

Structural flexibility versus rigidity of the aromatic unit of DNA ligands: Binding of aza- and azoniastilbene derivatives to duplex and quadruplex DNA

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1. Absorption and emission properties

Table S1. Absorption and Emission Properties of Aza- and Azoniastilbene Derivatives **2a–c** in Different Solvents.

	2a				2b				2c			
	$\lambda_{\text{abs}}^{\text{a}}$ / nm	$\lg \epsilon^{\text{b}}$	$\lambda_{\text{fl}}^{\text{c}}$ / nm	$\Phi_{\text{fl}}^{\text{d}}$ / 10^{-2}	$\lambda_{\text{abs}}^{\text{a}}$ / nm	$\lg \epsilon^{\text{b}}$	$\lambda_{\text{fl}}^{\text{c}}$ / nm	$\Phi_{\text{fl}}^{\text{d}}$ / 10^{-2}	$\lambda_{\text{abs}}^{\text{a}}$ / nm	$\lg \epsilon^{\text{b}}$	$\lambda_{\text{fl}}^{\text{c}}$ / nm	$\Phi_{\text{fl}}^{\text{d}}$ / 10^{-2}
DMSO	333	4.51	406	34	396	4.62	499	22	369	4.51	–	≤ 0.1
MeCN	327	4.51	397	40	390	4.60	492	21	366	4.60	462	31
MeOH	327	4.36	398	45	393	4.58	491	21	366	4.59	454	1.0
Water	325	4.35	420	19 ^e	379	4.58	496	18	366	4.58	454	22
BPE	329	4.56	414	18 ^e	379	4.56	491	19	366	4.55	454	19

^a Maximum of the long-wavelength absorption, $c = 25 \mu\text{M}$. ^b Molar extinction coefficient ϵ in $\text{cm}^{-1} \text{M}^{-1}$. ^c Fluorescence emission maximum (Abs. = 0.10 at λ_{ex}); **2a**: $\lambda_{\text{ex}} = 334 \text{ nm}$, **2b**: $\lambda_{\text{ex}} = 390 \text{ nm}$, **2c**: $\lambda_{\text{ex}} = 320 \text{ nm}$. ^d Fluorescence quantum yield rel. to Coumarin 102 (**2b**) and Coumarin 120 (**2a** and **2c**); estimated error: $\pm 10\%$. ^e Because of low solubility DMSO (5% v/v) was added as a co-solvent.

2. Spectrometric titrations

The titrations were performed in BPE buffer (pH = 7.0; **2a,b**: with 5% v/v DMSO) with ct DNA and in K-phosphate buffer (pH = 7.0; **2a,b**: 5% v/v DMSO) with quadruplex DNA. To avoid the dilution of the solution of the ligand during titration, the titrant solution contained the same ligand concentration as the analyte solution. In fluorimetric titrations the excitation wavelength usually corresponded to an isosbestic point or to an absorption wavelength that does not change significantly during titration, as determined by photometric titrations. After recording the absorption or fluorescence spectrum of the solution of the ligand, the respective DNA was added stepwise until no more significant changes of the absorption or fluorescence intensity could be observed. The equilibration time after each addition was 3 min.

From the fluorimetric or photometric titrations, the binding constants were calculated by fitting the experimental data to the theoretical model (eq. S1 and eq. S2).¹

$$\frac{F}{F_0} = 1 + \frac{Q-1}{2} [A + 1 + x - \sqrt{(A + 1 + x)^2 - 4x}] \quad (\text{eq. S1})$$

$$A = \frac{1}{K_B \times c_{\text{dye}}}, x = n \frac{c_{\text{DNA}}}{c_{\text{dye}}}, Q = \frac{F_{\text{Max}}}{F_0} \quad (\text{eq. S2})$$

F_0 = the background fluorescence(absorption intensity of ligand in the absence of DNA)

F_{max} = fluorescence intensity/absorption upon saturation

n = putative number of ligand molecules binding to a given DNA strand

The parameters Q and A were evaluated with the Levenberg–Marquardt non-linear curve fitting algorithm as implemented in the Origin 8.5 software.

In the case of ligand **2c**, the titration data with ct DNA were presented as Scatchard plots, *i.e.* r/c vs. r values, and numerically fitted to the neighbor exclusion model of McGhee and von Hippel (eq. S3).^{2,3} The numerical fitting was performed with the Levenberg–Marquardt non-linear curve fitting algorithm as implemented in the Origin 8.5 software.

¹ F. H. Stootman, D. M. Fisher, A. Rodger, and J. R. Aldrich-Wright, *Analyst*, 2006, **131**, 1145.

² McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* 1974, **86**, 469.

³ A. Granzhan, H. Ihmels and G. Viola, *J. Am. Chem. Soc.* 2007, **129**, 1254.

$$\frac{r}{c} = K (1 - nr) \left(\frac{1 - nr}{1 - (n-1)r} \right)^{n-1} \quad (\text{eq. S3})$$

$$r = \text{ratio of bound ligand molecules per DNA base pair: } r = \frac{c_b}{c_{\text{DNA}}} \quad (\text{eq. S4})$$

$$c = \text{concentration of the unbound ligand: } c = c_L - c_b \quad (\text{eq. S5})$$

$$c_b: \text{concentration of the DNA-bound ligand: } c_b = c_L \times \frac{A_f - A}{A_f - A_b} \quad (\text{eq. S6})$$

A_f is the absorbance/emission of the free ligand at a given wavelength, A_b is the absorbance/emission of the bound ligand, and A is the absorbance/emission at a given ligand-to-DNA ratio.



Figure S1. A: Fluorimetric titration of **2c** with ct DNA ($c = 1.0$ mM in base pairs) in K-phosphate buffer (pH = 7.0, 5% v/v DMSO), $T = 20$ °C; $\lambda_{\text{ex}} = 342$ nm. The arrows indicate the development of the emission bands during the titration. Inset: Plot of relative emission intensity I / I_0 versus c_{DNA} . B: Corresponding Scatchard plot of r / c versus r and fit of the experimental data to the theoretical model (eq. S3).

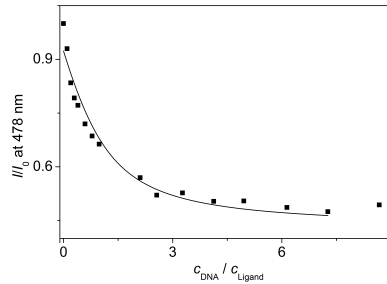


Figure S2. Plot of relative emission intensity I / I_0 versus $c_{\text{DNA}} / c_{\text{Ligand}}$ from spectrofluorimetric titration of ct DNA to **2a** in BPE butter (pH = 7.0, 5% v/v DMSO), $T = 20$ °C.



Figure S3. A: Plot of absorption $\text{Abs.}/\text{Abs}_0$ versus $c_{\text{DNA}} / c_{\text{Ligand}}$ from spectrophotometric titration of ct DNA to **2b** in BPE buffer (*cf.* Figure 2, A2) and fit of the experimental data to the theoretical model (eq. S1). B: Scatchard plot of r / c versus r from spectrophotometric titration of ct DNA to **2c** in BPE buffer (*cf.* Figure 2, A3) and fit of the experimental data to the theoretical model (eq. S3).

3. Thermal DNA melting analysis (FRET melting)

Separate stock solutions of the dye-labeled oligonucleotides **F21T** (5'-fluorescein-dGGGTTA-GGGTTAGGGTTAGGG-tetramethylrhodamine) or **Fa2T** (5'-fluorescein-dACAGGGGTGTG-GGACAGGGGTGTGGGG-tetramethylrhodamine) (50 μ M) and the ligands (20 μ M) were prepared, respectively, in cacodylate buffer (10 mM sodium cacodylate, 100 mM LiCl, 90 mM LiCl and 10 mM KCl or 90 mM NaCl and 10mM LiCl, pH 7.2). The ligand solution was diluted to concentrations of 0.2, 0.5, and 1.0 μ M, respectively, and the samples were placed in an ultrasonic bath for 10 min. For each experiment, DNA solution was added to the analyte solution to obtain a DNA concentration of 0.2 μ M. To record the melting curves, the following experimental settings were used:

Excitation wavelength	470 nm
Emission wavelength	515 nm
Excitation slit	5 nm
Emission slit	5 nm

The following heating-cooling sequence was used:

- 1) heating from 20 °C to 90 °C at 2.5 °C min⁻¹;
- 2) holding the max. temperature for 5 min;
- 3) cooling to 10 °C at 1.0 °C min⁻¹;
- 4) heating from 10 °C to 99 °C at 0.2 °C min⁻¹; the fluorescence was detected during the latter ramp.

The normalized fluorescence intensities were plotted versus temperature. The melting temperature was determined as the maximum of the first derivative of the melting curve approximated by the Gaussian function. The shift of the melting temperature was calculated as $\Delta T_m = T_m(\text{DNA-Ligand}) - T_m(\text{DNA})$.

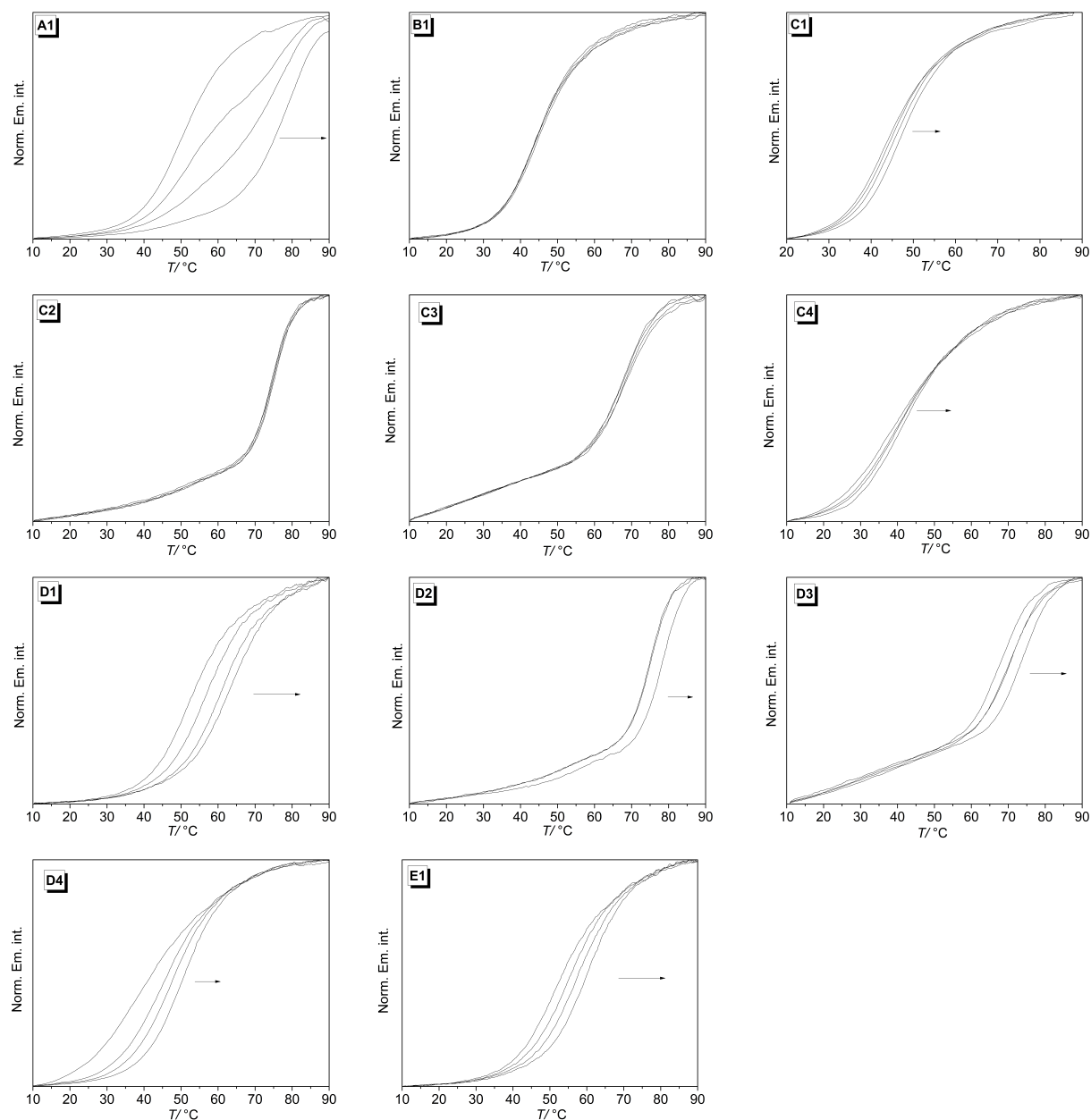


Figure S4. Normalized emission intensities of the G4-DNA **F21T** (1), **Fa2T** (2), **FmycT** (3) and **FkrasT** (4) (0.2 μM) ($\lambda_{\text{ex}} = 470 \text{ nm}$ and $\lambda_{\text{fl}} = 515 \text{ nm}$) plotted versus temperature in the presence of ligands **1d** (A), **2a** (B), **2b** (C) and **2c** (D and E; E in the presence of **ds26**) in Na-cacodylate buffer (10 mM K^+ , pH = 7.2); **2a**: plus DMSO (1% v/v). The arrows indicate the development of the melting curves with increasing *LDR* (0, 1.3, 2.5, and 5.0 molar equivalents).

4. CD-spectroscopic analysis

Solutions of DNA, the ligand and BPE buffer were measured after an equilibration time of 30 min. The CD spectra were determined with the following experimental settings:

Wavelength range	220–550 nm
Bandwidth	1.0 nm
Scan rate	1.0 nm/s
Time per point	0.5 s
Temperature	20 °C

5. NMR Spectra

NMR spectra were recorded on a Varian VNMR-S600 with Triple Resonance HCN inverse probe (3 mm) in 2 mm (50 μ L) or 3 mm (150 μ L) capillary tubes. Solvent suppression: *WET 1d* with 1.5 or 2 s relaxation time, 256 Scans (1D).

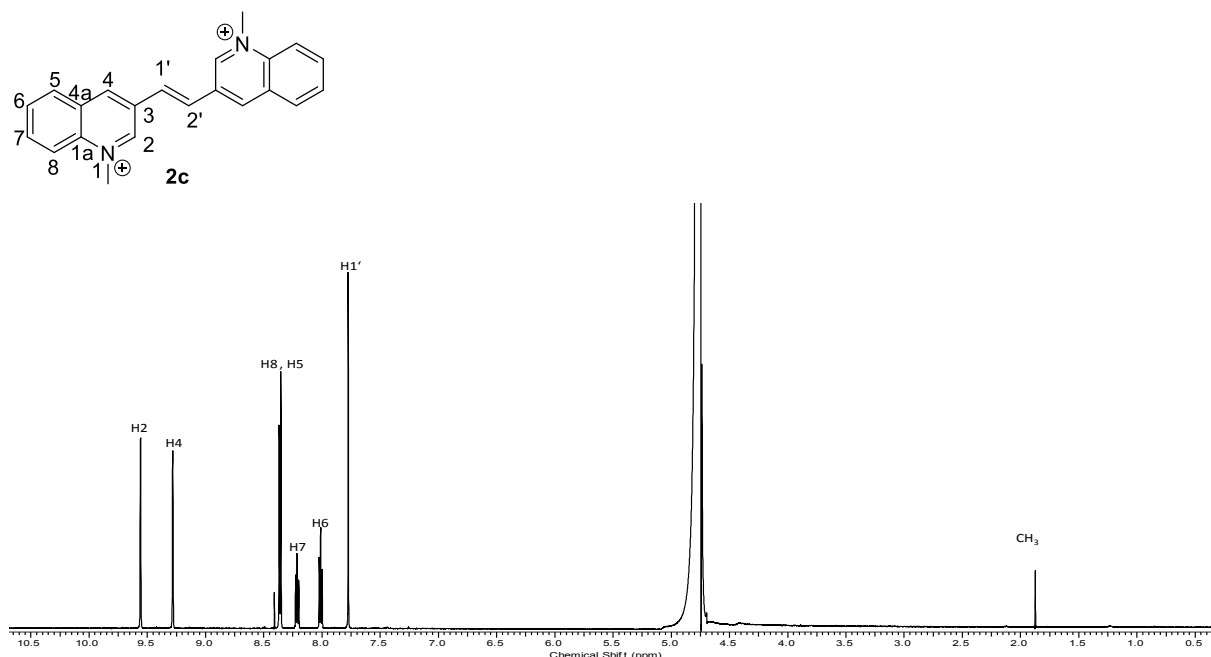


Figure S5. ¹H-NMR spectrum (600 MHz) of **2c** in K-phosphate buffer (H₂O:D₂O = 9:1; 95 mM, pH 7.0; *T* = 25 °C).

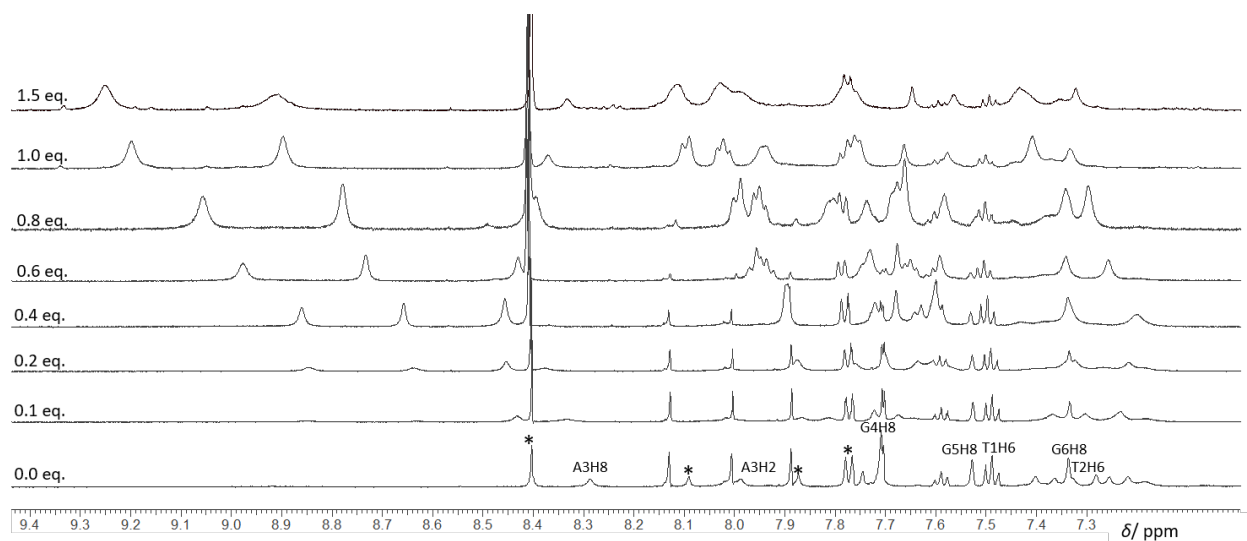


Figure S6. ¹H-NMR spectra (600 MHz) of **Tel6** (2 mM in bases) in K-phosphate buffer (H₂O:D₂O = 9:1; 95 mM, pH 7.0; *T* = 25 °C) with increasing amount of **2c**; * = monomeric quadruplex.

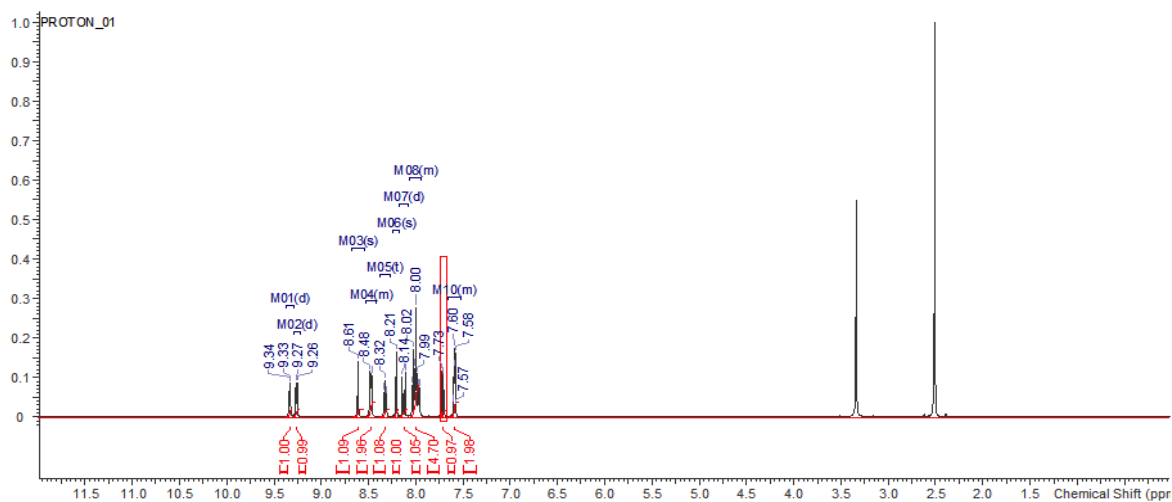


Figure S7. ^1H -NMR spectrum (600 MHz) of **2b** in $\text{DMSO}-d_6$.

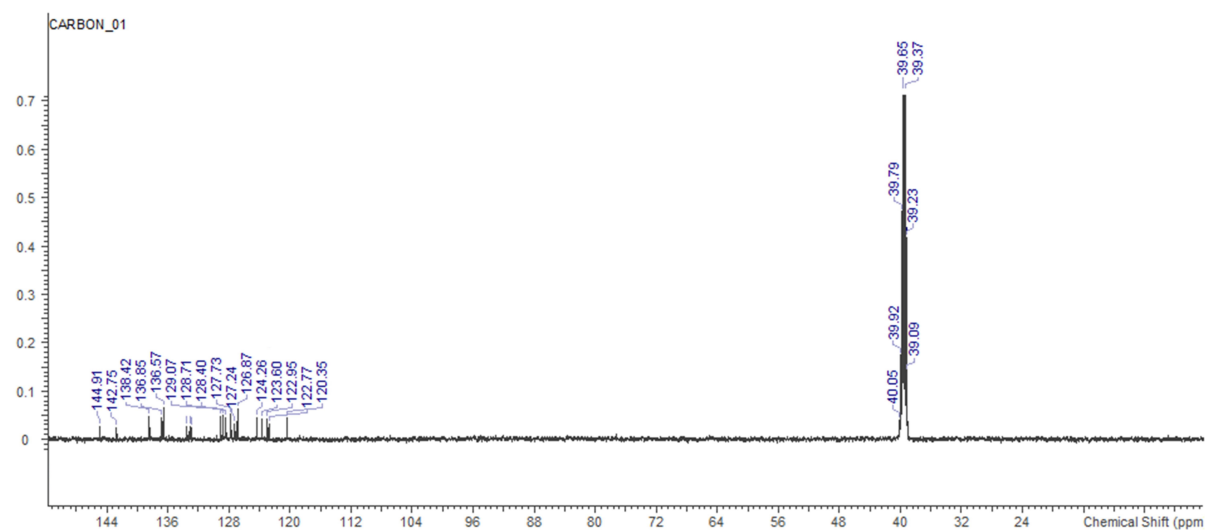


Figure S8. ^{13}C -NMR spectrum (150 MHz) of **2b** in $\text{DMSO}-d_6$.

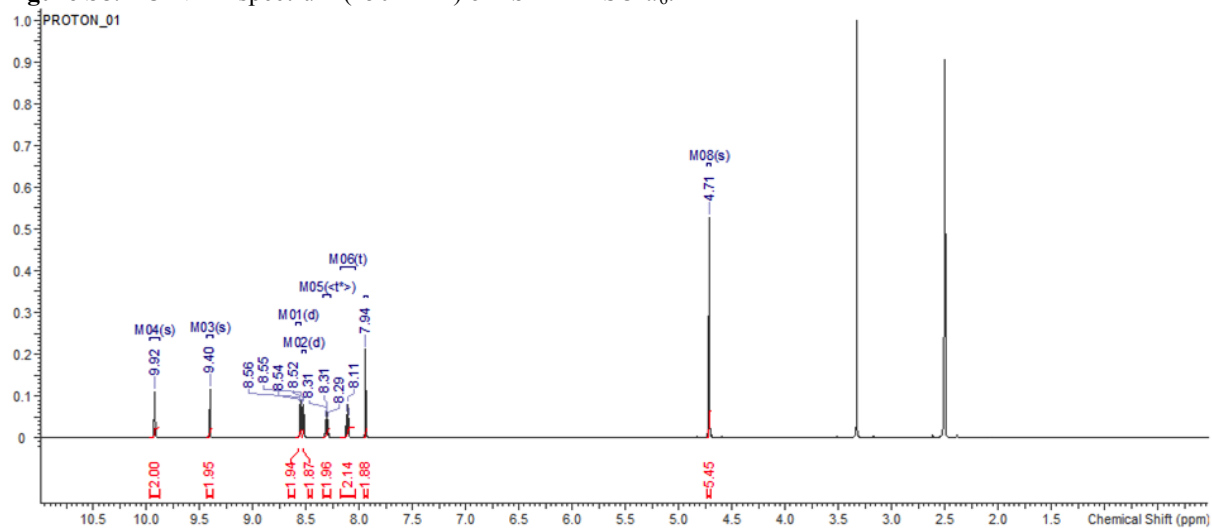


Figure S9. ^1H -NMR spectrum (600 MHz) of **2c** in $\text{DMSO}-d_6$.

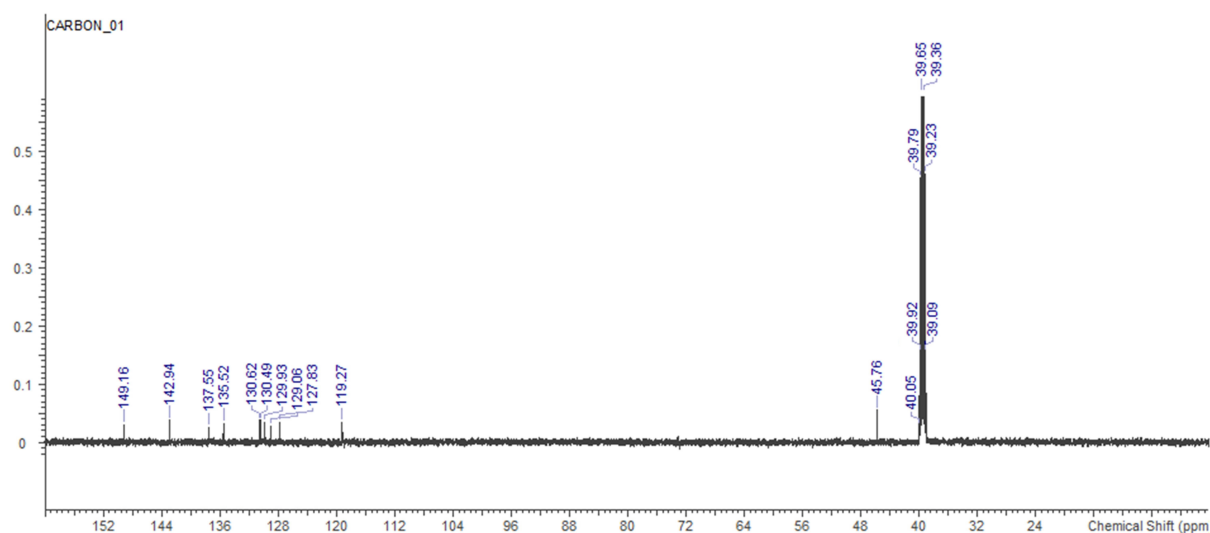


Figure S10. ^{13}C -NMR spectrum (150 MHz) of **2c** in $\text{DMSO-}d_6$.

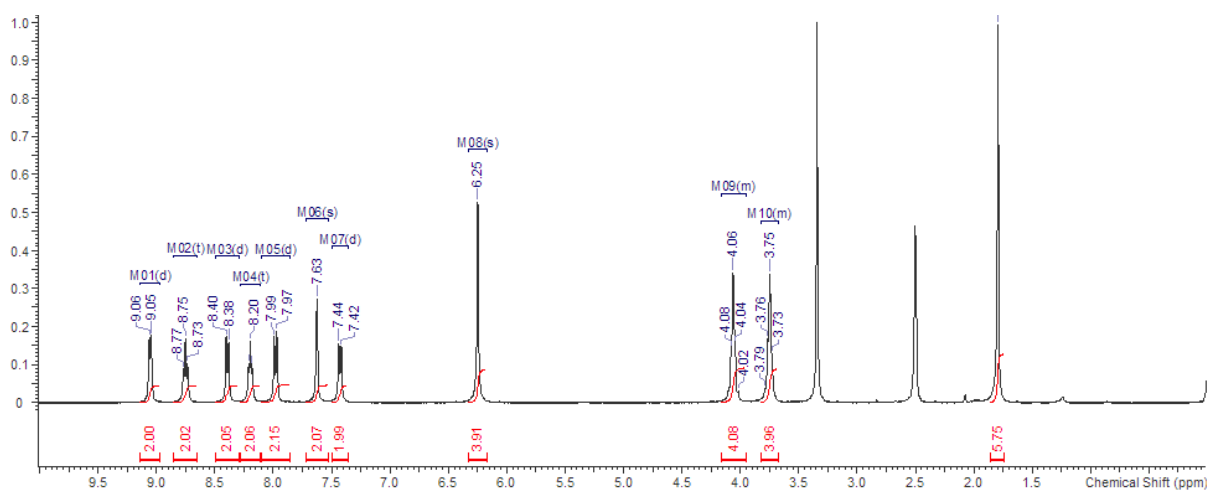


Figure S11. ^1H -NMR spectrum (600 MHz) of **7** in $\text{DMSO-}d_6$.

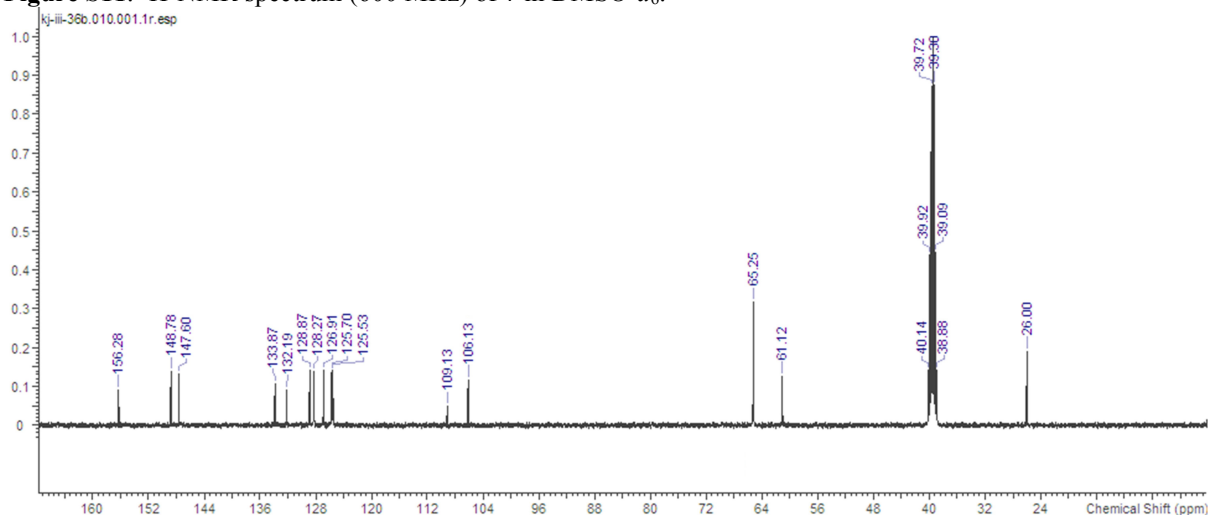


Figure S12. ^{13}C -NMR spectrum (150 MHz) of **7** in $\text{DMSO-}d_6$.

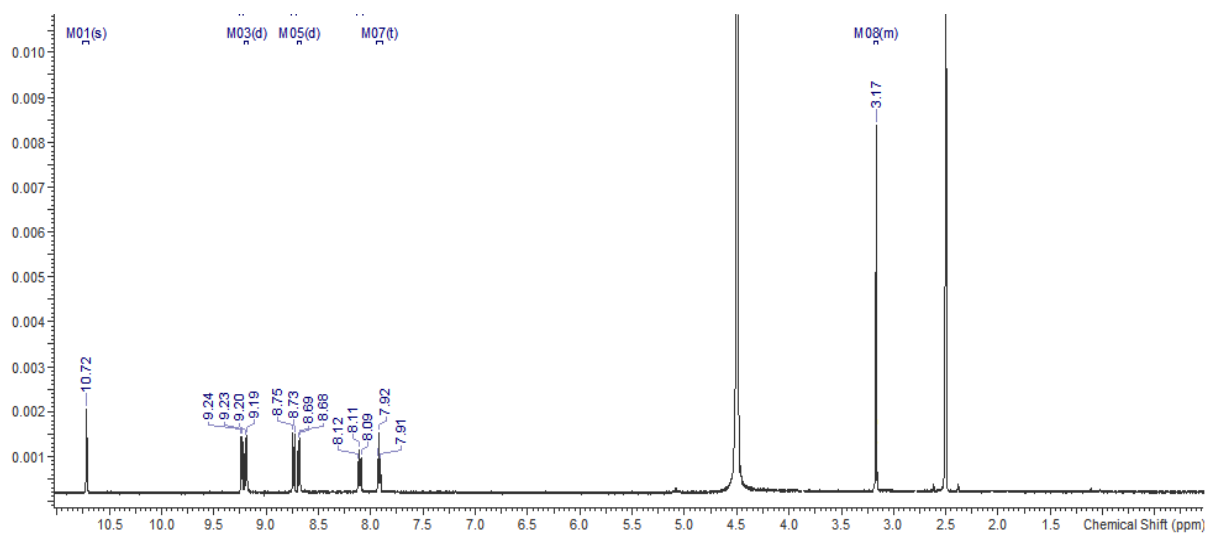


Figure S13. ¹H-NMR spectrum (600 MHz) of **1d** in DMSO-*d*₆.

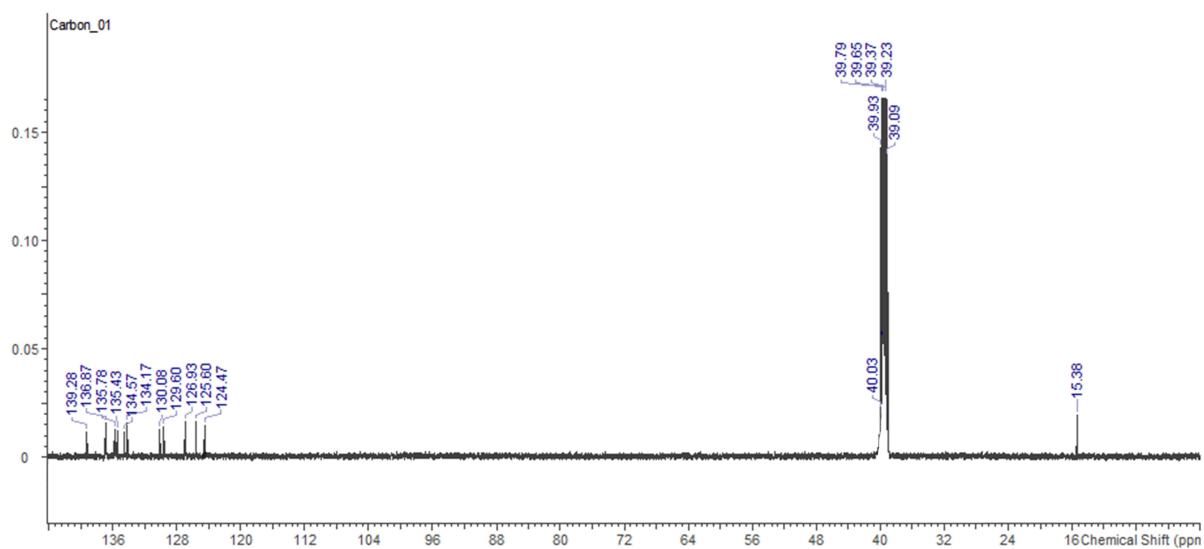


Figure S14. ¹³C-NMR spectrum (150 MHz) of **1d** in DMSO-*d*₆.