Oligo Modifications

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

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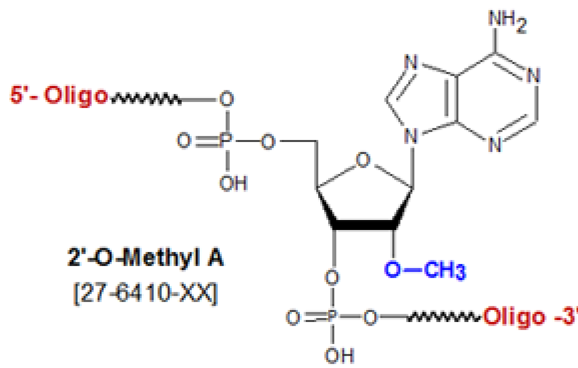
Modificaton Code List

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

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 Tm of its duplex with RNA, relative to that formed by an unmodified anti-sense DNA oligo, **of 1.3^{o}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 [Tm 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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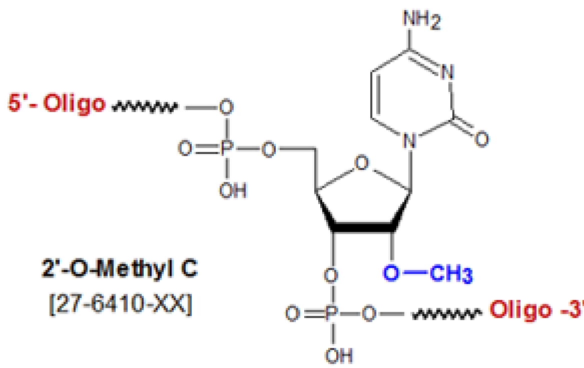
2'-O methyl C

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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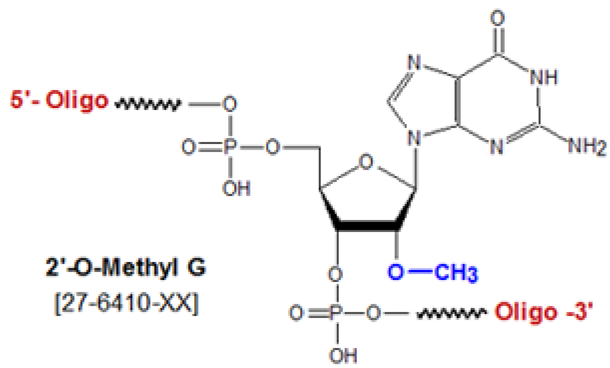
2'-O methyl G

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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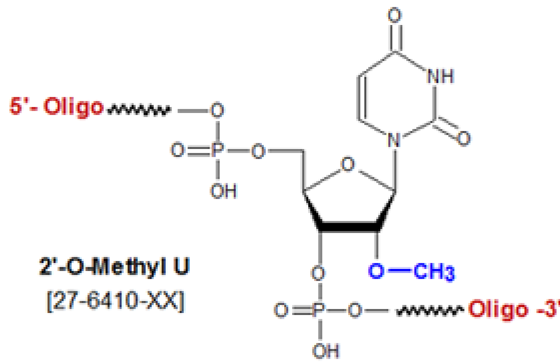
2'-O methyl U

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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5-Me dC

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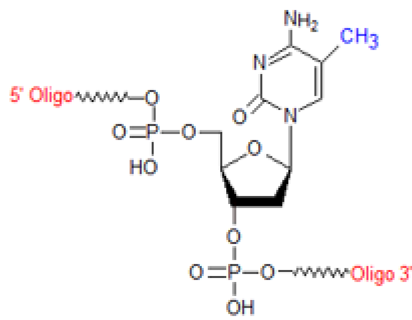
3 Prime	Y
Internal	Y
Molecular Weight(mw)	303.21



5-methyl deoxycytosine (Me-dC)
[26-6413-XX]

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 Tm 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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Duplex Stability [Tm Increase]

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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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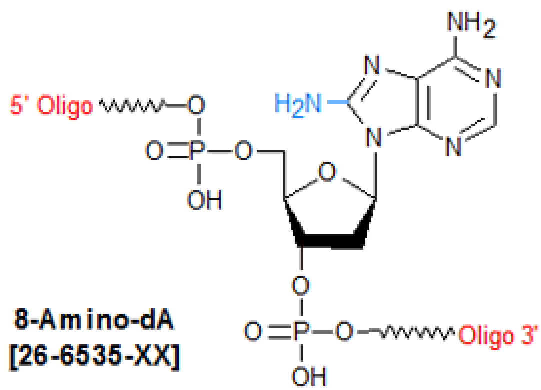
8-amino-dA

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-dA
[26-6535-XX]

8-Amino-dG

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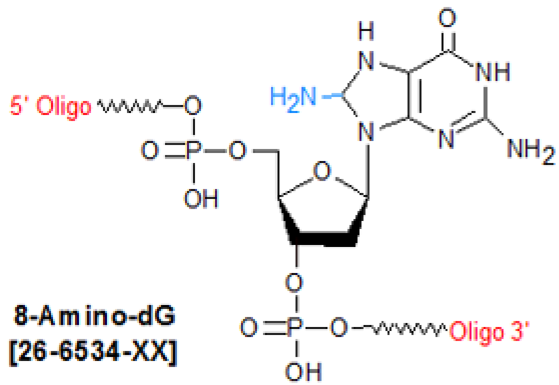
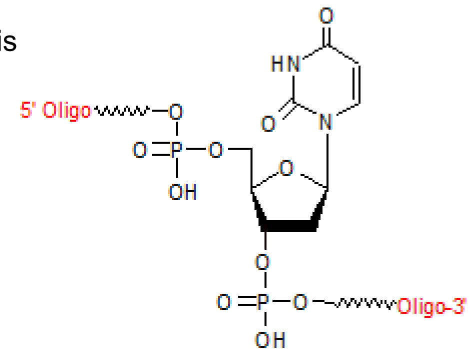
3 Prime	Y
Internal	Y
Molecular Weight(mw)	344.22



8-Amino-dG
[26-6534-XX]

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
[26-6408-XX]

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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propyne dU

Category	Antisense
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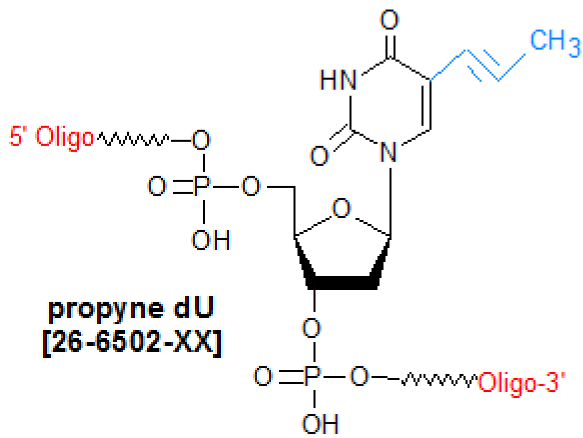
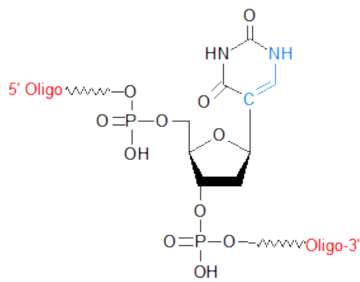
3 Prime	Y
Internal	Y
Molecular Weight(mw)	328.22



pseudoU-2'deoxy

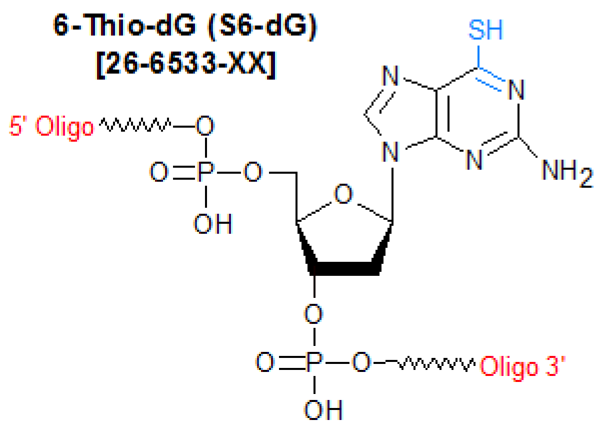
Category	Minor Bases
Modification Code	psi-dU
Reference Catalog Number	26-6531
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	290.17



pseudoUridine-2'deoxy
[26-6531-XX]

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-deoxyGuanosine (6-Thio-dG) is a nucleoside that, when incorporated into either DNA or RNA in the cell, exhibits potent cytotoxicity. Such cytotoxicity is most likely due to the 6-Thio-dG either inducing strand breakage or cross-linking to both DNA and proteins (1). The cytotoxic properties of 6-Thio-dG make it an effective cytotoxic agent for treating human leukemias. Its ability to photochemically cross-link to both nucleic acids and proteins also make 6-Thio-dG-modified oligonucleotides desirable reagents for use in studying binding interactions between DNA and DNA-binding proteins. In one study, 6-Thio-dG was shown to efficiently cross-link with EcoRV endonuclease and methyltransferase (2). Cross-linking was achieved with 340 nm UV light; because this wavelength is considerably removed from the UV absorbance maxima of the natural bases (260 nm), cross-linking can be achieved without additional UV damage to the DNA.

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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