Water-Soluble Neutral Calix[4]arene−Lanthanide Complexes: Synthesis and Luminescence Properties

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Water-soluble calix[4]arenes **10a**,**b** with chromophores ("antenna") attached to the lower rim via a short spacer are described. In the neutral lanthanide complexes of **10a**,**b** photoexcitation of the antenna induces lanthanide emission via intramolecular energy transfer. Calix[4]arene **10b** with a chrysene moiety as sensitizer shows strong lanthanide emission for Eu^{3+} with an excitation maximum at $\lambda = 363$ nm.

Introduction

There is considerable interest in the synthesis of molecular probes for imaging or bioassay purposes.[1−3] Particularly interesting are lanthanide probes because of their unique characteristics like exceptionally large Stoke shifts (∼200−300 nm), narrow emission line spectra, long-wavelength emission (∼500−600 nm), and long luminescence lifetimes (∼600−1000 ms).[4,5] This explains the strong motivation for the synthesis of ligands for these lanthanide ions that form complexes with high kinetic stability and that are soluble in water for coupling reactions to biomolecules.[6−13]

Various calix[4]arene derivatives are suitable for the complexation of trivalent cations, but in most cases they are not soluble in water.[14] Only Sabbatini et al.[15] reported the encapsulation of a lanthanide ion by a tetraamide calix[4]arene to give a water-soluble system, while Shinkai et al.[16] prepared lanthanide complexes via complexation of the phenolic oxygen atoms of water-soluble calix[*n*]arenes. However, such lanthanide complexes based on calix[4]arenes are positively charged, which might lead to aspecific binding in biological systems.[17]

Recently, we described calixarene derivatives that are substituted with three carboxylic acid groups and which form *neutral* complexes with lanthanide cations.[18] When we attached a triphenylene antenna chromophore to such calix[4]arenes they showed a strong sensitizing ability toward Eu^{3+} and Tb^{3+}, allowing the excitation of Eu^{3+} and Tb^{3+} with wavelengths extending to 350 nm.[19]

For a simple coupling to biomolecules, these calix[4]-arene lanthanide probes should be water-soluble.[20] In the literature, different types of water-soluble calix[4]-arenes have been reported that have hydrophilic substituents such as SO_3^-,[21] COO^-, PO_3^-,[22] $SO_2N(CH_2CH_2OH)_2$,[23] N^+Me_3,[24] or polyethyleneoxy chains.[25,26] Recently, we have reported neutral water-soluble calix[4]arenes by

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Scheme 1

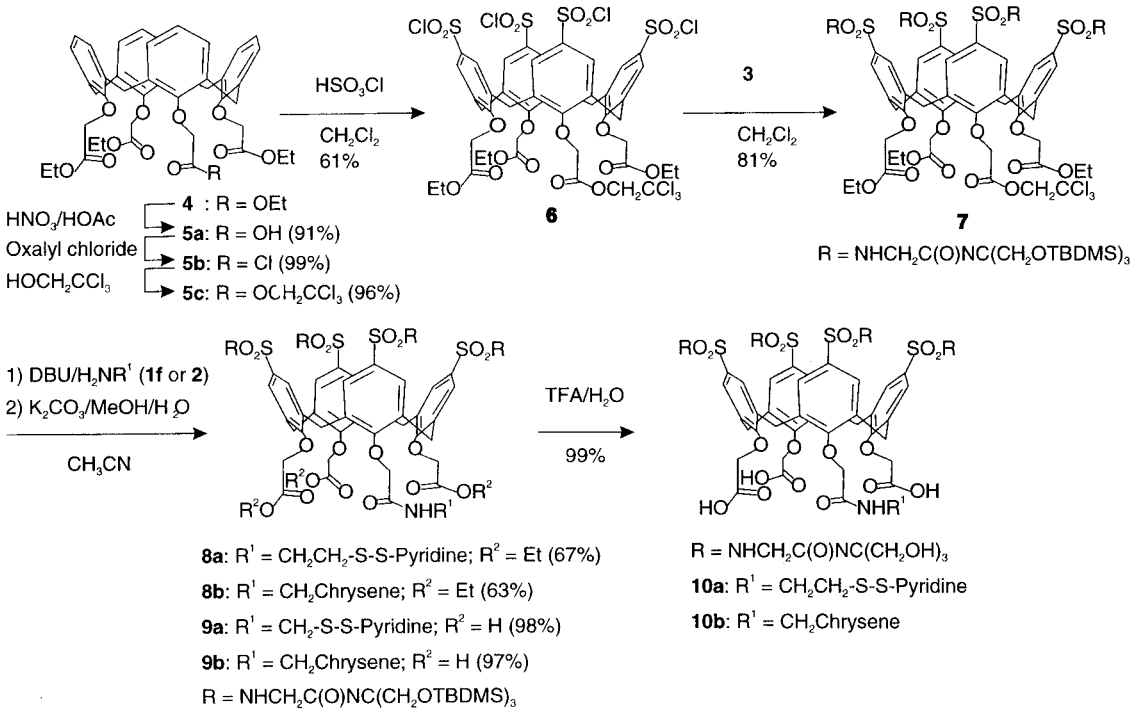
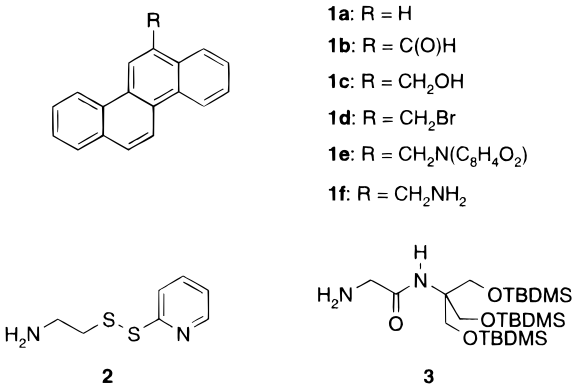


Chart 1



the attachment of polyalcohol residues at the *upper rim* of the calix[4]arene.[27]

In this paper, we report the synthesis and photophysical characterization of neutral, water-soluble Eu^{3+} and Tb^{3+} complexes substituted with two aromatic sensitizer groups (chrysene or 2-pyridyl disulfide).

Results and Discussion

Synthesis. The synthesis of water-soluble ionophores **10a**,**b** is depicted in Scheme 1. The water-solubility is introduced only in the last step of the synthesis because this facilitates handling and purification of the intermediates during the synthesis.

Reaction of triester−monoacid chloride calix[4]arene **5b**, prepared from the monocarboxylic acid **5a**,[19] with 2,2,2-trichloroethanol[28] in CH_2Cl_2 gave the trichloroethyl ester **5c** in 96% yield. Reaction of trichloroethyl ester **5c** with chlorosulfonic acid in $CHCl_3$[29] afforded the tetrasulfonyl calix[4]arene **6** in 61% yield.[30] *N*-[Tris-[[(*tert*-butyldimethylsilyl)oxy]methyl]methane]-2-aminoacetamide (**3**)[27] (Chart 1) was coupled to calix[4]arene **6** to afford the tetrasulfonamide **7** in 82% yield. This intermediate can be used to introduce different amino-substituted residues. Substitution[31] of the trichloroethyl group in **7** with 6-(aminomethyl)chrysene (**1f**) or *S*-(2-pyridylthio)cysteamine (**2**)[32] (Chart 1) and DBU as a base in acetonitrile gave the corresponding amides **8a**,**b** in 63 and 67% yield, respectively. These sulfonamides (**8a**,**b**) were purified by chromatography over a NaCl-saturated silica column.[33]

6-(Aminomethyl)chrysene (**1f**) was prepared from the known 6-(bromomethyl)chrysene[34] (**1d**) via the phthalimide **1e**. A Gabriel reaction of **1d** afforded the phthalimide **1e**, which after deprotection with hydrazine monohydrate gave the desired 6-(aminomethyl)chrysene (**1f**) in an overall yield of 95%. The precursor of **1d**, 6-(hydroxymethyl)chrysene (**1c**),[35] was obtained in 94% yield

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(31) Reductive removal of the trichloroethyl protecting group using Zn in a 1.0 M buffered Na_2PO_4/$NaHPO_4$/THF solution (1:5) yielded the carboxylic acid derivative of **7** in 86% yield. Attempts to activate the carboxylic acid group with various activating agents like DCC or CDI (and catalysts: HOBT (1-hydroxybenzatriazole) or dimethylaminopyridine (DMAP)) in reaction with *S*-(2-pyridylthio)cysteamine (**2**) were unsuccessful. The ^1H NMR spectrum of this carboxylic acid derivative shows two characteristic signals for the *tert*-butyl groups and dimethyl groups, indicating a partial cone conformation of the calix[4]arene, which could explain the reduced reactivity of the carboxylic acid functionality.

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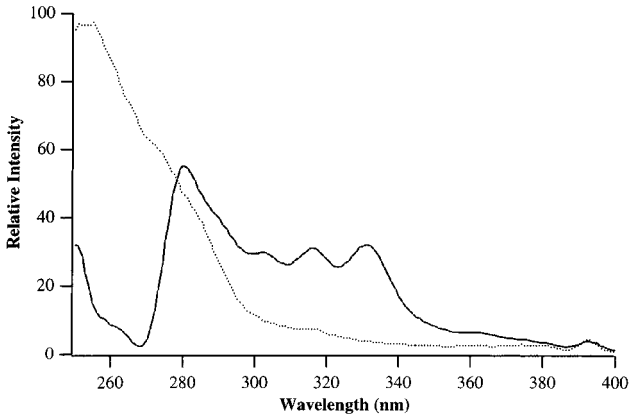

Figure 1. Corrected excitation spectra of Eu(**10a**) (···) and Eu(**10b**) (—) in Tris−HCl buffer (pH 8.0); $\lambda_{em} = 615$ nm. The spectra are normalized on the $^7F_0$–$^5L_6$ Eu^{3+} transition around 393 nm.

in an alternative way via reduction of 6-chrysenecarboxaldehyde (**1b**)[36] with BH_3·THF.

The selective hydrolysis of the three ethyl esters without cleavage of the amide bonds in **8a**,**b**, was achieved with K_2CO_3 in refluxing MeOH−H_2O−THF (5:1:2) to give the triacid derivatives **9a**,**b** in quantitative yield. Removal of the TBDMS-protective groups in **9a**,**b** with trifluoroacetic acid (TFA) gave the water-soluble derivatives **10a**,**b** in quantitative yield. Satisfactory mass spectra could be obtained with FRIT-FAB mass spectrometry. The ^{1}H NMR spectra of compounds **10a**,**b** are broad at room temperature but exhibit sharp signals at 80 °C, showing the characteristic AB system of the methylene bridges. This points to aggregation of the calix[4]arenes **10a**,**b** at room temperature.[37,38] The maximum solubility of **10a** in water, determined by UV spectroscopy, is 8.0 mM at room temperature, and this is sufficient to couple **10a** to biomolecules.[20]

Photophysical Properties. In order to investigate the sensitizing ability of **10a** and **10b** in aqueous solution, equivalent amounts of lanthanide ions (in the form of Eu$(NO_3)_3$·$5H_2O$ or Tb$(NO_3)_3$·$5H_2O$) were added to a 1.5×10^{-4} M solution of **10a** or **10b** in Tris−HCl buffer pH 8.0. After deoxygenation by purging with argon for 15 min, the excitation spectra of these solutions were measured with the detection set at the emission wavelength of the lanthanide ion (615 nm for Eu^{3+} and 545 nm for Tb^{3+}). The results for the Eu^{3+} complexes are shown in Figure 1. In this case, the excitation spectra in fact closely match the respective absorption spectra. Thus, efficient energy transfer occurs from both the 2-pyridyl disulfide and the chrysene "antenna" leading to population of the Eu^{3+} emissive state. The chrysene antenna in **10b** is particularly interesting because it allows excitation at wavelengths compatible with readily available light sources (e.g., a mercury arc) and with standard fluorescence microscope optics.

The spectral excitation region for compound Eu(**10b**) extends to the 0−0 transition of the chrysene chromophore around 365 nm (see Figure 1). As we discussed before,[19] this value must come close to the longest excitation wavelength possible for sensitization of Eu^{3+}, since after excitation of the chromophore first intersystem crossing to the lowest triplet state is assumed to occur, which can subsequently give energy transfer to the luminescent lanthanide level (see Figure 2). For organic (poly)aromatic systems, a minimal single−triplet energy gap ($^1E_{00}$−$^3E_{00}$) of 5000 cm^{-1} may be assumed (in chrysene this is 7800 cm^{-1}), while the triplet must still have high enough energy to make the energy transfer fast and to exclude possible (thermal) back-energy transfer. For chrysene the triplet energy is 20 000 cm^{-1},[39] only 2500 cm^{-1} above the luminescent Eu^{3+} level, implying that in compound Eu(**10b**) probably already some back energy transfer occurs at room temperature.

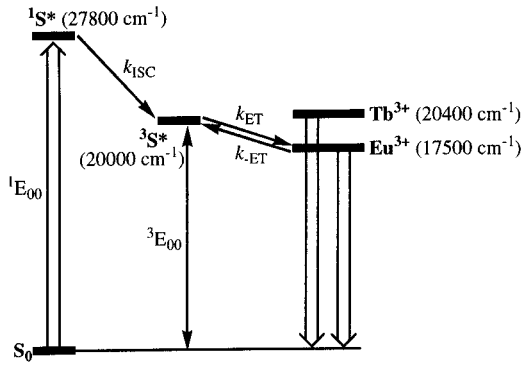

Figure 2. Energy scheme for the sensitized emission of Eu^{3+} and Tb^{3+} by chrysene. Depicted are the main luminescent levels of Eu^{3+} ($^5D_0$) and Tb^{3+} ($^5D_4$).

Not unexpectedly, with Tb^{3+} it was found that **10b** does not allow sensitized luminescence to be observed. This again can readily be explained from the energy diagram depicted in Figure 2, assuming that energy transfer to the lanthanide ion occurs from the antenna triplet.

Interestingly, in the case of **10b** the effect of complexation with lanthanide ions is not restricted to quenching of the chrysene triplet. This becomes evident from the effect of adding Eu$(NO_3)_3$·$5H_2O$ to an aqueous solution of **10b** (8.5×10^{-5} M) on the total luminescence spectrum (see Figure 3a). In the absence of Eu^{3+}, **10b** displays the typical fluorescence spectrum of the chrysene unit ($\Phi_{fluor} = 0.15$, $\Phi_{ISC} = 0.85$ reported for chrysene[39]). Upon addition of Eu^{3+}, the sensitized lanthanide emission appears, and its concentration dependence (see Figure 3b) nicely confirms formation of a 1:1 complex between **10b** and Eu^{3+}. However, at the same time, the chrysene fluorescence diminishes to about 10% of its orginal intensity. This fluorescence quenching seems to occur via nonspecific (dynamic) quenching and is not limited to a 1:1 stoichiometry. For the fluorescence quenching both direct energy transfer from the chrysene singlet excited state to the lanthanide ion and enhanced intersystem crossing of chrysene under the influence of a "heavy atom effect" of the lanthanide are viable mechanisms.[40] However, charge-transfer interaction between the singlet state of chrysene and the lanthanide also may be involved. The excited-state oxidation potential of chrysene is estimated to be $^1E_{ox}^* = -2.1$ V (calculated as the ground state oxidation potential $E_{ox} = 1.35$ V vs

(36) 6-Chrysenecarboxaldehyde (**1b**) was prepared in 71% yield from chrysene (**1a**) and α,α′-dichloromethyl methyl ether/$TiCl_4$. An alternative to prepare the aldehyde **1b** is the oxidation of 6-(hydroxymethyl)-chrysene. Akiyama, S.; Nakagawa, M. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 259.

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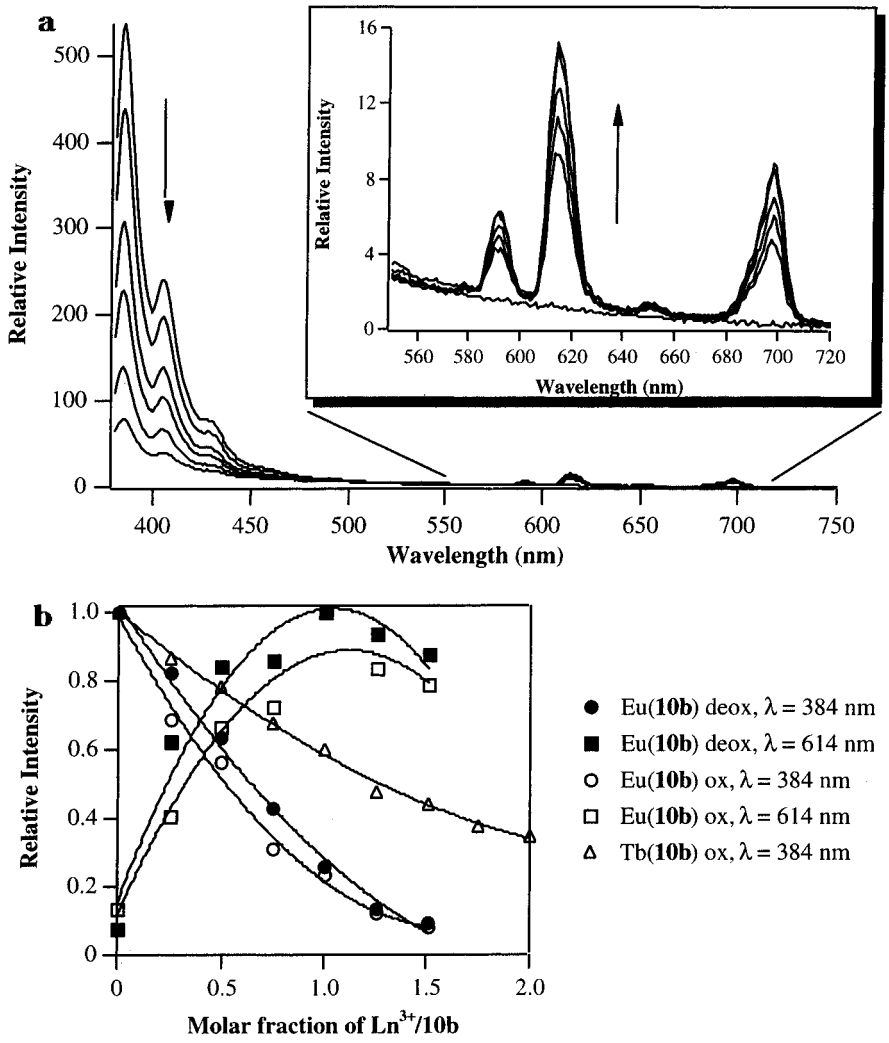

Figure 3. (a) Total luminescence spectra of **10b** (8.5×10^{-5} M) upon addition of increasing amounts of $Eu(NO_3)_3 \cdot 5H_2O$ in Tris−HCl buffer (pH 8.0); deoxygenated, $\lambda_{ex} = 363$ nm. (b) Relative luminescence intensities of the chrysene fluorescence (384 nm) and Eu^{3+} luminescence (614 nm) upon addition of increasing amounts of $Eu(NO_3)_3 \cdot 5H_2O$ or $Tb(NO_3)_3 \cdot 5H_2O$ to **10b** in Tris−HCl buffer (pH 8.0) for both aerated (ox) and deoxygenated (deox) solutions; $\lambda_{ex} = 363$ nm.

SCE minus the 0−0 energy $E_{00} = 3.45$ V),[39] which allows electron transfer to Eu^{3+} ($E_{red} = -0.6$ V vs SCE)[41] to occur. This conclusion is supported by the fact that the fluoresence of **10b** is quenched to a much lesser degree by Tb^{3+} (see Figure 3b). Tb^{3+} is very hard to reduce ($E_{red} = -3.9$ V vs SCE)[41] and also very hard to oxidize ($E_{ox} = 2.9$ V vs SCE),[41] making photoinduced electron transfer between Tb^{3+} and ^{1}chrysene* impossible.

We have reported previously on sensitized luminescence of calix[4]arene/lanthanide complexes containing an antenna chromophore attached to the lower rim of the calixarene cage, to a complexed lanthanide ion, and employing three carboxylic acid groups as the complexing moieties, which resulted in overall neutral lanthanide complexes.[18,19] Depending on the chromophore used, excitation wavelengths up to 350 nm could be achieved. However, these complexes could only be studied in organic solvents (e.g., methanol) because of their insolubility in aqueous media.

The present lanthanide complexes combine long-wavelength excitation (i.e., **10b**) and overall electroneutrality with water solubility via introduction of substituents at the upper rim of the calix[4]arene cage. The luminescent lifetime for Eu(**10b**) is 0.11 and 0.14 ms in aerated and deoxygenated aqueous solutions, respectively. These lifetimes are shorter than those observed previously for calix[4]arene/Eu^{3+} complexes in methanol (0.23−1.3 ms),[18,19] thus demonstrating the stronger quenching ability of water. However, the present luminescence lifetimes are also certainly useful in schemes employing time-gated emission measurements to suppress the influence of the general background fluorescence of biological material, which usually has a lifetime in the nanosecond region.

While the 2-pyridyl disulfide group in **10a** only allows short wavelength excitation, it can be used as a coupling reagent toward SH functionalities in biological material.

Conclusions

We have developed a facile route to neutral, water-soluble calix[4]arene-based lanthanide complexes. Moreover, our approach has the potential for variation of substituents in the carboxamido fragment at the lower rim. Chrysene gave high energy transfer from a 1:1 complex ligand/Eu^{3+} at wavelengths extending to 363 nm. Currently, we are investigating the possibility of introducing both a sensitizer and a handle for coupling to biomolecules in the synthesis route.

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Experimental Section

Synthesis. General Procedure. Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ with Me_4Si as the internal standard unless stated otherwise. Preparative column chromatography separations were performed on Merck silica gel 60 (230−400 mesh), while precoated silica gel plates (Merck, 60 F_{254}) were used for analytical TLC. FAB mass spectra were performed with *m*-nitrobenzyl alcohol as a matrix unless stated otherwise. $CHCl_3$, used in the chlorosulfonylation reaction, was flushed over an Al_2O_3 column and dried on molecular sieves (4 Å). All other chemicals were analytically pure and were used without further purification. Hexane refers to the fraction with bp 40−60 °C. All reactions were carried out under an argon atmosphere. Standard workup means that the organic layers were finally washed with water, dried over magnesium sulfate ($MgSO_4$), filtered, and concentrated *in vacuo*. The presence of solvents in the analytical samples was confirmed by ^{1}H NMR spectroscopy or potentiometric titrations. Fast atom bombardment (FAB) mass spectrometry for the water-soluble calix[4]arenes **10a**,**b** was carried out in a matrix solution (glycerol, thioglycerol, *m*-nitrobenzyl alcohol) onto a stainless steel probe and bombarded with xenon atoms with an energy of 3 KeV. During the high-resolution FRIT-FAB measurements a resolving power of 10.000 (10% valley definition) was used. Cesium iodide and/or glycerol were used to calibrate the mass spectrometer. *S*-(2-Pyridylthio)cysteamine hydrochloride,[32] de-*tert*-butylated calix[4]arene, and calix[4]arene tetraethyl ester were prepared according to literature procedures.[42]

Chrysene-6-carboxaldehyde (1b). To a solution of chrysene (**1a**) (2.0 g, 8.76 mmol) and freshly distilled $TiCl_4$ (3.30 g, 17.4 mmol) in dry CH_2Cl_2 (75 mL) was added dropwise bis(chloromethyl) ether (10.0 g, 86.8 mmol) at −10 °C. The reaction mixture was stirred at rt for 30 min. The reaction was quenched by the addition of crushed ice (15 g). The organic layer was washed with 2 M HCl (2 × 100 mL), followed by standard workup. The crude reaction product was purified by flash column chromatography (CH_2Cl_2/hexanes, 50:50). The product was crystallized from CH_2Cl_2/hexane to obtain a yellow colored solid: yield 71%; mp 165−168 °C (lit.[36] mp 164−166 °C).

6-(Hydroxymethyl)chrysene (1c). To a solution of **1b** (1.0 g, 3.9 mmol) in dry THF (75 mL) was added BH_3·THF (3 mL, 1.0 M solution) at 0 °C. The cooling bath was removed, and the solution was stirred for 2 h at rt. HCl (1 M, 75 mL) was added, and the precipitate was filtered off. The product was triturated with CH_2Cl_2 to give pure **1c**: yield 94%; mp 200−203 °C (lit.[35] mp 200−202 °C).

6-(Phthalimidomethyl)chrysene (1e). To a solution of **1d** (0.50 g, 1.56 mmol) in DMF (25 mL) was added potassium phthalimide (0.32 g, 1.72 mmol). The solution was stirred for 5 h at 50 °C. The solvent was removed *in vacuo* and the residue dissolved in CH_2Cl_2 (50 mL) and washed with 0.1 M NaOH (3 × 50 mL). The product was obtained as a white solid in quantitative yield: mp 243−245 °C; ^{1}H NMR (DMSO-d_6) δ 9.1−9.0 (m, 1 H), 8.91 (d, 1 H, J = 8.4 Hz), 8.86 (s, 1 H), 8.84 (d, 1 H, J = 8.4 Hz), 8.5−8.4 (m, 1 H), 8.2−8.1 (m, 2 H), 8.0−7.86 (m, 4 H), 7.86−7.7 (m, 4 H); ^{13}C NMR (DMSO-d_6) δ 166.28 (s), 42.69 (t); mass spectrum (EI) *m*/*z* 387.1 (M^+, calcd for $C_{27}H_{17}NO_2$ 387.1).

6-(Aminomethyl)chrysene (1f). A solution of **1e** (0.50 g, 1.29 mmol) and hydrazine monohydrate (0.32 g, 6.46 mmol) in EtOH (50 mL) was refluxed for 2 h. The solvent was removed *in vacuo*, and H_2O (50 mL) and CH_2Cl_2 (50 mL) were added to the residue. The organic layer was washed with $NaHCO_3$ (saturated aqueous solution, 50 mL), followed by standard workup: yield 95%; mp 204−206 °C; ^{1}H NMR δ 8.84 (d, 2 H, J = 8.3 Hz), 8.73 (d, 1 H, J = 7.5 Hz), 8.70 (d, 1 H, J = 7.5 Hz), 8.22 (d, 1 H, J = 7.5 Hz), 8.00 (d, 2 H, J = 8.3 Hz), 7.8−7.5 (m, 4 H), 4.52 (s, 2 H), 1.69 (broad s, 2 H); ^{13}C NMR δ 44.79; mass spectrum (EI) *m*/*z* 257.2 (M^+, calcd 257.1). Anal. Calcd for $C_{19}H_{15}N$·0.4H_2O: C, 86.26; H, 5.72; N, 5.29. Found: C, 86.20; H, 5.49; N, 5.12.

(42) Gutsche, C. D.; Lin, L.-G. *Tetrahedron* **1986**, *42*, 1633.

25,26,27-Tris[(ethoxycarbonyl)methoxy]-28-[(hydroxycarbonyl)methoxy]calix[4]arene (5a) was prepared similarly to a literature procedure for the *p*-*tert*-butylcalix[4]arene analogue:[19] white solid; yield 91%; mp 65−66 °C; ^{1}H NMR δ 7.13 (d, 4 H, J = 8.3 Hz), 7.0−6.8 (m, 2 H), 6.6−6.5 (m, 2 H), 6.5−6.4 (m, 4 H), 4.90 (d, 2 H, J = 15.0 Hz), 4.90, 4.85 (s, 4 H), 4.8−4.6 (m, 4 H), 4.40 (d, 2 H, J = 15.9 Hz), 4.3−4.2 (m, 6 H), 3.33 (d, 2 H, J = 12.5 Hz), 3.30 (d, 2 H, J = 12.5 Hz), 1.31 (t, 9 H, J = 8.3 Hz); FAB mass spectrum *m*/*z* 764.0 ([M + Na]$^+$, calcd 764.3), *m*/*z* 739.5 (M^+, calcd 739.3). Anal. Calcd for $C_{42}H_{44}O_{12}$: C, 68.10; H, 5.99. Found: C, 67.80; H, 5.95.

25,26,27-Tris[(ethoxycarbonyl)methoxy[-28-[[(2,2,2-trichloroethoxy)carbonyl]methoxy]calix[4]arene (5c). To a solution of triester monoacid **5a** (4.49 g, 6.9 mmol) in dry CH_2Cl_2 (75 mL) was added oxalyl chloride (5 mL), and the resulting reaction mixture was refluxed for 1 h. The solvent and excess of oxalyl chloride were removed *in vacuo*, whereupon 2,2,2-trichloroethanol (1.05 g, 7.0 mmol) and triethylamine (2.1 g, 20.8 mmol) were added. The mixture was stirred overnight at rt. Water (75 mL) was added, followed by standard workup. The crude product was separated by column chromatography (CH_2Cl_2/hexane 3/1) to give **5c** as a white solid: yield 96%; mp 124−125 °C; ^{1}H NMR δ 6.7−6.5 (m, 6 H), 6.47 (s, 6 H), 4.90 (s, 2 H), 4.89−4.65 (m, 4 H), 4.75, 4.70 (s, 4 H), 4.61 (d, 4 H, J = 6.7 Hz), 4.2−4.0 (m, 6 H), 3.18 (d, 2 H, J = 12.5 Hz), 3.17 (d, 2 H, J = 12.5 Hz), 1.3−1.2 (m, 9 H); FAB mass spectrum *m*/*z* 895.1 ([M + Na]$^+$, calcd 895.2). Anal. Calcd for $C_{44}H_{45}Cl_3O_{12}$: C, 60.59; H, 5.20. Found: C, 60.51; H, 5.19.

5,11,17,23-Tetrakis(chlorosulfonyl)-25,26,27-tris[(ethoxycarbonyl)methoxy]-28-[[(2,2,2-trichloroethoxy)carbonyl]methoxy]calix[4]arene (6). To a solution of trichloroethyl ester calix[4]arene **5c** (5.0 g, 5.7 mmol) in CH_2Cl_2 (100 mL) was added dropwise HSO_3Cl (18.7 mL, 0.23 mol) at −10 °C. When the addition was complete, the cooling bath was removed, and the reaction mixture was stirred at rt for 3 h. The solution was poured into a separation funnel filled with crushed ice (50 g). To the white suspension formed was added CH_2Cl_2 (100 mL), and the layers were allowed to separate for 24 h. The organic layer was concentrated, and the residue was purified by flash column chromatography (CH_2Cl_2/ethyl acetate (or THF), 90/10) to afford pure **6** as a white solid: yield 81%; mp 95−97 °C; ^{1}H NMR δ 7.60 (s, 2 H), 7.59 (s, 2 H), 7.27 (s, 4 H), 5.15 (d, 2 H, J = 12.5 Hz), 5.11 (d, 2 H, J = 12.5 Hz), 4.92 (s, 2 H), 4.81 (s, 6 H), 4.5−4.1 (m, 8 H), 3.55 (d, 2 H, J = 12.5 Hz), 3.52 (d, 2 H, J = 12.5 Hz), 1.4−1.2 (m, 9 H); FAB mass spectrum *m*/*z* 1289.4 ([M + Na]$^+$, calcd 1289.2). Anal. Calcd for $C_{44}H_{41}Cl_7O_{20}S_4$: C, 41.74; H, 3.26. Found: C, 41.82; H, 3.21.

5,11,17,23-Tetrakis[[[[[[tris[[(*tert*-butyldimethylsilyl)oxy]methyl]methyl]amino]carbonyl]methyl]amino]sulfonyl]-25,26,27-tris[(ethoxycarbonyl)methoxy]-28-[[(2,2,2-trichloroethoxy)carbonyl]methoxy]calix[4]arene (7). To a solution of tetrasulfonyl calix[4]arene **6** (1.62 g, 1.3 mmol) was added dropwise a mixture of amine **3** (3.30 g, 6.3 mmol) and triethylamine (0.92 g, 9.1 mmol). The solution was stirred overnight. The solution was washed with 1 M HCl (50 mL) followed by standard workup. The crude solid was purified by column chromatography (CH_2Cl_2/ethyl acetate 97/3) to give **7** as a white solid: yield 79%; mp 102−104 °C; ^{1}H NMR δ 7.36 (broad s, 4 H), 6.72 (broad s, 2 H), 6.65 (broad s, 2 H), 6.48 (broad s, 4 H), 5.05 (broad s, 2 H) 5.0−4.6 (m, 12 H), 4.18 (q, 6 H, J = 6.7 Hz), 3.81 (broad s, 24 H), 3.4−3.1 (m, 12 H), 1.3−1.2 (m, 9 H), 0.83 (s, 108 H), 0.00 (s, 72 H); FAB mass spectrum *m*/*z* 3226.0 ([M + Na]$^+$, calcd 3226.4). Anal. Calcd for $C_{140}H_{261}Cl_3N_8O_{36}S_4Si_{12}$·$H_2O$: C, 52.16; H, 8.24; N, 3.48. Found: C, 52.02; H, 8.21; N, 3.45.

5,11,17,23-Tetrakis[[[[[[tris[[(*tert*-butyldimethylsilyl)oxy]methyl]methyl]amino]carbonyl]methyl]amino]sulfonyl]-25,26,27-tris[(ethoxycarbonyl)methoxy]-28-[[[[2′-(pyridinedithio)ethyl]amino]carbonyl]methoxy]calix[4]arene (8a). A solution of **7** (0.65 g, 0.2 mmol), *S*-(2-pyridylthio)cysteamine (**2**) (0.17 g, 0.2 mmol), triethylamine (0.03 g, 0.2 mmol), and DBU (0.28 g, 1.8 mmol) in acetonitrile (50 mL) was heated at 40 °C for 30 min. After removal of the

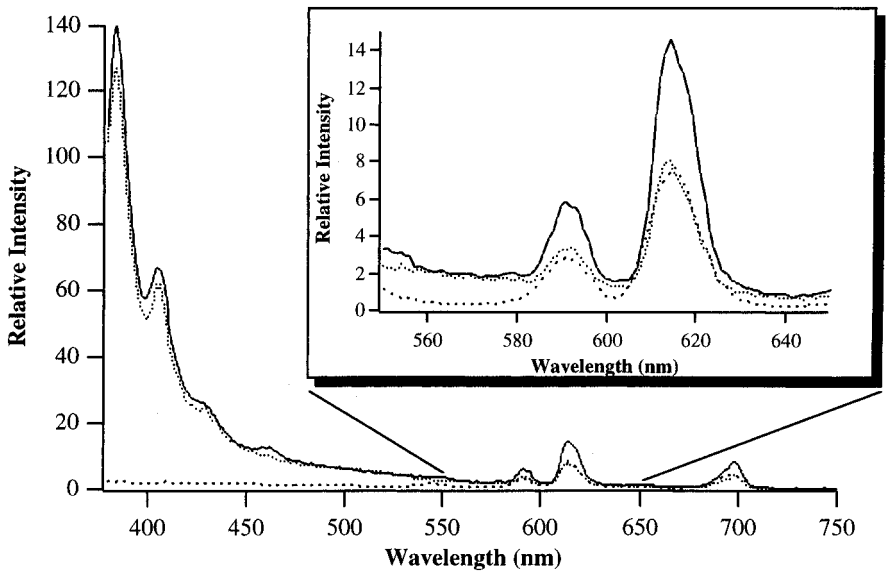

Figure 4. Corrected emission spectra of Eu(**10a**) (- - -; λ_{ex} = 250 nm) and Eu(**10b**) (aerated: ···; deoxygenated: —; λ_{ex} = 363 nm) in Tris−HCl buffer (pH 8.0) at room temperature.

solvent, the residue was dissolved in CH_2Cl_2 (50 mL) and the solution washed with 1 M HCl (50 mL), followed by standard workup. After column chromatography (silica gel saturated with NaCl, ethyl acetate), the product was obtained as a slightly yellow solid: yield 67%; mp 95−98 °C; ^{1}H NMR δ 8.43 (t, 1 H, J = 4.2 Hz), 8.05 (broad s, 1 H), 7.7−7.5 (m, 3 H), 7.4−7.1 (m, 8 H), 6.69 (broad s, 4 H), 6.58 (broad s, 4H), 5.0−4.6 (m, 16 H), 4.22 (q, 6 H, J = 8.3 Hz), 3.81 (s, 24 H), 3.69 (m, 2 H), 3.4−3.1 (m, 12 H), 2.99 (t, 2 H, J = 6.7 Hz), 1.3−1.2 (m, 9 H), 0.83 (s, 108 H), 0.00 (s, 72 H); FAB mass spectrum (ONPOE) m/z 3238.0 (M^+, calcd 3237.5), 3128.6 ([M^+ − *S*-pyridine]). Anal. Calcd for $C_{145}H_{268}N_{10}O_{35}S_6Si_{12}$: C, 53.44; H, 8.36; N, 4.30. Found: C, 53.30; H, 8.51; N, 4.17.

5,11,17,23-Tetrakis[[[[[[tris[[(*tert*-butyldimethylsilyl)oxy]methyl]methyl]amino]carbonyl]methyl]amino]-sulfonyl]-25,26,27-tris[(ethoxycarbonyl)methoxy]-28-[[[(9-chrysenemethyl)amino]carbonyl]methoxy]calix[4]-arene (8b) was prepared similarly as compound **8a** starting from the trichloroethyl ester **7** (1.65 g, 0.5 mmol), 6-(aminomethyl)chrysene (**1f**) (0.15 g, 0.6 mmol) and DBU (0.71 g, 4.6 mmol). The crude solid was purified by column chromatography (silica gel saturated with NaCl, ethyl acetate/CH_2Cl_2 5/95): yield 63%; mp 115−117 °C; ^{1}H NMR δ 8.8−8.7 (m, 3 H), 8.66 (d, 1 H, J = 9.3 Hz), 8.17 (d, 1 H, J = 7.5 Hz), 8.09 (broad s, 1 H), 8.0−7.9 (m, 2 H), 7.8−7.5 (m, 4 H), 7.4−7.2 (m, 4 H coincides with $CDCl_3$), 6.8−6.5 (m, 8 H), 6.40 (broad s, 4 H), 5.20 (d, 2 H, J = 6.2 Hz), 4.9−4.4 (m, 12 H), 4.07 (q, 6 H, J = 7.2 Hz), 3.81 (broad s, 24 H), 3.4−3.2 (broad s, 12 H), 1.08 (t, 9 H, J = 7.1 Hz), 0.85 (s, 108 H), 0.01 (s, 72 H); FAB mass spectrum m/z 3333.6 ([M − H]$^+$, calcd 3333.6). Anal. Calcd for $C_{157}H_{273}N_9O_{35}S_4Si_{12}$·2$H_2O$: C, 56.32; H, 8.34; N, 3.76. Found: C, 55.93; H, 8.41; N, 3.58.

General Procedure for the Preparation of Triacid Calix[4]arenes 9a,b. To a solution of **8a**,**b** (5 mmol) in MeOH/H_2O 5/1 (50 mL) and a minimum amount of THF, for solubility reasons, was added K_2CO_3 (50 mmol). After the solution was refluxed for 15 min, CH_2Cl_2 (50 mL) was added, and the solution was acidified with 1 M HCl to pH 7. The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined layers were dried over $MgSO_4$, filtered, and concentrated in *vacuo* to give the pure products.

The characterization of the derivatives **9a**,**b** with ^{1}H NMR spectroscopy was difficult because of line broadening. Additionally, the relative intensity of the ^{1}H CH_3-resonances (TBDMS) compared to the other ^{1}H-resonances is large. No satisfactory mass spectrum (FAB and FRIT-FAB) could be obtained for the triacid derivatives **9a**,**b**. Therefore, characterization was done by melting point determination and elemental analysis.

5,11,17,23-Tetrakis[[[[[[tris[[(*tert*-butyldimethylsilyl)oxy]methyl]methyl]amino]carbonyl]methyl]amino]-sulfonyl]-25,26,27-tris[(hydroxycarbonyl)methoxy]-28-[[[[2′-(pyridinedithio)ethyl]amino]carbonyl]methoxy]-calix[4]arene (9a): yield 98%; mp 143−144 °C. Anal. Calcd for $C_{139}H_{256}N_{10}O_{35}S_6Si_{12}$: C, 52.89; H, 8.17; N, 4.44. Found: C, 52.87; H, 8.34; N, 4.30.

5,11,17,23-Tetrakis[[[[[[tris[[(*tert*-butyldimethylsilyl)oxy]methyl]methyl]amino]carbonyl]methyl]amino]-sulfonyl]-25,26,27-tris[(hydroxycarbonyl)methoxy]-28-[[[(9-chrysenemethyl)amino]carbonyl]methoxy]calix[4]-arene (9b): yield 97%; mp 160−162 °C. Anal. Calcd for $C_{151}H_{261}N_9O_{35}S_4Si_{12}$·3.5$H_2O$: C, 55.10; H, 8.11; N, 3.83. Found: C, 54.80; H, 8.10; N, 3.72.

General Procedure for the Preparation of Water-Soluble Calix[4]arenes 10a,b. A solution of triacid calix[4]arenes **9a**,**b** (0.5 g) in trifluoroacetic acid (TFA)/H_2O (9/1, 25 mL) was stirred overnight, whereupon toluene (50 mL) was added. The solution was concentrated *in vacuo*. Again, toluene (50 mL) was added, and the mixture was concentrated *in vacuo*. This procedure was repeated three times. The crude white solid was dissolved in a minimum amount of H_2O (5 mL). To this solution was added dropwise acetonitrile (**10a**) or acetone (**10b**) until the product started to precipitate. After the suspension was stirred for 30 min, the product was filtered. The product was freeze-dried and analyzed. Prior to the luminescence measurements, solutions of compounds **10a**,**b** in water were dialyzed (benzoylated cellulose membrane) to remove excess trifluoroacetic acid and again freeze-dried.

5,11,17,23-Tetrakis[[[[[tris[(hydroxymethyl)methyl]-amino]carbonyl]methyl]amino]sulfonyl]-25,26,27-tris[(hydroxycarbonyl)methoxy]-28-[[[[2′-(pyridinedithio)ethyl]-amino]carbonyl]methoxy]calix[4]arene (10a). The crude solid was triturated with EtOAc/H_2O to obtain pure **10a**: yield 99%; during melting point determination the compound decomposed; ^{1}H NMR (400 MHz, D_2O, 50 °C) δ 8.57 (s, 1 H), 8.0−7.9 (m, 3 H), 7.8−7.2 (m, 8 H), 5.4−4.9 (m, 12 H), 4.6−4.4 (m, 8 H), 4.2−3.6 (m, 24 H), 3.40 (t, 2 H, J = 7.4 Hz), 3.28 (d, 4 H, J = 11.4 Hz), 1.50 (t, 2 H, J = 7.4 Hz); FAB mass spectrum (glycerol) m/z 1785.7 [M^+, calcd 1785.8]. Anal. Calcd for $C_{67}H_{88}N_{10}O_{35}S_6$·3TFA: C, 41.40; H, 5.20; N, 6.20. Found: C, 41.27; H, 5.05; N, 6.31.

5,11,17,23-Tetrakis[[[[[tris[(hydroxymethyl)methyl]-amino]carbonyl]methyl]amino]sulfonyl]-25,26,27-tris-[(hydroxycarbonyl)methoxy]-28-[[[(9-chrysenemethyl)-amino] carbonyl]methoxy]calix[4]arene (10b). The crude solid was triturated with ethyl acetate/H_2O to obtain **10b** as a white pure solid: yield 99%; during melting point determination the compound decomposed; ^{1}H NMR (D_2O, 80 °C) δ 9.0−

7.2 (m, 19 H), 5.4−4.8 (m, 18 H), 4.69 (broad s, 24 H), 3.82 (d, 4 H, J = 9.3 Hz), 3.27 (d, 4 H, J = 11.5 Hz); FAB mass spectrum (glycerol) m/z 1857.8 [$(M + H)^+$, calcd 1856.8]; exact FRIT-FAB m/z 1856.4735 [M^+, calcd 1856.4761], the fragmentation pattern corresponds with the proposed structure. Anal. Calcd for $C_{79}H_{93}N_9O_{35}S_4$·4TFA: C, 45.10; H, 4.01; N, 5.43. Found: C, 45.18; H, 4.23; N, 5.45.

Luminescence Measurements. Continuous emission and excitation spectra were recorded on a Spex Fluorolog 2 spectrofluorimeter. Figure 4 gives representative results of such spectra.

Time-resolved emission spectra were obtained using a Lumonics EX700 XeCl excimer laser (308 nm) as excitation source. The resulting luminescence was observed by a gated, intensified CCD camera from Princeton Instruments. Spectra were taken with a typical gatewidth of 25 μs, an initial delay of about 0.5 μs relative to the laser pulse maximum, and 50 μs increment delay between spectra. At each delay, spectra were averaged over 50 laser shots to improve the signal to noise ratio. From these data, luminescent lifetimes were calculated by fitting the wavelength integrated signal in time. Monoexponential decay was observed in all cases.

All measurements were done in Tris−HCl buffer (0.05 M, pH 8.0).

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