perpendicular to the phenylene plane, and there is no sign of shortening of the bonds connecting the nitrogen and oxygen atoms with the phenylene ring (1.49 and 1.41 Å, respectively, values typical of anisoles and phenyl nitroxides).[21] The C–C bond lengths of the *m*-phenylene ring are within 1.36–1.39 Å. Thus, the possibility of **3** is discounted.

Conclusion

The 4,6-dimethoxy-*m*-phenylene unit in **1** is not a ferromagnetic coupling unit; the two nitroxide groups couple in an antiferromagnetic fashion strongly in crystals and weakly in solid solutions. Lower π-spin polarization on the *m*-phenylene ring and a possible antiferromagnetic through-space interaction between the spins rather localized on the nitroxide radicals at a distance of 5.74 Å (between the two middle points of the N–O bonds) in conformation **1b** may be responsible for the nonferromagnetic coupling in **1**.[22] In toluene and PVC solid solutions, **1** is presumed to take other conformations,[10] e.g., a more planar conformation, or the anti form **1a**, in which the antiferromagnetic coupling is less effective. Much care should be taken in the proper choice of a combination of spins and ferromagnetic couplers for designing high-spin polymers.

Experimental Section

EPR Spectroscopy and Magnetic Measurements. EPR spectra were recorded by using a Bruker ESP 300 X-band (9.4 GHz) spectrometer equipped with a Hewlett-Packard 5350B microwave frequency counter. An Air Products LTD-3-110 liquid helium transfer system was attached for the low-temperature measurements. The magnetic susceptibility was measured by the Faraday balance method at 2 T on an Oxford Instruments magnetic susceptibility system with a 7-T superconducting solenoid as described previously.[3e] A quantum design SQUID susceptometer/magnetometer was also used for a microcrystalline sample. Accurately measured ca. 15 mg samples of **1** and main fields of 2 and 0.5 T were used for the balance and SQUID measurements, respectively.

X-ray Analysis. A single crystal of approximate dimensions 0.02625 mm^3 was mounted on a glass fiber support. Diffraction data were obtained on a Rigaku AFC-5R four-circle diffractometer with $2q$(max) = 55.1° using graphite-monochromated Mo Kα radiation (2.64 cm^{-1}) at 23 °C. The structure was solved in $P2_1$ (No. 4) by direct methods and converged by full-matrix least-squares analysis using the TEXSAN Version 2.0 program (Molecular Structure Corporation). Crystal data were as follows: $C_{16}H_{26}N_2O_4$, M = 310.39, monoclinic, space group $P2_1$ (No. 4), a = 9.8914 (8) Å, b = 7.904 (3) Å, c = 11.4547 (6) Å, β = 106.258 (5)°, V = 859.8 (3) Å^3, Z = 2, D_{calcd} = 1.199 g cm^{-3}. All non-hydrogen atoms were refined anisotropically, and hydrogen atoms were included at standard positions (C–H = 0.96 Å; C–C–H = 109.5°, 120°, or 180°) and refined isotropically using a rigid model. Refinement converged at R = 0.040 and R_w = 0.041 for 1153 unique reflections with $I > 3\sigma(I)$ and 198 variables.

Other Instrumentation. ^{1}H (270 MHz) and ^{13}C (68.0 MHz) NMR spectra were obtained on a JEOL GX-270 spectrometer. IR and mass spectra were obtained on Hitachi 270-30 and JEOL JMS D-300 spectrometers, respectively.

Materials. Solvents diethyl ether, tetrahydrofuran, 2-methyltetrahydrofuran, benzene, and toluene that were used for the reactions and spectral measurements were all distilled under high-purity N_2 after they were dried with sodium/benzophenone ketyl. All reaction mixtures were stirred under an atmosphere of N_2. Anhydrous magnesium sulfate was used as drying agent.

4,6-Dimethoxy-1,3-phenylenebis(*N*-*tert*-butyl-*N*-hydroxyamine). To a solution of 1.48 g (5.0 mmol) of 1,3-dibromo-4,6-dimethoxybenzene[14] in 75 mL of anhydrous ether was added 6.3 mL (10.0 mmol) of 1.6 M *n*-butyllithium in hexane at −78 °C. The mixture was allowed to warm up to ambient temperature, after which 0.97 g (11.0 mmol) of 2-methyl-2-nitrosopropane in 10 mL of ether was added at −78 °C. The mixture was stirred overnight at room temperature and then decomposed with aqueous ammonium chloride. Column chromatography on silica gel (Wako Gel C 200) gave 0.66 g (42%) of bis(hydroxyamine) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.18 (s, 18 H), 3.83 (s, 6 H), 5.73 (br, 2 H), 6.36 (s, 1 H), 7.55 (s, 1 H).

4,6-Dimethoxy-1,3-phenylenebis(*N*-*tert*-butyl nitroxide). A solution of 0.50 g of the bis(hydroxyamine) in 20 mL of ether was treated with 3 equiv of freshly prepared Ag_2O. The red solids thus obtained were recrystallized from ether to give red needles. Anal. Calcd for $C_{16}H_{26}N_2O_4$: C, 61.94; H, 8.39; N, 9.03. Found: C, 61.73; H, 8.27; N, 8.94.

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Supplementary Material Available: Thermal ellipsoid plot and tables of crystal and intensity collection data, atomic coordinates, torsion angles, and anisotropic displacement parameters for **1** (6 pages); tables of observed and calculated structure factors for **1** (8 pages). Ordering information is given on any current masthead page.

(21) Hanson, A. W. *Acta Crystallogr.* **1953**, *6*, 32.

(22) The results obtained in this paper, namely, that the 4,6-dimethoxy-*m*-phenylene unit is not a ferromagnetic coupling unit at least for *tert*-butyl nitroxides, and perhaps in general, are quite in contrast with those for other congested *m*-phenylene couplers reported in the literature.[9,11] It is surprising that the triarylmethyl-type diradicals containing 4,6-dimethyl-, 4,6-diisopropyl-, and 2,4,6-trimethyl-*m*-phenylene couplers[9] all have triplet ground states. The observed μ_{eff} values of 2.2, 2.5, and 2.2 μ_B, respectively, for these diradicals are considerably lower than the 2.83-μ_B value of ground-state triplets and would require purities of the samples of only 79, 89, and 79%, respectively. These reported data might alternatively be regarded as being closer to the 2.45-μ_{eff} value expected for diradicals in nearly degenerate singlet/triplet states.

Efficient Pyrimidine Dimer Radical Anion Splitting in Low Polarity Solvents

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Abstract: Photosensitized pyrimidine dimer splitting by a covalently linked methoxybenzene exhibited a strong solvent dependence. Fluorescence of the chromophore was quenched by the attached dimer, which was indicative of electron transfer from excited chromophore to dimer. The quantum efficiency of splitting of the dimer radical anion in the linked dimer$^{\cdot-}$–chromophore$^{\cdot+}$ was calculated from the observed quantum yields of splitting and the degree of fluorescence quenching. The quantum efficiency of dimer radical anion splitting was remarkably dependent on solvent polarity, ranging from 0.05 in water to ~0.5 in low polarity solvent mixtures (e.g., heptane/1,4-dioxane, 95:5). The results were rationalized in terms of competition of splitting and back electron transfer within the charge-separated species. The latter pathway may be slowed due to its exergonicity in low polarity media, in accord with Marcus inverted behavior. Photolyases may be effective for splitting dimers by providing catalytic groups and a medium in which both dimer radical anion formation and splitting are efficient.

DNA repair has received heightened attention in recent years as ozone depletion threatens to significantly increase DNA damage by UV-B radiation (280–320 nm). Among the major lesions formed in DNA by this radiation are pyrimidine dimers (cyclo-

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butadipyrimidines). The splitting of dimers into pyrimidines by the light-utilizing enzymes, the DNA photolyases, constitutes one form of DNA repair.[1] Studies of photolyases are increasingly pointing toward a mechanism in which an electron is transferred from a photoexcited, enzyme-bound cofactor to the enzyme-bound dimer.[2,3] Model studies of electron-donating systems[4-7] offer the opportunity to evaluate a variety of parameters one at a time that may influence dimer splitting efficiency, which allows intermediates in dimer splitting to be identified.[8] These studies inspire the search for similar species in the enzyme-catalyzed process.

Among the electron-donating systems that photosplit dimers by electron donation are indoles,[5,7,9] anilines[4,6] and dihydroflavins.[3,10] Although the latter is the type of sensitizer utilized by photolyases, model studies are hampered by the low quantum yield of splitting, the difficulty of manipulating the readily oxidized dihydroflavins, the failure of these species to luminesce at room temperature (unbound by enzyme[11]), and the paucity of knowledge about their excited states. Thus, model systems involving other electron donors have been devised, and their mechanism of inducing the splitting of dimers has been investigated.

Earlier model studies of photosensitized dimer splitting suggested that dimer radical anion splitting was intrinsically less efficient than dimer radical cation splitting in aqueous solution,[12] which posed the problem of how the photolyases might work so efficiently if they employed an electron-donating reduced flavin sensitizer. It has been recently shown,[4,5] however, that linked dimer–chromophore systems split relatively efficiently (Φ_{OBS} = 0.4) in low polarity media and that unlinked dimer/chromophore mixtures in water do likewise (quantum efficiency of dimer radical anion[6] was 0.4 at pH 12). Studies of the natural systems themselves have also led to the belief that dimer anions are plausible splitting intermediates in photolyase-mediated repair of DNA.[13,14]

To mimic the virtually intramolecular electron transfer from enzyme-bound sensitizer to enzyme-bound dimer, we have constructed several covalently linked systems consisting of a sensitizer and a dimer. These systems offer useful insights into the electron-transfer and bond-breaking processes involved in photosensitized dimer splitting. Forward and back electron-transfer rates involving sensitizer and dimer are relevant to the efficiency of splitting, and the effects of pH,[10] solvent polarity,[4,5] temperature,[15] stereochemistry,[4,16] and isotopic substitution[4,17] on splitting efficiency can be rationalized in terms of the effects on electron-transfer rates. Particularly relevant questions involve the importance of back electron transfer in limiting the efficiency of dimer radical anion splitting and the possible importance of deprotonation of the sensitizer prior to or after the initial electron transfer.[10,13]

We report here the design and synthesis of two linked dimer–chromophore systems. One contains a dimethoxybenzene. The other is an analogue in which one of the methoxy substituents is replaced by a hydroxy substituent, which is capable of being deprotonated. Chromophore fluorescence quenching and dimer splitting efficiency in these dimer–chromophore systems were measured as functions of solvent polarity. The results offer insights into the mechanistic features of dimer splitting by the electron donation pathway.

Experimental Section

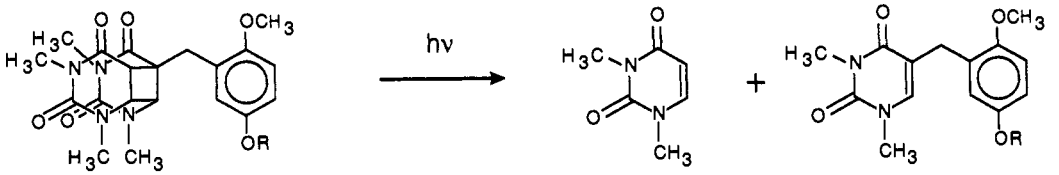
General Methods. *N,N*-Dimethylformamide (DMF) was dried as previously described.[5] Bis(trimethylsilyl)sodium amide and trimethylsilyl iodide were from Aldrich. Tetrahydrofuran was predried with Na^+ benzophenone$^{\cdot-}$ and then distilled under an N_2 atmosphere. Alcohols (1-propanol, 1-butanol, 1-pentanol, and 1-hexanol) were dried overnight with K_2CO_3 and were distilled after removal of the K_2CO_3. Water was deionized and subsequently double distilled. Acetonitrile and isopentane were spectroscopic grade from Aldrich and used without further purification. 1,4-Dioxane, cyclohexane, and heptane were used from freshly opened bottles or were distilled as previously described.[5] NMR spectra were recorded with a Varian 300-MHz or a Bruker AM-400 spectrometer.

Preparation of the 2,5-dimethoxybenzyl bromide was accomplished as follows. Reduction of 2,5-dimethoxybenzaldehyde (Aldrich) (5.2 g, 0.03 mol) with $NaBH_4$ (1.3 g, 0.03 mol) in 95% ethanol (75 mL) produced the 2,5-dimethoxybenzyl alcohol. The reaction was quenched with 1 N HCl, water was added, and the solution was extracted with $CHCl_3$. The organic layer was dried over anhydrous $MgSO_4$. Evaporation of the solvent in vacuo afforded the pure alcohol as a liquid: UV λ_{max} (CH_3OH) 205, 228, 292 nm; thin-layer chromatography R_f = 0.52 ($CHCl_3$/CH_3OH, 97:3); ^1H NMR (300 MHz, $CDCl_3$) δ 2.61 (1 H, s, CH_2OH), 3.74 (3 H, s, OCH_3), 3.78 (3 H, s, OCH_3), 4.62 (2 H, s, CH_2OH), 6.76–6.88 (3 H, m, phenyl); ^{13}C NMR (75.46 MHz; acetonitrile-d_3) δ 55.70, 55.99, 59.87, 111.97, 112.80, 114.60, 132.03, 151.82, 154.58; EI mass spectrum m/z 168 (M^+), 139, 125, 110, 93. The pure alcohol (2.1 g, 0.01 mol) was dissolved in methylene chloride and converted to the bromide by dropwise addition of PBr_3 (2.2 mL, 0.01 mol) in 100 mL of methylene chloride (N_2 atmosphere; dropping funnel equipped with a drying tube). The reaction was quenched by pouring the solution into ice water. Extraction with methylene chloride and washing with saturated aqueous sodium bicarbonate was followed by drying the extracts over anhydrous $MgSO_4$. Evaporation of the solvent yielded a waxy solid. The solid was recrystallized from $CHCl_3/CH_3OH$ to yield (2.06 g) of pure 2,5-dimethoxybenzyl bromide as colorless needles: mp 75–77 °C; UV λ_{max} 208, 234 (sh), 306 nm; thin-layer chromatography R_f = 0.68 (silica, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 3.75 (3 H, s, OCH_3), 3.83 (3 H, s, OCH_3), 4.51 (2 H, s, CH_2Br), 6.79–6.88 (3 H, m, phenyl); ^{13}C NMR (75.46 MHz; $CDCl_3$) δ 29.13, 56.08, 56.52, 112.72, 115.58, 116.93, 127.52, 152.35, 154.11; mass spectrum m/z 231 (M^+), 230, 151, 121, 91, 51.

Synthesis of 1. Preparation of **1** was accomplished by alkylation of the enolate formed by deprotonation[4,5,18] of C-5 of the cis-syn cyclobutane dimer of 1,3-dimethyluracil (DMUD). In a round-bottom flask equipped with a micro-Claisen head, a stirred solution of DMUD[19] (209 mg, 0.75 mmol) in 8 mL of dry DMF was treated with bis(trimethylsilyl)sodium amide ($[(CH_3)_3Si]_2NNa$) (0.82 mL, 0.82 mmol) transferred via syringe. The solution was stirred for 1 h at 0 °C under N_2. After addition of solid 2,5-dimethoxybenzyl bromide (190 mg, 0.82 mmol), the solution was stirred for an additional hour. The reaction was quenched by addition of saturated aqueous NH_4Cl. Evaporation of the solvent in vacuo afforded a crude product that was subjected to column chromatography (silica, $CHCl_3/CH_3OH$, 97:3). Further purification was accomplished by preparative layer silica gel (1 mm) (Analtech) chromatography ($CHCl_3/CH_3OH$, 97:3). Extraction with three methanol washings and a final washing with ethyl acetate yielded, after evaporation of the solvent, 137 mg (43%) of racemic **1** as a white solid: mp 139–141 °C; UV λ_{max} (CH_3OH) 207, 224 (sh), 295 nm (approximately 6 nm to the red

(1) Myles, G. M.; Sancar, A. *Chem. Res. Toxicol.* **1989**, *2*, 197–226. Sancar, G. B. *Mutat. Res.* **1990**, *236*, 147–160.
(2) Payne, G.; Sancar, A. *Biochemistry* **1990**, *29*, 7715–7727. Kim, S. T.; Sancar, A.; Essenmacher, C.; Babcock, G. T. *J. Am. Chem. Soc.* **1992**, *114*, 4442–4443.
(3) Jorns, M. S. *J. Am. Chem. Soc.* **1987**, *109*, 3133–3136.
(4) Kim, S. T.; Rose, S. D. *J. Photochem. Photobiol. B: Biol.* **1992**, *12*, 179–191.
(5) Kim, S. T.; Hartman, R. F.; Rose, S. D. *Photochem. Photobiol.* **1990**, *52*, 789–794.
(6) Yeh, S.-R.; Falvey, D. E. *J. Am. Chem. Soc.* **1991**, *113*, 8557–8558. Yeh, S.-R.; Falvey, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 7313–7314.
(7) Hélène, C.; Charlier, M. *Photochem. Photobiol.* **1977**, *25*, 429–434.
(8) Young, T.; Kim, S. T.; Van Camp, J. R.; Hartman, R. F.; Rose, S. D. *Photochem. Photobiol.* **1988**, *48*, 635–641.
(9) Van Camp, J. R.; Young, T.; Hartman, R. F.; Rose, S. D. *Photochem. Photobiol.* **1987**, *45*, 365–370.
(10) Hartman, R. F.; Rose, S. D. *J. Am. Chem. Soc.* **1992**, *114*, 3659–3660.
(11) Jordan, S. P.; Jorns, M. S. *Biochemistry* **1988**, *27*, 8915–8923.
(12) Lamola, A. A. *Mol. Photochem.* **1972**, *4*, 107–133. Roth, H. D.; Lamola, A. A. *J. Am. Chem. Soc.* **1972**, *94*, 1013–1014. Pac, C.; Kubo, J.; Majima, T.; Sakurai, H. *Photochem. Photobiol.* **1982**, *36*, 273–282.
(13) Okamura, T.; Sancar, A.; Heelis, P. F.; Begley, T. P.; Hirata, Y.; Mataga, N. *J. Am. Chem. Soc.* **1991**, *113*, 3143–3145.
(14) Heelis, P. F.; Okamura, T.; Sancar, A. *Biochemistry* **1990**, *29*, 5694–5698.
(15) Kim, S. T.; Rose, S. D. *Photochem. Photobiol.* **1988**, *47*, 725–729.
(16) Groy, T. L.; Kim, S. T.; Rose, S. D. *Acta Crystallogr.* **1991**, *C47*, 1898–1902. Groy, T. L.; Kim, S. T.; Rose, S. D. *Acta Crystallogr.* **1991**, *C47*, 1287–1290.
(17) McMordie, R. A. S.; Begley, T. P. *J. Am. Chem. Soc.* **1992**, *114*, 1886–1887.
(18) Hartman, R. F.; Van Camp, J. R.; Rose, S. D. *J. Org. Chem.* **1987**, *52*, 2684–2689.
(19) Elad, D.; Rosenthal, I.; Sasson, S. *J. Chem. Soc. C* **1971**, 2053–2057.

Scheme I



1: R = CH_3
2: R = H

of 2,5-dimethoxytoluene); thin-layer chromatography R_f = 0.35 (silica, $CHCl_3/CH_3OH$, 97:3); ^1H NMR (300 MHz, acetonitrile-d_3) δ 2.70 (3 H, s, N-CH_3), 2.81 (3 H, s, N-CH_3), 2.87 (3 H, s, N-CH_3), 3.01 (3 H, s, N-CH_3), 3.11 (1 H, d, J = 13 Hz, uracilyl-C(5)C*H*H'), 3.19 (1 H, d, J = 13 Hz, uracilyl-C(5)CH*H'*), 3.29 (1 H, d, J = 7.2 Hz, uracilyl-C(5)H), 3.68 (3 H, s, OCH_3), 3.73 (3 H, s, OCH_3), 3.90 (1 H, dd, J = 4.5, 7.2 Hz, uracilyl-C(6)H), 3.97 (1 H, d, J = 4.7 Hz, uracilyl-C(6)H), 6.60 (1 H, d, J = 3.2 Hz, phenyl-C(6)H), 6.77 (1 H, dd, J = 3.0, 8.8 Hz, phenyl-C(4)H), 6.86 (1 H, d, J = 8.8 Hz, phenyl-C(3)H); ^{13}C NMR (75.46 MHz; $CDCl_3$) δ 27.89, 28.27, 35.28, 35.66, 36.90, 40.56, 54.53, 54.82, 54.08, 56.34, 56.12, 112.25, 114.75, 118.14, 124.25, 152.30, 152.92, 153.72, 154.41, 165.95, 170.73; EI mass spectrum m/z 430 (M^+), 290, 259, 151, 121, 96. The structure of compound **1** was also verified by single-crystal X-ray diffraction (data not shown).

Synthesis of 2. This synthesis was achieved by the monodemethylation of **1**. In a flame-dried, 5-mL graduated reaction vial equipped with a microcondenser, a drying tube connected to an N_2 source, and a spinvane, compound **1** (25 mg, 0.06 mmol) was dissolved in $CDCl_3$ (2 mL). Addition of trimethylsilyl iodide [$(CH_3)_3SiI$] (0.34 mL, 0.24 mmol) via syringe produced a pale yellow solution. The reaction mixture was refluxed for 70 h, and the progress of the reaction was monitored by ^1H NMR for the disappearance of the methoxy signal of **1** at δ 3.7. At the completion of the reaction, the intermediate trimethylsilyl ether was methanolyzed to the corresponding alcohol by addition of the mixture to methanol (3 equiv). The solvents were evaporated, and the red residue was dissolved in $CHCl_3$. The solution was extracted with brine and saturated aqueous sodium bisulfite. The $CHCl_3$ layer was dried over anhydrous $MgSO_4$ and filtered, and the solvent was evaporated to give a white residue. Purification by preparative layer chromatography on silica gel ($CHCl_3/CH_3OH$, 97:3) afforded racemic **2** as a white solid: mp >200 °C dec; UV λ_{max} (CH_3OH) 207, 220 (sh), 298 nm; thin-layer chromatography R_f = 0.23 (silica, $CHCl_3/CH_3OH$, 97:3); ^1H NMR (300 MHz, acetonitrile-d_3) δ 2.70 (3 H, s, N-CH_3), 2.81 (3 H, s, N-CH_3), 2.88 (3 H, s, N-CH_3), 3.01 (3 H, s, N-CH_3), 3.10 (2 H, two d, J = 13 Hz, uracilyl-C(5)-CH_2), 3.28 (1 H, d, J = 7.2 Hz, uracilyl-C(5)H), 3.72 (3 H, s, OCH_3), 3.91 (1 H, dd, J = 7.1, 4.6 Hz, uracilyl-C(6)H), 3.94 (1 H, d, J = 4.6 Hz, uracilyl-C(6)H), 6.50 (1 H, d, J = 3.0 Hz, phenyl-C(6)H), 6.57 (1 H, s (br), phenyl-OH), 6.65 (1 H, dd, J = 8.7, 3.0 Hz, phenyl-C(4)H), 6.78 (1 H, d, J = 8.8 Hz, phenyl-C(3)H); ^{13}C NMR (75.46 MHz; $CDCl_3$) δ 27.96, 28.35, 35.30, 35.72, 36.29, 40.09, 54.57, 54.91, 56.36, 57.84, 112.0, 112.6, 116.0, 120.0, 124.2, 150.6, 152.2; mass spectrum m/z 416 (M^+) 415, 276, 245, 204, 136, 83.

To ascertain which of the methyl groups of **1** remained attached in **2**, NOE difference spectroscopy was employed. Irradiation of the phenyl-C(3)H signal at δ 6.77 had an NOE effect on the methoxy resonance at δ 3.72 (3.4%). Also, enhancement of the phenyl-C(4)H signal at δ 6.55 was observed. Complementarily, irradiation of the methoxy resonance at δ 3.72 enhanced the phenyl-C(3)H signal at δ 6.77 (13.5%). Irradiation of the phenyl-C(4)H signal at δ 6.55 enhanced the phenyl-C(3)H at δ 6.77 (5.0%) and the methoxy signal at δ 3.72 (1.7%). Furthermore, no enhancement of the phenyl-C(6)H signal at δ 6.49 was observed when the methoxy signal was irradiated, thereby showing that structure **2** is correctly formulated as the product of demethylation of the 5-methoxy group of **1**.

Quantum Yield Measurements. The photolysis apparatus used for quantum yield determinations has been previously described.[5] In general, light from an Oriel 500-W Hg-Xe lamp was passed through a monochromator, and the emerging beam was transmitted to a capped quartz cuvette containing a stirred, air-equilibrated solution of compound **1** or **2**. Irradiation of samples (3 mL) at 302 nm was carried out for 2 min in triplicate. Potassium ferrioxalate actinometry was performed in triplicate before and after sample irradiation to monitor the light intensity.

The extent of dimer splitting was monitored by the increase in absorbance at 270 nm due to the regeneration of the 5,6-double bonds of the pyrimidines. The absorbance spectra were recorded with a Perkin-Elmer 552 UV/vis spectrophotometer or a Beckman DU 7400 spectrophotometer. The value of ϵ_{270} employed was 1.64×10^4 M^{-1} cm^{-1}.

Identification of Photoproducts. In a quartz NMR tube, a solution of **1** (3.7 mg; 8.6 μmol) in acetonitrile-d_3 (1.0 mL) was irradiated at 302 nm for 8 h, during which time the spectrum was recorded. The only products detected were the products of splitting (84% conversion), which were separated by thin-layer chromatography (silica, $CHCl_3/CH_3OH$, 96:4) and individually identified by NMR spectroscopy. The NMR spectrum of one of the photoproducts, 1,3-dimethyluracil (**3**), matched that of an authentic sample (Aldrich): ^1H NMR (300 MHz; $CDCl_3$) δ 3.32 (3 H, s, N-CH_3), 3.37 (3 H, s, N-CH_3), 5.72 (1 H, d, J = 7.8 Hz, uracilyl-C(5)H), 7.10 (1 H, d, J = 7.8 Hz, uracilyl-C(6)H). The only other detectable photoproduct was 1,3-dimethyl-5-(2,5-dimethoxybenzyl)uracil (**4**): ^1H NMR (300 MHz; $CDCl_3$) δ 3.28 (3 H, s, N-CH_3), 3.34 (3 H, s, N-CH_3), 3.61 (2 H, s, uracilyl-C(5)CH_2), 3.74 (3 H, s, OCH_3), 3.76 (3 H, s, OCH_3), 6.78 (1 H, s, uracilyl-C(6)H), 6.72-6.81 (3 H, m, phenyl); mass spectrum m/z 290 (M^+), 153, 96.

The photolysis of compound **2** (1.6 mg; 3.9 μmol) in 0.8 mL of acetonitrile-d_3 as above for 5 h resulted in complete conversion to 1,3-dimethyluracil (**3**) along with the other expected product of photosplitting, 1,3-dimethyl-5-(5-hydroxy-2-methoxybenzyl)uracil (**5**): ^1H NMR (300 MHz; acetonitrile-d_3) δ 3.21 (3 H, s, N-CH_3), 3.24 (3 H, s, N-CH_3), 3.48 (2 H, s, uracilyl-C(5)CH_2), 3.73 (3 H, s, phenyl-OCH_3), 6.59-6.82 (3 H, m, phenyl), 7.08 (1 H, s, uracilyl-C(6)H).

Steady-State Fluorescence Emission of 1 and 2. Fluorescence emission spectra were measured at room temperature on a SPEX Fluorolog-2 1681 spectrofluorometer or a JASCO Model FP-777 spectrofluorometer. To determine percent quenching of fluorescence of **1** and **2**, fluorescence intensity of **1** or **2** was compared to the fluorescence intensity of the corresponding chromophore without a dimer attached (e.g., 2,5-dimethoxytoluene for **1** and 5-methoxy-2-hydroxybenzene for **3**). The absorbance at 280 nm (λ_{ex}) of the dimer–chromophore and the simple chromophore were made equal.

Results

Dimer Photosplitting of 1 and 2. Irradiation of **1** or **2** at 302 nm, which is not significantly absorbed by the dimer portion of the molecules, resulted in the photolysis products shown in Scheme I. Splitting was monitored by the increase in absorbance due to the regeneration of the 5,6 double bonds in the pyrimidine photoproducts.[5]

Quantum Yields of Splitting of 1 and 2. The observed quantum yield of dimer splitting [Φ_{OBS} = (number of dimers split)/(number of photons absorbed)] is a measure of the efficiency of the sensitizer to induce cycloreversion of the dimers. As shown in Table I, the quantum yields of splitting (λ_{irr} = 302 nm) of **1** and **2** were found to be solvent dependent. For **1**, values of Φ_{OBS} increased from approximately 0.05 in H_2O to 0.29 in 1-butanol, and then they decreased with a further decrease in solvent dielectric constant. The behavior of **2** was comparable, with Φ_{OBS} maximizing for solvents of intermediate polarity. These results demonstrate that dimer splitting efficiency decreases in low polarity solvent mixtures, which suggests that electron transfer to the dimer may be less efficient in low polarity media.

No concentration dependence was found when the quantum yield of splitting of **1** was measured in methanol over a 6-fold range of concentrations (data not shown). These results rule out the involvement of intermolecular photosensitization, i.e., the chromophore of one molecule of **1** being responsible for the splitting of the dimer of another molecule of **1**.

The quantum yields of splitting of **1** in three representative solvents were virtually unchanged by N_2-purging prior to irradiation. The values for air and N_2 (in parentheses) were as follows:

Table I. Solvent Dependence of Splitting Efficiency of Compounds **1** and **2**

solvent[a]	compound 1			compound 2		
	Φ_{OBS}	Q^b	ϕ_{SPL}	Φ_{OBS}	Q^b	ϕ_{SPL}
H_2O	0.05	99	0.05	0.02	99	0.02
acetonitrile	0.26	96	0.27	0.26	97	0.27
methanol	0.24	99	0.24	0.17	99	0.17
dioxane/H_2O (9:1)	0.26	96	0.27	0.23	97	0.24
ethanol	0.27	93	0.29	0.22	96	0.23
1-propanol	0.28	98	0.29	0.23	97	0.24
1-butanol	0.29	96	0.31		96	
1-pentanol	0.23	95	0.24	0.26	96	0.27
1-hexanol	0.25	93	0.27	0.25	94	0.27
tetrahydrofuran	0.26	73	0.36	0.28	75	0.37
diethyl ether	0.20	51	0.39	0.22	50	0.44
dioxane	0.24	64	0.38	0.24	55	0.44
cyclohexane/dioxane (95:5)	0.14	35	0.40	*c*	*c*	
isopentane/dioxane (95:5)	0.14	33	0.42	*c*	*c*	
heptane/dioxane (95:5)	0.13	31	0.45	*c*	*c*	
cyclohexane	0.13	24	0.54	*c*	*c*	

[a] Arranged in order of decreasing dielectric constant. [b] $Q = [1 - (F_{D-Chr}/F_{Chr})] \times 100$. [c] Insolubility precluded measurement.

H_2O, 0.05 (0.05); CH_3OH, 0.24 (0.24); and 1,4-dioxane, 0.24 (0.23).

Since **2** is a phenol, the possible effect of prior deprotonation of the phenolic hydroxyl group of the sensitizer was explored. The quantum yield of splitting of **2** was not pH dependent in aqueous solution over the range pH 5 to 12 (potassium phosphate buffer). The quantum yield of splitting was found to be 0.024 ± 0.001. In ethanol, however, Φ_{OBS} decreased from 0.22 to 0.16 upon deprotonation by ethoxide.

Steady-State Fluorescence Emission of 1 and 2. Table I shows that the extent of fluorescence quenching by the covalently attached dimer in **1** and **2** was highly sensitive to solvent polarity. In polar solvents, fluorescence quenching was highly efficient. Quenching became highly inefficient as the polarity decreased. Quenching was not a result of significant absorption of exciting light by the dimer in **1**, as judged by a comparison of the extinction coefficients of 2,5-dimethoxytoluene and DMUD in water ($\epsilon_{280} = 2.2 \times 10^3$ and 55 M^{-1} cm^{-1}, respectively), methanol ($\epsilon_{280} = 2.0 \times 10^3$ and 90 M^{-1} cm^{-1}, respectively), and 1,4-dioxane ($\epsilon_{280} = 2.2 \times 10^3$ and 40 M^{-1} cm^{-1}, respectively).

Figure 1a shows the fluorescence emission of **1** (spectrum A) compared to the fluorescence emission of free 2,5-dimethoxytoluene (spectrum B) in 1,4-dioxane at room temperature. The corresponding results for **1** and 2,5-dimethoxytoluene in methanol are shown in Figure 1b. The fluorescence emission maximizing at 320 nm upon excitation at 280 nm was 64% quenched in 1,4-dioxane and 99% quenched in methanol. Fluorescence quenching of **2** followed the same trend as **1** (Table I). These results imply that electron transfer from excited chromophore to the linked dimer is less efficient in lower polarity solvents, which results in less efficient splitting (vide infra).

Discussion

The variation of the observed quantum yield of dimer splitting (Φ_{OBS}) with solvent polarity allowed the evaluation of the effect of solvent polarity on the quantum efficiency of splitting (ϕ_{SPL}) of the intermediate dimer radical anion. This is an important parameter because it directly reflects the competition between splitting and charge recombination (by back electron transfer), processes that undoubtedly have counterparts in the enzymatic repair process. The significant dependence of observed dimer splitting efficiency on solvent polarity for **1** and **2** is shown in Table I. The dimer anion splitting efficiencies (ϕ_{SPL}) are also shown and were derived from Φ_{OBS} and the fluorescence quenching results, as described below.

To evaluate these processes, a simple mechanistic scheme was formulated (Scheme II). Excitation of the dimer–chromophore (D–Chr) produces an excited state of the chromophore. This excited state has relaxation pathways such as fluorescence (ϕ_F), internal conversion, and intersystem crossing (together represented by ϕ_{NR}). In addition, there is formulated a nonradiative decay pathway consisting of electron transfer to the dimer (ϕ_{ET}). These efficiencies sum to 1, since it is assumed that excitation produces the excited singlet state of the chromophore with a quantum yield of 1 (i.e., that there is no photochemistry from higher electronic or vibrationally excited states).

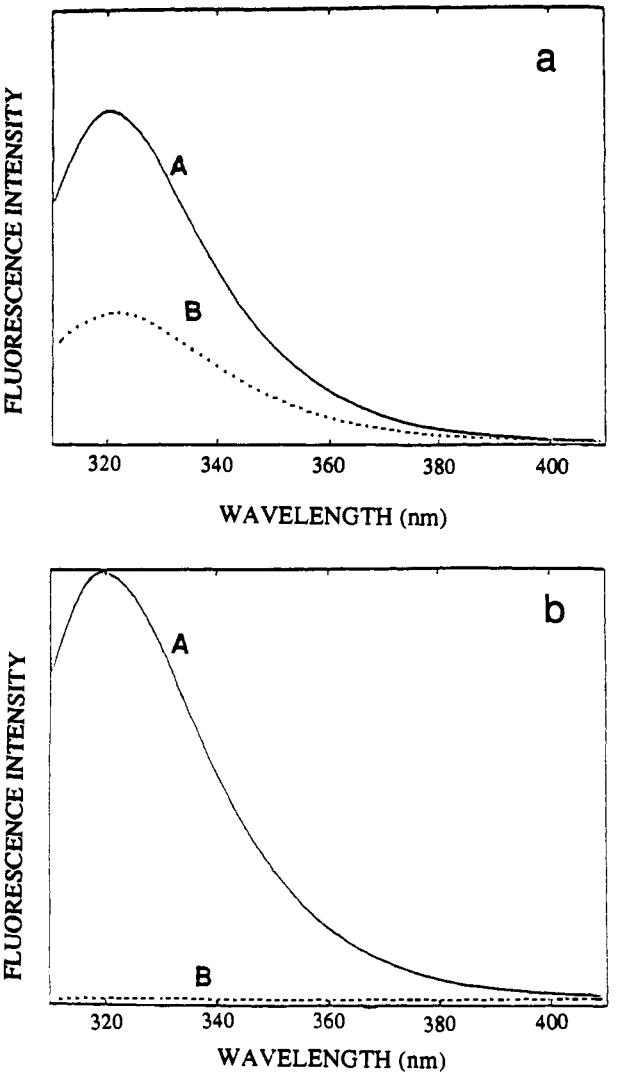
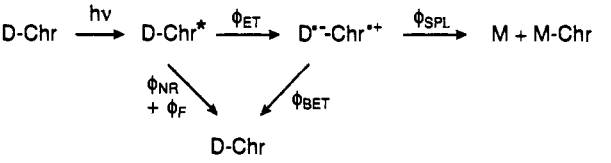


Figure 1. Relative fluorescence emission intensities of 2,5-dimethoxytoluene (curve A) and **1** (curve B); (a) in 1,4-dioxane and (b) in methanol.

Scheme II



Fluorescence quenching is probably a consequence of intramolecular electron transfer from excited chromophore to dimer.[20] Since there is almost no overlap of chromophore emission and DMUD absorption spectra (Figure 2), singlet–singlet energy transfer[21] is an improbable mode of fluorescence quenching in **1**. Also, a decrease in fluorescence of the chromophore in **1** due to an internal filter effect (i.e., absorption of exciting light by the dimer portion of **1**) is not significant (see Results). Participation of other modes of fluorescence quenching or varying degrees of

(20) Hamada, T.; Nishida, A.; Matsumoto, Y.; Yonemitsu, O. *J. Am. Chem. Soc.* **1980**, *102*, 3978–3980.
(21) Turro, N. J. *Modern Molecular Photochemistry*; Benjamin/Cummings: Menlo Park, CA, 1978; pp 296–328.

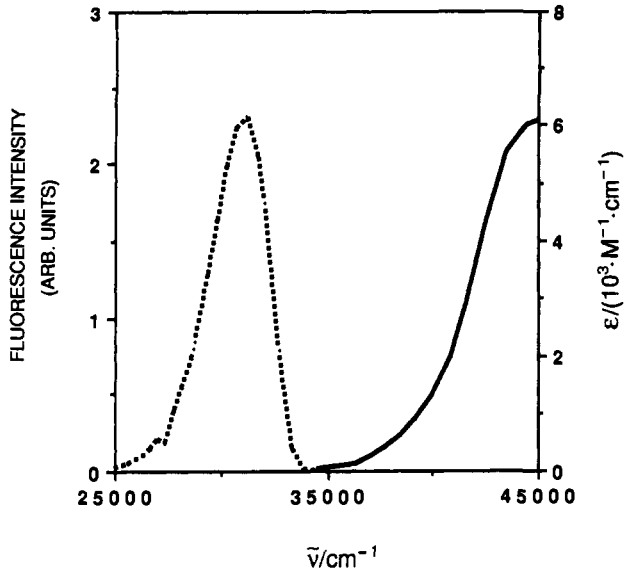


Figure 2. Emission spectrum of 2,5-dimethoxytoluene (dashed line) and absorption spectrum of DMUD (solid line) in methanol, showing lack of significant overlap. Results for water and 1,4-dioxane were very similar (not shown).

participation of different modes in different solvents cannot, however, be strictly excluded.

If electron transfer is indeed the predominant mode of fluorescence quenching in **1** and **2**,[22] then it can be concluded from the quenching data in Table I that electron transfer from excited chromophore to dimer becomes less efficient as solvent polarity decreases. Formation of the charge-separated species might reasonably be expected to be slowed in low polarity media, which would allow fluorescence and the nonradiative decay processes to predominate.

There is much indirect evidence for the existence of a charge-separated species (dimer$^{\bullet-}$–chromophore$^{\bullet+}$) in dimer splitting.[6,8,17,24] This species can undergo two processes, splitting (ϕ_{SPL}) or back electron transfer (ϕ_{BET}), and these efficiencies sum to 1 (Scheme II). The relative quantum efficiencies of these processes affect the observed quantum yield of splitting (Φ_{OBS}), which is given by eq 1.

$$\Phi_{OBS} = \phi_{ET}\phi_{SPL} \quad (1)$$

The fluorescence quenching (Q) of the excited chromophore in **1** (in percent, relative to free chromophore) is given by eq 2

$$Q = (1 - F_{D\text{-}Chr}/F_{Chr}) \times 100 \quad (2)$$

where fluorescence intensities of chromophore and **1** are given in eqs 3 and 4, respectively:

$$F_{D\text{-}Chr} = k_F/(k_F + k_{NR} + k_{ET}) \quad (3)$$

$$F_{Chr} = k_F^0/(k_F^0 + k_{NR}^0) \quad (4)$$

A reasonable assumption is that the rate constants for radiative and nonradiative relaxation pathways are unaltered by attachment of the dimer to the chromophore (i.e., $k_F = k_F^0$ and $k_{NR} = k_{NR}^0$) and that electron transfer (k_{ET}) can occur in excited D–Chr. Use of this assumption and eqs 1–4 gives the dependence of ϕ_{SPL} on the observed quantum yield of splitting and the observed fluorescence quenching as:

$$\phi_{SPL} = \Phi_{OBS} \times 100/Q \quad (5)$$

Use of eq 5 allowed the calculation of ϕ_{SPL}. Thus, for **1** it can be seen (Table I) that the quantum efficiency of splitting of the dimer radical anion within dimer$^{\bullet-}$–chromophore$^{\bullet+}$ is highly solvent dependent and, remarkably, ranges from 0.05 in water to ~0.5 in the lowest polarity solvents employed.

A possible explanation for the increased efficiency of splitting of the dimer radical anion in dimer$^{\bullet-}$–chromophore$^{\bullet+}$ (ϕ_{SPL}) *relative to back electron transfer* (ϕ_{BET}) is that back-electron transfer is slowed in the low polarity solvents. It is well known that electron transfer becomes *slow* when the driving force becomes *highly favorable* and exceeds the solvent reorganization energy, which is low for low polarity solvents (i.e., in the Marcus inverted region). Charge recombination in a nonpolar environment would certainly be expected to be highly exergonic and can result in Marcus inverted behavior,[25] so a slowing of back electron transfer in dimer$^{\bullet-}$–chromophore$^{\bullet+}$ in low polarity solvents might well be expected to allow splitting to increase. A solvent effect on splitting may also contribute to the observed trend, if, for example, charge delocalization is greater in the splitting transition state than in the dimer radical anion itself.[18]

An explanation based primarily on back electron transfer rates was likewise invoked for linked dimer–indole and dimer–arylamine systems,[4,5] in which it was found that the observed quantum yield of splitting increased with decreasing solvent polarity to a maximum value of 0.3–0.4, in contrast to the present study in which Φ_{OBS} increases and then decreases with decreasing solvent polarity. In those systems, splitting was highly inefficient in water ($\Phi_{OBS} \lesssim 0.05$). Unlinked mixtures of thymine dimers and an arylamine, in which the geminate radical ion pair can presumably dissociate, exhibit a higher quantum efficiency[6] of dimer radical anion splitting in water (ϕ_{SPL} = 0.1 at pH 7 and 0.4 at pH 12).

It was found that **2** exhibits some of the same behavior as **1** in higher polarity solvents, but insolubility prevented the determinations in the low polarity solvent mixtures. Deprotonation of **2** had little effect on the observed quantum yield of splitting in H_2O (e.g., Φ_{OBS} = 0.02 from pH 5 to 12) and caused a slight decrease in the case of ethanol (0.22 vs 0.16). Deprotonation of **2** prior to excitation may have had little effect on Φ_{OBS} because deprotonation of the phenolic radical cation might be so fast[26] that essentially the same state is reached (i.e., dimer$^{\bullet-}$–aryl–O$^{\bullet}$) before back electron transfer can occur whether or not deprotonation of the ground state was carried out.

The photolyase from *Escherichia coli* splits pyrimidine dimers in DNA with a quantum yield[2] of approximately 0.7. It is not known how the enzyme effects splitting, but electron transfer from an enzyme-bound cofactor ($FADH_2$) to the enzyme-bound dimer is a highly likely first step.[3,10,13,14,27] Binding to dimers results in efficient fluorescence quenching[11,24] indicative of efficient electron transfer. The dimer radical anion then splits efficiently in competition with back electron transfer to the donor and/or other decay pathways, with a splitting efficiency ϕ_{SPL} estimated at 0.8–1.[2,24] What may be unique about photolyases is that they may provide an environment, possibly of considerable hydrophobic nature,[28,29] that facilitates the splitting of the dimer radical anion while providing the features necessary for efficient forward electron transfer from excited dihydroflavin to dimer. The detailed manner in which this might be accomplished requires further study.

Acknowledgment. We gratefully acknowledge financial support from the National Institutes of Health (CA49729) and an instrument grant from the Del E. Webb Foundation.

(22) Use of $k_{ET} = 1/\tau - 1/\tau_0$ and the published[23] value of $\tau_0 = 2.7 \pm 0.5$ ns for 1,4-dimethoxybenzene in acetonitrile allows a very approximate estimate of $k_{ET} \approx 7 \times 10^8$ s^{-1} for **1** in 1,4-dioxane if it is also assumed that 64% quenching is due to a shortening of the lifetime of the chromophore in a single, predominant conformation of **1** by a factor of 0.36.

(23) Carroll, F. A.; McCall, M. T.; Hammond, G. S. *J. Am. Chem. Soc.* **1973**, *95*, 315–318.

(24) Kim, S. T.; Heelis, P. F.; Okamura, T.; Hirata, Y.; Mataga, N.; Sancar, A. *Biochemistry* **1991**, *30*, 11262–11270. Kim, S. T.; Sancar, A. *Biochemistry* **1991**, *30*, 8623–8630.

(25) Closs, G. L.; Miller, J. R. *Science* **1988**, *240*, 440–447.

(26) Ebbesen, T. W.; Previtali, C. M. *J. Photochem.* **1985**, *31*, 193–197.

(27) Kim, S. T.; Sancar, A.; Essenmacher, C.; Babcock, G. T. *J. Am. Chem. Soc.* **1992**, *114*, 4442–4443.

(28) Eker, A. P. M.; Hessels, J. K. C.; Dekker, R. H. *Photochem. Photobiol.* **1986**, *44*, 197–205.

(29) Li, Y. F.; Sancar, A. *Biochemistry* **1990**, *29*, 5698–5706.