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

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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
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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$_3$CN, 25°C): δ 8.43 (s, 2H, H$^A_3$), 8.41 (d, $J = 6.0$ Hz, 2H, H$^B_2$), 8.12 (d, $J = 8.0$ Hz, 2H, H$^D_2$), 7.73 (dd, $J = 7.5$, 7.0 Hz, 1H, H$^D_4$), 7.67 (dd, $J = 8.0$, 7.0 Hz, 2H, H$^D_3$), 7.47-7.37 (m, 10H, H$^C_2$, C$^3$, C$^4$, C$^5$, C$^6$), 7.25 (d, $J = 5.5$ Hz, 2H, H$^D_1$); $^{13}$C NMR (126 MHz, CD$_3$CN, 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$_2$BF$_4$): 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$_3$CN, 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$^{D3}$, D$^4$); $^{13}$C NMR (126 MHz, CD$_3$CN, 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$_2$BF$_4$): 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 \(1 \times 10^{-3}\) M phosphate buffer (pH 7) containing \(7.8 \times 10^{-6}\) M compound and \(2 \times 10^{-3}\) 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 \(1 \times 10^{-3}\) M phosphate buffer (pH = 7) and \(1 \times 10^{-2}\) M NaCl. The compound solution ((1.5—2.5) \(\times 10^{-4}\) M), containing also DNA (6.0 \(\times 10^{-4}\) M) at the same concentration as that in the viscometer, has been delivered in increments of 90–190 \(\mu\)L from a micropipette. Reduced viscosities have been calculated by established methods and plotted as \(\ln \eta/\eta_0\) against \(\ln (1+r)\) for rod–like DNA (600 base pairs) (\(\eta = \) reduced viscosity of the biopolymer solution in the presence of compound; \(\eta_0 = \) 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.

Supplementary Material (ESI) for Chemical Communications
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The data of the spectrophotometric titrations have been analyzed by a nonlinear least-squares fitting program, applied to McGhee and von Hippel equation. 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.

$$1 + h\nu + O_2 \rightarrow 2 + 2 \text{H}_2\text{O} \quad (1)$$

**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 $[\text{DNA}]/[\text{compound}] = 3$ 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: $1.1 \times 10^{-2} \text{M} ([\text{NaCl}] = 1.0 \times 10^{-2} \text{M}; [\text{phosphate buffer}]_{\text{pH}=7} = 1.0 \times 10^{-3} \text{M}); T = 298 \text{ K}$. Concentration of 1 or 2 is $2.7 \times 10^{-5} \text{M}$. 7
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: $1.1 \times 10^{-2} \text{ M}$ ($\left[\text{NaCl}\right] = 1.0 \times 10^{-2} \text{ M}$; $\left[\text{phosphate buffer}\right]_{\text{pH}=7} = 1.0 \times 10^{-3} \text{ M}$); $T = 298 \text{ K}$. Concentration of 1 or 2 is $2.7 \times 10^{-5} \text{ M}$.

References: