An Approach to Photolabile, Fluorescent Protecting Groups

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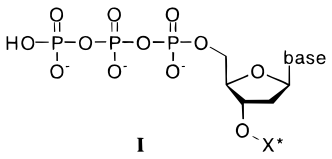
Received February 12, 1997[⊗]

The photolabile and fluorescent system **1** was prepared via a convergent route which involved thymidine 3′-functionalized with a proline residue. Photodecomposition of **1** was examined using 360 nm irradiation. Without any additives, 5′-silylated thymidine was not formed, but the N_{α}-deprotected cyclization precursor **II** accumulated instead. However, in the presence of ethanolamine, 5′-silylated thymidine formed at a rate which increases with the concentration of ethanolamine.

For several years we[1] and others[2−6] have been working on closely related chemical methods for sequencing DNA. Central to our approach are nucleoside triphosphates **I**. These are identical to the natural deoxynucleosides, except that they bear chemically labile 3′-protecting groups that also fluoresce at distinctive wavelengths to spectroscopically encode for the aromatic heterocyclic component of the nucleoside. Ultimately, we hope to establish that solid-supported DNA can be sequenced by a series of addition, detection, and deprotection steps. Specifically, the primer-template complex would be subjected to (i) biocatalytic addition of the appropriate nucleoside triphosphate analog; (ii) fluorescence detection to determine which base added to the primer (hence the complement in the template strand); and (iii) 3′-deprotection. Each cycle of these three steps would extend the primer by one base. Increased throughput of sequence information would follow if 100−300 bases could be sequenced because many DNA fragments could be processed in parallel on a single chip. The time and labor intensive aspects of gel electrophoresis in sequencing thereby would be circumvented.

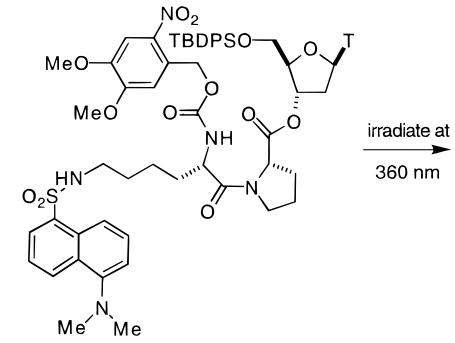


I

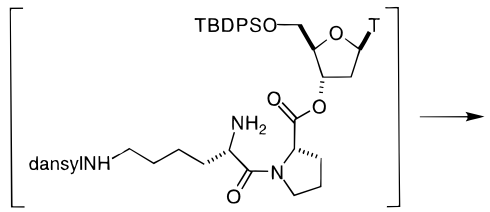
where X* has fluorescence that enables the base to be identified, and has chemical lability so that it can be removed from the 3'-*O* under conditions which do not denature double stranded DNA

base = adenine, cytosine, guanine, or thymine

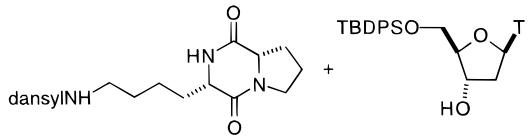
Scheme 1. Photochemical Activation of a Fluorescently-Tagged Safety Catch Mechanism



3'{NVOC-Lys(dansyl)-Pro}-T **1**



II



2 **3**

Many hurdles must be surmounted to realize the sequencing method outlined above. First amongst these are construction of nucleoside triphosphates **I** with labile and fluorescent 3′-protecting groups. This paper describes a synthesis of compound **1** which has a fluorescent tag supported on a safety catch linker that can be rendered labile via a photochemical trigger. The anticipated sequence of events that would follow photochemical activation are shown in Scheme 1. Irradiation at approximately 360 nm was anticipated to remove the NVOC (3,4-dimethoxy-6-nitrobenzyloxycarbonyl) protecting group giving intermediate **II** which would tend to ring close to give the diketopiperazine **2** and the free nucleoside **3**. In this way the 3′-terminus of the nucleoside would be unmasked and the fluorescent groups would be cleaved in a mild photochemical process that would not significantly perturb DNA strands.

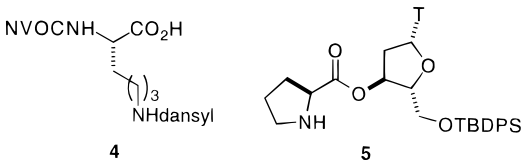
Our synthesis of the target molecule **1** consisted of two branches that converged in the final step. The first branch began with a series of routine manipulations of lysine protected at the α- and *ω*-termini by *tert*-butyloxycarbonyl and benzyl groups, respectively {*i.e.* BOC-Lys(Bn)}. Hydrogenolysis to remove the benzyl group and dansylation gave BOC-Lys(dansyl) in 91% overall yield. Removal of the BOC protecting group using TFA and

[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1997.

(1) Metzker, M. L.; Raghavachari, R.; Richards, S.; Jacutin, S. E.; Civitello, A.; Burgess, K.; Gibbs, R. A. *Nucl. Acids Res.* **1994**, *22*, 4259−67.
(2) Cheeseman, P. C. U.S. Patent US 5,302,509, 1994.
(3) Wilhelm, A. German Patent DE 41 41 178 A1, 1993.
(4) Rosenthal, A.; Close, K.; Brenner, S. WO 93/21340, 1993.
(5) Tsien, R. Y.; Ross, P.; Fahnestock, M.; Johnston, A. J. WO 91/06678, 1991.
(6) Canard, B.; Sarfati, R. S. *Gene* **1994**, *148*, 1−6.

S0022-3263(97)00260-0 CCC: $14.00

replacement of this with an NVOC group[7,8] (Na_2CO_3, dioxane) gave NVOC-Lys(dansyl) **4** in 54% yield. The second branch of the synthesis involved coupling 5′-(*tert*-butyldiphenylsilyl)thymidine (5′-TBDPS-T) with *N*-(α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl)-L-proline (DDZ-Pro) and then deprotection (5% TFA in CH_2Cl_2) to give compound **5** in 50% yield. It was necessary to use the relatively acid labile DDZ group[9] so that the proline nitrogen could be deprotected cleanly; BOC protection and TFA cleavage gave some desilylation of the nucleoside fragment. The synthesis culminated with the coupling of fragments **4** and **5** (EDCI, HOBt, NMM, CH_2Cl_2, 89%) to give the target molecule **1**.



4 **5**

Photolysis of compound **1** was performed using a relatively inexpensive strip light that generates irradiation in the 360 nm region; these very useful lamps are available from Southern New England Ultraviolet Company. Progress of the reaction in 7:3 acetonitrile:water was monitored by analytical HPLC. Using our weak light source, the half life for decomposition of compound **1** was determined to be approximately 30 min. Analysis of the products revealed that neither 5′-TBDPS-T or the diketopiperazine **2** was formed even after 5 h irradiation. In fact, the only new peak on the HPLC trace was an "intermediate" with a retention time and UV spectrum very similar to 5′-TBDPS-T. Formation of 5′-TBDPS-T**3** and the diketopiperazine **2** were observed (as proven by HPLC analyses of authentic samples), however, when the irradiation was performed with ethanolamine as an additive. Figure 1 shows HPLC traces reflecting the composition of the mixture immediately after 30 min irradiation times in the presence of 0 to 100 equiv of ethanolamine. As the amount of ethanolamine in the solution increases, so does the amount of 5′-TBDPS-T and diketopiperazine **2**. When 100 equiv of ethanolamine were used, no significant amount of the intermediate was observed. In other experiments we observed that decay of the intermediate and concomitant increase of 5′-TBDPS-T and diketopiperazine **2** occurs in the absence of light, *i.e.* the transformation is a thermal one. Conversion of the intermediate to 5′-TBDPS-T was complete after 4 h in the presence of 10 equiv of ethanolamine or more.

Table 1 relates the number of equivalents of ethanolamine used to the percentage of the starting material **1** that was consumed after 30 min irradiation and 4 h equilibration and the percentage conversion of that material into 5′-TBDPS-T. Several conclusions can be drawn from these data. First, the extent of photodecomposition of **1** was 50 ± 4% throughout and does not depend on the amount of ethanolamine used. Similarly, the percent conversion of **1** into 5′-TBDPS-T after thermal equilibration is 55−60%, irrespective of the ethanolamine concentration. However, 5′-TBDPS-T is not formed in the absence of ethanolamine, and the rate of its formation increases with the concentration of that base (Figure 1). In other work, we have observed that hydrazine also promotes conversion of the same intermediate into 5′-TBDPS-T, though not so effectively.

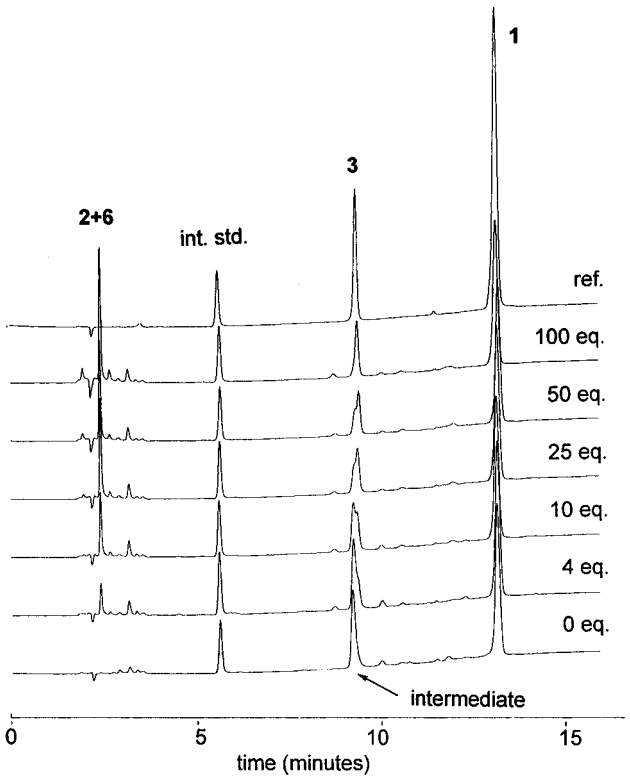


Figure 1. HPLC traces showing the photodecomposition of **1** in the presence of different amounts of ethanolamine. All samples were taken at an irradiation time corresponding approximately $t_{1/2}$ for the photodecomposition (30 min).

Table 1. Photodecomposition of 1 into 5′-TBDPS-T as a Function of Added Ethanolamine

ethanolamine (equiv relative to **1**)	photodecomposition of **1** (%)[a]	conversion of **1** into 5′-TBDPS-T (%)[a,b]
0	47	*c*
4	54	*d*
10	52	55
25	48	58
50	48	60
100	52	57

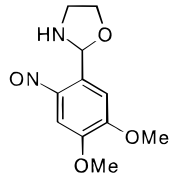
[a] After 30 min irradiation and 4 h equilibration time (to allow the intermediate to decompose into 5′-TBDPS-T); throughout the table, the data quoted is for the average of two experiments measured with 5-nitroindole as an internal standard (shown to be inert under these conditions). [b] Conversion is based upon percentage of photodecomposed **1** that is transformed into TBDPS-T. [c] 5′-TBDPS-T was not formed in this experiment. [d] Conversion of the intermediate formed after photolysis into 5′-TBDPS-T was incomplete after 4 h.

Photolysis of compound **1** gave the free amine intermediate **II** as demonstrated by HPLC and MALDI-MS analyses. Intermediate **II** was shown to be stable for at least three days in $MeCN_{(aq)}$ at ambient temperature. Addition of ethanolamine to this intermediate, however, rapidly generated the diketopiperazine **2** and 5′-TBDPS-T **3**. These experiments, coupled with those features in Figure 1 and Table 1, indicate that addition of ethanolamine is necessary for the formation of the products **2** and **3** and that it does not matter if the amine is added before or after photolysis.

At this stage we hypothesized that ethanolamine promotes the cyclization of the intermediate **II** and simultaneously reacts with the nitrosoaldehyde byprod-

(7) Patchornik, A.; Amit, B.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 6333−5.
(8) Amit, B.; Zehavi, U.; Patchornik, A. *J. Org. Chem.* **1974**, *39*, 192−6.
(9) Birr, C.; Lochinger, W.; Stahnke, G.; Lang, P. *Liebigs Ann. Chem.* **1972**, *763*, 162−72.

uct to form the oxazolidine **6**. To probe for the final byproduct from the photodecomposition of **1** (which we presume is the oxazolidine **6**), *N*-(3,4-dimethoxy-6-nitrobenzyloxycarbonyl)ethanolamine **7** was synthesized and then photolyzed in the presence of ethanolamine. This solution was analyzed by HPLC and was also coinjected with a sample from the photolysis of compound **1**. In this way it was shown that the diketopiperazine **2** and the putative oxazolidine **6** coeluted under the HPLC conditions that were used throughout this study.



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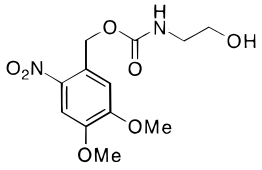


7

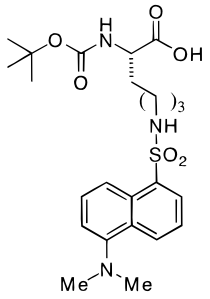
UV spectra of the diketopiperazine **2** and the putative oxazolidine **6** give additional information. The two compounds have different spectra; that of the diketopiperazine **2** shows a λ_{max} at 220 nm, while oxazolidine **6** has an absorbance minimum at 220 nm and a λ_{max} at 264 nm. The HPLC peak detected in the photolysis of compound **1** has a UV spectrum similar to that of pure diketopiperazine **2**. However, we suspect that this peak is really derived from coelution of **2** and **6**, but oxazolidine **6** has a much lower molar absorptivity (ϵ). Unfortunately, an attempt to isolate and characterize the oxazolidine was unsuccessful, so we were unable to measure the relative molar absorptivities of **2** and **6**.

Safety-catch deprotection via formation of diketopiperazines is not novel to this study since Bray and co-workers have used a similar scheme to generate acid labile linkers for syntheses of peptides on pins.[10,11] This work is the first, however, to demonstrate proof of concept for photochemical triggering of a safety catch bearing a fluorescent flag. With respect to our proposed sequencing procedure, the dansylated system is a prototype with convenient physical properties for the chemical synthesis. However, the fluorescence of the dansyl group is relatively weak, so this is a suboptimal choice with respect to detection sensitivity. Moreover, compound **1** does not afford 5′-TBDPS-T with high conversion (55−65%). Though it is possible that diminished yields in the conversion of the protected nucleoside into the 3′-unprotectected was due to inefficient conversion to the intermediate **II**, we regard photoreactions of the dansyl group prior to the desired sequence of events to be a more likely explanation. The reason for this conclusion is that dansyl groups are known to decompose to highly reactive species under these conditions,[12] and because we were unable to observe any stable byproducts other than those already discussed. Our thoughts are now focused upon refinements of **1** wherein the dansyl functionality is replaced by other fluorescent groups with higher extinction coefficients and for which more efficient photodeprotection can be achieved.

Experimental Section

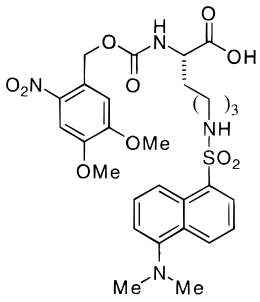
General Procedures. High field NMR spectra were recorded on a Varian XL-400 (^{1}H at 400 MHz), UnityPlus 300 (^{1}H at 300 MHz, ^{13}C at 75.4 MHz), or Varian XLAA 200 (^{1}H at 200 MHz, ^{13}C at 50 MHz) spectrometer. ^{1}H and ^{13}C chemical shifts are reported in δ (ppm). Multiplicities in ^{1}H NMR are reported as (br) broad, (s) singlet, (d) doublet, (t) triplet, and (m) multiplet. Thin layer chromatography was performed using silica gel 60 F_{254} plates from Whatman. Flash chromatography was performed using SP Silica Gel 60 (230−600 mesh ASTM). CH_2Cl_2 was distilled from $LiAlH_4$. Other chemicals were purchased from commercial suppliers and used as received unless otherwise specified.

***N*$_\alpha$-(*tert*-Butoxycarbonyl)-*N*$_\epsilon$-(5-(dimethylamino)-1-naphthalenesulfonyl)-L-lysine.** Boc-Lys-OH (4 g, 14 mmol) was dissolved in 50 mL of CH_2Cl_2. Et_3N (6 mL, 41.1 mmol, 3 equiv) was added at 25 °C, followed by dansyl chloride (4.4 g, 16 mmol, 1.2 equiv). After 12 h stirring under N_2, the reaction mixture was concentrated under vacuum, and the residue was purified by flash column chromatography (eluant 1:10 MeOH/



$CHCl_3$, $R_f = 0.28$) to give 6.6 g (91%) of the product Boc-Lys-(dansyl)-OH. ^{1}H NMR (300 MHz, CD_3OD) δ 8.53 (d, $J = 8.7$ Hz, 1H), 8.34 (d, $J = 8.7$ Hz, 1H), 8.17 (d, $J = 7.5$ Hz, 1H), 7.65−7.5 (m, 2H), 7.25 (d, $J = 7.5$ Hz, 1H), 4.01 (br, 1H), 3.95−3.75 (m, 1H), 3.25−3.08 (m, 2H), 2.86 (s, 6H), 2.9−2.78 (m, 2H), 1.78 (br, 1H), 1.69−1.15 (m, 5H), 1.41 (s, 9H). ^{13}C NMR (75 MHz, CD_3OD) δ 176.1, 157.9, 152.9, 136.9, 131, 130.8, 130.1, 129, 124.3, 120.5, 116.4, 79.4, 54.6, 45.8, 43.5, 32.1, 30, 28.7, 23.8. IR (KBr) 3324, 2977, 2867, 1698, 1575, 1506, 1367, 1317, 1162, 1143 cm^{-1}. FAB-MS *m*/*z* 479 [M]$^+$.

***N*$_\alpha$-(3,4-Dimethoxy-6-nitrobenzyloxycarbonyl)-*N*$_\epsilon$-(5-(dimethylamino)-1-naphthalenesulfonyl)-L-lysine (4).** Boc-Lys(dansyl)-OH (0.1 g, 0.19 mmol) was dissolved in 0.5 mL of CH_2Cl_2, and 2 mL of 50% TFA/CH_2Cl_2 was added at 25 °C. After 2 h of stirring, the reaction mixture was concentrated under vacuum. Repeated evaporation with MeOH/CH_2Cl_2 gave 0.14 g of the crude N_α-deprotected amino acid. This was used in the following step without further purification.



The amino acid formed as described above (0.14 g, 0.4 mmol) was dissolved in 7 mL of H_2O and $NaHCO_3$ (0.09 g, 1.1 mmol, 3 equiv) was added. NVOCCl (3,4-dimethoxy-6-nitrobenzyloxycarbonyl chloride)[8] (0.2 g, 0.7 mmol, 2 equiv) in dioxane (10 mL) was added at 25 °C under N_2, and the solution was stirred for 12 h. The solution was concentrated under high vacuum. The residue was dissolved in EtOAc and then acidified with 10 mL of 0.5 N HCl, and the organic layer was washed with water then brine and dried over Na_2SO_4. After evaporation of solvent, the residue was purified via flash chromatography (eluant 1:10 MeOH/$CHCl_3$, $R_f = 0.14$) to give a 0.12 g of the product **4** (52% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 8.53 (d, $J = 8.7$ Hz, 1H), 8.33 (d, $J = 8.7$ Hz, 1H), 8.17 (d, $J = 7.2$ Hz, 1H), 7.73 (s, 1H), 7.6−7.5 (m, 2H), 7.25 (d, $J = 8.1$ Hz, 1H), 7.17 (s, 1H), 5.47 (d, $J = 15.6$ Hz, 1H), 5.40 (d, $J = 15.6$ Hz, 1H), 3.98−3.83 (m, 2H), 3.92 (s, 3H), 3.89 (s, 3H),

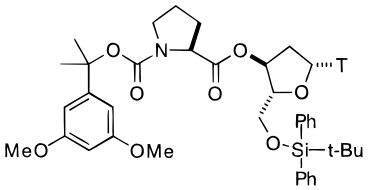
(10) Bray, A. M.; Maeji, N. J.; Valerio, R. M.; Campbell, R. A.; Geysen, H. M. *J. Org. Chem.* **1991**, *56*, 6659−66.

(11) Bray, A. M.; Lagniton, L. M.; Valerio, R. M.; Maeji, N. J. *Tetrahedron Lett.* **1994**, *35*, 9079−82.

(12) Pillai, V. N. R. In *Organic Photochemistry*; Padwa, A., Ed.; Marcel Dekker, Inc.: New York, 1987; Vol. 9, pp 225−323.

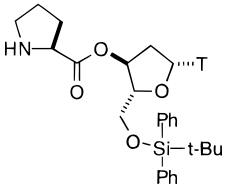
3.69−3.52 (m, 1H), 2.86 (s, 6H), 2.9−2.75 (m, 2H), 1.75−1.57 (br, 1H), 1.58−1.15 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 175.3, 155.9, 153.6, 151.4, 147.8, 139.1, 134.6, 130.2, 129.5, 129.4, 129.2, 128.1, 123.2, 123.1, 118.8, 115.2, 109.7, 107.9, 77.2, 63.8, 56.4, 56.2, 53.9, 45.3, 42.6, 28.7, 22. IR (KBr) 3330, 2941, 1711, 1582, 1519, 1463, 1439, 1328, 1278, 1220, 1161, 1142, 1069 cm^{-1}. MS *m/z* 619 [M + H]$^+$. HRMS (FAB, NaI): calcd for [M + Na]$^+$ 641.1893; found 641.1924.

3′-*O*-{*N*-(α,α-Dimethyl-3,5-dimethoxybenzyloxycarbonyl)-L-proline}-5′-*O*-(*tert*-butyldiphenylsilyl)thymidine. DDZ-Pro-OH (0.5 g, 1.5 mmol) and 5′-TBDPS protected thymidine (0.78 g, 1.6 mmol, 1.1 equiv) were dissolved in 10 mL of CH_2Cl_2, and then *N*-hydroxybenzotriazole (0.4 g, 3 mmol, 2.0 equiv) and *N*-methylmorpholine (0.81 mL, 5 equiv) were added. The solution was stirred for 5 min under N_2 at 0 °C, and then dicyclohexylcarbodiimide (0.61 g, 2.9 mmol, 2.0 equiv) was added and reaction mixture was stirred 12 h at 25 °C. A colorless precipitate formed (presumably a urea derivative) and was removed by filtration. The solvent was evaporated, the residue was dissolved in 10 mL of EtOAc and acidified with 0.5 N HCl, and the organic layer was washed with water then brine and dried over Na_2SO_4. After evaporation of the solvent,



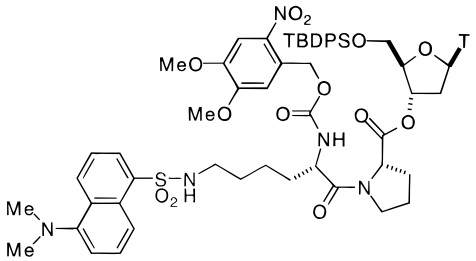
the residue was purified by flash chromatography (1:10 MeOH/$CHCl_3$, R_f = 0.51) to give a 0.6 g (50%) of the product, 3′-*O*-(DDZ-Pro)-5′-*O*-TBDPS-T as a mixture of two rotamers {^{1}H and ^{13}C NMR spectra show two sets of peaks in $CDCl_3$ (1:1) and in CD_3OD (3:1)}. ^{1}H NMR (300 MHz, CD_3OD) δ 7.82 (s, 1H), 7.72−7.62 (m, 4H), 7.52 (s, 1H), 7.48−7.33 (m, 6H), 6.49 (s, 1H), 6.48 (s, 1H), 6.36−6.22 (m, 2H), 5.59−5.42 (m, 1H), 4.5−4.42 (m, 1H), 4.12−4.04 (m, 1H), 4.02−3.88 (m, 2H), 3.71 (s, 3H), 3.69 (s, 3H), 3.8−3.32 (m, 2H), 2.46−2.16 (m, 3H), 2.14−1.8 (m, 3H), 1.75 (s, 3H), 1.67 (d, *J* = 14.1 Hz, 3H), 1.59 (d, *J* = 55.8 Hz, 3H), 1.07 (s, 9H). ^{13}C NMR (75 MHz, CD_3OD) δ 173.6 166.1, 162.2, 162.1, 154.8, 152.1, 150.1, 136.8, 136.6, 136,4, 134.3, 133.5, 131.3, 131.2, 129.1, 129, 111.9, 103.9, 99.6, 99.3, 85.9, 85.6, 83.5, 79.5, 76.8, 65.3, 60.4, 60.3, 55.8, 55.7, 47.8, 38.6, 30.2, 27.5, 24.5, 20.2, 12.3. IR (KBr) 3072, 2958, 2935, 2856, 1747, 1668, 1596, 1486, 1398, 1157, 1114 cm^{-1}. FAB-MS *m/z* 822 [M + Na]$^+$. HRMS (FAB, NaI): calcd for [M + Na]$^+$ 822.3398; found 822.3409.

3′-*O*-L-Prolinyl-5′-(*O*-*tert*-butyldiphenylsilyl)thymidine (5). 3′-*O*-(DDZ-Pro)-5′-*O*-TBDPS-T (0.22 g, 0.3 mmol) in 1 mL of CH_2Cl_2 and 5% TFA/CH_2Cl_2 (4.4 mL) were mixed at 25 °C. After 1 h stirring, the reaction was concentrated under



vacuum. The residue was purified via flash chromatography (1:10 MeOH/$CHCl_3$, R_f = 0.31) to give 0.15 g (97%) of the product **5**. ^{1}H NMR (300 MHz, CD_3OD) δ 7.89 (s, 1H), 7.76−7.6 (m, 4H), 7.53 (s, 1H), 7.49−7.32 (m, 6H), 6.32(dd, *J* = 5.7, 8.7 Hz, 1H), 5.61 (brd, *J* = 6.3 Hz, 1H), 4.49 (t, *J* =7.8 Hz, 1H), 4.19 (brd, *J* = 1.8 Hz, 1H), 4.08−3.95 (m, 2H), 3.46−3.33 (m, 2H), 2.61−2.34 (m, 3H), 2.22−1.96 (m, 3H), 1.5 (s, 3H), 1.13 (s, 9H). ^{13}C NMR (75 MHz, CD_3OD) δ 169.9, 166.1, 152.2, 136.9, 136.7, 136.4, 134.3, 133.5, 131.3, 131.2, 129.1, 112.1, 86.0, 85.9, 78.5, 65.4, 60.6, 47.3, 38.4, 29.3, 27.5, 24.6, 20.2, 12.2. IR (KBr) 3382, 3052, 2958, 2931, 2857, 1686, 1477, 1203, 1134, 1113 cm^{-1}. FAB-MS *m/z* 578 [M + H]$^+$.

3′-*O*-{N_α-(3,4-Dimethoxy-6-nitrobenzyloxycarbonyl)-N_ϵ-(5-(dimethylamino)-1-naphthalenesulfonyl)-L-lysine-L-proline}-5′-*O*-(*tert*-butyldiphenylsilyl)thymidine (1). The acid **4** (0.16 g, 0.27 mmol, 1.05 equiv), amine **5** (0.15 g, 0.25 mmol), and *N*-hydroxybenzotriazole (0.07 g, 0.5 mmol, 2 equiv) were dissolved in 2 mL of CH_2Cl_2, and *N*-methylmorpholine (0.08 mL, 0.75 mmol, 3 equiv) was added. The solution was stirred at 0 °C under N_2 for 5 min, and then 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (0.01 g, 0.5 mmol, 2 equiv) was added. Reaction mixture was stirred for 12 h at 25 °C. The solvent was evaporated, and the residue was dissolved in 10 mL of EtOAc, washed with water then brine, and dried over Na_2SO_4. The solvent was removed, and the residue was purified via flash chromatography (1:10



MeOH/$CHCl_3$, R_f = 0.61) to give a 0.26 g (89%) of the product **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (br, 1H), 8.51 (d, *J* = 8.4 Hz, 1H), 8.33 (d, *J* = 8.8 Hz, 1H), 8.18 (d, *J* = 7.2 Hz, 1H), 7.68 (s, 1H), 7.67−7.58 (m, 4H), 7.54−7.32 (m, 9H), 7.21 (d, *J* = 6.4 Hz, 1H), 6.99(s, 1H), 6.38−6.32 (m, 1H), 5.86−5.77 (m, 2H), 5.57−5.4 (m, 3H), 4.5−4.4 (m, 1H), 4.08−4.0 (br, 1H), 4.0−3.95 (br, 2H), 3.93 (s, 3H), 3.92 (s, 3H), 3.95−3.82 (m, 1H), 3.8−3.64 (m, 2H), 2.92 (s, 6H), 2.95−2.77 (m, 2H), 2.35−1.9 (m, 6H), 1.82 (br, 1H), 1.7−1.32 (m, 5H), 1.46 (s, 3H), 1.06 (s, 9H). IR (KBr) 3072, 2933, 2854,1737, 1673, 1519, 1438, 1203, 1178, 1137 cm^{-1}. MS *m/z* 1178 [M + H]$^+$. HRMS (MALDI/TOF): calcd for [M + Na]$^+$ 1200.4395; found 1200.4403.

General Procedure for the Photolysis Experiments. Throughout the study, the solvent used was a 7:3 acetonitrile: water mixture. 5-Nitroindole was used as an internal standard prior to photolysis; other experiments showed 5-nitroindole was inert under the reaction conditions. The sample was placed in an Eppendorf tube (Brinkmann Instruments, Inc., Westbury, NY) and irradiated at *ca.* 1 cm above the UV-fluorescent tube. After photolysis, each sample was analyzed by HPLC immediately and analyzed again after 4 h equilibration time.

General Procedure for the HPLC Analyses. All samples were analyzed on an analytical HPLC (SSI, State College, PA) equipped with a Reliasil C18 column (25 cm × 4.6 mm, 5 μm particle size) (Column Engineering, Inc., Ontario, CA); a diode-array UV detector was used, although most of the measurements were made at 220 nm. The separations were performed using a linear gradient (mobile phase A: 5% B in 0.1% TFA/water; mobile phase B: 0.1% TFA/acetonitrile; 50 to 100% B in 10 min, then at 100% B for 6 min) with a flow rate of 1 mL/min.

Acknowledgment. We thank Mr Alex J. Zhang for performing the MALDI-MS measurements. Financial support was provided by The Texas Advanced Technology Program and The Robert A. Welch Foundation, and K.B. thanks the NIH for a Research Career Development Award and the Alfred P. Sloan Foundation for a fellowship. NMR facilities were provided by the NSF via the chemical instrumentation program.

Supporting Information Available: Synthesis scheme of **1**, **4**, and **5**; copies of ^{1}H NMR spectra for all the compounds; data for photodecomposition of **1** giving $t_{1/2}$; HPLC traces showing thermal decomposition of the intermediate; HPLC traces showing the coelution of compounds **2** and **6**; UV spectra of **2**, **6**, and the HPLC peak derived from coelution of **2** and **6** upon photolysis of **1** (10 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.

JO9702608