Supporting Information (11 pages)

Photochemistry of *N*-Isopropoxy-Substituted 2(1H)-Pyridone and 4-*p*-Tolylthiazole-2(3H)-thione: Alkoxyl-Radical Release (Spin-Trapping, EPR and Transient Spectroscopy) and its Significance in the Photooxidative Induction of DNA Strand Breaks

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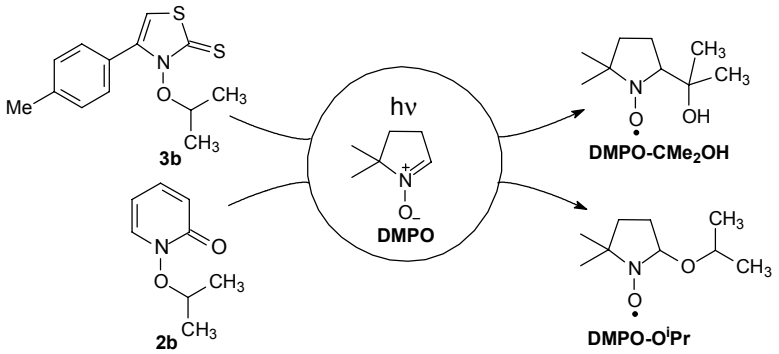

Experimental Section

Instrumentation. ^{1}H-NMR spectra were recorded with a Bruker 250 (200 MHz) and a Varian VXR-500 (500 MHz) spectrometer. ^{13}C-NMR (125 MHz) spectra were collected on a Bruker AC 200 (50 MHz) and a Varian VXR-500 spectrometer. IR spectra were measured on a JASCO FT-IR 5000 and Perkin-Elmer FTR (model 1605) spectrophotometer, UV spectra on a Hitachi U-3200 spectrophotometer and EPR spectra on a Bruker ESP 300 spectrometer (Bruker, ER 160 FC). The medium-pressure liquid chromatography (MPLC) system consisted of a Chemco Low-Prep 91-M-2 pump, equipped with an EYRA UV-D2 detector. The HPLC instrumentation consisted of two Bischoff HPLC pumps, model 2200 (Bischoff GmbH, Leonberg, Germany), equipped with a Rheodyne loop injector, model 7125 (Berkly, CA, USA) and a Waters 994 photodiode array detector (Waters GmbH, Eschborn, Germany).

Materials. Pyridone **2b**[23,24] and thiazolethione **3b**[25] were prepared by the methods reported previously and recrystallized before use. Supercoiled pBR 322 DNA was purchased from Pharmasia Biotech Europe GmbH and 5,5-dimethyl-1-pyrroline *N*-oxide (**DMPO**) was obtained from Fulka Chemie AG. Ethidium bromide and boric acid were obtained from Merck KGaA. Tris(hydroxymethyl)aminomethane (Tris base) was purchased from Sigma-Aldrich Chemie GmbH, and agarose from Serva Feinbiochemica GmbH.

Irradiation System. For the irradiation experiments of the pyridone **2b**, were used a Rayonet photoreactor (Southern New England Ultraviolet Company, Brandford, CT), equipped with 16 UV lamps (300 nm, each 21 W) or a black-light lamp (312 nm, 30 W, Itf Labortechnik, Wasserburg).

The irradiation experiments of the thiazolethione **3b** also employed a Rayonet photoreactor, equipped with 16 UV lamps (350 nm, each 24 W), and additionally a 450-W high-pressure Hg lamp.

Transient Spectroscopy. The details of the laser-flash photolysis technique have been provided elsewhere.[S1] Excitation was achieved by using the 308-nm output of a Lambda Physik EMG 101 MSC excimer laser (pulse width 20 ns, pulse energy ca. 75 mJ).

Before photolysis, the samples were thoroughly degassed by three freeze-pump-thaw cycles.

Quantum yields of the N-O bond scission were determined by actinometry applying the equation below, where $\Phi_{T(BP)}$ is the quantum yield for benzophenone as reference, to form the triplet state

$$\Phi_{N\text{-}O} = \Phi_{T(BP)} \times [A_R / A_{T(BP)}] \times [\varepsilon_{T(BP)} / \varepsilon_R]$$

$[\Phi_{T(BP)} = 1.0$ in benzene$]$[S2] with the molar absorption coefficient $\varepsilon_{T(BP)} = 7220\ M^{-1}cm^{-1}$ at 530 nm[S3], and $A_{T(BP)}$ is the experimentally determined absorption of the triplet state at 530 nm after laser excitation (308 nm). The subscript R refers to the corresponding values of the radicals produced by the excitation of the reagents **2b** and **3b** for which A_R was determined at 390 and 470 nm. The $\varepsilon_{\mathbf{A}}$ value[26] of the pyridyloxyl radical **A** is 680 $M^{-1}cm^{-1}$, the $\varepsilon_{\mathbf{B}}$ value[20] of the thiyl radical **B** was estimated to be 600 $M^{-1}cm^{-1}$. In these experiments, optically matched solutions of benzophenone and the radical sources were used (absorbance at $\lambda_{exc} = 308$ nm was 0.165).

Synthesis of Bis-[4-(4-methylphenyl)-2-thiazyl]disulfide (5). A solution of 100 mg (450 μmol) of 3-amino-4-hydroxy-4-(p-methylphenyl)thiazoline-2-thione[S4] and 86.0 mg (450 μmol) *p*-tosyl chloride in pyridine was stirred for 1.5 h at 20 °C. The mixture was diluted with 4.5 mL of water

and the precipitate was collected and recrystallized from ethanol to give 70.0 mg (170 μmol, 38%) of colorless plates, m. p. 158-160 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.38 (s, 6H), 7.22 (s, 4H), 7.45 (d, J = 8.3 Hz, 2H), 7.76 (d, J = 8.3 Hz, 4H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 20.9, 107.2, 115.1, 126.5, 129.8, 131.3, 138.8, 157.8, 159.2, 165.2; HRMS (EI) m/z calcd for $C_{20}H_{16}N_2S_4$ 412.0196, found 412.0197.

Photoproducts of the Pyridone 2b. A Pyrex tube (5 x 30 cm), provided with a cooling jacket, was charged with a solution of the pyridone **2b** (260 mg, 1.70 mmol) in 50 mL of a 40 : 60 mixture of H_2O and MeCN and irradiated in a Rayonet photoreactor at 300 nm and 5 °C for 55 min. The solvent was evaporated (40 °C, 20 Torr) and the residue was purified by silica-gel chromatography (EtOAc as eluent). 2-Pyridone **4a** and 3-isopropoxy-2-pyridone **4b** were isolated as photoproducts; the latter was crystallized from a CH_2Cl_2-hexane mixture. To determine the yield of the photoproducts, 14.7 mg (96.0 μmol) of the *N*-isopropoxylpyridone **2b** in D_2O were irradiated at 300 nm (Rayonet photoreactor) and 5 °C for 14.5 min in a sealed NMR tube. After evaporation of the solvent (40 °C, 20 Torr), the residue was dissolved in $CDCl_3$ and the conversion and the ratio of products **4a** and **4b** was determined with CH_2Cl_2 as internal standard, which was added immediately before the NMR analysis.

2(1H)-Pyridone (4a): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.45 (m, 2H, H-4, H-6), 6.59 (d, *J* = 9.2 Hz, 1H, H-3), 6.30 (ddd, *J* = 6.6 Hz, 6.6 Hz, 1.1 Hz, 1H, H-5).

3-Isopropoxyl-2(1H)-pyridone (4b): Colorless plates, mp 210-211 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.03 (dd, *J* = 6.6 Hz, 1.6 Hz, 1H, H-6'), 6.77 (dd, *J* = 7.5, 1.6 Hz, 1H, H-4'), 6.19 (dd, *J* = 7.5, 6.6 Hz, 1H, H-5'), 4.53 (h, *J* = 6.1 Hz, 1H, H-1), 1.39 (d, *J* = 6.1 Hz, 6H, H-2);

^{13}C NMR (50 MHz, $CDCl_3$) δ 161.3 (C-2'), 148.2 (C-3'), 125.6 (C-6'), 117.6 (C-4'), 106.5 (C-5'),

70.5 (C-1), 21.2 (C-2); IR (KBr) ν 1677 cm^{-1} (C=O), 1385 and 1370 cm^{-1} [$CH(CH_3)_2$]; HRMS (EI) m/z calcd for $C_8H_{11}NO_2$ 153.079, found 153.079.

The ^1H and ^{13}C NMR peaks were assigned by the HMBC technique (Figure S1).

Photoproducts of the Thiazolethione 3b. A solution of thiazolethione **3b** (200 mg, 0.745 mmol) in 100 mL of a 15 : 85 mixture of H_2O and MeCN was irradiated at 0 °C for 1 h with a 450-W high-pressure Hg lamp, provided with a glass filter (> 350 nm). The solvent was evaporated (35 °C, 20 Torr) and the residue was purified by silica-gel chromatography (1 : 3 to 1 : 1 CH_2Cl_2 : petroleum ether as eluent). Subsequent MPLC separation (silica gel, 1 : 3 to 1 : 1 CH_2Cl_2 : petroleum ether as eluent) afforded the disulfide **5** (10%), bithiazyl **6** (5%), thiazole **7** (10%) and isothiocyanate **8** (27%) as photoproducts. Since 22.0 mg of unreacted thiazolethione **3b** was recovered (convn 89%), the yields were calculated on the basis of consumed thiazolethione **3b**. The disulfide **5** and the bithiazyl **6** could not be separated by means of silica-gel chromatography and were characterized by ^1H-NMR and mass-spectral methods; the yields were determined directly from the ^1H-NMR spectrum of the reaction mixture.

Bis-[4-(4-methylphenyl)-2-thiazyl]disulfide (5): ^1H NMR (500 MHz, $CDCl_3$) δ 7.79 (m, 4H), 7.45 (s, 2H), 7.22 (m, 4H), 2.39 (s, 6H); MS (EI) *m/z* (relative intensity) 412 (M^+, 7.5).

4,4'-(*p*-tolyl)-2,2'-bithiazyl (6): ^1H NMR (500 MHz, $CDCl_3$) δ 7.70 (m, 4H), 7.41 (s, 2H), 7.19 (m, 4H), 2.37 (s, 6H); MS (EI) *m/z* (relative intensity) 348 (M^+, 2.2).

4-(4-Methylphenyl)thiazole (7): Colorless plates mp 55-56 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.87 (d, 2H, *J* = 2.0 Hz), 7.83 (m, 2H), 7.48 (d, 1H, *J* = 2.0 Hz), 7.25 (m, 2H), 2.39 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.4, 152.7, 138.1, 131.5, 126.3, 111.8, 21.3; IR (KBr) ν_{max} 1485 cm^{-1} (C=N); MS (EI) *m/z* (relative intensity) 175 (M^+, 27).

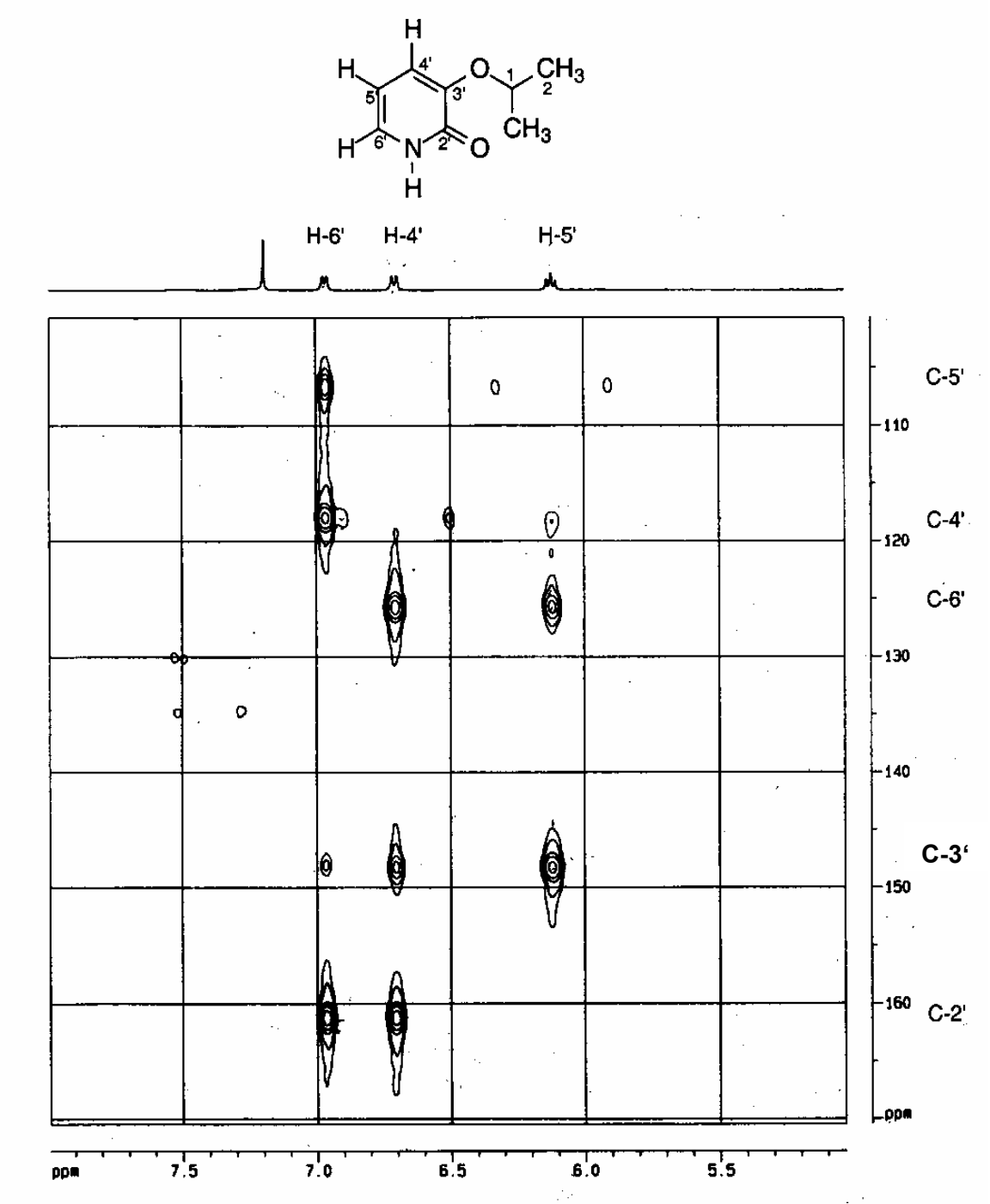

Figure S1. HMBC spectrum of pyridone **4b**

(*Z*)-1-(4-Methylphenyl)-2-[4-(4-methylphenyl)-2-thiazylsulfanyl]ethenyl-1-isothiocyanate (8): Pale yellow needles, mp 85 – 86 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (m, 2H, H-2'), 7.44 (m, 2H, H-2"), 7.40 (s, 1H, H-5), 7.26 (s, 1H, H-b), 7.24 (m, 2H, H-3'), 7.23 (m, 2H, H-3"), 2.39 (s, 6H, Me-4',4"); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.0 (C-2), 156.1 (C-4), 139.5 (C-4"), 138.8 (C-c), 138.4 (C-4'), 131.1, 131.0 (C-1', C-1"), 129.6 (C-3'), 129.5 (C-3"), 128.6 (C-a), 126.2 (C-2"), 124.9 (C-2'), 116.8 (C-b), 112.9 (C-5), 21.3, 21.2 (Me-4',4") ; IR (KBr) ν_{max} 2074 cm^{-1} (N=C=S); MS (EI) *m/z* (relative intensity) 380 (M^+, 13), 322 [100, $(M-NCS)^+$]; HRMS (FAB) m/z calcd for $C_{20}H_{17}N_2S_3$ 381.0554, found 381.0554. The ^{1}H and ^{13}H NMR peaks were assigned by HMBC technique (Figure S2). The *Z* configuration of the C_a-C_b double bond of the isothiocyanate **8** was established by the NOSY technique (Figure S3).

Quantum Yields for the Photolysis of Pyridone 2b and Thiazolethione 3b. The photon fluence for the light sources was determined by the potassium ferrioxalate actinometer.[31] Solutions of pyridone **2b** and thiazolethione **3b** in H_2O-MeCN (60 : 40) or H_2O were irradiated with a black-light lamp (312 nm) or the Xe lamp of a fluorescence photometer (308 nm) at 20 °C. The consumption of **3b** was followed by absorption spectroscopy and that of pyridone **2b** by HPLC analysis with UV detection (300 nm). The conversion of the pyridone **2b** was determined on a 250 x 4.6 mm (i. d.) Eurospher 100 C18 5-µm column (Knaur GmbH, Berlin, Germany) by using a 95 : 5 mixture of CH_3CN and water as eluent.

EPR Studies. For the detection of the radicals formed in the photolysis of pyridone **2b** and Thiazolethione **3b**, solutions of the pyridone **2b** or thiazolethione **3b** (3 mM) in benzene, water or H_2O-MeCN (60 : 40) were irradiated in a Rayonet photoreactor (300 nm for **2b**, 350 nm for **3b**) in

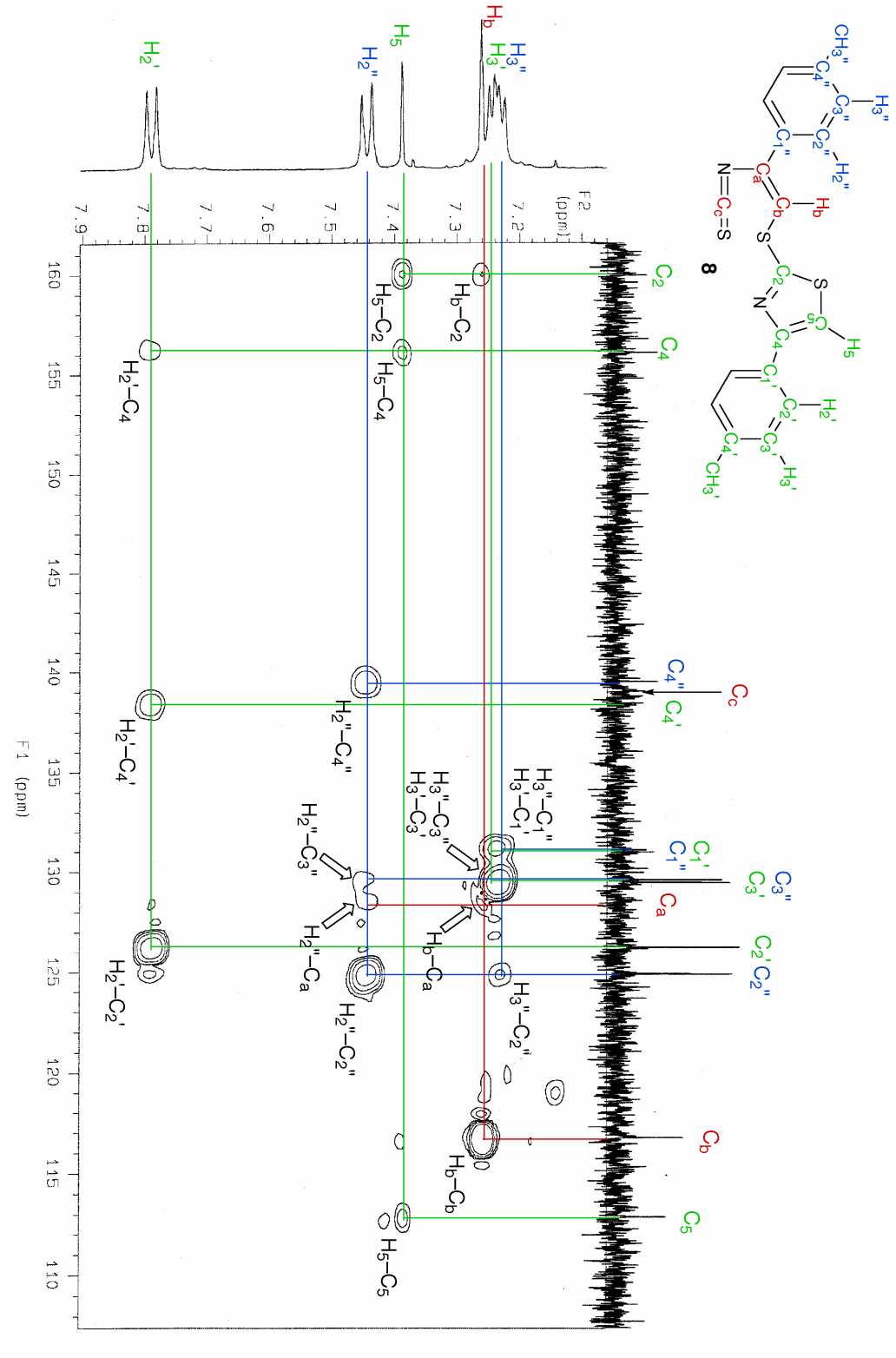

Figure S2. HMBC spectrum of the isothiocyanate **8** ($CDCl_3$).

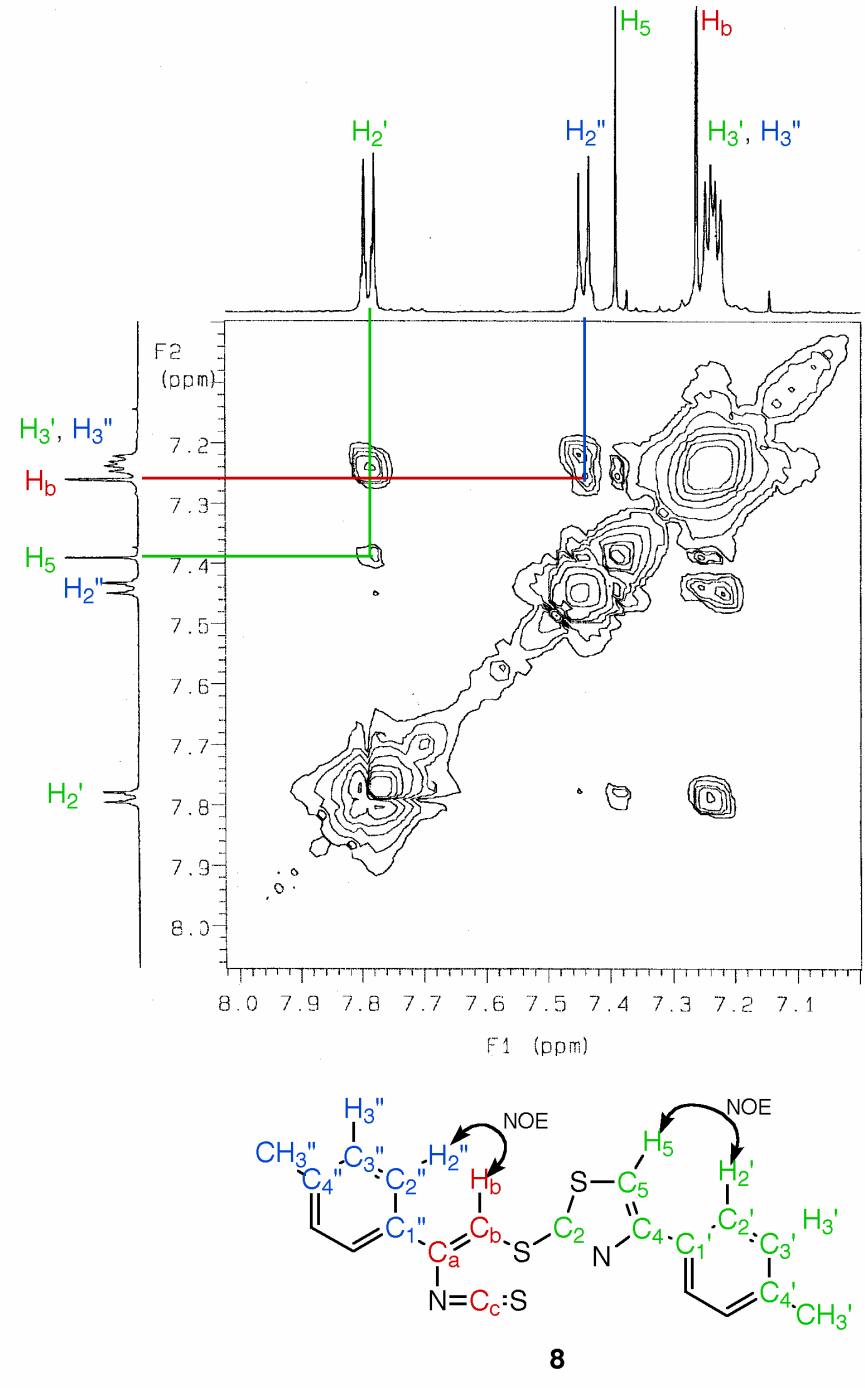

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Figure S3. NOESY spetrum of the isothiocyanate **8** ($CDCl_3$).

the presence of **DMPO** (9.0 – 90 mM) at 10 °C for 5 min. In the experiments performed without oxygen, the samples were degassed by three freeze-pump-thaw cycles before irradiation. Immediately after irradiation, the photolysates were submitted to EPR spectroscopy in a flat Quartz cell. The authentic spin adduct of **DMPO** with the 2-hydroxyprop-2-yl radical was generated by photolysis (300 nm) of acetone (480 mM) in H_2O-MeCN (60 : 40) in the presence of **DMPO** (90 mM) and isopropanol (600 mM).

For the scavenging experiments, the photoreaction of the pyridone **2b** (1 mM) was carried out in the presence of **DMPO** (27 mM) and thiazolethione **3b** (1 mM) or disulfide **9** (< 0.5 mM) in H_2O-MeCN (60 : 40), as described above and the photolysates were analyzed by EPR spectroscopy.

Modification of pBR 322 DNA. The reactions were carried out under atmospheric conditions in Eppendorf vials with supercoiled pBR 322 DNA (10 μg/mL) and pyridone **2b** or thiazolethione **3b** (4.0 mM) in 5.0 mM KH_2PO_4 buffer (pH 7.4) : MeCN (60 : 40). The samples (10 μL final volume) were prepared from stock solutions of pBR 322 DNA (33.3 μg/mL in 15.7 m*M* KH_2PO_4 buffer, pH 7.4) and pyridone **2b** or thiazolethione **3b** (10 mM in H_2O or MeCN). The charged Eppendorf vials were irradiated from above with a black-light lamp (312 nm) for 30 min at 0 °C. The control experiments were carried out in the presence of isopropanol (10 vol%), to assess the involvement of radicals in the photolysis.

Detection of Strand Breaks. To the photolysate was added 2.5 μL of bromophenol gel-loading solution and an 8-μL aliquot of the resulting mixture was transfered onto a 1% agarose gel, stained with 0.5 μg/mL ethidium bromide. Electrophoresis was carried out in Tris buffer (18.0 m*M* Tris

base, 18.0 m*M* boric acid, and 10.0 m*M* EDTA, pH 8.0) at 78 V for ca. 2 h in a Pharmacia horisontal apparatus (GNA 100), equipped with a power supply (GPS 200/400). The fluorescent spots of the DNA were detected by exposure to a UV transilluminator at 254 nm. Photographs of the gels were taken with a Herolab camera E. A. S. Y. 429K, which was connected to a personal computer, equipped with Herolab E. A. S. Y. software. The ratio of open-circular and the supercoiled DNA was determined from the fluorescence intensities of the spots.

Additional References

(S1) Zhang, X.; Erb, C.; Flammer, J.; Nau, W. M. *Photochem. Photobiol.* **2000**, *71*, 524-533.
(S2) Inbar, S.; Linschitz, H.; Cohen, S. G. *J. Am. Chem. Soc.* **1981**, *103*, 1048-1054.
(S3) Hurley, J. K.; Sinai, N.; Linschitz, H. *Photochem. Photobiol.* **1983**, *38*, 9-14.
(S4) Usui, Y. *Yakugaku Zasshi* **1969**, *89*, 689-698.