VOLUME 5, NUMBER 1 JUNE, 1992

The Glen Report

2'-OMe-RNA - THE LOGICAL RNA ALTERNATIVE?

Over the past 12 months, growth in the synthesis of oligoribonucleotides has increased dramatically, resulting in a serious strain on supplies of RNA monomers. As well as causing discomfort to the producers of RNA monomers, this growth is an indication of rapidly expanding research interest in oligoribonucleotides for a variety of research purposes, including physical studies, antisense experiments, and ribozyme studies. Increases in RNA synthesis experience have also focused attention on such factors as limitations in current monomer protecting groups: isobutyryl (*i*Bu) on the exocyclic amino group of Guanosine may be insufficiently labile while phenoxyacetyl (Pac) protection of the same group may be labile to exchange with acetate during the capping step; and *t*-butyl-dimethylsilyl (TBDMS) may be incompletely removed with *t*-butylammonium fluoride in tetrahydrofuran (THF).

Oligoribonucleotides containing 2'-OMe-RNA linkages are being studied for a variety of applications which require the higher binding of RNA-RNA interaction relative to DNA-RNA, along with greatly enhanced nuclease resistance[1]. Recent reports have included the use of biotinylated 2'-OMe-RNA for affinity purification of small nuclear ribonucleoprotein particles[2], the use of a complementary 2'-OMe-RNA sequence to inhibit mRNA processing by interaction with small nuclear ribonucleoprotein particles[3], and incorporation of 2'-OMe-RNA modified with cholesterol into liposomes[4]. It is clear that 2'-OMe-RNA is becoming a powerful research tool in its own right.

However, where "natural" RNA linkages are not necessary for a particular research application, we believe that 2'-OMe-RNA may be a useful alternative. In Table 1 on Page 4, we have collected a comparison of the advantages and disadvantages of substituting 2'-OMe-RNA synthesis for RNA synthesis. The main advantages lie in higher coupling efficiency and simplicity of handling which are more similar to DNA than RNA. On Page 4, we have also illustrated the purity by HPLC of a 2'-OMe-RNA sequence DMT-on immediately after synthesis (Figure 1) and after purification on a Poly-Pak™ cartridge (Figure 2).

We are able to conclude that, in situations where specific RNA linkages are not required, there are considerable practical benefits for the substitution of 2'-OMe-RNA linkages. The handling of 2'-OMe-RNA is much simpler, being essentially equivalent to DNA, because of nuclease resistance. While the cost of such a substitution is higher, it is not prohibitively so. It is clear that the main benefit achieved by the substitution of 2'-OMe-RNA linkages for RNA linkages is in time saved. Even when scientist time can not be valued monetarily, the time saved can be spent on items of more interest to the researcher, namely original research.

References:

1) B.S. Sproat, A.I. Lamond, B. Beijer, P. Neuner, and U. Ryder, *Nucleic Acids Res.*, 1989, 17, 3373.
2) H.O. Smith, K. Tabiti, G. Schaffner, D. Soldati, U. Albrecht, and M.L. Birnstiel,

(Continued on Page 4)

Inside

PSORALEN C2 PHOSPHORAMIDITE

Psoralen derived from plant extracts is an ancient drug used to treat skin diseases like psoriasis. The mode of activity of psoralen is related to its abilty to intercalate between the two strands of DNA. When exposed to long-wavelength UV light, intercalated psoralen reacts with thymidine residues to form a covalent photoadduct. When attached to an oligonucleotide, psoralen reacts to form interstrand crosslinks. The photoadducts can be reversed by short-wavelength UV light.

The use of a psoralen phosphoramidite[1] has been described for the introduction of psoralen at the 5'-terminus of oligonucleotides. Psoralen so attached is an effective intercalator as well as cross-linking reagent and applications abound in the areas of structure elucidation as well as antisense studies[2]. Future development will likely lead to the introduction of a psoralen phosphoramidite with a longer spacer arm designed to crosslink specifically with a triple strand allowing researchers to probe triplex formation[3].

References:

1) U. Pieles and U. Englisch, *Nucleic Acids Res.*, 1989, **17**, 285.
2) J. Woo and P.B. Hopkins, *J. Amer. Chem. Soc.*, 1991, **113**, 5457-5459.
3) M. Takasugi, A. Guendouz, M. Chassignol, J.L. Decout, J. Lhomme, N.T. Thuong, and C. Helene, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 5602-5606.

DIRECT FLUORESCEIN LABELLING OF OLIGONUCLEOTIDES

After biotin labelling, the label most often requested has been fluorescein for use in both diagnostic development and sequencing applications. Synthesis of fluorescein labelled sequencing primers is likely to be the primary use for a fluorescein phosphoramidite. A phosphoramidite based on the methyl ester of fluorescein has been described [1]. However, we preferred to design a phosphoramidite which would produce, on deprotection and isolation of the derived oligonucleotide, the same fluorescein-type structure as had been previously prepared using fluorescein isothiocyanate (FITC). Our fluorescein phosphoramidite also contains a DMT group to allow quantification of coupling.

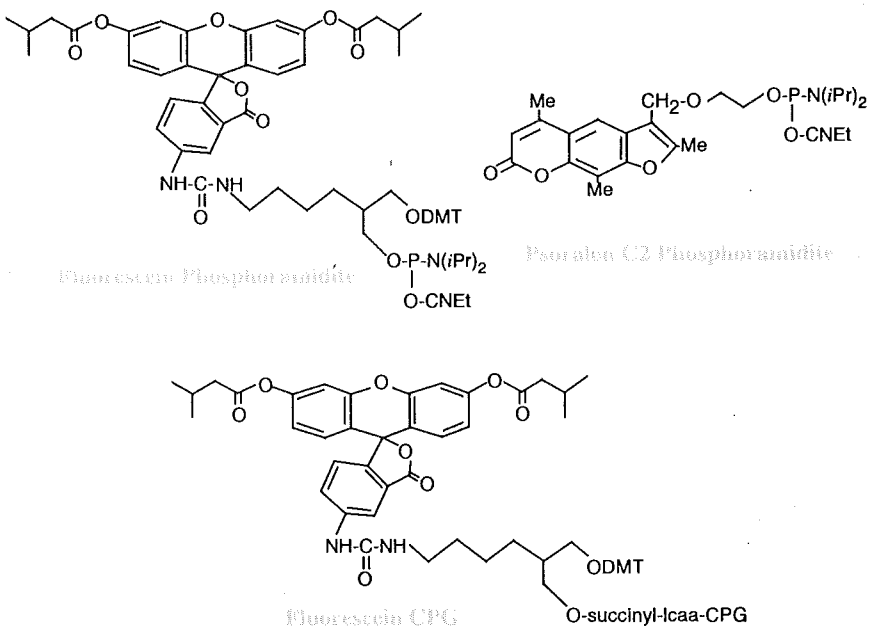
Fluorescein phosphoramidite is used in a manner identical to normal nucleoside phosphoramidites. We recommend that the synthesis be carried out DMT-off to allow the determination of the level of fluorescein labelling. Oligonucleotides are deprotected with ammonium hydroxide as normal; the protecting pivaloyl groups are removed and the linkage to fluorescein is stable under these conditions.

Fluorescein labelled oligonucleotides may be purified by gel electrophoresis or HPLC. However, the product, when used for labelling at the 5'-terminus, is designed to be compatible with reverse phase purification techniques, including disposable cartridges. We recommend following the normal DMT-on cartridge purification procedure but omitting the 2% TFA wash (which normally removes the DMT group). The product can be eluted with aqueous acetonitrile to remove all fluorescence from the cartridge (1 - 2 mL is usually sufficient).

Reference:

1) F. Schubert, K. Ahlert, D. Cech, and A. Rosenthal, *Nucleic Acids Res.*, 1990, **18**, 3427.

STRUCTURES OF FLUORESCEIN AND PSORALEN PHOSPHORAMIDITES



BIOTEG - THE ULTIMATE BIOTIN PHOSPHORAMIDITE?

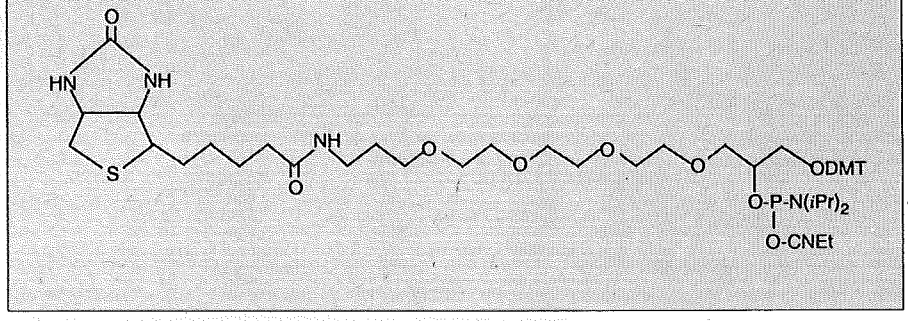
At Glen Research, we have been continuing our search for the ultimate biotin phosphoramidite for direct labelling of synthetic oligonucleotides. The following criteria have directed our efforts:

1. The product must be soluble in acetonitrile at concentrations useful for DNA synthesis.
2. The product must include a DMT group for cartridge purifications. This requirement is imperative in the design of biotinylated PCR primers because of the potential for cross contamination in HPLC purifications.
3. The product must also be capable of branching to allow multiple biotins to be introduced at the 3'- or 5'-terminus for probe development.
4. Oligonucleotides or double stranded DNA labelled with this biotin phosphoramidite must be adequately captured by streptavidin magnetic beads.

A recent paper described[1] a product which seemed to meet our design criteria. The product is soluble in acetonitrile and is capable of being inserted in multiple additions at the 3' or 5' terminus. This product is similar in concept to our 5'-Branched Modifier C3[2] in that, if inserted in the oligonucleotide sequence, the internucleotide linkage contains 2 carbon atoms. (The natural 2'-deoxyribose linkage contains 3 carbons.) Our current biotin phosphoramidite produces a more natural internucleotide linkage containing 3 carbon atoms and has been used successfully and productively.

If inserted at the 5' terminus, the spacer arm of most commercially available biotin phosphoramidites, including our original product, consists of 6 atoms. There are indications that for some applications, diagnostic probes and

STRUCTURE OF BIOTEG PHOSPHORAMIDITE



capture of long amplified DNA sequences, it is possible that a longer spacer arm would be desirable. A recent patent[3] discloses functionalizing reagents useful for the introduction of thiol and amino groups into oligonucleotides using a long hydrophilic spacer arm. Using a similar strategy, we have designed and now introduce BioTEG Phosphoramidite which contains a 15 atom mixed polarity spacer arm based on a triethylene glycol. This product would seem to meet all of our design criteria and may, in time, deserve to be referred to as the ultimate biotin phosphoramidite.

BioTEG phosphoramidite is now available in a variety of pack sizes to suit every need. Use a 50 μmole pack to add a single biotin to the 5' terminus or one of the two larger packs for several additions. The product is used in a manner identical to normal nucleoside phosphoramidites with the exception that a longer coupling step (10-15 minutes) is needed to give high coupling efficiency.

For use at the 3'-terminus, we are happy to introduce BioTEG CPG. Again the use of this reagent is totally compatible with automated synthesizers.

References:

1) K. Misiura, I. Durrant, M.R. Evans, and M.J. Gait, *Nucleic Acids Res.*, 1990, **18**, 4345.
2) P.S. Nelson, R. Sherman-Gold, and R. Leon, *Nucleic Acids Res.*, 1989, **17**, 7179.
3) C. Levenson, C. Chang, and F.T. Oakes, *US Patent 4,914,210*, 1990.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
BioTEG Phosphoramidite	10-1955-95	50 μmole	165.00
	10-1955-90	100 μmole	295.00
	10-1955-02	0.25g	675.00
BioTEG CPG	20-2955-01	0.1g	120.00
Pack of Four 1 μmole columns	20-2955-41	Pk/4	200.00
Pack of Four 0.2 μmole columns	20-2955-42	Pk/4	120.00
Fluorescein Phosphoramidite	10-1963-95	50 μmole	165.00
	10-1963-90	100 μmole	295.00
	10-1963-02	0.25g	675.00
Fluorescein CPG	20-2963-01	0.1g	120.00
Pack of Four 1 μmole columns	20-2963-41	Pk/4	200.00
Pack of Four 0.2 μmole columns	20-2963-42	Pk/4	120.00
Psoralen C2 Phosphoramidite	10-1982-90	100 μmole	195.00
	10-1982-02	0.25g	495.00

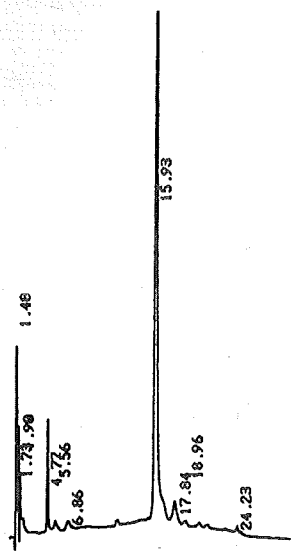
(2'-OMe-RNA - The Logical RNA Alternative? References contd.)

Proc. Natl. Acad. Sci. USA, 1991, **88**, 9784-9788.

3) M. Cotten, B. Oberhauser, H. Brunar, A. Holzner, G. Issakides, C.R. Noe, G. Schaffner, E. Wagner, and M.L. Birnstiel, *Nucleic Acids Res.*, 1991, **19**, 2629-2635.

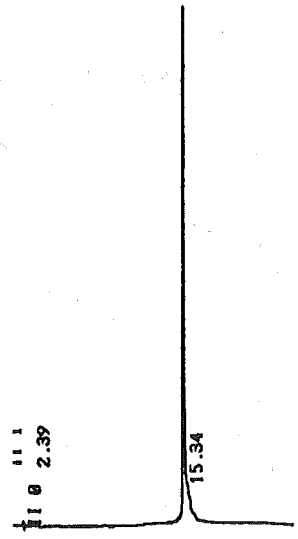
4) B. Oberhauser and E. Wagner, *Nucleic Acids Res.*, 1992, **20**, 533-538.

Figure 1: 2'-OMe-RNA DMT-on



RP HPLC of 2'-OMe-RNA oligonucleotide DMT-on using the conditions in Table 2.

Figure 2: 2'-OMe-RNA DMT-off



RP HPLC of 2'-OMe-RNA oligonucleotide DMT-off after Poly-Pak™ purification using the conditions in Table 3.

TABLE 1: COMPARISON OF 2'-OMe-RNA WITH RNA SYNTHESIS

	2'-OMe-RNA	RNA
Coupling Time	6 min.	12 min.
Coupling Efficiency	>99%	97 - 98%
Deprotection Procedure	ammonium hydroxide 6h. (or same as DNA)	a) ammonium hydroxide/ ethanol 17h. b) *t*-butylammonium fluoride/ THF 24h. c) desalt with sterile column
Purification	Poly-Pak™ Cartridge (or same as DNA)	gel electrophoresis or ion-exchange HPLC
Sterile Conditions	No (same as DNA)	Yes (after step b above)
Yield	10 ODU / 0.2 µmole synthesis	10 ODU / 1 µmole synthesis
Time for Isolation	1 day (or same as DNA)	3 -4 days
Scientist Time / 4 Oligos	1h.	10h.
Reagent Cost / 4 Oligos	monomers - $1,440 columns - $75	monomers - $600 columns - $100
Scientist Cost / $60/h	$60	$600
Total Cost / 4 Oligos	$1,575	$1,300
Cost to Scientist	Experiments proceed	Experimental time lost

RP HPLC Conditions for 2'-OMe-RNA DMT-on

Time	0.1M TEAA(%)	Acetonitrile(%)
0	93	7
20	75	25
30	75	25
35	93	7

Column: Spherisorb ODS-2 (150X4.6mm)
Flow Rate: 1mL/min.
Detector: UV at 254nm

RP HPLC Conditions for 2'-OMe-RNA DMT-off

Time	0.1M TEAA(%)	Acetonitrile(%)
0	95	5
7	95	5
21	81	19
22	81	19

Column: Spherisorb ODS-2 (150X4.6mm)
Flow Rate: 1mL/min.
Detector: UV at 254nm

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
2'-OMe-A-CE Phosphoramidite	10-3100-90	100 µmole	150.00
	10-3100-02	0.25g	360.00
2'-OMe-C-CE Phosphoramidite	10-3110-90	100 µmole	150.00
	10-3110-02	0.25g	360.00
2'-OMe-G-CE Phosphoramidite	10-3121-90	100 µmole	150.00
	10-3121-02	0.25g	360.00
2'-OMe-U-CE Phosphoramidite	10-3130-90	100 µmole	150.00
	10-3130-02	0.25g	360.00
2'-OMe-A-RNA 500	20-3600-02	0.25g	95.00
Pack of Four 1 µmole columns	20-3700-41	Pk/4	100.00
Pack of Four 0.2 µmole columns	20-3700-42	Pk/4	75.00
2'-OMe-C-RNA 500	20-3610-02	0.25g	95.00
Pack of Four 1 µmole columns	20-3710-41	Pk/4	100.00
Pack of Four 0.2 µmole columns	20-3710-42	Pk/4	75.00
2'-OMe-G-RNA 500	20-3620-02	0.25g	95.00
Pack of Four 1 µmole columns	20-3720-41	Pk/4	100.00
Pack of Four 0.2 µmole columns	20-3720-42	Pk/4	75.00
2'-OMe-U-RNA 500	20-3630-02	0.25g	95.00
Pack of Four 1 µmole columns	20-3730-41	Pk/4	100.00
Pack of Four 0.2 µmole columns	20-3730-42	Pk/4	75.00

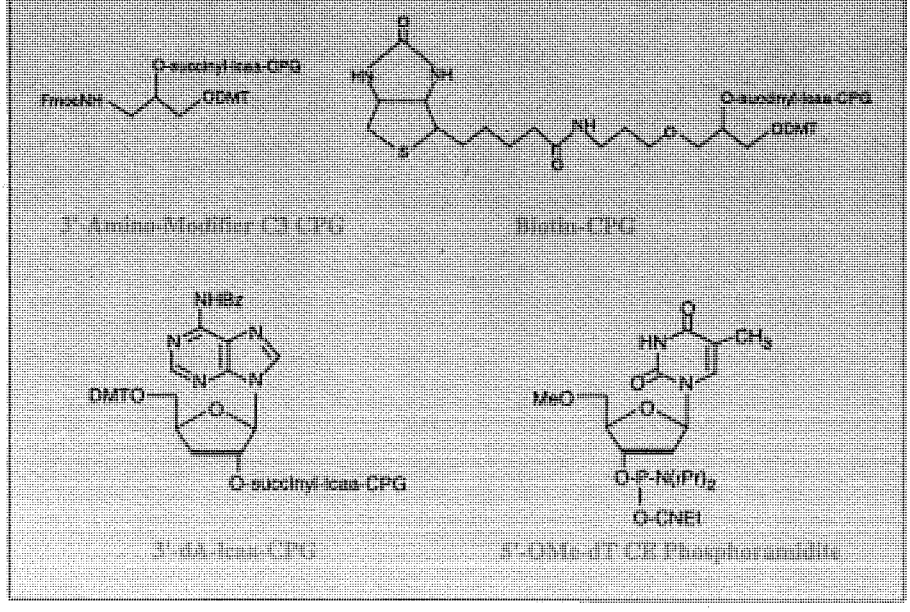
BLOCKING THE TERMINI OF OLIGONUCLEOTIDES

Exonuclease activity, predominantly at the 3'-terminus of oligonucleotides is the primary source of degradation of oligonucleotides designed as therapeutic agents. It is possible to enhance the stability of oligonucleotides to 3' exonuclease by modification of the 3'-terminus. Although 3'-Amino-Modifier C3 CPG was designed primarily as a reagent for allowing the introduction of a label at the 3'-terminus, it has been found to be particularly useful for modifying the 3'-terminus to block exonuclease degradation[1]. In a similar manner, biotin-CPG may be used to block the 3'-terminus from exonuclease digestion. However, it is more often used to block the 3'-terminus from extension during PCR while offering a tag for later capture by steptavidin.

The 3'-terminus may be blocked from polymerase extension by the introduction of a 3'-deoxynucleoside. Cordycepin (3'-deoxyAdenosine) CPG has been used effectively for this purpose.

Blocking of the 5'-terminus from 5' exonuclease activity is of lesser importance *in vivo* but may be useful as a research tool to protect defined sequences. 5'-OMe-dT-CE phosphoramidite has been designed to block the 5'-terminus from both digestion and extension.

STRUCTURES OF BLOCKERS



Reference:

1) J.G. Zendegui, K.M. Vasquez, J.H. Tinsley, D.J. Kessler, and M.E. Hogan, *Nucleic Acids Res.*, 1992, **20**, 307-314.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
3'-Amino-Modifier C3 CPG	20-2950-01	0.1g	85.00
Pack of Four 1 μmole columns	20-2950-41	Pk/4	125.00
Pack of Four 0.2 μmole columns	20-2950-42	Pk/4	75.00
Biotin-CPG	20-2951-01	0.1g	120.00
Pack of Four 1 μmole columns	20-2951-41	Pk/4	200.00
Pack of Four 0.2 μmole columns	20-2951-42	Pk/4	120.00
3'-dA-lcaa-CPG	20-2104-01	0.1g	300.00
Pack of Four 1 μmole columns	20-2104-41	Pk/4	600.00
Pack of Four 0.2 μmole columns	20-2104-42	Pk/4	200.00
5'-OMe-dT-CE Phosphoramidite	10-1031-90	100 μmole	135.00
	10-1031-02	0.25g	355.00

LITERATURE REVIEW

Synthesis by Post-Synthetic Substitution of Oligomers Containing Guanine Modified at the 6-Position with S-Derivatives, N-Derivatives and O-Derivatives

Using a common intermediate, a 6-(2,4-dinitrophenyl)-thioguanine derivative, the authors describe routes to oligos containing 6-thio-dG, 2,6-diaminopurine, 2-amino-6-methylaminopurine, and O6-methyl-dG. The intermediate CE phosphoramidite is protected at the N2 position with phenylacetyl in preference to isobutyryl which is more difficult to remove. All reactions are carried out on the DMT-on oligo for 2 days at room temperature.

This is clearly a versatile synthetic route to modified oligos which are difficult and costly to prepare otherwise.

Product Oligo Containing	*Deprotection*
6-thio-dG	1-mercaptoethanol/ ammonium hydroxide
2,6-diaminopurine	tetramethylguanidine/ 2-nitrobenzaldoxime/ ammonium hydroxide
2-amino-6-methylaminopurine	aqueous methylamine
O6-methyl-dG	methanol/DBU

Reference:

1) Y.Z. Xu, Q. Zheng, and P.F. Swann, *Tetrahedron*, 1992, **48**, 1729-1740.

THIOL MODIFICATION OF OLIGONUCLEOTIDES

The conjugation of enzymes, especially alkaline phosphatase and horseradish peroxidase, to oligonucleotides has become very significant in the production of diagnostic probe systems. These developments, along with continued interest in labelling with thiol-specific tags, have prompted us to rethink routes to thiol-modified oligonucleotides, as described below.

Thiol-Modifiers for the production of sulfhydryl groups at the 5'-terminus have been readily available commercially, including from Glen Research. These products are based[1,2,3] on blocking the thiol group during synthesis with a trityl protecting group. Although this procedure has been used successfully, several problems exist including a low level of oxidative detritylation during oligonucleotide synthesis and the use of silver nitrate during final deblocking. A recent note[4] describes a procedure to modify a 5'-amino-oligonucleotide to a thiol using N-acetyl-DL-homocystein thiolactone.

Synthetic routes to oligonucleotides containing 3'-thiols have also been described[5,6,7] utilizing solid supports containing disulfide linkages. 3'-Thiol-modified oligonucleotides are especially interesting in cases where a different label is desired for the 5'-terminus.

Thiol Group at the 5'-Terminus

a) DMT-off Synthesis

Add Thiol-Modifier C6 S-S at the 5'-terminus of the oligonucleotide in the automated DMT-off synthesis mode. The DMT release from the last cycle can be used to determine coupling efficiency. To the standard ammonium hydroxide used for deprotection, add dithiothreitol (DTT) to a concentration of 0.05M. Carry out deprotection in the normal manner (typically 55°C/16h). This procedure removes the base protecting groups and cleaves the disulfide linkage to generate the 5'-thiol. Isolate, desalt, and, if necessary, purify the thiol-modified oligo using standard procedures.

b) DMT-on Synthesis

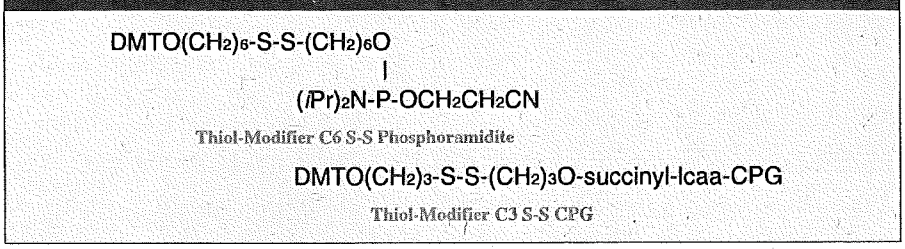
Add Thiol-Modifier C6 S-S at the 5'-terminus of the oligonucleotide in the automated DMT-on synthesis mode. Carry out deprotection in the normal manner. Purify the trityl containing oligonucleotide by HPLC or on a Poly-Pak™ cartridge omitting the 2% TFA step. Evaporate the product solution to dryness. Cleave the disulfide linkage using DTT (0.04M in 0.17M phosphate buffer, pH8) for 16h. Desalt the oligonucleotide on a cartridge or column. (The DMT containing thiol will remain attached to the desalting matrix.)

Thiol Group at the 3'-Terminus

Add Thiol-Modifier C6 S-S to any nucleoside support or use Thiol-Modifier C3 S-S CPG to synthesize the desired oligonucleotide. To the standard ammonium hydroxide used for deprotection, add dithiothreitol (DTT) to a concentration of 0.05M. Carry out deprotection at 55°C for 16h. This procedure removes the base protecting groups and cleaves the disulfide linkage to generate the 3'-thiol. Isolate, desalt and, if necessary, purify the thiol-modified oligonucleotide using standard procedures.

Note: Thiol-modified oligonucleotides should be kept either under an inert atmosphere or in a solution containing DTT (0.01M) to avoid oxidative disulfide formation. If necessary, the DTT can be extracted from the solution using ethyl acetate. Continue to the conjugation reaction.

STRUCTURE OF THIOL-MODIFIER S-S



References:

1) B.A. Connolly and R. Rider, *Nucleic Acids Res.*, 1985, **13**, 4485.
2) B.A. Connolly, *Nucleic Acids Res.*, 1987, **15**, 3131-3139.
3) N.D. Sinha and R.M. Cook, *Nucleic Acids Res.*, 1988, **16**, 2659.
4) A. Kumar, S. Advani, H. Dawar, and G.P. Talwar, *Nucleic Acids Res.*, 1991, **19**, 4561.
5) R. Zuckermann, D. Corey, and P. Schultz, *Nucleic Acids Res.*, 1987, **15**, 5305.
6) K.C. Gupta, P. Sharma, S. Sathyanarayana, and P. Kumar, *Tetrahedron Lett.*, 1990, **31**, 2471-2474.
7) U. Asseline, E. Bonfils, R. Kurfurst, M. Chassignol, V. Roig, and N.T. Thuong, *Tetrahedron*, 1992, **48**, 1233-1254.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Thiol-Modifier C6 S-S	10-1936-90	100 μmole	150.00
	10-1936-02	0.25g	360.00
3'-Thiol-Modifier C3 S-S CPG	20-2933-01	0.1g	85.00
Pack of Four 1 μmole columns	20-2933-41	Pk/4	125.00
Pack of Four 0.2 μmole columns	20-2933-42	Pk/4	75.00

OLIGO SOFTWARE

Software at Glen Research has previously meant chemical supplies for hardware called DNA synthesizers. We are now venturing into new territory as we offer, for the first time, computer software. As a supplier of products used for oligonucleotide synthesis and purification, the design of primers has always been of interest to us; faulty primer selection leads to poor performance, followed rapidly by quality questions.

Primer selection is now elegantly accomplished with the use of OLIGO, ver. 4.0 for Macintosh computers. OLIGO searches DNA and RNA sequence files (GenBank, EMBL, ASCII and most other sequence files) to select stable, unique primers within a specified range for PCR, sequencing, and hybridization experiments. While these are the features of OLIGO which are most appealing, the program does much more.

OLIGO uses "nearest neighbor" thermodynamic algorithms to calculate with accuracy optimal annealing temperatures and primer/product Tm and Td. Other PCR conditions are also predicted. A unique feature is OLIGO's choice of primers with relatively less stable 3' ends and more stable 5' ends in order to achieve greater specificity.

This is obviously a very sophisticated program based on the author's published [1] work and experiments [2]. However, version 4.0 for Macintosh computers is simple to use as soon as it is loaded. "HELP" functions throughout the program take care of most user questions and an excellent manual is included with the package. Once the user becomes comfortable with the program, further study will reveal that OLIGO offers many powerful performance features. A demo disk is available for use on all Macs.

References:

(1) W. Rychlik and R.E. Rhoads, *Nucleic Acids Res.*, 1989, **17**, 8543.

(2) W. Rychlik, W.J. Spencer, and R.E. Rhoads, *Nucleic Acids Res.*, 1990, **18**, 6409.

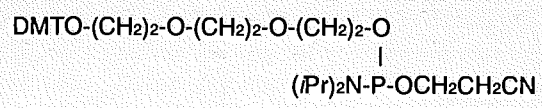
SPACER PHOSPHORAMIDITE

The need for fast and simple labelling of oligonucleotides has led to the development of CE phosphoramidite derivatives of the marker compounds, *e.g.*, fluorescein and biotin. While convenient for automated synthesis, the introduction of these products has led to situations where the spacer arm included in the phosphoramidite is of insufficient length for a specific application. Some of the most popular applications include:

1) The development of diagnostic probes where the label is included at the 3'- or 5'-terminus.
2) The development of sequencing and diagnostic techniques based on biotin at the 5'-terminus of a primer prior to amplification. The derived biotinylated DNA is then trapped using streptavidin magnetic beads.
3) The development of potential therapeutics containing labels which are also intercalating molecules.

The Spacer Phosphoramidite can be added once or in multiple additions between the oligonucleotide and the label during automated synthesis to form a 9 atom, or multiples thereof, mixed polarity spacer arm.

STRUCTURE OF SPACER PHOSPHORAMIDITE



Another application[1] is found in the preparation of oligonucleotides containing a hairpin loop made of polyethylene glycol. Naturally occurring hairpin and cruciform structures are known to occur frequently in regions which function as regulation and promotion sites. Polyethylene glycol loops are chosen because of their good solubility due to the presence of ether linkages. The loop length can be varied by consecutive additions of Spacer Phosphoramidite.

Reference:

(1) M. Durard, K. Chevrie, M. Chassignol, N.T. Thuong, and J.C. Maurizot, *Nucleic Acids Res.*, 1990, **18**, 6353.

ORDERING INFORMATION

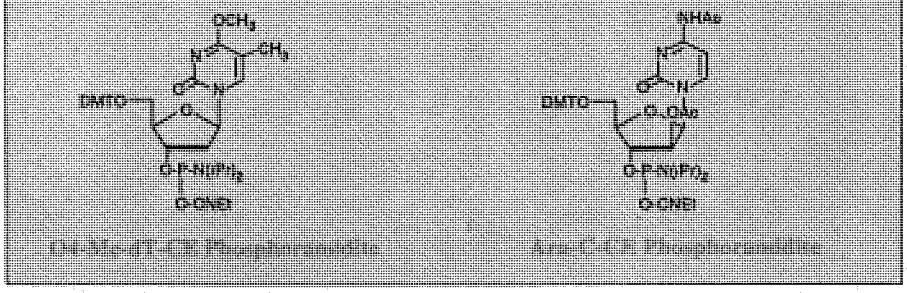
Item	Catalog No.	Pack	Price($)
Spacer Phosphoramidite	10-1909-90	100 μmole	75.00
	10-1909-02	0.25g	240.00
OLIGO ver. 4.0 Mac (Industry)	80-1001-01	ea.	800.00
OLIGO ver. 4.0 Mac (Non-profit)	80-1002-01	ea.	640.00
OLIGO ver. 4.0 Mac (Demo)	80-1000-01	ea.	No Charge

UNUSUAL MONOMERS

Over the years Glen Research has provided a large number of unusual monomers for oligonucleotide synthesis. In this issue, we have described several under specific headings. As usual though, we have a couple of items that defy being placed in an obvious category - unusual monomers.

Adenosine and Cytosine Arabinosides have been used as antiviral and anti-leukemia drugs, respectively. Ara-C is of special interest because of its effect as an inhibitor of DNA replication. It is desirable, therefore, to incorporate Ara-C into specific sites in DNA to investigate the mode of activity. Ara-C-CE Phosphoramidite[1] is designed for this purpose. Methylation of DNA occurs predominantly at the O6 position of dG and the O4 position of dT. Oligonucleotides containing

STRUCTURES OF MONOMERS



O6-Me-dG can be prepared using the phosphoramidite or *in situ* (see Literature Review on Page 5). We are now able to offer O4-Me- dT-CE Phosphoramidite also.

1) G.P. Beardsley, T. Mikita, M.M. Klaus, and A.L. Nussbaum, *Nucleic Acids Res.*, 1988, **16**, 9165.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
O4-Me-dT-CE Phosphoramidite	10-1032-90	100 μmole	135.00
	10-1032-02	0.25g	355.00
Ara-C-CE Phosphoramidite	10-4010-90	100 μmole	135.00
	10-4010-02	0.25g	355.00

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