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5' LABELLING - FLUORESCEIN AND CYANINE DYES, BIOTIN

INTRODUCTION

Traditional radioactive labelling of oligonucleotides is being rapidly supplanted by non-isotopic procedures. Increased awareness of potential operator exposure to radiation, along with the increasing cost of proper disposal of radioactive waste, has clearly mandated some change. At the same time, diagnostic procedures using non-isotopically labelled oligonucleotides can be carried out in a much wider variety of testing laboratories. Currently, non-radioactive labelling is broken into two main segments: labelling using fluorescent tags which are detected directly at their emission wavelength; and labelling using tags like biotin or digoxigenin which are detected indirectly after capture or association with a suitably substituted enzyme or antibody.

Since the majority of oligonucleotides are destined for sequencing and amplification experiments, which require the 3'-terminus to be available for extension, labelling of the 5'-terminus is the most popular technique. While our future development of new labels will concentrate on 5' labelling, it would be imprudent of us to neglect labelling of the 3'-terminus which can be especially useful in probe applications. However, the primary focus of this article is on a series of new 5' labels, including fluorescent and non-fluorescent tags shown in Figure 1.

FLUORESCEIN DYES

Fluorescein labelled oligonucleotides have found applications in DNA sequencing and amplification, as well as techniques for genetic analysis. In the forefront of this development has been our current fluorescein phosphoramidite, Figure 1 (1), which has proved to be a popular, versatile and effective reagent for the preparation of labelled oligonucleotides. Using this product or the related support (2), fluorescein molecules may be placed anywhere within the sequence. Although the branched structure of the spacer allows a degree of versatility, it can cause some minor complications in use. Due to steric hindrance of the phosphoramidite, an increased coupling time of 15 minutes is optimal. Also, purified oligonucleotides labelled with this product exhibit multiple peaks, especially in reverse phase (RP) HPLC, even though the product is prepared from the 5-carboxyfluorescein isomer alone. (Fluorescein's interesting numbering system is detailed in Figure 2.) This multiplicity is the result of diastereomer formation due to the chiral center in the spacer structure, along with the standard pH dependent structural variation of fluorescein as shown in Figure 2. A typical RP HPLC profile of an oligonucleotide labelled with this product is shown in Figure 3. Although not problematical in the usage of the labelled oligonucleotide, a product containing multiple peaks is always worrisome to customers. We are happy to maintain the supply of this product but clearly a fluorescein phosphoramidite with a simpler structure designed only for 5' labelling would be desirable.

And so we introduce the 5'-fluorescein phosphoramidite (3) whose sole role is to label the 5'-terminus during oligonucleotide synthesis. The product contains no 4,4'-dimethoxytrityl (DMT) group and can be added only once at the 5'-terminus, thereby

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DMT-on Phosphorylation

Photolabile Support

Transcription Terminator

2- and 4-Thio-dT

Ethylthiotetrazole

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terminating synthesis. Although this product is prepared using the 6-carboxyfluorescein derivative, its spectral characteristics are identical to those derived from product (1) in Figure 1. 5'-Fluorescein phosphoramidite can be used with the standard cycle of all DNA synthesizers. However, as with all minor bases and most labelling reagents, it will benefit from an extended coupling time of 3 minutes.

To take advantage of the multicolor detection capability of modern DNA sequencers and genetic analyzers, further derivatives of 5'-fluorescein phosphoramidite with shifted absorbance and emission maxima would obviously be of interest. We are therefore happy to introduce the tetrachloro (4) and hexachloro (5) phosphoramidites as the first two in a series of fluorescein analogues. The use of these products is identical to their fluorescein parent, as described above. The spectral characteristics of these dyes are detailed in Table 1 and typical RP HPLC traces are shown in Figure 4.

RHODAMINE DYES

The fluorescein structure is very compatible with oligonucleotide synthesis because it is resistant to hydrolysis with ammonium hydroxide, even when elevated temperatures and extended deprotection times are used.

Unfortunately, the same does not apply to rhodamine derivatives which are not sufficiently stable to survive conventional deprotection. These must be attached to amino-modified oligonucleotides using post-synthesis labelling techniques. Typically, an activated carboxylate, usually N-hydroxysuccinimide (NHS) ester, of the dye in solution in DMF or DMSO is conjugated with the amino-modified oligonucleotide in sodium carbonate/bicarbonate buffer at pH 9. Although this technique is time consuming and places demands on the final purification to remove unconjugated dye, it is nevertheless routine and successful.

We feel that our role at Glen Research is to offer interesting products as phosphoramidites but will offer the most popular rhodamine derivative (tetramethylrhodamine, TAMRA) as an NHS ester (6) as an interim step.

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FIGURE 1: STRUCTURES OF 5'-LABELS

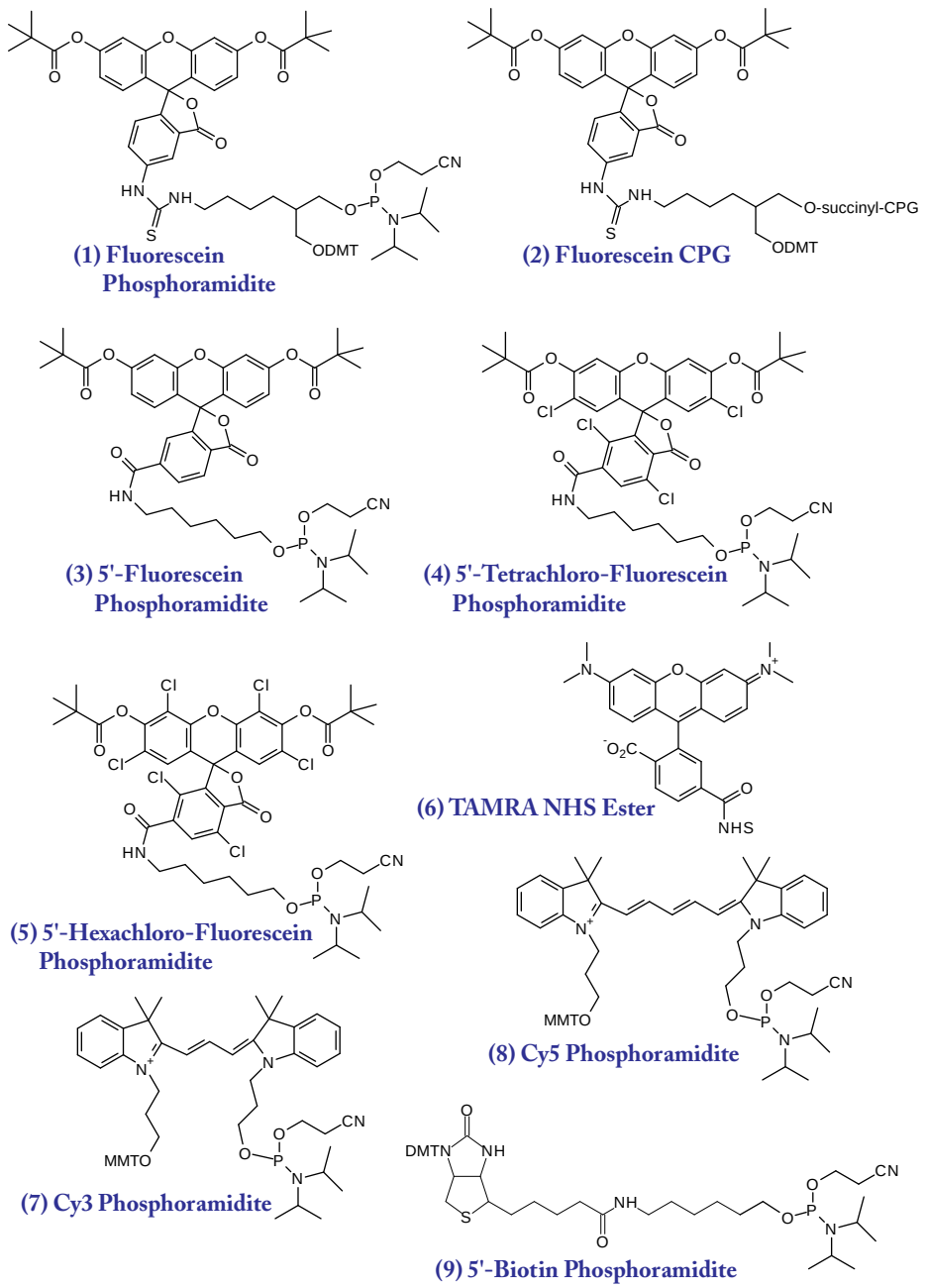

FIGURE 2: FLUORESCEIN NUMBERING

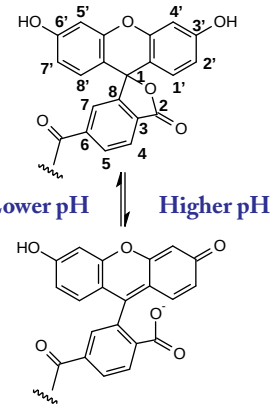

TABLE1: FLUORESCENT DYES

	Absorbance Maximum	*Emission Maximum*	*Color*
Fluorescein	494nm	525nm	Green
Tetrachloro-Fluorescein	521nm	536nm	Orange
Hexachloro-Fluorescein	535nm	556nm	Pink
TAMRA	565nm	580nm	Rose
Cy3	552nm	570nm	Red
Cy5	643nm	667nm	Violet

(Continued from Page 2)

CYANINE DYES

Some of our customers have expressed an interest in labelling with cyanine dyes, as well as admiration for their performance in labelled oligonucleotides. The commercial ownership of these dyes is a story in itself but we are happy to introduce them here (see SMALL PRINT page 4) and hope to maintain supply while stimulating development of new uses for them. The two cyanine derivatives we are introducing are Cy3™ (7) and Cy5™ (8) which differ in structure simply by the number of carbons in the conjugated poly-ene linkage.

Although these products have a 4-monomethoxytrityl (MMT) group and, in principal, could be added in multiple additions, we expect them to be added only once to the 5'-terminus. The MMT group should therefore be removed on the synthesizer. The absorbance of the MMT cation (yellow) is noticeably different from the DMT cation (orange), and so, absorbance-based trityl monitors will detect it incorrectly as a low coupling. On the other hand, conductivity detectors will interpret the release more correctly.

Deprotection of oligos containing Cy3 and Cy5 may be carried out with ammonium hydroxide at room temperature, regardless of the base protecting groups on the monomers used. If there is a need to use ammonium hydroxide at elevated temperature, Cy3 is more stable than Cy5, but it is always prudent to use monomers with base labile protecting groups to limit the exposure time to 2 hours or less at 55°C. Typical RP HPLC traces of Cy3- and Cy5-labelled oligonucleotides are shown in Figure 5.

(Continued on Page 4)

FIGURE 3: FLUORESCEIN OLIGO

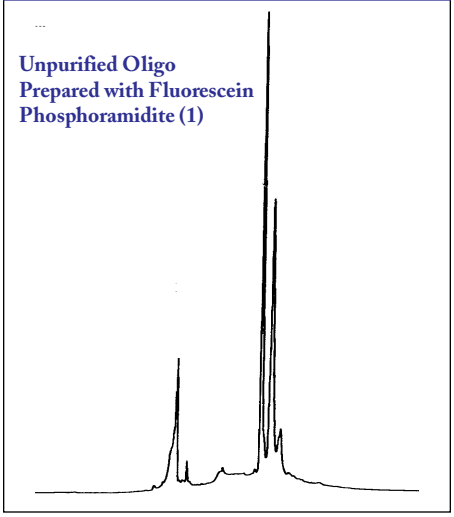

TABLE 2: HPLC CONDITIONS

The following HPLC conditions were used to produce the chromatograms shown on Pages 3,4 and 6:

Column: Spherisorb ODS2 (150X4.6mm)
Detector: UV at 254nm
Flow: 1mL/min
Gradient:

Time	0.1M TEAA(%)	Acetonitrile(%)
0	97	3
5	97	3
20	60	40
30	60	40

FIGURE 4: 5'-FLUORESCEIN DYE LABELLING

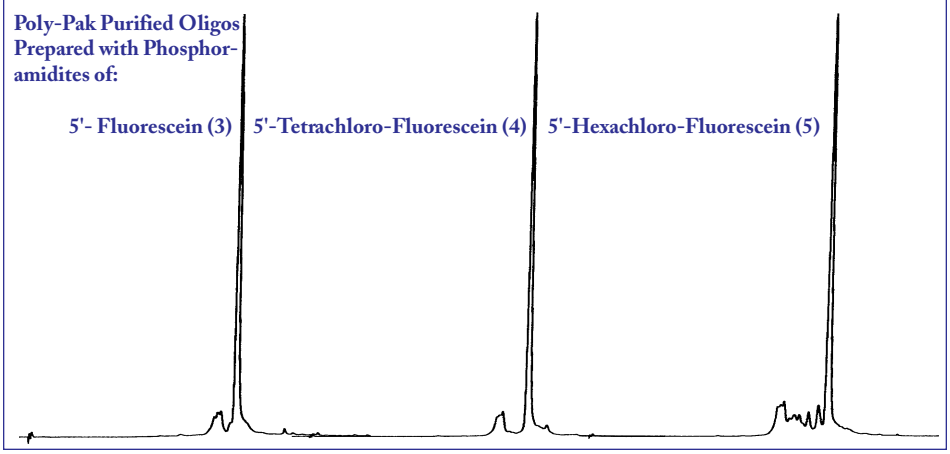

TABLE 3: USES FOR BIOTIN PHOSPHORAMIDITES AND SUPPORT

Biotin Product	Cat. No.	Spacer	Uses
BiotinTEG CPG	20-2955	15 Atom mixed polarity with triethylene glycol	3'-Biotinylation. Use with biotinTEG phosphoramidite to create high sensitivity probes with multiple biotins on the 3'-terminus.
Biotin Phosphoramidite	10-1953	6 Carbon	5'-Biotinylation with simple DMT-on purification. Can be used for inserting multiple biotins at the 3'- or 5'-terminus.
BiotinTEG Phosphoramidite	10-1955	15 Atom mixed polarity with triethylene glycol	5'-Biotinylation with simple DMT-on purification where longer spacer length is required for optimized detection or capture. Can be used for inserting multiple biotins at the 3'- or 5'-terminus.
Biotin dT	10-1038	10 Atom	Biotinylation within the sequence by replacing an existing dT residue. Does not affect hybridization.
5'-Biotin Phosphoramidite	10-5950	6 Atom hydrocarbon	5'-Biotinylation ONLY. Compatible with DMT-on purification techniques.

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5'-BIOTIN

Another biotin phosphoramidite, when Glen already has three such monomers available! Why?

It is fair to say that we have had considerable interest in our new 5'-biotin phosphoramidite (9) for a long time. However, it is covered by patent and, although we could have purchased the product and offered it under license, we were totally dissatisfied with the purity of the available product.

We have now obtained manufacturing rights to this product and we can at last offer this 5'-biotin phosphoramidite under license (see SMALL PRINT). While the product which was available previously clearly performed well, the chromatographic purity was substantially less than 90%. We feel that 90% should be the absolute minimum purity for a quality product and the 5'-biotin phosphoramidite from Glen Research will always meet this specification. Higher purity should translate into constant performance with better lifetime on an instrument once the product is dissolved.

Why has this particular biotin phosphoramidite interested us so much? Its performance features are listed below:

- It is, of course, freely soluble in acetonitrile.
- It can be used with the standard cycle of all instruments (although it will benefit from up to a 3 minute coupling time).
- Because of the short coupling time, it is more robust than our other biotin products with better performance in higher moisture situations and, therefore, has a better lifetime on the synthesizer.
- Because of the DMT group on the biotin, the coupling yield can still be determined on the synthesizer.
- ***CAUTION!*** 5'-Biotin phosphoramidite can be added only once to the 5'-terminus of an oligonucleotide. However, the DMT group on the biotin can be used in RP cartridge and HPLC purification techniques.

For reference, in Table 3 on the previous page, we compare, contrast and suggest usage for our team of biotin labels.

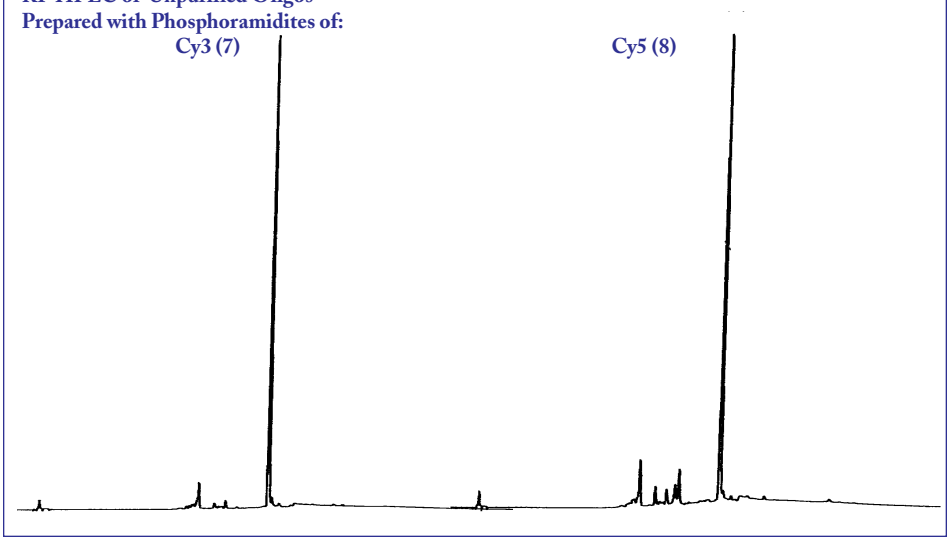
PURIFICATION

All of the products described above are amenable to purification using RP cartridge and HPLC techniques. Poly-Pak procedures are provided with each product on delivery. They are also available for review or download on the worldwide web: http://www.glenres.com/PolyPak.html/PPlabel.html.

SMALL PRINT

Cy3 and Cy5 are trademarks of Biological Detection Systems, Inc..
5'-Biotin Phosphoramidite is sold under license from Zeneca PLC.
Poly-Pak is a trademark of Glen Research Corporation.

FIGURE 5: 5'-CYANINE DYE LABELLING



ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
5'-Fluorescein Phosphoramidite	10-5901-95	50 µmole	165.00
(6-FAM)	10-5901-90	100 µmole	325.00
	10-5901-02	0.25g	875.00
5'-Hexachloro-Fluorescein	10-5902-95	50 µmole	165.00
Phosphoramidite	10-5902-90	100 µmole	325.00
(HEX)	10-5902-02	0.25g	875.00
5'-Tetrachloro-Fluorescein	10-5903-95	50 µmole	165.00
Phosphoramidite	10-5903-90	100 µmole	325.00
(TET)	10-5903-02	0.25g	875.00
TAMRA NHS Ester	50-5910-66	60 µL	200.00
(Solution in anhydrous DMSO)			
Cy3™ Phosphoramidite	10-5913-95	50 µmole	165.00
	10-5913-90	100 µmole	325.00
	10-5913-02	0.25g	875.00
Cy5™ Phosphoramidite	10-5915-95	50 µmole	165.00
	10-5915-90	100 µmole	325.00
	10-5915-02	0.25g	875.00
5'-Biotin Phosphoramidite	10-5950-95	50 µmole	125.00
	10-5950-90	100 µmole	225.00
	10-5950-02	0.25g	650.00

5'-PHOSPHORYLATION - NOW COMPATIBLE WITH DMT-ON PURIFICATION

Oligonucleotides containing a 5'-phosphate group find use in molecular biology for a variety of purposes: e.g., as linkers and adapters, in cloning and gene construction, and in the ligase chain reaction. The venerable T4 polynucleotide kinase has served researchers well by phosphorylating the 5'-terminus using ATP as the phosphate source. A chemical alternative to kinase, the sulfonyl ethyl phosphoramidite, Chemical Phosphorylation Reagent, (1) CPR in Figure 2, has become increasingly popular over the years since it is convenient to use on the synthesizer and the yield of 5'-phosphate is generally much higher than with kinase.[1] This reagent includes a dimethoxytrityl (DMT) protecting group which can be removed on the synthesizer to allow a determination of phosphorylation efficiency. However, the DMT protecting group can not be used for DMT-on purification. If the DMT group is intentionally left on the oligonucleotide, it is eliminated along with the sulfonyl ethyl group to produce the 5'-phosphate during the ammonium hydroxide deprotection.

Other reagents containing lipophilic groups have been used as phosphorylation reagents which are amenable to reverse phase (RP) purification techniques. However, none have offered straightforward, mild reaction conditions to eliminate the side chain to give the 5'-monophosphate. The most likely candidates for RP compatible phosphorylation reagents are: 2-(triphenylsilyl)ethyl phosphoramidite[2] which requires elimination using tetrabutyl ammonium fluoride at elevated temperature; and 2-tritylthioethyl phosphoramidite[3] which requires oxidative cleavage with iodine or silver nitrate, followed by reaction with dithiothreitol under basic conditions.

A novel phosphorylation reagent, (2)CPRII in Figure 2, has been described[4] which seems to meet all criteria for a successful product. It contains a DMT group which can be removed on the synthesizer to determine phosphorylation yield. The side chain is then completely eliminated during ammonium hydroxide deprotection. Alternatively, with this reagent, the DMT group can be left on the oligonucleotide and used for RP purification. The DMT group is removed with aqueous acid and the side chain is eliminated after brief treatment with aqueous ammonium hydroxide to yield the 5'-phosphate. The reaction scheme is shown in Figure 1. This novel phosphorylation reagent clearly offers great potential for rapid purification of oligonucleotide 5'-phosphates based on the popular DMT-on technique using disposable cartridges like Poly-Pak™ cartridges available from Glen Research.

We are happy to offer CPRII in cooperation with the researchers at the University of Turku in Finland.

FIGURE 1: PROPOSED SCHEME FOR 5'-PHOSPHORYLATION

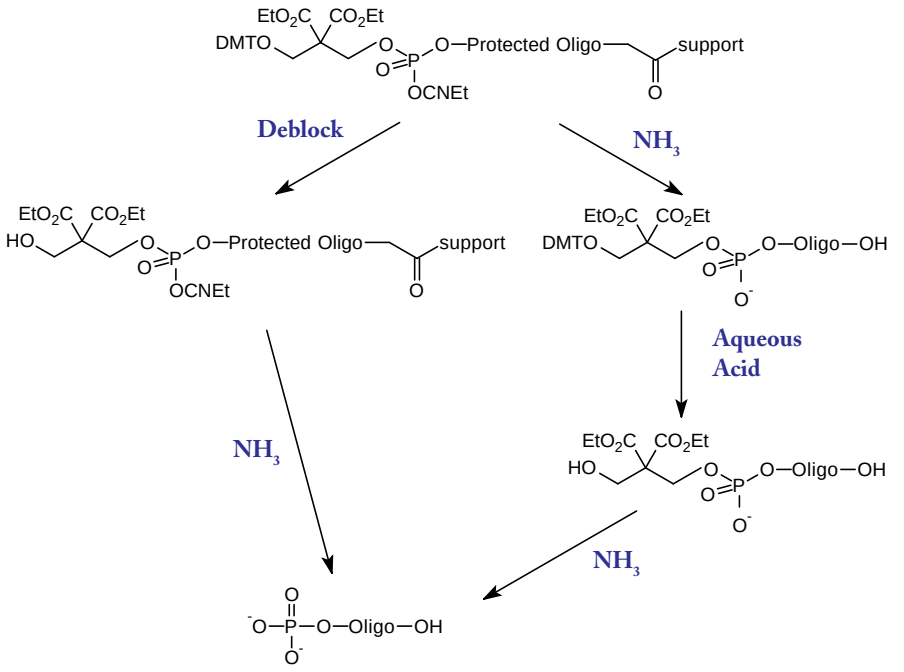
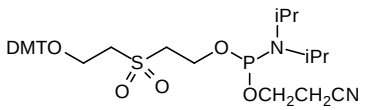
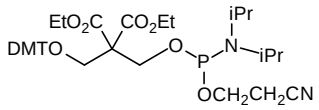


FIGURE 2: CPR STRUCTURES



(1) CPR



(2) CPR II

References:

(1) T. Horn and M. Urdea, *Tetrahedron Lett.*, 1986, **27**, 4705.

(2) J.E. Celebusky, C. Chan, and R.A. Jones, *J. Org. Chem.*, 1992, **57**, 5535-5538.

(3) B.A. Connolly and R. Rider, *Nucleic Acids Res.*, 1985, **13**, 4485.

(4) A. Guzaev, H. Salo, A. Azhayev, and H. Lonnberg, *Tetrahedron*, 1995, **51**, 9375-9384.

Poly-Pak is a trademark of Glen Research Corporation.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Chemical Phosphorylation Reagent II	10-1901-90	100 µmole	60.00
	10-1901-02	0.25g	200.00

PHOTOLABILE SUPPORT FOR THE PREPARATION OF 3'-CARBOXYLATES

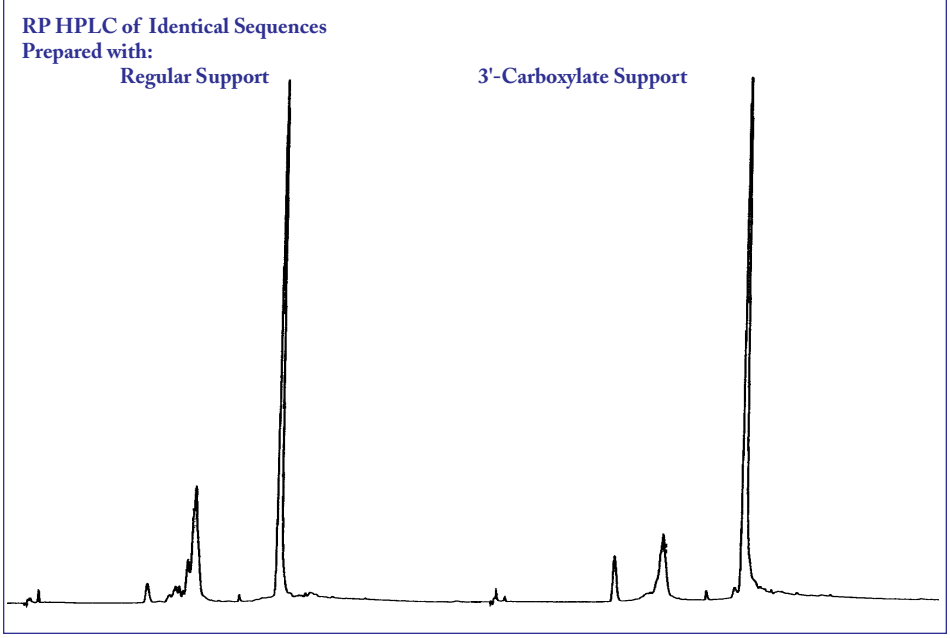
A variety of techniques exist for preparing oligonucleotides modified at the 5'-terminus or within the sequence but 3'-modification remains limited to amino and thiol (sulfhydryl) groups. Moreover, the vast majority of currently available commercial supports lead to the removal of at least a portion of the nucleobase and phosphate protecting groups during cleavage of the oligonucleotide from the support. (The exception is the ribonucleoside supports for DNA modification[1,2] and oxidizable solid support[3] which yield a fully base-protected oligonucleotide 3'-phosphate after oxidative cleavage and ß-elimination.) We are now introducing a universal, photolabile support for the preparation of an oligonucleotide 3'-carboxylate *with or without* the base protecting groups. The development of a commercially viable photolabile support extends from research carried out by Marc Greenberg and his group at Colorado State University.[4]

The research goals which culminated in the development of the support (1) are detailed below:

- Oligonucleotides containing 3'-terminal carboxylic acids should be isolated in normal yield by ammonium hydroxide deprotection.
- Photolytic cleavage from the support should result in the release of a fully protected oligonucleotide 3'-carboxylic acid.
- Photolytic cleavage from the support should occur under conditions which lead to minimal damage of the product oligonucleotide.
- The support should be independent of the base at the 3'-terminus, i.e., a universal support, the first 3'-base being added during the first phosphoramidite coupling cycle.

An oligonucleotide synthesized on the support (1) was compared to the equivalent oligonucleotide with no 3'-substitution. After ammonium hydroxide cleavage and deprotection, the products were analyzed by reverse phase (RP) HPLC, as shown in Figure 1. The mobility change when using the photolabile support is indicative of the formation of the desired 3'-carboxylate.

FIGURE 1: OLIGONUCLEOTIDE WITH AND WITHOUT 3'-CARBOXYLATE

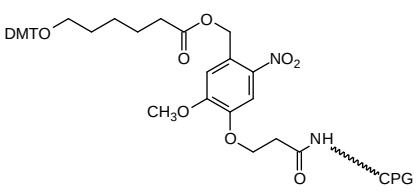


To compare photochemical cleavage with ammonium hydroxide cleavage, the same oligonucleotide was cleaved by photolysis from the support. The oligonucleotide was then deprotected with ammonium hydroxide and analyzed. The 3'-carboxylate products were found to be identical.[4]

The conditions chosen for irradiation of the product oligonucleotides have been shown to cause less than 1% thymidine dimer formation, as a measure of photoinduced damage. The yields of the oligonucleotides isolated by photo cleavage are reported to be about 30% less than those from ammonium hydroxide cleavage. To date, our experiments have been carried out using a TLC transilluminator (long wavelength UV) rather than a Hg/Xe lamp at 400nm.

Glen Research is happy to offer this photolabile carboxylate C6 support under license from Colorado State University.

FIGURE 2: PHOTOLABILE SUPPORT



(1) 3'-Carboxylate Photolabile C6 CPG 500

References:

(1) M. Lemaitre, C. Bisbal, B. Bayard, and B. Lebleu, *Nucleosides & Nucleotides*, 1987, **6**, 311-315.

(2) M. Lemaitre, B. Bayard, and B. Lebleu, *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 648-652.

(3) R. Lohrmann, L. Arnold, and J.L. Ruth, *DNA*, 1984, **3**, 122.

(4) D.J. Yoo and M.M. Greenberg, *J. Org. Chem.*, 1995, **60**, 3358-3364.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
3'-Carboxylate Photolabile C6 CPG 500	20-4090-01	0.1g	120.00
	20-4090-10	1.0g	975.00
1 µmole columns	20-4090-41	Pack of 4	200.00
0.2 µmole columns	20-4090-42	Pack of 4	120.00
40 nmole columns	20-4090-45	Pack of 4	120.00

TRANSCRIPTION TERMINATOR, 2-THIO- AND 4-THIO-THYMIDINE

Transcription Terminator

Chemical synthesis of RNA is still in an early stage of development: monomers are expensive to produce; coupling yields per cycle are substantially lower than in DNA; effective synthesis scales remain quite small; and post-synthetic deprotection steps are quite involved and time consuming. Consequently, a large percentage of RNA synthesis is carried out enzymatically using the method known as runoff transcription.[1] However, problems also exist in this enzymatic technique. In copying the DNA template, the polymerase enzyme sometimes fails to stop at the last coding base and the desired RNA sequence is contaminated with sequences containing one to several additional bases. This situation gives rise to a purification challenge since the contaminating RNA sequences are similar in length. Also, if the target RNA is to be isotopically labelled, the purification results in loss of expensive label.

The need to terminate RNA synthesis by somehow interrupting the polymerase led to the evaluation of several non-polar, non-hybridizing nucleoside analogues by a group at the University of Rochester.[2] When the indole derivative (1) was placed at the 5'-terminus of DNA templates, a significant improvement in quality of the transcribed RNA resulted. Without termination, longer undesired RNA sequences amounted to about 45% of the desired product RNA. This was reduced by more than 2-fold to below 20% when the template had the indole base analogue at the 5'-terminus. The indole derivative (1) has also been shown to be even more efficient at terminating enzymatic DNA synthesis, cutting the level of n+1mer by approximately 10-fold.[2]

The phosphoramidite of the 4-methylindole derivative (2) is now available from Glen Research, under license from the University of Rochester.

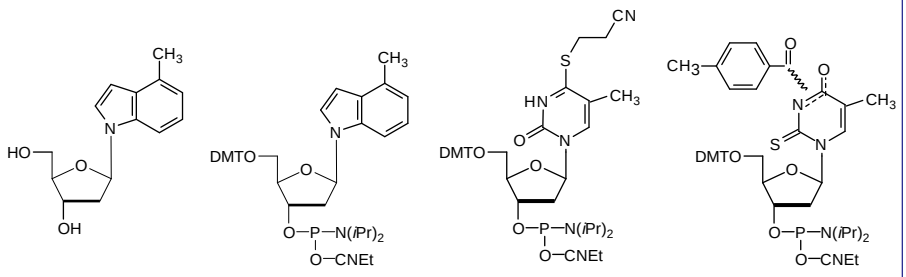
Sulfur Analogues of Thymidine

Incorporation of modified bases into synthetic oligonucleotides offers researchers the ability to study unnatural DNA functionality. Experiments may be designed to evaluate the effect of a given modification on DNA-DNA, DNA-RNA or DNA-protein interaction. In this way, researchers can examine hybridization or conformation of nucleic acids or elucidate the mechanism of, say, nuclease or polymerase interactions. One versatile and simple modification is the replacement of oxygen with sulfur on nucleobases. We now offer two thio derivatives of thymidine for investigation of oligonucleotide structure and activity.

4-Thio-dT is a very useful modification which can be used for photo-crosslinking and photoaffinity labelling experiments.[3] The thiocarbonyl group is also amenable to post-synthetic modification.[4] Although oligos containing 4-thio-dT can be accessed using our convertible dT monomer, routine preparation is significantly simplified using the protected monomer (3). Although synthesis with this monomer proceeds conventionally, we recommend a modification of the deprotection step to preserve the thiocarbonyl group. To the standard ammonium hydroxide solution, sodium hydrosulfide (NaSH) is added to a concentration of 50mM. This minimizes ammonolysis of the S-cyanoethyl group.

Similarly, oligos containing 2-thio-dT are useful in examining protein-DNA interaction by acting as photolabile probes. The thiocarbonyl group in 2-thio-dT is especially interesting in that it is available to react with compounds associating with the minor groove of DNA. A monomer protecting scheme which prevents desulfurization and degradation of 4-thio-dT by oxidation during oligonucleotide synthesis has been described.[5] The rather strange looking monomer (4) uses a toluyl protecting group which is located at the N3 or O4 position. This group is removed quantitatively during standard deprotection with ammonium hydroxide.

FIGURE 1: STRUCTURE OF TERMINATORS AND THIO-THYMIDINES



(1) (2) (3) (4)

References:

(1) J.F. Milligan, D.R. Groebe, G.W. Witherell, and O.C. Uhlenbeck, *Nucleic Acids Res.*, 1987, **15**, 8783-8798.

(2) S. Moran, X.-F. Ren, C.J. Sheils, S. Rumney IV, and E.T. Kool, *Nucleic Acids Res.*, manuscript submitted.

(3) T.T. Nikiforov and B.A. Connolly, *Nucleic Acids Res.*, 1992, **20**, 1209-1214.

(4) R.S. Coleman and E.A. Kesicki, *J. Amer. Chem. Soc.*, 1994, **116**, 11636-11642.

(5) R.G. Kuimelis and K.P. Nambiar, *Nucleic Acids Res.*, 1994, **22**, 1429-1436.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
4-Methylindole-CE Phosphoramidite	10-1045-90	100 µmole	125.00
	10-1045-02	0.25g	325.00
4-Thio-dT	10-1034-95	50 µmole	165.00
	10-1034-90	100 µmole	295.00
	10-1034-02	0.25g	675.00
2-Thio-dT	10-1036-95	50 µmole	165.00
	10-1036-90	100 µmole	295.00
	10-1036-02	0.25g	675.00

RAMBO ACTIVATOR - ETHYLTHIOTETRAZOLE

Suddenly, the use of 5-ethylthio-1H-tetrazole (S-ethyltetrazole) is increasing by leaps and bounds. What's going on here? Is a new rambo activator being born? This product has been lurking in our catalog for some time, so why the sudden interest now? Well, the explanation is fairly simple. Several articles have recently been published extolling its virtues as an activator.[1,2,3] But what particular attributes make it special compared to the tried and trusty veteran, 1H-tetrazole? The presence of the ethylthio group makes it significantly more acidic than tetrazole and this improves its ability as an activator. Ethylthiotetrazole is also considerably more soluble in acetonitrile than tetrazole. A more concentrated solution offers the possibility of better activator performance. However, concentrations from 0.25M to 0.75M are reported in the literature, so what is the optimal concentration?

We have now compared the performance of ethylthiotetrazole at various concentrations in acetonitrile with our standard tetrazole product in a series of low scale (≤1 μmole) syntheses. The water content of the solutions was always < 25ppm. DNA synthesis performance was determined by DMT assay and RP HPLC analysis of the resulting oligonucleotides, while RNA synthesis performance was determined by DMT yield alone. We found that a 0.25M solution of ethylthiotetrazole performs better than 0.45M tetrazole solution in DNA synthesis but a marked improvement was observed in the results of RNA syntheses. Results of this study are shown in a report on our world wide web site: http://www.glenres.com/NewNews/rambo.html.

Throughout the year, supplies of ethylthiotetrazole from the manufacturers have been plagued with high moisture content and particulate contamination. We will continue to offer the powder only if it will allow acceptable solutions to be produced. We will also be offering a solution at 0.25M concentration with a water content specification of ≤ 50ppm for better control and performance.

References:

(1) B. Sproat, et al., *Nucleosides and Nucleotides*, 1995, **14**, 255-273.

(2) D. Tsou, A. Hampel, A. Andrus, and R. Vinayak, *Nucleosides & Nucleotides*, 1995, **14**, 1481-1492.

(3) F. Wincott, et al., *Nucleic Acids Res.*, 1995, **23**, 2677-2684.

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Visit Glen Research at http://www.glenres.com. Find what's new, obtain catalog numbers, and access technical support information.