Glen Research

REPORT

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Introduction

In this issue of the *Glen Research Report*, we introduce many new products, including monomers for RNA and 2'-O-methyl-RNA synthesis, novel minor bases, and biotin phosphoramidites. However, there are some additional points worthy of note.

H-Phosphonate Chemistry

We have at last completed our Review of H-Phosphonate Chemistry which brings up to date events using that chemistry. Call us if you are interested in receiving a copy. Glen Research continues to carry out work on the application of this chemistry to large-scale synthesis and we will continue to supply H-phosphonate monomers. In this issue, we also review a paper with an interesting slant on the potential use of H-phosphonates for the simultaneous synthesis of multiple oligonucleotides. The authors also describe procedures for hydrolysis of the H-phosphonate active intermediates and recovery of excess monomers.

Amino-Modifier-dT

Amino-Modifier-dT has proved to be the best product by far for labelling oligonucleotides internally. The success of the product has allowed us to scale up manufacture and we are able to offer the item at a *25% lower price.*

RNA Synthesis - Options for 2'-OH Protection

For the last four years, we have been aware of the need for suitable solid-phase synthesis procedures to supplement the enzymatic techniques used to produce oligoribonucleotides. We have watched with interest as RNA phosphoramidites became commercially available but were disappointed to hear of problems encountered by end-users - cloudy monomer solutions, low monomer purities, and low coupling efficiencies - which, in some cases, led to some disappointing results. We do not make these comments simply to be critical of current suppliers - quite the contrary. The scale-up of the synthesis of RNA phosphoramidites is truly complex and it is to the great credit of the manufacturers that supplies have been maintained. Indeed, we know, to our cost, just how difficult the processes are! So, what are the problems in the production of RNA monomers and in automated RNA synthesis?

Protection Schemes

The only difference in a ribonucleoside relative to the corresponding 2'-deoxyribonucleoside is, obviously, the presence of the 2'-hydroxyl group. What can possibly happen at the 2' position[1] to make RNA synthesis so

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much more complex than DNA synthesis? First of all, a protecting group must be selected for the 2' position which will allow the preparation of the phosphoramidite monomer without migration of the 2' protecting group to the 3' position. Assuming this can be achieved, the protecting group must be stable to the conditions of automated synthesis without its loss or migration. Indeed, if it is lost during synthesis, chain scission may occur at the adjacent phosphodiester linkage during the ammonium hydroxide removal of the base protecting groups. Finally, the protecting group must be efficiently removed under mild conditions to generate the product oligoribonucleotide.

Most synthetic strategies have been designed to carry out automated DNA and RNA synthesis in the 3' to 5' direction. The 5'-hydroxyl group is usually protected with the 4,4'-dimethoxytrityl (DMT) group which is labile to dilute organic acid and generates a colored cation which can be monitored to give a measure of synthesis efficiency. While the combination of acid-labile DMT groups at the 5' position and base-labile protecting groups on the bases, as used routinely for DNA synthesis, is very convenient, it places severe constraints on the nature of the group used for 2' protection.

Protecting groups which are stable in the presence of acid and base are clearly prime candidates for use in 2' protection. For example, the 2-nitrobenzyl group[2] can be removed photochemically and the 3,4-dimethoxybenzyl[3] group can be oxidatively cleaved. However, in both cases, incomplete deblocking has been observed in the synthesis of long RNA fragments. The *t*-butyldimethylsilyl (TBDMS) group, extensively investigated by Ogilvie and coworkers[4] over many years, is also acid and base stable and can be removed relatively conveniently with fluoride ion. Indeed, 2'-O-TBDMS protection is the basis for all RNA monomers which are currently commercially available.

The 2'-acetal protecting groups, tetrahydropyranyl (THP) and methoxytetrahydropyranyl (MTHP), are cleaved under aqueous conditions at pH2 . As such, these groups are potentially very convenient since they allow a simple deprotection and work up just prior to use of the synthetic RNA fragments. However, both groups have proved to be too unstable[5] to acid to survive many detritylation cycles.

Protection of RNA monomers with 2'-O-MTHP in conjunction with base-labile 9-fluorenylmethoxycarbonyl (Fmoc) protection of the 5'-hydroxyl groups offers an interesting alternative synthetic strategy[6]. The coupling efficiency per cycle could still be monitored by measuring the release of the Fmoc group spectrophotometrically. Monomers for this approach have not yet been made available commercially.

Further refinements of 2'-acetal protection strategies have led to the development of 1-(2-chloro-4-methylphenyl)-4-methoxypiperidin-4-yl (CTMP)[1] and 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (FPMP)[7] by Reese and coworkers. These 2' protecting groups are unaffected by DCA deprotection during the normal synthesis cycles, but can be conveniently removed at pH 2 just prior to the use of the synthetic RNA fragment. Other 2'-acetal protecting

References:

(1) C.B. Reese, *Nucleosides & Nucleotides*, 1987, **6**, 121-129.
(2) T. Tanaka, S. Tamatsukuri, and M. Ikehara, *Nucleic Acids Res.*, 1987, **15**, 7235.
(3) H. Takaku, T. Ito, and K. Imai, *Chemistry Letters*, 1986, 1005.
(4) T. Wu and K.K. Ogilvie, *J. Org. Chem.*, 1990, **55**, 4717, and references cited therein.
(5) C. Christodoulou, S. Agrawal, and M.J. Gait, *Tetrahedron Lett.*, 1986, **27**, 1521.
(6) C. Lehmann, Y.Z. Xu, C. Christodoulou, Z.K. Tan, and M.J. Gait, *Nucleic Acids Res.*, 1989, **17**, 2379.
(7) C.B. Reese and E.A. Thompson, *J. Chem. Soc. Perkin Trans. 1*, 1988, 2881.

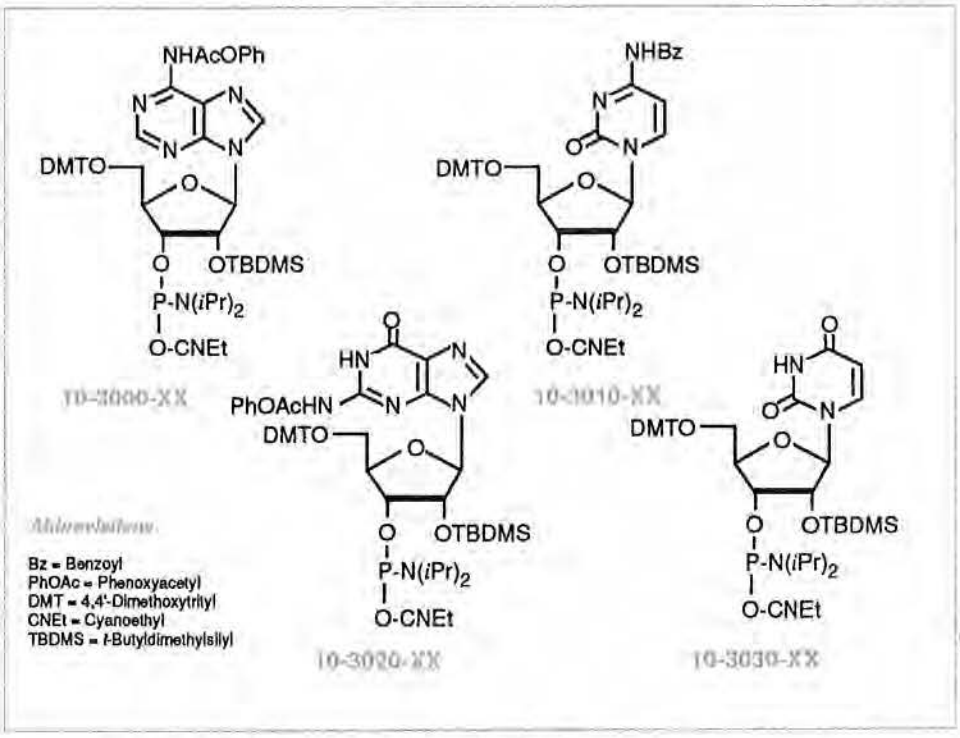

Figure 1: Structure and Catalog Numbers of RNA Monomers

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groups which have been evaluated include 3-methoxy-1,5-dicarbo-ethoxypentyl[8] and 1-(2-chloro-ethoxy)ethyl[9]. It is disappointing that monomers using this approach have not been made available commercially since this protection scheme seems to be close to optimal for automated RNA synthesis and facile isolation of the product RNA with reduced risk of RNase degradation. A recent paper by Sproat and coworkers[10] provides an excellent overview of the state of play in 2' protection, while opting for the use of FPMP.

Base Labile Protection

While the merits and demerits of various protection schemes can be debated at length, there is little doubt that most experience in RNA synthesis has been gained using the 2'-O-TBDMS protected monomers, described by Ogilvie and coworkers, since these have been commercially available for some time. The main problem with the use of these monomers for RNA synthesis has been the partial loss of the 2' protecting group leading to chain scission during the extended ammonium hydroxide deprotection necessary for removal of the standard base protecting groups. These problems have been largely overcome[11] by the use of base-labile protecting groups similar to those proposed for DNA synthesis[12].

At this point in the development of automated RNA synthesis, the combination of highly base-labile protecting groups along with 2'-O-TBDMS protection seems to us to offer the best approach currently available. Glen Research is happy to offer monomers based on this approach to RNA synthesis (Figure 1). Moreover, we are committed to providing only monomers which adhere to strict performance criteria, including >97% coupling and >97% purity by HPLC. We guarantee that we will adhere strictly to these quality specifications to avoid wasting researchers' time wrestling with poor quality products.

RNA Synthesis - Problems in Deprotection

Having carried out automated synthesis of oligoribonucleotides, the researcher is faced with the following difficulties.
1) How are the base protecting groups to be removed without affecting the 2' protecting groups? Remember, if the 2' protecting group is lost, the ammonium hydroxide, necessary to remove the base protecting groups, may cause chain cleavage at the adjacent phosphodiester linkage.
2) How are the 2' protecting groups removed quantitatively to give biologically active RNA?
3) What are the options for purification of the synthetic RNA?

Assuming the use of good quality monomers, RNA synthesis proceeds in a manner virtually identical to its DNA counterpart, the exception being the coupling time which is extended to 15 minutes to compensate for the presence of the bulky 2' protecting group. It is possible to use a more acidic activator, *e.g.*, 4-nitrophenyl-tetrazole[10], to speed up the coupling step and we will be examining a selection of these in the coming months. However, changes in the normal synthetic DNA deprotection scheme become essential.

Base Deprotection

Using standard base protecting groups and 2'-O-*t*-butyldimethylsilyl (TBDMS) protection, it was found[13] that the standard ammonia solution used for deprotection in DNA synthesis has to be modified for RNA synthesis because aqueous ammonia solutions cause significant loss of the silyl protecting groups which, in turn, may lead to chain scission. When the ammonia solution was modified by using a mixture of ammonium hydroxide - ethanol (3:1) for 17 hours at 55°C, chain cleavage was lowered to less than 1%. However, the authors note that the use of more base-labile protecting groups would be advantageous.

The harsh conditions involving the use of aqueous ethanolic ammonium hydroxide can be moderated if base-labile protecting groups are used to replace the groups normally used (isobutyryl for G, and benzoyl for A and C). Phenoxyacetyl protection for A and G has been described[12] for use in DNA synthesis. This protection scheme, along with similar base-labile protection for C, was evaluated by Ogilvie and coworkers[11]. Using ammonium hydroxide-ethanol (3:1) at room temperature, it was found that desilylation could be markedly reduced, although it was still observed. Also, the C monomer was found to be sparingly soluble in acetonitrile, and so, N-benzoyl-C was used for further studies. The authors then evaluated the use of anhydrous methanolic ammonia for base deprotection. It was found that deacylation proceeded rapidly (8 hours) at room temperature and completely eliminated desilylation and

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References:

(8) A. Sandstrom, M. Kwiatkowski, and J.B. Chattopadhayaya, *Acta Chem. Scand.*, 1985, **B39**, 273.

(9) S. Yamakage, O. Sakatsume, E. Furuyama, and H. Takaku, *Tetrahedron Lett.*, 1989, **30**, 6361.

(10) B. Beijer, I. Sulston, B.S. Sproat, P. Rider, A.I. Lamond, and P. Neuner, *Nucleic Acids Res.*, 1990, **18**, 5143-5151.

(11) T. Wu, K.K. Ogilvie, and R.T. Pon, *Nucleic Acids Res.*, 1989, **17**, 3501.

(12) J.C. Schulhof, D. Molko, and R. Teoule, *Nucleic Acids Res.*, 1987, **15**, 397.

(13) J. Stawinski, R. Stromberg, M. Thelin, and E. Westman, *Nucleic Acids Res.*, 1988, **16**, 9285.

(14) S.A. Scaringe, C. Francklyn, and N. Usman, *Nucleic Acids Res.*, 1990, **18**, 5433-5441.

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resulting chain scission.

A recent report[14] extends the use of anhydrous alcoholic ammonia to oligoribonucleotides containing the standard (isobutyryl for G, and benzoyl for A and C) base protecting groups. Ethanolic ammonia, being less volatile than methanolic ammonia, was used for deprotection at elevated temperatures (16 hours at 55ºC). Again, no silyl loss was observed.

Deprotection of 2'-O-TBDMS

The removal of the TBDMS group from the 2' position is accomplished[11] without phosphate migration or cleavage using 1M tetrabutyl-ammonium fluoride (TBAF) in THF (24 hours at room temperature). The excess TBAF is normally removed by desalting, but a simple procedure using an ion exchange cartridge has also been described[14].

Purification of Synthetic RNA

Polyacrylamide gel electrophoresis (PAGE) is the method of choice for purification of synthetic RNA. Elution from the gel is followed by desalting and lyophilization of the product. Purification by ion exchange or reverse phase (RP) HPLC has been described less frequently, presumably because of the danger of ribonuclease contamination in these procedures. RP cartridge purification would clearly have similar drawbacks. Analysis of synthetic RNA can be carried out using PAGE but rigorous confirmation that the product contains only 3'-5' linkages (rather than 2'-5' linkages produced by migration before, during or after synthesis) requires phospho-diesterase digestion of a sample of the product, followed by HPLC analysis of the nucleotides formed. Having made it this far, how do you avoid losing the precious synthetic RNA to ubiquitous ribonuclease enzymes? Once the 2' protecting groups have been removed, RNA must be worked with under scrupulously sterile conditions. Details of acceptable sterilization procedure and handling techniques are contained in standard molecular biology texts.

RNA Synthesis - 2'-O-Methyl Analogues

Oligonucleotides consisting of 2'-O-methyl-ribonucleotides (2'-OMe RNA) are arousing considerable interest as antisense probes since 2'-OMe RNA forms a stable duplex with RNA, while being resistant to RNase H activity[1]. At the same time, the 2'-OMe RNA is fully resistant to various RNase and DNase activities[2]. Of specific interest to researchers is the use of 2'-OMe RNA as antisense probes for studying site-specific inhibition of pre-mRNA splicing. The preparation of mixed oligoribonucleotides containing 2'-OMe ribonucleotides at specific points has been described[3], along with their significance in the study of RNA - protein interactions and their application to the synthesis of stable ribozymes.

As with all seemingly exciting developments, there are significant hurdles to overcome before the full potential of these techniques may be realized. The production of the CE Phosphoramidite monomers for use in 2'-OMe RNA synthesis is very difficult, with the G derivative (and to only a slightly lesser extent the A derivative) being particularly challenging. For a flavor of the synthesis of the purine monomers, check out the most up-to-date description[4] in which the G monomer is produced in a tortuous 12-step synthesis and the A monomer in a mere 7 steps! The pyrimidine

References:

(1) A. Mayeda, Y. Hayase, H. Inoue, E. Ohtsuka, and Y. Ohshima, *J. Biochem.*, 1990, **108**, 399.

(2) B.S. Sproat, A.I. Lamond, B. Beijer, P. Neuner, and U. Ryder, *Nucleic Acids Res.*, 1989, **17**, 3373.

(3) B. Beijer, I. Sulston, B.S. Sproat, P. Rider, A.I. Lamond, and P. Neuner, *Nucleic Acids Res.*, 1990, **18**, 5143-5151.

(4) B.S. Sproat, B. Beijer, and A. Irabarren, *Nucleic Acids Res.*, 1990, **18**, 41.

(5) A. Nyilas and J. Chattopadhyaya, *Acta Chem. Scand.*, 1986, **B40**, 826.

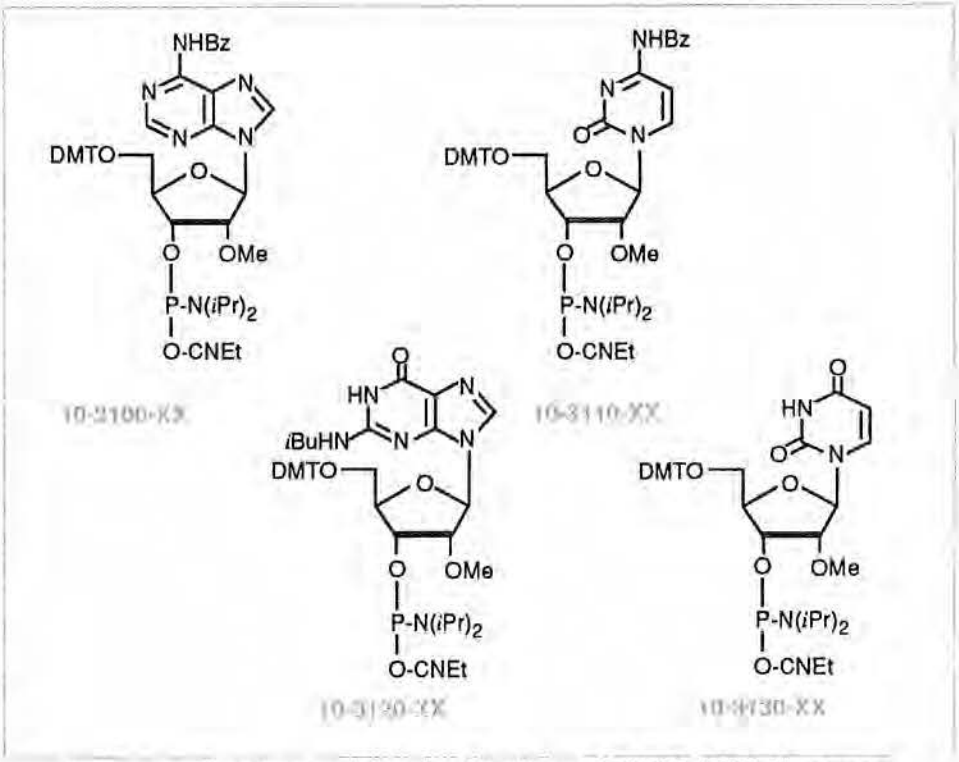

Figure 2: Structure and Catalog Numbers of 2'-OMe RNA Monomers

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monomers are, at least, slightly easier to produce[5].

The difficulties in synthesis of these monomers do not, fortunately, extend to the use of the monomers. Synthesis of 2'-OMe RNA proceeds in a manner analogous to RNA synthesis with a coupling time of 15 minutes. The deprotection steps are identical to those used for DNA synthesis since the 2'-OMe groups remain intact. Also, since the sequences are resistant to RNase hydrolysis, purification can be effected by the techniques normal to DNA synthesis, without the need for sterile conditions.

Because of the long and difficult synthesis of 2'-OMe RNA monomers, we are unable to guarantee that stocks will be sufficient to meet projected needs. However, we do guarantee that we will not compromise on quality and performance of monomers.

Literature Review

Simultaneous Synthesis of Multiple Oligonucleotides using Nucleoside-H-Phosphonate Intermediates

With the current heightened interest in DNA sequencing , it is probably timely to remember the potential of the segmented synthesis technique in which many oligonucleotides can be prepared simultaneously. A recent paper[1] applies H-phosphonate chemistry to this technique. The resulting oligonucleotides are prepared rapidly and efficiently, reminding us again of the simplicity and efficiency of H-phosphonate chemistry. The authors also describe a key advantage of H-phosphonate monomers in that excess activated monomer can be simply regenerated by hydrolysis and purified for re-use. The monomers are shown to be active and unmodified by the regeneration process.

Reference:

(1) H. Seliger and R. Rosch, *DNA and Cell Biology*, 1990, **9**, 691.

ORDERING INFORMATION

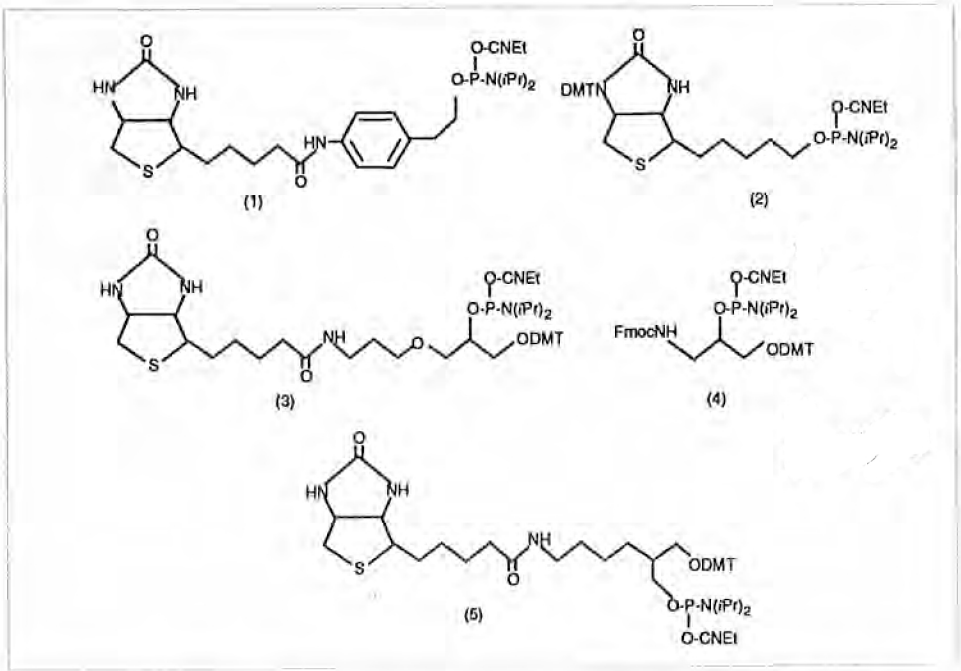
Item	Catalog No.	Pack	Price($)
RNA Synthesis Monomers			
Pac-A-CE Phosphoramidite	10-3000-02	0.25g	75.00
	10-3000-05	0.5g	150.00
	10-3000-10	1.0g	275.00
Bz-C-CE Phosphoramidite	10-3010-02	0.25g	75.00
	10-3010-05	0.5g	150.00
	10-3010-10	1.0g	275.00
Pac-G-CE Phosphoramidite	10-3020-02	0.25g	75.00
	10-3020-05	0.5g	150.00
	10-3020-10	1.0g	275.00
U-CE Phosphoramidite	10-3030-02	0.25g	75.00
	10-3030-05	0.5g	150.00
	10-3030-10	1.0g	275.00
RNA Synthesis Supports and Columns			
Pac-A-RNA 500	20-3300-02	0.25g	95.00
Pack of 4 1µmole columns	20-3400-41	Pk/4	100.00
Pack of 4 0.2µmole columns	20-3400-42	Pk/4	75.00
C-RNA 500	20-3310-02	0.25g	95.00
Pack of 4 1µmole columns	20-3410-41	Pk/4	100.00
Pack of 4 0.2µmole columns	20-3410-42	Pk/4	75.00
Pac-G-RNA 500	20-3320-02	0.25g	95.00
Pack of 4 1µmole columns	20-3420-41	Pk/4	100.00
Pack of 4 0.2µmole columns	20-3420-42	Pk/4	75.00
U-RNA 500	20-3330-02	0.25g	95.00
Pack of 4 1µmole columns	20-3430-41	Pk/4	100.00
Pack of 4 0.2µmole columns	20-3430-42	Pk/4	75.00
2'-OMe-RNA Synthesis Monomers			
2'-OMe-A-CE Phosphoramidite	10-3100-90	100µM	150.00
	10-3100-02	0.25g	360.00
2'-OMe-C-CE Phosphoramidite	10-3110-90	100µM	150.00
	10-3110-02	0.25g	360.00
2'-OMe-G-CE Phosphoramidite	10-3120-90	100µM	150.00
	10-3120-02	0.25g	360.00
2'-OMe-U-CE Phosphoramidite	10-3130-90	100µM	150.00
	10-3130-02	0.25g	360.00
2'-OMe-RNA Synthesis Supports and Columns			
2'-OMe-A-RNA 500	20-3600-02	0.25g	95.00
Pack of 4 1µmole columns	20-3700-41	Pk/4	100.00
Pack of 4 0.2µmole columns	20-3700-42	Pk/4	75.00
2'-OMe-C-RNA 500	20-3610-02	0.25g	95.00
Pack of 4 1µmole columns	20-3710-41	Pk/4	100.00
Pack of 4 0.2µmole columns	20-3710-42	Pk/4	75.00
2'-OMe-G-RNA 500	20-3620-02	0.25g	95.00
Pack of 4 1µmole columns	20-3720-41	Pk/4	100.00
Pack of 4 0.2µmole columns	20-3720-42	Pk/4	75.00
2'-OMe-U-RNA 500	20-3630-02	0.25g	95.00
Pack of 4 1µmole columns	20-3730-41	Pk/4	100.00
Pack of 4 0.2µmole columns	20-3730-42	Pk/4	75.00

Biotin Phosphoramidites

In the four years since we introduced our range of Amino-Modifiers, it has become clear that their most common use was as precursors for the introduction of biotin by reaction with the appropriate biotin NHS ester. While these methods have proved to be relatively successful, the only satisfactory means of purifying biotinylated oligonucleotides produced in this manner is by reverse phase (RP) HPLC. The process is time-consuming and suffers from the possibility of cross-contamination which is potentially troublesome in PCR applications.

More than a year ago, we set out to design and produce a biotin phosphoramidite which met the following performance criteria:
1) The biotin derivative must be soluble in acetonitrile at concentrations useful for DNA synthesis.
2) The product must be capable of being added in multiple additions to allow the introduction of several biotin molecules to enhance the sensitivity of detection of labelled probes.
3) The derivative must contain a DMT group to allow either quantification of the level of biotinylation or for use in RP cartridge purification techniques. This latter attribute is especially significant for the disposable cartridge purification of PCR primers where HPLC purification can lead to cross contamination.

Several descriptions of biotin phosphoramidites have appeared in the literature. A report from a researcher at Du Pont described[1] the biotin phosphoramidite (1) containing a phenethyl amide linkage to biotin. This reagent has been commercially available for some time. The main disadvantage of this reagent is its lack of solubility in acetonitrile which necessitates the use of anhydrous, amine-free DMF/dichloromethane to effect solution. Although lacking a DMT group, the phenethyl linkage is itself relatively hydrophobic and assists in reverse phase purification.



At about the same time, an alternative biotin phosphoramidite was reported[2]. The reagent (2) does contain a DMT group, is soluble in acetonitrile, but would not be suitable for the addition of multiple biotin molecules. So far, this reagent does not seem to have been made available commercially in the United States. A biotin phosphoramidite, "Biotin-dX", is available from MCRC, Midland, TX. The company does not reveal the structure but the main disadvantage of the product is that it can only be added once at the 5' terminus.

A more recent report describes[3] the preparation and use of the biotin phosphoramidite (3) which clearly meets all of our design criteria. Unfortunately, the chemical synthesis of this biotin phosphoramidite is quite tricky and, as described, is not very amenable to scale up. This product is similar in nature to 5' Branched Modifier C3 (4)[4] which we have been supplying for some time. (The disadvantage of the use of this branched modifier is that the level of biotinylation of the derived multiple amino groups is quite low, necessitating HPLC purification of a confusing array of multiple biotinylated species.)

Several groups have noted that the branched modifier (4) produces a linkage similar in structure to natural internucleotide linkages when inserted into an oligonucleotide. A further modification of a branched hydrocarbon backbone with extension of the biotin away from the branching site led to the design of an other biotin

References:
(1) A.J. Cocuzza, *Tetrahedron Lett.*, 1989, **30**, 6287-6290.
(2) A.M. Alves, D. Holland, and M.D. Edge, *Tetrahedron Lett.*, 1989, **30**, 3089.
(3) K. Misiura, I. Durrant, M.R. Evans, and M.J. Gait, *Nucleic Acids Res.*, 1990, **18**, 4345-4354.
(4) P.S. Nelson, R. Sherman-Gold, and R. Leon, *Nucleic Acids Res.*, 1989, **17**, 7179.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Biotin Phosphoramidite	10-1953-95	50 µmole	165.00
	10-1953-90	100 µmole	295.00
	10-1953-02	0.25g	675.00

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phosphoramidite (5). This product meets all of our design criteria and can be added in multiple steps at the 3' or 5' terminus, can be inserted at any position in the sequence, or can be added once at the 5' terminus for subsequent reverse phase purification.

Consequently, we are happy to offer for sale this biotin phosphoramidite. In addition, we are currently investigating an Fmoc equivalent for use in labelling oligonucleotides with other suitable tags.

The chromatogram shown in Figure 3 below demonstrates the use of our biotin phosphoramidite to label the 5' terminus of an oligonucleotide. The biotinylated oligonucleotide was simply purified by the DMT-on technique on a Poly-Pak™ cartridge. This product would certainly be of sufficient purity for use as a biotinylated PCR primer or probe.

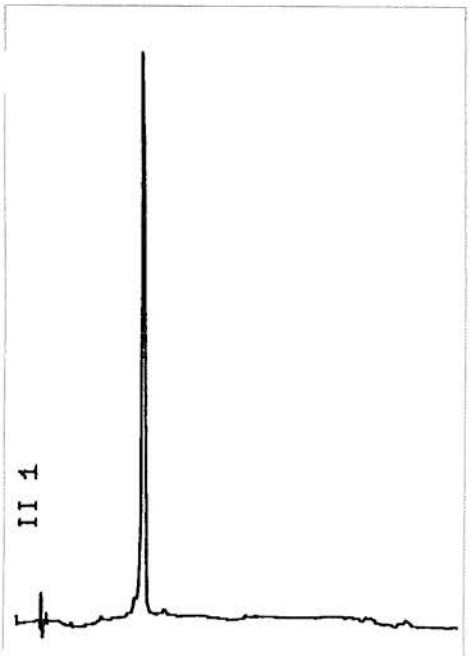


Figure: RP HPLC of Poly-Pak™ purified biotin labelled oligonucleotide using the conditions below:.

Time	0.1M TEAA(%)	Acetonitrile(%)
0	93	7
20	75	25
30	75	25
35	93	7
40	93	7

Column: Spherisorb ODS-2 (150X4.6mm)
Flow Rate: 1mL/min.
Detector: UV at 254nm

Sulfurization of Oligonucleoti

Oligonucleoside phosphorothioates (S-Oligos) are generating considerable interest as potential therapeutic agents because of the nuclease resistance of the internucleotide linkages. The synthesis of phosphorothioate linkages can be carried out with phosphoramidite chemistry using elemental sulfur[1] for sulfurization. This procedure is in routine use in automated synthesis despite several significant disadvantages: the reaction is slow (7.5 minutes); the sulfur may clog solenoid valves; and the solvent contains carbon disulfide which is noxious and potentially explosive. An alternative approach to the preparation of oligophosphorothioates has been the use of H-phosphonate chemistry. We use this technique routinely since the sulfurization is carried out after the synthesis is complete and is done off the instrument. The oligophosphorothioate is purified effectively using a Poly-Pak™ cartridge. Indeed this may be the technique of choice for the preparation of radioactive phosphorothioate linkages[2].

Recently, it was reported[3] that the thiosulfonate (1), more correctly named 3*H*-1,2-benzodithiole-3-one, 1,1-dioxide, was a much improved sulfurizing reagent. The compound exhibited the following desirable attributes:
1) It is reliably soluble in acetonitrile, making it safe to use on automated synthesizers.
2) The sulfurization reaction is fast (30 seconds), making the process convenient on small scales and readily amenable to scale-up.
3) The process is efficient, with better than 96% of the linkages being phosphorothioate and the remainder being phosphodiester.
4) The thiosulfonate reagent is stable for at least 1 month in solution.

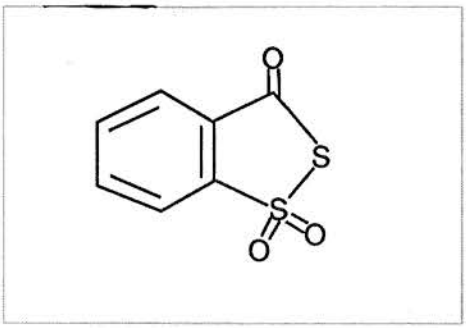


Figure: Structure of Sulfurizing Reagent (1)

One significant advantage in the use of the thiosulfonate is that it allows researchers to include both sulfurizing and oxidizing solutions on the instrument at the same time. (The instrument must have an available port and the researcher must be adept at programming.) With this set up, hybrid molecules containing phosphorothioate and phosphodiester linkages can be prepared.

Glen Research is happy to make this sulfurizing reagent commercially available. The reagent will be supplied in solid form in 1g and 2g packs in silanized bottles suitable for direct attachment to commercial synthesizers. Simply add 100mL or 200mL anhydrous acetonitrile.

References:

(1) C.A. Stein, C. Subasinghe, K. Shinozuka, and J.S. Cohen, *Nucleic Acids Res.*, 1988, **16**, 3209.
(2) C.A. Stein, P.L. Iversen, C. Subasinghe, J.S. Cohen, W.J. Stec, and G. Zon, *Anal. Biochem.*, 1990, **188**, 11.
(3) R.P. Iyer, W. Egan, J.B. Regan, and S.L. Beaucage, *J. Am. Chem. Soc.*, 1990, **112**, 1253-1254.

ORDERING INFORMATION

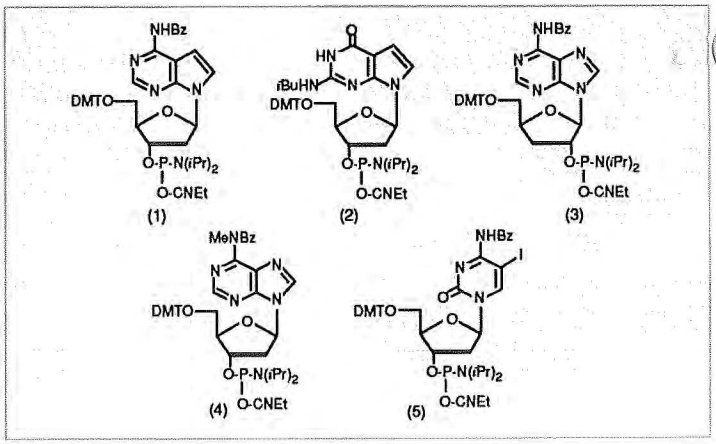
Item	Catalog No.	Pack	Price($)
Sulfurizing Reagent	40-4036-10	1.0g	95.00
"Beaucage Reagent"	40-4036-20	2.0g	190.00
(Please specify instrument when ordering.)			

Deoxynucleoside Analogues

Glen Research is dedicated to the production of analogues of the standard deoxy-nucleosides as phosphoramidites for oligonucleotide synthesis. To our existing line, we are happy to add the following five monomers which have been most requested by researchers. It would be short sighted for us to predict all of the promising applications for these monomers, but we will attempt to give some indication of their potential uses.

The 7-deaza analogues of 2'-deoxy-Adenosine (1) and 2'-deoxyGuanosine (2) have been requested for physical studies related to the interaction of enzymes and DNA. Further uses may be found in the examination of DNA structure, especially with the current interest in the formation and structure of triplex DNA.

Cordycepin (3'-deoxyAdenosine) (3) offers some potential interest for the formation of non standard 2'-5' linkages which may exhibit resistance to nucleases and restriction enzymes. The product will also be available attached to CPG to produce oligonucleotides with cordycepin at the 3' terminus. Oligos with cordycepin at the 3' terminus may be useful in blocking chain extension by polymerases.



Structure of Phosphoramidites of Deoxynucleoside Analogues

N6-Methyl-2'-deoxyAdenosine (4) may find its utility in carcinogenesis studies for which we currently offer the O6-methyl-2'-deoxyGuanosine analogue. 5-Iodo-2'-deoxyCytidine (5) joins our already extensive list of halogenated deoxynucleosides. The iodo analogues seem to be most interesting for structural studies using X-ray crystallography.

We are now maintaining a comprehensive list of analogues separate from our catalog since the availability of existing products may vary while new products are always in planning. Request our Minor Bases Catalog Insert for an up-to-date listing of 2'-deoxynucleoside analogues.

Glen Research

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