



## 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.

### Duplex Stability Introduction

Many molecular biology applications require oligonucleotide primers and/or probes with enhanced duplex stability, that is, a higher affinity for their complementary sequences. Examples include antisense, siRNA, and SNP detection experiments. For these and other applications, enhancing primer/probe-target duplex stability increases both the selectivity and sensitivity of the oligo for its target. An oligo's binding affinity for its target can be manipulated by incorporating various modifications into it, either on the ends or at an internal position (1). Examples include 2'-OMethyl base, 2-Amino-dA, 5-methyl-dC and 2'-Fluoro bases. Changes in duplex stability incurring from such modifications is indicated by changes in the melting temperature ( $T_m$ ) of the duplex formed between the modified oligo and its target over that formed by the unmodified oligo. Depending on the modification,  $T_m$  can be increased over a range of 0.5C to 3.0C per base substitution.

# Duplex Stability Design Protocols

## Modulating Oligonucleotide Duplex Stability--Design Considerations.

Specific and stable hybridization of an oligo to its complementary sequence is the desired outcome of a successful hybridization protocol. The melting temperature ( $T_m$ ) of an oligo indicates the strength of the affinity, and thus the stability, of the hybridization. Manipulation of the oligo sequence to increase its duplex stability or, in some cases, to decrease the duplex stability in certain loop structures will lead to oligos with increased affinity for the target molecule.

Duplex stability can be modulation by incorporation of modifications that are altered on the nucleotide base (e.g., 5-methyl-dC) or sugar (e.g., 2'-F or 2'-O-Methyl RNA bases). In addition, the phosphodiester backbone also can be altered (e.g., by phosphorothiolation).

For oligonucleotides having the same base sequences, duplex stability (and  $T_m$ ) proceeds in the following order:

DNA:DNA < DNA:RNA < RNA:RNA < RNA:2'-OMethyl RNA

For a DNA:DNA duplex, the sugars are 2'-endo, resulting in a B-form duplex. By contrast, in RNA:RNA duplexes, the sugars are 3'-endo, and the resulting duplex is A-form. The latter is more stable than the former. DNA:RNA duplexes have an intermediate sugar conformation, and thus a stability between those of DNA:DNA and RNA:RNA, with the specifics dependent on the composition and ratio of deoxyribo- to ribonucleotides (5).

2'-O-methyl and 2'-fluoro RNA bases are common duplex-stabilizing modifications that serve to modify the sugars of an oligo. 2'-O-methyl RNA bases are particularly useful in anti-sense applications, where their stabilizing effect can counteract the de-stabilizing effect of phosphorothiolation, which is used to confer nuclease resistance onto an oligo. Incorporating combinations of 2'-O-methyl and 2'-fluoro RNA bases is often a cost-effective way to significantly increase duplex stability in lieu of using highly expensive modifications.

5-methyl-dC, 2-amino-dA, and C5-pyrimidines are commonly used duplex stabilizing moieties that are modified on the nucleotide base.

Phosphorothiolation and methylphosphonate linkages are two ways of modifying the backbone of an oligonucleotide that affect the stability of a duplex. Unlike the other modifications however, these two, when incorporated into an oligo, actually lower the  $T_m$  of the corresponding duplex, and thus are de-stabilizing, with the methylphosphonate being the more de-stabilizing of the two. These two modifications can potentially be used in conjunction with other modifications to "fine-tune" the  $T_m$  of a duplex formed by a probe and its target (6,7). More commonly, phosphorothiolate and methylphosphonate linkages are used to confer nuclease resistance to an oligo slated for in vivo studies (e.g., anti-sense work). The duplex de-stabilizing effect of these modifications must be taken into account when designing such oligos.

## Duplex Stability Applications

For antisense and siRNA work, incorporation of modifications that enhance duplex stability may be needed to counteract the destabilizing effect of phosphorothiolation (which confers nuclease resistance on an oligo). In addition, increasing the duplex stability can augment the ability of an oligo to suppress gene expression, as a highly stable DNA-RNA duplex can effectively block translation via steric hindrance (2). Both C5-propyne base analogs and 2'-O-substituted bases (for example, 2'-OMethyl RNA bases) have been used for these purposes. For SNP detection assays, incorporation of duplex-stability-enhancing modifications into SNP detection probes lead to improvements in mis-match discrimination. 2'-fluoro RNA bases, for example, increase duplex  $T_m$  by 1.8C per modified residue (3), and thus can provide an excellent low-cost substitute for LNA bases in SNP detection assays. 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 (4).

## References

- (1) Swayze, E.E., Balkrishen, B. The Medical Chemistry of Oligonucleotides. In: Antisense drug technology: principles, strategies, and applications, 2nd Ed., Crooke, S.T. (Ed), CRC Press, Boca Raton (FL), 2008, 143-182.
- (2) Kurreck, J. Antisense technologies. Improvement through novel chemical modifications. *Eur. J. Biochem.* (2003), 270: 1628-1644.
- (3) Schulz, R.G., Gryaznov, S.M. Oligo-2'-fluoro-2'-deoxynucleotide N3'–P5'phosphoramidites: synthesis and properties. *Nucleic Acids Res.* (1996), 24: 2966-2973.
- (4) 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.
- (5) Saenger, W. in *Principles of Nucleic Acid Structure*, C.R. Cantor, Ed., Springer Advanced Texts in Chemistry, Springer-Verlag, New York, 1984.
- (6) Tidd, David M. Specificity of antisense oligonucleotides. in *Perspectives in Drug Discovery and Design*, Vol. 4, ESCOM Science Publishers, B.V., 1996, pp. 51-60.
- (7) Takagi-Sato, M., Tokuhiro, S., Kawaida, R., Koizumi, M. Fine-Tuning of ENA Gapmers as Antisense Oligonucleotides for Sequence-Specific Inhibition. *Oligonucleotides* (2007), 17: 291-301.

## 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
2-Amino dA	[2Am-dA]	26-6525
5-Carboxy dC	[5-ca-dC]	26-6891
5-methyl deoxycytosine [5mdC]	[5mdC]	26-6413
Anthraquinone-C2-dT	[AQ-dT]	26-6613
AP-dC G-Clamp (Aminoethyl-Phenoxazine-dC (AP-dC))	[AP-dC]	26-6920
d5SICS	[d5SICS]	26-6562
dDs (7-(2-thienyl)-imidazo[4,5-b]pyridine)	[dDs]	26-6565
dDss	[dDss]	26-6566
dNaM	[dNaM]	26-6561
dPa pyrrole-2-carbaldehyde (Pa)	[dPa]	26-6564
Locked Analog A (LNA-A)	[+A]	26-6517
Locked Analog G (LNA-G)	[+G]	26-6518

Locked Analog mC (LNA-mC)	[+mC]	26-6519
Locked Analog T (LNA-T)	[+T]	26-6520
MGB 3' CDPI3	[MGB-CDPI3-3]	26-6456
MGB-5' CDPI3	[MGB-CDPI3-5]	26-6457
N4-Ethyl dC [N4-Et-dC]	[N4-Et-dC]	26-6685
N6-Methyl dA (m6dA)	[m6dA]	26-6601
Nebularine deoxy	[dNeb]	26-6508
propyne dC	[pdC]	26-6501
propyne dU	[pdU]	26-6502
5'-Pyrene Cap (5')	[Pyr-Cap]	26-6949
Spermine Oligo Cationic Tail (ZNA-Oligos)	[Spm]	26-6454
Stilbene Cap: Trimethoxystilbene Cap (5')	[Stb-Cap]	26-6989
2-Thio-dT (S2dT)	[S2dT]	26-6537
Uaq Cap (3')	[Uaq-Cap]	26-6950



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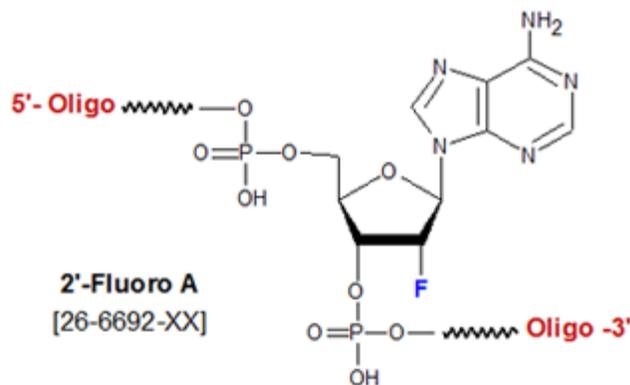
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### 2'-F-A

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.**

2'-Fluoro-deoxyadenosine (2'-F-A) is classified as a 2'-Fluoro RNA monomer. The substitution of a highly electronegative fluorine atom for the 2'-OH group of the ribose results in the ring of a 2'-F-ribonucleoside having a C3'-endo (i.e., RNA-type) conformation. Consequently, an RNA oligonucleotide containing a 2'-F RNA monomer adopts the more thermodynamically stable A-form helix on hybridization to a target (1).

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.

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(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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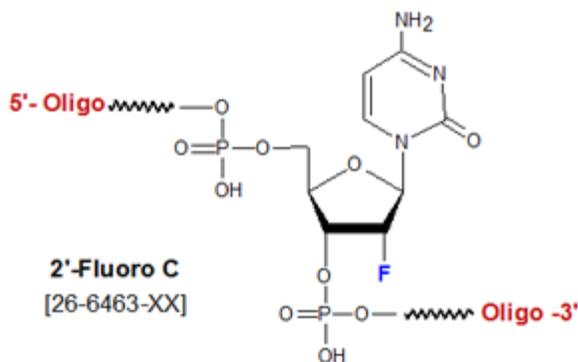
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### 2'-F-C

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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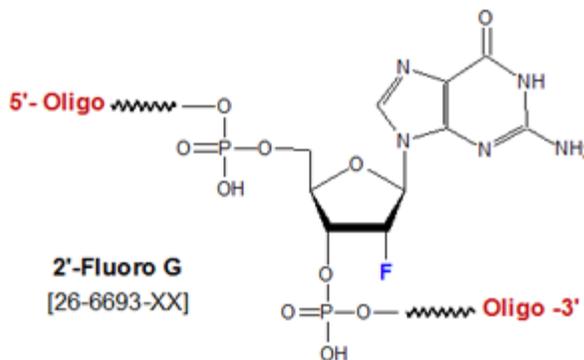
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### 2'-F-G

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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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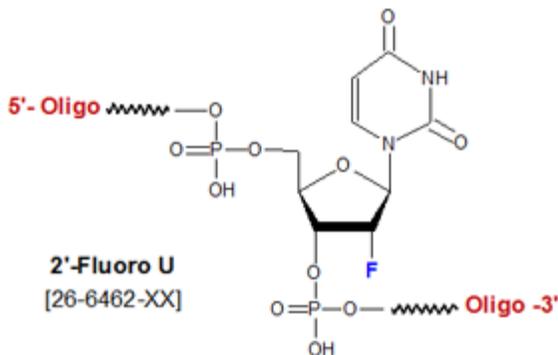
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5 Prime	Y
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Internal	Y
Molecular Weight(mw)	308.16



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(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:**

1. Kawasaki, A.M., et al., Uniformly modified 2'-deoxy-2'-fluoro phosphorothioate oligonucleotides as nuclease resistant antisense compounds with high affinity and specificity for RNA targets, *Journal of Medicinal Chemistry* (1993), **36**: 831-841.
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.
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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

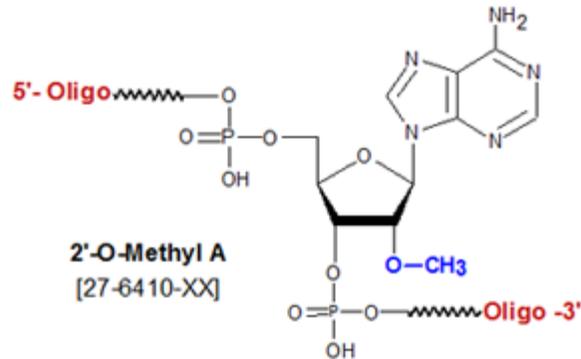
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## Oligo Modifications

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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



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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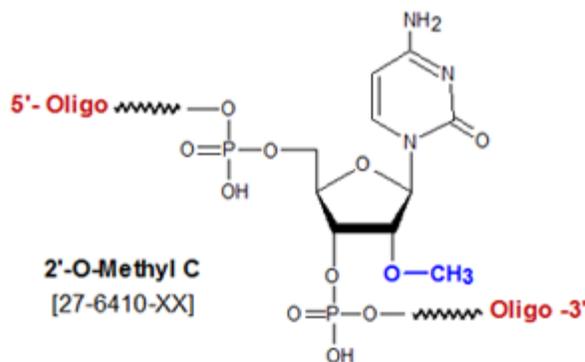
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## Oligo Modifications

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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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Modifications Increasing Duplex Stability and Nuclease Resistance

Modification

Duplex Stability [ $T_m$  Increase]

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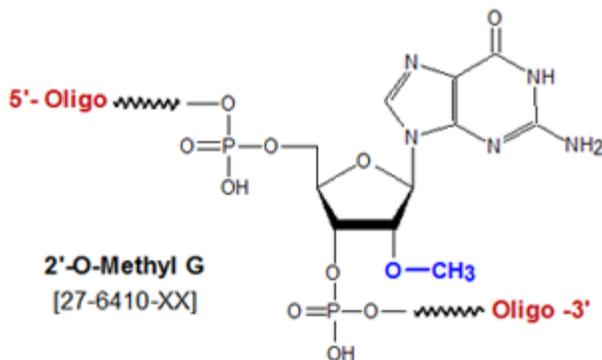
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## Oligo Modifications

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

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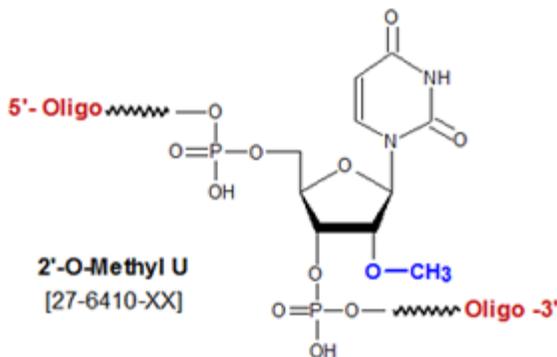
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## Oligo Modifications

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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

Modification

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

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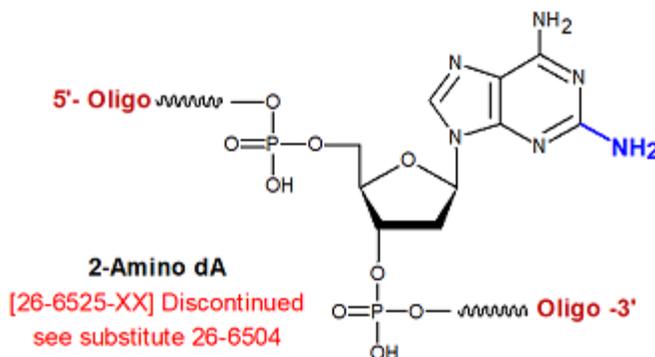
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## Oligo Modifications

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### 2-Amino dA

Category	Duplex Stability
Modification Code	2Am-dA
Reference Catalog Number	26-6525
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	328.22



2-Amino dA, Catalog#: 26-6525 has been discontinued effective June 25, 2025 and replaced by a more stable identical final modification of 2-6-diaminopurine (2-Amino dA) Catalog#: 26-6504. The original 2-Amino dA catalog number 26-6525 precursor base was labile and required mild synthesis conditions with low yield. The substitute is more stable.

#### 2-6-diaminopurine (2-Amino dA) Catalog: 26-6504-XX.

2-Amino-deoxyadenosine dA (also known as 2-6 diamino purine) can be used to **improve the ability of an oligo to hybridize to its target**. The 2-Amino-A nucleotide base forms three hydrogen bonds with thymine (T), compared with only two H-bonds between unmodified A and T. 2-Amino A:T base pairs thus have the same number of H-bonds as G:C base pairs do. Consequently, when a 2-Amino-dA oligo binds to its unmodified target, the T<sub>m</sub> of the duplex **is raised by 3oC per 2-Amino-dA residue added**, compared with the unmodified case (1). In addition, 2-Amino-A also destabilizes A-G wobble mismatches, presumably due to a steric clash between the 2-amino on A and the 2-amino on G. Thus 2-Amino-dA modified oligos show better specificity for a target than their unmodified counterparts.

Modifications Increasing Duplex Stability and Nuclease Resistance

Modification

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

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2-Amino dA has been shown to be particularly useful in the following oligo-based applications:

(a)Strong-binding PCR primers: 2-Amino-dA-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 72°C), 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. 2-Amino-dA 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)Selective Binding Complementary (SBC) Oligos: SBC oligos are complementary pairs of oligos that contain one or more modified base pairs (that is, each member of the pair is modified). Each individual modified base does not form a stable base pair with its modified partner, but does form a particularly stable base pair with its natural (unmodified) counterpart. Thus, two complementary SBC oligos do not form a stable duplex with each other, but each individual SBC oligo does form a very stable duplex with an unmodified complementary target. This property enables an SBC duplex to effectively bind with both the sense and anti-sense strands of a DNA or RNA duplex target. SBC oligos are useful as probes for investigation of branching secondary structures, and as anti-sense reagents against mRNA that has significant secondary structure.

An excellent pair of SBC oligos can be made by substituting 2-Amino-dA for A, and 2-Thio-dT for T. Because 2-Amino-dA only forms one hydrogen bond with 2-Thio-dT, these modified base pairs are very weak, and the corresponding duplex is unstable. However, both 2-Amino-dA and 2-Thio-dT bind effectively with T and A bases, respectively. In a classic study, SBC 20mers annealed against a DNA 20mer target exhibited  $T_m$  values 10° higher than the corresponding DNA-DNA hybrid, whereas the SBC-SBC hybrid yielded  $T_m$  values 30° lower (2). **References**

Lebedev, Y., *et al.* Oligonucleotides containing 2-aminoadenine and 5-methylcytosine are more effective as primers for PCR amplification than their unmodified counterparts. *Genetic Analysis: Biomolecular Engineering* (1996), **13**: 15-21.

Kutyavin, I.V.; Rhinehart, R.L.; Lukhtanov, E.A.; Gorn, V.V.; Meyer, R.B.; Gamper, H.B. *Oligonucleotides Containing 2-Aminoadenine and 2-Thiothymine Act as Selectively Binding Complementary Agents*. *Biochemistry* (1996), **35**: 11170-11176.



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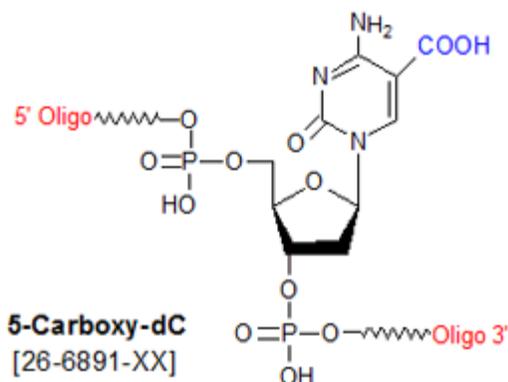
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### 5-ca dC

Category	Epigenetics
Modification Code	5-ca-dC
Reference Catalog Number	26-6891
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	333.19



5-carboxy deoxycytosine (5-carboxy-dC) was first synthesized in 2007 by Sumino et al (1) as an anion carrier that, when incorporated into oligonucleotides, would result in increased duplex and triplex stabilities. Initial studies with 5-carboxy-dC modified oligos 13mer and 14mer in length showed that this modified base does indeed increase duplex stability over the corresponding unmodified oligos, but forms relatively unstable triplexes with their target dsDNA (1). 5-carboxy-dC thus potentially could serve as a duplex stabilizing moiety for oligonucleotides slated for any application requiring formation of duplexes with higher  $T_m$ .

However, current research interest in 5-carboxy-dC is focused on its potential role as an intermediate in a putative (active) oxidative demethylation pathway for conversion of 5-Me-dC to dC. Demethylation of 5-Me-dC is necessary for epigenetic control of gene expression in the cell, and plays a key role in cellular reprogramming, embryogenesis, establishment of maternal and paternal methylation patterns in the genome (2), and also in certain autoimmune disorders and cancer (3). Recent observations of the presence of 5-hydroxymethyl-dC (5hm-dC) in a variety of tissues, most notably neuronal cells (4,5), and the discovery of an enzymatic pathway for conversion of 5-Me-dC to 5hm-dC, mediated by the enzyme Tet1 (6), has spurred efforts to determine whether or not 5-hm-dC is then subsequently converted to dC through a 5-carboxy-dC intermediate. In 2011, Ito and co-workers showed that Tet enzymes are able to convert 5hm-dC to 5-carboxyl-dC, and also observed the presence of 5-carboxy-dC in mouse embryonic stem cells and various mouse organ tissues. Genomic content of both 5hm-dC and 5-carboxy-dC can be modulated through overexpression or depletion of Tet proteins in these tissues (7). These experiments provide strong supporting evidence for DNA demethylation occurring via a Tet-mediated enzymatic pathway involving 5-carboxy-dC as a key intermediate. 5-carboxy-dC modified oligos can serve as important research tools for probing the DNA demethylation process.

1. Sumino, M., Ohkubo, A., Taguchi, H., Seio, K., Sekine, M. Synthesis and properties of oligodeoxynucleotides containing 5-carboxy-2'-deoxycytidines. *Bioorg. Med. Chem. Lett.* (2008), **18**: 274-277.

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# Product Specifications

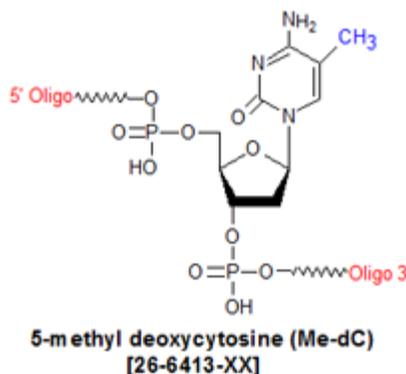
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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

### 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.  
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](#)

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**

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## Product Specifications

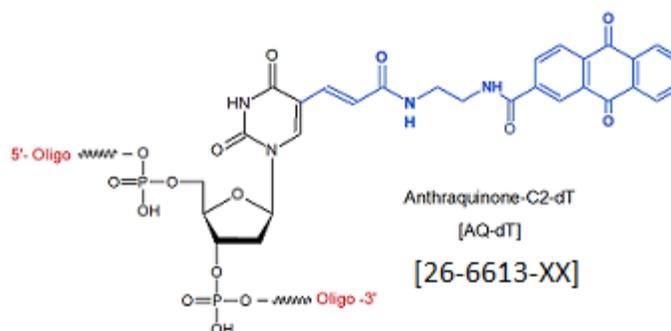
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## Oligo Modifications

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

### Anthraquinone-C2-dT

Category	Redox Electrochemical
Modification Code	AQ-dT
Reference Catalog Number	26-6613
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	1077





## Product Specifications

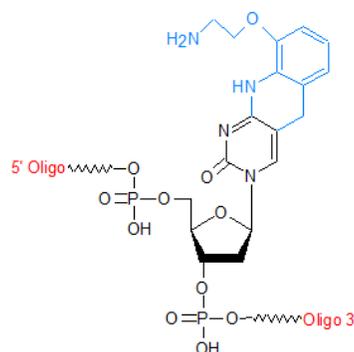
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## Oligo Modifications

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

### AP-dC

Category	Duplex Stability
Modification Code	AP-dC
Reference Catalog Number	26-6920
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	438.33



AP-dC G-Clamp (Aminoethyl-Phenoxazine-dC (AP-dC))  
[26-6920-XX]

Aminoethyl-Phenoxazine-deoxycytosine (AP-dC), sometimes referred to as “G-clamp”, pairs with dG, and when substituted for dC in an oligonucleotide, is able to form both Watson-Crick and Hoogsteen hydrogen bonds with the guanine base. A total of four hydrogen bonds form between AP-dC and dG: the usual three Watson-Crick hydrogen bonds and a Hoogsteen hydrogen bond between the protonated amine of AP-dC’s aminoethyl side-chain and the O6 position of the dG. As a result, an AP-dC:dG base pair significantly increases the stability of the resulting duplex relative to the comparable unmodified form. The increase in stability can be quite dramatic; in one study, a single incorporation of AP-dC in a 10-mer polypyrimidine oligonucleotide raised the T<sub>m</sub> of the corresponding duplex by 18 degC over a control duplex containing 5-Me-dC at the same position (1). Moreover, the additional, specific presence of the Hoogsteen hydrogen bond leads to high specificity of AP-dC for dG over the other three bases (1). Thus, AP-dC may be useful in any application in which the ability to discriminate dG in a target is necessary.

Flanagan and co-workers tested AP-dC for its utility in anti-sense oligos. Based on studies of AP-dC-modified anti-sense oligos for sequence-context dependence, activity mismatch, sensitivity, RNase-H cleavage, and hybridization kinetics, they concluded that AP-dC is a very potent, mis-match sensitive analog for dC, with high potential for improving the potency of anti-sense oligonucleotides (2). In another study, oligos containing one AP-dC at the 3'-end confer resistance to 3'-exonuclease digestion (3).

AP-dC is an excellent choice of modification whenever a large increase in duplex stability and/or specificity for dG in a target is required. **References**

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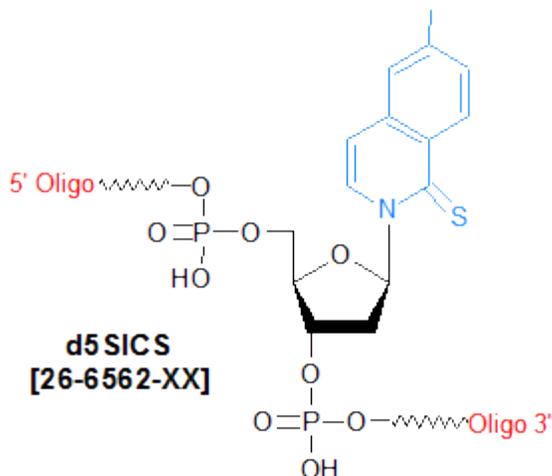
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## Oligo Modifications

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

### d5SICS

Category	Duplex Stability
Modification Code	d5SICS
Reference Catalog Number	26-6562
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	353.11





## Product Specifications

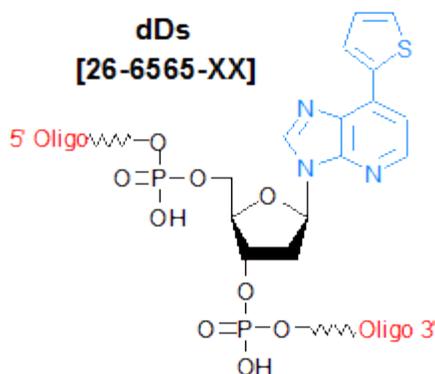
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## Oligo Modifications

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

### dDs

Category	Duplex Stability
Modification Code	dDs
Reference Catalog Number	26-6565
5 Prime	N
3 Prime	N
Internal	N
Molecular Weight(mw)	395.33



**This modification is discontinued. Consider dNaM [26-6561] and d5SICS [26-6562] as substitutes. See related products.** Base Pair Recognition Through Hydrophobic Interactions

The unnatural base pair between 7-(2-thienyl)-imidazo[4,5-b]pyridine (Ds) and pyrrole-2-carbaldehyde (Pa) is formed by specific hydrophobic shape complementation. The shape of the Ds-Pa pair is different from those of the natural A-T and G-C pairs, but the Ds-Pa pair works together with the natural pairs in in vitro replication and transcription. Pa also functions as a template base for incorporating another unnatural base, 2-amino-6-(2-thienyl)purine (s), into RNA. The s base also acts as a unique fluorescent base analog in DNA and RNA fragments. dDs is strongly fluorescent and is useful as a fluorescent tag for DNA detection. dDs also forms a base pair with dPa. Biotin PaTP can be site-specifically incorporated into RNA, opposite dDs at a desired position in DNA templates, by T7 transcription. Similarly, the fluorescent s base can be site-specifically incorporated into RNA opposite dPa in DNA templates.

The dNaM and d5SICS matched pair appears to be novel base pair. These unnatural C-nucleosides have pair recognition that rivals the A-T and G-C pairing in the natural genetic alphabet. In addition, they have been shown to be well-replicated by DNA polymerases under steady-state conditions, regardless of sequence. The fidelity and efficiency of dNaM and d5SICS replication approach those of natural synthesis. Both dNaM and d5SICS are also efficiently transcribed by T7 RNA polymerase in either direction.

See Glen Report for details: Unnatural Bases

#### References

1. Seo, Y.J.; Matsuda, S.; Romesberg, F.E. J. Am. Chem. Soc., 2009, 131(14), 5046-7.
2. Seo, Y.J.; Hwang, G.T.; Ordoukhanian, P.; Romesberg, F.E. J. Am. Chem. Soc., 2009, 131(9), 3246-52.
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5. Hari, Y.; Hwang, G.T.; Leconte, A.M.; Joubert, N.; Hocek, M.; Romesberg, F.E. ChemBioChem, 2008, 9(17), 2796-9.
6. Hwang, G.T.; Romesberg, F.E. J. Am. Chem. Soc., 2008, 130(44), 14872-82.
7. Leconte, A.M.; Hwang, G.T.; Matsuda, S.; Capek, P.; Hari, Y.; Romesberg, F.

E. J. Am. Chem. Soc., 2008, 130 (7), 2336-43.



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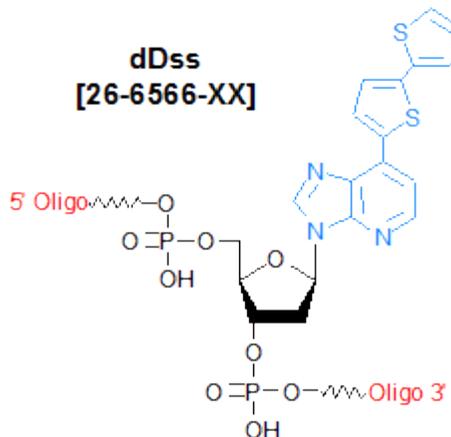
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## Oligo Modifications

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

### dDss

Category	Duplex Stability
Modification Code	dDss
Reference Catalog Number	26-6566
5 Prime	N
3 Prime	N
Internal	N
Molecular Weight(mw)	461.45



**This modification is discontinued. Consider dNaM [26-6561] and d5SICS [26-6562] as substitutes. See related products.** Base Pair Recognition Through Hydrophobic Interactions

The unnatural base pair between 7-(2-thienyl)-imidazo[4,5-b]pyridine (Ds) and pyrrole-2-carbaldehyde (Pa) is formed by specific hydrophobic shape complementation. The shape of the Ds-Pa pair is different from those of the natural A-T and G-C pairs, but the Ds-Pa pair works together with the natural pairs in in vitro replication and transcription. Pa also functions as a template base for incorporating another unnatural base, 2-amino-6-(2-thienyl)purine (s), into RNA. The s base also acts as a unique fluorescent base analog in DNA and RNA fragments. dDss is strongly fluorescent and is useful as a fluorescent tag for DNA detection. dDss also forms a base pair with dPa. Biotin PaTP can be site-specifically incorporated into RNA, opposite dDs at a desired position in DNA templates, by T7 transcription. Similarly, the fluorescent s base can be site-specifically incorporated into RNA opposite dPa in DNA templates.

The dNaM and d5SICS matched pair appears to be novel base pair. These unnatural C-nucleosides have pair recognition that rivals the A-T and G-C pairing in the natural genetic alphabet. In addition, they have been shown to be well-replicated by DNA polymerases under steady-state conditions, regardless of sequence. The fidelity and efficiency of dNaM and d5SICS replication approach those of natural synthesis. Both dNaM and d5SICS are also efficiently transcribed by T7 RNA polymerase in either direction.

See Glen Report for details: Unnatural Bases

#### References

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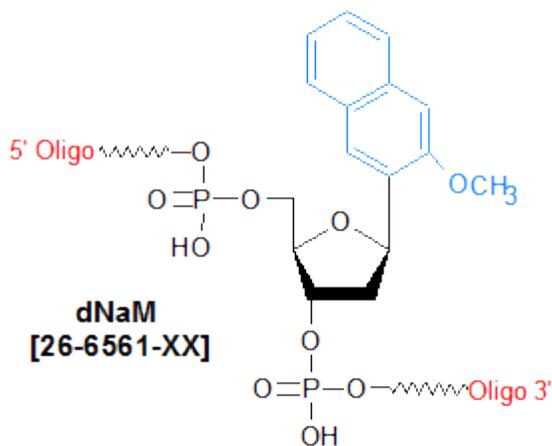
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## Oligo Modifications

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

### dNaM

Category	Duplex Stability
Modification Code	dNaM
Reference Catalog Number	26-6561
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	336.74





## Product Specifications

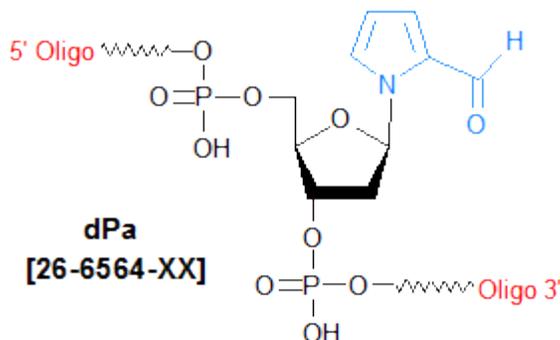
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## Oligo Modifications

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

### dPa (pyrrole-2-carbaldehyde)

Category	Duplex Stability
Modification Code	dPa
Reference Catalog Number	26-6564
5 Prime	N
3 Prime	N
Internal	N
Molecular Weight(mw)	0



**This modification is discontinued. Consider dNaM [26-6561] and d5SICS [26-6562] as substitutes. See related products.** Base Pair Recognition Through Hydrophobic Interactions

The unnatural base pair between 7-(2-thienyl)-imidazo[4,5-b]pyridine (Ds) and pyrrole-2-carbaldehyde (Pa) is formed by specific hydrophobic shape complementation. The shape of the Ds-Pa pair is different from those of the natural A-T and G-C pairs, but the Ds-Pa pair works together with the natural pairs in in vitro replication and transcription. Pa also functions as a template base for incorporating another unnatural base, 2-amino-6-(2-thienyl)purine (s), into RNA. The s base also acts as a unique fluorescent base analog in DNA and RNA fragments. dDs is strongly fluorescent and is useful as a fluorescent tag for DNA detection. dDs also forms a base pair with dPa. Biotin PaTP can be site-specifically incorporated into RNA, opposite dDs at a desired position in DNA templates, by T7 transcription. Similarly, the fluorescent s base can be site-specifically incorporated into RNA opposite dPa in DNA templates.

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See Glen Report for details: Unnatural Bases

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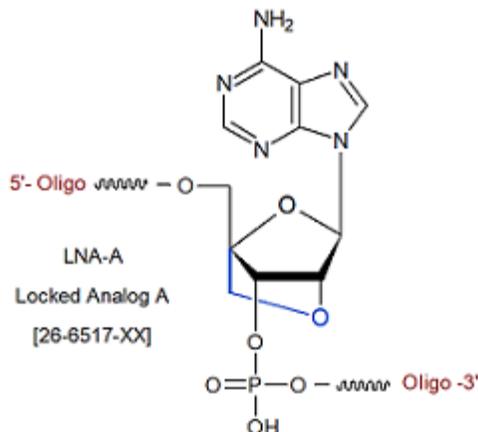
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## Oligo Modifications

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

### Locked Analog A

Category	Duplex Stability
Modification Code	+A
Reference Catalog Number	26-6517
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	341.21



Antisense Oligos (ODN) & siRNA Oligo Modifications

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

#### General Locked Nucleic Acid Analog Oligonucleotide Design Guidelines

1. Locked Nucleic Acid Analog (LNA) should be introduced at the positions where specificity and discrimination is needed (e.g. 3'-end in allele specific PCR and in the SNP position in allele specific hybridization probes).
2. Avoid stretches of more than 4 LNA bases. LNA hybridizes very tightly when several consecutive residues are substituted with Locked Nucleic Acid Analog bases.
3. Avoid LNA self-complementarity and complementarity to other LNA containing oligonucleotides in the assay. LNA binds very tightly to other LNA residues.
4. Typical primer length of 18mer should not contain more than 6-8 LNA bases.
5. Each LNA bases increases the  $T_m$  by approximately 2-4°C.
6. Do not use blocks of LNA near the 3'-end.
7. Keep the GC-content between 30-60 %.
8. Avoid stretches of more than 3 G DNA or LNA bases.
9.  $T_m$  of the primer pairs should be nearly equal.

LNA is a bicyclic nucleic acid where a ribonucleoside is linked between the 2'-oxygen and the 4'-carbon atoms with a methylene unit. Locked Nucleic Acid (LNA) was first described by Wengel and co-workers in 1998 (8-10) as a novel class of conformationally restricted oligonucleotide analogues.

The design and ability of oligos containing locked nucleic acids (LNAs) to bind supercoiled, double-stranded plasmid DNA in a sequence-specific manner has been described by Hertoghs et al (6) for the first time. The main mechanism for LNA oligos binding plasmid DNA is demonstrated to be by strand displacement. LNA oligos are more stably bound to plasmid DNA than similar peptide nucleic acid (PNA) 'clamps' for procedures such as particle mediated DNA delivery (gene gun). It is shown that LNA oligos remain associated with plasmid DNA after cationic lipid-mediated transfection into mammalian cells. LNA oligos can bind to DNA in a sequence-specific manner so that binding does not interfere with plasmid conformation or gene expression (6).

LNA Oligonucleotides exhibit unprecedented thermal stabilities towards complementary DNA and RNA, which allow excellent mismatch discrimination (8).

The high binding affinity of LNA oligos allows for the use of short probes in antisense protocols and LNA is recommended for use in any hybridization assay that requires high specificity and/or reproducibility, e.g., dual labeled probes, in situ hybridization probes, molecular beacons and PCR primers. Furthermore, LNA offers the possibility to adjust  $T_m$  values of primers and probes in multiplex assays. Each LNA base addition in an oligo increases the  $T_m$  by approximately 8°C. As a result of these significant characteristics, the use of LNA-modified oligos in antisense drug development is now coming under investigation, and recently the therapeutic potential of LNA has been reviewed (11).

#### Modifications Increasing Duplex Stability and Nuclease Resistance

##### Modification

##### Duplex Stability [ $T_m$ Increase]

Nuclease Resistance Locked Analog Bases Increased [2- 4°C per substitution] Increased 2-Amino-dA Increased [3.0°C per substitution] Similar to DNA C-5 propynyl-C Increased [2.8°C per substitution] Increased C-5 propynyl-U Increased [1.7°C per substitution] Increased 2'-Fluoro Increased [1.8°C per substitution] Increased 5-Methyl-dC Increased [1.3°C per substitution] Similar to DNA 2'-O Methyl Increased Increased Phosphorothioate Slightly decreased Increased [Click here for complete list of duplex stability modifications](#)

The synthesis and incorporation of LNA bases can be achieved by using standard DNA synthesis chemistry. Detailed research results have not yet concluded as to the amount of LNA bases and regular DNA base combination in successful antisense and gene delivery experiments. The investigator can elect to substitute individual bases in the oligo to LNA bases or use a combination. Due to the high affinity and thermal stability of the LNA: DNA duplex it is not advised to have more than 15 LNA bases in an oligo; this induces strong self-hybridization. The use of LNA C base requires special synthesis and post synthesis protocols. LNA-containing oligonucleotides can be purified and analyzed using the same methods employed for standard DNA. LNA can be mixed with DNA and RNA, as well as other nucleic acid analogues, modifiers and labels. LNA oligonucleotides are water soluble, and can be separated by gel electrophoresis and precipitated by ethanol.



## Product Specifications

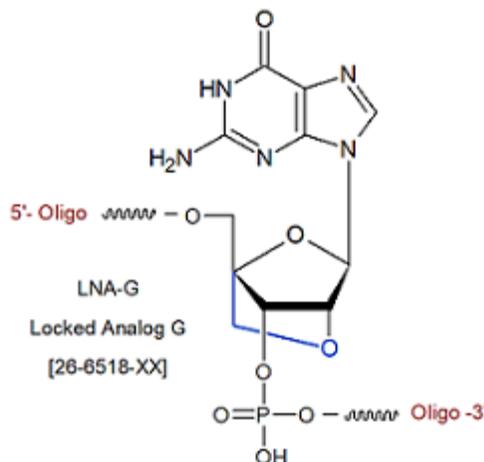
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## Oligo Modifications

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### Locked Analog G

Category	Duplex Stability
Modification Code	+G
Reference Catalog Number	26-6518
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	357.22



Antisense Oligos (ODN) & siRNA Oligo Modifications

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

#### General Locked Nucleic Acid Analog Oligonucleotide Design Guidelines

1. Locked Nucleic Acid Analog (LNA) should be introduced at the positions where specificity and discrimination is needed (e.g. 3'-end in allele specific PCR and in the SNP position in allele specific hybridization probes).
2. Avoid stretches of more than 4 LNA bases. LNA hybridizes very tightly when several consecutive residues are substituted with Locked Nucleic Acid Analog bases.
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#### Modifications Increasing Duplex Stability and Nuclease Resistance

##### Modification

##### Duplex Stability [ $T_m$ Increase]

Nuclease Resistance Locked Analog Bases Increased [2- 4°C per substitution] Increased 2-Amino-dA Increased [3.0°C per substitution] Similar to DNA C-5 propynyl-C Increased [2.8°C per substitution] Increased C-5 propynyl-U Increased [1.7°C per substitution] Increased 2'-Fluoro Increased [1.8°C per substitution] Increased 5-Methyl-dC Increased [1.3°C per substitution] Similar to DNA 2'-O Methyl Increased Increased Phosphorothioate Slightly decreased Increased [Click here for complete list of duplex stability modifications](#)

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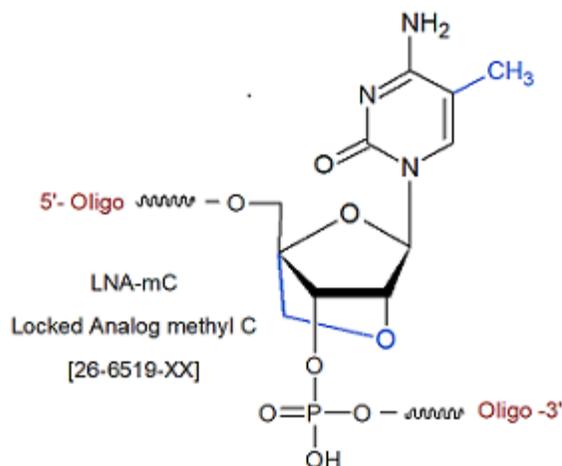
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## Oligo Modifications

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### Locked Analog mC

Category	Duplex Stability
Modification Code	+mC
Reference Catalog Number	26-6519
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	331.22



Antisense Oligos (ODN) & siRNA Oligo Modifications

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

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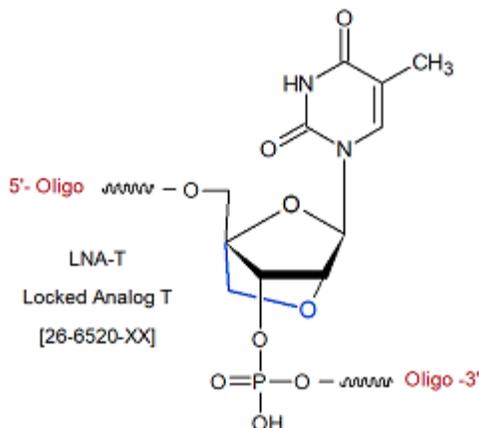
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## Oligo Modifications

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### Locked Analog T

Category	Duplex Stability
Modification Code	+T
Reference Catalog Number	26-6520
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	332.2



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## Oligo Modifications

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### MGB 3' CDPI3

Category	Duplex Stability
Modification Code	MGB-CDPI3-3
Reference Catalog Number	26-6456
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	813.27

#### **MGB Probe Design: 5'-Fluorophore** 5'-[Fluorophore]...probe sequence...[internal quencher][MGB]-3'

In the 5'-Fluorophore MGB probe design an internal quencher for example BHQ1-dT or BHQ2-dT is placed before the MGB at the 3' end.

#### **MGB Probe Design: 3'-Fluorophore** 5'-[MGB] [internal quencher]...probe sequence...[Fluorophore]-3'

In the 3'-Fluorophore MGB probe design an internal quencher for example BHQ1-dT or BHQ2-dT is placed after the MGB at the 5' end.

**MGB Probe Pricing** MGB probe pricing is the total of the price for [MGB] + [internal quencher]+ ..probe sequence +[Fluorophore]-3'

The tripeptide of dihydropyrroloindole-carboxylate (CDPI3) is a minor groove binding (MGB) moiety derived from the natural product CC-1065 with strong DNA binding properties. Synthetic oligonucleotides with covalently-attached CDPI3 have enhanced DNA affinity and have improved the hybridization properties of sequence-specific DNA probes. Short CDPI3-oligonucleotides hybridize with single-stranded DNA to give more stable DNA duplexes than unmodified ODNs of similar length. CDPI3 MGB-oligonucleotide conjugates have been found to be useful in the following applications:

- Arrest of primer extension and PCR blockers
- Short and fluorogenic PCR primers
- Real-time PCR probes
- miRNA Inhibitors

The simplest approach to MGB probe design is to use an MGB support, add a quencher molecule as the first addition and complete the synthesis with a 5'-fluorophore. Alternatively, a fluorophore support could be used with the 5' terminus containing a quencher molecule followed by a final MGB addition at the 5' terminus.

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## Oligo Modifications

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### MGB-5' CDPI3

Category	Duplex Stability
Modification Code	MGB-CDPI3-5
Reference Catalog Number	26-6457
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	872.96

#### **MGB Probe Design: 5'-Fluorophore** 5'-[Fluorophore]...probe sequence...[internal quencher][MGB]-3'

In the 5'-Fluorophore MGB probe design an internal quencher for example BHQ1-dT or BHQ2-dT is placed before the MGB at the 3' end.

#### **MGB Probe Design: 3'-Fluorophore** 5'-[MGB] [internal quencher]...probe sequence...[Fluorophore]-3'

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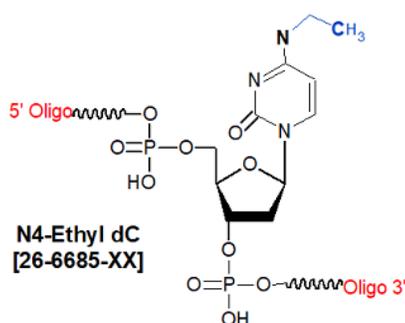
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## Oligo Modifications

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### N4-Ethyl dC [N4-Et-dC]

Category	Duplex Stability
Modification Code	N4-Et-dC
Reference Catalog Number	26-6685
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	317.42



N4-Ethyl-deoxycytidine (N4-Et-dC) is typically used to minimize the deleterious effect of large variations in GC content in target/probe sequences on the results produced by techniques involving simultaneous hybridization of many sequences, for example, DNA chip or reverse hybridization protocols (1). Due to the higher thermal stability of C:C base pairs, high-GC content sequences may contain mis-matches yet still stably hybridize to a probe or target (resulting in false positives), while low-GC content sequences may perfectly match to probe or target but the strands may dissociate upon washing (resulting in false negatives). This problem can be particularly acute in cases where the probes are short oligos (octamers, nonamers, etc.) A clever solution to this problem is to modify oligonucleotide probes to equalize (normalize) the thermal stability of G:C and A:T base pairs formed upon hybridization to the target, thereby making hybridization dependent only on oligo length and not on base composition. N4-Et-dC base pairs with dG, but the N4-Et-dC : dG base pair has a thermal stability similar to an A:T base pair instead of a C:G base pair. The dramatic effect on thermal stability was shown in two hybridization studies in which different sets of probes having GC content ranging from 0% to 100% were hybridized to their respective natural targets, and the  $T_m$  of the duplexes measured. For these unmodified probes, the  $T_m$  range was 39degC and 52degC, respectively. When N4-Et-dC was substituted for dC in these probes, the  $T_m$  range of the duplexes was only 7degC and 16degC, respectively (2,3).

N4-Et-dC-modified oligos have also been used in structure-function studies to better understand how CpG-containing oligos stimulate the innate immune system, and which structural elements in cytosine and guanine bases are required for recognition of, and interaction with, protein/receptor factors responsible for immunostimulation (4). **References**

1. Saiki, R.K, Walsh, P.S., Levenson, C.H., Erlich, H.A. Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes. *Proc. Natl Acad. Sci. USA* (1989), **86**: 6230-6234.
2. Nguyen, H-K, Auffray, P., Asseline, U., Dupret, D., Thuong, N.T. Modification of DNA duplexes to smooth their thermal stability independently of their base content for DNA sequencing by hybridization. *Nucleic Acids Res.* (1997), **25**: 3059-3065.
3. Nguyen, H-K.

, Bonfils, E., Auffay, P., Costaglioli, P., et al. The stability of duplexes involving AT and/or G4EtC base pairs is not dependent on their AT/G4EtC ratio content. Implication for DNA sequencing by hybridization. *Nucleic Acids Res.* (1998), **26**: 4249-4258.

4. Kandimalla, E.R., Yu, D., Zhao, Q., Agrawal, S. Effect of chemical modifications of cytosine and guanine in a CpG-motif of oligonucleotides: structure-immunostimulatory activity relationships. *Bioorg. Med. Chem.* (2001), **9**: 807-813.



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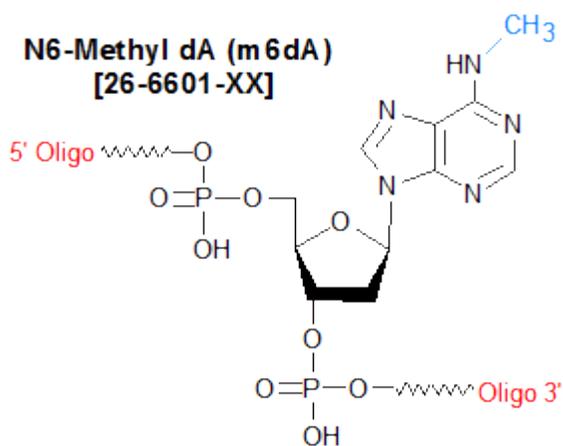
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### N6-Methyl dA (m6dA)

Category	Epigenetics
Modification Code	m6dA
Reference Catalog Number	26-6601
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	327.24





## Product Specifications

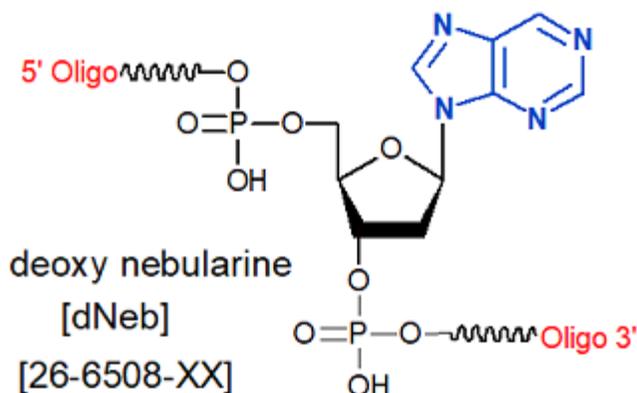
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### Nebularine deoxy

Category	Duplex Stability
Modification Code	dNeb
Reference Catalog Number	26-6508
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	298.19



#### [Click here for a complete list of Degenerate Bases & Spiking Oligo Modifications](#)

The design of primers is frequently complicated by the degeneracy of the genetic code. Three strategies are now available to confront this problem. In the first, a mixed base addition (N) is used to form the degenerate site. This approach is best if the number of degenerate sites is small. A second option is the use of 2'-deoxyInosine or 2'-deoxyNebularine which exhibit low, but unequal, hydrogen bonding to the other four bases. The third option is the use of a universal nucleoside. In this strategy, the base analog does not hybridize significantly to the other four bases and makes up some of the duplex destabilization by acting as an intercalating agent. 3-Nitropyrrole 2'-deoxynucleoside (M) is the first example of a set of universal bases. Subsequently, 5-nitroindole was determined to be an effective universal base and to be superior to 3-nitropyrrole, based on duplex melting experiments. The modified bases designated P and K show considerable promise as degenerate bases. The pyrimidine derivative P, when introduced into oligonucleotides, base pairs with either A or G, while the purine derivative K base pairs with either C or T. A dP+dK mix also can serve as a mixed base with much less degeneracy than dA+dC+dG+dT (N).

5-Nitroindole is a hydrophobic aromatic compound, and can be used as a **universal base analog** in oligonucleotides. The term "universal base" refers to a base with the ability to replace any of the four natural bases without significantly destabilizing either neighboring base-pair interactions or disrupting the expected functional capability of the resulting modified oligonucleotide. Incorporation of universal bases into oligos is desirable in cases when either imprecise or random base-pairing is required, and the resulting "mis-matched" complements need to be stable. Examples of such situations include reverse-translation of known protein sequence for oligo design (oligos to be used as primers or probes), development of an *in vitro* or *in vivo* oligo probe able to hybridize to related but distinct genes (for example, viral sub-strains or allelic variants—SNPs, indels, etc.), *in vitro* mutagenesis and motif cloning.

5-Nitroindole does not discriminate between the four natural nucleotide bases in duplex formation.

This is in contrast to deoxyinosine (dl), which, although often used as a "universal" base, actually shows fairly strong base-pair bias. 5-Nitroindole's lack of discrimination ("universality") is a consequence of the fact that it does not form hydrogen bonds with natural bases. Instead, 5-nitroindole stabilizes the duplex via base-stacking interactions, and is superior in this regard than 3-nitropyrrole, which also has been used as a universal base (1,2). Much research work on 5-nitroindole has focused on its potential as an alternative to the used of mixed bases in PCR or Sanger sequencing primers. The ability of 5-nitroindole to function in this way, however, is strongly dependent on where it is located in the primer. Based on the results of several research studies (reviewed in 3), the following guidelines may be useful in optimizing the utility of this base analog for these applications: 1. Substitution of 5-nitroindole as a universal base is less destabilizing towards the ends of oligos than towards the center. 2. Grouped substitutions are more easily tolerated than spaced ones, that is, contiguous rather than codon third substitutions. 3. If **more than two codon third substitutions** are incorporated into a PCR/sequencing primer, priming generally is poor. 4. Up to four contiguous substitutions in the middle or 5'-end of a PCR/sequencing primer generally yield acceptable amounts of PCR product or sequencing ladder. Above that priming is poor, due to the run of 5-nitroindoles forming undesirable secondary structures in the primer. 5. Substitutions at, or within eight bases of, the 3'-end produce primers that generally are ineffective at priming.

Beyond primers, nested sets of universal oligonucleotide probes containing 5-nitroindole have been successfully used to target regions of rRNA in a variety of microorganism species, in order to ensure equal probe specificity in all target organisms (4). 5-nitroindole has also been used to examine protein-DNA interactions relevant to nucleotide excision repair (NER) (5) and RNA polymerase binding (6). In addition, the potential of 5-nitroindole to enhance the stability of duplexes formed between a target DNA and octa-/decanucleotide probes immobilized on DNA microarrays has been investigated (7). Finally, 5-nitroindole often has been used to stabilize DNA structures of different types (3). **References**

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## Product Specifications

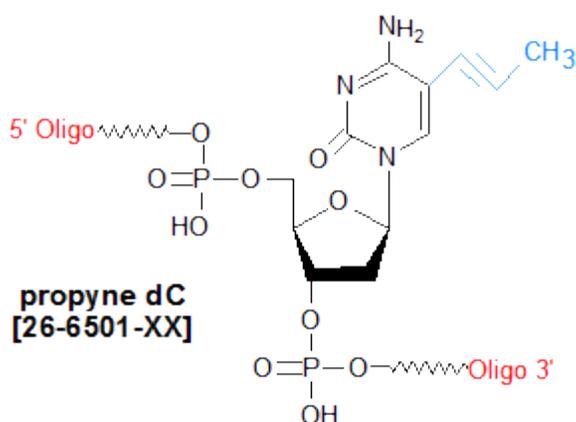
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## Oligo Modifications

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

### 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

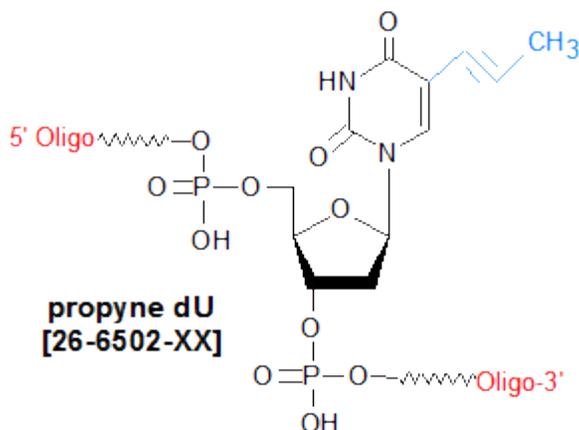
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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

### 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





## Product Specifications

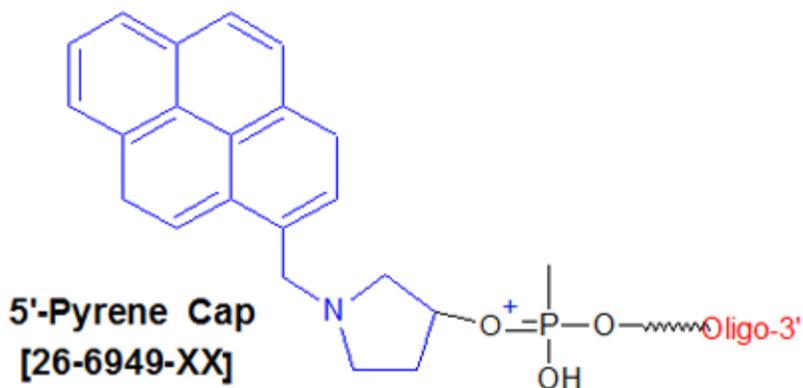
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## Oligo Modifications

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### Pyrene Cap (5')

Category	Duplex Stability
Modification Code	Pyr-Cap
Reference Catalog Number	26-6949
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	363.35



#### 5' and 3' Caps for Increased Duplex Stability.

Pyrene, Stilbene and 3'-Uaq caps favor the formation of stable Watson-Crick duplexes by stacking on the terminal base pair.

Melting point increases of over 10°C per modification can be realized for short duplexes

The caps fit canonical Watson-Crick base pairs and do not stack well on mismatched base pairs. This leads to increased base pairing selectivity at the terminal and the penultimate position of oligonucleotides featuring the caps. Base pairing fidelity is usually low at the termini, where fraying occurs frequently in the absence of caps. The beneficial effects of the caps are also realized when longer target strands are bound, so there is no need for blunt ends for the duplexes formed.<sup>2</sup> The caps, when attached to the terminus of an oligonucleotide, also facilitate purification as their lipophilicity leads to prolonged retention on reversed phase columns or cartridges. Finally, capping of termini may discourage the degradation of oligonucleotides by exonucleases.

#### 5' Pyrene Cap

5'-Pyrene cap (Pyrenylmethylpyrrolindol) will produce a cap that is more lipophilic than the trimethoxystilbene. The aromatic stacking moiety is linked to the terminus of the DNA through a more rigid, cyclic linker than in the case of trimethoxystilbene. This feature may prove advantageous for researchers interested in exploiting the special photophysical properties of the pyrenyl substituent. The pyrrolindol linker is stereoregular, leading to a single product that can be readily purified by HPLC. The pyrenyl cap is the lead compound discovered in a recent combinatorial study that evaluated over 40 different caps. The cap proved particularly successful for hybridization probes with a 5'-terminal deoxyadenosine residue. Again, its duplex-stabilizing effect does not require blunt ends. The tertiary amino group can be expected to be protonated at physiological pH, producing a cationic functionality that may help to attract target strands electrostatically. The five membered ring presenting the pyrenyl stacking unit mimics the deoxyribose of natural nucleosides, making duplexes terminating in this cap more similar in shape to unmodified DNA than those capped with the trimethoxystilbene.

#### Trimethoxystilbene cap

Stilbenes have been successfully employed for covalently bridging the termini of oligonucleotide hairpins. The trimethoxystilbene cap that is the result of a recent study that focused on stilbenes that are covalently linked to only one of the two strands forming a duplex.

The three methoxy substituents interact with the 2'-methylene group of the nucleoside in the target strand. Together with the stacking on the terminal base pair, this leads to much-improved mismatch discrimination. The effect is observed for any of the four possible base pairs at the terminus.

Trimethoxystilbene cap increases the signal for the fully complementary target strand when used for hybridization probes immobilized on a glass surface in the form of a DNA microarray. This feature is particularly important for A/T-rich sequences that often cause false negatives. The selective stabilization of neighboring Watson-Crick base pairs helps to suppress cross hybridization that would otherwise lead to stronger false positive results.

### 3' Uaq Cap

3'-Uaq Cap is available as a cap structure for the 3' of an oligo. It is a Uridine support modified with a 2'-anthraquinone residue and is the most effective oligonucleotide cap known to date. For short hybrid duplexes between DNA probes and RNA target strands, the increase in  $T_m$  is up to 18<sup>0</sup>C and the modification is effective in increasing the  $T_m$  of DNA:DNA, RNA:RNA, and DNA:RNA hybrid duplexes. 3'-Uaq Cap also increases probe specificity by depressing the melting point of terminal mismatches.

### References

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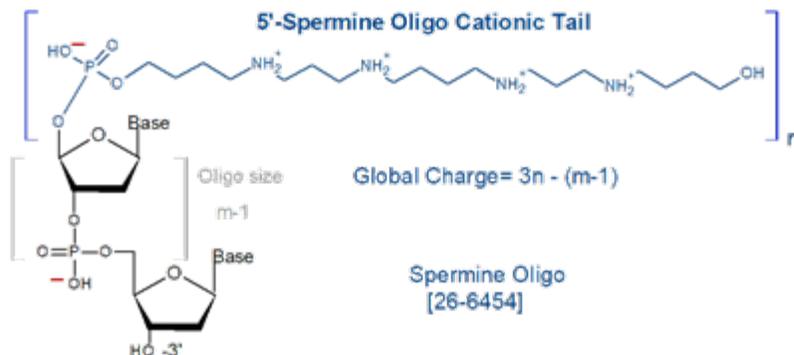
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## Oligo Modifications

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

### Spermine Oligo

Category	Duplex Stability
Modification Code	Spm
Reference Catalog Number	26-6454
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	408.52



**Solubility of oligos with 4-40 Spermine sites (ZNA Oligos).** Gene Link supplies all oligos as lyophilized/dried state. Oligos with more than 4 spermine sites have lower solubility in aqueous solutions. Reconstitute these oligos in 100 mM Ammonium hydroxide. Spermine oligos with more solubility concerns may be resolved by adding 50 mM ammonium hydroxide drop wise until the ZNA goes into solution in water OR by dissolving the ZNA in concentrated phosphate buffer saline.

Spermine phosphoramidite is used to produce oligospermine-oligonucleotide conjugates - Zip Nucleic Acids (ZNA) Oligos. The name reflects the presumed mode of action. The conjugates are believed to use the oligospermine to seek out and move along (scan) oligonucleotide strands until the probe complementary sequence is located. The oligospermine then performs the function of stabilizing the formed duplex by reducing electrostatic repulsion, thereby leading to significantly increased binding affinities. ZNA Oligos have found use in the following applications: Multiplex PCR; PCR of AT-rich Regions; RT qPCR; Detection of MicroRNA; Improved SNP Discrimination; and Antisense and Antigene Effects. Spermine phosphoramidite is simple to use in oligonucleotide synthesis and can be added multiple times at the 3' or 5' terminus. Deprotection and isolation are also straightforward. HPLC analysis of the conjugates requires high pH to suppress the ionization of the spermine residues.

By selecting the number of cationic units, the global charge of the ZNA molecules can be modulated which defines their field of applications. When negatively charged, ZNA are potent tools for molecular biology and diagnostic applications. Their design is essentially based on the expected and predictable  $T_m$  of the oligonucleotide which depends on the number of conjugated cationic units. When positively charged, the cationic conjugates become self-delivering oligonucleotides into cells and resistant to nucleases which make them very attractive molecules for antisense or RNA interference applications. With an increase in spermine content, the solubility of ZNA oligonucleotides may be noticeably less than unmodified DNA or RNA counterparts. This is typically observed when re-dissolving dried-down purified ZNA in water. In this case, drop wise addition of 50 mM ammonium hydroxide brings ZNA molecules into solution. Alternatively, dissolving ZNA oligos in concentrated phosphate buffered saline (2.5x PBS, pH 7).

4) has also been found to resolve solubility issues.

**Recommended Further Reading**

Glen Reports GR24-11. Spermine Phosphoramidite: A potent modification with many applications.

Glen Reports GR24-11. Zip Nucleic Acids (ZNA) are powerful cationic oligonucleotides for molecular biology, diagnostic and therapeutic applications.

**INTELLECTUAL PROPERTY**

"Spermine phosphoramidite" synthon is the subject matter of U.S. Patent Application No. 12/086.599, European Patent Application No. EP20060847298 and foreign equivalents for which Polyplus-transfection is the co-owner. Product is sold for research purposes only. Product shall not be used to manufacture oligonucleotide-oligospermine conjugates for use in diagnostics, clinical or commercial applications including use in humans. There is no implied license to manufacture oligospermine-oligonucleotide conjugates for diagnostic, clinical or commercial applications, including but not limited to contract research. Please contact Polyplus-transfection at [licensing@polyplus-transfection.com](mailto:licensing@polyplus-transfection.com) to obtain a license for such use.



## Product Specifications

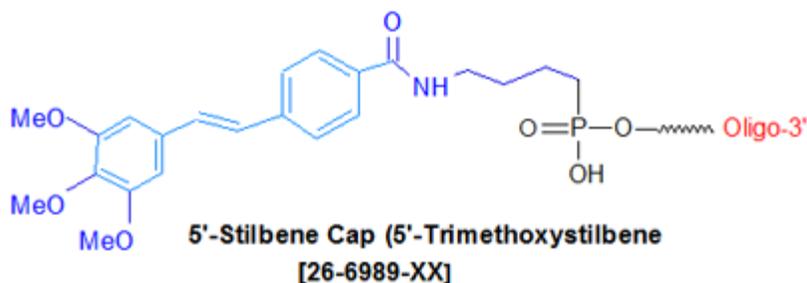
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## Oligo Modifications

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

### Stilbene Cap

Category	Duplex Stability
Modification Code	Stb-Cap
Reference Catalog Number	26-6989
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	433.39



#### 5' and 3' Caps for Increased Duplex Stability.

Pyrene, Stilbene and 3'Uaq caps favor the formation of stable Watson-Crick duplexes by stacking on the terminal base pair. Melting point increases of over 10 degrees C per modification can be realized for short duplexes

The caps fit canonical Watson-Crick base pairs and do not stack well on mismatched base pairs. This leads to increased base pairing selectivity at the terminal and the penultimate position of oligonucleotides featuring the caps. Base pairing fidelity is usually low at the termini, where fraying occurs frequently in the absence of caps. The beneficial effects of the caps are also realized when longer target strands are bound, so there is no need for blunt ends for the duplexes formed.<sup>2</sup> The caps, when attached to the terminus of an oligonucleotide, also facilitate purification as their lipophilicity leads to prolonged retention on reversed phase columns or cartridges. Finally, capping of termini may discourage the degradation of oligonucleotides by exonucleases.

#### Trimethoxystilbene cap

Stilbenes have been successfully employed for covalently bridging the termini of oligonucleotide hairpins. The trimethoxystilbene cap that is the result of a recent study that focused on stilbenes that are covalently linked to only one of the two strands forming a duplex. The three methoxy substituents interact with the 2' methylene group of the nucleoside in the target strand. Together with the stacking on the terminal base pair, this leads to much-improved mismatch discrimination. The effect is observed for any of the four possible base pairs at the terminus.

Trimethoxystilbene cap increases the signal for the fully complementary target strand when used for hybridization probes immobilized on a glass surface in the form of a DNA microarray. This feature is particularly important for A/T-rich sequences that often cause false negatives. The selective stabilization of neighboring Watson-Crick base pairs helps to suppress cross hybridization that would otherwise lead to stronger false positive results.

#### 5' Pyrene Cap

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The pyrrolindol linker is stereoregular, leading to a single product that can be readily purified by HPLC. The pyrenyl cap is the lead compound discovered in a recent combinatorial study that evaluated over 40 different caps. The cap proved particularly successful for hybridization probes with a 5' terminal deoxyadenosine residue. Again, its duplex-stabilizing effect does not require blunt ends. The tertiary amino group can be expected to be protonated at physiological pH, producing a cationic functionality that may help to attract target strands electrostatically. The five membered ring presenting the pyrenyl stacking unit mimics the deoxyribose of natural nucleosides, making duplexes terminating in this cap more similar in shape to unmodified DNA than those capped with the trimethoxystilbene.

### 3' Uaq Cap

3' Uaq Cap is available as a cap structure for the 3' of an oligo. It is a Uridine support modified with a 2' anthraquinone residue and is the most effective oligonucleotide cap known to date. For short hybrid duplexes between DNA probes and RNA target strands, the increase in T<sub>m</sub> is up to 18 C and the modification is effective in increasing the T<sub>m</sub> of DNA:DNA, RNA:RNA, and DNA:RNA hybrid duplexes. 3' Uaq Cap also increases probe specificity by depressing the melting point of terminal mismatches.

### References

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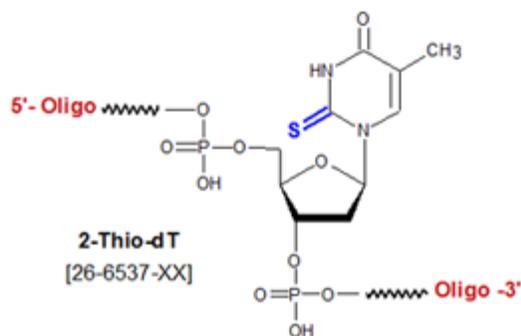
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## Oligo Modifications

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### Thio-2-dT (S2dT)

Category	Duplex Stability
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Reference Catalog Number	26-6537
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	320.26





## Product Specifications

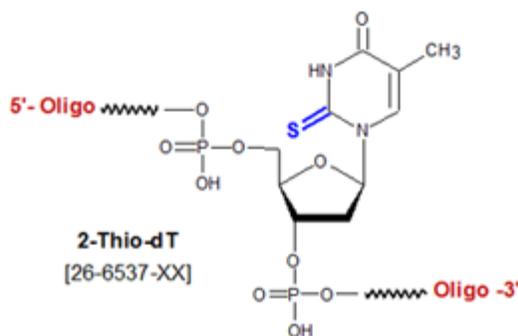
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## Oligo Modifications

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### Uaq Cap (3')

Category	Duplex Stability
Modification Code	Uaq-Cap
Reference Catalog Number	26-6950
5 Prime	n
3 Prime	Y
Internal	N
Molecular Weight(mw)	539.39



#### 5' and 3' Caps for Increased Duplex Stability.

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#### 3' Uaq Cap

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