Supplementary data

(6-4)-Photolyase activity requires a charge shift reaction

Thorsten Stafforst and Ulf Diederichsen*

General methods: For buffer preparation highest grade chemicals and deionised water (18 MΩ/cm) was used at 10 mM concentration: citric acid (pH 2, 3 and 5, each 0.10 M NaCl), sodium bishydrogenphosphate (pH 7, 0.10 M NaCl), borax (pH 9, 0.10 M NaCl), glycine (pH 11, 0.10 M NaCl) and perchloric acid (pH 1, 1.0 % $HClO_4$ MeOH/water 1:1, 0.10 M NaCl).
Infrared spectra were recorded on a Perkin Elmer FT-IR 1600. NMR spectra were recorded on a Varian Unity 300 or Varian Inova 600 spectrometer. UV absorption spectra were collected by a Jasco V-550 spectrometer. ESI mass spectra were collected by a Finnigan LCQ iontrap spectrometer. High resolution mass spectra (HRESI) were recorded on a Bruker FTMS-7 APEX® IV 70e FT-ICR spectrometer. Reversed phase HPLC was performed on a Pharmacia Biotech Äkta Basic 900 system (eluents: A: water + 0.1 % TFA; B: acetonitrile/water 90:10 + 0.1 % TFA). Analytical HPLC was done on a J'sphere ODS-H80, 150 x 4.6 mm ID, S-4 µm, 8 nm, C18 (YMC) column (1 ml min^{-1}). Preparative HPLC was done on a J'sphere ODS-H80, 250 x 20 mm ID, S-4 µm, 8 nm, C18 (YMC) column (10 ml/min).

Irradiation experiments: Irradiation experiments were performed on a Jasco FP-6200 spectrofluorometer at 450 nm with 5 nm bandwidth. The samples were prepared in a 10 mm quartz cuvette in a mixture of methanol/buffer 1:1. The concentration was adjusted to 20 µmol/l by measuring the absorbance at 450 nm (oxidised form). During the exposure time the solutions were bubbled continuously with oxygen-free, water-saturated nitrogen (reductive) or water saturated oxygen (oxidative), respectively. For the reductive process the samples were reduced with 100 equiv. (10 µl) of a 0.2 M sodium dithionite solution. The reduction was easily seen from the total depletion of fluorescence. At return of fluorescence the reduction was repeated as described above. Seven to eight samples (20 - 30 µl) were taken during a period of 75 % conversion of the starting material and were analysed by RP-HPLC. The relative starting material concentration was calculated from baseline corrected areas of the UV absorption peaks at 450 nm with relative starting material concentration = area starting material / [area starting material + area product]. The absolute error for the determination of each area was estimated to 0.04 mAu min. The errors for each value of ln[starting material] were between 2 % and 5 %. They were plotted as error bars. The error of the rates was determined graphically from the error bar and was usually between 4 % and 7 %. Reference experiments proved the stability of the starting material in the dark and were performed for all compounds under all pH conditions.

Actinometry:[1] The quantum yields were determined using 6.0 mM ferrioxalate at 450 nm under the same conditions (cuvette, volume, 5 nm bandwidth and purging with nitrogen) as the irradiation conditions. All solutions were produced freshly following literature protocols.[1] A calibrating curve was taken for the determination of iron(II) concentrations. A light flow of 89 x 10^{-9} erg/min was obtained. Quantum yields were calculated from the lifetimes and the extinction coefficients by the formula beneath. The extinction coefficients were estimated to 800 cm²/mol for the reduced and 12200 cm²/mol for the oxidised form at 450 nm as taken from literature for riboflavin.[2] The UV spectra of the flavin-containing compounds were identical with the UV spectra for riboflavin under the same conditions. The extinction coefficients were not corrected for their pH dependence. The absorptance (α) of the oxidised species (absorbance 450 nm ~ 0.25) was assumed to be 1.70 x absorbance, and that of the reduced species was assumed to be 2.3 x absorbance.

Analytical data: The NMR analysis of the flavin containing compounds suffered from a high rotation barrier caused by the tertiary amide bond of the *N*-acyl-(2-aminoethyl)glycine, which led to broadening and partly doubling of ^{1}H and ^{13}C NMR signals.[3] Nevertheless, by 2D-NOESY NMR experiments the constitutional integrity of a single compound was confirmed. A complete set of NMR assignments was obtained separately for both rotamers based on H,H-COSY, HSQC and HMBC experiments.

$$\Phi / \% = \frac{1 - \exp\left(-\frac{\ln 2}{\tau_{1/2}}\right)}{89 \times \varepsilon \times \alpha} \times 10^{8}$$

[ε] = cm²/mol, [τ] = min, α = 1.70 (oxidised species) or 2.3 (reduced species), respectively

Flavin-T<$OxPh_2$> **1**: HPLC (preparative): 30 - 85 % B in 25 min, t_R = 20.8 min; m.p. 230 °C; two isomers A and B were obtained in ratio A/B = 0.4 as determined by NMR; isomer A: ^{1}H NMR (600 MHz, CD_3OD, 50 °C): δ = 7.95 (s, 1H; flavin CH), 7.62 (s, 1H; flavin CH), 7.36-7.32 (m, 3.5H; phenyl CH), 7.29-7.24 (m, 3.5H; phenyl CH), 7.16-7.08 (m, 3H; phenyl CH), 5.59 (d, $^{2}J_{H,H}$ = 15.6 Hz; 1H; flavin CH_2), 5.43 (d, $^{2}J_{H,H}$ = 15.6 Hz, 1H; flavin CH_2), 4.86 (s, 1H; thymine CH), 4.42 (s, 2H; CH_2), 4.29 (d, $^{2}J_{H,H}$ = 16.8 Hz, 1H; CH_2), 3.80 (m, 1H; CH_2), 3.47 (m, $^{2}J_{H,H}$ = 16.2

Hz, 2H; CH_2), 3.33 (m, 2H; CH_2), 2.56 (s, 3H; flavin CH_3), 2.45 (s, 3H; flavin CH_3), 1.63 (s, 3H; thymine CH_3), 1.58 (s, 9H; $C(CH_3)_3$); ^{13}C NMR (150 MHz, CD_3OD, 50 °C): δ = 172.0, 170.9, 170.4, 168.1, 161.8, 158.3, 153.3, 151.8, 150.0, 145.3 (phenyl C), 140.6 (phenyl C), 138.8, 137.5, 136.0, 133.1, 132.7 (flavin CH), 129.4-128.9 (6 phenyl CH), 127.6-126.6 (4 phenyl CH), 117.4 (flavin CH), 92.7 (C(phenyl)$_2$), 84.8 ($C(CH_3)_3$), 77.8 (thymine C-5), 67.3 (thymine CH-6), 51.5, 49.7, 49.5, 48.2, 38.4, 28.4 ($C(CH_3)_3$), 23.6 (thymine CH_3), 21.2 (flavin CH_3), 19.3 (flavin CH_3); isomer B: ^{1}H NMR (600 MHz, CD_3OD, 50 °C): δ = 7.95 (s, 1H; flavin CH), 7.59 (s, 1H; flavin CH), 7.36-7.32 (m, 3.5H; phenyl CH), 7.29-7.24 (m, 3.5H; phenyl CH), 7.16-7.08 (m, 3H; phenyl CH), 5.83 (d, $^2J_{H,H}$ = 16.2 Hz, 1H; flavin CH_2), 5.59 (d, $^2J_{H,H}$ = 15.6 Hz; 1H; flavin CH_2), 4.85 (s, 1H; thymine CH), 4.56 (d, $^2J_{H,H}$ = 16.2 Hz, 1H; CH_2), 4.14 (d, $^2J_{H,H}$ = 17.4 Hz, 1H; CH_2), 4.01 (d, $^2J_{H,H}$ = 17.4 Hz, 1H; CH_2), 3.93 (d, $^2J_{H,H}$ = 17.4 Hz, 1H; CH_2), 3.80 (m, 1H; CH_2), 3.70 (m, 2H; CH_2), 3.57 (m, 1H; CH_2), 2.53 (s, 3H; flavin CH_3), 2.45 (s, 3H; flavin CH_3), 1.71 (s, 3H; thymine CH_3), 1.43 (s, 9H; $C(CH_3)_3$); ^{13}C NMR (150 MHz, CD_3OD, 50 °C): δ = 172.5, 171.0, 170.0, 167.6, 161.9, 158.3, 153.5, 151.7, 149.9, 145.1 (phenyl C), 140.1 (phenyl C), 138.8, 137.3, 136.1, 133.2, 132.5 (flavin CH), 129.4-128.9 (6 phenyl CH), 127.6-126.6 (4 phenyl CH), 117.8 (flavin CH), 92.8 (C(phenyl)$_2$), 83.5 ($C(CH_3)_3$), 77.9 (thymine C-5), 68.1 (thymine CH-6), 50.4, 50.3, 49.5, 48.0, 38.6, 28.3 ($C(CH_3)_3$), 23.5 (thymine CH_3), 21.2 (flavin CH_3), 19.3 (flavin CH_3); IR (KBr): ν = 3433, 2367, 1636, 1546, 1157, 557 cm^{-1}; UV (MeOH/water 1:1): λ_{max} = 445, 367, 266, 222 nm; fluorescence: λ_{exc} = 450 nm, λ_{max} = 525 nm; HR-ESI MS: *m/z* $[M+H]^+$ = calcd 805.33040 for $C_{42}H_{45}N_8O_9$, found 805.33022.

Flavin-T **2**: HPLC (preparative): 0 – 100 % B in 30 min, t_R = 19.6 min; m.p. 212 °C; two isomers A and B were obtained in ratio A/B = 1.8 as determined by NMR; isomer A: ^{1}H NMR (600 MHz, [D_6]DMSO, 35 °C): δ = 11.38 (s, 1H; NH), 11.19 (s, 1H; NH), 8.37 (br t, $^3J_{H,H}$ = 5.0 Hz, 1H; NH), 7.92 (s, 1H; flavin CH), 7.50 (s, 1H; flavin CH), 7.41 (s, 1H; thymine CH), 5.71 (br s, 2H; flavin CH_2), 4.52 (s, 2H; CH_2), 3.98 (s, 2H; CH_2), 3.64 (br s, 2H; CH_2), 3.46 (br s, 2H; CH_2), 2.45 (s, 3H; flavin CH_3), 2.38 (s, 3H; flavin CH_3), 1.68 (s, 3H; thymine CH_3), 1.37 (s, 9H; $C(CH_3)_3$); ^{13}C NMR (150 MHz, [D_6]DMSO, 35 °C): δ = 167.9, 167.8, 165.0, 164.3 (thymine C-4), 159.6, 155.5, 151.0 (thymine C-2), 150.1, 146.5, 142.3 (thymine CH-6), 136.7, 136.0, 133.6, 131.2, 130.5 (flavin CH), 116.8 (flavin CH), 107.8 (thymine C-5), 80.9 ($C(CH_3)_3$), 49.5, 48.5, 46.6, 45.6, 37.1, 27.6 ($C(CH_3)_3$), 20.4 (flavin CH_3), 18.7 (flavin CH_3), 11.8 (thymine CH_3); isomer B: ^{1}H NMR (600 MHz, [D_6]DMSO, 35 °C): δ = 11.34 (s, 1H; NH), 11.19 (s, 1H; NH), 8.11 (br t, $^3J_{H,H}$ = 5.0 Hz, 1H; NH), 7.92 (s, 1H; flavin CH), 7.49 (s, 1H; flavin CH), 7.37 (s, 1H; thymine CH), 5.45 (br s, 2H; flavin CH_2), 4.42 (s, 2H; flavin CH_2), 4.26 (s, 2H; CH_2), 3.37 (br s, 2H; CH_2), 3.33 (covered, 2H; CH_2), 2.45 (s, 3H; flavin CH_3), 2.38 (s, 3H; flavin CH_3), 1.68 (s, 3H; thymine CH_3), 1.53 (s, 9H; $C(CH_3)_3$); ^{13}C NMR (150 MHz, [D_6]DMSO, 35 °C): δ = 169.2, 167.2, 165.2, 164.3 (thymine C-4), 159.6 (thymine C-2), 155.2, 151.0, 150.2, 146.8, 142.2 (thymine CH-6), 136.8, 136.0, 133.4, 131.1, 130.5 (flavin CH), 116.0 (flavin CH), 107.9 (thymine C-5), 82.3 ($C(CH_3)_3$), 50.0, 49.3, 47.2, 46.6, 36.1, 27.7 ($C(CH_3)_3$), 20.3 (flavin CH_3), 18.6 (flavin CH_3), 11.7 (thymine CH_3); IR (KBr): ν = 3438, 1665, 1550, 1242, 1160 cm^{-1}; UV (MeOH/water 1:1): λ_{max} = 445, 367, 266, 222 nm; fluorescence: λ_{exc} = 450 nm, λ_{max} = 525 nm; HR-ESI MS: *m/z* $[M+H]^+$ = calcd 623.25724 for $C_{29}H_{35}N_8O_8$, found 623.25717.

Carbazole-T<OxPh$_2$> **3**: HPLC (preparative): 30 – 100 % B in 20 min, t_R = 18.54 min; m.p. 243 °C; ^{1}H NMR (300 MHz, [D_6]-DMSO, 35 °C): δ = 10.42 (s, 1H; thymine-NH), 8.26 (t, $^3J_{H,H}$ = 5.1 Hz, 1H; NH), 8.14 (d, $^3J_{H,H}$ = 7.5 Hz, 2H; carbazole H-4, H-5), 8.09 (t, $^3J_{H,H}$ = 5.1 Hz, 1H; NH), 7.51 (d, $^3J_{H,H}$ = 8.4 Hz, 2H; carbazole H-1, H-8), 7.42 (t, $^3J_{H,H}$ = 8.4 Hz, 2H; carbazole H-2, H-7), 7.36-7.25 (m, 10H; oxetane phenyl CH), 7.20 (t, $^3J_{H,H}$ = 7.5 Hz, 2H; carbazole H-3, H-6), 4.98 (s, 2H; carbazole CH_2), 4.79 (s, 1H; thymine H-6), 4.23 (d, $^2J_{H,H}$ = 16.5 Hz, 1H; thymine CH_2), 3.50 (d, $^2J_{H,H}$ = 16.5 Hz, 1H; thymine CH_2), 3.26-3.10 (m, 4H; CH_2-CH_2), 1.59 (s, 3H; thymine CH_3); ^{13}C NMR (75.6 MHz, [D_6]-DMSO, 35 °C): δ =169.7 (thymine C-4), 167.6 (carbazole CH_2CO), 167.4 (thymine CH_2CO), 151.2 (thymine C-2), 144.31 (oxetane phenyl C), 140.6 (2 carbazole C), 139.5 (oxetane phyenl C), 128.3-127.6 (5 oxetane phenyl CH), 125.6 (carbazole C-2, C-8), 125.5-124.9 (5 oxetane phenyl CH), 122.2 (2 carbazole C), 120.0 (carbazole C-4, C-5), 118.9 (carbazole C-3, C-6), 109.2 (carbazole C-1, C-8), 90.7 (oxetane C), 76.1 (thymine C-5), 65.2 (thymine C-6), 48.44 (thymine CH_2), 45.6 (carbazole CH_2), 38.4 (CH_2-CH_2), 38.3 (CH_2-CH_2), 23.0 (thymine CH_3); IR (KBr): ν = 3410, 1688, 1535, 1462, 1268, 970, 750 cm^{-1}; UV (MeOH): λ_{max} = 258, 290, 324, 340 nm; fluorescence: λ_{exc} = 335 nm, λ_{max} = 347, 360 nm; HR-ESI: m/z $[M+H]^+$= calcd 616.25545 for $C_{36}H_{33}N_5O_5$, found 616.25522.

Carbazole-T **4**: HPLC (preparative): 30 – 80 % B in 15 min, t_R = 11.85 min; m. p. 295 °C; ^{1}H NMR (300 MHz, [D_6]-DMSO, 35 °C): δ = 11.15 (s, 1H; thymine-NH), 8.29 (tbr, 1H; NH), 8.18 (tbr, 1H; NH), 8.14 (d, $^3J_{H,H}$ = 7.5 Hz, 2H; carbazole H-4, H-5), 7.50 (d, $^3J_{H,H}$ = 8.4 Hz, 2H; carbazole H-1, H-8), 7.43 (t, $^3J_{H,H}$ = 8.4 Hz, 2H; carbazole H-2, H-7), 7.35 (s, 1H; thymine H-6), 7.20 (t, $^3J_{H,H}$ = 7.5 Hz, 2H; carbazole H-3, H-6), 5.00 (s, 2H; carbazole CH_2), 4.25 (s, 2H; thymine CH_2), 3.18-3.16 (m, 4H; CH_2-CH_2), 1.73 (s, 3H; thymine CH_3); ^{13}C NMR (150 MHz, [D_6]-DMSO, 35 °C): δ =167.5 (carbazole CH_2CO), 167.0 (thymine CH_2CO), 164.3 (thymine C-4), 151.0 (thymine C-2), 142.1 (thymine C-6), 140.6 (2 carbazole C), 125.6 (carbazole C-2, C-8), 122.2 (2 carbazole C), 120.0 (carbazole C-4, C-5), 118.9 (carbazole C-3, C-6), 109.2 (carbazole C-1, C-8), 108.0 (thymine C-5), 49.24 (thymine CH_2), 45.5 (carbazole CH_2), 38.2 (2 CH_2), 11.7 (thymine CH_3); IR (KBr): ν = 3433, 1662, 565 cm^{-1}; UV (MeOH): λ_{max} = 258, 290, 324, 340 nm; fluorescence: λ_{exc} = 335 nm, λ_{max} = 344, 361 nm; HR-ESI: m/z $[M+H]^+$= calcd 434.18228 for $C_{23}H_{23}N_5O_4$, found 434.18268.

T<ox>Ph$_2$ **5**: M.p. 220 °C; ^{1}H NMR (300 MHz, [D_6]DMSO, 35 °C): δ = 12.90 (s, 1H; OH), 10.42 (s, 1H; NH), 7.43-7.23 (m, 10H; phenyl CH), 4.89 (s, 1H; H-6), 4.27 (d, $^2J_{H,H}$ = 17.7 Hz, 1H; CH_2), 3.86 (d, $^2J_{H,H}$ = 17.7 Hz, 1H; CH_2), 1.59 (s, 3H; CH_3); ^{13}C NMR (75 MHz, [D_6]DMSO, 35 °C): δ = 169.9, 169.8, 151.1 (C-2), 144.3 (phenyl C), 139.5 (phenyl C), 128.3 (2 phenyl CH), 128.0 (2 phenyl CH), 127.5 (2 phenyl CH), 125.4 (2 phenyl CH), 125.0 (2 phenyl CH), 90.8 (C(phenyl)$_2$), 76.0 (C-5), 65.2 (CH-6), 47.8, 23.2 (flavin CH_3); IR (KBr): ν = 3432, 1723, 1666, 1492,

1404, 1287, 1228, 750, 703 cm^{-1}; UV (MeOH/water 1:1): λ_{max} = 225 nm; HR-ESI MS: *m/z* $[M+H]^+$ = calcd 367.12885 for $C_{20}H_{19}N_2O_5$, found 367.12908.

Dipl. Chem. Thorsten Stafforst and Prof. Dr. Ulf Diederichsen*
Institut für Organische und Biomolekulare Chemie, Georg-August-Universität Göttingen, Tammannstrasse 2, D-37077 Göttingen, Germany. Fax: +49 (0)551 392944; Tel: +49 (0)551 393221; E-mail: udieder@gwdg.de

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