Photochemically driven intercalation of small molecules into DNA by *in situ* irradiation

Fausto Puntoriero, Maria Letizia Di Pietro, Fabien Tuyéras, Philippe Ochsenbein, Philippe P. Lainé and Sebastiano Campagna

SUPPORTING INFORMATION

Characterization of compounds 1 and 2

(including ^{1}H and ^{13}C NMR and mass spectra of **1** and **2** and X-ray - Ortep drawing - of **2**)

Materials and methods

Schematization of the photochemical process

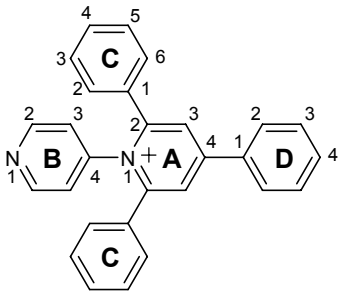
Circular dichroism spectra

Comparison between absorption spectra

References

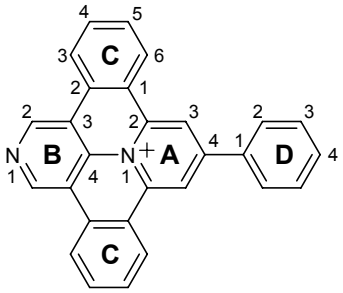
Characterization

Characterization of compounds **1** and **2** (for convenience, the structural formulae of the compounds and numbering schemes are also shown).



py-DPH$_2$-Ph$^+$, BF$_4^-$ (1)

^{1}H NMR (500 MHz, CD_3CN, 25°C): δ 8.43 (s, 2H, H^{A3}), 8.41 (d, J = 6.0 Hz, 2H, H^{B2}), 8.12 (d, J = 8.0 Hz, 2H, H^{D2}), 7.73 (dd, J = 7.5, 7.0 Hz, 1H, H^{D4}), 7.67 (dd, J = 8.0, 7.0 Hz, 2H, H^{D3}), 7.47-7.37 (m, 10H, $H^{C2, C3, C 4, C 5, C 6}$), 7.25 (d, J = 5.5 Hz, 2H, H^{B3}); ^{13}C NMR (126 MHz, CD_3CN, 25°C): δ 158.5, 157.2, 152.0, 147.2, 134.6, 134.0, 133.3, 131.7, 130.9, 130.8, 129.7, 129.6, 127.0, 124.2; ESI-MS (*m/z)*: $[M]^+$ calcd. for $C_{28}H_{21}N_2$, 385.17; found, 385.33; analysis (calcd., found for $C_{28}H_{21}N_2BF_4$): C (71.21, 71.17), H (4.48, 4.42), N (5.93, 5.99).



dBQNTH$_2$-Ph$^+$,BF$_4^-$ (2)

^{1}H NMR (500 MHz, CD_3CN, 25°C): δ 10.21 (s, 2H, H^{B2}), 9.55 (s, 2H, H^{A3}), 9.12 (d, J = 9.0 Hz, 2H, H^{C6}), 8.96 (d, J = 8.5 Hz, 2H, H^{C3}), 8.36-8.34 (m, 2H, H^{D2}), 8.18 (dd, J = 8.0, 7.5 Hz, 2H, H^{C4}), 8.06 (dd, J = 8.5, 7.5 Hz, 2H, H^{C5}), 7.79-7.78 (m, 3H, $H^{D3, D4}$); ^{13}C NMR (126 MHz, CD_3CN, 25°C): δ 153.2, 146.2, 144.8, 135.7, 135.6, 133.3, 133.2, 132.4, 130.84, 130.78, 130.0, 128.3, 125.6, 124.2, 120.3, 119.3; ESI-MS (*m/z*): $[M]^+$ calcd. for $C_{28}H_{17}N_2$, 381.14; found, 381.40; analysis (calcd., found for $C_{28}H_{17}N_2BF_4$): C (71.82, 71.78), H (3.67, 3.47), N (5.98, 6.06).

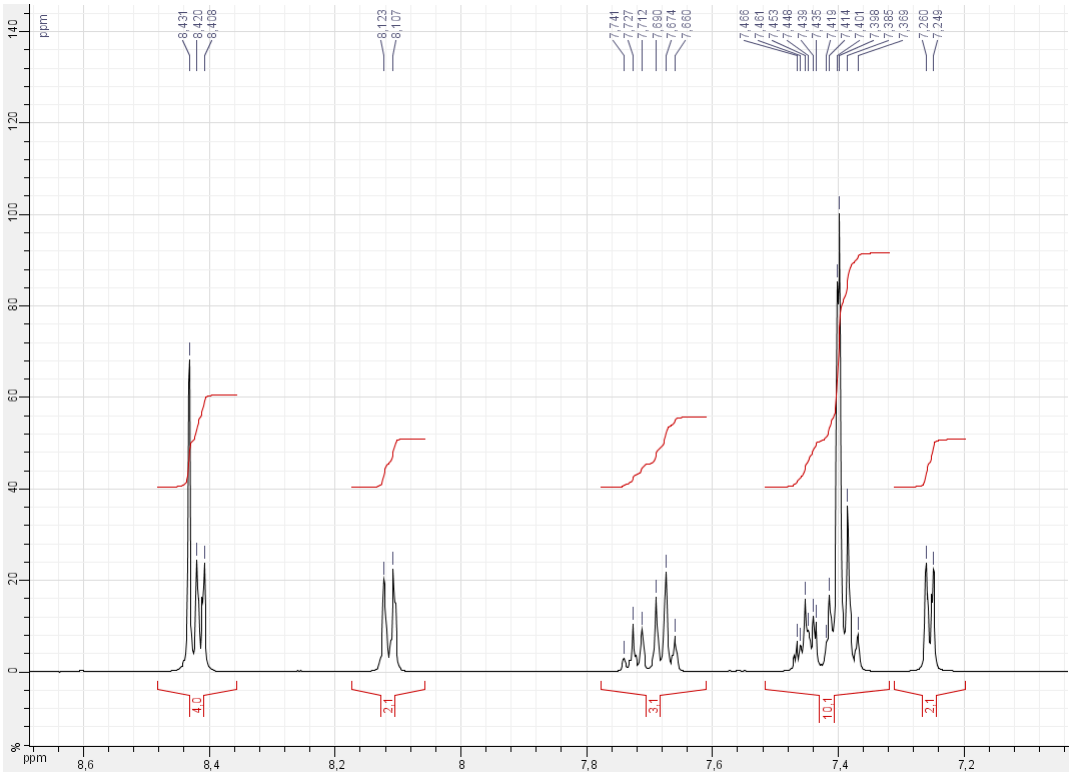


Figure S1. ^{1}H NMR spectrum of **1** in acetonitrile.

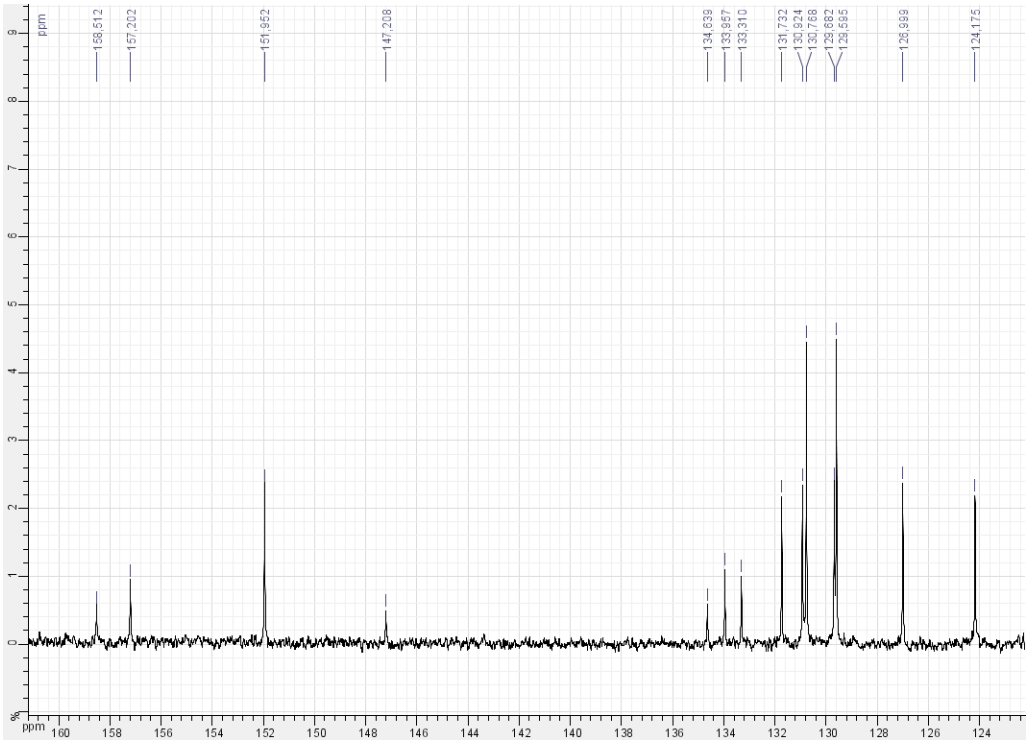


FIGURE S2. ^{13}C NMR spectrum of **1** in acetonitrile.

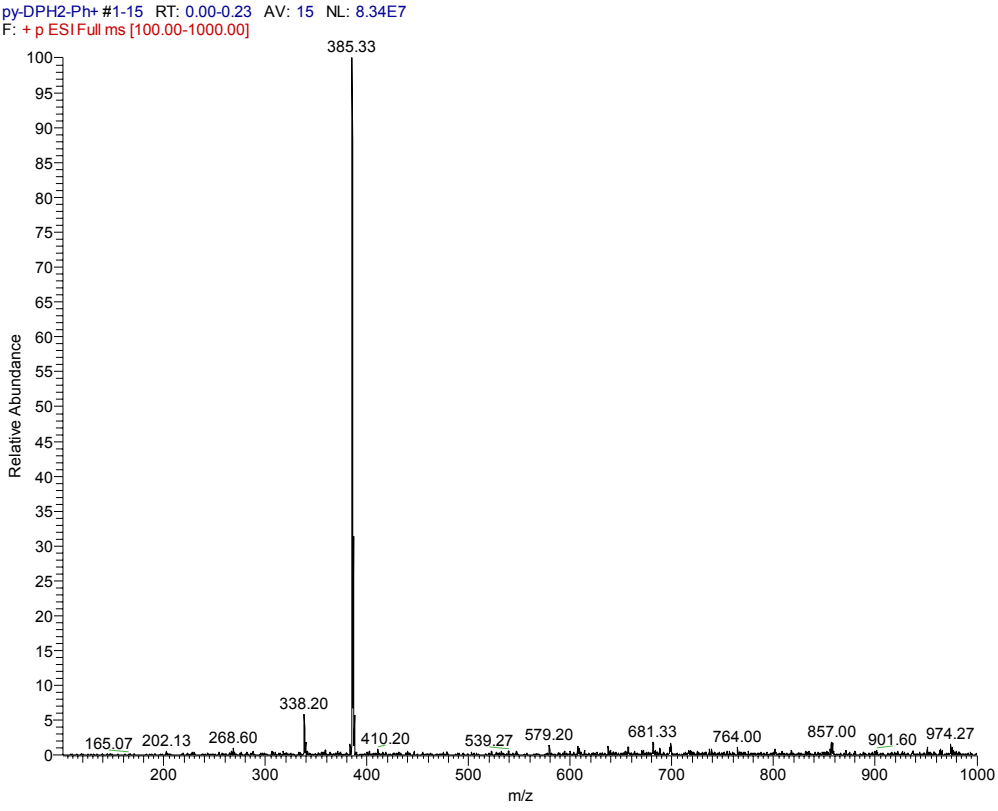


Figure S3. Mass spectrum of **1**.

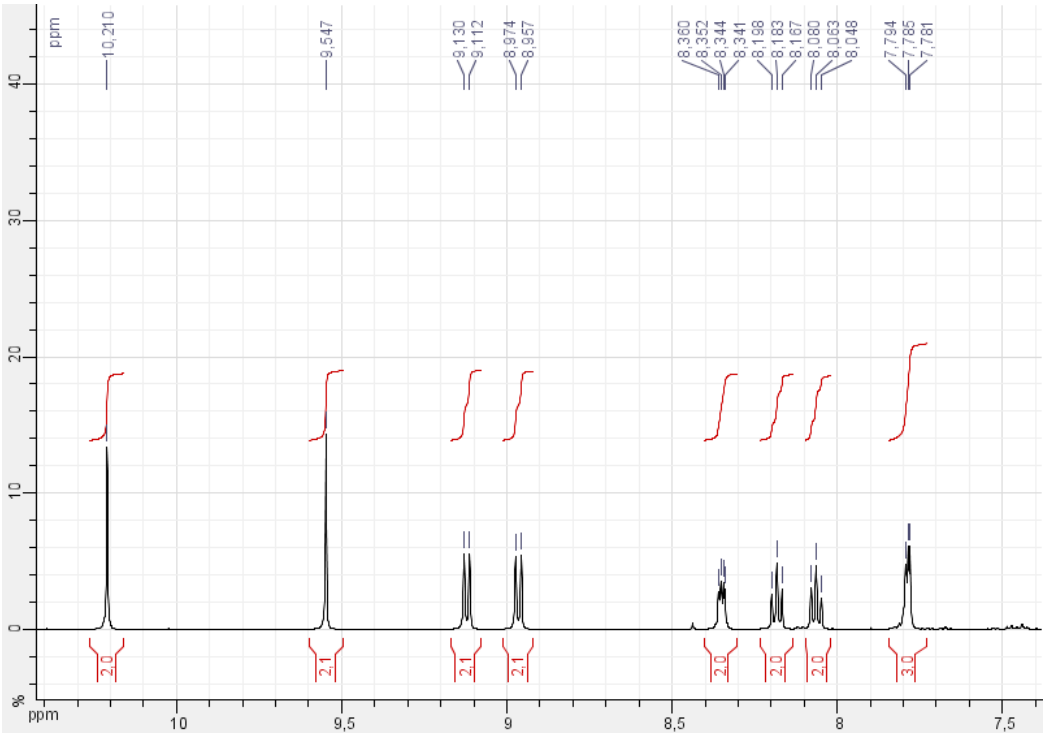


Figure S4. ^{1}H NMR spectrum of **2** in acetonitrile.

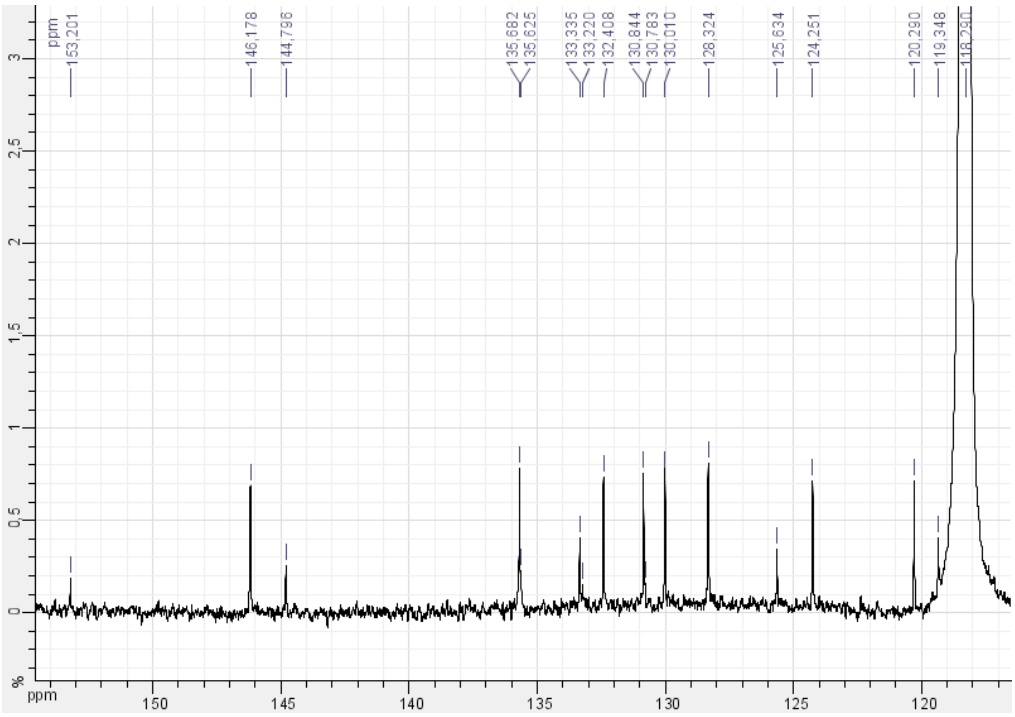


Figure S5. ^{13}C NMR spectrum of **2** in acetonitrile.

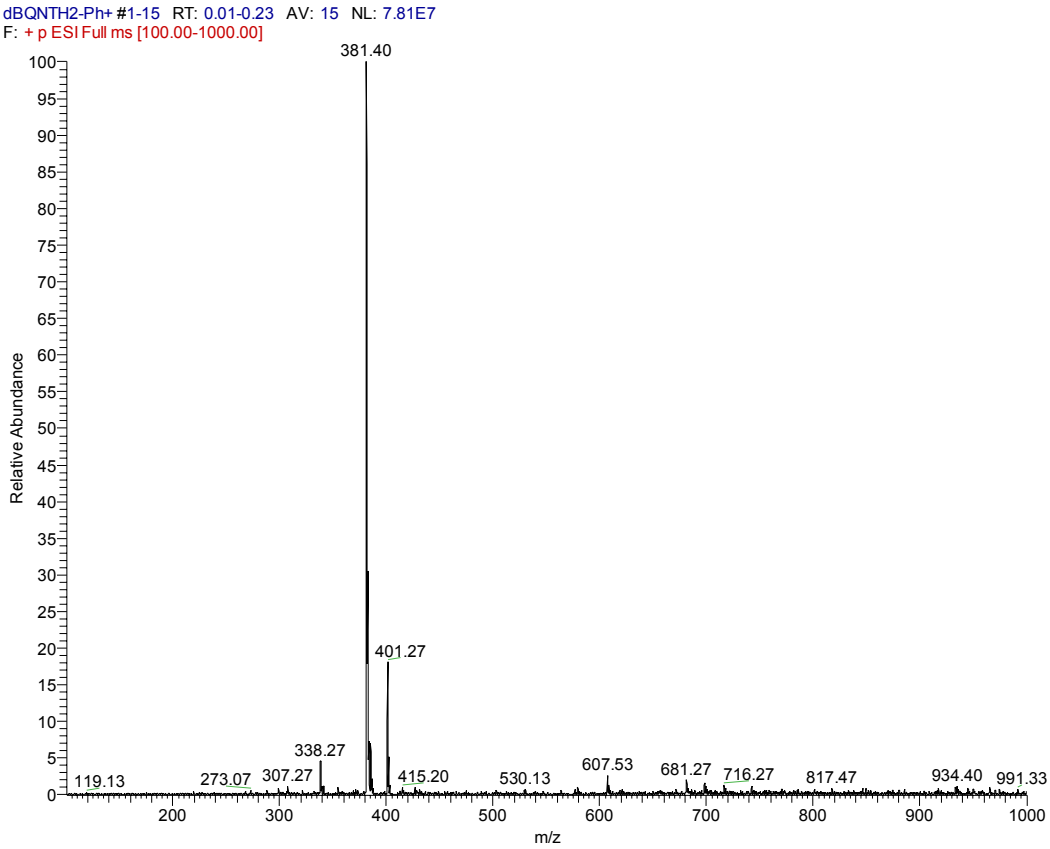


Figure S6. Mass spectrum of **2**.

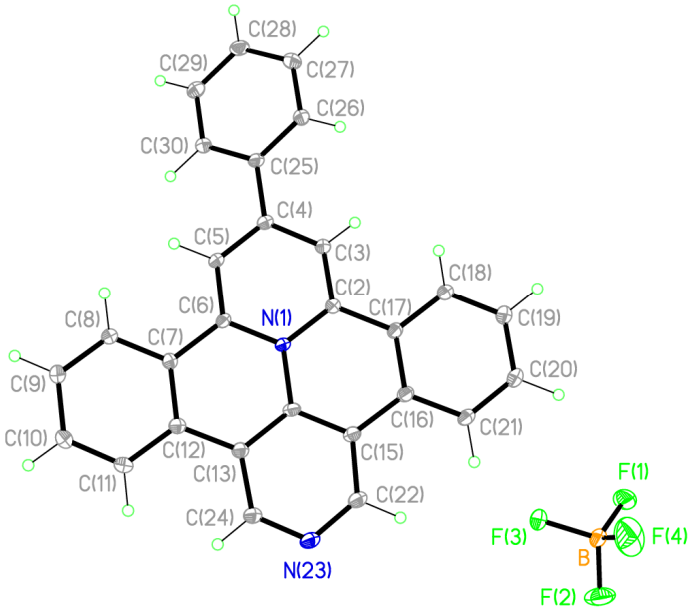


Figure S7. X-ray structure of **2** : ORTEP drawing with thermal ellipsoids (40% probability). For further details, CCDC-743209 contains the supplementary crystallographic data. These latter data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Materials and Methods

Absorption spectra have been obtained by a JASCO 560 and a Cintra 20 GBC spectrophotometers. Luminescence spectra have been recorded with a Horiba Jobin-Yvon Spex Fluoromax P fluorimeter equipped with a Hamamatsu R928 photomultiplier. Luminescence lifetimes have been obtained by an Edinburgh OB900 time-correlated single-photon-counting spectrometer (excitation pulse obtained by a laser diode at 308 nm; pulse width, 59 ps) and analyzed by Marquadt algorithm and deconvolution procedures supplied by the manufacturer. Luminescence quantum yields have been calculated with the optically diluted method[1] and photoreaction quantum yields have been calculated by using Aberchrome 540 as the standard.[2] Circular dichroism experiments have been performed by a Jasco J-810 spectropolarimeter. The thermal denaturation temperature of compound–DNA mixtures (1:10) has been determined in 1×10^{-3} M phosphate buffer (pH 7) containing 7.8×10^{-6} M compound and 2×10^{-3} M NaCl. Melting curves have been recorded at 260 nm. The temperature has been increased at a rate of 0.5 K/min by using a PTP-1 Peltier system. For the photochemical reaction, we used a Xenon lamp (150 W) as excitation source. The excitation wavelength was selected by employing a monochromator.

Viscosity titrations have been performed by means of a Cannon-Ubbelhode semi-micro-dilution viscometer (Series No. 75, Cannon Instrument Co.), thermostatically maintained at 298 K in a water bath. The viscometer contained 2 mL of sonicated DNA solution, in 1×10^{-3} M phosphate buffer (pH = 7) and 1×10^{-2} M NaCl. The compound solution ((1.5—2.5) $\times 10^{-4}$ M), containing also DNA (6.0×10^{-4} M) at the same concentration as that in the viscometer, has been delivered in increments of 90–190 μL from a micropipette. Reduced viscosities have been calculated by established methods and plotted as ln η/η_0 against ln (1+r) for rod–like DNA (600 base pairs) (η = reduced viscosity of the biopolymer solution in the presence of compound; η_0 = reduced viscosity of the biopolymer solution in the absence of compound; r = $[\text{compound}]_{\text{bound}}/[\text{biopolymer}]_{\text{tot}}$). For the viscometric titrations, in the case of irradiated **1** the amount of bound compound has been assumed to be equivalent to the amount of **1** in solution before irradiation. This is based on the quantitative photoinduced transformation of **1** into **2**. The same is assumed also for all the experiments when the concentration of irradiated **1** has to be considered.

The data of the spectrophotometric titrations have been analyzed by a nonlinear least-squares fitting program, applied to McGhee and von Hippel equation.[3] The binding constant, K_B, has been determined by the program, using the extinction coefficient of the compounds, the free compound concentration and the ratio of bound compound per mole of DNA.

Schematization of the photochemical biscyclization reaction

The photochemical reaction is schematized in equation 1.

$$\mathbf{1} + h\nu + O_2 \rightarrow \mathbf{2} + 2\ H_2O \qquad (1)$$

Circular dichroism

Generally, an achiral species acquires an induced CD signal only if it specifically binds a chiral molecule, like DNA. For our compounds, only compound **2** shows CD signals in the presence of excess DNA in the region where the compound-DNA supramolecular assembly absorbs. Circular dichroism spectra of the mixture [DNA]/[compound] = 3 are shown in Fig. S8.

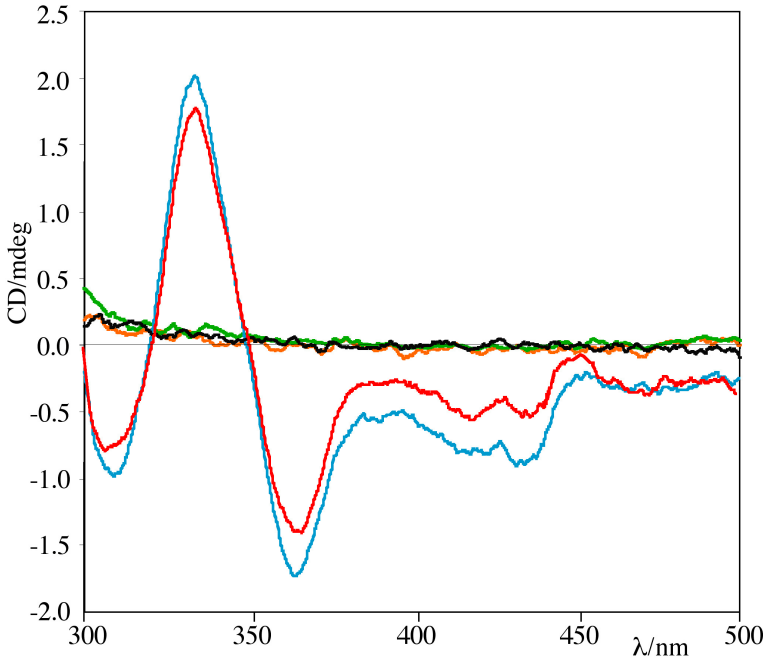


Figure S8. Circular dichroism spectra in buffered solution. The spectra of **1** (black), **2** (orange), and **1** in the presence of three-fold excess of DNA (green) are overlapped at CD signal around 0 mdeg. Light blue curve is **2** in the presence of DNA. Red curve is **1** in the presence of DNA after irradiation (30 min at 320 nm). Ionic strength: 1.1 x 10^{-2} M ([NaCl] = 1.0 x 10^{-2} M; $[\text{phosphate buffer}]_{pH=7}$ = 1.0 x10^{-3} M); T = 298 K. Concentration of **1** or **2** is 2.7 x 10^{-5} M.

Comparison between absorption spectra of 2 and irradiated 1 in the presence of DNA

Figure S9 shows a comparison between the absorption spectra of **2** and irradiated **1** in the presence of DNA. It can be noted that the spectra are practically identical. As **2** intercalates between base pairs (see main text and Fig. 1 in the paper), this experiment indicates that irradiated **1** behaves as **2**, as expected, and that the photoreaction is essentially quantitative, also in the presence of DNA.

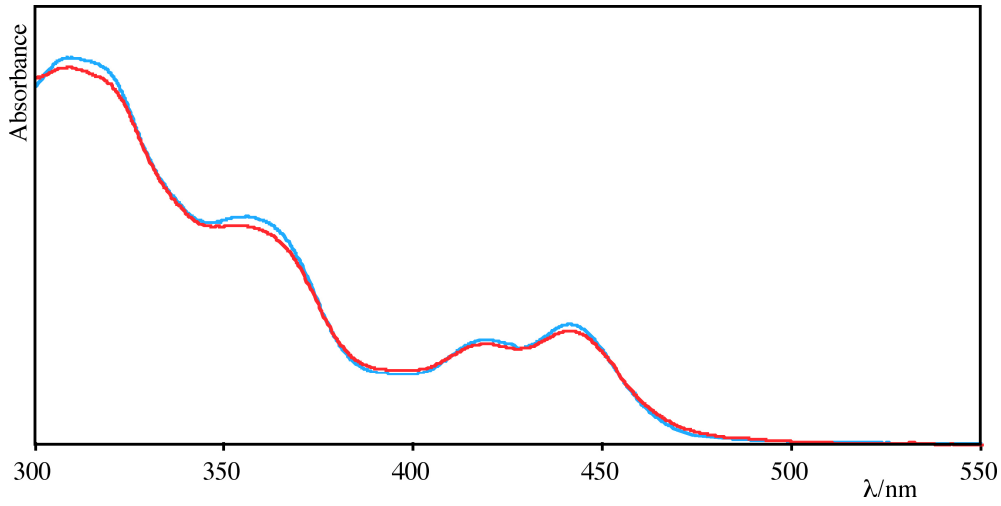


Figure S9. Absorption spectra of **2** in the presence of DNA (blue line) and of irradiated **1** in the presence of a three-fold excess of DNA (red line). Ionic strength: 1.1 x 10^{-2} M ([NaCl] = 1.0 x 10^{-2} M; $[\text{phosphate buffer}]_{pH=7}$ = 1.0 x10^{-3} M); T = 298 K. Concentration of **1** or **2** is 2.7 x 10^{-5} M.

References:

1. G. A. Crosby and J. N. Demas, *Phys. Chem.*, 1971, **75**, 991–1024.
2. Handbook of Photochemistry - Third Edition (Eds.: M. Montalti, A. Credi, L. Prodi and M. T. Gandolfi, CRC, Boca Raton, FL, 2006.
3. J. D. McGhee and P. H. von Hippel, *J. Mol. Biol.*, 1974, **86,** 469-489.