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NOVEL UNIVERSAL SUPPORT FEATURES RAPID AMIDE-ASSISTED DEPHOSPHORYLATION

The vast majority of oligonucleotide syntheses are carried out on supports to which the first nucleoside has been pre-attached. Following routine cleavage and deprotection, this nucleoside becomes the terminal nucleoside of the target oligonucleotide. For standard DNA and RNA synthesis, this requires an inventory of four deoxynucleoside and four ribonucleoside supports. A universal support (a support without the first nucleoside attached) offers the following advantages:

- eliminates the need for an inventory of nucleoside supports.
- minimizes the possibility of error in the selection of the correct support type.
- reduces time and eliminates the possible error in the generation of an array of nucleoside supports in 96 well synthesizers.
- allows the preparation of oligonucleotides containing a 3'-terminal nucleoside which is not available as a support.

Since these are major benefits, why is the use of a universal support not, well, universal? Indeed, universal-type supports are standard in the synthesis of amino-, thiol-, and other modified oligonucleotides. The major hurdle to overcome is to find conditions to eliminate the terminal phosphate, produced from the first nucleoside phosphoramidite addition cycle, to the required terminal hydroxyl group. Let's look at the problem in detail.

Universal supports are based on the ribonucleoside elimination model exemplified by our Universal Support (1) (Figure 1, Page 4), originally introduced in 1997 and known as the McLean support.[1] The instability of RNA to strongly basic conditions is caused by the proximity of the 2'-OH group to the phosphodiester group. Attack of the 2'-OH on the adjacent phosphorus gives rise to an energetically favorable 5-membered transition state, which can open up again to form a mixture of 2'- and 3'-phosphodiester internucleotide linkages, or can lead to chain scission by elimination of the 3'- or 5'-hydroxyl group (Scheme 1, Page 2). In the case of our Universal Support (1), cleavage from the support by hydrolysis of a succinate or, better, the hydroquinone-O,O'-diacetate (Q) linkage, generates a hydroxyl group adjacent to the terminal phosphodiester linkage. Additional base treatment leads to the elimination of the terminal phosphate group and formation of the desired 3'-OH (Scheme 2, Page 2). A similar strategy using a neighboring hydroxyl group to facilitate elimination was described by Wengel and coworkers.[2] A neat variation by Lyttle[3] and the

(Continued on Page 2)

Universal Support II

- ***Fast*** *- cleavage and dephosphorylation in 20 minutes at room temperature*
- ***Mild*** *- cleavage reagent is 2M ammonia in methanol*
- ***Compatible*** *with UltraMild, normal, and UltraFast deprotection*
- ***Cost-effective*** *- comparable in price to regular 2'-deoxynucleoside supports*

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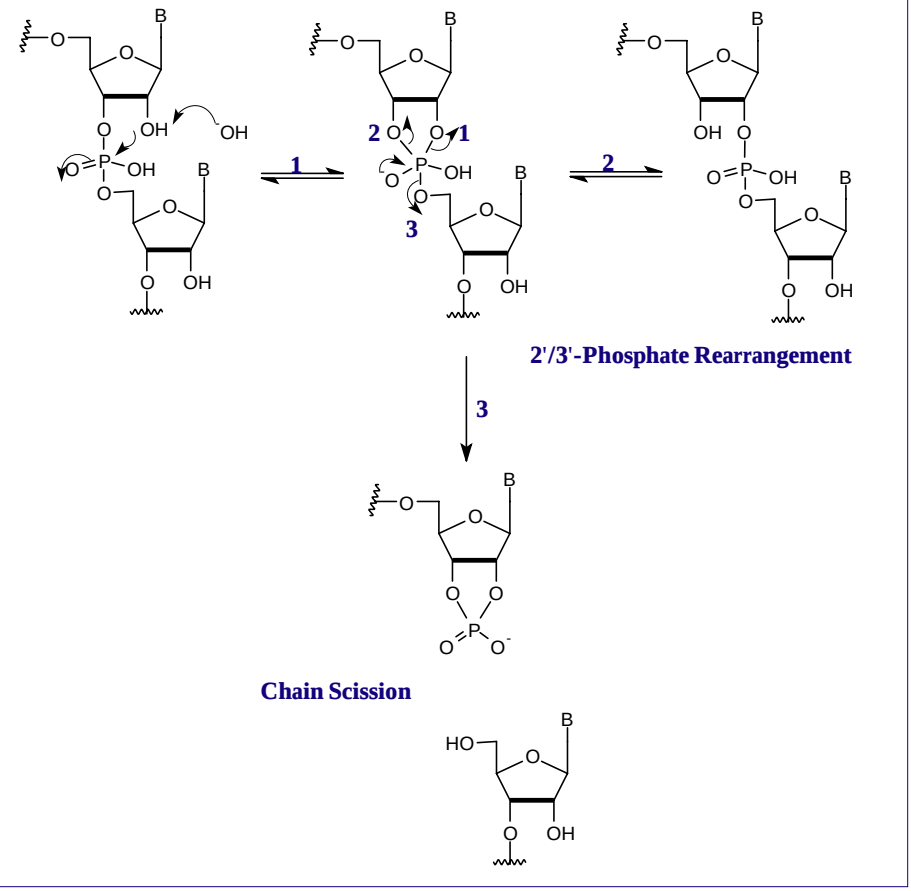
group at Biosearch Technologies uses a linkage to a polymeric support that is not hydrolyzed by base, so that extended base treatment releases the dephosphorylated oligo, while leaving any undesired by-product still attached to the support.

The main impediment to the universal adoption of a universal support has been the aggressively basic conditions required to complete the elimination reaction to release the terminal hydroxyl group. The standard reagents used in oligonucleotide deprotection are ammonium hydroxide and aqueous methylamine, which are popular since they are completely volatile. Using these reagents to carry out the elimination reaction requires either high temperature, with attendant high pressure, or extended reaction times. The situation can be improved by adding metal ions to the mix, and Li^+, Na^+ and Zn^{2+} have all been used to speed up the elimination reaction, presumably by stabilizing the 5-membered transition state. However, the speed and simplicity of evaporation of the deprotection solution to give the crude oligonucleotide without desalting is not possible with these ionic additives.

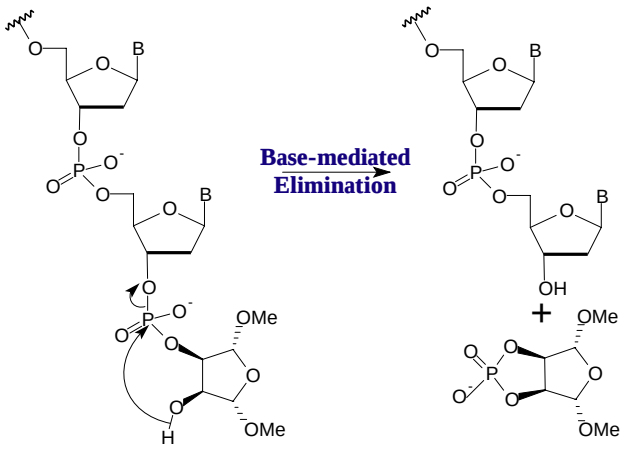
Considerably improved universal supports, using neighboring aminomethyl or diamino-ethyl groups to assist the elimination reaction, were recently described by Azhayev.[4] Using volatile ammonium hydroxide or aqueous methylamine, terminal dephosphorylation was significantly speeded up and even could be achieved using aqueous zinc chloride. We felt that this setup offered genuine advantages, especially if the oligonucleotides contained base-labile components, but was not quite ideal for mainstream applications.

Azhayev's group then went a step further in the investigation of neighboring group assistance in the dephosphorylation reaction. Amide groups may be considered to be weak N-H acids and can display basic properties in ammonium hydroxide or aqueous methylamine. (±)-3-Amino-1,2-propanediol was acylated to form several amide structures followed by dimethoxytritylation at the 1-position. The 2-hydroxyl was used to attach to supports via succinate or Q linkages and oligonucleotide synthesis was carried out as normal. Hydrolysis and dephosphorylation in a variety of basic solutions was investigated using T_6 as the target oligo. Yields of 75-88% (relative to T_6 prepared normally) were achieved using the Q-support, trifluoroacetamide and 2-9M ammonia in methanol-toluene or methanol-dioxane. Interestingly, aqueous ammonium hydroxide led to only 43% yield of T_6 with the remainder being the side product formed by ß-elimination of the cyanoethyl phosphate protecting group rather than the desired 3'-OH

SCHEME 1: BASE HYDROLYSIS OF RNA



SCHEME 2: BASE-MEDIATED ELIMINATION OF UNIVERSAL SUPPORT



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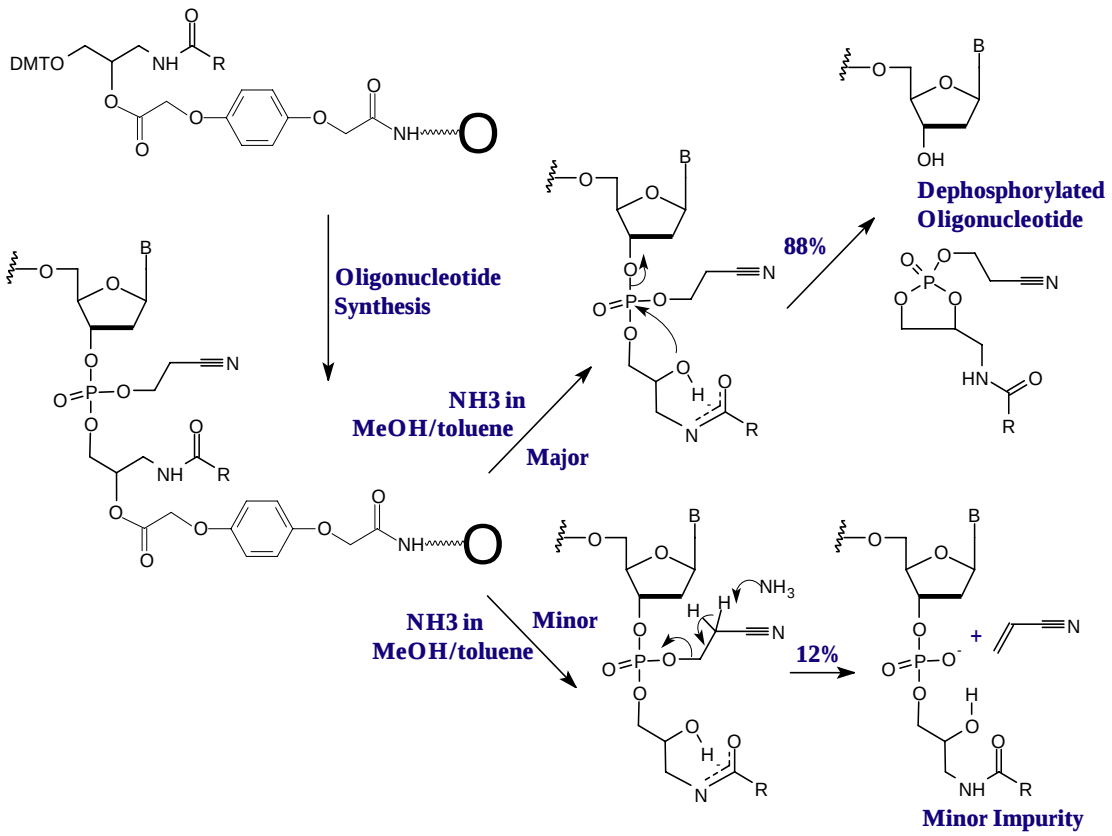
(Scheme 3, Page 3).

It was observed that the Q-linker was far superior to the equivalent succinate, indicating that faster generation of the 2-OH, relative to the β-elimination of the cyanoethyl phosphate protecting group is essential for successful dephosphorylation. Moreover, it was apparent that the acyl group on the amine had very little effect on the dephosphorylation reaction. Azhayev reasoned that using a succinate linker to attach the 3-amino group to the support and protecting the 2-OH with a labile protecting group should set up the identical amide assisted elimination in mildly basic conditions on the solid support. In this way, the dephosphorylation reaction would eliminate the desired 3'-OH oligonucleotide into solution and the product of any β-elimination competing side reaction would remain bound to the support (Scheme 4, Page 4).

SCHEME 3: BASE-MEDIATED DEPHOSPHORYLATION OF POTENTIAL UNIVERSAL SUPPORT



A variety of 2-O esters were tested to determine the optimal protecting group in conjunction with various mild hydrolysis conditions. The combination of 2-O formyl ester, Universal Support II (2, R=H)(Figure 1, Page 4), and hydrolysis with commercially available 2M ammonia in methanol for 1 hour at room temperature led to the optimal yield (>80%) and equivalent purity compared to T_6 prepared normally[5], as shown in Figures 2 and 3.

Once the cleavage and dephosphorylation reactions are complete, the deprotection of the standard bases can be achieved as follows:

- UltraMild – add potassium carbonate in methanol or ammonium hydroxide and leave for a further 4 hours at room temperature.
- Regular – add ammonium hydroxide and incubate for the normal time and temperature.
- UltraFast – add AMA and deprotect as normal.

Universal Support II has been shown to generate oligonucleotides with the same efficacy in polymerase extension

(Continued on Page 4)

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Universal Support II	20-5010-01	0.1g	11.00
	20-5010-02	0.25g	25.00
	20-5010-10	1.0g	95.00
ABI Format (not LV)			
1 µmole columns	20-5110-41	Pack of 4	60.00
0.2 µmole columns	20-5110-42	Pack of 4	40.00
40 nmole columns	20-5110-45	Pack of 4	40.00
10 µmole column (TWIST Format)	20-5110-13	Pack of 1	100.00
Expedite Format			
1 µmole columns	20-5210-41	Pack of 4	60.00
0.2 µmole columns	20-5210-42	Pack of 4	40.00
40 nmole columns	20-5210-45	Pack of 4	40.00
15 µmole column (TWIST Format)	20-5210-14	Pack of 1	150.00

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reactions as regular oligos. Despite the mild elimination reaction, oligonucleotides up to 50mer in length can be prepared routinely without substantial loss of oligo during the synthesis cycles.

References:

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

(2) C. Scheuer Larsen, C. Rosenbohm, T.J.D. Jorgensen, and J. Wengel, *Nucleosides & Nucleotides*, 1997, **16**, 67-80.

(3) M.H. Lyttle, D.J. Dick, D. Hudson, and R.M. Cook, *Nucleosides & Nucleotides*, 1999, **18**, 1809-1824.

(4) A.V. Azhayev, *Tetrahedron*, 1999, **55**, 787-800.

(5) A.V. Azhayev and M. Antopolsky, Personal Communication. Patent Pending.

SCHEME 3: BASE-MEDIATED DEPHOSPHORYLATION OF UNIVERSAL SUPPORT II

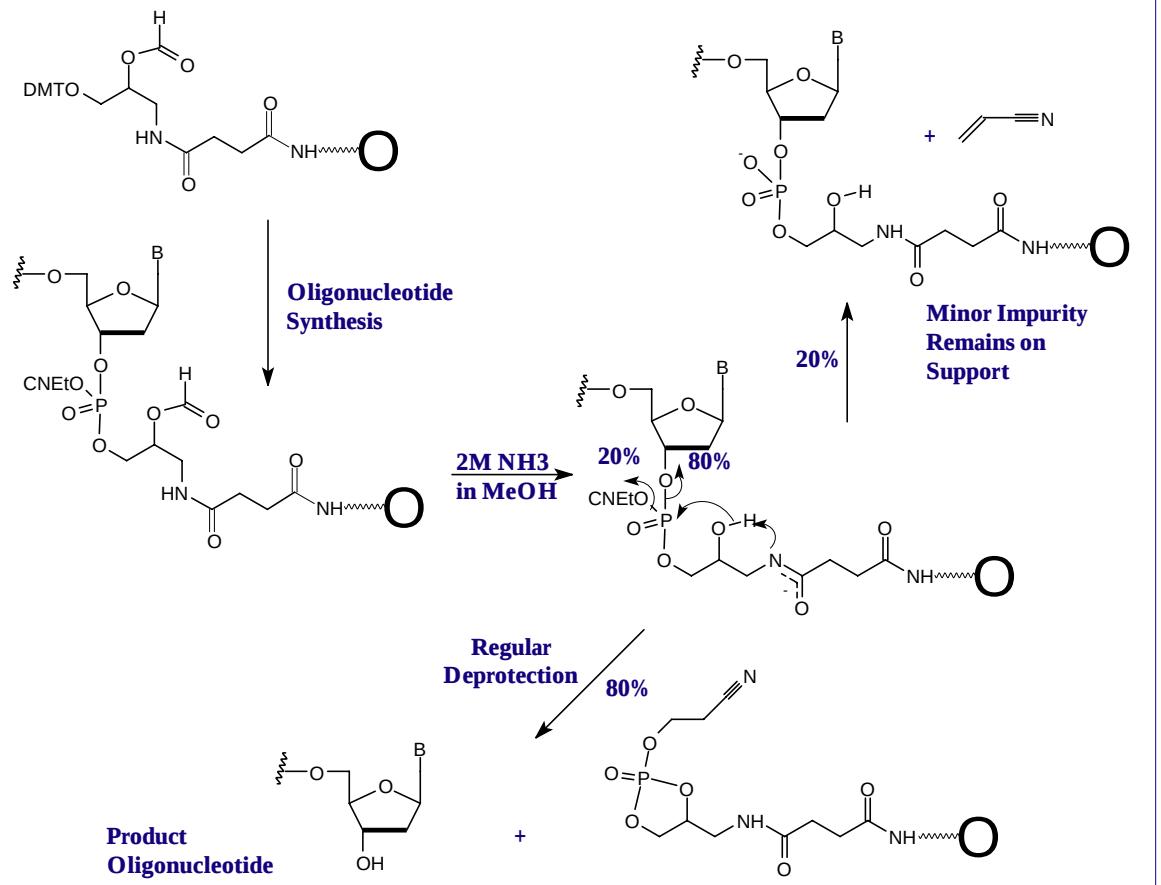
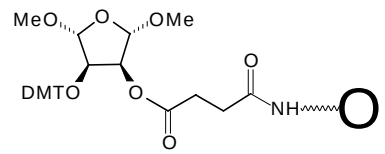
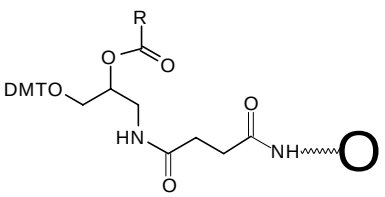


FIGURE 1: UNIVERSAL SUPPORTS



(1) Universal Support



(2) Universal Support II

FIGURE 2: IEX HPLC OF 20mer PRODUCED USING US II SUPPORT

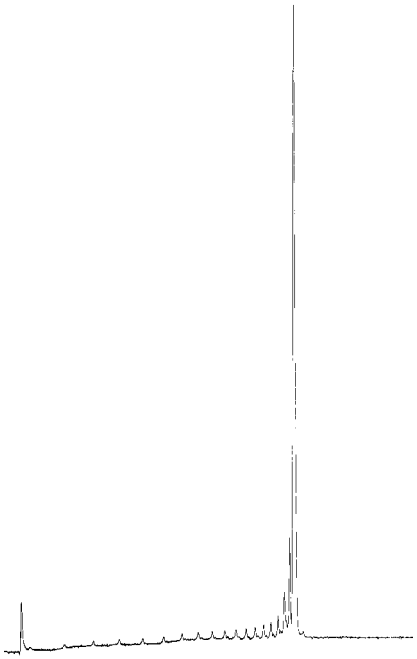
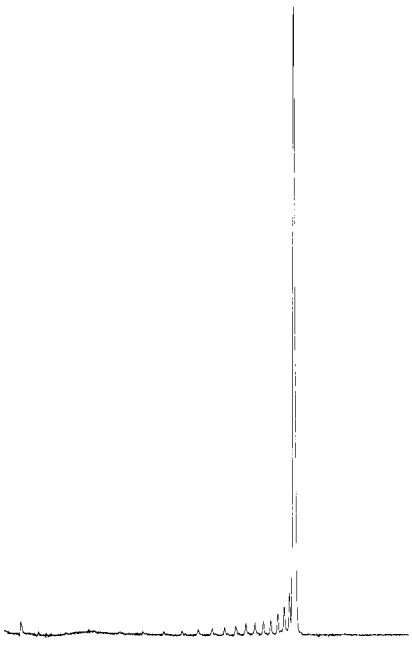


FIGURE 3: IEX HPLC OF 20mer PRODUCED USING T SUPPORT



A NEW SIMPLIFIED 3'-AMINO-MODIFIER CPG

Introduction

The use of oligonucleotides modified with aliphatic amino groups continues to grow as immobilization to surfaces becomes even more important than labelling with tags. The selection of commercially available 3'-amino-modifiers has been dominated by two products[1, 2] containing branched linkers in which the amino group is protected with the fluorenylmethoxycarbonyl (Fmoc) group. These are supplied by Glen Research and are the products 3'-Amino-Modifier C3 CPG (1) and 3'-Amino-Modifier C7 CPG (2), as shown in Figure 1. Since the Fmoc group is base-labile, it offers some advantages but also disadvantages.

Advantages

- The Fmoc group is quite stable and allows synthesis to proceed without significant loss.
- The Fmoc group can be removed specifically from the support to allow solid-phase conjugation of the desired tag. This can be done before or after the oligonucleotide synthesis.
- These supports have been used effectively and successfully for more than 10 years.

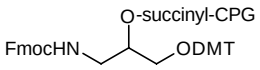
Disadvantages

- If the support is not handled properly, some loss of Fmoc group can occur. The free amino group is then capped with acetic anhydride and the resulting acetyl group is not removed during deprotection, leading to lower conjugation yields.
- Because of their branched structures, the linkers each contain a chiral center which generates a pair of diastereomers after oligo synthesis and these may be separated during HPLC analysis and purification.

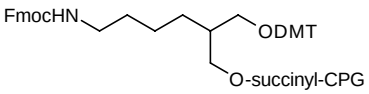
DMT-Amino C6-CPG

An amino support without an Fmoc group has been described by Lyttle and coworkers[3] and is now commercially available (Biosearch Technologies, Inc.).

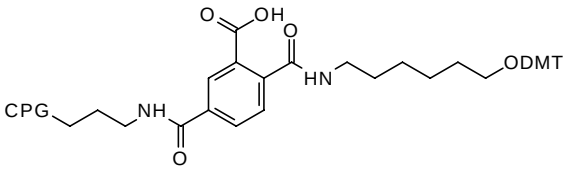
FIGURE 1: STRUCTURES OF PC PRODUCTS



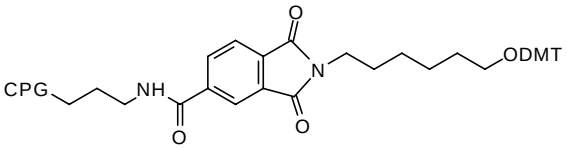
(1) 3'-Amino-Modifier C3 CPG



(2) 3'-Amino-Modifier C7 CPG



(3) DMT-Amino C6-CPG



(4) 3'-PT Amino-Modifier C6 CPG

Under normal circumstances, an aliphatic amide cannot be simply hydrolyzed under conditions appropriate for oligo-nucleotide deprotection. The novel support (3) contains an amide group which is hydrolyzed to give the aliphatic amine because of the participation in the hydrolysis of the neighboring carboxylic acid group. This approach does require extended cleavage conditions (ammonium hydroxide at 55°C for 17 hours) but the deprotection of the bases is obviously achieved simultaneously.

3'-PT-Amino-Modifier C6 CPG

Another interesting approach was described in 1992[4] and has remained commercially dormant since then. In this approach, the nitrogen destined to become the 3'-amino group is included in a phthalimide (PT) group which is attached to the support through an amide group attached to the aromatic ring. The structure of the support (4) is shown in Figure 1. This simple linkage is very stable to all conditions of oligonucleotide synthesis and contains no chiral center. Again, using an extended ammonium hydroxide treatment (55°C for 17 hours), the cleavage of the amine from the phthalimide is accomplished along with the deprotection of the oligonucleotide.

A comparison of the yields of crude oligonucleotides produced with 3'-Amino-Modifier C7 CPG (2) and 3'-PT-Amino-Modifier C6 CPG is shown in Table 1 and the HPLC purity of the oligonucleotides is shown in Figure 2 and Figure 3. The results indicate that the yield of product from the 3'-PT-Amino-Modifier C6 CPG is about 20% lower if deprotected with ammonium hydroxide. However, the purity of amino-modified product is significantly higher due to the absence of the acetyl capped product.

References:

(1) P.S. Nelson, R. Sherman-Gold, and R. Leon, *Nucleic Acids Res.*, 1989, **17**, 7179-86.

(2) P.S. Nelson, M. Kent, and S. Muthini, *Nucleic Acids Res.*, 1992, **20**, 6253-6259.

(3) M.H. Lyttle, H. Adams, D. Hudson, and R.M. Cook, *Bioconjugate Chemistry*, 1997, **8**, 193-198.

(4) C.R. Petrie, M.W. Reed, A.D. Adams, and R.B. Meyer, Jr., *Bioconjugate Chemistry*, 1992, **3**, 85-7.

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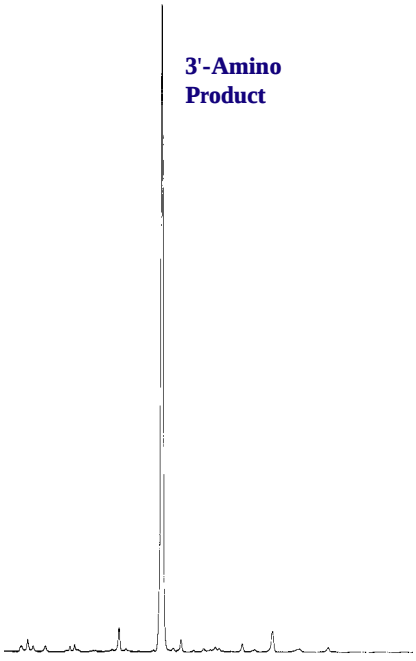
TABLE 1: COMPARISON OF YIELDS FROM 3'-AMINOMODIFIER SUPPORTS

Support	*Synthesis Scale*	*Oligo Length*	*Yield (A_{260} Units)*
3'-Amino-Modifier C7 CPG	1µmole	20mer	119
	1µmole	20mer	115
	1µmole	20mer	106
3'-PT Amino-Modifier C6 CPG	1µmole	20mer	91
	1µmole	20mer	97
	1µmole	20mer	81

FIGURE 2: IEX HPLC OF 20mer USING Fmoc AMINO SUPPORT

FIGURE 3: IEX HPLC OF 20mer USING PT AMINO SUPPORT



Item	Cat. No.	Pack	Price ($)
3'-Amino-Modifier C3 CPG 500	20-2950-01	0.1g	85.00
	20-2950-10	1.0g	600.00
1 µmole columns	20-2950-41	Pack of 4	125.00
0.2 µmole columns	20-2950-42	Pack of 4	75.00
10 µmole column (ABI)	20-2950-13	Pack of 1	225.00
15 µmole column (Expedite)	20-2950-14	Pack of 1	350.00
3'-Amino-Modifier C7 CPG 500	20-2957-01	0.1g	95.00
	20-2957-10	1.0g	675.00
1 µmole columns	20-2957-41	Pack of 4	140.00
0.2 µmole columns	20-2957-42	Pack of 4	85.00
10 µmole column (ABI)	20-2957-13	Pack of 1	250.00
15 µmole column (Expedite)	20-2957-14	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

PREPARATION OF OLIGONU

Background

Hydrolysis of nucleoside residues in DNA occurs naturally to generate abasic sites. Most commonly, dA sites are hydrolyzed causing depurination and leading to abasic residues. Although this process is slow under physiological conditions, the reaction is faster at lower pH and especially if the bases are already oxidatively damaged. Damaged bases are also removed enzymatically by the action of DNA N-glycosylases. The abasic residue (dR) exists predominantly in the cyclic form and the structure is shown (1) in Figure 1. The abasic site exhibits poor stability, especially in basic medium. This is caused by the instability of the aldehyde, the tautomeric form of the cyclic structure, to ß-elimination. This degradation reaction leads to chain scission at the abasic site with formation of a 5'-phosphate segment and a 3'-modified segment.

Because of the instability of the abasic residue, it has not been simple to prepare this variant by chemical synthesis. However, some excellent results have been generated using the stable dSpacer tetrahydrofuran analogue.[1, 2] This variant, (2) in Figure 1, is missing the 1'-OH of dR and is stable during oligo synthesis, purification and storage. It is possible to produce the genuine abasic site enzymatically using N-uracil glycosylase to remove uracil base from a 2'-deoxyuridine residue. A potentially very useful chemical method was described by Rayner.[3] In this method, the abasic site is protected with a photolabile 2-nitrobenzyl group, (3) in Figure 1, during oligonucleotide synthesis and purification. The 2-nitrobenzyl group is then eliminated by photolysis to produce the abasic site. As always, there is the concern of thymine dimer formation during phototolysis. A quick literature check shows that several other methods[4, 5, 6, 7] have been used to generate abasic sites but in all cases to date the synthesis of the monomer is fairly challenging and, in our opinion, the subsequent chemistry to generate the abasic site is hardly routine.

UCLEOTIDES CONTAINING ABASIC SITES

Synthesis

A new chemical method has been described[8] which allows the generation of abasic sites in double and single stranded oligonucleotides using very mild specific conditions and with very low probability of side reactions. A protected 3-deoxyhexitol is used as the monomer, (4) in Figure 1. Following oligonucleotide synthesis under standard conditions, the silyl protecting groups of the residue, (1) in Figure 2, are removed with aqueous acid. (This can be done in conjunction with trityl removal in the last step of a DMT-on purification.) The diol, (2) in Figure 2, so formed is then treated with aqueous sodium periodate to form the aldehyde, (3) in Figure 2, plus formaldehyde. The aldehyde (3) then immediately cyclizes to its preferred structure, the abasic cyclic sugar (dR). The process is illustrated in Figure 2.

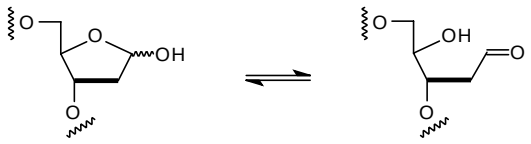
Oligonucleotide Stability

With the availability of oligonucleotides containing abasic sites, detailed stability information is now available.[8] The abasic site is stable almost indefinitely in 0.2M triethylammonium acetate buffer (pH6) at 5°C or less. However, the site is less stable at room temperature (half-life of around 30 days) and quite unstable at 55° (half-life of about 7 hours). Interestingly, the abasic site is completely degraded during evaporation to dryness.

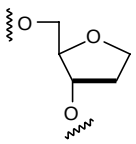
Structural Characteristics

Melting behavior of oligonucleotides containing the abasic site was examined[8] and it was found to behave like a complete mismatch opposite the 4 natural bases, with characteristics almost identical to those of dSpacer, which has been used extensively as a model abasic site. Other physical characteristics of oligonucleotides containing abasic sites have been examined, as well as their implication in DNA damage and repair.[6, 7, 9]

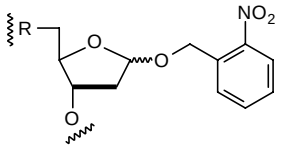
FIGURE 1: STRUCTURES OF PRODUCTS



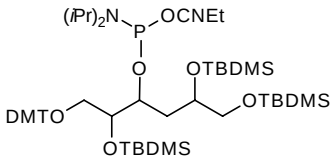
(1) Tautomers of Abasic Residue (dR)



(2) dSpacer

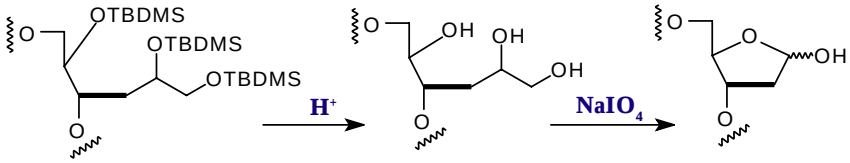


(3) Photolabile Residue



(4) Abasic Phosphoramidite

FIGURE 2: FORMATION OF ABASIC SITE WITHIN AN OLIGONUCLEOTIDE



(1) **(2)** **(3)**

References:

(1) M. Takeshita, C.N. Chang, F. Johnson, S. Will, and A.P. Grollman, *J. Biol. Chem.*, 1987, **262**, 10171-10179.

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(3) D. Péoc'h, A. Meyer, J.-L. Imbach, and B. Rayner, *Tetrahedron Lett.*, 1991, **32**, 207.

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(6) J.T. Hwang, K.A. Tallman, and M.M. Greenberg, *Nucleic Acids Res.*, 1999, **27**, 3805-10.

(7) M. Jourdan, J. Garcia, E. Defrancq, M. Kotera, and J. Lhomme, *Biochemistry*, 1999, **38**, 3985-95.

(8) I.G. Shishkina and F. Johnson, *Chem Res Toxicol*, 2000, **13**, 907-912.

(9) J. Lhomme, J.F. Constant, and M. Demeunynck, *Biopolymers*, 1999, **52**, 65-83.

ORDERING INFORMATION

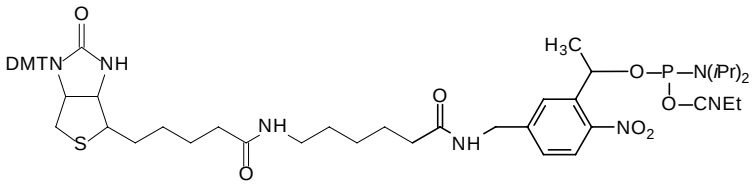
Item	Catalog No.	Pack	Price($)
Abasic Phosphoramidite	10-1924-95	50 µmole	105.00
	10-1924-90	100 µmole	210.00
	10-1924-02	0.25g	475.00

PC BIOTIN AND RELATED PHOTOCLEAVABLE MODIFIERS

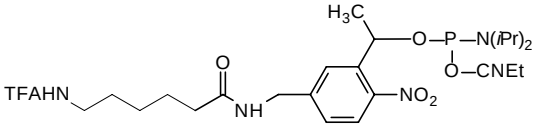
DNA researchers have long sought effective ways to capture oligonucleotides or PCR products from a crude mixture and then to release them in a pure, biologically active form. Now such a technique is available using PC Biotin and related photocleavable (PC) modifiers. After capturing biotin labelled DNA with streptavidin beads or attaching modified DNA to a surface, the DNA can be released into solution by simply illuminating with a hand-held UV light source.[1] Moreover, once freed from its tether the DNA is biologically active and ready for further action, e.g., direct ligation.

There have been other attempts in the past to break the association of the biotin – streptavidin complex. For example, by incorporating a disulfide linkage between the oligonucleotide and the biotin, the linkage can be cleaved later using dithiothreitol (DTT).[2] However, DTT is known to damage some enzymes and DNA – protein complexes, by reducing critical disulfide bonds in the protein.

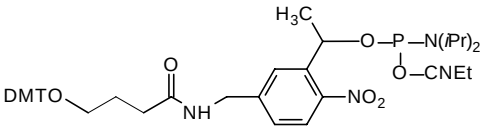
FIGURE 1: STRUCTURES OF PC PRODUCTS



(1) PC Biotin Phosphoramidite



(2) PC Amino-Modifier Phosphoramidite



(3) PC Spacer Phosphoramidite

PC Biotin Phosphoramidite, (1) in Figure 1, exhibits[3] similar properties to our popular 5'-biotin phosphoramidite:

1. It is fully compatible with all forms of DNA synthesis, cleavage and deprotection.
2. It contains a DMT group, which allows quantification of the coupling efficiency when removed on the synthesizer, or it can be used as a purification tag in the DMT-ON purification technique.
3. As an alternative to DMT-ON purification, simple capture of the PC Biotin oligo effects purification from failure sequences, which contain no biotin.
4. PC Biotin is rapidly and quantitatively cleaved from the 5'-terminus of the oligonucleotide using near-UV light at 300 – 350nm.
5. After photocleavage, a 5'-phosphate is generated on the DNA, rendering it suitable for further biological transformations, like gene construction and cloning after ligation.

Amino- and thiol-modified oligonucleotides have proved to be very useful for the attachment of a variety of haptens and fluorophores, as well as for the tethering of the oligonucleotides to a diversity of beads and surfaces. Clearly, the addition of a photocleavable linkage to these products would be desirable. PC Amino-Modifier[4] Phosphoramidite, (2) in Figure 1, brings the same versatility as PC Biotin to its field of endeavor. For the final word in versatility, PC Spacer Phosphoramidite, (3) in Figure 1, can be used as an intermediary to attach any modification reagent, available as a phosphoramidite, to the terminus of oligonucleotides. And, as always in the PC family, subsequent photocleavage is fast and efficient, providing DNA appropriate for further biological determination or transformation.[5]

The tiny levels of, for example, oligonucleotide probes on a DNA chip surface make further analysis very challenging. Of course, the PC family provides a convenient answer. MALDI-TOF mass spectrometry allows detailed analysis of miniscule amounts of organic molecules attached to surfaces. And separation based on molecular weight allows the detection of many analytes simultaneously. The photocleavable linker in the PC products is cleaved[6] during UV-MALDI analysis, opening the way for a variety of precise DNA-based assays. It is easy to envisage the rapid and precise analysis of multiple DNA samples in parallel, DNA and RNA sequence analysis, and detection of single nucleotide polymorphisms (SNPs), as examples. By using PC Amino-Modifier to prepare oligonucleotide-peptide conjugates, molecules containing DNA hybridization probe segment are combined with a peptide segment with a photocleavable linkage. UV-MALDI analysis reveals the result of the hybridization experiment along with the peptide fragments as photocleavable mass markers (PCMM).[7] By incorporating a photocleavable spacer at the 3'-terminus of an oligonucleotide undergoing synthesis, it would be possible to take

aliquots of the support during synthesis to examine progress directly using UV-MALDI analysis of the beads.

Another exciting possible use of this new family of reagents is to 'cage' oligonucleotides. Caging allows the biological activity of a species to be suppressed until released by an external agent - in this case a flash of UV light. This would give researchers exquisite spatial and temporal control of the concentration of the active species. For example, one of the new PC reagents could be used to tether an antisense oligo to a dextran which is too large to pass through the nuclear pores. Photocleavage would then be used to release the oligo, thereby decoupling antisense effects within the cytoplasm from antisense effects within the nucleus.

Glen Research is happy to offer these bright new products in association with AmberGen Inc. and Link Technologies, Ltd.

References:

(1) J. Olejnik, S. Sonar, E. Krzymanska-Olejnik, and K.J. Rothschild, *Proc Natl Acad Sci U S A*, 1995, **92**, 7590-4.
(2) G.A. Soukup, R.L. Cerny, and L.J. Maher, *Bioconjugate Chemistry*, 1995, **6**, 135-138.
(3) J. Olejnik, E. Krzymanska-Olejnik, and K.J. Rothschild, *Nucleic Acids Res*, 1996, **24**, 361-6.
(4) J. Olejnik, E. Krzymanska-Olejnik, and K.J. Rothschild, *Nucleic Acids Res*, 1998, **26**, 3572-3576.
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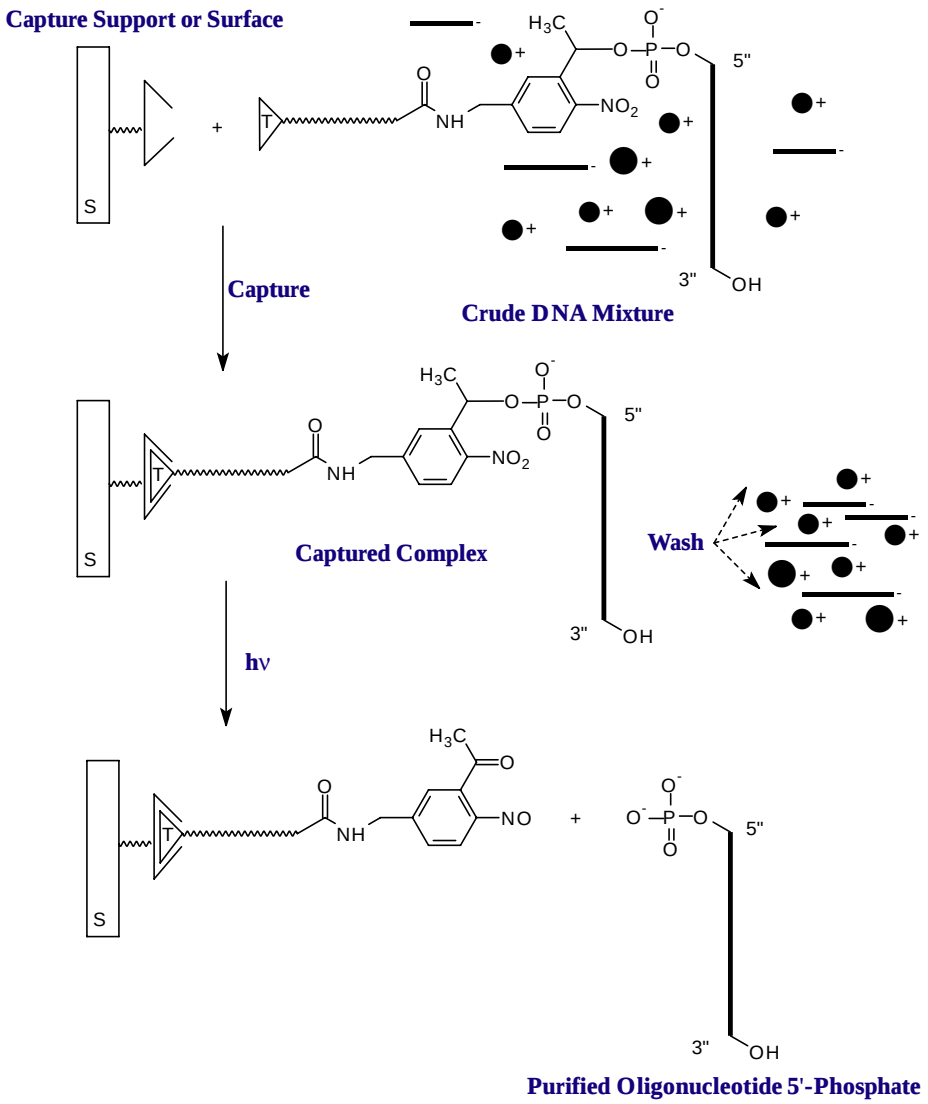
(6) S. Hahner, J. Olejnik, H.C. Ludemann, E. Krzymanska-Olejnik, F. Hillenkamp, and K.J. Rothschild, *Biomol Eng*, 1999, **16**, 127-133.
(7) J. Olejnik, H.C. Ludemann, E. Krzymanska-Olejnik, S. Berkenkamp, F. Hillenkamp, and K.J. Rothschild, *Nucleic Acids Res.*, 1999, **27**, 4626-4631.

FIGURE 2: PC CONJUGATES AND PHOTOCLEAVAGE



ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
PC Biotin Phosphoramidite	10-4950-95	50 µmole	135.00
	10-4950-90	100 µmole	250.00
	10-4950-02	0.25g	675.00
PC Amino-Modifier Phosphoramidite	10-4906-90	100 µmole	135.00
	10-4906-02	0.25g	395.00
PC Spacer Phosphoramidite	10-4913-90	100 µmole	135.00
	10-4913-02	0.25g	395.00

NEW OPeC™ REAGENTS FOR SYNTHESIS BY NATIVE LIGATION OF

Introduction

DNA-peptide conjugates have, in recent years, been identified as valuable tools in molecular biology. In particular, they have become increasingly important due to the identification of peptides as viable carriers for enhancing the cell delivery of oligonucleotides.[1]

Unfortunately, the hitherto cumbersome and inefficient methods of preparing DNA-peptide conjugates have hampered the systematic study of the sequence and structural requirements for good cell penetration. Total step-wise solid-phase synthesis on a single support has posed serious difficulties because of the incompatibilities in peptide and oligonucleotide protection and assembly chemistries. Increasingly, then, it has become clear that a post-assembly conjugation strategy offers the widest possibilities.

Awareness of this has led to the introduction of a new range of OPeC™ reagents for the synthesis of Oligonucleotide-Peptide Conjugates. These reagents have been developed by Link Technologies Ltd in Scotland and now Glen Research is pleased to be able to supply these products to our customers.

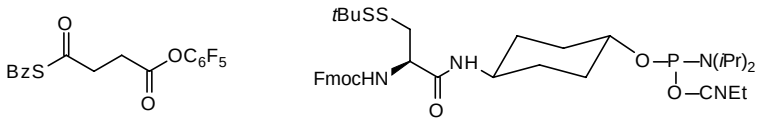
In OPeC™ conjugation strategies, peptide and oligonucleotide units are easily assembled separately on their own supports using conventional synthesizers and methodology. Each biomolecule is designed to carry a reactive functionality that is released upon full deprotection and cleavage from the support. The components can then be conjugated in aqueous/organic solution by selective reaction of these functionalities.

Advantages of OPeC™ over Existing Conjugation Methods

Prior to the emergence of OPeC™ technology, only a limited number of post-assembly conjugations had been reported. The range of methodologies used include the formation of a disulfide bond[2,3,4], reaction of a cysteine peptide with a maleimido oligonucleotide[5,6,7], and a bromoacetyl peptide with a thiol-functionalized oligonucleotide.[8] There are a number of important disadvantages of these methods however. For example, a disulfide bond is unstable to reducing agents that may be present under many assay conditions or within cells. The maleimide-thiol route requires a functionalization step on the oligonucleotide portion after release from the solid support. In addition, and perhaps most seriously of all, all three routes may sometimes lead to inefficient conjugation due to secondary structure or poor stability of certain peptide components in aqueous solution.

The "native ligation" strategy[9] of OPeC™ reagents has many attractions. First, the reagents used for the functionalization of the peptide and oligonucleotide components are stable, easy to handle solids. Second, the reagents are used in standard protocols of solid-phase synthesis and couple to their respective peptide or DNA/RNA partners in high yield. Third, assuming the peptide and oligonucleotide fragments are themselves prepared efficiently, the modified components may be used in the unpurified form for conjugation without the need for further manipulation following deprotection and release from their respective solid supports.

FIGURE 1: STRUCTURES OF OPeC™ REAGENTS



(1) Peptide Modifying Reagent (PMR)

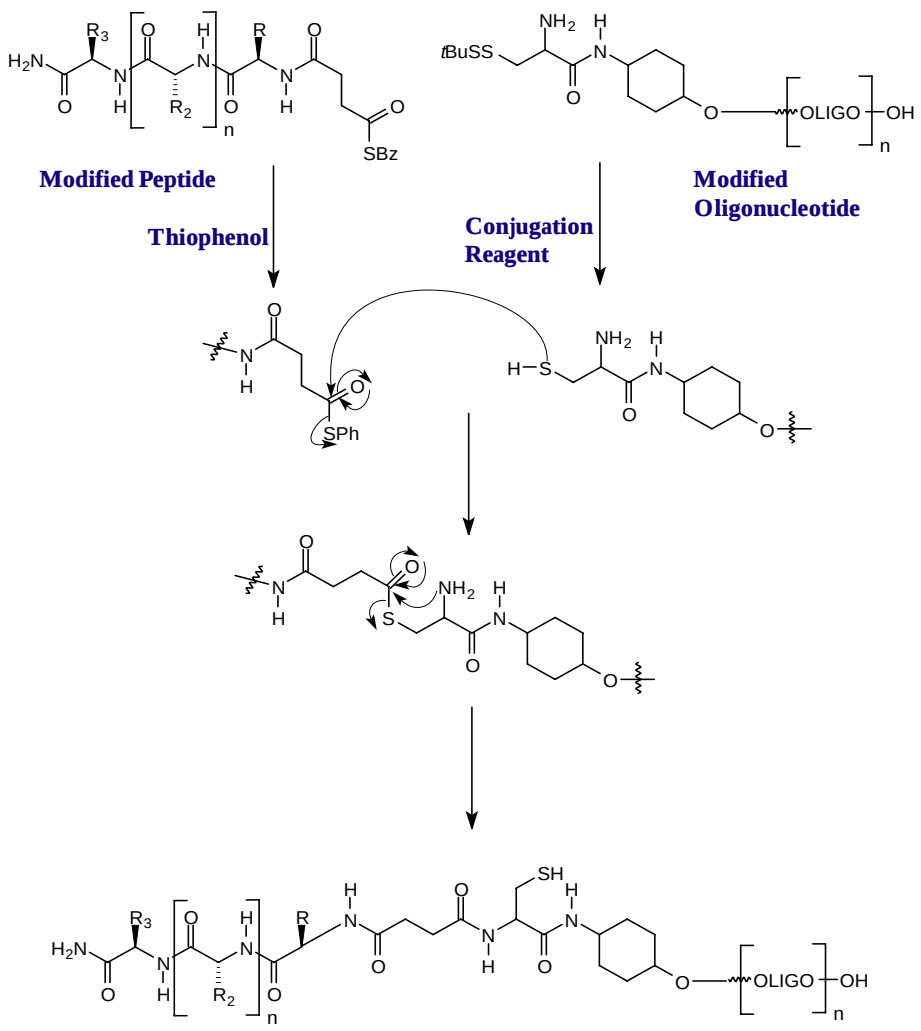
(2) Oligonucleotide Modifying Reagent (OMR)

The OPeC™ System

- *The method is based on the principle of template-free "native ligation"*
- *OPeC reagents are stable, easy-to-handle solids*
- *OPeC reagents are used seamlessly in normal DNA and peptide protocols using standard techniques*
- *All components of the OPeC system are conveniently provided in kit form*
- *OPeC modified oligonucleotides can, in most cases, be conjugated without further purification*
- *Conjugations are carried out under mild conditions*
- *The conjugation proceeds with both high yield and selectivity*
- *Conjugates can be purified easily using standard techniques*

OLIGONUCLEOTIDE-PEPTIDE CONJUGATES

SCHEME 1: OLIGONUCLEOTIDE-PEPTIDE CONJUGATION BY NATIVE LIGATION



Technical Description of the Conjugation Reaction

The OPeC™ method is based on the "native ligation" of an N-terminal thioester-functionalized peptide to a 5'-cysteinyl oligonucleotide. Pentafluorophenyl S-benzylthiosuccinate, Peptide Modifying Reagent (PMR), (1) in Figure 1, is used in the final coupling step in standard Fmoc-based solid-phase peptide assembly. Deprotection with trifluoroacetic acid generates, in solution, peptides substituted with an N-terminal S-benzylthiosuccinyl group (*Modified Peptide* in Scheme 1).

O-*trans*-4-(N-α-Fmoc-S-*tert*-butylsulfenyl-L-cysteinyl)amino-cyclohexyl O-2-cyanoethyl-N,N-diisopropylphosphoramidite, (2) Oligonucleotide Modifying Reagent (OMR), (2) in Figure 1, is used in the final coupling step in standard phosphoramidite solid-phase oligonucleotide assembly. Deprotection with aqueous ammonia solution generates in solution 5'-S-tert-butylsulfenyl-L-cysteinyl functionalized oligonucleotides (*Modified Oligonucleotide* in Scheme 1).

The thiobenzyl terminus of the *Modified Peptide* is converted to the thiophenyl analogue by the use of thiophenol, whilst the *Modified Oligonucleotide* is reduced using the tris(carboxyethyl)phosphine (*Conjugation Reagent* in Scheme 1). Coupling of these two intermediates, followed by the "native ligation" step, leads to formation of the *Oligonucleotide-Peptide Conjugate*.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Oligonucleotide-Peptide Conjugation Kit (OPeC™)	50-1000-01	each	495.00

Each OPeC kit consists of:

Oligo Modifying Reagent	150µmole
Peptide Modifying Reagent	450µmole
TCEP (Conjugation Reagent)	230mg

References:

(1) For a review of this area see: C.-H. Tung and S. Stein, *Bioconjugate Chem.*, 2000, **11**, 605-618.
(2) E. Vives and B. Lebleu, *Tetrahedron Lett.*, 1997, **38**, 1183-1186.
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(4) J.P. Bongartz, A.M. Aubertin, P.G. Milhaud, and B. Lebleu, *Nucleic. Acids Res.*, 1994, **22**, 4681-4688.
(5) C.-H. Tung, M.J. Rudolph, and S. Stein, *Bioconjugate Chem.*, 1991, **2**, 461-465.
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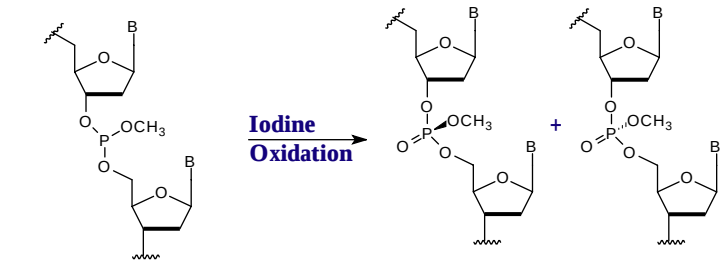
(8) K. Arar, A.-M. Aubertin, A.-C. Roche, M. Monsigny, and M. Mayer, *Bioconjugate Chem.*, 1995, **6**, 573-577.
(9) For an example of the use of the native ligation technique see: D.A. Stetsenko and M.J. Gait, *J. Organic Chem.*, 2000, **65**, 4900-4908.

PRODUCT UPDATE - HOW ARE METHYL TRIESTER LINKAGES PREPARED?

For many years, Glen Research has supplied methyl phosphoramidites in addition to ß-cyanoethyl (CE) phosphoramidites for the few situations where the more labile cyanoethyl group is not an advantage. Some of our customers, probably remembering that the methyl group was removed specifically with thiophenol, have tried to use these monomers to prepare the interesting, uncharged, and nuclease-resistant methyl phosphotriester linkage. Unfortunately, this linkage is labile to ammonium hydroxide and the regular phosphodiester linkage is formed (along with a small amount of chain scission).

Now we offer UltraMild methyl phosphoramidites! Oligos produced from these can be deprotected with potassium carbonate in methanol to produce methyl phosphotriester linkages. Since these linkages are diastereomeric and uncharged, the oligos may be hard to handle. Instead, it is likely that chimeras will be produced using these monomers along with the regular UltraMild CE phosphoramidites.

FIGURE 1: STRUCTURE OF METHYL PHOSPHOTRIESTER LINKAGES



Phosphite Triester

Phosphate Triester (Phosphotriester) Diastereomers

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Pac-dA-Me Phosphoramidite	10-1301-02	0.25g	25.00
	10-1301-05	0.5g	50.00
	10-1301-10	1.0g	100.00
Ac-dC-Me Phosphoramidite	10-1315-02	0.25g	25.00
	10-1315-05	0.5g	50.00
	10-1315-10	1.0g	100.00
iPr-Pac-dG-Me Phosphoramidite	10-1321-02	0.25g	25.00
	10-1321-05	0.5g	50.00
	10-1321-10	1.0g	100.00

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