A highly-selective probe for detection of zinc(II) in neutral aqueous solution

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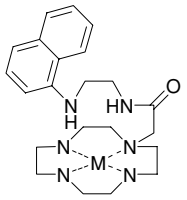
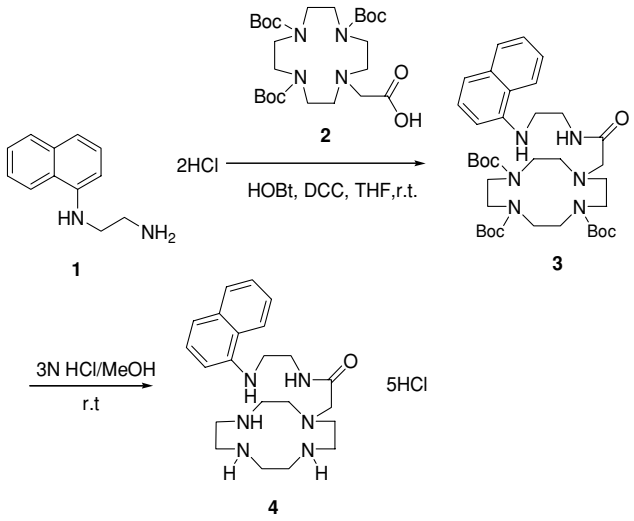
A novel fluorescent chemosensor for the selective detection of zinc(II) in neutral aqueous solution has been synthesised by attaching the fluorophore into a macrocyclic polyamine (1,4,7,10-tetraazacyclododecane), and ion selectivity has been studied by fluorescence spectrocopy.

Keywords: fluorescence spectroscopy, chemosensor, zinc ion

In recent years, there have been intensive investigations of fluorescent chemosensors for the detection of metal ions.[1] Among various metal ions, zinc ions play a vital role in biosystems.[2a] During the past few years, rapid improvements have been made in the development of Zn^{2+}-specific sensor molecules,[2b] but their mechanisms are not clear at present. Therefore, finding a new class of chemosensor for the selective detection of Zn^{2+} is challenging work. Fluorescent chemosensors are a strong tool for the detection of metal ions. Classic fluorescent chemosensors comprise two parts: the recognition site and the readout system.[1a,3] The readout system is often a fluorophore. In these systems, quenching is ascribed to a photoinduced metal-to-fluorophore electron-transfer (PET) mechanism.[4,5] However, when Zn^{2+} binds to a macrocyclic polyamine, it may hinder PET and enhance the fluorescence.[4] Macrocyclic polyamines, such as cyclen (1,4,7,10-tetraazacyclododecane), are well-known ligands which can strongly chelate to transition metals ions such as Zn^{2+} and Cu^{2+}. It is reported that macrocyclic polyamines attached with fluorophores, such as 9-anthrylmethylamino[6a], TSQ[4], 7-amino-4-trifluoromethylcoumarin[7b] and dansyl amidoethyl[7c] can be used as fluorescent chemosensors for Zn^{2+} through formation of pendant structures in base conditions. Different metal ions can result in quenching or enhancement of the fluorescence.[6-12] However, design of high selective chemosensors which can distinguish Zn^{2+} from its competitive ions (*e.g.* Cd^{2+}, Pb^{2+} and Cu^{2+}) is also challenging. In this paper, we have designed and synthesised a new fluorescent chemosensor, compound **4**, with a naphthyl group as fluorophore and cyclen as metal ion chelator (Schemes 1 and 2). The selective behaviour for different ions was detected by fluorescence spectroscopy.



Scheme 1 Binding model of compound **4** with metal ion.



Scheme 2 Synthesis of fluorescent chemosensor, compound **4**.

Experiment

^{1}H NMR spectra were measured on a JEOL 300 M Hz spectrometer with TMS as the internal standard. Mass spectra were acquired in positive ion mode using a Bruker ESQUIRE-LCTM ion trap spectrometer equipped with a gas nebuliser probe, capable of analysing ions up to *m/z* 20000. Solvents were purified and dried by standard procedures. Compound **2** was synthesised according to refs 13–15.

Synthesis of compound **4**

To a 25 ml round-bottomed flask with magnetic stirring, was added N-(1-naphthyl)ethylenediamine dihydrogen chloride (0.487 g, 1.88 mmol),[4,7,10-tris(*t*-butoxycarbonyl)-1,4,7,10- tetraazacyclododecan-1-yl] acetic acid (1.0 g, 1.88 mmol), Et_3N (0.3 g, 3 mmol), HOBt (0.27 g, 2 mmol) and dry THF (10 ml). In an ice-bath, DCC (0.413 g, 2mmol) in dry THF (5 ml) was added slowly (within 10 min) to the mixture thus obtained. Then the reaction mixture was stirred with the temperature rising to room temperature. The mixture was then stirred at room temperature for about 24 h. The solvent was then removed, and saturated $NaHCO_3$ solution (20 ml) and ethyl acetate (20 ml) were added. The aqueous phase was extracted with ethyl acetate twice (each time by 20 ml). The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. After removal of solvent, column chromatography (silica gel, ethyl acetate/petroleum ether) gave a solid (1.0 g, yield 77 %). After treatment with a solution of HCl /methanol (3 M), a brown solid was obtained.

^{1}H NMR (300 MHz, D_2O, δ ppm): 7.98–7.42 (m, 7H), 3.73 (t, *J*=5.5Hz, 2H), 3.54 (t, *J*=5.6Hz, 2H), 3.19 (s, 2H), 3.08–2.80 (m, broad, 16H); HRMS found [M+1]**:** 399.2861 (M+H), Calcd for $C_{22}H_{35}N_6O$ 399.2867. ESI-MS for complex solution: 399.4 [M+H], 461.2 [M-H] Zn, 497.2 [M+Zn] Cl

Results and discussion

Fluorescence emission spectra of compound **4** (10.4 μM) in HEPES buffer (pH7.06, 10 mM) in the presence of various concentrations of Zn^{2+} are shown in Fig.1. Upon addition of Zn^{2+}, the fluorescence intensity of compound **4** was increased 4-fold without any change in the position of the maximum emission peak (λ_{em}=440nm). During the titration of Zn^{2+}, a pendant macrocyclic tetraamine structure is formed with high stability.[6c] Interestingly, a plot of I/I_0 versus the concentration of Zn^{2+} indicated that compound **4** and Zn^{2+} form a 1:2 complex. This structure will be studied fully in the future.

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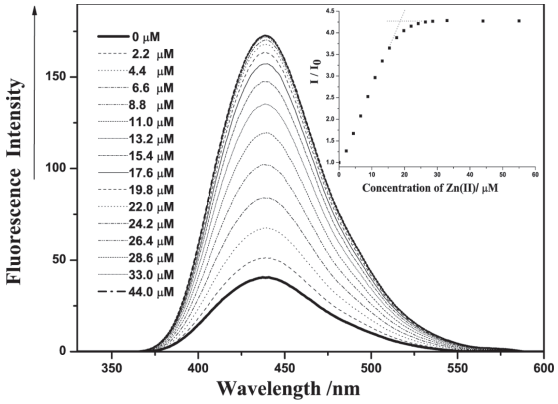

Fig. 1 Fluorescence emission spectra of compound **4** (10.4 μM) in the presence of increasing concentrations of Zn^{2+}. Inset: fluorescence intensity at λ_{em}= 440 nm as a function of zinc ion concentration. Excitation is selected at 287 nm. Measured at 25 °C in HEPES buffer (pH=7.06, 10 mM).

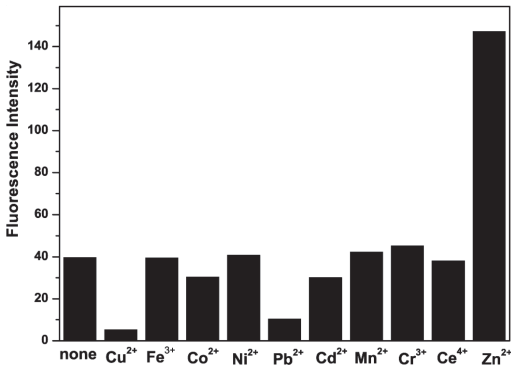

Fig. 2 Fluorescence emission spectra of **4** (10.4 μM) in the presence of different ions at the same concentration (22 μM). Fluorescence intensity at λem= 440 nm as a function of metal ions concentration. Excitation is selected at 287 nm. Measured at 25 °C in HEPES buffer (pH=7.06, 10 mM).

The fluorescent intensities of compound **4** (10.4 μM) with various metal cations at pH 7.06 are shown in Figs 2 and 3. The fluorescence behaviour of Zn^{2+} showed remarkable differences from other ions used in the fluorescence spectroscopy experiment. In all experiments, nitrate salts were employed and the counterion cannot affect the result of fluorescence experiments.The fluorescence of compound **4** was changed little by addition of Fe^{3+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Mn^{2+}, Cr^{3+} and Ce^{4+} under the same conditions. It is noted that addition of Cu^{2+} and Pb^{2+} can quench the intensity of the fluorescence of compound **4** about 8-fold by the formation of a complex with a ratio of 1:1. (Figs 2 and 3) From the literature,[16] if a quenching metal ion (*e.g.* open-shell, paramagnetic, large or easily reducible cation) binds tightly to the macrocyclic polyamine derivatives, intracomplex quenching takes place. Cu^{2+} or Pb^{2+} display this type of behaviour. Compound **4** probably forms complexes with Cu^{2+} or Pb^{2+} via the lone pair on nitrogen adjacent to the naphthyl ring and the fluorescence quenching most likely occurs by means of an electron or energy transfer between the metal cation and the fluorophore. While Zn^{2+} binds to macrocyclic polyamine, chelation-enhanced fluorescence (*CHEF*) is observed, which is probably ascribed to lowering the HOMO level.[4] Cd^{2+} does not display this behavior as in the literature.[6c, 16]

Addition of Zn^{2+} causes the enhancement of the fluorescence intensity of compound **4** (Figs 2 and 4). When Co^{2+}, Ni^{2+}, Cu^{2+}, $Pb^{2+}$and Cd^{2+} are added, the fluorescence of compound **4** is also enhanced in the presence of Zn^{2+} (20 μM), which indicates that compound **4** can bind Zn^{2+} tightly. However, upon addition of Cu^{2+} (40 μM) and Pb^{2+}(20 μM) in the prescence of Zn^{2+}(20 μM), the fluorescence intensity of compound **4** is increased only 2-fold.

In addition, fluorescence titration experiments (Fig.3) show that the special behaviour of compound **4** for different ions can be used to distinguish Zn^{2+} ion from its competitive ions.

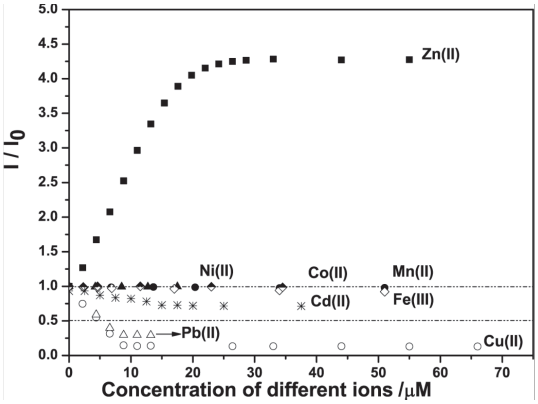

Fig. 3 Fluorescence emission spectra of 4 (10.4μM) in the presence of increasing concentrations of different ions. Fluorescence intensity at λ_{em}= 440 nm as a function of metal ions concentration. Excitation is selected at 287 nm. Measured at 25 °C in HEPES buffer (pH=7.06, 10 mM)

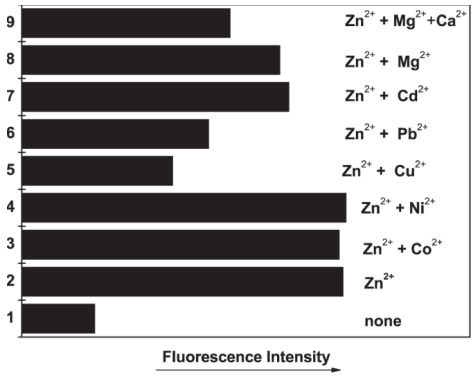

Fig. 4 Relative fluorescence intensity at 440 nm of compound **4** (10.0 μM) towards different metal ions. Lane 1: free compound **4**; lane 2: Zn^{2+} at 20 μM; lanes 3–9: Zn^{2+} (20 μM) mixed with different ions (Co^{2+}20 μM, Ni^{2+}20 μM, Cu^{2+}40 μM, Pb^{2+}20 μM, Cd^{2+}20 μM, Mg^{2+} 1 mM, Ca^{2+} 1 mM). Excitation is selected at 287 nm. Measured at 25 °C in HEPES buffer (pH=7.06, 10 mM)

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