Sensitized Near-Infrared Emission from Nd^{3+} and Er^{3+} Complexes of Fluorescein-Bearing Calix[4]arene Cages

Manon P. Oude Wolbers, Frank C. J. M. van Veggel,* Frank G. A. Peters, Eric S. E. van Beelen, Johannes W. Hofstraat, Frank A. J. Geurts, and David N. Reinhoudt*

Abstract: Three novel, fluorescein-containing calix[4]arenes bearing three carboxylic acid or three diethylamide functions were synthesized for the complexation of Nd^{3+} or Er^{3+}. The typical Nd^{3+} luminescence in the near-infrared region of the electromagnetic spectrum is increased up to seven times upon excitation via fluorescein relative to calix[4]arene excitation. The calix[4]arene moiety itself already acts as a sensitizer; however, fluorescein is not only a more efficient sensitizer, but also makes excitation in the visible part of the electromagnetic spectrum (at approximately 500 nm) possible. In contrast, Er^{3+} luminescence could only be observed upon fluorescein excitation. The luminescence intensity is strongly dependent on the conformational properties of the ligand, the solvent, and the length of the spacer between the calix[4]arene and fluorescein. The luminescence lifetimes are solvent-dependent but independent of the length of the flexible spacer between the sensitizer and the calix[4]arene. They were determined as 1.23–1.26 µs for calix[4]arene complex $\mathbf{1}\cdot\mathbf{Nd^{3+}}$ and 1.63–1.71 µs for $\mathbf{1}\cdot\mathbf{Er^{3+}}$ in DMSO solutions.

Keywords: calixarenes · fluorescence · lanthanides · luminescence · sensitizers

Introduction

Recently, we have reported the luminescence properties of various lanthanoid ion complexes.[1] We were mainly interested in long luminescence lifetimes of the lanthanoid ion complexes obtained by shielding the lanthanoid ions from high-energy vibrational modes in their chemical environment and efficient excitation. From the use of systematic deuteration, it became clear that the quenching by the encapsulating ligand is important in the case of a low concentration of O–H oscillators around the lanthanoid ions. Our overall aim is the application of organic lanthanoid ion complexes in polymer-based optical amplifiers that operate at 1.3 µm and 1.5 µm, the second and third telecommunication windows, respectively. For this purpose, Nd^{3+} and Er^{3+} can be used in inorganic matrices.[2] To achieve optical amplification, population inversion (more lanthanoid ions in the excited state than in the ground state) is required, which is facilitated by efficient excitation and long luminescence lifetimes. However, despite the optimization of the environment surrounding the lanthanoid ion, the luminescence intensities of the lanthanoid ions in inorganic matrices remain low because of the relatively inefficient direct excitation of the ions. The absorption coefficients of lanthanoid ions are low relative to organic chromophores: the absorption coefficients of conjugated organic molecules are typically 3–4 orders of magnitude higher than those of lanthanoid ions.[3] In addition, the absorption bands of chromophores are much broader, so that there is a wider choice of light sources for excitation. Therefore, organic molecules that can be excited very efficiently and may transfer the excitation energy to lanthanoid ions are often used as sensitizers. This so-called sensitized emission is often used in fluoroimmunoassays. Some examples of efficient sensitizers for Eu^{3+} and/or Tb^{3+} taken from the literature are carbostyril-124,[4] 4-(phenylethynyl)pyridine,[5] and triphenylene.[6] The efficiency of the energy transfer to lanthanoid ions is, amongst other things, dependent on the luminescent level of the complexed lanthanoid ion and on the energy level of the triplet state of the sensitizer, which can be influenced by structural modification of the sensitizer. Moreover, the energy transfer rate is largely influenced by the distance R between the sensitizer and the lanthanoid ion ($k \propto R^{-6}$ or e^{-R}). The use of sensitizers

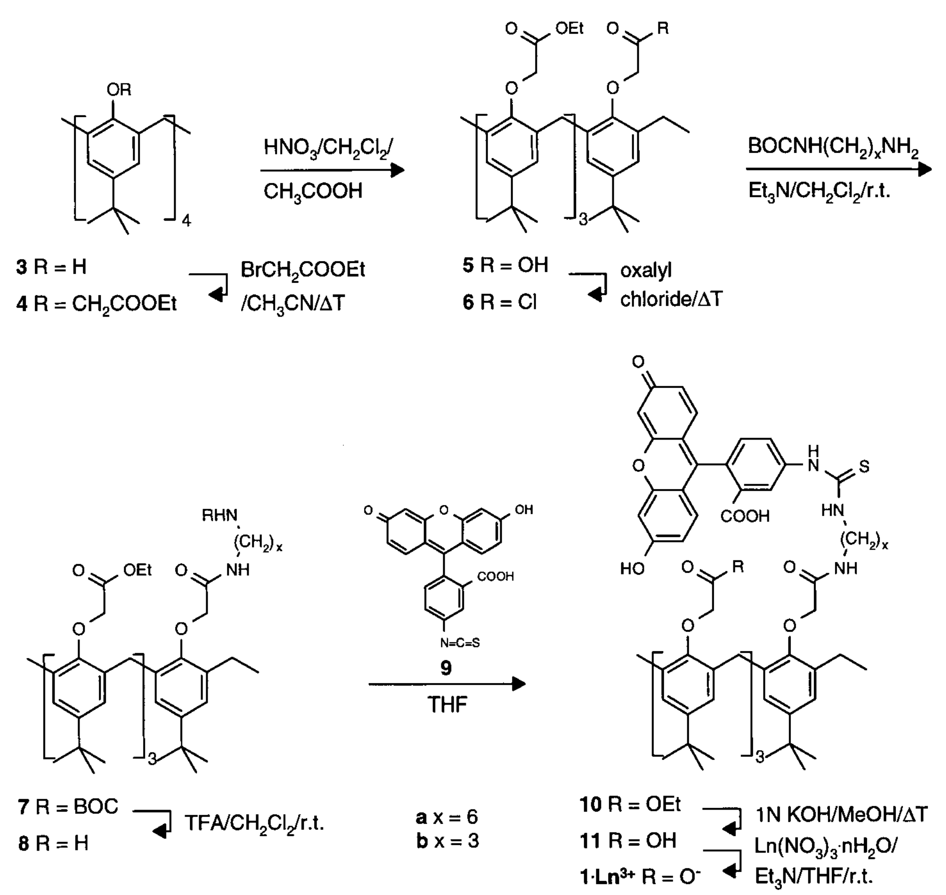
---

[*] Dr. ir. F. C. J. M. van Veggel, Dr. ir. M. P. Oude Wolbers, F. G. A. Peters, E. S. E. van Beelen, Prof. D. N. Reinhoudt
Department of Supramolecular Chemistry and Technology
MESA Research Institute, University of Twente
P.O. Box 217, NL-7500 AE Enschede (The Netherlands)
Fax: (+31)53-489-4645
E-mail: d.n.reinhoudt§ct.utwente.nl

Dr. J. W. Hofstraat, Dr. F. A. J. Geurts
Akzo Nobel Central Research b.v.
P.O. Box 9300, NL-6800 SB Arnhem (The Netherlands)

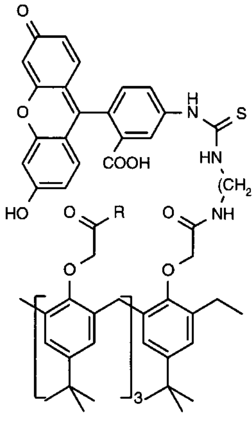
0947-6539/98/0405-0772 $ 17.50+.50/0

significantly reduces the required pump power that is needed to achieve population inversion in lanthanoid ions. Optical gain calculations[7] yield the pump power, which decreases by approximately three orders of magnitude as a result of the high absorption coefficient of the sensitizer. Another advantage is the applicability of various pump wavelengths as a result of the broad absorption bands of sensitizers. For application in telecommunications systems we are mainly interested in excitation with red light because lasers operating in this part of the spectrum are available at low cost.



Scheme 1. The synthesis of fluorescein-containing calix[4]arene-based triacids **11** and of their Ln complexes.

The energy transfer from fluorescein to Eu^{3+} and from its tetrabromo derivative, eosin, to Nd^{3+} or Yb^{3+} have been reported in solution.[8a,b] In both cases no ligands were present for complexation of the lanthanoid ions, apart from the carboxylic acid of the sensitizer. Meshkova and coworkers used (2-pyridazo)-2-naphthol for the luminescent detection of ytterbium.[8c,d] The present paper describes the covalent attachment of fluorescein to functionalized calix[4]arenes that can complex lanthanoid ions (**1** and **2**). It is assumed that in a preorganized supramolecular system, the sensitizer can be forced into close proximity to the lanthanoid ion, which will enhance the energy transfer rate. Furthermore, the calix[4]arene will shield the lanthanoid ion against effective quenchers, like O–H groups, in the first coordination sphere. The synthesis and luminescence properties of the Nd^{3+} and Er^{3+} complexes of the fluorescein-containing calix[4]arenes are discussed. To the best of our knowledge this is the first example of an organic sensitizer which is *covalently linked* to 1:1 complexes (polydentate ligand:lanthanoid ion) leading to sensitized emission in the near-infrared part of the electromagnetic spectrum. Er^{3+} emission originating from 1:1 organic complexes has not been reported before and has only recently been observed in our laboratories. Parker and coworkers have recently published details of the luminescence properties of ytterbium complexes on either direct excitation or sensitized emission with co-dissolved chromophores.[9] Horrocks and co-workers have recently described the sensitized emission of ytterbium complexed in a protein by means of tryptophan moieties.[10]



1 R = O$^-$, **a** x = 6, **b** x = 3
2 R = NEt$_2$, x = 6

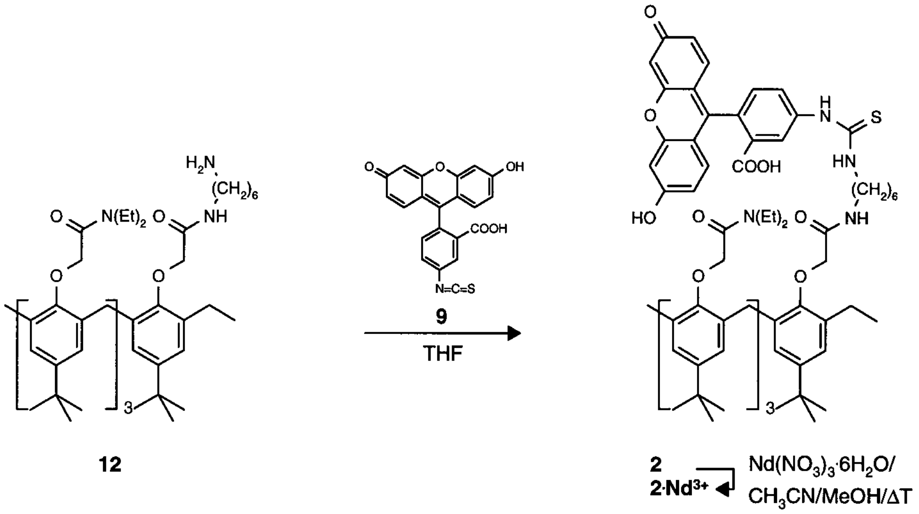
Results and Discussion

Synthesis: Three different organic ligands containing fluorescein as sensitizer were synthesized for the complexation of lanthanoid ions. Two bear three carboxylic acid groups as coordinating sites, whereas the other has three diethylamide functions. The fluorescein moiety is attached to the calix[4]arene by another amide function. Upon deprotonation and complexation, the carboxylic acid-containing ligands give overall neutral complexes, which only differ in the length of the spacer between the calix[4]arene and the fluorescein derivative.

The synthesis of both fluorescein-containing calix[4]arene-based triacids **11** (Scheme 1) starts from *p-tert*-butylcalix[4]arene (**3**), which is converted to the triethylester monoacid chloride **6** by literature procedures.[11] Compound **6** was coupled to one equivalent of the appropriate mono-Boc-protected diamine ($x = 6$ or 3) in dichloromethane in the presence of triethylamine as a base. Both products **7a** and **7b** were obtained in ≈80% yield and were pure (elemental analysis). Formation of the amide was evident from ^{1}H NMR spectroscopy; both spectra show a broad singlet at $\delta = 8.3-8.4$ for the amide hydrogen atom and a singlet at $\delta = 1.4$ which can be attributed to the *tert*-butyl hydrogen atoms of the Boc group. Subsequently, the Boc group was removed by addition of trifluoroacetic acid to a solution of **7** in dry dichloromethane. The free amines **8** were obtained in 80% to quantitative yield after basic work-up. Deprotection was obvious from ^{1}H NMR spectroscopy and from FAB mass spectra showing the highest peak at a value that can be attributed to $[(M+\mathrm{H})^+]$ for both products, that is, 1063.7 for **8a** ($C_{64}H_{91}N_2O_{11}$: 1063.7) and 1021.5 for **8b** ($C_{61}H_{85}N_2O_{11}$: 1021.6).

The free amines[12] were treated with the isothiocyanate-functionalized fluorescein (**9**) in THF at room temperature. After purification by Sephadex column chromatography, the products **10a** and **10b** were obtained in 88% and 70% yield, respectively, and were pure according to elemental analysis. The covalent attachment of the fluorescein was evident from IR spectroscopy; a vibrational band at 3360 cm^{-1} corresponding to the N–H stretch vibration of the thiourea was clearly visible, together with a band at 1611–1612 cm^{-1} which may be attributed to the C=S stretch vibration.[13] The highest peaks in the FAB mass spectra were observed at 1475.2 for **10a** and at 1432.7 for **10b**, corresponding to the calculated value for $[(M+\mathrm{Na}+\mathrm{H})^+]$ (1475.7 and 1432.6, respectively). Moreover, the ^{1}H NMR spectra show a singlet at $\delta = 8.2$, a double doublet at $\delta = 7.9$ (^3J(H,H) = 8.3 Hz, ^4J(H,H) = 2.0 Hz), and a doublet at $\delta = 6.9$ (^3J(H,H) = 8.3–8.6 Hz) for the hydrogen atoms on the aromatic ring of fluorescein that is directly connected to the thiourea unit.[14] The triesters **10** were hydrolyzed with KOH (1N) in refluxing methanol. From the ^{1}H NMR spectra it was evident that the ethyl groups were cleaved off, and the FAB mass spectra show peaks at $m/z = 1368.6$ for **11a** and $m/z = 1325.1$ for **11b**, corresponding to $[(M+\mathrm{H})^+]$ (1368.6 and 1325.5, respectively). The triacids were deprotonated with triethylamine in THF and complexed with one equivalent of the appropriate lanthanoid nitrate. The complex precipitated from the reaction mixture only after addition of four equivalents of triethylamine, whereas the solution remained clear after the addition of three equivalents. This implies that the acid at the fluorescein is also deprotonated in the precipitate, with Et_3NH^+ as the counterion. The complexes were isolated by centrifugation and subsequently washed with THF. The complete deprotonation to give carboxylate groups was also evident from the IR spectra, which showed vibrations at 1581–1597 cm^{-1} for the carboxylate groups. The C=O stretch vibrations of the triacids **11** absorb at 1754–1762 cm^{-1}. No satisfactory mass spectra could be obtained for **1**·**Nd**$^{3+}$, although the typical Nd^{3+} isotope pattern was clearly observed in the fragments. MALDI-TOF mass spectra of **1**·**Er**$^{3+}$ show peaks at 1526 $[(\mathbf{1a}\cdot\mathbf{Er}^{3+}-\mathrm{CO}+\mathrm{Na})^+]$, 1503 $[(\mathbf{1a}\cdot\mathbf{Er}^{3+}-\mathrm{CO})^+]$, and 1446 $[(\mathbf{1a}\cdot\mathbf{Er}^{3+}-\mathrm{CO}-\mathrm{CH_2COO})^+]$ for **1a**·**Er**$^{3+}$, and at 1483 $[(\mathbf{1b}\cdot\mathbf{Er}^{3+}-\mathrm{CO}+\mathrm{Na})^+]$, 1460 $[(\mathbf{1b}\cdot\mathbf{Er}^{3+}-\mathrm{CO})^+]$, 1428 $[(\mathbf{1b}\cdot\mathbf{Er}^{3+}-\mathrm{CO}-\mathrm{CH_2COO}+\mathrm{Na})^+]$, and 1405 $[(\mathbf{1b}\cdot\mathbf{Er}^{3+}-\mathrm{CO}-\mathrm{CH_2COO})^+]$ for **1b**·**Er**$^{3+}$. All peaks clearly have the characteristic isotope pattern of erbium. The loss of a CH_2COO fragment was also observed for terphenyl-based ligands.[1] In the FAB mass spectra of the preceding triacids **11** the $[(M-\mathrm{CO})^+]$ peaks were also observed.

The synthesis of calix[4]arene triamide **2** bearing a fluorescein moiety as sensitizer was performed via precursor **12** (Scheme 2), the synthesis of which was described in a previous paper.[15] The isothiocyanate-functionalized fluorescein **9** was added to calix[4]arene **12** in THF at room temperature. The product was obtained in 93% yield and was pure according to elemental analysis. Formation of the product was evident from FAB mass spectrometry, which showed peaks at $m/z = 1572.9$ and 1556.9 that can be attributed to $[(M+\mathrm{H}+\mathrm{K})^+]$ and $[(M+\mathrm{H}+\mathrm{Na})^+]$, respectively. Moreover, in the IR spectrum bands are present at 3429 cm^{-1} for the N–H stretch vibration, and at 1633 cm^{-1} for the C=S stretch vibration. The C=O stretch vibrations were observed at 1761 and 1669 cm^{-1} for the fluorescein moiety and the amide groups, respectively. Furthermore, the ^{1}H NMR spectrum shows a singlet at $\delta = 8.22$ and two doublets at $\delta = 7.73$ and 6.93 (^3J(H,H) = 8.2 Hz) for the hydrogen atoms at the fluorescein ring directly connected to the thiourea spacer. The triamide **2** was dissolved in a mixture of acetonitrile and methanol (v:v ≈ 3:1) and one equivalent of $Nd(NO_3)_3\cdot 5H_2O$ in acetonitrile was added. After refluxing overnight the reaction mixture remained clear and the photophysical studies were performed in situ.



Scheme 2. The synthesis of calix[4]arene triamide **2** bearing a fluorescein moiety as sensitizer.

Photophysical studies: Since Nd^{3+} and Er^{3+} have luminescence bands in the near-infrared (near-IR) part of the spectrum, at 1.3 μm and 1.5 μm, respectively, that is, just in the two wavelength regions relevant for optical telecommunication, these lanthanoid ions were used for complexation. Photophysical studies were performed on these complexes in [D$_4$]methanol and in DMSO. The efficiency of energy transfer from the fluorescein moiety to the lanthanoid ions was studied relative to the calix[4]arene moiety, which itself also acts as a sensitizer as is evidenced by the excitation spectra of calix[4]arene-based Eu^{3+} complexes.[6] Direct excitation of Nd^{3+} at 800 nm was not performed, whereas after direct excitation of Er^{3+} at 488 nm it is not possible to discriminate between direct and sensitized emission due to the significant absorption of fluorescein at this wavelength.

Neodymium: The typical Nd^{3+} luminescence consists of a relatively intense emission band at 1060 nm and weaker ones at 880 and at 1350 nm (corresponding to the ${}^4F_{3/2}-{}^4I_{11/2}$, ${}^4F_{3/2}-{}^4I_{9/2}$, and ${}^4F_{3/2}-{}^4I_{13/2}$ transition, respectively). The excitation spectra of 5×10^{-5} M solutions of **1a** · **Nd^{3+}** and **1b** · **Nd^{3+}** in [D$_4$]methanol[16] and DMSO, detected at 1060 nm, are depicted in Figure 1a. The excitation band around 500 nm proves the occurrence of sensitized emission through the fluorescein moiety. The Nd^{3+} emission at 1060 nm is a result of the excitation of fluorescein, followed by energy transfer to Nd^{3+}. In DMSO solutions the excitation band is broader, allowing more red-shifted excitation.[17]

Figure 1b shows the emission spectra of **1a** · **Nd^{3+}** and **1b** · **Nd^{3+}** in DMSO, which are normalized for the absorption coefficient and the lamp intensity at the excitation wavelength. The spectra illustrate the significant increase in luminescence intensity upon excitation via the fluorescein moiety relative to excitation via the calix[4]arene for both complexes. In addition, the calix[4]arene itself also acts as a sensitizer. The same effects were observed in [D$_4$]methanol (data not shown). The intensity increase upon excitation of fluorescein is more pronounced for the C$_6$ spacer (approximately seven times higher, compared with a twofold increase for the C$_3$ spacer). However, the highest luminescence intensity is obtained for **1b** · **Nd^{3+}**, which might be due to a difference in the energy transfer efficiency in **1b** · **Nd^{3+}** and **1a** · **Nd^{3+}**. Figure 2a, in which the relative intensities of sensitized emission for the complexes with the C$_6$ and C$_3$ spacer are depicted, also indicates the more intense luminescence in case of the shorter spacer.

The luminescence lifetimes of **1a** · **Nd^{3+}** and **1b** · **Nd^{3+}** in [D$_4$]methanol and DMSO solutions were determined after laser excitation at 337 nm (Table 1). The lifetimes of both complexes are equal within the experimental error, and are longer in DMSO than in [D$_4$]methanol (1.23–1.26 µs and 0.80–0.86 µs, respectively). This implies that the deactivation by the high-energy vibrational modes of both the surrounding organic ligand and the solvent is similar for **1a** · **Nd^{3+}** and **1b** · **Nd^{3+}** and, therefore, cannot be the cause of the differences in luminescence intensities. On the other hand, the lifetimes are significantly longer in DMSO; this might be the result of conformational changes upon changing the solvent, rendering deactivation by the organic ligand less efficient in DMSO, or by shielding of the lanthanoid ion from strongly quenching O–H groups by complexing DMSO molecules. Moreover, deactivation by [D$_4$]methanol might be relatively efficient because the first overtone of the O–D vibration ($\tilde{\nu}_{O\text{-}D}\approx 2400\,cm^{-1}$) is more or less resonant with the energy gap between the luminescent excited state and a lower-lying state of Nd^{3+} ($\Delta E_{Nd^{3+}}\approx 5300\,cm^{-1}$).[18]

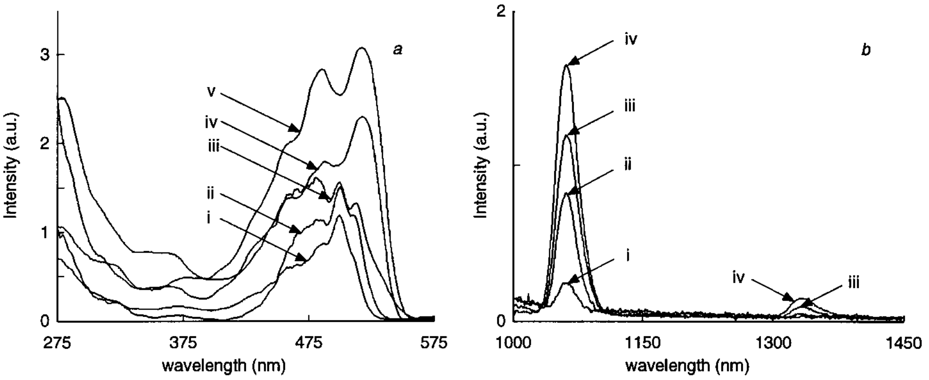


Figure 1. a) Excitation spectra of i) **1a** · **Nd^{3+}** in [D$_4$]methanol, ii) **1b** · **Nd^{3+}** in [D$_4$]methanol, iii) **2** · **Nd^{3+}** in acetonitrile, iv) **1a** · **Nd^{3+}** in DMSO, and v) **1b** · **Nd^{3+}** in DMSO, detected at 1060 nm; b) emission spectra of DMSO solutions of i) **1a** · **Nd^{3+}** excited at 310 nm, ii) **1b** · **Nd^{3+}** excited at 310 nm, iii) **1a** · **Nd^{3+}** excited at 515 nm, and iv) **1b** · **Nd^{3+}** excited at 515 nm.

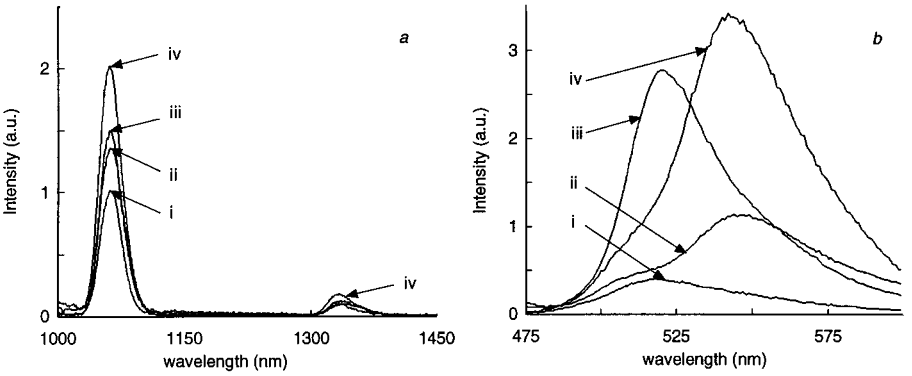


Figure 2. a) Emission spectra of i) **1a** · **Nd^{3+}** in [D$_4$]methanol, ii) **1b** · **Nd^{3+}** in [D$_4$]methanol, iii) **1a** · **Nd^{3+}** in DMSO, and iv) **1b** · **Nd^{3+}** in DMSO, excited at 500 nm and b) fluorescence spectra of i) **1b** · **Nd^{3+}** in [D$_4$]methanol, ii) **1b** · **Nd^{3+}** in DMSO, iii) **1a** · **Nd^{3+}** in [D$_4$]methanol, and iv) **1a** · **Nd^{3+}** in DMSO, excited at 300 or 310 nm.

Furthermore, the more intense luminescence of **1b** · **Nd^{3+}** is corroborated by the less intense fluorescence observed for fluorescein for **1b** · **Nd^{3+}** relative to **1a** · **Nd^{3+}** in both [D$_4$]methanol and DMSO solutions (Figure 2b). The reduction in fluorescein intensity is most likely caused by an increase of the singlet-to-triplet intersystem crossing induced by the lanthanoid ions (a heavy

Table 1. Lifetimes (in μs) of Nd^{3+} and Er^{3+} complexes of **1a** and **1b** after excitation at 337 nm.[a]

	1a·**Nd**$^{3+}$	**1b**·**Nd**$^{3+}$	**1a**·**Er**$^{3+}$	**1b**·**Er**$^{3+}$
CD_3OD	0.86	0.80	0.76	0.88
DMSO	1.23	1.26	1.71	1.63

[a] Curve fitting was performed only for the decay part of the luminescence; the lifetime of **1b**·**Er**$^{3+}$ in DMF is 0.63 μs (error approximately 10%).

atom effect), which indicates that the sensitizer would be closer to and more efficient in exciting the lanthanoid ion in **1b**·**Nd**$^{3+}$ relative to **1a**·**Nd**$^{3+}$. The decrease in fluorescence intensity in going from the C_6 to the C_3 spacer is not proportional to the increase in Nd^{3+} emission. This might be the result of a difference in energy transfer efficiency, or in the population and/or lifetime of the triplet level. The fluorescence maximum is red-shifted in DMSO to 550 nm, compared with 525 nm in [D$_4$]methanol, which indicates the solvent-dependent positions of the energy levels of fluorescein. Since the quantum yield of the triplet state of fluorescein is very low ($\phi = 0.02$), it might be possible that the singlet state is involved in the energy transfer process, either by reabsorption[19] or by dipolar interactions. However, it has been observed that the energy transfer from fluorescein to a Nd^{3+} ion that is complexed by a polyaminocarboxylate to which the fluorescein moiety is covalently attached is significantly deactivated by $^3O_2$.[20] This observation suggests that the energy transfer from fluorescein to lanthanoid ions most probably occurs via the triplet state.[6] Also, the reduction of the fluorescence of fluorescein in the presence of the lanthanoid ion suggests that the triplet state may be involved in the energy transfer process. It is likely that the quantum yield of the triplet state of fluorescein is much larger in the complex compared to free fluorescein. The luminescence of the Nd^{3+} complexes **1a**·**Nd**$^{3+}$ and **1b**·**Nd**$^{3+}$ is not deactivated by $^3O_2$, as is demonstrated by the absence of the $^1O_2$ emission band at 1275 nm and the equal lifetimes of **1a**·**Nd**$^{3+}$ and **1b**·**Nd**$^{3+}$ in aerated and in deaerated solutions. Therefore, it can be concluded that the energy transfer from fluorescein to Nd^{3+} must be very fast, since the rate of energy transfer to $^3O_2$ ($\approx 10^{10}$ dm^3 mol^{-2} s^{-1}) is limited by diffusion.

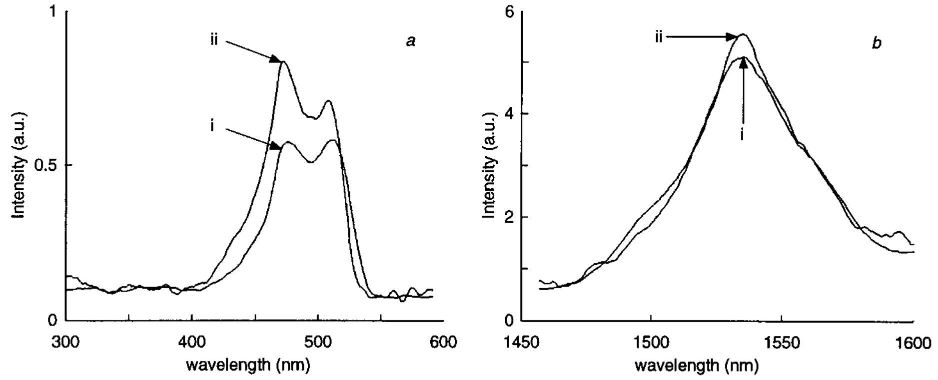


Figure 4. a) Excitation spectra detected at 1535 nm and b) emission spectra after excitation at 515 nm, both of DMSO solutions of i) **1a**·**Er**$^{3+}$ and ii) **1b**·**Er**$^{3+}$.

The increase in luminescence intensity upon excitation of fluorescein was also observed for a solution of **2**·**Nd**$^{3+}$ in acetonitrile. The excitation spectrum shows a similar excitation band at 500 nm, as was observed for **1a**·**Nd**$^{3+}$ and **1b**·**Nd**$^{3+}$ (Figure 1a), and a large increase in the Nd^{3+} emission intensity was observed upon excitation in this band (Figure 3). The luminescence intensity after excitation at 500 nm was only slightly affected by the addition of triethylamine to the solution, even in the case of a large excess of triethylamine. Since only the mono- and dianions of fluorescein are fluorescent,[21] this implies that the degree of deprotonation of fluorescein during the complexation is sufficient to obtain a highly fluorescent organic ligand. The lifetime of **2**·**Nd**$^{3+}$ in acetonitrile was determined as 0.73 μs after laser excitation at 337 nm. No $^1O_2$ emission was observed around 1300 nm, giving strong evidence that also in this complex the energy transfer is fast and *intramolecular*.

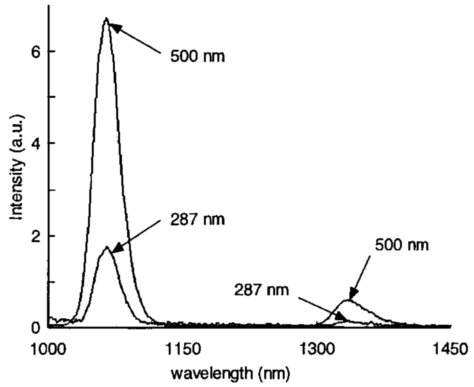


Figure 3. Emission spectra of **2**·**Nd**$^{3+}$ in acetonitrile after excitation at 287 nm and 500 nm.

Erbium: The same photophysical studies were performed for the Er^{3+} complexes of **1a** and **1b**; however, only spectra of 5×10^{-5} M DMSO solutions could be recorded, as these complexes are poorly soluble in [D$_4$]methanol. The excitation spectra of **1a**·**Er**$^{3+}$ and **1b**·**Er**$^{3+}$in DMSO, detected at 1535 nm, the $^4I_{13/2}-^4I_{15/2}$ transition (Figure 4a), show the excitation band at 500 nm due to sensitized emission via fluorescein. In the Er^{3+} complexes, the luminescence intensity of the organic ligand containing the shorter C_3 spacer is slightly more intense, probably resulting from a more efficient energy transfer from fluorescein to the Er^{3+} ion.

This is further illustrated by Figure 4b, which displays the relative intensities for sensitized emission for **1a**·**Er**$^{3+}$ and **1b**·**Er**$^{3+}$. The excitation via the calix[4]arene moiety at 310 nm did not lead to the typical Er^{3+} emission at 1535 nm, which is also obvious from the excitation spectra. This implies that sensitized emission occurs only via fluorescein. The fluorescence spectra of fluorescein itself are depicted in Figure 5 and show a higher intensity for **1a**·**Er**$^{3+}$ relative to

0947-6539/98/0405-0776 $ 17.50+.50/0

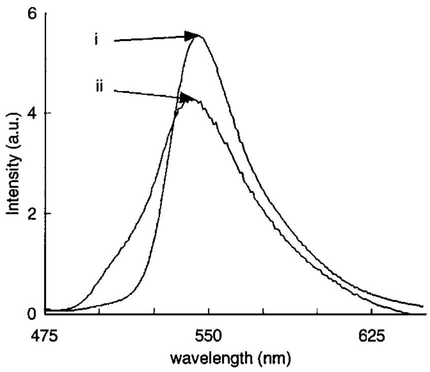


Figure 5. Fluorescence spectra of i) $\mathbf{1a}\cdot\mathbf{Er}^{3+}$ and ii) $\mathbf{1b}\cdot\mathbf{Er}^{3+}$ in DMSO after excitation at 310 nm.

$\mathbf{1b}\cdot\mathbf{Er}^{3+}$; this is in agreement with the difference in energy transfer efficiency between the two complexes as was also observed for the Nd^{3+} complexes. The broader fluorescence band of $\mathbf{1b}\cdot\mathbf{Er}^{3+}$ might be the effect of small conformational changes.

The lifetime measurements were performed for both DMSO and [D_4]methanol solutions (Table 1). The lifetimes of $\mathbf{1a}\cdot\mathbf{Er}^{3+}$ and $\mathbf{1b}\cdot\mathbf{Er}^{3+}$ are equal within experimental error, and the increase in lifetime by a factor of approximately 2 upon changing the solvent from [D_4]methanol to DMSO is even more pronounced for the Er^{3+} complexes than for the Nd^{3+} complexes, indicating the solvent dependency of the composition of the first coordination sphere of the lanthanoid ion. Deactivation by $^3O_2$ was also negligible for the Er^{3+} complexes; this indicates that the energy transfer from fluorescein to Er^{3+} is also fast.

Concluding Remarks

Fluorescein that is covalently attached to a lanthanoid ion complex based on a functionalized calix[4]arene is able to act as a sensitizer for Nd^{3+} and Er^{3+}. The lanthanoid luminescence intensities are significantly increased upon excitation via fluorescein compared to excitation via the calix[4]arene. The energy transfer is fast for both spacers on the luminescence timescale, but is more efficient in case of the longer C_6 spacer, which may be attributed to structural changes. Moreover, fluorescein can be excited in the visible part of the electromagnetic spectrum with relatively inexpensive light sources, which is an important advantage from a commercial point of view. The highest luminescence intensity was observed for the Nd^{3+} complex with the shorter C_3 spacer, probably as a result of a more effective interaction, and consequently a shorter distance, between the carboxylic acid group of the fluorescein moiety and the Nd^{3+} ion. On the other hand, the spacer length does not influence the luminescence lifetimes. The solvent has a significant effect on the luminescence properties; the luminescence lifetimes are significantly longer in DMSO solutions compared to [D_4]methanol solutions, which might be the result of the solvent-dependent position of the energy levels of fluorescein, conformational changes due to solvent complexation, or efficient luminescence quenching by the O–D modes of [D_4]methanol. The lifetimes of these complexes are relatively long for organic near-IR luminescent lanthanoid ion complexes: up to 1.23–1.26 μs for the Nd^{3+} complexes and 1.63–1.71 μs for the Er^{3+} complexes dissolved in DMSO. These results indicate that emission in the near-IR region of the spectrum is relatively easily achieved by the use of a proper sensitizer.

Experimental Section

General: Melting points were determined with a Reichert melting-point apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ unless otherwise stated, with Me_4Si as internal standard, on a Bruker AC250 spectrometer. For those ^1H NMR and ^{13}C NMR spectra that were recorded in THF, the solvent contained a drop of D_2O to exchange the protons for deuterium. Mass spectra were recorded with a Finnigan MAT 90 spectrometer with *m*-NBA (nitrobenzyl alcohol) as a matrix, unless otherwise stated. IR spectra were obtained with a Biorad 3200 or a Nicolet 5SXC FT-IR spectrophotometer. CH_2Cl_2 was distilled from $CaCl_2$ and THF from Na/benzophenone ketyl prior to use. MeOH was dried over molecular sieves (3 Å) for at least 3 days. Fluorescein isothiocyanate isomer I 90% was bought from Aldrich and purified prior to use by Sephadex LH20 column chromatography using THF/CH_2Cl_2 (v:v = 1:1) as eluent. All other chemicals were of reagent grade and were used without further purification. All reactions were carried out under an argon atmosphere, and syntheses concerning fluorescein derivatives were performed in the dark. 25-Mono[(carboxylato)methoxy]-5,11,17,23-tetrakis(1,1-dimethylethyl)-26,27,28-tris[(ethoxycarbonyl)methoxy]calix[4]arene (**5**) was prepared according to literature procedures.[11]

Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry was carried out using a PerSeptive Biosystems Voyager-DE-RP MALDI-TOF mass spectrometer.

25-{*N*-[6-(1,1-Dimethylethoxy)carbonylamino]hexyl(aminocarbonyl)methoxy}-5,11,17,-23-tetrakis(1,1-dimethylethyl)-26,27,28-tris[(ethoxycarbonyl)methoxy]calix[4]arene (**7a**): Triester monoacid **5** (1.0 g, 1.0 mmol) was refluxed in oxalyl chloride (10 mL) for 2 h. After evaporation of the remaining oxalyl chloride, the monoacid chloride was dissolved in dichloromethane (30 mL) under an argon atmosphere. A solution of *N*-Boc-1,6-hexanediamine (0.26 g, 1.0 mmol) and triethylamine (0.29 mL, 2.1 mmol) in dichloromethane (50 mL) was slowly added. The reaction mixture was stirred overnight at room temperature and subsequently quenched with an aqueous acetic acid solution (5%, 50 mL). The layers were separated and the organic layer was washed twice with water (50 mL), followed by evaporation of the solvent. Compound **7a** was obtained as white solid in 81% yield. M.p: 85–87 °C; ^1H NMR ([D_8]THF/D_2O): δ = 8.33 (br s, 1 H; NH), 6.75, 6.72, 6.71 (s, 8 H; ArH), not observed (NH^{Boc}), 4.83 and 4.56 [AB-q, $^3J(H,H) = 16.1$ Hz, 4 H; $OCH_2C(O)$], 4.69, 4.60, 3.18, 3.16 (AB-q, $^3J(H,H) = 13.1$ Hz, 8 H; $ArCH_2Ar$), 4.65, 4.46 [s, 4 H; $OCH_2C(O)$], 4.13 (q, $^3J(H,H) = 7.1$ Hz, 6 H; OCH_2CH_3), 3.4–3.2 (m, 2 H; NCH_2^{spacer}), 3.1–3.0 (m, 2 H; NCH_2^{spacer}), 1.37 [s, 9 H; $C(CH_3)_3$], 1.6–1.2 (m, 8 H; CH_2^{spacer}), 1.20 (t, $^3J(H,H) = 7.1$ Hz, 9 H; OCH_2CH_3), 1.04, 1.00, 0.98 [s, 36 H; $C(CH_3)_3$]; ^{13}C NMR ([D_8]THF/D_2O): δ = 170.5, 170.4, 170.1 [s, C(O)], 153.3, 153.0, 152.8 (s, ArC–O), 145.5, 145.3, 145.1 (s, ArC–*t*Bu), 133.4–132.6 (s, ArC), 125.7–125.3 (d, ArC–H), 74.5, 71.8, 71.3 [t, $OCH_2C(O)$], 60.7, 60.5, 60.3 (t, OCH_2CH_3), 39.3 (t, NCH_2^{spacer}), 33.9, 33.8 [s, $C(CH_3)_3$], 32.0 (t, $ArCH_2Ar$), 31.4, 31.3 [q, $C(CH_3)_3$], 29.9, 26.9, 26.6 (t, CH_2^{spacer}), 28.4 [q, $C(CH_3)_3^{Boc}$], 14.2 (q, OCH_2CH_3); MS (FAB): *m*/*z* = 1163.7 [$(M+H)^+$], 1185.6 [$(M+Na)^+$]; $C_{69}H_{98}N_2O_{13}$ (1162.7): calcd C 71.23, H 8.49, N 2.41; found C 71.10, H 8.69, N 2.51.

25-{*N*-[3-(1,1-Dimethylethoxy)carbonylamino]propyl(aminocarbonyl)methoxy}-5,11,17,-23-tetrakis(1,1-dimethylethyl)-26,27,28-tris[(ethoxycarbonyl)methoxy]calix[4]arene (**7b**): The white solid **7b** was prepared analogously to **7a** and was obtained in 79% yield from **6** (1.0 g, 1.0 mmol), oxalyl chloride (10 mL), *N*-Boc-1,3-propanediamine (0.18 g, 1.0 mmol), Et_3N (0.30 mL, 2.2 mmol), and CH_2Cl_2 (50 mL). M.p. 79–81 °C. ^1H NMR ([D_8]THF/D_2O): δ = 8.44 (br s, 1 H; NH), 6.82, 6.79, 6.74 (s, 8 H; ArH), 5.65 (br s, 1 H; NH^{Boc}), 4.91 and 4.63 [AB-q, $^3J(H,H) = 16.2$ Hz, 4 H; $OCH_2C(O)$], 4.8–4.5 (m, 4 H; $ArCH_2Ar$), 4.70, 4.55 [s, 4 H; $OCH_2C(O)$], 4.20 (q,

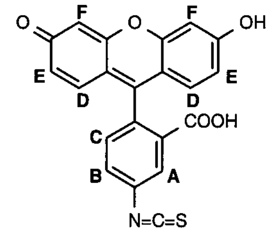
0947-6539/98/0405-0777 $ 17.50+.25/0

^3J(H,H) = 7.1 Hz, 6H; OC*H*$_2$CH$_3$), 3.5–3.4 (m, 2H; NCH$_2^{\mathrm{spacer}}$), 3.25, 3.23 (2 parts of AB-q, ^3J(H,H) = 13.1 Hz, 4H; ArCH$_2$Ar), 3.3–3.1 (m, 2H; NCH$_2^{\mathrm{spacer}}$), 1.8–1.7 (m, 2H; CH$_2^{\mathrm{spacer}}$), 1.43 [s, 9H; C(CH$_3$)$_3$], 1.4–1.2 (m, 9H; OCH$_2$C*H*$_3$), 1.11, 1.07, 1.05 [s, 36H; C(CH$_3$)$_3$]; ^{13}C NMR ([D$_8$]THF/D$_2$O): δ = 170.4, 170.3 [s, C(O)], 153.2, 152.8 (s, ArC–O), 145.7, 145.6, 145.4 (s, ArC–*t*Bu), 133.2–132.6 (s, ArC), 125.8–125.5 (d, ArC–H), 74.2, 71.8, 71.4 [t, OCH$_2$C(O)], 60.8, 60.6 (t, O*C*H$_2$CH$_3$), 37.2, 36.0 (t, NCH$_2^{\mathrm{spacer}}$), 33.9, 33.8 [s, *C*(CH$_3$)$_3$], 31.9 (t, ArCH$_2$Ar), 31.4, 31.3, 31.0 [q, C(*C*H$_3$)$_3$], 30.5 (t, CH$_2^{\mathrm{spacer}}$), 28.4 [q, C(*C*H$_3$)$_3^{\mathrm{Boc}}$], 14.2, 14.1 (q, OCH$_2$*C*H$_3$); MS (FAB): *m*/*z* = 1143.4 [(*M* + Na)$^+$], 1121.7 [(M + H)$^+$]; C$_{66}$H$_{92}$N$_2$O$_{13}$ (1120.7): calcd C 69.57, H 8.31, N 2.46; found C 69.80, H 8.11, N 2.46.

25-[*N*-(6-Amino)hexyl(aminocarbonyl)methoxy]-5,11,17,23-tetrakis(1,1-dimethylethyl)-26,27,28-tris[(ethoxycarbonyl)methoxy]calix[4]arene (8a): Compound **7a** (0.50 g, 0.43 mmol) was dissolved in CH$_2$Cl$_2$ (25 mL), and the solution was cooled to 0 °C under an argon atmosphere. Subsequently, a solution of trifluoroacetic acid (1.16 mL, 15 mmol) in CH$_2$Cl$_2$ (5 mL) was added over a period of 20 minutes. The reaction mixture was allowed to warm to room temperature and subsequently stirred for an additional 4 h. The organic solution was evaporated and the residue was stripped with toluene (3 × 50 mL). The crude product was taken up in CH$_2$Cl$_2$ and washed twice with a 5% aqueous K$_2$CO$_3$ solution (100 mL) and once with water (100 mL). After drying over MgSO$_4$ and evaporation of the solvent, a white solid was obtained in 83% yield. This product has to be stored at low temperature under an argon atmosphere. M.p. 83–84 °C. ^{1}H NMR ([D$_8$]THF/D$_2$O): δ = 8.31 (brs, 1H; NH) (not observed: NH$_2$), 6.76, 6.72, 6.67 (s, 8H; ArH), 4.83 and 4.56 [AB-q, ^3J(H,H) = 16.2 Hz, 4H; OCH$_2$C(O)], 4.69, 4.59, 3.18, 3.16 (AB-q, ^3J(H,H) = 13.0 Hz, 8H; ArCH$_2$Ar), 4.65, 4.47 [s, 4H; OCH$_2$C(O)], 4.13 (q, ^3J(H,H) = 7.1 Hz, 6H; OC*H*$_2$CH$_3$), 3.4–3.3 (m, 2H; NCH$_2^{\mathrm{spacer}}$), 2.62 (t, ^3J(H,H) = 6.7 Hz, 2H; NCH$_2^{\mathrm{spacer}}$), 1.5–1.2 (m, 8H; CH$_2^{\mathrm{spacer}}$), 1.20 [t, ^3J(H,H) = 7.1 Hz, 9H; OCH$_2$C*H*$_3$], 1.05, 1.00, 0.98 [s, 36H; C(CH$_3$)$_3$]; ^{13}C NMR ([D$_8$]THF/D$_2$O): δ = 170.4, 170.3, 170.1 [s, C(O)], 153.2, 152.9, 152.8 (s, ArC–O), 145.5, 145.3 (s, ArC–*t*Bu), 133.2–132.6 (s, ArC), 125.7–125.5 (d, ArC–H), 74.4, 71.8, 71.3 [t, O*C*H$_2$C(O)], 60.7, 60.5 (t, O*C*H$_2$CH$_3$), 42.0, 39.4 (t, NCH$_2^{\mathrm{spacer}}$), 33.8 [s, *C*(CH$_3$)$_3$], 31.9 (t, ArCH$_2$Ar), 31.3 [q, C(*C*H$_3$)$_3$], 30.0, 27.0, 26.6 (t, CH$_2^{\mathrm{spacer}}$), 14.2 (q, OCH$_2$*C*H$_3$); MS (FAB): *m*/*z* = 1063.7 [(*M* + H)$^+$], 1085.6 [(*M* + Na)$^+$]; C$_{64}$H$_{90}$N$_2$O$_{11}$ (1062.7).

25-[*N*-(3-Amino)propyl(aminocarbonyl)methoxy]-5,11,17,23-tetrakis(1,1-dimethylethyl)-26,27,28-tris[(ethoxycarbonyl)methoxy]calix[4]arene (8b): Compound **7b** (0.78 g, 0.70 mmol) was deprotected in the same way as described for **7a**, by means of trifluoroacetic acid (2.0 mL, 26 mmol) and CH$_2$Cl$_2$ (50 mL). A white solid was obtained in quantitative yield. M.p. 102–104 °C. ^{1}H NMR ([D$_8$]THF/D$_2$O): δ = 8.31 (brt, ^3J(H,H) = 6.2 Hz, 1H; NH), 6.98 (brs, 2H; NH$_2$), 6.75, 6.73, 6.66 (s, 8H; ArH), 4.85 and 4.57 [AB-q, ^3J(H,H) = 16.2 Hz, 4H; OCH$_2$C(O)], 4.68, 4.60, 3.18, 3.16 (AB-q, ^3J(H,H) = 13.1 Hz, 8H; ArCH$_2$Ar), 4.64, 4.47 [s, 4H; OCH$_2$C(O)], 4.13 (q, ^3J(H,H) = 7.1 Hz, 6H; OC*H*$_2$CH$_3$), 3.43 (q, ^3J(H,H) = 6.7 Hz, 2H; NCH$_2^{\mathrm{spacer}}$), 2.71 (t, ^3J(H,H) = 6.5 Hz, 2H; NCH$_2^{\mathrm{spacer}}$), 1.8–1.7 (m, 2H; CH$_2^{\mathrm{spacer}}$), 1.19 [t, ^3J(H,H) = 7.1 Hz, 9H; OCH$_2$C*H*$_3$], 1.04, 1.01, 0.97 [s, 36H; C(CH$_3$)$_3$]; ^{13}C NMR ([D$_8$]THF/D$_2$O): δ = 170.4 [s, C(O)], 153.2, 152.8 (s, ArC–O), 145.5, 145.3 (s, ArC–*t*Bu), 133.2–132.5 (s, ArC), 125.8–125.5 (d, ArC–H), 74.4, 71.7, 71.4 [t, O*C*H$_2$C(O)], 60.7, 60.5 (t, O*C*H$_2$CH$_3$), 39.4, 36.4 (NCH$_2^{\mathrm{spacer}}$), 33.8 [s, *C*(CH$_3$)$_3$], 32.0 (t, ArCH$_2$Ar), 31.3 [q, C(*C*H$_3$)$_3$] (not observed: CH$_2^{\mathrm{spacer}}$), 14.2 (q, OCH$_2$*C*H$_3$); MS (FAB): *m*/*z* = 1021.5 [(*M* + H)$^+$], 1043.6 [(*M* + Na)$^+$]; C$_{61}$H$_{84}$N$_2$O$_{11}$: 1020.6.

5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26,27-tris[(ethoxycarbonyl)methoxy]-28-{*N*-[6-fluorescein(thioureido)]hexyl(aminocarbonyl)methoxy}-calix[4]arene (10a): A solution of fluorescein isothiocyanate (**9**, 86 mg, 0.22 mmol) in freshly distilled THF (10 mL) was added dropwise to a solution of precursor **8a** (0.20 g, 0.19 mmol) in freshly distilled THF (15 mL) at room temperature. The reaction mixture was stirred overnight at room temperature, followed by evaporation of the solvent. The crude product was dissolved in CH$_2$Cl$_2$ and washed several times with an aqueous 1N HCl solution (20 mL, repeated until pH ≈ 3–4) and twice with water (20 mL). The product was dried over MgSO$_4$, and after evaporation of the solvent, the yellow powder was purified by Sephadex LH20 column chromatography (THF:CH$_2$Cl$_2$ = 1:1). The pure product (TLC, cellulose MeOH:CH$_2$Cl$_2$ = 1:9) was obtained in 88% yield. M.p. 155–157 °C. ^{1}H NMR ([D$_8$]THF/D$_2$O): δ = 8.17 (s, 1H; H$_A^{\mathrm{fluor}}$), 7.91 (dd, ^3J(H,H) = 8.5 Hz, ^4J(H,H) = 2.0 Hz, 1H; H$_B^{\mathrm{fluor}}$), 6.89 (d, ^3J(H,H) = 8.5 Hz, 1H; H$_C^{\mathrm{fluor}}$), 6.83, 6.77, 6.73 (s, 8H; ArH), 6.54 (d, ^3J(H,H) = 8.6 Hz, 2H; H$_D^{\mathrm{fluor}}$), 6.50 (d, ^3J(H,H) = 2.3 Hz, 2H; H$_F^{\mathrm{fluor}}$), 6.40 (dd, ^3J(H,H) = 8.6 Hz, ^4J(H,H) = 2.3 Hz, 2H; H$_E^{\mathrm{fluor}}$), 4.79 [part of AB-q, ^3J(H,H) = 16.8 Hz, 2H; OCH$_2$C(O)], 4.7–4.4 [m, 10H; OCH$_2$C(O) and ArCH$_2$Ar], 4.2–4.0 (m, 6H; OC*H*$_2$CH$_3$), 3.6–3.4 [m, 2H; CH$_2$NC(S)], 3.4–3.3 (m, 2H; NCH$_2^{\mathrm{spacer}}$), 3.3–3.0 (m, 4H; ArCH$_2$Ar), 1.7–1.5 and 1.4–1.3 (m, 8H; CH$_2^{\mathrm{spacer}}$), 1.15 (t, ^3J(H,H) = 7.1 Hz, 9H; OCH$_2$C*H*$_3$), 1.05, 1.00, 0.98 [s, 36H; C(CH$_3$)$_3$]; ^{13}C NMR ([D$_8$]THF/D$_2$O): δ = 183.9 [C(O)$^{\mathrm{fluor}}$], 172.4, 172.3, 170.5 [C(O)], 161.9 [C(O)$^{\mathrm{fluor}}$], 155.7, 155.5, 154.9 (ArC–O), 147.3 (ArC–*t*Bu), 144.2 (ArC–O$^{\mathrm{fluor}}$), 136.1–135.5, 131.4–125.9 (ArC), 114.4, 113.2, 104.6 (C$^{\mathrm{fluor}}$), 76.7, 74.2, 73.7 [O*C*H$_2$C(O)], 62.7, 62.4 (O*C*H$_2$CH$_3$), 46.1, 40.9 (NCH$_2^{\mathrm{spacer}}$), 36.0, 35.9 [*C*(CH$_3$)$_3$], 34.1 (ArCH$_2$Ar), 33.7, 33.2 (CH$_2^{\mathrm{spacer}}$), 33.2 [C(*C*H$_3$)$_3$], 32.0, 30.7, 30.1 (CH$_2^{\mathrm{spacer}}$), 16.0 (q, OCH$_2$*C*H$_3$); IR (KBr): $\tilde{\nu}$ = 3357 [NH(C=S)], 1758 (C=O), 1611 (C=S) cm^{-1}; MS (FAB): *m*/*z* = 1475.2 [(*M* + Na + H)$^+$], 1452.9 [(*M* + H)$^+$]; C$_{85}$H$_{101}$N$_3$O$_{16}$S · MeOH: calcd C 69.57, H 7.13, N 2.83; found C 69.65, H 7.36, N 2.71.



9

5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26,27-tris[(ethoxycarbonyl)methoxy]-28-{*N*-[3-fluorescein(thioureido)]propyl(aminocarbonyl)methoxy}-calix[4]arene (10b): The reaction of **8b** with fluorescein isothiocyanate (**9**) occurred as described for **10a**, using **8b** (0.22 g, 0.22 mmol), **9** (99 mg, 0.25 mmol), and freshly distilled THF (25 mL). An orange solid was obtained in 70% yield. M.p. 196–198 °C. ^{1}H NMR ([D$_8$]THF/D$_2$O): δ = 8.18 (s, 1H; H$_A^{\mathrm{fluor}}$), 7.89 (dd, ^3J(H,H) = 8.3 Hz, ^4J(H,H) = 2.0 Hz, 1 H; H$_B^{\mathrm{fluor}}$), 6.90 (d, ^3J(H,H) = 8.3 Hz, 1H; H$_C^{\mathrm{fluor}}$), 6.81, 6.76, 6.73 (s, 8 H; ArH), 6.54 (d, ^3J(H,H) = 2.3 Hz, 2H; H$_F^{\mathrm{fluor}}$), 6.52 (d, ^3J(H,H) = 8.7 Hz, 2H; H$_D^{\mathrm{fluor}}$), 6.40 (dd, ^3J(H,H) = 8.7 Hz, ^4J(H,H) = 2.3 Hz, 2H; H$_E^{\mathrm{fluor}}$), 4.83 [part of AB-q, ^3J(H,H) = 16.1 Hz, 2H; OCH$_2$C(O)], 4.7–4.4 [m, 10H; OCH$_2$C(O) and ArCH$_2$Ar], 4.1–4.0 (m, 6H; OC*H*$_2$CH$_3$), 3.6–3.5 [m, 2H; CH$_2$NC(S)], 3.4–3.3 (m, 2H; NCH$_2^{\mathrm{spacer}}$), 3.3–3.0 (m, 4H; ArCH$_2$Ar), 1.9–1.8 (m, 2H; CH$_2^{\mathrm{spacer}}$), 1.8–1.6 (m, 9H; OCH$_2$C*H*$_3$), 1.04, 0.99, 0.97 [s, 36H; C(CH$_3$)$_3$]; ^{13}C NMR ([D$_8$]THF/D$_2$O): δ = [not observed: C(O)$^{\mathrm{fluor}}$] 173.2, 172.5, 170.9 [C(O)], 162.1 [C(O)$^{\mathrm{fluor}}$], 155.4, 154.9 (ArC–O), 147.4 (ArC–*t*Bu), 144.3 (ArC–O$^{\mathrm{fluor}}$), 135.9–135.3, 131.1–125.7 (ArC), 114.4, 112.9, 104.5 (C$^{\mathrm{fluor}}$), 76.4, 74.2, 73.6 [O*C*H$_2$C(O)], 63.0, 62.7 (O*C*H$_2$CH$_3$) (not observed: NCH$_2^{\mathrm{spacer}}$), 35.9 [*C*(CH$_3$)$_3$], 34.0 (ArCH$_2$Ar), 33.3, 33.2 [C(*C*H$_3$)$_3$], 32.0 (CH$_2^{\mathrm{spacer}}$), 16.0 (q, OCH$_2$*C*H$_3$); IR (KBr): $\tilde{\nu}$ = 3364 [NH(C=S)], 1754 (C=O), 1612 (C=S) cm^{-1}; MS (FAB): *m*/*z* = 1432.7 [(*M* + Na + H)$^+$], 1408.4 ([*M*$^-$]); C$_{82}$H$_{94}$N$_3$O$_{16}$S (1408.6) · 2 MeOH: calcd C 68.45, H 6.98, N 2.85; found C 68.10, H 6.97, N 3.01.

5,11,17,23-Tetrakis(1,1-dimethylethyl)-25-{*N*-[6-fluorescein(thioureido)]-hexyl(amino-carbonyl)methoxy}-26,27,28-tris[(hydroxycarbonyl)methoxy]calix[4]arene (11a): A KOH solution (1N, 1 mL) was added to a solution of triester **10a** (80 mg, 55 µmol) in methanol (10 mL). The reaction mixture was heated to reflux, stirred for 1 h, and cooled to room temperature. Subsequently, the solvent was evaporated and the residue was dissolved in CH$_2$Cl$_2$ (25 mL). The crude product was washed twice with an aqueous HCl solution (1N) and dried over MgSO$_4$. A yellow solid was obtained after evaporation of the solvent in 90% yield. M.p. > 300 °C (decomp.). ^{1}H NMR ([D$_8$]THF/D$_2$O): δ = 8.15 (s, 1H; H$_A^{\mathrm{fluor}}$), 7.92 (dd, ^3J(H,H) = 8.3 Hz, ^4J(H,H) = 2.0 Hz, 1H; H$_B^{\mathrm{fluor}}$), 7.02 (s, 4H; ArH), 6.89 (d, ^3J(H,H) = 8.3 Hz, 1H; H$_C^{\mathrm{fluor}}$), 6.73, 6.60 (s, 4H; ArH), 6.54 (d, ^3J(H,H) = 2.3 Hz, 2H; H$_F^{\mathrm{fluor}}$), 6.6–6.5 (m, 2H; H$_D^{\mathrm{fluor}}$), 6.40 (dd, ^3J(H,H) = 8.7 Hz, ^4J(H,H) = 2.3 Hz, 2H; H$_E^{\mathrm{fluor}}$), 4.9–4.8 [m, 2H; OCH$_2$C(O)], 4.7–4.5 [m, 8H; OCH$_2$C(O) and ArCH$_2$Ar], 4.18 [s, 2H; OCH$_2$C(O)], 3.6–3.5 [m, 8H; CH$_2$NC(S)], 3.4–3.3 (m, 2H; NCH$_2^{\mathrm{spacer}}$), 3.3–3.1 (m, 4H; ArCH$_2$Ar), 1.7–1.6 and 1.5–1.4 (m, 8H; CH$_2^{\mathrm{spacer}}$), 1.14, 0.91, 0.81 [s, 36H; C(CH$_3$)$_3$]; ^{13}C NMR ([D$_8$]THF/D$_2$O): δ = 183.9 [C(O)$^{\mathrm{fluor}}$], 173.0–170.5 [C(O)], 161.9 [C(O)$^{\mathrm{fluor}}$], 155.4–153.5 (ArC–O), 148.5–147.5 (ArC–*t*Bu), 144.1 (ArC–O$^{\mathrm{fluor}}$), 137.2–134.6, 131.4–125.9 (ArC), 114.4, 113.2, 104.6 (C$^{\mathrm{fluor}}$), 77.3, 74.5, 74.2 [O*C*H$_2$C(O)], 46.4, 40.9 (NCH$_2^{\mathrm{spacer}}$), 36.2, 35.9 [*C*(CH$_3$)$_3$], 33.4, 33.1 [C(*C*H$_3$)$_3$], 33.2 (ArCH$_2$Ar), 32.8–28.5 (CH$_2^{\mathrm{spacer}}$); IR (KBr): $\tilde{\nu}$ = 3380 [NH(C=S)], 1762 (C=O), 1610 (C=S) cm^{-1}; MS (FAB): *m*/*z* = 1368.6 [(*M* + H)$^+$], 1337.6 [(*M* − CO)$^+$], 1367.1 [*M*$^-$]; C$_{79}$H$_{89}$N$_3$O$_{16}$S (1368.6) · 3H$_2$O · CH$_2$Cl$_2$: calcd C 63.73, H 6.48, N 2.79; found C 63.44, H 6.68, N 2.54.

5,11,17,23-Tetrakis(1,1-dimethylethyl)-25-{*N*-[1-fluorescein(thioureido)]-propyl(amino-carbonyl)methoxy}-26,27,28-tris[(hydroxycarbonyl)methoxy]calix[4]arene (11b): The triester **10b** (0.14 g, 0.099 mmol) was hydrolyzed in the same way as described for **11a**, with a KOH methanol solution (1N, 2 mL) and methanol (20 mL). An orange solid was obtained in 70% yield. M.p. > 300 °C. ^{1}H NMR ([D_8]THF/D_2O): $\delta = 8.21$ (s, 1H; $\mathrm{H_A^{fluor}}$), 7.91 (d, ^3J(H,H) = 8.3 Hz, 1H; $\mathrm{H_B^{fluor}}$), 7.01 (s, 4H; ArH), 6.89 (d, ^3J(H,H) = 8.3 Hz, 1H; $\mathrm{H_C^{fluor}}$), 6.73, 6.63 (s, 4H; ArH), 6.54 (d, ^3J(H,H) = 2.3 Hz, 2 H; $\mathrm{H_F^{fluor}}$), 6.6–6.5 (m, 2 H; $\mathrm{H_D^{fluor}}$), 6.41 (dd, ^3J(H,H) = 8.7 Hz, ^4J(H,H) = 2.3 Hz, 2H; $\mathrm{H_E^{fluor}}$), 4.72 [part of AB-q, ^3J(H,H) = 15.6 Hz, 2H; $OCH_2C(O)$], 4.7–4.4 [m, 6H; $OCH_2C(O)$ and $ArCH_2Ar$], 4.52, 4.26 [s, 4H; $OCH_2C(O)$], 3.7–3.5 [m, 2H; $CH_2NC(S)$], 3.5–3.4 (m, 2 H; $\mathrm{NCH_2^{spacer}}$), 3.2–3.0 (m, 4H; $ArCH_2Ar$), 1.9–1.8 (m, 2H; $\mathrm{CH_2^{spacer}}$), 1.13, 0.91, 0.83 [s, 36H; $C(CH_3)_3$]; ^{13}C NMR ([D_8]THF/D_2O): $\delta = 183.6$ [$\mathrm{C(O)^{fluor}}$], 173.3–171.0 [C(O)], 162.2 [$\mathrm{C(O)^{fluor}}$], 155.3–153.8 (ArC–O), 148.4–147.6 (ArC–*t*Bu), 144.3 (ArC–$\mathrm{O^{fluor}}$), 136.9–134.8, 131.3–125.7 (ArC), 114.4, 113.1, 112.8, 104.5 ($\mathrm{C^{fluor}}$), 77.1, 74.4, 74.2 [$OCH_2C(O)$], 43.3, 38.5 ($\mathrm{NCH_2^{spacer}}$), 36.1, 35.9 [$C(CH_3)_3$], 33.3, 33.1 [$C(CH_3)_3$], 33.1 ($ArCH_2Ar$), 31.9 ($\mathrm{CH_2^{spacer}}$); IR (KBr): $\tilde{\nu} = 3398$ [NH(C=S)], 1754 (C=O), 1611 (C=S) cm^{-1}; MS (FAB): $m/z = 1325.1$ [$(M+\mathrm{H})^+$], 1349.2 [$(M+\mathrm{Na}+\mathrm{H})^+$], 1295.0 [$(M-\mathrm{CO})^+$]; $C_{76}H_{82}N_3O_{16}S$ (1324.5) · 3MeOH: calcd C 66.74, H 6.66, N 2.96; found C 66.93, H 6.80, N 2.95.

General procedure for the complexation of $\mathbf{Ln^{3+}}$: The precursor **11** (22 μmol) was dissolved in THF (3 mL), after which Et_3N (4 equiv) was added with a microsyringe. Subsequently, a solution of $Ln(NO_3)_3 \cdot nH_2O$ (1.2 equiv) in a minimal amount of THF was added and the reaction mixture was stirred for 2 h at room temperature. The tiny particles formed were collected by means of centrifugation and washed twice with THF. The complex was obtained in quantitative yield as a slightly orange powder. Analytical data are reported in Table 2.

25,26,27-Tris[(*N,N*-diethylaminocarbonyl)methoxy]-5,11,17,23-tetrakis(1,1-dimethyl-ethyl)-28-{*N*-[6-fluorescein(thioureido)]hexyl(aminocarbonyl)methoxy}calix[4]arene (2): The reaction of **16** with **9** was performed in the same way as described for the synthesis of **10**. An orange solid was obtained in 93% yield. M.p. > 300 °C. ^{1}H NMR ([D_4]methanol): $\delta = 8.22$ (s, 1H; $\mathrm{H_A^{fluor}}$), 7.73 (d, ^3J(H,H) = 8.2 Hz, 1H; $\mathrm{H_B^{fluor}}$), 7.34 (m, 8H; ArH), 6.93 (d, ^3J(H,H) = 8.2 Hz, 1H; $\mathrm{H_C^{fluor}}$), 6.62 (d, ^3J(H,H) = 2.3 Hz, 2H; $\mathrm{H_F^{fluor}}$), 6.56 (d, ^3J(H,H) = 8.7 Hz, 2H; $\mathrm{H_D^{fluor}}$), 6.46 (dd, ^3J(H,H) = 8.7 Hz, ^4J(H,H) = 2.3 Hz, 2H; $\mathrm{H_E^{fluor}}$), 4.79, 4.61 [brs, 8H; $OCH_2C(O)$], 4.11 (part of AB-q, ^3J(H,H) = 12.4 Hz, 4H; $ArCH_2Ar$), 3.6–3.5 [m, 10H; $ArCH_2Ar$ and NCH_2CH_3], 3.4–3.1 [m, 10H; NCH_2CH_3 and $CH_2NC(S)$ and $\mathrm{NCH_2^{spacer}}$], 1.7–1.6, 1.5–1.4 (m, 8H; $\mathrm{CH_2^{spacer}}$), 1.3–1.2 (m, 9H; NCH_2CH_3), 1.2–1.0 [m, 45H; NCH_2CH_3 and $C(CH_3)_3$]; ^{13}C NMR ([D_4]methanol): $\delta = 182.4$ [$\mathrm{C(O)^{fluor}}$], 173.0, 171.5, 171.4 [C(O)], 162.3 [$\mathrm{C(O)^{fluor}}$], 154.5, 151.5, 150.5, 150.4 (ArC–O and ArC–*t*Bu), 142.9 (ArC–$\mathrm{O^{fluor}}$), 136.2, 136.0, 130.5–125.9 (ArC), 114.4, 111.9, 103.7 ($\mathrm{C^{fluor}}$), 78.0, 73.5 [$OCH_2C(O)$], 45.1, 42.8, 42.6, 41.1 (NCH_2CH_3 and $\mathrm{NCH_2^{spacer}}$), 35.5 [$C(CH_3)_3$] (not observed: $ArCH_2Ar$), 31.7 [$C(CH_3)_3$], 31.2–27.6 ($\mathrm{CH_2^{spacer}}$), 14.1, 13.5 (q, OCH_2CH_3); IR (KBr): $\tilde{\nu} = 3429$ [NH(C=S)], 1761 (C=$\mathrm{O^{fluor}}$), 1669 (C=$\mathrm{O^{amide}}$), 1633 (C=S) cm^{-1}; MS (FAB): $m/z = 1572.9$ [$(M+\mathrm{K}+\mathrm{H})^+$], 1556.9 [$(M+\mathrm{Na}+\mathrm{H})^+$]; $C_{91}H_{116}N_6O_{13}S$ (1532.9) · 4CH_2Cl_2: calcd C 60.90, H 6.67, N 4.49; found C 61.36, H 6.42, N 4.62.

Photophysical studies: Steady-state luminescence measurements were performed with a PTI (Photon Technology International) Alphascan spectrofluorimeter. For excitation a 75 W quartz–tungsten–halogen lamp followed by a SPEX 1680 double monochromator was used. A PTI 0.25 m single monochromator was used for separation of the emitted light that was detected at an angle of 90° from the excitation light. The signal from a Hamamatsu R928 photomultiplier was fed to a photon-counting interface. For time-resolved luminescence measurements, an Edinburgh Analytical Instruments LP900 system was used, which consisted of a pulsed Xe–lamp (μs-pulsed output) followed by a 0.25 m monochromator for excitation, and another 0.25 m monochromator, used for the separation of light, positioned at an angle of 90° with respect to the first one. The luminescence lifetimes were determined with a LTB MSG 400 nitrogen laser, with pulse duration of 500 ps at a wavelength of 337 nm. The photons were transformed to electric signals by means of a NorthCoast EO817P liquid nitrogen cooled germanium detector, and fed to a Tektronix fast digital oscilloscope. The maximum of the luminescence decay curve was significantly shifted with respect to the detector signal; therefore, the luminescence lifetimes were determined only for the decay part of the luminescence. Because of the sensitivity of the luminescence lifetimes and intensities to the water content of the solutions, DMSO, methanol, and acetonitrile were dried over molecular sieves (3 Å) prior to use and the lifetimes and luminescence spectra were recorded with freshly prepared samples. The spectra that are used to compare the efficiency of energy transfer from the fluorescein moiety to the lanthanoid ion are all corrected for the absorption at the excitation wavelength, lamp intensity, concentration, and baseline.

Acknowledgments: We gratefully thank R. Fokkens and N. Nibbering (University of Amsterdam, The Netherlands) for recording the MALDI-TOF spectra.

Received: October 8, 1997 [F848]

---

[1] a) M. P. Oude Wolbers, F. C. J. M. van Veggel, B. H. M. Snellink-Ruël, J. W. Hofstraat, F. A. J. Geurts, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1997**, *119*, 138–144; b) M. P. Oude Wolbers, F. C. J. M. van Veggel, J. W. Hofstraat, F. A. J. Geurts, D. N. Reinhoudt *J. Chem. Soc. Perkin Trans. 2* **1997**, 2275–2282; c) M. P. Oude Wolbers, F. C. J. M. van Veggel, B. H. M. Snellink-Ruël, J. W. Hofstraat, F. A. J. Geurts, D. N. Reinhoudt, *Perkin Trans. 2, 19*, **1998**, in press.

[2] R. J. Mears, S. R. Baker, *Opt. Quantum Electron.* **1992**, *24*, 517–538.

[3] W. De W. Horrocks, Jr., M. Albin, *Prog. Inorg. Chem.* **1984**, *31*, 1–104.

[4] M. Li, P. R. Selvin, *J. Am. Chem. Soc.* **1995**, *117*, 8132–8138.

[5] H. Takalo, I. Hemmilä, T. Sutela, M. Latva, *Helv. Chim. Acta* **1996**, *79*, 789–802.

[6] F. J. Steemers, W. Verboom, D. N. Reinhoudt, E. B. van der Tol, J. W. Verhoeven, *J. Am. Chem. Soc.* **1995**, *117*, 9408–9414.

[7] These calculations have been reported in a separate paper: L. H. Slooff, A. Polman, M. P. Oude Wolbers, F. C. J. M. van Veggel, D. N. Reinhoudt, J. W. Hofstraat, *J. Appl. Phys.* **1997**, *83*, 497–503.

[8] a) C. M. Briskina, V. V. Grigoryants, M. E. Zhabotinski, V. F. Zolin, M. A. Samokhina, *J. Chem. Phys. Tome* **1967**, *64*, 13; b) T. A. Shakhverdov, P. A. Shakhverdov, E. B. Sveshnikova, *SPIE Proceedings* **1994**, *2388*, 280; c) Y. V. Korovin, S. B. Meshkova, N. S. Poluektov, *Zh. Anal. Khim.* **1984**, *39*, 300; d) N. S. Poluektov, S. B. Meshkova, Y. V. Korovin, *Ukr. Khim. Zn.* **1987**, *53*, 844.

[9] A. Beeby, R. S. Dickins, S. Faulkner, D. Parker, J. A. G. Williams, *Chem. Commun.* **1997**, 1401–1402.

[10] W. D. Horrocks, Jr., J. P. Bolender, W. D. Smith, R. M. Supkowski, *J. Am. Chem. Soc.* **1997**, *119*, 5972–5973.

[11] F. Arnaud-Neu, E. M. Collins, M. Deasy, G. Ferguson, S. J. Harris, B. Kaitner, A. J. Lough, M. A. McKervey, E. Marques, B. L. Ruhl, M. J. Schwing-Weill, E. M. Seward, *J. Am. Chem. Soc.* **1989**, *111*, 8681-8691;

Table 2. Characterization of the lanthanoid ion complexes.

	1a · Nd^{3+}	**1b · Nd^{3+}**	**1a · Er^{3+}**	**1b · Er^{3+}**	**2 · Nd^{3+}**
m.p. [°C]	280 (decomp)	270 (decomp)	280 (decomp)	270 (decomp)	–[a]
IR [cm^{-1}]	3406, 1590	3430, 1581	3433, 1584	3438, 1597	3422, 1669,[b] 1635
MS (MALDI-TOF)	Nd^{3+} isotope pattern –[c]	Nd^{3+} isotope pattern –[c]	Er^{3+} isotope pattern 1526 [$(M-\mathrm{CO}+\mathrm{Na})^+$] 1503 [$(M-\mathrm{CO})^+$] 1446 [$(M-\mathrm{CO}-\mathrm{CH_2COO})^+$]	Er^{3+} isotope pattern 1483 [$(M-\mathrm{CO}+\mathrm{Na})^+$] 1460 [$(M-\mathrm{CO})^+$] 1428 [$(M-\mathrm{CO}-\mathrm{CH_2COO}+\mathrm{Na})^+$] 1405 [$(M-\mathrm{CO}-\mathrm{CH_2COO})^+$]	–[d]

[a] Not isolated. [b] Weak band. [c] No satisfactory data were obtained. [d] Not performed.

b) V. Böhmer, W. Vogt, S. J. Harris, R. G. Leonard, E. M. Collins, M. Deasy, M. A. McKervey, M. Owens, *J. Chem. Soc. Perkin Trans. 1* **1990**, 431 – 433.

[12] The corresponding compound from *o*-phenylenediamine has been made, but the reactivity of the aromatic amino group was too low in the subsequent step.

[13] I. Kräuter, W. Herrmann, G. Wenz, *J. Incl. Phenom. Mol. Recognit. Chem.* **1996**, *25*, 93 – 96.

[14] The triesters containing the fluorescein derivatives eosin (tetrabromofluorescein) or erythrosine (tetraiodofluorescein) instead of fluorescein were also synthesized and characterized. The quantum yields of triplet states of these sensitizers are significantly higher (0.33 and 0.83, respectively) than that of fluorescein (0.02). Moreover, the energy of the triplet states is lower, rendering more red-shifted excitation possible. However, hydrolysis of these compounds to the triacids was not successful until now.

[15] M. P. Oude Wolbers, F. C. J. M. van Veggel, R. H. M. Heeringa, J. W. Hofstraat, F. A. J. Geurts, G. J. van Hummel, S. Harkema, D. N. Reinhoudt, *Liebigs Ann./Receuil* **1997**, 2587 – 2600.

[16] The exact concentration of the [D$_4$]methanol solutions is not known, owing to the low solubility of the complexes in organic solvents.

[17] The excitation wavelengths used for the [D$_4$]methanol solutions were 287 and 500 nm. However, DMSO strongly absorbs light of 287 nm, and therefore 310 nm excitation was used for these solutions, whereas 515 nm excitation was used in the visible part of the electromagnetic spectrum because of the red-shifted excitation band.

[18] G. Stein, E. Würzberg, *J. Chem. Phys.* **1975**, *62*, 208 – 213.

[19] Since Nd^{3+} has an absorption band at 500 nm, reabsorption may occur.

[20] M. H. V. Werts, F. A. J. Geurts, J. W. Hofstraat, J. W. Verhoeven, *Chem. Phys. Lett.* **1997**, *276*, 196 – 201.

[21] R. P. Haugland, *Handbook of Fluorescent Probes and Research Chemicals*, 6th ed., Molecular Probes, Leiden, **1996**, p. 552.

0947-6539/98/0405-0780 $ 17.50+.50/0