Potential MRI Contrast Agents Based on Micellar Incorporation of Amphiphilic Bis(alkylamide) Derivatives of $[(Gd-DTPA)(H_2O)]^{2-}$

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DTPA-bisamide derivatives with alkyl chains containing 14, 16 and 18 carbon atoms were synthesized and complexes of various trivalent lanthanide ions (Ln = Gd, La, Pr, Eu) were formed. Variable temperature proton NMR spectroscopy of paramagnetic praseodymium(III) and europium(III) complexes revealed that long aliphatic substituents considerably increase the energy barrier for the intramolecular rearrangement around the lanthanide ion. The gadolinium(III) complexes were incorporated into mixed micelles, and photon correlation spectroscopy showed that the mean sizes of all the micelles were within the same range. The NMRD curves of all three DTPA-bisamide-gadolinium complexes incorporated in mixed micelles display higher relaxivity values than the commercially available Gd–DTPA contrast agent. The higher relaxivity obtained for the micellar DTPA-bisamide-gadolinium complexes with C_{14} and C_{16} chains relative to the micellar DTPA-bisamide-Gd^{III} C_{18} chain complex could be attributable to the fact that the alkyl chain containing 18 carbon atoms is longer than the alkyl chain of the major component of the micelles, DPPC, in which it is inserted. This would allow increased mobility of the polar head and hence a lower relaxivity.

Introduction

Complexes of lanthanides with diethylenetriamine-*N*,*N*,*N*′,*N*′′,*N*′′-pentaacetate ($DTPA^{5-}$) and with its amide derivatives have attracted considerable attention in recent years. The anionic $[Gd(DTPA)(H_2O)]^{2-}$, the first contrast agent approved for use in humans, is nowadays routinely used in clinical magnetic resonance imaging for contrast enhancement under the name Magnevist® (Schering, Berlin, Germany). This paramagnetic complex contains one inner-sphere water molecule[1−4] that exchanges rapidly[5] with the bulk water in the human body, therefore providing an efficient mechanism for the enhancement of the relaxation rates of the water protons. The negative charge of the complex, however, results in a relatively high osmolality of the clinical formulation. In order to neutralize two negative charges of $[Gd(DTPA)(H_2O)]^{2-}$, numerous bis-amide derivatives of DTPA have been developed. The bis-methylamide derivative of Gd−DTPA, Gd−DTPA-BMA, [H_3DTPA-BMA = *N*, *N*′′-bis(methylcarbamoylmethyl)−carboxymethyliminobis(ethylenediamino) diacetic acid] has been in clinical use nearly as long as its parent complex.[6] The practical application of Gd−DTPA-BMA has stimulated extensive research on the synthesis and study of new DTPA amide derivative ligands. In general, the methyl groups of DTPA-BMA have been replaced by various alkyl or aryl groups,[7−16] and in addition, macrocyclic bis(amide) derivatives of DTPA have also been successfully synthesized.[17−19] X-ray crystal structures of the lanthanide(III) complexes of some DTPA bis(amide) derivatives have revealed that the ligands coordinate to the lanthanide ion in an octadentate mode, while the ninth coordination site is occupied by a water molecule.[9,10,14,16] In solution, the presence of H_2O molecule in the inner sphere was detected in the europium(III) complex by luminescence lifetime measurements[3,20] and for some dysprosium(III) complexes by ^{17}O NMR studies.[11]

In attempt to achieve higher proton relaxivity, the Gd^{III} complexes of (DTPA-bisamide) alkyl copolymers with alkyl chains containing six, ten, or twelve $-CH_2$ groups have been synthesized.[21] The unexpectedly high proton relaxivities of these polymers were attributed to the formation of rigid, micelle-like structures in solution, these structures forming as a consequence of hydrophobic interactions between long alkyl chains. Detailed ^{17}O NMR and EPR spectroscopic studies showed that the rates and mechanisms of water exchange of all Gd (DTPA-bisamide) alkyl copolymers are the same as in the monomer chelate unit, and the proton relaxivity difference was explained in terms of differences in global motion.

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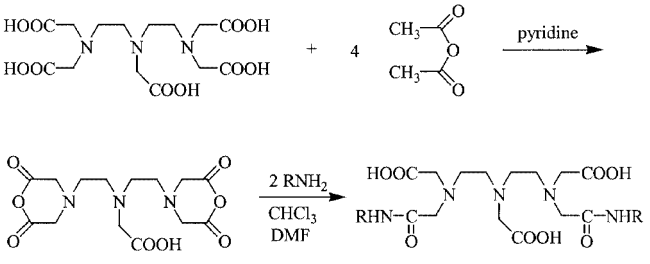
Bis(amide) derivatives of Gd−DTPA containing long alkyl chains were also incorporated into the membranes of liposomal vesicles, yielding gadolinium-labelled liposomes. These liposomes, containing paramagnetic amphiphilic agents, significantly enhanced the proton relaxivity and were shown to be especially suitable for contrast enhancement in the liver, where the highly soluble parent Gd−DTPA complex does not accumulate efficiently.[22] Other attempts to slow down the rotational motion of gadolinium-based contrast agents, and thus to improve their relaxation efficiency, have included the synthesis of macromolecular gadolinium(III) chelates such as dendrimers,[23] linear polymers[24−26] or proteins.[27−29] The strategy proved rather disappointing, since the relaxivity gain obtained by increasing the molecular size is often far less than expected, as a result of internal flexibility or non-rigid attachment of the chelate to the macromolecule.[23−30] An interesting approach to high-relaxivity contrast agents is to synthesize amphiphilic gadolinium(III) complexes which can self-assemble into micelles.[30] An example is the Gd−DOTA derivative (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) with a long alkyl chain, reported by André et al.[31]

In this study we describe the synthesis of neutral, amphiphilic bisamide derivatives of DTPA with alkyl chains containing 14, 16 and 18 carbon atoms. The gadolinium complexes of these ligands were incorporated into mixed micelles to produce supramolecular structures with decreased rotational motion, and the NMRD profiles of the paramagnetic micelles are reported. The solution dynamics of europium and praseodymium complexes were investigated by NMR, and comparison was made with the related Ln-DTPA complexes.

Results and Discussion

The Ligands and Complexes

The ligands DTPA-bis-tetradecylamide (DTPA-BC14), DTPA-bis-hexadecylamide (DTPA-BC16) and DTPA-bis-octadecylamide (DTPA-BC18) were synthesized in high yields according to Scheme 1.



DTPA-BC14, R = $C_{14}H_{29}$
DTPA-BC16, R = $C_{16}H_{33}$
DTPA-BC18, R = $C_{18}H_{37}$

Scheme 1. Synthesis of DTPA bis(amide) derivatives

Under slightly alkaline solutions, these all readily formed complexes with lanthanide(III) ions (Ln = Gd, La, Eu, Pr), as shown in Figure 1. IR spectroscopic data of all ligands showed strong absorptions in the 3350−3360 and 1625−1670 cm^{-1} regions, corresponding to NH and CO stretching modes, respectively.[32] Shifts of around 20 cm^{-1} to lower energy were observed for the carbonyl stretching frequencies upon complexation, indicating amide oxygen coordination to the lanthanide ion. These findings are consistent with previous studies, which have shown that DTPA bis(amide) derivatives coordinate to Ln^{III} through three acetate oxygens, three nitrogen atoms and two carbonyl oxygens of the amide group, while the ninth coordination site is occupied by a water molecule.[9,10,14,16]

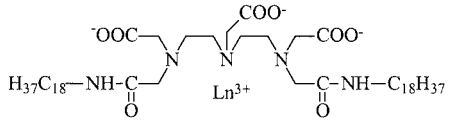


Figure 1. Schematic representation of the Ln−DTPA-BC18 complex; the ligand coordinates to lanthanide ion through three carboxylate oxygens, three nitrogen atoms and two carbonyl oxygens of the amide group, while a water molecule occupies the ninth coordination site

Solution Structures and Dynamics of Ln−DTPA-BC14, DTPA-BC16 and DTPA-BC18 Complexes

The coordination polyhedron of the nine-coordinate lanthanide complexes can best be described as a tricapped trigonal prism,[33] with the bis-amide-DTPA ligand arranged around lanthanide ion in such a way that eight different enantiomers are possible. The eight possible enantiomers of the bound ligand give rise to the complex geometries, in which four are the mirror images of the other four. Thus, in theory, four NMR signals could be detectable for each nucleus in the bis-amide-DTPA ligand. However, rapid interconversion of the bis-amide-DTPA complexes on the NMR timescale very often results in a reduction of the number of NMR signals.[11] In order to examine the solution dynamics of the lanthanide complexes with DTPA-BC14, DTPA-BC16 and DTPA-BC18, the ^1H NMR spectra were recorded at various temperatures. Unfortunately, due to the low solubility of all the complexes in the common NMR solvents we were unable to collect ^{13}C NMR spectra of good quality.

The ^1H NMR spectra of lanthanum(III) complexes with DTPA-BC14, DTPA-BC16 and DTPA-BC18 ligands at room temperature were quite complex, due to the broadening and overlapping of proton resonances originating from various isomers. This made the interpretation of the spectra quite difficult. The proton NMR spectra of the praseodymium(III) complexes were much more informative, however, since the presence of the paramagnetic ion increased the chemical shift range and resulted in separate signals for CH_2 protons. At room temperature (298 K), the praseodymium(III) complexes with DTPA-BC14, DTPA-BC16 and DTPA-BC18 show eighteen resonances for ten acetate and

eight ethylenic protons in the range from −35 to +35 ppm. Exact distinction between ethylenic and acetate resonances was not possible, since this would require use of partially deuterated ligand. It is reasonable to assume that, analogously to the previously assigned proton NMR spectra of the parent Pr−DTPA and Eu−DTPA complexes, the acetate resonances should in general experience significant downfield shifts, while the ethylenic protons should be shifted upfield.[34]

We also extended our observation to the DTPA-BC14, DTPA-BC16 and DTPA-BC18 complexes of europium(III), because we expected that increased charge/size ratio should

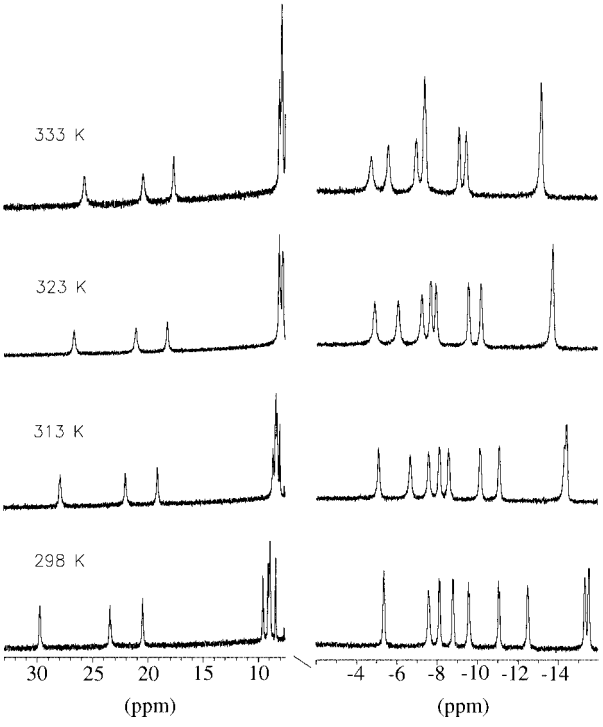


Figure 2. Variable temperature NMR spectra of Eu−DTPA-BC16 in $CDCl_3/CD_3OH$ solution; only the regions containing resonances of acetate protons (at low field) and ethylenic protons (at high field) are shown; the relative assignment was based on the assignment of the parent Eu−DTPA complex reported in ref.[34] the region containing solvent peaks as well as aliphatic proton resonances was omitted for clarity

favour even higher rigidity of the system. As Figure 2 shows, in the proton NMR spectrum of Eu-DTPA-BC16 the "frozen" structures could already be detected at 298 K. In theory, eight possible enantiomers of the bound ligand are possible, and since four of those geometries are mirror images of the other four, four signals can be expected for each group of magnetically equivalent protons in the Eu−DTPA-BC16 ligand. Examination of the NMR spectra shown in Figure 2 suggests that the spectra are consistent with the occurrence either of two static structures or of two pairs of interconverting isomers. To test this, the temperature of the solution was increased to 333 K, but only the coalescence of two pair of peaks was detected. Unfortunately, the chloroform/methanol solution in which the complexes were dissolved did not allow us to record high temperature spectra in order to observe the coalescence process. However, the broadening of the resonances supports the view that the observed spectral pattern is due to the existence of interconverting isomers.

This dynamic behaviour of the praseodymium and europium complexes with DTPA-BC14, DTPA-BC16 and DTPA-BC18 is rather surprising, since the parent Pr−DTPA and Eu−DTPA complexes are known to undergo fast intramolecular rearrangement at room temperature, so that "frozen" structures could be detected only at temperatures below 268 K, while the exchange process leading to coalescence was already observed at 338 K.[34]

Detailed kinetic study on bispropylamide-DTPA complex of neodymium(III) showed that the activation barrier for the conformational interconversion at the middle nitrogen atom in the diethylenetriamine backbone (so called "wagging" process) was virtually the same as the activation barrier of the parent DTPA complex, indicating that the replacement of two carboxylate groups by the relatively weak coordinating amide groups has no influence on this process.[11] However, the inversion of the terminal diethylenetriamine nitrogen atoms, which is characterized by a higher activation barrier, requires partial decomplexation of the ligand. It is likely that, for steric reasons, the long aliphatic substituents on DTPA-BC14, DTPA-BC16 and DTPA-BC18 ligands considerably alter the rate of this inversion, thus increasing the energy barrier for the intramolecular rearrangement around the lanthanide ion.

Mixed Micelle Formation and Size Distribution

The gadolinium(III) complexes of DTPA-BC14, DTPA-BC16 and DTPA-BC18 ligands were incorporated into mixed micelles made of phospholipid (DPPC), a surfactant (Tween 80) and the gadolinium complex. The micelle size was determined at room temperature by photon correlation spectroscopy. All micelles were monodisperse (i.e., there was only one main kind of particles in the size distribution histogram). As can be seen from Figure 3, the mean diameters of the micelles are all within the same range (15−20 nm) and can be regarded as similar. This indicates that the size of the micelles is virtually independent of the length of the bisamide chain, and that the factor that determines the size is the phospholipid DPPC.

Nuclear Magnetic Relaxation Dispersion (NMRD) Measurements

Figure 4 shows the proton NMRD profiles of the relaxivity (defined as the longitudinal relaxation rate enhancement produced by one millimole per litre of Gd complex) of Gd−DTPA-BC14, Gd−DTPA-BC16 and Gd−DTPA-BC18, incorporated in micelles, at 37 °C. As would be expected, the inclusion of the gadolinium complexes into micelles results in an increased relaxivity relative to the parent complex Gd−DTPA and in a maximum at high magnetic fields, induced on the one hand by the decreased rotational mobility of the paramagnetic complex and on the other hand by the increase in the electronic longitudinal relax-

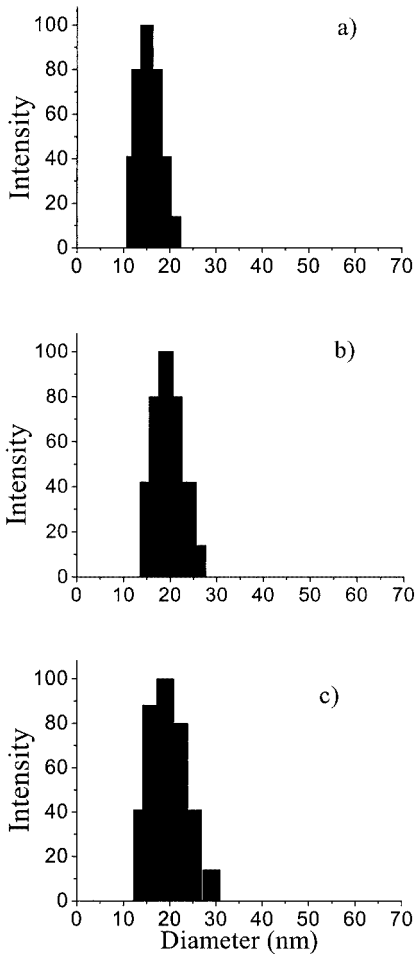


Figure 3. Histogram representing micelle size distribution, as measured by photon correlation spectroscopy: a) Gd−DTPA-BC14; b) Gd−DTPA-BC16; c) Gd−DTPA-BC18

ation time at high fields. The relaxivities of micellar Gd−DTPA-BC14 and Gd−DTPA-BC16 are quite similar, and are larger than for micellar Gd−DTPA-BC18.

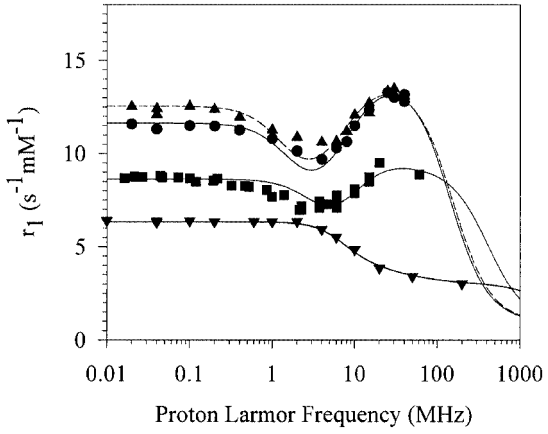


Figure 4. NMRD profile of micellar Gd−DTPA-BC14 (circles), Gd−DTPA-BC16 (triangles up) and Gd−DTPA-BC18 (squares) at 37 °C; the NMRD profile of Gd−DTPA (triangles down) has been added for comparison[47]

The data were analysed by use of the classical inner sphere[35,36] and outer sphere[37] theories. The distance of closest approach was set to 0.36 nm, the relative diffusion constant was fixed to 2.93 10^{-9} m^2s^{-1}, the distance for the inner sphere interaction was set to 0.31 nm, and the number of coordinated water molecule was assumed to be equal to 1 by analogy with other bisamide derivatives of Gd−DTPA.[35,38] The parameters describing the electronic relaxation of Gd (τ_{SO} is the electronic relaxation rate at very low magnetic field and τ_V the correlation time modulating the electronic relaxation) as well as the "global" rotational correlation time (τ_R) and the residence time of the coordinated water molecule (τ_M) were simultaneously fitted. The results are shown in Table 1.

Table 1. Parameters obtained from the theoretical fitting of the proton NMRD data (T = 37 °C)

	τ_R (ns)	τ_M (ns)	τ_{SO} (ps)	τ_V (ps)
Gd−DTPA-BC14	3.05	1300	175	30
Gd−DTPA-BC16	2.97	1280	209	30
Gd−DTPA-BC18	0.62	1470	101	27
Gd−DTPA[a]	0.059	143	82	23
Gd−DTPABMA[b]	0.047	1050	85	24

[a] From ref.[47] τ_R, τ_{SO} and τ_V obtained from the fitting of the proton NMRD data, τ_M obtained from the fitting of the ^{17}O transverse relaxation rates. [b] Calculated at 37 °C with the parameters obtained from the simultaneous fitting of EPR, ^{17}O NMR and NMRD data by Powell et al.[39]

The parameters obtained for Gd−DTPA-BC14 and Gd−DTPA-BC16 are quite similar, with a rotational correlation time of about 3 ns, a value two order of magnitude lower than the rotational correlation time calculated by the Stokes-Einstein theory for a rigid sphere (τ_R ranging between 285 and 675 ns depending on the micelle size). The observed difference can be related to the local motion of the Gd complex within the micellar structure. For micelles made of compound Gd−DTPA-BC18, which contains hydrophobic chains longer than that of DPPC, the fitted τ_R is smaller, indicating an increased local mobility in relation to the other complexes. The τ_M values are similar for the three complexes and larger than for the parent compound Gd−DTPA. Such increases in the residence time of the coordinated water molecule have already been reported for numerous Gd−DTPA bisamide derivatives.[39,40] Moreover, the insertion of both hydrophobic chains of the Gd complex in the micellar structure could also prevent the exchange of the water molecule.

Conclusions

Amphiphilic Gd−DTPA bis(amide) complexes were incorporated into mixed micelles in order to obtain supramolecular structures with decreased mobility. As already reported for other gadolinium complexes incorporated into mixed micelles,[41,42] the relaxivities of the micellar solutions

of the three complexes Gd−DTPA-BC14, Gd−DTPA-BC16 and Gd−DTPA-BC18 are higher than that seen for the hydrophilic Gd−DPTA, as a result of the reduced mobility of the gadolinium complexes. In the case of Gd−DTPA-BC18, the immobilization inside the micellar structure is less effective, probably because the aliphatic chains of the complex are longer than the alkyl chain of DPPC, in which it is inserted. The increase in relaxivity can be related to the lower mobility of the paramagnetic complex inside the micelle. We are currently developing other amphiphilic complexes of gadolinium, which will be incorporated into micelles with the goal of achieving higher increases in relaxivity.

Experimental Section

Chemicals: Reagents were obtained from Aldrich Chemical Co. Inc., Acros Organics and Fluka, and were used without further purification. DTPA was obtained from Koch Light Laboratories. 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was obtained from Genzyme Pharmaceuticals (Switzerland). Gadolinium chloride hexahydrate was obtained from GFS chemicals (USA).

Instruments: Elemental analysis was performed on a CE Instruments EA-1110 elemental analyser. ^{1}H NMR spectra were run on a Bruker Avance 300, operating at 300 MHz. IR spectra were measured on a Bruker IFS66 FT IR spectrometer, in KBr discs. Mass spectra were run on a Q-tof 2 (Micromass, Manchester UK). Samples for the mass spectra were prepared as follows: the complex (2 mg) was dissolved in methanol (1 mL), and 200 μL of this solution was added to 800 μL of a mixture of a 50:50 water methanol solution. The resulting solution was injected, with a flow rate of 5 μL/min.

Synthesis of the Ligands: DTPA bisanhydride was prepared by the published procedure.[43] DTPA-BC14, DTPA-BC16 and DTPA-BC18 were prepared by the method of Jasanda and Nepveu.[44]

Analytical Data for the Ligands

DTPA-BC14: ^{1}H NMR (CF_3COOD, 298 K, ref. CF_3COOD at δ = 11.62 ppm): δ = 0.88 (t, 6 H, 2 × CH_3), 1.31 (m, 44 H, 22 × CH_2), 1.61 (m, 4 H, 2 × CH_2), 3.39 (t, 4 H, 2 × CH_2), 3.80 (m, 4 H, 2 × CH_2), 4.08 (m, 4 H, 2 × CH_2), 4.14 (s, 2 H, CH_2), 4.52 (s, 4 H, 2 × CH_2), 4.54 (s, 4 H, 2 × CH_2) ppm. IR: $\tilde{\nu}$ = 3350 cm^{-1} (N−H amide), 2960, 2920, 2850 (C−H alkyl), 1655 (CO, amide I), 1625 (COOH), 1550 (amide II). $C_{42}H_{81}N_5O_8$ (784.1): calcd. C 64.33, H 10.41, N 8.93; found C 64.42, H 10.39, N 8.99.

DTPA-BC16: ^{1}H NMR (CF_3COOD, 298 K, ref. CF_3COOD at δ = 11.62 ppm): δ = 0.91 (t, 6 H, 2 × CH_3), 1.34 (m, 52 H, 26 × CH_2), 1.64 (m, 4 H, 2 × CH_2), 3.42 (t, 4 H, 2 × CH_2), 3.88 (m, 4 H, 2 × CH_2), 4.12 (m, 4 H, 2 × CH_2), 4.18 (s, 2 H, CH_2), 4.56 (s, 4 H, 2 × CH_2), 4.58 (s, 4 H, 2 × CH_2) ppm. IR: $\tilde{\nu}$ = 3356 cm^{-1} (N−H amide), 2960, 2918, 2849 (C−H alkyl), 1666 (CO, amide I), 1624 (COOH), 1541 (amide II). $C_{46}H_{89}N_5O_8$ (840.2): calcd. C 65.76, H 10.68, N 8.34; found C 65.80, H 10.64, N 8.35.

DTPA-BC18: ^{1}H NMR (CF_3COOD, 298 K, ref. CF_3COOD at δ = 11.62 ppm): δ = 0.90 (t, 6 H, 2 × CH_3), 1.34 (m, 60 H, 30 × CH_2), 1.63 (m, 4 H, 2 × CH_2), 3.41 (t, 4 H, 2 × CH_2), 3.82 (m, 4 H, 2 × CH_2), 4.11 (m, 4 H, 2 × CH_2), 4.18 (s, 2 H, CH_2), 4.56 (s, 4 H, 2 × CH_2), 4.58 (s, 4 H, 2 × CH_2) ppm. IR: $\tilde{\nu}$ = 3350 cm^{-1} (N−H amide), 2960, 2920, 2850 (C−H alkyl), 1655 (CO, amide I), 1625 (COOH), 1550 (amide II). $C_{50}H_{97}N_5O_8$ (896.3): calcd. C 66.99, H 10.90, N 7.81; found C 66.82, H 10.85, N 7.77.

Synthesis of the Ln^{III} Complexes: All complexes were synthesized by the General Procedure as follows: a solution of hydrated $LnCl_3$ salt (1.1 mmol) in H_2O (1 mL) was added to the ligand (1 mmol) dissolved in pyridine (30 mL), and the mixture was heated at 70 °C for 3 hours. The solvents were evaporated under reduced pressure and the crude product was then heated at reflux in ethanol for 1 hour. After the mixture had cooled to room temperature, the complex was filtered off and dried in vacuo. The absence of free gadolinium was checked with xylenol orange indicator.[45]

Analytical Data for the Ln^{III} Complexes

Gd−DTPA-BC14: $C_{42}H_{78}N_5GdO_8$ (938.4): calcd. C 53.76, H 8.37, N 7.32; found C 53.49, H 8.64, N 7.28. IR: $\tilde{\nu}$ = 3093 cm^{-1} (N−H amide), 2919, 2850 (C−H alkyl), 1667 (CO, amide I), 1617 (COO^- assym. stretch), 1394 (COO^- sym. stretch). ES-MS+: *m/z* = 939 ($[M + H]^+$), 961 ($[M + Na]^+$), 1430 ($[3M + 2Na]^{2+}$).

Eu-DTPA-BC14: $C_{42}H_{80}N_5EuO_9$ (951.1): calcd. C 52.89, H 8.48, N 7.36; found C 53.04, H 8.38, N 7.25. IR: $\tilde{\nu}$ = 3093 cm^{-1} (N−H amide), 2923, 2854 (C−H alkyl), 1631 (CO, amide I), 1600 (COO^- assym. stretch), 1394 (COO^- sym. stretch). ES-MS+: *m/z* = 956.5 ($[M + Na]^+$), 1422 ($[3M + 2Na]^{2+}$), 1890 ($[2M + Na]^+$).

Pr-DTPA-BC14: $C_{42}H_{78}N_5O_8Pr$ (922.0): calcd. C 54.71, H 8.53, N 7.60; found C 55.00, H 8.63, N 7.56. IR: $\tilde{\nu}$ = 3088 cm^{-1} (N−H amide), 2920, 2850 (C−H alkyl), 1640 (CO, amide I), 1600 (COO^- assym. stretch), 1386 (COO^- sym. stretch). ES-MS+: *m/z* = 945 ($[M + Na]^+$), 1405 ($[3M + 2Na]^{2+}$), 1867 ($[2M + Na]^+$).

La-DTPA-BC14: $C_{42}H_{78}LaN_5O_8$ (920.0): calcd. C 54.83, H 8.54 , N 7.61; found C 54.37, H 8.39, N 7.44. IR: $\tilde{\nu}$ = 3089 cm^{-1} (N−H amide), 2926, 2850 (C−H alkyl), 1641 (CO, amide I), 1600 (COO^- assym. stretch), 1386 (COO^- sym. stretch). ES-MS+: *m/z* = 943 ($[M + Na]^+$), 1403 ($[3M + 2Na]^{2+}$), 1862 ($[2M + Na]^+$).

Gd−DTPA-BC16: $C_{46}H_{86}GdN_5O_8$ (994.5): calcd. C 55.56, H 8.72, N 7.04; found C 56.39, H 8.74, N 7.02. IR: $\tilde{\nu}$ = 3093 cm^{-1} (N−H amide), 2960, 2918, 2849 (C−H alkyl), 1666 (CO, amide I), 1624 (COO^- assym. stretch), 1395 (COO^- sym. stretch). ES-MS+: *m/z* = 1017 ($[M + Na]^+$), 1514 ($[3M + 2Na]^{2+}$).

Eu-DTPA-BC16: $C_{46}H_{88}EuN_5O_9$ (1007.2): calcd. C 54.86, H 8.81, N 6.95; found C 54.53, H 8.73, N 6.80. IR: $\tilde{\nu}$ = 3090 cm^{-1} (N−H amide), 2919, 2850 (C−H alkyl), 1635 (CO, amide I), 1601 (COO^- assym. stretch), 1391 (COO^- sym. stretch). ES-MS+: *m/z* = 1035 ($[M + Na]^+$), 1540 ($[3M + 2Na]^{2+}$), 2047 ($[2M + Na]^+$).

Pr-DTPA-BC16: $C_{46}H_{86}N_5O_8Pr$ (978.1): calcd. C 56.49, H 8.86, N 7.16, found C 56.61, H 8.74, N 7.09. IR: $\tilde{\nu}$ = 3094 cm^{-1} (N−H amide), 2921, 2844 (C−H alkyl), 1635 (CO, amide I), 1596 (COO^- assym. stretch), 1395 (COO^- sym. stretch). ES-MS+: *m/z* = 1001 ($[M + Na]^+$), 1490 ($[3M + 2Na]^{2+}$), 1977 ($[2M + Na]^+$).

La-DTPA-BC16: $C_{46}H_{88}LaN_5O_9$ (994.1): calcd. C 55.58, H 8.92, N 7.04; found C 55.50, H 8.73, N 6.90. IR: $\tilde{\nu}$ = 3085 cm^{-1} (N−H amide), 2920, 2850 (C−H alkyl), 1667 (CO, amide I), 1624 (COO^- assym. stretch), 1385 (COO^- sym. stretch). ES-MS+: *m/z* = 999 ($[M + Na]^+$), 1486 ($[3M + 2Na]^{2+}$), 1975 ($[2M + Na]^+$).

Gd−DTPA-BC18: $C_{50}H_{96}GdN_5O_9$ (1068.6): calcd. C 56.14, H 9.05, N 6.55; found C 56.09, H 9.55, N 6.38. IR: $\tilde{\nu}$ = 3094 cm^{-1} (N−H amide), 2960, 2920, 2850 (C−H alkyl), 1633 (CO, amide I), 1596 (COO^- assym. stretch), 1390 (COO^- sym. stretch). ES-MS+: *m/z* = 1073 ($[M + Na]^+$), 1599 ($[3M + 2Na]^{2+}$).

Eu-DTPA-BC18: $C_{50}H_{100}EuN_5O_{11}$ (1099.3): calcd. C 54.62, H 9.16, N 6.37; found C 54.58, H 9.87, N 6.23. IR: $\tilde{\nu}$ = 3105 cm^{-1} (N−H amide), 2916, 2851 (C−H alkyl), 1634 (CO, amide I), 1595 (COO^- assym. stretch), 1386 (COO^- sym. stretch). ES-MS+: *m*/*z* = 1068 ($[M + Na]^+$)

Pr-DTPA-BC18: $C_{50}H_{96}N_5O_9Pr$ (1052.2): calcd. C 57.07, H 9.20, N 6.66; found C 57.46, H 9.19, N 6.57. IR: $\tilde{\nu}$ = 3089 cm^{-1} (N−H amide), 2915, 2844 (C−H alkyl), 1635 (CO, amide I), 1597 (COO^- assym. stretch), 1386 (COO^- sym. stretch). ES-MS+: *m*/*z* = 1057 ($[M + Na]^+$)

La-DTPA-BC18: $C_{50}H_{98}LaN_5O_{10}$ (1068.3): calcd. C 56.22, H 9.24, N 6.56; found C 56.19, H 8.82, N 6.39. IR: $\tilde{\nu}$ = 3083 cm^{-1} (N−H amide), 2925, 2850 (C−H alkyl), 1640 (CO, amide I), 1596 (COO^- assym. stretch), 1385 (COO^- sym. stretch). ES-MS+: *m*/*z* = 1055 ($[M + Na]^+$)

Sodium and Potassium Ion Content Measurements: Before the compounds were used for incorporation into micelles, the potassium and sodium ion contents were checked by flame photometry (IL, 943, Instrumentation Laboratories, Massachusetts, USA). The Na^+ and K^+ ion contents were measured on samples obtained by extraction of the solutions of complex (1.7 mg) dissolved in a 1:1 chloroform/methanol mixture with 500 μL of water. Na^+ contents (mmol:mol of the complex): (Gd−DTPA-BC14) 0.10, (Gd−DTPA-BC16) 0.15, (Gd−DTPA-BC18) 0.15. K^+ contents: no K^+ was found in any of the complexes.

Preparation of Micelles: 1,2-Dipalmitoyl *sn-glycero*-3 phosphatidyl choline (DPPC, 225 mg) and the complex (25 mg) were dissolved in a 1:1 solution of chloroform/methanol (50 mL). Evaporation under reduced pressure yielded a thin film, which was then rehydrated with hot water (5 mL, 70 °C). This solution was sonicated for 15 min with a 70-W sonicator while the temperature was maintained at 65 °C with a thermostatic bath. After the sonication, 75 mg of a surfactant was added (Tween 80® − polyoxyethylene sorbitan monooleate), followed by another 15 minutes of sonication.

Determination of Micelle Size: The mean micelle sizes were determined by photon correlation spectroscopy performed with a BIC multiangle laser light scattering system at room temperature and with a 90° scattering angle (Brookhaven Instruments Corporation, Holtsville, USA). The intensity weighted micellar diameter was measured on diluted suspensions and calculated by a non-negatively constrained least-squares (multiple pass) routine. The diameters obtained are as follows: micelles with (Gd−DTPA-BC14): 15 nm, with (Gd−DTPA-BC16): 20 nm, with (Gd−DTPA-BC18): 19 nm.

T_1 Measurements: Proton Nuclear Magnetic Relaxation Dispersion (NMRD) profiles were recorded between 0.24 mT and 1.2 T with Field Cycling Relaxometers (Field Cycling Systems, Oradell, New Jersey, USA or Stelar Spinmaster FFC-2000, Stelar S.R.L., Mede, Italy) on 0.6 mL solutions contained in 10 mm o.d. tubes. Proton relaxation rates were also measured at 0.47 T and 1.5 T on Minispec PC-120 and mq-60 instruments (Bruker, Karlsruhe, Germany). All samples were diluted 10 times and the temperature was maintained at 37 °C. ^1H NMRD data were fitted according to the theoretical inner-sphere model described by Solomon[35] and Bloembergen[36] and to the outer-sphere contribution described by Freed.[37] Calculations were performed with the aid of previously described software.[46−48]

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