VOLUME 11
NUMBER 2
DECEMBER 1998

ADVANCES IN RNA SYNTHESIS AND STRUCTURAL ANALYSIS

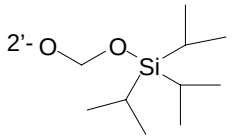
RNA Synthesis

In March 1991, we reviewed[1] the state of RNA synthesis and concluded that the monomers we offered at that time, with 2′-O-t-butyldimethylsilyl (TBDMS) and labile base protecting groups, were close to being optimal. In the intervening years, we have continued to supply these monomers and some incremental improvements have been made, including the use of ethylthiotetrazole[2, 3] as a more efficient activator, acetyl protected C monomer along with methylamine[2, 4] for deprotection, and triethylamine trihydrofluoride[5] for desilylation. With these modifications, RNA synthesis is currently very healthy but the process is still not as dependable or as routine as DNA synthesis.

However, two recent innovations promise to push RNA synthesis to new levels, as well as introduce two new acronyms, ACE and TOM, to the field.

The key to a new synthesis strategy[6] is the use of 2′-O-bis(2-acetoxyethoxy)methyl (ACE) orthoester protection which requires substitution of DMT with silyl ethers for 5′ protection. Although substantial cycle and reagent changes are required in this strategy, its great advantage is the stability of the 2′-O-protected RNA which can be purified and stored. The 2′-protection is then efficiently removed by incubation in aqueous buffers. This strategy holds distinct promise and we look forward to the further optimization and commercialization of this chemistry.

Although the TBDMS group has served well for 2′ protection over many years, a deceptively simple change to 2′-O-triisopropylsilyloxymethyl (TOM)[7] protection offers very significant advantages which are detailed in an account from Xeragon AG beginning on Page 2. Glen Research has contracted with Xeragon to supply TOM RNA monomers exclusively worldwide.



TOM-Protecting-Group™

NAIM

In addition to these recent developments in the field of RNA synthesis, a powerful and elegant new technique is now available for probing the structure and function of RNA molecules. Nucleotide Analog Interference Mapping[8] (NAIM) allows one to probe the effect of substituting an analog for a particular nucleotide in all positions within an RNA molecule simultaneously. The NAIM process is reviewed in detail by Scott Strobel beginning on Page 6.

NAIM is a radical departure from the conventional means of determining the contribution of a functional group to the activity of an RNA. Current techniques have relied upon exhaustive analysis of a series of substituted RNAs prepared by either chemical[9, 10] or semi-synthetic[11] methods. Rather than using such a brute force approach, NAIM utilizes a combinatorial strategy in which a population of substituted

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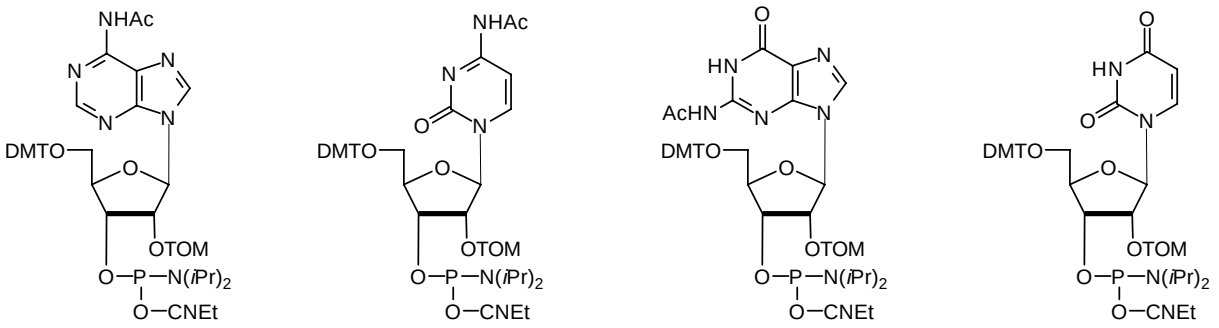
RNA Synthesis
Expedite Columns
NAIM
Expedite Alternatives
Novel Monomers

TOM-PROTECTING-GROUP™ - A MAJOR IMPROVEMENT IN RNA SYNTHESIS

Patrick Weiss, Xeragon AG

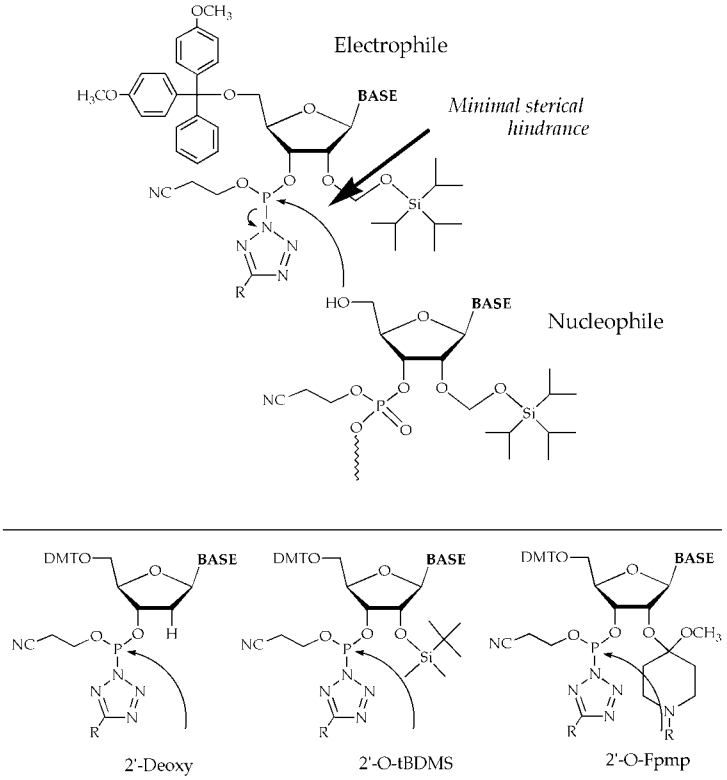
Xeragon AG and Glen Research Corporation introduce a novel and superior 2'-O-protecting group for automated RNA-synthesis, the TOM-Protecting-Group™ (patents pending):

FIGURE 1: STRUCTURE OF TOM-RNA MONOMERS



The TOM-Protecting-Group™ is structurally related to the, so far, most successful tBDMS-Group introduced by *Ogilvie and Usman* in the early Eighties and is fully compatible to the established tBDMS-Chemistry used worldwide. This compatibility has the advantage that one can still use all known and already available modifications. Amidites, shown in Figure 1, containing the TOM-Protecting-Group™ are characterized by the features illustrated in Figures 2-5.

FIGURE 2: HIGH PERFORMANCE DUE TO MINIMIZED STERIC DEMAND



Coupling Yields in [%] (Detritylation Assay)

Nucleophile \ Electrophile	A	C	G	U
A	99.6	99.1	99.5	99.5
C	99.0	99.3	99.5	99.1
G	99.1	98.4	99.9	98.4
U	99.5	99.5	99.9	99.9

Conditions (1.5μmol scale):

- 2 min. coupling time
- 6 molequiv. TOM-Phosphoramidites
- 0.25M benzylthiotetrazole

The TOM-Protecting-Group™ solves the problems encountered in automated RNA-synthesis due to the presence of a suitable spacer between the nucleoside and the silyl-group. This minimized steric demand of the TOM-Protecting-Group™ results in excellent coupling yields under DNA-coupling conditions, as illustrated in Figure 2 above.

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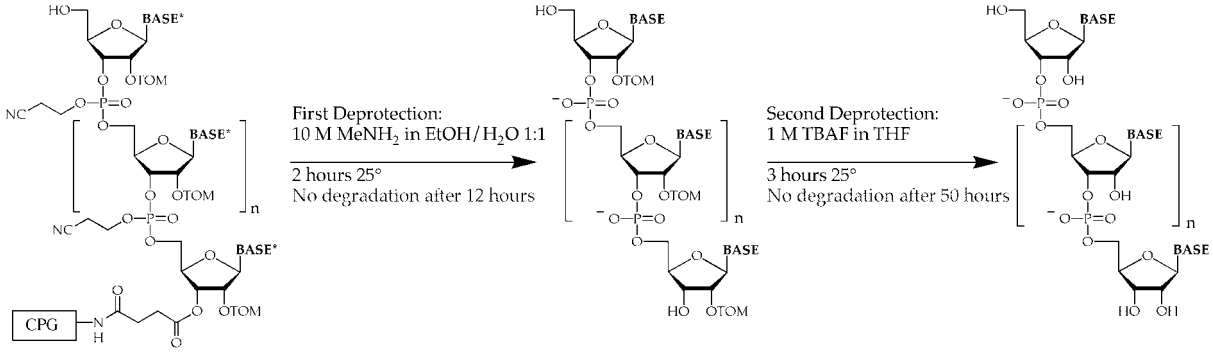
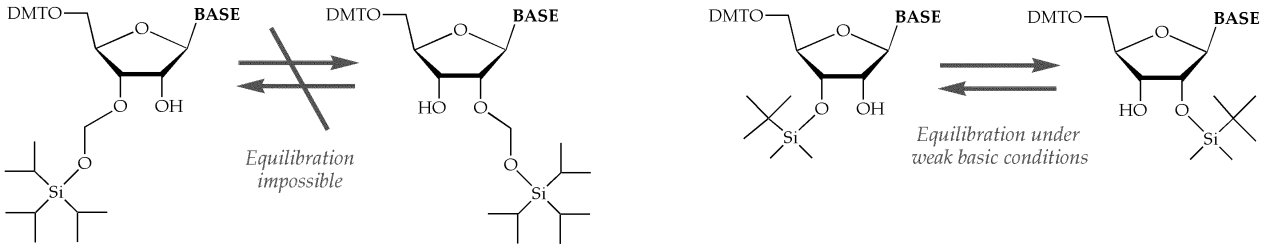
FIGURE 3: FAST AND RELIABLE DEPROTECTION



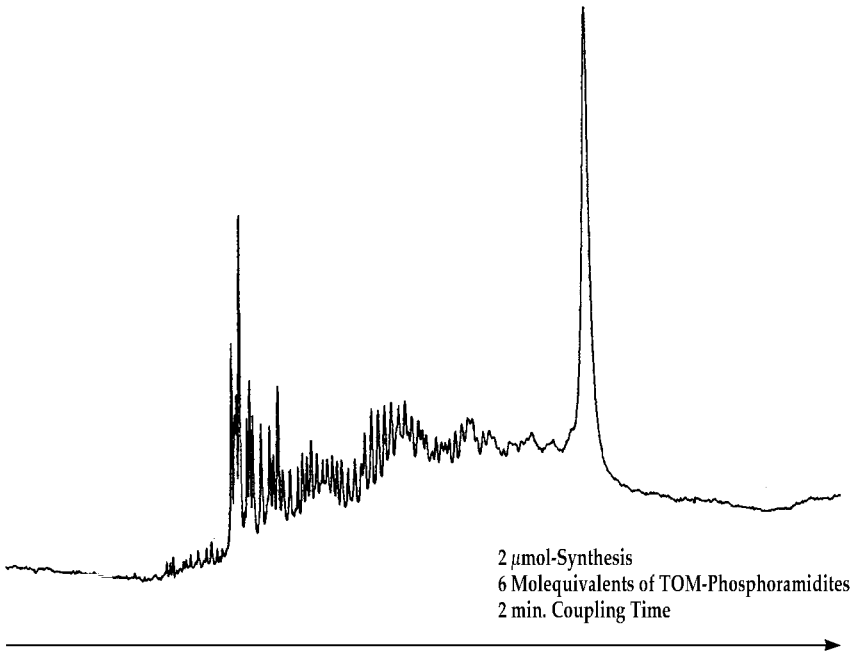
The TOM-protecting group is removed easily and completely under mild conditions leaving the RNA completely intact, as shown in Figure 3. The short deprotection times result in minimized breaking of the strand during deprotection.

FIGURE 4: ABSENCE OF 2'-5' PHOSPHODIESTER LINKAGES IN THE OLIGO



The acetal structure of our TOM-Protecting-Group™ makes it completely stable towards basic and weakly acidic conditions and specifically prevents its migration from 2′-O to 3′-O (which would result in isomeric RNA, containing 2′-5′-phosphodiester linkages).

FIGURE 5: CRUDE PRODUCT OF AN 84mer ON CAPILLARY ELECTROPHORESIS



The synthesis of long and high yielding RNA-Molecules now becomes possible, as illustrated above.

(Continued on Page 4)

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Conclusion

The TOM-Protecting-Group™ is fully compatible with the established RNA-Chemistry, having the advantage of higher coupling yields and shorter coupling times. By making the TOM-Chemistry™ available to a large number of people, we hope to contribute to the field of RNA research and related areas.

References:

On the TOM-Protecting-Group™:

Stefan Pitsch, Patrick A. Weiss and Luzi Jenny, "Ribonucleoside-Derivative and Method for Preparing the Same", Swiss Patent Application 01 931/97, August 18, 1997.

Stefan Pitsch, Xiaolin Wu, Patrick A. Weiss and Luzi Jenny, 1998, in Brow, D., Gesteland, R., Krämer, A. and Pyle A. (eds), RNA '98: The Third Annual Meeting of the RNA Society, Program & Abstract, University of Wisconsin, p. 554.

Stefan Pitsch, Patrick A. Weiss, Xiaolin Wu and Luzi Jenny, "Easy and Efficient Chemical Synthesis of Oligoribonucleotides (RNA) from 2'-O-(Triisopropyl)silyloxymethyl-protected Nucleoside Building Blocks", *in preparation*.

Stefan Pitsch, Patrick A. Weiss, Xiaolin Wu, Damian Ackermann and Thomas Honegger, "Fast and Reliable Automated Synthesis of Oligoribonucleotides Based on Two Novel Orthogonal Protecting Groups for the 2'-O-Position of the Nucleosides", *in preparation*.

Using the TOM-Protecting-Group™:

Xiaolin Wu and Stefan Pitsch, "Synthesis and Pairing Properties of Oligoribonucleotide Analogues Containing a Metal-Binding Site Attached to b-D-Allofuranosyl Cytosine", 1998, *Nucleic Acids Res.*, **26**, 4315-4323.

Martin Huenges, Christian Rölz, Ruth Gschwind, Ralph Peteranderl, Fabian Berglechner, Gerald Richter, Adelbert Bacher, Horst Kessler and Gerd Gemmecker, "Solution Structure of the Antitermination Protein NusB of Escherichia Coli: A Novel All-Helical Fold for an RNA-Binding Protein", 1998, *EMBO J.*, **17**, 4092-4100.

Ronald Micura, "Cyclic Oligoribonucleotides (RNA) by Solid-Phase Synthesis", *in preparation*.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
A-TOM-CE Phosphoramidite	10-3004-02	0.25g	75.00
	10-3004-05	0.5g	150.00
	10-3004-10	1.0g	275.00
C-TOM-CE Phosphoramidite	10-3014-02	0.25g	75.00
	10-3014-05	0.5g	150.00
	10-3014-10	1.0g	275.00
G-TOM-CE Phosphoramidite	10-3024-02	0.25g	75.00
	10-3024-05	0.5g	150.00
	10-3024-10	1.0g	275.00
U-TOM-CE Phosphoramidite	10-3034-02	0.25g	75.00
	10-3034-05	0.5g	150.00
	10-3034-10	1.0g	275.00
A-TOM-RNA 1000	20-3304-02	0.25g	95.00
1 µmole columns	20-3404-41	Pack of 4	100.00
0.2 µmole columns	20-3404-42	Pack of 4	75.00
10 µmole column (ABI)	20-3404-13	Pack of 1	225.00
15 µmole column (Biosearch)	20-3404-14	Pack of 1	300.00
C-TOM-RNA 1000	20-3314-02	0.25g	95.00
1 µmole columns	20-3414-41	Pack of 4	100.00
0.2 µmole columns	20-3414-42	Pack of 4	75.00
10 µmole column (ABI)	20-3414-13	Pack of 1	225.00
15 µmole column (Biosearch)	20-3414-14	Pack of 1	300.00
G-TOM-RNA 1000	20-3324-02	0.25g	95.00
1 µmole columns	20-3424-41	Pack of 4	100.00
0.2 µmole columns	20-3424-42	Pack of 4	75.00
10 µmole column (ABI)	20-3424-13	Pack of 1	225.00
15 µmole column (Biosearch)	20-3424-14	Pack of 1	300.00
U-TOM-RNA 1000	20-3334-02	0.25g	95.00
1 µmole columns	20-3434-41	Pack of 4	100.00
0.2 µmole columns	20-3434-42	Pack of 4	75.00
10 µmole column (ABI)	20-3434-13	Pack of 1	225.00
15 µmole column (Biosearch)	20-3434-14	Pack of 1	300.00

Related Publications:

Pitsch S., "An Efficient Synthesis of Enantiomeric Ribonucleic Acids from D-Glucose", 1997, *Helv. Chim. Acta*, **80**, 2286-2314.

NEW COLUMNS FOR EXPEDITE AND LV APPLICATIONS

Expedite Synthesizer

PE Biosystems has announced that the Expedite 8909 synthesizer and the 16-column add-on MOSS unit will continue to be produced and enhanced. The Expedite synthesizer currently uses Biosearch crimped columns which have not changed since the mid-1980s, despite some clear design shortcomings:

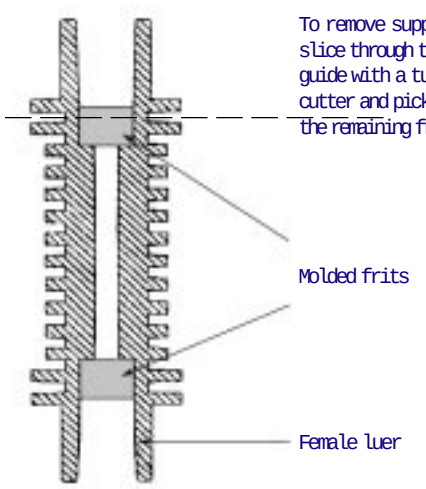
- The sealing surfaces are small, making occasional liquid leaks almost inevitable.
- The polypropylene fabric filter at each end may be a little frayed and a stray fiber could cause very occasional leaks of powder.
- The aluminum crimped seals may relax with time, compromising the seal.
- The crimps are difficult to remove when necessary.

Radical improvements are clearly overdue and we are happy to present the Glen Research version of the Expedite column.

- A single-piece body removes the need for seals and guarantees that liquid leaks can not occur.
- Polyethylene frits are precision-molded for accurate fit to ensure that no powder leaks can occur and for reproducible porosity to give accurate flows.
- For those researchers who wish to open the column, guide rings are provided to slice the end off the column with a tubing cutter (Cole Palmer Part No. 06438-90), allowing access to the frit for easy removal.

Our testing of these columns over many syntheses has confirmed that their performance is at least equal to that of the previous columns while column to column reproducibility is considerably improved. Both 1 µmole and 0.2 µmole/ 40 nmole versions are available in the new format.

FIGURE 1: 0.2 MICROMOLE AND LV COLUMN



Low Volume (LV) Columns

The Expedite synthesizer is extremely frugal in its use of reagents, enjoying a well-deserved reputation for providing low-cost oligos. This situation allows efficient use of monomers, especially expensive, unusual bases. In addition to use on the Expedite, testing of the new columns on ABI 394 synthesizers has allowed us to recommend the use of the 0.2 µmole and 40 nmole columns on the LV200 and LV40 cycles, respectively. It has been our conclusion for some time that the best synthesis cycle is the LV200 cycle and we are happy to now offer our unusual supports in columns which allow use of this cycle.

Catalog Numbers

Effective immediately, our regular Biosearch columns, shown on Page 7 of the 1998 catalog, will be replaced with the new columns. Catalog numbers and pricing of the regular support columns will remain the same. Pricing remains the same for empty columns and replacement frits. New catalog numbers are shown below:

For accurate ordering of unusual supports in these new columns, we will add the letter E (for Expedite) to the regular catalog numbers. For example, 1 µmole and 0.2 µmole dabcyl columns would have catalog numbers as shown:

Pack of four 3'-Dabcyl-CPG columns (1µm) 20-5911-41E
Pack of four 3'-Dabcyl-CPG columns (0.2µm) 20-5911-42E

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Empty Synthesis Columns (40nm, 0.2µm)	20-0021-02	10	48.00
Empty Synthesis Columns (1µm)	20-0021-01	10	48.00
Replacement Filters (40nm, 0.2 or 1µm)	20-0021-0F	20	20.00

NUCLEOTIDE ANALOGS FOR INTERFERENCE MAPPING OF RNA

Scott Strobel
Dept. of Molecular Biophysics and Biochemistry, Yale University

Introduction

Single-site nucleotide analog substitution has been a commonly used approach for gaining atomic resolution biochemical information about RNA function. In this approach, a specific chemical group is modified or deleted at a defined position within an RNA prepared by solid phase chemical synthesis. The oligoribonucleotide is then assayed for its ability to perform the biochemical function of the native molecule. Although a powerful method, it is limited by the technical difficulty of synthesizing and characterizing a series of singly substituted RNAs. This is particularly true of longer RNA molecules (>40 nucleotides), which cannot be routinely synthesized as a single oligoribonucleotide. Here we describe an alternate approach to rapidly screen *in vitro* transcribed RNAs for biochemical effects resulting from functional group modifications.

Interference Mapping

Nucleotide Analog Interference Mapping (NAIM) is a chemogenetic approach that makes it possible to simultaneously, yet individually, probe the contribution of a particular functional group at almost every RNA nucleotide position in a single experiment[1]. The method utilizes a series of 5'-*O*-(1-thio)nucleoside analog triphosphates in a modification interference procedure that is as simple as RNA sequencing. In a NAIM experiment the smallest mutable unit is not the base pair, but rather the individual functional groups that comprise the nucleotides. Because the modification or deletion of a particular functional group within an RNA can severely affect its activity, this approach makes it possible to efficiently determine the chemical basis of RNA structure and function.

Instead of synthesizing a series of RNAs with chemical substitutions at specific sites, NAIM utilizes a combinatorial approach. Each nucleotide analog is prepared as a triphosphate for incorporation into the RNA during DNA templated *in vitro* transcription. The nucleotide analogs used in NAIM include a specific chemical alternation to the base or sugar, and an α-phosphorothioate substitution which serves as a chemical tag. The nucleotide analog triphosphate is randomly incorporated into an RNA transcript,

FIGURE 1: DIAGRAMATIC REPRESENTATION OF A NAIM EXPERIMENT

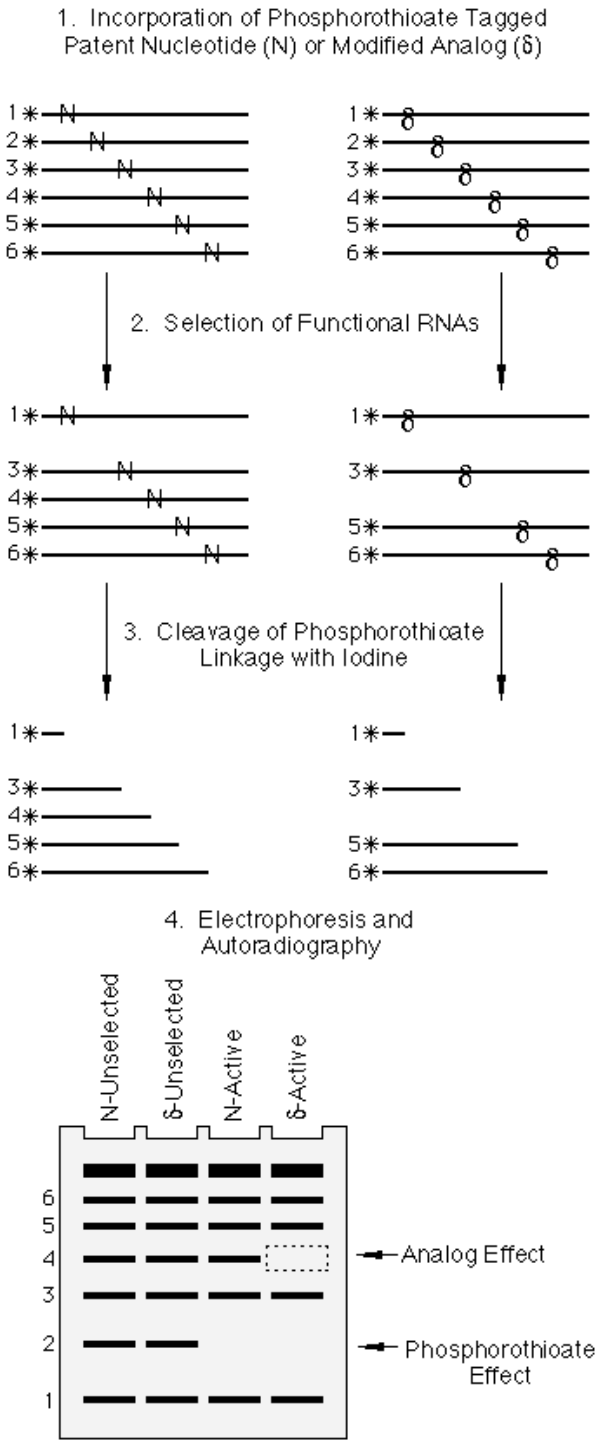


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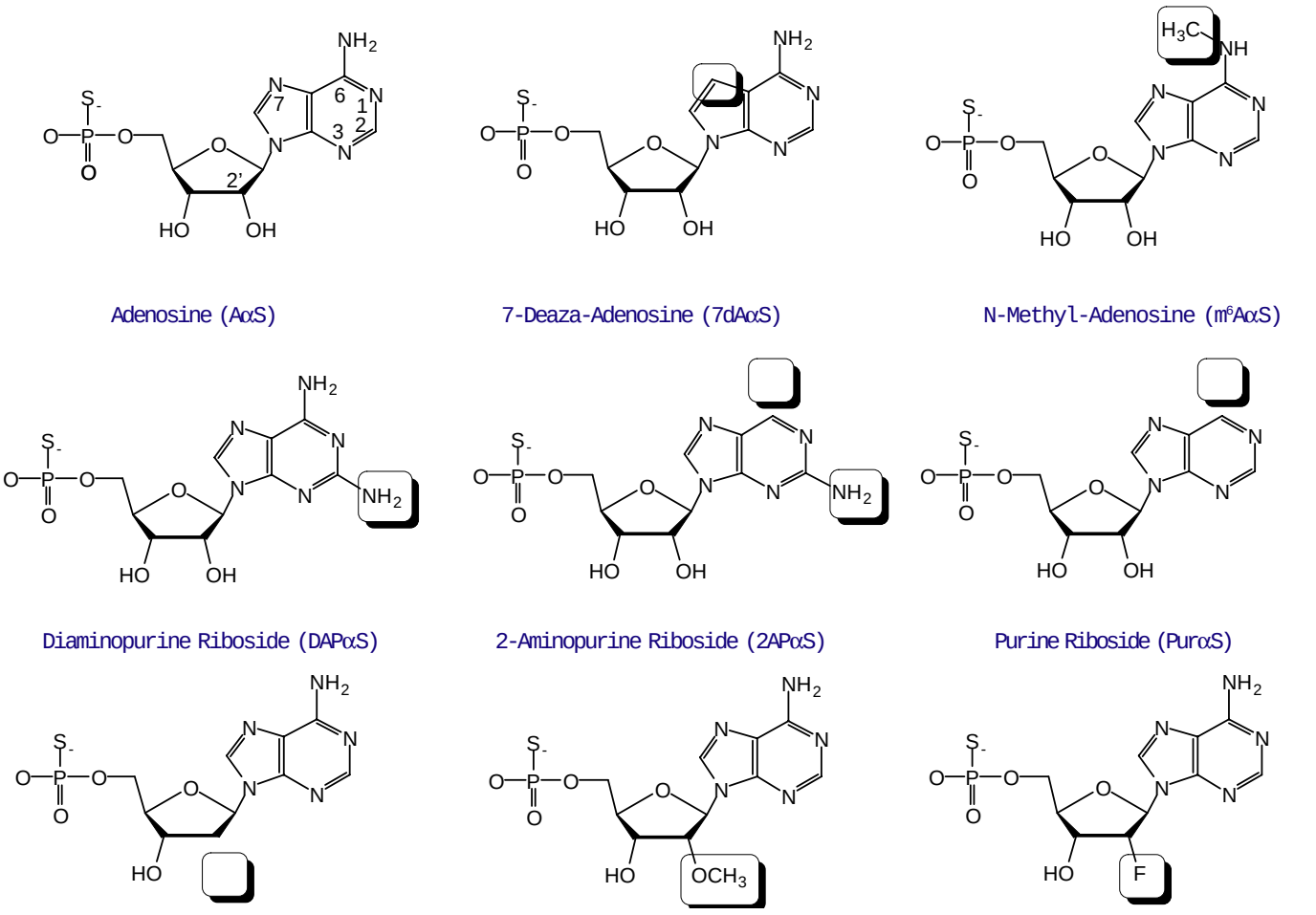
FIGURE 2: STRUCTURES OF ADENOSINE AND ANALOG α-THIOTRIPHOSPHATES



(Continued from Page 6)

where the phosphorothioate linkage can be selectively cleaved by the addition of I_2 to produce a series of RNA cleavage products whose lengths correspond to the sites of analog incorporation[2]. By radioactively or fluorescently tagging one end of the RNA transcript, cleaving the RNA with I_2, and resolving the cleavage products on a denaturing polyacrylamide gel, the sites of analog incorporation throughout the RNA can be individually assayed and used for interference analysis. The phosphorothioate tagged nucleotide analogs make it possible for all of the positions in the RNA to be assayed individually for functional group modification in a single experiment.

Because the phosphorothioate chemical tag is independent of the nucleotide analog whose location it reports, NAIM is generalizable to any analog that can be incorporated into a transcript by an RNA polymerase. A typical NAIM experiment is comprised of four steps (Fig. 1). (i) The phosphorothioate tagged nucleotide analog is randomly incorporated throughout the RNA to create a family of transcripts, each of which contains only a few substitutions. A different transcription reaction is performed for each analog. (ii) The functional RNA variants in the population are separated from the inactive transcripts. The exact nature of the activity assay is specific for the RNA being studied, but could include affinity chromatography, native gel electrophoresis, filter binding, selective radiolabeling, etc. (iii) The phosphorothioate linkages in the active and unselected RNA populations are cleaved by I_2 addition to mark the sites of analog incorporation within each molecule. (iv) The individual RNA fragments are resolved by gel electrophoresis and visualized by autoradiography. Sites of analog substitution that are detrimental to function are scored as gaps in the sequencing ladder among the active RNA variants (Fig. 1). Because every position in the sequence is a unique and independent band on the sequencing gel, a single screen can define the effect a particular analog has at every

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incorporated position within the RNA. The approach is applicable to any RNA that can be transcribed *in vitro* and has an assayable function that can be used to distinguish active and inactive variants. RNA functions that are amenable to this approach include catalysis, folding, protein or ligand binding, and the ability to act as a reaction substrate.

Nucleotide Analogs

NAIM utilizes α-phosphorothioate tagged nucleotide analogs, each of which includes an incremental chemical alteration in the base or ribose sugar. The most completely developed set of analogs are those of adenosine, for which eight different analogs have been utilized in NAIM (Fig. 2)[3]. Five analogs modify the nucleotide base and three modify the ribose sugar. The base analogs include purine riboside (PuroαS), N6-methyladenosine (m^6AoαS), tubercidin (7dAoαS), diaminopurine riboside (DAPoαS), and 2-aminopurine riboside (2APoαS). The ribose sugar analogs all modify the 2'-OH group and include 2'-deoxyadenosine (dAoαS), 2'-deoxy-2'-fluoroadenosine (^{F}AoαS), and 2'-*O*-methyladenosine (^{OMe}AoαS). All of the analogs can be randomly incorporated into an RNA transcript at an ideal 5% level of efficiency using either the wild-type T7 RNA polymerase or a Y639F RNA polymerase point mutant[4]. Each of these analogs provides specific information about the chemical basis of RNA activity at almost every incorporated position in the transcript. A similar collection of analogs can be utilized for the other three nucleotides G, C, and U.

PuroαS, 2APoαS and m^6AoαS measure the effect of modifications to the N6 exocyclic amine of adenosine. PuroαS and 2APoαS delete the amine, and m^6AoαS replaces one proton of the amine with a methyl group. m^6AoαS interference indicates that either both hydrogen atoms of the amine are necessary, or that there is insufficient space in the local structure to accommodate the additional methyl group. PuroαS and 2APoαS interference identifies sites where the amine is important for activity.

Interference with 7dAoαS is indicative of an important major groove contact to the ring nitrogen, as this nucleotide replaces the N7 nitrogen with a CH group. Interference with PuroαS, m^6AoαS, and 7dAoαS are strong indicators of Hoogsteen hydrogen bonding.

DAPoαS and 2APoαS both add an additional amine to the C2 position of adenosine. In general, DAPoαS and 2APoαS show interference in areas of close packing in the minor groove of RNA. Another characteristic effect observed with DAPoαS is enhancement of activity when paired with a U in regions of the molecule where duplex stability is important for function. ^{OMe}AoαS also serves as a probe for tight packing in the minor groove.

dAoαS interference identifies the 2'-OH groups important for RNA function, while ^{F}AoαS delineates the role these 2'-OH groups play as either hydrogen bond donor or hydrogen bond acceptors. If a 2'-OH shows interference with both analogs, it suggests that the 2'-OH is a hydrogen bond donor. If instead the position has dAoαS, but not ^{F}AoαS interference, it argues that the 2'-OH at this site is a hydrogen bond acceptor.

In a few cases ^{F}AoαS interference can also provide indirect information about the conformation of the ribose sugar for a given nucleotide. ^{F}AoαS interference at sites lacking dAoαS interference suggests that the 2'-OH does not make a direct contribution to activity. Instead, it argues that there is an indirect effect due to the chemical nature of the fluorine substitution. The 2'-fluoro group is highly electronegative, and as such, the ribose sugar favors the C3'-endo conformation[5]. This indirect effect is consistent with sites of C2'-endo ribose sugar conformation in a defined RNA system[3], and may provide a biochemical signature for sites with unusual sugar pucker in other RNAs.

As our initial entry into this new collection of reagents, we are glad to offer a set of the adenosine analogs shown in Fig. 2. The collection will be expanded in the future to include a series of analogs for each of the nucleotides. We expect these molecules will become powerful reagents for the identification of the chemical groups important for a wide variety of RNA and DNA functions.

References:

1. S. A. Strobel and K. Shetty, *Proc. Natl. Acad. Sci. U.S.A.*, 1997, **94,** 2903-2908.
2. G. Gish and F. Eckstein, *Science*, 1988, **240,** 1520-1522.
3. L. Ortoleva-Donnelly, A. A. Szewczak, R. R. Gutell and S. A. Strobel, *RNA*, 1998, **4,** 498-519.
4. R. Sousa and R. Padilla, *EMBO J.*, 1995, **14,** 4609-4621.
5. S. Uesugi, H. Miki, M. Ikehara, H. Iwahashi and Y. Kyogoku, *Tetrahed. Lett.*, 1979, **42,** 4073-4076.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Adenosine α-thiotriphosphate (0.5mM)	80-3000-01	100 µL	75.00
7-Deaza-Adenosine α-thiotriphosphate (10mM)	80-3303-01	100 µL	75.00
N6-Me-Adenosine α-thiotriphosphate (4mM)	80-3302-01	100 µL	75.00
Diaminopurine riboside α-thiotriphosphate (0.25mM)	80-3305-01	100 µL	75.00
2-Aminopurine riboside α-thiotriphosphate (20mM)	80-3304-01	100 µL	75.00
Purine riboside α-thiotriphosphate (20mM)	80-3301-01	100 µL	75.00
2'-deoxyAdenosine α-thiotriphosphate (15mM)	80-1000-01	100 µL	75.00
2'-OMe-Adenosine α-thiotriphosphate (20mM)	80-1101-01	100 µL	75.00
2'-Fluoro-Adenosine α-thiotriphosphate (10mM)	80-1102-01	100 µL	75.00

α-Thiotriphosphates are sodium salts in TE buffer, pH7, 10X concentrates. The concentrations shown are optimal for incorporation during polymerase reactions.

ALTERNATIVES TO EXPEDITE MONOMERS

Expedite Monomers

The monomers protected with t-butyl-phenoxyacetyl groups (Expedite monomers) have proved to be popular and are used predominantly for the two applications covered below. With their very base-labile protecting groups, oligonucleotides produced using Expedite monomers can be deprotected under mild conditions using ammonium hydroxide for 2 hours at room temperature, or rapidly in 15 minutes at 55°C. Glen Research supplies systems that are equivalent or superior in performance, as described below.

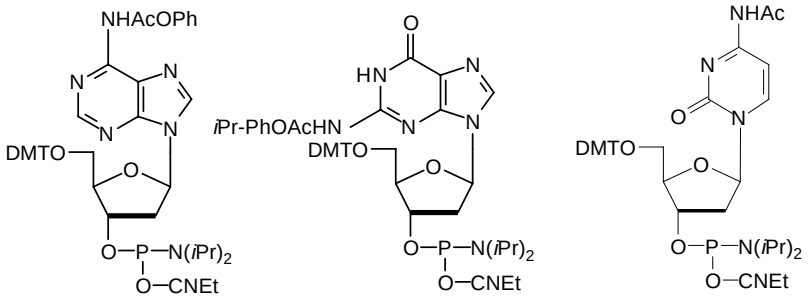
UltraMILD Deprotection

The synthesis of labelled oligonucleotides has become a standard procedure in many laboratories and many labelling reagents are now available as phosphoramidites. In some instances the labelling reagents are not stable to the alkaline conditions required for removal of the base protecting groups on the standard dA, dC and dG monomers. Glen Research is pleased to offer a set of monomers using phenoxyacetyl (Pac) protected dA, 4-isopropyl-phenoxyacetyl (iPr-Pac) protected dG, and acetyl (Ac) protected dC. These monomers can be used with sensitive labelling reagents such as TAMRA, Cy5 and HEX since cleavage and deprotection can be carried out in 2 hours at room temperature with ammonium hydroxide or 0.05M potassium carbonate in anhydrous methanol. For additional ordering information, see Page 32 of the 1998 Catalog under UltraMILD Synthesis.

UltraFAST Deprotection

The Glen Research line of reagents for DNA synthesis includes Ac-dC-CE Phosphoramidite and the corresponding Ac-dC support, the basis for the UltraFAST cleavage and deprotection system that allows 10 minute deprotection of oligonucleotides. This system requires that the normal benzoyl (Bz) protection of the dC monomer be replaced with acetyl (Ac). The three other monomers remain unchanged.

FIGURE 1: MONOMER STRUCTURES



This seemingly minor change in protecting group leads to oligonucleotides which can be cleaved and deprotected in 10 minutes using AMA which is a 50:50 mixture of aqueous **A**mmonium hydroxide and aqueous **M**ethyl**A**mine. With AMA the cleavage of the oligonucleotide from the support is accomplished in 5 minutes at room temperature. The deprotection step is carried out at 65°C for a further 5 minutes. Deprotection can also be carried out at lower temperatures as follows. In all cases, no base modification has been observed.

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

Further details of UltraFAST cleavage and deprotection are on Page 3 of the 1998 catalog.

Please note the new lower prices for the UltraMILD dA and dG monomers.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Pac-dA-CE Phosphoramidite	10-1601-02	0.25g	15.00
	10-1601-05	0.5g	30.00
	10-1601-10	1.0g	60.00
Ac-dC-CE Phosphoramidite	10-1015-02	0.25g	12.50
	10-1015-05	0.5g	25.00
	10-1015-10	1.0g	50.00
iPr-Pac-dG-CE Phosphoramidite	10-1621-02	0.25g	15.00
	10-1621-05	0.5g	30.00
	10-1621-10	1.0g	60.00

MORE NOVEL MONOMERS - EDTA-C2-dT, 7-Deaza-dX, 2'-DEOXYPSEUDOURIDINE

As always, several new phosphoramidites and supports have made a break from our labs. Some never become products but other more enterprising candidates make it into The Glen Report. Here are several new products that we hope will brighten your research prospects.

EDTA-C2-dT

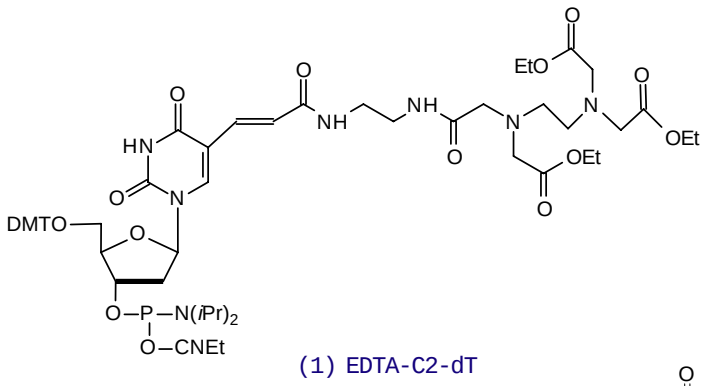
About time too! The Dervan group at Caltech described the cleavage of DNA with oligos containing EDTA-Fe at the C-5 position of Thymidine over 10 years ago.[1] Their phosphoramidite contained the triethyl ester of EDTA and our version is shown, (1) in Figure 1. Coupling of EDTA-dT is normal but cleavage and deprotection should be carried out with sodium hydroxide in aqueous methanol (0.4M NaOH in methanol/water 4:1) overnight at room temperature.

The cleavage reaction is only initiated once Fe(II) and dithiothreitol are added and so is readily controlled. The Dervan group has described sequence-specific cleavage[1] of single-stranded DNA by a probe containing EDTA, as well as cleavage[2] of double-stranded DNA following triplex formation by the EDTA-oligo. A later publication described[3] the application of the latter cleavage reaction to investigate the tertiary structure of RNA.

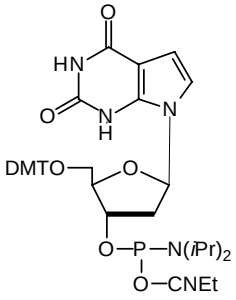
7-Deaza-2'-deoxyXanthosine

As a purine analogue of Thymidine, 7-deaza-2'-deoxyXanthosine (7-deaza-dX) promises to have interesting effects on DNA structure. Previously, 7-deaza-dX has been shown[4] to form stable 7-deaza-X:A-T triplets, allowing triplex formation to occur in the anti-parallel motif. 7-Deaza-dX has also been investigated[5] for its ability to form a non-standard base pair with a 2,4-diaminopyrimidine nucleoside analogue. In both cases, 7-deaza-dX was added to the sequence using H-phosphonate chemistry. We offer the phosphoramidite, as shown, (2) in Figure 1, which we have successfully incorporated into oligonucleotides. In a

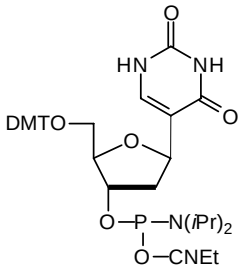
FIGURE 1: STRUCTURES OF NEW PRODUCTS



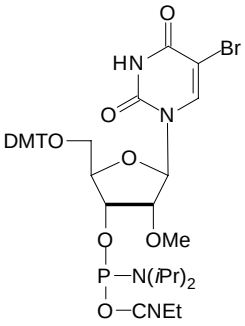
(1) EDTA-C2-dT



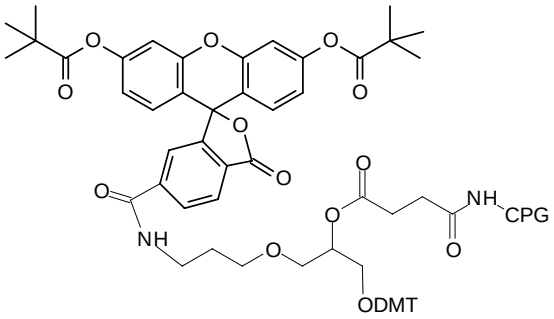
(2) 7-deaza-dX



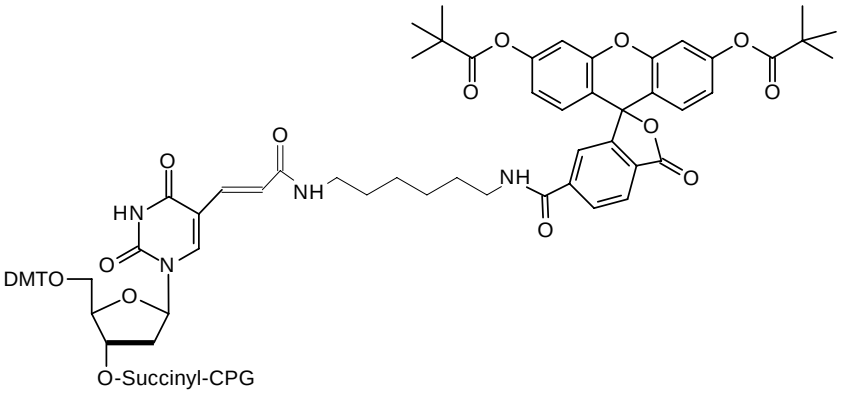
(3) dpseudoU



(4) 2'-OMe-5-Br-U



(5) 3'-(6-FAM) CPG



(6) 3'-Fluorescein-dT CPG

5-Br-2'-OMe-U, 6-FAM CPG, FLUORESCEIN-dT CPG

manner analogous to 7-deaza-dG, 7-deaza-dX requires the use of (10-camphorsulfonyl)oxaziridine (CSO)[6] in place of the regular iodine oxidation if more than two insertions are to be made into an oligonucleotide.

2′-Deoxypseudouridine

Another nucleoside analogue with the potential for affecting DNA structure is the C-nucleoside 2′-deoxypseudo-uridine. Again this analogue has been investigated[7] for its behavior in triple helix formation and it has been shown to form stable triplexes with the 2′-deoxypseudouridine in the second strand (C:pseudoU-A triplets). Triplex formation was not observed in the same system when U was substituted for pseudoU. The phosphoramidite (3), Figure 1, is incorporated into oligonucleotides with no changes from standard procedures.

2′-OMe-5-Bromo-U

5-Bromo-2′-deoxyUridine (Br-dU) is the most actively used halogenated pyrimidine analogue in our catalog. Its uses range from crystallographic studies due to the heavy atom to cross-linking because of its photolability. We are happy to extend this action to the 2′-OMe-RNA series by the addition of 2′-OMe-5-bromo-U phosphoramidite (4) in Figure 1, to our product range.

Fluorescein Supports

Our existing product, 3′-fluorescein CPG, has proved to be very useful for insertion of a fluorescein group at the 3′-terminus of an oligonucleotide. Indeed the use of 3′-fluorescein CPG is probably the simplest way to prepare a fluorescein-labelled oligonucleotide. However, a significant drawback has been the difficulty in producing a really pure oligonucleotide from this support. Several mechanisms, and probably the structure itself, lead to several fluorescein-containing species which in turn lead to multiple peaks on RP HPLC. It is difficult to explain to a customer that several isomers exist in this oligo and all these peaks are "the product". But when researchers moved on to labelling at the 3′ terminus with fluorescein and the 5′ terminus with a second dye, purification became truly messy.

We now offer two fluorescein supports to help clarify the situation. Both are derivatives of 6-carboxyfluorescin (6-FAM) and are single isomers. Both use an amide linkage which is stable during cleavage and deprotection and does not allow isomer formation. 3'-(6-FAM) CPG, (5) in Figure 1, effectively blocks the 3′-terminus from polymerase extension as well as exonuclease digestion, while fluorescein-dT CPG, (6) in Figure 1, allows both of these enzymatic activities to proceed. We believe these two supports are the perfect complements for our existing 5′-fluorescein (6-FAM) phosphoramidite for 5′-terminal labelling and fluorescein-dT for internal labelling.

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ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
EDTA-C2-dT-CE Phosphoramidite	10-1059-95	50 µmole	250.00
	10-1059-90	100 µmole	495.00
	10-1059-02	0.25g	975.00
7-deaza-dX-CE Phosphoramidite	10-1076-95	50 µmole	177.50
	10-1076-90	100 µmole	355.00
	10-1076-02	0.25g	975.00
2'-deoxypseudoU-CE Phosphoramidite	10-1055-95	50 µmole	177.50
	10-1055-90	100 µmole	355.00
	10-1055-02	0.25g	975.00
2'-OMe-5-Br-U-CE Phosphoramidite	10-3190-90	100 µmole	225.00
	10-3190-02	0.25g	675.00
3'-(6-FAM) CPG	20-2961-01	0.1g	120.00
	20-2961-10	1.0g	995.00
1 µmole columns (ABI)	20-2961-41	Pack of 4	200.00
0.2 µmole columns (ABI)	20-2961-42	Pack of 4	120.00
10 µmole column (ABI)	20-2961-13	Pack of 1	300.00
1 µmole columns (Expedite)	20-2961-41E	Pack of 4	200.00
0.2 µmole columns (Expedite)	20-2961-42E	Pack of 4	120.00
15 µmole columns (Expedite)	20-2961-14	Pack of 1	450.00
3'-Fluorescein-dT CPG	20-2056-01	0.1g	120.00
	20-2056-10	1.0g	995.00
1 µmole columns (ABI)	20-2056-41	Pack of 4	200.00
0.2 µmole columns (ABI)	20-2056-42	Pack of 4	120.00
10 µmole column (ABI)	20-2056-13	Pack of 1	300.00
1 µmole columns (Expedite)	20-2056-41E	Pack of 4	200.00
0.2 µmole columns (Expedite)	20-2056-42E	Pack of 4	120.00
15 µmole columns (Expedite)	20-2056-14	Pack of 1	450.00

(Continued from Front Page)

RNAs is generated and analyzed. By using α-thiotriphosphate nucleotide analogs, the sites affected by the substitution, exhibiting either inhibition or enhancement of activity, are determined without anything more esoteric than a sequencing gel. At present, NAIM has been used to refine the structural basis for the activity of *Tetrahymena* group I intron[12, 13, 14], but may be readily extended in any system where a selection process between the active and inactive species is possible including catalysis, protein or ligand interactions, and folding for RNA and even DNA molecules.

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