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

(1) Juliano, R., Alam, Md.R., Dixit, V., Kang, H. Mechanisms and strategies for effective delivery of antisense and siRNA oligonucleotides. Nucleic Acids Res. (2008), 36: 4158-4171.
(2) Juliano, R.L. Biological Barriers to Nanocarrier-Mediated Delivery of Therapeutic and Imaging Agents. In: Niemeyer, C.M., Mirkin, C.A., editors. Nanobiotechnology II. Weinheim, Germany: Wiley-VCH; 2007. pp. 263-278.
(3) Soutschek, J., Akinc, A., Bramlage, B., Charisse, K., Constien, R., Donoghue, M., Elbashir, S., Geick, A., Hadwiger, P., Harborth, J., et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature (2004), 432: 173-178.
(4) Wolfrum, C., Shi, S., Jayaprakash, K.N., Jayaraman, M., Wang, G., Pandey, R.K., Rajeev, K.G., Nakayama, T., Charrise, K., Ndungo, E.M., et al. Mechanisms and optimization of in vivo delivery of lipophilic siRNAs. Nat. Biotechnol. (2007), 25: 1149-1157.
(5) van Vlerken, L.E., Vyas, T.K., Amiji, M.M. Poly(ethylene-glycol)-modified nanocarriers for tumor-targeted and intracellular delivery. Pharm. Res. (2007), 24: 1405-1414.
(6) Debart, F., Abes, S., Deglane, G., Moulton, H.M., Clair, P., Gait, M.J., Vasseur, J., Lebleu, B. Chemical Modifications to Improve the Cellular Uptake of Oligonucleotides. Curr. Top. Med. Chem. (2007), 7: 727-737.
(7) Manoharan, M. Oligonucleotide conjugates as potential antisense drugs with improved uptake, biodistribution, targeted delivery, and mechanism of action. Antisense Nucleic Acid Drug Dev. (2002), 12: 103-128.
(8) Chaltin, P., Margineanu, A., Marchand, D., Van Aerschot, A., et al. Delivery of antisense oligonucleotides using cholesterol-modified sense dendrimers and cationic lipids. Bioconj. Chem. (2005), 16: 827-836.
(9) Lorenz, C., Hadwiger, P., John, M., Vornlocher, H.P., Unverzagt, C. Steroid and lipid conjugates of siRNAs to enhance cellular uptake and gene silencing in liver cells. Bioorg. Med. Chem. Lett. (2004), 14: 4975-4977.

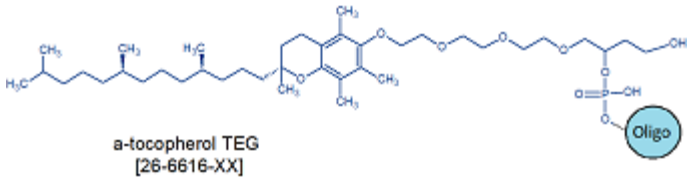
Modificaton Code List

Modification	Code	Catalog Number
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)	[CholTEG]	26-6602
Cholesterol TEG-3' (15 atom triethylene glycol spacer)	[3-CholTEG]	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
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	[3-Pal-C16]	26-6621
Palmityl C16 (5') LMO	[5-Pal-C16]	26-6622
Palmityl C16 Oligo (NHS) LMO	[Pal-C16-N]	26-6619
Propionyl (C3) PA LMO	[3-PA-C3]	26-6781
Puromycin	[Puro]	26-6603
Spacer 18 (hexaethyleneglycol)	[Sp18]	26-6447
Spermine Oligo Cationic Tail (ZNA-Oligos)	[Spm]	26-6454
Stearyl C18 LMO LMO 3'	[3-Str-C18]	26-6623
Stearyl C18 LMO Oligo 5'	[5-Str-C18]	26-6617

a-Tocopherol TEG

Category	Antisense
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]

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.
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).

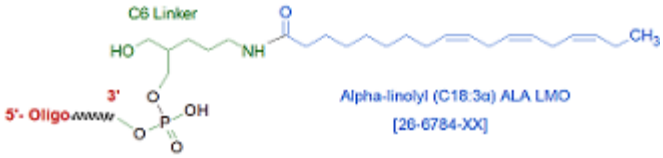
Recommended Further Reading

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.

Alpha-linolenic acid (C18:3α) Oligo

Category	Antisense
Modification Code	3-ALA-C18-3
Reference Catalog Number	26-6784
5 Prime	Y
3 Prime	Y
Internal	Y
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.).
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).

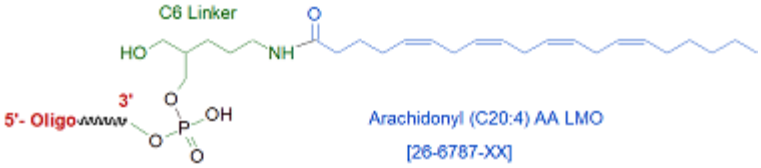
Recommended Further Reading

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.

Arachidonic acid (C20:4) Oligo

Category	Antisense
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.).
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).

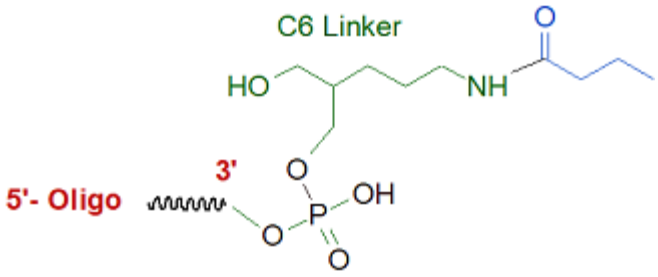
Recommended Further Reading

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.

Butyl (C4) BA LMO

Category	Antisense
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.).
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).

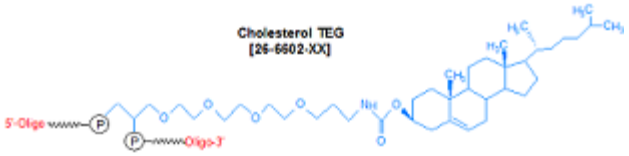
Recommended Further Reading

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.

Cholesterol TEG

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



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.

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.

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.
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).

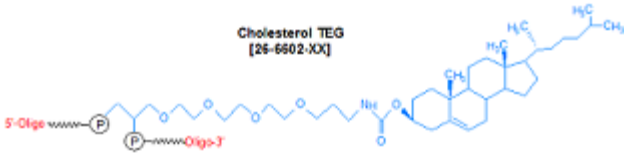
Recommended Further Reading

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.

Cholesterol TEG-3'

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



Antisense Oligos (ODN) & siRNA Oligo Cellular Delivery Modifications
Click here for more information on antisense modifications, design & applications.

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.

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.

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.

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

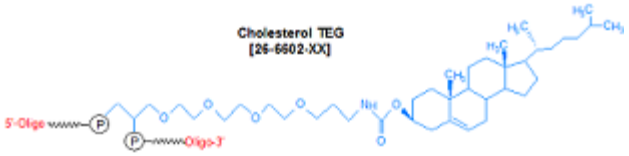
N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

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.

d-Tocopherol TEG

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



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.
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).

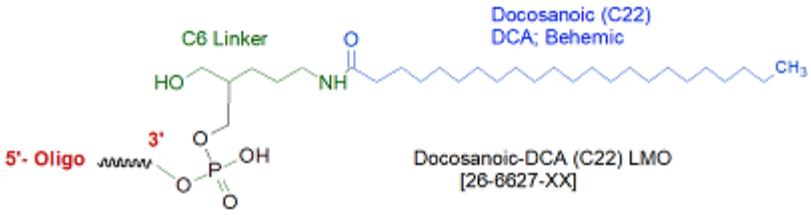
Recommended Further Reading

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.

DCA (C22) LMO

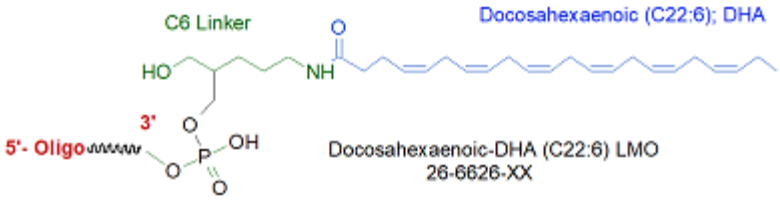
Category	Antisense
Modification Code	3-DCA-C22
Reference Catalog Number	26-6627
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	517



-Docosanoic-DCA (C22) Lipid Modified Oligo (LMO)

DHA (C22:6) LMO

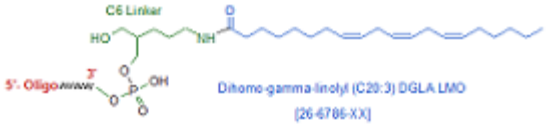
Category	Antisense
Modification Code	3-DHA-C22-6
Reference Catalog Number	26-6626
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	505



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

Dihomo-γ-linolenic acid (C20:3) Oligo

Category	Antisense
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

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).

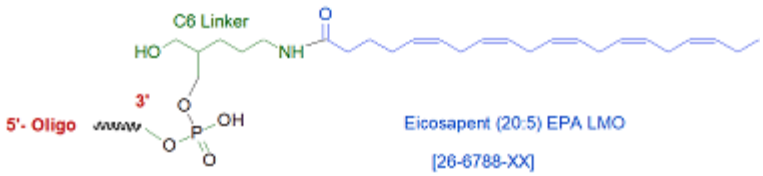
Recommended Further Reading

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.

Eicosapentaenoic acid (20:5) Oligo

Category	Antisense
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).

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).

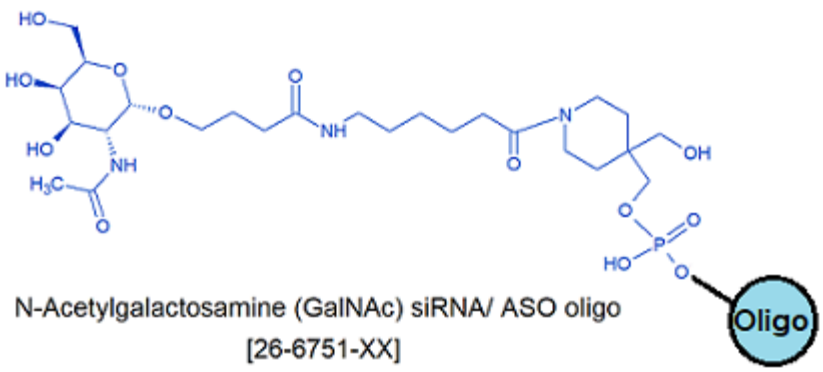
Recommended Further Reading

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.

GalNAc

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



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

siRNA Oligo Cellular Delivery Modifications
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 for the quoted/invoiced price of GalNAc component
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.

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).

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.

Recommended Further Reading

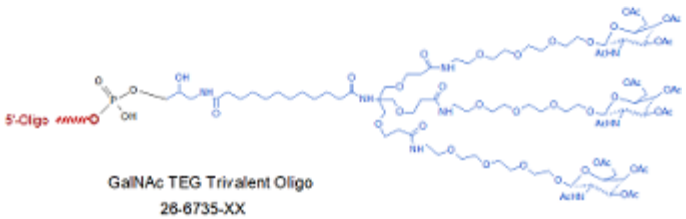
N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

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.

GalNAc TEG Trivalent 3'

Category	Antisense
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 on antisense and siRNA modifications, design & applications.

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.

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).

-

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.

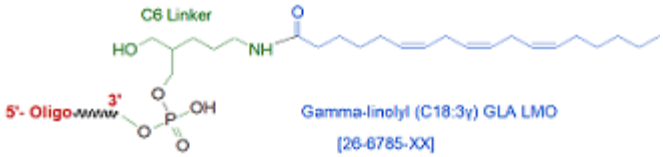
Recommended Further Reading

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.

Gamma-linolenic acid (C18:3γ) Oligo

Category	Antisense
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.).
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).

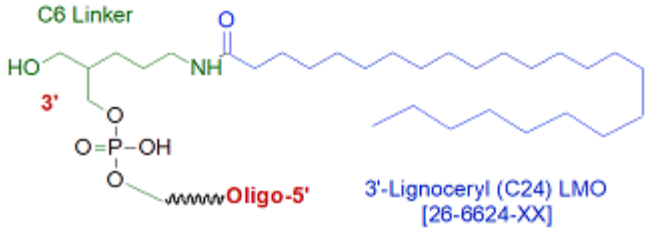
Recommended Further Reading

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.

Lignoceryl C24 (3') LMO

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



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 **YIELD** Approximate polyacrylamide gel purified yield for various scales are given below.
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.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis
~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
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).

Recommended Further Reading

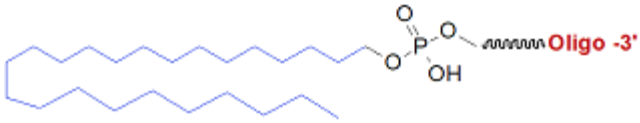
N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

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.

Lignoceryl C24 (5') LMO

Category	Antisense
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

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 **YIELD**
Approximate polyacrylamide gel purified yield for various scales are given below.
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.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis
~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
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).

Recommended Further Reading

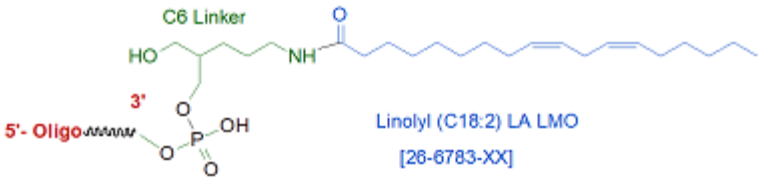
N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

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.

Linoleic acid (C18:2) Oligo

Category	Antisense
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.).
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).

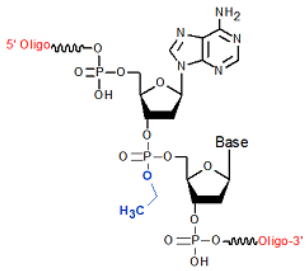
Recommended Further Reading

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.

P-Ethoxy dA

Category	Antisense
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

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

1. Sarin, P.S., Agrawal, S., Civeira, M.P., Goodchild, J., Ikeuchi, T., Zamecnik, P.C. Inhibition of acquired immunodeficiency syndrome virus by oligodeoxynucleoside methylphosphonates. (1988) *Proc. Natl. Acad. Sci. USA* **85**: 7448-7451.
2. Blake, K.R., Murakami, A., Spitz, S.A., Glave, S.A., Reddy, M.P., Ts'o, P.O., Miller, P.S. Hybridization arrest of globin synthesis in rabbit reticulocyte lysates and cells by oligodeoxyribonucleoside methylphosphonates. (1985) *Biochemistry*, **24**: 6139-6145.
3. Kibler-Herzog, L., Zon, G., Uznanski, B., Whittier, G, Wilson, W.D. Duplex stabilities of phosphorothioate, methylphosphonate, and RNA analogs of two DNA 14-mers. (1991) *Nucleic Acids Res.* **19**: 2979-2986.
4. Walder, J. Antisense DNA and RNA: progress and prospects. (1988) *Genes Dev.* **2**: 502-504.
5. Prater, C.E., Miller, P.S. 3'-Methylphosphonate-Modified Oligo-2'-O-methylribonucleotides and Their Tat Peptide Conjugates: Uptake and Stability in Mouse Fibroblasts in Culture. (2004) *Bioconjugate Chem.* **15**: 498-507.
6. Niu, H., Xia, J., Lue, N.F. Characterization of the Interaction between the Nuclease and Reverse Transcriptase Activity of the Yeast Telomerase Complex. (2000) *Mol. Cell. Biol.* **20**: 6806-6815.
7. Temsamani, J., Agrawal, S., Pederson, T. Biotinylated Antisense Methylphosphonate Oligodeoxynucleotides-Inhibition of Spliceosome Assembly and Affinity Selection for U1 and U2 Small Nuclear RNPs. (1991) *J. Biol. Chem.* **266**: 468-472.
8. Thiviyanathan, V., Vyazovkina, K.V., Gozansky, E.K., Bichenchova, E., Abramova, T.V., Luxon, B.A., Lebedev, A.V., Gorenstein, D.G. (2002) Structure of Hybrid Backbone Methylphosphonate DNA Heteroduplexes: Effect of R and S Stereochemistry. *Biochemistry*. **416**: 827-838.

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).

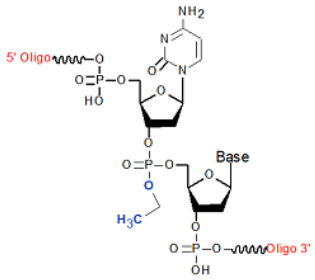
Mesyl Phosphoramidate (Ms, u) References 1. Miroshnichenko, S.K., Patutina, O.A., Burakova, E.A., Chelobanov, B.P., Fokina, A.A., Vlassov, V.V., Altmanb, A., Zenkova, M.A., Stetsenko, D. A. Mesyl phosphoramidate antisense oligonucleotides as an alternative to phosphorothioates with improved biochemical and biological properties. *PNAS* 2019. **116** : 1229-1234.
2. Prokhorova, D. V., Chelobanov, B.P., Burakova, E.A., Fokina, A.A., Stetsenko, D.A. (2017) New oligodeoxyribonucleotide derivatives bearing internucleotide N-tosyl phosphoramidate groups: Synthesis and complementary binding to DNA and RNA. Russ. J. Bioorganic Chem. 43:38-42.
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.
5. Taber, D.F., Ruckle, R.E. Jr., Hennessy, M.J., (1986) Mesyl Azide: A Superior Reagent for Diazo Transfer. J. Org. Chem., 51:4077-4078

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
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

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

1. Sarin, P.S., Agrawal, S., Civeira, M.P., Goodchild, J., Ikeuchi, T., Zamecnik, P.C. Inhibition of acquired immunodeficiency syndrome virus by oligodeoxynucleoside methylphosphonates. (1988) *Proc. Natl. Acad. Sci. USA* **85**: 7448-7451.
2. Blake, K.R., Murakami, A., Spitz, S.A., Glave, S.A., Reddy, M.P., Ts'o, P.O., Miller, P.S. Hybridization arrest of globin synthesis in rabbit reticulocyte lysates and cells by oligodeoxyribonucleoside methylphosphonates. (1985) *Biochemistry*, **24**: 6139-6145.
3. Kibler-Herzog, L., Zon, G., Uznanski, B., Whittier, G, Wilson, W.D. Duplex stabilities of phosphorothioate, methylphosphonate, and RNA analogs of two DNA 14-mers. (1991) *Nucleic Acids Res.* **19**: 2979-2986.
4. Walder, J. Antisense DNA and RNA: progress and prospects. (1988) *Genes Dev.* **2**: 502-504.
5. Prater, C.E., Miller, P.S. 3'-Methylphosphonate-Modified Oligo-2'-O-methylribonucleotides and Their Tat Peptide Conjugates: Uptake and Stability in Mouse Fibroblasts in Culture. (2004) *Bioconjugate Chem.* **15**: 498-507.
6. Niu, H., Xia, J., Lue, N.F. Characterization of the Interaction between the Nuclease and Reverse Transcriptase Activity of the Yeast Telomerase Complex. (2000) *Mol. Cell. Biol.* **20**: 6806-6815.
7. Temsamani, J., Agrawal, S., Pederson, T. Biotinylated Antisense Methylphosphonate Oligodeoxynucleotides-Inhibition of Spliceosome Assembly and Affinity Selection for U1 and U2 Small Nuclear RNPs. (1991) *J. Biol. Chem.* **266**: 468-472.
8. Thiviyanathan, V., Vyazovkina, K.V., Gozansky, E.K., Bichenchova, E., Abramova, T.V., Luxon, B.A., Lebedev, A.V., Gorenstein, D.G. (2002) Structure of Hybrid Backbone Methylphosphonate DNA Heteroduplexes: Effect of R and S Stereochemistry. *Biochemistry*. **416**: 827-838.

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).

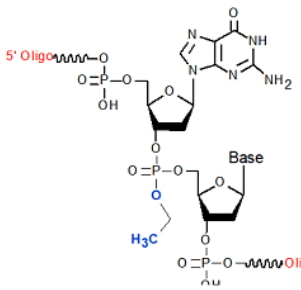
Mesyl Phosphoramidate (Ms, u) References 1. Miroshnichenko, S.K., Patutina, O.A., Burakova, E.A., Chelobanov, B.P., Fokina, A.A., Vlassov, V.V., Altmanb, A., Zenkova, M.A., Stetsenko, D. A. Mesyl phosphoramidate antisense oligonucleotides as an alternative to phosphorothioates with improved biochemical and biological properties. *PNAS* 2019. **116** : 1229-1234.
2. Prokhorova, D. V., Chelobanov, B.P., Burakova, E.A., Fokina, A.A., Stetsenko, D.A. (2017) New oligodeoxyribonucleotide derivatives bearing internucleotide N-tosyl phosphoramidate groups: Synthesis and complementary binding to DNA and RNA. Russ. J. Bioorganic Chem. 43:38-42.
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.
5. Taber, D.F., Ruckle, R.E. Jr., Hennessy, M.J., (1986) Mesyl Azide: A Superior Reagent for Diazo Transfer. J. Org. Chem., 51:4077-4078

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 dG

Category	Antisense
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]

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

1. Sarin, P.S., Agrawal, S., Civeira, M.P., Goodchild, J., Ikeuchi, T., Zamecnik, P.C. Inhibition of acquired immunodeficiency syndrome virus by oligodeoxynucleoside methylphosphonates. (1988) *Proc. Natl. Acad. Sci. USA* **85**: 7448-7451.
2. Blake, K.R., Murakami, A., Spitz, S.A., Glave, S.A., Reddy, M.P., Ts'o, P.O., Miller, P.S. Hybridization arrest of globin synthesis in rabbit reticulocyte lysates and cells by oligodeoxyribonucleoside methylphosphonates. (1985) *Biochemistry*, **24**: 6139-6145.
3. Kibler-Herzog, L., Zon, G., Uznanski, B., Whittier, G, Wilson, W.D. Duplex stabilities of phosphorothioate, methylphosphonate, and RNA analogs of two DNA 14-mers. (1991) *Nucleic Acids Res.* **19**: 2979-2986.
4. Walder, J. Antisense DNA and RNA: progress and prospects. (1988) *Genes Dev.* **2**: 502-504.
5. Prater, C.E., Miller, P.S. 3'-Methylphosphonate-Modified Oligo-2'-O-methylribonucleotides and Their Tat Peptide Conjugates: Uptake and Stability in Mouse Fibroblasts in Culture. (2004) *Bioconjugate Chem.* **15**: 498-507.
6. Niu, H., Xia, J., Lue, N.F. Characterization of the Interaction between the Nuclease and Reverse Transcriptase Activity of the Yeast Telomerase Complex. (2000) *Mol. Cell. Biol.* **20**: 6806-6815.
7. Temsamani, J., Agrawal, S., Pederson, T. Biotinylated Antisense Methylphosphonate Oligodeoxynucleotides-Inhibition of Spliceosome Assembly and Affinity Selection for U1 and U2 Small Nuclear RNPs. (1991) *J. Biol. Chem.* **266**: 468-472.
8. Thiviyanathan, V., Vyazovkina, K.V., Gozansky, E.K., Bichenchova, E., Abramova, T.V., Luxon, B.A., Lebedev, A.V., Gorenstein, D.G. (2002) Structure of Hybrid Backbone Methylphosphonate DNA Heteroduplexes: Effect of R and S Stereochemistry. *Biochemistry*. **416**: 827-838.

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).

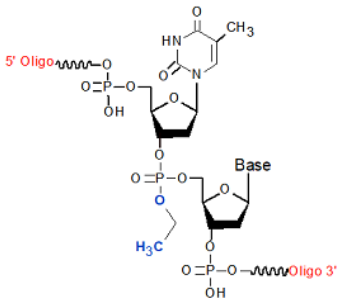
Mesyl Phosphoramidate (Ms, u) References 1. Miroshnichenko, S.K., Patutina, O.A., Burakova, E.A., Chelobanov, B.P., Fokina, A.A., Vlassov, V.V., Altmanb, A., Zenkova, M.A., Stetsenko, D. A. Mesyl phosphoramidate antisense oligonucleotides as an alternative to phosphorothioates with improved biochemical and biological properties. *PNAS* 2019. **116** : 1229-1234.
2. Prokhorova, D. V., Chelobanov, B.P., Burakova, E.A., Fokina, A.A., Stetsenko, D.A. (2017) New oligodeoxyribonucleotide derivatives bearing internucleotide N-tosyl phosphoramidate groups: Synthesis and complementary binding to DNA and RNA. Russ. J. Bioorganic Chem. 43:38-42.
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.
5. Taber, D.F., Ruckle, R.E. Jr., Hennessy, M.J., (1986) Mesyl Azide: A Superior Reagent for Diazo Transfer. J. Org. Chem., 51:4077-4078

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 dT

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



P-Ethoxy dT / Ethoxy Phosphate dT Oligo
[26-6641T-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

1. Sarin, P.S., Agrawal, S., Civeira, M.P., Goodchild, J., Ikeuchi, T., Zamecnik, P.C. Inhibition of acquired immunodeficiency syndrome virus by oligodeoxynucleoside methylphosphonates. (1988) *Proc. Natl. Acad. Sci. USA* **85**: 7448-7451.
2. Blake, K.R., Murakami, A., Spitz, S.A., Glave, S.A., Reddy, M.P., Ts'o, P.O., Miller, P.S. Hybridization arrest of globin synthesis in rabbit reticulocyte lysates and cells by oligodeoxyribonucleoside methylphosphonates. (1985) *Biochemistry*, **24**: 6139-6145.
3. Kibler-Herzog, L., Zon, G., Uznanski, B., Whittier, G, Wilson, W.D. Duplex stabilities of phosphorothioate, methylphosphonate, and RNA analogs of two DNA 14-mers. (1991) *Nucleic Acids Res.* **19**: 2979-2986.
4. Walder, J. Antisense DNA and RNA: progress and prospects. (1988) *Genes Dev.* **2**: 502-504.
5. Prater, C.E., Miller, P.S. 3'-Methylphosphonate-Modified Oligo-2'-O-methylribonucleotides and Their Tat Peptide Conjugates: Uptake and Stability in Mouse Fibroblasts in Culture. (2004) *Bioconjugate Chem.* **15**: 498-507.
6. Niu, H., Xia, J., Lue, N.F. Characterization of the Interaction between the Nuclease and Reverse Transcriptase Activity of the Yeast Telomerase Complex. (2000) *Mol. Cell. Biol.* **20**: 6806-6815.
7. Temsamani, J., Agrawal, S., Pederson, T. Biotinylated Antisense Methylphosphonate Oligodeoxynucleotides-Inhibition of Spliceosome Assembly and Affinity Selection for U1 and U2 Small Nuclear RNPs. (1991) *J. Biol. Chem.* **266**: 468-472.
8. Thiviyanathan, V., Vyazovkina, K.V., Gozansky, E.K., Bichenchova, E., Abramova, T.V., Luxon, B.A., Lebedev, A.V., Gorenstein, D.G. (2002) Structure of Hybrid Backbone Methylphosphonate DNA Heteroduplexes: Effect of R and S Stereochemistry. *Biochemistry*. **416**: 827-838.

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).

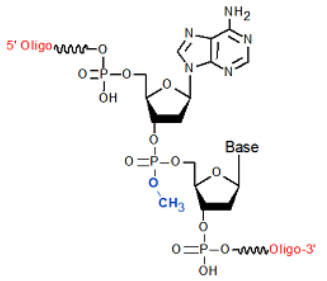
Mesyl Phosphoramidate (Ms, u) References 1. Miroshnichenko, S.K., Patutina, O.A., Burakova, E.A., Chelobanov, B.P., Fokina, A.A., Vlassov, V.V., Altmanb, A., Zenkova, M.A., Stetsenko, D. A. Mesyl phosphoramidate antisense oligonucleotides as an alternative to phosphorothioates with improved biochemical and biological properties. *PNAS* 2019. **116** : 1229-1234.
2. Prokhorova, D. V., Chelobanov, B.P., Burakova, E.A., Fokina, A.A., Stetsenko, D.A. (2017) New oligodeoxyribonucleotide derivatives bearing internucleotide N-tosyl phosphoramidate groups: Synthesis and complementary binding to DNA and RNA. Russ. J. Bioorganic Chem. 43:38-42.
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.
5. Taber, D.F., Ruckle, R.E. Jr., Hennessy, M.J., (1986) Mesyl Azide: A Superior Reagent for Diazo Transfer. J. Org. Chem., 51:4077-4078

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-Methoxy dA

Category	Antisense
Modification Code	MoP-dA
Reference Catalog Number	26-6642A
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	327.24



P-Methoxy dA / Methoxy Phosphate dA Oligo
[26-6642A-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

1. Sarin, P.S., Agrawal, S., Civeira, M.P., Goodchild, J., Ikeuchi, T., Zamecnik, P.C. Inhibition of acquired immunodeficiency syndrome virus by oligodeoxynucleoside methylphosphonates. (1988) *Proc. Natl. Acad. Sci. USA* **85**: 7448-7451.
2. Blake, K.R., Murakami, A., Spitz, S.A., Glave, S.A., Reddy, M.P., Ts'o, P.O., Miller, P.S. Hybridization arrest of globin synthesis in rabbit reticulocyte lysates and cells by oligodeoxyribonucleoside methylphosphonates. (1985) *Biochemistry*, **24**: 6139-6145.
3. Kibler-Herzog, L., Zon, G., Uznanski, B., Whittier, G, Wilson, W.D. Duplex stabilities of phosphorothioate, methylphosphonate, and RNA analogs of two DNA 14-mers. (1991) *Nucleic Acids Res.* **19**: 2979-2986.
4. Walder, J. Antisense DNA and RNA: progress and prospects. (1988) *Genes Dev.* **2**: 502-504.
5. Prater, C.E., Miller, P.S. 3'-Methylphosphonate-Modified Oligo-2'-O-methylribonucleotides and Their Tat Peptide Conjugates: Uptake and Stability in Mouse Fibroblasts in Culture. (2004) *Bioconjugate Chem.* **15**: 498-507.
6. Niu, H., Xia, J., Lue, N.F. Characterization of the Interaction between the Nuclease and Reverse Transcriptase Activity of the Yeast Telomerase Complex. (2000) *Mol. Cell. Biol.* **20**: 6806-6815.
7. Temsamani, J., Agrawal, S., Pederson, T. Biotinylated Antisense Methylphosphonate Oligodeoxynucleotides-Inhibition of Spliceosome Assembly and Affinity Selection for U1 and U2 Small Nuclear RNPs. (1991) *J. Biol. Chem.* **266**: 468-472.
8. Thiviyanathan, V., Vyazovkina, K.V., Gozansky, E.K., Bichenchova, E., Abramova, T.V., Luxon, B.A., Lebedev, A.V., Gorenstein, D.G. (2002) Structure of Hybrid Backbone Methylphosphonate DNA Heteroduplexes: Effect of R and S Stereochemistry. *Biochemistry*. **416**: 827-838.

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).

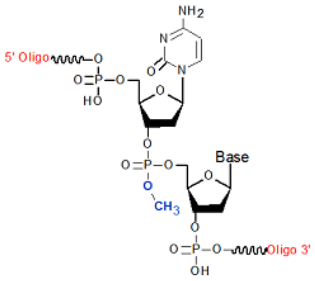
Mesyl Phosphoramidate (Ms, u) References 1. Miroshnichenko, S.K., Patutina, O.A., Burakova, E.A., Chelobanov, B.P., Fokina, A.A., Vlassov, V.V., Altmanb, A., Zenkova, M.A., Stetsenko, D. A. Mesyl phosphoramidate antisense oligonucleotides as an alternative to phosphorothioates with improved biochemical and biological properties. *PNAS* 2019. **116** : 1229-1234.
2. Prokhorova, D. V., Chelobanov, B.P., Burakova, E.A., Fokina, A.A., Stetsenko, D.A. (2017) New oligodeoxyribonucleotide derivatives bearing internucleotide N-tosyl phosphoramidate groups: Synthesis and complementary binding to DNA and RNA. Russ. J. Bioorganic Chem. 43:38-42.
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.
5. Taber, D.F., Ruckle, R.E. Jr., Hennessy, M.J., (1986) Mesyl Azide: A Superior Reagent for Diazo Transfer. J. Org. Chem., 51:4077-4078

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-Methoxy dC

Category	Antisense
Modification Code	MoP-dC
Reference Catalog Number	26-6642C
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	317.46



P-Methoxy dC / Methoxy Phosphate dC Oligo
[26-6642C-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

1. Sarin, P.S., Agrawal, S., Civeira, M.P., Goodchild, J., Ikeuchi, T., Zamecnik, P.C. Inhibition of acquired immunodeficiency syndrome virus by oligodeoxynucleoside methylphosphonates. (1988) *Proc. Natl. Acad. Sci. USA* **85**: 7448-7451.
2. Blake, K.R., Murakami, A., Spitz, S.A., Glave, S.A., Reddy, M.P., Ts'o, P.O., Miller, P.S. Hybridization arrest of globin synthesis in rabbit reticulocyte lysates and cells by oligodeoxyribonucleoside methylphosphonates. (1985) *Biochemistry*, **24**: 6139-6145.
3. Kibler-Herzog, L., Zon, G., Uznanski, B., Whittier, G, Wilson, W.D. Duplex stabilities of phosphorothioate, methylphosphonate, and RNA analogs of two DNA 14-mers. (1991) *Nucleic Acids Res.* **19**: 2979-2986.
4. Walder, J. Antisense DNA and RNA: progress and prospects. (1988) *Genes Dev.* **2**: 502-504.
5. Prater, C.E., Miller, P.S. 3'-Methylphosphonate-Modified Oligo-2'-O-methylribonucleotides and Their Tat Peptide Conjugates: Uptake and Stability in Mouse Fibroblasts in Culture. (2004) *Bioconjugate Chem.* **15**: 498-507.
6. Niu, H., Xia, J., Lue, N.F. Characterization of the Interaction between the Nuclease and Reverse Transcriptase Activity of the Yeast Telomerase Complex. (2000) *Mol. Cell. Biol.* **20**: 6806-6815.
7. Temsamani, J., Agrawal, S., Pederson, T. Biotinylated Antisense Methylphosphonate Oligodeoxynucleotides-Inhibition of Spliceosome Assembly and Affinity Selection for U1 and U2 Small Nuclear RNPs. (1991) *J. Biol. Chem.* **266**: 468-472.
8. Thiviyanathan, V., Vyazovkina, K.V., Gozansky, E.K., Bichenchova, E., Abramova, T.V., Luxon, B.A., Lebedev, A.V., Gorenstein, D.G. (2002) Structure of Hybrid Backbone Methylphosphonate DNA Heteroduplexes: Effect of R and S Stereochemistry. *Biochemistry*. **416**: 827-838.

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).

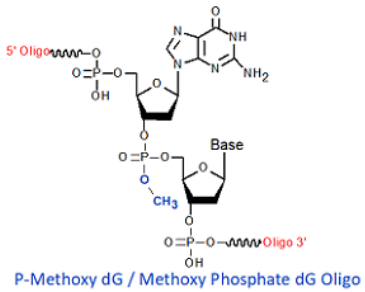
Mesyl Phosphoramidate (Ms, u) References 1. Miroshnichenko, S.K., Patutina, O.A., Burakova, E.A., Chelobanov, B.P., Fokina, A.A., Vlassov, V.V., Altmanb, A., Zenkova, M.A., Stetsenko, D. A. Mesyl phosphoramidate antisense oligonucleotides as an alternative to phosphorothioates with improved biochemical and biological properties. *PNAS* 2019. **116** : 1229-1234.
2. Prokhorova, D. V., Chelobanov, B.P., Burakova, E.A., Fokina, A.A., Stetsenko, D.A. (2017) New oligodeoxyribonucleotide derivatives bearing internucleotide N-tosyl phosphoramidate groups: Synthesis and complementary binding to DNA and RNA. Russ. J. Bioorganic Chem. 43:38-42.
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.
5. Taber, D.F., Ruckle, R.E. Jr., Hennessy, M.J., (1986) Mesyl Azide: A Superior Reagent for Diazo Transfer. J. Org. Chem., 51:4077-4078

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-Methoxy dG

Category	Antisense
Modification Code	MoP-dG
Reference Catalog Number	26-6642G
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	343.24



P-Methoxy dG / Methoxy Phosphate dG Oligo
[26-6642G-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

1. Sarin, P.S., Agrawal, S., Civeira, M.P., Goodchild, J., Ikeuchi, T., Zamecnik, P.C. Inhibition of acquired immunodeficiency syndrome virus by oligodeoxynucleoside methylphosphonates. (1988) *Proc. Natl. Acad. Sci. USA* **85**: 7448-7451.
2. Blake, K.R., Murakami, A., Spitz, S.A., Glave, S.A., Reddy, M.P., Ts'o, P.O., Miller, P.S. Hybridization arrest of globin synthesis in rabbit reticulocyte lysates and cells by oligodeoxyribonucleoside methylphosphonates. (1985) *Biochemistry*, **24**: 6139-6145.
3. Kibler-Herzog, L., Zon, G., Uznanski, B., Whittier, G, Wilson, W.D. Duplex stabilities of phosphorothioate, methylphosphonate, and RNA analogs of two DNA 14-mers. (1991) *Nucleic Acids Res.* **19**: 2979-2986.
4. Walder, J. Antisense DNA and RNA: progress and prospects. (1988) *Genes Dev.* **2**: 502-504.
5. Prater, C.E., Miller, P.S. 3'-Methylphosphonate-Modified Oligo-2'-O-methylribonucleotides and Their Tat Peptide Conjugates: Uptake and Stability in Mouse Fibroblasts in Culture. (2004) *Bioconjugate Chem.* **15**: 498-507.
6. Niu, H., Xia, J., Lue, N.F. Characterization of the Interaction between the Nuclease and Reverse Transcriptase Activity of the Yeast Telomerase Complex. (2000) *Mol. Cell. Biol.* **20**: 6806-6815.
7. Temsamani, J., Agrawal, S., Pederson, T. Biotinylated Antisense Methylphosphonate Oligodeoxynucleotides-Inhibition of Spliceosome Assembly and Affinity Selection for U1 and U2 Small Nuclear RNPs. (1991) *J. Biol. Chem.* **266**: 468-472.
8. Thiviyanathan, V., Vyazovkina, K.V., Gozansky, E.K., Bichenchova, E., Abramova, T.V., Luxon, B.A., Lebedev, A.V., Gorenstein, D.G. (2002) Structure of Hybrid Backbone Methylphosphonate DNA Heteroduplexes: Effect of R and S Stereochemistry. *Biochemistry*. **416**: 827-838.

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).

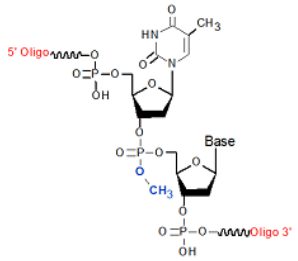
Mesyl Phosphoramidate (Ms, u) References 1. Miroshnichenko, S.K., Patutina, O.A., Burakova, E.A., Chelobanov, B.P., Fokina, A.A., Vlassov, V.V., Altmanb, A., Zenkova, M.A., Stetsenko, D. A. Mesyl phosphoramidate antisense oligonucleotides as an alternative to phosphorothioates with improved biochemical and biological properties. *PNAS* 2019. **116** : 1229-1234.
2. Prokhorova, D. V., Chelobanov, B.P., Burakova, E.A., Fokina, A.A., Stetsenko, D.A. (2017) New oligodeoxyribonucleotide derivatives bearing internucleotide N-tosyl phosphoramidate groups: Synthesis and complementary binding to DNA and RNA. Russ. J. Bioorganic Chem. 43:38-42.
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.
5. Taber, D.F., Ruckle, R.E. Jr., Hennessy, M.J., (1986) Mesyl Azide: A Superior Reagent for Diazo Transfer. J. Org. Chem., 51:4077-4078

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-Methoxy dT

Category	Antisense
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

1. Sarin, P.S., Agrawal, S., Civeira, M.P., Goodchild, J., Ikeuchi, T., Zamecnik, P.C. Inhibition of acquired immunodeficiency syndrome virus by oligodeoxynucleoside methylphosphonates. (1988) *Proc. Natl. Acad. Sci. USA* **85**: 7448-7451.
2. Blake, K.R., Murakami, A., Spitz, S.A., Glave, S.A., Reddy, M.P., Ts'o, P.O., Miller, P.S. Hybridization arrest of globin synthesis in rabbit reticulocyte lysates and cells by oligodeoxyribonucleoside methylphosphonates. (1985) *Biochemistry*, **24**: 6139-6145.
3. Kibler-Herzog, L., Zon, G., Uznanski, B., Whittier, G, Wilson, W.D. Duplex stabilities of phosphorothioate, methylphosphonate, and RNA analogs of two DNA 14-mers. (1991) *Nucleic Acids Res.* **19**: 2979-2986.
4. Walder, J. Antisense DNA and RNA: progress and prospects. (1988) *Genes Dev.* **2**: 502-504.
5. Prater, C.E., Miller, P.S. 3'-Methylphosphonate-Modified Oligo-2'-O-methylribonucleotides and Their Tat Peptide Conjugates: Uptake and Stability in Mouse Fibroblasts in Culture. (2004) *Bioconjugate Chem.* **15**: 498-507.
6. Niu, H., Xia, J., Lue, N.F. Characterization of the Interaction between the Nuclease and Reverse Transcriptase Activity of the Yeast Telomerase Complex. (2000) *Mol. Cell. Biol.* **20**: 6806-6815.
7. Temsamani, J., Agrawal, S., Pederson, T. Biotinylated Antisense Methylphosphonate Oligodeoxynucleotides-Inhibition of Spliceosome Assembly and Affinity Selection for U1 and U2 Small Nuclear RNPs. (1991) *J. Biol. Chem.* **266**: 468-472.
8. Thiviyanathan, V., Vyazovkina, K.V., Gozansky, E.K., Bichenchova, E., Abramova, T.V., Luxon, B.A., Lebedev, A.V., Gorenstein, D.G. (2002) Structure of Hybrid Backbone Methylphosphonate DNA Heteroduplexes: Effect of R and S Stereochemistry. *Biochemistry*. **416**: 827-838.

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).

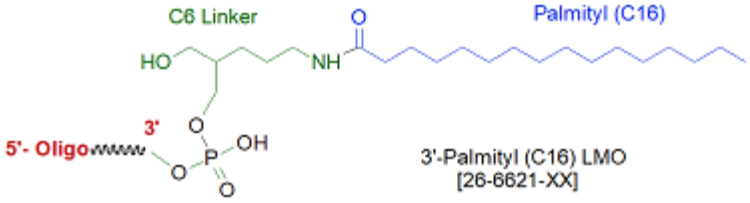
Mesyl Phosphoramidate (Ms, u) References 1. Miroshnichenko, S.K., Patutina, O.A., Burakova, E.A., Chelobanov, B.P., Fokina, A.A., Vlassov, V.V., Altmanb, A., Zenkova, M.A., Stetsenko, D. A. Mesyl phosphoramidate antisense oligonucleotides as an alternative to phosphorothioates with improved biochemical and biological properties. *PNAS* 2019. **116** : 1229-1234.
2. Prokhorova, D. V., Chelobanov, B.P., Burakova, E.A., Fokina, A.A., Stetsenko, D.A. (2017) New oligodeoxyribonucleotide derivatives bearing internucleotide N-tosyl phosphoramidate groups: Synthesis and complementary binding to DNA and RNA. Russ. J. Bioorganic Chem. 43:38-42.
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.
5. Taber, D.F., Ruckle, R.E. Jr., Hennessy, M.J., (1986) Mesyl Azide: A Superior Reagent for Diazo Transfer. J. Org. Chem., 51:4077-4078

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.

Palmityl C16 (3') LMO

Category	Antisense
Modification Code	3-Pal-C16
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. 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 B>YIELD Approximate polyacrylamide gel purified yield for various scales are given below.
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.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis
~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
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.

Palmityl C16 (5') LMO

Category	Antisense
Modification Code	5-Pal-C16
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. 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 B>YIELD Approximate polyacrylamide gel purified yield for various scales are given below.
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.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis
~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
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

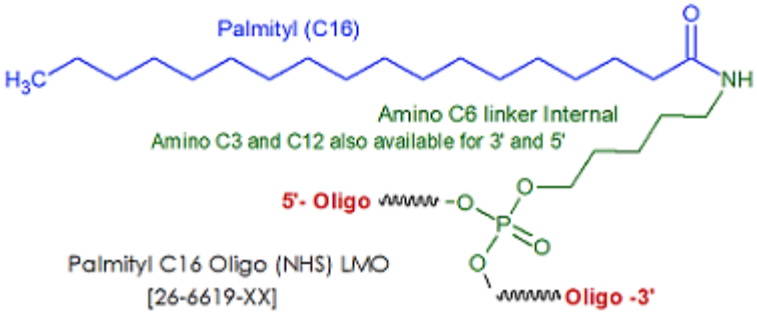
N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

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.

Palmityl C16 Oligo (NHS) LMO

Category	Antisense
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.
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.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis
~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

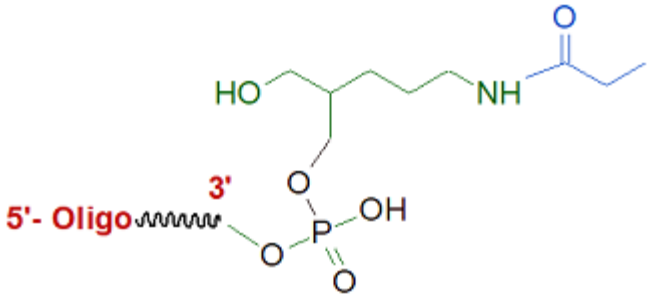
N-acetylgalactosamine (GalNAc) Oligo Application Note: Glen Report 29.14: N-acetylgalactosamine (GalNAc) Oligonucleotide Conjugates

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.

Propionic acid (C3) Modified Oligo

Category	Antisense
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.).
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).

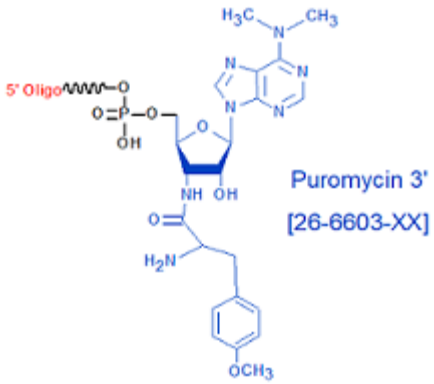
Recommended Further Reading

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.

Puromycin

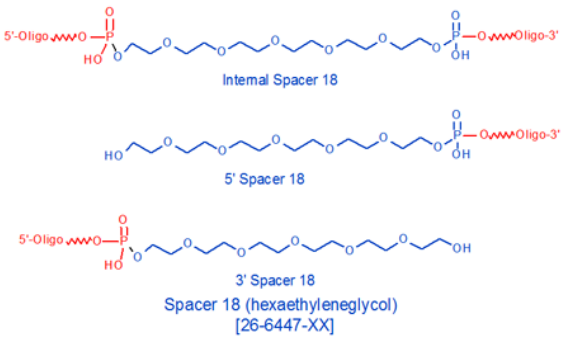
Category	Others
Modification Code	Puro
Reference Catalog Number	26-6603
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	533.48



Puromycin can be attached to the 3' end of RNA and DNA oligos. Puromycin is an antibiotic that mimics transfer RNA. Puromycin binds in the ribosome's A site and forms a peptide bond with the growing peptide chain to block peptide elongation. By linking puromycin to synthetic RNA; a peptide-RNA fusion product can be formed. An application example is the use of 3'Puromycin to synthesize d(A27CC)-puromycin to which various mRNA sequences were then ligated. The mRNA sequence information was then translated in a reticulocyte lysate system. As the ribosome reached the poly-dA sequence, translation was stalled. Puromycin entered the ribosome A site and a peptide bond formed between the C-terminal of the synthesized peptide and the RNA encoding the peptide structure. The poly-dA sequence serves two purposes, first it stalls the ribosome thereby allowing puromycin to enter the A site and second it acts as a future capture site for oligo-dT-biotin. References: (1) S. Borman, C&EN, Feb. 12, 1996, 29-54. (2) R.W. Roberts and J.W. Szostak, Proc. Natl. Acad. Sci. USA, 1997, 94, 12297-302

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

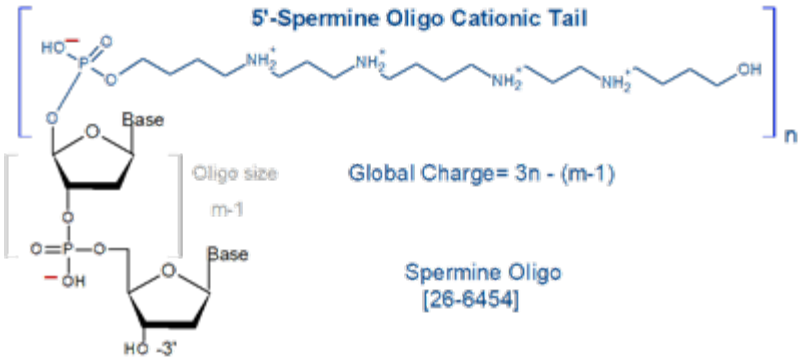


Spacer 18 is a hexaethylene glycol chain that is 18 atoms long (12 carbons + 6 oxygens), and is used to incorporate a long spacer arm into an oligonucleotide. Spacer 18 can be incorporated in consecutive additions whenever a longer spacer is required. Spacer 18 had been used to form bold folds and hairpin 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). **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.

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, dropwise 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

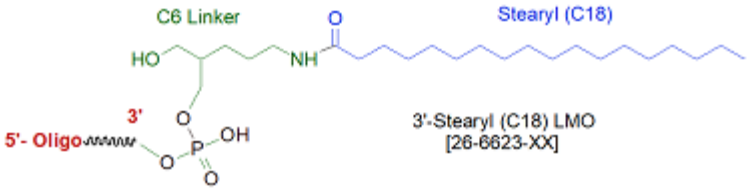
Glen Reports GR24-11. Spermine Phosphoramidite: A potent modification with many applications.
Glen Reports GR24-11. Zip Nucleic Acids (ZNA®) are powerful cationic oligonucleotides for molecular biology, diagnostic and therapeutic applications.

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



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.
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
Modification Code	5-Str-C18
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).

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.