Photochemically and Photoenzymatically Cleavable DNA

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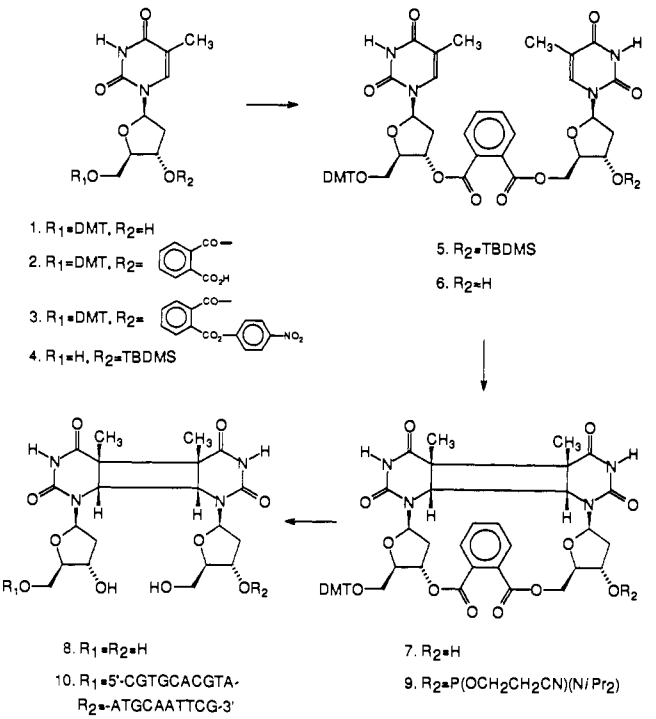
Abstract: Methodology for the synthesis of site-specifically modified DNA has facilitated the physical, chemical, and enzymatic manipulation of DNA, as well as the study of important biological processes and the development of new classes of reagents and drugs. Herein we report the design, synthesis, and characterization of a DNA synthesis building block for the site-specific introduction of a photochemically and photoenzymatically cleavable linkage that is based on the photochemistry and photoenzymology of the cis-syn thymidine dimer. The phosphoramidite building block was synthesized in six steps from 5'-*O*-DMT-thymidine and 3'-*O*-TBDMS-thymidine and used to prepare a 22-mer containing a central dimer by standard automated DNA synthesis. Direct 254-nm photolysis of the 22-mer led to formation of the expected cleavage products in quantitative yield, as did visible light photolysis in the presence of *Escherichia coli* photolyase. An appreciable amount of cleavage (20%) by photolyase could also be induced by a single camera flash. The ability to photochemically and photoenzymatically cleave DNA site-specifically might find useful applications in the study of nucleic acid conformational transitions and enzymatic reactions, as well as the activation of prodrug forms of antisense oligonucleotides and ribozymes.

The ability to synthesize site-specifically modified DNA has greatly aided the study of DNA–protein interactions[1] and mutagenesis[2] and has led to the design and synthesis of new classes of reagents and drugs.[3] DNA can also be site-specifically modified to facilitate its physical, chemical, and enzymatic manipulation. Recently, DNA synthesis building blocks have been developed for the site-specific incorporation of chemoselectively cleavable internucleotide linkages, such as phosphorothioates[4] and phosphoramidates.[5] Cleavage of the former linkage results in oligomers with sulfhydryl termini, whereas cleavage of the latter linkage results in oligomers with amino termini, both of which would be undesirable for applications requiring further enzymatic processing. A DNA synthesis building block has been developed for incorporating a photochemically inducible, base cleavable abasic site by taking advantage of nitroveratrole photochemistry.[6] This method, though leading to enzymatically processible termini, would be unsuitable for applications requiring instantaneous strand cleavage or the absence of side products. Herein, we report a DNA synthesis building block (compound **9**, Scheme I) for the site-specific introduction of a photochemically and photoenzymatically cleavable linkage that has such attributes and, in addition, leads to no side products, making it potentially useful for in vivo applications.

The design of the building block was based on the known photochemical and photoenzymatic reversibility of cis-syn cyclobutane pyrimidine dimer formation[7] (Figure 1) and the realization that reversal of a dimer lacking the intradimer phosphate would result in strand cleavage. That photochemical reversal of such a dimer would indeed lead to strand cleavage was demonstrated a number of years ago by Lewis and Hanawalt, who were investigating the possibility that pyrimidine dimer formation might have functioned as a primitive form of DNA ligation.[8] They demonstrated that irradiation with >290-nm light caused the "ligation" of dT_{10} on a poly(dA) template and that subsequent irradiation with 254-nm light resulted in cleavage of the UV-induced linkages. They were unable, however, to detect photocleavage of these linkages by the cis-syn dimer-specific repair enzyme, *Escherichia coli* photolyase.[9] More recently it has been found that photolyase can repair a cis-syn thymine dimer which lacks the sugar and phosphate moieties with low efficiency,[10] and that it does not appear to contact the phosphate between the two thymidines of a cyclobutane dimer in duplex DNA.[11] Together, these results suggested that there might be conditions under which a dimer lacking the internucleotide phosphate could indeed be photoenzymatically cleaved.

Scheme I



Experimental Section[12]

General Procedures. All reactions were conducted under anhydrous conditions and an argon atmosphere. Tetrahydrofuran, methylene chloride, and pyridine were dried by distillation from the appropriate drying agents. ^1H NMR and ^{13}C NMR spectra were acquired on either a Varian Gemini or XR-600 spectrometer, and ^{31}P NMR spectra were

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(12) Abbreviations: DMAP, 4-(dimethylamino)pyridine; DMT, 4,4'-dimethoxytrityl; TBDMS, *tert*-butyldimethylsilyl; TBAF, tetrabutylammonium fluoride; THF, tetrahydrofuran; TpT, thymidylyl-(3'→5')-thymidine.

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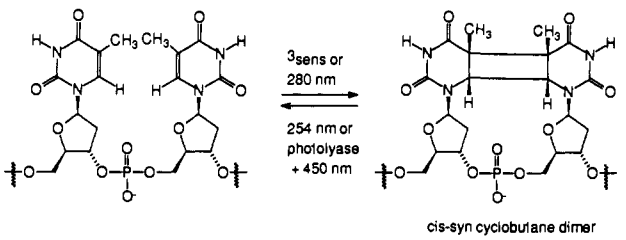

Figure 1. Photochemistry and photoenzymology of a TpT site in DNA.

acquired on a Varian XL-300 spectrometer. Elemental analyses were conducted by Galbraith Laboratories, TN.

Ester Acid 2. 4-(Dimethylamino)pyridine (DMAP) (1.22 g, 10 mmol) and phthalic anhydride (1.48 g, 10 mmol) were added to 5′-*O*-DMT-thymidine[13] (**1**) (4.0 g, 7.34 mmol) dissolved in dry pyridine (15 mL), and the mixture was stirred overnight at room temperature. The pyridine was removed under reduced pressure, and the residue was dissolved in ethyl acetate, washed with saturated aqueous NaCl, dried with $MgSO_4$, and evaporated to dryness under reduced pressure. The crude product was flash chromatographed on a silica gel column (1:9 methanol/ethyl acetate), to give the desired ester acid **2** (3.19 g, 4.62 mmol) as a white powder in 63% yield: ^{1}H NMR (300 MHz, acetone-d_6, referenced to CHD_2COCD_3 at 2.04 ppm) δ 8.58 (m, 1 H, NH), 7.91 (m, 1 H, Ar), 7.76–7.64 (m, 4 H, Ar), 7.52–7.50 (m, 2 H, Ar), 7.40–7.32 (m, 6 H, Ar), 7.28–7.26 (m, 1 H, Ar), 6.92 (d, J = 9 Hz, 4 H, DMT), 6.37 (t, J = 7 Hz, 1 H, H1′), 5.73 (m, 1 H), 4.38 (m, 1 H), 3.78 (s, 6 H, ArOMe), 3.56 (dd, J = 10.5, 3 Hz, 1 H), 3.47 (dd, J = 10, 3 Hz, 1 H), 2.63 (dd, J = 7, 4 Hz, 1 H), 2.63 (dd, J = 7, 4 Hz, 2 H), 1.40 (s, 3 H, TCH_3); IR (KBr) 3061, 1692, 1608, 1508, 1461, 1362, 1251, 1177, 1083, 1033, 829, 755, 701 cm^{-1}; MS (FAB) m/z 692 (M^+, 100), 460 (76), 399 (29), 373 (30). Anal. Calcd for $C_{39}H_{36}O_{10}N_2$: C, 67.62; H, 5.23; N, 4.04. Found: C, 66.81; H, 5.41; N, 4.52.

***p*-Nitrophenyl Ester 3.** A solution of dicyclohexylcarbodiimide (DCC) (1.30 g, 5 mmol) and *p*-nitrophenol (695 mg, 5 mmol) in methylene chloride (10 mL) was added to the acid **2** (4 mmol, 2.77 g) in dry pyridine (10 mL) and stirred overnight at room temperature (15 h). The solvent was then removed under reduced pressure and the residue dissolved in ethyl acetate (35 mL). After removal of the insoluble material by filtration, the ethyl acetate solution was extracted with saturated aqueous NaCl and dried with $MgSO_4$, and the solvent was removed under reduced pressure. The residue was flash chromatographed on a silica gel column (4:1 ethyl acetate/hexane) to give the desired product **3** (2.14 g, 2.64 mmol) as a white foam in 66% yield: R_f = 0.51 (3:2 ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$, referenced to TMS) δ 9.43 (br, s, 1 H, NH), 8.28 (d, J = 9 Hz, 2 H, $ArNO_2$), 7.95 (dd, J = 6, 3.5 Hz, 1 H, phth), 7.86 (dd, J = 5.5, 3 Hz, 1 H, phth), 7.71–7.66 (m, 2 H, Ar), 7.65 (s, 1 H, TH6), 7.45 (d, J = 9 Hz, 2 H, $ArNO_2$), 7.42–7.25 (m, 1 H, Ar), 6.84 (d, J = 9 Hz, 4 H, DMT), 6.51 (dd, J = 9, 5.5 Hz, 1 H, H1′), 5.76 (d, J = 6 Hz, 1 H, H3′), 4.38 (br, s, 1 H), 3.79 (s, 6 H, $ArOCH_3$), 3.51 (m, 2 H), 2.65 (dd, J = 8.5, 6.5 Hz, 1 H), 2.54 (m, 1 H), 1.39 (s, 3 H, TCH_3); ^{13}C NMR (75 MHz, $CDCl_3$, referenced to $CDCl_3$ at 77.0 ppm) δ 166.7, 165.2, 164, 158.9, 155.5, 150.8, 145.7, 144.3, 135.4, 135.2, 135.2, 132.4, 132.1, 131.5, 130.7, 130.2, 130.1, 129.5, 129.5, 128.1, 127.3, 125.5, 122.5, 113.3, 111.9, 87.1, 84.2, 83.8, 63.6, 55.0, 37.4, 11.3; MS (FAB) m/z 814.1 (M + 1, 84), 621.9 (100), 551 (32), 527.6 (65), 454.5 (42), 438.4 (40), 391 (61). Anal. Calcd for $C_{45}H_{35}O_{12}N_3$: C, 66.41; H, 4.83; N, 5.16. Found: C, 65.88; H, 5.20; N, 4.99.

Diester 5. To a well-cooled suspension of sodium hydride (60% by weight in oil, 360 mg, 9 mmol) in dry THF (3 mL) at 0 °C was added a THF solution (5 mL) of 3′-TBDMS-thymidine[14] (**4**) (642 mg, 1.80 mmol). After the mixture was stirred for 45 min at room temperature, a THF solution (5 mL) of the *p*-nitrophenyl ester **3** (1.46 g, 1.8 mmol) was injected, and the reaction mixture was stirred at room temperature for an additional 1.5 h. The excess sodium hydride was quenched at 0 °C with saturated aqueous ammonium chloride solution (2 mL). The reaction mixture was diluted with ethyl acetate, extracted with saturated aqueous NH_4Cl, dried with $MgSO_4$, and evaporated under reduced pressure. The residue was flash chromatographed on a silica gel column (3:2 ethyl acetate/hexane) to give the desired product **5** (940 mg, 0.91 mmol) as a white foam in 51% yield: R_f = 0.43 (4:1 ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$, referenced to TMS) δ 9.12 (s, 2 H, NH), 7.77–7.73 (m, 2 H, phth), 7.65 (s, 1 H, TH6), 7.62–7.56 (m, 2 H, phth), 7.42–7.22 (m, 9 H, DMT), 6.85 (d, J = 9 Hz, 4 H, DMT), 6.44 (dd, J = 9.5, 5.5 Hz, 1 H, H1′), 6.30 (t, J = 6.5 Hz, 1 H, H1′), 5.71 (d, J = 5.5 Hz, 1 H), 4.61 (dd, J = 12, 4 Hz, 1 H), 4.52 (dd, J = 12, 3.5 Hz, 1 H), 4.43 (m, 1 H), 4.35 (s, 1 H), 4.18 (dd, J = 8, 4 Hz, 1 H), 3.80 (s, 6 H, $ArOCH_3$), 3.53 (m, 2 H), 2.65 (dd, J = 13.5, 5.5 Hz, 1 H), 2.53 (m, 1 H), 2.31 (m, 1 H), 2.14 (m, 1 H), 1.67 (s, 3 H, TCH_3), 1.34 (s, 3 H, TCH_3), 0.87 (s, 9 H, *t*-Bu), 0.06 (s, 6 H, $(CH_3)_2Si$); ^{13}C NMR (75 MHz, $CDCl_3$, referenced to $CDCl_3$ at 77.0 ppm) δ 167.1, 166.8, 164.0, 159.0, 150.7, 150.4, 144.3, 135.6, 135.5, 135.3, 135.2, 132.0, 132.0, 131.6, 131.2, 130.2, 129.4, 128.8, 128.3, 128.2, 127.4, 113.4, 111.8, 111.1, 87.2, 85.0, 84.4, 84.3, 83.3, 71.8, 64.7, 63.7, 55.1, 40.5, 37.3, 25.4, 17.6, 12.0, 11.2, −5.0, −5.2; IR (KBr) 2954, 1696, 1608, 1580, 1509, 1466, 1364, 1178, 1035, 780, 704 cm^{-1}; MS (FAB) m/z 1030 (M − 1, 24), 843 (20), 527 (26), 454 (25), 438 (27), 378 (74), 356 (100). Anal. Calcd for $C_{55}H_{62}O_{14}N_4Si$: C, 64.06; H, 6.06; N, 5.43. Found: C, 63.76; H, 6.07; N, 5.34.

Diester 6. Tetrabutylammonium fluoride in THF (1.0 M, 2.5 mL) was added to a solution of diester **5** (400 mg, 0.38 mmol) in dry THF (5 mL) and stirred at room temperature. After 5 min, TLC indicated that the reaction was complete. The solvent was removed under reduced pressure, and the residue was flash chromatographed on a silica gel column (5:95 methanol/ethyl acetate), to give the desired product in 98% yield (338 mg, 0.37 mmol): R_f = 0.54 (1:9 methanol/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$, referenced to TMS) δ 10.35 (s, 1 H, NH), 9.70 (s, 1 H, NH), 7.81–7.71 (m, 2 H, Ar), 7.65 (m, 2 H, TH6), 7.64–7.53 (m, 2 H, Ar), 7.42–7.26 (m, 9 H, DMT), 6.85 (d, J = 8.5 Hz, 4 H, DMT), 6.42 (dd, J = 10.5, 5 Hz, 1 H, H1′), 6.37 (t, J = 7 Hz, 1 H, H1′), 5.69 (d, J = 5 Hz, 1 H), 4.66 (d, J = 5 Hz, 1 H), 4.48 (m, 1 H), 4.43 (d, J = 3.5 Hz, 1 H), 4.35 (m, 1 H), 4.30 (dd, J = 7.5, 4 Hz, 1 H), 3.80 (s, 6 H, $ArOCH_3$), 3.53 (m, 2 H), 2.71 (dd, J = 13.5, 5.5 Hz, 1 H), 2.51 (m, 2 H), 2.12 (m, 1 H), 1.80 (s, 3 H, TCH_3), 1.34 (s, 3 H, TCH_3); ^{13}C NMR (75.0 MHz, $CDCl_3$, referenced to $CDCl_3$ at 77.0 ppm) δ 167.5, 166.9, 164.4, 163.9, 159.0, 151.6, 151.0, 144.3, 135.6, 135.3, 135.2, 132.5, 132.2, 131.5, 130.9, 130.3, 130.3, 129.6, 129.1, 128.3, 128.3, 127.4, 113.4, 112.5, 111.5, 87.3, 84.9, 84.4, 84.4, 83.2, 71.0, 65.3, 63.8, 55.1, 39.6, 36.8, 12.2, 11.2; IR (KBr) 3063, 1695, 1608, 1509, 1468, 1363, 1254, 1178, 1069, 1033, 830, 755, 704 cm^{-1}; MS (FAB) m/z 915 (M − 1, 12), 767 (19), 613 (24), 531 (25), 460 (100), 341 (52). Anal. Calcd for $C_{49}H_{48}O_{14}N_4$: C, 64.24; H, 5.27; N, 6.11. Found: C, 63.86; H, 5.10; N, 6.06.

Cis-Syn Dimer 7. Diester **6** (360 mg, 0.39 mmol) and acetophenone (600 μL) were dissolved in 1:0.75 distilled water/acetonitrile (350 mL) in a Pyrex immersion well photochemical reactor and purged with argon for 30 min prior to irradiation with a 450-W Hanovia medium-pressure mercury vapor lamp. After the mixture was irradiated for 7 h, the solvent was removed under reduced pressure and the residue flash chromatographed on a silica gel column (5:95 methanol/ethyl acetate) to give the desired dimer **7** as a white solid in 25% yield (90 mg, 98 μmol); R_f = 0.52 (5:95 methanol/ethyl acetate); ^{1}H NMR (600 MHz, acetone-d_6, referenced to CHD_2COCD_3 at 2.04 ppm) δ 8.91 (s, 1 H, NH), 8.80 (s, 1 H, NH), 7.89 (dd, J = 6, 1.5 Hz, 1 H), 7.68 (m, 3 H, phth), 7.40–7.10 (m, 9 H), 6.80 (overlapping d, J = 9 Hz, 4 H), 6.36 (dd, J = 12.5, 4 Hz, 1 H, 3′-TH1′), 5.37 (m, J = 9.9, 5.3 Hz, 1 H, 5′-TH1′), 5.28–5.23 (m, 2 H, 5′-TH3′, 3′-TH5′ or 5″), 4.64 (d, J = 6.9, 1 H, 3′-TO3′H), 4.33 (m, 1 H, 3′-TH3′), 4.23 (d, J = 11.9, 1 H, 3′-TH5″ or 5′), 4.12 (d, J = 5.3, 1 H, 3′-TH6), 3.98 (d, J = 5.5 Hz, 1 H, 5′-TH6), 3.90 (m, 1 H, 5′-TH4′), 3.84 (t, J = 5.5 Hz, 1 H, 3′-TH4′), 3.77 (s, 6 H, $ArOCH_3$), 3.31 (m, 2 H, 5′-TH5′,H5″), 3.02 (m, 1 H, 5′-TH2′), 2.29 (m, 1 H, 3′-TH2′ or 2″), 2.20 (m, 2 H, 5′-TH2″, 3′-TH2″ or H2′), 1.52 (s, 3 H, 5′-TCH_3), 1.44 (s, 3 H, 3′-TCH_3); ^{13}C NMR (75 MHz, methanol-d_4, referenced to methanol at 49.00 ppm) δ 174.0, 171.4, 170.2, 167.5, 160.4, 153.2, 152.3, 147.0, 137.7, 135.3, 133.5, 132.5, 132.5, 131.6, 131.6, 131.0, 130.8, 129.6, 129.0, 127.9, 114.3, 92.0, 87.5, 85.0, 84.2, 82.0, 79.7, 70.9, 66.5, 65.1, 64.8, 55.7, 55.4, 53.1, 44.2, 39.8, 31.2, 18.2, 18.1; IR (KBr) 3233, 1716, 1607, 1509, 1447, 1391, 1178, 1069, 1035, 971, 830, 746, 703 cm^{-1}; MS (FAB) m/z 916 (M^+, 61), 810 (20), 597 (47), 554 (100), 534 (95), 460 (94), 423 (55). Anal. Calcd for $C_{49}H_{48}O_{14}N_4$: C, 64.18; H, 5.27; N, 6.11. Found: C, 63.29; H, 5.55; N, 5.97.

Cis-Syn Thymidine Dimer 8. Diester **7** (40 mg, 43 μmol) was stirred at room temperature with 80% acetic acid (5 mL) for 5 h. The acetic acid was then removed under reduced pressure, and the residue was passed through a short silica gel column (5:95 methanol/ethyl acetate, R_f = 0.28) to give the detritylated product in 75% yield (20 mg, 32.5 μmol). This product was then dissolved in concentrated ammonium hydroxide solution (3 mL) and heated in a sealed tube for 12 h at 55 °C, after which the ammonia solution was removed under reduced pressure. The residue was flash chromatographed on a short silica gel column (3:7 methanol/ethyl acetate, R_f = 0.44) to give a product in 72% yield (11.3 mg, 25.5 μmol) that was assigned as compound **8** by comparison of its ^{1}H NMR (600 MHz, D_2O) to that previously reported for the cis-syn cyclobutane dimer of thymidine.[15]

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(14) Ogilvie, K. K. *Can. J. Chem.* **1973**, *51*, 3799–3807.

Cis-Syn Thymidine Dimer Building Block 9. *N,N*-Diisopropylmethylphosphonamidic chloride (150 μL, 0.75 mmol) was added to a solution of cis-syn dimer **7** (60 mg, 65 μmol) and *N,N*-diisopropylethylamine (200 μL, 2 mmol) in dry methylene chloride (4 mL). The reaction was judged by TLC to be complete after 5 min. The mixture was then extracted with methylene chloride/saturated aqueous sodium carbonate, dried with $MgSO_4$, and evaporated under reduced pressure. The residue was flash chromatographed on a silica gel column (3:2 ethyl acetate/hexane, R_f = 0.43) to give the product **9** in 78% yield (55 mg, 51 μmol): R_f = 0.77 (4:1 ethyl acetate/hexane); ^{31}P NMR (121.5 MHz, acetone-d_6, referenced to TMP) δ 146.9, 146.7; ^1H NMR (300 MHz, acetone-d_6, referenced to CHD_2COCD_3 at 2.04 ppm) δ 9.01 (br, s, 1 H, NH), 8.83 (br, s, 1 H, NH), 7.92–7.88 (m, 1 H, phth), 7.72–7.63 (m, 3 H, phth), 7.47–7.45 (m, 2 H, DMT), 7.36–7.10 (m, 7 H, DMT), 6.82 (d, J = 9 Hz, 4 H, DMT), 6.42 (m, 1 H, H1′), 5.34 (m, 2 H), 5.25 (m, 1 H), 4.6–3.4 (m, 7 H), 3.77 (s, 6 H, $ArOCH_3$), 3.52 (m, 2 H, OCH_2CH_2CN), 3.01 (m, 2 H), 2.85 (m, 2 H, $COCH_2CH_2CN$), 2.40 (m, 2 H), 2.20 (m, 1 H), 1.52 (d, J = 2.5 Hz, 3 H, TCH_3), 1.43 (overlapping d, J = 2.5 Hz, 3 H, TCH_3), 1.27 (overlapping d, J = 6 Hz, 12 H, $^iPrCH_3$).

Cis-Syn Thymidine Dimer-Containing 22-mer 10. The oligonucleotide was synthesized on a 0.2-μmol scale on an ABI Model 380B synthesizer utilizing standard β-cyanoethyl phosphoramidite chemistry.[16] The oligonucleotide was purified by anion-exchange chromatography on a Nucleogen DEAE 60-7 column with a 45 min, 2 mL/min, 0–0.8 M KCl/20% acetonitrile/20 mM KH_2PO_4/K_2HPO_4 (pH 7.0) gradient. The fraction eluting at 25.5 min was desalted on a C-18 chromatography column by flushing with doubly distilled water, followed by a 0–30% acetonitrile/water gradient to give the oligonucleotide in 31% yield, as quantified by UV absorbance at 260 nm. The dimer-containing 22-mer was 5′-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase in kinase buffer (70 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 5 mM DTT) according to standard procedures.[17]

Photochemical Cleavage Experiments. The 5′-end-labeled single-stranded oligonucleotide **10** (0.05 μM) in 50 μL of 1/20 kinase buffer was placed on a plastic weigh boat and exposed to 254-nm-light irradiation from a low-pressure mercury vapor lamp at a fluence of ≈0.25 mW·cm^{-2}. Aliquots of about 4 μL were removed after 1, 2, 4, 8, 16, and 32 min of irradiation, denatured, electrophoresed on a denaturing 15% polyacrylamide gel, and exposed to Kodak XAR-5 film at −80 °C.

Photoenzymatic Cleavage Experiments. Double-stranded substrate was prepared by annealing the 5′-end-labeled 22-mer to the complementary 34-mer d(CATGATTACGAATTGCATAATACGTGCAGGCATG). For the photocleavage experiments, a 50-μL solution of the double-stranded substrate (20 nM) and 10 pmol of *E. coli* photolyase (a gift from A. Sancar) in 100 mM NaCl, 50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 20 mM DTT, 0.1 mg/mL acetylated BAS, 4% glycerol was prepared in the dark. The solution was then transferred to a plastic weigh boat, covered with the lid of a plastic Petri dish to prevent any exposure to short-wavelength light, and irradiated with an incandescent 135-W light bulb (General Electric) at a distance of about 5 cm. Aliquots of about 4 μL were taken out after 1, 2, 4, 16, 32, and 64 min of irradiation, denatured, and electrophoresed. The same substrate was irradiated with visible light for 30 min in the absence of photolyase to serve as a control experiment. For flash photolysis experiments, a solution (20 μL) containing 10 nM double-stranded substrate and 40 or 200 nM photolyase was subjected to a single flash from a Nikon SB-15 photographic flash unit at a distance of about 1 cm. For the T4 *denV* endonuclease V cleavage experiments, 0.2 pmol of the double-stranded substrate was incubated with 18 ng of endonuclease (a gift from S. Lloyd) at 37 °C for 2 h in 10 μL of 25 mM sodium phosphate buffer (pH 6.8) and 0.1 mg/mL bovine serum albumin (Promega).

Results and Discussion

Building Block Synthesis. The synthetic route to a DNA building block for the cis-syn thymidine dimer was based on previous routes that we developed for the synthesis of building blocks for the cis-syn dimers of dipyrimidine sites[18] (Scheme I). Ester linkages were selected to tether the two thymidines together because of their stability to standard solid-phase DNA synthesis steps and their lability to standard deprotection conditions.[16] A tether was used to facilitate dimer formation and to maximize the yield of the desired cis-syn cyclobutane stereochemistry by forcing the photodimerization reaction to take place intramolecularly. Thus, 5′-*O*-DMT-thymidine[12,13] (**1**) was treated with phthalic anhydride and catalytic DMAP in pyridine to give the ester acid **2**, which was then converted to the *p*-nitrophenyl ester **3**. The p-nitrophenyl ester **3** was transesterified with the sodium salt of 3′-*O*-TBDMS-thymidine[14] (**4**) in THF to give the diester **5**, which was desilylated with TBAF in THF to give diester **6**. Triplet-sensitized photolysis of **6** with acetophenone and Pyrex-filtered light gave the cis-syn dimer **7** in 25% yield. The synthesis of the building block was completed by converting the cis-syn dimer **7** to the corresponding phosphoramidite **9**.

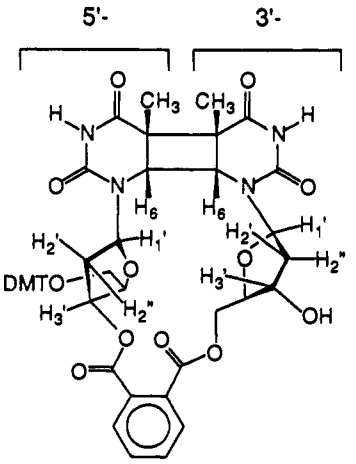

Figure 2. Conformation and stereochemistry of the cis-syn dimer **7** based on molecular model building constrained by NOE and chemical shift data.

The structure and stereochemistry of the thymine dimer unit of the building block were verified by complete deprotection of **7** to give the cyclobutane photodimer of thymidine **8**, whose NMR spectrum matched that previously reported for the cis-syn isomer.[15] Additional evidence for the cis-syn stereochemistry and evidence that the absolute stereochemistry of the cyclobutane ring is the same as that of the major cis-syn dimer of TpT[19] and of DNA[20] (5′-5*R*,6*R*, 3′-5*S*,6*S*) (Figure 1) were obtained by analysis of the ROESY spectrum of **7** following assignment of the ^1H spectrum by TOCSY. NOEs between 3′-TH6 and both methyl groups, along with coupling between the two H6 protons, confirm the cis-syn stereochemistry (Figure 2). NOEs between H6, H2′, and H3′ of the 3′-thymidine subunit indicate that the 3′-glycosyl bond is in an anti conformation, while their absence in the 5′-thymidine subunit, along with an NOE between H6 and H1′, indicates that the 5′-glycosyl bond is in a syn conformation (Figure 2). Additional evidence in support of an anti 3′-glycosyl conformation is the 1.0 ppm downfield shift of the H1′ signal of the 3′-thymidine relative to that of the 5′-thymidine which can be attributed to the deshielding effect of the adjacent 3′-C2 carbonyl group. The syn 5′-glycosyl conformation is likewise supported by the 0.7–0.8 ppm downfield shift of the 5′-H2′ proton relative to the other H2′ and H2″ proton signals, attributable to the deshielding effect of the 5′-C2 carbonyl group.[19b-d,21] These same 5′-syn, 3′-anti glycosyl bond conformations are also observed in the crystal structure of the β-cyanoethyl phosphate ester of the cis-syn dimer of TpT.[22]

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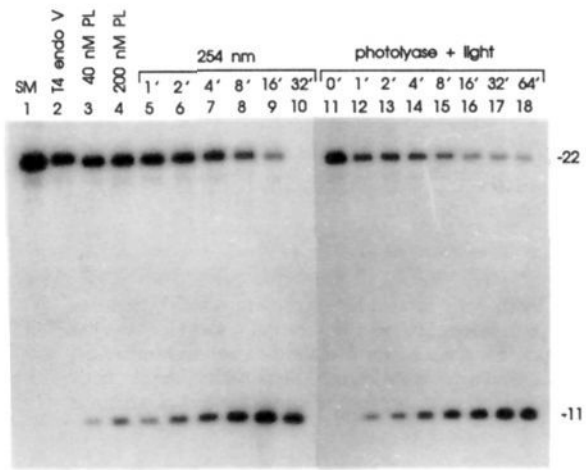

Figure 3. Autoradiogram of a 15% denaturing polyacrylamide electrophoresis gel of the photochemical and photoenzymatic cleavage of the 5′-^{32}P-end-labeled 22-mer containing a central thymidine dimer (**10**): lane 1, untreated single-stranded 22-mer; lane 2, duplex 22-mer treated with T4 *denV* endonuclease V; lanes 3 and 4, photolysis of the duplex 22-mer with a single camera flash in the presence of 40 and 200 nM *E. coli* photolyase, respectively; lanes 5–10, direct photolysis of the single-stranded 22-mer with 254-nm light for the times (min) indicated; lane 11, photolysis of the duplex 22-mer with visible light for 30 min in the absence of photolyase; lanes 12–18, photolysis of the duplex 22-mer in the presence of 200 nM photolyase and visible light for the times (min) indicated.

Model-building experiments indicate that this set of glycosyl bond conformations are only compatible with the stereochemistry for **7** shown in Figure 2 and are not compatible with a 5′-5*S*,6*S*, 3′-5*R*,6*R* structure.

Photocleavage. To demonstrate that the cis-syn thymidine dimer building block **9** can be used to introduce a photocleavable site, a 22-mer oligodeoxynucleotide with the cis-syn thymidine linker in the center (**10**) was synthesized by standard automated synthesis (Scheme I).[16] Oligonucleotide **10** was purified by anion-exchange HPLC following heating of the crude synthesis product with ammonium hydroxide for 12 h at 55 °C in a sealed tube to remove the phthalate linker and the protecting groups. Irradiation of a 5′-^{32}P-end-labeled sample at 254 nm led to a new band corresponding to an 11-mer as the only labeled product, indicative of cleavage at the thymidine dimer site (Figure 3, lanes 5–10). The 22-mer was completely cleaved following extended photolysis (lane 10).

To test whether the thymidine dimer could be photoenzymatically cleaved, the end-labeled 22-mer was annealed to a complementary 34-mer strand and subjected to visible light in the presence and absence of *E. coli* photolyase. While the oligonucleotide remained intact in the absence of photolyase (lane 11), it was cleaved in the presence of photolyase, yielding the same 11-mer that was produced by direct photolysis (lanes 12–18). The oligonucleotide could also be cleaved by photolyase with a single pulse of light from a camera flash unit (lanes 3 and 4). Increasing the concentration of photolyase from a 4-fold to a 20-fold excess under otherwise identical conditions increased the amount of cleavage from 8% to 20% following a single camera flash. While the cis-syn thymidine dimer is a substrate for *E. coli* photolyase, it does not appear (lane 2) to be a substrate for another dimer-specific repair enzyme, T4 *denV* endonuclease V,[23] suggesting that this enzyme may recognize dimers by a different mechanism.

Conclusion. The ability to induce site-specific cleavage of nucleic acids with a pulse of light may find application in the study of structural transitions or enzymatic reactions of nucleic acids, such as those triggered by the release of supercoiling.[24,25] The ability to photocleave nucleic acids might also find practical applications in the activation of prodrug forms of antisense oligonucleotides and ribozymes.[3]

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