Cellular Delivery Introduction

Oligonucleotides are single-stranded nucleic acid molecules, and due to their phosphodiester backbones, are predominantly hydrophilic. While this is usually not a problem for in vitro applications, for in vivo work, the hydrophobic nature of the cell membrane makes it difficult for oligos to permeate it and enter the cell. The need to modify oligos to improve their membrane permeability and cellular uptake can be critical in anti-sense, siRNA, or in vivo diagnostic applications. Generally speaking, such modifications need to be both hydrophobic and relatively non-toxic. Modification of oligos at the 5'- or 3'-ends with cholesterol or PEG are strategies commonly used to achieve this.

Cellular Delivery Design Protocols

Cell Membrane Permeation Design Considerations

Unmodified oligonucleotides generally show poor bioavailability in vivo, due to their inability to effectively penetrate cell membranes. The primary reason for this is that the polyanionic character of unmodified oligonucleotides makes them highly hydrophilic, but since the surfaces of cell membranes are hydrophobic, the former cannot effectively enter into the cell.

To reduce this polyanionic effect and improve delivery efficacy, one approach is to use an oligonucleotide complexing agent as a delivery vehicle, for example, cationic lipid formulations, cyclodextrins, etc. Such complexing agents have been shown to increase the efficiency of oligo cellular uptake relative to that observed with naked oligonucleotides, but because the agent is not covalently bound to the oligo and an excess of the agent must be used, the actual amount of oligo that is actually available for delivery into the cell typically is low (6).

Alternatively, oligonucleotides can be directly conjugated to a lipophilic moiety that will make it easier for the oligo to penetrate cell membranes. Cholesterol and polyethylene glycol (PEG) are two such moieties that are commonly used as modifications for this purpose (7). An important advantage of this approach is that the amounts of lipophile-oligo conjugates actually available for delivery into the cell tend to be significantly higher than those observed with oligo-complexing delivery vehicles. At the same time, it is important to be aware that, in some cases, covalent attachment of the lipophilic moiety to the oligo negatively impacts the latter's intra-cellular distribution or ability to hybridize to its target. In such cases, a combination of the two approaches (complexation and direct conjugation) may be necessary to resolve the problem (6).

A related approach is to conjugate the lipophilic moiety to a "delivery" oligo that is complementary to the oligo slated for delivery into the cell. In duplex form, the modified "delivery" oligo thus serves to assist passage of the other oligo through the cell membrane. The effect is often enhanced when the duplex is mixed with a cationic lipid formulation or other oligo complexing agent before either being applied to cell culture or injected into the organism. An attractive feature of this "oligo-assisted oligo delivery" method is that because the lipophilic moiety is not conjugated to the oligo slated for delivery, it does not affect either that oligo's intra-cellular distribution or its ability to hybridize to its target. This strategy has been employed, using cholesterol as the lipophilic moiety, in both anti-sense and siRNA applications (8, 9).

Cellular Delivery Applications

For in vivo studies, it is important to keep in mind that oligonucleotides, and their delivery agents, almost always are taken up by the cell via endocytosis, and need to exit from the endosome to reach their designated cellular target. Thus, in order to obtain both good uptake and effective delivery of an oligo to its target in vivo, the multiple routes of endocytosis and the molecular trafficking pathways of cells need to be considered (1), as well as clearance issues at the whole organism level (for example, excretion by the kidney or uptake by phagocytes that may sequester the oligo in the spleen or liver) (2). Conjugation of cholesterol to siRNA or anti-sense oligos increases the resulting conjugate's association with serum proteins, which often has a positive effect on their pharmacokinetics (for example, elimination half-life and tissue accumulation), biodistribution and effects on gene expression (3). For cholesterol-linked siRNAs, both high density lipoprotein (HDL) and low density lipoprotein (LDL) were their primary carriers in serum. Uptake of the oligos occurs via both LDL- and HDL-receptors, with LDL-receptors being the predominant receptor utilized in liver (4). Cell entry appears to occur by a complex receptor-mediated endocytosis mechanism that includes transfer of the cholesterol-oligo conjugate from the receptor to a plasma membrane protein Sid1 along the way (4). Oligos can also be conjugated with PEG (PEGylated) to improve in vivo uptake. PEGylated oligo conjugates are less susceptible to phagocytes, since they poorly absorb to plasma proteins (opsonins) that enhance phagocytosis. This significantly increases the elimination half-life of the oligos in circulation, thereby facilitating cellular uptake (5).

References

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Modificaton Code List

Modification	Code	Catalog Number
2'-O-C16 Adenosine siRNA LMO: 2'-O-hexadecyl (C16)	[C16-2-O-A]	27-6541A
2'-O-C16 Cytidine siRNA LMO: 2'-O-hexadecyl (C16)	[C16-2-O-C]	27-6541C
2'-O-C16 Guanosine siRNA LMO: 2'-O-hexadecyl (C16)	[C16-2-O-G]	27-6541G
2'-O-C16 Uridine siRNA LMO: 2'-O-hexadecyl (C16)	[C16-2-O-U]	27-6541U
2'-O-C22 Adenosine siRNA LMO: 2'-O-docosyl (C22)	[C22-2-O-A]	27-6542A
2'-O-C22 Cytidine siRNA LMO: 2'-O-docosyl (C22)	[C22-2-O-C]	27-6542C
2'-O-C22 Guanosine siRNA LMO: 2'-O-docosyl (C22)	[C22-2-O-G]	27-6542G
2'-O-C22 Uridine siRNA LMO: 2'-O-docosyl (C22)	[C22-2-O-U]	27-6542U
Alpha Tocopherol TEG (Vitamin E)	[a-toco-TEG]	26-6616
Alpha-linolyl (C18:3α) ALA LMO	[3-ALA-C18-3]	26-6784
Arachidonyl (C20:4) AA LMO	[3-AA-C20-4]	26-6787
butyric acid (C4) Modified Oligo	[3-BA-C4]	26-6782
Cholesterol TEG (15 atom triethylene glycol spacer)5' LMO	[CholTEG-5]	26-6602
Cholesterol TEG-3' (15 atom triethylene glycol spacer) LMO	[CholTEG-3]	26-6570
Delta Tocopherol TEG (Vitamin E)	[d-toco-TEG]	26-6628
Docosanoic (C22) DCA LMO	[3-DCA-C22]	26-6627
Docosahexaenoic (C22:6) DHA LMO	[3-DHA-C22-6]	26-6626
Dihomo-gamma-linolyl (C20:3) DGLA LMO	[3-DGLA-C20-3]	26-6786
Eicosapent (20:5) EPA LMO	[3-EPA-C20-5]	26-6788
Folic Acid NHS (Vitamin B9)	[Folate-N]	26-6631

GalNAc Oligo N-Acetylgalactosamine C3	[GalNAc]	26-6751
GalNAc Trivalent TEG	[GalNAc-TEG3X]	26-6735
Gamma-linolyl (C18:3γ) GLA LMO	[3-GLA-C18-3]	26-6785
Lignoceryl C24 (3') LMO	[3-Lig-C24]	26-6624
Lignoceryl C24 (5') LMO	[5-Lig-C24]	26-6625
Linolyl (C18:2) LA LMO	[3-LA-C18-2]	26-6783
Ethoxy Phosphate dA	[EoP-dA]	26-6641A
Ethoxy Phosphate dC	[EoP-dC]	26-6641C
Ethoxy Phosphate dG	[EoP-dG]	26-6641G
Ethoxy Phosphate dT	[EoP-dT]	26-6641T
Methoxy Phosphate dA	[MoP-dA]	26-6642A
Methoxy Phosphate dC	[MoP-dC]	26-6642C
Methoxy Phosphate dG	[MoP-dG]	26-6642G
Methoxy Phosphate dT	[MoP-dT]	26-6642T
Palmityl C16 (3') LMO	[Pal-C16-3]	26-6621
Palmityl C16 (5') LMO	[Pal-C16-5]	26-6622
Palmityl C16 Oligo (NHS) LMO	[Pal-C16-N]	26-6619
Pomalidomide-C3-NHS	[PDM-C3-N]	26-6634
Pomalidomide-PEG1-C2-Azide	[PDM-PEG1-C2-N3]	26-6635
Propionyl (C3) PA LMO	[3-PA-C3]	26-6781
Puromycin	[Puro-3]	26-6603

Spacer 18 (hexaethyleneglycol, PEG6)	[Sp18]	26-6447
Spermine Oligo Cationic Tail (ZNA-Oligos)	[Spm]	26-6454
Stearyl C18 LMO LMO 3'	[Str-C18-3]	26-6623
Stearyl C18 LMO Oligo 5'	[Str-C18-5]	26-6617

2'-O-C16 Adenosine siRNA LMO

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2'-O-C16: 2'-O-hexadecyl (C16) & 2'-O-C22: 2'-O-docosyl (C22)Lipid Modified Oligo (LMO)

The 2'-O-alkyl modification introduces an alkyl ether linkage at the 2'-hydroxyl group of the ribose sugar of one or more nucleotides within the siRNA strand. This modification serves a dual purpose: it confers nuclease resistance (a property shared with other 2'-O-methyl and 2'-fluoro modifications) and it introduces hydrophobic character proportional to chain length.

2'-O-C16 (hexadecyl): A 16-carbon saturated aliphatic chain appended via an ether bond at the 2' position. C16 is equivalent in chain length to palmitic acid and has been used to drive association with cell membranes and high-density lipoprotein (HDL) particles.

Application: The use of 2'-O C-16 bases (C16-siRNA) has been reported by Brown et. Al (1) as a suitable and effective delivery modification in extrahepatic tissues, such as the central nervous system (CNS), eye and lung. Multiple CNS diseases, The C16-siRNA was shown with potential clinical benefits of siRNA-based therapeutics in the lung, enhanced delivery and siRNA uptake into the alveolar and bronchiolar epithelium. The combination of a C16 lipophilic modification with our fully chemically modified, metabolically stable siRNAs achieves efficient delivery to the CNS, eye and lung, resulting in a robust and durable gene silencing in rodents.

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Modifications can be placed at internal or terminal positions of the antisense strand (guide strand), often in conjunction with phosphorothioate backbone modifications to further enhance stability. The site and number of lipid conjugations are optimized empirically to balance hydrophobicity, potency, and biodistribution.

The 2'-O-C16 and 2'-O-C22 lipid modifications represent a chemically elegant and biologically effective strategy for enhancing siRNA cellular delivery. By harnessing endogenous lipoprotein transport pathways and exploiting hydrophobic membrane interactions, these LMO-siRNA constructs achieve gymnotic uptake and gene silencing in relevant cell types without the need for exogenous delivery vehicles. C16 modifications preferentially associate with HDL and suit certain extrahepatic applications, while C22 modifications exhibit stronger LDL binding and potent hepatic silencing activity. Together, these platforms expand the therapeutic window for RNA interference-based medicines.

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References

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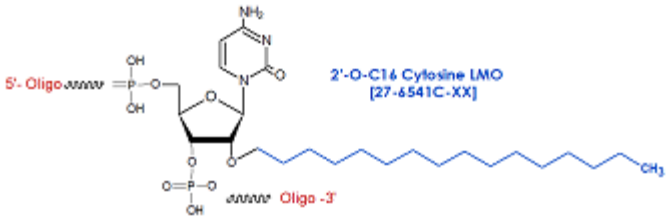
GalNAc Trivalent Modification

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The GalNAc ligand originally used by Alnylam is the triantennary ligand would seem to lend itself to formation by post synthesis conjugation to the 3' terminus but a complex trivalent GalNAc support would also be perfectly applicable, if challenging to produce. However, an alternative approach using a monovalent GalNAc support with two additions of a monovalent GalNAc phosphoramidite was also described and yielded a trivalent GalNAc structure. This (1+1+1) trivalent GalNAc structure led to GalNAc modified siRNA oligos with potency equal to the equivalent siRNA with the triantennary GalNAc ligand both in vitro and in vivo.
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A further report on antisense oligonucleotides demonstrated (5) the effectiveness of modifying at the 5' terminus using monovalent GalNAc ligands. Up to five GalNAc monomers were added in a serial manner (Figure 3) and it was shown that activity of the antisense oligonucleotides improved as the number of GalNAc units increased. The authors also showed that phosphodiester linkages between the GalNAc units were preferable to phosphorothioate linkages in their testing (5).
References. Adapted from Glen Research Reports. http://www.glenresearch.com/GlenReports/GR29-14.html
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2'-O-C16 Cytidine siRNA LMO

Category	Antisense & siRNA
Modification Code	C16-2-O-C
Reference Catalog Number	27-6541C
5 Prime	Y
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Internal	Y
Molecular Weight(mw)	530.61



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2'-O-C16 Guanosine siRNA LMO

Category	Antisense & siRNA
Modification Code	C16-2-O-G
Reference Catalog Number	27-6541G
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	570.64



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siRNA Oligo Cellular Delivery Modifications

2'-O-C16: 2'-O-hexadecyl (C16) & 2'-O-C22: 2'-O-docosyl (C22)Lipid Modified Oligo (LMO)

The 2'-O-alkyl modification introduces an alkyl ether linkage at the 2'-hydroxyl group of the ribose sugar of one or more nucleotides within the siRNA strand. This modification serves a dual purpose: it confers nuclease resistance (a property shared with other 2'-O-methyl and 2'-fluoro modifications) and it introduces hydrophobic character proportional to chain length.

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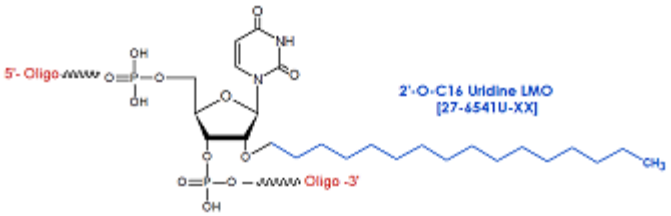
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Internal	Y
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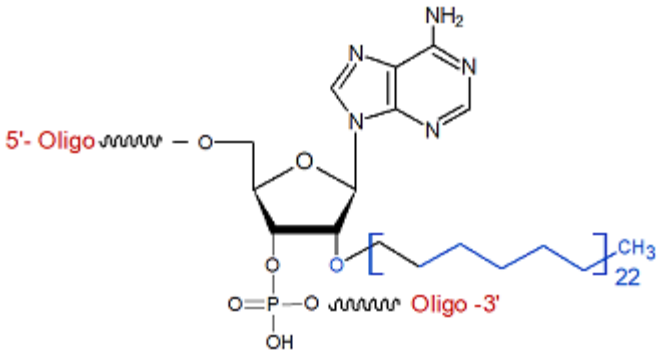
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Category	Antisense & siRNA
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Reference Catalog Number	27-6542A
5 Prime	Y
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Molecular Weight(mw)	639.24



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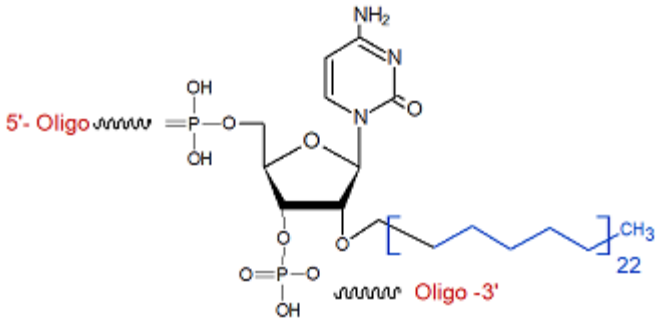
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2'-O-C22 Cytidine siRNA LMO

Category	Antisense & siRNA
Modification Code	C22-2-O-C
Reference Catalog Number	27-6542C
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	615.21



2'-O-C22 Cytosine LMO
[27-6542C-XX]

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siRNA Oligo Cellular Delivery Modifications

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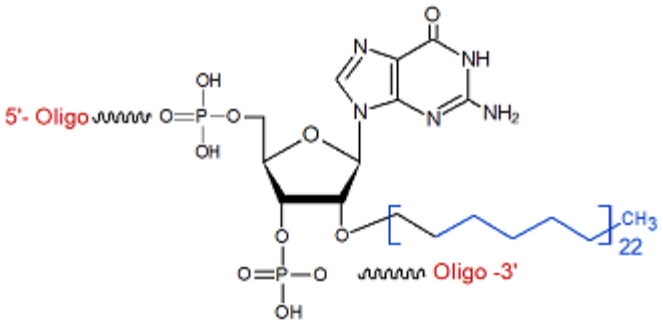
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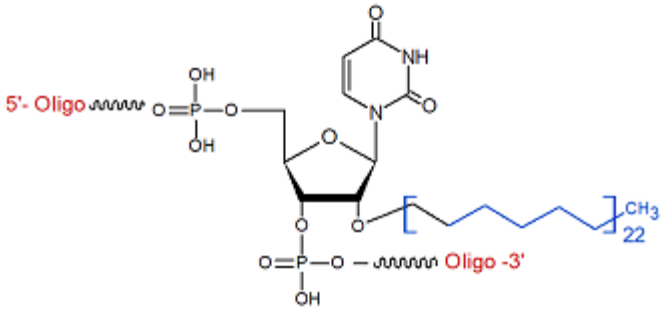
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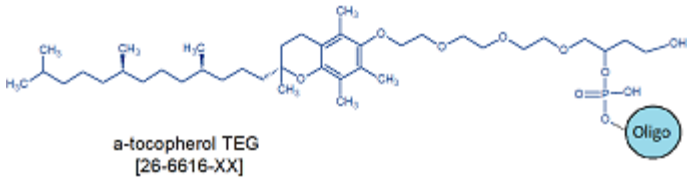
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References. Adapted from Glen Research Reports. http://www.glenresearch.com/GlenReports/GR29-14.html
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3. K.G. Rajeev, et al., Chembiochem, 2015, 16, 903-8.
4. I. Cedillo, et al., Molecules, 2017, 22.
5. T. Yamamoto, M. Sawamura, F. Wada, M. Harada-Shiba, and S. Obika, Bioorganic & Medicinal Chemistry, 2016, 24, 26-32.

a-Tocopherol TEG (LMO)

Category	Antisense & siRNA
Modification Code	a-toco-TEG
Reference Catalog Number	26-6616
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	698.91



a-tocopherol TEG
[26-6616-XX]

Click here for a list Cellular Delivery Modifications.
Oligonucleotides are predominantly hydrophilic species and require help in permeating cell membranes. One strategy to improve cellular uptake of therapeutic oligonucleotides is to conjugate them with non-toxic, lipophilic molecules. Gene Link offers cholesteryl TEG, alpha-tocopherol and stearyl labelling of oligonucleotides and this strategy has proved to be useful for delivering therapeutic oligonucleotides to a broad distribution of targets.

alpha-tocopherol TEG Modification
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Vitamin B12 (Cyanocobalamin) Oligo

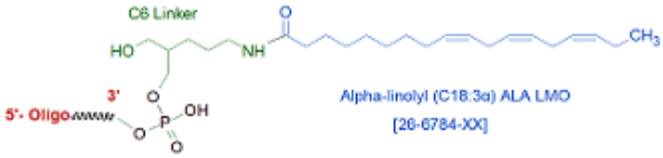
The vitamin B12-Cobalmine conjugated oligo may help in cellular delivery of siRNA to the brain and nervous system. Cyanocobalamin (commonly known as Vitamin B12) is a highly complex, essential vitamin, owing its name to the fact that it contains the mineral, cobalt. This vitamin is produced naturally by bacteria, and is necessary for DNA synthesis and cellular energy production. Vitamin B12 has many forms, including the cyano-, methyl-, deoxyadenosyl- and hydroxy-cobalamin forms. The cyano form, is the most widely used form in supplements and prescription drugs.

Vitamin B12-NHS Ester is a derivative of Vitamin B12 which is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. The NHS ester is utilized to conjugate to amino derivatized oligo.

References. Adapted from Glen Research Reports. http://www.glenresearch.com/GlenReports/GR29-14.html
1. J.K. Nair, et al., J Am Chem Soc, 2014, 136, 16958-61.
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Alpha-linolenic acid (C18:3α) Oligo

Category	Antisense & siRNA
Modification Code	3-ALA-C18-3
Reference Catalog Number	26-6784
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	454.57



Gene Link offers a wide range of lipid modified oligos for cellular delivery. Click here to see the complete list.

Lipid modified oligos (LMO; lignoceric, palmitic, cholesterol, Propionyl (C3) PA, Butyl (C4) BA, Linolyl (C18:2) LA, Alpha□linolyl (C18:3α) ALA, Gamma□linolyl (C18:3γ) GLA, Dihomo□gamma□linolyl (C20:3) DGLA, Arachidonyl (C20:4) AA, Eicosapent (20:5) EPA etc.).
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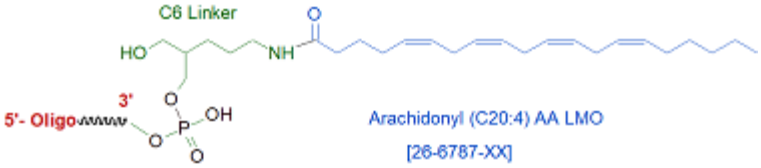
Recommended Further Reading

N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

References. Adapted from Glen Research Reports. http://www.glenresearch.com/GlenReports/GR29-14.html
1. J.K. Nair, et al., J Am Chem Soc, 2014, 136, 16958-61.
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Arachidonic acid (C20:4) Oligo

Category	Antisense & siRNA
Modification Code	3-AA-C20-4
Reference Catalog Number	26-6787
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	480.6



Gene Link offers a wide range of lipid modified oligos for cellular delivery. Click here to see the complete list.

Lipid modified oligos (LMO; lignoceric, palmitic, cholesterol, Propionyl (C3) PA, Butyl (C4) BA, Linolyl (C18:2) LA, Alpha□linolyl (C18:3α) ALA, Gamma□linolyl (C18:3γ) GLA, Dihomo□gamma□linolyl (C20:3) DGLA, Arachidonyl (C20:4) AA, Eicosapent (20:5) EPA etc.).
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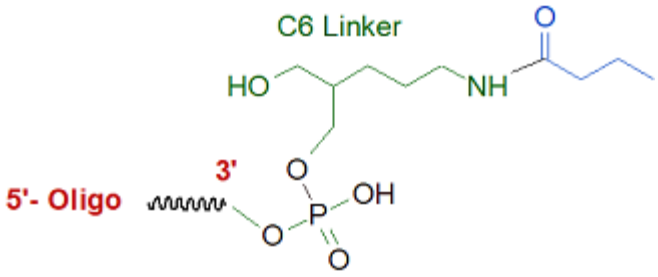
Recommended Further Reading

N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

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4. T. Yamamoto, M. Sawamura, F. Wada, M. Harada-Shiba, and S. Obika, Bioorganic & Medicinal Chemistry, 2016, 24, 26-32.

Butyl (C4) BA LMO

Category	Antisense & siRNA
Modification Code	3-BA-C4
Reference Catalog Number	26-6782
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	264.24



Butyl (C4) BA LMO
[26-6782-XX]

Gene Link offers a wide range of lipid modified oligos for cellular delivery. Click here to see the complete list.

Lipid modified oligos (LMO; lignoceric, palmitic, cholesterol, Propionyl (C3) PA, Butyl (C4) BA, Linolyl (C18:2) LA, Alpha□linolyl (C18:3α) ALA, Gamma□linolyl (C18:3γ) GLA, Dihomo□gamma□linolyl (C20:3) DGLA, Arachidonyl (C20:4) AA, Eicosapent (20:5) EPA etc.).
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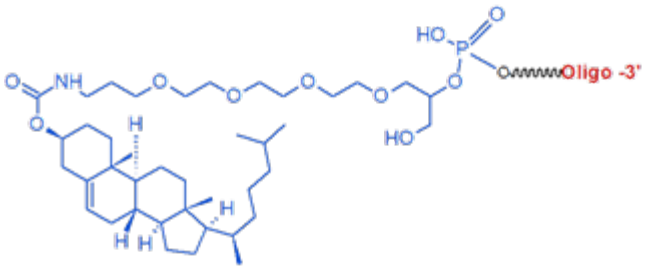
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N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

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Cholesterol TEG 5'

Category	Antisense & siRNA
Modification Code	CholTEG-5
Reference Catalog Number	26-6602
5 Prime	Y
3 Prime	N
Internal	Y
Molecular Weight(mw)	755.97



Cholesterol TEG 5' (15 atom triethylene glycol spacer)
26-6602F-XX

Click here for a list Cellular Delivery Modifications.

Modification Code Change Effective May 12, 2025 the code for Cholesterol 5' was changed from [CholTEG] to [CholTEG-5].
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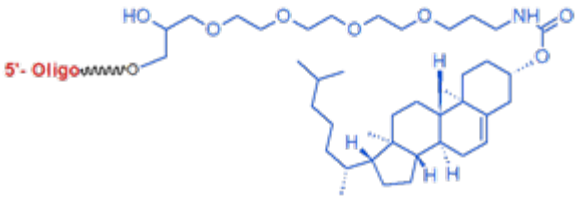
N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

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Cholesterol TEG-3'

Category	Antisense & siRNA
Modification Code	CholTEG-3
Reference Catalog Number	26-6570
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	755.97



Cholesterol TEG 3' (15 atom triethylene glycol spacer)
26-6570-XX

Click here for a list Cellular Delivery Modifications.

Modification Code Change Effective May 12, 2025 the code for Cholesterol 3' was changed from [3-CholTEG] to [CholTEG-3].
Antisense Oligos (ODN) & siRNA Oligo Cellular Delivery Modifications
Click here for more information on antisense modifications, design & applications.

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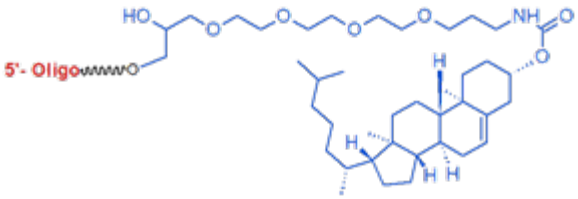
N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

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d-Tocopherol TEG (LMO)

Category	Antisense & siRNA
Modification Code	d-toco-TEG
Reference Catalog Number	26-6628
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	672



Cholesterol TEG 3' (15 atom triethylene glycol spacer)
26-6570-XX

Click here for a list Cellular Delivery Modifications.
Oligonucleotides are predominantly hydrophilic species and require help in permeating cell membranes. One strategy to improve cellular uptake of therapeutic oligonucleotides is to conjugate them with non-toxic, lipophilic molecules. Gene Link offers cholesteryl TEG, alpha-tocopherol and stearyl labelling of oligonucleotides and this strategy has proved to be useful for delivering therapeutic oligonucleotides to a broad distribution of targets.

alpha-tocopherol TEG Modification
alpha-tocopherol (vitamin E) is both lipophilic and non-toxic even at high doses so would be an excellent candidate as a lipophilic carrier for oligonucleotides. Similar to cholesterol TEG, the TEG liker arm facilitates solubility issues of the oligo making it soluble in aqueous buffers.

Cholesterol TEG Modification
Cholesterol TEG Modification is a lipophilic modification aiding in cellular delivery. The TEG liker arm facilitates solubility issues of the oligo making it soluble in aqueous buffers.

Stearyl Modification
Stearyl Modification is C18 lipid, it is an economical and effective carrier molecule. We envisage that the 5'-stearyl group will become a favored lipophilic carrier for experimentation with synthetic oligonucleotides.

GalNAc
A more directed approach to the delivery of therapeutic oligonucleotides specifically to the liver has been to target the asialoglycoprotein receptor (ASGPR) using a suitable glycoconjugate. Indeed, ASGPR is the ideal target for delivery of therapeutic oligonucleotides to the liver since it combines tissue specificity, high expression levels and rapid internalization and turnover. The use of oligonucleotide glycoconjugates has led to significant advances in therapeutic delivery as evidenced by the work of Alnylam Pharmaceuticals which has developed multivalent N-acetylgalactosamine (GalNAc) conjugated siRNAs that bind at nanomolar levels to ASGPR (1). A similar strategy has been applied at Ionis Pharmaceuticals directed at the development of antisense oligonucleotide therapeutics (2).
The GalNAc ligand originally used by Alnylam is the triantennary ligand would seem to lend itself to formation by post synthesis conjugation to the 3' terminus but a complex trivalent GalNAc support would also be perfectly applicable, if challenging to produce.

However, an alternative approach using a monovalent GalNAc support with two additions of a monovalent GalNAc phosphoramidite was also described and yielded a trivalent GalNAc structure. This (1+1+1) trivalent GalNAc structure led to GalNAc modified siRNA oligos with potency equal to the equivalent siRNA with the triantennary GalNAc ligand both in vitro and in vivo.

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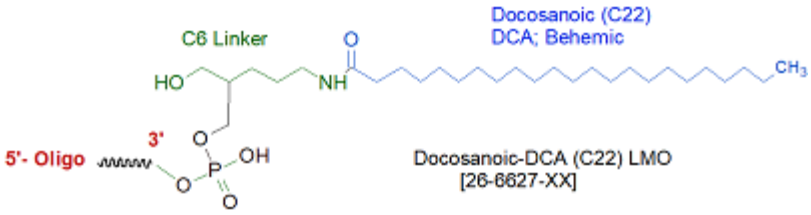
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DCA (C22) LMO

Category	Antisense & siRNA
Modification Code	3-DCA-C22
Reference Catalog Number	26-6627
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	517



Click here for a list Cellular Delivery Modifications.
Click here for more information on siRNA and antisense oligo modifications, design & applications.
siRNA Oligo Cellular Delivery Modifications

-Docosahexaenoic-DHA (C22:6) Lipid Modified Oligo (LMO) Delivery

DHA (docosahexaenoic acid) and DCA (docosanoic acid) are fatty acids that can be conjugated to siRNA molecules to improve their delivery and efficacy, particularly in extrahepatic tissues. DHA conjugation enhances siRNA distribution in the brain and promotes cellular uptake by neurons and astrocytes. DCA conjugation facilitates efficient and sustainable gene silencing in skeletal and cardiac muscles after systemic injection.

References

Nikan, M., Osborn, M.F., Coles, A.H., Godinho, B.M., Hall, L.M., Haraszti, R.A., Hassler, M.R., Echeverria, D., Aronin, N., and Khvorova, A. (2016). Docosahexaenoic acid conjugation enhances distribution and safety of siRNA upon local administration in mouse brain. Mol. Ther. Nucleic Acids 5, e344. Biscans,A., Caiazzi, J., McHugh, N., Hariharan, V., Muhuri, M., and Anastasia Khvorova (2020) Docosanoic acid conjugation to siRNA enables functional and safe delivery to skeletal and cardiac muscles. Mol. Ther. 29:4

2'-O-hexadecyl (C16) Modifications

RNA interference (RNAi) is a natural, conserved biological process in which small RNA molecules suppress gene expression by targeting specific mRNA molecules for degradation or translational repression.
RNA interference (RNAi) therapeutics use an endogenous mechanism whereby short interfering RNAs (siRNAs) direct the RNA-induced silencing complex (RISC) to sequence matched target transcripts for knockdown. Various cellular delivery modifications have been used, for instance lipid nanoparticles and N-acetylgalactosamine (GalNAc) conjugates are clinically validated and approved delivery strategies for liver targets. In addition, various other cellular delivery modifications have been reported that include alpha-tocopherol, DHA, DCA, Folic acid etc.

The use of 2'-O C-16 bases (C16-siRNA) has been reported by Brown et. Al (1) as a suitable and effective delivery modification in extrahepatic tissues, such as the central nervous system (CNS), eye and lung. Multiple CNS diseases, The C16-siRNA was shown with potential clinical benefits of siRNA-based therapeutics in the lung, enhanced delivery and siRNA uptake into the alveolar and bronchiolar epithelium. The combination of a C16 lipophilic modification with our fully chemically modified, metabolically stable siRNAs achieves efficient delivery to the CNS, eye and lung, resulting in a robust and durable gene silencing in rodents.
Brown et. Al (1) have reported as part of the preclinical evaluation of first development candidate for a CNS disease, ALN-APP, to investigate the impact of a C16-siRNA targeting the amyloid beta precursor protein (APP) gene transcript in a mouse model of Alzheimer's disease. The treatment led to potent and durable knock down in the CNS and ameliorated physiological and behavioral deficits. The sustained knockdown observed suggests that infrequent dosing of C16-siRNAs could be feasible in humans, which is especially important considering the IT route of administration. ALN-APP targeting APP for the treatment of early onset Alzheimer's Disease (EOAD) and cerebral amyloid angiopathy (CAA) is now in clinical development.

References

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Vitamin B12 (Cyanocobalamin) Oligo

The vitamin B12-Cobalmine conjugated oligo may help in cellular delivery of siRNA to the brain and nervous system. Cyanocobalamin (commonly known as Vitamin B12) is a highly complex, essential vitamin, owing its name to the fact that it contains the mineral, cobalt. This vitamin is produced naturally by bacteria, and is necessary for DNA synthesis and cellular energy production. Vitamin B12 has many forms, including the cyano-, methyl-, deoxyadenosyl- and hydroxy-cobalamin forms. The cyano form, is the most widely used form in supplements and prescription drugs.
Vitamin B12-NHS Ester is a derivative of Vitamin B12 which is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. The NHS ester is utilized to conjugate to amino derivatized oligo.

Vitamin E. alpha-tocopherol TEG Modification

alpha-tocopherol (vitamin E) is both lipophilic and non-toxic even at high doses so would be an excellent candidate as a lipophilic carrier for oligonucleotides. Similar to cholesterol TEG, the TEG liker arm facilitates solubility issues of the oligo making it soluble in aqueous buffers.

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Stearyl Modification

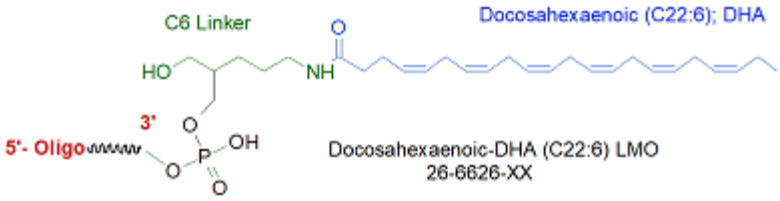
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DHA (C22:6) LMO

Category	Antisense & siRNA
Modification Code	3-DHA-C22-6
Reference Catalog Number	26-6626
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	505



Click here for a list Cellular Delivery Modifications.
Click here for more information on siRNA and antisense oligo modifications, design & applications.
siRNA Oligo Cellular Delivery Modifications

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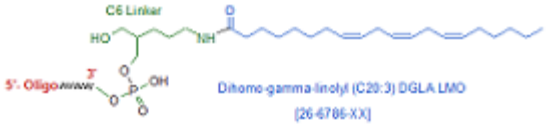
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Dihomo-γ-linolenic acid (C20:3) Oligo

Category	Antisense & siRNA
Modification Code	3-DGLA-C20-3
Reference Catalog Number	26-6786
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	482.62



Gene Link offers a wide range of lipid modified oligos for cellular delivery. Click here to see the complete list.

Lipid modified oligos (LMO; lignoceric, palmitic, cholesterol, Propionyl (C3) PA, Butyl (C4) BA, Linolyl (C18:2) LA, Alpha□linolyl (C18:3α) ALA, Gamma□linolyl (C18:3γ) GLA, Dihomo□gamma□linolyl (C20:3) DGLA, Arachidonyl (C20:4) AA, Eicosapent (20:5) EPA etc.).
Oligonucleotides are predominantly hydrophilic species and require help in permeating cell membranes. One strategy to improve cellular uptake of therapeutic oligonucleotides is to conjugate them with non-toxic, lipophilic molecules. Gene Link offers cholesteryl TEG, alpha-tocopherol and stearyl labelling of oligonucleotides and this strategy has proved to be useful for delivering therapeutic oligonucleotides to a broad distribution of targets.

Stearyl Modification

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Cholesterol TEG Modification

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alpha-tocopherol TEG Modification

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GalNAc

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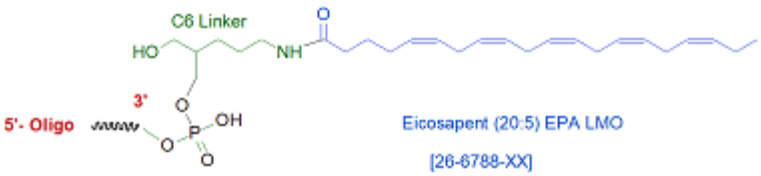
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Eicosapentaenoic acid (20:5) Oligo

Category	Antisense & siRNA
Modification Code	3-EPA-C20-5
Reference Catalog Number	26-6788
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	478.58



Gene Link offers a wide range of lipid modified oligos for cellular delivery. Click here to see the complete list.

Lipid modified oligos (LMO; lignoceric, palmitic, cholesterol, Propionyl (C3) PA, Butyl (C4) BA, Linolyl (C18:2) LA, Alpha□linolyl (C18:3α) ALA, Gamma□linolyl (C18:3γ) GLA, Dihomo□gamma□linolyl (C20:3) DGLA, Arachidonyl (C20:4) AA, Eicosapent (20:5) EPA etc.).
Oligonucleotides are predominantly hydrophilic species and require help in permeating cell membranes. One strategy to improve cellular uptake of therapeutic oligonucleotides is to conjugate them with non-toxic, lipophilic molecules. Gene Link offers cholesteryl TEG, alpha-tocopherol and stearyl labelling of oligonucleotides and this strategy has proved to be useful for delivering therapeutic oligonucleotides to a broad distribution of targets.

Stearyl Modification

Stearyl Modification is C18 lipid, it is an economical and effective carrier molecule. We envisage that the 5'-stearyl group will become a favored lipophilic carrier for experimentation with synthetic oligonucleotides.

Cholesterol TEG Modification

Cholesterol TEG Modification is a lipophilic modification aiding in cellular delivery. The TEG liker arm facilitates solubility issues of the oligo making it soluble in aqueous buffers.

alpha-tocopherol TEG Modification

Similar to cholesterol TEG, alpha-tocopherol (vitamin E) is both lipophilic and non-toxic even at high doses so would be an excellent candidate as a lipophilic carrier for oligonucleotides. The TEG liker arm facilitates solubility issues of the oligo making it soluble in aqueous buffers.

GalNAc

A more directed approach to the delivery of therapeutic oligonucleotides specifically to the liver has been to target the asialoglycoprotein receptor (ASGPR) using a suitable glycoconjugate. Indeed, ASGPR is the ideal target for delivery of therapeutic oligonucleotides to the liver since it combines tissue specificity, high expression levels and rapid internalization and turnover. The use of oligonucleotide glycoconjugates has led to significant advances in therapeutic delivery as evidenced by the work of Alnylam Pharmaceuticals which has developed multivalent N-acetylgalactosamine (GalNAc) conjugated siRNAs that bind at nanomolar levels to ASGPR (1).

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The GalNAc ligand originally used by Alnylam is the triantennary ligand would seem to lend itself to formation by post synthesis conjugation to the 3' terminus but a complex trivalent GalNAc support would also be perfectly applicable, if challenging to produce. However, an alternative approach using a monovalent GalNAc support with two additions of a monovalent GalNAc phosphoramidite was also described and yielded a trivalent GalNAc structure. This (1+1+1) trivalent GalNAc structure led to GalNAc modified siRNA oligos with potency equal to the equivalent siRNA with the triantennary GalNAc ligand both in vitro and in vivo.

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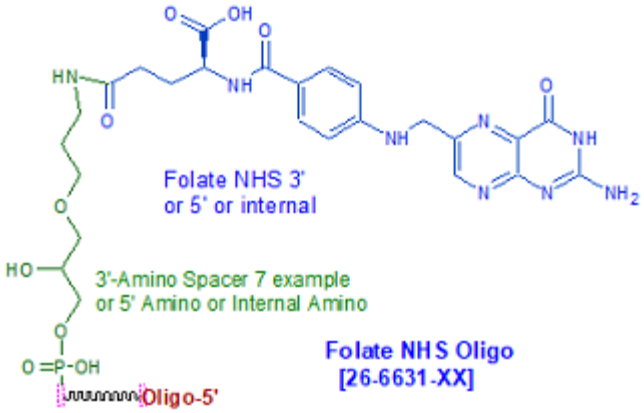
N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

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Folic Acid NHS (Vitamin B9)

Category	Antisense & siRNA
Modification Code	Folate-N
Reference Catalog Number	26-6631
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	424.4



Click here for a list Cellular Delivery Modifications.
Click here for more information on siRNA and antisense oligo modifications, design & applications.

Folate Oligo Modification: Vitamin B9 (Folic Acid)-Based Drug Delivery

Folic acid (FA), also known as vitamin B9, is a water-soluble vitamin involved in DNA synthesis, repair, and methylation. In recent years, FA has garnered significant attention in nanomedicine and targeted drug delivery due to its high affinity for the folate receptor (FR), which is overexpressed in various cancer cells (e.g., ovarian, breast, lung, colorectal, and kidney cancers), but minimally expressed in most normal tissues.

Mechanism of Targeting via Folate Receptor

FA binds with high affinity (Kd ≈ 10□⁹ M) to the folate receptor α (FR-α), a glycosylphosphatidylinositol-anchored cell surface protein. Upon binding, the FA-conjugated drug delivery system is internalized by receptor-mediated endocytosis, leading to the release of the drug inside the target cells. This mechanism enhances selectivity and reduces off-target toxicity in chemotherapy

Advantages of Folic Acid in Targeted Delivery

High receptor binding specificity Small size and chemical stability Non-immunogenic Easy chemical conjugation Effective targeting of various solid tumors

References

1. Liu Y, et al. (2007). Folate receptor-targeted delivery of siRNA using PEG-PEI. Mol Pharmaceutics, 4(5):695-705.
2. Chen Y, et al. (2010). Tumor-targeted siRNA delivery via folate-receptor mediated endocytosis. Biomaterials, 31(3):524-537.
3. Zhang Y, et al. (2012). Folic acid-chitosan nanoparticles for siRNA delivery to breast cancer. Int J Nanomedicine, 7:2227-2238.
4. Kim J, et al. (2010). Multifunctional gold nanoparticles for siRNA delivery and imaging. ACS Nano, 4(7):3689-3696.
5. Kesharwani P, et al. (2015). Dendrimer-based targeted delivery of siRNA. Drug Discov Today, 20(5):536-547.

siRNA Oligo Cellular Delivery Modifications

Vitamin B12 (Cyanocobalamin) Oligo

The vitamin B12-Cobalmine conjugated oligo may help in cellular delivery of siRNA to the brain and nervous system. Cyanocobalamin (commonly known as Vitamin B12) is a highly complex, essential vitamin, owing its name to the fact that it contains the mineral, cobalt. This vitamin is produced naturally by bacteria, and is necessary for DNA synthesis and cellular energy production. Vitamin B12 has many forms, including the cyano-, methyl-, deoxyadenosyl- and hydroxy-cobalamin forms. The cyano form, is the most widely used form in supplements and prescription drugs.
Vitamin B12-NHS Ester is a derivative of Vitamin B12 which is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. The NHS ester is utilized to conjugate to amino derivatized oligo.

Vitamin E. alpha-tocopherol TEG Modification

alpha-tocopherol (vitamin E) is both lipophilic and non-toxic even at high doses so would be an excellent candidate as a lipophilic carrier for oligonucleotides. Similar to cholesterol TEG, the TEG liker arm facilitates solubility issues of the oligo making it soluble in aqueous buffers.

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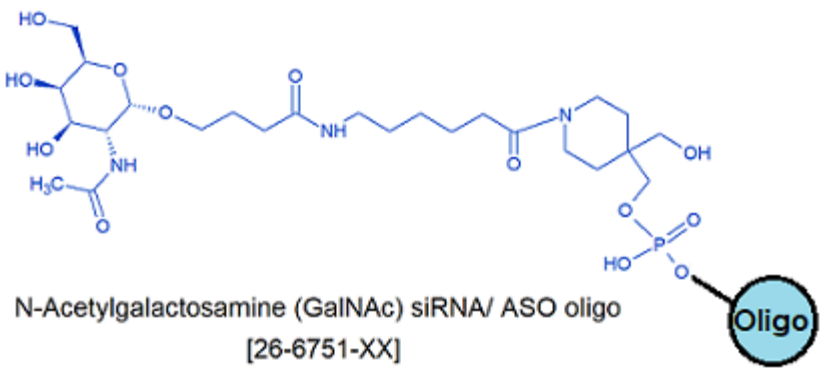
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GalNAc

Category	Antisense & siRNA
Modification Code	GalNAc
Reference Catalog Number	26-6751
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	609.61



N-Acetylgalactosamine (GalNAc) siRNA/ ASO oligo
[26-6751-XX]

siRNA Oligo Cellular Delivery Modifications

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Click here for more information on antisense modifications, design & applications.

Sold under license from AM Chemicals LLC for Research Use Only. An additional royalty fee of 8% will be added as a line item [RY-GLNC-00] for the quoted/invoiced price of GalNAc component
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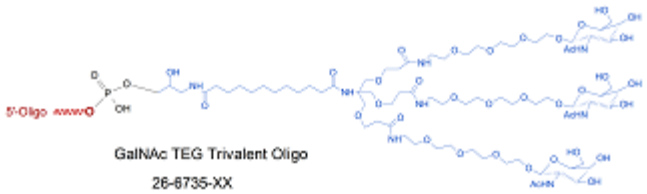
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GalNAc TEG Trivalent 3'

Category	Antisense & siRNA
Modification Code	GalNAc-TEG3X
Reference Catalog Number	26-6735
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	1820



GalNAc TEG Trivalent Oligo

26-6735-XX

siRNA Oligo Cellular Delivery Modifications

Click here for a list Cellular Delivery Modifications.
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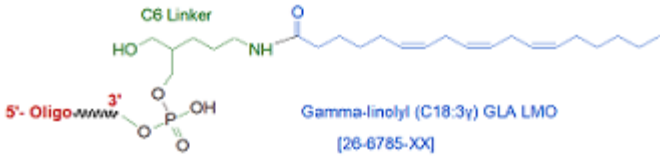
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Gamma-linolenic acid (C18:3γ) Oligo

Category	Antisense & siRNA
Modification Code	3-GLA-C18-3
Reference Catalog Number	26-6785
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	454.57



Gene Link offers a wide range of lipid modified oligos for cellular delivery. Click here to see the complete list.

Lipid modified oligos (LMO; lignoceric, palmitic, cholesterol, Propionyl (C3) PA, Butyl (C4) BA, Linolyl (C18:2) LA, Alpha□linolyl (C18:3α) ALA, Gamma□linolyl (C18:3γ) GLA, Dihomo□gamma□linolyl (C20:3) DGLA, Arachidonyl (C20:4) AA, Eicosapent (20:5) EPA etc.).
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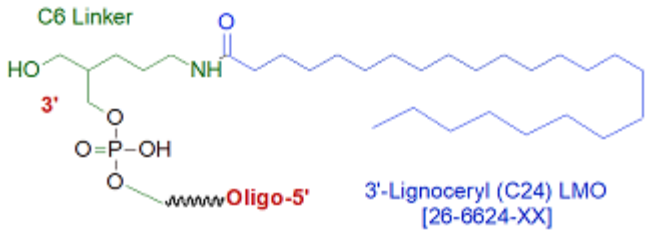
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Lignoceryl C24 (3') LMO

Category	Antisense & siRNA
Modification Code	3-Lig-C24
Reference Catalog Number	26-6624
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	544.77



We offer custom oligo synthesis of your designed sequences for conjugation to lignoceric acid and palmitic acid for MULTI-seq: sample multiplexing for single-cell RNA sequencing **YIELD** Approximate polyacrylamide gel purified yield for various scales are given below for an oligo below 50mer.
Yield given below are for oligos shorter than 50mer. Please see longer oligos yield at this link Long Oligo Typical Yield.
~2 nmol final yield for 50 nmol scale synthesis.
~8 nmol final yield for 200 nmol scale synthesis.
~25 nmol final yield for 1 umol scale synthesis
~45nmol final yield for 2 umol scale synthesis
~110nmol final yield for 5 umol scale synthesis
~180 nmol final yield for 10 umol scale synthesis
~260 nmol final yield for 15 umol scale synthesis
Click here to order stock MULTI-Seq LMO Lig-Anchor oligos.

Lipid modified oligos (LMO; lignoceric, palmitic, cholesterol etc.); These LMO rapidly and stably incorporate into the plasma membrane of live cells by step-wise assembly. McGinnis, C. et al. (1) adapted LMOs into MULTI-seq: scRNA-seq (single-cell) and snRNA-seq (single-nucleus) sample multiplexing using lipid-tagged indices. MULTI-seq localizes sample barcodes to live cells and nuclei regardless of species or genetic background while preserving cell viability and endogenous gene expression patterns.

MULTI-Seq LMO Lig Anchor and MULTI-Seq LMO Palm Co-Anchor oligos are lignoceric and palmitic acid conjugated oligos as described by McGinnis, C. et al. (1)
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Lignoceric acid, or tetracosanoic acid, is long C24 chain saturated fatty acid. It is found in wood tar, various cerebrosides, and in small amounts in most natural fats. The fatty acids of peanut oil contain small amounts of lignoceric acid. This fatty acid is also a byproduct of lignin production.
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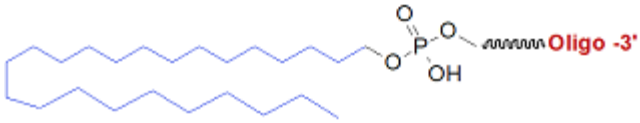
N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

References. Adapted from Glen Research Reports. http://www.glenresearch.com/GlenReports/GR29-14.html

1. J.K. Nair, et al., J Am Chem Soc, 2014, 136, 16958-61.
2. T.P. Prakash, et al., Bioorganic & Medicinal Chemistry Letters, 2015, 25, 4127-4130.
3. K.G. Rajeev, et al., Chembiochem, 2015, 16, 903-8.
4. T. Yamamoto, M. Sawamura, F. Wada, M. Harada-Shiba, and S. Obika, Bioorganic & Medicinal Chemistry, 2016, 24, 26-32.

Lignoceryl C24 (5') LMO

Category	Antisense & siRNA
Modification Code	5-Lig-C24
Reference Catalog Number	26-6625
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	415.61



Lignoceryl (C24) 5'-LMO
[26-6625-XX

We offer custom oligo synthesis of your designed sequences for conjugation to lignoceric acid and palmitic acid **YIELD**
Approximate polyacrylamide gel purified yield for various scales are given below for an oligo below 50mer.
Yield given below are for oligos shorter than 50mer. Please see longer oligos yield at this link Long Oligo Typical Yield.
~2 nmol final yield for 50 nmol scale synthesis.
~8 nmol final yield for 200 nmol scale synthesis.
~25 nmol final yield for 1 umol scale synthesis
~45nmol final yield for 2 umol scale synthesis
~110nmol final yield for 5 umol scale synthesis
~180 nmol final yield for 10 umol scale synthesis
~260 nmol final yield for 15 umol scale synthesis
Click here to order stock MULTI-Seq LMO Lig-Anchor oligos.

Lipid modified oligos (LMO; lignoceric, palmitic, cholesterol etc.); These LMO rapidly and stably incorporate into the plasma membrane of live cells by step-wise assembly. McGinnis, C. et al. (1) adapted LMOs into MULTI-seq: scRNA-seq (single-cell) and snRNA-seq (single-nucleus) sample multiplexing using lipid-tagged indices. MULTI-seq localizes sample barcodes to live cells and nuclei regardless of species or genetic background while preserving cell viability and endogenous gene expression patterns.

MULTI-Seq LMO Lig Anchor and MULTI-Seq LMO Palm Co-Anchor oligos are lignoceric and palmitic acid conjugated oligos as described by McGinnis, C. et al. (1)
1. McGinnis, C. et al. MULTI-seq: sample multiplexing for single-cell RNA sequencing using lipid-tagged indices. Nat. Methods 16, 619-626 (2019).

Lignoceric acid, or tetracosanoic acid, is long C24 chain saturated fatty acid. It is found in wood tar, various cerebrosides, and in small amounts in most natural fats. The fatty acids of peanut oil contain small amounts of lignoceric acid. This fatty acid is also a byproduct of lignin production.
Oligonucleotides are predominantly hydrophilic species and require help in permeating cell membranes. One strategy to improve cellular uptake of therapeutic oligonucleotides is to conjugate them with non-toxic, lipophilic molecules. Gene Link offers cholesteryl TEG, alpha-tocopherol and stearyl labelling of oligonucleotides and this strategy has proved to be useful for delivering therapeutic oligonucleotides to a broad distribution of targets.

Stearyl Modification

Stearyl Modification is C18 lipid, it is an economical and effective carrier molecule. We envisage that the 5'-stearyl group will become a favored lipophilic carrier for experimentation with synthetic oligonucleotides.

Cholesterol TEG Modification

Cholesterol TEG Modification is a lipophilic modification aiding in cellular delivery. The TEG liker arm facilitates solubility issues of the oligo making it soluble in aqueous buffers.

alpha-tocopherol TEG Modification

Similar to cholesterol TEG, alpha-tocopherol (vitamin E) is both lipophilic and non-toxic even at high doses so would be an excellent candidate as a lipophilic carrier for oligonucleotides. The TEG liker arm facilitates solubility issues of the oligo making it soluble in aqueous buffers.

GalNAc

A more directed approach to the delivery of therapeutic oligonucleotides specifically to the liver has been to target the asialoglycoprotein receptor (ASGPR) using a suitable glycoconjugate. Indeed, ASGPR is the ideal target for delivery of therapeutic oligonucleotides to the liver since it combines tissue specificity, high expression levels and rapid internalization and turnover. The use of oligonucleotide glycoconjugates has led to significant advances in therapeutic delivery as evidenced by the work of Alnylam Pharmaceuticals which has developed multivalent N-acetylgalactosamine (GalNAc) conjugated siRNAs that bind at nanomolar levels to ASGPR (1). A similar strategy has been applied at Ionis Pharmaceuticals directed at the development of antisense oligonucleotide therapeutics (2).

The GalNAc ligand originally used by Alnylam is the triantennary ligand would seem to lend itself to formation by post synthesis conjugation to the 3' terminus but a complex trivalent GalNAc support would also be perfectly applicable, if challenging to produce. However, an alternative approach using a monovalent GalNAc support with two additions of a monovalent GalNAc phosphoramidite was also described and yielded a trivalent GalNAc structure. This (1+1+1) trivalent GalNAc structure led to GalNAc modified siRNA oligos with potency equal to the equivalent siRNA with the triantennary GalNAc ligand both in vitro and in vivo.

A further report on antisense oligonucleotides demonstrated (4) the effectiveness of modifying at the 5' terminus using monovalent GalNAc ligands. Up to five GalNAc monomers were added in a serial manner (Figure 3) and it was shown that activity of the antisense oligonucleotides improved as the number of GalNAc units increased. The authors also showed that phosphodiester linkages between the GalNAc units were preferable to phosphorothioate linkages in their testing (4).

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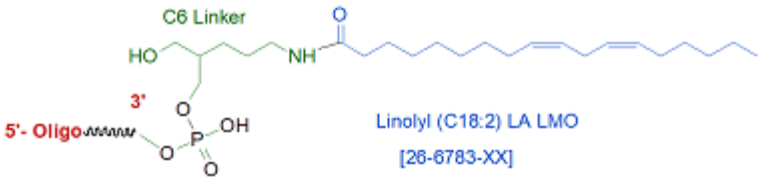
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Linoleic acid (C18:2) Oligo

Category	Antisense & siRNA
Modification Code	3-LA-C18-2
Reference Catalog Number	26-6783
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	456.58



Gene Link offers a wide range of lipid modified oligos for cellular delivery. Click here to see the complete list.

Lipid modified oligos (LMO; lignoceric, palmitic, cholesterol, Propionyl (C3) PA, Butyl (C4) BA, Linolyl (C18:2) LA, Alpha□linolyl (C18:3α) ALA, Gamma□linolyl (C18:3γ) GLA, Dihomo□gamma□linolyl (C20:3) DGLA, Arachidonyl (C20:4) AA, Eicosapent (20:5) EPA etc.).
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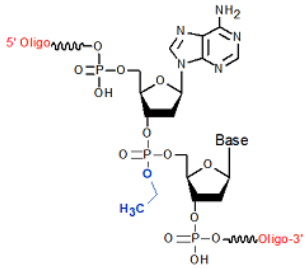
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P-Ethoxy dA

Category	Antisense & siRNA
Modification Code	EoP-dA
Reference Catalog Number	26-6641A
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	341.49



P-Ethoxy dA / Ethoxy Phosphate dA Oligo
[26-6641A-XX]

P-Methoxy (Methoxy Phosphate)[MoP] and P-Ethoxy (Ethoxy Phosphate) [EoP] modification has a setup charge of $250.00 per order for special synthesis reagents.

P-Methoxy (Methoxy Phosphate)[MoP] and P-Ethoxy (Ethoxy Phosphate) [EoP] modified backbone oligos

P-Methoxy (Methoxy Phosphate), P-Ethoxy (Ethoxy Phosphate) and methyl phosphonate [mp] modified backbone oligos makes the phosphodiester linkage neutral charged. The solubility of the oligo in aqueous solutions slowly decreases with increasing modified linkages; consider incorporating as many standard phosphodiester linkages as well in the oligo. Increasing percentage of DMSO from 0.5 to 10% may be used to solubilize the oligo.
These oligonucleotides with neutral backbone displayed high nuclease resistance and improved cellular uptake (1). These are one of the favorable properties of antisense oligonucleotides. In addition to being neutral charge but also impart lipophilic character to the modified oligo.
. Gutierrez-Puente et al (2) used a P-ethoxy oligonucleotide (oligo), 20 bases long and specific for the translation initiation site of human Bcl-2 mRNA. This was incorporated into liposomes to increase its intracellular delivery. This oligo selectively inhibited Bcl-2 protein expression and induced growth inhibition in t(14;18)-positive transformed follicular lymphoma (FL) cell lines. They studied the inhibitory effects of shorter liposomal P-ethoxy oligos (7, 9, 11 or 15 mer) in order to determine the activity of different oligo chain lengths targeted to the same Bcl-2 mRNA. At 12 µM, all the oligos inhibited the growth of a FL cell line. They compared the 7-mer oligo with the 20-mer oligo. The two oligos inhibited Bcl-2 protein expression similarly: 66% and 60% for the 7- and 20-mer, respectively. The uptake and retention of both oligos were also very similar. Their results indicate that the Bcl-2 inhibitory activity is maintained with P-ethoxy antisense oligos ranging from 7 to 20 bases.

P-Methoxy (Methoxy Phosphate), P-Ethoxy (Ethoxy Phosphate) References

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Leukemia & Lymphoma 44: 1979-1985

Methyl phosphonoamidites are deoxynucleoside amidites modified such that, when incorporated into an oligonucleotide, that base position will have a (electrically neutral) methyl phosphonate backbone linkage instead of the standard (negatively charged) phosphodiester linkage. Oligos containing one or more methyl phosphonate linkages will be resistant to nuclease degradation at those positions, and the lack of charge improves intracellular transport. Because of these properties, methyl phosphonolated oligos have been explored as anti-sense reagents (1). However, since methyl phosphonate linkages lower the oligo's cellular uptake (2) as well as the Tm of the duplex formed with its RNA target (3), and, most importantly, also interferes with activation of RNase H activity (4), considerable care must taken in choosing which, and how many, methyl phosphonate linkages to incorporate into a putative anti-sense oligo. In that regard, we note that 2'-O-Methyl RNA oligos containing a single 3'-end methyl phosphonate "cap" (to eliminate 3'-exonuclease degradation) have been successfully used as anti-sense reagents (5). In addition, DNA extension primers containing such a "cap" have been used to characterize the nuclease activity of the yeast telomerase complex (6). Methylphosphonolated anti-sense oligos have also been used successfully to "mask" sites in U1 and U2 snRNPs required for spliceosome formation, and thus interfere with mRNA splicing (7). Many of the unique properties of methyl phosphonate oligos are due to the introduction of chirality into the phosphodiester backbone by the methyl group (8).

Methyl phosphonate (mp) References

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Phosphorothioate Phosphorothioate modification is to the backbone linkage modifying the phosphodiester linkage to phosphorothioate. This imparts considerable nuclease resistance and is used widely in the design of antisense oligonucleotides (ODN).
An antisense oligonucleotide refers to a short, synthetic DNA or RNA strand that is complementary in sequence to a short target sequence on a particular mRNA strand, which upon specific hybridization to its target induces inhibition of gene expression. The mechanism of inhibition is based on two properties: first, the physical blocking of the translation process by the presence of the short double-stranded region, and second, in the case of antisense DNA, the resulting DNA-RNA duplex is susceptible to cleavage by cellular RNase H activity, which degrades the mRNA and prevents proper translation. The latter property is the classic mode of action for antisense oligos. The former property can be used when it is necessary to design an antisense oligo with certain modifications that result in it not supporting RNase-H activity (1,2).

Phosphorothioate References 1. Sazani, P., Kole, R. Therapeutic potential of antisense oligonucleotides as modulators of alternative splicing. (2003) J. Clin. Invest., 112: 481-486.
2. Juliano, R., Alam, Md.R., Dixit, V., Kang, H.(2008) Mechanisms and strategies for effective delivery of antisense and siRNA oligonucleotides. Nucleic Acids Res., 36: 4158-4171.
3. Chan, J.H., Lim, S., Wong, W.S. Antisense oligonucleotides: from design to therapeutic applications. (2006) Clin. Exp. Pharmacol. Physiol., 33: 533-540.
4. Kurreck, J. Antisense technologies. Improvement through novel chemical modifications. (2003) Eur. J. Biochem., 270: 1628-1644.
5. Crooke, S.T. (2004) Progress in antisense technology. Annu. Rev. Med., 55: 61-95.

Mesyl Phosphoramidate (Ms, u) Forty years of research have shown that antisense oligonucleotides have great potential to target mRNAs of disease-associated genes and noncoding RNAs. Among the vast number of oligonucleotide backbone modifications, phosphorothioate modification is the most widely used in research and the clinic. However, along with their merits are notable drawbacks of phosphorothioate oligonucleotides, including decreased binding affinity to RNA, reduced specificity, and increased toxicity. Here we report the synthesis and in vitro evaluation of the DNA analog mesyl phosphoramidate oligonucleotide. This oligonucleotide type recruits RNase H and shows significant advantages over phosphorothioate in RNA affinity, nuclease stability, and specificity in inhibiting key processes of carcinogenesis. Thus, mesyl phosphoramidate oligonucleotides may be an attractive alternative to phosphorothioates (1).
DNA analog in which the mesyl (methanesulfonyl) phosphoramidate group is substituted for the natural phosphodiester group at each internucleotidic position (2-5), the oligomers show significant advantages over the often-used DNA phosphorothioates in RNA binding affinity, nuclease stability, and specificity of their antisense action, which involves activation of cellular RNase H enzyme for hybridization-directed RNA cleavage. Biological activity of the oligonucleotide analog was demonstrated with respect to pro-oncogenic miR-21. A 22-nt anti-miR-21 mesyl phosphoramidate oligodeoxynucleotide specifically decreased the miR-21 level in melanoma B16 cells, induced apoptosis, reduced proliferation, and impeded migration of tumor cells, showing superiority over isosequential phosphorothioate oligodeoxynucleotide in the specificity of its biological effect. Lower overall toxicity compared with phosphorothioate and more efficient activation of RNase H are the key advantages of mesyl phosphoramidate oligonucleotides, which may represent a promising group of antisense therapeutic agents (1).

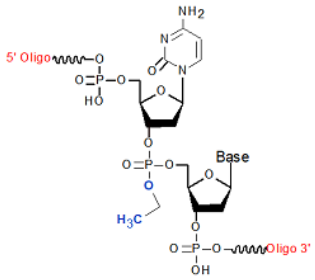
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3. Chelobanov,B.P., Burakova,E.A., Prokhorova, D.V., Fokina, A.A., Stetsenko,D.A. (2017) New oligodeoxynucleotide derivatives containing N-(methanesulfonyl)-phosphoramidate (mesyl phosphoramidate) internucleotide group. Russ. J. Bioorganic Chem. 43:664-668.
4. Boyer, J. H.; Mack, C. H.; Goebel, W.; Morgan, L. R. (1959) Reactions of Sodium Phenylacetylide and Sodium Alkoxide with Tosyl and Mesyl Azides. Jr. J. Org. Chem., 23: 1051-1053.
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ASO's and siRNA Delivery. The development of effective delivery systems for antisense oligonucleotides is essential for their clinical therapeutic application. The most common delivery system involves a relatively hydrophobic molecule that can cross the lipid membrane. Cholesterol TEG, alpha-Tocopherol TEG (a natural isomer of vitamin E), stearyl and GalNAc modifications have been shown to effective for delivery of ASO's and siRNA in addition to cell penetrating peptides.

Click this link to view these modifications.

P-Ethoxy dC

Category	Antisense & siRNA
Modification Code	EoP-dC
Reference Catalog Number	26-6641C
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	303.21



P-Ethoxy dC / Ethoxy Phosphate dC Oligo
[26-6641C-XX]

P-Methoxy (Methoxy Phosphate)[MoP] and P-Ethoxy (Ethoxy Phosphate) [EoP] modification has a setup charge of $250.00 per order for special synthesis reagents.

P-Methoxy (Methoxy Phosphate)[MoP] and P-Ethoxy (Ethoxy Phosphate) [EoP] modified backbone oligos

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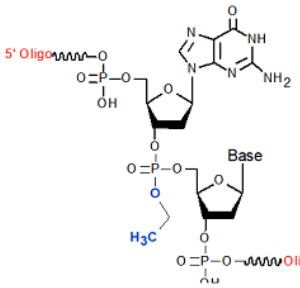
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P-Ethoxy dG

Category	Antisense & siRNA
Modification Code	EoP-dG
Reference Catalog Number	26-6641G
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	357.49



P-Ethoxy dG / Ethoxy Phosphate dG Oligo
[26-6641G-XX]

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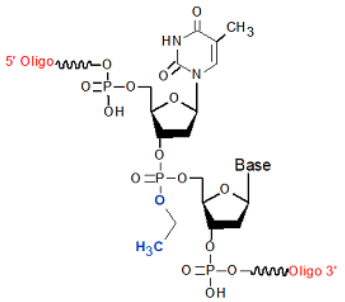
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P-Ethoxy dT

Category	Antisense & siRNA
Modification Code	EoP-dT
Reference Catalog Number	26-6641T
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	332.48



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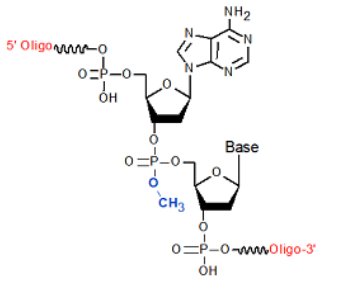
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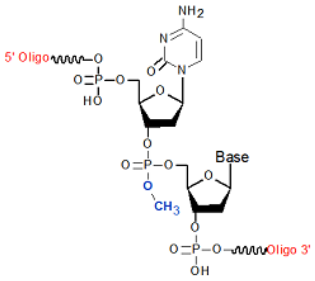
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P-Methoxy dC

Category	Antisense & siRNA
Modification Code	MoP-dC
Reference Catalog Number	26-6642C
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	303.19



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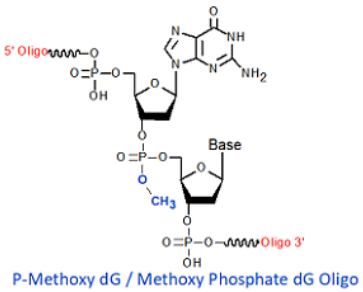
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Internal	Y
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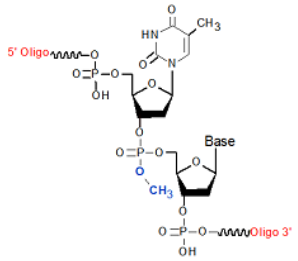
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Click this link to view these modifications.

P-Methoxy dT

Category	Antisense & siRNA
Modification Code	MoP-dT
Reference Catalog Number	26-6642T
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	318.23



P-Methoxy dT / Methoxy Phosphate dT Oligo
[26-6642T-XX]

P-Methoxy (Methoxy Phosphate)[MoP] and P-Ethoxy (Ethoxy Phosphate) [EoP] modification has a setup charge of $250.00 per order for special synthesis reagents.

P-Methoxy (Methoxy Phosphate)[MoP] and P-Ethoxy (Ethoxy Phosphate) [EoP] modified backbone oligos

P-Methoxy (Methoxy Phosphate), P-Ethoxy (Ethoxy Phosphate) and methyl phosphonate [mp] modified backbone oligos makes the phosphodiester linkage neutral charged. The solubility of the oligo in aqueous solutions slowly decreases with increasing modified linkages; consider incorporating as many standard phosphodiester linkages as well in the oligo. Increasing percentage of DMSO from 0.5 to 10% may be used to solubilize the oligo.
These oligonucleotides with neutral backbone displayed high nuclease resistance and improved cellular uptake (1). These are one of the favorable properties of antisense oligonucleotides. In addition to being neutral charge but also impart lipophilic character to the modified oligo.
. Gutierrez-Puente et al (2) used a P-ethoxy oligonucleotide (oligo), 20 bases long and specific for the translation initiation site of human Bcl-2 mRNA. This was incorporated into liposomes to increase its intracellular delivery. This oligo selectively inhibited Bcl-2 protein expression and induced growth inhibition in t(14;18)-positive transformed follicular lymphoma (FL) cell lines. They studied the inhibitory effects of shorter liposomal P-ethoxy oligos (7, 9, 11 or 15 mer) in order to determine the activity of different oligo chain lengths targeted to the same Bcl-2 mRNA. At 12 µM, all the oligos inhibited the growth of a FL cell line. They compared the 7-mer oligo with the 20-mer oligo. The two oligos inhibited Bcl-2 protein expression similarly: 66% and 60% for the 7- and 20-mer, respectively. The uptake and retention of both oligos were also very similar. Their results indicate that the Bcl-2 inhibitory activity is maintained with P-ethoxy antisense oligos ranging from 7 to 20 bases.

P-Methoxy (Methoxy Phosphate), P-Ethoxy (Ethoxy Phosphate) References

1. Roberts, T. C.; Langer, R.; Wood, M. J. A. (2020) Advances in oligonucleotide drug Delivery. Nature Reviews Drug Discovery 19: 673-694.
2. Gutierrez-Puente, Y.; Tari, A.M.; Ford, R.J.; Tamez-Guerra, R.; Mercado-Hernandez, R.; Santoyo-Stephano, M.; Lopez-Berestein, G. (2009) Cellular Pharmacology of P-ethoxy Antisense Oligonucleotides Targeted to Bcl-2 in a Follicular Lymphoma Cell Line.

Leukemia & Lymphoma 44: 1979-1985

Methyl phosphonoamidites are deoxynucleoside amidites modified such that, when incorporated into an oligonucleotide, that base position will have a (electrically neutral) methyl phosphonate backbone linkage instead of the standard (negatively charged) phosphodiester linkage. Oligos containing one or more methyl phosphonate linkages will be resistant to nuclease degradation at those positions, and the lack of charge improves intracellular transport. Because of these properties, methyl phosphonolated oligos have been explored as anti-sense reagents (1). However, since methyl phosphonate linkages lower the oligo's cellular uptake (2) as well as the Tm of the duplex formed with its RNA target (3), and, most importantly, also interferes with activation of RNase H activity (4), considerable care must taken in choosing which, and how many, methyl phosphonate linkages to incorporate into a putative anti-sense oligo. In that regard, we note that 2'-O-Methyl RNA oligos containing a single 3'-end methyl phosphonate "cap" (to eliminate 3'-exonuclease degradation) have been successfully used as anti-sense reagents (5). In addition, DNA extension primers containing such a "cap" have been used to characterize the nuclease activity of the yeast telomerase complex (6). Methylphosphonolated anti-sense oligos have also been used successfully to "mask" sites in U1 and U2 snRNPs required for spliceosome formation, and thus interfere with mRNA splicing (7). Many of the unique properties of methyl phosphonate oligos are due to the introduction of chirality into the phosphodiester backbone by the methyl group (8).

Methyl phosphonate (mp) References

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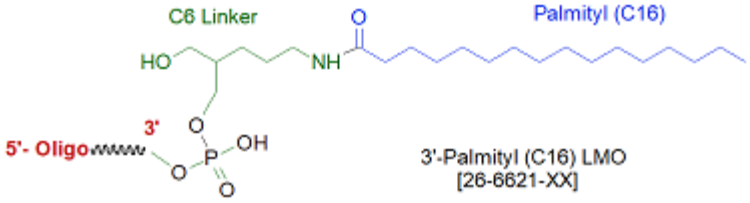
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Click this link to view these modifications.

Palmityl C16 (3') LMO

Category	Antisense & siRNA
Modification Code	Pal-C16-3
Reference Catalog Number	26-6621
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	432.56



This Palmitic C16 is only for 3' modification. We offer custom oligo synthesis of your designed sequences for conjugation to lignoceric acid and palmitic acid B>YIELD Approximate polyacrylamide gel purified yield for various scales are given below.
YIELD Approximate polyacrylamide gel purified yield for various scales are given below for an oligo below 50mer.
Yield given below are for oligos shorter than 50mer. Please see longer oligos yield at this link Long Oligo Typical Yield.
~2 nmol final yield for 50 nmol scale synthesis.
~8 nmol final yield for 200 nmol scale synthesis.
~25 nmol final yield for 1 umol scale synthesis
~45nmol final yield for 2 umol scale synthesis
~110nmol final yield for 5 umol scale synthesis
~180 nmol final yield for 10 umol scale synthesis
~260 nmol final yield for 15 umol scale synthesis
Click here to order stock MULTI-Seq LMO Lig-Anchor oligos.

Lipid modified oligos (LMO; lignoceric, palmitic, cholesterol etc.); These LMO rapidly and stably incorporate into the plasma membrane of live cells by step-wise assembly. McGinnis, C. et al. (1) adapted LMOs into MULTI-seq: scRNA-seq (single-cell) and snRNA-seq (single-nucleus) sample multiplexing using lipid-tagged indices. MULTI-seq localizes sample barcodes to live cells and nuclei regardless of species or genetic background while preserving cell viability and endogenous gene expression patterns.

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Palmitic acid, or hexadecanoic acid in IUPAC nomenclature, is the most common saturated fatty acid found in animals, plants and microorganisms. It is a C16 long chain saturated fatty acid. Palmitic acid is found naturally in palm oil and palm kernel oil, as well as in butter, cheese, milk and meat.
Oligonucleotides are predominantly hydrophilic species and require help in permeating cell membranes.

One strategy to improve cellular uptake of therapeutic oligonucleotides is to conjugate them with non-toxic, lipophilic molecules. Gene Link offers cholesteryl TEG, alpha-tocopherol and stearyl labelling of oligonucleotides and this strategy has proved to be useful for delivering therapeutic oligonucleotides to a broad distribution of targets.

Stearyl Modification

Stearyl Modification is C18 lipid, it is an economical and effective carrier molecule. We envisage that the 5'-stearyl group will become a favored lipophilic carrier for experimentation with synthetic oligonucleotides.

Cholesterol TEG Modification

Cholesterol TEG Modification is a lipophilic modification aiding in cellular delivery. The TEG liker arm facilitates solubility issues of the oligo making it soluble in aqueous buffers.

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GalNAc

A more directed approach to the delivery of therapeutic oligonucleotides specifically to the liver has been to target the asialoglycoprotein receptor (ASGPR) using a suitable glycoconjugate. Indeed, ASGPR is the ideal target for delivery of therapeutic oligonucleotides to the liver since it combines tissue specificity, high expression levels and rapid internalization and turnover. The use of oligonucleotide glycoconjugates has led to significant advances in therapeutic delivery as evidenced by the work of Alnylam Pharmaceuticals which has developed multivalent N-acetylgalactosamine (GalNAc) conjugated siRNAs that bind at nanomolar levels to ASGPR (1). A similar strategy has been applied at Ionis Pharmaceuticals directed at the development of antisense oligonucleotide therapeutics (2).

The GalNAc ligand originally used by Alnylam is the triantennary ligand would seem to lend itself to formation by post synthesis conjugation to the 3' terminus but a complex trivalent GalNAc support would also be perfectly applicable, if challenging to produce. However, an alternative approach using a monovalent GalNAc support with two additions of a monovalent GalNAc phosphoramidite was also described and yielded a trivalent GalNAc structure. This (1+1+1) trivalent GalNAc structure led to GalNAc modified siRNA oligos with potency equal to the equivalent siRNA with the triantennary GalNAc ligand both in vitro and in vivo.

A further report on antisense oligonucleotides demonstrated (4) the effectiveness of modifying at the 5' terminus using monovalent GalNAc ligands. Up to five GalNAc monomers were added in a serial manner (Figure 3) and it was shown that activity of the antisense oligonucleotides improved as the number of GalNAc units increased. The authors also showed that phosphodiester linkages between the GalNAc units were preferable to phosphorothioate linkages in their testing (4).

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Palmityl C16 (5') LMO

Category	Antisense & siRNA
Modification Code	Pal-C16-5
Reference Catalog Number	26-6622
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	303.4



Palmityl (C16) 5'-LMO
[26-6622-XX

This Palmitic C16 is only for 5' modification. We offer custom oligo synthesis of your designed sequences for conjugation to lignoceric acid and palmitic acid **YIELD** Approximate polyacrylamide gel purified yield for various scales are given below for an oligo below 50mer.
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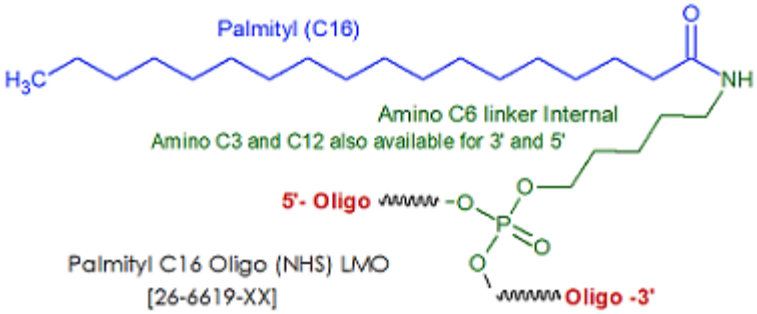
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Palmityl C16 Oligo (NHS) LMO

Category	Antisense & siRNA
Modification Code	Pal-C16-N
Reference Catalog Number	26-6619
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	256.62



This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield: NHS based modifications are post synthesis conjugation performed using a primary amino group. The yield is lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.
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~32 nmol final yield for 2 umol scale synthesis
~160 nmol final yield for 10 umol scale synthesis
~240 nmol final yield for 15 umol scale synthesis

Lipid Modified Oligo (LMO) Cell Tagging in Single Cell RNAseq/MULTI-Seq : Palmitic Oligo [C16] & Lignoceric Oligo [C24]
We offer custom oligo synthesis of your designed sequences for conjugation to lignoceric acid and palmitic acid for MULTI-seq: sample multiplexing for single-cell RNA sequencing
Click here to order stock MULTI-Seq LMO Lig-Anchor oligos.

Lipid modified oligos (LMO; lignoceric, palmitic, cholesterol etc.); These LMO rapidly and stably incorporate into the plasma membrane of live cells by step-wise assembly. McGinnis, C. et al. (1) adapted LMOs into MULTI-seq: scRNA-seq (single-cell) and snRNA-seq (single-nucleus) sample multiplexing using lipid-tagged indices. MULTI-seq localizes sample barcodes to live cells and nuclei regardless of species or genetic background while preserving cell viability and endogenous gene expression patterns.

MULTI-Seq LMO Lig Anchor and MULTI-Seq LMO Palm Co-Anchor oligos are lignoceric and palmitic acid conjugated oligos as described by McGinnis, C. et al. (1)
1. McGinnis, C. et al. MULTI-seq: sample multiplexing for single-cell RNA sequencing using lipid-tagged indices. Nat.

Methods 16, 619-626 (2019).

Palmitic acid, or hexadecanoic acid in IUPAC nomenclature, is the most common saturated fatty acid found in animals, plants and microorganisms. It is a C16 long chain saturated fatty acid. Palmitic acid is found naturally in palm oil and palm kernel oil, as well as in butter, cheese, milk and meat.

Oligonucleotides are predominantly hydrophilic species and require help in permeating cell membranes. One strategy to improve cellular uptake of therapeutic oligonucleotides is to conjugate them with non-toxic, lipophilic molecules. Gene Link offers cholesteryl TEG, alpha-tocopherol and stearyl labelling of oligonucleotides and this strategy has proved to be useful for delivering therapeutic oligonucleotides to a broad distribution of targets.

Stearyl Modification

Stearyl Modification is C18 lipid, it is an economical and effective carrier molecule. We envisage that the 5'-stearyl group will become a favored lipophilic carrier for experimentation with synthetic oligonucleotides.

Cholesterol TEG Modification

Cholesterol TEG Modification is a lipophilic modification aiding in cellular delivery. The TEG liker arm facilitates solubility issues of the oligo making it soluble in aqueous buffers.

alpha-tocopherol TEG Modification

Similar to cholesterol TEG, alpha-tocopherol (vitamin E) is both lipophilic and non-toxic even at high doses so would be an excellent candidate as a lipophilic carrier for oligonucleotides. The TEG liker arm facilitates solubility issues of the oligo making it soluble in aqueous buffers.

GalNAc

A more directed approach to the delivery of therapeutic oligonucleotides specifically to the liver has been to target the asialoglycoprotein receptor (ASGPR) using a suitable glycoconjugate. Indeed, ASGPR is the ideal target for delivery of therapeutic oligonucleotides to the liver since it combines tissue specificity, high expression levels and rapid internalization and turnover. The use of oligonucleotide glycoconjugates has led to significant advances in therapeutic delivery as evidenced by the work of Alnylam Pharmaceuticals which has developed multivalent N-acetylgalactosamine (GalNAc) conjugated siRNAs that bind at nanomolar levels to ASGPR (1). A similar strategy has been applied at Ionis Pharmaceuticals directed at the development of antisense oligonucleotide therapeutics (2).

The GalNAc ligand originally used by Alnylam is the triantennary ligand would seem to lend itself to formation by post synthesis conjugation to the 3' terminus but a complex trivalent GalNAc support would also be perfectly applicable, if challenging to produce. However, an alternative approach using a monovalent GalNAc support with two additions of a monovalent GalNAc phosphoramidite was also described and yielded a trivalent GalNAc structure. This (1+1+1) trivalent GalNAc structure led to GalNAc modified siRNA oligos with potency equal to the equivalent siRNA with the triantennary GalNAc ligand both in vitro and in vivo.

A further report on antisense oligonucleotides demonstrated (4) the effectiveness of modifying at the 5' terminus using monovalent GalNAc ligands. Up to five GalNAc monomers were added in a serial manner (Figure 3) and it was shown that activity of the antisense oligonucleotides improved as the number of GalNAc units increased. The authors also showed that phosphodiester linkages between the GalNAc units were preferable to phosphorothioate linkages in their testing (4).

Recommended Further Reading

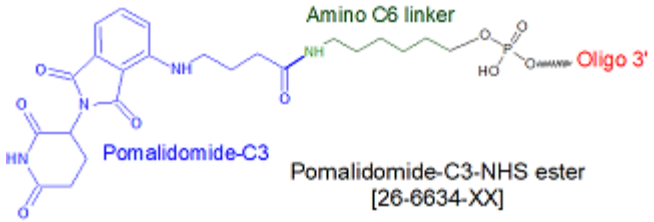
N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

References. Adapted from Glen Research Reports. http://www.glenresearch.com/GlenReports/GR29-14.html

1. J.K. Nair, et al., J Am Chem Soc, 2014, 136, 16958-61.
2. T.P. Prakash, et al., Bioorganic & Medicinal Chemistry Letters, 2015, 25, 4127-4130.
3. K.G. Rajeev, et al., Chembiochem, 2015, 16, 903-8.
4. T. Yamamoto, M. Sawamura, F. Wada, M. Harada-Shiba, and S. Obika, Bioorganic & Medicinal Chemistry, 2016, 24, 26-32.

Pomalidomide-C3-NHS

Category	Affinity Ligands
Modification Code	PDM-C3-N
Reference Catalog Number	26-6634
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	317.32



Gene Link offers two versions of Pomalidomide modification. 1. Pomalidomide-C3-NHS is for oligo modified with an amino group and this version contains a C3 linker. 2. Pomalidomide-PEG1-C2-N3 is a an azide for click chemistry to DBCO or BCN. At Gene Link we use BCN to conjugate the azide version to oligos.

The use of a Pomalidomide modification represents a shift in how we approach targeted protein degradation (TPD). Pomalidomide modification enables the creation of oligonucleotide-based PROteolysis Targeting Chimeras (Oligo-PROTACs) and aptamer-PROTAC conjugates. Pomalidomide acts as a highly effective recruitment ligand for the Cereblon (CRBN) E3 ubiquitin ligase, facilitating the ubiquitination and subsequent proteasomal degradation of target proteins. This modification is particularly valuable for engaging historically "undruggable" targets, such as transcription factors and RNA/DNA-binding proteins, by utilizing the programmable sequence recognition of the oligonucleotide.

By anchoring the pomalidomide payload to a targeting aptamer sequence, the degradation engine is effectively cloaked and selectively steered only to cells overexpressing tumor-specific surface biomarkers. This receptor-driven delivery logic ensures that the degrader remains inert until it internalizes into the target cancer cell, significantly boosting in vivo potency while sparing healthy tissues from unwanted side effects.

Tsujimura et al. (2023) utilized a 5' pomalidomide modification by linking this pomalidomide handle to a specialized DNA aptamer sequence targeting the estrogen receptor alpha (ERα). They engineered a bifunctional chimera capable of bringing the target protein and the cellular degradation machinery into close physical proximity. This targeted alignment successfully induces the selective ubiquitination and subsequent proteasomal destruction of the receptor, proving that pomalidomide-functionalized oligonucleotides can effectively expand the scope of targeted protein degradation to challenging intracellular targets.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol * The yield will be lower for oligos longer than 50mer.

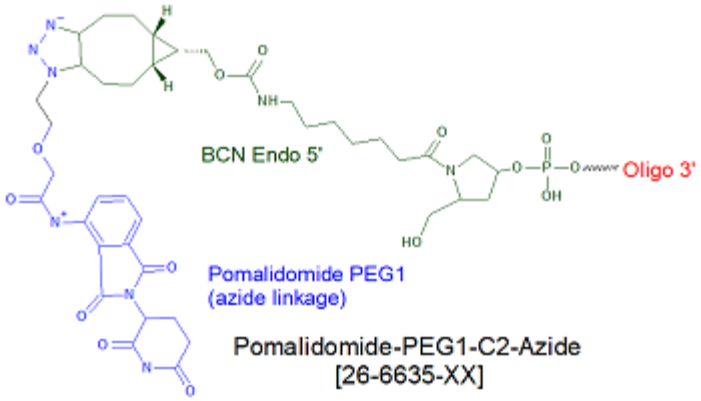
Click here for yield table of long oligos. * Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites.
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References

He, S., Dong, G., & Sheng, C. (2021). Aptamer-PROTAC Conjugates (APCs) for Tumor□Specific Targeting in Breast Cancer. Angewandte Chemie International Edition, 60(43), 23299-23305. Watanabe, D., Terauchi, H., Osawa, H., & Demizu, Y. (2025). Phosphorothioate-modified DNA aptamer-based PROTACs for targeted degradation of estrogen receptor α. Bioconjugate Chemistry. Advance online publication. Tsujimura, H., Naganuma, M., Ohoka, N., Inoue, T., Naito, M., Tsuji, G., & Demizu, Y. (2023). Development of DNA aptamer-based PROTACs that degrade the estrogen receptor. ACS Medicinal Chemistry Letters, 14(6), 827-832.

Pomalidomide-PEG1-C2-N3

Category	Affinity Ligands
Modification Code	PDM-PEG1-C2-N3
Reference Catalog Number	26-6635
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	386.4



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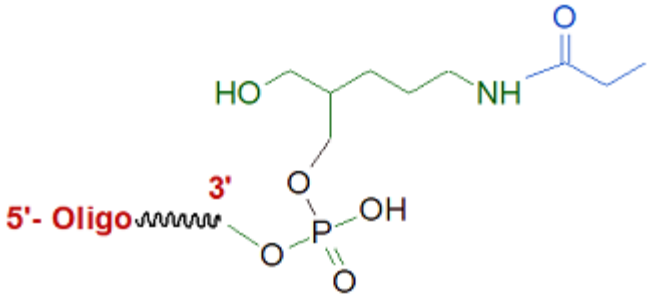
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Propionic acid (C3) Modified Oligo

Category	Antisense & siRNA
Modification Code	3-PA-C3
Reference Catalog Number	26-6781
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	250.21



3'-Propionyl (C3) PA LMO

[26-6781-XX]

Propionyl (C3) PA LMO
Gene Link offers a wide range of lipid modified oligos for cellular delivery. Click here to see the complete list.

Lipid modified oligos (LMO; lignoceric, palmitic, cholesterol, Propionyl (C3) PA, Butyl (C4) BA, Linolyl (C18:2) LA, Alpha□linolyl (C18:3α) ALA, Gamma□linolyl (C18:3γ) GLA, Dihomo□gamma□linolyl (C20:3) DGLA, Arachidonyl (C20:4) AA, Eicosapent (20:5) EPA etc.).
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Recommended Further Reading

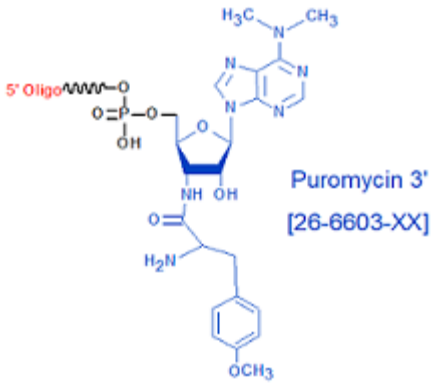
N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

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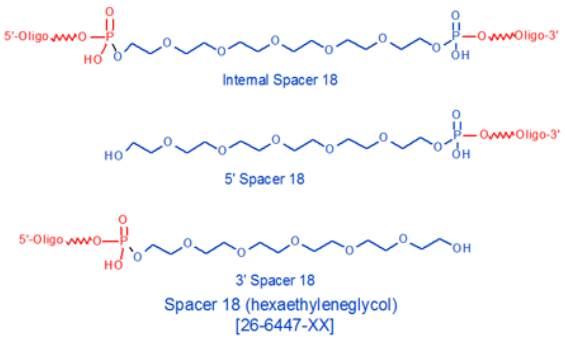
Puromycin

Category	Others
Modification Code	Puro-3
Reference Catalog Number	26-6603
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	533.48



Spacer 18

Category	Spacers
Modification Code	Sp18
Reference Catalog Number	26-6447
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	344.3



Spacer 18 also known as PEG6 is a hexaethylene glycol chain that is 18 atoms long (12 carbons + 6 oxygen's), and is used to incorporate a long spacer arm into an oligonucleotide. Spacer 18 can be incorporated in consecutive additions whenever a longer spacer is required. Spacer 18 had been used to form bold folds and hairpin loops in oligonucleotides (1,2), and for solid-phase immobilization of hybridization probes (3). Spacer 18 has also been used to modify random primers used in whole genome amplification (WGA)-based applications, as a way to eliminate self-priming events that form spurious DNA products (that is, false-positive amplification) in the PCR reactions (4).
Gene Link offers spacers of various length, examples C2, C3, C6, C12 and Spacer 9 and 18. These can be inserted multiple times to yield a total much longer spacer length. **References**

1. Salunkhe, M., Wu, T.F., Letsinger, R.L. Control of folding and binding of oligonucleotides by use of non-nucleotide linker. *J. Am. Chem. Soc.* (1992), **114**: 8768-8772.
2. Durand, M., Chevrie, K., Chassignol, M., Thuong, N.T., Maurizot, J. Circular dichroism studies of an oligodeoxyribonucleotide containing a hairpin loop made of a hexaethylene glycol chain: conformation and stability.*Nucleic Acids Res.* (1990), **18**: 6353-6359.
3. Zhang, Y., Coyne, M.Y., Will, S.G., Levenson, C.H., Kawasaki, E.S. Single-base mutational analysis of cancer and genetic diseases using membrane bound modified oligonucleotides. *Nucleic Acids Res.* (1991), **19**: 3929-3933.
4. Brukner, I., Paquin, B., Belouchi, M., Labuda, D., Krajinovic, M. Self-priming arrest by modified random oligonucleotides facilitates the quality control of whole genome amplification.*Anal. Biochem.* (2005), **339**: 345-347.

Oligonucleotide PEGylation : Spacers vs. PEGylation Gene Link offers short PEG3 and PEG6 as direct coupling using automated chemistry. The PEG3 is termed as Spacer 9 and PEG6 as spacer 18. These are also used to introduce space between adjacent sequence and modifications. These can be inserted multiple times to increase the PEG units.
Larger 2, 5, 10 and 20 kDa PEGylation of oligonucleotides is inserted at any site of an oligonucleotide using a post synthesis amino group on the oligo with PEG-NHS.
PEGylation is the covalent attachment of polyethylene glycol (PEG) to oligonucleotides such as DNA, RNA, antisense, siRNA and aptamers.

It improves pharmacokinetics, reduces renal clearance, increases nuclease stability, and decreases immunogenicity. (1) The way PEG shields its conjugated payload offers new challenges and opportunities for oligonucleotide PEGylation. Other than aptamers, the target of most oligonucleotides is a complementary sequence.
Comparison of PEGylation Size & Biological Outcome

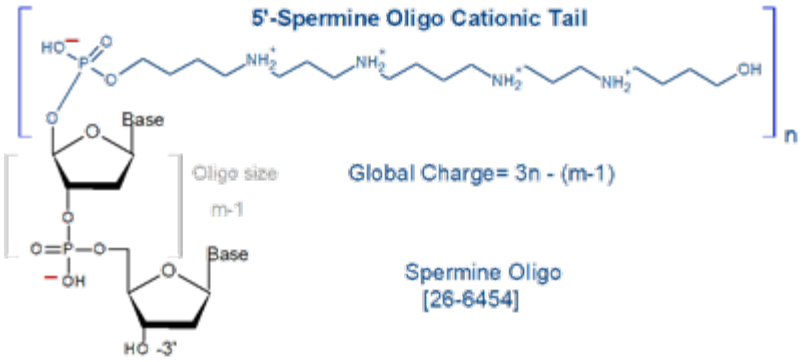
PEG Size (Ethylene Glycol Units)

Hydrodynamic Effect

Typical Outcome 2 kDa (~44) Minimal Slight stability increase 5 kDa (~114) Moderate Partial half-life improvement 10 kDa (~227) Strong Reduced renal clearance 20 kDa (~455) Very strong Long circulation 40 kDa (~910) Extreme Depot-like behavior Pharmacokinetics depend on hydrodynamic diameter, not molecular weight of the oligo. Unmodified 20-mer oligo ≈ ~7 kDa. Kidney filtration cutoff ≈ 40-60 kDa hydrodynamic equivalent Oligo PEG size controls circulation time versus tissue penetration. Optimal design balances exposure and activity
Messenger RNA (mRNA) delivery strategies are required to protect biologically fragile mRNA from ribonuclease (RNase) attacks to achieve efficient therapeutic protein expression. To tackle this issue, most mRNA delivery systems have used cationic components.
A cation-free delivery strategy by hybridization of PEGylated RNA oligonucleotides with mRNA. The PEG strands on the mRNA sterically and electrostatically shields the mRNA, improving mRNA nuclease stability 15-fold and the PEGylated mRNA induced nearly 20-fold higher efficiency of reporter protein expression than unhybridized mRNA in cultured cells (2).
PEGylation has been used to improve the biopharmaceutical properties of protein drugs since the 1990s, and over a dozen PEGylated pharmaceuticals are currently on the market (2). PEG creates a large hydration shell, which sterically blocks other biomacromolecules from penetrating through the polymer layer and binding with the interior substrate (3, 4). Binding requires displacing the PEG by the incoming molecule, generally making such binding less thermodynamically favorable. These properties usually result in weaker interactions between the receptor and the conjugated molecule, but increased drug solubility, prolonged blood circulation, and increased drug stability often offset by the reduced binding affinity. PEGylated oligonucleotides can be an exception to this generalization, with increased binding to a complementary sequence compared to unmodified ONs. The effect is attributed to macromolecular volume exclusion (6).
PEGylation References
1. Li WJ; Zhan P; De Clercq E; Lou HX; Liu XY Current drug research on PEGylation with small molecular agents. Prog. Polym. Sci 2013, 38, 421-444.
2. Yoshinaga, N; Naito, M; Tachihara, Y; Boonstra, E; Osada, K; Cabral, H and Uchida, S. PEGylation of mRNA by Hybridization of Complementary PEG-RNA Oligonucleotides Stabilizes mRNA without Using Cationic Materials. Pharmaceutics 2021, 13, 800.
3. Harris JM; Chess RB Effect of pegylation on pharmaceuticals. Nat. Rev. Drug Discov 2003, 2, 214-221. [PubMed: 12612647]
4. Harris JM; Martin NE; Modi M Pegylation. Clin. Pharmacokinet 2001, 40, 539-551. [PubMed: 11510630]
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6. Nakano S-I; Karimata H; Ohmichi T; Kawakami J; Sugimoto N The effect of molecular crowding with nucleotide length and cosolute structure on DNA duplex stability. J. Am. Chem. Soc 2004, 126, 14330-14331. [PubMed: 15521733]

Spermine Oligo

Category	Duplex Stability
Modification Code	Spm
Reference Catalog Number	26-6454
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	408.52



Solubility of oligos with 4-40 Spermine sites (ZNA Oligos). Gene Link supplies all oligos as lyophilized/dried state. Oligos with more than 4 spermine sites have lower solubility in aqueous solutions. Reconstitute these oligos in 100 mM Ammonium hydroxide. Spermine oligos with more solubility concerns may be resolved by adding 50 mM ammonium hydroxide drop wise until the ZNA goes into solution in water OR by dissolving the ZNA in concentrated phosphate buffer saline.

Spermine phosphoramidite is used to produce oligospermine-oligonucleotide conjugates - Zip Nucleic Acids (ZNA) Oligos. The name reflects the presumed mode of action. The conjugates are believed to use the oligospermine to seek out and move along (scan) oligonucleotide strands until the probe complementary sequence is located. The oligospermine then performs the function of stabilizing the formed duplex by reducing electrostatic repulsion, thereby leading to significantly increased binding affinities. ZNA Oligos have found use in the following applications: Multiplex PCR; PCR of AT-rich Regions; RT qPCR; Detection of MicroRNA; Improved SNP Discrimination; and Antisense and Antigene Effects. Spermine phosphoramidite is simple to use in oligonucleotide synthesis and can be added multiple times at the 3' or 5' terminus. Deprotection and isolation are also straightforward. HPLC analysis of the conjugates requires high pH to suppress the ionization of the spermine residues.
By selecting the number of cationic units, the global charge of the ZNA molecules can be modulated which defines their field of applications. When negatively charged, ZNA are potent tools for molecular biology and diagnostic applications. Their design is essentially based on the expected and predictable Tm of the oligonucleotide which depends on the number of conjugated cationic units. When positively charged, the cationic conjugates become self-delivering oligonucleotides into cells and resistant to nucleases which make them very attractive molecules for antisense or RNA interference applications.
With an increase in spermine content, the solubility of ZNA oligonucleotides may be noticeably less than unmodified DNA or RNA counterparts. This is typically observed when re-dissolving dried-down purified ZNA in water. In this case, drop wise addition of 50 mM ammonium hydroxide brings ZNA molecules into solution. Alternatively, dissolving ZNA oligos in concentrated phosphate buffered saline (2.5x PBS, pH 7.

4) has also been found to resolve solubility issues.

Recommended Further Reading

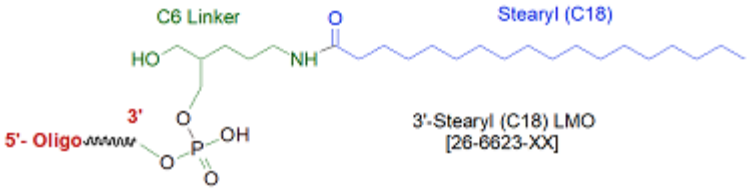
Glen Reports GR24-11. Spermine Phosphoramidite: A potent modification with many applications.
Glen Reports GR24-11. Zip Nucleic Acids (ZNA) are powerful cationic oligonucleotides for molecular biology, diagnostic and therapeutic applications.

INTELLECTUAL PROPERTY

"Spermine phosphoramidite" synthon is the subject matter of U.S. Patent Application No. 12/086.599, European Patent Application No. EP20060847298 and foreign equivalents for which Polyplus-transfection is the co-owner. Product is sold for research purposes only. Product shall not be used to manufacture oligonucleotide-oligospermine conjugates for use in diagnostics, clinical or commercial applications including use in humans. There is no implied license to manufacture oligospermine-oligonucleotide conjugates for diagnostic, clinical or commercial applications, including but not limited to contract research. Please contact Polyplus-transfection at licensing@polyplus-transfection.com to obtain a license for such use.

Stearyl C18 (3') LMO

Category	Affinity Ligands
Modification Code	Str-C18-3
Reference Catalog Number	26-6623
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	460.61



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GalNAc

A more directed approach to the delivery of therapeutic oligonucleotides specifically to the liver has been to target the asialoglycoprotein receptor (ASGPR) using a suitable glycoconjugate. Indeed, ASGPR is the ideal target for delivery of therapeutic oligonucleotides to the liver since it combines tissue specificity, high expression levels and rapid internalization and turnover. The use of oligonucleotide glycoconjugates has led to significant advances in therapeutic delivery as evidenced by the work of Alnylam Pharmaceuticals which has developed multivalent N-acetylgalactosamine (GalNAc) conjugated siRNAs that bind at nanomolar levels to ASGPR (1). A similar strategy has been applied at Ionis Pharmaceuticals directed at the development of antisense oligonucleotide therapeutics (2).

The GalNAc ligand originally used by Alnylam is the triantennary ligand would seem to lend itself to formation by post synthesis conjugation to the 3' terminus but a complex trivalent GalNAc support would also be perfectly applicable, if challenging to produce. However, an alternative approach using a monovalent GalNAc support with two additions of a monovalent GalNAc phosphoramidite was also described and yielded a trivalent GalNAc structure.

This (1+1+1) trivalent GalNAc structure led to GalNAc modified siRNA oligos with potency equal to the equivalent siRNA with the triantennary GalNAc ligand both in vitro and in vivo.
Researchers at Ionis have developed antisense oligonucleotides containing the GalNAc cluster. In their case, they were able to show2 that moving the triantennary GalNAc ligand to the 5' terminus led to improved potency in vitro and in vivo. As may be expected, such a large complex ligand lends itself to solution phase chemistry to produce GalNAc modified antisense oligos. However, a solid phase synthetic approach was also described, and compared to the solution phase approach structure of the 5'-GalNAc triantennary ligand (4).
A further report on antisense oligonucleotides demonstrated (5) the effectiveness of modifying at the 5' terminus using monovalent GalNAc ligands. Up to five GalNAc monomers were added in a serial manner (Figure 3) and it was shown that activity of the antisense oligonucleotides improved as the number of GalNAc units increased. The authors also showed that phosphodiester linkages between the GalNAc units were preferable to phosphorothioate linkages in their testing (5).

Recommended Further Reading

N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

References. Adapted from Glen Research Reports. http://www.glenresearch.com/GlenReports/GR29-14.html
1. J.K. Nair, et al., J Am Chem Soc, 2014, 136, 16958-61.
2. T.P. Prakash, et al., Bioorganic & Medicinal Chemistry Letters, 2015, 25, 4127-4130.
3. K.G. Rajeev, et al., Chembiochem, 2015, 16, 903-8.
4. I. Cedillo, et al., Molecules, 2017, 22.
5. T. Yamamoto, M. Sawamura, F. Wada, M. Harada-Shiba, and S. Obika, Bioorganic & Medicinal Chemistry, 2016, 24, 26-32.

Stearyl C18 (5') LMO

Category	Antisense & siRNA
Modification Code	Str-C18-5
Reference Catalog Number	26-6617
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	332.46



Stearyl (C18) 5'-LMO
[26-6617-XX]

Oligonucleotides are predominantly hydrophilic species and require help in permeating cell membranes. One strategy to improve cellular uptake of therapeutic oligonucleotides is to conjugate them with non-toxic, lipophilic molecules. Gene Link offers cholesteryl TEG, alpha-tocopherol and stearyl labelling of oligonucleotides and this strategy has proved to be useful for delivering therapeutic oligonucleotides to a broad distribution of targets.

Stearyl Modification

Stearyl Modification is C18 lipid, it is an economical and effective carrier molecule. We envisage that the 5'-stearyl group will become a favored lipophilic carrier for experimentation with synthetic oligonucleotides.

Cholesterol TEG Modification

Cholesterol TEG Modification is a lipophilic modification aiding in cellular delivery. The TEG liker arm facilitates solubility issues of the oligo making it soluble in aqueous buffers.

alpha-tocopherol TEG Modification

Similar to cholesterol TEG, alpha-tocopherol (vitamin E) is both lipophilic and non-toxic even at high doses so would be an excellent candidate as a lipophilic carrier for oligonucleotides. The TEG liker arm facilitates solubility issues of the oligo making it soluble in aqueous buffers.

GalNAc

A more directed approach to the delivery of therapeutic oligonucleotides specifically to the liver has been to target the asialoglycoprotein receptor (ASGPR) using a suitable glycoconjugate. Indeed, ASGPR is the ideal target for delivery of therapeutic oligonucleotides to the liver since it combines tissue specificity, high expression levels and rapid internalization and turnover. The use of oligonucleotide glycoconjugates has led to significant advances in therapeutic delivery as evidenced by the work of Alnylam Pharmaceuticals which has developed multivalent N-acetylgalactosamine (GalNAc) conjugated siRNAs that bind at nanomolar levels to ASGPR (1). A similar strategy has been applied at Ionis Pharmaceuticals directed at the development of antisense oligonucleotide therapeutics (2).

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