



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.

Triple Helix Stability Introduction

Triple-Helix Forming Oligonucleotides (TFO) are a DNA sequence-specific tool that binds to a polypurine:polypyrimidine region of a DNA duplex, resulting in a triple-helix structure at that location. Their sequence-specificity enables them to be used for directing cleaving/cross-linking reagents, transcription factors, nucleases to specific regions of a DNA duplex target (1). They also can be used as tools for altering or controlling gene expression via site-directed mutagenesis or DNA recombination (2), or as part of biochemical assays, e.g. monitoring topoisomerase activity or protein translocation (3,4).

TFOs have several natural limitations associated with them which must be addressed by the researcher in order for TFOs to be effective in in vitro or in vivo contexts. First, TFOs bind in the major groove of the duplex via Hoogsteen or reverse Hoogsteen hydrogen bonds, which are weaker than the Watson-Crick hydrogen bonds between the two strands of duplex itself. In addition, because all three strands of a triple helix are negatively charged under physiological conditions, strand repulsion is significantly and destabilizing. Moreover, in the cell, there are additional concerns: the need for the TFO to be nuclease-resistant, be able to form a triple-helix at physiological pH (7.2), not be locked in a stable secondary structure, and form a triple-helix stable enough to successfully interfere with the targeted biological process working on DNA (1).

Overcoming these limitations involves incorporating different kinds of modifications, depending on the particular TFO application. Modifications can be in the base, the ribose sugar, or the phosphodiester backbone (1).

Triple Helix Stability Design Protocols

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Triple Helix Stability Applications

I. in vitro/In vivo Applications as Gene Modulators

A. Transcription Modulation

TFOs can be used as effective transcription modulators. The binding of a TFO in the major groove of DNA can alter gene expression in several ways: interfering with transcription factor binding (5), interfering with initiation complex formation (6), or arresting transcription elongation (7). In the latter case, the TFO used can be either unmodified or conjugated to psoralen or other DNA-damaging agents. Gene expression can be both downregulated or upregulated (8).

B. Replication Inhibition

Binding of TFOs upstream or downstream of the replication initiation site can induce inhibition of DNA polymerase elongation (9). Triple helices also bind to purine-rich triple-helix DNA binding proteins that are involved in replication. For example, in *S. cerevisiae*, the CDP1 gene codes for a triple-helix DNA binding protein that is involved in chromosome condensation/de-condensation. Condensation is facilitated by triple-helix binding, and de-condensation by triple-helix release (10).

C. Site-Specific Mutagenesis

TFOs coupled to DNA-damaged agents can be used to induce site-specific DNA damage for site-specific mutagenesis and recombination applications. Diverse damaging agents can be coupled to the TFO, as needs require. Examples include psoralen, Fe-EDTA, orthophenantroline, metalloporphyrins.

References

- (1) Duca, M., Vekhoff, P., Oussedik, K., Halby, L., Arimondo, P.B. The triple helix: 50 years later, the outcome. *Nucleic Acids Res.* (2008), 36: 5123-5138. (2) Vasquez, K.M., Narayanan, L., Glazer, P.M. Specific mutations induced by triplex forming oligonucleotides in mice. *Science* (2000), 290: 530-533. (3) Maxwell, A., Burton, N.P., O'Hagen, N. High-throughput assays for DNA gyrase and other topoisomerases. *Nucleic Acids Res.* (2006), 34: e104. (4) Levy, O., Ptacin, J.L., Pease, P.J., Gore, J., Eisen, M.B., Bustamante, C., Cozzarelli, N.R. Identification of oligonucleotide sequences that direct the movement of the *Escherichia coli* FtsK translocase. *Proc. Natl. Acad. Sci. USA* (2005), 102: 17618-17623. (5) Svinarchuk, F., Nagibneva, I., Cherry D., Ait-Si-Ali, S., Pritchard, L.L., Robin, P., Malvy, C., Harel-Bellan, A., Chern, D. Recruitment of transcription factors to the target site by triplex-forming oligonucleotides. *Nucleic Acids Res.* (1997), 25: 3459-3464. (6) Karympalis, V., Kalopita, K., Zarros, A., Carageorgiou, H. Regulation of gene expression via triple helical formations. *Biochemistry* (2004), 69: 855-860. (7) Giovannangeli, C., Helene, C. Triplex-forming molecules for modulation of DNA information processing. *Curr. Opin. Mol. Theor.* (2000), 2: 288-296. (8) Song, J., Intody, Z., Li, M., Wilson, J.H. Activation of gene expression by triplex-directed psoralen crosslinks. *Gene* (2004), 324: 183-190.

Modificaton Code List

Modification	Code	Catalog Number
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
5-methyl deoxycytosine [5mdC]	[5mdC]	26-6413
8-amino-dA	[8-am-dA]	26-6535
8-Amino-dG	[8-Am-dG]	26-6534
deoxyuridine dU	[dU]	26-6408
propyne dU	[pdU]	26-6502
pseudoUridine-2'deoxy (psi-dU)	[psi-dU]	26-6531
6-Thio-dG (S6-dG)	[S6-dG]	26-6533



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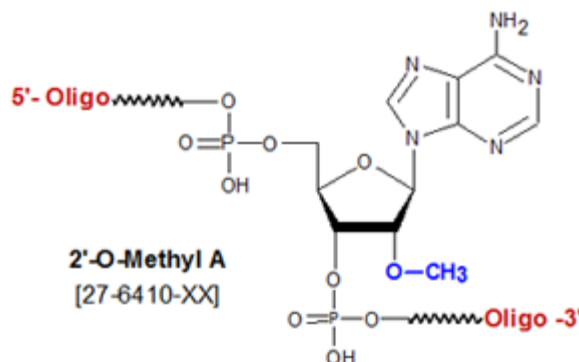
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2'-O methyl A

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



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The hydrogen bonding behavior of a 2'-O-Methyl RNA/RNA base pair is closer to that of an RNA/RNA base pair than a DNA/RNA base pair. Consequently, the presence of 2'-O-Methyl nucleotides **improves duplex stability**. Indeed, incorporation of a 2'-O-Methyl nucleotide into an anti-sense oligo (resulting in a 2'-O-Methyl RNA/DNA chimeric), lead to a **increase** in the T_m of its duplex with RNA, relative to that formed by an unmodified anti-sense DNA oligo, **of 1.3°C per 2'-O-Methyl RNA residue added** (2). Moreover, from a synthesis standpoint, the coupling efficiency of 2'-O-Methyl phosphoramidites are higher than those of RNA monomers, resulting in higher yield of full-length oligos.

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

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ASO's and siRNA Modifications.

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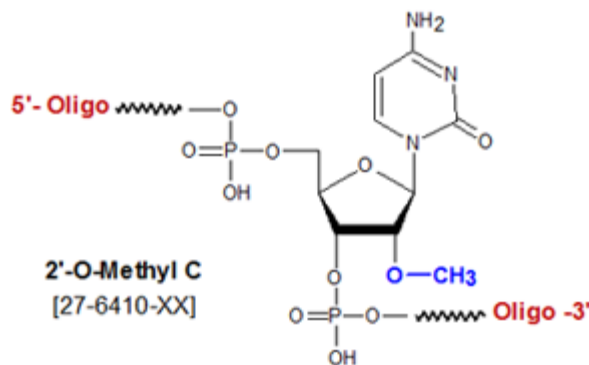
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2'-O methyl C

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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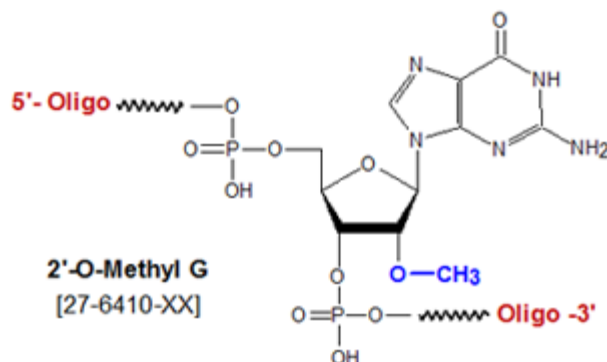
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2'-O methyl G

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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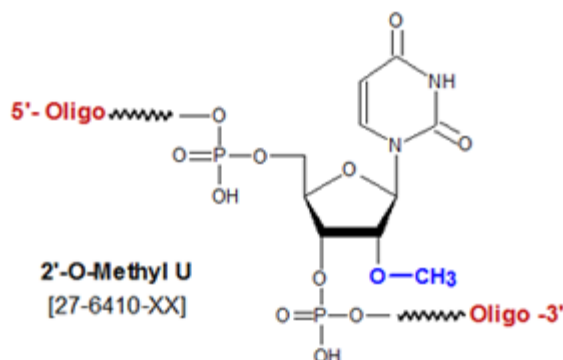
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2'-O methyl U

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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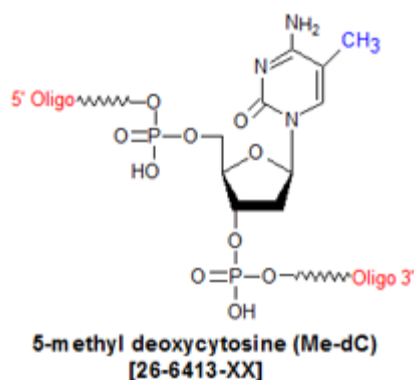
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5-Me dC

Category	Epigenetics
Modification Code	5mdC
Reference Catalog Number	26-6413
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	303.21



5-methyl deoxycytosine (5-Me-dC) pairs with dG, and when substituted for dC in an oligonucleotide, increases the stability of the resulting duplex relative to the comparable unmodified form, raising the T_m by 1.3degC per 5-Me-dC residue added (1,2). 5-Me-dC thus can be used to **improve the ability of an oligo to hybridize to its target**. The presence of the hydrophobic 5-methyl group presumably acts to exclude water molecules from the duplex.
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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

5-Me-dC is particularly useful in the following applications:

(a)Strong-binding PCR primers: 5-Me-dC-modified PCR primers have been shown to prime far better than their unmodified counterparts in PCR reactions, consistently yielding more product per cycle, permitting amplification at very high annealing temperatures (as high as 72degC), and interestingly, allowing excellent priming from within palindromic sequences (1). The improvement in priming efficiency could significantly reduce the number of amplification-related mutations in PCR products.

5-Me-dC primers also could be useful in several PCR applications, *e.g.*, when short, specific primers are required, when only a limited quantity of template is available (*e.g.* ancient DNA), when DNA secondary structure in the primer binding site prevents binding of an unmodified primer, or when primer extension is blocked by downstream DNA secondary structure in the template.

(b) Anti-sense: Anti-sense oligonucleotides containing a CpG motif induce pro-inflammatory responses after *in vivo* administration to animals, including human, via activation of Toll-like receptor 9 (TLR9). Substitution of 5-Me-dC for dC in these motifs can prevent or sharply reduce these undesirable immune responses (3).

(b) DNA methylation studies: Methylation of dC to 5-methyl-dC, when it occurs in CpG sites near promoters is associated with gene silencing, and is an important epigenetic mechanism in living organisms. Oligonucleotides incorporating 5-Me-dC have been used by a number of research groups as research tools to study the epigenetic effects of DNA methylation in such areas as tumorigenesis and the effects of cocaine on fetal heart development (4-6). **References**

1. Lebedev, Y.; Akopyants, N.; Azhikina, T.; Shevchenko, Y.; Potapov, V.; Stecenko, D.; Berg, D.; Sverdlov, E.. Oligonucleotides containing 2-aminoadenine and 5-methylcytosine are more effective as primers for PCR amplification than their nonmodified counterparts. *Genet Anal.* (1996), **13**: 15-21.
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3. Henry, S.P.; Stecker, K.; Brooks, D.; Monteith, D.; Conklin, B.; Bennett, C.F. Chemically modified oligonucleotides exhibit decreased immune stimulation in mice. *J. Pharmacol. Exp. Ther.* (2000), **292**: 468-479.
4. Mancini, D.N.; Singh, S.M.; Archer, T.K.; Rodenhiser, D.I. Site-specific DNA methylation in the neurofibromatosis (NF1) promoter interferes with binding of CREB and SP1 transcription factors. *Oncogene.* (1999), **18**: 4108-4119.
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6. Ishii, T.; Fujishiro, M.; Masuda, M.; Teramoto, S.; Matsuse, T. A methylated oligonucleotide induced methylation of GSTP1 promoter and suppressed its expression in A549 lung adenocarcinoma cells. *Cancer Letters* (2004), **212**: 211-223.



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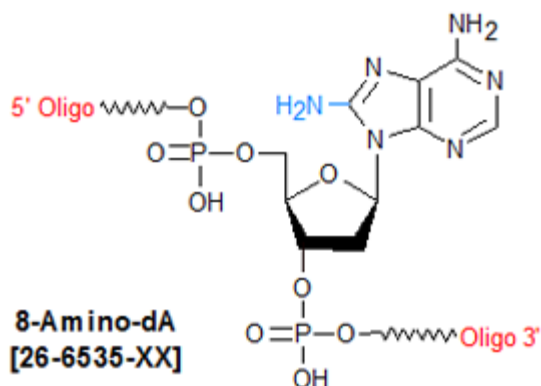
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8-amino-dA

Category	Duplex Stability
Modification Code	8-am-dA
Reference Catalog Number	26-6535
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	328.22



8-Amino-deoxyAdenosine (8-Amino-dA) is an 8-amino-purine that is most commonly used to study the structural and functional properties of triple helices. The 8-amino group is able to form an additional Hoogsteen purine-pyrimidine hydrogen bond, which serves to increase triple helix stability (1). Moreover, it has been shown that triple-helix-forming oligos (TFO) containing 8-amino-dA form stable helices at neutral pH, instead of under acidic conditions (2). This property may be relevant for design of TFOs slated for in vivo work, which generally occurs under physiological pH (7.3-7.4) conditions. **References**
 1. Cubero, E., Avino, A., de la Torre, B.G., Frieden, M., Eritja, R., Luque, F.J., Gonzalez, C., Orozco, M. Hoogsteen-based parallel-stranded duplexes of DNA. The effect of 8-amino derivatives. *J. Am. Chem. Soc.* (2002), **124**: 3133-3142.
 2. Zhu, Q., Delaney, M.O., Greenberg, M.M. Observation and elimination of N-acetylation of oligonucleotides prepared using fast-protecting phosphoramidites and ultra-mild deprotection. *Bioorg. Med. Chem. Lett.* (2001), **11**: 11105-1107.



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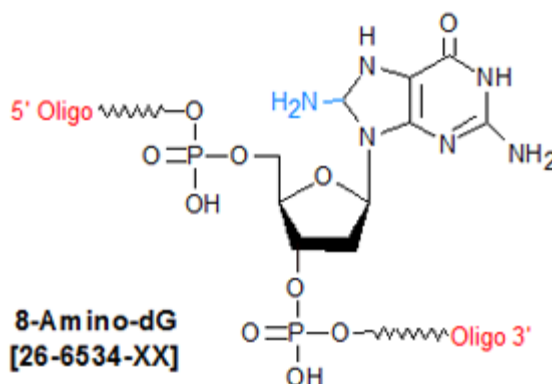
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8-Amino-dG

Category	Structural Studies
Modification Code	8-Am-dG
Reference Catalog Number	26-6534
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	344.22



8-Amino-deoxyGuanosine (8-Amino-dG) is an 8-amino-purine that is most commonly used to study the structural and functional properties of triple helices. The 8-amino group is able to form an additional Hoogsteen purine-pyrimidine hydrogen bond, which serves to increase triple helix stability (1). 8-Amino-dG-modified oligonucleotides are also used in oxidative DNA damage/repair studies. The industrial solvent 2-nitropropane generates 8-Amino-dG as a DNA lesion, which in this initial study appears to be weakly mutagenic (2). **References**

1. Cubero, E., Avino, A., de la Torre, B.G., Frieden, M., Eritja, R., Luque, F.J., Gonzalez, C., Orozco, M. Hoogsteen-based parallel-stranded duplexes of DNA. The effect of 8-amino derivatives. *J. Am. Chem. Soc.* (2002), **124**: 3133-3142.
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Product Specifications

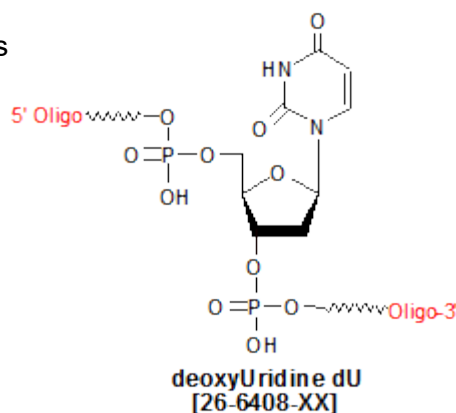
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

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

dU

Category	DNA Oligo Synthesis
Modification Code	dU
Reference Catalog Number	26-6408
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	290.17



Deoxyuridine (dU) is a pyrimidine deoxyribonucleoside, and a derivative of the nucleoside uridine, with the only difference being that, in dU, a hydrogen (-H) group is substituted for uridine's -OH group located at the 2'-position of the ribose. dU is generated in cellular DNA as a deamination product of dC (deoxycytidine), with the deamination process catalyzed by the enzyme AID (activation-induced cytidine deaminase) (1). AID is a B cell-specific gene that is necessary for antibody gene diversification via class-switch recombination and somatic hypermutation (2, 3). The dC-to-dU conversion(s) by AID occurs in the IgG locus, with various gene diversification pathways arising from the different DNA repair mechanisms used by B-cells to repair the dU lesion (1).

dC-to-dU conversion via cytidine deamination is also implicated in innate immunity to retroviruses. Here deamination of dC is mediated by the enzyme APOBEC3G, which is present in T cells, acting on the first (minus) strand cDNA of retroviruses. Generation of dU produces a dU /dG mismatch in the retroviral cDNA duplex, resulting in a dC-to-dT transition mutation on the minus-strand cDNA, and a dG-to-dA transition on the plus-strand (4). The presence of dU in the minus-strand cDNA could lead to innate immunity by one or more of the following: (a) hypermutation capable of disabling viral functions, (b) degradation by BER (base excision repair), (c) plus-strand cDNA mis-replication (5). dU can be used to modify oligos for use in studies of DNA damage and associated repair mechanisms.

Oligos modified with dU can serve as effective research tools for mechanistic studies of both adaptive and innate immunity in animal systems. 1. Neuberger, M.S., Harris, R.S., Di Noia, J., Petersen-Mahrt, S.K. Immunity through DNA deamination. *Trends Biochem. Sci.* (2003), **28**: 305-312.

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Product Specifications

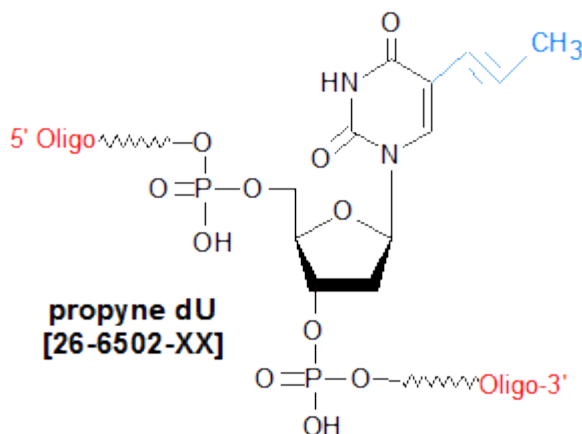
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

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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



It has been shown that C-5 propyne analogs of dC and dT when substituted in phosphorothioate oligonucleotide imparts greater inhibition of gene expression due to increased binding affinity to the target mRNA and increased stability. Based on the above information antisense oligonucleotide could either be Phosphorothioated at all diester linkages or combined with substitutions of dC and dT by C-5 propyne analogs pdC and pdU.
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 The use of propyne analogs is covered by patents and licensing agreements. The sale of propyne-modified oligos is for research use only. See license agreement*



Product Specifications

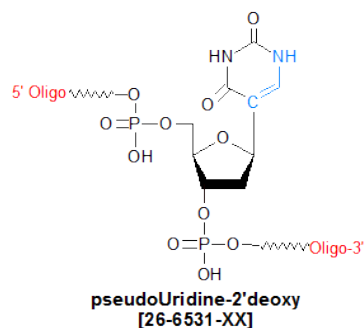
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

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pseudoU-2'deoxy

Category	Minor Bases
Modification Code	psi-dU
Reference Catalog Number	26-6531
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	290.17



Pseudouridine-2'-deoxy ("psi") is a C-glycoside isomer of uridine, and is the most common modified nucleoside found in structural RNA, such as tRNA, rRNA, snRNA, and snoRNA (1,2). Psi-modified RNA can be used as research tools for studies into the roles of this residue in RNA structure and function in the cell. Currently, the role of psi in RNA is a subject of active research, with some things now known. Psi can coordinate a water molecule through its free N1 hydrogen, thereby inducing a modest increase in rigidity on the nearby sugar-phosphate backbone. The presence of psi also enhances base-stacking. Such effects have been proposed as explanations for the deleterious functional effects observed in mutant bacterial strains that lack certain psi residues in tRNA or rRNA (2). Also, based on recent studies, it has been proposed that psi may offer RNA molecules protection from radiation (3). **References**

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Product Specifications

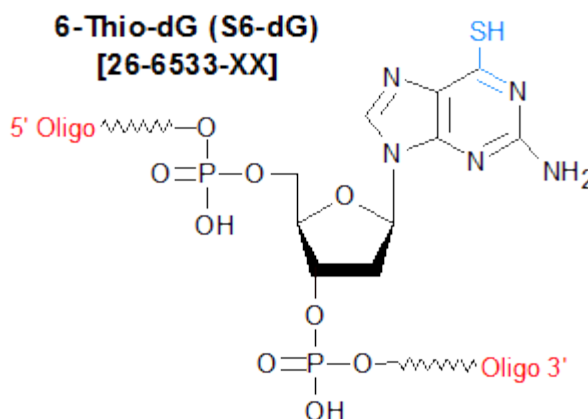
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Oligo Modifications

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Thio 6-dG (s6dG)

Category	Structural Studies
Modification Code	S6-dG
Reference Catalog Number	26-6533
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	345.26



6-Thio-dG can also be used to study the properties of G-rich triple-helix forming oligonucleotides. For example, substitution of 6-Thio-dG for some or all dGs in such oligos results in inhibition of both oligo self-association and G-quartet formation, thereby favoring normal formation of triple helices (3).

In addition, because the thiol group of 6-Thio-dG is active, incorporation of this modified nucleoside into an oligo also incorporates a reactive thiol at that position, which can be utilized to selectively alkylate the sulfur at that position (4).

References

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