

Selective Ratiometric Detection of Mercury(II) Ions in Water with an Acridizinium-Based Fluorescent Probe

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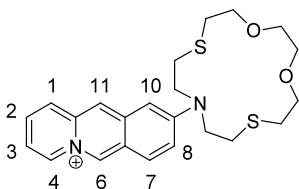
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General Instrumentations and Materials. All commercially available chemicals were reagent grade and used without further purification. The melting point were determined with a Büchi 510K melting point apparatus and are not corrected. Mass spectra (ESI in the positive-ion mode, source voltage 6 kV) were recorded with a Finnigan LCQ Deca instrument; only m/z values in the range of 100–2000 units were analyzed. NMR spectra were measured on Bruker Avance 400 (^1H : 400 MHz, ^{13}C : 100 MHz) and Varian NMR System 600 (^1H : 600 MHz, ^{13}C : 150 MHz) spectrometers at 20 °C; chemical shifts are given in ppm (δ) relative to TMS ($\delta = 0.00$ ppm). Unambiguous proton NMR assignments were established by $\{^1\text{H}, ^1\text{H}\}$ -COSY, HSQC and HMBC experiments. Elemental microanalysis of the new compound was performed with a HEKAtech EuroEA combustion analyzer by Mr. H. Bodenstedt (Organische Chemie I, Universität Siegen). TLC analyses were performed on silica gel sheets (Macherey-Nagel Polygram Sil G/UV254), eluent: $\text{CHCl}_3/\text{MeOH}/\text{AcOH}$ 90:10:1, v/v. Ion exchange resin was purchased from SERVA Electrophoresis (DOWEX[®] 1 \times 8, 50–100 mesh, pract. grade, Cl-form). Purified water with resistivity $\geq 18 \text{ M}\Omega \text{ cm}^{-1}$ was used for spectrometric measurements. 9-Fluoroacridizinium bromide, 9-aminoacridizinium chloride and 1,4-dioxa-7,13-dithia--10-aza-cyclopentadecane were prepared according to literature procedures.¹

Synthesis of 9-(1',4'-dioxo-7',13'-dithia-10'-azacyclopentadecyl)acridizinium (1c).



A solution of 9-fluoroacridizinium bromide (417 mg, 1.50 mmol) and 1,4-dioxo-7,13-dithia-10-azacyclopentadecane (754 mg, 3.00 mmol) in EtOH (5 mL) was stirred under reflux for 5 h (TLC control, CHCl₃/MeOH/AcOH 90:10:1, R_f = 0.3) and cooled to room temp. The reaction mixture was passed through a chloride-saturated ion-exchange resin. The product was obtained as orange prisms by crystallization from the solution at 4 °C. The crystals were collected and washed with ethyl acetate (EtOAc) and diethyl ether to give the product **1c** (330 mg, 47%) as chloride salt. An analytically pure sample was obtained by recrystallization from MeOH/EtOAc. M.p. 227-229 °C. ¹H-NMR (400 MHz, CD₃OD): δ = 2.82 (t, ³ J = 5 Hz, 4H, CH₂), 3.02 (t, ³ J = 8 Hz, 4H, CH₂), 3.67 (s, 4H, CH₂), 3.80 (t, ³ J = 5 Hz, 4H, CH₂), 4.00 (t, ³ J = 8 Hz, 4H, CH₂), 7.05 (d, ⁴ J = 2 Hz, 1H, CH_{ar}, H-10), 7.37-7.43 (m, 1H, CH_{ar}, H-3), 7.60-7.71 (m, 2H, CH_{ar}, H-2, H-8), 8.02 (dd, ³ J = 9 Hz, ⁴ J = 1 Hz, 1H, CH_{ar}, H-1), 8.19 (d, ³ J = 10 Hz, 1H, CH_{ar}, H-7), 8.32 (s, 1H, CH_{ar}, H-11), 8.71 (d, ³ J = 7 Hz, 1H, CH_{ar}, H-4), 9.60 (s, 1H, CH_{ar}, H-6); ¹³C-NMR (100 MHz, CD₃OD): δ = 30.7 (2CH₂), 32.9 (2CH₂), 53.5 (2CH₂), 71.7 (2CH₂), 75.0 (2CH₂), 101.7 (CH_{ar}), 119.4 (CH_{ar}), 120.0 (CH_{ar}), 122.1 (C_q), 123.5 (CH_{ar}), 126.8 (CH_{ar}), 130.5 (CH_{ar}), 131.4 (CH_{ar}), 134.3 (CH_{ar}), 139.0 (CH_{ar}), 139.3 (C_q), 140.0 (C_q), 153.3 (C_q); MS (ESI⁺): m/z (%) = 429 [M]⁺; El. Anal. calcd. (%) for C₂₃H₂₉ClN₂O₂S₂ × H₂O (483.09): C, 57.18; H, 6.47; N, 5.80; S, 13.28. Found C, 57.02; H, 6.54; N, 5.81; S, 12.85. The corresponding perchlorate salt was obtained by the addition of a saturated aqueous solution of NaClO₄ (5 mL) to the aqueous solution of the chloride salt, and subsequent recrystallization of the precipitate from CH₃CN/EtOAc: Yellow prisms; m.p. 177-179 °C; El. Anal. calcd. (%) for C₂₃H₂₉ClN₂O₂S₂ (529.07): C, 52.21; H, 5.52; N, 5.29; S, 12.12. Found C, 52.19; H, 5.56; N, 5.22; S, 11.79.

Spectrophotometric measurements. Absorption spectra were recorded on a Varian Cary 100 double-beam spectrophotometer; emission spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. All spectrophotometric measurements were performed in thermostated quartz sample cells at 20 °C. Solutions for analysis were prepared by dilution of stock solutions (1.0×10^{-3} M in water) immediately before the experiments. The solution concentrations were 50 μ M for absorption spectroscopy and 10 μ M for fluorescence spectroscopy. Spectrophotometer slit widths were kept 2 nm for absorption spectroscopy and 5/5 nm for emission spectroscopy. The aliquots of Hg(OAc)₂ solution containing the appropriate concentration of compound **1c** (to avoid dilution effects) were added to a cuvette containing a constant amount of compound **1c** in water and analyzed photometrically. The titration was continued until no further changes in the spectrum were observed. Spectrofluorimetric titrations were performed just as described for the spectrophotometric titrations with $c(\mathbf{1c}) = 10 \mu\text{M}$. In control experiments with other metal cations the concentration of the ligand **1c** and of the metals was $c = 10 \mu\text{M}$. The excitation wavelength corresponded to the isosbestic point determined from the UV/Vis titrations.

For control experiments, all solutions of metal salts were prepared by dilution of freshly prepared stock solutions (1.0×10^{-2} M in water) to the appropriate concentrations. The salts employed were Ca(ClO₄)₂, Cd(ClO₄)₂, Zn(ClO₄)₂, Ni(ClO₄)₂, Cr(ClO₄)₃, Cu(OAc)₂, Pb(NO₃)₂, Co(ClO₄)₂, Fe(ClO₄)₃, and AgClO₄.

All spectrophotometric measurements were performed at least three times to ensure reproducibility.

Table S1. Absorption and Emission Properties of **1c** in Water and Acetonitrile

Solvent	λ_{abs}^a	$\lg \varepsilon^b$	λ_{fl}^c	ϕ_{fl}^d
H ₂ O	399	4.29	523	0.22
MeCN	402	4.33	531	0.27

^aLong-wavelength absorption maximum, in nm; $c = 50 \mu\text{M}$; ^bextinction coefficient, $\text{cm}^{-1}\text{M}^{-1}$; ^cfluorescent emission maximum, $c = 10 \mu\text{M}$; excitation wavelength $\lambda_{\text{ex}} = 390$ nm; ^dfluorescence quantum yield relative to Coumarin 153, estimated error $\pm 10\%$.

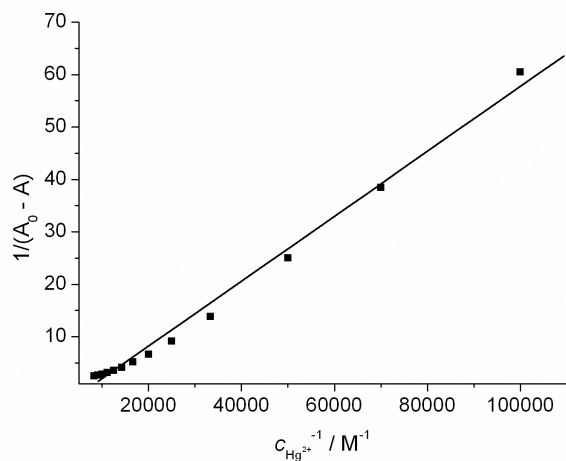


Figure S1 Benesi-Hildebrand plot from photometric titration of Hg^{2+} to **1c** ($R^2 = 0.990$). The resulting association constant is $1.5 \times 10^4 \text{ M}^{-1}$. Another independent calculation by computer program SPECFIT gave the association constant of $1.6 \times 10^4 \text{ M}^{-1.2}$.

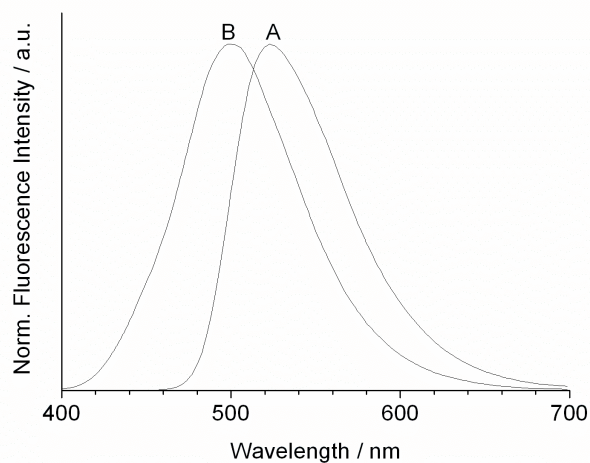


Figure S2 Normalized fluorescence of **1c** absence (A) and presence (B) of 10 equiv. $\text{Hg}(\text{OAc})_2$; $c(\mathbf{1c}) = 10 \mu\text{M}$, $\lambda_{\text{ex}} = 388 \text{ nm}$.

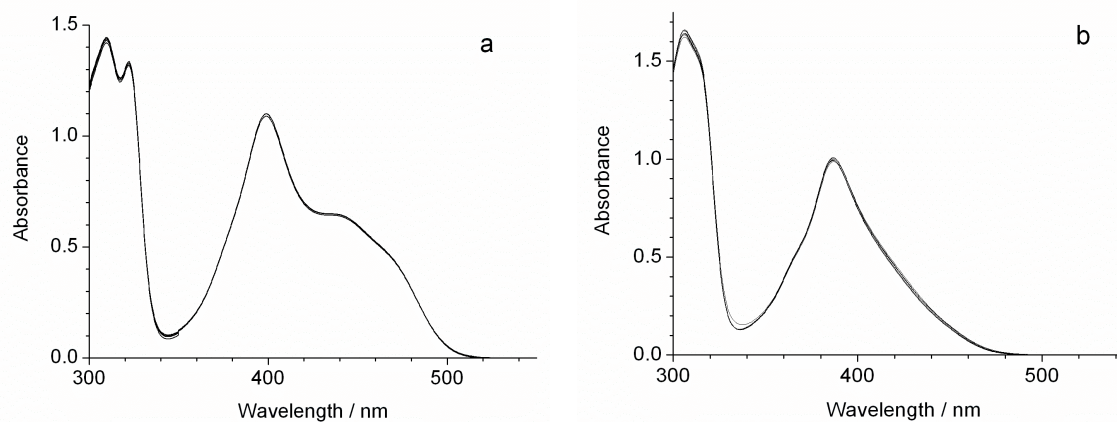


Figure S3. a) Absorption spectra of free **1c** ($50 \mu\text{M}$) with different pH (7.1, 5.2, 3.3, 1.6 and 1.1) in Britton-Robinson buffer;³ b) absorption spectra of **1c** ($50 \mu\text{M}$) in the presence of Hg^{2+} (0.1 mM) with different pH (6.4, 5.9, 5.5, 5.0, 4.5, 4.1, 3.6 and 3.1) in acetate buffer (50 mM).

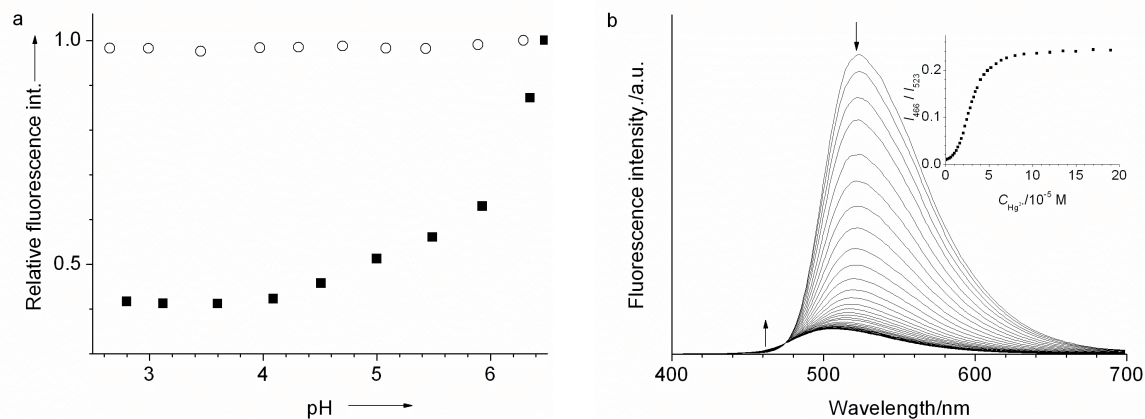


Figure S4 a) The relative fluorescence intensity of compound **1c** (10^{-5} M) in the absence (\circ) and in the presence (\blacksquare) of Hg^{2+} (0.1 mM) at different pH values in acetate buffer (50 mM); $\lambda_{\text{ex}} = 388$ nm, $\lambda_{\text{em}} = 523$ nm ; b) Spectrofluorimetric titration of an aqueous solution of $\text{Hg}(\text{OAc})_2$ to compound **1c** at pH 4.0 (acetate buffer 50 mM, $\lambda_{\text{ex}} = 388$ nm; $c = 10$ μM). Arrows on top of the spectra indicate the development of the emission band upon addition of $\text{Hg}(\text{OAc})_2$. Inset: the ratiometric analysis of I_{466}/I_{523} vs $C_{\text{Hg}^{2+}}$

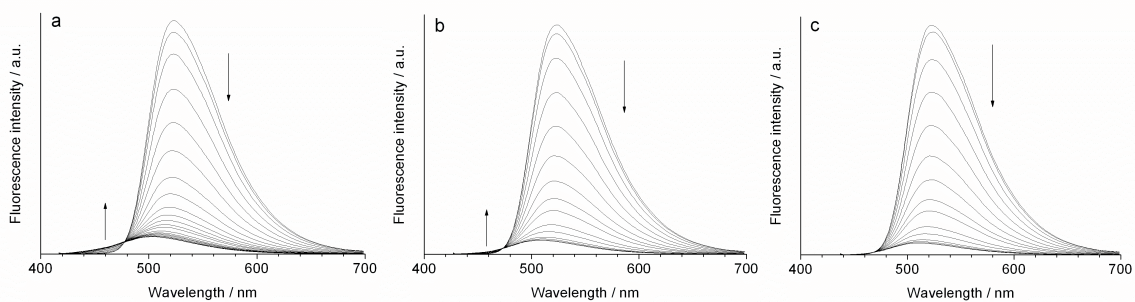


Figure S5. Spectrofluorimetric titrations of an aqueous solution of $\text{Hg}(\text{OAc})_2$ to compound **1c** ($c = 10$ μM) with different excitation wavelength (a: $\lambda_{\text{ex}} = 410$ nm; b: $\lambda_{\text{ex}} = 420$ nm; c: $\lambda_{\text{ex}} = 430$ nm); arrows indicate the changes of the fluorescence intensity upon addition of $\text{Hg}(\text{OAc})_2$.

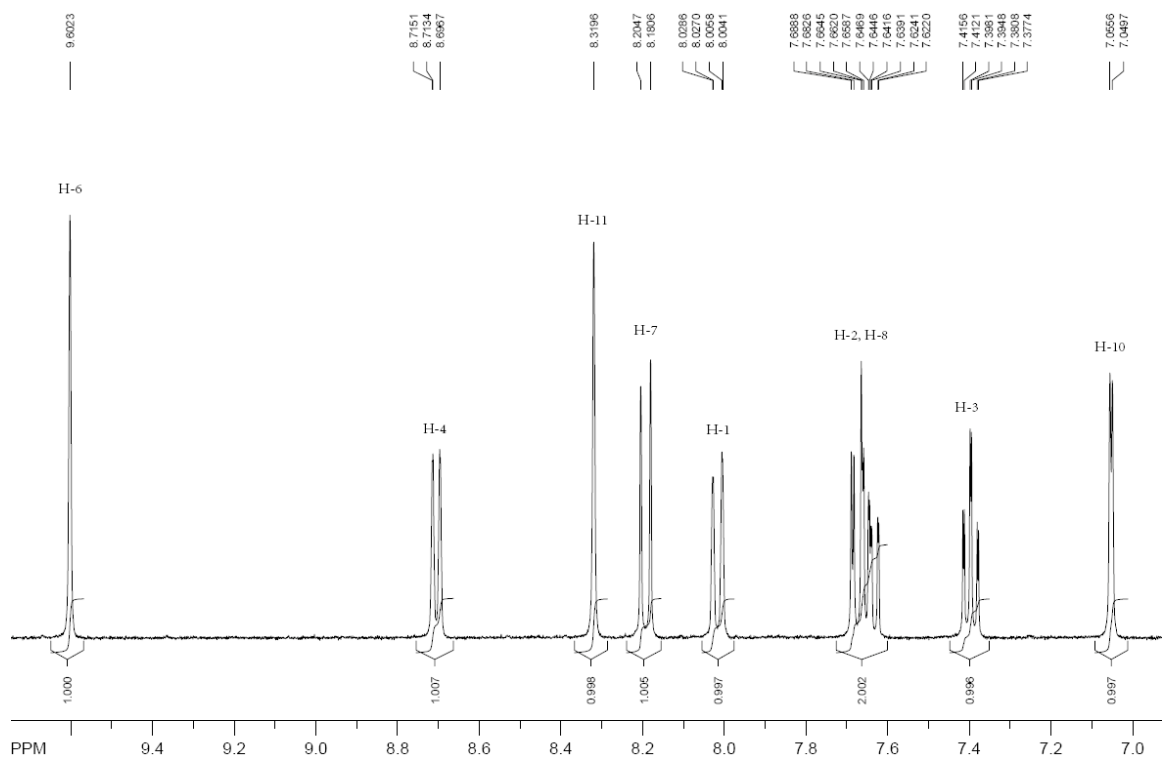
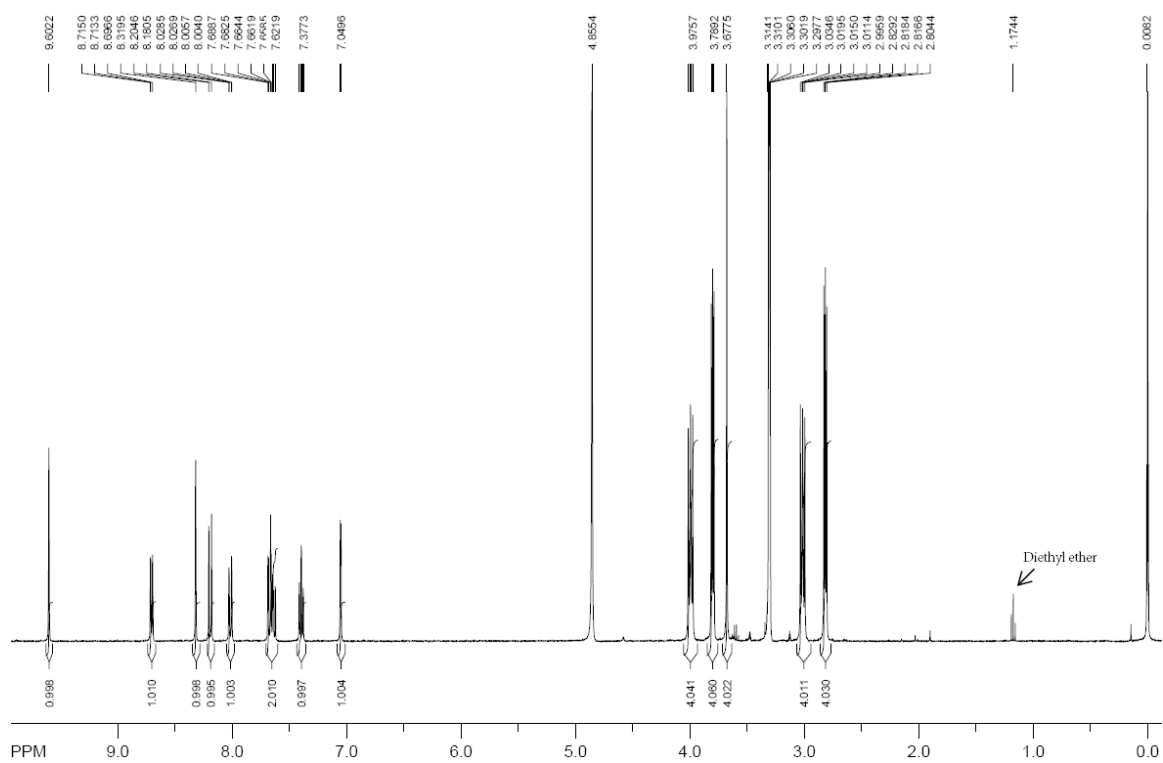


Figure S6. ^1H -NMR spectrum of **1c** in MeOD

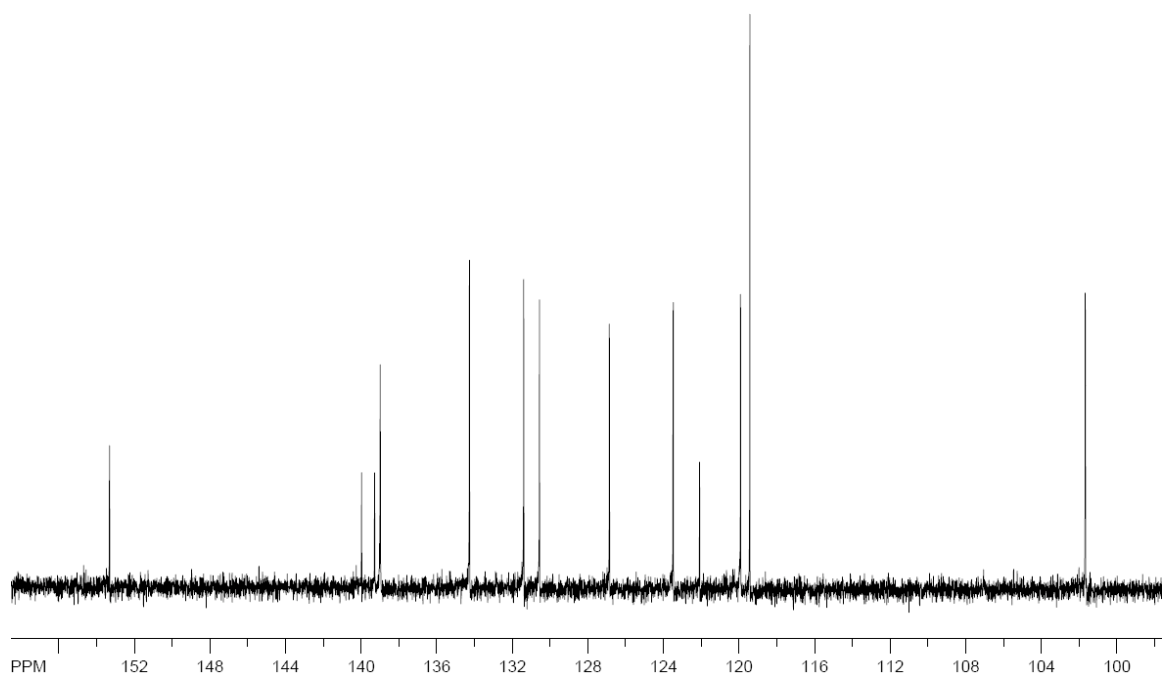
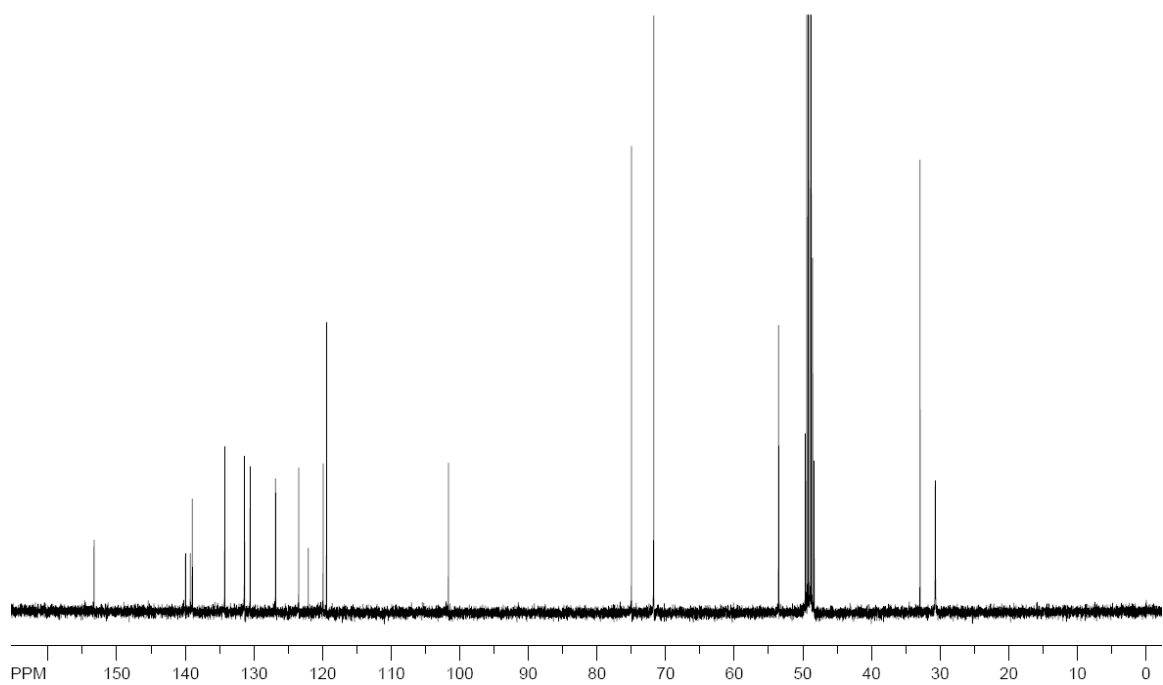


Figure S7. ^{13}C -NMR spectrum of **1c** in MeOD

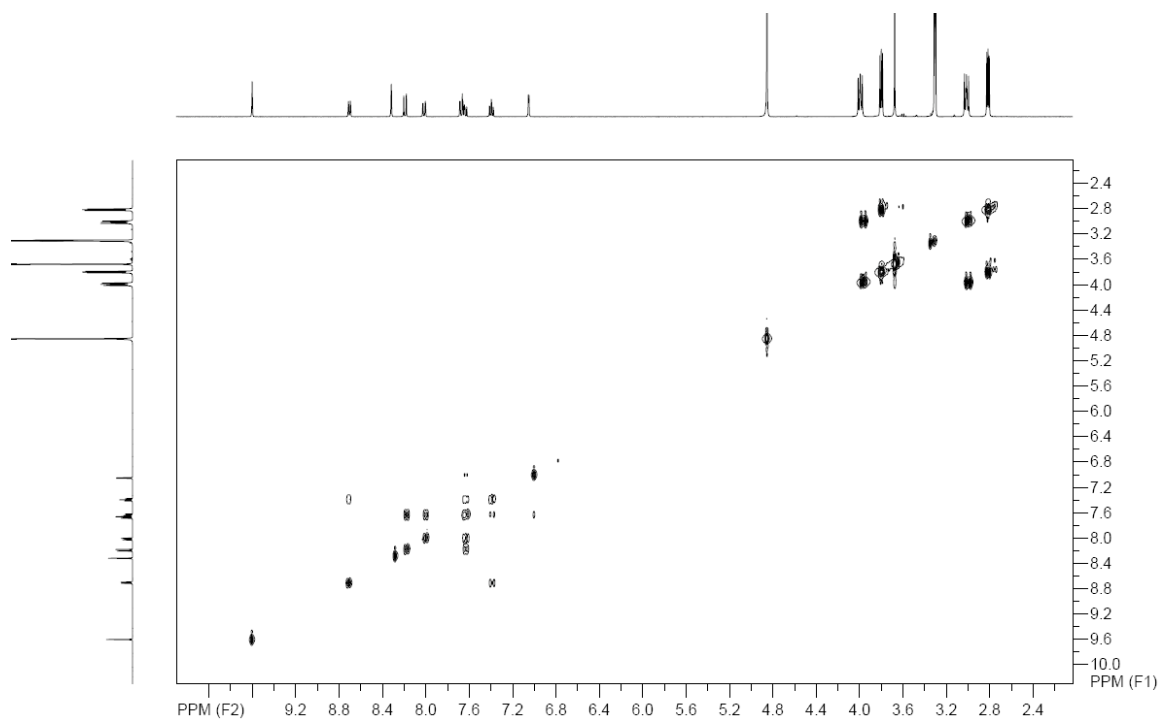


Figure S8. $\{^1\text{H}, ^1\text{H}\}$ -COSY spectrum of **1c** in MeOD

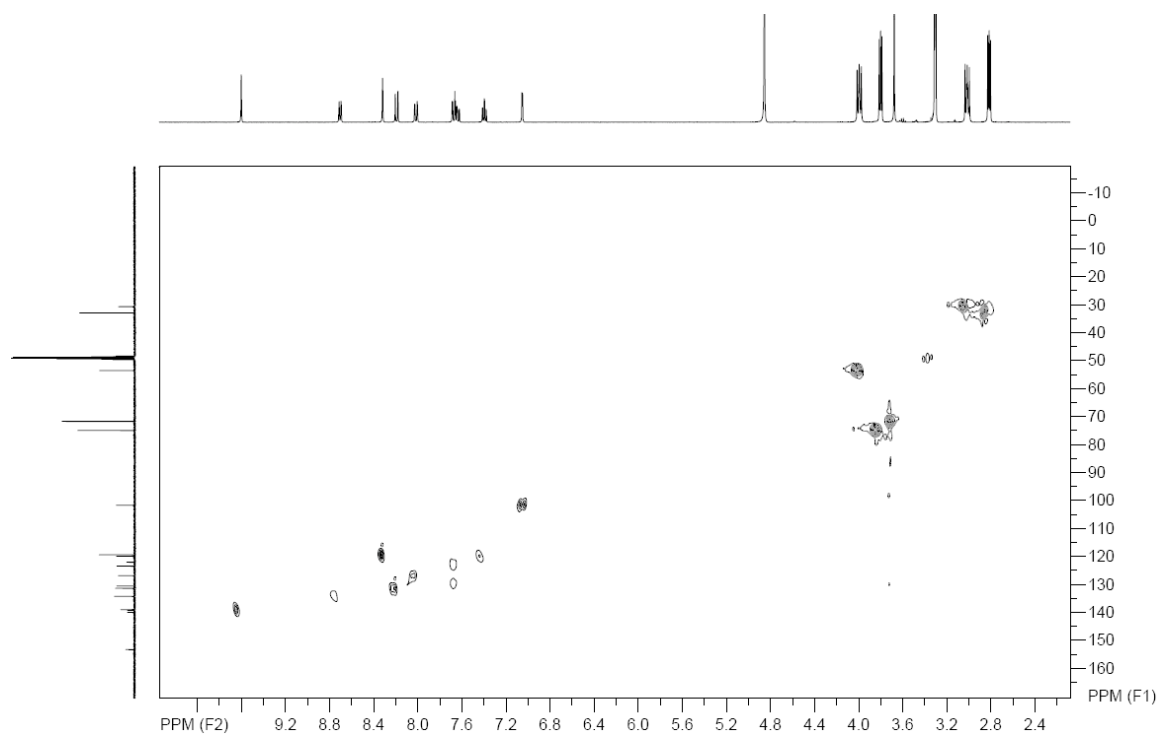


Figure S9. HSQC spectrum of **1c** in MeOD

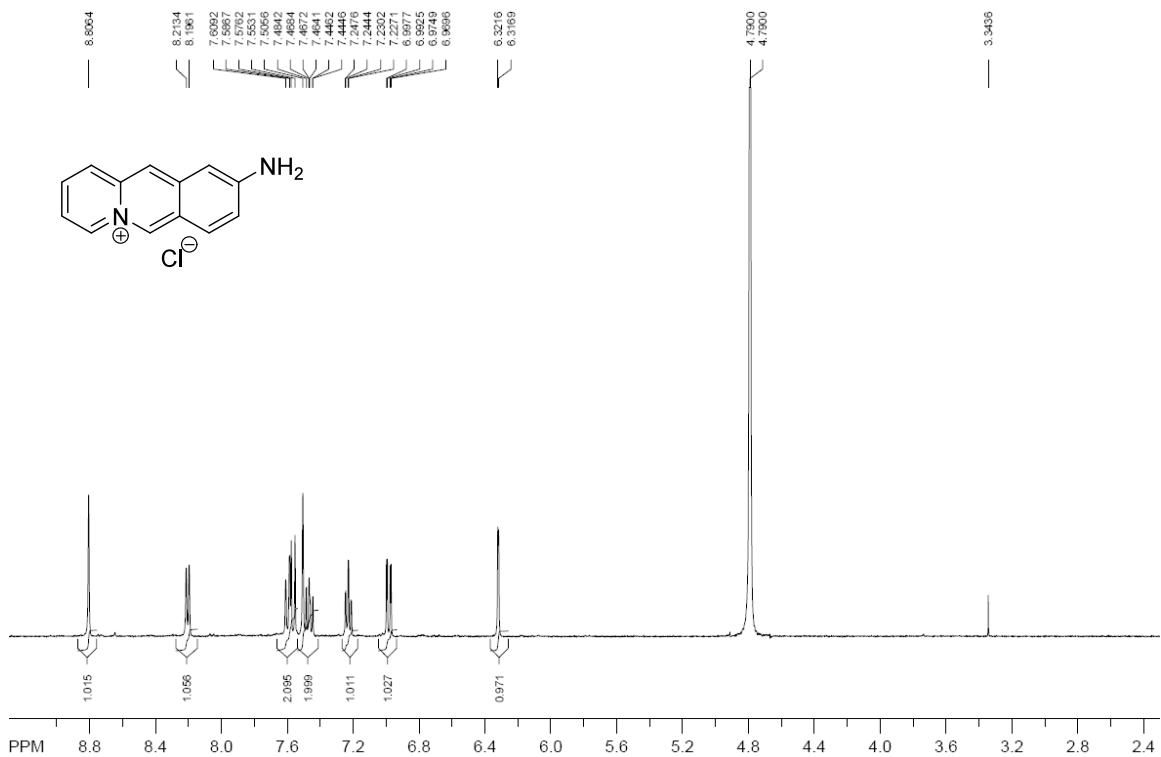


Figure S10. ¹H-NMR spectrum of 9-aminoacridizinium chloride in D₂O

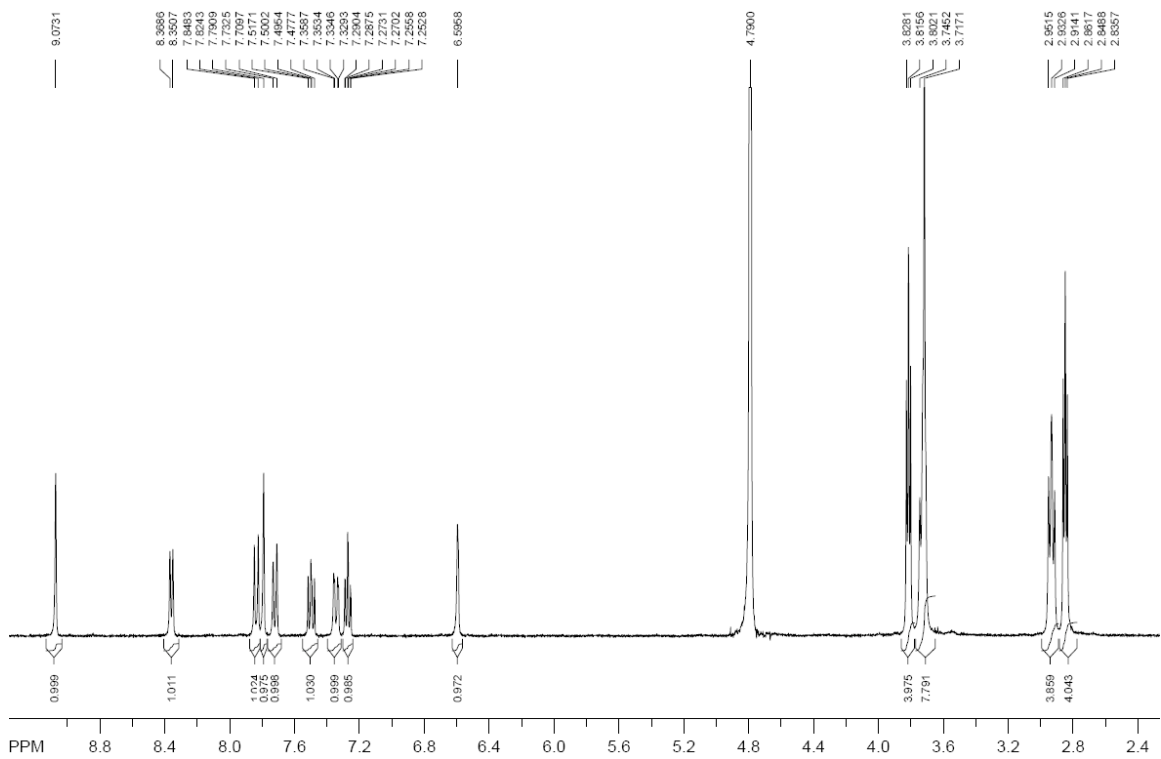


Figure S11 ¹H-NMR spectrum of 1c in D₂O

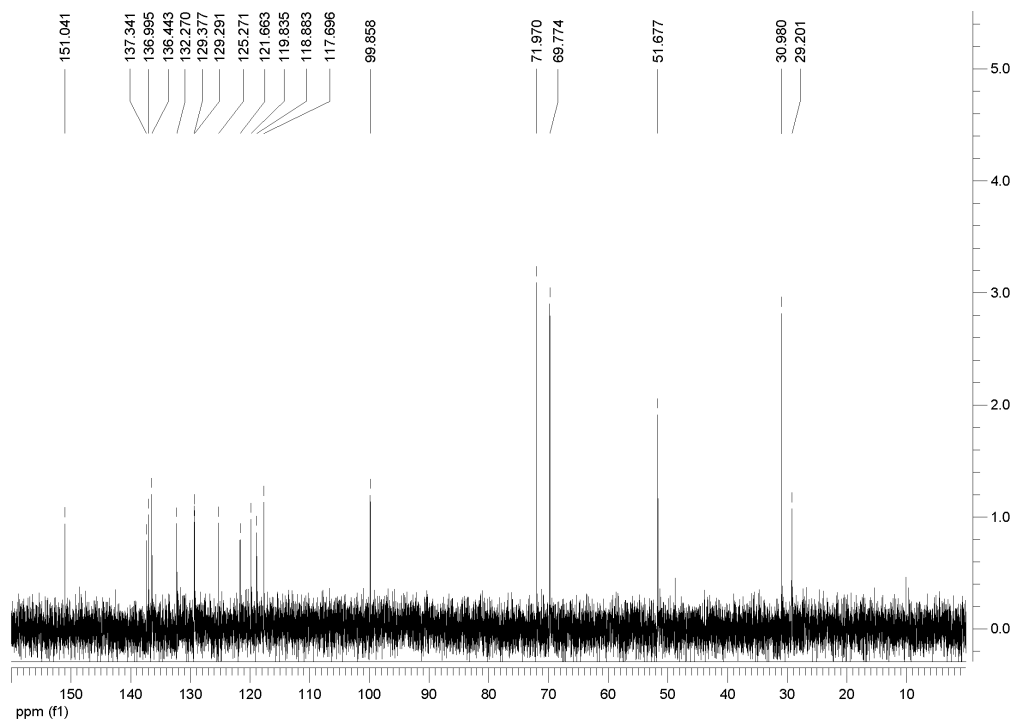


Figure S12. ^{13}C -NMR spectrum of **1c** in D_2O

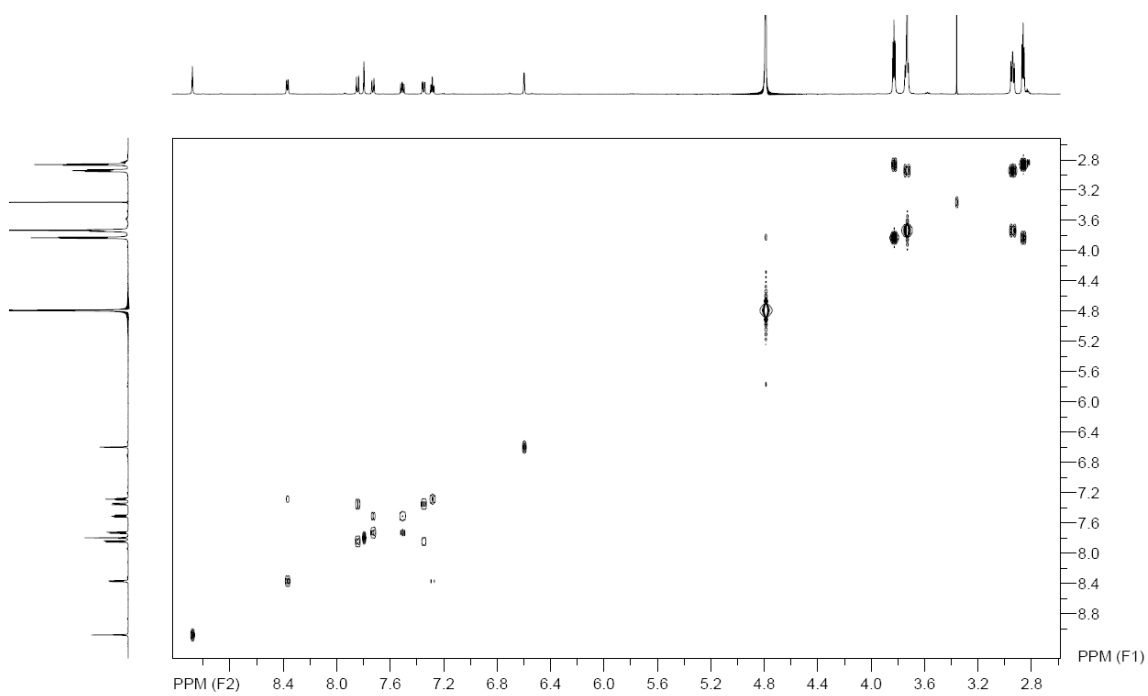


Figure S13. $\{^1\text{H}, ^1\text{H}\}$ -COSY spectrum of **1c** in D_2O

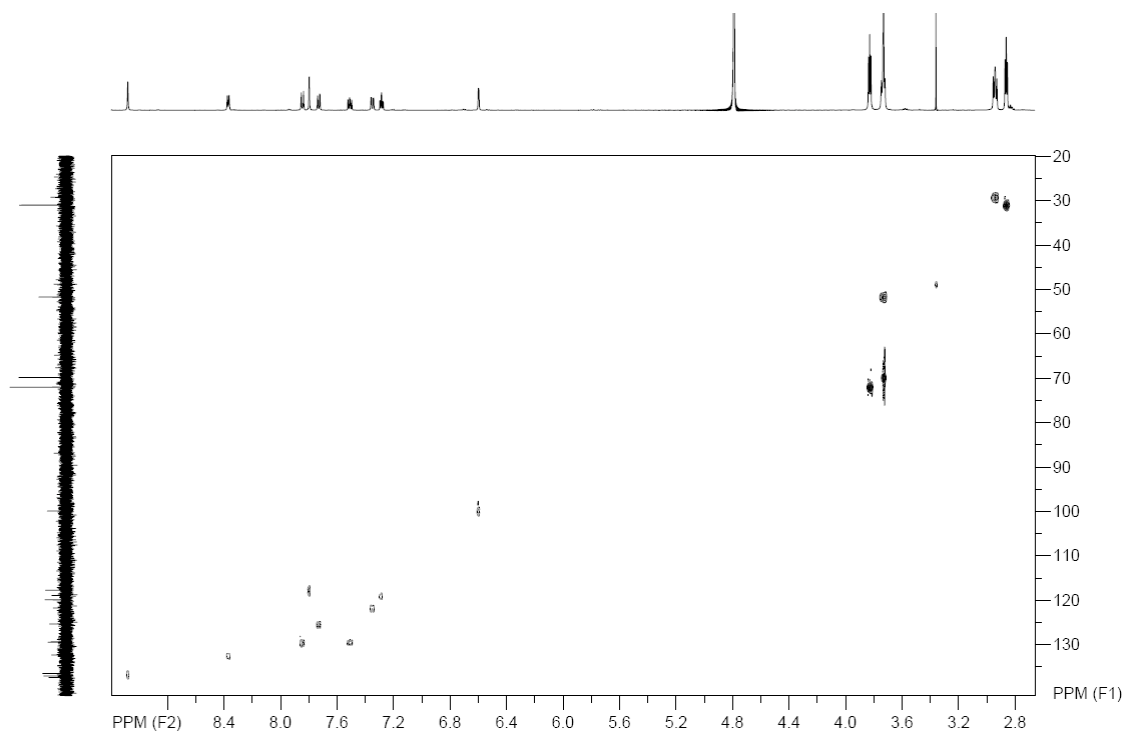


Figure S14. HSQC spectrum **1c** in D₂O

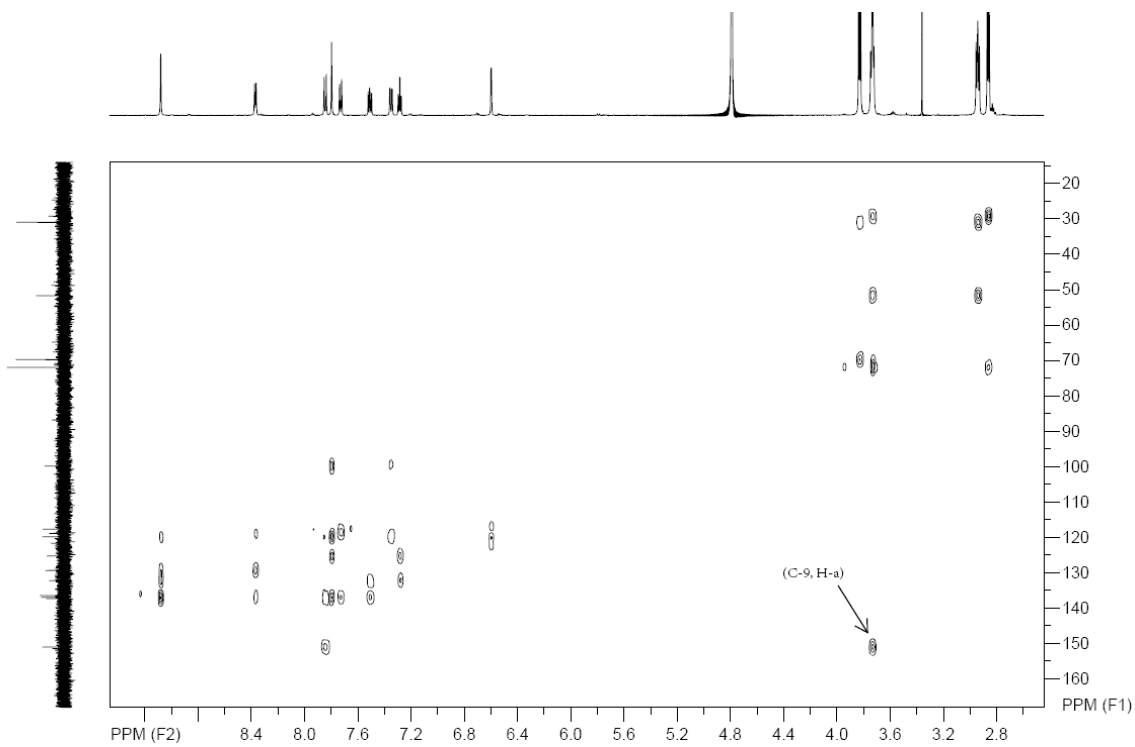


Figure S15. HMBC spectrum **1c** in D₂O

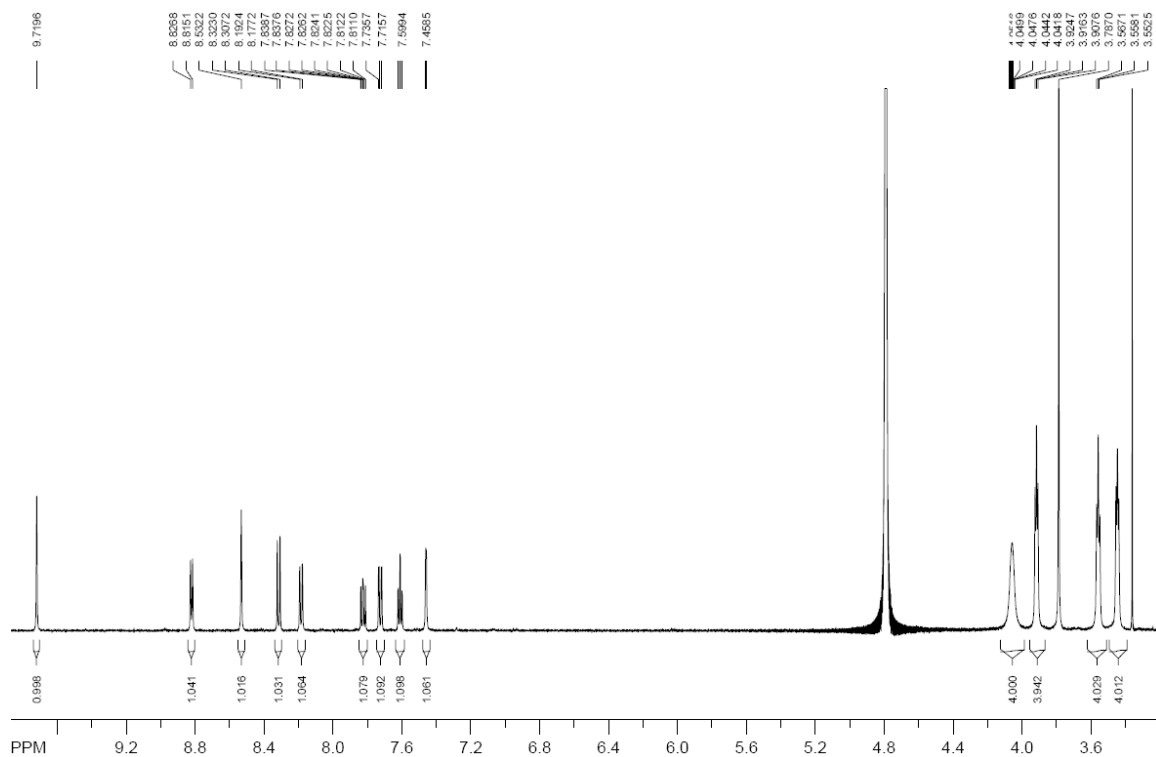


Figure S16. ^1H -NMR spectrum of **1c** + 1 equiv. $\text{Hg}(\text{OAc})$ in D_2O

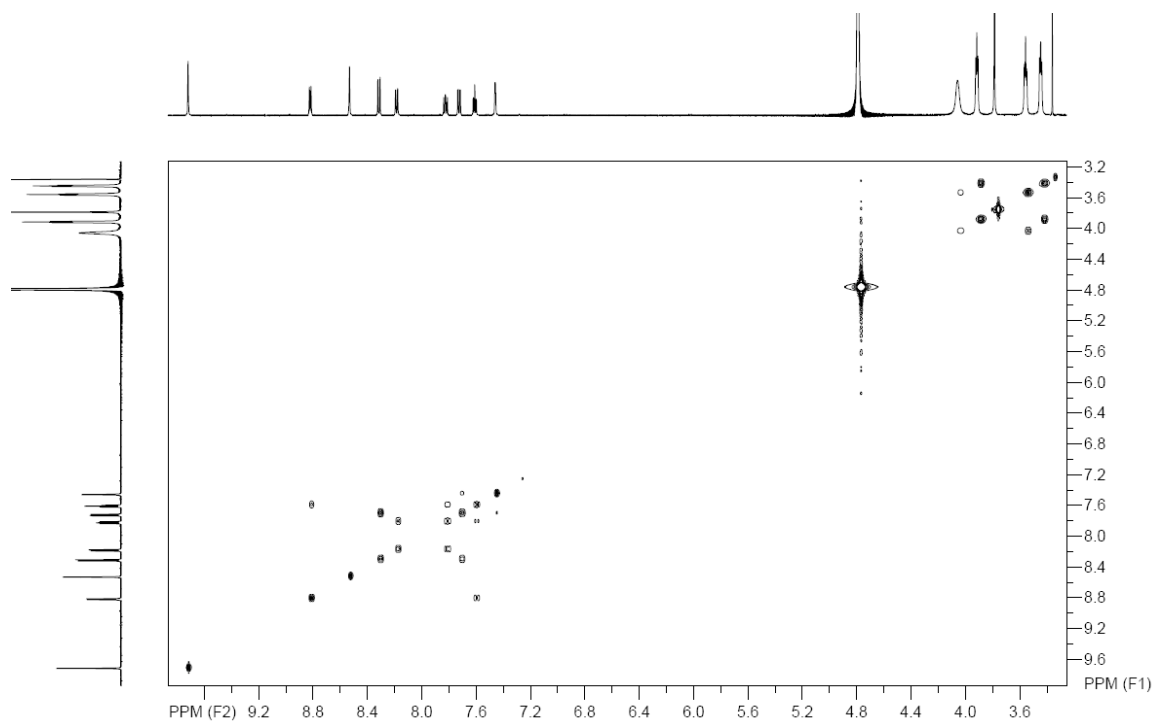


Figure S17. $\{^1\text{H}, ^1\text{H}\}$ -COSY spectrum of **1c** + 1 equiv. $\text{Hg}(\text{OAc})$ in D_2O

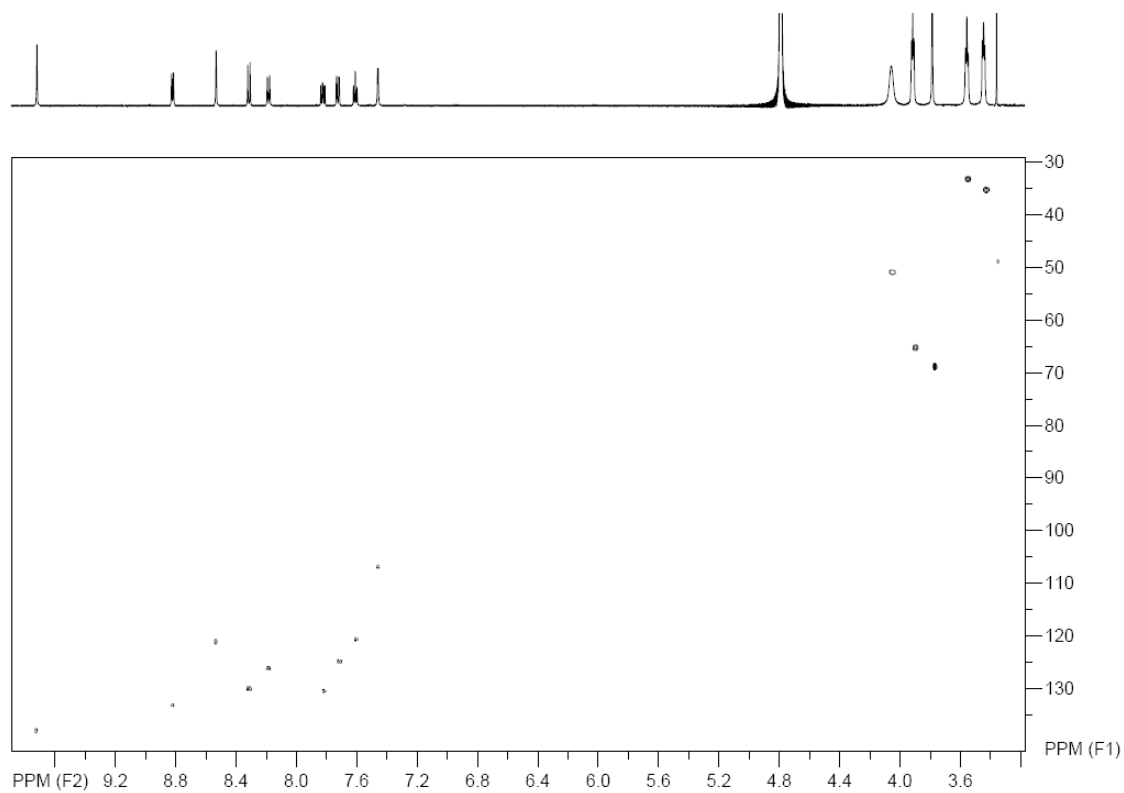


Figure S18. HSQC spectrum of **1c** + 1 equiv. Hg(OAc) in D₂O

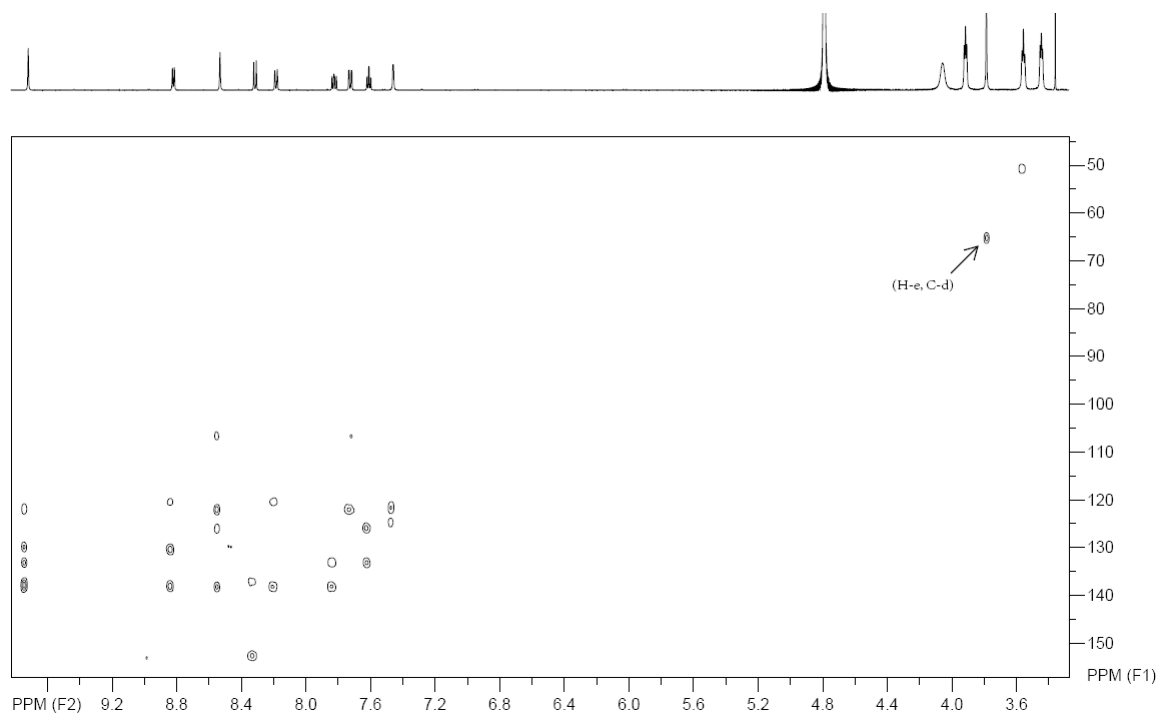


Figure S19. HMBC spectrum of **1c** + 1 equiv. Hg(OAc) in D₂O

¹ a) C. K. Bradsher, J. P. Sherer, J. H. Parham, *J. Chem. Eng. Data* **1965**, *10*, 180; b) H. Ihmels, B. Engels, K. Faulhaber, C. Lennartz, *Chem. Eur. J.* **2000**, *6*, 2854; c) M. Tanaka, M. Nakamura, T. Ikeda, K. Ikeda, H. Ando, Y. Shibutani, S. Yajima, K. Kimura, *J. Org. Chem.* **2001**, *66*, 7008.

² Association constant was obtained using the computer program SPECFIT, Global Analysis system, Version 3.0.32

³ Britton, H. T. S.; Robinson, R. A. *J. Chem. Soc.* **1931**, 458.