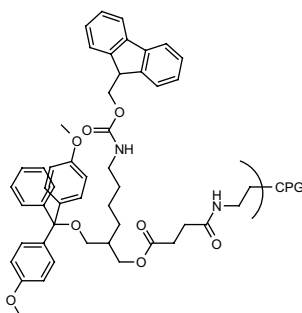


# Phthalimidyl Protected 3' Amine Modifying Reagent Enhances Yield of Conjugated Product

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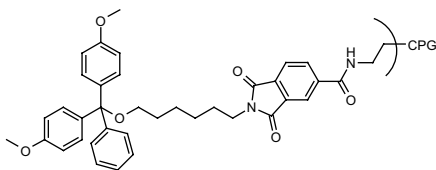
The reagent most commonly used to modify the 3' terminus of an oligonucleotide is the 3' Amino Modifier C-7 available through several distributors (Figure 1). It is based on a branched alkyl amine with two hydroxyl groups, one for linkage to a solid support and the other for coupling with a nucleoside phosphoramidite. Unfortunately, it suffers one serious drawback; the flourenylmethoxycarbonyl (Fmoc) group protecting the primary amine is unstable making the amine susceptible to irreversible acetylation during the capping step of the DNA synthesis cycle.



**Figure 1:** Structure of 3'-Amino Modifier C-7 CPG

An interesting alternative, which placed the amine within the actual link to the solid support using a base labile phthalimide (Figure 2), was published several years ago (Petrie, *et al.* 1992). However, after the initial report, this innovative reagent was not seen in the literature again.

Always seeking new ways to make higher quality modified oligonucleotides, we synthesized the phthalimide protected amino support and compared it to the 3' Amino Modifier C-7 support. The Phthalimidyl-3'-Amino-CPG was synthesized by TriLink's organic chemistry group according to the reported procedure with slight modification. The 3' Amino Modifier C-7 CPG and the Phthalimidyl-3'-Amino-CPG are available through Glen Research, Sterling, VA.



**Figure 2:** Structure of Phthalimide 3'-Amino Modifier C-6 CPG

The two types of 3' amino labeling support (1  $\mu$ mole each) were loaded into synthesis columns and a 20mer phosphodiester oligonucleotide, 5'-GTC-ATC-TGA-TAG-CAC-GTC-GA-(Linker)-NH<sub>2</sub>-3', was prepared using an Expedite 8909 and standard conditions. Three columns of each support were synthesized. The oligonucleotides were deprotected by overnight treatment at 55°C with 1.5 ml of fresh concentrated aqueous ammonium hydroxide in 4 ml capped vials. After deprotection the oligonucleotides were

decanted, the beads rinsed, and the combined solutions dried. The samples were ethanol precipitated and aliquots containing 30 OD<sub>260</sub> units of each of the six samples were placed into separate 1.5 ml microtubes and dried in preparation for conjugation.

Each of the samples was conjugated to DABCYL succinimidyl ester for 6 hours under identical conditions. The mixtures were then run through a 10 ml G-25 column using dH<sub>2</sub>O as the eluent to remove excess dye. The conjugated oligonucleotides were analyzed by reverse-phase (RP) HPLC.

The results of the conjugations are shown in Table 1 below. The HPLC chromatograms shown in Figures 3 and 4 are typical of the set and clearly illustrate the difference in product quality between the two supports.

In order to substantiate the hypothesis that acetylation of the amine functionality is the root cause of the lower conjugation efficiency, an additional 1  $\mu$ mole scale column

	Synthesis	Crude Yield (in OD <sub>260</sub> units)	Percent Conjugated
<b>Phthalimidyl-CPG</b>	1a	90	83%
	1b	91	82%
	1c	93	82%
<b>C-7 3'-Amino-Modifier</b>	2a	103	65%
	2b	109	68%
	2c	111	67%

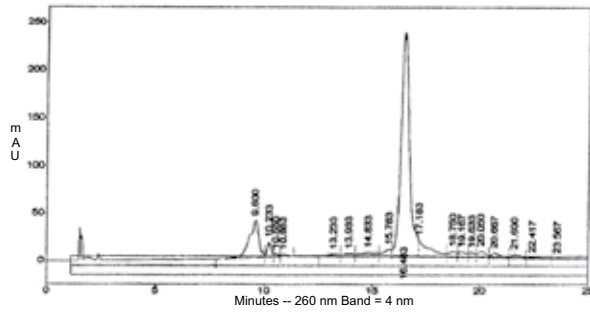
**Table 1:** DABCYL conjugation results

of the same sequence as above was synthesized with each support, with the dimethoxytrityls left on. These oligonucleotides were purified by RP-HPLC on a Waters  $\mu$ Bondapak C-18 column (8x100 mm) using 0.05 M triethylammonium acetate (pH 7.2) and a gradient of acetonitrile. After pooling and drying the fractions containing the entire DMT-on product, the trityl protecting groups were removed and the oligonucleotide dried, and then repurified using the same RP-HPLC conditions. The oligonucleotides were then analyzed by mass spectroscopy (see Figures 5 and 6). The product isolated from the Amino Modifier C-7 support contains an additional major peak (~20%) at +42 au (6368). This corresponds to the mass of an acetyl capping group.

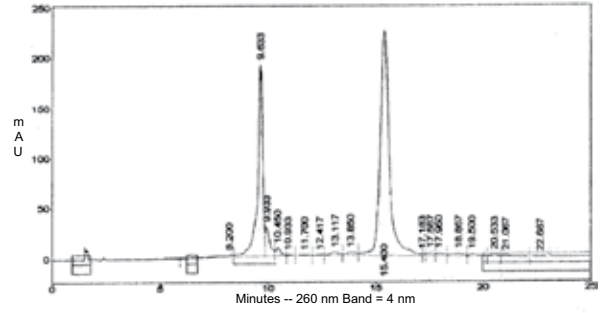
In summation, the Phthalimide-3'Amino-CPG is superior to the 3' Amino Modifier C-7 for most applications. By its very nature, the conjugation efficiency is not susceptible to lot-to-lot variations or to synthesis issues. It is therefore a more reliable reagent for use in high throughput and microspotting applications where reproducibility and high conjugation efficiencies are very desirable. As a less expensive reagent with superior properties, it is the obvious choice for 3' amino labeling of oligonucleotides.

## References

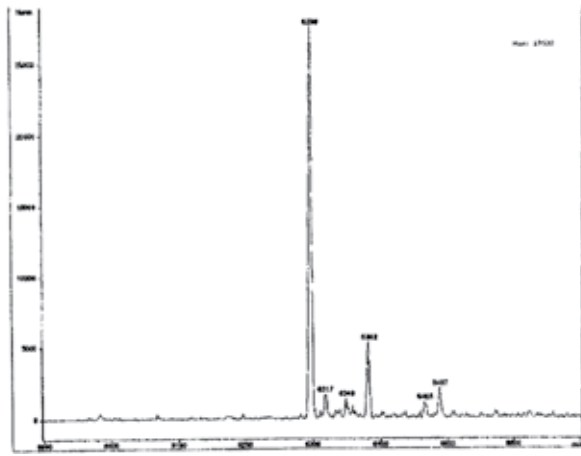
- Petrie, Charles R., Reed, Michael W., Adams, David A., Meyer, Rich B. Jr., An Improved CPG Support for the Synthesis of 3' -Amine-Tailed Oligonucleotides, 1992, Bioconjugate Chem., Volume 3, 85-87.
- Reed, Michael W., Meyer, Jr., Rich B., Petrie, Charles R., Tabone, John C. U.S. Patent 5,419,966. 1995.



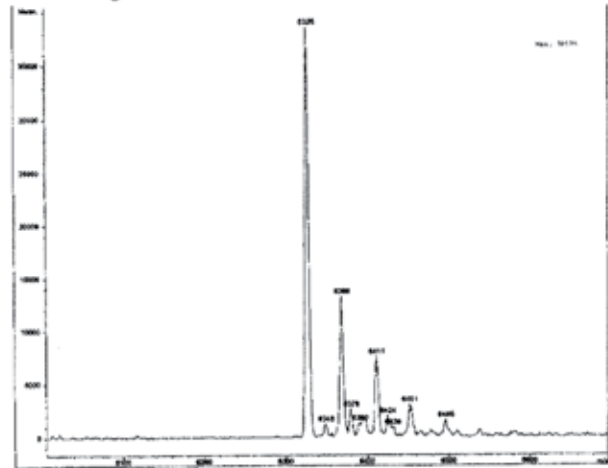
**Figure 3:** RP-HPLC chromatogram of crude conjugation mixture with Phthalimidyl 3'-Amino support. Conjugation product run at ~16.5 mins.



**Figure 4:** RP-HPLC chromatogram of crude conjugation mixture with 3'-Amino Modifier C7 support. Conjugation product run at ~15.4 mins.



**Figure 5:** Electrospray mass spectroscopy analysis of HPLC purified oligonucleotide synthesized off of phthalimide-3' CPG.



**Figure 6:** Electrospray mass spectroscopy analysis of HPLC purified oligonucleotide synthesized off of 3'-Amino Modifier C-7 CPG.