Antisense Oligonucleotides

Background

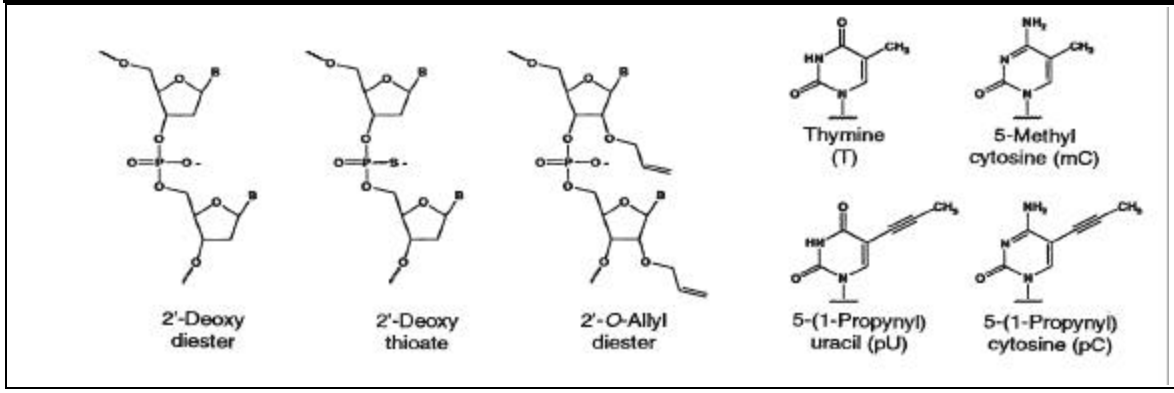
Antisense oligonucleotides refer to short, synthetic oligonucleotides which are complementary in sequence and upon specific hybridization to its cognate gene product induces inhibition of gene expression. Oligonucleotides, as short as a 15 mer has the required specificity to inhibit gene expression of 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).

Oligonucleotide Design

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 phoshodiester 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.

Internucleotide Linkages & C5 Propyne Analogs
Natural diester linkage, Thioate (S-Oligo) linkage & C5 propyne analogs of dC and dT



The sulfur substituted oligonucleotides have a phosphorothiote 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.

Recently 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.

References

1. Milligan, J.F., Matteucci, M.D. and Martin, J.C. (1993) Current concepts in antisense drug design. J. Medicinal Chem. 36:1923-1937.
2. Helene, C., Toulme, J. (1990) Specific regulation of gene expression by antisense, sense and antigene nucleic acids. Biochim. Biophys. Acta. 1049: 99-125.
3. Weintraub, H. M. (1990) Antisense RNA and DNA. Sci. Amer. 262:40-46.
4. Iyer, R.P., Egan. W., Regan, J.B and Beaucage, S.L. (1990) J. Am. Chem. Soc.112; 1253-1254.
5. Wagner, R.W., Matteucci, M.D., Lewis, J.G., Gutierrez, A.J., Moulds, C. and Froehler, B.C. (1993) Antisense gene inhibition by oligonucleotides containing C-5 propyne pyrimidines. Science 260:1510-1513.

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Custom Antisense Oligonucleotide Synthesis

Ordering Information

	Synthesis Scale, $/base NO SET CHARGES			
Modification	**200 nmol**	**1 µmol**	**10 µmol**	**15 µmol**
Phosphorothioate	$4.25	$6.50	$50.00	$55.00
C-5 propyne dC (pdC)	$130/3 sites each additional site @ $25.00	$200/3 sites each additional site @ $35.00	$600/3 sites each additional site @ $100.00	$650/3 sites each additional site @ $100.00
C-5 propyne dU (pdU)	$130/3 sites each additional site @ $25.00	$200/3 sites each additional site @ $35.00	$600/3 sites each additional site @ $100.00	$650/3 sites each additional site @ $100.00

Purification*				
	200 nmol	1 µmol	10 µmol	15 µmol
Gel Purification	$75.00	$150.00	$600.00	$600.00
Reverse Phase Cartridge	$30.00	$90.00	$240.00	$240.00
*Desalted and Lyophilized at no extra charge				

Prices subject to change without notice

All Gene Link products are for research use only.