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INSIDE

THYMINE DIMERS - DNA LESIONS INDUCED BY SUNLIGHT
CIS-SYN THYMINE DIMER PHOSPHORAMIDITE NOW AVAILABLE

One of the major sources of DNA damage in all organisms is the UV component of sunlight. The predominant reaction induced by UV light on DNA is dimerization of adjacent pyrimidine bases leading to cyclobutane dimers (CPDs) and 6-4 photoproducts. The dimers formed in the most significant quantity are the *cis-syn* cyclobutane dimer of two thymine bases (1) and the corresponding 6-4 photoproduct (3). The *trans-syn* thymine dimer (2) is formed at a much lower level in single and double stranded DNA. In sunlight, the 6-4 photoproduct is converted to its Dewar isomer (4) by absorption of long-wave UV light at around 325nm.[1]

Although formed routinely, these dimer products are, fortunately for us, efficiently excised and repaired enzymatically (nucleotide excision repair) or the dimerization is reversed by photolase enzymes. These lesions have been connected to the formation of squamous cell carcinomas. In addition, humans who lack ability to repair CPD lesions with high efficiency may be genetically predisposed to Xeroderma Pigmentosa (XP), a disease characterized by extreme sensitivity to sunlight and high frequency of skin cancer. Polymerases encountering unrepaired CPD lesions are quite error-prone, presumably leading to incorrect base insertions and subsequent mutations.[2]

The literature covering the chemistry of thymidine dimers and other CPDs is replete with references from John-Stephen Taylor's group at Washington University in St. Louis. Any article on thymine dimer phosphoramidites has to acknowledge the work of Professor Taylor and his co-workers. Their contribution[3] to this field has been outstanding.

It has been clear to us for some time that researchers into DNA damage and repair would value the ability to produce oligonucleotides containing *cis-syn* thymine dimer at specific locations within the sequence. Unfortunately, the chemical processes required to produce *cis-syn* thymine dimer phosphoramidite are very tortuous[4,5] and it was only recently that we were able to obtain this

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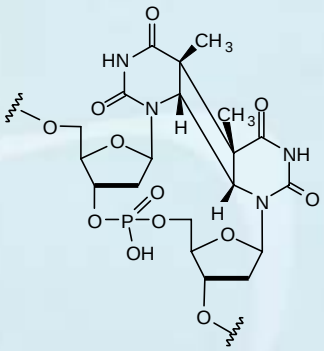
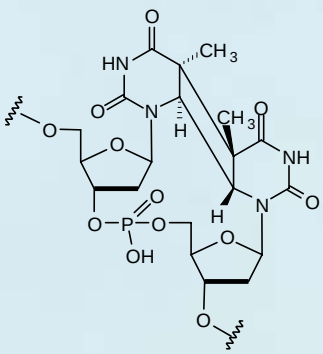
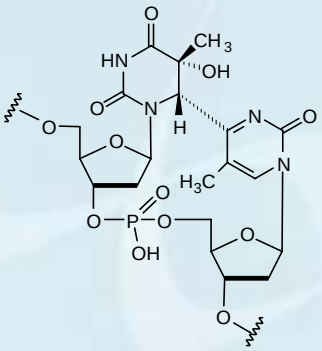
FIGURE 1: **PHOTO-INDUCED THYMINE DIMERS**



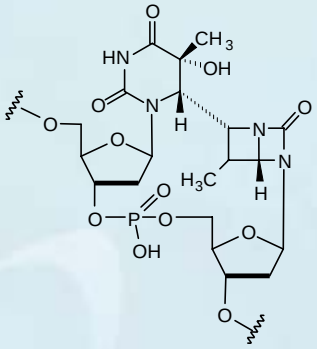
(1) Cis-syn Thymine Dimer



(2) Trans-syn Thymine Dimer



(3) 6-4 Thymine Dimer



(4) Dewar Thymine Dimer

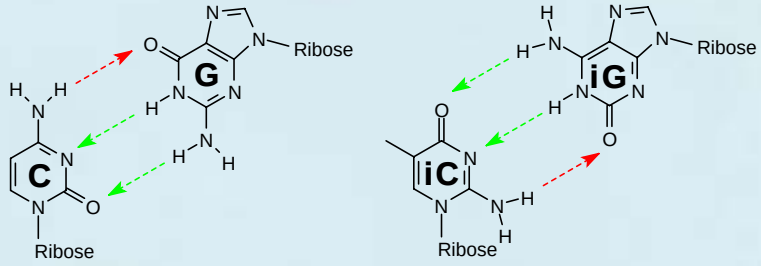
ERAGEN BIOSCIENCES TECHNOLOGY (courtesy of EraGen Biosciences)

In natural DNA, two complementary strands are joined by a sequence of Watson-Crick base pairs. These obey two rules of complementarity: size (large purines pair with smaller pyrimidines) and hydrogen bonding (hydrogen bond donors from one nucleobase pair with hydrogen bond acceptors from the other). The former is necessary to permit the structure that underlies enzyme recognition. The latter achieves the specificity that gives rise to the simple rules for base pairing ("A pairs with T, G pairs with C") that underlie genetics and molecular biology. No other class of natural products has reactivity that obeys such simple rules. Nor is it obvious how one designs a class of chemical substances that does so much so simply.

Some time ago, Professor Steven Benner noticed that the DNA alphabet need not be limited to the four standard nucleotides known in nature. Rather, twelve nucleobases forming six base pairs joined by mutually exclusive hydrogen bonding patterns might be possible within the geometry of the Watson-Crick base pair. EraGen has exclusively licensed this idea and the inventions to follow in order to build more robust recognition and analysis systems.

Today, EraGen technology consists of two additional bases (isoCytosine (iC) and isoGuanosine (iG) that form the third base pair, as shown in Figure 1. EraGen has spent years optimizing the production of all needed reagents to allow the technology to reach its full potential. EraGen technology is being used in both solution and solid phase detection as described below.

FIGURE 1: C-G AND iC-iG BASE PAIRS



Schematic of the natural pair Cytosine (C) and Guanosine (G) next to the EraGen pair isoCytosine (iC) and isoGuanosine (iG). Note the directionality of hydrogen bonding (depicted by arrows) is different between the two pairs.

FIGURE 2: EFFECT OF BASE PAIRING ON THERMAL STABILITY OF A 14MER DUPLEX

	G	C	A	T	iG	iC
C	**58**	46	49	44	54	44
G	47	**58**	48	46	52	44
T	49	44	**56**	45	53	41
A	47	45	43	**53**	46	43
iC	45	44	42	41	**62**	44
iG	51	53	48	51	48	**58**

5'-CACPACTTTCTCCT-3'
3'-GTGQTGAAAGAGGA-5'

P = column, Q = row

Thermal melting points for 14-mer with single substitution of nucleotide at P (column) and Q (row) position. Correct match is shown on the diagonal in Red and underlined.

Details

Research has shown that the complementarity rules for EraGen technology are analogous to those of natural DNA. Therefore, any researcher can exploit the rule-based molecular recognition properties to design molecules built from EraGen components that will bind to other molecules built from the corresponding binding partner.

Since the recognition is specific, EraGen provides a molecular recognition system that is "orthogonal" to that provided by natural DNA. The table shown in Figure 2 is constructed so that the Tm value for duplexes obeying the extended Watson-Crick pairing rules are found along the diagonal (values are underlined). In each case, the underlined value is higher than any of the Tm values associated with the mismatches. Especially important is the observation that the Tm values for mismatches with standard nucleobases are lower than the Tm values for mismatches with the correct EraGen component.

State-Of-The-Art Products

EraGen is providing scientific professionals with new options to address their research and molecular diagnostic needs. Under license from EraGen, Bayer Diagnostics has incorporated EraGen Technology into their third generation branched DNA assays (Versant™) to achieve previously unattainable levels of sensitivity and specificity for quantitative viral load testing in clinical labs.

EraGen is currently commercializing two platforms with optimized reagent sets: GENE-CODE®, a real time PCR alternative to Taq-Man® and SYBR Green; and MULTI-CODE®, a multiplexed genotyping analysis system. The platform technologies utilize the iC and iG amidites and newly developed triphosphates to capture assay products and incorporate signaling molecules.

Figure 3 shows how these advanced reagents are used to perform Gene-Code. Overall, the advantages of the Gene-Code real-time PCR system are as follows:

- Implemented with any current primer designs
- High sensitivity and specificity
- Multiplex capability
- Implemented on most real time instrumention
- Excellent reproducibility

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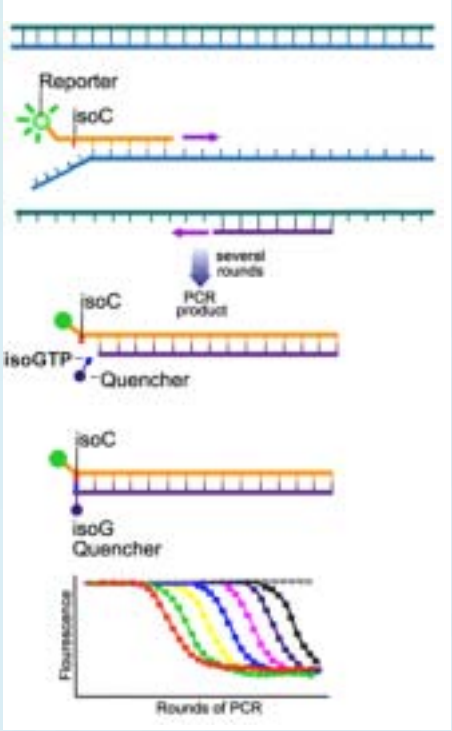
FIGURE 3: GENE-CODE ASSAY



Gene-Code assay system with a single labeled primer containing an iC with a natural reverse primer. During PCR amplification, iGTP-quencher is specifically incorporated opposite the iC, causing a dramatic and rapid decrease in fluorescence as shown in the bottom panel. Multiplexing is achieved by the use of multiple dyes that do not spectrally overlap.

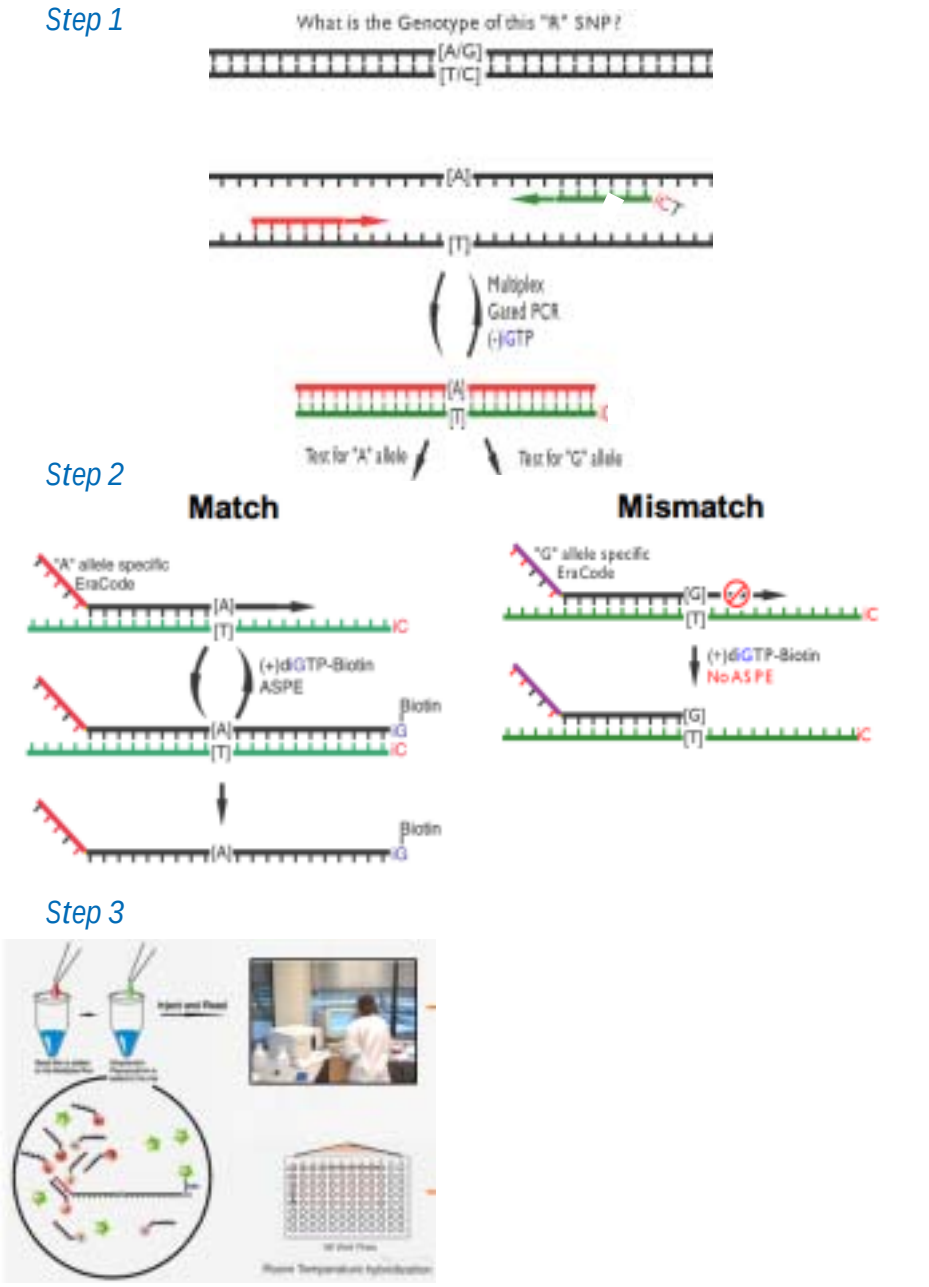
- Post PCR thermal melt – Control for correct amplified sequence
- Long shelf life

The unique characteristics of EraGen technology were also used to develop Multi-Code, a high throughput genotyping system (Figure 4). Overall, the advantages of the Multi-Code multiplexing platform are as follows:

- Flexibility
- No washing, no centrifugation, no filtration
- High sensitivity and specificity
- High multiplexing capability
- Implemented on Luminex instrumentation

Employing EraGen technology in this fashion allows all Multi-Code assays to be performed in a single reaction vessel requiring less plastic and allowing for easier automation.

FIGURE 4: MULTI-CODE ASSAY STEPS



The three steps of the Multi-Code system are shown below. Step one is standard multiplexed PCR using a primer with an iC at the 5' end in the absence of iG-biotin, which results in a "gated" amplicon. Primer extenders and iG-biotin are added for the allelic-specific primer extension step, which results in extended primers with a label and specific capture code. In the third and final step, SAPE and Luminex microparticles are added just prior to injection onto the Luminex instrument. Data analysis is performed on EraGen's proprietary software.

Summary

The EraGen Technology is being implemented in some of the most impressive new platforms to hit the market recently. This new paradigm shift in fundamental biology will allow scientists to greatly simplify genetic analysis. With these new reagents and their improved characteristics, EraGen Biosciences is proud to be working with Glen Research to provide today's researcher with exciting new alternatives in molecular diagnostics and genetic analysis tools. For more information please visit www.eragen.com or reference recent publications:

Nucleic Acids Research, 2003, **31**(17), 5048-5053, and *Clinical Chemistry*, 2003, **49**(3), 407-414.

ACTIVATORS, COLUMNS AND PLATES

Benzylthiotetrazole (BTT)

The first activator described for phosphoramidite chemistry was 1*H*-tetrazole (1), which has served well over the years. Nevertheless, more potent activators, 5-methylthio-1*H*-tetrazole (2) and 5-nitrophenyl-1*H*-tetrazole (3), have popped up but fallen down. More recently, 5-ethylthio-1*H*-tetrazole (ETT) (4) has gained considerable acceptance as a more acidic activator. ETT has the added advantage of being more soluble in acetonitrile than 1*H*-tetrazole (up to 0.75M versus 0. 5M solution in acetonitrile). Less acidic but much more nucleophilic is 4,5-dicyanoimidazole (DCI) (5). DCI is even more soluble in acetonitrile (up to 1.2M solution in acetonitrile). ETT and DCI have proved popular for high throughput synthesizers since they do not tend to crystallize and block the fine outlet nozzles.

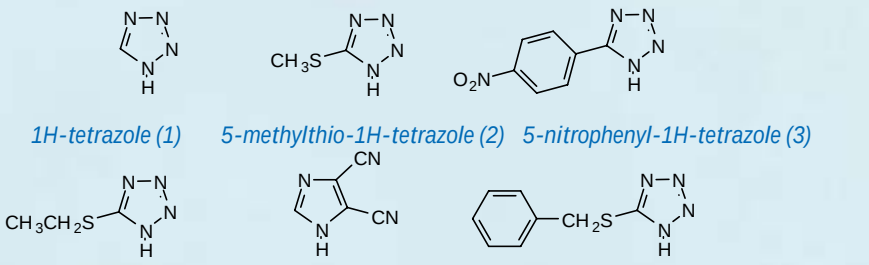
The renewed interest in RNA synthesis due to the explosion of siRNA technology has led us to evaluate 5-benzylthio-1*H*-tetrazole (BTT) (6), which was described[1] several years ago as an ideal activator for RNA synthesis using TOM-protected RNA phosphoramidites[2] and recently[3] for TBDMS-protected monomers.

Our experiments with BTT have shown that its maximum solubility is about 0.33M in acetonitrile The optimal coupling time for TOM-protected RNA phosphoramidites was found to be 90 seconds and for TBDMS-protected RNA phosphoramidites it was 3 minutes. However, we were concerned that the increased acidity of BTT (pKa 4.08 vs ETT pKa 4.28 vs 1*H*-tetrazole pKa 4.89)[3] could potentially detritylate the monomer during the coupling step leading to a dimer addition. If this process were significant, the full-length oligo would be contaminated with n+1 insertion mutations. However, by examining oligonucleotides by ion-exchange HPLC, we find that n+1 peaks are no more significant using BTT with lower coupling times than ETT with a 6 minute coupling or 1*H*-tetrazole with a 12 minute coupling.

References:

(1) X. Wu and S. Pitsch, *Nucleic Acids Res*, 1998, **26**, 4315-23.
(2) S. Pitsch, P.A. Weiss, L. Jenny, A. Stutz, and X.L. Wu, *Helv Chim Acta*, 2001, **84**, 3773-3795.
(3) R. Welz and S. Muller, *Tetrahedron Lett*, 2002, **43**, 795-797.

FIGURE 1: ACTIVATOR STRUCTURES



Columns and Plates

We are happy to announce the availability of polystyrene columns fully compatible with the Applied Biosystems 3900 synthesizer. Initially, we are offering the regular 4 nucleoside supports, Bz-dA, Bz-dC, dmf-dG and dT. In the near future, we will be adding several of our most popular supports, including RNA supports, for this platform.

We are also adding 96 well plates containing both of our CPG-based universal supports, as well as dT. Universal Support 1000 is suitable for preparing unmodified oligonucleotides. Any regular or modified oligonucleotide, including RNA, can be prepared using Universal Support II. The dT support should find favor among researchers preparing siRNA oligos. These plates are offered initially filled at the 40 nmole level. The support is held tightly in the wells with porous frits, which help to disperse the liquid flow more evenly through the support bed, while avoiding the splashing of support that is virtually inevitable in loosely filled wells.

ORDERING INFORMATION

Item		Catalog No.	Pack	Price($)
5-Benzylthio-1H-tetrazole (BTT)		30-3070-10	1g	35.00
(Dissolve 1g in 21.3mL anhydrous		30-3070-20	2g	60.00
acetonitrile for a 0.25M solution)		30-3070-25	25g	500.00
0.25M 5-Benzylthio-1H-tetrazole in Acetonitrile		30-3170-45	45mL	40.00
(Applied Biosystems)		30-3170-52	200mL	100.00
		30-3170-57	450mL	200.00
		30-3170-62	2L	760.00
(Expedite)		30-3172-66	60mL	50.00
		30-3172-52	200mL	100.00
		30-3170-57	450mL	200.00
AB 3900				
dA PS	200 nmole columns	26-2600-62	Pack of 200	825.00
	40 nmole columns	26-2600-65	Pack of 200	825.00
dC PS	200 nmole columns	26-2610-62	Pack of 200	825.00
	40 nmole columns	26-2610-65	Pack of 200	825.00
dmf-dG PS	200 nmole columns	26-2629-62	Pack of 200	825.00
	40 nmole columns	26-2629-65	Pack of 200	825.00
dT PS	200 nmole columns	26-2630-62	Pack of 200	825.00
	40 nmole columns	26-2630-65	Pack of 200	825.00
96 Well Format				
Universal Support 1000		96-5001-40	each	200.00
Universal Support II		96-5010-40	each	200.00
dT-CPG 1000		96-2031-40	each	200.00

LOCKED NUCLEIC ACID (LNA™) PHOSPHORAMIDITES

Locked Nucleic Acid (LNA) was first described by Wengel and co-workers in 1998[1] as a novel class of conformationally restricted oligonucleotide analogues. LNA is a bicyclic nucleic acid where a ribonucleoside is linked between the 2'-oxygen and the 4'-carbon atoms with a methylene unit. The structures are detailed in Figure 1. Under licence from Exiqon A/S (Denmark), and in association with Link Technologies Ltd (Scotland), we are now able to offer the four standard LNA phosphoramidites.

Oligonucleotides containing LNA exhibit unprecedented thermal stabilities towards complementary DNA and RNA[2], which allows excellent mismatch discrimination. In fact the high binding affinity of LNA oligos allows for the use of short probes in, for example, SNP genotyping[3], allele specific PCR and mRNA sample preparation. In fact, LNA is recommended for use in any hybridization assay that requires high specificity and/or reproducibility, e.g., dual labelled probes, *in situ* hybridization probes, molecular beacons and PCR primers. Furthermore, LNA offers the possibility to adjust Tm values of primers and probes in multiplex assays.

As a result of these significant characteristics, the use of LNA-modified oligos in antisense drug development is now coming under investigation[4], and recently the therapeutic potential of LNA has been reviewed.[5]

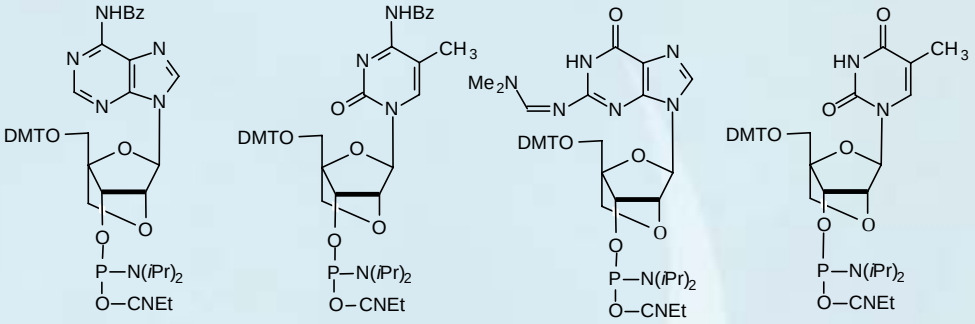
In general, LNA oligonucleotides can be synthesized by standard phosphoramidite chemistry using automated DNA synthesizers. The phosphoramidites can be dissolved in anhydrous acetonitrile to standard concentrations, except for the 5-Me-C variant which requires the use of a 25% THF/acetonitrile solution. They are more sterically hindered compared to standard DNA phosphoramidites and therefore require a longer coupling time. 180 seconds and 250 seconds coupling times are recommended for ABI and Expedite synthesizers, respectively.

The oxidation of the phosphite after LNA coupling is slower compared to the similar DNA phosphite, and therefore a longer oxidation time is suggested. Using standard iodine oxidation procedures, 45 seconds has been found to be the optimal oxidation time on both ABI and Expedite instruments. LNA-containing oligonucleotides are deprotected following standard protocols. It is, however, advisable to avoid the use of methylamine when deprotecting oligos containing Me-Bz-C-LNA since this can result in introduction of an *N*4-methyl modification.

LNA-containing oligonucleotides can be purified and analyzed using the same methods employed for standard DNA. LNA can be mixed with DNA and RNA, as well as other nucleic acid analogues, modifiers and labels. LNA oligonucleotides are water soluble, and can be separated by gel electrophoresis and precipitated by ethanol.

Locked-nucleic Acid (LNA) phosphoramidites are protected by EP Pat No. 1013661, US Pat No. 6,268,490 and foreign applications and patents owned by Exiqon A/S. Products are made and sold under a license from Exiqon A/S. Products are for research purposes only. Products may not be used for diagnostic, clinical, commercial or other use, including use in humans. There is no implied license for commercial use, including contract research, with respect to the products and a license must be obtained directly from Exiqon A/S for such use.

FIGURE 1: **LNA MONOMER STRUCTURES**



References:

(1a) A.A. Koshkin, S.K. Singh, P. Nielsen, V.K. Rajwanshi, R. Kumar, M. Meldgaard, C.E. Olsen, and J. Wengel, *Tetrahedron*,1998, **54**, 3607-3630.

(1b) S.K. Singh, P. Nielsen, A.A. Koshkin, and J. Wengel, *Chem. Comm.*, 1998, (4), 455-456.

(2) L. Kværnø and J. Wengel, *Chem. Comm.*, 1999, (7), 657-658.

(3) P. Mouritzen, A.T. Nielsen, H.M. Pfundheller, Y. Choleva, L. Kongsbak, and S. Møller, *Expert Review of Molecular Diagnostics*, 2003, **3**(1), 27-38.

(4a) J. Kurreck, E. Wyszko, C. Gillen, and V.A. Erdmann, *Nucleic Acids Res.*, 2002, **30**, 1911-1918.

(4b) H. Ørum and J. Wengel, *Curr. Opinion in Mol. Therap.*, 2001, **3**, 239-243.

(5a) M. Petersen and J. Wengel, *Trends in Biotechnology*, 2003, **21**(2), 74-81.

(5b) D.A. Braasch, D.R. Corey, *Biochemistry*, 2002, **41**, 4503-4510.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Bz-A-LNA-CE Phosphoramidite	10-2000-90	100 µm	120.00
	10-2000-02	0.25g	225.00
	10-2000-05	0.5g	450.00
5-Me-Bz-C-LNA-CE Phosphoramidite	10-2011-90	100 µm	120.00
	10-2011-02	0.25g	225.00
	10-2011-05	0.5g	450.00
dmf-G-LNA-CE Phosphoramidite	10-2029-90	100 µm	120.00
	10-2029-02	0.25g	225.00
	10-2029-05	0.5g	450.00
T-LNA-CE Phosphoramidite	10-2030-90	100 µm	120.00
	10-2030-02	0.25g	225.00
	10-2030-05	0.5g	450.00

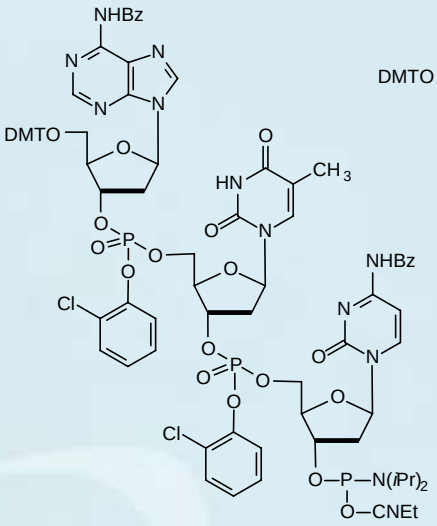
TRIMER (CODON) PHOSPHORAMIDITES SIMPLIFY LIBRARY PREPARATION

Oligonucleotide-directed mutagenesis is probably the most popular approach for the preparation of proteins with variations at specific sites. This protein engineering technique uses oligonucleotides of mixed sequences to generate libraries of proteins for screening potential improvements in specific biological function. It is certainly possible to produce the mixed oligonucleotide sequences by opening the synthesis columns, splitting the supports, and recombining the supports after coupling. This procedure is surely labor-intensive and coupling efficiency is always affected by the splitting and recombination process. The technique is also limited in that the complexity desired may be greater than the number of particles of support in the columns. Another technique is to use mixtures of monomers to generate codon mixtures but the degeneracy of the genetic code guarantees that redundancies and stop codons will be generated. Mutagenesis generating substoichiometric amounts of codons at specific positions has been described[1, 2] using a mixture of trimer and monomer phosphoramidites. A further refinement of this strategy has been described[3] using two sets of monomers, one set with 5'-DMT protection and one set with base-labile 5'-Fmoc protected monomers.

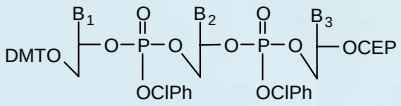
In principle, the simplest approach for oligonucleotide-directed mutagenesis would be the use of trimer phosphoramidites. Of the 64 possible combinations of codons, only 20 codons would be required to cover the 20 amino acids, although, in practice, several codons will likely be duplicated depending on the organism. Several reports describing[4-6] the synthesis of trimer phosphoramidites have been published. We prefer the approach described[7-9] by Kayushin *et al* and our trimers use their protection scheme.

Quality control of trimer phosphoramidites is very challenging. We normally use RP HPLC for purity and identity determination of our regular phosphoramidites. However, trimer phosphoramidites have chiral centers at all three phosphorus positions. There are, therefore, $2^3 = 8$ diastereomers in each phosphoramidite, which are at least partially separated on RP HPLC, rendering the technique questionable for purity and identity determination. There is also the concern that the sequence of the trimers has to be verified. For example, CAT coding

FIGURE 1: STRUCTURE OF TRIMER PHOSPHORAMIDITES



Detailed Structure of ATC



General Structure of Trimer Phosphoramidites, where B=A^{bz}, C^{bz}, G^{ibu}, T

TABLE 1: TRIMER CODING AND PHYSICAL PARAMETERS

Trimer	Amino Acid	MW	RF	MWxRF	mg/10μmol (adjusted for RF)
AAA	Lys	1911.5	1.1	2102.65	21.0 (11)
AAC	Asn	1887.5	1.0	1887.50	18.9 (10)
ACT	Thr	1774.5	1.3	2306.85	23.1 (13)
ATC	Ile	1774.5	1.2	2129.40	21.3 (12)
ATG	Met	1780.5	1.3	2314.65	23.1 (13)
CAG	Gln	1869.5	2.0	3739.00	37.4 (20)
CAT	His	1774.5	1.3	2306.85	23.1 (13)
CCG	Pro	1845.5	1.8	3321.90	33.2 (18)
CGT	Arg	1756.5	1.1	1932.15	19.3 (11)
CTG	Leu	1756.5	1.2	2107.80	21.1 (12)
GAA	Glu	1893.5	1.9	3597.65	36.0 (19)
GAC	Asp	1869.5	1.3	2430.35	24.3 (13)
GCT	Ala	1756.5	1.5	2634.75	26.3 (15)
GGT	Gly	1762.5	1.1	1938.75	19.4 (11)
GTT	Val	1667.5	1.9	3168.25	31.7 (19)
TAC	Tyr	1774.5	1.6	2839.20	28.4 (16)
TCT	Ser	1661.4	1.3	2159.82	21.6 (13)
TGC	Cys	1756.5	1.5	2634.75	26.3 (15)
TGG	Try	1762.5	5.7	10046.25	100.5 (57)
TTC	Phe	1661.4	2.2	3655.08	36.6 (22)
					=592.6 mg

Example of Preparation of Trimer Mixture

Prepare 592.6 mg of the trimer mix, taking the amount (mg) for each trimer from the right column. Dissolve the trimer mix in dichloromethane (highest grade possible; acid-free). Evaporate to dryness to produce a homogenous mixture of all 20 trimers.

Example of Preparation of Trimer Mixture for the Synthesizer

Dissolve 592.6 mg, which is equivalent to 20X10 μmoles (normalized for RF) of the trimer mix in 2.0 mL of acetonitrile-dichloromethane mixture, 1:3 v/v to produce a 0.10N solution of trimers, ready for use in a synthesizer.

for histidine, has to be differentiated from TAC, coding for tyrosine. These two trimers have virtually identical lipophilicity and their identity cannot be clearly confirmed by HPLC. This problem has been solved[10] using HPLC electrospray mass spectrometric analysis of the trimers, which provides data confirming molecular weight and sequence.

In Table 1, the trimers, their coding amino acid and their reaction factor (RF) are listed. The reaction factor is critical since the trimers will likely be mixed and they have differing reactivity in the coupling reaction. RF for AAC is 1.0 and for TAC is 1.6. Therefore, 1.6 equivalents of TAC are needed for every 1.0 equivalent of AAC for equal coupling. Mixtures can easily be made using equimolar solutions or the molecular weight of each trimer has to be used to generate the appropriate weights of each trimer to use if mixing by weight. An example of the preparation of a mixture of all 20 trimers is shown in the right column of Table 1 and completed in the footnotes.

All of the trimers are now available individually so that researchers can prepare custom mixtures. A mixture of all 20 trimers designed to produce equal coupling of all 20 is also available. If you require custom production of a specific mixture, please e-mail support@glenresearch.com for a quotation and projected delivery.

References:

(1) J. Sondek and D. Shortle, *Proc Natl Acad Sci U S A*, 1992, **89**, 3581-3585.
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ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
AAA Trimer Phosphoramidite	13-1000-95	50 µm	350.00
	13-1000-90	100 µm	700.00
AAC Trimer Phosphoramidite	13-1001-95	50 µm	350.00
	13-1001-90	100 µm	700.00
ACT Trimer Phosphoramidite	13-1013-95	50 µm	350.00
	13-1013-90	100 µm	700.00
ATC Trimer Phosphoramidite	13-1031-95	50 µm	350.00
	13-1031-90	100 µm	700.00
ATG Trimer Phosphoramidite	13-1032-95	50 µm	350.00
	13-1032-90	100 µm	700.00
CAG Trimer Phosphoramidite	13-1102-95	50 µm	350.00
	13-1102-90	100 µm	700.00
CAT Trimer Phosphoramidite	13-1103-95	50 µm	350.00
	13-1103-90	100 µm	700.00
CCG Trimer Phosphoramidite	13-1112-95	50 µm	350.00
	13-1112-90	100 µm	700.00
CGT Trimer Phosphoramidite	13-1123-95	50 µm	350.00
	13-1123-90	100 µm	700.00
CTG Trimer Phosphoramidite	13-1132-95	50 µm	350.00
	13-1132-90	100 µm	700.00
GAA Trimer Phosphoramidite	13-1200-95	50 µm	350.00
	13-1200-90	100 µm	700.00
GAC Trimer Phosphoramidite	13-1201-95	50 µm	350.00
	13-1201-90	100 µm	700.00
GCT Trimer Phosphoramidite	13-1213-95	50 µm	350.00
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GGT Trimer Phosphoramidite	13-1223-95	50 µm	350.00
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GTT Trimer Phosphoramidite	13-1233-95	50 µm	350.00
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TAC Trimer Phosphoramidite	13-1301-95	50 µm	350.00
	13-1301-90	100 µm	700.00
TCT Trimer Phosphoramidite	13-1313-95	50 µm	350.00
	13-1313-90	100 µm	700.00
TGC Trimer Phosphoramidite	13-1321-95	50 µm	350.00
	13-1321-90	100 µm	700.00
TGG Trimer Phosphoramidite	13-1322-95	50 µm	350.00
	13-1322-90	100 µm	700.00
TTC Trimer Phosphoramidite	13-1331-95	50 µm	350.00
	13-1331-90	100 µm	700.00
Trimer Phosphoramidite Mix 1 (Mix of above 20 trimers)	13-1991-95	50 µm	375.00
	13-1991-90	100 µm	750.00

STILL MORE NEW PRODUCTS

PC Linker Phosphoramidite

Photo-triggered DNA cleavage is a major tool used for studying conformational changes and strand breaks, as well as for studying activation of nucleic-acid-targeted drugs, such as antisense oligonucleotides. A versatile photocleavable DNA building block has been described by researchers in Washington University, Missouri and used in phototriggered hybridization.[1] In association with Link Technologies Ltd (Scotland) this phosphoramidite is now available from Glen Research.

This reagent has also been used in the design of multifunctional DNA and RNA conjugates[2] for the *in vitro* selection of new molecules catalyzing biomolecular reactions. Researchers at Bruker Daltonik in Germany have also developed genoSNIP, a method for single-nucleotide polymorphism (SNP) genotyping by MALDI-TOF mass spectrometry.[3] This method uses size reduction of primer extension products by incorporation of the photocleavable linker for phototriggering strand breaks near to the 3' end of the extension primer.

Similar to the 3 other available PC monomers, the general design of the PC Linker is based on an α-substituted 2-nitrobenzyl group. The photo-reactive group is derivatized as a β-cyanoethyl phosphoramidite and the non-nucleoside PC Linker can be incorporated into oligonucleotides at any position by standard automated DNA synthesis methodology. Coupling efficiencies >95% are achieved using an extended coupling time of 15 minutes. For ease of use, the product contains a dimethoxytrityl group. The β-cyanoethyl group is removed under normal synthesis conditions using 25% aqueous ammonia solution.

Upon irradiating a PC-modified oligo with near-UV light, the phosphodiester bond between the linker and the phosphate is cleaved, resulting in the formation of a 5'-monophosphate on the released oligonucleotide. Unlike other photocleavable spacers, the PC Linker Phosphoramidite has the added advantage in that photocleavage results in monophosphate fragments at both the 3'- and 5'-termini (see Figure 2).

FIGURE 1: PHOTOCLEAVABLE MODIFIERS

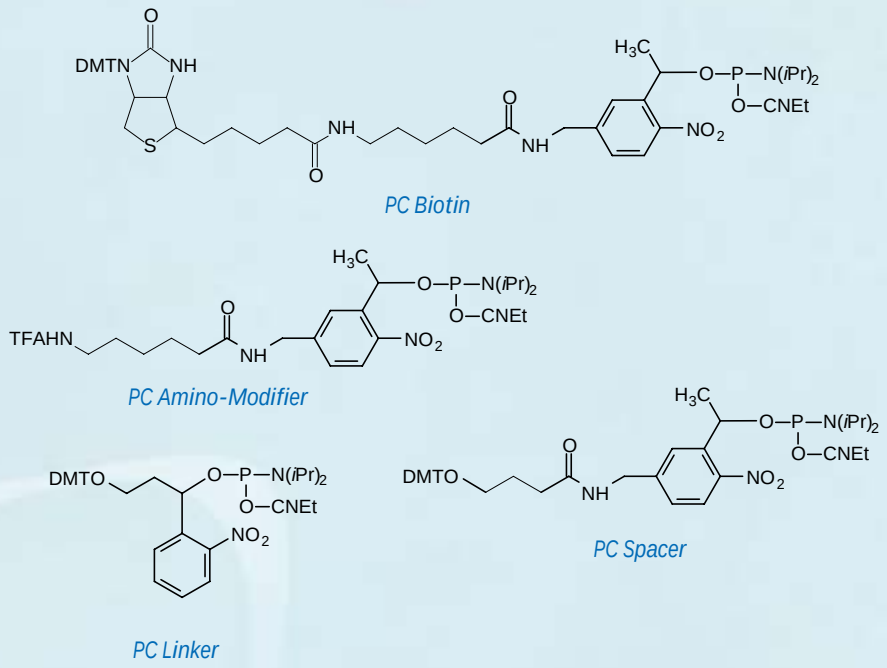
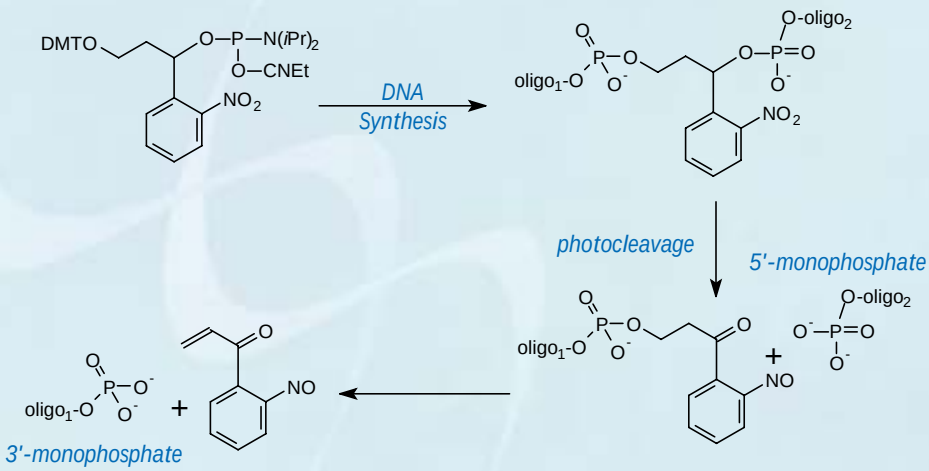


FIGURE 2: PHOTO-CLEAVAGE USING PC LINKER PHOSPHORAMIDITE



References:

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(3) T. Wenzel, T. Elssner, K. Fahr, J. Bimmler, S. Richter, I. Thomas, and M. Kostrzewa, *Nucleosides, Nucleotides & Nucleic Acids*, 2003, **22**, 1579-1581.

(Continued on Next Page)

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
PC Linker Phosphoramidite	10-4920-90	100 µm	170.00
	10-4920-02	0.25g	500.00

(Continued from Previous Page)

DesthiobiotinTEG

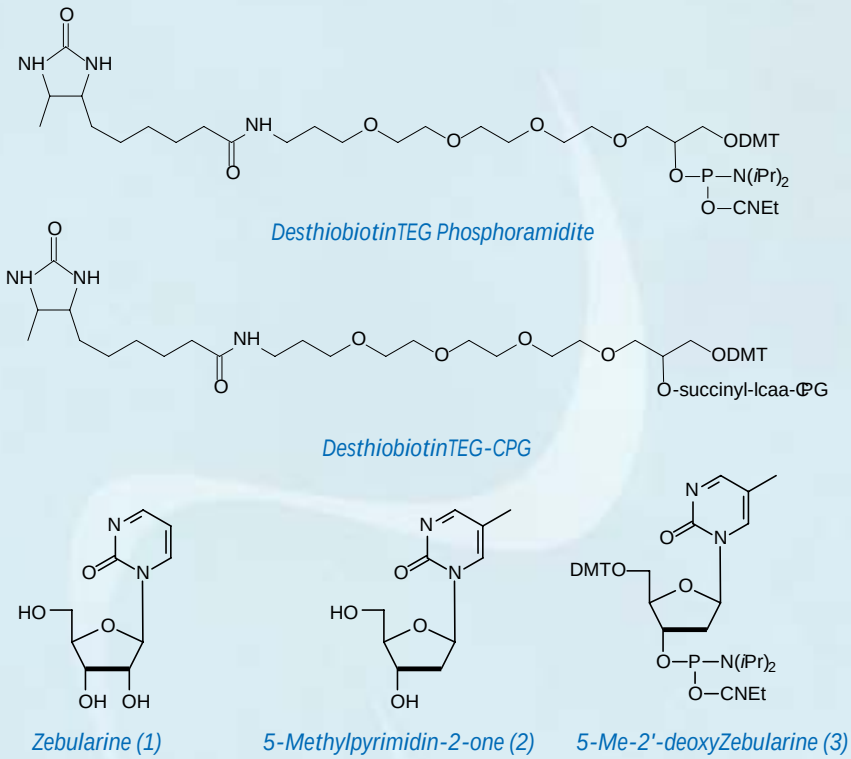
Avidin, streptavidin and other biotin-binding proteins have the ability to form an intense association with biotin-containing molecules. This association has been used for many years to develop systems designed to capture biotinylated biomolecules. In the oligonucleotide field, probably the most common modification is biotinylation and reagents are available to modify oligos at the termini and within the sequence. A wide variety of tests and techniques are in routine use to exploit the extraordinary affinity of these biotin-binding proteins for biotinylated biomolecules. However, the intense affinity of biotin-binding proteins for biotin is also the biggest drawback in that the association is essentially irreversible. Indeed, extremely low pH and highly concentrated chaotropic reagents are required to break the association and these conditions are not entirely compatible with oligonucleotides. 2-Iminobiotin has been used as a reversibly binding biotin reagent since its association with biotin-binding proteins can be broken at pH4. However, 2-iminobiotin is not stable to the conditions of oligonucleotide deprotection. Another biotin analogue that exhibits lower binding to biotin-binding proteins like streptavidin is desthiobiotin (or dethiobiotin). This biotin analogue is lacking the sulfur group from the molecule and has a dissociation constant (Kd) several orders of magnitude less than biotin/streptavidin. As a result, biomolecules containing desthiobiotin are dissociated from streptavidin simply in the presence of buffered solutions of biotin.[1] We believe that these characteristics will allow another biotin product to prosper.

Our most versatile biotin products are the biotinTEG products which can be added singly or in multiple additions anywhere within an oligonucleotide. In addition, the triethyleneglycol (TEG) section of the structure separates the biotin from the oligonucleotide in such a way that it is more readily captured by streptavidin. We have used the same structure to offer desthiobiotinTEG phosphoramidite and the corresponding CPG.

Reference:

(1) J.D. Hirsch, L. Eslamizar, B.J. Filanoski, N. Malekzadeh, R.P. Haugland, and J.M. Beechem, *Anal Biochem*, 2002, **308**, 343-57.

FIGURE 1: DESTHIOBIOTIN MODIFIERS



5-Me-2'-deoxyZebularine

Zebularine (pyrimidin-2-one ribonucleoside) (1) is a cytidine analog that acts as a DNA demethylase inhibitor, as well as a cytidine deaminase inhibitor. This structure is very active biologically and Zebularine is now used as a potent anti-cancer drug. A 2'-deoxynucleoside analogue of Zebularine, 5-methyl-pyrimidin-2-one, 2'-deoxy-nucleoside (2), has been used[1] to probe the initiation of the cellular DNA repair process by making use of its mildly fluorescent properties. We believe that this combination of biological activity and fluorescence properties would make the phosphoramidite (3) a strong addition to our array of nucleoside analogue phosphoramidites.

Reference

(1) S.F. Singleton, et al., *Org Lett*, 2001, **3**, 3919-22.

ORDERING INFORMATION

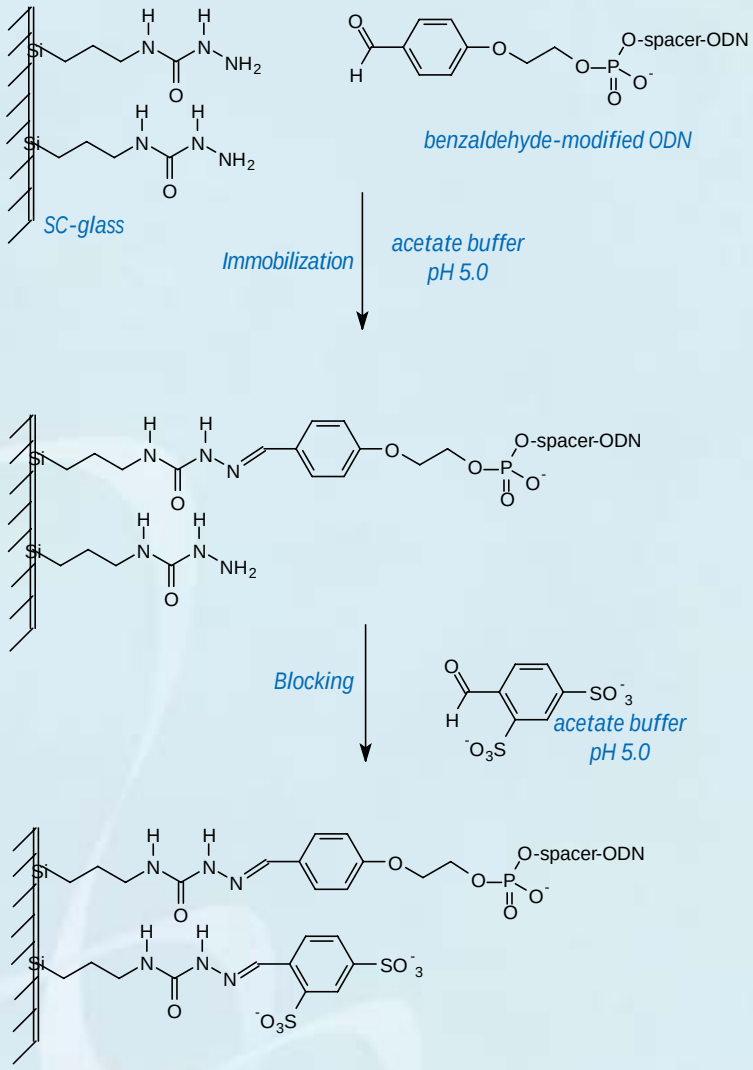
Item	Catalog No.	Pack	Price($)
DesthiobiotinTEG Phosphoramidite	10-1952-95	50 µm	185.00
	10-1952-90	100 µm	335.00
	10-1952-02	0.25g	775.00
DesthiobiotinTEG-CPG	20-2952-01	0.1g	140.00
	20-2952-10	1.0g	1150.00
0.2 µmole columns	20-2952-42	Pack of 4	140.00
1 µmole columns	20-2952-41	Pack of 4	230.00
10 µmole column (ABI)	20-2952-13	Pack of 1	345.00
15 µmole column (Expedite)	20-2952-14	Pack of 1	520.00
5-Me-2'-deoxyZebularine-CE Phosphoramidite	10-1061-95	50 µm	200.00
	10-1061-90	100 µm	400.00
	10-1061-02	0.25g	975.00

NEW EPOCH PRODUCTS - 5'-ALDEHYDE-MODIFIER C2 PHOSPHORAMIDITE

Oligonucleotide conjugation reactions are predominantly carried out using a nucleophilic group on the oligonucleotide to attach to an electrophilic group on a tag or support. This strategy presumably predominates since oligonucleotide deprotection schemes are carried out by bases, which are inherently nucleophilic. Indeed, there are many strategies to produce oligonucleotides modified with amine and thiol groups at a variety of positions. On the other hand, there are situations where researchers wish to conjugate a variety of nucleophilic groups to oligonucleotides. In this vein, convenient carboxy-modifiers have been described recently. 5'-Carboxy-Modifier C10 (1) which contains a carboxylate NHS ester (available from Glen Research) allows convenient solid-phase conjugation of amino compounds to oligonucleotides on the solid support prior to deprotection.[1] Similarly, another reagent containing a protected carboxylic acid and suitable for solid-phase conjugation reactions has been described[2] recently. A phosphoramidite containing an electrophilic chloroacetyl group has been described.[3] In the latter case, the oligonucleotide can also be derivatized on solid phase, but the chloroacetyl group is also stable to deprotection with potassium carbonate in methanol and oligonucleotides modified with this reagent can also be used for solution-phase conjugations.

Aldehyde modifiers would be attractive electrophilic substitutions in oligonucleotides since they are able to react with amino groups to form a Schiff's base, with hydrazino groups to form hydrazones, and with semicarbazides to form semicarbazones. The Schiff's base is unstable and must be reduced with sodium borohydride to form a stable linkage but hydrazones and semicarbazides are very stable linkages. Similarly to activated carboxylic acids, aldehydes are generally unstable to oligonucleotide deprotection conditions. Many strategies have used post synthetic oxidation of a glycol with sodium periodate to generate an aldehyde group. However, a recent report describes[4] the use of a formylindole nucleoside analog (2) to generate an aldehyde group directly in an oligonucleotide without the need for a protecting group on the aldehyde. Our collaboration with Epoch Biosciences has allowed us to adopt the protected benzaldehyde derivative (3) as our first 5'-aldehyde modifier.[5] The acetal protecting group is sufficiently hydrophobic for use in RP HPLC and cartridge purification and is readily removed after oligonucleotide synthesis under standard oligonucleotide detritylation conditions with 80% acetic acid or 2% TFA after cartridge purification.

SCHEME 1: IMMOBILIZATION OF 5'-ALDEHYDE-MODIFIED OLIGONUCLEOTIDE TO SC-GLASS



Scheme 1 illustrates the utility of this 5'-aldehyde modifier in the preparation of oligonucleotide arrays on glass slides. First, glass slides were derivatized with semicarbazidopropyltriethoxysilane, a reagent readily prepared by the reaction of isocyanopropyltriethoxysilane with hydrazine. The slides were washed, dried and cured at 110°C to produce SC-glass slides. The aldehyde-modified oligonucleotides were prepared with a Spacer 18 molecule between the benzaldehyde group and the oligonucleotide to help separate the oligonucleotides from the glass surface for efficient hybridization with the target. Arrays can be prepared by spotting the 5'-aldehyde-modified oligonucleotides in acetate buffer at pH 5 onto the plates and maintaining the slides in a humid environment for 3 hours at 37°C. The slides were washed to remove excess oligonucleotide and then excess semicarbazide residues on the slide were blocked with 5-formyl-1,3-benzene-disulfonic acid disodium salt. The slides were then ready for hybridization experiments.

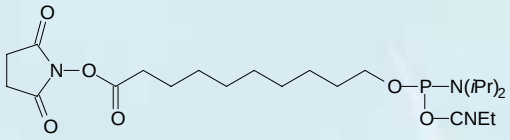
AND GIG HARBOR GREEN™ PHOSPHORAMIDITE

Epoch Gig Harbor Green (4) and 6-FAM (5) are based on the same fluorescein core structure. The difference between the two molecules is the linker strategy. Therefore, there should be little difference between Gig Harbor Green and FAM in terms of absorbance and emission spectra. It is well known that the fluorescence of fluorescein-based dyes depends on the degree of ionization of the phenolic groups and, therefore, is pH dependent. Gig Harbor Green is more pH dependent than FAM, which means that at pH below 7.5 fluorescence will drop slightly faster for Gig Harbor Green than for FAM. On the other hand, at higher pH such as 8.5 (PCR conditions) both dyes are completely ionized and Gig Harbor Green is 15-20% brighter than FAM.[6]

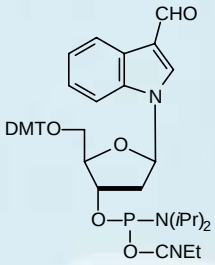
5'-Aldehyde-Modifier and Gig Harbor Green are offered in collaboration with Epoch Biosciences, Inc. Please read the following statement before choosing to use these products commercially.

"These Products are for research purposes only, and may not be used for commercial, clinical, diagnostic or any other use. The Products are subject to proprietary rights of Epoch Biosciences, Inc. and are made and sold under license from Epoch Biosciences, Inc. There is no implied license for commercial use with respect to the Products and a license must be obtained directly from Epoch Biosciences, Inc. with respect to any proposed commercial use of the Products. "Commercial use" includes but is not limited to the sale, lease, license or other transfer of the Products or any material derived or produced from them, the sale, lease, license or other grant of rights to use the Products or any material derived or produced from them, or the use of the Products to perform services for a fee for third parties (including contract research)."

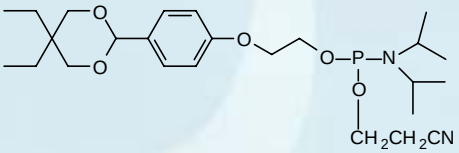
FIGURE 1: ELECTROPHILIC MODIFIERS AND EPOCH GIG HARBOR GREEN



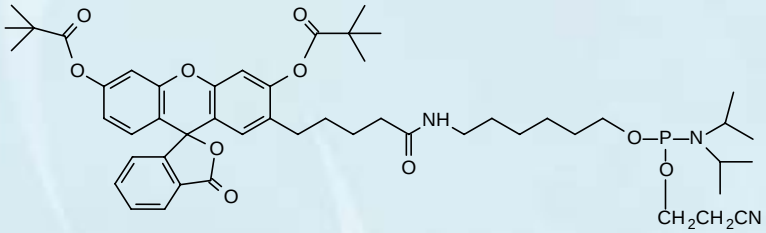
(1) 5'-Carboxy-Modifier C10



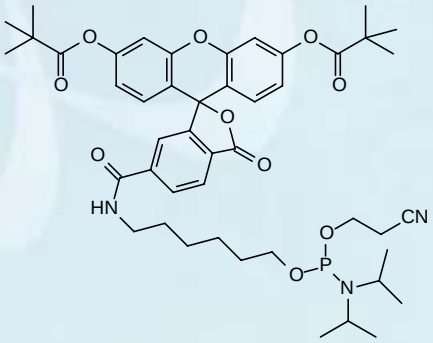
(2) 3-Formylindole



(3) Epoch 5'-Aldehyde-Modifier C2



(4) Epoch Gig Harbor Green



(5) 6-FAM

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
5'-Aldehyde-Modifier C2 Phosphoramidite	10-1933-90	100 µm	85.00
	10-1933-02	0.25g	325.00
Epoch Gig Harbor Green Phosphoramidite	10-5922-95	50 µm	165.00
	10-5922-90	100 µm	325.00
	10-5922-02	0.25g	875.00

References:

(1) RI Hogrefe and MM Vaghefi, 2001, US Patent No. 6,320,041.
(2) A.V. Kachalova, D.A. Stetsenko, E.A. Romanova, V.N. Tashlitsky, M.J. Gait, and T.S. Oretskaya, *Helv Chim Acta*, 2002, **85**, 2409-2416.
(3) A. Guzaev and M. Manoharan, *Nucleos Nucleot*, 1999, **18**, 1455-1456.
(4) A. Okamoto, K. Tainaka, and I. Saito, *Tetrahedron Lett*, 2002, **43**, 4581-4583.
(5) M.A. Podyminogin, E.A. Lukhtanov, and M.W. Reed, *Nucleic Acids Res*, 2001, **29**, 5090-8.
(6) E. Lukhtanov, Epoch Biosciences, Personal Communication.

(Continued from Front Page)

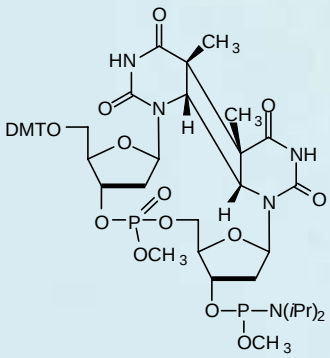
phosphoramidite in sufficient quantity to offer it for sale. Due to the strategy used to synthesize our version of this phosphoramidite, it has methyl protecting groups on phosphorus, rather than the more usual cyanoethyl. This monomer exhibits high purity, performance and stability but requires the use of thiophenoxide (or another phosphate demethylating reagent) prior to regular deprotection.

Cis-syn thymine dimer phosphoramidite (5) is by far the most expensive product we have offered for sale. We are packaging it initially in standard ABI and Expedite vials, as well as amber V vials compatible with the Expedite synthesizer. Using this V vial containing 50 micromoles, it is possible to prepare 6 oligos on a 0.2 μmole scale each containing a single insertion, or 4 oligos on a 1 μmole scale. Similar results should be possible using the LV cycles in Applied Biosystems instruments. Given the high price of this phosphoramidite, if only one or two oligos are required, it makes sense to have a custom oligo house prepare them. A custom oligo house may also wish to group oligos for several clients for most eficient use of this phosphoramidite. Contact us for the names of custom oligo services active in preparing oligos containing *cis-syn* thymine dimer.

References:

(1) C.A. Smith and J.-S. Taylor, *J Biol Chem*, 1993, **268**, 11143-51.
(2) H. Ling, F. Boudsocq, B.S. Plosky, R. Woodgate, and W. Yang, *Nature*, 2003, **424**, 1083-7.
(3) L. Sun, et al., *Biochemistry*, 2003, **42**, 9431-7, and references cited therein.
(4) J.-S. Taylor, I. Brockie, and C. O'Day, *J Am Chem Soc*, 1987, **109**, 6735-6742.
(5) T. Murata, S. Iwai, and E. Ohtsuka, *Nucleic Acids Research*, 1990, **18**, 7279-86.

***FIGURE 2: CIS-SYN* THYMINE DIMER**



Cis-syn Thymine Dimer Phosphoramidite (5)

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Item	Catalog No.	Pack	Price($)
Cis-syn Thymine Dimer Phosphoramidite	11-1330-95	50 μm	2100.00
(ABI septum vial is standard vial.	11-1330-90	100 μm	4200.00
Add E to catalog no. for Expedite vial	11-1330-02	0.25g	10200.00
or V to catalog no. for Expedite V vial)			

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