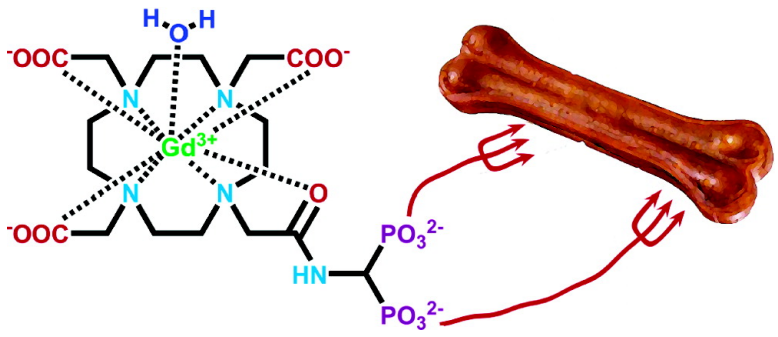
Article

A Bisphosphonate Monoamide Analogue of DOTA: A Potential Agent for Bone Targeting

Vojtch Kubc#ek, Jakub Rudovsk, Jan Kotek, Petr Hermann, Luce Vander Elst, Robert N. Muller, Zvonimir I. Kolar, Hubert Th. Wolterbeek, Joop A. Peters, and Ivan Luke

J. Am. Chem. Soc., **2005**, 127 (47), 16477-16485• DOI: 10.1021/ja054905u • Publication Date (Web): 04 November 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 13 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 11/04/2005

A Bisphosphonate Monoamide Analogue of DOTA: A Potential Agent for Bone Targeting

Vojtěch Kubíček,[†,‡] Jakub Rudovský,[‡] Jan Kotek,[‡] Petr Hermann,[‡] Luce Vander Elst,[§] Robert N. Muller,[§] Zvonimir I. Kolar,[||] Hubert Th. Wolterbeek,[||] Joop A. Peters,*[,†] and Ivan Lukeš*[,‡]

Contribution from the Laboratory of Organic Chemistry and Catalysis, Delft University of Technology, Julianalaan 136, 2628 BL Delft, The Netherlands, Department of Inorganic Chemistry, Charles University, Hlavova 8, 128 40 Prague, Czech Republic, Department of Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons-Hainaut, B-7000, Mons, Belgium, and Department of Radiation, Radionuclides & Reactors, Faculty of Applied Sciences, Delft University of Technology, 2629 JB Delft, The Netherlands

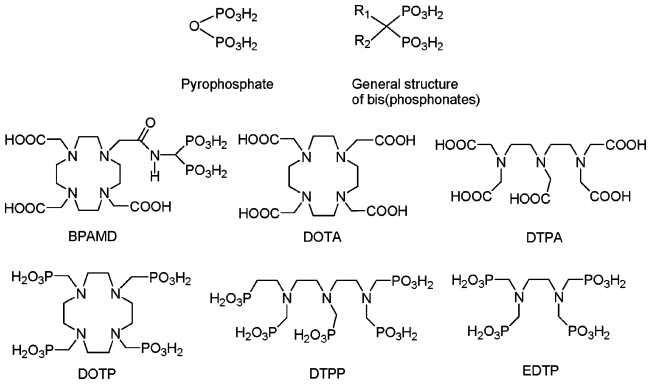
Received July 21, 2005; E-mail: J.A.Peters@tnw.tudelft.nl; Lukes@natur.cuni.cz

Abstract: A new macrocyclic DOTA-like ligand (BPAMD) for bone imaging and therapy containing a monoamide bis(phosphonic acid) bone-seeking group was designed and synthesized. Its lanthanide(III) complexes were prepared and characterized by ^{1}H and ^{31}P NMR spectroscopy. The Gd(III)−BPAMD complex was investigated in detail by ^{1}H and ^{17}O relaxometric studies to inspect parameters relevant for its potential application as an MRI contrast agent. Sorption experiments were conducted with Gd(III) and Tb(III) complexes using hydroxyapatite (HA) as a model of bone surface. Very effective uptake of the Gd−BPAMD complex by the HA surface was observed in NMR experiments. Radiochemical studies with the (^{160}Tb−BPAMD)−HA system proved the sorption to be remarkably fast and strong on one hand and fully reversible on the other hand. The strong (Gd−BPAMD)−HA interaction was also supported by ^{1}H NMRD measurements in the presence of a hydroxyapatite slurry, which showed an increase of the rotational correlation time upon adsorption of the complex on the HA surface, resulting in a significant relaxivity enhancement. The amide-bis(phosphonate) moiety is the only factor responsible for the binding of the complex to HA.

Introduction

Magnetic resonance imaging (MRI) is one of the most powerful noninvasive diagnostic methods in medicine. It relies on variations of the water content in different tissues and on differences in the composition of body liquids causing variations of water proton relaxation rates at the clinically used magnetic fields (0.5−2 T). Paramagnetic compounds can affect relaxation rates, which have been exploited extensively to increase the contrast of MRI images. Among the MRI contrast agents (CAs) routinely used, the most widespread are based on the Gd(III) ion, which is favorable because of its high magnetic moment and long electron spin relaxation time. Due to the toxicity of unbound lanthanide(III) ions, Gd(III) cannot be used as the aqua-ion, but it has to be encapsulated in a thermodynamically and kinetically stable complex. Polyaminocarboxylate ligands derived from DOTA and DTPA (Chart 1) are commonly used for medical applications. However, sequestering of the Gd(III) ion reduces its ability to enhance the water ^{1}H relaxation rate (usually expressed as the relaxivity r_1,[1] which is the relaxation rate enhancement of a millimolar solution of the Gd(III) complex under study). As the contrast gained is usually limited, injection of relatively high doses of CA (up to 5 g of CA for each examination) is necessary so far.[1−3] There are two major ways to decrease the administrated dose of CA without losing

Chart 1. Structure of Ligands Discussed



[†] Laboratory of Organic Chemistry and Catalysis, Delft University of Technology.
[‡] Charles University.
[§] University of Mons-Hainaut.
[||] Department of Radiation, Radionuclides & Reactors, Delft University of Technology.

(1) Merbach, A. E., Tóth, E., Eds. *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; John Wiley and Sons: New York, 2001.
(2) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293−2352.

contrast: (i) by employing new Gd(III) complexes endowed with much higher relaxivities; (ii) by improvement of the biodistribution of CAs.[4−6] The latter can be achieved by conjugating the metal complex with a targeting group. Commonly, this is a biologically active compound, which exhibits a high and specific affinity for a particular tissue. Hence, after administration, the CA accumulates in the tissue concerned, resulting in local contrast enhancement.

In medical treatment of bone diseases, geminal bis(phosphonates) have been widely used during the last 20 years (Chart 1).[7] Their success can be ascribed to the high affinity of the bis(phosphonic acid) group, which is a non-hydrolyzable analogue of natural pyrophosphate, to the bone surface. The two other substituents in the bis(phosphonates) are attached to the central carbon atom (Chart 1), and these groups are responsible for the clinical effect of the drug (e.g., by affecting osteoclasts and/or osteocysts—cells responsible for bone formation and desorption).[7] Bis(phosphonates) are adsorbed preferentially to the active parts of bones (areas of growing and pathological changes),[7] which further enhances the desired effect. More recently, CAs for MRI with phosphonates and bis(phosphonates) as targeting group have been developed.[8−11] Introduction of the bis(phosphonate) group into the molecule of a Gd(III) complex of a suitable polyaminocarboxylic ligand results in a strong affinity of the resulting species for the desired part of the bone surface, which may be reflected in an increase of contrast of this tissue. Strong interaction of such species with calcified tissues has been demonstrated for the bis(phosphonate)-containing amides of DTPA.[9,10] Unfortunately, these compounds lack sufficient stability of their Ln(III) complexes for in vivo applications.[12] Furthermore, bis(phosphonates) attached to superparamagnetic iron oxide (SPIO) particles have been reported as promising bone-targeting CAs.[11] Another approach to bone-targeting CAs involves phosphonate analogues of polyaminocarboxylate ligands (DOTP, DTPP, EDTP; Chart 1), which exhibit a strong adsorption on HA. However, upon binding of the Gd(III) complex of DOTP, the relaxivity is practically silent due to expulsion of second-sphere water molecules upon adsorption.[13,14] In the case of DTPP and EDTP, the strong interaction of the phosphonate groups with the surface of HA weakens the binding of the Gd(III) ion, resulting in a decrease in stability of these complexes.[13]

Here, we report on a new DOTA-like ligand ((4-{[(bis(phosphonomethyl))carbamoyl]methyl}-7,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododec-1-yl)acetic acid (BPAMD; Chart 1)) containing a bis(phosphonic acid) pendant arm. The properties of its lanthanide(III) complexes with respect to its potential application as a bone-targeted CA were evaluated. The sorption and MRI contrast−enhancement abilities of the Ln(III) complexes were tested using hydroxyapatite (HA) as a model of bone surface. The sorption was studied using the ^{160}Tb radioisotope (half-life 72.1 days, β^- 0.6 and 1.7 MeV, γ 879, 299, and 966 keV) as a model for its neighbor in the periodical system, gadolinium. This isotope was effectively produced by neutron activation of ^{159}Tb (100% natural abundance) and could be easily handled because of its long half-life.

Experimental Section

Materials. Commercially available chemicals had synthetic purity and were used as received. Acetonitrile and toluene were dried by distillation with P_2O_5. *t*-Bu$_3$DO3A·HBr was prepared according to a published procedure.[15] Hydroxyapatite (HA) was purchased from Fluka (cat. number: 55496); the specific surface area is 53 m^2/g as determined by N_2 adsorption by means of a Quantachrome Autosorb-6B apparatus. Thin-layer chromatography was performed on TLC aluminum sheets with silica gel 60 F254 (Merck KGaA, Germany). For the detection, iodine vapors were used. Elemental analyses were done in the Institute of Macromolecular Chemistry (Academy of Sciences of the Czech Republic, Prague).

NMR Spectroscopy. ^{1}H (300 MHz),^{13}C (75.5 MHz), ^{17}O (40.7 MHz), and ^{31}P (121.5 MHz) NMR spectra were recorded on a Varian Unity Inova-300 spectrometer using 5 mm sample tubes. Unless stated otherwise, NMR experiments were performed at 298 K. Chemical shifts are given in ppm (δ-scale). For the measurements in D_2O, *tert*-butyl alcohol was used as an internal standard with the methyl signal referenced to 1.2 ppm (^{1}H) or 31.2 ppm (^{13}C). Water was used as an external chemical shift reference for ^{17}O resonances. The ^{31}P chemical shifts were measured with respect to 1% H_3PO_4 in D_2O as an external standard (substitution method). The pH values of the samples were measured at ambient temperature using a Corning 125 pH meter with a calibrated combined microelectrode purchased from Aldrich Chemical Co. The pH values of the solutions were adjusted using dilute aqueous solutions of NaOH and HCl.

Mass Spectrometry. Mass spectra were recorded on a Bruker Esquire 3000 spectrometer equipped with an electrospray ion source and an ion trap. All measurements were carried out in the positive mode.

Activity Measurements. The radioactivity of ^{160}Tb in samples was determined by measuring the intensity of emitted γ rays using a NaI-(Tl)-scintillation detector based γ-ray counter (Wallac).

Tetraethyl (*N*,*N*-Dibenzyl)aminomethyl-bis(phosphonate) (2).[16] Triethyl orthoformate (10.6 g, 71 mmol), diethyl phosphite (25.6 g, 186 mmol), and dibenzylamine (11.8 g, 59.9 mmol) were mixed in a 100 mL three-necked round-bottom flask. The solution was refluxed under argon atmosphere (150 °C in an oil bath). After 5 h, the cooler was removed and the solution was heated for 24 h at 160 °C under argon flux to remove the ethanol. After cooling to RT, $CHCl_3$ (300 mL) was added and the solution was washed with 3 × 60 mL of 5% aqueous NaOH and 2 × 75 mL of brine. The organic fraction was dried with anhydrous Na_2SO_4, and then the solvents were removed by rotary evaporation. The resulting oil was purified by column chromatography on silica gel (hexane−EtOH, from 100:0 to 0:100). Yield: 12.4 g (43%) of a viscous, colorless oil. R_f (hexane−EtOAc, 1:1) = 0.2−0.3. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.32 (td, 12H, −CH_3, $^3J_{HH}$ = 7.2 Hz, $^4J_{PH}$ = 2.0 Hz), 3.55 (t, 1H, P−CH−P, $^2J_{PH}$ = 25.2 Hz), 4.07 (m, 4H, N−CH_2−Ph), 4.15 (m, 8H, O−CH_2−), 7.20−7.45 (m, 10H, Ar−H). ^{31}P NMR ($CDCl_3$, 300 MHz): δ 20.7 (d, $^2J_{HP}$ = 25.2 Hz). MS: calcd 506.5, obsd 506.7 (M + Na$^+$).

(3) Aime, S.; Botta, M.; Fasano, M.; Terreno, E. *Chem. Soc. Rev.* **1998**, *27*, 19−29.
(4) Weissleder, R.; Mahmood, U. *Radiology* **2001**, *219*, 316−333.
(5) Jacques, V.; Desreux, J. F. *Top. Curr. Chem.* **2002**, *221*, 123−164.
(6) Aime, S.; Cabella, C.; Colombatto, S.; Crich, S. G.; Gianolio, E.; Maggioni, F. *J. Magn. Reson. Imaging* **2002**, *16*, 394−406.
(7) Fleisch, H. *Bisphosphonates in Bone Disease*, 4th ed.; Academic Press: London, 2000.
(8) Adzamli, I. K.; Gries, H.; Johnson, D.; Blau, M. *J. Med. Chem.* **1989**, *32*, 139−144.
(9) Adzamli, I. K.; Johnson, D.; Blau, M. *Invest. Radiol.* **1991**, *26*, 143−148.
(10) Adzamli, I. K.; Blau, M.; Pfeffer, M. A.; Davis, M. A. *Magn. Reson. Med.* **1993**, *29*, 505−511.
(11) Greb, W.; Blum, H.; Roth, M. PCT WO2003/097074, 2003; *Chem. Abstr.* **2003**, 931193.
(12) Adzamli, I. K.; Blau, M. *Magn. Reson. Med.* **1991**, *17*, 141−148.
(13) Bligh, S. W. A.; Harding, C. T.; McEwen, A. B.; Sadler, P. J.; Kelly, J. D.; Marriott, J. A *Polyhedron* **1994**, *13*, 1937−1943.
(14) Alves, F. C.; Donato, P.; Sherry, A. D.; Zaheer, A.; Zhang, S.; Lubag, A. J. M.; Merritt, M. E.; Lenkiski, R. E.; Frangioni, J. V.; Neves, M.; Prata, M. I. M.; Santos, A. C.; de Lima, J. J. P.; Geraldes, C. F. G. C. *Invest. Radiol.* **2003**, *38*, 750−760.
(15) Dadabhoy, A.; Faulkner, S.; Sammes, P. G. *J. Chem. Soc., Perkin Trans. 2* **2002**, 348−357.

Tetraethyl Aminomethyl-bis(phosphonate) (3). A 250 mL two-necked round-bottom flask was flushed with argon and charged with 10% Pd/C (0.8 g), and then a solution of **2** (4.0 g, 8.3 mmol) in absolute EtOH (150 mL) was added. The mixture was refluxed with vigorous stirring under a hydrogen atmosphere for 24 h. After filtration and evaporation of volatiles, the product was obtained as a colorless oil. Yield: 2.4 g (96%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.36 (t, 12H, $-CH_3$, $^3J_{HH} = 6.8$ Hz), 3.43 (t, 1H, P$-CH-$P, $^2J_{PH} = 20.4$ Hz), 4.23 (m, 8H, O$-CH_2-$). ^{31}P NMR ($CDCl_3$, 121.5 MHz): δ 20.8 (d, $^2J_{HP} = 20.4$ Hz). MS: calcd 326.2, obsd 326.2 ($M + Na^+$).

Chloroacetamide (4). A solution of **3** (2.4 g, 7.9 mmol) in dry acetonitrile (50 mL) was slowly dropped into a suspension of Na_2CO_3 (5.2 g, 49 mmol) in a solution of chloroacetyl chloride (2.9 g, 25.6 mmol) in dry acetonitrile (50 mL), which was cooled at -40 °C. After that, the mixture was allowed to warm to room temperature and stirred overnight. After treatment with charcoal and filtration, the excess of chloroacetyl chloride was removed by repeated evaporation with toluene. Traces of chloroacetic acid were removed by stirring with K_2CO_3 (1.0 g, 9.4 mmol) in dry toluene (50 mL) overnight, followed by filtration and evaporation of the volatiles. The product was obtained as a colorless oil in a yield of 3.1 g (96%). R_f (concentrated aqueous ammonia$-$MeOH$-$EtOAc, 1:4:14) = 0.8$-$0.9. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.34 (td, 12H, $-CH_3$, $^3J_{HH} = 7.2$ Hz, $^4J_{PH} = 4.8$ Hz), 4.09 (s, 2H, Cl$-CH_2-$CO), 4.21 (m, 8H, O$-CH_2-$), 5.02 (td, 1H, P$-CH-$P, $^2J_{PH} = 21.6$ Hz, $^3J_{HH} = 10.0$ Hz), 7.50 (bd, 1H, N*H*, $^3J_{HH} = 10$ Hz). ^{31}P NMR ($CDCl_3$, 121.5 MHz): δ 16.1 (d, $^2J_{HP} = 21.6$ Hz). MS: calcd 402.7, obsd 402.4 ($M + Na^+$).

Ligand BPAMD (1). A solution of **4** (3.1 g, 7.6 mmol) in dry acetonitrile (50 mL) was slowly dropped into a solution of *t*-Bu_3DO3A·HBr (2.6 g, 4.4 mmol) in a suspension of K_2CO_3 (3.5 g, 25 mmol) in dry acetonitrile (50 mL), and then the mixture was stirred for 24 h. After treatment with charcoal, filtration, and rotary evaporation of the filtrate, the resulting oil was purified by column chromatography on silica gel (concentrated aqueous ammonia$-$MeOH$-$EtOAc, from 1:8:14 to 1:8:0). The yellow oil obtained after the chromatography was dissolved in 30% HBr in dry AcOH (50 mL) and stirred for 24 h at room temperature. After removal of volatiles on a rotary evaporator, the resulting solid was suspended in dry THF (100 mL). The white solid was filtered off, dissolved in water (20 mL), and purified on a strongly acidic cation-exchange resin (Dowex 50×4, elution with water followed by 10% aqueous pyridine). After rotary evaporation, the remaining pyridine was removed on a weakly acidic cation-exchange resin (Amberlite CG50, elution with water). The product-containing fractions were combined and treated with charcoal, solids were filtered off, and the filtrate was concentrated on a rotary evaporator to a final volume of 25 mL. The product was obtained as a white fine powder on standing for 5 days. The precipitate was filtered, washed with EtOH, and dried over P_2O_5 at RT. The yield was 1.2 g of **1** as BPAMD·7.5H_2O (39%). R_f (concentrated aqueous ammonia$-$MeOH$-$EtOAc, 1:4:14) = 0.2$-$0.3. Anal. Calcd for $C_{17}H_{48}N_5O_{20.5}P_2$: C, 28.66; H, 6.79; N, 9.83. Found: C, 28.75; H, 6.84; N, 9.72. ^{1}H NMR (D_2O, 300 MHz): δ 3.78 (bs, 8H, cyclen-CH_2-N), 3.87 (bs, 8H, cyclen-CH_2-N), 4.31 (s, 4H, N$-CH_2-$CO), 4.44 (s, 2H, N$-CH_2-$CO), 4.47 (s, 2H, N$-CH_2-$CO), 5.14 (t, 1H, P$-CH-$P, $^2J_{PH} = 19.8$ Hz). ^{13}C{^{1}H} NMR (D_2O, 75.5 MHz): δ 45.75 (1C, t, P$-CH-$P, $^1J_{PC} = 130.2$ Hz), 47.40 (2C, cyclen$-CH_2-$N), 47.71 (2C, cyclen$-CH_2-$N), 48.32 (4C, cyclen$-CH_2-$N), 52.61 (1C, N$-CH_2-$pendant), 52.77 (1C, N$-CH_2-$pendant), 53.32 (2C, N$-CH_2-$pendant), 166.18 (1C, $-CON-$), 169.17 (2C, $-COO-$), 170.24 (1C, $-COO-$). ^{31}P NMR (D_2O, 121.5 MHz): δ 13.8 (d, $^2J_{HP} = 19.8$ Hz). MS: calcd 578.4, obsd 578.2 ($M + H^+$).

Preparation of the Lanthanide(III) Complexes. General Procedure. The hydrate of BPAMD (100 mg, 0.140 mmol) was suspended in H_2O (0.4 mL). A solution of NaOH (30% in H_2O) was slowly added until all ligand was dissolved. The pH of the resulting solution was 8$-$9. This mixture was slowly dropped into a solution of hydrated lanthanide(III) chloride (0.9 equiv, 0.127 mmol) in H_2O (0.2 mL). Then, the pH value was readjusted to 9 with 30% NaOH in H_2O and the solution obtained was heated at 80 °C overnight. After cooling to RT, the pH value and the concentration of the sample were adjusted to the desired values. Sometimes, a precipitate was formed after mixing of the ligand and the metal salt solutions; however, upon increase of the pH value, this precipitate dissolved.

Preparation of Solutions of ^{160}Tb$-$BPAMD-Containing Tb$-$BPAMD (^{160}Tb-containing materials and their solutions in following text are denoted as "labeled"; other Tb-containing materials are denoted as "nonlabeled"). Labeled $Tb(NO_3)_3·5H_2O$ was prepared by thermal neutron irradiation of $Tb(NO_3)_3·5H_2O$ target (20 mg, 46 mmol) in a nuclear reactor (thermal neutron flux = 4.49×10^{12} cm^{-2} s^{-1}, irradiation time = 1.5 h) (Reactor Institute Delft, Faculty of Applied Sciences, Delft University of Technology, Delft, The Netherlands). This material (specific activity A = 65 GBq mol^{-1}) was used without further purification.

The hydrate of BPAMD (180 mg, 252 mmol) was suspended in H_2O (3 mL). A solution of NaOH (20% in H_2O) was slowly added until all ligand was dissolved (pH = 8$-$9). This mixture was slowly dropped into a solution of $Tb(NO_3)_3·5H_2O$ (10 mg, 23 mmol of labeled; 99 mg, 228 mmol of nonlabeled salts) in H_2O (2 mL). After the addition was completed, the pH was adjusted to 9 (with 20% NaOH in H_2O), after which the solution was heated at 80 °C for 12 h. The solution of the complex obtained was transferred into a 25 mL volumetric flask and diluted to a final concentration of 0.01 mol L^{-1} (A = 60 MBq L^{-1}), and the pH was readjusted to the desired value (pH = 7.5).

Estimation of the Time Required To Establish the Thermodynamic Equilibrium. In a 10 mL vial, HA (50 mg) was suspended in a TRIS buffer solution (0.10 mol L^{-1}, pH = 7.5). To this solution was added the solution of the labeled Tb(III)$-$BPAMD complex (the final concentration of labeled Tb(III) in samples was 1.00 mmol L^{-1}, the total volume of the solution was 3 mL), and the resulting mixture was gently shaken. After incubation (1, 3, 5, 7, 24, 48, 72 h; one vial for each point), the suspension was filtered through a Millipore filter (0.22 μm) and 1 mL of the filtrate was counted.

Reversibility of Tb$-$BPAMD Adsorption. In a 10 mL vial, HA (50 mg) was suspended in a TRIS buffer solution (0.10 mol L^{-1}, pH = 7.5). The solution of labeled Tb(III)$-$BPAMD complex was added, and the mixture was gently shaken for 3 days. Then, a solution of nonlabeled complex was added (final concentration of labeled Tb(III) in the samples was 0.33 mmol L^{-1}, the final concentration of nonlabeled Tb(III) in the samples was 1.50 mmol L^{-1}, the total volume of the solution was 3 mL), and the mixture obtained was shaken for another 3 days. The suspension was then filtered through a Millipore filter (0.22 μm), and 1 mL of the filtrate was counted.

Adsorption of Tb$-$BPAMD on HA. In a 10 mL vial HA (50 mg) was suspended in a TRIS buffer solution (0.10 mol L^{-1}, pH = 7.5). The solution of labeled Tb(III)$-$BPAMD complex was added (final concentration of labeled Tb(III) in samples was 0.17$-$2.00 mmol L^{-1}, the total volume of the solution was 3 mL), and the mixture obtained was gently shaken for 3 days. The suspension was then filtered through a Millipore filter (0.22 μm), and 1 mL of the filtrate was counted.

Desorption of Tb$-$BPAMD from HA by Methylenediphosphonic Acid. In a 10 mL vial HA (50 mg) was suspended in a TRIS buffer solution (0.10 mol L^{-1}, pH = 7.5). The solution of labeled Tb(III)$-$BPAMD complex was added, and the mixture was gently shaken for 3 days. Then, a solution of methylenediphosphonic acid (MDP) was added (the final concentration of labeled Tb(III) in the samples was 0.33 mmol L^{-1}, the final concentration of MDP in the samples was 0$-$33.33 mmol L^{-1}, the total volume of the solution was 3 mL), and the mixture obtained was shaken for another 3 days. The suspension was then filtered through a Millipore filter (0.22 μm), and 1 mL of the filtrate was counted.

Competitive Sorption of Ligand BPAMD and Tb$-$BPAMD Complex on HA. In a 10 mL vial HA (50 mg) was suspended in a TRIS buffer solution (0.10 mol L^{-1}, pH = 7.5). A solution of labeled

Tb(III)−BPAMD complex and a solution of ligand BPAMD were added (final concentration of labeled Tb−BPAMD complex in samples was 0.55 mmol L^{-1} (experiment A), 1.10 mmol L^{-1} (experiment B), final concentration of free ligand in samples was 0−1.67 mmol L^{-1} (experiment A), 0−3.33 mmol L^{-1} (experiment B), the total volume of the solution was 3 mL), and the mixture obtained was gently shaken for 3 days. The suspension was then filtered through a Millipore filter (0.22 μm), and 1 mL of the filtrate was counted.

Preparation of the Slurry of HA with Adsorbed Gd−BPAMD. In a 5 mL plastic vial, HA (0.3 g) was suspended in a TRIS buffer solution (1.5 mL, 0.1 mol L^{-1}, pH = 7.5). A solution of Gd−BPAMD or Gd−DTPA (0.08 mL, 20 mmol L^{-1}) was added, and the mixture was shaken for 24 h. The suspension was then transferred into an NMR tube. After standing for 4 h, the ratio clear solution/slurry remained unchanged. The solution (~0.75 mL) was decanted and filtered into a clean NMR tube, and then relaxometric measurements with both sample tubes were performed.

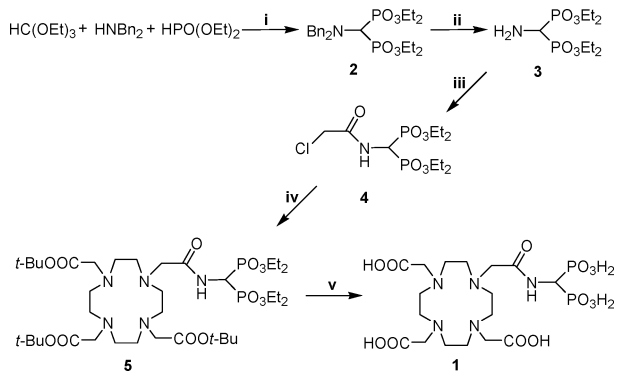
Variable-Temperature ^{17}O NMR Study of Gd−BPAMD. An 80 mM solution of Gd−BPAMD was prepared as described above, and the pH value of this solution was adjusted to 7.5. Then, 10 μL of ^{17}O-enriched water (10% $H_2{}^{17}O$) was added. The exact concentration of Gd(III) was determined by the bulk magnetic susceptibility shift measurement (BMS) as described previously.[17] All NMR spectra were acquired without frequency lock. To correct the ^{17}O NMR shift for a contribution of the BMS, the difference between chemical shifts of proton signals of *tert*-butyl alcohol in the paramagnetic sample and in pure water was measured according to the procedure described previously.[18] Transversal ($1/T_2$) relaxation rates were obtained by the Carr−Purcell−Meiboom−Gill pulse sequence.[19]

Measurement of $1/T_1$ ^{1}H NMRD Profiles. A 2 mM sample solution at pH 7.5 was prepared as described above. The exact concentration of Gd(III) was determined using the BMS method.[17] The $1/T_1$ NMRD profiles were measured at 298 K at magnetic field strengths between 4.7×10^{-4} and 0.35 T using a Stelar SpinMaster FFC-2000 relaxometer. Measurements at 0.47 and 1.42 T were performed on a Bruker Minispec PC-20 and Bruker Minispec mq60, respectively. The experimental data were fitted simultaneously with ^{17}O NMR data by means of a least-squares fitting procedure using the Micromath Scientist program version 2.0 (Salt Lake City, UT) as described previously.[20]

Results and Discussion

Synthesis of the Ligand. Ligand BPAMD (**1**) was synthesized from the tris(*tert*-butyl ester) of DO3A (*t*-Bu_3DO3A) and bis(phosphonate) **4** (Scheme 1). The latter was prepared by reaction of dibenzylamine, triethylorthoformate, and diethyl phosphite to give intermediate **2**, followed by debenzylation with H_2 and Pd/C as the catalyst.[16] The yield of the condensation was relatively low (43%) due to the formation of many byproducts (monophosphonate derivatives, formic acid amides and esters). The deprotected aminomethyl-bis(phosphonate) (**3**) was converted quantitatively into compound **4** by means of a substitution reaction with an excess of chloroacetyl chloride. The chloroacetylamide (**4**) was used to alkylate the tris(*tert*-butyl ester) of DO3A. The conversion was almost quantitative; however the yield after purification was only 65% due to some decomposition of the product during chromatography. Finally, ligand **1** was obtained by hydrolysis of ester **5** in a solution of HBr in dry AcOH.

Scheme 1. Synthesis of the Ligand BPAMD[a]



[a] (i) Argon atmosphere, 160 °C; (ii) H_2, Pd/C, EtOH, reflux; (iii) $ClCH_2C(O)Cl$, acetonitrile, Na_2CO_3, −40 °C, (iv) *t*-Bu_3DO3A, acetonitrile, K_2CO_3, RT; (v) 30% HBr in dry AcOH.

Preparation and Characterization of Lanthanide(III) Complexes of BPAMD. The complexation process was monitored in the case of the Eu(III) complex by ^{31}P NMR. Immediately after mixing of solutions of Eu(III) and the ligand BPAMD, the resulting pH was 2−4 and the resonances in the ^{31}P spectrum were so broad that they disappeared in the baseline. At this stage, sometimes a precipitate was formed that dissolved after the solution was rendered alkaline to pH 9. Then, the spectrum displayed a broad resonance (δ = 60.2 ppm, $\Delta\nu_{1/2}$ = 7310 Hz) in addition to the resonance for the excess of free ligand. Upon heating the solution at 80 °C overnight, two sharp resonances belonging to Eu−BPAMD complex of about equal intensity were observed at 19.1 and 15.1 ppm (see Supporting Information, Figure S1).

Most likely, these phenomena can be ascribed to a complex formation mechanism involving several intermediates. When the components are mixed (at pH 2−4), only bis(phosphonate) oxygen atoms will be coordinated to the lanthanide(III) ion, whereas the carboxylate pendant arms and macrocyclic amine groups remain protonated.[21,22] The incidental precipitation may be due to formation of poorly soluble lanthanide salts typical for bis(phosphonate) complexes with divalent and trivalent metal ions.[23] Once the pH value is set to 9, most likely, the pathway that has been established for the parent compound DOTA[24] is followed. Thus initially, an "out-of-cage" complex is formed, in which both the carboxylate and phosphonate oxygen atoms bind the Ln(III) ion and the macrocyclic nitrogen atoms are still protonated. Then in a last and rate-determining step the nitrogen atoms are deprotonated, while the Ln(III) ion moves into the cage. This rearrangement is strongly pH dependent, as reflected in the complex formation rate. At pH 6.5 the complex formation takes several days, whereas at pH 8.5, it is completed overnight.

The ^{1}H NMR spectra of the Ln−BPAMD complexes show close similarity with those of the corresponding Ln−DOTA complexes,[25,26] although they are much more complex due to

(16) Kantoci, D.; Denike, J. K.; Wechter W. J. *Synth. Commun.* **1996**, *26*, 2037−2043.
(17) Corsi, D. M.; Platas-Iglesias, C.; van Bekkum, H.; Peters, J. A. *Magn. Reson. Chem.* **2001**, *39*, 723−726.
(18) Zitha-Bovens, E.; Vander Elst, L.; Muller, R. N.; van Bekkum, H.; Peters, J. A. *Eur. J. Inorg. Chem.* **2001**, 3101−3105.
(19) Meiboom, S.; Gill, D. *Rev. Sci. Instrum.* **1958**, *29*, 688−691.
(20) Kotek, J.; Lebdušková, P.; Hermann, P.; Vander Elst, L.; Muller, R. N.; Maschmeyer, T.; Lukeš, I.; Peters, J. A. *Chem. Eur. J.* **2003**, *9*, 5899−5915.
(21) Moreau, J.; Guillon, E.; Pierrard, J.-C.; Rimbault, J.; Port, M.; Aplincourt, M. *Chem. Eur. J.* **2004**, *10*, 5218−5232.
(22) Bollinger, J. E.; Roundhill, D. M. *Inorg. Chem.* **1993**, *32*, 2821−2826.
(23) Zeevaart, J. R.; Jarvis, N. V.; Louw, W. K. A.; Jackson, G. E.; Cukrowski, I.; Mouton, C. J. *J. Inorg. Biochem.* **1999**, *73*, 265−272.
(24) Tóth, É.; Brücher, E.; Lazar, I.; Tóth, I. *Inorg. Chem.* **1994**, *33*, 4070−4076.
(25) Aime, S.; Botta M.; Ermondi, G. *Inorg. Chem.* **1992**, *31*, 4291−4299.

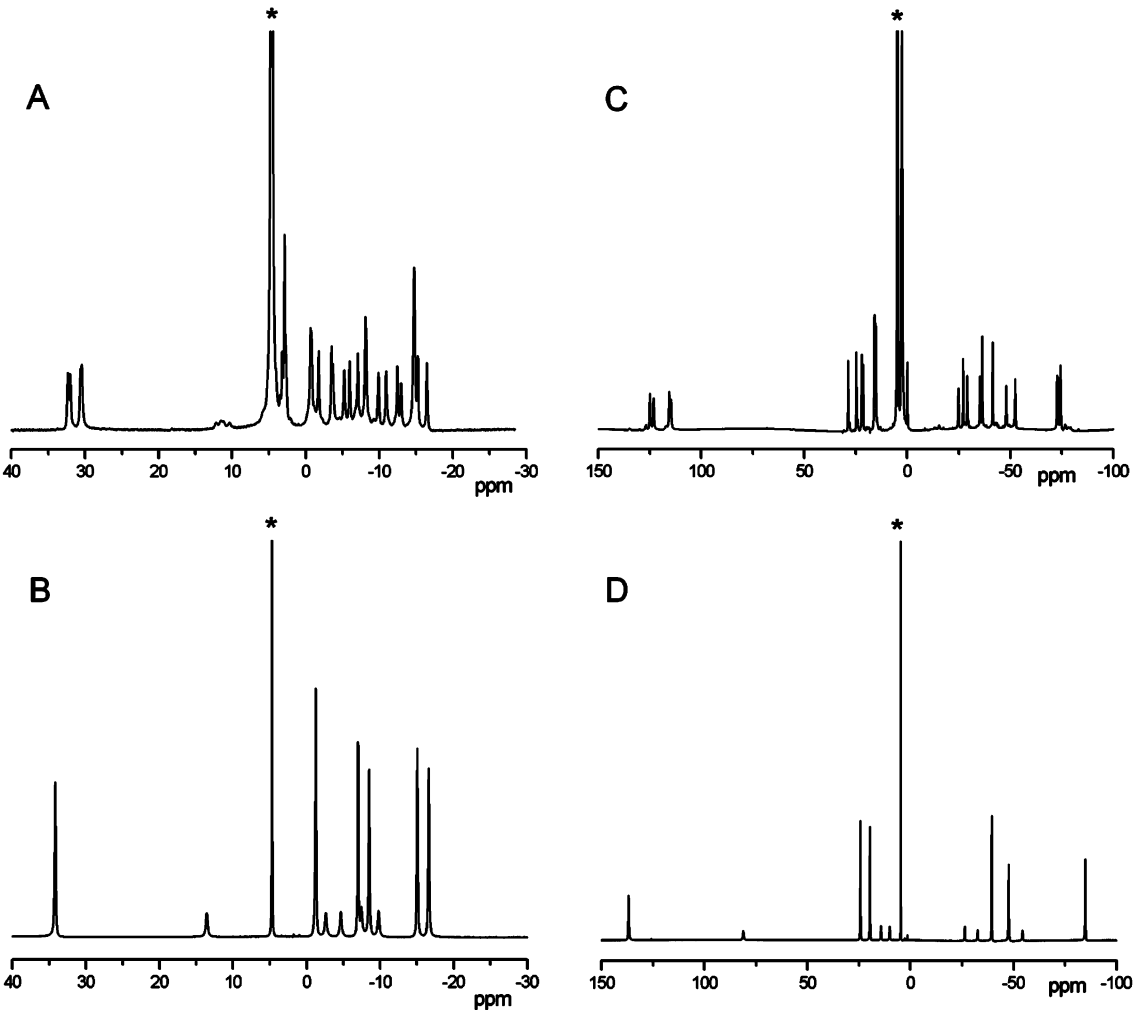


Figure 1. ^{1}H NMR spectra (400 MHz, pH = 7, 25 °C) of (A) Eu−BPAMD, (B) Eu−DOTA, (C) Yb−BPAMD, and (D) Yb−DOTA (see also Supporting Information). The signals of solvents are marked with asterisks.

the lower symmetry of the complexes (C_1 rather than C_4). Typically, two sets of spectra were observed, in which the lanthanide-induced ^{1}H shifts for one of these sets were significantly larger than for the other one. This can be explained by the presence of two diastereomeric structures as has been well established for Ln(III) complexes of DOTA derivatives. From a number of solid state and solution studies on the lanthanide(III) complexes of DOTA-like ligands it is known that these complexes occur in solution as a mixture of two diastereomeric forms which differ in mutual orientation of the "nitrogen" and "oxygen" planes of the coordination polyhedron: the square-antiprismatic (usually labeled *M*) and twisted square-antiprismatic (usually labeled *m*).[25] The ratio of *m*/*M* commonly changes along the lanthanide series; the smaller ions at the end of the series prefer the *M* form. From ^{1}H NMR spectra of Ln−BPAMD, it can be concluded that the *m*/*M* ratios in solution parallel those reported for Ln−DOTA complexes.[25,27] In the ^{1}H spectrum of the Yb−BPAMD complex a single set of signals corresponding to the *M* isomer was found (Figure 1C). However, the spectra of the Eu−BPAMD complex (Figure 1A and Figure S2) displayed two sets of signals for *m* and *M* with a ratio of intensities of 0.18:0.82, which is in accord with the *m*/*M* ratio of 0.21:0.79 reported for the Eu−DOTA complex.[27]

Interestingly, two separate ^{31}P NMR resonances with approximate intensities in a ratio 1:1 were observed for the BPAMD complexes for Eu(III), Dy(III), and Yb(III). Since the ^{1}H NMR spectra of Dy^{3+} and Yb^{3+} complexes showed that both occur exclusively as the *M* isomer, the occurrence of two ^{31}P resonances with equal intensities must be ascribed to a nonequivalent spatial orientation of the two phosphorus atoms, which are in slow interconversion on the NMR time scale (see Supporting Information, Figure S3). This was nicely demonstrated with ^{31}P spectra of the Eu(III) complex when the two ^{31}P signals coalesced when the sample was heated at 80 °C (Figure S4). A similar behavior was previously reported for other phosphonate-containing DOTA monoamides, where it was accounted for in terms of hindered rotation around the amidic bond.[28] Alternatively, this phenomenon can be explained with a single rotamer and hindered rotation of the bis(phosphonate) moiety due to the formation of a hydrogen bond between a phosphonate oxygen and the Ln(III)-bound water molecule. Molecular models indicate that one of the phosphonate groups can be close enough to the coordinated water to allow such a

(26) Howard, J. A. K.; Kenwright, A. M.; Moloney, J. M.; Parker, D.; Port, M.; Navet, M.; Rousseau, O.; Woods, M. *Chem. Commun.* **1998**, *13*, 1381−1382.
(27) Aime, S.; Botta, M.; Fasano, M.; Marques, M. P. M.; Geraldes, C. F. G. C.; Pubanz, D.; Merbach, A. E. *Inorg. Chem.* **1997**, *36*, 2059−2068.
(28) Aime, S.; Botta, M.; Garino, E.; Geninatti Crich, S.; Giovenzana, G.; Pagliarin, R.; Palmisano, G.; Sisti, M. *Chem. Eur. J.* **2000**, *6*, 2609−2617.

hydrogen bond. An inspection of molecular models also indicates that the P atoms are about 6 Å from the Ln(III) ion. This is in agreement with Ln(III)-induced ^{31}P longitudinal relaxation rate measurements on these complexes. For example, for the Dy(III) complex the $1/T_1$ values measured were 9.95 and 12.93 s^{-1}. With the use of an equation taking into account both the dipolar and the Curie relaxation,[20,29] the distances between the Dy(III) ion and the two P atoms were estimated to be 6.5 and 6.2 Å, respectively. Anyway, these data show that the phosphonate groups are not bound to the Ln(III) ion.

The hydration number of the complex was determined by measuring the ^{17}O NMR chemical shifts of water induced by the presence of the dysprosium complex. For its suitable magnetic properties, Dy(III) is generally selected for this purpose. Previously, it has been shown that the Dy(III)-induced water ^{17}O shift of a coordinated water molecule in a Dy(III) complex is almost independent of the other ligands coordinated to the Dy(III) ion.[29−31] Consequently, the Dy(III)-induced shift (DIS) can be used to determine the hydration numbers of the Dy(III) complexes. The DIS measured for the Dy−BPAMD system at 300 K (extrapolated to a molar ratio of Dy(III)/water = 1) was −2253 ppm. The DIS determined for an aqueous solution of $DyCl_3$ under the same conditions amounts to −19256 ppm. If it is assumed that in the absence of organic ligands Dy(III) is coordinated to eight water ligands, it can be concluded from these data that $q = 1$ for Dy−BPAMD and that the Ln(III) ions in the Ln−BPAMD complexes are nine-coordinated, which is consistent with a general coordination mode of this class of compounds.[1,2] Therefore complexes of the title ligand should be defined as $[Ln(H_2O)(H_xBPAMD]^{(4-x)-}$ (at pH = 7, the bis(phosphonate) group is mono- or diprotonated[32,33]).

Relaxometric Studies of Gd(III) Complex. To evaluate parameters governing the relaxivity of the Gd−BPAMD complex, variable-temperature ^{17}O transversal relaxation rates (T_2) and a ^1H NMRD profile were measured (Figure 2). These data were fitted simultaneously with a set of equations usually used to evaluate these parameters (see Supporting Information).[1,20] Since these equations contain a large number of parameters, it was necessary to fix some of them either to independently determined values or to values estimated for related complexes of DOTA-like ligands.[1,34] The number of Ln(III)-coordinated water molecules (q), a crucial parameter, was determined by the DIS experiment to be $q = 1$ (see above) and fixed during the calculation. The hyperfine coupling constant of Gd−O interaction, $A/\hbar$, was fixed at -3.6×10^6 rad s^{-1}, which is the value generally observed for complexes with $q = 1$.[1,29] The diffusion coefficient D_{GdH}, needed for the calculation of the outer-sphere contribution to the relaxation, was taken to be equal to 2.0×10^{-9} m^2 s^{-1}. The distances of gadolinium to the water

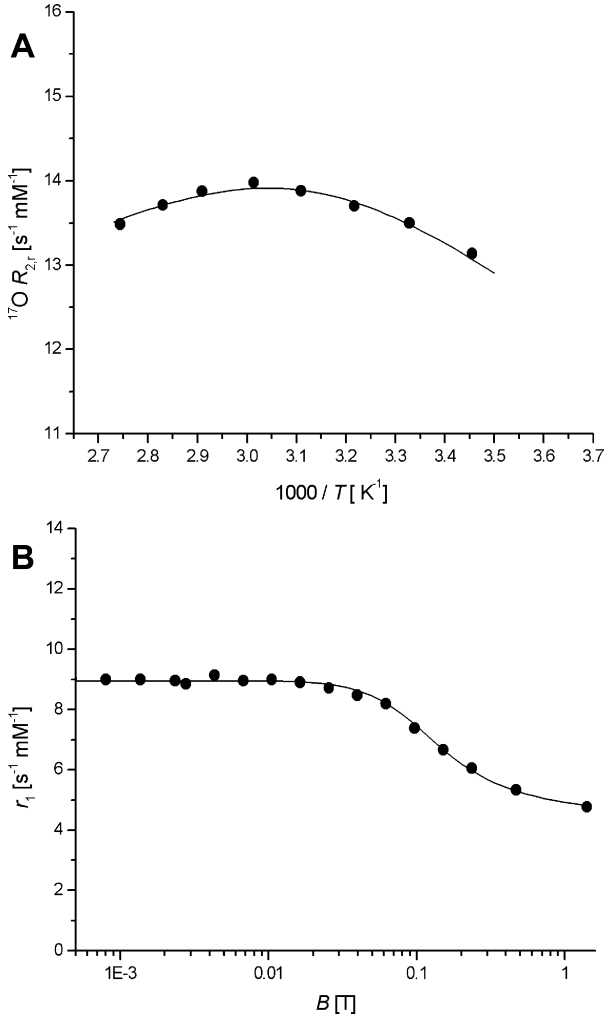


Figure 2. (A) ^{17}O R_{2r} temperature dependence (80 mM, 300 MHz); (B) ^1H NMRD profile (2 mM, 298 K) of Gd−BPAMD in aqueous solution (pH = 7.5); the solid line represents the best simultaneous fit of the data (see Table 1).

Table 1. Comparison of the Relaxometric Parameters (298 K) Found for the Gd(III) Complex of the Title Ligand, DOTA, and Related Monoamides (R^1, R^2 are groups attached to the amide nitrogen atom)

ligand	Δ^2 [$s^{-2}/10^{19}$]	τ_v [ps]	τ_r [ps]	τ_M [μs]	r_1 (20 MHz) [s^{-1} mM^{-1}]
BPAMD	3.7 ± 0.2	17 ± 1	88 ± 3	1.18 ± 0.06	5.3
DOTA[a]	1.6	11	77	0.244	4.8
L_1[b]	1.8	21	97	1.6	6.2
L_2[b]	1.6	23	91	1.6	6.0
L_3[b]	3.3	17	78	1.5	5.6

[a] Ref 34. [b] Ref 28; L_1: $R^1 = -CH_2PO_3H_2$; $R^2 = -CH_2PO_3H_2$; L_2: $R^1 = -CH_2COOH$; $R^2 = -CH_2PO_3H_2$; L_3: $R^1 = -H$; $R^2 = -CH_2PO_3H_2$.

O atom and H atom (R_{GdO} and R_{GdH}) were fixed at 2.5 and 3.1 Å, respectively, whereas the distance of closest approach of a bulk water molecule to gadolinium was taken as 3.65 Å. The most relevant parameters obtained are compared with parameters reported for other Gd(III) complexes of DOTA-like ligands in Table 1 (for complete fitting results, see Supporting Information, Table S1).

As can be seen, the values of these parameters are fully comparable with those reported on similar systems. The values of parameters determining the electronic relaxation, the mean square zero-field splitting energy (Δ^2), and the correlation time

(29) Peters, J. A.; Huskens, J.; Raber, D. J. *Prog. NMR Spectrosc.* **1996**, *28*, 283−350.
(30) Kieboom, A. P. G.; van der Toorn, J. M.; Peters, J. A.; Bovée, W. M. M. J.; Sinnema, A.; Vijverberg, C. A. M.; van Bekkum, H. *Recl. Trav. Chim. Pays-Bas* **1978**, *97*, 247.
(31) Alpoim, M. C.; Urbano, A. M.; Geraldes, C. F. G. C.; Peters, J. A. *J. Chem. Soc., Dalton Trans.* **1992**, 463.
(32) Dyba, M.; Kozlowksi, H.; Tlalka, A.; Leroux, Y.; El Manouni, D. *Pol. J. Chem.* **1998**, *72*, 1148−1153.
(33) Vaňura, P.; Jedináková-Křížová, V.; Hakenová, L.; Munesawa, Y. *J. Radioanal. Nucl. Chem.* **2000**, *246*, 689−692.
(34) Powell, D. H.; Dhubhghaill, O. M. N.; Pubanz, D.; Helm, L.; Lebedev, Y. S.; Schlaepfer, W.; Merbach, A. E. *J. Am. Chem. Soc.* **1996**, *118*, 9333−9346.

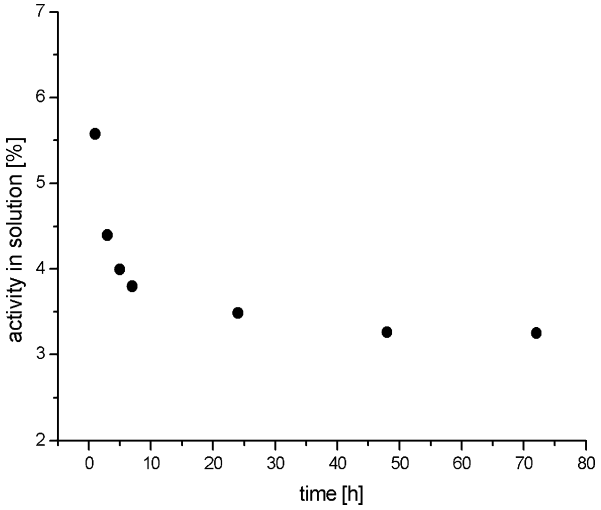


Figure 3. Determination of the time required to establish the thermodynamic equilibrium of sorption of Tb−BPAMD on HA.

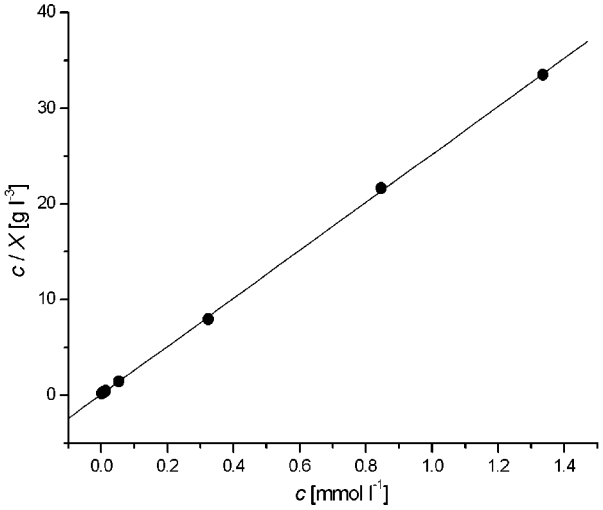


Figure 4. Adsorption isotherm of labeled Tb−BPAMD on the surface of HA. The solid line is obtained by linear regression.

of the zero-field splitting modulation ($^{298}\tau_v$) for the title ligand are close to those reported for similar ligands (Table 1). The values for the low-field limit of the electronic relaxation time, τ_{s0}, can be calculated to be 132 and 473 ps for Gd−BPAMD and Gd−DOTA, respectively. This difference can be ascribed to the difference in symmetry between these two complexes.[1,28] The rotational correlation time ($^{298}\tau_r$) was found to be 88 ps, as should be expected for a Gd(III) complex of this size. The relaxivity (5.34 s^{-1} mM^{-1} at 20 MHz, 298 K, pH = 7.5) was found to be somewhat higher than that for the Gd−DOTA complex despite the rather long water residence time ($^{298}\tau_M$ = 1.2 μs) and the short electronic relaxation time. The negative effect of these two parameters on the relaxivity is compensated by an increase in relaxivity due to the somewhat larger τ_r.

Complexes of related ligands often have a substantial contribution to the relaxivity by water molecules in the second sphere as a result of an extensive hydrogen-bond network around the phosphonic/phosphinic acid moieties.[20,35−37] Introduction of second-sphere parameters into the model did not result in better fits of the experimental data, which suggests that the second-sphere contribution is not important in the present case.

In addition, we measured the pH dependence of the relaxivity to obtain the typical shape for relaxivity influenced by prototropic exchange[38,39] of water protons in both acidic and basic regions of the profile (for more details, see Supporting Information and Figure S5).

Sorption Experiments with Tb−BPAMD. Since the title ligand was designed for bone targeting, an investigation of its sorption abilities was of primary importance. As a model system of bone tissue, we choose an aqueous suspension of hydroxyapatite (HA). The sorption of the Tb−BPAMD complex labeled with the ^{160}Tb radioisotope proved to be remarkably fast: more than 95% of the complex was adsorbed during the first hour of the experiment (Figure 3).

Definitely, the reversibility of the sorption process is one of the crucial points, since it determines the in vivo applicability of Gd−BPAMD as MRI CA. To verify the reversibility, we performed a competition experiment between already adsorbed labeled complex and free nonlabeled complex. After 3 days, the equilibrium activity was the same as that of a mixture of labeled/nonlabeled complex of the same concentrations in the presence of fresh apatite. Having confirmed the full reversibility of the sorption/desorption process, we treated the corresponding data on the basis of a linearized Langmuir adsorption isotherm:[40]

$$c/X = 1/KX_m + c/X_m$$

where c represents the equilibrium concentration and X is the equilibrium-specific adsorbed amount (Figure 4). Linear regression afforded the affinity constant K and the maximal adsorption capacity X_m (application of this model is reasonable due to negligible dissociation of the Ln(III) DOTA-like complexes in the presence of HA[41]). Comparison of the data obtained with those reported for related systems (Table 2) indicates that the sorption of labeled Tb−BPAMD is extremely favorable: K was found to be 2.1 × 10^5 L mol^{-1}, which is about 2 orders of magnitude higher than the values reported for other simple bis(phosphonates).[42] To estimate the thermodynamic stability of the (Tb−BPAMD)−HA adduct, desorption experiments with methylenediphosphonic acid (MDP) were carried out. Importantly, the presence of a 100-fold molar excess of MDP resulted in only 6% desorption of labeled Tb−BPAMD from the HA surface.

Table 2. Affinity Constants of Bis(phosphonates) with HA (298 K)

	$K/10^3$ [mol^{-1} L]
HEDP[a,b]	3.3
MDP[a,b]	0.7
Tb−BPAMD	210

[a] HEDP = 1-hydroxyethane-1,1-bis(phosphonic acid); MDP = methylenediphosphonic acid. [b] Ref 42.

(35) Botta, M. *Eur. J. Inorg. Chem.* **2000**, 399−407.
(36) Rudovský, J.; Cígler, P.; Kotek, J.; Hermann, P.; Vojtíšek, P.; Lukeš, I.; Peters, J. A.; Vander Elst, L.; Muller, R. N. *Chem. Eur., J.* **2005**, *11*, 2373−2384.
(37) Rudovský, J.; Kotek, J.; Hermann, P.; Lukeš, I.; Mainero, V.; Aime, S. *Org. Biomol. Chem.* **2005**, *3*, 112−117.
(38) Aime, S.; Botta, M.; Fasano, M.; Terreno, E. *Acc. Chem. Res.* **1999**, *32*, 941−949.
(39) Aime, S.; Gianolio, E.; Barge, A.; Kostakis, D.; Plakatouras, I. C.; Hadjiljadis, N. *Eur. J. Inorg. Chem.* **2003**, 2045−2048.
(40) Langmuir, I. *J. Am. Chem. Soc.* **1918**, *40*, 1361−1403.
(41) Li, W. P.; Ma, D. S.; Higginbotham, C.; Hoffman, T.; Ketring, A. R.; Cutler, C. S.; Jurisson, S. S. *Nucl. Med. Biol.* **2001**, *28*, 145−154.
(42) Claessens, R. A. M. J.; Kolar, I. Z. *Langmuir* **2000**, *16*, 1360−1367.

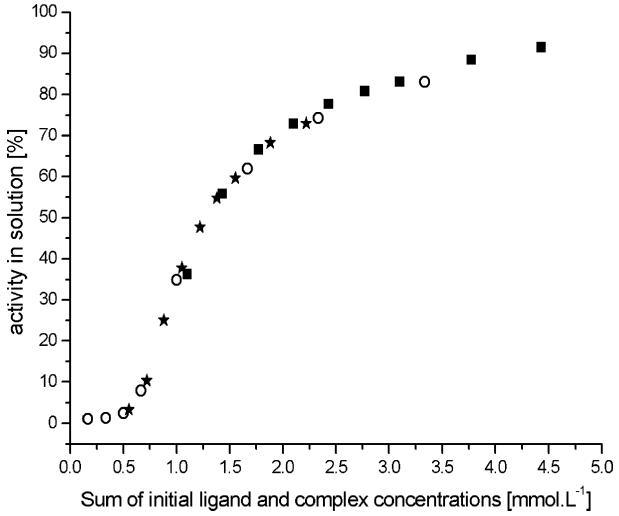


Figure 5. Competitive sorption experiment of the ligand BPAMD and labeled Tb−BPAMD complex: (★) initial concentrations: labeled Tb−BPAMD 0.55 mmol L^{-1}, BPAMD 0−1.67 mmol mL^{-1}; (■) initial concentrations: labeled Tb−BPAMD 1.1 mmol L^{-1}, BPAMD 0−3.33 mmol L^{-1}; (○) initial concentration: labeled Tb−BPAMD 0.17−3.33 mol L^{-1}.

The area on the HA surface occupied by one molecule of the complex was estimated by combining the fitted value of the maximal adsorption capacity ($X_m = 4.0 \times 10^{-5}$ mol g^{-1}) with the known active surface size of the HA sample (53 m^2 g^{-1}). The calculated value 221 $Å^2$ is in excellent agreement with an estimation obtained with molecular modeling:[43] by assuming free rotation of the complex around the anchored bis(phosphonic) group, the surface occupied by one molecule was calculated to be 227 $Å^2$. Hence, it can be concluded that a monomolecular layer was formed on HA by sorption of the Tb−BPAMD complex.

To further elucidate the origin of the favorable sorption ability of the ligand BPAMD, we designed a competitive co-sorption experiment between free BPAMD ligand and labeled Tb−BPAMD complex at variable concentrations of the components in the mixture. Plots of the activity obtained versus the sum of initial ligand and complex concentrations were almost identical for all ratios of free ligand and complex applied (Figure 5). This reveals that the affinity constants and maximum adsorption capacity of both species are similar. It may be concluded that the bis(phosphonate) moiety attached to the macrocycle via the amide nitrogen atom is almost exclusively responsible for the unusually strong adsorption of BPAMD complexes to HA.

Relaxometric Experiments with Gd−BPAMD Adsorbed on the HA Surface. As a complementary method for monitoring the sorption processes of Gd−BPAMD, we applied a slightly modified relaxometric procedure used for qualitative characterization of the interaction of Gd−DOTP interaction with HA by Sherry et al.[14] A solution of the Gd−BPAMD complex in TRIS buffer was shaken overnight with HA. The suspension was then transferred into an NMR tube. After sedimentation (completed in 4 h), the supernatant was decanted off and filtered into another NMR tube. The relaxivities of both the supernatant and the remaining slurry were measured. The experiment was arranged to obtain approximately the same volume of the slurry and the supernatant, allowing a direct comparison of the

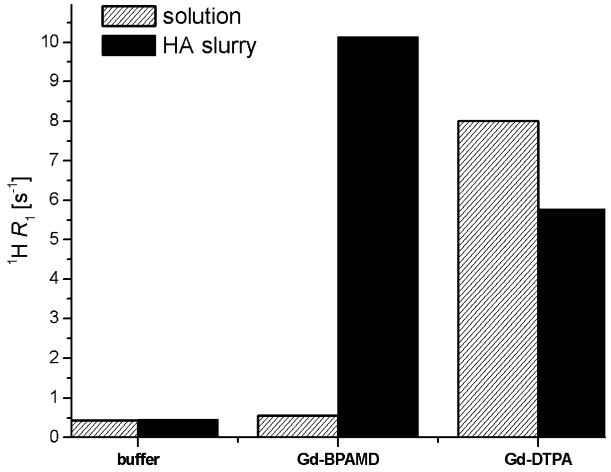


Figure 6. Longitudinal ^{1}H relaxation rates of the solution and the HA layer obtained after sedimentation of an equilibrated suspension of HA containing Gd−BPAMD and Gd−DTPA in water (298 K, 300 MHz).

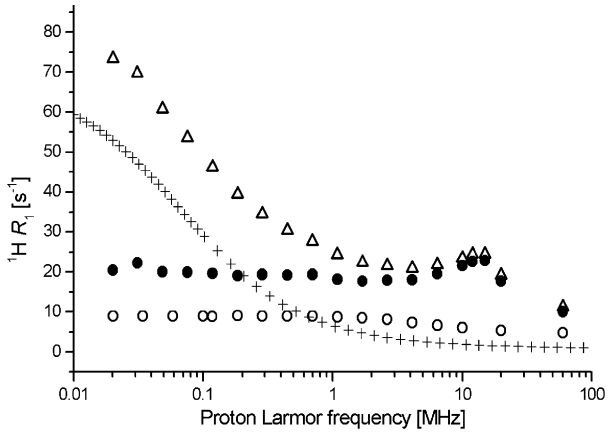


Figure 7. ^{1}H NMRD profiles of pure HA (+), (Gd−BPAMD)−HA (△), the paramagnetic contribution of the (Gd−BPAMD)−HA slurry (●), and Gd−BPAMD complex in aqueous solution (○). The Gd(III) concentration in the two latter samples is 0.8 mM.

relaxation rates of both samples. The results show that the Gd−BPAMD complex was almost quantitatively adsorbed on the HA surface (Figure 6). The concentration of Gd−BPAMD in the supernatant was very low (0.06 mmol L^{-1}, i.e., 6% of the initial concentration), which was confirmed by a measurement of the Gd(III)-induced bulk magnetic susceptibility shift.[17] Additionally, control samples of Gd−DTPA under the same conditions were measured (negligible sorption abilities of the Gd−DTPA complex were previously reported[41]). The slightly different relaxation rates of Gd−DTPA in the slurry and the supernatant (Figure 6) should be ascribed only to the different physicochemical environments of the Gd−DTPA in both phases.

To further investigate the impact of sorption on the relaxivity of Gd−BPAMD, the ^{1}H NMRD profile of the HA slurry upon sorption was measured. In fact, the profile obtained is a superposition of a paramagnetic contribution coming from the Gd(III) complex and a diamagnetic contribution coming from HA (Figure 7). The paramagnetic contribution to the ^{1}H NMRD profile was obtained by subtraction of the ^{1}H NMRD profile measured on a slurry of pure HA.[44] The resulting profile showed a local maximum at 10−20 MHz, which is typical for Gd(III) complexes with a relatively large τ_r. Notably, upon adsorption of the Gd−BPAMD on the HA surface, the relaxivity at 20 MHz is 24.0 s^{-1} mM^{-1}, which is 4.5 times higher than that for

(43) *HyperChem Release 7.5 for Windows*, Molecular Modeling System; Hypercube, Inc., 2002.

"free" complex. This observation is in contrast with results reported for Gd−DOTP (tetraphosphonated analogue of DOTA with no water molecule in the first sphere), where an expulsion of the second-sphere water molecules damped its relaxivity totally.[14]

Conclusion

We prepared the new DOTA monoamide analogue with a distant bis(phosphonic acid) moiety and a series of its Ln(III) complexes. The ligation process proceeds stepwise. Initially, the Ln(III) ion is coordinated to the ligand through the phosphonic acid groups. In a second step, insertion of the metal ion into the macrocyclic cavity takes place. This step was found to be strongly dependent on the pH and is reasonably fast only in alkaline solutions. The solution structure of the Ln(III) complexes resembles that of complexes of DOTA monoamides: an octadentate binding fashion of the organic ligand, one water molecule in the first coordination sphere, and similar *m*/*M* ratios.

The relaxometry study of the Gd(III) complex revealed a relatively slow water exchange ($^{298}\tau_M = 1.2$ μs) typical for DOTA monoamides, but nevertheless a relaxivity ($r_1 = 5.3$ s^{-1} mM^{-1}, 20 MHz, 25 °C) that is slightly higher than that for DOTA.

Sorption experiments with hydroxyapatite demonstrated that both the free BPAMD and its Ln(III) complexes are adsorbed swiftly and strongly. Additionally, the sorption of the complexes was demonstrated to be reversible. The ^{1}H NMRD of Gd−BPAMD immobilized on HA afforded a profile with a high-field dispersion typical for a slow-tumbling Gd(III) complex. This significant increase of rotational correlation time upon binding of the Gd−BPAMD complex on a hydroxyapatite surface results in a significant increase in relaxivity. Upon adsorption, the relaxivity is 24.0 s^{-1} mM^{-1} (20 MHz, 25 °C).

These in vitro results demonstrate that BPAMD is a promising ligand for bone targeting. By virtue of favorable properties, its lanthanide(III) complexes have great potential for applications in MRI, radiodiagnosis, and radiotherapy.

Acknowledgment. Thanks are due to the EU for financial support via a Marie Curie training site host fellowship (QLK5-CT-2000-60062). Supports from the Grant Agency of the Czech Republic (No. 203/03/0168) and from FNRS of the French Community of Belgium are acknowledged. This work was done in the frame of COST D18 Action and the EU Network of Excellence (NoE) "European Molecular Imaging Laboratory" (EMIL).

Supporting Information Available: ^{31}P NMR spectra of Eu−BPAMD measured shortly after mixing of the components, ^{1}H EXSY spectrum of the Eu−BPAMD complex, ^{31}P spectra of the Dy and Yb complexes, variable-temperature ^{31}P NMR spectra of the Eu complex, equations used for the fitting of the NMRD and ^{17}O NMR data, and full list of best-fit parameters for Gd−BPAMD and a discussion on the pH dependence of the relaxivity of Gd−BPAMD. This material is available free of charge via the Internet at http://pubs.acs.org.

JA054905U

(44) The high relaxivity of HA at low field can be explained by the long correlation time modulation of dipole−dipole relaxation of protons. This very long correlation time is due to the adsorption equilibrium of the water molecules on the surface of HA and to their subsequent slow motion. The same trend is observed with colloidal suspensions of silica beads. Roose, P.; van Craen, J.; Finsy, R.; Eisendraht, H. *J. Magn. Reson. A* **1995**, *115*, 20.