The Glen Report

22825 DAVIS DRIVE • STERLING, VIRGINIA • 20164

GLEN RESEARCH

VOLUME 20

NUMBER 1

MAY 2008

INSIDE

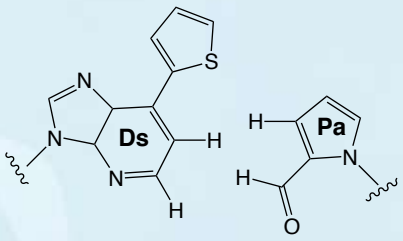
AN UNNATURAL BASE PAIR SYSTEM FOR THE EXPANSION OF GENETIC INFORMATION

Expansion of the genetic alphabet by unnatural base pair systems is an attractive future biotechnology.[1] DNA fragments containing unnatural base pairs function as templates for the site-specific incorporation of extra components into nucleic acids and proteins by biosyntheses, according to the Central Dogma of molecular biology - replication, transcription, and translation. Nucleic acids and proteins with extra components at specific positions could exhibit novel or increased functionality, which would be useful for both basic research and applied sciences. Several researchers have been actively developing such unnatural base pairs, some of which display unique abilities in duplex DNA and in nucleic acid and protein biosyntheses.[2-5] Glen Research is already a source for nucleoside derivatives related to unnatural base pairs, such as dP, dK, 5-Me-isodC, and isodG. Now we are adding to our lineup the amidites of dDs, dPa, and ds, the elements for a new unnatural base pair system.

DS--PA BASE PAIR

The unnatural base pair between 7-(2-thienyl)-imidazo[4,5-b]pyridine (Ds) and pyrrole-2-carbaldehyde (Pa) is formed by specific hydrophobic shape complementation. The shape of the Ds-Pa pair is different from those of the natural A-T and G-C pairs, but the Ds-Pa pair works together with the natural pairs in *in vitro* replication and transcription (Figures 1 and 2 on Page 2).[6] The Ds-Pa pair complementarily functions as a template base in DNA fragments for exonuclease-proficient (exo+) DNA polymerases, such as the Klenow fragment and Vent DNA polymerase for replication, as well as T7 RNA polymerase for transcription. In replication by the Klenow fragment (exo+), the triphosphate substrates of the unnatural bases, dDsTP and dPaTP, are incorporated into DNA opposite the respective Pa and Ds bases in the template strands under the conventional conditions. In addition, the DNA fragments containing the Ds-Pa pair can be amplified by PCR using Vent DNA polymerase. This PCR amplification requires[6] modified substrates, γ-amidotriphosphates of Ds and A, instead of their corresponding triphosphate substrates, to increase the fidelity. Under specific conditions, the total mutation rate of the Ds-Pa site in DNA fragments after 10 PCR cycles was estimated to be ~1%.

UNNATURAL DS-PA BASE PAIR



Base Pair Relies on Hydrophobicity and Shape

The Ds-Pa pair also complementarily mediates[6] the site-specific incorporation of Ds, Pa, and modified Pa bases into RNA by conventional T7 transcription. For example, the ribonucleoside triphosphate of Pa (PaTP) or biotin-linked Pa (Biotin-PaTP) can be incorporated at desired positions in RNA molecules opposite Ds in DNA templates by T7 RNA polymerase. Biotin-linked Pa is used for the immobilization of RNA molecules on avidin supports and also as a chemiluminescent marker, using streptavidin coupled to alkaline phosphatase, to detect RNA molecules and their interactions with other molecules.

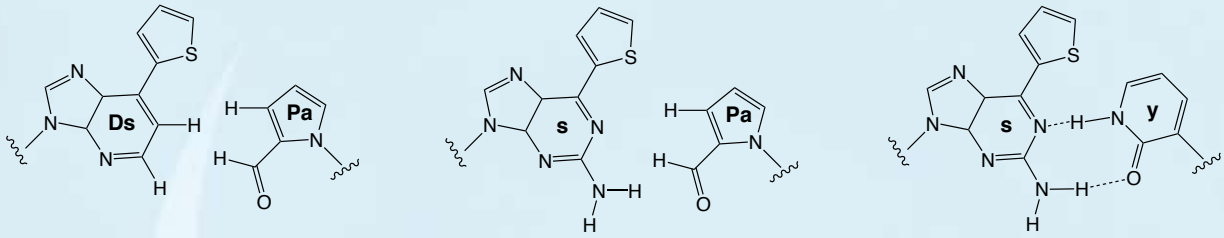
S--PA BASE PAIR

Pa also functions as a template base for incorporating another unnatural base (Figures 1 and 2 on Page 2), 2-amino-6-(2-thienyl)purine (s), into RNA.[6] As described below, the s base acts as a unique fluorescent base analog in DNA and RNA fragments.

(Continued on Page 2)

(Continued from Front Page)

FIGURE 1: THE UNNATURAL DS-PA, S-PA, AND S-Y PAIRS.

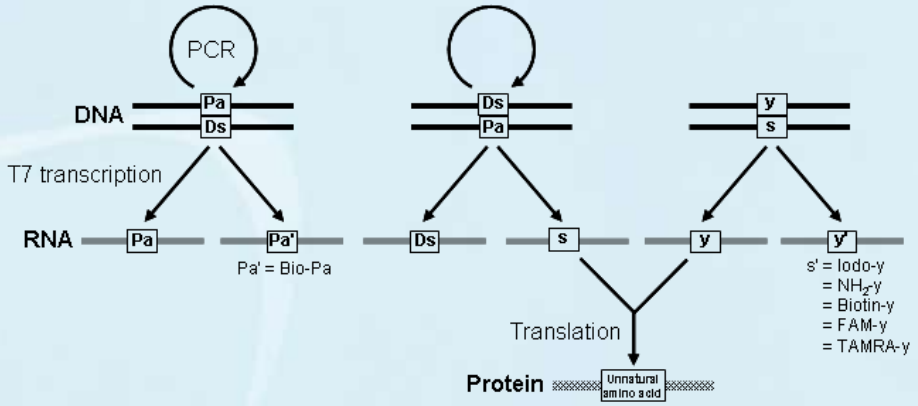


S--Y BASE PAIR

The s base was originally developed as a pairing partner of 2-oxopyridine (y), and the s-y pair is compatible with both transcription and translation (Figures 1 and 2).[7] The substrate of y (yTP) for transcription is site-specifically incorporated into RNA opposite s in DNA templates by T7 RNA polymerase. Modified y bases, such as iodo-, aminoalkyl-, biotin-linked, and fluorophore-linked y bases, can also be incorporated into RNA by specific transcription using s-containing DNA templates.[8] Iodo-y is a photosensitive component that is capable of cross-linking upon irradiation at 312 nm,[9] and aminoalkyl-y is useful for post-transcriptional modification at the aminoalkyl-y incorporation site in RNA molecules.[10] Biotin- or fluorophore-linked y was used in an RNA aptamer to facilitate the detection of the interaction between the aptamer and its target molecules.[10,11]

RNA molecules containing y at a specific position can be used as mRNA for the expansion of the genetic code, in which novel codons containing y are assigned to unnatural amino acids. The combination of this specific transcription with *in vitro* translation systems enables protein synthesis incorporating extra amino acids with functional groups of interest. In the translation system, tRNA with an s-containing anticodon is required to interact with the corresponding y-containing codon in mRNA. The preparation of the s-containing tRNA molecules is accomplished by T7 transcription using Pa-containing DNA templates and sTP by mediating the s-Pa pairing, in which Pa is the more efficient template base than y for the s incorporation into RNA.[12]

FIGURE 2: AN UNNATURAL BASE PAIR SYSTEM FOR REPLICATION, TRANSCRIPTION, AND TRANSLATION.



FLUORESCENCE PROPERTIES OF S BASE

Interestingly, the s base itself is strongly fluorescent (excitation: 299 or 352 nm; emission: 434 nm; quantum yield: 41%).[12,13] The fluorescent intensity of s in oligonucleotides decreases upon stacking with neighboring bases, and thus the fluorescent profiles reflect the local structure at the labeling site of the oligonucleotides. In addition, fluorescence resonance energy transfer (FRET) is observed between a low-fluorescence state of s and a high-fluorescence state of another fluorophore-linked y, such as 5-carboxyfluorescein-linked y (FAM-y), in oligonucleotides.[12] Using these abilities of s, researchers can design a variety of ingenious structural analyses of DNA and RNA molecules.

OLIGONUCLEOTIDE SYNTHESIS

DNA fragments containing Ds or Pa are prepared by DNA chemical synthesis using the dDs- or dPa-amidite by the usual phosphoramidite methods.

DNA fragments containing s are prepared by DNA chemical synthesis using the ds-amidite, in which the amino group is protected with a phenoxyacetyl group. We, therefore, recommend the use of phenoxyacetic anhydride (Pac_2O) in Cap A for the capping process during DNA synthesis. This is because the use of the usual acetic anhydride capping reagent exchanges some of the phenoxyacetyl protecting groups on s with the acetyl groups. RNA fragments containing s are prepared by T7 transcription using sTP and Pa-containing DNA templates.[13]

Initially, Glen Research will supply researchers with dDs-, dPa-, and ds-amidites (Figure 3 on Page 3) for DNA chemical synthesis. Glen Research is indebted to TagCyx Biotechnologies for incorporating these phosphoramidites into our Agreement. TagCyx Biotechnologies has licenses from RIKEN and The University of Tokyo (for Ds and Pa) and from Japan Science and Technology Agency (for s).

We are indebted to Professor Hirao for providing us with text for this article.

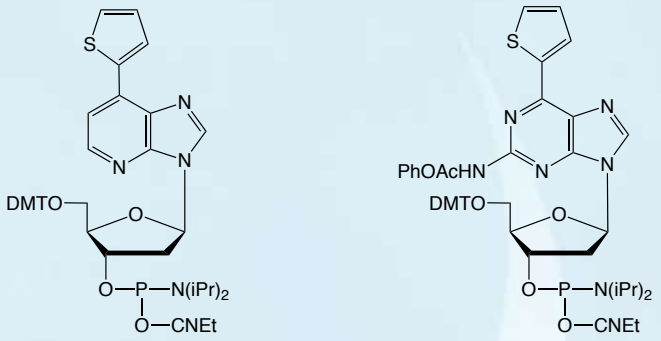
It is our pleasure to inform our readers that some triphosphates mentioned in this

article will shortly be available from Glen Research. Specifically, sTP and Biotin-PaTP will be added to our catalog in the near future. Some others will follow at a later date. Please check our web site for further updates. Also, subscribe to our e-mail newsletter by sending an e-mail to custserv@glenres.com to learn immediately about the release of these triphosphates.

REFERENCES:

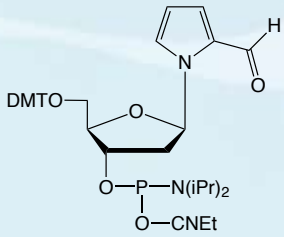
1. S.A. Benner, P. Burgstaller, T.R. Battersby, and S. Jurczyk, Did the RNA world exploit an expanded genetic alphabet? In *The RNA World*, Gesteland. R.F.; Cech T.R.; Atkins J.F., Eds. Cold Spring Harbor Laboratory Press: 1999; pp 163-181.
2. J.D. Bain, C. Switzer, A.R. Chamberlin, and S.A. Benner, *Nature*, 1992, **356**, 537-9.
3. E.T. Kool, *Curr Opin Chem Biol*, 2000, **4**, 602-608.
4. A.A. Henry, and F.E. Romesberg, *Curr Opin Chem Biol*, 2003, **7**, 727-33.
5. I. Hirao, *Curr Opin Chem Biol*, 2006, **10**, 622-7.
6. I. Hirao, et al., *Nat Methods*, 2006, **3**, 729-35.
7. I. Hirao, et al., *Nat Biotechnol*, 2002, **20**, 177-82.
8. I. Hirao, *Biotechniques*, 2006, **40**, 711-717.
9. M. Kimoto, et al., *Chem Biol*, 2004, **11**, 47-55.
10. R. Kawai, et al., *J Am Chem Soc*, 2005, **127**, 17286-95.
11. K. Moriyama, M. Kimoto, T. Mitsui, S. Yokoyama, and I. Hirao, *Nucl. Acids Res.*, 2005, **33**, e129.
12. T.Mitsui, M.Kimoto, R.Kawai, S.Yokoyama, and I.Hirao, *Tetrahedron*, 2007, **35**, 5360-5369.
13. M. Kimoto, et al., *Nucl. Acids Res.*, 2007, **35**, 5360-5369.

FIGURE 3: **STRUCTURES OF NEW DNA PHOSPHORAMIDITES OF UNNATURAL BASES**



dDs

ds



dPa

ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
dDs-CE Phosphoramidite	10-1521-90	100 µmole	145.00
	10-1521-02	0.25g	420.00
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

INTELLECTUAL PROPERTY

This product is covered by patents or patents pending owned by TagCyx Biotechnologies. Purchase of this product includes a limited license to use this product solely for research. This license specifically excludes: (a) therapeutic or diagnostic applications (including products or services that incorporate this product), (b) any *in vivo* toxicity/safety study in support of an investigational new drug application (or foreign counterpart), (c) resale, or (d) gene functionalization activities (including products or services that incorporate data derived from gene functionalization activities) if such activities have commercial application. All of the above require a separate license from TagCyx Biotechnologies. Neither this product nor any product created through its use may be used in human clinical trials.

THIOPHOSPHORAMIDITES AND THEIR USE IN SYNTHESIZING OLIGONUCLEOTIDE PHOSPHORODITHIOATE LINKAGES

Xianbin Yang
AM Biotechnologies, 6023 Ave S.
Galveston, Texas 77551

INTRODUCTION

Replacing two non-bridging oxygen atoms with sulfur atoms in a DNA phosphodiester linkage creates a phosphorodithioate (PS2) linkage.[1] Like natural DNA, the phosphorodithioate linkage is achiral at phosphorus. Additionally, recent research has demonstrated that this analog is completely resistant to nuclease degradation and forms complexes with DNA and RNA with somewhat reduced stabilities.[2] Moreover, it has been found that PS2-ODNs bind proteins with a higher affinity than their phosphodiester analogues[2-6] suggesting that PS2-ODNs may have additional utility in the form of sulfur-modified phosphate ester aptamers (thioaptamers)[3,6-8] for therapeutic and diagnostic applications. The biological interest and promise of the PS2-ODNs has spawned a variety of strategies for synthesizing, isolating, characterizing and purifying this compound over the past two decades.[9] However, the thiophosphoramidite building blocks necessary to synthesize a PS2-ODN have only now become commercially available after recent work at AM Biotechnologies (www.thioaptamer.com). This article highlights a working protocol that enables the widespread use of thiophosphoramidites for the synthesis of PS2-ODNs.

MATERIALS AND METHODS

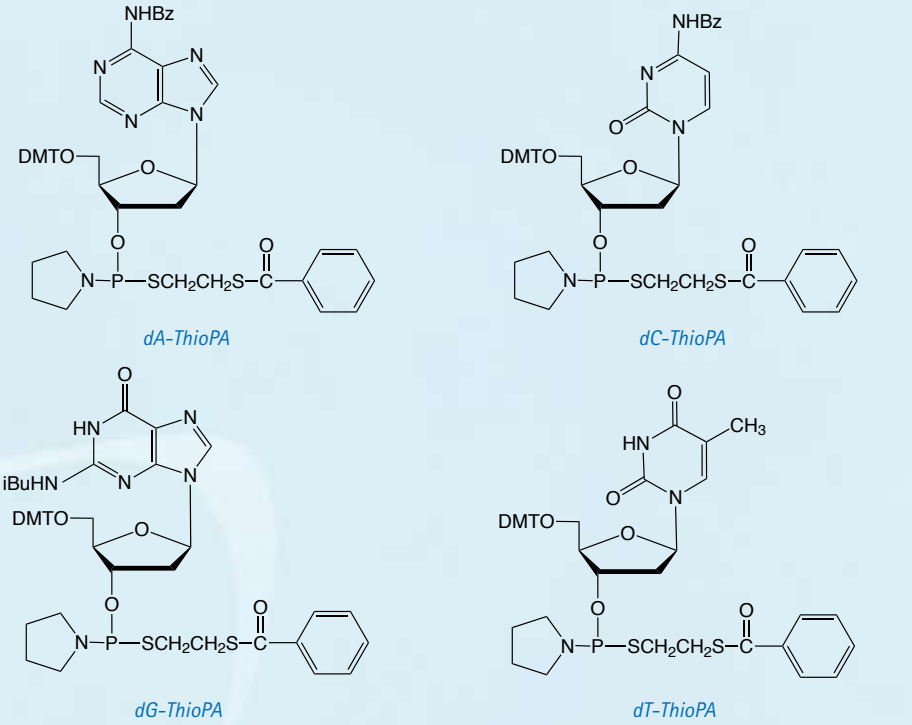
Chemicals

Synthesis columns, thiophosphoramidites, and synthesis reagents were obtained from Glen Research, Sterling, VA. Dichloromethane (DCM) anhydrous (#270997), 3Å Molecular Sieve beads (8-12 mesh, #208582), DL-dithiothreitol (#D9779-1G), Ammonium hydroxide (#338818), HPLC grade ethanol (#459828) were from Sigma-Aldrich.

Protocol or guidelines

The structures of thiophosphoramidites (thioPA) are shown in Figure 1. Like normal DNA and RNA phosphoramidites, the dried solid form of the thioPAs is very stable at -20°C for at least one year based on ^{31}P-NMR analysis with no observed reduction in reactivity for synthesizing the PS2 linkage. However, a few simple modifications to standard synthesis protocols are necessary when using the thioPAs.

FIGURE 1: **STRUCTURES OF THE THIOPHOSPHORAMDITES (THIOPA) DA, DC, DG AND DT**



(1) Unlike normal DNA phosphoramidites, the thioPAs are not completely soluble in anhydrous acetonitrile diluent. Rather, 10% DCM (v/v) in acetonitrile is an ideal diluent for all four of the thioPAs for a final amidite concentration of 0.15 M.

(2) Additionally, while normal DNA phosphoramidites are very stable in anhydrous acetonitrile at room temperature, the thioPAs are somewhat less stable in anhydrous acetonitrile containing 10% DCM; however, the coupling efficiency of all four thioPAs is not reduced after two days in solution at room temperature. Therefore, for best results the thioPA solution should be used within two days.

(3) To avoid the slight chance of some solid precipitant in the reagent bottle, the thioPA bottle on the synthesizer should be replaced with one containing acetonitrile diluent shortly after the completion of the synthesis. Flushing the synthesizer line with acetonitrile is highly recommended.

A SYNTHESIS CYCLE FOR A PS2 LINKAGE

A typical cycle for the solid-phase synthesis of a PS2 linkage is different from a standard cycle for the synthesis of normal phosphate linkages. For the PS2 linkage synthesis, following 3% TCA detritylation of a 2'-deoxynucleoside linked to the solid support, the thioPA is coupled to the free 5' hydroxyl group on the support using an activator such as DCI or tetrazole. The resulting thiophosphite triester is then sulfurized by either 3-ethoxy-1,2,4-dithiazolidine-5-one (EDITH) or 3-((N,N-dimethyl-aminomethylidene) amino)-3H-1,2,4-dithiazole-5-thione (DDTT) reagent. Unreacted support-linked 2'-deoxynucleoside is then capped, which completes the cycle for addition of the nucleotide.

THIOPHOSPHORAMIDITE ACTIVATORS

Tetrazole has been commonly used as an activator of choice for phosphoramidite

chemistry. However, unpublished studies have shown that tetrazole is not the most efficient activator for the thioPA coupling reaction. It is imperative to use an efficient activator during commercial PS2 production.

Activators that are commercially available include 5-ethylthio-1H-tetrazole (ETT), 5-benzylthio-1H-tetrazole (BTT), 4,5-dicyanoimidazole (DCI), and 5-(bis-3.5-trifluoromethylphenyl)-1H-tetrazole (Activator-42). The coupling efficiency of all of these activators during PS2 synthesis was compared on an Expedite 8909 DNA synthesizer. Significant differences were observed, which suggests that the thioPA activation mechanism differs from that of the normal phosphoramidite. Therefore, a practical protocol for use on an Expedite 8909 DNA synthesizer was developed for the commonly used tetrazole activator; however, another protocol for the DCI activator, which is more efficient for PS2 linkage synthesis, was also developed.

(a) Use of Tetrazole as a thioPA Activator

(http://www.glenresearch.com/Technical/Expedite_8909_PS2_Tetrazole_Protocol.PDF)

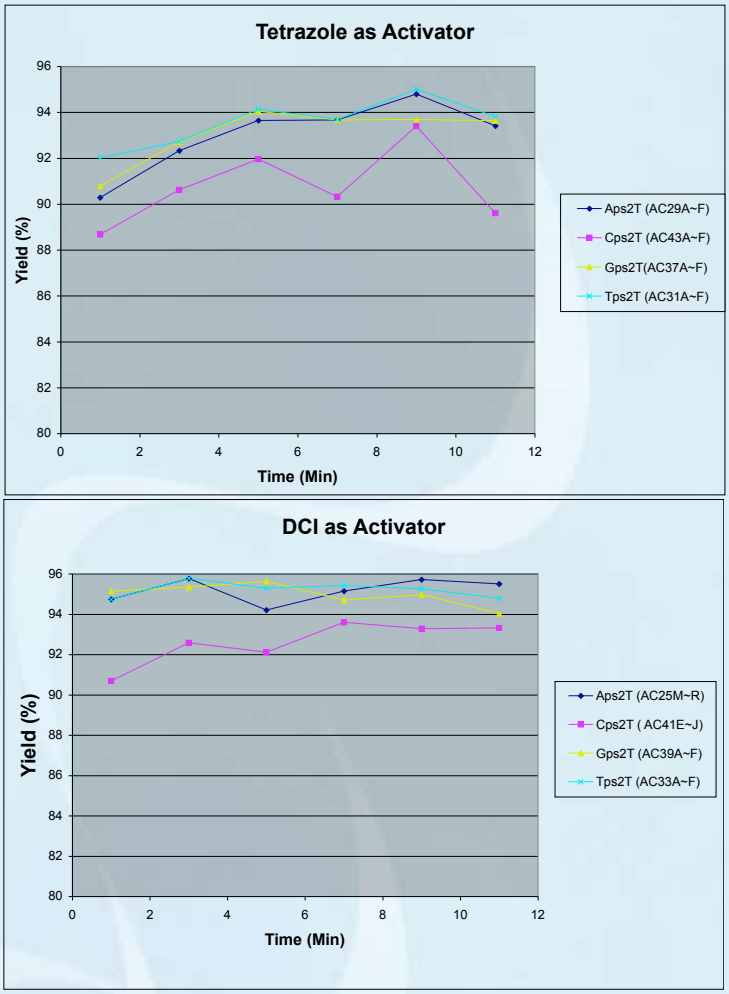
A dimer model sequence, 5'-X_{PS2}T-3', was used to study the thioPA coupling efficiency. Tetrazole was used as the activator and DDTT as a sulfurizing reagent. The experiments demonstrated that to achieve > 93% coupling yield for all of the thioPAs, a coupling time of 9 minutes was required. Even the dC-thioamidite exhibited a very slow rate (Figure 2) of PS2 linkage formation using tetrazole.

(b) Use of DCI as a thioPA Activator

(http://www.glenresearch.com/Technical/Expedite_8909_PS2_DCI_Protocol.PDF

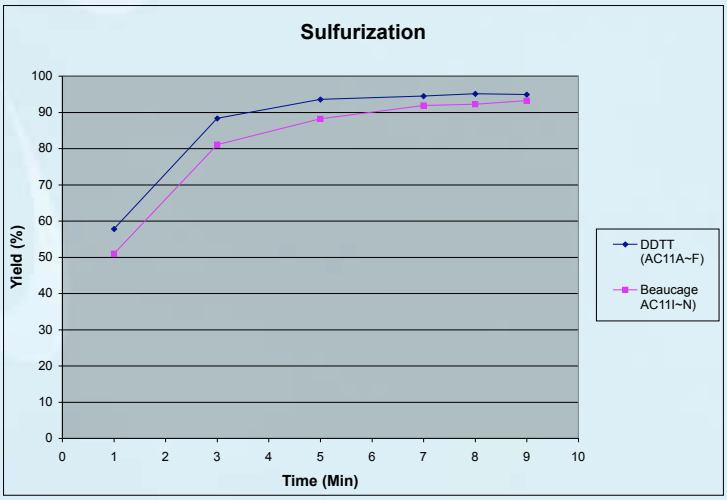
DCI is emerging as an alternative activator to tetrazole. DCI is soluble in acetonitrile up to 1.1 M at room temperature and experiments with thioPAs have shown that DCI decreases the time required to achieve up to 96% coupling yield by a factor of three as compared with tetrazole (Figure 2).

FIGURE 2: COUPLING EFFICIENCY USING TETRAZOLE OR DCI AS ACTIVATOR.



Coupling efficiency using Tetrazole or DCI as activator. ThioPA concentration: 0.15 M in acetonitrile containing 10% dichloromethane; the volume of thioPA: 0.125 mL.

FIGURE 3: SULFURIZATION EFFICIENCY COMPARISON USING DDTT *VS* BEAUCAGE REAGENT.



Sulfurization efficiency comparison for the synthesis of 5'-G_{PS2}T-3' by using DDTT vs Beaucage Reagent. dG-thiophosphoramidite concentration: 0.15 M in CH_3CN containing 10% DCM; the volume of thioPA: 0.125 mL.

SULFURIZING REAGENTS IN THE SYNTHESIS OF PS2 LINKAGE

Beaucage Reagent is a very popular sulfurizing agent for the synthesis of phosphoromonothioate linkages using normal phosphoramidites. When Beaucage Reagent was used with the thioPAs to synthesize PS2 linkages, it was observed that the by-product formed in the sulfurization reaction oxidizes the thiophosphite triester. This oxidation leads to phosphoromonothioate by-products, thus lowering the desired product yield and complicating the purification of the desired product. In addition, Beaucage Reagent has a tendency to precipitate from solution and clog the solvent and reagent transfer lines of a DNA synthesizer. DDTT is another sulfurizing agent primarily used to synthesize RNA phosphoromonothioates; however, it can be used to synthesize DNA phosphoromonothioates as well. Comparing DDTT efficiency to Beaucage Reagent in sulfurizing PS2 linkages, it was found that DDTT has slightly better sulfurizing reactivity than Beaucage Reagent (Figure 3); however, importantly, DDTT reduces the formation of phosphoromonothioate linkages during the synthesis of PS2 linkages thus increasing product yield.

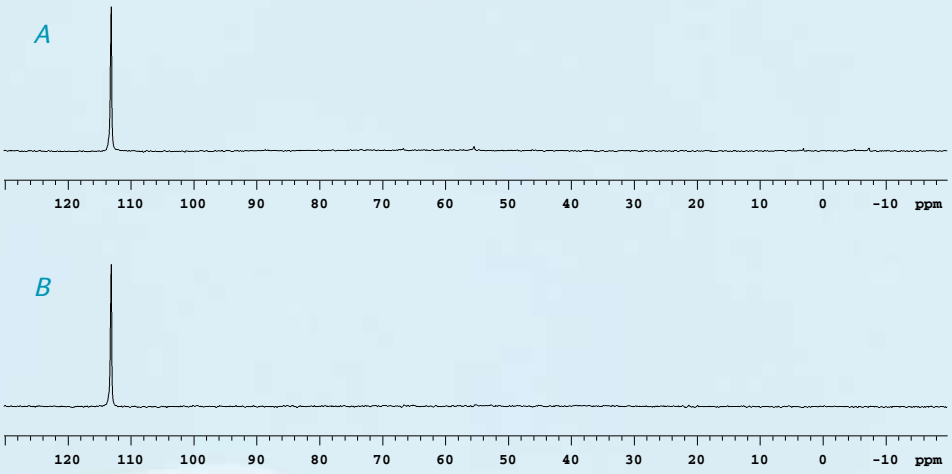
CLEAVAGE FROM THE SUPPORT AND REMOVAL OF BASE, PHOSPHATE AND THIOPHOSPHATE PROTECTING GROUPS

Upon completion of the automated synthesis, the support was removed from the synthesizer and dried with argon. The support was transferred into a 4 mL sealable vial where 1 mL of concentrated ammonia:ethanol (3:1, V:V) mix containing 20 mM DTT was added to the vial. The vial was sealed and incubated at 55 °C for 15-16 h. After the vial was removed from the oven and cooled to room temperature, the solution was transferred to a larger vial and 4~5 mL of distilled water was added. Solvents were removed by lyophilization.

EXAMPLE

The above protocol was used to synthesize a model sequence: 5'-$T_{PS2}T_{PS2}T_{PS2}T_{PS2}T_{PS2}T$-3'. The ^{31}P-NMR spectra are provided in Figure 4.

FIGURE 4: ^{31}P-NMR SPECTRA OF 5'-$T_{PS2}T_{PS2}T_{PS2}T_{PS2}T_{PS2}T$-3'.



^{31}P-NMR spectra of 5'-$T_{PS2}T_{PS2}T_{PS2}T_{PS2}T_{PS2}T$-3'. T-thiophosphoramidite concentration: 0.15M ; T-thiophosphoramidite volume: 0.125ml ; sulfurization reagent : DDTT ; coupling time : 3 minutes ; Activator: (A) Tetrazole and (B) DCI.

SYNTHESIS OF CHIMERIC DNA CONTAINING PS2 LINKAGE(S)

It should be noted that the Expedite 8909 oxidizing time as well as the oxidizing reagent volumes found in the manufacturer's protocol are not sufficient to synthesize chimeric DNA containing PS2 linkage(s). The protocols referenced above correct these problems.

ABI SYNTHESIS CYCLE

Glen Research has also developed a PS2 synthesis cycle for the ABI394 DNA synthesizer using regular tetrazole or 0.25M DCI activators.

(http://www.glenresearch.com/Technical/ABI_394_PS2_Protocol.PDF)

REFERENCES

1. J. Nielsen, W.K.D. Brill, and M.H. Caruthers, *Tetrahedron Letters*, 1988, **29**, 2911-2914.
2. L. Cummins, D. Graff, G. Beaton, W.S. Marshall, and M.H. Caruthers, *Biochemistry*, 1996, **35**, 8734-41.
3. X. Yang, and D.G. Gorenstein, *Curr Drug Targets*, 2004, **5**, 705-15.
4. W.S. Marshall, and M.H. Caruthers, *Science*, 1993, **259**, 1564-70.
5. J.L. Tonkinson, et al., *Antisense Research and Development*, 1994, **4**, 269-278.
6. X. Yang, et al., *Bioorg Med Chem Lett*, 1999, **9**, 3357-62.
7. X. Yang, et al., *Ann N Y Acad Sci*, 2006, **1082**, 116-9.
8. X. Yang, et al., *Nucleic Acids Res*, 2002, **30**, e132.
9. W.T. Wiesler, W.S. Marshall, and M.H. Caruthers, *Methods Mol Biol*, 1993, **20**, 191-206.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
dA-Thiophosphoramidite	10-1700-90	100 µmole	150.00
	10-1700-02	0.25g	360.00
dC-Thiophosphoramidite	10-1710-90	100 µmole	150.00
	10-1710-02	0.25g	360.00
dG-Thiophosphoramidite	10-1720-90	100 µmole	150.00
	10-1720-02	0.25g	360.00
dT-Thiophosphoramidite	10-1730-90	100 µmole	150.00
	10-1730-02	0.25g	360.00

TECHNICAL BRIEF - PURIFICATION OF 6-FAM LABELLED OLIGOS USING GLEN-PAK™ CARTRIDGES

The Glen-Pak™ purification cartridges introduced at the end of 2007 have been very well received and we continue to add product specific protocols for use with these versatile tools for oligonucleotide (DNA and RNA) purification.

The most powerful feature of these columns is their high affinity for dimethoxytrityl (DMT)-On oligonucleotides along with their concurrent inefficient binding of DMT-Off fragments. Recently work was done with 6-Fluorescein Phosphoramidite (10-1964-xx), which conveniently has a DMT group that can be utilized for successful purification on the Glen-Pak DNA cartridge.

A mixed base 18 mer oligonucleotide was synthesized at the 1.0µmole scale and the 6-Fluorescein was added at the 5' end (DMT-On) using standard methods as described in its product profile (http://www.glenresearch.com/ProductFiles/10-1964.html).

The oligonucleotide was cleaved in 1.0mL NH_4OH and deprotected overnight at 55°C. After cooling, a small portion of the crude sample was detritylated and analyzed using ion-exchange HPLC to illustrate the base protecting groups and the number of failure sequences present (Figure 1).

The balance of the deprotected oligo was diluted with an equal volume of 100mg/mL NaCl and loaded on a properly prepared Glen-Pak DNA purification cartridge (60-5200-01). After rinsing with 2 mL of salt wash solution, 2 mL of 2% TFA, and 3 mL of deionized water, the purified oligo was eluted in 1 mL of 50% Acetonitrile /Water containing 0.5% NH_4OH. The eluent was dried in a speedvac, reconstituted in 0.5% NH_4OH in deionized water and analyzed by ion-exchange HPLC.

Figure 2 details the dramatic enhancement in purity of the oligo post Glen-Pak purification. The crude sample contained only 77% full-length product while the final product measured 99% pure with virtually quantitative recovery (data not shown).

We plan to continue developing new protocols and confirming the use of various modifiers/labels with the Glen-Pak DNA and RNA cartridges. Feel free to contact the technical support team for advice or to give feedback about this exciting addition to our purification product line.

FIGURE 1: **IEX HPLC ANALYSIS OF A CRUDE, DETRITYLATED 18 MER**

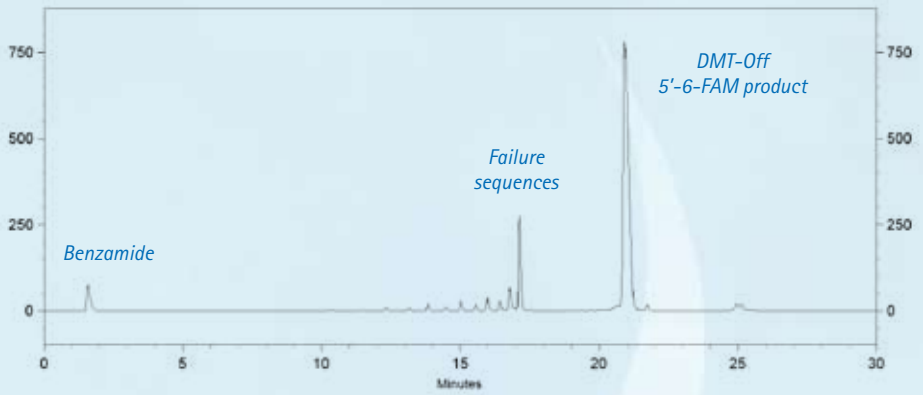
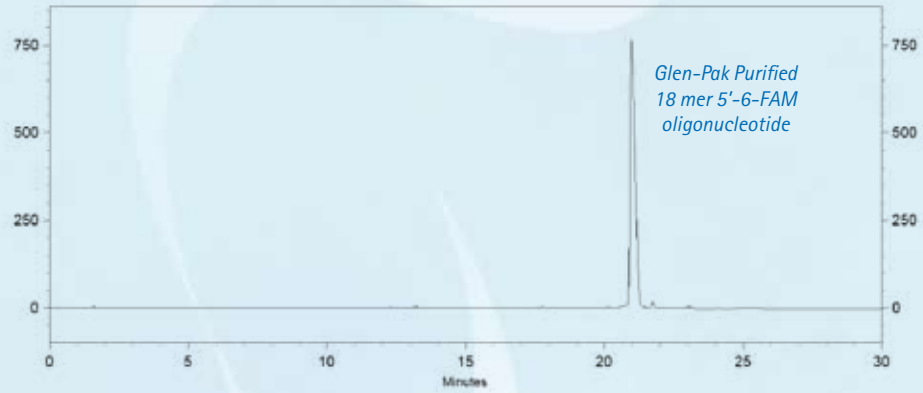


FIGURE 2: **IEX HPLC ANALYSIS OF SAME 6-FAM-LABELLED 18 MER GLEN-PAK PURIFIED**

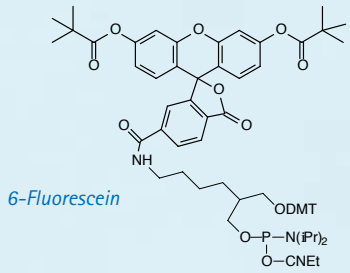


IEX HPLC Conditions

Column: Dionex DNAPac PA200 250 x 4mm
Buffers: A - 25mM Tris, pH 8, 5% ACN;
B - 25mM Tris, pH 8, 5% ACN,
1.0M NH_4Cl, pH 8
Gradient: 0-90% Buffer B at a flow rate of 1mL/min.

Glen-Pak™ is a trademark of Glen Research Corporation.

FIGURE 3: **STRUCTURE OF 6-FLUORESCEIN AMIDITE**



ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
Glen-Pak™ DNA Purification Cartridge	60-5100-10	Pack of 10	80.00
(For use in vacuum manifolds	60-5100-30	Pack of 30	200.00
and high-throughput devices)	60-5100-96	Pack of 96	475.00
Glen-Pak™ DNA Purification Cartridge	60-5200-01	each	8.00
(For use with disposable syringes)	60-5200-10	Pack of 10	80.00
Glen-Pak™ RNA Purification Cartridge	60-6100-10	Pack of 10	95.00
(For use in vacuum manifolds	60-6100-30	Pack of 30	225.00
and high-throughput devices)	60-6100-96	Pack of 96	575.00
Glen-Pak™ RNA Purification Cartridge	60-6200-01	each	9.50
(For use with disposable syringes)	60-6200-10	Pack of 10	95.00
RNA Quenching Buffer	60-4120-82	250mL	80.00
	60-4120-80	1L	200.00

MORE CLICK CHEMISTRY

The term 'Click Chemistry' has been coined by Sharpless[1] to describe the [3+2] cycloaddition[2] between alkynes and azides, a reaction which has allowed remarkable selectivity in conjugation reactions in biological samples (Figure 1). Click chemistry is a very hot topic these days and click chemistry applied to oligonucleotides is developing rapidly with citations more than doubling each of the last two years. Without providing a definitive "Click" bibliography, here are some topics that have caught our interest recently: oligonucleotide cyclization with a microwave assist[3]; peptide-DNA conjugation[4]; oligonucleotide immobilization[5, 6]; fluorescent labelling[7]; oligonucleotides on gold nanoparticles[8]; and labeling following PCR with alkyne modified nucleoside triphosphates[9].

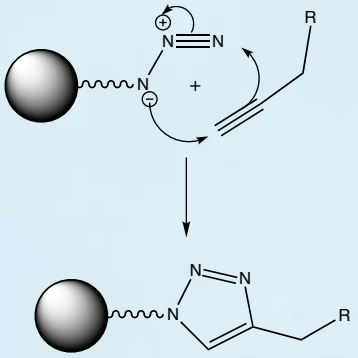
In the Glen Report 19.1 (April 2007), we launched the 5'-Hexynyl Phosphoramidite (1) as an introduction to the application of click chemistry to oligonucleotides. This product has been well received but our offering was unbalanced in that the azide options were limited. However, we reported then that we were able to produce a 5'-azide by quantitatively converting a 5'-iodo modified oligonucleotide (prepared using 5'-Iodo-dT) or a 5'-dabcyl modified oligonucleotide (prepared using 5'-dabcyl-dT) using sodium azide. We are now happy to propose two new very simple ways for introducing an azido group into oligonucleotides.

For these new additions to our catalogue, we have been inspired by some publications and presentations from late 2007. In a presentation given during the 3rd meeting of the Oligonucleotide Therapeutics Society (Berlin, Germany, Oct 4-6, 2007), Tom Brown presented the work of his laboratory.[10] He showed that very short cyclic oligonucleotides can be prepared in high yield on a multi-micromolar scale. They give rise to very stable duplexes that are potential tools for biophysical and biological studies. Cyclic oligonucleotides are very stable in serum for several days.

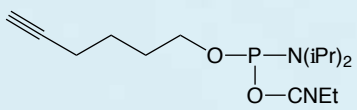
Almost at the same time, a French team at the Max Mousseron Institute for Biomolecules (Montpellier) developed the use of a bromohexyl phosphoramidite and different solid supports to allow conjugation and modifications.[11]

Our first proposal is to carry out the conjugation post-synthesis of an amino-modified oligonucleotide with an azide N-hydroxysuccinimide (NHS) ester, Azidobutyrate NHS Ester (2). This method is efficient for azido-modification of amines at either the 3'-end or the 5'-end of an oligo and it can even be used for internal modification on an Amino-Modifier-C6 dX residue within the sequence. The second approach, specific to the 5'-terminus, consists of adding 5'-Bromohexyl Phosphoramidite (3) in the last cycle. This modifier can then be easily transformed into a 5'-azido group by displacement of bromide using sodium azide. The first method can be performed in solution while the other allows the azide addition to be performed in solid phase on the synthesis column prior to cleavage and deprotection of the oligonucleotide. These products are easy to use and should prove valuable additions to our catalog.

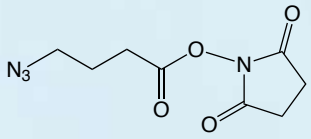
FIGURE 1: **CLICK CHEMISTRY STRUCTURES**



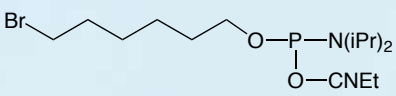
[3+2] Cycloaddition or 'Click'



5'-Hexynyl Phosphoramidite (1)



Azidobutyrate NHS Ester (2)



5'-Bromohexyl Phosphoramidite (3)

AZIDOBUTYRATE USE

In this case, oligonucleotides are synthesized as usual on any synthesizer. Amino groups have to be introduced either at the 3' or at the 5' end of the oligonucleotide. After cleavage and deprotection, the azido group can be introduced as a modification of the amine. For example, the oligonucleotide in sodium carbonate/sodium bicarbonate buffer (pH 8.75) was incubated at room temperature with succinimidyl-4-azidobutyrate in DMSO. The final oligo is then purified using HPLC or precipitated with ethanol or butanol.

BROMOHEXYL USE

The 5'-Bromohexyl Phosphoramidite is used to make 5'-bromohexyl oligonucleotides with the same phosphoramidite elongation cycle used for the coupling as the regular 2'-deoxynucleoside phosphoramidites. Bromohexyl ODNs were then converted into azidohexyl ODNs on the column by treatment of the CPG with a solution of sodium azide and sodium iodide in dry DMF at 65 °C. Finally, the CPG with the 5'-azido oligo is treated with ammonia affording 5'-azidohexyl-ODNs in solution.[11]

CYCLIZATION AND LIGATION OF OLIGOS USING CLICK CHEMISTRY.

To exemplify the use and possibilities of this chemistry, we reference some work carried out in Professor Tom Brown's laboratory at the University of Southampton (UK).[10] This paper describes a template-directed oligonucleotide strand ligation, a covalent intramolecular DNA circularization and a catenation experiment using Click Chemistry. Tom Brown's group also described the formation of a very stable cyclic DNA mini-duplex with just two base pairs, as an extension of this work.[12]

These experiments rely on an efficient and simple method of oligonucleotide click ligation that produces an unnatural extended DNA backbone linkage. One of the reacting ODNs contains a 3'-azide and the other a 5'-alkyne. The 5'-alkyne

can be introduced using our 5'-Hexynyl Phosphoramidite (1).

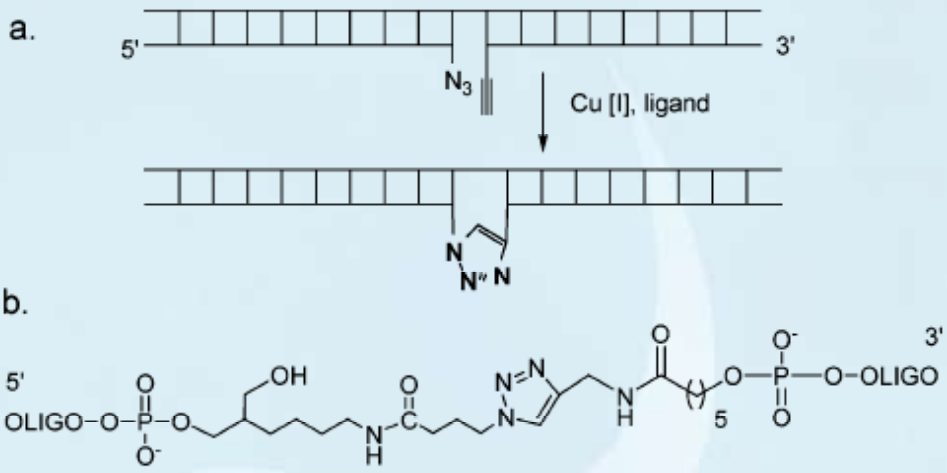
For the oligonucleotide ligation reactions, the authors[10] decided to use a strategy where a Cu[I] click catalyst was prepared *in situ* from Cu[II] sulfate and sodium ascorbate. All ligation reactions were carried out in 0.2 M aqueous sodium chloride to ensure complete formation of a duplex with the template. Unfortunately, under these conditions extensive degradation of all ODNs occurred, even with degassed buffers. However, the water-soluble tris-triazolylamine Cu[I]-binding ligand[13] greatly reduced degradation and, when greater than 5-fold excess of ligand relative to Cu[I] was employed, very little decomposition was observed.

The ligated oligonucleotide product is obtained within two hours at room temperature using equimolar ratios of the two participating ODNs. The ligation can be forced to proceed with or without a template simply by varying the concentrations of the reactants. If the alkyne and azide are located in the same oligonucleotide, circularization occurs even in the absence of a template ODN. The circularized ODN can then be used in the formation of a double strand DNA catenane.

Examples with 12mer, 70mer and 72mer oligos were described.[10] Click ligated single-strand cyclic oligos were purified by denaturing polyacrylamide gel electrophoresis. Circular ssDNA oligos were also prepared in this manner at 1.0 and 4.0 µmolar concentrations. Click ligation reactions of the same oligos were carried out at 10.0, 2.0, and 0.4 µmolar concentrations with and without a template oligonucleotide. Dilution was carried out by increasing the volume of 0.2 M aqueous NaCl. The authors observed that click chemistry can be conducted in a template-mediated manner over a wide concentration range, and below 2.0 µmolar the reaction does not proceed in the absence of a template ODN.

The authors analyzed the properties of the click ligation junction by conducting melting experiments in a quantitative PCR thermocycler (ROCHE Light-Cycler) in the presence of SYBR-Green.

FIGURE 2: **TEMPLATE-MEDIATED CLICK LIGATION**



Template-mediated click ligation of two ODNs: (a) schematic and (b) chemical structure at ligation point. (Illustration Courtesy of Reference 10)

CONCLUSION

Click chemistry is an excellent approach to carry out template-mediated ligation of two oligonucleotide strands against a complementary template. The methodology has also been shown to be efficient for the synthesis of a covalently closed ssDNA circle and a dsDNA pseudohexagon with sides of ca. 4 nm in length. This method is likely to be of value in nanotechnology applications involving DNA scaffolds. In addition, the use of single-stranded covalently closed DNA circles in biological applications warrants further investigation. Such constructs are likely to have increased *in vivo* stability, as they will be resistant to exonuclease degradation.

This click ligation may be useful in stabilizing structures like decoy oligos or aptamers. And such a fast and easy way of connecting two complementary oligo strands could be advantageous when compared to the synthesis of a single hairpin oligo of twice the length.

REFERENCES:

1. V.V. Rostovtsev, Green, L.G., Fokin, V.V. and Sharpless, K.B., *Angew Chem Int Ed*, 2002, **41**, 2596-2599.
2. R. Huisgen, *Angew Chem Int Ed*, 1963, **2**, 565-598.
3. J. Lietard, A. Meyer, J.J. Vasseur, and F. Morvan, *Journal of Organic Chemistry*, 2008, **73**, 191-200.
4. K. Gogoi, M.V. Mane, S.S. Kunte, and V.A. Kumar, *Nucleic Acids Res.*, 2007, **35**, e139.
5. M.V. Kvach, et al., *Nucleosides Nucleotides & Nucleic Acids*, 2007, **26**, 809-813.
6. D.I. Rozkiewicz, et al., *Chembiochem*, 2007, **8**, 1997-2002.
7. Y.H. Zhang, et al., *Tetrahedron*, 2007, **63**, 6813-6821.
8. M. Fischler, et al., *Chem Commun (Camb)*, 2008, 169-71.
9. P.M. Gramlich, C.T. Wirges, J. Gierlich, and T. Carell, *Org Lett*, 2008, **10**, 249-51.
10. R. Kumar, et al., *Journal of the American Chemical Society*, 2007, **129**, 6859-6864.
11. J. Lietard, A. Meyer, J.J. Vasseur, and F. Morvan, *Tetrahedron Letters*, 2007, **48**, 8795-8798.
12. A.H. El-Sagheer, et al., *Chembiochem*, 2008, **9**, 50-2.
13. T.R. Chan, R. Hilgraf, K.B. Sharpless, and V.V. Fokin, *Org. Lett.*, 2004, **6**, 2853-2855.

ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
5'-Hexynyl Phosphoramidite	10-1908-90	100 µmole	60.00
	10-1908-02	0.25g	200.00
Azidobutyrate NHS Ester	50-1904-23	2.3mg	60.00
(Dissolve 2.3mg in 60µL of DMSO)	50-1904-24	23mg	300.00
5'-Bromohexyl Phosphoramidite	10-1946-90	100 µmole	60.00
	10-1946-02	0.25g	200.00

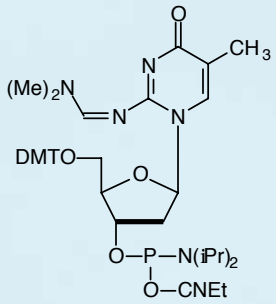
TECHNICAL BRIEF - NEW APPLICATION OF 5-ME-ISO-DC AND ISO-DG

In a paper published in the November 2007 issue of Biotechniques[1], researchers from the Siemens Medical Solutions Diagnostic Lab in Berkeley provided an interesting new way to solve the problem of designing degenerate primers and/or probes in PCR situations in which, for instance, equivalent quantification across subtype samples is desired.

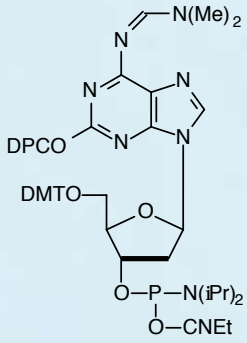
In the paper under reference, the authors showed that the iso-bases, 5-Me-iso-dC (F) and iso-dG (J) (our products 10-1065 and 10-1078, respectively) can actually be used as kind of universal bases. In the example described in the paper, they developed probes binding to a region of HCV transcripts containing 2 genotype-specific polymorphic sites. And they showed that degenerate probes containing iso-bases provide much closer cycle thresholds (CtS) for targets of both genotypes than other approaches aimed at creating degenerate probes.

It is already well established that iso-bases can increase specificity of nucleic acid hydridization when introduced as a third base pair. Now these authors have demonstrated that iso-bases 5-Me-iso-dC and iso-dG performed as degenerate pyrimidine and purine bases, respectively. Iso-dG further functioned as a degenerate base opposite B (C, T, and G) ambiguous sites.

FIGURE 1: **STRUCTURES OF 5-ME-ISO-DC AND ISO-DG PHOSPHORAMIDITES**



dmf-5-Me-isodC



dmf-isodG

Iso-bases are supplied under license from EraGen Biosciences, Inc. US Patents 5,432,272, 6,001,983, 6,037,120, and 6,140,496.

REFERENCE:

1. M. Abraham, Maria Albalos, Toumy Guettouche, Michel J. Friesenhahn, and T. Battersby, *Biotechniques*, 2007, **43**, 617-624.

TECHNICAL BRIEF - NEW APPLICATION FOR 5'-OME-DT PHOSPHORAMIDITE

In a paper published in the February 2008 issue of RNA, a group of researchers has shown[1] that 5'-OMe modification of a strand of a ds-siRNA can control guide strand selection and targeting specificity.

It is known that siRNA- and miRNA-containing ribonucleoprotein silencing complexes (RISC) are formed from short double-stranded RNA. Duplex siRNA is basically symmetric. However, only one strand enters the RISC and there is a bias for the preferred loading of one of the two duplex-forming strands into the RISC. Recruitment of the "wrong" strand can reduce expected activity and generate off-target silencing.

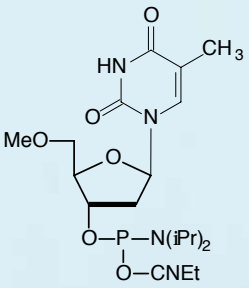
In their *RNA* paper, the authors have shown that the 5'-phosphorylation status within a duplex siRNA is an important determinant of strand incorporation into RISC. They also demonstrated that selective asymmetric 5'-O-methylation of one strand of an siRNA duplex can be used to direct the non-methylated strand to be incorporated into RISC. The off-targeting activity of the 5'-O-methylated strand was reduced, while the off-targeting activity of the phosphorylated strand was enhanced. They concluded that asymmetric 5' modification of siRNA duplexes can be extremely useful for controlling targeting specificity.

This simple and effective modification of siRNA can be readily achieved using our product (5'-O-Me-dT phosphoramidite; 10-1031).

REFERENCE:

1. P.Y. Chen, et al., *RNA*, 2008, **14**, 263-274.

FIGURE 2: **STRUCTURE OF 5'-OME-DT**



5'-OMe-dT

ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
dmf-5-Me-isodC-CE Phosphoramidite	10-1065-90	100 µmole	100.00
	10-1065-02	0.25g	255.00
dmf-isodG-CE Phosphoramidite	10-1078-90	100 µmole	165.00
	10-1078-02	0.25g	355.00
5'-OMe-dT-CE Phosphoramidite	10-1031-90	100 µmole	135.00
	10-1031-02	0.25g	355.00

NEW PRODUCT - DEUTERATED 2′-DEOXYGUANOSINE PHOSPHORAMIDITE

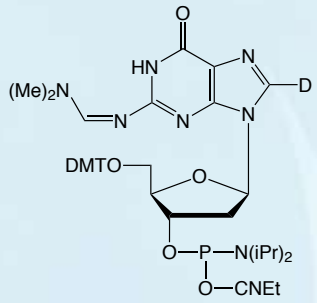
For several decades, perdeuteration and selective deuteration have been useful approaches for the simplification of NMR spectra and for other structural studies of large biomolecules. Consequently, deuteration of nucleic acids and proteins has found widespread applications especially in multinuclear multidimensional solution NMR studies of these complex molecular systems.

Researchers have focused attention on the efficient syntheses of nucleosides and nucleotides containing deuterium (^{2}H, D) labeled base residues. These reactions and the subsequent purification of the final products are easier to perform and much more cost-effective than multi-step chemical and biochemical procedures for sugar deuteration. According to Huang[1], in typical two dimensional (2D) NOESY spectra of oligonucleotides, structural information is primarily contained in the region that connects base with sugar protons (as shown in Figure 2). If the selected base protons, especially H8 in purines and H6 in pyrimidines, are replaced with ^{2}H, all the pertinent cross peaks correlating the base to sugar protons will disappear.

Also, according to Chirakul[2], many reported structures represent ground states that may bear little resemblance to the structures of the transition states. Therefore, structural information about ground states needs to be augmented with knowledge about the movements of functionally important residues. Solid state ^{2}H NMR is a valuable tool to study motions of specific sites in nucleic acids. Researchers who are interested in studying the role of dynamics in the interaction of proteins with DNA by solid state ^{2}H NMR require site-specific incorporation of deuterium-labeled nucleotides. To study the base motion of dA in DNA, Chirakul[2] incorporated ^{2}H into the 2- or 8-position of the adenine base.

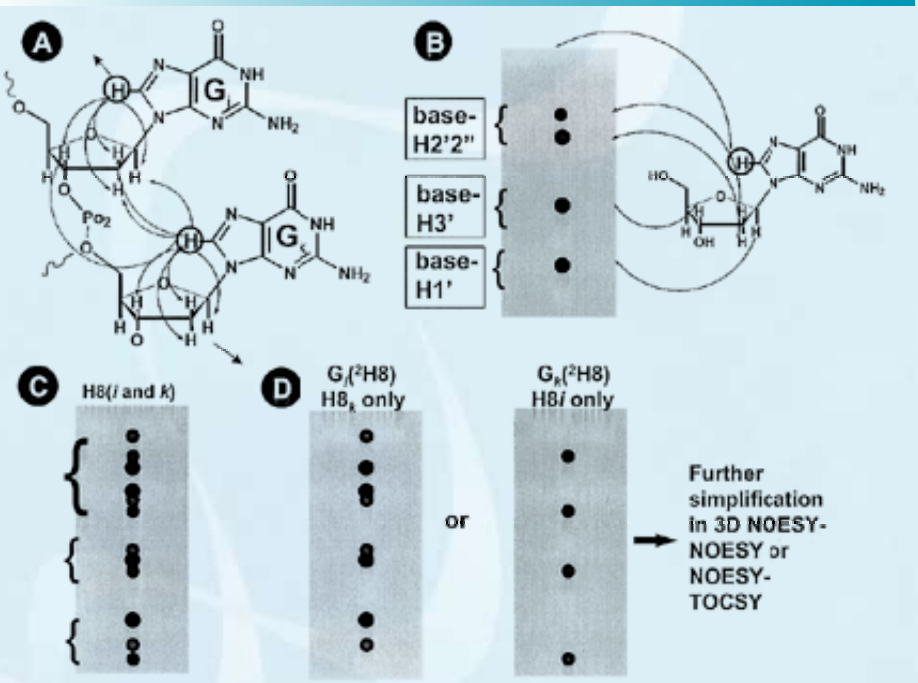
We are happy to offer the first commercially available ^{2}H modified phosphoramidite, 8-deutero-2′-deoxyGuanosine phosphoramidite (8-D-dmf-dG) (Figure1), to our customers involved in NMR spectroscopy. This phosphoramidite can be used on any DNA synthesizer using standard protocols. However, it is necessary to deprotect oligos containing 8-D-dmf-dG with 25% deuterated ammonium hydroxide (ND_4OD) for 40 hours at room temperature to prevent deuterium exchange.[1]

FIGURE 1: **STRUCTURE OF 8-DEUTERO-DG-CE PHOSPHORAMIDITE**



8-D-dmf-dG

FIGURE 2: **EFFECT OF 2H-LABELING IN NOESY**



Schematic illustration of the effect of base 2H-labeling in NOESY spectrum.
(A) The conventional intra- and inter-residue connectivities in a right handed helix of nucleic acids.
(B) The corresponding 2D NOE connectivities between the protons of a base and a sugar residue and
(C) An ideal NOESY spectrum in the base to sugar proton region with the two base proton resonances superimposed.
(D) The predicted simplified NOESY spectra using site-specifically 2H-labeled samples.
(Illustration Courtesy of Reference 1)

REFERENCES:

1. X. Huang, P. Yu, E. LeProust, and X. Gao, *Nucleic Acids Res.*, 1997, **25**, 4758-4763.
2. P. Chirakul, J.R. Litzer, and S.T. Sigurdsson, *Nucleosides, Nucleotides and Nucleic Acids*, 2001, **20**, 1903-1913.

ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
8-D-dmf-dG-CE Phosphoramidite	10-1520-90	100 µmole	90.00
	10-1520-02	0.25g	240.00

SOLID CPR II

CPR II (1) has become[1] a very popular chemical phosphorylation reagent (CPR) for phosphorylating oligos at the 5' terminus. While CPR II is most commonly used DMT-on to allow simple cartridge purification of the oligos produced, it can also be used DMT-off if the 5'-phosphorylated oligos can be used without purification in the same way as our original CPR (2).[2] One minor drawback in the usage of these two CPRs is the fact that they are both viscous oils. We offer these products prepackaged in septum-capped vials but it is sometimes useful in high throughput situations to be able to weigh powder into a bottle in the exact quantity needed for the synthesis session. The answer is simple – Solid CPR II (3).

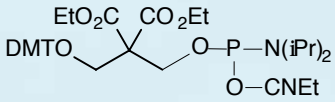
Solid CPR II is the dimethylamide analogue of CPR II so it is more stable than CPR II to the conditions of oligonucleotide synthesis. Consequently, it can be used at the 5' terminus without the need to remove the capping step in the last cycle, which is the case for CPR II. It can also be used at the 3' terminus in situations where CPR is too labile for the synthesis cycles and any special manipulations during the synthesis. For example, this amide structure was used[3] for the synthesis of a long oligo in which a silyl protecting group had to be removed with a fluoride reagent, which proved to be too basic for our standard 3'-phosphate support.

A 6 minute coupling time using tetrazole as activator is recommended for Solid CPR II. When used for DMT-on purification, the DMT is removed conventionally followed by a basic elimination to the phosphate. In solution, ammonium hydroxide will eliminate the diamide to the desired 5'-phosphate. On a reverse phase cartridge, the elimination can be carried out using a 1M sodium chloride solution at pH 12, followed by normal elution of the purified 5'-phosphate.

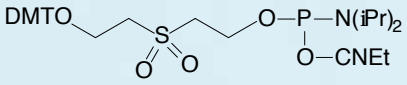
REFERENCES:

1. A. Guzaev, H. Salo, A. Azhayev, and H. Lonnberg, *Tetrahedron*, 1995, **51**, 9375-9384.
2. T. Horn, and M. Urdea, *Tetrahedron Lett.*, 1986, **27**, 4705.
3. P.J. Brooks, et al., *J Biol Chem*, 2000, **275**, 22355-62.

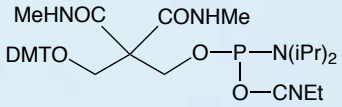
FIGURE 1: STRUCTURES OF PHOSPHORYLATION REAGENTS AND ALDEHYDE MODIFIERS



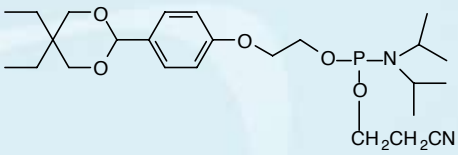
(1) Chemical Phosphorylation Reagent II



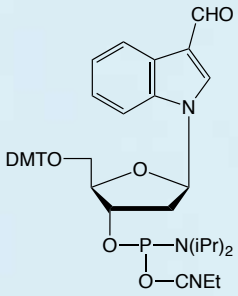
(2) Chemical Phosphorylation Reagent



(3) Solid Chemical Phosphorylation Reagent II



(4) 5'-Aldehyde-Modifier C2



(5) Formylindole

FORMYLINDOLE – ALDEHYDE MODIFIER

Conjugation of biopolymers using aldehyde intermediates is becoming increasingly popular[1,2] since the reaction of an aldehyde with oximes, hydrazines and semicarbazides is fast and highly specific. In 2003, Glen Research launched our first aldehyde modifier, 5'-aldehyde modifier C2 (4).[3] This patented product is effective and easy to use and is offered by Glen Research under a license agreement with Epoch/ Nanogen. While this product may be used for all research and development purposes, IVD developers would usually prefer to use a product with no intellectual property (IP) issues. At the time of our review of aldehyde modifiers, we also evaluated the formylindole phosphoramidite (5), which had just been described[4] by researchers at Kyoto University. As the interest in aldehyde modifiers grows, we have concluded that this product has a place in our catalog and we are happy to make it available to our customers.

This product is simplicity personified and there is no need to change any of the regular synthesis, cleavage and deprotection conditions when using it. The aldehyde is stable during deprotection and the DMT group is available for DMT-on purification, if desired. The aldehyde is still sufficiently active to conjugate extremely well with reagents containing oximes, hydrazines and semicarbazides.

REFERENCES:

1. T.S. Zatsepin, D.A. Stetsenko, M.J. Gait, and T.S. Oretskaya, *Bioconjugate Chemistry*, 2005, **16**, 471-489.
2. T.S. Zatsepin, D.A. Stetsenko, M.J. Gait, and T.S. Oretskaya, *Tetrahedron Lett*, 2005, **46**, 3191-3195.
3. M.A. Podyminogin, E.A. Lukhtanov, and M.W. Reed, *Nucleic Acids Res*, 2001, **29**, 5090-8.
4. A. Okamoto, K. Tainaka, and I. Saito, *Tetrahedron Lett*, 2002, **43**, 4581-4583.

NEW PRODUCT

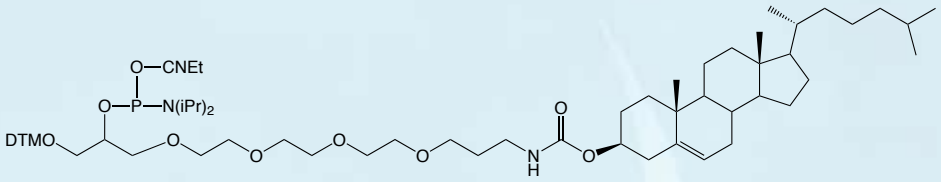
5′-CHOLESTERYL-TEG PHOSPHORAMIDITE

Cholesteryl labelling of oligos continues to find favor, especially in the field of therapeutics: antisense[1-3] and siRNA[4-7]. Since oligonucleotides are predominantly hydrophilic, they tend to have difficulty permeating cell membranes. In order to improve cellular uptake, one strategy is to conjugate to oligonucleotides molecules that are non-toxic and hydrophobic, such as cholesterol. And it is relatively simple to modify oligonucleotides at the 3' or 5' terminus with cholesteryl-TEG.

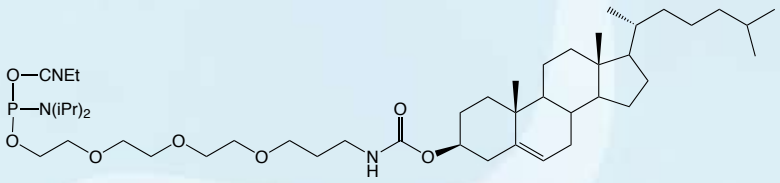
Several of our customers have asked for a change in the structure of our existing product, Cholesteryl-TEG phosphoramidite (1). This product uses the popular tetraethylene glycol (TEG) branched spacer originally introduced many years ago, which allows multiple insertions of the attached tag. The problem is that the cholesterol molecule is so hydrophobic that one addition per oligo terminus is more than enough. Consequently, Cholesteryl-TEG Phosphoramidite is normally added only once at the 5' terminus and there is no need for its capacity for multiple additions. The DMT group and the underlying 1,2-diol then become a liability rather than an asset. A minor structural adjustment leads us to 5'-Cholesteryl-TEG Phosphoramidite (2).

5'-Cholesteryl-TEG Phosphoramidite is dissolved in acetonitrile, which is in contrast with Cholesteryl-TEG Phosphoramidite which requires 10% THF in acetonitrile for solubility. A coupling time of 3 minutes with tetrazole as activator is optimal for 5'-Cholesteryl-TEG Phosphoramidite. Cholesterol is VERY hydrophobic so oligos prepared using 5'-Cholesteryl-TEG Phosphoramidite are easily purified by reverse phase techniques, including reverse phase cartridges.

FIGURE 1: **STRUCTURE OF CHOLESTERYL-TEG PHOSPHORAMIDITES**



(1) Cholesteryl-TEG



(2) 5'-Cholesteryl-TEG

REFERENCES:

1. M.K. Bijsterbosch, et al., *Nucleic Acids Res.*, 2000, **28**, 2717-2725.
2. M.K. Bijsterbosch, et al., *J. Pharmacol. Exp. Ther.*, 2002, **302**, 619-626.
3. M. Manoharan, Antisense *Nucleic Acid Drug Dev*, 2002, **12**, 103-28.
4. M. Manoharan, *Curr Opin Chem Biol*, 2004, **8**, 570-9.
5. J. Krutzfeldt, et al., *Nature*, 2005, **438**, 685-9.
6. J. Krutzfeldt, et al., *Nucleic Acids Res.*, 2007, **35**, 2885-2892.
7. C. Wolfrum, et al., *Nat Biotechnol*, 2007, **25**, 1149-57.

ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
Chemical Phosphorylation Reagent	10-1900-90	100 µmole	50.00
	10-1900-02	0.25g	160.00
Chemical Phosphorylation Reagent II (CPR II)	10-1901-90	100 µmole	60.00
	10-1901-02	0.25g	200.00
Solid Chemical Phosphorylation Reagent II (Solid CPR II)	10-1902-90	100 µmole	60.00
	10-1902-02	0.25g	200.00
5'-Aldehyde-Modifier C2 Phosphoramidite	10-1933-90	100 µmole	85.00
	10-1933-02	0.25g	325.00
Formylindole CE Phosphoramidite	10-1934-90	100 µmole	85.00
	10-1934-02	0.25g	325.00
Cholesteryl-TEG Phosphoramidite	10-1975-95	50 µmole	165.00
	10-1975-90	100 µmole	295.00
	10-1975-02	0.25g	675.00
5'-Cholesteryl-TEG Phosphoramidite	10-1976-95	50 µmole	150.00
	10-1976-90	100 µmole	260.00
	10-1976-02	0.25g	675.00

IMPROVING UNIVERSAL SUPPORT II FOR OLIGONUCLEOTIDE SYNTHESIS

In 2001, Azhayev and his group at the University of Kuopio designed and reported[1] on several new universal supports for oligonucleotide synthesis, which lack the limitations of similar and previously reported solid phases.[2-4] Several properties of these new solid phases make them rather attractive in industrial high output multi-well synthesizers. Our results have demonstrated that these new matrices are:

- Fast: cleavage and dephosphorylation in 20 minutes at room temperature;
- Mild: cleavage reagent is 2 M ammonia in methanol;
- Compatible with UltraMild, normal, and UltraFast deprotection; and
- Cost-effective: comparable in price to regular 2'-deoxynucleoside supports.

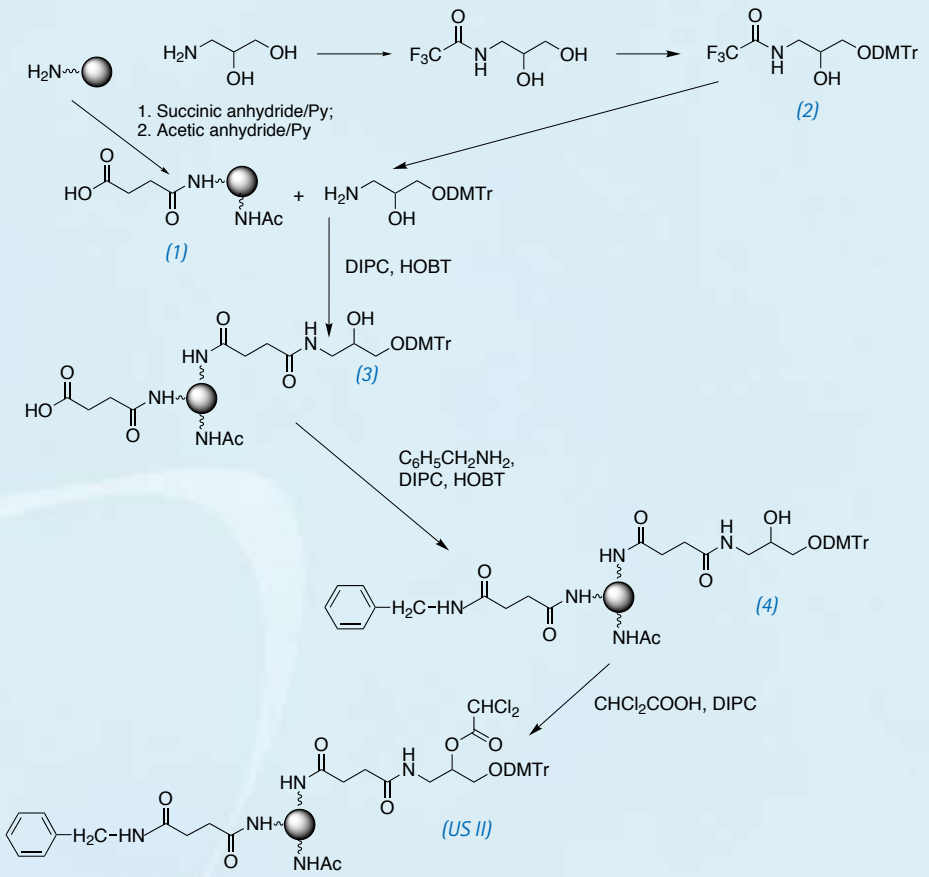
Scheme 1 describes a procedure for the preparation of USII support. Initially, long chain alkylamino controlled pore glass (lcaaCPG) or Macroporous Aminomethyl-polystyrene is succinylated and capped to give succinylamido-support (1). (±)-3-Amino-1-(4,4'-dimethoxytriphenylmethyl)-2-propanediol (2) is obtained in 3 steps and then attached to support (1) in the presence of N,N'-di-isopropylcarbodiimide and N-hydroxybenzotriazole to give solid support (3). The unreacted carboxyl groups of support (3) are capped with benzylamine, and only then the free hydroxyl group of support (4) is dichloroacetylated, to give the final support, Universal Support II (US II).

The preparation of the linker precursor (2) consists of 3 steps. The synthesis of US II, therefore, consists of 6 steps, 3 of which take place on solid phase.

Scheme 2 shows a procedure for the preparation of the new Universal Support III (US III). The Carbomoylation Linker (5) for US III preparation is synthesized in 3 simple steps in 80-85% yield and then directly attached to the Macroporous Aminomethyl-polystyrene, employing a new carbomoylation procedure.[5] Finally, the unreacted aminomethyl groups of the Aminomethyl-polystyrene are capped with dichloroacetyl imidazole to give the USIII support.

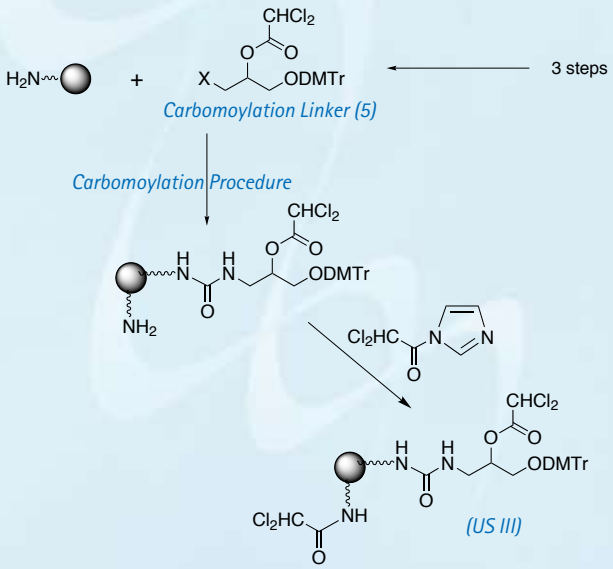
Therefore, the preparation of the Carbomoylation Linker consists of 3 steps. And the preparation of US III takes only 2 steps of modification on solid phase – attachment of linker and a capping step.

SCHEME 1: SYNTHESIS OF UNIVERSAL SUPPORT II



R=Cl2CH-; DIPC: N,N'-di-isopropylcarbodiimide; HOBT: N-hydroxybenzotriazole; Support = aminomethyl-polystyrene or long chain alkylamino-CPG.

SCHEME 2: SYNTHESIS OF UNIVERSAL SUPPORT III



Support = aminomethyl-polystyrene.

The synthesis of US II is a lengthy and laborious procedure - 3 steps to the linker (2) and 3 further reactions on solid phase. Preparation of US III, comprising only 2 steps of solid phase modification, is a much more straightforward procedure. Especially noteworthy is the fact that the required loading of the Carbomoylation Linker, (5) in Scheme 2, on solid phase is much easier to achieve when using the carbomoylation procedure.

Taken together, these facts make US III an improved product when compared to US II. Moreover, the new US III appears the same as, if not better than, US II in terms of performance as the truly universal solid support for oligonucleotide synthesis.

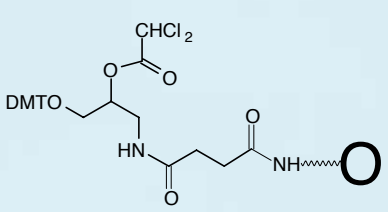
Because the universal linker is unchanged and the succinate or urea groups remain attached to the support, we use the same catalog numbers for US II and III. Using Universal Support II or III, an oligo yield of > 80% can be achieved on CPG supports and > 95% on polymeric supports, with purity equivalent to the same oligo prepared normally.

In addition, the carbomoylation process using the Carbomoylation Linker, (5) in Scheme 2, is now available for license to those who would prefer to produce US III on their own solid matrices.

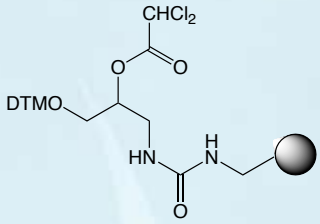
REFERENCES:

1. A.V. Azhayev, and M.L. Antopolsky, *Tetrahedron*, 2001, **57**, 4977-4986.
2. S. Scott, P. Hardy, R.C. Sheppard, and M.J. McLean *A universal support for oligonucleotide synthesis*, In Innovations and Perspectives in Solid Phase Synthesis, 3rd International Symposium, 1994; R. Epton, Ed. Mayflower Worldwide: 1994; pp 115-124.
3. M.H. Lyttle, D. Hudson, and R.M. Cook, *Nucleic Acids Res*, 1996, **24**, 2793-8.
4. A.V. Azhayev, *Tetrahedron*, 1999, **55**, 787-800.
5. The new carbomoylation chemistry, resulting in the stable urea fragment bridging the universal linker and aminoalkylated solid phase, is subject to proprietary rights of Glen Research Corporation and Metkinen Chemistry (U.S. Patent Application Serial No 60/854,721; International Patent Application No. PCT/FI2007/050575). Universal Support II and III are covered by the following intellectual property: US Patent No.: 6,770,754 and European Patent No.: 1404695.

FIGURE 1: **UNIVERSAL SUPPORT STRUCTURES**



Universal Support II



Universal Support III

DIFFERENCES BETWEEN UNIVERSAL SUPPORTS II AND III

What are the structural differences between US II and US III?

The universal linker, the top left section of structures 1 and 2 in Figure 1, is identical in Universal Support II and Universal Support III. The difference is in the attachment of the universal linker to the support. In the case of Universal Support II, the attachment is through a succinoyl diamide linkage while in Universal Support III it is a urea linkage. The amide assisted dephosphorylation reaction that releases the oligo-3'-OH into solution takes place in the universal linker section and the remainder of the attachment remains with the support after release of the oligonucleotide. So the structural differences have no effect on the function of the two supports and have no impact on the product oligonucleotide.

Why was the change necessary?

The synthetic changes described in this article were made to improve the control of the production of the universal support. In the case of Universal Support II, three reactions on solid phase are required and the quality analysis can only be carried out after these three steps. In the case of Universal Support III, the loading reaction is followed by simple capping of the support. The support can be analyzed after both steps. Clearly, the production of Universal Support III is under much more control and is also more amenable to scale up.

Why are the catalog numbers the same?

We have retained the same catalog numbers for Universal II and III since the difference only affects the support. (The situation is similar to lcaa-CPG, the core of most CPG supports, where individual manufacturers may use different structures for the long chain alkylamine (lcaa) linker.) All of our polymeric supports of this type have already been changed to the Universal Support III structure and the name and structure on the analytical data reflect this change. In future, CPG supports may also be changed if the benefits of the change are the same for CPG as for polymeric supports.

Can I make my own support?

The Carbomoylation Linker, (5) in Scheme 2, will be available commercially very shortly. The carbomoylation chemistry used to make the urea linkage is proprietary and details will be released as soon as the patent process is complete. In the meantime, the technology, Universal Support III and the carbomoylation technology required to produce the supports is available for licensing. Contact support@glenres.com for more information.

Has the performance of the support been affected?

Universal Support III performs identically to Universal Support II. However, since tighter control of production is possible, Universal Support III will always perform at the highest level previously achieved by Universal Support II.

Has the change affected my cost?

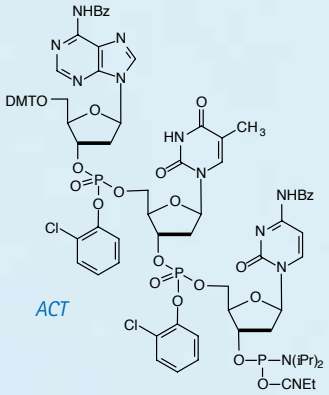
Cost is always paramount in everyone's mind when related to high throughput or large-scale oligonucleotide synthesis. The much improved synthesis of Universal Support III using the carbomoylation procedure also improved the cost structure. Universal Support III can be offered at about the same price as regular 2'-deoxynucleoside supports and at a lower price than ribonucleoside supports. Please contact us for a quotation.

With the incredible affinity and specificity that they afford, antibodies have become an indispensable tool for biological research, in vitro diagnostics, and proteomics.

Traditionally, hybridoma technology[1] has been used to produce these antibodies – a labor-intensive and time-consuming process that typically involves the immortalization of murine B cells in mice that have been inoculated with an antigen. However, there is a powerful alternative technique that is growing in popularity - phage display.[2] In phage display, a bacteriophage - typically T7 or M13 - is engineered to express antigen-binding fragments (Fabs) on the protein coat of the viral particle. These viral particles can act essentially as artificial antibodies with K_d values often below 1 nM. However, unlike standard antibodies, these viral particles contain the DNA sequence defining the binding motif, allowing for easy sequence analysis and manipulation. Typically, a large phage library is enriched in clones that bind the antigen of interest by affinity sorting - going through multiple rounds of selection and amplification under increasingly stringent conditions. Happily, these procedures are easily amenable to high-throughput approaches with phage display systems.

Fundamental to the success of phage display, however, is generating high diversity in the library itself for which Trimer Phosphoramidites are ideally suited. Using high-throughput techniques, Fellouse and colleagues[3] were able to generate a phage display library with a theoretical diversity of 1030 clones. When tested against a panel of 14 diverse proteins with low sequence homology, they were able to find high affinity phage expressing Fabs against all of the target proteins tested. Remarkably 83% of the clones exhibited K_d values lower than 20 nM and 10% lower than 1 nM.[3] In a second paper, the more traditional NNK-generated phage library was compared to those generated by Trimer Phosphoramidites. The Trimer Phosphoramidite-generated library was found to be an order of magnitude more diverse and had increased amino acid uniformity,[4] clearly illustrating the utility of the Trimer Phosphoramidite technology.

FIGURE : **TRIMER PHOSPHORAMIDITE STRUCTURE**



REFERENCES:

1. G. Kohler, and C. Milstein, *Nature*, 1975, **256**, 495-7.
2. H.R. Hoogenboom, et al., *Immunotechnology*, 1998, **4**, 1-20.
3. F.A. Fellouse, et al., *J Mol Biol*, 2007, **373**, 924-40.
4. L.R. Krumpe, K.M. Schumacher, J.B. McMahon, L. Makowski, and T. Mori, *BMC Biotechnol*, 2007, **7**, 65.

GLEN RESEARCH

22825 DAVIS DRIVE
STERLING, VIRGINIA
20164