J|A|C|S
ARTICLES
Published on Web 02/07/2002

Noncovalent Binding of Sensitizers for Lanthanide(III) Luminescence in an EDTA-bis(β-cyclodextrin) Ligand

Jasper J. Michels, Jurriaan Huskens,* and David N. Reinhoudt*

Contribution from the Laboratory of Supramolecular Chemistry and Technology, MESA+ Research Institute, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands

Received September 7, 2001. Revised Manuscript Received December 5, 2001

Abstract: EDTA-linked β-cyclodextrin dimer **3** was synthesized from EDTA bis(anhydride) **1** and mono-(propylamino)-appended β-cyclodextrin **2**. *p-tert*-Butylbenzoate **5**, bound by the β-cyclodextrin cavities of **3** with an association constant of 10^4 M^{-1} in water, acts as a sensitizer for the Eu^{III} and Tb^{III} complexes of **3**. Luminescence spectroscopy, microcalorimetry, and Gd^{III}-induced NMR relaxation rate measurements prove that **3** forms a 1:2 complex with **5** and that one of the β-cyclodextrin-bound sensitizers coordinates to the EDTA-encapsulated Ln^{III} ion. The Eu^{III} complex of **3** forms strong 1:1 complexes ($K \approx 10^7$ M^{-1}) with bis(propylamido adamantyl)-functionalized biphenyl sensitizers **7** and **8** in water. Both β-cyclodextrins of **3** are involved in the binding of these guests. The amide functionality adjacent to the biphenyl unit in **7** and **8** coordinates to the EDTA-encapsulated Ln^{III} ion. For these biphenyl-based antennae both binding to β-cyclodextrin and coordination to the Ln^{III} center are crucial for efficient sensitization.

Introduction

Most trivalent lanthanide (Ln^{III}) ions have long-lived luminescent 4f states which exhibit sharp emission bands at characteristic wavelengths ranging from green (Tb^{III}) to near-infrared light (Nd^{III}, Er^{III}). This renders Ln^{III} ions interesting for application in fluoroimmunoassays[1−4] and optical amplification.[1,5] Because of the forbidden nature of the optical intra-4f transitions, Ln^{III} ions are usually excited via organic antenna chromophores (sensitizers). This energy transfer process usually involves an electron exchange (Dexter) mechanism which requires a physical overlap of the antenna orbitals and the Ln^{III} 4f shells.[6] Hence, the effectiveness is strongly distance dependent and limits practical Ln^{III}-antenna distances to <5 Å. A common strategy is to link the chromophore covalently to a multidentate Ln^{III}-complexing ligand such as diethylenetriaminepentaacetate (DTPA),[7] tetraazacyclododecanetetraacetate (DOTA),[8] or terpyridyl[9] and terphenyl[10] derivatives. Ethylenediaminetetraacetate (EDTA) is well known to complex Ln^{III} ions strongly,[7b] but is less commonly used in combination with a covalently linked chromophore.

β-Cyclodextrin derivatives covalently functionalized with a crown ether,[11,12] DOTA,[13] or DTPA[14] moiety to complex Eu^{III} or Tb^{III} have been exploited as luminescent sensing molecules for the detection of aromatic guests. The noncovalent capture of a guest sensitizer in the β-cyclodextrin cavity enables sensitized lanthanide luminescence and, thus, detection of the guest. The guest binding of the crown ether-based systems is decreased relative to β-cyclodextrin because of an increased polarity of the β-cyclodextrin cavity due to the charge of the crown-complexed Ln^{III} ion. The DOTA and DTPA ligands effectively shield the Ln^{III} ion from water because they occupy (almost) all coordination sites. This reduces the quenching of the lanthanide luminescence by the OH vibrations of the water molecules, but also prohibits coordination of a sensitizer to the Ln^{III} center. This leads to a relatively inefficient energy transfer.[13,15]

Ideally, one would like a lanthanide ion to be well shielded but still with a few coordination sites free for coordination of a sensitizer. The smaller EDTA, in comparison to DTPA and DOTA, seems suitable for this purpose. In general, lanthanides prefer hard donor atoms such as oxygen; therefore, carboxylates

* To whom correspondence should be addressed. Phone: +31 53 4892980. Fax: +31 53 4894645. E-mail: smct@ct.utwente.nl.

(1) Sabbatini, N.; Guardigli, M.; Lehn, J.-M. *Coord. Chem. Rev.* **1990**, *123*, 201.
(2) (a) Mukkala, V.-M.; Helenius, M.; Hemmilä, I.; Kankare, J.; Takalo, H. *Helv. Chim. Acta* **1993**, *76*, 1361; (b) Takalo, H.; Hemmilä, I.; Sutela, T.; Latva, M. *Helv. Chim. Acta* **1996**, *79*, 789.
(3) Mathis, G. *Clin. Chem.* **1993**, *39*, 1953.
(4) Werts, M. H. V.; Woudenberg, R. H.; Emmerink, P. G.; van Gassel, R.; Hofstraat, J. W.; Verhoeven, J. W. *Angew. Chem., Int. Ed.* **2000**, *39*, 4542.
(5) Desurvire, E. *Phys. Today* **1994**, *97*, 20.
(6) Dexter, D. L. *J. Phys. Chem.* **1953**, *21*, 836.
(7) (a) Werts, M. H. V.; Hofstraat, J. W.; Geurts, F. A. J.; Verhoeven, J. W. *Chem. Phys. Lett.* **1997**, *276*, 196. (b) Werts, M. H. V.; Verhoeven, J. W.; Hofstraat, J. W. *J. Chem. Soc., Perkin Trans. 2* **2000**, 433. (c) Ozaki, H.; Suda, E.; Nagano, T.; Sawai, H. *Chem. Lett.* **2000**, 312.
(8) (a) Govenlock, L. J.; Mathieu, C. E.; Maupin, C. L.; Parker, D.; Riehl, J. P.; Siligardi, G.; Williams, J. A. G. *Chem. Commun.* **1999**, 1699. (b) Gunnlaugsson, T.; MacDónail, D. A.; Parker, D. *Chem. Commun.* **2000**, 93.
(9) Cooper, M. E.; Sammes, P. G. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1695.
(10) (a) Klink, S. I.; Hebbink, G. A.; Grave, L.; Peters, F. G. A.; Van Veggel, F. C. J. M.; Reinhoudt, D. N.; Hofstraat, J. W. *Eur. J. Org. Chem.* **2000**, 1923. (b) Klink, S. I.; Keizer, H.; van Veggel, F. C. J. M. *Angew. Chem., Int. Ed.* **2000**, *39*, 4319.
(11) Pikramenou, Z.; Nocera, D. G. *Inorg. Chem.* **1992**, *31*, 532.
(12) (a) Pikramenou, Z.; Johnson, K. M.; Nocera, D. G. *Tetrahedron Lett.* **1993**, *34*, 3531. (b) Pikramenou, Z.; Nocera, D. G. *Proc. Int. Symp. Cyclodextrins, 6th* **1993**, 259.
(13) Skinner, P. J.; Beeby, A.; Dickins, R. S.; Parker, D.; Aime, S.; Botta, M. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1329.
(14) Mortellaro, M. A.; Nocera, D. G. *J. Am. Chem. Soc.* **1996**, *118*, 7414.
(15) Rudzinski, C. M.; Engebretson, D. S.; Hartmann, W. K.; Nocera, D. G. *J. Phys. Chem. A* **1998**, *102*, 7442.

10.1021/ja017025y CCC: $22.00

seem appropriate as the coordinating site on the sensitizer. However, complexation of a carboxylate requires the removal of a water molecule of the first coordination sphere and is intrinsically weak, especially in the presence of another shielding ligand such as EDTA. Therefore, we designed a ligand with cyclodextrin binding sites to complex the organic sensitizer and bring it in close proximity to the Ln^{III} ion so that the increase in effective molarity may lead to efficient coordination to a vacant coordination site.

Here we report the synthesis and guest binding of an EDTA-based β-cyclodextrin dimer and its Eu^{III} and Tb^{III} complexes. The EDTA ligand provides six donor atoms and does not saturate the coordination sites on the Ln^{III} ion. Consequently, sensitizing guests having both hydrophobic binding sites for β-cyclodextrin *and* a Ln^{III}-coordinating functionality are of particular interest. Complexation of small and large sensitizers was studied. Luminescence spectroscopy, microcalorimetry, and Gd^{III}-induced ^{13}C relaxation rate enhancement measurements were performed to study the complexes and to probe possible coordination to the Ln^{III} center. Such a ditopic mode of binding[16] is especially the subject of investigation as this may lead to the development of cyclodextrin systems with both strong binding and efficient energy transfer. Furthermore, covalent and noncovalent combinations of lanthanide complexes and cyclodextrins are of interest for magnetic resonance imaging (MRI) applications as they combine a good thermodynamic stability of Ln^{III} complexation with a high molecular weight and thus high relaxivity.[17]

Experimental Section

General. Chemicals were obtained from commercial sources and used as such. β-Cyclodextrin was dried in vacuo at 80 °C in the presence of P_2O_5 for at least 5 h before use. Solvents were dried using standard laboratory procedures. NMR spectra were recorded using a Varian Inova 300 NMR spectrometer. ^1H NMR chemical shifts (300 MHz) are given relative to residual $CHCl_3$ (7.25 ppm), CHD_2OD (3.30 ppm), DMSO-h,d_5 (2.50 ppm), or HDO (4.65 ppm) unless mentioned otherwise. ^{13}C chemical shifts (75 MHz) are given relative to $CDCl_3$ (77.0 ppm), CD_3-OD (49.0 ppm), or DMSO-d_6 (39.5 ppm) unless mentioned otherwise. Mass spectra were recorded with a Finnigan MAT 90 spectrometer using *m*-nitrobenzyl alcohol (NBA) as the matrix. Elemental analyses were carried out with a model 1106 Carlo-Erbu Strumentazione elemental analyzer. Molecular modeling was performed using HYPERCHEM.

EDTA-Based β-Cyclodextrin Dimer (3). A solution of EDTA bisanhydride **1** (2.6 mg, 0.10 mmol) in CH_2Cl_2 was added dropwise to a solution of TBDMS-protected mono(2-*O*-aminopropyl)-β-cyclodextrin **2**[21] (410 mg, 0.21 mmol) and Et_3N (29 μL, 0.021 mmol) in CH_2Cl_2 at room temperature. The reaction mixture was stirred for 30 min, after which the solution was diluted with CH_2Cl_2 and washed with 0.1 M HCl and brine. The organic layer was dried over Na_2SO_4. The product was purified over silica gel using EtOAc/EtOH/H_2O = 6/2/1 as the eluent (R_f = 0.5) and used as such in the deprotection step. The TBDMS-protected precursor of **3** (262 mg, 0.062 mmol) was dissolved in THF at room temperature, and 1.25 mL (1.23 mmol) of a 1 M solution of tetrabutylammonium fluoride (TBAF) in THF was added. The reaction mixture was refluxed (66 °C) overnight. The solvent was evaporated in vacuo, and the residue was diluted with water. The aqueous layer was washed twice with diethyl ether. The excess of TBAF was removed by eluting the product four times over an Amberlite MB3 mixed H^+/OH^- ion exchange column. EDTA-based β-cyclodextrin dimer **3** was obtained in 127 mg yield (48% overall) as a white solid. ^1H NMR (D_2O): δ 5.08 (d, 2H, J = 3.3 Hz, H1 (CD) spacer attached), 4.94 (d, 12H, J = 3.6 Hz, H1 (CD) free glucose ring), 3.90 (t, 2H, J = 9.3 Hz, H3 (CD) spacer attached), 3.84 (t, 12H, J = 9.3 Hz, H3 (CD) free glucose ring), 3.75−3.60 (m, 46H), 3.60−3.33 (m, 36H), 3.26 (t, 4H, J = 6.0 Hz, $NCH_2CH_2CH_3$), 3.18 (s, 4H, NCH_2CH_2N), 1.72 (t, 4H, J = 6.3 Hz, OCH_2CH_2). ^{13}C NMR (D_2O, ref CH_3OD): δ 172.0, 168.5, 101.7, 99.9, 81.4, 81.0, 80.1, 72.9, 72.3, 71.9, 71.6, 69.8, 60.1, 56.3, 51.2, 36.4, 28.3. MALDI-TOF-MS Calcd for $C_{100}H_{166}O_{76}N_4$: m/z = 2639.0. Found: m/z = 2662.9 ($[M + Na]^+$). Anal. Calcd for $C_{100}H_{166}O_{76}N_4 \cdot 5H_2O$: C, 43.99; H, 6.50; N, 2.05. Found: C, 43.96; H, 6.20; N, 2.05.

Bis(1-propylammonium) Salt of EDTA-bis(propylamide) (4). EDTA-bis(*N*-propylamide) bis(propylammonium) salt **4** was synthesized analogous to a literature procedure[22] by dropwise addition of a solution of EDTA bisanhydride **1** in CH_2Cl_2 to a CH_2Cl_2 solution containing a large excess of *n*-propylamine and subsequent evaporation in vacuo. The product was obtained as a colorless oil. ^1H NMR (CD_3-OD): δ 3.20−3.14 (m, 12H, O=CCH_2 + $NCH_2CH_2CH_3$), 2.85 (t, 4H, J = 7.5 Hz, $NCH_2CH_2CH_3$), 2.72 (s, 4H, NCH_2CH_2N), 1.67 (sx, 4H, J = 7.6 Hz, CH_2CH_3), 1.54 (sx, 4H, J = 7.4 Hz, CH_2CH_3), 1.00 (t, 6H, J = 7.5 Hz, CH_3), 0.92 (t, 6H, J = 7.4 Hz, CH_3). ^{13}C NMR (CD_3-OD): δ 178.7, 174.2, 60.9, 60.2, 54.5, 42.4, 42.0, 23.7, 22.3, 11.9, 11.2. FAB-MS Calcd for $C_{16}H_{30}N_4O_6$: m/z = 374.2. Found: m/z = 375.2 ($[M + H]^+$), 373.1 ($[M - H]^-$).

Biphenyl-4,4′-dicarboxylic acid-bis(1-adamantanemethylamide) (7). Biphenyl-4,4′-dicarboxylic acid (47 mg, 0.20 mmol) was stirred overnight in an excess of $SOCl_2$ at 80 °C. The excess of thionyl chloride was removed by evaporation in vacuo, and the product was redissolved in CH_2Cl_2. A solution of 1-adamantanemethylamine **11** (67 mg, 0.41 mmol) and Et_3N (57 μL, 0.57 mmol) in CH_2Cl_2 was added dropwise at room temperature. The solution was stirred for 30 min at room temperature. Subsequently, the turbid reaction mixture was filtered through a Millipore filter (∅ 0.23 μm) and purified over silica gel using CH_2Cl_2:MeOH = 20:1 as the eluent (R_f = 0.4). Product **7** was obtained in 54 mg yield (50%) as a white solid. ^1H NMR (DMSO-d_6): δ 8.36 (t, 2H, J = 6.5 Hz, NHC=O), 7.96 (d, 4H, J = 8.7 Hz, ArH), 7.81 (d, 4H, J = 8.4 Hz, ArH), 3.01 (d, 4H, J = 6.3 Hz, NCH_2), 1.93 (s, 6H, adamantyl $CHCH_2$), 1.63 (br q, 12H, J = 11.6 Hz, adamantyl $CHCH_2$), 1.51 (d, 12H, J = 2.1 Hz, adamantyl $CHCH_2$). ^{13}C NMR (DMSO-d_6): δ 166.3, 141.6, 134.2, 128.1, 126.7, 50.6, 36.6, 34.4, 27.8. FAB-MS Calcd for $C_{36}H_{44}N_2O_2$: m/z = 536.4. Found: m/z = 537.4 ($[M + H]^+$). Anal. Calcd for $C_{36}H_{44}N_2O_2 \cdot 0.1MeOH$: C, 80.30; H, 8.29; N, 5.19. Found: C, 80.02; H, 8.19; N, 5.49.

Biphenyl-4,4′-dicarboxylic acid-bis(1-adamantylcarboxylic acid 3-amidopropyl-amide) (8). Biphenyl-4,4′-dicarboxylic acid (50 mg, 0.21 mmol) and amine **12** (98 mg, 0.41 mmol; see below) were reacted analogous to the preparation of **7**, after which the mixture was washed with 0.1 M HCl and brine. The organic layer was dried over Na_2SO_4. The product was purified over silica gel using CH_2Cl_2:MeOH = 9:1

(16) (a) Willner, I.; Goren, Z. *J. Chem. Soc., Chem. Commun.* **1983**, 1469. (b) Tabushi, I.; Kuroda, Y. *J. Am. Chem. Soc.* **1984**, *106*, 4580.
(17) (a) Aime, S.; Botta, M.; Frullano, L.; Geninatti Crich, S.; Giovenzana, G. B.; Pagliarin, R.; Palmisano, G.; Sisti, M. *Chem.-Eur. J.* **1999**, *5*, 1253. (b) Fatin-Rouge, N.; Tóth, É.; Perret, D.; Backer, R. H.; Merbach, A. E.; Bünzli, J.-C. G. *J. Am. Chem. Soc.* **2000**, *122*, 10810. (c) Skinner, P. J.; Beeby, A.; Dickins, R. S.; Parker, D.; Aime, S.; Botta, M. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1329. (d) Zitha-Bovens, E.; Van Bekkum, H.; Peters, J. A.; Geraldes, C. F. G. C. *Eur. J. Inorg. Chem.* **1999**, 287.
(18) Canet, D.; Levy, G. C.; Peat, I. R. *J. Magn. Reson.* **1975**, *18*, 199.
(19) Although a luminescence increase was observed in H_2O upon addition of biphenyl guests **7** and **8**, titrations of **7**−**10** were carried out in D_2O. The higher signal-to-noise ratio obtained in D_2O allowed more accurate determination of association constants at the low experimental host concentrations.
(20) No difference in the value for τ_{obs} was obtained when the Ln^{III} ion was excited directly or via the sensitizer (λ_{ex} = 280 nm). This confirms that energy transfer is fast (microsecond region) as compared to the lanthanide luminescence decay (millisecond region).
(21) Nelissen, H. F. M.; Schut, A. F. J.; Venema, F.; Feiters, M. C.; Nolte, R. J. M. *Chem. Commun.* **2000**, 577.
(22) Coates, J.; Sammes, P. G.; West, R. M. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1283.

as the eluent ($R_f = 0.6$). Product **8** was obtained in 86 mg yield (60%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.96 (d, 4H, $J = 8.7$ Hz, ArH), 7.69 (d, 4H, $J = 8.4$ Hz, ArH), 7.51 (t, 2H, $J = 6.3$ Hz, NHC═O), 6.21 (t, 2H, $J = 6.5$ Hz, NHC═O), 3.46 (q, 4H, $J = 6.0$ Hz, NCH_2), 3.38 (q, 4H, $J = 6.1$ Hz, NCH_2), 2.05 (s, 6H, adamantyl C*H*CH_2), 1.88 (d, 12H, $J = 2.4$ Hz, adamantyl CHC*H*$_2$), 1.77−1.67 (m, 16H, NCH_2CH_2 + adamantyl CHC*H*$_2$). ^{13}C NMR ($CDCl_3$): δ 179.2, 167.4, 142.9, 133.7, 127.7, 127.1, 40.7, 39.2, 36.4, 35.9, 35.5, 29.9, 28.1. FAB-MS Calcd for $C_{42}H_{54}N_4O_4$: $m/z = 678.4$. Found: $m/z = 679.1$ ($[M + H]^+$). Anal. Calcd for $C_{42}H_{54}N_4O_4 \cdot 0.46CHCl_3$: C, 69.50; H, 7.48; N, 7.63. Found: C, 69.21; H, 7.31; N, 7.61.

Biphenyl-4,4′-dicarboxylic acid-bis(1-adamantanemethylsulfonamide) (9). A solution of biphenyl-4,4′-disulfonyl chloride (125 mg, 0.36) in acetonitrile was added dropwise to a solution of 1-adamantanemethylamine **11** (124 mg, 0.76 mmol) and Et_3N (106 μL, 0.76 mmol) in acetonitrile at room temperature. Upon the addition a white precipitate formed, which was collected by filtration. The white solid was washed twice with acetonitrile and subsequently dried in vacuo. The product **9** was obtained in 183 mg yield (83%). ^{1}H NMR (DMSO-d_6): δ 7.95 (d, 4H, $J = 9.0$ Hz, ArH), 7.89 (d, 4H, $J = 8.4$ Hz, ArH), 7.57 (br, 2H, SNH), 2.41 (br s, 4H, NCH_2), 1.91 (br s, 6H, adamantyl C*H*CH_2), 1.61 (q, 12H, $J = 13.0$ Hz, adamantyl CHC*H*$_2$), 1.42 (s, 12H, adamantyl CHC*H*$_2$). ^{13}C NMR (DMSO-d_6): δ 142.1, 140.7, 127.9, 127.3, 54.4, 39.6, 36.5, 33.0, 27.7. FAB-MS Calcd for $C_{34}H_{44}N_2O_4S_2$: $m/z = 608.3$. Found: $m/z = 609.3$ ($[M + H]^+$). Anal. Calcd for $C_{34}H_{44}N_2O_4S_2$: C, 67.07; H, 7.28; N, 4.60; S 10.53. Found: C, 66.95; H, 7.30; N, 4.81; S, 10.39.

Biphenyl-4,4′-dicarboxylic acid-bis(1-adamantylcarboxylic acid 3-amidopropyl-sulfonamide) (10). A mixture of free amine **12** (138 mg, 0.58 mmol; see below) and Et_3N (81 mL, 0.58 mmol) in acetonitrile was added dropwise to a solution of biphenyl-4,4′-disulfonyl chloride (98 mg, 0.28) in acetonitrile at room temperature. The reaction mixture was stirred for 30 min, after which the solvent was evaporated, and the remaining white solid was recrystallized twice from MeOH. Product **10** was obtained in 147 mg yield (70%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 7.96 (d, 4H, $J = 8.4$ Hz, ArH), 7.88 (d, 4H, $J = 8.7$ Hz, ArH), 7.60 (br, 2H, SNH), 7.30 (t, 2H, $J = 5.7$ Hz, NHC═O), 3.01 (q, 4H, $J = 5.7$ Hz, NCH_2), 2.71 (t, 4H, $J = 7.1$ Hz, NCH_2), 1.87 (s, 6H, adamantyl C*H*CH_2), 1.64 (d, 12H, $J = 3.0$ Hz, adamantyl CHC*H*$_2$), 1.59−1.47 (m, 16H, NCH_2CH_2 + adamantyl CHC*H*$_2$). ^{13}C NMR (DMSO-d_6): δ 177.1, 142.1, 140.0, 127.8, 127.4, 40.4, 39.8, 38.7, 36.1, 35.9, 29.2, 27.6. FAB-MS Calcd for $C_{40}H_{54}N_4O_6S_2$: $m/z = 750.4$. Found: $m/z = 751.4$ ($[M + H]^+$). Anal. Calcd for $C_{40}H_{54}N_4O_6S_2$: C, 63.97; H, 7.25; N, 7.46; S, 8.54. Found: C, 63.89; H, 7.34; N, 7.48; S, 8.61.

1-Adamantylcarboxylic acid 3-aminopropylamide (12). A solution of 1-adamantanecarboxylic acid (1.18 g, 6.55 mmol) and a large excess of $(COCl)_2$ in CH_2Cl_2 was stirred for 4 h at 40 °C. The mixture was evaporated to dryness in vacuo, and CH_2Cl_2 was added to the remaining white solid. A mixture of *N*-Boc-1,3-diaminopropane (1.15 g, 6.60 mmol) and Et_3N (1.0 mL, 7.21 mmol) in CH_2Cl_2 was added dropwise at room temperature. The solution was stirred for 30 min at room temperature. Subsequently, the reaction mixture was washed with 0.01 M HCl and brine. The organic layer was dried over $MgSO_4$. The product was purified over silica gel using CH_2Cl_2:MeOH = 25:1 as the eluent. Trifluoroacetic acid (1.2 mL, 15.5 mmol) was added to a solution of the *N*-Boc-protected precursor of **12** (524 mg, 1.55 mmol) in CH_2Cl_2. The reaction mixture was stirred overnight at room temperature. The solvent was evaporated in vacuo leaving a colorless oil. The residue was repeatedly dissolved in CH_2Cl_2/toluene 2:1 (v/v) and evaporated to dryness in vacuo until precipitation of colorless crystals was observed. The TFA salt of **12** was obtained as a white solid in 511 mg yield (89%). ^{1}H NMR (D_2O): δ 3.17 (t, 2H, $J = 6.6$ Hz, CH_2NC═O), 2.85 (t, 2H, $J = 7.7$ Hz, C*H*$_2$NH$_2$), 1.90 (s, 3H, adamantyl C*H*CH_2), 1.74 (qn, 2H, $J = 7.2$ Hz, NCH_2CH_2), 1.69 (d, 6H, $J = 2.1$ Hz, adamantyl CHC*H*$_2$), 1.59 (br q, 6H, $J = 12.1$ Hz, adamantyl CHC*H*$_2$). ^{13}C NMR (D_2O, ref CH_3OD): δ 40.7, 38.6, 37.0, 35.9, 27.8, 26.9. FAB-MS Calcd for $C_{14}H_{24}N_2O$: $m/z = 236.2$. Found: $m/z = 237.3$ ($[M + H]^+$). Anal. Calcd for $C_{16}H_{25}N_2O_3F_3$: C, 54.84; H, 7.21; N, 8.00. Found: C, 54.79; H, 7.13; N, 8.31.

Eu^{III} and Tb^{III} Complexes of 3. All measurements were carried out at pH 7 in doubly distilled water (Q2) at 25 °C, unless mentioned otherwise. The lanthanide(III) complexes of EDTA-based β-cyclodextrin dimer **3** were prepared by adding a solution of $EuCl_3$ or $TbCl_3$ in water to a solution of **3**, adjusting the pH to 7 with NaOH. A slight excess of **3** relative to Ln^{III} (1.03:1) was used, ensuring quantitative complexation of the lanthanide ion.

^{13}C NMR Relaxation Time Measurements. Gd^{III}-induced ^{13}C relaxation rate enhancement measurements were performed on a Varian Unity WB NMR spectrometer at 100.6 MHz using the inversion recovery pulse sequence. The longitudinal ^{13}C relaxation times were calculated using a nonlinear least squares three parameter curve fitting routine.[18] To a solution containing a constant high concentration of benzoate **5** (0.5 M), an increasing amount of **3**·Gd^{III} was added determining the longitudinal ^{13}C NMR relaxation times (T_1)$_i$ of **5** after each addition. Values for ρ_L (=[**3**·Gd^{III}]/[**5**]) of 0, 5.0×10^{-5}, 1.0×10^{-4}, 1.9×10^{-4}, and 5.0×10^{-4} were applied.

Photophysical Studies. Luminescence titrations were carried out using an Edinburgh Analytical Instruments FS900 fluorescence spectrophotometer. Absorption spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. For all excitation wavelengths used the optical density remained below 0.1, as determined by UV−vis spectroscopy. For the titrations of *p-tert*-butylbenzoate **5** to the Eu^{III} and Tb^{III} complexes of EDTA-based β-cyclodextrin dimer **3**, aliquots of a 11.5 mM solution of **5** in H_2O were added to 3.0 mL of the 0.1 mM **3**·Ln^{III} complex in H_2O in a quartz cuvette, recording the luminescence spectrum between 550 and 725 nm or 450 and 650 nm, for **3**·Eu^{III} or **3**·Tb^{III}, respectively ($\lambda_{ex} = 280$ nm). For the titrations of biphenyl compounds **7**−**10** to the Eu^{III} complex of **3**, 0.45 mM stock solutions of **7**−**10** in DMSO were prepared. Aliquots of this solution were added to a quartz cuvette containing 2.75 mL of a 6.0 μM solution of the **3**·Eu^{III} complex in D_2O,[19] recording the Eu^{III} luminescence spectrum between 550 and 725 nm ($\lambda_{ex} = 285$ nm). During the titration, the amount of DMSO present in the solution remained below 9%, which is assumed not to affect the guest binding strength of β-cyclodextrin. Luminescence lifetime measurements of the **3**·Eu^{III} and **3**·Tb^{III} complexes were performed in duplo using an Edinburgh Analytical Instruments LP900 fluorescence spectrophotometer monitoring the luminescence decay upon direct excitation of the Ln^{III} ion[20] ($\lambda_{ex} = 393$ and 484 nm for **3**·Eu^{III} and **3**·Tb^{III}, respectively). The luminescence emission was monitored at 617 nm for **3**·Eu^{III} and 545 nm for **3**·Tb^{III}.

Calorimetric Titrations. Calorimetric measurements were carried out using a Microcal VP-ITC microcalorimeter with a cell volume of 1.4115 mL. For studying the complexation of *p-tert*-butylbenzoate **5** to the free EDTA-based β-cyclodextrin dimer **3** and the **3**·Eu^{III} complex, aliquots of a 20.0 mM solution of **5** in the buret were added to a 0.97 mM solution of **3** or **3**·Eu^{III} in the calorimetric cell, monitoring the heat change after each addition. For the accurate determination of binding constants, **5** was also titrated to **3**·Eu^{III} under more dilute conditions: 2.0 mM of **5** (titrant) to 0.10 mM of **3**·Eu^{III} (cell). For studying the complexation of adamantyl derivative **12** to β-cyclodextrin, aliquots of a 5.4 mM solution of β-cyclodextrin were added to a 0.53 mM solution of the TFA salt of **12**. Dilution experiments showed that at the experimental concentrations none of the species described above showed significant aggregation behavior in water nor (de)protonation.

Results and Discussion

Synthesis. EDTA-based β-cyclodextrin dimer **3** was synthesized in two steps starting from the commercially available EDTA bisanhydride **1** and β-cyclodextrin derivative **2** (Scheme 1). The latter was synthesized according to a literature proce-

Scheme 1

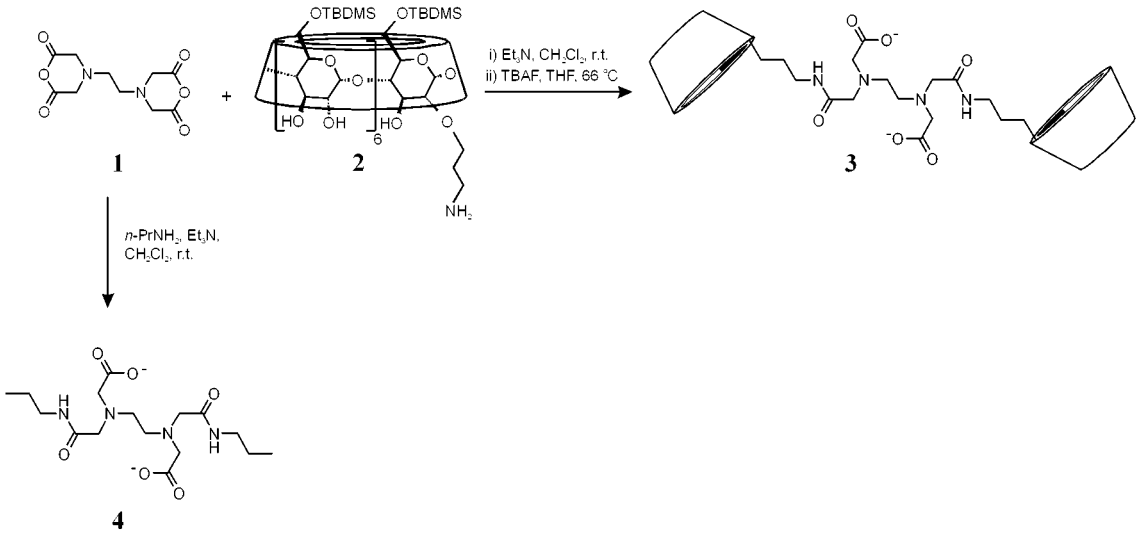
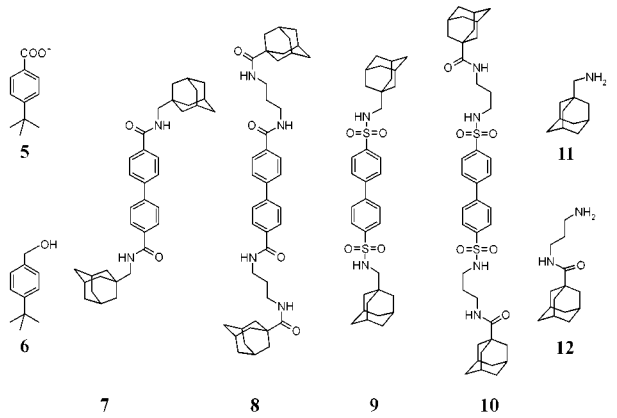


Chart 1



dure.[21] Two equivalents of **2** were reacted with EDTA bisanhydride **1** in the presence of triethylamine in dichloromethane. After acidic workup and purification by silica column chromatography, the TBDMS-protected precursor of EDTA-based β-cyclodextrin dimer **3** was obtained. Subsequent deprotection using tetrabutylammonium fluoride (TBAF) in refluxing THF and removal of the excess of TBAF by ion exchange chromatography yielded the water-soluble host **3**.

EDTA-bis(*N*-propylamide) **4** (Scheme 1) was used as a reference compound and synthesized analogous to a literature procedure[22] by reaction of EDTA bisanhydride **1** with 1-aminopropane in dichloromethane. The product was obtained as the bis(propylammonium) salt after removal of the solvent.

The triplet state energy of *p-tert*-butylbenzoate **5** (Chart 1) was assumed comparable to that of 4-methylbenzoic acid (E_T = 26 880 cm^{-1}),[23] which is high enough to sensitize Eu^{III} and Tb^{III} luminescence.[24] The energies of the receiving $^5D_0$ and $^5D_1$ states of Eu^{III} are 17 500 and 19 000 cm^{-1}, respectively. The receiving $^5D_4$ state of Tb^{III} is at 20 500 cm^{-1}. Antenna molecule **5** has both a guest site (the *p-tert*-butylphenyl moiety) for binding to β-cyclodextrin[25] and a Ln^{III}-coordinating carboxylate group. This may allow coordination to the Ln^{III} center of the **3**·Ln^{III} complex and thus lead to efficient energy transfer for sensitized luminescence. *p-tert*-Butylbenzyl alcohol **6**, that complexes as strong to β-cyclodextrin as **5** but lacks a Ln^{III}-coordinating site, was used as a reference. The triplet state energy of **6** was assumed to be comparable to that of **5** and therefore suitable as a sensitizer for both Eu^{III} and Tb^{III} luminescence.

Because of the presence of two cyclodextrin moieties in **3**, we also envisaged using both cavities for the binding of an antenna molecule to reach close proximity between the Ln^{III} ion and the sensitizer. Chart 1 shows four biphenyl-based sensitizers (**7**−**10**), equipped with two adamantyl units as binding sites for β-cyclodextrin. Sensitizers with different spacer lengths between the biphenyl moiety and the adamantyl groups were prepared. Biphenyl derivatives have triplet state energies[23] typically in the range 21 000−23 000 cm^{-1} and are therefore suitable antennae for the sensitization of Eu^{III} luminescence.

Conversion of 4,4′-biphenyldicarboxylic acid into the corresponding diacid chloride and subsequent reaction with an amine-appended adamantane (**11** or **12**) yielded the bis(adamantyl)-biphenyl derivatives **7** and **8**, respectively. *N*-Propylamino-appended adamantane **12** was prepared by coupling *N*-Boc-1,3-diaminopropane to 1-adamantylcarbonyl chloride and subsequent removal of the Boc protecting group with trifluoroacetic acid (TFA). Sulfonamides **9** and **10** were obtained by reaction of **11** or **12** with 4,4′-biphenyldisulfonyl chloride.

Binding of *p-tert*-Butylbenzoate. When EDTA-based β-cyclodextrin dimer **3** was added to a solution of $EuCl_3$ in H_2O at pH 7, the Eu^{III} luminescence emission spectrum showed significant changes upon direct excitation of the Eu^{III} ion at 393 nm. First, the overall Eu^{III} luminescence intensity increased upon addition of **3**, owing to displacement of water molecules from the first coordination sphere of the Eu^{III} ion upon complexation by the EDTA unit. Second, the relative intensity of the emission band centered around 615 nm, corresponding to the hypersensitive (electric dipole) $^5D_0 \rightarrow {}^7F_2$ transition, increased as this transition is favored upon desymmetrization of the first coordination sphere by complexation to the EDTA unit.[26]

(23) Murov, S. L.; Carmichael, I.; Hug, G. L. *Handbook of Photochemistry*, 2nd ed.; Marcel Dekker: New York, 1993.
(24) The triplet energy of the sensitizer should be at least 2000 cm^{-1} higher than the receiving luminescent state(s) of the Ln^{III} ion for irreversible energy transfer to occur. See: Sato, S.; Wada, M. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 1955.
(25) Hofler, Th.; Wenz, G. *J. Inclusion Phenom.* **1996**, *25*, 81.

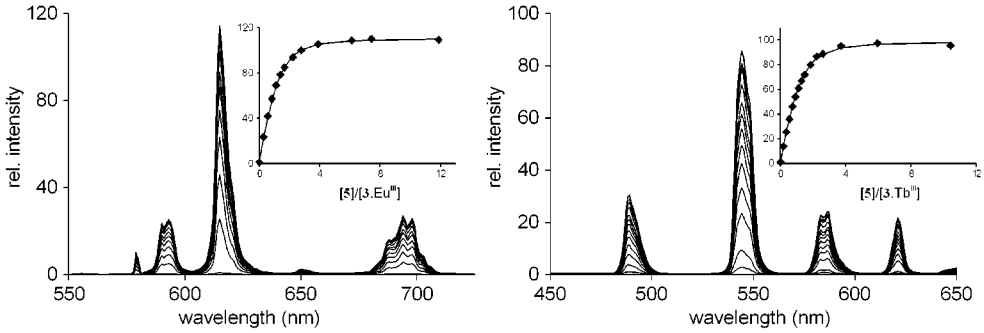


Figure 1. Increase in luminescence intensity of **3**·Eu^{III} (left) and **3**·Tb^{III} (right) in H_2O upon addition of *p-tert*-butylbenzoate **5** (λ_{ex} = 280 nm). The intensities are given relative to the intensity of **3**·Ln^{III} in the absence of sensitizer at 617 and 545 nm for **3**·Eu^{III} and **3**·Tb^{III}, respectively. Inset: luminescence intensities, monitored at 617 and 545 nm, plotted as a function of the [**5**]/[**3**·Ln^{III}] ratio; the lines represent the fit to a 1:1 binding model.

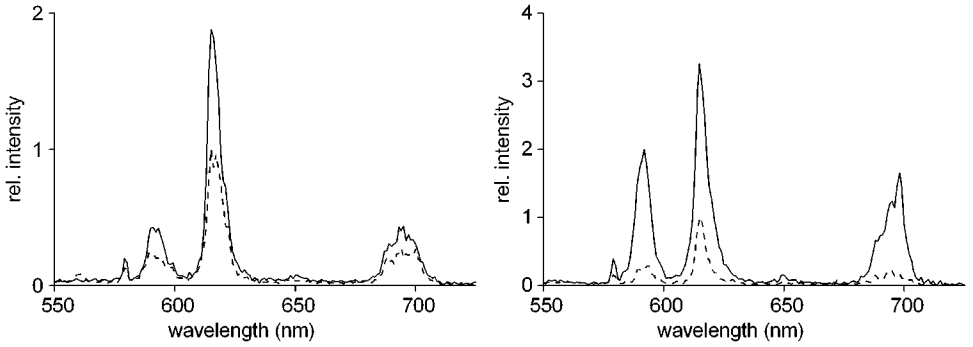


Figure 2. Luminescence emission spectra of a 1.0×10^{-4} M solution of **3**·Eu^{III} (left, dashed line) and **4**·Eu^{III} (right, dashed line) in H_2O and after the addition of 0.86 equiv of *p-tert*-butylbenzyl alcohol **6** (left, solid line) or *p-tert*-butylbenzoate **5** (right, solid line). Excitation of **5** and **6** occurred at 250 and 265 nm, respectively.

The luminescence intensities of the **3**·Eu^{III} or **3**·Tb^{III} complexes in H_2O strongly increased upon addition of *p-tert*-butylbenzoate **5** and excitation at 280 nm (Figure 1). Maximum amplifications of factors 110 and 95 were achieved for **3**·Eu^{III} and **3**·Tb^{III}, respectively. When sensitizer **5** was excited at 250 nm, the luminescence intensity could even be raised by a factor of 350 for **3**·Eu^{III} and 310 for **3**·Tb^{III}. This is 8 times more than the amplification obtained for a previously reported β-cyclodextrin-DTPA-Tb^{III} couple using biphenyl as the sensitizer.[15] The excitation spectra of **3**·Eu^{III} (λ_{em} = 617 nm) and **3**·Tb^{III} (λ_{em} = 545 nm) in the presence of **5** showed the same spectral features (shape and position of the bands) as the absorption spectrum of **5**. These observations show that **5** acts as a sensitizer for Eu^{III} and Tb^{III} luminescence.

The insets of Figure 1 show the data points obtained from the titrations of **3**·Eu^{III} (left; monitored at 617 nm) and **3**·Tb^{III} (right; monitored at 545 nm) with **5** and the fits to a 1:1 binding model. An apparent 1:1 association constant of 2.9×10^4 M^{-1} was obtained for both the **3**·Eu^{III}·**5** and the **3**·Tb^{III}·**5** complexes.

p-tert-Butylbenzyl alcohol **6**, which has a comparable binding affinity to that of β-cyclodextrin but lacks a Ln^{III}-coordinating functionality, shows much less effective sensitization of the luminescence of **3**·Eu^{III} (Figure 2, left). The luminescence intensity of **3**·Eu^{III} was increased only by a factor 2 upon addition of 0.86 equiv of **6**. The luminescence intensity of the europium(III) complex of EDTA-bis(propylamide) **4**, which lacks the β-cyclodextrin cavities, was enhanced by a factor of 3.3 upon addition of 0.86 equiv of sensitizer **5** (Figure 2, right).

Depite the fact that EDTA-based β-cyclodextrin dimer **3** has two binding sites for *p-tert*-butylbenzoate **5**,[25] the complex stoichiometry inferred from the luminescence titrations is apparently 1:1. Complexation of *p-tert*-butylbenzoate **5** by **3** and **3**·Eu^{III} was also studied by microcalorimetry. Figure 3 depicts the (exothermic) heat profiles obtained from the calorimetric titrations of the Ln^{III} free β-cyclodextrin dimer **3** (left) and europium(III) complex **3**·Eu^{III} (right) with *p-tert*-butyl benzoate **5** in water. In contrast to the luminescence experiments, the inflection points of the binding isotherms indicate a 1:2 (host:guest) stoichiometry.[27]

Table 1 shows the thermodynamic parameters for the complexation of *p-tert*-butylbenzoate **5** to **3** and to **3**·Eu^{III}. The stability constants for the first complexation step of *p-tert*-butylbenzoate to **3** and **3**·Eu^{III} ($K_1 = 1.3 \times 10^4$ and 5.0×10^4 M^{-1}, respectively) are comparable to those of the strongest complexes of previously reported systems in which a Ln^{III}-complexing unit is covalently functionalized with β-cyclodextrin.[13,14] The binding strength of *p-tert*-butylbenzoate **5** by EDTA-based β-cyclodextrin dimer **3** is comparable to the complexation by native β-cyclodextrin.[25] The stability of the **3**·Eu^{III}·**5** complex is enhanced by the presence of the Eu^{III} ion, which indicates coordination of **5** to the Ln^{III} center.

The complexation of the *p-tert*-butylbenzyl part of **5** by the β-cyclodextrin cavities of **3** or **3**·Eu^{III} is largely exothermic and results in exothermic overall heat changes and binding enthalpies. The binding enthalpy for **3**·Eu^{III} is less exothermic than

(26) (a) Kirby, A. F.; Foster, D.; Richardson, F. S. *Chem. Phys. Lett.* **1983**, *95*, 507. (b) Kirby, A. F.; Richardson, F. S. *J. Phys. Chem.* **1983**, *87*, 2544.

(27) For the titration of **5** to β-cyclodextrin dimer **3**, the data points were fitted accurately to a 1:2 model assuming independent binding sites ($K_1 = 4K_2$ and $\Delta H_1 = \Delta H_2$). The data obtained for the complexation of **5** to **3**·Eu^{III} gave an accurate fit only to a model assuming nonequivalent binding sites.

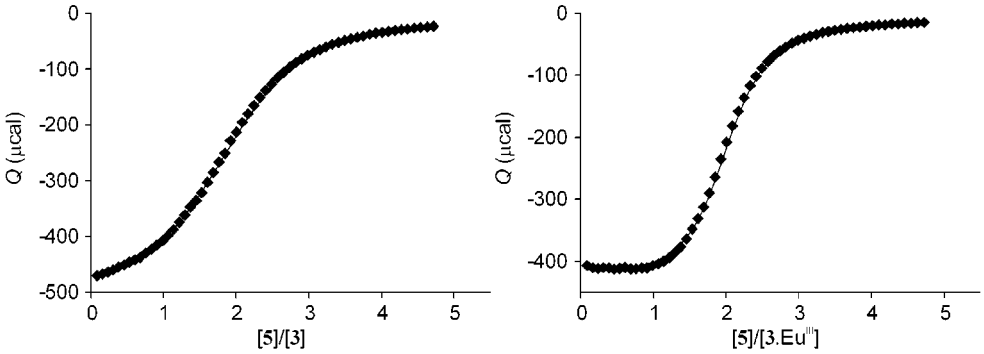


Figure 3. Heat evolved per injection plotted against the [**5**]/[**3**] ratio for the calorimetric titrations of *p-tert*-butylbenzoate **5** to **3** (left) and **3**·Eu^{III} (right) in water.

Table 1. Thermodynamic Parameters of the Complexation of *p-tert*-Butylbenzoate **5** to **3** and **3**·Eu^{III}, As Determined by Microcalorimetry

host	stoichiometry (host:guest)	K (M^{-1})	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$T\Delta S°$ (kcal/mol)
3	1:1	1.3×10^4	−5.6	−5.1	0.5
	1:2	3.2×10^3	−4.8	−5.1	−0.3
3·Eu^{III}	1:1	5.0×10^4	−6.4	−4.1	2.3
	1:2	1.1×10^4	−5.5	−4.4	1.1

Table 2. Luminescence Lifetimes in H_2O and D_2O and the Number of Water Molecules in the First Coordination Sphere, n (Eq 1), of the Ln^{III} Complexes of **3**, in the Absence and Presence of **5** (λ_{em} = 617 and 545 nm, λ_{ex} = 393 and 484 nm for Eu^{III} and Tb^{III}, Respectively)

Ln^{III} complex	τ_{H_2O} (ms)	τ_{D_2O} (ms)	n^a
3·Eu^{III}	0.29	2.2	2.9
3·Tb^{III}	0.93	2.9	2.9
3·Eu^{III}·$\mathbf{5}_2$	0.33	2.2	2.4
3·Tb^{III}·$\mathbf{5}_2$	0.98	2.6	2.5

[a] $n \pm 0.5$ or ± 1.5 for Eu^{III} and Tb^{III}, respectively.

that for **3**, which supports coordination of **5** to the Eu^{III} center of **3**·Eu^{III} upon binding by a β-cyclodextrin cavity, as the coordination of carboxylates to lanthanide(III) ions is known to be slightly endothermic.[28] Also the increased binding entropy for **3**·Eu^{III} as compared to that of **3** supports coordination, as Ln^{III}-coordinated water is liberated upon binding of the carboxylate sensitizer. In conclusion, all thermodynamic parameters (K, $\Delta H°$, and $T\Delta S°$) indicate the coordination of the carboxylate group of **5** to the Ln^{III} center in the **3**·Ln^{III}·**5** complex.

The luminescence data for **3**·Eu^{III} and **3**·Tb^{III} (Figure 1) were also fitted well to a 1:2 model using the values obtained for K_1 and K_2 from microcalorimetry. Fixing these numbers in the fitting procedure while independently varying the luminescence intensities of the complexes led to a luminescence increase of only about 10% upon complexation of the second sensitizer **5** to both Ln^{III} complexes. This explains why the luminescence data fit equally well to a 1:1 binding model. This finding suggests that one cyclodextrin-bound sensitizer quantitatively coordinates to the lanthanide and that energy transfer almost exclusively occurs upon coordination.

Ditopic binding of guests in cyclodextrin derivatives has been shown before,[16] but the novelty here lies in the fact that it is used for efficient energy transfer from the guest to the lanthanide center. Thus, the cyclodextrin unit is used for efficient complexation, while the carboxylate ensures a close enough proximity of the guest to the Ln^{III} ion. The microcalorimetry experiments described above show that the intrinsic interaction of the coordination of sensitizer **5** to the Ln^{III} center is weak (in the order of $1-10\ M^{-1}$). Still, coordination of one sensitizer **5** takes place quantitatively because the cyclodextrins of **3** increase the effective concentration of **5** in the vicinity of the Ln^{III} center.

To estimate the number of water molecules (n) in the first coordination sphere of a lanthanide(III) ion, and thus indirectly the number of coordination sites occupied by other ligands, an empirical relationship has been derived (eq 1).[29]

$$n = q(1/\tau_{H_2O} - 1/\tau_{D_2O} - k_{corr}) \qquad (1)$$

Here, q is a constant with a value that depends on the lanthanide ion; q is 1.05 and 4.2 ms for Eu^{III} and Tb^{III} complexes, respectively. τ_{H_2O} and τ_{D_2O} are the luminescence lifetimes of the Ln^{III} complexes in H_2O and D_2O, and k_{corr} (0.25 and 0.05 ms^{-1} for Eu^{III} and Tb^{III}, respectively) corrects for closely diffusing second sphere water molecules.

The luminescence lifetimes τ_{H_2O} and τ_{D_2O} (Table 2) for the **3**·Eu^{III} and **3**·Tb^{III} complexes were determined in the absence and presence of an excess of sensitizer **5** using time-resolved luminescence spectroscopy. Substitution of the lifetimes in eq 1 showed that, in the absence of sensitizer, the water coordination number is three, indicating the occupation of six of the, in total, nine Ln^{III} coordination sites by the EDTA unit, that is, via the two nitrogens and all four carboxylic oxygens, similar to that observed for Ln^{III} complexes of native EDTA.[30] Upon complexation of **5**, effectively 0.5 water molecule[31] is displaced from the first coordination sphere of the Ln^{III} center, indicating coordination of only one sensitizer to the Ln^{III} center. The fact that $\Delta n < 1$ can be attributed to expansion of the first coordination sphere due to electrostatic repulsion between multiple negatively charged ligands (in this case the EDTA moiety of **3** and carboxylate **5**).[30,32]

(28) (a) Huskens, J.; van Bekkum, H.; Peters, J. A.; Choppin, G. R. *Inorg. Chim. Acta* **1996**, *245*, 51. (b) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum Press: New York, Vol. 3, **1977**; Vol. 5, **1982**; Vol. 6, **1989**.

(29) (a) Kropp, J. L.; Windsor, M. W. *J. Chem. Phys.* **1965**, *42*, 1599. (b) Horrocks, W. DeW., Jr.; Sudnick, D. R. *Acc. Chem. Res.* **1981**, *14*, 384. (c) Holz, R. C.; Chang, C. A.; Horrocks, W. DeW., Jr. *Inorg. Chem.* **1991**, *30*, 3270. (d) Beeby, A.; Clarkson, J. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; de Souza, A. S.; Williams, J. A. G.; Woods, M. *J. Chem. Soc., Perkin Trans. 2* **1999**, 493.

(30) Huskens, J.; Kennedy, A. D.; Peters, J. A.; Van Bekkum, H. *J. Am. Chem. Soc.* **1995**, *117*, 375.

(31) This value is not corrected for the fact that the luminescence quenching by the amide protons of the EDTA ligand is canceled in D_2O due to proton−deuteron exchange.

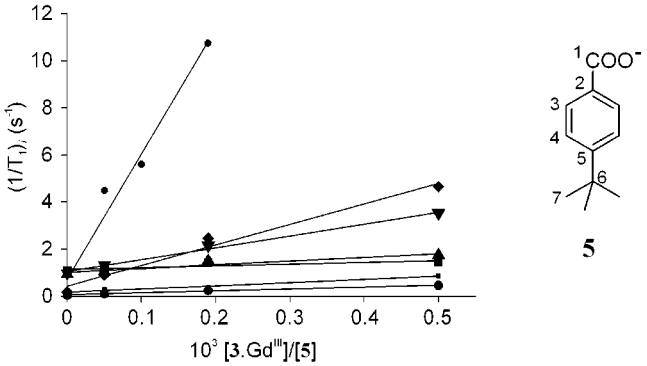


Figure 4. Longitudinal ^{13}C NMR relaxation rate plotted as a function of the [**3**·Gd^{III}]/[**5**] ratio for all carbon atoms of **5**.

Table 3. Experimental and Calculated **3**·Gd^{III}-Induced ^{13}C Relaxation Rate Enhancements of Carboxylate **5**, and Estimates for the Ci−Gd^{III} Distances for Two Different Coordination Modes, Based on Molecular Modeling

Ci	R_i (10^3 s^{-1}) experimental	R_i (10^3 s^{-1}) calculated	r_i (Å) bidentate	r_i (Å) monodentate
1	53.12	53.12	2.83	3.76
2	8.72	8.72	4.23	4.63
3	5.00	4.98	5.11	4.53, 6.00
4	1.58	1.71	6.47	5.81, 7.01
5	1.32	1.19	7.09	6.97
6	0.81	0.81	8.62	8.37
7	0.74	0.75	9.38	8.57, 9.20, 9.23
Gd^{III} − O			2.50	2.50

Further evidence for coordination of the carboxylate group of **5** to Ln^{III} in the **3**·Ln^{III}·$\mathbf{5}_2$ complex was obtained using Gd^{III}-induced ^{13}C NMR relaxation rate enhancement measurements, a technique which previously has been successfully applied to elucidate Ln^{III} complex geometries[28a,33] and allows application of Ln^{III} ions in MRI.[34−37] Figure 4 depicts the longitudinal ^{13}C NMR relaxation rates $(1/T_1)_i$ of sensitizer **5** as a function of the [**3**·Gd^{III}]/[**5**] ratio (ρ_L). The Gd^{III}-induced relaxation rate enhancement R_i for the carbon atoms of **5** is obtained by dividing the slopes of the lines in Figure 4 by the number of coordinating sensitizers.[28a,34c] Only one sensitizer coordinates, indicated by the microcalorimetric and luminescence titrations.

The values for R_i of the carbon atoms of carboxylate **5** are listed in Table 3. Relaxation rate enhancement is most pronounced for the carboxylic carbon atom (C1) of **5**. R_i decreases strongly for the other carbon atoms of **5** in the order indicated in Table 3, which proves coordination of the sensitizer to the Gd^{III} center. Table 3 also lists molecular modeling estimates for the distances r_i between the carbon atoms of benzoate **5** and the Gd^{III} center in the complex, calculated for bidentate ($r_{b,i}$) and monodentate ($r_{m,i}$) coordination of the carboxylate group.[28a] Hence, R_i is a weighted average of the enhancements of the individual modes. Normalized values for the individual relaxation rate enhancement contributions for the coordination modes ($R_{b,i}$ and $R_{m,i}$) can then be calculated with eqs 2 and 3 using an initial estimate for $R_{m,1}$, the rate enhancement of the nucleus closest to the metal ion with the ligand in the monodentate coordination mode.

$$R_{m,i} = (r_{m,1}/r_{m,i})^6 \times R_{m,1} \quad (2)$$

$$R_{b,i} = (r_{m,1}/r_{b,i})^6 \times R_{m,1} \quad (3)$$

The fractions of complexed carboxylate ligand in the mono- and bidentate coordination mode f_m and f_b (=1 − f_m) can be obtained by fitting the experimental values for R_i to eq 4 using $R_{m,1}$, f_m, and the nonspecific relaxation rate enhancement[34c] R_{inter} as independently varied fitting parameters. The fractions of **5**

$$R_i = f_m R_{m,i} + (1 - f_m)R_{b,i} + R_{inter} \quad (4)$$

coordinating to the Gd^{III} ion in a monodentate and bidentate fashion were found to be 73% and 27%, respectively, with $R_{m,1}$ = 23.6 × 10^3 s^{-1} and R_{inter} = 0.6 × 10^3 s^{-1}. It shows that the majority binds in a monodentate fashion.

If diamagnetic contributions are assumed to be negligible, the relaxation rates can be related to the distance r_i between nucleus i and the Gd^{III} ion via[38,39]

$$R_{m,i} = C/r_i^6 \quad (5)$$

with

$$C = \frac{2}{5}\left(\frac{\mu_0}{4\pi}\right)^2 \gamma^2 \mu_{eff}^2 \beta^2 \tau_c \quad (6)$$

Here, $\mu_0/4\pi$ is the magnetic permeability of a vacuum, μ_{eff} is the effective magnetic moment of Gd^{III}, γ is the magnetogyric ratio, β is the Bohr magneton, and τ_c is the correlation time. For small Ln^{III}-carboxylate complexes (MW ≈ 400), C has been estimated to be about 1.1 × 10^7 $Å^6$ s^{-1}.[28a] The rotational correlation time τ_R for the present case can be estimated from the Debye−Stokes−Einstein equation (eq 7).

$$\tau_R = \frac{4\pi a^3 \eta}{3kT} \quad (7)$$

Here, a is the hydrodynamic radius of the complex, and η is the viscosity of the solvent. Equation 7 shows that τ_R correlates linearly with the volume, and thus the molecular weight, of the complex. Since under the conditions employed here $\tau_c \approx \tau_R$,[34c] C is estimated to be 8 × 10^7 $Å^6$ s^{-1} for the **3**·Gd^{III}·$\mathbf{5}_2$ complex (MW ≈ 3000). Therefore, $R_{m,1}$ was estimated to be 28 × 10^3 s^{-1}, which corresponds well to the observed $R_{m,1}$ value and thus to quantitative coordination of one carboxylate group to the Gd^{III} center.

Both luminescence and NMR data strongly point to the lack of coordination of the second guest molecule to the Ln^{III} center. Most likely, this is due to both steric and electrostatic reasons. It would require bringing both cyclodextrin cavities close to the EDTA unit, as well as expelling an extra water molecule

(32) Näslund, J.; Lindqvist-Reis, P.; Persson, I.; Sandström, M. *Inorg. Chem.* **2000**, *39*, 4006.
(33) (a) Peters, J. A.; Kieboom, A. P. G. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 381. (b) Vijverberg, C. A. M.; Peters, J. A.; Kieboom, A. P. G.; van Bekkum, H. *Tetrahedron* **1986**, *42*, 167. (c) van Westrenen, J.; Peters, J. A.; Kieboom, A. P. G.; van Bekkum, H. *J. Chem. Soc., Dalton Trans.* **1988**, 2723.
(34) (a) Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901. (b) Parker, D. *Chem. Soc. Rev.* **1990**, *19*, 271. (c) Peters, J. A.; Huskens, J.; Raber, D. J. *Prog. Nucl. Magn. Reson. Spectrosc.* **1996**, *28*, 283.
(35) Fatin-Rouge, N.; Tóth, É.; Perret, D.; Backer, R. H.; Merbach, A. E.; Bünzli, J.-C. G. *J. Am. Chem. Soc.* **2000**, *122*, 10810.
(36) André, J. P.; Tóth, É.; Fischer, H.; Seelig, A.; Mäcke, H. R.; Merbach, A. E. *Chem.-Eur. J.* **1999**, *5*, 2977.
(37) Anelli, P. L.; Bertini, I.; Fragai, M.; Lattuada, L.; Luchinat, C.; Parigi, G. *Eur. J. Inorg. Chem.* **2000**, 625.
(38) Bloembergen, N. *J. Chem. Phys.* **1957**, *27*, 572.
(39) Solomon, I. *Phys. Rev.* **1955**, *99*, 559.

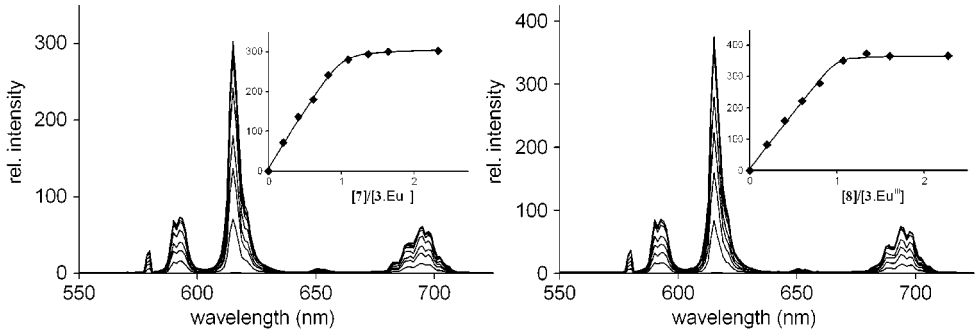


Figure 5. Increase in luminescence intensity of **3**·Eu^{III} in D_2O upon addition of adamantyl-appended biphenyl sensitizers **7** (left) and **8** (right) (λ_{ex} = 285 nm). The intensities are given relative to the intensity of **3**·Eu^{III} at 615 nm in the absence of sensitizer. Inset: luminescence intensity at 615 nm, plotted against the [**7**]/[**3**·Eu^{III}] (left) and [**8**]/[**3**·Eu^{III}] (right) ratios; the lines represent the fit to a 1:1 binding model.

from the first coordination sphere of the Ln^{III} ion, which becomes progressively more difficult. Furthermore, the first coordination sphere becomes more negatively charged, thus repelling the coordination of another guest molecule. As shown above, the coordination of the first carboxylate is already weak, and binding of the second is therefore probably too weak to be observed, even though the complexation by the second cyclodextrin cavity is efficient and increases the effective molarity of the guest.

Using the Stern−Volmer equation (eq 8), an estimate of the energy transfer rate can be calculated if deactivation of the antenna triplet state via oxygen quenching is assumed to be the only process competing with energy transfer. I_{deox} and I_{ox} are

$$I_{deox}/I_{ox} = 1 + k_{diff}\tau_T[O_2] \qquad (8)$$

the luminescence intensities in the absence and presence of oxygen, k_{diff} is the diffusion-controlled quenching rate constant, and τ_T is the lifetime of the triplet state of the sensitizer. If deactivation of the antenna triplet is dominated by energy transfer to the Ln^{III} center, the energy transfer rate constant (k_{ET}) can be calculated by

$$k_{ET} \approx 1/\tau_T \qquad (9)$$

When k_{diff} and [O_2] are assumed to be 10^{10} M^{-1} s^{-1} and 1.39 mM, respectively,[23,40] it follows that $k_{ET,Eu}$ and $k_{ET,Tb}$ are both in the order of 10^6 s^{-1}.[41] This energy transfer rate is 1−2 orders of magnitude lower than that previously reported.[42,43] Still, the energy transfer in the **3**·Ln^{III}·**5**$_2$ complexes is faster than that in a β-cyclodextrin-DTPA-Tb^{III} couple with biphenyl as the sensitizer ($k_{ET,Tb} = 5.8 \times 10^4$ s^{-1}).[15]

Binding of Bisadamantyl Biphenyl Sensitizers. The binding of bisadamantyl-appended biphenyl guests **7**−**10** to the Eu^{III} complex **3**·Eu^{III} of EDTA-based β-cyclodextrin dimer **3** in D_2O was studied using luminescence spectroscopy.[19] Because of the very low solubility of **7**−**10** in water, stock solutions of these guests were prepared in DMSO. Addition of adamantylamido-appended biphenyl derivatives **7** and **8** to a 6.0 μM solution of the **3**·Eu^{III} complex in water had a strong effect on the luminescence intensity (Figure 5, left and right, respectively) upon excitation of **7** or **8** at 285 nm. Amides **7** and **8** act as sensitizers for Eu^{III} luminescence as proven by the luminescence increase and by the fact that the absorbance spectra of **7** and **8** show the same spectral features (shape and position of the bands) as the excitation spectra of the **3**·Eu^{III}·**7** and **3**·Eu^{III}·**8** complexes (λ_{em} = 617 nm).

In the insets of Figure 5 the Eu^{III} luminescence intensity at 617 nm is plotted as a function of the [**7**]/[**3**·Eu^{III}] (Figure 5, left) or [**8**]/[**3**·Eu^{III}] (Figure 5, right) ratios. A sharp inflection point at a 1:1 host:guest ratio is observed for both titration curves, indicative of 1:1 binding stoichiometry. Least-squares fitting to a 1:1 binding model gave high stability constants (9.9×10^6 and $>1.0 \times 10^7$ M^{-1} for the **3**·Eu^{III}·**7** and **3**·Eu^{III}·**8** complexes, respectively). These values are about 200 times higher than that of the association constant for the binding of monoadamantyl derivative **12** by native β-cyclodextrin in water ($K = 5.4 \times 10^4$ M^{-1}), as determined by microcalorimetry (enthalpogram not shown). The high association constants and the 1:1 binding stoichiometry suggest that both β-cyclodextrin cavities of dimer **3** are involved in the complexation of bisadamantyl-biphenyl sensitizers **7** and **8**. The stabilities of the **3**·Eu^{III}·**7** and **3**·Eu^{III}·**8** complexes are >1000 times higher than those of the complexes reported by Parker et al.[13] and Nocera et al.[11,12,14,15] and are comparable to Breslow's 1:1 complex of a bisadamantyl-functionalized phosphate ester and a 2,2′-bipyridyl-spaced β-cyclodextrin dimer.[44]

Apparently, the length of the spacers between the biphenyl unit and the adamantyl groups does not affect the complexation strength of the bisadamantyl guest by β-cyclodextrin dimer **3** significantly. The excitation spectra of **3**·Eu^{III} (λ_{em} = 617 nm) recorded upon the addition of equimolar amounts of bisadamantyl-biphenyl sensitizers **7** and **8** in water show that also the luminescence enhancing properties of the biphenyl sensitizers are not significantly affected by the spacer length. This is supported by the small difference in maximum intensity amplification achieved for **7** and **8** (300 and 360 times the initial intensity in the absence of sensitizer, see Figure 5).

In contrast to amides **7** and **8**, addition of sulfonamides **9** and **10** to **3**·Eu^{III} in D_2O did not increase the luminescence intensity. The fluorescence spectra of amide **8** and sulfonamide **10** showed that the singlet state energy of the sulfonamide

(40) The solubility of molecular oxygen in water at 20 °C and 1 bar is 1.39 mM, see: *Handbook of Chemistry and Physics*, 76th ed.; CRC Press: New York, 1995; p 6-4.
(41) The luminescence spectra in the absence and presence of oxygen were recorded several times to obtain reliable values for the ratio I_{deox}/I_{ox}.
(42) Van der Tol, E. B.; Van Ramesdonk, H. J.; Verhoeven, J. W.; Steemers, F. J.; Kerver, E. G.; Verboom, W.; Reinhoudt, D. N. *Chem.-Eur. J.* **1998**, *4*, 2315.
(43) Steemers, F. J.; Verboom, W.; Hofstraat, J. W.; Geurts, F. A. J.; Reinhoudt, D. N. *Tetrahedron Lett.* **1998**, *39*, 7583.
(44) (a) Zhang, B.; Breslow, R. *J. Am. Chem. Soc.* **1993**, *115*, 9353. (b) Zhang, B.; Breslow, R. *J. Am. Chem. Soc.* **1997**, *119*, 1676.

sensitizer **10** (31 500 cm^{-1}) is comparable to that of the amide sensitizer **8** (30 300 cm^{-1}), both high enough to allow energy transfer to the Eu^{III} center, provided that the singlet−triplet energy gap is small enough in both cases. This suggests that, although sulfonamides **9** and **10** probably bind to the **3**·Eu^{III} complex via accommodation of the adamantyl units by the β-cyclodextrin cavities, the biphenyl moiety is too far away from the Eu^{III} center for efficient energy transfer to occur.

Most likely, one of the amide functionalities adjacent to the biphenyl moiety of **7** or **8** coordinates to the Eu^{III} center of **3**·Eu^{III}, thus allowing energy transfer, whereas the sulfonamide groups of **9** and **10** do not coordinate. It has been shown that the coordination strength of amides to Ln^{III} ions is comparable to that of carboxylates,[45] whereas sulfonamides coordinate significantly weaker. The geometry of the 1:1 complex, in which both β-cyclodextrins accommodate an adamantyl group, probably does not allow coordination of the amide functionalities adjacent to the adamantyl units in **8** and **10**. The lack of coordination of the sulfonamide groups of **9** and **10** explains the absence of the spacer length effect on the luminescence enhancement of **7** and **8**. These observations show that also for large sensitizers binding to both β-cyclodextrins of the **3**·Ln^{III} complex, coordination (weak) of the antenna to the lanthanide(III) center is of utmost importance for efficient sensitization of Ln^{III} luminescence.

Conclusions

This work demonstrates that two β-cyclodextrins covalently linked to an EDTA unit cooperate with a ligated luminescent Ln^{III} ion in the noncovalent binding of antenna molecules for sensitized luminescence. This phenomenon requires the presence of cyclodextrins on the host, a Ln^{III}-coordinating site on the antenna guest, and a free coordination site on the Ln^{III} ion. Despite the weak interaction between a carboxylate sensitizer and the EDTA-encapsulated Ln^{III} ion in water, quantitative coordination takes place because the β-cyclodextrins increase the effective concentration of sensitizer in the vicinity of the Ln^{III} center. Coordination of carboxylate sensitizer **5** occurs predominantly in a monodentate fashion and ensures relatively efficient energy transfer as compared to similar systems in which the sensitizer does not coordinate to the Ln^{III} ion.[13,15]

Very strong binding is achieved when both β-cyclodextrin cavities of the host are involved in the complexation of a tailor-made biphenyl sensitizer functionalized with two adamantyl groups. The association constants found are at least 3 orders of magnitude higher than earlier reported values for similar systems.[11−14] Also for these complexes coordination of the sensitizer to the Ln^{III} center is a prerequisite for efficient energy transfer. This indicates that the restriction of motional freedom of the two β-cyclodextrins of a host by complexing to the same guest molecule does not bring the antenna moiety in close enough proximity to the Ln^{III} center.

Acknowledgment. The Netherlands Organization of Scientific Research (NWO-CW) is gratefully acknowledged for financial support. We thank Ms. Hanny Visser for performing the ^{13}C NMR relaxation rate enhancement measurements.

JA017025Y

(45) (a) Brücher, E.; Sherry, A. D. In *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Merbach, A. E., Tóth, E., Eds.; Wiley: Weinheim, 2001. (b) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293.