The Glen Report

POST-SYNTHETIC SUBSTITUTION - CONVERTIBLE NUCLEOSIDES

The ability to produce oligonucleotides containing modified bases has stimulated research in a variety of areas, including carcinogenesis studies, investigation of DNA and RNA activity, and especially research into protein - DNA interaction. As most researchers are aware, Glen Research offers a wide variety of minor and modified base CE phosphoramidites which are introduced into oligonucleotides directly during synthesis. However, to satisfy an even wider group of research activities, we are now offering synthons for purine derivatives modified at the 6-position and pyrimidines modified at the 4-position. These synthons have been termed "Convertible Nucleosides"[1], and the process has been referred to as "Post-Synthetic Substitution"[2].

Purine Modification

Several schemes have been applied to the production of convertible pyrimidine deoxynucleosides but fewer choices are available for purine modification. A recent article[3] describes the use of 6-O-(pentafluorophenyl)-2'-deoxyguanosine as a versatile synthon for the production of oligonucleotides containing purine residues modified at the 6-position with N and O derivatives. This route is not applicable to the formation of 6-thioguanine derivatives which are interesting in medical research since 6-thioguanine has been used for many years as an anti-leukemia drug. Two groups have recently reported[4,5] the preparation of 6-thio-dG monomers for DNA synthesis with S6-cyanoethyl protection.

A more versatile synthon has been described[2] which allows the preparation of guanine derivatives modified at the 6-position with S, N and O derivatives by post-synthetic substitution. This convertible nucleoside, a derivative of 6-(2,4-dinitrophenyl)-thio-2'-deoxyGuanosine (S6-DNP-dG), gives ready access to oligonucleotides containing: thioguanine for use in medical studies and for DNA structural research; 2,6-diaminopurine also for DNA structural research since it forms an additional hydrogen bond to T, thereby enhancing duplex stability; O6-alkyl derivatives for carcinogenesis studies; and ^{13}C, ^{15}N and ^{17}O labels for NMR experiments. The protecting group chosen by the authors for the N2 position (phenylacetyl) is very base-labile and removes some of the problems encountered in the past with the slow removal of isobutyryl from that position in 6-substituted purine derivatives.

Oligonucleotide Synthesis

The authors[2] recommend a coupling time of 3 minutes for the CE phosphoramidite of S6-DNP-dG. *In our hands, a coupling time of 1 minute was sufficient to obtain >99% coupling efficiency.* The general synthetic scheme for carrying out these substitutions is shown in Figure 1.

Deprotection

Oligonucleotides containing 6-thio-dG
Deprotect the oligonucleotide with 10% mercaptoethanol in concentrated ammonium hydroxide for 48h at room temperature.

Oligonucleotides containing 2,6-diaminopurine (2-amino-dA)
Deprotect the oligonucleotide with concentrated ammonium hydroxide containing 65mM tetramethyl-guanidine and 75mM 2-nitro-benzaldoxime for

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Convertible Nucleosides
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48h at room temperature.

Oligonucleotides containing 2-amino-6-methylaminopurine
Deprotect the oligonucleotide with 40% aqueous methylamine for 48h at room temperature.

Oligonucleotides containing O6-methylguanine
Deprotect the oligonucleotide with a solution of 10% v/v 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) in anhydrous methanol for 48h at room temperature in the dark. Lyophilize the solution to an oil and dissolve the oil in 10mM aqueous sodium hydroxide.

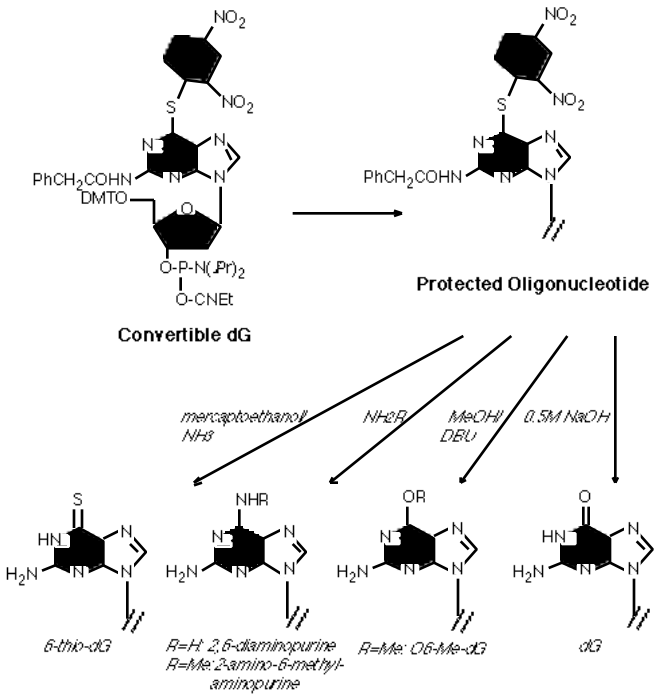
Oligonucleotides containing guanine
Deprotect the oligonucleotide with a solution of 0.5M aqueous sodium hydroxide for 48h at room temperature.

Pyrimidine Modification

Modification of pyrimidine bases has been predominantly carried out prior to the preparation of the phosphoramidite for oligonucleotide synthesis. This necessitates the preparation of a variety of monomers some of which can be quite tricky to handle. The alternative post-synthetic substitution strategy is equally valid for pyrimidine bases and has been described[1] for a convertible nucleoside, 4-O-(2,4,6-trimethyl-phenyl)- 2'-deoxyuridine (TMP-dU). TMP-dU was successfully used for the addition of a variety of functionalized amino derivatives to the final oligonucleotide. In this way, the authors were able to introduce the following functionalities to the oligonucleotide: amine, disulfide, thiol, and carboxyl groups. Oligonucleotides containing 4-thio-pyrimidine nucleosides have been prepared from monomers containing a variety of protecting groups for the 4-thio residue including cyanoethyl[6,7], pivaloyloxymethyl[8], and nitrophenylethyl[9].

A more universal approach to pyrimidine modification has been suggested[10] in which the 4-position is modified by a triazolyl group. This substitution with a triazolyl group was previously known but the addition of the conversion to the 4-thio derivative

FIGURE 1: POST SYNTHETIC SUBSTITUTION OF CONVERTIBLE dG



makes this synthon much more versatile. Although these procedures were described for thymidine, it is likely to be equally applicable to other pyrimidine nucleosides. These triazolyl pyrimidine convertible nucleosides allow oligonucleotides to be produced containing: 4-thio derivatives for cross linking reactions; 4-amino derivatives for DNA structural research; O4-alkyl derivatives for carcinogenesis studies; and offer the potential for the introduction of ^{13}C, ^{15}N and ^{17}O labels for NMR experiments.

Oligonucleotide Synthesis

The Convertible dT and dU-CE phosphoramidites couple to the same extent and with the same coupling time as normal monomers. The general synthetic scheme for carrying out these substitutions on the Thymidine derivative is shown in Figure 2. The authors[10] recommend the use of a mild deprotection scheme or monomers with base-labile protection for these modified oligonucleotides to avoid unwanted hydrolysis at the modified sites.

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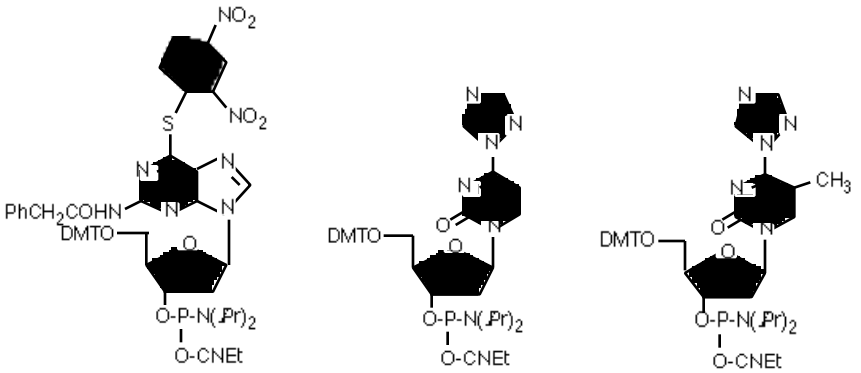
STRUCTURES OF CONVERTIBLE NUCLEOSIDES



S6-DNP-dG-CE Phosphoramidite

O4-Triaz.-dU-CE Phosphoramidite

O4-Triaz.-dT-CE Phosphoramidite

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Deprotection

Oligonucleotides containing 4-thio-pyrimidines
Deprotect the oligonucleotide on the support with 10% thiolacetic acid in acetonitrile for 24h at room temperature. Wash the reagents from the column with acetonitrile and deprotect with 10% DBU in anhydrous methanol for 16h at room temperature in the dark. (This avoids hydrolysis of the thio group with ammonia.) Lyophilize the solution to an oil and dissolve the oil in 10mM aqueous sodium hydroxide prior to purification.

Oligonucleotides containing 4-amino-pyrimidines
Deprotect the oligonucleotide with concentrated ammonium hydroxide for 24h at room temperature.

Oligonucleotides containing 4-dimethylhydrazino-pyrimidines
Deprotect the oligonucleotide on the support with 10% 1,1-dimethyl-hydrazine in acetonitrile for 3h at room temperature. Wash the reagents from the column with acetonitrile and deprotect with 0.5M aqueous sodium hydroxide for 16h at room temperature.

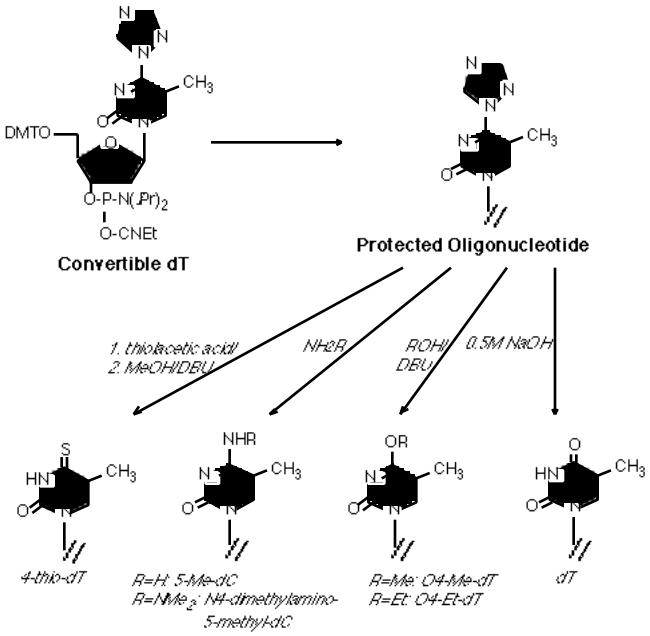
Oligonucleotides containing O4-alkyl-pyrimidines
Deprotect the oligonucleotide with a solution of 10% v/v DBU in anhydrous methanol for 24h or in anhydrous ethanol for 48h at room temperature in the dark. Lyophilize the solution to an oil and dissolve the oil in 10mM aqueous sodium hydroxide.

Oligonucleotides containing pyrimidines
Deprotect the oligonucleotide with a solution of 0.5M aqueous sodium hydroxide for 24h at room temperature.

Purification and Analysis

Purification and isolation of the modified oligonucleotides can be carried out using standard procedures. We prefer to carry out the oligonucleotide synthesis DMT-ON, deprotect as described, dilute the organic content to <5% with water, and follow the standard Poly-Pak purification scheme. Base composition analysis[10] should be carried out to confirm the presence of the desired modified nucleoside.

FIGURE 2: POST SYNTHETIC SUBSTITUTION OF CONVERTIBLE dT



Acknowledgment

We wish to thank Dr. Y.Z. Xu for his help and encouragement in commercializing these useful products. The original research was funded by Cancer Research Campaign (U.K.) and this support is gratefully acknowledged.

References:

(1) A.M. MacMillan and G.L. Verdine, *Tetrahedron*, 1991, **14**, 2603-2616.
(2) Y.Z. Xu, Q. Zheng, and P.F. Swann, *Tetrahedron*, 1992, **48**, 1729-1740.
(3) H. Gao, R. Fathi, B.L. Gaffney, B. Goswami, P.P. Kung, Y. Rhee, R. Jin, and R.A. Jones, *J. Org. Chem.*, 1992, **57**, 6954-6959.
(4) M.S. Christopherson and A.D. Broom, *Nucleic Acids Res.*, 1991, **19**, 5719-5724.
(5) T.S. Rao, K. Jayaraman, R.H. Durland, and G.R. Revankar, *Tetrahedron Lett.*, 1992, **33**, 7651-7654.
(6) R.S. Coleman and J.M. Siedlecki, *J. Amer. Chem. Soc.*, 1992, **114**, 9229-9230.
(7) T.T. Nikifirov and B.A. Connolly, *Tetrahedron Lett.*, 1992, **33**, 2379-2382.
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(9) T.T. Nikifirov and B.A. Connolly, *Tetrahedron Lett.*, 1991, **32**, 3851-3854.
(10) Y.Z. Xu, Q. Zheng, and P.F. Swann, *J. Org. Chem.*, 1992, **57**, 3841.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
S6-DNP-dG-CE Phosphoramidite *(Convertible dG)*	10-1071-95	50 µmole	227.50
	10-1071-90	100 µmole	455.00
	10-1071-02	0.25g	975.00
O4-Triaz.-dT-CE Phosphoramidite *(Convertible dT)*	10-1033-90	100 µmole	105.00
	10-1033-02	0.25g	255.00
O4-Triaz.-dU-CE Phosphoramidite *(Convertible dU)*	10-1051-90	100 µmole	135.00
	10-1051-02	0.25g	355.00

2'-OMe-RNA UPDATE

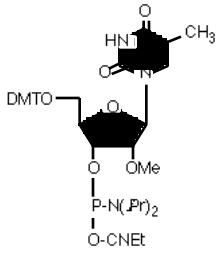
In the last year, oligonucleotides containing 2'-OMe-RNA linkages have been examined in a variety of circumstances which exploit their enhanced RNase and DNase resistance and the increased thermal stability of their duplexes and triplexes. Some potentially very interesting results have been generated in the fields of antisense, triplex and ribozyme research. Their RNase and DNase resistance was exploited in experiments demonstrating the effective incorporation of 2'-OMe RNA into liposomes through cholesteryl modification[1]. This resistance was also used to protect ribozymes from hydrolysis by converting the regions flanking the hammerheads to 2'-OMe linkages. Surprisingly, this modification also served to increase the ribozyme activity of the hybrid oligonucleotide[2].

One area of particular interest has been the formation of triple helices which are strongly stabilized by 2'-OMe-RNA relative to DNA sequences. Further modification of these triplex structures with intercalating or cleavage reagents[3] may offer great potential in therapeutic development.

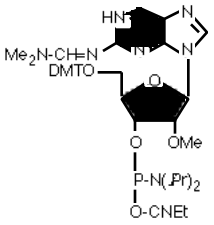
To aid in the evaluation of structural analyses of complexes containing 2'-OMe-RNA sequences, we are introducing the following CE phosphoramidites: 2'-OMe-5-Methyluridine (2'-OMe-T)[4] for triplex studies; 2'-OMe-2-aminopurine for ribozyme[5] and protein interaction studies; and 2'-OMe-5-F-C (by post-synthetic substitution with ammonia) and 2'-OMe-5-F-U for structural studies.

As the scale of synthesis of the four normal 2'-OMe-RNA monomers grows, we are able to offer an improvement in cost which we are delighted to pass on to researchers. Effective June 1, therefore, we are lowering their prices as shown. They are still not inexpensive but we hope this price reduction will give researchers better access to these exciting RNA derivatives. The structutes of these monomers are shown on Page 27 of the 1993 Catalog.

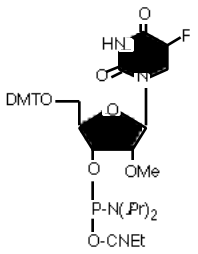
STRUCTURES OF NEW 2'-OMe-RNA MONOMERS



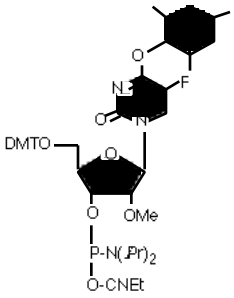
2'-OMe-5-Me-U-CE Phosphoramidite



2'-OMe-2-AP-CE Phosphoramidite



2'-OMe-5-F-U-CE Phosphoramidite



2'-OMe-TMP-5-F-U-CE Phosphoramidite

References:

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

(2) J. Goodchild, *Nucleic Acids Res.*, 1992, **20**, 4607-4612.

(3) C. Giovannangeli, N.T. Thuong, and C. Helene, *Nucleic Acids Res.*, 1992, **20**, 4275-4281.

(4) C. Giovannangeli, M. Rougee, T. Garestier, N.T. Thuong, and C. Helene, *Proc. Natl. Acad. Sci. U.S.A.*, 1992, **89**, 8631-8635.

(5) J.A. Doudna, J.W. Szostak, A. Rich, and N. Usman, *J. Org. Chem.*, 1990, **55**, 5547-5549.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
2'-OMe-A-CE Phosphoramidite	10-3100-90	100 µmole	75.00
	10-3100-02	0.25g	180.00
2'-OMe-C-CE Phosphoramidite	10-3110-90	100 µmole	75.00
	10-3110-02	0.25g	180.00
2'-OMe-G-CE Phosphoramidite *(N-dmf-G)*	10-3121-90	100 µmole	75.00
	10-3121-02	0.25g	180.00
2'-OMe-U-CE Phosphoramidite	10-3130-90	100 µmole	75.00
	10-3130-02	0.25g	180.00
2'-OMe-5-Me-U- CE Phosphoramidite *(2'-OMe-T)*	10-3131-90	100 µmole	150.00
	10-3131-02	0.25g	360.00
2'-OMe-2-Aminopurine- CE Phosphoramidite *(N-dmf-AP)*	10-3123-95	50 µmole	177.50
	10-3123-90	100 µmole	355.00
	10-3123-02	0.25g	975.00
2'-OMe-TMP-5-F-U- CE Phosphoramidite *(2'-OMe-5-F-C Precursor)*	10-3111-95	50 µmole	177.50
	10-3111-90	100 µmole	355.00
	10-3111-02	0.25g	975.00
2'-OMe-5-F-U-CE Phosphoramidite	10-3132-95	50 µmole	177.50
	10-3132-90	100 µmole	355.00
	10-3132-02	0.25g	975.00

DEOXYURIDINE DERIVATIVES FOR INTERNAL MODIFICATION

Amino-Modifier C6 dT[1] and its corresponding biotinylated product Biotin-dT have proved to be optimal for amino-modifying and biotinylating oligonucleotides within the sequence. The resulting labelled oligonucleotide has standard hybridization characteristics[2] and is ideal for attaching large molecules like alkaline phosphatase[3,4].

To this useful line of dU derivatives, we have added Amino-Modifier C2 dT and Carboxy-dT. In contrast to Amino-Modifier C6 dT where the label is designed to be placed where it cannot interact with the double stranded oligonucleotide, the C2 version is designed for the attachment of molecules like EDTA or alkylating reagents which can cut the complementary strand or double strand. The methyl ester of Carboxy-dT is hydrolyzed during standard deprotection and can be coupled directly to a molecule containing a primary amino group by a standard peptide coupling or *via* the intermediate N-hydroxysuccinimide (NHS) ester.

References:

(1) J.L. Ruth, C. Morgan, and A. Pasko, *DNA*, 1985, **4**, 93.
(2) J. Telser, K.A. Cruickshank, L.E. Morrison, and T.L. Netzel, *J. Am. Chem. Soc.*, 1989, **111**, 6966-6976.
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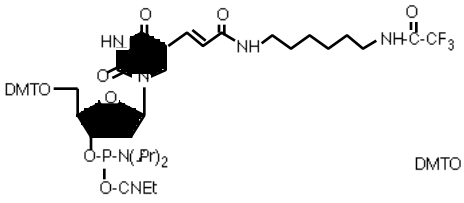
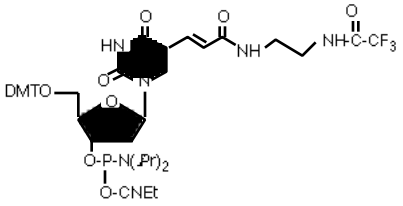
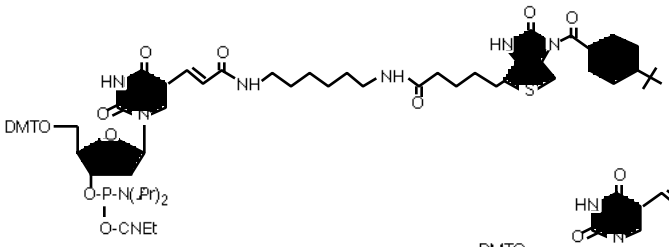
STRUCTURES OF dU DERIVATIVES



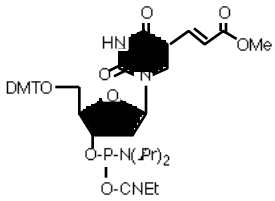
Amino-Modifier C6 dT



Amino-Modifier C2 dT



Biotin-dT



Carboxy-dT

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Amino-Modifier C6 dT	10-1039-90	100 µmole	180.00
	10-1039-02	0.25g	360.00
	10-1939-05	0.5g	720.00
Amino-Modifier C2 dT	10-1037-90	100 µmole	180.00
	10-1037-02	0.25g	360.00
	10-1937-05	0.5g	720.00
Biotin-dT	10-1038-95	50 µmole	180.00
	10-1038-90	100 µmole	325.00
	10-1938-02	0.25g	675.00
Carboxy-dT	10-1035-90	100 µmole	180.00
	10-1035-02	0.25g	360.00
	10-1935-05	0.5g	720.00

MILD DEPROTECTION PROCEDURE

Some linkages, modified bases, or modifiers may require more gentle deprotection conditions than the normal assault with ammonium hydroxide. The following procedure, described for a 0.2 µmole synthesis, is mild and leads logically to Poly-Pak type DMT-ON purification.

1. Carry out the synthesis of oligonucleotides containing modified bases DMT-on, and oligonucleotides labelled with, for example, psoralen or acridine DMT-off.
2. Open the synthesis column and transfer the support to a suitable reaction vial.
3. Treat the support with 1mL of 0.4M methanolic sodium hydroxide (methanol:water, 4:1) for 17h at room temperature.
4. Pipette the supernatant from the support and neutralize with 1.5mL of 2M triethylammonium acetate.
5. *Either:* Desalt the oligonucleotide using normal procedures, lyophilize the resulting product and store the oligonucleotide at -20°C.

6a. *Or:* Dilute the neutralized solution with 13.5mL of water (to bring the methanol content to 5%). Apply the diluted oligonucleotide solution to a prepared purification cartridge and carry out the standard purification scheme. (If the oligonucleotide is labelled and contains no DMT group, skip the 2% TFA wash.)

6b. Elute the purified oligonucleotide and lyophilize the resulting product. Store the oligonucleotide at -20°C.

5'→3' SYNTHESIS

Oligonucleotide synthesis is routinely carried out from the 3' to the 5' terminus for no other reason than the ease of synthesis of the monomer units. The 5'-hydroxyl group, a primary hydroxyl, is significantly more reactive than the secondary 3'-hydroxyl (or 2'-hydroxyl) group, making it straightforward to protect with the DMT group and leaving the 3'-hydroxyl available to form the phosphoramidite. However, a few situations make it necessary to synthesize oligonucleotides in the opposite sense.

Parallel Stranded Oligos (Hairpin)

Oligonucleotides containing a hairpin loop are used routinely for structural studies of duplex formation. The hairpin loop allows the oligonucleotide to bend back on itself thereby forming a duplex in an antiparallel format. The hairpin may be nucleosidic or it may consist of a polyethylene glycol spacer[1]. By using 5'-phosphoramidites for part of the synthesis, oligos with hairpin loops can be formed in which the strands are parallel[2]. These parallel stranded oligos can be readily prepared with 5'→3' or 3'→5' sense (Figure 1).

Nuclease Resistant Linkages

The design of antisense oligonucleotides as therapeutics has stimulated significant research activity on backbone modification. Modifying the natural phosphodiester linkage is essential to protect the oligonucleotide from intracellular nuclease degradation. The structural variety of these backbone modifications is diverse and they will not be detailed here. However, an interesting addition to the protection of antisense oligonucleotides is to modify the terminal linkages from the natural 3'-5' to 3'-3' and/or 5'-5' linkages. In this way, the oligonucleotides are protected against exonuclease activity, especially 3'-exonuclease activity which is by far the most significant enzymatic degradation route. Moreover, once degradation has occurred, the products are normal nucleosides with no toxicity concerns. Although this strategy has been applied successfully[3] to the protection of internal linkages using alternating α,β nucleosides to maintain effective hybridization, the most simple strategy is to modify only the linkage at the 3' terminus[4]. This is conveniently carried out as shown in Figure 2 and results in effective resistance with minimal disruption of hybridization.

FIGURE 1: PARALLEL STRANDS

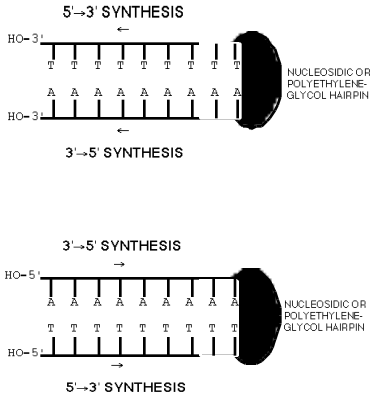
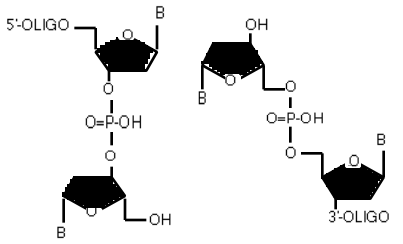


FIGURE 2: 3'→3' & 5'→5' LINKAGES



5'→3' Synthesis

Other areas where synthesis in the opposite sense may be of interest would be:

i) The preparation of oligos containing a base at the 3' terminus which is unsuitable for attachment to CPG, e.g., 2',3'-ddT and ddI.
ii) The synthesis of oligonucleotides on a support using a non-hydrolyzable linkage such that the 3' terminus is then available for polymerase extension.

Pricing for these monomers and supports is shown on Page 13 of the 1993 Catalog.

References:

(1) M. Durand, S. Peloille, N.T. Thuong, and J.C. Maurizot, *Biochemistry*, 1992, **31**, 9197-9204.
(2) J.H.v.d. Sande, N.B. Ramsing, M.W. Germann, W. Elhorst, B.W. Kalisch, E.v. Kitzing, R.T. Pon, R.C. Clegg, and T.M. Jovin, *Science*, 1988, **241**, 551-557.
(3) M. Koga, M.F. Moore, and S.L. Beaucage, *J. Org. Chem.*, 1991, **56**, 3757.
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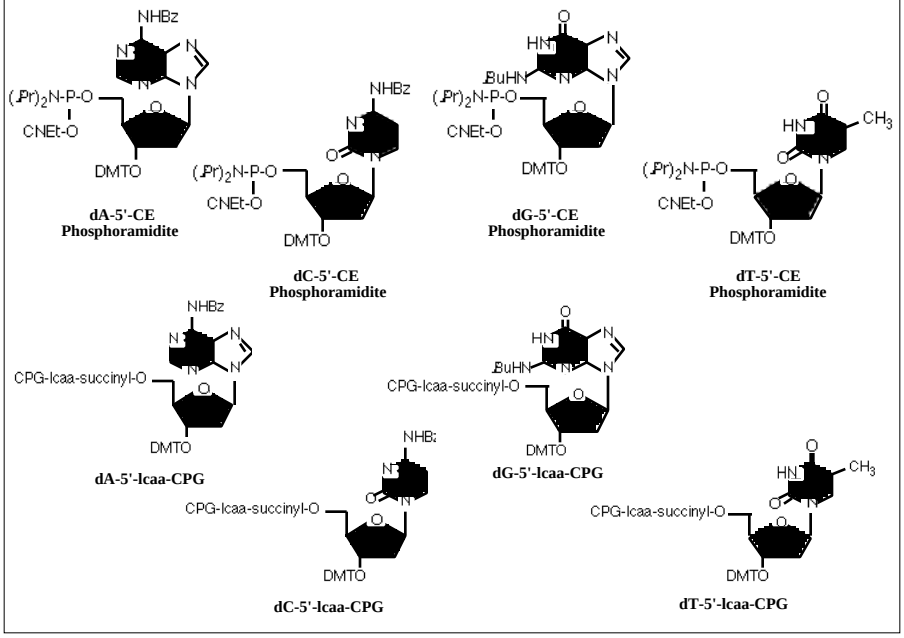
STRUCTURES OF 5'-PHOSPHORAMIDITES AND 5'-CPG



NEW LABELLING REAGENTS

Cholesterol, 2,4-dinitrophenol and psoralen had nothing whatsoever in common - until now. CE phosphoramidites containing these molecules are now available and their diverse purposes are briefly discussed below.

Cholesteryl-TEG Phosphoramidite

Potential therapeutic oligonucleotides must permeate the cell membrane for optimal activity. The addition of lipophilic groups to an oligonucleotide would be expected to enhance activity. The use of cholesteryl oligos and the consequent improvement in activity has been described[1,2]. We have designed our Cholesteryl Phosphoramidite with our branched triethyleneglycol (TEG) spacer for maximum solubility in acetonitrile as well as for applications requiring multiple labels.

DNP-TEG Phosphoramidite

A new analytical test based on detection of 2,4-dinitrophenyl (DNP) labelled oligonucleotides with anti-DNP antibodies has been proposed[3]. Again, we have chosen the branched TEG spacer in our version of DNP phosphoramidite since it can be added once or several times to the 3' or 5' terminus.

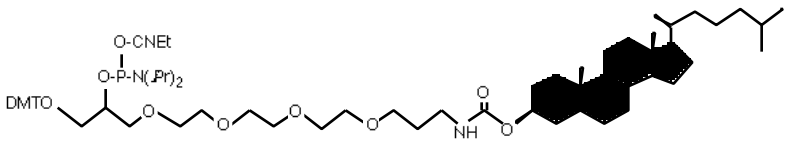
Psoralen C2 and C6 Phosphoramidites

The use of Psoralen C2 Phosphoramidite for addition to the 5' terminus and to serve as an intercallating and cross-linking reagent in double-stranded oligonucleotides has been described[4]. This reagent has proved to be both successful and popular.

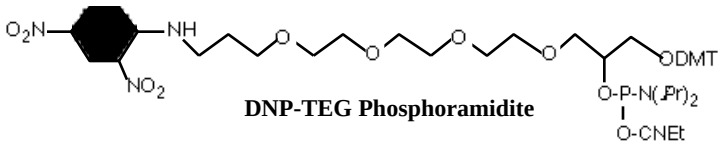
However, to complement current psoralen C2 labelling, it is necessary to add to our product line a further psoralen phosphoramidite with a 6 atom spacer arm. In this way, the psoralen molecule can intercallate and cross-link with a triplex oligonucleotide strand[5].

We are, therefore, happy to introduce the Psoralen C6 Phosphoramidite which can be added to the 5' terminus and indeed to specific locations within the sequence. The utility of psoralen labelling has been demonstrated in several recent[6,7,8,9] publications.

STRUCTURES OF NEW LABELLING REAGENTS



Cholesteryl-TEG Phosphoramidite



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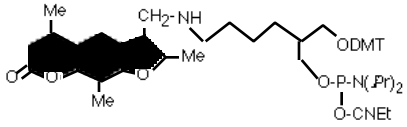
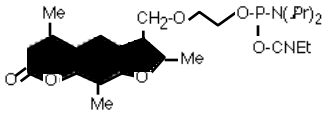


Psoralen C6 Phosphoramidite



Psoralen C2 Phosphoramidite

References:

(1) C. Mackellar, D. Graham, D.W. Will, S. Burgess, and T. Brown, *Nucleic Acids Res.*, 1992, **20**, 3411-3417.

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(4) U. Pieles and U. Englisch, *Nucleic Acids Res.*, 1989, **17**, 285.

(5) M. Takasugi, A. Guendouz, M. Chassignol, J.L. Decout, J. Lhomme, N.T. Thuong, and C. Helene, *Proc. Natl. Acad. Sci. U.S.A.*, 1991, **88**, 5602-5606.

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ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Cholesteryl-TEG Phosphoramidite	10-1975-95	50 µmole	165.00
	10-1975-90	100 µmole	295.00
	10-1975-02	0.25g	675.00
DNP-TEG Phosphoramidite	10-1985-95	50 µmole	165.00
	10-1985-90	100 µmole	295.00
	10-1985-02	0.25g	675.00
Psoralen C6 Phosphoramidite	10-1986-95	50 µmole	165.00
	10-1986-90	100 µmole	295.00
	10-1986-02	0.25g	675.00
Psoralen C2 Phosphoramidite	10-1982-90	100 µmole	195.00
	10-1982-02	0.25g	495.00

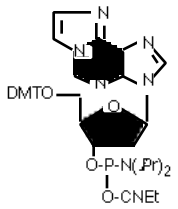
ETHENO-DA AND ETHENO-A - FLUORESCENT MONOMERS

Fluorescent derivatives are produced by reaction of chloroacetaldehyde with adenosine forming the corresponding 1,N6-etheno compounds. The resulting extension of conjugation allows specific excitation of etheno-A residues in the presence of proteins and nucleic acids. Also, the derived emission can be detected in the presence of protein fluorescence[1].

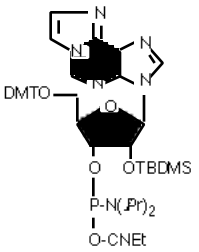
The availability of CE phosphoramidites of etheno-dA and etheno-A allows these residues to be specifically located within sequences of interest. Applications may be found in carcinogenesis studies as well as in studies of DNA and RNA structures.

Etheno-dA and etheno-A residues are base sensitive and mild procedures should be employed for deprotection of the derived oligonucleotides. We recommend deprotection with ammonium hydroxide for 24h at room temperature for DNA sequences and the use of fast deprotecting monomers and anhydrous methanolic ammonia at room temperature for RNA sequences.

STRUCTURES OF MONOMERS



Etheno-dA-CE Phosphoramidite



Etheno-A-CE Phosphoramidite

Reference:

(1) J.A. Secrist, J.R. Barrio, N.J. Leonard, and G. Weber, *Biochemistry*, 1972, **11**, 3499-3506.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Etheno-dA-CE Phosphoramidite	10-1006-90	100 µmole	105.00
	10-1006-02	0.25g	255.00
Etheno-A-CE Phosphoramidite	10-3006-90	100 µmole	150.00
	10-3006-02	0.25g	360.00