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Supramolecular Chemistry

Publication details, including instructions for authors and subscription information:

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First published on: 17 February 2010

To cite this Article Tian, Demei, Yan, Huijuan and Li, Haibing(2010) 'A selective fluorescent probe of Hg²⁺ based on triazole-linked 8-oxyquinoline calix[4]arene by click chemistry', *Supramolecular Chemistry*, 22: 4, 249 – 255, First published on: 17 February 2010 (iFirst)

To link to this Article: DOI: 10.1080/10610270903410504

URL: <http://dx.doi.org/10.1080/10610270903410504>

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A selective fluorescent probe of Hg²⁺ based on triazole-linked 8-oxyquinoline calix[4]arene by click chemistry

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(Received 3 August 2009; final version received 11 October 2009)

Calixarene compound **1** with two 8-oxyquinoline subunits was synthesised using ‘click’ chemistry. Compound **1** shows strong fluorescence quenching by Hg²⁺ and lower pH. Using the fluorescence behaviour of **1** towards Hg²⁺ and lower pH, a NOR (not an OR) logic gate was established.

Keywords: calixarene; 8-oxyquinoline; fluorescent probe; click chemistry; logic gate

1. Introduction

Mercury ion is considered as a highly toxic environmental pollutant (1). Despite a reduction in its industrial use, high concentrations of mercury are still present in many environmental compartments. Thus, a number of highly sensitive and selective Hg²⁺ sensors have been developed (2–7). Fluorescent mercuric sensors have been designed using crown ether (8), porphyrin (9) and other types of small molecules (10–12).

Calixarene has also been thoroughly investigated as the framework of many fluorescent chemosensors due to its unique cavity and easy modification (13). Lately, fluorescent chemosensors based on the calix[4]arene scaffold have been coming up as good candidates for the detection of various metal ions, especially Hg²⁺, Cu²⁺, Pb²⁺, Zn²⁺, Ni²⁺, etc. (14–17). In general, the methods of linking fluorophore to calixarenes were by ethers (18, 19), amide (20–23), esters (24–28), crown esters (29–33), etc. Thus, the synthesis of calixarene chemosensors via the new linking methods has attracted more and more attention (34). Nowadays, ‘click’ reaction is developed as an efficient synthesis method because of its modularity, it is tolerant of a wide range of solvents and functional groups, simple to perform and very high yielding (35). With respect to a chemosensor, the triazole formed during the click reaction could act as a good receptor for some metal ions (36–40).

In the present work, we report the syntheses of a new chemosensor (**1**) with two 8-oxyquinoline subunits linked to the lower rims of the calix[4]arene by bridging triazole moieties and its analogue (**2**) with one 8-oxyquinoline subunit on the calix[4]arene (shown in Scheme 1), and report the fluorescent behaviour of **1** upon complexation

with transitional metal ions. The result showed that the fluorescence of **1** was strongly quenched by Hg²⁺ and lower pH. Taking into account of this, a NOR (not an OR) logic gate can be established (41–43).

2. Experimental section

2.1 Instruments

¹H and ¹³C NMR spectra were recorded in CDCl₃ (or in CD₃CN) on a Varian Mercury VX600 (600 MHz) spectrometer. ESI-MS data were obtained from a Finnigan LCQ Advantage mass spectrometry service. Elemental analyses were performed on a VarioEL III instrument. Fluorescence spectra were recorded on a Fluoromax-P luminescence spectrometer.

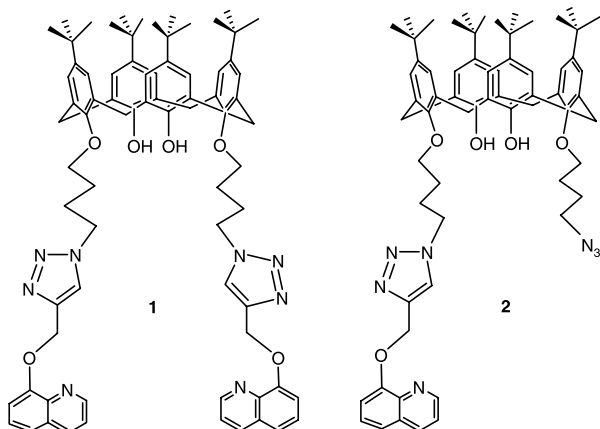
2.2 Materials

25,27-Bis-(4-bromobutanyl)-4-tert-butyl-calix[4]arene was synthesised according to the literature procedure (44). All other chemicals were commercially available and used without further purification. Petroleum ether refers to the fraction with b.p. 60–90°C.

2.3 Synthesis of 8-propyloxyquinoline (8-POQL)

A mixture of 8-hydroxyquinoline (1.45 g, 0.01 mol), 3-bromo-1-propyne (1.5 ml, 0.02 mol), K₂CO₃ (3.5 g, 0.02 mol) and acetone (100 ml) was stirred for 12 h at room temperature and then filtered. The filtrate was concentrated, dissolved in 50 ml ether and washed with 10% K₂CO₃ solution and water three times, respectively. The ether layer was dried over anhydrous Na₂SO₄, and concentrated to give a brown solid (1.68 g, 92% yield).

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Scheme 1. The structure of compounds **1** and **2**.

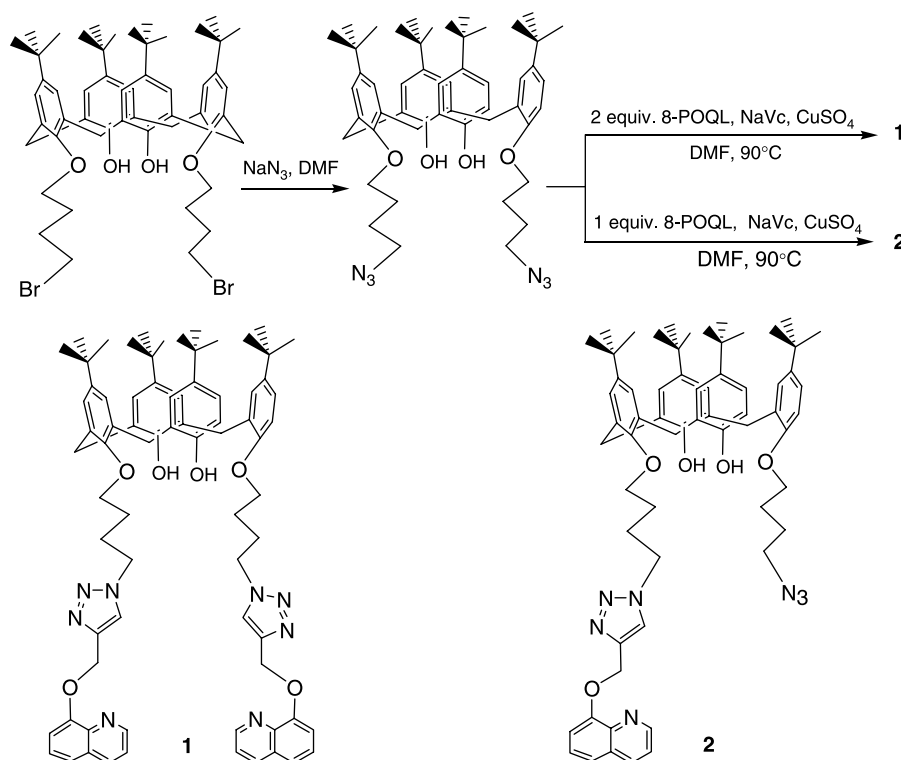
^1H NMR (CDCl_3 , 600 MHz) δ : 8.856 (dd, 1H), 8.150 (dd, 1H), 7.507–7.458 (m, 3H), 7.266 (m, 1H), 5.036 (s, 2H), 2.546 (s, 1H) (see S6, Supporting Information).

2.4 Synthesis of compound **1**

25,27-Bis-(4-bromobutanyl)-4-tert-butyl-calix[4]arene (4.58 g, 5 mmol), NaN_3 (1.3 g, 20 mmol) and 20 mg CsCl were dissolved in 30 ml DMF and reacted at 90°C and controlled by TLC. The reaction was cooled to room temperature after about 2 h when 25,27-bis-(4-bromobutanyl)-4-tert-butyl-calix[4]arene was disappeared.

Then, this mixture was poured into 200 ml of water and a pale powder was collected by filtration, washed with water for three times and obtained 25,27-bis-(4-azidobutanyl)-4-tert-butyl-calix[4]arene (3.82 g, 93%) as a pale white solid, m.p. $188\text{--}192^\circ\text{C}$.

A mixture of 8-POQL (366 mg, 2 mmol) and 25,27-bis-(4-azidobutanyl)-4-tert-butyl-calix[4]arene (842 mg, 1 mmol) in 15 ml of DMF was stirred after the reactants were dissolved. Then, sodium ascorbate (180 mg, 0.904 mmol) and $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ (25 mg, 0.0904 mmol) were added. The mixture was allowed to react at 90°C for 2 h. After cooled to room temperature, the reaction mixture was poured into 50 ml of water, and then extracted with ethyl acetate for three times. The organic layer was collected, and dried over anhydrous Na_2SO_4 . The crude product was purified by column chromatography with $V(\text{ethyl acetate})\text{--}V(\text{methanol}) = 6:1$ as the eluent to give 725 mg of **1** as a pale white solid in 60% yield. ^1H NMR (CDCl_3 , 600 MHz, TMS, ppm) δ : 8.875 (s, 2H), 8.76 (d, 4H), 7.986 (s, 2H, triazole-H), 7.382–7.326 (m, 4H), 7.072 (s, 2H), 7.034 (s, 4H, ArH, calix[4]arene), 6.713 (s, 4H, ArH, calix[4]arene), 5.523 (s, 4H, $\text{CH}_2\text{Oquinoline}$), 4.502 (t, 4H, CH_2OAr), 4.085–4.107 (d, $J = 12.6$ Hz, 4H, ArCH_2Ar), 3.828 (br, 4H, CH_2), 3.246–3.225 (d, $J = 12.6$ Hz, 4H, ArCH_2Ar), 2.162 (t, 4H, CH_2), 1.729 (t, 4H, CH_2), 1.298 (s, 18H, $-\text{C}(\text{CH}_3)_3$), 0.897 (s, 18H, $-\text{C}(\text{CH}_3)_3$); ^{13}C NMR (CDCl_3 , 150 MHz) δ : 153.9, 150.4, 149.5, 149.2, 147.0, 143.8, 141.7, 140.3, 135.9, 132.2,

Scheme 2. Synthetic scheme for **1** and **2**.

129.4, 127.7, 126.7, 125.5, 125.1, 124.0, 121.6, 120.0, 110.0, 75.9, 62.8, 50.0, 33.9, 33.8, 31.7, 31.4, 30.9, 27.6, 26.6; ESI-MS m/z : 1209.1 (M^+). Anal. calcd for $C_{76}H_{88}N_8O_6$: C 75.47, H 7.33, N 9.26; found: C 75.55, H 4.44, N 9.31.

2.5 Synthesis of compound 2

A mixture of 8-POQL (183 mg, 1 mmol) and 25,27-bis-(4-azidobutanyl)-4-tert-butyl-calix[4]arene (842 mg, 1 mmol) was reacted at the similar condition of compound 1. The crude product was purified by column chromatography with $V(\text{petroleum})-V(\text{ethyl acetate}) = 1:2$ as the eluent to give 510 mg of 2 as a pale white solid in 50% yield. ^1H NMR (CDCl_3 , 600 MHz, TMS, ppm) δ : 9.055 (s, br, 1H), 8.310 (s, br, 2H), 7.549 (br, 2H), 7.476 (s, 2H), 7.285 (s, 1H, triazole-H), 7.042 (d, d, $J = 2.4$ Hz, 4H, ArH, calix[4]arene), 6.762 (s, 4H, ArH, calix[4]arene), 5.597 (s, 2H, $\text{CH}_2\text{Oquinoline}$), 4.657 (t, 2H, CH_2OAr), 4.208–4.155 (d, d, $J = 12.6$ Hz, 4H, ArCH_2Ar), 3.984 (t, 2H, CH_2), 3.912–3.852 (br, 2H, CH_2), 3.314–3.255 (d, d, $J = 12.6$ Hz, 4H, ArCH_2Ar), 2.383 (t, 2H, CH_2), 1.988 (t, 2H, CH_2), 1.909–1.818 (br, 4H, CH_2), 1.289 (s, 18H, $\text{ArC}(\text{CH}_3)_3$), 0.937 (9H, $-\text{C}(\text{CH}_3)_3$), 0.932 (s, 9H, $-\text{C}(\text{CH}_3)_3$); ESI-MS m/z : 1026.7 (M^+). Anal. calcd for $C_{64}H_{79}N_7O_5$: C 74.89, H 7.76, N 9.55; found: C 74.94, H 7.78, N 9.44.

3. Results and discussion

3.1 Synthesis and characterisation

The synthetic pathway for the target compounds is shown in Scheme 2. Under our selected conditions, chemosensor 1 and its analogue 2 were synthesised via ‘click’ chemistry. At first, 25,27-bis-(4-azidobutanyl)-4-tert-butyl-calix[4]-

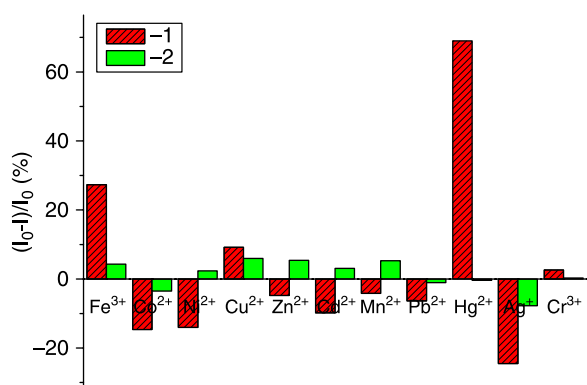


Figure 1. Fluorescence intensity changes ($(I_0 - I)/I_0 \times 100\%$) of 1 and 2 (each of 10^{-5} M) in $\text{MeCN}-\text{H}_2\text{O}$ (3:1, v/v, upon addition of various metal ions, 10 equiv.). Excitation wavelength was at 310 nm. I_0 is the fluorescent emission intensity at 390 nm of each free host and I is the fluorescent intensity after adding metal ions.

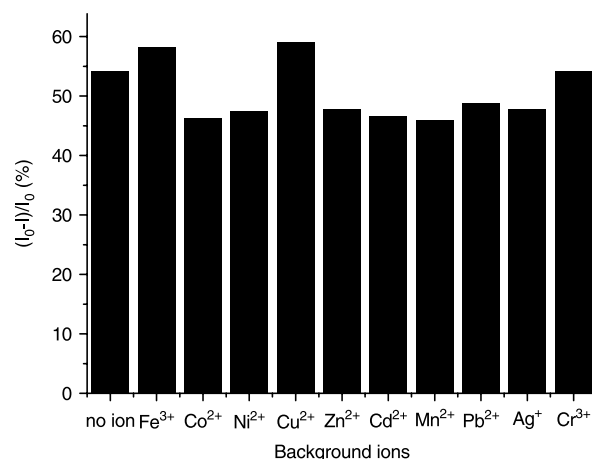


Figure 2. Quench ratio $(I_0 - I)/I_0 \times 100\%$ of fluorescence intensity of 1 (1.0×10^{-5} M) upon addition of 2 equiv. Hg^{2+} and 10 equiv. background ions in $\text{MeCN}-\text{H}_2\text{O}$ (3:1, v/v).

arene was obtained by the substitution of 25,27-bis-(4-bromobutanyl)-4-tert-butyl-calix[4]arene (17) with NaN_3 at 90°C in DMF. If CsCl was added into the reaction mixture, the reaction time can be decreased from 6 to 2 h and the yield was also improved from 89 to 93%. Then, 25,27-bis-(4-azidobutanyl)-4-tert-butyl-calix[4]arene was reacted with one or two equivalents of 8-POQL in DMF at 90°C in the presence of sodium ascorbate (VcNa) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to give 2 and 1 in 50 and 60% yields, respectively.

The structure of 1 and 2 was identified by ESI-MS, elemental analysis, ^1H and ^{13}C NMR (see S1–S5, Supporting Information). The ESI-MS spectrum of 1 shows the expected molecular ion peak, which indicates two 8-oxyquinolines attached onto the calixarene moiety via triazole groups. In the ^1H NMR spectra of 1, two doublets of $\text{Ar}-\text{CH}_2-\text{Ar}$ in AB patterns, the relevantly

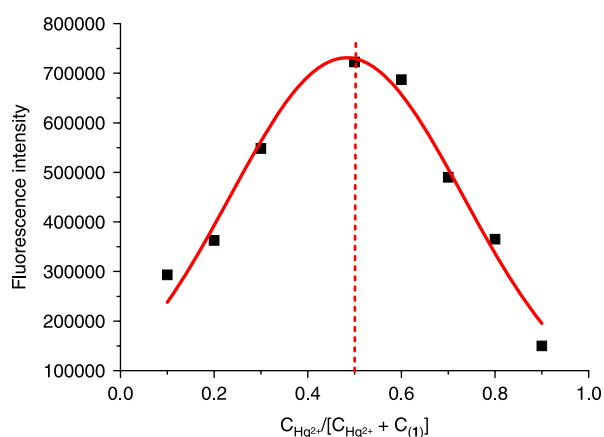


Figure 3. Job plot of a 1:1 complex of 1 and Hg^{2+} ion, where the emission at 390 nm was plotted against the mole fraction of 1 at an invariant total concentration of $20 \mu\text{M}$ in $\text{MeCN}-\text{H}_2\text{O}$ (v/v, 3:1) (excitation 310 nm).

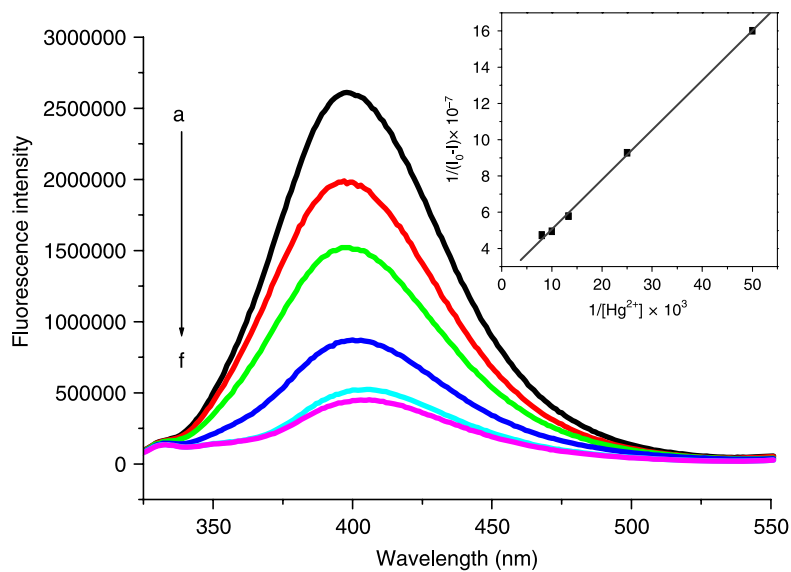


Figure 4. Fluorescence titration of **1** with Hg^{2+} in MeCN– H_2O (3:1, v/v, $[\mathbf{1}] = 1 \times 10^{-5} \text{ mol l}^{-1}$, $[\text{Hg}^{2+}] = 1 \times 10^{-3} \text{ mol l}^{-1}$ a–f: 0, 2.0, 4.0, 7.5, 10, 12.5, equiv., $\lambda_{\text{ex}} = 310 \text{ nm}$). Inset: the plot of $1/(I_0 - I)$ vs. $1/[\text{Hg}^{2+}]$.

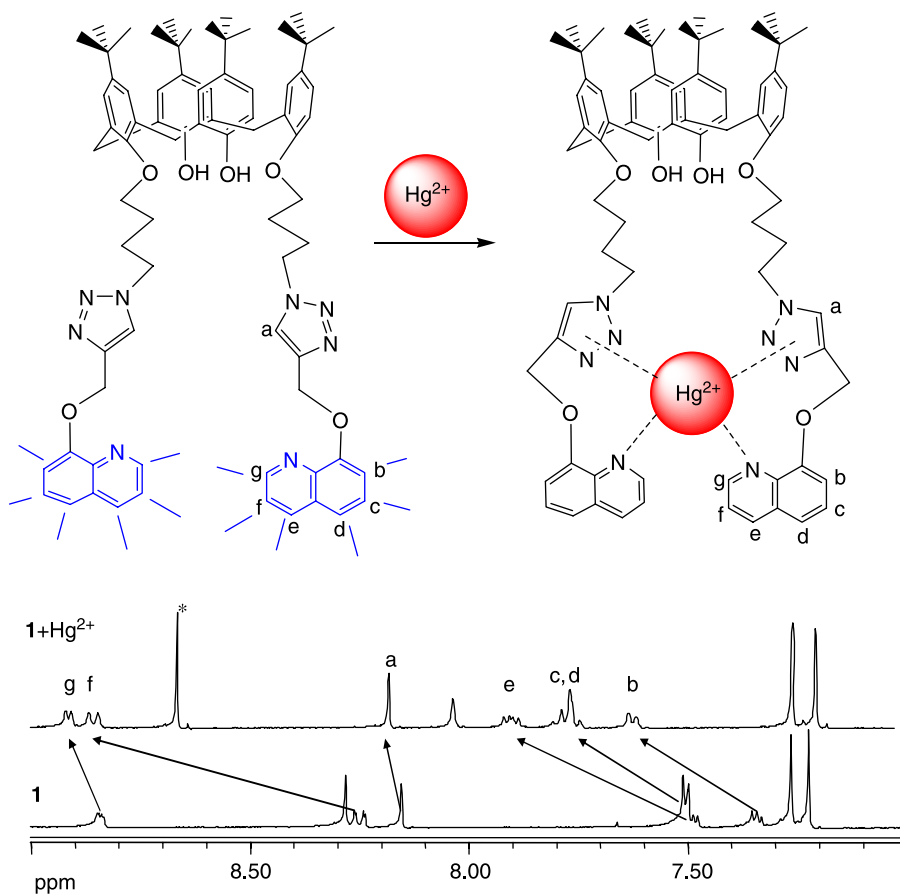


Figure 5. The partial ^1H NMR spectra of **1** in CD_3CN and in the presence of Hg^{2+} (* the protons of picrate).

two singlets (1:1) for $-\text{C}(\text{CH}_3)_3$, indicate that **1** exhibits a cone conformation (45). The ESI-MS spectrum of **2** shows the molecular ion peak at 1026.7, which indicates that there is only one triazole-linked 8-oxyquinoline group at the lower rim of the calix[4]arene. The ^1H NMR spectrum of **2** shows three singlets (2:1:1) for $-\text{C}(\text{CH}_3)_3$, and two pairs of doublets (1:1) for $\text{Ar}-\text{CH}_2-\text{Ar}$, which supports that **2** adopts a cone conformation at ambient temperature.

3.2 Fluorescence behaviours

To evaluate the metal ion binding properties of **1** and **2**, we investigated the fluorescent experiment upon the addition of the nitrate salt of alkali metal, alkaline earth metal and transitional metal in $\text{MeCN}-\text{H}_2\text{O}$ (3:1, v/v). The results are shown in Figure 1. (The data on alkali metal and alkaline earth metal have not been shown here because of little fluorescence changes.) The fluorescence intensity of **1** with two triazole-linked 8-oxyquinoline subunits was strongly quenched by Hg^{2+} and modestly quenched by Fe^{3+} . Addition of fluoride ions to form the complex FeF_6^{3-} can eliminate the interference of Fe^{3+} (46). However, compound **2** with one triazole-linked 8-oxyquinoline subunit shows little significant spectral change upon addition of any of the metal ions.

Table 1. Truth table for logic gate L.

Gate	Chemical input		Output luminescence
	In1 (pH)	In2 (Hg^{2+} M)	
L	0 (pH 7)	0	1 (1.0)
L	0 (pH 7)	1 ($\text{Hg}^{2+} = 2.5 \times 10^{-3}$)	0 (0.17)
L	1 (pH 2)	0	0 (0.14)
L	1 (pH 2)	1 ($\text{Hg}^{2+} = 2.5 \times 10^{-3}$)	0 (0.15)

To further explore the selectivity of **1** for Hg^{2+} , competition experiments were also performed for **1** in the presence of Hg^{2+} mixed with background metal cations such as Fe^{3+} , Co^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Mn^{2+} , Pb^{2+} , Ag^+ , Ni^{2+} and Cr^{3+} , respectively (see Figure 2). We found that other metal ions have no obvious disturbance to the selective sensing of **1** towards Hg^{2+} . This indicates that **1** has selectivity for Hg^{2+} over other studied ions.

In order to determine the stoichiometry of the $\mathbf{1}\cdot\text{Hg}^{2+}$ complex, the method of continuous variations (Job's methods (47)) was employed (see Figure 3). In the Job plot, a maximum fluorescence change was observed when the molar fraction of **1** vs. Hg^{2+} was 0.5, which indicated the formation of a 1:1 complex.

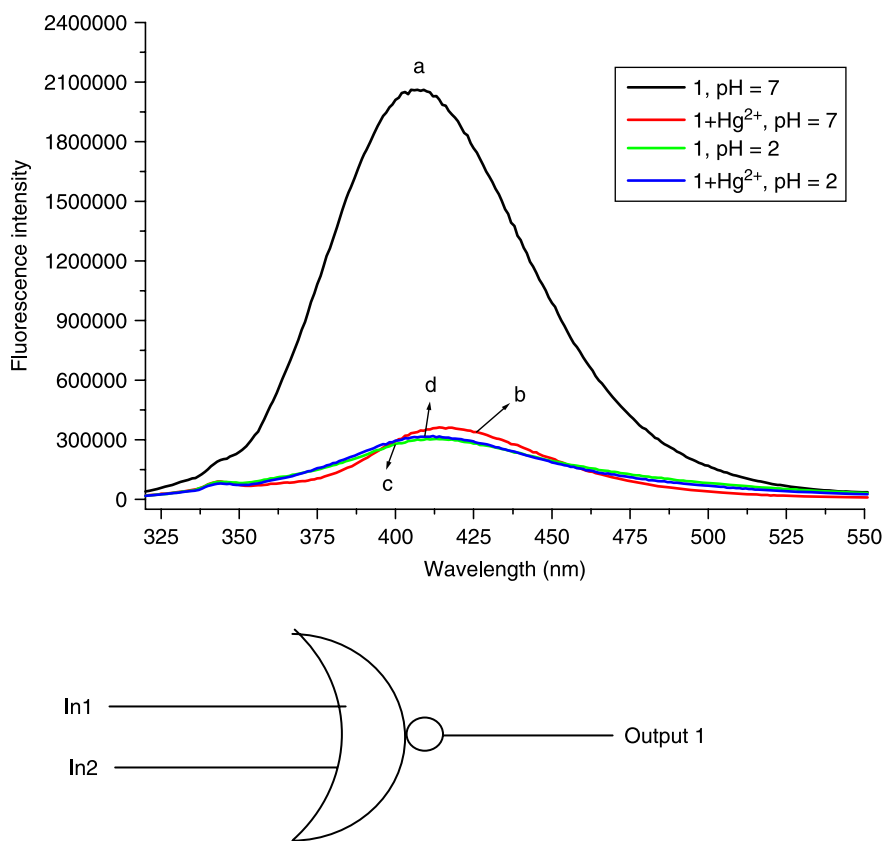


Figure 6. Fluorescence emission spectra for L under different input conditions (a, blank; b, $\text{In2: Hg}^{2+} = 2.5 \times 10^{-3}$ M; c, In1: pH 2 ; d, $\text{In1 and In2: Hg}^{2+} = 2.5 \times 10^{-3}$ M and pH 2).

To further study the sensitivity of **1** towards Hg^{2+} , fluorescence titration experiments were carried out (see Figure 4). The fluorescence of **1** was efficiently quenched over more than 70% by 12.5 equiv. Hg^{2+} . The association constant of Hg^{2+} to **1** was calculated to be 8.527×10^3 litre mol^{-1} by the Benesi–Hildebrand equation (see S7, Supporting Information) (48). The association constants of Cu^{2+} and Fe^{3+} were found to be 1.21×10^3 and 4.42×10^3 litre mol^{-1} , respectively. These results implied that **1** showed selectivity towards Hg^{2+} .

3.3 ^1H NMR study of the complex

To study the complexation mode of **1** for Hg^{2+} , the ^1H NMR experiment was carried out (Figure 5). In the presence of Hg^{2+} , the peaks of protons of quinoline units and triazole units were found to be downfield shifted. These results suggest that the two triazole units and two 8-oxyquinoline units of **1** are an efficient and selective metal ion binding site, which leads to form the stable complexation of Hg^{2+} . Thus, a severe fluorescence quenching with photo-induced electron transfer induced by Hg^{2+} occurred. Clearly, compound **2** obtaining only one triazole-linked 8-oxyquinoline side arm is unable to form effective Hg^{2+} binding.

3.4 Construction of logic gate

Recently, the urge for miniaturisation has been encouraging the design of molecular-level electronic devices (49). Therefore, various molecular logic gates (AND, OR, XOR, INHIBIT, NAND, half-adder, half-subtractor) have been well studied (50). To further study the fluorescent property, we found that the fluorescence of **1** was quenched when pH is lower (see S8, Supporting Information). The fluorescence quenching of **1** towards Hg^{2+} and lower pH provide us a promising structure to NOR logic gates. The output signal is off (0) when either Hg^{2+} or lower pH is present. In other words, only in the absence of Hg^{2+} and higher pH, the output signal is on (1). Thus, this process could be viewed as a NOR logic gate (see Table 1 and Figure 6).

4. Conclusions

In summary, a selective fluorescent probe of Hg^{2+} based on two triazole-linked 8-oxyquinoline subunits with calix[4]arene **1** shows strong fluorescence quenching by Hg^{2+} and lower pH. A logic gate was constructed using the response of the fluorescence of **1** towards Hg^{2+} and lower pH.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (20602015 and 20772038),

Program for Distinguish Young Scientist of Hubei Province (2007ABB017) and Program for Chenguang Young Scientist for Wuhan (200750731283).

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