



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

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
2'-O-C16 Adenosine siRNA LMO: 2'-O-hexadecyl (C16)	[C16-2-O-A]	27-6541A
2'-O-C16 Cytidine siRNA LMO: 2'-O-hexadecyl (C16)	[C16-2-O-C]	27-6541C
2'-O-C16 Guanosine siRNA LMO: 2'-O-hexadecyl (C16)	[C16-2-O-G]	27-6541G
2'-O-C16 Uridine siRNA LMO: 2'-O-hexadecyl (C16)	[C16-2-O-U]	27-6541U
Alpha Tocopherol TEG (Vitamin E)	[a-toco-TEG]	26-6616
Alpha-linolyl (C18:3 α) ALA LMO	[3-ALA-C18-3]	26-6784
Arachidonyl (C20:4) AA LMO	[3-AA-C20-4]	26-6787
butyric acid (C4) Modified Oligo	[3-BA-C4]	26-6782
Cholesterol TEG (15 atom triethylene glycol spacer)5' LMO	[CholTEG-5]	26-6602
Cholesterol TEG-3' (15 atom triethylene glycol spacer) LMO	[CholTEG-3]	26-6570
Delta Tocopherol TEG (Vitamin E)	[d-toco-TEG]	26-6628
Docosanoic (C22) DCA LMO	[3-DCA-C22]	26-6627
Docosahexaenoic (C22:6) DHA LMO	[3-DHA-C22-6]	26-6626
Dihomo-gamma-linolyl (C20:3) DGLA LMO	[3-DGLA-C20-3]	26-6786
Eicosapent (20:5) EPA LMO	[3-EPA-C20-5]	26-6788
Folic Acid NHS (Vitamin B9)	[Folate-N]	26-6631
GalNAc Oligo N-Acetylgalactosamine C3	[GalNAc]	26-6751
GalNAc Trivalent TEG	[GalNAc-TEG3X]	26-6735
Gamma-linolyl (C18:3 γ) GLA LMO	[3-GLA-C18-3]	26-6785
Lignoceryl C24 (3') LMO	[3-Lig-C24]	26-6624

Lignoceryl C24 (5') LMO	[5-Lig-C24]	26-6625
Linolyl (C18:2) LA LMO	[3-LA-C18-2]	26-6783
Ethoxy Phosphate dA	[EoP-dA]	26-6641A
Ethoxy Phosphate dC	[EoP-dC]	26-6641C
Ethoxy Phosphate dG	[EoP-dG]	26-6641G
Ethoxy Phosphate dT	[EoP-dT]	26-6641T
Methoxy Phosphate dA	[MoP-dA]	26-6642A
Methoxy Phosphate dC	[MoP-dC]	26-6642C
Methoxy Phosphate dG	[MoP-dG]	26-6642G
Methoxy Phosphate dT	[MoP-dT]	26-6642T
Palmityl C16 (3') LMO	[Pal-C16-3]	26-6621
Palmityl C16 (5') LMO	[Pal-C16-5]	26-6622
Palmityl C16 Oligo (NHS) LMO	[Pal-C16-N]	26-6619
Propionyl (C3) PA LMO	[3-PA-C3]	26-6781
Puromycin	[Puro-3]	26-6603
Spacer 18 (hexaethyleneglycol)	[Sp18]	26-6447
Spermine Oligo Cationic Tail (ZNA-Oligos)	[Spm]	26-6454
Stearyl C18 LMO LMO 3'	[Str-C18-3]	26-6623
Stearyl C18 LMO Oligo 5'	[Str-C18-5]	26-6617



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

2'-O-C16 Adenosine siRNA LMO

Category Antisense

Modification Code C16-2-O-A

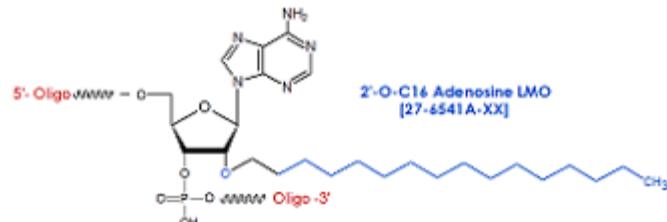
Reference Catalog Number 27-6541A

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 554.64



[Click here for a list Cellular Delivery Modifications.](#)

[Click here for more information on siRNA and antisense oligo modifications, design & applications.](#)

2'-O-C16: 2'-O-hexadecyl (C16) Lipid Modified Oligo (LMO) siRNA Cellular Delivery

RNA interference (RNAi) is a natural, conserved biological process in which small RNA molecules suppress gene expression by targeting specific mRNA molecules for degradation or translational repression.

RNA interference (RNAi) therapeutics use an endogenous mechanism whereby short interfering RNAs (siRNAs) direct the RNA-induced silencing complex (RISC) to sequence matched target transcripts for knockdown. Various cellular delivery modifications have been used, for instance lipid nanoparticles and N-acetylgalactosamine (GalNAc) conjugates are clinically validated and approved delivery strategies for liver targets. In addition, various other cellular delivery modifications have been reported that include alpha-tocopherol, DHA, DCA, Folic acid etc.

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

Brown et. Al (1) have reported as part of the preclinical evaluation of first development candidate for a CNS disease, ALN-APP, to investigate the impact of a C16-siRNA targeting the amyloid beta precursor protein (APP) gene transcript in a mouse model of Alzheimer's disease. The treatment led to potent and durable knock down in the CNS and ameliorated physiological and behavioral deficits. The sustained knockdown observed suggests that infrequent dosing of C16-siRNAs could be feasible in humans, which is especially important considering the IT route of administration.

ALN-APP targeting APP for the treatment of early onset Alzheimer's Disease (EOAD) and cerebral amyloid angiopathy (CAA) is now in clinical development.

References

1. Brown, K.M. et al. Expanding RNAi therapeutics to extrahepatic tissues with lipophilic conjugates. *Nat. Biotechnol.* 40, 1500-1508 (2022).
2. Setten, R. L., Rossi, J. J. & Han, S. P. The current state and future directions of RNAi-based therapeutics. *Nat. Rev. Drug Discov.* 18, 421-446 (2019).
3. Alterman, J. F. et al. A divalent siRNA chemical scaffold for potent and sustained modulation of gene expression throughout the central nervous system. *Nat. Biotechnol.* 37, 884-894 (2019).
4. Nair, J. K. et al. Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. *J. Am. Chem. Soc.* 136, 16958-16961 (2014).
5. Jayaraman, M. et al. Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angew. Chem. Int. Ed. Engl.* 51, 8529-8533 (2012).
6. Zimmermann, T.S. et al. RNAi-mediated gene silencing in non-human primates. *Nature* 441, 111-114 (2006).
7. Alterman, J.F. et al. Hydrophobically Modified siRNAs Silence Huntingtin mRNA in Primary Neurons and Mouse Brain. *Molecular therapy. Nucleic acids* 4, e266 (2015).
8. Soutschek, J. et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* 432, 173-178 (2004).
9. Finkel, R.S. et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. *The New England journal of medicine* 377, 1723-1732 (2017).

siRNA Oligo Cellular Delivery Modifications

Vitamin B12 (Cyanocobalamin) Oligo

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

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

Vitamin E. alpha-tocopherol TEG Modification

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

Staryl Modification

Staryl 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 Trivalent Modification

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).

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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

2'-O-C16 Cytidine siRNA LMO

Category Antisense

Modification Code C16-2-O-C

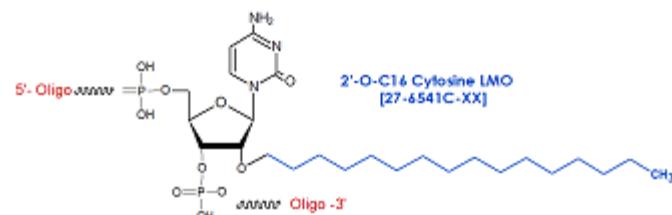
Reference Catalog Number 27-6541C

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 530.61



[Click here for a list Cellular Delivery Modifications.](#)

[Click here for more information on siRNA and antisense oligo modifications, design & applications.](#)

2'-O-C16: 2'-O-hexadecyl (C16) Lipid Modified Oligo (LMO) siRNA Cellular Delivery

RNA interference (RNAi) is a natural, conserved biological process in which small RNA molecules suppress gene expression by targeting specific mRNA molecules for degradation or translational repression.

RNA interference (RNAi) therapeutics use an endogenous mechanism whereby short interfering RNAs (siRNAs) direct the RNA-induced silencing complex (RISC) to sequence matched target transcripts for knockdown. Various cellular delivery modifications have been used, for instance lipid nanoparticles and N-acetylgalactosamine (GalNAc) conjugates are clinically validated and approved delivery strategies for liver targets. In addition, various other cellular delivery modifications have been reported that include alpha-tocopherol, DHA, DCA, Folic acid etc.

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

Brown et. Al (1) have reported as part of the preclinical evaluation of first development candidate for a CNS disease, ALN-APP, to investigate the impact of a C16-siRNA targeting the amyloid beta precursor protein (APP) gene transcript in a mouse model of Alzheimer's disease. The treatment led to potent and durable knock down in the CNS and ameliorated physiological and behavioral deficits. The sustained knockdown observed suggests that infrequent dosing of C16-siRNAs could be feasible in humans, which is especially important considering the IT route of administration.

ALN-APP targeting APP for the treatment of early onset Alzheimer's Disease (EOAD) and cerebral amyloid angiopathy (CAA) is now in clinical development.

References

1. Brown, K.M. et al. Expanding RNAi therapeutics to extrahepatic tissues with lipophilic conjugates. *Nat. Biotechnol.* 40, 1500-1508 (2022).
2. Setten, R. L., Rossi, J. J. & Han, S. P. The current state and future directions of RNAi-based therapeutics. *Nat. Rev. Drug Discov.* 18, 421-446 (2019).
3. Alterman, J. F. et al. A divalent siRNA chemical scaffold for potent and sustained modulation of gene expression throughout the central nervous system. *Nat. Biotechnol.* 37, 884-894 (2019).
4. Nair, J. K. et al. Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. *J. Am. Chem. Soc.* 136, 16958-16961 (2014).
5. Jayaraman, M. et al. Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angew. Chem. Int. Ed. Engl.* 51, 8529-8533 (2012).
6. Zimmermann, T.S. et al. RNAi-mediated gene silencing in non-human primates. *Nature* 441, 111-114 (2006).
7. Alterman, J.F. et al. Hydrophobically Modified siRNAs Silence Huntingtin mRNA in Primary Neurons and Mouse Brain. *Molecular therapy. Nucleic acids* 4, e266 (2015).
8. Soutschek, J. et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* 432, 173-178 (2004).
9. Finkel, R.S. et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. *The New England journal of medicine* 377, 1723-1732 (2017).

siRNA Oligo Cellular Delivery Modifications

Vitamin B12 (Cyanocobalamin) Oligo

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

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

Vitamin E. alpha-tocopherol TEG Modification

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

Staryl Modification

Staryl 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 Trivalent Modification

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).

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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

2'-O-C16 Guanosine siRNA LMO

Category Antisense

Modification Code C16-2-O-G

Reference Catalog Number 27-6541G

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 570.64



[Click here for a list Cellular Delivery Modifications.](#)

[Click here for more information on siRNA and antisense oligo modifications, design & applications.](#)

2'-O-C16: 2'-O-hexadecyl (C16) Lipid Modified Oligo (LMO) siRNA Cellular Delivery

RNA interference (RNAi) is a natural, conserved biological process in which small RNA molecules suppress gene expression by targeting specific mRNA molecules for degradation or translational repression.

RNA interference (RNAi) therapeutics use an endogenous mechanism whereby short interfering RNAs (siRNAs) direct the RNA-induced silencing complex (RISC) to sequence matched target transcripts for knockdown. Various cellular delivery modifications have been used, for instance lipid nanoparticles and N-acetylgalactosamine (GalNAc) conjugates are clinically validated and approved delivery strategies for liver targets. In addition, various other cellular delivery modifications have been reported that include alpha-tocopherol, DHA, DCA, Folic acid etc.

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

Brown et. Al (1) have reported as part of the preclinical evaluation of first development candidate for a CNS disease, ALN-APP, to investigate the impact of a C16-siRNA targeting the amyloid beta precursor protein (APP) gene transcript in a mouse model of Alzheimer's disease. The treatment led to potent and durable knock down in the CNS and ameliorated physiological and behavioral deficits. The sustained knockdown observed suggests that infrequent dosing of C16-siRNAs could be feasible in humans, which is especially important considering the IT route of administration.

ALN-APP targeting APP for the treatment of early onset Alzheimer's Disease (EOAD) and cerebral amyloid angiopathy (CAA) is now in clinical development.

References

1. Brown, K.M. et al. Expanding RNAi therapeutics to extrahepatic tissues with lipophilic conjugates. *Nat. Biotechnol.* 40, 1500-1508 (2022).
2. Setten, R. L., Rossi, J. J. & Han, S. P. The current state and future directions of RNAi-based therapeutics. *Nat. Rev. Drug Discov.* 18, 421-446 (2019).
3. Alterman, J. F. et al. A divalent siRNA chemical scaffold for potent and sustained modulation of gene expression throughout the central nervous system. *Nat. Biotechnol.* 37, 884-894 (2019).
4. Nair, J. K. et al. Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. *J. Am. Chem. Soc.* 136, 16958-16961 (2014).
5. Jayaraman, M. et al. Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angew. Chem. Int. Ed. Engl.* 51, 8529-8533 (2012).
6. Zimmermann, T.S. et al. RNAi-mediated gene silencing in non-human primates. *Nature* 441, 111-114 (2006).
7. Alterman, J.F. et al. Hydrophobically Modified siRNAs Silence Huntingtin mRNA in Primary Neurons and Mouse Brain. *Molecular therapy. Nucleic acids* 4, e266 (2015).
8. Soutschek, J. et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* 432, 173-178 (2004).
9. Finkel, R.S. et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. *The New England journal of medicine* 377, 1723-1732 (2017).

siRNA Oligo Cellular Delivery Modifications

Vitamin B12 (Cyanocobalamin) Oligo

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

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

Vitamin E. alpha-tocopherol TEG Modification

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

Staryl Modification

Staryl 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 Trivalent Modification

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).

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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

2'-O-C16 Uridine siRNA LMO

Category Antisense

Modification Code C16-2-O-U

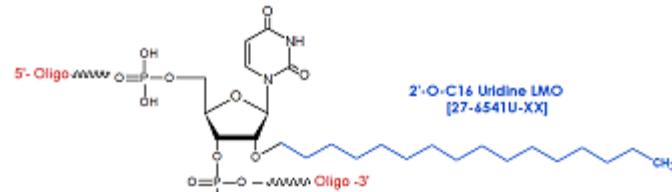
Reference Catalog Number 27-6541U

5' Prime Y

3' Prime Y

Internal Y

Molecular Weight(mw) 531.6



[Click here for a list Cellular Delivery Modifications.](#)

[Click here for more information on siRNA and antisense oligo modifications, design & applications.](#)

2'-O-C16: 2'-O-hexadecyl (C16) Lipid Modified Oligo (LMO) siRNA Cellular Delivery

RNA interference (RNAi) is a natural, conserved biological process in which small RNA molecules suppress gene expression by targeting specific mRNA molecules for degradation or translational repression.

RNA interference (RNAi) therapeutics use an endogenous mechanism whereby short interfering RNAs (siRNAs) direct the RNA-induced silencing complex (RISC) to sequence matched target transcripts for knockdown. Various cellular delivery modifications have been used, for instance lipid nanoparticles and N-acetylgalactosamine (GalNAc) conjugates are clinically validated and approved delivery strategies for liver targets. In addition, various other cellular delivery modifications have been reported that include alpha-tocopherol, DHA, DCA, Folic acid etc.

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

Brown et. Al (1) have reported as part of the preclinical evaluation of first development candidate for a CNS disease, ALN-APP, to investigate the impact of a C16-siRNA targeting the amyloid beta precursor protein (APP) gene transcript in a mouse model of Alzheimer's disease. The treatment led to potent and durable knock down in the CNS and ameliorated physiological and behavioral deficits. The sustained knockdown observed suggests that infrequent dosing of C16-siRNAs could be feasible in humans, which is especially important considering the IT route of administration.

ALN-APP targeting APP for the treatment of early onset Alzheimer's Disease (EOAD) and cerebral amyloid angiopathy (CAA) is now in clinical development.

References

1. Brown, K.M. et al. Expanding RNAi therapeutics to extrahepatic tissues with lipophilic conjugates. *Nat. Biotechnol.* 40, 1500-1508 (2022).
2. Setten, R. L., Rossi, J. J. & Han, S. P. The current state and future directions of RNAi-based therapeutics. *Nat. Rev. Drug Discov.* 18, 421-446 (2019).
3. Alterman, J. F. et al. A divalent siRNA chemical scaffold for potent and sustained modulation of gene expression throughout the central nervous system. *Nat. Biotechnol.* 37, 884-894 (2019).
4. Nair, J. K. et al. Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. *J. Am. Chem. Soc.* 136, 16958-16961 (2014).
5. Jayaraman, M. et al. Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angew. Chem. Int. Ed. Engl.* 51, 8529-8533 (2012).
6. Zimmermann, T.S. et al. RNAi-mediated gene silencing in non-human primates. *Nature* 441, 111-114 (2006).
7. Alterman, J.F. et al. Hydrophobically Modified siRNAs Silence Huntingtin mRNA in Primary Neurons and Mouse Brain. *Molecular therapy. Nucleic acids* 4, e266 (2015).
8. Soutschek, J. et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* 432, 173-178 (2004).
9. Finkel, R.S. et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. *The New England journal of medicine* 377, 1723-1732 (2017).

siRNA Oligo Cellular Delivery Modifications

Vitamin B12 (Cyanocobalamin) Oligo

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

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

Vitamin E. alpha-tocopherol TEG Modification

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

Staryl Modification

Staryl 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 Trivalent Modification

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).

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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

a-Tocopherol TEG (LMO)

Category Antisense

Modification Code a-toco-TEG

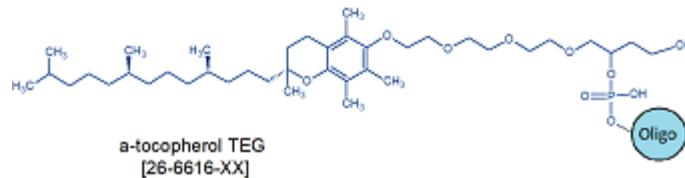
Reference Catalog Number 26-6616

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 698.91



Click here for a list Cellular Delivery Modifications.

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

alpha-tocopherol TEG Modification

alpha-tocopherol (vitamin E) is both lipophilic and non-toxic even at high doses so would be an excellent candidate as a lipophilic carrier for oligonucleotides. Similar to cholesterol TEG, the TEG like 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 like 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).

Vitamin B12 (Cyanocobalamin) Oligo

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

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

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

1. J.K. Nair, et al., J Am Chem Soc, 2014, 136, 16958-61.
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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Alpha-linolenic acid (C18:3 α) Oligo

Category Antisense

Modification Code 3-ALA-C18-3

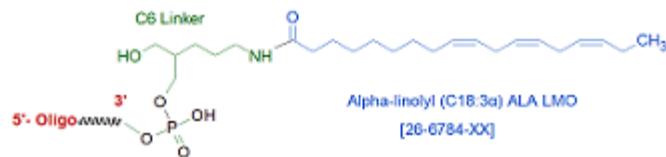
Reference Catalog Number 26-6784

5 Prime N

3 Prime Y

Internal N

Molecular Weight(mw) 454.57



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

Lipid modified oligos (LMO; lignoceric, palmitic, cholesterol, Propionyl (C3) PA, Butyl (C4) BA, Linolyl (C18:2) LA, Alpha-linolyl (C18:3 α) ALA, Gamma-linolyl (C18:3 γ) 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 cholesterol 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 linker 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 linker 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Arachidonic acid (C20:4) Oligo

Category Antisense

Modification Code 3-AA-C20-4

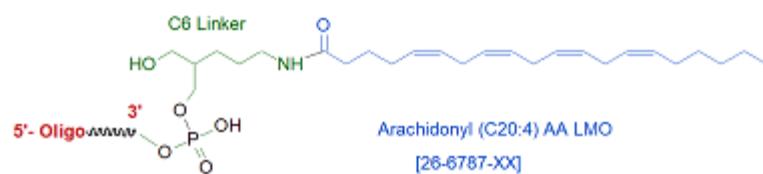
Reference Catalog Number 26-6787

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 (C18:3 α) ALA, Gamma linolyl (C18:3 γ) GLA, Dihomo γ gammalinolyl (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 cholesterol 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 linker 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 linker 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.



Product Specifications

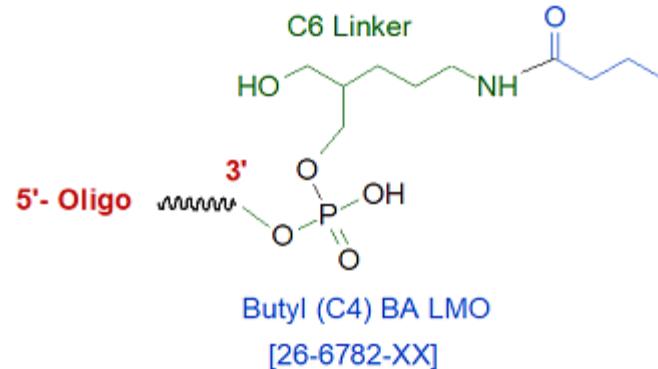
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

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



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 ω linolyl (C18:3 α) ALA, Gamma ω linolyl (C18:3 γ) GLA, Dihomo ω gamma ω 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 cholesterol 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 linker 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 linker 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Cholesterol TEG 5'

Category Antisense

Modification Code CholTEG-5

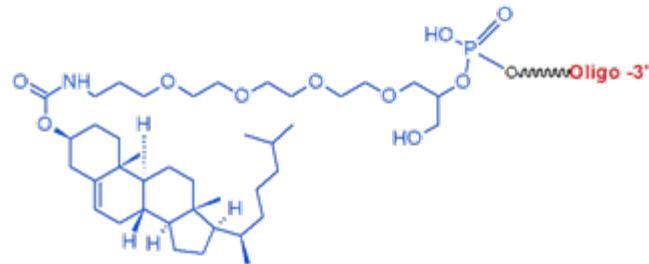
Reference Catalog Number 26-6602

5 Prime Y

3 Prime N

Internal Y

Molecular Weight(mw) 755.97



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

[Click here for a list Cellular Delivery Modifications.](#)

Modification Code Change Effective May 12, 2025 the code for Cholesterol 5' was changed from [CholTEG] to [CholTEG-5]. 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 cholestryol 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 linker 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 linker 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Cholesterol TEG-3'

Category Antisense

Modification Code CholTEG-3

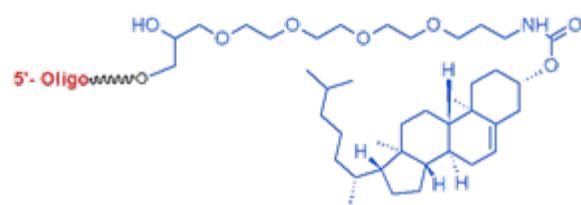
Reference Catalog Number 26-6570

5 Prime N

3 Prime Y

Internal N

Molecular Weight(mw) 755.97



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

[Click here for a list Cellular Delivery Modifications.](#)

Modification Code Change Effective May 12, 2025 the code for Cholesterol 3' was changed from [3-CholTEG] to [CholTEG-3].
Antisense Oligos (ODN) & siRNA Oligo Cellular Delivery Modifications

[Click here for more information on antisense modifications, design & applications.](#)

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 linker 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 linker 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

d-Tocopherol TEG (LMO)

Category Antisense

Modification Code d-toco-TEG

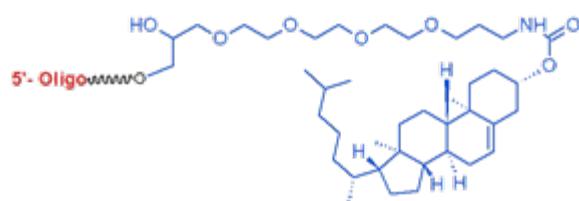
Reference Catalog Number 26-6628

5' Prime Y

3' Prime Y

Internal Y

Molecular Weight(mw) 672



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

[Click here for a list Cellular Delivery Modifications.](#)

Oligonucleotides are predominantly hydrophilic species and require help in permeating cell membranes. One strategy to improve cellular uptake of therapeutic oligonucleotides is to conjugate them with non-toxic, lipophilic molecules. Gene Link offers cholestryol 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 like 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 like 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

DCA (C22) LMO

Category Antisense

Modification Code 3-DCA-C22

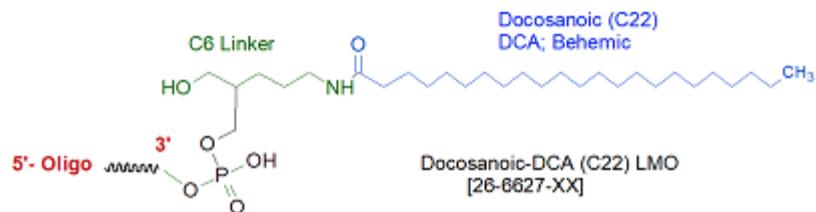
Reference Catalog Number 26-6627

5 Prime N

3 Prime Y

Internal N

Molecular Weight(mw) 517





Product Specifications

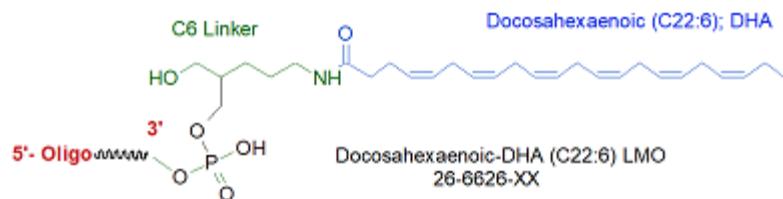
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates, Locked Nucleic Acids (LNA): 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

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





Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

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

Category Antisense

Modification Code 3-DGLA-C20-3

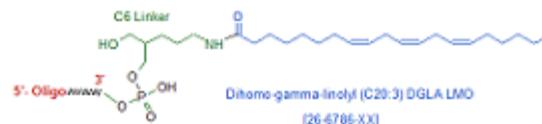
Reference Catalog Number 26-6786

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 ω linolyl (C18:3 α) ALA, Gamma ω linolyl (C18:3 γ) GLA, Dihomo ω gamma ω 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 cholesterol 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 linker 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 linker 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Eicosapentaenoic acid (20:5) Oligo

Category Antisense

Modification Code 3-EPA-C20-5

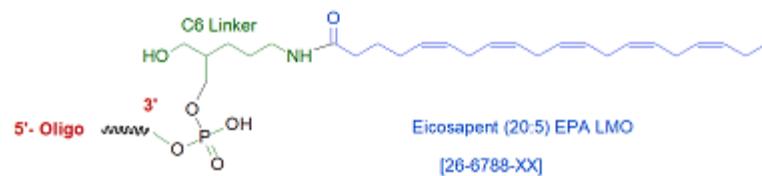
Reference Catalog Number 26-6788

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 ω linolyl (C18:3 α) ALA, Gamma ω linolyl (C18:3 γ) GLA, Dihomo ω gamma ω 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 cholesterol 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 linker 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 linker 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Folic Acid NHS (Vitamin B9)

Category Antisense

Modification Code Folate-N

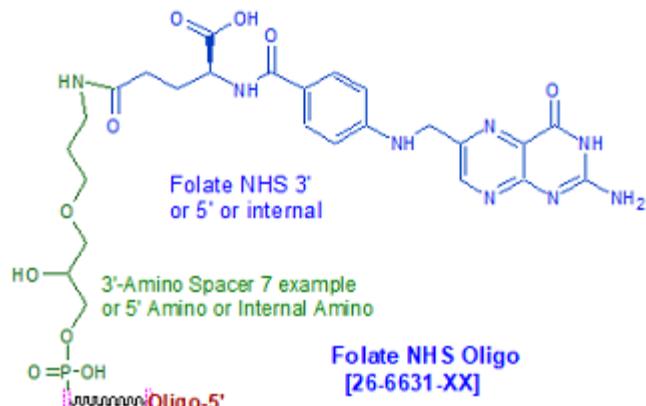
Reference Catalog Number 26-6631

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 424.4



[Click here for a list Cellular Delivery Modifications.](#)

[Click here for more information on siRNA and antisense oligo modifications, design & applications.](#)

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

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

Mechanism of Targeting via Folate Receptor

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

Advantages of Folic Acid in Targeted Delivery

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

References

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

siRNA Oligo Cellular Delivery Modifications

Vitamin B12 (Cyanocobalamin) Oligo

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

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

Vitamin E. alpha-tocopherol TEG Modification

alpha-tocopherol (vitamin E) is both lipophilic and non-toxic even at high doses so would be an excellent candidate as a lipophilic carrier for oligonucleotides. Similar to cholesterol TEG, the TEG linker 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 linker 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 Trivalent Modification

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).

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.



Product Specifications

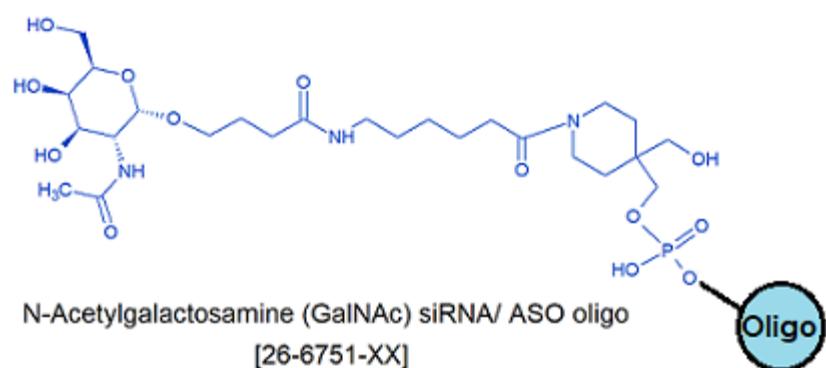
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

GalNAc

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



siRNA Oligo Cellular Delivery Modifications

[Click here for a list 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 like 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 like 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

GalNAc TEG Trivalent 3'

Category Antisense

Modification Code GalNAc-TEG3X

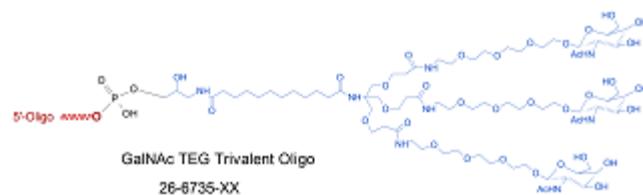
Reference Catalog Number 26-6735

5 Prime N

3 Prime Y

Internal N

Molecular Weight(mw) 1820



siRNA Oligo Cellular Delivery Modifications

[Click here for a list 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 cholestryl 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 like 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 like 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Gamma-linolenic acid (C18:3 γ) Oligo

Category Antisense

Modification Code 3-GLA-C18-3

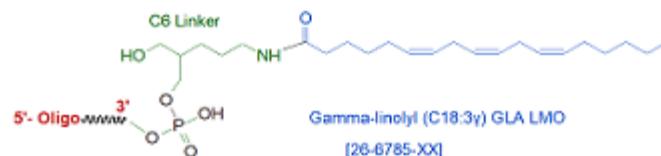
Reference Catalog Number 26-6785

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 ω linolyl (C18:3 α) ALA, Gamma ω linolyl (C18:3 γ) GLA, Dihomo ω gamma ω 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 cholesterol 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 linker 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 linker 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

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



We offer custom oligo synthesis of your designed sequences for conjugation to lignoceric acid and palmitic acid for MULTI-seq: sample multiplexing for single-cell RNA sequencing **YIELD** Approximate polyacrylamide gel purified yield for various scales are given below for an oligo below 50mer.

Yield given below are for oligos shorter than 50mer. Please see longer oligos yield at this link Long Oligo Typical Yield.

~2 nmol final yield for 50 nmol scale synthesis.

~8 nmol final yield for 200 nmol scale synthesis.

~25 nmol final yield for 1 umol scale synthesis

~45nmol final yield for 2 umol scale synthesis

~110nmol final yield for 5 umol scale synthesis

~180 nmol final yield for 10 umol scale synthesis

~260 nmol final yield for 15 umol scale synthesis

[Click here to order stock MULTI-Seq LMO Lig-Anchor oligos.](#)

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

MULTI-Seq LMO Lig Anchor and MULTI-Seq LMO Palm Co-Anchor oligos are lignoceric and palmitic acid conjugated oligos as described by McGinnis, C. et al. (1)

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 cholestryol 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.

Staryl Modification

Staryl 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 linker 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 linker 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Lignoceryl C24 (5') LMO

Category Antisense

Modification Code 5-Lig-C24

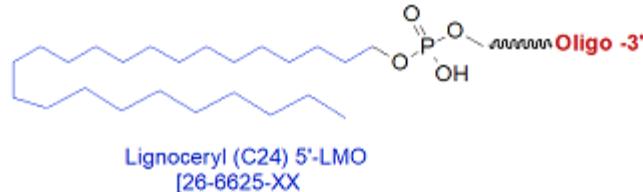
Reference Catalog Number 26-6625

5 Prime Y

3 Prime N

Internal N

Molecular Weight(mw) 415.61



We offer custom oligo synthesis of your designed sequences for conjugation to lignoceric acid and palmitic acid **YIELD** Approximate polyacrylamide gel purified yield for various scales are given below for an oligo below 50mer.

Yield given below are for oligos shorter than 50mer. Please see longer oligos yield at this link [Long Oligo Typical Yield](#).

~2 nmol final yield for 50 nmol scale synthesis.

~8 nmol final yield for 200 nmol scale synthesis.

~25 nmol final yield for 1 umol scale synthesis

~45nmol final yield for 2 umol scale synthesis

~110nmol final yield for 5 umol scale synthesis

~180 nmol final yield for 10 umol scale synthesis

~260 nmol final yield for 15 umol scale synthesis

[Click here to order stock MULTI-Seq LMO Lig-Anchor oligos.](#)

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

MULTI-Seq LMO Lig Anchor and MULTI-Seq LMO Palm Co-Anchor oligos are lignoceric and palmitic acid conjugated oligos as described by McGinnis, C. et al. (1)

1. McGinnis, C. et al. MULTI-seq: sample multiplexing for single-cell RNA sequencing using lipid-tagged indices. *Nat. Methods* 16, 619-626 (2019).

Lignoceric acid, or tetracosanoic acid, is long C24 chain saturated fatty acid. It is found in wood tar, various cerebrosides, and in small amounts in most natural fats. The fatty acids of peanut oil contain small amounts of lignoceric acid. This fatty acid is also a byproduct of lignin production.

Oligonucleotides are predominantly hydrophilic species and require help in permeating cell membranes. One strategy to improve cellular uptake of therapeutic oligonucleotides is to conjugate them with non-toxic, lipophilic molecules. Gene Link offers cholestry 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.

Staryl Modification

Staryl 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 linker 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 linker 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

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



Linolyl (C18:2) LA LMO
[26-6783-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 (C18:3 α) ALA, Gamma linolyl (C18:3 γ) GLA, Dihomo γ 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 cholesterol 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 linker 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 linker 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

P-Ethoxy dA

Category Antisense

Modification Code EoP-dA

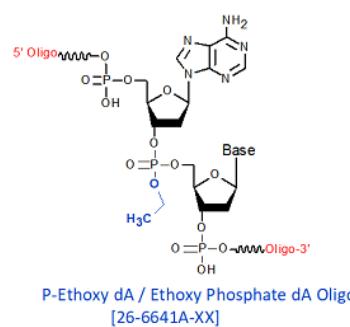
Reference Catalog Number 26-6641A

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 μ M, 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.

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. Thivyanathan, 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), staryl and GaINAc 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

P-Ethoxy dC

Category

Modification Code

Reference Catalog Number

5 Prime

3 Prime

Internal

Molecular Weight(mw)

Antisense

EoP-dC

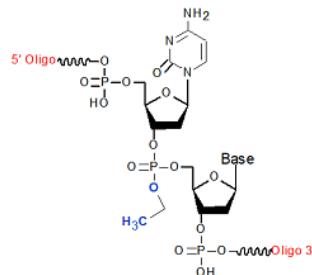
26-6641C

Y

Y

Y

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 μ M, 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.

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 T_m 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. Thivyanathan, 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), staryl and GaINAc 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

P-Ethoxy dG

Category

Modification Code

Reference Catalog Number

5 Prime

3 Prime

Internal

Molecular Weight(mw)

Antisense

EoP-dG

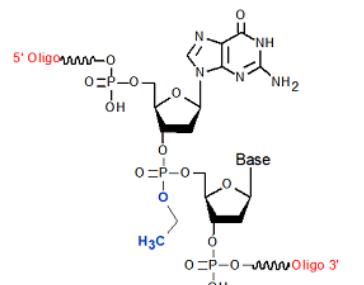
26-6641G

Y

Y

Y

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 μ M, 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.

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 T_m 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. Thivyanathan, 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*. **41**: 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), staryl and GaINAc 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

P-Ethoxy dT

Category

Modification Code

Reference Catalog Number

5 Prime

3 Prime

Internal

Molecular Weight(mw)

Antisense

EoP-dT

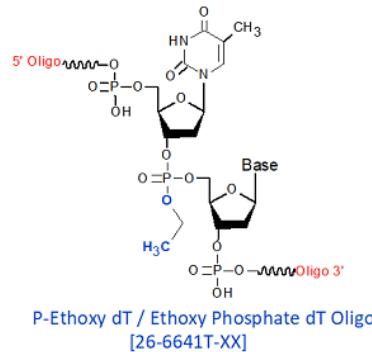
26-6641T

Y

Y

Y

332.48



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 μ M, 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.

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 T_m 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. Thivyanathan, 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), staryl and GaINAc 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

P-Methoxy dA

Category

Antisense

Modification Code

MoP-dA

Reference Catalog Number

26-6642A

5 Prime

Y

3 Prime

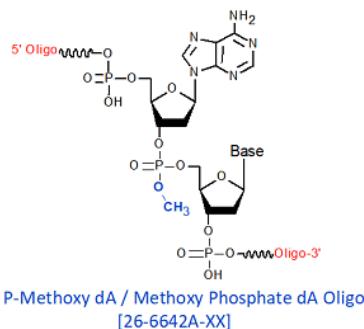
Y

Internal

Y

Molecular Weight(mw)

327.24



P-Methoxy (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 μ M, 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.

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 T_m 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. Thivyanathan, 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), staryl and GaINAc 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.



Product Specifications

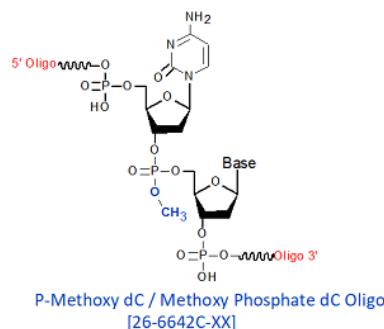
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

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)	303.19



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 μ M, 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.

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 T_m 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. Thivyanathan, 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), staryl and GaINAc 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

P-Methoxy dG

Category Antisense

Modification Code MoP-dG

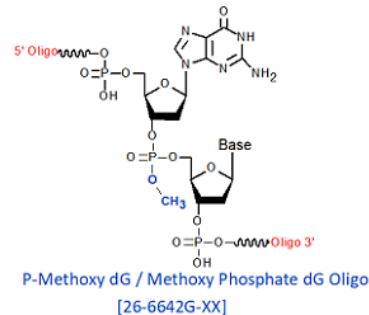
Reference Catalog Number 26-6642G

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 343.24



P-Methoxy (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 μ M, 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.

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 T_m 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. Thivyanathan, 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), staryl and GaINAc 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

P-Methoxy dT

Category Antisense

Modification Code MoP-dT

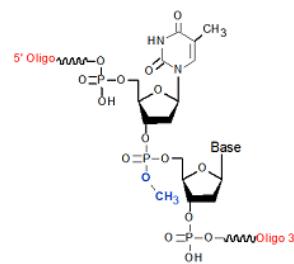
Reference Catalog Number 26-6642T

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 μ M, 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.

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 T_m 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. Thivyanathan, 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), staryl and GaINAc 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Palmityl C16 (3') LMO

Category Antisense

Modification Code Pal-C16-3

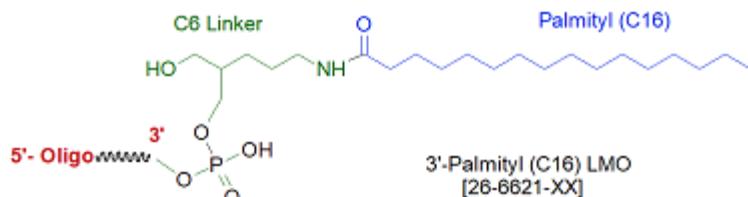
Reference Catalog Number 26-6621

5 Prime N

3 Prime Y

Internal N

Molecular Weight(mw) 432.56



3'-Palmityl (C16) LMO
[26-6621-XX]

This Palmitic C16 is only for 3' modification. We offer custom oligo synthesis of your designed sequences for conjugation to lignoceric acid and palmitic acid B>YIELD Approximate polyacrylamide gel purified yield for various scales are given below.

YIELD Approximate polyacrylamide gel purified yield for various scales are given below for an oligo below 50mer.

Yield given below are for oligos shorter than 50mer. Please see longer oligos yield at this link Long Oligo Typical Yield.

~2 nmol final yield for 50 nmol scale synthesis.

~8 nmol final yield for 200 nmol scale synthesis.

~25 nmol final yield for 1 umol scale synthesis

~45nmol final yield for 2 umol scale synthesis

~110nmol final yield for 5 umol scale synthesis

~180 nmol final yield for 10 umol scale synthesis

~260 nmol final yield for 15 umol scale synthesis

[Click here to order stock MULTI-Seq LMO Lig-Anchor oligos.](#)

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

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 cholestryl 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 linker 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 linker 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Palmityl C16 (5') LMO

Category Antisense

Modification Code Pal-C16-5

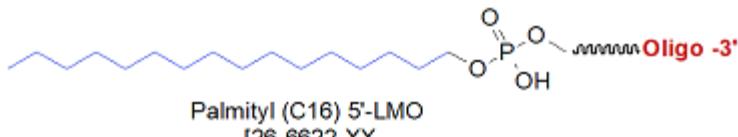
Reference Catalog Number 26-6622

5 Prime Y

3 Prime N

Internal N

Molecular Weight(mw) 303.4



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

Yield given below are for oligos shorter than 50mer. Please see longer oligos yield at this link [Long Oligo Typical Yield](#).

~2 nmol final yield for 50 nmol scale synthesis.

~8 nmol final yield for 200 nmol scale synthesis.

~25 nmol final yield for 1 umol scale synthesis

~45nmol final yield for 2 umol scale synthesis

~110nmol final yield for 5 umol scale synthesis

~180 nmol final yield for 10 umol scale synthesis

~260 nmol final yield for 15 umol scale synthesis

[Click here to order stock MULTI-Seq LMO Lig-Anchor oligos.](#)

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

MULTI-Seq LMO Lig Anchor and MULTI-Seq LMO Palm Co-Anchor oligos are lignoceric and palmitic acid conjugated oligos as described by McGinnis, C. et al. (1)

1. McGinnis, C. et al. MULTI-seq: sample multiplexing for single-cell RNA sequencing using lipid-tagged indices. *Nat. Methods* 16, 619-626 (2019).

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 linker 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 linker 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.



Product Specifications

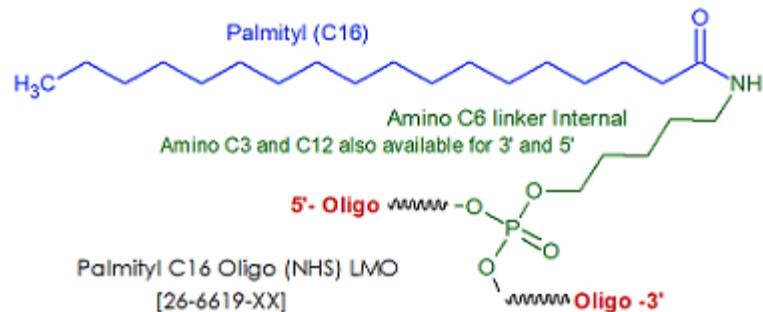
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

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 oligo 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.

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 cholestryol 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.

Staryl Modification

Staryl 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 linker 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 linker 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.



Product Specifications

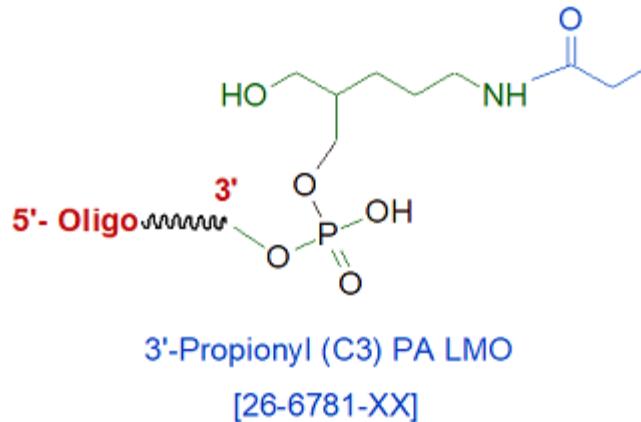
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

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



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 ω linolyl (C18:3 α) ALA, Gamma ω linolyl (C18:3 γ) GLA, Dihomo ω gamma ω 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 cholestryl 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 linker 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 linker 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.



Product Specifications

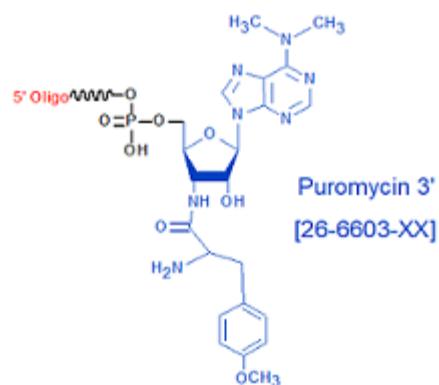
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Puromycin

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





Product Specifications

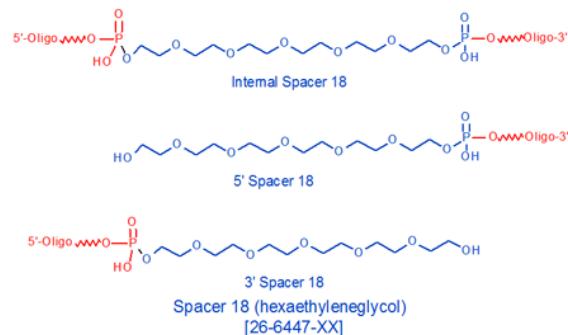
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

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





Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Spermine Oligo

Category

Modification Code

Reference Catalog Number

5 Prime

3 Prime

Internal

Molecular Weight(mw)

Duplex Stability

Spm

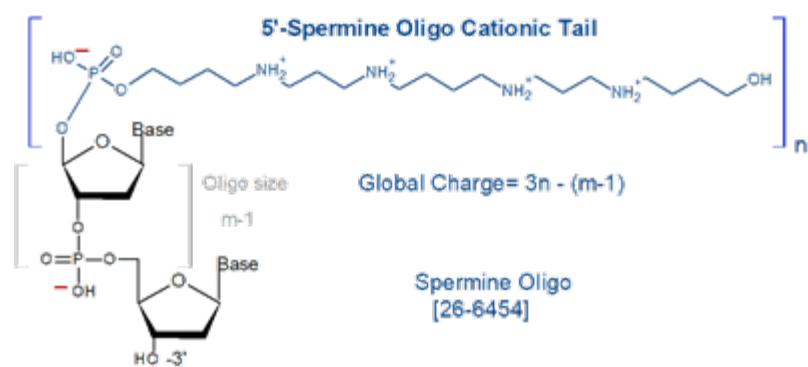
26-6454

Y

Y

Y

408.52



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

Spermine phosphoramidite is used to produce oligospermine-oligonucleotide conjugates - Zip Nucleic Acids (ZNA) Oligos. The name reflects the presumed mode of action. The conjugates are believed to use the oligospermine to seek out and move along (scan) oligonucleotide strands until the probe complementary sequence is located. The oligospermine then performs the function of stabilizing the formed duplex by reducing electrostatic repulsion, thereby leading to significantly increased binding affinities. ZNA Oligos have found use in the following applications: Multiplex PCR; PCR of AT-rich Regions; RT qPCR; Detection of MicroRNA; Improved SNP Discrimination; and Antisense and Antigene Effects. Spermine phosphoramidite is simple to use in oligonucleotide synthesis and can be added multiple times at the 3' or 5' terminus. Deprotection and isolation are also straightforward. HPLC analysis of the conjugates requires high pH to suppress the ionization of the spermine residues.

By selecting the number of cationic units, the global charge of the ZNA molecules can be modulated which defines their field of applications. When negatively charged, ZNA are potent tools for molecular biology and diagnostic applications. Their design is essentially based on the expected and predictable Tm of the oligonucleotide which depends on the number of conjugated cationic units. When positively charged, the cationic conjugates become self-delivering oligonucleotides into cells and resistant to nucleases which make them very attractive molecules for antisense or RNA interference applications. With an increase in spermine content, the solubility of ZNA oligonucleotides may be noticeably less than unmodified DNA or RNA counterparts. This is typically observed when re-dissolving dried-down purified ZNA in water. In this case, drop wise addition of 50 mM ammonium hydroxide brings ZNA molecules into solution. Alternatively, dissolving ZNA oligos in concentrated phosphate buffered saline (2.5x PBS, pH 7).

4) has also been found to resolve solubility issues.

Recommended Further Reading

Glen Reports GR24-11. Spermine Phosphoramidite: A potent modification with many applications.

Glen Reports GR24-11. Zip Nucleic Acids (ZNA) are powerful cationic oligonucleotides for molecular biology, diagnostic and therapeutic applications.

INTELLECTUAL PROPERTY

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



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Stearyl C18 (3') LMO

Category

Affinity Ligands

Modification Code

Str-C18-3

Reference Catalog Number

26-6623

5 Prime

N

3 Prime

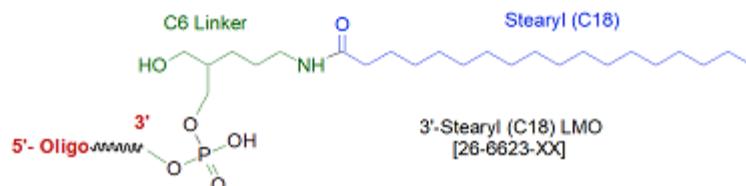
Y

Internal

N

Molecular Weight(mw)

460.61



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 linker 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 linker 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.



Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Stearyl C18 (5') LMO

Category Antisense

Modification Code Str-C18-5

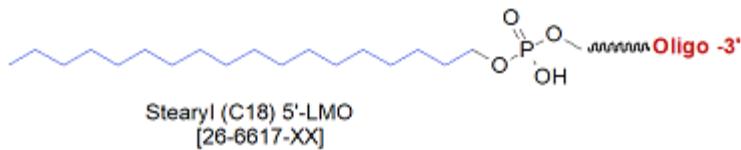
Reference Catalog Number 26-6617

5 Prime Y

3 Prime N

Internal N

Molecular Weight(mw) 332.46



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 cholestryl 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 linker 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 linker 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.