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

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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 ($^1$H: 400 MHz, $^{13}$C: 100 MHz) and Varian NMR System 600 ($^1$H: 600 MHz, $^{13}$C: 150 MHz) spectrometers at 20 °C; chemical shifts are given in ppm ($\delta$) relative to TMS ($\delta = 0.00$ 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/AcOH 90:10:1, v/v. Ion exchange resin was purchased from SERVA Electrophoresis (DOWEX® 1 × 8, 50–100 mesh, pract. grade, Cl-form). Purified water with resistivity $\geq$ 18 MΩ 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'-dioxa-7',13'-dithia-10'-azacyclopentadecyl)acridizinium (1c).

A solution of 9-fluoroacridizinium bromide (417 mg, 1.50 mmol) and 1,4-dioxo-7,13-dithio-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, Rᵥ = 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, 3J = 5 Hz, 4H, CH₂), 3.02 (t, 3J = 8 Hz, 4H, CH₂), 3.67 (s, 4H, CH₂), 3.80 (t, 3J = 5 Hz, 4H, CH₂), 4.00 (t, 3J = 8 Hz, 4H, CH₂), 7.05 (d, 4J = 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, 3J = 9 Hz, 4J = 1 Hz, 1H, CH_ar, H-1), 8.19 (d, 3J = 10 Hz, 1H, CH_ar, H-7), 8.32 (s, 1H, CH_ar, H-11), 8.71 (d, 3J = 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 µM for absorption spectroscopy and 10 µM 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(1c) = 10 µM. In control experiments with other metal cations the concentration of the ligand 1c and of the metals was c = 10 µM. 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

<table>
<thead>
<tr>
<th>Solvent</th>
<th>λₘₐₓ a</th>
<th>lg ε b</th>
<th>λₘₐₓ c</th>
<th>φₘ d</th>
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<tr>
<td>H₂O</td>
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<td>4.29</td>
<td>523</td>
<td>0.22</td>
</tr>
<tr>
<td>MeCN</td>
<td>402</td>
<td>4.33</td>
<td>531</td>
<td>0.27</td>
</tr>
</tbody>
</table>

a Long-wavelength absorption maximum, in nm; c = 50 µM; b extinction coefficient, cm⁻¹M⁻¹; c fluorescent emission maximum, c = 10 µM; excitation wavelength λₑₓ = 390 nm; d fluorescence quantum yield relative to Coumarin 153, estimated error ±10%.
**Figure S1** Benesi-Hildebrand plot from photometric titration of Hg$^{2+}$ to 1c ($R^2 = 0.990$). The resulting association constant is $1.5 \times 10^4$ M$^{-1}$. Another independent calculation by computer program SPECFIT gave the association constant of $1.6 \times 10^4$ M$^{-1}$.

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

**Figure S3.** a) Absorption spectra of free 1c (50 µM) with different pH (7.1, 5.2, 3.3, 1.6 and 1.1) in Britton-Robinson buffer; b) absorption spectra of 1c (50 µM) 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 (○) and in the presence (■) of Hg²⁺ (0.1 mM) at different pH values in acetate buffer (50 mM); λ_{ex} = 388 nm, λ_{em} = 523 nm; b) Spectrofluorimetric titration of an aqueous solution of Hg(OAc)₂ to compound 1c at pH 4.0 (acetate buffer 50 mM, λ_{ex} = 388 nm; c = 10 µM). Arrows on top of the spectra indicate the development of the emission band upon addition of Hg(OAc)₂. Inset: the ratiometric analysis of I₄₆₆/I₅₂₃ vs c_{Hg²⁺}.

Figure S5. Spectrofluorimetric titrations of an aqueous solution of Hg(OAc)₂ to compound 1c (c = 10 µM) with different excitation wavelength (a: λ_{ex} = 410 nm; b: λ_{ex} = 420 nm; c: λ_{ex} = 430 nm); arrows indicate the changes of the fluorescence intensity upon addition of Hg(OAc)₂.
Figure S6. $^1\text{H}$-NMR spectrum of 1c in MeOD
Figure S7. $^{13}$C-NMR spectrum of 1c in MeOD
Figure S8. $^1$H, $^1$H}-COSY spectrum of 1c in MeOD

Figure S9. HSQC spectrum of 1c in MeOD
Figure S10. $^1$H-NMR spectrum of 9-aminoacridizinium chloride in D$_2$O

Figure S11. $^1$H-NMR spectrum of 1c in D$_2$O
Figure S12. $^{13}$C-NMR spectrum of 1c in $D_2O$

Figure S13. {1H, 1H}-COSY spectrum of 1c in $D_2O$
Figure S14. HSQC spectrum 1c in D₂O

Figure S15. HMBC spectrum 1c in D₂O
Figure S16. $^1$H-NMR spectrum of 1c + 1 equiv. Hg(OAc) in D$_2$O

Figure S17. {$^1$H, $^1$H}-COSY spectrum of 1c + 1 equiv. Hg(OAc) in D$_2$O
**Figure S18.** HSQC spectrum of 1c + 1 equiv. Hg(OAc) in D$_2$O

**Figure S19.** HMBC spectrum of 1c + 1 equiv. Hg(OAc) in D$_2$O

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