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PC BIOTIN AND RELATED PHOTOCLEAVABLE MODIFIERS

Introduction

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.

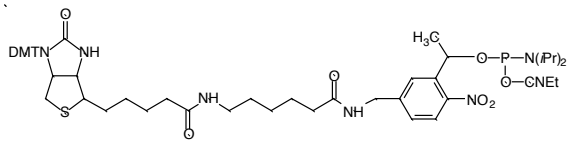
Photocleavable Modifiers

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

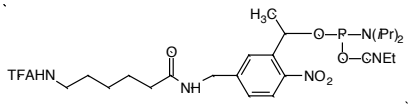
1. It is fully compatible with all forms of DNA synthesis, cleavage and deprotection.
2. It contains a DMT group, which allows quantification of the coupling efficiency when removed on the synthesizer, or it can be used as a purification tag in the DMT-ON purification technique.
3. As an alternative 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

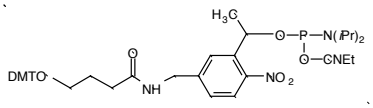
FIGURE 1: **STRUCTURES OF PC PRODUCTS**



(1) PC Biotin Phosphoramidite



(2) PC Amino-Modifier Phosphoramidite



(3) PC Spacer Phosphoramidite

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]

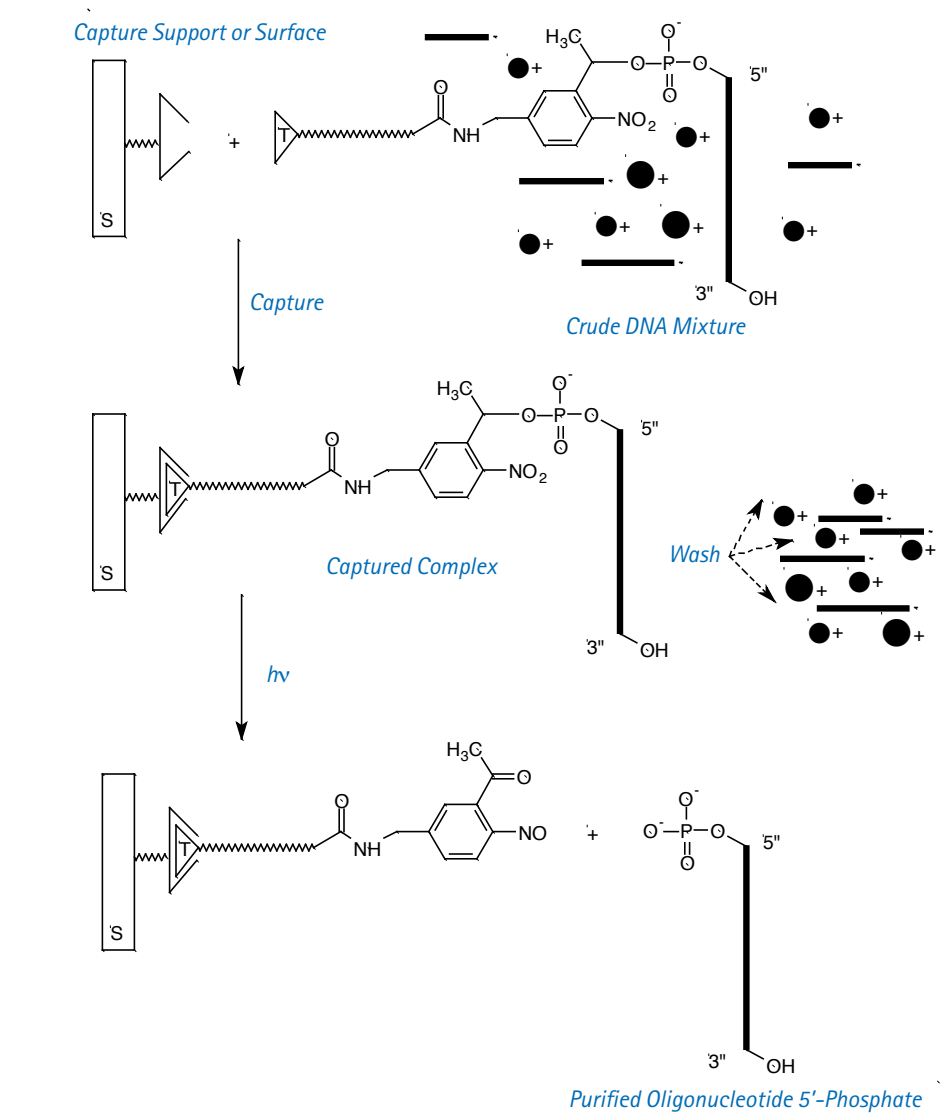
The tiny levels of, for example, oligonucleotide probes on a DNA chip surface make further analysis very challenging. Of course, the PC family provides a convenient answer. MALDI-TOF mass spectrometry allows detailed analysis of miniscule amounts of organic molecules attached to surfaces. And separation based on molecular weight allows the detection of many analytes simultaneously.

The photo-cleavable 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 offers PC Biotin, PC Amino-Modifier and PC Spacer products in association with AmberGen, Inc. and Link Technologies, Ltd. For a commercial application license, please contact AmberGen, Inc., +617-975-0680, http://www.ambergen.com/.

FIGURE 2: **PC CONJUGATES AND PHOTOCLEAVAGE**



References:

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(4) J. Olejnik, E. Krzymanska-Olejnik, and K.J. Rothschild, *Nucleic Acids Res*, 1998, **26**, 3572-3576.

(5) J. Olejnik, E. Krzymanska-Olejnik, and K.J. Rothschild, *Methods Enzymol*, 1998, **291**, 135-54.

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