
**Product Profile**

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dye labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

# Antisense Oligonucleotides

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

**Phosphorothioate Oligos**

**Propyne dC and dU labeled Oligos**

**2'O methyl Oligos**

**Locked Nucleic Acids (LNA)Oligos**

**2'-5' linked Oligos**

**Chimeric Oligos**

Gene Link™

# Antisense Oligonucleotides

## Background

Antisense oligonucleotides refer to short, synthetic oligonucleotide that are complementary in sequence and upon specific hybridization to its cognate gene product induces inhibition of gene expression. Oligonucleotides, as short as 15 mer have the required specificity to inhibit gene expression of a particular gene by annealing to the cellular mRNA (1,2). The mechanism of gene expression is based on two properties; the first is the physical blocking of the translation process by the presence of the short double stranded region, secondly the presence of the RNA-DNA duplex is susceptible to cellular RNase H activity. RNase H cleaves the RNA-DNA duplex region of the mRNA thus preventing the faithful translation of the mRNA (3).

The stability of the RNA-DNA duplex in terms of hybridization and half-life is crucial to successful gene inhibition. Vigorous research activity in the area of nucleic acid chemistry has been devoted in developing novel base analogs that are resistant to degradation and that possess strong hybridization properties. This product profile aims at listing some analogs that meet the above criteria and are amenable to be synthesized by currently available standard DNA synthesis chemistry. This includes the classical phosphorothioate linkages (4), propyne analogs (5) and the latest locked nucleic acid (LNA) base analogs (6). We believe from cited reports that LNA substituted oligos with phosphorothioate linkages presents the most stable hybridization and are least susceptible to nuclease degradation (6).

At Gene Link in addition to the synthesis of these modified oligos, we routinely assist customers in the design of the oligos that are particularly suited to their application.

## Oligonucleotide Design & Modifications

### Phosphorothioate

The driving force for the search for novel chemical modification groups compatible with Watson-Crick hybridization of oligonucleotide was based on the observation of the short stability of naturally occurring oligonucleotides with phosphodiester bonds. Oligonucleotides with natural phosphodiester bonds are highly susceptible to rapid degradation by cellular nucleases. Cellular nucleases have endonuclease activity as well such that 3' and 5' end caps are not sufficient to prevent from degradation.

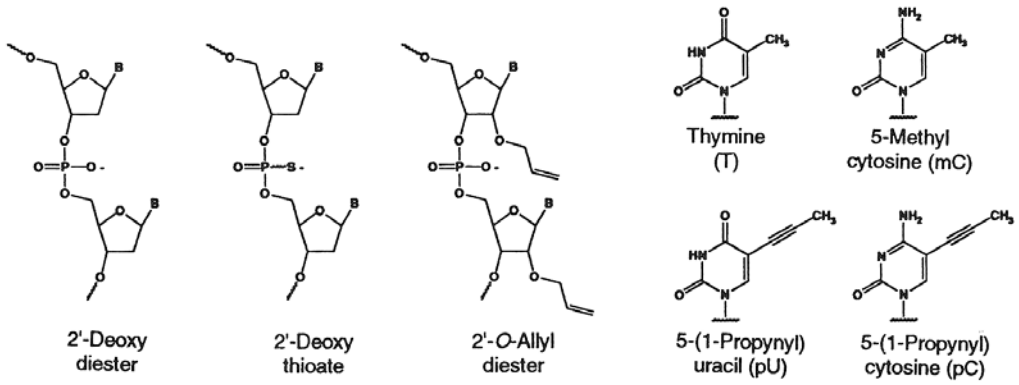
Modification of the phosphodiester bond by replacing one of the non-bridging oxygen by sulfur imparts resistance to nuclease degradation, but in general hybridize to the target sequences with lesser affinity than the phosphodiester counter part. *This can be minimized by the use of LNA and 2'-5' linked oligos as described in the section below.* The sulfur-substituted oligonucleotides have a phosphorothioate linkage and are termed as **phosphorothioates** or simply as **S-oligo.** Phosphorothioate oligos are synthesized by Gene Link using the Beaucage (4) sulfurizing reagent. The sulfurization reaction is rapid and is performed on automated DNA synthesizers yielding greater than 96% phosphorothioate linkages; the remainder are phosphodiester linkages. 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.

### Propyne* Analogs

It has been shown that C-5 propyne analogs of dC and dT when substituted in phosphorothioate oligonucleotide imparts greater inhibition of gene expression due to increased binding affinity to the target mRNA and increased stability (5). Based on the above information antisense oligonucleotide could either be Phosphorothioated at all diester linkages or combined with substitutions of dC and dT by C-5 propyne analogs pdC and pdU.

The use of propyne analogs is covered by patents and licensing agreements. The sale of propyne-modified oligos is for research use only. See license agreement below*.

**Internucleotide Linkages & C5 Propyne Analogs**




**Natural diester linkage, Thioate (S-Oligo) linkage & C5 propyne analogs of dC and dT**

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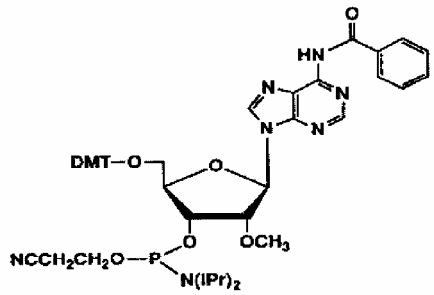
## 2'-O-methyl RNA oligonucleotides

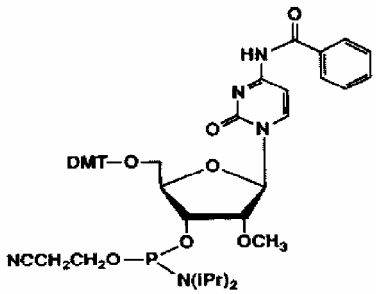
RNA oligos are susceptible to degradation to the same extent as native RNA extracted from various sources. An attractive alternate to prevent degradation from nucleases is the use of 2'-O- methyl RNA bases, when specific 2'OH is not required. The 2'-O- methyl oligonucleotides confer considerable nuclease resistance and are similar in hydrogen bonding properties to RNA/RNA than the lower RNA/DNA binding property (7). 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 phosphodiester 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. 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.

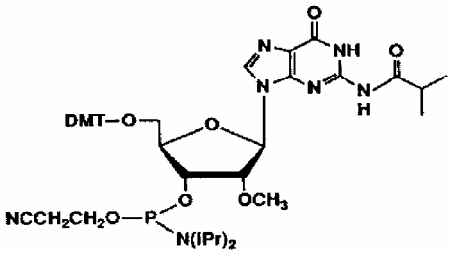
## 2'O methyl phosphoramidite bases



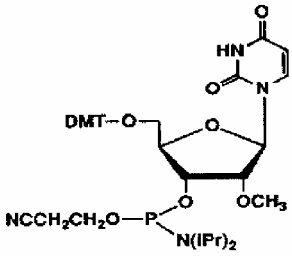
*N⁶-Benzoyl-5'-O-DMT-2'-O-Methyl -Adenosine-3'-(β-cyanoethyl)- Phosphoramidite*



*N⁴-Benzoyl-5'-O-DMT-2'-O-Methyl -Cytidine-3' -(β-cyanoethyl)- Phosphoramidite*



*N²-Isobutyryl-5'-O-DMT-2'-O-Metnyl -Guanosine-3' -(β-cyanoethyl)- Phosphoramidite*

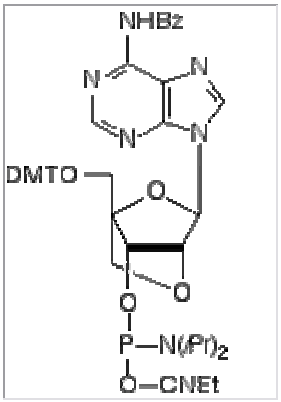
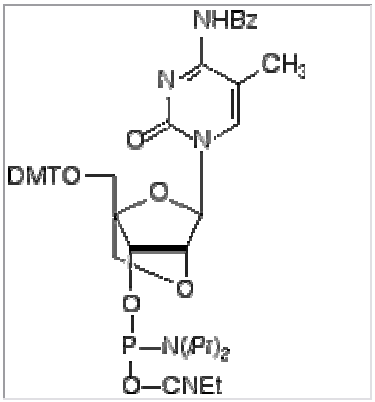
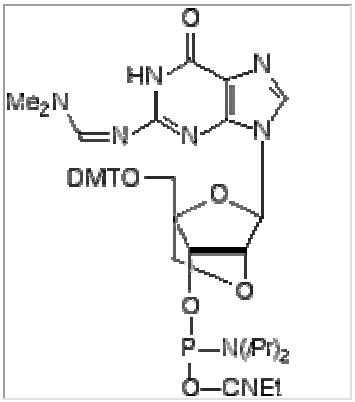
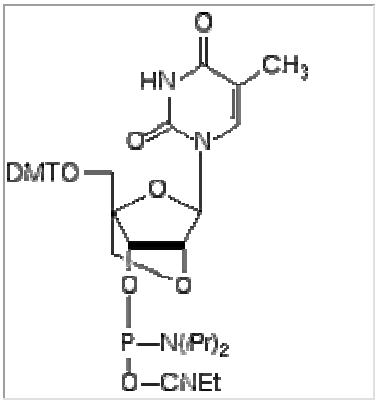


*5'-O-DMT-2'-O-Methyl-Uridine- 3' -(β-cyanoethyl)-Phosphoramidi'*

## Locked Nucleic Acids (LNA)

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

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

| LNA MONOMER STRUCTURES | | | |
|---|---|---|---|
|  |  |  |  |
| Bz-A-LNA | 5-Me-Bz-C-LNA | dmf-G-LNA | T-LNA |

LNA Oligonucleotides exhibit unprecedented thermal stabilities towards complementary DNA and RNA, which allow excellent mismatch discrimination (8). The high binding affinity of LNA oligos allows for the use of short probes in antisense protocols and LNA is recommended for use in any hybridization assay that requires high specificity and/or reproducibility, e.g., dual labeled probes, in situ hybridization probes, molecular beacons and PCR primers. Furthermore, LNA offers the possibility to adjust Tm values of primers and probes in multiplex assays. Each LNA base addition in an oligo increases the Tm by approximately 8$^{o}$C. As a result of these significant characteristics, the use of LNA-modified oligos in antisense drug development is now coming under investigation, and recently the therapeutic potential of LNA has been reviewed (11).

The synthesis and incorporation of LNA bases can be achieved by using standard DNA synthesis chemistry. Detailed research results have not yet concluded as to the amount of LNA bases and regular DNA base combination in successful antisense and gene delivery experiments. The investigator can elect to substitute individual bases in the oligo to LNA bases or use a combination. Due to the high affinity and thermal stability of the LNA: DNA duplex it is not advised to have more than 15 LNA bases in an oligo; this induces strong self-hybridization

The use of LNA C base requires special synthesis and post synthesis protocols. LNA-containing oligonucleotides can be purified and analyzed using the same methods employed for standard DNA. LNA can be mixed with DNA and RNA, as well as other nucleic acid analogues, modifiers and labels. LNA oligonucleotides are water soluble, and can be separated by gel electrophoresis and precipitated by ethanol.

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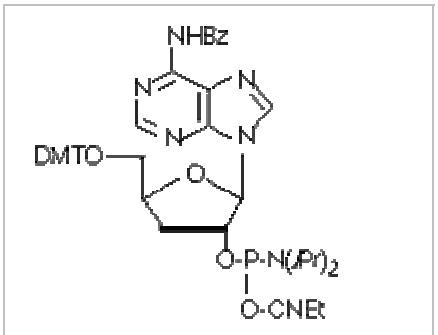
## 2'-5' Linked Oligonucleotides

Cellular DNA and RNA are made up of ribo- and 2'-deoxyribonucleic acids linked together via 3'-5' phosphodiester linkages and by far comprise the bulk of polynucleic acids found in cells. Much less common are oligonucleotides which have 2'-5' linkages. However, a unique feature of 2'-5' linked oligonucleotides is their ability to bind selectively to complementary RNA (12-13). These features suggest a number of interesting uses for 2'-5' linked oligos such as their use as RNA specific probes or in antisense oligos.

Chimeric oligos have been synthesized using 3'-deoxy-2'-phosphoramidites and 2'-deoxy-3'-phosphoramidites. Using these amidites the authors synthesized phosphorothioate oligos with 2'-5' linkages and chimeras with 2'-5' linked ends and 3'-5' linked central regions. They found that 2'-5' phosphorothioate oligos: 1) bind selectively to complementary RNA with the same affinity as phosphodiester oligos; 2) exhibit much nonspecific binding to cellular proteins; 3) do not activate RNase H. In experiments with Chinese hamster ovary cells transfected with human 5a-reductase-II (5aR-II), chimeric antisense oligos complementary to the 5' untranslated region of 5aR-II, containing seven 3'-5' linkages in the center, were effective in inhibiting 5aR-II protein in a dose dependent manner. The same oligos with 2'-5' linkages only were ineffective in inhibiting 5aR -II protein synthesis (14).




**3'-dA-CE Phosphoramidite**

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## Ordering Information

| Custom Antisense Oligo Synthesis | | | | | | |
|---|---|---|---|---|---|---|
| Product | Catalog No. | 50 nmol scale (XX=05) | 200 nmol scale (XX=02) | 1 µmol scale (XX=01) | 10 µmol scale (XX=10) | 15 µmol scale (XX=15) |
| Phosphorothioates | 26-6401-XX | 3.50 | 4.25 | 6.50 | 50.00 | 65.00 |
| Propyne dC or propyne dU* | 26-6408-XX | 130.00 | 130.00 | 200.00 | 1600.00 | 2000.00 |
| 2'O methyl bases | 27-6410-XX | na | 14.00 | 20.00 | 200.00 | 250.00 |
| Locked Nucleic Acid (LNA) | 26-6417-XX | 190.00 | 190.00 | 250.00 | 2000.00 | 2500.00 |
| 2'-5' linked bases | 26-6414-XX | 275.00 | 275.00 | 300.00 | 2400.00 | 3000.00 |
| 5-Me-dC | 26-6413-XX | 75.00 | 75.00 | 125.00 | 825.00 | 1250.00 |
| Please inquire about volume discounts | | | | | | |

| Purification | | | | | | |
|---|---|---|---|---|---|---|
| Product | Catalog No. | 50 nmol scale (XX=05) | 200 nmol scale (XX=02) | 1 µmol scale (XX=01) | 10 µmol scale (XX=10) | 15 µmol scale (XX=15) |
| Gel Purification | 26-6400-XX | 75.00 | 75.00 | 150.00 | 1500.00 | 1500.00 |
| Reverse Phase Cartridge | 26-6400-XX | 30.00 | 30.00 | 90.00 | 750.00 | 750.00 |

## Related Products Ordering Information

| Fluorophore* | Color | Absorbance max (nm) | Emission max (nm) | $, 200 nmol scale | $, 1 µmol scale |
|---|---|---|---|---|---|
| Dabcyl (Quencher) | | 453 | | 290.00 | 350.00 |
| BHQ-1** (Quencher) | | 534 | | 290.00 | 350.00 |
| BHQ-2** (Quencher) | | 579 | | 290.00 | 350.00 |
| BHQ-3** (Quencher) | | 672 | | 290.00 | 350.00 |
| 6-FAM (Fluorescein) | Green | 494 | 525 | 390.00 | 450.00 |
| TET | Orange | 521 | 536 | 390.00 | 450.00 |
| HEX | Pink | 535 | 556 | 390.00 | 450.00 |
| Cy 3 | Red | 552 | 570 | 490.00 | 550.00 |
| Cy 3.5 | Purple | 588 | 604 | 490.00 | 550.00 |
| Cy 5 | Violet | 643 | 667 | 490.00 | 550.00 |
| Cy 5.5 | Blue | 683 | 707 | 490.00 | 550.00 |
| Tetramethylrhodamine | Rose | 565 | 580 | 540.00 | 600.00 |
| Alexa Dye Series | Varies | Varies | Varies | 540.00 | 600.00 |
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