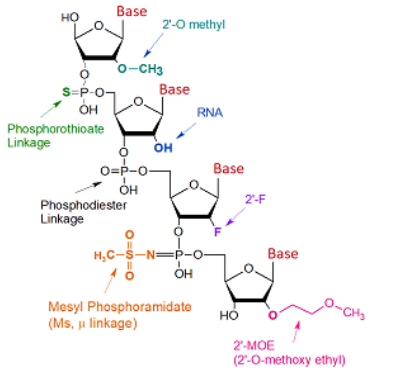
Mesyl Linkages

Category	Antisense
Modification Code	Ms
Reference Catalog Number	26-6914
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	77.1



Phosphodiester, Phosphorothioate and Mesyl Linkages in Oligo Modifications

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Custom Confirmed Orders Only & Pricing Structure **Mesyl Linkage Setup Charges:** The setup charge is $1,500.00 per order. The raw material for synthesizing oligos with mesyl linkages are procured for confirmed orders and the cost is added to the order in addition to the per linkage pricing listed above.

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

Phosphorothioate References 1. Sazani, P., Kole, R. Therapeutic potential of antisense oligonucleotides as modulators of alternative splicing. (2003) J. Clin. Invest., 112: 481-486.
2. Juliano, R., Alam, Md.R., Dixit, V., Kang, H.(2008) Mechanisms and strategies for effective delivery of antisense and siRNA oligonucleotides. Nucleic Acids Res., 36: 4158-4171.
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Methyl phosphonate (mp) Methyl phosphonate (mp) modification makes the phoshodiester linkage neutral charged. The solubility of the oligo in aqueous solutions slowly decreases with increasing mp 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.

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 methylphosphonolated oligos are due to the introduction of chirality into the phosphodiester backbone by the methyl group (8).

Methyl phosphonate (mp) References

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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.
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ASO's and siRNA Delivery. The development of effective delivery systems for antisense oligonucleotides is essential for their clinical therapeutic application. The most common delivery system involves a relatively hydrophobic molecule that can cross the lipid membrane. Cholesterol TEG, alpha-Tocopherol TEG (a natural isomer of vitamin E), stearyl and GalNAc modifications have been shown to effective for delivery of ASO's and siRNA in addition to cell penetrating peptides. Click this link to view these modifications.