The Glen Report

22825 DAVIS DRIVE • STERLING, VIRGINIA • 20164

GLEN RESEARCH

VOLUME 16

NUMBER 1

FEBRUARY 2003

INSIDE

THYMIDINE GLYCOL

2'-Se-Me-URIDINE

DISULFIDE GOLD ARRAY

NOVEL MONOMERS

PYRROLO-dCTP

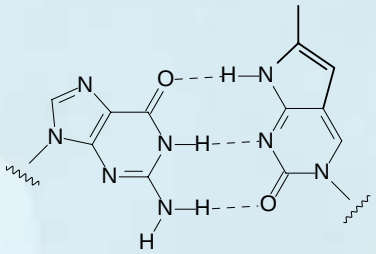
PYRROLO-C - A FLUORESCENT NUCLEOSIDE BASE ANALOGUE THAT CODES EFFICIENTLY AS C

The fluorescence of a nucleoside base is highly dependent on the environment of the base and the measurement of its fluorescence is a powerful and sensitive tool for the analysis of DNA and RNA structure. It is especially useful for analyzing the interaction of DNA and RNA with their corresponding binding proteins. Fluorescence measurements allow real-time probing of these structural interactions. Unfortunately, the intrinsic fluorescence of the regular bases is extremely low to non-existent, so fluorescent analogues have been sought for a long time.

For probing DNA structure, the ideal fluorescent nucleoside:

- Should have bright fluorescence, which is sensitive to its environment, and a large Stokes shift;
- Should be amenable to phosphoramidite preparation for incorporation into oligo-nucleotides by solid-phase synthesis;
- Should not disrupt duplex formation and should mimic one of the regular nucleosides;
- Should behave as a regular nucleoside in its interaction with proteins and enzymes; and
- Should be capable of being converted to the triphosphate and be incorporated into DNA with high efficiency by current commercial polymerases.

Several fluorescent nucleoside analogues have been prepared as phosphoramidites in recent years. Etheno-A (1) and etheno-C (2)[1] (Figure 1 on Page 2) are two readily accessible fluorescent structures but these molecules are both non-hybridizing. Other notable fluorescent base analogues are the pteridine nucleoside analogues actively being investigated by Pfleiderer, Hawkins and co-workers. The most promising analogue described to date[2] is the adenosine analogue (3) but guanosine and other analogues have also been investigated.[3-5]



G ..... Pyrrolo-C Base Pair

HIGHLIGHTS

Pyrrolo-dC is a fluorescent deoxycytidine analog that is an ideal probe of DNA structure and dynamics.

- *It base-pairs as a normal dC nucleotide. An oligo fully substituted with pyrrolo-dC has the same T_m as the control dC oligo with the same specificity for dG.*
- *Its small size does not perturb the structure of the DNA helix and it is well tolerated by a number of DNA and RNA polymerases.*
- *It is highly fluorescent and its excitation and emission are well to the red of most fluorescent nucleotide analogs, which eliminates or reduces background fluorescence from proteins.*

2-Aminopurine (4) is a fluorescent base which has found significant use in probing DNA structures. It is especially useful in that it is capable of hybridizing to T.[6]

In 2001, Glen Research introduced furano-T (5) as a novel fluorescent nucleoside. It quickly became apparent that furano-T is unstable during cleavage and deprotection steps, but forms another fluorescent nucleoside on treatment with ammonium hydroxide. Mass spec data supported the conclusion that furano-T had been transformed

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by ammonium hydroxide in the cleavage and deprotection to the equivalent amino compound, pyrrolo-C (6) (Figure 1). Furano-dT-CE Phosphoramidite (7) (Figure 2) was discontinued and Pyrrolo-dC-CE Phosphoramidite (8) (Figure 2), the fluorescent dC analogue, was introduced early in 2002. Pyrrolo-C CE Phosphoramidite (9) (Figure 2) for RNA synthesis has now also been synthesized and we foresee potential applications in RNA structural analysis.

Chemistry

Pyrrolo-dC is stable to all DNA synthesis cycles and reagents, with the exception of strong iodine oxidizer solutions. Routinely, 0.02M iodine oxidizer solutions are now used and these solutions have no effect on pyrrolo-dC. However, some older instruments and cycles use 0.1M iodine or stronger solutions and these cause degradation of pyrrolo-dC each cycle. These strong iodine solutions should not be used with pyrrolo-dC.

Pyrrolo-dC is stable to most cleavage and deprotection conditions, including UltraMild with potassium carbonate in methanol or ammonium hydroxide at room temperature, and regular with ammonium hydroxide, preferably at room temperature. This analogue contrasts with the pyrrolo-dC derivative, lacking only the methyl group, described[7] by Epoch researchers, which was unstable to deprotection conditions. They also observed that the non-methylated version of the ring system formed a mismatch with G, which differs from our observations for the pyrrolo-C - G base pair.

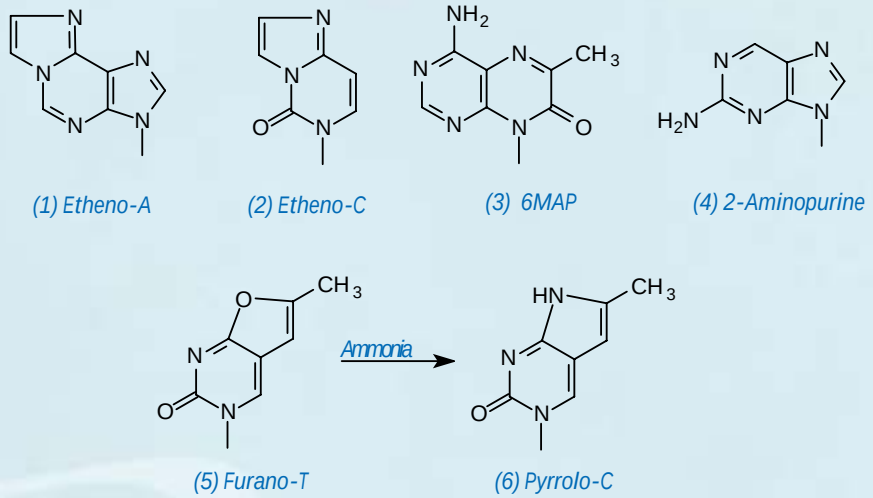
Spectral Properties

The spectral properties of pyrrolo-dC, coupled with its unique base-pairing ability, make this fluorescent analog extremely valuable in probing DNA structure. When the pyrrolo-dC is base-paired, its fluorescence is significantly quenched through what is most likely base stacking or dG interactions.

	QY	***λ***	***ε***(L/mol.cm)
single-stranded	0.07	260nm	4000
		347nm	3700
double-stranded	0.02		

(QY determined relative to quinine sulfate in 0.5M H_2SO_4)

FIGURE 1: STRUCTURES OF FLUORESCENT BASES



(1) Etheno-A *(2) Etheno-C* *(3) 6MAP* *(4) 2-Aminopurine*

(5) Furano-T *(6) Pyrrolo-C*

The quantum yield of fluorescence for pyrrolo-dC is quite sensitive to its hybridization state, making it ideally suited[8,9] for probing the dynamic structure of DNA. Work by Liu and Martin has shown[9] that, when the pyrrolo-dC is mismatched in an otherwise duplex hybrid, the fluorescence is higher than the single-stranded species when the mismatched base is adenosine. This most likely arises from efficient energy transfer from the adenosine to the pyrrolo-dC. This unusual behavior also allows differentiation *in situ* between a DNA-DNA duplex and a DNA-RNA heteroduplex.

Biology

The quenching of pyrrolo-dC allows local structural changes to be probed with great sensitivity. Using pyrrolo-dC, Liu and Martin[8] have characterized the transcription bubble in elongation complexes of T7 RNA Polymerase to single-base resolution by observing roughly a two-fold increase in fluorescence as the polymerase induces melting. By starving the T7 RNA Polymerase of specific nucleoside triphosphates, the enzyme could be stalled at specific sites, producing 'fluorescence snapshots' of the complex, and yielding detailed information on the nature of the transcription bubble and heteroduplex.

Work is still progressing in evaluating the effect of this modified fluorescent nucleoside in biological systems and will be reported in detail later. However, a few comments on our findings to date may be of interest. Oligonucleotides containing pyrrolo-dC act as efficient primers and the PCR products appear to be identical for primers with 0 to 5 pyrrolo-dC residues replacing dC. Preliminary data indicate that

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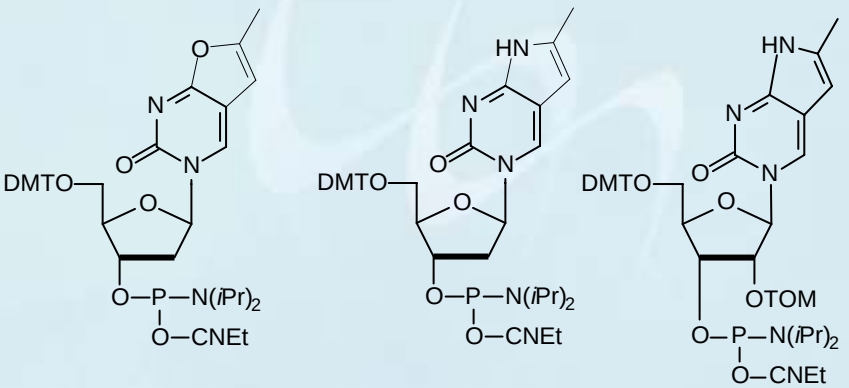
FIGURE 2: STRUCTURES OF FURANO-dT, PYRROLO-dC AND PYRROLO-C



(7) Furano-dT-CE Phosphoramidite *(8) Pyrrolo-dC-CE Phosphoramidite* *(9) Pyrrolo-C-CE Phosphoramidite*

DTPA - IMMOBILIZE OLIGOS TO GOLD SURFACES BY MULTIPLE THIOL ANCHORAGES

We are grateful to Dr. Peter Frischmann of FRIZ Biochem in Munich for providing us with the following information.

A prerequisite for reproducible and reliable results on DNA-arrays on gold substrates is an optimized immobilization chemistry. Although the gold-sulfur bond (with a bond energy of 30–40 kcal/mol) is a relatively strong anchor between a surface and biopolymer, it is well known that monofunctional thiolated oligonucleotides are slowly displaced from the gold surface at temperatures between 60–90 °C and at high salt concentration buffers (1 M NaCl). Especially drastic effects are detected with biological buffer systems containing dithiothreitol or mercaptoethanol, which lead to complete displacement of the oligonucleotides. For DNA-arrays on gold substrates, it is critical to guarantee the stability of the anchor during the washing steps (mechanical stress). In addition, the lateral diffusion of monofunctional anchored molecules on gold surfaces has to be prevented.

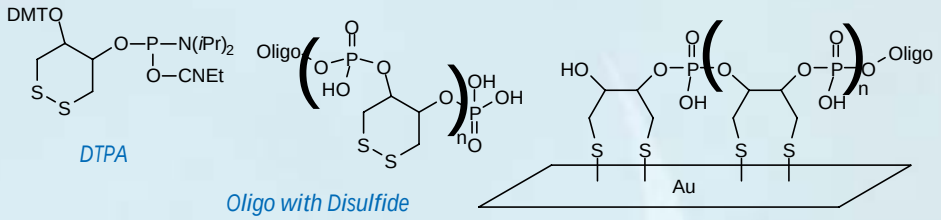
A special reagent for multiple anchorage:

In order to immobilize biopolymers on gold surfaces, a stable anchor is essential to avoid decomposition. FRIZ Biochem has developed a new compound with a performance far superior to commercially available components. The stable immobilization of oligonucleotides on DNA-arrays is a prerequisite for reliable and reproducible results. By modifying the capture probes with this new compound, FRIZ's research has detected no loss of oligonucleotides for the range of conditions used for array technologies (*i.e.*, mechanical stress caused by washing or hybridization procedures). This increased stability is the basis for robust DNA-chips on gold substrates.

Superior performance with Dithiol-Phosphoramidite(DTPA)

FRIZ Biochem's proprietary DTPA is a disulfide-containing phosphoramidite to modify synthetic DNA or RNA with multiple thiol groups (Figure 1). This novel product can be incorporated at any position of the oligonucleotide. The number of monomers, each forming two gold sulfur bonds, can be adapted to the demands of the application. The stability of the oligonucleotides tethered to the surface is increased by the multiple gold-anchors formed. Three units of the Disulfide-Modifier have proved to offer optimal stability, where the bonding is stressed by washing steps. Of course, this modifier can also be used for multiple reactions with maleimides and other thiol-specific derivatives.

FIGURE 1: **STRUCTURES OF DTPA AND OLIGOS FORMED FROM DTPA**



DTPA

Oligo with Disulfide Modifiers at the 3'-Terminus

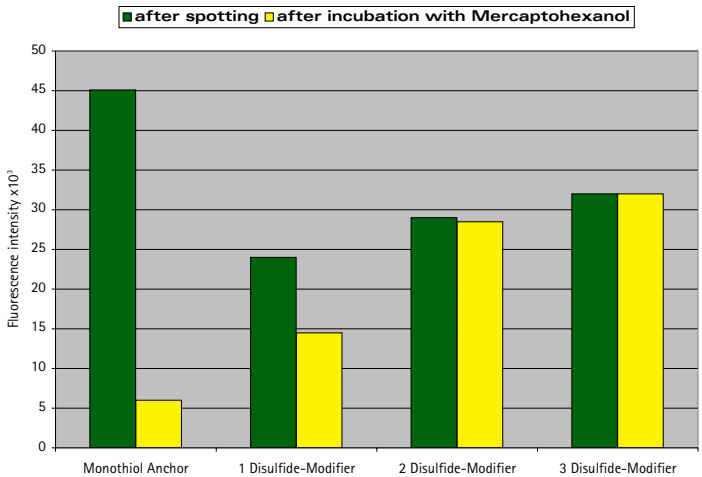
Anchorage of Biopolymers by DTPA Units

Stability tests of immobilized oligonucleotides on gold

The stability of thiolanchored oligonucleotides has been determined through fluorophore labelling studies. In FRIZ's experiments, commercially available monothiol modified oligonucleotides were compared with oligonucleotides immobilized with this new Disulfide-Modifier. A fluorophore (fluorescein) was attached at the 5' end of each oligonucleotide in order to study the immobilization and the stability with a fluorescence scanner. After the spotting of the oligonucleotides and washing with deionized water, the fluorescence intensities are shown in Figure 2 (green columns). To improve the performance of a bioassay, exposed bare gold surfaces should be avoided. In this case, FRIZ treated the surface with 1 mM mercaptohexanol. During this process, immobilized oligonucleotides are displaced according to the stability of their anchorage on the surface. The yellow columns in Figure 2 show the fluorescence intensities after this exposure. Up to 90% of oligonucleotides with one thiol modification were displaced from the gold. Under these conditions, the incorporation of three Disulfide-Modifiers (six gold-sulfur bonds) was shown to result in a stable bond – no oligonucleotides were displaced.

DTPA is offered by Glen Research under license from FRIZ Biochem.

FIGURE 2: **FLUORESCENCE INTENSITIES OF OLIGOS ANCHORED ON A GOLD SURFACE**



Fluorescence intensities of oligonucleotides anchored on a gold surface by different immobilization groups before and after mercaptohexanol (1mM) treatment.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Dithiol Phosphoramidite (DTPA)	10-1937-90	100 µm	200.00
	10-1937-02	0.25g	480.00

MINOR BASE AND RELATED NOVEL PHOSPHORAMIDITES

With over 100 nucleoside analogues already in our catalog, it becomes more and more difficult to find new and exciting nucleoside products. Of course, the most accessible modified nucleosides have already been produced as phosphoramidites. In the last year, we have continued to target some of our favorite applications - DNA damage and repair, modulation of hybridization, fluorescent products, and novel labelling procedures. In conjunction with our chemistry collaborators at Berry and Associates, we have evaluated more than a dozen new products in the last year. Unfortunately, the majority did not make the cut, but we hope the set that follows will find an interested audience.

Thymidine Glycol

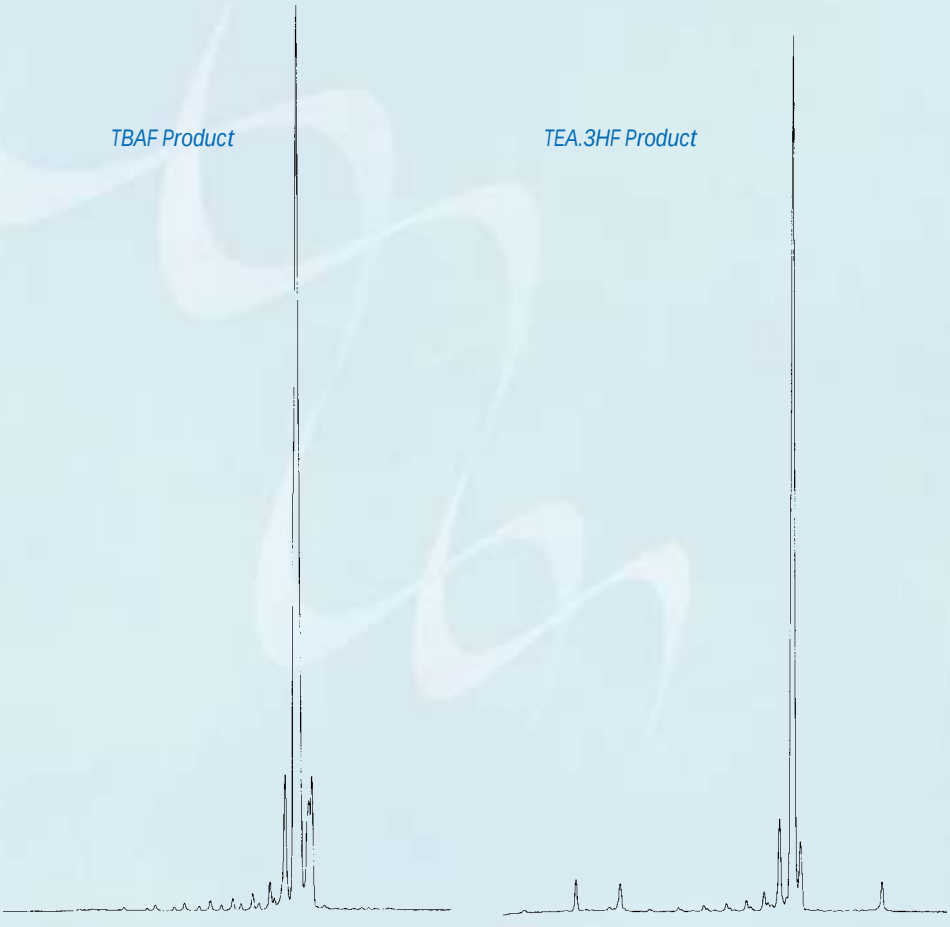
Thymine glycol (5,6-dihydroxy-5,6-dihydrothymine) (1) is formed when thymine is subjected to oxidative stress, including ionizing radiation. Oxidation of the 5,6 double bond of Thymidine generates two chiral centers at C5 and C6. The cis-5R,6S form (1) is generated as the predominant product along with the other diastereomer, the cis-5S,6R form (2). The presence of thymidine glycol in DNA has significant biological consequences and many organisms possess specific repair enzymes for the excision of this lesion. Previously, oligonucleotides containing thymidine glycol were formed by subjecting them to post synthetic oxidation using potassium permanganate or osmium tetroxide. Yields of the desired thymidine glycol lesion were low, specificity was poor and the desired products were difficult to isolate. Despite the biological significance of this mutation, a procedure for incorporating this oxidatively damaged monomer into oligonucleotides was only recently described.[1]

We are happy to introduce Thymidine Glycol (Tg) CE Phosphoramidite (3) with the glycol protected with a pair of t-butyl-dimethylsilyl (TBDMS) protecting groups. Using this monomer and phosphoramidites from our UltraMild group of products, Pac-dA, Ac-dC, and iPr-Pac-dG, oligonucleotide synthesis proceeds routinely. UltraMild cleavage and deprotection using ammonium hydroxide at room temperature for 2 hours gave a clean oligonucleotide product containing thymidine glycol still protected with TBDMS groups.

Our initial attempts to remove the TBDMS groups with t-butylammonium fluoride in tetrahydrofuran (TBAF) or triethylamine trihydrofluoride (TEA.3HF) overnight at room temperature generated products that looked very good by reverse phase HPLC, but analysis by ion-exchange HPLC revealed multiple species, albeit with a major component. Electrospray mass spectroscopic analysis of the products revealed that the TBAF product included lower molecular weight products, indicating decomposition of the deprotected thymidine glycol, while the TEA.3HF product had higher molecular weight species, indicating incomplete desilylation. In an attempt to mediate the TBAF reaction, the TBAF solution was first dried over activated molecular sieves.[2] The deprotection of a simple 12 mer overnight at room temperature with dry TBAF again gave an excellent product by RP HPLC. Ion exchange HPLC (Figure 1 - TBAF) revealed a much purer product and MALDI TOF MS analysis showed that the product was predominantly the correct dihydrothymine structure with some 5-methyl-5-hydroxy-hydantoin decomposition product (6) also present. However, the best result was achieved using TEA.3HF at 40°C overnight. RP HPLC showed a pure product while ion-exchange HPLC (Figure 1 - TEA.3HF) and MS data indicated that the product was essentially pure.

It has been reported[3] that epimerization at the 6-position of thymine glycol can occur, so we must assume that the products from oligonucleotide deprotection are equilibrium mixtures of diastereomers. Recently, Iwai[4] and Wang[5] have published further data indicating that the equilibrium after oligonucleotide synthesis is approximately 87% cis-5R,6S (1) to 13% trans-5R,6R (4).

FIGURE 1: **ION-EXCHANGE HPLC OF 12mer CONTAINING ONE THYMIDINE GLYCOL RESIDUE**



8-Amino-dG

In the last Glen Report, we introduced 8-Amino-dA (7) and we now offer its partner 8-Amino-dG (8). 8-Amino-purines are especially interesting for their properties in triple helices. The additional amino group is effective in increasing the stability of the triple helix by the addition of a Hoogsteen purine-pyrimidine hydrogen bond, as well as integrating an amino group in the "spine of hydration" in the minor-major groove of the triple helix.[6] In addition to its use in triplex oligos, 8-Amino-dG will find use in mutagenesis studies. 8-Amino-G (9) is formed along with 8-oxo-G (10), as the major mutagenic lesions formed in DNA damage caused by 2-nitropropane. 2-Nitropropane is an industrial solvent and a component of paints, dyes and varnishes, and is also present in cigarette smoke.[7]

The use of 8-Amino-dG in oligonucleotide synthesis is straightforward, requiring no changes from regular procedures, with the exception of the addition of 2-mercaptoethanol to the cleavage and deprotection solutions to avoid further oxidative damage.

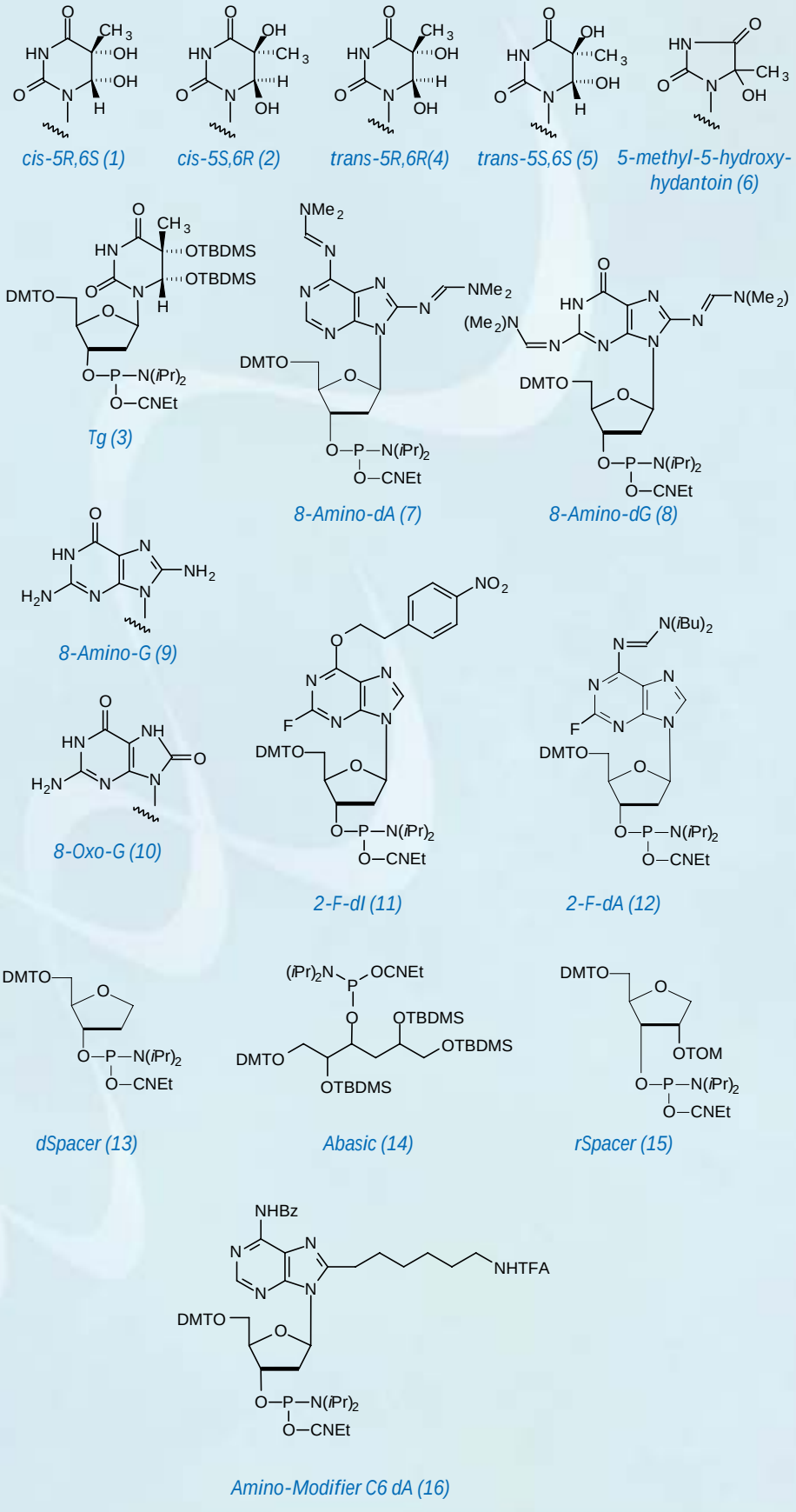
2-F-dA

We also described in the previous Glen Report the use of 2-F-dI (11) as a convertible nucleoside for the preparation of 2'-dG derivatives following the displacement of the 2-fluorine by primary amines.[8] Following an analogous procedure, 2-F-dA (12) can be used as a convertible nucleoside to form 2-amino-dA derivatives. The fluorine in 2-F-dA is much more resistant to displacement than in 2-F-dI. However, we have successfully displaced the fluorine with dansyl cadaverine on solid phase and with methylamine in solution. Work is continuing to optimize the displacement reaction and the information on our web site will give the most up-to-date procedures.

rSpacer

Abasic sites are produced in DNA by a variety of mechanisms, including oxidative damage, and depurination or depyrimidination by chemical and enzymatic means. DNA researchers are well served

FIGURE 2: STRUCTURES OF MODIFIED BASE ANALOGUES



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because of the availability of precursors to produce an abasic site in oligos, including our dSpacer (13)[9,10], which leads to a reduced and base-stable analogue of the true abasic site and Abasic Phosphoramidite (14),used to form a true abasic site.[11] Abasic sites in RNA are not so easily generated because of the greater stability of RNA to depurination, but the growing interest in RNA would seem to justify the introduction of an abasic ribose analogue for incorporation into RNA.

Abasic sites do indeed have an effect on RNA structure and activity. An example has been described using the hairpin ribozyme, which catalyzes a phosphodiester cleavage reaction. When abasic sites are introduced in various positions of the ribozyme core, ribozyme activity is greatly reduced. Interestingly, ribozyme activity could be rescued at least partially by the addition of nucleobases and the relative ability of the nucleobases to restore ribozyme activity could be used to probe ribozyme function and structure.[12]

We are happy to introduce rSpacer (15), the ribo-equivalent of dSpacer. For 2'-protection, we have chosen the TOM protecting group, which will make this novel monomer compatible with monomers with TBDMS or TOM[13] protecting groups.

2'-Se-Me-U

We are delighted to introduce 2'-selenomethyl-U CE Phosphoramidite, the initial product derived from our association with Dr. Zhen Huang from Brooklyn College. Dr. Huang has kindly provided us with some notes on applications of 2'-Se-Me-U for X-ray crystallography. His review is on the opposite page.

Amino-Modifier C6 dA

Usually, when we introduce a new product at Glen Research, we have good reasons to believe that it contains the most likely structural features for success. In the case of Amino-Modifier C6 dA, (16) (Figure 1 on Page 5), we already know that the structure is not optimal, in that attachment of groups to the 8 position of dA will destabilize the base pair to T. A better strategy is to attach the group at the 7-position of a 7-deaza-dA and probably the best strategy is to use 7-deaza-8-aza-dA, which also has the same electronic attributes as dA. However, these two options will yield a product which is enormously difficult to synthesize and, therefore, very expensive. Our tests with oligos containing Amino-Modifier C6 dA do indeed indicate that duplexes are destabilized by 2°C per insertion. However, Amino-Modifier C6 dA still codes specifically as dA. Although possibly not ideal, we feel that Amino-Modifier C6 dA offers a reasonable combination of performance and price.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Thymidine Glycol CE Phosphoramidite	10-1096-95	50 µm	180.00
	10-1096-90	100 µm	360.00
	10-1096-02	0.25g	975.00
8-Amino-dA-CE Phosphoramidite	10-1086-95	50 µm	177.50
	10-1086-90	100 µm	355.00
	10-1086-02	0.25g	975.00
8-Amino-dG-CE Phosphoramidite	10-1079-95	50 µm	177.50
	10-1079-90	100 µm	355.00
	10-1079-02	0.25g	975.00
2-F-dA-CE Phosphoramidite	10-1087-95	50 µm	180.00
	10-1087-90	100 µm	360.00
	10-1087-02	0.25g	975.00
2-F-dI-CE Phosphoramidite	10-1082-95	50 µm	180.00
	10-1082-90	100 µm	360.00
	10-1082-02	0.25g	975.00
dSpacer CE Phosphoramidite	10-1914-90	100 µm	85.00
	10-1914-02	0.25g	295.00
Abasic Phosphoramidite (dR Precursor)	10-1924-95	50 µm	105.00
	10-1924-90	100 µm	210.00
	10-1924-02	0.25g	475.00
rSpacer CE Phosphoramidite	10-3914-95	50 µm	90.00
	10-3914-90	100 µm	180.00
	10-3914-02	0.25g	495.00
Amino-Modifier C6 dA	10-1089-90	100 µm	205.00
	10-1089-02	0.25g	455.00
	10-1089-05	0.5g	910.00

References:

(1) S. Iwai, *Angew Chem Int Ed*, 2000, **39**, 3874+.
(2) R.I. Hogrefe, A.P. Mccaffrey, L.U. Borozdina, E.S. Mccampbell, and M.M. Vaghefi, *Nucleic Acids Res*, 1993, **21**, 4739-4741.
(3) M.J. Lustig, J. Cadet, R.J. Boorstein, and G.W. Teebor, *Nucleic Acids Res*, 1992, **20**, 4839-45.
(4) S. Iwai, *Chemistry*, 2001, **7**, 4343-51.
(5) Y.S. Wang, *Chem Res Toxicol*, 2002, **15**, 671-676.
(6) E. Cubero, et al., *J Am Chem Soc*, 2002, **124**, 3133-3142.
(7) L. Venkatarangan, A. Sivaprasad, F. Johnson, and A.K. Basu, *Nucleic Acids Res*, 2001, **29**, 1458-63.
(8) R. Eritja, A.R. Diaz, and E. Saison Behmoaras, *Helv Chim Acta*, 2000, **83**, 1417-1423.
(9) M. Takeshita, C.N. Chang, F. Johnson, S. Will, and A.P. Grollman, *J. Biol. Chem.*, 1987, **262**, 10171-10179.
(10) M.W. Kalnik, C.N. Chang, A.P. Grollman, and D.J. Patel, *Biochemistry*, 1988, **27**, 924-931.
(11) I.G. Shishkina and F. Johnson, *Chem Res Toxicol*, 2000, **13**, 907-912.
(12) A. Peracchi, L. Beigelman, N. Usman, and D. Herschlag, *Proc Natl Acad Sci U S A*, 1996, **93**, 11522-7.
(13) S. Pitsch, P.A. Weiss, L. Jenny, A. Stutz, and X.L. Wu, *Helv Chim Acta*, 2001, **84**, 3773-3795.

2'-SE-URIDINE PHOSPHORAMIDITE FOR RNA AND DNA DERIVATIZATION IN X-RAY CRYSTALLOGRAPHY

Zhen Huang, Ph.D.
Department of Chemistry
Brooklyn College
2900 Bedford Ave., Brooklyn, NY 11210
Phone: (718) 951-5746, Fax: (718) 951-4607
E-mail: zhuang@brooklyn.cuny.edu

Determination of the three-dimensional structures of RNA molecules, RNA-protein and DNA-protein complexes with high resolution is invaluable for gaining understanding of biological systems at the molecular level. X-ray crystallography is the most direct and powerful tool for structure determination of these macromolecules. However, derivatization with heavy atoms for phase determination, a long-standing problem in nucleic acid X-ray crystallography, has slowed down the structural determination process. It can take years just to prepare derivatives and to determine the required phase information.

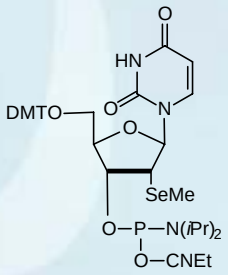
Recently, we have successfully demonstrated a novel derivatization strategy via selenium replacement of nucleotide oxygen.[1-3] Unlike conventional halogen derivatization (Br or I), where halogens are primarily placed on the 5-position of deoxyuridine (a mimic of thymidine), selenium can be selectively introduced to a variety of positions via oxygen replacement (*e.g.*, 2'-, 3'-, 5'-ribose oxygen, furan ring oxygen, non-bridging phosphate oxygen, or oxygen on nucleobases). Choice of positioning can avoid disruption of structure and function caused by modification. As the Multiwavelength Anomalous Dispersion (MAD) signal of selenium is as strong as that of bromine, selenium MAD phasing can be an alternative to the current bromine MAD phasing in nucleotide X-ray crystallography.

Our research results have shown that diffraction quality crystals of the 2'-selenium-derivatized oligonucleotides, such as 5'-GU$_{Se}$GTACAC-3' (Se-octamer containing 2'-Me-Se-uridine) and 5'-GCGTAU$_{Se}$ACGC-3' (Se-decamer containing 2'-Me-Se-uridine), were identified, and X-ray fluorescence spectra confirmed the presence of selenium in crystals.[2] MAD data of the Se-decamer to 1.2 Å resolution were collected and the diffraction data were successfully phased on the basis of the selenium anomalous signal.[3] Likewise, diffraction data of the octamer to 1.8 Å resolution were collected, and the structure of the octamer was determined by the molecular replacement technique. These X-ray crystal structures also confirmed the presence of the 2'-methylseleno group at the α-position of the uridine.

In both structures, the 2'-Me-Se-substituted furanoses display C3'-endo puckers, consistent with the A-form geometry of the unmodified decamer and octamer duplexes, which is adopted by RNA and A-form DNA. As previously established for 2'-O-methylated nucleotides and other 2'-O-modified ribonucleotide analogs, the methyl groups of the methylseleno moieties are directed into the minor groove and the C3'-C2'-Se-Me torsion angles adopt an antiperiplanar conformation. As the 2'-α-position selenium derivatization retains the native C3'-*endo* conformation of A-Form DNA and RNA molecules, this 2'-selenium label approach is suitable for RNA and A-Form DNA derivatization for X-ray crystallography. So far, our progress in this project of selenium derivation on nucleic acids has demonstrated for the first time a new strategy to covalently derivatize nucleotides with selenium for phase and structure determination in X-ray crystallography, and have shown that the MAD phasing technique works with this selenium derivatization strategy.

As one selenium atom enables phase determination for RNAs or DNAs up to 30 nt. (based on an approximate calculation of X-ray phasing power) and the frequency of a building block in a nucleotide sequence is high (25% average rate), selenium-labeled dU (mimic of T) and U building blocks should meet most of the needs for DNA and RNA derivatization. Here we present you a product that can serve as both selenium-labeled dU and U building blocks for RNA and DNA Derivatization in X-ray Crystallography.

FIGURE : **STRUCTURE OF 2'-Se-Me-U**



2'-Se-Me-U

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
2'-Se-Me-U-CE Phosphoramidite	10-3730-95	50 µm	175.00
	10-3730-90	100 µm	350.00
	10-3730-02	0.25g	975.00

References:

1. Nicolas Carrasco, Dov Ginsburg, Quan Du, and Zhen Huang, "Synthesis of Selenium-Derivatized Nucleosides and Oligonucleotides for X-ray Crystallography", *Nucleosides, Nucleotides, & Nucleic Acids*, **2001**, Vol. *20*, issue 9, 1723-1734.

2. Quan Du, Nicolas Carrasco, Marianna Teplova, Christopher J. Wilds, Martin Egli, and Zhen Huang, "Internal Derivatization of Oligonucleotides with Selenium for X-ray Crystallography Using MAD", J*ournal of American Chemical Society*, **2002**, vol. *124*, No. 1, 24-25.

3. Marianna Teplova, Christopher J. Wilds, Quan Du, Nicolas Carrasco, Zhen Huang, and Martin Egli, "Covalent Incorporation of Selenium into Oligonucleotides for X-ray Crystal Structure Determination via MAD: Proof of Principle", *Biochimie*, **2002**, vol. *84*, No. 9, 849-858.

(Continued from Page 2)

pyrrolo-dC codes as dC in PCR experiments. And very preliminary evidence indicates that pyrrolo-dC triphosphate is incorporated efficiently by Taq polymerase and is incorporated specifically opposite dG.

We are happy to introduce pyrrolo-dCTP and look forward to its use in biological assay development.

The pyrrolo-dC project is a joint development by Berry and Associates (http://www.berryassoc.com) and Glen Research. Patents covering this modified fluorescent base and its uses are currently pending.

References

(1) S.C. Srivastava, S.K. Raza, and R. Misra, *Nucleic Acids Res*, 1994, **22**, 1296-304.
(2) M.E. Hawkins, W. Pfleiderer, O. Jungmann, and F.M. Balis, *Anal Biochem*, 2001, **298**, 231-240.
(3) M.E. Hawkins, W. Pfleiderer, F.M. Balis, D. Porter, and J.R. Knutson, *Anal Biochem*, 1997, **244**, 86-95.
(4) S.L. Driscoll, M.E. Hawkins, F.M. Balis, W. Pfleiderer, and W.R. Laws, *Biophys J*, 1997, **73**, 3277-86.

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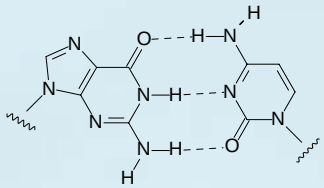
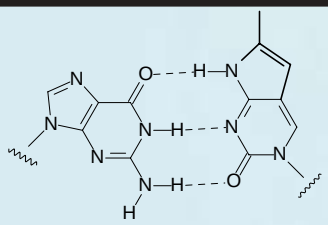
FIGURE 3: **BASE PAIRS G - C AND PYRROLO-C - G**



G ..... C Base Pair



G ..... Pyrrolo-C Base Pair

(5) R. Charubala, et al., *Nucleos Nucleot*, 1997, **16**, 1369-1378.
(6) J.M. Jean and K.B. Hall, *Proc Natl Acad Sci U S A*, 2001, **98**, 37-41.
(7) J. Woo, R.B. Meyer, Jr., and H.B. Gamper, *Nucleic Acids Res*, 1996, **24**, 2470-5.
(8) C. Liu and C.T. Martin, *J Mol Biol*, 2001, **308**, 465-75.
(9) C. Liu and C.T. Martin, *J Biol Chem*, 2002, **277**, 2725-31.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Pyrrolo-dC-CE Phosphoramidite	10-1017-95	50 µm	110.00
	10-1017-90	100 µm	220.00
	10-1017-02	0.25g	675.00
Pyrrolo-C-TOM-CE Phosphoramidite	10-3017-95	50 µm	212.50
	10-3017-90	100 µm	425.00
	10-3017-02	0.25g	975.00
Pyrrolo-dCTP (10 mM)	81-1017-01	100 µL	150.00

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