Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Electronic Supplementary Information

Diphenylaminostyryl-substituted quinolizinium derivatives are fluorescent light-up probes for duplex and quadruplex DNA

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1. Experimental Section

1.1 Equipment

Absorption spectra were recorded with a Varian Cary 100 Bio Spectrophotometer in quartz cells (10 mm x 10 mm) with baseline correction, and emission spectra were collected with a Varian Cary Eclipse spectrophotometer in quartz cells (10 mm x 10 mm) at 20 °C. NMR spectra were recorded with a Varian VNMR-S 600 (^{1}H: 600 MHz, ^{13}C: 150 MHz) at 25 °C (DMSO-d_6). NMR spectra were processed with the software MestReNova and referenced to the solvent DMSO-d_6 (^{1}H: δ = 2.50, ^{13}C: δ = 39.5). Elemental analyses data were determined with a HEKAtech EUROEA combustion analyser by Rochus Breuer, Organische Chemie I, Universität Siegen. Mass spectra (ESI) were recorded on a Finnigan LCQ Deca (U = 6 kV; working gas: Ar; auxiliary gas: N_2; temperature of the capillary: 200 °C). Circular dichroism (CD) spectra were recorded with a Chirascan CD spectrometer, Applied Photophysics. The melting points were determined with a BÜCHI 545 (BÜCHI, Flawil, CH) and are uncorrected.

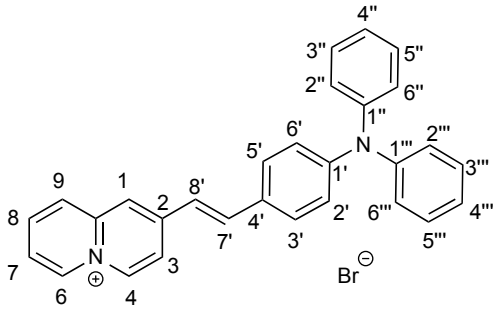
1.2 Materials

2-Methylquinolizinium (**1**), 4-(diphenylamino)benzaldehyde (**2a**) and 4,4′-diformyltriphenylamine (**2b**) were synthesized according to the published procedure.[1] Oligodeoxyribonucleotides (HPLC purified) d[A(GGGTAA)3GGG] (**22AG**) was purchased from Metabion Int. AG (Planegg/Martinsried). Calf thymus DNA (ct DNA, type I; highly polymerized sodium salt; ε = 12824 cm^{-1} M^{-1}) was purchased from Sigma Aldrich (St. Louis, USA). The nucleic acids were used without further purification. Concentration of **22AG** is given as $c_{oligonucleotide}$; concentration of ct DNA is given in base pairs (bp). The ct DNA was dissolved in BPE buffer solution. Solutions of oligonucleotide **22AG** were prepared by dissolving in K-phosphate buffer, heating to 95 °C for 5 min and cooling slowly to room temperature within 4 h. K-phosphate buffer: 25 mM K_2HPO_4, 70 mM KCl; adjusted with 25 mM KH_2PO_4 to pH 7.0; BPE (biphosphate EDTA) buffer: 6.0 mM Na_2HPO_4, 2.0 mM NaH_2PO_4, 1.0 mM Na_2EDTA; pH 7.0. All buffer solutions were prepared from purified water (resistivity 18 MΩ cm) and biochemistry-grade chemicals. The buffer solutions were filtered through a PVDF membrane filter (pore size 0.45 µm) prior to use.

[1] (a) T. Mallegol, S. Gmouh, M. A. A. Meziane, M. Blanchard-Desce and O. Mongin, *Synthesis*, 2005, **11**, 1771.
(b) A. Richards and T. S. Stevens. *J. Chem. Soc*., 1958, 3067; (c) O. F. Beumel, Jr.; W. N. Smith and B. Rybalka, *Synthesis*, 1974, **1**, 43.

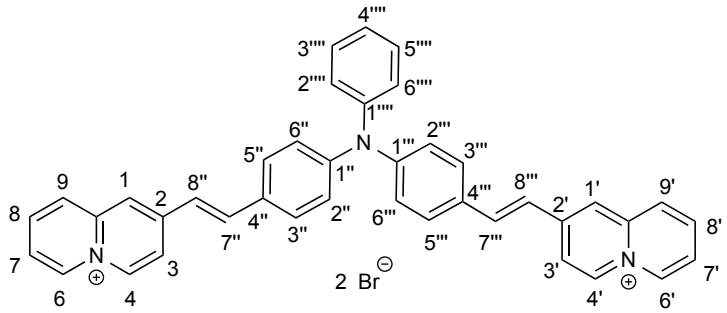
1.3 Synthesis

(E)-2-[1'-(Diphenylamino)styryl)quinolizinium bromide ***(3a)***



To a refluxing solution of 2-methylquinolizinium bromide (**1**) (100 mg, 0.45 mmol) and 4-(diphenylamino)benzaldehyde (**2a**) (244 mg, 0.89 mmol, 2.0 equiv.) in acetonitrile (10 ml) was added piperidine (57 mg, 66 µL, 0.7 mmol, 1.5 equiv.). The mixture was stirred under reflux for 6 h. After cooling of the mixture to room temp., diethyl ether (30 ml) was added to the resulting mixture and gradually an intense brown solid started to precipitate. The resulting brown precipitate was filtered, washed with ether, and dried in a vacuum to afford a dark brown solid that was further purified by column chromatography (SiO_2, $CHCl_3$/MeOH 95:5, R_f = 0.35) to give the product as dark brown amorphous solid (150 mg, 0.31 mmol, 70%); mp 234–235° C. – ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 6.96 (d, 2H, ^3J = 9.0 Hz, 3'-H, 5'-H, 2'-H, 6'-H), 7.12–7.08 (m, 4H, 6''-H, 2''-H, 6'''-H, 2'''-H), 7.15 (tt, 2H, ^3J = 7.8, ^4J = 0.6 Hz, 4''-H, 4'''-H), 7.35 (d, 1H, ^3J = 16.2 Hz, 7'-H, 8'-H), 7.39–7.35 (m, 4H, 5''-H, 3''-H, 5'''-H, 3'''-H), 7.63 (d, 2H, ^3J = 9.0 Hz, 2'-H, 6'-H, 3'-H, 5'-H), 7.88 (d, 1H, ^3J = 16.2 Hz, 8'-H, 7'-H), 7.92 (ddd, 1H, ^3J = 7.8 Hz , ^3J = 6.6 Hz, ^4J = 1.2 Hz, 7-H), 8.25 (ddd, 1H, ^3J = 7.8 Hz, ^3J = 6.6 Hz, ^4J = 0.6 Hz, 8-H), 8.40–8.35 (m, 2H, 3-H, 9-H), 8.48 (br. s, 1H, 1-H), 9.19 (d, 1H, ^3J = 6.6 Hz, 6-H), 9.24 (d, 1H, ^3J = 7.2 Hz, 4-H). – ^{13}C-NMR (150 MHz, DMSO-d_6): δ = 148.9 (C1'), 146.3 (C4'), 145.4 (C2), 142.7 (9a), 138.2 (C7'), 136.5 (C8), 136.4 (C6), 136.3 (C4), 129.7 (C8'), 129.2 (C3', C5'), 128.4 (C1'', C1'''), 126.6 (C9), 125.1 (C6'', C2'', C6''', C2'''), 124.2 (C4'', C4'''), 122.3 (C7), 122.0 (C1), 121.2 (C5'', C3'', C5''', C3'''), 121.0 (C2', C6'), 120.1 (C3). – MS (ESI$^+$): *m*/*z* = 399 (100) [M-Br]$^+$. – EI. Anal. for $C_{29}H_{23}BrN_2$ (478.10), calcd (%): C, 68.78; H, 5.17; N, 5.53; found (%): C, 68.46; H, 5.03; N, 5.51.

2,2'-{(Phenylimino)-bis[(*E*)-1'',1'''-styryl]}-bis[quinolizinium] dibromide (**3b**)



To a refluxing solution of 4,4′-diformyltriphenylamine (**2b**) (100 mg, 0.33 mmol) and 2-methylquinolizinium bromide (**1**) (370 mg, 1.65 mmol, 5 equiv.) in acetonitrile (15 ml) was added piperidine (254.3 mg, 2.98 mmol, 3 equiv.). The mixture was stirred at reflux for 12 h. The dark brown precipitate, that started to form after ca. 4 h, was filtered off, washed with ether, and dried i. vac. to afford a dark brown solid that was further purified by column chromatography (SiO_2, $CHCl_3$/MeOH, 90:10, R_f = 0.37) to give the product as brick red amorphous solid (160 mg, 0.23 mmol, 67%); mp > 300° C (dec.). – ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.07 (d, 4H, ^3J = 8.4, 3''-H, 5''-H, 3'''-H, 5'''-H, 2''-H, 6''-H, 2'''-H, 6'''-H), 7.14 (dd, 2H, ^3J = 0.6, ^4J = 0.6 Hz, 6''''-H, 2''''-H), 7.21 (tt, 1H, ^3J = 7.2 Hz, ^3J = 7.2 Hz, 4''''-H), 7.41 (d, 2H, ^3J = 7.8 Hz, 7''-H, 7'''-H, 8''-H, 8'''-H), 7.44–7.39 (m, 2H, 5''''-H, 3''''-H), 7.69 (d, 4H, ^3J = 9 Hz, 2''-H, 6''-H, 2'''-H, 6'''-H, 3''-H, 5''-H, 3'''-H, 5'''-H), 7.93 (d, 2H, ^3J = 1.2 Hz, 7-H, 7'-H), 7.95–7.94 (m, 2H, 8''-H, 8'''-H, 7''-H, 7'''-H), 8.27–8.25 (m, 2H, 8-H, 8'-H), 8.41 (d, 4H, ^3J = 9 Hz, 3-H, 3'-H, 9-H, 9'-H), 8.55 (s, 2H, 1-H, 1'-H), 9.26 (d, 2H, ^3J = 7.2 Hz, 6-H, 6'-H), 9.31 (d, 2H, ^3J = 7.2 Hz, 4-H, 4'-H). – ^{13}C NMR (150 MHz, DMSO-d_6): δ = 147.9 (C1'', C1'''), 145.7 (C4'', C4'''), 145.2 (C2, C2'), 142.6 (9a, 9'a'), 137.9 (7'', 7'''), 136.6 (C8, C8'), 136.4 (C6, C6'), 136.3 (C4, C4'), 130.0 (C1''''), 129.9 (C8'', C8'''), 129.3 (C3'', C3''', C5'', C5'''), 126.6 (C9, C9'), 125.8 (C6'''', C2''''), 125.0 (C4''''), 123.0 (C2'', C2''', C6'', C6'''), 122.4 (C7, C7'), 122.2 (C1, C1'), 122.0 (C5'''', C3''''), 120.2 (C3, C3'). – MS (ESI$^+$): *m*/*z* = 276.9 (100) [M-Br]$^+$, 552.4 (30) [M-2Br]$^{2+}$. – EI. Anal. for $C_{40}H_{31}Br_2N_3$ (553.25), calcd (%): C, 64.88; H, 4.63; N, 5.67; found: C, 64.82; H, 4.29; N, 5.78.

1.4 Absorption and emission spectra of 3a and 3b in different solvents

Solutions were prepared for each measurement from stock solutions of the derivatives **3a** and **3b** in MeOH (*c* = 1.0 mM). Aliquots of the stock solution were evaporated under a stream of nitrogen and redissolved in the respective solvent or solvent mixture. For the detection of fluorescence spectra, the excitation and emission slits were adjusted to 5 nm and the excitation wavelengths were fixed to 460 nm for **3a** and 500 nm for **3b**.

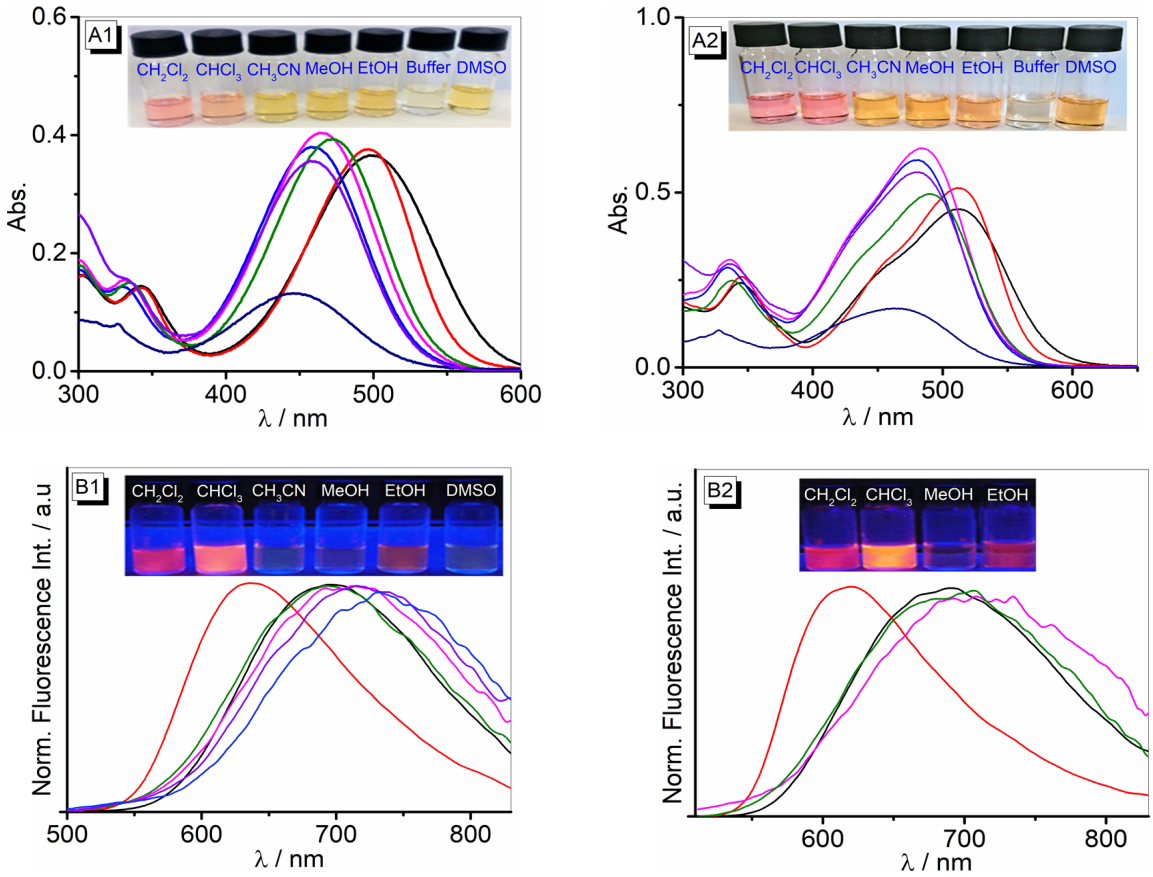


Figure S1. Absorption (A) and emission spectra (B) of **3a** (1) and **3b** (2) (*c* = 10 µM) in different solvents (black: CH_2Cl_2, red: $CHCl_3$, blue: CH_3CN, magenta: MeOH, green: EtOH, navy: aq. buffer, purple: DMSO; buffer: 10 mM BPE buffer solution at pH 7); **3a**: λ_{ex} = 460 nm; **3b**: λ_{ex} = 500 nm.

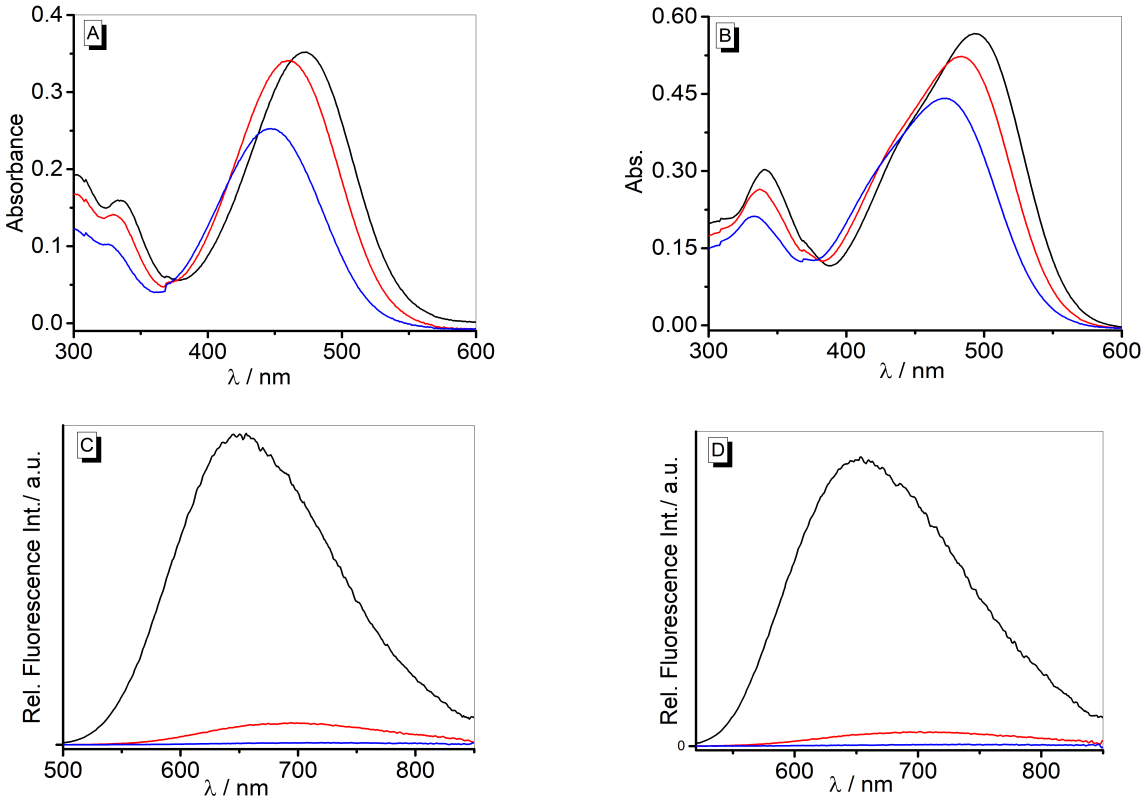


Figure S2. Absorption and emission spectra of **3a** (A, C; *c* = 10 μM) and **3b** (B, D;, *c* = 10 μM) in glycerol–H_2O mixtures; wt.% of glycerol in glycerol–H_2O mixtures: 0 (blue), 50 (red), 100 (black).

1.5 Determination of the fluorescence quantum yield

Solutions were prepared for each measurement from stock solutions of the derivatives **3a** and **3b** in MeOH (*c* = 1.0 mM). Aliquots of the stock solution were evaporated under a stream of nitrogen and redissolved in various solvents. For the detection of fluorescence spectra, the excitation and emission slits were adjusted to 2.5 nm. The relative fluorescence quantum yields of **3a** and **3b** were determined under identical conditions (detection wavelength, excitation wavelength, detector voltage, slit bandwidths, collection rate). The quantum yield Φ was measured according to equation 1.[2]

$$\Phi_x = \Phi_s (F_x / F_s)(A_s / A_x)(n_x^2/ n_s^2) \qquad \text{(equation 1);}$$

where, the indices X and S indicate the analyte and standard solution, respectively; Φ = emission quantum yield;

F = area under the emission curve;

A = absorbance at the excitation wave length;

[2] (a) B. Valeur and M. N. Berberan-Santos, *Molecular fluorescence. Principles and applications*, WileyVCH, Weinheim, 2nd ed., 2012; (b) G. A. Crosby and J. N. Demas, *J. Phys. Chem*., 1971, **75**, 991.

n = index of refraction of the solvent.

Measurements were performed with Rhodamine-B in ethanol as standard [Φ = 0.7]. The estimated error is ca.10% of the given values.

1.6 Photometric and fluorimetric DNA titrations

Different solutions were prepared for each of the measurements from stock solutions of **3a** and **3b** in MeOH (*c* = 1.0 mM). For photometric and fluorimetric titration of **3a** and **3b** with ct DNA, aliquots of the stock solution were evaporated under a stream of nitrogen, redissolved in DMSO (10% v/v) and BPE buffer to obtain a ligand concentration of *c* = 20 µM. The respective DNA solutions also contained the ligand at the same concentration in order to avoid dilution effects. For photometric and fluorimetric titration of **3a** and **3b** with **22AG**, no DMSO was used for initial solubilization of the ligand. For the detection of emission spectra, the excitation and emission slits were adjusted to 5 nm. The spectra were smoothed with the implemented moving average function by a factor of 5. Emission spectra in the range between 600 and 800 nm were corrected using an instrument-specific correction curve. Aliquots of the ligand solutions were placed into quartz cells and absorption or emission spectra were recorded. The titrations were performed until no more changes were observed in the absorption or emission spectra. All spectrometric titrations were performed at least two times to ensure reproducibility.

The binding constants, K_b, were determined from binding isotherms of the photometric titration spectra (Figure S3) by fitting of the experimental data to the theoretical model according to equation 2.[3]

$$\frac{I}{I_0} = 1 + \frac{Q-1}{2}\left(A + xn + 1 - \sqrt{(Q + xn + 1)^2 - 4xn}\right) \qquad \text{(equation 2)}$$

Here, Q = I / I_0 is the minimal absorbance in the presence of excess ligand; n is the number of independent binding sites per DNA;

A = 1 / (K_b × c_L);

x = c_{DNA} /c_L is the titration variable.

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

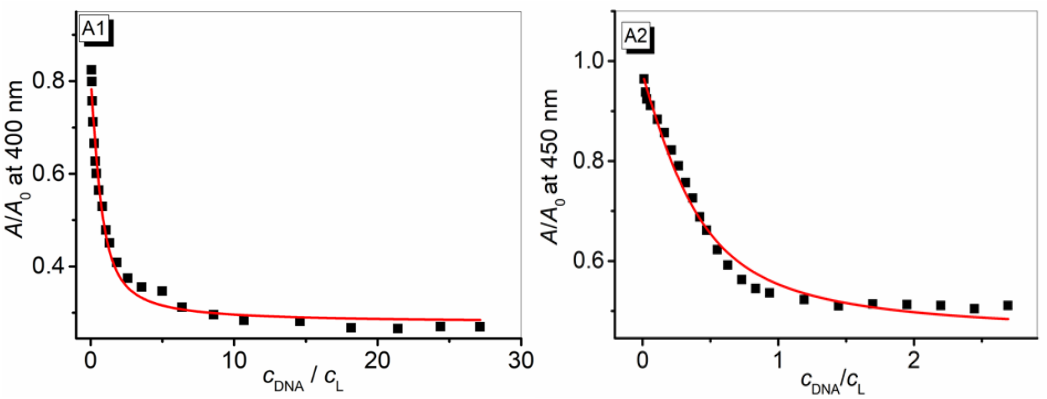


Figure S3. Fitting curves of binding isotherms resulting from spectrophotometric titrations of **3a** (A1) and **3b** (A2) for the determination of binding constants (K_b). Red lines represent the best fits to the theoretical model.[3]

1.7 Determination of the limit of detection

The limit of detection (LOD) values of **3a** and **3b** for ct DNA and **22AG** were determined from equation 3.[4]

$LOD = K \times Sb_1 / S$ (equation 3)

K = 3; Sb_1 is the standard deviation for multiple measurements of a blank solution; S is the slope derived from the linear range of the fluorimetric titrations of the two ligands **3a** and **3b** with ct DNA and **22AG**.

The corresponding values of standard deviation, linear range and detection limits are shown in Table S1 and calculated from the data shown in Fig. S3.

Table S1. Calculation of the Limit of Detection (LOD) from Fluorimetric Titrations.

Ligand	DNA	Sb_1 [a]	Linear range	LOD / μM
3a	ct DNA	0.09	0–1.25	0.02
3a	**22AG**	0.21	0–7.54	0.05
3b	ct DNA	0.79	0–82.6	0.79
3b	**22AG**	0.06	0–3.53	0.01

[a] Sb_1 = Standard deviation, LOD = Limit of Detection

[4] (a) A. R. Reddi, T. R. Guzman, R. M. Breece, D. L. Tiemey and B. R. Gibney, *J. Am. Chem. Soc.*, 2007, **129**, 12815; (b) C. R. Lohani, J.-M. Kim, S.-Y. Chung, J. Yoon and K.-H. Lee, *Analyst*, 2010, **135**, 2079.

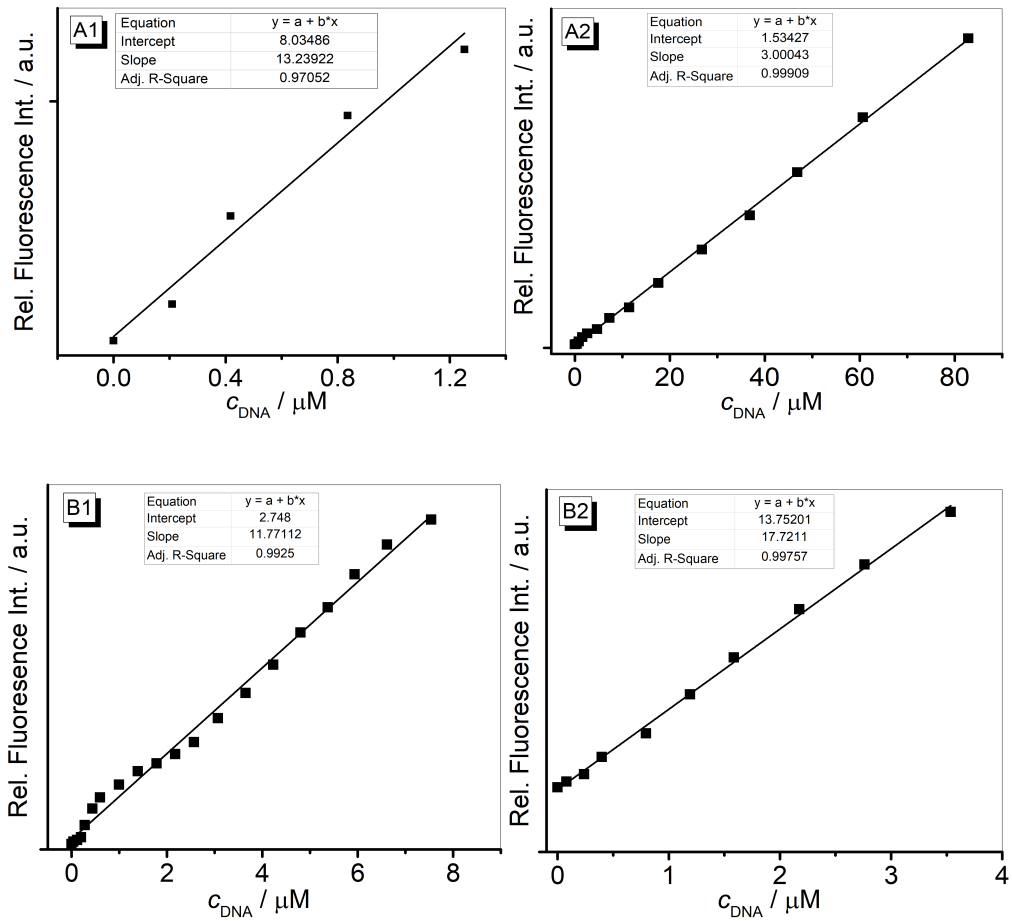


Figure S4. Plot of the fluorescence intensity of **3a** (1) and **3b** (2) (10 μM) versus concentration of ct DNA (A, *c* = 1.88 mM) and **22AG** (B, *c* = 200 μM).

1.8 Studies onthe influence of oxygen

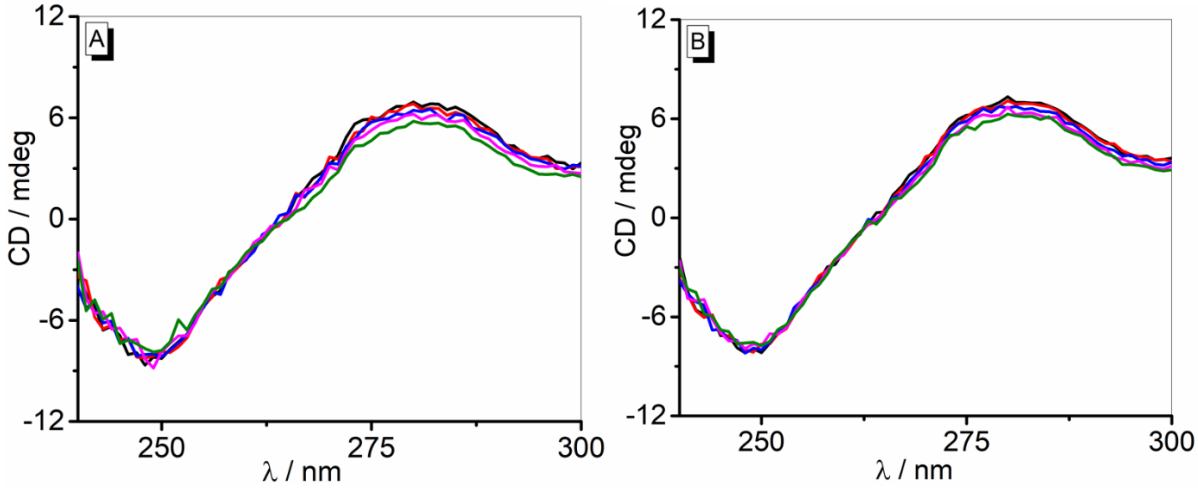


Figure S5. CD spectra of ligand **3b**-ct DNA complex (*LDR* = 0.3) after irradiation (A: 470 nm, B: 520 nm) at different time intervals; ligand-DNA complex (black), 5 min (red), 15 min (blue), 30 min (magenta), 1 h (green).

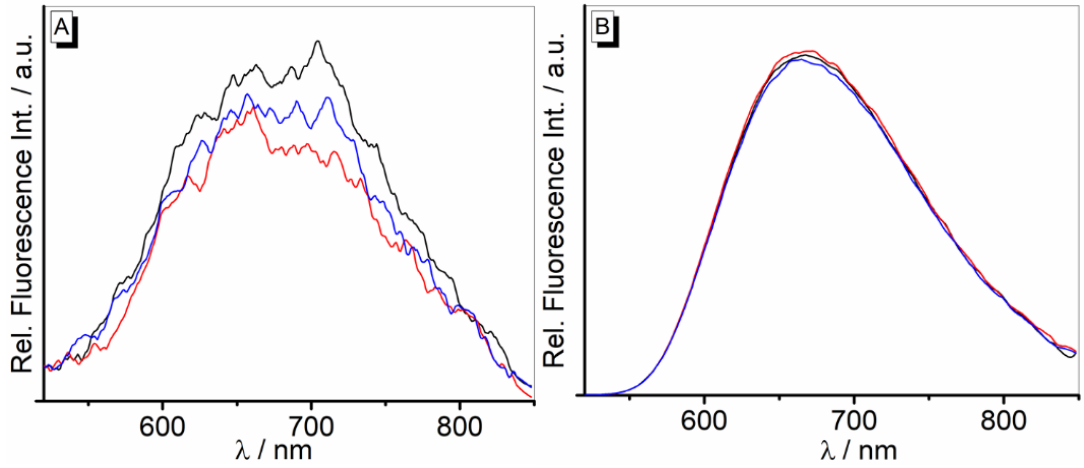


Figure S6. A: Fluorescence spectra of **3b** (A, *c* = 10 μM) and **3b**-ct DNA complex (B, c_{3b} = 10 μM; 5 mol. eq. DNA) under aerobic (black), anaerobic (red) and oxygen saturated condition (blue).

1.8 CD and LD spectroscopic analysis

CD and LD spectra were recorded in BPE buffer solution for ct DNA (c_{DNA} = 50.0 μM) and K-phosphate buffer solution for **22AG** (c_{DNA} = 20.0 μM) at different ligand-DNA ratios (*LDR*). The CD and LD measurements for ct DNA were performed at *LDR* = 0, 0.3, 0.5, 0.6, 0.8, 1.0 and for **22AG,** CD measurements were investigated at *LDR* = 0, 0.5, 1.0 and 2.0. CD signals were recorded with band width of 1 nm, recording speed of 1 nm/s and time per data point of 0.5 seconds. LD spectra were recorded on CD spectrometer equipped a High Shear Couette Cell

Accessory (Applied Photophysics). The LD samples were recorded in a rotating couette with a shear gradient of 1200 s^{-1}.

1.9 Thermal G4-DNA denaturation

The melting temperature of G4-DNA **22AG** was determined from the temperature-dependend CD spectra according to reported procedure.[5] CD spectra were recorded in the temperature range 35−95°C, by monitoring the ellipticity of **22AG** at 290 nm with increasing temperature. Spectra acquisition was performed in the absence and presence of 2 mol equiv. of compounds **3a** and **3b** to assess ligand-induced thermal stabilization. CD melting data were converted into fraction folded (θ) plots using equation 4.

$$\theta = \frac{CD_\lambda - CD_\lambda^{min}}{CD_\lambda^{max} - CD_\lambda^{min}} \qquad \text{(equation 4)}$$

CD is the ellipticity of the monitored wavelength at each temperature, CD^{min} is the lowest ellipticity and CD^{max} is the highest ellipticity. The melting temperatures (T_m's) were determined from a sigmoidal curve fit using the Boltzmann function, where the maximum of the Gaussian function denotes the melting temperature (Tm) which corresponds also to the midpoint value x0, and ΔT_m is the ligand-induced change in topology corresponding to the melting temperature.

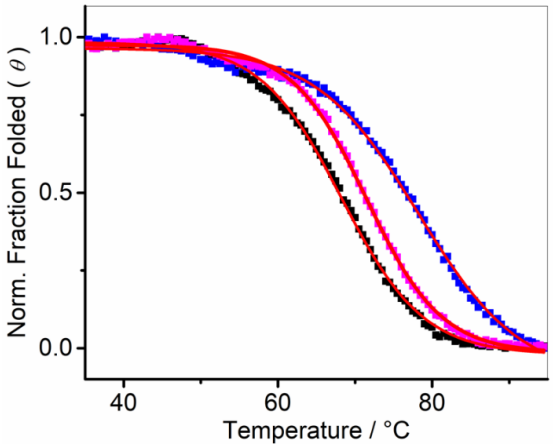


Figure S7. CD melting curves for **22AG** (20 μM in 10 mM K-phosphate buffer) in the absence and presence of 2 mol equiv of **3a** and **3b**. Fitting curve with black symbols (**22AG**), magenta symbols (**3a** + **22AG**), blue symbols (**3b**+**22AG**). B: Numerical first derivative of the melting curve of DNA; the red line denotes the Gaussian peak function, the maximum corresponds to the melting temperature Tm.

[5] J. Carvalho, J. A. Queiroz and C. Cruz, *J. Chem. Educ.*, 2017, **94**, 1547−1551.

2. NMR spectra

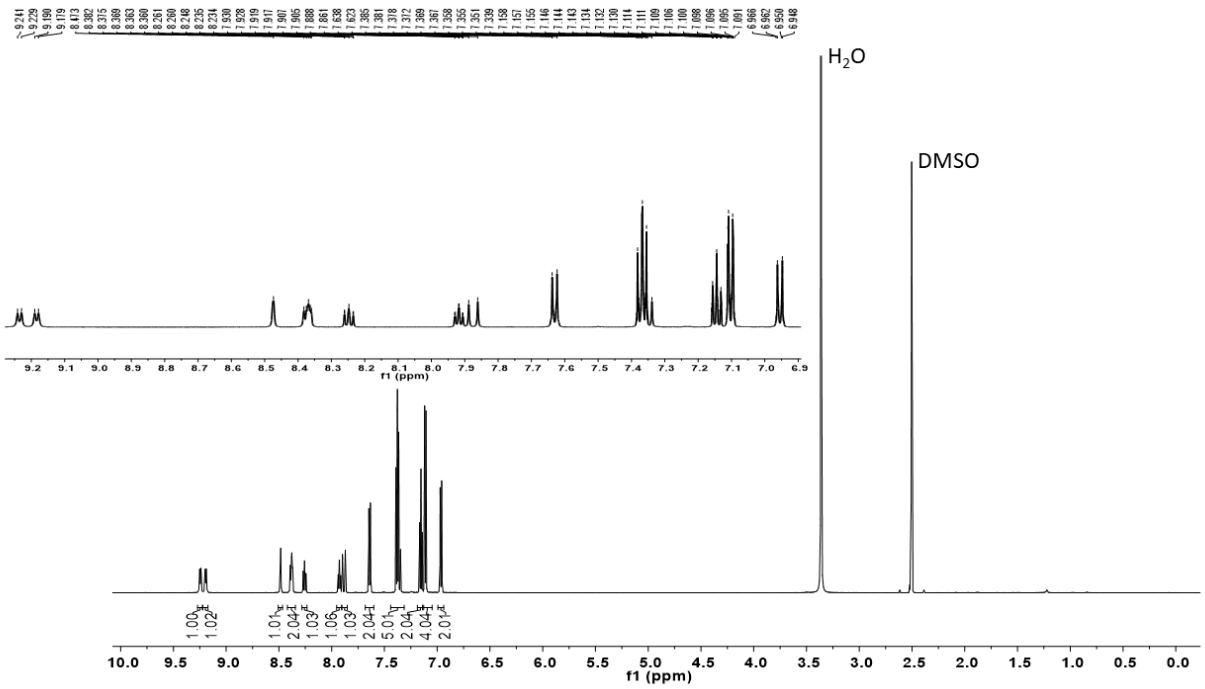


Figure S8. ^{1}H-NMR spectrum (600 MHz) of **3a** in DMSO-d_6. Inset: Magnified range of aromatic protons.

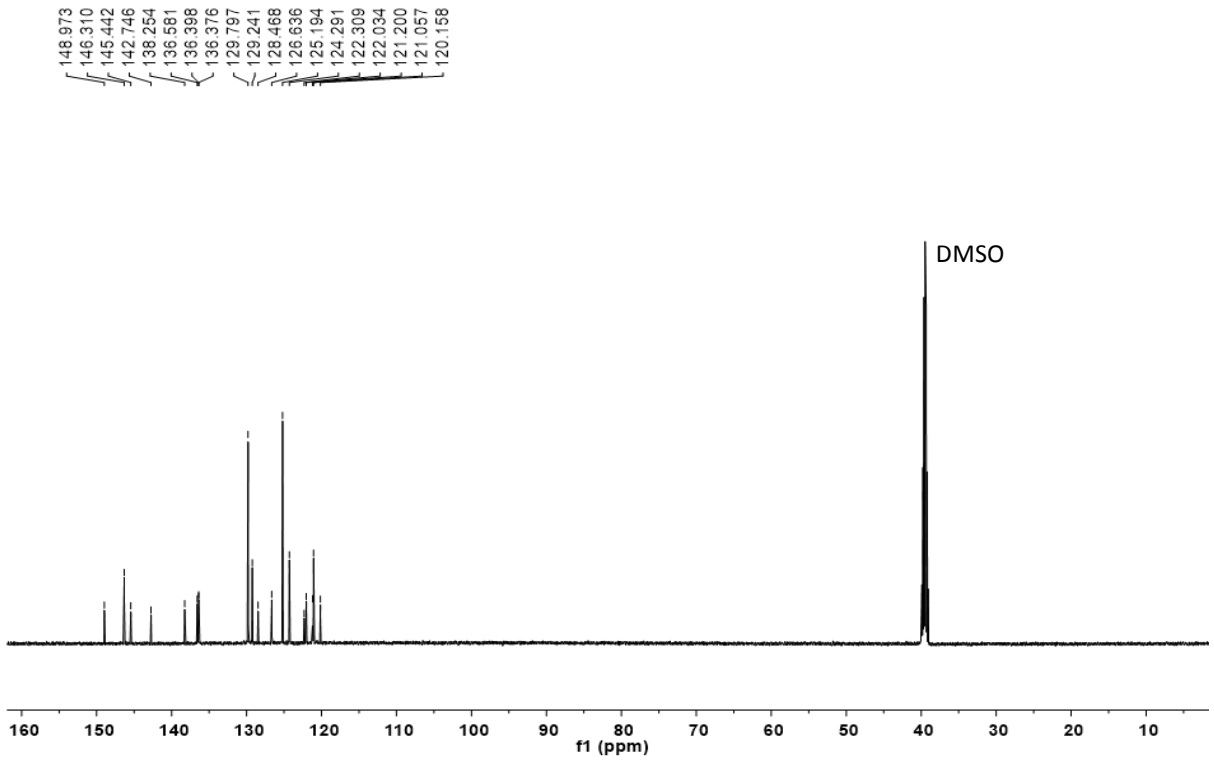


Figure S9. ^{13}C-NMR spectrum (150 MHz) of **3a** in DMSO-d_6.

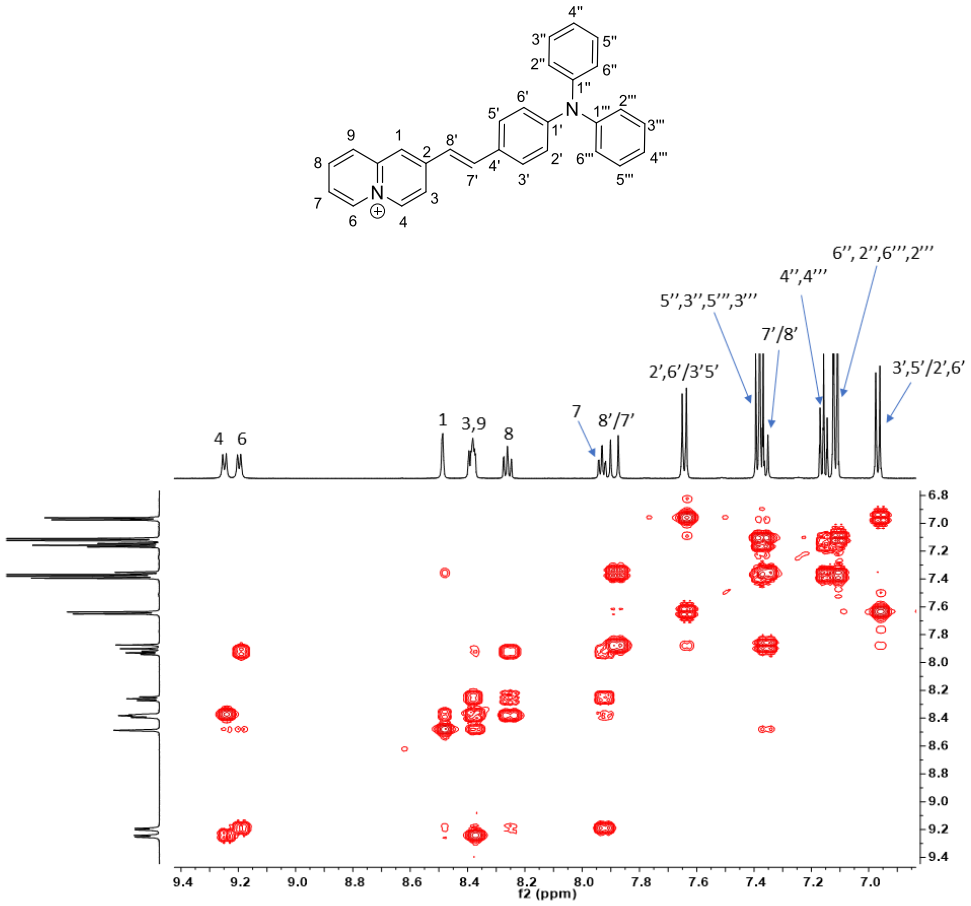


Figure S10. HH-COSY spectrum of **3a** in DMSO-d_6.

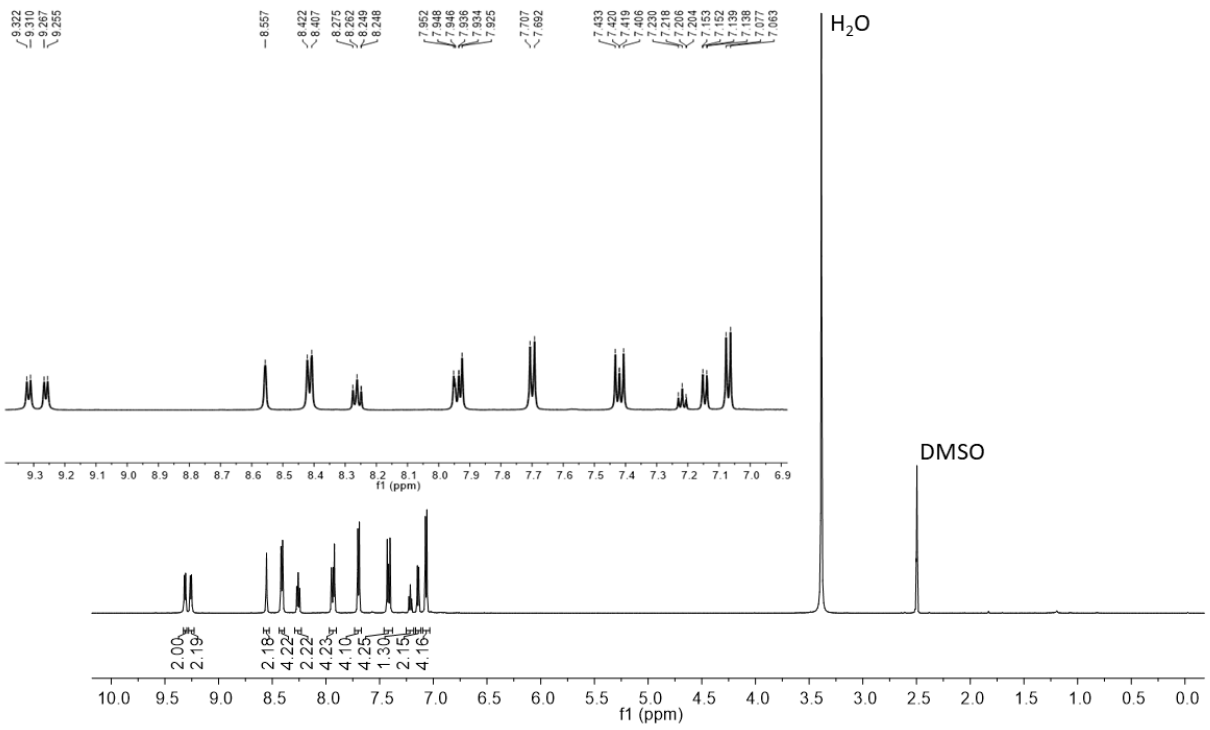


Figure S11. [1]H-NMR spectrum (600 MHz) of **3b** in DMSO-d_6. Inset: Magmified range of aromatic protons.

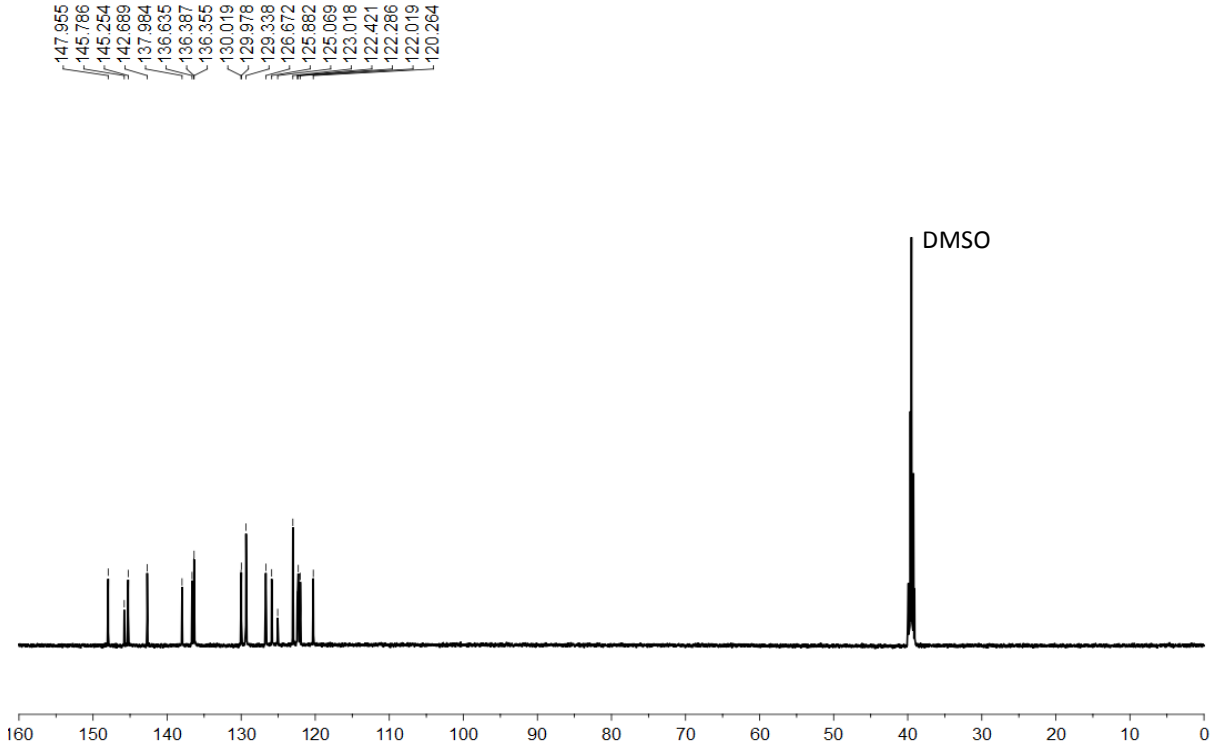


Figure S12. [13]C-NMR spectrum (150 MHz) of **3b** in DMSO-d_6.

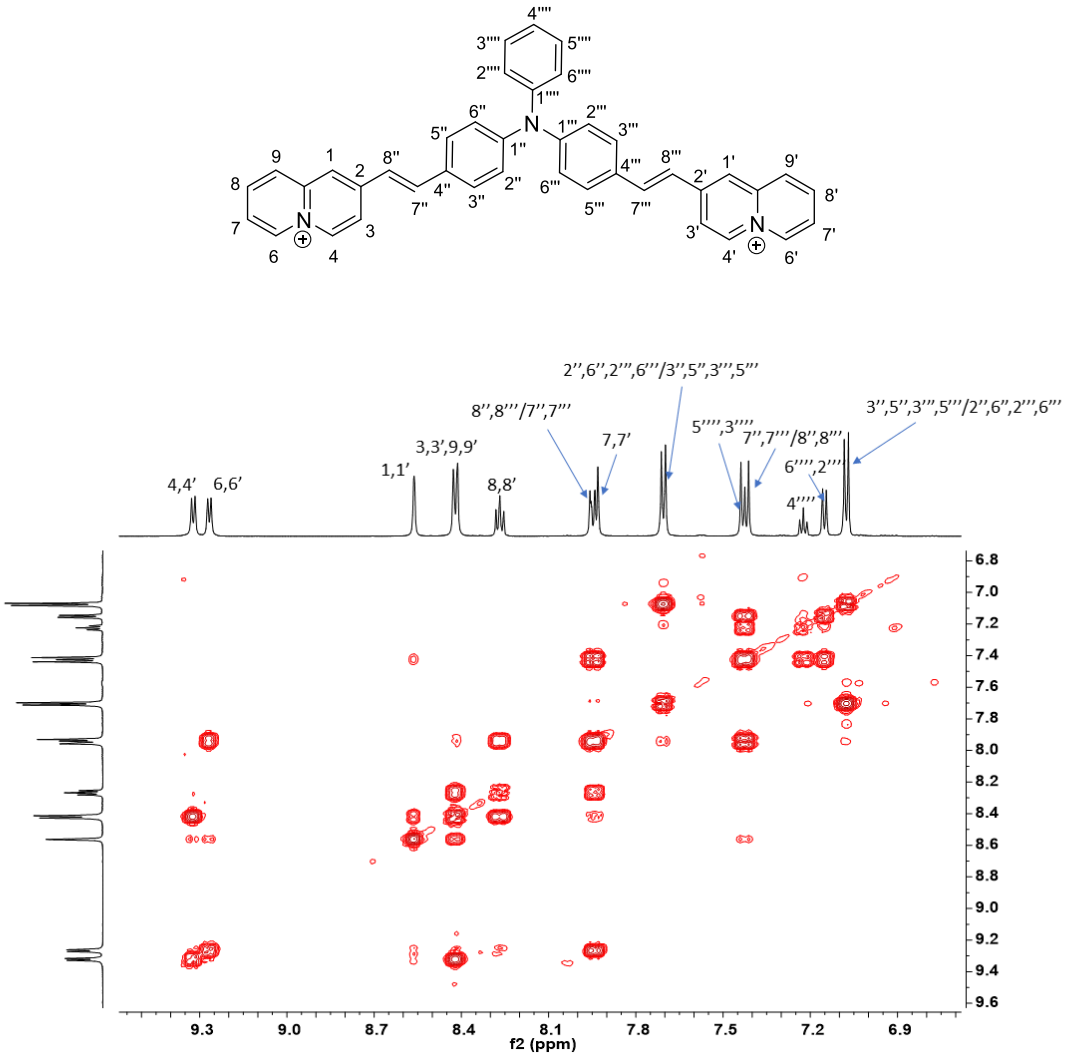


Figure S13. HH-COSY spectrum of **3b** in DMSO-d_6.