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22825 DAVIS DRIVE • STERLING, VIRGINIA • 20164

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INSIDE

PHOSPHONOACETATE (PACE) OLIGONUCLEOTIDES

INTRODUCTION

Over the years, Glen Research has been able to introduce many unique products for research and development. We are now especially pleased to offer Phosphonoacetate (PACE) Phosphoramidites, used to synthesize oligonucleotides that contain a phosphoacetate linkage in place of the standard phosphodiester linkage. Oligonucleotides containing this modification offer improved transfection characteristics, enhanced nuclease resistance, and they show considerable promise in siRNA research.

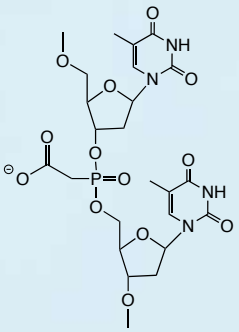
Phosphonocarboxylate oligonucleotides are recently-developed modifications that can be incorporated using "phosphoramidite-like" monomers.[1,2] The monomers, shown in Figure 1, Page 2, are formally called "phosphonamidites or phosphinocarboxylic acid amidites" because they contain a carbon atom attached to the phosphorus atom instead of a protected oxygen.

Phosphonocarboxylate is a broad term that refers to a modified phosphate in which one of the oxygen atoms has been replaced with a carboxylic acid. The two examples that have currently been reported are the phosphonoacetic acid derivative (PAA)[3], and the phosphonoformic acid (PFA) derivative[4], shown in Figure 2, Page 2. Of these two modified phosphates, the acetic acid version fits best into the standard phosphoramidite synthesis protocols due to its stability to typical DNA/RNA deprotection conditions such as methylamine. As a result, phosphonoacetate derived DNA can be conveniently produced in high yields using standard DNA synthesis protocols on any automated DNA synthesizer.

BACKGROUND

Phosphorus-modified DNA and RNA have been utilized for many years for the biochemical evaluation of DNA/DNA, DNA/RNA, RNA/RNA, protein/RNA, and protein/DNA interactions.[5]

PACE INTERNUCLEOTIDE LINKAGE



Phosphorothioates

One of the first chemically modified internucleotide bonds utilized for these studies was phosphorothioate modified oligonucleotides, as shown in Figure 3, Page 2. Methods for the synthesis of phosphorothioate modified DNA were originally developed over 30 years ago by Eckstein in order to probe DNA/protein interactions.[6] In the initial rationale for this modification, it was well understood that the negative charge on the internucleotide bond was an important recognition element for the binding of nucleic acids by other nucleic acids and proteins. This modification was particularly appealing because the simple substitution of a sulfur atom for a non-bridging oxygen did not significantly change the crystal structure or charge distribution around small molecule model compounds. Furthermore, the development of efficient and reliable sulfurization protocols[7] made the modification as simple to synthesize as normal DNA.

The one significant difference in the physical properties of these molecules compared to normal DNA is the change in hydrogen bonding that is associated with a transformation from oxygen to sulfur. Oxygen can act both as a hydrogen bond acceptor and a hydrogen bond donor; however, sulfur can only act as a weak hydrogen bond

(Continued on Page 2)

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donor and cannot act as a hydrogen bond acceptor. Therefore, sulfur substitutions on the phosphodiester internucleotide linkage change the ability of water to shield the negative charge on the phosphodiester through hydrogen bonding interactions. From a practical perspective, this means that phosphorothioate DNA has increased non-specific binding to proteins, which is usually overcome *in vitro* by increasing salt concentrations.

A second significant difference that accompanies this modification is that a sulfur substitution for oxygen in the non-bridging position of the phosphodiester internucleotide bond generates a stereo center, and the two phosphorus diastereomers show differential binding and recognition by proteins and nucleic acids. Even with these limitations, phosphorothioate modified DNA and RNA have been proven to be very useful tools for biochemistry, molecular biology, cell biology, and oligonucleotide therapeutics.

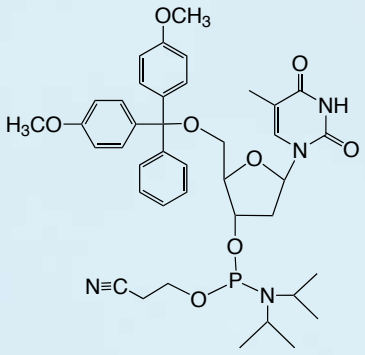
Methyl Phosphonates

The second significant phosphorus-modified internucleotide bond developed and used for biochemical studies was methylphosphonate DNA (Figure 3). These internucleotide modifications were first proposed by Miller and Tso[8] and were specifically intended to address characteristics desirable for therapeutic use of oligonucleotides.

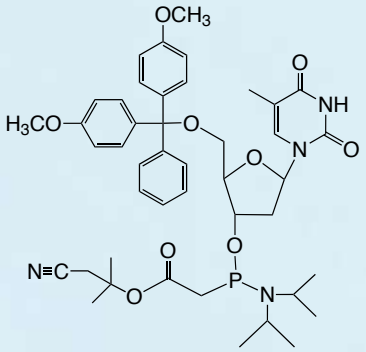
It had been widely observed that highly charged therapeutic molecules, even small molecules like nucleoside mono-phosphates, are not taken up by cells in culture or by animal tissues. The rationale for the methylphosphonate modification was that removing the negatively charged oxygen from the internucleotide bond and replacing it with a non-charged methyl group should increase the ability of these molecules to be taken up by cells in culture and simultaneously protect the resulting modified DNA from being degraded by phosphodiesterases.

What was observed with this modification is that by removing a required recognition element from the DNA mimic, *i.e.*, the negatively charged backbone, the resulting oligonucleotides become inactive in many biological roles. The internucleotide bond in methylphosphonate DNA is highly resistant to nucleases,

FIGURE 1: STANDARD CE VS PACE PHOSPHORAMIDITE

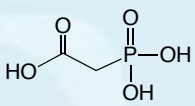


Standard CE Phosphoramidite

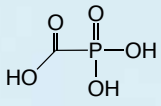


Phosphonoacetate (PACE) Monomer

FIGURE 2: PHOSPHONO CARBOXYLIC ACIDS

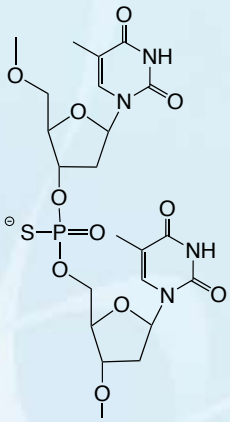


PhosphonoAcetic Acid (PAA)

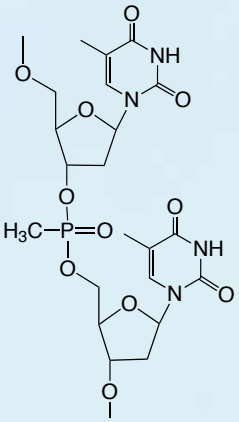


PhosphonoFormic Acid (PFA)

FIGURE 3: PHOSPHOROTHIOATE AND METHYL PHOSPHONATE INTERNUCLEOTIDE LINKAGES



Phosphorothioate Linkage



Methyl Phosphonate Linkage

and reported to give enhanced cellular uptake in various assays. The modification became more broadly accepted when it was demonstrated that it could be incorporated using "phosphoramidite-like" monomers on a typical DNA synthesizer and is now most often incorporated along with natural internucleotide bonds.[9]

Other Backbones

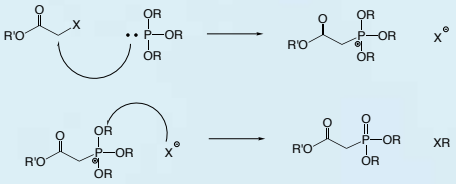
There have been several additional phosphorus-modified oligonucleotides reported in the scientific literature, most notably phosphorodithioate DNA[10], boron phosphonate DNA[11], and finally phosphoramidate DNA[12]. Each of these modifications has demonstrated quite unique and potentially useful structural and biological properties. However, none of these newer modifications has demonstrated significantly enhanced cellular uptake.

PHOSPHONOCARBOXYLATES

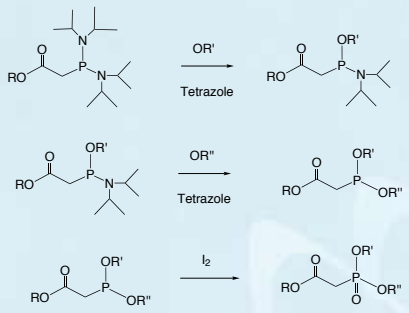
Small molecule phosphonocarboxylates, both PAA and PFA (Figure 2), are well known and quite old mimics of phosphate. PFA, as the acid derivative, was originally synthesized over 100 years ago by Arbuzov. Phosphonoformate and Phosphonoacetate are both pretty good mimics of the phosphate group: the 3-dimensional crystal structure is strikingly similar and the charge density distribution is similar. The carboxylic acid functional group of PFA and PAA is effective at hydrogen bonding in both the protonated and charged forms. However, one significant difference is that the ionization constant

is changed to that of a weaker acid in the diester form. The diester salt of PAA has a pKa of ≈ 5.0 while the diester salt of phosphate has a pKa of ≈ 2.0. Therefore, when a phosphodiester internucleotide bond is produced with the acetate in the non-bridging position, the protonated and non-protonated forms should be in equilibrium at neutral to mildly acidic pH, Figure 4. This internucleotide bond would be negatively charged, but the charge density would be decreased by the acid/base equilibrium.

Asymmetric diesters of phosphonocarboxylates were historically quite difficult to produce. These compounds were typically produced using an Arbuzov reaction, whereby a halide was reacted at elevated temperature with a trialkyl phosphite in a two-step, sequential, oxidative transformation.



Using this synthetic approach, it was difficult to produce anything but symmetrical diesters. The introduction of phosphinoacetic acid diamidites has significantly changed the synthesis and availability of this class of molecules.[3] Using acid activation of phosphonamidites with azole acids (such as tetrazole) phosphonoacetic acid diesters could be produced in greater than 95% stepwise yields; also resulting in the synthesis of phosphonoacetic acid modified DNA.



PHOSPHONOCARBOXYLATE OLIGONUCLEOTIDES

The original rationale for the synthesis of phosphonocarboxylate oligonucleotides was to evaluate whether they could be taken into cells through a receptor mediated endocytosis pathway similar to that which had been described for certain carboxylic acid containing glycopeptides.[13] It was thought that certain macromolecular glycopeptides are allowed to penetrate into the cytoplasm due to a high concentration of acid neutralizable carboxylate groups on their exterior. These macromolecules would first bind to cell surface receptors and be taken into an endosome. Acidification of the endocytic vesicles after fusion with lysosomes would result in protonation of carboxylic acid chains on the glycopeptides and this pH dependent charge neutralization would in turn allow for penetration of the macromolecule into the cytoplasm. The protonation of carboxylic acid residues that occurs during the acidification of endocytic vesicles has been implicated as a necessary step in various processes including receptor recycling, virus penetration, and the entry of diphtheria toxin into cells.[14] The question was whether this proposed mechanism for cellular uptake would function for carboxylic acid modified DNA sequences.

FIGURE 4: PHOSPHONOACETATE INTERNUCLEOTIDE LINKAGE

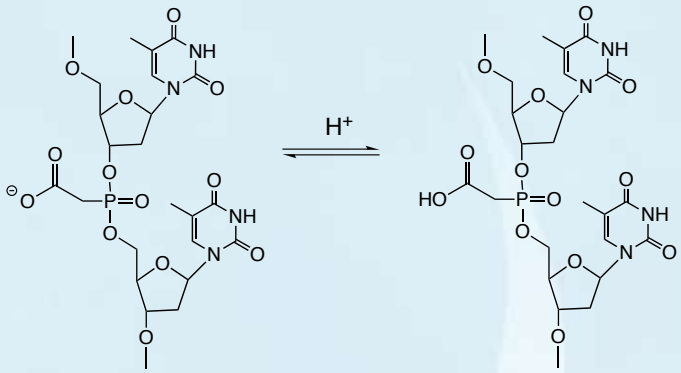
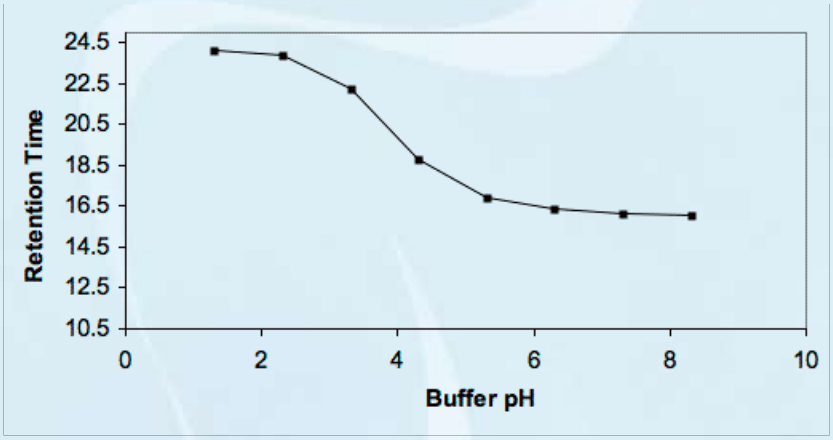


FIGURE 5: PLOT OF HPLC RETENTION TIME VS pH



PHOSPHONOACETATE OLIGONUCLEOTIDES

Mixed sequence phosphonoacetate oligonucleotides were first synthesized and characterized using dimethylcyanoethyl (DMCE) protected phosphinoacetic acid amidites.[15] The oligonucleotides were characterized by Sheehan, *et. al.*[16] and shown to have well behaved biochemical properties: they were highly soluble, hybridized in a sequence specific manner to both DNA and RNA, and were highly nuclease resistant.

This existence of the protonated and non-protonated forms in equilibrium on a PACE oligonucleotide was demonstrated by decreasing the pH with mild acid, resulting in greatly increased retention of PACE DNA on a reverse-phase HPLC column. The plot of HPLC retention time as a function of pH gave a sigmoidal curve similar to a titration curve (Figure 5).

A comparison of melting temperatures (ΔTm expressed as a change in °C per linkage) showed a loss of heteroduplex stability for PACE-DNA/DNA and PACE-

DNA/RNA relative to natural DNA/DNA and DNA/RNA duplexes, respectively. Losses of approximately 1.3° C per linkage for PACE-DNA/RNA duplexes and approximately 0.3° C for PACE-DNA/DNA duplexes were observed.

The most remarkable observation on PACE DNA was that the fully modified mixed sequence oligonucleotides, 18 to 21 nucleotides in length, demonstrated significantly enhanced uptake with cells in culture.[17] There were three specific observations made in these experiments.

First, in comparison to phosphorothioate DNA, or methylphosphonate DNA, a significant amount of PACE DNA was taken up by several cell types in the absence of cationic lipids (all cell types tested). This result was reproducible and verified using Fluorescence Assisted Cell Sorting (FACS) and confocal microscopy. Under these conditions, there was no reproducible cell uptake seen with either fluorescently labelled phosphorothioate DNA or fluorescently labelled methyl phosphonate DNA.

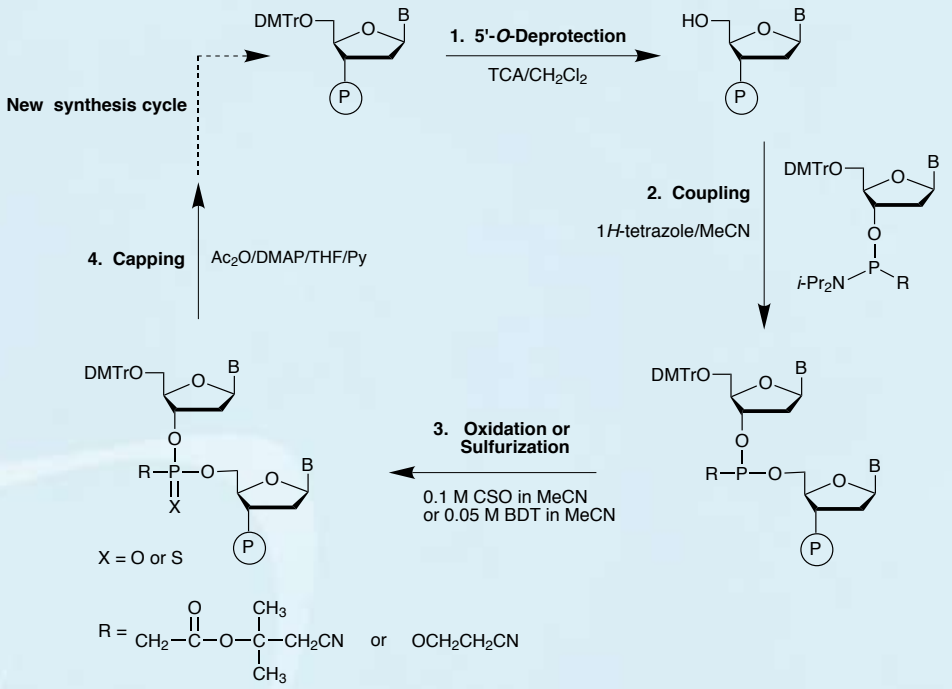
Second, in the presence of cationic lipids, PACE DNA sequences were taken up to a greater extent and with lower lipid concentration than required for normal DNA or phosphorothioate DNA. In most cases, the N to P ratio (the N/P ratio is a measure of the ionic balance of the transfection reagent/DNA complexes, referring to the number of nitrogen residues (ammonium) of the transfection reagent per DNA phosphate) using PACE DNA was allowed to be lowered to the point that little or no cell death could be observed with many cell types while retaining effective transfections.

Third, esterification of the carboxylic acid greatly enhanced the cell uptake of PACE DNA. Remarkably, in order to achieve complete transfections in all cell types evaluated, including JURKAT cells, only 50% of the PACE oligonucleotides needed to be esterified.

CONCLUSION

Phosphonoacetate (PACE) modified oligonucleotides show great potential as biological modifiers in a wide variety of research applications. Oligonucleotides containing this modification are easy to synthesize. The monomers can be easily incorporated into complex oligonucleotides and are compatible with a wide variety of other sugar or heterobase modifications. PACE DNA can be conjugated through the carboxylic acid functional group. They have been shown to be active in siRNA duplexes and accelerate the initial rate of cleavage by RNase H-1 when incorporated with phosphorothioates. However, the most interesting observation to date is that they exhibit an unprecedented enhancement in penetration of cultured cells.

FIGURE 6: PHOSPHONOACETATE SYNTHESIS CYCLE



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12. Gryaznov, S. M., Skorski, T., Cucco, C., Nieborowska-Skorska, M., Chiu, C. Y., Chen, J. K., Koziolkiewicz, M. Calabretta, B., *Proc. Natl Acad. Sci. USA*, 1995, **92**, 5798–5802.
13. Maxfield, F. R., Cell., 1982, **28**, 643-651; Tartakoff, A. M., *Cell.*,1983, **32**, 1026-1028.
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17. Yamada, C. M., Dellinger, D. J., Caruthers, M. H., *Nucleosides, Nucleotides, and Nucleic Acids*, 2007, **26**, 539–546.

SYNTHESIS, CLEAVAGE AND DEPROTECTION OF PACE OLIGONUCLEOTIDES

Synthesis

The general scheme for synthesis is shown in Figure 6, Page 4. The structures of the four monomers are shown in Figure 7. The phosphonoacetates are fully soluble in acetonitrile at a recommended concentration of 0.1M and are compatible with standard DNA synthesizers. A recommended coupling time of 33.3 minutes with 1H-Tetrazole is necessary when using the standard protocol. A modified LV cycle for AB instruments that reduces coupling time to 15 minutes with 1H-Tetrazole is available on our website.

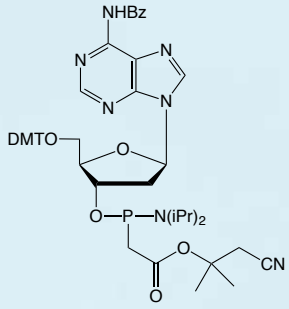
Oxidation must precede capping in the synthesis cycle. Reagents for oxidation depend on the type of synthesis. For fully modified oligos, we recommend the non-aqueous oxidizer camphorsulfonyloxaziridine (CSO) as a 0.1M solution. For mixed phosphodiester and phosphonoacetate modified oligos, a 0.5M CSO solution is recommended. Low water oxidizer, 40-4032, is an alternative oxidizing reagent although it has been reported that this can result in conversion of a small percentage of the phosphonoacetate to the phosphodiester. We also recommend the use of the Cap Mix B with DMAP (40-4020) instead of the standard Cap Mix B containing 1-Methylimidazole.

Cleavage and Deprotection

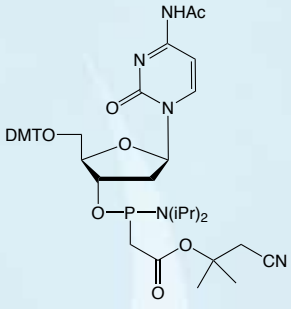
The standard protocol for cleavage and deprotection requires a two step method with pretreatment using 1,8-Diazabicyclo[5.4.0] undec-7-ene (DBU) and subsequent cleavage using methylamine. The DBU is used to deprotect the dimethylcyanoethyl (DMCE) protecting groups and to prevent alkylation of the bases during deprotection. Cleavage with 40% methylamine in water is recommended and we have also had good results when using AMA deprotection.

1. Prepare a 1.5% DBU solution in anhydrous acetonitrile.
2. Load a 1 mL plastic syringe with 1mL of solution.
3. Connect to one luer fitting of the synthesis column.
4. Connect a clean 1 mL syringe to the other luer fitting.
5. Carefully pass the solution through the column a few times.
6. Allow to stand at room temperature for 60 minutes.
7. Discard solution from syringe into waste.
8. Rinse with 3 x 5 mL of anhydrous acetonitrile and dry under stream of argon.
9. Carefully transfer CPG to clean 4 mL vial.
10. Add 1 mL 40% aqueous methylamine and heat to 55°C for 15 minutes. Avoid prolonged deprotection times.

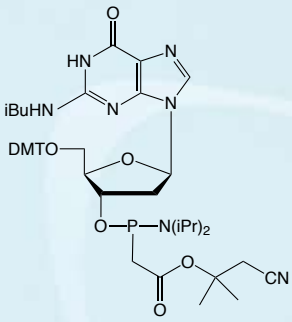
FIGURE 7: PHOSPHONOACETATE (PACE) MONOMERS



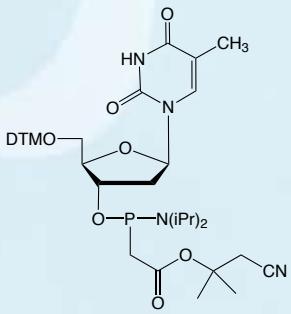
dA-PACE Phosphoramidite



Ac-dC-PACE Phosphoramidite



dG-PACE Phosphoramidite



dT-PACE Phosphoramidite

Purification and Desalting

DMT-On oligos can be purified using the standard Glen-Pak™ protocol. DMT-Off Oligos can be desalted using ethanol or butanol precipitation. The phosphonoacetate modified oligos form diastereomers that can make purification difficult by Ion-Exchange or RP HPLC. An oligo containing multiple incorporations will produce broad peaks on the chromatogram. If HPLC purification is desired, we recommend DMT-On purification by reverse phase HPLC with subsequent removal of the DMT protecting group.

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ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
dA-PACE Phosphoramidite	10-1140-02	0.25g	100.00
	10-1140-05	0.5g	200.00
	10-1140-10	1.0g	400.00
Ac-dC-PACE Phosphoramidite	10-1150-02	0.25g	100.00
	10-1150-05	0.5g	200.00
	10-1150-10	1.0g	400.00
dG-PACE Phosphoramidite	10-1160-02	0.25g	100.00
	10-1160-05	0.5g	200.00
	10-1160-10	1.0g	400.00
dT-PACE Phosphoramidite	10-1170-02	0.25g	100.00
	10-1170-05	0.5g	200.00
	10-1170-10	1.0g	400.00

NOVEL REAGENTS FOR MODIFICATION AND LABELLING

Reagents for modification and labelling of oligonucleotides have become ever more popular since these reagents allow synthetic oligonucleotides to be tagged at a variety of positions for a multitude of purposes. Our most popular non-nucleosidic phosphoramidites for modification and labelling are based on two structural types as shown in Figure 1: 1,2-diols and 1,3-diols. Figure 1 also shows BiotinTEG Phosphoramidite, an example of a structure based on a 1,2-diol backbone, and Biotin Phosphoramidite, an example of a 1,3-diol based structure. Products based on a 1,2-diol backbone were first described to allow amino-modification[1] and biotin labelling[2]. Of course, Glen Research was in the forefront of these developments, and rapidly offered products of this type for 3'-amino-modification, as well as a family of products for biotin, cholesterol, DNP and fluorescein labelling. Glen Research also introduced a family of products based on the competing 1,3-diol backbone[3], including a support for 3'-amino-modification and phosphoramidites and supports for acridine, biotin and fluorescein labelling.

Technically, the 1,2-diol backbone has some drawbacks relative to the 1,3-diol backbone. As shown in Figure 2, the 1,2-diol backbone can participate in a dephosphorylation reaction since the 1,2-diol can form a favored 5-membered cyclic phosphate intermediate. This reaction is competitive with simple hydrolysis of the protecting groups and leads to some loss of label. However, the degree of loss at the 3' terminus can be limited by the removal of the cyanoethyl protecting group using DBU or diethylamine prior to the cleavage and deprotection steps. Similarly, loss at the 5' terminus can be eliminated by retaining the DMT group until the oligo is fully deprotected. Fortunately, the elimination reaction is virtually non-existent in the 1,3-diol backbone since the cyclic intermediate would be a 6-membered ring which is not favored for a cyclic phosphate intermediate.

This large variety of products covering the modification and labelling spectrum addresses most of our customers' needs. However, recently, several of our IVD customers have requested a new backbone based on a 1,3-diol that would overcome any technical or IP issues surrounding our current products. The goal has been to

FIGURE 1: **STRUCTURES OF 1,2- AND 1,3- DIOL BACKBONES**

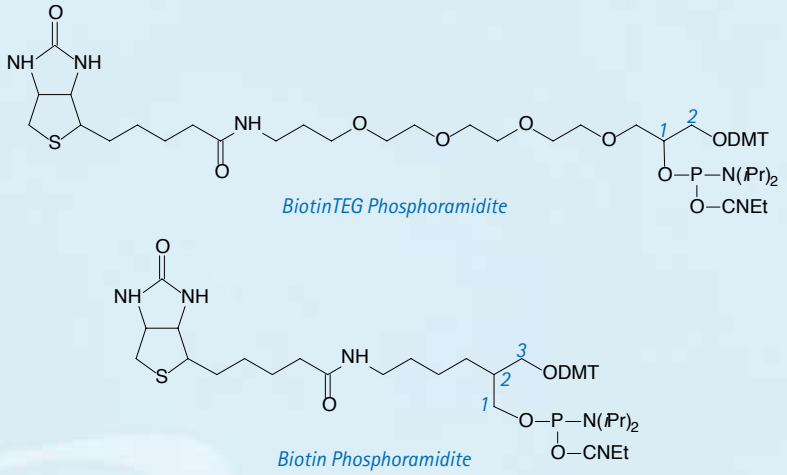
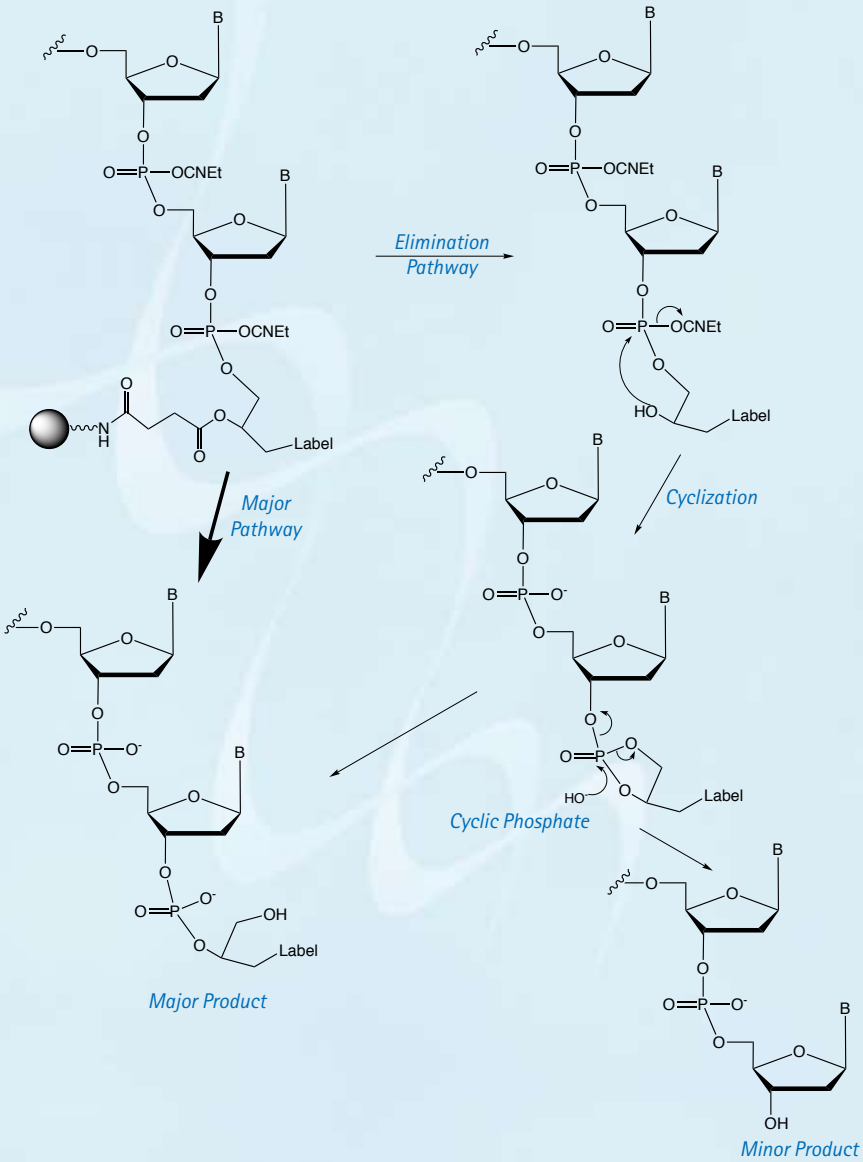


FIGURE 2: **COMPETITIVE ELIMINATION IN 1,2-DIOL BASED PRODUCTS**



replace the existing 1,3-diol product line with another of equivalent quality and performance with no IP concerns for IVD developers. At the same time, the new biotin structures should be protected to avoid any branching from the biotin molecule itself. Branching from biotin is not very significant for small-scale synthesis but becomes very significant at the larger synthesis scales required by IVD developers.

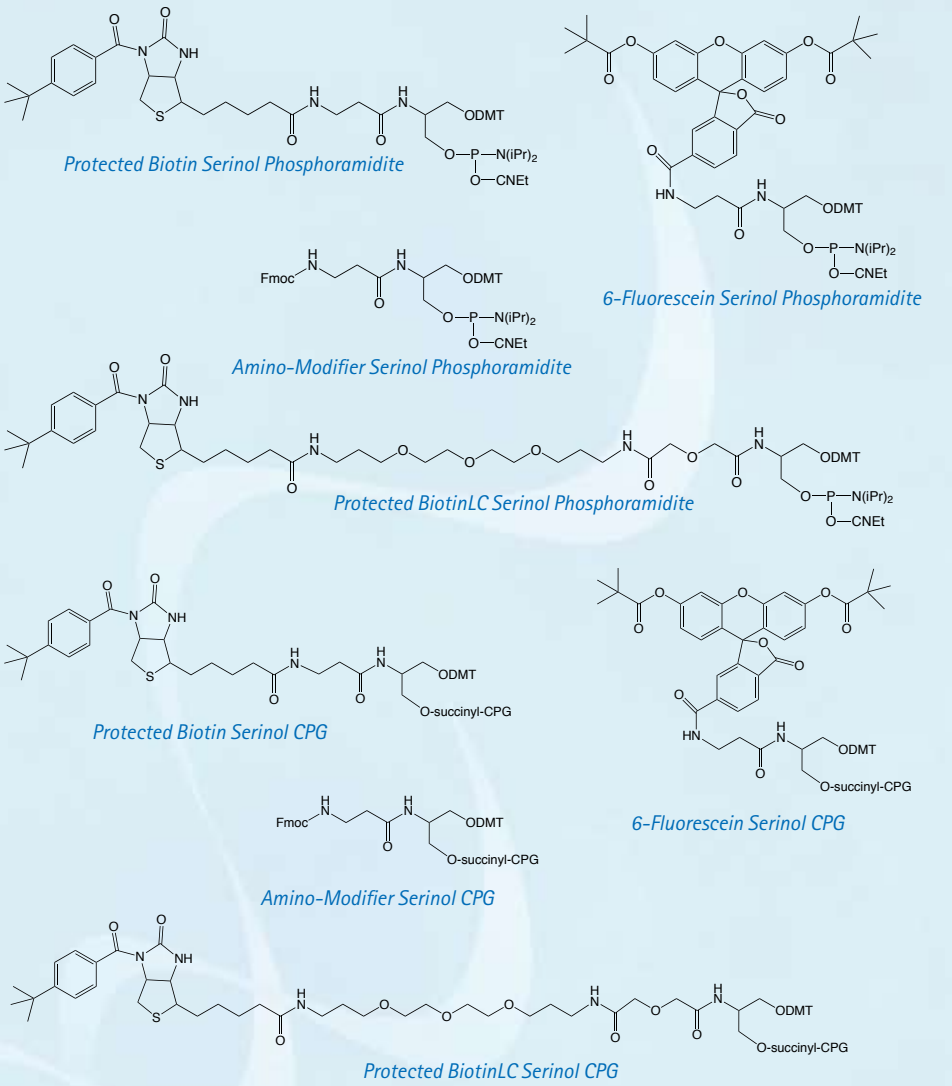
Our response is the line of products, shown in Figure 3, based on the serinol backbone. These products have been developed in close collaboration between Glen Research and Nelson Biotechnologies. At this early stage, we are offering phosphoramidites and supports for Amino-Modification, and Biotin and Fluorescein labelling. These products all exhibit the high purity and performance that is standard for all Glen Research products.

Protected Biotin Serinol Phosphoramidite and CPG are protected with a *t*-butylbenzoyl group on the biotin ring.[4] This group is designed to stop any phosphoramidite reactions at this active position in biotin. This protection avoids branching when using nucleophilic activators like DCI. The protecting group is easily removed during oligonucleotide cleavage and deprotection. The BiotinLC versions are similarly protected and should be useful for the synthesis of highly sensitive biotinylated probes. 6-Fluorescein Serinol Phosphoramidite and CPG are designed to prepare oligonucleotides containing one or several 6-Fluorescein (6-FAM) residues. Amino-Modifier Serinol Phosphoramidite and CPG are used to add amino groups into one or several positions in oligonucleotides. The amino group is protected with Fmoc, which may be removed on the synthesis column prior to solid-phase conjugation to the amino groups, or which may be removed during deprotection for subsequent solution phase conjugation to the amino groups. Further products based on this novel serinol-derived linker may be added in the future.

References:

1. P.S. Nelson, R. Sherman-Gold, and R. Leon, *Nucleic Acids Res.*, 1989, **17**, 7179-86.
2. K. Misiura, I. Durrant, M.R. Evans, and M.J. Gait, *Nucleic Acids Res.*, 1990, **18**, 4345-4354.
3. P.S. Nelson, M. Kent, and S. Muthini, *Nucleic Acids Res.*, 1992, **20**, 6253-6259.
4. U. Pieles, B.S. Sproat, and G.M. Lamm, *Nucleic Acids Res.*, 1990, **18**, 4355-4360.

FIGURE 3: **STRUCTURES OF SERINOL BASED MONOMERS AND SUPPORTS**



ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
Protected Biotin Serinol Phosphoramidite	10-1993-95	50 µmole	165.00
	10-1993-90	100 µmole	295.00
	10-1993-02	0.25g	675.00
6-Fluorescein Serinol Phosphoramidite	10-1994-95	50 µmole	165.00
	10-1994-90	100 µmole	295.00
	10-1994-02	0.25g	595.00
Protected BiotinLC Serinol Phosphoramidite	10-1995-95	50 µmole	205.00
	10-1995-90	100 µmole	365.00
	10-1995-02	0.25g	675.00
Amino-Modifier Serinol Phosphoramidite	10-1997-95	50 µmole	125.00
	10-1997-90	100 µmole	225.00
	10-1997-02	0.25g	595.00

(Continued on Back Page)

DEPROTECTION - VOLUME 1 - DEPROTECT TO COMPLETION

INTRODUCTION

This article offers practical information that will help newcomers to the field of oligo synthesis to understand the various considerations before choosing the optimal deprotection strategy, as well as the variety of options that are available for deprotection. It is not the intent of these articles to provide a comprehensive, fully referenced review of deprotection strategies in oligonucleotide synthesis - they are simply guidelines. For more detailed information, see, for example, the review[1] by Beaucage and Iyer. This is the first of a series of articles on deprotection that will be posted on our web site.

Oligo deprotection can be visualized in three parts: cleavage, phosphate deprotection, and base deprotection. Cleavage is removal from the support. Phosphate deprotection is the removal of the cyanoethyl protecting groups from the phosphate backbone. Base deprotection is the removal of the protecting groups on the bases or modifier. There are many considerations when approaching oligo deprotection, as shown in the Box on the right. However, when reviewing the procedures available to deprotect any oligonucleotide, you must heed the primary consideration: *First, Do No Harm*. You can then proceed with confidence to *Deprotect to Completion*.

FIRST, DO NO HARM!

Determination of the appropriate deprotection scheme should start with a review of the components of the oligonucleotide to determine if any group is sensitive to base and requires a mild deprotection or if there are any pretreatment requirements. Sensitive components are usually expensive components so it is imperative to follow the procedure we recommend for any individual component. For example, the presence of a dye like TAMRA or HEX will require a different procedure from regular unmodified oligonucleotides. Similarly, an oligo containing a base-labile monomer like 5,6-dihydro-dT will have to be treated according to the procedure that is noted on the product insert (Analytical Report, Certificate of Analysis, or Technical Bulletin). Occasionally, some products require a special pretreatment to prevent unwanted side reactions. For example, amino modifiers use a special diethylamine pretreatment to improve the overall yield of the amino-labelled oligo. If the oligo has several unusual components, you must follow the mildest procedure recommended and, yes, things can get complicated fast. Volume 2 will focus on this complex topic.

RNA deprotection is unique because of the necessity to retain the 2' protecting group during cleavage and base deprotection. 2'-OMe-RNA and 2'-F-RNA, however, are virtually identical to DNA during deprotection. But, if a hybrid oligonucleotide contains even a single RNA linkage (with the exception of a 3'-ribonucleoside linkage), the oligo must be treated as RNA. See the appropriate RNA deprotection protocols:
http://www.glenresearch.com/GlenReports/GR19-22.html
http://www.glenresearch.com/Technical/TB_TOM.pdf

Another consideration for potential harm is loss of trityl group during vacuum concentration of the oligo solution prior to purification, which will reduce product yield. During evaporation the heat should be turned off the vacuum concentrator to avoid loss of the DMT group. It should be noted that most DMT-on purification protocols, including Poly-Pak™ and Glen-Pak™, do not require evaporation of the deprotection solution prior to purification.

A unique case for potential harm is an oligonucleotide containing a 5'-amine protected with the MMT protecting group (e.g., 10-1906). In this situation, deprotection should not be carried out at > 37°C to avoid thermal loss of the MMT group.

CLEAVAGE

On classic synthesizers from Applied Biosystems, the cleavage of the oligo from the synthesis support can be carried out separately on the machine, prior to deprotection. As a result, many researchers still carry out the cleavage reaction separately and so the time required to do this is mentioned at the beginning of each Deprotection section. However, most researchers do a one step cleavage/deprotection reaction, which has the advantage of ensuring optimal yields. The only downside to this strategy is the fact that the basic solution at elevated temperatures will dissolve a small amount of silica from CPG and a white insoluble residue will be apparent if the deprotection solution is evaporated to dryness. However, any residual silicate is easily removed by filtration, desalting or any purification procedure.

VOLUME 1: DEPROTECT TO COMPLETION

1) Do I have very special components in my oligo or not?
2) Am I in a rush or not?
3) Do I have one or many oligos to treat?
4) Do I need/want to purify my oligo after deprotection or not?
5) Does my oligo contain RNA, 2'-OMe-RNA, or 2'-F-RNA linkages?

DEPROTECT TO COMPLETION

The rate-determining step in oligonucleotide synthesis is more than likely the removal of the protecting group on the G base. Ignore this at your peril since, traditionally, one of the most common reasons for poor performance of oligonucleotides is the presence of a small percentage of the G protecting groups remaining in the final product oligonucleotide. Chromatographic methods may miss the presence of the G protecting groups but these are readily revealed by mass spectral analysis. What are the options with attendant pros and cons for oligonucleotide deprotection?

Regular Deprotection

The cleavage reaction with concentrated ammonium hydroxide (28 to 33% NH_3 in water), if carried out separately, is normally considered to be 1 hour at room temperature. Deprotection using ammonium hydroxide is the most traditional method and dates back to the earliest days of oligonucleotide synthesis. One of the critical issues when using ammonium hydroxide, which is water saturated with ammonia gas, is to keep the solution fresh. We aliquot and store ammonium hydroxide in the refrigerator in portions appropriate for use in 1 week. Using an old bottle of ammonium hydroxide is false economy since the resulting oligos are not going to be completely deprotected.

Table 1

dG Protection	Temperature	Time
iBu-dG	RT	36h
	55°C	16h
	65°C	8h
dmf-dG , Ac-dG	RT	16h
	55°C	4h
	65°C	2h
iPr-Pac-dG	RT	2h
	55°C	0.5h

Table 1, Page 8 shows the various times and temperatures appropriate for deprotection with FRESH ammonium hydroxide.

UltraFAST Deprotection

Using the UltraFAST procedure, cleavage of the oligonucleotide from the support is performed using AMA[2] which is a 1:1 mixture (v/v) of aqueous Ammonium hydroxide and aqueous MethylAmine. If carried out separately, it is accomplished in 5 minutes at room temperature.

UltraFAST deprotection allows 5-10 minute deprotection of oligonucleotides using AMA. It is important to note that the UltraFAST system requires acetyl (Ac) protected dC to avoid base modification at the C base if Bz-dC is used. The three other monomers remain unchanged and the system works equally well with iBu-, Ac-, or dmf-dG, the last being our preferred dG phosphoramidite.

The deprotection step is carried out at 65°C for 5 minutes. Deprotection can also be carried out at lower temperatures as shown in Table 2. In all cases, no base modification has been observed.

Figure 1 illustrates the differences in RP HPLC between partially and fully deprotected oligos, DMT-off and DMT-on.

Table 2

dG Protection	Temperature	Time
iBu-dG, dmf-dG or	RT	120 min.
Ac-dG	37°C	30 min.
	55°C	10 min.
	65°C	5 min.

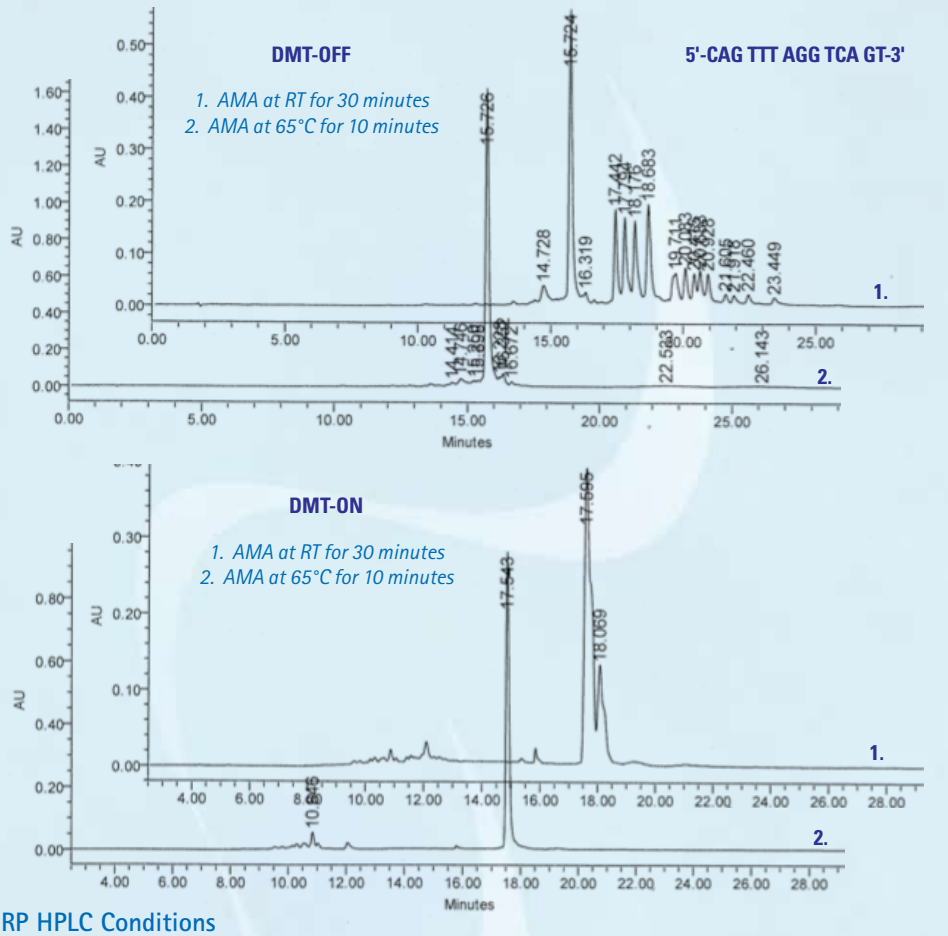
Note: UltraFAST system requires acetyl (Ac) protected dC to avoid base modification at the C base.

UltraMILD Deprotection

Cleavage is not carried out separately when using UltraMILD techniques. Since many of our nucleosides and dye products are not stable to deprotection with ammonium hydroxide or AMA, the procedure to deprotect the labelled oligonucleotide must be changed.

We often recommend using the UltraMILD monomers (Pac-dA, Ac-dC and iPr- Pac-dG) and deprotection with potassium carbonate in methanol. In this way, some of these very sensitive oligonucleotides can be conveniently isolated. If capping is carried out using Cap A containing phenoxyacetic anhydride, it is possible to deprotect UltraMILD oligonucleotides in 4 hours at RT with 0.05M potassium carbonate in methanol or 2 hours at RT with ammonium hydroxide. Alternatively, using the regular Cap A containing acetic anhydride, it is necessary to deprotect overnight at room temperature to remove any Ac-dG formed during the capping step. For TAMRA containing oligonucleotides, an alternative deprotection[3] may be carried out using t-butylamine/methanol/water (1:1:2) overnight at 55°C. Another option that we have found to be excellent uses t-butylamine/water (1:3) for 6 hours at 60°C. In this case, the regular protecting groups on the monomers may be used. An even milder approach has been described as "Ultra-UltraMild".[4] In this technique, Q-supports[5] are combined with UltraMild monomers to allow extremely gentle deprotection. After completion of the synthesis, the solid support is dried and treated overnight at 55°C with a solution containing 10% (v/v) diisopropylamine (iPr_2NH) in 0.25 M ß-mercaptoethanol in MeOH.

FIGURE 1: **RP HPLC OF AN OLIGO 1. PARTIALLY AND 2. FULLY DEPROTECTED, DMT-OFF AND DMT-ON**



RP HPLC Conditions

Column: Waters X-Bridge C18, 250 x 4mm; Buffers: A - ACN; B - 0.1M TEAA , pH 7.
DMT-off Gradient: 3-15% B over 15 min. DMT-on Gradient: 3-40% B over 15 min. Flow rate: 1mL/min.

SUMMARY

Successful oligonucleotide cleavage and deprotection require consideration of the deprotection conditions for each product and some products may require pretreatment or special deprotection conditions. Each synthesis should be reviewed to ensure the products have compatible deprotection conditions. Special deprotection requirements can be found on our Analytical Reports, Certificates of Analysis, Technical Bulletins, and Website: http://www.glenresearch.com.

References:

(1) S.L. Beaucage and R.P. Iyer, *Tetrahedron*, 1992, **48**, 2223-2311.
(2) M.P. Reddy, N.B. Hanna, and F. Farooqui, *Nucleos Nucleot*, 1997, **16**, 1589-1598.
(3) B. Mullah and A. Andrus, *Tetrahedron Lett*, 1997, **38**, 5751-5754.
(4) L.C.J. Gillet, J. Alzeer, and O.D. Scharer, *Nucleic Acid Res*, 2005, **33**, 1961-1969.
(5) R.T. Pon, and S.Y. Yu, *Nucleic Acids Res*, 1997, **25**, 3629-3635.

NEW UNIVERSAL SUPPORT - GLEN UNYSUPPORT

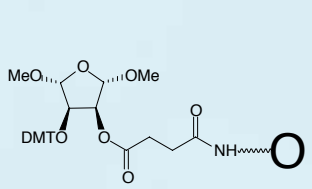
In 1997, Glen Research introduced our first Universal Support, popularly known as the McLean support, (1) in Figure 1.[1] In principle, this support allows every oligonucleotide synthesis to take place on a single support without the need for a support for every desired 3' nucleoside. The support found favor in high-throughput oligonucleotide synthesis platforms and became the universal support of choice for deprotection using gaseous methylamine. However, since the phosphate elimination (dephosphorylation) reaction to yield a free 3'-hydroxyl group requires quite aggressive conditions, as outlined in Table 1, this product has been somewhat limited in its applications. The main problem was the fact that the product could not be used to prepare oligos with some of our most popular nucleoside analogues at the 3' terminus since many of these are not compatible with the required aggressive dephosphorylation conditions. Hence, we felt it was time to investigate other supports to determine if an alternative would offer better overall properties.

Our original Universal Support (1) is one of a family of universal supports where the cleavage from the support is followed by deprotection and dephosphorylation.[2] Usually in this type of support (Type 1), dephosphorylation is the slowest step. The process is illustrated in Figure 2.

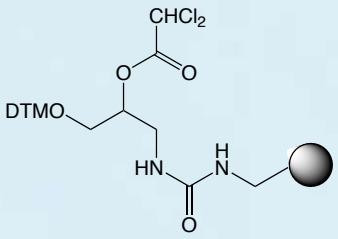
Glen Research's work on universal supports has culminated in the development of Universal Support III, (2) in Figure 1. US III is unique among universal supports in that the dephosphorylation reaction occurs first and leads to cleavage from the support (Type 2).[2] This reaction occurs with mild anhydrous methanolic ammonia, or even with the UltraMild deprotection solution of anhydrous potassium carbonate in methanol. Oligonucleotide deprotection can then be carried out using your favorite procedure - AMA, ammonium hydroxide, and all other common methods. The process is illustrated in Figure 3, Page 11.

Why is there a need for any universal support other than our US III? After all, US III is truly universal in that it can be used for ANY oligonucleotide synthesis, from DNA with very sensitive bases, through siRNA, to exceptionally sensitive labelled probes. However, the one minor issue with US III is that it is not readily compatible with gas phase cleavage and deprotection using anhydrous methylamine gas, and this strategy is used in many high-throughput

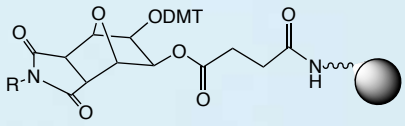
FIGURE 1: UNIVERSAL SUPPORT STRUCTURES



Universal Support (1)



Universal Support III (2)



UnyLinker™ (R=Ph)
Glen UnySupport (R=Me) (3)

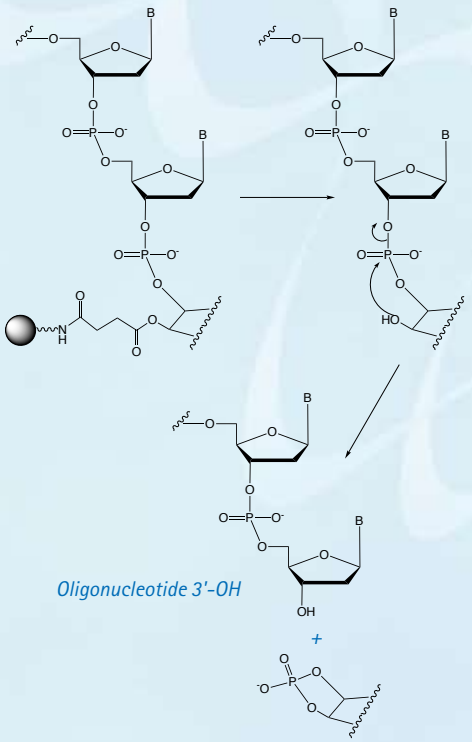
TABLE 1: ELIMINATION CONDITIONS - UNIVERSAL SUPPORT (1)

Reagent	Conditions
Ammonium hydroxide	80°C/8h min.
Ammonium hydroxide/ 40% Methylamine (AMA)	55°C/17h 80°C/3h min.
40% Methylamine	55°C/8h
0.4M NaOH in methanol/water (4:1)	80°C/0.5h 55°C/3h RT/17h

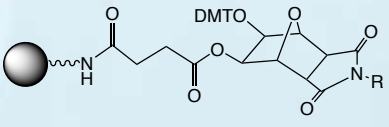
TABLE 2: ELIMINATION CONDITIONS - GLEN UNYSUPPORT (3)

Reagent	Conditions
Ammonium hydroxide	80°C/2h 55°C/8h
Ammonium hydroxide/ 40% Methylamine (AMA)	80°C/0.5h 65°C/1h 55°C/2h

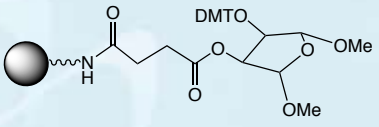
FIGURE 2: ELIMINATION MECHANISM FOR TYPE 1 UNIVERSAL SUPPORTS



Oligonucleotide 3'-OH



UnyLinker™ (R=Ph)
Glen UnySupport (R=Me) (3)



Universal Support (1)

situations. It appears that the elimination of cyanoethyl protecting groups is favored in anhydrous methylamine gas. Once the cyanoethyl protecting group is eliminated to give the phosphodiester group, the amide-assisted dephosphorylation reaction to give the desired 3'-OH grinds to a halt. The yield of isolated oligonucleotide therefore suffers.

Our original Universal Support (1) is compatible with deprotection using anhydrous methylamine gas and has been used extensively, but the time for complete dephosphorylation is longer than most companies wish to allocate. A recent development has been the use of a support based on a molecule which is "conformationally preorganized" to accelerate the dephosphorylation reaction.[3,4] By using a rigid bicyclic molecule on the support (3) in Figure 1, Page 10, it was hoped that the molecule's conformation would facilitate the formation of the cyclic phosphate transition state, thereby stimulating the rate of dephosphorylation, as shown in Figure 2, Page 10. And, indeed, it was found that the rate of elimination is markedly faster than the original Universal Support (1), as shown in Table 2, Page 10.

The structure of Glen UnySupport is shown, (3) in Figure 1, Page 10. The phenyl version, developed at Isis Pharmaceuticals as UnyLinker™, is available from several companies for large scale oligo synthesis. Glen UnySupport is the methyl version, which is preferred for high throughput oligonucleotide synthesis since methylamine rather than aniline is formed on deprotection. We are happy to offer Glen UnySupport in a variety of popular formats under license from Isis Pharmaceuticals.

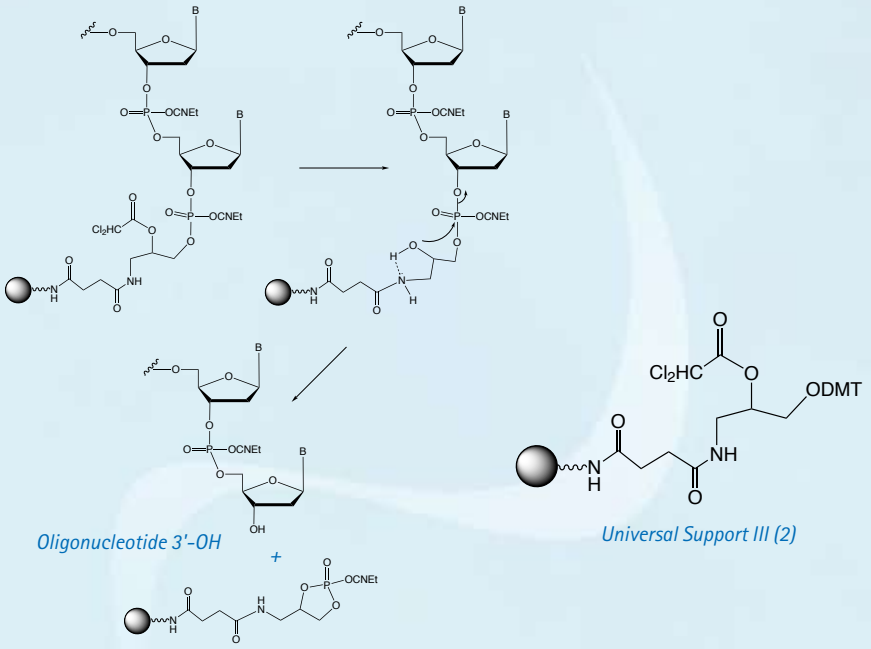
References:

1. S. Scott, P. Hardy, R.C. Sheppard, and M.J. McLean, in A universal support for oligonucleotide synthesis, Innovations and Perspectives in Solid Phase Synthesis, 3rd International Symposium, 1994; R. Epton, Ed. Mayflower Worldwide: 1994; pp 115-124.
2. A. Azhayev, M. Antopolsky, T.M. Tennila, H. Mackie, and J.B. Randolph, *GEN*, 2005, **25**.
3. A.P. Guzaev, and M. Manoharan, ***J Am Chem Soc***, 2003, **125**, 2380-2381.
4. R.K. Kumar, A.P. Guzaev, C. Rentel, and V.T. Ravikumar, *Tetrahedron*, 2006, **62**, 4528.

INTELLECTUAL PROPERTY

This product is covered by US Patent 7,202,264 owned by Isis Pharmaceuticals, Inc..

FIGURE 3: **AMIDE ASSISTED ELIMINATION OF TYPE 2 UNIVERSAL SUPPORT III**



ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
Bulk Support			
Glen UnySupport	20-5040-01	0.1g	11.00
(500Å CPG)	20-5040-02	0.25g	25.00
	20-5040-10	1.0g	95.00
ABI Format (not LV)			
1 µmole columns	20-5140-41	Pack of 4	60.00
0.2 µmole columns	20-5140-42	Pack of 4	40.00
40 nmole columns	20-5140-45	Pack of 4	40.00
10 µmole column (TWIST Format)	20-5140-13	Pack of 1	100.00
AB 3900 Format			
Glen UnySupport PS			
1 µmole columns	26-5140-51	Pack of 10	150.00
0.2 µmole columns	26-5140-52	Pack of 10	100.00
40 nmole columns	26-5140-55	Pack of 10	100.00
Expedite Format			
1 µmole columns	20-5240-41	Pack of 4	60.00
0.2 µmole columns	20-5240-42	Pack of 4	40.00
40 nmole columns	20-5240-45	Pack of 4	40.00
15 µmole column (TWIST Format)	20-5240-14	Pack of 1	150.00
96 Well Format (MerMade, etc.)			
1 µmole columns	20-5140-91	Pack of 96	375.00
0.2 µmole columns	20-5140-92	Pack of 96	250.00
40 nmole columns	20-5140-95	Pack of 96	250.00
Bulk Support			
Glen UnySupport	20-5041-01	0.1g	11.00
(1000Å CPG)	20-5041-02	0.25g	25.00
	20-5041-10	1.0g	95.00

NEW PRODUCTS FOR ATTACHMENT OF OLIGONUCLEOTIDES ON GOLD SURFACES

There has been a lot of development during recent years directed at the application of miniaturization technologies to molecular biology. The ultimate goal is to perform fast molecular tests on microchips. Such microarrays can be fabricated using a variety of technologies, including printing with fine-pointed pins onto glass slides, photolithography with or without pre-made masks, ink-jet printing, or electrochemistry on a microelectrode. These varying technologies allow the manufacturing of microarrays with hundreds, thousands or even millions of specific sequences on solid surfaces that can be glass, silica or gold layered on plastic supports with an area of only 1–2 cm^2.[1,2]

The common factors among all microarrays are:

(i) Oligonucleotides must be irreversibly attached to the solid surface.

(ii) Hybridization events must be capable of measurement with high accuracy, sensitivity and selectivity, and, if possible in a quantitative way.

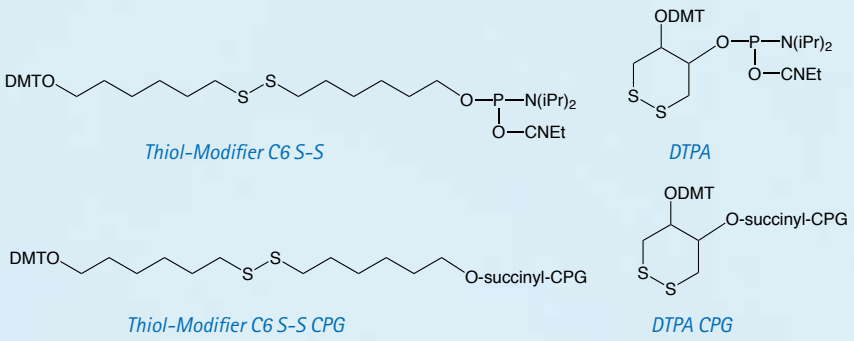
Typically, high-density arrays use fluorescence to detect hybridization and such setups are widely commercially available. But there is another technology progressively emerging, which is electrical biosensor detection.[3] Osmetech, for example, received FDA clearance in July 2008 for its Warfarin Sensitivity Test and new eSensor® XT-8 platform that uses electrochemical detection and oligos attached to gold surfaces.[4]

In most cases, electrochemical detection implies that oligos are attached on a gold surface. Typically, oligos are attached on a gold surface using thiol groups forming a self-assembled monolayer (SAM).[5] SAMs are molecular layers formed on a surface when it is immersed in a solution containing molecules that specifically interact with this surface. Although different molecules can be immobilized (silanes, carboxylic acids, pyridines, sulphites and thiols) on different surfaces (gold, silver, platinum, copper, mercury and glass), thiols are the most commonly used especially in conjunction with gold surfaces. The stability and organization of the SAMs depend on the forces of attraction between the immobilized molecules, the interaction between terminal groups and their local environment, and the binding force between the surface and the binding group. Oligonucleotide SAMs can be formed directly on the surface when the oligonucleotides contain a pendant thiol group. Or, attachment can be to reactive and previously formed SAMs via EDC. The direct strategy reduces the number of steps required for immobilization and avoids the EDC reaction. However, the indirect strategy (SAMs + EDC) is an alternative when non-saturated monolayers are desired.[6,7,8]

This immobilization of nucleic acids onto substrates is complex and crucial to the performance of the microarray since (i) the capture probe has to form a stable bond to the substrate, (ii) the spacing of the capture probes has to allow specific binding of the target, (iii) nonspecific adsorption of the material to be arrayed has to be prevented. Consequently, chemisorption of thiols on gold (electrodes) is a common and simple procedure to immobilize probes on a surface. The gold–sulfur bond with a binding energy of about 30–45 kcal/mol (cf. at least 100–150 kcal/mol for a covalent bond) is relatively weak in order to anchor a biopolymer onto a surface.[9] As reported, mono-functional thiol-terminated oligonucleotides immobilized on a surface are slowly lost at temperatures between 60 and 90 °C and in the presence of buffers with high salt concentration[10] and are almost completely displaced from the surface when treated with biological buffer systems containing, *e.g.*, dithiothreitol or mercaptoethanol.[11,12]

In 2003, Glen Research introduced Dithiol Phosphoramidite (DTPA, 10-1937) as a way to attach more than one thiol residue at the extremity of an oligonucleotide. Considering the success of this product, we have now decided to offer a solid support for the easy introduction of this molecule at the 3'-end of an oligonucleotide. Combining the support and the amidite, it is possible

FIGURE 1: STRUCTURES OF DISULFIDE REAGENTS



Thiol-Modifier C6 S-S *DTPA*

Thiol-Modifier C6 S-S CPG *DTPA CPG*

FIGURE 2: SYNTHESIS OF MULTIPLE DTPA UNITS AT THE 3' TERMINUS

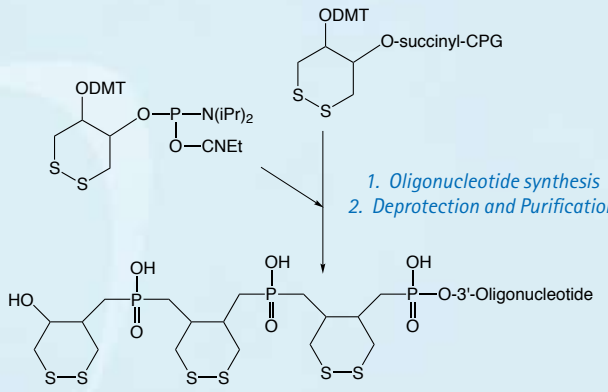
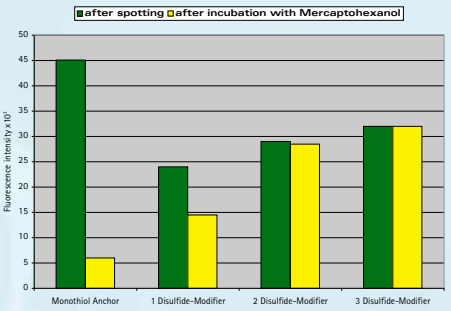


FIGURE 3: OLIGO FLUORESCENCE INTENSITIES



Fluorescence intensities of oligonucleotides anchored on a gold surface by different immobilization groups before and after mercaptohexanol (1mM) treatment.[9]

to introduce more than one residue, so multiple dithiol residues, conferring an order of magnitude greater stability to the oligo-gold link, can be easily produced. This has been studied in detail[9] and is illustrated in Figure 3, Page 12. This support can be used as any other support and is available on 1000Å CPG. As shown in Figure 2, Page 12, by combining this support and the existing phosphoramidite, it is easy to create a dithiol tail at the 3'-end of an oligonucleotide. The bond between DTPA and the gold surface is formed spontaneously without need of prior reduction of the disulfide bond. That is the beauty of this product. However, it is important that the gold surface is very clean. Piranha solutions (FRIZ Biochem) may be used for cleaning gold electrodes.

We are also pleased to provide a product that completes our roster, 3'-Thiol-Modifier C6 S-S CPG. We have offered Thiol-Modifier C6 S-S Phosphoramidite (10-1936) for many years, but we only offered the C3 S-S support. We are now introducing the C6 S-S CPG support, also on 1000Å CPG, for convenient 3'-thiol labelling of oligonucleotides. The structure is shown in Figure 1, Page 12.

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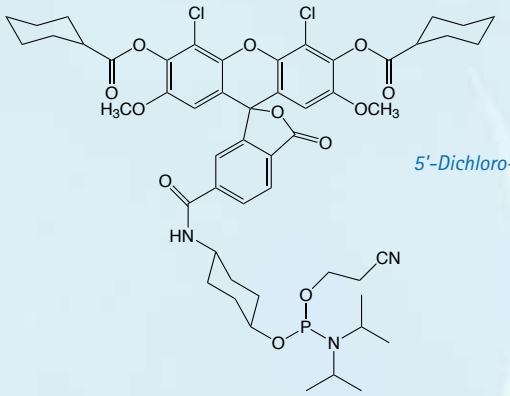
5'-DICHLORO-DIMETHOXY-FLUORESCEIN (JOE™) PHOSPHORAMIDITE

FIGURE 1: **STRUCTURE OF DICHLORO-DIMETHOXY-FLUORESCEIN PHOSPHORAMIDITE**



5'-Dichloro-dimethoxy-Fluorescein

Fluorescent labelling is now at the forefront of DNA detection and sequencing. As such, Glen Research is always happy to expand our repertoire of fluorescent phosphoramidite reagents in order to help our customers find a fluorophore with just the spectral characteristics they desire. The latest addition to our line of dyes is 5'-Dichloro-dimethoxy-fluorescein Phosphoramidite.

The 5'-Dichloro-dimethoxy-fluorescein dye is more commonly known as JOE™ to those familiar with the dye sets used in Applied Biosystems DNA PRISM sequencers - e.g., Dye Set 20 and 32, which contain FAM/JOE/TAMRA/ROX and FAM/JOE/NED/ROX, respectively.

As a dye, it gained popularity because its emission is nicely resolved from both FAM and TAMRA, falling squarely between them. This allows for multiplex detection without too much signal bleed through into other channels, making it extremely useful in automated DNA sequencing. In addition, because of the electron-withdrawing groups on the xanthene ring, the 5'-Dichloro-dimethoxy-fluorescein dye is less prone to quenching due to protonation. As such, its fluorescence is much less pH sensitive than its popular cousin, fluorescein. Even at pH 5, our in-house testing indicates 5'-Dichloro-dimethoxy-fluorescein's signal dropped by only 30%.

With its high extinction coefficient of 75,000 L/mol.cm, a quantum yield of fluorescence of 0.58[1], and excellent stability to standard deprotection conditions in ammonium hydroxide, we are sure the 5'-Dichloro-dimethoxy-fluorescein Phosphoramidite will be a popular addition to our ever-expanding selection of fluorophore phosphoramidites.

Spectral Characteristics

E260 12,000 L/mol.cm Absorbance 525 nm
Emax 75,000 L/mol.cm Emission 548 nm
QY 0.58

Reference:

1. J. Ju, C. Ruan, C.W. Fuller, A.N. Glazer, and R.A. Mathies, *Proc Natl Acad Sci U S A*, 1995, **92**, 4347-51.

ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
3'-Thiol-Modifier C6 S-S CPG	20-2936-01	0.1g	85.00
	20-2936-10	1.0g	600.00
1 µmole columns	20-2936-41	Pack of 4	125.00
0.2 µmole columns	20-2936-42	Pack of 4	75.00
10 µmole column (ABI)	20-2936-13	Pack of 1	225.00
15 µmole column (Expedite)	20-2936-14	Pack of 1	350.00
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	20-2937-10	1.0g	895.00
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0.2 µmole columns	20-2937-42	Pack of 4	110.00
10 µmole column (ABI)	20-2937-13	Pack of 1	335.00
15 µmole column (Expedite)	20-2937-14	Pack of 1	520.00
5'-Dichloro-dimethoxy-Fluorescein Phosphoramidite (JOE)	10-5904-95	50 µmole	175.00
	10-5904-90	100 µmole	340.00
	10-5904-02	0.25g	775.00

SITE-SPECIFIC INCORPORATION OF FUNCTIONAL COMPONENTS INTO RNA BY TRANSCRIPTION USING UNNATURAL BASE PAIR SYSTEMS

Introduction

RNA-based research and biotechnology are growing rapidly and extensively. In the process, researchers have long sought a method for allowing the site-specific incorporation of extra components, with a functional group of interest, into desired positions of RNA molecules. Such a method could be provided by creating extra, unnatural base pairs to augment the natural A-T and G-C pairs of DNA, and to expand the genetic alphabet[1-4]. Hirao's group has now developed the unnatural base pairs between 7-(2-thienyl)-imidazo[4,5-b] pyridine (Ds) and pyrrole-2-carbaldehyde (Pa)[5], and 2-amino-6-thienylpurine (s) and Pa[6], which can be utilized in transcription for the site-specific, enzymatic incorporation of functional components into RNA by T7 RNA polymerase. The Ds-Pa pair complementarily mediates the incorporation of the triphosphate substrates of Ds (DsTP) and Pa (PaTP) into RNA by T7 transcription. A series of modified Pa bases, bearing functional groups attached to position 4 of Pa via an aminopropynyl linker, is also incorporated into RNA opposite Ds in DNA templates. Furthermore, Pa can be used as a template base for the enzymatic incorporation of the fluorescent s base, as a triphosphate substrate (sTP), into RNA. Glen Research has already begun offering the amidites of dDs and dPa for DNA template synthesis. (See Figure 1 and *Glen Report*, Vol. **20**, No. 1 in 2008). We are now supplying Biotin PaTP and sTP for their incorporation by T7 transcription (Figure 1).

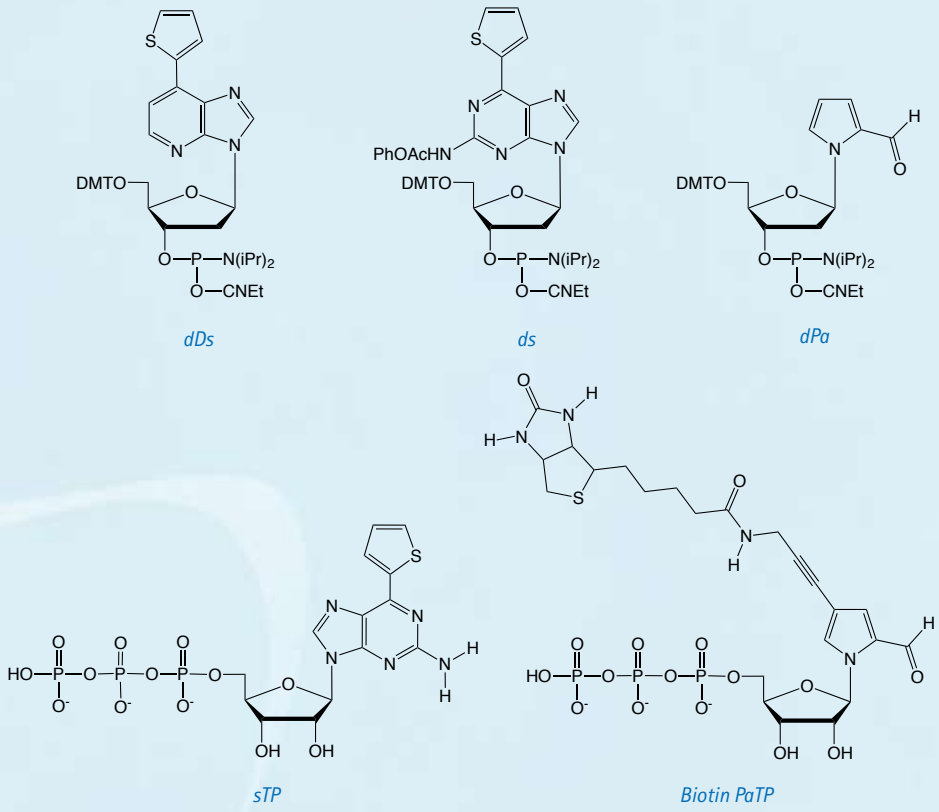
Biotin PaTP

Biotinylated RNA molecules are routinely used for immobilization on avidin supports or for chemiluminescent detection, using streptavidin coupled to alkaline phosphatase. Biotin PaTP can be site-specifically incorporated into RNA, opposite dDs at a desired position in DNA templates, by T7 transcription[5]. Thus, this method facilitates the immobilization and detection of a target RNA without the loss of the intrinsic activity of the RNA molecule.

sTP

The s base is strongly fluorescent, absorbing at 299 and 352 nm and emitting at 434 nm, with a quantum yield of 0.41 at pH 7.07. The fluorescent intensity of

FIGURE 1: **STRUCTURES OF PHOSPHORAMIDITES AND TRIPHOSPHATES OF UNNATURAL BASES**



APPLICATIONS OF NON-NATURAL NUCLEOSIDES

Local Structural Analysis of DNA

- Use ds Amidite
- Introduce s base in single stranded DNA
- Fluorescence intensity of ds decreases when ds-containing oligo hybridizes
- Fluorescence ds probing is useful for analyzing oligonucleotide behavior in many practical applications
- FRET experiments possible
- *Tetrahedron*, 2007, **63**, 3528-3537.

Local Structural Analysis of RNA

- sTP / dPa system
- Make DNA Template containing dPa using amidite
- Produce fluorescent RNA using sTP during transcription
- Fluorescence intensity of s decreases when s-containing RNA hybridizes or forms structures, allowing local structure analysis
- *Nucleic Acids Res.*, 2007, **35**, 5360-5369.

Site-specific Biotinylation of RNA

- The dDs / Biotin PaTP system
- PCR amplify a target RNA using a pair of primers - one primer introduces T7 promoter and the other contains dDs analogue
- PCR amplification produces a DNA template with dDs
- T7 transcription using Biotin PaTP produces RNA containing a Biotin at the specific position of Ds
- *Nature Methods*, 2006, **3**, 729-735.

s in DNA and RNA molecules changes according to the structural environment, and is quenched depending on the degree of stacking interactions with adjacent bases[6,7]. Since the stacking manner in RNA molecules directly reflects their tertiary structure, site-specific s labeling is useful for studying the dynamics of the local structural features and changes of RNA molecules. The fluorescent s base can be site-specifically incorporated into RNA opposite dPa in DNA templates, by T7 transcription.

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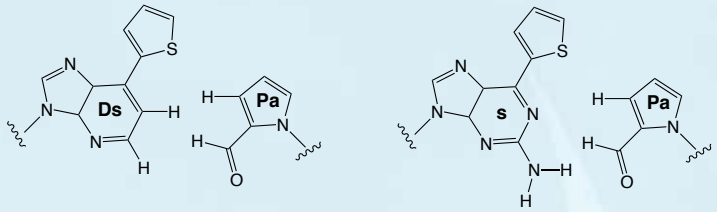
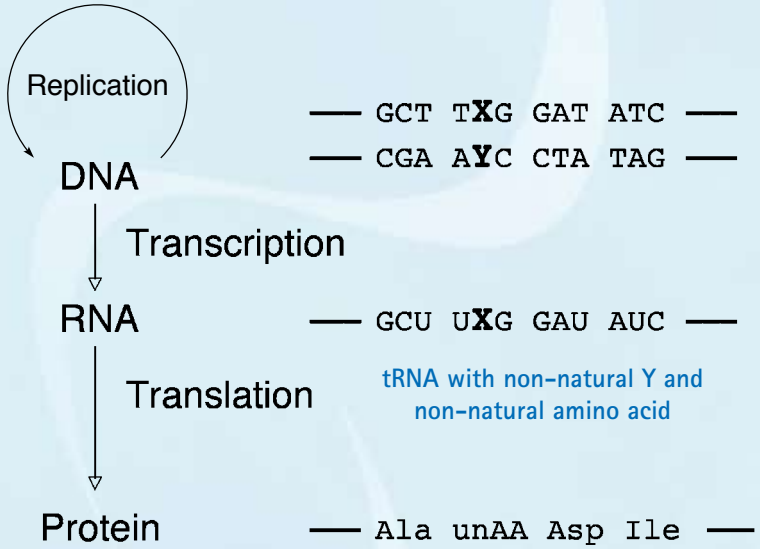
FIGURE 2: UNNATURAL BASE PAIRS



POTENTIAL APPLICATIONS OF NON-NATURAL NUCLEOSIDES



- With an unnatural base pair [X-Y] functioning in replication, transcription and translation, it becomes possible to incorporate nucleotide analogs at specific sites into RNA and introduce amino acid analogues (unAA) into proteins.
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- *Nucleic Acids Res.*, 2002, **30**, 4692-4699.

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Item	*Catalog No.*	*Pack*	*Price($)*
dDs-CE Phosphoramidite	10-1521-90	100 µmole	145.00
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Pac-ds-CE Phosphoramidite	10-1522-90	100 µmole	170.00
	10-1522-02	0.25g	420.00
dPa-CE Phosphoramidite	10-1523-90	100 µmole	130.00
	10-1523-02	0.25g	420.00
sTP 10mM	81-3522-02	25 µL	350.00
Biotin PaTP 10mM	81-3525-02	25 µL	450.00

(Continued from Page 5)

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0.2 µmole columns	20-2993-42	Pack of 4	120.00
1 µmole columns	20-2993-41	Pack of 4	200.00
10 µmole column (ABI)	20-2993-13	Pack of 1	300.00
15 µmole column (Expedite)	20-2993-14	Pack of 1	450.00
3'-6-Fluorescein Serinol CPG	20-2994-01	0.1g	120.00
	20-2994-10	1.0g	995.00
0.2 µmole columns	20-2994-42	Pack of 4	120.00
1 µmole columns	20-2994-41	Pack of 4	200.00
10 µmole column (ABI)	20-2994-13	Pack of 1	300.00
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3'-Protected BiotinLC Serinol CPG	20-2995-01	0.1g	120.00
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1 µmole columns	20-2995-41	Pack of 4	200.00
10 µmole column (ABI)	20-2995-13	Pack of 1	300.00
15 µmole column (Expedite)	20-2995-14	Pack of 1	450.00
3'-Amino-Modifier Serinol CPG	20-2997-01	0.1g	95.00
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