Synthesis and Solution Structure Analysis of a Bispyrenyl Bishydroxamate Calix[4]arene-Based Receptor, a Fluorescent Chemosensor for Cu^{2+} and Ni^{2+} Metal Ions

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A bispyrenyl calix[4]arene-based receptor, **1**, incorporating two hydroxamic acid functionalities has been designed for the optical detection of Cu^{2+} and Ni^{2+} metal ions in solution. The synthetic route is based on the utilization of pyrene-labeled, *O*-protected hydroxylamines that represent versatile building blocks for the generation of fluorescent siderophore-based chelators. Compound **1** is shown to exist in CDCl$_3$ solution at room temperature as a mixture of mainly two conformers, namely, the cone and partial-cone species (partial-cone/cone ratio ca. 57/43). The full assignment of the proton and carbon resonances of the calix[4]arene core for both conformers is described. Ligand **1** exhibited in solution a dual fluorescence emission spectrum composed of monomer and excimer contributions, the latter resulting from intramolecular interactions between pyrene nuclei in the excited state. In methanol/water (80/20 v/v), the fluorescence intensity was shown to be sensitive to proton concentration and to the presence of transition-metal ions. Addition of Cu^{2+} and Ni^{2+} metal cations induced a dramatic quenching of fluorescence of **1** depending on the value of −log[H$^+$]. The excimer-forming photoresponsive ligand **1** belongs to a new class of sensitive chemosensors for the selective detection of transition-metal species in aqueous media.

Introduction

The design and the synthesis of fluorescent chemosensors for the detection of metal ions in aqueous and nonaqueous media is an active and fascinating area of supramolecular chemistry.[1−3] The generation of new molecular systems for the optical detection of *transition-metal ions* is currently a task of prime importance for environmental or biological applications.[4−14]

Recently, we reported[12,13,15] on a series of siderophore-based hydroxamate chelators incorporating the pyrene chromophore that were shown to display remarkable fluorescence emission properties sensitive to inherently quenching metal ions, such as Fe^{3+} or Cu^{2+}, but also to nonquenching cations such as Ga^{3+}.[12,13,15] Furthermore, selective optical discrimination between competing metals in aqueous methanol solution could be achieved by varying proton concentration.[13] In connection with this project, we report here on a tetra-*p*-(*tert*-butyl)calix[4]-arene derivative, **1**, substituted at the lower rim by two 1,3-distal hydroxamate-based complexing arms each incorporating a pyrene fluorescent reporter group.[16]

Calixarenes provide three-dimensional versatile platforms on which to anchor a variety of binding units, which affords a wide family of molecular receptors endowed with recognition properties toward ions and neutral molecules.[17,18] Photosensitive calixarene-based complexing molecules were shown to present a great potential as metal sensory systems.[19−22] Especially a bispyrenyl calix[4]arene, to which compound **1** is structurally related, was shown to respond to solvent polarity and alkali-metal ions.[19] The tetradentate ligand **1** was

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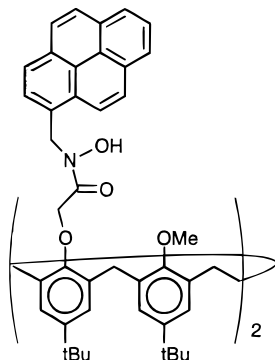 (12) Fages, F.; Bodenant, B.; Weil, T. *J. Org. Chem.* **1996,** *61,* 3956−3961.
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Figure 1. Corrected fluorescence emission spectra ($\lambda_{exc} = 340$ nm) of the free ligand **1** in nondegassed solution (5×10^{-7} M, 25 °C): ···, diethyl ether; - - -, methanol; —, methanol/water 80/20 (v/v, $-\log[H^+] = 7.4$).

Scheme 1.   Synthesis of Ligand 1

designed to provide a coordination environment suitable for divalent metal ions, such as copper(II) and nickel(II), according to the high affinity of hydroxamic acids toward these metal species in solution.[23-25] Only a few examples of calixarenes incorporating hydroxamate functionalities have been reported so far,[18c,26,27] and they were revealed to display promising features as metal binders. It thus occurred to us that siderophore-like calixarenes bearing photosensitive units could be of great interest in the field of metal sensing.

Here we describe the synthesis of compound **1** and its ^{1}H and ^{13}C NMR study in CDCl$_3$ solution, together with its fluorescence emission properties in aqueous methanol solution in the absence and in the presence of Cu^{2+} or Ni^{2+} metal cations, and at various proton concentrations.

Results and Discussion

Synthesis. For the synthesis of ligand **1**, we followed a synthetic route outlined in a previous paper.[12] Condensation of the *O*-benzoyl-*N*-substituted-hydroxylamine derivative, **4**,[12] with the diacid chloride[26a,28] **3** afforded the *O*-protected calix[4]arene-based ligand, **2**, in 21% isolated yield. Treatment of the latter with methanolic ammonia at room temperature allowed **1** (40% yield) to be obtained as a beige solid (Scheme 1). Compound **1** gave statisfactory analytical and spectral data.

Fluorescence Emission Properties of Free Ligand 1 in Solution. Fluorescence emission spectra of **1** were recorded in two solvents, diethyl ether and methanol. They showed the contribution of both monomer and excimer emissions, the value of the excimer-to-monomer intensity ratio being dependent on the nature of the solvent (Figure 1). In methanol, a weaker excimer contribution ($I_E = 23\%$)[29] was noticed as compared to diethyl ether as solvent ($I_E = 78\%$). These results are in

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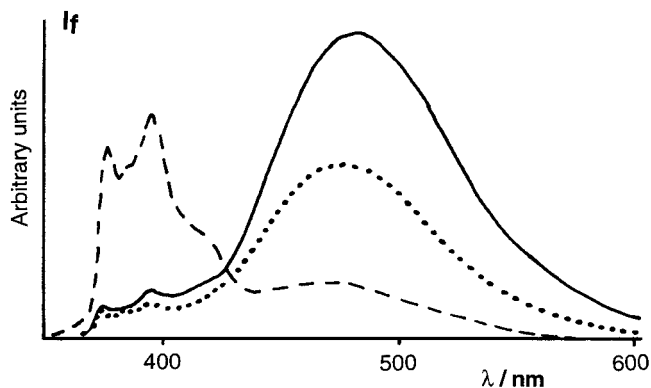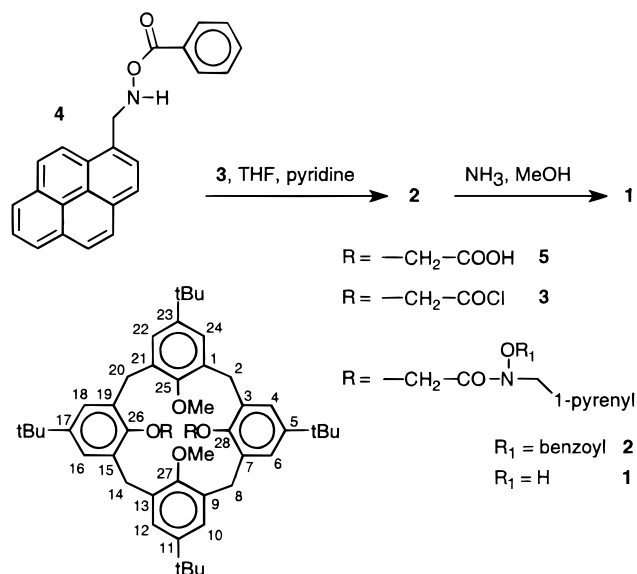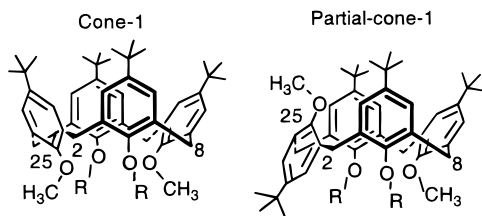
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(29) I_E represents the relative peak intensity of the excimer emission: I_E (%) $= 100 \times I_{excimer}/(I_{monomer} + I_{excimer})$.

Scheme 2. Cone and Partial-Cone Conformers of Ligand 1

Figure 2. $-\log[H^+]-$fluorescence profiles of the pyrene-labeled bishydroxamate ligand **1**: (a) free ligand, (b) in the presence of one molar equivalent of Cu(II), (c) in the presence of one molar equivalent of Ni(II) (methanol/water 80/20 (v/v), $-\log[H^+] = 7.4$ (HEPES, 10 mM), $\mu = 0.1$ (KCl), 25 °C).

full agreement with those reported by Shinkai for the related bispyrenyl calix[4]arene containing two ester linkers.[19] The latter compound was shown to exist as an interconverting mixture of mainly two sets of conformers in the ground state, namely, the cone and partial-cone structures, the value of the cone/partial-cone molar ratio being dependent on the polarity of the solvent. For steric reasons, the interactions between pyrene moieties were believed by the authors to occur more easily in the partial-cone rather than in the cone species. In the polar solvent methanol, the cone conformer was reported to predominate owing to its higher dipole moment relative to the partial-cone structure, which accounted for the low excimer fluorescence contribution in methanol relative to less polar solvents.[19]

In the case of ligand **1**, the occurrence in solution of the cone and partial-cone conformers (Scheme 2) was indeed confirmed by the NMR study (vide infra). Molecular mechanics calculations were used in an attempt to estimate the intramolecular plane-to-plane distance between the two pyrene moieties in the cone and the partial-cone structures, and ultimately to confirm the effect of the geometry of the calixarene core on the excimer fluorescence behavior. The average distance was found to be slightly shorter in the former (ca. 4.1−4.3 Å) relative to the latter (ca. 4.6−4.8 Å), which gave no strong evidence for the existence of ground-state structural factors favoring mutual interactions between pyrenes in one conformer relative to the other. In fact, other parameters should be taken into account, such as the quality of π overlap between pyrenes in the excited state which is crucial for excimer emission to occur efficiently.[30]

In the methanol/water (80/20 v/v) solvent system ($-\log[H^+] = 7.4$), the value of the excimer-to-monomer ratio was observed to increase considerably, the excimer emission being predominantly observed to that of the monomer ($I_{excimer}/I_{monomer}$ ca. 17 instead of 0.3 in methanol). In the aqueous solvent, hydrophobic forces are expected to enforce the pyrenyl groups to associate intramolecularly, leading to the generation of excimer species preformed in the ground state.[30] Pyrene aggregation was indeed revealed by spectral broadening and red-shifting (ca. +1 nm) of the ^1L_a absorption band as compared to nonaqueous solvents.[30] When a solution of **1** and excess strong acid in methanol/water (80/20) was titrated with a standard KOH solution, the total quenching of the fluorescence emission of the ligand was observed beyond $-\log[H^+] = 8$, the fluorescence intensity (I_f) vs $-\log[H^+]$ profile being given in Figure 2. This behavior was attributed to the formation of the electron-rich, deprotonated hydroxamato species, which were previously demonstrated to quench the pyrene fluorescence via a photoinduced electron-transfer mechanism.[13]
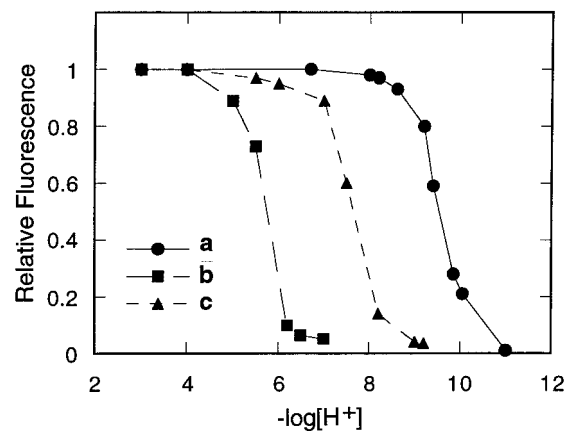
The dihydroxamic acid ligand, **1** (H₂L), has two dissociable hydrogen ions per molecule. The two corresponding protonation constants could be roughly evaluated[13] from the first-derivative curve of the spectrofluorimetric profile (pK_1, pK_2 ca. 9.5, 10.2), and were found in agreement with values determined for dihydroxamic acid compounds.[24]

NMR Study. ^1H and ^{13}C NMR spectra of free ligand **1** were recorded at room temperature in CDCl₃ as solvent (ca. 15 mM). The total assignment of the proton and carbon resonances of the calix[4]arene unit of **1** was performed by the combined use of COSY, HOHAHA, HMQC, and HMBC techniques.

The ^1H NMR spectrum at room temperature (Figure 3) shows a series of numerous, well-resolved resonance patterns lying from ca. δ 1 to δ 8.5. Given our synthetic conditions[31] and according to literature data,[19] compound **1** is expected to exist as a mixture of mainly two conformers in solution, namely, the cone species and the partial-cone conformer in which an anisole group is inverted relative to the other alkyloxyaryl units (Scheme 2). Indeed, in the *tert*-butyl region, five sharp singlets are recorded. Three of these, present in a 1/2/1 ratio (δ 1.22, 1.06, and 1.51, respectively), correspond to the signals of the partial-cone conformation.[32] The two others (δ 0.99 and 1.13 ppm) appear in a 1/1 integration ratio and correspond to a species bearing two sets of magnetically equivalent *tert*-butyl groups, which is expected for either of the 1,2-alternate, 1,3-alternate, or cone conformations. Actually, the latter of the three possibilities is shown to occur (vide infra). The ratio partial-cone/cone was found to be about 57/43.

Partial-Cone Conformer. Starting from the three aforementioned *tert*-butyl signals, HOHAHA experiments allowed the aryl protons to be assigned. In the partial-cone conformation which has only a plane of symmetry, the methoxyphenyl rings are not equivalent and two singlets appear at δ 7.14 and 7.70 for the aromatic protons, $H-C(10)$, $H-C(12)$ and $H-C(22)$, $H-C(24)$, respectively (Scheme 2). The pyrene-bearing rings exhibit two signals at δ 6.94 and 7.01, corresponding to $H-C(4)$,

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(31) The calix[4]arene derivative, **5**, diametrically substituted with two carboxylic acid groups, was obtained in the cone conformation.[28,33]
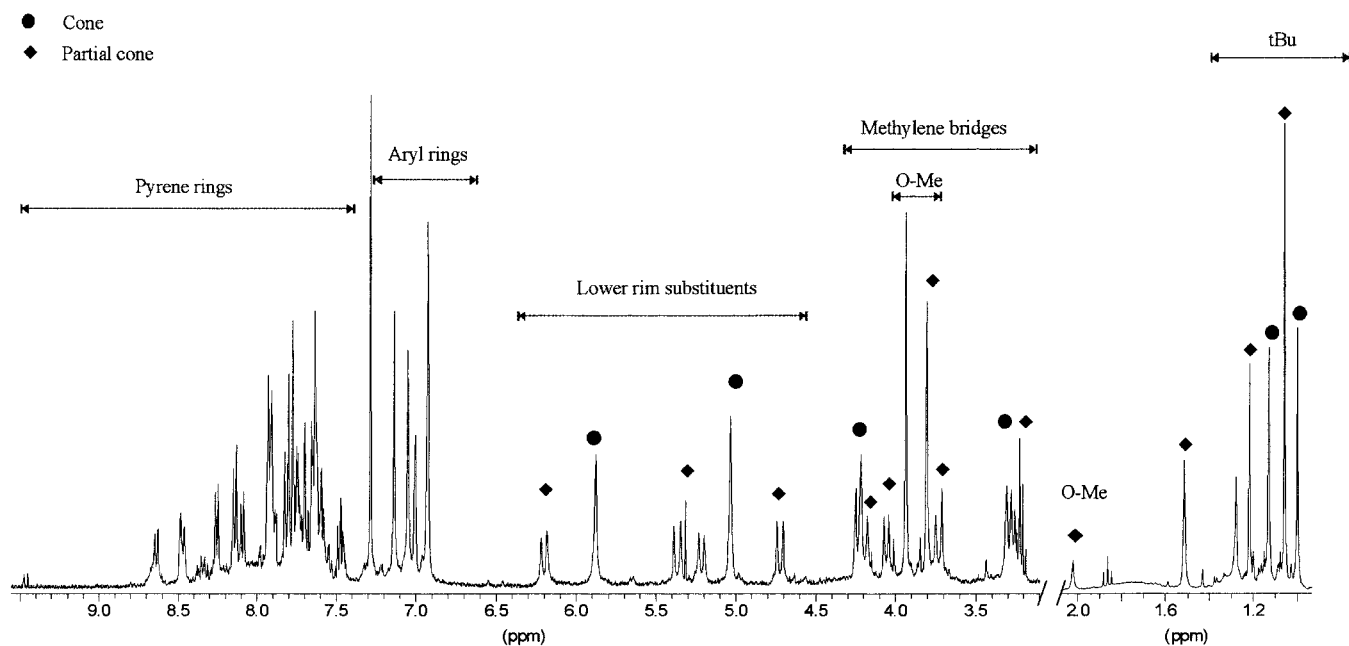(32) Gutsche, C. D.; Dhawan, B.; Levine, J. A.; Hyun, K.; Bauer, L. J. *Tetrahedron* **1983**, *39*, 409−426.

Figure 3. ^{1}H NMR spectrum (400 MHz, CDCl$_3$, 298 K) of calix[4]arene **1**.

Table 1. ^{1}H and ^{13}C NMR Data[a] for Calix[4]arene Ligand 1

		phenyl groups	methylene bridges	*tert*-butyl groups	lower-rim substituents
cone-**1**	^{1}H	6.93 C(4,6,16,18)H 7.05 C(10,12,22,24)H	4.23, 3.29 ($J = 12.5$ Hz) C(2,8,14,20)H_2	0.99 C(5,17)-C(CH_3)$_3$ 1.13 C(11,23)-C(CH_3)$_3$	3.93 C(25,27)-OCH_3 5.05 C(26a,28a)H_2 5.88 C(26c,28c)H_2
	^{13}C	126.0 C(10,12,22,24)H 126.2 C(4,6,16,18)H 134.5, 134.9 C(1,21,9,13), C(3,7,15,19) 148.1 C(5,17) 148.0 C(11,23) 152.0 C(25,27) 148.3 C(26,28)	30.5 C(2,8,14,20)H$_2$	31.4 C(5,17)-C(CH$_3$)$_3$ 34.5 C(5,17)-C(CH$_3$)$_3$ 31.7 C(11,23)-C(CH$_3$)$_3$ 34.6 C(11,23)-C(CH$_3$)$_3$	63.9 C(25,27)-OCH$_3$ 73.8 C(26a,28a)H$_2$ 169.6 C(26b,28b) 50.7 C(26c,28c)H$_2$
partial-cone-**1**	^{1}H	6.94, 7.01 ($J = 2.3$ Hz) C(4,6,16,18)H 7.14 C(10,12)H 7.70 C(22,24)H	3.73, 4.20 ($J = 12$ Hz) C(2,20)H_2 3.24, 4.05 ($J = 12$ Hz) C(8,14)H_2	1.22 C(11)-C(CH_3)$_3$ 1.51 C(23)-C(CH_3)$_3$ 1.06 C(5,17)-C(CH_3)$_3$	2.02 C(25)-OCH_3 3.80 C(27)-OCH_3 4.72, 5.36 ($J = 16.7$ Hz) C(26a,28a)H_2 5.20, 6.20 ($J = 16.7$ Hz) C(26c,28c)H_2
	^{13}C	126.3, 126.9 C(4,6,16,18)H 125.8 C(10,12)H 125.9 C(22,24)H 134.3, 135.6 C(1,21,3,19) 134.2, 134.8 C(7,15,9,13) 147.8 C(5,17) 148.1 C(11), 147.9 C(23) 154.7 C(25), 152.5 C(27) 149.8 C(26,28)	38.2, C(2,20)H$_2$ 30.3, C(8,14)H$_2$	31.5 C(5,17)-C(CH$_3$)$_3$ 34.4 C(5,17)-C(CH$_3$)$_3$ 31.8 C(11)-C(CH$_3$)$_3$ 34.6 C(11)-C(CH$_3$)$_3$ 32.1 C(23)-C(CH$_3$)$_3$ 35.1 C(23)-C(CH$_3$)$_3$	58.8 C(25)-OCH$_3$ 63.5 C(27)-OCH$_3$ 71.3 C(26a,28a)H$_2$ 169.2 C(26b,28b) 50.6 C(26c,28c)H$_2$

[a] Recorded at 298 K in CDCl$_3$.

H−C(18), H−C(6), and H−C(16). HMQC and HMBC experiments enabled then the total assignment of all proton and carbon resonances (Table 1). Figure 4 represents the long-range ^{1}H−^{1}H and ^{1}H−^{13}C correlations (from HOHAHA and HMBC spectra).

The proton spectrum shows two AX systems for the methylene bridge protons: one at δ 3.73 and 4.20 (H−C(2) and H−C(20), $J = 12$ Hz), and another at δ 3.24 and 4.05 (H−C(8) and H−C(14), $J = 12$ Hz). The ^{13}C NMR signals for the methylene carbon atoms were recorded at δ 38.2 (C(2), C(20)) and at δ 30.3 (C(8), C(14)), which are in good agreement with values reported[34] by

Jaime et al. for the partial-cone conformation of a series of calix[4]arenes. The methoxy groups appear as two singlets at δ 2.02 (CH_3−C(25)) and 3.80 (CH_3−C(27)), which clearly indicates that the methoxy group at the C(25) position is oriented inside the cavity. The corresponding carbon atom resonances are detected at δ 58.8 (CH_3−C(25)) and 63.5 (CH_3−C(27)).

Cone Conformer. Following the same approach as that for the partial-cone conformer, the phenolic protons could be assigned as two singlets at δ 6.93 (H−C(4,6, 16,18)) and 7.05 (H−C(10,12,22,24)), which confirmed the presence of two planes of symmetry characteristic of the

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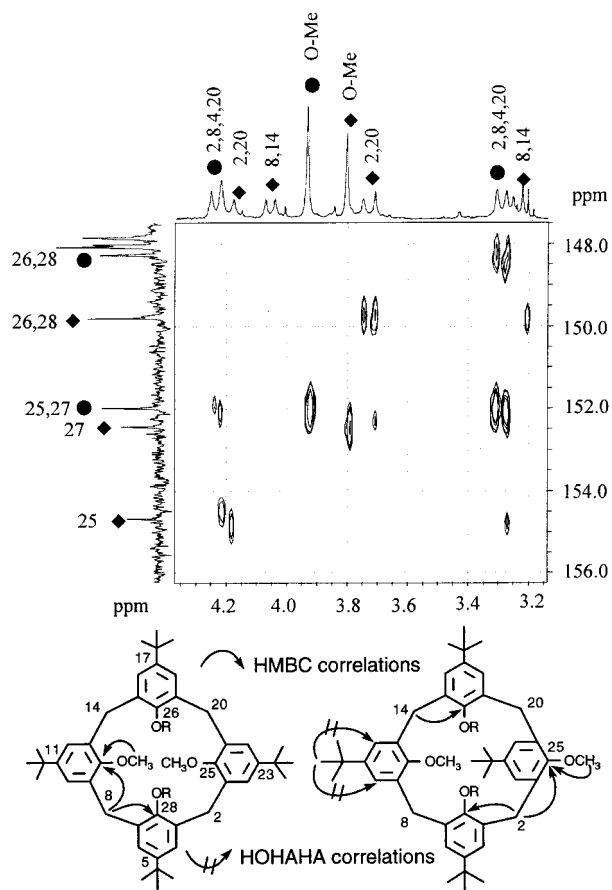
Figure 4. Part of the 400 MHz HMBC spectrum of compound **1** (CDCl₃, room temperature) showing characteristic long-range correlations between the methylene bridge protons and carbon atoms of the aryl rings for the cone (●) and partial-cone (◆) conformers.

cone species. From these data, the total ¹H and ¹³C assignment could be obtained (Figure 4), and the chemical shift values are collected in Table 1. The AX spectrum of the methylene bridges is found at δ 3.29 and 4.23 (J = 12.5 Hz), and a single resonance line at δ 30.5 is recorded for the methylene carbon atoms. According to literature data,[34,35] the latter values provide further evidence for the occurrence of the cone conformation in CDCl₃ solution. Finally, the methoxy group protons show a singlet at δ 3.93.

The methylene protons of the pyrene-bearing pendant chains give rise to two singlets at δ 5.05 (O—C*H*₂—C=O) and 5.88 (N—C*H*₂—pyrenyl) in the case of the cone conformer. For the partial-cone species, these protons appear as two AX systems (J = 16.7 Hz) at δ 5.36 and 4.72 ((O—C*H*₂—C=O) and at δ 6.20 and 5.20 (N—C*H*₂—pyrenyl). The ¹H and ¹³C resonances of the pyrene nuclei were not assigned due to severe resonance overlap.

Finally it is worth mentionning that compound **1** shows sharp signals at room temperature in CDCl₃, indicating that conformational interconversion is slower than for related calixarene derivatives.[36] Conformational rigidification could arise from intramolecular hydrogen-bond formation involving the hydroxamic acid functionalities,

and/or stabilizing π stacking interactions between pyrene rings. Consistently, in the polar, H-bond acceptor solvent DMSO, peak broadening was observed, a coalescent spectrum being recorded at room temperature.

Bishydroxamate Receptor 1 as a Fluorescent Chemosensor for Cu(II) and Ni(II) ions. The addition of one molar equivalent of Cu(II) metal ion to a HEPES-buffered solution of ligand **1** was followed by the quantitative quenching of fluorescence. Such behavior is in accord with the well-known ability of transition metal ions to induce the nonradiative deactivation of a proximate aromatic moiety due to the existence of metal-centered low-lying excited states and/or redox activity. Electronic energy transfer and photoinduced electron transfer are usually considered as the two main radiationless decay processes in chromophore−quencher complexes.[15,37] Under the same experimental conditions, the addition of Ni(II) salt did not induce any effect on the fluorescence of the ligand, but when the same experiment was carried out at −log[H⁺] = 8, on/off switching of the pyrene fluorescence was then observed, similarly in the case of copper. Furthermore, fluorimetric titrations established 1/1 binding stoichiometry for both Cu²⁺ and Ni²⁺ metal ions. These features suggested that a proton-induced discrimination between these two metals could be achieved using the fluorescent bishydroxamate chelator **1**.[6] This is illustrated by the I_f/−log[H⁺] profiles obtained for **1** in the presence of one molar equivalent of Cu(II) or Ni(II) (Figure 1). The sigmoidal curves are centered at ca. 5.5 and 7.5 for the copper(II) and nickel(II) complexes, respectively, which is consistent with the expected greater stability of the former chelate relative to the latter. Moreover, these results suggest that proton-assisted decomplexation of Cu(II) and Ni(II) metal ions is (i) effected selectively at pH values differing by ca. two log units and (ii) accompanied by the rekindling of pyrene fluorescence, the ligand then functioning in the off/on switching mode.

Spectrofluorimetric −log[H⁺] titrations of compound **1** in the presence of other divalent metal ions, such as Fe(II), Co(II), or Zn(II), did not lead to any effect on the photophysical properties of the pyrene chromophore. This is due to the fact that these ions are not chelated by the bishydroxamate ligand under these experimental conditions as they do not profit from the ligand field stabilization energy, as Cu(II) and Ni(II) do consistently with the Irving−Williams series.[24,25,38] Finally, it is found that the fluorescence and coordination properties of the tetradentate ligand bearing negatively charged oxygen atoms is in keeping with that reported for a series of anthracene-containing polyaza receptors.[6]

Conclusion

The fluorescent calix[4]arene-based metal binder **1** has been synthesized by following a modular design approach which has been previously developed for the generation of pyrene-containing hydroxamate chelators. We have shown that this excimer-forming photoresponsive ligand could act as a selective fluorescent chemosensor capable

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(36) For the bispyrenyl compound related to **1**, ¹H NMR spectroscopy was performed at −30 °C.[19]

(37) In the case of ligand **1** in the presence of Cu²⁺ or Ni²⁺ ions, the nature of the quenching mechanism was not investigated. However, for a series of related pyrene-containing hydroxamate binders, photo-induced electron transfer was inferred to account to a large extent for the Cu(II)-promoted pyrene singlet-state deactivation.[13]

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of discriminating copper(II) and nickel(II) from other divalent transition-metal ions. Furthermore, proton-induced decomplexation is likely to provide a versatile means of distinguishing Cu(II) and Ni(II) ions in solution via a fluorescence emission enhancement effect. Finally, the calix[4]arene platform appears as a promising ligand anchoring unit for the generation of photoresponsive molecules endowed with transition-metal ion sensing properties. A molecular engineering approach based on the introduction at the phenolic oxygen positions of different kinds of functional arms should allow the fine-tuning of complexation and photophysical properties of these supramolecular structures.

Experimental Section

General Procedures. Physical and spectroscopic methods described elsewhere.[39] Spectrometric grade solvents were purchased commercially and used without purification. THF was refluxed over and distilled from sodium benzophenone ketyl. Dichloromethane was distilled from calcium hydride. Methanol was refluxed with and distilled over magnesium turnings. Thin layer chromatographic analyses were conducted as reported in a previous paper.[12] The following materials were used: 1,3-dimethoxy-4-*tert*-butylcalix[4]arene (Janssen), copper(II) sulfate pentahydrate (Prolabo), nickel(II) sulfate hexahydrate (Merck), and iron(III) nitrate nonahydrate (Aldrich). Potentiometric studies of compound **1** in the absence and in the presence of metal ions were carried out with an Orion model 720A pH meter according to a procedure described elsewhere.[40] Fluorimetric measurements ([**1**] = 5×10^{-7} M, λ_{exc} = 340 nm) were performed at room temperature in methanol/water (80/20 v/v) as solvent using HEPES (10 mM, $-\log[H^+]$ = 7.4) as buffer. The ionic strength (μ) was adjusted to 0.1 M (KCl). Molecular mechanics calculations were performed on a SGI Indy 4400SC platfrom running Macromodel version 5.0. Conformational minima were found using the modified MM2* (1987 parameters) force field. The cone and partial-cone conformers were submitted to a 0.5-ns dynamics run using stochastic dynamics at T = 300 K. The pyrene–pyrene distance was continuously monitored and averaged. Due to the lack of parameters, the hydroxamic acid moiety was replaced by an amide functionality.

NMR Spectroscopy. 1D and 2D spectra were recorded on a Bruker DPX400 spectrometer at 400.13 and 100.6 MHz for proton and carbon nuclei, respectively, equipped with an inverse 5-mm broad-band probe. Chemical shifts are given vs Me$_4$Si. The parameters used for ^{1}H–^{1}H COSY, HOHAHA, HMQC, and HMBC experiments are given elsewhere.[41]

5,11,17,23-Tetra(*tert*-butyl)-26,28-bis[*N*-(benzoyloxy)-*N*-(1-pyrenylmethyl)aminocarbonylmethoxy]-25,27-dimethoxycalix[4]arene (2). To a solution of compound **4** (0.5 g, 1.4 mmol) and pyridine (1 mL) in THF (25 mL) at room temperature was added dropwise a solution of diacid chloride **3**, obtained[26a] from the corresponding diacid calix[4]arene derivative **5**[28] (0.52 g, 0.65 mmol), in THF (10 mL) over a period of 1 h, under a nitrogen atmosphere and with vigorous stirring. The mixture was allowed to stir for one night at room temperature. After evaporation of THF, the residue was taken up in 200 mL of dichloromethane. The solution was washed with 200 mL of 10% HCl and 3 × 200 mL of water and then dried over MgSO$_4$. After evaporation to dryness, chromatography of the crude product on a silica gel column, eluting with CH$_2$Cl$_2$/MeOH (98/2–90/10 v/v), followed by a crystallization in aqueous ethanol yielded the bis-*O*-protected receptor **2** (200 mg, 21%) as a beige solid. ^{1}H NMR (CDCl$_3$): δ 8.6–7.2 (m, 28H, pyrenyl, benzoyl), 7.04, 6.36 (s, 8H, aryl), 5.91 (br s, 4H, C*H*$_2$–pyrenyl), 4.55 (s, 4H, O–C*H*$_2$–C=O), 4.40, 3.05 (dd, *J* = 12.8 Hz, 8H, ArC*H*$_2$Ar), 4.25 (s, 6H, OC*H*$_3$), 1.32, 0.75 (s, 18H, –C(C*H*$_3$)$_3$).[42] FT-IR (KBr pellet): 1764, 1692 cm^{-1}. MS (FAB): *m/z* 1460.7 (calcd for M + H$^+$ 1460.8), 1482.7 (calcd for M + Na$^+$ 1482.8). Anal. Calcd for C$_{98}$H$_{94}$O$_{10}$N$_2$·4H$_2$O: C, 76.84; H, 6.71. Found: C, 76.78; H, 6.49.

5,11,17,23-Tetra(*tert*-butyl)-26,28-bis[*N*-hydroxy-*N*-(1-pyrenylmethyl)aminocarbonylmethoxy]-25,27-dimethoxycalix[4]arene (1). Compound **1** (0.15 g, 0.10 mmol) was dissolved in 100 mL of MeOH, and the solution was saturated with NH$_3$ gas for 30 min. The solution was concentrated on a rotary evaporator until it became slightly cloudy. After the solution stood at 5 °C for one night, a beige solid was deposited, which was filtrated. Recrystallization in MeOH afforded **1** (50 mg, 40%) as a beige solid. FT-IR (KBr pellet): 1652 cm^{-1}. MS (FAB): *m/z* 1273.8 (calcd for M + Na$^+$ 1274.6). Anal. Calcd for C$_{84}$H$_{86}$O$_8$N$_2$·5H$_2$O: C, 75.19; H, 7.21. Found: C, 75.15; H, 6.99.

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(42) At room temperature in CDCl$_3$, compound **2** was observed to exist mainly as the cone conformer.