VOLUME 11
NUMBER 1
JULY 1998

USING MODIFIED BASES TO OPTIMIZE HYBRIDIZATION

Introduction

The vast majority of synthetic oligonucleotides today are destined for use as primers in PCR or in sequencing applications, and current systems – from primers to amplification techniques - seem to work very well indeed. Of course, optimization of base pairing is always desirable and, as long as the cost does not outweigh the benefits, may be the answer to some challenging research problems.

2-Amino-dA

As shown in Figure 1, A-T base pairs have two hydrogen bonds whereas G-C base pairs have three hydrogen bonds. The simplest approach to improving primers would be to substitute A sites with 2-amino-A which forms three hydrogen bonds with T on hybridization.[1] 2-Amino-A also destabilizes A-G wobble mismatches, thus increasing specificity. Although 2-amino-dA monomers have been commercially available, they have had two severe drawbacks: the protection scheme and the cost. Because 2-amino-dA is very susceptible to depurination during the acidic deblocking step of DNA synthesis, mild deprotecting groups like PAC to protect both amino groups should not be used. The combination of N2-isobutyryl and N6-formamidine protecting groups in our earlier monomer (1, Figure 2) stabilized the monomer to depurination but made it very slow to deprotect, requiring 7 days in ammonium hydroxide at 55° or 17 hours at 55° in AMA for complete removal. After a significant development effort, we are happy to announce a new 2-amino-dA monomer (2) which appears to solve all of the earlier problems: deprotection is fast and effective in ammonium hydroxide; it is stabilized to depurination during synthesis; and the cost is only about 20% of the earlier monomer.

Pyrimidine Analogues

Substitution of 5-Me-dC[1,2] (3) or, better, C-5 propynyl-dC (4) for dC[3] and C-5 propynyl-dU (5) for dT[3] are effective strategies to enhance base pairing. This increase in hybridization efficiency is due to the hydrophobic nature of the groups at the C-5 position which helps to exclude water molecules from the duplex.

Duplex Stabilization

Using these base substitutions, duplex stability and therefore melting temperatures are raised by the approximate amounts shown below:

2-Amino-A	3.0° per substitution
5-Methyl-C	1.3° per substitution
C-5 propynyl-C	2.8° per substitution
C-5 propynyl-U	1.7° per substitution

While these modifications would also have a desirable effect on antisense oligonucleotides, the increased costs associated with most of them may limit their use. However, primers are less cost-sensitive because of the smaller scale, so the effects of the modified bases may be more generally useable. Potential improvements

Novel Monomers

Affinity Supports

TAMRA-dT

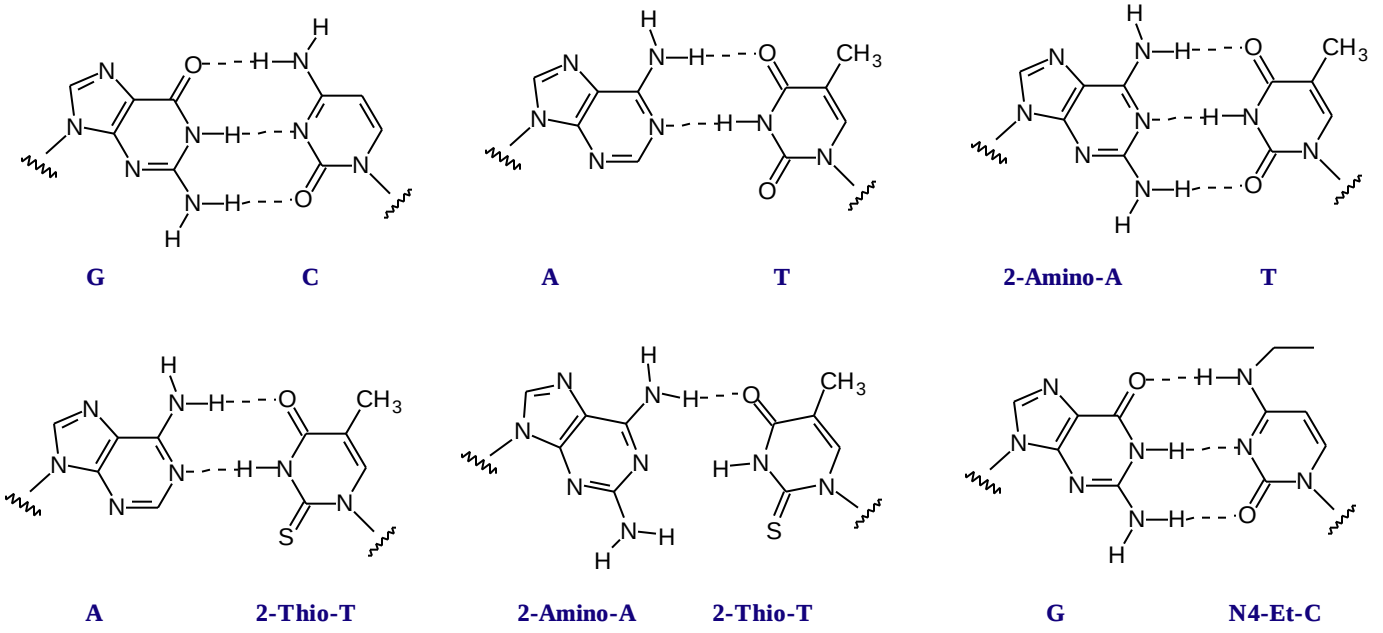
Dabcyl-dT

Puromycin CPG

(Continued on Page 2)

(Continued from Front Page)

FIGURE 1: HYDROGEN BONDING PATTERNS FOR REGULAR AND MODIFIED BASES



would include: the ability to use shorter oligos when sequence information is incomplete; higher melting temperatures, which should minimize the frequency of mutations; and enhanced binding, which should break any secondary structure in the target.

SBC Oligos

Selectively Binding Complementary (SBC) oligonucleotides[4] have the unique property of being able to simultaneously bind to both the sense and antisense strands of a DNA or RNA duplex. This should make them extremely useful for investigating secondary structures such as Holliday junctions and other branching moieties. They may also prove useful as antisense agents where the mRNA target exhibits significant secondary structure. SBC oligos possess this unique ability because, although they exhibit high affinity for natural oligonucleotides, they show little affinity for other SBC oligos even of a complementary sequence.

Oligos in which A has been replaced with 2-amino-A and T with 2-thio-T represent an excellent example of SBC oligos.[4] While 2-amino-A forms a very stable base pair with T containing three hydrogen bonds, the stability of the base pair with 2-thio-T is greatly diminished. Model building suggests that steric interactions between the 2-thio group of thymidine and the 2-amino group of adenine tilt the bases relative to each other yielding a base pair that contains only a single hydrogen bond.[4] However, 2-thio-T base pairs perfectly well with A, as shown in Figure 1. But the real proof is not in models: SBC 20mers annealed against a DNA 20mer target exhibited T_m values 10° higher than the corresponding DNA-DNA hybrid, whereas the SBC-SBC hybrid yielded T_m values 30° lower.[4]

Because of the resistance to hydrolysis of the protecting groups of our earlier 2-amino-dA monomer, the aggressive deprotection was not

(Continued on Page 3)

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
2-Amino-dA-CE Phosphoramidite (2,6-diaminopurine)	10-1085-95	50 µmole	55.00
	10-1085-90	100 µmole	100.00
	10-1085-02	0.25g	200.00
5-Me-dC-CE Phosphoramidite	10-1060-90	100 µmole	75.00
	10-1060-02	0.25g	185.00
pdC-CE Phosphoramidite	10-1014-90	100 µmole	85.00
	10-1014-02	0.25g	245.00
	10-1014-05	0.5g	490.00
pdU-CE Phosphoramidite	10-1054-90	100 µmole	65.00
	10-1054-02	0.25g	195.00
	10-1054-05	0.5g	390.00
2-Thio-dT-CE Phosphoramidite	10-1036-95	50 µmole	165.00
	10-1036-90	100 µmole	295.00
	10-1036-02	0.25g	675.00
N4-Et-dC-CE Phosphoramidite	10-1068-95	50 µmole	125.00
	10-1068-90	100 µmole	225.00
	10-1068-02	0.25g	675.00

(Continued from Page 2)

compatible with the presence of 2-thio-dT in the same oligo. Fortunately, our new 2-amino-dA monomer is rapidly deprotected in ammonium hydroxide and is compatible with 2-thio-dT (6). Now SBC oligos containing 2-amino-dA and 2-thio-dT can be easily prepared.

Hybridization Independent of Base Composition

Any technique that involves hybridization of multiple sequences simultaneously, as in DNA chip and reverse hybridization technologies, is subject to inaccuracies due to differences in GC content. Sequences with high GC content may contain mismatches and still hybridize, whereas a low GC content probe may match perfectly and yet disassociate from the target, leading to false positives and negatives respectively.

An elegant way of circumventing this problem would be to use a modified base that normalized the stability of the GC and AT base pairs. With this goal in mind, a series of modified dC bases was evaluated to develop a system where hybridization was independent of base composition and only dependent upon oligonucleotide length.[5] It was found that the N4-ethyl analogue (N4-Et-dC) hybridizes specifically to natural dG but the stability of the base pair is reduced to about the level of an AT base pair. In a series of probes whose GC content ranged from 0 to 100%, the range in T_m values when N4-Et-dC was used was only 7°; when dC was used, that range was 39°. We are happy to offer N4-Et-dC (7) for oligo synthesis.

References

(1) Y. Lebedev, et al., *Genetic Analysis - Biomolecular Engineering*, 1996, **13**, 15-21.

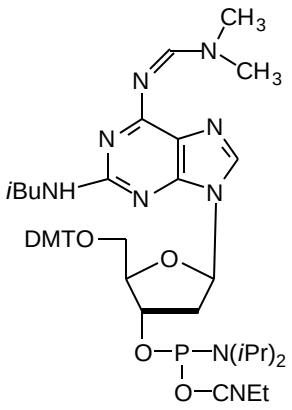
(2) L.E. Xodo, G. Manzini, F. Quadrifoglio, G.A.v.d. Marel, and J.H.v. Boom, *Nucleic Acids Res.*, 1991, **19**, 5625-5631.

(3) B.C. Froehler, S. Wadwani, T.J. Terhorst, and S.R. Gerrard, *Tetrahedron Lett.*, 1992, **33**, 5307-5310.

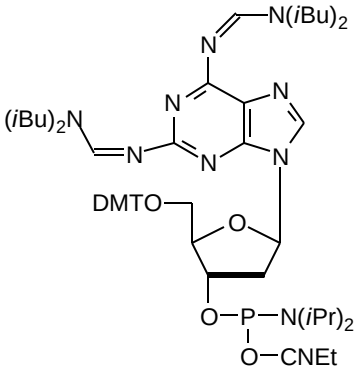
(4) I.V. Kutyavin, R.L. Rhinehart, E.A. Lukhtanov, V.V. Gorn, R.B. Meyer, and H.B. Gamper, *Biochemistry*, 1996, **35**, 11170-11176.

(5) H.K. Nguyen, P. Auffray, U. Asseline, D. Dupret, and N.T. Thuong, *Nucleic Acids Res.*, 1997, **25**, 3059-65.

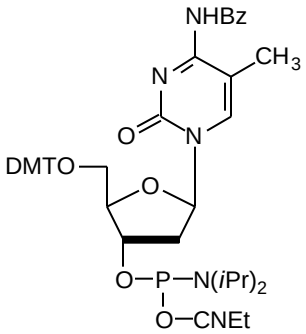
FIGURE 2: MONOMER STRUCTURES



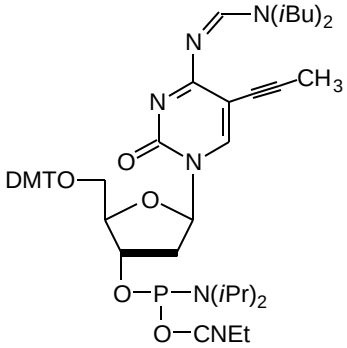
(1) 2-Amino-dA (old)



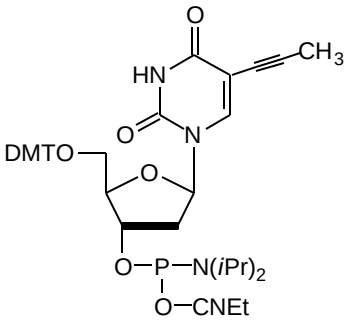
(2) 2-Amino-dA (new)



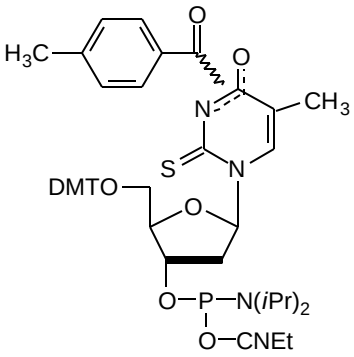
(3) 5-Me-dC



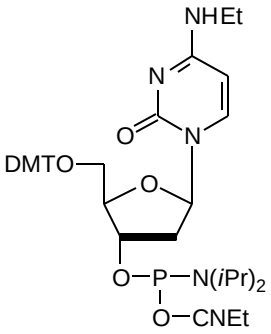
(4) C-5 Propynyl-dC



(5) C-5 Propynyl-dU



(6) 2-Thio-dU



(7) N4-Et-dC

OLIGO AFFINITY SUPPORTS

More than eight years ago, we introduced an oligo affinity support (OAS)[1] which allowed normal oligonucleotide synthesis but on treatment with ammonium hydroxide, the fully-deprotected oligonucleotide remained attached to the support. After annealing the complementary strand to the support-bound oligonucleotide, an affinity support for the purification of DNA binding proteins was generated.[2, 3] Unfortunately, the process used to make the support proved to be hazardous and we had to discontinue the product.

In the meantime, highly effective affinity supports could still be prepared by a two-step process in which the oligonucleotide is amino-modified and reacted with an activated support.[4, 5] However, we have remained intrigued by the elegance of direct production of the support and we continued evaluating potential supports. The criteria for a successful affinity support are simple:

1. The support must allow high quality oligonucleotide synthesis using organic solvents.
2. The support must also be suitable for use in the aqueous medium of affinity chromatography.
3. The support must exhibit low non-specific binding of the intended affinity target.

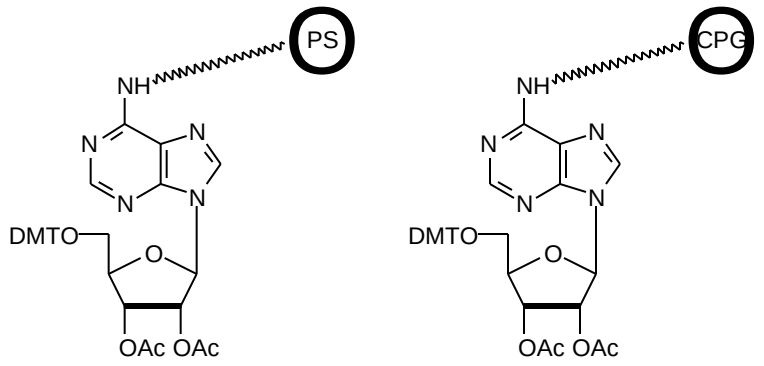
We are now happy to offer two oligo affinity supports: polystyrene/ polyethyleneglycol copolymer (PS), designed primarily for affinity purification of biomolecules; and controlled pore glass (CPG), which is less suitable for purification of proteins but ideal for chromatographic applications.

While these supports may find immediate use in the preparation of affinity matrices, we also envisage other potential applications, including enzymatic reactions with ligase and kinase. Also, synthesis in the 5' to 3' sense with 5'-phosphoramidites would yield a supported oligonucleotide with the 3'-terminus available for extension with polymerases.

Oligonucleotide Synthesis

Synthesize the oligonucleotide using standard cycles. (The 3'-base should be entered into the synthesizer as A but it is non-coding. The real 3'-terminal nucleoside is the first monomer added.)

FIGURE 1: OLIGO AFFINITY SUPPORT STRUCTURES



OAS PS **OAS CPG**

Deprotection

Treat OAS PS with ammonium hydroxide or other deprotecting solutions as normal. OAS CPG can be deprotected with ammonium hydroxide at elevated temperatures but stronger bases may damage the silica structure of the support. In either case, decant or pipet the liquid from the support and wash the support with water until neutral pH is achieved. DO NOT DISCARD THE SUPPORT. The support can be air-dried for storage but it should not be lyophilized.

Ordering Information is provided on the Back Page.

References

(1) R. Lohrmann, L. Arnold, and J.L. Ruth, *DNA*, 1984, **3**, 122.
(2) C.H. Duncan and S.L. Cavalier, *Analytical Biochemistry*, 1988, **169**, 104.
(3) J.T. Kadonaga, *Methods Enzymol*, 1991, **208**, 10-23.
(4) H.C. Kirch, H. Kruger, and H.S. Holthausen, *Nucleic Acids Res.*, 1991, **19**, 3156.
(5) C.L. Larson and G.L. Verdine, *Nucleic Acids Res.*, 1992, **20**, 3525.

Cleavage of Oligos from OAS (for Quality Determination)

After synthesis, oligonucleotides are not cleaved from the support by basic media. However, occasionally it may be necessary to cleave the oligonucleotide from the support for analytical purposes. The attachment to the support is through the N6 position of Adenosine and the oligonucleotide can be cleaved specifically from the support by periodate oxidation followed by ß-elimination yielding the 3'-phosphate.

Periodate Oxidation of the Ribose Ring to the Di-aldehyde

1. Treat the support with 1 mL of 100 mM sodium periodate in 10 mM (PS) or 100 mM (CPG) sodium phosphate buffer (pH 5-6). If the DMT group is to be retained on the oligonucleotide, adjust the pH to 7.2-7.5.
2. Stir or agitate the support in the dark for 6 hours at room temperature.
3. Decant or pipet the liquid from the support and wash the support with water (2X2 mL).

ß-Elimination to the 3'-Phosphate

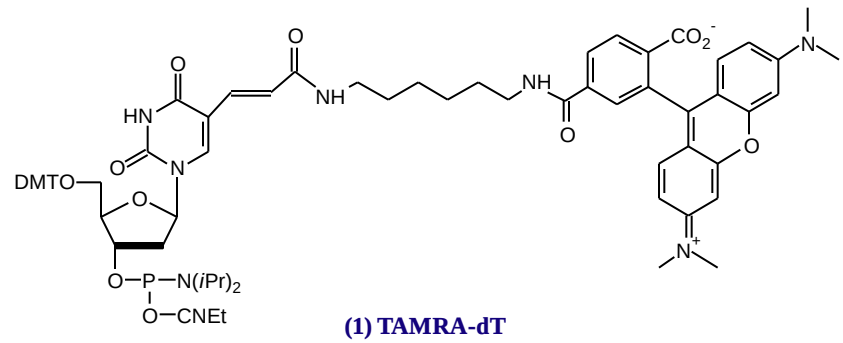
4. Prepare solution A by mixing water (2.4 mL), acetonitrile (0.6 mL), and propylamine (0.3 mL)
5. Submerge the support in 1 mL of solution A and heat the solution at 50° for 3 hours.
6. Decant or pipet the supernatant which now contains the oligonucleotide. Rinse the support with water (2X1 mL) and combine with the supernatant.
7. Lyophilize to obtain the product oligonucleotide 3'-phosphate.

NEW FLUORESCENT REAGENTS - TAMRA-dT, DABCYL-dT

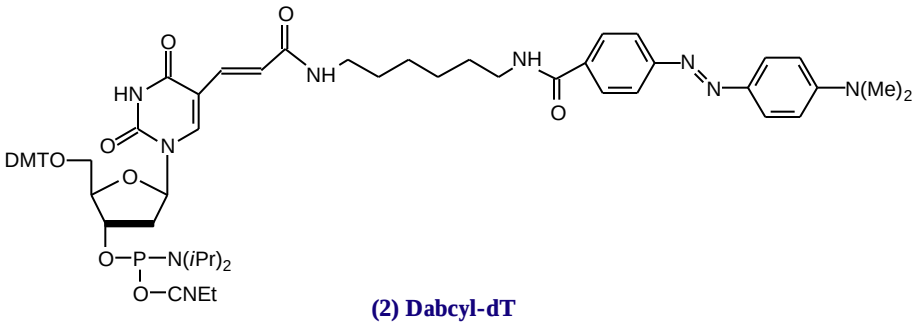
At Glen Research, customer service extends beyond looking after our customers' orders. We also listen to our customers' needs and try to take every opportunity to streamline their production processes where possible. Especially in the area of fluorescent labelling, post-synthesis conjugation of dyes to amino- or thiol-modified oligonucleotides is inefficient and imposes a heavy purification burden to eliminate unconjugated excess dye. We are happy to introduce two new dT derivatives which streamline oligo production currently carried out by post-synthesis conjugation.

In our reviews of current literature, we have previously observed that product applications in the area of fluorescent labelling are extensively covered by patents. We would, therefore, request that our customers make themselves aware of any patents covering the processing of fluorescent oligonucleotides and the uses that might apply to their work.

FIGURE 1: STRUCTURES OF NEW FLUORESCENT PRODUCTS



(1) TAMRA-dT



(2) Dabcyl-dT

TAMRA-dT

To supplement the tetramethylrhodamine (TAMRA) NHS ester and TAMRA-CPG we currently supply, we are offering TAMRA-dT for adding TAMRA within the oligo sequence. Because TAMRA is not stable to ammonium hydroxide, the procedure to cleave and deprotect the labelled oligonucleotide must be changed. We recommend using the UltraMILD monomers and deprotection with potassium carbonate in methanol. In this way, TAMRA oligonucleotides can be conveniently isolated.

A recent publication[1] describes an alternative TAMRA support, along with a procedure[2] to allow the use of regular monomers rather than our UltraMILD set. Cleavage and deprotection is carried out using *t*-butylamine/methanol/water (1:1:2).

Dabcyl-dT

Molecular beacon probes[3] have come to rely on the fluorescence quenching properties of the dabcyl molecule. A standard molecular beacon has a stem loop structure with a fluorophore like fluorescein at the 5'-terminus along with the quencher, usually a dabcyl group, at the 3'-terminus.

A further very elegant application of the quenching properties of the dabcyl group was described recently.[4] Again the oligos contained the fluorophore at the 5'-terminus but the dabcyl group was located within the sequence. The structure of the primer should allow it to form a hairpin in such a way that the fluorophore is spatially adjacent to the quencher in the hairpin stem. The oligos can then be used as PCR primers. The fluorescence intensity of the amplified product correlates with the amount of incorporated primers since the hairpin no longer exists in the double-stranded amplified product. Indeed, the fluorescent signal is only present when the primers are incorporated into the double-stranded amplified product. The authors note that this technology eliminates the risk of carry-over contamination, simplifies the amplification assay, and opens up new possibilities for the real-time quantification of the amplified DNA over an extremely wide dynamic range.

The hairpin primers were produced by substituting an appropriate dT residue with Amino-Modifier C6-dT and incorporating fluorescein at the 5'-terminus with a 5'-fluorescein phosphoramidite. The dabcyl group was added post-synthetically by conjugating the Amino-Modifier C6-dT with dabcyl-NHS ester. We now simplify the production process by offering Dabcyl-dT phosphoramidite for direct incorporation into the quencher site.

References:

(1) B. Mullah and A. Andrus, *Tetrahedron Lett*, 1997, **38**, 5751-5754.
(2) S.L. Woo, S.M. Menchen, and S. Fung, 1993, US Patent No. 5,231,191.
(3) S. Tyagi and F.R. Kramer, *Nature Biotechnology*, 1996, **14**, 303-308.
(4) I.A. Nazarenko, S.K. Bhatnagar, and R.J. Hohman, *Nucleic Acids Res.*, 1997, **25**, 2516-21.

Ordering Information is provided on the Back Page.

THE USE OF PUROMYCIN CPG IN COMBINATORIAL CHEMISTRY

New methods in the field of combinatorial chemistry make it possible to synthesize and screen large libraries of synthetic molecules for biological activity. Classically, well-defined molecules were systematically modified and the product compounds analyzed for improved biological activity. Newer combinatorial chemistry methods allow a chemist to synthesize a large population of similar compounds and then select for biological activity. (For a review[1] of issues concerning combinatorial chemistry see the February 1996 issue of C&E News.) In order for the new combinatorial chemistry to be an effective tool in evaluating structure-activity relationships three fundamental criteria must be met:

- A technique must be developed to synthesize a random series of structures.
- A high-throughput screening assay must be available to evaluate the large number of synthesized molecules.
- A method must be developed to capture and analyze the structure of the selected compounds.

Random Structure Generation

Combinatorial chemistry is well suited to studying peptides and oligonucleotides. Libraries of these compounds can be easily synthesized using solid-phase chemistry. Sequence degeneracy can be incorporated during the synthesis using either split synthesis or parallel synthesis.

1. In the split synthesis approach, the solid support is divided into portions prior to each coupling step. A different synthon is then coupled to each portion. All portions are recombined after coupling and the synthesis cycle is completed. This "split and mix" approach has the advantage of yielding a unique sequence on each support bead and variability in synthon reactivity can be corrected by varying the coupling conditions. Peptide synthesis, where variations in reactivity between amino acid synthons are significant, requires the "split and mix" approach. Oligonucleotide monomers on the other hand have similar reactivity. Degeneracy can be incorporated into oligonucleotides simply by using equimolar mixes of amidites at each coupling step. Of course, this variation does not yield one sequence per bead.

2. In the parallel synthesis approach, different compounds are synthesized in multiple reaction vessels. Parallel synthesis has been performed using microtiter plates as well as in specific locations on solid surfaces that are reactive, such as the DNA chip technology developed by Affymetrix.

High-Throughput Screening

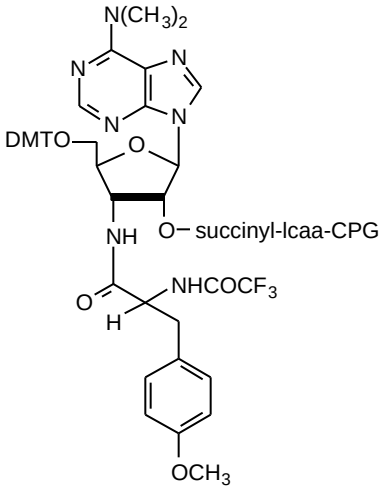
Following random structure generation, there must be a method to isolate structures that exhibit enhanced activity. Protein-binding and enzyme-activity assays are common methods that have been used to select for active structures from the library of synthesized compounds.

Capture and Analysis

One of the most challenging requirements associated with combinatorial chemistry is the recovery of sequence information of the oligonucleotide or peptide selected by the screening assay. A recent paper[2] describes a novel approach to this challenge. The authors have developed a method to generate a fusion product between mRNA and the polypeptide it encodes using *in vitro* translation of synthetic RNAs 3'-labeled with puromycin, an antibiotic that mimics transfer RNA. Puromycin binds in the ribosome's A site, forms a peptide bond with the growing peptide chain, and blocks further peptide elongation. By linking puromycin to mRNA, a peptide-RNA fusion product results from the translation of the message linking the encoding mRNA with its peptide product.

Exploiting this observation, a new support was created by attaching protected puromycin to controlled pore glass (Puromycin-CPG) (Figure 1). This support was then used to synthesize $d(A_{27}CC)$-puromycin to which various mRNA sequences were then ligated. The mRNA sequence information was then translated in a reticulocyte lysate system. As the ribosome reached the poly-dA sequence, translation was stalled. Puromycin entered the ribosome A site and a peptide bond formed between the C-terminal of the synthesized peptide and the RNA encoding the peptide structure. The poly-dA sequence serves two purposes, first it stalls the ribosome thereby allowing puromycin to enter the A site and second it acts as a future capture site for oligo-dT-biotin.

FIGURE 1: PUROMYCIN CPG

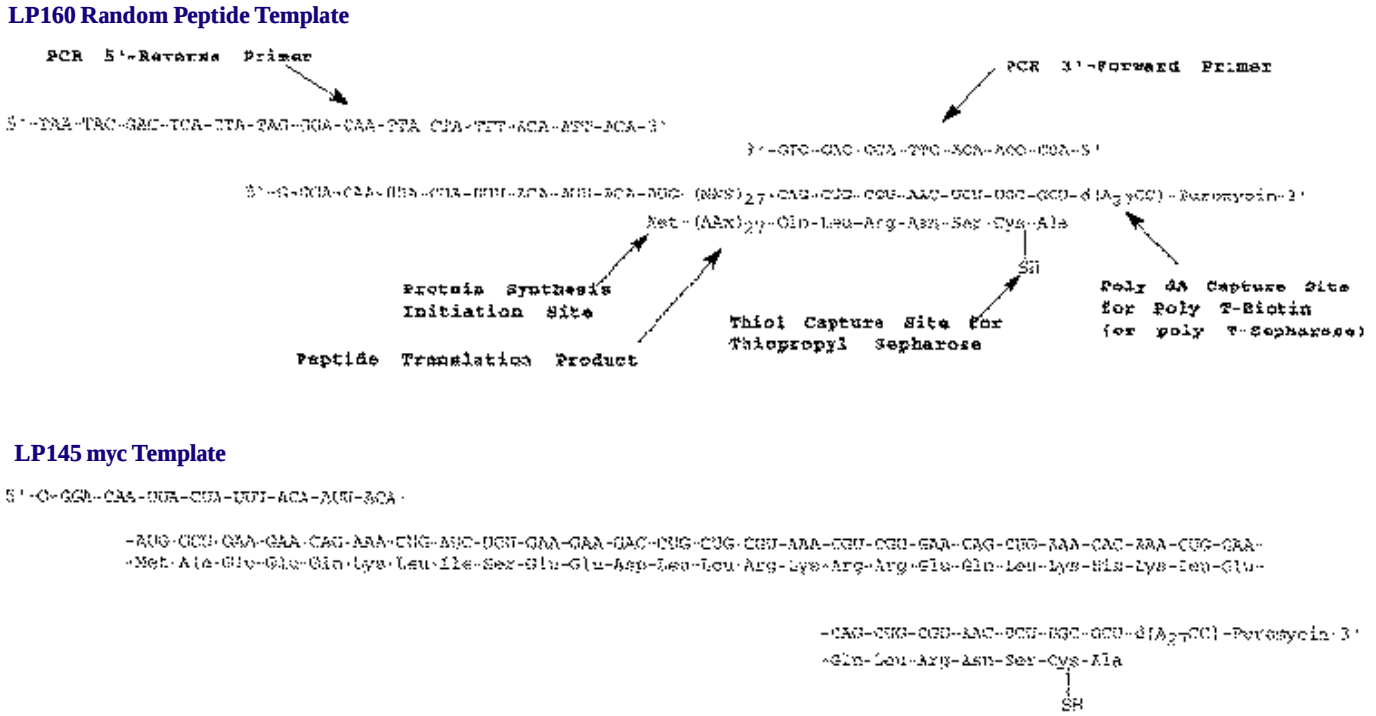


Method Verification

To demonstrate that the method could be used for selection enrichment, the authors synthesized two mRNA-puromycin peptide templates; LP160 random peptide template and LP154 myc template (Figure 2). A number of common features were built into each template:

- A 5' sequence that promotes ribosome binding and translation initiation.
- An AUG codon to initiate polypeptide synthesis.
- A mRNA coding sequence for either a known (myc) or random peptide.
- A cysteine residue to introduce a thiol

FIGURE 2: SELECTION ENRICHMENT STRATEGY



LP145 myc Template

capture site, only seen in a successfully translated peptide, for capture by thiopropyl Sepharose.

- A poly dA capture site for oligo-dT-biotin.
- Forward and reverse priming sites for PCR amplification of the reverse transcribed DNA sequences.

The templates were then translated in a reticulocyte lysate system to yield the peptide-RNA fusion product which was then isolated by sequential purification using dT_{25}-biotin or dT_{25}-Sepharose followed by thiopropyl Sepharose. The RNA was reverse transcribed using Superscript reverse-transcriptase to give a RNA-DNA duplex. Peptides synthesized using the LP154 myc template were selected using an anti-myc antibody. The selectivity of this method was demonstrated by immunoprecipitation of myc-RNA fusion product from up to a 200-fold excess of LP160-fusion product. Following selection the RNA-DNA duplex was heated in the presence of ammonium hydroxide to degrade the RNA. After removal of ammonium hydroxide by evaporation, PCR primers were added and the DNA amplified by PCR for subsequent sequencing.

Conclusion

Highly complex libraries of random peptide sequences linked to their encoding RNAs can be easily synthesized. The RNA-peptide fusion products carry their structural identity in a form that can be easily recovered following an activity-specific selection protocol. The ability to synthesize very specific RNA sequences from chemically or enzymatically synthesized oligonucleotides along with the availability of puromycin-CPG open up this exciting technique to everyone.

References:

(1) S. Borman, *C&EN*, Feb. 12, 1996, 29-54.
(2) R.W. Roberts and J.W. Szostak, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 12297-302.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Puromycin CPG	20-4040-01	0.1g	120.00
	20-4040-10	1.0g	995.00
1 µmole columns	20-4140-41	Pack of 4	200.00
0.2 µmole columns	20-4140-42	Pack of 4	120.00
10 µmole column (ABI)	20-4140-13	Pack of 1	360.00
15 µmole columns (Biosearch)	20-4140-14	Pack of 1	540.00

UPDATE - UNIVERSAL SUPPORTS, Q-SUPPORTS

Universal Support

Until our recent addition of a Universal Support, procedures in oligonucleotide synthesis required that the solid support contain the first nucleoside. This situation therefore required that an inventory of all four regular nucleoside supports be maintained. At the same time, oligonucleotides with unusual nucleosides, available as phosphoramidites but not as supports, at the 3'-terminus could not be readily prepared. However, the most worriesome aspect of this situation is the potential for a mistake to be made in the selection of the column containing the 3'-nucleoside. This potential for error may be fairly low in regular column-type synthesizers, but it is especially significant in the new generation of parallel synthesizers where 96 or 192 wells may contain all four supports in a defined grid.

Our initial choice as Universal Support[1], shown in Figure 1, allows the detritylation of the support to be carried out under normal conditions, as is the addition of the first nucleoside monomer. The remainder of the oligonucleotide synthesis also proceeds without any changes from the regular cycles.

The base-mediated elimination of the terminal phosphodiester group in the oligonucleotide - the mechanism is shown in Figure 2 - is the crucial step in this strategy. The elimination must proceed promptly under conditions comparable with routine deprotection strategies, using reagents which are also standard. Preferably the reagents should be volatile for simple evaporation (ammonium hydroxide or aqueous methylamine) or readily desalted (aqueous sodium hydroxide).

Optimization of conditions for the elimination of this structure to give 3'-OH at the terminus has led to a selection of conditions which have been readily adopted in many labs (Table 1). Using ammonium hydroxide, oligonucleotide deprotection and elimination to the 3'-OH is carried out at 80°C for a minimum of 8 hours, but preferably overnight. As long as acetyl protected dC monomer (Ac-dC) is being used, deprotection and elimination can be carried out in AMA at 55°C overnight, or at 80°C for a minimum of 3 hours. Both of these cleavage and elimination solvents are completely volatile and standard procedures can be applied for desalting and/or purification.

Again as long as Ac-dC monomer is being used, very fast deprotection and elimination occurs in aqueous alcoholic sodium hydroxide at 80°C for 30 minutes. Since this solution contains no volatile gases, very little pressure build-up occurs at 80°C. This mixture can be diluted with water and desalted using standard techniques.

Alternatively, 0.2 µmole or greater syntheses can be ethanol precipitated and the desalted, partially-purified sodium salt of the oligo can be quickly isolated by centrifugation.

FIGURE 1: UNIVERSAL AND Q-SUPPORT STRUCTURES

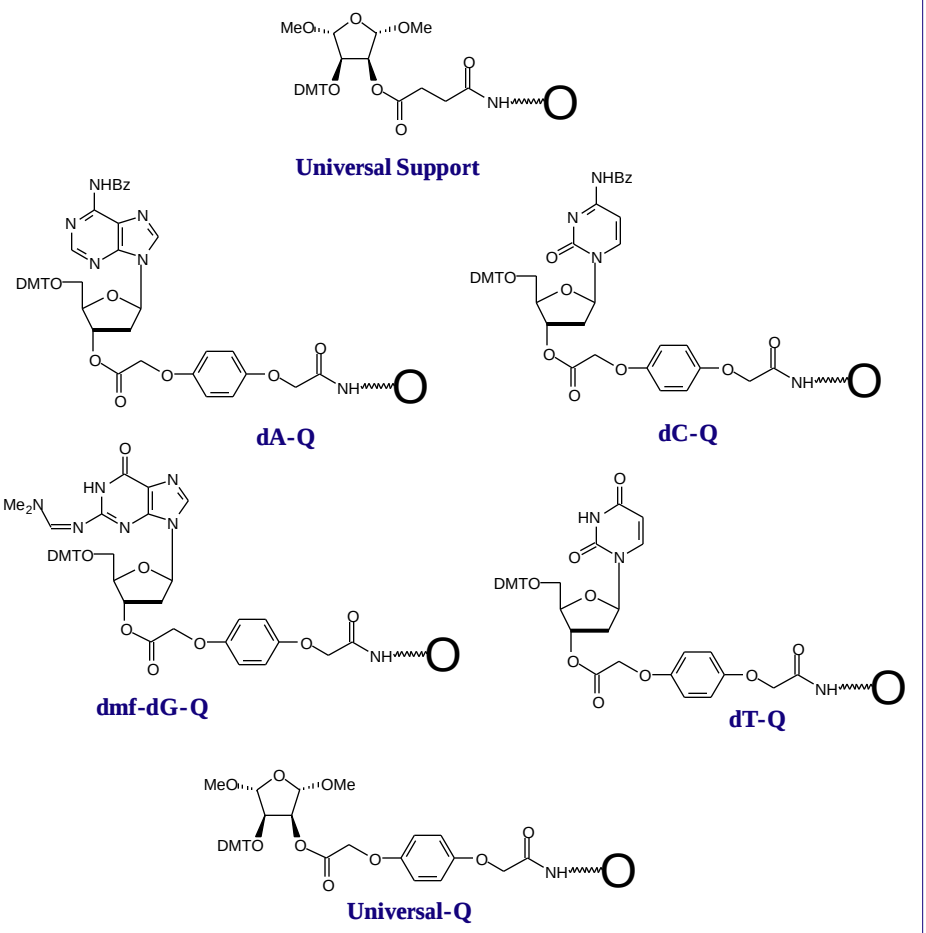


TABLE 1: ELIMINATION CONDITIONS

Reagent	Conditions
Ammonium hydroxide	80°C/8h min.
Ammonium hydroxide/ 40% Methylamine (AMA)*	55°C/17h
Ammonium hydroxide/ 40% Methylamine (AMA)*	80°C/3h min.
40% Methylamine*	55°C/5h
0.5M NaOH in ethanol/water (1:1)*	80°C/0.5h

* Requires the use of Ac-dC monomer to prevent base modification of dC residues by methylamine or hydrolysis of dC to dU residues with NaOH.

Using the Universal-Q support, the oligonucleotide can be cleaved from the support in a matter of a few minutes using any of the above deprotection and elimination solutions. The elimination reaction must then be continued for the indicated time. The Universal Support (1) is sold under license from Zeneca PLC.

Q-Supports

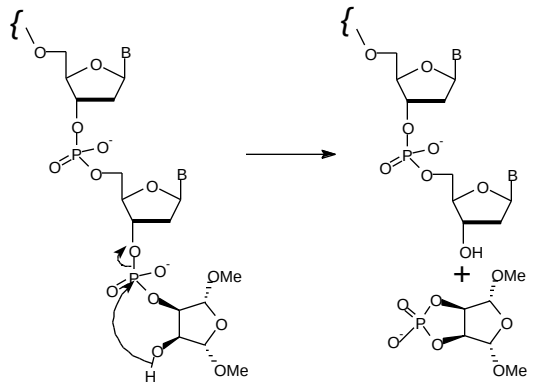
Cleavage of the succinate linkage between the the oligo and the support using ammonium hydroxide, either manually or on the synthesizer, is a major bottleneck in productivity for many synthesis groups. It consumes one hour of precious time while releasing only about 80% of the oligonucleotide. Richard Pon's group has identified[2] hydroquinone-O,O'-diacetic acid as the most satisfactory alternative to the succinate group. Nucleosides with this linker arm (Q-linker) are attached to supports with the same ease as the succinyl linker arm. The cleavage time in ammonium hydroxide at room temperature was found to be a mere 2 minutes while the linkage was shown to be very stable to the reagents of routine oligonucleotide synthesis.

The Q-linker is absolutely compatible with all hydrolytic cleavage procedures, especially mild procedures like potassium carbonate in methanol. Glen Research has been offering Q-supports under license from the University of Calgary for around 6 months. Initially, we have made available Q-linkers on 500Å CPG in 0.2 and 1 µmole scales in both Applied Biosystems and Biosearch formats with the four most popular nucleosides being offered, as well as the Universal Support.

The response to date has been quite gratifying. Interestingly, the main usage of the Q-supports so far has been in Biosearch columns where cleavage has normally been carried out manually. Clearly, reducing the manual step from 1 hour to 2 minutes has been the critical selling point for Biosearch customers. We look forward to increased use of Q-supports to increase throughput on the popular Expedite instruments with 16 column add-ons.

Although in use on Applied Biosystems' instruments by several groups, the lack of columns in the low volume format is clearly impeding adoption of the Q-supports. We are actively working on a low volume Q-support column for LV40 and LV200 cycles and expect columns to be available in the fourth quarter of this year.

FIGURE 2: MECHANISM OF ELIMINATION



References:

(1) S. Scott, P. Hardy, R.C. Sheppard, and M.J. McLean, *Innovations and Perspectives in Solid Phase Synthesis, 3rd International Symposium,* **1994**, Ed. Roger Epton, Mayflower Worldwide, 115-124.

(2) R.T. Pon and S.Y. Yu, *Tetrahedron Lett.*, 1997, **38**, 3327-3330.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Universal Support 500			
500Å Bulk Support	20-5000-01	0.1g	11.00
	20-5000-02	0.25g	25.00
	20-5000-10	1.0g	95.00
ABI Format (not LV)			
1 µmole columns	20-5100-41	Pack of 4	60.00
0.2 µmole columns	20-5100-42	Pack of 4	40.00
40 nmole columns	20-5100-45	Pack of 4	40.00
10 µmole column (TWIST Format)	20-5100-13	Pack of 1	100.00
Biosearch Format			
1 µmole columns	20-5200-41	Pack of 4	60.00
0.2 µmole columns	20-5200-42	Pack of 4	40.00
40 nmole columns	20-5200-45	Pack of 4	40.00
15 µmole column (TWIST Format)	20-5200-14	Pack of 1	150.00

Catalog No. dA-Q	Catalog No. dC-Q	Catalog No. dmf-dG-Q	Catalog No. dT-Q	Catalog No. Universal-Q	Pack	Price($)
500Å Bulk Support						
21-2000-01	21-2010-01	21-2029-01	21-2030-01	21-5000-01	0.1g	11.00
21-2000-02	21-2010-02	21-2029-02	21-2030-02	21-5000-02	0.25g	25.00
21-2000-10	21-2010-10	21-2029-10	21-2030-10	21-5000-10	1.0g	95.00
ABI Format (not LV)						
21-2100-41	21-2110-41	21-2129-41	21-2130-41	21-5100-41	Pk/4	60.00
21-2100-42	21-2110-42	21-2129-42	21-2130-42	21-5100-42	Pk/4	40.00
Biosearch Format						
21-2200-41	21-2210-41	21-2229-41	21-2230-41	21-5200-41	Pk/4	60.00
21-2200-42	21-2210-42	21-2229-42	21-2230-42	21-5200-42	Pk/4	40.00

MORE NOVEL MONOMERS - 6-THIO-dG, 2'-AMINO-RNA, 5-HYDROXY-dC,dU

6-Thio-dG

The purine 6-thioguanine[1] has been used for many years in the treatment of human malignancies, especially leukemias. It is likely that purine is converted into the nucleoside 6-thioguanosine in DNA and RNA and the cytotoxic effects may be due to damage caused by strand cleavage or cross-linking to DNA and proteins. The physico-chemical basis for these biological effects is the reactive thiocarbonyl group and oligonucleotide researchers have exploited this reactivity in several ways.

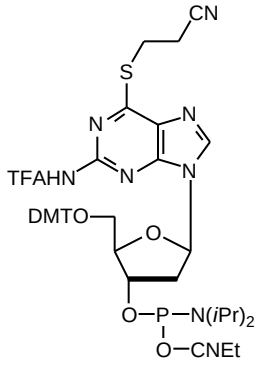
Cross-linking

The interaction of DNA with DNA binding proteins can be studied using photochemically induced cross-linking. Predominantly, studies have been carried out using 5-bromo- and 5-iodo-pyrimidines but 4-thiopyrimidines have also been used. With the advent of phosphoramidites of thionucleosides, photoaffinity labelling experiments using these modified bases are now readily possible. For example, 4-thiothymidine and 6-thiodeoxyguanosine (S6-dG) were shown[2] to cross-link effectively with Eco RV endonuclease and methyltransferase. The advantages of using the thio derivatives for photo cross-linking include their similarity to the natural structures, and the wavelength required, 340 nm, which is removed from the maxima of the regular bases and should cause no other damage. In addition to its photochemical reactivity, nucleobases containing thiocarbonyl groups can also be chemically modified selectively at the sulfur position by alkylating reagents.[3]

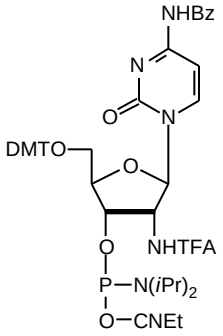
Triplex

6-Thiodeoxyguanosine has also been incorporated into G-rich triple helix-forming oligonucleotides. Replacement of all or some G residues in G-rich oligonucleotides with S6-dG has been shown[4] to inhibit self association and formation of G tetrads, especially in potassium buffers. This allows triple helix formation to take place normally.

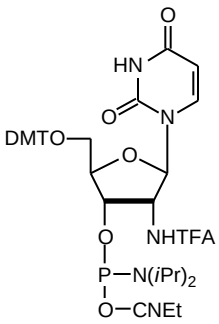
FIGURE 1: STRUCTURES OF NEW FLUORESCENT PRODUCTS



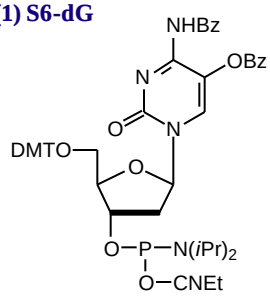
(1) S6-dG



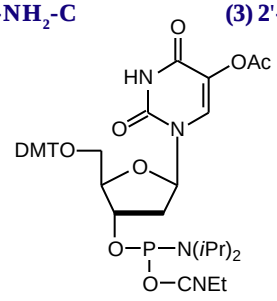
(2) 2'-NH_2-C



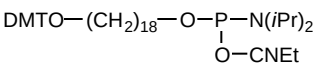
(3) 2'-NH_2-U



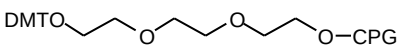
(4) 5-OH-dC



(5) 5-OH-dU



(6) Spacer C18



(7) Spacer 9-CPG

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
6-Thio-dG-CE Phosphoramidite	10-1072-95	50 µmole	177.50
	10-1072-90	100 µmole	355.00
	10-1072-02	0.25g	975.00
2'-NH_2-C-CE Phosphoramidite	10-3510-90	100 µmole	195.00
	10-3510-02	0.25g	500.00
2'-NH_2-U-CE Phosphoramidite	10-3530-90	100 µmole	175.00
	10-3530-02	0.25g	500.00
5-OH-dC-CE Phosphoramidite	10-1063-90	100 µmole	225.00
	10-1063-02	0.25g	675.00
5-OH-dU-CE Phosphoramidite	10-1053-90	100 µmole	225.00
	10-1053-02	0.25g	675.00
Spacer C18 CE Phosphoramidite	10-1928-90	100 µmole	95.00
	10-1928-02	0.25g	240.00
3'-Spacer 9 CPG	20-2919-01	0.1g	70.00
	20-2919-10	1.0g	480.00
1 µmole columns	20-2919-41	Pack of 4	100.00
0.2 µmole columns	20-2919-42	Pack of 4	60.00
10 µmole column (ABI)	20-2919-13	Pack of 1	180.00
15 µmole columns (Biosearch)	20-2919-14	Pack of 1	280.00

SPACER C18, SPACER 9-CPG

Deprotection

Our new S6-dG monomer (1, Figure 1) has the S6 position protected with cyanoethyl and N2 with trifluoroacetyl protecting groups. After normal synthesis, the synthesis column with oligonucleotides containing S6-dG should be treated[5] with 1M 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in anhydrous acetonitrile at room temperature for 5 hours to remove the S6-cyanoethyl protecting group. The oligo deprotection is completed with 50 mM sodium hydrosulfide (NaSH) in ammonium hydroxide at room temperature for 24 hours.

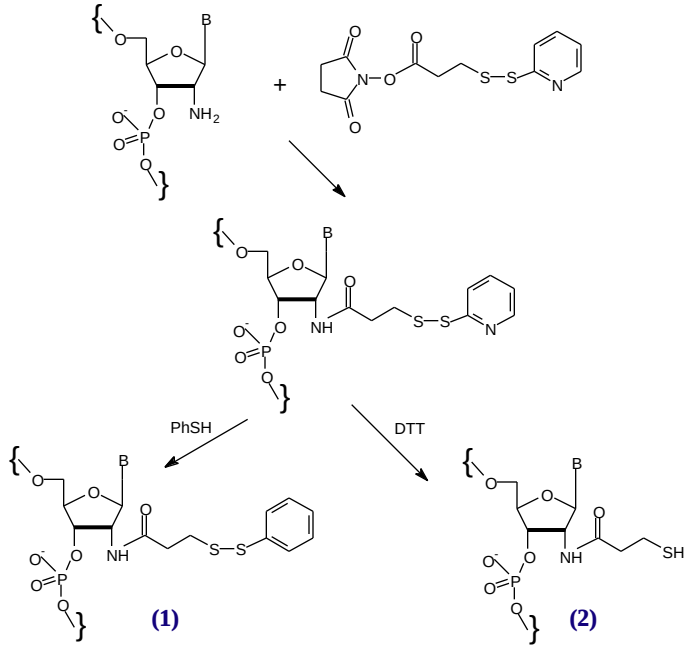
2'-Amino-RNA

RNA modification is currently in vogue for such applications as antisense and ribozymes. Interesting changes in RNA activity can be effected by substituting the 2'-hydroxyl with 2'-fluoro or 2'-O-alkyl groups. A further obvious substitution would be the 2'-amino group.

The thermal stability of duplexes containing 2'-amino-RNA has been determined[6] and it was reported that 2'-amino-C substitutions destabilized by about 4° relative to RNA C. It was also further reported that 2'-amino-RNA linkages are nuclease-resistant.

The pKa of the 2'-amino group is quite low at 6.2 but this retains sufficient nucleophilicity to allow conjugation reactions to take place. It is therefore possible to label a 2'-amino group with a fluorophore like rhodamine. This activity has been used rather elegantly to investigate[7] thermal motion in a large ribozyme. The 2'-position within an RNA duplex is directed towards the outside of the helix in a location which is very amenable to interhelix contact. The researchers were able to conjugate a disulfide group to the 2'-amino group via an activated ester to yield intermediates (1) or (2), as shown in Figure 2. An exchange reaction between the activated disulfide and a thiol in the complementary section or strand neatly forms a disulfide cross-link.

FIGURE 2: PREPARATION OF CROSSLINK PRECURSORS



It is worthy of note that complex oligonucleotides containing 2'-amino-RNA residues benefit considerably from the use of 4,5-dicyanoimidazole (DCI)[8] as activator.

We are happy to offer 2'-Amino-C (2) and 2'-Amino-U (3) monomers conveniently protected with base-labile groups which are removed by regular treatment with ammonium hydroxide.

5-Hydroxy-Pyrimidine Nucleosides

Free-radically induced DNA damage and attendant enzymatic repair systems continue to be research topics of more than passing interest. Glen Research has made it a goal to introduce oxidatively damaged deoxynucleosides in forms suitable for automated DNA synthesis. Our latest introductions are the oxidized cytidine nucleoside 5-Hydroxy-2'-deoxyCytidine (5-OH-dC) and its deamination product 5- hydroxy-2'-deoxyUridine (5-OH-dU) as phosphoramidites. These lesions are observed in liver, kidney and brain DNA, at fairly constant levels, indicating that repair systems are not totally effective with obvious implications to the aging process. The enzymatic repair of these two lesions, as well as their role in mutagenesis, are clearly worthy of study.

The synthesis of these two monomers and their incorporation into oligos was recently described[9] by a group from MIT. The reactive 5-OH group was protected in both cases with acyl protecting groups and this simple protection scheme proved to be perfectly compatible with regular synthesis and deprotection.

New Spacers

Sometimes we prepare products for which there is no known use and little logic for their creation. Here are two spacer products which are looking for good homes. Spacer 9 CPG is absolutely the ideal product for preparing an oligonucleotide with its 3'-terminus blocked with a triethyleneglycol group. So, if you need to block polymerase extension with a mixed polarity polyether, you need look no further. Spacer C18 Phosphoramidite promises to be the product of choice for adding a very hydrophobic section to an oligonucleotide. There, we just knew a silent minority of our customers was fervently awaiting these below-average innovations.

(Continued on Back Page)

(Continued from Previous Page)

(Ordering Information is Continued from Pages 4 and 5)

References:

(1) M.S. Christopherson and A.D. Broom, *Nucleic Acids Res.*, 1991, **19**, 5719-5724.
(2) T.T. Nikiforov and B.A. Connolly, *Nucleic Acids Res.*, 1992, **20**, 1209-1214.
(3) R.S. Coleman and R.M. Pires, *Nucleic Acids Res.*, 1997, **25**, 4771-4777.
(4) T.S. Rao, R.H. Durland, D.M. Seth, M.A. Myrick, V. Bodepudi, and G.R. Revankar, *Biochemistry*, 1995, **34**, 765-772.
(5) R.S. Coleman, J.C. Arthur, and J.L. McCary, *Tetrahedron*, 1997, **53**, 11191-11202.
(6) H. Aurup, T. Tuschl, F. Benseler, J. Ludwig, and F. Eckstein, *Nucleic Acids Res.*, 1994, **22**, 20-4.
(7) S.B. Cohen and T.R. Cech, *J. Amer. Chem. Soc.*, 1997, **119**, 6259-6268.
(8) C. Vargeese, et al., *Nucleic Acids Res.*, 1998, **26**, 1046-1050.
(9) M.L. Morningstar, D.A. Kreutzer, and J.M. Essigmann, *Chem. Res. Toxicol.*, 1997, **10**, 1345-1350.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Oligo-Affinity Support (PS) (OAS PS)	26-4001-01	0.1g	180.00
	26-4001-02	0.25g	425.00
	26-4001-10	1.0g	1590.00
Oligo-Affinity Support (PS) 1 µmole TWIST columns	26-4101-41	Pack of 4	300.00
Oligo-Affinity Support (CPG) (OAS CPG)	20-4001-01	0.1g	240.00
	20-4001-02	0.25g	570.00
	20-4001-10	1.0g	2130.00
Oligo-Affinity Support (CPG) 1 µmole TWIST columns	20-4101-41	Pack of 4	400.00
TAMRA-dT	10-1057-95	50 µmole	250.00
	10-1057-90	100 µmole	495.00
	10-1057-02	0.25g	975.00
Dabcyl-dT	10-1058-95	50 µmole	250.00
	10-1058-90	100 µmole	495.00
	10-1058-02	0.25g	975.00

GLEN RESEARCH ON THE WORLD WIDE WEB

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