Selective Detection of Hg^{2+} in the Microenvironment of Double-Stranded DNA with an Intercalator Crown-Ether Conjugate

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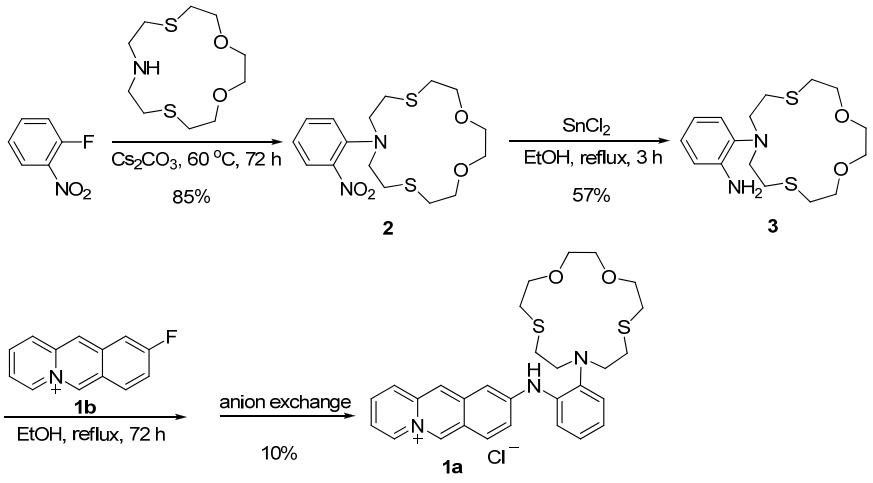
Electronic Supplementary Information (ESI)

General Instrumentations and Materials

All commercially available chemicals were reagent grade and used without further purification. Culf thymus (ct) DNA was purchased from Aldrich and yeast RNA was purchased from Acros Organics. The melting points 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 (^{1}H: 400 MHz, ^{13}C: 100 MHz) spectrometer at 20 °C; chemical shifts are given in ppm (δ) relative to TMS (δ = 0.00 ppm). Unambiguous proton NMR assignments were established by {1H, 1H}-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: $CHCl_3$/MeOH 9:1, v/v. Purified water with resistivity ≥ 18 MΩ cm^{-1} was used for spectrometric measurements. 9-fluorobenzo[*b*]quinolizinium bromide **1b** and 1,4-dioxa-7,13-dithia-10-azacyclopentadecane were prepared according to literature procedures.[1,2]

Synthesis



***N*-(2-Nitrophenyl)-1,4-dioxa-7,13-dithia-10-azacyclopentadecane (2)**: A suspension of 2-fluoronitrobenzene (4.12 g, 29.2 mmol), Cs_2CO_3 (2.98 g, 9.14 mmol) and 1,4-dioxa-7,13-dithia-10-azacyclopentadecane (0.96 g, 3.80 mmol) was stirred at 60 °C under nitrogen atmosphere for 72 h. After cooling the reaction mixture to room temperature, water (100 mL) was added and the aqueous phase was extracted with chloroform (3 × 100 mL). The combined organic layers were washed with brine (100 mL) and dried over $MgSO_4$. After evaporation of the solvent in vacuo, the product was islated by column chromatography (SiO_2, dichloromethane/hexane 1:1, R_f: 0.5). Evaporation of the eluent gave the product (1.31 g, 92%) as orange-colored oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 2.72 (t, ^3J = 5 Hz, 4 H, CH_2), 2.84-2.88 (m, 4 H, CH_2), 3.39-3.41 (m, 4H, CH_2), 3.67 (s, 4 H, CH_2), 3.77 (t, ^3J = 5 Hz, 4 H, CH_2), 6.95-6.99 (m, 1 H, CH_{ar}), 7.18 (dd, ^4J = 1 Hz, ^4J = 1 Hz, 1 H, CH_{ar}), 7.39-7.44 (m, 1 H, CH_{ar}), 7.67 (dd, ^4J = 1 Hz, ^4J = 1 Hz, 1 H, CH_{ar}); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 30.5 (2 CH_2), 31.8 (2 CH_2), 53.6 (2 CH_2), 70.9 (2 CH_2), 74.1 (2 CH_2), 121.2 (CH_{ar}), 123.0 (CH_{ar}), 125.9 (CH_{ar}), 133.0 (CH_{ar}), 143.6 (C_q), 144.4 (C_q); MS (ESI^+): *m/z* (%) 165.3 (100) [*M*-$C_8H_{16}O_2S_2]^+$, 373.6 (69) [*M*+H]$^+$; El. Anal. calcd. (%) for $C_{16}H_{24}N_2O_4S_2$ (372.12): C, 51.59; H, 6.49; N, 7.52; S, 17.22. Found: C, 51.30; H, 6.54; N, 7.66; S, 17.22.

***N*-(2-Aminophenyl)-1,4-dioxa-7,13-dithia-10-azacyclopentadecane (3)**: A suspension of *N*-(2-nitrophenyl)-1,4-dioxa-7,13-dithia-10-azacyclopentadecane (1.20 g, 3.22 mmol) and tinchloride dihydrate (4.28 g, 19.0 mmol) in ethanol (40 mL) was stirred under nitrogen atmosphere at 90 °C for 3 h. After cooling the reaction mixture to room temperature the solvent was removed in vacuo and ethylacetate (150 mL) was added. An aqueous solution of Na_2CO_3 (5%) was added to ajust the pH value to 8. The aqueous solution was extracted with ethylacetate (3 x 100 mL) and the combined organic layers were washed with brine (100 mL) and dried with $MgSO_4$. The solvent was removed in vacuo and crystallization from ethylacetate/hexane gave the product (0.65 g, 57%) as orange-colored needles; m.p. 101-103 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 2.75-2.81 (m, 8 H, CH_2), 3.18-3.22 (m, 4 H, CH_2), 3.69 (s, 4 H, CH_2), 3.79 (t, ^3J = 6 Hz, 4 H, CH_2), 4.10-4.32 (br s, 2 H, NH_2), 6.69-6.72 (m, 2 H, CH_{ar}), 6.91-6.95 (m, 1 H, CH_{ar}), 7.04 (dd, ^4J = 1Hz, ^4J = 1Hz, 1 H, CH_{ar}). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 31.0 (2 CH_2), 32.0 (2 CH_2), 53.8 (2 CH_2), 71.1 (2 CH_2), 73.3 (2 CH_2), 115.4 (CH_{ar}), 118.2 (CH_{ar}), 123.1 (CH_{ar}), 125.5 (CH_{ar}), 137.1 (C_q), 143.8 (C_q); MS (ESI^+): *m/z* (%) 365.7 (100) [*M*+Na]$^+$, 343.6 (60) [*M*+H]$^+$; El. Anal. calcd. (%) for $C_{16}H_{26}N_2O_2S_2$ (342.14): C, 56.11; H, 7.65; N, 8.18; S, 18.72. Found: C, 56.27; H, 7.83; N, 8.14; S, 18.89.

9-[2-(1,4-Dioxa-7,13-dithia-10-azacyclopentadecyl)phenyl]aminobenzo[*b*]quinolizinium chloride (1a): A solution of 9-fluorobenzo[*b*]quinolizinium bromide (487 mg, 1.75 mmol) and *N*-(2-aminophenyl)-1,4-dioxa-7,13-dithia-10-azacyclopentadecane (598 mg, 1.75 mmol) in ethanol (5 mL) was stirred under reflux for 72 h under nitrogen atmosphere. After cooling the reaction mixture to room temperature, the reaction mixture was passed through an ion exchange resin (DOWEX®1×8 Cl^-). The product was purified by a column chromatography (SiO_2, $CHCl_3$/MeOH 10:1, R_f: 0.4) and subsequent recrystallization from chloroform/ethylacetate gave **1a (**116 mg, 10%) as orange-red needles; m.p. 240-241°C. It's demonstrated by ^{1}H-NMR spectrometric and elemental analysis data that the crystal contained one equivalent of chloroform molecule.; ^{1}H-NMR (400 MHz, MeOD): δ = 2.68-2.75 (m, 8 H, CH_2), 3.25-3.28 (m, 4 H, CH_2), 3.64 (s, 4 H, CH_2), 3.72 (t, ^3J = 5 Hz, 4 H, CH_2), 7.19-7.24 (m, 2 H, CH_{ar}, Ph-H), 7.32-7.34 (m, 1 H, CH_{ar}, Ph-H), 7.41 (d, ^4J = 2 Hz, 1 H, 10-H), 7.45-7.47 (m, 1 H, CH_{ar}, 3-H), 7.56-7.58 (m, 1 H, CH_{ar}, Ph-H), 7.67-7.78 (m, 2 H, 2-H, H-8), 8.06 (d, ^3J = 9 Hz, 1 H, 1-H), 8.20 (d, ^3J = 9 Hz, 1 H, 7-H), 8.35 (s, 1 H, 11-H), 8.77 (d, ^3J = 7 Hz, 1 H, H-4), 9.67 (s, 1 H, H-6); ^{13}C-NMR (100 MHz, MeOD): δ = 31.8 (CH_2), 32.7 (CH_2), 55.1 (CH_2), 71.9 (CH_2), 74.1 (CH_2), 102.0 (CH_{ar}), 120.0 (CH_{ar}), 120.4 (CH_{ar}), 123.3 (CH_{ar}), 123.6 (CH_{ar}), 124.3 (CH_{ar}), 125.7 (CH_{ar}), 126.8 (CH_{ar}), 126.9 (CH_{ar}), 127.7 (CH_{ar}), 130.7 (CH_{ar}), 130.9 (CH_{ar}), 134.4 (CH_{ar}), 135.9 (C_q), 138.9 (C_q), 139.5 (CH_{ar}), 140.5 (C_q), 145.2 (C_q), 151.5 (C_q). MS (ESI^+): *m/z* (%) 520.3 (100) $[M]^+$; El. Anal. calcd. (%) for $C_{29}H_{33}N_3ClS_2O_2 \times CHCl_3$ (675.56): C, 53.34; H, 5.22; N, 6.22; S, 9.49. Found: C, 53.14; H, 5.22; N, 6.09; S, 9.42 (the presence of one molar equivalent of chloroform, as lattice solvent, was confirmed by ^{1}H-NMR spectroscopy).

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. Circular dichroism (CD) spectra were recorded on a Chirascan (Applied Photophysics Limited, UK) spectrometer. 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 μM for absorption or CD spectroscopy, 10 μM for fluorescence spectroscopy and 2.5 μM for determination of quantum yields. Water-free ethanol was used as the solvent for the fluorescence quantum yield standard Coumarin 153, whose quntum yield was reported to be 0.38[3]. The quantum yields were determined according to the standard method.[4] Spectrophotometer slit widths were kept 2 nm for absorption spectroscopy and 5/5 nm for emission spectroscopy. Titrations with Hg^{2+} or DNA: The solution of the titrants $Hg(OAc)_2$ or ct DNA contained the appropriate concentration of compound **1a** (to avoid dilution effects) and were added to a cuvette containing a solution of free **1a** or **1a** with appropriate amount of ct DNA in HEPES buffer. The titration was monitored by absorption, emission or CD spectrosopy. The titration was continued until no further changes in the spectrum were observed. All spectrophotometric measurements were performed at least three times to ensure reproducibility.

The limit of detection (LOD) was determined following the established procedures.[5] Thus, the signal (fluorescence intensity) of 20 reference samples (10 μM of **1a** in the presence of 0.15 mM of DNA) was measured whose standard deviation δ was determined.

The limit of detection is then defined as LOD = 3 δ / S, where S is the slope of the calibration curve, which is obtained from the fluorimetric titrations (inset of Figure 3A).

Table S1 Absorption and Emission Properties of **1a**.

Solvent	λ_{abs} [a]	lg ε [b]	λ_{fl} [c]	ϕ_{fl} [d] / × 10^{-2}
H_2O	400	4.30	514	0.06
MeOH	405	4.39	523	0.02
MeCN	404	4.36	528	0.08
DMSO	403	4.36	532	0.12
CH_2Cl_2	412	4.36	525	0.05
$CHCl_3$	409	4.37	533	0.10

[a]Long-wavelength absorption maximum, in nm; [b]ε = molar absorption coefficient, in $cm^{-1}M^{-1}$; [c]fluorescene maximum, in nm; excitation wavelength λ_{ex} = 384 nm; [d]fluorescence quantum yield relative to Coumarin 153.[6]

Table S2 Emission Quantum Yields of **1a** in Different Solvents in the Absence or in the Presence of Hg^{2+} Ions

Solvents	ϕ_{fl} [a] / × 10^{-2} (w/o Hg^{2+})	ϕ_{fl} [a] / × 10^{-2} (with Hg^{2+})[d]
Water	0.06	0.08
Methanol	0.02	0.02
DMSO	0.12	0.12
PEG (40% w/v)[b]	0.12	0.13
PEG (40% w/v)[b,c]	0.12	0.82

[a]Fluorescence quantum yield relative to Coumarin 153. [b]In HEPES buffer. [c]In the prescence of DNA (0.15 mM). [d] c = 10 μM.

Table S3 Emission Quantum Yields of **1a**-DNA in the Presence of Different Cations.

Cation	ϕ_{fl} / × 10^{-2} [a]			
	Control	Hg^{2+}	DNA	Hg^{2+} + DNA
Control [b]	0.06	0.08	0.08	0.68
Cd^{2+}	0.08	0.07	0.06	0.70
Cu^{2+}	0.05	0.05	0.09	0.62
Ca^{2+}	0.05	0.04	0.08	0.68
Co^{2+}	0.06	0.06	0.07	0.61
Ni^{2+}	0.07	0.06	0.08	0.68
Zn^{2+}	0.09	0.07	0.10	0.65
Mg^{2+}	0.06	0.08	0.09	0.65
K^{+}	0.06	0.06	0.05	0.64
Pb^{2+}	0.04	0.08	0.06	0.69
Ag^{+}	0.06	0.08	0.62	0.70

[a] Fluorescence quantum yield relative to Coumarin 153. All experiments performed in HEPES buffer. c_{Hg2+} = 10 μM, c_{DNA} = 0.15 mM; [b]For Cu^{2+}, Co^{2+}, Cd^{2+}, Ag^{+}, Zn^{2+}, Ni^{2+}, Pb^{2+} and Ca^{2+}, c = 0.1 mM; c_{Mg2+} = 10 mM, c_{K+} = 100 mM.

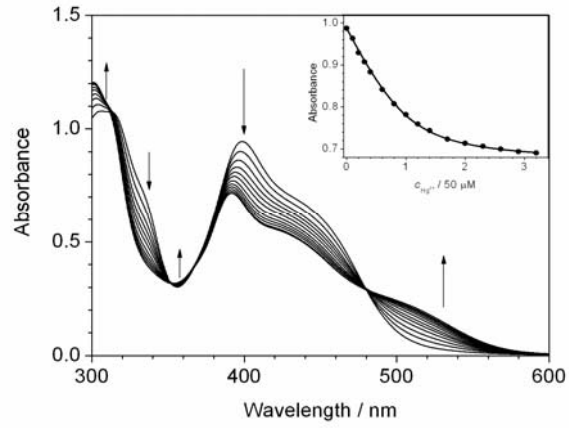


Figure S1 Spectrophotometric titration of Hg^{2+} to **1a** with in aqueous buffer (HEPES, 25 mM, pH 7.0). The arrows indicate the changes of the absorption bands upon addition of Hg^{2+}. Inset: Plot of absorbance at 398 nm versus concentration of Hg^{2+}; fit corresponds to the theoretical binding isostherm with a 1:1 stoichiometry ($K_b = 1.0 \times 10^5$ M^{-1}).

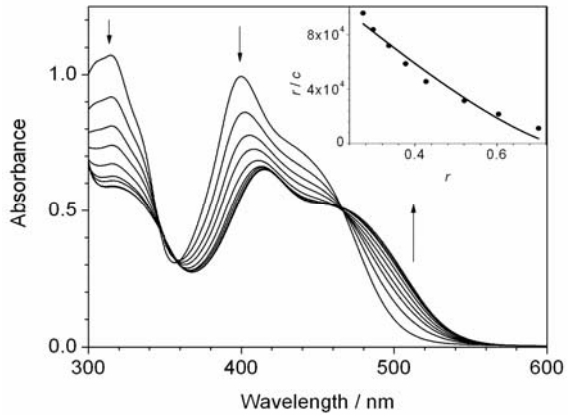


Figure S2 Spectrophotometric titration of ct DNA to **1a** (c = 50 μM) in aqueous buffer solution (HEPES, 25 mM, pH 7.0). The arrows indicate the changes of the bands upon addition of ct DNA. Inset: the Scatchard plot (r/c vs r; r = ligand-to-DNA ratio) fitted to the neighbor-exclusion model of McGhee and von Hippel;[7] $K = (1.6 \pm 0.1) \times 10^5$ M^{-1}; binding site size $n = 1.3 \pm 0.1$.

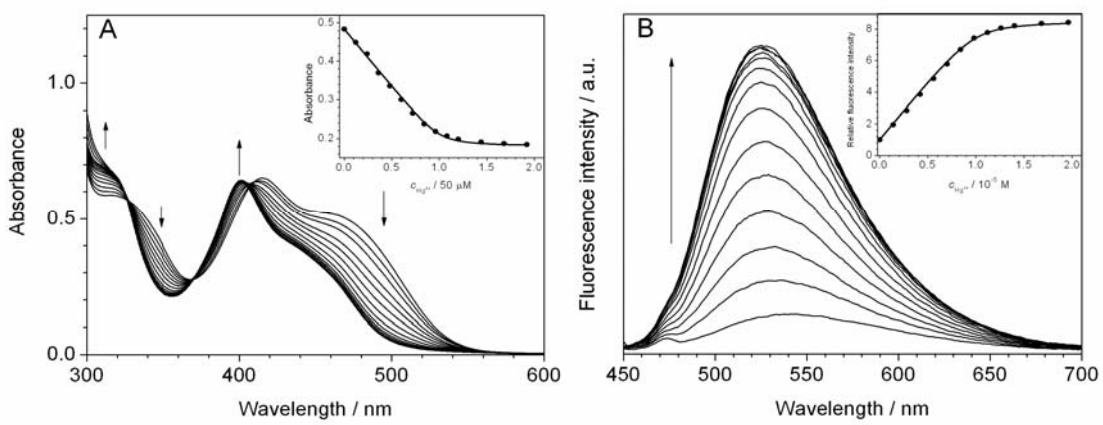


Figure S3 A: Spectrophotometric titration of Hg^{2+} to **1a** in the prescence of ct DNA in aqueous buffer (HEPES, 25 mM, pH 7.0, $c_{\mathbf{1a}}$ = 50 μM, c_{DNA} = 0.3 mM); B: spectrofluorimetric titration of Hg^{2+} to **1a** in the presence of ct DNA in aqueous buffer (λ_{ex} = 407 nm, $c_{\mathbf{1a}}$ = 10 μM, c_{DNA} = 0.15 mM). The arrows indicate the changes of the absorption or fluorescence during the titrations. Insets: plot of the absorption at 475 nm (A) and emission intensity at 525 nm (B) versus concentration of Hg^{2+}; numerical fit (A) calculated for $K_b = 1.5 \times 10^6$ M^{-1}.

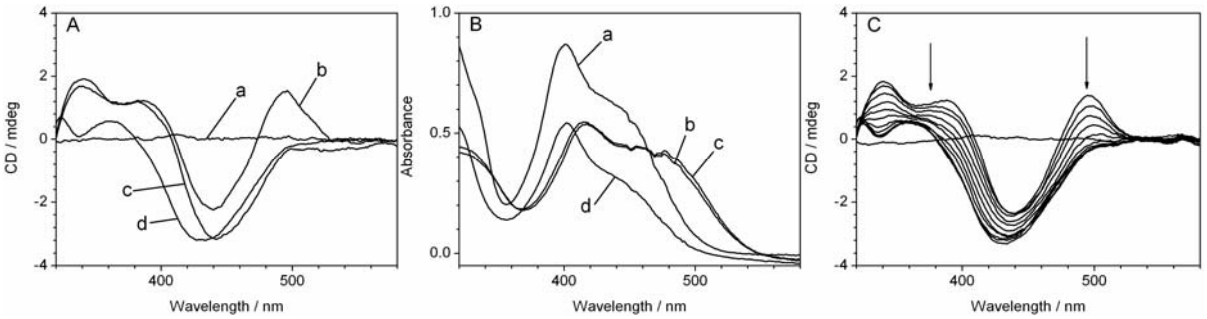


Figure S4 A: CD spectra of **1a** (c = 5 × 10^{-5} M) with ct DNA; a: control; b: c_{ligand}/c_{DNA} = 0.2; c: [ligand]/[DNA] = 0.1; d: [ligand]/[DNA] = 0.2 and subsequent addition of Hg^{2+} (c_{Hg} = 5 × 10^{-5} M). B: The corresponding absorption spectra during the experiments. C: Titration of Hg^{2+} (c_{Hg} = 0–6.5 × 10^{-5} M) into the solution of **1a** (c = 5 × 10^{-5} M) in the presence of DNA (c_{ligand}/c_{DNA} = 0.2). A-C: In HEPES buffer, 25 mM, pH 7.0; T = 20 °C).

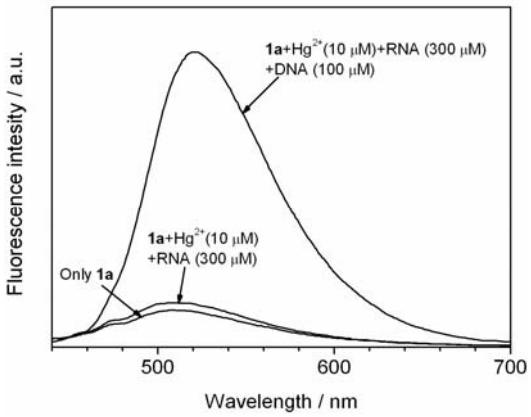


Figure S5 Emission spectra of **1a** (c = 10 μM), in the presence of RNA and Hg^{2+} (300 μM and 10 μM resp.), or RNA, Hg^{2+} and DNA (300 μM, 10 μM and 100 μM resp.).

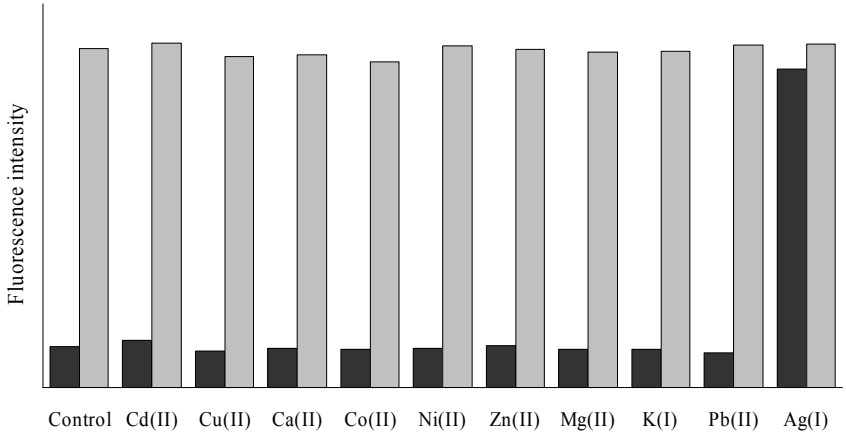


Figure S6 Black bars: relative fluorescence intensities (λ_{em} = 525 nm, λ_{ex} = 407 nm) of **1a** ($c_{\mathbf{1a}}$ = 10 μM) in the presence of ct DNA (c_{DNA} = 0.15 mM) in HEPES buffer (25 mM, pH 7.0) in the absence (control) and in the presence of Cu^{2+}, Co^{2+}, Cd^{2+}, Ag^{+}, Zn^{2+}, Ni^{2+}, Pb^{2+}, Ca^{2+} (c = 0.1 mM), Mg^{2+} (c = 10 mM) and K^{+} (c = 100 mM).

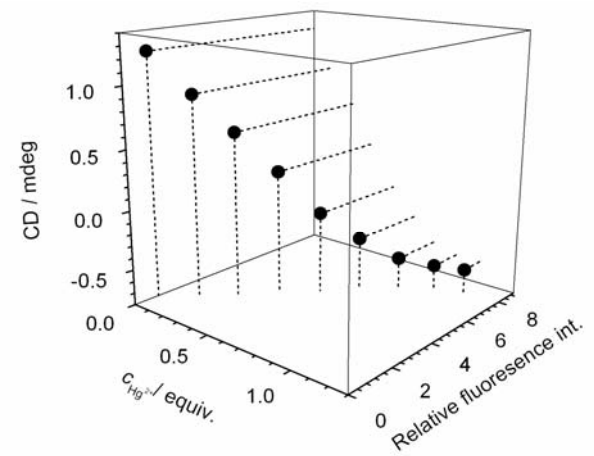


Figure S7 Three dimensional plot of the fluorescence intensity (λ = 525 nm) and CD intensity (λ = 490 nm) of **1a** in the presence of ct DNA (0.15 mM) versus concentration of Hg^{2+} in aqueous buffer (HEPES, 25 mmol, pH 7).

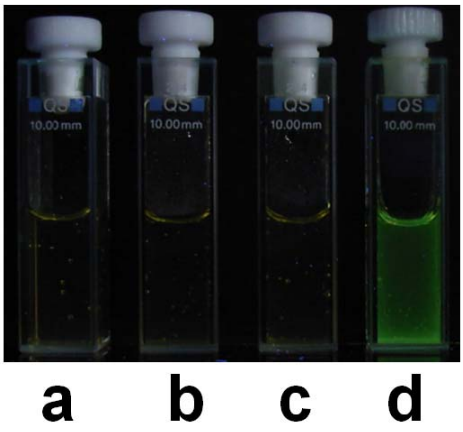
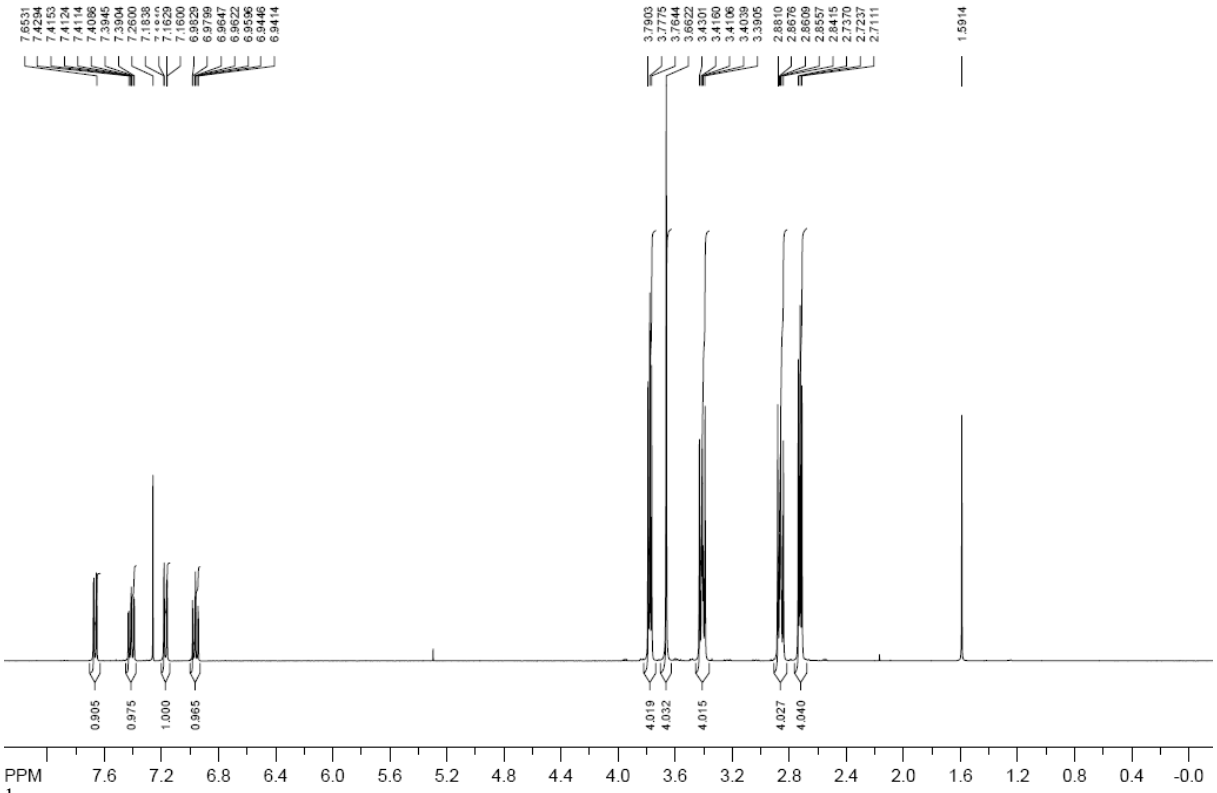
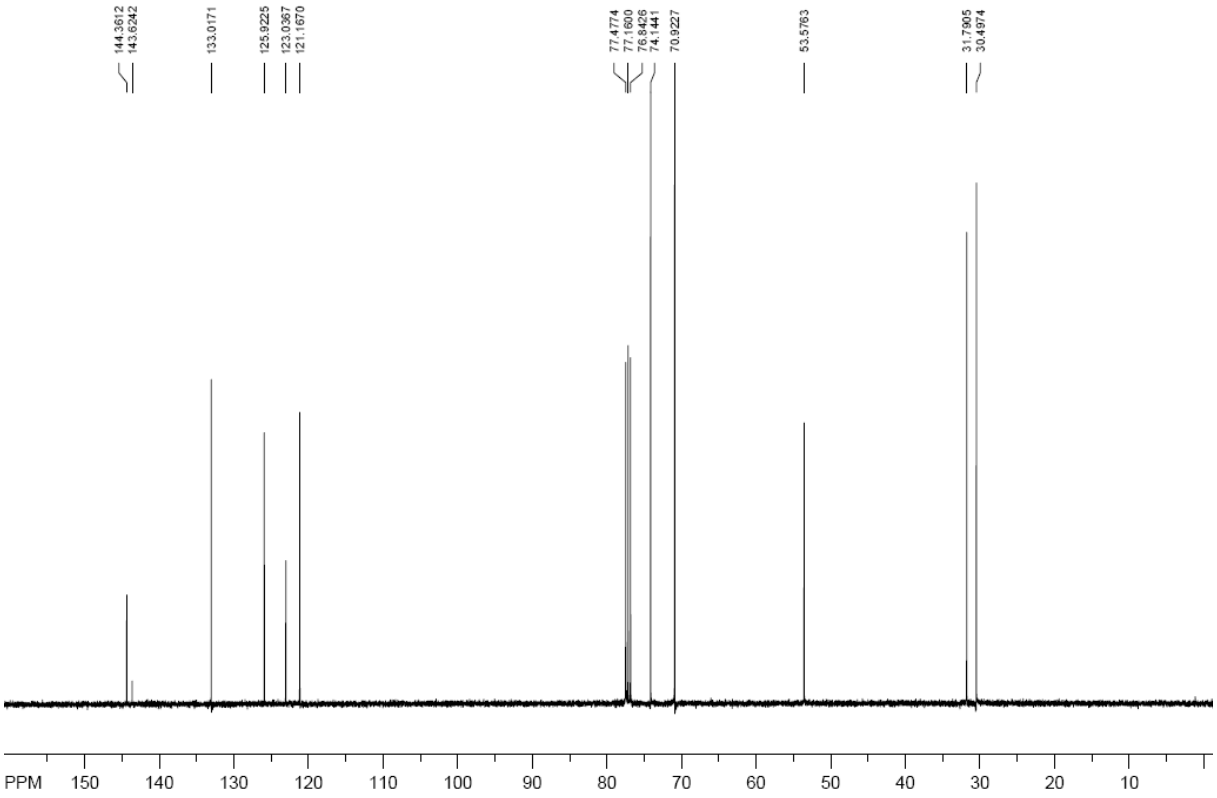


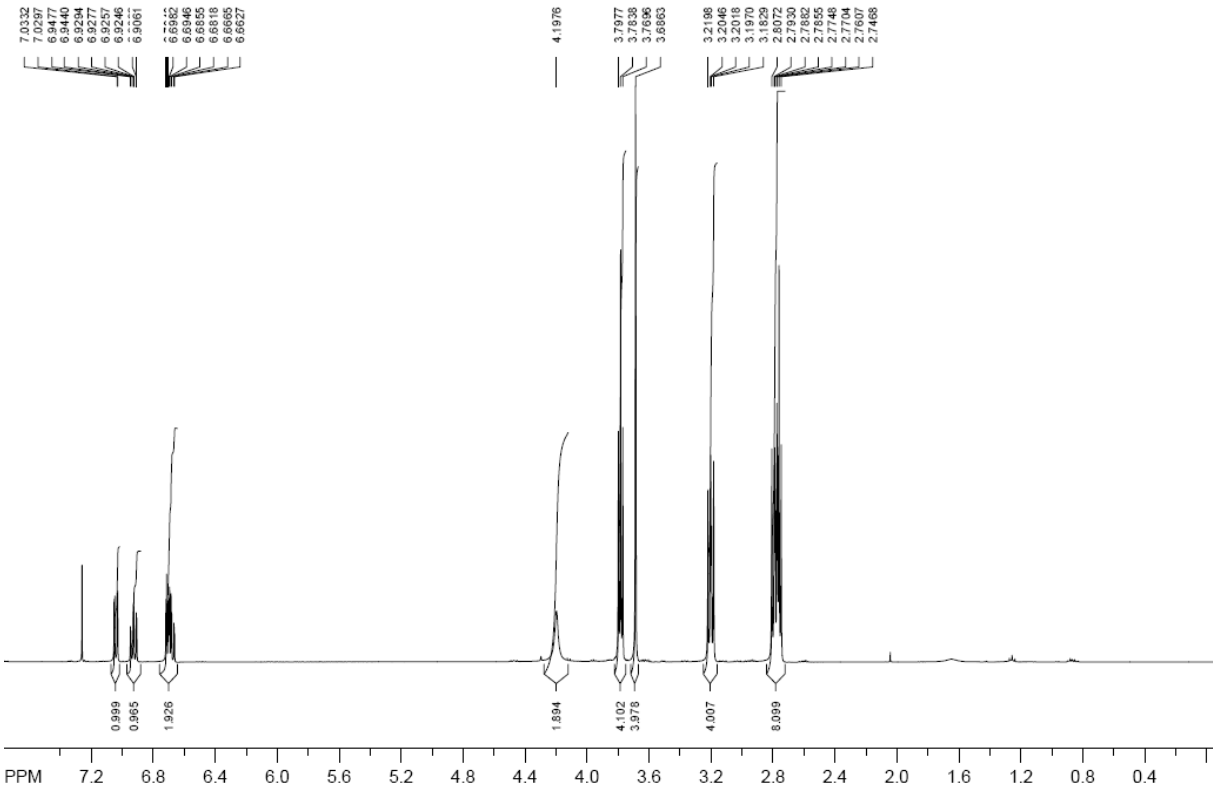
Figure S8 Image of solutions of **1a** (c ≈ 50 µM) in aqueous buffer solutions (HEPES, 25 mM, pH 7.0): only **1a** (a), in the presence of DNA (b, c_{DNA} = 0.3 mM), in the presence of Hg^{2+} (c, c_{Hg} = 50 µM), and in the presence of both DNA and Hg^{2+} (d, c_{DNA} = 0.3 mM, c_{Hg} = 50 µM) under UV illumination (λ = 312 nm).



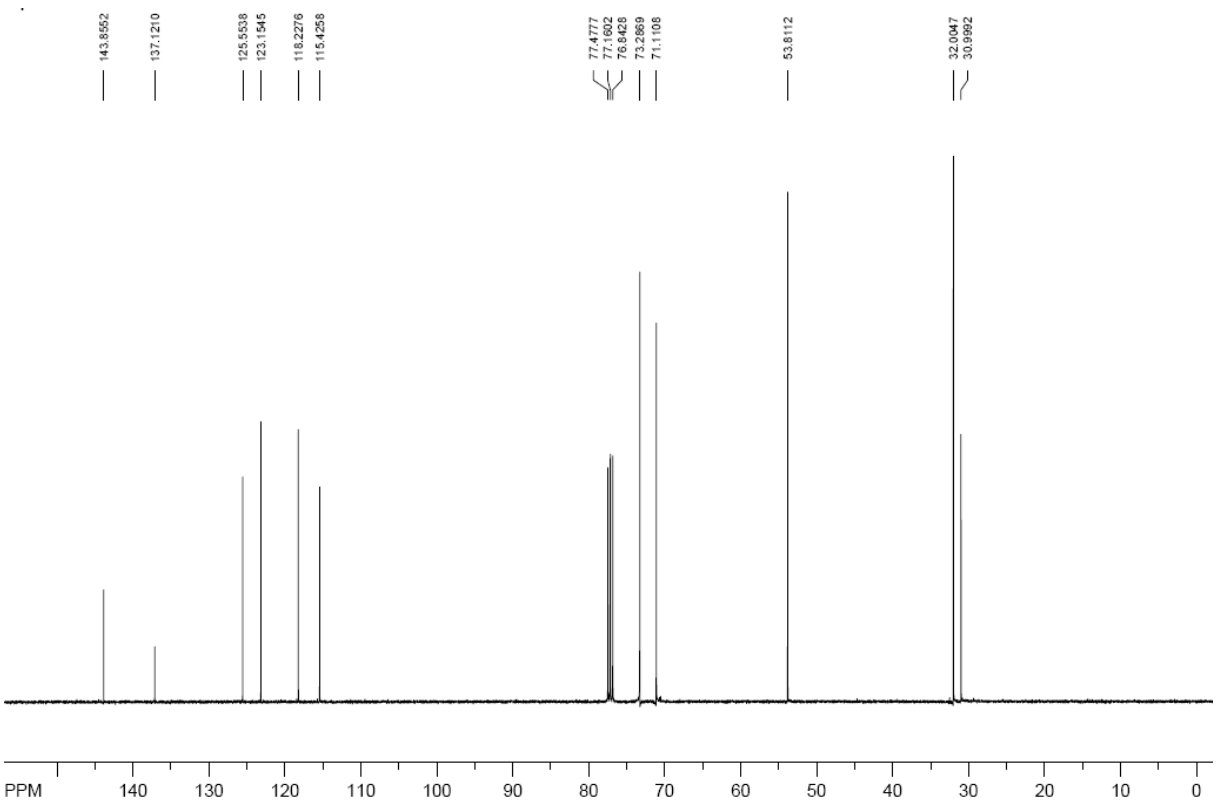
^{1}H-NMR spectrum of **2** in $CDCl_3$



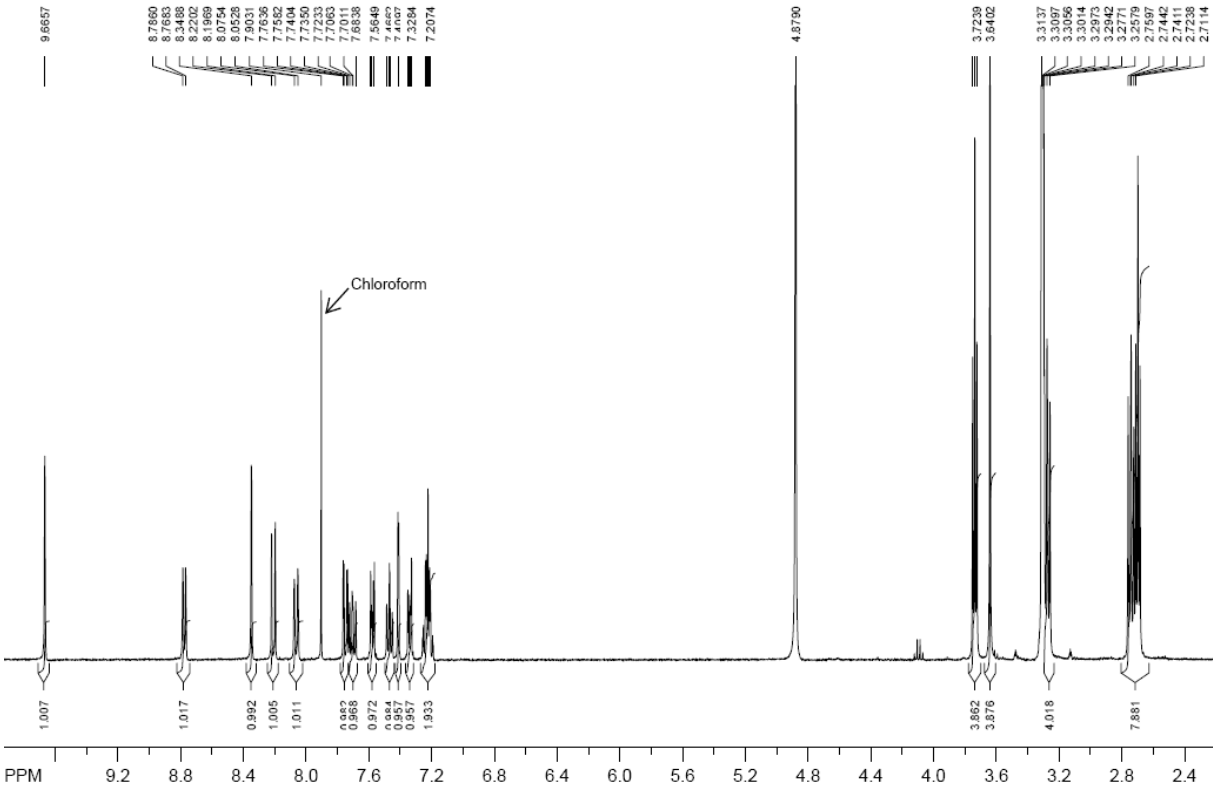
^{13}C-NMR spectrum of **2** in $CDCl_3$.



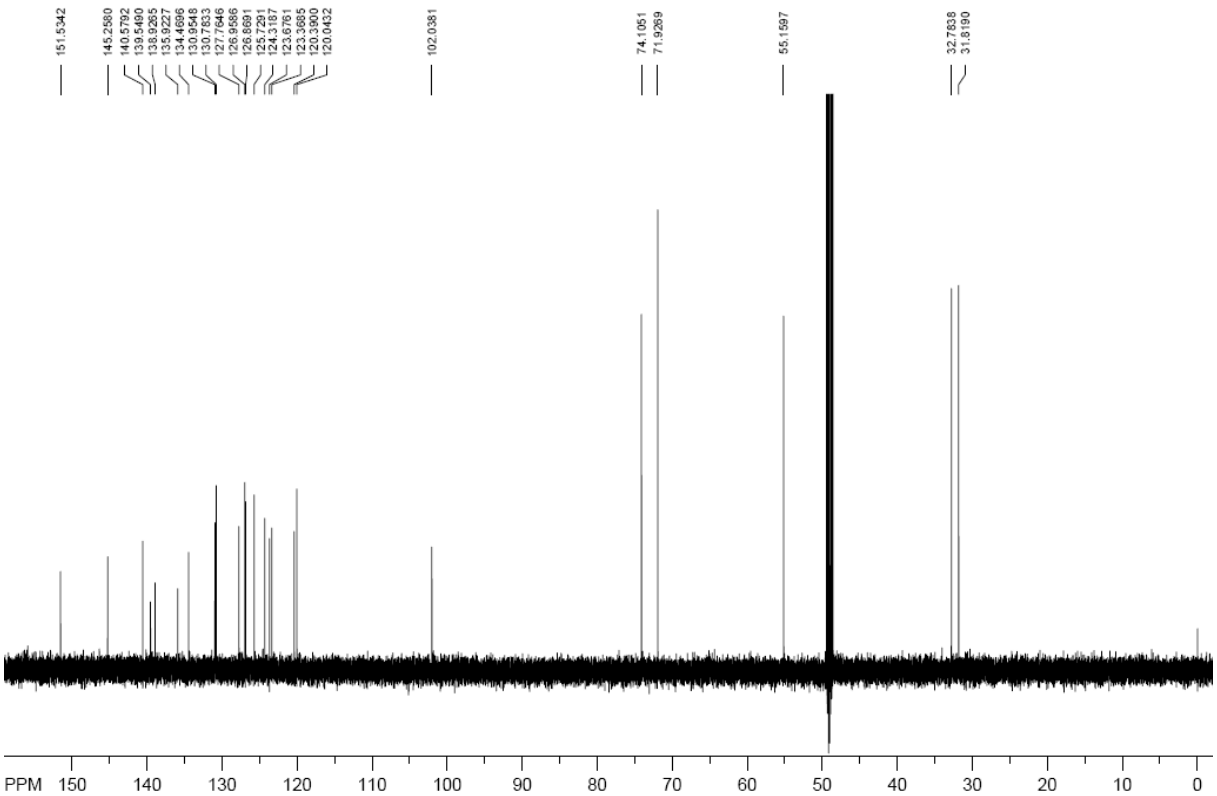
^{1}H-NMR spectrum of **3** in $CDCl_3$.



^{13}C-NMR spectrum of **3** in $CDCl_3$.



^{1}H-NMR spectrum of **1a** in MeOD.



^{13}C-NMR spectrum of **1a** in MeOD.

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