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The Design of a Highly Selective Fluorescent Chemosensor for Cu(II) within Wide pH Region and a Molecular Switch Controlled by pH

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Abstract

A new calix[4]arene-based fluorescent compound with two thio-ether groups as ionophore and one 3-alkoxy-2-naphthoic acid moiety as fluorophore has been designed, which exhibited highly selective binding of Cu^{2+} over alkali, alkaline earth and some transition metal ions, including Co^{2+}, Ni^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Ag^{+}, Cu^{+}, in CH_3OH–H_2O (2:1) within wide pH region. Moreover, the change of pH induces the consecutive quenching/revival of the fluorescence, with a concomitant distinct difference of the fluorescence quantum yield. In consequence, this system could be considered as a molecular switch.

Introduction

Cu^{2+} is a significant environmental pollutant and an essential trace element in biological systems [1]. Obviously, recognition of Cu^{2+} in different environments is an exciting subject in analytical and supramolecular chemistry. Sensors directed toward the detection and measurement of Cu^{2+} have enjoyed particular attention. Synthetic Cu^{2+} sensors generally choose monoamide-diamine [2], diamide-diamine [3–5], triamine [6], tetraamine [7, 8], hydroxamic acid [9], *O*-acylhydroxylamine [10], tripeptide [11], or dansylated peptide [12] based motifs as the ionophores, and only in one case – tetrathia 14–crown-4 [13] – a thio-ether moiety has been used. More recently, a two thio-ether and three amine units based chemosensor has been reported [14]. However, owing to protonation under acidic conditions, amino-based fluorescent sensors could show sensitive recognition for Cu^{2+} only within narrow pH region, mostly under neutral condition. We also have reported two fluorescent chemosensors based on calix[4]arene, which can selectively discriminate transition metal cations, including Cu^{2+} [15]. In connection with this project, we report here a new fluorescent chemosensor **5**, which not only selectively recognizes Cu^{2+} in a wider pH range (especially under acidic condition), but also acts as a molecular-level light switch, which operates by a change of pH.

The design of selective ion sensors that exploit the calixarene framework has been one of the most active research areas in supramolecular chemistry [16]. The methodology provides considerable flexibility of design. However, the recognition is hard to be fully detected if the fluorophore does not directly contact the bound metal ion. To achieve Cu^{2+} selective ionophores, the potential of mixed ligating sites (S, N, O) has been taken into account. We choose thio-ether groups as the preorganized ionophore to selectively recognize Cu^{2+} on calixarenes, together with a fluorophore of 3-alkoxy-2-naphthoic acid, which can not only transfer the recognition signal, but also be directly involved in the interaction with Cu^{2+}. Therefore the chemosensor **5** was designed.

In order to prove that the fluorophore in the chemosensor **5** is a part of the recognition sites and two thio-ether groups as ionophore are necessary, the fluorescent studies on the undesirable recognition behavior of compounds **4** and **7** to Cu^{2+} were also carried out.

The experiment on the effect of pH on the fluorescence of **8** and 8-Cu^{2+} helps us clarify the recognition behavior of compound **5** to Cu^{2+} under basic conditions.

Experimental

General

Melting points were determined on an electrothermal melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were obtained at, 300 and 75 MHz ($CDCl_3$, TMS as internal standard) respectively on a Bruker DMX300 NMR. MALDI-TOF MS were recorded on a Bruker BIFLEXIII mass spectrometer with CCA (2-cyano-4′-hydroxycinnamic acid) as the matrix. Elemental analyses were performed by the Analytical Laboratory of the Institute. IR Spectra were

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recorded on a JASCO 480 spectrometer. NaH (60% in oil, ACROS) was washed twice with petroleum ether (30–60 °C) and stored in a desiccator. All other chemicals were reagent grade and were used without further purification. Preparative column chromatography was performed with silica gel (200–300 mesh). Petroleum ether for column chromatography refers to that of 60–90 °C boiling range. DMF and benzene were dried over 4 Å molecular sieve. Compound **2** was prepared according to literature procedures [17].

5,11,17,23-Tetra-tert-*butyl-25,27-di(2′-ethylthioethoxy)calix[4]arene* (*3*)

Ethanethiol (222 μl, 3 mmol) was dissolved in DMF (25 ml) and benzene (50 ml) under N_2. To this solution was added KOH (168 mg, 3 mmol). After the mixture was stirred at reflux temperature for 10 min, compound **2** (430 g, 0.5 mmol) was added. The reaction was held at reflux temperature for 8 h. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (50 ml). This solution was washed with 1 M HCl (2 × 50 ml) and brine (50 ml). The organic layer was dried over Na_2SO_4 and evaporated. The residue was crystallized from CH_2Cl_2 /CH_3OH to give 268 mg **3** as a white solid.

3: Yield: 65%; m.p.: 75–76 °C; ^{1}H NMR: δ 7.06 (s, 4H, Ar*H*), 7.02 (s, 2H, ArO*H*), 6.76 (s, 4H, Ar*H*), 4.31 (d, $J = 13.0$ Hz, 4H, ArCH_2Ar), 4.12 (t, $J = 7.2$ Hz, 4H, ArOCH_2), 3.32 (d, $J = 13.1$ Hz, 4H, ArCH_2Ar), 3.10 (t, $J = 7.4$ Hz, 4H, SCH_2CH$_2$O), 2.70 (q, $J = 7.4$ Hz, 4H, SCH_2CH$_3$), 1.32 (t, $J = 7.4$ Hz, 6H, SCH$_2$CH_3),1.30 (s, 18H, C(CH_3)$_3$), 0.93 (s, 18H, C(CH_3)$_3$); ^{13}C NMR: δ 150.5, 149.7, 146.9, 141.5, 132.4, 127.8, 125.5, 125.1, 75.7, 33.8, 31.6, 31.5, 31.2, 31.0, 30.7, 26.6, 15.0; IR(KBr): ν 3442, 2961,1484 cm^{-1}; MALDI-TOF MS: m/z 847 [(M + Na)$^+$]; 863 [(M + K)$^+$]. Elemental analysis calcd. for $C_{52}H_{72}O_4S_2$: C, 75.68; H 8.79. Found: C, 75.79; H 8.77.

5,11,17,23-Tetra-tert-*butyl-25,27-di-(2′-ethylthioethoxy)-26-[2′-(3″-methoxycarbonyl-2″-naphthyloxy)ethoxy]calix[4]arene* (*4*)

The mixture of compound **3** (1 mmol, 824 mg), and NaH (1 mmol, 40 mg) in DMF (30 ml) was stirred at room temperature for 1 h under N_2. Methyl 3-(2-bromo-ethoxy) naphthalene-2-carboxylate (1 mmol, 309 mg) was then added and the temperature was kept at 0 °C for 3 h. The reaction mixture was quenched with water (10 ml) and stirred for another 1 h. After removal of the solvent under reduced pressure, 10% HCl (30 ml) was added. The aqueous solution was extracted with CH_2Cl_2 (230 ml). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (SiO_2, 2:1 petroleum ether/CH_2Cl_2) to give 736 mg **4** as a white solid.

4: Yield: 70%; m.p.: 123–125 °C; ^{1}H NMR: δ 8.31 (s, 1H, naph-*H*), 7.85 (d, $J = 4.3$ Hz, 1H, naph-*H*), 7.82 (d, $J = 4.1$ Hz, 1H, naph-*H*), 7.59 (s, 1H, naph-*H*), 7.51 (t, $J = 7.3$ Hz, 1H, naph-*H*), 7.37 (t, $J = 7.3$ Hz, 1H, naph-*H*), 7.18 (s, 2H, Ar*H*), 7.12 (s, 2H, Ar*H*), 6.53 (s, 4H, Ar*H*), 5.67 (s, 1H, ArO*H*), 5.05–5.00 (m, 2H, ArOCH_2), 4.49–4.47 (m, 2H, ArOCH$_2$CH_2), 4.41 (d, $J = 13.3$ Hz, 4H, ArCH_2Ar), 3.92–3.88 (m, 4H, ArOCH_2), 3.90 (s, 3H, ArCOOCH_3), 3.30 (d, $J = 13.3$ Hz, 2H, ArCH_2Ar), 3.23 (d, $J = 12.8$ Hz, 2H, ArCH_2Ar), 2.82 (t, $J = 7.4$ Hz, 4H, SCH_2CH$_2$O), 2.22 (q, $J = 7.2$ Hz, 4H, SCH_2CH$_3$), 1.36 (s, 18H, C(CH_3)$_3$), 0.94 (t, $J =$ 7.4 Hz, 6H, SCH$_2$CH_3), 0.82 (s, 18H, C(CH_3)$_3$); ^{13}C NMR: 167.2, 155.2, 150.9, 150.8, 146.3, 145.7, 141.7, 136.4, 135.8, 132.6, 132.1 131.7, 129.2, 128.6, 128.2, 127.5, 126.9, 125.8, 125.2, 125.0, 124.9, 124.2, 108.2, 74.6, 71.0, 67.6, 52.1, 34.2, 33.9, 33.7, 31.8, 31.7, 31.3, 31.2, 31.0, 26.2, 14.5; IR(KBr): 3531, 2958, 1736, 1631, 1479 cm^{-1}; MALDI-TOF MS: m/z 1075[(M + Na)$^+$], 1091 [(M + K)$^+$]; Elemental analysis calcd. for $C_{66}H_{84}O_7S_2$ C, 75.24; H, 8.04. Found: C, 75.46; H, 8.28.

5,11,17,23-Tetra-tert-*butyl-25-[2′-(3″-methoxycarbonyl-2″-naphthyloxy)ethoxy]calix[4]arene* (*6*)

$Ba(OH)_2{\cdot}8H_2O$ (0.88 mmol, 280 mg) and BaO (0.88 mmol, 135 mg) was added to a mixture of **1** (0.77 mmol, 500 mg) and methyl 3-(2-bromo-ethoxy)naphthalene-2-carboxylate (1.06 mmol, 310 mg) in DMF (15 ml). The reaction mixture was kept at room temperature overnight and then quenched with a small amount of 10% HCl. After removal of the solution under reduced pressure, the residue was partitioned between water and CH_2Cl_2.The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatograpy (SiO_2, 1:2 petroleum ether/CH_2Cl_2) to give 337 mg **6** as a white solid.

6: Yield: 50%; m.p.: 189–190 °C; ^{1}H NMR: δ 10.03 (s, 1H, ArO*H*), 9.40 (s, 2H, ArO*H*), 8.24 (s, 1H, naph-*H*), 7.78 (d, $J = 8.3$ Hz, 2H, naph-*H*), 7.51 (t, $J = 7.1$ Hz, 1H, naph-*H*), 7.35 (t, $J = 8.8$ Hz, 2H, naph-*H*), 7.06 (s, 2H, Ar*H*), 7.01 (s, 2H, Ar*H*), 6.92 (s, 2H, Ar*H*), 6.89 (s, 2H, Ar*H*), 4.72–4.74 (m, 2H, ArOCH_2), 4.55–4.57 (m, 2H, ArOCH$_2$CH_2), 4.47 (d, $J = 13.0$ Hz, 2H, ArCH_2Ar), 4.03 (d, $J = 13.6$ Hz, 2H, ArCH_2Ar), 3.41 (d, $J = 13.1$ Hz, 2H, ArCH_2Ar), 3.34 (s, 3H, ArCOOCH_3), 3.28 (d, $J = 13.7$ Hz, 2H, ArCH_2-Ar), 1.14 (s, 36H, C(CH_3)$_3$); ^{13}C NMR: δ 166.6, 154.4, 148.8, 148.4, 148.1, 147.7, 143.3, 143.1, 136.0, 133.7, 132.8, 128.7, 128.5, 128.1, 128.0, 127.8, 127.7, 126.7, 126.5, 125.7, 124.7, 122.2, 107.9, 74.0, 67.6, 51.8, 34.3, 33.9, 32.9, 31.9, 31.5, 31.2, 31.1, 29.7; IR (KBr): 3280, 2957, 1735, 1630, 1484 cm^{-1}; MALDI-TOF MS: m/z 899 [(M + Na)$^+$], 915 [(M + K)$^+$]; Elemental analysis calcd. for $C_{58}H_{68}O_7$: C, 79.42; H, 7.81. Found: C, 79.00; H, 7.86.

Hydrolysis of compounds 4 and 6 – General procedure

Compound **4** or **6** (0.5 mmol) in C_2H_5OH (30 ml) was treated with NaOH (2.5 mmol) in water (10 ml) at reflux temperature for 5 h. After removal of the solvent, 10% HCl (15 ml) was added. The aqueous solution was extracted with CH_2Cl_2 (2 × 20 ml). The organic layer was dried over Na_2SO_4 and evaporated. The residue was crystallized from CH_2Cl_2/CH_3OH to give compound **5** or **7**.

5,11,17,23-Tetra-tert-*butyl-25, 27-di(2′-ethylthioethoxy)-26-[2′-(3″-hydroxycarbonyl-2″-naphthyloxy)ethoxy] calix[4]arene* (*5*)

5: Yield: 90%; m.p.: 117–119 °C; ^{1}H NMR: δ 8.83 (s, 1H, naph-*H*), 7.95–7.88 (m, 2H, naph-*H*), 7.76 (s, 1H, naph-*H*), 7.59 (t, $J = 7.1$ Hz, 1H, naph-*H*), 7.46 (t, $J = 7.1$ Hz, 1H, naph-*H*), 7.21 (s, 2H, Ar*H*), 7.15 (s, 2H, Ar*H*), 6.54 (s, 2H, Ar*H*), 6.51(s, 2H, Ar*H*), 5.48 (s, 1H, ArO*H*), 5.37–5.31 (m, 2H, ArOCH_2), 4.57–4.52 (m, 2H, ArOCH$_2$CH_2), 4.43 (d, $J = 12.8$ Hz, 2H, Ar*C*H_2-Ar), 4.42 (d, $J = 13.2$ Hz, 2H, ArCH_2Ar), 3.99–3.88 (m, 4H, ArOCH_2), 3.33 (d, $J = 13.5$ Hz, 2H, ArCH_2Ar), 3.27 (d, $J = 13.5$ Hz, 2H, ArCH_2Ar), 2.76 (t, $J = 6.6$ Hz, 4H, SCH_2CH$_2$O), 2.22 (q, $J = 7.2$ Hz, 4H, SCH_2CH$_3$), 1.37 (s, 18H, C(CH_3)$_3$), 0.95 (t, $J = 7.4$ Hz, 6H, SCH$_2$CH_3), 0.82 (s, 18H, C(CH_3)$_3$); ^{13}C NMR: δ 165.6, 154.0, 152.9, 150.9, 150.7, 147.0, 145.9, 142.1, 136.9, 136.0, 135.8, 131.9, 131.5, 129.4, 128.3, 127.1, 126.1, 125.3, 125.2, 124.9, 117.9, 108.7, 74.7, 69.9, 68.8, 34.3, 33.9, 33.7, 31.8, 31.7, 31.6, 31.2, 31.1, 31.0, 26.3, 14.4; IR (KBr): ν 3531, 3291, 2959, 1742, 1631, 1481 cm^{-1}; MALDI-TOF MS: *m*/*z*. 1059 [(M + Na)$^+$], 1075 [(M + K)$^+$]; Elemental analysis calcd. for $C_{65}H_{82}O_7S_2$: C, 75.10; H, 7.95. Found: C, 75.09; H, 7.91.

5,11,17,23-Tetra-tert-*butyl-25-[2′-(3″-hydroxycarbonyl-2″-naphthyloxy)ethoxy]calix[4]arene* (*7*)

7: Yield: 50%; m.p.: 121–122 °C; ^{1}H NMR: δ 11.99 (s, 1H, naph-COO*H*), 9.93 (s, 1H, ArO*H*), 9.44 (s, 2H, ArO*H*), 8.75 (s, 1H, naph-*H*), 7.94 (d, $J = 8.2$ Hz, 1H, naph-*H*), 7.87 (d, $J = 8.0$ Hz, 1H, naph-*H*), 7.62 (t, $J = 7.0$ Hz, 1H, naph-*H*), 7.51 (s, 1H, naph-*H*), 7.49 (t, $J = 7.0$ Hz, 1H, naph-*H*), 7.14 (s, 2H, Ar*H*), 7.06 (s, 2H, Ar*H*), 7.01 (s, 2H, Ar*H*), 6.98 (s, 2H, Ar*H*), 5.01–4.98 (m, 2H, ArOCH_2), 4.72–4.70 (m, 2H, ArOCH$_2$CH_2), 4.39 (d, $J = 13.0$ Hz, 2H, ArCH_2Ar), 4.15 (d, $J = 13.7$ Hz, 2H, ArCH_2Ar), 3.50 (d, $J = 13.1$ Hz, 2H, ArCH_2Ar), 3.40 (d, $J = 13.8$ Hz, 2H, ArCH_2Ar), 1.21 (s, 36H, C(CH_3)$_3$); ^{13}C NMR: δ 165.9, 153.4, 148.9, 148.6, 148.0, 147.5, 143.6, 143.4, 136.4, 135.9, 133.4, 129.4, 129.3, 128.6, 128.0, 127.9, 127.8, 126.8, 125.9, 125.7, 125.5, 119.4, 108.4, 73.8, 68.7, 34.3, 33.9, 33.7, 32.9, 32.3, 31.4, 31.2, 28.9; IR (KBr): ν 3377, 2958, 1696, 1632, 1483 cm^{-1}; MALDI-TOF MS: *m*/*z*. 885 [(M + Na)$^+$], 901 [(M + K)$^+$]; Elemental analysis calcd. for $C_{57}H_{66}O_7$: C, 79.32; H, 7.71. Found: C, 79.05; H, 7.88.

5,11,17,23-Tetra-tert-*butyl-25,27-di(2′-ethylthioethoxy)-26-[2′-(3″-hydroxycarbonyl-2″-naphthyloxy)ethoxy]-28-methoxyl-calix[4]arene* (*8*)

Compound **4** (0.086 mmol, 90 mg) in DMF (10 ml) was treated with NaH (0.43 mmol, 12 mg) under N_2 at 60 °C for 30 min, and then CH_3I (0.43 mmol, 26 μl) was added to the mixture. After the reaction was hold at 60 °C for 3 h, NaOH (0.43 mmol, 17 mg) in H_2O (1 ml) was added, and the mixture was stirred for 3 h. After removal of the solution under reduced pressure, the residue was partitioned between water and CH_2Cl_2.The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatograpy (SiO_2, 10:1 petroleum ether/acetone) to give 40 mg **8** as a white solid.

8: Yield: 40%; m.p.: 70–71 °C; ^{1}H NMR: δ 8.80 (s, 1H, naph-*H*), 7.93–7.91 (m, 2H, naph-*H*), 7.80 (s, 1H, naph-*H*), 7.60 (t, J = 7.0 Hz, 1H, naph-*H*), 7.45 (t, J = 7.3 Hz, 1H, naph-*H*), 7.14 (s, 4H, Ar*H*), 6.60 (s, 4H, Ar*H*), 5.10 (bs, 1H, ArOCH_2), 4.68 (bs, 1H, ArOCH_2), 4.38–4.34 (m, 4H, Ar*C*H_2Ar), 4.22–4.17 (m, 2H, ArOCH_2), 4.01–3.91 (m, 5H, ArOCH_2, ArOCH_3), 3.82–3.77 (m, 2H, ArOCH_2) 3.22–3.18 (m, 4H, Ar*C*H_2-Ar), 2.90 (bs, 4H, SCH_2CH$_2$O), 2.44 (bs, 4H, SCH_2CH$_3$), 1.30 (s, 18H, C(CH_3)$_3$), 0.97–0.89 (m, 6H, SCH$_2$CH_3), 0.77 (s, 18H, C(CH_3)$_3$); ^{13}C NMR: δ 165.3, 153.8, 152.5, 146.1, 144.7, 136.5, 136.3, 134.8, 133.8, 132.5, 132.0, 129.5, 129.4, 128.4, 126.5, 125.9, 125.3, 118.8, 118.2, 108.3, 74.2, 73.4, 69.0, 59.8, 34.1, 33.7, 31.7, 31.6, 31.2, 29.7, 26.4, 22.7, 14.8; IR (KBr): ν 3446, 2961, 1743, 1480 cm^{-1}; MALDI-TOF MS: *m*/*z*. 1075 [(M + Na)$^+$],1091 [(M + K)$^+$]; Elemental analysis calcd. for $C_{65}H_{82}O_7S_2$: C,75.24; H, 8.04. Found: C, 74.97; H, 8.03.

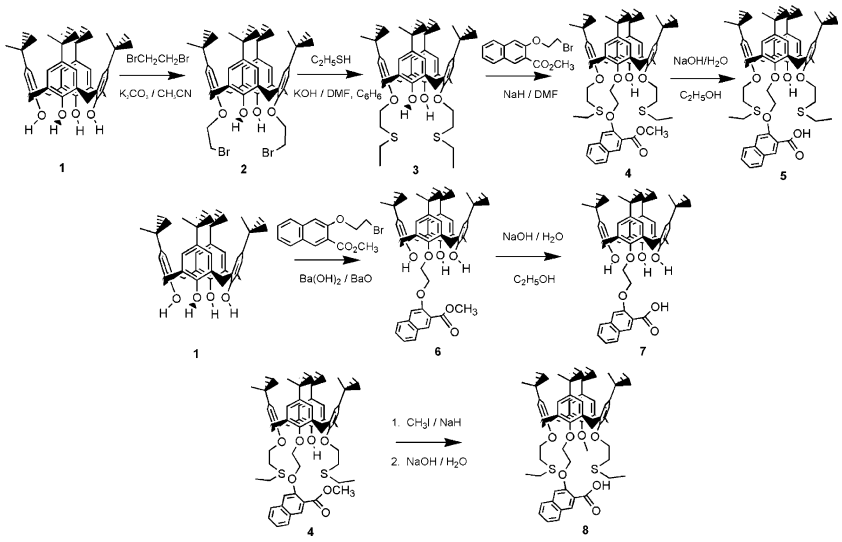
UV–Vis and fluorescence measurements

UV–Vis spectra were recorded with Shimazu 2401 spectrophotometer, and fluorescence spectra were measured using a HITACHI F-4500 instrument. UV–Vis spectral and fluorescence spectral measurements were obtained by adding the concentrated solution of metal ion to a solution of ligand in a 1 cm path length cuvette by aliquot. No more than 50 μl of metal ion solution was added in 2 ml of the measuring solution for UV–Vis and fluorescence measurements.

Results and discussion

Synthesis

The chemosensor **5** could be conveniently synthesized starting from the *t*-butylcalix[4]arene **1**. Thus, the bromide **2** was prepared according to the literature



Scheme 1.

[17], and then reacted with ethanethiol in the presence of potassium hydroxide to give the bis-thioether compound **3** in 65% yield. Compound **4** was obtained in 70% yield from the reaction of **3** with methyl 3-(2-bromoethoxy)-naphthalene-2-carboxylate in the presence of sodium hydride. Finally, the saponification of **4** by sodium hydroxide, followed by acidification provided the target compound **5** in almost quantitative yield. Compound **7** could be obtained from the monoalkylated compound **6** in 90% yield. Compound **8** was synthesized by alkylation of **4** with CH_3I, successive saponification, and then acidification in 40% yield. (Scheme 1). The chemical structures of **3**, **4, 5, 6, 7** and **8** were identified by FT-IR, ^{1}H NMR, ^{13}C NMR, MALDI-TOF MS and elemental analyses, which indicated that the calix[4]arene backbone of these compounds adopted the cone conformation.

The UV spectrophotometric titration of 5 with Cu^{2+}

In the absorption spectrum of **5**, maximum bands were observed at 231, 278 and 330 nm. Addition of $Cu(ClO_4)_2$ resulted in a decrease of the absorption intensity at 231 nm and an increase of the intensity at 278 and 330 nm with the isobestic point at 233 nm, which indicates complex formation. The titration of **5** (10 μM) with Cu^{2+} showed a gradual increase in absorption at 330 nm over a Cu^{2+} concentrations range of 10–50 μM, above which a plateau was achieved (Figure 1). So, **5** can act as a chromoionophore and detect 10–50 μM Cu^{2+} by UV–Vis spectroscopy.

The fluorometric titration of 5 with Cu^{2+}

Like other acid-based fluorescent sensors, Compound **5** is also pH sensitive. The fluorescent spectrum of **5** remains unaffected between pH 6.0 and 4.0 (Figure 6). Thus, fluorescence measurements were carried out at pH 5.25, adjusted with $HClO_4$ solution. The fluorescence emission is directly proportional to the concentration of **5** (1–10 μM), which showed that **5** was not susceptible to self-quenching or to aggregation in the concentration range explored.

In the fluorescent spectrum of **5**, the maximum excitation and emission wavelengths were observed at 328 and 392 nm, respectively. The titration of **5** (10 μM) with Cu^{2+} showed gradual quenching in fluorescence between 1 and 10 μM of Cu^{2+} and achieved a plateau (Figure 2). The stoichiometry of the complexation is determined through Job plot by fluorescence spectroscopy and was found to be a 1:1 Cu^{2+}-**5** complex. According to the Stern–Volmer plot for fluorescence quenching, we could deduce that the binding constant between **5** and Cu^{2+} under this condition was about 2.26×10^5 M^{-1}.

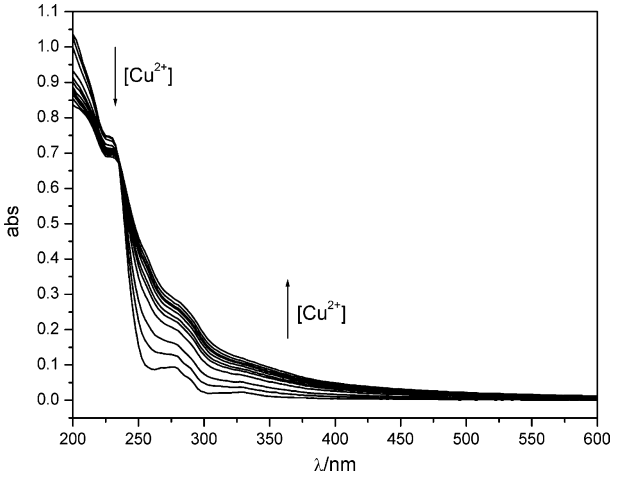


Figure 1. Changes in the absorption spectrum of CH_3OH–H_2O (2:1, v/v) solution of **5** (10 μM) in $HClO_4$, pH = 5.25, with the concentration of $Cu(ClO_4)_2$ increasing. The concentration of $Cu(ClO_4)_2$ (μM): 0; 10; 20; 30; 40; 50; 70; 100; 150; 200; 300; 400.

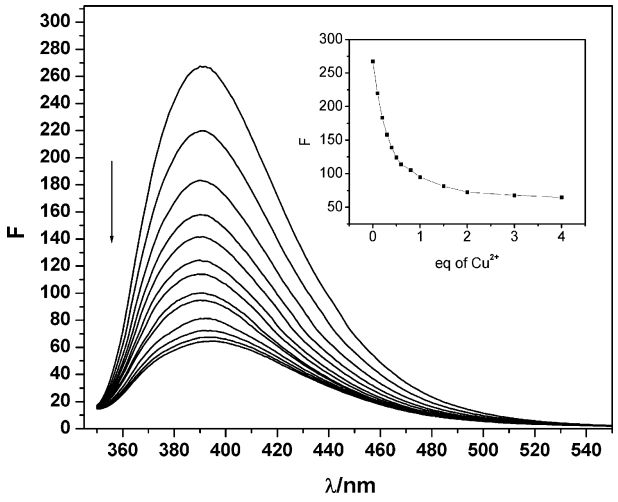


Figure 2. Fluorescent emission spectrum changes of **5** (10 μM) in CH_3OH–H_2O (2:1, v/v) quenched by $Cu(ClO_4)_2$ ($\lambda_{ex} = 328$ nm) in $HClO_4$ solution, pH = 5.25. The concentration of $Cu(ClO_4)_2$ (μM): 0; 1; 2; 3; 4; 5; 6; 8; 10; 15; 20; 30; 40. Inset: the fluorescence intensity *versus* eq. of Cu^{2+} profile of **5**.

Fluorescent responses of 5 to other metal ions

Similar measurements for several metal ions, such as Co^{2+}, Ag^{+}, Ni^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+}, Pb^{2+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, K^{+} and Cu^{+} were examined. However, no noticeable spectral changes of **5** were observed upon the addition of these cations under the same conditions (Figure 3). So it can also be concluded that Compound **5** has higher selectivity for recognition of Cu^{2+}. Such selectivity is likely to originate from the recognition ability of the thio-ether groups together with the interaction between fluorophore and ionophore.

Fluorescent studies of 4, 7 and 6 to Cu^{2+}

In the fluorescent spectrum of **4**, the maximum excitation and emission wavelengths were observed at 340 and 409 nm, respectively. The fluorescence intensity of **4** in the presence of increasing amounts of Cu^{2+} is shown in Figure 4. The intensity decreased continually upon the addition of Cu^{2+}, and when the concentration of Cu^{2+} increased to 35 equivalents, the intensity changed to

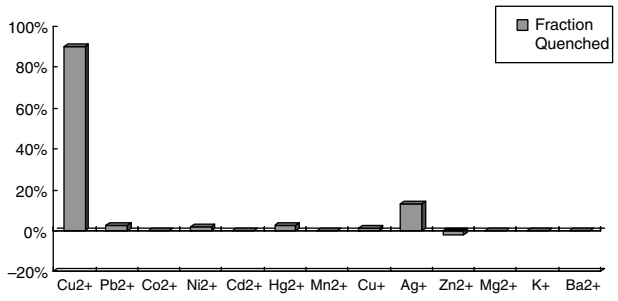


Figure 3. The percentage of emission of **5** quenched by adding metal ions (40 μM) to the solution of compound **5** (10 μM) in CH_3OH–H_2O (2:1, v/v).

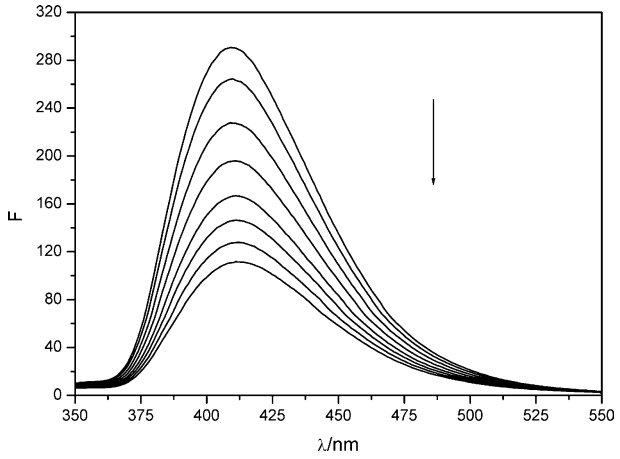


Figure 4. Fluorescent emission spectrum changes of **4** (10 μM) in CH_3OH–H_2O (2:1, v/v) quenched by $Cu(ClO_4)_2$ ($\lambda_{ex} = 340$ nm) in $HClO_4$ solution, pH = 5.25. The concentration of $Cu(ClO_4)_2$ (μM): 0; 50; 100; 150; 200; 250; 300; 350.

39% of its initial value. The binding constant between **4** and Cu^{2+} was about 5100 M^{-1}.

Compared with Compound **5**, Compound **4** with a 3-alkoxy-2-naphthoic ester as fluorophore has a lower sensitivity for Cu^{2+}. This is because there is no direct interaction between the fluorophore and Cu^{2+}. In this aspect, an ideal fluorescent probe will be one whose fluorescent unit is directly involved in the interaction with metal ions.

The fluorescent spectrum of **7** shows little change under acid condition. In the fluorescent spectrum of **7**, maximum excitation and emission wavelengths were observed at 330 and 408 nm, respectively. The titration of **7** (10 μM) with Cu^{2+} showed gradual enhancement in the fluorescence intensity between 10 and 100 μM of Cu^{2+} and then achieved a plateau. (Figure 5) The Scatchard-type equation was used to calculate the binding constant between **7** and Cu^{2+}, which was about 6890 M^{-1}. Moreover, addition of Ni^{2+} and Zn^{2+} can induce similar emission spectral changes in **7**.

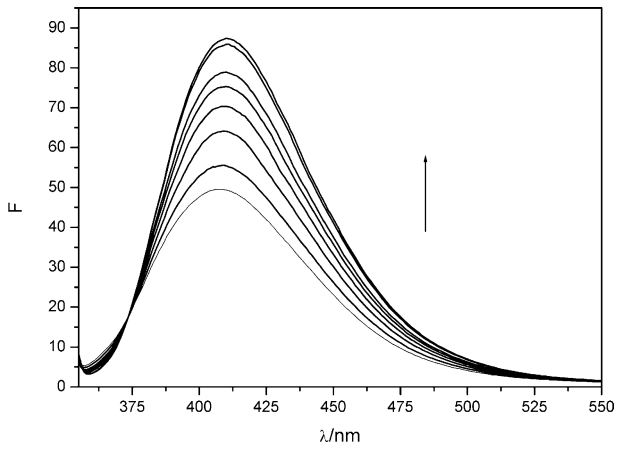


Figure 5. Fluorescent emission spectral changes of **7** (10 μM) in CH_3OH–H_2O (2:1, v/v) increased by $Cu(ClO_4)_2$ ($\lambda_{ex} = 330$ nm) solution in $HClO_4$, pH = 5.25. The concentration of $Cu(ClO_4)_2$ (μM): 0; 10; 20; 30; 40; 50; 100; 150.

Compound **7** without two thio-ether groups has a lower sensitivity and selectivity for Cu^{2+} than compound **5** bearing two thio-ether groups as ionophore. This indicates the two thio-ether groups in compound **5** are crucial to selectively recognize Cu^{2+}.

We also tested the fluorescent response of **6** to Cu^{2+} under the same conditions. The changes in the fluorescence spectra could be scarcely detected, which confirms the influence of the carboxylic group on the recognition.

*Compound **5** as a molecular switch controlled by pH*

To investigate the range of pH in which chemosensor **5** can effectively detect Cu^{2+}, the titration curve of fluorescence intensity *versus* pH in CH_3OH–H_2O (2:1, v/v) was measured (Figure 6). From curve (a), the free **5** exhibited a balanced fluorescence at pH = 4–6. When the pH was higher than 6, the fluorescence intensity began to increase and reached a maximum at pH = 8.5. From curve (b), along with increasing the pH, the fluorescent intensity of the **5**-Cu^{2+} complex slightly decreased, and reached a minimum at pH 5.25, which indicated the binding ability was the strongest at pH 5.25 under acidic conditions. Then the curve rose slowly at pH = 5.25–6.5 and when the pH was higher than 7.0, the fluorescence markedly increased, adjacent to the original value of **5** at pH = 8.5.

The effect of pH on the fluorescence of **5**-Cu^{2+} in CH_3OH–H_2O (2:1, v/v) exhibited a different feature from that of free ligand **5** (Figure 6). The obvious difference was observed in the range of pH = 4–7.5 in which the fluorescence intensity was strongly quenched. Evidently, the binding between the thio-ether groups and Cu^{2+} caused this pronounced quenching, and electron energy transfer (ET) between Cu^{2+} and the naphthyl ring would be the major factor affecting the emission intensity. When pH is higher than 7.5, the fluorescence intensity decreases slightly.

To make an explanation about the weakly quenched fluorescence under basic conditions, we carried out the titration on fluorescence intensity of **8** and 8-Cu^{2+} *versus* pH in CH_3OH–H_2O (3:1, v/v) (Figure 7). A similar

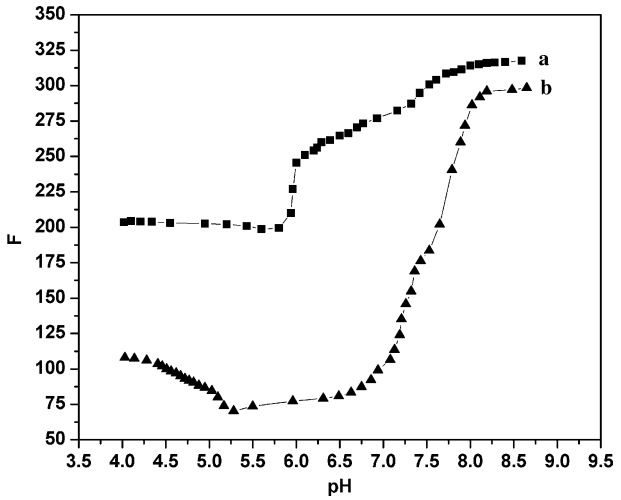


Figure 6. Fluorescence intensity of calixarene **5** at different pH (a) free **5**, (b) **5** and Cu^{2+}. Concentration of **5** is kept constant at 10 μM. Concentration of Cu^{2+} is 10 μM. (excitation at 340 nm).

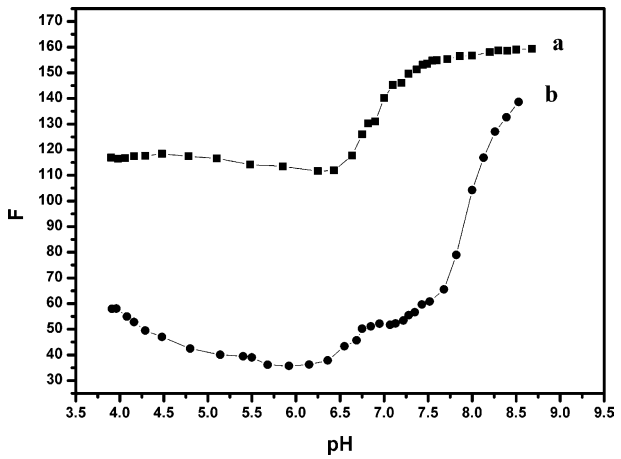


Figure 7. Fluorescence intensity of calixarene **8** at different pH (a) free **8**, (b) **8** and Cu^{2+}. Concentration of **8** is kept constant at 10 μM. Concentration of Cu^{2+} is 20 μM. (excitation at 328 nm).

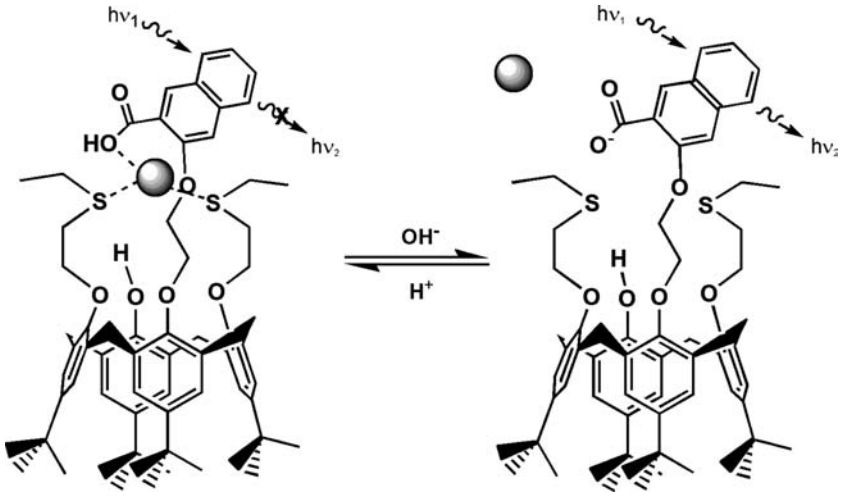


Figure 8. On/off molecular switching behavior through the translocation of Cu^{2+} by a change of pH.

feature on the pH titration curve is that Cu^{2+} also induced a slight quenching, which implied that the recognition behavior of **5** to Cu^{2+} was not related to the phenol group.

Considering that the pK_a values for the carboxyl group and the phenol group are about 4.1 and 10.0, respectively, the carboxyl group present in **5** can be deprotonated at pH = 9.0, while the phenol group in **5** is not easily deprotonated under the same conditions.

According to the above research, we deduce that Cu^{2+} can combine with the thio-ether groups and the carboxyl group under acidic conditions, nevertheless, along with increasing the pH, Cu^{2+} can be gradually displaced from the recognition sites and be bound by OH^- in solution under basic conditions (Figure 8).

The fluorescence quantum yields for the complex **5**-Cu^{2+} under different conditions were determined using anthracene as a standard in cyclohexane ($\Phi_f = 0.36$). At pH = 5.25, the fluorescence quantum yield was 0.120. After adjusting pH to 8.5 on addition of $(CH_3)_4N^+OH^-$, the fluorescence quantum yield increased to 0.812. So, this system could be considered as a molecular switch, which rests at the off state under acid conditions and can be turned on under basic conditions. Moreover, the on/off process of this system is reversible by controlling pH.

Conclusion

We have presented a new fluorescent chemosensor with two thio-ether groups as ionophore based on a calix[4]arene derivative, which can detect Cu^{2+} with an excellent selectivity and molecular sensitivity within relatively wide pH region. Moreover, this system could act as a molecular switch of fluorescence, which operates by a change of pH.

Acknowledgements

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