Water-soluble neutral calix[4]arene lanthanide complexes; synthesis and luminescence properties
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Published in:
Journal of Organic Chemistry

Citation for published version (APA):
Water-soluble neutral 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. Particularly interesting are lanthanide probes because of their unique characteristics like exceptionally large antenna, long-wavelength emission \((\sim 200\text{–}300\text{ nm})\), narrow emission line spectra, and long luminescence lifetimes \((\sim 500\text{–}600\text{ nm})\). 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. Several calix[4]arene derivatives are suitable for the complexation of trivalent cations, but in most cases they are not soluble in water. Only Sabbatini et al. reported the encapsulation of a lanthanide ion by a tetramide calix[4]arene to give a water-soluble system, while Shinkai et al. 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.

Recently, we described calixarene derivatives that are substituted with three carboxylic acid groups and which form neutral complexes with lanthanide cations. 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.

For a simple coupling to biomolecules, these calix[4]-arene lanthanide probes should be water-soluble. In the literature, different types of water-soluble calix[4]arenes have been reported that have hydrophilic substituents such as SO\(_3\)^{−}, COO\(^{−}\), PO\(_4\)^{−}, SO\(_3\)(CH\(_2\))\(_{12}\)OH\(_2\), N\(^\circ\)Me\(_3\) or polyethyleneoxy chains. Recently, we have reported neutral water-soluble calix[4]arenes by...
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-trichloroethanol28 in CH$_2$Cl$_2$ 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-(

Chart 1

![Chart](image)

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[(tert-butyl(dimethyl)silyl)oxy]methyl)methane]-2-aminoacetamide (3)27 (Chart 1) was coupled to calix[4]arene 6 to afford the tetrasm sulfonamide 7 in 82% yield. This intermediate can be used to introduce different amino-substituted residues. Substitution31 of the trichloroethyl group in 7 with 6-(aminomethyl)chrysene (1f) or S-(2-pyridylthiocysteamine (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)chrysene34 (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


in an alternative way via reduction of 6-chrysenecarboxylic acid derivatives. The selective hydrolysis of the three ethyl esters without cleavage of the amide bonds in 8a,b, was achieved with \( \text{K}_2\text{CO}_3 \) in refluxing MeOH. 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. 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.

**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\(_2\)O or Tb(NO\(_3\))\(_3\)-5H\(_2\)O) were added to a 1.5 \( \times \) 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\(^{3+}\)). 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,39 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 \((\Delta E_{00} - \Delta E_{00})\) 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.

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\(_2\)O to an aqueous solution of 10b (8.5 \( \times \) 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 original 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 \(E_{ox} = -2.1\) V (calculated as the ground state oxidation potential \(E_{ox} = 1.35\) V vs

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(36) 6-Chrysenecarboxaldehyde (1b) was prepared in 71% yield from chrysene (1a) and dimethyl sulfoxide ethereal ether/Ticls. 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.


SCE minus the $0-0$ energy $E_{00} = 3.45$ V),
which allows electron transfer to Eu$^{3+}$ ($E_{\text{red}} = -0.6$ V vs SCE)$^{41}$ to occur. This conclusion is supported by the fact that the fluorescence of 10b is quenched to a much lesser degree by Tb$^{3+}$ (see Figure 3b). Tb$^{3+}$ is very hard to reduce ($E_{\text{red}} = -3.9$ V vs SCE)$^{41}$ and also very hard to oxidize ($E_{\text{ox}} = 2.9$ V vs SCE)$^{41}$ making photoinduced electron transfer between Tb$^{3+}$ and 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 insolvency 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, watersoluble 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. H NMR and 13C NMR spectra were recorded in CDCl3 with Me4Si 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 F254) were used for analytical TLC. FAB mass spectra were performed with m-nitrobenzyl alcohol as a matrix unless stated otherwise. CHCl3, used in the chlorosulfonylation reaction, was flushed over an Al2O3 column and dried on molecular sieves (4 Å). All organic solvents were dried over molecular sieves (4 Å) and removed in vacuo (50 mL) was refluxed for 2 h. The solvent was removed, and the residue was purified by column chromatography (CH2Cl2/hexanes, 50:50). All solvent ratios are given in volume.

The reaction mixture was stirred at rt for 30 min. The reaction was quenched by the addition of crushed ice (15 g). The cooling bath was removed, and the reaction mixture was stirred overnight. The solvent was removed, and the residue was stirred at rt for 3 h. The solvent was poured into a separation funnel filled with crushed ice (50 g). To the white suspension formed was added CH2Cl2 (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 (CH2Cl2/hexanes, 50:50). The product was purified by column chromatography (CH2Cl2/hexanes, 50:50). The product was crystallized from CH2Cl2/hexane to obtain a yellow colored solid: yield 71%; mp 165–168 °C (lit.18 mp 164–166 °C).

5.25, 26, 27-Tris[(ethoxycarbonyl)carbonyl]methoxy]-28-[[(hydroxy carbonyl)carbonyl]calix[4]arene (1f). To a solution of triester monoacid 3a (4.49 g, 6.9 mmol) in dry CH2Cl2 (75 mL) was added oxalyl chloride (5 mL), and the solution was stirred overnight at rt. Water (75 mL) was added, followed by standard workup. The crude product was separated by column chromatography (CH2Cl2/hexanes, 50:50) to give 2f as a white solid: yield 94%; mp 200–203 °C (lit.18 mp 200–202 °C).

solvent, the residue was dissolved in CH₂Cl₂ (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; ¹H 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, 1 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 3233.6 ([M + H]⁺, calcd 3238.0). Anal. Calcd for C₁₄₅H₂₆₈N₁₀O₃₅S₆Si₁₂: C, 53.44; H, 8.51; N, 4.17. Found: C, 53.30; H, 8.51; N, 4.17.

Figure 4. Corrected emission spectra of Eu(10a) (---; λ_exc = 250 nm) and Eu(10b) (- -; λ_exc = 363 nm) in Tris–HCl buffer (pH 8.0) at room temperature.

5,11,17,23-Tetrakis[tris[(tert-butyldimethylsilyl)oxy]methyl]amino]calix[4]arene (9a). A solution of trichloroethyl ester 7 (1.65 g, 0.55 mmol), 6-(aminomethyl)chrysene (1f) (0.15 g, 0.6 mmol) and DBU (0.71 g, 4.6 mmol) in CH₂Cl₂ (50 mL) was stirred overnight. The suspension was stirred for 30 min, the product was filtered. The aqueous layer was extracted with CH₂Cl₂ (2 × 33 mL) and the solution was acidified with 1 M HCl to pH 7. The suspension was stirred for 15 min, CH₂Cl₂ (50 mL) was added, and the solution was acidified with 1 M HCl to pH 7. The aqueous layer was extracted with CH₂Cl₂ (2 × 50 mL). The combined layers were dried over MgSO₄, filtered, and concentrated in vacuo to give the pure products.

The characterization of the derivatives 9a,b with ¹H NMR spectroscopy was difficult because of line broadening. Additionally, the relative intensity of the ¹H CH₂-sonances (TBDMs) compared to the other ¹H-resonances is large. No satisfactory mass spectrum (FAB and FRIT-FAB) could be obtained for the triad derivatives 9a,b. Therefore, characterization was done by melting point determination and elemental analysis.


5,11,17,23-Tetrakis[tris[(tert-butyldimethylsilyl)oxy]methyl]amino]calix[4]arene (9a): 1H 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, 1 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 3233.6 ([M + H]⁺, calcd 3238.0). Anal. Calcd for C₁₄₅H₂₆₈N₁₀O₃₅S₆Si₁₂: C, 53.44; H, 8.51; N, 4.17. Found: C, 53.30; H, 8.51; N, 4.17.

General Procedure for the Preparation of Water-Soluble Calix[4]arenes 10a,b. A solution of trichloroethyl ester 7 (0.5 g) in trifluoroacetic acid (TFA)/H₂O (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 H₂O (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[(tert-butyldimethylsilyl)oxy]methyl]amino]calix[4]arene (9a): 1H 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, 1 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 3233.6 ([M + H]⁺, calcd 3233.6). Anal. Calcd for C₁₄₅H₂₆₈N₁₀O₃₅S₆Si₁₂: C, 53.44; H, 8.51; N, 4.17. Found: C, 55.93; H, 8.34; N, 3.76.

General Procedure for the Preparation of Triacid Calix[4]arenes 9a,b. To a solution of 8a,b (5 mmol) in MeOH/H₂O 5/1 (50 mL) and a minimum amount of THF, for solubility reasons, was added K₂CO₃ (50 mmol). After the solution was refluxed for 15 min, CH₂Cl₂ (50 mL) was added, and the solution was acidified with 1 M HCl to pH 7. The aqueous layer was extracted with CH₂Cl₂ (2 × 50 mL). The combined layers were dried over MgSO₄, filtered, and concentrated in vacuo to give the pure products.
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 C79H93N9O35S4': 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).

Acknowledgment. The research described in this paper was supported by the Technology Foundation (S.T.W.), Technical Science Branch of the Netherlands Organization for Scientific Research (NWO).