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# The Glen Report

## PROPYNYL-2'-OMe-RNA, Br-U, I-U

### - ANTISENSE PROBES, CROSSLINKING

Researchers have continued to explore the potential uses of C-5 propynyl pyrimidine derivatives of oligonucleotides. Caltech investigators[1] have examined the effect of C-5 propynyl-dU on triple helix formation. Groups from Gilead Sciences and The Agouron Institute[2] have measured the specific effect of a series of C-5 propyne oligonucleotides on HIV mRNA targets.

Froehler and coworkers[3] have already examined the effect of C-5 propynyl pyrimidine modifications in the behavior of 2'-O-allyl-RNA. Since our main focus in RNA monomer supply is on 2'-OMe-RNA, we believe that the enhanced binding of C-5 propynyl groups in 2'-OMe-RNA would be beneficial in the preparation and use of antisense RNA probes. We, therefore, have prepared C5-propynyl-2'-OMe-C and C5-propynyl-2'-OMe-U-CE Phosphoramidites. Structures and Ordering Information are shown on the Back Page. *It should be cautioned that the U analogue is quite insoluble in acetonitrile and we recommend the use of THF as solvent and/or manual coupling for this monomer.*

### *Br-U, I-U-CE Phosphoramidites*

Interest in crosslinking experiments has not been restricted to DNA protein interactions and we have been asked to provide halogenated RNA monomers. The two Uridine derivatives 5-Br-U and 5-I-U[4] are now available from Glen Research.

*(Continued on Back Page)*

## ULTRAFAST CHEMISTRY

### - SOME QUESTIONS ANSWERED

Late last year, Glen Research introduced an Acetyl protected dC (Ac-dC) monomer for oligonucleotide synthesis which allows UltraFAST (10 minutes versus the normal 6-7 hours) cleavage and deprotection. This simple but elegant modification of the standard protection scheme was developed by Beckman Instruments and patents are pending.[1] With minimal change (replacement of benzoyl by acetyl for the dC monomer - other monomers remain unchanged) to the tried and true DNA monomer protection scheme, oligonucleotide synthesis proceeds normally. However, by using AMA (a 50:50 mixture of ammonium hydroxide and aqueous methylamine), the time-consuming cleavage and deprotection steps are reduced to a mere 10 minutes.

Since the introduction of the UltraFAST system, we have been asked many questions which are worth reproducing here along with answers which

*(Continued on Page 4)*

## *Inside*

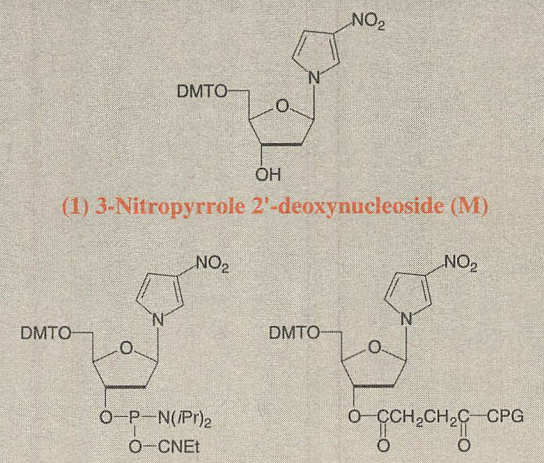
# UNIVERSAL NUCLEOSIDE

The design of oligonucleotide primers and probes is frequently made more complicated by the degeneracy of the genetic code or incomplete peptide sequence data. To confront this problem, two strategies are popular but both have significant drawbacks. The use of a mixed base addition (N) at specific points is a logical approach and is achieved either by delivery of equal amounts of monomer on the synthesizer or using the monomers premixed in equivalent amounts. This strategy is more successful the fewer the number of degenerate sites, probably simply due to the smaller number of oligonucleotides formed. (Remember that x degenerate sites lead to $4^x$ oligonucleotides, e.g., 4 fully degenerate sites lead to 256 oligonucleotides of which only one is the desired sequence.) An alternative approach is to substitute 2'-deoxyInosine (dI) at the degenerate sites. Hydrogen bonding of dI to each of the other bases is low but, unfortunately, is not completely equivalent and this factor can cause problems in PCR and sequencing experiments.

There clearly exists a need for a new strategy, preferably one that involves a universal base which substitutes each unknown or degenerate site during construction of oligonucleotides. These modified oligonucleotides must still have the ability to generate accurate sequencing data or participate in the amplification of the correct DNA.

A recent publication[1] describes the properties of 3-nitropyrrole 2'-deoxynucleoside (1) (designated M) when used as a universal nucleoside. The authors' strategy was to design and evaluate molecules which would maximize stacking while minimizing hydrogen-bonding interactions. In this way, base-pairing specificity of the target molecule with the 4 normal bases should be minimized while the duplex should be stabilized by the enhanced stacking interactions. For maximum polarity and base stacking, the authors first chose to evaluate 3-substituted pyrrole derivatives as candidates for universal nucleosides based on weakened hydrogen bonding. M was selected from the candidate molecules because of its electronic similarity to p-nitroaniline derivatives which are the smallest known intercalating molecules.

*FIGURE 1:* UNIVERSAL NUCLEOSIDE AND DERIVATIVES




(1) 3-Nitropyrrole 2'-deoxynucleoside (M)

(2) 3-Nitropyrrole CE Phosphoramidite (3) 3-Nitropyrrole CPG

### *Sequencing*

The CE phosphoramidite (2) of 3-nitropyrrole 2'-deoxynucleoside was used to prepare oligonucleotides with several unknown sites substituted with M. The correct sequencing primer in a dideoxy sequencing experiment was compared with the equivalent primers modified at the third position in 4 codons with N (a 256-fold degenerate primer), with dI, and with M. The sequencing ladder obtained using N was unreadable, using dI was only partially readable, but using M was an exact match of the correct oligonucleotide primer. Even more impressively, 17 base oligonucleotide primers with three, six and nine contiguous substitutions of M also generated accurate sequencing data. Both double and single stranded templates were successfully sequenced.

### *PCR*

Promising results were also demonstrated in initial PCR experiments amplifying from total RNA using primers containing three M substitutions. Amplification of the correct product resulted.

### *Melting Behavior*

The ability of an oligonucleotide containing M to function as a sequencing primer would indicate that a duplex is being successfully formed with the complementary strand. Indeed, a normal pattern was observed from the DNA double strand to single strand transition. The Tm values of oligonucleotides containing M-X base pairs (where X=A,C,G or T) all fell within a 3° range. In contrast, duplexes containing dI opposite the other bases vary in Tm by as much as 15°.

### *Other Potential Applications*

As usual, alert and ingenious researchers have already suggested other applications for the use of M: So far, experiments involving ligase chain reaction, in situ hybridization, mutagenesis, motif cloning, and even in restriction fragment length polymorphism (RFLP) have been proposed. We await feedback with interest.

As always, Glen Research is dedicated to offering research-oriented products in a timely manner. We are, therefore, happy to offer M as its CE phosphoramidite and the corresponding support (3) under license from the University of Michigan and Purdue University.

### *Reference:*

(1) R. Nichols, P.C. Andrews, P. Zhang, and D.E. Bergstrom, *Nature*, 1994, **369**, 492-493.

## ORDERING INFORMATION

| Item | Catalog No. | Pack | Price($) |
|---|---|---|---|
| 3-Nitropyrrole-CE Phosphoramidite | 10-1043-90 | 100 µmoles | 125.00 |
| | 10-1043-02 | 0.25g | 325.00 |
| 3-Nitropyrrole CPG | 20-2043-01 | 0.1g | 60.00 |
| (Bulk) | 20-2043-10 | 1.0g | 600.00 |
| 1 µmole columns | 20-2143-41 | Pk/4 | 200.00 |
| 0.2 µmole columns | 20-2143-42 | Pk/4 | 120.00 |

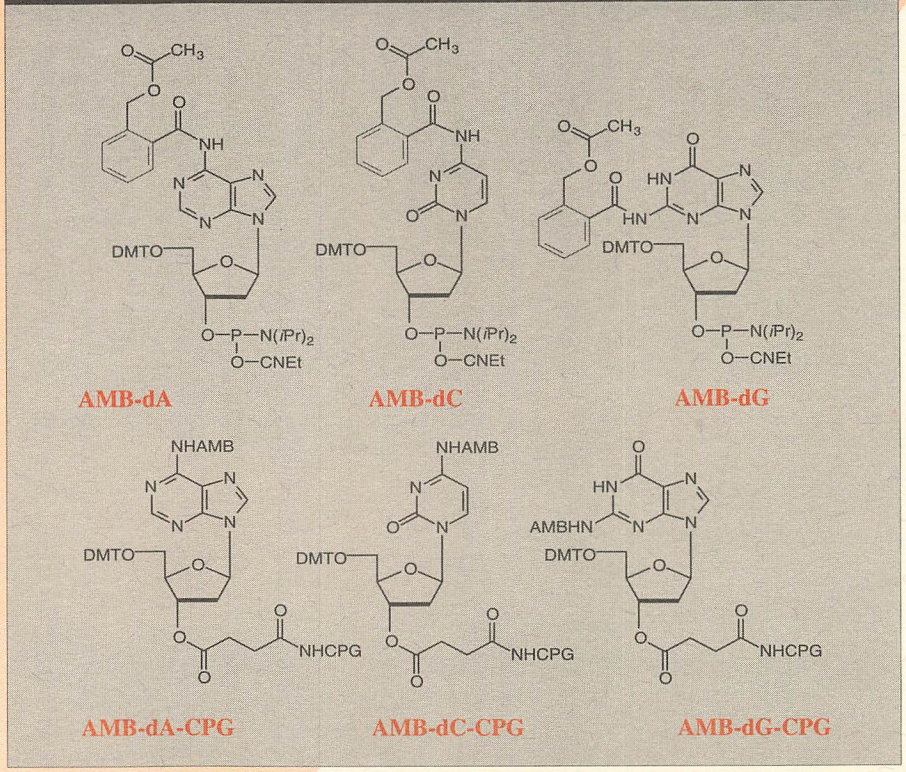
# ULTRAMILD DNA SYNTHESIS

In recent years, the synthesis of labelled oligonucleotides has become virtually a standard procedure in many labs. Many labelling reagents, e.g., biotin, fluorescein are now available as ß-cyanoethyl (CE) phosphoramidites and this provides a rapid means of producing the appropriate oligonucleotides directly. Prior to the availability of these labelling reagents as CE phosphoramidites, conjugations were carried out using the solution phase reaction of amino-or thiol-modified oligonucleotides with the appropriately functionalized label. Labels which are currently available as CE phosphoramidites have one property in common - they must be stable to strongly alkaline conditions required for removal of the base protecting groups. This property is lacking in several interesting dyes and labels and so we have been seeking an alternative protecting scheme for the normal CE phosphoramidites which allows UltraMILD deprotection and should not react with a wider variety of tags and labels.

Expedite™ chemistry, as offered by Biosearch, does allow for much milder deprotection (ammonium hydroxide/ 2h/RT) but even this is too harsh for some prospective CE phosphoramidite labels. Our goal, therefore, was to find a synthesis system employing monomer protecting groups which can be removed without the use of harsh basic reaction conditions.

We were prompted[1] to look at work which had been carried out to prepare base-labile backbones, e.g., methyl phosphotriesters[2] and methyl phosphonates[3]. As described by Dutch researchers[2,3], the 2-(acetoxy-methyl)benzoyl (AMB) group is used for base protection and later removed using anhydrous potassium carbonate in methanol (90 minutes/RT). Structures of the AMB protected monomers and supports are shown in Figure 1 and a suggested mechanism for their removal is shown in Figure 2.

*FIGURE 1:* 2-(ACETOXYMETHYL) BENZOYL (AMB) PROTECTED MONOMERS



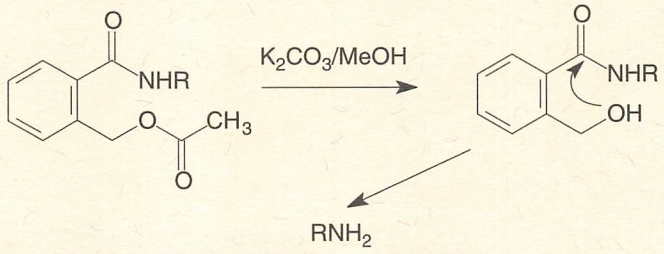

*References:*


(1) L.J. Marnett, Vanderbilt University Medical Center, *Personal Communication.*

(2) W.H.A. Kuijpers, J. Huskens and C.A.A. Van Boeckel, *Tetrahedron Lett.*, 1990, **31**, 6729-6732.

(3) W.H.A. Kuijpers, E. Kuyl-Yeheskiely, J.H. Van Boom, and C.A.A. Van Boeckel, *Nucleic Acids Res.*, 1993, **21**, 3493-3500.


*FIGURE 2:* MECHANISM OF AMB DEPROTECTION WITH $K_2CO_3$/MeOH




## ORDERING INFORMATION

| Item | Catalog No. dA | Catalog No. dC | Catalog No. dG | Pack | Price($) |
|---|---|---|---|---|---|
| AMB-CE Phosphoramidite | 10-1600-02 | 10-1610-02 | 10-1620-02 | 0.25g | 25.00 |
| | 10-1600-05 | 10-1610-05 | 10-1620-05 | 0.5g | 50.00 |
| | 10-1600-10 | 10-1610-10 | 10-1620-10 | 1.0g | 100.00 |
| AMB-CPG (Bulk) | 20-2600-02 | 20-2610-02 | 20-2620-02 | 0.25g | 40.00 |
| | 20-2600-10 | 20-2610-10 | 20-2620-10 | 1.0g | 150.00 |
| (ABI Columns) | 20-2710-42 | 20-2720-42 | 20-2730-42 | 4X0.2 µmole | 60.00 |
| | 20-2710-41 | 20-2720-41 | 20-2730-41 | 4X1 µmole | 60.00 |
| | 20-2710-13 | 20-2720-13 | 20-2730-13 | 10 µmole | 100.00 |
| (Biosearch Columns) | 20-2810-42 | 20-2820-42 | 20-2830-42 | 4X0.2 µmole | 60.00 |
| | 20-2810-41 | 20-2820-41 | 20-2830-41 | 4X1 µmole | 60.00 |
| | 20-2810-14 | 20-2820-14 | 20-2830-14 | 15 µmole | 150.00 |

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illustrate why this deceptively simple concept is proving so valuable.

***Is the replacement of the normal dC monomer with Ac-dC really the only change necessary?***

Yes. The only change on the synthesizer is the substitution with the Ac-dC monomer. The other monomers remain unchanged. Of course, if the target oligonucleotide contains dC at the 3' terminus, an Ac-dC column must be used.

***What is AMA and how do I get it?***

AMA contains strong ammonium hydroxide traditionally used for deprotection along with 40% aqueous methylamine which is available from Aldrich, Catalog No. M2,775-1. Prepare the AMA reagent by mixing equal volumes of the two reagents. *(Caution: observe the recommended safety precautions provided by the suppliers.)* The resulting AMA solution is stable and can be stored refrigerated in the same way as ammonium hydroxide.

***Can Ac-dC be used in situations where labile groups like fluorescein are in the oligonucleotide?***

AMA is a very active reagent and potentially could damage sensitive molecules like fluorescein. However, the Ac-dC monomer is so versatile, it is completely compatible with traditional ammonium hydroxide deprotection. Indeed, if an expensive label or minor base has been included in the oligonucleotide, there is no need to risk your investment by using AMA. Simply use the standard ammonium hydroxide deprotection.

***Can long oligos be produced using this chemistry?***

Ac-dC couples with an efficiency at least as high as the normal Bz-dC monomer, so long oligos can be readily prepared. Although there is no evidence that AMA causes base modification at a level which may prove to be significant in oligos greater than 50mer, it would again seem unwise to take the risk. The Ac-dC protection is completely compatible with ammonium hydroxide deprotection so why risk problems in later time-consuming experiments?

***Can the AMA reagent be put on the synthesizer for automated cleavage?***

The only incompatibility known for the AMA reagent is with rubber which may be weakened or swell. Vials which use rubber O-rings to seal and syringes with rubber plunger seals should not be used. The components of a DNA synthesizer which contact the reagents should be unharmed by AMA even on extended use. If the AMA reagent is attached to the synthesizer, the cleavage time may be reduced to 5 minutes.

***Expedite monomers from Biosearch also can be cleaved and deprotected in 15 minutes, what are the differences between these two new techniques?***

A "fair" comparison is shown in Table 1. The assertion by Biosearch that oligos prepared using Expedite™ monomers can be cleaved and deprotected in 15 minutes is accurate only if the user opens the column and exposes the support matrix directly to the deprotection reagent. This may be easy for the membrane based columns which can be opened to allow the membrane to be directly deprotected. However, to break open the aluminum seals on the Biosearch CPG columns prior to heating the CPG support in ammonium hydroxide is no easy task. This process leads to a product containing silica which must be, at least, desalted prior to use. A separate on-column cleavage step takes 45 minutes.

***Can the unpurified oligonucleotide be used directly for PCR or sequencing?***

The crude oligonucleotide isolated by evaporating the AMA reagent dissolves completely in aqueous solution and can be used directly. This contrasts with the crude oligonucleotide produced by Expedite chemistry which contains *t*-butyl-phenoxyacetamide which is not water-soluble and requires a centrifugation step before use. Significant product loss has also been reported to occur.

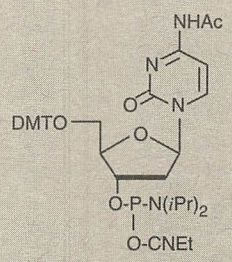
***Do any other reagents need to be changed?***

The standard synthesis reagents are appropriate for UltraFAST chemistry, with the exception of the Ac-dC monomer change. Again, this contrasts with Expedite chemistry which requires an alternative capping reagent, and a change in the dA, dC and dG monomers.

***How and why does this UltraFast chemistry work?***

AMA is an aggressive base which is much more reactive than ammonium

*FIGURE 1:* STRUCTURE OF Ac-dC




Ac-dC-CE Phosphoramidite

*TABLE 1:* COMPARISON OF UltraFAST AND EXPEDITE TECHNIQUES

| | *UltraFAST* | *Expedite* |
|---|---|---|
| Monomer Changes Necessary | dC only | dA, dC and dG |
| Solubility Problem (0.1M solution)? | None | dG |
| Reagent Changes Necessary | None | Cap A |
| Deprotection Reagent | AMA | ammonium hydroxide |
| Cleavage Time | 5 minutes | 45 minutes |
| Deprotection at 55° | 10 minutes | 15 minutes |
| Total Time (Cleavage and Deprotection) | 15 minutes | 60 minutes |
| Can Crude Oligo be Used Directly? | Yes | No |
| Compatible with Poly-Pak Purification? | Yes | Yes |
| Compatible with Base Labile Groups? | Use ammonium hydroxide | Yes |

*(Continued from Previous Page)*

**TABLE 2:** DEPROTECTION WITH AMA

| Time | Temperature |
|---|---|
| 5 min | 65° |
| 10 min | 55° |
| 30 min | 37° |
| 90 min | 25° |

*Notes:*
1. If a dry heating block is used, increase the times by 5 minutes to allow equilibration.
2. AMA attacks most rubber materials in tubes and syringes. See Ordering Information for sources of compatible products.

hydroxide alone. The cleavage of the oligonucleotide from the support is simply speeded up, reducing the reaction time from 45 minutes to 5 minutes. However, the critical aspect is in the rate of hydrolysis of the base protecting groups. The acetyl group on the dC residues is hydrolyzed almost instantaneously, followed by the benzoyl groups from dA sites, and slowest, the *i*Bu groups from the dG sites. The significance of the UltraFAST hydrolysis of the acetyl group from the dC sites is illustrated in Figure 2.

### *Where can compatible deprotection tubes be purchased?*

In the previous *Glen Report*, these tubes were incorrectly sourced. The catalog number was correct but the supplier is Rainin.

## ORDERING INFORMATION

| Item | Catalog No. | Pack | Price($) |
|---|---|---|---|
| Ac-dC-CE Phosphoramidite *(AB and compatible)* | 10-1015-02 | 0.25g | 25.00 |
| | 10-1015-05 | 0.5g | 50.00 |
| | 10-1015-10 | 1.0g | 100.00 |
| | 10-1015-20 | 2.0g | 200.00 |
| Ac-dC-CE Phosphoramidite *(Beckman Oligo 1000)* | 10-1015-B2 | 0.25g | 25.00 |
| | 10-1015-B5 | 0.5g | 50.00 |
| | 10-1015-1B | 1.0g | 100.00 |
| Ac-dC-CE Phosphoramidite *(Biosearch Expedite and Cyclone)* | 10-1015-C2 | 0.25g | 25.00 |
| | 10-1015-C5 | 0.5g | 50.00 |
| | 10-1015-1C | 1.0g | 100.00 |
| Ac-dC-lcaa-CPG *(Bulk)* | 20-2015-01 | 0.1g | 18.00 |
| | 20-2015-02 | 0.25g | 40.00 |
| | 20-2015-10 | 1.0g | 150.00 |
| *(AB and compatible columns)* | 20-2115-41 | 4X1 µmole | 60.00 |
| | 20-2115-42 | 4X0.2 µmole | 60.00 |
| | 20-2115-45 | 4X40 nmole | 60.00 |
| | 20-2115-13 | 1X10 µmole | 100.00 |
| *(Biosearch columns)* | 20-2215-41 | 4X1 µmole | 60.00 |
| | 20-2215-42 | 4X0.2 µmole | 60.00 |
| | 20-2215-45 | 4X40 nmole | 60.00 |
| | 20-2215-14 | 1X15 µmole | 150.00 |
| Methylamine (40% solution) | Aldrich M2,775-1 | | |
| Syringes (1mL) | Aldrich Z23072-3 | | |
| Deprotection tubes | Rainin ST336-5S | | |

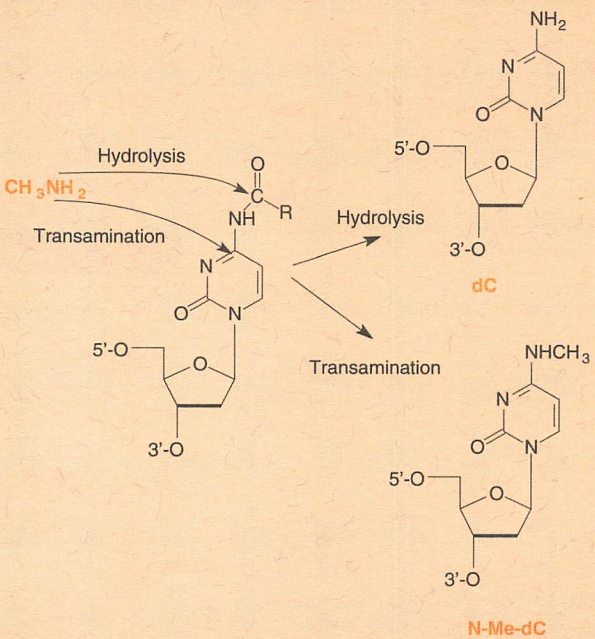
### *References:*


(1) a. G. Sasaki, J.J. Dih, and M.P. Reddy, *Technical Information Bulletin T-1792*, Beckman Instruments, Inc. (1993).
b. M.P. Reddy and N. Hanna (patents pending).
c. M.P. Reddy, N. Hanna, and F. Farooqui, *Tetrahedron Lett.*, 1994, **35**, 4311.


**FIGURE 2:** COMPARISON OF THE RATE OF HYDROLYSIS OF ACYL PROTECTING GROUPS ON dC

When the exocyclic amine of 2'-deoxyCytidine is protected for oligonucleotide synthesis as an amide with, for example, benzoyl, it is susceptible to a transamination reaction on deprotection with alkylamines. The mechanism of this reaction is shown. The reaction leads to N-alkyl-2'-deoxyCytidine mutations in the product oligonucleotide. Base composition analysis of the digested oligonucleotide indicated levels of N-methyl-dC as shown in the following table:

| *Protected dC Monomer* | *Time Required for Deprotection with AMA* | *Amount of N-Me-dC Formed* |
|---|---|---|
| N-benzoyl-dC | 1 hour | 10.0% |
| N-isobutyryl-dC | 2 min. | 0.7% |
| N-propionyl-dC | 1 min. | 0.05% |
| N-acetyl-dC | <<1min. | 0.0% |




We thank M.P. Reddy for providing us with these data.

# NEW MINOR BASES

### *8-Br-dA and 8-Br-dG*

Photoactive bases may be used to probe the structure of protein-DNA complexes. Currently, photoactive analogues of dC and dT (5-bromo- and 5-iodo-dC and dU) are commercially available. However, photo cross-linking studies to examine base to amino acid contact pairs require all 4 bases to be available in photoactive form. We are now happy to introduce 8-Br-dA[1] and 8-Br-dG as photoactive bases to complete the set of 4 commercially available bases. These halogenated bases are also suitable for X-ray crystallography studies.

*Please note that our studies on the stability of halogenated bases to ammonium hydroxide have led us to recommend the use of mild deprotection to avoid ammonolysis of the halogenated site. Our data indicate that this undesirable side reaction is virtually eliminated by carrying out the deprotection at room temperature for 24 hours.*

### *8-oxo-dA and 8-oxo-dG*

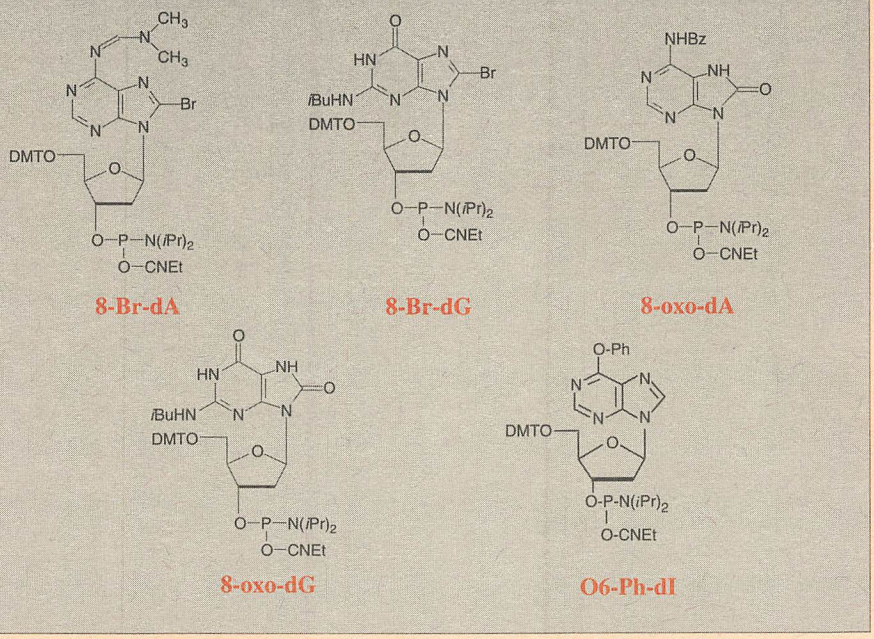
Recently, there has been significant interest in the purine deoxynucleosides with an oxo group at the 8-position. This type of modification of DNA is formed naturally by oxidation or ionizing radiation. The resulting 8-oxo modification is significant in mutagenesis and ultimately carcinogenesis. To investigate this type of lesion in DNA, it is clearly desirable to have access to the 8-oxo-dA- and 8-oxo-dG-CE Phosphoramidite monomers which allow users to prepare oligonucleotides containing 8-oxo-dA[2, 3] or 8-oxo-dG[3] site specifically.

*Synthetic oligonucleotides containing 8-oxo-dG must be cleaved and deprotected with ammonium hydroxide containing 0.25M 2-mercaptoethanol to avoid oxidative degradation of 8-oxo-dG sites.*

### *O6-Ph-dI (Convertible dA)*

With the unfortunate demise of Glen Research's Oligo Affinity Support, we are frequently asked to supply an alternative and we continue to test

## STRUCTURES OF NEW MINOR BASES




candidates. In the meantime, it is possible to produce a support for affinity purification by attaching an oligonucleotide amino-modified at the 5'- or 3'-terminus to an activated carboxy support like NHS-agarose. While this form of amino-modification is relatively convenient, it does not allow self ligation of double stranded DNA containing the desired restriction site. However, this may be accomplished using an oligonucleotide internally amino-modified with Amino-Modifier C6-dT or using O6-phenyl-2'-deoxyInosine (O6-Ph-dI). This latter compound, which should be thought of as a convertible dA, is modified after *mild* ammonium hydroxide deprotection with a suitable diamine in a post synthetic substitution reaction.[4] The authors successfully substituted the convertible dA positions with 1,4-diaminobutane, annealed the complementary strand, and ligated the DNA to produce a duplex containing multiple amino residues. The DNA was then attached to NHS-Sepharose to form an affinity matrix. The high capacity affinity column so formed was used for the purification of transcription factor H2TF1 which had resisted purification attempts by conventional methods.

### *References:*


(1) J. Liu and G.L. Verdine, *Tetrahedron Lett.*, 1992, **33**, 4265-4268.

(2) A. Guy, A.M. Duplaa, P. Harel, and R. Teoule, *Helvetica Chimica Acta*, 1988, **71**, 1566-1572.

(3) V. Bodepudi, S. Shibutani, and F. Johnson, *Chem. Res. Toxicol.*, 1992, **5**, 608-617.

(4) C.L. Larson and G.L. Verdine, *Nucleic Acids Res.*, 1992, **20**, 3525.


## ORDERING INFORMATION

| Item | Catalog No. | Pack | Price($) |
|---|---|---|---|
| 8-Br-dA-CE Phosphoramidite | 10-1007-90 | 100 µmoles | 115.00 |
| | 10-1007-02 | 0.25g | 295.00 |
| 8-Oxo-dA-CE Phosphoramidite | 10-1008-90 | 100 µmoles | 135.00 |
| | 10-1008-02 | 0.25g | 355.00 |
| 8-Br-dG-CE Phosphoramidite | 10-1027-90 | 100 µmoles | 105.00 |
| | 10-1027-02 | 0.25g | 255.00 |
| 8-Oxo-dG-CE Phosphoramidite | 10-1028-95 | 50 µmoles | 177.50 |
| | 10-1028-90 | 100 µmoles | 355.00 |
| | 10-1028-02 | 0.25g | 975.00 |
| O6-Ph-dI-CE Phosphoramidite | 10-1042-90 | 100 µmoles | 135.00 |
| | 10-1042-02 | 0.25g | 355.00 |

# NEW MODIFICATION REAGENTS AND SUPPORTS

### *Spacer 18*

Spacer molecules have been used to introduce non-nucleotide segments into oligonucleotides. These have been used to form folds and hairpins[1,2] to bridge sections of oligonucleotides where no appropriate binding is possible, as well as simply to space tags further away from the oligonucleotide. We are pleased to add Spacer 18 to our existing line of spacer phosphoramidites.

### *dSpacer*

Abasic sites in DNA are generated chemically or enzymatically by selective hydrolysis of the glycosidic linkage. The resulting apurinic/apyrimidinic sites lack coding information and lead to mis-incorporation of bases by polymerases and may play an important role in mutagenesis. Abasic sites are rather unstable and are susceptible to ß-elimination causing chain scission. The instability of the natural abasic site can be eliminated by the substitution of a tetrahydrofuran derivative[3,4] with a methylene group modifying the 1 position of 2'-deoxyribose. The CE phosphor-amidite of the tetrahydrofuran derivative is offered as a new spacer phosphoramidite which we have named dSpacer .

### *Glyceryl CPG*

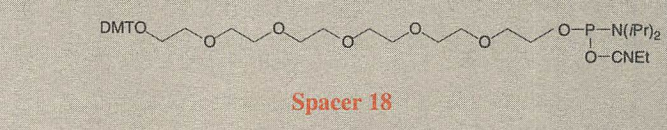
A support has been described[5] for the preparation of oligonucleotides with a 3'-phosphoglyceryl terminus. The terminus is readily oxidized by sodium periodate to form a 3'-phospho-glycaldehyde. The aldehyde may be further oxidized to the corresponding carboxylic acid. Either the aldehyde or the carboxylate may be used for subsequent conjugation to amine-containing products. The authors were specifically interested in the products from the radical cleavage of oligonucleotides by DNA targeting molecules like bleomycin, enediyne antibiotics and transition metal complexes.

### *Cholesteryl CPG*

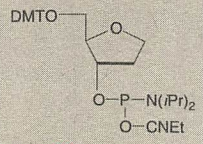
Cholesteryl conjugated oligo-nucleotides have been the subject of substantial interest in antisense and other studies due to the lipophilicity and ready availability of cholesterol. Cholesteryl CE phosphoramidites tend to be rather insoluble in acetonitrile, making them inconvenient to use in automated synthesis. Even our cholesteryl-TEG phosphoramidite has to be dissolved in 5% THF in acetonitrile. A simple solution is to use a support with cholesterol attached as was described recently.[6] The authors started with our 3'-amino-modifier C7-CPG, removed the Fmoc protecting group with DBU in acetonitrile, and conjugated the resulting amine with cholesteryl chloroformate. We offer cholesteryl-TEG CPG as a more direct solution.

*Note:* Supplementary information is available on these items on request.

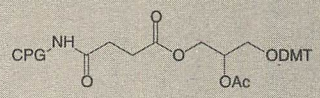
## STRUCTURES OF PRODUCTS DESCRIBED



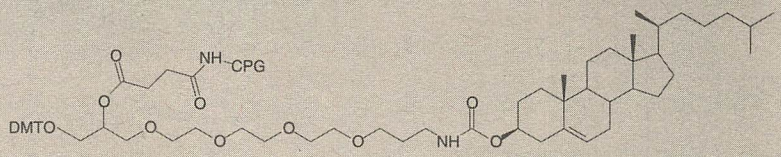

Spacer 18




dSpacer




Glyceryl CPG




Cholesteryl-TEG CPG

### *References:*


(1) M. Salunkhe, T.F. Wu, and R.L. Letsinger, *J. Amer. Chem. Soc.*, 1992, **114**, 8768-8772.

(2) N.G. Dolinnaya, M. Blumenfeld, I.N. Merenkova, T.S. Oretskaya, N.F. Krynetskaya, M.G. Ivanovskaya, M. Vasseur, and Z.A. Shabarova, *Nucleic Acids Res.*, 1993, **21**, 5403-5407.

(3) M. Takeshita, C.N. Chang, F. Johnson, S. Will, and A.P. Grollman, *J. Biol. Chem.*, 1987, **262**, 10171-10179.

(4) M.W. Kalnik, C.N. Chang, A.P. Grollman, and D.J. Patel, *Biochemistry*, 1988, **27**, 924-931.

(5) H. Urata and M. Akagi, *Tetrahedron Lett.*, 1993, **34**, 4015-4018.

(6) C.J. Marasco, N.J. Angelino, B. Paul, and B.J. Dolnick, *Tetrahedron Lett.*, 1994, **35**, 3029-3032.


## ORDERING INFORMATION

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| 1 µmole columns | 20-2902-41 | Pk/4 | 125.00 |
| 0.2 µmole columns | 20-2902-42 | Pk/4 | 75.00 |
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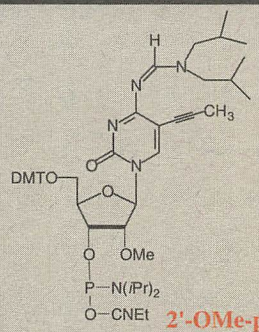
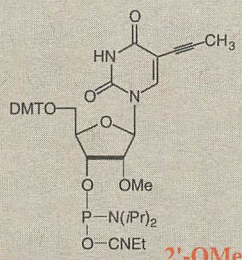
| (Bulk) | 20-2975-10 | 1.0g | 995.00 |
| 1 µmole columns | 20-2975-41 | Pk/4 | 200.00 |
| 0.2 µmole columns | 20-2975-42 | Pk/4 | 120.00 |

*(Continued from Front Page)*

## STRUCTURES OF NEW RNA ANALOGUES




2'-OMe-pC

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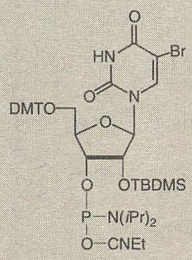
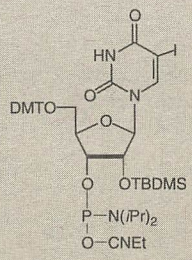



5-Br-U




5-I-U

*References:*

(1) N. Colocci and P.B. Dervan, *J. Amer. Chem. Soc.*, 1994, **116**, 785-786.

(2) S.D. Fenster, R.W. Wagner, B.C. Froehler, and D.J. Chin, *Biochemistry*, 1994, **33**, 8391-8398.

(3) B.C. Froehler, R.J. Jones, X.D. Cao, and T.J. Terhorst, *Tetrahedron Lett.*, 1993, **34**, 1003-1006.

(3) K. Shah, H. Wu, and T.M. Rana, *Bioconjugate Chemistry*, In Press.

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