Nucleic Acids Research, 1996, Vol. 24, No. 2 361–366

# Photocleavable biotin phosphoramidite for 5′-end-labeling, affinity purification and phosphorylation of synthetic oligonucleotides

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Received August 17, 1995; Revised and Accepted November 20, 1995

## ABSTRACT

**We report the design, synthesis and evaluation of a non-nucleosidic photocleavable biotin phosphoramidite (PCB-phosphoramidite) which provides a simple method for purification and phosphorylation of oligonucleotides. This reagent introduces a photocleavable biotin label (PCB) on the 5′-terminal phosphate of synthetic oligonucleotides and is fully compatible with automated solid support synthesis. HPLC analysis shows that the PCB moiety is introduced predominantly on full-length sequences and is retained during cleavage of the synthetic oligonucleotide from the solid support and during subsequent deprotection with ammonia. The full-length 5′-PCB-labeled oligonucleotide can then be selectively isolated from the crude oligonucleotide mixture by incubation with immobilized streptavidin. Upon irradiation with 300–350 nm light the 5′-PCB moiety is cleaved with high efficiency in <4 min, resulting in rapid release of affinity-purified 5′-phosphorylated oligonucleotides into solution. 5′-PCB-labeled oligonucleotides should be useful in a variety of applications in molecular biology, including cassette mutagenesis and PCR. As an example, PCB-phosphoramidite has been used for the synthesis, purification and phosphorylation of 50- and 60mer oligonucleotides.**

## INTRODUCTION

Biotin is widely used for non-radioactive DNA/RNA detection (1) due to the extremely high affinity of the biotin–streptavidin interaction (association constant $10^{15}$/M) (2). A biotin moiety can be introduced into the oligonucleotide: (i) during its solid support synthesis using biotinyl phosphoramidite (3–7); (ii) enzymatically with biotinylated nucleoside triphosphate analogs, such as biotin-11-dUTP (8); (iii) through post-synthetic modifications with suitable biotinylation reagents such as biotin *N*-hydroxysuccinimide ester, biotin hydrazide (1,9) or photobiotin (10). If this biotinylation could be reversed, thereby releasing the oligonucleotide from the streptavidin–biotin complex, biotinylation would provide a much more general approach in molecular biology. For example, oligonucleotides could be isolated using streptavidin affinity media and then released for subsequent use in recombinant DNA methods. A particularly useful application is the isolation of full-length oligonucleotides from failure sequences that result during automated solid support synthesis. A second application is the isolation of PCR products resulting from incorporation of biotinylated primers.

The most common approach for the removal of biotinyl moieties is the introduction of a chemically cleavable spacer arm. For example, a biotinylated nucleoside triphosphate analog containing a disulfide bond has been synthesized (11) and used in a variety of applications (12–14). Alternatively, a biotin reagent with a cleavable spacer arm can be used to label 5′-amino-modified oligonucleotides (15). However, release of the oligonucleotide requires a reducing agent, such as dithiotreitol (DTT), which can damage enzymes and DNA–protein complexes and also leaves a modified base which can interfere with subsequent use of the oligonucleotide. Nucleosidic phosphoramidites containing an acid-cleavable spacer arm on the 5′-end have also been described (16,17). However, these require long cleavage times (>3 h) under acidic conditions (80% acetic acid).

We report here the synthesis of a photocleavable biotin phosphoramidite (PCB-phosphoramidite). This phosphoramidite incorporates a recently described photocleavable biotin moiety (PCB) (18) on the 5′-end of a synthetic oligonucleotide. We show that: (i) the PCB-phosphoramidite reagent is fully compatible with automated DNA/RNA synthesizers using phosphoramidite chemistry; (ii) the 5′-PCB moiety is retained during cleavage from the solid support and deprotection of the oligonucleotide with ammonia; (iii) the 5′-PCB moiety allows streptavidin affinity purification of the oligonucleotide from failure sequences; (iv) the PCB moiety is rapidly and quantitatively photocleaved from the 5′-end upon irradiation with near-UV light (300–350 nm) to give a 5′-phosphorylated oligonucleotide. Importantly, PCB-phosphoramidite provides a rapid method for the purification, isolation and phosphorylation of synthetic oligonucleotides. As an example, we have used PCB-

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phosphoramidite to synthesize, purify and phosphorylate 50- and 60mer oligonucleotides.

## MATERIALS AND METHODS

All chemicals used in the synthesis were purchased from Aldrich Chemical Co. (Milwaukee, WI). $^1$H NMR spectra were recorded in $CDCl_3$ on a Varian (Palo Alto, CA) Unity Plus spectrometer at 400 MHz with chemical shifts (δ, p.p.m.) reported relative to a tetramethylsilane internal standard. $^{31}$P NMR spectra were recorded in $CDCl_3$ on a JEOL (Peabody, MA) JNM-GSX270 spectrometer at 109.36 MHz with chemical shifts (δ, p.p.m.) reported relative to an 85% $H_3PO_4$ external standard. Oligonucleotide synthesis was performed on an Applied Biosystems (Foster City, CA) DNA/RNA synthesizer model 392. Samples were irradiated with a Blak Ray XX-15 UV lamp (Ultraviolet Products Inc., San Gabriel, CA) at a distance of 15 cm (emission peak 365 nm, 300 nm cut-off, 1.1 mW intensity at 31 cm). UV-visible spectra were recorded on a Shimadzu 2101PC spectrophotometer. HPLC analysis was performed on a Waters (Milford, MA) system consisting of a U6K injector, 600 Controller, Novapak C18 (3.9 × 150 mm) column and a 996 photodiode array detector. Buffer A, 0.1 N triethylamine acetate, pH 6.0; buffer B, acetonitrile. Elution was performed using a linear gradient (8–45%) of buffer B in buffer A over 45 min at a flow rate of 1 ml/min. Preparative purification of 5′-PCB-$(dT)_7$ was achieved on a Waters Novapak C18 RCM cartridge (8× 100 mm) using conditions as specified above, except for flow rate, which was increased to 2 ml/min. Fractions were then analyzed, pooled and freeze dried. No special precautions were necessary to protect the reagent and the 5′-PCB-labeled oligonucleotides from light.

### PCB-phosphoramidite synthesis

*1-N-(4,4′-dimethoxytrityl)-5-(6-biotinamidocaproamidomethyl)-2-nitroacetophenone (compound **2**).* 5-(6-Biotinamidocaproamidomethyl)-2-nitroacetophenone (**1**) (18) (0.5 g, 0.94 mmol) was dried by co-evaporation with anhydrous pyridine (3 × 2 ml) and then dissolved in 5 ml of the latter. To this solution was added 4,4′-dimethoxytrityl chloride (DMTr-Cl) (0.634 g, 1.87 mmol) followed by 4-dimethylaminopyridine (0.006 g, 0.046 mmol). The reaction mixture was stirred at room temperature for 5 h and then an additional 0.317 g DMTr-Cl was added. After 24 h the reaction was quenched with methanol (1 ml), poured into 100 ml 0.1 M sodium bicarbonate and extracted with methylene chloride (3 × 50 ml). Evaporation of the combined extracts gave a yellow oil, which was further purified on a silica gel column using a step gradient of methanol in dichloromethane, 0.2% triethylamine. Appropriate fractions were pooled and evaporated to give compound **2** as a white foam (0.73 g, 93% yield). TLC, $CHCl_3$:MeOH 9:1 v/v; $R_f$ = 0.45. $^1$H NMR: 7.87–7.85 (d,2H), 7.23–7.20 (m,5H), 7.17–7.15 (d,1H), 7.11–7.05 (m,5H), 6.75–6.71 (m,4H), 5.92–5.86 (t,1H), 5.61 (s,1H), 4.43–4.36 (m,2H), 4.05–3.85 (m,2H), 3.73 (s,6H), 3.70 (s,3H), 3.40–3.30 (m,1H), 3.12–3.02 (m,2H), 2.98–2.89 (m,1H), 2.48 (s,1H), 2.32–2.20 (m,2H), 2.11–2.03 (m,4H), 1.63 (s,3H), 1.60–1.34 (m,7H), 1.26–1.23 (m,2H). Elemental analysis (%): calculated ($C_{46}H_{53}N_5O_8S$), C 66.09, H 6.39, N 8.38; found, C 65.85, H 6.23, N 8.05.

*1-N-(4,4′-dimethoxytrityl)-5-(6-biotinamidocaproamidomethyl)-1-(2-nitrophenyl)-ethanol (compound **3**).* 1-*N*-(4,4′-Dimethoxytrityl)-5-(6-biotinamidocaproamidomethyl)-2-nitroacetophenone (**2**) (0.85 g, 1.016 mmol) was dissolved in 7 ml ethanol and sodium borohydride (0.028 g, 0.74 mmol) was added with stirring. After 1 h the reaction was quenched with 4 ml acetone and evaporated under reduced pressure to give a yellow oil, which was redissolved in 10 ml methanol and the solution added to 120 ml water. The precipitate was isolated by centrifugation (7000 r.p.m., 45 min) and dried *in vacuo* over KOH to give compound **3** (0.7 g, 82%). TLC, $CHCl_3$:MeOH 9:1 v/v; $R_f$ = 0.39. $^1$H NMR: 7.83–7.74 (m,2H), 7.32 (t,1H), 7.24–7.17 (m,5H), 7.13–7.00 (m,4H), 6.98 (d,1H), 6.73–6.69 (m,4H), 5.99–5.91 (d,1H), 5.86–5.78 (m,1H) (OH), 5.49–5.45 (m,1H), 4.50–4.24 (m,4H), 3.74 (s,6H), 3.58–3.25 (m,1H), 3.07–3.01 (m,1H), 3.00–2.85 (m,1H), 2.29–2.12 (m,2H), 2.01–1.96 (m,2H), 1.80–1.75 (m,1H), 1.64 (s,3H), 1.53–1.43 (m,12H), 1.38–1.11 (m,1H). Elemental analysis (%): calculated ($C_{46}H_{55}N_5O_8S$), C 65.93, H 6.62, N 8.36; found, C 65.97, H 6.52, N 8.10.

*[1-N-(4,4′-dimethoxytrityl)-5-(6-biotinamidocaproamidomethyl)-1-(2-nitrophenyl)-ethyl]-2-cyanoethyl-N,N-diisopropylaminophosphoramidite (compound **4**).* 1-*N*-(4,4′-Dime t h o x y trityl)-5-(6-biotinamidocaproamidomethyl)-1-(2-nitrophenyl) ethanol (**3**) (0.186 g, 0.22 mmol) was placed in an oven-dried flask with a magnetic stirring bar, sealed with a septum and dried for at least 6 h *in vacuo*. Anhydrous acetonitrile (0.003% water) (1 ml) was added through a septum under argon. Subsequently *N*,*N*-diisopropylethylamine (0.15 ml, 0.88 mmol) was added, followed by 2-cyanoethoxy-*N*,*N*-diisopropylchlorophosphine (0.052 g, 0.22 mmol). After 1 h another 0.5 eq. phosphine was added. After an additional 2 h at room temperature the reaction mixture was treated with 0.3 ml ethyl acetate, followed by a saturated saline solution (10 ml), and extracted with methylene chloride (3 × 10 ml). The organic layer was washed with water, dried over sodium sulfate and evaporated under reduced pressure and purified on a silica gel column using a step gradient (0–3%) of triethylamine in acetonitrile. Appropriate fractions were pooled and evaporated to give compound **4** as a white foam (0.144 g, 62% yield). TLC, MeCN:$Et_3N$, 95:5 v/v; $R_f$ = 0.48. $^1$H NMR (p.p.m.): 7.79–7.32 (m,1H), 7.65–7.61 (m,1H), 7.25–7.19 (m,5H), 7.13–7.05 (m,4H), 6.91–6.85 (m,1H), 6.75–6.73 (m,4H), 5.75–5.66 (br s,1H), 5.54–5.43 (m,1H), 5.22s, 5.12d (1H), 4.38–4.26 (m,3H), 4.23–4.11 (m,2H), 3.88–3.77 (m,1H), 3.73 (s,1H), 3.66–3.54 (m,2H), 3.46–3.37 (m,1H), 3.29–3.21 (m,1H), 3.10–3.02 (m,2H), 2.65–2.60 (m,1H), 2.54–2.44 (m,1H), 2.40–2.32 (m,1H), 2.26–2.20 (dd,1H), 2.10–2.11 (app. t,1H), 2.08–2.01 (m,2H), 1.62–1.58 (m,6H), 1.55–1.45 (m,4H), 1.39–1.35 (t,2H), 1.31–1.27 (t,2H), 1.16–1.07 (m,9H), 0.87–0.83 (dd,3H). $^{31}$P NMR (p.p.m.): 146.7, 147.9. Elemental analysis (%): calculated ($C_{55}H_{72}N_7O_9PS$), C 63.63, H 6.99, N 9.44; found, C 63.11, H 6.79, N 9.20.

### 5′-PCB-oligonucleotide synthesis

A 0.1 M solution of the PCB-phosphoramidite (**4**) in anhydrous acetonitrile was attached to the extra port of the Applied Biosystems 392 DNA/RNA synthesizer. The syntheses were carried out on a 0.2 µmol scale using cyanoethyl phosphoramidites. For the last coupling (introduction of **4**) the coupling time was increased by 120 s, as recommended for conventional biotin phosphoramidite (19). Typical coupling efficiency (as deter-

mined by trityl cation conductance) was between 95 and 97%. Standard detritylation ('trityl-off' option) as well as cleavage and deprotection procedures were used. Control 5′-phosphorylated sequences were synthesized using chemical phosphorylation reagent Phosphalink™ (Applied Biosystems) according to the manufacturer's instructions (20).

### Affinity purification and photocleavage

Crude 5′-PCB-oligonucleotide (16 nmol) was added to a suspension of streptavidin–agarose beads (700 µl, 24 nmol) (Sigma, Milwaukee, WI) and the suspension incubated at room temperature for 1 h. It was then spin-filtered (5 min, 5000 r.p.m.) using a 0.22 µm Ultrafree MC filter (Millipore, Bedford, MA). Beads on the filter were washed with 100 µl phosphate buffer, pH 7.2, and spin-filtered (three times). Finally, the beads were resuspended in 700 µl phosphate buffer and irradiated for 5 min. After irradiation the suspension was spin-filtered, the beads were washed with phosphate buffer (3 × 100 µl) and the combined filtrate volume was adjusted to 1 ml and analyzed by UV absorption spectroscopy or HPLC.

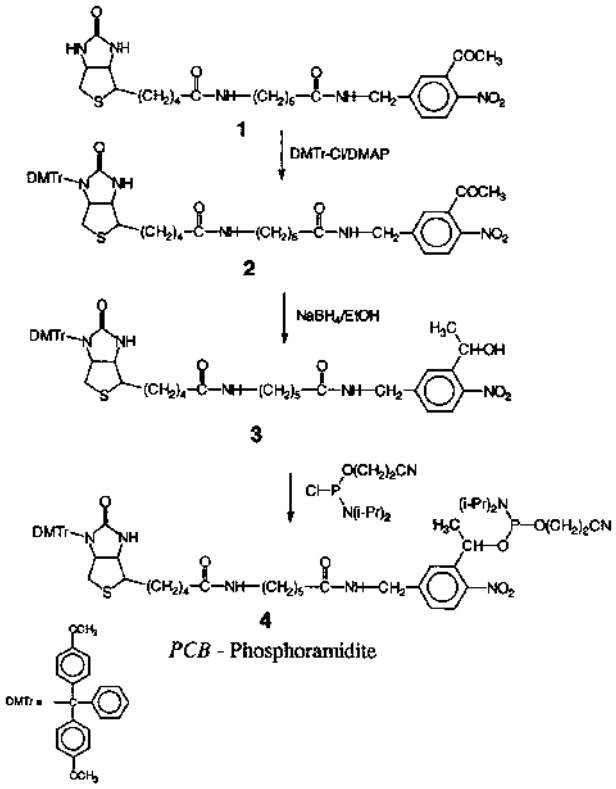
### Time dependence of the photocleavage

In order to calculate the time dependence of the photocleavage, HPLC-purified 5′-PCB-$(dT)_7$ (48 nmol) was incubated with 1.5 eq. streptavidin–agarose beads for 1 h. The beads were spin-filtered, washed, resuspended in phosphate buffer, pH 7.2, and irradiated. Aliquots (200 µl each) were withdrawn after 0, 0.25, 0.5, 1, 2, 4, 6 and 10 min irradiation, spin-filtered and washed as described above. The filtrate volume was adjusted to 1 ml and the absorbance at 260 nm measured. A sample of 700 µl streptavidin which had not been incubated with oligonucleotide was spin-filtered and the UV absorption measured, serving as background. A similar measurement was made on a sample of oligonucleotide not incubated with streptavidin (16 nmol, 700 µl phosphate buffer, serving as 100% control). The molar extinction coefficient at 260 nm for the PCB moiety was determined separately (4700/M/cm) and this value subtracted from the estimated (assuming a molar extinction coefficient equal to 12 000 for each dT) molar extinction coefficient of 5′-PCB-$(dT)_7$ (88 700) for photorelease efficiency calculations. In order to determine the time course of photocleavage in solution $(dT)_7$-5′-PCB (1 $OD_{260}$) was dissolved in 1 ml phosphate buffer and irradiated at 300–350 nm. Aliquots (10 µl) were withdrawn after 0, 0.25, 0.5, 1, 2, 4, 6 and 10 min irradiation and injected onto an HPLC column. The percent conversion was calculated from the ratio of the area of the particular peak (i.e. 5′-PCB-$(dT)_7$ or 5′-p-$(dT)_7$) over the sum of the areas of the component peaks, with molar extinction coefficients of the components adjusted as described above.

## RESULTS

### Design and synthesis of PCB-phosphoramidite

The synthesis of PCB-phosphoramidite (**4**) is depicted in Scheme 1 (see Materials and Methods for more details). The compound consists of a protected biotin moiety linked through a spacer arm (6-aminocaproic acid) to a photoreactive 1-(2-nitrophenyl)ethyl moiety (21), which is derivatized with *N,N*′-diisopropyl-2-cyanoethyl-phosphoramidite. The starting material, 5-(6-biotinamidocaproamidomethyl)-2-nitroacetophenone (**1**) was synthesized as



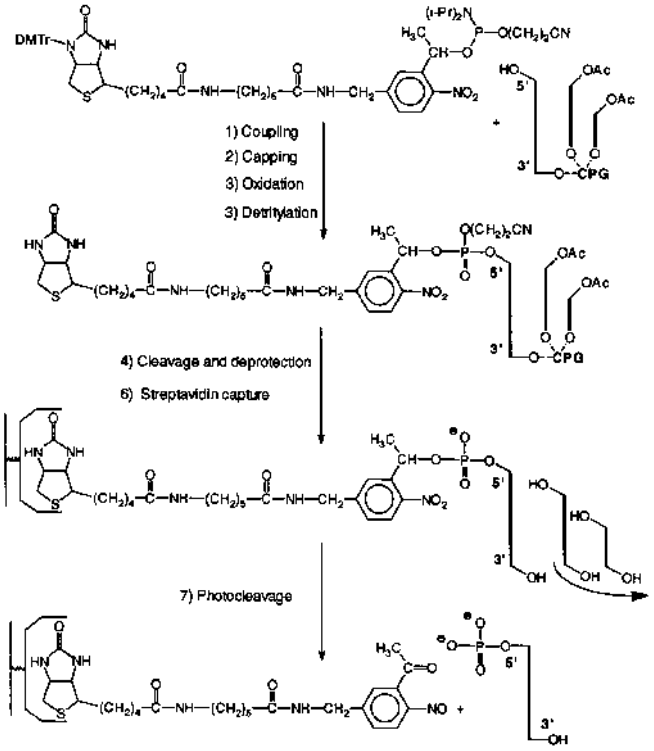

**Scheme 1.**

described previously (18). The 4,4′-dimethoxytrityl (DMTr) group was introduced selectively onto the N1 nitrogen of biotin (3,6). The intermediate (**2**) was then selectively reduced using sodium borohydride to give compound **3** and, finally, the resulting hydroxyl group was phosphitylated using 1.5 eq. 2-cyanoethoxy-*N,N*-diisopropylchlorophosphine. No phosphitylation of biotin nitrogen N2 was observed under the reaction conditions.

PCB-phosphoramidite (**4**) was designed for direct use in any automated DNA/RNA synthesizer employing standard phosphoramidite chemistry. As shown in Scheme 2, the selective reaction of compound **4** with the free 5′-OH group of a full-length oligonucleotide results in the introduction of a phosphodiester group linked to a photocleavable biotin moiety. In contrast, all capped failure sequences which lack a free 5′-OH group do not react with the PCB-phosphoramidite. The biotinyl moiety thus allows selective isolation of only full-length sequences through streptavidin affinity media. Upon irradiation with near-UV light the phosphodiester bond between the PCB moiety and the phosphate is cleaved, resulting in the formation of a 5′-monophosphate on the released oligonucleotide. The 1-(2-nitrophenyl)ethyl moiety is converted into a 2-nitrosoacetophenone derivative.

### Synthesis and evaluation of PCB-oligonucleotides

The heptamer 5′-PCB-$(dT)_7$ was assembled using PCB-phosphoramidite (**4**) in an automated DNA/RNA synthesizer. The unmodified sequence 5′-OH-$(dT)_7$ and a 5′-phosphorylated sequence, 5′-p-$(dT)_7$, were prepared using standard procedures (see Materials and Methods). Figure 1 shows the HPLC trace of




**Scheme 2.**

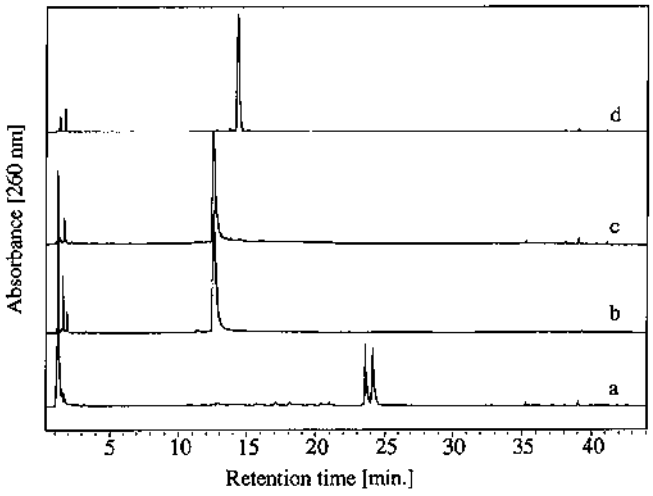



**Figure 1.** HPLC traces of (**a**) 5′-PCB-$(dT)_7$, (**b**) 5′-PCB-$(dT)_7$ complexed with streptavidin after 4 min irradiation, (**c**) 5′-p-$(dT)_7$ and (**d**) 5′-OH-$(dT)_7$. See Materials and Methods for more details.

5′-PCB-$(dT)_7$ (trace a). Two main peaks are observed in this trace, with retention times of 23.7 and 24.3 min. These two peaks can be attributed to the two diastereoisomers generated by introduction of the PCB moiety onto the 5′-end of the oligonucleotide (22). Compared with the unmodified oligonucleotide 5′-OH-$(dT)_7$ (trace d, retention time 14.5 min) the PCB-modified oligonucleotide (trace a) shows an increased retention time, which is typical for biotinylated oligonucleotides (5,16). We conclude from these data that the 5′-PCB moiety is retained during cleavage and deprotection of the oligonucleotide with ammonia [5′-phosphorylated oligonucleotide is not present in the 5′-PCB-$(dT)_7$ sample].

Interaction of the PCB-modified oligonucleotide with streptavidin and photorelease of the oligonucleotide were evaluated by incubating 5′-PCB-$(dT)_7$ with streptavidin–agarose beads, separating the beads from the solution by spin-filtering and irradiating the resuspended beads with 300–350 nm light. The effects of irradiating the resuspended beads for 4 min are shown in Figure 1. The two peaks assigned to 5′-PCB-$(dT)_7$ (trace a) disappear and a single peak appears (trace b) with a retention time of 12.5 min. Importantly, the retention time of this peak is almost identical to that of the reference 5′-phosphorylated sequence, i.e. 5′-p-$(dT)_7$ (trace c, retention time 12.6 min). These data conclusively show that irradiation causes cleavage of the PCB moiety and release of 5′-phosphorylated oligonucleotide into solution.

## Time dependence of photocleavage and oligonucleotide release

We measured the time dependence of the photoconversion of 5′-PCB-$(dT)_7$ into 5′-p-$(dT)_7$ in solution. For this purpose a 5′-PCB-$(dT)_7$ solution was subjected to irradiation with 300–350 nm light and the reaction mixture was analyzed by reversed phase HPLC after different irradiation times (Fig. 2). It can be seen from the decrease in the intensity of peaks at 23.7 and 24.3 min, assigned to 5′-PCB-$(dT)_7$, and the increase in the intensity of the single peak at 12.6 min, assigned to 5′-p-$(dT)_7$, that the photoreaction is complete in ~4 min. The appearance of additional small peaks with a retention time of ~33 min can be attributed to formation of the biotinyl-2-nitrosoacetophenone derivative and other minor photoproducts identified previously (23,24).

The time dependence and efficiency of photocleavage of 5′-PCB-$(dT)_7$ complexed with streptavidin–agarose beads was determined by measuring the absorbance of the supernatant at 260 nm, which reflects the amount of 5′-p-$(dT)_7$ released into solution. The initial $A_{260}$ value at 0 min (Fig. 2, inset) corresponds to <3% of the 5′-PCB-$(dT)_7$ prior to complexation with streptavidin–agarose beads. This result shows that 5′-PCB-$(dT)_7$ is almost quantitatively (97%) complexed with streptavidin–agarose. Upon irradiation photorelease is very rapid (4 min) and reaches 92% of the estimated absorption due to 5′-PCB-$(dT)_7$ prior to complexation (see Materials and Methods). No further increase in absorbance was observed after 6 min irradiation. In a separate experiment synthetic 5′-p-$(dT)_7$ was incubated with streptavidin–agarose beads. It was found that ~8% of 5′-p-$(dT)_7$ binds non-specifically to the streptavidin–agarose beads (data not shown). Thus the incomplete release of 5′-p-$(dT)_7$ appears to be due to non-specific binding and not to incomplete photocleavage.

## PCB-mediated purification and phosphorylation

In order to evaluate the usefulness of PCB-phosphoramidite for synthesis and affinity purification/phosphorylation of longer oligonucleotides two 5′-PCB-labeled sequences, a 50mer and a 60mer, were prepared. After deprotection the crude 5′-PCB-oligonucleotides were separately incubated with streptavidin–agarose beads. The beads were then washed, resuspended and finally irradiated to obtain the full-length phosphorylated oligo-

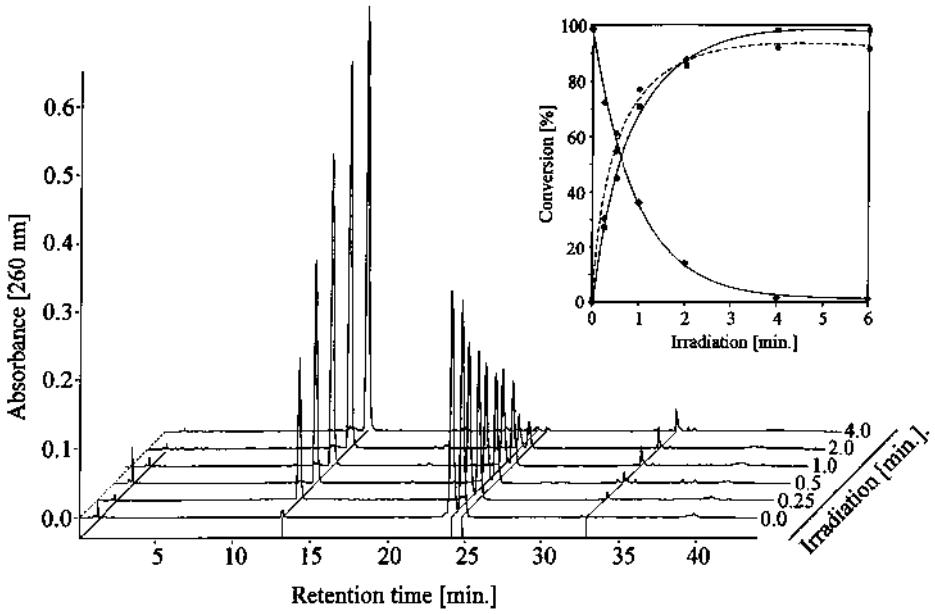



**Figure 2.** HPLC traces of 5′-PCB-$(dT)_7$ after increasing times of irradiation. See Materials and Methods for more details. (Inset) Time dependence of the photocleavage reaction 5′-PCB-$(dT)_7$(5′-p-$(dT)_7$ in solution (solid lines); ♦, concentration of 5′-PCB-$(dT)_7$; ■, concentration of 5′-p-$(dT)_7$. For comparison the time dependence for photorelease of 5′-p-$(dT)_7$ from the 5′-PCB-$(dT)_7$–streptavidin–agarose beads complex is shown (dashed line, ●).

nucleotides. Figure 3 shows the results of polyacrylamide gel electrophoresis (PAGE) of the crude 50mer (lane 1) and 60mer (lane 4) oligonucleotides and the affinity-purified and photocleaved oligonucleotides (50mer, lane 2; 60mer, lane 5). PAGE of the supernatant obtained after isolation of the oligonucleotides with streptavidin–agarose beads is also shown (50mer, lane 3; 60mer, lane 6). In agreement with earlier studies, the biotinylated oligonucleotides migrate more slowly than non-biotinylated oligonucleotides, while 5′-phosphorylated sequences migrate faster than sequences with 5′-OH (3). It can be further seen that affinity purification and photocleavage results in a compact band, indicative of high purity and homogeneity, in contrast to the crude material, which exhibits a much broader band with additional material appearing at lower molecular weight. Since this latter material did not bind to streptavidin, it is likely to correspond to the failure sequences.

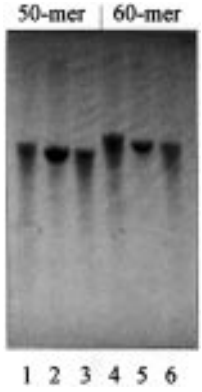



**Figure 3.** Polyacrylamide electrophoresis gel (20%) of the 50mer (lanes 1–3) and 60mer (lanes 4–6) visualized by UV shadowing, each lane representing 0.25 $OD_{260}$ units of material. Lanes 1 and 4, crude 5′-PCB-oligonucleotides; lanes 2 and 5, PCB affinity-purified and phosphorylated oligonucleotides; lanes 3 and 6, filtrate containing sequences not bound to streptavidin.

## DISCUSSION

In a previous paper (18) we described a photocleavable biotin *N*-hydroxysuccinimide (PCB-NHS) derivative which reacts with primary aliphatic amino groups and can be used for isolation of biomolecules. In the present work we further expand this approach and describe the synthesis of a phosphoramidite reagent which incorporates a photocleavable biotin moiety on the 5′-end of an oligonucleotide during solid support DNA synthesis.

The addition of a 5′-PCB moiety onto oligonucleotides provides a *photoremovable* affinity tag for fast and efficient purification and phosphorylation of synthetic DNA/RNA. The wavelength and the low intensity of the light used for photocleavage minimizes possible irradiation-induced damage to DNA, which typically occurs at wavelengths below 300 nm (25). The approach described is especially important for the removal of failure sequences in synthetic DNA, which are formed as a result of imperfections during the synthesis cycle. Note, however, that this method would not eliminate deletion sequences, which are also known to occur (26,27).

Current methods for purification and analysis of synthetic oligonucleotides include reversed phase high performance liquid chromatography (HPLC) (28), ion exchange HPLC (29), high performance capillary electrophoresis (HPCE) (30) and PAGE (31). Alternatively, a DMTr group on the 5′-end of an oligonucleotide can facilitate purification by non-specific adsorption on a reversed-phase silica gel (32). However, none of these methods is able to remove failure sequences quantitatively. In contrast, 5′-PCB provides an efficient and simple method for isolation of full-length product and removal of failure sequences.

The ability to 5′-phosphorylate an oligonucleotide through photocleavage is a second important advantage of this approach. Many applications of synthetic oligonucleotides require phosphorylation on the 5′-end, including gene construction, cloning, oligonucleotide ligation assay (33), the ligation chain reaction (34) and total cDNA sequencing. Typically, 5′-phosphorylation is achieved by either enzymatic or chemical methods. The use of enzymes involves several time consuming steps and often results in non-quantitative phosphorylation. Chemical phosphorylation is possible during oligonucleotide synthesis using commercially available phosphoramidites such as Phosphalink™ (20), however, the resulting product still requires additional purification.

In addition to PCB-mediated affinity purification and phosphorylation, PCB-oligonucleotides should also be useful in a number of other applications. For example, they could be used as primers for PCR, thereby simplifying the streptavidin-mediated affinity purification of PCR products from a reaction mixture containing template DNA, polymerases and other components. Importantly, this procedure would yield unmodified amplified fragments suitable for sequencing or cloning. Other possible applications include isolation of DNA/RNA macromolecular complexes (11–15) and controlled photorelease of oligonucleotides for the triggering of DNA–protein interactions and for therapeutic purposes.

## ACKNOWLEDGEMENTS

We thank Lincoln Scott for help with $^{31}$P NMR experiment. We also thank Sanjay Sonar and Matthew Coleman for helpful discussions and Cheryl and Gary Ludlam for critical reading of the manuscript. This work was supported by a grant from the Army Research Office (ARO) (DAAL03-92-G-0172) to KJR. JO was supported by a J.William Fulbright post-doctoral fellowship.

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