instance, methods and compositions of the invention can be used in veterinary applications as well as in human prevention and treatment regimens. In some embodiments of the invention, a vertebrate subject is a mammal.

30 In certain embodiments of the invention, a multivalent ligand cluster/pharmaceutical agent complex of the invention is delivered to and contacted with a cell. In some embodiments of the invention, a contacted cell is in culture, and in other embodiments a contacted cell is in a subject. Types of cells that may be contacted with a multivalent ligand

cluster/pharmaceutical agent complex of the invention include, but are not limited to, liver cells, muscle cells, cardiac cells, circulatory cells, neuronal cells, glial cells, fat cells, skin cells, hematopoietic cells, epithelial cells, sperm, oocytes, muscle cells, adipocytes, kidney cells, hepatocytes, or pancreas cells. In some embodiments, the cell contacted with a
5 multivalent ligand cluster/pharmaceutical agent complex of the invention is a liver cell.

Dosage

Dosage levels for the medicament and pharmaceutical compositions that may be delivered using a multivalent ligand cluster/pharmaceutical agent complex of the present
10 disclosure can be determined by those skilled in the art by routine experimentation. In at least some embodiments, a unit dose may contain between about 0.01 mg/kg and about 100 mg/kg body weight of siRNA. Alternatively, the dose can be from 10 mg/kg to 25 mg/kg body weight, or 1 mg/kg to 10 mg/kg body weight, or 0.05 mg/kg to 5 mg/kg body weight, or 0.1 mg/kg to 5 mg/kg body weight, or 0.1 mg/kg to 1 mg/kg body weight, or 0.1 mg/kg to 0.5
15 mg/kg body weight, or 0.5 mg/kg to 1 mg/kg body weight, or 1 mg/kg to 3 mg/kg body weight.

The pharmaceutical composition may be a sterile injectable aqueous suspension or solution, or in a lyophilized form. The pharmaceutical compositions and medicaments of the present disclosure may be administered to a subject in a pharmaceutically effective dose.
20

Administration Methods

A variety of administration routes for a multivalent ligand cluster/pharmaceutical agent complex of the invention are available. The particular delivery mode selected will depend upon the particular condition being treated and the dosage required for therapeutic
25 efficacy. Methods of this invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of treatment without causing clinically unacceptable adverse effects. In some embodiments of the invention, a multivalent ligand cluster/pharmaceutical agent complex of the invention may be administered via an oral, enteral, mucosal, percutaneous, and/or parenteral route. The
30 term “parenteral” includes subcutaneous, intravenous, intramuscular, intraperitoneal, and intracisternal injection, or infusion techniques. Other routes include but are not limited to nasal (e.g., via a gastro-nasal tube), dermal, vaginal, rectal, and sublingual. Delivery routes of the invention may include intrathecal, intraventricular, or intracranial. In some embodiments

of the invention, a multivalent ligand cluster/pharmaceutical agent complex of the invention may be placed within a slow release matrix and administered by placement of the matrix in the subject.

A multivalent ligand cluster/pharmaceutical agent complex of the invention may be administered in formulations, which may be administered in pharmaceutically acceptable solutions, which may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, adjuvants, and optionally other therapeutic ingredients. According to methods of the invention, the multivalent ligand cluster/pharmaceutical agent complex may be administered in a pharmaceutical composition.

10 In general, a pharmaceutical composition comprises the multivalent ligand cluster/pharmaceutical agent complex of the invention and a pharmaceutically-acceptable carrier. Pharmaceutically acceptable carriers are well known to the skilled artisan and may be selected and utilized using routine methods. As used herein, a pharmaceutically-acceptable carrier means a non-toxic material that does not interfere with the effectiveness of the

15 biological activity of the active ingredients (e.g., the ability of the delivered nucleic acid, for example the siRNA to prevent and/or treat a disease or condition to which it is directed).

Pharmaceutically acceptable carriers may include diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials that are well-known in the art. Illustrative pharmaceutically acceptable carriers are described in U.S. Pat. No. 5,211,657 and others are

20 known by those skilled in the art. Such preparations may routinely contain salt, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and

25 pharmaceutically-acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

30 In some embodiments of the invention, a multivalent ligand cluster/pharmaceutical agent complex of the invention may be administered directly to a tissue. Direct tissue administration may be achieved by direct injection, or other art-known means. A multivalent ligand cluster/pharmaceutical agent complex of the invention may be administered once, or

alternatively may be administered in a plurality of administrations. If administered multiple times, a multivalent ligand cluster/pharmaceutical agent complex of the invention may be administered via different routes. For example, the first (or the first few) administrations may be made directly into an affected tissue or organ while later administrations may be systemic.

5 A multivalent ligand cluster/pharmaceutical agent complex of the invention, when it is desirable to have it administered systemically, may be formulated for parenteral administration by injection (e.g., by bolus injection or continuous infusion). Formulations for injection may be presented in unit dosage form (e.g., in ampoules or in multi-dose containers), with or without an added preservative. The pharmaceutical compositions may take such
10 forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing, and/or dispersing agents.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such
15 as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions, or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives
20 may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Lower doses will result from other forms of administration, such as intravenous administration. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. Multiple doses
25 per day may be used as needed to achieve appropriate systemic or local levels of one or more multivalent ligand cluster/pharmaceutical agent complexes of the invention, to result in a desired level of the pharmaceutical agent, for example a desired level of the siRNA.

Both non-biodegradable and biodegradable polymeric matrices can be used to deliver one or more multivalent ligand cluster/pharmaceutical agent complexes of the invention to a
30 cell and/or subject. In some embodiments, a matrix may be biodegradable. Matrix polymers may be natural or synthetic polymers. A polymer can be selected based on the period of time over which release is desired, generally in the order of a few hours to a year or longer. Typically, release over a period ranging from between a few hours and three to twelve

months can be used. The polymer optionally is in the form of a hydrogel that can absorb up to about 90% of its weight in water and further, optionally is cross-linked with multivalent ions or other polymers.

In certain embodiments of the invention, a multivalent ligand cluster/pharmaceutical agent complex of the invention may be delivered using the bioerodible implant by way of diffusion, or by degradation of the polymeric matrix. Illustrative synthetic polymers for such use are well known in the art. Biodegradable polymers and non-biodegradable polymers can be used for delivery of one or more of a multivalent ligand cluster/pharmaceutical agent complex of the invention using art-known methods. Such methods may also be used to deliver one or more multivalent ligand cluster/pharmaceutical agent complexes of the invention for treatment. Additional suitable delivery systems can include time-release, delayed release or sustained-release delivery systems. Such systems can avoid repeated administrations of a multivalent ligand cluster/pharmaceutical agent complex of the invention, increasing convenience to the subject and the health-care provider. Many types of release delivery systems are available and known to those of ordinary skill in the art. [See for example: U.S. Pat. Nos. 5,075,109; 4,452,775; 4,675,189; 5,736,152; 3,854,480; 5,133,974; and 5,407,686 (the teachings of each of which are incorporated herein by reference)]. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

Use of a long-term sustained release implant may be particularly suitable for prophylactic treatment of subjects and for subjects at risk of developing a recurrent disease or condition to be prevented and/or treated with an siRNA delivered using a multivalent ligand cluster of the invention. Long-term release, as used herein, means that the implant is constructed and arranged to delivery therapeutic levels of the active ingredient for at least 30 days, 60 days, 90 days, or longer. Long-term sustained release implants are well-known to those of ordinary skill in the art and include some of the release systems described above.

Therapeutic formulations of one or more multivalent ligand cluster/pharmaceutical agent complexes of the invention may be prepared for storage by mixing the multivalent ligand cluster/pharmaceutical agent complex having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers [Remington's Pharmaceutical Sciences 21st edition, (2006)], in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as

phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN[®], PLURONICS[®] or polyethylene glycol (PEG).

The multivalent ligand cluster/pharmaceutical agent complexes of the present disclosure may be formulated as pharmaceutical compositions. The pharmaceutical compositions may be used as medicaments, alone or in combination with other agents. The multivalent ligand cluster/pharmaceutical agent complex of the present disclosure can also be administered in combination with other therapeutic compounds, either administrated separately or simultaneously (e.g., as a combined unit dose). In at least some embodiments, the present disclosure includes a pharmaceutical composition comprising one or more multivalent ligand cluster/pharmaceutical agent complex according to the present disclosure in a physiologically/pharmaceutically acceptable excipient, such as a stabilizer, preservative, diluent, buffer, and the like.

A pharmaceutical composition of the invention may be administered alone, in combination with each other, and/or in combination with other drug therapies, or other treatment regimens that are administered to subjects with a disease or condition. Pharmaceutical compositions used in the embodiments of the invention preferably are sterile and contain an effective amount of a multivalent ligand cluster/pharmaceutical agent complex to prevent or treat a disease or condition, to which the pharmaceutical agent, for example a siRNA, is directed.

The dose or doses of a pharmaceutical composition of the invention that are sufficient to treat a disease or condition when administered to a subject can be chosen in accordance with different parameters, in particular in accordance with the mode of administration used and the state of the subject. Other factors may include the desired period of treatment. In the

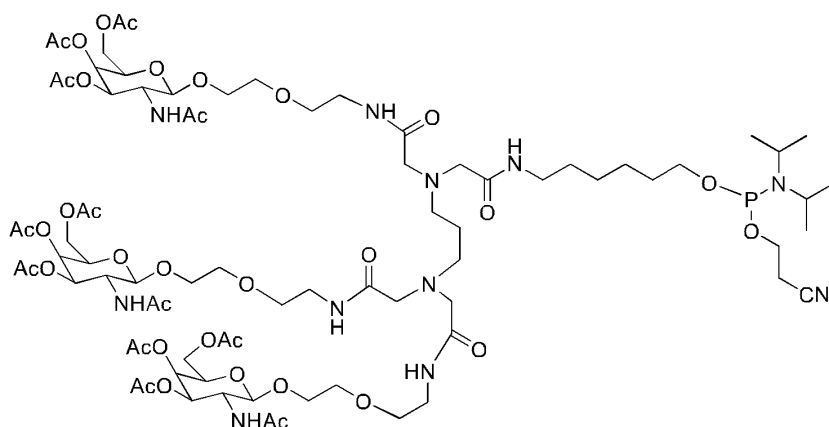
event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. In some embodiments of the invention, dosing is used that has been determined using routine means such as in clinical trials.

5

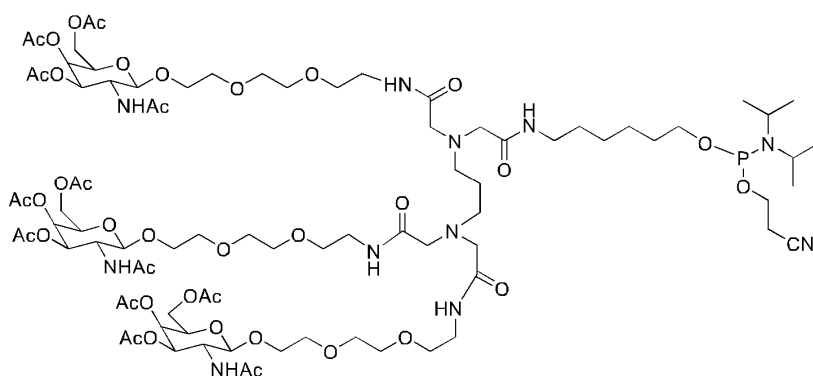
Examples

Example 1. Multivalent Ligand Clusters Comprising GalNAc Targeting Ligands

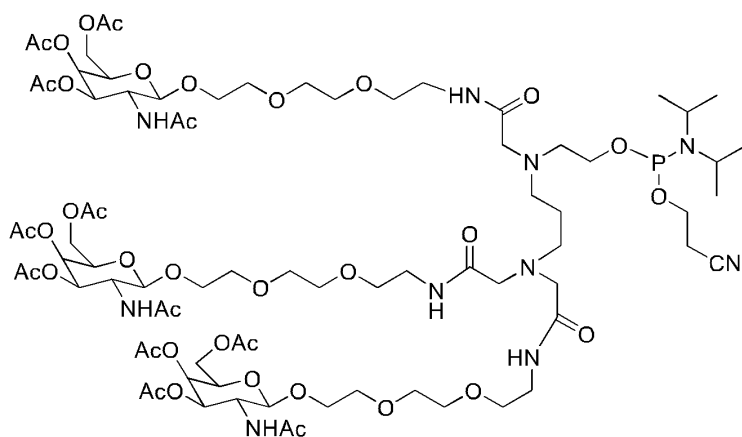
In some embodiments of the invention, a multivalent ligand cluster may comprise GalNAc targeting ligands. The following are example compounds of multivalent ligand clusters comprising acetyl group protected GalNAc targeting ligands, core C2 and C3 diamines, branching acetyl and propanoyl amides, PEG2 and PEG3 linkerAs, various linkerBs as described herein, and various functional groups capable of linking to one or more pharmaceutical agents, as described herein. The acetyl protecting group on the below GalNAc ligands can be readily removed after conjugation is completed to generate GalNAc targeting ligands.



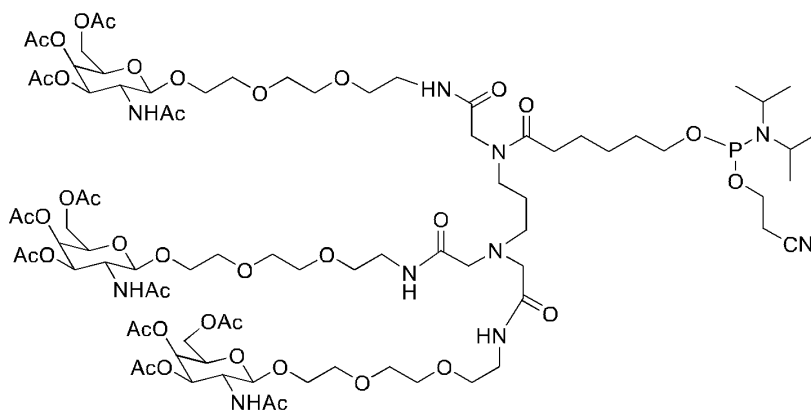
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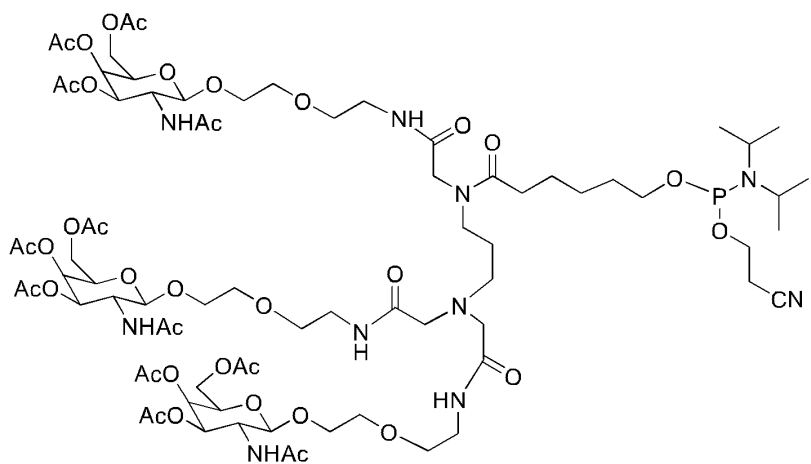
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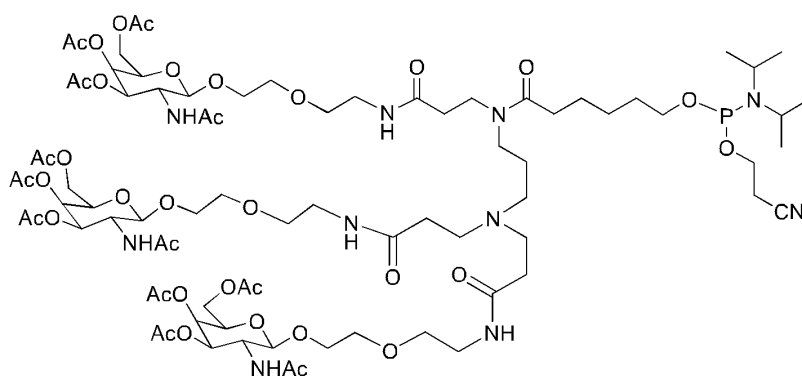
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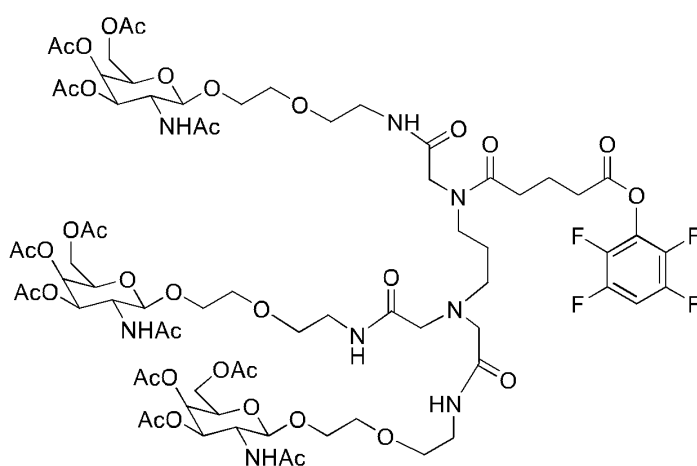
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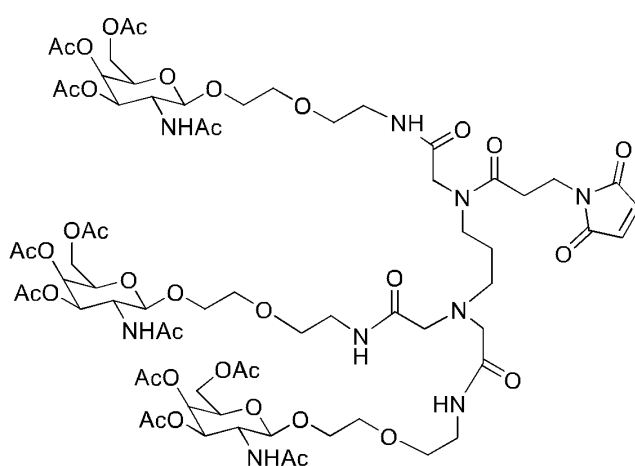
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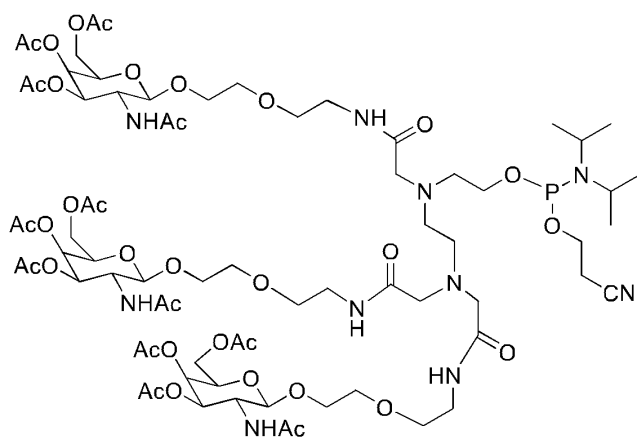
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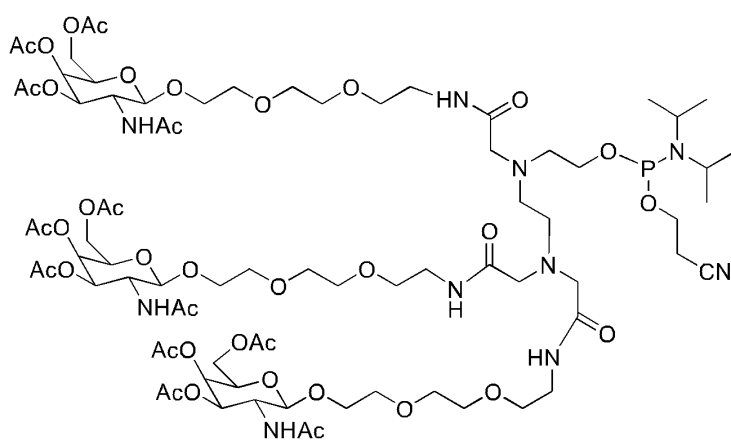
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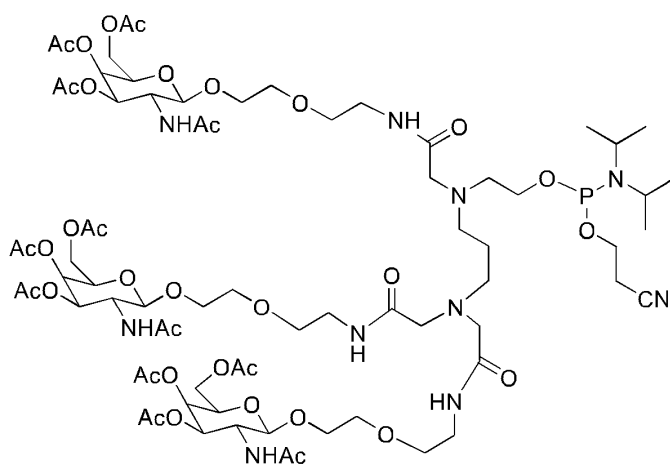
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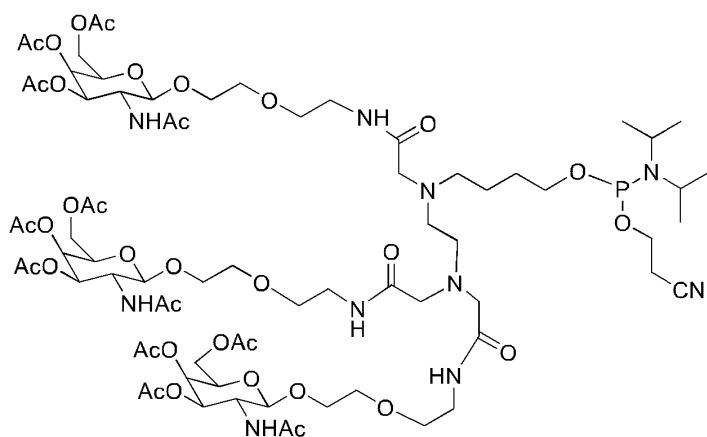
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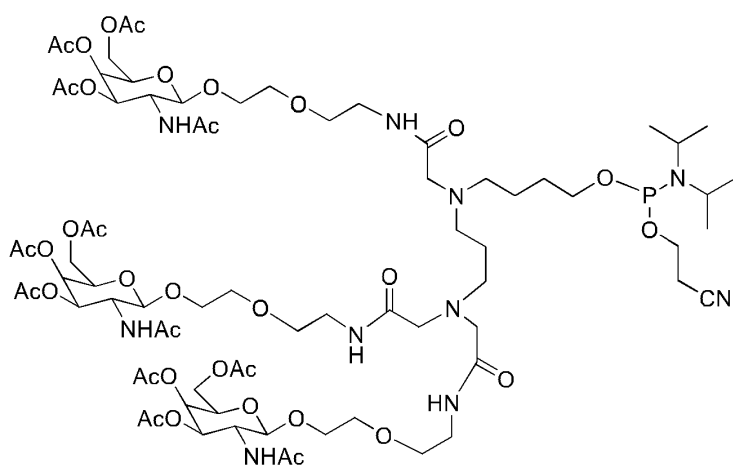
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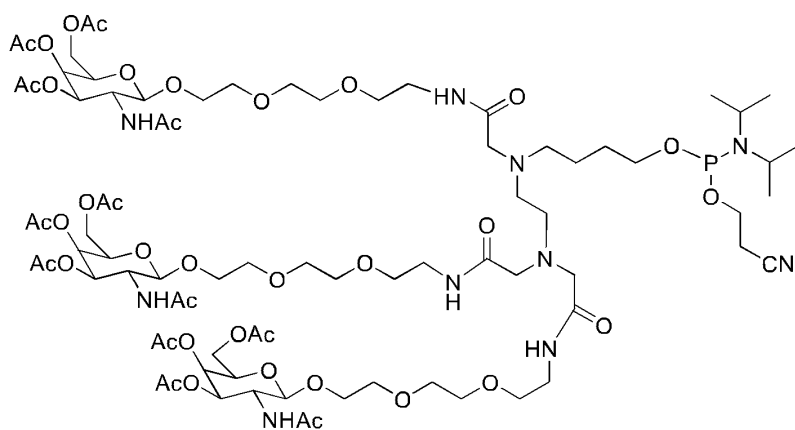
Compound



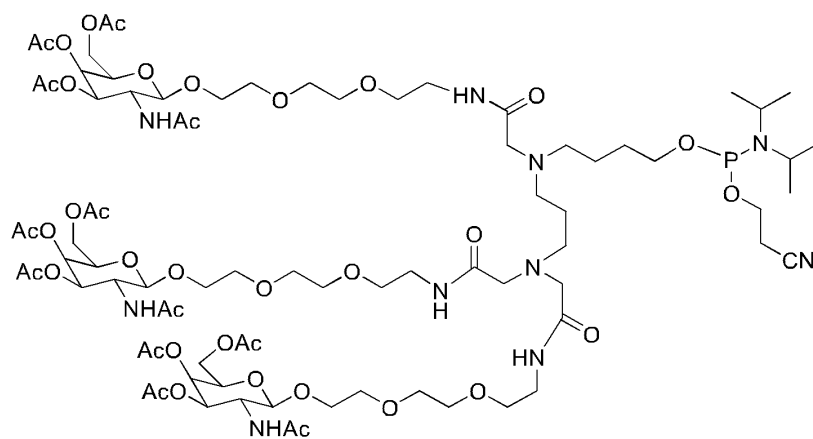
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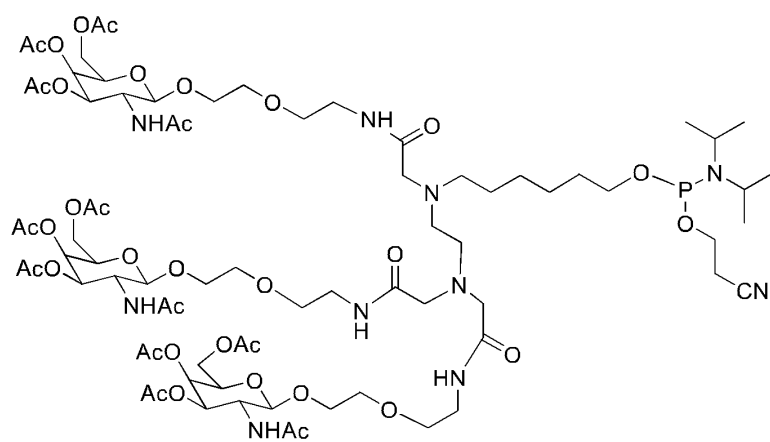
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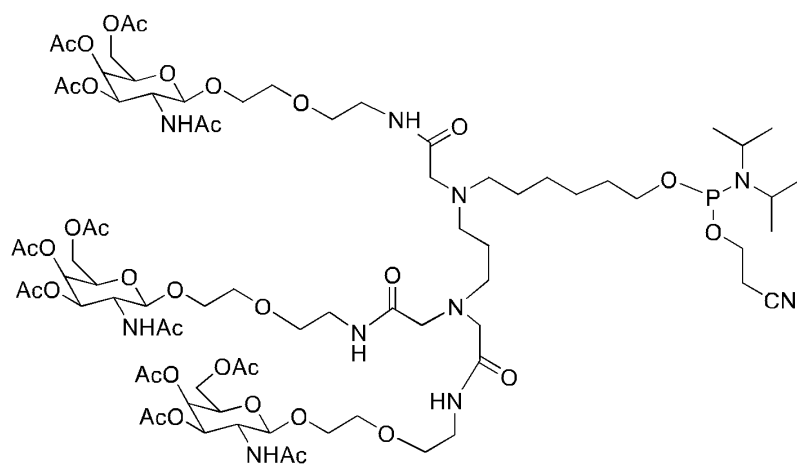
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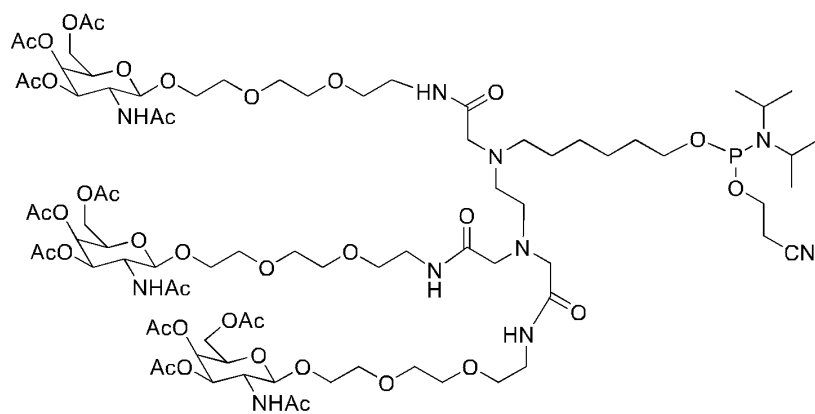
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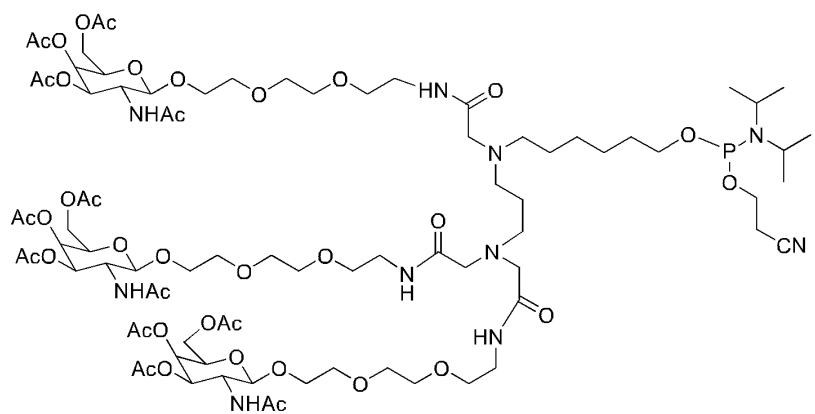
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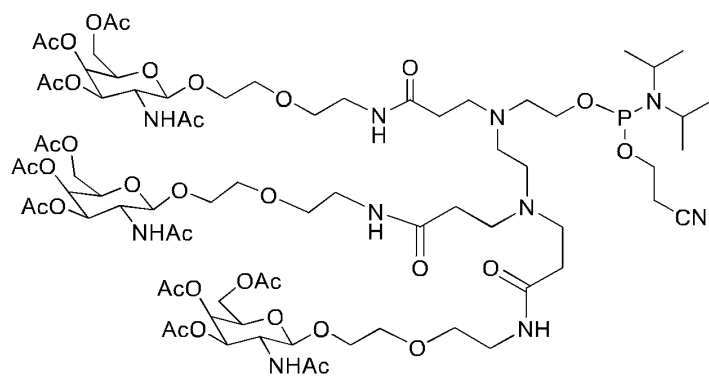
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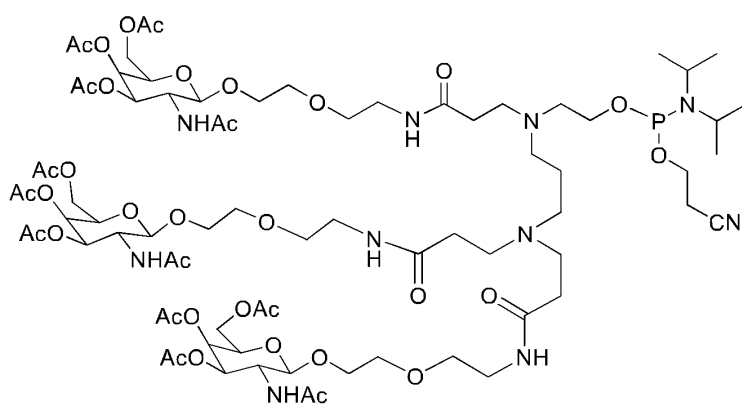
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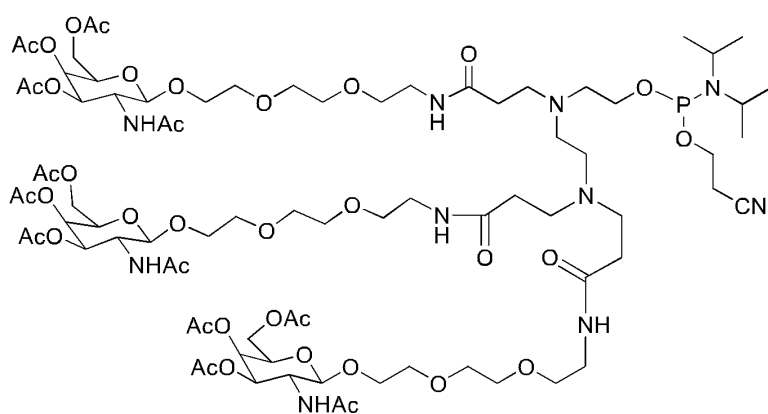
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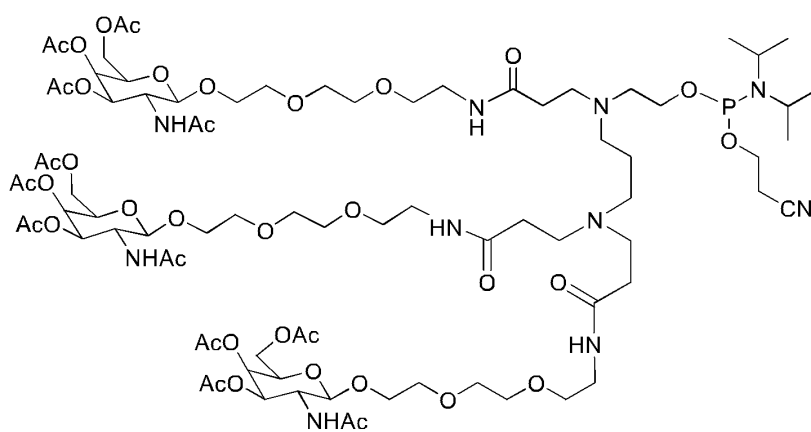
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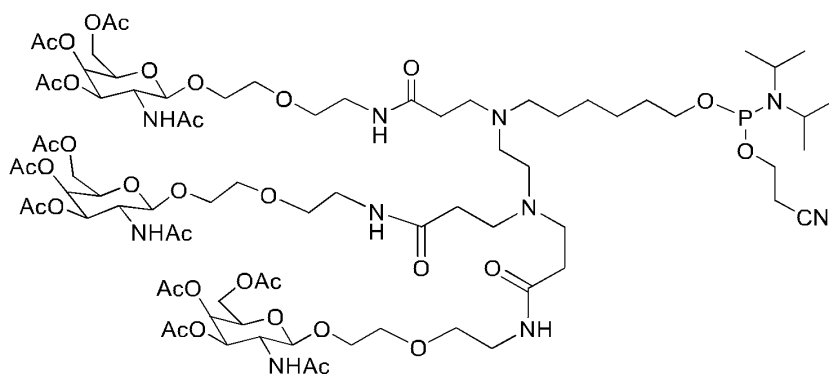
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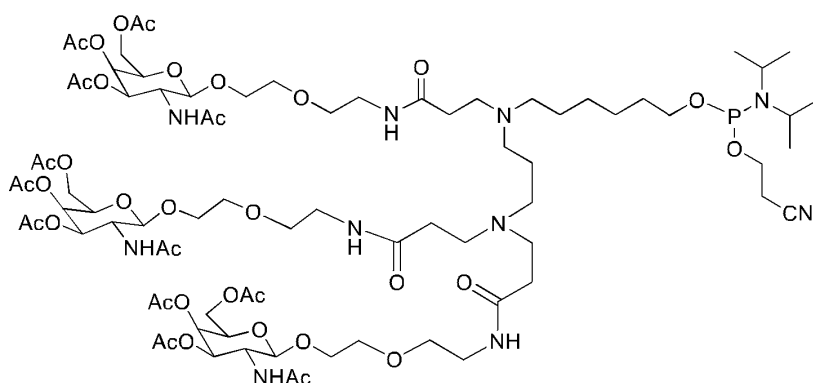
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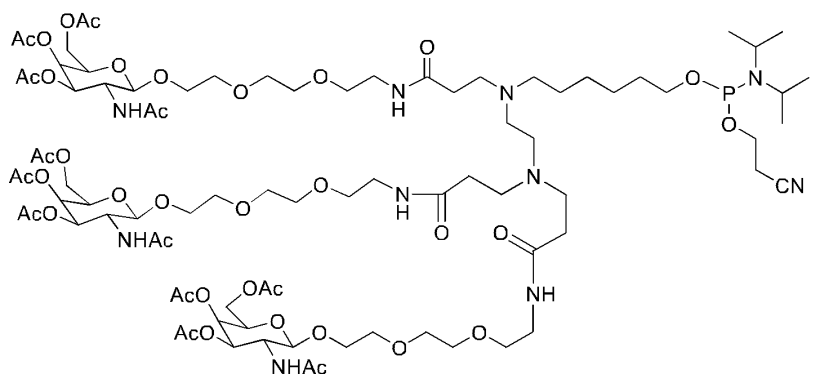
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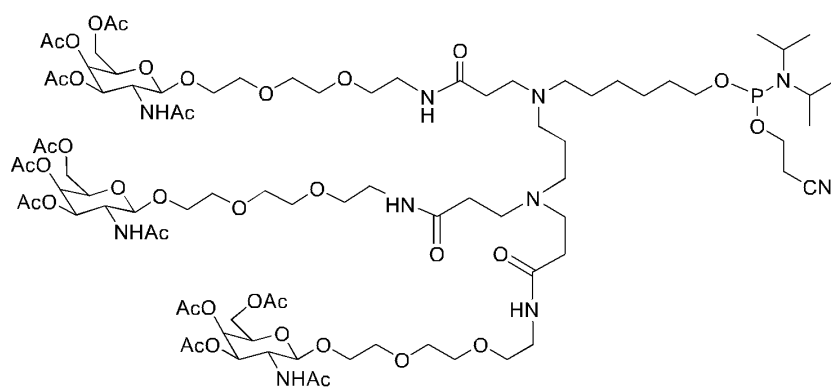
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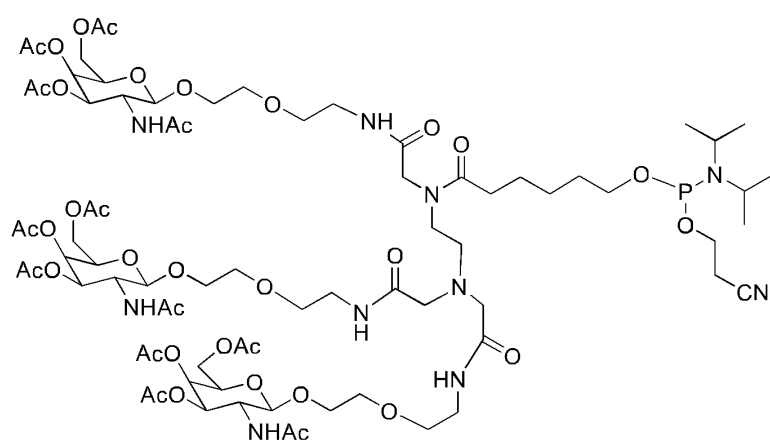
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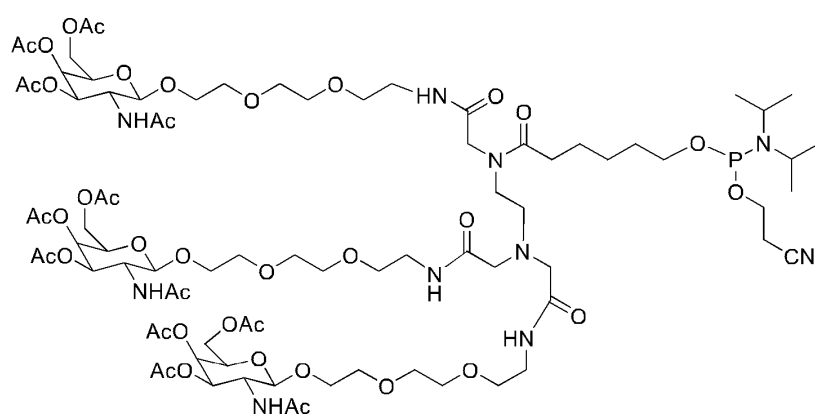
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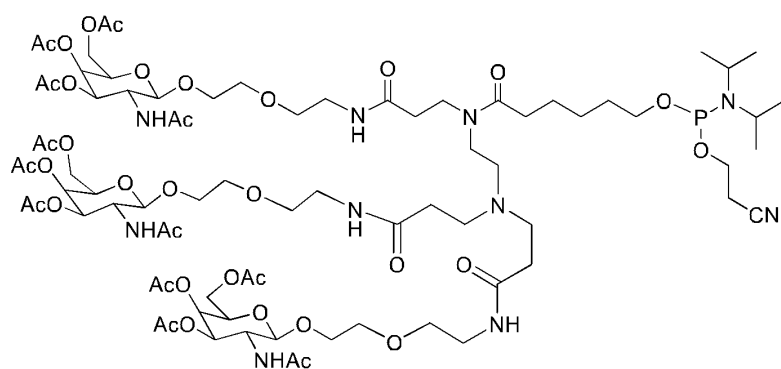
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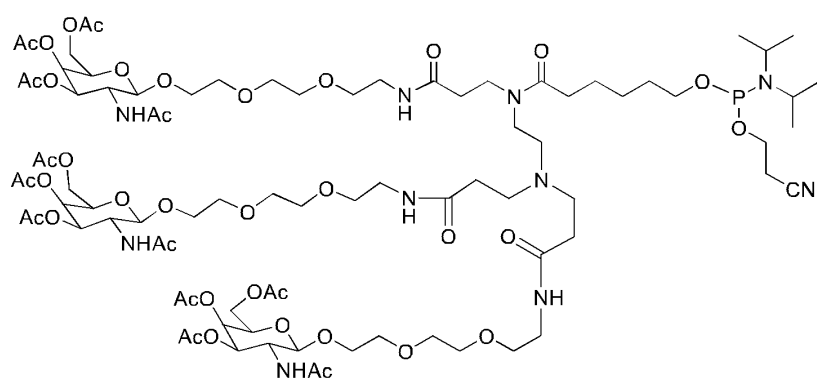
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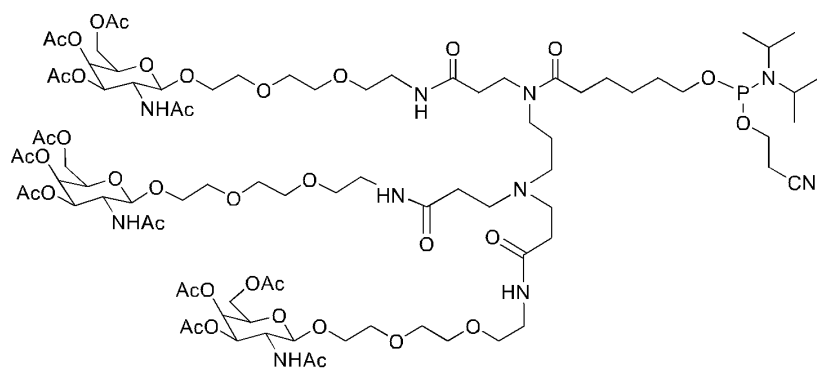
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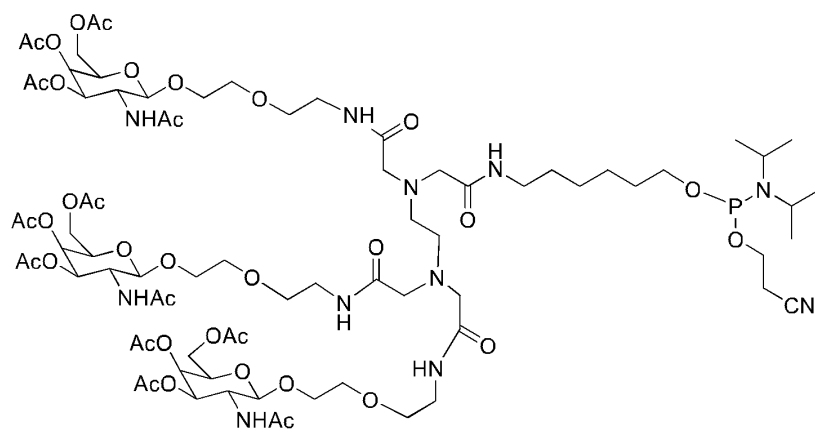
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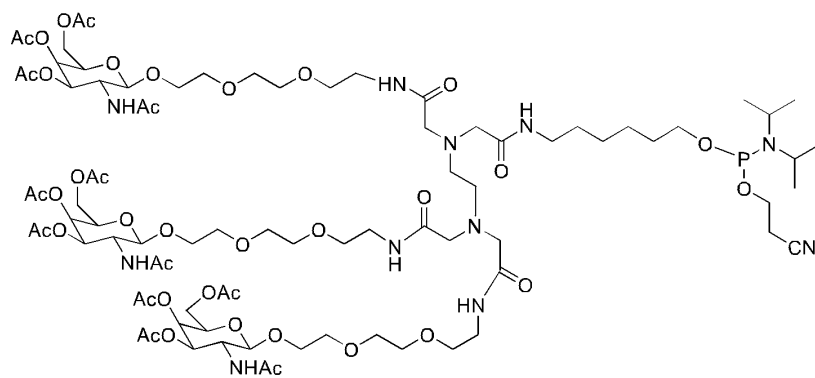
Compound 31



Compound 32

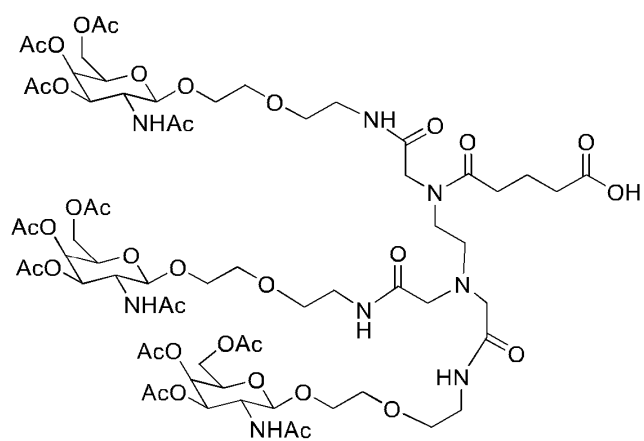


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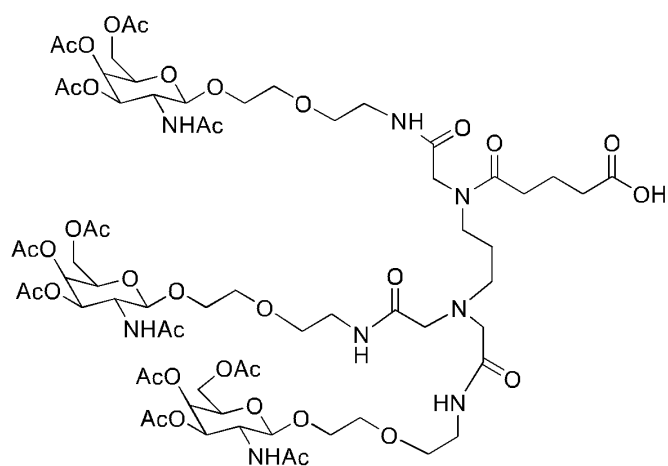


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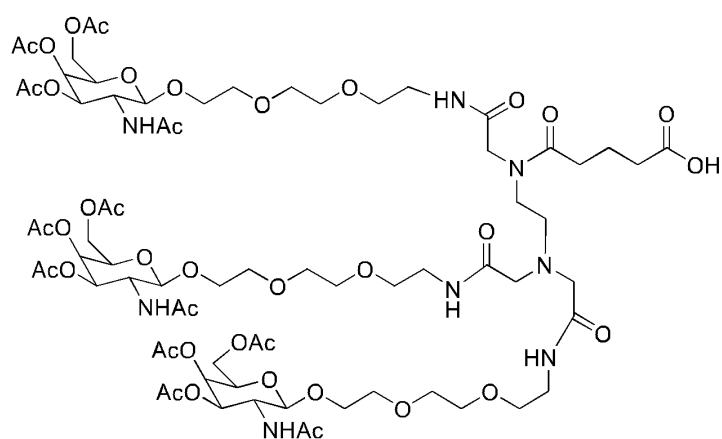
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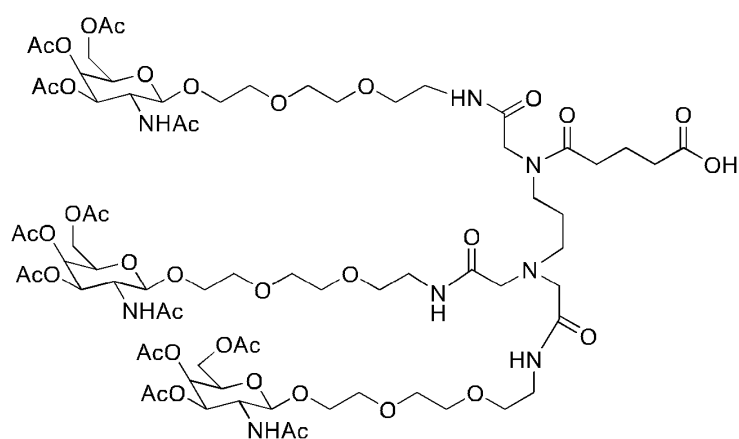
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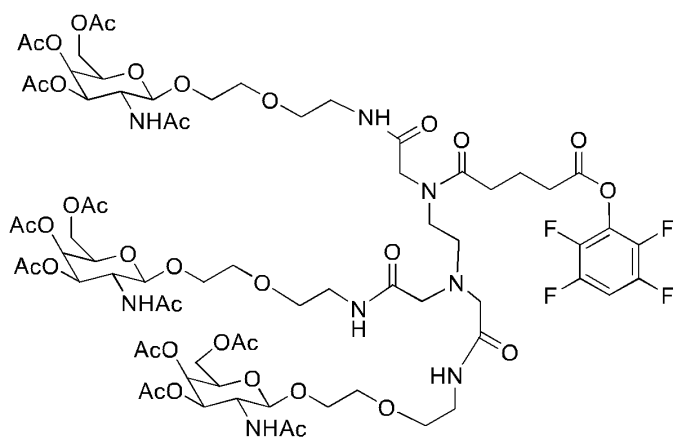
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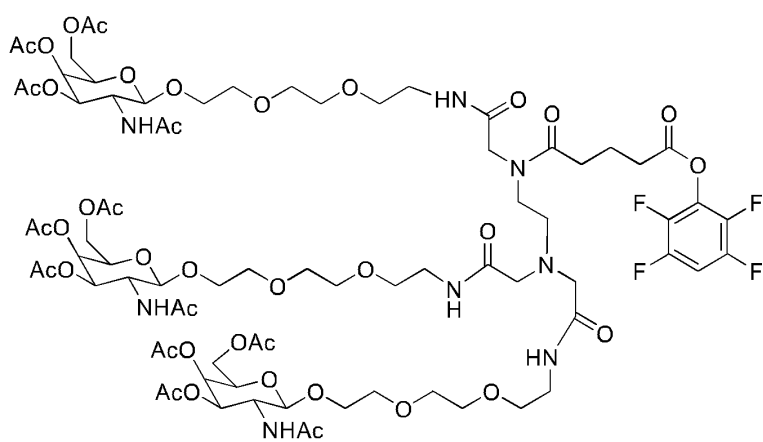
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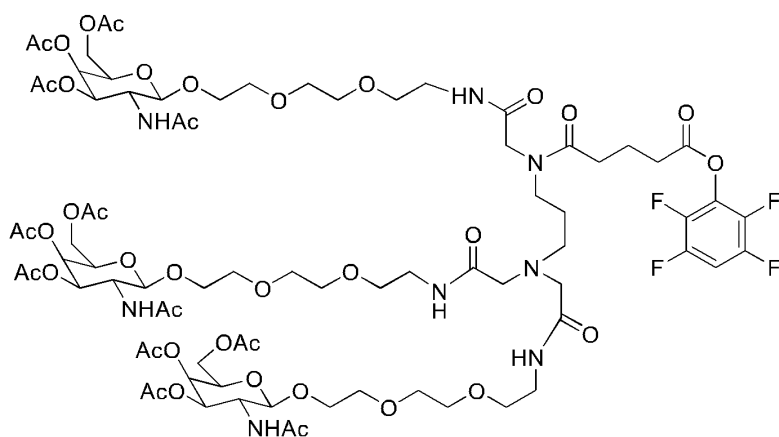
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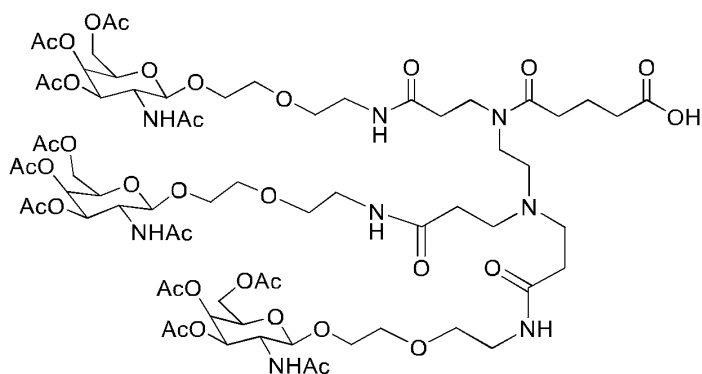
Compound 39



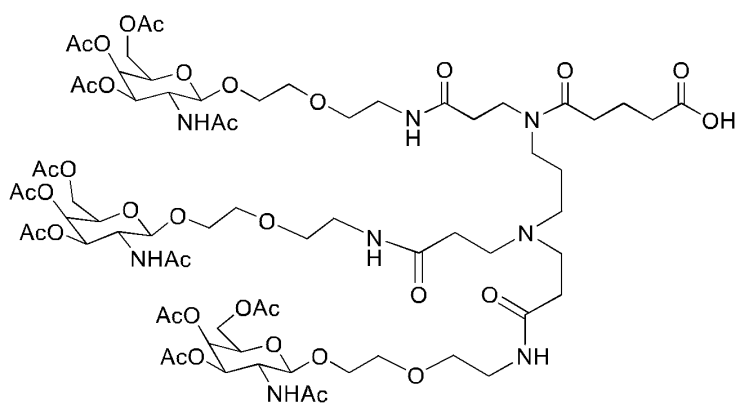
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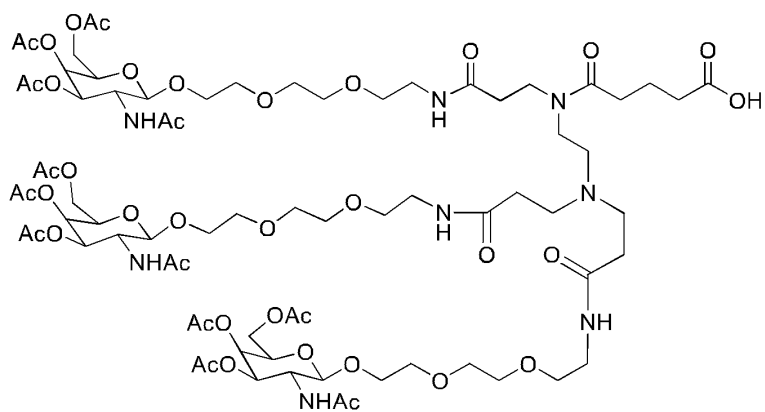
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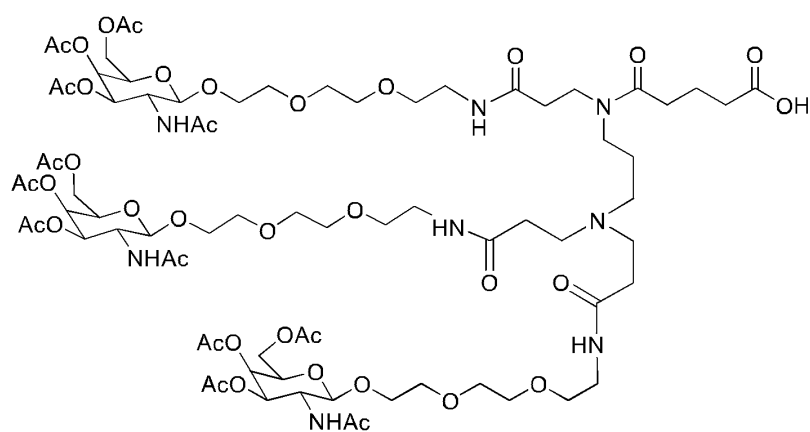
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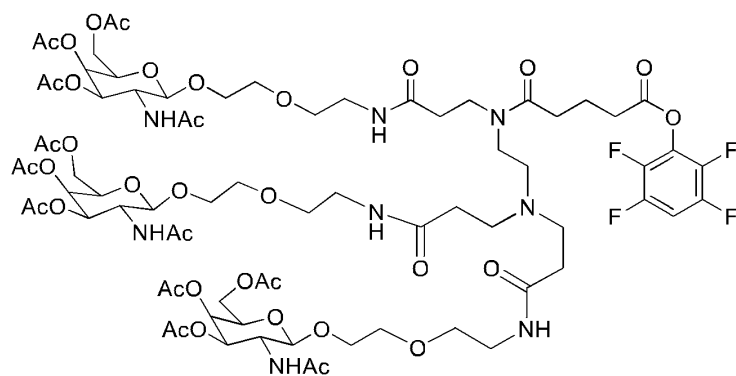
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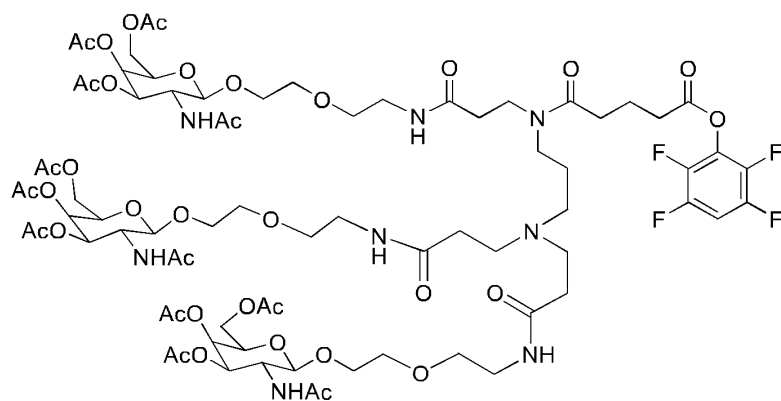
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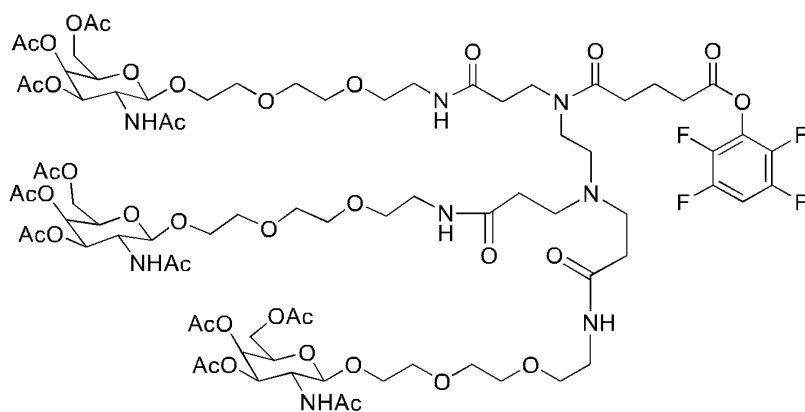
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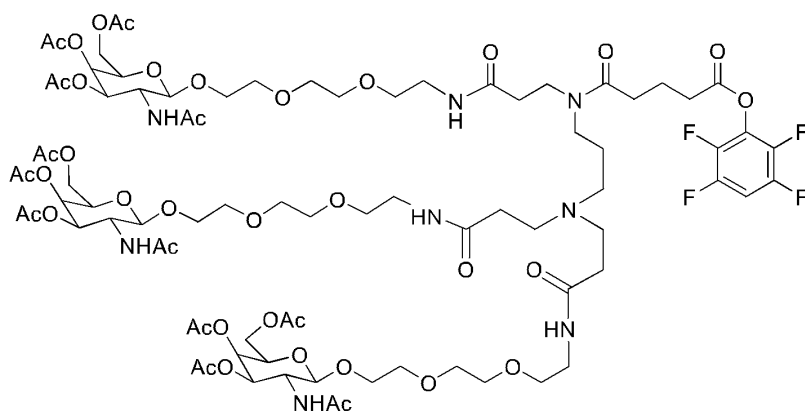
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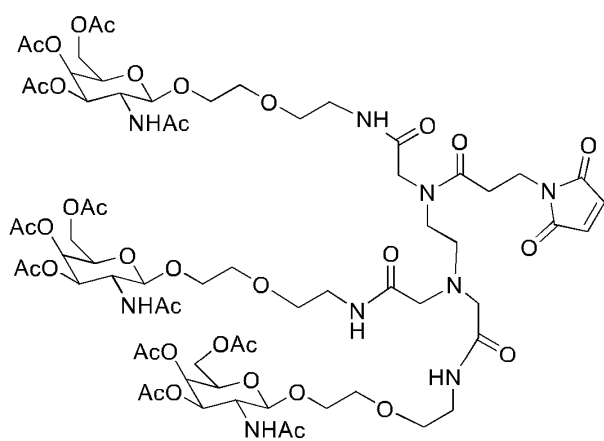
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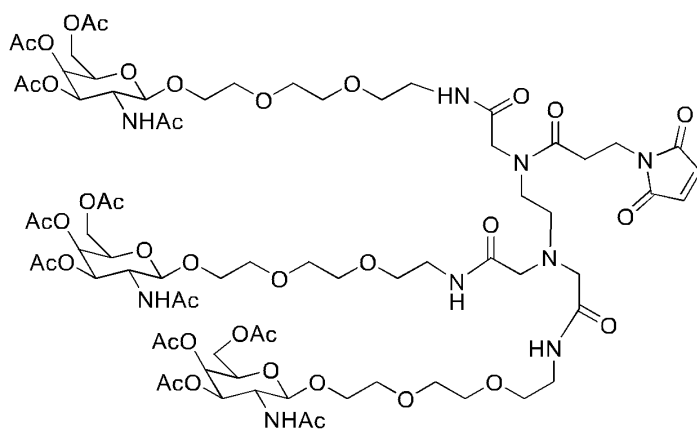
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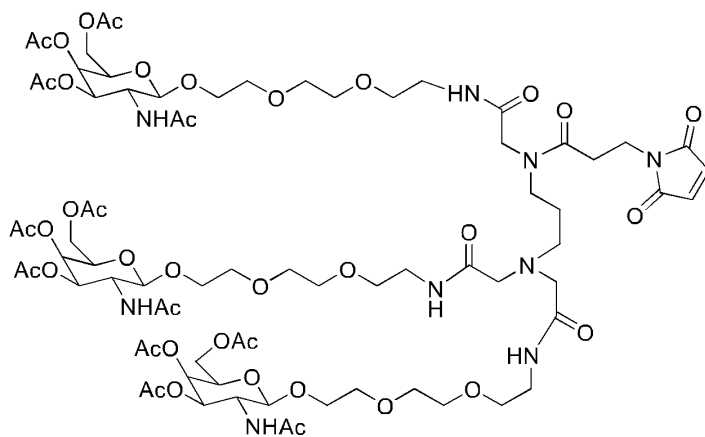
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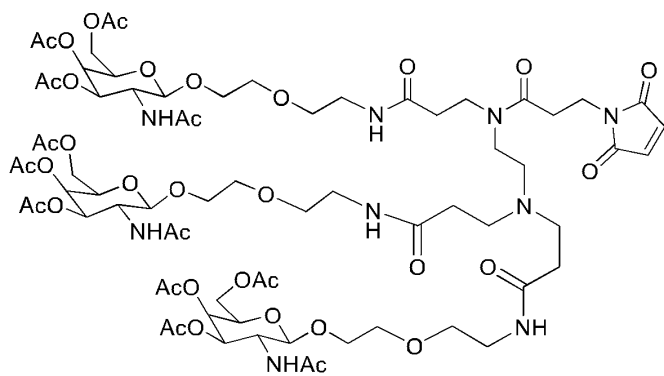
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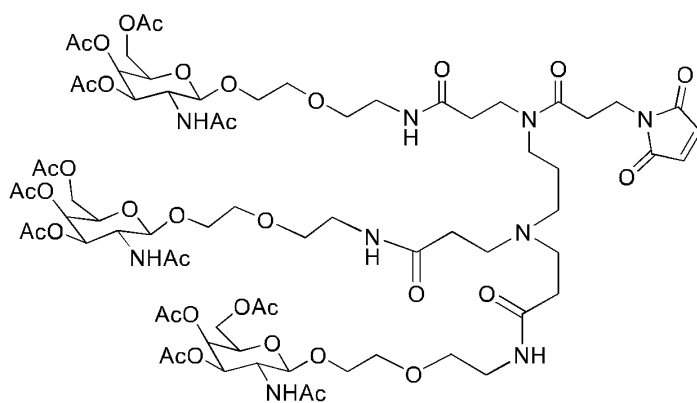
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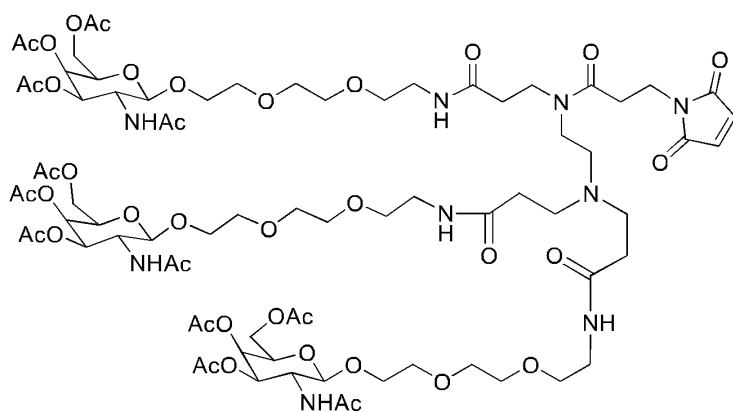
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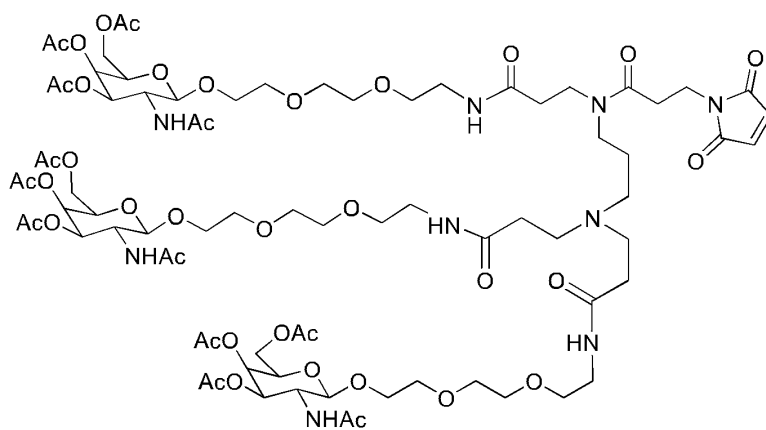
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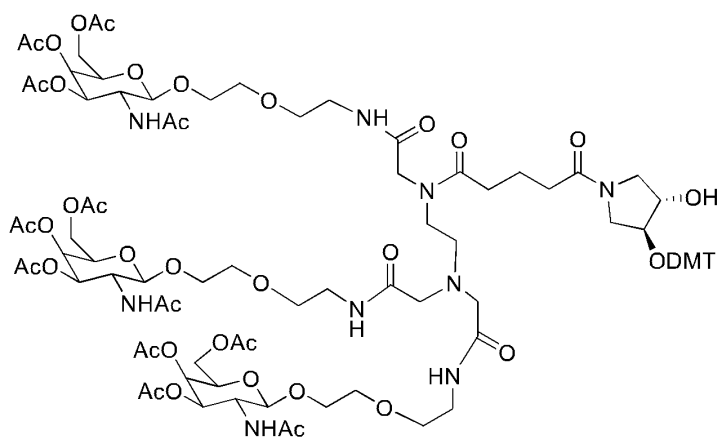
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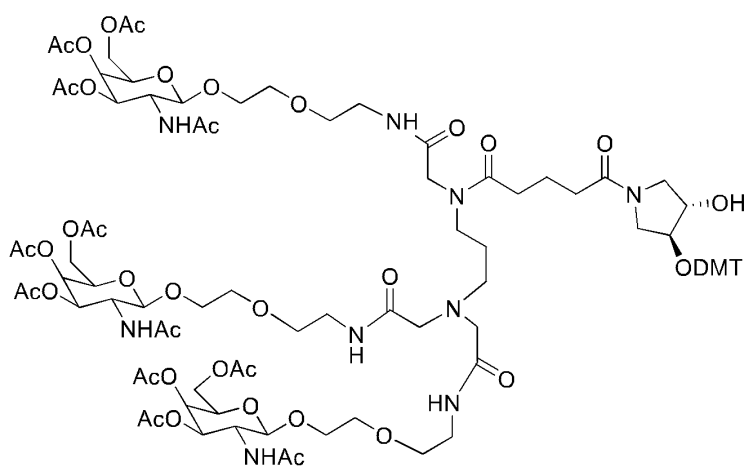
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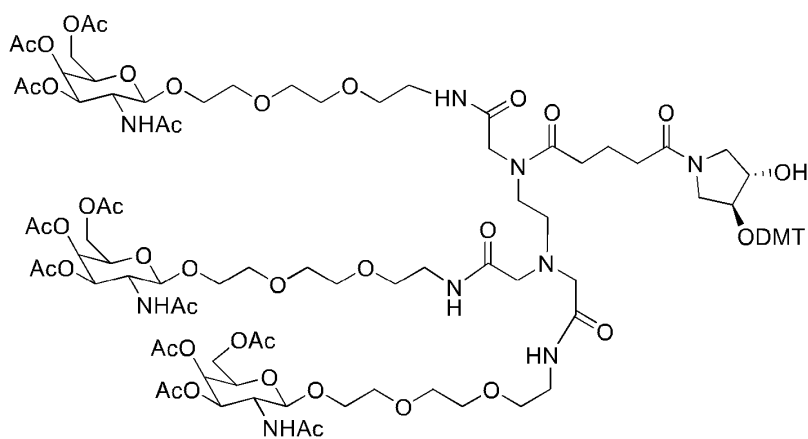
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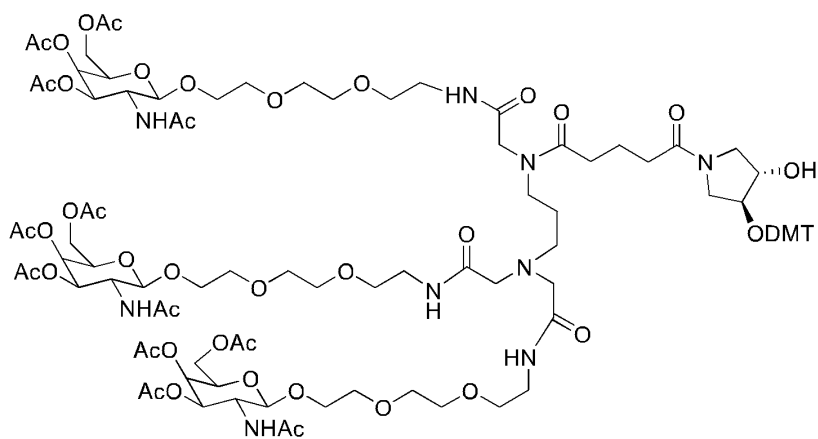
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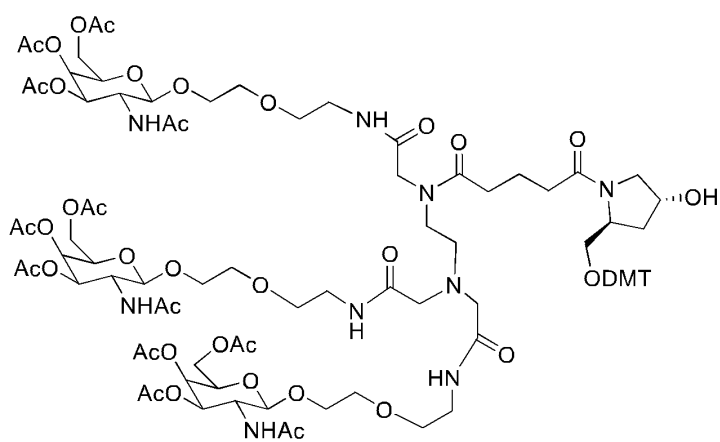
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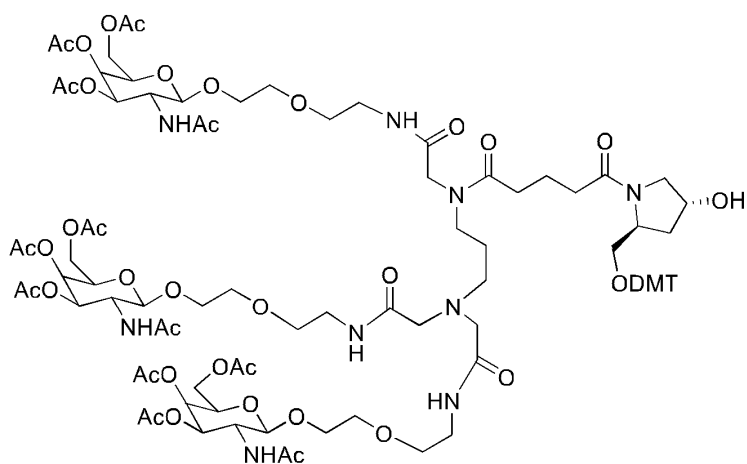
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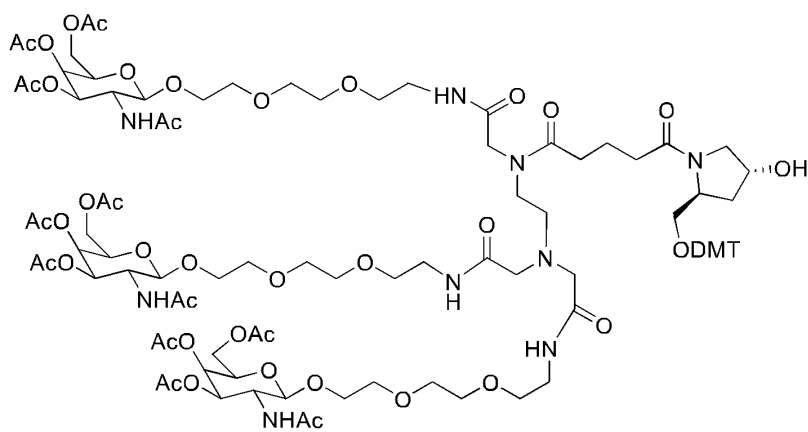
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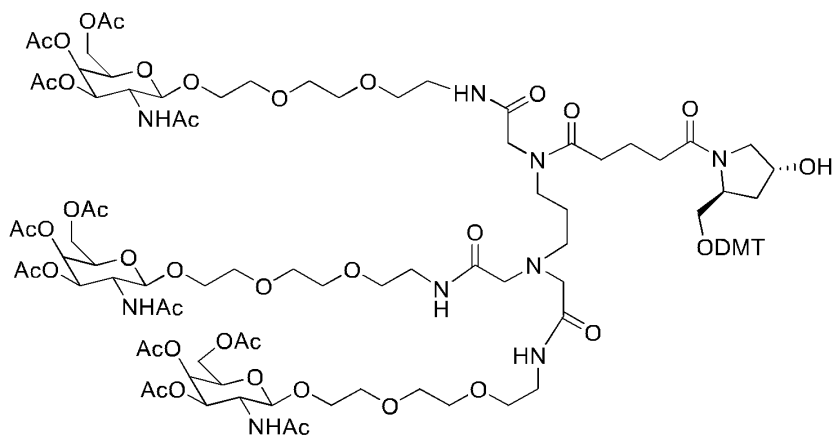
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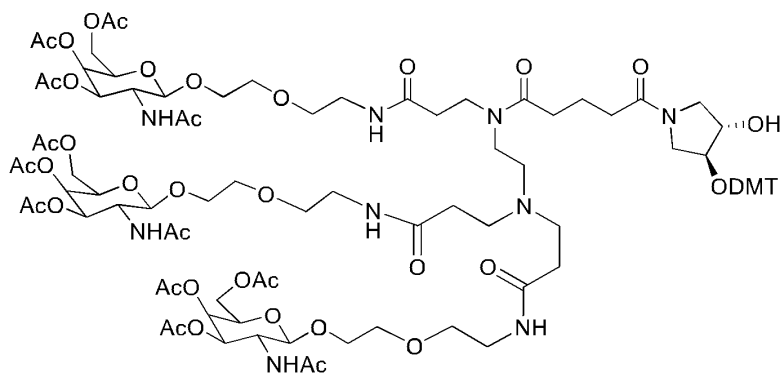
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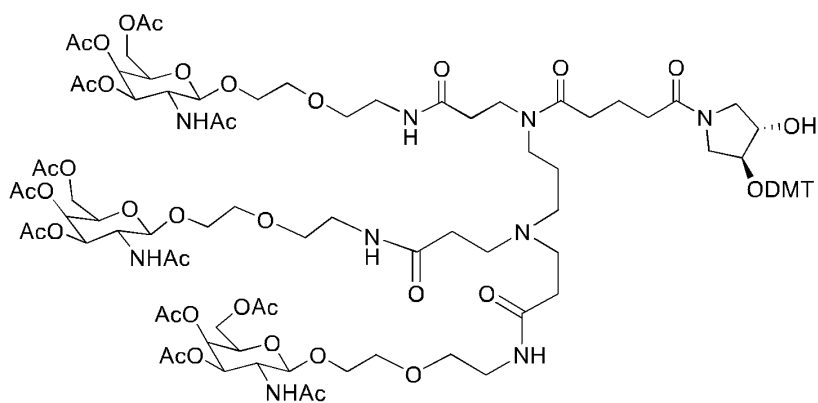
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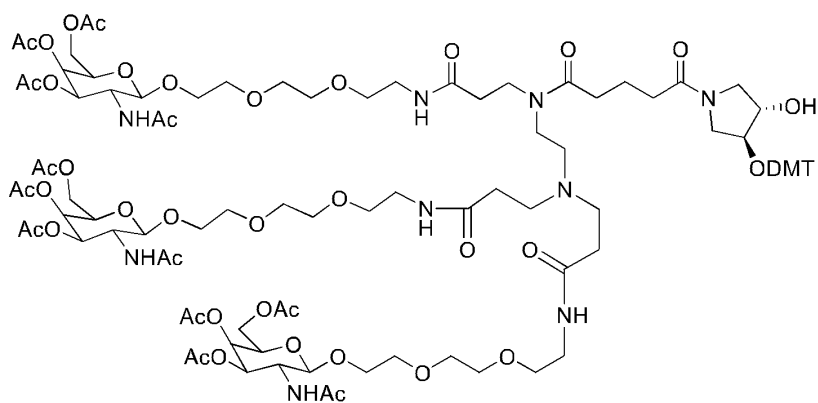
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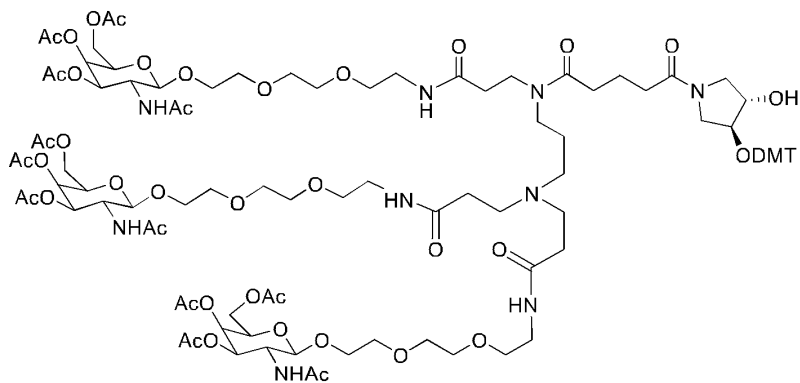
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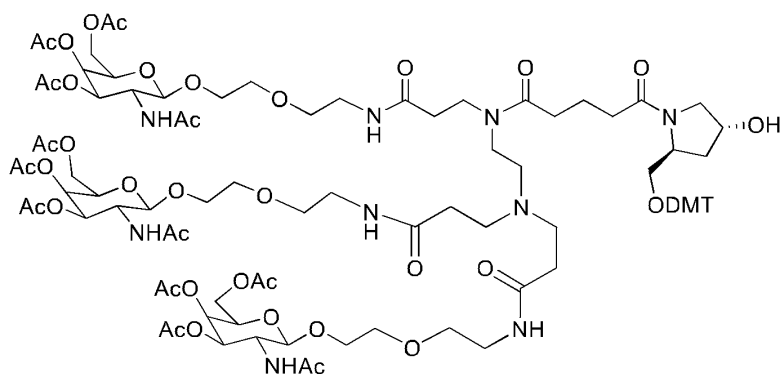
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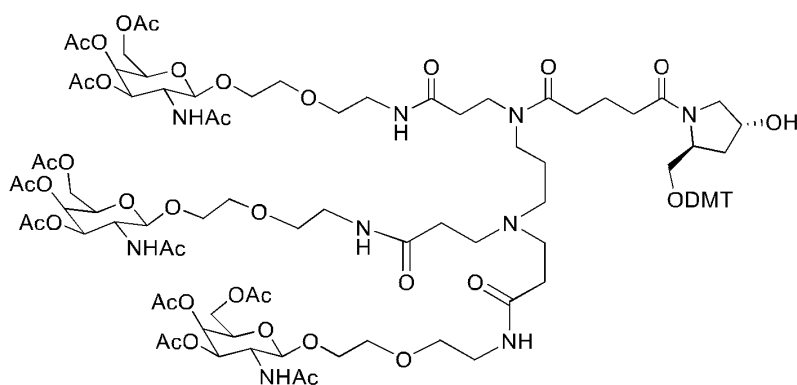
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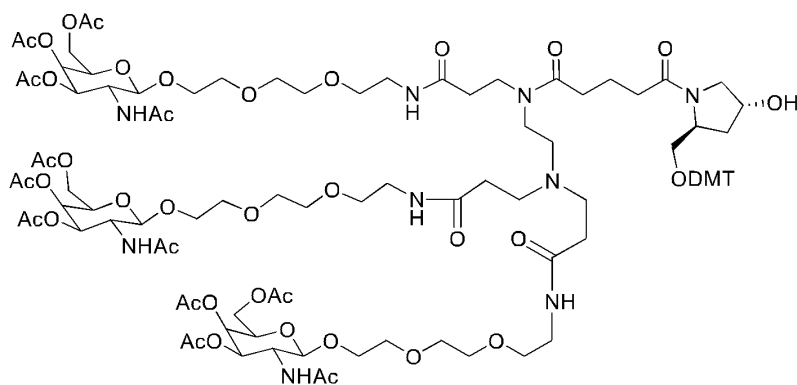
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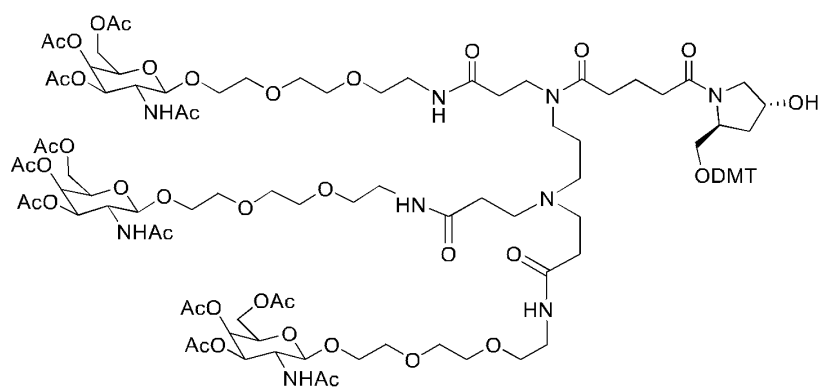
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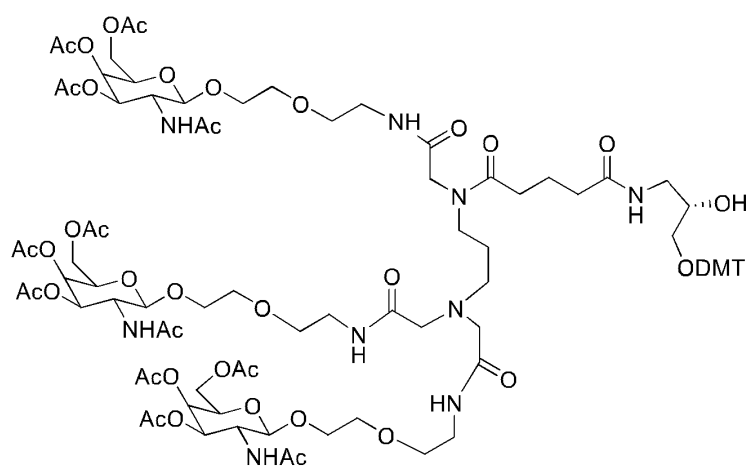
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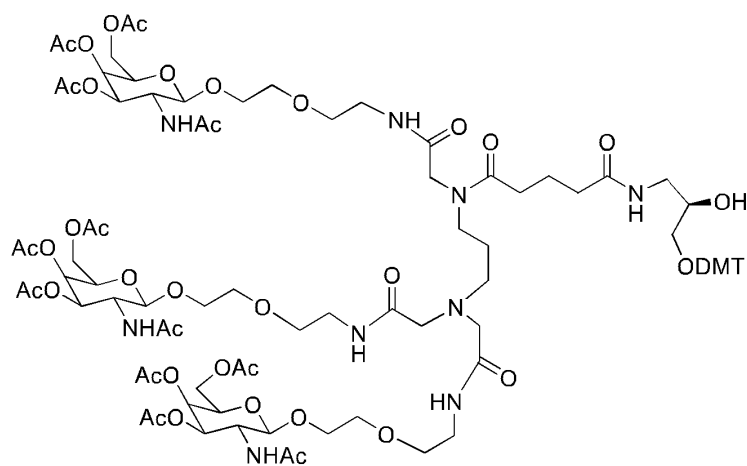
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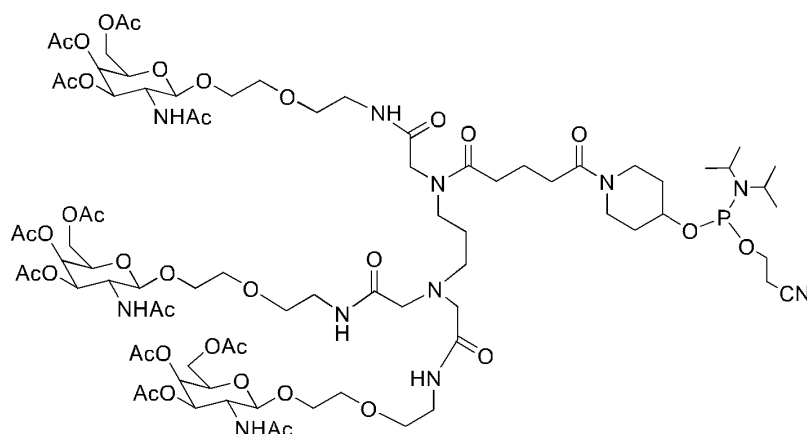
Compound 72



Compound 73



Compound 74



Compound 75

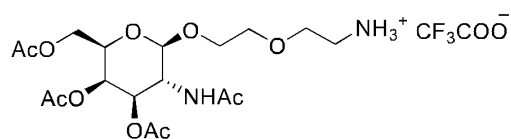
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Example 2. Preparation of Intermediate Compounds

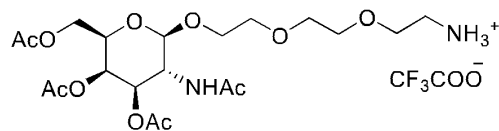
In Scheme 3 below, Intermediate-A was synthesized by treating commercially available galactosamine pentaacetate (Compound I) with trimethylsilyl trifluoromethanesulfonate (TMSOTf) in dichloromethane (DCM). This was followed by glycosylation with Cbz protected 2-(2-aminoethoxy)ethan-1-ol to give Compound II. The Cbz protecting group was removed by hydrogenation to afford Intermediate-A as a trifluoroacetate (TFA) or HCl salt. Intermediate B was synthesized based on the same scheme except Cbz protected 2-(2-(2-aminoethoxy)ethoxy)ethan-1-ol was used as the starting material. Scheme 3 allows access to variation of linkerA, as well as variation of targeting

10

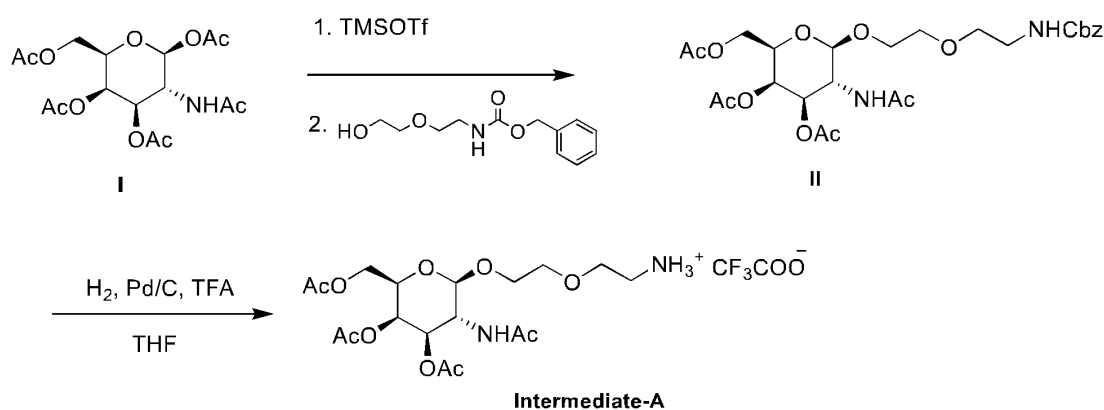
15



Intermediate A



Intermediate B



Scheme 3

To a solution of Compound I (20.0 g, 51.4 mmol) in DCE (100 mL) was added
 5 TMSOTf (17.1 g, 77.2 mmol). The resulting reaction solution was stirred at 60 °C for 2 hrs,
 and then at 25 °C for 1 hr. Cbz protected 2-(2-aminoethoxy)ethan-1-ol (13.5 g, 56.5 mmol) in
 DCE (100 mL) dried over 4 Å powder molecular sieves (10 g) was added dropwise to the
 abovementioned reaction solution at 0 °C under N₂ atmosphere. The resulting reaction
 mixture was stirred at 25 °C for 16 hrs under N₂ atmosphere. The reaction mixture was
 10 filtered and washed with sat. NaHCO₃ (200 mL), water (200 mL) and sat. brine (200 mL).
 The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced
 pressure to give a crude product, which was triturated with 2-Me-THF/heptane (5/3, v/v, 1.80
 L) for 2 hrs, filtered and dried to give Compound II (15.0 g, 50.3% yield) as a white solid.

To a dried and argon purged hydrogenation bottle was carefully added 10% Pd/C
 15 (1.50 g), followed by THF (10 mL) and then a solution of Compound II (15.0 g, 26.4 mmol)
 in THF (300 mL) and TFA (3.00 g, 26.4 mmol). The resulting mixture was degassed and
 purged with H₂ three times and stirred at 25 °C for 3 hrs under H₂ (45 psi) atmosphere. TLC
 (DCM: MeOH = 10:1) indicated Compound II was consumed completely. The reaction
 mixture was filtered and concentrated under reduced pressure. Residue was dissolved in
 20 anhydrous DCM (500 mL) and concentrated. This process was repeated 3 times to give
 Intermediate-A (14.0 g, 96.5% yield) as a foamy white solid. ¹H NMR (400 MHz DMSO-*d*₆):
 δ ppm 7.90 (d, *J* = 9.29 Hz, 1 H), 7.78 (br s, 3 H), 5.23 (d, *J* = 3.26 Hz, 1 H), 4.98 (dd, *J* =
 11.29, 3.26 Hz, 1 H), 4.56 (d, *J* = 8.53 Hz, 1 H), 3.98 - 4.07 (m, 3 H), 3.79 - 3.93 (m, 2 H),
 3.55 - 3.66 (m, 5 H), 2.98 (br d, *J* = 4.77 Hz, 2 H), 2.11 (s, 3 H), 2.00 (s, 3 H), 1.90 (s, 3 H),
 25 1.76 (s, 3 H).

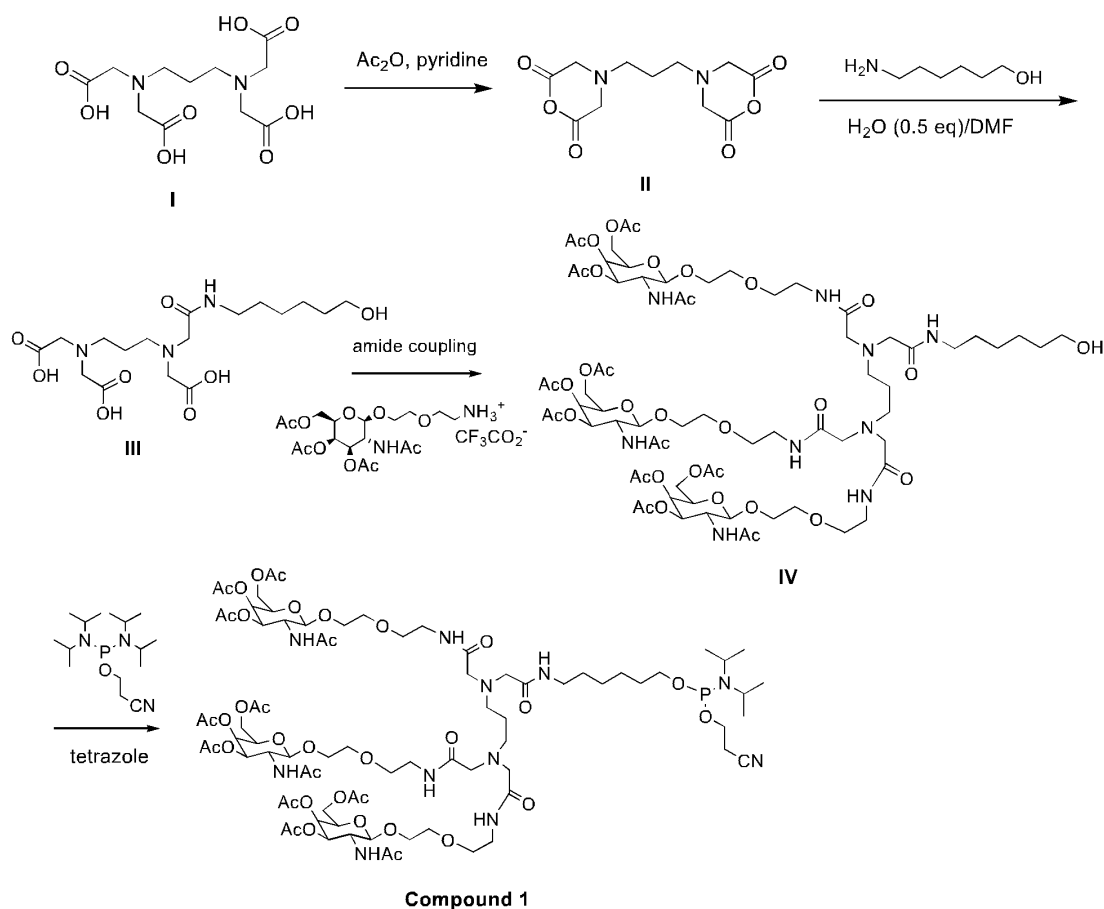
Intermediate-B was synthesized using similar procedures for synthesis of Intermediate-A. ^1H NMR (400 MHz DMSO- d_6): δ ppm 7.90 (br d, $J = 9.03$ Hz, 4 H), 5.21 (d, $J = 3.51$ Hz, 1 H), 4.97 (dd, $J = 11.1$ Hz, 1 H), 4.54 (d, $J = 8.53$ Hz, 1 H), 3.98 - 4.06 (m, 3 H), 3.88 (dt, $J = 10.9$ Hz, 1 H), 3.76 - 3.83 (m, 1 H), 3.49 - 3.61 (m, 9 H), 2.97 (br s, 2 H), 2.10 (s, 3 H), 1.99 (s, 3 H), 1.88 (s, 3 H), 1.78 (s, 3 H). Mass calc. for $\text{C}_{20}\text{H}_{34}\text{N}_2\text{O}_{11}$: 478.22; found: 479.3 ($\text{M}+\text{H}^+$).

Example 3. Preparation of Compound 1

Scheme 4 below was used to prepare Compound 1 identified in Example 1 above.

10 Commercially available 2,2',2'',2'''-(propane-1,3-diylbis(azanetriyl))tetraacetic acid (Compound I in Scheme 4) was converted to dianhydride Compound II. Upon treatment of 6-aminohexan-1-ol followed by hydrolysis, Compound II was converted to triacid Compound III. Amide coupling between Compound III and Intermediate-A afforded Compound IV.

15 Treatment of Compound IV with 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite and a catalytic amount of 1H-tetrazole afforded the phosphoramidite Compound 1.



Scheme 4

To a stirring solution of Ac_2O (8.83 g, 86.5 mmol) and pyridine (193 mg, 2.45 mmol) was added tetraacid Compound I (5.0 g, 16.3 mmol). After purging with N_2 for 3 times, the reaction mixture was stirred at 65 °C for 12 hrs under N_2 atmosphere. After cooling, the reaction mixture was filtered to remove insoluble solid. Filtrate was concentrated in vacuum. Toluene was added to the residue and volatiles were evaporated. This process was repeated 3 times to give compound II (2.20 g, 49.8% yield) as a yellow oil. ^1H NMR (400 MHz $\text{DMSO}-d_6$): δ ppm 3.65 (s, 8 H), 2.46 (br t, $J = 7.19$ Hz, 4 H), 1.55 - 1.65 (m, 2 H).

To a mixture of Compound II (1.80 g, 6.66 mmol) and imidazole (3.63 g, 53.2 mmol) in DMF (18 mL) was added 6-aminohexan-1-ol (624 mg, 5.33 mmol) and pyridine (263 mg, 3.33 mmol) sequentially. The mixture was left stirring at 50 °C for 5 hrs under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure. Residue was purified by reversed phase prep-HPLC. Compound III was obtained (1.90 g, contains 8.4% DMF and 63.2% imidazole by weight). ^1H NMR (400 MHz $\text{DMSO}-d_6$): δ ppm 4.00 (br t, $J = 6.50$ Hz, 1

H), 3.44 - 3.56 (m, 6 H), 3.34 - 3.39 (m, 3 H), 3.20 - 3.27 (m, 2 H), 3.02 - 3.12 (m, 2 H), 2.80 - 2.86 (m, 1 H), 2.79 - 2.87 (m, 1 H), 2.64 - 2.70 (m, 2 H), 1.49 - 1.69 (m, 3 H), 1.33 - 1.73 (m, 4 H), 1.18 - 1.46 (m, 3 H).

To a solution of Compound III (950 mg, purity 28.3% 0.66 mmol) and Intermediate-A (1.01 g, 2.32 mmol) in DMF (10 mL) was added DIEA (385 mg, 2.99 mmol), HOBt (358 mg, 2.65 mmol) and EDC (508 mg, 2.65 mmol) sequentially. The resulting reaction mixture was stirred at 25 °C for 3 hrs under N₂ atmosphere. LC-MS indicated desired product. The reaction mixture was purified by reverse phase prep-HPLC. Fractions containing desired product were combined and concentrated to afford Compound IV (200 mg, 18.2% yield) as a white solid. ¹H NMR (400 MHz DMSO-*d*₆): δ ppm 8.03 - 8.11 (m, 3 H), 7.84 (d, *J* = 9.26 Hz, 3 H), 5.21 (d, *J* = 3.38 Hz, 3 H), 4.97 (dd, *J* = 11.19, 3.31 Hz, 3 H), 4.54 (d, *J* = 8.50 Hz, 3 H), 4.34 (br t, *J* = 4.88 Hz, 1 H), 4.03 (s, 9 H), 3.82 - 3.92 (m, 3 H), 3.73 - 3.81 (m, 3 H), 3.44 - 3.60 (m, 10 H), 3.38 - 3.43 (m, 8 H), 3.19 - 3.27 (m, 6 H), 3.01 - 3.07 (m, 8 H), 2.39 - 2.48 (m, 6 H), 2.10 (s, 9 H), 2.00 (s, 9 H), 1.89 (s, 9 H), 1.78 (s, 9 H), 1.55 (br s, 2 H), 1.33 - 1.44 (m, 4 H), 1.23 (br s, 4 H). LCMS: [M+2H⁺]/2, 828.0.

To a solution of compound IV (200 mg, 120 μmol) in anhydrous DCM (2.0 mL) was added diisopropylammonium tetrazolide (22.9 mg, 132 μmol), followed by dropwise addition of 3-bis(diisopropylamino)phosphanyloxypropanenitrile (145 mg, 483 μmol) at 25 °C under N₂. The reaction mixture was stirred at 25 °C for 2 hrs. LC-MS indicated compound IV was consumed completely. The reaction was quenched by addition of a mixture of brine and saturated NaHCO₃ solution (1:1, 5 mL) at -20 °C and the resulting mixture was stirred at 0 °C for 1 min. Layers were separated. The aqueous phase was extracted with additional DCM (5 mL). The combined organics were washed with brine / saturated aq. NaHCO₃ solution (1:1, 5 mL), dried over Na₂SO₄, filtered and concentrated to ~ 1 mL of volume. This solution was added dropwise to MTBE (20 mL) while stirring. This resulted in formation of white solid, which was isolated by centrifuge. This process was repeated one more time. Solid was then dissolved in anhydrous CH₃CN and volatiles were removed. This process was repeated 3 times to give Compound 1 (103 mg, 45.9% yield) as a colorless oil. ¹H NMR (CDCl₃): δ ppm 7.74 - 7.88 (m, 3 H), 6.70 - 7.02 (m, 3 H), 5.37 (br s, 3 H), 5.14 - 5.27 (m, 3 H), 4.77 (br d, *J* = 7.78 Hz, 3 H), 4.13 - 4.27 (m, 6 H), 3.95 (br s, 10 H), 3.71 - 3.82 (m, 4 H), 3.47 - 3.70 (m, 20 H), 3.42 (br s, 3 H), 3.13 - 3.29 (m, 10 H), 2.63 - 2.68 (m, 6 H), 2.15 - 2.23 (m, 9 H), 2.07 (s, 9 H), 2.02 (s, 9 H), 1.89 (s, 9 H), 1.53 - 1.77 (m, 6 H), 1.37 - 1.18 (m, 16 H). ³¹P NMR (CDCl₃): δ ppm 147.14.

Example 4. Preparation of Compound 2

Compound 2 (in Example 1) was synthesized using the same procedure based on

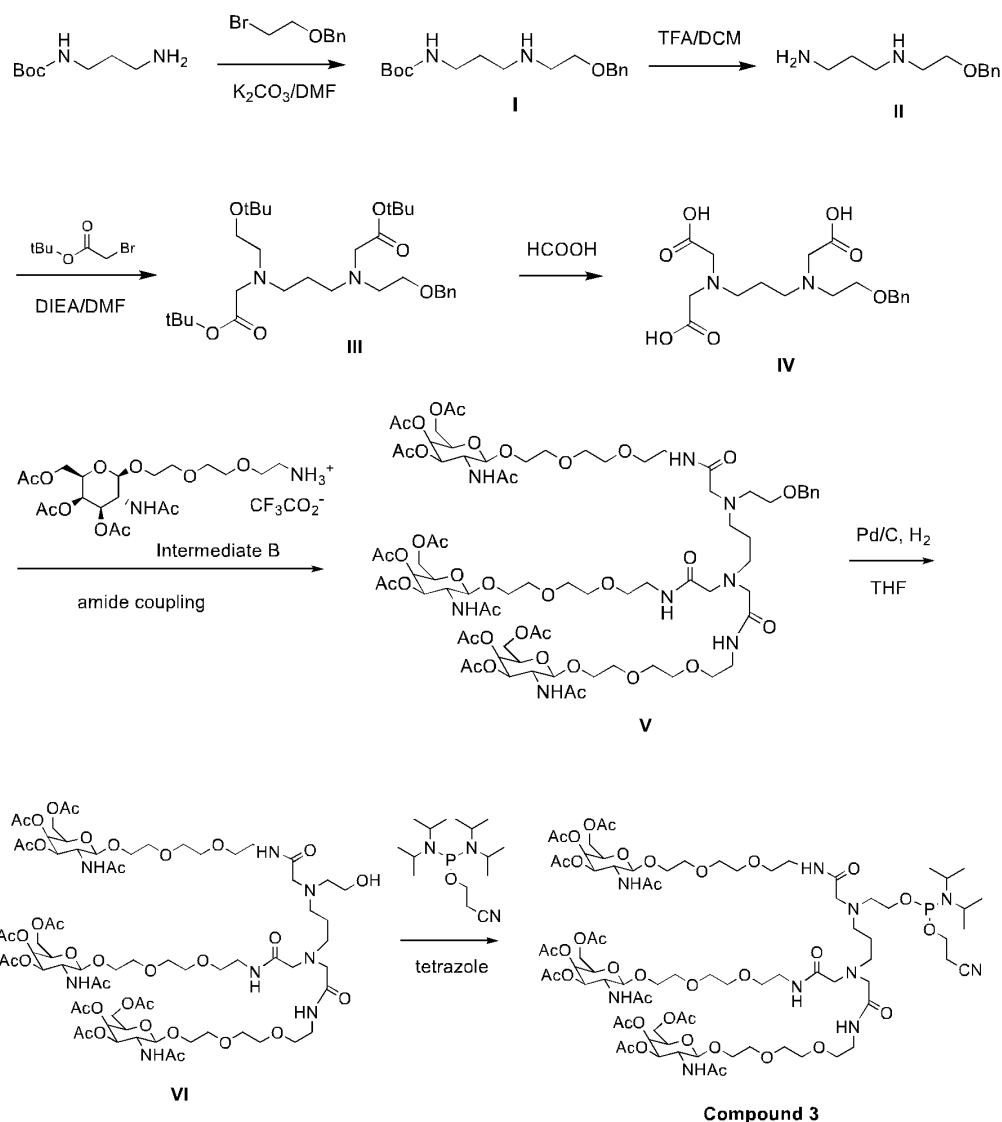
Scheme 4 above except Intermediate B was used instead of Intermediate A. ¹H NMR

5 (CDCl₃): δ ppm 8.01 - 8.09 (m, 1 H), 7.59 - 7.61 (m, 2 H), 7.21 - 7.23 (m, 1 H), 6.66 - 6.85 (m, 3 H), 5.35 (br s, 3 H), 5.06 - 5.25 (m, 3 H), 4.72 - 4.84 (m, 3 H), 4.05 - 4.25 (m, 10 H), 3.76 - 4.00 (m, 12 H), 3.46 - 3.62 (m, 32 H), 3.20 (br s, 10 H), 2.61 - 2.68 (m, 6 H), 2.16 - 2.18 (m, 9 H), 2.05 (s, 9 H), 1.96 - 2.02 (m, 18 H), 1.61 - 1.66 (m, 4 H), 1.52 (br s, 2 H), 1.36 (br s, 4 H), 1.17 - 1.19 (m, 12 H). ³¹P NMR (CDCl₃): δ ppm 147.07.

10

Example 5. Preparation of Compound 3

Scheme 5 below can be used to prepare Compound 3 identified in Example 1 above.

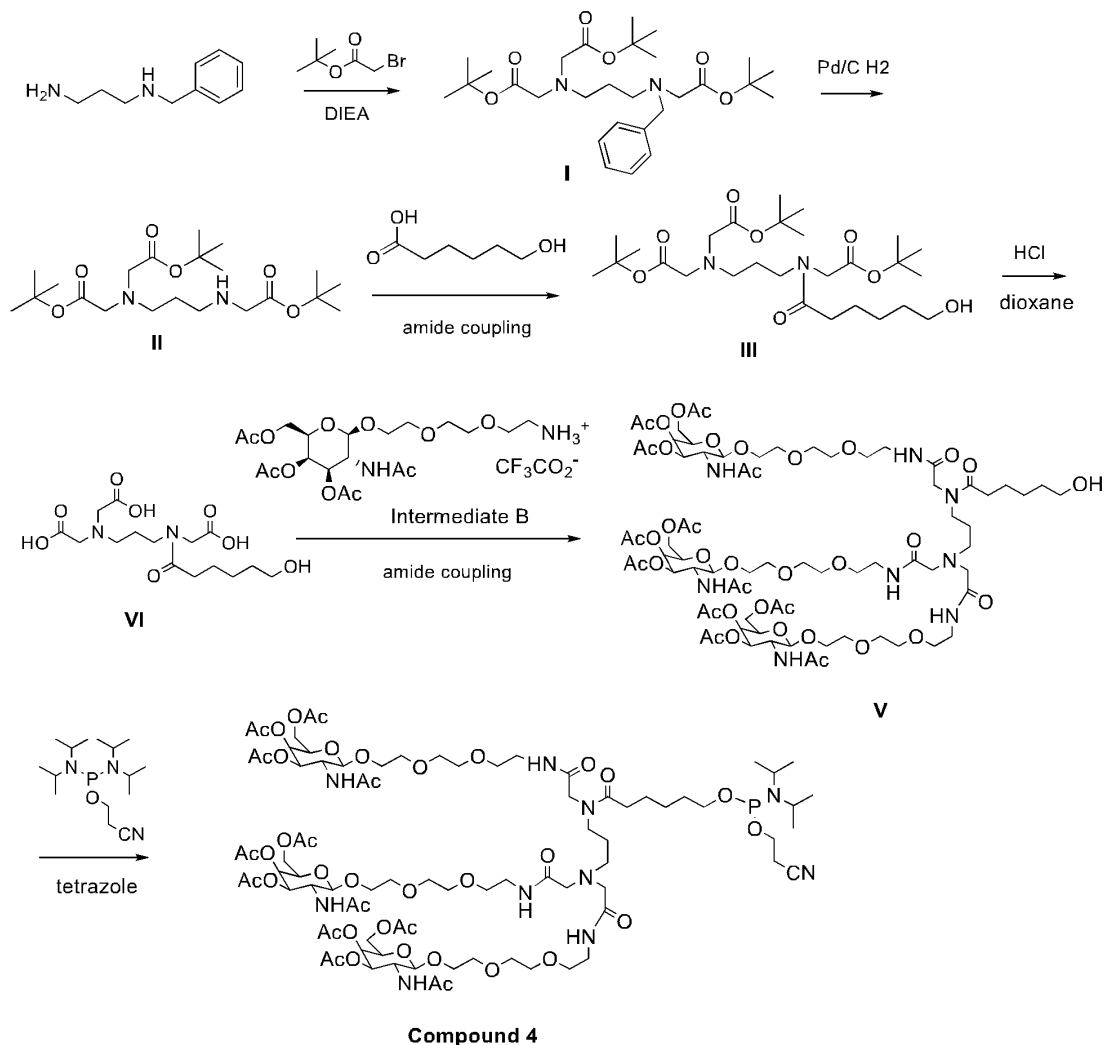


Scheme 5

Starting from *tert*-butyl (3-aminopropyl)carbamate, it can be alkylated with benzyl protected 2-bromoethanol (S_N2 substitution) to give Compound I. The Boc group can then be removed under acidic condition to afford Compound II, which can be alkylated with *tert*-butyl 2-bromoacetate to afford triester Compound III. *tert*-Butyl protecting groups can then be removed upon treatment of formic acid to generate triacid Compound IV. Amide coupling with Intermediate-A affords Compound V. The benzyl protecting group may then be removed by hydrogenation to afford compound VI. Phosphoramidite Compound 3 can be synthesized by treating Compound VI with 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite and a catalytic amount of 1H-tetrazole.

Example 6. Preparation of Compound 4

Scheme 6 below was used to prepare Compound 4 identified in Example 1 above.



5

Scheme 6

Starting from benzyl protected propane-1,3-diamine, it was alkylated with *tert*-butyl 2-bromoacetate to afford triester Compound I. The benzyl protecting group was removed by hydrogenation to afford secondary amine Compound II. Amide coupling with 6-hydroxyhexanoic acid afforded Compound III. *tert*-Butyl protecting groups were then removed upon treatment of HCl in dioxane to generate triacid Compound IV. Amide coupling between triacid compound IV and Intermediate-A was performed to afford Compound V. Phosphoramidite Compound 4 was synthesized by phosphitylation of

Compound V with 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite and a catalytic amount of 1H-tetrazole.

To a solution of N¹-benzylpropane-1,3-diamine (5.00 g, 30.4 mmol) in DMF (100 mL) was added *tert*-butyl 2-bromoacetate (23.7 g, 121 mmol), followed by addition of DIEA (23.61 g, 182 mmol) dropwise. The resulting reaction mixture was stirred at 25-30 °C for 16 hrs. LCMS showed N¹-benzylpropane-1,3-diamine was consumed completely. Reaction mixture was diluted with H₂O (500 mL) and extracted with EtOAc (500 mL x 2). The combined organics were washed with sat. brine (1 L), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to give crude product, which was purified by silica gel column chromatography (gradient: petroleum ether:ethyl acetate from 20:1 to 5:1). Compound I (12.1 g, 78.4% yield) was obtained as a colorless oil. ¹H NMR (400 MHz, CDCl₃): δ ppm 7.26 - 7.40 (m, 5 H), 3.79 (s, 2 H), 3.43 (s, 4 H), 3.21 (s, 2 H), 2.72 (dt, *J* = 16.9, 7.34 Hz, 4 H), 1.70 (quin, *J* = 7.2 Hz, 2 H), 1.44 - 1.50 (m, 27 H).

A dried hydrogenation bottle was purged with Argon three times. Pd/C (200 mg, 10%) was added, followed by MeOH (5 mL) and then a solution of Compound I (1.00 g, 1.97 mmol) in MeOH (5 mL). The reaction mixture was degassed under vacuum and refilled with H₂. This process was repeated three times. The mixture was stirred at 25°C for 12 hrs under H₂ (15 psi) atmosphere. LCMS showed Compound I was consumed completely. The reaction mixture was filtered under reduced pressure under N₂ atmosphere. Filtrate was concentrated under reduced pressure to give Compound II (655 mg, 79.7% yield) as yellow oil, which was used for the next step without further purification. ¹H NMR (400 MHz, CDCl₃): δ ppm 3.44 (s, 4 H), 3.31 (s, 2 H), 2.78 (t, *J* = 7.1 Hz, 2 H), 2.68 (t, *J* = 6.9 Hz, 2 H), 1.88 (br s, 1 H), 1.69 (quin, *J* = 7.03 Hz, 2 H), 1.44 - 1.50 (s, 27 H).

A mixture of Compound II (655 mg, 1.57 mmol), 6-hydroxyhexanoic acid (249 mg, 1.89 mmol), DIEA (1.02 g, 7.86 mmol), EDCI (904 mg, 4.72 mmol), and HOBt (637 mg, 4.72 mmol) in DMF (6 mL) was degassed and purged with N₂ three times, and then was stirred at 25°C for 3 hrs under N₂ atmosphere. LCMS indicated desired product. The reaction mixture was diluted with H₂O (10 mL) and extracted with EtOAc 20 mL (10 mL x 2). Organics were combined and washed with sat. brine (20 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated to give crude product, which was purified by silica gel column chromatography (gradient: petroleum ether:ethyl acetate from 5:1 to 1:1) to afford Compound III (650 mg, 77.8% yield) as a yellow oil. ¹H NMR (400 MHz, CDCl₃): δ ppm 3.90 - 3.95 (s, 2 H), 3.63 (t, *J* = 6.40 Hz, 2 H), 3.38 - 3.45 (m, 6 H), 2.72 (t, *J* = 6.65 Hz, 2 H),

2.40 (t, $J = 7.28$ Hz, 2 H), 1.55 - 1.75 (m, 8 H), 1.44 (s, 27 H). Mass calc. for $C_{27}H_{50}N_2O_8$: 530.36; found: 531.3 ($M+H^+$).

A mixture of Compound III (5.5 g, 10.3 mmol) in HCl/dioxane (2M, 55 mL) was stirred at 25 °C for 3 hrs. LCMS showed complete consumption of Compound III. Reaction mixture was filtered, washed with EtOAc (50 mL), and dried reduced pressure to give crude product. It was dissolved in CH_3CN (50 mL), volatiles were removed under vacuum. This process was repeated three times to give Compound IV (2.05 g, 54.5% yield) as a white solid. 1H NMR (400 MHz, D_2O): δ ppm 4.21 (s, 1 H), 4.07 (d, $J = 4.5$ Hz, 4 H), 3.99 (s, 1 H), 3.45 - 3.52 (m, 3 H), 3.42 (t, $J = 6.5$ Hz, 1 H), 3.32 - 3.38 (m, 1 H), 3.24 - 3.31 (m, 1 H), 2.37 (t, $J = 7.4$ Hz, 1 H), 2.24 (t, $J = 7.4$ Hz, 1 H), 1.99 (dt, $J = 15.5, 7.53$ Hz, 1 H), 1.85 - 1.94 (m, 1 H), 1.85 - 1.94 (m, 1 H), 1.39 - 1.56 (m, 4 H), 1.19 - 1.31 (m, 2 H).

A mixture of Compound IV (150 mg, 0.413 mmol), Intermediate-B (693 mg, 1.45 mmol), DIEA (267 mg, 2.07 mmol), EDCI (277 mg, 1.45 mmol), and HOBt (195 mg, 1.45 mmol) in DMF (2.6 mL) was stirred at 25°C for 3 hrs under N_2 atmosphere. LCMS indicated desired product. The reaction mixture was purified by reversed phase prep-HPLC to afford Compound V as a white solid after lyophilizing (186 mg, 0.106 mmol, 25.7% yield). 1H NMR (400 MHz, $CDCl_3$): ppm δ 7.91 - 8.13 (m, 1 H), 7.70 (br s, 1 H), 7.02 (br s, 1 H), 6.54 - 6.84 (m, 3 H), 5.27 (br d, $J = 3.0$ Hz, 2 H), 5.26 - 5.30 (m, 1 H), 4.99 - 5.15 (m, 3 H), 4.66 - 4.76 (m, 3 H), 3.98 - 4.17 (m, 10 H), 3.83 - 3.95 (m, 8 H), 3.63 - 3.76 (m, 4 H), 3.46 - 3.60 (m, 30 H), 3.40 (br s, 6 H), 3.12 - 3.18 (m, 4 H), 2.56 (br d, $J = 7.2$ Hz, 2 H), 2.22 - 2.39 (m, 2 H), 2.09 (s, 9 H), 1.98 (s, 9 H), 1.87 - 1.95 (m, 18 H), 1.69 (br d, $J = 6.25$ Hz, 2 H), 1.50 (br s, 2 H), 1.37 (br d, $J = 7.0$ Hz, 2 H).

To a solution of Compound V (180 mg, 0.103 mmol) in anhydrous DCM (3.6 mL) was added diisopropylammonium tetrazolide (19.44 mg, 0.114 mmol), followed by dropwise addition of 3-bis(diisopropylamino)phosphanyloxypropanenitrile (124 mg, 0.412 mmol) at ambient temperature under N_2 . The reaction mixture was stirred at 20 ~ 25 °C for 2 hrs. LCMS indicated Compound V was consumed completely. After cooling to -20 °C, the reaction mixture was added to a stirred solution of brine/saturated aq. $NaHCO_3$ (1:1, 5 mL) at 0 °C. After stirring for 1 min, DCM (5 mL) was added. Layers were separated. Organics were washed with brine/saturated aq. $NaHCO_3$ solution (1:1.5 mL), dried over Na_2SO_4 , filtered, and concentrated to ~ 1 mL of volume. The residue solution was added dropwise to 20 mL MTBE with stirring. This resulted in precipitation of white solid. The mixture was centrifuged, and solid was collected. This process was repeated one more time. The solid

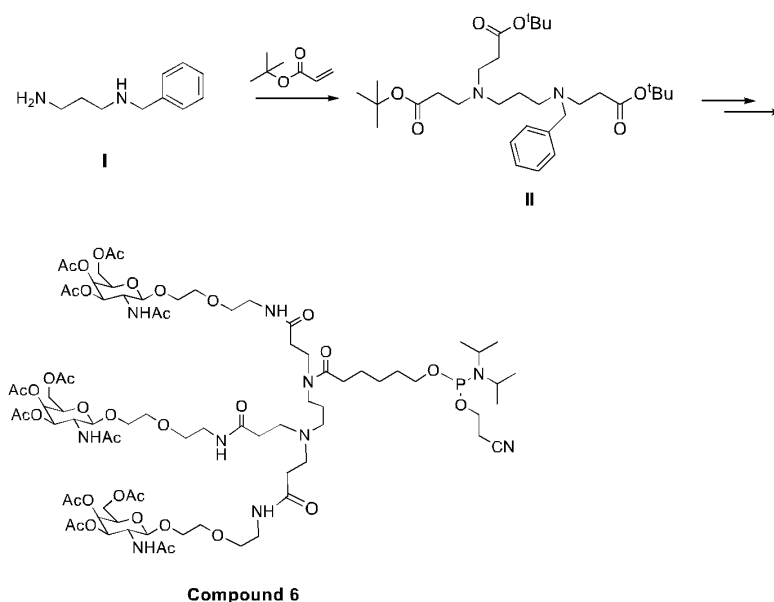
collected was dissolved in anhydrous CH₃CN. Volatiles were removed. This process was repeated two more times to afford Compound 4 (106 mg, 52.8% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃): ppm δ 7.94 - 8.18 (m, 1 H), 7.69 (br s, 1 H), 6.66 - 7.10 (m, 3 H), 5.35 (d, *J* = 3.5 Hz, 3 H), 5.07 - 5.25 (m, 3 H), 4.76 - 4.86 (m, 3 H), 4.01 - 4.31 (m, 10 H), 3.91 - 4.01 (m, 8 H), 3.74 - 3.86 (m, 4 H), 3.52 - 3.71 (m, 30 H), 3.42 - 3.50 (m, 6 H), 3.15 - 3.25 (m, 4 H), 2.52 - 2.70 (m, 4 H), 2.22 - 2.45 (m, 2 H), 2.15 - 2.22 (s, 9 H), 2.06 (s, 9 H), 1.95 - 2.03 (m, 18 H), 1.77 (br s, 2 H), 1.58 - 1.66 (m, 4 H), 1.40 (m, 2 H), 1.08 - 1.24 (m, 12 H). ³¹P NMR (CDCl₃): ppm δ 147.12.

Example 7. Preparation of Compound 5

Compound 5 was synthesized using the same procedure based on Scheme 6 except Intermediate A was used instead of Intermediate B. ¹H NMR (400 MHz, CDCl₃): ppm δ 7.71 - 8.06 (m, 2 H), 7.36 - 7.49 (m, 0.5 H), 6.59 - 7.14 (m, 3 H), 6.34 - 6.43 (m, 0.5 H), 5.36 (br d, *J* = 3.01 Hz, 3 H), 5.10 - 5.31 (m, 3 H), 4.57 - 4.85 (m, 3 H), 3.85 - 4.22 (m, 18 H), 3.29 - 3.81 (m, 30 H), 3.13 - 3.26 (m, 4 H), 2.61 - 2.68 (m, 4 H), 2.26 - 2.42 (m, 2 H), 2.13 - 2.19 (m, 9 H), 2.05 (s, 9 H), 1.97 - 2.01 (m, 9 H), 1.94 - 1.96 (m, 9 H), 1.63 (br s, 4 H), 1.35 - 1.46 (m, 2 H), 1.16 - 1.19 (m, 12 H). ³¹P NMR (CDCl₃): ppm δ 147.15.

Example 8. Preparation of Compound 6

Scheme 7 below can be used to prepare Compound 6 identified in Example 1 above.



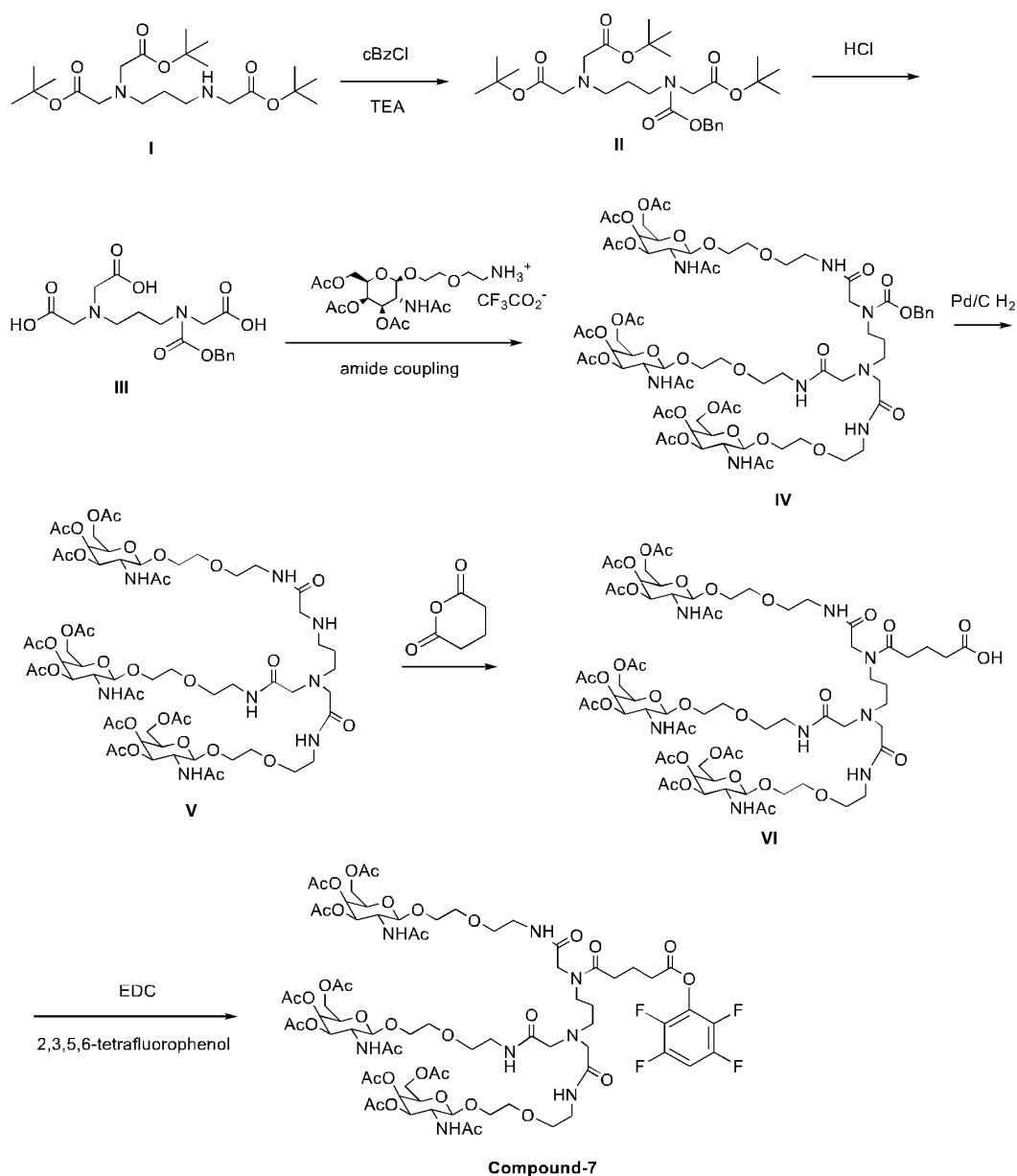
Scheme 7

Starting from the benzyl protected propane-1,3-diamine (Compound I), a Michael addition reaction with tert-butylacrylate may be performed to afford triester Compound II. Once Compound II is synthesized, the same procedure for synthesis of Compound 4 in Scheme 6 may be followed for remaining steps to synthesize Compound 6.

5

Example 9. Preparation of Compound 7

Scheme 8 below can be used to prepare Compound 7 identified in Example 1 above.



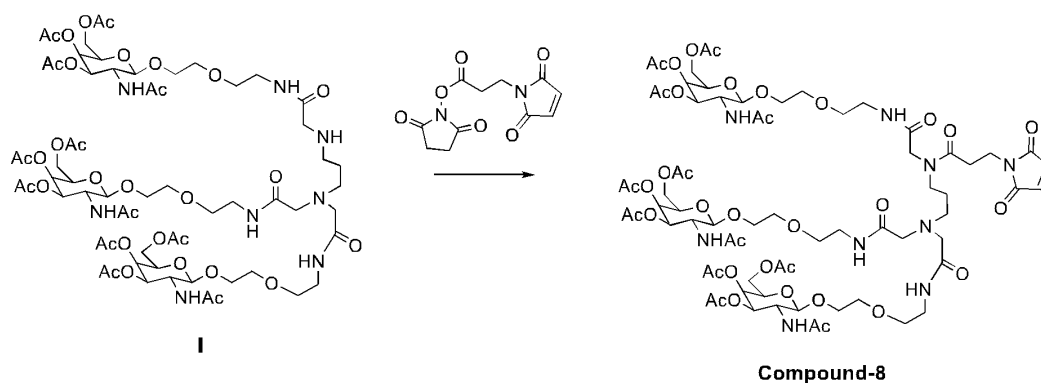
Scheme 8

Starting from secondary amine Compound I (Compound II in Scheme 6), Cbz protection may be used to afford Compound II. The tert-Butyl groups of Compound II may be removed by treatment with acid to give triacid Compound III. Amide coupling of Compound III with Intermediate-A may afford Compound IV. The Cbz protecting group of Compound IV may be removed by hydrogenation to afford secondary amine Compound V, which may react with glutaric anhydride to afford carboxylic Compound VI. The carboxylic acid of Compound VI may be converted to tetrafluorophenyl ester by standard procedure to afford Compound 7.

10

Example 10. Preparation of Compound 8

Scheme 9 below can be used to prepare Compound 8 identified in Example 1 above.



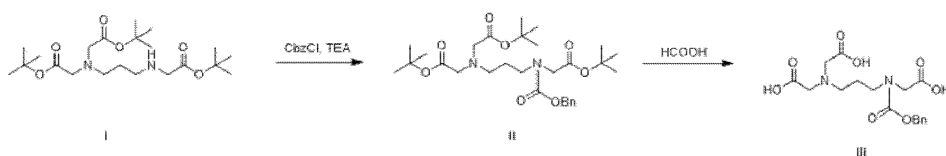
Scheme 9

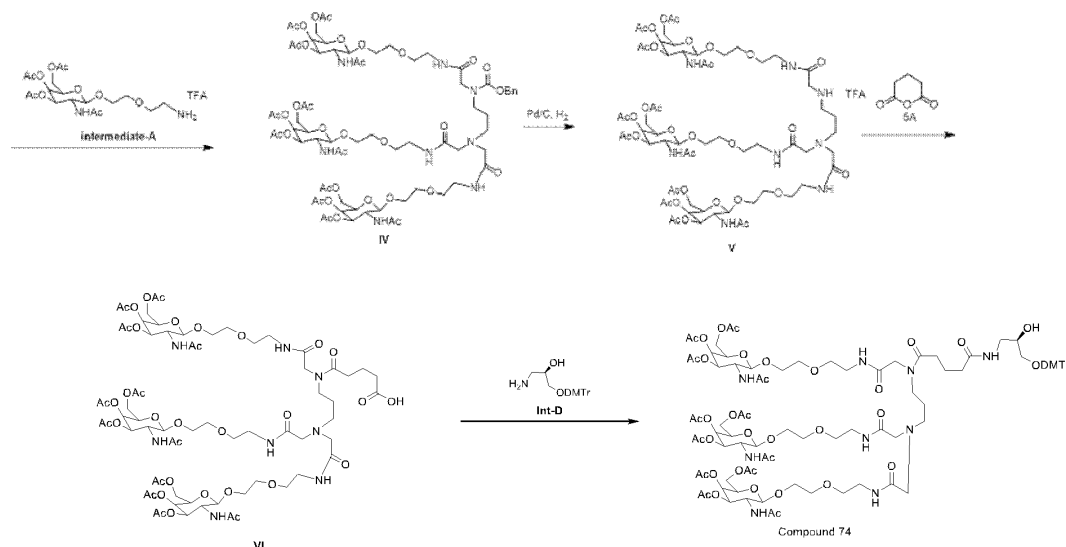
Compound I (Compound V in Scheme 8) may be reacted with NHS conjugated maleimide compound 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate to afford Compound 8.

20

Example 11. Preparation of Compound 74

Scheme 10 below can be used to prepare Compound 74 identified in Example 1 above.





Scheme 10

- 5 Starting from compound I (as same from example 6 Compound II in Scheme 6).

To a solution of Compound I (275 g, 660 mmol, 1.00 eq.) in DCM (2.75 L) was added TEA (133 g, 1.32 mol, 2.00 eq.), then Cbz-Cl (169 g, 990 mmol, 1.50 eq.) was added dropwise into the reaction mixture. The mixture was stirred at 25 °C for 2 hrs. LCMS showed Compound I was consumed completely and one main peak with desired mass was detected.

- 10 The reaction mixture was diluted with NaHCO₃ (800 mL) and extracted. The combined organic layers were washed with brine 500 mL (500 mL * 1), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give crude product, which was purified by column chromatography (SiO₂, PE/EA=100/1 to 5/1) to give Compound II (290 g, 527 mmol, 75.7% yield) as a colorless oil.
- 15 ¹H NMR: 400 MHz, DMSO-d₆ δ ppm 7.23 - 7.40 (m, 5 H), 5.00 - 5.12 (m, 2 H), 3.86 - 3.95 (m, 2 H), 3.23 - 3.39 (m, 6 H), 2.55 - 2.67 (m, 2 H), 1.56 - 1.64 (m, 2 H), 1.31 - 1.46 (m, 27 H).

- To a solution of Compound II (145 g, 263 mmol, 1.00 eq) in HCOOH (2.9 L). The mixture was stirred at 60 °C for 12 hrs under air atmosphere. LCMS showed Compound III
- 20 was consumed completely and one main peak with desired mass was detected. The reaction was diluted with toluene and acetonitrile (ACN, 1500 mL each), and the mixture was concentrated in vacuum to remove the formic acid azeotropically. The residue was diluted with 1:1 ACN : toluene (~750 mL) and concentrated. The residue was diluted with ACN (1000 mL), and concentrated. This process was repeated one more time to give crude product

as a solid. The crude product was triturated with ACN (700 mL) at 60 °C for 2 hrs, filtered and dried to give compound **III** (210 g, quantitative yield) as a white solid.

¹H NMR: 400 MHz, DMSO-d₆ δ ppm 7.26 - 7.40 (m, 5 H), 5.02 - 5.10 (m, 2 H), 3.89 - 4.00 (m, 2 H), 3.36 - 3.45 (m, 4 H), 3.24 - 3.34 (m, 2 H), 2.59 - 2.72 (m, 2 H), 1.40 (s, 2 H).

5 To a solution of Compound **III** (100 g, 261 mmol, 1.00 *eq.*), intermediate A (502 g, 915. mmol, 3.50 *eq.*, TFA) in DMF (1.00 L) was added TBTU (327 g, 1.02 mol, 3.90 *eq.*), TEA (212 g, 2.09 mol, 291 mL, 8.00 *eq.*). The mixture was stirred at 25 °C for 1 hr. LCMS showed Compound **III** was consumed completely and one main peak with desired mass was detected. The reaction mixture was added into H₂O (4000 mL). The resulting mixture was
10 extracted with MTBE (2000 mL *2) to remove impurities. The remaining aqueous portion was extracted with DCM (3000 mL * 2). The combined DCM extracts were washed with 10% citric acid (2000 mL * 2), saturated NaHCO₃ (2000 mL * 2), brine 2000 mL, dried over Na₂SO₄, filtered and concentrated under reduced pressure to give Compound **IV** (260 g, 159 mmol, 60.9% yield) as a white solid.

15 **¹H NMR:** 400 MHz, DMSO-d₆ δ ppm 7.99 - 8.08 (m, 2 H), 7.93 (br d, *J*=5.50 Hz, 1 H), 7.79 - 7.86 (m, 3 H), 7.26 - 7.39 (m, 5 H), 5.22 (d, *J*=3.13 Hz, 3 H), 4.95 - 5.08 (m, 5 H), 4.54 (br d, *J*=8.38 Hz, 3 H), 4.03 (s, 9 H), 3.81 - 3.93 (m, 5 H), 3.76 (br d, *J*=4.88 Hz, 3 H), 3.44 - 3.62 (m, 10 H), 3.34 - 3.43 (m, 6 H), 3.24 (br d, *J*=6.13 Hz, 7 H), 3.02 - 3.09 (m, 4 H), 2.40 - 2.47 (m, 2 H), 2.10 (s, 9 H), 1.99 (s, 9 H), 1.89 (s, 9 H), 1.77 (s, 9 H), 1.57 - 1.68 (m, 2 H).

20 The 2.00 L hydrogenation bottle was purged with Ar for 3 times and added dry Pd/C (9 g) carefully. Then MeOH (50 mL) was added to wet the Pd/C completely, followed by the solution of Compound **IV** (90 g, 55.1 mmol, 1.00 *eq.*) and TFA (6.29 g, 55.1 mmol, 1.00 *eq.*) in MeOH (850 mL) slowly under Ar atmosphere. The resulting mixture was degassed and purged with H₂ for 3 times, and then the mixture was stirred at 25 °C for 10 hrs under H₂
25 atmosphere. LCMS showed Compound **IV** was consumed completely and one main peak with desired mass. The reaction mixture was filtered under reduced pressure carefully under N₂ atmosphere. The filtrate was concentrated under reduced pressure to give compound **V** (160 g, 90.2% yield).

¹H NMR: 400 MHz, DMSO-d₆ δ ppm 9.12 (br s, 2 H), 8.50 (br t, *J*=5.19 Hz, 1 H), 8.10 (br t, *J*=5.50 Hz, 2 H), 7.85 - 7.91 (m, 3 H), 5.22 (d, *J*=3.25 Hz, 3 H), 4.95 - 5.01 (m, 3 H), 4.52 -
30

4.58 (m, 3 H), 4.03 (s, 9 H), 3.84 - 3.93 (m, 3 H), 3.75 - 3.83 (m, 3 H), 3.39 - 3.61 (m, 17H), 3.23 - 3.32 (m, 7H), 3.15 - 3.18 (m, 3 H), 2.97 - 3.05 (m, 2 H), 2.54 - 2.61 (m, 2 H), 2.10 (s, 9 H), 2.00 (s, 9 H), 1.89 (s, 9 H), 1.77 - 1.80 (m, 9 H), 1.70 - 1.76 (m, 2 H).

To a solution of compound V (5.0 g, 3.10 mmol, 1.0 eq, TFA salt) in DCM (50 mL) was added glutaric anhydride compound 5A, 531 mg, 4.65 mmol, 1.5 eq) at 25 °C, then TEA (1.26 g, 12.4 mmol, 1.73 mL, 4.0 eq.) was added to the mixture dropwise. The mixture was stirred at 25 °C for 1.0 hr. LC-MS showed compound V was consumed completely and a main peak with desired product mass. The resulting reaction mixture was triturated with isopropyl ether for two times (50 mL * 2) vacuum dried to afford compound VI (crude, 5.5 g) as a brown solid.

To a solution of compound VI (2.6 g, 1.61 mmol, 1.0 eq) in DMF (26 mL) was added int-D (protected (R)-3-aminopropane-1,2-diol) (952 mg, 2.42 mmol, 1.5 eq), TBTU (1.04 g, 3.23 mmol, 2.0 eq) and DIEA (625.53 mg, 4.84 mmol, 843.03 uL, 3.0 eq). The mixture was stirred at 25 °C for 1.0 hr. LC-MS showed compound int-D (protected (R)-3-aminopropane-1,2-diol) was consumed completely. The resulting reaction mixture was triturated with isopropyl ether (260 mL) to afford crude product. It was purified by column chromatography (SiO₂, DCM/MeOH = 100/1 to 10/1, 0.1% Et₃N) to give compound **74** (900 mg, 28.0% yield) as a white solid.

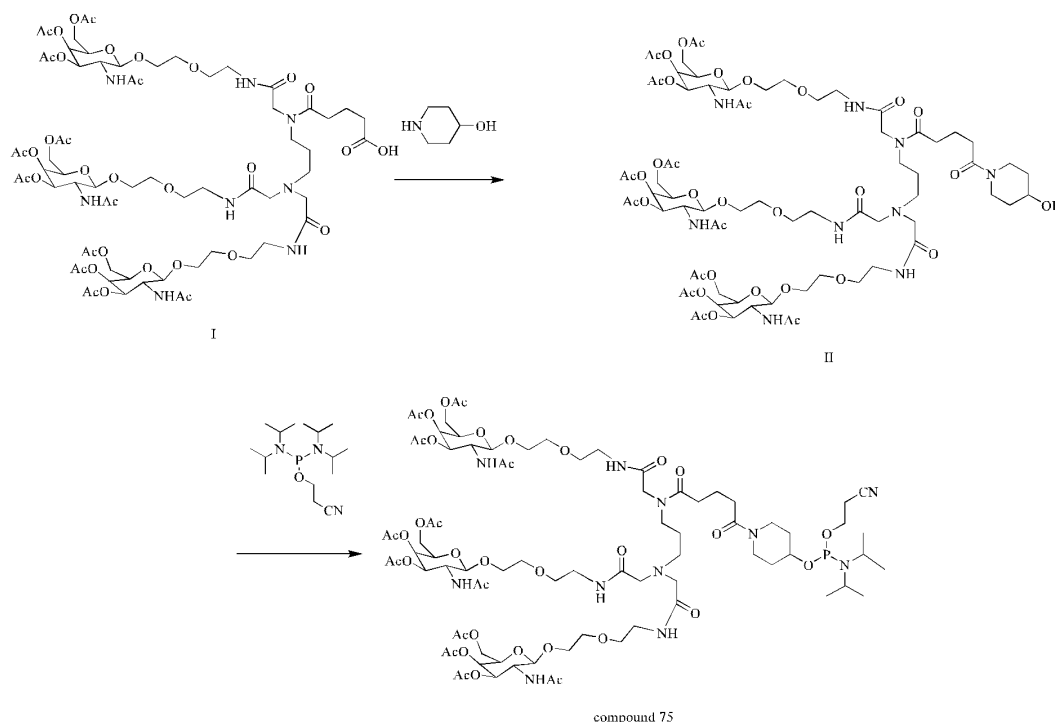
Compound **74** ¹H NMR: (400 MHz, DMSO-*d*₆) δ ppm 8.06 (br d, J=6.00 Hz, 2 H), 7.83 (br d, J=8.50 Hz, 3 H), 7.66 (dt, J=10.22, 5.21 Hz, 1 H), 7.39 (br d, J=7.75 Hz, 2 H), 7.20 - 7.30 (m, 8 H), 6.87 (br d, J=8.63 Hz, 4 H), 5.21 (br d, J=3.00 Hz, 3 H), 4.98 (br dd, J=11.07, 2.81 Hz, 4 H), 4.49 - 4.59 (m, 3 H), 4.02 (br s, 9 H), 3.83 - 3.91 (m, 5 H), 3.75 - 3.80 (m, 3 H), 3.73 (s, 6 H), 3.54 - 3.59 (m, 5 H), 3.48 (br d, J=7.25 Hz, 8 H), 3.24 (br d, J=5.63 Hz, 9 H), 3.07 (br d, J=13.13 Hz, 4 H), 2.87 - 2.97 (m, 7 H), 2.43 (br d, J=7.38 Hz, 2 H), 2.30 (br d, J=6.38 Hz, 1 H), 2.16 (br d, J=7.50 Hz, 1 H), 2.09 (s, 9 H), 1.99 (s, 9 H), 1.89 (s, 9 H), 1.77 (s, 9 H), 1.65 (br dd, J=12.76, 6.25 Hz, 3 H), 1.50 - 1.57 (m, 1 H).

Compound **73** could be prepared per the procedure described in compound **74**, except protected (S)-3-aminopropane-1,2-diol was used instead of the protected (R)-3-aminopropane-1,2-diol.

30

Example 12. Preparation of Compound 75

Scheme 11 below can be used to prepare Compound 75 identified in Example 1 above.



5 Scheme 11

Compound II was synthesized based on Scheme 11. Starting from compound I (compound VI in Scheme 10), coupling with piperidine-4-ol afforded compound II. Phosphoramidite Compound 75 was synthesized by treating Compound II with 2-Cyanoethyl N,N diisopropylchlorophosphoramidite and a catalytic amount of 1H-tetrazole.

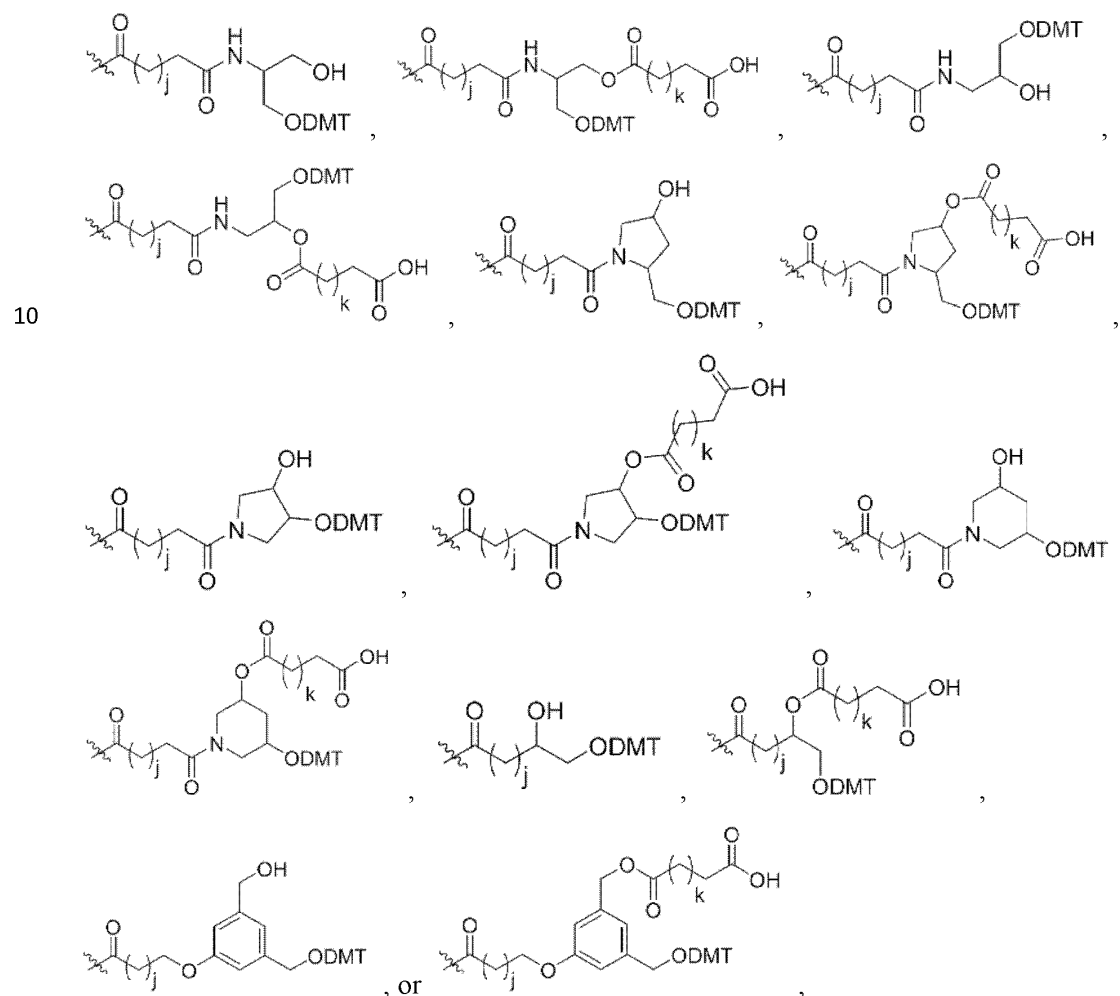
- 10 Compound 75 ¹H NMR (400 MHz in DMSO-d₆): δ ppm 8.05 (br d, J = 6.50 Hz, 2 H), 7.81 (br d, J=9.01 Hz, 3 H), 5.22 (d, J=3.25 Hz, 3 H), 4.98 (dd, J=11.26, 3.25 Hz, 3 H), 4.55 (br d, J=8.50 Hz, 3 H), 4.03 (s, 9 H), 3.64 - 3.97 (m, 12 H), 3.55 - 3.63 (m, 6 H), 3.50 (br s, 5 H), 3.40 (br d, J=6.13 Hz, 6 H), 3.17 - 3.30 (m, 9 H), 3.07 (br d, J=14.26 Hz, 4 H), 2.76 (t, J=5.82 Hz, 2 H), 2.18 - 2.47 (m, 6 H), 2.10 (s, 9 H), 1.99 (s, 9 H), 1.89 (s, 9 H), 1.78 (s, 9 H),
 15 1.52 - 1.74 (m, 6 H), 1.12 - 1.19 (m, 12 H). ³¹P NMR (DMSO-d₆): ppm δ 145.25.

Example 13. LinkerBs Attached to Functional Groups Capable of Linking to One or More Pharmaceutical Agents

- It will be appreciated that various linkerB's of the present disclosure may be attached
 20 to various functional groups (W) capable of linking to one or more pharmaceutical agents. In

particular, linkerB may comprise a diol moiety in which one of the alcohols is protected as a DMT ether, while the other alcohol may be linked directly or indirectly to a solid phase synthesis solid support material. Upon removal of the DMT group, the free alcohol is generated, which can be phosphitylated by reacting with a phosphoramidite to initiate

5 oligonucleotide chain growth. Thus, target ligand clusters of the present disclosure can be attached at the 3'-end of an oligonucleotide. A non-limiting list of linkerB's of the present disclosure that may be attached to the 3'-end of an oligonucleotide includes the structures represented below and their stereoisomers:



where:

- 15 j is an integral number between 0 and 12, and
 k is an integral number between 0 and 12.

All target ligand clusters of the present disclosure can also be attached at the 5'- end of the oligonucleotide, including but not limited to the 5'- end of the sense strand in dsRNA, or the 5' -end of the antisense strand in dsRNA:

Example 14. Preparation of Ligand Cluster Conjugated siRNA

- 5 Sense and antisense strand sequences of siRNA were synthesized on oligonucleotide synthesizers using a well-established solid phase synthesis method based on phosphoramidite chemistry. Oligonucleotide chain propagation is achieved through 4-step cycles: a condensation, a capping, an oxidation, and a deprotection step for addition of each nucleotide. Syntheses were performed on a solid support made of controlled pore glass (CPG, 1000 Å).
- 10 Monomer phosphoramidites were purchased from commercial sources. Ligand cluster attached phosphoramidites were synthesized according to the procedures of Examples 3-12 herein. 5-Ethylthio-1H-tetrazole was used as an activator. I₂ in THF/Py/H₂O and phenylacetyl disulfide (PADS) in pyridine/MeCN was used for oxidation and sulfurization reactions, respectively. After the final solid phase synthesis step, solid support bound oligomer was
- 15 cleaved and protecting groups were removed by treating with a 1:1 volume solution of 40 wt. % methylamine in water and 28% ammonium hydroxide solution. Crude single strand product was isolated by lyophilization and purified by ion pairing reversed phase HPLC (IP-RP-HPLC). Purified single strand oligonucleotide product from IP-RP-HPLC was converted to sodium salt by dissolving in 1.0 M NaOAc and precipitation by addition of ice cold EtOH.
- 20 Annealing of equimolar complementary sense strand and antisense strand oligonucleotide in water was performed to form the double strand siRNA product, which was lyophilized to afford a fluffy white solid.

Example 15. In Vivo Evaluation of GalNAc Ligand Cluster Conjugated siRNAs

- 25 To evaluate delivery efficacy, GalNAc ligand clusters were conjugated to the 5' end or 3' end of a sense strand of a known active FXII siRNA from literature. *See Liu et al.* "An investigational RNAi therapeutic targeting Factor XII (ALN-F12) for the treatment of hereditary angioedema". RNA. 2019 Feb;25(2):255-263. doi: 10.1261/rna.068916.118. Sequence and modification information of this FXII siRNA is summarized in Table 1. Shown
- 30 in Table 1 are also six examples of GalNAc ligand clusters conjugated to FXII siRNA. Conjugation of GalNAc cluster to 5'-end or 3' end sense strand of FXII siRNA was carried out as part of solid phase synthesis outlined in Example 14. Their structure drawings

are shown below Table 1. Mass of these six compounds and the positive control compound is summarized in Table 2.

These compounds were tested for efficacy of knocking down mouse FXII. FXII is a secreted protein mainly produced in hepatocyte. Reduction of FXII expression in plasma after siRNA treatment correlates strongly with reduction of FXII mRNA in hepatocyte. Since these GalNAc ligand clusters are conjugated to the same FXII siRNA with known activity, delivery efficacy can be assessed and compared by measuring degree of reduction of FXII expression in plasma for each conjugate.

Mice were given a single subcutaneous injection of siRNA compounds (see Table 1 below) at 0.5 or 1 mg/kg or PBS. The literature compound (GalNAc ligand cluster conjugated to 3'-end of sense strand) was included in this study as the positive control. Plasma samples were collected pre-dosing, and at day 7, 14 and /or 28 post-dosing. Concentration of mouse FXII protein was measured by ELISA assay following literature procedure. *See Liu et al.* "An investigational RNAi therapeutic targeting Factor XII (ALN-F12) for the treatment of hereditary angioedema". RNA. 2019 Feb;25(2):255-263. doi: 10.1261/rna.068916.118). Knockdown activity was calculated for percent reduction of FXII protein in mouse plasma normalized to PBS treated group and is summarized in Table 3. FXII siRNA conjugated with GalNAc ligand GLS-1 and GLS-2 showed significant activity knocking down mouse FXII protein expression in mouse plasma at both dosages at day 7, 14 and/or 28 post-dosing. This activity compares favorably to the positive control. The data confirms GalNAc ligand clusters based on diamine scaffold attached to 5'-end of sense strand are highly efficacious in delivering siRNA into hepatocyte in vivo.

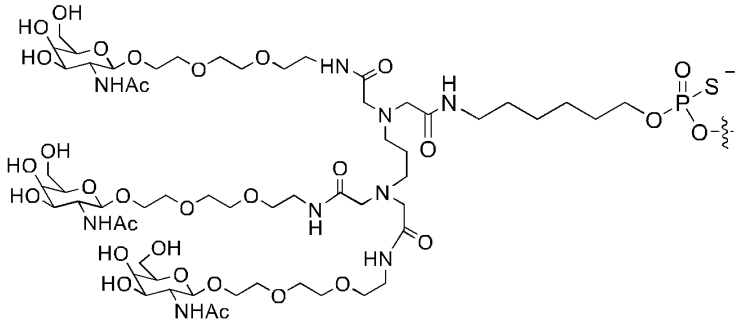
Table 1. FXII siRNA compounds. Upper case letters: 2'-deoxy-2'-fluoro (2'-F) ribonucleotide; lower case letters: 2'-O-methyl (2'-OMe) ribonucleotide; (*) indicates PS linkage. L96 is the trivalent GalNAc ligand cluster from literature. (GalNAc3 as in Jayaprakash, et al., (2014) J. Am. Chem. Soc., 136, 16958–16961)

Compound	Sense Sequence 5'→3'	SEQ ID NO	Antisense Sequence 5'→3'	SEQ ID NO
Positive control AD00127	a*a*cucaauAaAgUgcuuuga*a-L96	1	u*U*caaaGcacuUuAuUga g*u*u	8
AD00130	GLS-1-*a*a*cucaauAaAgUgcuuuga*a	2		
AD00131	GLS-2-*a*a*cucaauAaAgUgcuuuga*a	3		
AD00197	GLS-5-*a*a*cucaauAaAgUgcuuuga*a	4		
AD00448	GLS-15-*(Invab)* aacucaauAaAgUgcuuuga*(Invab)	5		

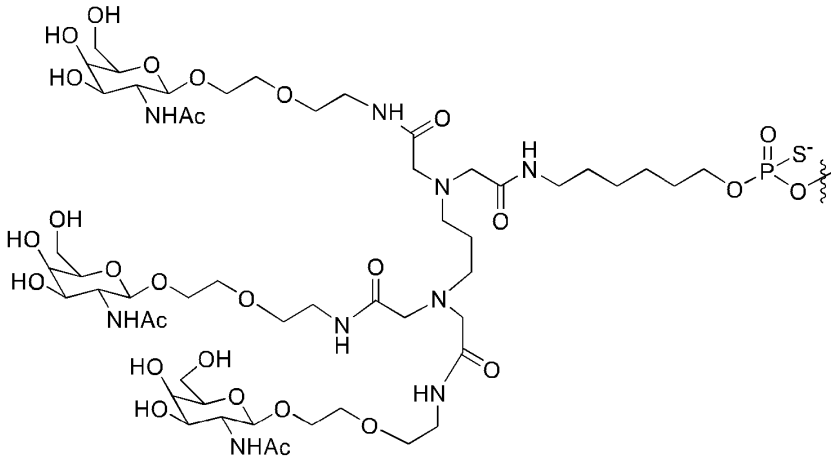
AD00449	GLS-15-*a*acucaauAaAgUgcuuga*a	6		
AD00831	a*a*cucaauAaAgUgcuuga*a-GLS-14	7		

Table 2. Mass of FXII siRNA compounds

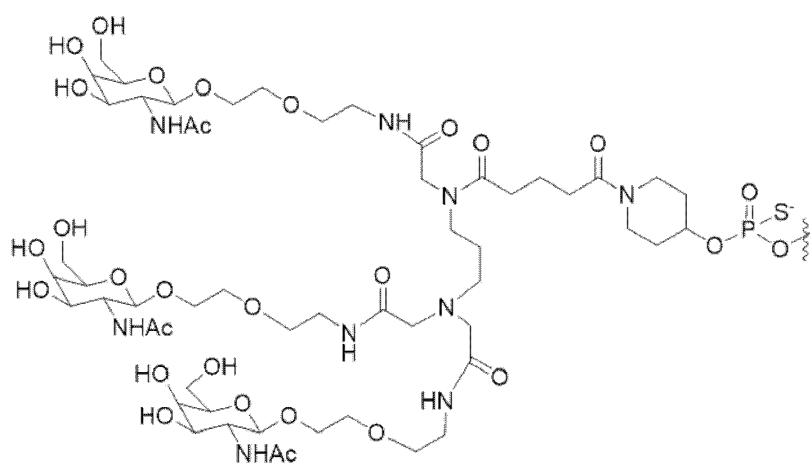
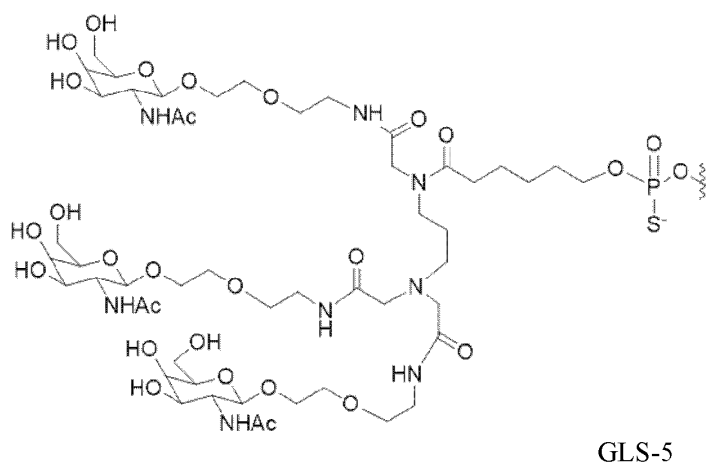
Compound	Calculated Mass		Observed Mass	
	Sense Strand	Antisense Strand	Sense Strand	Antisense Strand
Positive control	8784.68	6918.66	8785.31	6918.95
AD00130	8482.28		8482.74	
AD00131	8350.12		8350.60	
AD00197	8307.05	6918.66	8307.84	6918.89
AD00448	8734.265	6918.66	8734.45	6918.76
AD00449	8374.075	6918.66	8374.49	6918.76
AD00831	8364.04	6918.66	8364.70	6918.44



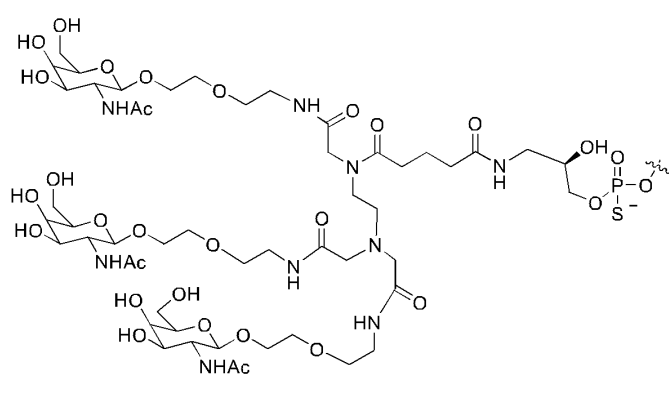
GLS-1



GLS-2



5



GLS-14

Table 3. Percent reduction of FXII protein in mouse plasma normalized to PBS treated group.

Compound ID	Dosage (mg/kg)	Day 7		Day 14		Day 28	
		Knockdown	STD	Knockdown	STD	Knockdown	STD
Positive control AD00127	1	74%	0.011	81%	0.011	67%	0.011
AD00130	0.5	67%	0.044	62%	0.041	45%	0.041
	1	82%	0.030	82%	0.016	71%	0.016
AD00131	0.5	61%	0.075	72%	0.034	51%	0.067
	1	84%	0.048	80%	0.045	66%	0.020

Example 16. In Vivo Evaluation of GalNAc Ligand Cluster Conjugated siRNAs

Mice were given a single subcutaneous injection of siRNA compounds at 1 mg/kg or PBS. Plasma samples were collected at day 14 post-dosing. Concentration of mouse FXII protein was measured by ELISA assay following literature procedure. *See Liu et al.* “An investigational RNAi therapeutic targeting Factor XII (ALN-F12) for the treatment of hereditary angioedema”. RNA. 2019 Feb;25(2):255-263. doi: 10.1261/rna.068916.118). Knockdown activity was calculated for percent reduction of FXII protein in mouse plasma normalized to PBS treated group and is summarized in Table 4. FXII siRNA conjugated with GalNAc ligand GLS-5 and GLS-15 showed significant activity knocking down mouse FXII protein expression in mouse plasma. The data confirms GalNAc ligand clusters based on diamine scaffold attached to 5'-end of sense strand are highly efficacious in delivering siRNA into hepatocyte in vivo.

there also found that when linkerB contains a six-membered ring fragment, when it is used as targeted delivery of pharmaceutical agents, especially a 4-Hydroxypiperidinyl group, such as the compound AD00448、AD00449, it shows better in vivo stability and activity. Table 4. Percent reduction of FXII protein in mouse plasma normalized to PBS treated group.

Compound ID	Dosage (mg/kg)	Day 14	
		Knockdown	STD
AD00197	1	83.1%	0.015
AD00448	1	86.0%	0.013
AD00449	1	80.0%	0.031

Example 17. In Vivo Evaluation of GalNAc Ligand Cluster Conjugated siRNAs AD00831

Mice were given a single subcutaneous injection of siRNA compounds at 2 mg/kg or PBS. Plasma samples were collected at day 7 and 14 post-dosing. Concentration of mouse FXII protein was measured by ELISA assay following literature procedure. *See Liu et al.* “An investigational RNAi therapeutic targeting Factor XII (ALN-F12) for the treatment of hereditary angioedema”. RNA. 2019 Feb;25(2):255-263. doi: 10.1261/rna.068916.118).

Knockdown activity was calculated for percent reduction of FXII protein in mouse plasma normalized to PBS treated group. The percent of knockdown at days 7 and 14 post dosing was 87% and 88%. The data confirms GalNAc ligand clusters based on diamine scaffold attached to 3'-end of sense strand are highly efficacious in delivering siRNA into hepatocyte in vivo.

Example 18

In Vivo testing of ANGPTL3 siRNA Duplexes

At 14 days before dosing of siRNAs, female C57BL/6J mice were infected by intravenous administration of a solution of adeno-associated virus 8 (AAV8) vector encoding human ANGPTL3 and luciferase gene. At day 0, mice were subcutaneously administered a single dose of AD00112-2 (Table 5.) at 1, 3 or 10 mg/kg or PBS. Blood samples were collected at day 0, before dosing of siRNA and at day 7, at termination. Serum samples were isolated and luciferase activity of serum samples was measured per manufacturer's recommended protocol. Since expression of human ANGPTL3 level correlates with expression level of luciferase, measurement of luciferase activity is the surrogate for measuring ANGPTL3 expression. Percent remaining of luciferase activity was calculated by comparing luciferase activity in samples from pre- (day 0) and post (day 7) treatment of siRNA for each mouse and normalized by the change of luciferase activity in the samples from the control treated mice during the same period of time. Result is summarized in Table 6. AD00112-2 demonstrated dose dependent activity suppressing expression of ANGPTL3, which confirms again GalNAc ligand clusters based on diamine scaffold are highly efficacious in delivering siRNA into hepatocyte in vivo.

Table 5. ANGPTL3 siRNA compounds. Upper case letters: 2' -deoxy-2' -fluoro (2' -F) ribonucleotide; lower case letters: 2' -O-methyl (2' -OMe) ribonucleotide; (*) indicates PS linkage.

Compound	Sense Sequence 5'->3'	SEQ ID NO	Antisense Sequence 5'->3'	SEQ ID NO
AD00112-2	(GLS-15)*(Invab)* gaauggaaGgUuAuacucuaa*(Invab)	9	u*U*agagUauaaCcUuCcau *u*c	10

Table 6 provides experimental results of in vivo studies (percent reduction of luciferase activity). The duplex sequence and modification of AD00112-2 is shown in Table 5.

Duplex AD#	Dose (mg/kg)	Day 7, relative to PBS (Mean $\pm$ SD)
AD00112-2	1	0.33 $\pm$ 0.06
AD00112-2	3	0.20 $\pm$ 0.09
AD00112-2	10	0.11 $\pm$ 0.03

Equivalents

5 Although several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be illustrative and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings of the present invention is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual feature, system, article, material, and/or method described herein.

10 In addition, any combination of two or more such features, systems, articles, materials, and/or methods, if such features, systems, articles, materials, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

25 All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

 The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

30 The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, *i.e.*, elements that are conjunctively present in some cases and disjunctively present in other cases. Other elements

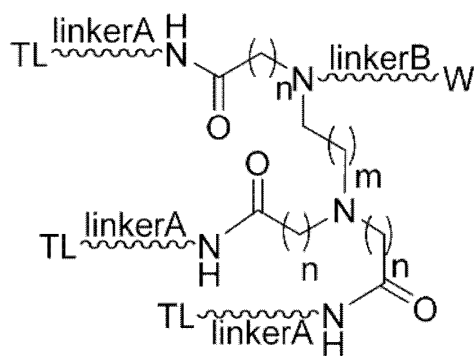
may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified, unless clearly indicated to the contrary.

5 All references, patents and patent applications, and publications that are cited or referred to in this application are incorporated by reference herein in their entirety.

CLAIMS

What is claimed is:

1. A compound for targeted delivery of one or more pharmaceutical agents, the
 5 compound having the formula:



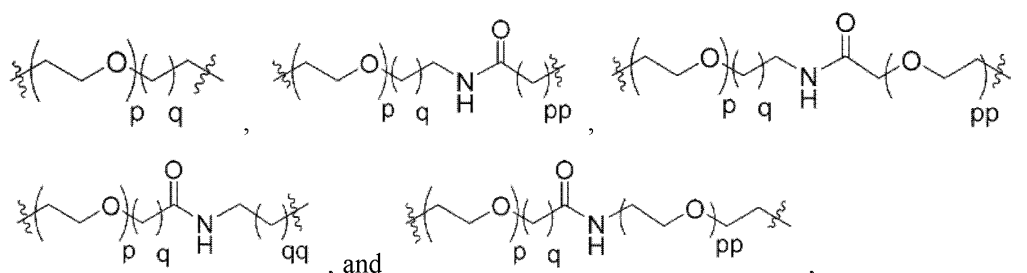
wherein:

- each TL is an independently selected targeting ligand,
 m is an integral number between 1 and 10,
 10 each n is an independently selected integral number between 1 and 10,
 each linkerA is an independently selected spacer,
 linkerB is a spacer, and
 W is either the one or more pharmaceutical agents or a functional group capable of
 linking to the one or more pharmaceutical agents.
 15
2. The compound of claim 1, wherein m is 1.
3. The compound of claim 1, wherein m is 2.
- 20 4. The compound of any one of claims 1-3, wherein at least one of the independently
 selected TLs is capable of binding to one or more cell receptors, cell channels, and cell
 transporters capable of facilitating endocytosis.
5. The compound of claim 4, wherein at least one of the independently selected TLs
 25 comprises at least one small molecule ligand.

6. The compound of claim 5, wherein the at least one small molecule comprises at least one of N-acetylgalactosamine, galactose, galactosamine, N-formyl-galactosamine, N-propionylgalactosamine, N-butanoylgalactosamine, and N-iso-butanoylgalactosamine, a macrocycle, a folate molecule, a fatty acid, a bile acid, and a cholesterol.
- 5 7. The compound of claim 4, wherein at least one of the independently selected TLs comprises at least one peptide.
8. The compound of claim 7, wherein at least one of the independently selected TLs
10 comprises at least one cyclic peptide.
- 9 The compound of claim 4, wherein at least one of the independently selected TLs comprises at least one aptamer.
- 15 10. The compound of any one of claims 4-9, wherein at least one of the independently selected TLs is capable of binding to at least one Asialoglycoprotein receptor (ASGPR).
11. The compound of any one of claims 4-9, wherein at least one of the independently selected TLs is capable of binding to at least one transferrin receptor.
- 20 12. The compound of any one of claims 4-9, wherein at least one of the independently selected TLs is capable of binding to at least one integrin receptor.
13. The compound of any one of claims 4-9, wherein at least one of the independently
25 selected TLs is capable of binding to at least one folate receptor.
14. The compound of any one of claims 4-9, wherein at least one of the independently selected TLs is capable of binding to at least one G-protein-coupled receptor (GPCR).
- 30 15. The compound of any one of claims 1-14, wherein at least one of the independently selected linkerAs comprises at least one of polyethylene glycol, an alkyl group, a cycloalkyl group, an alkenyl group, a cycloalkenyl group, an alkynyl group, an aryl group, an aralkyl group, an aralkenyl group, and an aralkynyl group.

16. The compound of any one of claims 1-15, wherein at least one of the independently selected linkerAs comprises at least one heteroatom.
- 5 17. The compound of claim 16, wherein the at least one heteroatom comprises at least one of oxygen, nitrogen, sulfur, or phosphorous.
18. The compound of any one of claims 1-17, wherein at least one of the independently selected linkerAs comprises at least one aliphatic heterocycle.
- 10 19. The compound of claim 18, wherein the at least one aliphatic heterocycle comprises at least one of tetrahydrofuran, tetrahydropyran, morpholine, piperidine, piperazine, pyrrolidine, and azetidine.
- 15 20. The compound of any one of claims 1-19, wherein at least one of the independently selected linkerAs comprises at least one heteroaryl group.
21. The compound of claim 20, wherein the at least one heteroaryl group comprises at least one of imidazole, pyrazole, pyridine, pyrimidine, triazole, and 1,2,3-triazole.
- 20 22. The compound of any one of claims 1-21, wherein at least one of the independently selected linkerAs comprises at least one amino acid.
23. The compound of any one of claims 1-22, wherein at least one of the independently selected linkerAs comprises at least one nucleotide.
- 25 24. The compound of any one of claims 1-23, wherein at least one of the independently selected linkerAs comprises at least one saccharide.
- 30 25. The compound of claim 24, wherein the at least one saccharide comprises at least one of glucose, fructose, mannose, galactose, ribose, and glucosamine.

26. The compound of any one of claims 1-25, wherein at least one of the independently selected linkerAs comprises one or more of:



5 wherein:

- p is an integral number between 0 and 12,
- pp is an integral number between 0 and 12,
- q is an integral number between 1 and 12, and
- qq is an integral number between 1 and 12.

10

27. The compound of any one of claims 1-26, wherein linkerB comprises at least one of a polyethylene glycol, an alkyl group, a cycloalkyl group, an alkenyl group, a cycloalkenyl group, an alkynyl group, an aryl group, an aralkyl group, an aralkenyl group, and an aralkynyl group.

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28. The compound of any one of claims 1-27, wherein linkerB comprises at least one heteroatom.

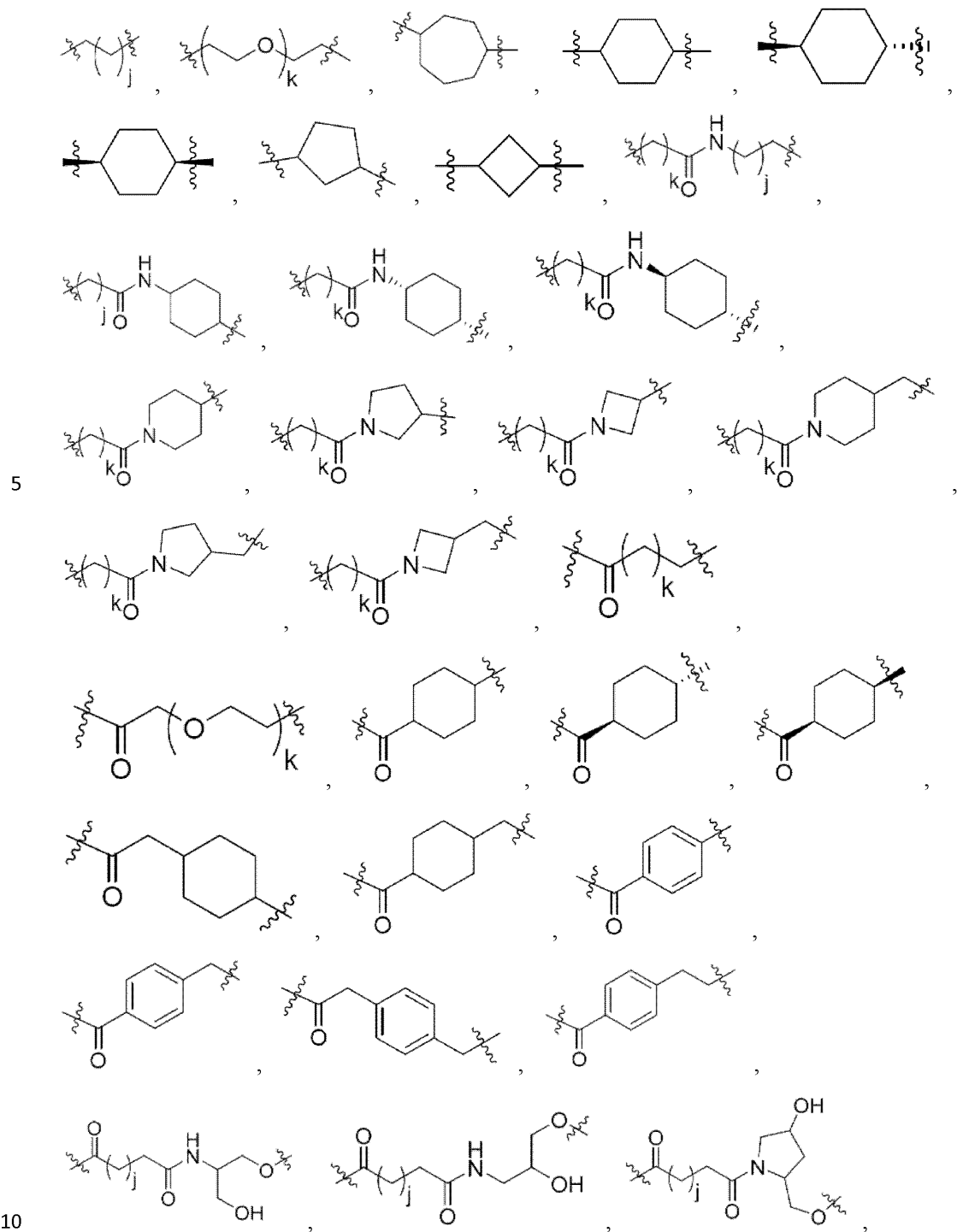
29. The compound of claims 28, wherein the at least one heteroatom comprises at least
20 one of oxygen, nitrogen, sulfur, and phosphorous.

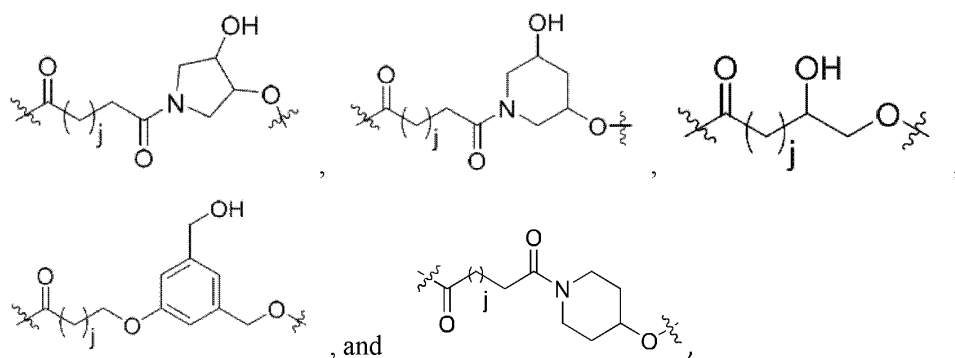
30. The compound of any one of claims 1-29, wherein linkerB comprises at least one aliphatic heterocycle.

25 31. The compound of claim 30, wherein the at least one aliphatic heterocycle comprises at least one of tetrahydrofuran, tetrahydropyran, morpholine, piperidine, piperazine, pyrrolidine, and azetidine.

32. The compound of any one of claims 1-31, wherein linkerB comprises at least one heteroaryl group.
33. The compound of claim 32, wherein the at least one heteroaryl group comprises at least one of imidazole, pyrazole, pyridine, pyrimidine, triazole, and 1,2,3-triazole.
34. The compound of any one of claims 1-33, wherein linkerB comprises at least one amino acid.
35. The compound of any one of claims 1-34, wherein linkerB comprises at least one nucleotide.
36. The compound of claim 35, wherein the at least one nucleotide comprises at least one of an abasic nucleotide and an inverted abasic nucleotide.
37. The compound of claim 36, wherein the abasic nucleotide is an abasic deoxyribonucleic acid.
38. The compound of claim 36, wherein the inverted abasic nucleotide is an inverted abasic deoxyribonucleic acid.
39. The compound of claim 36, wherein the abasic nucleotide is an abasic ribonucleic acid.
40. The compound of claim 36, wherein the inverted abasic nucleotide is an inverted abasic ribonucleic acid.
41. The compound of any one of claims 1-40, wherein linkerB comprises at least one saccharide.
42. The compound of claim 41, wherein the at least one saccharide comprises at least one of glucose, fructose, mannose, galactose, ribose, and glucosamine.

43. The compound of any one of claims 1-42, wherein linkerB comprises at least one of:



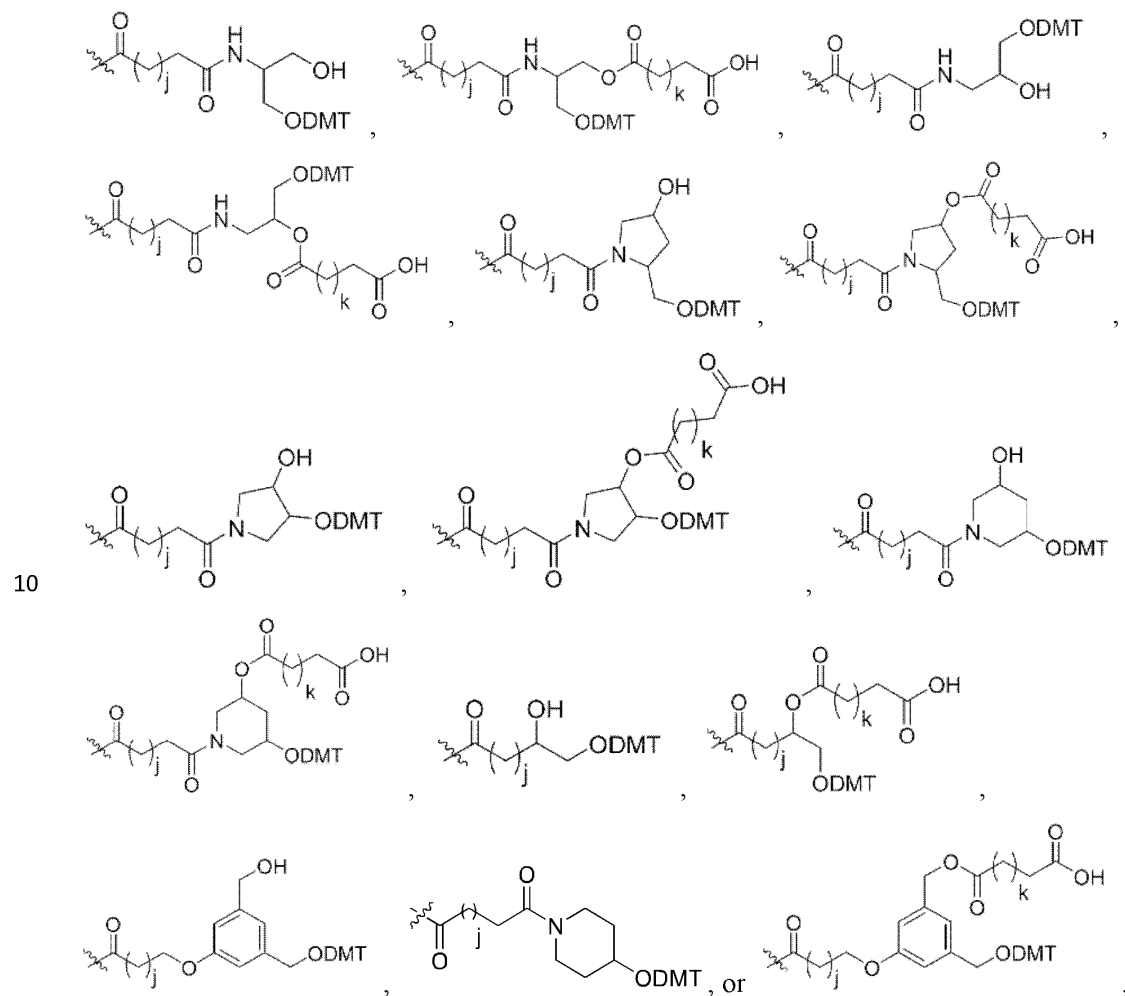


wherein:

j is an integral number between 1 and 12, and

5 k is an integral number between 0 and 12.

44. The compound of any one of claims 1-26, wherein linkerB-W is:



wherein:

j is an integral number between 0 and 12, and

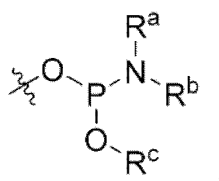
k is an integral number between 0 and 12.

45. The compound of any one of claims 1-43, wherein W is a hydroxy group.

46. The compound of any one of claims 1-43, wherein W is a protected hydroxy group.

47. The compound of claim 46, wherein the protected hydroxy group is protected using at least one of 4,4'-dimethoxytrityl (DMT), monomethoxytrityl (MMT), 9-(*p*-methoxyphenyl)xanthen-9-yl (Mox), and 9-phenylxanthen-9-yl (Px).

48. The compound of any one of claims 1-43, wherein W is a phosphoramidite group having the formula:



wherein:

R^a is a C1 to C6 alkyl, C3 to C6 cycloalkyl, an isopropyl group, or R^a joins with R^b through a nitrogen atom to form a cycle,

R^b is a C1 to C6 alkyl, C3 to C6 cycloalkyl, an isopropyl group, or R^b joins with R^a through a nitrogen atom to form a cycle, and

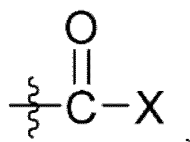
R^c is a phosphite protecting group, phosphate protecting group, or a 2-cyanoethyl group.

49. The compound of claim 48, wherein the phosphite protecting group comprises at least one of methyl, allyl, 2-cyanoethyl, 4-cyano-2-butenyl, 2-cyano-1,1-dimethylethyl, 2-(trimethylsilyl)ethyl, 2-(*S*-acetylthio)ethyl, 2-(*S*-pivaloylthio)ethyl, 2-(4-nitrophenyl)ethyl, 2,2,2-trichloroethyl, 2,2,2-trichloro-1,1-dimethylethyl, 1,1,1,3,3,3-hexafluoro-2-propyl, fluorenyl-9-methyl, 2-chlorophenyl, 4-chlorophenyl, and 2,4-dichlorophenyl.

50. The compound of claim 48, wherein the phosphate protecting group comprises at least one of methyl, allyl, 2-cyanoethyl, 4-cyano-2-butenyl, 2-cyano-1,1-dimethylethyl, 2-(trimethylsilyl)ethyl, 2-(S-acetylthio)ethyl, 2-(S-pivaloylthio)ethyl, 2-(4-nitrophenyl)ethyl, 2,2,2-trichloroethyl, 2,2,2-trichloro-1,1-dimethylethyl, 1,1,1,3,3,3-hexafluoro-2-propyl, 5 fluorenyl-9-methyl, 2-chlorophenyl, 4-chlorophenyl, and 2,4-dichlorophenyl.

51. The compound of any one of claims 1-43, wherein W is a carboxyl group.

52. The compound of claim 51, wherein W is an activated carboxyl group having the 10 formula:

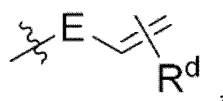


wherein X is a leaving group.

53. The compound of claim 52, wherein the leaving group is selected from the group 15 consisting of carboxylate, sulfonate, chloride, phosphate, imidazole, hydroxybenzotriazole (HOBt), N-hydroxysuccinimide (NHS), tetrafluorophenol, pentafluorophenol, and *para*-nitrophenol.

54. The compound of any one of claims 1-43, wherein W is a Michael acceptor. 20

55. The compound of claim 54, wherein the Michael acceptor has the formula:



wherein:

E is an electron withdrawing group; and
25 R^d is hydrogen or a C1-C6 alkyl substitution group on olefin.

56. The compound of claim 55, wherein the electron withdrawing group is carboxamide or an ester.

57. The compound of claim 55 or 56, wherein E and the carbon-carbon double bond are part of maleimide.

58. The compound of any one of claims 1-43, wherein W is an oligonucleotide.

5

59. The compound of claim 58, wherein the oligonucleotide is a single-stranded oligonucleotide.

60. The compound of claim 58, wherein the oligonucleotide is a double-stranded oligonucleotide.

10

61. The compound of claim 58, wherein the oligonucleotide comprises at least 3 independently selected nucleotides.

62. The compound of claim 61, wherein the oligonucleotide comprises between 16 and 23 independently selected nucleotides.

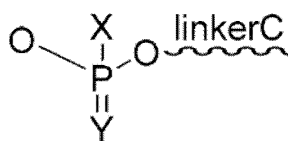
15

63. The compound of claim 61, wherein the oligonucleotide comprises about 100 independently selected nucleotides.

20

64. The compound of claim 61, wherein the oligonucleotide comprises up to fourteen thousand independently selected nucleotides.

65. The compound of any one of claims 1-43, wherein W is:



25

wherein:

linkerC is absent or a spacer attached to a 3' or 5' end of an oligonucleotide,

X is a methyl group, oxygen, sulfur, or an amino group, and

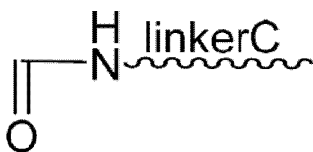
Y is oxygen, sulfur, or an amino group.

30

66. The compound of claim 65, wherein linkerC comprises at least a heterocyclic compound.

67. The compound of claim 66, wherein the heterocyclic compound is an abasic nucleotide or an inverted abasic nucleotide.

68. The compound of any one of claims 1-43, wherein W is:



wherein linkerC is a spacer attached to a 3' or 5' end of an oligonucleotide.

69. The compound of claim 68, wherein linkerC comprises at least one of polyethylene glycol (PEG), an alkyl group, and a cycloalkyl group.

70. The compound of claim 68 or 69, wherein linkerC comprises at least one heteroatom.

71. The compound of claim 70, wherein the at least one heteroatom comprises at least one of oxygen, nitrogen, sulfur, and phosphorous.

72. The compound of any one of claims 68-71, wherein linkerC comprises at least one aliphatic heterocycle.

73. The compound of claim 72, wherein the at least one aliphatic heterocycle comprises at least one of tetrahydrofuran, tetrahydropyran, morpholine, piperidine, piperazine, pyrrolidine, and azetidine.

74. The compound of any one of claims 68-73, wherein linkerC comprises at least one heteroaryl group.

75. The compound of claim 74, wherein the at least one heteroaryl group comprises at least one of imidazole, pyrazole, pyridine, pyrimidine, triazole, and 1,2,3-triazole.

76. The compound of any one of claims 68-75, wherein linkerC comprises at least one amino acid.

5 77. The compound of any one of claims 68-76, wherein linkerC comprises at least one nucleotide.

78. The compound of claim 77, wherein the at least one nucleotide comprises at least one of an abasic nucleotide and an inverted abasic nucleotide.

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79. The compound of claim 78, wherein the abasic nucleotide is an abasic deoxyribonucleic acid (DNA).

80. The compound of claim 78, wherein the inverted abasic nucleotide is an inverted abasic deoxyribonucleic acid (DNA).

15

81. The compound of claim 78, wherein the abasic nucleotide is an abasic ribonucleic acid (RNA).

20 82. The compound of claim 78, wherein the inverted abasic nucleotide is an inverted abasic ribonucleic acid (RNA).

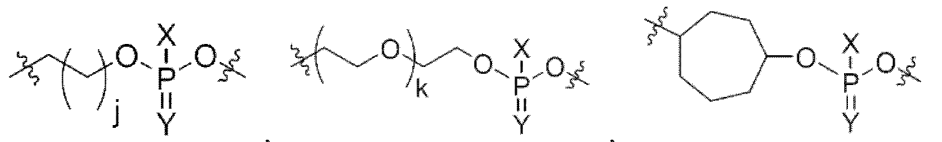
83. The compound of any one of claims 68-82, wherein linkerC comprises at least one saccharide.

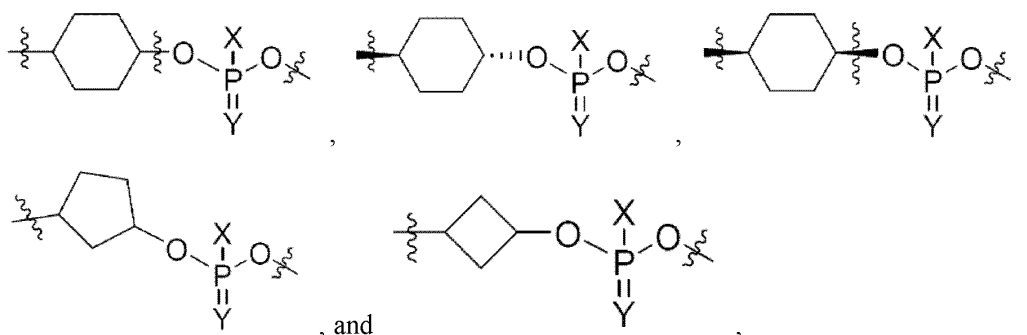
25

84. The compound of claim 83, wherein the at least one saccharide comprises at least one of glucose, fructose, mannose, galactose, ribose, and glucosamine.

85. The compound of any one of claims 68-84, wherein linkerC comprises one or more of:

30



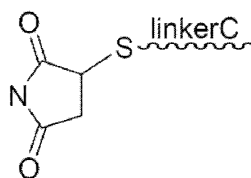


wherein:

j is an integral number between 1 and 12, and

5 k is an integral number between 0 and 12.

86. The compound of any one of claims 1-43, wherein W is:

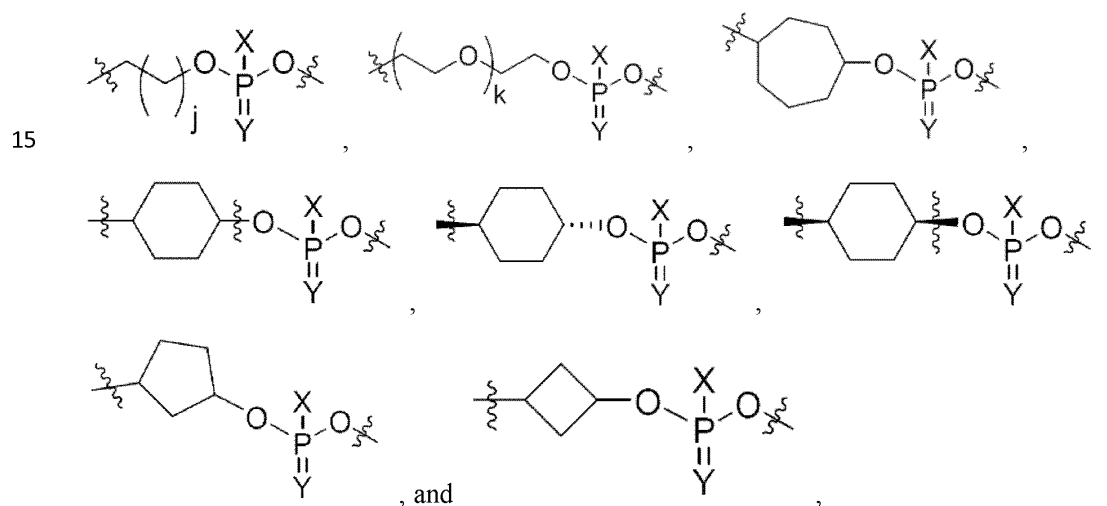


wherein linkerC is a spacer attached to a 3' or 5' end of an oligonucleotide.

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87. The compound of claim 86, wherein linkerC comprises at least one of polyethylene glycol (PEG), an alkyl group, and a cycloalkyl group.

88. The compound of claim 86 or 87, wherein linkerC comprises one or more of:



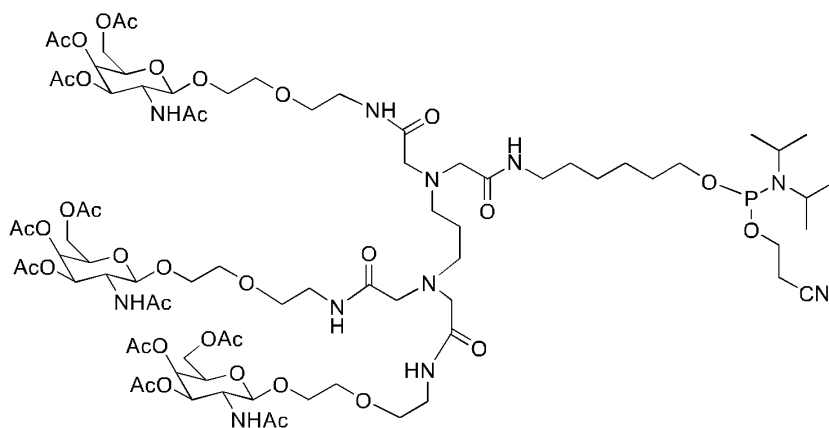
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wherein:

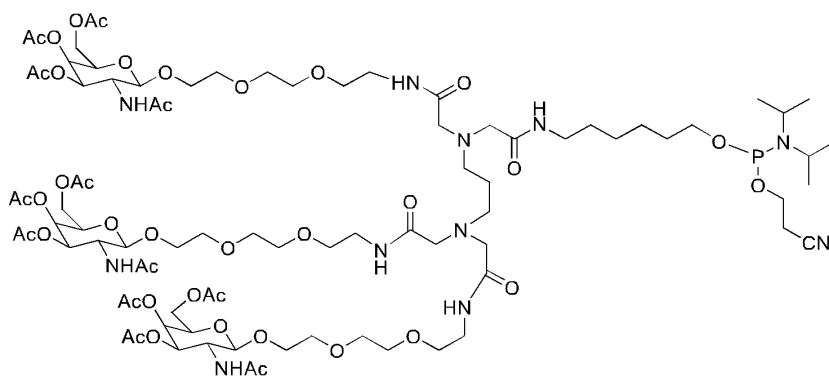
j is an integral number between 1 and 12, and

k is an integral number between 0 and 12.

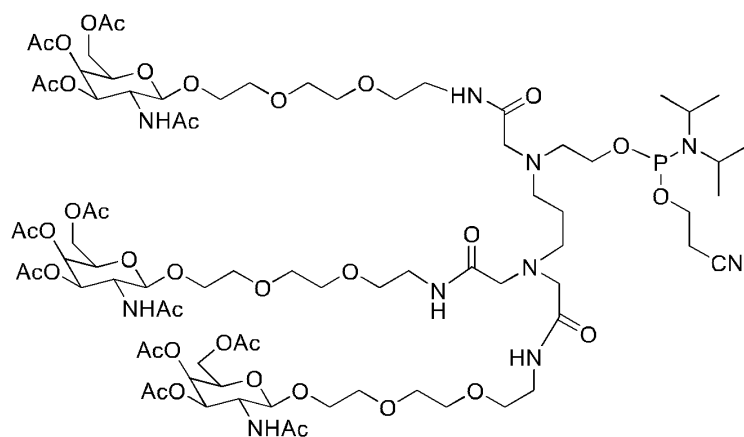
- 5 89. The compound of any one of claims 1-88, wherein the compound is selected from the group consisting of:



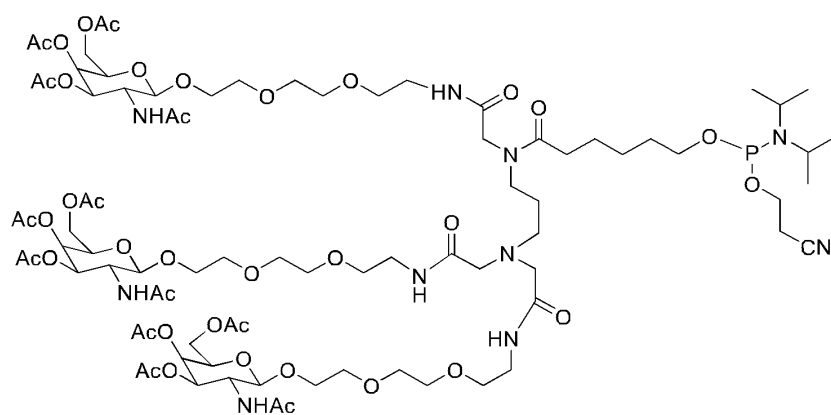
Compound 1;



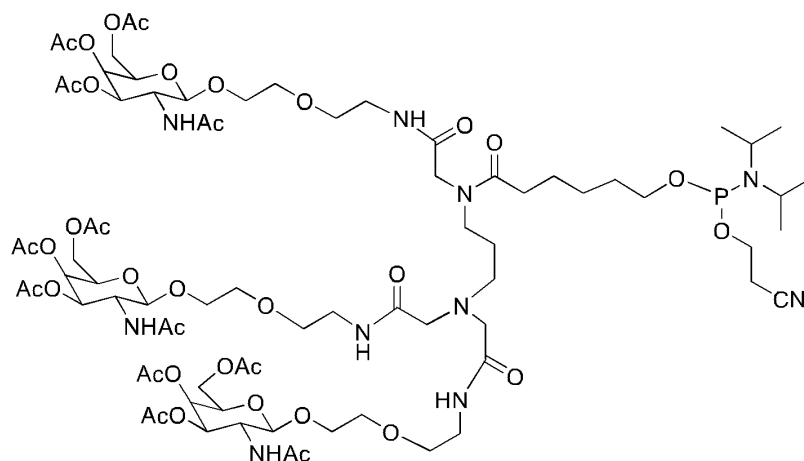
Compound 2;



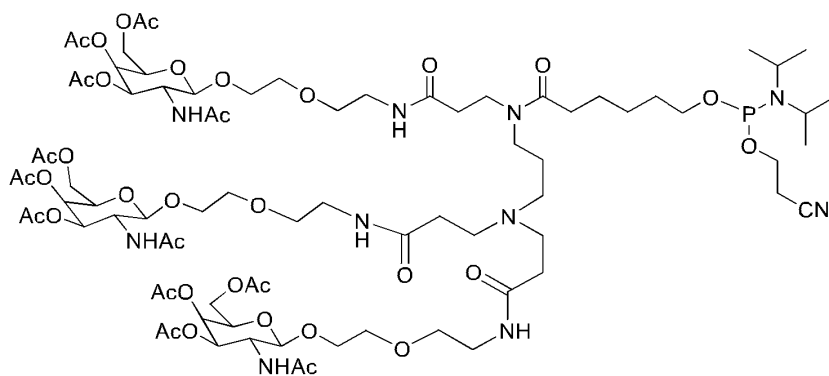
Compound 3;



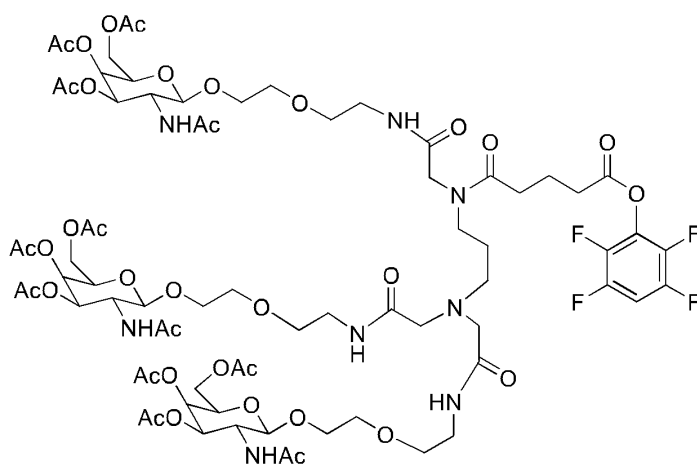
Compound 4;



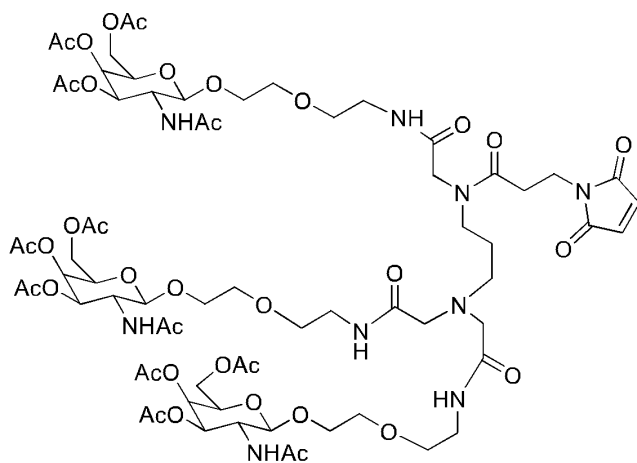
Compound 5;



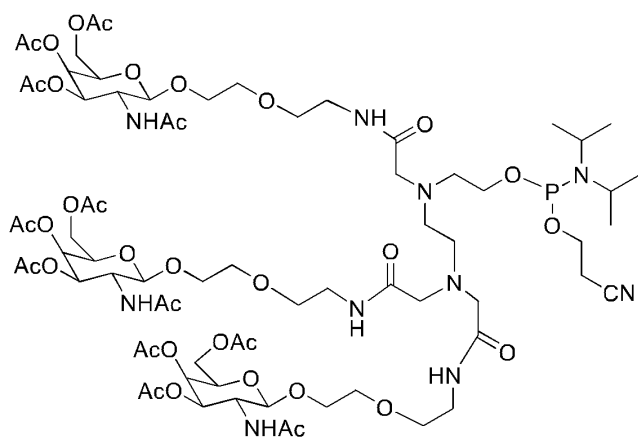
Compound 6;



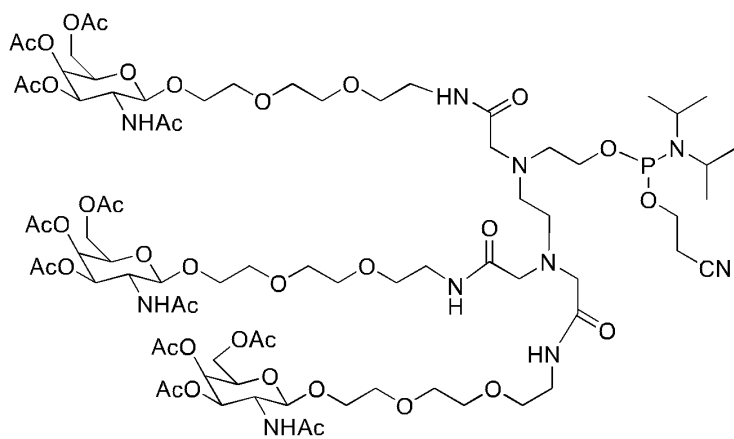
Compound 7;



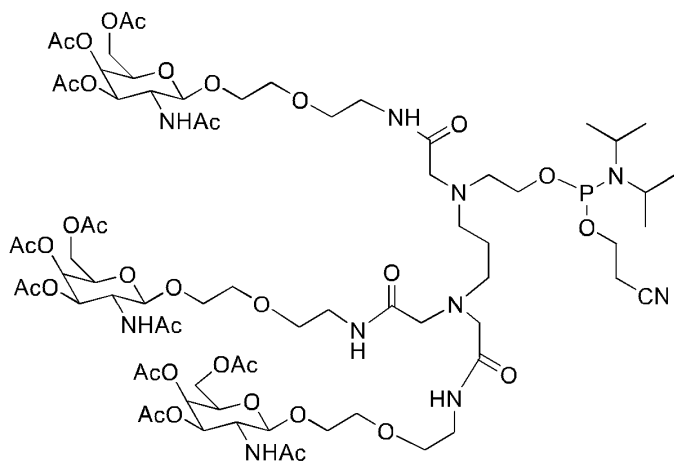
Compound 8;



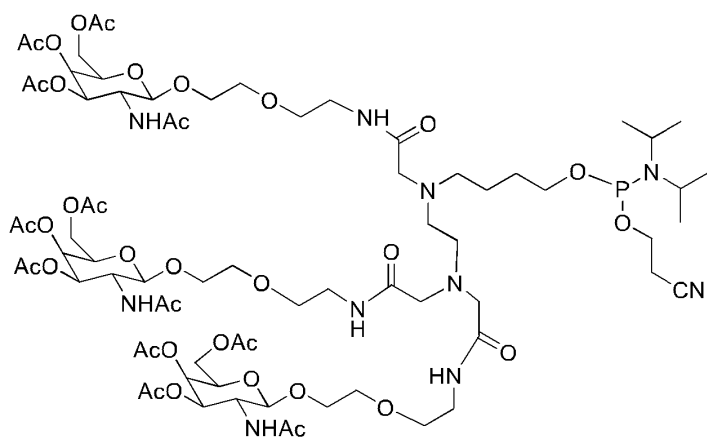
Compound 9;



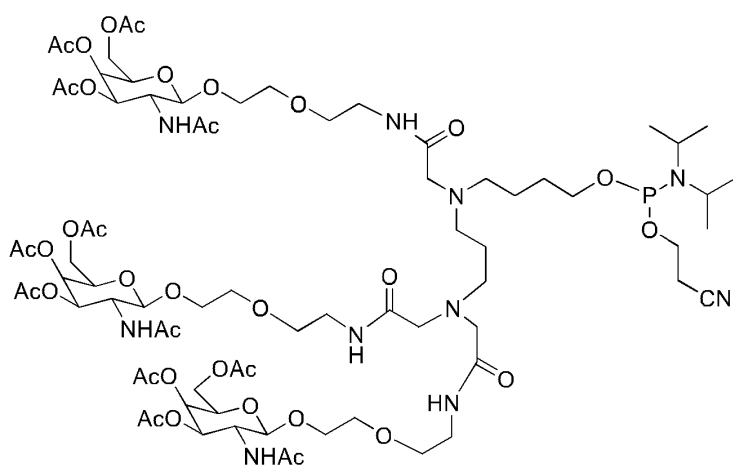
Compound 10;



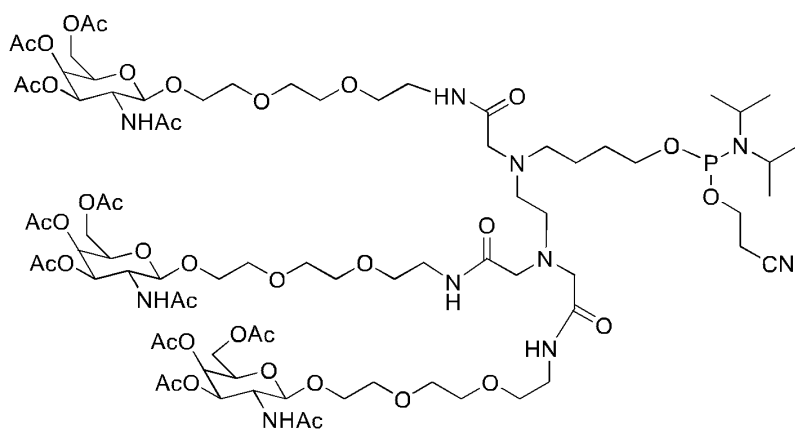
Compound



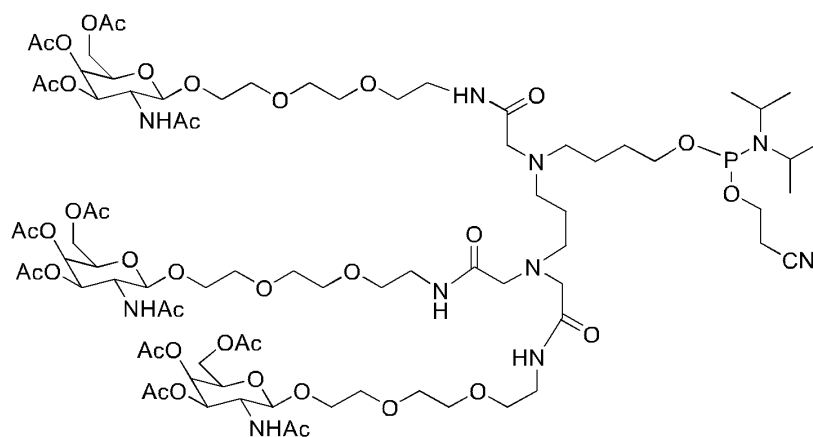
Compound 12;



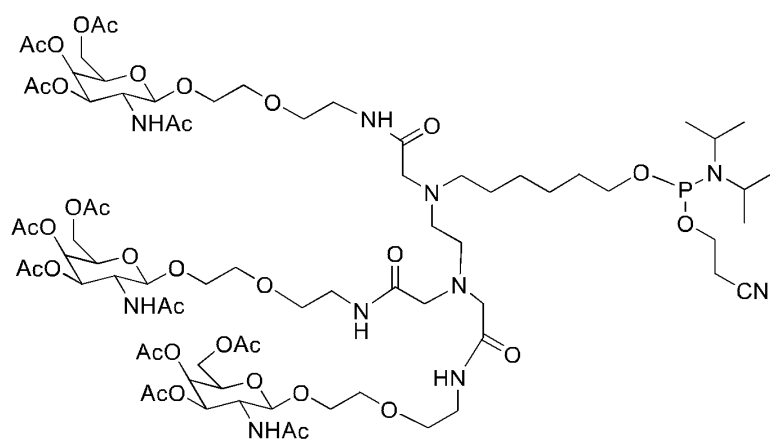
Compound 13;



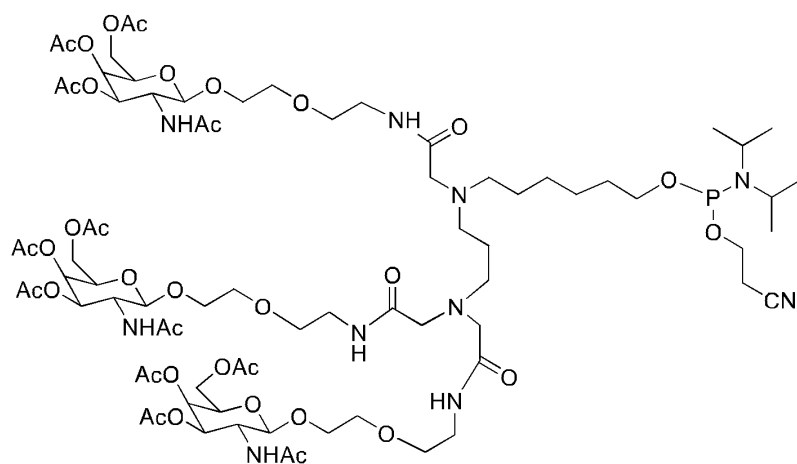
Compound 14;



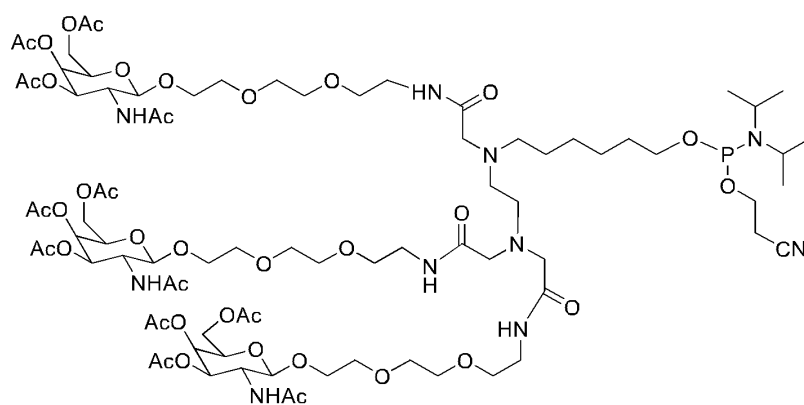
Compound 15;



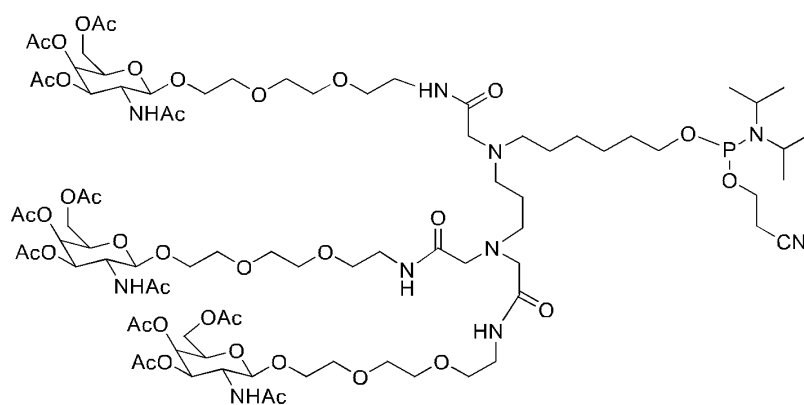
Compound 16;



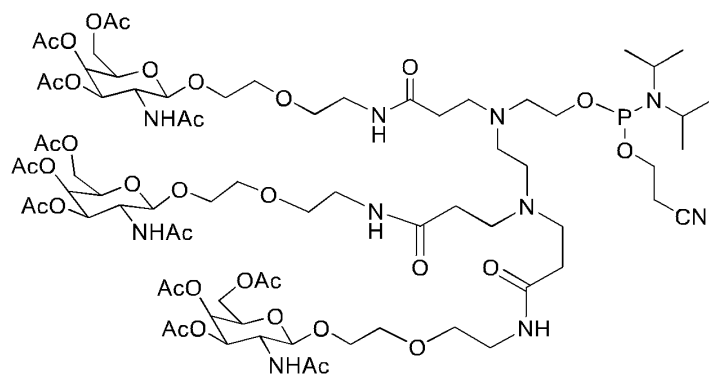
Compound 17;



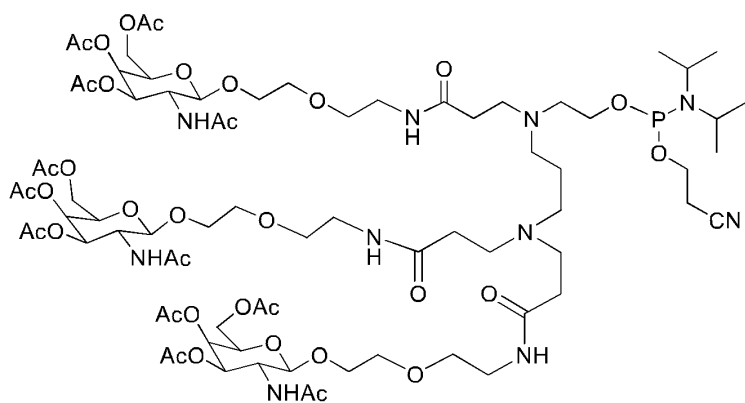
Compound 18;



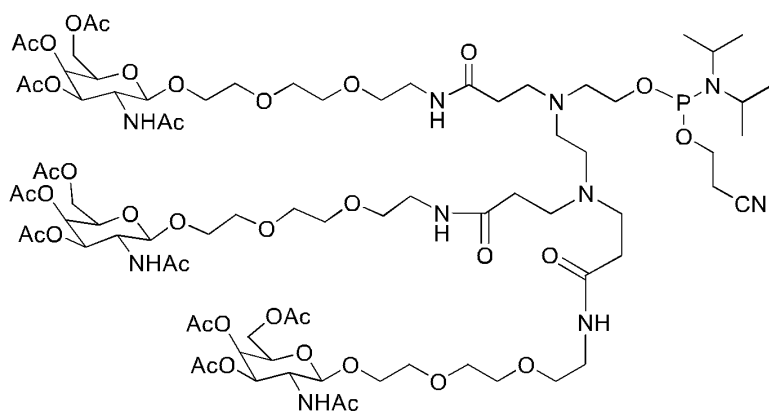
Compound 19;



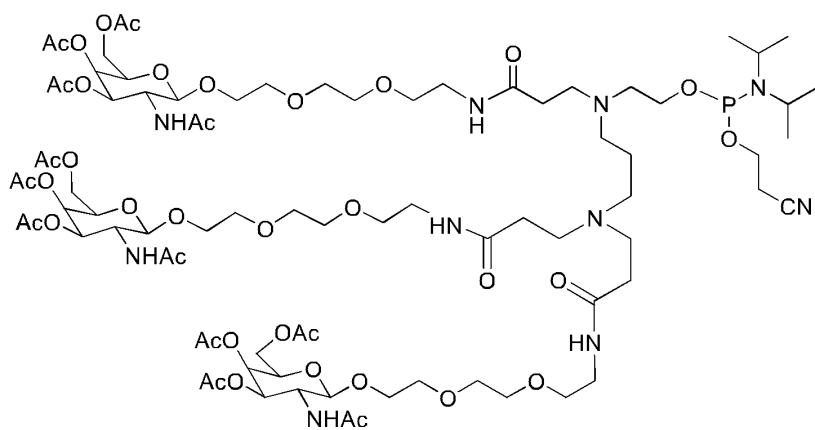
Compound 20;



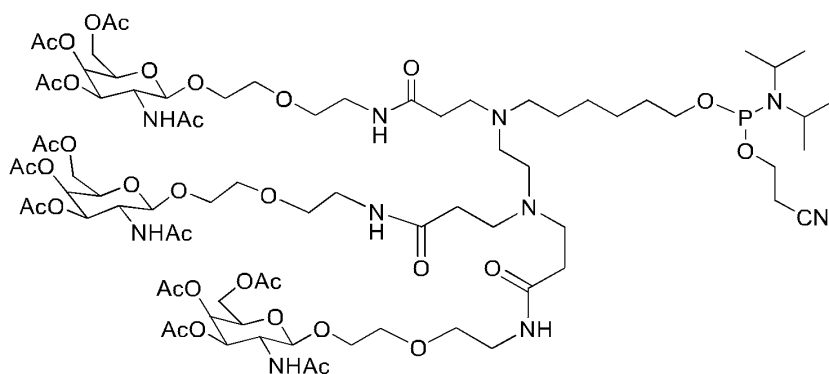
Compound 21;



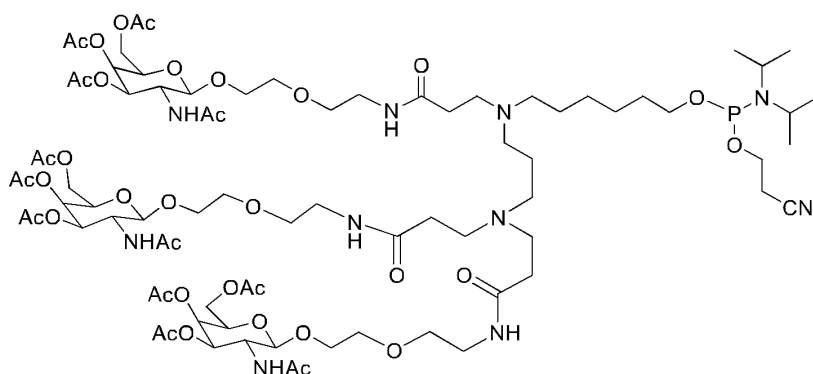
Compound 22;



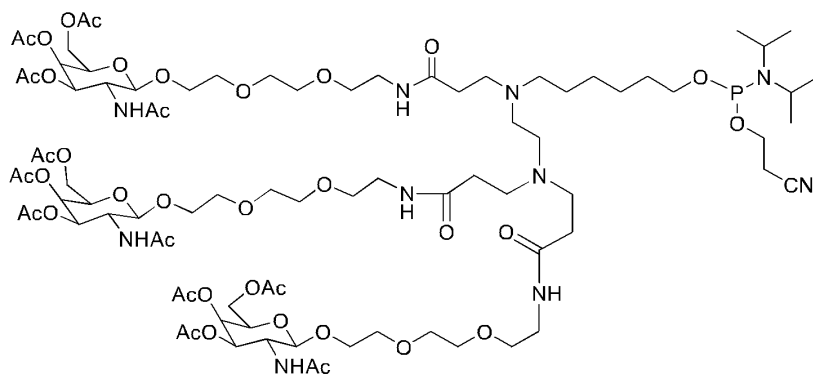
Compound 23;



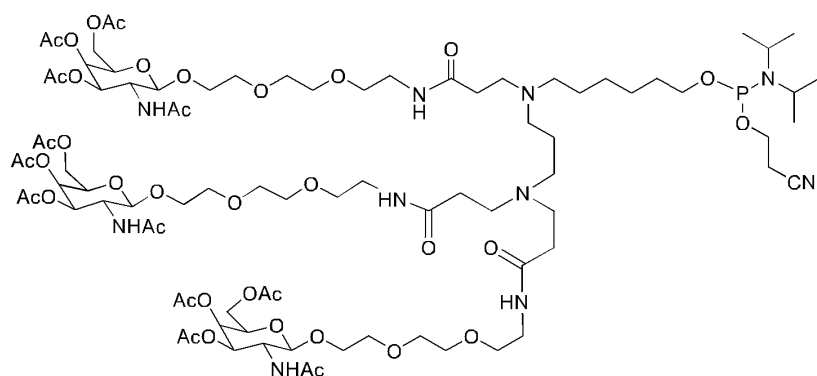
Compound 24;



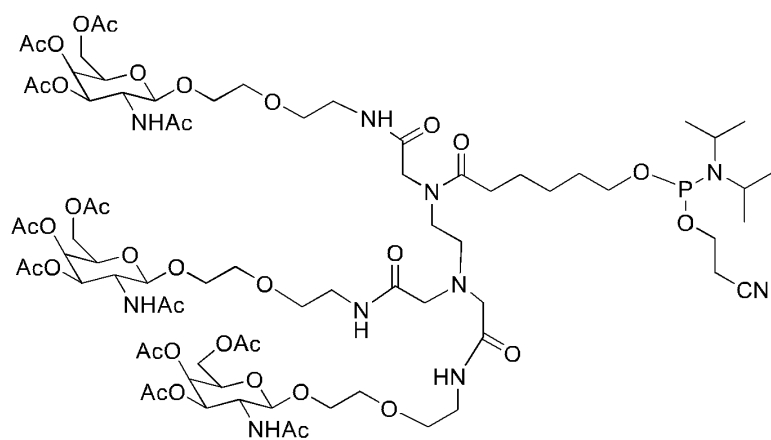
Compound 25;



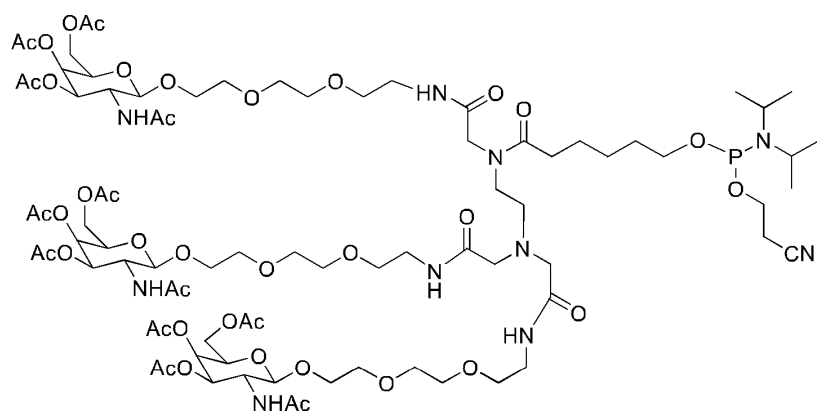
Compound 26;



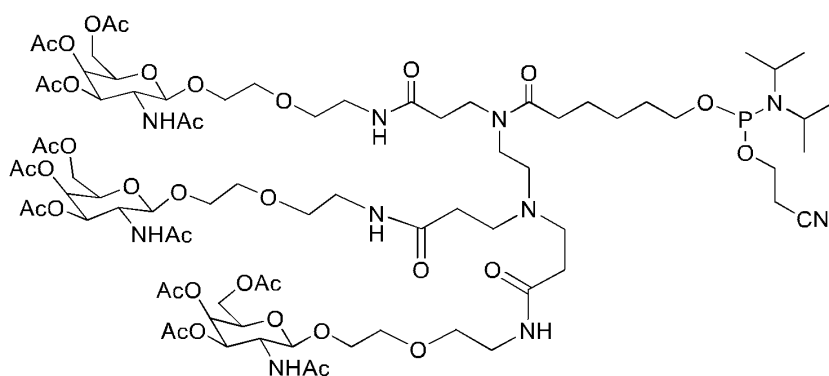
Compound 27;



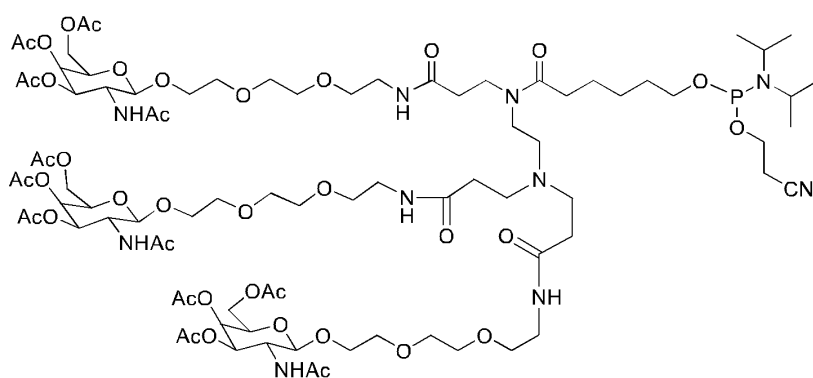
Compound 28;



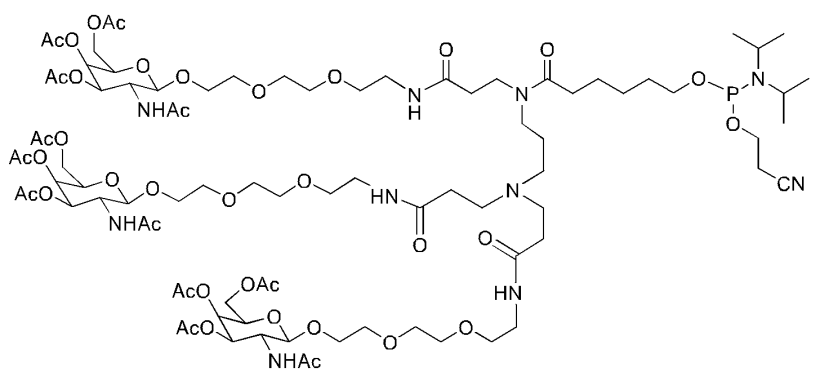
Compound 29;



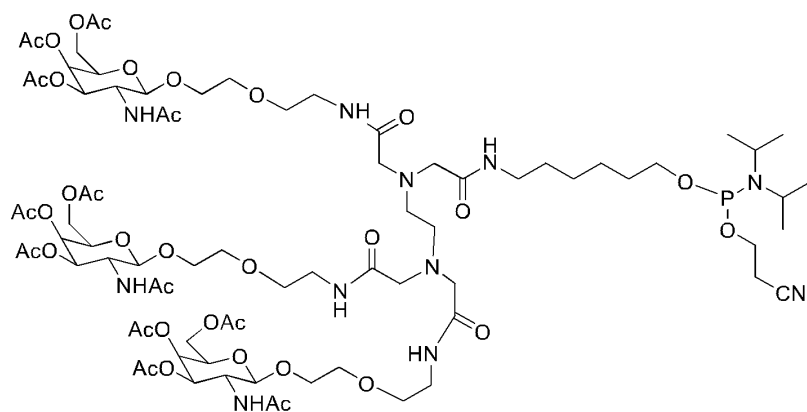
Compound 30;



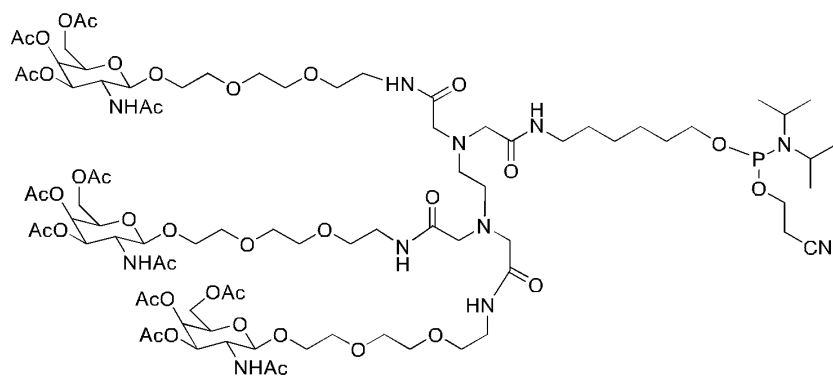
Compound 31;



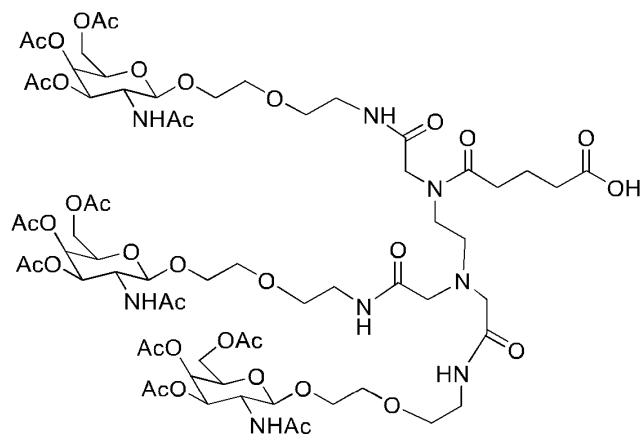
Compound 32;



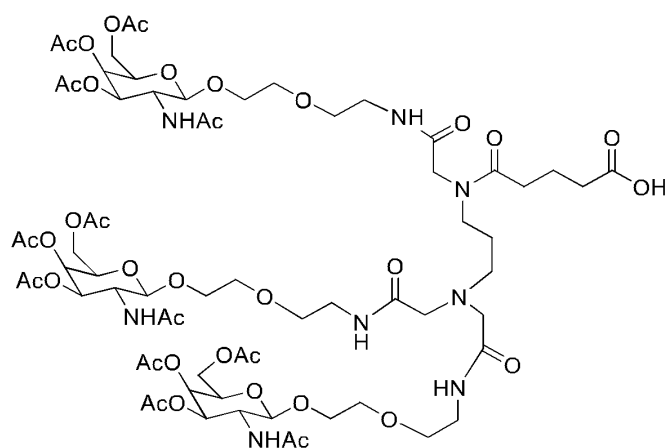
Compound 33;



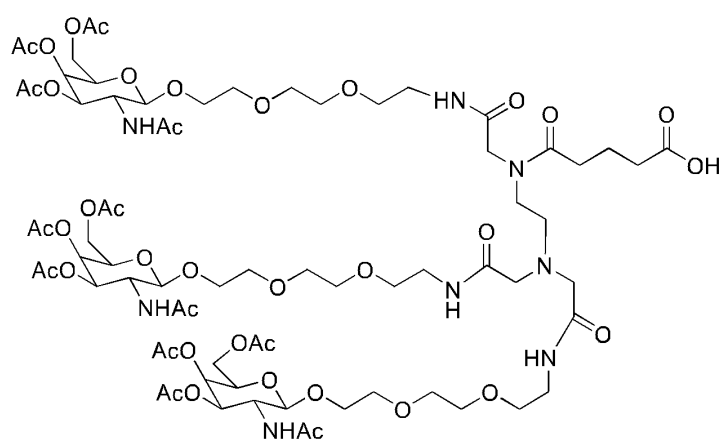
Compound 34;



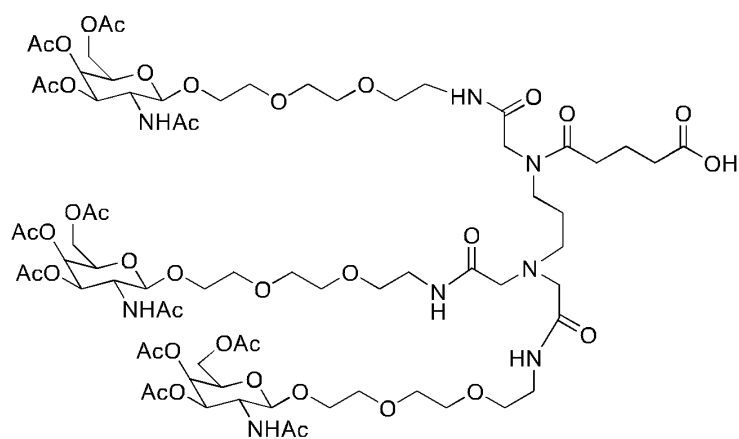
Compound 35;



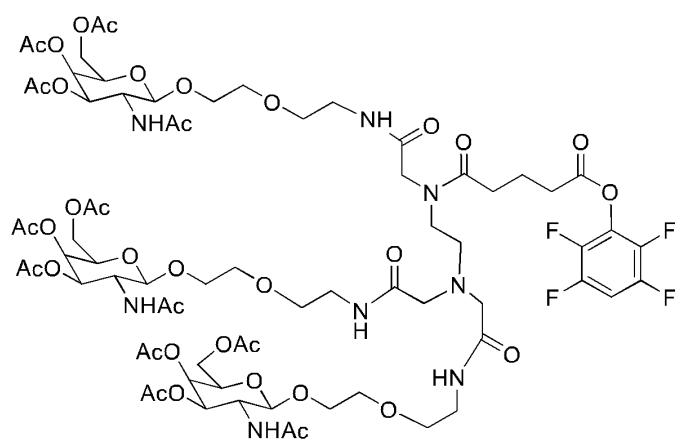
Compound 36;



Compound 37;

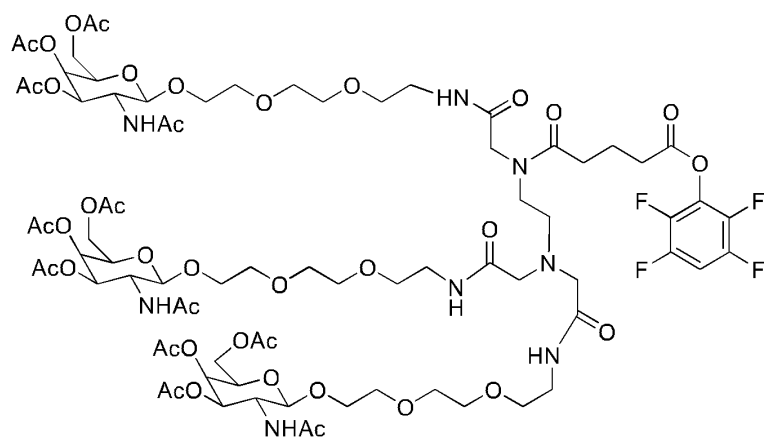


Compound 38;



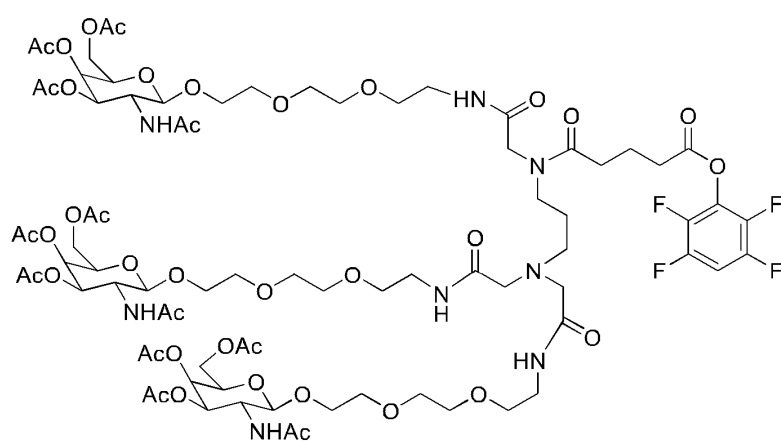
39;

Compound

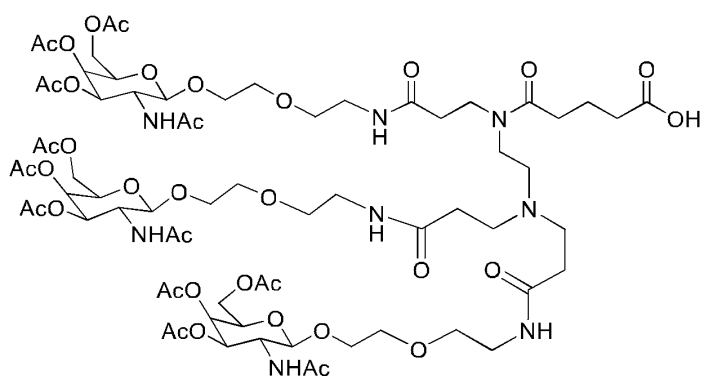


Compound 40;

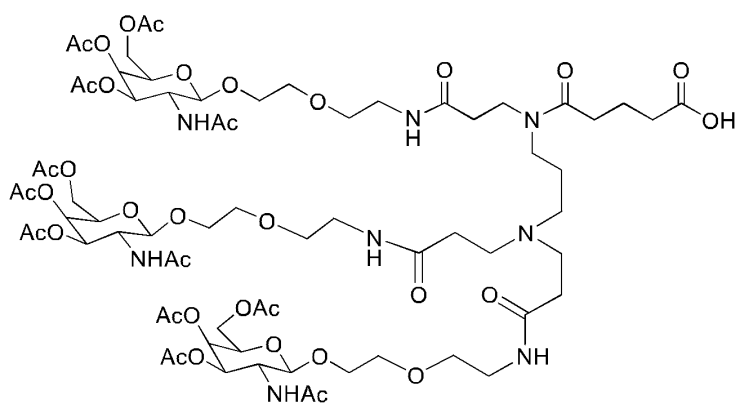
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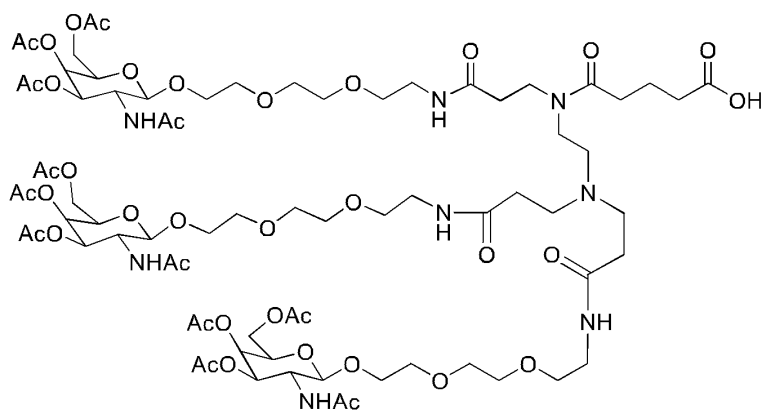
Compound 41;



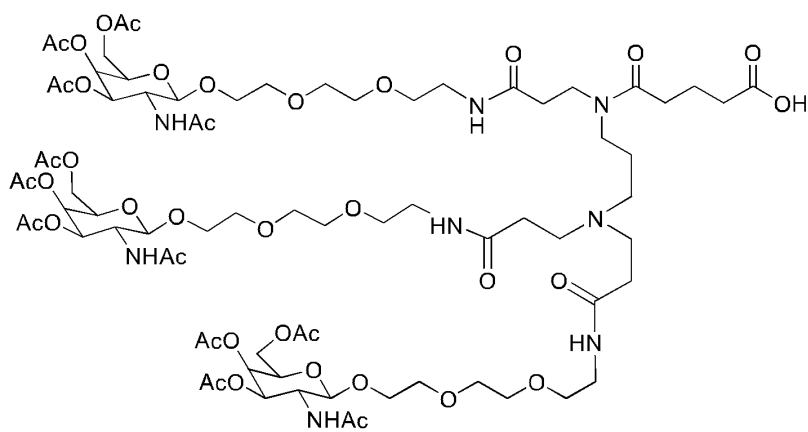
Compound 42;



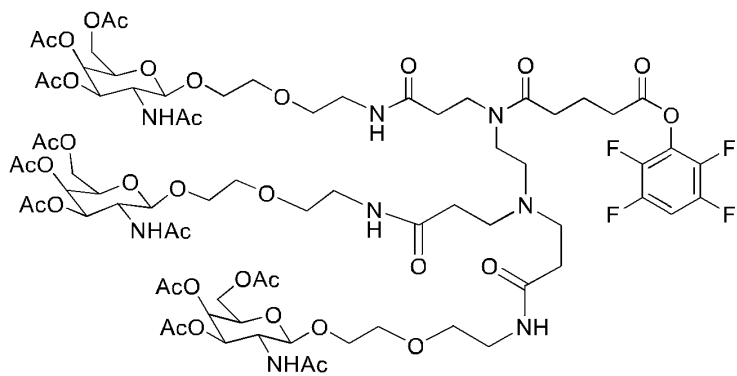
Compound 43;



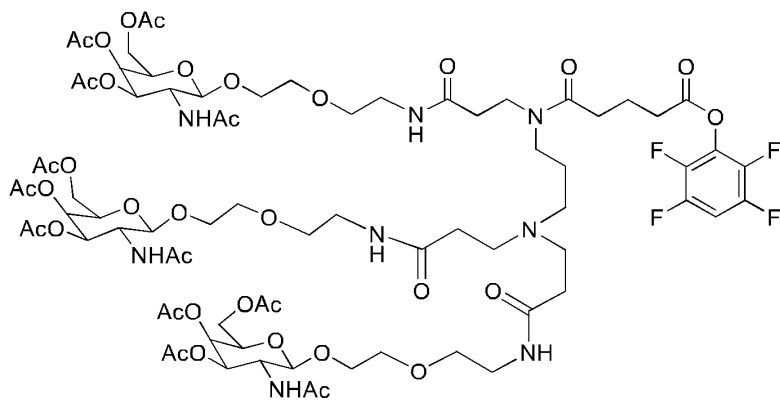
Compound 44;



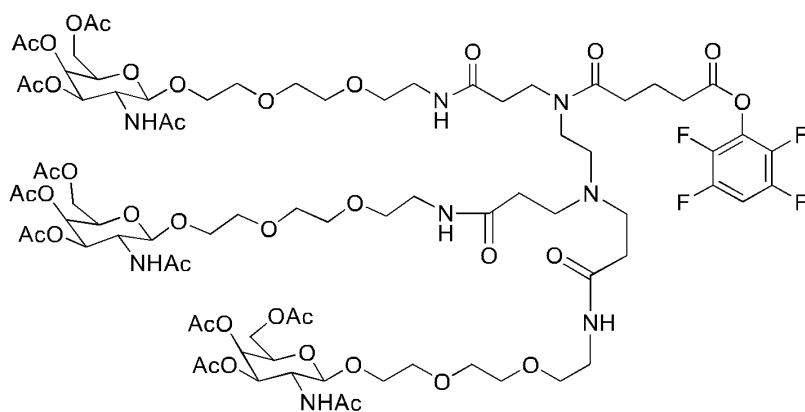
Compound 45;



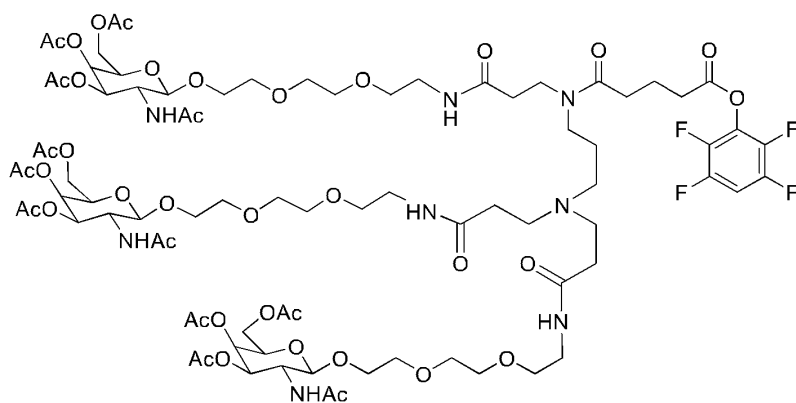
Compound 46;



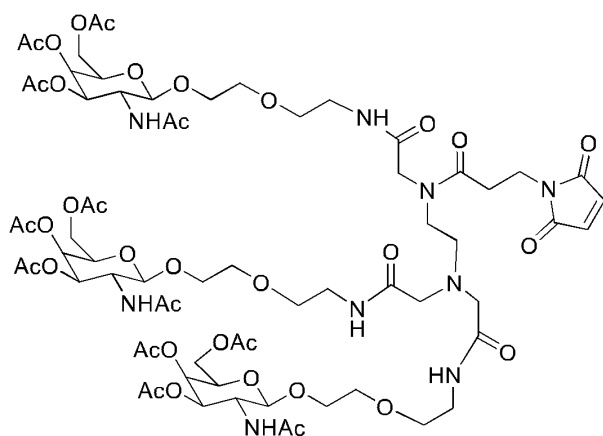
Compound 47;



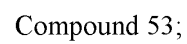
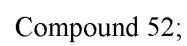
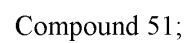
Compound 48;

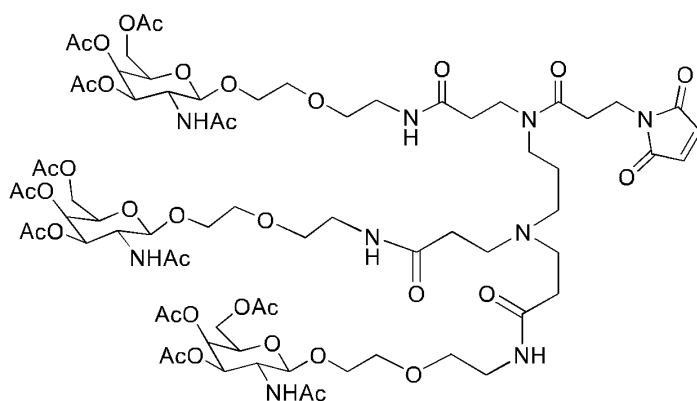


Compound 49;

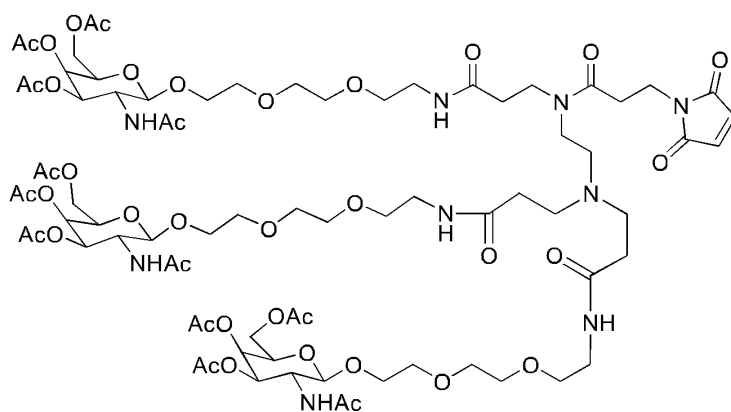


Compound 50;

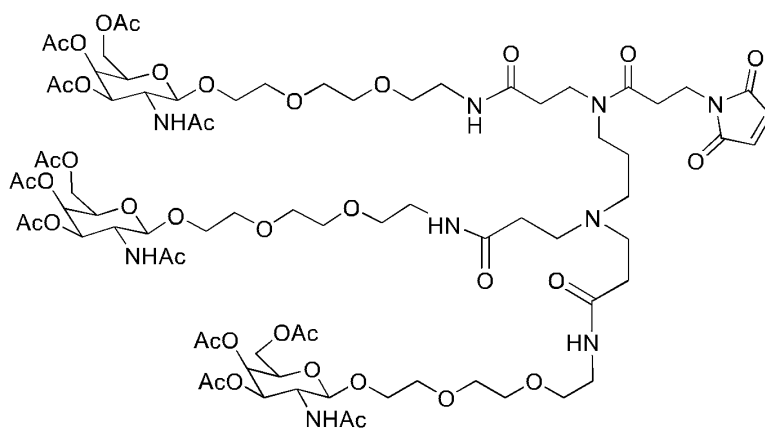




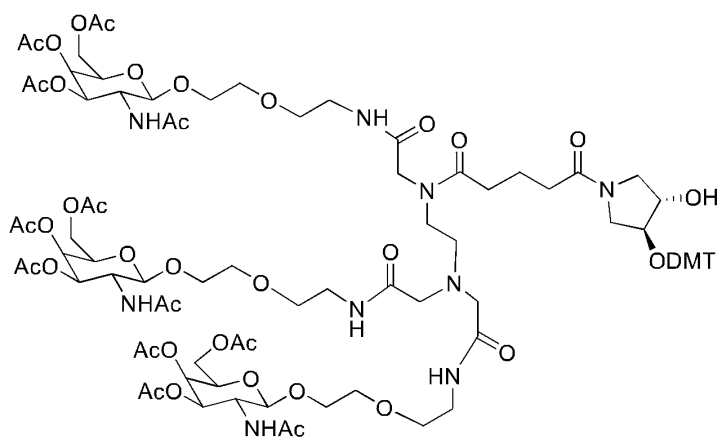
Compound 54;



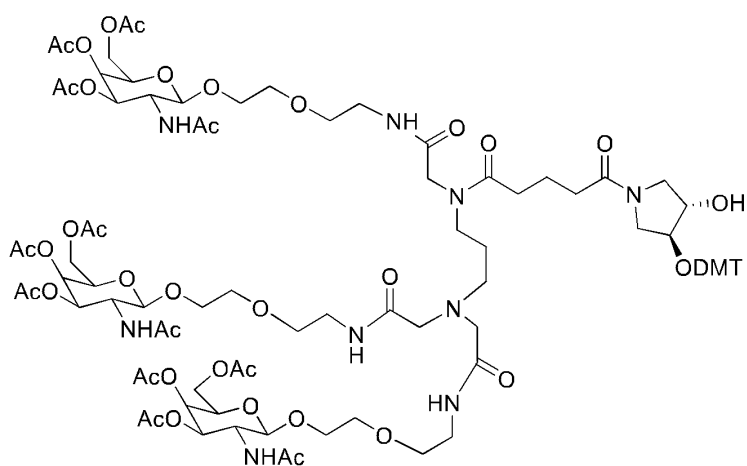
Compound 55;



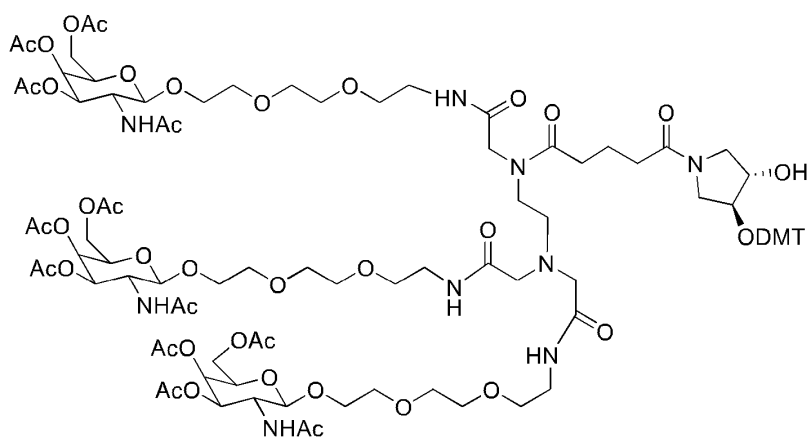
Compound 56;



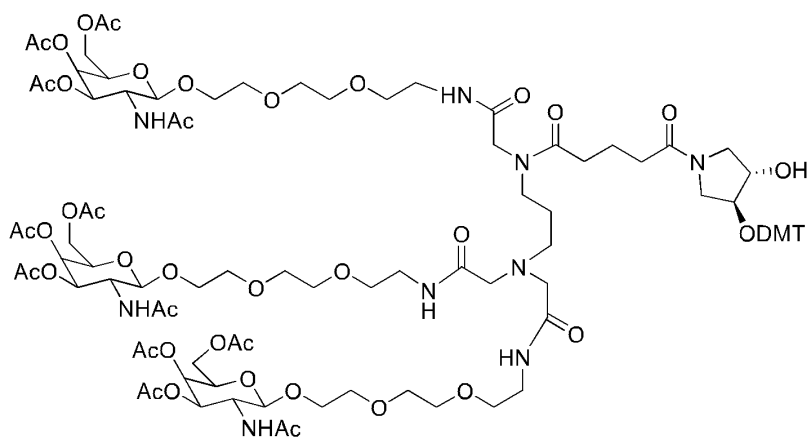
Compound 57;



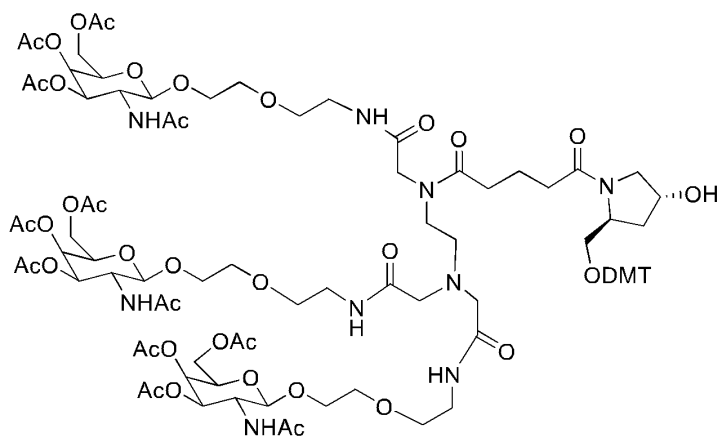
Compound 58;



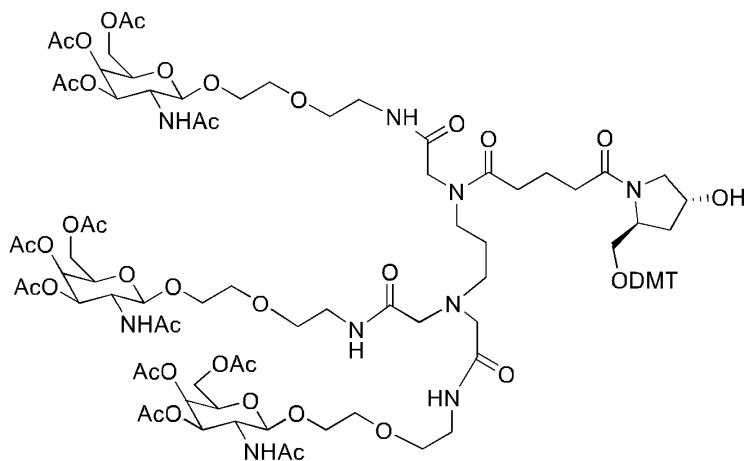
Compound 59;



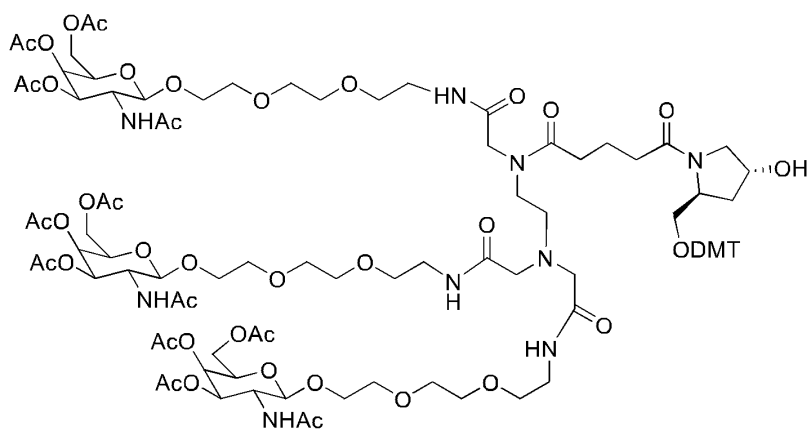
Compound 60;



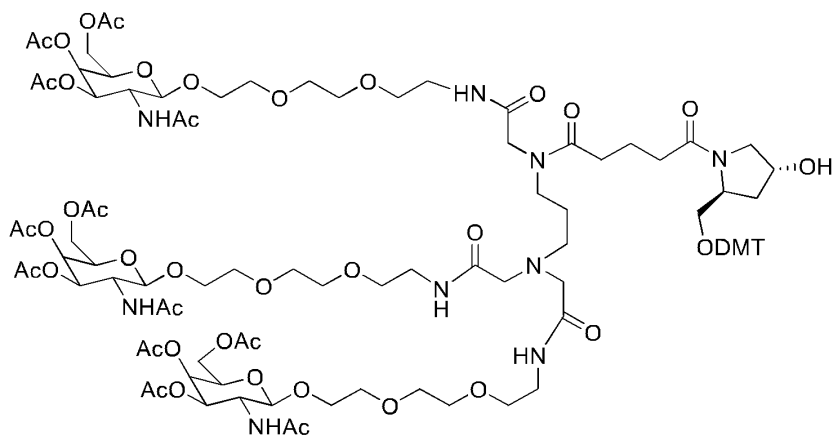
Compound 61;



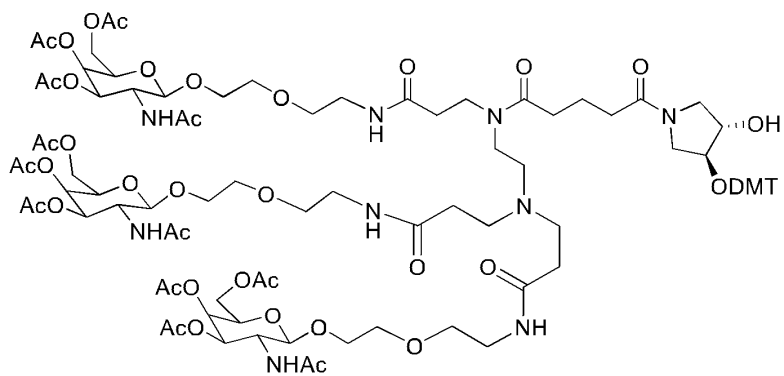
Compound 62;



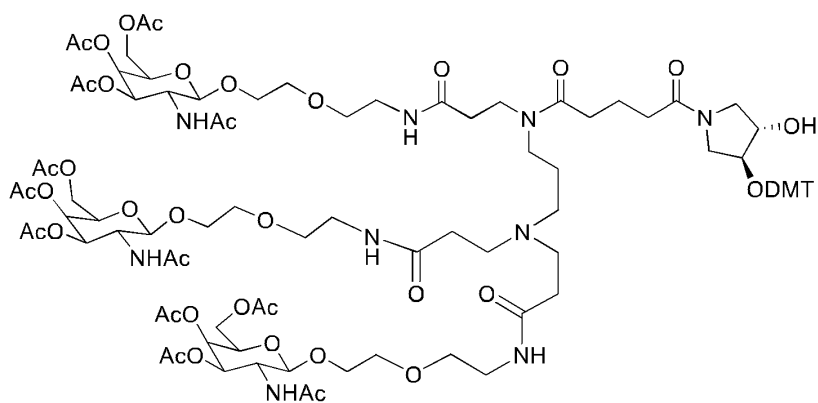
Compound 63;



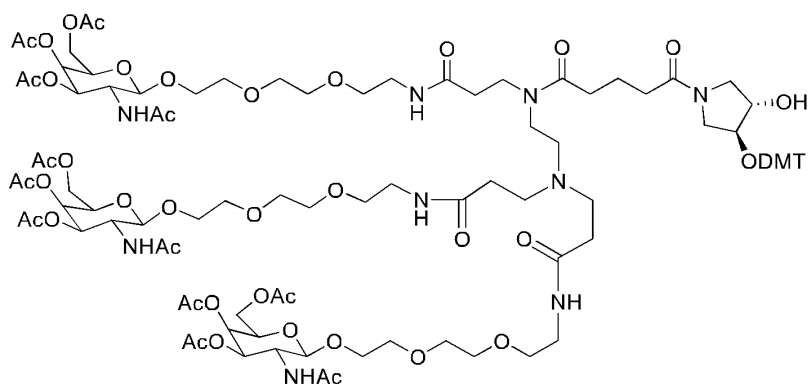
Compound 64;



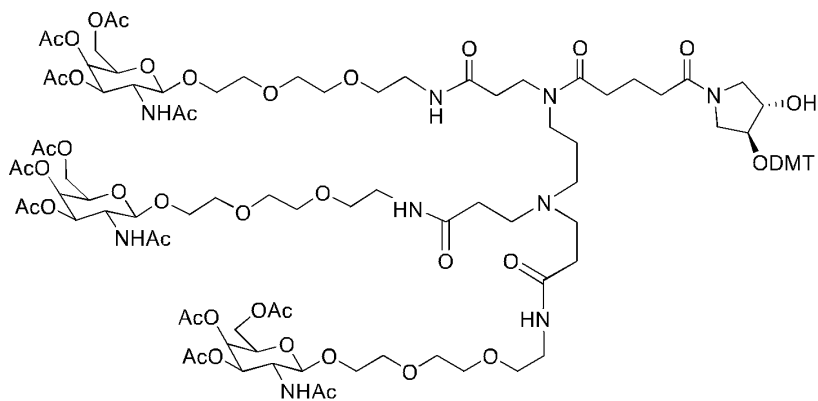
Compound 65;



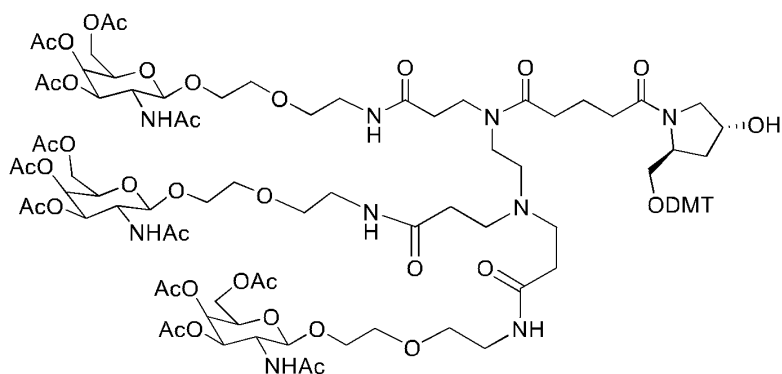
Compound 66;



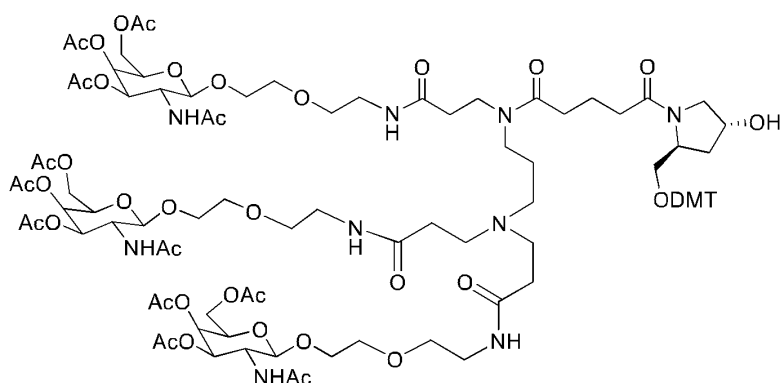
Compound 67;



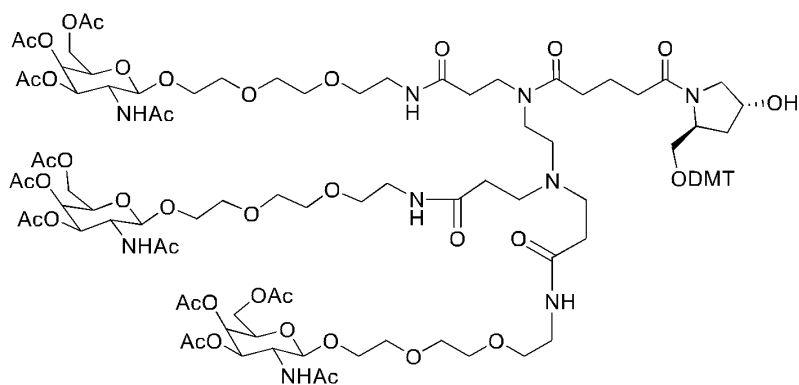
Compound 68;



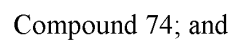
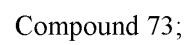
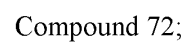
Compound 69;

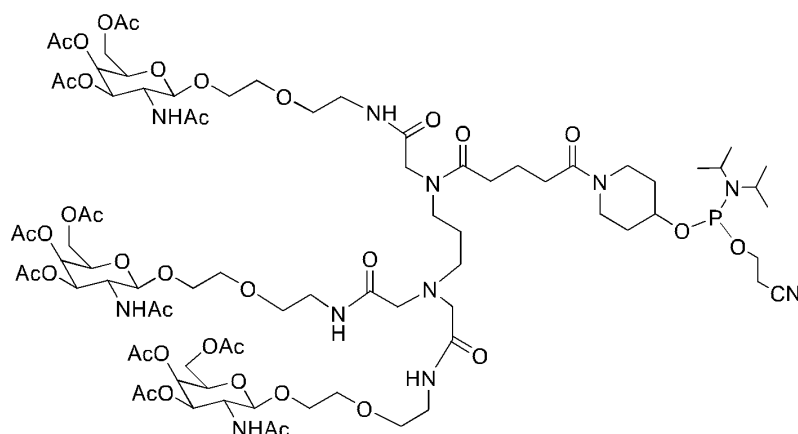


Compound 70;



Compound 71;





Compound 75.

90. The compound of claim 89, wherein the compound is a stereoisomer of one of Compound 1-75.

5

91. The compound of any one of claims 1-90, wherein W is the one or more pharmaceutical agents.

92. The compound of claim 91, wherein the one or more pharmaceutical agents comprises at least one of a small interfering RNA (siRNA), a single strand siRNA, a double stranded siRNA, a small activating RNA, an RNAi, a microRNA (miRNA), an antisense oligonucleotide, a short guide RNA (gRNA), a single guide RNA (sgRNA), a messenger RNA (mRNA), a ribozyme, a plasmid, an immune-stimulating nucleic acid, an antagomir, and an aptamer.

15

93. The compound of claim 92, wherein the double stranded siRNA comprises at least one modified ribonucleotide.

94. The compound of claim 92, wherein substantially all ribonucleotides of the double stranded siRNA are modified.

20

95. The compound of claim 92, wherein all ribonucleotides of the double stranded siRNA are modified.

96. The compound of any one of claims 92-95, wherein the modified ribonucleotide comprises a 2'-O-methyl nucleotide, 2'-Fluoro nucleotide, 2'-deoxy nucleotide, 2'3'-seco nucleotide mimic, locked nucleotide, 2'-F-Arabino nucleotide, 2'-methoxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted abasic nucleotide, inverted 2'-OMe nucleotide, inverted 2'deoxy nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, mopholino nucleotide, and 3'-OMe nucleotide, a nucleotide comprising a 5'-phosphorothioate group, or a 5'-(E)-vinyl phosphonate nucleotide (antisense strand only), or a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, a phosphoramidate, or a non-natural base comprising nucleotide.
97. The compound of any one of claims 92-96, wherein at least one strand of the double-stranded siRNA comprises at least one phosphorothioate linkage.
98. The compound of any one of claims 92-97, wherein at least one strand of the double-stranded siRNA comprises up to 6 phosphorothioate linkages.
99. The compound of any one of claims 92-98, wherein the double-stranded siRNA comprises at least one locked nucleic acid.
100. The compound of any one of claims 92-99, wherein the double-stranded siRNA comprises at least one unlocked nucleic acid.
101. The compound of any one of claims 92-100, wherein the double-stranded siRNA comprises at least one glycerol nucleic acid.
102. A pharmaceutical composition comprising the compound of any one of claims 1-101.
103. The pharmaceutical composition of claim 102, wherein W is the one or more pharmaceutical agents.
104. The pharmaceutical composition of claim 103, further comprising one or more therapeutic agents.

105. The pharmaceutical composition of claim 103 or 104, further comprising a pharmaceutically acceptable carrier.

- 5 106. A composition for targeted delivery of one or more pharmaceutical agents, the composition comprising the compound of any one of claims 1-90, wherein W is the one or more pharmaceutical agents.

107. The composition of claim 106, wherein the one or more pharmaceutical agents
10 comprises at least one of a small interfering RNA (siRNA), a single strand siRNA, a double stranded siRNA, a small activating RNA, a microRNA (miRNA), an antisense oligonucleotide, a short guide RNA (gRNA), a single guide RNA (sgRNA), a messenger RNA (mRNA), a ribozyme, a plasmid, an immune stimulating nucleic acid, an antagomir, and an aptamer.

15

108. The composition of claim 107, wherein the double-stranded siRNA comprises at least one modified ribonucleotide in one or both strands of the siRNA.

109. The composition of claim 108, wherein substantially all ribonucleotides of the double-
20 stranded siRNA are modified.

110. The composition of claim 108, wherein all ribonucleotides of the double-stranded siRNA are modified.

25 111. The composition of any one of claims 108-110, wherein the modified ribonucleotide comprises: a 2'-O-methyl nucleotide, 2'-Fluoro nucleotide, 2'-deoxy nucleotide, 2'3'-seco nucleotide mimic, locked nucleotide, 2'-F-Arabino nucleotide, 2'-methoxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted abasic nucleotide, inverted 2'-OMe nucleotide, inverted 2'deoxy nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified
30 nucleotide, morpholino nucleotide, and 3'-OMe nucleotide, a nucleotide comprising a 5'-phosphorothioate group, or a 5'-(E)-vinyl phosphonate nucleotide (antisense strand only), or a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group,

a 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, a phosphoramidate, or a non-natural base comprising nucleotide.

112. The composition of any one of claims 107-111, wherein at least one strand of the-
5 double stranded siRNA comprises at least one phosphorothioate linkage.

113. The composition of claim 112, wherein at least one strand of the double-stranded siRNA comprises up to 6 phosphorothioate linkages.

10 114. The composition of any one of claims 107-113, wherein the double-stranded siRNA comprises at least one locked nucleic acid.

115. The composition of any one of claims 107-114, wherein the double-stranded siRNA comprises at least one unlocked nucleic acid.

15

116. The compound of any one of claims 107-115, wherein the double-stranded siRNA comprises at least one glycerol nucleic acid.

117. A pharmaceutical composition comprising the composition of any one of claims 106-
20 116.

118. The pharmaceutical composition of claim 117, further comprising one or more therapeutic agents.

25 119. The pharmaceutical composition of claim 117 or 118, further comprising a pharmaceutically acceptable carrier.

120. A method for making a compound for targeted delivery of one or more pharmaceutical agents, the method comprising:
30 receiving a first compound comprising a diamine, the diamine comprises a first nitrogen and a second nitrogen, the first nitrogen being a primary amine, the second nitrogen being a secondary amine comprising a protecting group;

producing a second compound by coupling a plurality of protected carboxylic acids to the first compound, the first nitrogen in the second compound being a tertiary amine comprising a first protected carboxylic acid and a second protected carboxylic acid, the second nitrogen of the second compound being a tertiary amine comprising the protecting group and a third protected carboxylic acid;

producing a third compound by deprotecting the second nitrogen of the second compound, resulting in the second nitrogen becoming a secondary amine comprising the third protected carboxylic acid;

producing a fourth compound by attached a moiety comprising a hydroxy group to the second nitrogen of the third compound, resulting in the second nitrogen becoming a tertiary amine or an amide comprising the third protected carboxylic acid and the moiety comprising the hydroxy group;

producing a fifth compound by converting the protected carboxylic acids of the fourth compound into carboxylic acids; and

producing a sixth compound by performing an amide coupling reaction using the fifth compound, the first nitrogen in the sixth compound being a tertiary amine comprising a first amide and a second amide, the second nitrogen in the sixth compound being a tertiary amine comprising the moiety comprising the hydroxy group and a third amide, wherein the first amide, the second amide, and the third amide are each coupled to an independently selected targeting ligand.

121. The method of claim 120, wherein the protecting group is selected from the group consisting of a benzyl group and a triphenylmethyl group.

122. The method of claim 120 or 121, wherein producing the second compound comprises performing a S_N2 substitution reaction using the first compound.

123. The method of claim 120 or 121, wherein producing the second compound comprises performing a reductive amination reaction using the first compound.

124. The method of claim 120 or 121, wherein producing the second compound comprises performing a Michael addition reaction using the first compound.

125. The method of any one of claims 120-124, wherein:
the protecting group is a benzyl group; and
producing the third compound comprises performing a hydrogenation reaction using
the second compound.

5

126. The method of any one of claims 120-124, wherein:
the protecting group is a triphenylmethyl group; and
producing the third compound comprises reacting the second component with at least
one acid.

10

127. The method of any one of claims 120-126, wherein producing the fourth compound
comprises performing a S_N2 substitution reaction using the third compound.

128. The method of any one of claims 120-126, wherein producing the fourth compound
comprises performing a reductive amination reaction using the third compound.

129. The method of any one of claims 120-126, wherein producing the fourth compound
comprises performing a Michael addition reaction using the third compound.

20 130. The method of any one of claims 120-126, wherein producing the fourth compound
comprises performing an amide coupling reaction using the third compound.

131. The method of any one of claims 120-126, wherein producing the fourth compound
comprises performing a nucleophilic addition reaction using the third compound.

25

132. The method of any one of claims 120-131, wherein the moiety comprising the
hydroxy group is attached to the second nitrogen using the linkerB of any one of claims 24-
40.

30 133. The method of any one of claims 120-132, wherein producing the fifth compound
comprises reacting the fourth compound with at least one acid.

134. The method of claim 133, wherein the at least one acid comprises at least one of hydrochloric acid, hydrobromic acid, trifluoroacetic acid, and formic acid.
135. The method of any one of claims 120-134, wherein producing the fifth compound
5 comprises performing a hydrogenation reaction using the fourth compound.
136. The method of any one of claims 120-134, wherein producing the fifth compound comprises performing a hydrolysis reaction using the fourth compound.
- 10 137. The method of any one of claims 120-136, wherein the first amide, the second amide, and the third amide are each coupled to the independently selected targeting ligand using the independently selected linkerA of any one of claims 12-23.
138. The method of any one of claims 120-137, wherein the independently selected
15 targeting ligand is independently selected to be the targeting ligand of any one of claims 4-11.
139. The method of any one of claims 120-138, further comprising converting the hydroxy group to a phosphoramidite group using a phosphitylation reaction.
- 20 140. The method of claim 139, wherein converting the hydroxy group to the phosphoramidite group is performed after performing the amide coupling reaction to produce the sixth compound.
141. A method for making a compound for targeted delivery of one or more
25 pharmaceutical agents, the method comprising:
receiving a first compound comprising a diamine, the diamine comprising a first nitrogen and a second nitrogen, the first nitrogen being a secondary amine comprising a first protecting group, the second nitrogen being an amine comprising a second protecting group;
producing a second compound by coupling a first protected carboxylic acid to the first
30 nitrogen of the first compound, resulting in the first nitrogen becoming a tertiary amine;
removing the first protecting group from the first nitrogen of the second compound to produce a third compound comprising the first nitrogen and the second nitrogen, the first

nitrogen being a secondary amine comprising the first protected carboxylic acid, the second nitrogen being an amine comprising the second protecting group;

producing a fourth compound by coupling a second protected carboxylic acid to the first nitrogen of the third compound, resulting in the first nitrogen becoming a tertiary amine;

5 removing the second protecting group from the fourth compound to produce a fifth compound comprising the first nitrogen and the second nitrogen, the first nitrogen being a tertiary amine comprising the first protected carboxylic acid and the second protected carboxylic acid, the second nitrogen being a primary amine;

10 producing a sixth compound by coupling a third protected carboxylic acid to the second nitrogen of the fifth compound, resulting in the second nitrogen becoming a secondary amine;

producing a seventh compound by attaching a moiety comprising a hydroxy group to the second nitrogen of sixth compound, resulting in the second nitrogen becoming a tertiary amine;

15 producing an eighth compound by converting the third protected carboxylic acid of the seventh compound into a first carboxylic acid;

producing a ninth compound by performing an amide coupling reaction using the eighth compound, the first nitrogen of the ninth compound comprising the first protected carboxylic acid and the second protected carboxylic acid, the second nitrogen of the ninth compound comprising the a first amide having a first targeting ligand coupled thereto and the moiety comprising the hydroxy group;

producing a tenth compound by converting the second protected carboxylic acid of the ninth compound into a second carboxylic acid;

25 producing an eleventh compound by performing an amide coupling reaction using the tenth compound, the first nitrogen of the eleventh compound comprising the first protected carboxylic acid and a second amide having a second targeting ligand coupled thereto, the second nitrogen of the eleventh compound comprising the first amide having the first targeting ligand coupled thereto and the moiety comprising the hydroxy group;

30 producing a twelfth compound by converting the first protected carboxylic acid of the eleventh compound into a third carboxylic acid; and

producing a thirteenth compound by performing an amide coupling reaction using the twelfth compound, the first nitrogen of the thirteenth compound comprising the second amide having the second targeting ligand coupled thereto and a third amide having a third targeting

ligand coupled thereto, the second nitrogen of the thirteenth compound comprising the first amide having the first targeting ligand coupled thereto and the moiety comprising the hydroxy group.

- 5 142. The method of claim 141, wherein:
 the first protecting group is a benzyl group; and
 the second protecting group is a *tert*-butyloxycarbonyl (Boc) group.
- 10 143. The method of claim 141 or 142, wherein producing the second compound comprises performing a S_N2 substitution reaction using the first compound.
144. The method of claim 141 or 142, wherein producing the second compound comprises performing a reductive amination reaction using the first compound.
- 15 145. The method of claim 141 or 142, wherein producing the second compound comprises performing a Michael addition reaction using the first compound.
146. The method of any one of claims 141-145, wherein producing the third compound
20 comprises performing a hydrogenation reaction using the second compound.
147. The method of any one of claims 141-146, wherein producing the fourth compound
 comprises performing a S_N2 substitution reaction using the third compound.
- 25 148. The method of any one of claims 141-146, wherein producing the fourth compound
 comprises performing a reductive amination reaction using the third compound.
149. The method of any one of claims 141-146, wherein producing the fourth compound
 comprises performing a Michael addition reaction using the third compound.
- 30 150. The method of any one of claims 141-146, wherein producing the fourth compound
 comprises performing an amide coupling reaction using the third compound.

151. The method of any one of claims 141-146, wherein producing the fourth compound comprises performing a nucleophilic addition reaction using the third compound.

152. The method of any one of claims 141-151, wherein producing the fifth compound
5 comprises reacting the fourth compound with at least one acid.

153. The method of claim 152, wherein the at least one acid comprises at least one of hydrochloric acid and trifluoroacetic acid.

10 154. The method of any one of claims 141-153, wherein producing the sixth compound comprises performing a S_N2 substitution reaction using the fifth compound.

155. The method of any one of claims 141-153, wherein producing the sixth compound comprises performing a reductive amination reaction using the fifth compound.

15

156. The method of any one of claims 141-153, wherein producing the sixth compound comprises performing a Michael addition reaction using the fifth compound.

157. The method of any one of claims 141-156, wherein producing the seventh compound
20 comprises performing a S_N2 substitution reaction using the sixth compound.

158. The method of any one of claims 141-156, wherein producing the seventh compound comprises performing a reductive amination reaction using the sixth compound.

25 159. The method of any one of claims 141-156, wherein producing the seventh compound comprises performing a Michael addition reaction using the sixth compound.

160. The method of any one of claims 141-156, wherein producing the seventh compound comprises performing an amide coupling reaction using the sixth compound.

30

161. The method of any one of claims 141-156, wherein producing the seventh compound comprises performing a nucleophilic addition reaction using the sixth compound.

162. The method of any one of claims 141-161, wherein the first amide is coupled to the first targeting ligand using an independently selected linkerA of any one of claims 12-23.
163. The method of any one of claims 141-162, wherein the second amide is coupled to the second targeting ligand using an independently selected linkerA of any one of claims 12-23.
164. The method of any one of claims 141-163, wherein the third amide is coupled to the third targeting ligand using an independently selected linkerA of any one of claims 12-23.
165. The method of any one of claims 141-164, wherein the first targeting ligand, the second targeting ligand, and the third targeting ligand are independently selected to be one or more of the targeting ligands of any one of claims 4-11.
166. The method of any one of claims 141-165, wherein the hydroxy group is coupled to the second nitrogen using the linkerB of any one of claims 24-40.
167. The method of any one of claims 141-166, further comprising converting the hydroxy group to a phosphoramidite group using a phosphorylation reaction.
168. The method of claim 167, wherein converting the hydroxy group to the phosphoramidite group is performed after producing the thirteenth compound.
169. A method for delivering a pharmaceutical agent to a subject, the method comprising:
administering, to the subject,
(a) the compound of any one of claims 1-90, wherein W is the one or more pharmaceutical agents, or
(b) the composition of any one of claims 106-116.
170. The method of claim 169, wherein the subject is a vertebrate.
171. The method of claim 169, wherein the subject is a mammal.
172. The method of claim 169, wherein the mammal is a human.

173. The method of any one of claims 169-172, wherein the compound is administered in a pharmaceutically acceptable carrier.

5 174. A method for delivering a pharmaceutical agent to a subject, the method comprising: administering, to the subject, a pharmaceutical composition of any one of claims 102, 103, 104, 105, 116, 117, 118, or 119.

175. The method of claim 174, wherein the subject is a vertebrate.

10

176. The method of claim 174, wherein the subject is a mammal, optionally the mammal is a human.

177. The method of claim 174, wherein the one or more pharmaceutical agents comprises
15 at least one of a small interfering RNA (siRNA), a single strand siRNA, a double stranded siRNA, a small activating RNA, a microRNA (miRNA), an antisense oligonucleotide, a short guide RNA (gRNA), a single guide RNA (sgRNA), a messenger RNA (mRNA), a ribozyme, a plasmid, an immune stimulating nucleic acid, an antagomir, and an aptamer.

20 178. The method of claim 177, wherein the double-stranded siRNA comprises at least one modified ribonucleotide in one or both strands of the siRNA.

179. The method of claim 178, wherein substantially all ribonucleotides of the double-stranded siRNA are modified.

25

180. The method of claim 178, wherein all ribonucleotides of the double-stranded siRNA are modified.

181. The method of any one of claims 178-180, wherein the modified ribonucleotide
30 comprises: a 2'-O-methyl nucleotide, 2'-Fluoro nucleotide, 2'-deoxy nucleotide, 2'3'-seco nucleotide mimic, locked nucleotide, 2'-F-Arabino nucleotide, 2'-methoxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted abasic nucleotide, inverted 2'-OMe nucleotide, inverted 2'-deoxy nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified

nucleotide, morpholino nucleotide, and 3'-OMe nucleotide, a nucleotide comprising a 5'-phosphorothioate group, or a 5'-(E)-vinyl phosphonate nucleotide (antisense strand only), or a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, a phosphoramidate, or a non-natural base comprising nucleotide.

182. The method of any one of claims 177-181, wherein at least one strand of the double stranded siRNA comprises at least one phosphorothioate linkage.

183. The method of claim 182, wherein at least one strand of the double-stranded siRNA comprises up to 6 phosphorothioate linkages.

184. The method of any one of claims 177-183, wherein the double-stranded siRNA comprises at least one locked nucleic acid.

185. The method of any one of claims 177-184, wherein the double-stranded siRNA comprises at least one unlocked nucleic acid.

186. The method of any one of claims 177-185, wherein the double-stranded siRNA comprises at least one glycerol nucleic acid.

187. The method of any one of claims 174-186, wherein the pharmaceutical composition further comprises one or more therapeutic agents.

188. The compound of claim 1, wherein n is 1 or 2.