Electronic Supplementary Material (ESI) for Chemical Communications

A Ratiometric Naphthalimide Sensor for Live Cell Imaging of Copper(I)

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Electronic Supporting Information

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General experimental methods. The NMR experiments were carried out at 27° C on a Varian UNITY Inova 500 MHz spectrometer (^{1}H at 499.88 MHz, ^{13}C-NMR at 125.7 MHz) equipped with pulse field gradient module (Z axis) and a tuneable 5 mm Varian inverse detection probe (ID-PFG). ESI mass spectra were acquired on a ES-MS Thermo-Finnigan LCQ-DECA using MeOH (positive ion mode). A JASCO V-560 UV-vis spectrophotometer equipped with a 1 cm path-length cell was used for the measurement of Job's plots and for the UV-vis measurements. Luminescence measurements were carried out using a Cary Eclipse Fluorescence spectrophotometer with a λ_{ex} of 335 nm and a 0.5 nm resolution, at room temperature. The emission was recorded at 90° with respect to the exciting line beam using 10:10 slit-widths for all measurements. All chemicals were reagent grade and were used without further purification. Millipore water was used to prepare all aqueous solutions. All spectroscopic measurements in solution were performed in 20 mM HEPES buffer (with 50%$_{v/v}$ of CH_3CN), pH 7.2.

Procedure for ^{1}H NMR titration. Two mother solutions of **Naphtyl-CS1** (receptor) and Cu^+ (guest) (7.0 x 10^{-3} M) in CD_3CN were prepared in order to get different solutions with different receptor/guest ratios as reported below, and ^{1}H NMR spectra were recorded at 27±1°C.

Procedure for fluorescence titration. Two mother solutions of **Naphtyl-CS1** (receptor) and Cu^+ (guest) (7.0 x 10^{-3} M) in 20 mM HEPES buffer (with 50%$_{v/v}$ of CH_3CN), pH 7.2, were prepared. From these, different solutions with different ratio receptor/guest were prepared as reported below, and emission spectra, normalized to eliminate dilution effect, were recorded. Fluorescence titration was carried out using λ_{exc} 335 nm and recording at λ_{em} 470 and 550 nm at 25°C. The stability constants for the aqueous Cu^+–CH_3CN system have been accurately determined[1] and can be used in the nonlinear least-squares data analysis to account for competitive binding by CH_3CN.[2] With this data treatment, the apparent binding affinity of **Naphtyl-CS1** was estimated to be log β (12.1 ± 0.6), using HypSpec (version 1.1.33),[3] a software designed to extract equilibrium constants from potentiometric and/or spectrophotometric titration data. HypSpec starts with an assumed complex formation scheme and uses a least-squares approach to derive the spectra of the complexes and the stability constants. χ^2 test (chi-square) was applied, where the residuals follow a normal distribution (for a distribution approximately normal, the χ^2 test value is around 12 or less). In all of the cases, $\chi^2 \leq 10$ were found, as obtained by 3 independent measurements sets. The Φ_F value **of Naphtyl−CS1** change from 0.075 to 0.088 by addition of 1 equivalent of Cu^+, with a 2-fold emission turn-on detected at 470 nm (see S10). Fluorescence quantum yields (Φ_F) were estimated by using *N*-butyl-4-butylamino-1,8-naphthalimide as a standard (Φ_F = 0.81).[4]

Determination of Stoichiometry. Stoichiometry of the complex was investigated by the Job's plot method using spectrophotometric measurements. The samples were prepared by mixing equimolecular stock solutions (2 x 10^{-4} M) of **Naphtyl-CS1** and Cu^+ to cover the whole range of molar fractions keeping constant the total concentration (1×10^{-5} M). The fluorescence spectra were acquired using λ_{ex} 335 nm and recording at λ_{em} 470 nm at 25°C. The changes in emission, compared to uncomplexed receptor species ($\Delta I \times \chi^{-1}$) were calculated and reported versus the receptor mole fraction (χ). These plot show invariably a maximum at 0.5 mol fraction of receptor indicating its 1:1 complex formation with the Cu^+ ion.

Determination of Selectivity

The solutions of the metal ions were prepared from $NiCl_2 \cdot 2H_2O$, $CaCl_2$, $FeCl_2 \cdot 4H_2O$, $MgCl_2$, $CuSO_4$, $Co(OAc)_2 \cdot 4H_2O$, KPF_6, $ZnCl_2 \cdot 2H_2O$, NaCl and $[Cu(CH_3CN)]PF_6$, respectively.

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[1] Kamau, P.; Jordan, R. B. *Inorg. Chem.* **2001**, *40*, 3879–3883

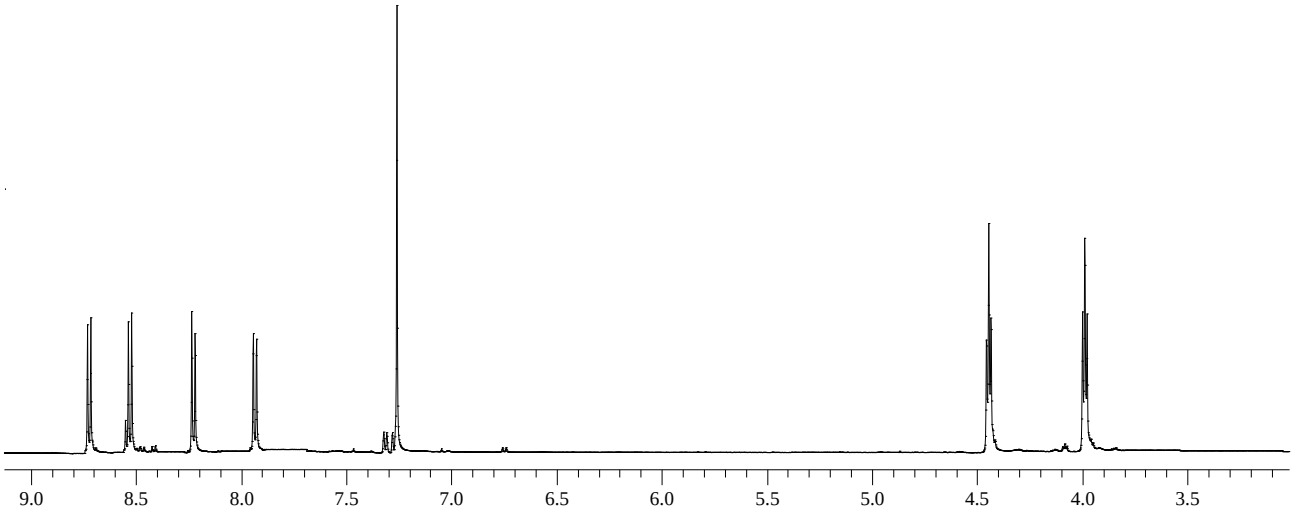
[2] Yang, L. ; McRae, R. ; Henary, M. M.; Patel, R. ; Lai, B.; Vogt, S.; Fahrni, C. J. *Proc. Nat. Acad. Sci.*, **2005**, *102*, 11179–11184

[3] (a) Jennings, A. R.; Son, D. Y. *Tetrahedron Lett.* **2012**, *53*, 2181−2184. (b) Pappalardo, A.; Ballistreri, F. P.; Li Destri, G.; Mineo, P. G.; Tomaselli, G. A.; Toscano, R. M.; Trusso Sfrazzetto, G. *Macromolecules* **2012**, *45*, 7549−7556. (c) Pappalardo, A.; Amato, M. E.; Ballistreri, F. P..; Tomaselli, G. A.; Toscano, R. M.; Trusso Sfrazzetto, G. *J. Org. Chem.* **2012**, *77*, 7684-7687.

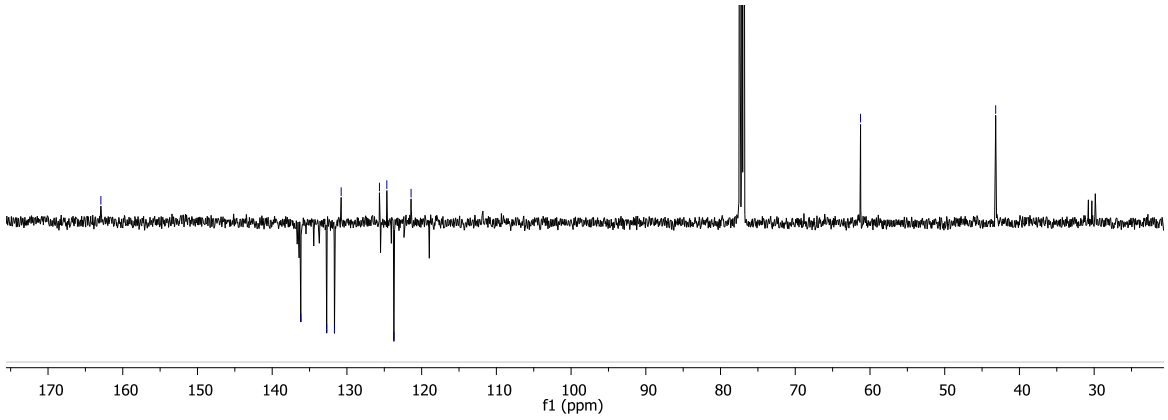
[4] Guo, X. Qian, L. Jia, *J. Am. Chem. Soc.* **2004**, *126*, 2272−2273.

Synthesis of *N*-(4-hydroxyethyl)-4-bromo-5-nitro-1,8-naphthalimide (2)

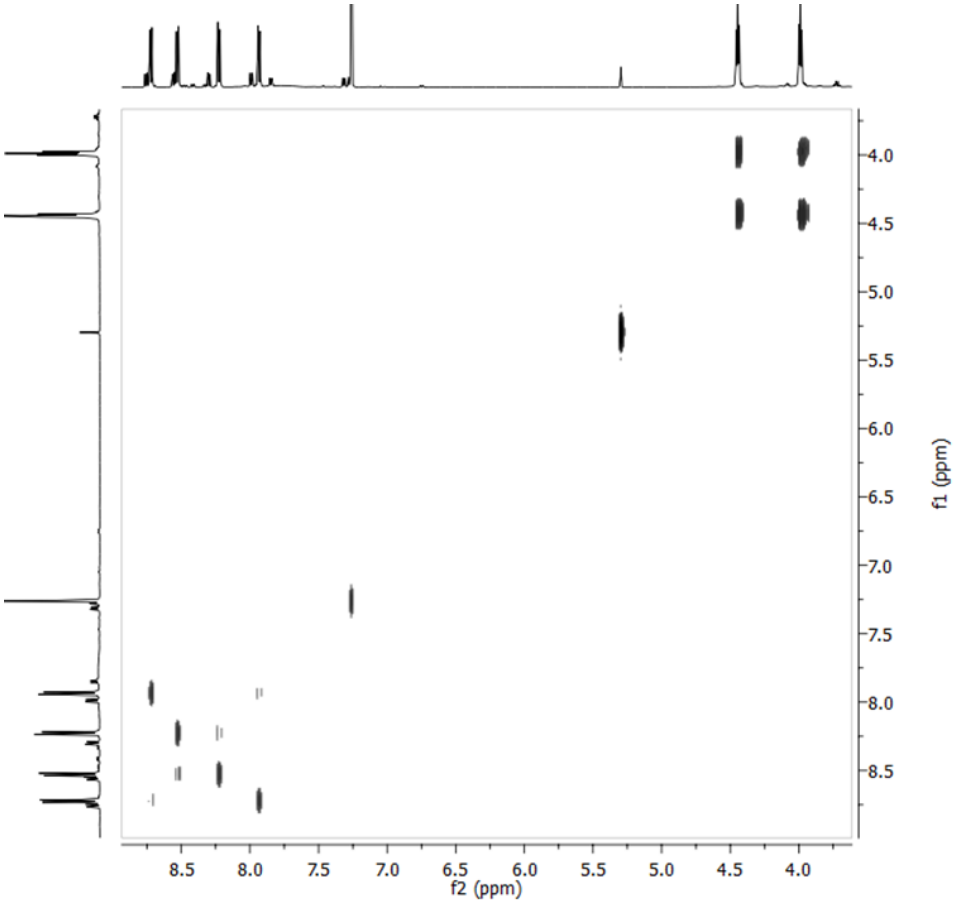
A suspension of 1g (3.12 mmol) of 4-bromo-5-nitro-1,8-naphthalic anhydride (**1**) in ethanol (30 mL) was stirred at 45°C under nitrogen for 1 h. 2.1 g (3.30 mmol) of ethanolamine was added dropwise and the reaction was stirred at 50°C for 24 h. Then, the solvent was removed under reduced pressure and CH_2Cl_2 (50 mL) was added to the crude product. The solid was filtered, and the organic solution was concentrated to afford the pure compound **2** (855 mg, 72% yield). ^{1}H NMR δ = 8.72 (d, *J* = 8 Hz, 1H), 8.53 (d, *J* = 8 Hz, 1H), 8.23 (d, *J* = 8 Hz, 1H), 7.94 (d, *J* = 8 Hz, 1H), 4.45 (t, *J* = 5 Hz, 2H), 3.99 (t, *J* = 5 Hz, 2H). ^{13}C NMR δ = 162.93, 136.17, 132.71, 131.67, 130.77, 125.65, 124.66, 123.71, 121.42, 61.27, 43.18. ESI MS *m/z* 364.0 $[M+H]^+$. Anal. Calcd. For $C_{14}H_9BrN_2O_5$: C, 46.05; H, 2.48; Br, 21.88; N, 7.67; O, 21.91. Found: C, 46.01; H, 2.46; Br, 21.81; N, 7.62.



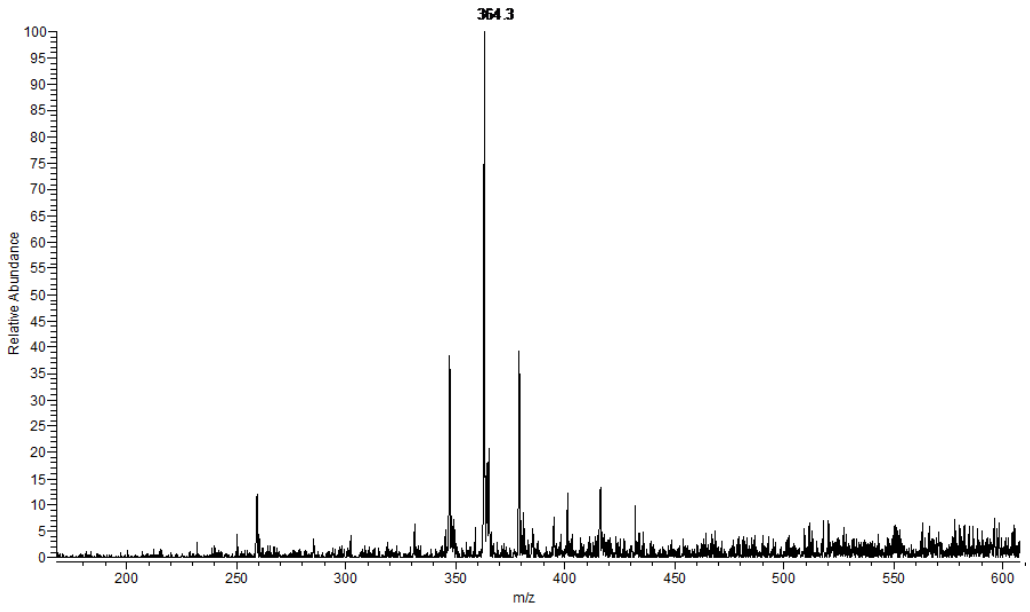
^{1}H NMR spectrum of **2** in $CDCl_3$



APT spectrum of **2** in $CDCl_3$



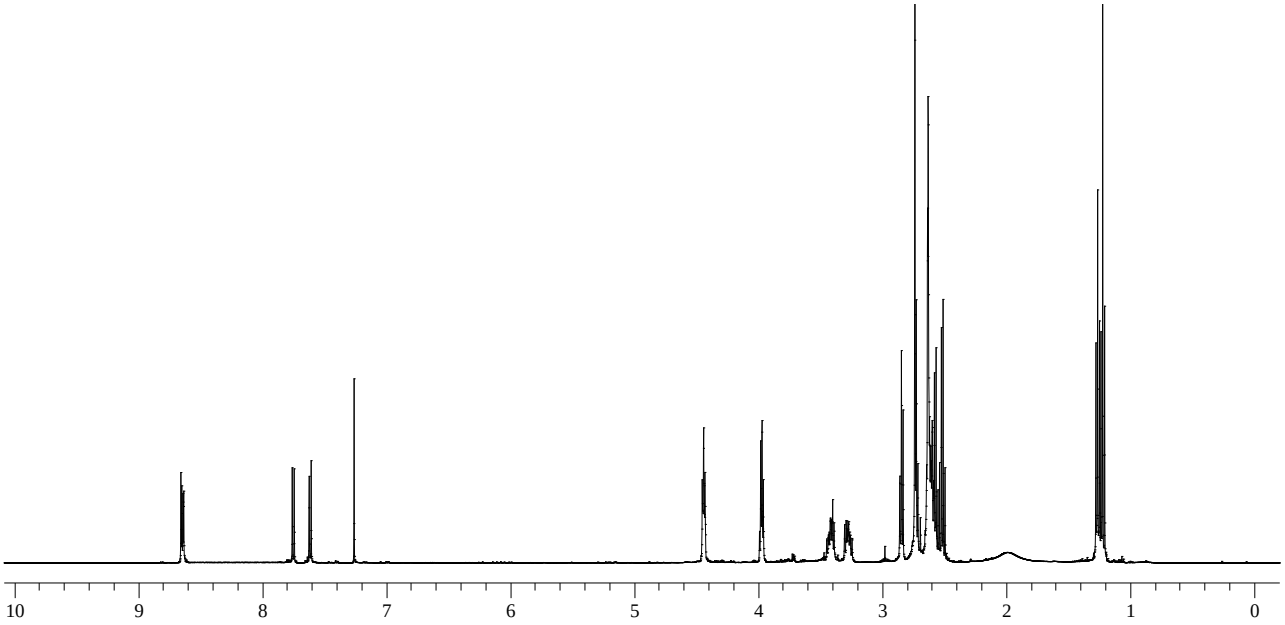
g-COSY spectrum of **2** in $CDCl_3$



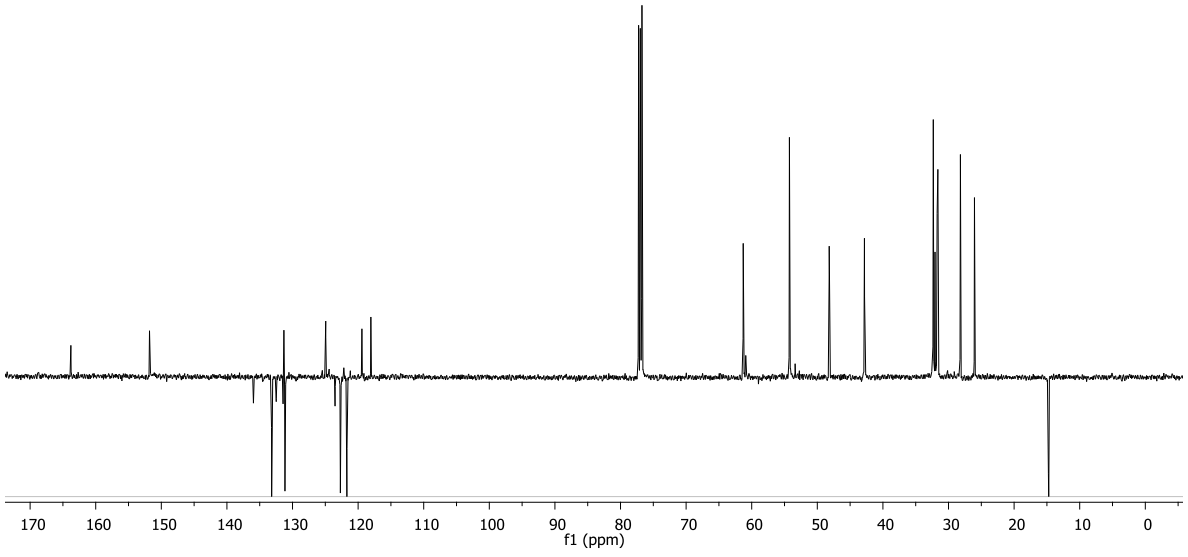
ESI-MS spectrum of **2**

Synthesis of Naphtyl-CS1

A solution of **2** (202 mg, 0.55 mmol) and 3,6,12,15-Tetrathia-9-monoazaheptadecane[5] (170 mg, 0.50 mmol) in 2-methoxyethanol (8mL) was refluxed under nitrogen atmosphere for 24h. The solvent was removed under reduced pressure and the crude product was separated by flash chromatography (SIO_2, eluent: Hexane/EtOAc 90:10) affording **Naphtyl-CS1** as a red oil (85 mg, 26% yield). ^{1}H NMR δ = 8.65 (d, *J* = 8 Hz, 1H), 8.64 (d, *J* = 8 Hz, 1H), 7.75 (d, *J* = 8 Hz, 1H), 7.61 (d, *J* = 8 Hz, 1H), 4.44 (t, *J* = 5 Hz, 2H), 3.97 (t, *J* = 5 Hz, 2H), 3.39-3.45 (m, 1H), 3.24-3.30 (m, 2H), 2.85 (t, *J* = 6 Hz, 2H), 2.71-2.75 (m, 6H), 2.60-2.64 (m, 6H), 2.57 (q, *J* = 7 Hz, 2H), 2.52 (q, *J* = 7 Hz, 2H), 1.26 (t, *J* = 7 Hz, 3H), 1.22 (t, *J* = 7 Hz, 3H). ^{13}C NMR δ = 163.79, 151.80, 135.96, 133.18, 132.48, 131.31, 131.15, 124.94, 123.52, 122.70, 121.71, 119.42, 118.06, 61.29, 54.25, 48.18, 42.82, 32.32, 32.18, 32.06, 31.74, 31.62, 28.19, 26.04, 14.77, 14.72. ESI MS *m/z* 598 $[M+H]^+$, 620 $[M+Na]^+$, 636 $[M+K]^+$. Anal. Calcd. For $C_{26}H_{35}N_3O_5S_4$: C, 52.24; H, 5.90; N, 7.03; O, 13.38; S, 21.45. Found: C, 52.19; H, 5.87; N, 7.00; S, 21.41.

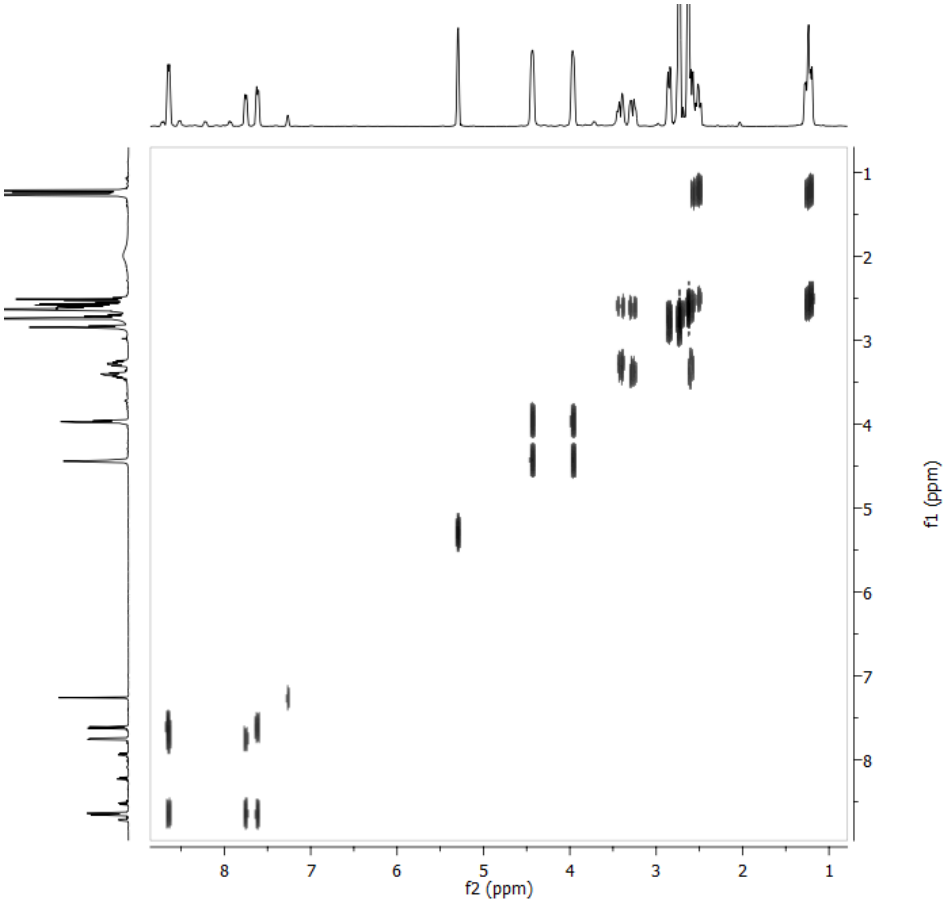


^{1}H NMR spectrum of **Naphtyl-CS1** in $CDCl_3$

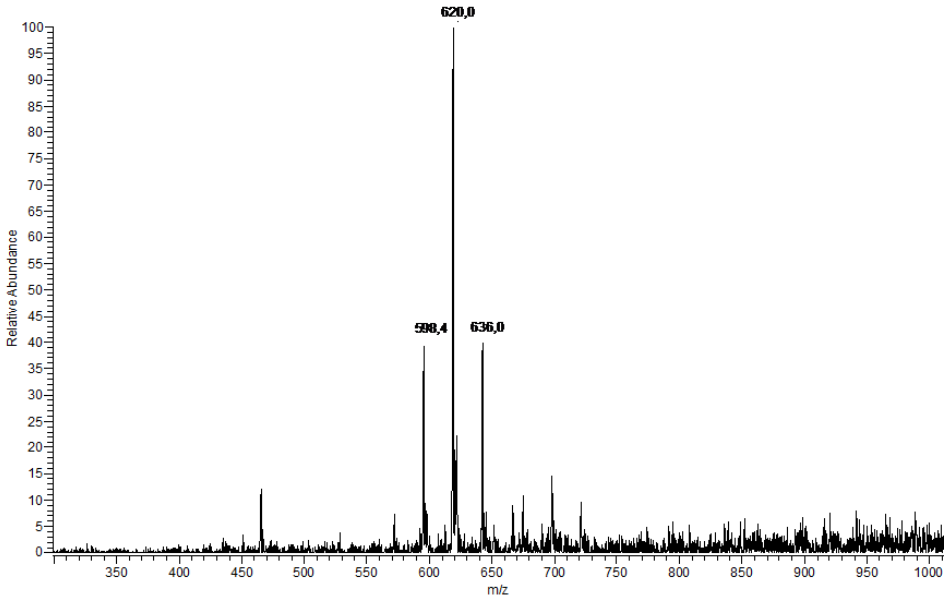


APT spectrum of **Naphtyl-CS1** in $CDCl_3$

[5] L. Zeng, E. W. Miller, A. Pralle, E. Y. Isacoff, C. J. Chang, *J. Am. Chem. Soc.*, **2006**, *128*, 10−11

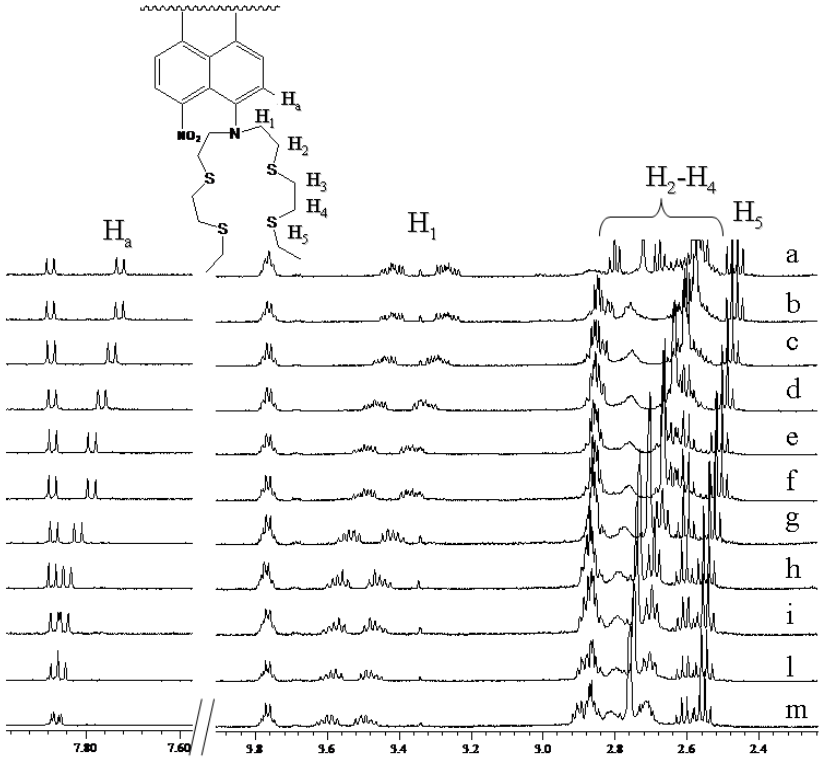


g-COSY spectrum of **Naphtyl-CS1** in $CDCl_3$



ESI-MS spectrum of **Naphtyl-CS1**

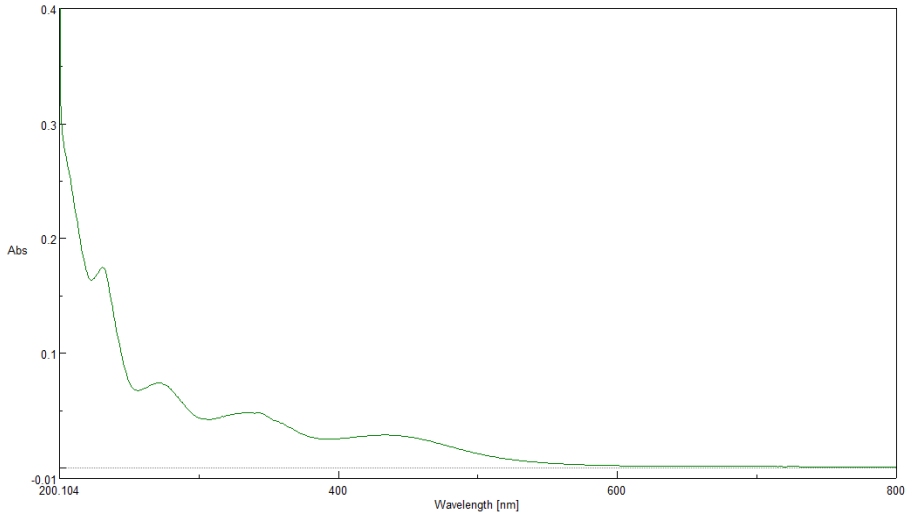
^{1}H NMR titration of Naphtyl-CS1 and Cu^+

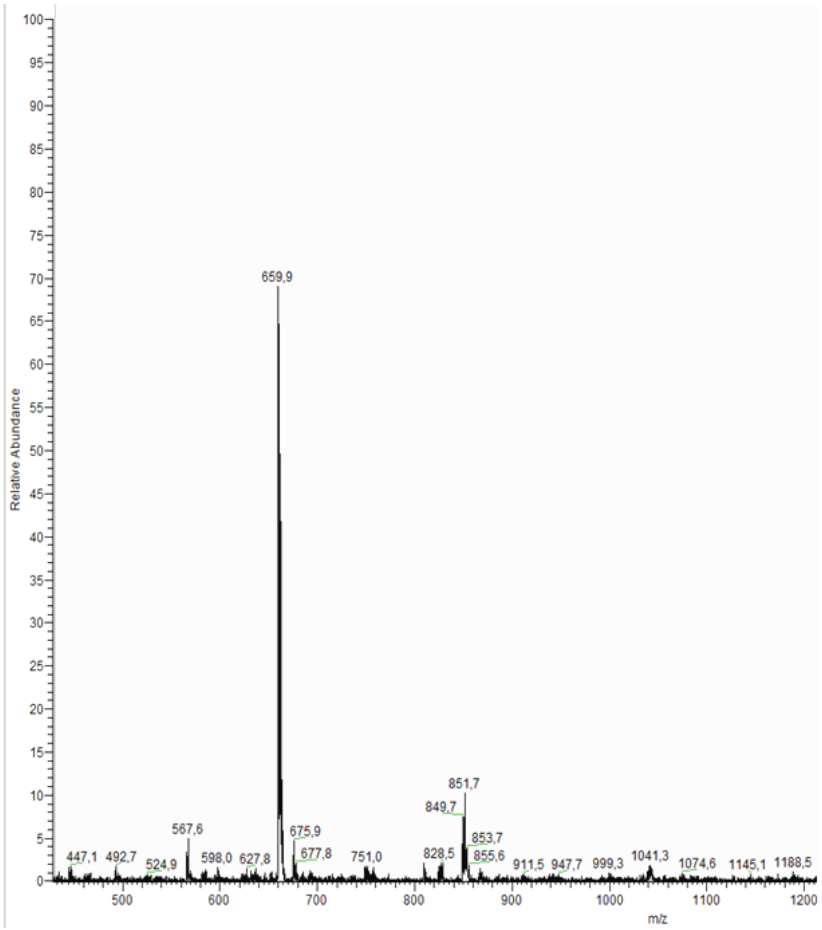


Selected regions of ^{1}H NMR titration between **Naphtyl-CS1** and Cu^+ in CD_3CN at 27°C. Initial concentration of receptor (2.00×10^{-3} M) with addition of various equivalents of Cu^+: (a) receptor, (b) 0.2 eq., (c) 0.4 eq., (d) 0.6 eq., (e) 0.8 eq., (f) 1.0 eq., (g) 1.2 eq. (h) 1.4 eq., (i) 1.6 eq., (l) 1.8 eq., (m) 2.0 eq.

UV-Vis spectrum of Naphtyl-CS1 (1.00 x 10^{-5}M) in 20 mM HEPES buffer (with 50%$_{v/v}$ of CH_3CN), pH 7.2.

The UV−Vis spectrum of **Napthyl−CS1** shows 4 bands respectively at 232, 272, 338 and 435 nm.

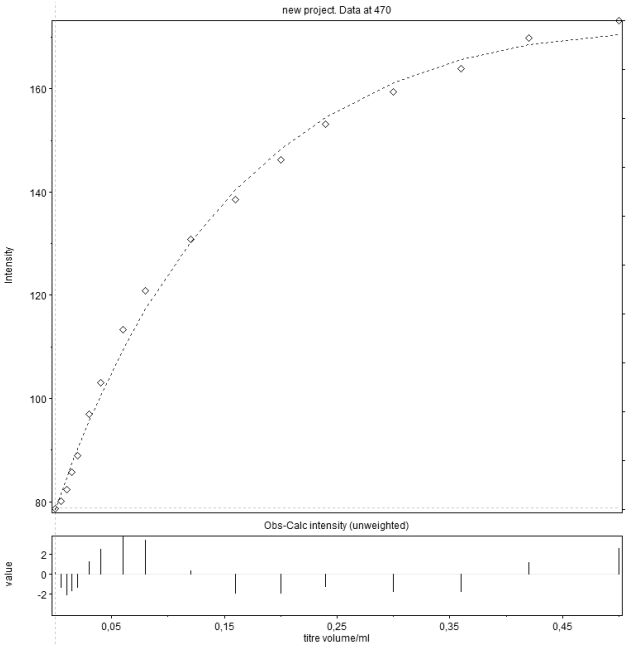




ESI-MS spectrum of [Naphtyl-CS1 + Cu]$^+$

Fluorescence titrations of Naphtyl-CS1 and Cu^{+}

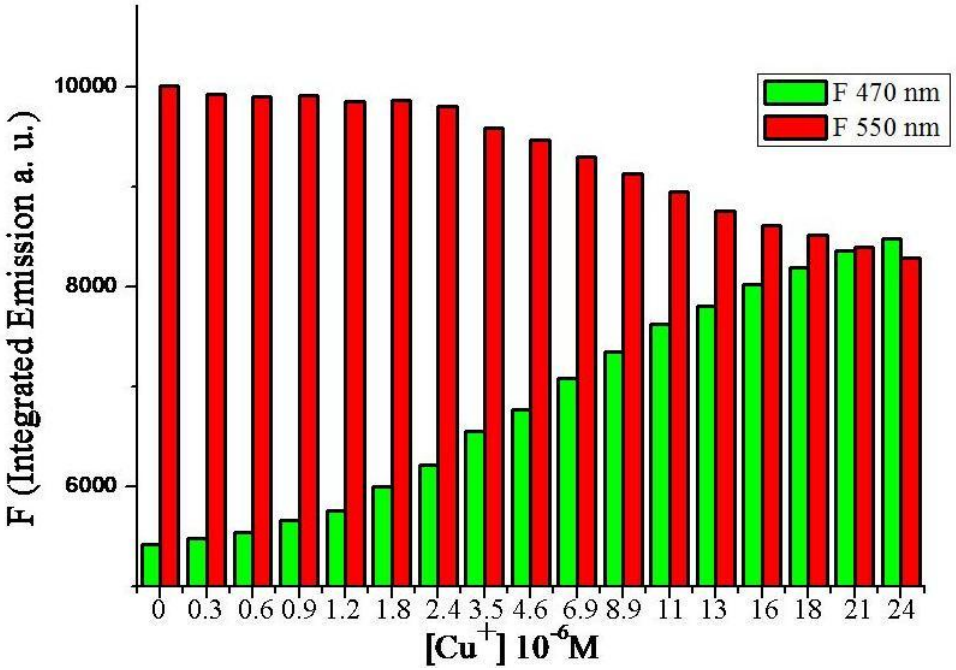
HypSpec plot for binding constant determination:



Converged in 1 iterations with sigma = 4,6888

Log beta	value	standard deviation
BC	2,63	
BC2	4,02	
BC3	4,3	
AB	12.0634	0.6424

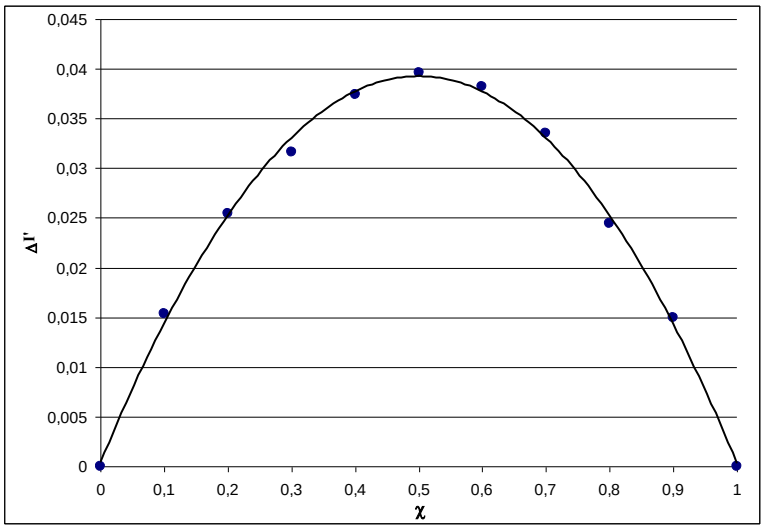
Fluorescence Emission Ratio of Naphtyl-CS1



The integrated fluorescence emission of **Naphtyl-CS1** (10μM) after addition of different amounts of Cu^{+}. Excitation was provided at 335 nm and the emission was collected over 450-800 nm in a HEPES buffer solution (pH = 7.2). Red and green bars represent the integrated emission from 459-510 nm (F 470) and 510-700 nm (F 550), respectively.

Job's Plot of Naphtyl-CS1 and Cu^{+}

χ	I	Δl'
0	79,13	0
0,1	72,64	0,015408
0,2	71,28	0,025478
0,3	69,64	0,031612
0,4	68,45	0,037453
0,5	66,52	0,039651
0,6	63,42	0,038192
0,7	58,22	0,033477
0,8	46,44	0,024472
0,9	19,01	0,01497



Cell culture

Human neuroblastoma SH-SY5Y cells were propagated as undifferentiated cells in DMEM-F-12 (1:1) medium, supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 2 mM l-glutamine and maintained in a humidified incubator at 37°C in 5% CO_2. Before the experiment cells were plated into glass-bottom dishes at a density of $1x10^5$ cells/dish and medium were replaced every three days with freshly-prepared DMEM-F-12 containing decreasing concentration of heat inactivated fetal bovine serum (FBS). To induce differentiation of SH-SY5Y into neuronal-like cells, 5 μM of All-trans retinoic acid (RA) were added to medium containing 1% of FBS, for up to 5 days.

Treatment of cells with copper

$CuSO_4$ from stock solution (1 mM in ultrapure water) was applied to the differentiated cells at a concentration of 100 μM for 6 hours. Cells were washed twice with 10 mM HEPES after the
treatment to remove extra-cellular Cu before imaging.

Laser scanning confocal microscopy (LSM)

LSM observations were carried out by using an Olympus FV1000 confocal laser-scanning microscope, equipped with UV/visible lasers: 405nm (50mW), 20mW Multiline Argon laser (457nm, 488nm, 515nm, Total 30mW), HeNe(G) laser (543nm, 1mW), HeNe(R) laser (633nm, 1mW); oil immersion objective (60xO PLAPO) and spectral filtering system. Excitation wavelengths were set at 405 nm, 488 nm and 633 nm, and emitted light was detected in sequential mode in the spectral windows respectively of blue (440-510 nm), green (500-530 nm) and red (650-700 nm). The detector gain was fixed at a constant value and images were taken for all of the samples at random locations throughout the area of the well.

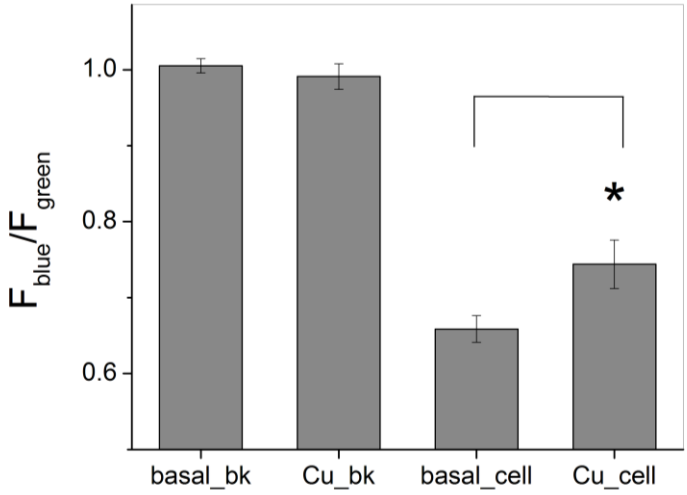
Live cell imaging

For the detection of **Napthyl-CS1** fluorescence in live cell imaging, a 1 mM stock concentration in DMSO was added to the cells for 5 minutes at a final working concentration of 500 nM. After the incubation period, the cells were rinsed and analyzed in HEPES buffer.

For staining of mitochondria and trans-Golgi organelles, cells were treated respectively with Mitotracker Deep Red (Molecular Probes, 50 nM) and BODIPY FL C5-ceramide-BSA complex (Molecular Probes, 2.25 μM) trackers for 15 minutes, rinsed with HEPES and immediately observed at the confocal microscope.

Quantitative image analysis was performed with IMAGEJ software (1.43m version). Specifically, after preliminary background subtraction, mean of the median value for n fields of cells with standard error of means is reported, and statistical analyses were performed with a one-way Anova test in Microcal Origin (8.6 version) software.

Integrated fluorescence ratio in living cells



Results of LSM image analysis of **Naphtyl-CS1** (500 nM)-treated samples in terms of the integrated fluorescence response ratio for 440-510 nm emission (F_{blue}) over the emission in the 500-530 nm range (F_{green}). The considered regions of interest for each acquired field are the background (bk) and the intracellular areas (cell) for both basal and copper-supplemented cells. (*= the means difference is significant at the 0.01 level, as determined by Anova test).