Gd(III) complex of a monophosphinate-bis(phosphonate) DOTA analogue with a high relaxivity; Lanthanide(III) complexes for imaging and radiotherapy of calcified tissues†

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A new phosphinic-acid DOTA-like ligand, DO3AP^{BP}, containing a geminal bis(phosphonic acid) moiety as a highly effective bone-seeking group, was synthesized in high yield. Its crystal structure was determined by X-ray analysis. Complexation with lanthanide(III) ions occurs under mild conditions (pH = 8–9, 25 °C, 2–3 h). ^{1}H, ^{31}P, and ^{17}O NMR spectroscopy show that DO3AP^{BP} forms nine-coordinated lanthanide(III) complexes with one water molecule in the first coordination sphere except for Ln = Er–Lu, which have in addition a species without lanthanide(III)-bound water. Selective formation of only two diastereomers (out of four possible) suggests that the coordinated phosphinate phosphorus atom occurs exclusively in one of the enantiomeric forms. The ratio of the twisted square antiprism (TSA) and square antiprism (SA) diastereomers changes along the lanthanide series; the gadolinium(III) complex has about 35% of the TSA species. The bis(phosphonate) moiety remains free for anchoring to osseous tissue. The ^{1}H longitudinal relaxivity of the Gd-DO3AP^{BP} complex (r_1 = 7.4 s^{-1} mM^{-1}, 20 MHz, 25 °C, pH = 7.5) is unexpectedly high compared to that of other monohydrated chelates of similar size thanks to a significant contribution from the second hydration sphere. The water residence time τ_M^{298} is 198 ns. Further increase in the relaxivity was observed in the presence of Zn(II), Mg(II) or Ca(II) ions, due to formation of coordination polymers. Slowing down of the tumbling rate of the Gd-DO3AP^{BP} complex upon adsorption on hydroxyapatite also leads to an increase of the relaxivity (r_1 = 17 s^{-1} mM^{-1}, 20 MHz, 25 °C, pH = 7.5).

Introduction

Bone tissue is predominantly composed of an extracellular matrix, which mainly consists of hydroxyapatite (HA). The high amount of mineral substance (about 75 wt%) gives it a unique character and makes it a challenging target for selective delivery of therapeutic and diagnostic agents.[1] Several types of compounds exhibit a strong affinity to bone tissue including heavy metals, tetracyclines, polymalonic acid, the hexapeptide of L-aspartic acid, 4-carboxy-3-hydroxy-1,2-pyrazoles, and geminal bis(phosphonates).[2,3] The latter are known to seek calcified tissues with a high turnover (*i.e.* parts of natural growth and pathological changes).[3] Bis(phosphonates) may act as antiresorptive agents in several types of bone diseases like osteoporosis, Paget's disease, rheumatoid arthritis, and osteolytic bone tumours.[4,5] Some of them induce tumour cell apoptosis and exhibit anticancer activity.[6] Their complexes with radionuclides have been widely used as radiodiagnostics (^{99m}Tc, ^{186}Re), radiotherapeutics (^{90}Y, ^{153}Sm, ^{166}Ho), and for palliative treatment of bone metastases (^{153}Sm, ^{186}Re).[7] Additionally, geminal bis(phosphonates) have been utilized as building blocks and generic carriers in the delivery of compounds of interest to bones.[2,5,8]

At present, clinical imaging of bone, in contrast to that of soft tissues, is based solely on methods using high-energy radiation (X-ray computed tomography, CT; single-photon emission computed tomography, SPECT; positron-emission tomography, PET).

A near infrared fluorescent bis(phosphonate) is commercially available for *in vivo* imaging of bone growth and remodelling in animals and has been tested successfully for imaging of osteoblastic activity in a mouse model.[9] Gadolinium(III) complexes

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† Electronic supplementary information (ESI) available: Complexation of the DO3AP^{BP} monitored by ^{31}P{^{1}H} NMR; ^{31}P{^{1}H}, ^{1}H NMR spectra of Ln-DO3AP^{BP} complexes; ^{31}P{^{1}H} NMR spectra of Eu- and Yb-DO3AP^{BP} complexes at different temperatures; ^{31}P{^{1}H} NMR titration curves of SA and TSA species in the Yb-DO3AP^{BP} complex and distribution diagrams; pH dependence of the relaxivity; the temperature dependence of reduced ^{17}O NMR chemical shifts, R_1, R_2, and ^{1}H NMRD profiles of the Gd-DO3AP^{BP} complex at pH = 6; τ_R as a function of concentration of the La-DO3AP^{BP} complex; kinetic stability of the Eu-DO3AP^{BP} complex against transmetallation tested by luminescence measurements; paramagnetic contribution to ^{1}H NMRD profiles of the Gd(III) complexes of the ligands DO3AP^{BP}, BPAMD, BPAPD upon adsorption on HA; the geometrical parameters found in the crystal structure of DO3AP^{BP}·2.25H$_2$O and corresponding cif file; the relaxivity r_1 and parameters governing it in the Gd-DO3AP^{BP} complex at pH 6 and 7.5 as obtained from the simultaneous fitting of ^{17}O NMR and ^{1}H NMRD data. CCDC reference number 710108. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b820705d

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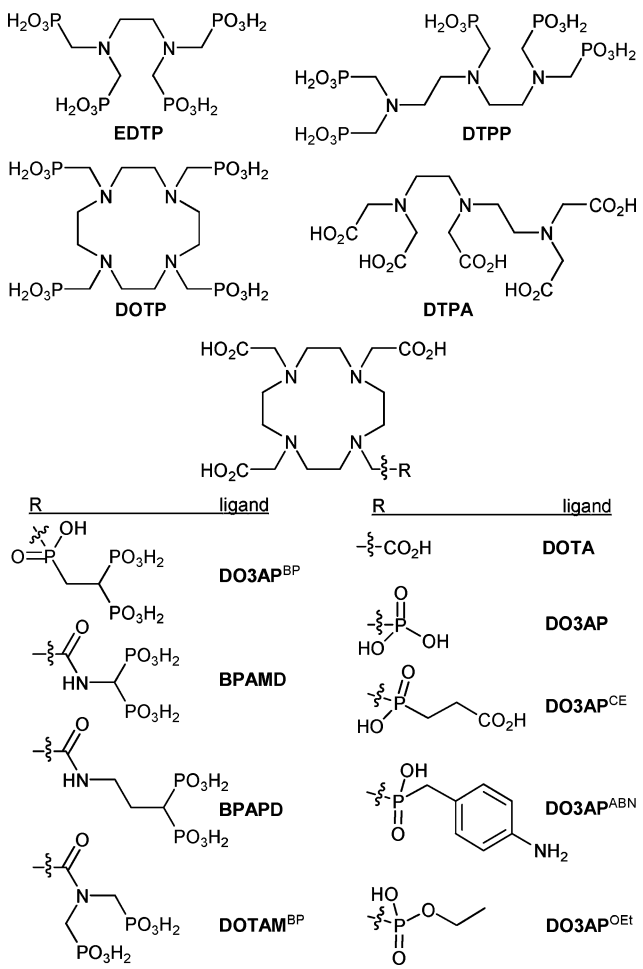
of acyclic phosphonate ligands as EDTP or DTPP (Scheme 1) have been proposed as bone targeted contrast agents (CAs) for magnetic resonance imaging (MRI), but unfortunately they show a low stability because extensive interaction of the phosphonate groups with the surface of HA causes release of the Gd(III) ion.[10] Complexes of the bis(phosphonate)-containing monoamides of DTPA (Scheme 1)[11] are expected not to be sufficiently kinetically inert for *in vivo* applications and the same holds for other open-chain DTPA analogues.[12] The macrocyclic Gd-DOTP complex (Scheme 1) is thermodynamically and kinetically stable. The complex has no water molecule in the inner coordination sphere ($q = 0$), and it acts as a positive MRI contrast agent due to water molecules in the second sphere. But it fails as an MRI CA for calcified tissues since its water ^{1}H relaxation enhancing ability is quenched upon adsorption on the HA surface due to expulsion of the water in the second sphere of the Gd(III) ion.[13] Recently, a gadofullerene, $Gd@C_{82}O_2(OH)_{16}(C(PO_3Et_2)_2)_{10}$, has been proposed as a potential bone-seeking CA; but its efficacy of binding to HA remains to be tested.[14]

We have previously reported on two bis(phosphonate)-containing DOTA monoamides, BPAMD and BPAPD (Scheme 1).[15–17] The lanthanide(III) complexes of both ligands were designed as potential bone-targeting radioimaging or radiotherapeutical agents, for pain palliation and, in the case of the Gd(III) complexes, as T_1 CAs for MRI. These DOTA-type complexes with an encapsulated lanthanide(III) ion in the macrocyclic cavity are thermodynamically and kinetically stable.[18,19] The complexes exhibit a high affinity for and a swift adsorption on HA[15,17] and bones.[16] In addition, the Gd(III) complexes showed a significant increase in ^{1}H longitudinal relaxivity (r_1, the longitudinal NMR relaxation rate enhancement of water protons expressed in s^{-1} per mM Gd(III)) resulting from an increase in the rotational correlation time (τ_R) upon adsorption on HA. *In vivo* tests on a rat model demonstrated the high affinity of these complexes for bones, particularly for parts of natural growth (growth plates and teeth).[16] However, these compounds have some drawbacks. Their syntheses are laborious and have relatively low yields. In addition, their relaxivities are suboptimal due to the relatively long residence time of a coordinated water molecule in these complexes ($\tau_M = 1.1$ μs),[15,16] which is typical for complexes of DOTA-monoamides.[20] Recent studies on complexes of monophosphonate/phosphinate DOTA analogues have shown them to have significantly lower values of τ_M (14–60 ns).[21–23] These τ_M values are very close to the optimum desired for MRI CAs at Larmor frequencies of 20–60 MHz (0.5–1.5 T). At higher magnetic field strengths, the optimum is at considerably lower τ_M values.[24]

In this paper, we describe the synthesis and the physical-chemical evaluation of a new monophosphinate DOTA-type ligand, DO3AP^{BP} (Scheme 1), bearing the bis(phosphonate) moiety on a side chain.

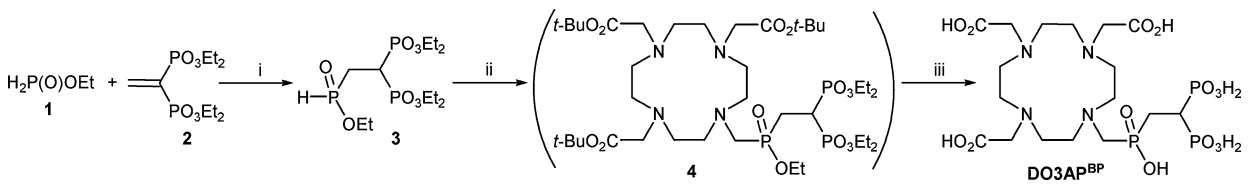


Scheme 1 Structures of ligands discussed.

Results and discussion

Synthesis

The ligand DO3AP^{BP} was prepared in a high yield (75% based on *t*-Bu$_3$DO3A·HBr) (Scheme 2). Compound **3** was the precursor for the phosphinate-bearing pendant arm and was prepared by reaction of an excess of ethyl hypophosphite **1**[25] with vinylidenebis(phosphonate) **2**.[26] The addition is *anti*-Markovnikov; the bulky –P(H)(O)OEt moiety attacks regioselectively to the C2 carbon atom. Compound **3** was attached to the tris(*t*-butylester) of DO3A[27] *via* a Mannich reaction in anhydrous toluene; this



Scheme 2 Synthesis of the DO3AP^{BP}. (i) Tetrahydrofuran, DIPEA, 25 °C, 12 h; (ii) *t*-Bu$_3$DO3A·HBr, $(CH_2O)_x$, toluene, 90–100 °C, 15–24 h; (iii) 6 M HCl, reflux, 12 h.

reaction proceeded swiftly and there was no need to shift the equilibrium by removing the water produced. The resulting ester **4** was exceptionally pure and was, therefore, hydrolyzed without further purification. The final product, DO3AP^{BP}, was purified on cation exchange resins and was finally crystallized from hot water. Thanks to this simple and efficient purification, the ligand DO3AP^{BP} can be prepared in large quantities (gram scale) in a short time.

Crystal structure of DO3AP^{BP}

The ligand DO3AP^{BP} crystallized at pH ~1.5 as a hydrate in white prisms (for the molecular structure see Fig. 1; experimental and structural parameters are listed in Tables S1 and S2†).[28] In the crystal structure, one ligand molecule, two water molecules with full occupancy, and a third one, which was best refined with an occupancy of 0.25, were found in the independent unit.

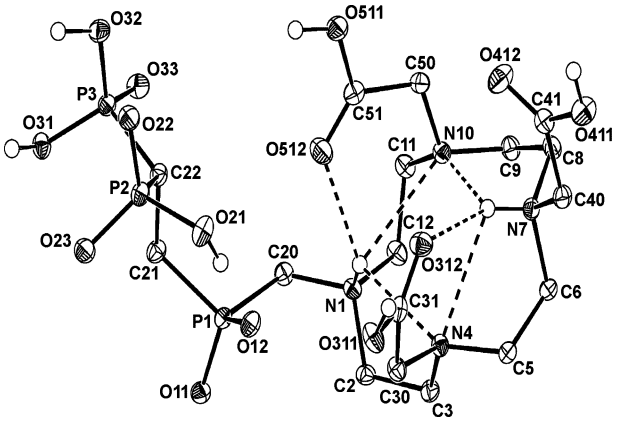


Fig. 1 Molecular structure of the DO3AP^{BP} in DO3AP^{BP}·2.25H_2O with the atom-numbering scheme. The hydrogen atoms attached to the carbon atoms are omitted for the sake of clarity. The dashed lines represent the intramolecular hydrogen bonds. Thermal ellipsoids probability level 60%.

The ligand crystallized in the zwitterionic form. All three carboxylic pendant arms of the ligand are protonated. The phosphinate group is deprotonated and the bis(phosphonate) moiety is triprotonated. The N1 nitrogen atom, bearing the phosphinate pendant arm, and the N7 nitrogen atom ('*trans*' with respect to N1) are both protonated. The protonation of the N1 nitrogen atom is rather unexpected, because, as a consequence of the stronger electron-withdrawing character of the phosphinate group, a nitrogen atom bearing a phosphinate functionality usually has a lower basicity than a nitrogen atom bearing a carboxylate pendant arm.[19] Probably the phosphinate effect is compensated for by the electron repulsion from the bis(phosphonate) moiety. In addition, hydrogen bonds and/or crystal packing effects may play a role. Similar effects have been observed in the solid-state structures of DO3AP^{CE} which also has a bifunctional phosphinate pendant arm (Scheme 1).[29] The molecular conformation is stabilized by intramolecular hydrogen bonds between protonated (N1 and N7) and unprotonated (N4 and N10) nitrogen atoms (distances N···N 2.85–3.02 Å, angles N–H···N′ about 110°). The acetate groups attached to the non-protonated nitrogen atoms N4 and N10 are turned inwards the macrocycle, and participate in an intramolecular hydrogen bond system (d(O512···N1) = 2.96 Å, angle O512···H11–N1 = 132°; d(O312···N7) = 2.97 Å, angle O312···H71–N7 = 133°). The remaining acetate group as well as the phosphinate-bis(phosphonate) pendant arm are turned outwards from the macrocycle cavity.

The whole structure is stabilized by an extensive intermolecular hydrogen-bonding network between carboxylate and bis(phosphonate) functions of ligand molecules and water solvate molecules (Table S2†).

Solution structures of lanthanide(III) DO3AP^{BP} complexes

Lanthanide(III) complexes of DO3AP^{BP} were prepared by mixing of aqueous solutions of the ligand (pH = 8–9) and of the appropriate lanthanide(III) chloride at room temperature (RT) followed by adjustment of the pH. Although the complexation occurred under slightly alkaline conditions, no lanthanide(III) hydroxide precipitated, which may be attributed to the strongly chelating bis(phosphonate) moiety. At pH = 8–9, the complexation was completed within 2–3 h at RT. Initially, the $^{31}P\{^1H\}$ NMR spectra measured during the complexation (Fig. S1†) showed broad lines next to the resonances of the final lanthanide(III) complex, which gradually disappeared. Most likely, the complexation occurs through an initial 'out of cage' complex, which rearranges into the final product *via* several intermediates similar to those proposed for DOTA[30] and BPAMD.[15] The complexation rate for DO3AP^{BP} is relatively high, probably due to the unique combination of a coordinating phosphinate group in close proximity to both the bis(phosphonate) moiety and the macrocyclic cavity, which results in an easier conversion of the 'outer-' into the 'inner-cage' complex. By contrast, the complexation of the ligands BPAMD[15] and BPAPD[16] containing the weakly coordinating amide moiety instead of phosphinate group requires heating.

The number of water molecules in the inner coordination sphere (q) of the Eu(III) ion in the Eu-DO3AP^{BP} complex,[31] was determined by the luminescence decay measurements. From the lifetimes (τ_{H2O} = 710, 741 and 776 μs; τ_{D_2O} = 1880, 1880 and 2040 μs at pH = 6, 7.5 and 10, respectively) and reported equations,[32] q was calculated to be 0.7–0.9.

The ^1H NMR spectra of the various paramagnetic Ln-DO3AP^{BP} complexes showed great similarity with those of corresponding lanthanide(III) complexes of DOTA derivatives with C_1 symmetry (see ESI†). Therefore, it may be concluded that the lanthanide(III) ion is coordinated in a similar fashion as in the parent DOTA system: through the four macrocyclic nitrogen atoms, an oxygen atom of each of the three acetate pendant arms, and an oxygen atom of the phosphinate function in the fourth pendant arm; the last coordination site is occupied by a water molecule in most cases. Since the total coordination number of lanthanide complexes of this type is usually 9, this suggests that the bis(phosphonate) moiety is not coordinated to the lanthanide(III) ion and, therefore, remains free for anchoring to osseous tissue or for interaction with other ions. This is consistent with the rather small lanthanide induced shifts of the ^{31}P nuclei in the bis(phosphonate) moiety as compared to those of the ^{31}P nuclei in the lanthanide(III)-coordinated phosphinate function (see ESI†). Additionally, distances between the Ho(III) ion and each phosphorus atom in the Ho-DO3AP^{BP} complex were estimated from ^{31}P longitudinal relaxation rates according to a reported equation taking into account both the dipolar and the Curie relaxation.[33] As the

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distances between the Ho(III) ion and the phosphorus atoms of the bis(phosphonate) moiety are much larger (5.6 and 5.4 Å) compared to that of the coordinating phosphinate moiety (3.3 Å), one can assume that the phosphonate groups are not involved in coordination of the Ho(III) ion.

Lanthanide(III) complexes of DOTA and its derivatives are known to form two diastereomers in solution, arising from the conformation of the macrocyclic ethylene groups (λ/δ) and the helicity of the acetate pendant arms (Λ/Δ).[34] This leads to two coordination geometries: square antiprismatic (SA, also called 'major' M, ΔλλλλΛ/ΛδδδδΔ) and twisted square antiprismatic (TSA, also called 'minor' m, ΔδδδδΔ/ΛλλλλΛ). For DO3AP^{BP}, the prochiral phosphorus atom of the phosphinate function in the pendant arm creates, upon complexation, another chiral centre (*R*/*S*), so that for lanthanide(III) complexes of the ligand, in principle, four enantiomeric pairs of diastereomers may exist.

At 25 °C, the $^{31}P\{^{1}H\}$ NMR spectra of Ln-DO3AP^{BP} complexes show a single set of resonances for Ln = La and Ce, two sets for Ln = Pr → Er and three sets for Ln = Tm, Yb and Lu (see Fig. 2 and Figs. S2–S15†). By comparison of these spectra with those of other lanthanide(III) complexes with P-containing pendant arms,[21,22,23,35] the resonances were assigned to one SA and two TSA diastereomers (denoted as TSA_1 and TSA_2). For the Pr(III), Nd(III), Eu(III), Ho(III) and Yb(III) complexes, the assignment was supported by comparison of the ^{1}H NMR spectra with those of lanthanide(III) DOTA complexes, which made it possible to assign the resonances with the largest lanthanide induced shifts to the axial protons in the TSA/SA enantiomers.[36] Apparently, the phosphinate phosphorus atom in the SA geometry occurs exclusively in one of the enantiomeric forms *R*/*S*. The same holds for the TSA diastereomers for Ln = La → Er. For Ln = Tm, Yb and Lu both enantiomeric forms of the TSA are populated with a high preference for one of the two possible phosphorus arrangements. A similar behaviour has been reported for lanthanide(III) complexes of DO3AP^{ABn} (Scheme 1),[21] and its ditopic derivative.[35]

Fig. 2 gives a typical example of these NMR spectra. The ^{1}H NMR spectrum of the Eu-DO3AP^{BP} complex at 25 °C (Fig. S7†) exhibits only two sets of axial proton resonances corresponding to the TSA_1 (δ = 8–20 ppm, 42%) and SA (δ = 25–35 ppm, 58%) diastereomers. The corresponding $^{31}P\{^{1}H\}$ NMR spectrum shows two resonances of the phosphinate moiety assigned to the

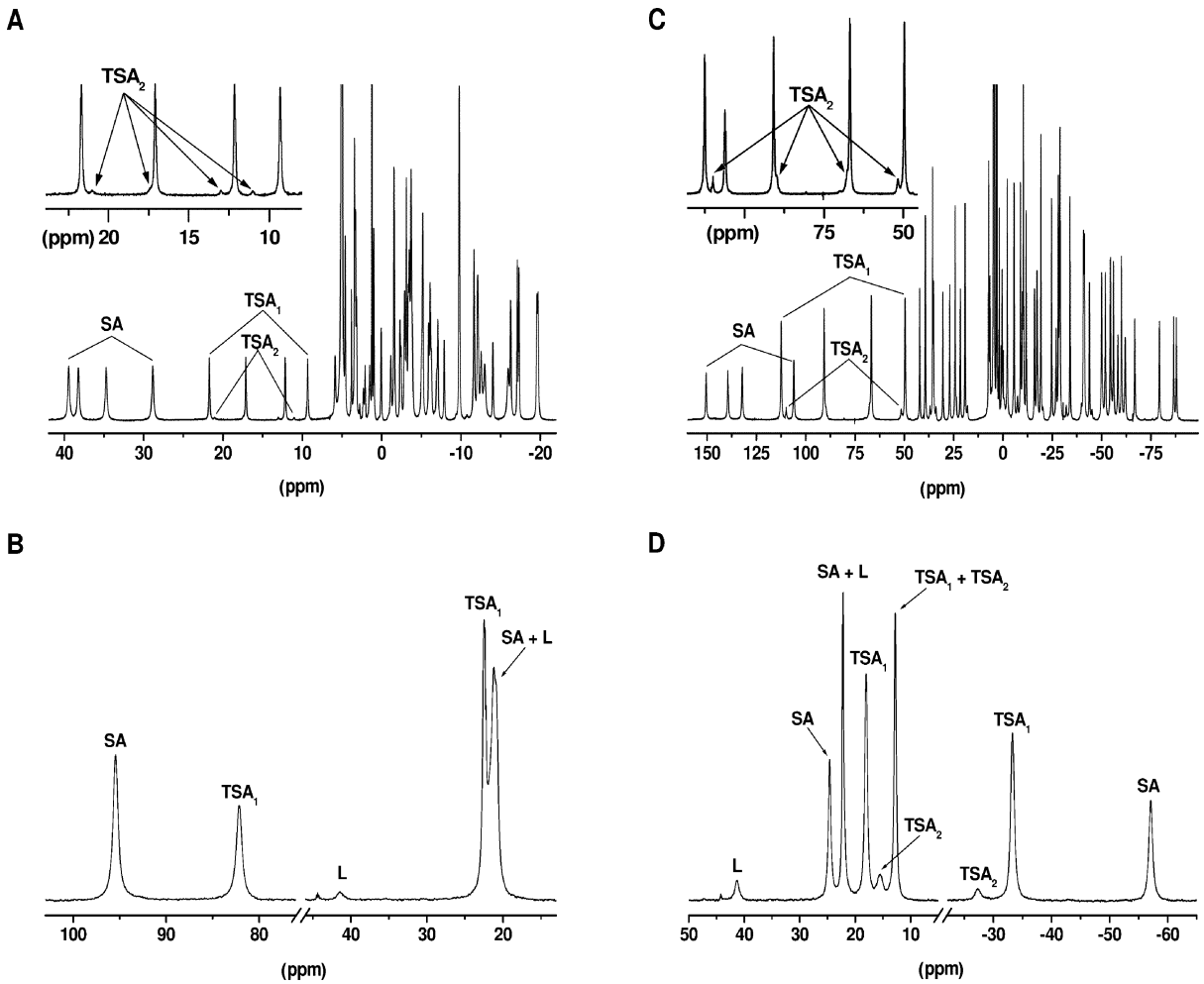

Fig. 2 (A) ^{1}H NMR spectrum of the Eu-DO3AP^{BP} complex (5 °C, pH = 7); (B) $^{31}P\{^{1}H\}$ NMR spectrum of the Eu-DO3AP^{BP} complex (25 °C, pH = 7); (C) ^{1}H NMR spectrum of the Yb-DO3AP^{BP} complex (25 °C, pH = 7); (D) $^{31}P\{^{1}H\}$ NMR spectrum of the Yb-DO3AP^{BP} complex (25 °C, pH = 7). $^{31}P\{^{1}H\}$ resonances of the free ligand in excess are labelled with 'L'.

diastereomers (TSA_1, $\delta = 82$ ppm and SA, $\delta = 95$ pm), and two other resonances around $\delta = 20$ ppm of the bis(phosphonate) moiety (Fig. 2B). At lower temperature (5 °C), a third set of signals due to presence of a tiny amount of the TSA_2 ($\delta = 11$–21 ppm, <2%) diastereomer appears (Fig. 2A); in the $^{31}P\{^1H\}$ NMR spectrum, the TSA_2 diastereomer is not distinguishable due to the large line widths.

The ^1H NMR spectrum of the Yb-DO3AP^{BP} complex shows three sets of signals, which can be assigned, to the TSA_1 ($\delta =$ 49–113 ppm, 56%), TSA_2 ($\delta = 51$–110 ppm, ~6%) and SA ($\delta = 106$–151 ppm, 38%) diastereomers even at 25 °C (Fig. 2C). Similarly, the $^{31}P\{^1H\}$ NMR spectrum of the Yb-DO3AP^{BP} complex exhibits three resonances of the phosphinate moiety of the three diastereomers (SA, $\delta = -57$ ppm; TSA_1, $\delta =$ −33 ppm and TSA_2, $\delta = -27$ ppm) and two resonances of each diastereomer between $\delta = 12$–25 ppm corresponding to chemically non-equivalent phosphorus atoms of the bis(phosphonate) moiety (Fig. 2D).

The observed non-equivalence of the phosphonate groups is given by the chirality of the macrocyclic complex (see Fig. S8–S10, S12–S14).

Fig. 3 shows a plot of the molar fractions of the SA and TSA diastereomers of the Ln-DO3AP^{BP} complexes, as determined from the integrals in the $^{31}P\{^1H\}$ NMR spectra as function of ion radii. The abundance of the TSA and SA diastereomers changes along the lanthanide series. A decrease of the molar fraction of the TSA species was observed going from Ce(III) to Ho(III) complexes. A similar trend, although with a steeper decrease of the molar fraction of the TSA diastereomer along the lanthanide series, was observed for the lanthanide(III) DOTA complexes.[34] It is known that the TSA species leave more space between N4- and O4-planes than the SA diastereomers and, therefore, the TSA arrangement is preferred for complexes with the larger lanthanide(III) ions.[19,37] The less steep decrease in the mole fraction of TSA upon decreasing the Ln(III) ionic radius for Ln-DO3AP^{BP} as compared to that observed for the lanthanide(III) DOTA complexes may be attributed to the larger steric demands of the phosphorus-containing pendant arm.[18,23] The increase in abundance of the TSA species near the end of the lanthanide series (Er → Lu) may be explained by the formation of eight-coordinated TSA complexes without a Ln(III) coordinated water molecule ($q = 0$).[23,38]

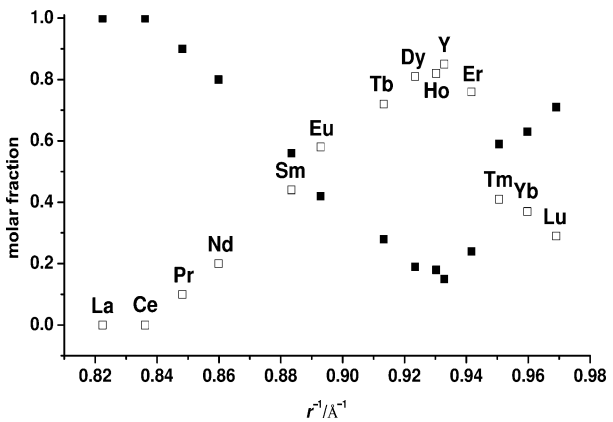

Fig. 3 Molar fractions of the TSA (■) and SA (□) diastereomers of the lanthanide(III) DO3AP^{BP} complexes as function of ion radii (25 °C, pH = 7).

Table 1 $\log\beta$ and pK_a values of the bis(phosphonate) moiety of two major diastereomers (SA, TSA_1) of the Yb-DO3AP^{BP} complex in water (25 °C)

	SA (M)		TSA_1 (m)	
Equilibrium[a]	$\log\beta$	pK_a	$\log\beta$	pK_a
$H^+ + A^{5-} \rightleftharpoons HA^{4-}$	11.2(1)	11.2	11.0(1)	11.0
$H^+ + HA^{4-} \rightleftharpoons H_2A^{3-}$	18.2(1)	7.0	18.3(1)	7.3

[a] A^{5-} denotes the fully deprotonated Yb-DO3AP^{BP} complex.

Coalescence of the resonances for the diastereomers (TSA and SA) was observed in $^{31}P\{^1H\}$ NMR spectra of the Eu(III) and Yb(III) DO3AP^{BP} complexes at high temperature (Fig. S16†).

The acid–base properties of the bis(phosphonate) moiety were studied for the Yb-DO3AP^{BP} complex. The pK_a values were determined for the two major diastereomers (SA and TSA_1) from the pH dependence of the $^{31}P\{^1H\}$ NMR chemical shifts of the phosphinate group and the two non-equivalent phosphorus atoms of bis(phosphonate) moiety (Fig. S17†). Only the two highest pK_a values for each diastereomer could be determined (Table 1). Below pH 4, severe line broadening occurred and, therefore, the remaining pK_a values could not be determined. The line broadening may be ascribed to a dramatic change of the intramolecular hydrogen-bond network upon further protonation. In addition, a partial decomplexation can be expected at pH below 3 as indicated by an increase of the relaxivity in samples of the Gd-DO3AP^{BP} complex under those conditions (Fig. S18†). The two highest pK_a values can be assigned to the bis(phosphonate) group and they have about the same values as those reported for the Yb-BPAPD complex[16] and other bis(phosphonate) compounds.[39] This once again confirms that the bis(phosphonate) group in the Yb-DO3AP^{BP} complex is not coordinated to the Yb(III) ion, because coordination would have resulted in a decrease of these pK_a values.

Relaxometric study of the Gd-DO3AP^{BP} complex

The efficacy of the Gd-DO3AP^{BP} complex as a potential contrast agent (CA) for MRI of calcified tissue was investigated by relaxometric measurements. The parameters governing the ^1H relaxivity were determined from variable-temperature ^{17}O longitudinal, transversal relaxation times (T_1, T_2) and ^{17}O NMR chemical shifts of water, and from ^1H NMRD profiles at pH 7.5 (Fig. 4). These data were fitted simultaneously to theoretical equations according to an established procedure.[40] To evaluate the effect of the protonation state of the bis(phosphonate) group on the relaxivity, the measurements were performed also at pH 6 (see Table S3†). As usual for phosphonate or phosphinate containing chelates,[22,23,21,35,41] not only inner-sphere (IS) and outer-sphere (OS) but also second-sphere (SS) contributions to the overall relaxivity had to be taken into account to achieve a satisfactory fit. To limit the number of adjustable parameters in the fitting procedure, some of them were fixed. The diffusion coefficient of the complex was fixed at the value obtained with a semi-empirical method from its molecular weight and the corresponding activation energy was fixed at 18.2 kJ mol^{-1}.[42] The distance of a water proton to the Gd(III) nucleus (r_{GdH}) was fixed at 3.1 Å, the same value as in most of the previous studies. The water molecules in the second coordination sphere are assumed to be hydrogen bonded

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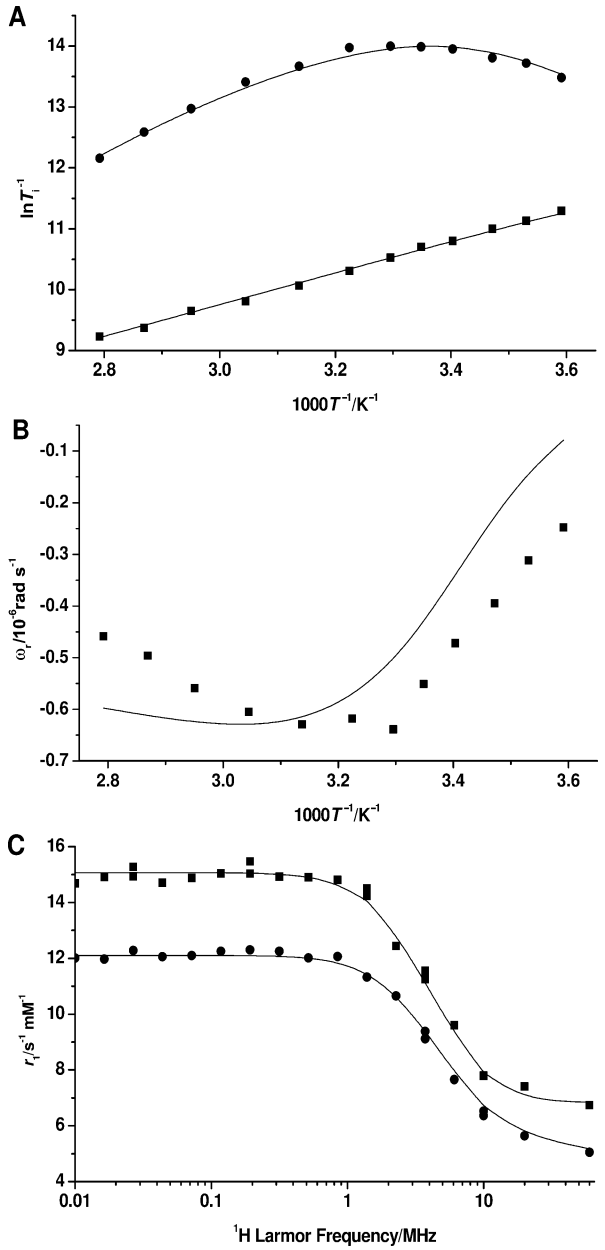

Fig. 4 The temperature dependence of longitudinal T_1 (A, ■) and transverse T_2 relaxation times (A, ●) and reduced ^{17}O NMR chemical shifts ω_r (B) of water for the Gd-DO3AP^{BP} complex (93 mM, pH = 7.5, 400 MHz). ^{1}H NMRD profiles of the Gd-DO3AP^{BP} complex in aqueous solution at 25 °C (C, ■) and 37 °C (C, ●) (2.5 mM, pH = 7.5). The curves represent the simultaneous least-squares fits.

to the Gd(III) coordinated O-atoms of the ligands. Accordingly, the distance between Gd(III) and these protons (R_{GdHss}) was fixed at 3.5 Å. The residence time of the second-sphere water molecules, τ_{Mss} was taken as 56 ps, the value calculated by Borel *et al.* for [Gd(DOTP)]$^{5-}$ by molecular dynamics;[43] the corresponding activation energy was fixed at 30 kJ mol^{-1}. The experimental data for the Gd-DO3AP^{BP} complex at pH = 7.5 are depicted in Fig. 4 together with the calculated curves, the best-fit values for the adjustable parameters are compared in Table 2 with the data for the Gd-DOTA complex,[40] and some other Gd(III) complexes of phosphinate- and phosphonate-containing chelates. For full sets of the fitted parameters at pH = 6 and 7.5, see Table S3; the data at pH = 6 are shown in Fig. S19.†

Table 2 The relaxation parameters of the Gd-DO3AP^{BP} and similar complexes (25 °C, pH = 7–7.5, $q = 1$); see Scheme 1. Unless otherwise stated, the r_1 values correspond to 20 MHz and 25 °C

Ligand	τ_M/ns	τ_R/ps	τ_v/ps	$\Delta^2/10^{19}$ s^{-2}	r_1/s^{-1} mM^{-1}
DO3AP^{BP}	198 ± 34	134.0 ± 7.7	7.1 ± 1.3	6.0 ± 1.5	7.4[a]
DOTA[40]	240	77	11	1.6	4.8
BPAMD[15,16]	1100	95	22	2.6	5.3
BPAPD[16]	1100	86	27	1.2	5.0
DOTAM^{BP} [47]	1600	97	21	1.8	6.2
DO3AP^{ABn} [21]	16.2	88	11.2	2.5	6.7 (10 MHz)
DO3AP[22]	14	83	3.9	20.7	4.6 (37 °C)

[a] Value of the experimental point; the best-fit value is 6.9.

The best-fit value for the residence time of the coordinated water molecule, $\tau_M^{298} = 198$ ns, is much shorter than that for the previously studied complexes of monoamide DOTA analogues bearing bis(phosphonate) groups, Gd-DOTAM^{BP} (Scheme 1),[47] Gd-BPAMD and Gd-BPAPD (Table 2).[15,16] This can be rationalized by the increase in negative charge and in steric strain near the coordinated water-binding site upon going from an amide function to the more bulky and negatively charged phosphinate. Since this type of lanthanide(III) complexes generally shows a dissociative water exchange mechanism,[44] these alterations result in an enhancement of the water exchange rate. On the other hand, the value of τ_M^{298} is larger than that observed previously for complexes of other monophosphonate/phosphinate DOTA-like ligands, which are in the range of 14–60 ns (Table 2).[21,22,23,35]

It should be mentioned that, for Gd(III) complexes of DOTA derivatives, the water exchange on the TSA diastereomers is much faster than that on the SA ones.[45] In the case of the Gd-DO3AP^{BP} complex, the estimated molar fraction of the TSA isomer is 0.35 (Fig. 3), which is more than twice as high as for the Gd-DOTA complex, but about the same as for the Gd-DO3AP^{OEt} complex[23] (Scheme 1). Therefore, the relatively slow water exchange on the Gd-DO3AP^{BP} complex compared to that of Gd-DO3AP^{OEt} cannot be attributed to a difference in molar ratio TSA/SA. It is more likely that charge or electronic effects and/or hydrogen bond between hydrogen atom of coordinated water molecule and oxygen atom of phosphonate group are responsible for this effect.

The parameters determining the electronic relaxation, the mean square zero-field splitting energy (Δ^2) and the correlation times of the zero-field splitting modulation (τ_v^{298}), are close to those reported for the complexes of similar ligands.[23]

The rotational correlation time of the Gd-DO3AP^{BP} complex was determined independently from the deuterium longitudinal relaxation times of the (diamagnetic) La(III) complex of partly deuterated ligand.[46] In such a diamagnetic system, the deuterium relaxation depends only on quadrupolar interactions and is given by eqn (1)

$$\frac{1}{T_1} = \frac{3}{8}\left(\frac{e^2qQ}{\hbar}\right)^2 \tau_R \quad (1)$$

The quadrupolar coupling constant ($e^2qQ/\hbar$) has a value of 170 × 2π kHz for an sp^3-hybridized C–^{2}H bond. It has been demonstrated that τ_R values obtained in this way agree well with those obtained from ^{1}H NMRD profiles.[46] The τ_R value as

estimated from an extrapolation of the ^{2}H T_1 data as a function of the complex concentration (see Fig. S20†) to 1 mM (τ_R^{298} = 162 ps, pH = 7.5) is in agreement with that obtained from the fitting of ^{1}H NMRD and ^{17}O NMR data (τ_R^{298} = 134 ps, pH = 7.5). The relatively high τ_R value as compared to Gd(III) complexes of other bis(phosphonate) DOTA derivatives, BPAMD and BPAPD (Table 2), may be ascribed to a higher negative charge and rich solvation shell of the Gd-DO3AP^{BP} complex.

The relaxivity of the Gd-DO3AP^{BP} complex (r_1 = 7.4 s^{-1} mM^{-1} at 20 MHz, 25 °C, and pH = 7.5) is high for a low-molecular-weight chelate with q = 1 (Table 2). This is mainly thanks to a significant contribution (1.2 s^{-1} mM^{-1} at 20 MHz, ~15%) from the SS water molecules (q_{ss} ~ 2), as well as to the relatively large value of τ_R. The relatively high relaxivity in solution could be also ascribed to a formation of coordination oligomers and polymers (COP) as it is known for strongly chelating bis(phosphonates) in solution as well as in the solid state.[48] In the case of the negatively charged Ln-DO3AP^{BP} complexes, the formation of COP is determined mainly by the counter-ions used and the stability constants of their complexes with the bis(phosphonate) moiety. This was tested by relaxometry on Gd-DO3AP^{BP} upon addition of some monovalent and divalent metal ions. Because no changes in relaxivities of the Gd-DO3AP^{BP} complex were observed going from samples containing the non-coordinating Me_4N^+ ion to those containing weekly coordinating Na(I) or K(I) ions,[39] it seems that COP formation is most likely negligible in the presence of monovalent metal ions. Furthermore, it should be noted that the relaxivity of the Gd-DO3AP^{BP} complex is independent of the protonation state of the bis(phosphonate) moiety in range of pH = 3–12 (Fig. S18†).

On the other hand, a significant increase in relaxivity of the Gd-DO3AP^{BP} complex was observed in presence of divalent metal ions (*e.g.* for 3 equiv. of Zn(II) ions, r_1 = 22.4 s^{-1} mM^{-1} at 20 MHz, 37 °C). This cannot be attributed to transmetallation with Zn(II) ions as no significant changes in luminescence lifetimes and emission spectra of the Eu-DO3AP^{BP} complex were observed in the presence of Zn(II) and phosphate ions even after several days (Table S4 and Fig. S21†). Thus, one can conclude that Ln-DO3AP^{BP} complexes are kinetically inert against transmetallation. Therefore, the enhanced relaxivity indicates an increase of the τ_R value as a consequence of formation of COP. This was independently supported by a two- to three-fold increase of the τ_R value determined from ^{2}H T_1 data of the deuterated La-DO3AP^{BP} complex in presence of 0.5 equiv. of Zn(II) ions. The relaxivity enhancement upon addition of divalent metal ions is concentration and pH dependent. It may be concluded that the relaxivity is not affected by the presence of Na(I) or K(I) ions as the formation of COP seems to be negligible due to the very low stability constants of their complexes with bis(phosphonates). From preliminary results of experiments with divalent metal ions it seems that the Gd-DO3AP^{BP} complex could be a promising candidate for sensing of these ions and this is a subject of the further study.

Adsorption on hydroxyapatite

The adsorption behaviour of Ln-DO3AP^{BP} complexes was evaluated by *in vitro* experiments with hydroxyapatite (HA) as a model of bone. Recently, an adsorption study using the ^{160}Tb-DO3AP^{BP} complex confirmed that this complex has a strong affinity to the HA (affinity constant K = 1.29 × 10^5 L mol^{-1}). The complex adsorbs reversibly in a monomolecular layer on the HA surface (maximum adsorption capacity X_m = 0.778 μmol m^{-2}).[17]

In Fig. 5 the ^{1}H NMRD profile of a slurry of HA in the presence of the Gd-DO3AP^{BP} complex is compared with profiles for adsorbed Gd-BPAMD and Gd-BPAPD complexes under the same conditions (the estimated HA surface coverage is about 40% under the conditions applied). The ^{1}H NMRD profiles of all these slurries were measured after settlement and removing of the supernatant. These profiles can be described as the sum of the contributions coming from the paramagnetic complexes and the profile for HA. The latter compound has a high relaxivity at low field,[15] which can be explained by the long correlation time for the modulation of the dipole–dipole relaxation of the protons.[49] 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 paramagnetic contributions to the ^{1}H NMRD profiles were evaluated by subtraction of the relaxation rates for HA covered with an equivalent amount of the corresponding diamagnetic La(III) complex (compare Fig. 5 and Fig. S22†). These diamagnetic profiles appeared to be identical for the various complexes studied. The resulting corrected ^{1}H NMRD profiles are not flat at low Larmor frequencies (<1 MHz), which is uncommon and which indicates that the estimated diamagnetic contributions were somewhat too high; apparently, the La(III) complexes are not good probes for the diamagnetic contribution in this case, or the presence of a different lanthanide ion can change physical properties—the settling and packing—of the HA. The ^{1}H NMRD profiles have a local maximum between 10–20 MHz indicating a long τ_R, due to reduced mobility of the complex upon adsorption on the HA surface (Fig. 5).[50]

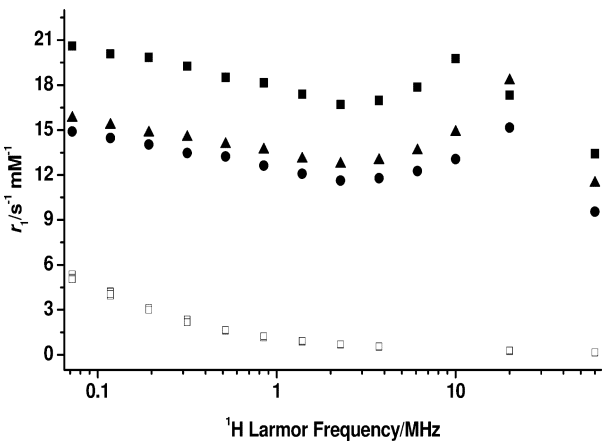

Fig. 5 ^{1}H NMRD profiles of the Gd-DO3AP^{BP} (■), Gd-BPAMD (●) and Gd-BPAPD (▲) complexes and ^{1}H NMRD profiles of diamagnetic La(III) complexes of desired ligands (□). All complexes were adsorbed on HA under analogous conditions (pH = 7.5, 25 °C). The diamagnetic contribution is ligand independent.

Conclusions

A bis(phosphonate) containing DOTA analogue, DO3AP^{BP}, was prepared by means of a simple procedure. The Gd(III) complex of this ligand has a higher relaxivity than previously reported Gd(III) complexes of bis(phosphonates) thanks to a higher τ_R value, and

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to a significant contribution to the relaxivity by second-sphere water molecules. The bis(phosphonate) function, which is at some distance of the Gd(III) centre is free for interaction with bone (or its model HA) and upon this interaction the coordinated water molecules are not expelled. The studies with the model HA showed that the relaxivity of Gd-DO3AP^{BP} is high (about 17 s^{-1} mM^{-1} at 20 MHz and 25 °C). Therefore, it may be concluded that this compound has potential as a positive MRI contrast agent for bone and for other calcified tissues.

This complex shows a high affinity for divalent cations resulting in the formation of coordination oligomers and polymers, which is accompanied by a significant increase of the relaxivity due to the decrease of the molecular tumbling rate. This phenomenon may be applied in the development of *in vitro* and *in vivo* responsive contrast agents for these ions.

Experimental

Materials

All commercially available reagents had synthetic purity and were used as received. THF and toluene were dried by distillation from Na and P_2O_5, respectively. *t*-Bu_3DO3A·HBr,[27] ethylhypophosphite **1**,[25] and tetraethyl vinylidene-1,1-bis(phosphonate) **2**[26] were synthesized according to reported procedures. HA was purchased from Fluka (Cat. No. 55496; specific surface area 63 m^2 g^{-1}).

NMR spectroscopy

^{1}H (399.95 MHz), ^{13}C (100.58 MHz), ^{31}P (161.9 MHz), ^{17}O (54.22 MHz) and ^{2}H (61.395 MHz) NMR spectra were recorded on a Varian Unity Inova-400 spectrometer, using 5 mm sample tubes. For the measurements in $CDCl_3$, TMS 0.00 ppm (^{1}H), and $CDCl_3$ 77.0 ppm (^{13}C) were used as internal standards. For the measurements in D_2O, *t*-BuOH was used as an internal standard with the methyl signal referenced to 1.25 ppm (^{1}H) or 31.2 ppm (^{13}C). 1% H_3PO_4 in D_2O was used as an external reference to ^{31}P NMR. Unless stated otherwise, the NMR spectra were recorded at 25 °C. *J* values are given in Hz.

The pK_a values of the bis(phosphonate) moiety in the Yb-DO3AP^{BP} complex were determined from ^{31}P{^{1}H} NMR titration data. The pH values of the samples were adjusted using aqueous HCl and NaOH. The pH values were measured at 25 °C using a Corning 125 pH-meter with a combined electrode (Aldrich Chemical Co.). For the calibration of the pH-meter standard buffer solutions were used (pH = 4.01, 7.00 and 10.01). The pH values >12 were calculated from the known concentration of NaOH in the samples.

For the ^{17}O NMR measurements, 30 μl of ^{17}O-enriched water (10% $H_2{}^{17}O$) was added to the samples, and H_2O (pH = 6 and 7.5) was used as an external chemical shift reference. The pH was adjusted with aqueous KOH. Variable-temperature ^{17}O NMR measurements (*i.e.* chemical shifts, T_1 and T_2 relaxation times) of solutions of the Gd-DO3AP^{BP} complex (87–93 mM, pH = 6 and 7.5) were performed without spin and frequency lock in the temperature range 5–85 °C. ^{17}O NMR chemical shifts were corrected for the bulk magnetic susceptibility (BMS) contribution by measurement of the difference between chemical shifts of ^{1}H NMR signals of *t*-BuOH in the paramagnetic sample and in a sample of *t*-BuOH in pure water.[51]

^{31}P{^{1}H} T_1 relaxation times of the Ho-DO3AP^{BP} complex in D_2O (110 mM, pH = 7.0) were measured without spin and frequency lock, with gain set to zero at 25 °C. Obtained data were not corrected for a diamagnetic contribution.

^{2}H T_1 relaxation times of aqueous solutions of partially deuterated La-DO3AP^{BP} complexes (20–250 mM, pH = 7.5; and 50 mM, pH = 7.5 in the presence of 0.5 equiv. of $Zn(NO_3)_2$) were measured without spin and frequency lock, with gain set to zero and 25 °C.

The T_1 and T_2 relaxation times were obtained by the inversion recovery method[52] and the Carr-Purcell-Meiboom-Gil pulse sequence[53], respectively. Unless otherwise stated, the concentrations of lanthanide(III) ions were determined by bulk magnetic susceptibility measurements.[54]

Mass spectrometry

ESI/MS spectra were recorded on a Bruker Esquire 3000 spectrometer equipped with an electrospray ion source and ion-trap detection.

Synthesis of DO3AP^{BP}

Tetraethyl{[(ethoxyhydrophosphoryl)methyl]methylene}bis(phosphonate) 3. Ethylester **1** was generated *in situ* from solid H_3PO_2 (1.58 g, 23.9 mmol) and $EtOSiMe_3$ (5.65 g, 47.8 mmol) in dried tetrahydrofuran (30 ml) at RT in 2 h (conversion about 60%, according to ^{31}P NMR spectroscopy).[25] Then vinylidenebis(phosphonate) **2** (3.00 g, 9.96 mmol) and DIPEA (3.09 g, 23.91 mmol) were added in one portion and the reaction mixture was stirred at RT overnight. Volatiles were evaporated under vacuum. The remaining oil was dissolved in CH_2Cl_2 (10 ml) and extracted with H_2O (15 ml). The aqueous phase was re-extracted with CH_2Cl_2 (2 × 10 ml). The combined organic phases were dried with anhydrous Na_2SO_4 and then filtered. Solvents were evaporated under vacuum to give crude **3** as a colourless viscous oil (3.62 g, >90% purity according to ^{1}H and ^{31}P NMR spectra), which was used in the next step without further purification.

A portion of compound **3** was purified by column chromatography (silica gel, EtOAc : EtOH 3 : 1, R_F = 0.4); δ_H (400 MHz; $CDCl_3$) 1.37 (15H, m, –CH_3), 2.31 (2H, m, –CH_2–CH), 2.84 (1H, m, P–CH–P), 4.18 (10H, m, –CH_2–CH_3), 7.34 (1H, d, P–H, $^1J_{PH}$ 577); $\delta_{C\{H\}}$ (100.6 MHz; $CDCl_3$) 15.72 (5C, m, –CH_3), 24.36 (1C, dt, –CH_2–CH, $^1J_{PC}$ 94.5, $^2J_{PC}$ 5.0), 30.08 (1C, td, P–CH–P, $^1J_{PC}$ 135.0, $^2J_{PC}$ 3.5), 62.07 (1C, d, HPO–CH_2–, $^2J_{PC}$ 6.4), 62.36 (4C, m, CH–PO–CH_2–); $\delta_{P\{H\}}$ (161.9 MHz; $CDCl_3$) 22.0 (1P, d, *P*–CH–P, $^3J_{PP}$ 34), 22.2 (1P, d, *P*–CH–P, $^3J_{PP}$ 21), 35.8 (1P, dd, *P*–H, $^3J_{PP}$ 21, $^3J_{PP}$ 34); δ_P (161.9 MHz; $CDCl_3$) 22.1 (2P, m, *P*–CH–*P*), 35.8 (1P, dm, *P*–H, $^1J_{PH}$ 577); ESI/MS(+) *m*/*z* 394.8 (M + H^+), 416.9 (M + Na^+), 432.7 (M + K^+), $C_{12}H_{29}O_8P_3$ requires 394.1.

10-{[(2,2-Bisphosphonoethyl)hydroxyphosphoryl]methyl}-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, DO3AP^{BP}. Crude **3** (3.62 g, 9.18 mmol) was dissolved in dried toluene (100 ml). *t*-Bu_3DO3A·HBr (0.5 equiv., 2.73 g, 4.58 mmol) and paraformaldehyde (1 equiv., 0.28 g, 9.33 mmol) were added and the reaction mixture was heated at 90–100 °C for 15–24 h.

The reaction was monitored by ESI/MS in the positive mode for the disappearing of the *t*-Bu_3DO3A signal at *m/z* 515 ($M + H^+$); ESI/MS(+) *m/z* of the compound **4**: 921.1 ($M + H^+$), 943.5 ($M + Na^+$), $C_{33}H_{61}N_4O_{14}P_3$ requires 920.5). Volatiles were evaporated under vacuum and the oily residue (**4**) was hydrolyzed directly by aqueous HCl (6 M) under reflux overnight. After evaporation of volatiles, the excess of HCl was removed by repetitive co-evaporation with water. The resulting oil was purified on a strong cation exchange resin (Dowex 50, H^+-form, 100/50 mesh, Fluka, elution with water followed by 10% aqueous pyridine), and then on a weak cation exchange resin (Amberlite CG 50, H^+-form, 160/200 mesh, Fluka, elution with H_2O). The purity of fractions containing $DO3AP^{BP}$ was monitored by ^{31}P and ^1H NMR spectroscopy. Crystallization of $DO3AP^{BP}$ by slow evaporation of aqueous solutions gave white rod crystals suitable for X-ray analysis. The yield of $DO3AP^{BP}\cdot1.5H_2O$ was 2.19 g (75% based on *t*-Bu_3DO3A·HBr) (Found: C, 31.79; H, 5.90; N, 8.62. $C_{17}H_{35}N_4O_{14}P_3\cdot1.5H_2O$ requires C, 31.94; H, 5.99; N, 8.77%); δ_H (400 MHz; D_2O; 90 °C; pH ~1.5) 2.35 (2H, m, $-CH_2-$CH), 2.68 (1H, m, P–C*H*–P), 3.33 (12H, m, $-CH_2-$N–pendant), 3.49 (2H, d, –N–CH_2–P, $^2J_{PH}$ 7.2), 3.50 (4H, m, $-CH_2-$N–pendant), 3.85 (4H, s, $-CH_2-$COOH), 3.90 (2H, s, $-CH_2-$COOH); $\delta_{C\{H\}}$ (100.6 MHz; D_2O; 60 °C; pH = 10) 31.16 (1C, d, $-CH_2-$CH, $^1J_{PC}$ 85.), 35.87 (1C, t, P–*C*H–P, $^1J_{PC}$ 113.6), 52.28 (2C, s, $-CH_2-$N–pendant), 52.96 (2C, s, $-CH_2-$N–pendant), 53.17 (2C, s, $-CH_2-$N–pendant), 53.47 (2C, s, $-CH_2-$N–pendant), 55.35 (1C, d, –N–CH_2–P, $^1J_{PC}$ 88.5), 59.27 (2C, s, $-CH_2-$COOH), 59.45 (1C, s, $-CH_2-$COOH), 178.38 (2C, s, –*C*OOH), 178.81 (1C, s, –*C*OOH); δ_P (161.9 MHz; D_2O; 90 °C; pH ~1.5) 23.4 (2P, m, $-PO_3H_2$), 39.6 (1P, bs, –*P*–CH_2); ESI/MS(–) *m/z* (611.2 ($M - H^+$), 633.2 ($MNa^+ - 2H^+$), 649.2 ($MK^+ - 2H^+$), $C_{17}H_{35}N_4O_{14}P_3$ requires 612.1.

Partial deuteration of $DO3AP^{BP}$

The $DO3AP^{BP}$ sample (50 mg) was heated at 90 °C in D_2O (1 ml) in the presence of annealed K_2CO_3 (pH = 10) for 5 days, analogous to a published procedure.[55] During this time, the solvent was evaporated off under vacuum twice, after which a fresh portion of D_2O was added. The degree of deuteration of the $-CH_2$ functions of the acetate groups of the ligand $DO3AP^{BP}$ was 86% as determined by ^1H NMR spectroscopy. Strong and weak cation exchange resins (Dowex 50, Amberlite CG 50) were used as described above to obtain the pure product (40 mg).

Synthesis of lanthanide(III) complexes: general procedure

The investigated solutions of lanthanide(III) complexes contained 10% molar excess of free ligand as we were not able to remove the excess of lanthanide(III) ions in the presence of the strongly chelating geminal bis(phosphonate) moiety. The ligand $DO3AP^{BP}$ was dissolved in water at pH 8–9. An aqueous solution of lanthanide(III) chloride was added (0.9 equiv.). The mixture was stirred 2 h at RT. Then the pH was adjusted to the required value by addition of 20% aqueous solution of NaOH or KOH, if necessary.

Assessment of the hydration number

The luminescence measurements of Eu(III) were performed on a Luminescence Thermo Spectronic spectrometer AMINCO Bowman Series 2. The luminescence spectra were obtained after excitation at the $^5L_6 \leftarrow {}^7F_0$ band of the Eu(III) band (λ = 396 nm). All measurements were performed at RT. Luminescence lifetimes of an aqueous solution (H_2O and D_2O) of the Eu-$DO3AP^{BP}$ complex (90 mM) were measured at different pH values (6, 7.5, 10).

Complex stability in the presence of Zn^{2+} ions

Luminescence lifetimes and emission spectra of the Eu-$DO3AP^{BP}$ complex (62 mM in H_2O, pH = 7) in the presence of phosphate (67 mM) and $ZnCl_2$ (195 mM) were measured. As a reference sample, the Eu-$DO3AP^{BP}$ complex (62 mM in H_2O, pH = 7) in the presence of phosphate (67 mM) was used. The measurements were performed after mixing the chemicals and after standing at RT for a few hours up to three days.

Proton NMRD profiles

All ^1H NMRD profiles were measured at magnetic field range 4.7×10^{-4}–0.35 T (0.02–15 MHz) using a Stelar SpinMaster FFC-2000 relaxometer. Measurements at 0.47 and 1.42 T (20 and 60 MHz) were performed with a Bruker Minispec mq20 and Bruker Minispec mq60, respectively. Aqueous solutions of the Gd-$DO3AP^{BP}$ complex (2.5 mM, pH = 6 and 7.5) were measured at 25 and 37 °C.

The ^1H T_1 relaxation times of the Gd-$DO3AP^{BP}$ complexes (2 mM, pH ~7, prepared using NaOH, KOH or Me_4NOH for pH adjustment; and 2 mM, pH = 2–12, using NaOH and HCl for pH adjustment) were measured at 25 °C, 20 MHz.

The ^1H T_1 relaxation time of the Gd-$DO3AP^{BP}$ complex (2 mM, pH = 7.1–7.4) were measured upon addition of $Zn(NO_3)_2$ (3 equiv.) at 37 °C, 20 MHz. For pH adjustment KOH was used.

Adsorption on hydroxyapatite: general procedure

An increase of r_1 upon adsorption on HA was tested similarly as described in the literature.[15,16] HA (1.0 g) was treated with aqueous solution of the Gd(III) complex (1 ml, 25 mM) in a TRIS·HCl buffer solution (4.0 ml, 0.1 M, pH = 7.5) at RT for 3 days. The suspension settled, the supernatant was removed, and ^1H NMRD profile of the HA slurry was measured at 25 °C. A correction for the diamagnetic contribution to r_1 was applied using ^1H NMRD data of HA slurry with the adsorbed La(III) complex under analogous conditions. Concentration of Gd in both the HA slurry and the supernatant was determined with an ICP-AES spectrometer VistaPro (Varian) in axial plasma configuration, equipped with an autosampler SPS-5 (Australia), an inert parallel flow nebuliser, an inert spray chamber and a demountable torch with an inert injector tube. The HA sample was digested in concentrated HNO_3 and measured after dilution as solutions in 5% HNO_3.

Data evaluation

The experimental ^1H NMRD data were fitted simultaneously with ^{17}O NMR data according to the established procedure[40] by means of a least square fitting procedure using the Micromath Scientist program version 2.0 (Salt Lake City, UT).[56] The pK_a values were calculated from the dependence of $^{31}P\{^1H\}$ NMR chemical shifts of the phosphinate group and two non-equivalent phosphorus

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atoms of bis(phosphonate) moiety in the Yb-DO3AP^{BP} complex on the solution pH using the OPIUM program package.[57]

X-Ray diffraction

Single crystals of DO3AP^{BP} were obtained by slow evaporation of aqueous solutions. The diffraction data were collected at 150 K (Cryostream Cooler, Oxford Cryosystem) using a Nonius Kappa CCD diffractometer and Mo-Kα radiation (λ = 0.71073 Å) and analyzed using the HKL DENZO program package.[58] The structures were solved by direct methods (SIR92),[59] and refined by full-matrix least-squares techniques (SHELXL97).[60] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were located in difference map of electron density; however, they were treated in theoretical (C–H) or original (O–H, N–H) positions with thermal parameters $U_{eq}(H) = 1.2\ U_{eq}(X)$ as their free refinement led to some unrealistic bond lengths. In the crystal structure, one ligand molecule, two water molecules with full occupancy, and a third one, which was best refined with occupancy of 0.25, were found in the independent unit.

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