Electronic Supplementary Information

Photoinduced in situ generation of a DNA-binding benzothiazoloquinolinium derivative

Daria Berdnikova, Olga Fedorova, Elena Gulakova, and Heiko Ihmels

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1. Materials and equipment

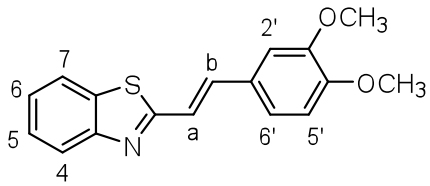
All reagents and solvents were obtained from commercial sources and used as received. Purified water with resistivity ≥ 18 MΩ cm^{-1} was used for preparation of buffer solutions and spectrometric measurements. BPE buffer (6.0 mM Na_2HPO_4, 2.0 mM NaH_2PO_4, 1.0 mM Na_2EDTA; total Na^+ concentration 16.0 mM; pH 7.0) was used for DNA titrations and thermal denaturation studies. Phosphate buffer (5.8 mM Na_2HPO_4, 4.2 mM NaH_2PO_4; total Na^+ concentration 15.8 mM; pH 7.0) was used for photochemical reactions.

NMR spectra were recorded at 293 K on a Bruker DRX-600 spectrometer using 5 mm tubes. Chemical shifts were determined with an accuracy of 0.01 ppm (^1H) and 0.1 ppm (^{13}C) and are given relative to the residual signal of the solvent that was used as internal reference. Spin–spin coupling constants were determined with accuracy of 0.1 Hz. Elemental analysis was performed at the Laboratory of Microanalysis of Nesmeyanov Institute of Organoelement Compounds RAS.

2. Synthesis

2-(3,4-dimethoxystyryl)benzothiazole **1** was prepared according to literature procedures.[1] 2,3-(Dimethoxy)[1,3]benzothiazolo[3,2-*a*]quinolin-1*a*-ium hydroxide **2** was obtained by irradiation of **1** in acetonitrile solution with the full light of a high-pressure Hg-lamp according to published protocols.[2] The photoproduct **2** was fully characterized as perchlorate salt as obtained by recrystallization from methanol with addition of $HClO_4$ (corresponding data marked as **2***).

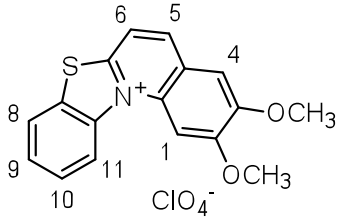
*2-[(E)-2-(3,4-Dimethoxyphenyl)vinyl]-1,3-benzothiazole (**1**)*



M.p. 143–146 ºC; ^1H-NMR (CD_3CN): δ = 3.84 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.97 (d, 1H, H-5', J = 8.4 Hz), 7.21 (dd, 1H, H-6', ^3J = 8.4 Hz, ^4J = 1.8 Hz), 7.29 (s, 1H, H-2'), 7.37-7.41 (m, 2H, H-5, H-b), 7.48 (t, 1H, H-6, J = 7.0 Hz, J = 7.3 Hz), 7.54 (d, 1H, H-a, J_{trans} = 16.3 Hz), 7.93 (d, 2H, H-7, J = 7.9 Hz), 7.96 (d, 2H, H-4, J = 7.7 Hz); ^{13}C-NMR (CD_3CN): δ = 56.8 and 56.8 ($2OCH_3$), 111.0 (C-2'), 112.9 (C-5'), 121.3 (C-b), 123.1 (C-4, C-6'), 123.8 (C-7), 126.6 (C-5), 127.7 (C-6),

129.7 (C-3'), 135.6 (C_{ar}), 139.0 (C-a), 150.9 (C-4'), 152.1 (C-1'), 155.4 (C_{ar}), 168.7 (C-2); elemental analysis calcd. (%) for $C_{17}H_{15}NO_2S$: C 68.66, H 5.08 N 4.71; found: C 68.59, H 5.02, N 4.75.

2,3-(Dimethoxy)[1,3]benzothiazolo[3,2-a]quinolin-1a-ium perchlorate ***(2*)***



M.p. 224–226 ºC. ^{1}H-NMR (CD_3CN): δ = 4.07 (s, 3H, OCH_3), 4.24 (s, 3H, OCH_3), 7.76 (s, 1H, H-4), 7.92 (t, 1H, H-9, J = 7.3 Hz; J = 8.1 Hz), 8.00 (t, 1H, H-10, J = 8.5 Hz; J = 8.8 Hz), 8.28 (d, 1H, H-6, J = 8.8 Hz), 8.37 (d, 1H, H-8, J = 8.1 Hz), 8.44 (s, 1H, H-1), 8.62 (d, 1H, H-5, J = 9.0 Hz), 9.05 (d, 1H, H-11, J = 8.8 Hz); ^{13}C NMR (CD_3CN): δ = 57.4 and 58.0 ($2OCH_3$), 101.2 (C-1), 110.4 (C-4), 117.5 (C-6), 120.9 (C-11), 124.5 (C_q), 125.9 (C-8), 130.2 (C-10), 130.3 (C_q), 130.5 (C-9), 135.3 (C_q), 139.8 (C_q), 140.3 (C-5), 151.8 (C_q), 156.7 (C_q), 156.7 (C_q); elemental analysis calcd. (%) for $C_{17}H_{14}ClNO_6S$: C 51.59, H 3.57, N 3.54; found: C 51.62, H 3.59, N 3.51.

3. Spectrophotometric measurements

Absorption spectra were recorded using a Varian Cary 100 Bio spectrophotometer, fluorescence spectra were recorded on a Varian Cary Eclipse spectrofluorometer. Spectrophotometric measurements were performed in thermostated quartz sample cells of 10 mm pathlength at 20 °C. Fluorescence quantum yield of **2** were determined in water at 20 °C relative to Coumarin 6 in EtOH (φ = 0.78).[3] Solutions for analysis were prepared from stock solutions in acetonitrile shortly before the experiments. To avoid effects from the co-solvents, aliquots of the stock solution of the ligand were pipetted into vials and the the solvent was evaporated. The residue was dissolved in phosphate buffer. In the case of **1** 10 vol.% of DMSO was added to provide sufficient solubility. To avoid dilution of the analyte solutions during titration, the titrant solutions contained ct DNA at an approximate concentration c_{DNA} = 1–2 mM (bp) and the ligand at the same concentration as in the analyte solution. The actual concentrations of DNA samples were determined photometrically using the extinction coefficient ε_{260} = 12824 cm^{-1} M^{-1} (bp). Aliquots of the analyte solutions were placed into quartz cells and titrated with the titrant solutions in intervals of 0.5-2 equivalent, and absorption spectra were recorded. The titrations were stopped after no changes were observed in absorption spectra upon addition of at least three two-equivalent portions of the titrant. All spectrophotometric titrations were performed at least three times to ensure the reproducibility.

Spectrofluorimetric titrations were performed just as described for the spectrophotometric titrations. The excitation wavelength corresponds to the isobestic point determined from the photometric titrations.

Preparation of solutions and all experiments with photosensitive compounds were performed in a dark room under red light.

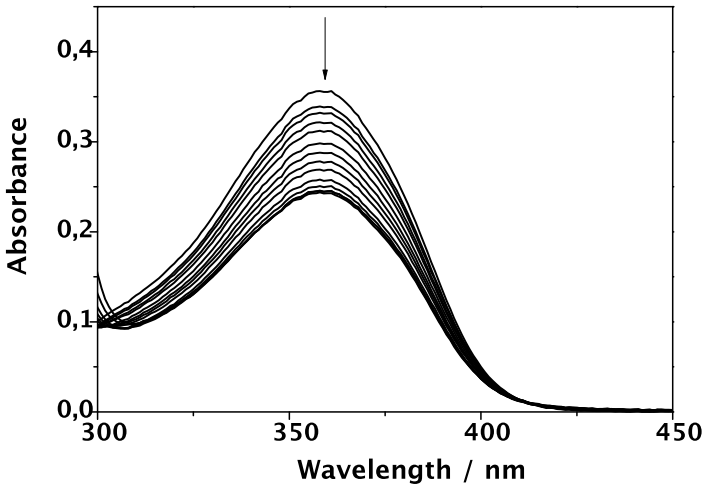


Fig. S1. Spectrophotometric titration of ct DNA to **1** (c = 10 µM in BPE with 10% DMSO, c_{DNA}/c_{lig} = 0–12.3)

4. CD measurements

Circular dichroism spectra were measured with a Chirascan CD spectrometer (Applied Photophysics) in thermostated quartz sample cells of 10 mm pathlength at 20 °C.

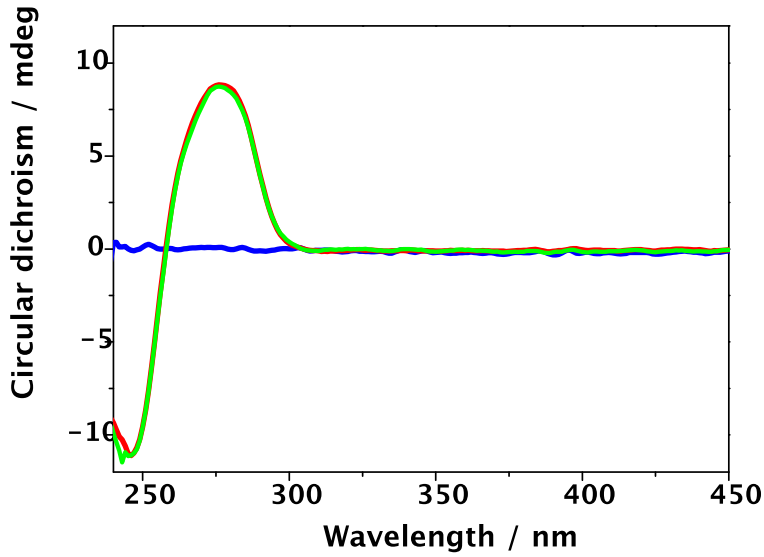


Fig. S2. CD spectra of mixture of ct DNA (c = 60 µM in base pairs) with ligand **1** in phosphate buffer with 10% DMSO (v/v); blue: **2**; red: ct DNA, green: r = 0.17.

5. Thermal DNA denaturation

Aliquots of the stock solution of the ligand **2** in acetonitrile were pipetted into Eppendorf vials and the latter were left open until all solvent evaporated. The residue was dissolved in phoshate buffer. The volume of the buffer solution was calculated based on the concentration of the DNA stock solution, to establish a final volume of 1.00 mL. The samples were degassed in an ultrasonic bath for 15 min. Fixed amounts of DNA (c_{final} = 40 μM in bp) were added; the samples were mixed briefly and transferred into semi-micro quartz cells (pathlength ℓ = 1 cm). DNA melting curves were recorded with a Varian Cary 100 Bio spectrophotometer equipped with a thermoelectric temperature controller. Samples were heated from 20.0 to 97.0 °C at a rate of 0.2 deg min^{-1}, while the absorbance was monitored at 260 nm at each temperature. The melting curves were presented as plots of normalized absorbance change, $\hat{A}$ (Eq. S1), versus temperature.

$$\hat{A} = \frac{A_T - A_{60°C}}{A_{max} - A_{60°C}} \qquad \text{(Eq. S1)}$$

Here A_T is absorbance at 260 nm at a given temperature, $A_{60\,°C}$ is absorbance at 60 °C, and A_{max} is the maximum absorbance in the range of interest (60–90ºC).

The ligand-induced shifts of DNA melting transitions (ΔT_m) were calculated according to Eq. S2 and plotted as a function of ligand-to-DNA ratio, $r = c_L / c_{DNA}$.

$$\Delta T_m = T_m(\text{DNA-Ligand}) - T_m(\text{DNA}) \qquad \text{(Eq. S2)}$$

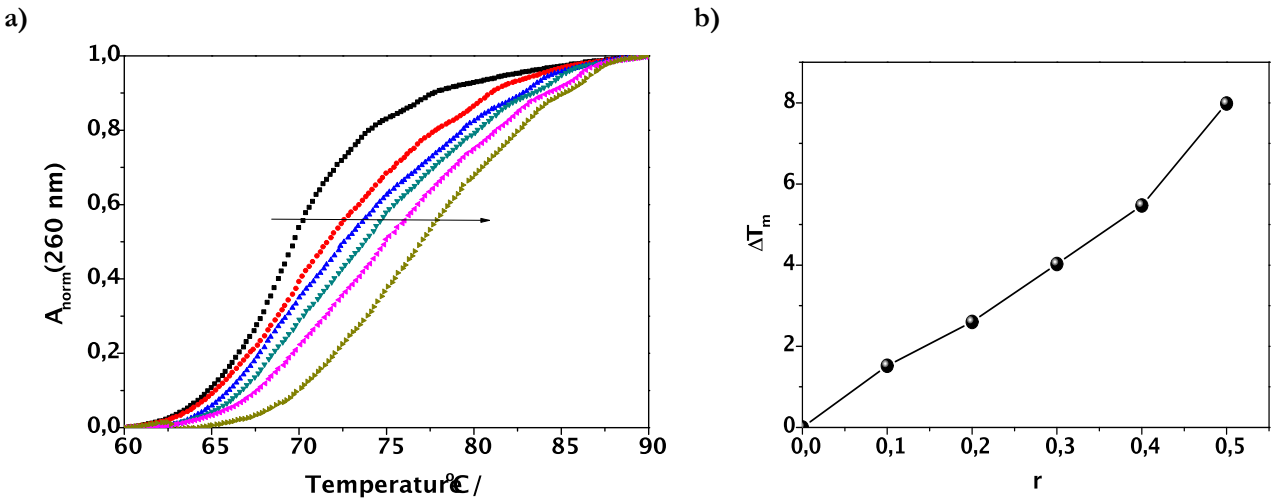


Fig. S3. a) Normalized thermal denaturation curves of ct DNA in the presence of compound **2** at ligand-to-DNA ratio r = 0, 0.1, 0.2, 0.3, 0.4, 0.5; and b) plot of induced T_m shifts, C_{DNA} = 40 μM bp in BPE buffer.

6. Viscometric titration

Aliquots of the solution of the ligand in BPE buffer were added to the ct DNA solution (1 mM base pair in the BPE buffer). Flow times were measured after a thermal equilibration period of 5 min. Each flow time was measured three times and an average value was calculated. As a reference ethidium bromide was employed under identical conditions. The relative viscosity was presented as $(\eta/\eta_o)^{1/3}$.[4] The value η is the viscosity of the solution in the presence of a compound, calculated from the time for the sample (t_L) to flow through the viscometer subtracted by the time measured for the buffer (t_0) only (eq. S3).

$$\eta = (t_L - t_0) / t_0 \qquad \text{(Eq. S3)}$$

η_0 is the viscosity of the ct DNA solution alone according to eq. S4.

$$\eta_0 = (t - t_0) / t_0 \qquad \text{(Eq. S4)}$$

The relative viscosity $(\eta/\eta_o)^{1/3}$ was plotted as a function of ligand-to-DNA ratio, $r = c_L \ / \ c_{DNA}$.

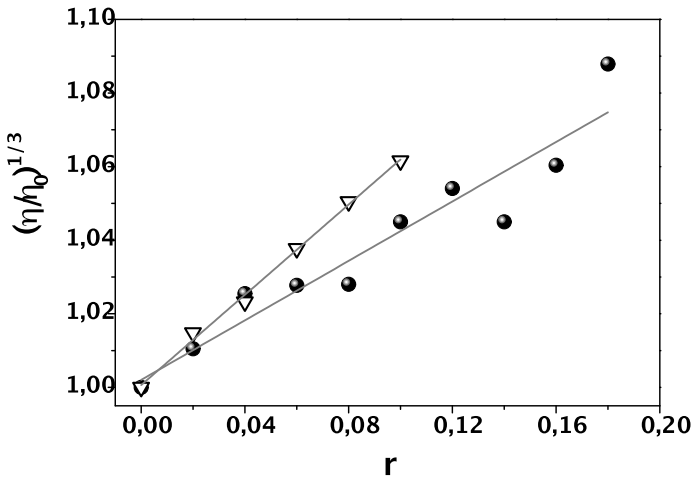


Fig. S4. Relative specific viscosity of ct DNA in the presence of **2** (circles) and ethidium bromide (triangles) as a function of the ligand-to-DNA ratio, c_{DNA} = 1 mM bp in phosphate buffer.

7. Photocyclization of 1

Photochemical reactions were carried out with a Hg high-pressure lamp (145 W) in phosphate buffer with addition of DMSO. The use of optical filter (λ >320 nm) allowed to avoid the photoinduced destruction of ct DNA and DMSO. During the photoreaction the UV-Vis and CD spectra were recorded at 25 °C.

8. NMR spectroscopy data

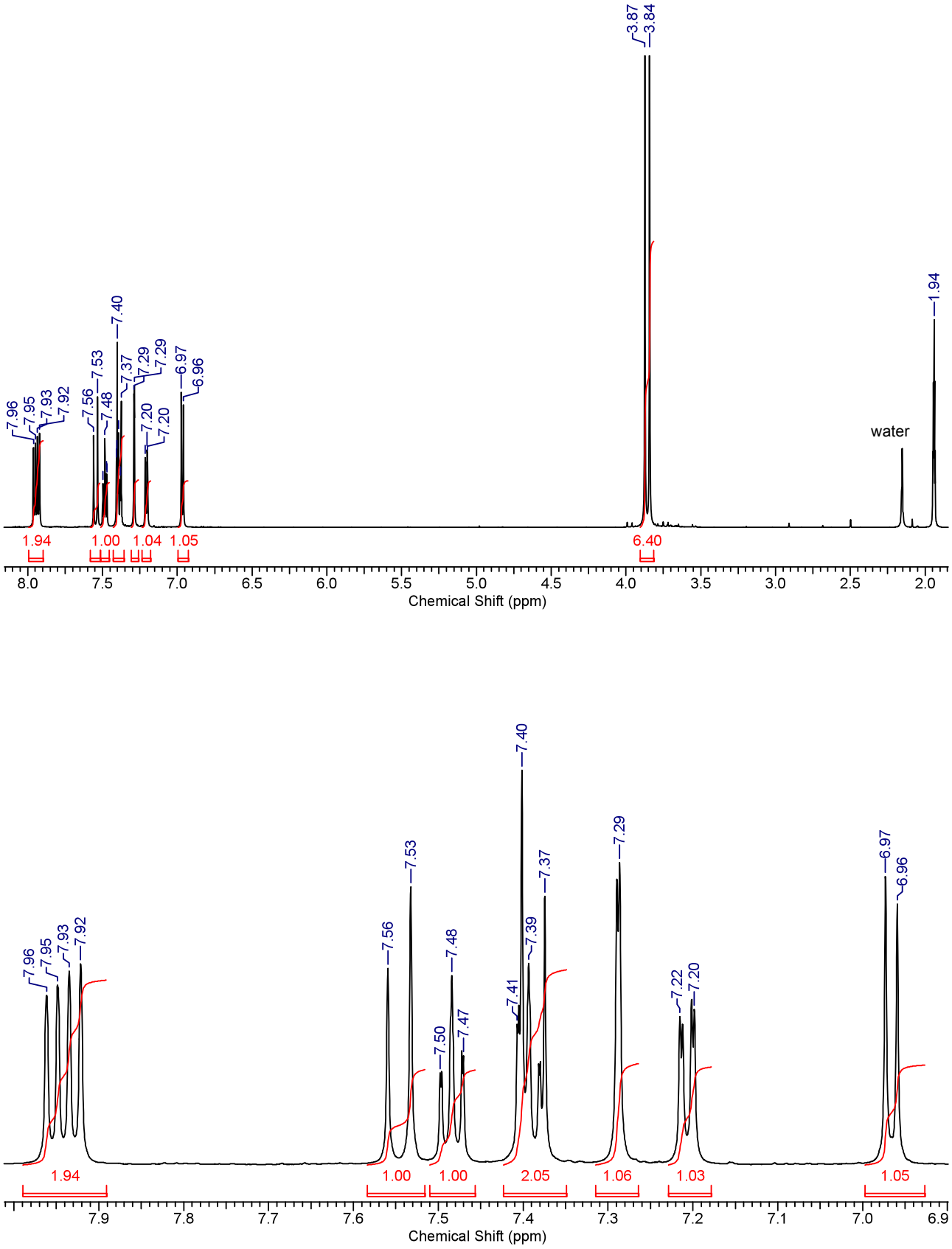


Fig. S5. ^{1}H-NMR spectrum of **1** in CD_3CN

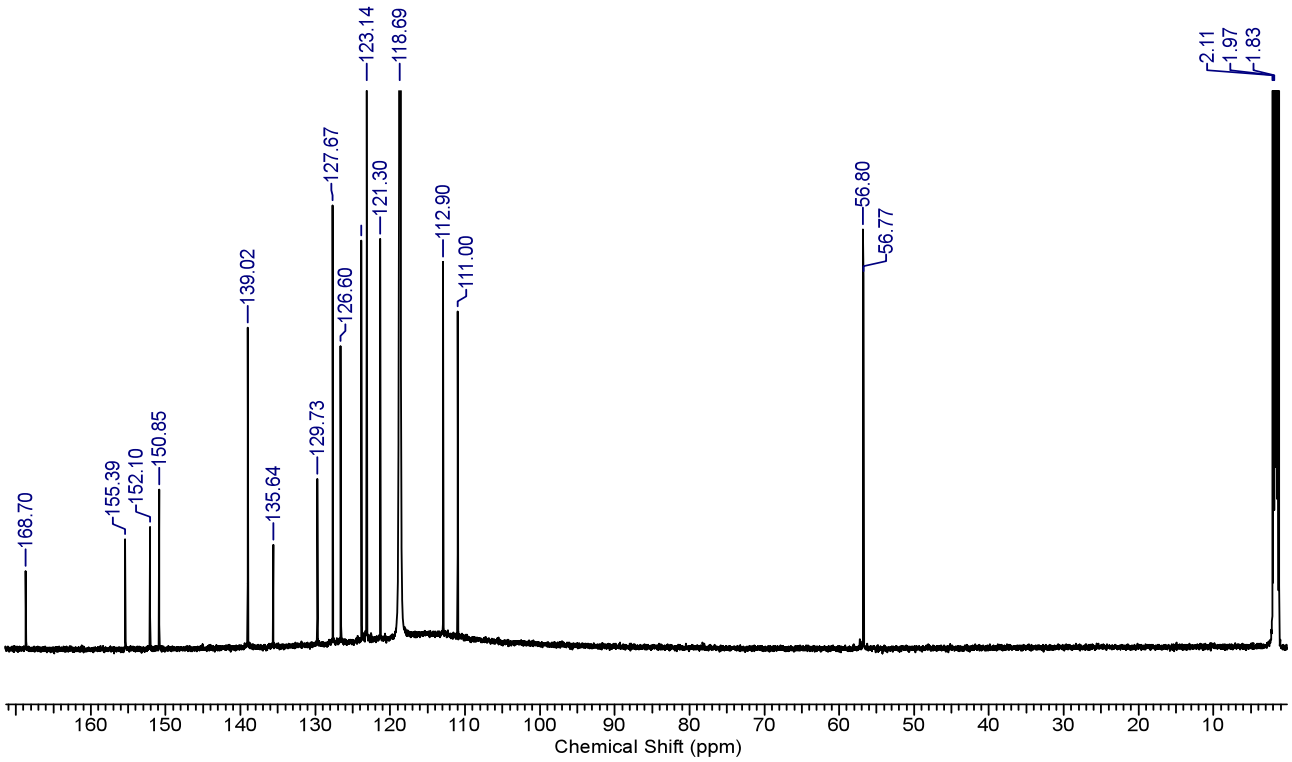


Fig. S6. ^{13}C-NMR spectrum of **1** in CD_3CN

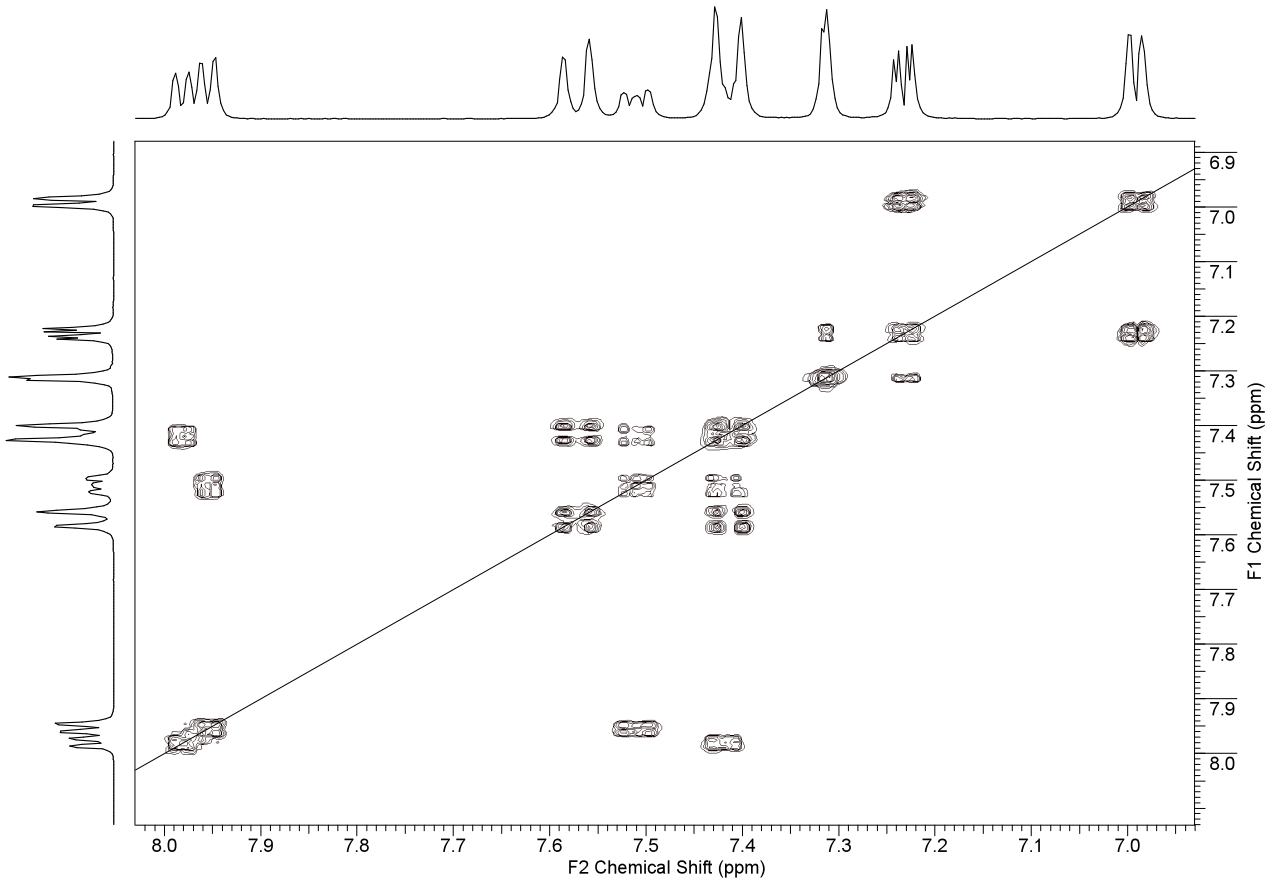


Fig. S7. {1H, 1H}-COSY spectrum of **1** in CD_3CN (range of aromatic protons)

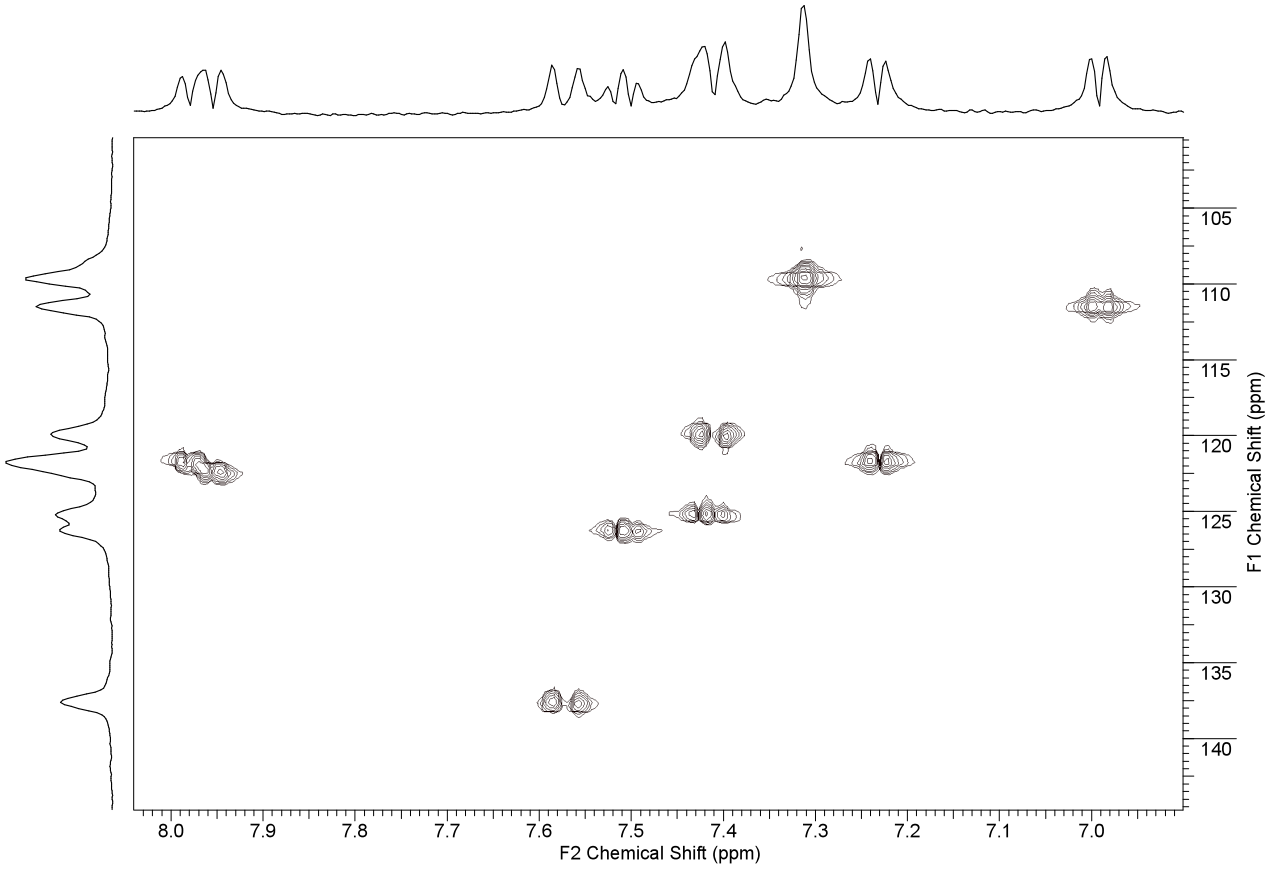


Fig. S8. HSQC spectrum of **1** in CD_3CN (range of aromatic protons)

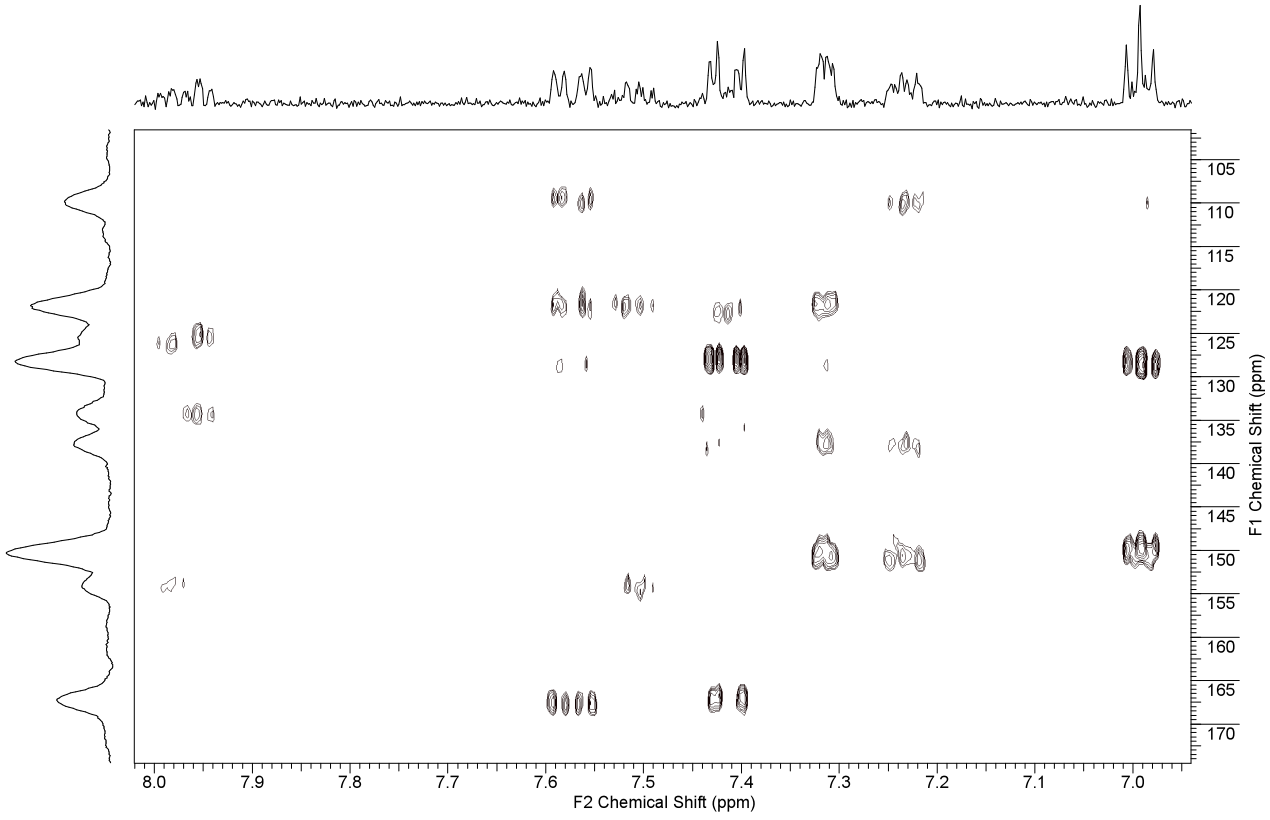


Fig. S9. HMBC spectrum of **1** in CD_3CN (range of aromatic protons)

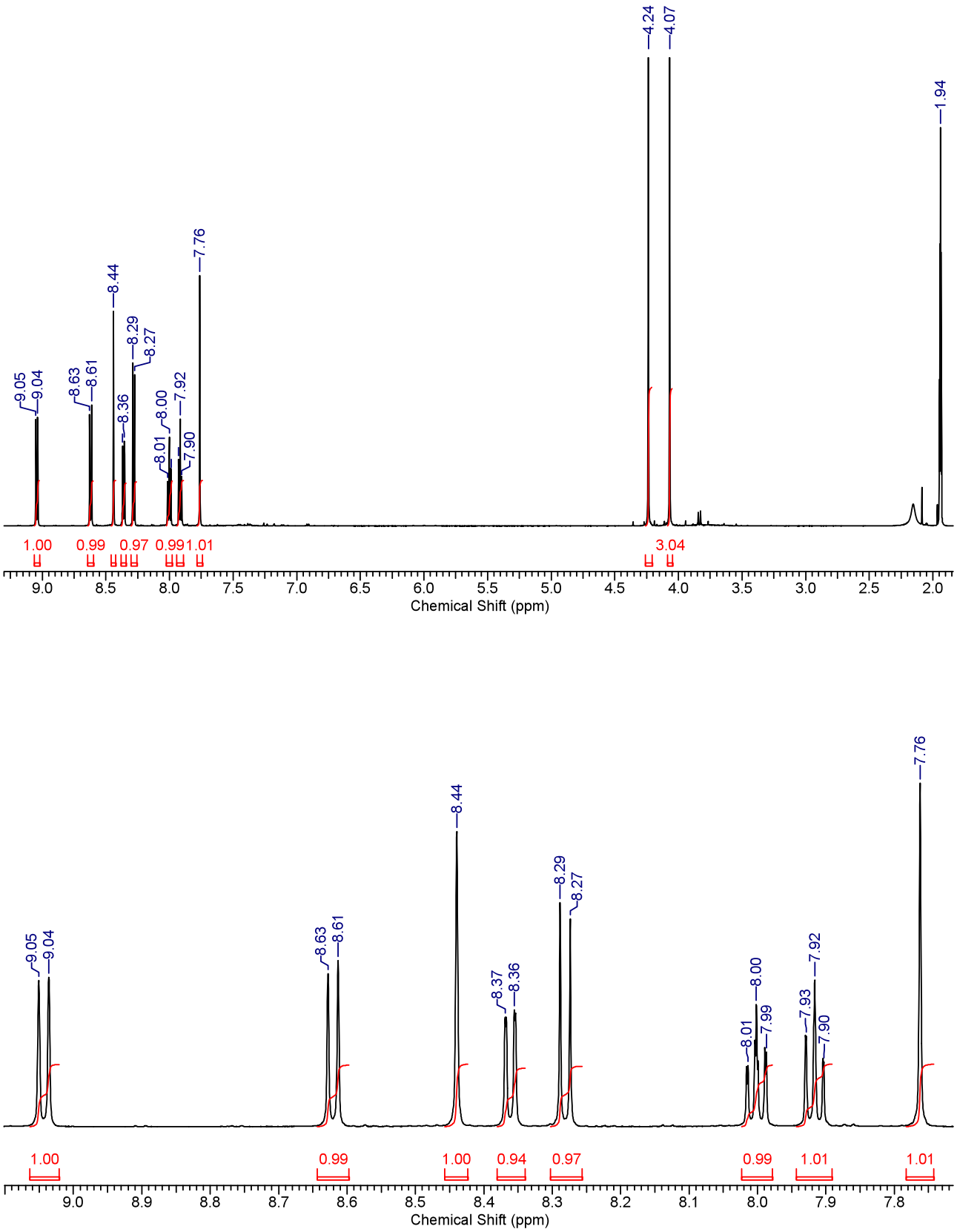


Fig. S10. ^{1}H-NMR spectrum of **2*** in CD_3CN

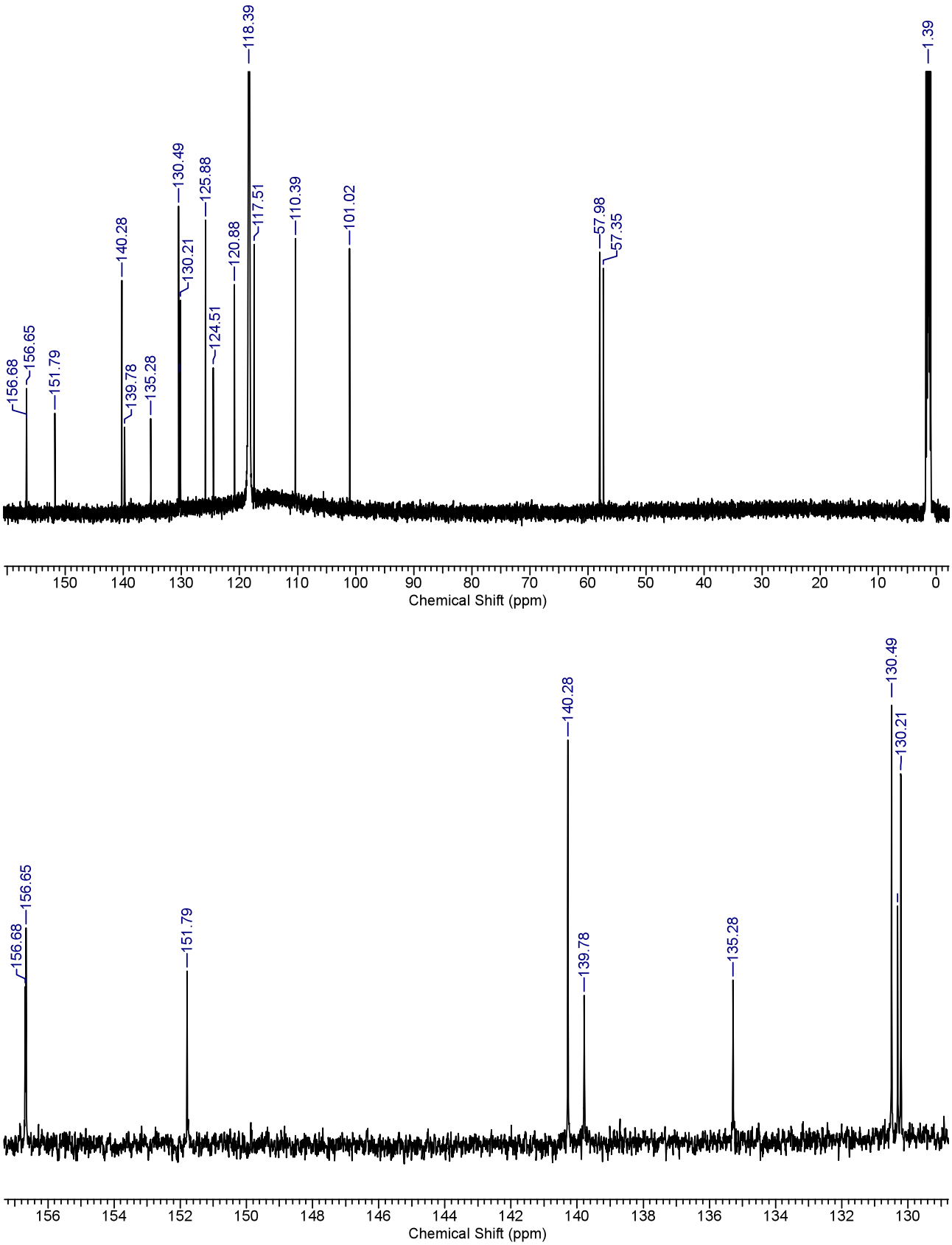


Fig. S11. ^{13}C-NMR spectrum of **2*** in CD_3CN

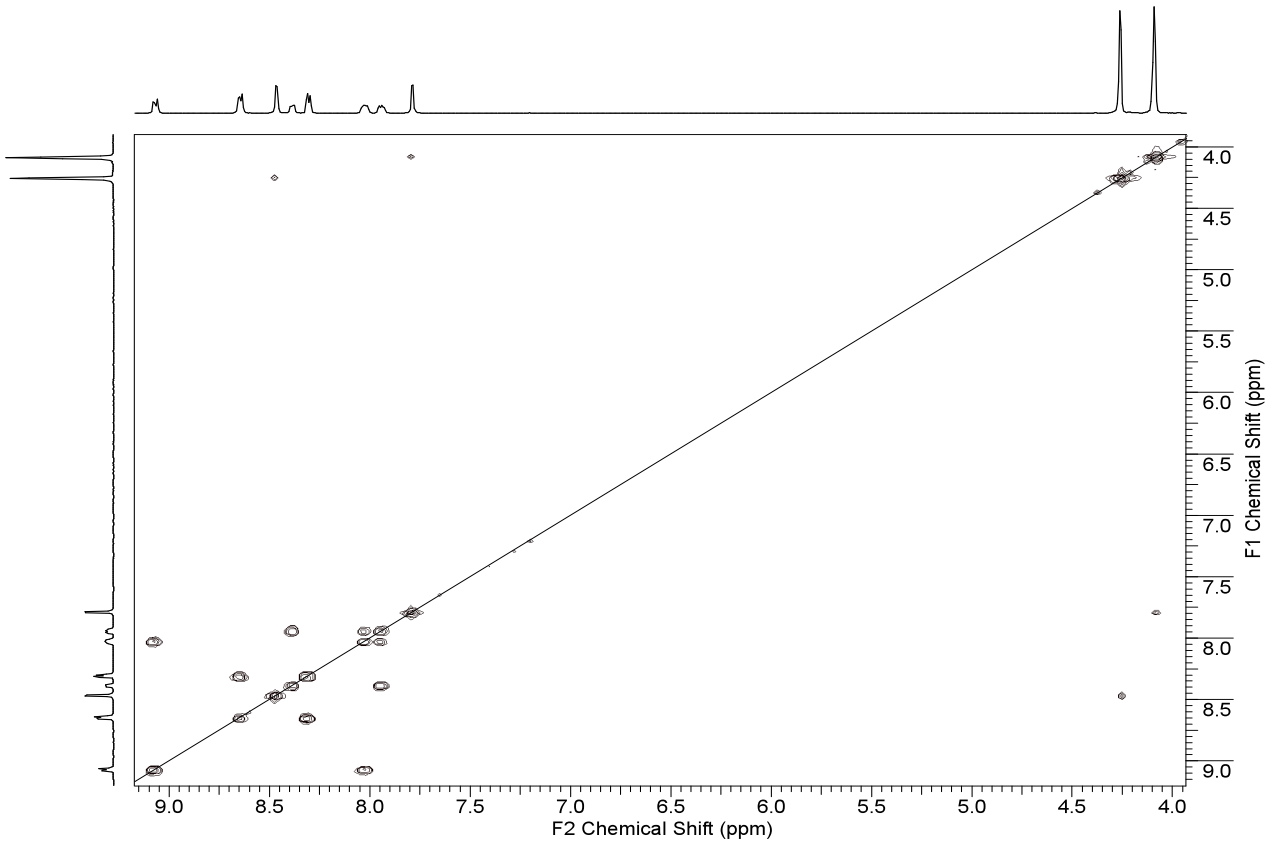


Fig. S12. {1H, 1H}-COSY spectrum of **2*** in CD_3CN

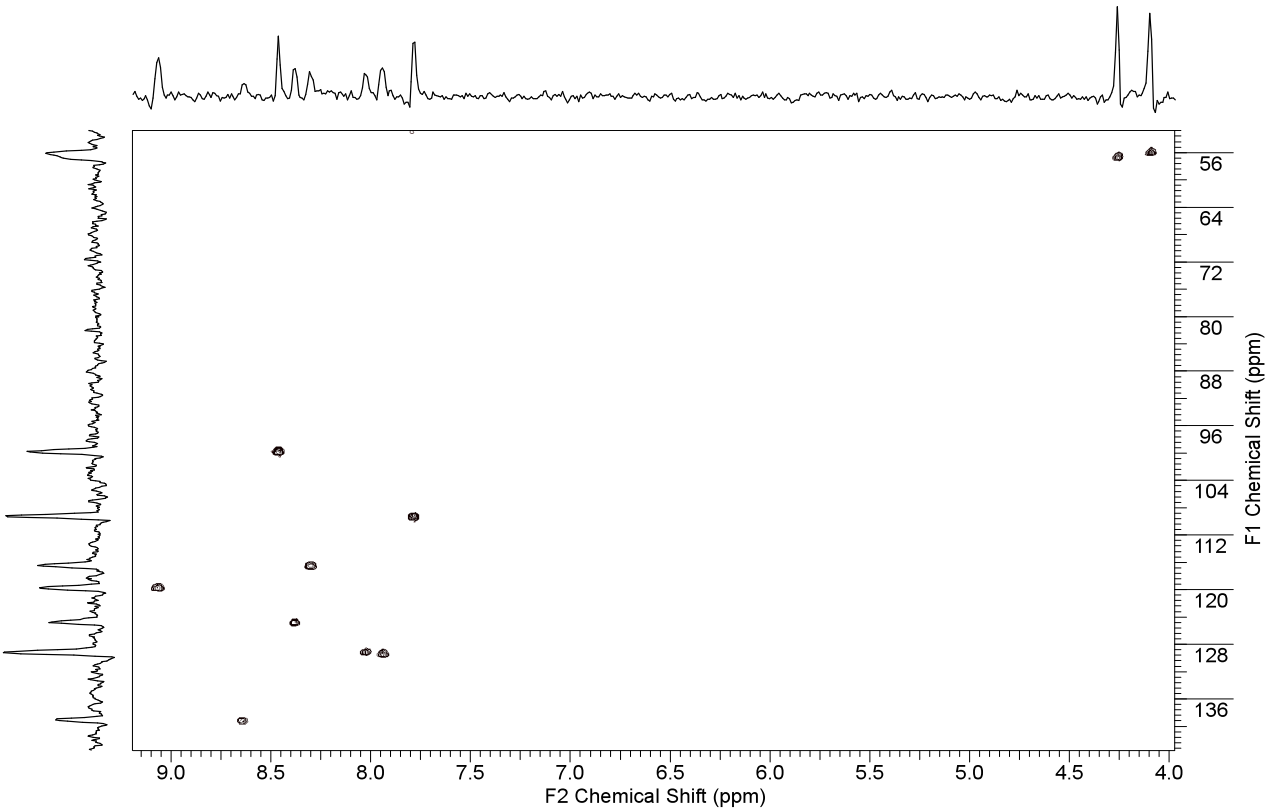


Fig. S13. HSQC spectrum of **2*** in CD_3CN

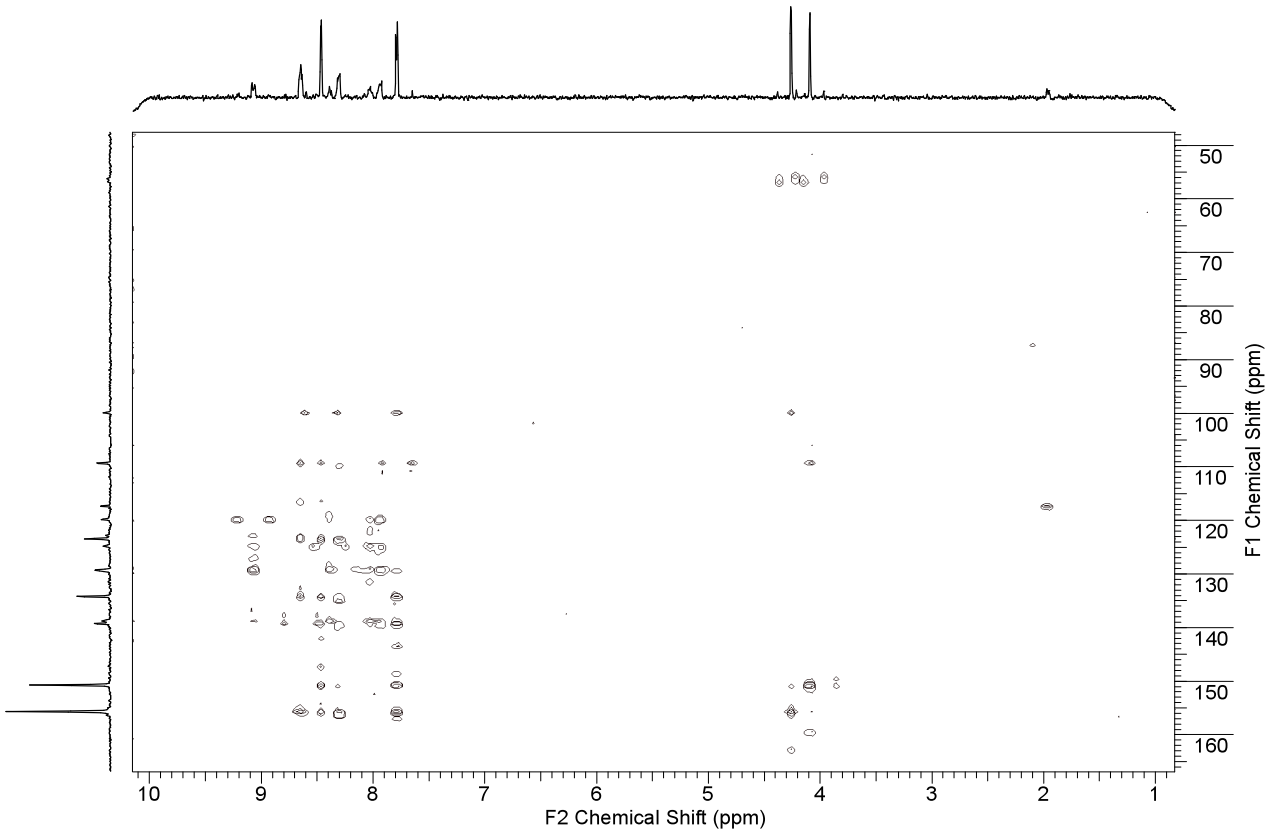


Fig. S14. HMBC spectrum of **2*** in CD_3CN

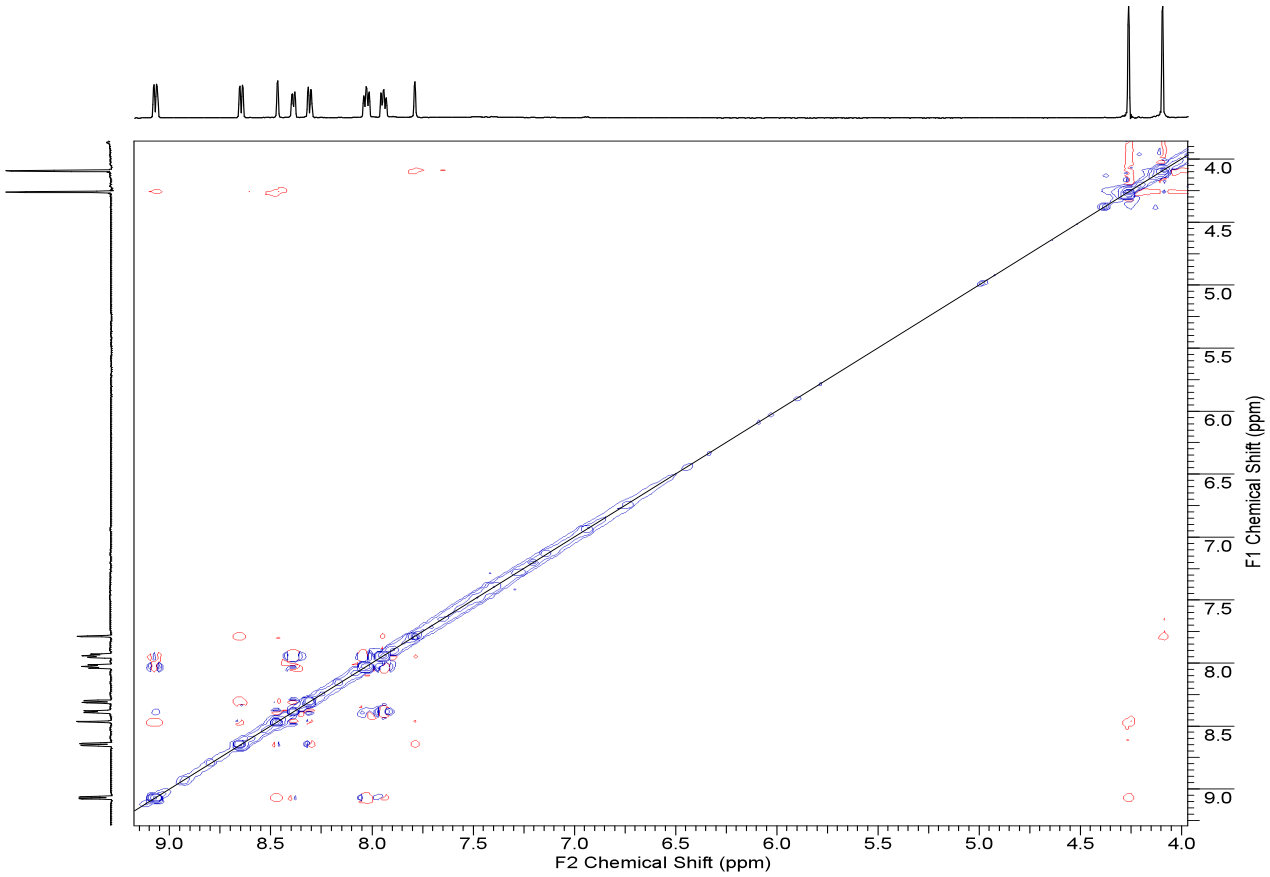


Fig. S15. NOESY spectrum of **2*** in CD_3CN

[1] O.A. Fedorova, E.N. Andryukhina, S.P. Gromov, *Synthesis* **2003**, *3*, 371-375.

[2] O.A. Fedorova, E.N. Gulakova, Yu.V. Fedorov, I.E. Lobazova, M.V. Alfimov and G. Jonusauskas, *J. Photochem. Photobiol. A: Chem.* **2008**, *196*, 239-245.

[3] G.A. Reynolds, K.H. Drexhage, *Optics Commun.* **1975**, *13*, 222-225.

[4] G. Cohen, H. Eisenberg, *Biopolymers* **1969**, *8*, 45–55.