The Glen Report

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INSIDE

NEW PRODUCT - (5'S)-5',8-CYCLO-dG - DNA DAMAGE AND REPAIR

Radiation-induced damage of DNA has been shown to lead to bridged cyclo-nucleosides, with the cyclo-purines, cyclo-dA and cyclo-dG, predominantly formed.

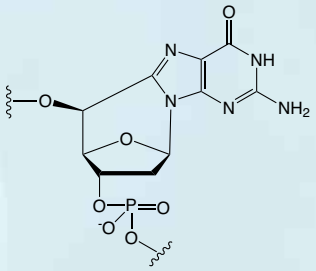
In Glen Report 21.1, Page 10, we introduced 5',8-Cyclo-dA CE Phosphoramidite, (1) on Back Page, in an article that described the repair of DNA damage by Nucleotide Excision Repair (NER) and Base Excision Repair (BER) mechanisms, while focusing specifically on the repair of cyclo-purine lesions. In this article, we update the biological effects of cyclo-purines in DNA while introducing a new Cyclo-dG-CE Phosphoramidite monomer.

The cyclo-purines are formed when a free radical is induced at the C5' of the deoxyribose sugar by attack by hydroxyl radicals, photolysis or other ionizing processes. The C5' free radical inserts into the C8-N7 double bond of the adjacent purine residue to form 5',8-cyclo-purine as either a 5'R or 5'S diastereomer. Of the two diastereomers, the (5'S) cyclo-purine appears to be the most cytotoxic.[1] Cyclo-dA is more prevalent than cyclo-dG in DNA damage. Cyclo-purines have been shown to cause significant distortion of the regular DNA helix and these lesions are repaired not by BER but by NER.[2,3]

In the absence of repair by NER proteins, cyclo-purines accumulate in cells and can block mammalian RNA polymerase II and replicative DNA polymerases.[4,5] The distortion in nucleoside structure caused by the C5'-C8 covalent bond perturbs regular hydrogen bonding with the complementary base and has the potential to disrupt normal enzymatic activity in cells. Such DNA lesions may cause polymerase enzymes to be blocked and the efficiency and fidelity of DNA replication may be compromised. The potential for mutations to occur in cells is consequently magnified.[6]

More recent research on the structure of duplexes containing cyclo-dG has confirmed that

(5'S)-5',8-CYCLO-dG IN OLIGO



base stacking is perturbed at the cyclo-dG site, as well as at adjacent base pairs. Further work[7] revealed that mis-insertion of dT or dA opposite the cyclo-dG site can occur. The authors also showed[8] that a cyclo-dG...dT mismatch occurs and adopts a wobble base pairing providing a rationale for the observed cyclo-dG -> dA transitions. However, no hydrogen bonding was detected between the cyclo-dG...dA base pair, which is consistent with the observed low levels of cyclo-dG -> dT transitions.

Interestingly, Saccharomyces Cerevisiae and human polymerase eta (pol eta) are able to insert the correct complementary bases opposite cyclo-purines and their adjacent 5' nucleosides at fidelities and efficiencies that are similar to those of their respective undamaged nucleosides. Pol eta's accurate and efficient bypass of the cyclo-purines contrasts with the mutagenic bypass by other polymerases. The results suggest that pol eta may function in cells to alleviate the cellular burden of endogenously induced DNA lesions, including cyclo-dA and cyclo-dG.[9]

It is clear that interest in cyclo-dA and cyclo-dG lesions remains high for investigation of cellular DNA damage and repair. We have offered the cyclo-dA monomer since 2009 and we are now happy to add cyclo-dG to our DNA damage and repair toolbox.

Unfortunately, conventional DMT protection of cyclo-dG is not possible at this time due to severe

(Continued on Back Page)

ANTISENSE TRIMER PHOSPHORAMIDITES - UPDATE

In our previous Glen Report, GR24.2, we were very pleased to announce the introduction of new Antisense Trimer Phosphoramidites which allow the simultaneous mutation of multiple, distal sites on a gene to be introduced in a facile manner.

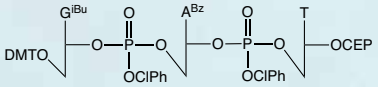
However at that time, we did not have any sequencing data that could be used to determine their relative reaction factors (RFs). The RFs are important to compensate for differences between the trimers' relative coupling rates. Trimers that are found to couple slower are given a commensurately larger RF which, in turn, leads to a higher concentration of that trimer phosphoramidite in the trimer mix. The higher concentration of trimer increases the rate of reaction, allowing one to hit the desired target percentage of each codon at the mutation site despite differences in rates of reaction. In the meantime, a value of 1.425 was used, which is the average RF of the well-characterized sense trimers.

In collaboration with Oblique Bio Inc., which specializes in sequence determination, we were able to determine RFs for the new Antisense Trimer Phosphoramidites.

To do so, first a pool of oligos was synthesized with ten incorporations of a twelve-codon trimer mix: 9 antisense trimers and 3 sense trimers for which the RF values are well established. On either side of the block of trimers were fixed flanking sequences for cloning purposes. A primer was annealed to a flanking region and PCR was then used to create a double-stranded pool. These were then cloned into a linearized pGEM vector. After re-circularization and transformation into a DH5 alpha cell line, the clones were plated out onto antibiotic-treated agar plates. The colonies were transferred to liquid media, grown for 18 hours at 37 °C and the plasmids purified by miniprep using the Omega BioTek MagBind purification kit and then sequenced on an ABI 3730xL sequencer. The results are shown in Table 1.

With the exception of GGT, the predicted percentages of the sense trimers were quite accurate. The disparity between the observed and the theoretical number of incorporations for a particular trimer may be due to under-sampling or quirks in either the cell line, polymerase, or miniprep, giving a positive or negative selection pressure for that trimer. For this reason, we consider these RF values to be preliminary until we have more sequencing data based upon a variety of amplification and cloning systems. As such, we will refine these values once more sequencing data becomes available and consistent trends are observed. At present, though, we are happy to provide our best estimates for the Antisense Trimer RFs, which are given in Table 2, along with the RFs for the Sense Trimer Phosphoramidites.

TRIMER PHOSPHORAMIDITE



Abbreviated Structure of Trimer Phosphoramidite GAT

Acknowledgment:

We thank Lance Larka of Oblique Bio Inc. for his assistance with the cloning and sequencing experiments.

TABLE 1: **DETERMINATION OF RF OF ANTISENSE TRIMER PHOSPHORAMIDITES**

Trimer	*RF*	*Observed Incorporated (%)*	*Theoretical Incorporated (%)*	*Ratio Obs/Theor.*	*Corrected RFs*
ACC	1.0	9.7	8.3	1.16	0.9
AGA	1.0	6.1	8.3	0.73	1.4
CCA	1.0	7.6	8.3	0.91	1.1
CGG	1.0	10.9	8.3	1.31	0.8
GAT	1.0	5.8	8.3	0.70	1.4
GCA	1.0	8.7	8.3	1.04	1.0
GCG	1.0	13	8.3	1.56	0.6
GGT*	1.1	10.9	8.3	1.31	0.8
GTA	1.0	5.7	8.3	0.68	1.5
TAC*	1.6	8.4	8.3	1.01	1.6
TCT*	1.3	8	8.3	0.96	1.4
TTT	1.0	4.9	8.3	0.59	1.7

**sense trimer controls*

TABLE 2: **RF OF TRIMER PHOSPHORAMIDITES**

Sense codons (5'->3')	*Reaction Factor (RF)*	*Antisense codons (5'->3')*	*Reaction Factor (RF)*
AAA (Lys)	1.10	**TTT**	**1.70**
AAC (Asn)	1.00	GTT	1.90
ACT (Thr)	1.60	GGT	1.10
ATC (Ile)	1.50	**GAT**	**1.40**
ATG (Met)	1.30	CAT	1.30
CAG (Gln)	2.00	CTG	1.20
CAT (His)	1.30	ATG	1.30
CCG (Pro)	1.80	**CGG**	**0.80**
CGT (Arg)	1.40	**GCG**	**0.60**
CTG (Leu)	1.20	CAG	2.00
GAA (Glu)	1.40	TTC	1.30
GAC (Asp)	1.60	ATC	1.50
GCT (Ala)	1.50	TGC	1.50
GGT (Gly)	1.10	**ACC**	**0.90**
GTT (Val)	1.90	AAC	1.00
TAC (Tyr)	1.60	**GTA**	**1.50**
TCT (Ser)	1.30	**AGA**	**1.40**
TGC (Cys)	1.50	**GCA**	**1.00**
TGG (Trp)	1.10	**CCA**	**1.10**
TTC (Phe)	1.30	GAA	1.40

Fluorescein in its most popular 6-carboxy-fluorescein (FAM) form is one of the most ubiquitous fluorescent dyes used to label DNA. With its high molar extinction coefficient, high quantum yield of fluorescence and good stability toward DNA synthesis and deprotection chemistries, FAM continues to be one of the most popular fluorophores on the market.

However, there is a bit of a chemical mystery associated with it - under certain conditions, a late-eluting peak is observed in oligos that exhibits no absorbance in the visible spectrum. We found that the impurity appeared when using AMA (ammonium hydroxide/40% aqueous methylamine 1:1 v/v) to deprotect a FAM-labelled oligo and was present whether the oligo was deprotected at room temperature or at 65 °C. Figure 1a contains the RP HPLC of FAM coupled to a T6 oligo when deprotected in AMA for 10 minutes at 65 °C, which shows the later eluting impurity at a concentration of around 5%.

Two other observations should be noted. The first is that FAM is perfectly stable when deprotected in concentrated ammonium hydroxide even for 17 hours at 55 °C - a chemical stability which is rare for fluorophores. The second is that the relative amount of this later eluting impurity was the same whether the oligo is deprotected in AMA for 10 minutes or for 60 minutes at 65 °C. So, it appears that the FAM is stable to the AMA solution - but only after the pivaloyl protecting groups of the 3' and 6' hydroxyls have been removed, at which point the FAM is no longer susceptible to degradation by AMA.

To identify the impurity, a FAM-labelled oligo deprotected in AMA was analyzed by Electrospray MS. The FAM side product had a molecular weight (mw) of +13 Da. The structure that is consistent with the mw of the impurity as well as its lack of absorbance in the visible spectrum is shown in Figure 1a. We propose that a nucleophilic attack by the methylamine may occur at position C1 which ultimately leads to a non-hydrolyzable amide. A proposed mechanism is shown in Figure 2.

Why the analogous reaction does not occur with ammonia is not clear. We can only surmise that, with the greater nucleophilicity of methylamine, the relative rate of nucleophilic attack at the C1 of the spiro-carbon of the cyclic lactone is significant compared to the rate of hydrolysis of the pivaloyl ester. With ammonia, the rate of nucleophilic attack at the C1 must be low, making the amount of the non-fluorescent lactam insignificant.

This difference can be used to advantage, though, by first treating the protected FAM-labelled oligo with ammonium hydroxide while it is still on the support. Once the yellow-green color of the fluorescein is evident, which indicates the the pivaloyl groups have been removed, the methylamine solution can be safely added. Shown in Figure 1b is the chromatogram of FAM-T6 from the same synthesis as shown in Figure 1a. However, this time the oligo was first briefly deprotected in ammonium hydroxide and then the 40% methylamine was added to complete the deprotection. Note the rate of removal of the pivaloyl protecting groups by ammonia will depend upon the length of the oligo and the location of the FAM - i.e., whether it is 3' or 5'.

FIGURE 1: **FAM OLIGO DEPROTECTED WITH AMA WITH AND WITHOUT AMMONIUM HYDROXIDE TREATMENT**

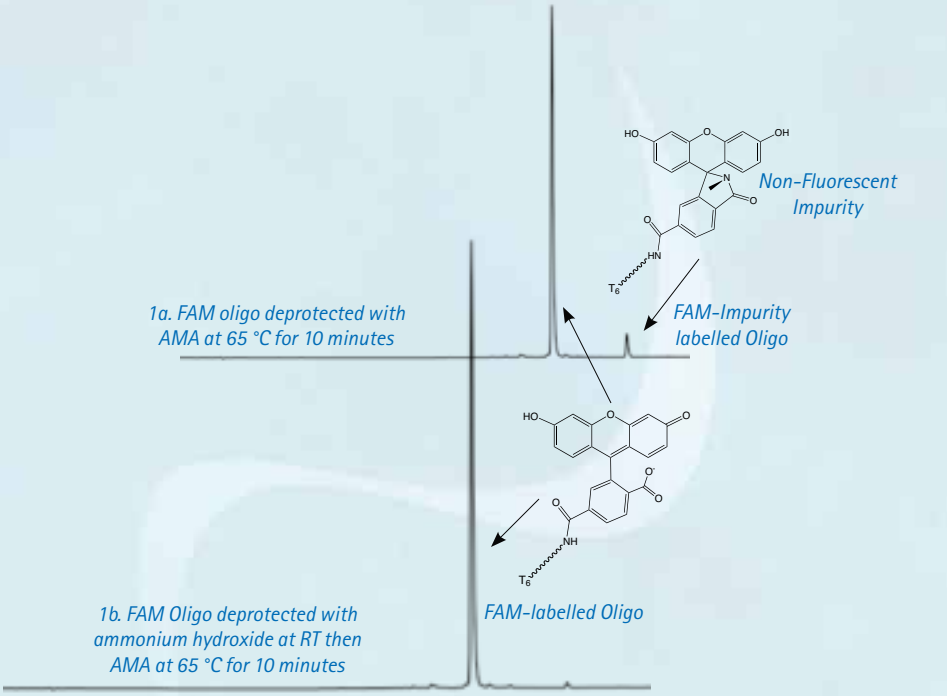
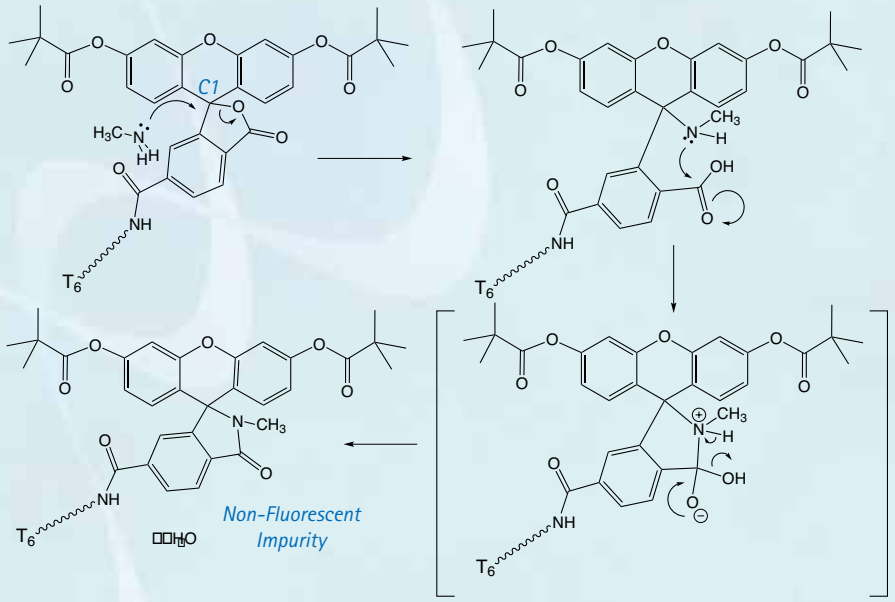


FIGURE 2: **PROPOSED MECHANISM FOR THE SIDE REACTION OF METHYLAMINE WITH FAM**



U.S. PATENT NO. 8,394,948

Novel reagents utilizing a serinol scaffold for labeling synthetic oligonucleotides

We are pleased to announce that the above titled U.S. Patent was approved and issued March 12, 2013. The inventors are Paul Nelson, Hugh Mackie and Andrew Murphy and the patent has been assigned to Glen Research Corporation and Nelson Biotechnologies, Inc.

The abstract states the following:

"Novel CE-phosphoramidites and CPG reagents have been synthesized from a serinol backbone. These reagents are useful to introduce functional groups or directly label oligonucleotides. The versatile serinol scaffold allows for labeling at any position (5' or 3' termini, or any internal position) during automated DNA synthesis. Multiple labels or functional groups can be achieved by repetitive coupling cycles. Optimal spacer arms and protected label moieties have been specially designed. Further, the natural 3-carbon atom internucleotide phosphate distance is retained when inserted internally."

Our serinol-based line of products for modification and labeling has been available for some time and the products have proved to be very popular in a variety of research applications. Although these products are now covered by U.S. Patent, there will only be the usual limitation in the use of these products. All of Glen Research's products are primarily for research use only and this applies to the serinol line regardless of whether oligos containing them are prepared by the end user or a core facility or a custom oligo company. Clearly, we wish to encourage the use of these valuable products. It is also our intent that IVD companies, for example, would be able to use these products to overcome any IP issues associated with alternative products for modification and labeling.

The significance of this product line is described in the background of the invention.

Current methods to introduce chemical modifications into oligonucleotides have limitations. Many non-nucleosidic phosphoramidite reagents are limited to

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

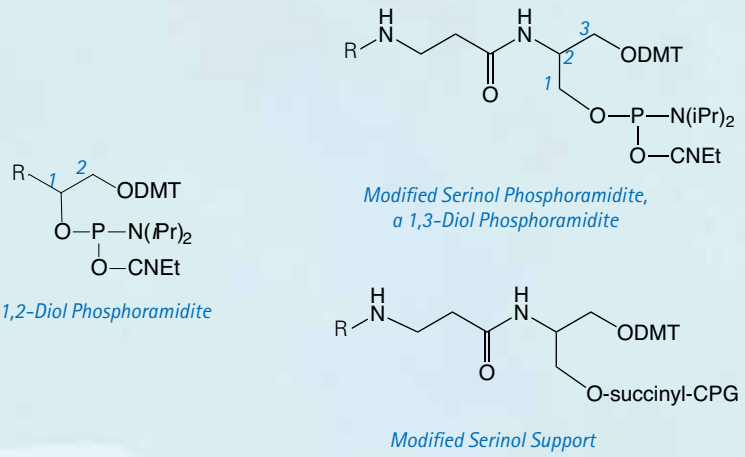
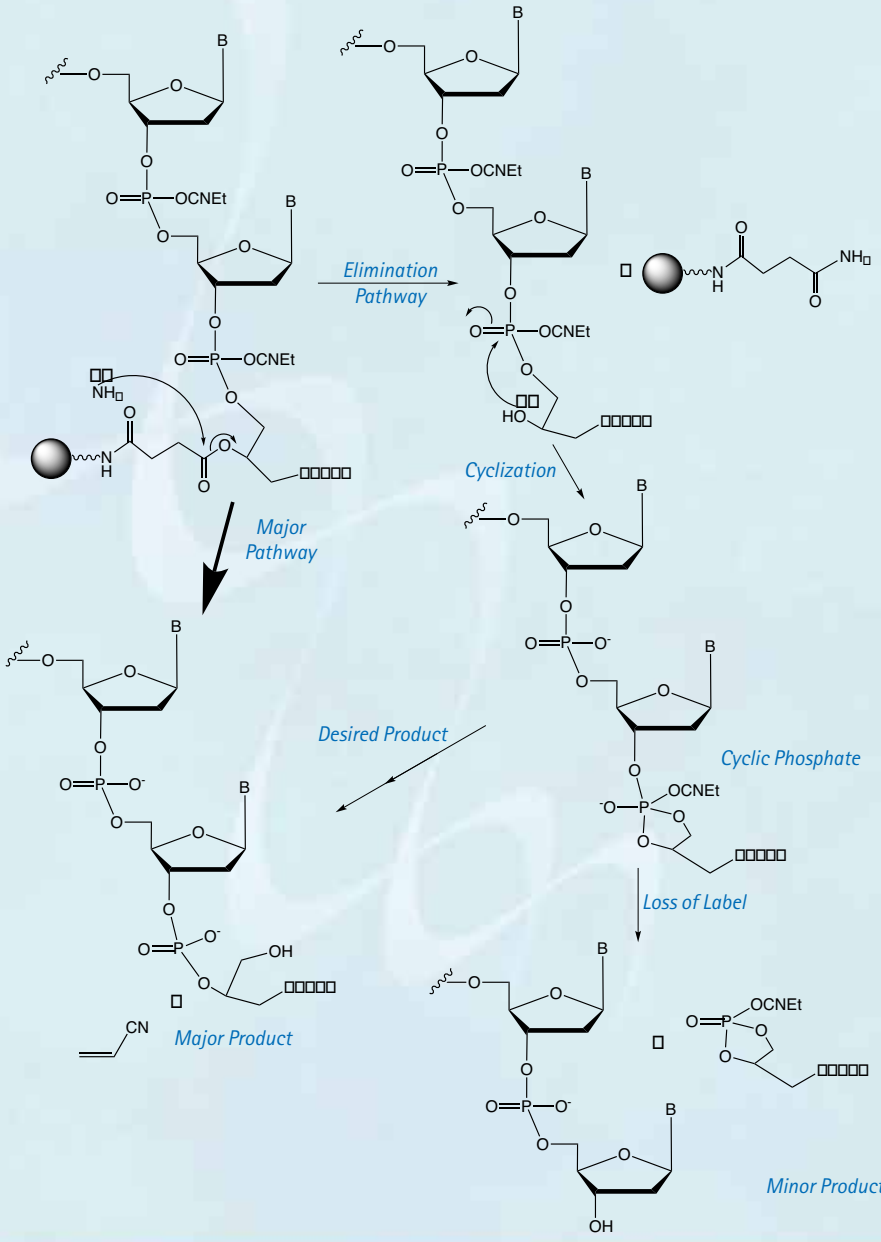


FIGURE 2: COMPETITIVE ELIMINATION IN 1,2-DIOL BASED PRODUCTS



single modifications at the 5' terminus, thus terminating chain elongation at the point of introduction. Those designed for multiple incorporations, such as 1,2-diol backbone phosphoramidite reagents, also suffer some drawbacks. The internucleotide distance, when incorporated internally, results in a constricted internucleotide phosphate distance one carbon atom shorter than the natural DNA structure. Further, the 1,2-diol backbone can participate in a dephosphorylation reaction due to a highly favorable 5-membered cyclic phosphate intermediate, resulting in cleavage of the label. Other reagents suffer from poor design in protecting label moieties. For example, some biotin phosphoramidite reagents do not protect its urea moiety. Hence, phosphoramidites can react at this active position of biotin in unwanted side reactions.

The 1,3 diol reagents of Nelson et al have proven to be superior, overcoming the above disadvantages, albeit, improved protection of label moieties has not been addressed. The subject of this invention builds upon the advantages of the 1,3 diol reagents by utilizing a serinol backbone. This backbone is versatile, readily available, and allows for convenient preparation of reagents. The purpose of this invention is to overcome the disadvantages encountered in the prior art by providing improved reagents to directly modify or label oligonucleotides via automated DNA synthesis.

The general structure of our serinol products is shown in Figure 1 and the wide variety of products we offer is shown in the Ordering Information table on the right.

In Figure 2, we illustrate the side reaction inherent in 1,2-diol based products that leads to significant label loss during deprotection. This reaction is competitive with simple hydrolysis of the protecting groups and leads to some loss of label. Fortunately, the elimination reaction is virtually non-existent in the 1,3-diol backbone since the cyclic intermediate would be a 6-membered ring which is not favored for a cyclic phosphate intermediate.

Glen Research is pleased to offer to our research customers these stable and efficient reagents for modifying and labeling oligonucleotides.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Alkyne-Modifier Serinol Phosphoramidite	10-1992-95	50 µmole	100.00
	10-1992-90	100 µmole	185.00
	10-1992-02	0.25g	575.00
Protected Biotin Serinol Phosphoramidite	10-1993-95	50 µmole	165.00
	10-1993-90	100 µmole	295.00
	10-1993-02	0.25g	675.00
6-Fluorescein Serinol Phosphoramidite	10-1994-95	50 µmole	165.00
	10-1994-90	100 µmole	295.00
	10-1994-02	0.25g	595.00
Protected BiotinLC Serinol Phosphoramidite	10-1995-95	50 µmole	205.00
	10-1995-90	100 µmole	365.00
	10-1995-02	0.25g	675.00
Amino-Modifier Serinol Phosphoramidite	10-1997-95	50 µmole	125.00
	10-1997-90	100 µmole	225.00
	10-1997-02	0.25g	595.00
3'-Alkyne-Modifier Serinol CPG	20-2992-01	0.1g	105.00
	20-2992-10	1.0g	800.00
0.2 µmole columns	20-2992-42	Pack of 4	100.00
1 µmole columns	20-2992-41	Pack of 4	175.00
10 µmole column (ABI)	20-2992-13	Pack of 1	260.00
15 µmole column (Expedite)	20-2992-14	Pack of 1	390.00
3'-Protected Biotin Serinol CPG	20-2993-01	0.1g	120.00
	20-2993-10	1.0g	995.00
0.2 µmole columns	20-2993-42	Pack of 4	120.00
1 µmole columns	20-2993-41	Pack of 4	200.00
10 µmole column (ABI)	20-2993-13	Pack of 1	300.00
15 µmole column (Expedite)	20-2993-14	Pack of 1	450.00
3'-6-Fluorescein Serinol CPG	20-2994-01	0.1g	120.00
	20-2994-10	1.0g	995.00
0.2 µmole columns	20-2994-42	Pack of 4	120.00
1 µmole columns	20-2994-41	Pack of 4	200.00
10 µmole column (ABI)	20-2994-13	Pack of 1	300.00
15 µmole column (Expedite)	20-2994-14	Pack of 1	450.00
3'-Protected BiotinLC Serinol CPG	20-2995-01	0.1g	120.00
	20-2995-10	1.0g	995.00
0.2 µmole columns	20-2995-42	Pack of 4	120.00
1 µmole columns	20-2995-41	Pack of 4	200.00
10 µmole column (ABI)	20-2995-13	Pack of 1	300.00
15 µmole column (Expedite)	20-2995-14	Pack of 1	450.00
3'-Amino-Modifier Serinol CPG	20-2997-01	0.1g	95.00
	20-2997-10	1.0g	675.00
0.2 µmole columns	20-2997-42	Pack of 4	85.00
1 µmole columns	20-2997-41	Pack of 4	140.00
10 µmole column (ABI)	20-2997-13	Pack of 1	250.00
15 µmole column (Expedite)	20-2997-14	Pack of 1	375.00

TECHNICAL BRIEF - ALDEHYDE AND AMINOOXY CONJUGATIONS

In our December 2011 Glen Report 23.2, we introduced AminoOxy-Modifier-11 CE Phosphoramidite (1) as a convenient reagent for labelling DNA with aldehydes to form stable oxime-labelled oligonucleotides. Historically, oxime conjugations are performed under mildly acidic conditions, pH 4.5, to catalyze the reaction for rapid conjugation.[1] At pH 7.4, however, the oxime conjugation can be much slower and a catalyst may be required to achieve a reasonable rate of reaction. Aniline has been used as a catalyst for oxime conjugations and is typically used at significant excess to achieve reasonable effects.[2]

Recent work by the Kool group at Stanford has identified alternative catalysts that can be used at lower concentrations and still achieve a reasonable reaction rate.[3,4] Of the catalysts reviewed by the Kool group, 5-methoxyanthranilic acid (5MA) was the most promising as it is soluble in aqueous buffer at pH 7.4, it is commercially available, and it is relatively inexpensive. Interestingly, they also report that the choice of aldehyde can have a significant effect on the overall rate of reaction. We chose to evaluate two different aldehydes, cinnamaldehyde and nonanal, for conjugation efficiency in the presence or absence of the catalyst. The results and experimental conditions are shown in Table 1.

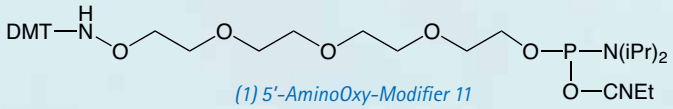
The choice of aldehyde, alkyl versus aryl, can have a significant effect on the overall rate of reaction. This is consistent with previous reports that the equilibration concentration of the aldehyde/ aminooxy addition complex along with the rate of dehydration determine the overall rate of reaction.[1] In one hour without catalyst, the nonanal labelling reaction was 82% complete compared to 3% labelling with cinnamaldehyde. In one hour with catalyst, the nonanal oligo was 86% labelled in 1 hour compared to 13% labelled for cinnamaldehyde. At higher concentrations of nonanal (10 equivalents) the labelling reaction is essentially complete (95%) in one hour, whereas cinnamaldehyde required 24 hours for complete reaction.

These results indicate that fluorophores and other aldehyde labels for oxime conjugations should have an alkyl linker to dramatically improve the kinetics of the reaction. This may have practical benefits for intracellular labelling, *in situ* labelling, and surface labelling where high concentrations of catalyst may not be feasible yet high labelling efficiency is required. For general oxime conjugations, the catalysts aniline and 5-methoxyanthranilic acid can be used to reduce the time and increase the overall efficiency of the labelling reactions.

For aryl aldehydes, we recommend 5-10 equivalents of aldehyde, 25 equivalents of 5MA, and reaction overnight at room temperature. For alkyl aldehydes, we recommend 10 equivalents of aldehyde and a 2 hour reaction at room temperature.

In summary, oxime labelling offers a mild, efficient, aqueous method for the labelling of oligonucleotides at acidic and neutral pH. The oxime is stable to standard oligonucleotide deprotection conditions and the reaction is bioorthogonal. Conjugation efficiency is based on the structure of the aldehyde, pH, and the presence of catalyst.

FIGURE 1: STRUCTURE OF 5'-AMINOOXY-MODIFIER



(1) 5'-AminoOxy-Modifier 11

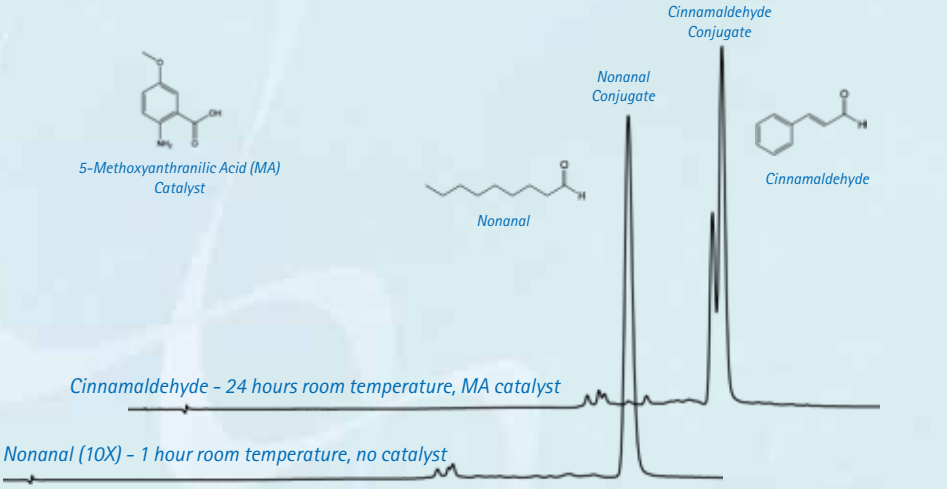
TABLE 1: ALDEHYDE AND AMINOOXY CONJUGATIONS WITH AND WITHOUT CATALYST

Room Temperature	Nonanal No Catalyst	Nonanal With Catalyst	Nonanal (10X) No Catalyst	Cinnamaldehyde No Catalyst	Cinnamaldehyde Catalyst
1 hour	82.0%	85.7%	94.6%	3.4%	12.5%
2 hours	84.9%	86.2%	94.9%	10.4%	26.1%
5.5 hours	88.5%	88.0%	94.2%	22.1%	54.6%
24 hours	92.7%	92.2%	94.5%	72.4%	94.6%

Experimental

Prepare a 125 mM stock solution of 5MA in PBS, adjusted to pH 7.4 using 2M aqueous sodium hydroxide. (It should be noted that the 5MA is poorly soluble at acidic pH but is fully soluble at pH 7.4.) Add 0.125 mmoles of 5'-aminooxy-modified DNA in PBS and 0.625 mmoles of aldehyde in DMSO/Water (1:1) to a reaction tube. For the reactions with no catalyst, dilute to 0.4 mL with PBS. For the reactions with catalyst, add 3.125 mmoles of catalyst and dilute to 0.4 mL with PBS. Sample 60 µL and dilute to 150 µL with water. Load onto a prepared Glen Gel-Pak™ 0.2 cartridge and pre-elute with 200µL of water. Elute with 300µL of water and inject directly on HPLC.

FIGURE 2: CINNAMALDEHYDE CONJUGATION WITH AND WITHOUT CATALYST



References:

1. W.P. Jencks, *J Amer Chem Soc*, 1959, **81**, 475-481.
2. A. Dirksen, and P.E. Dawson, *Bioconjugate Chemistry*, 2008, **19**, 2543-2548.
3. P. Crisalli, and E.T. Kool, *J Org Chem*, 2013, **78**, 1184-9.
4. P. Crisalli, and E.T. Kool, *Org Lett*, 2013, **15**, 1646-9.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
5'-AminoOxy-Modifier 11	10-1919-95	50 µmole	140.00
	10-1919-90	100 µmole	265.00
	10-1919-02	0.25g	895.00

Fortunately, phosphoramidites are very stable products and generally require no special protection even for summer shipping since domestic and international packages are usually delivered very promptly. However, one significant exception is our amino-modifiers with trifluoroacetate (TFA) protecting groups. These products are viscous oils and trace impurities seem to be able to cause some degradation if transit times are extended especially during summer shipping. These products also have no DMT or MMT group and so confirming coupling efficiency is more difficult. We ship these products in containers containing blue ice to help protect them from degradation. However, occasionally international shipments can be held up in the customs clearance process so we advise international customers to place an order for projected summer needs in late spring before high temperatures are encountered in their area.

How do you detect if some degradation has occurred in transit?

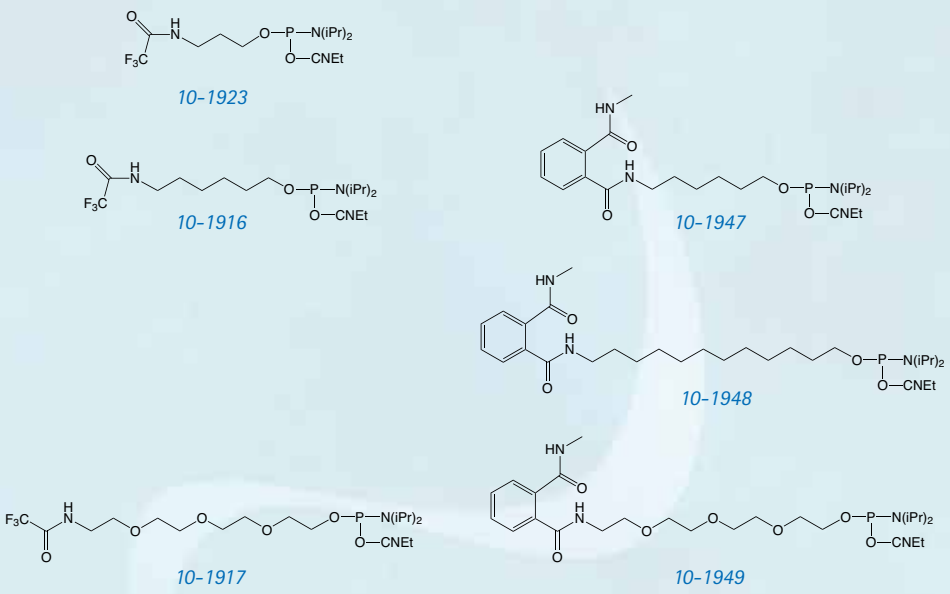
As noted above, these products are clear oils and this can be verified by holding the vial up to the light. If any crystals are observed in the clear oil, significant degradation has occurred and the vial should not be used. Indeed, complete degradation of these products to the corresponding H-phosphonate analogue leads to complete crystallization of the product.

How can you avoid this situation?

These products are all stable in freezer storage so, as noted above, we advise our overseas customers to place an order for projected summer needs in late spring before high temperatures are encountered.

An alternative is to use the equivalent amino-modifiers protected with phthalic diamide (PDA) protecting groups. 5'-PDA-Amino-Modifiers[1], which were developed by Stefan Pitsch along with Stefan Berger of ReseaChem in Switzerland, were introduced by Glen Research in the spring of 2012. In contrast to the other protected amino modifiers which are viscous oils, the analogous PDA protected compounds are granular powders. This important property of these compounds allows straightforward handling, storage and aliquoting and leads to a significant increase in stability.

FIGURE 1: STRUCTURES OF 5'-AMINO-MODIFIERS



These products are, therefore, fully stable even for extended summer shipping. They are also priced equivalently so there would be no additional cost involved in using the PDA products.

It has to be noted that PDA protection is rapidly removed with methylamine and mixtures like AMA. In fact, 20 minutes at room temperature is sufficient to remove >95% of the PDA protecting group with AMA. However, deprotection with ammonium hydroxide does not proceed to completion and a significant percentage of the protected amine will remain.

Our TFA amino-modifiers are shown in Figure 1 along with the PDA products. At this point, we do not offer the C3 version of the PDA-Amino-Modifiers but we do offer a C12 option.

KEY POINTS FOR PDA-AMINO-MODIFIERS

- *Oligonucleotides containing PDA-amino-modified oligonucleotides must be treated with aqueous methylamine or AMA for complete deprotection.*
- *If ammonium hydroxide must be used, reaction at 55°C will yield around 80% of the deprotected amino group, even with extended deprotection times.*

1. Developed by Stefan Pitsch and ReseaChem GmbH (S. Berger), Patent pending.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
5'-Amino-Modifier C3-TFA	10-1923-90	100 µmole	50.00
	10-1923-02	0.25g	175.00
5'-Amino-Modifier C6-TFA	10-1916-90	100 µmole	30.00
	10-1916-02	0.25g	100.00
5'-Amino-Modifier TEG	10-1917-90	100 µmole	115.00
	10-1917-02	0.25g	500.00
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

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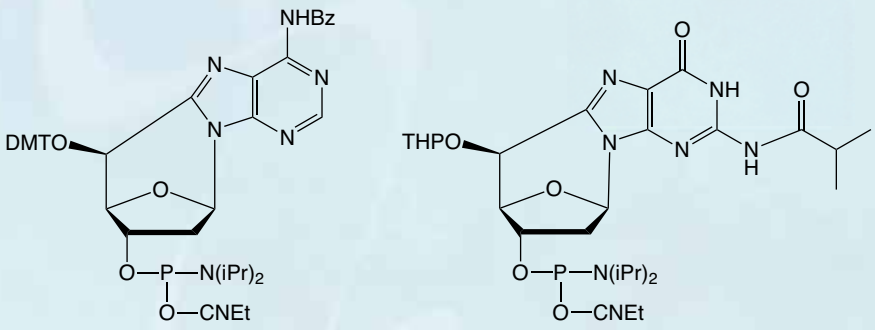
difficulties encountered in preparing that protected monomer. Instead, 5',8-Cyclo-dG CE Phosphoramidite (2) is offered with a 5'-tetrahydropyran (THP) protecting group. This group is removed by extended treatment with the regular deblock reagent and the subsequent coupling has to be increased to a 15 minute coupling time.

References:

1. I. Kuraoka, et al., *J. Biol. Chem.*, 2001, **276**, 49283-49288.
2. P. Jaruga, J. Theruvathu, M. Dizdaroglu, and P.J. Brooks, *Nucleic Acids Res*, 2004, **32**, e87.
3. P. Jaruga, and M. Dizdaroglu, *DNA Repair (Amst)*, 2008, **7**, 1413-25.
4. P.J. Brooks, et al., *J Biol Chem.*, 2000, 275, 22355-62.
5. B. Yuan, J. Wang, H. Cao, R. Sun, and Y. Wang, *Nucleic Acids Res*, 2011, **39**, 5945-54.
6. C. Marietta, and P.J. Brooks, *EMBO Rep*, 2007, **8**, 388-93.
7. H. Huang, R.S. Das, A.K. Basu, and M.P. Stone, *J Am Chem Soc*, 2011, **133**, 20357-68.
8. H. Huang, R.S. Das, A.K. Basu, and M.P. Stone, *Chem Res Toxicol*, 2012, **25**, 478-90.
9. A.L. Swanson, J. Wang, and Y. Wang, *Chem Res Toxicol*, 2012, **25**, 1682-91.

***FIGURE 1:* STRUCTURES OF (5'S)-5',8-CYCLO-PURINES**



5',8-Cyclo-dA-CE Phosphoramidite (1)

5',8-Cyclo-dG-CE Phosphoramidite (2)

ORDERING INFORMATION

Item	*Catalog No.*	*Pack*	*Price($)*
5',8-Cyclo-dA CE Phosphoramidite	10-1098-95	50 µmole	950.00
	10-1098-90	100 µmole	1850.00
	10-1098-02	0.25g	5350.00
5',8-Cyclo-dG CE Phosphoramidite	10-1598-95	50 µmole	1250.00
	10-1598-90	100 µmole	2450.00