Tetrahedron Letters 50 (2009) 2735–2739

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Fluorescence switch-on sensor for Cu^{2+} by an amide linked lower rim 1,3-bis(2-picolyl)amine derivative of calix[4]arene in aqueous methanol

Roymon Joseph, Balaji Ramanujam, Amitabha Acharya, Chebrolu P. Rao *

Bioinorganic Laboratory, Department of Chemistry, Indian Institute of Technology Bombay, Mumbai 400 076, India

ARTICLE INFO

Article history:
Received 14 January 2009
Revised 7 March 2009
Accepted 14 March 2009
Available online 20 March 2009

Keywords:
Cu^{2+} sensor
Fluorescence switch-on
Calix[4]arene appended with bis-(2-picolyl)amine
Nano structure

ABSTRACT

A highly selective fluorescence switch on sensor, L for detecting Cu^{2+} has been synthesized by introducing a bis-(2-picolyl)amine moiety at the lower rim of a calix[4]arene platform via amide linkage. Binding properties of L toward ten different biologically relevant M^{n+} ions have been studied by fluorescence and absorption spectroscopy in methanol and aqueous methanol. L was found to detect Cu^{2+} selectively down to a concentration of 196 and 341 ppb, respectively, in methanol and 1:1 aqueous methanol even in the presence of other metal ions. The composition of the complex has been found to be 1:1 based on the Job plot and is further confirmed by ESI MS. The role of calix[4]arene platform as well as the pre-organized binding core in the selective recognition of Cu^{2+} has been demonstrated by studying appropriate reference molecules. The possible modes of binding of L with Cu^{2+} have been modeled by computational calculations. L and its Cu^{2+} complex could very well be differentiated based on the nano-structural features observed in SEM and AFM.

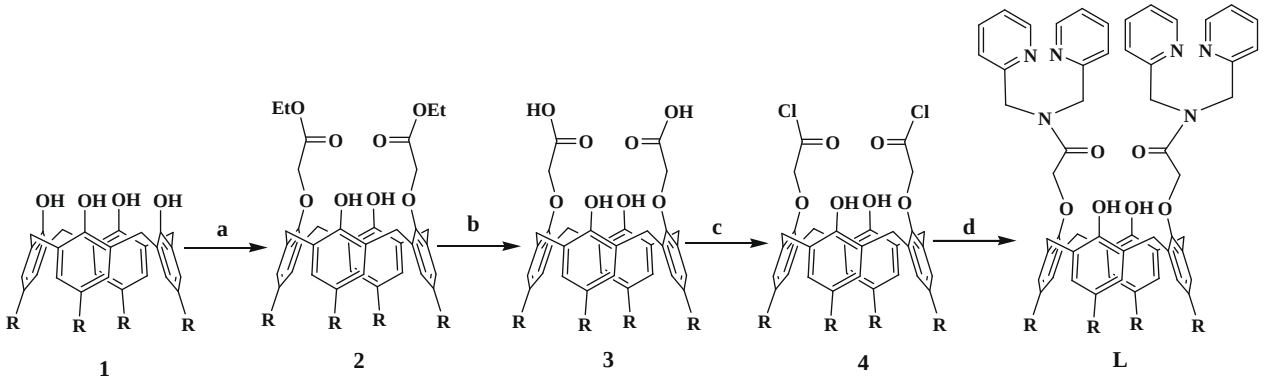
Detections of cations, anions and molecular species are some of the challenging areas of current research relevant to the receptor design and development. Copper is one of the essential trace elements[1] of transition metals which serves as a cofactor by taking an active part in a large variety of enzymes.[2] As copper can easily access both +1 and +2 oxidation states, it can act as electron carrier.[3] Both excessive copper intake and copper deficiency lead to several disorders.[2b,2c,4] Therefore, the detection of copper by various synthetic receptors is an emerging area of current research. Enhancement in the fluorescence intensity as a result of metal ion binding is more attractive than quenching. There are several reports in the literature in which appropriately functionalized calixarenes[5] have been used as host molecules for ions and neutral molecular species[6], among them those for Cu^{2+} are rather limited. To our knowledge, only an upper rim functionalized calix[4]arene having an aminoquinoline moiety connected through schiff's base exhibited a fluorescence enhancement with Cu^{2+} in CH_3CN[7], while others exhibited quenching.[8] Therefore in this Letter we report selective ion recognition features of 1,3-di-derivative of calix[4]arene containing a bis-(2-picolyl)amine moiety at its lower rim connected by an amide linkage toward Cu(II).

The receptor molecule, L, viz., 5,11,17,23-*tert*-butyl-25,27-bis-((*N*,*N*-bis(pyridine-2-yl)methylamine)carbonylmethoxy)-26,28-dihydroxycalix[4]arene, was synthesized by four known steps[9] starting from *p*-*tert*-butyl calix[4]arene (Scheme 1, SI 01). All the molecules including L were characterized satisfactorily by ^1H NMR, ^{13}C NMR, ESI MS, IR, and elemental analysis.[10] The cone conformation of L has been confirmed by ^1H NMR spectroscopy.

The metal ion binding studies of L and its control molecules were demonstrated by fluorescence spectroscopy. The metal ions, viz., Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Na^+, K^+, Ca^{2+}, and Mg^{2+}, were subjected to recognition studies with L and the control molecules. The studies were carried out by exciting the solutions at 285 nm and recording the fluorescence spectra in the range of 295–420 nm in methanol as well as in 1:1 aqueous methanol (SI 02). During the titration in methanol, the fluorescence intensity of L increases as a function of Cu^{2+} addition (Fig. 1a) and shows about 7 fold enhancement and saturates around 2 equiv of Cu^{2+} addition (Fig. 1b). Thus the titration of L with Cu^{2+} results in a stoichiometric reaction. However, when similar titrations were carried out with other metal ions, such as, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Na^+, K^+, Ca^{2+}, and Mg^{2+}, only a minimal enhancement or minimal quenching has been observed (Fig. 1c). Since aqueous solutions are of paramount importance in order to have a wide range of applicability of the receptor, similar titrations were carried out in 1:1 aqueous methanol and a ~12 fold increase in the fluorescence intensity of L with Cu^{2+} (Fig. 1b and c) was found though the saturation was found around 5–6 equiv. The observed enhancement in the fluorescence intensity can be explained to the reversal of PET when Cu^{2+} binds to the nitrogens of pyridyl moieties. The binding constant of L with Cu^{2+} was calculated by Benesi–Hildebrand equation and the corresponding association constant, K_a was found to be 17,547 ± 1000 and 30,221 ± 1600 M^{-1}, respectively, in methanol and 1:1 aqueous methanol. The quantum yield of L and its complex with Cu^{2+} were found to be 0.0118 and 0.0559 in methanol and

* Corresponding author. Tel.: +91 22 2576 7162; Fax: +91 22 2572 3480.
E-mail address: cprao@iitb.ac.in (C.P. Rao).

0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.03.109



Scheme 1. Synthesis of lower rim calix[4]arene-1,3-di-derivatives, L: (a) bromoethylacetate/K_2CO_3/acetone; (b) NaOH/C_2H_5OH, reflux; (c) $SOCl_2$/benzene, reflux; (d) bis(2-picolyl)amine/Et_3N/THF. R = *tert* butyl.

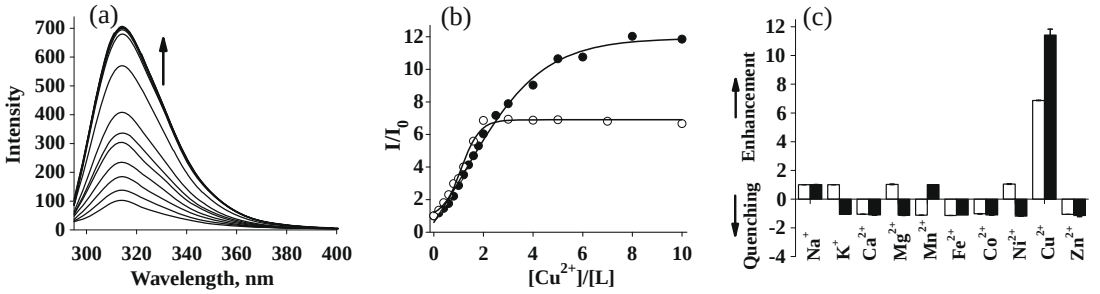


Figure 1. Fluorescence titration of L with different metal ions: (a) Spectral traces during the titration of L with Cu^{2+} in methanol, (b) plot of relative fluorescence intensity versus number of equivalents of Cu^{2+} added in methanol (unfilled) and in 1:1 aqueous methanol (filled) and (c) histogram representing the fluorescence enhancement and quenching fold exhibited by L with different metal ions studied in methanol (unfilled) and in 1:1 aqueous methanol (filled).

0.0127 and 0.0980 in aqueous methanol with respect to naphthalene as standard.

In order to support the binding of L by Cu^{2+}, absorption titrations were carried out. The spectral changes were suggestive of binding of Cu^{2+} with L (Fig. 2). Absorption spectra recorded at higher concentrations exhibited a d–d transition band at 655 nm which also demonstrates the interaction of ligand with metal ion (Fig. 2a, inset, SI 03). Plot of absorbance versus added [Cu^{2+}] clearly indicated the formation of the complex (Fig. 3a) and the complex formed was found to be 1:1 based on Job's plot (Fig. 3b) in both the solvent systems.

The 1:1 stoichiometry of the complex has been further supported based on the molecular ion peak observed at *m*/*z* value of 1189 in ESI MS titration (Fig. 3c). The isotopic peak pattern provides an unambiguous assignment to this peak by confirming the presence of Cu^{2+} in the complex. The minimum concentration at which L can detect Cu^{2+} has been found to be 196 ppb in methanol and 341 ppb in aqueous methanol (SI 04).

The competition of Cu^{2+} toward L against other metal ions has been explored by titrating a solution containing L and M^{n+} in 1:30 ratio in the case of Na^+, K^+, Ca^{2+}, Mg^{2+} and 1:5 ratio in the case of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+} with Cu^{2+} of different concentrations. These studies resulted in no significant change in the fluorescence intensity observed with the {L+Cu^{2+}} species and thereby revealed that the Cu^{2+} could replace M^{n+}. This clearly conforms to the strong binding nature of L toward Cu^{2+} in the presence of other M^{n+} ions (Fig. 4).

The role of calix[4]arene platform and the pre-organized nitrogen core in the recognition process has been proven by studying fluorescence properties of the reference molecules (Fig. 5), viz., L_1 and L_2 with different metal ions. The control molecule L_1[11] possessing the calixarene moiety has been prepared by the same method

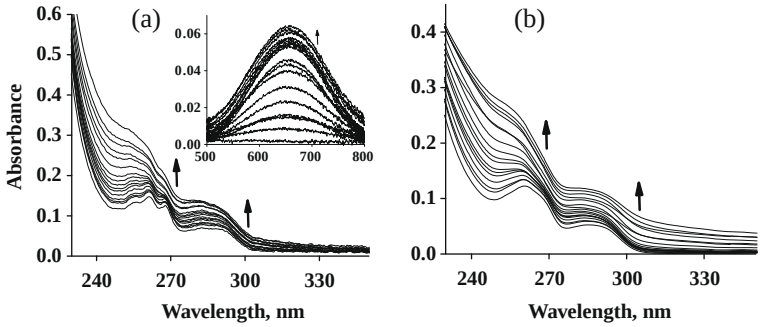


Figure 2. Absorption spectral titration of L with Cu^{2+}: (a) spectral traces observed during the titration in the region 230–350 nm, inset shows the spectral traces in the region 500–800 nm as measured at a higher concentration in methanol and (b) spectral traces observed in aqueous methanol medium.

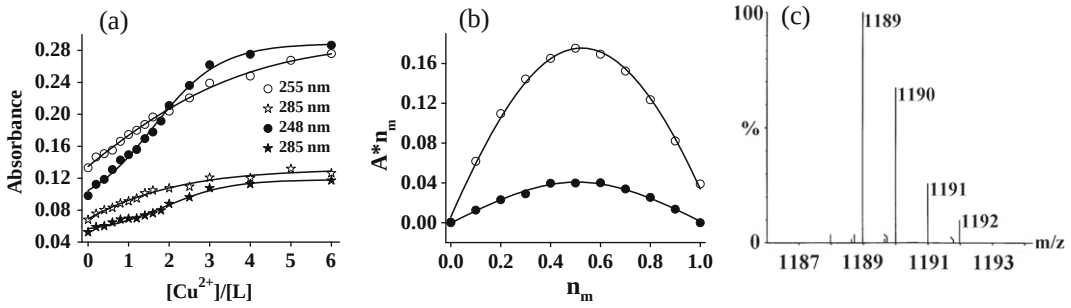


Figure 3. (a) Absorbance versus mole ratio of $[Cu^{2+}]/[L]$ added in methanol (unfilled) and in 1:1 aqueous methanol (filled), (b) Job's plot of n_m versus $A * n_m$, where n_m is mole fraction of the metal ion added and A is absorbance as studied in methanol (unfilled) and aqueous methanol (filled) and (c) Molecular ion peak indicating the isotopic peak pattern for the Cu^{2+} complex of L as obtained from ESI mass spectrum.

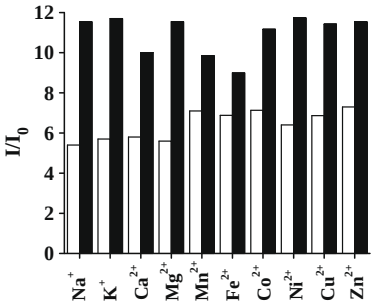


Figure 4. Relative fluorescence intensity of L upon the addition of 3 equiv of Cu^{2+} in the presence of 30 equiv of Na^+, K^+, Ca^{2+}, and Mg^{2+} and 5 equiv of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+}, carried out in methanol (unfilled) and in 1:1 aqueous methanol (filled).

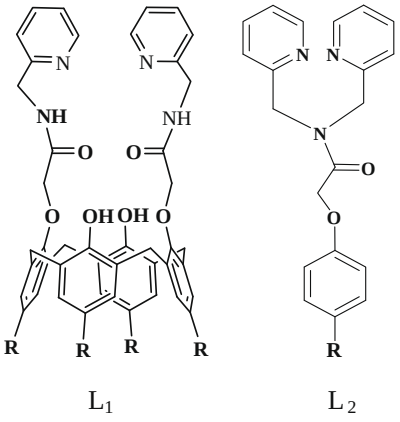


Figure 5. Schematic structures of the control molecules L_1 and L_2. R = *tert* butyl.

that has been discussed for L by coupling diacid chloride derivative of calix[4]arene, **4**, with 2-aminomethyl pyridine. On the other hand, L_2[11,12], a molecular system that has only one strand of L without any calixarene platform, has been synthesized by using *p-tert*-butyl phenol as starting material instead of calix[4]arene (SI 01).

The structural features of the control molecule L_1 has already been reported by us.[12] The L_1 contains a methyl pyridine moiety instead of two pyridine moieties that were present in L. The role of calix[4]arene platform in the selective binding of Cu^{2+} with L has been further established by studying the metal ion binding properties of L_2. The fluorescence titration studies of L_1 showed no selectivity toward any metal ions, while Fe^{2+}, Zn^{2+}, and Cu^{2+} exhibited a fluorescence quenching (Fig. 6a). Though Cu^{2+} exhibited a minimal fluorescence quenching of 2.8 fold, such minimal response is not sufficient enough to detect a particular M^{n+} ion and hence reflects on the lack of pre-organized binding core. The lack of calix[4]arene platform makes L_2 a non-selective molecule toward all the metal ions studied (Fig. 6b). The results obtained from the control molecules suggest that a pre-organized hetero core is required for Cu^{2+} binding. However, L contains two picolyl moieties and a calixarene platform that makes it suitable for selective binding.

In order to understand the structural features of the 1:1 complex formed between L and Cu^{2+}, computational calculations were carried out at HF/3-21G followed by HF/6-31G levels using GAUSSIAN 03[13] package. The computations were initiated by taking the coordinates of L from its crystal structure[14] and by replacing the *tert*-butyl moiety by a hydrogen atom to result in L′. The L′ has been optimized at both HF/3-21G and HF/6-31G before carrying out the computation for the complex (SI 05.). Even the DFT level computations carried out with 6-31G basis set exhibited same conformation as that obtained at HF/6-31G level. Computations for the complex species have been initiated by placing the Cu^{2+} at a non-interacting distance that is well above the pyridyl core of

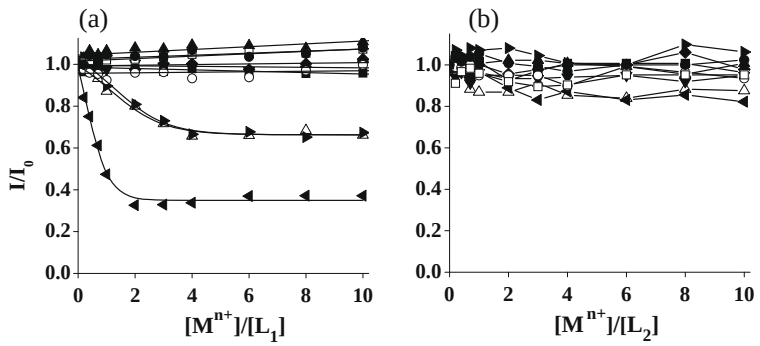


Figure 6. Plots of (I/I_0) as a function of metal to the ligand mole ratio during the fluorescence titration in methanol, (a) L_1, (b) L_2. The symbols corresponds to ■ = Mn^{2+}; △ = Fe^{2+}; ▲ = Co^{2+}; ▼ = Ni^{2+}; ◄ = Cu^{2+}; ► = Zn^{2+}; ♦ = Na^+; ○ = K^+; □ = Ca^{2+}; ● = Mg^{2+}.

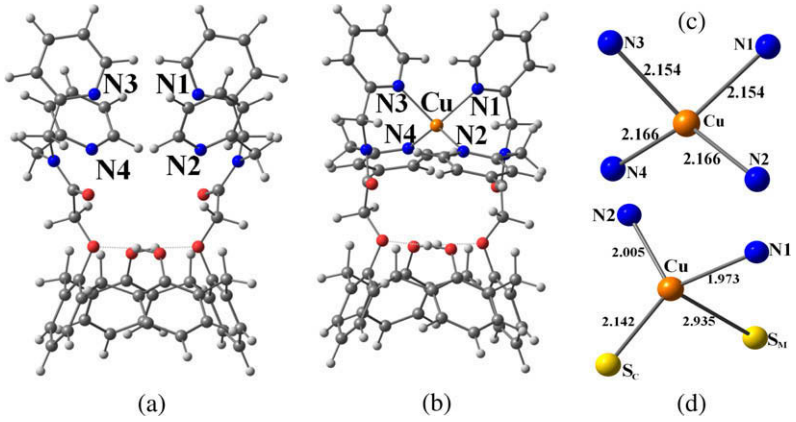


Figure 7. HF/6-31G optimized structure of (a) L′ and (b) $[CuL']^{2+}$; (c) Cu^{2+} coordination site as in (b), and (d) Cu^{2+} coordination site from plastocyanin (PDB id: 1BXU). Coordination core angles in (°) for Cu^{2+} in (c): N1–Cu–N2 = 89.8; N1–Cu–N3 = 92.1; N1–Cu–N4 = 128.4; N2–Cu–N3 = 128.4; N2–Cu–N4 = 127.2 and N3–Cu–N4 = 89.8. Coordination core angles in (°) for Cu^{2+} in (d): N1–Cu–N2 = 101.4; N1–Cu–S_M = 98.8; N1–Cu–S_C = 121.4; N2–Cu–S_M = 86.0; N2–Cu–S_C = 131.0 and S_M–Cu–S_C = 107.7.

the derivative and optimized at HF/3-21G level and the output from this has been taken through HF/6-31G. Based on these calculations it has been found that the formation of the complex has been accompanied by an energy stabilization of −422.8 kcal/mol at HF/6-31G. The optimization brought the N_4 core of the pyridyl moieties in L′ into the coordination range (Fig. 7a and b) and resulted in a tetra-coordinated Cu^{2+} center where all the four pyridyl moieties were involved in binding. The coordination of Cu^{2+} center is highly distorted tetrahedral where the angles range from 89° to 128° that is very much similar to that observed for the same in blue copper proteins, viz., plastocyanin (Fig. 7c and d). Such highly distorted tetrahedral center observed for Cu^{2+} in blue copper proteins has been interpreted to the ease with which the protein could fulfill the coordination requirement for Cu^{1+} during the electron transfer process.

Further, in order to confirm the structural changes that exist at nano level between the receptor L and its Cu^{2+} complex, studies were carried out by scanning electron microscopy (SEM) and atomic force microscopy (AFM). While L shows rod-like structure of length varying from 4 to 20 μm, its Cu^{2+} complex shows a smooth surface of smaller particles (1–2 μm) of irregular shape though these are approximately closer to spherical ones (SI 06). AFM of L shows spherical particles of three different sizes (Fig. 8). While the smallest one has a size of 37 nm and a height of 4 nm, the medium- and large-sized particles are exactly double and triple to this, respectively, indicating that the smallest unit shows little aggregation. However, this aggregation is severe in the Cu^{2+} complex of L leading to the formation of large-sized clusters with size >250 nm and height >35 nm. Thus L and its Cu^{2+} complex are distinguishable based on their SEM and AFM features.

In this Letter, we demonstrate the synthesis and characterization of a receptor molecule L and its selective binding ability toward Cu^{2+} by *switch-on* fluorescence, among the ten metal ions, viz., Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Na^+, K^+, Ca^{2+}, and Mg^{2+} studied. Fluorescence titration of L with Cu^{2+} has been found to be stoichiometric and the observed enhancement provided a handle for the detection and quantification of this ion by L even in aqueous solutions. Convincing evidences were provided for the determination of the stoichiometry of the complex based on absorption and ESI MS and the complex has been found to be 1:1 between L and Cu^{2+}. Based on the studies of the competitive metal ion titration, it is possible to conclude that Cu^{2+} can be sensed even in the presence of some biologically relevant ions in aqueous solutions. Both the calix[4]arene platform and the pyridyl binding core are required for selective recognition of Cu^{2+}, as established upon comparing the results obtained with the relevant control molecules. The computationally obtained structure for Cu^{2+} complex exhibited a tetra-coordinated geometry that was found even in the blue copper protein, viz., plastocyanin. The structural differences observed in SEM and AFM are good enough to differentiate the receptor from its complex with Cu^{2+}. Thus the selective recognition of Cu^{2+} by L has been demonstrated by a more variety of methods than the corresponding upper rim based quinoline derivative that appeared in the literature recently.[7]

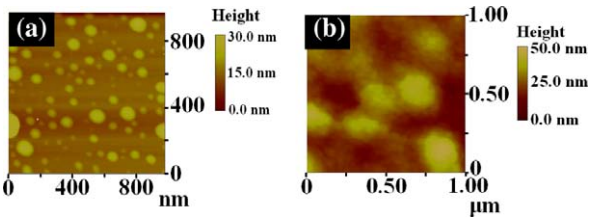


Figure 8. AFM images of (a) L and (b) Cu^{2+} complex of L.

Acknowledgments

C.P.R. acknowledges the financial support by DST, CSIR and BRNS-DAE. R.J. thanks UGC and A.A. thanks CSIR for SRF. The authors thank SAIF, IIT Bombay for ESI MS data and also IIT Bombay for AFM (DST-FIST, Department of Physics) and SEM (at MEMS) facilities.

References and notes

1. Mertz, W. *Science* **1981**, *213*, 1332.
2. (a) Frausto da Silva, J. J. R.; Williams, R. J. P. *The Biological Chemistry of the Elements: The Inorganic Chemistry of Life*; Clarendon Press: Oxford, 1991; (b) Lonnerdal, B. *Am. J. Clin. Nutr.* **1996**, *63*, 821S; (c) Linder, M. C.; Hazegh-Azam, M. *Am. J. Clin. Nutr.* **1996**, *63*, 797S.
3. (a) Solomon, E. I.; Chen, P.; Metz, M.; Lee, S.-K.; Palmer, A. E. *Angew. Chem., Int. Ed.* **2001**, *40*, 4570; (b) Mirica, L. M.; Ottenwaelder, X.; Stack, T. D. P. *Chem. Rev.* **2004**, *104*, 1013; (c) Klinman, J. P. *Chem. Rev.* **1996**, *96*, 2541; (d) Roat-Malone, R. *Bioinorganic chemistry: a short course*; John Wiley & Sons: Hoboken, New Jersey, 2002.
4. (a) Pang, Y.; MacIntosh, D. L.; Ryan, P. B. *J. Nutr.* **2001**, *131*, 2171; (b) Scheinberg, I. H.; Sternlieb, I. *Am. J. Clin. Nutr.* **1996**, *63*, 842S; (c) Harris, Z. L.; Gitlin, J. D. *Am. J. Clin. Nutr.* **1996**, *63*, 836S; (d) Olivares, M.; Uauy, R. *Am. J. Clin. Nutr.* **1996**, *63*, 846S; (e) Wu, J.; Ricker, M.; Muench, J. *J. Am. Board. Fam. Med.* **2006**, *19*, 191; (f) Kumar, N. *Mayo. Clin. Proc.* **2006**, *81*, 1371.
5. (a) Gutsche, C. D. *Calixarenes*; Royal Society of Chemistry: Cambridge, U.K., 1989; (b) Mandolini, L.; Ungaro, R. *Calixarenes in Action*; Imperial College Press: London, 2000.

6. (a) Lee, J. Y.; Kim, S. K.; Jung, J. H.; Kim, J. S. *J. Org. Chem.* **2005**, *70*, 1463; (b) Ho, I.-T.; Lee, G.-H.; Chung, W.-S. *J. Org. Chem.* **2007**, *72*, 2434; (c) Chang, K.-C.; Su, I.-H.; Leeb, G.-H.; Chung, W.-S. *Tetrahedron Lett.* **2007**, *48*, 7274; (d) Kim, J. S.; Quang, D. T. *Chem. Rev.* **2007**, *107*, 3780; (e) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515; (f) Ji, H.-F.; Brown, G. M.; Dabestani, R. *Chem. Commun.* **1999**, 609; (g) Leray, I.; O'Reilly, F.; Habib Jiwan, J.-L.; Soumillionb, J.-Ph.; Valeur, B. *Chem. Commun.* **1999**, 795; (h) Bodenant, B.; Weil, T.; Businelli-Pourcel, M.; Fages, F.; Barbe, B.; Pianet, I.; Laguerre, M. *J. Org. Chem.* **1999**, *64*, 7034; (i) Kim, H. J.; Bok, J. H.; Vicens, J.; Suh, I.-H.; Ko, J.; Kim, J. S. *Tetrahedron Lett.* **2005**, *46*, 8765; (j) Métivier, R.; Leray, I.; Valeur, B. *Chem. Commun.* **2003**, 996; (k) Metivier, R.; Leray, I.; Valeur, B. *Chem. Eur. J.* **2004**, *10*, 4480; (l) Kim, S. K.; Kim, S. H.; Kim, H. J.; Lee, S. H.; Lee, S. W.; Ko, J.; Bartsch, R. A.; Kim, J. S. *Inorg. Chem.* **2005**, *44*, 7866; (m) Kim, J. S.; Kim, H. J.; Kim, H. M.; Kim, S. H.; Lee, J. W.; Kim, S. K.; Cho, B. R. *J. Org. Chem.* **2006**, *71*, 8016; (n) Buie, N. M.; Talanov, V. S.; Butcher, R. J.; Talanova, G. G. *Inorg. Chem.* **2008**, *47*, 3549; (o) Bu, J.-H.; Zheng, Q.-Y.; Chen, C.-F.; Huang, Z.-T. *Org. Lett.* **2004**, *6*, 3301; (p) Kim, H. J.; Kim, S. K.; Lee, J. Y.; Kim, J. S. *J. Org. Chem.* **2006**, *71*, 6611; (q) Schazmann, B.; Alhashimy, N.; Diamond, D. *J. Am. Chem. Soc.* **2006**, *128*, 8607; (r) Miao, R.; Zheng, Q.-Y.; Chen, C.-F.; Huang, Z.-T. *Tetrahedron Lett.* **2004**, *45*, 4959; (s) Beer, P. D.; Drew, M. G. B.; Hesek, D.; Shadea, M.; Szemes, F. *Chem. Commun.* **1996**, 2161; (t) Zheng, Y.-S.; Zhang, C. *Org. Lett.* **2004**, *6*, 1189; (u) Liu, S.-Y.; He, Y.-B.; Wu, J.-L.; Wei, L.-H.; Qin, H.-J.; Meng, L.-Z.; Hu, L. *Org. Biomol. Chem.* **2004**, *2*, 1582.
7. Li, G.-Ke.; Xu, Z.-X.; Chen, C.-F.; Huang, Z.-T. *Chem. Commun.* **2008**, 1774.
8. (a) Bodenant, B.; Weil, T.; Businelli-Pourcel, M.; Fages, F.; Barbe, B.; Pianet, I.; Laguerr, M. *J. Org. Chem.* **1999**, *64*, 7034; (b) Liu, J.-M.; Zheng, Q.-Y.; Yang, J.-L.; Chen, C.-F.; Huang, Z.-T. *Tetrahedron Lett.* **2002**, *43*, 9209; (c) Cao, Y.-D.; Zheng, Q.-Y.; Chen, C.-F.; Huang, Z.-T. *Tetrahedron Lett.* **2003**, *44*, 4751; (d) Chang, K.-C.; Luo, L.-Y.; Diau, E. W.-G.; Chung, W.-S. *Tetrahedron Lett.* **2008**, *49*, 5013; (e) Quang, D. T.; Jung, H. S.; Yoon, J. H.; Lee, S. Y.; Kim, J. S. *Bull. Korean Chem. Soc.* **2007**, *28*, 682; (f) Liang, Z.; Liu, Z.; Jiangc, L.; Gao, Y. *Tetrahedron Lett.* **2007**, *48*, 1629; (g) Xu, Z.; Kim, S.; Kim, H. N.; Han, S. J.; Lee, C.; Kim, J. S.; Qian, X.; Yoon, J. *Tetrahedron Lett.* **2007**, *48*, 9151; (h) Kumar, R.; Bhalla, V.; Kumar, M. *Tetrahedron* **2008**, *64*, 8095; (i) Bhalla, V.; Kumar, R.; Kumar, M.; Dhir, A. *Tetrahedron* **2007**, *63*, 11153; (j) Dhir, A.; Bhalla, V.; Kumar, M. *Tetrahedron Lett.* **2008**, *49*, 4227.
9. (a) Rao, P. V.; Rao, C. P.; Kolehmainen, E.; Wegelius, E. K.; Rissanen, K. *Chem. Lett.* **2001**, 1176; (b) Ali, A.; Salunke-Gawali, S.; Rao, C. P.; Linares, J. *Inorg. Chem. Commun.* **2004**, *7*, 1298; (c) Kraf, D.; Loon, J.-D.; Owens, M.; Verboom, W.; Vogt, W.; McKervey, M. A.; Boihmer, V.; Reinhoudt, D. N. *Tetrahedron Lett.* **1990**, *31*, 4941; (d) Collins, E. M.; McKervey, M. A.; Madigan, E.; Moran, M. B.; Owens, M.; Ferguson, G.; Harris, S. J. *J. Chem. Soc., Perkin Trans. 1* **1991**, 3137; (e) Bohmer, V.; Ferguson, G.; Gallagher, J. F.; Lough, A. J.; McKervey, M. A.; Madigan, E.; Moran, M. B.; Phillips, J.; Williams, G. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1521.
10. Yield (35%, 0.52 g) $C_{72}H_{82}N_6O_6$ (1127.50): Anal. (% found) C, 75.18; H, 7.34; N, 7.34, $C_{72}H_{82}N_6O_6$. C_2H_5OH (% requires) C, 75.71; H, 7.56; N, 7.16). FTIR: (KBr, cm^{-1}): 1641 ($\nu_{C=O}$), 3394 (ν_{OH}). ^1H NMR: ($CDCl_3$, δ ppm): 0.93 (s, 18H, $C(CH_3)_3$), 1.27 (s, 18H, $C(CH_3)_3$), 3.27 (d, 4H, Ar-CH_2-Ar, J = 13.14 Hz), 4.34 (d, 4H, Ar-CH_2-Ar, J = 13.14 Hz), 4.71, 4.94 (s, 8H, NCH_2), 4.97 (s, 4H, OCH_2), 6.76 (s, 4H, Ar-*H*), 7.02 (s, 4H, Ar-*H*), 7.04 (t, 2H, Py-*H*, J = 6.40 Hz), 7.13 (t, 2H, Py-H, J = 6.42 Hz), 7.25–7.27 (m, 2*H*, Py-*H*), 7.40 (d, 2H, Py-*H*, J = 7.90 Hz), 7.51–7.58 (m, 6H, Py-*H* and O*H*), 8.41 (d, 2H, Py-*H*, J = 4.88 Hz) 8.52 (d, 2H, Py-*H*, J = 4.88 Hz). ^{13}C NMR: ($CDCl_3$, 100 MHz δ ppm): 31.1, 31.8 ($C(CH_3)_3$), 31.9 (Ar-CH_2-Ar), 33.9, 34.0 ($C(CH_3)_3$), 51.4, 52.2 (NCH2), 74.5 (OCH_2CO), 122.2, 122.25, 122.5, 125.1, 125.7, 127.9, 132.7, 136.7, 136.9, 141.3, 147.3, 148.9, 149.9, 150.8, 156.4, 157.3 (Py-*C* and calix-Ar-*C*), 169.2 (*C*=O). m/z (ES-MS) 1127.78 ($[M]^+$ 70%), 1128.80 ($[M+H]^+$ 40%,).
11. Joseph, R.; Ramanujam, B.; Acharya, A.; Khutia, A.; Rao, C. P. *J. Org. Chem.* **2008**, *73*, 5745.
12. Yield (43%, 0.40 g) $C_{24}H_{27}N_3O_2$ (389.48): Anal. (% found) C, 73.80; H, 6.89; N, 11.20, $C_{24}H_{27}N_3O_2$ (% requires) C, 74.00; H, 6.98; N, 10.78). FTIR: (KBr, cm^{-1}): 1660 ($\nu_{C=O}$. ^1H NMR: ($CDCl_3$, δ ppm): 1.21 (s, 9*H*, $C(CH_3)_3$), 4.68 (d, 4H, NCH_2, J = 8.55 Hz), 4.88 (s, 2H, OCH_2), 6.79 (d, 2H, Ar-*H*, J = 9.17 Hz), 7.08 (t, 1H, Py-*H*, J = 5.04 Hz), 7.13 (t, 2H, Py-*H*, J = 6.87 Hz), 7.16–7.21 (m, 3H, Ar-*H* and Py-*H*), 7.50 (t, 1H, Py-*H*, J = 7.63 Hz), 7.55 (t, 1H, Py-*H*, J = 7.79 Hz), 8.40 (d, 1H, Py-*H*, J = 6.42 Hz), 8.50 (d, 1H, Py-*H*, J = 5.81 Hz). ^{13}C NMR: ($CDCl_3$, 100 MHz δ ppm): 31.6 ($C(CH_3)_3$), 34.2 ($C(CH_3)_3$), 51.5, 52.4 (NCH2), 67.6 (OCH_2), 114.3, 121.7, 122.5, 122.7, 126.8, 126.3, 136.8, 144.2, 149.2, 150.0, 155.9, 156.3, 157.1 (Ar-*C* and Py-*C*) 169.4 (*C*=O). m/z (ES-MS) 390.16 ($[M+H]^+$ 100%).
13. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H.P.; Cross, J.B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G.A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *GAUSSIAN 03, Revision C.02*; Gaussian: Wallingford, CT, 2004.
14. Joseph, R.; Rao, C. P. Unpublished crystal structure data.