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A new Hg^{2+} fluorescent sensors based on 1,3-alternate thiacalix[4]arene (L) and the complex of $[L+Hg^{2+}]$ as turn-on sensor for cysteine

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ABSTRACT

A new thiacalix[4]arene derivative in a 1,3-alternate conformation bearing four naphthalene groups through crown-3 chains has been synthesized, which exhibits high selectivity toward Hg^{2+} by forming a 1:2 complex, among other metal ions (Na^+, K^+, Mg^{2+}, Ba^{2+}, Ca^{2+}, Sr^{2+}, Cs^+, Mn^{2+}, Fe^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Li^+, and Zn^{2+}) with a low detection limit (3.30×10^{-7} M). The metal ion-binding properties were studied by fluorescence, AFM, and ^1H NMR spectroscopy. The in situ prepared $[Hg^{2+}+L]$ complex shows well recognition ability for cysteine with a low detection limit (2.23×10^{-7} M) through fluorescence turning on. The mechanism of fluorescence turning on is the host L releasing from $[L+Hg^{2+}]$ for $[Cys+Hg^{2+}]$ complex formed. Thus the paper reports secondary-sensor design: Hg^{2+} as a first sensor for $[L+Hg^{2+}]$ form, cysteine as a second sensor for Hg^{2+} releasing from the $[L+Hg^{2+}]$ complex after cysteine adding in.

1. Introduction

The development of highly selective and sensitive fluorescent sensors, which are capable with dual monitoring of metal ions and amino acids, have attracted considerable attention because of their wide impacts on environment and biological monitoring.[1] Among the metal ions, Hg^{2+} is attracted much more attention because it could cause a variety of symptoms in vivo, including digestive, cardiac, kidney, and neurological diseases.[2] Generally, the damage of Hg^{2+} mainly takes place through the process of complex interactions between mercury and sulfur, which provided by amino acids and peptides in vivo.[3] In particular, Hg^{2+} ions are inactive toward other amino acids except cysteine in terms of the previous reports.[4] As one of the indispensable amino acids, cysteine plays vital and important roles in human body.[5] Therefore, detecting and sensing mercury and cysteine based on one fluorescent sensor with dual functionality is feasible, and which is more economical, convenient and also of great significance. There are several reports upon recognizing Hg^{2+} and cysteine individually. For the sake of Hg^{2+} detection, plenty of receptors have been applied, such as calix[4]crowns,[6] rhodamine,[7] calix[4]arene-diaza-crown ether,[8] and dipyrene-diamide.[9] Fluorescent sensors based on amino recognition also have been reported.[10] Therefore, detecting and sensing mercury and cysteine based on one fluorescent sensor with dual functionality is feasible, and which is more economic, convenient, and also of great significance. Keeping these in mind and encouraged by the recent brilliant achievements of competitive binding assays, such as Mirkin's.[11] As a result, a specific host for Hg^{2+} recognition and second detection of cysteine is quite demanded. Being famous for its excellent skeleton structure serving as molecular platform,[12] calixarene based on numerous derivative molecules have achieved wonderful recognization performances. Especially combined with the universal and popular 'click chemistry' technology, which endows the calixarene more recognization sites, such as triazole groups.[13] On the basis of the wonderful cooperative interactions between triazole and metal ions,[14] to achieve the Hg^{2+} recognization and secondary detection of cysteine by a clicked fluorescent calixarene is reasonable and meaningful. And to the best of knowledge, such a secondary sensor of cysteine recognization followed by Hg^{2+} detection has been no report.

Herein, we exploited a novel functionalized thiacalix[4]arene (L) by means of the classic 'click chemistry' methodology.[15] 1-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)naphthalene were chosen to graft onto the thiacalix[4]arene for the following two reasons: (i) thiacalix[4]arene considered as a desirable platform, the pre-formed triazole groups provided binding sites. (ii) The naphthalene groups offered fluorescent signals for the sensitive fluorescent detection. The prepared L was characterized by ^1H NMR, MS, elementary analysis. The verification performance of L toward recognition of metal ions is subsequently investigated. High selectivity and sensitivity upon Hg^{2+} was accomplished. Most significantly, the formed $[Hg^{2+}+L]$ complex in the first step shows specific recognition of cysteine with high selectivity and sensitivity. The characteristic changes observed

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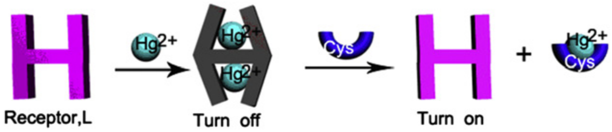
in the fluorescence spectroscopy during the sensing of Hg^{2+} as well cysteine are represented schematically in Scheme 1.



Scheme 1. Schematic representation of the primary and secondary sensing properties of L.

2. Results and discussion

2.1. Synthesis

The receptor molecule, L, has been synthesized as given in Fig. 1. All of these molecules including L were characterized satisfactorily by ^1H NMR, ^{13}C NMR, HRMS, and elemental analysis. The compound **1** with 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)naphthalene was carried out in DMF at 90 °C, with copper(II) sulfate and sodium ascorbate, to afford L (Fig. S4). A well-defined and simple ^1H NMR spectrum showed a highly symmetric structure for receptor L. The ^{t}Bu groups and aromatic protons of the thiacalix[4]arene moiety appeared as singlets at δ 1.08 ppm and δ 7.41 ppm, respectively. These δ values are in good agreement with the values obtained for 1,3-alternate conformers reported elsewhere.[16]

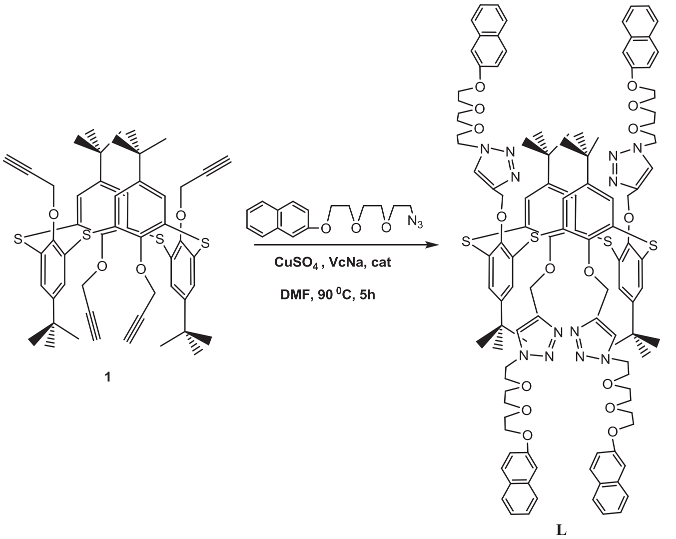


Fig. 1. Synthesis of the receptor L.

2.2. The properties of L in the selective recognition of Hg^{2+}

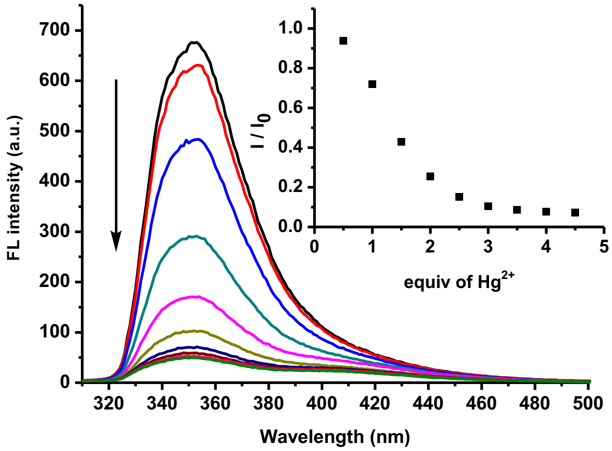


Fig. 2. Fluorescence spectra (λ_{exc}=300 nm) of L (7×10^{-6} M) in CH_3CN/H_2O (v/v, 3/1) with increasing amount of Hg^{2+} (0–3 equiv) in CH_3CN/H_2O (v/v, 3/1). Inset shows variation of fluorescence intensity against equivalents of Hg^{2+}. The excitation wavelength was 300 nm.

2.2.1. Fluorescence titration studies. The fluorescence spectrum of L (λ_{exc}=300 nm) in CH_3CN/H_2O (v/v, 3/1) exhibited a characteristic emission band at 354 nm. Upon addition of increasing amounts of Hg^{2+} to a solution of L in CH_3CN/H_2O (v/v, 3/1), quenching of fluorescence at 354 nm was observed instantaneously. Fluorescence titration of L (7×10^{-6} M) was conducted in CH_3CN/H_2O (v/v, 3/1) by the addition of Hg^{2+} from 0 to 2.1×10^{-5} M (Fig. 2). About 2 equiv of Hg^{2+} makes the quenched fluorescence reach a minimum. As the result, excess Hg^{2+} cannot achieve further quenching of fluorescence. Based on the mole ratio method, mole ratio between L and Hg^{2+} is 1:2, and meanwhile, the job's plots highly indicated a 1:2 mol ratio (Fig. S8). The detection of Hg^{2+} is about 3.3×10^{-7} M. Given the binding between the host and the guest, the binding constants could be calculated from the fluorescence titration experiments. The titration curves were analyzed by the Hyperquad 2003 program[17] and the binding constants were calculated to be log K_{11}=8.75(2) and log K_{12}=14.23(4).

Under the above conditions, as shown in Fig. 3, the fluorescence of L (7×10^{-6} M) at 354 nm was strongly quenched by Hg^{2+}, no significant spectral changes occurred in the presence of 4 equiv each of Na^+, K^+, Mg^{2+}, Ba^{2+}, Ca^{2+}, Sr^{2+}, Cs^+, Mn^{2+}, Fe^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Li^+, and Zn^{2+}. To find out whether L can detect Hg^{2+} selectively even in the presence of other metal ions, competitive metal ion titrations were carried out. Of practical significance is that even 14 equiv each of these metal ions did not interfere in the sensing of Hg^{2+}, and the results of the competition experiments are shown in Fig. 4. The origin of the fluorescence quenching may result from the electron or energy transfer from the excited naphthalene fluorescence to the Hg^{2+} ion. Alkali and alkaline earth metal ions showed no interaction with L, which may be due to their hard acid properties. Other transition and heavy metal ions produced insignificant fluorescence changes. Thus, while Hg^{2+} ion can be detected quantitatively in the presence of a number of biologically relevant Mn^+ ions.

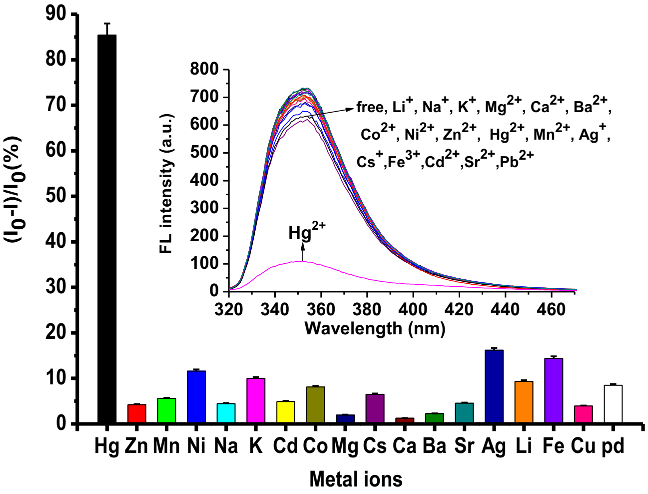


Fig. 3. Quench ratio $[(I_0-I)/I_0]$ of L (7×10^{-6} M) in CH_3CN/H_2O (v/v, 3/1) upon addition of 4 equiv of metal ions. The excitation wavelength was 300 nm. I_0 is the fluorescent emission intensity of the host at 354 nm, I is the fluorescent intensity after adding metal ions. Inset shows fluorescence intensity changes for L in CH_3CN/H_2O upon addition of metal ions.