Electronic Supplementary Material (ESI) for RSC Advances

A cyclam-type "turn on" fluorescent sensor selective for merury ions in aqueous media

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Supporting Information Available:

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II) Spectral Data (^{1}H NMR, ^{13}C NMR, DART-TOF-Mass spectra, and ESI-Mass spectra and HRMS)

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V) DFT geometry optimization for the **5**- Hg^{2+} and **3**-Hg^{2+} complexes

VI) Detection limit of probe **3** for Hg^{2+}ions.

I) Experimental Section

General Procedures: All reactions were carried out under anhydrous conditions in dry solvents, using argon or nitrogen in flame-dried glassware. Reactions were monitored by thin-layer chromatography (TLC) using silica gel plates from Merck (60F254), which were visualized under a UV-Vis Lamp (254 and 366 nm, respectively) or with a 7% ethanolic solution of phosphomolybdic acid. Flash column chromatography was performed in silica gel 60 from Merck (230-400 mesh). NMR spectra were taken on an AMX500 Bruker FT-NMR or a MSL300 Bruker FT-NMR spectrometer; proton chemical shifts are reported in relative to tetramethylsilane. Fluorescence spectra were recorded on an Aminco Bowman spectrofluorimeter (Spectronics Co., USA). Mass spectrometric experiments were performed using DART-TOF-MS (JEOL: JMS T100TD) technique[1] and ESI-Mass spectrometer. HRMS were taken at ProFI, Foundation for Research and Technology-Hellas (ITE), Heraklion, Greece. Ultra-pure water was collected from a PURELAB Ultra instrument by ELGA.

Tri-BOC-cyclam 2: To a solution of cyclam **1** (200 mg, 0.998 mmol) in 14 ml $CHCl_3$, was added dropwise – within 0.5 h - a solution of $(BOC)_2O$ (653 mg, 2.994 mmol) in 20 ml $CHCl_3$, at -40 ^oC. After the completion of the reaction, as indicated by TLC (50% ethyl acetate in petroleum ether), the solvent was distilled in high vacuum and product **2** was isolated through flash liquid gradient chromatography, using initially 50% ethyl acetate in petroleum ether, then 100% ethyl acetate and sequentially 5% and 10% ethyl acetate in methanol as solvents. The product was white-coloured with foamy texture. Chromatography yielded 435 mg of tri-BOC-cyclam **2** (87 %). ^1H NMR (500 MHz, $CDCl_3$): δ 3.26-3.37 (m, 12H), 2.78 (m, 2H), 2.61 (m, 2H), 1.88 (m, 2H), 1.69 (m, 2H), 1.41 (bs, 27H); ^{13}C NMR (300 MHz, $CDCl_3$ / 1 drop CD_3OD): 160.38, 159.67, 83.90, 83.73, 53.59, 51.56, 50.47, 49.49, 48.42, 33.51, 32.25. HRMS: calculated for $C_{25}H_{49}N_4O_6$: 501.3652, found 501.3658.

7-Isothiocyanato-4-methyl-2*H*-1-benzopyran-2-one, (1): To a solution of 7-amino-4-methylcoumarin (Aldrich) (245 mg, 1.4 mmol), in 14 ml CH_2Cl_2 was added triethylamine (390 μl, 2.8 mmol) and the solution was stirred at 35^o C for 10.0 min. After cooling the system to room temperature, thiophosgene (320 μl, 4.2 mmol) was added dropwise and the mixture was stirred at 35^o C for 20.0 min. The reaction was completed after 20.0 min, as indicated by TLC (20% acetone in toluene). Excess of $CSCl_2$ was removed by distillation in high vacuum. The orange precipitate was dissolved in 10 ml CH_2Cl_2, filtered through silica gel for the removal of triethylamine hydrochloride, washed with 3 ml dichloromethane, and dried in high vacuum to yield 301 mg (98%) of **1** as a light orange solid. ^1H NMR (500 MHz, $CDCl_3$): δ 7.54 (d, *J*=5.5 Hz, 1H), 7.23 (s, 1H), 7.11 (d, *J*=5 Hz, 1H), 7.08 (s, 1H), 2.40 (s, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): 160.32, 154.43, 151.98, 139.09, 134.87, 126.36, 122.43, 119.39, 115.65, 114.18, 19.27. HRMS: calculated for $C_{11}H_6NO_2S$: 218.0275, found 218.0272.

Tri-BOC-cyclam coumarin probe 3: (1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylic acid,11-[[(4-methyl-2-oxo-2*H*-1-benzopyran-7-yl)amino] carbonothioyl] -tris(1,1-dimethylethyl) ester**:** To a solution of isothiocyanate **1** (62 mg, 0.286 mmol) in 5 ml $CHCl_3$ was added tri-BOC-cyclam **2** (143 mg, 0.286 mmol) and the solution was heated at 50 ^oC for 30.0 min. After the completion of the reaction, as indicated by TLC (20% acetone in toluene), solvent was distilled in high vacuum and the isolation

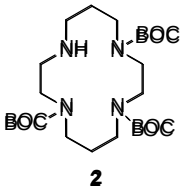
of probe **3** was achieved through flash liquid step elution chromatography, using 20, 30, 50%, 100% ethyl acetate in toluene as solvents. Chromatography yielded 199 mg probe **3** (97 %). TLC: R_f: 0.61 (EtOAc/Toluene/MeOH: 49/49/2. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.10 (br.s, 1H, thioamide), 7.70 (d, *J*=9.0 Hz, 1H, C_5-H), 7.39 (overlapping s & d, *J*=9.0 Hz, 2H, C_8-H & C_6-H), 6.32 (s, 1H, C_3-H), 3.91 (br.s., 2H, -(CO)N-$\underline{CH_2}CH_2CH_2$-N(CO)), 3.77 (br.s., 2H, (CO)N -$CH_2CH_2\underline{CH_2}$- N(CO)), 3.60(br.s., 2H, (C=S)N -$CH_2CH_2\underline{CH_2}$- N(CO)), 3.42-3.25 (m, 10H, (CO)N -$\underline{CH_2CH_2}$-N(CO)), 3.30-3.21 & (C=S)N -$\underline{CH_2}CH_2CH_2$- N(CO)), 2.43 (s, 3H, aryl methyl), 1.88 (m, 2H, $CH_2\underline{CH_2}CH_2$-), 1.67 (m, 2H, -$CH_2\underline{CH_2}CH_2$-), 1.37 (s, 27H, $C(CH_3)_3$); ^{13}C NMR (300 MHz, $CDCl_3$): 180.90 (C=S), 161.20 (C=O, coumarin), 156.00 (C-8a), 155.75 (C-4), 155.53, 153.67, 152.44 (BOC, C=O), 143.65 (C-7, coumarin), 124.05 (C-5), 121.06 (C-6), 116.65 (C-4a), 113.63 (C-3), 111.97 (C-8), 80.10 [O-$\underline{C}(CH_3)_3$], 50.46 (-$\underline{C}H_2$-N-S=O), 48.39, 46.49 (-$\underline{CH_2}CH_2\underline{CH_2}$-N-C=O), 47.75 (N-$\underline{CH_2CH_2}$-N), 28.38 [-C($\underline{C}H_3)_3$], 27.63 (-$CH_2\underline{CH_2}CH_2$- N-C=O), 18.63 (vinylic methyl). ESI- MS: m/z 740 $(M+Na)^+$, 756 $(M+K)^+$. HRMS calcd for $C_{36}H_{55}N_5O_8S$+H, 718.3844; found: 718.3846.

Cyclam coumarin probe 5: (*N*-(4-methyl-2-oxo-2*H*-1-benzopyran-7-yl)-1,4,8,11-tetraazacyclotetradecane-1-carbothioamide).

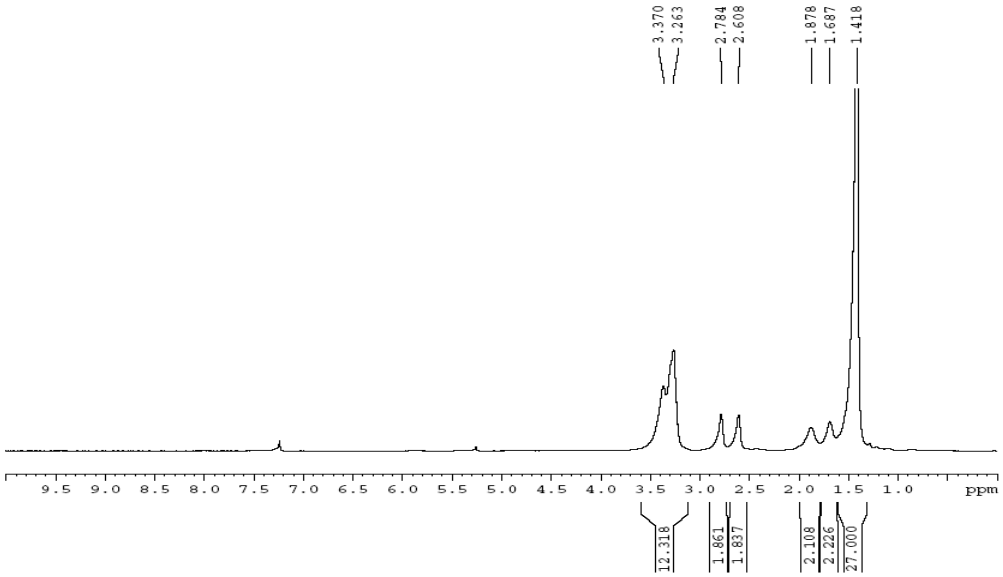
To a solution of cyclam (184,0 mg, 0.92 mmol) in 25 ml $CHCl_3$ cooled at -50 ^{o}C was added isothiocyanate **1** (100,0 mg, 0.46 mmol) dissolved in 5 ml $CHCl_3$ dropwise in a 1h period. The solution was then allowed to reach room temperature and was further stirred for 0.5h. After the completion of the reaction, as indicated by TLC (20% acetone in toluene), the solvent was distilled *in vacuo* and the isolation of probe **5** was achieved through repeated suspensions of the reaction mixture in ethanol followed by centrifugation. It must be noticed at this point that, when methanol was used as solvent, the purity of the resulting solid product was very low. This procedure yielded 134 mg of probe **5** (70.0 %). TLC: R_f: 0.61 (0,1% CH_2Cl_2 in MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.65 (d, *J*=8.5 Hz, 1H, H-5), 7.47 (s, 1H, H-8), 7.41 (d, *J*=8.5 Hz 1H, H-6), 6.23 (s, 1H, H-3), 3.54 (br.s., 3H, N-H), 3.79 (m, 2H, (C=S)-N-CH_2-), 3.34 (br.s, 12H, -$\underline{CH_2}CH_2\underline{CH_2}$- & -C(=S)-N-$CH_2\underline{CH_2}$-N) & -NH-$\underline{CH_2}CH_2$-NH), 2.93, (br.s., 2H, -NH-$\underline{CH_2}CH_2$-NH) 2.71 (br.s., 2H, -$CH_2\underline{CH_2}CH_2$-), 2.41 (s, 3H, CH_3) 1.94 (br.s, 2H, -$CH_2\underline{CH_2}CH_2$). ESI- MS (negative ionization): m/z 416 $(M-H)^-$. HRMS (positive mode) calcd for $C_{21}H_{31}N_5O_2S$+H, 418.2271; found: 418.2271.

II) Spectral Data for Compounds 1, 2, 3 and 5.

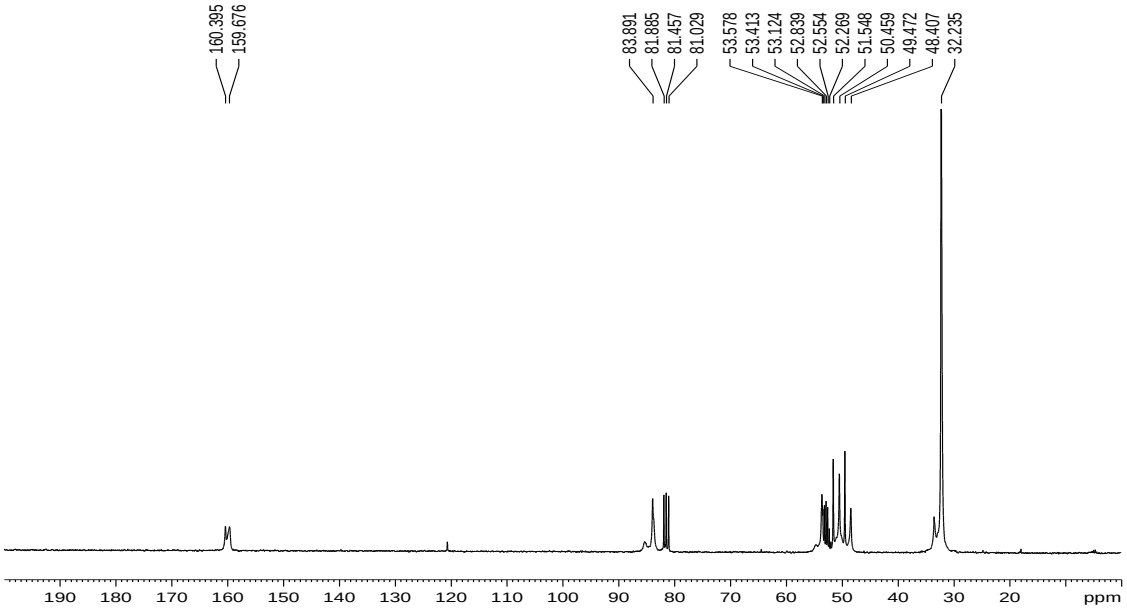
^{1}H NMR spectrum of compound 2 (500 MHz, $CDCl_3$)



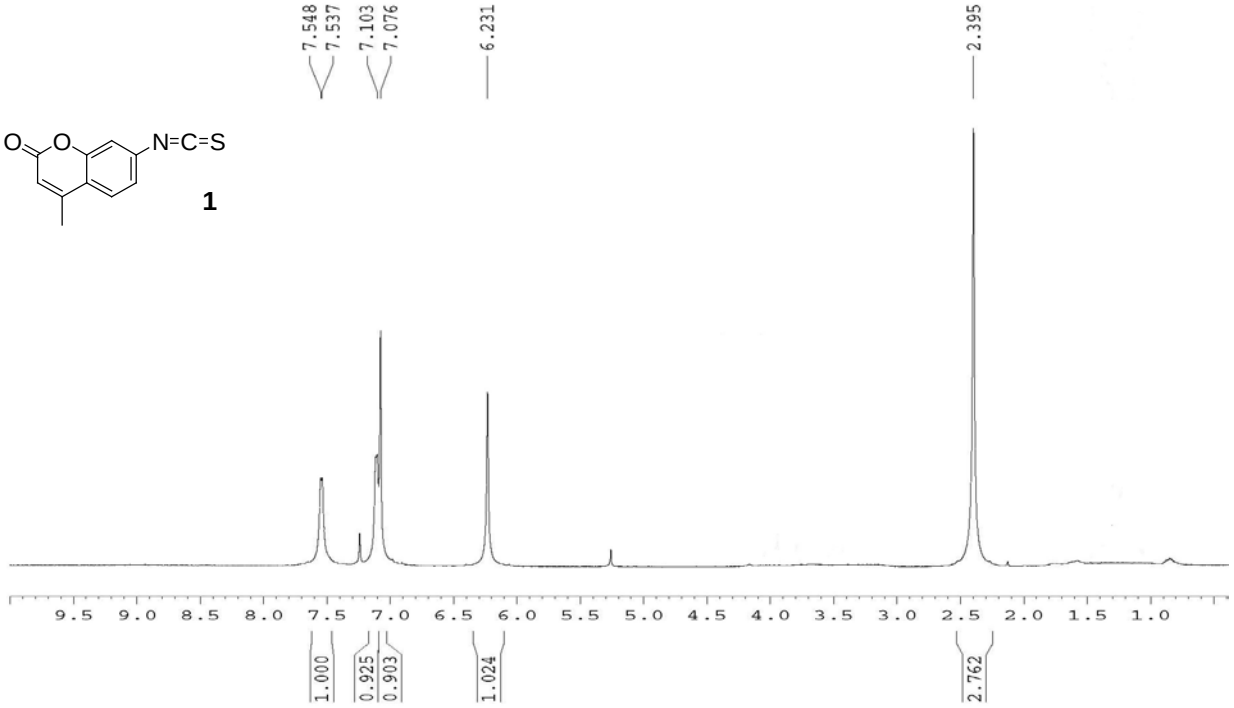
2



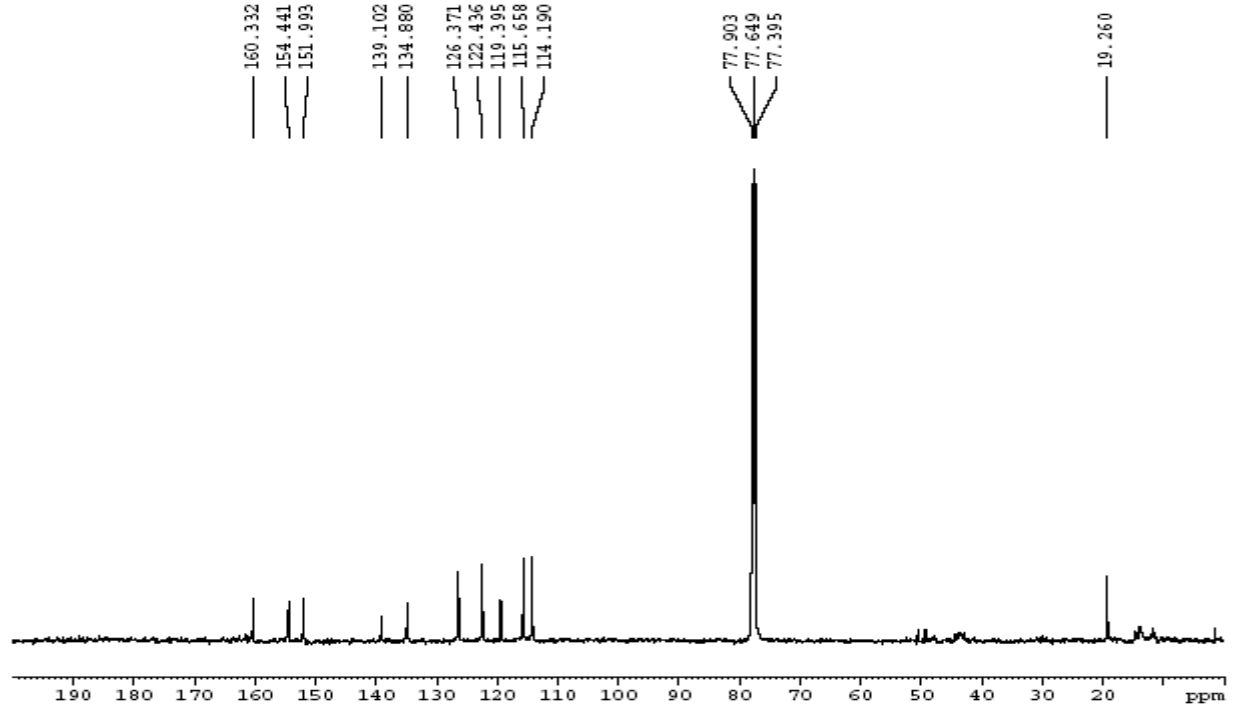
^{13}C NMR spectrum of compound 2 (300 MHz, $CDCl_3$ / 1 drop CD_3OD)



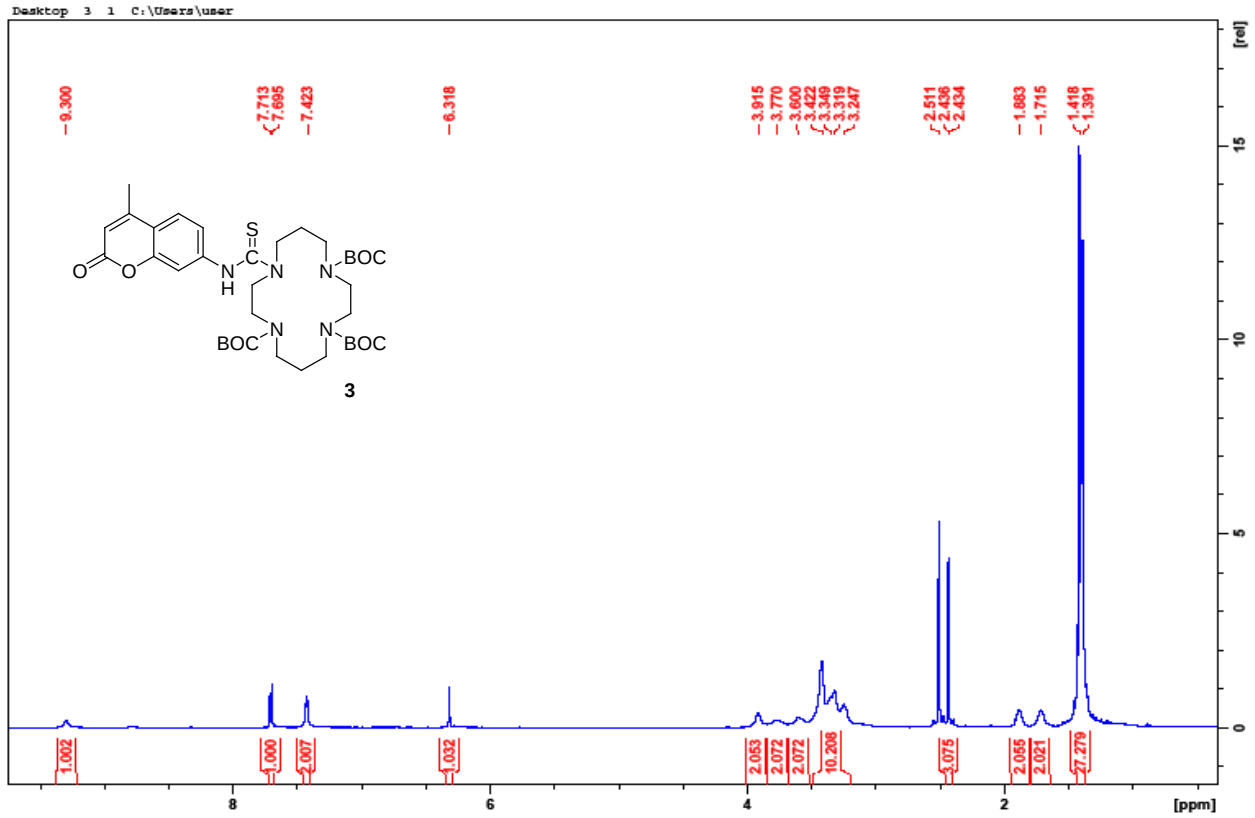
^{1}H NMR spectrum of compound 1 (500 MHz, $CDCl_3$)



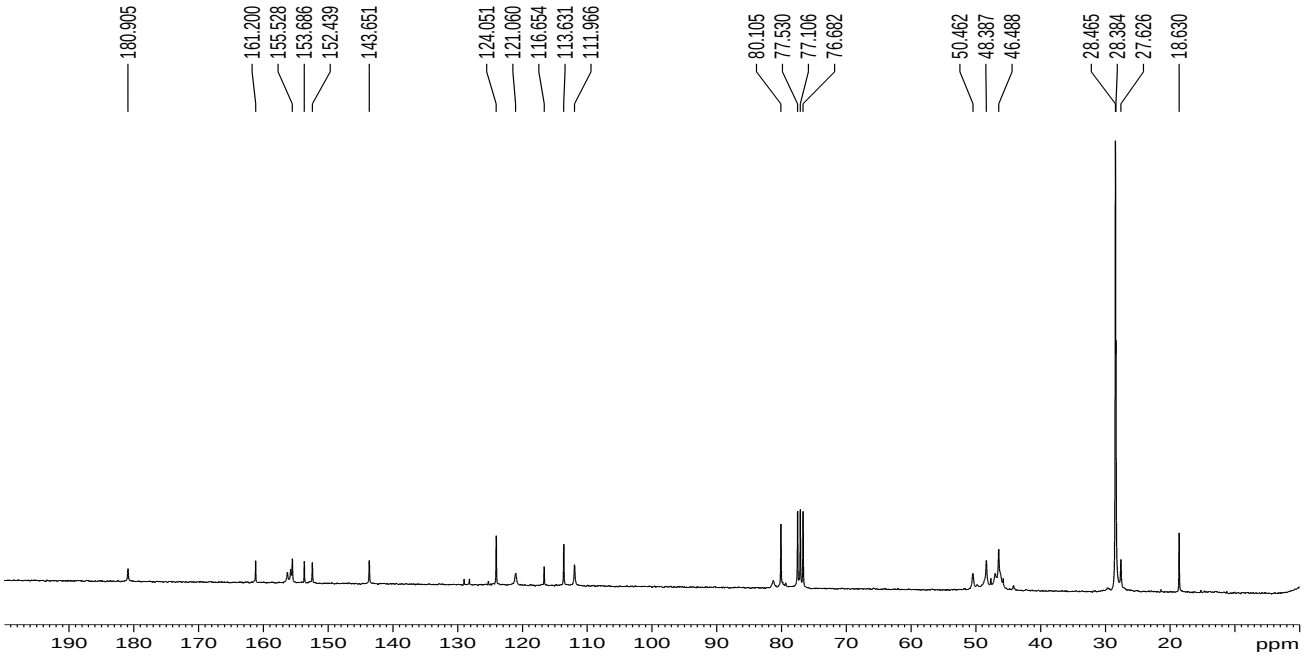
^{13}C NMR spectrum of compound 1 (500 MHz, $CDCl_3$)



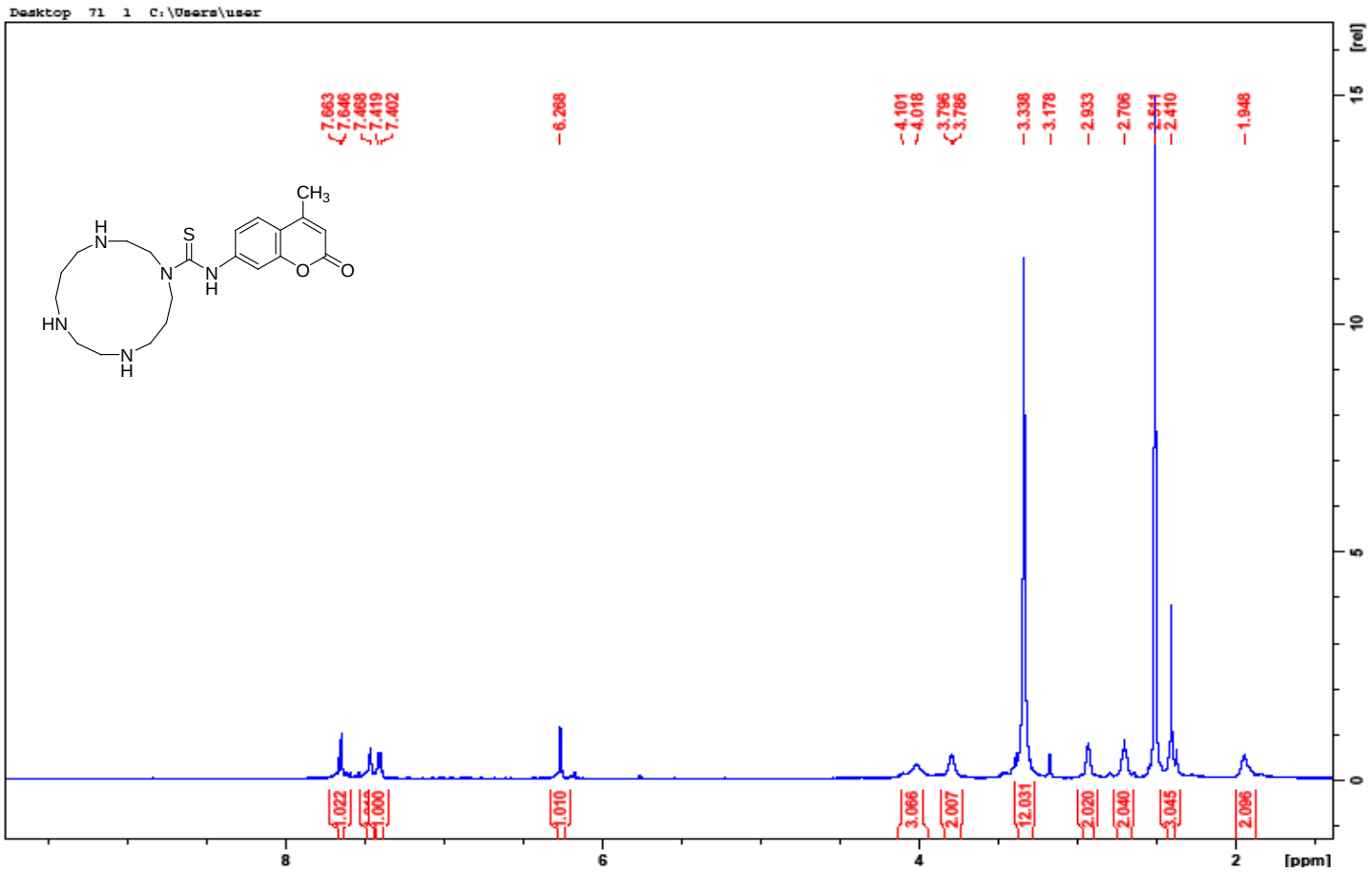
^{1}H NMR spectrum of compound 3 (500 MHz, DMSO-d_6)



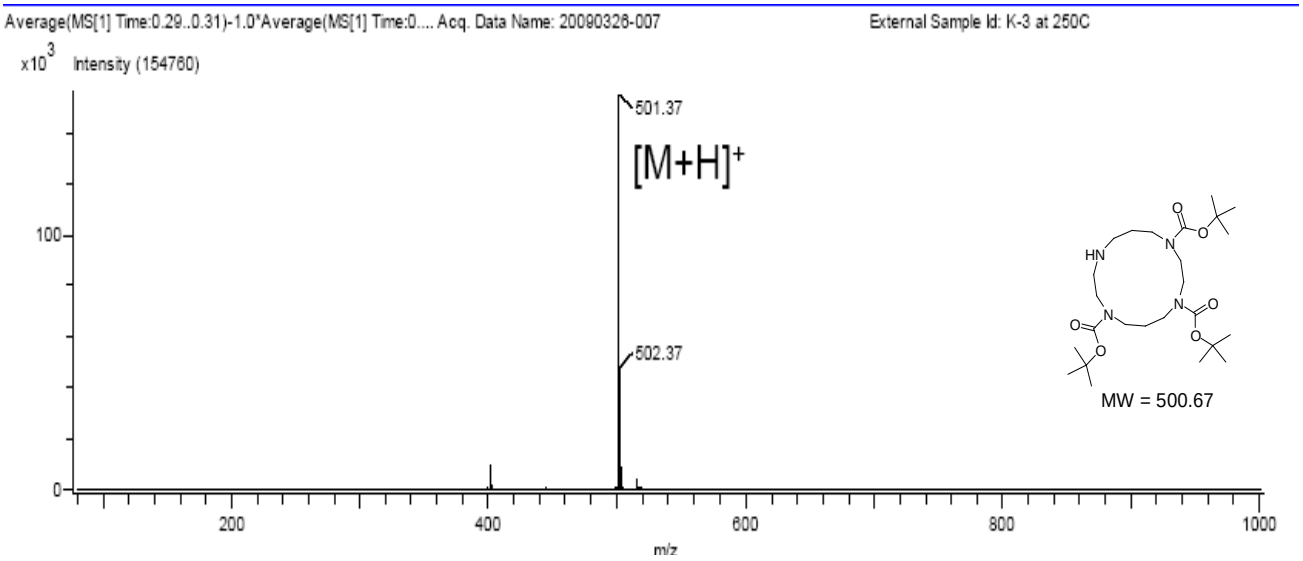
^{13}C NMR spectrum of compound 3 (500 MHz, $CDCl_3$)



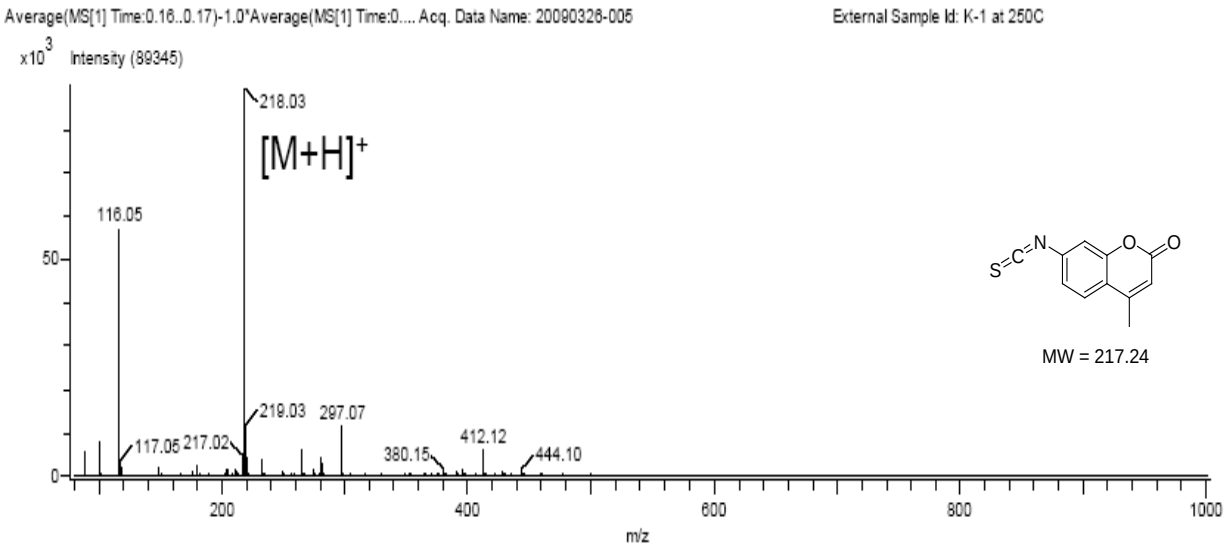
^{1}H NMR spectrum of compound 5 (500 MHz, $CDCl_3$)



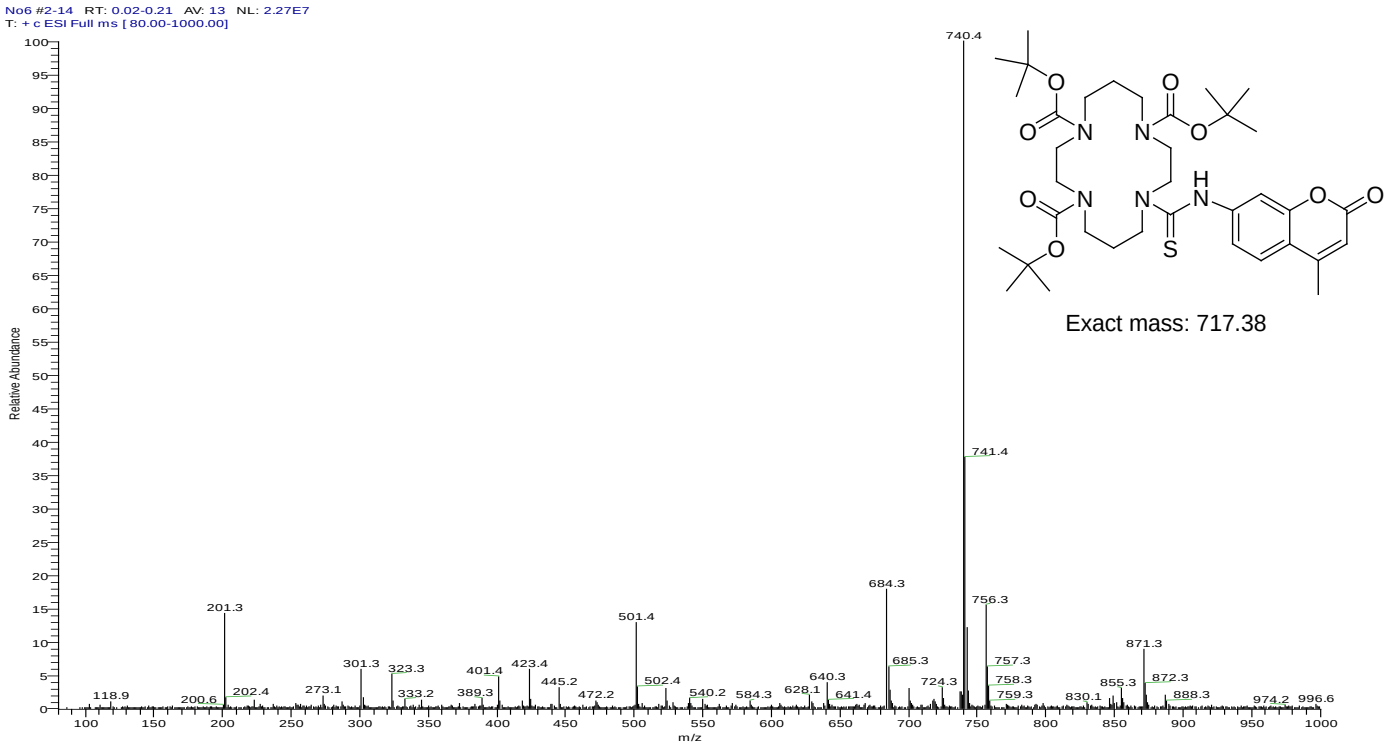
DART-TOF-Mass spectrum of compound 2



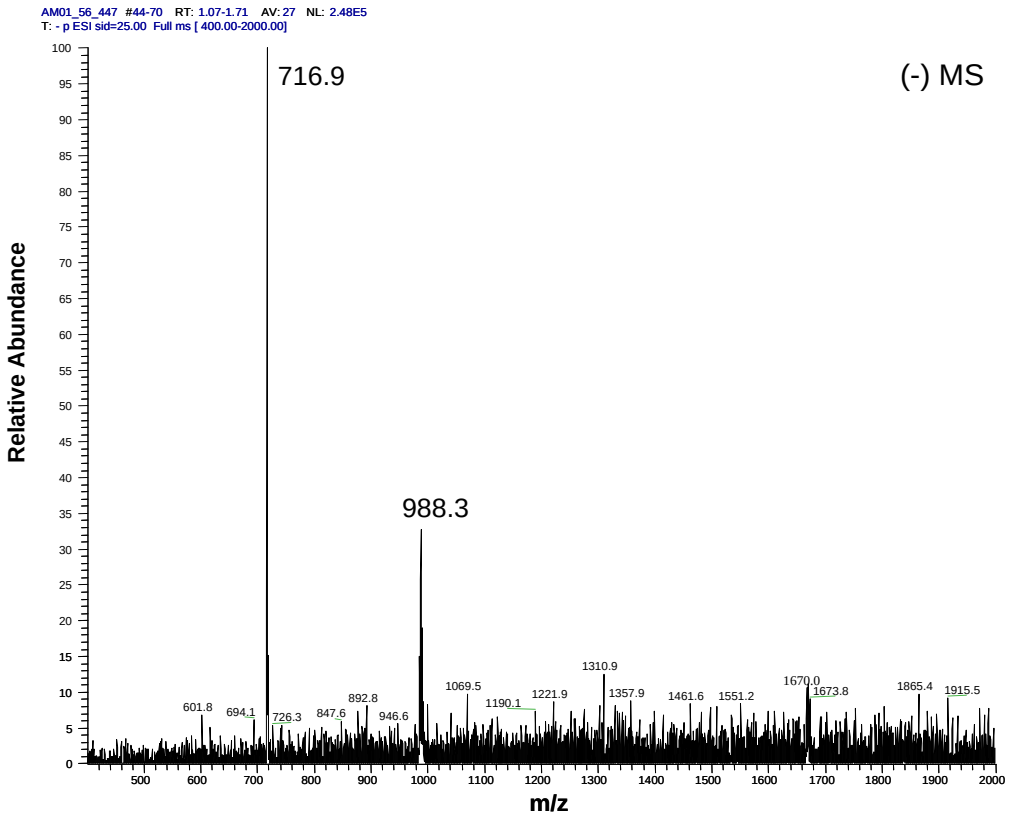
DART-TOF-Mass spectrum of compound 1



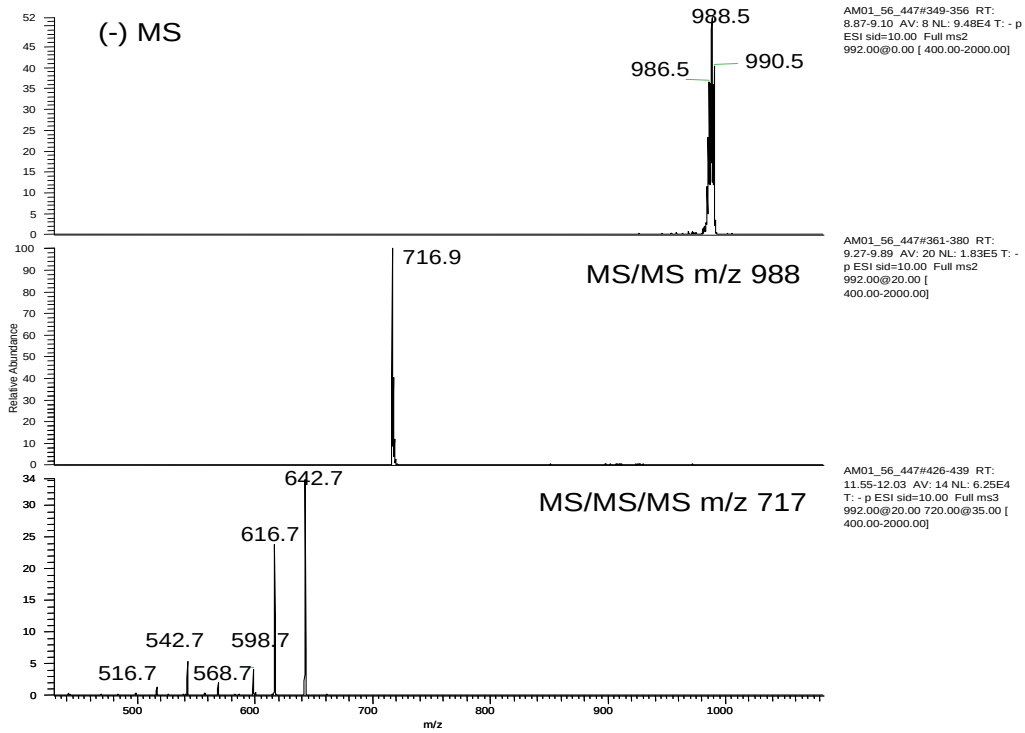
ESI-Mass spectrum of probe 3



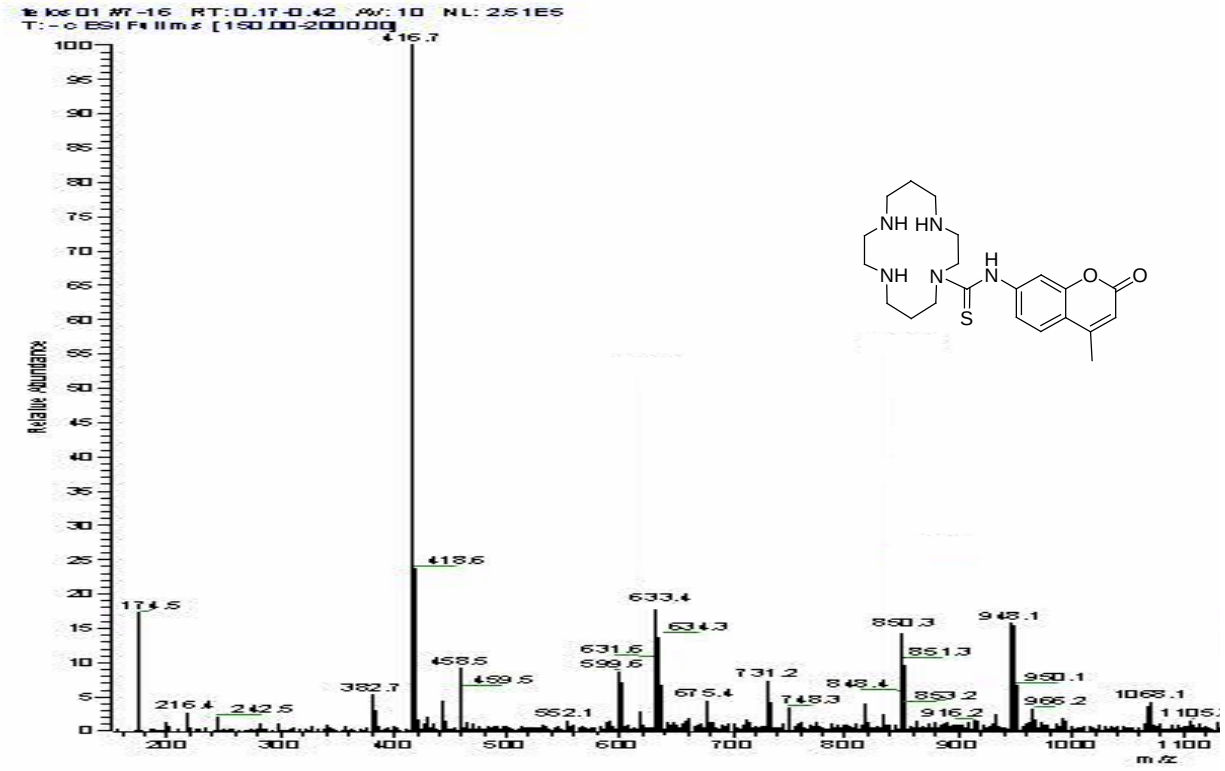
ESI-Mass spectrum of probe 3 and Hg^{2+} mixed at equal concentrations (negative ionization)



ESI-MS/MS of probe 3-Hg^{2+} complex



ESI-Mass spectrum of probe 5



III) Fluorescence Spectra for 3 in the free and Hg^{2+}-bound form

Preparation of indicators solutions

A stock indicator solution was prepared in DMSO (≥99.5%) at 10 mM concentration. An aliquot of this stock solution was then added to ultra-pure water, to make a final indicator concentration of 10 μM. Hg^{2+}-containing solutions were prepared by adding Hg^{2+} (0.01 M $HgCl_2$ solutions prepared in nanopure water) to the final indicator solution, to give Hg^{2+} concentrations ranging from 10 μM to 120 μM. The compounds are insensitive to lower concentrations and addition of higher concentrations than the maximum one resulted in quenching of fluorescence.

Ion competition study was performed by adding an aliquot of the stock indicator solution in nanopure water to make a final indicator concentration of 10 μM. To this solution metal was added (0.01 M stock metal solutions prepared in nanopure water from the corresponding chlorides) to make a final metal concentration of 120 μM and fluorescence emission was measured.

Quantum yields were calculated as described in reference 14 using 10 μM solutions of dyes and 120 μM Hg^{2+} ion concentrations.

Ion Competition Study for dye 3

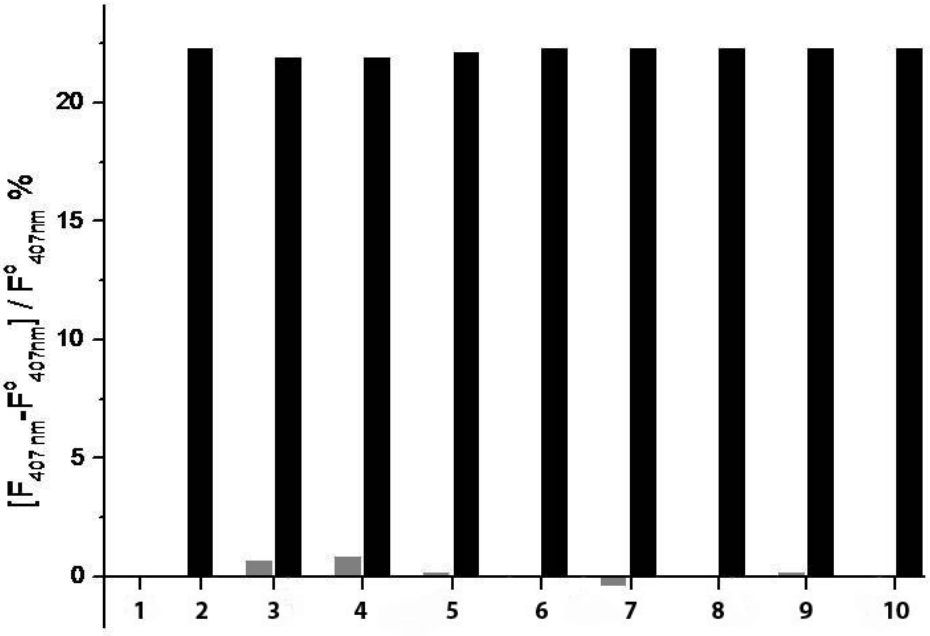


Fig. S1. Ion competition study for dye **3**. First measurement: Fluorescence ratio for the free probe (zero). Second measurement: increase of fluorescence ratio $[F_{(407)}-F^{o}_{(407)}]/F^{o}_{(407)}$ in the presence of 120 μM Hg^{2+} (black bar). Third measurement set: fluorescence ratio in the presence of 120 μM Zn^{2+} - grey bar (almost zero)- and increase of fluorescence ratio in the presence of both 120 μM Zn^{2+} and 120 μM Hg^{2+} - black bar. Likewise, the rest of the measurement sets indicate a fluorescence ratio of the dye in the presence of 120 μM of metal ions (grey bars) vs. ratio after addition of 1 equiv. of Hb^{2+} to the sample (black bars). 1:free dye, 2:Hg^{2+}, 3:Zn^{2+}, 4:Cd^{2+}, 5:Pb^{2+}, 6:Ca^{2+}, 7:Ni^{2+}, 8:Co^{2+}, 9:Cu^{2+}, 10:Na^{2+}

IV) Representative measurements of fluorescence enhancement of probe 5 in the presence of ions.

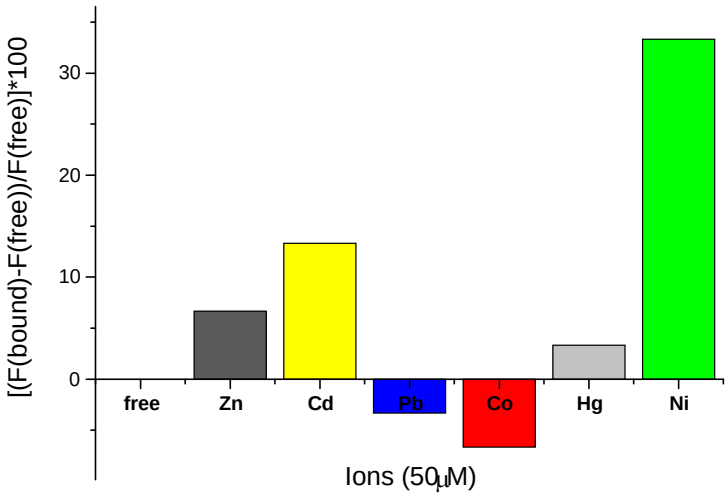
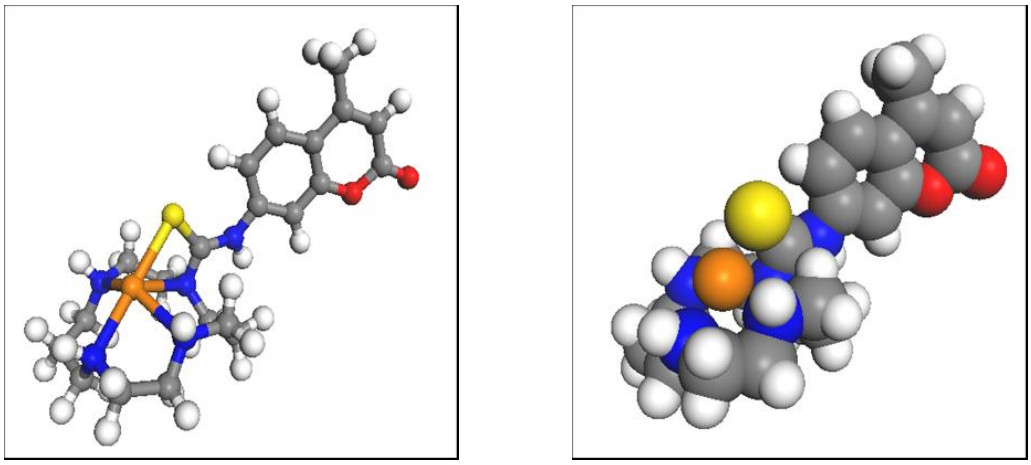


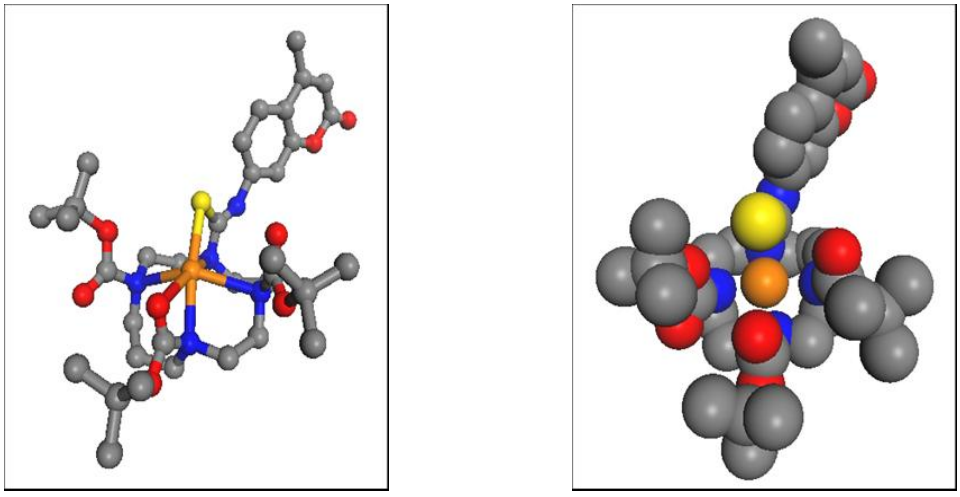
Fig. S2. Indicative measurements of fluorescence enhancement, upon binding of probe **5** with a host of ions. Experiments were performed using buffer (10mM HEPES, 140mM NaCl, 2.5 mM KCl, pH= 7.5) and final ion concentration 50 μM. The results verify the total absence of selectivity of **5** for the ions tested. Ni^{2+} ions are known to form stable complexes even with multi-cyclam ligands.[2]

V) DFT geometry optimization for the 5- Hg^{2+} and 3-Hg^{2+} complexes



A **B**

Fig. S3. DFT geometry optimization for the **5**-Hg^{2+} complex (sensor without butyloxycarbonyl-groups). Atoms are represented in colours; mercury: orange, sulphur: yellow, nitrogen: blue, oxygen: red, carbon: grey, hydrogen: white. In Fig. **S3B**, CPK visualization method was used, in which atoms are rendered as spheres with radii related to the van der Waals (VDW) radii. The VDW radii for all atoms can be found in references 3 and 4.[3,4]



A **B**

Fig. S4. DFT geometry optimization for the **3**-Hg^{2+} complex (sensor with butyloxycarbonyl-groups). Atoms are represented in colours; mercury: orange, sulphur: yellow, nitrogen: blue, oxygen: red, carbon: grey. Hydrogen atoms are not shown for simplicity. In Fig. **S4B**, CPK visualization method was used, in which atoms are rendered as spheres with radii related to to the van der Waals (VDW) radii. The VDW radii for all atoms can be found in references 3 and 4.

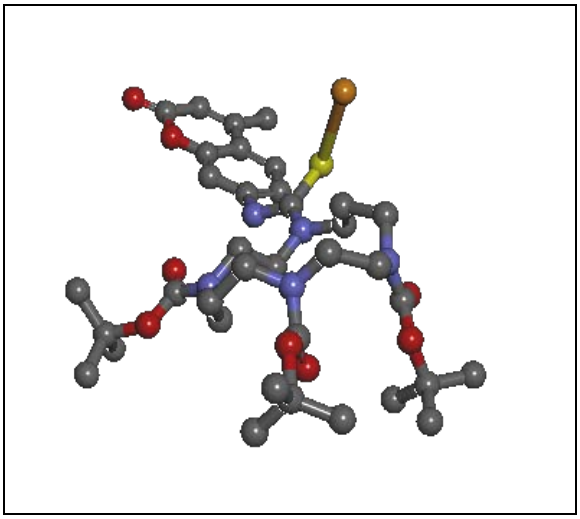


Fig. S5 DFT geometry optimization for the **3**-Hg^{2+} complex. Atoms are represented in colours; mercury: orange, sulphur: yellow, nitrogen: blue, oxygen: red, carbon: grey. Hydrogen atoms are not shown for simplicity. Figure **S5** shows geometry optimizations with all three butyloxycarbonyl-groups "facing away" from the thiourea sulphur atom. In this case coordination with the cyclam nitrogens is lost and the mercury ion is linked only to the sulphur.

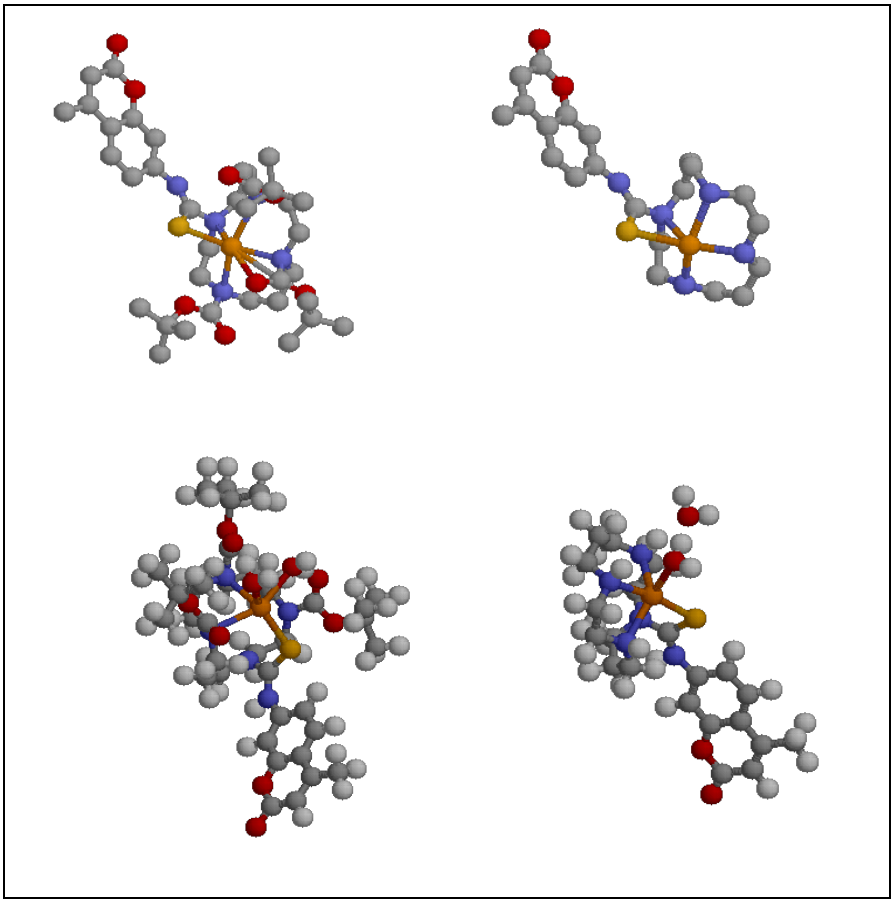


Fig. S6 COSMO geometry optimizations for the **3**-Hg^{2+} (upper left) and **5**-Hg^{2+} (upper right) complexes in "aqueous solution". Hydrogen atoms are not shown for simplicity. No significant changes in the Hg-N bond distances were observed. A second set of DFT calculations on **3**-Hg^{2+} and **5**-Hg^{2+} were performed in "gas phase" in the presence of two molecules of water. The results indicated that in **5**-Hg^{2+} (lower right), one of the water molecules is coordinated with the mercury ion, whereas the second one forms a hydrogen bond with a N-H hydrogen in the cyclam system. No significant differences were observed in Hg-N bond distances. In **3**-Hg^{2+} (lower left) both water molecules are coordinated with the mercury ion and Hg-N bond distances increased distinctly for two of the cyclam nitrogens. These results support the hypothesis that in aqueous solution, water may compete with the ligand and as a result weaker ligand coordination would be expected.

Atoms are represented in colours; mercury: orange, sulphur: yellow, nitrogen: blue, oxygen: red, carbon: grey, hydrogen: white.

Table S1 Bond lengths calculated in DFT geometry optimizations for complexes

Distances (Å)	**S-Hg**	**N1-Hg**	**N2-Hg**	**N3-Hg**	**N4-Hg**
Probe 5					
"Gas phase"	2.58	2.40	2.47	2.70	2.47
"Gas phase"-2H_2O	2.61	2.415	2.50	2.82	2.455
Solvation model-COSMO	2.65	2.39	2.42	2.78	2.40
Probe 3					
"Gas phase"	2.53	2.39	2.655	2.77	2.68
"Gas phase"-2H_2O	2.51	2.48	2.76	3.30	3.58
Solvation model-COSMO	2.50	2.33	2.71	2.735	2.70

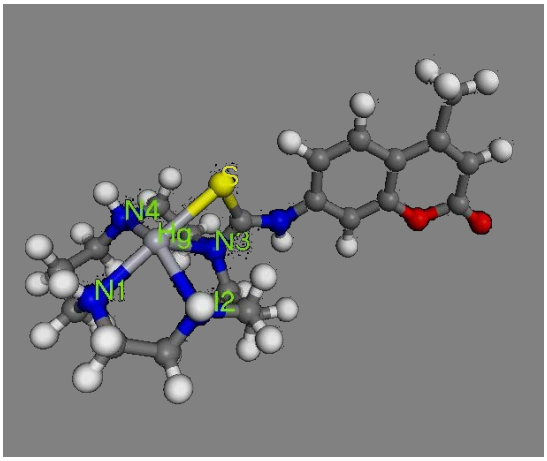


Table S2 Differences in binding energies of Hg^{2+} and Zn^{2+} ions with probes **3** and **5**.

$E_{binding}$ X- $E_{binding}$ Y	ΔE (kcal/mol)
$E_{binding}$ (Zn^{2+}-**3**)- $E_{binding}$ (Hg^{2+}-**3**)	20.0
$E_{binding}$ (Zn^{2+}-**5**)- $E_{binding}$ (Hg^{2+}-**5**)	31,4
$E_{binding}$ (Hg^{+2}-**5**)- $E_{binding}$ (Hg^{+2}-**3**)	33,0
$E_{binding}$ (Zn^{2+}-**5**)- $E_{binding}$ (Zn^{2+}-**3**)	44,8

VII) Detection limit of probe 3 for Hg^{2+}ions.

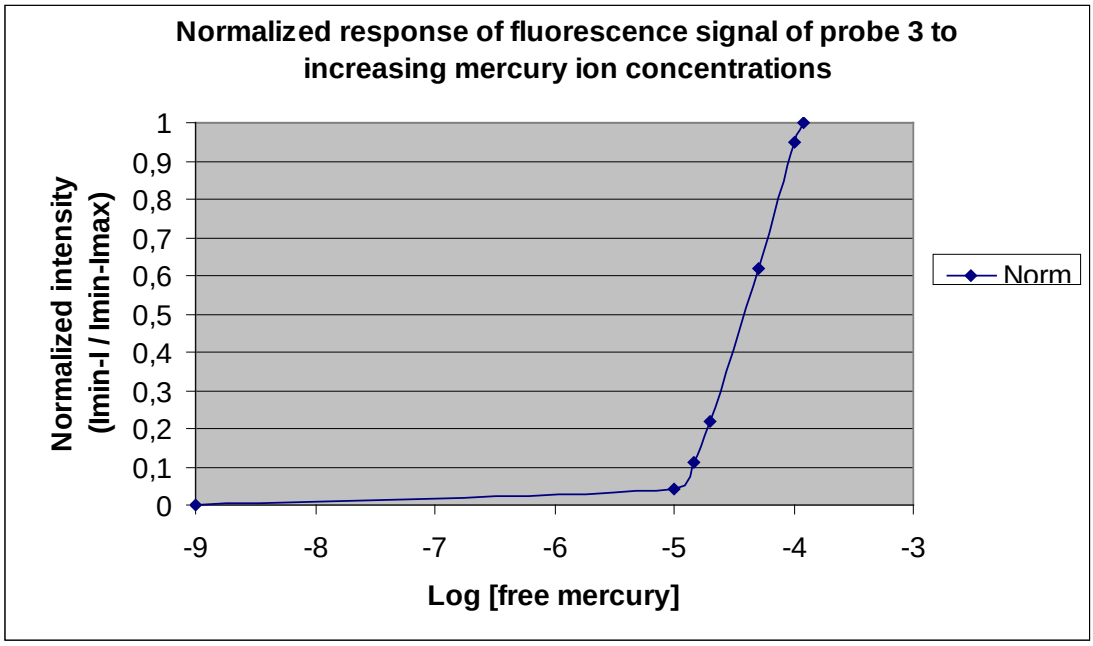


Fig. S6. Fluorescence intensity of the sensor at each concentration of mercury ion, normalized between the minimum fluorescence intensity, found at zero free mercury (shown on the graph as 1,0 nM), and the maximum fluorescence intensity, found at 120,0 μM. A linear regression curve was fitted to the five intermediate values (10,0 μM -100,0 μM) of **Fig. S6**. The point at which this line crossed the ordinate axis was taken as the detection limit and equalled approximately 21 μM.[5]

LITERATURE

[1] G. G. Morlock, and Y. Ueda, *J. Chromatogr. A*, , 2007, **1143**, 243-251.

[2] Y. Dong , L. F. Lindoy , P. Turner and G. Wei *Dalton Trans.*, 2004, 1264-1270

[3] A.Bondi, *J. Phys. Chem.* 1964, **68**, 441-451.

[4] M. Mantina, A. C. Chamberlin, R. Valero, C. J. Cramer, and D. G. Truhlar, *J. Phys. Chem. A*, 2009, **113**, 5806-5812

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