

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.



1 Westchester Plaza, Suite 126, Elmsford, NY 10523 | Tel: 914-769-1192 | Email : support@genelink.com | www.genelink.com | RNA Oligo Modifications | Print Date Version : October 20, 2025 | Page 1

RNA Oligo Modifications Design Protocols

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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.



RNA Oligo Modifications Applications

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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).

- 2'-F RNA modifications have been shown to be particularly useful in the following oligo-based applications:
- (a) <u>Anti-sense DNA oligos</u>: 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.
- (b) <u>Aptamers</u>: 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) <u>siRNA</u>: siRNA synthesized with 2'-F pyrimidines have been shown to have greatly increased stability in plasma compared to 2'-OH siRNA (4,5). In one study, levels of inhibition for 2'-F siRNA and 2'-OH siRNA, in cell culture and *in vivo* using BALB/c mice transfected with pGL3 luciferase, were similar over time (4). In another study, siRNA fully substituted with both 2'-F RNA and 2'-O-Methyl RNA nucleotides displayed not only enhanced stability in plasma, but also >500-fold increase in capability to down-regulate gene expression compared with the corresponding unmodified siRNA (5).



(d) <u>LNA Alternative</u> : 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. 2. Khati, M.; Schuman, M.; Ibrahim, J.; Sattentau, Q.; Gordon, S.; James, W. Neutralization of infectivity of diverse R5 clinical isolates of human immunodeficiency virus type 1 by gp120-binding 2'F-RNA aptamers. *J Virol*, (2003). **77**: 2692-8. 3. 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.
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- 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.



Modification 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)	[fl]	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	[ml]	27-64101
2'-O methyl uridine U	[mU]	27-6410U
2-Amino Purine ribose	[2-A-rP]	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 rlnosine (2'-5' linked)	[3Oml]	27-64581
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-lodo ribocytosine (5-l C)	[5-l-rC]	27-6553
5-lodo ribouridine (5-l U)	[5-l-rU]	27-6554
5-methyl-Cytosine [5mrC]	[5mrC]	27-6945
5-Methyl-Uridine (m5U)	[m5U]	27-6557
8-Azanebularine ribo (8-azaN)	[8-AzaN]	27-6606
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 [rl]	[rl]	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 [m6-6A]	[m6-6A]	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



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2'-F Bases

Category	Nuclease Resistance		NH ₂
Modification Code	fN		N N
Reference Catalog Number	26-6692N	5'- Oligo	
5 Prime	Υ	0=P-	0-700
3 Prime	Υ	ОН	
Internal	Υ	2'-Fluoro A	0 F
Molecular Weight(mw)	323.43	[26-6692-XX]	0=P-0- www Oligo -3'
			OH

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

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

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

(a) <u>Anti-sense oligos & Nuclease Resistance</u>: 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) <u>Aptamers</u>: 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) <u>siRNA & Nuclease Resistance</u>: siRNA synthesized with 2'-F pyrimidines have been shown to have greatly increased stability in plasma compared to 2'-OH siRNA (4,5). In one study, levels of inhibition for 2'-F siRNA and 2'-OH siRNA, in cell culture and *in vivo* using BALB/c mice transfected with pGL3 luciferase, were similar over time (4). In another study, siRNA fully substituted with both 2'-F RNA and 2'-O-Methyl RNA nucleotides displayed not only enhanced stability in plasma, but also >500-fold increase in capability to down-regulate gene expression compared with the corresponding unmodified siRNA (5).
- (d) <u>LNA Alternative</u>: The increased thermal stability and nuclease resistance provided by 2'-F RNA residues make them attractive as lower-cost substitutes in any application involving LNA-modified oligos.

ASO's and siRNA Modifications.

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

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

REFERENCES:

- 1. Kawasaki, A.M., et al., Uniformly modified 2'-deoxy-2'-fluoro phosphorothioate oligonucleotides as nuclease resistant antisense compounds with high affinity and specificity for RNA targets, *Journal of Medicinal Chemistry* (1993), **36**: 831-841. 2. Khati, M.; Schuman, M.; Ibrahim, J.; Sattentau, Q.; Gordon, S.; James, W. Neutralization of infectivity of diverse R5 clinical isolates of human immunodeficiency virus type 1 by gp120-binding 2'F-RNA aptamers. *J Virol*, (2003). **77**: 2692-8. 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.
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2'-F-A

Category	Nuclease Resistance		NH ₂
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Reference Catalog Number	26-6692	5'- Oligo	
5 Prime	Υ	0=P-	0-700
3 Prime	Υ	ОН	
Internal	Υ	2'-Fluoro A) F
Molecular Weight(mw)	331.2	[26-6692-XX]	0=P-0- www Oligo -3'
			OH

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2'-F-ANA-Bases

Category	Antisense	
Modification Code	FANA-N	
Reference Catalog Number	27-6605N	5'- Oligo www — o Base
5 Prime	Υ	°=
3 Prime	Υ	он 🗾
Internal	Υ	2'-Fluoro ANA Base 2'-deoxy-2'-fluoroarabinoside Base
Molecular Weight(mw)	323.43	[27-6605N-XX] O=P-0- MMM Oligo -3'
		ОН

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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°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°C and in a 2'-F-ANA - DNA duplex a DTm of -3.9°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).



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Oligo Modifications

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2'-F-C

Category	Nuclease Resistance		
Modification Code	fC		NH ₂
Reference Catalog Number	26-6463	5'- Oligo	N
5 Prime	Υ	0=P-	-0-7 0 N
3 Prime	Υ	он	
Internal	Υ	2'-Fluoro C	
Molecular Weight(mw)	307.17	[26-6463-XX]	0=P-0- www Oligo -3'

Antisense Oligos (ODN) & siRNA Oligo Modifications

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

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

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

(a) <u>Anti-sense oligos & Nuclease Resistance</u>: 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 [1.8C per substitution] Increased [1.8C per substitution] Increased Increased [1.8C per substitution] Increased Increased



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) <u>Aptamers</u>: 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) <u>siRNA & Nuclease Resistance</u>: siRNA synthesized with 2'-F pyrimidines have been shown to have greatly increased stability in plasma compared to 2'-OH siRNA (4,5). In one study, levels of inhibition for 2'-F siRNA and 2'-OH siRNA, in cell culture and *in vivo* using BALB/c mice transfected with pGL3 luciferase, were similar over time (4). In another study, siRNA fully substituted with both 2'-F RNA and 2'-O-Methyl RNA nucleotides displayed not only enhanced stability in plasma, but also >500-fold increase in capability to down-regulate gene expression compared with the corresponding unmodified siRNA (5).
- (d) <u>LNA Alternative</u>: The increased thermal stability and nuclease resistance provided by 2'-F RNA residues make them attractive as lower-cost substitutes in any application involving LNA-modified oligos.

ASO's and siRNA Modifications.

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

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

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Oligo Modifications

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2'-F-G

Category	Nuclease Resistance		
Modification Code	fG		🕯
Reference Catalog Number	26-6693	5'- Oligo	NH
5 Prime	Υ		O- N-NH2
3 Prime	Υ	он	
Internal	Υ	2'-Fluoro G	
Molecular Weight(mw)	347.16	[26-6693-XX]	0=P-0- www Oligo -3'
			OH

Antisense Oligos (ODN) & siRNA Oligo Modifications

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

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

(a) <u>Anti-sense oligos & Nuclease Resistance</u>: 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

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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 [1.8C per substitution] Increased [1.8C per substitution] Increased Increased [1.8C per substitution] Increased Increased



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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Oligo Modifications

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2'-F-Inosine

Category	Nuclease Resistance	2'-Fluoro-Inosine O
Modification Code	fl	NH NH
Reference Catalog Number	26-6618	5' Oligo www-O
5 Prime	Υ	
3 Prime	Υ	ОН
Internal	Υ	O F
Molecular Weight(mw)	332.18	0=P-0-~~~~Oligo 3'
		OH

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2-Fluoro Inosine (fl) 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. dl 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 dl 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 dl 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 dl is at the 3'-position, it may be advisable to use primers with and without dl 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.



- 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 **References** 1. Oda, Y, Uesugi, S., Ikehara, M., Kawase, Y., Ohtsuka, E. NMR studies for identification of dl: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.



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Oligo Modifications

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2'-F-U

Category	Nuclease Resistance		
Modification Code	fU		Ů
Reference Catalog Number	26-6462	5'- Oligo • • •	NH
5 Prime	Υ	0=	N N
3 Prime	Υ		OH C
Internal	Υ	2'-Fluoro U	
Molecular Weight(mw)	308.16	[26-6462-XX]	0=P-0- www Oligo -3'
			OH

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Oligo Modifications

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2'-MOE-Bases

Category	Antisense		N N
Modification Code	MOE-N	5'- Oligo www — O	
Reference Catalog Number	27-6450N	0=P-	
5 Prime	Υ	ОН	()
3 Prime	Υ	2'-MOE A [27-6450A-XX]	CH ₃
Internal	Υ		\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \
Molecular Weight(mw)	386.55		o=
			ÓН

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

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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 NH_2

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



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Oligo Modifications

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2'-MOE-5mC

Category	Antisense	NH ₂	
Modification Code	MOE-mC	H ₃ C	
Reference Catalog Number	27-6450mC	5'- Oligo www —o	
5 Prime	Υ		
3 Prime	Υ	ОН	
Internal	Υ	2'-MOE 5me C	1
Molecular Weight(mw)	377.3	[27-6450mC-XX] 0=P-0- www Oligo -3	,
		I OH	





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

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2'-MOE-5mU

Category	Antisense		o II
Modification Code	MOE-mU		NH
Reference Catalog Number	27-6450mU	5'- Oligo AMM —O	
5 Prime	Υ	0=P-	
3 Prime	Υ	ОН	
Internal	Υ	2'-MOE 5mU 0 0 [27-6450U-XX] 0=P-0-	CH ₃
Molecular Weight(mw)	378.3		0=P-0
			OH





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

Oligo Modifications

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2'-MOE-A

Category	Antisense		NH ₂
Modification Code	MOE-A	5'- Oligo www.—o	N
Reference Catalog Number	27-6450A		
5 Prime	Υ) OH	"
3 Prime	Υ	2'-MOE A [27-6450A-XX]	
Internal	Υ		OO_CH3
Molecular Weight(mw)	387.3		0=P-0
			он





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

Oligo Modifications

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2'-MOE-G

Category	Antisense		9
Modification Code	MOE-G		N NH
Reference Catalog Number	27-6450G	5'- Oligo www —o	
5 Prime	Υ	o=P—c	NH2
3 Prime	Υ	ОН	
Internal	Υ	2'-MOE G	CH3
Molecular Weight(mw)	403.3	[27-6450G-XX]	0=P-0- www Oligo -3'
			OH





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Oligo Modifications

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2'-O Me-5-Me-C

Category	Structural Studies		NHa
Modification Code	m5mC		H ₃ C NH ₂
Reference Catalog Number	27-6508	5'- Oligo www —o	
5 Prime	Υ		
3 Prime	Υ	он	
Internal	Υ	2'-O-Methyl 5 methyl C	0—CH ₂
Molecular Weight(mw)	333.24		0=P-0 Oligo -3'
			Ьн

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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

Modifications Increasing Duplex Stability and Nuclease Resistance

can prevent or sharply reduce these undesirable immune responses (2,3).

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

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Oligo Modifications

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2'-O methyl A

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Oligo Modifications

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2'-O methyl bases

Category	Antisense	
Modification Code	mN	NH ₂
Reference Catalog Number	27-6410N	5'- Oligoo
5 Prime	Υ	
3 Prime	Υ	óн
Internal	Υ	2'-O-Methyl A
Molecular Weight(mw)	334.97	[27-6410-XX] 0=-0Oligo -3'

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

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Duplex Stability [Tm Increase]

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Oligo Modifications

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2'-O methyl C

Category Nuclease Resistance NH₂ mC Modification Code Reference Catalog Number 27-6410C 5'- Oligo www 5 Prime OH 3 Prime Υ Internal Υ 2'-O-Methyl C Molecular Weight(mw) 319.21 [27-6410-XX] www Oligo -3' ÓН

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Oligo Modifications

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2'-O methyl G

Category Nuclease Resistance Modification Code mG Reference Catalog Number 27-6410G 5'- Oligo www. 5 Prime 3 Prime Υ Internal Υ 2'-O-Methyl G Molecular Weight(mw) 359.24 [27-6410-XX] www Oligo -3' ÓН

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Oligo Modifications

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2'-O methyl Inosine

Category	Others	2'-O-methyl-inosine O [27-6410I-XX N
Modification Code	ml	5' Oligo VVVV—O
Reference Catalog Number	27-64101	O P O N
5 Prime	Υ	OH O
3 Prime	Υ	
Internal	Υ	о́ о́сн ₃
Molecular Weight(mw)	344.22	O≔P−O~~~~Oligo 3' OH

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Oligo Modifications

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2'-O methyl U

Category Nuclease Resistance Modification Code mU Reference Catalog Number 27-6410U 5'- Oligo www 5 Prime 3 Prime Υ Internal Υ 2'-O-Methyl U Molecular Weight(mw) 320.2 [27-6410-XX] www Oligo -3' ÓH

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Oligo Modifications

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2-Amino Purine ribose

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 Jean JM, Hall KB (2001). "2-Aminopurine fluorescence quenching and lifetimes: role of base stacking".



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

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- 4. Kimura, T.; Kawai, K.; Fujitsuka, M.; Tetsuro, M. Monitoring G-quadruplex structures and G-quadruplex-ligand complex using 2-aminopurine modified oligonucleotides. *Tetrahedron* (2007), **63**: 3585-3590.
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Oligo Modifications

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3'-O methyl bases

Category	Antisense	5'- Oligo www - o Base
Modification Code	3OmN	$\langle ^{\circ} \rangle$
Reference Catalog Number	27-6458N	2'
5 Prime	Υ	CH3 O=P-O - www Oligo -3'
3 Prime	Υ	но
Internal	Υ	2'-5' linkage for internal sites
Molecular Weight(mw)	386.55	3'-O-Methyl Base [27-6458N-XX]

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

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





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

Oligo Modifications

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

3'-O methyl rA

Category	Antisense	
Modification Code	3OmA	NH ₂
Reference Catalog Number	27-6458A	N
5 Prime	Υ	5'- Oligo
3 Prime	Υ	2'
Internal	Υ	CH3 O=P-Ovww Oligo -3' HÓ
Molecular Weight(mw)	343.24	2'-5' linkage for internal sites
·····/		3'-O-Methyl A [27-6458A-XX]

Antisense Oligos (ODN) & siRNA Oligo Modifications

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Oligo Modifications

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3'-O methyl rC

Category	Antisense	
Modification Code	3OmC	NH ₂ ↓
Reference Catalog Number	27-6458C	(N
5 Prime	Υ	5'- Oligo
3 Prime	Υ	2' 0 5'
Internal	Υ	ĊH3 O≕P-O — www Oligo -3' HÓ
Molecular Weight(mw)	Veight(mw) 319.21	2'-5' linkage for internal sites
wordan weight(IIIw)	010.21	3'-O-Methyl C
		[27-6458C-XX]

Antisense Oligos (ODN) & siRNA Oligo Modifications

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

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Oligo Modifications

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3'-O methyl rG

Antisense Oligos (ODN) & siRNA Oligo Modifications

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Oligo Modifications

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3'-O methyl rl

Category	Antisense	
Modification Code	3Oml	ů II
Reference Catalog Number	27-64581	NH
5 Prime	Υ	5'- Oligo vavav – o
3 Prime	Υ	2' 0 5'
Internal	Υ	ĆH3 O≕P-O — www Oligo -3' HÓ
Molecular Weight(mw)	343.24	2'-5' linkage for internal sites
wolecular weight(IIIW)	J4J.Z4	3'-O-Methyl Inosine [27-6458I-XX]

Antisense Oligos (ODN) & siRNA Oligo Modifications

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Oligo Modifications

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3'-O methyl rU

Category	Antisense	
Modification Code	3OmU	Ů
Reference Catalog Number	27-6458U	NH
5 Prime	Υ	5'- Oligo
3 Prime	Υ	2' 5'
Internal	Υ	ĊH3 O≕P-O — ✓ Oligo -3'
Molecular Weight(mw)	320.2	2'-5' linkage for internal sites
,		3'-O-Methyl U [27-6458U-XX]

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Oligo Modifications

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

Category	Minor Bases		NH ₂
Modification Code	3rA2-5		N N
Reference Catalog Number	26-6682	5' Oligo *****-O	
5 Prime	Υ	0=P-0-	~ 1 N
3 Prime	Υ	ОН	
Internal	Υ	C	DH O
Molecular Weight(mw)	329.24	3'-rA (2'-5' linked) [26-6682-XX]	0 = P - 0 - \(\text{Oligo-3}' \) OH



Oligo Synthesis

Product Specifications

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

Oligo Modifications

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

Category	Minor Bases		NH ₂
Modification Code	3rC2-5		N
Reference Catalog Number	26-6911	5' Oligo	
5 Prime	Υ	0=P-0-	
3 Prime	Υ	НО	
Internal	Υ		OH O
Molecular Weight(mw)	305.18	3'-rC (2'-5' linked) [26-6911-XX]	O=P-O-vvvOligo-3*





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

Oligo Modifications

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

Category	Minor Bases		0
Modification Code	3rG2-5		N NH
Reference Catalog Number	26-6912	5' Oligonmon—O	
5 Prime	Υ	0=P-0-	
3 Prime	Υ	ОН	
Internal	Υ		OH O
Molecular Weight(mw)	345.21	3'-rG (2'-5' linked) [26-6912-XX]	0=P-0-*****Oligo-3*





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

Oligo Modifications

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

Category	Minor Bases	0	
Modification Code	3rU2-5	NH	
Reference Catalog Number	26-6913	5' Oligoww-Q	
5 Prime	Υ	o=!-o¬ _o. j	
3 Prime	Υ	но	
Internal	Υ	ОНО	
Molecular Weight(mw)	306.17	3'-rU (2'-5' linked) [26-6913-XX]	Oligo-3'





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

Oligo Modifications

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5-Br rC

Category	RNA Oligo Synthesis	S
Modification Code	5-Br-rC	NH ₂
Reference Catalog Number	27-6551	N Br
5 Prime	Υ	5' Oligonum-O
3 Prime	Υ	0=P-0-0-0-1
Internal	Υ	O OH
Molecular Weight(mw)	384.08	5-Br-rC O OH O

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).

- 1. Hendrickson, W.; Ogata, C. Phase determination from multiwavelength anomalous diffraction measurements. *Meth. Enzymol.*. (1997), **276**: 494-523.
- 2. Walsh M.A.; Evans G.; Sanishvili R.; Dementieva I.; Joachimiak, A. MAD data collection current trends. *Acta Cryst.* (1999), **D55**: 1726-1732.
- 3. Herbert, A.G.; Rich, A. A method to identify and characterize Z-DNA binding proteins using a linear oligodeoxynucleotide. *Nucleic Acids Res.* (1993), **21**: 2669-2672.
- 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).







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

Oligo Modifications

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5-Br rU

Category RNA Oligo Synthesis Modification Code 5-Br-rU Reference Catalog Number 27-6552 5' Oligonnon 5 Prime 3 Prime Υ ΗÓ Internal Υ Molecular Weight(mw) 385.07 5-Br-U -vvvvOligo-3' [27-6552-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

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5-I C

Category	RNA Oligo Synthesi	S NH ₂
Modification Code	5-I-rC	5' Oligonnun-O
Reference Catalog Number	27-6553	0=P-0-0 N
5 Prime	Υ	но
3 Prime	Υ	ОН
Internal	Υ	O=P-O-//Oligo 3'
Molecular Weight(mw)	431.08	OH 5-lodo-Cytosine (5-l-rC) [27-6553-XX]

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- 3. Herbert, A.G.; Rich, A. A method to identify and characterize Z-DNA binding proteins using a linear oligodeoxynucleotide. *Nucleic Acids Res.* (1993), **21**: 2669-2672.
- 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).







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

Oligo Modifications

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

5-I U

Category	RNA Oligo Synthesi	
Modification Code	5-I-rU	5' Oligomw-O
Reference Catalog Number	27-6554	0=1-0-1.0.
5 Prime	Υ	но
3 Prime	Υ	OH OH
Internal	Υ	O≕P−O−-wwwOligo-3' OH
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).

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).

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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).







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

Oligo Modifications

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

5-Me rC [5mrC]

Category	Epigenetics		NH ₂
Modification Code	5mrC		N CH3
Reference Catalog Number	27-6945	5' Oligo ·····O	
5 Prime	Υ	o=	-∘¬ັດ Ï
3 Prime	Υ	но	
Internal	Υ		ООН
Molecular Weight(mw)	319.21	5-Methyl rC [27-6945-XX]	0=P-0-*****Oligo-3*

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.



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Oligo Modifications

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

5mU (m5U)

Category	Minor Bases		Ŭ CH₃
Modification Code	m5U	51.01	O N N
Reference Catalog Number	27-6557	5' Oligo^^^^ O =P-	
5 Prime	Υ	HO HO	
3 Prime	Υ	110	
Internal	Υ	5me rU (m5U)	O OH
Molecular Weight(mw)	320.19	[27-6557]	0=P-0-****Oligo 3'
			Óн





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

Oligo Modifications

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8-Azanebularine ribo

Category	Others	N N
Modification Code	8-AzaN	5' Oligowww—O
Reference Catalog Number	27-6606	
5 Prime	Υ	он 🗸 🗡
3 Prime	Υ	8-Azanebularine
Internal	Υ	9 A==N
Molecular Weight(mw)	315.17	0-Azain 0=P-0-wwwOligo 3' [27-6606-XX] OH





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

Oligo Modifications

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8-Br rG

Category	RNA Oligo Synthesis	s 🐰 🗓
Modification Code	8-Br-rG	5' Oligo WWO Br
Reference Catalog Number	27-6446	O=P-O¬ NNNNH2
5 Prime	Υ	OH O
3 Prime	Υ	
Internal	Υ	8-Bromo Guanosine I I OH
Molecular Weight(mw)	424.11	0=P-0-Oligo 3'
		ÓН





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Oligo Modifications

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8-Oxo rG [8-Oxo-rG]

N NH
O NH2
Т Т о он
Î P—O~******* <mark>Oligo 3'</mark> I OH





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

Oligo Modifications

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Adenylation-5' (rApp)

Category Conjugation Chemistry

Modification Code 5rApp

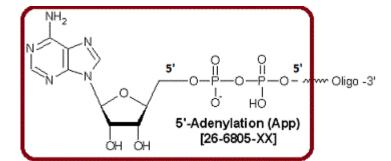
Reference Catalog Number 26-6805

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



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- q., 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.

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Oligo Modifications

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Amino Allyl rU

Category	Others		HN
Modification Code	AmAll-rU		H ₂
Reference Catalog Number	27-6548	5'- Oligo	_
5 Prime	Υ	OH	O Amino Allyl rU
3 Prime	Υ		[27-6548-XX]
Internal	Υ		0 но
Molecular Weight(mw)	363.79	0	=P-0 - www Oligo -3'
			OH





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

Oligo Modifications

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Amino C6 U

Category	Minor Bases	NH ₂
Modification Code	AmC6U	HN NH V
Reference Catalog Number	27-6422	5' Oligo
5 Prime	Υ	Amino C6 U (RNA)
3 Prime	Υ	OH [27-6422-XX]
Internal	Υ	о он
Molecular Weight(mw)	474.4	О=Р-О





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

Oligo Modifications

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dihydro rU (5-6 DH rU)

		0,1
Category	Minor Bases	HN
Modification Code	5-6-DHrU	5' Oligonono-O
Reference Catalog Number	27-6683	o=ḥ—o— 。
5 Prime	Υ	но 🗸
3 Prime	Υ	—
Internal	Υ	dihydrorU (5-6 DHU) ┆ ŮH
Molecular Weight(mw)	308.19	[27-6683-XX] O=P-O
		ÓH





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

Oligo Modifications

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Inosine ribo [rl]

Category	Others	Inosine ribo O
Modification Code	rl	N NH
Reference Catalog Number	27-6421	5' Oligo
5 Prime	Υ	1 20
3 Prime	Υ	ОН
Internal	Υ	ÓОН
Molecular Weight(mw)	330.19	0=P-0-///Oligo 3'
		ÓН





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

Oligo Modifications

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Inverted rA (reverse linkage)

Category Minor Bases Modification Code Inv-rA Inverted rA-5' (reverse linkage) Reference Catalog Number 27-6441 [27-6441-XX] 5 Prime 3 Prime Υ Internal Υ Molecular Weight(mw) 329.21 ^///^/Oligo 3

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.

Construct Examples A 5'-NNNNNNN-3'-3'-NNNNNNN-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. B 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. C 5'-NNNNNNNN-3'-5'--5'-NNNNNNNN-3'

The same construct is shown above 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.

The CE phosphoramidite of 5'-3' reverse dA has the dimethoxytrityl (DMT) and phosphoramidite groups reversed from the normal case, that is, the DMT-group is attached to the 3'-OH, and the phosphoramidite attached to the 5'-OH, of the ribose. This 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. <u>Formation of nuclease resistant (5'-5', 3'-3') linkages</u>. 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 ddl. References
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Oligo Modifications

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Inverted rC (reverse linkage)

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.

Construct Examples A 5'-NNNNNNN-3'-3'-NNNNNNN-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. B 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. C 5'-NNNNNNN-3'-5'--5'-NNNNNNN-3'

The same construct is shown above 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.

The CE phosphoramidite of 5'-3' reverse dA has the dimethoxytrityl (DMT) and phosphoramidite groups reversed from the normal case, that is, the DMT-group is attached to the 3'-OH, and the phosphoramidite attached to the 5'-OH, of the ribose. This 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.



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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. <u>Formation of nuclease resistant (5'-5', 3'-3') linkages</u>. 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 ddl. 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.

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

Oligo Modifications

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

Inverted rG (reverse linkage)

Category Minor Bases Inverted rG (reverse linkage) Modification Code Inv-rG [27-6443-XX] Reference Catalog Number 27-6443 5 Prime 3 Prime Υ Internal Υ Molecular Weight(mw) 329.21 -^vvv<mark>Oligo-3</mark>

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.

Construct Examples A 5'-NNNNNNN-3'-3'-NNNNNNN-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. B 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. C 5'-NNNNNNN-3'-5'--5'-NNNNNNN-3'

The same construct is shown above 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.

The CE phosphoramidite of 5'-3' reverse dA has the dimethoxytrityl (DMT) and phosphoramidite groups reversed from the normal case, that is, the DMT-group is attached to the 3'-OH, and the phosphoramidite attached to the 5'-OH, of the ribose. This 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).

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- 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.

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Oligo Modifications

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

Inverted rU (reverse linkage)

5'-Oligo AAAAA-Category Minor Bases Inverted rU (reverse linkage) Modification Code Inv-rU [27-6444-XX] Reference Catalog Number 27-6444 OH 5 Prime 3 Prime Υ Internal Υ Molecular Weight(mw) 329.21 VVVVVVOligo 3

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.

Construct Examples A 5'-NNNNNNN-3'-3'-NNNNNNN-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. B 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. C 5'-NNNNNNN-3'-5'--5'-NNNNNNN-3'

The same construct is shown above 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.

The CE phosphoramidite of 5'-3' reverse dA has the dimethoxytrityl (DMT) and phosphoramidite groups reversed from the normal case, that is, the DMT-group is attached to the 3'-OH, and the phosphoramidite attached to the 5'-OH, of the ribose. This 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:

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- 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.

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Oligo Modifications

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

L-RNA rA

Category	Others	NH ₂	
Modification Code	L-rA	N N	5, 6,
Reference Catalog Number	27-6941	N N N	0—/////5' Oligo
5 Prime	Υ	0	OH
3 Prime	Υ		Off
Internal	Υ	он о	L-RNA rA [27-6941-XX]
Molecular Weight(mw)	329.2	Oligo 3' ////OP==O	[2: 55:17.04]
		ÓН	

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

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

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

L-DNA Applications

- References

- 1) Damha M.J., Giannaris P.A., Marfey P. Antisense L/D-oligonucleotide chimeras: nuclease stability, base-pairing properties, and activity at directing ribonuclease H. Biochemistry. 1994;33:7877?7885.<
- 2) Utilising the left-helical conformation of L-DNA for analysing different marker types on a single universal microarray platform Nicole C. Hauser, Rafael Martinez, Anette Jacob, Steffen Rupp, J"rg D. Hoheisel, Stefan Matysiak Nucleic Acids Res. 2006 October; 34(18): 5101-5111. Published online 2006 September 20. doi:ÿ10.1093/nar/gkl671
- 3) Superior structure stability and selectivity of hairpin nucleic acid probes with an L-DNA stem. Youngmi Kim, Chaoyong James Yang, Weihong Tan Nucleic Acids Res. 2007 December; 35(21): 7279?7287. Published online 2007 October 24. doi:ÿ10.1093/nar/gkm771
- 4) Hayashi G., Hagihara M., Nakatani K. Application of L-DNA as a molecular tag. Nucleic Acids Symp. Ser. 2005;49:261?262
- 5) Utilizing the bioorthogonal base-pairing system of L-DNA to design ideal DNA nanocarriers for enhanced delivery of nucleic acid cargos Kyoung-Ran Kim, Taemin Lee, Byeong-Su Kim and Dae-Ro Ahn.



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





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

Oligo Modifications

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

L-RNA rC

Category	Others	NH ₂	
Modification Code	L-rC	∬ N	51.01
Reference Catalog Number	27-6942	N O O	0 — ^^^,5' Oligo —P=0
5 Prime	Υ	0	OH
3 Prime	Υ		
Internal	Υ	OH O	L-RNA rC [27-6942-XX]
Molecular Weight(mw)	305.19	Oligo 3'	
		он	

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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- 4) Hayashi G., Hagihara M., Nakatani K. Application of L-DNA as a molecular tag. Nucleic Acids Symp. Ser. 2005;49:2617262
- 5) Utilizing the bioorthogonal base-pairing system of L-DNA to design ideal DNA nanocarriers for enhanced delivery of nucleic acid cargos Kyoung-Ran Kim, Taemin Lee, Byeong-Su Kim and Dae-Ro Ahn.



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





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Oligo Modifications

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

L-RNA rG

Category	Others	O 	
Modification Code	L-rG	HN	
Reference Catalog Number	27-6943	H ₂ N N N PO	0-///5' Oligo
5 Prime	Υ	0 0	i
3 Prime	Υ		OH
Internal	Υ	OH O	L-RNA rG [27-6943-XX]
Molecular Weight(mw)	345.21	Oligo 3'	
		ОН	

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

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





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Oligo Modifications

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L-RNA rU

Category	Others	O NIII	
Modification Code	L-rU	NH	El Ol:
Reference Catalog Number	27-6944	N O	0—////,5' Oligo
5 Prime	Υ	0	−P=0
3 Prime	Υ		OH -II
Internal	Υ	OH Q	L-RNA rU [27-6944-XX]
Molecular Weight(mw)	306.17	Oligo-3' VVVVO-P=O	-
		ОН	

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

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L-DNA Applications

- References

- 1) Damha M.J., Giannaris P.A., Marfey P. Antisense L/D-oligonucleotide chimeras: nuclease stability, base-pairing properties, and activity at directing ribonuclease H. Biochemistry. 1994;33:7877?7885.<
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- 4) Hayashi G., Hagihara M., Nakatani K. Application of L-DNA as a molecular tag. Nucleic Acids Symp. Ser. 2005;49:261?262
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Chem. Sci., 2014,5, 1533-1537



27-6532.pdf

Product Specifications

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

Oligo Modifications

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N1-methyl pseudoUridine (m1-psi rU)

Category	Structural Studies	
Modification Code	m1-psi-rU	СН3
Reference Catalog Number	27-6532	5' Oligoww-O
5 Prime	Υ	0=0-0-0
3 Prime	Υ	ОН
Internal	Υ	O OH O=P-O-/wwwOligo-3'
Molecular Weight(mw)	320.19	ÓH N1-methyl pseudoUridine (m1-psi rU)
		[27-6532-XX]



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

Oligo Modifications

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N1-Methyl rA (m1A)

Category	Structural Studies	N1-Methyl rAdenosine [27-6559-XX] NH ₂ CH ₃
Modification Code	m1A	, N
Reference Catalog Number	27-6559	5' Oligo \\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\
5 Prime	Υ	_0 _
3 Prime	Υ	ОН
Internal	Υ	ОН
Molecular Weight(mw)	344.24	0=P-0Oligo 3'
		OH

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



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Oligo Modifications

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N3-methyl-rU [m3U]

Category	Others	H ₃ C
Modification Code	m3U	N N
Reference Catalog Number	27-6558	5' Oligo//// O =P-O -
5 Prime	Υ	но
3 Prime	Υ	
Internal	Υ	N3-Methyl-rU OH OH
Molecular Weight(mw)	320.19	0 = P - 0 - \(\text{Oligo 3'}
		óн





3. Nguyen, H-K.

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.

N4-Ethyl rC [N4-Et-rC]

Category **Duplex Stability** N4-Et-rC Modification Code Reference Catalog Number 27-6685 5 Prime 5' Oligonmo-O 3 Prime Υ НÓ Internal Υ N4-Ethyl rC [27-6685-XX] Molecular Weight(mw) 333.4 -wwwOligo 3'

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.



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, 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.

Oligo Synthesis

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.

N6-Benzyl Adenosine (bn6A)

Category RNA Oligo Synthesis Modification Code bn6A 5' Oligowww 27-6604 Reference Catalog Number 5 Prime 3 Prime Υ -√///M/M Oligo 3 Internal Υ 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.
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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.



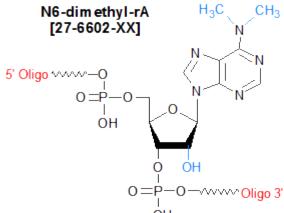
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Oligo Modifications

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

N6-dimethyl rA [m6-6A]

		N6-dim
Category	Others	[27-66
Modification Code	m6-6A	
Reference Catalog Number	27-6602	5' Oligo
5 Prime	Υ	0 =
3 Prime	Υ	
Internal	Υ	
Molecular Weight(mw)	333.2	





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Oligo Modifications

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

N6-isopentenyl-rA (i6a)

Category	RNA Oligo Synthesis	N6-isopentenyl rA (i6A) [27-6603-XX]	1
Modification Code	N6-i6A	N N	
Reference Catalog Number	27-6603	5' Oligo www—O	
5 Prime	Υ	OH CO	
3 Prime	Υ	ÿ \	
Internal	Υ	ÓР	
Molecular Weight(mw)	398.33	O=P-O- ~~~~Oligo 3'	
		ÓН	



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

Oligo Modifications

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

N6-Methyl rA (m6A)

Category	Epigenetics	N6-Methyl rA (m6A) [27-6601-XX]
Modification Code	m6A	N
Reference Catalog Number	27-6601	5' Oligo \\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\
5 Prime	Υ	OH OH
3 Prime	Υ	
Internal	Υ	o oh
Molecular Weight(mw)	343.23	0=P-0-///Oligo 3'
		OH

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.



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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.



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.

pseudoUridine (psi rU)

Category	Affinity Ligands	HN NH
Modification Code	psi-rU	
Reference Catalog Number	27-6531	5' Oligo ~~~~ O
5 Prime	Υ	
3 Prime	Υ	ОН
Internal	Υ	pseudoUridine [27-6531-XX] OH
Molecular Weight(mw)	306.17	0=P-0\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\
		ÓН



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Oligo Modifications

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

rA RNA Base

Category	RNA Oligo Synthesis	riboadenosine (rA) NH ₂	
Modification Code	rA	N N	
Reference Catalog Number	27-6400A	5' Oligo \\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	
5 Prime	Υ	OH O	
3 Prime	Υ		
Internal	Υ	ÓОН	
Molecular Weight(mw)	329.21	O=P−O//-Oligo 3'	
		ОН	



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

Oligo Modifications

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

rC RNA Base

Category	RNA Oligo Synthesis	ribocytosine rC [27-6400-XX]	NH ₂
Modification Code	rC		N
Reference Catalog Number	27-6400C	5' Oligo \\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	o N
5 Prime	Υ	О_Р_О= НО	
3 Prime	Υ	110	
Internal	Υ		O OH
Molecular Weight(mw)	305.18	0:	=P-0-///Oligo 3'
			OH



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

Oligo Modifications

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

rG RNA Base

Category	RNA Oligo Synthesis	s o
Modification Code	rG	N II ŅH
Reference Catalog Number	27-6400G	O N N NH ₂
5 Prime	Υ	5'- Oligo
3 Prime	Υ	O OH
Internal	Υ	0=P-0 Oligo -3'
Molecular Weight(mw)	345.21	OH OH
· , ,		riboguanosine (rG) [27-6400-XX]



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

Oligo Modifications

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0

rSpacer

Category	Spacers	5'-Oligoww-O-P-O OH
Modification Code	rABS	
Reference Catalog Number	26-6442	0=P-0-
5 Prime	Υ	ОН
3 Prime	Υ	rSpacer 0=P-0 Base [26-6442-XX]
Internal	Υ	OH OH
Molecular Weight(mw)	196.09	0 0=P-0-//\\Oligo
		· · · · · · · · · · · · · · · · · · ·





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.

rU RNA Base

Category	RNA Oligo Synthes	s is o
Modification Code	rU	NH NH
Reference Catalog Number	27-6400U	5'- Oligo O-P-O-
5 Prime	Υ	OH OH
3 Prime	Υ	он О
Internal	Υ	0=P-0-
Molecular Weight(mw)	306.17	ÖH ribouridine (rU) [27-6400-XX]



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.

Thio 6-rG (s6rG)

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

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- 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. 3. Rao, T.S., Durland, R.H., Seth, D.M., Myrick, M.A., Bodepudi, V., Revankar, G.



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- 4. Coleman, R.S., Pires, R.M. Covalent cross-linking of duplex DNA using 4-thio-2'-deoxyuridine as a readily modifiable platform for introduction of reactive functionality into oligonucleotides. Nucleic Acids Res. (1997), 25: 4771-4777.



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.

Thio-2-rU (s2U)

Category	RNA Oligo Synthes	is o
Modification Code	s2U	NH
Reference Catalog Number	27-6537	5' Oliganww-O
5 Prime	Υ	HO 0
3 Prime	Υ	O OH
Internal	Υ	O=P-O
Molecular Weight(mw)	322.22	ÓH 2-Thio-Uridine (s2U) [27-6537-XX]





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

SH

Oligo Modifications

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

Thio-4-rU (s4U)

Category	Minor Bases	NH
Modification Code	s4U	5' Oligo
Reference Catalog Number	27-6445	o=p-o
5 Prime	Υ	но
3 Prime	Υ	4-Thio-Uridine (s4U)
Internal	Υ	[27-6445-XX] O OH
Molecular Weight(mw)	322.22	0 = P - 0 - \www\Oligo-3'
		OH





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.

Thymidine Ribo

Category	Minor Bases	[27-6946-XX] CH ₃
Modification Code	rT	- HN
Reference Catalog Number	27-6946	5' Oligo VVVV—O
5 Prime	Υ	0=P-0 HO
3 Prime	Υ	
Internal	Υ	ООН
Molecular Weight(mw)	321.07	0=P−0-///Oligo 3'
		ÓН





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.

Zebularine ribo

Category	Structural Studies	ĺ , Ň
Modification Code	rZ	5' Oligo y
Reference Catalog Number	27-6435	5'- Oligo w 0 N O
5 Prime	Υ	riboZebularine
3 Prime	Υ	[27-6435] O OH
Internal	Υ	0=P-0- ww Oligo -3'
Molecular Weight(mw)	290.17	он

