Photoswitchable DNA-binding properties of a photochromic spirooxazine derivative

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Electronic Supplementary Information

1. *Materials*

Purified water with resistivity ≥ 18 MΩ cm^{-1} was used for the preparation of buffer solutions and spectrometric measurements. BPE buffer (6.0 mM Na_2HPO_4, 2.0 mM NaH_2PO_4, 1.0 mM Na_2EDTA; total Na^+ concentration 16.0 mM; pH 7.0) was used for photometric and viscometric DNA titrations and for CD spectroscopic studies. KCl-Na-cacodylate-buffer (100 mM KCl, 10 mM Na-cacodylate; pH 7.2 – 7.3) was used for the fluorescence indicator displacement (FID) experiments. Spiro[indolinephenanthrolinoxazine] (**1**) was prepared according to literature protocols.[1]

2. *Synthesis*

*Spiro[indoline-1-methyl-1,10-phenanthrolinoxazine] iodide (**2**^{SO}**-I**).* A mixture of spiro[indoline-phenanthrolinoxazine] (**1**) (380 mg, 1.00 mmol) and iodomethane (10 ml) was stirred at 25 °C for 24 hours. After stirring at room temperature for 24 hours the crude product was obtained. Filtration over silica yielded 501 mg (0.95 mmol, 96%) of the product as a blue solid; mp 276 °C (dec.). ^{1}H-NMR (500 MHz, $CDCl_3$) δ = 1.40 (6H, s, CH_3), 3.06 (3H, s, CH_3), 4.39 (3H, s, CH_3), 6.61-6.68 (1H, d, ^3J = 7.8 Hz, ArH, indoline), 6.97-7.01 (1H, t, ^3J = 7.5 Hz, ArH, indoline), 7.05-7.07 (2H, m, ArH, indoline), 7.51-7.57 (1H, dd, ^3J = 8.2, 4.3 Hz, ArH, phenanthroline), 7.68-7.74 (1H, dd, ^3J=8.3, 4.3 Hz, ArH, phenanthroline), 7,.91 (1H, s, N = CH), 8.44-8.49 (1H, dd, ^3J = 8.3, 1.8 Hz, ArH, phenanthroline), 8.99-9.02 (1H, dd, ^3J = 8.3, 1.8 Hz, ArH, phenanthroline), 9.09-9.14 (1H, dd, ^3J = 4.4, 1.8 Hz, ArH, phenanthroline), 9.13-9.19 (1H, dd, ^3J = 4.2, 1.7 Hz, ArH, phenanthroline). ^{13}C-NMR (500 MHz, $CDCl_3$) δ= 18.2 (CH_3), 35.2 (CH_3), 48.2 (C-(CH_3)$_2$), 51.3 (CH_3), 107.1 (ArH, indoline), 117.2 (spiro C), 117.7 (ArH, indoline), 121.8 (ArH, phenanthroline), 121.6 (ArH, phenanthroline), 122.1 (ArH, phenanthroline), 123.4 (ArC, phenanthroline), 124.4 (ArH, phenanthroline), 125.9 (C-N, phenanthroline), 126.5 (ArH, indoline), 127.5 (ArH, indoline), 129.8 (ArH, phenanthroline), 137.3 (ArC, indoline), 137.1 (ArH, phenanthroline), 141.7 (ArH, phenanthroline), 145.3 (ArC, phenanthroline), 147.7 (ArH, phenanthroline), 148.5 (ArC, indoline), 149.1 (ArH, phenanthroline), 151.6 (C-O, phenanthroline), 163.8.(C=N). ESI-HRMS: *m/z* calc. for $C_{25}H_{23}N_4O^+$ 395.18753 [M]+, found: 395.18723.

[1] a) J.-L. Pozzo, A. Samat, R. Guglielmetti and D. De Keukeleire, J. *Chem. Soc., Perkin Trans. 2*, 1993, 1327. b) M. M. Paquette, R. A. Kopelman, E. Beitler and N. L. Frank, *Chem. Commun*., 2009, 5424. c) W. Paw and R. Eisenberg, *Inorg. Chem*, 1997, **36**, 2287.

*Spiro[indoline-1-methyl-1,10-phenanthroliniumoxazine] hexafluorophosphate (**2**^{SO}).* 501 mg (0.95 mmol) of spiro[indoline-1-methyl-1, 10-phenanthrolinoxazine] iodide **2**^{SO}**-I** were eluted over a columm with a 10 % solution of ammonium hexafluorophosphate in acetone. The solvent was removed *in vacuo* by and the solid was solved in chloroform and washed three times with water. After removing the solvent *in vacuo* by 489 mg (0.9 mmol, 94 %) of (**2**^{SO}) was obtained. ^{1}H-NMR (500 MHz, $CDCl_3$) δ = 1.41 (6H, s, CH_3), 3.06 (3H, s, CH_3), 4.57 (3H, s, CH_3), 6.65 (1H, d, ^{3}J = 7.8 Hz, ArH, indoline), 6.99 (1H, t, ^{3}J = 7.5 Hz, ArH, indoline), 7.06-7.08 (2H, m, ArH, indoline), 7.54 (1H, dd, ^{3}J = 8.2, 4.3 Hz, ArH, indoline), 7.71 (1H, dd, ^{3}J = 8.3, 4.3 Hz, ArH, indoline), 7.91 (1H, s, N=CH), 8.46 (1H, dd, ^{3}J = 8.3, 1.8 Hz, ArH, phenanthroline), 9.01 (1H, dd, ^{3}J = 8.3, 1.8 Hz, ArH, phenanthroline), 9.13 (1H, dd, ^{3}J = 4.3, 1.8 Hz, ArH, phenanthroline), 9.13-9.19 (1H, dd, ^{3}J = 4.2, 1.7 Hz, ArH, phenanthroline). ^{13}C-NMR (500 MHz, $CDCl_3$) δ = 18.4 (CH_3), 35.3 (CH_3), 49.4 (C-(CH_3)$_2$), 51.3 (CH_3), 106.9 (Ar-CH, indoline), 117.5 (spiro-C), 117.8 (Ar-CH, indoline), 121.8 (Ar-CH, phenanthroline), 121.6 (Ar-CH, phenanthroline), 122.2 (Ar-CH, phenanthroline), 123.4 (Ar-C_q, phenanthroline), 124.4 (Ar-CH, phenanthroline), 125.9 (C-N, phenanthroline), 126.5, 127.5 (Ar-CH, indoline), 129.9 (Ar-CH, phenanthroline), 137.3 (Ar-C_q, indoline), 137.1 (Ar-CH, phenanthroline), 141.7 (Ar-CH, phenanthroline), 145.3 (Ar-CH, phenanthroline), 148.1 (Ar-CH, phenanthroline), 148.8 (Ar-C_q, indoline), 149.1 (Ar-CH, phenanthroline), 151.6 (C-O, phenanthroline), 163.9 (C=N). HI: Es fehlen quartäre C-Atome! ESI-HRMS: *m*/*z* calc. for $C_{25}H_{23}N_4O^+$ 395.18647 [M]+, found: 395.18763.

3. *Spectrophotometric measurements*

Absorption spectra were recorded on a Varian Cary 100 Bio spectrophotometer. The pH of aqueous solutions was determined with a calibrated pH-meter (Qph 70, VWR). Spectrophotometric measurements were performed in thermostated quartz cells (10 mm pathlength) at 20 °C. Solutions were freshly prepared from stock solutions (1 mM in DMSO or methanol). Aliquots of the stock solution of the ligand were pipetted into vials, the solvent was evaporated and the residue was dissolved in the corresponding buffer. To provide sufficient solubility in the case of **2**^{SO} of DMSO was added (10 vol.%). Concentrations of ct DNA samples were determined photometrically (ε_{260} = 12824 cm^{-1} M^{-1}, in base pairs). To avoid dilution during the titration experiments, titrant solutions of ct DNA contained the same concentration of ligand as the analyte solution. The analyte solutions (100 µM) in BPE buffer were titrated with the titrant solutions in intervals of 0.5–2 equivalents and subsequently analyzed by absorption or fluorescence spectroscopy, respectively. All spectrophotometric titrations were performed at least two times to ensure the reproducibility.

The binding constant was determined by fitting of the experimental data from the photometric DNA titration to the theoretical model equations 1–3.[2]

$$y = \frac{(A+B+n\cdot x-\sqrt{(A+B+n\cdot x)^2-4\cdot B\cdot n\cdot x}}{2\cdot B} \qquad \text{eq. 1}$$

$$y = (Abs - Abs_0) / (Abs_{sat} - Abs_0) \qquad \text{eq. 2}$$

$$A = 1 / K_b \qquad \text{eq. 3}$$

Abs is the absorption at a given wavelength at a particular ligand concentration; Abs_0 is the absorption of the solution of the ligand, Abs_{sat} is the absorption of the solution at saturation; B is the concentration of the ligand; *n* is the binding site size; $x = c_{ligand}$ (titration variable).

4. *Fluorescent intercalator displacement (FID) assay*

A stock solution of the duplex DNA was prepared from the oligonucleotides 5'-$C_2AGT_2CGTAGTA_2C_3$-3' and 3'-$G_2TCA_2GCATCAT_2G_3$-5' in KCl-Na-cacodylate buffer and incubated (30 min) with TO (c_{DNA} = 0.25 µM; c_{TO} = 0.5 µM). The emission spectrum was recorded from 510 nm to 750 nm (λ_{ex} = 501 nm). A solution of the ligand (c = 50 µM) was added stepwise (0 µL, 6 x 2.5 µL, 3 x 5 µL, 2 x 10 µl), and 3 min after each addition an emission spectrum was recorded. For analysis, the fluorescence area *FA* (510-750 nm) was calculated by integration of each emission signal. The percentage displacement *PD* was determined according to equation 4.

$$PD = 100 - \left(\frac{FA}{FA_0} \cdot 100\right) \qquad \text{eq. 4}$$

FA_0 is the integrated area of the emission signal before the addition of the ligand solution. PD was plotted as a function of the ligand concentration.

5. *CD spectroscopic experiments*

Circular dichroism spectra were recorded on a Chirascan CD spectrometer (Applied Photophysics) in thermostated quartz sample cells (pathlength 2 mm) at 20 °C. Mixtures of ct DNA (50 µM in base pairs) and the ligand in BPE buffer at different ligand-to-DNA ratios (0, 0.1, 0.5, 1) were prepared and CD spectra were recorded after an equilibration period of 30 min.

[2] F. H. Stootman, D. M. Fisher, A. Rodger, J. R. Aldrich-Wright, *Analyst* **2006**, *131*, 1145.

6. *Viscometric studies*

Aliquots of the solution of the ligand **2**^{SO} in BPE buffer were added to a solution of ct DNA (c = 1 mM, in base pairs) in BPE buffer. Flow times were measured in an Ubbelohde viscosimeter after a thermal equilibration period of 5 min. Each flow time was measured three times. The relative viscosity $(\eta/\eta_0)^{1/3}$ was calculated from the average values according to equations 5 and 6. The value η is the viscosity of the DNA solution in the presence of the ligand, calculated from the flow time through the viscometer (t_L) minus the flow time of the pure buffer (t_0) (eq. 5).

$$\eta = (t_L - t_0) / t_0 \qquad \text{eq. 5}$$

η_0 is the viscosity of the ct DNA solution alone according to equation 3.

$$\eta_0 = (t - t_0) / t_0 \qquad \text{eq. 6}$$

The relative viscosity $(\eta/\eta_0)^{1/3}$ was plotted as a function of ligand-to-DNA ratio.

7. *NMR spectroscopy*

NMR spectra were measured at room temperature on a Bruker DRX 500 spectrometer (500 MHz). $CDCl_3$ was used as an internal standard for all NMR-spectra.

8. *Figures*

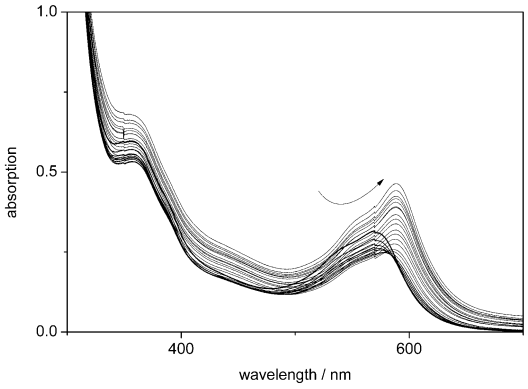

Figure S1. Spectrophotometric titration of ct DNA (c = 0–1.2 mM) to a solution of **2**^{PM} (c = 0.1 mM) in BPE buffer; thick lines: absorption spectra at ligand : DNA > 1.10; thin lines: absorption spectra at ligand : DNA < 0.80.

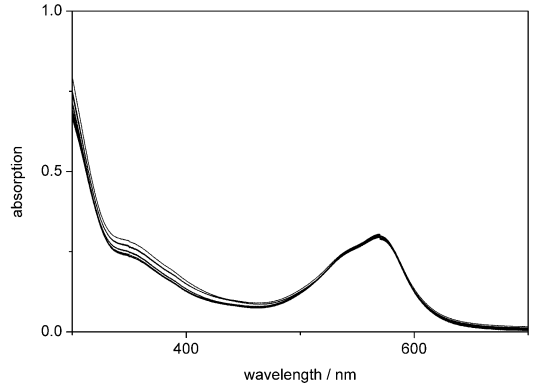

Figure S2. Spectrophotometric titration of [poly(dA-dT)]$_2$ to a solution of **2**$^{\mathbf{PM}}$ (c = 0.1 mM) in BPE buffer.

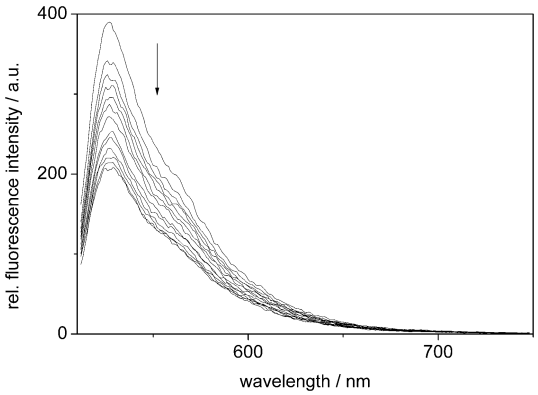

Figure S3. Fluorimetric detection of the displacement of TO (0.5 µM) from duplex DNA (0.25 µM in base pairs) by ligand **2**$^{\mathbf{SO}}$.

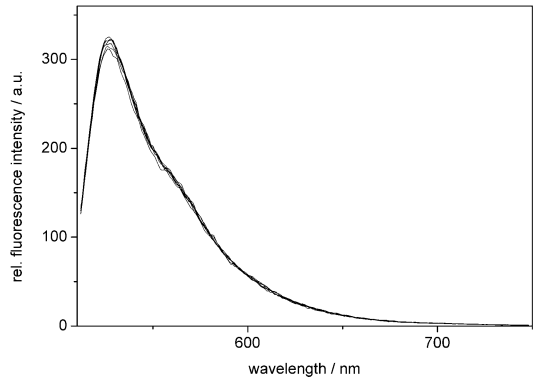

Figure S4. Fluorimetric detection of the displacement of TO (0.5 µM) from duplex DNA (0.25 µM in base pairs) by ligand **2**$^{\mathbf{PM}}$.

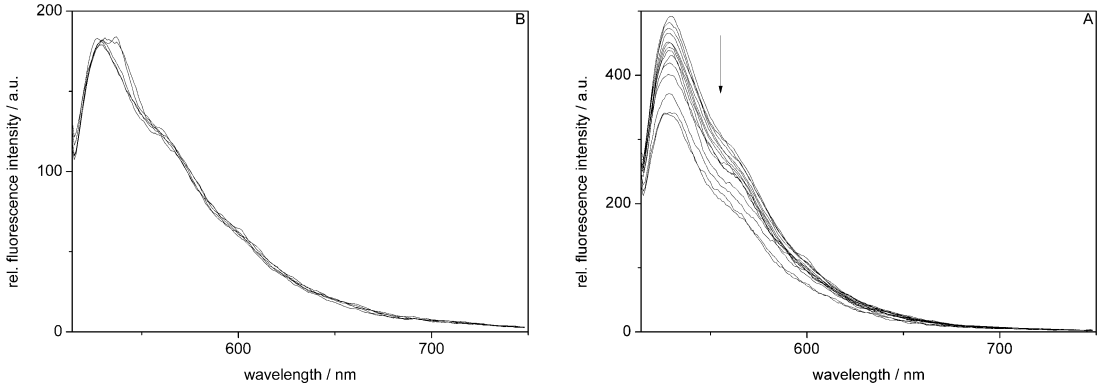

Figure S5. Fluorimetric detection of the displacement of TO (0.5 µM) from $[poly(dA\text{-}dT)]_2$ (left) and $[poly(dG\text{-}dC)]_2$ (right) by ligand $\mathbf{2}^{\mathbf{SO}}$; *c*(DNA) = 0.25 µM in base pairs.