



## Product Specifications

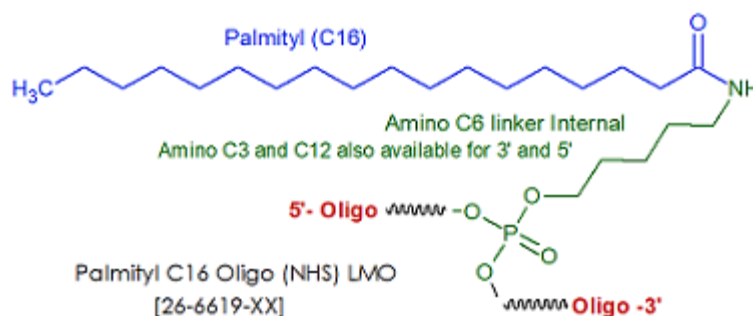
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## 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 oligos yield at this link Long Oligo Typical Yield.

~2 nmol final yield for 50 nmol scale synthesis.

~5 nmol final yield for 200 nmol scale synthesis.

~16 nmol final yield for 1 umol scale synthesis

~32 nmol final yield for 2 umol scale synthesis

~160 nmol final yield for 10 umol scale synthesis

~240 nmol final yield for 15 umol scale synthesis

Lipid Modified Oligo (LMO) Cell Tagging in Single Cell RNAseq/MULTI-Seq : Palmitic Oligo [C16] & Lignoceric Oligo [C24]

We offer custom oligo synthesis of your designed sequences for conjugation to lignoceric acid and palmitic acid for

MULTI-seq: sample multiplexing for single-cell RNA sequencing

Click here to order stock MULTI-Seq LMO Lig-Anchor oligos.

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

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

1. McGinnis, C. et al. MULTI-seq: sample multiplexing for single-cell RNA sequencing using lipid-tagged indices. Nat.

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.