Gadolinium DTPA-Monoamide Complexes Incorporated into Mixed Micelles as Possible MRI Contrast Agents

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Four monoamide derivatives of Gd–DTPA with alkyl chains consisting of 12, 14, 16, or 18 carbon atoms were synthesized. The gadolinium(III) complexes with chain lengths of 14, 16 or 18 carbon atoms were efficiently incorporated into mixed micelles whereas the complex with a chain length of 12 carbon atoms was not incorporated into a micellar structure. The size distribution of the micelles was measured by photon correlation spectroscopy. The mean sizes of the micelles for all the complexes lay within a narrow range, typically between 11 and 20 nm. The NMRD curves of the gadolinium(III) DTPA-monoamide complexes incorporated into mixed micelles display higher relaxivity values than the commercially available Gd–DTPA contrast agent. Moreover, micelles with gadolinium DTPA-monoamide complexes showed higher relaxivities than micelles containing the corresponding gadolinium DTPA-bis(amide) complexes, most likely because of a more efficient exchange of the coordinated water molecule.

Introduction

MRI (magnetic resonance imaging) contrast agents are routinely used in medicine because they provide reliable results that assist in the rapid clinical interpretation of MRI images. Most of the commonly used contrast agents achieve their effect by enhancing the relaxation rate of water protons in tissues. In general, contrast agents consist of a paramagnetic metal centre, typically gadolinium(III), which must be complexed to a strong chelating ligand, since the free metal ions are toxic at the concentrations needed for diagnosis. Water-soluble, anionic $[Gd(DTPA)(H_2O)]^{2-}$ was the first contrast agent approved for use in humans and is currently in routine use as a clinical magnetic resonance imaging agent under the name Magnevist® (Schering, Berlin, Germany). This complex contains one inner-sphere water molecule[1–4] that exchanges rapidly with bulk water,[5] providing an efficient relaxation of the surrounding water protons. However, $[Gd(DTPA)(H_2O)]^{2-}$ is a nonspecific contrast agent since its hydrophilicity results in an efficient enhancement of contrast only through its preferential distribution in the bloodstream. In recent years, contrast agents with improved characteristics, such as increased efficacy and organ specificity, have been sought.

Several different approaches to slow the rotational motion of gadolinium-based contrast agents, and thus to improve their relaxation efficiency, have been reported in the literature. These include synthesis of covalently or noncovalently bound macromolecular gadolinium(III) chelates such as dendrimers,[6] linear polymers[7–9] or proteins.[10,11] Some of these strategies proved to be rather disappointing since the relaxivity gain obtained by increasing the molecular size was often far less than expected as a result of some internal flexibility or the nonrigid attachment of the chelate to the macromolecule.[6–11]

An increase in the relaxivity could also be achieved by the synthesis of molecules with an efficient targeting system that is able to provide interactions between the agent and specific macromolecular receptors. For these purposes various hydrophobic groups have been introduced into the DTPA molecule through amidation of monoacid tetraester DTPA derivatives. The paramagnetic complexes of these compounds were investigated for the detection of tumors and myocardial infections.[12–14] In addition, incorporation of certain amphipathic gadolinium(III) complexes into liposomal membranes yielded paramagnetically labeled liposomes which significantly enhanced proton relaxivity.[15–21] Liposomes bearing gadolinium(III) complexes with long alkyl chains are expected to be less toxic and more tissue-specific, and their potential to deliver various MRI contrast

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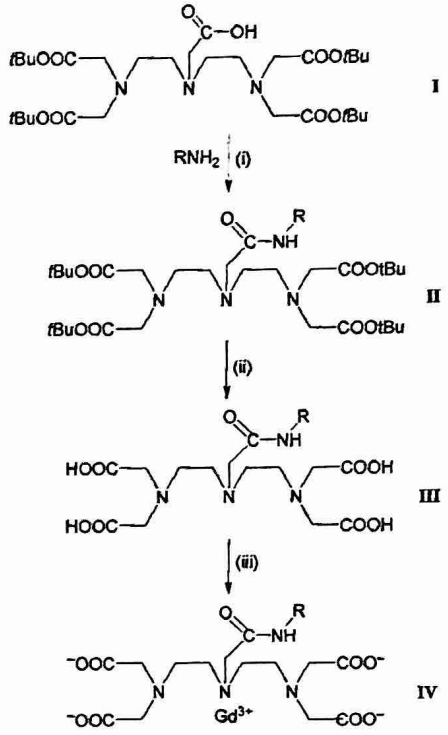
enhancement agents to selective sites has also been investigated.[15–21]

Another interesting strategy for the development of new types of contrast reagents is the synthesis of amphiphilic gadolinium(III) complexes which can self-assemble to form micelles.[22,23] One example of this is the Gd–DOTA derivative (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) with a long alkyl chain, which was reported by André et al.[24] This compound displayed high relaxation rates because of an increase in the rotational correlation time. Alternatively, the lipophilic gadolinium(III) complexes can be incorporated into mixed micelles in order to obtain supramolecular systems with a better control of the size.[25] We recently described the synthesis of amphiphilic bis(amide) derivatives of DTPA with alkyl chains consisting of 12, 14, 16, and 18 carbon atoms, whose gadolinium(III) complexes were incorporated into mixed micelles.[26] The resulting supramolecular structures showed an increase in relaxivity relative to Gd–DTPA, because of the lower mobility of the paramagnetic complex inside the micelle. In order to investigate the effects of the structure of the lipophilic gadolinium(III) complex on the overall relaxivity of the micelles, we have now synthesized amphiphilic monoamide derivatives of DTPA with alkyl chains of various lengths. The gadolinium complexes of these ligands were incorporated into mixed micelles and the NMRD profiles of these paramagnetic micelles are reported.



Scheme 1. Synthesis of DTPA monoamide derivatives; reaction conditions: (i) DEPC, TEA, DMF; (ii) 6 M HCl; (iii) pyridine, $GdCl_3$

Results and Discussion

Ligands and Complexes

The ligands DTPA-monolaurylamide (DTPA–MC12), DTPA-monomyristylamide (DTPA–MC14), DTPA-monopalmitylamide (DTPA–MC16) and DTPA-monostearylamide (DTPA–MC18) were synthesized according to Scheme 1.

Infrared spectroscopic data of all the ligands showed strong absorptions in the regions around 3410 and 1745–1670 cm^{-1}, corresponding to NH and CO stretching modes, respectively.[27] Upon complexation with gadolinium(III), the CO stretching vibrations of the free acid groups disappear, while the CO stretching mode of the amide shifts ca. 40 cm^{-1}, indicating amide oxygen coordination to the lanthanide ion. Although the negative-mode electron-spray mass spectra were not very informative, the positive-mode electron-spray mass spectra of Gd–DTPA–MC12, Gd–DTPA–MC14, Gd–DTPA–MC16 and Gd–DTPA–MC18 in chloroform/methanol solutions showed cluster peaks at *m/z* values of 716, 744, 772, and 800, respectively, which indicate coordination of gadolinium(III) to the ligand. These results are consistent with the formation of complexes in which DTPA–MC12, DTPA–MC14, DTPA–MC16 and DTPA–MC18 act as octadentate ligands, and where the ninth coordination site of gadolinium(III) is occupied by a water molecule (Figure 1). Initially, it might seem unusual that these negatively charged complexes were detected in the positive-mode electron-spray mass spectra. However, we used the positive ESI-MS for the best sensibility of the technique. In the ESI spectra, we observed protonated molecular ions or adducts of sodium. This can be explained by the fact that the molecules contain basic groups (carboxylate and amine groups) that can be protonated readily. Moreover, these groups can exhibit Lewis basicity, forming adducts with sodium ions.

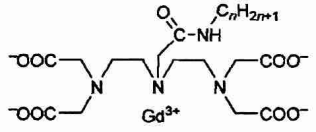


Figure 1. Schematic representation of the Gd–DTPA-monoamide complex: the ligand coordinates to the gadolinium(III) ion through four acetate oxygen atoms, three nitrogen atoms and one carbonyl oxygen atom of the amide group, while a water molecule occupies the ninth coordination site

^1H NMR Studies of the Complexes

The low solubility of the lanthanide(III) complexes of DTPA–MC14, DTPA–MC16 and DTPA–MC18 limited the scope of solution studies. A range of deuterated solvents including D_2O, $CDCl_3$, CD_2Cl_2, $[D_6]DMSO$, CD_3OH, CD_3CN, $(CD_3)_2CO$, and various combinations of these solvents, were used in attempts to obtain solutions of the complexes suitable for NMR measurements. Unfortunately, the concentration of most of the solutions was too low to obtain good-quality NMR spectra. However, in the case of

the La–DTPA–MC18 complex, it was possible to obtain a relatively well-resolved proton NMR spectrum in [D_6]DMSO/CD_3OH solution. This spectrum showed that the DTPA resonances of the complex are significantly broadened compared to the free ligand. As previously described for the bis(amide)-DTPA complexes, the broadening of DTPA resonances indicates the presence of several interconverting isomers in solution.[26] Variable-temperature NMR studies on this complex did not provide more information about the exchange process since the strong solvent peaks at δ = 2.61 ppm ([D_6]DMSO) and 3.28 ppm (CD_3OH) obscured some of the DTPA resonances.

Mixed Micelle Formation and Size Distribution

A phospholipid (DPPC) and a surfactant (Tween 80), whose structures are given in the Supporting Information, together with the gadolinium(III) complexes of DTPA–MC12, DTPA–MC14, DTPA–MC16, and DTPA–MC18 ligands were used to prepare mixed micelles. The micelle size was determined at room temperature by photon correlation spectroscopy. All micelles were monodisperse. The mean diameters of the micelles were all within a narrow range (11–20 nm, see Supporting Information), indicating that their size is virtually independent of the length of the monoamide chain, and that the phospholipid DPPC determines micelle size. A schematic representation of a mixed micelle incorporating a gadolinium(III) complex is shown in Figure 2.

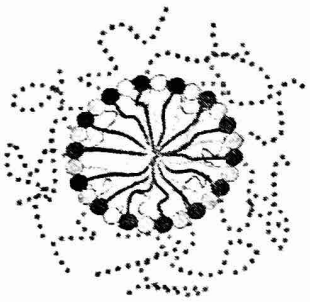


Figure 2. Schematic representation of the amphiphilic gadolinium(III) complex incorporated into a mixed micelle: gadolinium(III) complex (black circles and lines), DPPC phospholipid (gray circles and lines), Tween® 80 (gray dots)

Nuclear Magnetic Relaxation Dispersion (NMRD) Measurements

Figure 3 shows the proton NMRD profiles of the relaxivity of Gd–DTPA–MC12, Gd–DTPA–MC14, Gd–DTPA–MC16 and Gd–DTPA–MC18 in micellar solution at 37 °C. As expected, the inclusion of the gadolinium(III) complexes Gd–DTPA–MC14, Gd–DTPA–MC16 and Gd–DTPA–MC18 into micelles results in an increased relaxivity when compared with the parent complex Gd–DTPA. The hump at higher field strengths (20–60 MHz), which is typical for supramolecular structures, is also seen here. As explained previously, this is caused by a decrease in the rotational mobility of the paramagnetic complex.[26] On the other hand, the proton NMRD profile of Gd–DTPA–MC12 in micellar solution is much less intense and does not reach a maximum at high magnetic fields. This behaviour is typical of a hydrophilic gadolinium(III) complex of low molecular weight and suggests that this complex is very loosely bound or not incorporated at all into the micellar structure.

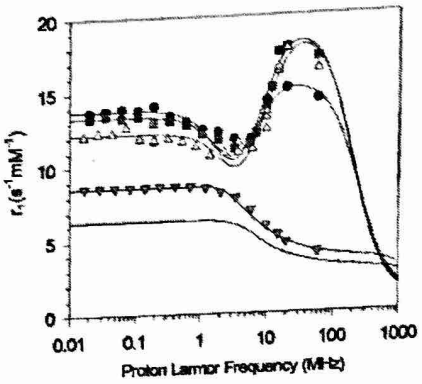


Figure 3. NMRD profiles of micellar solutions of Gd–DTPA–MC12 (triangles down), Gd–DTPA–MC14 (circles), Gd–DTPA–MC16 (squares) and Gd–DTPA–MC18 (triangles up), at 37 °C; the NMRD profile of Gd–DTPA (line underneath) has been added for comparison[32]

The data were analysed using the classical inner-sphere[28–29] and outer-sphere[30] theories. The distance of closest approach was set at 0.36 nm, the relative diffusion constant was fixed at 3.3×10^{-9} m^2s^{-1} and the distance for the inner-sphere interaction was set at 0.31 nm. The number of coordinated water molecules was assumed to be equal to one by analogy with other derivatives of Gd–DTPA.[31–33] The parameters describing the electronic relaxation of Gd (the electronic relaxation time at very low magnetic field, τ_{SO}, and the correlation time modulating the electronic relaxation, τ_V) 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)

Compound	τ_R [ns]	τ_M [ns]	τ_{SO} [ps]	τ_V [ps]
Gd–DTPA–MC12	0.105	310	210	40
Gd–DTPA–MC14	0.91	680	194	41
Gd–DTPA–MC16	1.12	510	154	39
Gd–DTPA–MC18	1.07	500	127	38
Gd–DTPA[a]	0.059	143	82	23
Gd–DTPA–BMA[b]	0.064	967	76	23

[a] From ref.[32]: 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] From ref.[33]

The parameters obtained for Gd–DTPA–MC14, Gd–DTPA–MC16 and Gd–DTPA–MC18 are quite similar, having a rotational correlation time of about 1 ns.

This value is at least two orders of magnitude lower than the rotational correlation time calculated by the Stokes–Einstein theory for a rigid sphere (τ_R ranging between 112 and 673 ns depending on the micelle size). The observed difference can be related to the local motion of the gadolinium(III) complex within the micellar structure. The values of τ_M are in the same range for the three complexes, and are larger than for the parent compound Gd–DTPA but smaller than that of Gd–DTPA–BMA [a hydrophilic bis(amide) derivative of Gd–DTPA]. Indeed it is well known that Gd–DTPA derivatives with amide groups are characterized by a decreased exchange rate of the coordinated water molecule and that τ_M is related to the number of amide functions and their substituents.[34]

The values for τ_R are smaller than the ones reported for the corresponding bis(amide) derivatives incorporated into the same micellar structure. This is probably related to a more efficient immobilisation of the paramagnetic part of the bis(amide) derivatives caused by the incorporation of both hydrophobic chains into the micellar membrane. These smaller τ_R values are not reflected by a subsequent decrease but by an increase in relaxivity because of the smaller τ_M values, which induce an opposing effect. Moreover, the relaxivity of the micellar solution of the Gd–DTPA–MC18 complex bearing an amide chain of 18 carbon atoms, and thus longer than the DPPC chains, is similar to that of its analogue with 16 carbon atoms, at the magnetic field strengths used for imaging.

The τ_R value obtained for the complex Gd–DTPA–MC12 in the micellar solution is much lower than those of the other complexes, which indicates that this complex is either very loosely incorporated inside the micellar membrane or not incorporated at all. It seems that the aliphatic chain of this complex is too short and the hydrophobic character of the complex too low to allow efficient incorporation into the micellar structures.

Conclusion

While it appears that the gadolinium(III) complex of Gd–DTPA–MC12 does not form mixed micelles with DPPC and Tween 80, the gadolinium(III) complexes of DTPA–MC14, DTPA–MC16, and DTPA–MC18 ligands were successfully incorporated into mixed micelles to obtain supramolecular structures with a decreased mobility. The relaxivities of solutions containing these micelles are significantly higher than of solutions of Gd–DTPA as a result of the reduced mobility of the gadolinium(III) complexes. At imaging fields (20–60 MHz), all micellar solutions show similar relaxivities, which is in contrast to the bis(amide) complexes in which the lowest relaxivities were observed for the complex containing the longest side chains.[26] This observation suggests that the insertion of the monoamide compound Gd–DTPA–MC18 is similar to that of the homologues with shorter chain length despite being inserted into micelles composed of DPPC, whose alkyl chain is shorter. By a comparison of the relaxivity data of the monoamide compounds with those of the previously reported bis(amide) compounds, we can conclude that the relaxivity gain, relative to Gd–DTPA, is higher for mixed micelles in which monoamide complexes are inserted than for the micelles in which bis(amide) compounds of the same chain length are inserted.

Experimental Section

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

Instruments: ^{1}H NMR spectra were obtained using a Bruker Avance 300 operating at 300 MHz. IR spectra (KBr pellets) were measured with a Bruker IFS66 FTIR spectrometer. Mass spectra were obtained using a Q-tof 2 (Micromass, Manchester UK). Samples (0.1 g) were dissolved in methanol/water (50:50) and infused at a rate of 5 μL/min.

Synthesis of the Ligands

***N,N*-Bis(2-{bis[2-(1,1-dimethylethoxy)-2-oxoethyl]amino}ethyl)-glycine (Product I in Scheme 1):** This compound was prepared according to a published procedure.[35] Yield 45%. IR: $\tilde{\nu}$ = 2977, 2935 (C–H alkyl stretch); 1733 (CO, ester); 1646 (COOH) cm^{-1}. ^{1}H NMR ([D$_6$]DMSO): δ = 1.45 (s, 36 H, 12 × CH$_3$), 2.98 (t, 4 H, 2 × CH$_2$), 3.10 (br., 4 H, 2 × CH$_2$), 3.36 (s, 8 H, 4 × CH$_2$), 3.58 (s, 2 H, CH$_2$) ppm. ES-MS$^+$: *m/z* = 618 [M + H]$^+$ (C$_{30}$H$_{55}$N$_3$O$_{10}$, $M_{calcd.}$ = 617).

DTPA-Monoamides: The alkylamine (4.72 mmol) was added to a solution of *N,N*-bis(2-{bis[2-(1,1-dimethylethoxy)-2-oxoethyl]-amino}ethyl)glycine (4.72 mmol) in DMF (80 mL). After the dropwise addition of DEPC [(EtO)$_2$P(O)CN] (0.8 mL, 5.28 mmol), the reaction mixture was kept at 3 °C by cooling in an ice bath. Triethylamine (5.92 mmol) was then added slowly to the solution. After 10 min, the amine had fully reacted and the solution was warmed to room temperature and allowed to stand for 30 min. DMF was then removed by evaporation under reduced pressure. The crude product dissolved in ethyl acetate (132 mL) and washed with saturated aqueous sodium hydrogen carbonate (2 × 100 mL) then saturated aqueous sodium chloride solution (2 × 80 mL) to obtain product **II** in Scheme 1. Product **II** (838 mg, 1.05 mmol) was dissolved in 6 N HCl (8 mL). The mixture was stirred at room temperature for 1 h and then washed with dichloromethane (2 × 10 mL). The aqueous phase was concentrated to give DTPA-monamide (product **III** in Scheme 1).

Analytical Data for the Ligands

DTPA–MC12: Yield 73% (1.93 g). IR: $\tilde{\nu}$ = 3422 (N–H stretch), 2938, 2851 (C–H alkyl stretch), 1745 (CO acid), 1674 (CO, amide I) cm^{-1}. ^{1}H NMR (CDCl$_3$): δ = 0.80 (t, 3 H, CH$_3$), 1.1–1.3 (m, 20 H, 10 × CH$_2$), 2.7 (t, 4 H, 2 × CH$_2$), 2.8 (t, 4 H, 2 × CH$_2$), 3.35 (t, 2 H, CH$_2$), 3.4 (s, 2 H, CH$_2$), 3.9 (s, 8 H, 4 × CH$_2$), 4.4 (s, 4 H, 4 × OH) ppm. ES-MS$^+$: 561 [M + H]$^+$ (C$_{26}$H$_{48}$N$_4$O$_9$, $M_{calcd.}$ = 560).

DTPA–MC14: Yield 14% (390 mg). IR: $\tilde{\nu}$ = 3422 (N–H stretch), 2941, 2851 (C–H alkyl stretch), 1745 (CO acid), 1674 (CO, amide I) cm^{-1}. ^{1}H NMR (MeOD, CDCl$_3$): δ = 0.89 (t, 3 H, CH$_3$), 1.27

(s, 22 H, 11 × CH_2), 1.55 (m, 2 H, CH_2), 2.89 and 3.02 (m, 8 H, 4 × CH_2), 3.45 (m, 4 H, 2 × CH_2), 3.92 (s, 8 H, 4 × CH_2) ppm. ES-MS$^+$: 589 $[M + H]^+$ ($C_{28}H_{52}N_4O_9$, $M_{calcd.}$ = 588).

DTPA–MC16: Yield (PA) 12% (349 mg). IR: $\tilde{\nu}$ = 3424 (N–H stretch), 2932, 2851 (C–H alkyl stretch), 1745 (CO acid), 1674 (CO, amide I) cm^{-1}. ^{1}H NMR (MeOD, $CDCl_3$): δ = 0.83 (t, 3 H, CH_3), 1.27 (s, 26 H, 13 × CH_2), 1.39 (m, 2 H, CH_2), 2.70 and 3.05 (m, 8 H, 4 × CH_2), 4.12 (s, 8 H, 4 × CH_2) ppm. ES-MS$^+$: 617 $[M + H]^+$ ($C_{30}H_{56}N_4O_9$, $M_{calcd.}$ = 616).

DTPA–MC18: Yield (SA) 22% (669 mg). IR: $\tilde{\nu}$ = 3432 (N–H stretch), 2921, 2843 (C–H alkyl stretch), 1745 (CO acid), 1671 (CO, amide I) cm^{-1}. ^{1}H NMR (MeOD/$CDCl_3$): δ = 0.89 (t, 3 H, CH_3), 1.27 (s, 30 H, 15 × CH_2), 1.55 (m, 2 H, CH_2), 2.89 and 3.02 (m, 8 H, 4 × CH_2), 3.45 (m, 4 H, 2 × CH_2), 3.92 (s, 8 H, 4 × CH_2) ppm. ES-MS$^+$: 667 $[M + Na]^+$, 645 $[M + H]^+$ ($C_{32}H_{60}N_4O_9$, $M_{calcd.}$ = 644).

Synthesis of the Gadolinium(III) Complexes (Product IV in Scheme 1): The complexes were synthesized according to the general procedure as follows: A solution of the hydrated $GdCl_3$ 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 h. The solvents were evaporated under reduced pressure and the crude product was then refluxed in ethanol for 1 h. After cooling to room temperature, the complex was filtered off and dried in vacuo. The absence of free gadolinium was confirmed using a xylenol orange indicator.[36]

Analytical Data for the Complexes

Gd–DTPA–MC12: Yield 74% (529 mg). IR: $\tilde{\nu}$ = 3408 (N–H stretch), 3092 (N–H amide), 2930, 2850 (C–H alkyl stretch), 1634 (CO, amide I/COO^- assym. stretch) cm^{-1}. ES-MS$^+$: 716 $[M + H]^+$ ($C_{26}H_{45}GdN_4O_9$, $M_{calcd.}$ = 715).

Gd–DTPA–MC14: Yield 85% (631 mg). IR: $\tilde{\nu}$ = 3410 (N–H stretch), 3090 (N–H amide), 2931, 2856 (C–H alkyl stretch), 1632 (CO, amide I/COO^- assym. stretch) cm^{-1}. ES-MS$^+$: 744 $[M + H]^+$($C_{28}H_{49}GdN_4O_9$, $M_{calcd.}$ = 743).

Gd–DTPA–MC16: Yield 61% (470 mg). IR: $\tilde{\nu}$ = 3410 (N–H stretch), 3094 (N–H amide), 2922, 2849 (C–H alkyl stretch); 1630 (CO, amide I/COO^- assym stretch) cm^{-1}. ES-MS$^+$: 772 $[M + H]^+$ ($C_{30}H_{53}GdN_4O_9$, $M_{calcd.}$ = 771).

Gd–DTPA–MC18: Yield 67% (535 mg). IR: $\tilde{\nu}$ = 3401 (N–H stretch), 3094 (N–H amide), 2926, 2848 (C–H alkyl stretch), 1630 (CO, amide I/COO^- assym. stretch) cm^{-1}. ES-MS$^+$: 800 $[M + H]^+$ ($C_{32}H_{57}GdN_4O_9$, $M_{calcd.}$ = 799).

Sodium/Potassium Content: Prior to incorporation into micelles, the potassium and sodium ion contents of the compounds were checked by flame photometry (IL, 943, Instrumentation Laboratories, Massachusetts, USA). The Na^+ and K^+ ion contents were measured using a sample that had been obtained by extracting a solution of complex (1.6 mg) in chloroform/methanol (10:1) with 2 mL of water. Na^+ content (mmol/mol of the complex): Gd–DTPA–MC12: 3.0; Gd–DTPA–MC18: 0.4; Gd–DTPA–MC16: 0.1; Gd–DTPA–MC14: 0.4. K^+ content: no K^+ was found in any of the complexes.

Preparation of Micelles: 1,2-Dipalmitoyl-*sn*-glycero-3 phosphatidylcholine (DPPC) (225 mg) and the complex (25 mg) were dissolved in chloroform/methanol (1:1) (50 mL). Concentration 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 using a 70-W sonicator while maintaining the temperature at 65 °C with a thermostatic bath. After sonication, 75 mg of a surfactant was added (Tween 80®, polyoxyethylene sorbitan monooleate), followed by another 15 min 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 micelle diameter was measured on diluted suspensions and calculated by a nonnegatively constrained least-squares (multiple pass) routine. The error is of the order of 20%. The diameters obtained are as follows: micellar solutions with Gd–DTPA–MC12: 20 nm, with Gd–DTPA–MC14: 13 nm, with Gd–DTPA–MC16: 18 nm, with Gd–DTPA–MC18: 11 nm.

T_1 Measurements: Proton Nuclear Magnetic Relaxation Dispersion (NMRD) profiles were recorded between 0.24 mT and 1.2 T with a Field Cycling instrument (Stelar Spinmaster FFC-2000, Stelar S.R.L., Mede, Italy) on 0.6 mL solutions in 10-mm (o.d.) tubes. Proton relaxation rates were also measured at 0.47 T, and 1.5 T with Minispec PC-120, and mq-60 (Bruker, Karlsruhe, Germany). All samples were diluted 10 times and the temperature was maintained at 37 °C. ^{1}H NMRD data were fitted according to the theoretical inner-sphere model described by Solomon[28] and Bloembergen[29] and to the outer-sphere contribution described by Freed.[30] Calculations were performed with a previously described software package.[37,38]

Acknowledgments

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