An Efficient Synthesis of a Furan-Side Furocoumarin Thymidine Monoadduct

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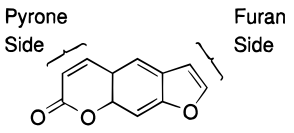
The construction of oligonucleotides containing a modified nucleoside at a predefined site has provided new insights into the mechanisms of DNA repair and mutagenesis.[1] Organic chemists have played a key role by synthesizing nucleosides damaged by UV and ionizing radiation, chemical carcinogens, and chemotherapeutic agents.[2] Further incorporation of these modified nucleosides into oligonucleotides has enabled the direct study of biologically relevant nucleoside adducts in any desired DNA sequence. Recent studies on anticancer drug−DNA adducts have suggested a new generation of agents that have diminished mutagenic potential, while preserving the desired cytotoxic properties necessary for therapeutic activity.[3] Psoralen (**1**) and its derivatives are linear furocoumarins that are highly effective therapeutic agents to combat skin disorders[4] and cutaneous T-cell lymphoma.[5] Psoralen reacts primarily with thymidines in helical DNA by a [2 + 2] photocycloaddition. The key mechanistic step required for reactivity is the intercalation of psoralen between the bases in double-stranded DNA. The intercalation event positions psoralen in an orientation to form a *cis*-syn four-membered ring with thymidine. Once intercalated, irradiation with long wave UV-light (320−400 nm) results in a photoreaction between psoralen and thymidine to give a monoadduct. If the reaction occurs on the furan-side of psoralen, the initial adduct can absorb a second photon and react with an adjacent thymidine in the opposite strand forming an interstrand crosslink. Pyrone-side adducts do not possess a chromophore that can absorb long wave UV-light and therefore do not form crosslinks. Hearst and co-workers have isolated and characterized a large number of



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psoralen-DNA adducts.[6] Out of the monoadducts, the furan-side adduct is the most appealing for genetic and DNA repair studies because it contains an intact chromophore that can still photoreact in double stranded DNA to form a crosslink. Therefore, it was of use to synthesize a furocoumarin thymidine monoadduct for its eventual site-specific incorporation into oligonucleotides.

The total synthesis of any psoralen−thymidine or furocoumarin−thymidine monoadduct has proved particularly difficult owing to the synthetically challenging *cis*-syn stereochemistry in the product. In the absence of a DNA helix, furocoumarins photodimerize adding an additional complication.[7] Consequently, previous attempts to synthesize furocoumarin−thymidine monoadducts have resulted in photoproducts with the incorrect stereochemistry[8] or a mixture of isomers from which only small quantities of the desired isomer could be isolated.[9] We have recently synthesized a 2-carbomethoxypsoralen−thymidine furan-side adduct by utilizing a benzofuran derivative in the key photochemical step followed by a pyrone ring annulation to afford the desired product.[10] This "two-stage" strategy eliminated photodimerization and yielded a single photoproduct containing a *cis*-syn geometry. While this approach gave the desired monoadduct in reasonable yield, it involved many steps with a delicate saturated pyrimidine. In this note, we describe a more direct approach utilizing a full furocoumarin derivative; this approach is more appealing because it shortens the synthetic scheme and eliminates all but one reaction with a saturated pyrimidine. At the outset, a major concern in using an intact furocoumarin was that it could lead to self-dimerization instead of a thymidine−furocoumarin photoproduct. Our earlier studies suggested, however, that a 2′-ester linkage could favor the intramolecular reaction between the furocoumarin and thymidine over furocoumarin photodimerization. We now report our further exploration of this linkage by using an intact furocoumarin derivative.

The strategy involves linking a 2′-carboxypsoralen to the 5′-hydroxyl of thymidine to control the stereochemistry of the photoreaction. 2′-Carboxypsoralen derivatives have been previously synthesized to improve photoreactivity and water solubility of furocoumarins.[11] On

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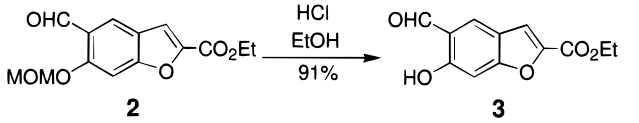
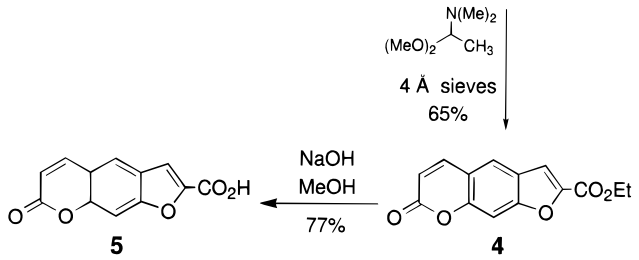
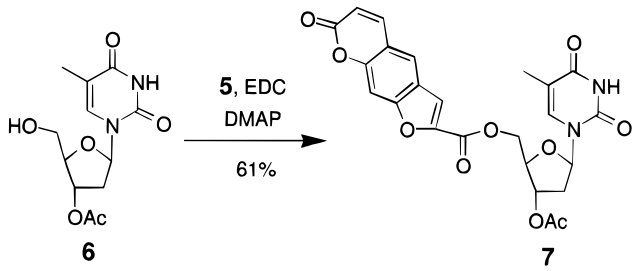
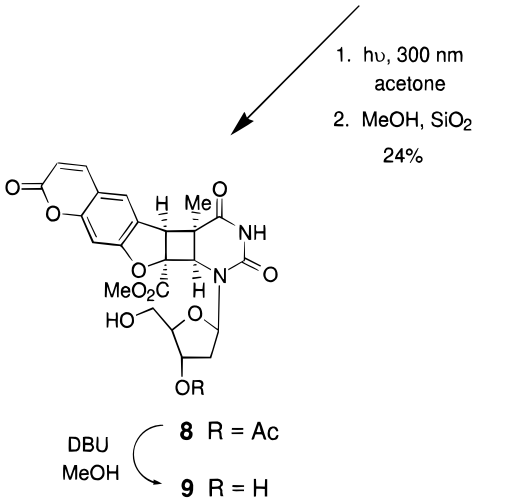
Scheme 1



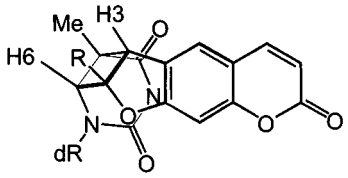


Scheme 2





the basis of our earlier work with benzofuran−thymidine photoproducts,[10] we developed a new method for the construction of 2′-carboethoxypsoralen shown in Scheme 1. Removal of the MOM-protecting group from **2**[10] with HCl in ethanol yielded phenol **3**. Cyclization of compound **3** with dimethylacetamide dimethyl acetal in the presence of 4 Å sieves gave 2′-(ethoxycarbonyl) psoralen (**4**) in 65% yield.[12,13] Saponification of **4** afforded acid **5**. The 2-carboxypsoralen was then attached to a suitably protected thymidine. As shown in Scheme 2, coupling of psoralen **5** with thymidine **6**[8] yielded photoprecursor **7**. Attempts to effect the photochemical reaction by using



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R = CO_2Me

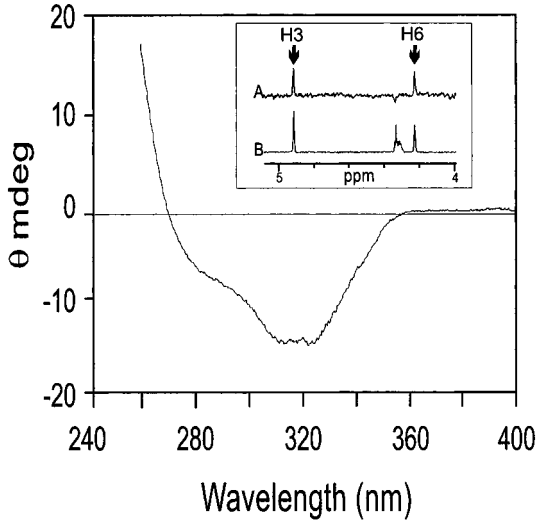

Figure 1. (Above) Structure of the furocoumarin−thymidine photoproduct **8**, where dR = 2′-deoxy-3′-*O*-acetylribose. (Below) Circular dichroism spectrum of photoproduct **8**. (Inset) Nuclear Overhauser enhancements for photoproduct **8**. (A) Difference spectrum between sample irradiated at the thymidine methyl resonance (1.76 ppm) and spectrum B; (B) 4.0−5.0 ppm region of the proton NMR spectrum.

long wave UV light (366 nm) produced only photodegradation of the starting material, compound **7**. However, the use of 300 nm light and acetone as a photosensitizer yielded one photoproduct, which underwent a transesterification with methanol and silica gel to afford **8** in 24% yield. The low yield was presumably due to some photodimerization even though the reactions were performed at low concentration (1 mM). Photoproduct **8** has been previously converted into the nucleoside **9**.[10]

The regio- and stereochemistry of photoproduct **8** was assigned by NMR and CD spectroscopy. The regiochemistry of **8** was determined by the coupling constant between the H6 proton of thymidine and the H4 proton of the furocoumarin. Only long range coupling (2 Hz) was observed, which is indicative of the syn regioisomer.[14] Irradiation of the thymidine methyl group in a difference NOE experiment resulted in positive signals for both protons on the four-membered ring assigning the stereochemistry as *cis* (Figure 1, inset). Determination of the facial selectivity of the photoreaction was ascertained by CD spectroscopy (Figure 1). Hearst et al. have shown that the CD spectrum of the two major furan-side monoadducts behave as enantiomers in the presence of polarized light.[15] The CD spectrum of **8** was identical to the photoproduct generated by our earlier method[10] and

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(13) We have found that the addition of 4 Å sieves to remove the methanol generated during the reaction affords higher yields at room temperature.

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to an isolated psoralen−thymidine photoproduct.[6f,15] The synthesized adduct is the equivalent to the biologically generated photoadduct occurring at a 5′-TpA-3′ site in DNA.

We have described a straightforward synthesis of a furocoumarin−thymidine furan-side adduct. A 2-carboxypsoralen tethered to the 5′-hydroxyl of thymidine controlled the stereochemical outcome of the photochemical reaction to give the desired *cis*-syn isomer. In addition, this linkage also helped minimize photodimerization. This simple approach to furocoumarin−thymidine adducts should expedite the incorporation of these adducts into oligonucleotides. The advantage of this total synthetic approach is that it will allow for the first time the selective placement of a furocoumarin−thymidine adduct in sequences where multiple sites of adduction are possible. Many such sites are mutational hot spots that occur in proto-oncogenes and tumor suppressor genes and are thought to play a key role in neoplastic transformation.[16] Incorporation of this adduct into DNA and its biological ramifications will reported in due course.

Experimental Section

General Procedures. Unless otherwise specified, materials were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran (THF) was distilled from potassium/benzophenone ketyl. Methanol (MeOH) was distilled from sodium metal and used immediately after distillation. Anhydrous solvents were otherwise obtained from Aldrich. Column chromatography was performed on Merck silica gel (230−400 mesh). ^{1}H NMR spectra were recorded at 500 or 300 MHz on superconducting FT spectrometers. ^{13}C NMR spectra were proton decoupled and were recorded at 125 or 75 MHz. Coupling constants are measured in hertz. Melting points (Pyrex capillary) are uncorrected.

2-Carbethoxy-5-formyl-6-hydroxybenzofuran (3). A solution of 1.02 g (3.67 mmol) of compound **2**, 2 mL of concentrated HCl, and 100 mL of ethanol was heated at reflux for 1 h. After cooling to room temperature, the solution was neutralized with saturated $NaHCO_3$ and diluted with ethyl acetate. The layers were separated, and the aqueous portion was washed twice with ethyl acetate. The combined organic washes were washed with brine and dried over Na_2SO_4. Removal of the solvents *in vacuo* and purification by silica gel chromatography eluting with a gradient of 10:1 to 2:1 hexanes/ethyl acetate afforded 0.78 g (91%) of a yellow-white solid. Recrystallization from hexanes/ethyl acetate afforded yellow plates: mp 158−159 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (t, 3 H, J = 7.2), 4.42 (q, 2 H, J = 7.2), 7.09 (s, 1 H), 7.49 (s, 1 H), 7.88 (s, 1 H), 9.95 (s, 1 H), 11.23 (s, 1 H); ^{13}C NMR δ 14.3, 61.7, 100.1, 113.7, 119.2, 120.5, 129.9, 147.0, 158.9, 160.2, 161.8, 195.9; HRMS (EI) calcd for $C_{12}H_{10}O_5$ $(M)^+$ 234.0528, found 234.0529. Anal. Calcd for $C_{12}H_{10}O_5$: C, 61.54; H, 4.30. Found: C, 61.17; H, 4.11.

2-Carbethoxypsoralen (4). To a solution of 95 mg (0.41 mmol) of compound **3** in 4 mL of dry THF were added 4 Å sieves and 120 μL (0.82 mmol) of *N*,*N*-dimethylacetamide dimethyl acetal. The solution was stirred for 5 h at room temperature under an argon atmosphere. The reaction mixture was diluted with ethyl acetate, washed with saturated 1 N HCl, saturated $NaHCO_3$, and brine and then dried over Na_2SO_4. The crude product was purified by silica gel chromatography eluting with a gradient of 3:1 to 1:1 hexanes/ethyl acetate to afford 69 mg (65%) of a white solid. Recrystallization from hexanes/ethyl acetate afforded white needles: mp 221−222 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (t, 3 H, J = 7.2), 4.46 (q, 2 H, J = 7.2), 6.42 (d, 1 H, J = 9.6), 7.55 (s, 1 H), 7.57 (s, 1 H), 7.79−7.82 (m, 2 H); ^{13}C NMR δ 14.3, 61.9, 100.6, 113.2, 115.5, 116.5, 121.8, 124.2, 143.5, 147.6, 153.7, 156.8, 158.9, 160.2; HRMS (EI) calcd for $C_{14}H_{10}O_5$ $(M)^+$ 258.0528, found 258.0529. Anal. Calcd for $C_{14}H_{10}O_5$: C, 65.12; H, 3.90. Found: C, 65.05; H, 3.72.

2′-Carboxypsoralen (5). A solution of 0.27 g (1.06 mmol) of compound **4** and 10 mL of aqueous 1 N NaOH in 10 mL of methanol was heated to reflux for 30 min. The solvents were removed, and the crude product was dissolved in water and acidified to pH 1 with 1 N HCl. The resulting precipitate was filtered, washed with cold 1 N HCl and ether, and then dried overnight to afford 0.19 g (77%) of a yellowish-white solid: mp 264−266 °C dec; ^{1}H NMR (300 MHz, DMF-d_7) δ 6.51 (d, 1 H, J = 9.6), 7.79 (s, 1 H), 7.83 (s, 1 H), 8.23 (s, 1 H), 8.26 (d, 1 H, J = 9.6); ^{13}C NMR δ 100.9, 114.4, 116.0, 117.8, 124.1, 125.7, 145.7, 149.7, 154.9, 157.8, 160.9, 160.9; HRMS (EI) calcd for $C_{12}H_6O_5$ $(M)^+$ 230.0215, found 230.0214.

5′-*O*-[2′-Psoralenyl-]-3′-*O*-acetylthymidine (7). To a solution of 80 mg (0.35 mmol) of compound **5** in 1.5 mL of dry DMF and 1.5 mL of dry pyridine was added 150 mg (0.53 mmol) of 3′-*O*-acetylthymidine (**6**), and the suspension was sonicated for 5 min. To the milky white solution were added 170 mg (0.88 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and 5 mg (0.35 mmol) of DMAP. The solution was stirred at room temperature under an argon atmosphere for 24 h. The solvent was concentrated, dissolved in CH_2Cl_2, washed with 1 N HCl and saturated $NaHCO_3$, and then dried over $MgSO_4$. The crude product was purified by silica gel chromatography, eluting with a gradient of 100:1 to 50:1 dichloromethane/methanol to afford 106 mg (61%) of a white foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.70 (d, 3 H, J = 1.0), 2.14 (s, 3 H), 2.39 (ddd, 1 H, J = 6.6, 8.7, 14), 2.55 (ddd, 1 H, J = 1.4, 5.5, 14), 4.34−4.40 (m, 1 H), 4.64−4.71 (m, 2 H), 5.36 (app d, 1 H, J = 6.4), 6.40 (dd, 1 H, J = 5.5, 8.7), 6.43 (d, 1 H, J = 9.7), 7.39 (d, 1 H, J = 1.2), 7.48 (s, 1 H), 7.67 (s, 1 H), 7.80 (d, 1 H, J = 9.7), 7.83 (s, 1 H), 9.01 (br s, 1 H); ^{13}C NMR δ 12.4, 20.9, 37.4, 65.1, 74.6, 82.1, 85.1, 100.4, 111.5, 114.6, 115.9, 116.9, 122.2, 123.9, 134.7, 143.3, 146.5, 150.3, 154.2, 156.8, 158.1, 159.9, 163.3, 170.5; UV_{max} (MeOH) 334, 264 nm; HRMS (FAB^+, NBA) calcd for $C_{24}H_{20}O_{10}N_2$ $(M + H)^+$ 497.1196, found 497.1203.

2-Carbomethoxypsoralen−3′-*O*-Acetylthymidine *Cis*-Syn Photoproduct 8. A solution of 20 mg (0.04 mmol) of compound **7** in 40 mL of dry CH_3CN was deaerated with argon bubbling for 30 min. Acetone (2 mL) was added, and the solution was irradiated with 300 nm light in a 16-bulb Rayonet photoreactor for 3 h at room temperature. Removal of the solvents and adsorption of the crude material onto silica gel using methanol as the solvent effected lactone ring opening. The absorbed compound was loaded on top of a silica gel column and purified by eluting with a gradient of 100:1 to 50:1 dichloromethane/methanol to afford 6 mg (24%) of a clear foam: ^{1}H NMR (300 MHz, acetone-d_6) δ 1.76 (s, 3 H), 2.01−2.05 (m, 1 H), 2.07 (s, 3 H), 2.13 (ddd, 1 H, J = 2.0, 5.9, 14), 3.71−3.73 (m, 2 H), 3.88 (s, 3 H), 3.90−3.92 (m, 1 H), 4.11 (t, 1 H, J = 5.4, (-OH)), 4.24 (s, 1 H), 4.97 (d, 1 H, J = 2.0), 5.20−5.22 (m, 1 H), 6.20 (dd, 1 H, J = 5.9, 9.8), 6.24 (d, 1 H, J = 9.3), 6.90 (s, 1 H), 7.39 (s, 1 H), 7.90 (d, 1 H, J = 9.3); ^{13}C NMR ($CDCl_3$) δ 21.0, 22.9, 35.8, 46.6, 53.7, 55.0, 57.3, 62.5, 74.3, 84.1, 84.9, 87.8, 99.3, 113.8, 114.4, 121.5, 125.8, 143.2, 150.4, 156.3, 160.5, 164.0, 169.2, 169.7, 170.7; UV_{max} (MeOH) 324, 293; HRMS (FAB^+, 3-NBA) calcd for $C_{25}H_{24}O_{11}N_2$ $(M + H)^+$ 529.1458, found 529.1456.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **5**, **7**, and **8** (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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