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**Tatjana N. Parac-Vogt · Kristof Kimpe
Sophie Laurent · Corinne Piérart · Luce Vander Elst
Robert N. Muller · Koen Binnemans**

Paramagnetic liposomes containing amphiphilic bisamide derivatives of Gd-DTPA with aromatic side chain groups as possible contrast agents for magnetic resonance imaging

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Abstract Three amphiphilic DTPA bisamide derivatives containing long-chain phenylalanine esters (with 14, 16 and 18 carbon atoms in the alkyl chain) were synthesized and their corresponding gadolinium(III) complexes were prepared. The attempts to form paramagnetic micelles carrying the gadolinium(III) complexes yielded unstable or polydisperse micelles implying that the presence of the bulky aromatic side groups in the amphiphilic Gd-DTPA bisamide complexes results in an inefficient packing of the paramagnetic complex into micelles. All complexes were efficiently incorporated into liposomes consisting of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), yielding stable and monodisperse paramagnetic liposomes. All liposomes had a comparable size, typically between 120 and 160 nm. As a result of the reduced mobility of the gadolinium(III) complexes, solutions of these supramolecular structures show a higher relaxivity than solutions of Gd-DTPA. However, the relaxivity gain is lower compared to compounds consisting of purely aliphatic chains of the same length, most likely due to the less efficient packing or increased local mobility of the gadolinium(III) complex. In the case of the Gd-DTPA bisamide complex with 18 carbon atoms, the immobilization inside the liposomal structure is less effective, probably because the aliphatic chains of the complex are longer than the alkyl chains of the DPPC host, resulting in a relatively high local mobility. The paramagnetic liposomes containing the Gd-DTPA bisamide complexes with 14 carbon atoms showed the highest relaxivity because the optimal length match between the hydrophobic chains of the DPPC and the ligand allowed very efficient packing of the paramagnetic complex into the liposome.

Keywords Imaging · Liposome · Liposome formation and characterization · Relaxation

T. N. Parac-Vogt (✉) · K. Kimpe · K. Binnemans
Department of Chemistry, Katholieke Universiteit Leuven,
Celestijnenlaan 200F, 3001 Leuven, Belgium
E-mail: Tatjana.Vogt@chem.kuleuven.ac.be
Fax: +32-16-327992

S. Laurent · C. Piérart · L. V. Elst · R. N. Muller
NMR and Molecular Imaging Laboratory,
Department of Organic and Biomedical Chemistry,
University of Mons-Hainaut, 7000 Mons, Belgium
E-mail: robert.muller@umh.ac.be

Introduction

The fast development of magnetic resonance imaging (MRI) as a diagnostic technique has stimulated a large interest in gadolinium(III) complexes as potential contrast agents. The anionic gadoliniumdiethylenetriaminepentaacetate complex, Gd-DTPA, the first contrast agent approved for use in human beings, is nowadays routinely used in clinical MRI for contrast enhancement under the name Magnevist® (Schering, Berlin, Germany). Its solubility and rapid excretion by the kidneys make the Gd-DTPA complex an ideal agent for imaging leakage through the blood brain barrier (Kaiser et al. 1989; Higgins and Hricak 1987), but unfortunately, Gd-DTPA is of little value for imaging the liver or spleen since this hydrophilic complex does not accumulate in these organs (Carr et al. 1986; Strich et al. 1985).

The design and development of novel organ-specific contrast agents has attracted much attention and a variety of contrast agents aimed at specific targets are currently being investigated. In addition, to perform magnetic resonance angiography, the technique that is used for the imaging of blood vessels, the administered contrast agent must stay in the blood stream for a long time. In order to prolong the intravascular retention of a contrast agent, several different strategies have been proposed so far. Attachment of a protein-binding group such as diphenylcyclohexyl to a gadolinium(III) chelate via a phosphodiester linkage, like in the MS-325 contrast agent, results in reversible binding of MS-325 to human serum albumin in plasma (Caravan et al. 2002). The binding to human serum albumin reduces the extravasation of the contrast agent out of the vascular system

and also leads to a high increase in relaxivity. Due to these properties MS-325 provides strong, persistent enhancement of blood vessel images (Lauffer et al. 1996; Grist et al. 1998). Other attempts to design blood pool contrast agents include synthesis of macromolecular gadolinium(III) chelates such as dendrimers, linear polymers, or proteins, which stay confined into blood vessels because of their large size. This strategy proved to be rather disappointing: 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 (for more information, see for instance Aime et al. 1998).

Another strategy employs supramolecular systems like liposomes or micelles as carriers for the contrast agents. Several studies describe the encapsulation of a paramagnetic substance into liposome vesicles or its immobilization in the liposome membrane (Navon et al.1986). The compounds obtained by this approach exhibit longer residence times in the blood than the water-soluble metal complexes. It has been shown that the paramagnetic liposomes were suitable for contrast enhancement of the liver, spleen, bone marrow and other organs rich in macrophage activity (Kabalka et al 1991). Alternatively, lipophilic paramagnetic complexes can be incorporated into mixed micelles by using a non-ionic surfactant and an amphipatic phospholipid (Anelli et al. 2001). When these micelles are dispersed in an aqueous liquid, they form stable colloidal dispersions, i.e. the micelles resist agglomeration or aggregation into larger aggregates for a long period of time.

We recently reported the synthesis of amphiphilic Gd-DTPA derivatives with alkyl chains consisting of 14, 16 and 18 carbon atoms (Gd-DTPA-BC14, Gd-DTPA-BC16 and Gd-DTPA-BC18) (Kimpe et al. 2003; Parac-Vogt et al. 2004). The gadolinium(III) complexes of these ligands were incorporated into mixed micelles to obtain supramolecular structures that had decreased rotational motion and enhanced relaxivity. Since the gadolinium(III) complex is an essential part of paramagnetic micelles and liposomes, it is very important to determine the influence of the paramagnetic complex on their stability and relaxivity properties. A recent study has shown that the nature of Gd-chelate proved to be the most important in order to obtain a high degree of incorporation (Gløgård et al. 2002).

In this paper we describe three different gadolinium(III) complexes containing amphiphilic DTPA bisamide derivatives based on phenylalanine derivatives. The gadolinium(III) complexes of these ligands were incorporated into mixed micelles and liposomes to examine the influence of the aromatic side chain on micelle and liposome formation. Relaxometric studies were performed in order to determine the parameters that govern the relaxation properties. An understanding of the effect of liposomal contrast agents on water proton relaxation is pertinent to their design and to the interpretation of their effect on the MR image.

Materials and methods

Chemicals: Reagents were obtained from Aldrich, Acros Organics, or Fluka, and used without further purification. DTPA was obtained from Koch Light Laboratories. DPPC was obtained from Genzyme Pharmaceuticals (Switzerland). Gadolinium(III) 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 FT IR-spectrometer Bruker IFS66, using KBr discs. Mass spectra were run on a Q-tof 2 (Micromass, Manchester, UK). Samples for the mass spectra were prepared as follows: 2 mg of the complex was dissolved in 1 ml of methanol. This solution (200 µl) 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 according to a published procedure (Montembault et al. 1996). Esters of L-phenylalanine were synthesized following the procedure described by Penney et al. (1985). DTPA bisamides derived from L-phenylalanine esters were prepared using the procedure of Zhao (Zhao et al. 1997).

Analytical data for the ligands

Diethylenetriamine-N,N‴-di(acetyl-L-phenylalanineoctadecylester)-N,N″,N‴-triacetic acid (DTPA-BPO)

Yield: 22%; ^{1}H-NMR (DMSO-d_6): 0.89 (t, 6 H, 2×CH_3), 1.27 (s, 60 H, 30×CH_2), 1.59 (m, 4 H, 2×CH_2), 2.98 (m, 4 H, 2×CH_2), 3.09 (m, 4 H, 2×CH_2), 3.34 (m, 12 H, CHNHC = O + N–CH_2C = O), 4.08 (m, 4 H, 2×CH_2), 7.27 (m, 10 H, 2×Ph).

IR: ν (cm^{-1}): 3,390 (N–H stretch); 3,088 (N–H amide stretch II); 3,031 (C–H stretch, phenyl); 2,926, 2,854 (C–H alkyl stretch); 1,740 (CO acid, ester); 1,688 (bending, amide I). Elem. Anal. Calcd. (found): $C_{68}H_{113}N_5O_{12}$ (H_2O): C: 67.46 (67.56); H, 9.57 (9.69); N, 5.78 (5.57)%.

Diethylenetriamine-N,N‴-di(acetyl-L-phenylalaninehexadecylester)-N,N″,N‴-triacetic acid (DTPA-BPH)

Yield: 15%; ^{1}H-NMR (CD_3OD/$CDCl_3$): 0.89 (t, 6 H, 2×CH_3), 1.27 (m, 52 H, 26×CH_2), 1.59 (m, 4 H, 2×CH_2), 2.98 (m, 4 H, 2×CH_2), 3.18 (m, 8 H, 4×CH_2), 4.08 (m, 12 H, CHNHC = O + N–CH_2C = O), 4.59 (m, 4 H, 2×CH_2), 7.27 (m, 10 H, 2×Ph).

IR: ν (cm^{-1}): 3,390 (N–H stretch); 3,088 (N–H amide stretch II); 3,031 (phenyl, C–H stretch); 2,926, 2,854 (C–H alkyl stretch); 1,740 (CO acid, ester); 1,688 (bending, amide I). Elem. Anal. Calcd. (found): $C_{64}H_{105}N_5O_{12}(-H_2O)$: C: 66.57 (66.12); H, 9.34 (9.26); N, 6.05 (5.88)%.

Diethylenetriamine-N,N‴-di(acetyl-L-phenylalaninetetradecylester)-N,N‴,N‴-triacetic acid (DTPA-BPT)

Yield: 23.2%; ^{1}H-NMR ($CD_3OD/CDCl_3$): 0.88 (t, 6 H, 2×CH_3), 1.27 (s, 44 H, 22×CH_2), 1.60 (m, 4 H, 2×CH_2), 2.98 (m, 4 H, 2×CH_2), 3.20 (m, 8 H, 4×CH_2), 4.09 (m, 12 H, CHNHC=O+N–CH_2C=O), 4.74 (m, 4 H, 2×CH_2), 7.28 (m, 10 H, 2×Ph). IR: ν (cm^{-1}): 3073 (N–H amide stretch II); 3,031 (phenyl, C–H stretch) 2,922, 2,849 (C–H alkyl stretch); 1735 (CO acid, ester); 1,688 (bending, amide I). Elem. Anal. Calcd. (found): $C_{64}H_{97}N_5O_{12}(H_2O)_3$: C: 61.10 (61.55); H, 8.55 (9.06); N, 5.94 (5.94)%.

Synthesis of the gadolinium(III) complexes

All complexes were synthesized according to the general procedure as follows: a solution of $GdCl_3.6H_2O$ 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 vacuum. The absence of free gadolinium was checked with the xylenol orange indicator (Brunisholz and Randin 1959).

Analytical Data for the gadolinium(III) complexes

Gd-DTPA-BPO

Yield: 88%; ES-MS+: 1,347.9 $[M+H]^+$, (M = $GdC_{68}H_{110}N_5O_{12}$, M_{cal} = 1,346.9) IR: ν (cm^{-1}): 2,932, 2,842 (C–H alkyl stretch); 1,745 (COO ester); 1,620 (bending, amide I); 1,599 and 1,500 (phenyl). Elem. Anal. Calcd. (found): $GdC_{68}H_{110}N_5O_{12}(H_2O)_5$: C: 55.6 (55.11); H, 8.23 (7.97); N, 4.76 (5.12)%.

Gd-DTPA-BPH

Yield: 87%; ES-MS+: 1,291.7 $[M+H]^+$; 1,313.7 $[M+Na]^+$, (M = $GdC_{64}H_{102}N_5O_{12}$, M_{cal} = 1,290.7); IR: ν (cm^{-1}): 3,068 (N–H stretch II); 2,932, 2,842 (C–H, alkyl stretch); 1,740 (COO ester); 1,630 (bending, amide I); 1,599 (phenyl). Elem. Anal. Calcd. (found): $GdC_{64}H_{102}N_5O_{12}(H_2O)_5$: C, 55.67 (55.66); H, 8.17 (7.9); N, 5.07 (4.94)%.

Gd-DTPA-BPT

Yield: 32%; ES-MS+: 1,235.7 $[M+H]^+$; 1,267.8 $[M+Na]^+$, (M = $GdC_{60}H_{94}N_5O_{12}$, M_{cal} = 1234.7); IR: ν (cm^{-1}): 2,916, 2,850 (C–H alkyl stretch); 1,738 (COO ester); 1,627 (bending, amide I); 1,601 and 1,490 (phenyl). Elem. Anal. Calcd. (found): $GdC_{60}H_{94}N_5O_{12}(-H_2O)_7$: C, 52.96 (52.73); H, 7.99 (8.50); N, 5.15 (5.71)%.

Sodium and potassium ion content measurements

Before the compounds were used for incorporation into micelles, the potassium and sodium ion content was checked by flame photometry (IL 943, Instrumentation Laboratories, Massachusetts, USA). The Na^+ and K^+ ion content was measured on a sample that was obtained by extracting with 500 μL of water the solution of the complex (1.7 mg) which was dissolved in a 1:1 chloroform/methanol mixture. Na^+ content (mmol:mol of the complex): Gd(DTPA-BPO): 0, Gd(DTPA-BPH): 0, Gd(DTPA-BPT): 3.3. K^+ content: no K^+ was found in any of the complexes.

Preparation of micelles

1,2-Dipalmitoyl-sn-glycero-3-phosphatidyl choline (225 mg) and the gadolinium(III) 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 using a 70-W sonicator while maintaining the temperature 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 min of sonication.

Preparation of liposomes

Four milligrams of the complex and 15 mg of DPPC were mixed to form a homogeneous lipid film that was rehydrated with an aqueous solution containing 5% glucose. The mixture was placed on a vortex and was then heated to 55°C. The obtained liposomes were extruded ten times through filters with a porosity of 400 nm.

Determination of micelle/liposome size

The mean micelle sizes were determined by photon correlation spectroscopy performed on 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.

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, NJ, 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 (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 and Bloembergen and to the outer sphere contribution described by Freed (Solomon 1955; Bloembergen 1957; Freed 1978). Calculations were performed with a previously described software (Muller et al.1990).

Results and discussion

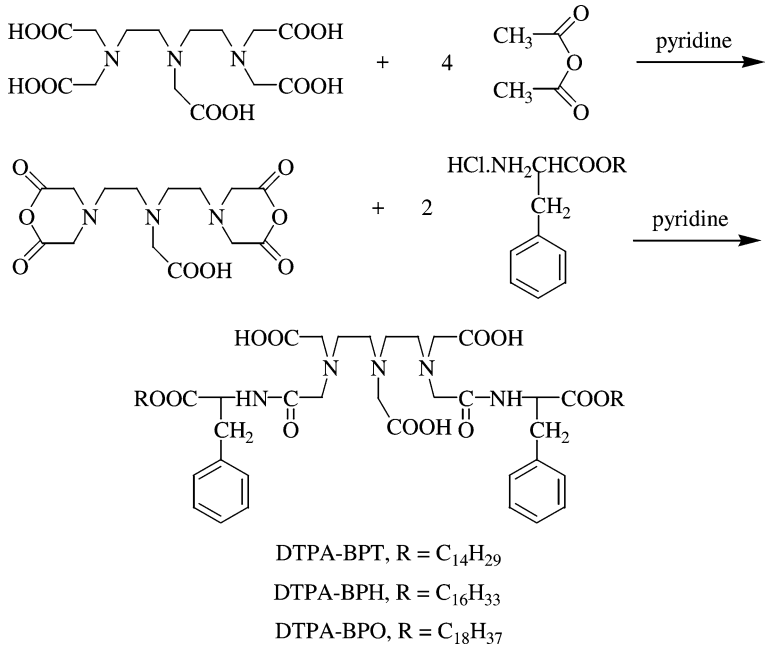
The ligands diethylenetriamine-*N,N‴*-di(acetyl-L-phenylalanineoctadecylester)-*N,N″,N‴*-triacetic acid (DTPA-BPO), diethylenetriamine-*N,N‴*-di(acetyl-L-phenylalaninehexadecylester)-*N,N″,N‴*triacetic acid (DTPA-BPH) and diethylenetriamine-*N,N‴*-di(acetyl-L-phenylalaninetetradecylester)-*N,N″,N‴*-triacetic acid were synthesized according to Scheme 1.

Under slightly alkaline solutions, they all readily formed complexes with the gadolinium(III) ion, as shown in Fig. 1. IR spectral data of all ligands showed strong absorptions in the regions around 3,375 and 1,740–1,650 cm^{-1}, corresponding to NH and CO stretching modes, respectively (Pretsch et al. 1976). Shifts of ca. 60 cm^{-1} to lower energy were observed for the carbonyl stretching frequencies upon complexation, indicating amide oxygen coordination to gadolinium(III) ion. These findings are consistent with previous studies which have shown that DTPA bisamide derivatives coordinate to lanthanide(III) ions via 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 (Geraldes et al. 1991; Ehnebom et al. 1992; Bligh et al. 1995; Aime et al. 1997; Wang et al. 1999).

The gadolinium(III) complexes of DTPA-BPO, DTPA-BPH and DTPA-BPT ligands were incorporated into mixed micelles made of a phospholipid (DPPC) and a surfactant (Tween 80®). The micelle size was determined at room temperature by photon correlation spectroscopy. Unfortunately, the measurements showed that all the micelles formed were polydisperse. Moreover, the micelles were also unstable; thus the micelle size could not be determined accurately. Previous studies have shown that micelles containing analogous bis(alkyl)amide derivatives of Gd-DTPA with aliphatic chains consisting of 14, 16 and 18 carbon atoms were stable and monodisperse (Kimpe at al. 2003). This suggests that the presence of the bulky aromatic side chains obstructs efficient packing of gadolinium(III) complexes of DTPA-BPO, DTPA-BPH and DTPA-BPT into the micelles.

As the next step, the gadolinium(III) complexes of DTPA-BPO, DTPA-BPH and DTPA-BPT ligands were incorporated into liposomes made of the phospholipic

Scheme 1 Synthesis of bisamide derivatives of DTPA with aliphatic derivatives of phenylalanine esters



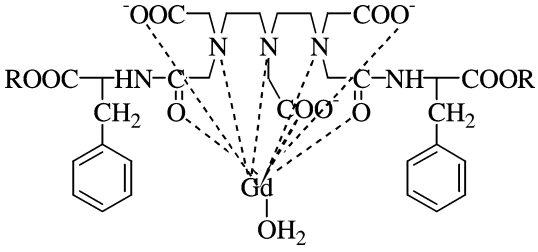


Fig. 1 Schematic representation of the gadolinium(III) complex. The ligand coordinates to the gadolinium ion via three acetate oxygens, three nitrogen atoms and two carbonyl oxygens of the amide group, while a water molecule occupies the ninth coordination site

DPPC. Liposomes are vesicles which can carry paramagnetic complexes for magnetic resonance applications either by encapsulating them into the interior aqueous volume or by incorporating paramagnetic moieties into the membranes of liposomal vesicles (Fossheim et al. 1999; Tilcock et al. 1992). In the latter case the paramagnetic amphiphilic gadolinium(III) complex becomes an integral part of the liposomal lamella. The schematic representation of a liposome carrying paramagnetic gadolinium(III) complexes is shown in Fig. 2.

The photon correlation spectroscopy measurements indicated that the liposomes incorporating Gd-DTPA-BPT, Gd-DTPA-BPH and Gd-DTPA-BPO were monodisperse. As can be seen from Fig. 3, the mean diameters of liposomes increase with increasing chain

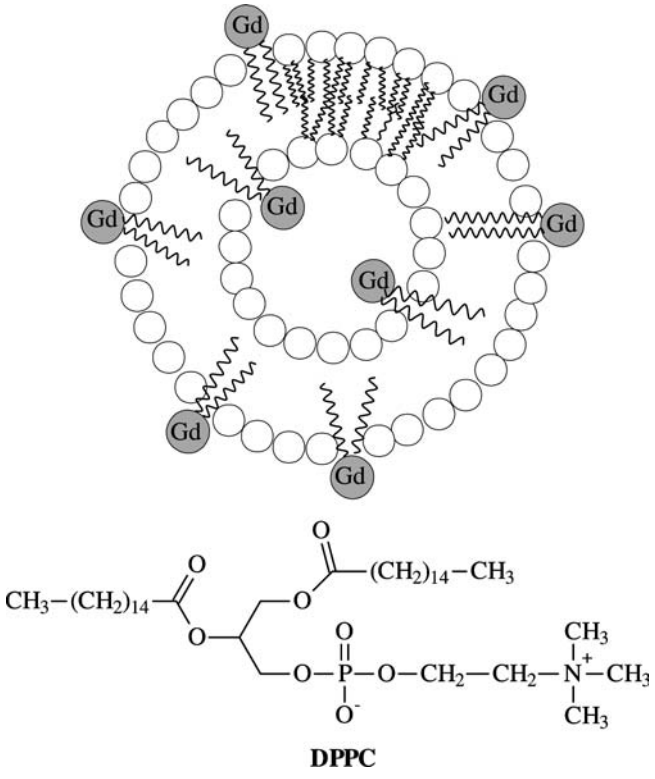


Fig. 2 Schematic representation of the paramagnetic liposome made of DPPC and a paramagnetic gadolinium(III) complex

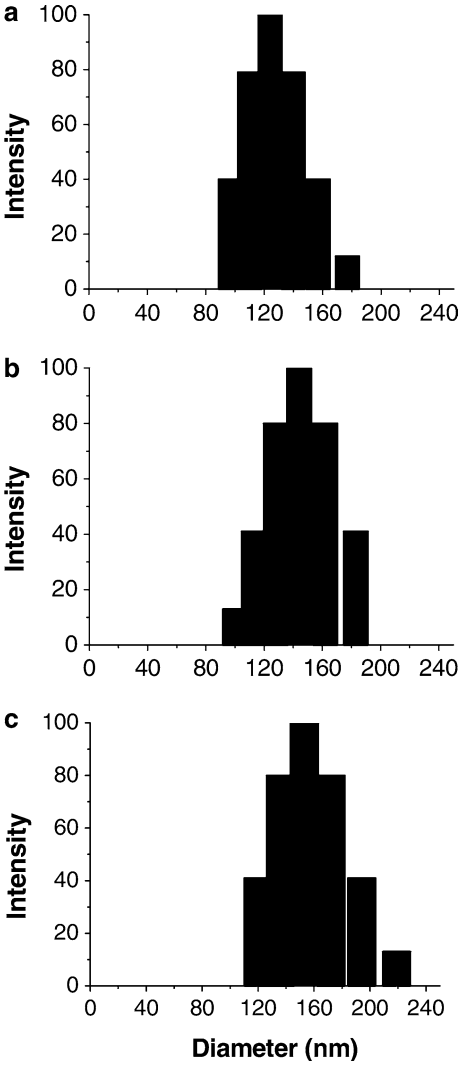


Fig. 3 Histogram representing liposome size distribution, as measured by photon correlation spectroscopy (PCS): **a** Gd-DTPA-BPT; **b** Gd-DTPA-BPH; **c** Gd-DTPA-BPO

length of the amphiphilic ligand, from about 127 nm (variance 0.025) for compound Gd-DTPA-BPT to 144 nm (variance 0.024) and 157 nm (variance 0.025) for compounds Gd-DTPA-BPH and Gd-DTPA-BPO, respectively.

The paramagnetic relaxivity profiles of liposomes solutions at 37°C containing Gd-DTPA-BPT, Gd-DTPA-BPH and Gd-DTPA-BPO are shown in Fig. 4. As the NMRD profiles show, the inclusion of the gadolinium(III) complexes into liposomes results in an increased relaxivity as compared to the parent complex Gd-DTPA. The hump at higher field strengths, corresponding to proton Larmor frequencies of 20–60 MHz, is induced by a combination of decreased rotational mobility (the value of τ_R increases) of the paramagnetic complex and an increase of the electronic longitudinal relaxation time at high fields. This observation is typical for supramolecular structures. The relaxivities decrease

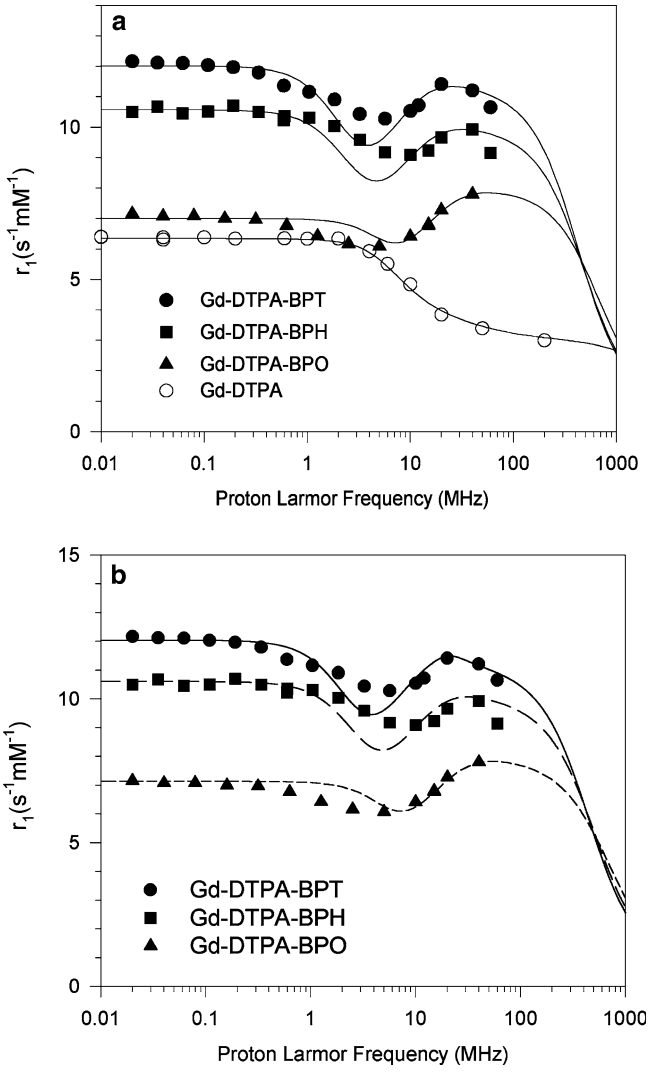


Fig. 4 NMRD profile of micellar Gd-DTPA-BPT (hexagonals), Gd-DTPA-BPH (*squares*) and Gd-DTPA-BPO (*triangles*) at 37°C. The NMRD profile of Gd-DTPA (*open circles*) has been added for comparison (Laurent et al. 2000). The theoretical fittings using the classical model (model 1) are shown in the top graph whereas the fittings using model 2 are shown in the *bottom graph*

with increasing length of the amino acid ester chain arm of the compound by which the liposome is labelled.

Data were analysed using the classical inner sphere (Solomon 1955; Bloembergen 1957) and outer sphere (Freed 1978) theories. The assumption was made that either the complex is included only in the outer layer of the liposome membrane or that the complex is present in both layers with a very fast water exchange rate between the inside and outside liposomal compartments. Since this is most likely not true, only approximate values can be obtained; but even if these values do not have much physical relevance, they will allow to try to explain the relaxivity differences between the three samples. The distance of closest approach was set to the usual value of 0.36 nm (Laurent et al. 2000; Parac-Vogt et al. 2004), the relative diffusion constant was fixed to 3.3×10^{-9} m^2 s^{-1} (Vander Elst et al. 2005) and the distance for the inner sphere interaction was set to 0.31 nm (Powell et al.1996; Laurent et al. 2000). The number of coordinated water molecules was assumed to be equal to one, by analogy with other Gd-DTPA bisamide derivatives (Alpoim et al. 1992). The parameters describing the electronic relaxation of gadolinium(III) (τ_{SO}, the electronic relaxation rate at very low magnetic field and τ_V, the correlation time modulating the electronic relaxation) as well as the "effective" 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.

The rotational correlation time τ_R decreases with increasing length of the amino acid ester chain arm. The most dramatic change occurs when altering the amino acid ester chain length from 16 carbon atoms to 18, resulting in a 33% decrease of τ_R, causing a significant decrease of relaxivity. The latter liposomes contain hydrophobic chains longer than that of DPPC, resulting in an increased local mobility as compared to the other complexes. The τ_M values are similar for the three complexes and larger than that for the parent compound Gd-DTPA. Such increase of the residence time of the coordinated water molecule has already been reported for numerous Gd-DTPA bisamide derivatives, such as for example gadolinium(III) diethylenetriaminepentaacetate-bis(methylamide) Gd-DTPABMA (Powell et al. 1996; Botteman et al. 2002). Moreover, the insertion of both hydrophobic chains of the gadolinium(III) complex in the liposome structure could also prevent the exchange of the water molecule.

A Lipari-Szabo model of the inner sphere relaxivity was also used to take into account the rotational motion of the liposomal structure and that of the Gd-complex (Dunand et al 2001; Nicolle et al. 2002). The rotational correlation time value of the liposome (τ_g) calculated from the PCS diameter is of the order of 10^{-4} s. This value was fixed during the fittings as well as τ_M that was fixed at 500 ns in order to reduce the number of fitted parameters. The local rotational correlation time of the complex τ_R^l, τ_{SO}, τ_V and the Lipari-Szabo parameter S^2 were fitted. The results (Table 1 and Fig. 4) show that S^2 is very low ($<10^{-3}$) for Gd-DTPA-BPH and Gd-DTPA-BPO indicating thus a nearly fully isotopic internal motion. For Gd-DTPA-BPT, S^2 is somewhat larger but is still very low. As a result, the τ_R values show the same trend with the largest value for the Gd-DTPA-BPT.

In order to elucidate the solution dynamics of the lanthanide(III) complexes NMR studies were performed on diamagnetic lanthanum(III)-DTPA-BPO and on paramagnetic praseodymium(III)-DTPA-BPO complexes. Similar to other bisamide-DTPA complexes, the coordination sphere of the nine-coordinate lanthanide complexes with DTPA-BPO can be best described as a tricapped trigonal prism, with the bisamide-DTPA ligand arranged around the lanthanide ion in such a way that eight different enantiomers are possible (Geraldes

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

Compound	Model [c]	τ_R (ns)	τ_M (ns)	τ_{SO} (ps)	τ_V (ps)
Gd-DTPA-BPT	1	0.47	640	159	40
	2	0.40	500(fixed)	150	43.5
Gd-DTPA-BPH	1	0.42	790	135	32
	2	0.34	500(fixed)	120	33.5
Gd-DTPA-BPO	1	0.28	730	60	25
	2	0.24	500(fixed)	60	23.5
Gd-DTPA[a]	1	0.059	143	82	23
Gd-DTPABMA[b]		0.047	1,050	85	24

[a]From (Tournier et al. 2002): 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. (1996)
[c]Model 1 includes the classical inner sphere and outer sphere theories with a single τ_R value whereas model 2 uses the Lipari-Szabo theory with two τ_R values, a "global" τ_R fixed to 10^{-4} s (see text) and a fitted "local" τ_R shown in the table

et al. 1991). The room temperature NMR spectra of the lanthanum(III) and praseodymium(III) complexes of DTPA-BPO contained very broad peaks, which points to a fast interconversion of the various isomers on the NMR time scale. This behaviour was rather surprising, because analogous complexes with aliphatic DTPA-BC14, DTPA-BC16 and DTPA-BC18 ligands undergo slow exchange, so that "frozen" structures could be observed at room temperature (Kimpe et al. 2003). Only upon lowering the temperature of the Pr-DTPA-BPO solution to 278 K the detection of relatively sharp peaks in the range from −40 to 40 ppm could be observed (Fig. 5). The large peak at around 1 ppm could be assigned to protons in the aliphatic chains, while the peaks which are shifted up-field or down-field correspond to ethylenic and acetate protons of DTPA. Like in the case of amphiphilic Gd-DTPA-bisamide complexes, the exact distinction between ethylenic and acetate resonances was not possible since this would require the use of a partially deuterated ligand. However, the comparison with previously assigned proton NMR spectra of the parent Pr-DTPA complexes suggests that the acetate resonances may experience significant down-field shift, while the ethylenic peaks are usually shifted up-field (Aime and Botta 1990). NMR measurements suggest that the activation barrier for the interconversion of lanthanide(III) complexes of DTPA-BPO isomers is lower than those for the analogous lanthanide(III) complexes consisting of aliphatic chains with the same number of carbon atoms. The presence of bulky aromatic groups close to the coordination sites most likely affects the binding to the lanthanide(III) ion, due to the steric and electronic demands of the aromatic substituents.

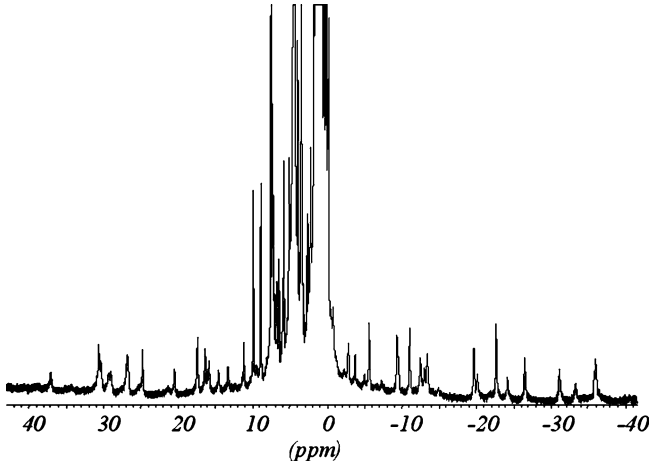


Fig. 5 Proton NMR spectrum of Pr-DTPA-BPO in the CD_3OH/CD_3Cl (1:1) mixture at 278 K

Conclusion

The presence of the bulky aromatic side groups in the amphiphilic Gd-DTPA-bisamide complexes results in an inefficient packing of the compounds into micelles, yielding unstable or polydisperse micelles. On the other hand, incorporation of the complexes into liposomes gave stable and monodisperse liposomes. The relaxivities of solutions of these supramolecular structures are higher than of solutions of Gd-DTPA as a result of the reduced mobility of the gadolinium complexes. However, the increase in relaxivity gain is lower compared to compounds consisting of aliphatic chains of the same length, most likely due to less efficient packing or increased local mobility. In the case of Gd-DTPA-BPO, the immobilization inside the liposomal structure is less effective, probably because the aliphatic chains of the complex are longer than the alkyl chains of DPPC, in which it is inserted, resulting in a relatively high local mobility. The paramagnetic liposomes containing the Gd-DTPA-BPT showed the highest relaxivity, most likely because the optimal length match between the hydrophobic chains of the DPPC and the DTPA-BPT ligand allowed very efficient packing of the paramagnetic complex into the liposome.

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