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.

RNA Oligo Modifications Introduction

Gene Link specializes in the synthesis of complex RNA modified oligos, siRNA, RNA fluorescent probes with quenchers, chimeric oligos containing various combinations of DNA, 2'O methyl bases, 2'F bases and phosphorothioate linkages.

RNA Oligo Modifications Design Protocols

Gene Link specializes in the synthesis of complex RNA modified oligos, RNA fluorescent probes with quenchers, chimeric oligos containing various combinations of DNA, 2'O methyl bases, 2'F bases and phosphorothioate linkages.

RNA and 2'-O-methyl RNA oligonucleotide synthesis is performed at Gene Link using the b -cyanoethyl chemistry and state of the art synthesizers. These involve proprietary software protocols with long coupling times and specialized cycles to obtain ultra clean oligos.
RNA oligos are susceptible to degradation to the same extent as native RNA extracted from various sources. An attractive alternate to prevent degradation from nucleases is the use of 2'F and 2'-O- methyl RNA bases, when specific 2'OH is not required. The 2'-O- methyl oligonucleotides confer considerable nuclease resistance and are similar in hydrogen bonding properties to RNA/RNA than the lower RNA/DNA binding property. The coupling efficiency of 2'-O- methyl phosphoramidite is also higher than the RNA monomers resulting in higher yield of full length oligos.
Gene Link also offers custom synthesis of RNA and DNA chimeric oligos with investigator specified ribo or deoxy bases or 2'-O-methyl bases. The chimeric oligos can also be synthesized with the regular phoshodiester bonds or substituted with phosphorothioate linkages. The combination of 2'-O- methyl RNA bases with phosphorothioate internucleotide linkages imparts these oligos greater nuclease resistance which is particularly useful for antisense studies (please refer to our technical sheet on Antisense Oligonucleotides for other modifications). Custom phosphorothioate oligonucleotides synthesized by Gene Link can be specified to have all the diester bonds substituted or only some selected diester linkages depending upon the researchers experimental requirement. Substitution of all diester linkage is recommended to provide greater nuclease resistance.

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2'-Fluoro-deoxy bases are 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).

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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.

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(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.

References

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.
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3. Goringer, H.U.; Adler, Annette; Forster, Nicole; Homann, Matthias. Post-SELEX Chemical Optimization of a Trypanosome-Specific RNA Aptamer. *Combinatorial Chemistry & High Throughput Screening* (2008), **11**: 16-23.
4. Layzer, J.M.; McCaffrey, A.P.; Tanner, A.K.; Huang, Z.; Kay, M.A.; Sullenger, B.A. In vivo activity of nuclease-resistant siRNAs. *RNA* (2004), **10**, 766-71.
5. Allerson, C.R.; et al. Fully 2'-modified oligonucleotide duplexes with improved in vitro potency and stability compared to unmodified small interfering RNA. *Journal of Medicinal Chemistry* (2005), **48**: 901-904.

Modificaton Code List

Modification	Code	Catalog Number
2'-Fluoro Bases	[fN]	26-6692N
2'-Fluoro deoxyadenosine (2'-F-A)	[fA]	26-6692
2'-fluoroarabinoside-Base (FANA-N)	[FANA-N]	27-6605N
2'-Fluoro deoxycytosine (2'-F-C)	[fC]	26-6463
2'-Fluoro deoxyguanosine (2'-F-G)	[fG]	26-6693
2'-F Inosine (2'-F-I)	[fI]	26-6618
2'-Fluoro deoxyuridine (2'-F-U)	[fU]	26-6462
2'-O-methoxy-ethyl Bases (2'-MOE)	[MOE-N]	27-6450N
2'-O-methoxy-ethyl 5me Cytidine-(2'-MOE meC)	[MOE-mC]	27-6450mC
2'-O-methoxy-ethyl 5me Uridine-(2'-MOE 5me U)	[MOE-mU]	27-6450mU
2'-O-methoxy-ethyl Adenosine-(2'-MOE rA)	[MOE-A]	27-6450A
2'-O-methoxy-ethyl Guanosine-(2'-MOE rG)	[MOE-G]	27-6450G
2'-O Me-5-Me-C	[m5mC]	27-6508
2'-O methyl adenosine A	[mA]	27-6410A
2'-O methyl bases	[mN]	27-6410N
2'-O methyl cytosine C	[mC]	27-6410C
2'-O methyl guanosine G	[mG]	27-6410G
2'-O methyl Inosine	[mI]	27-6410I
2'-O methyl uridine U	[mU]	27-6410U
2-Amino Purine ribose	[2-APr]	27-6505

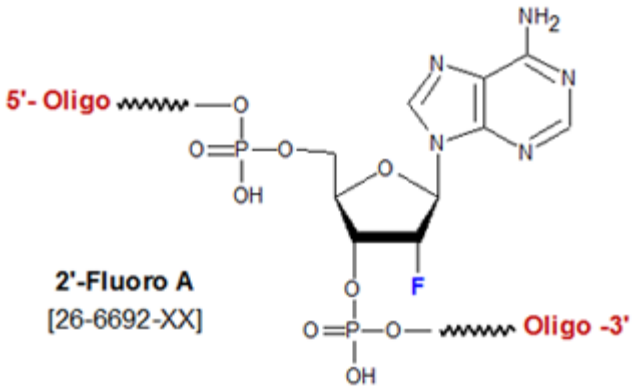
3'-O methyl bases (2'-5' linked)	[3OmN]	27-6458N
3'-O methyl rA (2'-5' linked)	[3OmA]	27-6458A
3'-O methyl rC (2'-5' linked)	[3OmC]	27-6458C
3'-O methyl rG (2'-5' linked)	[3OmG]	27-6458G
3'-O methyl rInosine (2'-5' linked)	[3OmI]	27-6458I
3'-O methyl rU (2'-5' linked)	[3OmU]	27-6458U
3'-rA (2'-5' linked)	[3rA2-5]	26-6682
3'-rC (2'-5' linked)	[3rC2-5]	26-6911
3'-rG (2'-5' linked)	[3rG2-5]	26-6912
3'-rU (2'-5' linked)	[3rU2-5]	26-6913
5-bromo rC (5-Br rC)	[5-Br-rC]	27-6551
5-bromo rU (5-Br rU)	[5-Br-rU]	27-6552
5-Iodo ribocytosine (5-I C)	[5-I-rC]	27-6553
5-Iodo ribouridine (5-I U)	[5-I-rU]	27-6554
5-methyl-Cytosine [5mrC]	[5mrC]	27-6945
5-Methyl-Uridine (m5U)	[m5U]	27-6557
8-Bromo rG (8-Br rG)	[8-Br-rG]	27-6446
8-Oxo rG riboguanosine [8-Oxo-rG]	[8-Oxo-rG]	27-6434
Adenylation-5' (rApp)	[5rApp]	26-6805
Aminoallyl rU	[AmAll-rU]	27-6548
Amino C6 U (RNA)	[AmC6U]	27-6422

dihydro rUracil (5-6 DH rU)	[5-6-DHrU]	27-6683
Inosine ribo [rI]	[rI]	27-6421
Inverted rA (reverse linkage)	[Inv-rA]	27-6441
Inverted rC (reverse linkage)	[Inv-rC]	27-6442
Inverted rG (reverse linkage)	[Inv-rG]	27-6443
Inverted rU (reverse linkage)	[Inv-rU]	27-6444
L-RNA rA	[L-rA]	27-6941
L-RNA rC	[L-rC]	27-6942
L-RNA rG	[L-rG]	27-6943
L-RNA rU	[L-rU]	27-6944
N1-methyl pseudoUridine (m1-psi rU)	[m1-psi-rU]	27-6532
N1-Methyl rAdenosine (m1A)	[m1A]	27-6559
N3-methyl-rU [m3U]	[m3U]	27-6558
N4-Ethyl rC [N4-Et-rC]	[N4-Et-rC]	27-6685
N6-Benzyl Adenosine (bn6A)	[bn6A]	27-6604
N6-dimethyl rA [m6-2A]	[m6-2A]	27-6602
N6-isopentenyl-rA (i6a)	[N6-i6A]	27-6603
N6-Methyl rA (m6A)	[m6A]	27-6601
pseudoUridine ribo (psi rU)	[psi-rU]	27-6531
riboadenosine rA	[rA]	27-6400A
ribocytosine rC	[rC]	27-6400C

riboguanosine rG	[rG]	27-6400G
rAbasic Site (rSpacer abasic furan)	[rABS]	26-6442
ribouridine rU	[rU]	27-6400U
6-Thio-rG (S6-rG)	[S6-rG]	27-6533
2-Thio-Uridine (s2U)	[s2U]	27-6537
4-Thio-Uridine (s4U)	[s4U]	27-6445
Thymidine Ribo	[rT]	27-6946
rZebularine	[rZ]	27-6435

2'-F Bases

Category	Nuclease Resistance
Modification Code	fN
Reference Catalog Number	26-6692N
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	323.43



Mixed base 2' Fluoro N has a setup charge of $250.00 per order .

Antisense Oligos (ODN) & siRNA Oligo Modifications
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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 Click here for complete list of duplex stability modifications

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

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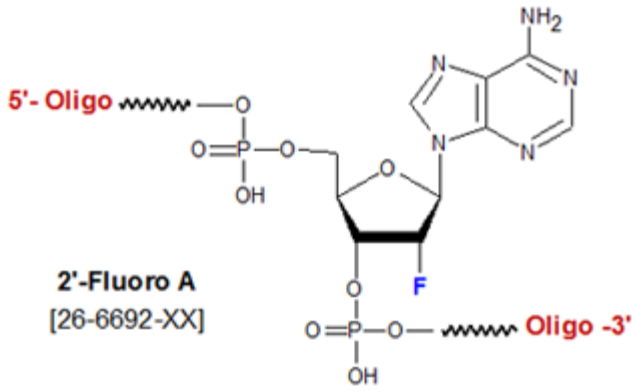
2'-F-A

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



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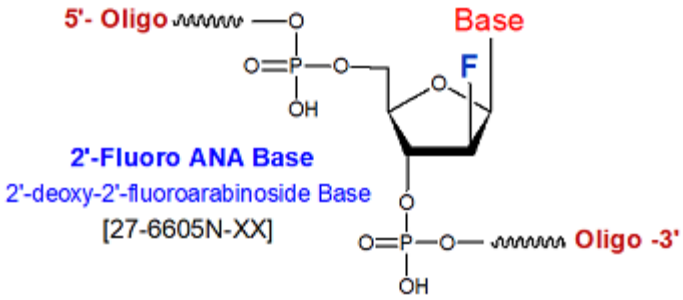
2'-F-ANA-Bases

Category	Antisense
Modification Code	FANA-N
Reference Catalog Number	27-6605N
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	323.43



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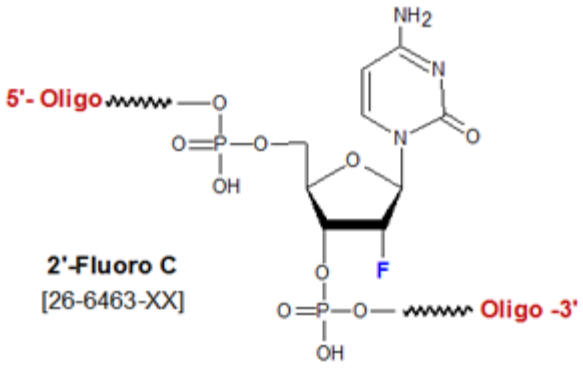
Arabinonucleosides are epimers of ribonucleosides with the chiral switch being at the 2' position of the sugar residue. 2'-F-ANA adopts a more DNA-like B-type helix conformation, not through the typical C2-endo conformation but, rather, through an unusual O4'-endo (east) pucker. However, the presence of the electronegative fluorine leads to a still significant increase (DTm1.2^{O}C/mod) in melting temperature per modification (1). 2'-F-ANA-containing oligonucleotides exhibit very high binding specificity to their targets. Indeed, a single mismatch in a 2'-F-ANA-RNA duplex leads to a DTm of -7.2^{O}C and in a 2'-F-ANA - DNA duplex a DTm of -3.9^{O}C (2).
The presence of fluorine at the 2' position in 2' F-ANA leads to increased stability to hydrolysis under basic conditions relative to RNA and even 2'-F-RNA (1,3). The stability of 2'-F-ANA to nucleases also makes this a useful modification for enhancing the stability of oligonucleotides in biological environments (2). 2' F-ANA hybridizes strongly to target RNA and, unlike most 2' modifications, induces cleavage of the target by RNase H. Phosphorothioate (PS) 2' F-ANA is routinely used in these applications due to its increased nuclease resistance. Alternating 2' F-ANA and DNA units provide among the highest potency RNase H-activating oligomers. Both the "altimer" and "gapmer" strand architectures consistently outperform PS-DNA and DNA/RNA gapmers (4).
siRNA oligos were found to tolerate the presence of 2'-F-ANA linkages very well. High potency gene silencing was demonstrated5 with siRNA chimeras containing 2'-F-RNA and/or LNA and 2'-F-ANA. The high efficacy of these chimeras was attributed to the combination of the rigid RNA-like properties of 2'-F-RNA and LNA with the DNA-like properties of 2'-F-ANA.
Additional Recommended Reading Glen Report 22.13.
References E. Viazovkina, M.M. Mangos, M.I. Elzagheid, and M.J. Damha, Curr Protoc Nucleic Acid Chem, 2002, Chapter 4, Unit 4 15. J.K. Watts, and M.J. Damha, Can. J.

Chem., 2008, 86, 641-656. J.K. Watts, A. Katolik, J. Viladoms, and M.J. Damha, Org Biomol Chem, 2009, 7, 1904-10. A. Kalota, et al., Nucleic Acids Res., 2006, 34, 451. G.F. Deleavey, et al., Nucleic Acids Res., 2010, 38, 4547-4557, J.K. Watts, et al., Nucleic Acids Res., 2007, 35, 1441-1451, T. Dowler, et al., Nucleic Acids Res., 2006, 34, 1669-1675. Intellectual Property 2'-F-ANA is covered by intellectual property. Key patents covering siRNA and antisense applications are as follows: WO/2009/146556 (siRNA); WO 03064441 and WO 0220773 (antisense).

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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(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 Tm 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 [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 Click here for complete list of duplex stability modifications

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

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

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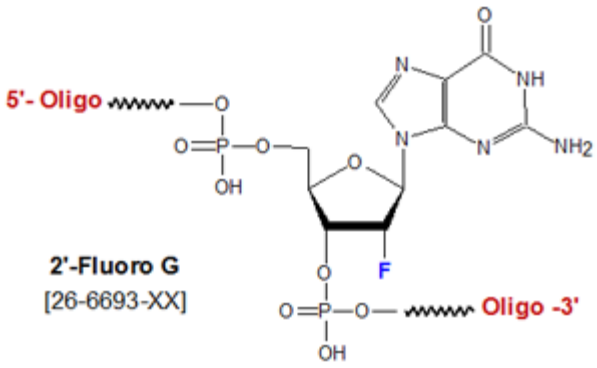
2'-F-G

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



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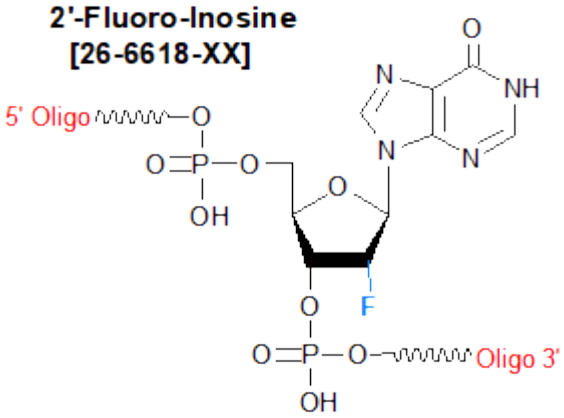
2'-F-Inosine

Category	Nuclease Resistance
Modification Code	fI
Reference Catalog Number	26-6618
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	332.18



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2-Fluoro Inosine (fI) is classified as a nucleotide base analog; it is structurally similar to guanosine, but is missing the 2-amino group and in addition the 2' hydroxyl is replaced by fluoro imparting robust nuclease resistance. Because it is able to form two hydrogen bonds with each of the four natural nucleotide bases (1), it is often used by researchers as a "universal" base meaning that it can base pair with all the naturally-occurring bases--in synthetic oligos. dI typically is substituted for the nucleoside at the third ('wobble') position of codons, in order to reduce the complexity of mixed oligo PCR primers/hybridization probes needed to deal with degenerate codons in the target DNA (2, 3). However, it is important to remember that dI does not base pair equally well with the naturally-occurring bases, with the order of thermodynamic stability being I-C >: I-A > I-G ~ I-T. Thermodynamic stability of inosine-containing duplexes is also affected by neighboring bases (4). Consequently, when using dI as an alternative to mixed-base degeneracy at a particular oligo position, keep in mind that the above base-pairing bias may lead to differences in the oligo's priming or hybridization efficiency in the corresponding degenerate regions of the target. Because the effect could be more pronounced when dI is at the 3'-position, it may be advisable to use primers with and without dI at the 3'-end, in order to maximize diversity of PCR products (5).
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.

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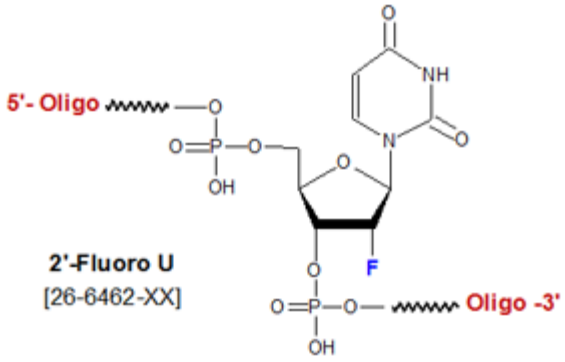
1. Oda, Y, Uesugi, S., Ikehara, M., Kawase, Y., Ohtsuka, E. NMR studies for identification of dI:dG mismatch base-pairing structure in DNA. *Nucleic Acids Res.* (1991), **19**: 5263-5267.
2. Liu, H., Nichols, R. PCR amplification using deoxyinosine to replace entire codon and at ambiguous positions.*Biotechniques.* (1994), **16**: 24-26.
3. Ohtsuka, E., Matsuki, S., Ikehara, M., Takahashi, Y., Matsubara, K. An alternative approach to deoxynucleotides as hybridization probes by insertion of deoxyinosine at ambiguous codon positions. *J. Biol. Chem.* (1985), **260**: 2605-2608.
4. Martin, F.H., Castro, M.M., Aboul-ela, F., Tinoco, I. Base pairing involving deoxyinosine: implications for probe design. *Nucleic Acids Res.* (1985), **13**: 8927-8938.
5. Ben-Dov, E., Shapiro, O.H., Siboni, N., Kushmaro, A. Advantage of Using Inosine at the 3’ Termini of 16S rRNA Gene Universal Primers for the Study of Microbial Diversity. *Appl. Environ. Microb.* (2006), 72: 6902-6906.

2'-F-U

Category	Nuclease Resistance
Modification Code	fU
Reference Catalog Number	26-6462
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	308.16



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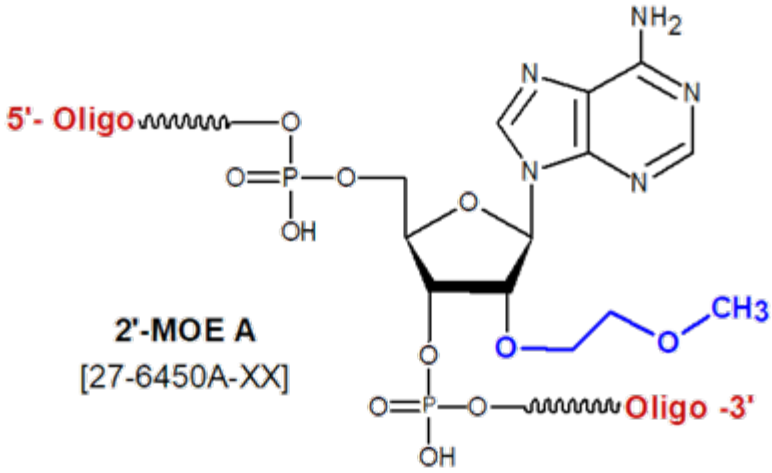
2'-MOE- Bases

Category	Antisense
Modification Code	MOE-N
Reference Catalog Number	27-6450N
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	386.55



Mixed base 2'-MOE N has a setup charge of $250.00 per order .

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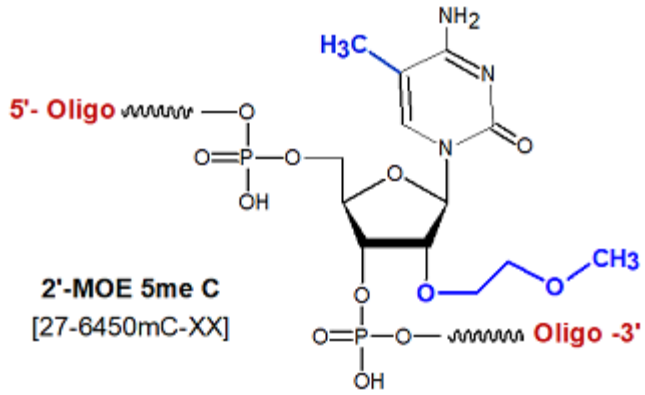
asp?modid=431"> Click this link to view these modifications.

Oligo Modifications

2'-MOE-5mC

Category	Antisense
Modification Code	MOE-mC
Reference Catalog Number	27-6450mC
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	377.3



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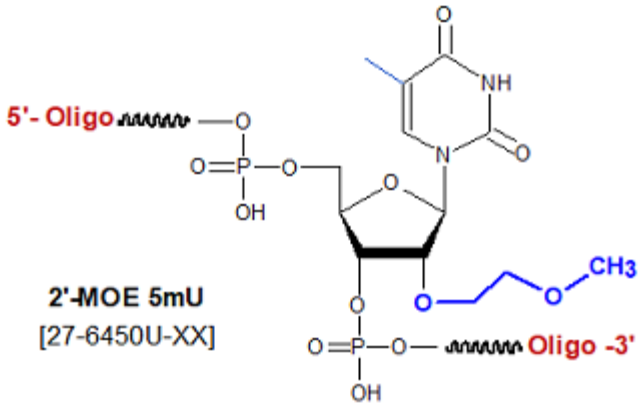
2'-MOE-5mU

Category	Antisense
Modification Code	MOE-mU
Reference Catalog Number	27-6450mU
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	378.3



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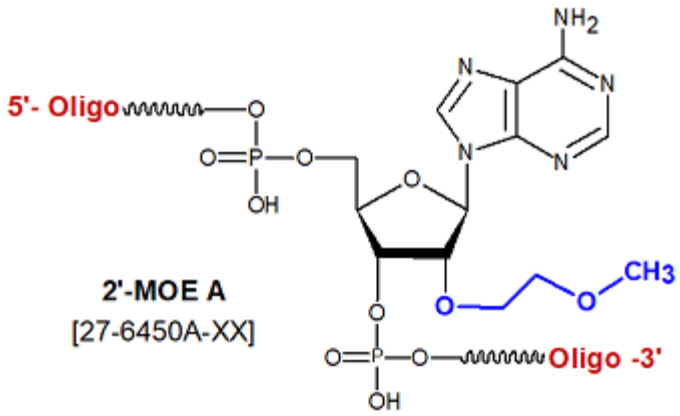
Oligo Modifications

2'-MOE-A

Category	Antisense
Modification Code	MOE-A
Reference Catalog Number	27-6450A
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	387.3



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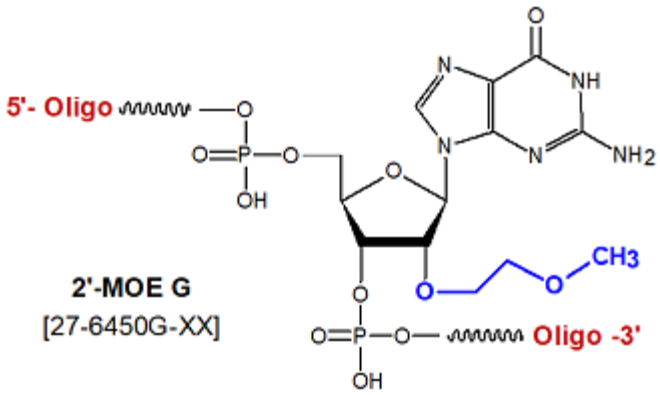
2'-MOE-G

Category	Antisense
Modification Code	MOE-G
Reference Catalog Number	27-6450G
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	403.3



Antisense Oligos (ODN) & siRNA Oligo Modifications
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Antisense oligonucleotides (ASOs) and small interfering RNA (siRNA) are both recognized therapeutic agents for the silencing of specific genes at the posttranscriptional level. Chemical modifications, particularly 2'-O-(2-Methoxyethyl)-oligoribonucleotides (2'-O-MOE bases) and 2'-O-Methyl bases are commonly used to confer nuclease resistance to an oligo designed for anti-sense, siRNA or aptamer-based research, diagnostic or therapeutic purposes, when specific 2'-OH is not required.
Nuclease resistance can be further enhanced by phosphorothiolation of appropriate phosphodiester linkages within the oligo. These modifications confers nuclease resistance, high binding affinity towards complementary RNA, reduced unspecific protein binding and extended half-life in tissues.
Gapmers.
Currently, the mainstream of the ASO is gapmer design ASOs. Gapmer design oligonucleotides, contain two to five chemically modified nucleotides'(LNA, 2'-O methyl or 2'-O-MOE RNA) as 'wings' at each terminus flanking a central 5- to 10-base 'gap' of DNA, enable cleavage of the target mRNA by RNase H, which recognizes DNA/RNA heteroduplexes. Usually all the phosphodiester linkages are converted to phosphorothioate.

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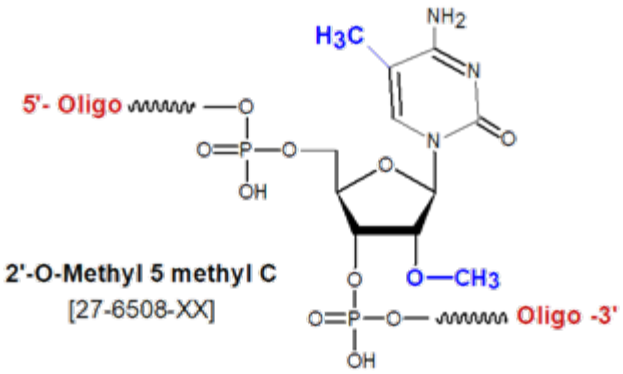
ASO's and siRNA Delivery. The development of effective delivery systems for antisense oligonucleotides is essential for their clinical therapeutic application. The most common delivery system involves a relatively hydrophobic molecule that can cross the lipid membrane. Cholesterol TEG, alpha-Tocopherol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

2'-O Me-5-Me-C

Category	Structural Studies
Modification Code	m5mC
Reference Catalog Number	27-6508
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	333.24



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2'-OMethyl-5-methyl cytosine (2'-OMe-5-Me-C) is an RNA monomer that pairs with G, and when substituted for C in an oligonucleotide, both increases the stability of the resulting duplex relative to the comparable unmodified form, and confers nuclease resistance at that position(1). This "double-methylated"-modified cytosine thus is an excellent choice for incorporation into anti-sense oligos, where both properties are particularly desirable. Furthermore, because anti-sense oligonucleotides containing a CpG motif are known to induce pro-inflammatory responses after *in vivo* administration to animals, including human, via activation of Toll-like receptor 9 (TLR9), substitution of 2'-OMe-5-Me-dC for C in these motifs can prevent or sharply reduce these undesirable immune responses (2,3).
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

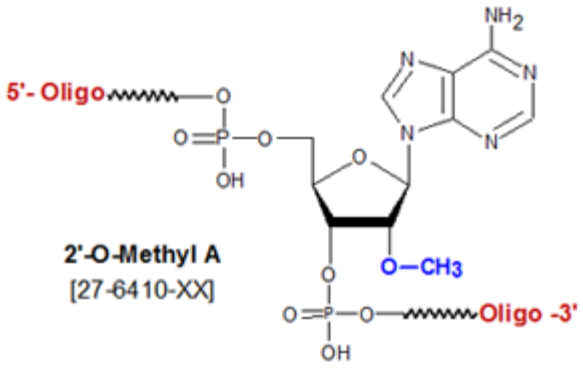
asp?mod_sp_cat_id=19 >Click here for complete list of duplex stability modifications **References**

1. Bundock, P.; de Both, M.T.J.; Hogers, R.C.J. 2006. Alternative nucleotides for improved targeted nucleotide exchange. Patent No. 2007073149, filed Dec 22. 2005, issued June 28, 2007. 2. Henry, S.P.; Stecker, K.; Brooks, D.; Monteith, D.; Conklin, B.; Bennett, C.F. Chemically modified oligonucleotides exhibit decreased immune stimulation in mice. *J. Pharmacol. Exp. Ther.* (2000), **292**: 468-479.

3. Yu, D.; Wang, D.; Zhu, F.-G.; Bhagat, L.; Dai, M.; Kandimalia, E.R.; Agrawal, S. Modifications Incorporated in CpG Motifs of Oligodeoxynucleotides Lead to Antagonist Activity of Toll-like Receptors 7 and 9. *J. Med. Chem.* (2009), **52**: 5108-5114.

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.
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Modification

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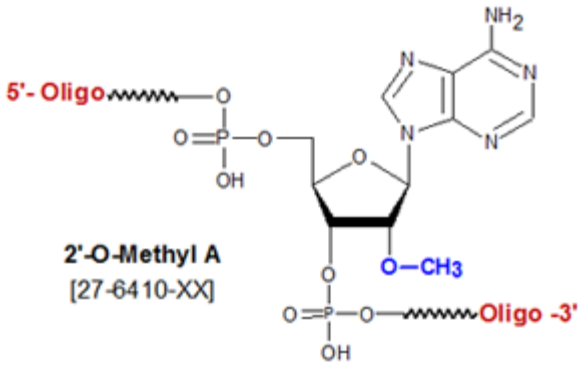
2'-O methyl bases

Category	Antisense
Modification Code	mN
Reference Catalog Number	27-6410N
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	334.97



Mixed base 2'-O methyl N has a setup charge of $250.00 per order.

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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]

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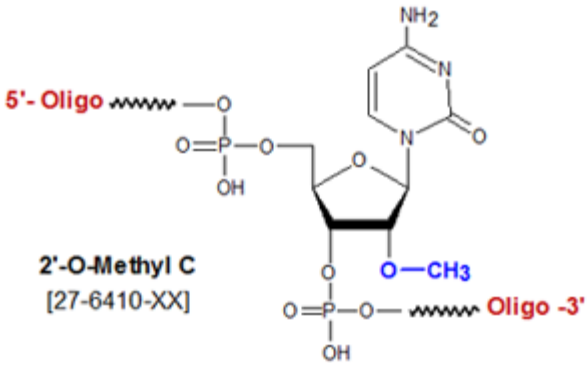
2'-O methyl C

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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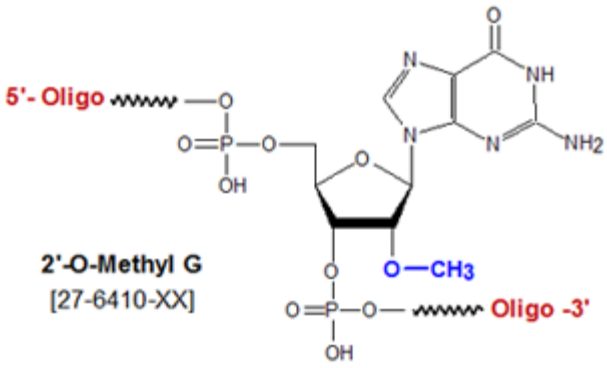
2'-O methyl G

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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Modifications Increasing Duplex Stability and Nuclease Resistance

Modification

Duplex Stability [Tm Increase]

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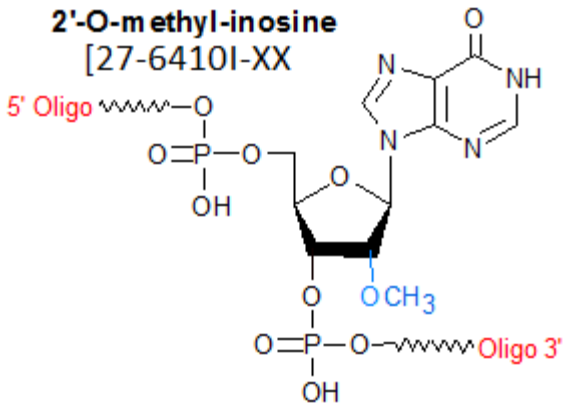
2'-O methyl Inosine

Category	Others
Modification Code	mI
Reference Catalog Number	27-6410I
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	344.22



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Modification

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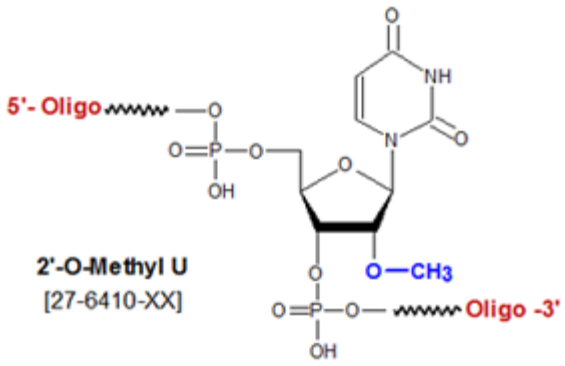
2'-O methyl U

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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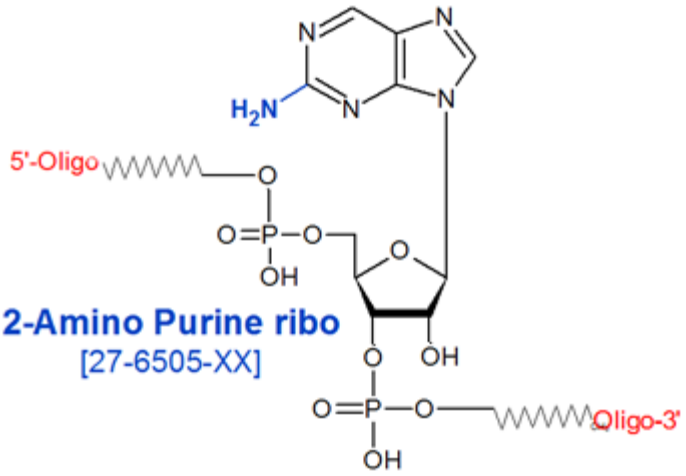
Oligo Modifications

2-Amino Purine ribose

Category	RNA Oligo Synthesis
Modification Code	2-APr
Reference Catalog Number	27-6505
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	329.21



Deletion of the O6 carbonyl group of guanosine results in 2-aminopurine riboside (2-AP). The hydrogen bonding pattern of the 2-aminopurine nucleobase (N1 acceptor, H-N2 donor) is isomeric with that of adenosine (N1 acceptor, H-N6 donor). 2-Amino Purine (2-AP) is a fluorescent molecule that is classified as an adenine and guanine analog, and thus can pair with both thymine and cytosine bases (1). It is an attractive choice for use as a probe in nucleic acid secondary structural studies, both because its fluorescence is highly sensitive to the nature of the local environment, and because it usually does not significantly affect duplex stability (2). Examples include the hairpin-loop structure of the (CAG)8 repeat, involved in several neurodegenerative disorders—2AP substituted for A (3), the G-quadruplex telomeric structure [AGGG(TTAGGG)3]—2AP substitute for A (4). 2-AP also has been used to characterize the effects of DNA mismatch repair on mutagenesis induced by several different nucleoside analogs (5).

2-Amino purine nucleoside allows the study of the role of exocyclic functional groups, base stacking, and hydrogen bonding patterns in purine-containing nucleic acids. For example, replacement of guanosine residues with 2-AP in the core region of hammerhead ribozymes was useful in determining their role in stabilizing the transition state of ribozyme cleavage (6). The nature of hydrogen-bonding between G-A mismatches in RNA internal loops was studied with 2-AP (7). The role of hydrogen-bonding and stacking interactions in the stability of GNRA loops was probed using 2-AP substitutions (8). The thermodynamic parameters for RNA loops of the type (A)n were determined using time-resolved spectrofluorimetry on RNAs bearing 2-AP residues in place of A residues, since 2-AP is blue fluorescent and was found to have properties in the (A)n region that were otherwise very similar to adenosine (9). In this sense, 2-AP can be used as a non-invasive conformational probe in RNA studies. Of the different phosphoramidites that have been used for 2-aminopurine riboside incorporation into RNA oligonucleotides (6-10), we have chosen to offer 2-Aminopurine riboside CEP in the particular form shown (6,9) which appears to offer the best results in RNA synthesis yield and purity. **References**

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Proc. Natl. Acad. Sci. U.S.A. 98 (1): 37-41. doi:10.1073/pnas.011442198.
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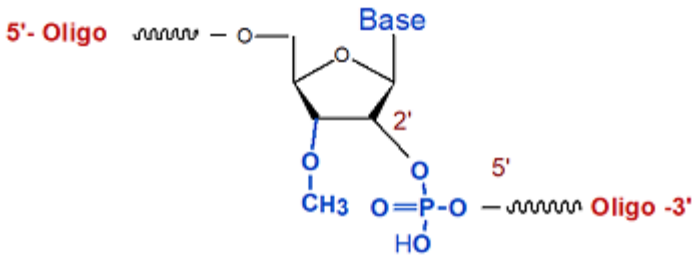
3'-O methyl bases

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



2'-5' linkage for internal sites

3'-O-Methyl Base
[27-6458N-XX]

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

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

3'-O methyl bases (3'-Ome)-(2'-5' linked), are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2', 5'-phosphodiester linkages selectively bind to complementary single-stranded 3', 5'- RNA over comparable 3', 5'- DNA. Presumably this selectively is a consequence of the 2', 5'- linkages destabilizing duplexes formed with 3', 5'- DNA more than those formed with 3', 5'-RNA, leading to 2'-5'- RNA:3', 5'- DNA duplexes having much lower Tm than the corresponding 2'-5'-RNA:3'- 5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.
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Nuclease resistance can be further enhanced by phosphorothiolation of appropriate phosphodiester linkages within the oligo. These modifications confers nuclease resistance, high binding affinity towards complementary RNA, reduced unspecific protein binding and extended half-life in tissues.
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Usually all the phosphodiester linkages are converted to phosphorothioate.
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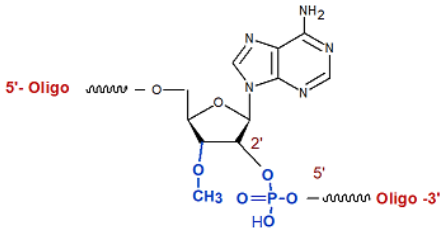
Cholesterol
Tocopherol (alpha-tocopherol, a natural isomer of vitamin E)
PEG

3'-O methyl rA

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



2'-5' linkage for internal sites

3'-O-Methyl A
[27-6458A-XX]

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3'-O methyl bases (3'-Ome)-(2'-5' linked), are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2', 5'-phosphodiester linkages selectively bind to complementary single-stranded 3', 5'- RNA over comparable 3', 5'- DNA. Presumably this selectively is a consequence of the 2', 5'- linkages destabilizing duplexes formed with 3', 5'- DNA more than those formed with 3', 5'-RNA, leading to 2'-5'- RNA:3', 5'- DNA duplexes having much lower Tm than the corresponding 2'-5'- RNA:3'- 5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.
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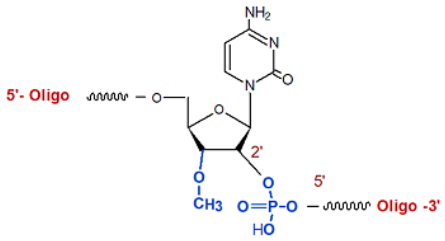
Cholesterol
Tocopherol (alpha-tocopherol, a natural isomer of vitamin E)
PEG

3'-O methyl rC

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



2'-5' linkage for internal sites

3'-O-Methyl C

[27-6458C-XX]

Antisense Oligos (ODN) & siRNA Oligo Modifications

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

3'-O methyl bases (3'-Ome)-(2'-5' linked), are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2', 5'-phosphodiester linkages selectively bind to complementary single-stranded 3', 5'- RNA over comparable 3', 5'- DNA. Presumably this selectively is a consequence of the 2', 5'- linkages destabilizing duplexes formed with 3', 5'- DNA more than those formed with 3', 5'-RNA, leading to 2'-5'- RNA:3', 5'- DNA duplexes having much lower Tm than the corresponding 2'-5'- RNA:3'- 5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

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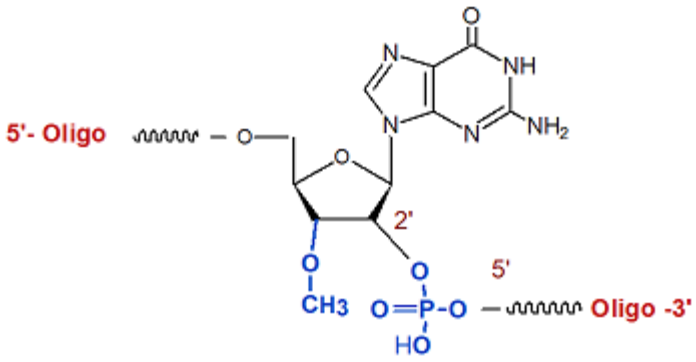
Cholesterol
Tocopherol (alpha-tocopherol, a natural isomer of vitamin E)
PEG

3'-O methyl rG

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



2'-5' linkage for internal sites

3'-O-Methyl G

[27-6458G-XX]

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3'-O methyl bases (3'-Ome)-(2'-5' linked), are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2', 5'-phosphodiester linkages selectively bind to complementary single-stranded 3', 5'- RNA over comparable 3', 5'- DNA. Presumably this selectively is a consequence of the 2', 5'- linkages destabilizing duplexes formed with 3', 5'- DNA more than those formed with 3', 5'-RNA, leading to 2'-5'- RNA:3', 5'- DNA duplexes having much lower Tm than the corresponding 2'-5'-RNA:3'- 5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

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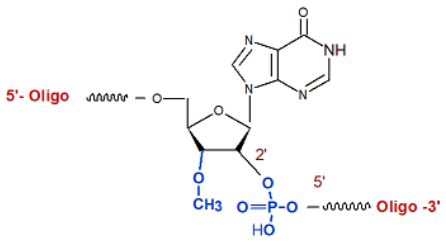
Cholesterol
Tocopherol (alpha-tocopherol, a natural isomer of vitamin E)
PEG

3'-O methyl rI

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



2'-5' linkage for internal sites

3'-O-Methyl Inosine

[27-6458I-XX]

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3'-O methyl bases (3'-Ome)-(2'-5' linked), are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2', 5'-phosphodiester linkages selectively bind to complementary single-stranded 3', 5'- RNA over comparable 3', 5'- DNA. Presumably this selectively is a consequence of the 2', 5'- linkages destabilizing duplexes formed with 3', 5'- DNA more than those formed with 3', 5'-RNA, leading to 2'-5'- RNA:3', 5'- DNA duplexes having much lower Tm than the corresponding 2'-5'- RNA:3'- 5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

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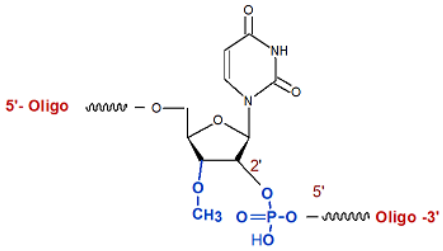
Tocopherol (alpha-tocopherol, a natural isomer of vitamin E)
PEG

3'-O methyl rU

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



2'-5' linkage for internal sites

3'-O-Methyl U
[27-6458U-XX]

Antisense Oligos (ODN) & siRNA Oligo Modifications
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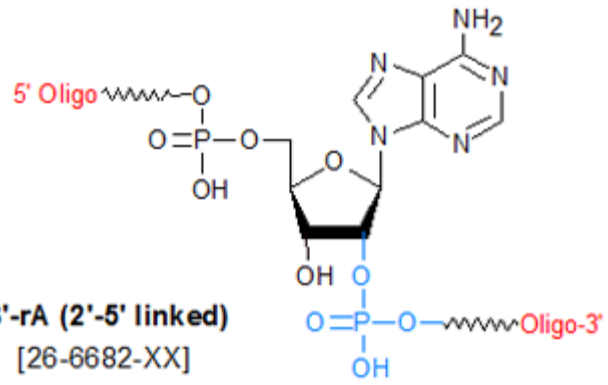
Cholesterol
Tocopherol (alpha-tocopherol, a natural isomer of vitamin E)
PEG

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

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



3'-rA (2'-5' linked)
[26-6682-XX]

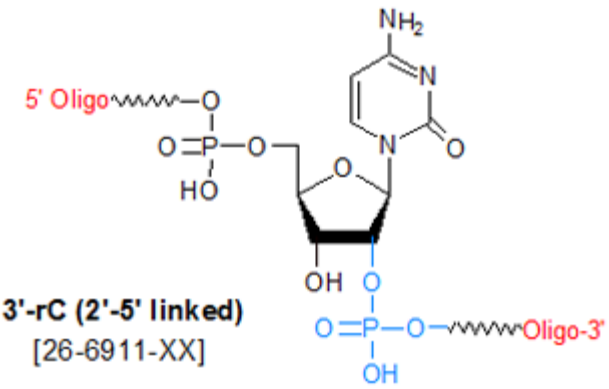
3'-riboadenosine (3'-rA)-(2'-5' linked), and the other three 3'-ribonucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA (1). Presumably this selectively is a consequence of the 2',5'-linkages destabilizing duplexes formed with 3',5'-DNA more than those formed with 3',5'-RNA, leading to 2'5'-RNA:3',5'-DNA duplexes having much lower Tm than the corresponding 2'5'-RNA:3',5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

A interesting application of the 3'-rA-(2',5'-linked) modification is as an activator for 2-5A dependent RNAse to direct it to cleave unique RNA sequences (2). In this approach, the 5'-phosphorylated, 2',5'-linked tetramer p5'A2'p5'A2'p5'A2'p5'A (abbreviated "2-5A" as covalently linked to anti-sense oligo, resulting in the chimera (2-5A:AS). The AS sequence of 2-5A:AS bound to a particular ssRNA target sequence, and he 2-5A activator sequence activated 2-5A-dependent RNA, causeing it to cleave the target after UpUp and UpA motifs. Selectively targeted destruction of ssRNA in vivo via this approach has potential applications for therapeutic control of gene expression in such diseases as cancer, viral infections, and certain genetic disorders. **References**

1. Giannaris, P.A.; Damha, M.J. Oligoribonucleotides containing 2',5'-phosphodiester linkages exhibit binding selectivity for 3',5'-RNA over 3',5'-ssDNA. *Nucleic Acids Res* (1993), **21**: 4742-4749.
2. Torrence, P.F.; Maitra, R.K.; Lesiak, K.; Khamnei, S.; Zhou, A.; Silverman, R.H. Targeting RNA for degradation with a (2'-5') oligoadenylate-antisense chimera. *Proc. Natl. Acad. Sci. USA* (1992), **90**: 1300-1304.

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

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



3'-rC (2'-5' linked)
[26-6911-XX]

3'-ribocytidine (3'-rC)-(2'-5' linked), and the other three 3'-ribonucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA (1). Presumably this selectively is a consequence of the 2',5'-linkages destabilizing duplexes formed with 3',5'-DNA more than those formed with 3',5'-RNA, leading to 2'5'-RNA:3',5'-DNA duplexes having much lower Tm than the corresponding 2'5'-RNA:3',5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

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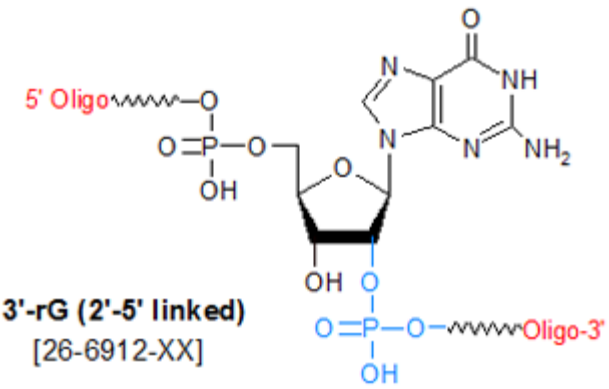
2. Torrence, P.F.; Maitra, R.K.; Lesiak, K.; Khamnei, S.; Zhou, A.; Silverman, R.H. Targeting RNA for degradation with a (2'-5') oligoadenylate-antisense chimera. *Proc. Natl. Acad. Sci. USA* (1992), **90**: 1300-1304.

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

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



3'-rG (2'-5' linked)
[26-6912-XX]

3'-riboguanosine(3'-rG)-(2'-5' linked), and the other three 3'-ribonucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA (1). Presumably this selectively is a consequence of the 2',5'-linkages destabilizing duplexes formed with 3',5'-DNA more than those formed with 3',5'-RNA, leading to 2'5'-RNA:3',5'-DNA duplexes having much lower Tm than the corresponding 2'5'-RNA:3',5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

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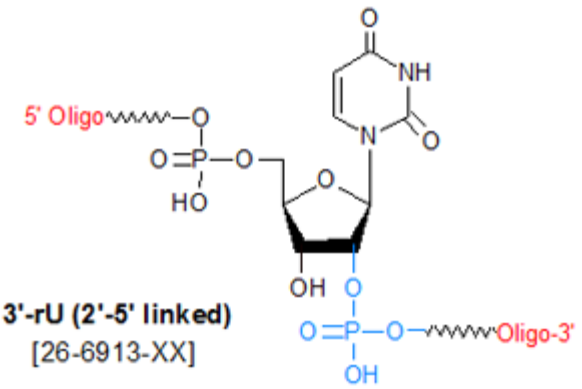
2. Torrence, P.F.; Maitra, R.K.; Lesiak, K.; Khamnei, S.; Zhou, A.; Silverman, R.H. Targeting RNA for degradation with a (2'-5') oligoadenylate-antisense chimera. *Proc. Natl. Acad. Sci. USA* (1992), **90**: 1300-1304.

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

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



3'-rU (2'-5' linked)
[26-6913-XX]

3'-ribouridine (3'-rU)-(2'-5' linked), and the other three 3'-ribonucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA (1). Presumably this selectively is a consequence of the 2',5'-linkages destabilizing duplexes formed with 3',5'-DNA more than those formed with 3',5'-RNA, leading to 2'5'-RNA:3',5'-DNA duplexes having much lower Tm than the corresponding 2'5'-RNA:3',5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

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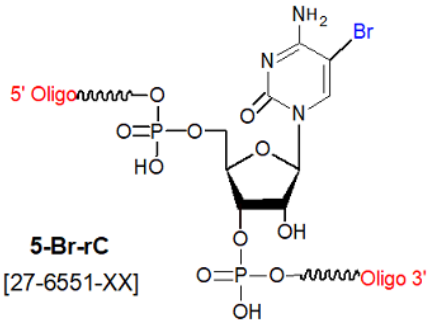
2. Torrence, P.F.; Maitra, R.K.; Lesiak, K.; Khamnei, S.; Zhou, A.; Silverman, R.H. Targeting RNA for degradation with a (2'-5') oligoadenylate-antisense chimera. *Proc. Natl. Acad. Sci. USA* (1992), **90**: 1300-1304.

5-Br rC

Category	RNA Oligo Synthesis
Modification Code	5-Br-rC
Reference Catalog Number	27-6551
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	384.08



5-halogenated rC, dC, rU and dU are primarily used to facilitate the determination of DNA and RNA structure by X-ray crystallography (1). When incorporated into a DNA or RNA molecule, the multi-wavelength anomalous dispersion (MAD) technique can be applied to obtain the phase information necessary to correctly calculate the electron density for the unit cell of the molecule under study. Because the MAD technique allows for the measurement of all the diffraction data with the same sample, is a much simpler to use than the traditional multiple isomorphous replacement (MIR) method for phase determination, which requires the synthesis of, and collection of diffraction data from, multiple heavy-atom isomorphic derivatives of the original molecule (2).

Halogenated nucleotides are also photo-labile, and can be used in UV-crosslinking experiments to investigate the structure of protein-DNA complexes. For example, incorporation of 5-Br-dC (and 5-Br-dG) into a 22-base dC-dG oligo resulted in the oligo being able to readily flip into the Z-DNA conformation in 10 mM MgCl2. This oligo was used as a probe to detect Z-DNA binding proteins (3).

An intriguing use of 5-Br-dC is as a post-SELEX modification to convert a SELEX-identified aptamer into a photo-aptamer (4). In this case, 5-methyl-dC serves as a non-photoreactive “placeholder” in the candidate nucleotide mixture used for aptamer selection during SELEX. One or more of the 5-methyl-dC nucleotides is then replaced by photo-labile 5-Br-dC to generate the corresponding photo-aptamer. As substitution of bromine for methyl at the 5-position of the base does not significantly change the steric properties of the oligo, the photo-aptamer typically has nearly the same binding affinity for the target as that of the (non-photo-reactive) original. **References**

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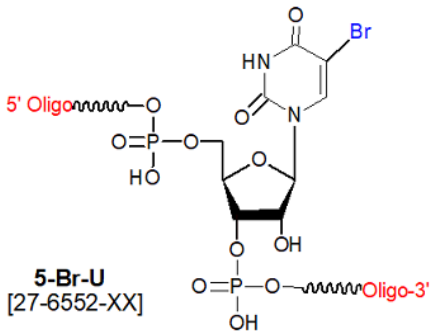
4. Schneider, D.J.; Wilcox, S.K.; Zichi, D.; Nieuwlandt, D.; Carter, J.; Gold, L. Improved SELEX and Photo-SELEX. (2008), PCT/US2008/070371 (WO/2009/012410).

5-Br rU

Category	RNA Oligo Synthesis
Modification Code	5-Br-rU
Reference Catalog Number	27-6552
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	385.07



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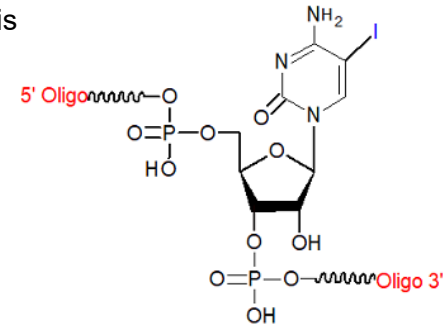
Oligo Modifications

5-I C

Category	RNA Oligo Synthesis
Modification Code	5-I-rC
Reference Catalog Number	27-6553
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	431.08



5-Iodo-Cytosine (5-I-rC)
[27-6553-XX]

5-halogenated rC, dC, rU and dU are primarily used to facilitate the determination of DNA and RNA structure by X-ray crystallography (1). When incorporated into a DNA or RNA molecule, the multi-wavelength anomalous dispersion (MAD) technique can be applied to obtain the phase information necessary to correctly calculate the electron density for the unit cell of the molecule under study. Because the MAD technique allows for the measurement of all the diffraction data with the same sample, is a much simpler to use than the traditional multiple isomorphous replacement (MIR) method for phase determination, which requires the synthesis of, and collection of diffraction data from, multiple heavy-atom isomorphic derivatives of the original molecule (2).

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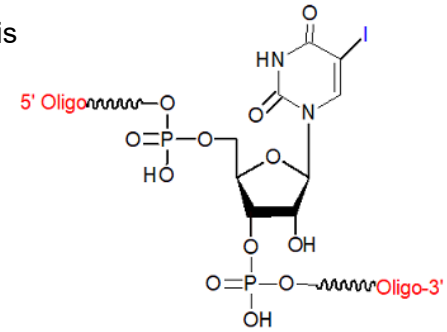
Oligo Modifications

5-I U

Category	RNA Oligo Synthesis
Modification Code	5-I-rU
Reference Catalog Number	27-6554
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	432.07



5-iodo-uridine (5-I-rU)

[27-6554-XX]

5-halogenated rC, dC, rU and dU are primarily used to facilitate the determination of DNA and RNA structure by X-ray crystallography (1). When incorporated into a DNA or RNA molecule, the multi-wavelength anomalous dispersion (MAD) technique can be applied to obtain the phase information necessary to correctly calculate the electron density for the unit cell of the molecule under study. Because the MAD technique allows for the measurement of all the diffraction data with the same sample, is a much simpler to use than the traditional multiple isomorphous replacement (MIR) method for phase determination, which requires the synthesis of, and collection of diffraction data from, multiple heavy-atom isomorphic derivatives of the original molecule (2).

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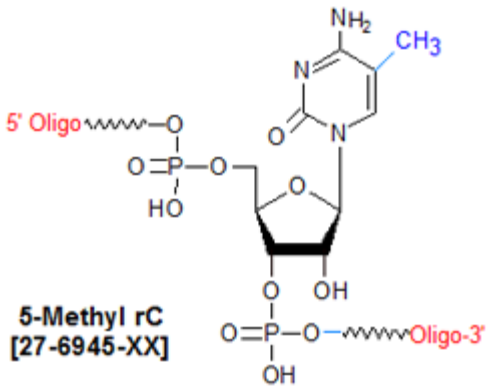
Oligo Modifications

5-Me rC [5mrC]

Category	Epigenetics
Modification Code	5mrC
Reference Catalog Number	27-6945
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	319.21



5-methyl cytosine (5-Me-rC) is a modified ribonucleotide which pairs with rG in an RNA duplex. 5-Me-rC forms a Watson-Crick base pair with rG in a normal manner. The presence of 5-Me-rC in cellular RNA is widespread, but its function is not well understood. 5-Me-rC has been observed in several base positions of eukaryotic and archaeal tRNA, most notably at positions 48/49, at the junction between the variable region and TphiC stem (1), suggesting an important structural role for it. The location of 5-Me-rC in rRNA from many organisms (bacterial to human) also appears to be fairly well-conserved, again hinting at an important structural role (2). Archael rRNA is an exception, however, as the number and location of 5-Me-rC is highly variable, complicating the picture (3,4). 5-Me-rRNA is found in the 5'-cap structure of mRNA, as well as in tRNA-like structures within other RNA molecules, such as viral RNA and SINE elements (5).

While there is a recognition that 5-methyl-rC plays a structural role in stabilizing tRNA, and appropriate binding of Mg2+ ions to it (6), little is known about how this modification's presence within tRNA and rRNA affects mRNA translation within the ribosome (2). Some evidence exists which suggests that the presence of 5-Me-rC, at least in yeast tRNA, is required to minimize translation errors, this is not definitive (7). In rRNA, its presence may assist with both tRNA recognition and peptidyl transfer (8).

One intriguing functional possibility for RNA methylation via 5-Me-rC is as a modulator of the innate human immune system. In one study, while a set of unmodified RNA strongly stimulated this system via Toll-like receptor activation, incorporation of 5-methyl-C into the oligos of this set dramatically reduced their stimulatory effect (9). These observations suggest that methylation interferes with the ability of the innate immune system to recognize RNA. Use of this principle may have therapeutic implications for a number of immune-system-related disorders.

The observation of RNA-dependent inheritance of certain phenotypes in mouse hints at a second possibility for RNA methylation: as a regulator of epigenetic inheritance patterns (10). The recent discovery that 5-Me-rC is widespread in both the coding and non-coding mRNA (esp. in the UTRs) of the human transcriptome supports this, and suggests that RNA methylation may play a much broader role in post-transcriptional control of cellular RNA than was previously believed (11), raising the possibility that RNA methylation may be critical to the ability of the cell to support various states of growth and differentiation.

References

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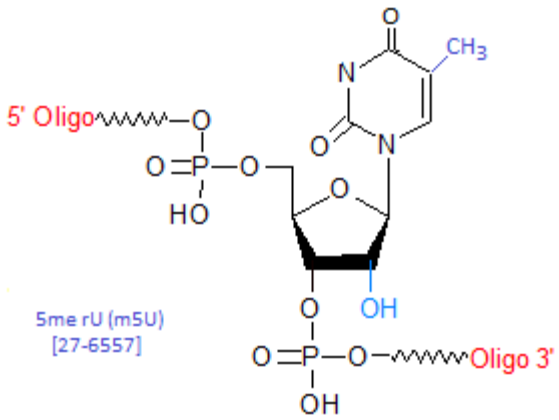
Oligo Modifications

5mU (m5U)

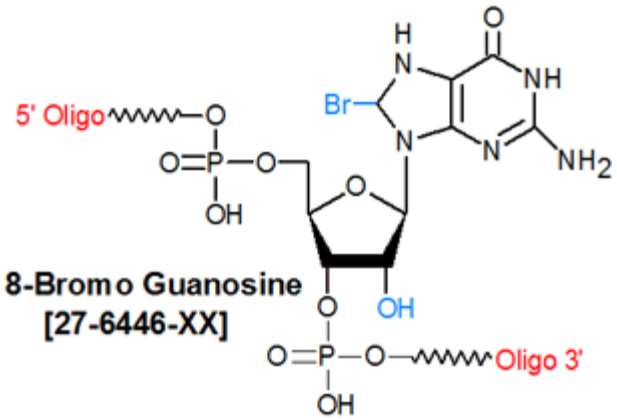
Category	Minor Bases
Modification Code	m5U
Reference Catalog Number	27-6557
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	320.19



5-Methyl-Uridine (m5U)are useful for analyzing RNA structure and activity relationships

8-Br rG

Category	RNA Oligo Synthesis
Modification Code	8-Br-rG
Reference Catalog Number	27-6446
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	424.11



8-Bromoguanosine is a brominated derivative of guanosine. Purine nucleobases with bromine at position eight are known to preferentially adopt the syn conformation as nucleosides and, thus, can be used to reduce the conformational heterogeneity of RNA to potentially enhance its function(1). It is reported to activate lymphocytes through an intracellular mechanism to exert immunostimulatory effects (2,3).

References

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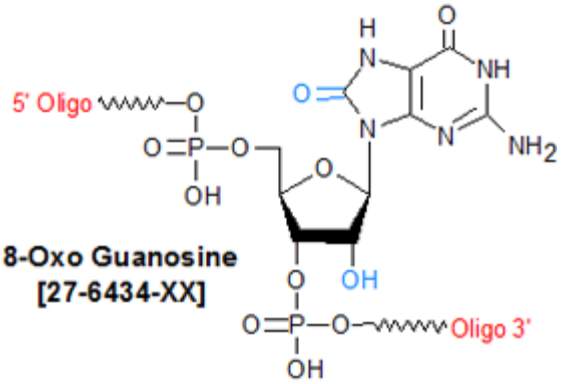
8-Oxo rG [8-Oxo-rG]

Category	Structural Studies
Modification Code	8-Oxo-rG
Reference Catalog Number	27-6434
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	361.21



8-Oxo-riboguanosine (8-Oxo-G) is classified as an oxidized ribonucleotide, and is primarily used in studies of oxidative RNA damage and associated RNA repair and RNA turnover mechanisms within the cell. In the cell, 8-Oxo-G RNA lesions are formed by reaction with reactive oxygen species (ROS) generated either via normal oxidative metabolic processes, UV ionizing radiation, or exposure to oxidative agents such as hydrogen peroxide, ethanol, ammonia and 2-nitropropane (an industrial solvent) (1,2). Oxidative RNA damage can lead to defects in protein synthesis, for example, decreased rates of protein synthesis and production of aggregated or truncated peptides (3,4), with important implications in aging and neurodegenerative disorders and artherosclerosis (5,6). Current understanding of cellular repair and turnover mechanisms for RNA 8-Oxo-G lesions is reviewed in reference 1. **References**

1. Wurtmann, E.J., Wolin, S.L. RNA under attack: Cellular handling of RNA damage. *Crit. Rev. Biochem. Mol. Biol.* (2009), **44**: 34-49.
2. Cadet J., Douki, T., Badouard, C., Favier, A., Ravanat-J.L. Oxidatively generated damage to cellular DNA: mechanistic aspects. *In: Oxidative Damage to Nucleic Acids (Evans, M.F., Cooke, M.S., ed.),* Landes Bioscience; Austin, TX (2007), pp.1-13.
3. Shan, X., Chang, Y., Lin, C.G. Messenger RNA oxidation is an early event preceding cell death and causes reduced protein expression.*FASEB J.* (2007), **21**: 2753-2764.
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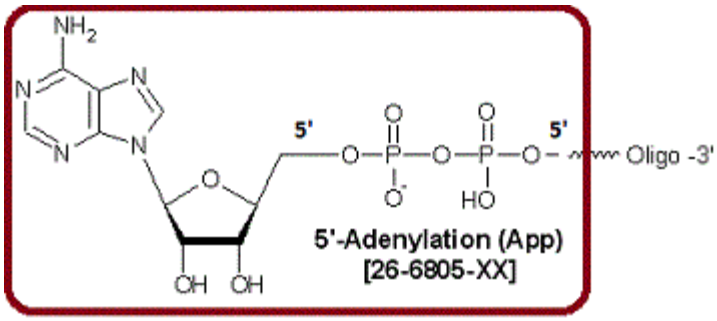
6. Martinet, W., de Meyer, G.R.Y., Herman, A.G., Kockx, M.M. Reactive oxygen species induce RNA damage in human atherosclerosis. *Eur. J. Clin. Invest.* (2004), **34**: 323-327.

Adenylation-5' (rApp)

Category	Conjugation Chemistry
Modification Code	5rApp
Reference Catalog Number	26-6805
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	358



YIELD 5' Adenylation is performed enzymatically and thus the yield obtained is lower than other chemically modified oligos.
~400 pmole (0.4nmol) final yield for 200 nmol scale
~1 nmole final yield for 1 umol scale

The ligation of two DNA or RNA molecules, by *T4 DNA ligase* or *T4 RNA ligase*, respectively, proceeds by a mechanism in which the enzyme uses ATP to place a 5',5'-adenyl pyrophosphoryl moiety (App) onto the 5'-end of a DNA/RNA oligonucleotide. Subsequent nucleophilic attack of the pyrophosphoryl linkage of this intermediate by the 3'-OH of a second oligonucleotide produces the concatenated product, with release of AMP (1,2). The key role that 5'-adenylated DNA/RNA plays in nucleic acid ligation means that robust synthesis of stable, pre-adenylated oligonucleotides (5'-App oligos), which act as substrates for T4 ligases in the absence of ATP, could be beneficial in any experimental study involving ligation.

Gene Link provides 5'-adenylation of **any** DNA or RNA oligonucleotide synthesized by us, as a custom service. To prevent the 5'-App oligo from self-ligating, the 3'-end is capped with a 3'-terminal blocking group, such as a dideoxy nucleotide or 3'-amino linker, which lack 3'-OH groups. The resulting 5'-App oligonucleotide is stable and ready for use in any ligation-based application. Examples of such applications include the following:

(a) miRNA library construction/next-generation sequencing: miRNAs processed *in vivo* are short (21-23 nt) and have 5'-phosphate and 3'-OH termini. Consequently, construction of high-quality miRNA libraries from cellular RNA is difficult, because attempts to ligate adaptors to miRNA ends, using *T4 RNA ligase* and ATP, results in a high level of undesirable miRNA self-ligation. This problem can be eliminated by first using the ligase to attach a 5'-App-modified adaptor to the 3'-OH end of the miRNA **in the absence of ATP**, and then attaching a second adaptor to the 5'-phosphate end of the resulting miRNA-3'-adaptor molecule using the ligase in the presence of ATP. The miRNA library will now have the appropriate adaptors at both ends, and can be cloned into a suitable vector for subsequent sequencing (3-5).

(b) activated nucleic acid substrates for *in vitro*-selected ribo/deoxyribozymes: Performing detailed structure-function studies on long, catalytically-active, naturally-occurring RNA (*e.*

g., group I and II introns, ribonuclease P) often requires incorporation of site-specific modifications. However, because such modification is currently not possible by *in vitro* transcription methods, various combinations of modified, chemically synthesized RNA oligos and RNA transcripts must be ligated together to generate the desired long, modified RNA molecule. Since T4 ligases often exhibit low yields and a limited range of possible substrates when used to form such RNAs by ligation, *in vitro*-selected ribozymes/deoxyribozymes are being developed to broaden the available selection of ligation strategies (6). 5'-App oligonucleotides, containing desired site-specific modifications, can be used as activated substrates for these ribo/deoxyribozymes, both during the initial selection process used to develop them, and during the subsequent construction of a particular long RNA to be used in a structure-function study.

(c) broaden the range of explorable RNA substrates for ribo/deoxyribozymes: The RNA variant 5'-triphosphorylated RNA (5'-pppRNA) is produced by *in vitro* transcription with *T7 RNA polymerase* (7). Both natural ribozymes and *in vitro*-selected ribo/deoxyribozymes can use 5'-pppRNA as a substrate for ligation, with the 5'-leaving group being pyrophosphate (PPi). However, for a 5'-pppRNA produced *in vitro* by *T7 RNA polymerase*, the 5'-pppNTP is 5'-pppG. 5'-AppRNA is structurally similar to 5'-pppRNA, and the 5'-AMP leaving group, like PPi, is also good. However, 5'-AppRNA can be synthesized with any nucleotide at the 5'-end, not only G. Thus, substitution of 5'-AppRNA for 5'-pppRNA broadens the range of sequences that can be explored as RNA substrates for both natural and *in vitro*-selected ribozymes/deoxyribozymes.

(d) activated 5'-pyrimidine-rich RNA: RNA that is pyrimidine-rich at the 5'-end is often difficult or even impossible to transcribe *in vitro* with phage polymerases. Consequently, obtaining pyrimidine-rich RNA that is 5'-phosphorylated, and thus suitable for ligation, is a major challenge (8). 5'-adenylation of such RNA molecules could prove to be a viable strategy for activating them for ligation.

(e) 5'-end labeling: The adenylate group attached to the oligo contains a ribose 2',3'-diol moiety that can be oxidized to aldehydes using sodium periodate. Consequently, a 5'-App nucleic acid could, after such oxidation, be labeled at the 5'-end with fluorescent dyes or other biophysical probes via reductive amination of the aldehydes (3,9). This labeling strategy would be useful in cases where insertion of a particular 5'-end modification using solid phase synthesis is either not possible or not desired.

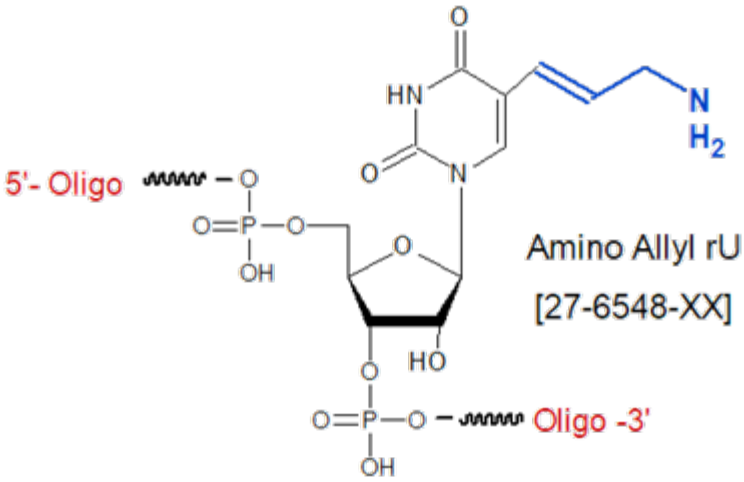
References

1. Lehman, I.R. DNA ligase: Structure, mechanism, and function, *Science* (1974), **186**: 790-797.
2. Ohtsuka, E., Nishikawa, S., Sugiura, M., Ikehara, M. Joining of ribooligonucleotides with T4 RNA ligase and identification of the oligonucleotide-adenylate intermediate, *Nucleic Acids Res.* (1976), **3**: 1613-1623.
3. Wang, Y., Silverman, S.K. Efficient RNA 5'-adenylation by T4 DNA ligase to facilitate practical applications *RNA* (2006), **12**: 1142-1146.
4. Pak, J., Fire A. Distinct Populations of Primary and Secondary Effectors During RNAi in C. elegans, *Science* (2007), **315**: 241-244.
5. Vigneault, F., Sismour, A.M., Church, G.M. Efficient microRNA capture and bar-coding via enzymatic oligonucleotide adenylation, *Nature Methods* (2008), **5**: 777-779.
6. Flynn-Charlebois, A., Wang, Y., Prior, T.K., Rashid, I., Hoadley, K.A., Coppins, R.L., Wolf, A.C., Silverman, S.K. Deoxyribozymes with 2'-5' RNA Ligase Activity. *J. Am. Chem. Soc.* (2003), **125**: 2444-2454.
7. Milligan, J.F., Groebe, D.R., Witherell, G.W., Uhlenbeck, O.C. (1987) Oligoribonucleotide synthesis using T7 RNA polymerase and synthetic DNA templates, *Nucl. Acids. Res.* (1987), **15**: 8783-8798. 8. Fukunaga, J., Gouda, M., Umeda, K., Ohno, S., Yokogawa, T., Nishikawa, K. Use of RNase P for efficient preparation of yeast tRNATyr transcript and its mutants, *J Biochem (Tokyo)* (2006), **139**: 123-127.
9. Proudnikov, D., Mirzabekov, A. Chemical methods of DNA and RNA fluorescent labeling, *Nucl. Acids Res.* (1996), **24**: 4535-4542.

Oligo Modifications

Amino Allyl rU

Category	Others
Modification Code	AmAll-rU
Reference Catalog Number	27-6548
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	363.79



Aminoallyl-dU and rU can be incorporated in custom oligos at any position. The aminoallyl base is similar to dUTP that is enzymatically incorporated into DNA with Reverse Transcriptases, Taq DNA polymerase, phi29 DNA Polymerase, Klenow Fragment, Klenow Fragment, exo- and DNA Polymerase I.
The custom oligos containing aminoallyl modifications can be similarly used to label with amino reactive NHS fluorescent dye, biotin or hapten. The custom oligos can be used as primers for PCR, reverse transcription, nick translation and any other similar application.
Gene Link specializes in the synthesis of long oligos up to 250mer; these oligos with multiple internal sites of aminoallyl modification subsequently labelled with appropriate dyes can be used for FISH as probes, multicolor FISH and comparative genome hybridization (CGH), dot blots and microarray applications.

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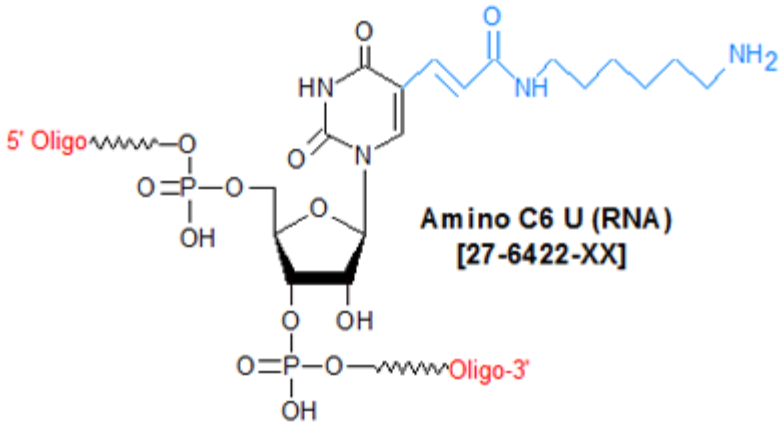
The primary amine labelled oligos can also be conjugated to carboxyl functional groups usually for solid supports applications using EDC mediated reaction as shown in the figure below.

Amino C6 U

Category	Minor Bases
Modification Code	AmC6U
Reference Catalog Number	27-6422
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	474.4



Amino C6-U can be used to internally incorporate an active primary amino group into either an RNA oligonucleotide or a chimeric oligo. The presence of the primary amino group allows the user to label the oligo with a variety of different ligands for affinity, reporter or protein moieties (as NHS esters or isothiocyanates), depending on the application. Examples include biotin, digoxigenin, and fluorescent dyes or quenchers, magnetic beads and enzymes (for example, alkaline phosphatase).

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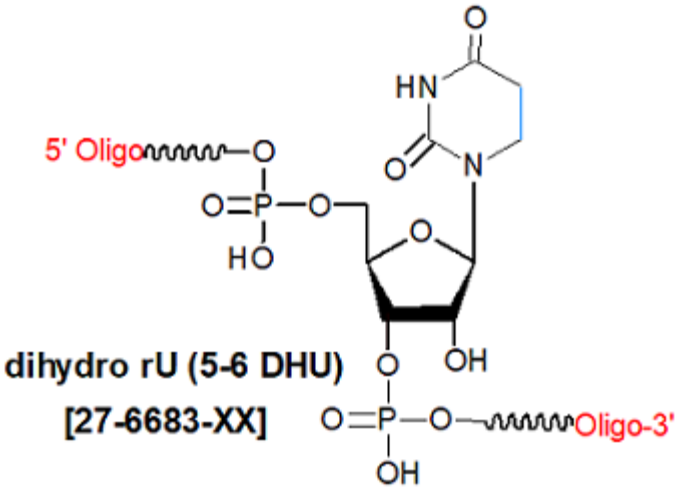
The primary amine labelled oligos can also be conjugated to carboxyl functional groups usually for solid supports applications using EDC mediated reaction as shown in the figure below.

dihydro rU (5-6 DH rU)

Category	Minor Bases
Modification Code	5-6-DHrU
Reference Catalog Number	27-6683
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	308.19



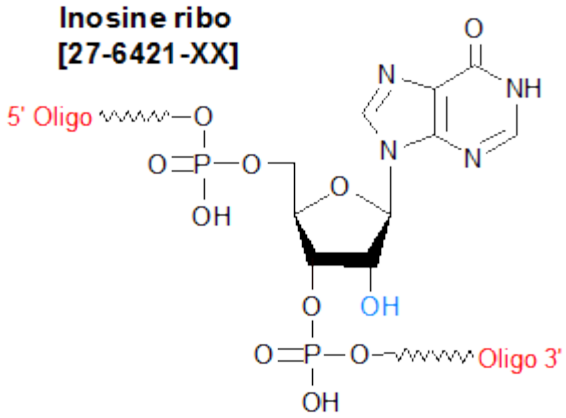
Dihydro dU (5,6-DHU) is primarily used in studies of irradiative DNA damage and associated repair mechanisms. In the cell, 5,6-DHU DNA lesions are formed by gamma irradiation of deoxycytosine under anoxic conditions, resulting in deamination followed by addition of hydrogen at C5 and C6 of the base. DHU is highly mutagenic, leading to C-to-T transitions at the mutation site (because DNA polymerase inserts A opposite the 5,6-DHU lesion) (1). Because DHU is recognized and removed by endonuclease III and other eukaryotic endo III homologs, DHU-modified oligos are used in model systems for studying DNA damage and repair mechanisms. **References**

1. Liu, J., Doetsch, P.W. Escherichia coli RNA and DNA polymerase bypasss of dihydrouracil: mutagenic potential via transcription and replication. *Nucleic Acids Res.* (1998), **26**: 1707-1712.

Oligo Modifications

Inosine ribo [rI]

Category	Others
Modification Code	rI
Reference Catalog Number	27-6421
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	330.19

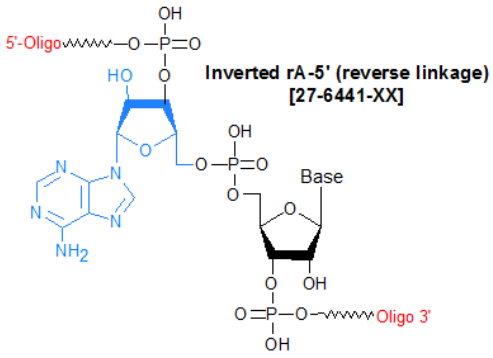


Inosine (dI & rI) is classified as a nucleotide base analog; it is structurally similar to guanosine, but is missing the 2-amino group. Because it is able to form two hydrogen bonds with each of the four natural nucleotide bases (1), it is often used by researchers as a "universal" base meaning that it can base pair with all the naturally occurring bases n synthetic oligos. Inosine typically is substituted for the nucleoside at the third ("wobble") position of codons, in order to reduce the complexity of mixed oligo PCR primers/hybridization probes needed to deal with degenerate codons in the target DNA (2, 3). However, it is important to remember that I does not base pair equally well with the naturally-occurring bases, with the order of thermodynamic stability being I-C >: I-A > I-G ~ I-T. Thermodynamic stability of inosine-containing duplexes is also affected by neighboring bases (4). Consequently, when using inosine as an alternative to mixed-base degeneracy at a particular oligo position, keep in mind that the above base-pairing bias may lead to differences in the oligo's priming or hybridization efficiency in the corresponding degenerate regions of the target. Because the effect could be more pronounced when dI is at the 3'-position, it may be advisable to use primers with and without I at the 3'-end, in order to maximize diversity of PCR products (5). **References**

1. Oda, Y, Uesugi, S., Ikehara, M., Kawase, Y., Ohtsuka, E. NMR studies for identification of dI:dG mismatch base-pairing structure in DNA. *Nucleic Acids Res.* (1991), **19**: 5263-5267.
2. Liu, H., Nichols, R. PCR amplification using deoxyinosine to replace entire codon and at ambiguous positions.*Biotechniques.* (1994), **16**: 24-26.
3. Ohtsuka, E., Matsuki, S., Ikehara, M., Takahashi, Y., Matsubara, K. An alternative approach to deoxynucleotides as hybridization probes by insertion of deoxyinosine at ambiguous codon positions. *J. Biol. Chem.* (1985), **260**: 2605-2608.
4. Martin, F.H., Castro, M.M., Aboul-ela, F., Tinoco, I. Base pairing involving deoxyinosine: implications for probe design. *Nucleic Acids Res.* (1985), **13**: 8927-8938.
5. Ben-Dov, E., Shapiro, O.H., Siboni, N., Kushmaro, A. Advantage of Using Inosine at the 3' Termini of 16S rRNA Gene Universal Primers for the Study of Microbial Diversity. *Appl. Environ. Microb.* (2006), 72: 6902-6906.

Inverted rA (reverse linkage)

Category	Minor Bases
Modification Code	Inv-rA
Reference Catalog Number	27-6441
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	329.21



Reverse synthesis can be achieved by incorporation modifications where the synthesis orientation can be changed as desired. Oligo can be designed for the production of 5'-5' or 3'-3' linkages or a combination of these in the same oligo. These modified phosphodiester linkage modified oligos are useful in antisense studies, or to synthesize oligonucleotide segments in the opposite sense from normal synthesis, for structural studies.

Having a single inverted base at the 3' position with a 3'-3' linkage imparts the oligo exonuclease resistance and prevents extension by polymerases as there is no free 3' hydroxyl group to initiate synthesis.

Construct Examples 5'-NNNNNNNN-3'-3'-NNNNNNNN-5'

The construct shown above starts at the right side in orange font 5' end with an inverted base, towards the left side is the 3' end. This orientation will continue with more sites of the inverted bases. Insertion of a standard bases shown in green font will have a 3'-3' phosphodiester linkage and to the left is the 5' end. 3'-NNNNNNNN-5'--3'-[Inv-dT]-5'--5'-NNNNNNNN-3'

The construct shown above is with a single [Inv-dT] to signify the orientation change point after the standard bases in green font; chemical synthesis starts from the 3' end. Note ALL bases shown in orange font after the first inverted bases towards the left will also be inverted bases to keep the reverse orientation.

The same construct is shown below but with standard orientation bases shown in green font inserted after the inverted base, this will reverse the polarity and thus the oligo will have a 5' and a 3' end. 5'-NNNNNNNN-3'--3'-[Inv-dT]-5'--5'-NNNNNNNN-3'

The reverse configuration allows for oligonucleotide synthesis in the 5' to 3' direction (instead of the standard 3' to 5' direction). Reverse synthesis is advisable in the following cases:

1. Formation of oligos containing hairpin loops with parallel strands. Oligos with hairpin loops are used for structural studies into duplex formation.

Typically the strands of the stem of the hairpin are anti-parallel. However, by switching to 5'-phosphoramidites for part of the synthesis of such an oligo (for example, initiating the switch during synthesis of the loop portion of the hairpin), the strands of the hairpin stem will be in parallel orientation (1).

2. Formation of nuclease resistant (5'-5', 3'-3') linkages. Anti-sense oligos containing terminal 5'-5' or 3'-3' linkages are highly resistant to exonuclease degradation. For the terminal 5'-5' linkage, the appropriate 5'-phosphoramidite is incorporated at the 5'-end in the final synthesis cycle. For the terminal 3'-3' linkage, the appropriate deoxynucleoside-5'-CPG is used as the solid support for the 3'-end, followed by synthesis of the oligo in the standard 3'-5' direction to make the terminal 3'-3' linkage (2).

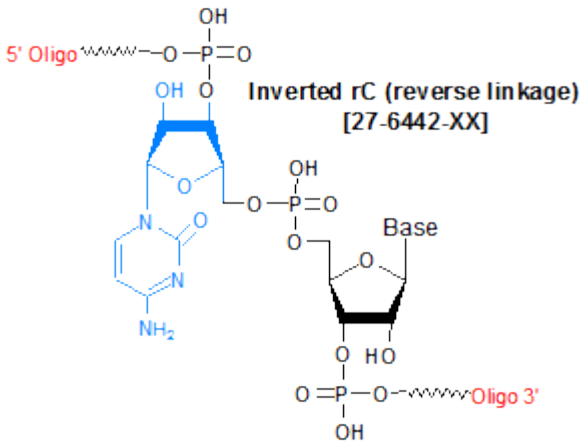
Having a single inverted base at the 3' position with a 3'-3' linkage imparts the oligo exonuclease resistance and prevents extension by polymerases as there is no free 3' hydroxyl group to initiate synthesis.

3. 3'-terminal base/moiety cannot be attached to a CPG. Examples include 2',3'-ddT or ddI. **References**

1. van de Sande, J.H., Ramsing, N.B., Germann, M.W., Flhorstn, W., Kalisch, B.W., Clegg, R.C., Pon, R.T., Jovin, T.M. Parallel-Stranded DNA. *Science* (1988), **241**: 551-557.
2. Ortigao, J.F.R., Rosch, H., Selter, H., Frohlich, A., Lorenz, A., Montenarh, M., Seliger, H. Antisense effect of oligodeoxynucleotides with inverted terminal internucleotidic linkages: a minimal modification protecting against nucleolytic degradation.*Antisense Res. Dev.* (1992), **2**: 129-146.

Inverted rC (reverse linkage)

Category	Minor Bases
Modification Code	Inv-rC
Reference Catalog Number	27-6442
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	329.21



Reverse synthesis can be achieved by incorporation modifications where the synthesis orientation can be changed as desired. Oligo can be designed for the production of 5'-5' or 3'-3' linkages or a combination of these in the same oligo. These modified phosphodiester linkage modified oligos are useful in antisense studies, or to synthesize oligonucleotide segments in the opposite sense from normal synthesis, for structural studies.

Having a single inverted base at the 3' position with a 3'-3' linkage imparts the oligo exonuclease resistance and prevents extension by polymerases as there is no free 3' hydroxyl group to initiate synthesis.

Construct Examples 5'-NNNNNNNN-3'-3'-NNNNNNNN-5'

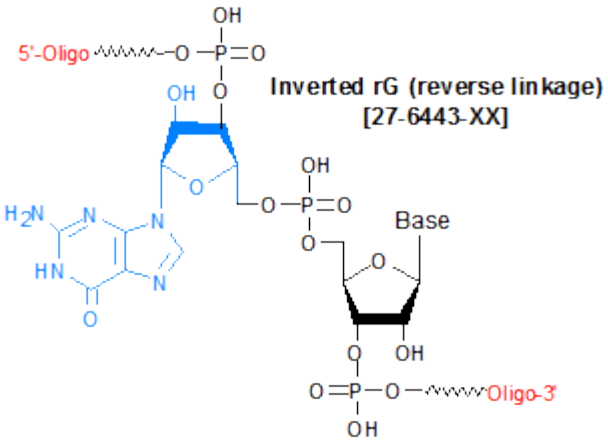
The construct shown above starts at the right side in orange font 5' end with an inverted base, towards the left side is the 3' end. This orientation will continue with more sites of the inverted bases. Insertion of a standard bases shown in green font will have a 3'-3' phosphodiester linkage and to the left is the 5' end. 3'-NNNNNNNN-5'--3'-[Inv-dT]-5'--5'-NNNNNNNN-3'

The construct shown above is with a single [Inv-dT] to signify the orientation change point after the standard bases in green font; chemical synthesis starts from the 3' end. Note ALL bases shown in orange font after the first inverted bases towards the left will also be inverted bases to keep the reverse orientation.

The same construct is shown below but with standard orientation bases shown in green font inserted after the inverted base, this will reverse the polarity and thus the oligo will have a 5' and a 3' end. 5'-NNNNNNNN-3'--3'-[Inv-dT]-5'--5'-NNNNNNNN-3'

Inverted rG (reverse linkage)

Category	Minor Bases
Modification Code	Inv-rG
Reference Catalog Number	27-6443
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	329.21



Reverse synthesis can be achieved by incorporation modifications where the synthesis orientation can be changed as desired. Oligo can be designed for the production of 5'-5' or 3'-3' linkages or a combination of these in the same oligo. These modified phosphodiester linkage modified oligos are useful in antisense studies, or to synthesize oligonucleotide segments in the opposite sense from normal synthesis, for structural studies.

Having a single inverted base at the 3' position with a 3'-3' linkage imparts the oligo exonuclease resistance and prevents extension by polymerases as there is no free 3' hydroxyl group to initiate synthesis.

Construct Examples 5'-NNNNNNNN-3'-3'-NNNNNNNN-5'

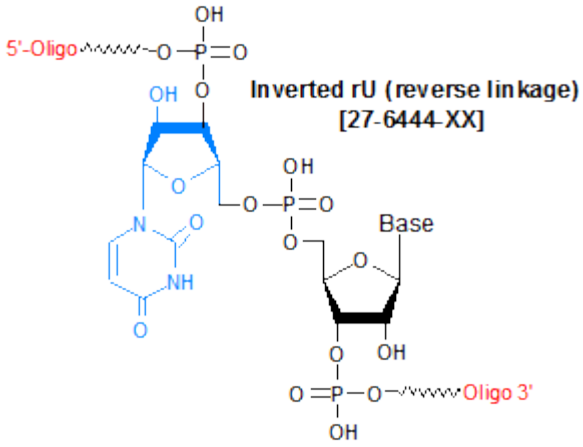
The construct shown above starts at the right side in orange font 5' end with an inverted base, towards the left side is the 3' end. This orientation will continue with more sites of the inverted bases. Insertion of a standard bases shown in green font will have a 3'-3' phosphodiester linkage and to the left is the 5' end. 3'-NNNNNNNN-5'--3'-[Inv-dT]-5'--5'-NNNNNNNN-3'

The construct shown above is with a single [Inv-dT] to signify the orientation change point after the standard bases in green font; chemical synthesis starts from the 3' end. Note ALL bases shown in orange font after the first inverted bases towards the left will also be inverted bases to keep the reverse orientation.

The same construct is shown below but with standard orientation bases shown in green font inserted after the inverted base, this will reverse the polarity and thus the oligo will have a 5' and a 3' end. 5'-NNNNNNNN-3'--3'-[Inv-dT]-5'--5'-NNNNNNNN-3'

Inverted rU (reverse linkage)

Category	Minor Bases
Modification Code	Inv-rU
Reference Catalog Number	27-6444
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	329.21



Reverse synthesis can be achieved by incorporation modifications where the synthesis orientation can be changed as desired. Oligo can be designed for the production of 5'-5' or 3'-3' linkages or a combination of these in the same oligo. These modified phosphodiester linkage modified oligos are useful in antisense studies, or to synthesize oligonucleotide segments in the opposite sense from normal synthesis, for structural studies.

Having a single inverted base at the 3' position with a 3'-3' linkage imparts the oligo exonuclease resistance and prevents extension by polymerases as there is no free 3' hydroxyl group to initiate synthesis.

Construct Examples 5'-NNNNNNNN-3'-3'-NNNNNNNN-5'

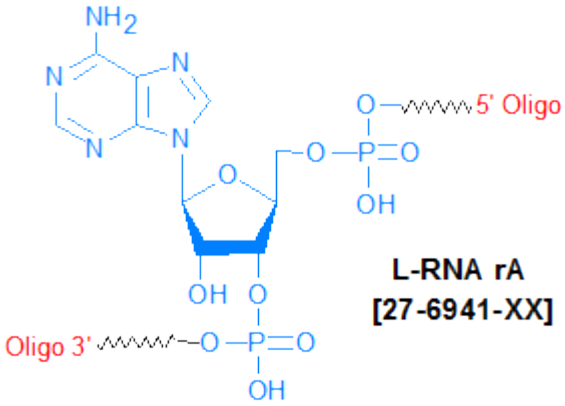
The construct shown above starts at the right side in orange font 5' end with an inverted base, towards the left side is the 3' end. This orientation will continue with more sites of the inverted bases. Insertion of a standard bases shown in green font will have a 3'-3' phosphodiester linkage and to the left is the 5' end. 3'-NNNNNNNN-5'--3'-[Inv-dT]-5'--5'-NNNNNNNN-3'

The construct shown above is with a single [Inv-dT] to signify the orientation change point after the standard bases in green font; chemical synthesis starts from the 3' end. Note ALL bases shown in orange font after the first inverted bases towards the left will also be inverted bases to keep the reverse orientation.

The same construct is shown below but with standard orientation bases shown in green font inserted after the inverted base, this will reverse the polarity and thus the oligo will have a 5' and a 3' end. 5'-NNNNNNNN-3'--3'-[Inv-dT]-5'--5'-NNNNNNNN-3' #799f84">NNNNNNNN-3'--3'-[Inv-dT]-5'--5'-NNNNNNNN-3'

L-RNA rA

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



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

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

L-DNA Applications

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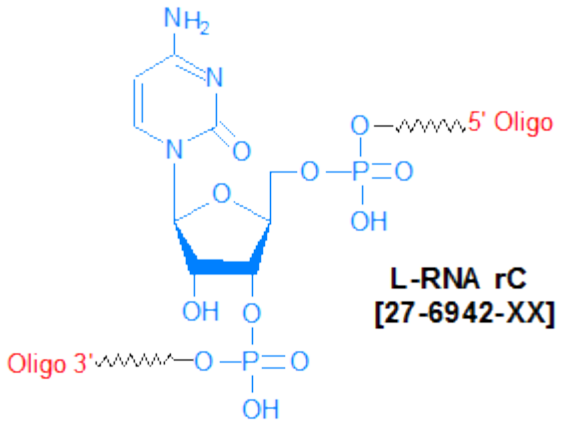
Oligo Modifications

L-RNA rC

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



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L-DNA Applications

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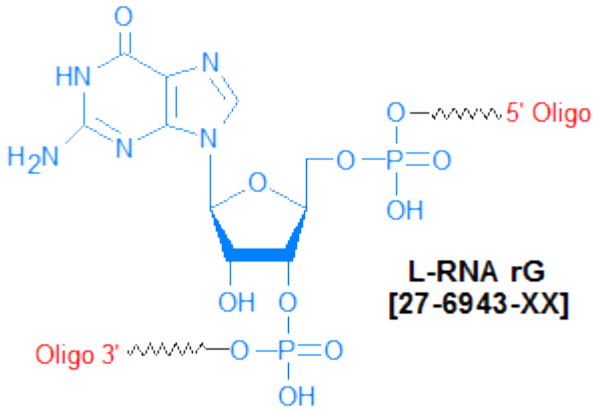
Chem. Sci., 2014,5, 1533-1537

L-RNA rG

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



L-RNA rG
[27-6943-XX]

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L-DNA Applications

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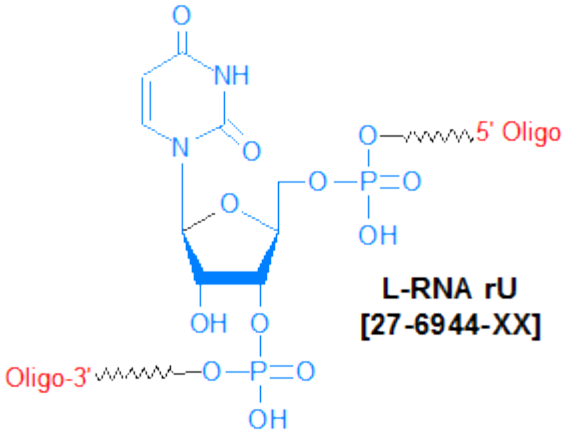
Chem. Sci., 2014,5, 1533-1537

L-RNA rU

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



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L-DNA Applications

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4) Hayashi G., Hagihara M., Nakatani K. Application of L-DNA as a molecular tag. Nucleic Acids Symp. Ser. 2005;49:261?262
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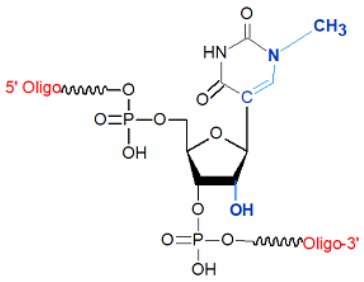
N1-methyl pseudoUridine (m1-psi rU)

Category	Structural Studies
Modification Code	m1-psi-rU
Reference Catalog Number	27-6532
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	320.19

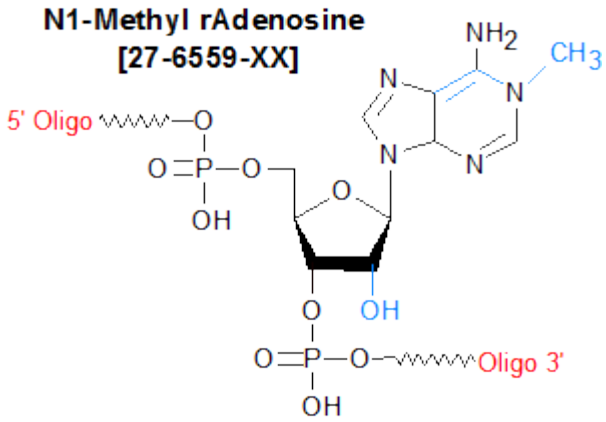


N1-methyl pseudoUridine (m1-psi rU)
[27-6532-XX]

RNA methylation occurs in a large selection of RNA nucleosides and this post transcriptional modification of RNA, carried out by a variety of RNA methyltransferases, appears in a wide variety of RNA species - including tRNA, mRNA, miRNA and RNA viruses. Over 90 methylated nucleosides have been found in tRNA and these play many significant roles in tRNA structure. In addition, methylation appears to mark the tRNA as mature, preventing its degradation as well as directing localization within the cell. mRNA, modified with 1-methylpseudouridine (1-Me-Ψ) alone or in combination with 5-methylcytidine (5-Me-C), significantly increases protein expression in cells and mouse models. 1-Me-Ψ is also a modified nucleobase that can greatly enhance the properties of mRNA by reducing immunogenicity and increasing stability.

N1-Methyl rA (m1A)

Category	Structural Studies
Modification Code	m1A
Reference Catalog Number	27-6559
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	344.24



Dimroth rearrangement of m1A to m6A under basic conditions.
Our Synthesis and deprotection scheme of mild conditions for deprotection of synthesized oligos for 60 hours at room temperature usually has no rearrangement.

Methylation of adenosine at position 1 produces a drastic functional change in the nucleobase. 1-Methyladenosine (pKa 8.25) is a much stronger base than adenosine (pKa 3.5). N-1 methylation excludes participation of the adenine base in canonical Watson-Crick base pairing and provides a positive charge to the nucleobase. This modification also alters the hydrophobicity of the base, the stacking properties, the ordering of water molecules and the chelation properties. The base may become involved in non-canonical hydrogen bonding, in electrostatic interactions and, in general, it may contribute to the conformational dynamics of the tRNA.

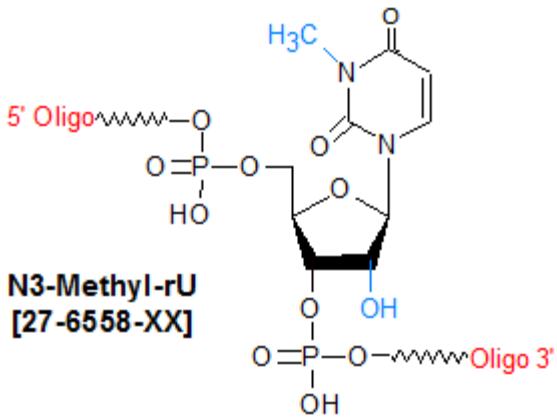
N1-Methyl riboadenosine (N1-Me-rA; also known as 1-Me-rA) is a methylated RNA nucleoside base, and is primarily used in the study of its role in tRNA folding. N1-Me-rA occurs in nature as a post-transcriptional modification, in which the N1 position of adenine is methylated by methyl-1-adenosine transferase (1). In tRNA, N1-Me-rA often is found at position 58 in the T loop, and position 14 in the D loop (2). Its presence introduces significant 3-D structural alteration to the tRNA (3); these alterations can be necessary for establishment of reverse transcription in virus-infected cells. For example, in order for HIV-1 to successfully infect cells, it must be able to divert tRNALys in the cell for reverse transcription; in order to do this, position 58 of the tRNA must be N3-Me-rA (4). **References**

(1) Anderson, J., Droogmans, L. Biosynthesis and function of 1-Me-Ado in tRNA. In *Topics in Current Genetics*, Springer Eds., 2005, 12: 121-139.
(2) Sierzputowska-Gracz, H., Gopal, D., Agris, P.F. Comparative structural analysis of 1-methyladenosine, 7-methylguanosine, ethenodenosine, and their protonated salts. IV. 1H, 13C and 15N NMR studies at natural isotope abundance. *Nucleic Acids Res.* (1986), **14**: 7783-7801.
(3) Helm, M., et al. The presence of modified nucleotides is required for cloverleaf folding of a human mitochondrial tRNA. *Nucleic Acids Res.* (1998), **26**: 1636-1643.
(4) Marquet, R., Dardel, F. Transfer RNA modifications and DNA editing in HIV-1 reverse transcription.

In *Topics in Current Genetics*, Springer Eds., 2005, 12: 401-429.

N3-methyl-rU [m3U]

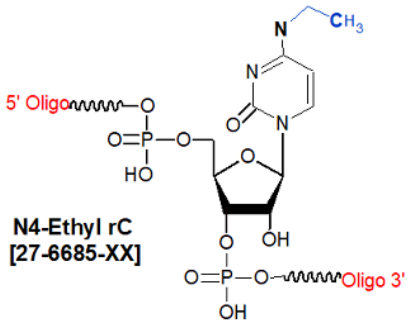
Category	Others
Modification Code	m3U
Reference Catalog Number	27-6558
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	320.19



N3-methyl-rU (m3U) are useful for analyzing RNA structure and activity relationships

N4-Ethyl rC [N4-Et-rC]

Category	Duplex Stability
Modification Code	N4-Et-rC
Reference Catalog Number	27-6685
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	333.4



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 Tm of the duplexes measured. For these unmodified probes, the Tm range was 39degC and 52degC, respectively. When N4-Et-dC was substituted for dC in these probes, the Tm range of the duplexes was only 7degC and 16degC, respectively (2,3).

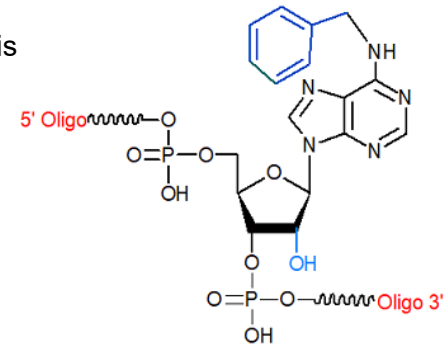
N4-Et-dC-modified oligos have also been used in structure-function studies to better understand how CpG-containing oligos stimulate the innate immune system, and which structural elements in cytosine and guanine bases are required for recognition of, and interaction with, protein/receptor factors responsible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

N6-Benzyl Adenosine (bn6A)

Category	RNA Oligo Synthesis
Modification Code	bn6A
Reference Catalog Number	27-6604
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	437.34



N6-Benzyl Adenosine (bn6A)
[27-6604-XX]

N6-methyl-riboadenosine (N6-methyl rA; m6A) is a common, fairly abundant RNA modification found in the mRNA of most eukaryotes (1,2); it has also been observed in tRNA, rRNA snRNA and in long non-coding RNA (3). While the biological importance of this modification remains poorly understood, results from a number of research studies suggest that regulation of m6A levels in mRNA may have significant effects on subsequent gene expression. The modification mainly appears in exons, 3'-UTRs and near stop codons. Within 3'-UTRs, N6-methyl-rA is associated with miRNA binding sites (4). The modification itself is catalyzed by a N6-methyl-rA methyltransferase complex that contains the METTL3 subunit (5). Silencing this methyltransferase dramatically affects N6-methyl-A cellular levels, gene expression and alternative RNA splicing patterns (6). The FTO and ALKBH5 genes, implicated in obesity risk, encode two different N6-methyl-rA demethylases; silencing of FTO with siRNA results in increased levels of N6-methyl-rA in poly(A) RNA (6), while FTO overexpression results in decreased levels (4). Moreover, modulation of the activities of these three enzymes can alter the expression of thousands of genes at the cellular level. This suggests that N6-methyl-rA plays an important role in RNA metabolism and as an epigenetic marker (7). **References**

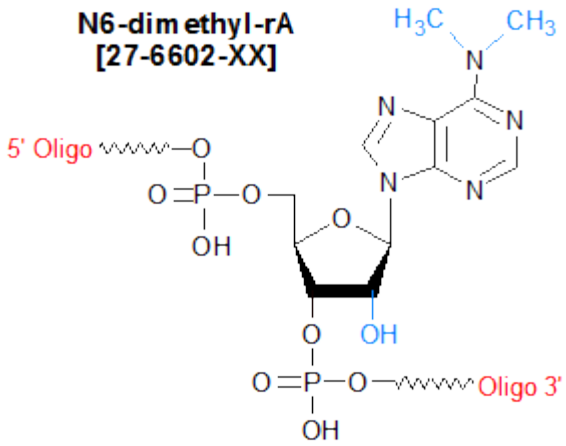
1. Tuck, M.T. The formation of internal 6-methyladenine residues in eucaryotic messenger RNA. *Int. J. Biochem.* (1992), **24**: 379-386.
2. Jia, G., Fu, Y., He, G. Reversible RNA adenosine methylation in biological regulation. *Trends Genet.* (2013), **29**: 108-115.
3. Pan, T. N6-methyl-adenosine modification in messenger and long non-coding RNA. *Trends Biochem. Sci.* (2013), **38**: 204-209.
4. Meyer, K.D., Saletore, Y., Zumbo, P., Elemento, O. Mason, C.E., Jaffrey, S.R. Comprehensive Analysis of mRNA Methylation Reveals Enrichment in 3' UTRs and near Stop Codons. *Cell* (2012), **149**: 1635-1646.
5. Bokar, J.A., Shambaugh, M.E., Polayes, D., Matera, A.G., Rottman, F.M. High-Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6-adenosine)-methyltransferase. *RNA* (1997), **3**: 1233-1247.
6. Dominissini, D., Moshitch-Moshkovitz, S., Schwartz, S., Salmon-Divon, M., Ungar, L., Osenberg, S., Cesarkas, K., Jacob-Hirsch, J., Amariglio, N., Kupiec, M., Sorek, R., Rechavi, G. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq.

Nature (2112), **485**: 201-206.
7. Niu, Y., Zhao, X., Wu, Y.S., Li, M.M., Wang, X.J., Yang, Y.G. N6-methyl-adenosine (m6A) in RNA: an old modification with a novel epigenetic function. *Genom. Proteom. Bioinform.* (2013), **11**: 8-17.

Oligo Modifications

N6-dimethyl rA [m6-2A]

Category	Others
Modification Code	m6-2A
Reference Catalog Number	27-6602
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	333.2



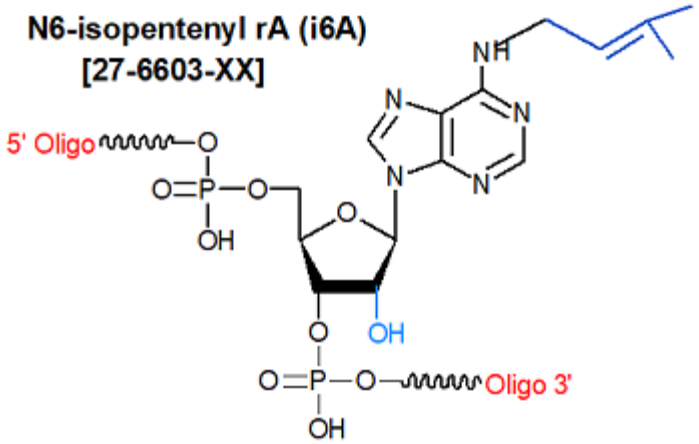
N6-dimethyl-riboadenosine (N6-dimethyl rA) is a minor RNA modification found primarily in rRNA (1), and recently, in Mycobacterium tRNA (2). N6-dimethyl rA appears to play a structural role in rRNA; in 16S RNA of E. coli, two successive N6-dimethyl rA modifications are present 24 and 25 residues from the 3' end, and nowhere else (3). These residues are located at the interface between the 30S and 50S subunits of 70S E. coli rRNA (1), but do not appear to play a major role in the binding of fMet-tRNA, or in initiation of protein synthesis (4). Its precise role in tRNA is yet unknown. **References**

1. Politz, S.M., Glitz, D.G. Ribosome structure: Localization of N6, N6-dimethyladenosine by electron microscopy of a ribosome-antibody complex.*Proc. Natl. Acad. Sci. USA.* (1997), **74**: 1468-1472.
2. Chan, C.T.Y., Chionh, Y.H., Ho, C-H., Lim, K.S., Babu, I.R., Ang, E., Wenwei, L., Alonso, S., Dedon, P.C. Identification of N6,N6-Dimethyladenosine in Transfer RNA from Mycobacterium bovis Bacille Calmette-Guerin. *Molecules* (2011), **16**: 5168-5181.
3. Ehresmann, C., Stiegler, P., Mackie, G.A., Zimmermann, R.A., Ebel, J.P., Fellner, P. Primary sequence of the 16S ribosomal RNA of Eschericia coli. *Nucleic Acids Res.* (1975), **2**: 265-278.
4. Studies on the Function of Two Adjacent N6,N6-Dimethyladenosines Near the 3' End of 16 S Ribosomal RNA of Escherichia coli. *J. Biol. Chem.* (1979), **254**: 9090-9094.

Oligo Modifications

N6-isopentenyl-rA (i6a)

Category	RNA Oligo Synthesis
Modification Code	N6-i6A
Reference Catalog Number	27-6603
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	398.33

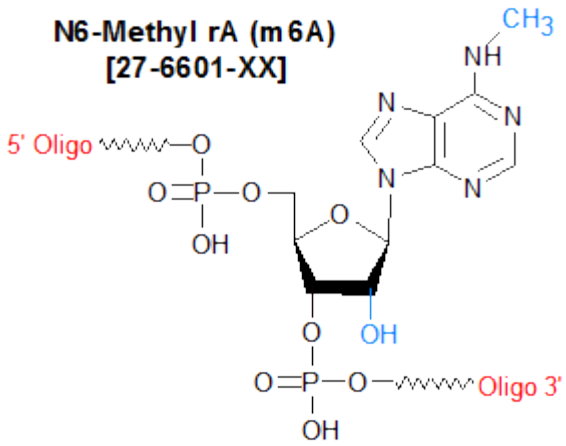


N6-isopentenyladenosine (i6A), a naturally occurring modified nucleoside, inhibits the proliferation of human tumor cell lines in vitro, but its mechanism of action remains unclear. Among the over 100 base modifications occurring in tRNA, many target the anticodon stem loop, in particular base 34 reading the wobble base and the dangling base 37 3′-adjacent to the anticodon. Base modifications present at base 37 include mostly bulky additions to adenosine, i.e. N6-isopentenyladenosine (i6A), 2-methyl-thio i6A (ms2i6A), 6-hydroxy ms2i6A (ms2io6A), N6-threonylcarbamoyladenosine (t6A), ms2t6A, and modification of guanosine, to N1-methylguanosine (m1G) or wybutosine (yW). The i6A modified base was one of the first hypermodified bases identified and has been found in bacteria and eukaryotes, but not in archeae. (1)
The enzyme tRNA-isopentenyltransferase-1 (E.C. 2.5.1.75), encoded by the putative tumor suppressor gene TRIT1 (1), catalyzes the transfer of an isopentenyl group from isopentenyl diphosphate to the adenosine in position 37 of selenocysteine-specific transfer RNA (tRNA) [2,3]. The resulting isopentenyladenosine-tRNA (i6A-tRNA) improves the reading frame maintenance during the synthesis of selenoproteins [4]. N6-isopentenyladenosine (i6A), which is a breakdown product of i6A-tRNA turnover, is found in mammalian cells and is excreted in the urine (1-4) References

1. Schweizer U, Bohleber S, and Fradejas-Villar N. The modified base isopentenyladenosine and its derivatives in tRNA. RNA Biology. 14 (2017) 1197–1208.
2. Spinola M., Galvan A., Pignatiello C., Conti B., Pastorino U., Nicander B., Paroni R., Dragani T.A. Identification and functional characterization of the candidate tumor suppressor gene TRIT1 in human lung cancer. Oncogene, 24 (2005) 5502-5509.
3. Golovko A., Hjälm G., Sitbon F., Nicander B.Cloning of a human tRNA isopentenyl transferase. Gene, 258 (2000) 85-93
4. Fradejas N., Carlson B.A., Rijntjes E., Becker N.P., Tobe R., Schweizer U. Mammalian Trit1 is a tRNA([ser]sec)-isopentenyl transferase required for full selenoprotein expression. Biochemical Journal, 450 (2013), pp. 427-432

N6-Methyl rA (m6A)

Category	Epigenetics
Modification Code	m6A
Reference Catalog Number	27-6601
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	343.23



N6-methyl-riboadenosine (N6-methyl rA; m6A) is a common, fairly abundant RNA modification found in the mRNA of most eukaryotes (1,2); it has also been observed in tRNA, rRNA snRNA and in long non-coding RNA (3). While the biological importance of this modification remains poorly understood, results from a number of research studies suggest that regulation of m6A levels in mRNA may have significant effects on subsequent gene expression. The modification mainly appears in exons, 3'-UTRs and near stop codons. Within 3'-UTRs, N6-methyl-rA is associated with miRNA binding sites (4). The modification itself is catalyzed by a N6-methyl-rA methyltransferase complex that contains the METTL3 subunit (5). Silencing this methyltransferase dramatically affects N6-methyl-A cellular levels, gene expression and alternative RNA splicing patterns (6). The FTO and ALKBH5 genes, implicated in obesity risk, encode two different N6-methyl-rA demethylases; silencing of FTO with siRNA results in increased levels of N6-methyl-rA in poly(A) RNA (6), while FTO overexpression results in decreased levels (4). Moreover, modulation of the activities of these three enzymes can alter the expression of thousands of genes at the cellular level. This suggests that N6-methyl-rA plays an important role in RNA metabolism and as an epigenetic marker (7). **References**

1. Tuck, M.T. The formation of internal 6-methyladenine residues in eucaryotic messenger RNA. *Int. J. Biochem.* (1992), **24**: 379-386.
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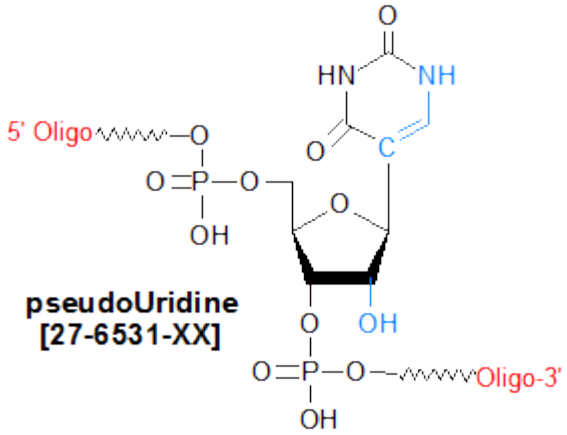
Nature (2112), **485**: 201-206.
7. Niu, Y., Zhao, X., Wu, Y.S., Li, M.M., Wang, X.J., Yang, Y.G. N6-methyl-adenosine (m6A) in RNA: an old modification with a novel epigenetic function. *Genom. Proteom. Bioinform.* (2013), **11**: 8-17.

pseudoUridine (psi rU)

Category	Affinity Ligands
Modification Code	psi-rU
Reference Catalog Number	27-6531
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	306.17



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

1. Hamma, T., Ferre-D-Amare, A.R. Pseudouridine synthases. *Chem. Biol.* (2006), **13**: 1125-1135.
2. Charette, M., Gray, M.W. Pseudouridine in RNA: what, where, how, and wny.*IUBMB Life* (2000), **49**: 341-351.
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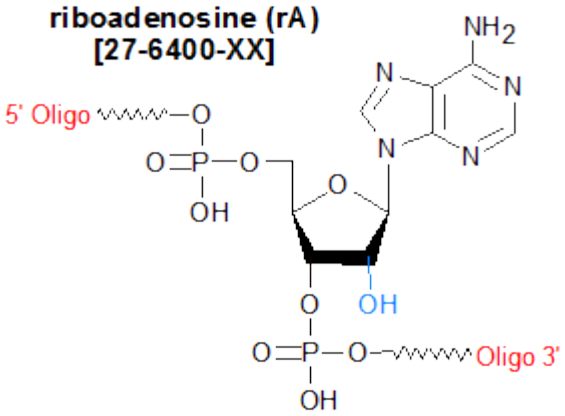
rA RNA Base

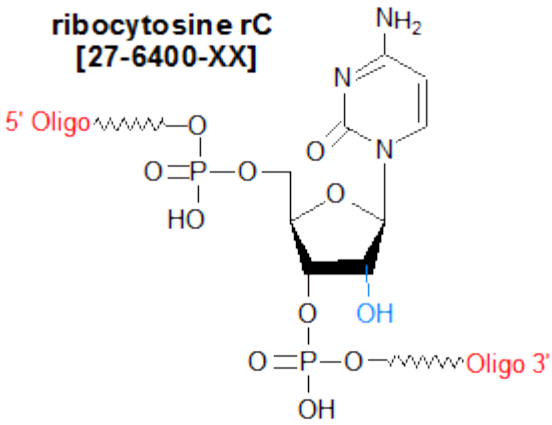
Category	RNA Oligo Synthesis
Modification Code	rA
Reference Catalog Number	27-6400A
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	329.21



Riboadenosine, or adenosine (rA) is a purine ribonucleoside, and is one of the four standard ribonucleosides that compose an RNA molecule. The presence of the -OH group at the 2'-position of the ribose results in RNA being less stable to DNA (which lacks -OH groups at this position), because this 2'-hydroxyl group can chemically attack the adjacent phosphodiester bond in the sugar-phosphate backbone of RNA, leading to cleavage of the backbone structure. rA forms a Watson-Crick base pair with rU (ribouridine/uridine) in RNA duplexes, and dT (deoxythymidine) in RNA-DNA duplexes.

rC RNA Base

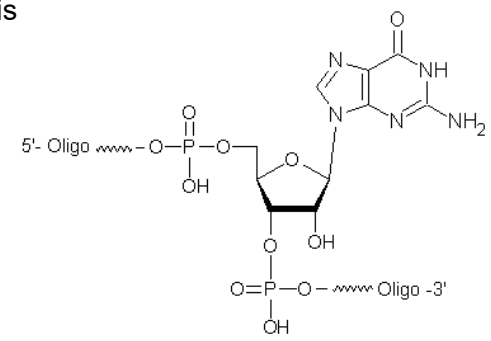
Category	RNA Oligo Synthesis
Modification Code	rC
Reference Catalog Number	27-6400C
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	305.18



Ribocytosine, or cytosine (rC) is a pyrimidine ribonucleoside, and is one of the four standard nucleosides that compose an RNA molecule. The presence of the -OH group at the 2'-position of the ribose results in RNA being less stable to DNA (which lacks -OH groups at this position), because this 2'-hydroxyl group can chemically attack the adjacent phosphodiester bond in the sugar-phosphate backbone of RNA, leading to cleavage of the backbone structure. rC forms a Watson-Crick base pair with rG (riboguanosine/guanosine) in RNA duplexes, or dG (deoxyriboguanosine) in RNA-DNA duplexes.

rG RNA Base

Category	RNA Oligo Synthesis
Modification Code	rG
Reference Catalog Number	27-6400G
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	345.21

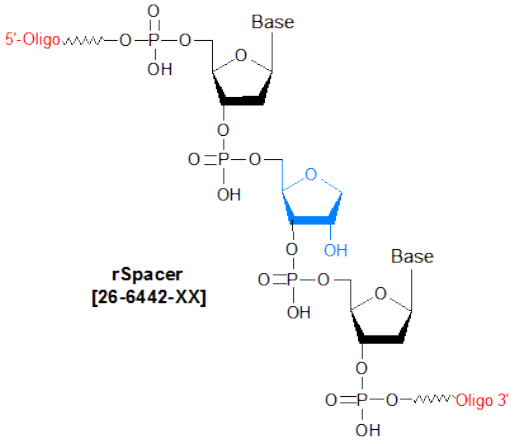


riboguanosine (rG)
[27-6400-XX]

Riboguanosine, or guanosine (rG) is a purine deoxyribonucleoside, and is one of the four standard nucleosides that compose an RNA molecule. The presence of the -OH group at the 2'-position of the ribose results in RNA being less stable to DNA (which lacks -OH groups at this position), because this 2'-hydroxyl group can chemically attack the adjacent phosphodiester bond in the sugar-phosphate backbone of RNA, leading to cleavage of the backbone structure. rG forms a Watson-Crick base pair with rC (ribocytosine/cytosine) in RNA duplexes, or dC (deoxyribocytosine) in RNA-DNA duplexes.

rSpacer

Category	Spacers
Modification Code	rABS
Reference Catalog Number	26-6442
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	196.09



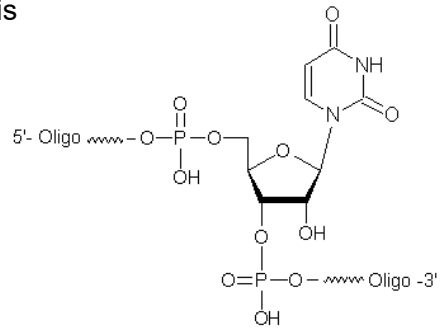
Ribo rAbasic Site (rSpacer abasic furan) RiboSpacer (rSpacer) is a tetrahydrofuran derivative, in which a methylene group occupies the 1 position of 2'-ribose. rSpacer is commonly used to mimic an abasic site in an RNA oligonucleotide. Naturally-occurring abasic sites in RNA are less common than in DNA, due to RNA being less susceptible to depurination (1). However, once generated, either spontaneously or via an enzymatic pathway, RNA abasic sites are about 15-fold more stable than DNA abasic sites; this fairly high level of stability could have important biological consequences for long-lived RNAs (for example, tRNAs or rRNA) (2). While such biological consequences have been largely unexplored thus far, abasic site effects on RNA structure and activity has been observed for the case of the hammerhead ribozyme, which catalyzes phosphodiester bond cleavage (3). Introduction of abasic sites at different positions of this ribozyme's core significantly reduced ribozyme activity. Interestingly, the activity was partially rescued for some abasic positions by exogenous addition of the missing base. rSpacer-modified oligonucleotides could serve as important research tools for elucidating the effects of abasic sites on the structure and function of long-lived RNAs and ribozymes. **References**

1. Kochetkov, N.K., Budovskii, E.I. Hydrolysis of N-glycosidic bonds in nucleosides, nucleotides and their derivatives. In *Organic Chemistry of Nucleic Acids* New York: Plenum; (1993). pp. 425-448.
2. Kupfer, P.A., Leumann, C.J. The chemical stability of abasic RNA compared to abasic DNA. *Nucleic Acids Res.* (2007), **35**: 58-68.
3. Peracchi, A., Beigelman, L., Usman, N., Herschlag, D. Rescue of abasic hammerhead ribozymes by exogenous addition of specific bases. *Proc. Natl. Acad. Sci. USA.* (1996), **93**: 11522-11527.

Oligo Modifications

rU RNA Base

Category	RNA Oligo Synthesis
Modification Code	rU
Reference Catalog Number	27-6400U
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	306.17

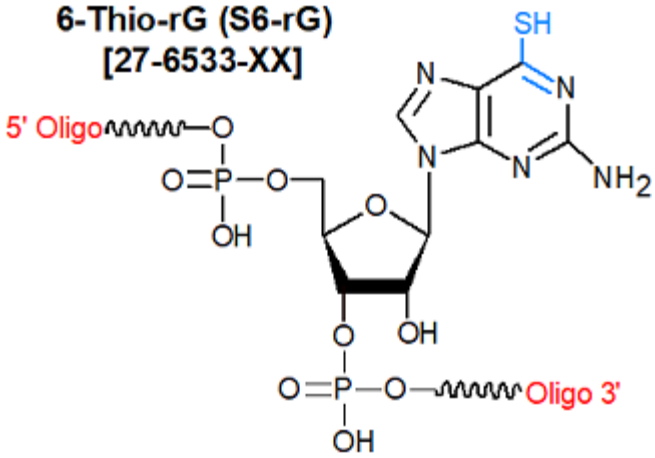


ribouridine (rU)
[27-6400-XX]

Ribouridine, or uridine (rU) is a purine ribonucleoside, and is one of the four standard ribonucleosides that compose an RNA molecule. The presence of the -OH group at the 2'-position of the ribose results in RNA being less stable to DNA (which lacks -OH groups at this position), because this 2'hydroxyl group can chemically attack the adjacent phosphodiester bond in the sugar-phosphate backbone of RNA, leading to cleavage of the backbone structure. rU forms a Watson-Crick base pair with rA (riboadensoine/adenosine) in RNA duplexes, and dA (deoxyadenosine) in RNA-DNA duplexes.

Thio 6-rG (s6rG)

Category	Structural Studies
Modification Code	S6-rG
Reference Catalog Number	27-6533
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	361.26



6-Thio-ribGuanosine (6-Thio-4G; s6rG) and 6-Thio-deoxyGuanosine (6-Thio-dG; s6dG) 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-ribGuanosine (6-Thio-4G; s6rG) and 6-Thio-deoxyGuanosine (6-Thio-dG; s6dG) can also be used to study the properties of G-rich triple-helix forming oligonucleotides. For example, substitution of 6-Thio-ribGuanosine (6-Thio-4G; s6rG) and 6-Thio-deoxyGuanosine (6-Thio-dG; s6dG) for some or all Gs 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-ribGuanosine (6-Thio-4G; s6rG) and 6-Thio-deoxyGuanosine (6-Thio-dG; s6dG) are 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

1. Christopherson, M.S., Broom, A.D. Synthesis of oligonucleotides containing 2'-deoxy-6-thioguanosine at a predetermined site. *Nucleic Acids Res.* (1991), **19**: 5719-5724.
2. Nikiforov, T.T., Connolly, B.A. Oligodeoxynucleotides containing 4-thiothymidine and 6-thiodeoxyguanosine as affinity labels for the Eco RV restriction endonuclease and modification methylase. *Nucleic Acids Res.* (1992), **20**: 1209-1214.
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R. Incorporation of 2'-Deoxy-6-thioguanosine into G-Rich Oligodeoxyribonucleotides Inhibits G-Tetrad Formation and Facilitates Triplex Formation.*Biochemistry* (1995), **34**: 765-772.
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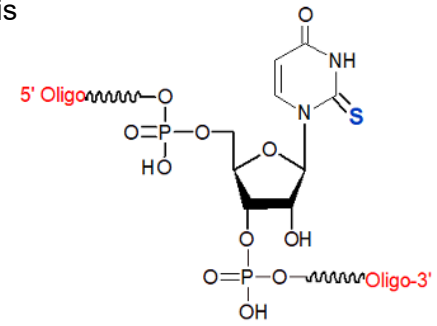
Thio-2-rU (s2U)

Category	RNA Oligo Synthesis
Modification Code	s2U
Reference Catalog Number	27-6537
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	322.22



2-Thio-Uridine (s2U)
[27-6537-XX]

- The effect of 2-thiouridine on sugar conformation and RNA duplex thermodynamics has been well documented (1,2). The presence of the 2-sulfur modification stabilizes the 3'-endo sugar conformation at the nucleoside and nucleotide level (1). Wobble base pair specificity can also be improved by substituting 2-thiouridine for uridine. Testa and co-workers (2) have shown the at S2U favors S2U-A pairing more than S2U-G pairing, and more than U favors U-A relative to U-G. The sulfur modification improves specificity while retaining other key uridine activities.
In addition, thiolated uridine has been shown to improve the rate and fidelity of both nonenzymatic (3) and ribozyme (4) catalyzed nucleotide addition in RNA synthesis.

References

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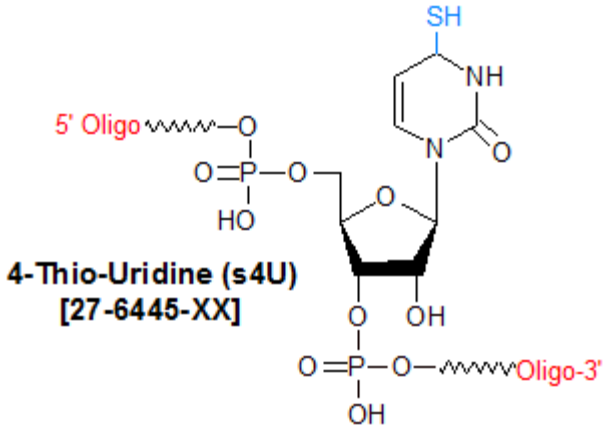
2. Testa, S.M.; Disney, M.D.; Turner, D.H.; Kierzek, R. Biochem. 1999, 38, 16655-16662.
3. Heuberger, B.D.; Pal, A.; Del Frate, F.; Topkar, V.V.; Szostak, J.W. J. Am. Chem. Soc., 2015, 137 (7), pp 2769–2775.
4. Prywes, N.; Michaels, Y.S.; Pal, A.; Oh, S.S., Szostak, J.W. Chem. Commun. 2016. 52, 6529-6532.
5. a) Kumar, R.K.; Davis, D.R. J. Org. Chem.1995, 60, 7726-7727. b) Gait, M.J. Oligonucleotide Synthesis-A Practical Approach 1990, Oxford University Press, London.

Thio-4-rU (s4U)

Category	Minor Bases
Modification Code	s4U
Reference Catalog Number	27-6445
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	322.22



4-Thio Uridine (s4U)) is a thiol-modified ribonucleoside, and is typically used to modify oligos slated for RNA, or RNA-protein, structural studies. A 4-thio-rU modified RNA pentamer was used to study the effect of this modification on codon-anticodon interaction when it is in the wobble position of tRNA (1). Because 4-thio-rU is photoreactive, 4-thio-rU modified RNA oligos have also been used as photoaffinity probes in the role of substrate analogs for characterizing the enzyme:substrate complex of tRNA:pseudouridine-5S synthase (2). 4-thio-rU modified oligos have also been used as modules for assembling U25 small nucleolar RNAs (U25snoRNA) by ligation. These snoRNAs were used in cross-linking studies to identify which proteins assembled on them in vivo in Xenopus oocytes (3). In addition, because the thiol group is chemically reactive, other moieties can be conjugated at the thiol group of 4-thio-rU. Such a strategy was used to introduce spin labels to 4-thio-rU-containing RNA oligos (4). **References**

1. Kumar, R.K., Davis, D.R. Synthesis and Studies on the Effect of 2-Thiouridine and 4-Thiouridine on Sugar Conformation and RNA Duplex Stability. *Nucleic Acids Res.* (1997), **25**: 1272-1280.
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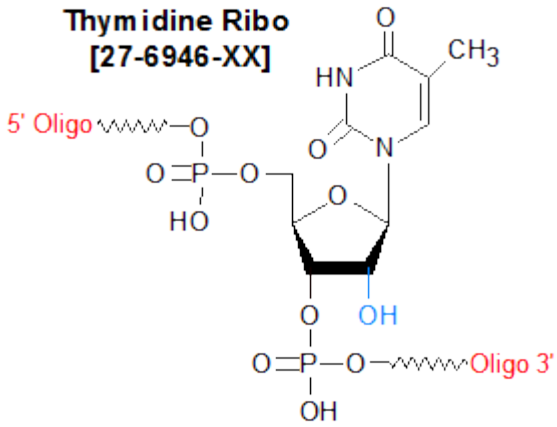
Thymidine Ribo

Category	Minor Bases
Modification Code	rT
Reference Catalog Number	27-6946
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	321.07



Thymidine Ribo (ribothymidine (rT), 5-methyl-rU) is the ribonucleotide version of thymidine, and is used to modify structural RNA, especially tRNA, for use in enzyme-RNA structural and kinetics studies. Ribothymidine is the most common methylated ribonucleoside found in prokaryotic tRNA, typically at the 23rd position from the 3'-end in the nucleotide sequence G-T-PseudoU-C-Purine (1). By contrast, in eukaryotic 'class D' tRNAs, unmodified uridine is always found at that position; the presence of rT there reduces protein synthesis efficiency in vitro (2). Roe and Tsen found that, for 'class C' mammalian tRNAs, that is, tRNAs having varying amounts of rT and U at position 23, the rate and extent of protein synthesis are proportional to the rT content of the tRNA, with an increase in rT leading to a proportional increase in the Vmax of the synthesis reaction (3). A recent review of work involving rT-modified RNA substrates is found in (4). **References**

1. Kim, S.H., Sussman, J.L., Suddath, F.L., Quigley, G.S., McPherson, A., Wang, A.M.J., Seeman, M.C., Rich, A. The general structure of transfer RNA molecules. *Proc. Natl. Acad. Sci. USA* (1974), **71**: 4970-4974.
2. Marcu, K.B., Dudock, B.S. Effect of ribothymidine in specific eukaryotic tRNAs on their efficiency in in vitro protein synthesis.*Nture* (1976), **261**: 159-162.
3. Roe, B.A., Tsen, H.Y. Role of ribothymidine in mammalian tRNAPhe *Proc. Natl. Acad. Sci. USA* (1977), **74**:3696-3700.
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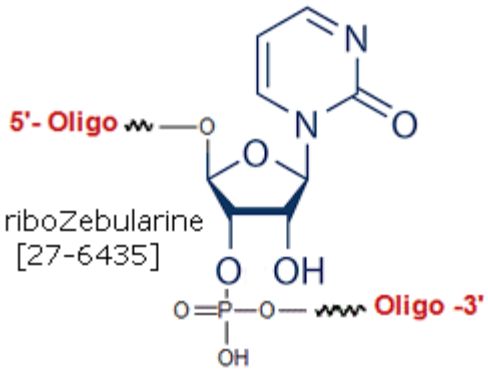
Zebularine ribo

Category	Structural Studies
Modification Code	rZ
Reference Catalog Number	27-6435
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	290.17



Zebularine (pyrimidin-2-one ribonucleoside) may be regarded as a Cytidine derivative lacking the exocyclic amino group. Zebularine and Pyridin-2-one Ribonucleoside, the 3-deaza analogue of Zebularine, are prime candidates for use in evaluating ribozyme activity and function. It should be noted that Zebularine is mildly fluorescent, absorbing at 298nm and emitting at 367nm.
Cytosine Arabanoside (Ara-C) is an anti-viral drug which has achieved limited use. Its effect on DNA structure and activity can be investigated by incorporating it into synthetic oligonucleotides.
Zebularine (pyrimidin-2-one ribonucleoside) is a cytidine analogue that acts as a DNA demethylase inhibitor, as well as a cytidine deaminase inhibitor. This structure is very active biologically and Zebularine is now used as a potent anti-cancer drug. A 2'-deoxynucleoside analogue of Zebularine, 5-methyl-pyrimidin-2-one, 2'-deoxynucleoside, has been used to probe the initiation of the cellular DNA repair process by making use of its mildly fluorescent properties. This combination of biological activity and fluorescence properties would make 5-Me-2'-deoxyZebularine a strong addition to our array of nucleoside analogues.

References

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