



Product Specifications

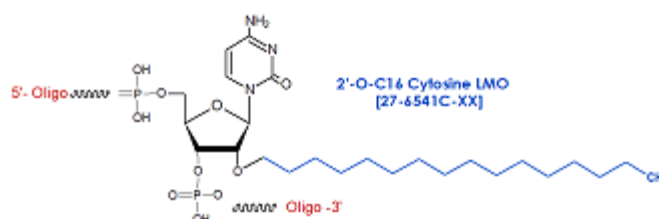
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

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

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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).
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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).
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siRNA Oligo Cellular Delivery Modifications

Vitamin B12 (Cyanocobalamin) Oligo

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

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

Vitamin E. alpha-tocopherol TEG Modification

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

Cholesterol TEG Modification

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

Stearyl Modification

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

GalNAc 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>

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3. K.G. Rajeev, et al., Chembiochem, 2015, 16, 903-8.
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