The Glen Report

22825 DAVIS DRIVE • STERLING, VIRGINIA • 20164

GLEN RESEARCH

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INSIDE

2′-F-ARABINONUCLEIC ACID (2′-F-ANA)

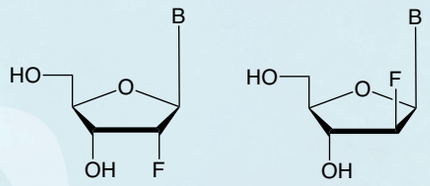
Arabinonucleosides are epimers of ribonucleosides with the chiral switch being at the 2' position of the sugar residue. In ribonucleosides, the 2'-hydroxyl group is in the bottom face (α) position and, in arabinonucleosides, the 2'-hydroxyl group is in the top face (β) position. The 2'-F versions of the nucleosides in RNA and ANA are shown in Figure 1.

2'-F RNA monomers have been available commercially for several years and oligonucleotides containing this modification have been very useful in antisense, siRNA and aptamer studies. Thanks to the pioneering work of Masad Damha and his group at the Department of Chemistry, McGill University in Montreal, 2'-F-ANA is now attracting considerable attention in the research community. In this article, we briefly review the properties of 2'-F-ANA and assess its promise in these fields of research.

STRUCTURAL INFORMATION

Modification at the 2' position of nucleosides has proved to be critical in adjusting the properties of oligonucleotides. It is also relatively straightforward to predict the effect of 2' modification on the conformational geometry of the sugar and its subsequent effect on duplex stability. For example, the electronegative fluorine in 2'-F-RNA causes the sugar to adopt the C3'-*endo* configuration, normally observed in RNA, which leads to an increase in duplex stability, while conformationally restricted analogues like LNA are fully constrained to that configuration. This leads to a significant increase in melting temperature for each position modified by 2'-F-RNA (ΔT_m 3.0 °C/mod) and LNA (ΔT_m 5.6° C/mod). In contrast, the 2'-F-ANA adopts a more DNA-like B-type helix conformation, not through the typical C2'-*endo* conformation but, rather, through an unusual O4'-*endo* (east) pucker. However, the presence of the electronegative fluorine leads to a still significant increase (ΔT_m 1.2° C/mod) in melting temperature per modification.[1]

FIGURE 1: **2'-FLUORO-2'-DEOXYNUCLEOSIDES**



2'-F-RNA *2'-F-ANA*

BASE PAIRING

High base pairing specificity is essential in such applications as diagnostics and therapeutics. 2'-F-ANA-containing oligonucleotides exhibit very high binding specificity to their targets. Indeed, a single mismatch in a 2'-F-ANA – RNA duplex leads to a ΔT_m of -7.2 °C and in a 2'-F-ANA-DNA duplex a ΔT_m of -3.9 °C.[2]

HYDROLYTIC AND NUCLEASE STABILITY

While adopting a DNA-like structure, 2'-F-ANA is much more stable to enzymatic and chemical hydrolysis. Indeed, the order of stability to snake venom phosphodiesterase has been reported to be phosphorothioate (PS) DNA >> 2'-F-ANA > RNA > 2'-F-RNA, while phosphodiester (PO) 2'-F-ANA > PO-DNA. The glycosidic bond in 2'-F-ANA is also remarkably stable to acidic hydrolysis due to the presence of the electronegative fluorine and 2'-F-ANA is stable at pH 1.2 at 37 °C for several days without cleavage. The presence of fluorine at the 2' position in 2'-F-ANA also leads to increased stability to hydrolysis under basic conditions relative to RNA and even 2'-F-RNA.[1,3] The stability of 2'-F-ANA to nucleases makes this a useful modification for enhancing the stability of oligonucleotides in biological environments.[2]

(Continued on Page 2)

RNASE H ACTIVITY AND ANTISENSE

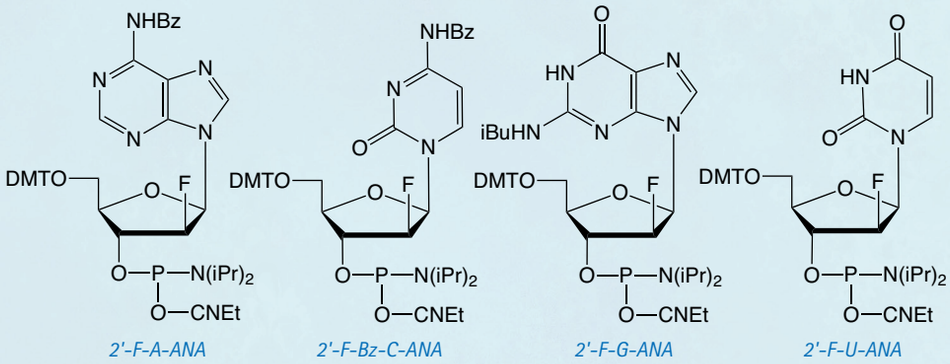
2'-F-ANA hybridizes strongly to target RNA and, unlike most 2' modifications, induces cleavage of the target by RNase H. PS 2'-F-ANA is routinely used in these applications due to its increased nuclease resistance. However, uniformly modified PS 2'-F-ANA, while still active, is not as well recognized by RNase H. Of course, the gapmer strategy of flanking a DNA core in the oligo with PS 2'-F-ANA segments is effective and unusually so. In this case, even a single deoxynucleoside in the gap restores high RNase H activity. Interestingly, the target RNA is cleaved throughout the chain rather than only at positions within the gap.[2] Furthermore, alternating 2'-F-ANA and DNA units provide among the highest potency RNase H-activating oligomers. Both the "altimer" and "gapmer" strand architectures consistently outperform PS-DNA and DNA/RNA gapmers.[4]

siRNA OPTIONS

Since 2'-F-ANA exhibits mostly DNA-like behavior and siRNA activity requires RNA-like behavior, is there a place for 2'-F-ANA in siRNA development? siRNA is recognized in mammalian cells by the multiprotein RNA-induced silencing complex (RISC) containing the exonuclease Argonaute2. When siRNA is introduced, the sense or passenger strand is removed, while the guide strand, antisense to the target mRNA, is incorporated into the RISC. Interestingly, siRNA oligos were found to tolerate the presence of 2'-F-ANA linkages very well. The whole sense strand can, in fact, be all 2'-F-ANA but potency is improved by incorporating 5 RNA residues towards the 3' terminus. This increased potency may be attributed to a slight destabilization of the duplex at the 3' sense terminus, which favors loading of the antisense strand into the RISC complex.[5] A small number of 2'-F-ANA are well tolerated in the antisense strand but modified antisense strands should be 5'-phosphorylated for proper potency. Also, modification of the 3' overhang of the antisense strand with 2'-F-ANA leads to increased potency. As can be expected, the serum half life of 2'-F-ANA modified siRNA is also significantly improved relative to unmodified siRNA.[2,5]

A recent publication[5] describes high potency gene silencing with siRNA chimeras containing 2'-F-RNA and/or LNA and 2'-F-ANA. The high efficacy of these chimeras was attributed to the combination of the rigid RNA-like properties of 2'-F-RNA and LNA with the DNA-like properties of 2'-F-ANA. There is clearly great potential for 2'-F-ANA to contribute to the continuing improvement of the efficacy of siRNA.

FIGURE 2: 2'-F-ANA PHOSPHORAMIDITES



OLIGONUCLEOTIDE SYNTHESIS AND DEPROTECTION

We are happy to introduce all four 2'-F-ANA monomers, as shown in Figure 2. 2'-F-ANA oligonucleotides are readily prepared with a 6 minute coupling time under otherwise standard conditions. Sulfurization, if desired, can be carried out using either of our Sulfurizing Reagents. The resulting oligonucleotides can be deprotected using ammonium hydroxide even at elevated temperatures. Oligonucleotides and chimeras can be purified using all standard methods.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
2'-F-A-ANA CE Phosphoramidite	10-3800-90	100 µmole	150.00
	10-3800-02	0.25g	375.00
2'-F-Bz-C-ANA CE Phosphoramidite	10-3810-02	0.25g	200.00
	10-3810-05	0.5g	400.00
2'-F-G-ANA CE Phosphoramidite	10-3820-90	100 µmole	165.00
	10-3820-02	0.25g	425.00
2'-F-U-ANA CE Phosphoramidite	10-3830-02	0.25g	125.00
	10-3830-05	0.5g	250.00

We thank Masad Damha for taking the time to review this article and for his encouragement to make these products available to the research community.

References:

1. E. Viazovkina, M.M. Mangos, M.I. Elzagheid, and M.J. Damha, *Curr Protoc Nucleic Acid Chem,* 2002, **Chapter 4**, Unit 4 15.
2. J.K. Watts, and M.J. Damha, *Can. J. Chem.,* 2008, **86**, 641-656.
3. J.K. Watts, A. Katolik, J. Viladoms, and M.J. Damha, *Org Biomol Chem,* 2009, **7**, 1904-10.
4. A. Kalota, *et al., Nucleic Acids Res.*, 2006, **34**, 451.
5. G.F. Deleavey, *et al., Nucleic Acids Res.,* 2010, **38**, 4547-4557, J.K. Watts, *et al., Nucleic Acids Res.*, 2007, **35**, 1441-1451, T. Dowler, *et al., Nucleic Acids Res.*, 2006, **34**, 1669-1675.

INTELLECTUAL PROPERTY

2'-F-ANA is covered by intellectual property. Key patents covering siRNA and antisense applications are as follows:

WO/2009/146556 (siRNA); WO 03064441 and WO 0220773 (antisense).

NEW PRODUCTS – DX AND FERROCENE-DT

2'-DEOXYXANTHOSINE

2'-DeoxyXanthosine (dX) is a naturally occurring nucleoside that may be derived from oxidative deamination of 2'-deoxyGuanosine (dG). dX has interested researchers for many years because of its potential to base pair with all four natural bases and it has been considered as a potential universal base.[1] dX featured in Benner's attempts to extend the genetic alphabet with a new base pair of dX and pyrimidine-2,4-diamine nucleoside.[2] dX has also interested researchers in the field of DNA damage and repair since it is a product of nitric oxide-induced mutagenesis.[3]

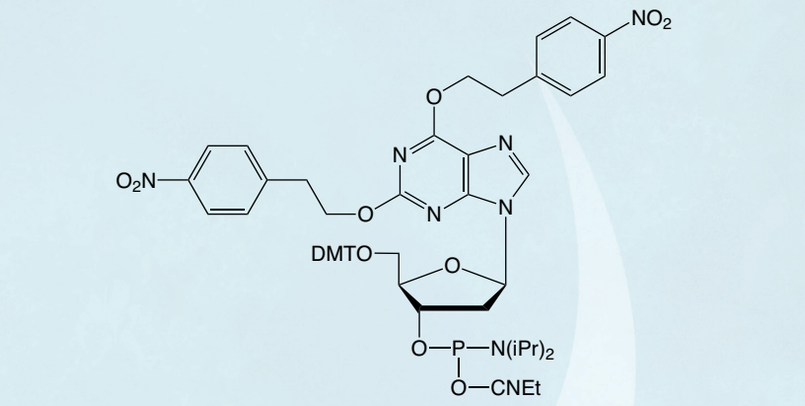
Unfortunately, dX is also a very insoluble nucleoside and it has proved difficult to carry out the chemistry required to make a useful phosphoramidite for incorporating dX into oligonucleotides. However, Benner's choice of protection for dX with dual nitrophenylethyl (NPE) protecting groups (1) has proved to be the most successful to date.[4] This protected dX monomer can be incorporated into oligonucleotides using conventional synthesis. The resulting oligonucleotides are cleaved and deprotected using ammonium hydroxide at room temperature with times dependent on the protecting groups on the regular bases (4 hours for UltraMild monomers, overnight for dmf-dG, and up to 36 hours for iBu-dG). After drying, the NPE protecting groups must be removed using 0.3M tetramethylguanidine/2-nitrobenzaldoxine in water dioxane (1:1) at 70 °C for 48 hours. After evaporation, the product oligonucleotide is desalted using standard techniques.[4]

FERROCENE-dT

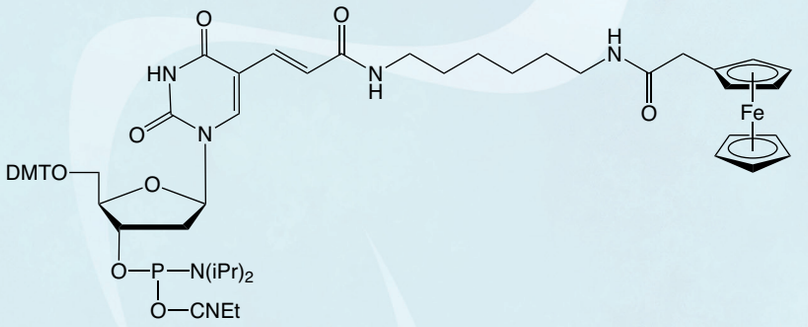
With an excellent stability profile, ferrocene has always attracted considerable interest for DNA labelling to generate probes for electrochemical detection.[5, 6] A variety of post-synthesis conjugation techniques has been used to attach ferrocene to oligonucleotides, with the most popular technique being the conjugation of amino-modified oligonucleotides with activated ferrocene carboxylates. Such post-synthesis conjugation techniques may be useful but they tend to be inconvenient as well as low yielding. Of course, the problems quickly escalate if several positions need to be conjugated.

We are happy to introduce ferrocene-dT (2) to our product range. Based on our Amino-Modifier C6-dT structure, this product is easily added to oligonucleotides with no disruption of regular hybridization behavior. Multiple incorporations into an oligonucleotide probe are also simply achieved. Oligonucleotides are deprotected using standard techniques. Ferrocene oligonucleotides should be stored under Argon and aqueous solutions should be degassed immediately.

***FIGURE 1:* NEW PRODUCT STRUCTURES**



(1) 2'-dX



(2) Ferrocene-dT

References:

1. R. Eritja, *et al.*, *Nucleic Acids Res,* 1986, **14**, 8135-53.
2. M.J. Lutz, H.A. Held, M. Hottiger, U. Hubscher, and S.A. Benner, *Nucleic Acids Res,* 1996, **24**, 1308-13.
3. G.E. Wuenschell, T.R. O'Connor, and J. Termini, *Biochemistry,* 2003, **42**, 3608-16.
4. S.C. Jurczyk, J. Horlacher, K.G. Devined, S.A. Benner, and T.R. Battersby, *Helv Chim Acta,* 2000, **83**, 1517-1524.
5. A.E. Navarro, *et al.*, *Nucleic Acid Res,* 2004, **32**, 5310-5319.
6. H. Brisset, A.E. Navarro, N. Spinelli, C. Chaix, and B. Mandrand, *Biotechnology Journal,* 2006, **1**, 95-98.

ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
2'-dX-CE Phosphoramidite	10-1537-95	50 µmole	105.00
	10-1537-90	100 µmole	200.00
	10-1537-02	0.25g	420.00
Ferrocene-dT-CE Phosphoramidite	10-1576-95	50 µmole	170.00
	10-1576-90	100 µmole	330.00
	10-1576-02	0.25g	670.00

NEW PRODUCT: 0.5M CSO FOR NON-AQUEOUS OXIDATION IN DNA SYNTHESIS

Iodine-based oxidizers have been the standard for DNA and RNA synthesis since the advent of automated synthesizers.[1] They are fast and efficient oxidizers, typically requiring less than 30 seconds for complete oxidation of phosphite triesters to phosphate triesters. However, while iodine-based oxidizers work well for most applications, there are some circumstances where non-aqueous oxidizers may be advantageous, especially where the bases or linkages being produced are sensitive to the presence of water and/or iodine during synthesis. For example, using low water oxidizers has been shown to improve the synthesis of oligos containing methyl phosphonates.[2,3] Non-aqueous oxidizers, typically peroxides, including tert-butyl hydroperoxide, cumene hydroperoxide, hydrogen peroxide, and bis-trimethylsilyl peroxide, among others, have also been employed in DNA synthesis.[5,6] These peroxides tend to be unstable, requiring that they be freshly formulated just prior to use, and so are difficult to use in routine automated synthesis.[7]

In 1996, we investigated the use of (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO)[4] as a non-aqueous oxidizer in DNA synthesis.[2] We found that a 0.5M solution of CSO in acetonitrile worked well as an oxidizer for the synthesis of oligos containing multiple incorporations of 7-deaza-dG, compared with iodine oxidation which caused substantial degradation.

More recently, CSO has been used for synthesizing oligos that incorporate the phosphonoacetate modification.[8] A solution of 0.1M CSO is recommended for the oxidation of PACE modifications as the phosphonite internucleotide linkage is more easily oxidized than the phosphite internucleotide linkage. When synthesizing DNA-phosphonoacetate chimeric oligos, a 0.5M CSO solution is recommended. CSO has also been used as an oxidizer in 2'-O-DMAOE modified siRNA oligo synthesis.[9]

COMPARISON OF CSO WITH IODINE

We prepared the following oligo using 0.5M CSO in acetonitrile and compared it to the same oligo synthesized using standard 0.02M Iodine in THF/Water/Pyridine.

5'-TTA TTG TTC TTA TTG TTC TT-3'

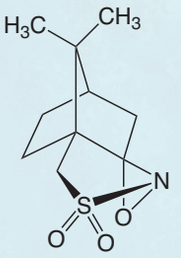
We found the products to be virtually identical on RP HPLC, as shown in Figure 1.

A second pair of oligos was synthesized incorporating NHS-carboxy-dT (10-1535) to compare the performance of 0.5M CSO in acetonitrile to the standard 0.02M iodine oxidizer in an oligo containing a very sensitive modified base.

5'-TTT TTT TTT TTT TTT XTT TTT TTT T – 3' (where X = NHS-Carboxy-dT)

The resulting oligonucleotides, shown in Figure 2, were of comparable purity by RP HPLC.

***CSO* STRUCTURE**



CSO

***FIGURE 1:* MIXED BASE OLIGO USING CSO OR I2**

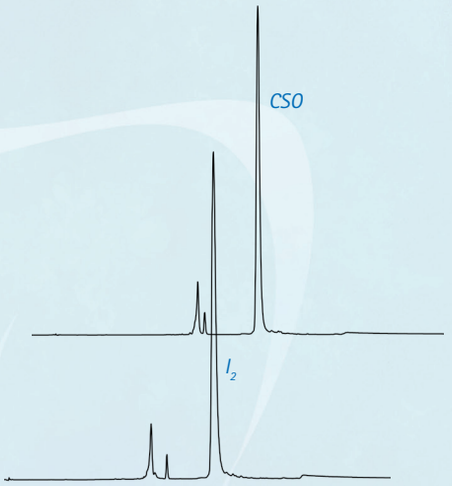
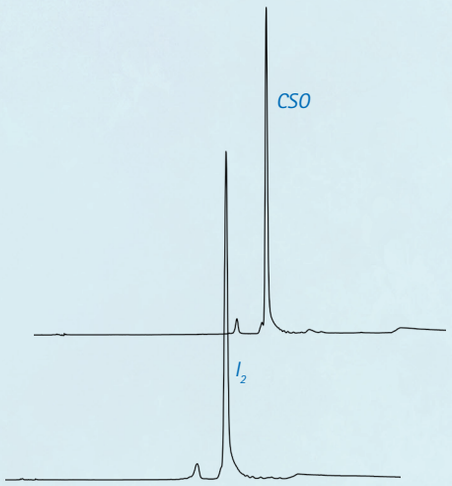


***FIGURE 2:* SENSITIVE OLIGO USING CSO OR I2**



We can conclude that CSO is an effective, stable, non-aqueous oxidizer. Despite the demonstrated effectiveness of CSO as a non-aqueous oxidizer, the cost and the quality of the CSO has been prohibitive for use in formulating CSO as an oxidizer for routine use. We are now pleased to offer 0.5M CSO in acetonitrile as an effective, stable, non-aqueous oxidizer for DNA synthesis. We also offer 0.1M CSO for PACE chemistry at a more affordable price.

Non-aqueous oxidizers may prove beneficial in applications where it is desirable to avoid exposure to iodine or water. This might include on-chip or chamber-based synthesizers, and with phosphoramidites that are sensitive to the presence of iodine or moisture.

ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
0.1M CSO in Anhydrous Acetonitrile (ABI)	40-4631-52	200mL	85.00
0.1M CSO in Anhydrous Acetonitrile (Expedite)	40-4631-52E	200mL	85.00
0.5M CSO in Anhydrous Acetonitrile (ABI)	40-4632-52	200mL	250.00
0.5M CSO in Anhydrous Acetonitrile (Expedite)	40-4632-52E	200mL	250.00

References:

1. M.H. Caruthers, *et al., Gene Amplif Anal,* 1983, **3**, 1-26.
2. M.A. Reynolds, *et al., Nucleic Acids Res,* 1996, **24**, 4584-4591.
3. *The Glen Report,* 1996, **9**, 8-9.
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NEW PRODUCT - UNIVERSAL TOSYL PHOSPHORAMIDITE

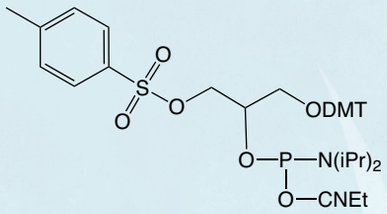
The reactivity of the tosyl functional group in biomolecule conjugation has now been demonstrated[1] in a number of publications. One of the first uses of a tosyl phosphoramidite was in the work by Herrlein, Nelson and Letsinger[2] in the early 1990s. They demonstrated the template-dependent chemical ligation of a 3'-phosphorothioate and a 5'-tosyl oligo to join two synthetic oligonucleotides together. This methodology was later used by Letsinger to demonstrate[3] the tolerance of the Taq polymerase in efficiency of amplification. In Letsinger's work, a 5'-tosyl phosphoramidite was prepared that lacked a DMT purification handle and was not extendable in oligonucleotide synthesis. In recent work by Mirkin *et al.*, a Universal Tosyl Phosphoramidite containing a DMT group was used[4] to prepare 3'-tosyl oligonucleotides. Antibodies were then coupled to the oligonucleotides via nucleophilic displacement of the tosyl group by an amine or thiol functional group on the antibody.

The Universal Tosyl Phosphoramidite can be used to insert a tosyl group at the 3' or 5' terminus of an oligonucleotide. The tosyl group may be displaced by nucleophilic substitution using, e.g., a thiol tag, on the synthesis column. Alternatively, the oligonucleotide can be cleaved and deprotected using UltraMild chemistry to yield the fully-deprotected tosyl oligonucleotide. The tosyl group can then be displaced in solution with a protein, antibody, or even an amino-modified nucleic acid of interest.

As described above[4], the Mirkin group has used the Universal Tosyl Phosphoramidite to functionalize the 3' terminus of oligonucleotides destined for conjugation with gold nanoparticles. These oligonucleotides are modified at the 5' terminus with disulfide residues prepared using Dithiol Phosphoramidite (DTPA) or using a Trebler phosphoramidite followed by Thiol-Modifier C6 S-S. Modified oligonucleotides attached to gold nanoparticles are described as "barcodes" and are used in a nanoparticle-based biobarcode assay.

Glen Research now offers this Universal Tosyl Phosphoramidite that can be coupled either at the 5' or the 3' end of an oligo and conveniently purified via standard reverse phase purification. This phosphoramidite also potentially may allow the introduction of other nucleophiles (azido, amino, cyano, etc.) via nucleophilic displacement of the tosyl group on the support. The use of UltraMild monomers is recommended in the synthesis of tosyl oligos, along with DTPA monomer for subsequent gold attachment chemistry.

***FIGURE 1:* UNIVERSAL TOSYL PHOSPHORAMIDITE**



Universal Tosyl Phosphoramidite

PREPARATION OF TOSYLATED OLIGONUCLEOTIDES

Oligonucleotides should be cleaved and deprotected using UltraMild chemistry to yield the fully-deprotected tosyl oligonucleotide.

CONJUGATION OF 3'-TOSYLATED 5'-DISULFIDE OLIGONUCLEOTIDES (BARCODES) TO GOLD NANOPARTICLES

Tosyl-oligonucleotide gold conjugates were prepared by addition of 1 O.D. of the barcode to 1mL of 30nm gold particles. The mixture was allowed to stand at room temperature for 24 hours. Following this initial incubation period, 10% SDS was introduced to a final concentration of 0.1%, followed by addition of sodium chloride to a final concentration of 0.1M using a 1M salt solution. The mixture was then allowed to stand at room temperature for 48 hours. The conjugates were harvested by centrifugation at 6800 rpm for 15 minutes using an Eppendorf bench top centrifuge, washed twice with nanopure water, and then finally suspended in nanopure water and refrigerated.

GENERAL METHOD FOR PREPARATION OF BIOBARCODE PROBES CO-LOADED WITH ANTIBODIES

The co-loaded probes were prepared by concentration of 3.0mL of the tosyl-oligonucleotide gold conjugates down to 60 µL by centrifugation and removal of the supernatant. To this concentrate was added 20 µL of a 0.2% Tween20 solution followed by 10 µg of the desired protein in 20µL of PBS buffer, pH 7.4. (In the case of PSA detection[4], Human Kallikrein 3/PSA Biotinylated Affinity Purified PAb, Goat IgG was used.) To this mixture, 100µL of a 0.2M borate buffer solution at pH 9.5 was added. The mixture was allowed to react at 37 °C for 24 hours at 550 rpm on an Eppendorf Thermomixer R. To this mixture 10µL of a 10% BSA solution was added and allowed to react for an additional 24 hours under the previous conditions. The probes were harvested by centrifugation at 5800 rpm for 15 minutes followed by washes using a pH 7.4 PBS buffer containing 0.1% BSA, 0.025% Tween20 (assay buffer), and finally re-suspended in 3mL of the assay buffer and refrigerated.

ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
Universal Tosyl Phosphoramidite	10-1929-90	100 µmole	80.00
	10-1929-02	0.25g	210.00

References:

1. P.A. Bertin, *et al.*, *J Amer Chem Soc,* 2006, **128**, 4168-9.
2. M.K. Herrlein, J.S. Nelson, and R.L. Letsinger, *J. Amer Chem. Soc.,* 1995, **117**, 10151-10152.
3. R.L. Letsinger, T.F. Wu, and R. Elghanian, *Nucleos Nucleot,* 1997, **16**, 643-652.
4. C.S. Thaxton, *et al.*, *Proc Natl Acad Sci U S A,* 2009, **106**, 18437-42.

NEW PRODUCTS - GLEN GEL-PAK™ DNA/RNA DESALTING AND 1000Å GLEN UNYSUPPORT™ FRITS

PRINCIPLES OF GLEN GEL-PAK™ DNA/RNA DESALTING

The principle of the Glen Research gel filtration column, Gel-Pak™, is based on size exclusion chromatography that separates molecules according to the hydrodynamic volume of the molecule in aqueous solutions. In gel filtration, the mobile phase for size exclusion is an aqueous solution and the stationary phase is a porous resin. The pores of the resin are sized such that they allow small molecules to enter the pores, yet exclude larger molecules from the pores. The small molecules, such as salts and hydrolyzed protecting groups, diffuse into the pores of the resin and move slowly through the column. The larger molecules, such as DNA or proteins, are excluded from the pores and move quickly through the column. The end result is that the larger molecules elute first in the column void volume while the small molecules are still flowing through the resin of the column.

Gel-Pak columns are ideal for desalting and reaction clean up. They can be used for removal of the ammonium hydroxide deprotection solution and hydrolyzed protecting groups after deprotection. The columns can also be used for the clean up of NHS-labelling reactions to separate the labelled oligo and unlabelled oligo from the unreacted NHS ester, the hydrolyzed label, and n-hydroxysuccinimide, thereby greatly simplifying the downstream purification steps.

There are many benefits to Gel-Pak columns:

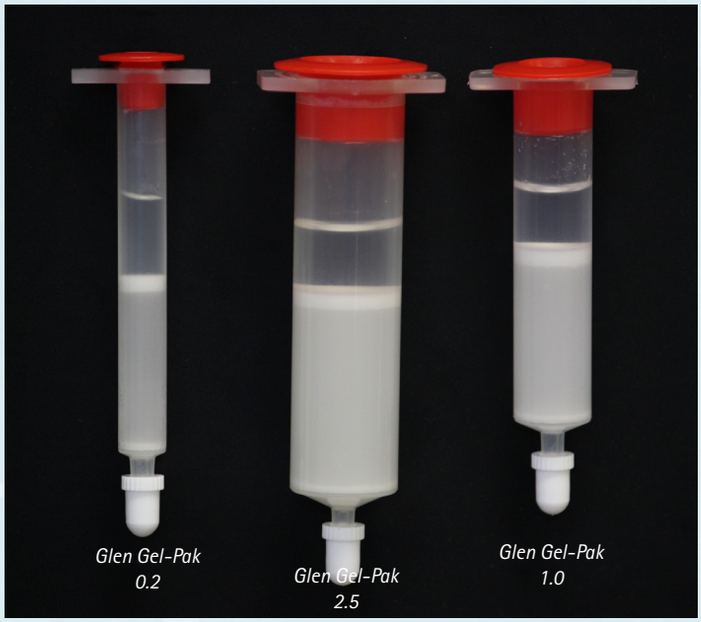
Versatility:

- Ability to directly desalt oligonucleotides deprotected in either 30% ammonium hydroxide OR 50:50 ammonium hydroxide/40% aqueous methylamine (AMA)
- Easily exchange buffers
- Simple clean-up of labelling reactions
- Mild method for purification from salts and solvents such as DMSO and DMF

Capacity:

- Multiple column sizes (0.2 mL, 1.0 mL and 2.5 mL) are available to match synthesis scale
- Ability to efficiently desalt short and long oligos at different scales using the same protocol
- Suitable for oligos >10mer in length

GLEN GEL-PAK™ DNA/RNA DESALTING COLUMNS



Gel-Pak columns are easy to use and available in three sizes for different sample volumes (see below).

GLEN UNYSUPPORT

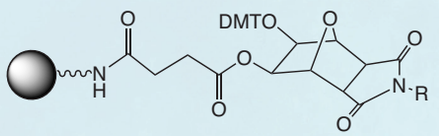
Glen UnySupport™ is a version of UnyLinker™, shown in Figure 1, and is preferred for high throughput oligonucleotide synthesis. We supply Glen UnySupport under license from Isis Pharmaceuticals.

GLEN UNYSUPPORT FRITS

Universal Supports are the supports of choice for high throughput synthesis since they remove the need for individual wells or columns to contain a unique support.

We now offer high density polyethylene (HDPE) frits with embedded 500Å or 1000Å Glen UnySupport for high throughput applications and to allow customers to make their own inexpensive columns. The frits contain 40 nanomoles of Glen UnySupport and fit directly into empty MerMade and AB3900 columns. They can also be used on ABI 394 and Expedite instruments when fitted into inexpensive female-female luer adapters.

***FIGURE 1:* UNIVERSAL SUPPORT STRUCTURES**



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

ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
Glen Gel-Pak™ 0.2 Desalting Column	61-5002-05	Pack of 5	30.00
(0.2 mL Capacity)	61-5002-50	Pack of 50	300.00
Glen Gel-Pak™ 1.0 Desalting Column	61-5010-05	Pack of 5	35.00
(1.0 mL Capacity)	61-5010-50	Pack of 50	350.00
Glen Gel-Pak™ 2.5 Desalting Column	61-5025-05	Pack of 5	45.00
(2.5 mL Capacity)	61-5025-25	Pack of 25	225.00
Glen UnySupport 500Å (40 nmole frits)	20-5440-95	Pack of 96	150.00
Glen UnySupport 1000Å (40 nmole frits)	20-5441-95	Pack of 96	150.00
Female-Female Luer Adapter	20-0060-00	Pack of 10	20.00

NEW PRODUCTS – AZIDE TAGS FOR CLICK CHEMISTRY

NEW CLICK TAGS

Glen Research is collaborating with baseclick Gmbh to offer a variety of interesting alkyne phosphoramidites. We offer some azide products and are now embarking on an expansion of our azide catalog. Our strategy is to offer first our most popular labels for general interest and, subsequently, we will add azide products that are not compatible with phosphoramidite chemistry.

Out first offering of azide tags for Click Chemistry, BiotinTEG, DesthiobiotinTEG, 6-FAM-TEG, and Dipivaloyl 6-FAM-TEG, is shown in Figure 1.

Biotin is still our most commonly used label and biotinTEG, with its hydrophilic triethylene glycol spacer, is the most popular biotin product. Desthiobiotin is a biotin analogue that is well captured by streptavidin but the captured product can be easily released by applying a biotin solution to the streptavidin beads. 6-FAM is our most popular fluorescein derivative and we offer azides of both 6-FAM and pivaloyl-protected 6-FAM for situations where subsequent reactions require the 6-FAM to be protected. In both 6-FAM products, the hydrophilic TEG spacer is again used. The azides are offered in 25 and 100 µmole packs for convenient oligonucleotide labelling.

Figure 2 illustrates the high efficiency of the click reaction between 6-FAM-TEG Azide and an oligonucleotide modified with C8-Alkyne-dT (10-1540) on a 0.8 µmole scale.

FIGURE 1: NEW AZIDE LABELS

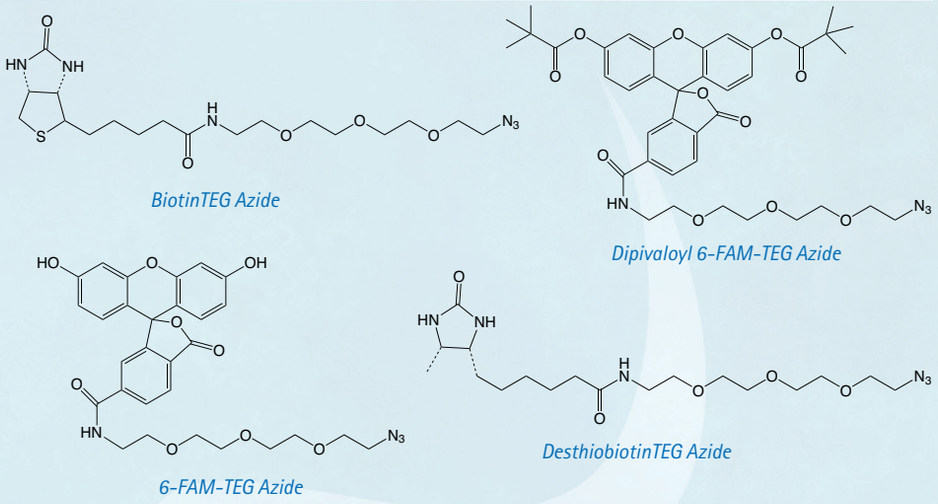
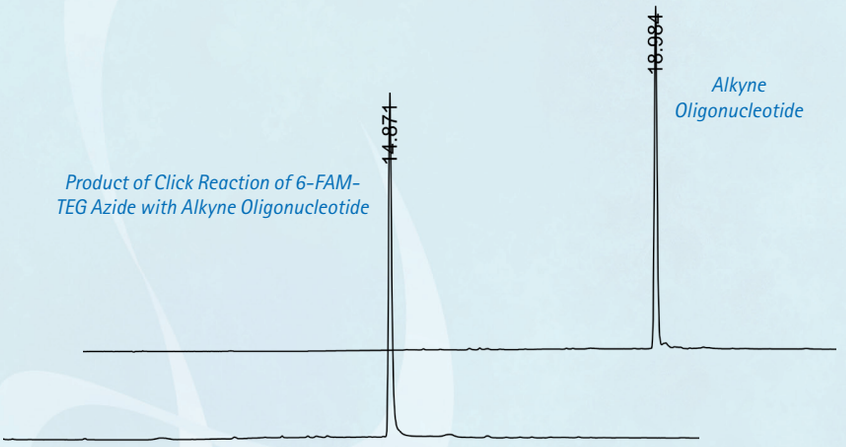


FIGURE 2: CLICK REACTION OF ALKYNE-LABELLED OLIGO WITH 6-FAM-TEG AZIDE



CLICK PROCEDURE

Notes on Click Chemistry:

The CuBr/TBTA solution must be made fresh, however, the 0.1 M TBTA solution in DMSO/t-butanol can be safely stored in the freezer. If the click reaction is performed on the column, note that the resulting triazole linkage is compatible with deprotection in AMA for 10 minutes at 65 °C or overnight deprotection in ammonium hydroxide at room temperature.

Materials:

1. 800 nmoles of Alkyne-labelled oligo in 80 µL water (10 mM)
2. 2 molar equivalents of azide label per alkyne moiety to Click
3. 3 mg CuBr
4. 240 µL 0.1 M TBTA in DMSO/t-butanol 3:1 (v/v)

Procedure:

1. Add the azide label to the alkyne-labelled oligo and vortex to dissolve.
2. Add the CuBr to the 0.1 M TBTA solution and vortex to dissolve. (Note: this solution must be made fresh.)
3. Combine the CuBr/TBTA and oligo/azide solutions, mix well and transfer to a heat block set at 45 °C.
4. Let react for 1.5 hours.
5. Dilute to 1 mL with water and desalt on a Glen Gel-Pak™ cartridge or equivalent. (Note: CuBr may precipitate upon the addition of water but this will not interfere with the desalting of the labelled oligo.)

ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
BiotinTEG Azide	50-2000-92	25 µmole	150.00
	50-2000-90	100 µmole	450.00
DesthiobiotinTEG Azide	50-2001-92	25 µmole	135.00
	50-2001-90	100 µmole	400.00
Dipivaloyl 6-FAM-TEG Azide	50-2002-92	25 µmole	230.00
	50-2002-90	100 µmole	690.00
6-FAM-TEG Azide	50-2003-92	25 µmole	180.00
	50-2003-90	100 µmole	540.00

The deprotection of oligonucleotides, especially for high-throughput syntheses, can be the rate-limiting step during the production of oligos and is often difficult to automate due to issues with liquid handling. To streamline the deprotection process, gas phase deprotection using ammonia or methylamine gas is often employed[1]. After the removal of the protecting groups is complete, the oligo is conveniently eluted directly in water or the buffer of choice. However, the equipment necessary to safely handle a pressurized, corrosive gas is expensive and the additional cost is not worthwhile for many smaller production facilities and research labs.

An alternative method that incorporates much of the convenience of gas-phase deprotection but still utilizes low-cost and simple equipment is On-Column deprotection. In this case, the nucleophilic amine used to remove the protecting groups is dissolved in a non-polar solvent, such as toluene, in which the deprotected oligonucleotide is insoluble. After the deprotection of the oligonucleotide is complete, the column is rinsed, allowed to briefly dry and the oligo, still bound to the support, is eluted in the aqueous buffer of choice, as described by Kempe[2]. A similar strategy was used by Damha for the deprotection of RNA on glass slides[3]. Based upon a protocol used to deprotect TC RNA monomers[4], we have developed a procedure for the deprotection of standard DNA as shown in the Procedure below:

In Figure 1, we show the results of an oligonucleotide synthesis that was split, with half being deprotected in standard aqueous AMA and the other half in an EDA/toluene solution. Both product oligos had the same molecular weight as determined by electrospray mass spectrometry. We also found there was no drop in yield from the On-Column deprotection compared with the standard aqueous deprotection.

When oligos of the same length but different molecular weights were synthesized on Glen UnySupport Frits and deprotected in the same EDA solution, we found there was no indication of any cross-contamination of oligos between the frits by mass spec analysis. This means that an entire 96 well plate can be conveniently deprotected in a single vessel. It should be noted, however, that the Glen UnySupport required 2 hours at 65 °C to be fully eliminated from the 3' terminus of the oligo in the EDA solution.

We have found that this method is compatible with PS supports as well as CPG supports. However, if there are hydrophobic labels on the product oligo, e.g., DMT or CyDyes, some oligo (10-20%) may be retained on a PS support. In this case, we recommend eluting the oligo in buffer containing 10-20% acetonitrile.

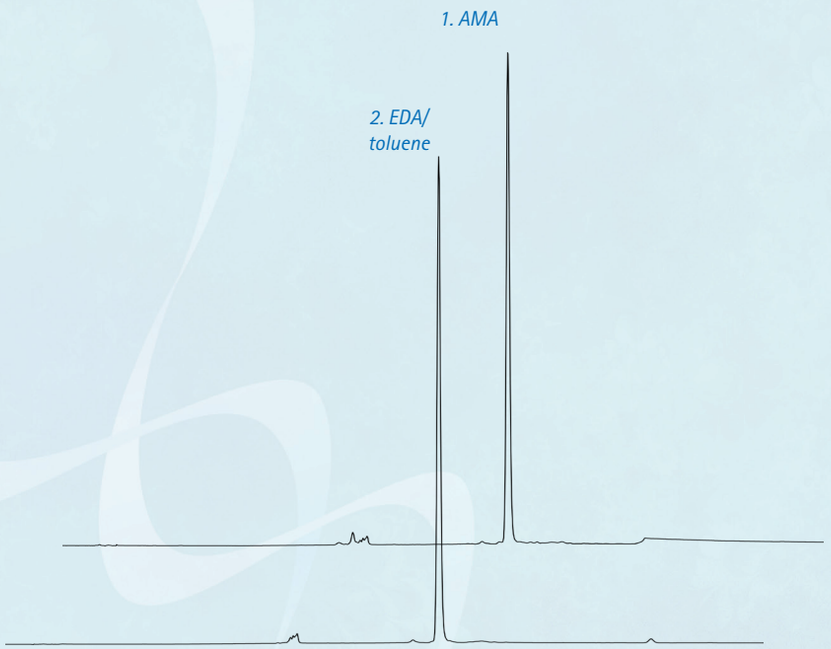
VOLUME 5: DEPROTECT TO COMPLETION IN ORGANIC SOLVENTS

1) *When should I use on-column deprotection?*
   When using high throughput synthesis.
2) *Do I need special monomers?*
   On-column deprotection requires the use of Ac-dC.
3) *Do I need to desalt after on-column deprotection?*
   No, the organic by-products remain on the synthesis column.

References:

1. J.H. Boal, *et al.*, *Nucleic Acids Res.*, 1996, **24**, 3115-3117.
2. T. Kempe, Anhydrous amine cleavage of oligonucleotides. United States Patent 5750672.
3. J.G. Lackey, D. Mitra, M.M. Somoza, F. Cerrina, and M.J. Damha, *J. Amer. Chem. Soc.*, 2009, **131**, 8496-502.
4. D.J. Dellinger, *personal communication*.

FIGURE 1: RP HPLC OF AN OLIGO DEPROTECTED WITH 1. AMA AND 2. EDA/TOLUENE



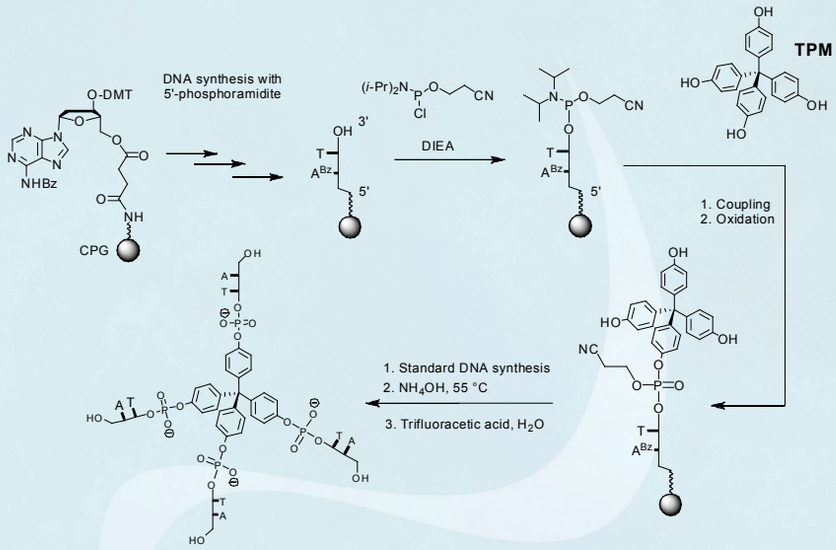
PROCEDURE : DEPROTECTION USING EDA/TOLUENE (1:1)

1. After the synthesis is complete, treat the support with 10% diethylamine in acetonitrile, slowly pushing the solution through the column to waste over a 3-5 minute period. This will remove the cyanoethyl protecting groups from the phosphate backbone. This initial treatment is critical to the success of the protocol.
2. Rinse the column with acetonitrile.
3. Briefly dry the CPG under vacuum.
4. Treat the column with Ethylenediamine (EDA)/Toluene solution 1:1 (v/v), pulling the EDA solution into the column so that the support is completely wetted. Use approximately 500 µL per µmole for small-scale syntheses.
5. Let the solution sit over the support for 2 hours at room temperature. Apply vacuum and remove the deprotection solution.
6. Rinse the column with Toluene (3x).
7. Briefly dry the support under vacuum.
8. Elute the oligo from the support in aqueous buffer of choice.

TECHNICAL BRIEF - SYMMETRICALLY BRANCHED FOUR-ARM DNA

Nature evolved DNA as a linear polymer. As a genetic material, this linear polymer is a wonderful compound to work with. However, for molecular construction and supramolecular chemistry, the linear structure of DNA is a severe limitation. Positioning structural elements at defined positions and creating densely cross-linked 3D materials requires branching points not found in natural oligonucleotides. Glen Research offers branching elements for custom DNA syntheses, such as 5-Me-dC Brancher Phosphoramidite and Trebler Phosphoramidite. These contain flexible alkyl chains that provide conformational flexibility to the resulting branched chains. Recently, Meng et al. reported that a more rigid, symmetrical branching element gives access to four-arm DNA hybrids with surprising properties.[1] When the four chains of a symmetrically branched oligonucleotide are linked by tetrakis(*p*-hydroxyphenyl) methane (**TPM**), the DNA hybrids obtained have an extreme propensity to assemble into three-dimensional networks.

SCHEME 1



Synthesis of symmetrically branched oligonucleotides with rigid core via two-stage solid-phase synthesis.

For these rigid hybrids to form, two strong base pairs per DNA arm will suffice to induce the formation of a solid upon addition of magnesium cations to micromolar solutions in conventional aqueous buffer.[1] The new nanoporous material has interesting properties and can readily be stained with intercalators. The synthesis of the rigid hybrids (Scheme 1) is more complicated than that of branched oligonucleotides with flexible chains, but it is still simpler than the solution phase syntheses of other symmetrical hybrids.[2] The combination of reversed or 5'-phosphoramidites (stage 1), on-support phosphitylation, and conventional 3'-phosphoramidites ensures that all chains are attached via the 3'-terminus, but some off-synthesizer steps are required. Glen Research will be happy to make **TPM** available to interested researchers upon request.

References:

1. M. Meng, *et al., Chembiochem,* 2009, **10**, 1335-9.
2. O. Plietzsch, *et al., Org Biomol Chem,* 2009, **7**, 4734-43.

TECHNICAL BRIEF - CROSSLINKING WITH CLICK CHEMISTRY

The click process is rapidly becoming the method of choice for key organic reactions useful in oligonucleotide modification.[1] The advent of Copper (Cu^1) catalyzed [3+2] azide-alkyne click chemistry (CuACC) has been the main reason for the surge in interest in this chemistry. CuACC reactions have simply improved the speed and performance of click chemistry. At the same time, the specificity of the reaction between an alkyne and an azide means that side reactions are very unlikely even in complex biological systems.

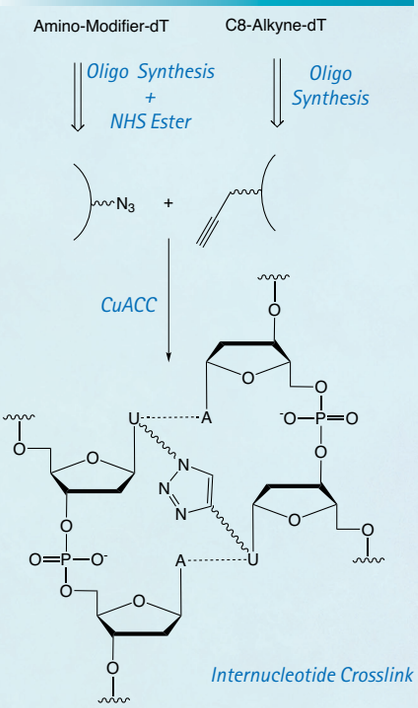
One of our most common questions from researchers is how to create a specific crosslink between the strands of an oligonucleotide duplex. Most procedures use light to create a crosslink using a free radical mechanism. Photolytic crosslinking is typically low yielding with many side reactions possible. Figure 1 shows click chemistry being used[2] to form such an internucleotide crosslink. One DNA strand is simply modified using C8-Alkyne-dT (10-1540). The complementary strand is first modified using Amino-Modifier C6-dT (10-1039). A post-synthesis conjugation with Azidobutyrate NHS ester (50-1904) leads to the azido-modified strand. A subsequent CuACC click reaction leads to the intranucleotide crosslink in high yield.

In a recent article, this crosslinking technique was extended to hairpin and hammerhead ribozymes.[3]

References:

1. A.H. El-Sagheer, and T. Brown, *Chem Soc Rev,* 2010, **39**, 1388-405.
2. P. Kocalka, A.H. El-Sagheer, and T. Brown, *Chembiochem,* 2008, **9**, 1280-1285.
3. A.H. El-Sagheer, and T. Brown, *Proc Natl Acad Sci U S A,* 2010, **107**, 15329-34.

***FIGURE 1:* CROSSLINKING WITH CLICK CHEMISTRY**



GLEN-PAK™ PRODUCT UPDATE: NEW PROTOCOLS FOR PURIFICATION

Glen-Pak™ purification has become a very important part of routine oligonucleotide purification. Our customers challenge the Technical Support group to adapt Glen-Pak techniques to their purification needs. As always, we enjoy the challenge.

In this article, we introduce a new cartridge designed for optimal purification of oligos produced on 200 nmole or lower scales in a high throughput environment. We have also developed procedures for purifying oligos containing 2'-OMe and/or 2'-F RNA linkages. Finally, we describe Glen-Pak purification of disulfide containing oligonucleotides, followed by optional reduction with dithiothreitol and desalting on a second Glen-Pak cartridge.

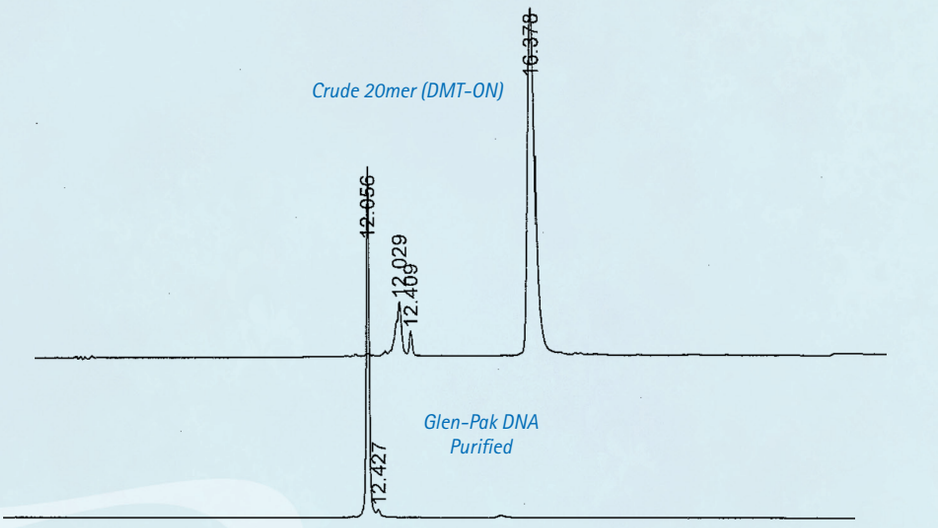
Step by step versions of these new protocols can be viewed in our latest update of the Glen-Pak User Guide found in the purification section of our website or by following this URL: http://www.glenresearch.com/Technical/GlenPak_User_Guide.pdf

50MG GLEN-PAK™ DNA CARTRIDGE FOR UP TO 200 NMOLE SCALE SYNTHESES:

The standard Glen-Pak DNA Purification cartridges (60-5100-xx and 60-5200-xx) have become indispensable tools for desalting and purification of oligos up to 1.0 µmole scale. This range of capacity is quite convenient for those customers who desire a one-cartridge solution to most of their small-scale production needs. However, some of our small scale, high throughput synthesis customers have been requesting a similar configuration with lower capacity and reagent volume requirements. We are happy to introduce the 50mg Glen-Pak DNA cartridge for use on manifolds and other existing high throughput cartridge purification systems.

The 50mg cartridge is used in the same manner as the standard sized Glen-Pak, but requires less volume for most of the protocol steps, potentially allowing single reagent additions. The lower volume requirements begin with cleavage and deprotection of syntheses at or below 200 nmole scale and extend through most of the protocol to the elution step. The 50mg Glen-Pak DNA cartridge is only offered in a vacuum manifold configuration due to its intended use with higher throughput applications.

FIGURE 1: **RP HPLC OF 20MER - CRUDE AND 50MG GLEN-PAK PURIFIED**



Some of the benefits of the 50mg cartridge include:

- Same cartridge configuration as standard sized Glen-Pak DNA cartridge.
- Same purification performance with short AND long oligonucleotides.
- Lower bed volume leaves more room for reagents in the column.
- Lower volume additions of reagents for all but the DMT removal step.
- Lower final elution volume of 0.5mL, which reduces sample drying time.
- More amenable to robotic applications due to single reagent addition capability.
- Less expensive.

PURIFICATION OF 2'-OME AND 2'-F RNA ON THE GLEN-PAK™ DNA PURIFICATION CARTRIDGE

The use of 2'-OMe and 2'-F RNA monomers has steadily increased over the past few years. The fact that these monomers are becoming cheaper and are used in synthesis in a similar manner to their DNA counterparts has made them very popular in therapeutics development. Customer requests for viable downstream processing protocols have driven us to see how best to utilize the Glen-Pak DNA cartridge for an easy, efficient purification of oligonucleotides containing these monomers.

In this experiment, we decided to use a hybrid siRNA oligo containing both 2'-F and 2'-OMe bases.

Oligo 1:
5' – AGCUGACCCUGAAGUUCAUTT – 3'

- A and G are derived from 2'-OMe RNA monomers 10-3100-xx and 10-3121-xx.
- C and U are derived from 2'-F RNA monomers 10-3415-xx and 10-3430-xx.
- T is derived from dT monomer 10-1030-xx.

The coupling time was defaulted to 6 minutes for all bases using 0.45M Tetrazole as the activator. Cleavage and deprotection were completed in 30% Ammonium Hydroxide/40% Methylamine 1:1 (AMA) for 2 hours at room temperature.

TABLE 1: **SCALE SUGGESTIONS FOR GLEN-PAK DNA PRODUCT LINE**

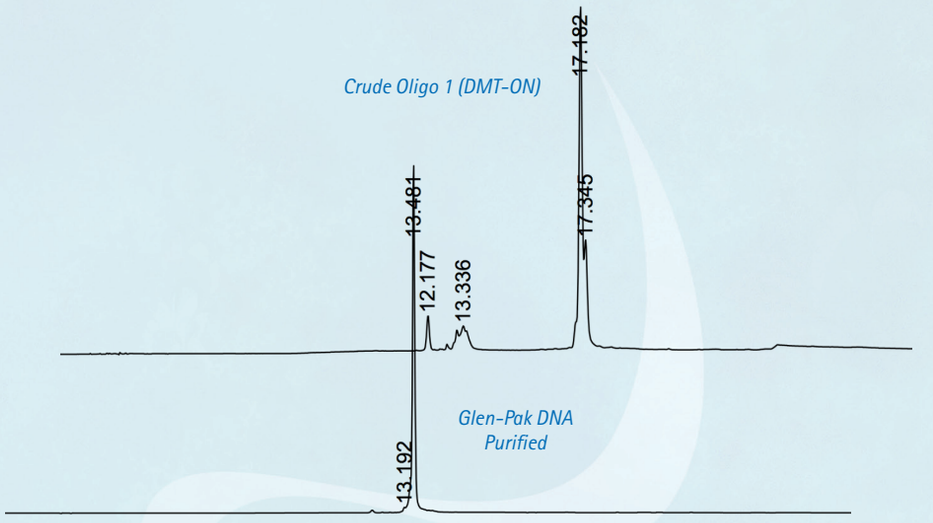
Glen-Pak DNA Product	*Catalog Number*	*Synthesis Scale Compatibility*
Glen-Pak DNA 50mg Purification Cartridge	60-5000-96	10 nmole – 200 nmole
Glen-Pak DNA Purification Cartridge	60-5100-XX and 60-5200-XX	10 nmole – 1.0 µmole
Glen-Pak DNA Cartridge 3G	60-5300-01	5 µmole – 20 µmole
Glen-Pak DNA 30 mg 96-Well Plate	60-5400-01	10 nmole – 50 nmole

The oligonucleotide was then filtered from the support in preparation for Glen-Pak purification and sampled for crude purity determination (see Figure 2).

As in normal Glen-Pak DNA cartridge purification, the deprotection mixture was diluted 1:1 in 100mg/mL sodium chloride. The one step that has been added to the standard DMT-ON purification protocol is a pre-load heating step. The salt/AMA/Oligo mixture was heated at 55 °C for 15 minutes and loaded while still warm onto the Glen-Pak cartridge. We have found when purifying long oligonucleotides, sequences containing higher C/G content, and designed hairpins, that heating the sample results in better binding of DMT-ON full-length products at the load step.

The remainder of the Glen-Pak purification protocol is exactly as described in the DMT-ON DNA purification section of the Glen-Pak User Guide. The final purified product is shown in Figure 2 in an overlay with the crude sample.

FIGURE 2: RP HPLC OF OLIGO-1 – CRUDE AND 50MG GLEN-PAK PURIFIED



GLEN-PAK™ PURIFICATION AND REDUCTION OF THIOL MODIFIED OLIGONUCLEOTIDES

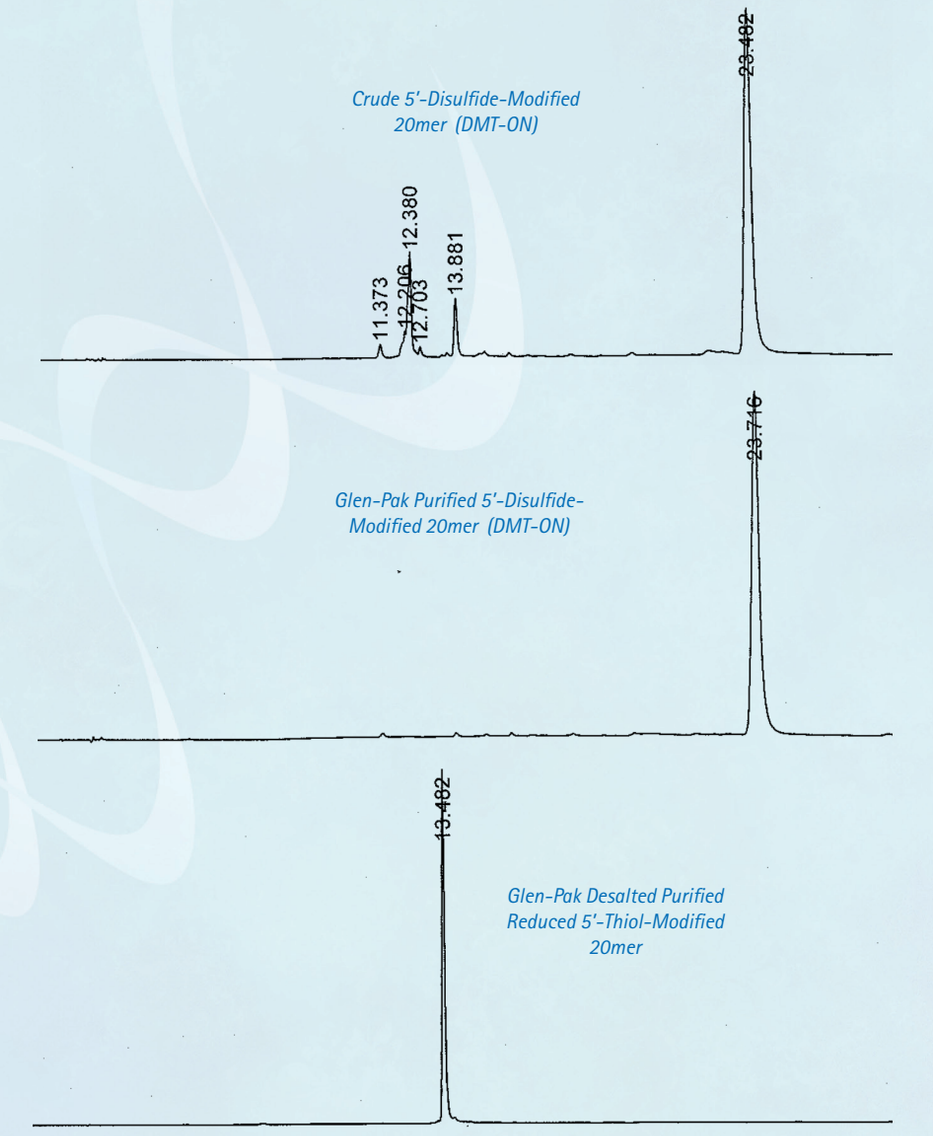
Thiol modification of oligonucleotides is important for labelling with iodoacetamides and maleimides, conjugation of enzymes such as horseradish peroxidase, and attachment to gold surfaces. The disulfide versions of these thiol-modifiers (including Thiol-Modifier C6 S-S, 10-1936-XX) have become very popular due to the simple reductive cleavage step using DTT or TCEP to generate a functional thiol group. Many customers have asked us to determine what protocol would be compatible with our Glen-Pak DNA Purification Cartridges.

In this experiment, a mixed base 20mer oligonucleotide was modified at the 5' terminus with Thiol-Modifier C6 S-S and the DMT was left ON for use in Glen-Pak DNA cartridge purification.

Cleavage and deprotection was completed in 30% Ammonium Hydroxide/40% Methylamine 1:1 (AMA) for 15 minutes at 55°C. The oligonucleotide was then filtered from the support in preparation for Glen-Pak purification and sampled for crude purity determination (see Figure 3).

Step one of the procedure follows a normal Glen-Pak DNA cartridge purification protocol for use with DMT-

FIGURE 3: RP HPLC OF 5'-THIOL-MODIFIED 20MER



GLEN RESEARCH

22825 DAVIS DRIVE
STERLING, VIRGINIA
20164

(Continued from Page 11)

ON oligonucleotides with one exception. There is no DMT-removal step using TFA, since the post purification process (Step 2) includes the reduction of the disulfide and removal of the DMT. The oligo, eluted in 50% acetonitrile with the DMT and disulfide intact, can be stored in this form for subsequent reduction and use if so desired (see Figure 3 on Page 11).

Step two of the procedure entails treatment of the Glen-Pak purified oligo with DTT to reduce the disulfide and remove the DMT group. It is accomplished by simply adding an equal volume (1mL) of 0.2M dithiothreitol in 0.1M phosphate buffer, pH 8.3-8.5, to the eluent from the Glen-Pak and allowing it to sit for 30 minutes at room temperature.

Step three is the final desalting and elution of the reduced thiol and is completed using a modified desalting protocol on a second Glen-Pak DNA purification cartridge. The full protocol for all three of these steps can be found in our Glen-Pak User Guide. Final elution of the oligonucleotide is done in 10% Acetonitrile in water (see Figure 3 on Page 11).

ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
DNA Purification Cartridges			
Glen-Pak™ 50mg DNA Purification Cartridge (For use in vacuum manifolds and high-throughput devices)	60-5000-96	Pack of 96	415.00
Glen-Pak™ DNA Purification Cartridge (For use in vacuum manifolds and high-throughput devices)	60-5100-10 60-5100-30 60-5100-96	Pack of 10 Pack of 30 Pack of 96	80.00 200.00 475.00
Glen-Pak™ DNA Purification Cartridge (For use with disposable syringes)	60-5200-01 60-5200-10	each Pack of 10	8.00 80.00
Glen-Pak™ DNA Cartridge 3g	60-5300-01	Pack of 1	150.00
Glen-Pak™ DNA 30mg 96-Well Plate	60-5400-01	Pack of 1	475.00
RNA Purification Cartridges			
Glen-Pak™ RNA Purification Cartridge (For use in vacuum manifolds and high-throughput devices)	60-6100-10 60-6100-30 60-6100-96	Pack of 10 Pack of 30 Pack of 96	95.00 225.00 575.00
Glen-Pak™ RNA Purification Cartridge (For use with disposable syringes)	60-6200-01 60-6200-10	each Pack of 10	9.50 95.00