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New coumarin-based sensor molecule for magnesium and calcium ions

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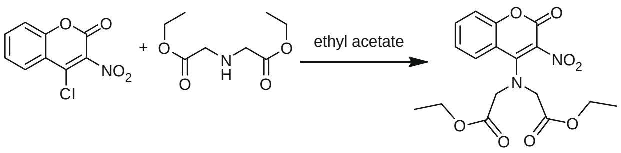
ABSTRACT

A new coumarin-based sensor molecule (**L$_1$**) has been synthesized and this was found to bind calcium and magnesium ions more effectively as compared to other alkali/alkaline earth/lanthanide and certain transition metal ions. A significant enhancement in fluorescence intensity was observed on binding to Ca^{2+} and Mg^{2+} ions; while a minor quenching was observed for weakly bound Hg^{2+}, Ni^{2+}, Fe^{3+}, and Co^{2+} ions. PET process, coupled with the ICT process, is proposed to explain the observed spectral response.

Fluorescent chemosensors which are capable of sensing biologically important analytes by different signal transduction mechanisms are of current interest.[1] In this regard, sensing of certain alkaline earth metal ions such as Ca^{2+} and Mg^{2+} is significant owing to their involvements in various biological processes.[2,3] Chemosensors are generally composed of a receptor unit that is selective for a specific analyte and covalently bound to a signaling unit. The changes induced by receptor–analyte binding in the form of output signals are to be generally probed through changes in either spectral (^{1}H NMR, electronic, or fluorescence) or redox behavior. Among chromogenic sensors, sensor with fluorescent signaling unit has an obvious edge over others owing to its ability to detect the desired analyte that is present in ultratrace quantity.[4] However, signal transduction mechanism associated with binding to the metal ion depends on the way the receptor and signaling units are covalently coupled to each other. For a system, where receptor and analyte are separated by a saturated spacer, photoinduced electron transfer (PET)[5] process is reported to be one of the dominant processes; while for a integrated system,[6] having an amino functionality conjugated to an electron-withdrawing functionality, ICT process usually is the prevailing one.[1h,6a] The main feature of the PET process is the appreciable change in the luminescence intensity on metal ion binding and ICT process is generally associated with spectral shift. Also, there are instances for integrated system where both processes (fluorescence enhancement and spectral shift) are operational.[7] In this Letter we report a new example of an integrated system and try to reveal the dominant pathway for the signal transduction.

There are many references available in the literature that describes fluorescence-based sensor molecules for Ca^{2+} and Mg^{2+} ions. However, syntheses of many of these molecules involve intricate methodologies and hence are relatively difficult to prepare. For example, synthesis of several receptors for alkaline earth metal ions which were derived from polyamino polycarboxylato/azacrown ether/calixarene derivatives/open chain ethers and were bound to squarene/squarine dye or other appropriate reporter functionality has been reported. Synthesis of these sensor molecules generally involves multistep procedures.[8] A comparative study reveals that among the various receptors, polyamino, polycarboxylate derivative-based receptor functionalities generally show relatively higher binding affinity toward these cations. To cite an example, Akkya and his co-workers have developed such a PET-based sensor molecule for Ca^{2+}, which works in non-aqueous medium with K_d of 240 nM.[2b] While a receptor unit with pendant ester functionality has shown an even higher affinity constant value in acetonitrile.[2c] This led us to design a sensor molecule (**L$_1$**) where a coumarin-based signaling unit is coupled directly to an amine functionality with ONO-donor set having pendant ester functionality and study its selectivity in binding to these two metal ions.

The diethyl-2,2′-(3-nitro-2-oxochroman-4-ylazanediyl) diacetate (**L$_1$**, Scheme 1), was synthesized in a single step with reasonably good yield and **L$_1$** was found to bind preferentially to Mg^{2+} and Ca^{2+} ions as compared to all other alkali, alkaline earth, lanthanides, and most transition metal ions (Supplementary data). Binding phenomena was probed by monitoring the changes either in fluorescence intensity or in optical density. Apart from these two metal ions, lower binding affinities of **L$_1$** toward Hg^{2+} and Ni^{2+} were also observed.



Scheme 1. Methodology followed for the synthesis of **L$_1$**.

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The new receptor molecule **L$_1$** was synthesized following a one-step procedure (Scheme 1). 4-Chloro-3-nitro coumarin was added in a dropwise manner to a diethyl iminodiacetate solution in ethyl acetate at 0 °C and the resultant reaction mixture was allowed to stir at 0 °C for 1 h. Then it was allowed to attain the room temperature and stirred for another 2 h. The solvent was removed under reduced pressure and the crude, thus obtained, was purified by column chromatography using neutral Al_2O_3 as the stationary phase and ethyl acetate as the eluent. Desired product was isolated in pure form with 80% yield. Analytical data (ESI-MS, ^{1}H NMR, ^{13}C NMR analysis) confirmed the proposed structure and purity of **L$_1$**.[9]

This newly synthesized receptor molecule with pendant ester (**L$_1$**) functionality showed absorption bands at 279 and 315 nm and the molar extinction coefficient was found to be 3348 M^{-1} cm^{-1} for absorption band at 315 nm. Emission maxima observed at 370 nm upon excitation at 315 nm was attributed to a charge transfer emission state—while amine and nitro coumarin units are donor and acceptor fragments, respectively. Further a red shift in emission maxima with associated enhancement in intensity was observed when polarity of the medium was systematically varied from 4.4 (for ethyl acetate, $\lambda_{max} = 365$ nm) to 6.4 (for DMF, $\lambda_{max} = 393$ nm). This also confirms the ICT nature of the emission band. However, involvement of the photoinduced electron transfer (PET)-based process for the **L$_1$** could not be completely ruled out. The observed weak fluorescence for acetonitrile solution of **L$_1$** could be attributed to PET-based process involving tertiary N-atom with non-bonding electrons and the acceptor coumarin moiety. Further, presence of the $-NO_2$ group in the acceptor functionality is expected to lower the energy for the coumarin-based LUMO and thus is expected to facilitate the PET process. Similar effect was also observed by other researchers.[10]

Preliminary spectral studies revealed an appreciable change in optical spectral pattern for **L$_1$** upon addition of Mg^{2+} and Ca^{2+} in acetonitrile media and are shown in Figure 1. No such changes were registered when similar experiments were repeated for other alkali, alkaline earth, and transition metal ions such as Li(ClO$_4$), Na(ClO$_4$), K(ClO$_4$), Cs(ClO$_4$), Ba(ClO$_4$)$_2$, Sr(ClO$_4$)$_2$·xH$_2$O, Hg(ClO$_4$)$_2$·xH$_2$O, Pb(ClO$_4$)$_2$·3H$_2$O, Co(ClO$_4$)$_2$·6H$_2$O, Ni(ClO$_4$)$_2$·6H$_2$O, Cu(ClO$_4$)$_2$·6H$_2$O, Fe(ClO$_4$)$_2$·xH$_2$O, and Cr(ClO$_4$)$_3$·6H$_2$O. Lanthanide ions also show an insignificant binding affinity toward **L$_1$**. This reveals a preferential binding of **L$_1$** to Mg^{2+} and Ca^{2+} ions. Upon addition of Ca^{2+} ion, the absorption at 279 nm was found to decrease; while an increase in absorption at 312 nm, with 8 nm red shift, was observed. Simultaneous occurrence of three distinct isobestic points at 264, 307, and 378 nm was evident during the systematic titration with increasing [Ca^{2+}]. Presence of the isosbestic points signified the presence of two species in equilibrium. Almost similar trends were observed for titrations with [Mg^{2+}]. Decrease in absorption at 264 nm and an increase in absorbance at 312 nm with appearance of a clear shoulder at ~330 nm and the associated isosbestic points appeared at 369, 307, and 264 nm. Titration profile indicated the formation of a 1:1 complex (**L$_1$**·M^{2+}) for both metal ions. Respective binding constant for formation of **L$_1$**·Ca^{2+} and **L$_1$**·Mg^{2+} was evaluated using Benesi–Hildebrand equation (Eq. 1)[11] and was found to be 1.50 × 10^7 M^{-1} and 5.05 × 10^5 M^{-1}.

$$\frac{1}{A - A_0} = \frac{1}{K(A_{max} - A_0)[M^{x+}]^n + \frac{1}{A_{max} - A_0}} \qquad (1)$$

Here A_0 is the absorbance of **L$_1$** in the absence of guest (M^{x+}), A is absorbance recorded in presence of added guest, A_{max} is absorbance in presence of added $[M^{n+}]_{max}$ and **K** is the association constant. The association constant (K) could be determined from the slope of the straight line of the plot of $1/(A - A_0)$ against $1/[M^{x+}]$. Linearity of this plot (Fig. 2) confirms a 1:1 complex formation between **L$_1$** and Ca^{2+}/Mg^{2+} ion. This further corroborates 1:1 complex formation that we have concluded based on the spectral titration profile. Based on the spectrophotometric studies, lowest detection limit for these two ions was ~50 ppb (signal to noise ratio of 3:1).

On addition of either Ca^{2+} or Mg^{2+} to acetonitrile solution of **L$_1$**, a significant enhancement in the emission intensity at 376 (for Ca^{2+}) or 377 (for Mg^{2+}) nm upon excitation at 315 nm (Fig. 3) was observed. Emission spectra for **L$_1$** in acetonitrile solution were recorded for other metal ions and are shown in Figure 4.

A very small enhancement in emission intensity was observed for Ba^{2+} and Cs$^+$ ions; while a small decrease in fluorescence intensity of **L$_1$** was observed in the presence of added Hg^{2+} ion. This decrease in emission intensity was even more insignificant in the presence of added Ni^{2+}, Fe^{3+}, and Co^{2+}. These small changes in emission intensities (enhancement or quenching) signified a weaker binding of the respective metal ions to **L$_1$**. No detectable change for other metal ions nullifies any possibility of any binding of **L$_1$** to other metal ions studied. Further, it is reported in the literature that carbonyl functionality of the alizarin moiety could coordinate to the Ca^{2+}/Mg^{2+} ion.[3c] We performed a control experiment in order to check any such binding of coumarin with Ca^{2+} or Mg^{2+} ions. Fluorescence and absorption spectra of coumarin in the absence and in the presence of Ca^{2+} or Mg^{2+} ions were recorded in acetonitrile solution. No change in electronic spectral pattern and a very little enhancement in the fluorescence spectra, as compared to that of **L$_1$**, nullified such a possibility.

Thus, a small red shift of 7/8 nm of the emission maxima for **L$_1$** was observed in the presence of Ca^{2+} and Mg^{2+} ions (Fig. 3). It is

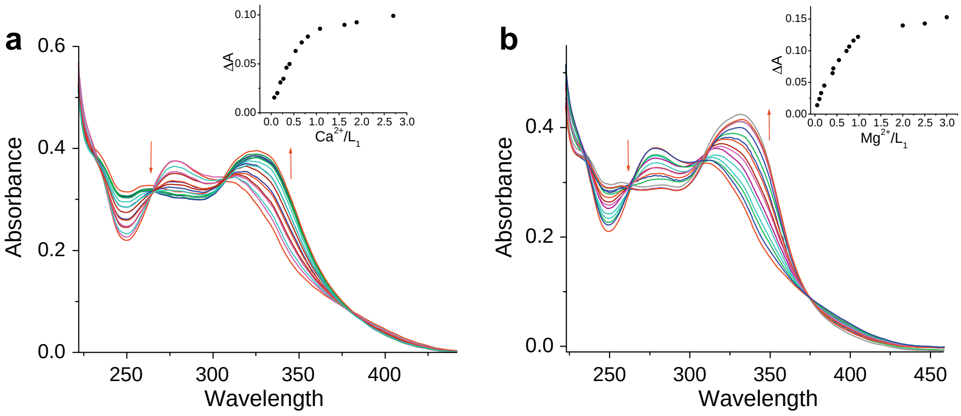

Figure 1. UV–vis spectra of **L$_1$** (5.90 × 10^{-5} M) upon addition of (a) 0–4.8 × 10^{-4} M of Ca^{2+} and (b) 0–5.6 × 10^{-4} M of Mg^{2+} in acetonitrile. Inset: Corresponding titration curves for Ca^{2+} and Mg^{2+} show the 1:1 stoichiometry.

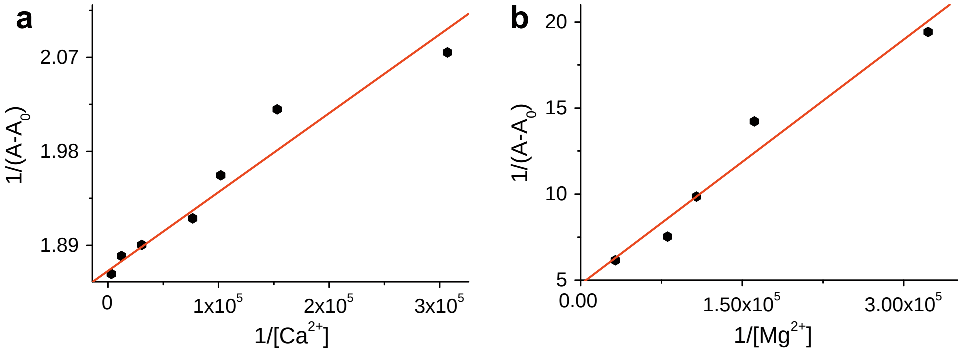

Figure 2. Benesi–Hildebrand method for calculating binding constants with (a) calcium and (b) magnesium.

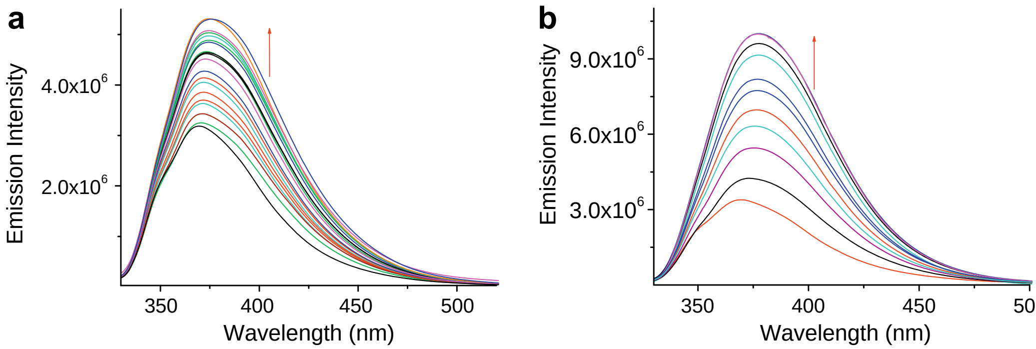

Figure 3. Fluorescence spectra of $\mathbf{L_1}$ (2×10^{-5} M) upon titration of (a) 0–3.1×10^{-4} M of Ca^{2+} and (b) 0–1.7×10^{-3} M of Mg^{2+} in acetonitrile. Excitation: 315 nm.

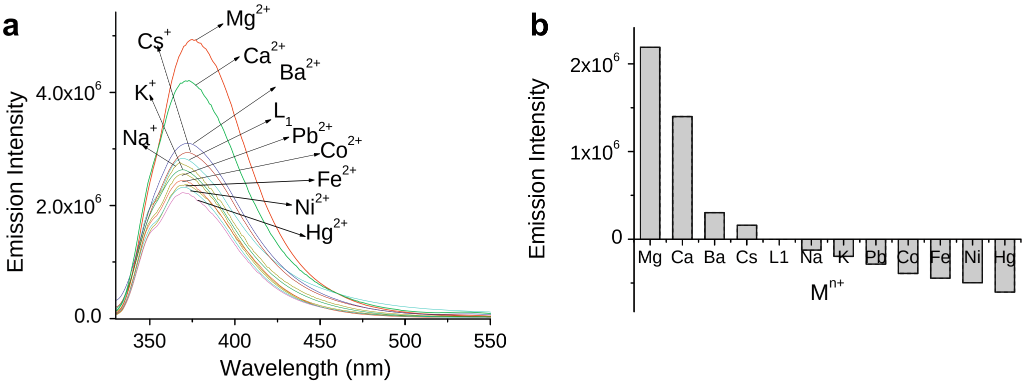

Figure 4. (a) Fluorescence spectra of $\mathbf{L_1}$ (2.0×10^{-5} M) upon addition of 1 equiv of different metal ions in acetonitrile media when excited at 315 nm. (b) Bar diagram shows the relative emission intensity.

well documented that for ICT-based emission processes, fluorescence spectrum is expected to be only slightly affected on metal ion binding.[1h,6a] While a more appreciable shift in electronic spectra (Fig. 1) could be detected in the presence of these ions. These observations are suggestive of the fact that ICT-based signal transduction process is operational. However, considerable enhancement of the emission intensity for $\mathbf{L_1}$ on binding to Mg^{2+} and Ca^{2+} ions tends to suggest that PET process is also operational along with ICT process. Relatively strong binding of $\mathbf{L_1}$ to these two metal ions involves the lone pair of electron of the tertiary N-atom and effectively interrupts the PET process and this could be accounted for the observed enhancement of the coumarin-based fluorescence. Respective binding constant for Ca^{2+} and Mg^{2+} was evaluated based on the systematic fluorescence titrations (Fig. 3) with varying $[M^{2+}]$ ($M^{2+} = Ca^{2+}/Mg^{2+}$) and was found to match nicely with that obtained from spectrophotometric titration. Changes in emission intensity for Hg^{2+} and Ni^{2+} were very small as compared to those for Ca^{2+}/Mg^{2+} (Fig. 5), however changes were sufficient for evaluation of respective binding constant for formation of $\mathbf{L_1{\cdot}Hg^{2+}}$ and $\mathbf{L_1{\cdot}Ni^{2+}}$. These values for Hg^{2+} and Ni^{2+} were found to much lower ($K_{Hg^{2+}} = 1.58 \times 10^4$ M^{-1} and $K_{Ni^{2+}} = 1.13 \times 10^3$ M^{-1}) as compared to those for Ca^{2+} and Mg^{2+}.

Time-resolved fluorescence decay studies for $\mathbf{L_1}$ were carried out in the absence and in the presence of Ca^{2+} and Mg^{2+} ions using TCSPC technique. A laser excitation source of 340 nm was used and decay luminescence kinetics was monitored at 370 nm. An air-equilibrated acetonitrile solution of $\mathbf{L_1}$ was used for studies. In the absence of any added Ca^{2+} or Mg^{2+} ions, fluorescence decay trace for $\mathbf{L_1}$ could be best fitted to single exponential time constant of $\tau = 1.09 \pm 0.01$ ns ($\chi^2 = 1.29$) (Fig. 6).

However, in the presence of Ca^{2+} and Mg^{2+} (1.3 equiv) fluorescence decay traces could be best fitted to 1.83 ± 0.01 ns

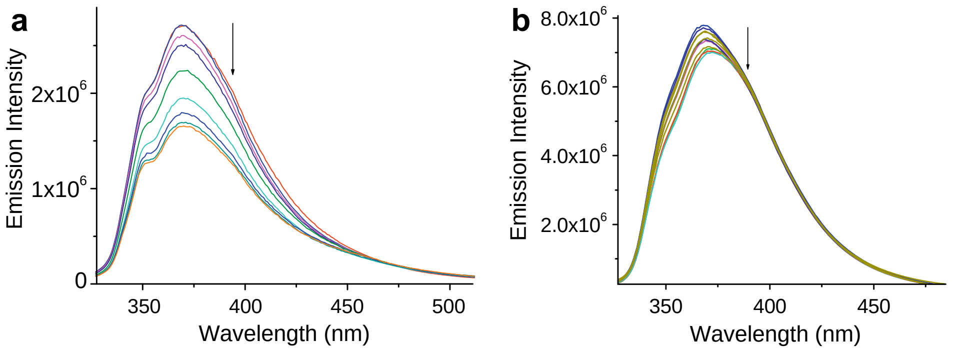

Figure 5. Fluorescence spectra of $\mathbf{L_1}$ (2×10^{-5} M) upon titration of (a) 0–1.25 equiv Hg^{2+} and (b) 0–1.25 equiv of Ni^{2+} in acetonitrile; λ_{Ext}: 315 nm.

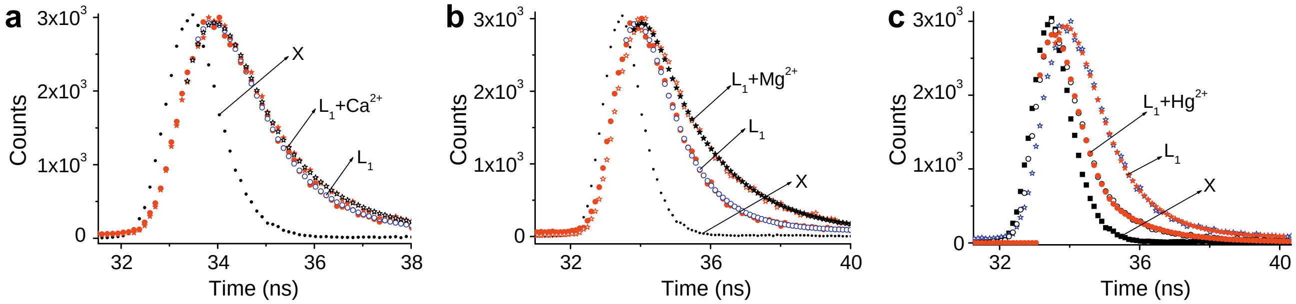

Figure 6. Fluorescence decay spectra of $\mathbf{L_1}$ with (a) Ca^{2+}, (b) Mg^{2+}, and (C) Hg^{2+} in acetonitrile. LED excitation source (340 nm) was used. **X** signifies the lamp response.

($\chi^2 = 1.16$) and 1.29 ± 0.02 ns ($\chi^2 = 1.27$), respectively. Similar time-resolved emission studies with Hg^{2+} shows bi-exponential decay with the life times of $\tau_1 = 1.26 \pm 0.06$ ns (39.5%) and $\tau_2 = 0.165 \pm 0.058$ ns (60.5%) ($\chi^2 = 1.058$). In the absence of any PET process, de-excitation process involves electronic transition from photo-excited LUMO to the HOMO of $M\mathbf{L_1}$ ($M = Ca^{2+}/Mg^{2+}$) and perhaps also accounts for longer lifetime. Changes are more prominent for Ca^{2+}, compared to Mg^{2+}, and this agreed well with the fact that Ca^{2+} binds strongly to $\mathbf{L_1}$. For Hg^{2+}, the minor component ($\tau_1 = 1.26 \pm 0.06$ ns) could be accounted for the uncomplexed coumarin derivative; while the faster and major component (0.165 ± 0.058 ns, 60.5%) was attributed to Hg^{2+}–$\mathbf{L_1}$ complex. The decay component that matches with free $\mathbf{L_1}$, reflects the presence of free ligand under comparable metal ion concentration (as compared to Ca^{2+} or Mg^{2+} ions) and a relatively weaker binding process. The faster decay could be explained on the basis of the spin–orbit coupling. Spin–orbit coupling is of relativistic origin and becomes significant for heavier metal ions such as Hg^{2+}. This generally lifts the degeneracy of otherwise orbitally degenerate states and can provide a mechanism to mix states of different states of different spin and thereby allowing otherwise spin forbidden transitions.[12] This mixing up of energy of states generally causes a faster deactivation of the excited state through a non-radiative pathway. Thus, time resolved studies also corroborate the results of the steady state spectral studies.

In conclusion, we have described a new sensor molecule which shows some selectivity toward calcium and magnesium in acetonitrile solution; while the lowest concentration that could be detected for these two ions was ∼50 ppb. Experimental data tend to suggest the involvement of both ICT and PET processes in signal transduction.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.07.141.

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9. ^{1}H NMR (500 MHz, $CDCl_3$), (ppm): 7.988 ($1H_g$, d, *J* = 8 Hz), 7.67 ($1H_i$, t, *J* = 7.5 Hz), 7.41 ($2H_{j,h}$, m), 4.22 ($8H_{b,d}$, m), 1.29 ($6H_a$, t, *J* = 6). ^{13}C NMR (500 MHz, $CDCl_3$), (ppm): *C*: 168.1, *e*: 155, *l*: 152.7, *k*: 151.8, *m*: 134.1, *i*: 130.3, *h*: 127.3, *g*: 125.3, *j*: 117.9, *f*: 116.3, b: 61.9, *d*: 53.6, *a*: 14.0. ESI-MS: *m*/*z*: 417.68 for [$\mathbf{L_1}$ + K^+], 401.71 [$\mathbf{L_1}$+Na^+], 379.73 for [$\mathbf{L_1}$+H^+]; calcd for $C_{17}H_{18}N_2O_8$, ($\mathbf{L_1}$) is 378.3.
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