Solid-Phase Synthesis of Oligonucleotides Containing a Site-Specific Psoralen Derivative

William R. Kobertz and John M. Essigmann*

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

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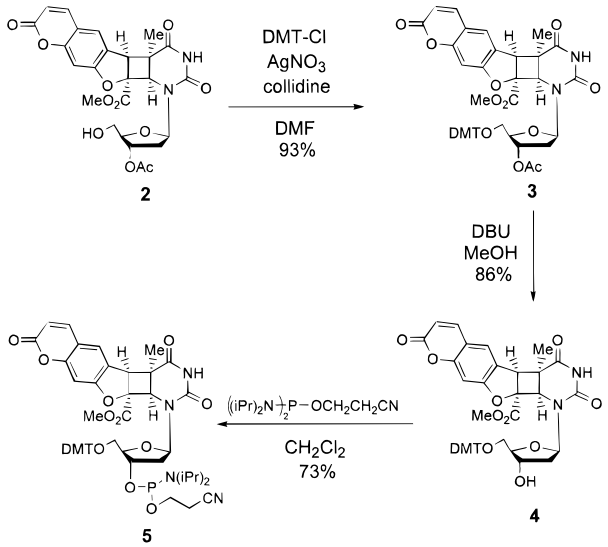
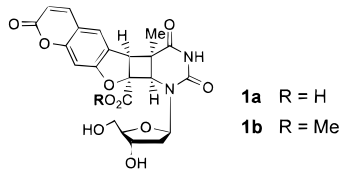
Psoralens are linear furocoumarins used for the treatment of skin diseases[1] and cutaneous T-cell lymphoma.[2] DNA is believed to be the cellular target for the therapeutic activity of the psoralens. In addition to medical and DNA diagnostic applications, psoralen-containing oligonucleotides have been useful probes for determining nucleic acid structure and function,[3] for studying the mechanisms of DNA repair,[4] and for arresting RNA transcription complexes.[5] Psoralens react via a [2 + 2] cycloaddition, in concert with long-wave UV light, to form photoproducts primarily with thymidines in 5′-TA-3′ sites in double-stranded DNA. This reaction is highly regio- and stereospecific, forming three major photoproducts with *cis-syn* stereochemistry: an interstand cross-link, a pyrone-side monoadduct, and a furan-side monoadduct.[6] Traditional methods for the synthesis of large amounts of psoralen-containing oligonucleotides require the utilization of high intensity lasers and are best applied when the desired psoralen-containing oligonucleotide contains only one 5′-TA-3′ site.[7] It would be desirable to have total synthetic procedures allowing for the preparation of psoralen adducts in any sequence context because recent studies show that context plays an important role in the replicative bypass, mutagenic, and genotoxic effects of DNA-damaging agents.[8] The inability to design psoralenated oligonucleotides in any desired sequence context has restricted study of the biological effects of these important therapeutic agents. Here, we report the synthesis of a 2-carboxypsoralen furan-side monoadduct phosphoramidite and its incorporation into DNA.

Synthesis of the furan-side monoadduct was chosen for two reasons. First, the furan-side adduct contains an intact coumarin chromophore, which when hybridized to a complementary strand can be converted into the cross-link upon irradiation with long-wave UV light.[9] Second, we believed that the furan-side adduct could survive the basic deprotection conditions used in solid-phase DNA synthesis, whereas the unsaturated lactone of the pyrone-side monoadduct is known to transesterify readily in the presence of mild aqueous base.[10] It is also known that furan-side monoadducts can be reversed by treatment with strong base at elevated temperatures;[10] this consideration therefore prompted us to explore deprotection conditions that avoided the use of harsh aqueous base. Commercially available PAC-phosphoramidites have protecting groups that are readily removed with 10% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in ethanol or methanol.[11] Nucleoside **1a** when treated with the aforementioned conditions remained intact for 24 h at room temperature.

Scheme 1





Another concern was the observation that nucleoside **1b** slowly hydrolyzes to the carboxylic acid derivative **1a** when stored in neutral aqueous solutions. This hydrolysis did not affect the photocross-linking capabilities of the psoralen−thymidine monoadduct; however, for purification purposes it was convenient to have a homogenous oligonucleotide. Therefore, reaction conditions were developed to saponify the alkyl ester without destroying the coumarin ring and the photocross-linking ability of the monoadduct. Treatment of **1b** with a 10 mM sodium carbonate solution at pH 9 for 12 h led to greater than 95% conversion to **1a**, alleviating purification problems.

The synthesis of the suitably protected psoralen−thymidine phosphoramidite is shown in Scheme 1. The synthesis of deoxynucleoside **2** has been previously described.[12] Protection of the 5′ hydroxyl of **2** with 4,4-dimethoxytrityl chloride (DMT-Cl) in the presence of silver nitrate led to rapid conversion to **3**.[13] Removal of the 3′ acetate with 5% DBU in freshly distilled methanol afforded **4**. Phosphitylation using diisopropylammonium tetrazolide as an activating agent yielded phosphoramidite **5**.

To demonstrate the flexibility of our approach, we chose a prototypical human TATA box sequence to incorporate **1a**. Runs

* Author to whom correspondence should be addressed.

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of 5′-TA-3′ sequences are known to be mutational hotspots for psoralen,[14] and it is possible that the therapeutic efficacy of psoralen might be due in part to the inability of transcriptional complexes to bind to modified TATA boxes. The modified oligodeoxynucleotide **6** was synthesized on a 1 μmol scale by using an automated DNA synthesizer. The coupling time for phosphoramidite **5** was extended to 15 min affording coupling yields of 90% based on DMT cation release. For unmodified phosphoramidites, yields are typically higher; our lower yield is presumably due to the increased bulk of the psoralen−thymidine phosphoramidite. The solid support was treated with 1 mL of 10% DBU in anhydrous ethanol in the presence of cetyltrimethyl ammonium bromide, which aided solubility.[15] After 24 h at room temperature, the deprotection solution was neutralized with aqueous acetic acid and the salts were removed by using a Na^+ exchange column. The crude oligonucleotide was treated with 50 mM sodium carbonate (pH 9.0) solution for 12 h to saponify the alkyl ester into the carboxylic acid. Neutralization followed by purification by reversed phase HPLC afforded oligonucleotide **6**.

The integrity of oligonucleotide **6** was established by enzymatic digestion and electrospray mass spectrometry. Enzymatic digestion and HPLC analysis of **6** yielded nucleoside ratios which were within experimental error of the theoretical composition of oligonucleotide **6**.[16] The peak corresponding to the modified nucleoside had identical HPLC retention characteristics and UV spectrum as the synthesized standard **1a**. Electrospray ionization mass spectrometry of **6** revealed the presence of ions at m/z 1058.05, 846.5, and 705.2 corresponding to 3−, 4−, and 5− ions, respectively. The calculated molecular weight was 4236.9, which agreed well with the calculated molecular weight of 4236.7.

One of the valuable applications of psoralen-containing oligonucleotides is their ability to cross-link to a hybridized strand.[9] The oligonucleotides described herein could be used as hybridization probes to form a cross-link to a target strand. Figure 1 shows such photocross-linking experiments of oligonucleotide **6** with its complementary strand **7**. By ^{32}P phosphate labeling the 5′ hydroxyl of only one of the two DNA stands in each experiment, the reactions of the two DNA stands can be independently analyzed by denaturing gel electrophoresis. Oligonucleotide **6** was labeled in lanes 2−7. Oligonucleotide **7** was labeled in lanes 8−13. The presence of the psoralen derivative caused oligonucleotide **6** to migrate slower than its unmodified counterpart **8** (lane 1 vs 2). Irradiation of the duplex with 366 nm light (9.0 J/m^2) afforded the slower moving interstrand cross-link in 5 min (lanes 4 and 10). In fact, irradiation with a hand-held 4 W long-wave UV lamp yielded the cross-link in 84% yield in 90 min.[16] A well-known chemical property of psoralen−DNA cross-links is their reversibility with 254 nm light[9] or their asymmetric conversion to the pyrone-side adduct by heating in the presence of base.[10] Treatment of the cross-linked duplex with 254 nm light (16.7 J/m^2) for 20 min resulted in photoreversion to the monoadduct (lanes 5 and 11) and eventually complete or near complete reversal to the unmodified strand (lanes 6 and 12). Again, a low wattage hand-held 254 nm lamp caused near complete reversal in 2 h.[16] Base-catalyzed reversal results in conversion of the interstrand cross-link into a pyrone-side adduct, essentially transferring the original psoralen furan-side monoadduct in **6** to the complementary strand **7**.[10] Treatment of a cross-linked duplex with 0.1 M NaOH at 90 °C for 30 min efficiently reversed the cross-link, affording unmodified oligonucleotide (lane 7) and primarily a pyrone-side monoadduct (lane 13). These experiments demonstrated that the synthesized psoralen oligonucleotides posses the useful hybridization/cross-linking/reversal properties of psoralen-containing oligonucleotides generated by traditional methods.

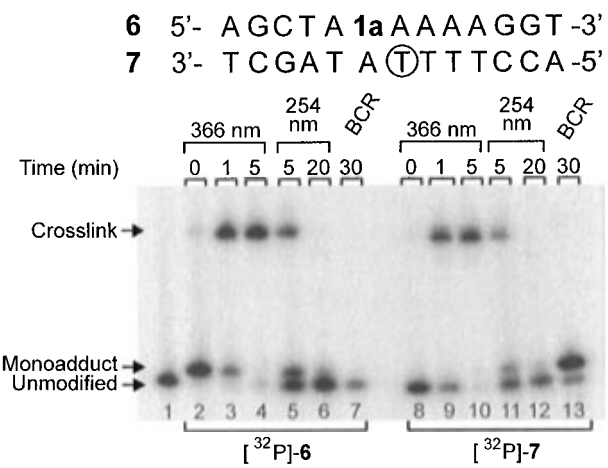

Figure 1. Photoreactions of the furan-side monoadduct containing oligonucleotide and the photoreversion and base-catalyzed reversal (BCR) of the cross-linked oligonucleotides. The photoreactive thymidine in oligonucleotide **7** is encircled. Oligonucleotide **6** and the complementary strand **7** were 5′ phosphate labeled with ^{32}P in lanes 2−7 and lanes 8−13, respectively: (lane 1) unmodified control oligonucleotide **8** labeled with ^{32}P at the 5′ terminus; (lanes 2−4 and 8−10) irradiation with 366 nm light; (lanes 5−6 and 11−12) photoreversal of the cross-link with 254 nm light; (lanes 7 and 13) base-catalyzed reversal of cross-link.

The successful site-specific incorporation of a *cis-syn* furan-side psoralen−thymidine monoadduct into oligonucleotides will enable the study of this therapeutic agent in DNA sequence contexts that contain multiple sites of reactivity. Such sites are known to be biologically important, and with this methodology, it will now be possible to design experiments to probe the details of how sequence context influences biological endpoints. In addition, this synthetic approach makes it feasible to utilize the valuable cross-linking properties of oligonucleotides containing furan-side monoadducts in hybridization assays and other experiments where site-specific cross-linking is desired.

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Supporting Information Available: Experimental details for compounds **3**−**6** and photocross-linking, photoreversal, base-catalyzed reversal, and enzymatic digestion conditions of oligonucleotide **6** (8 pages). See any current masthead page for ordering and Internet access instructions.

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(16) See Supporting Information Figures S1 and S2.