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A Versatile Photocleavable Bifunctional Linker for Facile Synthesis of Substrate−DNA Conjugates for the Selection of Nucleic Acid Catalysts

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Covalent photocleavable attachment of small molecules or peptides to oligonucleotides is an integral strategic element in the selection of novel nucleic acid enzymes. Here, we report the synthesis of a multipurpose, photocleavable bifunctional linker (PCBL) suitable for nucleic acid selections and other biotechnology applications. PCBL contains a photocleavable *O*-nitrobenzyl group flanked on one side by an *N*-hydroxysuccinimidyl ester (reactive toward primary amines) and on the other side by a sulfhydryl. To demonstrate the utility of PCBL, the linker was used to couple an analog of the antibiotic chloramphenicol (Cam) to the 5′ end of an amino-modified 8-mer DNA oligo. Coupling was confirmed by MALDI-TOF spectrophotometry. Decoupling was performed by irradiating the coupled species with near-UV light (approximately 360 nm), regenerating the original amino-modified oligo. Ligation of the Cam−PCBL−DNA conjugate to random-sequence RNA generated a diversity library appropriate for the selection of new ribozymes that catalyze reactions involving the tethered substrate. Coupling and decoupling of the Cam analog from the library was monitored on a trilayered organomercurial polyacrylamide gel. The coupling/decoupling strategy described here is readily generalized to many combinations of macromolecules and small molecules. For example, analogs of this small molecule−DNA conjugate can be generated as synthons for ligation to nucleic acid diversity libraries during each round of novel ribozyme selections, or they can be immobilized onto chips for addresssably reversible microarray analysis.

INTRODUCTION

Covalent modifications of nucleic acids have found broad application in chemistry and biology. In particular, there has been growing interest in the coupling of functional groups to nucleic acids. For example, conjugation of peptides to oligos has been used to improve biological stability, cellular uptake efficiency, and cell-specific targeting of antisense RNAs, aptamers, and siRNA (*1*−*4*). Nucleic acids have also been conjugated to chromophores for target detection or tertiary structural analysis, or to immobilizing agents for the production of high-density DNA arrays (*5*−*7*). Similar conjugation is also very useful in the *in vitro* selections of catalytic nucleic acids such as ribozymes that catalyze Michael addition, Diels−Alder cycloaddition, or oxidoreduction reactions (*8*, *9*).

The in vitro selection of ribozymes involves multiple cycles of partitioning and amplification of reactive nucleic acids from among a vast number of starting molecules (10^{14} or more). The partition often takes advantage of the conversion of a nucleic-acid-tethered substrate into a nucleic-acid-tethered product, followed by preferential purification and amplification of nucleic acids that are tethered to product. Each selection cycle increases the number of copies of catalytically active nucleic acid strands relative to nonspecifically recovered molecules, until the population is dominated by nucleic acids with autocatalytic activity. This strategy is highly effective at recovering reactive RNA species but can be limited by poor control of regiospecificity and cumbersome synthesis of substrate-linked nucleic acid libraries. Several selections that were originally intended to target nucleic-acid-tethered substrates have instead yielded ribozyme libraries dominated by species that modify themselves at internal sites on the RNA, such as 2′ hydroxyls, rather than modifying the intended substrate (*10*−*13*). This is especially problematic for catalysis of condensation reactions that label active nucleic acids with an affinity tag, such as biotin or a free sulfhydryl. Depending on the method used for recovering product-linked ribozymes, this unwanted background can be difficult to separate from the desired ribozymes.

A productive strategy for overcoming this limitation has been to introduce a cleavable moiety between the RNA and the tethered substrate, such that the desired product−RNA conjugates can be easily decoupled from unreacted substrate−RNA conjugates (*14*, *15*). In this strategy, the partition step of the selection includes three stages: recovery of all nucleic acid species that have become modified during the course of the reaction (including product−RNA conjugates and internally reacted species), cleavage of the tether joining the substrate/product to the ribozyme, and elimination of all RNA species that still retain internal covalent modifications. A second limitation is that the attachment of small-molecule substrates to the nucleic acids can be challenging in the absence of considerable synthetic expertise. Although there is a limited repertoire of commercially available mono- and oligonucleotides with covalently attached pendants, such as nucleoside analogs and fluorescent dyes, the use of such reagents is not readily generalizable to the attachment of new pendants. Importantly, most attachment strategies do not allow facile reversal of the coupling using benign conditions.

The present work addresses both of these concerns by introducing a photocleavable *O*-nitrobenzyl group (ONB) into the linker. ONB derivatives are especially convenient photocleavable moieties and have been used for a variety of applications, because they are synthetically tractable and photocleave upon irradiation in the near-UV at 330−370 nm. Examples include the covalent attachment of immobilizing agent to the

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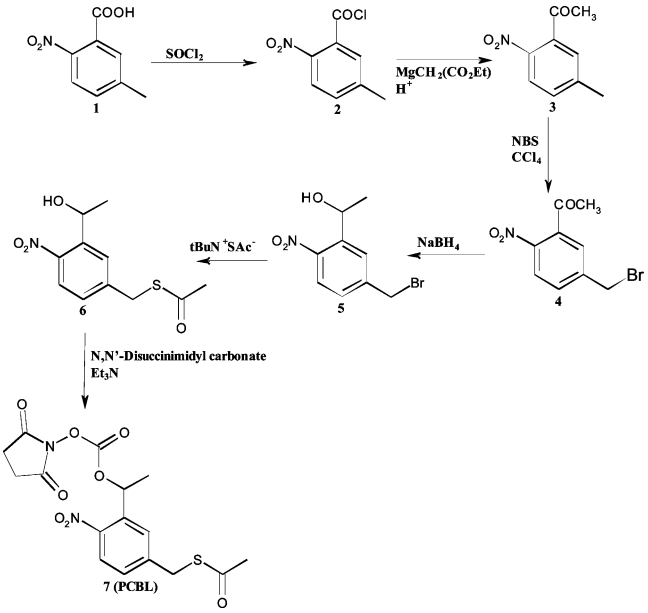
Scheme 1. Synthesis Scheme of Photocleavable Bifunctional Linker



termini of nucleic acids and proteins for substrate surface modifications and purification and analyses (*16*−*18*), conjugation of protein toxin to an antibody for cellular delivery (*19*), protection of amines and carboxylic acid groups during chemical synthesis of amino acids and nucleotides (*20*−*22*), synthesis of many caged fluorophores, proteins, and other caged compounds (*23*), and synthesis of multifunctional mono- and dinucleotide analogs (*15*). Photoreversible fluorescent chain terminators and fluorescent oligonucleotides have been generated for DNA sequencing by synthesis (*5*, *24*, *25*), and aptamers to hepatitis C virus RNA polymerase have been immobilized to affinity chromatography columns via ONB linkers to allow photoelution of the bound polymerase (*26*).

This paper reports a time-effective, inexpensive, easy-to-use general scheme for the synthesis and evaluation of a multipurpose, photocleavable, bifunctional linker (PCBL). The PCBL can be used to couple any pair of amine-containing and thiol-reactive species, and the linkage is labile to near-UV irradiation at 360 nm under conditions that are benign for nucleic acids. The utility of the PCBL is demonstrated experimentally by coupling an analog of the antibiotic chloramphenicol (Cam) to the 5′ end of a DNA oligonucleotide and to a random-sequence RNA library and subsequently decoupling it in both contexts by near-UV irradiation.

EXPERIMENTAL PROCEDURES

Synthesis of Photocleavable Bifunctional Linker (PCBL) (Scheme 1). Photocleavable bifunctional linker was synthesized as shown in Scheme 1.

Compound 2. 5-Methyl-2-nitrobenzoic acid (**1**; 1.81 g, 0.01 mmol) and 2 drops of DMF were added to 25 mL of $SOCl_2$ and refluxed at 80 °C for 3 h under inert atmosphere. The mixture was evaporated to a yellow oil, washed after dissolving in ether, and further evaporated to dryness.

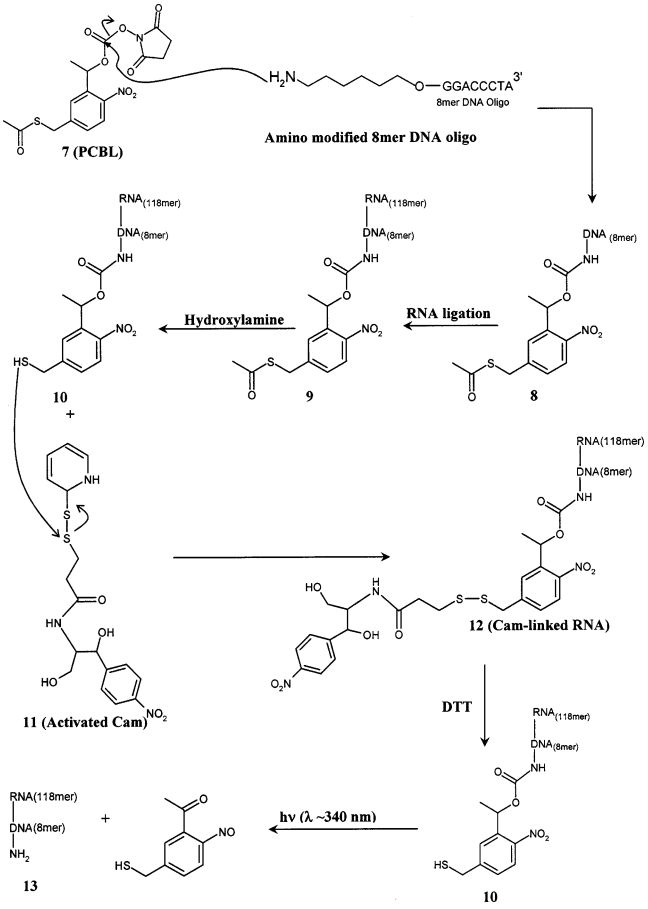
Compound 3. Compound **3** was prepared according to the procedure discussed by Reynolds et al. (*27*). Magnesium turnings (270 mg) were mixed with 0.25 mL of absolute ethanol and 0.025 mL of carbon tetrachloride in a three-neck, round-bottom flask and stirred at room temperature under inert (N_2) atmosphere. If the reaction did not start immediately (no effervescence), the flask was heated for a few minutes on a steam bath. The reaction was allowed to continue for 5 min, then 10 mL of ether was added slowly with constant stirring. A solution of 1.66 mL of diethyl malonate, 1 mL of absolute ethanol, and 1.25 mL of absolute ether was added at a rate that maintained constant boiling, and the mixture was refluxed over a steam bath until most of the magnesium was dissolved. Compound **2**, 5-methyl-2-nitrobenzoyl chloride, (0.01 mmol) was dissolved in 2.5 mL of ether and was very slowly added to the gray solution. Reflux was continued throughout the addition until the resulting green solution became too viscous to stir. The reaction mixture was cooled and dissolved in dilute sulfuric acid (0.69 mL of H_2SO_4 in 10 mL of H_2O). The ether layer was separated, and the aqueous layer was extracted with 2.06 mL of ether. Ether extracts were combined, and solvent was removed to yield crude diethyl-5-methyl-2-nitrobenzoylmalonate. A solution of 2 mL of glacial acetic acid and 0.38 mL of concentrated sulfuric acid was added, and the mixture was refluxed until no more carbon dioxide was evolved. The reaction mixture was cooled on an ice bath and made alkaline with NaOH solution, and the product 5-methyl-2-nitroacetophenone **3** was extracted with ether and purified by flash column chromatography on a column of silica (3 × 20 cm, grade 230−400 mesh, purchased from Scientific Adsorbance), using 20% ether in hexane as eluent. This product is characterized by ^{1}H NMR ($CDCl_3$) δ: 2.582 (s, 3H, CH_3), 2.637 (s, 3H, Ar−CH_3), 7.324 (s, H, ArH), 7.485 (d, H, ArH), 8.078 (d, H, ArH).

Compound 4. Synthetic protocols for compounds **4** through **6** were adapted from Senter et al. (*19*). 2 g (11 mmol) of compound **3**, 2.18 g (12.3 mmol) of *N*-bromosuccinimide, 27 mg (0.11 mmol) of benzoyl peroxide, and 20 mL of CCl_4 were mixed and refluxed for 6 h under Ar or until the succinimide produced in the reaction floated on the top of the solution. The reaction mixture was cooled and filtered, and the filtrate was evaporated to dryness. The product was further dissolved in CH_2Cl_2, washed with water, and dried. Formation of 5-Bromomethyl-2-nitroacetophenone (**4**) was confirmed by monitoring the new ^{1}H NMR (s, 2H, CH_2Br) peak at δ 4.573. Additional peaks in the NMR spectra indicated a small amount of unreacted starting material and dibrominated product, but further purification at this step was deemed unnecessary.

Compound 5. Compound **4** (2.0 g, 7.7 mmol) was dissolved in a mixture of 10 mL dioxane and 14 mL methanol and kept on ice. $NaBH_4$ (150 mg, 3.94 mmol) was added in the ice-cold solution of the reaction mixture and allowed to stir for 2 h at 0 °C. The reaction was quenched with acetone, and solvent was evaporated to dryness. The crude product was dissolved in 25 mL of $CHCl_3$, washed with brine water, and evaporated to a yellow oil. The formation of 1-(5-bromomethyl-2-nitrophenyl) ethanol (**5**) was confirmed by NMR without further purification. ^{1}H NMR ($CDCl_3$) δ: 1.582 (d, 3H, CH_3), 4.571 (s, 2H, Ar−CH_2Br), 5.458 (q, H, CHOH), 7.28 (s, H, ArH), 7.36 (d, H, ArH), 7.88 (d, H, ArH).

Compound 6. 3.28 mL (5.0 mmol) of 40% aq tetrabutylammonium hydroxide was slowly added to a solution of 0.35 mL (5.0 mmol) of thioacetic acid in 5 mL DMF at room temperature. A solution of compound **5** (1.0 g, 3.85 mmol) in 6.37 mL DMF was slowly added, and the mixture was stirred for 3 h. The solvent was evaporated, and the residue was redissolved in 25 mL ether and washed with saturated aqueous NaCl, then with saturated $NaHCO_3$, and dried under vacuum. 1-[5-(Methyl-3-*S*-acetylthioic acid ester)-2-Nitrophenyl] ethanol [**6**] was purified by silica flash column chromatography (grade 230−400 mesh, purchased from Scientific Adsorbance), using 35% ether in hexane as eluent. ^{1}H NMR ($CDCl_3$) δ: 1.582 (d, 3H, CH_3), 2.395 (s, 3H, $COCH_3$), 4.178 (s, 2H, Ar−CH_2Br), 5.458 (q, H, CHOH), 7.28 (s, H, ArH), 7.36 (d, H, ArH), 7.88 (d, H, ArH).

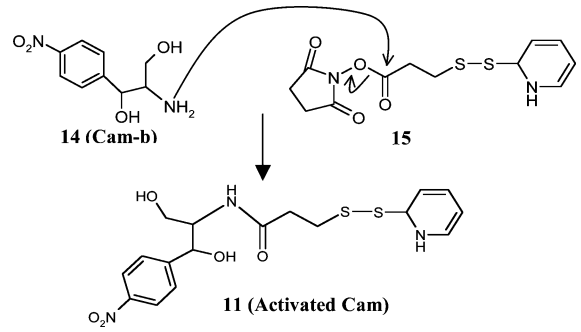
Scheme 2. Synthesis of Cam-Linked RNA Pool and Decoupling Reaction of Cam from RNA



PCBL, Final Compound (7). Compound **6** (50 mg, 0.20 mmol) was dissolved in 0.8 mL of DMF. 80 mg (0.33 mmol) of *N*,*N*′-disuccinimidyl carbonate was added, followed by 84.5 mL (0.6 mmol) of triethylamine, and the mixture was stirred for 6 h at room temperature. Solvent was evaporated to dryness, and the residue was washed thoroughly with 0.1 M $NaHCO_3$ and water. Finally, the crude product 1-[5-(methyl-3-*S*-acetyl-thioic acid ester)-2-nitrophenyl] ethyl-*N*-hydroxysuccinimidyl carbonate [**7**] was dissolved in minimum CH_2Cl_2 and purified over preparative TLC (250 μ glass-backed silica gel plate) using a solvent system of 70:30 ratio $CHCl_3/CH_3OH$. ^1H NMR ($CDCl_3$) δ: 1.79 (d, 3H, CH_3), 2.395 (s, 3H, $COCH_3$), 4.19 (s, 2H, Ar$-CH_2$Br), 6.41 (q, H, CHOH), 7.28 (s, H, ArH), 7.45 (d, H, ArH), 7.98 (d, H, ArH).

Synthesis of Conjugated RNA Pool (Scheme 2). 1 mM 5′ amino-modified DNA oligo was incubated with 5 mM PCBL in 80% DMSO for 12 h at room temperature. The product was purified on a 1 mL size-exclusion column (G25 Sephadex, purchased from Sigma), dried under vacuum, and resuspended in water. Ligation was performed by incubating 118 nt RNA (1 μM) carrying 70 random nucleotides (*13*) with purified PCBL-linked 8-mer DNA oligo (1.5 μM) and 24-mer DNA bridge (3 μM) in a buffer that contained 1 mM ATP and T4 DNA ligase at 37 °C for 5 h. RNA was internally radiolabeled by transcription in the presence of [α-^{32}P] UTP. The sequence of the DNA bridge (5′-GATTCGCTTTTCCCTAGGGTCC-3′) is complementary to both the 8-mer DNA oligo and to the 5′ terminal 14 nucleotides of the RNA. Deacylation was accomplished by incubating 10 μM hybrid RNA (**9**) with 25 mM hydroxylamine at pH 7.5 and purifying it on a trilayer APM gel. 10 μM of deacylated product (**10**) was incubated with 10 μM DTT (1 equiv) for 5 min. Activated Cam in 60% DMSO was added to a final concentration of 50 μM (5 equiv). The mixture was incubated for 4 h at room temperature, and the product Cam-linked RNA pool (**12**) was purified on an 8% denaturing (8 M urea) polyacrylamide gel. Activated Cam was synthesized by incubating 1 mM chloramphenicol base (Cam-b) (**14**) with 1 mM *N*-succinimidyl 3-(2-pyridyldithio)-propionate (SPDP) (**15**) in DMSO for 2 h at room temperature (Scheme 3).

Scheme 3. Activation of Cam Analog with Commercially Obtained Crosslinker



Photolysis of Conjugate from the RNA Pool. A 1 μM solution of DTT-treated, Cam-linked RNA pool in 25 mM phosphate buffer at pH 7.4 was irradiated with a hand-held, long-wavelength UV lamp (UVP, Inc., model no UVGL-25) at approximately 360 nm from a distance of 10 cm for 5 or 10 min, as indicated in the text.

RESULTS

PCBL Design and Synthesis. The photocleavable bifunctional linker (PCBL) carries two reactive groups separated by a photocleavable *O*-nitrobenzyl moiety. It was designed to permit reversible coupling of any amine-containing and sulfur-reactive species (Figure 1). The *N*-hydroxysuccinimide group is highly reactive toward primary amines, which react to form a stable carbamate. The sulfur at the other end of the PCBL is initially protected by an acetyl group to avoid premature reactivity or disulfide bond formation. Deacetylation of the thioester with hydroxylamine exposes the sulfhydryl, which is highly reactive toward numerous reagents. This PCBL reagent was synthesized as described in methods and summarized in Scheme 1.

Coupling to, and Photorelease from, a DNA Oligo. PBCL was dissolved in DMSO and allowed to react with 5′ amino-modified DNA oligo (Scheme 2). The PBCL−DNA conjugate (**8**) was purified from the reaction mixture containing DMSO and other contaminants by gel filtration on a size-exclusion column (pre-equilibrated with water), eluting with multiple 1 mL fractions of water. A second column was loaded in parallel with 5′ radiolabeled 8-mer DNA oligo to identify the elution fraction likely to contain the purified DNA. The fraction containing the DNA was concentrated, and product formation was confirmed by MALDI-TOF (Figure 2). A peak of molecular

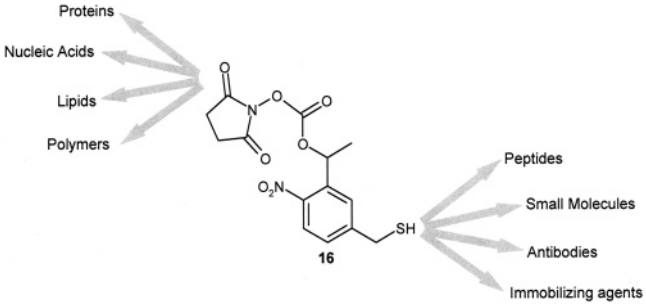


Figure 1. Some of the potential combinations of attachment of macromolecules with small molecules using PCBL [**7**].

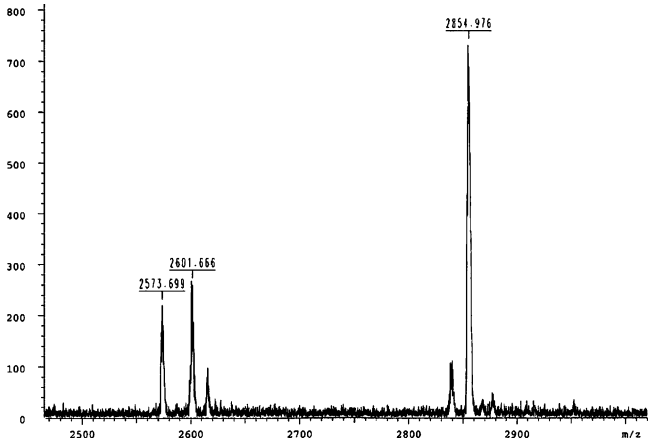


Figure 2. MALDI-TOF spectra of PBCL-linked 8-mer DNA oligo. The calculated molecular weight of the starting material (amino-linked 8-mer DNA oligo) is 2573. Reaction with PCBL is expected to increase the molecular weight by 282 (396 for PCBL minus 114 for released NHS), yielding a product with expected molecular weight of 2854. Peak at 2854.9 confirms the product formation.

weight 2573, which is the molecular weight of both the starting material and the photolytic product, was always present in the spectrum, generated by photolysis of the product by the 337 nm laser of the MALDI-TOF instrument. Essentially identical spectra were obtained irrespective of the amount of excess PCBL relative to DNA in the coupling reaction, suggesting that the peak at MW 2573 is due to photocleaved product and not to leftover starting material.

A Model Substrate-Linked RNA Library. For the PCBL to be useful in ribozyme and DNAzyme selections, it must be suitable for tethering potential substrates to a nucleic acid library, followed by releasing the substrate or product after the intended reaction. To this end, the PCBL−DNA conjugate (**8**) was enzymatically ligated to a random-sequence, radiolabeled RNA library (Scheme 2). The 118 nt input RNA and the 126 nt ligated product (**9**) were separately loaded onto a trilayer organomercurial polyacrylamide gel (*13*, *28*) in which the middle layer was doped with *N*-acryloylaminophenylmercuric chloride (APM) (*29*). The strong Hg−S interaction impedes the migration of nucleic acids carrying a free thiol. Because neither of these two input RNAs carries a free thiol, there was no appreciable material retained in the APM layer (lanes 1 and 2 of Figure 3a; columns 1 and 2 of Figure 3b). In contrast, exposing the sulfhydryl by deprotecting compound **9** with hydroxylamine to yield compound **10** resulted in retention of >60% of the input RNA in the APM layer (lane 3 of Figure 3a; column 3 of Figure 3b). PCBL attachment to the 5′ end of the DNA oligo did not affect ligation efficiency of T4 DNA ligase (data not shown). Compound **10** was purified from the APM layer and incubated with thiopropyl-activated Cam **11** (Scheme 3). To minimize the formation of disulfide via spontaneous oxidation, 1 equiv of dithiothreitol (DTT) was added to the RNA pool prior to incubating it with activated Cam. 5 equiv of activated Cam analog was used in the coupling reaction to minimize disulfide reduction of the product by unused DTT. Coupling of Cam to the library was confirmed by loading the product onto a trilayer APM gel. The Cam-coupled library (**12**) no longer carries a free thiol, and it again passed through the APM (lane 4 of Figure 3a; column 4 of Figure 3b). However, when this material was incubated with DTT to reduce the disulfide bond between the linker and the Cam, the free sulfhydryl is regenerated (**10**), and the reduced product was again retained at the APM layer (lane 5 of Figure 3a; column 5 of Figure 3b). Thus, Cam can be attached to an RNA library via ligation of a PCBL-modified oligo and released by reduction with DTT.

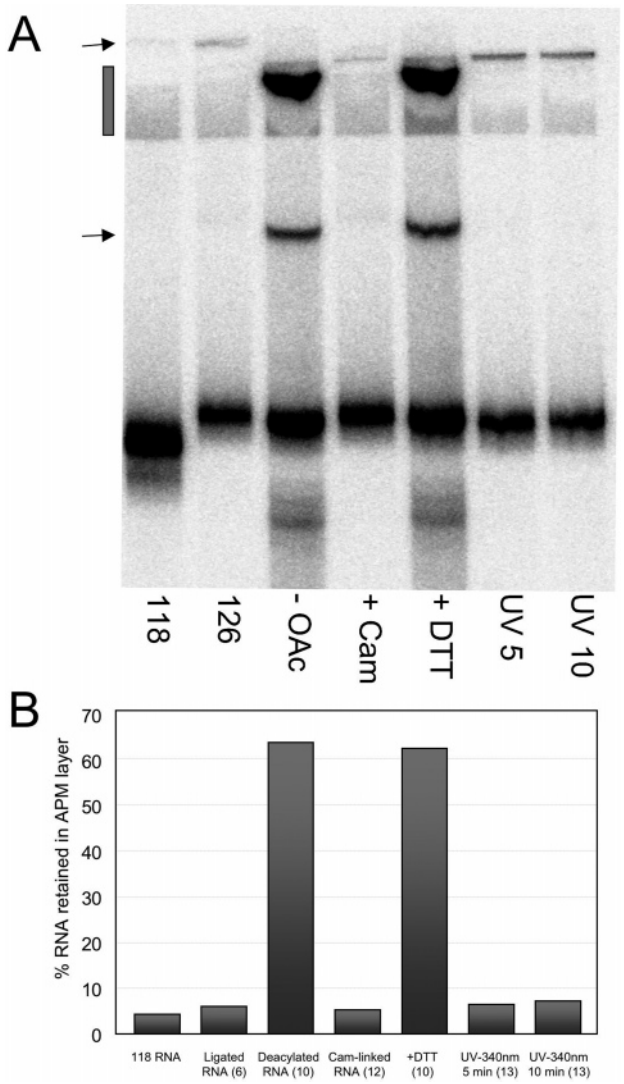


Figure 3. Attachment to, and decoupling from, random-sequence RNA library. (A) Trilayer APM gel of internally radiolabeled RNA treated as described in the text. Top arrow, wells; box, APM layer; bottom arrow, disulfide-oxidized side product. The sulfhydride can be retained in reduced form without interfering with APM electrophoresis by including tris-carboxyethyl phosphine (not shown) (*13*, *28*). (B) Percentage of internally radiolabeled RNA retained in the APM layer is plotted for individual constructs given under each column.

Photocleavage of Pendant from Library. While chemical reduction with DTT is convenient in some circumstances, it is not feasible in cases where the intended selection is based upon acquisition of a new sulfhydryl in the product or where the initial coupling is performed using thioether linkages (reaction with maleimides or iodoacetyl groups) or via interactions with inorganic compounds or surfaces (such as nanoparticles or chip arrays) rather than via a disulfide. Photocleavage is much more convenient in these cases.

To test the photodecoupling efficiency of the linker, compound **10** was purified from the APM layer and irradiated with near-UV light at 360 nm for 5 or 10 min. Decoupling of the *O*-nitrobenzyl moiety from the RNA removes the free sulfhydryl, allowing the RNA (**13**) to migrate through the APM layer. The reaction is complete within 5 min, with no additional product observed after 10 min of irradiation (lanes 6 and 7 of Figure 3a; columns 6 and 7 of Figure 3b). The results are in agreement with previous studies on the time requirements for *O*-nitrobenzyl derivative photocleavage (*16*, *17*).

DISCUSSION

We have successfully synthesized an *O*-nitrobenzyl-containing, photocleavable bifunctional linker (PCBL). This linker enabled coupling an analog of Cam to the 5′ end of an RNA library and its photorelease by near-UV irradiation. Analogs of this small molecule−DNA conjugate can be generated as synthons that are ligated to a nucleic acid diversity library during each round of a ribozyme selection. Alternatively, they could be annealed to immobilized oligonucleotides for chip array analysis. Diverse substrates can be readily coupled to nucleic acid libraries for use in novel nucleic acid enzyme selection using the PCBL. In this context, photochemical release of the tethered product (vs internal products) can be exploited to ensure regiospecific control of the reactions catalyzed by those selected ribozymes. Photocleavable, heterobifunctional crosslinkers such as PCBL and analogous compounds reported previously (*18*) are also highly versatile for applications in proteomics and nanotechnology, as they enable reversible coupling of a wide variety of other combinations of amine-containing species (oligonucleotides, peptides, proteins, fluorophores, and immobilizing agents) with thiol-reactive species (maleimides, iodoacetyl groups, activated disulfides, semiconductor surfaces, and organomercurial compounds and several metallic or semiconductor surfaces) (Figure 1).

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LITERATURE CITED

(1) Astriab-Fisher, A., Sergueev, D. S., Fisher, M., Shaw, B. R., and Juliano, R. L. (2000) Antisense inhibition of P-glycoprotein expression using peptide-oligonucleotide conjugates. *Biochem. Pharmacol. 60*, 83−90.

(2) Kwok, K. Y., Park, Y., Yang, Y., McKenzie, D. L., Liu, Y., and Rice, K. G. (2003) In vivo gene transfer using sulfhydryl cross-linked PEG-peptide/glycopeptide DNA co-condensates. *J. Pharm. Sci. 92*, 1174−85.

(3) McInnes, J. L., and Symons, R. H. (1989) *NucleicAcid Probes*, pp 33−80, CRC, Boca Raton.

(4) Rogers, F. A., Manoharan, M., Rabinovitch, P., Ward, D. C., and Glazer, P. M. (2004) Peptide conjugates for chromosomal gene targeting by triplex-forming oligonucleotides. *Nucleic Acids Res 32*, 6595−604.

(5) Bai, X., Li, Z., Jockusch, S., Turro, N. J., and Ju, J. (2003) Photocleavage of a 2-nitrobenzyl linker bridging a fluorophore to the 5′ end of DNA. *Proc. Natl. Acad. Sci. U.S.A. 100*, 409−13.

(6) Niemeyer, C. M., and Blohm, D. (1999) DNA microarrays. *Angew. Chem. Inter Ed. 38*, 2865−2869.

(7) Wojczewski, C., Stolze, K., and Engels, J. W. (1999) Fluorescent oligonucleotides - Versatile tools as probes and primers for DNA and RNA analysis. *Synlett* 1667−1678.

(8) Seelig, B., and Jaschke, A. (1999) A small catalytic RNA motif with Diels-Alderase activity. *Chem. Biol. 6*, 167−176.

(9) Tarasow, T. M., Tarasow, S. L., and Eaton, B. E. (1997) RNA-catalysed carbon-carbon bond formation. *Nature (London) 389*, 54−57.

(10) Curtis, E. A., and Bartel, D. P. (2005) New catalytic structures from an existing ribozyme. *Nat. Struct. Mol. Biol. 12*, 994−1000.

(11) Jenne, A., and Famulok, M. (1998) A novel ribozyme with ester transferase activity. *Chem. Biol. 5*, 23−34.

(12) Lorsch, J. R., and Szostak, J. W. (1994) In vitro evolution of new ribozymes with polynucleotide kinase activity. *Nature (London) 371*, 31−6.

(13) Saran, D., Nickens, D. G., and Burke, D. H. (2005) A trans acting ribozyme that phosphorylates exogenous RNA. *Biochemistry 44*, 15007−16.

(14) Eisenfuhr, A., Arora, P. S., Sengle, G., Takaoka, L. R., Nowick, J. S., and Famulok, M. (2003) A ribozyme with michaelase activity: synthesis of the substrate precursors. *Bioorg. Med. Chem. 11*, 235−49.

(15) Hausch, F., and Jäschke, A. (2001) Multifunctional dinucleotide analogs for the generation of complex RNA conjugates. *Tetrahedron 57*, 1261−1268.

(16) Bai, X., Kim, S., Li, Z., Turro, N. J., and Ju, J. (2004) Design and synthesis of a photocleavable biotinylated nucleotide for DNA analysis by mass spectrometry. *Nucleic Acids Res. 32*, 535−41.

(17) Olejnik, J., Sonar, S., Krzymanska-Olejnik, E., and Rothschild, K. J. (1995) Photocleavable biotin derivatives: a versatile approach for the isolation of biomolecules. *Proc. Natl. Acad. Sci. U.S.A. 92*, 7590−4.

(18) Yan, F., Chen, L., Tang, Q., and Wang, R. (2004) Synthesis and characterization of a photocleavable cross-linker and its application on tunable surface modification and protein photodelivery. *Bioconjugate Chem. 15*, 1030−6.

(19) Senter, P., Tansey, M., Lambert, J., and WA, B. (1985) Novel photocleavable protein crosslinking reagents and their use in the preparation of antibody-toxin conjugates. *Photochem. Photobiol. 42*, 231−237.

(20) Kaplan, J. H., Forbush, B., 3rd, and Hoffman, J. F. (1978) Rapid photolytic release of adenosine 5′-triphosphate from a protected analogue: utilization by the Na:K pump of human red blood cell ghosts. *Biochemistry 17*, 1929−35.

(21) McCray, J. A., Herbette, L., Kihara, T., and Trentham, D. R. (1980) A new approach to time-resolved studies of ATP-requiring biological systems; laser flash photolysis of caged ATP. *Proc. Natl. Acad. Sci. U.S.A. 77*, 7237−41.

(22) Patchornik, A., Amit, B., and Woodward, R. B. (1970) Photosensitive protecting groups. *J. Am. Chem. Soc. 92*, 6333−6335.

(23) Ottl, J., Gabriel, D., and Marriott, G. (1998) Preparation and photoactivation of caged fluorophores and caged proteins using a new class of heterobifunctional, photocleavable cross-linking reagents. *Bioconjugate Chem. 9*, 143−51.

(24) Ruparel, H., Bi, L., Li, Z., Bai, X., Kim, D. H., Turro, N. J., and Ju, J. (2005) Design and synthesis of a 3′-O-allyl photocleavable fluorescent nucleotide as a reversible terminator for DNA sequencing by synthesis. *Proc. Natl. Acad. Sci. U.S.A. 102*, 5932−7.

(25) Seo, T. S., Bai, X., Ruparel, H., Li, Z., Turro, N. J., and Ju, J. (2004) Photocleavable fluorescent nucleotides for DNA sequencing on a chip constructed by site-specific coupling chemistry. *Proc. Natl. Acad. Sci. U.S.A. 101*, 5488−93.

(26) Cho, S., Lee, S. H., Chung, W. J., Kim, Y. K., Lee, Y. S., and Kim, B. G. (2004) Microbead-based affinity chromatography chip using RNA aptamer modified with photocleavable linker. *Electrophoresis 25*, 3730−9.

(27) Reynolds, G. A., and Hauser, C. R. (1950) O-nitroacetophenone. *Org. Synth. 30*, 70.

(28) Rhee, S. S., and Burke, D. H. (2004) Tris(2-carboxyethyl)-phosphine stabilization of RNA: comparison with dithiothreitol for use with nucleic acid and thiophosphoryl chemistry. *Anal. Biochem. 325*, 137−143.

(29) Igloi, G. L. (1988) Interaction of transfer-RNAs and of phosphorothioate-substituted nucleic-acids with an organomercurial - probing the chemical environment of thiolated residues by affinity electrophoresis. *Biochemistry 27*, 3842−3849.