The Glen Report

22825 DAVIS DRIVE • STERLING, VIRGINIA • 20164

GLEN RESEARCH

VOLUME 26

NUMBER 1

MAY 2014

INSIDE

DNA CONJUGATION TO HALOTAGGED PROTEIN USING GLEN BROMOHEXYL LINKER

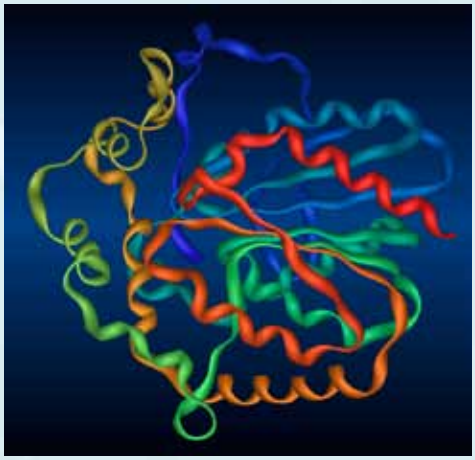
Vijay Singh and Eric T. Kool
Department of Chemistry
Stanford University
Stanford, CA 94305 (USA)

The strategy of small-molecule fluorescent labeling of genetically encoded proteins has become a popular alternative to GFP labeling. This is because the methodology offers the advantage of much greater temporal resolution as the dye can be added at any point during the cell cycle or stage of an organism's development.

Among the most widely used approaches is the HaloTag method developed by Promega, which utilizes a bacterial haloalkane dehalogenase. The enzyme removes halides from aliphatic hydrocarbons by a nucleophilic displacement mechanism to form a covalent ester linkage between the haloalkane and Asp106 in the enzyme. In the wild type haloalkane dehalogenase, the ester is quickly hydrolyzed by histidine 272 in the catalytic active site. However, by mutating the histidine to phenylalanine, the HaloTag variant renders the covalent ester bond stable toward hydrolysis, as shown in Figure 1.

The HaloTag domain is an exceedingly simple

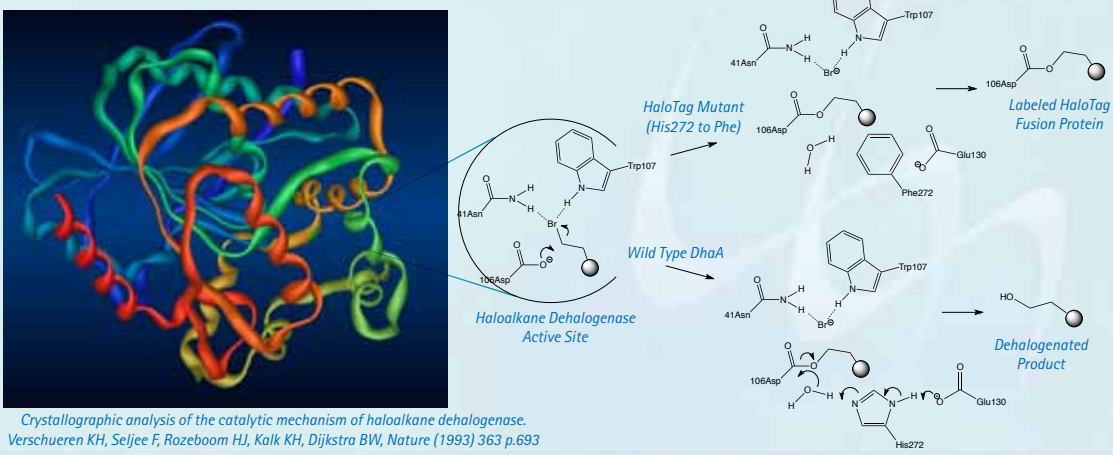
CRYSTAL STRUCTURE OF HALOALKANE DEHALOGENASE



way to form covalent bonds between a variety of different molecules and labels to essentially any protein of interest.[1] Standard ligands for HaloTag protein use lengthy chloroalkyl-substituted groups. Previously, we described a method for conjugating oligonucleotides to proteins by employing a specific phosphoramidite carrying a chloroalkyl linker which required several steps to prepare.[2]

(Continued on Next Page)

FIGURE 1: **MUTANT AND WILD TYPE HALOALKANE DEHALOGENASE ACTIVE SITE AND REACTION**



Crystallographic analysis of the catalytic mechanism of haloalkane dehalogenase. Verschueren KH, Seljee F, Rozeboom HJ, Kalk KH, Dijkstra BW, Nature (1993) 363 p.693

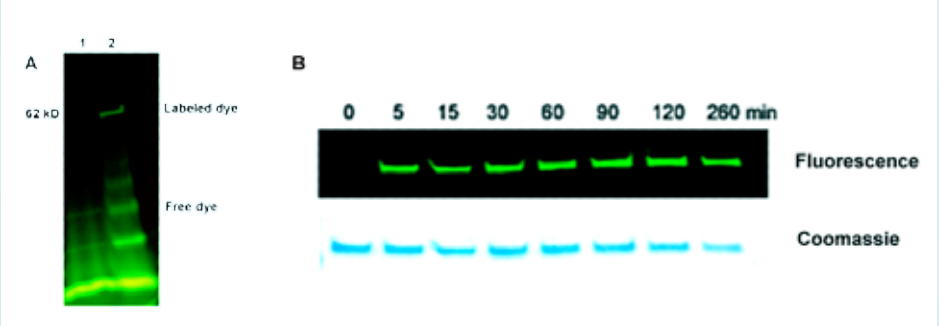
(Continued from Front Page)

To avoid these synthetic steps, we considered the possibility that a 5'-Bromohexyl Phosphoramidite could be used in combination with the Spacer Phosphoramidite 18 (a hexaethyleneglycol linker), both of which are commercially available from Glen Research, to produce a substrate for the HaloTag domain. We carried out preliminary experiments using the sequence, 5'-Bromohexyl-Spacer 18-(dT)15-Fluorescein-3' to provide a fluorescent label for visualization.

Oligonucleotides with and without bromohexyl linkers were tested for HaloTag protein labeling using a GST-HaloTag fusion protein in vitro, with the control oligo using a 5'-Hexynyl linker. The GST-HaloTag fusion protein (0.5 µg (1.0 µM)) was treated with the oligonucleotides at 5.0 µM in PBS at 37°C for 1h. The reaction mixture was then resolved by SDS-PAGE and analyzed by fluorescence gel imaging (Ex 488 nm). The labeling experiment showed robust HaloTag protein labeling only with the oligonucleotide having the bromohexyl linker; we observed no protein labeling with the control oligo having no bromohexyl linker (see Figure 2 A).

Next we evaluated the efficiency of protein labeling with the bromohexyl linker. In a time course experiment, 0.5 µg (1.0 µM) GST HaloTag fusion protein was incubated with 5.0 µM bromohexyl-modified oligo in PBS at 37°C and reaction was stopped at different time intervals and evaluated by gel with fluorescence imaging. The data showed rapid labeling of GST-HaloTag fusion protein with the 5'-Bromohexyl-modified oligo, with reaction apparently complete within 5 minutes (see Figure 2 B). Thus, the results from this initial test indicate the GST-HaloTag fusion protein is rapidly and efficiently conjugated to an oligo employing the 5'-Bromohexyl Phosphoramidite in combination with a hexaethylene glycol linker at the 5' end of an oligonucleotide.

FIGURE 2: DNA PROTEIN CONJUGATION



Testing DNA-protein conjugation between a GST-HaloTag fusion protein and a 5'-Bromohexyl-substituted dT15 oligo. A) Protein labeling experiment without (1) and with (2) the bromohexyl linker. B) Time course experiment with the Bromohexyl-Spacer 18 modified oligo: upper panel fluorescence scanning (Ex 488), lower panel coomassie blue staining.

References:

1. Los, G. V.; Encell, L. P.; McDougall, M. G.; Hartzell, D. D.; Karassina, N.; Zimprich, C.; Wood, M. G.; Learish, R.; Ohana, R. F.; Urh, M.; Simpson, D.; Mendez, J.; Zimmerman, K.; Otto, P.; Vidugiris, G.; Zhu, J.; Darzins, A.; Klaubert, D. H.; Bulleit, R. F.; Wood, K. V. ***HaloTag: a novel protein labeling technology for cell imaging and protein analysis.*** *ACS Chem. Biol.*, 2008, **3**, 373-382
2. Vijay Singh, Shenliang Wang, and Eric T. Kool, ***Genetically Encoded Multispectral Labeling of Proteins with Polyfluorophores on a DNA Backbone***. *J. Am. Chem. Soc.*, 2013, **16**, 6184-6191.

PREPARATION OF BROMOHEXYL-MODIFIED OLIGONUCLEOTIDES FOR HALOTAG CONJUGATION

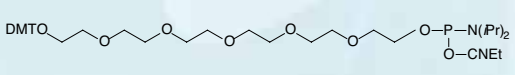
SYNTHESIS

To prevent elimination or displacement of the bromine, monomers that allow for UltraMILD deprotection must be used. (dA: 10-1601-xx, dC: 10-1015-xx, dG: 10-1621-xx, dT: 10-1030-xx). To avoid any exchange of the iPr-Pac group on the dG with acetyl, use the UltraMild Cap Mix A (40-4210-xx/ 40-4212-xx). Both the 5'-Bromohexyl and Spacer Phosphoramidite 18 are oils that require 5-10 minutes with occasional shaking to go fully into solution. A 3 minute coupling time is recommended for the 5'-Bromohexyl Phosphoramidite.

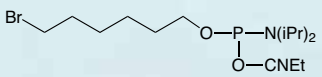
DEPROTECTION

Fit the synthesis column with two syringes and deprotect using 1 mL of 50 mM Potassium Carbonate in methanol for 4 hours at room temperature. Transfer the cleaved oligo solution into a clean vial and rinse the support with an additional 1 mL of 50 mM Potassium Carbonate in methanol and combine. Neutralize the solution using 12µL glacial acetic acid and dilute with an equal volume of 2 M TEAA, pH 7.0. Dilute to 10 mL and desalt using a Glen-Pak DNA cartridge.

FIGURE 1: STRUCTURES OF SPACER 18 AND 5'-BROMOHEXYL PHOSPHORAMIDITES



Spacer 18



5'-Bromohexyl

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Spacer Phosphoramidite 18	10-1918-90	100 µmole	95.00
	10-1918-02	0.25g	240.00
5'-Bromohexyl Phosphoramidite	10-1946-90	100 µmole	60.00
	10-1946-02	0.25g	200.00

NEW PRODUCT - 5-ME-DC NOW COMPATIBLE WITH AMA AND ULTRAMILD DEPROTECTION

One of the earliest minor bases introduced by Glen Research was 5-Me-dC-CE Phosphoramidite. 5-Me-dC has always been interesting to researchers because it stabilizes duplexes relative to dC by 1.5° per insertion. This desirable effect is attributed to the hydrophobic nature of the C5 methyl group which helps exclude water from the duplex. Antisense and diagnostic developers have long made use of 5-Me-dC as a simple and relatively inexpensive substitution for dC.

More recently, 5-Me-dC has generated interest in its own right in the burgeoning field of epigenetics. Methylation of dC usually occurs in areas with high concentration of CG bases (CpG islands) and the transformation of dC to 5-Me-dC is mediated by specialized DNA methyltransferase (DNMT) enzymes. Once methylated, 5-Me-dC can then be oxidized to form, consecutively, 5-hydroxymethyl-dC, 5-formyl-dC, and 5-carboxy-dC, all modified nucleosides of immense interest to epigenetics researchers.

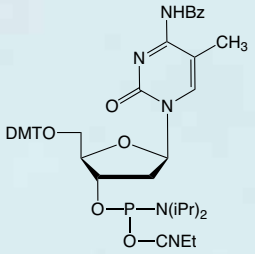
Our existing 5-Me-dC monomer (1) with the N4-amine protected with a benzoyl group has served the research community well for decades. However, this monomer is not compatible with deprotection using AMA. As shown in Figure 2, deprotection of a simple oligo containing benzoyl protected 5-Me-dC leads to around 7% of the N4-Me mutation caused by displacement of benzamide by methylamine. In addition, the benzoyl protecting group is not compatible with UltraMild deprotection since it is not removed by potassium carbonate in methanol.

To remedy these shortcomings, we are introducing the acetyl-protected 5-Me-dC monomer, Ac-5-Me-dC-CE Phosphoramidite (2). This monomer is fully compatible with AMA deprotection and none of the N4-Me mutation is observed on deprotection, as shown in Figure 2. The N4-acetyl protecting group is also completely removed under the conditions of UltraMild deprotection.

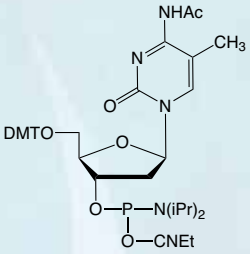
We are happy to offer the acetyl-protected monomer in addition to its older benzoyl-protected cousin.

Historically, we have offered a CPG support for the benzoyl-protected 5-Me-dC. However, the acetyl version is fully compatible with AMA deprotection and, therefore, a universal support like Glen UnySupport can be used.

FIGURE 1: **STRUCTURES OF 5-Me-dC MONOMERS**

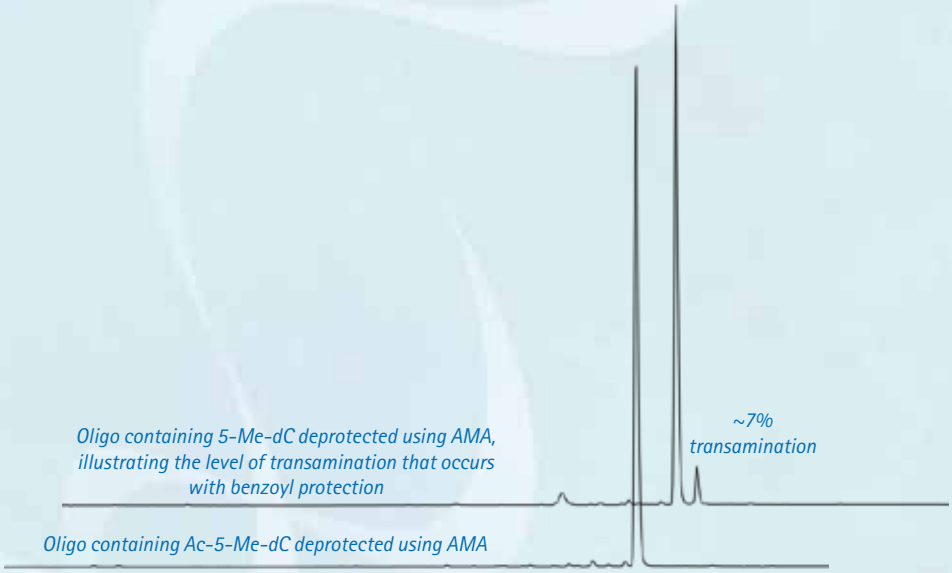


5-Me-dC (1)



Ac-5-Me-dC (2)

FIGURE 2: **RP HPLC ANALYSIS - OLIGO DEPROTECTION USING AMA WITH Bz-5-Me-dC AND Ac-5-Me-dC**



ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
5-Me-dC-CE Phosphoramidite	10-1060-90	100 µmole	50.00
	10-1060-02	0.25g	120.00
5-Me-dC-CPG	20-2060-01	0.1g	50.00
1 µmole columns	20-2160-41	Pack of 4	200.00
0.2 µmole columns	20-2160-42	Pack of 4	120.00
Ac-5-Me-dC-CE Phosphoramidite	10-1560-90	100 µmole	50.00
	10-1560-02	0.25g	120.00

TECHNICAL BRIEF - AMA – UBIQUITOUS REAGENT FOR OLIGO DEPROTECTION

INTRODUCTION

In December 1993 in Glen Report 6.2, we introduced our customers to UltraFAST oligonucleotide deprotection using AMA (Ammonium Hydroxide/40% aqueous MethylAmine 1:1 v/v), originally developed by MP Reddy and his group at Beckman Instruments.[1, 2] The presence of methylamine allows the deprotection of the exocyclic base protecting groups, including iBu in dG, in 10 minutes at 65°C. However, if AMA is used with benzoyl protected dC, hydrolysis to the desired dC is accompanied by transamination to form N4-Me-dC at a level of around 5%. With the use of acetyl protected dC, hydrolysis is almost instantaneous and no N-Me-dC is observed. The transamination reaction with Bz-dC is illustrated in Figure 1. HPLC analysis of oligonucleotides containing Bz-dC and Ac-dC and deprotected with AMA is shown in Figure 2.

As we noted in the article, the reduction in time of the deprotection step from many hours to a matter of minutes should relieve the production bottleneck when trying to manufacture large numbers of oligos each day. Our prediction was indeed correct and AMA or variations thereof (aqueous methylamine, methylamine gas, or propylamine in regions where methylamine is controlled) has become the principal method for deprotection in the high throughput synthesis of unmodified DNA oligos.

In this article, we will focus on the advantages that AMA offers in a variety of circumstances in oligonucleotide synthesis and modification. It should be stressed that the use of acetyl-protected C is mandatory in each application we describe.

FIGURE 1: TRANSAMINATION OF dC WITH METHYLAMINE

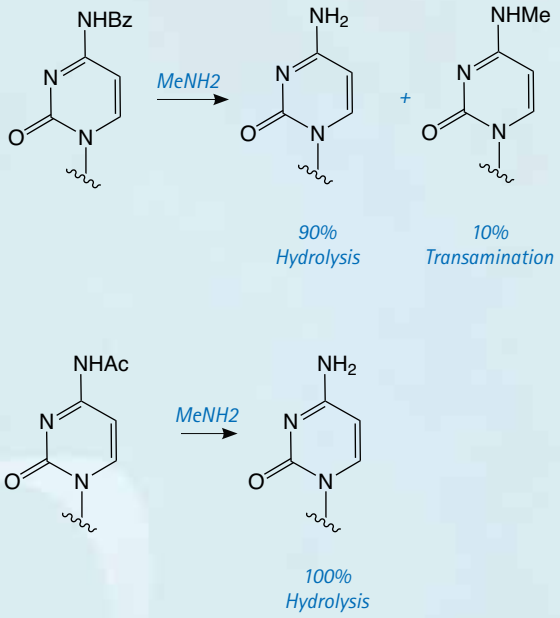
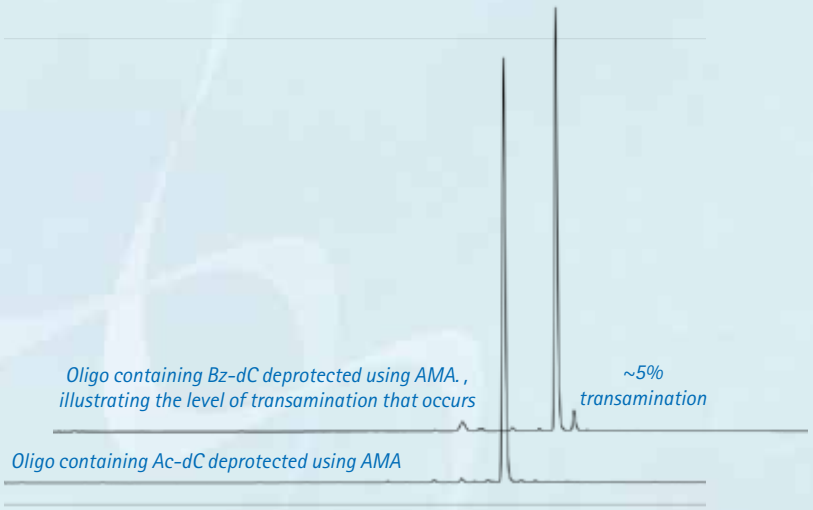


FIGURE 2: RP HPLC ANALYSIS - OLIGO DEPROTECTION USING AMA WITH Bz-dC AND Ac-dC



RNA SYNTHESIS

At the time of introduction of AMA, oligoribonucleotide synthesis was something of an art and it was regularly noted that the presence of the 2'-OH made RNA synthesis an order of magnitude more complex than DNA synthesis. In an early paper[3] on RNA synthesis, Reddy compared the use of AMA and aqueous methylamine with the most popular reagent for RNA deprotection at the time, ammonium hydroxide/ethanol 3:1 in this example. In addition to faster cleavage and deprotection, the authors also noted a significantly higher yield of full length product.[3]

After many years of development work in labs all over the world and much work in our own lab to confirm – Glen Research now recommends AMA as the optimal reagent for RNA deprotection. We offer RNA monomers protected with TBDMS or TOM[4] protecting groups at the 2'-OH and both types can be deprotected with AMA. We suggest carrying out the synthesis DMT-ON to allow the oligo to be purified with an RNA Glen-Pak. The simple procedure is outlined below:

1. Synthesize oligos DMT-ON.
2. Deprotect with AMA for 10 minutes at 65°C.
3. Evaporate to dryness.
4. Remove 2'-silyl protecting groups using TEA.3HF.
5. Dilute with RNA Quenching Buffer.
6. Purify using RNA Glen-Pak cartridges.
7. More details of this procedure were published in Glen Report 19.2.

TFA PROTECTED AMINO-MODIFIERS

One of the most perplexing issues that arose in the early days of amino-modifiers protected with a trifluoroacetyl (TFA) protecting group was partial inactivation of the amine after deprotection with ammonium hydroxide. We were able to

show that the amine, once deprotected, could be alkylated by acrylonitrile formed by elimination of the phosphate cyanoethyl protecting groups. More interestingly, the alkylamine formed was capable of deprotecting acyl protecting groups on adjacent bases to yield a varying percentage of inactive acyl capped amines. This situation is described in detail in the following link:

http://www.glenres.com/Technical/TB_avoidaminealkylation.html

These competing side reactions are minimized, though not eliminated, by using AMA for deprotection since the desired oligonucleotide alkylamine is simply swamped by the vastly higher concentration of methylamine.

3′-PT-AMINO-MODIFIERS

In these supports, the amino group that is destined to be the 3'-amino-modification is incorporated into a phthaloyl (PT) group and is fully protected throughout the synthesis procedure. The amino group is then fully hydrolyzed from the phthaloyl moiety by standard cleavage/deprotection with AMA at 65°C for 10 minutes. There are no side reactions and only pure 3'-alkylamine is released into solution. The structures of the 3'-PT-Amino supports are shown in Figure 3. Also shown in Figure 3 are the potential impurities during hydrolysis of the 3'-PT-Amino-Modifier C6, none of which have been observed with AMA deprotection.

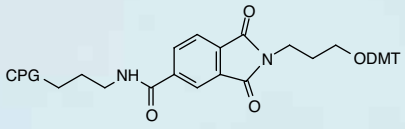
In contrast, other 3'-amino-modifiers, where the amine is protected with the Fmoc group, have several drawbacks in comparison to the 3'-PT-Amino-Modifiers, as described in Table 1.

We can conclude that, used with AMA, the 3'-PT-Amino supports are undoubtedly the best option for the preparation of 3'-amino-modified oligonucleotides. More details of the 3'-PT-Amino-Modifiers were provided in Glen Report 15.1.

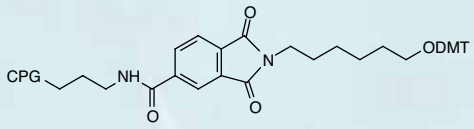
5′-PDA-AMINO-MODIFIERS

Phthalic acid diamide (PDA) 5'-amino-modifiers were developed by Stefan Pitsch and ReseaChem Gmbh (Stefan Berger) and were introduced by Glen Research in Glen Report 24.1. PDA phosphoramidites have the huge advantage that they are

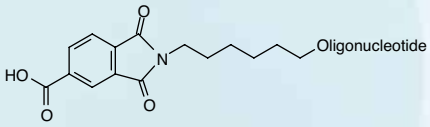
FIGURE 3: STRUCTURES OF 3'-PT-AMINO-MODIFIERS AND POTENTIAL IMPURITIES



3'-PT-Amino-Modifier C3 CPG



3'-PT-Amino-Modifier C6 CPG



Potential Impurities Formed by Incomplete Hydrolysis of the Phthaloyl Group

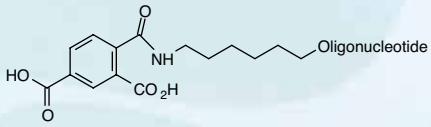
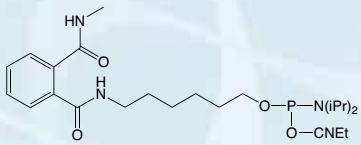


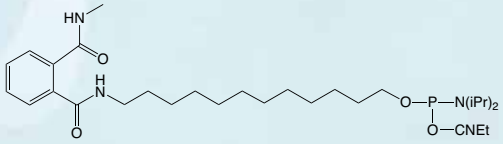
TABLE 1: COMPARISON OF FMOC AND PT PROTECTED AMINO MODIFIER SUPPORTS

Fmoc Protected	PT Protected
Loss of protection possible during synthesis and then the desired amine is irreversibly capped as acetate	No capped amine
Chiral center in the branched structure	No branch
Potential elimination of linker to 3′-OH during deprotection	No elimination

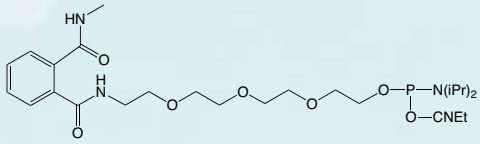
FIGURE 4: STRUCTURES OF 5'-AMINO-MODIFIER-PDA PHOSPHORAMIDITES AND DEPROTECTION WITH AMA



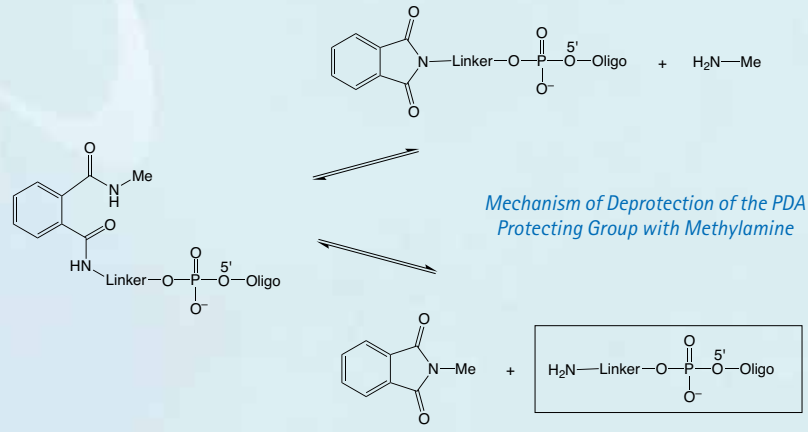
5'-Amino-Modifier C6-PDA



5'-Amino-Modifier C12-PDA



5'-Amino-Modifier TEG-PDA



Mechanism of Deprotection of the PDA Protecting Group with Methylamine

chemically stable powders and can be shipped with no thermal protection even in high summer. In contrast, the equivalent TFA-protected monomers are viscous oils that are not simply handled, stored, or split into smaller aliquots by the customer. They are also susceptible to thermal degradation. The PDA protecting group was designed with methylamine and AMA in mind and is removed under the normal AMA deprotection conditions of 65°C for 10 minutes with no evidence of any of the side reactions that can occur when using the TFA-protected monomers. In addition, the PDA group is removed in AMA in only 30 minutes at room temperature.

In situations where trityl-on purification of the resulting amino-modified oligos is not necessary, the 5'-PDA-Amino-Modifiers are the clear winners for 5'-amino-modification. A comparison of all of our 5'-amino-modifiers was published in Glen Report 24.2.

CONCLUSION

When we introduced the UltraFAST chemistry for DNA synthesis and deprotection in conjunction with Beckman Instruments, we envisaged that it would become popular for the synthesis of simple unmodified DNA oligos. In combination with Ac-dC, this has indeed transpired, with this approach favored for high throughput oligo synthesis in most situations. However, we have also seen the use of AMA grow in popularity in more esoteric situations, which we have illustrated in this article with reference to RNA synthesis and amino-modification.

References:

1. M.P. Reddy, N.B. Hanna, and F. Farooqui, *Tetrahedron Letters*, 1994, **35**, 4311-4314.
2. M.P. Reddy, N.B. Hanna, and F. Farooqui, *Nucleos Nucleot*, 1997, **16**, 1589-1598.
3. M.P. Reddy, F. Farooqui, and N.B. Hanna, *Tetrahedron Letters*, 1995, **36**, 8929-8932.
4. S. Pitsch, P.A. Weiss, L. Jenny, A. Stutz, and X.L. Wu, *Helv Chim Acta*, 2001, **84**, 3773-3795.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
DNA Synthesis			
Ac-dC-CE Phosphoramidite	10-1015-02	0.25g	12.50
	10-1015-05	0.5g	25.00
	10-1015-10	1.0g	50.00
	10-1015-20	2.0g	100.00
	10-1015-40	4.0g	200.00
TOM RNA Synthesis			
C-TOM-CE Phosphoramidite	10-3014-02	0.25g	75.00
	10-3014-05	0.5g	150.00
	10-3014-10	1.0g	275.00
TBDMS RNA Synthesis			
Ac-C-CE Phosphoramidite	10-3015-02	0.25g	40.00
	10-3015-05	0.5g	80.00
	10-3015-10	1.0g	160.00
RNA Purification Cartridges			
Glen-Pak™ RNA Purification Cartridge	60-6100-10	Pack of 10	95.00
(For use in vacuum manifolds	60-6100-30	Pack of 30	225.00
and high-throughput devices)	60-6100-96	Pack of 96	575.00
Glen-Pak™ RNA Purification Cartridge	60-6200-01	each	9.50
(For use with disposable syringes)	60-6200-10	Pack of 10	95.00
Reagents			
RNA Quenching Buffer	60-4120-82	250mL	80.00
	60-4120-80	1L	200.00
3'-PT Amino Supports			
3'-PT-Amino-Modifier C3 CPG	20-2954-01	0.1g	95.00
	20-2954-10	1.0g	675.00
1 µmole columns	20-2954-41	Pack of 4	140.00
0.2 µmole columns	20-2954-42	Pack of 4	85.00
10 µmole column (ABI)	20-2954-13	Pack of 1	250.00
15 µmole column (Expedite)	20-2954-14	Pack of 1	375.00
3'-PT-Amino-Modifier C6 CPG	20-2956-01	0.1g	95.00
	20-2956-10	1.0g	675.00
1 µmole columns	20-2956-41	Pack of 4	140.00
0.2 µmole columns	20-2956-42	Pack of 4	85.00
10 µmole column (ABI)	20-2956-13	Pack of 1	250.00
15 µmole column (Expedite)	20-2956-14	Pack of 1	375.00
3'-PT-Amino-Modifier C6 PS	26-2956-01	0.1g	125.00
	26-2956-10	1.0g	1025.00
200 nmole columns (AB 3900)	26-2956-52	Pack of 10	220.00
40 nmole columns (AB 3900)	26-2956-55	Pack of 10	220.00
PDA-Amino-Modifiers			
5'-Amino-Modifier C6-PDA	10-1947-90	100 µmole	30.00
	10-1947-02	0.25g	100.00
5'-Amino-Modifier C12-PDA	10-1948-90	100 µmole	65.00
	10-1948-02	0.25g	240.00
5'-Amino-Modifier-TEG-PDA	10-1949-90	100 µmole	105.00
	10-1949-02	0.25g	420.00

NEW PRODUCT - THPTA - A WATER SOLUBLE CLICK LIGAND

Click Chemistry has become a mainstay for bioorthogonal conjugation for three simple reasons: it is fast, efficient, and specific.

Classic Click Chemistry uses copper, Cu(I), to catalyze the 1,3-dipolar cycloaddition of an alkyne with an azide to form a 1,2,3-triazole.[1, 2] Sources of Cu(I) include copper(I) iodide, copper(I) bromide, copper turnings, or copper(II) sulfate ($CuSO_4$) and a reducing agent.[1]

An improvement in Click Chemistry uses the *in situ* preparation of Cu(I) from the reduction of $CuSO_4$ with sodium ascorbate and a Cu(I) stabilizing ligand, tris-(benzyltriazolylmethyl)amine (TBTA).[3] This leads to a more reliable click reaction by avoiding the oxidation of catalytic Cu(I) by dissolved oxygen. In a typical reaction, copper sulfate is pre-complexed with TBTA to form a brilliant blue solution. This complexed catalyst is mixed with the alkyne labeled oligonucleotide and the azide label, followed by the addition of sodium ascorbate to initiate the click reaction.

TBTA VERSUS THBTA

TBTA (1) covers most of the practical applications for Click Chemistry except for a completely aqueous conjugation reaction. The benefits of a completely aqueous reaction include the biological labeling of live cells or the labeling of proteins without the concern of denaturing secondary structures. The water-soluble tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) (2) click ligand further simplifies Click Chemistry by allowing the entire reaction to be run in water, affording biological compatibility for Click reactions. The THPTA ligand binds Cu(I), blocking the bioavailability of Cu(I) and ameliorating the potential toxic effects while maintaining the catalytic effectiveness in click conjugations. The THPTA ligand was effectively used to label live cells with high efficiency while maintaining cell viability.[4]

In our hands, we have found THPTA to be a highly efficient ligand for click chemistry with working ranges from 10nmol up to 1000nmol, in partially organic and completely aqueous reactions. Labeling is complete in as little as 15 minutes at room temperature. The ligand $CuSO_4$ complex exhibits no loss of activity when frozen for at least a month.

FIGURE 1: **STRUCTURES OF TBTA AND THPTA**

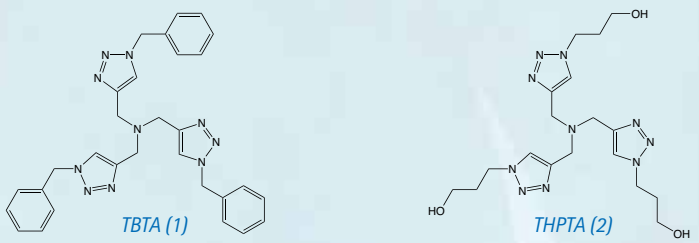


FIGURE 2: **RP HPLC ANALYSIS - 10 NMOLE REACTION WITH HEX AZIDE AND THPTA, 60 MINUTES**

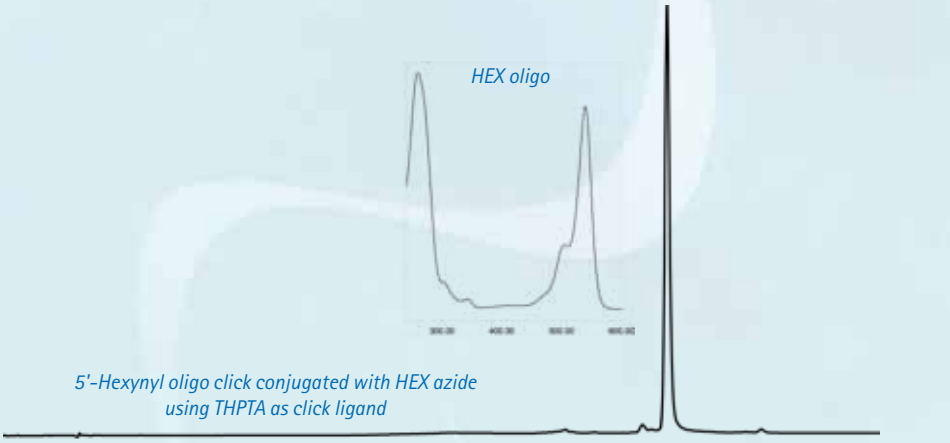


FIGURE 3: **RP HPLC ANALYSIS - 100 NMOLE REACTION WITH FAM AZIDE, THPTA AND TBTA, 15 MINUTES**

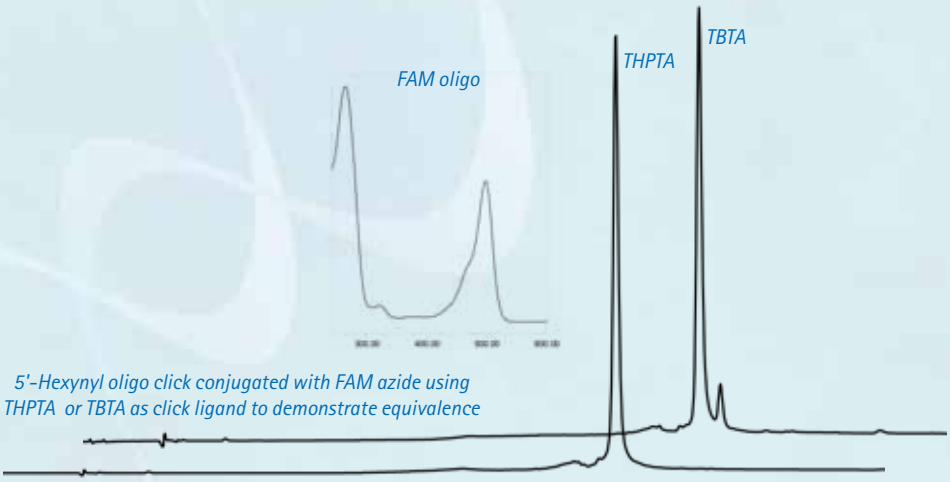
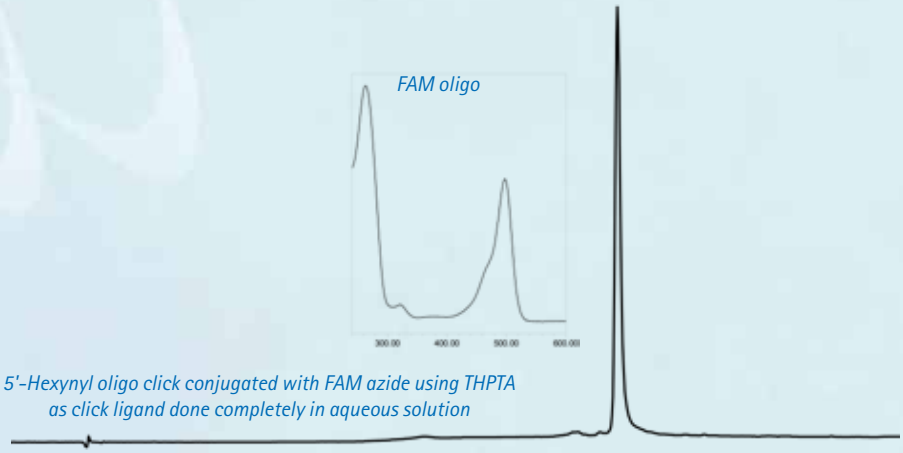


FIGURE 4: **RP HPLC ANALYSIS - 1 µMOLE AQUEOUS REACTION WITH FAM AZIDE AND THPTA, 30 MINUTES**



GLEN RESEARCH

22825 DAVIS DRIVE
STERLING, VIRGINIA
20164

All of the oligonucleotides used in this study were 5'-hexynyl modified 12mers. Figure 2 shows the result from the click conjugation of HEX Azide at the 10 nmole scale. In this case, the ligand $CuSO_4$ complex had been stored frozen for 1 month before use. We also compared the results of conjugations at the 100 nmole level using TBTA or THPTA as ligand. At all time points, the levels of conjugation were equivalent. The 15 minute time point is shown in Figure 3. These reactions were done in a mixture of organic and aqueous conditions to ensure the TBTA was in solution. In Figure 4, we illustrate the click conjugation in aqueous conditions using THPTA. The conjugation with 4 equivalents of FAM Azide (dissolved in water at slightly basic pH) was complete in 30 minutes at room temperature.

Our results indicate that THPTA performs at least as well as TBTA under equivalent conditions. However, THPTA can be used in aqueous conjugations where TBTA is insoluble, a distinct advantage. The simple procedure is as follows:

- Prepare the following click solutions: 0.2M THPTA ligand in water; 0.1M CuSO4 in water; alkyne labeled oligo in water; 0.1M sodium ascorbate in water; and 10mM azide in DMSO/tBuOH or water.
- Pre-chelate the CuSO4 with THPTA ligand in a 1:1 ratio several minutes before the reaction. This solution is stable for several weeks when frozen.
- To the oligo solution, add an excess of azide (4-50 eq).
- Add 25 equivalents of THPTA/CuSO4.
- Add 40 equivalents of sodium ascorbate.
- The solution can be degassed briefly with an inert gas.
- Let the reaction stand at room temperature for 15-60 minutes.
- Ethanol-precipitate the oligo or purify using Glen Gel-Pak.

Glen Research offers several options for the incorporation of an alkyne label into an oligonucleotide with a selection of nucleosidic and non-nucleosidic alkynes. We have several nucleosidic alkynes that are ideal for internally labeling oligonucleotides, and, with the use of our protected alkynes, oligos can be labeled with different reporters on the same oligo.[5] We also offer several non-nucleosidic alkynes that can be used for terminus or internal labeling using our 5'-Hexynyl Phosphoramidite or Alkyne Serinol Modifiers. A selection of azide labels is also available. Please see our web site for details.

We are now happy to add THPTA to our line of products for Click Chemistry.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
THPTA Ligand	50-1004-92	25 µmole	50.00
	50-1004-90	100 µmole	180.00

References:

1. V.V. Rostovtsev, Green, L.G., Fokin, V.V. and Sharpless, K.B., *Angew Chem Int Ed,* 2002, **41**, 2596-2599.
2. C.W. Tornøe, C. Christensen, and M. Meldal, *The Journal of Organic Chemistry,* 2002, **67**, 3057-3064.
3. T.R. Chan, R. Hilgraf, K.B. Sharpless, and V.V. Fokin, *Org. Lett.*, 2004, **6**, 2853-2855.
4. V. Hong, N.F. Steinmetz, M. Manchester, and M.G. Finn, *Bioconjugate Chemistry,* 2010, **21**, 1912-1916.
5. P.M.E. Gramlich, S. Warncke, J. Gierlich, and T. Carell, *Angewandte Chemie-International Edition,* 2008, **47**, 3442-3444.