The Glen Report

C-5 PROPYNE DERIVATIVES

- ANTISENSE BREAKTHROUGH?

Antisense oligodeoxynucleotides (ODNs) must be designed with the following properties necessary for optimal activity.

1) ODNs must be nuclease resistant before and during residence in cells.
2) ODNs must have the ability to cross the cellular membrane with some level of efficiency.
3) ODNs must demonstrate high binding affinity and specificity for the target sequence.

At first glance at least, nuclease resistance is fairly easy to achieve by modification of the normal phosphodiester backbone (*e.g.*, phosphorothioates, methyl phosphonates) or by use of a 3'-terminal cap (*e.g.*, 3'-aminopropyl modification or using a 3'-3' terminal linkage). Improved transport through the cellular membrane can be attained by use of a carrier molecule (*e.g.*, cholesterol, poly-lysine) or by backbone modification to more lipophilic linkages. Increasing the affinity and specificity of an oligonucleotide has been more difficult to achieve since this necessitates modifying the natural bases which are already almost perfectly set up for optimal hydrogen bonding. This article covers one way[1] in which advances have been made in improving affinity while maintaining specificity.

C-5 methylated pyrimidine deoxynucleosides are known to form more stable duplexes and triplexes than their corresponding pyrimidine derivatives. Consequently, thymidine and 5-methyl-2'-deoxyCytidine (5-Me-dC) form more stable complexes than 2'-deoxyUridine and 2'-deoxyCytidine (dC). For example, substitution of 5-Me-dC for dC in a duplex structure has

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ULTRAFAST DNA SYNTHESIS

- 10 MINUTE DEPROTECTION!

Commercial DNA synthesizers currently are capable of operating with fast cycles of 2 to 3 minutes. The synthesis of primers can, therefore, routinely be achieved in under 1 hour. However, a major bottleneck in processing the products is the cleavage and deprotection steps which can take anywhere from 1 to 7 hours. All this is before the isolation and/or purification strategies which take a minimum of 1 hour. It is clear that the DNA production process would benefit tremendously if cleavage and deprotection steps were streamlined.

Normal Protecting Groups

Alternative strategies have been proposed. Using the standard base protection scheme (benzoyl for dA and dC, isobutyryl for dG), deprotection can be speeded up by elevating temperature[1] or combining elevated temperature (80°) with the addition of triethylamine to the ammonium hydroxide[2]. In addition to the potential hazard of such a high temperature, in both cases the time for both cleavage and deprotection is still at least 1 hour. A proprietary procedure developed and recently introduced by Barrskogen, Inc.[3] allows cleavage and deprotection to occur in less than an hour but includes a potentially cumbersome precipitation step.

Base-Labile Protecting Groups

Using base-labile protecting groups, it is possible to reduce the deprotection time to as little as 15 minutes. However, each system introduced to date has exhibited flaws. Phenoxyacetyl (PAC) protection[4] is a reasonable alternative to the standard base protecting groups but a deprotection time of 30-60 minutes[5] does not include the cleavage step (normally at least 45

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The synthesis of 2'-OMe-RNA is as simple to perform as DNA synthesis and avoids most of the problems associated with RNA synthesis. Oligo-2'-OMe-nucleotides are resistant (but not totally resistant) to a variety of ribo- and deoxyribonucleases. As well as being stable to normal handling and nuclease resistant, oligo-2'-OMe-nucleotides form more stable hybrids with complementary RNA strands than equivalent DNA and RNA sequences. This combination of useful properties promises to make 2'-OMe-RNA sequences powerful research tools as has been noted in a recent review[1].

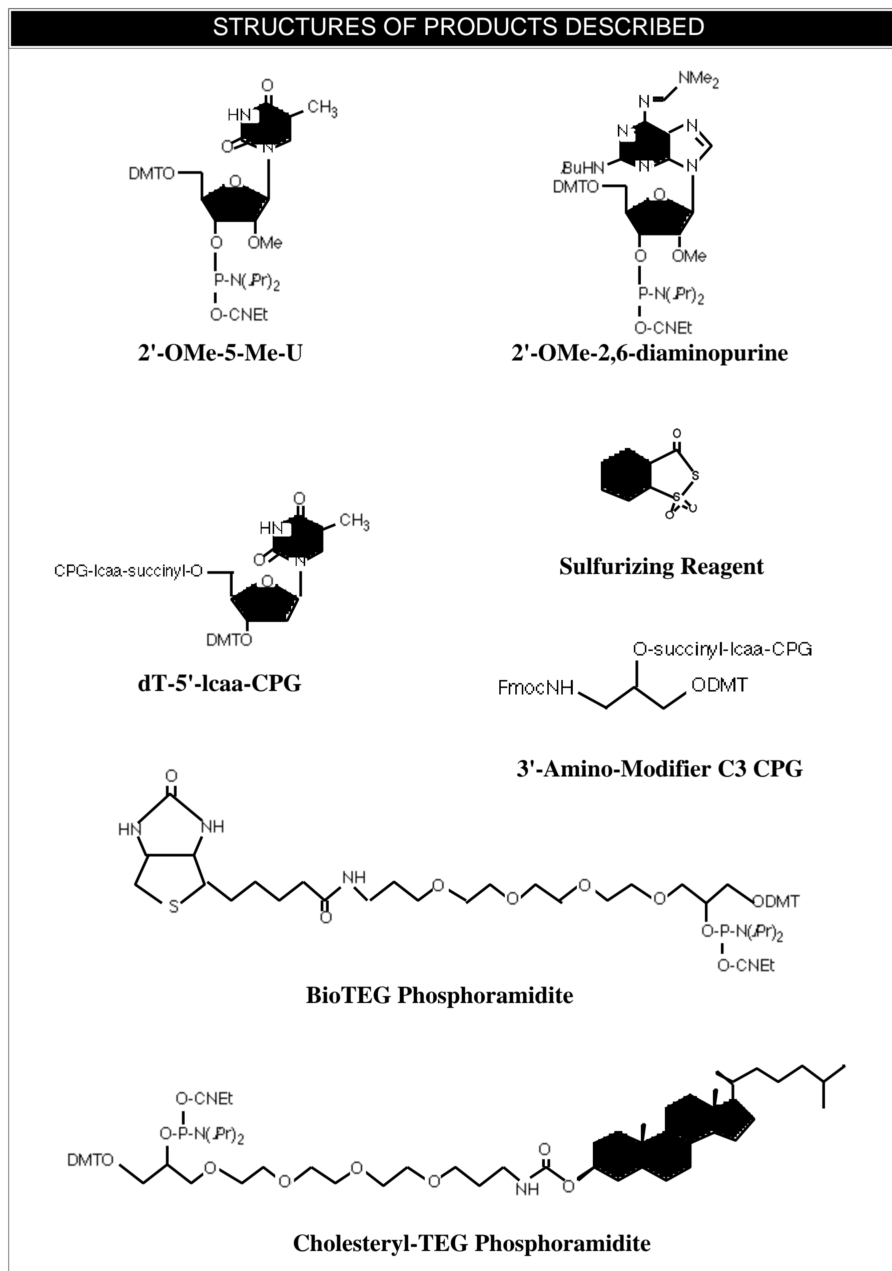
Antisense Activities

Oligonucleotides are believed to induce cleavage of RNA by RNase H, thereby reducing levels of the target RNA. Although 2'-OMe-RNA sequences exhibit a high affinity for RNA targets, these sequences are not substrates for RNase H. The combination of requirements of nuclease resistance, high affinity for target RNA, and being substrates for RNase H, has led to the evaluation of chimeric oligo-2'-OMe-nucleotides containing oligodeoxynucleotide gaps as antisense therapeutics. A group at Isis Pharmaceuticals has described[2] chimeric antisense oligonucleotides based on 2'-modified oligonucleotides containing oligodeoxynucleotide gaps with all internucleotide linkages modified to phosphorothioates for even better nuclease resistance. The results indicate that in the system tested, a minimum of five oligodeoxynucleotide linkages in the center are necessary for maintaining RNase H and antisense activity. An additional route to enhancing exonuclease resistance of antisense oligonucleotides is to invert the nucleoside at the 3'-terminus[3] with a 3'-3' linkage. This is readily accomplished using an appropriate support in which the nucleoside is attached using the 5'-hydroxyl and synthesis proceeds from the 3'-hydroxyl group. Eventual digestion of the modified oligonucleotide leads to only natural nucleosides. Similarly, the 3'-terminus can be blocked with an aminoalkyl group[4].

Enhanced Binding

(Continued on Page 3)

STRUCTURES OF PRODUCTS DESCRIBED



References:

(1) A.I. Lamond and B.S. Sproat, *FEBS Letters*, 1993, **325**, 123-127.
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(3) J.F.R. Ortigao, H. Rosch, H. Selter, A. Frohlich, A. Lorenz, M. Montenarh, and H. Seliger, *Antisense Res. & Dev.*, 1992, **2**, 129-146.
(4) J.G. Zendegui, K.M. Vasquez, J.H. Tinsley, D.J. Kessler, and M.E. Hogan, *Nucleic Acids Res.*, 1992, **20**, 307-314.
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(6) B.C. Froehler, R.J. Jones, X.D. Cao, and T.J. Terhorst, *Tetrahedron Lett.*, 1993, **34**, 1003-1006.
(7) B. Oberhauser and E. Wagner, *Nucleic Acids Res.*, 1992, **20**, 533-538.
(8) M. Cotten, B. Oberhauser, H. Brunar, A. Holzner, G. Issakides, C.R. Noe, G. Schaffner, E. Wagner, and M.L. Birnstiel, *Nucleic Acids Res.*, 1991, **19**, 2629-2635.
(9) H.O. Smith, K. Tabiti, G. Schaffner, D. Soldati, U. Albrecht, and M.L. Birnstiel, *Proc. Nat. Acad. Sc. USA*, 1991, **88**, 9784-9788.

(Continued from Page 2)

In situations where higher affinity for the target strand is sought, it is possible to substitute A sites with 2-amino-A (2,6-diaminopurine) which sets up an additional hydrogen bond to U. The Tm of the hybrid forms increases by 3° for every site modified[1]. This is especially important in cases where the target is U-rich[5]. Enhanced binding can be achieved by the simple substitution of Thymidine for U since the additional 5-methyl group leads to a more stable duplex. A further instance where substitution of a modified base to enhance duplex stabilization is found in the demonstration[6] that 5-propynyl-U and 5-propynyl-C (as the 2'-O-allyl derivatives) exhibit a higher affinity for the target RNA strand. Each U substitution enhances Tm by 0.3° while each C substitution enhances Tm by 0.7°. (This strategy is described in further detail beginning on the front page of this issue.)

Enhanced Cell Penetration

Transport of oligonucleotides into cells is a problem routinely faced by antisense researchers. This process would be expected to be improved by modifying the antisense oligonucleotides with suitable lipids. Recently, enhanced penetration of oligo-2'-OMe-nucleotides modified by the attachment of cholesterol (via a disulfide linkage) into liposomes was reported[7]. The attachment of cholesterol improved both affinity for and internalization of the oligo-2'-OMe-nucleotides.

Antisense Probes

The combination of properties offered by oligo-2'-OMe-nucleotides - nuclease resistance, stable hybrid formation, ease of synthesis - make them especially suitable for use as antisense probes. In this mode, RNase H resistant oligo-2'-OMe-nucleotides, labelled or unlabelled, can be used to block specific RNA functions[8] and, when attached to a suitable tag, usually biotin[9], can be used in affinity purification of RNA complexes. These activities are reviewed in detail by Lamond and Sproat[1] whose contribution to research in this area has been especially noteworthy.

ORDERING INFORMATION

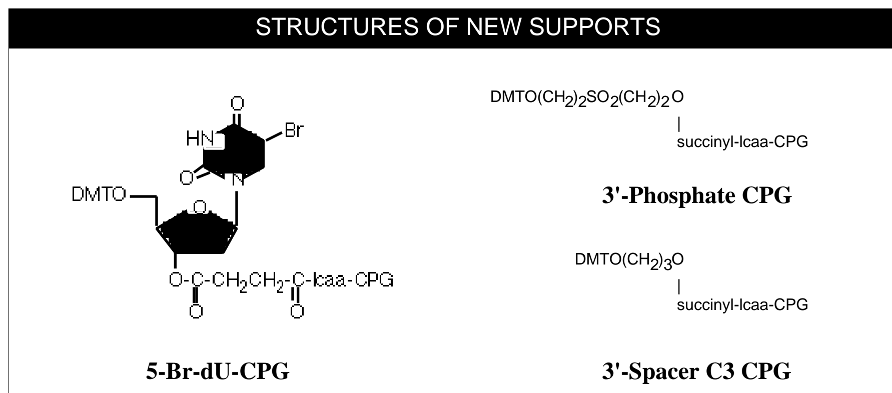
Item	Catalog No.	Pack	Price($)
2'-OMe-A-CE Phosphoramidite	10-3100-90	100 µmole	75.00
	10-3100-02	0.25g	180.00
2'-OMe-C-CE Phosphoramidite	10-3110-90	100 µmole	75.00
	10-3110-02	0.25g	180.00
2'-OMe-G-CE Phosphoramidite	10-3121-90	100 µmole	75.00
(N-dmf-G)	10-3121-02	0.25g	180.00
2'-OMe-U-CE Phosphoramidite	10-3130-90	100 µmole	75.00
	10-3130-02	0.25g	180.00
Sufurizing Reagent	40-4036-10	1.0g	95.00
	40-4036-20	2.0g	190.00
dT-5'-lcaa-CPG	20-0302-01	0.1g	50.00
1 µmole columns	20-0312-41	Pk/4	200.00
0.2 µmole columns	20-0312-42	Pk/4	120.00
3'-Amino-Modifier C3 CPG	20-2950-01	0.1g	85.00
(Bulk)	20-2950-10	1.0g	600.00
1 µmole columns	20-2950-41	Pk/4	125.00
0.2 µmole columns	20-2950-42	Pk/4	75.00
2'-OMe-2,6-Diaminopurine-	10-3124-95	50 µmole	177.50
CE Phosphoramidite	10-3124-90	100 µmole	355.00
(2-amino-A)	10-3124-02	0.25g	975.00
2'-OMe-5-Me-U-	10-3131-90	100 µmole	150.00
CE Phosphoramidite *(2'-OMe-T)*	10-3131-02	0.25g	360.00
Cholesteryl-TEG Phosphoramidite	10-1975-95	50 µmole	165.00
	10-1975-90	100 µmole	295.00
	10-1975-02	0.25g	675.00
Biotin-TEG Phosphoramidite	10-1955-95	50 µmole	165.00
(BioTEG)	10-1955-90	100 µmole	295.00
	10-1955-02	0.25g	675.00

Notes:
1. 5'-Supports are available for all four bases. See Catalog Page 13 for details.
2. Applied Biosystems-style columns are supplied unless otherwise specified.
3. Items listed below are described in detail on Page 4 of this issue.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
dU-lcaa-CPG 1000	20-2051-01	0.1g	50.00
1 µmole columns	20-2151-41	Pk/4	200.00
0.2 µmole columns	20-2151-42	Pk/4	120.00
5-Br-dU-lcaa-CPG	20-2090-01	0.1g	50.00
1 µmole columns	20-2090-41	Pk/4	200.00
0.2 µmole columns	20-2090-42	Pk/4	120.00
3'-Phosphate CPG	20-2900-01	0.1g	85.00
(Bulk)	20-2900-10	1.0g	600.00
1 µmole columns	20-2900-41	Pk/4	125.00
0.2 µmole columns	20-2900-42	Pk/4	75.00
3'-Spacer C3 CPG	20-2913-01	0.1g	85.00
(Bulk)	20-2913-10	1.0g	600.00
1 µmole columns	20-2913-41	Pk/4	125.00
0.2 µmole columns	20-2913-42	Pk/4	75.00

NEW SUPPORTS



Is there any need for DNA synthesis supports other than tried and true CPG? While the performance of CPG has stood the test of time, we feel that there is a need for a high loading support designed for larger-scale synthesis and a polymeric support suitable for smaller-scale synthesis.

High Loading CPG

Our high loading support is based on controlled pore silica and it retains the usual 500Å pores. The spacer is also conventional. The only significant difference is the loading which is in the range 80 - 130 µmoles/g or about 2.5 times the loading of normal 500Å CPG. Typical loadings are in the 100 - 120 µmoles/g range. As a consequence of the high loading, this support should not be used for sequences longer than 40mers. This high loading support is available in columns for most synthesizers. The 2.5 µmole column is identical to our standard 1 µmole column (with the exception of the loading). It should be used on occasions when greater than 1 µmole is desired but when a 10 or 15 µmole synthesis is too high. It should be run using the 1 µmole cycle. The 25 µmole column is identical to the 10 µmole column used on Applied Biosystems synthesizers. It is run using the 10 µmole cycle. The 35 µmole column is used as an alternative to the 15 µmole MilliGen column. Again no changes to the standard cycle are recommended. The support is of course available in bulk for use on large-scale synthesizers. A word of caution is in order. When using a column with a higher load than recommended by the instrument manufacturer, there is a much smaller margin for error. All reagents must be fresh and anhydrous diluent and activator must be used. Should you decide to prepare higher-loading columns, ensure that the molar excess of monomer to support nucleoside is at least 5X and preferably 10X.

Polymeric Support

Polymeric supports tend to be effective with nucleoside loadings lower than standard CPG. Consequently, they are especially useful for lower-scale synthesis columns. They are normally used on the 40 nmole scale and are ideal for the rapid synthesis of primers. Our polymeric support has a loading range of 20 - 30 µmoles/g. It is available in 40 nmole and 0.2 µmole configurations, as well as in bulk.

New Supports

In support of our line of minor bases and modifiers, we have added some supports which may prove useful for a variety of purposes. dU-CPG is now also available on 1000Å CPG for the synthesis of longer oligonucleotides. 5-Br-dU for cross-linking and antibody detection applications is now available on 500Å CPG. 3'-Phosphate CPG is added to our line of modifiers to allow direct preparation of oligonucleotides with a 3'-phosphate group. Finally, 3'-Spacer C3 CPG is now available as a blocker of exonuclease and polymerase activity at the 3'-terminus. Ordering Information for these new supports is collected on Page 3.

New Columns

In recent months, we have introduced several column types which have been redesigned to make them most unlikely to leak during synthesis. TWIST™ columns are compatible with a variety of synthesizers including those from Applied Biosystems. TWIST columns allow ready access to the support by removal of the cap. We have also made modifications to our Applied Biosystems-style 10 µmole columns and MilliGen-style 15 µmole columns to make them leak proof.

ORDERING INFORMATION

Item	Catalog No. dA	Catalog No. dC	Catalog No. dG	Catalog No. dT	Pack	Price($)
High-loading CPG	25-2100-46	25-2110-46	25-2120-46	25-2130-46	4X2.5 µmole	75.00
(Applied Biosystems format)	25-2100-17	25-2110-17	25-2120-17	25-2130-17	25 µmole	125.00
High-loading CPG	25-2200-46	25-2210-46	25-2220-46	25-2230-46	4X2.5 µmole	75.00
(MilliGen format)	25-2200-18	25-2210-18	25-2220-18	25-2230-18	35 µmole	185.00
High-loading CPG	25-2000-02	25-2010-02	25-2020-02	25-2030-02	0.25g	50.00
(Bulk)	25-2000-10	25-2010-10	25-2020-10	25-2030-10	1.0g	185.00
Low-loading PS	26-2100-45	26-2110-45	26-2120-45	26-2130-45	4X40 nmole	60.00
(Applied Biosystems format)	26-2100-42	26-2110-42	26-2120-42	26-2130-42	4X0.2 µmole	75.00
Low-loading PS	26-2200-45	26-2210-45	26-2220-45	26-2230-45	4X40 nmole	60.00
(MilliGen format)	26-2200-42	26-2210-42	26-2220-42	26-2230-42	4X0.2 µmole	75.00
Low-loading PS	26-2000-02	26-2010-02	26-2020-02	26-2030-02	0.25g	55.00
(Bulk)	26-2000-10	26-2010-10	26-2020-10	26-2030-10	1.0g	200.00

(Continued from Front Page)

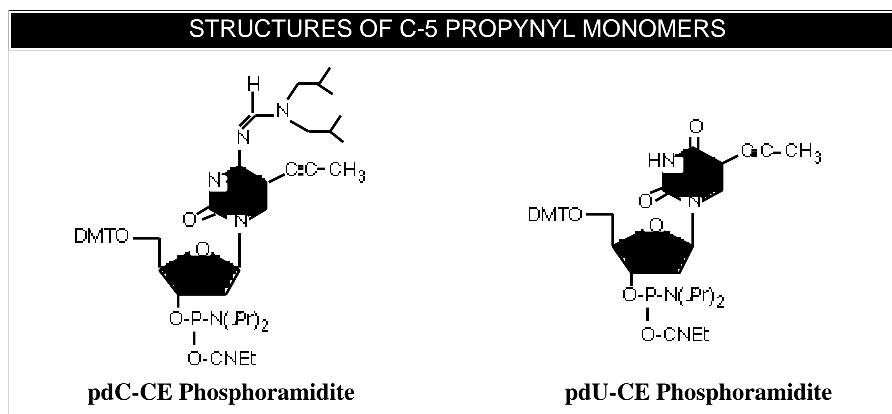
been shown[2] to increase Tm by 1.3° per substitution and in a triplex structure by 1.7° per substitution. The authors postulate that this effect is caused by entropic factors in that the presence of the methyl residues eliminates water molecules from the duplex structure.

Synthesis of 5-(1-propynyl)-2'-deoxy-Uridine (pdU) and 5-(1-propynyl)-2'-deoxyCytidine (pdC) monomers for oligonucleotide synthesis demonstrated[1] that both substitutions enhanced duplex stability while triplex binding was improved by substitution of pdU but destabilized by pdC. Substitution of methyl with 1-propyne at the C-5 position of pyrimidines allowed better stacking of the bases since the propyne group is planar with respect to the heterocyclic base. At the same time, propyne is more hydrophobic than methyl and this property contributed to a further increase in binding. The improved lipophilicity of the propyne group may also improve transport through cell walls. Duplex binding enhancement due to these modified bases was substantial (1.7° per pdU residue and 1.5° per pdC residue).

Similar results were found[3] using 5-propynyl-2'-O-allyl derivatives, paU and paC. These derivatives were found to bind with high affinity to RNA targets, enhancing binding relative to control by 1.7° per paU residue and 2.0° per paC residue. This significant increase results from a combination effect of both propynyl and allyl modifications.

Inhibition of gene expression by an antisense ODN containing C-5 propyne modifications has been examined[4] in detail. In this elegant study, researchers at Gilead Sciences were able to specifically measure antisense activity without interference from cellular uptake and intranuclear degradation using a rapid and controlled assay system. ODNs were introduced into cells by microinjection along with two plasmids that direct the expression of two separate proteins. Antisense inhibition of the RNA target sequence would arrest the production of one protein without affecting the expression of the second control protein. Protein production was monitored by fluorescence assay.

STRUCTURES OF C-5 PROPYNYL MONOMERS



pdC-CE Phosphoramidite **pdU-CE Phosphoramidite**

Thankfully for this chemist, the techniques used have been lucidly reviewed[5] elsewhere (page 1930 of the review article). However, the results were indeed dramatic in that the C-5 propyne substituted phosphorothioate ODN inhibited gene expression at the very low concentration of 0.05 μM. The modified phosphorothioate was 50X more effective than the corresponding phosphodiester ODN, presumably as a result of increased nuclease resistance. The corresponding 2'-O-allyl-RNA derivatives did not inhibit gene expression even though they exhibit higher affinity for the target RNA sequence. This would indicate that RNase H cleavage of the target RNA complex is necessary for potent antisense inhibition.

The authors[4] note that *"The C-5 propyne-substituted phosphorothioates represent a new class of ODNs that may prove to be universal reagents for the inhibition of gene expression".*

Glen Research is happy to offer pdU- and pdC-CE phosphoramidites by exclusive license from Gilead Sciences. It is clear that the article in Science[4] has generated a high level of interest in these products and we look forward to maintaining a routine, high-quality supply of them. In the near future, we hope to add the C-5 propynyl-2'-OMe-RNA derivatives which may prove to be useful tools for the evaluation of mechanisms of antisense inhibition. We also see applications for the C-5 propyne derivatives in PCR experiments, especially where the target is purine rich.

References:

(1) B.C. Froehler, S. Wadwani, T.J. Terhorst, and S.R. Gerrard, *Tetrahedron Lett.*, 1992, **33**, 5307-5310.
(2) L.E. Xodo, G. Manzini, F. Quadrifoglio, G.A.v.d. Marel, and J.H.v. Boom, *Nucleic Acids Res.*, 1991, **19**, 5625-5631.
(3) B.C. Froehler, R.J. Jones, X.D. Cao, and T.J. Terhorst, *Tetrahedron Lett.*, 1993, **34**, 1003-1006.
(4) R.W. Wagner, M.D. Matteucci, J.G. Lewis, A.J. Gutierrez, C. Moulds, and B.C. Froehler, *Science*, 1993, **260**, 1510-1513.
(5) J.F. Milligan, M.D. Matteucci, and J.C. Martin, *J. Med. Chem.*, 1993, **36**, 1923-1937.

ORDERING INFORMATION

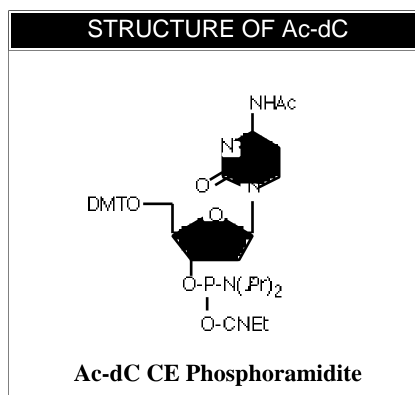
Item	Catalog No.	Pack	Price($)
pdC-CE Phosphoramidite	10-1014-90	100 μmole	85.00
	10-1014-02	0.25g	245.00
	10-1014-05	0.5g	490.00
pdU-CE Phosphoramidite	10-1054-90	100 μmole	65.00
	10-1054-02	0.25g	195.00
	10-1054-05	0.5g	390.00
pdC-lcaa-CPG 500	20-2014-01	0.1g	75.00
1 μmole columns	20-2114-41	Pk/4	250.00
0.2 μmole columns	20-2114-42	Pk/4	150.00
pdU-lcaa-CPG 500	20-2054-01	0.1g	75.00
1 μmole columns	20-2154-41	Pk/4	250.00
0.2 μmole columns	20-2154-42	Pk/4	150.00

minutes). The PAC-dG monomer is rather insoluble and the protecting group needs to be changed to produce better solubility. It is fair to note that PAC protection is certainly an excellent mild deprotection scheme. The use of dimethylformamidine (dmf) protected monomers[6] (also referred to as FOD[7,8]) again allows fast deprotection (1 hour at 55°) but the cleavage time is still at least 45 minutes. There are still questions about the stability of dmf-dA during synthesis and this group may need refinement. *t*-Butylphenoxyacetyl protected monomers[9] (known as Expedite™ monomers by Millipore) also offer very fast deprotection (15 minutes at 55°) but cleavage from the support is still 45 minutes. Expedite monomers require a different capping reagent (*t*-butylphenoxyacetic anhydride rather than the normal acetic anhydride) and it will be interesting to see how this affects subtle points of base modification[10] and loss of capping during synthesis. Only time and extensive use will reveal any undesirable side reactions. Nevertheless, Expedite monomers are our currently preferred choice in situations where very mild deprotection of oligonucleotides is necessary, *e.g.*, when preparing sequences containing labile nucleobases.

UltraFAST System

Relief of the production bottleneck is now at hand with the discovery of the UltraFAST cleavage and deprotection system by Beckman Instruments[11]. This system, for which patents are pending, requires the normal benzoyl protection of the dC monomer to be replaced with acetyl. All other monomer protecting groups remain the same. The chemical stability of the acetyl-dC monomer is equivalent to the normal benzoyl-dC monomer allowing it to be conveniently stored at controlled room temperature rather than 4° or below. This seemingly minor change in protecting group leads to an oligonucleotide which can be cleaved and deprotected in 10 minutes using an inexpensive reagent known as AMA. The AMA reagent 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° for a further 5 minutes. Deprotection can also be carried out at lower temperatures as shown in Table 1. In

STRUCTURE OF Ac-dC



Ac-dC CE Phosphoramidite

TABLE 1: DEPROTECTION WITH AMA

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

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

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Ac-dC-CE Phosphoramidite	10-1015-02	0.25g	25.00
	10-1015-05	0.5g	50.00
	10-1015-10	1.0g	100.00
	10-1015-20	2.0g	200.00
Ac-dC-lcaa-CPG	20-2015-01	0.1g	18.00
	20-2015-02	0.25g	40.00
	20-2015-10	1.0g	150.00
ABI columns	20-2115-4Y	Pk/4	60.00
MilliGen columns	20-2215-4Y	Pk/4	60.00
(10 µmole ABI)	20-2115-13	Pk/1	100.00
(15 µmole MilliGen)	20-2215-14	Pk/1	150.00

(Note: For 0.2 µmole columns, Y=2; For 1 µmole columns, Y=1.)

Item	Source
Methylamine (40% solution)	Aldrich M2,775-1
Syringes (1mL)	Aldrich Z23072-3
Deprotection tubes	BioRad ST336-5S

TABLE 2: COMPARISON OF FAST CLEAVAGE AND DEPROTECTION TECHNIQUES

Monomers	*Reagent*	*Cleavage Time*	*Fastest Deprotection Temp*	*Fastest Deprotection Time*	*Total Time*	*Notes*
Normal	NH3	45 min	80°	60 min	105 min	Well tried[1]
Normal	NH3/TEA	45 min	80°	30 min	75 min	Well tried[2]
Normal	Proprietary	15 min	85°	30 min	45 min	Novel method[3]
PAC	NH3	45 min	70°	30 min	75 min	Well tried[4,5]
FOD	NH3	45 min	55°	60 min	105 min	Problems with dA[6,7,8]
Expedite	NH3	45 min	55°	15 min	60 min	Best for mild techniques[9]
Ac-dC	AMA	5 min	65°	5 min	10 min	Not yet tested for mild techniques[11]

Expedite is a trademark of Millipore Corporation

FIGURE 1: DMT-ON (CRUDE) BY RP HPLC

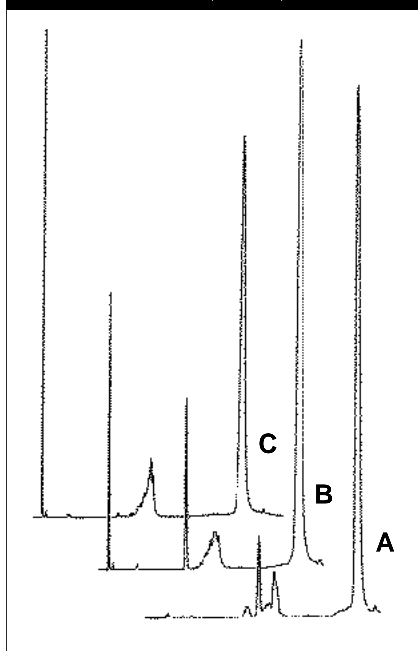


FIGURE 2: DMT-OFF BY CE (CRUDE)

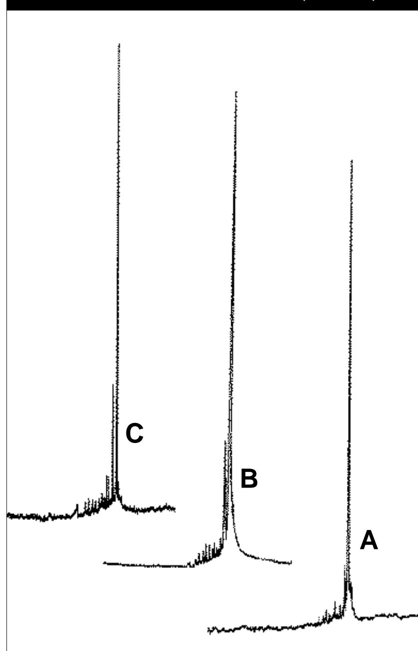
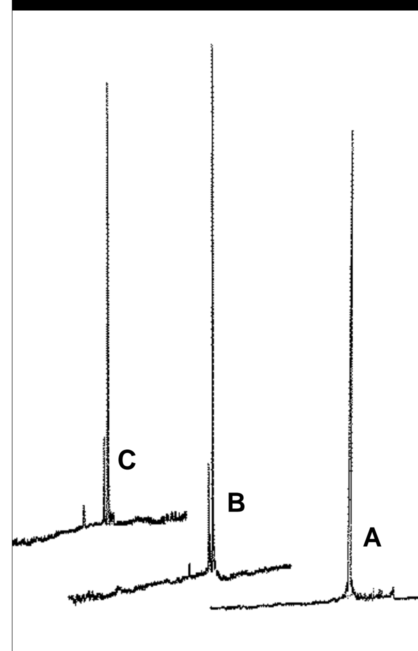


FIGURE 3: DMT-OFF BY CE AFTER POLY-PAK



all cases, no base modification has been observed.

In a comparative testing of the use of acetyl-dC with normal benzoyl-dC, no differences have been observed in the final oligonucleotides by analytical techniques of HPLC, capillary electrophoresis, base composition analysis, but even more importantly in the usage of the oligonucleotides as primers in PCR and sequencing. Indeed, hundreds of oligonucleotides produced from acetyl-dC monomers have been used successfully without a single adverse observation. After deprotection, there is no need to change the procedures of Poly-Pak™ purification so oligonucleotides can also be purified in a further 10 minutes with only 0.5mL of aqueous acetonitrile to evaporate prior to use. The use of Ac-dC is also compatible with ammonium hydroxide deprotection under normal conditions making this system the most ubiquitous so far offered.

An informal comparison of the purity of an oligonucleotide produced using acetyl-dC, benzoyl-dC, and Expedite monomers was carried out. In all cases, excellent products were obtained. Figures 1 and 2 demonstrate the high quality of the crude products while Figures 3 and 4 demonstrate that the products were upgraded to very high purity using Poly-Pak cartridges.

References:

(1) T.R. Reynolds and G.A. Buck, *Biotechniques*, 1992, **12**, 518.

(2) *Technical Bulletin No. 041R*, Cruachem Inc., (1991).

(3) *DNA Mate Reagents*, Barrskogen, Inc., (1993).

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

(5) *Analects Vol.21*, Pharmacia P-L Biochemicals, Inc., (1993).

(6) L.J. McBride, R. Kierzek, S.L. Beaucage, and M.H. Caruthers, *J. Amer. Chem. Soc.*, 1986, **108**, 2040.

(7) H. Vu, C. McCollum, K. Jacobson, P. Theisen, R. Vinayak, E. Spiess, and A. Andrus, *Tetrahedron Lett.*, 1990, **31**, 7269-7272.

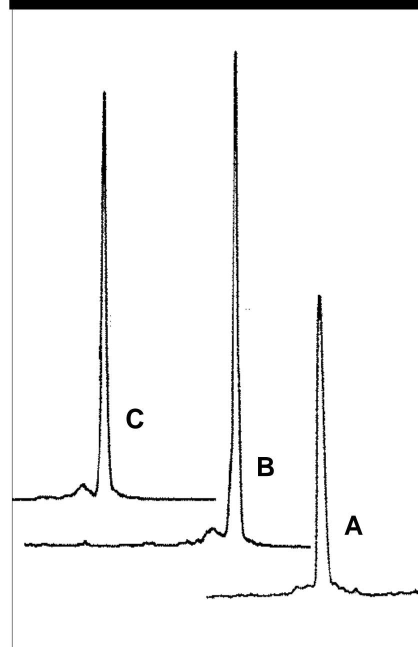
(8) *User Bulletin No. 57*, Applied Biosystems, Inc.. (1990).

(9) N.D. Sinha, P. Davis, N. Usman, J. Perez, R. Hodge, J. Kremsky, and R. Casale, *Biochimie*, 1993, **75**, 13-23.

(10) R.T. Pon, N. Usman, N.J. Damha, and K.K. Ogilvie, *Nucleic Acids Res.*, 1986, **14**, 6453-6470.

(11) a. G. Sasaki, J.J. Dih, and M.P. Reddy, *Technical Information Bulletin T-1792*, Beckman Instruments, Inc., (1993).
b. M.P Reddy and N. Hanna (patents pending).
c. M.P. Reddy, N. Hanna, and F. Farooqui (1993; manuscript in preparation).

FIGURE 4: ION EXCHANGE AFTER POLY-PAK



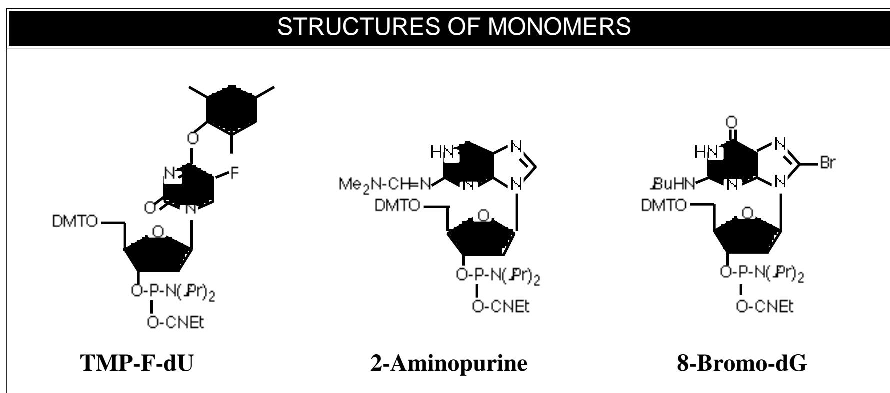
Notes:

1. Oligonucleotide shown is a 21mer. In Figures, oligo A used Ac-dC, B normal, and C Expedite monomers.
2. Figure 1 illustrates that the different protecting groups yield different amide by-products on deprotection as well as the desired products.
3. Figure 2 confirms the excellent quality of the unpurified oligos using capillary electrophoresis.
4. Figures 3 and 4 demonstrate the high purity of these oligos after DMT-on purification by Poly-Pak.
5. These chromatograms are not intended to be a formal comparison of the methods.
6. Chromatographic conditions are available on request.

Poly-Pak is a trademark of Glen Research Corporation

NEW MINOR BASES - 5-F-dC, 2-Aminopurine, 8-Br-dG

STRUCTURES OF MONOMERS



TMP-F-dU **2-Aminopurine** **8-Bromo-dG**

5-F-2'-deoxyCytidine

Oligonucleotides containing 5-fluoro-2'-deoxyCytidine are effective inhibitors of DNA methyltransferases. Two serious problems have impeded efforts to produce oligonucleotides containing F-dC. The presence of the fluorine residue makes acyl protection of the amino group very difficult. Also, the deoxynucleoside itself is very expensive. Both of these problems are circumvented by the use of a Convertible Nucleoside strategy[1]: 4-O-(2,4,6-trimethylphenyl)-5-fluoro-2'-deoxyUridine (TMP-F-dU) is converted into F-dC after oligonucleotide synthesis by the ammonium hydroxide used for the routine deprotection steps.

2-Aminopurine and 8-Bromo-dG

Also available for the first time are 2-aminopurine and 8-bromo-dG monomers. The former may be used for structural studies and the latter for cross-linking. We hope to add 8-oxo-dG, 8-bromo-dA, 8-oxo-dA, and convertible dI monomers soon.

Reference:

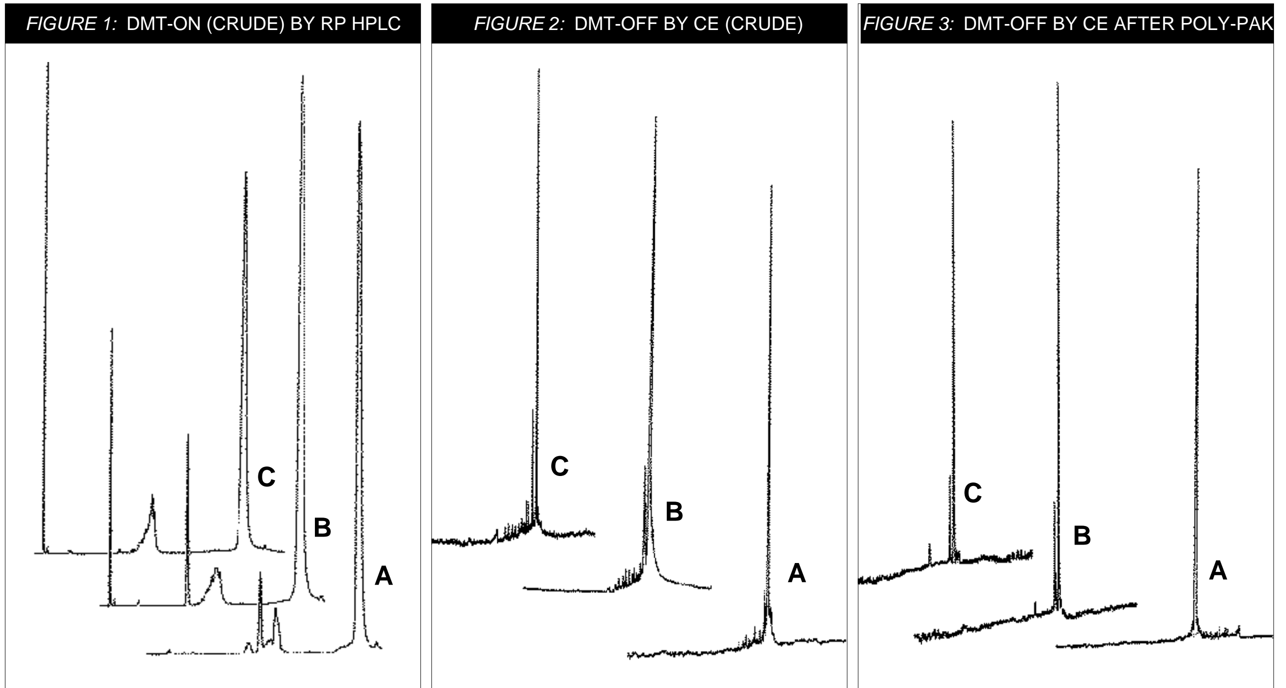
(1) A.M. MacMillan, L. Chen, and G.L. Verdine, *J. Org. Chem.*, 1992, **57**, 2989-2991.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
TMP-F-dU-CE Phosphoramidite	10-1016-90	100 µmole	195.00
(Convertible F-dC)	10-1016-02	0.25g	495.00
2-Aminopurine-CE Phosphoramidite	10-1046-90	100 µmole	135.00
	10-1046-02	0.25g	355.00
8-Br-dG-CE Phosphoramidite	10-1027-90	100 µmole	105.00
	10-1027-02	0.25g	255.00

NEW - Internet email: `support@glenres.com`

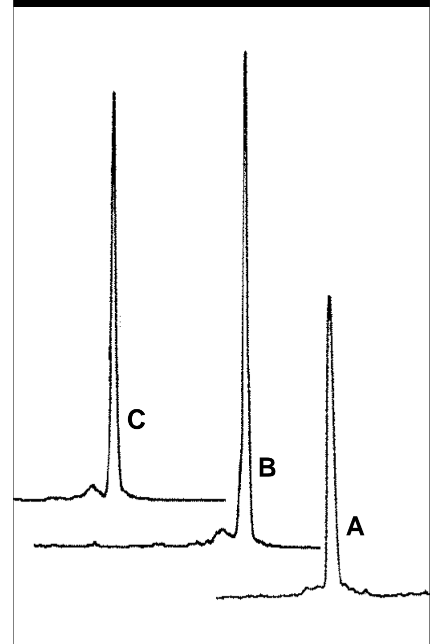
GR6.2 - CHROMATOGRAPHIC CONDITIONS



a. **RP HPLC**

Solvent A: Acetonitrile
Solvent B: 0.1M aqueous triethylammonium acetate
Column: Spherisorb ODS2 (150X4.6mm), 5 µm packing
Flow: 1 mL/min.
Detection: UV at 254nm
Gradient:

Time	%A	%B
0	3	97
5	3	97
20	40	60
30	40	60



b. **Ion Exchange HPLC**

Solvent A: 2mM aqueous sodium hydroxide + 0.2M sodium chloride
Solvent B: 2mM aqueous sodium hydroxide + 1M sodium chloride
Column: Dionex Nucleopac PA100 (250X4.0mm), 5 µm packing
Flow: 1 mL/min.
Detection: UV at 254nm
Gradient:

Time	%A	%B
0	100	0
30	10	90

c. **Capillary Electrophoresis**

Capillary: Beckman eCAP ssDNA Gel, 30cm
Field: 11.1kV
Temperature: 30°C
Detection: UV at 254nm