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

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.

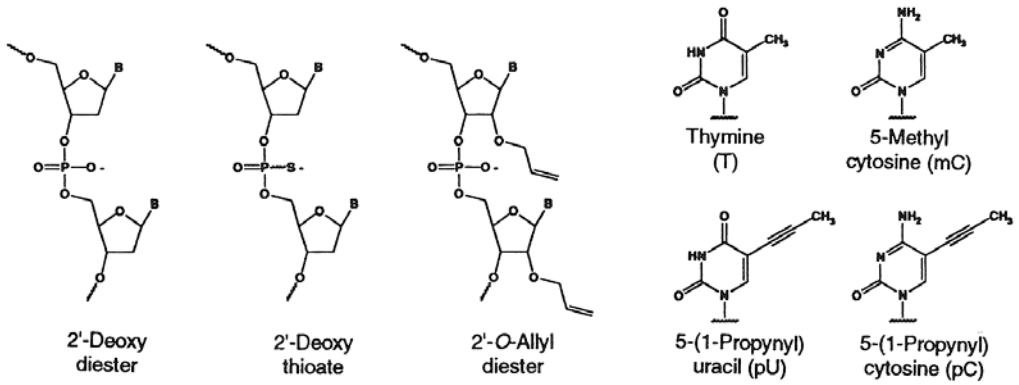
Modification of the phosphodiester bond by replacing one of the non-bridging oxygen by sulfur imparts resistance to nuclease degradation, but in general hybridize to the target sequences with lesser affinity than the phosphodiester counter part. *This can be minimized by the use of LNA and 2'-5' linked 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 is 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 researcher's 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

***Propyne Analog Use Agreement**

Our agreement with Glen Research who in turn has an agreement with Isis Pharmaceuticals, Inc. allows us to sell to you C-5 Propynes and G-clamps that are ultimately used for RESEARCH PURPOSES ONLY. In accordance with this agreement, we must inform you of the uses to which these products may be put, which are described below.

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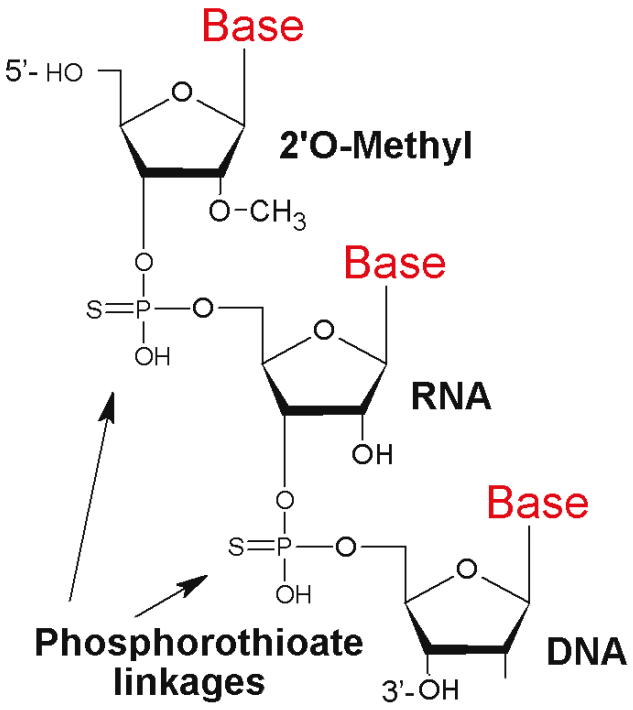
2'-O-methyl RNA oligonucleotides

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 researcher's experimental requirement. Substitution of all diester linkage is recommended to provide greater nuclease resistance



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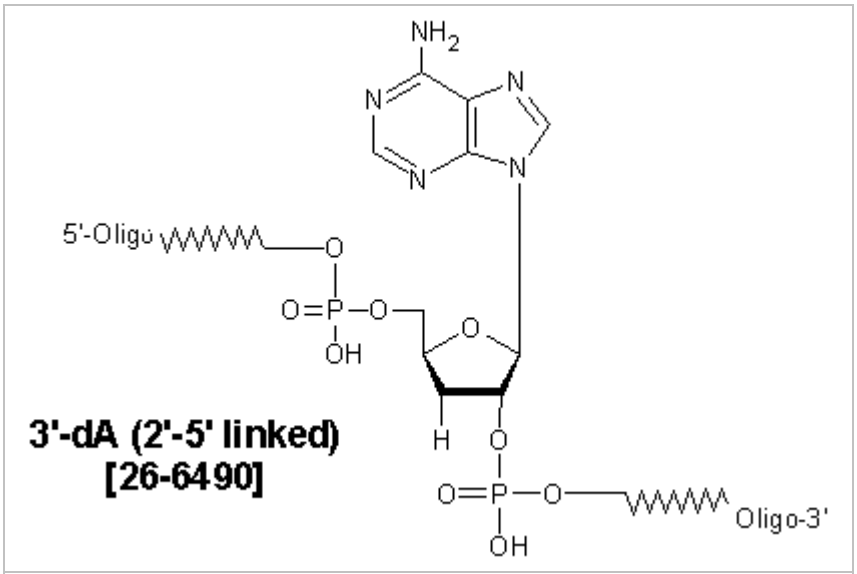
2'-5' Linked Oligonucleotides

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).



Duplex Stabilization

Using these base substitutions, duplex stability and therefore melting temperatures are raised by the approximate amounts shown below.

Modifications Increasing Duplex Stability and Nuclease Resistance		
Modification*	**Duplex Stability [Tm Increase]**	**Nuclease Resistance**
Phosphorothioate	Slightly decreased	Increased
2'-OMethyl	Increased	Increased
2'-Fluoro	Increased [1-2° per substitution]	Increased
2-Amino-dA	Increased [3.0° per substitution]	No effect
5-Methyl-dC	Increased [1.3° per substitution]	No effect
C-5 propynyl-C	Increased [2.8° per substitution]	Increased
C-5 propynyl-U	Increased [1.7° per substitution]	Increased
*Selected list of modifications available from Gene Link, Inc. are listed in this table. Visit www.genelink.com for complete offerings.		

References

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Ordering Information

Modified Bases & Linkages	
Product	**Catalog No.**
Phosphorothioates	26-6401-XX
Propyne dC or propyne dU*	26-6408-XX
Propyne dC or propyne dC	26-6501-XX
2'O methyl bases	27-6410-XX
2'-5' linked bases	26-6414-XX
5-Me-dC	26-6413-XX
2-Amino dA	26-6525-XX
Spacer 9	26-6440-XX
2'F A	26-6692-XX
2'F C	26-6463-XX
2'F G	26-6693-XX
2'F U	26-6462-XX
Spacer 9	26-6440-XX
2'O-Me-5-Me-C	26-6508-XX
Chimeric Linkage	26-6420-XX
3' dA (2'-5' linked)	26-6490-XX
3' dC (2'-5' linked)	26-6491-XX
3' dG (2'-5' linked)	26-6492-XX
3' dT (2'-5' linked)	26-6493-XX
XX in catalog number is replaced by the specific scale of synthesis	

Related Products Ordering Information

Fluorophore*	Color	Absorbance max (nm)	Emission max (nm)
Dabcyl (Quencher)		453	
BHQ-1** (Quencher)		534	
BHQ-2** (Quencher)		579	
BHQ-3** (Quencher)		672	
6-FAM (Fluorescein)	Green	494	525
TET	Orange	521	536
HEX	Pink	535	556
Cy 3	Red	552	570
Cy 3.5	Purple	588	604
Cy 5	Violet	643	667
Cy 5.5	Blue	683	707
Tetramethylrhodamine	Rose	565	580
Please see our complete list at www.genelink.com or call at 1800-436-3546			

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Gene Link, Inc.

Tel: (914) 769-1192
Email: support@genelink.com
www.genelink.com