



## 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.

## Nuclease Resistance Introduction

Like cellular DNA and RNA, synthetic oligonucleotides are prone to degradation once introduced into the cell or body fluids. Such degradation is due to the ubiquitous presence of nuclease enzymes (both exonucleases and endonucleases), as well as chemical instability (particularly for RNA). Under normal cellular conditions, this leads to fast in vivo degradation of oligos and a short half-life (1). In addition, the ease with which RNase, being very stable, can contaminate laboratory equipment and benchtop surfaces means that RNA is susceptible to degradation under normal laboratory conditions as well. To reduce or eliminate this susceptibility, nuclease-resistant modifications (for example, phosphorothiolation or 2'-OMethyl RNA bases) can be introduced into oligonucleotides slated for in vivo and/or regular benchtop work.

# Nuclease Resistance Design Protocols

## Nuclease Resistant Oligos for In vivo Applications and Design Considerations

While oligonucleotides are quickly degraded (typically within 15-30 minutes) by nucleases in both in vitro and in vivo contexts, the need to incorporate nuclease resistance into oligonucleotides is critically important for in vivo applications. Within serum or the cell, oligonucleotides can be degraded by both endo- and exonucleases. In serum, the 3'-exonucleases are of greatest concern (7), while within the cell, both 3'- and 5'-exonucleases are problematic (8). Endonucleases can also be an issue in those cases where the oligo contains a restriction site.

Based on the above, designing a nuclease-resistant oligonucleotide for in vivo applications primarily involves modifying it so as to protect it from exonucleases, while minimizing potential deleterious side-effects (such as reduced duplex stability, increased toxicity, or induction of off-target biological effects). The simplest and most cost-effective way to do this is to design the oligo as a 'gapmer', in which the linkages of the three end-most 5'- and 3'-bases are phosphorothiolated, with the remaining bases in the middle having native phosphodiester linkages. Such phosphorothiolated 'gapmers' are highly resistant to both 5'- and 3'-exonuclease degradation. In addition, because phosphorothiolation lowers the binding affinity of the oligo for its target ( $T_m$  of the oligo-target duplex is lowered between 0.5C and 1.5C per linkage, depending on sequence), use of only six such linkages often yields an acceptable balance between nuclease resistance and binding affinity (if increased binding affinity is required, other modifications can also be incorporated into the oligo, such as 2'-fluoro pyrimidines, 2'-O-methyl RNA bases, or C5-propyne pyrimidines). Finally, since large numbers of phosphorothiolate linkages can be toxic (due to the presence of sulfur), using only a small number of such linkages in an oligo minimizes cellular toxicity.

If phosphorothiolation is not desired, other modifications can be used. One option is to use methylphosphonates instead of phosphorothiolation for the 5'- and 3'-end positions of the 'gapmer'. Methylphosphonates lower an oligo's binding affinity more than phosphorothiolation, however, so the use of additional modifications, such as 2'-fluoro-pyrimidines, is advisable to counteract this effect. More commonly, the substitution of 2'-O-methyl RNA bases at some or all positions of an oligo is used as an alternative to phosphorothiolation. Since the nuclease resistance conferred by 2'-O-methyl RNA lies between that of standard bases (no resistance) and phosphorothiolation (highly resistant), extensive/complete 2'-O-methylation is frequently chosen when a high level of nuclease resistance is required. 2'-O-methylation also confers higher binding affinity (that is, higher duplex  $T_m$ ) to the oligo for its target, a desirable property, in many cases.

## Nuclease Resistance Applications

For antisense or RNAi applications, incorporation of modifications conferring nuclease resistance is essentially indispensable and such modifications are used intensely. The most popular modification used for this purpose is phosphorothiolation, in which a phosphodiester backbone linkage is replaced by a phosphothiolate linkage. Such linkages are highly resistant to nuclease degradation, but they also lower duplex stability by about 0.5°C per phosphorothioate linkage. However, judicious use of this modification (for example, placing them only at the three endmost bases of each end of the oligo to minimize exonuclease degradation) can produce excellent nuclease resistance while still maintaining reasonably good duplex stability (2). 2'-O Methyl (or other 2'-O-substituted) RNA bases also confer nuclease resistance to an oligo, and have the added benefit of increasing duplex stability as well (3,4). However, duplexes formed between oligos having 2'-OMethyl bases at all positions and RNA are incapable of activating RNase H activity (3), and this fact must be kept in mind if the user wishes to use such oligos for antisense applications. Recently, "mirror-image" (L)-nucleotide base phosphoramidites have become available as well. Oligonucleotides containing (L)-nucleotides are completely immune to nuclease attack at the incorporated positions (5). While (L)-nucleotides also do not base pair with natural (D)-nucleotides (6), they still could potentially be incorporated in, for example, the stems of molecular beacons to protect them from degradation by exonucleases.

## References

- (1) Cazenave, C., Chevrier, M., Nguyen, T.T., Helene, C. Rate of degradation of [alpha]- and [beta]-oligodeoxynucleotides in *Xenopus* oocytes. Implications for anti-messenger strategies. *Nucleic Acids Res.* (1987), 15: 10507-10521.
- (2) Pandolfi, D., Rauzi, F., Capobianco, M.L. Evaluation of different types of end-capping modifications on the stability of oligonucleotides toward 3'- and 5'-exonucleases. *Nucleosides Nucleotides* (1999), 18: 2051-2069.
- (3) Monia, B.P., Lesnik, E.A., Gonzalez, C., Lima, W.F., McGee, D., Guinosso, C.J., Kawasaki, A.M., Cook, P.D., Frier, S.M. Evaluation of 2'-Modified Oligonucleotides Containing 2'-Deoxy Gaps as Antisense Inhibitors of Gene Expression. *J. Biol. Chem.* (1993), 268: 14514-14522.
- (4) Monia, B.P., Johnston, J.F., Sasmor, H., Cummins, L.L. Nuclease Resistance and Antisense Activity of Modified Oligonucleotides Targeted to Ha-ras. *J. Biol. Chem.* (1996), 271: 14533-14540.
- (5) Sooter, L.J., Ellington, A.D. Reflections on a Novel Therapeutic Candidate. *Chem. & Biol.* (2002), 9: 857-858.
- (6) Urata, H., Ogura, E., Shinohara, K., Ueda, Y., Akagi, M. Synthesis and properties of mirror-image DNA. *Nucleic Acids Res.* (1992), 20: 3325-3332.
- (7) Eder, P.S., DeVine, R.J., Dagle, J.M., Walder, J.A. Substrate specificity and kinetics of degradation of antisense oligonucleotides by a 3' exonuclease in plasma, *Antisense Res. Dev.* (1991), 1: 141-151.
- (8) Dagle, J.M., Weeks, D.L., Walder, J.A. Pathways of degradation and mechanism of action of antisense oligonucleotides in *Xenopus laevis* embryos. *Antisense Res. Dev.* (1991), 1: 11-20.

## Modification Code List

Modification	Code	Catalog Number
2'-Fluoro deoxyadenosine (2'-F-A)	[fA]	26-6692
2'-Fluoro deoxycytosine (2'-F-C)	[fC]	26-6463
2'-Fluoro deoxyguanosine (2'-F-G)	[fG]	26-6693
2'-Fluoro deoxyuridine (2'-F-U)	[fU]	26-6462
2'-O methyl adenosine A	[mA]	27-6410A
2'-O methyl cytosine C	[mC]	27-6410C
2'-O methyl guanosine G	[mG]	27-6410G
2'-O methyl uridine U	[mU]	27-6410U
3'-dA (2'-5' linked)	[3dA2-5]	26-6490
3'-dC (2'-5' linked)	[3dC2-5]	26-6491
3'-dG (2'-5' linked)	[3dG2-5]	26-6492
3'-dT (2'-5' linked)	[3dT2-5]	26-6493
3'-O methyl bases (2'-5' linked)	[3OmN]	27-6458N
3'-O methyl rA (2'-5' linked)	[3OmA]	27-6458A
3'-O methyl rC (2'-5' linked)	[3OmC]	27-6458C
3'-O methyl rG (2'-5' linked)	[3OmG]	27-6458G
3'-O methyl rU (2'-5' linked)	[3OmU]	27-6458U
3'-rA (2'-5' linked)	[3rA2-5]	26-6682
3'-rC (2'-5' linked)	[3rC2-5]	26-6911
3'-rG (2'-5' linked)	[3rG2-5]	26-6912

3'-rU (2'-5' linked)	[3rU2-5]	26-6913
L-DNA dA (beta L)	[L-dA]	26-6941
L-DNA dC (beta L)	[L-dC]	26-6942
L-DNA dG (beta L)	[L-dG]	26-6943
L-DNA dT (beta L)	[L-dT]	26-6944
L-RNA rA	[L-rA]	27-6941
L-RNA rC	[L-rC]	27-6942
L-RNA rG	[L-rG]	27-6943
L-RNA rU	[L-rU]	27-6944
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
Phosphorothioate [Ps]	[Ps]	26-6401
propyne dC	[pdC]	26-6501
propyne dU	[pdU]	26-6502



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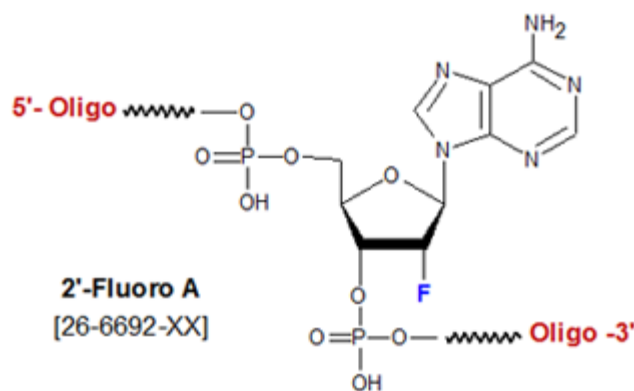
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### 2'-F-A

Category	Nuclease Resistance
Modification Code	fA
Reference Catalog Number	26-6692
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	331.2



Antisense Oligos (ODN) & siRNA Oligo Modifications

**Click here for more information on antisense modifications, design & applications.**

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2'-F RNA modifications have been shown to be particularly useful in the following oligo-based applications:

(a) **Anti-sense oligos & Nuclease Resistance:** When 2'-F RNA is incorporated into an anti-sense DNA oligo (resulting in a 2'-F RNA/DNA chimeric), the  $T_m$  of its duplex with RNA **increases** relative to that formed by an unmodified anti-sense DNA oligo **by 1.8°C per 2'-F RNA residue added** (1). The DNA/RNA duplex formed between a chimeric 2'-F-RNA/DNA anti-sense oligo and its RNA target also is a substrate for RNase H. With respect to nuclease resistance, while 2'-F RNA nucleotides do provide some nuclease resistance when incorporated into DNA, phosphorothiolation of the 2'-F RNA phosphodiester linkages is recommended, because it strongly enhances such resistance. This effect becomes particularly important if the 2'-F RNA nucleotide is to be incorporated at one or more of the first three positions of the 5'- or 3'-ends of the oligo.

Modifications Increasing Duplex Stability and Nuclease Resistance

Modification

Duplex Stability [ $T_m$  Increase]

Nuclease Resistance Locked Analog Bases Increased [2- 4C per substitution] Increased 2-Amino-dA Increased [3.0C per substitution] Similar to DNA C-5 propynyl-C Increased [2.8C per substitution] Increased C-5 propynyl-U Increased [1.

7C per substitution] Increased 2'-Fluoro Increased [1.8C per substitution] Increased 5-Methyl-dC Increased [1.3C per substitution] Similar to DNA 2'-O Methyl Increased Increased Phosphorothioate Slightly decreased Increased Click here for complete list of duplex stability modifications

(b) **Aptamers:** The addition of 2'-F pyrimidines (C, U) into RNA aptamers has been shown to provide them with both considerably increased nuclease resistance and equal or higher binding affinity for their ligands, compared to the corresponding unmodified aptamers (2,3). The incorporation of 2'-F RNA nucleotides into RNA aptamers as an optimization strategy is becoming an increasingly common practice.

(c) **siRNA & Nuclease Resistance:** siRNA synthesized with 2'-F pyrimidines have been shown to have greatly increased stability in plasma compared to 2'-OH siRNA (4,5). In one study, levels of inhibition for 2'-F siRNA and 2'-OH siRNA, in cell culture and *in vivo* using BALB/c mice transfected with pGL3 luciferase, were similar over time (4). In another study, siRNA fully substituted with both 2'-F RNA and 2'-O-Methyl RNA nucleotides displayed not only enhanced stability in plasma, but also >500-fold increase in capability to down-regulate gene expression compared with the corresponding unmodified siRNA (5).

(d) **LNA Alternative:** The increased thermal stability and nuclease resistance provided by 2'-F RNA residues make them attractive as lower-cost substitutes in any application involving LNA-modified oligos.

#### **ASO's and siRNA Modifications.**

Click this link to view ASO's and siRNA Modifications.

**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.

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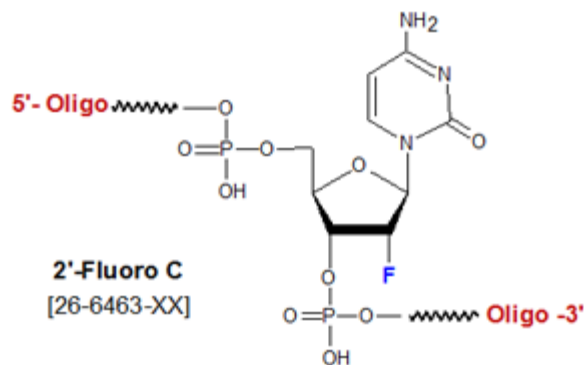
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### 2'-F-C

Category	Nuclease Resistance
Modification Code	fC
Reference Catalog Number	26-6463
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	307.17



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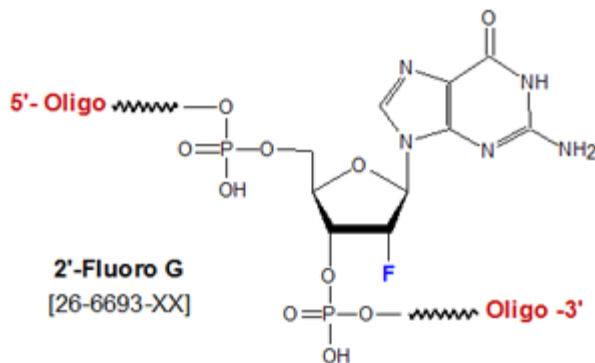
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### 2'-F-G

Category	Nuclease Resistance
Modification Code	fG
Reference Catalog Number	26-6693
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	347.16



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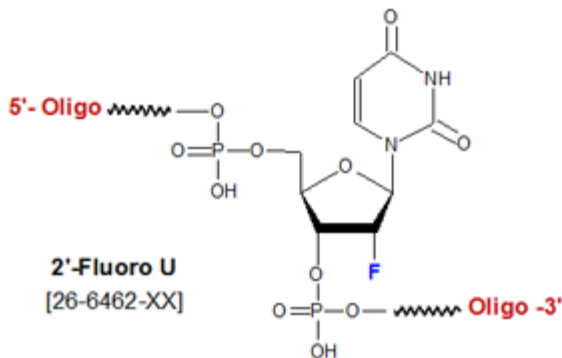
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Reference Catalog Number	26-6462
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	308.16



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Modifications Increasing Duplex Stability and Nuclease Resistance

Modification

Duplex Stability [ $T_m$  Increase]

Nuclease Resistance Locked Analog Bases Increased [2- 4C per substitution] Increased 2-Amino-dA Increased [3.0C per substitution] Similar to DNA C-5 propynyl-C Increased [2.8C per substitution] Increased C-5 propynyl-U Increased [1.

7C per substitution] Increased 2'-Fluoro Increased [1.8C per substitution] Increased 5-Methyl-dC Increased [1.3C per substitution] Similar to DNA 2'-O Methyl Increased Increased Phosphorothioate Slightly decreased Increased Click here for complete list of duplex stability modifications

(b) **Aptamers:** The addition of 2'-F pyrimidines (C, U) into RNA aptamers has been shown to provide them with both considerably increased nuclease resistance and equal or higher binding affinity for their ligands, compared to the corresponding unmodified aptamers (2,3). The incorporation of 2'-F RNA nucleotides into RNA aptamers as an optimization strategy is becoming an increasingly common practice.

(c) **siRNA & Nuclease Resistance:** siRNA synthesized with 2'-F pyrimidines have been shown to have greatly increased stability in plasma compared to 2'-OH siRNA (4,5). In one study, levels of inhibition for 2'-F siRNA and 2'-OH siRNA, in cell culture and *in vivo* using BALB/c mice transfected with pGL3 luciferase, were similar over time (4). In another study, siRNA fully substituted with both 2'-F RNA and 2'-O-Methyl RNA nucleotides displayed not only enhanced stability in plasma, but also >500-fold increase in capability to down-regulate gene expression compared with the corresponding unmodified siRNA (5).

(d) **LNA Alternative:** The increased thermal stability and nuclease resistance provided by 2'-F RNA residues make them attractive as lower-cost substitutes in any application involving LNA-modified oligos.

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#### **REFERENCES:**

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2. Khati, M.; Schuman, M.; Ibrahim, J.; Sattentau, Q.; Gordon, S.; James, W. Neutralization of infectivity of diverse R5 clinical isolates of human immunodeficiency virus type 1 by gp120-binding 2'-F-RNA aptamers. *J Virol*, (2003). **77**: 2692-8.
3. Goring, H.U.; Adler, Annette; Forster, Nicole; Homann, Matthias. Post-SELEX Chemical Optimization of a Trypanosome-Specific RNA Aptamer. *Combinatorial Chemistry & High Throughput Screening* (2008), **11**: 16-23.
4. Layzer, J.M.; McCaffrey, A.P.; Tanner, A.K.; Huang, Z.; Kay, M.A.; Sullenger, B.A. In vivo activity of nuclease-resistant siRNAs. *RNA* (2004), **10**, 766-71.
5. Allerson, C.R.; et al. Fully 2'-modified oligonucleotide duplexes with improved in vitro potency and stability compared to unmodified small interfering RNA. *Journal of Medicinal Chemistry* (2005), **48**: 901-904.



## Product Specifications

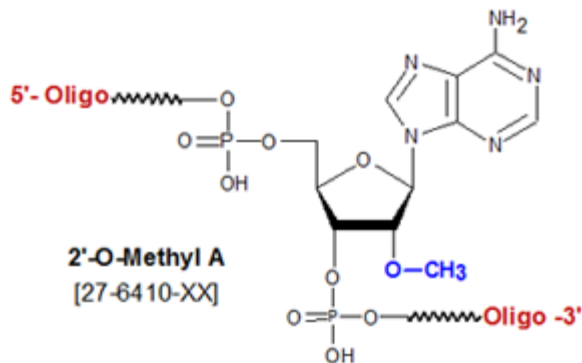
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## Oligo Modifications

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

### 2'-O methyl A

Category	Nuclease Resistance
Modification Code	mA
Reference Catalog Number	27-6410A
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	343.24



Antisense Oligos (ODN) & siRNA Oligo Modifications

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Modifications Increasing Duplex Stability and Nuclease Resistance

Modification

Duplex Stability [T<sub>m</sub> Increase]

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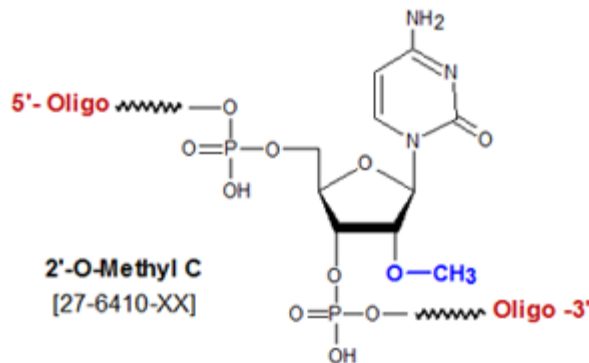
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## Oligo Modifications

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### 2'-O methyl C

Category	Nuclease Resistance
Modification Code	mC
Reference Catalog Number	27-6410C
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	319.21



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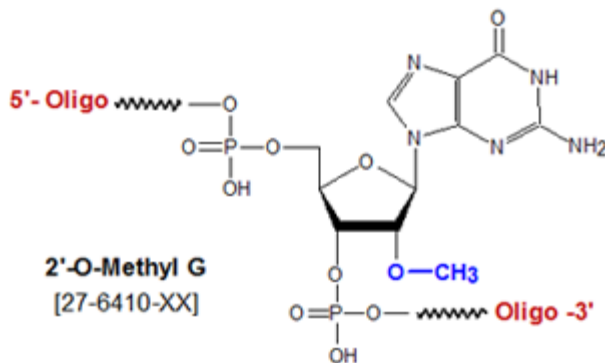
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## Oligo Modifications

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### 2'-O methyl G

Category	Nuclease Resistance
Modification Code	mG
Reference Catalog Number	27-6410G
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	359.24



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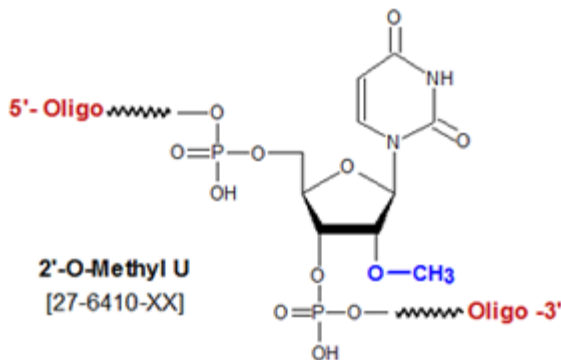
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## Oligo Modifications

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### 2'-O methyl U

Category	Nuclease Resistance
Modification Code	mU
Reference Catalog Number	27-6410U
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	320.2



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Modifications Increasing Duplex Stability and Nuclease Resistance

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Duplex Stability [T<sub>m</sub> Increase]

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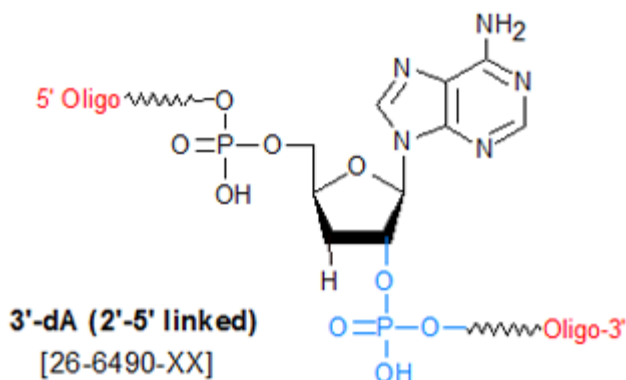
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## Oligo Modifications

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### 3'-dA (2'-5' linked)

Category	Antisense
Modification Code	3dA2-5
Reference Catalog Number	26-6490
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	313.21



3'-deoxy bases (2'-5' linked) are deoxy at the 3' position of the ribose, instead of at the usual 2'-position (**note: All four deoxy and ribo versions the 2'-5' linked A, C, G and T or U are available from Gene Link**). 3'-deoxynucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligo. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3,5'-DNA (1,2). This property means that DNA oligos containing such linkages could be useful in either anti-sense applications or as ssRNA-specific probes.

Bhan *et al.* (2) studied the potential for 2',5'-linked DNA oligos as anti-sense molecules. High selectivity for 3',5' RNA over 3',5' DNA was observed, presumably due to the 2',5'-linkages destabilizing duplexes formed with 3',5' DNA more than those formed with 3',5'-RNA (for 2',5' DNA:3',5' RNA duplexes, Delta Tm is only about ~0.5 deg C per 2',5' linkage substitution). Phosphorothiolation (which confers nuclease resistance) of 2'-5' linkages lowers the Tm of 2',5' DNA:3',5' RNA duplexes even less, ~0.2 deg C per phosphorothiolated 2',5'-linkage substitution. (by contrast, phosphorothiolation of a 3',5' linkage lowers the Tm of 3',5' DNA:RNA duplexes by 0.5 to 2.0 degC). Thus, 2',5'-linked DNA oligos show both high selectivity and good duplex stability for RNA target sequences. However, 2',5'-linked DNA oligos, whether phosphorothiolated or not, do not support RNase H activity when bound to complementary RNA. But, substitution of six or seven contiguous 3',5' phosphorothiolate linkages into a 2',5' phosphorothiolated oligo at an appropriate place (that is, making a 2',5'/3',5' phosphorothiolated chimera restores the oligo's ability to support RNase H activity. Furthermore, 2',5'-linked DNA oligos, whether phosphorothiolated or not, show little or no non-sequence specific binding to cellular proteins (by contrast, 3',5' DNA oligos show considerable levels of such binding).

In summary, this research suggests that 2',5'/3',5' phosphorothiolated chimeric oligos, in which 6-7 of the linkages are 3',5' to ensure that it can support RNase H activity, have considerable potential as anti-sense reagents, due to their high selectivity for complementary RNA targets, and minimal non-sequence specific binding to cellular proteins.

In 2004, Sinha and co-workers showed that 2',5'-linked DNA has some capability to function as a template for polymerase-directed DNA synthesis of the complementary strand (3).

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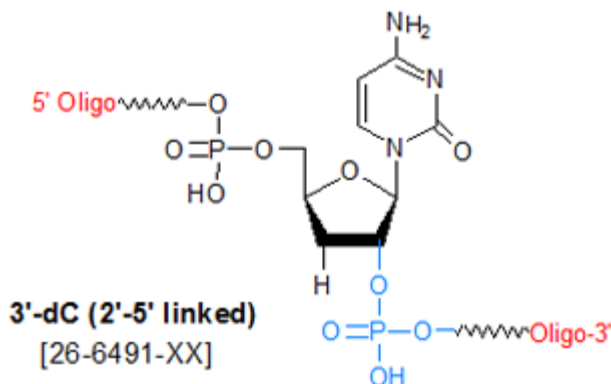
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### 3'-dC (2'-5' linked)

Category	Antisense
Modification Code	3dC2-5
Reference Catalog Number	26-6491
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	289.18



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## Product Specifications

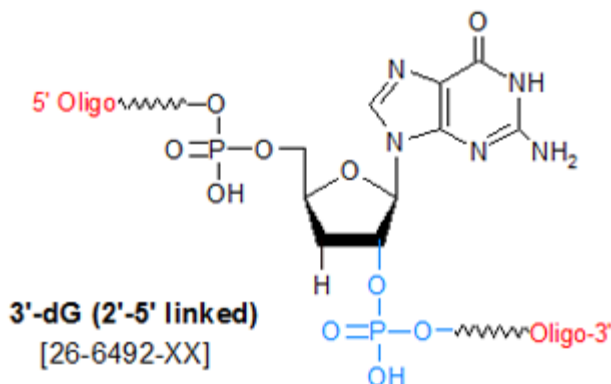
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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

### 3'-dG (2'-5' linked)

Category	Antisense
Modification Code	3dG2-5
Reference Catalog Number	26-6492
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	329.21



3'-deoxy bases (2'-5' linked) are deoxy at the 3' position of the ribose, instead of at the usual 2'-position (**note: All four deoxy and ribo versions the 2'-5' linked A, C, G and T or U are available from Gene Link**). 3'-deoxynucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligo. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3,5'-DNA (1,2). This property means that DNA oligos containing such linkages could be useful in either anti-sense applications or as ssRNA-specific probes.

Bhan *et al.* (2) studied the potential for 2',5'-linked DNA oligos as anti-sense molecules. High selectivity for 3',5' RNA over 3',5' DNA was observed, presumably due to the 2',5'-linkages destabilizing duplexes formed with 3',5' DNA more than those formed with 3',5'-RNA (for 2',5' DNA:3',5' RNA duplexes, Delta Tm is only about ~0.5 deg C per 2',5' linkage substitution). Phosphorothiolation (which confers nuclease resistance) of 2'-5' linkages lowers the Tm of 2',5' DNA:3',5' RNA duplexes even less, ~0.2 deg C per phosphorothiolated 2',5'-linkage substitution. (by contrast, phosphorothiolation of a 3',5' linkage lowers the Tm of 3',5' DNA:RNA duplexes by 0.5 to 2.0 degC). Thus, 2',5'-linked DNA oligos show both high selectivity and good duplex stability for RNA target sequences. However, 2',5'-linked DNA oligos, whether phosphorothiolated or not, do not support RNase H activity when bound to complementary RNA. But, substitution of six or seven contiguous 3',5' phosphorothiolate linkages into a 2',5' phosphorothiolated oligo at an appropriate place (that is, making a 2',5'/3',5' phosphorothiolated chimera restores the oligo's ability to support RNase H activity. Furthermore, 2',5'-linked DNA oligos, whether phosphorothiolated or not, show little or no non-sequence specific binding to cellular proteins (by contrast, 3',5' DNA oligos show considerable levels of such binding).

In summary, this research suggests that 2',5'/3',5' phosphorothiolated chimeric oligos, in which 6-7 of the linkages are 3',5' to ensure that it can support RNase H activity, have considerable potential as anti-sense reagents, due to their high selectivity for complementary RNA targets, and minimal non-sequence specific binding to cellular proteins.

In 2004, Sinha and co-workers showed that 2',5'-linked DNA has some capability to function as a template for polymerase-directed DNA synthesis of the complementary strand (3).

The authors showed several polymerases, and HIV reverse transcriptase, can successfully use a string of 2-4 2',5'-linked DNA nucleotides as a template to synthesize its complementary strand with high fidelity, and speculated that the polymerases were serving as a "template for the template", i.e., compensating for structural deficiencies in the 2',5'-linked DNA that, in non-enzymatic contexts, would preclude genetic information transfer for 2',5'-linked DNA. **References**

1. Giannaris, P.A.; Damha, M.J. Oligoribonucleotides containing 2',5'-phosphodiester linkages exhibit binding selectivity for 3',5'-RNA over 3',5'-ssDNA. *Nucleic Acids Res* (1993), **21**: 4742-4749.
2. Bhan, P.; Bhan, A.; Hong, M.K.; Hartwell, J.G.; Saunders, J.M.; Hoke, G.D. 2',5'-linked oligo-3'-deoxyribonucleoside phosphorothioate chimeras: thermal stability and antisense inhibition of gene expression. *Nucleic Acids Res.* (1997), **25**: 40-41.
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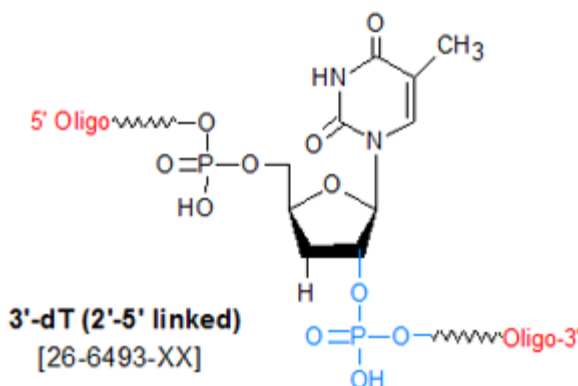
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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

### 3'-dT (2'-5' linked)

Category	Antisense
Modification Code	3dT2-5
Reference Catalog Number	26-6493
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	304.2



3'-deoxy bases (2'-5' linked) are deoxy at the 3' position of the ribose, instead of at the usual 2'-position (**note: All four deoxy and ribo versions the 2'-5' linked A, C, G and T or U are available from Gene Link**). 3'-deoxynucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligo. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3,5'-DNA (1,2). This property means that DNA oligos containing such linkages could be useful in either anti-sense applications or as ssRNA-specific probes.

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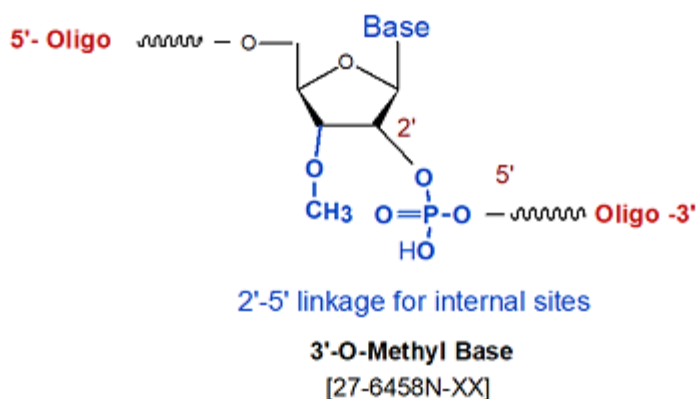
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## Oligo Modifications

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

### 3'-O methyl bases

Category	Antisense
Modification Code	3OmN
Reference Catalog Number	27-6458N
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	386.55



Mixed base N has a setup charge of \$250.00 per order.

Antisense Oligos (ODN) & siRNA Oligo Modifications

**Click here for more information on antisense modifications, design & applications.**

3'-O methyl bases (3'-Ome) form a 2'-5' phosphodiester linkage when placed internally. A single 3'-O methyl base modification at the 3' end will have a hydroxyl group at the 2' end and 3'-O methyl at the 3' end, this will prevent the oligo from extension by polymerases.

3'-O methyl bases (3'-Ome)-(2'-5' linked), are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2', 5'- phosphodiester linkages selectively bind to complementary single-stranded 3', 5'- RNA over comparable 3', 5'- DNA. Presumably this selectivity is a consequence of the 2', 5'- linkages destabilizing duplexes formed with 3', 5'- DNA more than those formed with 3', 5'-RNA, leading to 2'-5'- RNA:3', 5'- DNA duplexes having much lower T<sub>m</sub> than the corresponding 2'-5'- RNA:3'- 5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

Antisense oligonucleotides (ASOs) and small interfering RNA (siRNA) are both recognized therapeutic agents for the silencing of specific genes at the posttranscriptional level. Chemical modifications, particularly 2'-O-(2-Methoxyethyl)-oligoribonucleotides (2'-O-MOE bases) and 2'-OMethyl bases are commonly used to confer nuclease resistance to an oligo designed for anti-sense, siRNA or aptamer-based research, diagnostic or therapeutic purposes, when specific 2'-OH is not required.

Nuclease resistance can be further enhanced by phosphorothiolation of appropriate phosphodiester linkages within the oligo. These modifications confers nuclease resistance, high binding affinity towards complementary RNA, reduced unspecific protein binding and extended half-life in tissues.

**Gapmers.** Currently, the mainstream of the ASO is gapmer design ASOs. Gapmer design oligonucleotides, contain two to five chemically modified nucleotides (LNA, 2'-O methyl or 2'-O-MOE RNA) as 'wings' at each terminus flanking a central 5- to 10-base 'gap' of DNA, enable cleavage of the target mRNA by RNase H, which recognizes DNA/RNA heteroduplexes.

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**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. The following list of modifications are suitable for delivery system in addition to cell penetrating peptides.

Cholesterol

Tocopherol (alpha-tocopherol, a natural isomer of vitamin E)

PEG



## Product Specifications

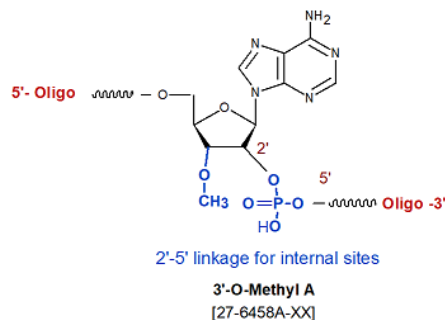
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## Oligo Modifications

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

### 3'-O methyl rA

Category	Antisense
Modification Code	3OmA
Reference Catalog Number	27-6458A
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	343.24



Antisense Oligos (ODN) & siRNA Oligo Modifications

**Click here for more information on antisense modifications, design & applications.**

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3'-O methyl bases (3'-Ome)-(2'-5' linked), are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2', 5'- phosphodiester linkages selectively bind to complementary single-stranded 3', 5'- RNA over comparable 3', 5'- DNA. Presumably this selectivity is a consequence of the 2', 5'- linkages destabilizing duplexes formed with 3', 5'- DNA more than those formed with 3', 5'-RNA, leading to 2'-5'- RNA:3', 5'- DNA duplexes having much lower T<sub>m</sub> than the corresponding 2'-5'- RNA:3'- 5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

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## Product Specifications

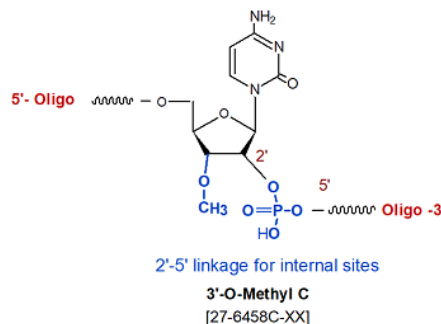
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## Oligo Modifications

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### 3'-O methyl rC

Category	Antisense
Modification Code	3OmC
Reference Catalog Number	27-6458C
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	319.21



Antisense Oligos (ODN) & siRNA Oligo Modifications

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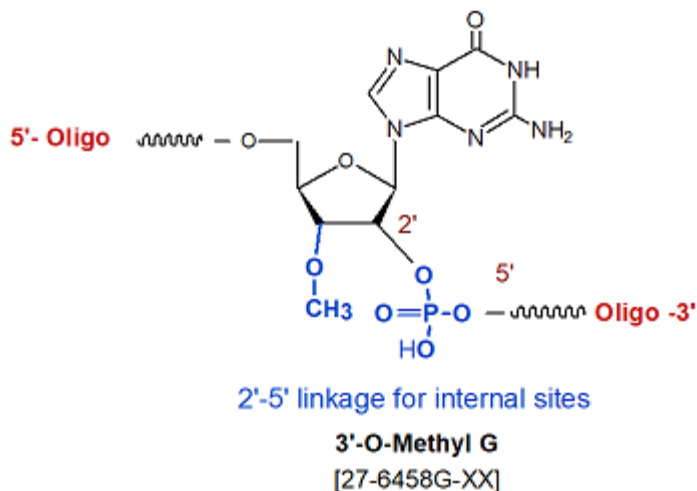
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## Oligo Modifications

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### 3'-O methyl rG

Category	Antisense
Modification Code	3OmG
Reference Catalog Number	27-6458G
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	359.24



Antisense Oligos (ODN) & siRNA Oligo Modifications

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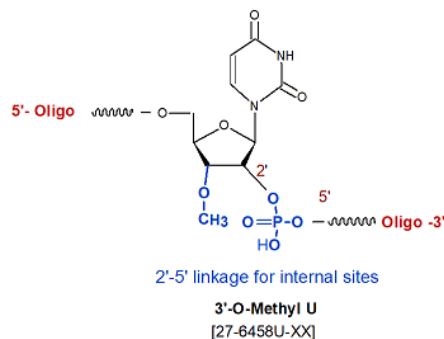
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## Oligo Modifications

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### 3'-O methyl rU

Category	Antisense
Modification Code	3OmU
Reference Catalog Number	27-6458U
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	320.2



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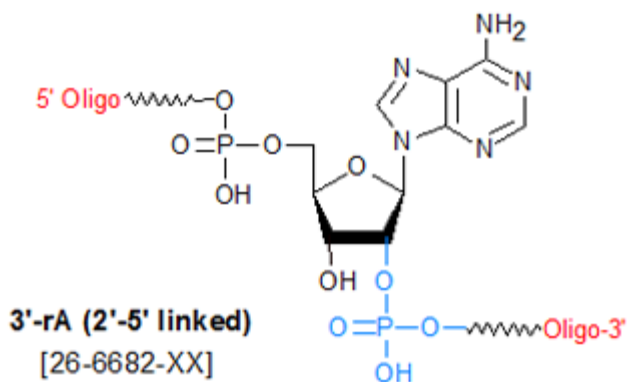
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## Oligo Modifications

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### 3'-rA (2'-5' linked)

Category	Minor Bases
Modification Code	3rA2-5
Reference Catalog Number	26-6682
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	329.24





## Product Specifications

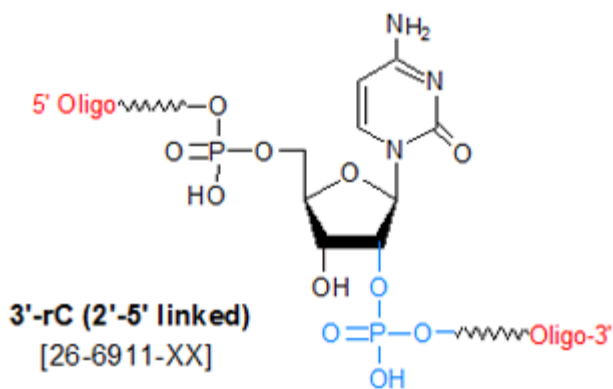
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## Oligo Modifications

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### 3'-rC (2'-5' linked)

Category	Minor Bases
Modification Code	3rC2-5
Reference Catalog Number	26-6911
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	305.18





## Product Specifications

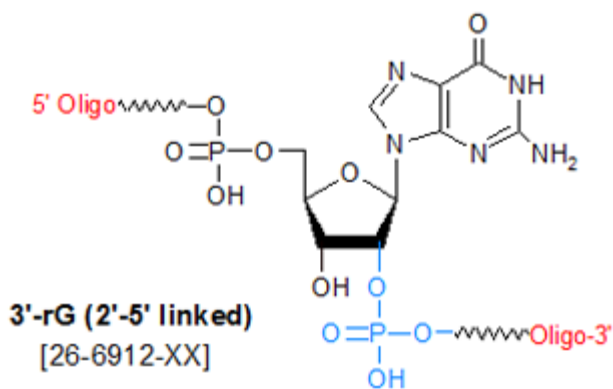
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## Oligo Modifications

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### 3'-rG (2'-5' linked)

Category	Minor Bases
Modification Code	3rG2-5
Reference Catalog Number	26-6912
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	345.21





## Product Specifications

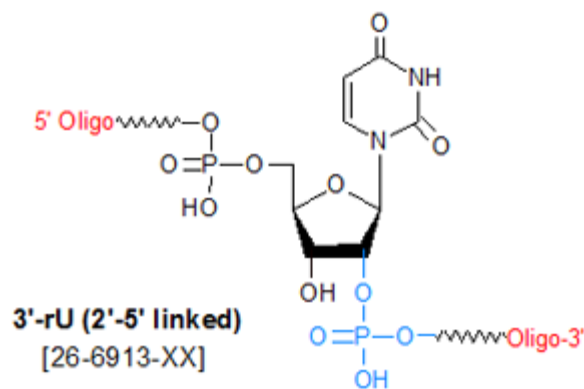
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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

### 3'-rU (2'-5' linked)

Category	Minor Bases
Modification Code	3rU2-5
Reference Catalog Number	26-6913
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	306.17





## Product Specifications

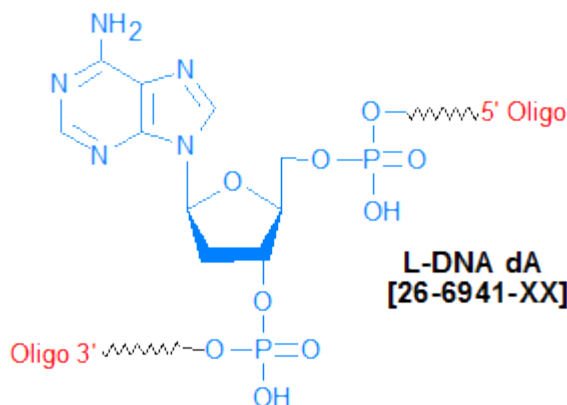
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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

### L-DNA dA

Category	Others
Modification Code	L-dA
Reference Catalog Number	26-6941
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	313.2



L-DNA (beta stereoisomer deoxyribose that is same as in D-DNA) is the left-turning and mirror image version of natural DNA, as opposed to the naturally occurring right-turning version called D-DNA. L-DNA is more stable than D-DNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-DNA but form a left-helical double-helix. Because of its chiral difference, L-DNA does not bind to its naturally occurring D-DNA counterpart.

One important aspect of L-DNA is that it is poor at hybridizing to D-DNA (2). This confers multiple uses, one being that the incorporation of L-DNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-DNA oligos with any combination of D-DNA bases including fluorescent dyes and all other available modifications.

#### L-DNA Applications References

- 1) Damha M.J., Giannaris P.A., Marfey P. Antisense L/D-oligonucleotide chimeras: nuclease stability, base-pairing properties, and activity at directing ribonuclease H. *Biochemistry*. 1994;33:7877-7885.
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- 4) Hayashi G., Hagihara M., Nakatani K. Application of L-DNA as a molecular tag. *Nucleic Acids Symp. Ser.* 2005;49:261-262
- 5) Utilizing the bioorthogonal base-pairing system of L-DNA to design ideal DNA nanocarriers for enhanced delivery of nucleic acid cargos Kyoung-Ran Kim, Taemin Lee, Byeong-Su Kim and Dae-Ro Ahn.





## Product Specifications

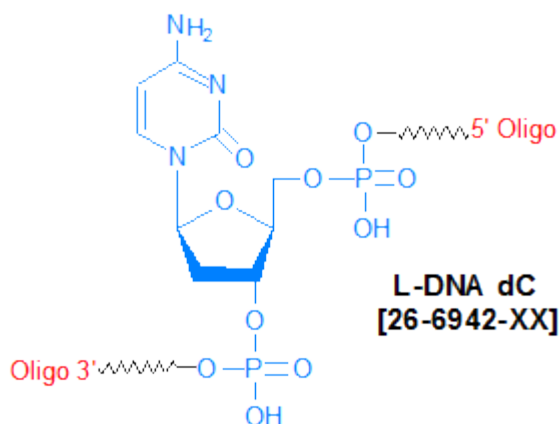
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## Oligo Modifications

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

### L-DNA dC

Category	Others
Modification Code	L-dC
Reference Catalog Number	26-6942
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	289.19



L-DNA (beta stereoisomer deoxyribose that is same as in D-DNA) is the left-turning and mirror image version of natural DNA, as opposed to the naturally occurring right-turning version called D-DNA. L-DNA is more stable than D-DNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-DNA but form a left-helical double-helix. Because of its chiral difference, L-DNA does not bind to its naturally occurring D-DNA counterpart.

One important aspect of L-DNA is that it is poor at hybridizing to D-DNA (2). This confers multiple uses, one being that the incorporation of L-DNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-DNA oligos with any combination of D-DNA bases including fluorescent dyes and all other available modifications.

#### L-DNA Applications References

- 1) Damha M.J., Giannaris P.A., Marfey P. Antisense L/D-oligonucleotide chimeras: nuclease stability, base-pairing properties, and activity at directing ribonuclease H. *Biochemistry*. 1994;33:7877-7885.
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- 4) Hayashi G., Hagihara M., Nakatani K. Application of L-DNA as a molecular tag. *Nucleic Acids Symp. Ser.* 2005;49:261-262
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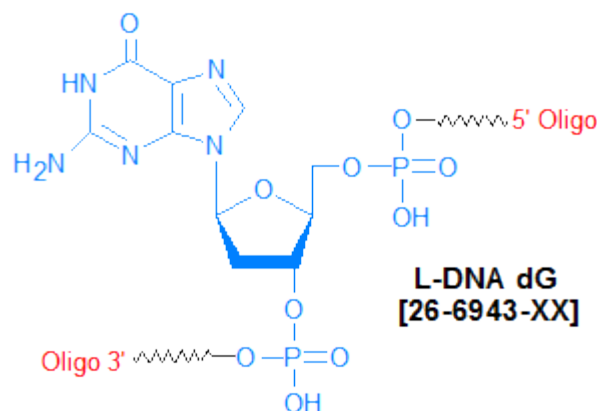
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## Oligo Modifications

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

### L-DNA dG

Category	Others
Modification Code	L-dG
Reference Catalog Number	26-6943
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	329.21



L-DNA (beta stereoisomer deoxyribose that is same as in D-DNA) is the left-turning and mirror image version of natural DNA, as opposed to the naturally occurring right-turning version called D-DNA. L-DNA is more stable than D-DNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-DNA but form a left-helical double-helix. Because of its chiral difference, L-DNA does not bind to its naturally occurring D-DNA counterpart.

One important aspect of L-DNA is that it is poor at hybridizing to D-DNA (2). This confers multiple uses, one being that the incorporation of L-DNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-DNA oligos with any combination of D-DNA bases including fluorescent dyes and all other available modifications.

#### L-DNA Applications References

- 1) Damha M.J., Giannaris P.A., Marfey P. Antisense L/D-oligonucleotide chimeras: nuclease stability, base-pairing properties, and activity at directing ribonuclease H. *Biochemistry*. 1994;33:7877-7885.
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## Product Specifications

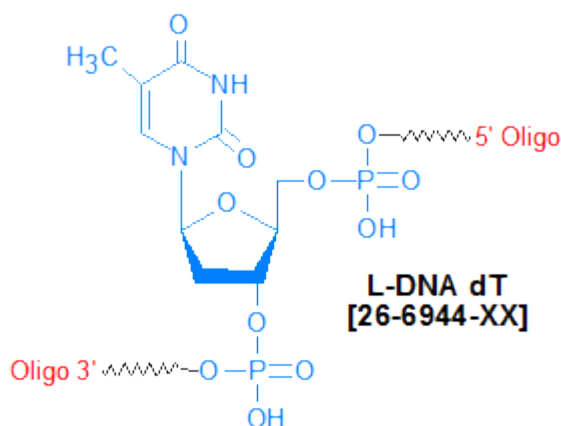
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## Oligo Modifications

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

### L-DNA dT

Category	Others
Modification Code	L-dT
Reference Catalog Number	26-6944
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	304.2



L-DNA (beta stereoisomer deoxyribose that is same as in D-DNA) is the left-turning and mirror image version of natural DNA, as opposed to the naturally occurring right-turning version called D-DNA. L-DNA is more stable than D-DNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-DNA but form a left-helical double-helix. Because of its chiral difference, L-DNA does not bind to its naturally occurring D-DNA counterpart.

One important aspect of L-DNA is that it is poor at hybridizing to D-DNA (2). This confers multiple uses, one being that the incorporation of L-DNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-DNA oligos with any combination of D-DNA bases including fluorescent dyes and all other available modifications.

#### L-DNA Applications References

- 1) Damha M.J., Giannaris P.A., Marfey P. Antisense L/D-oligonucleotide chimeras: nuclease stability, base-pairing properties, and activity at directing ribonuclease H. *Biochemistry*. 1994;33:7877-7885.
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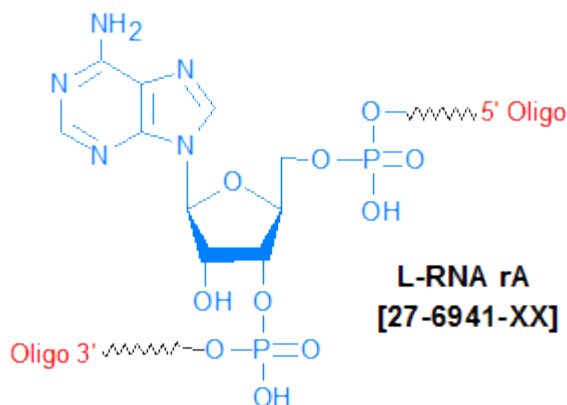
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## Oligo Modifications

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

### L-RNA rA

Category	Others
Modification Code	L-rA
Reference Catalog Number	27-6941
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	329.2



L-RNA (beta stereoisomer ribose that is same as in D-RNA) is the left-turning and mirror image version of natural RNA, as opposed to the naturally occurring right-turning version called D-RNA. L-RNA is more stable than D-RNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-RNA but form a left-helical double-helix. Because of its chiral difference, L-RNA does not bind to its naturally occurring D-RNA counterpart.

One important aspect of L-RNA is that it is poor at hybridizing to D-RNA (2). This confers multiple uses, one being that the incorporation of L-RNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-RNA oligos with any combination of D-RNA bases including fluorescent dyes and all other available modifications and chimeric bases.

#### L-DNA Applications

##### - References

- 1) Damha M.J., Giannaris P.A., Marfey P. Antisense L/D-oligonucleotide chimeras: nuclease stability, base-pairing properties, and activity at directing ribonuclease H. *Biochemistry*. 1994;33:7877-7885.
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## Product Specifications

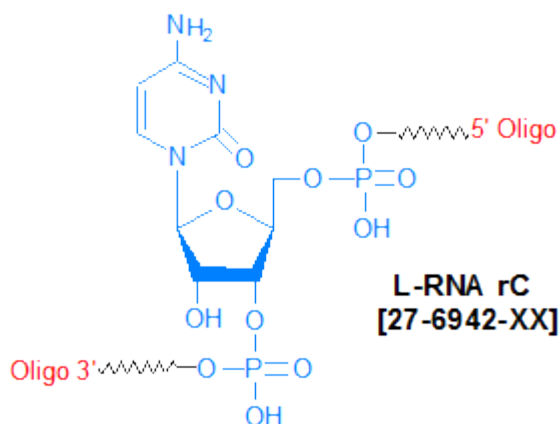
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## Oligo Modifications

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

### L-RNA rC

Category	Others
Modification Code	L-rC
Reference Catalog Number	27-6942
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	305.19



L-RNA (beta stereoisomer ribose that is same as in D-RNA) is the left-turning and mirror image version of natural RNA, as opposed to the naturally occurring right-turning version called D-RNA. L-RNA is more stable than D-RNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-RNA but form a left-helical double-helix. Because of its chiral difference, L-RNA does not bind to its naturally occurring D-RNA counterpart.

One important aspect of L-RNA is that it is poor at hybridizing to D-RNA (2). This confers multiple uses, one being that the incorporation of L-RNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-RNA oligos with any combination of D-RNA bases including fluorescent dyes and all other available modifications and chimeric bases.

#### L-DNA Applications

##### - References

- 1) Damha M.J., Giannaris P.A., Marfey P. Antisense L/D-oligonucleotide chimeras: nuclease stability, base-pairing properties, and activity at directing ribonuclease H. *Biochemistry*. 1994;33:7877-7885.<
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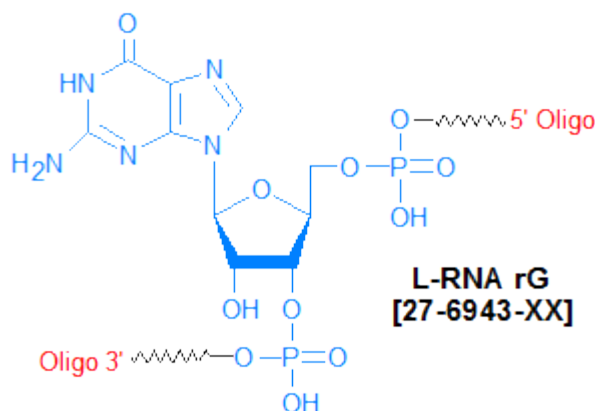
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## Oligo Modifications

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### L-RNA rG

Category	Others
Modification Code	L-rG
Reference Catalog Number	27-6943
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	345.21



L-RNA (beta stereoisomer ribose that is same as in D-RNA) is the left-turning and mirror image version of natural RNA, as opposed to the naturally occurring right-turning version called D-RNA. L-RNA is more stable than D-RNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-RNA but form a left-helical double-helix. Because of its chiral difference, L-RNA does not bind to its naturally occurring D-RNA counterpart.

One important aspect of L-RNA is that it is poor at hybridizing to D-RNA (2). This confers multiple uses, one being that the incorporation of L-RNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-RNA oligos with any combination of D-RNA bases including fluorescent dyes and all other available modifications and chimeric bases.

#### L-DNA Applications

##### - References

- 1) Damha M.J., Giannaris P.A., Marfey P. Antisense L/D-oligonucleotide chimeras: nuclease stability, base-pairing properties, and activity at directing ribonuclease H. *Biochemistry*. 1994;33:7877-7885.<
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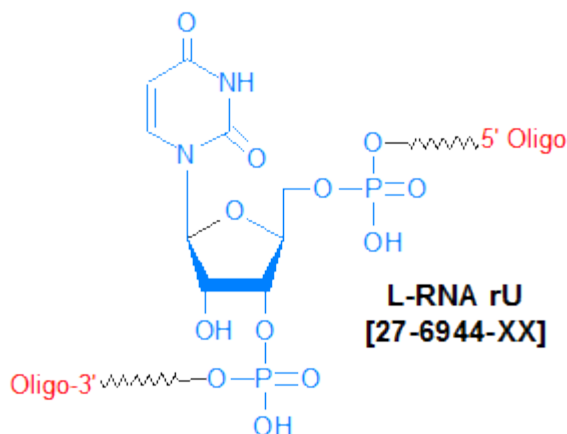
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## Oligo Modifications

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### L-RNA rU

Category	Others
Modification Code	L-rU
Reference Catalog Number	27-6944
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	306.17



L-RNA is the left-turning and mirror image version of natural RNA, as opposed to the naturally occurring right-turning version called D-RNA. L-RNA is more stable than D-RNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-RNA but form a left-helical double-helix. Because of its chiral difference, L-RNA does not bind to its naturally occurring D-RNA counterpart.

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Gene Link synthesizes L-RNA oligos with any combination of D-RNA bases including fluorescent dyes and all other available modifications and chimeric bases.

#### L-DNA Applications

##### - References

- 1) Damha M.J., Giannaris P.A., Marfey P. Antisense L/D-oligonucleotide chimeras: nuclease stability, base-pairing properties, and activity at directing ribonuclease H. *Biochemistry*. 1994;33:7877-7885.
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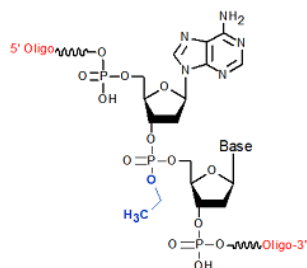
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## Oligo Modifications

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.

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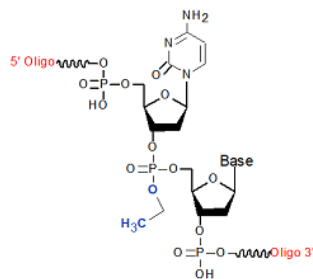
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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

### P-Ethoxy dC

Category	Antisense
Modification Code	EoP-dC
Reference Catalog Number	26-6641C
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	303.21



P-Ethoxy dC / Ethoxy Phosphate dC Oligo  
[26-6641C-XX]

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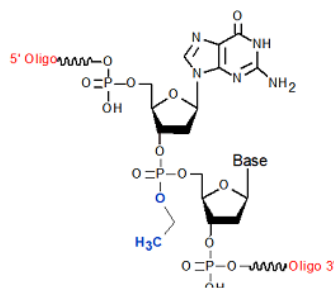
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

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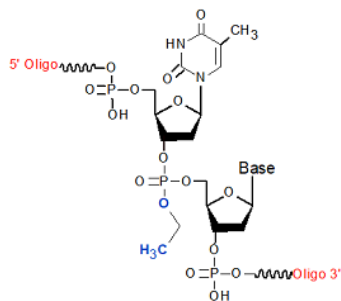
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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### P-Ethoxy dT

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



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

P-Methoxy (Methoxy Phosphate)[MoP] and P-Ethoxy (Ethoxy Phosphate) [EoP] modification has a setup charge of \$250.00 per order for special synthesis reagents.

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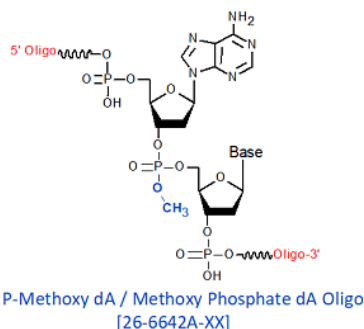
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

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



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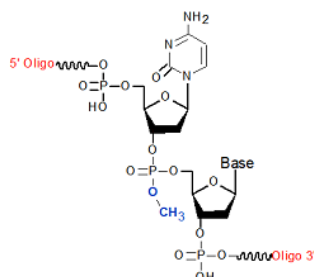
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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### 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 dC / Methoxy Phosphate dC Oligo  
[26-6642C-XX]

P-Methoxy (Methoxy Phosphate)[MoP] and P-Ethoxy (Ethoxy Phosphate) [EoP] modification has a setup charge of \$250.00 per order for special synthesis reagents.

#### P-Methoxy (Methoxy Phosphate)[MoP] and P-Ethoxy (Ethoxy Phosphate) [EoP] modified backbone oligos

P-Methoxy (Methoxy Phosphate), P-Ethoxy (Ethoxy Phosphate) and methyl phosphonate [mp] modified backbone oligos makes the phosphodiester linkage neutral charged. The solubility of the oligo in aqueous solutions slowly decreases with increasing modified linkages; consider incorporating as many standard phosphodiester linkages as well in the oligo. Increasing percentage of DMSO from 0.5 to 10% may be used to solubilize the oligo.

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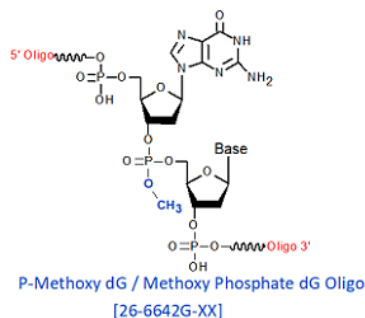
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### P-Methoxy dG

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



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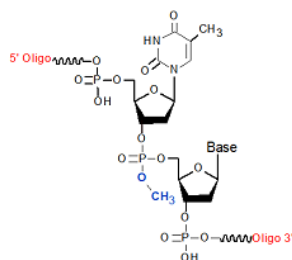
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5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	318.23



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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.
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**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).

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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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  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 Phenylacetylidyde and Sodium Alkoxide with Tosyl and Mesyl Azides. *Jr. J. Org. Chem.*, 23: 1051-1053.
  5. Taber, D.F., Ruckle, R.E. Jr., Hennessy, M.J., (1986) Mesyl Azide: A Superior Reagent for Diazo Transfer. *J. Org. Chem.*, 51:4077-4078

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

Click this link to view these modifications.



# Product Specifications

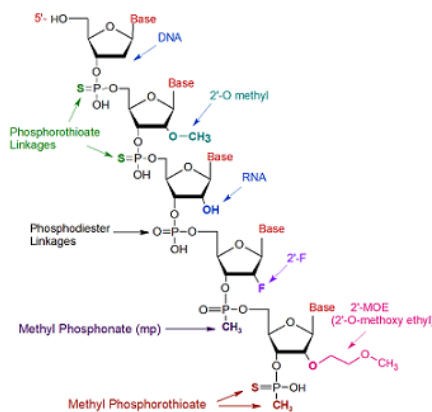
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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

### Phosphorothioate [Ps]

Category	Antisense
Modification Code	Ps
Reference Catalog Number	26-6401
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	16.06



The code for phosphorothioate linkage has been changed to [Ps] from [\*]. Please do not use an asterisk "\*" to denote phosphorothioate linkages. Standard phosphodiester linkages does not require a code. Antisense Oligos (ODN) & siRNA Oligo Modifications

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

**Minimum Pricing** Note that the above pricing for phosphorothioate linkages is for one site only. Minimum charges apply for 15 sites per oligo.

**Phosphorothioate** Phosphorothioate modification is to the backbone linkage modifying the phosphodiester linkage to phosphorothioate. Replacing one non-bridging oxygen atom with sulfur atom in a DNA phosphodiester linkage creates a phosphorothioate [PS] linkage. 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 Chiral Rp and Sp Isomers

Modification of the phosphodiester linkage to phosphorothioate by replacing a non-bridging oxygen atom creates additional stereocenters. Each phosphorothioate substitution can be either an 'Sp' or 'Rp' conformation. As such, a fully phosphorothioate 20 nt antisense oligonucleotide will be a mixture of more than half a million different molecules. Rp and Sp are the two stereoisomers of phosphorothioate [PS] linkages.

genelink.com/newsite/products/images/modificationimages/PO\_PS\_PS2\_Phosphorothioate\_Oligo\_Linkages\_V1.png" align="center">

- Phosphorothioate References**
1. Sazani, P., Kole, R. Therapeutic potential of antisense oligonucleotides as modulators of alternative splicing. (2003) *J. Clin. Invest.*, 112: 481-486.
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  4. Kurreck, J. Antisense technologies. Improvement through novel chemical modifications. (2003) *Eur. J. Biochem.*, 270: 1628-1644.
  5. Croke, S.T. (2004) Progress in antisense technology. *Annu. Rev. Med.*, 55: 61-95.

### Phosphorodithioate Linkages

Gene Link offers phosphorodithioate linkage modified DNA and 2'O methyl oligos. The phosphorodithioate linkage [PS2] are achiral and is a good substitute to obviate the disadvantage of phosphorothioate [PS] linkages that yield stereoisomers. Each phosphorothioate substitution can be either an "Sp" or "Rp" conformation. As such, a fully phosphorothioate 20 nt antisense oligonucleotide will be a mixture of more than half a million different molecules.

To avoid and/or reduce this type of complexity is to use achiral phosphorodithioates instead, where both non-bridged oxygen atoms are replaced with sulfurs. Phosphorodithioates share many of the desirable properties of phosphorothioates without the additional undesired stereocenters. (1,2) In addition, phosphorodithioates are even more stable to nucleases than their phosphorothioate counterparts.

Replacing two non-bridging oxygen atoms with sulfur atoms in a DNA phosphodiester linkage creates a phosphorodithioate (PS2) linkage (1). Like natural DNA, the phosphorodithioate linkage is achiral at phosphorus. This analog is completely resistant to nuclease degradation and forms complexes with DNA and RNA with somewhat reduced stabilities (2). Moreover, it has been found that PS2 oligos bind with a higher affinity than their phosphodiester analogue (2-6) suggesting that PS2 oligos may have additional utility in the form of sulfur-modified phosphate ester aptamers (thioaptamers) (3,6-8) for therapeutic and diagnostic applications.

The phosphorodithioate linkage (PS2) is both achiral and essentially resistant to nucleases. Previous studies have shown very interesting results which include observations that DNA with PS2 linkages activates RNase H in vitro, strongly inhibits human immunodeficiency virus (HIV) reverse transcriptase, induces B-cell proliferation and differentiation, and is completely resistant to hydrolysis by various nucleases. 2'OMe- RNA Thiophosphoramidites are RNA monomers designed to produce oligos combining the PS2 linkage with the 2'O-methyl ribose modification. These PS2-modified RNA oligos have potential for use in siRNAs and dithiophosphate aptamers (thioaptamers).

### Phosphorodithioate References

1. W.S. Marshall, and M.H. Caruthers, *Science*, 1993, 259, 1564-70.
2. X. Yang, and E. Mierzejewski, *New Journal of Chemistry*, 2010, 34, 805-819.
3. W.T. Wiesler, and M.H. Caruthers, *J Org Chem*, 1996, 61, 4272-4281.
4. *The Glen Report*, 2008, 20.1, 4-6.
5. *The Glen Report*, 2015, 27.2, 4-5.
6. N.D. Abeydeera, et al., *Nucleic Acids Res*, 2016, 44, 8052-64.
7. P. Amero, et al., *J Am Chem Soc*, 2021, 143, 7655-7670.
8. K. Bleicher, et al. Oligonucleotides comprising a phosphorodithioate internucleoside linkage. WO2019122279A1, Jun 27, 2019

**Methyl phosphonate (mp)** Methyl phosphonate (mp) modification makes the phosphodiester 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 phosphonated 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<sub>m</sub> of the duplex formed with its RNA target (3), and, most importantly, also interferes with activation of RNase H activity (4), considerable care must be 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). Methylphosphonated 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 methylphosphonated 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.
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[Click this link to view these modifications.](#)



## Product Specifications

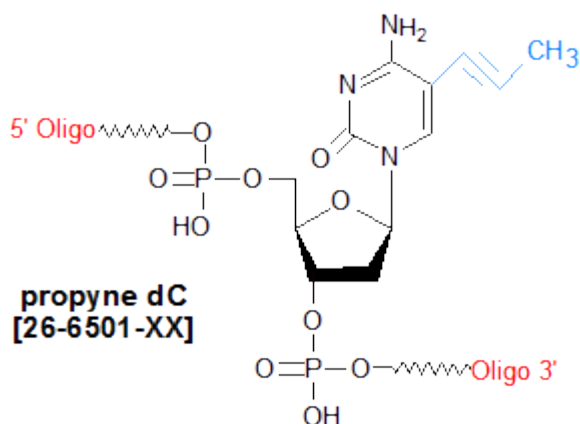
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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

### propyne dC

Category	Antisense
Modification Code	pdC
Reference Catalog Number	26-6501
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	327.23





## 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

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### propyne dU

Category	Antisense
Modification Code	pdU
Reference Catalog Number	26-6502
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	328.22

