Tetrahedron Letters 54 (2013) 2063-2066

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Novel fluorescein appended calix[4]arenes for preferential recognition of Cu²⁺ ions

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ARTICLE INFO

Article history: Received 24 October 2012 Revised 1 February 2013 Accepted 4 February 2013 Available online 13 February 2013

Keywords: Calixarenes Fluorescence Copper ion Recognition Chemosensor

ABSTRACT

Novel calix[4]arene derivatives with fluorescein appendages (7 and 8) were synthesized for application in preferential recognition of Cu^{2+} ions over other metal ions through enhancement of fluorescence intensity with a detection limit up to nanomolar level in acetonitrile. A profound color change from colorless to yellow in acetonitrile and colorless to purple in aqueous medium indicates specific copper ion recognition plausibly due to selective ring opening of fluorescein spirolactam functionality. The synthesized receptor shows high selectivity and reversibility required for developing efficient chemosensors for Cu^{2+} . © 2013 Elsevier Ltd. All rights reserved.

Development of organic molecules as receptors with the ability to bind and sense ions or neutral molecules through an optical response is an eminent area of interest due to their application in chemistry and biomedical research.^{1,2} Fluorescence sensors offer distinct benefits in terms of better sensitivity and high selectivity associated with the fluorescence based techniques.^{3–5}

Synthesis of a 'turn on' fluorescence sensor for Cu²⁺ is a challenging task as it is known to quench the fluorescence intensity due to its paramagnetic nature.⁶ Most of the reported 'turn on' fluorescent sensors for Cu²⁺ included coumarin, amino naphthalimide, picolino hydrazide, and BODIPY based molecular probes.⁷

In this Letter we describe our results on the synthesis and evaluation of novel calix[4]arene derivatives for recognition of copper ions which play a ubiquitous role in biological processes.⁸ Its deficiency may lead to bone disorders, anemia, and leucopenia,⁹ while its excess may lead to usher scourge of Wilson and Alzheimer diseases.¹⁰ The target receptors for copper ions were synthesized by linking the cone conformer of calix[4]arene with fluorescein fluorophore through a reaction sequence given in Scheme 1.¹¹ Choice of a fluorescein derivative was based upon the high absorption and emission wavelength,^{12,13} extinction coefficient, and quantum yield of such derivatives.^{14–16} Fluorescein hydrazide was prepared by refluxing fluorescein with hydrazine hydrate in ethanol.¹⁴ The required starting materials **2–6** were synthesized according to the literature.¹¹ The fluorescein hydrazide was then reacted with the formyl derivatives of calix[4]arene (**5**, **6**) in ethanol to give the corresponding fluorescein appended calix[4]arene $(7, 8)^{11d,e}$ as given in Scheme 1.

The structures of fluorescein appended calix[4]arenes (7, 8) were confirmed by ¹H NMR and ¹³C as well as IR and mass spectroscopy. For example, in the IR spectra, the appearance of strong absorption peaks in the range of 1650-1660 cm⁻¹ could be assigned to C=N stretching frequency. Moreover, the derivatives 7-8 also showed absorption peaks in the range of 3200-3400 and 1600–1610 cm⁻¹ which could be ascribed to –OH and aromatic C=C stretching frequencies respectively. Compound 7 exhibited a pair of doublets at 4.17 and 3.46 ppm for equatorial and axial protons respectively in the ¹H NMR spectrum and a distinct singlet at 30.2 ppm for the methylene bridge carbon in the ¹³C NMR spectrum indicated the cone conformation. Compound 7 exhibited a signal at δ 66.48 ppm in the ¹³C spectra which indicated the predominance of lactam ring. Similarly **8** exhibited a pair of doublets at 4.24 and 3.17 ppm in ¹H spectrum which could be ascribed to the endo and exo protons respectively of methylene bridge. The hydrazone formation in 7 and 8 was established by the appearance of a singlet at 8.93 and 8.38 ppm respectively which could be assigned to CH=N group.

The ion sensing ability of **7** in acetonitrile was examined by recording the change in the UV–vis spectum of 7 in the presence of various metal ions. The receptor (**7**) exhibited a peak at 330 nm which could be assigned to the closed spirolactam ring of the fluorescein. It was observed that upon gradual addition of Cu^{2+} to 4×10^{-5} M solution of **7**, the intensity of absorption band at 330 nm decreased with the appearance of two absorption bands centered at 430 and 520 nm. A typical variation of absorption spectrum of **7** upon the gradual addition of Cu^{2+} is shown in Figure 1.





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Scheme 1. Synthesis of novel fluorescein appended calixarenes. Reagents and conditions: (i) NH₂NH₂·2H₂O, ethanol, reflux; (ii) 1,3 dibromopropane, K₂CO₃, CH₃CN reflux; (iii) *p*-hydroxybenzaldehyde, K₂CO₃, CH₃CN reflux; (iv) Fluorescein hydrazine, acetic acid, ethanol reflux; (v) ethyl bromoacetate, K₂CO₃, CH₃CN, reflux; (vi) HMTA, TFA, reflux.

The isobestic point was observed at 363 nm. This was also accompanied by a change in color of solution from colorless to yellow. The addition of other cations (Mn^{2+} , K^+ , Co^{2+} , Hg^{2+} , Ag^+ , Ni^{2+} , Fe^{2+} , Na^+ , Zn^{2+} , Li^+ and Cd^{2+}) resulted in negligible changes in the UV–vis spectum of **7**. (SI, Fig. S5)

lon sensing ability of **7** was further assessed by the fluorescence titrations. Its emission spectrum was recorded from 350 to 620 nm by exciting it at 340 nm. It exhibited the emission maxima at 420 nm which could be assigned to a trace of the ring opened spirolactam form of fluorescein.

The effect of Cu^{2+} on the emission spectrum of **7** is shown in Figure 2. Inset shows relative change in the fluorescence intensity

of **7** with varying concentrations of Cu²⁺. Only Cu²⁺ displayed remarkable enhancement in the fluorescence intensity of **7** among various tested metal ions with a red shift of 40 nm from 420 nm to 460 nm in the emission spectrum. Addition of other metal ions did not produce any significant change in the emission spectrum of **7** (SI, Fig. S6).

Fluorescence intensity of **7** was determined to vary linearly with $[Cu^{2+}]$ upto 65 μ M (inset Fig. 2) and limit of detection for Cu^{2+} by **7** could be calculated by using calibration sensitivity (*m*) of relative fluorescence intensity versus $[Cu^{2+}]$ in the aforementioned range.¹⁷ To calculate LOD, minimum change in the fluorescence intensity due to the presence of Cu^{2+} was taken to be $3 \times s_0$

where s_0 represents standard deviation of Fo for 10 replicate measurements.¹⁸ Thus, the LOD is calculated using the formula LOD [Cu²⁺] = $3 \times s_0/m$, and it was found to be 10 nM (0.63 ppb).

Under identical conditions, addition of Cu^{2+} to **8** resulted in 25folds increase in the intensity of the receptor (SI, Fig. S7). This shows that **7** is more sensitive to Cu^{2+} .

In order to evaluate the sensitivity of **7** toward Cu²⁺, fluorescence titrations were performed with perchlorates of competitive metal ions as well. Figure 3 depicts relative change in the fluorescence intensity of **7** upon addition of various metal ions. It is quite evident from Figure 3 that other metal ions entail insignificant change in the emission spectra of **7**. Selectivity coefficients were calculated for all the studied metal ions using the formula $K_{\rm M}^{n+}, c_{\rm u}^{2+} = \Delta F_{\rm M}^{n+} / \Delta F_{\rm Cu}^{2+}$ where $\Delta F_{\rm M}^{n+}$ represents change in the fluorescence intensity of **7** in the presence of various metal ions and $\Delta F_{\rm Cu}^{2+}$ represents change in the fluorescence intensity of **7** in the presence of Cu²⁺. Selectivity coefficients for all the studied metal ions were found to be too small to pose any hindrance in the detection of Cu²⁺.

To further analyze the selectivity of the receptor, competitive experiment was performed with 1 mM Cu^{2+} in combination with 1 mM competitive metal ions. The data clearly showed that all

other metal ions have marginal effect on the fluorescence emission spectra of **7** (SI, Fig. S7).

To examine the plausible use of **7** in aqueous assay, its ion sensing properties were studied in aqueous medium ($CH_3CN/H_2O = 1/1$, v/v). Under these conditions, no new absorption peak in the visible region was observed. Successive addition of copper ions resulted in enhancement of the absorption of the peak at 540 nm with simultaneous change in the color of the solution from colorless to a purple solution (Fig. 4).

Fluorescence titration was also performed in aqueous solution (CH₃CN:H₂O = 1/1, v/v). A small quenching (24%) in the fluorescence emission intensity of **7** in the presence of Cu²⁺ was observed (SI, Fig. S9). The quenching observed might be due to the paramagnetic nature of Cu²⁺ metal ion or due to energy or electron transfer in the excited state.¹⁹

Selectivity of the synthesized probe **7** toward surveyed metal ions was examined by adding up to 4 equiv of other metal ions when no change in the absorption was observed but addition of Cu^{2+} to the solution of 7 resulted in a rapid enhancement in intensity of absorption in the UV-visible spectra of **7** (SI, Fig. S10).

The reversibility of the present sensing system was assessed by introducing ethylenediamine tetraacetic acid solution (4 equiv to Cu^{2+}) to **7** (20 μ M) that contained Cu^{2+} (200 μ l) in CH₃CN/H₂O (1:1, v/v) when the absorption spectrum regressed to the original



Figure 1. Change in the UV-vis spectra of 7 (40 $\mu M)$ upon addition of 4 equiv of Cu^{2+} (1 mM) in CH_3CN.



Figure 2. Enhancement in fluorescence intensity of **7** (40 μ M) in CH₃CN in the presence of Cu²⁺ (1 mM) ($\lambda_{excitation}$ = 340 nm); Inset shows change in the fluorescence intensity of **7** with varying concentration of Cu²⁺.



Figure 3. Change in the fluorescence intensity of **7** upon addition of 2.5 equiv of various metal ions to receptor in CH₃CN ($\lambda_{\text{excitation}} = 340 \text{ nm}$); Inset shows selectivity coefficients, $K_{\text{M}}^{n+}/_{\text{Cu}}^{2+} = \Delta F_{\text{M}}^{-n+}/\Delta F_{\text{Cu}}^{2+}$.



Figure 4. Change in the UV-vis spectra of the ligand (7) (40 μ M) on addition of 4 equiv of Cu²⁺ in CH₃CN/H₂O (1/1, v/v).

absorption spectrum of **7** probably due to release of copper from the sensing system due to demetalation. The experiments were repeated twice with the same final outcome (SI, Fig. S9).

According to Benesi–Hildebrand plot, linearity was observed between 1/(A-Ao) versus 1/[Cu²⁺] indicating the formation of 1:1 complex between **7** and target ion which was further confirmed by Job's continuous variation plot experiment. (SI, Fig. S10) The binding constant of **7** with Cu²⁺ in CH₃CN/H₂O (1/1/, v/v) was calculated to be 1.74×10^3 (R^2 = 0.9968).

The binding of Cu^{2+} with **7** could not be monitored through NMR titrations due to the paramagnetic nature of Cu^{2+} which leads to the broadening of NMR spectrum.

In conclusion, we have synthesized and characterized novel fluorescein appended calix[4]arene receptors for their potential utilization as chemical sensors. Among the two fluorescein appended calix[4]arene frameworks, **7** is found to be extremely sensitive toward Cu^{2+} and recognizes it through 1:1 stoichiometry with high binding affinity.

Acknowledgments

R.S. thanks CSIR, India for fellowship. Financial assistance from DST, MFPI, MOEF and MHRD is gratefully acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.02. 017.

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