Fluorescent, Siderophore-Based Chelators. Design and Synthesis of a Trispyrenyl Trishydroxamate Ligand, an Intramolecular Excimer-Forming Sensing Molecule Which Responds to Iron(III) and Gallium(III) Metal Cations

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Two pyrene-labeled hydroxylamines, **5-Bn** and **5-Bz**, *O*-protected with the benzyl and the benzoyl group, respectively, have been prepared for the generation of siderophore-based new chelators incorporating both the pyrene chromophore and the hydroxamic acid functionality. **5-Bz** formed the starting point toward the synthesis of the tripod-shaped trishydroxamate, **1**. That trichromophoric ligand displays remarkable fluorescence emission properties (dual emission: "monomer" and excimer type) which are markedly and selectively modified by binding $Fe(NO_3)_3$ and $Ga(NO_3)_3$. Ferric ions induce a quasi total quenching of the pyrene fluorescence, whereas the nonquenching Ga(III) cations are observed to affect the value of the excimer-to-monomer fluorescence intensity ratio. Ethylenediaminetetraacetic acid (EDTA) competition reactions yielded an estimated value of 3.8 for log K^* of the complex LFe in methanol/water (80/20 v/v), where $K^* = ([LFe][H^+]^3)/([LH_3][Fe^{3+}])$ and L is the ligand in its totally deprotonated form. Compound **1** is the prototype of a new class of photoresponsive molecular systems which could act as sensitive probes for metal cation detection and recognition.

There is an increasing interest in the synthesis of fluorescent receptors that are endowed with both metal cation recognition and optical signaling properties.[1] These are photoresponsive molecular or supramolecular systems that contain a chromophore center in close vicinity to a cation binding site, and that are designed[2] to spectrofluorimetrically detect the complexation of a guest cation. Given the inherent sensitivity of fluorescence, the use of such molecular probes is particularly attractive for the development of a new generation of optical methods that could enable the real-time, continuous, *in situ* monitoring of trace metals in various media.[3] One of the most successful practical applications of chemosensors for metal ions has been in biological research,[1,4] and for instance the decisive contribution of Tsien to intracellular calcium determination[5] is to be particularly emphasized. Besides, the growing need for such methods in environmental chemistry and oceanography represents an appealing direction for the organic chemist.[6]

Whereas a broad effort has been devoted over the last decade to the synthesis and the coordination studies of photosensitive ligands that respond to alkali- and alkaline-earth-metal ions,[1,2,7] it is only recently that a few examples of fluorescent chemosensors for transition metal ions and heavy metal ions[2c,4b,8] have been reported in the literature. Actually, the design of photoresponsive chelators enabling the detection, but also the speciation,[9] of these metals is a task of prime importance with respect to the essential role of these species in biological and/or environmental media.

In that connection, we undertook the design of new fluorescent receptors for the optical recognition of highly charged metal cations, *e.g.* transition metal such as Fe^{3+}, actinide elements, and group 13 metal ions such as Ga^{3+} and Al^{3+}.[9,6b] Recently, we reported[10] the synthesis of the iron(III) and gallium(III) complexes of ligand **3**, and their fluorescence emission features in acetonitrile were shown to depend remarkably on the nature of the chelated metal. It thus occurred to us that compound **1** (Scheme 1), a trichromophoric tripodal hydroxamate ligand, could

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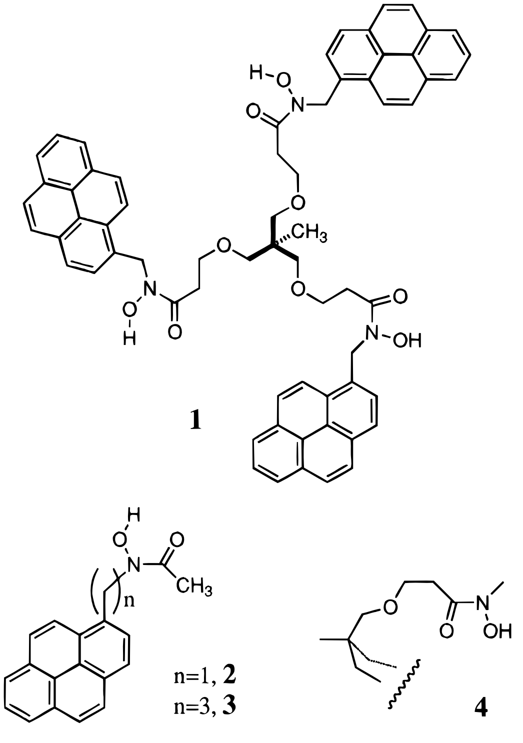
Scheme 1. Trispyrenyl Trishydroxamate Chelator 1[a]



[a] **2** and **3** are the monochromophoric bidentate ligands, and **4** is the nonfluorescent analogue[14] of **1**.

possess a promising prototype structure according to the two following main converging design objectives.

On one hand, trishydroxamate siderophores are naturally occurring and synthetic ion chelators[11] that exhibit a high affinity toward acidic metal cations,[12] with a marked selectivity for Fe^{3+}. This property was observed for tripod-shaped abiotic siderophores,[11,13] and particularly for compound **4**, the nonfluorescent analogue of **1,** synthesized by Martell *et al.*,[14] in which three terminal methyl groups replace the pyrenylmethyl moieties (Scheme 1). Therefore, a synthetic strategy based on a biomimetic approach, *e.g.* consisting of the incorporation of siderophore-like complexing structures in fluoroionophore molecules, was expected to afford systems displaying the remarkable features needed for iron(III) sensing applications such as high sensitivity and selectivity. It is worth to mention that during our investigations promising results were reported in the recent studies of two series of monochromophoric linear trishydroxamate siderophores, *e.g.* naturally-fluorescent pyoverdins[8g] and fluorescent derivatives[4b] of desferrioxamine. As iron(III) is an inherently-quenching metal (*vide infra*), the optical response of ligand **1** to iron complexation was expected to be a significant quenching of the fluorescence intensity.[10]

On the other hand, attaching a pyrenyl group to each of the tripodal chain extremities would lead to the generation of a conformationally-flexible trichromophoric molecular system whose fluorescence emission spectrum should display in solution, even at very low concentrations, both “monomer” and intramolecular excimer contributions.[15] Thus, we envisioned that compound **1** might behave as previously reported bispyrenyl sensors[16] that were shown to give rise to guest-influenced conformational rearrangements leading to excimer formation or disparition. If such were the case, these features could be particularly attractive in view of using **1** for detecting also nonquenching metal ions such as Ga^{3+} and Al^{3+}. Indeed, it has been reported that monomer *vs* excimer fluorescence intensity-ratio measurements were particularly well-suited for metal sensing applications.[2a,7,8e,16,17]

In this paper, we report the synthesis of the tripodlike receptor, **1**, and the synthesis of a monochromophoric bidentate reference compound, **2**, which both involved the preparation of two pyrene-labeled *O*-protected hydroxylamine synthons (Scheme 1).[18] Furthermore, we present a preliminary account of the fluorescence emission properties of these two ligands in solution, in the absence and in the presence of two metal cations, Fe^{3+} and Ga^{3+}, which were selected as representative examples of quenching and nonquenching metals, respectively.[19]

Results

Synthesis. The general stepwise procedure is outlined in Scheme 2. It involves the condensation of a pyrene-labeled hydroxylamine, **5-Bn** or **5-Bz**, with a selected carboxylic acid chloride derivative. This step is then followed by the removal of the hydroxamate *O*-substituent. By varying the nature of the carboxylic acid precursor in the first step, one can expect to generate so a family of siderophore-like receptors containing both the masked *N*-hydroxy-amide function and the pyrene chromophore.

The synthetic route that was followed for the preparation of **5-Bn** and **5-Bz** is given in Scheme 3. Carbamate alkylation were performed by treatment of **7-Bn**[20] and **7-Bz**[21] with the halide compound[22] **8** in the presence of NaH (60% in oil) in anhydrous DMF, leading to **6-Bn** (71% yield) and **6-Bz** (79% yield), respectively. Treatment of **6-Bn** with trifluoroacetic acid (in CH_2Cl_2), and of **6-Bz** with anhydrous HCl (in dioxane) afforded the corresponding *N*-deprotected derivatives in almost quantitative yields. Due to their inherent reactivity and

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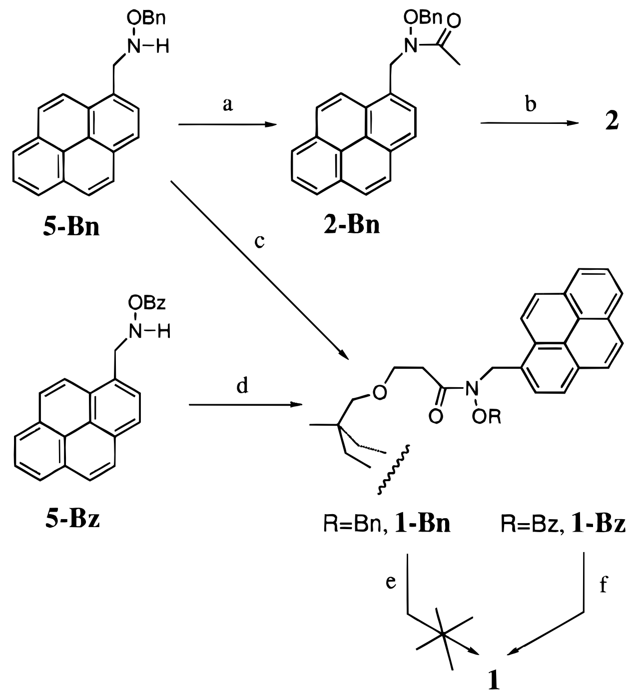
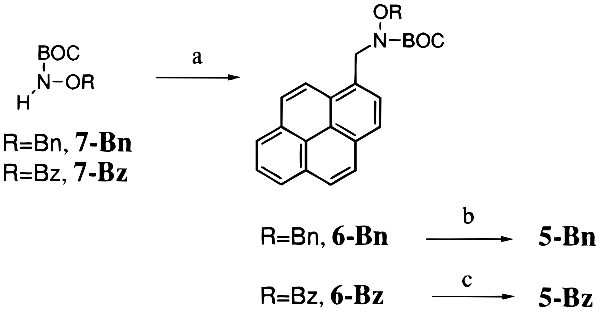
Scheme 2. Synthesis of Ligands 1 and 2[a]



[a] (a) CH_3COCl, Et_3N, toluene; (b) H_2, 10% Pd/C, THF/MeOH; (c) **9**, Et_3N, benzene; (d) **9**, pyridine, THF; (e) H_2, 10% Pd/C, THF/MeOH, or $BF_3{-}Et_2O$, EtSH, CH_2Cl_2; (f) NH_3 gas, $MeOH/CH_2Cl_2$.

Scheme 3. Synthesis of the Synthons 5-Bn and 5-Bz[a]



[a] (a) **8**, NaH, DMF; (b) TFA, CH_2Cl_2; (c) HCl gas, dioxane.

photosensitivity, the *N*-pyrenylmethyl-*O*-protected-hydroxylamines, **5-Bn** and **5-Bz**, were used directly without further purification.

The condensation of **5-Bn** with acetyl chloride in the presence of triethylamine afforded **2-Bn** (56% yield). Similarly, **5-Bn**, and **5-Bz** were condensed with the triacid trichloride derivative,[23] **9**, and the corresponding *O*-protected tripodal ligands, **1-Bn** (17% yield) and **1-Bz** (23% yield), were obtained, respectively (Scheme 2).

The standard treatment of **2-Bn** (H_2, Pd/C, in methanol), in order to effect the reductive debenzylation, generated the desired ligand, **2**, cleanly. On the other hand, the same method totally failed to afford the *O*-deprotected trishydroxamate, **1**, from **1-Bn**. The catalytic hydrogenolysis of **1-Bn** was observed to generate a complex mixture containing partially deprotected compounds and also products resulting from the hydrogenation of the aromatic moieties. Furthermore, attempts to cleave selectively the benzyl ether groups by treatment of **1-Bn** with a boron trifluoride etherate−ethanethiol reagent[24] were also unsuccessful. From these experiments, it occurred to us that the benzyl substituent as *O*-protecting group was of limited utility in the case of trishydroxamates bearing bulky aromatic moieties. Therefore, the benzoyl group was chosen because of its stability towards planned reactions and yet its ease of removal by methanolic ammonia at room temperature.[25] Indeed, reaction of **1-Bz** with ammonia in methanol allowed to obtain **1** in relatively good yield and with the high purity required in order to carry out fluorimetric measurements.

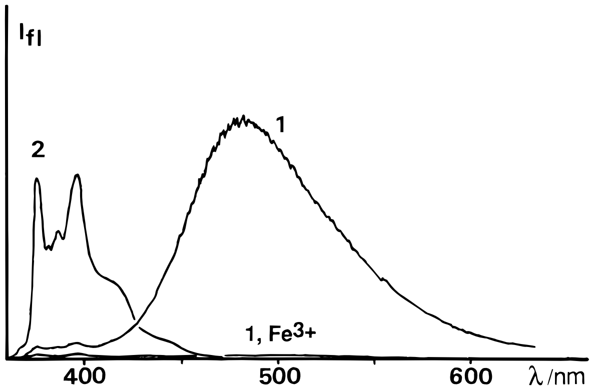


Figure 1. Corrected fluorescence emission spectra ($\lambda_{exc} = 340$ nm) of the free ligands **1** and **2** and of **1** in the presence of 1 mol equiv of $Fe(NO_3)_3$ in nondegassed solution ($MeOH/H_2O$ 80/20 (v/v), concn *ca.* 5×10^{-7} M, $-\log[H^+]$ *ca.* 7.4 (HEPES, 10 mM), $\mu = 0.1$ M ($NaNO_3$), 25 °C).

All new compounds described here gave analytical and spectral data in agreement with their structure. In particular, 250-MHz proton NMR spectroscopy fully confirmed the structure of the free ligands, **1** and **2**, in solution at room temperature. Furthermore, the ^1H NMR spectrum of **1** in DMSO-d_6 showed that the structure of that ligand is symmetrical on the NMR time scale, suggesting a random orientation of each of the tripod chains.

Fluorescence Emission. Compound **1** exhibited in solution (MeOH or $MeOH/H_2O$ 80/20 (v/v), concn $< 10^{-6}$ M) a fluorescence spectrum composed of the monomerlike emission band, and of a broad intense red-shifted band with an emission maximum at 485 nm (Figure 1). The latter was attributed to the emission of the pyrene excimer arising from the intramolecular interaction of a pyrene chromophore in its singlet excited state with a second one in the ground state.[26] In contrast, the monochromophoric reference compound, **2**, displayed only the monomer structured fluorescence spectrum characteristic of a 1-substituted derivative of pyrene (Figure 1).

The addition of 1 equiv of iron(III) nitrate to the solution of **1** was observed to cause the quantitative quenching of the fluorescence emission of both monomer and excimer species (Figure 1). The fluorescence emission properties of a solution of 1-ethylpyrene were not modified by the presence of 1 molar equiv of iron(III) salts, which indicated that the deactivation of the ligand excited state by diffusion-controlled intermolecular reaction with Fe^{3+} was not operative under these conditions.

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Addition of a great excess of desferrioxamine B methylsulfonate (Desferal) to the solution of **1** containing 2 equiv of iron(III) cations induced the reversed effect, restoring the original emission of the free ligand. The observed fluorescence quenching was ascribed to the formation a complex in the ground state between **1** and ferric cations. Indeed, the UV-visible absorption spectrum of **1** in the presence of an equimolar amount of iron in methanol (conc *ca.* 5×10^{-5} M) showed an absorption ($\lambda_{\max} = 470$ nm, $\epsilon_{\max} = 2700$ $M^{-1}cm^{-1}$) which could be indicative of the formation of a monoprotonated $\mathbf{1}\cdot FeH^+$ complex as observed[14] by Martell for the nonfluorescent receptor **4**. Addition of a base in excess (pyridine or sodium acetate) to that solution induced a blue shift of that absorption band which pointed then at 435 nm. Furthermore, UV-visible absorption titration of **1** with $Fe(NO_3)_3$ in methanol established 1:1 binding stoichiometry.

As expected from the results reported for previous examples of fluorescent siderophores,[4b,8g,27] the fluorescence emission of receptor **1** is markedly quenched upon complexation of Fe^{3+} cations. Actually, quenching of electronically excited state of aromatic hydrocarbons by ferric ions or iron(III) chelates is a known phenomenom that has been the subject of extensive investigations. It has been suggested that two main pathways could account for the efficient radiationless deactivation of the singlet excited state, *i.e.* electron transfer from the excited aromatic chromophore to the metal and/or to energy transfer from the excited pyrene to low-lying metal centered energy states.[28] Such processes could be particularly effective in the complex of **1** with Fe^{3+}, due probably to the fact that the chelated metal cation is held very close to the excited chromophore.

In contrast to the case of Fe^{3+}, the addition of Ga^{3+} cations to solutions of receptor **1** was not followed by the quenching of the total fluorescence emission. Instead, a marked modification of the shape of the spectrum was observed. In methanol, the value of the ratio of the fluorescence intensities of excimer and monomer (I_E/I_M), that equals 14 for the free ligand, dropped off to about 4 upon addition of two molar equivalents of $Ga(NO_3)_3$. The fluorimetric titration of ligand **1** in methanol with Ga^{3+} (Figure 2) illustrated well the pronounced effect of that trivalent metal ion on the I_E/I_M ratio. With increased Ga^{3+} concentration, the excimer emission intensity decreased while the monomer emission increased, and the presence of an isoemissive point was detected at *ca.* 430 nm. Addition of an excess of Desferal to the solution of **1** containing 2 equiv of gallium(III) cations restored completely the initial spectrum corresponding to that of the free ligand. From these results and by analogy with those obtained by Martell with siderophore **4**,[14] the effect of gallium might be ascribed to the formation of a 1:1 Ga(III)/ligand complex. Since Ga^{3+} is not redox active and does not possess any empty low-energy orbitals, no quenching interactions are likely to develop between that metal and the pyrene chromophore in the excited state. Rather, the chelation of the gallium cation might alter the conformational properties of the ligand, which could induce a rigidification of the tripodal structure and impede the intramolecular excimer formation. In the chelate, the pyrene nuclei at the extremities of the tripod chains would experience a poorer overlap and the monomer emission would be favored at the expense of that of the excimer. This interpretation lies on the assumption that conformational changes of the receptor molecule are associated with the complexation processes.[7,15,16] It is to be mentioned that, consistently with published observations for ferrichrome tripodal models,[13a] the 250-MHz ^1H NMR spectrum of **1** was strongly altered by the addition of slightly over 1 equiv of $Ga(NO_3)_3$. In methanol/water (80/20 (v/v)), the value of the I_E/I_M ratio was found to be equal to 17 and 11 for the free ligand and the ligand in the presence of Ga^{3+}, respectively. This would indicate a greater trend for the pyrene chromophores to interact intramolecularly in the excited state in both the free and the complex ligand in aqueous solution relative to neat methanol.[15a]

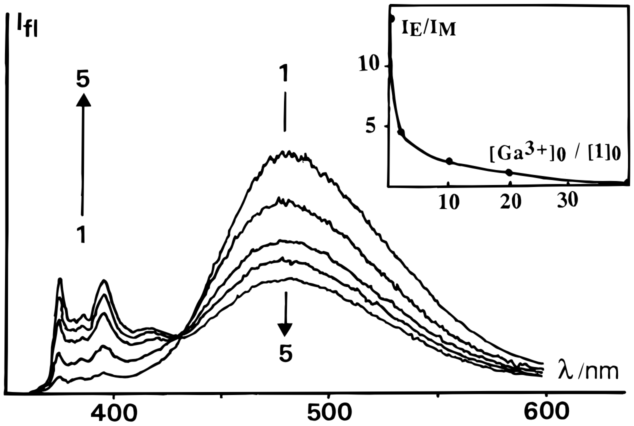

Figure 2. Corrected fluorescence emission spectra ($\lambda_{exc} = 340$ nm) of chelator **1** in the presence of gradual amounts of $Ga(NO_3)_3$ in methanol (non-degassed, 25 °C). $[\mathbf{1}]_0 = 1.3 \times 10^{-5}$ M. $[Ga^{3+}]_0 \times 10^5$ M: 1, 0; 2, 2.6; 3, 13; 4, 26; 5, 52. Inset: spectrofluorimetric titration curve.

The thermodynamic stability constant of the ferric complex of ligand **1** was estimated by spectrofluorimetric competitions against EDTA in methanol/water (80/20 v/v) at $-\log[H^+] = 4$.[29] Since the solubility of **1** was too low in aqueous methanol to allow the determination of its protonation constants, these experiments yielded the proton-dependent formation constant K^*:[30]

$$K^* = \beta_{110}{}^{FeL}/\beta_{013}{}^{LH_3}$$

where $\beta_{mlh} = [Fe_mL_lH_n]/([Fe]^m[L]^l[H]^n)$, Fe = ferric ion, L = ligand **1** in its deprotonated form, H = hydrogen ion.

A value of 3.8 was obtained for log K^*. It is interesting to note that this value is higher than that of the related ligand **4**[14] and in agreement with those observed for tris(hydroxamate) ligands[30,31] exhibiting effective iron binding abilities. However, it should be mentioned that the use of different experimental conditions in our study (*e.g.* solvent systems of lower polarity and of decreased solvation ability) renders the comparison with literature data difficult. Preliminary experiments showed that the proton-dependent stability constant of the gallium complex was 2 to 3 orders of magnitude smaller than that of the ferric complex.[32]

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In contrast to the behavior of receptor **1**, the fluorescence emission properties of a solution of the reference ligand **2** in methanol/water (80/20 (v/v); $-\log[H^+] = 7.4$) were not affected by the presence of gradual amounts of iron salts (up to 3 mol equiv). Still spectrophotometric titrations[32,33] confirmed ground-state metal complexation and indicated the formation of mixtures of iron(III) chelates with different ligand/metal stoichiometries. Similarly, the fluorescence of **2** was found to be insensitive to the addition of up to 5 mol equiv of Ga^{3+}.[33] These results underlined the necessity of designing molecular systems that combine both the chelating sites and the chromophoric subunits in a highly preorganized structure in order to ensure effective binding properties and sensitive optical responses to complexation.[34]

Conclusion

The main purpose of this paper is to provide a general synthetic approach toward the synthesis of fluorescent hydroxamic siderophores incorporating the pyrene chromophore. To this end, the synthesis of two pyrene-labeled *O*-protected hydroxylamines, **5-Bn** and **5-Bz**, is outlined, which should be versatile building-blocks for the elaboration of a wide array of fluorescent chelators. In this report, the *O*-benzoyl derivative has been used in order to obtain the new fluorescent tripod-shaped ligand, **1**. That compound is the first example of a siderophore-based chelator that displays a dual fluorescence emission (monomer- and excimerlike) which is sensitive to the presence of Fe^{3+} and Ga^{3+} metal cations. *The optical response is dependent on the nature of the complexed metallic species.* This work is a first step toward the development of a new class of fluorescent chemosensors that could be of potential interest for environmental applications.

Experimental Section

All reagents and solvents (spectrometric grade) were purchased commercially and used without further purification, except as below. Benzene, DMF, and dichloromethane were distilled from calcium hydride. THF was refluxed with and distilled from sodium benzophenone ketyl. Triethylamine and pyridine were distilled from and stored over KOH pellets. The purity of all compounds was examined by thin-layer chromatography, using a two-wavelength detection system (365 and 254 nm) and, in the case of the hydroxamate ligands, a visualization method ($FeCl_3$). 1,1,1-Tris((2-(chlorocarbonyl)ethoxy)methyl)ethane,[23] **9**, 1-(chloromethyl)pyrene,[22] **8**, *tert*-butyl *N*-(benzyloxy)carbamate,[20] **7-Bn**, and *tert*-butyl-*N*-(benzoyloxy)carbamate,[21] **7-Bz**, were prepared according to published procedures. The following abbreviations have been used: Bn: benzyl; Bz: benzoyl. $Fe(NO_3)_3 \cdot 9H_2O$ (98%) and gallium nitrate (99.999%) were purchased from Aldrich. Stock solutions of the metals (in *ca.* 0.1 M HCl) were standardized with EDTA by using Pyrocatechol violet as an indicator.[35] Desferrioxamine B methylsulfonate salt was used as a gift from Ciba-Geigy and used without further purification. The protonation constants of EDTA in aqueous methanol (80/20 v/v) were taken from literature data.[36]

Physical and spectroscopic methods were described elsewhere,[7] except that FT-IR spectra and fluorescence emission spectra were recorded on a Perkin Elmer Paragon 1000PC instrument and on a Hitachi F4500 spectrofluorimeter, respectively.

***O*-Benzyl-*N*-(*tert*-butoxycarbonyl)-*N*-(1-pyrenyl)hydroxylamine (6-Bn).** To a solution of *tert*-butyl-*N*-(benzyloxy)carbamate,[20] **7-Bn**, (10.1 g; 45 mmol) in DMF (200 mL) was added portionwise NaH (60% in oil, 2.2 g; 50 mmol NaH), and the mixture was stirred under N_2 atmosphere over 15 min. Then a solution of 1-(chloromethyl)pyrene[22] **8** (11.3 g; 45 mmol) in DMF (50 mL) was added dropwise at room temperature. The reaction mixture was heated at 80−90 °C for 3 h and stirred one additional night at room temperature. The solution was poured into 500 mL of ice-cooled water. After extraction with 4 × 250 mL of ethyl acetate, the usual workup was followed by a chromatography on a SiO_2 column, eluting with CH_2Cl_2/petroleum ether 70/30 (v:v). **6-Bn** was obtained (14 g; 71%) as an orange waxy product. $R_f = 0.64$ (CH_2Cl_2). ^1H NMR (250 MHz, $CDCl_3$): 1.45, 4.38, 5.41, 7.36, 8−8.6 ppm. MS (EI) *m/z* 437 (calcd 437). Anal. Calcd for $C_{29}H_{27}NO_3$: C, 79.61; H, 6.22; N, 3.20; O, 10.97. Found: C, 80.78; H, 6.16; N, 2.90; O, 10.15.

***O*-Benzoyl-*N*-(*tert*-butoxycarbonyl)-*N*-(1-pyrenyl)hydroxylamine (6-Bz).** By the same procedure as above, using 3 g (13 mmol) of *tert*-butyl-*N*-(benzoyloxy)carmate,[21] **7-Bz**, and 0.52 g of NaH (60% in oil, 13 mmol of NaH) in DMF (80 mL), and 3.5 g (14 mmol) of 1-(chloromethyl)pyrene,[22] **8**, in DMF (50 mL), **6-Bz** was obtained as a green-yellow solid (4.5 g, 79%): mp 118−120 °C; $R_f = 0.72$ (CH_2Cl_2). ^1H NMR (60 MHz, $CDCl_3$): 1.5, 5.45, 7.1−8.2 ppm. MS (EI) *m/z* 451 (calcd 451). Anal. Calcd for $C_{29}H_{25}NO_4$: C, 77.14; H, 5.58; N, 3.10. Found: C, 76.96; H, 5.66; N, 3.12.

***O*-Benzyl-*N*-(1-pyrenyl)hydroxylamine (5-Bn).** To a solution of **6-Bn** (8 g, 18 mmol) in 15 mL of CH_2Cl_2 was added 15 mL of trifluoroacetic acid. The reaction mixture was allowed to stir at room temperature for 4 h and then evaporated to dryness. The residue was dissolved in CH_2Cl_2 (200 mL), the organic layer was washed with 2 x 200 mL of 10% aqueous $NaHCO_3$ and then dried over K_2CO_3. The evaporation of CH_2Cl_2 yielded to a pasty orange-yellow product (5.5 g, 89%) which was used directly without further purification. ^1H NMR (60 MHz, $CDCl_3$): 4.9, 5.5, 7.6, 8−8.7 ppm.

***O*-Benzoyl-*N*-(1-pyrenyl)hydroxylamine (5-Bz).** A stream of HCl gas was passed through a solution of **6-Bz** (4 g, 9 mmol) in dioxane (150 mL), and the course of the reaction was followed by TLC. After the disparition of the starting product, the dioxane was evaporated and the residue was taken up into CH_2Cl_2. The organic layer was washed with 10% aqueous Na_2CO_3 and dried over $MgSO_4$. The evaporation of the solvent left a green solid (2.8 g, 89%) which was used without further purification. ^1H NMR (60 MHz, $CDCl_3$): 4.7, 7.1−8.1 ppm.

***N*-(Benzyloxy)-*N*-(1-pyrenyl)acetamide (2-Bn).** To a stirred solution of **5-Bn** (5.5 g, 16 mmol) and triethylamine (10 mL) in dried toluene (100 mL) was added dropwise acetyl chloride (2 mL) in toluene (50 mL). After the usual workup and chromatography on silica gel, eluting with CH_2Cl_2, **2-Bn** was obtained as a white solid (3.4 g, 56%): mp 140 °C, $R_f = 0.86$ (CH_2Cl_2/MeOH 98/2 (v/v)). ^1H NMR (250 MHz, $CDCl_3$): 2.25, 4.28, 5.49, 7.13, 7.6−8.6 ppm. Anal. Calcd for $C_{26}H_{21}NO_2$: C, 82.30; H, 5.58; N, 3.69; O, 8.43. Found: C, 82.10; H, 5.61; N, 3.59; O, 8.02.

1,1,1-Tris[[[N-(benzyloxy)-N-(1-pyrenylmethyl)amino]carbonyl]ethoxy]methyl] ethane (1-Bn). To a solution of **5-Bn** (3 g, 9 mmol) and triethylamine (10 mL) in benzene (200 mL) at room temperature was added a solution of triacid chloride[23] **9** (1.1 g, 3 mmol) in benzene (50 mL) over a period of 1 h, under a nitrogen atmosphere and with vigorous stirring. The mixture was allowed to stir an additional 48 h at room temperature. After washing with 3 x 200 mL water, the

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[32] The detailed description of the complexation properties of ligands **1** and **2** will be reported in a forthcoming paper, together with photophysical studies.

[33] Consistently, we observed that the pyrene fluorescence emission of the neutral iron(III) complex of ligand **3**, which is very weak in acetonitrile (see ref 10), was totally restored in methanol. The photophysical properties of $(\mathbf{2})_3M(III)$ and $(\mathbf{3})_3M(III)$ (M = Fe and Ga), and their dependence on the nature of the solvent, have been investigated (see ref 34). To be published.

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[36] Rorabacher, D. B.; MacKellar, W. J.; Shu, F. R.; Bonavita, S. M. *Anal. Chem.* **1971**, *43*, 561.

reaction mixture was dried over Na_2SO_4 and evaporated to dryness. Chromatography of the crude product on a silica gel column, eluting with CH_2Cl_2/MeOH 98/2 (v/v), followed by a crystallization in CH_2Cl_2/petroleum ether mixtures yielded to the tris-*O*-protected receptor **1-Bn** (0.64 g, 17%) as a pale yellow solid: mp 120 °C, $R_f = 0.31$ (CH_2Cl_2/MeOH 98/2 (v/v)). ^{1}H NMR (250 MHz, $CDCl_3$): 1.00, 2.82, 3.39, 3.82, 4.45, 5.53, 7.0−8.4 ppm. FT-IR (KBr pellet): 1650 cm^{-1}. MS (FAB) *m/z* 1294.5 (calcd for $M + H^+$ 1294.5); 1316.1 (calcd for $M + Na^+$ 1316.5). Anal. Calcd for $C_{86}H_{75}N_3O_9{\cdot}H_2O$: C, 78.70; H, 5.91; N, 3.20. Found: C, 78.79; H, 6.10; N, 3.39.

1,1,1-tris[[[[*N*-(Benzoyloxy)-*N*-(1-pyrenylmethyl)amino]carbonyl]ethoxy]methyl]ethane (1-Bz). Similarly, using **5-Bz** (4 g, 11.4 mmol) and pyridine (3 mL) in THF (50 mL), and **9**[23] (1.1 g, 3 mmol) in THF (20 mL), **1-Bz** was obtained as a pasty yellow solid (0.9 g, 23%): $R_f = 0.71$ (CH_2Cl_2/MeOH 98/2 (v/v)). ^{1}H NMR (250 MHz, $CDCl_3$): 1.28, 2.56, 3.17, 3.66, 5.71, 7.2−8.4 ppm. FT-IR (KBr pellet): 1750, 1660 cm^{-1}. MS (FAB) *m/z* 1336.5 (calcd for $M + H^+$ 1336.5), 1358.5 (calcd for $M + Na^+$ 1358.5). Anal. Calcd for $C_{86}H_{69}N_3O_{12}{\cdot}H_2O$: C, 76.25; H, 5.28; N, 3.10. Found: C, 76.02; H, 5.27; N, 3.01.

***N*-Hydroxy-*N*-(1-pyrenyl)acetamide (2).** **2-Bn** (0.7 g, 1.8 mmol) was dissolved in 80 mL of the THF/MeOH (50/50 v/v) solvent system, and 10% Pd/C (0.5 g) was added. The hydrogenation was carried out for 4 h at room temperature and atmospheric pressure. The filtration of the reaction mixture afforded a white solid which was crystallized in hot CH_2Cl_2 to give the free monohydroxamate **2** (0.45 g, 84%): mp 215−220 °C; $R_f = 0.48$ (CH_2Cl_2/ethyl acetate 50/50 (v/v)). ^{1}H NMR (250 MHz, DMSO-d_6): 2.10, 5.42, 8.0−8.5, 9.89 ppm. FT-IR (KBr pellet): 1605, 1590 cm^{-1}. MS (FAB) *m/z* 290.1 (calcd for $M + H^+$ 290.1), 312.0 (calcd for $M + Na^+$ 312.1). Anal. Calcd for $C_{19}H_{15}NO_2$: C, 78.87; H, 5.22; N, 4.84; O, 11.06. Found: C, 78.05; H, 5.51; N, 4.97; O, 11.00.

1,1,1-tris[[[[*N*-Hydroxy-*N*-(1-pyrenylmethyl)amino]carbonyl]ethoxy]methyl] ethane (1). The tris-*O*-protected ligand **1-Bz** (0.7 g, 5.2 mmol) was dissolved in 100 mL of the CH_2Cl_2/MeOH (20/80 v/v) solvent system at room temperature and the solution was saturated with NH_3 gas for 30 mn. The slightly cloudy solution was concentrated and left to stand at 0−5°C for 2 h. A beige solid was deposited, which was filtrated and dried under vacuum over P_2O_5, yielding **1** (0.2 g, 37%): mp 145−150 °C; $R_f = 0.58$ (CH_2Cl_2/MeOH 90/10 (v/v)). ^{1}H NMR (250 MHz, DMSO-d_6): 0.83, 2.69, 3.22, 3.64, 5.40, 7.9−8.5, 9.75 ppm. FT-IR (KBr pellet): 1640 cm^{-1}. MS (FAB) *m/z* 1024.5 (calcd for $M + H^+$ 1024.4), 1046.6 (calcd for $M + Na^+$ 1046.4). Anal. Calcd for $C_{65}H_{57}N_3O_9{\cdot}H_2O$: C, 74.91; H, 5.71; N, 4.03. Found: C, 74.96; H, 5.66; N, 3.92.

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