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Synthesis of a bifunctional monophosphinic acid DOTA analogue ligand and its lanthanide(III) complexes. A gadolinium(III) complex endowed with an optimal water exchange rate for MRI applications†

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A new bifunctional octa-coordinating ligand containing an aminobenzyl moiety, DO3AP^{ABn} (H_4DO3AP^{ABn} = 1,4,7,10-tetraazacyclododecane-4,7,10-triacetic-1-{methyl[(4-aminophenyl)methyl]phosphinic acid}), has been synthesized. Its lanthanide(III) complexes contain one water molecule in the first coordination sphere. The high-resolution ^{1}H and ^{31}P spectra of [Eu(H_2O) (DO3AP^{ABn})]$^-$ show that the twisted square-antiprismatic form of the complexes is more abundant in respect to the corresponding Eu(III)–DOTA complex. The ^{1}H NMRD and variable-temperature ^{17}O relaxation measurements of [Gd(H_2O)(DO3AP^{ABn})]$^-$ show that the water residence time is short ($^{298}\tau_M$ = 16 ns) and falls into the optimal range predicted by theory for the attainment of high relaxivities once this complex would be endowed by a slow tumbling rate. The relaxivity ($^{298}r_1$ = 6.7 mM^{-1} s^{-1} at 10 MHz) is higher than expected as a consequence of a significant contribution from the second hydration sphere. These results prompt the use of [Gd(H_2O)(DO3AP^{ABn})]$^-$ as a building block for the set-up of highly efficient macromolecular MRI contrast agents.

Introduction

Gadolinium(III) complexes of polyaminopolycarboxylates are widely used as contrast agents (CA) in magnetic resonance imaging (MRI).[1] The efficacy of a paramagnetic CA is primarily assessed by its relaxivity (r_1) that reports the T_1 relaxation enhancement of (tissue) water protons of the paramagnetic complex at 1 mM concentration. The search for systems endowed with high relaxivity continues to be an important task. It is even more important in view of applications in the field of molecular imaging where the low concentration of the targeting sites has to be compensated with an improved sensitivity of the imaging probes.[2] It was recognized early on that a significant increase of relaxivity can be expected when the reorientational motion (represented by the rotational correlation time τ_R) of the gadolinium(III) chelates is slowed down upon conjugation to suitable high-molecular weight synthons. However, such an expected enhancement of relaxivity is often "quenched" by the occurrence of an exceedingly long exchange lifetime of the coordinated water (water residence time τ_M).[1,3] It has been suggested that, at the imaging field of 0.5 T (corresponding to hydrogen Larmor resonance frequency of 20 MHz), an optimal value for $^{298}\tau_M$ is between 10 to 30 ns.[1,4] Most of the currently available macrocyclic systems display a residence lifetime too long for the attainment of high relaxivities (*e.g.* $^{298}\tau_M$ of [Gd(H_2O)(DOTA)]$^-$ has been reported to be 243 ns at 298 K; H_4DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracetic acid[5]) when their τ_R would have been slowed down to the nanosecond range.

As it has been shown that the exchange of the coordinated water in [Gd(H_2O)(DOTA)]$^-$ and similar complexes occurs through a dissociative pathway,[1] a possible route to accelerate water exchange rate can be envisaged by destabilizing the ground-state nonacoordinated structure. This approach has been shown to work successfully in the case of [Gd(H_2O)(TRITA)]$^-$ (H_4TRITA = 1,4,7,10-tetraazacyclotridecane-1,4,7,10-tetraacetic acid).[6] Further insights into the relationship between water exchange rates and solution structures have been gained from in-depth investigations on the structural isomers of lanthanide(III) DOTA complexes. It is known that DOTA-like ligands wrap around a lanthanide(III) ion yielding two coordination geometries, namely ***M*** (SA, square-antiprismatic) and ***m*** (TSA, twisted square-antiprismatic).[1,7] It was found that the ***m*** (TSA) isomers display 10 to 100 times faster exchange of the coordinated water molecule, both in amide and acid derivatives.[8,9] This effect was ascribed to an increased steric repulsion around the water-binding site in the case of ***m*** (TSA) isomers. Both the [Gd(HDOTP)]$^{4-}$ (H_8DOTP = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylphosphonic acid)) and complexes of the phosphinic acid analogs are characterized by a ***m***-type structure.[10,11] Interestingly, the bulkiness of the phosphorus acid groups in the complexes causes the total expulsion of the water molecule from the inner coordination sphere of the central lanthanide(III) ion. On the basis of these findings, we surmise that the design of a DOTA-like system endowed with a fast exchange of coordinated water can be pursued through a fine modulation of the steric constrain around the water-binding site.[6,12,13]

In this work, we show that the replacement of an acetic arm with a methylphosphinic acid moiety on DOTA structure destabilizes the coordinated water molecule yielding its exchange rate in the region of the optimal values for MRI applications. Moreover, the methylphosphinic acid arm is functionalized with a *p*-aminophenyl moiety that represents a site for further conjugation, for instance to a macromolecular system.

Results and discussion

Syntheses

The target ligand, DO3AP^{ABn} (H_4DO3AP^{ABn} = 1,4,7,10-tetraazacyclododecane-4,7,10-triacetic-1-{methyl[(4-aminophenyl)-methyl)]phosphinic acid}), was obtained by a multi-step

† Electronic supplementary information (ESI) available: additional figures, equations. See http://www.rsc.org/suppdata/ob/b4/b410103k/.

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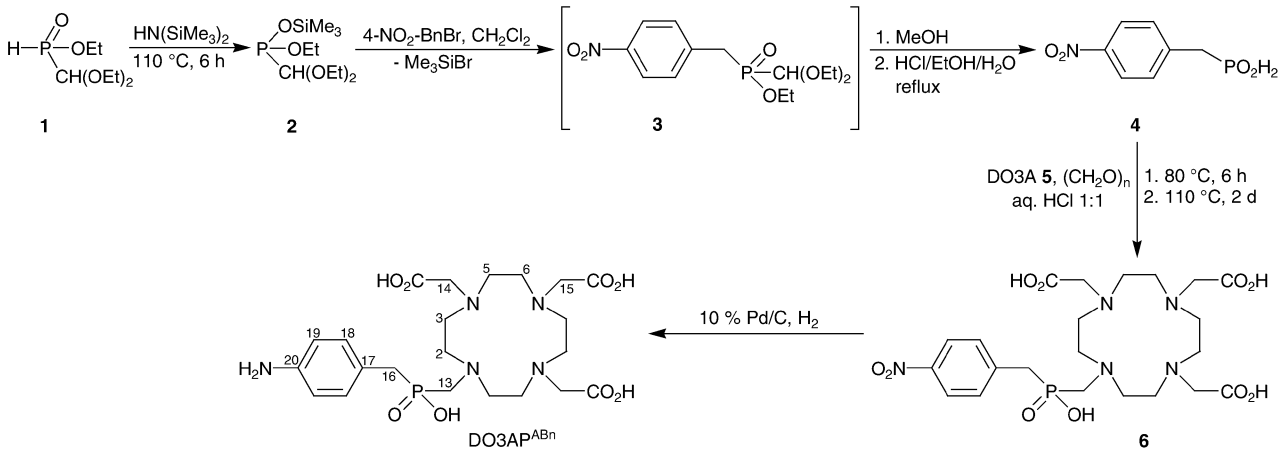

Scheme 1

synthesis starting from hypophosphorous acid and H_3DO3A **5** (Scheme 1). The key phosphorus-containing intermediate **2** was obtained according to literature procedure.[14,15] Arbuzov reaction of compound **2** and 4-nitrobenzyl bromide produced a complex reaction mixture as the by-product Me_3SiBr is able to remove the ester group as well as the P–H bond protecting the diethoxymethyl group from the intermediate **3**. After hydrolysis of this mixture and subsequent purification by extraction and recrystallization, the pure phosphonic acid **4** was isolated. Mannich reaction between the acid **4** and H_3DO3A **5** led to the macrocyclic intermediate **6**. As the last nitrogen atom of the cyclen ring has a low reactivity, a large excess of phosphinic acid **4** and formaldehyde, as well as a long reaction time, was used. Acyclic impurities were removed with strong cation exchange resin. An extensive chromatography on weak cation exchange resin gave the pure macrocycle **6**. Hydrogenation of **6** with Pd/C catalyst produced the target ligand isolated as stable trihydrate (according to elemental and thermal analyses).

Two procedures for preparation of lanthanide(III) complexes were used. In one method, the aqueous solution of the lanthanide(III) chloride was mixed with 10% molar excess of DO3AP^{ABn}. The solution pH was adjusted to 7 with 1.5 M KOH and the mixture was heated to 70 °C for a few minutes. No free lanthanide ions were present as assessed by the xylenol orange test. In the latter method, a slight excess of $LnCl_3$ was added to the solution of DO3AP^{ABn} followed by pH adjustment to 7. After pH stabilization, the mixture was heated at 50 °C overnight. The complexes were purified on Amberlite CG50 with water elution. The HCl released by the reaction was present in the first fraction, the pure complex was somehow delayed on column and eluted in later fractions. Any cations were taken up by the column. The purified complexes were characterized by ^{1}H and ^{31}P NMR spectroscopies.

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

First of all, the occurrence of a coordinated water molecule was assessed by measuring the water ^{17}O NMR chemical shift upon titration of 50 mM solution of DO3AP^{ABn} with Dy^{3+} ions. The method relies on the fact that the dysprosium(III) induced shift is dependent upon the number of water molecules present in the inner coordination sphere of the paramagnetic ion. As reported some years ago, one coordinated water molecule is responsible for the dysprosium(III) induced ^{17}O shift about −40 ppm mM^{-1}.[16] For the dysprosium(III)-DO3AP^{ABn} system, a value of −39 ppm mM^{-1} at 298 K was found. This is fully consistent with the presence of one water molecule in the inner coordination sphere of the lanthanide(III) ion.

Next, we assessed the solution structures of lanthanide(III) complexes of DO3AP^{ABn} by means of high-resolution ^{1}H and ^{31}P NMR spectroscopy. The ^{31}P NMR spectra are particularly useful as, from the number and relative intensity of the observed resonances, one can quickly determine the number and relative abundance of the species eventually present. As it is not possible to acquire the high-resolution NMR spectra of the gadolinium(III) complex (excessively broad lines), its solution structure

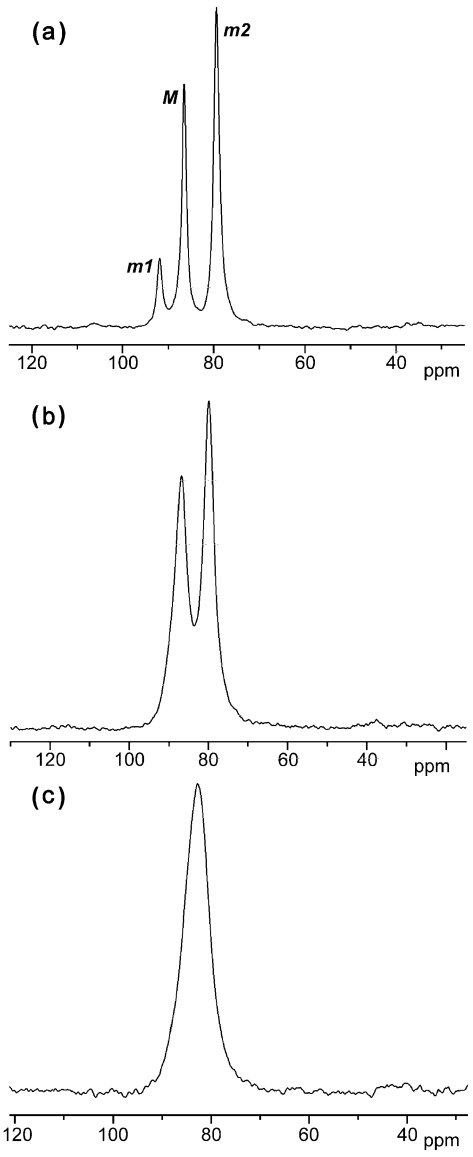

Fig. 1 Variable temperature ^{31}P NMR spectra of [Eu(H_2O)(DO3AP^{ABn})]$^-$ at pH 7: (a) 298 K, (b) 320 K and (c) 345 K.

can be surmised by investigating the europium(III) complex. The rt ^{31}P NMR spectrum of [Eu(H_2O)(DO3AP^{ABn})]$^-$ displays three resonances at 92.0, 86.6 and 79.5 ppm, respectively, in the relative intensity ratio of *ca.* 1 : 4 : 5 (Fig. 1a). As the temperature is increased, the two signals with higher δ_P broaden and collapse in a single resonance (T_C *ca.* 320 K, Fig. 1b). Then, at higher temperature, a new dynamic process leading to the coalescence of the two remaining resonances takes place (T_C *ca.* 345 K). The high-temperature limiting spectrum therefore consists of a single ^{31}P resonance to indicate the occurrence of a fast izomerization process (Fig. 1c). For full details refer to the supplementary information†. To exclude the possibility of complex decomposition this experiment was performed repeatedly on the same sample.

When the ^{1}H NMR spectrum of [Eu(H_2O)(DO3AP^{ABn})]$^-$ at 298 K is compared with the corresponding spectrum of [Eu(H_2O)(DOTA)]$^-$ and related derivatives,[7–9] the presence of three sets of resonances has been clearly identified. Focusing on the low-field region of the ^{1}H NMR spectrum (region of the axial hydrogens of the macrocyclic ring) (Fig. 2), one can assign the four most-shifted resonances (from 38 to 32 ppm) to one diastereoisomer with a square-antiprismatic structure (***M***) whereas the two sets of resonances, from 28 to 17 ppm, have to be ascribed to two ***m***-type isomers (twisted square-antiprismatic structure). The intensity ratio between (***m1*** plus ***m2***) and ***M*** resonances is about 6 : 4. Thus, we assign the ^{31}P signals at 92.0 and 79.5 ppm to two ***m***-type isomers and the ^{31}P resonance at 86.6 ppm to a ***M***-type species. In principle, due to the prochirality of the phosphinate moiety, four diastereoisomers would have been expected. Most likely, the ***M***-type structure displays a large preference for only one arrangement on the phosphorus atom. Thus, the dynamic behaviour shown in variable-temperature ^{31}P NMR spectra corresponds first to a ***m***–***M*** izomerization and then, at higher temperature, to an epimerization process of the phosphinate moiety. Corresponding variable-temperature ^{1}H NMR spectra parallel the behaviour observed in ^{31}P NMR spectra.

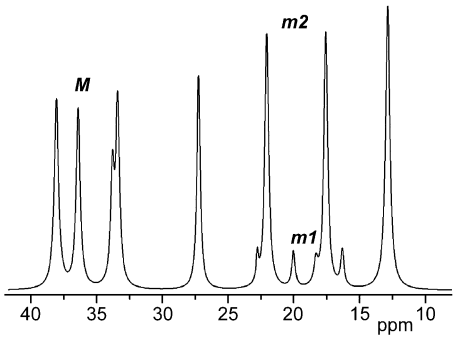

Fig. 2 Low-field region of the ^{1}H NMR spectrum of [Eu(H_2O)(DO3AP^{ABn})]$^-$ at 298 K (400 MHz).

Interestingly, the ***m***–***M*** isomer ratio for [Eu(H_2O)(DO3AP^{ABn})]$^-$ complex is much higher than the value found for the parent [Eu(H_2O)(DOTA)]$^-$ complex (*ca.* 0.27).[7a] It is most likely that the presence of the bulky phosphinate moiety shifts the ***M***↔***m*** equilibrium towards the less sterically demanding[17] ***m***-type structure.

^{1}H and ^{17}O NMR relaxometry of [Gd(H_2O)(DO3AP^{ABn})]$^-$ complex

The stability of the complex was qualitatively assessed by measurement of water proton relaxivity dependence on pH (see supplementary information†). The value of r_1 was constant over the studied pH region (2–11) showing that the complex is stable in acidic solution and protonation/deprotonation of the distant amino group (pK_a 4.76, ref. 18) does not have any effect on relaxometric properties of the complex. The water proton relaxivity of the complex is 6.7 mM^{-1} s^{-1} (25 °C, 10 MHz, pH 7.0). Such a value is about 15% higher than that of [Gd(H_2O)(DOTA)]$^-$ complex under the same conditions.[1a]

Further information about the determinants of the proton relaxivity has been acquired by measuring the temperature-dependent water ^{17}O T_{2r} data (Fig. 3) and ^{1}H NMRD profile (Fig. 4). The experimental data were treated on the basis of the Solomon–Bloembergen–Morgan (SBM) theory of paramagnetic relaxation[1,19] implemented in equations used for multiparametrical fitting (see supplementary information†). The simultaneous fitting of the ^{1}H NMRD profile and ^{17}O T_{2r} data have been carried out by fixing some parameters to the values previously found for related Gd(III) chelates and the relevant parameters obtained are presented in Table 1. Namely, the parameters E_v (1 kJ) and E_r (16.1 kJ) have been fixed to the values reported for Gd(III) DOTA complex.[5] The value of the hyperfine coupling constant $A/\hbar$ (-2.98×10^6 rad s^{-1}) was estimated from contact contribution to lanthanide induced ^{17}O chemical shift of water obtained in the presence of [Ln(H_2O)(DO3AP^{ABn})]$^-$ complexes (supplementary information†). The values of r_{GdH} and r_{GdO} were fixed to the values (r_{GdH} = 3.1 Å, r_{GdO} = 2.6 Å) reported in the literature for related complexes.[20] A good fit for the ^{1}H NMRD profiles (Figure 4) has been obtained only upon introducing a contribution to the overall relaxivity arising from one water molecule in the second coordination sphere (q_{ss} = 1), in addition to the usual contributions from the inner-sphere water molecule (q = 1) and from water molecules diffusing in the proximity of the paramagnetic complex (outer-sphere contribution). The Gd–H distance (R_{ss}) is the value estimated on the basis of the X-ray crystal structure of [Nd(H_2O)(HDO3AP)]$^-$ (H_5DO3AP = 1,4,7,10-tetraazacyclododecane-4,7,10-triacetic-1-methylphosphonic acid).[21] The residence lifetime of the second-sphere water used in the fitting procedure ($^{298}\tau_{mss}$ = 1 ns)

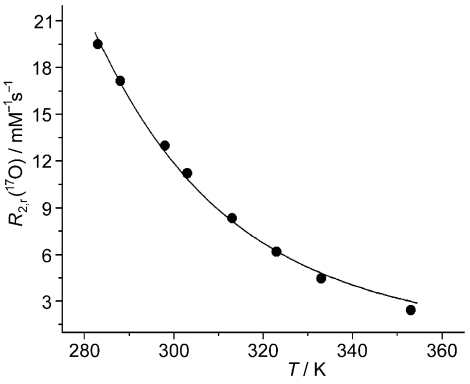

Fig. 3 Variable-temperature ^{17}O NMR transverse relaxation rates R_{2r} measurements in the presence of [Gd(H_2O)(DO3AP^{ABn})]$^-$ complex (pH 7.0). The curve shows the best simultaneous fit of experimental data.

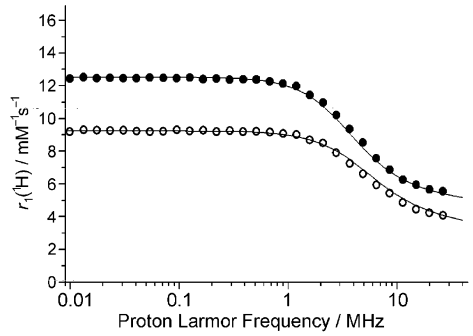

Fig. 4 ^{1}H NMRD profile of [Gd(H_2O)(DO3AP^{ABn})]$^-$ at pH 7 (25 °C: full circles; 37 °C: open circles). The curve represents the best fit of the data resulted from simultaneous fitting based on SBM equations.[1,19]

Table 1 Results from simultaneous fitting of ^{1}H NMRD and variable-temperature ^{17}O NMR R_{2r} data in presence of $[Gd(H_2O)(DO3AP^{ABn})]^-$. Value for E_v (1 kJ) was adjusted and fixed at $[Gd(H_2O)(DOTA)]^-$ values (see text for details)[5]

Parameter	$[Gd(H_2O)(DO3AP^{ABn})]^{-a}$	$[Gd(H_2O)(DOTA)]^-$
$^{298}r_1$/mmol^{-1} s^{-1} [b]	6.7	5.7
$\Delta^2/10^{20}$ s^{-2}	0.25 ± 0.01	0.16
$^{298}\tau_M$/ns	16.2 ± 0.1	244
ΔH_M/kJ mol^{-1}	20.6 ± 0.3	49.8
$^{298}\tau_R$/ps	88 ± 4	77
E_v/kJ	29 ± 1	16.1
$^{298}\tau_v$/ps	11.2 ± 0.1	11
$(A/\hbar)/10^6$ rad s^{-1} [c]	**−2.89**	−3.7
R_{GdO}/Å	**2.6**	2.38
R_{GdH}/Å	**3.1**	—
A/Å	**3.6**	—
ΔH_{mss}/kJ	**15**	—
$^{298}\tau_{rss}$/ps	9 ± 2	—
R_{ss}/Å	**3.6**	—
$^{298}\tau_{mss}$/ns	**1**	—
q [d]	**1**	**1**
q_{ss}	**1**	—

[a] Bold numbers were fixed during the fitting. [b] Millimolar relaxivity of $[Gd(H_2O)(DO3AP^{ABn})]^-$ was measured at 25 °C and 10 MHz. Corresponding value for $[Gd(H_2O)(DOTA)]^-$ was adopted from literature.[1a] [c] Value of hyperfine coupling constant was calculated from ^{17}O chemical shift measurement through the lanthanide series. [d] Hydration value q was obtained for Dy(III) induced shift data.

is similar to the value previously reported for $[Gd(HDOTP)]^{4-}$ (1–3 ns).[22]

The values of the electronic parameters Δ^2 (0.25×10^{20} s^{-2}) and τ_v (11 ps) as well as the value of rotation correlation time $^{298}\tau_R$ (88 ps) obtained from the best fit are comparable to those found for the $[Gd(H_2O)(DOTA)]^-$ complex. A very important result concerns the water residence lifetime ($^{298}\tau_M$) of the inner-sphere water molecule. From variable-temperature ^{17}O NMR transverse relaxation time measurements, it has been found that $^{298}\tau_M$ in $[Gd(H_2O)(DO3AP^{ABn})]^-$ is 16 ns, *i.e.* a value which is one order of magnitude lower than that found for $[Gd(H_2O)(DOTA)]^-$ ($^{298}\tau_M = 243$ ns).[5] The value obtained corresponds to an average value of water residence lifetime for the two isomers. In principle, one should have expected that the curve of ^{17}O T_{2r} *vs* temperature would reflect the contributions from the two ***m*** and the ***M*** isomers. The observation of a curve characterized by a single component (Fig. 3) would suggest that the ***M*** isomer has a τ_M value similar to those of ***m***-type isomers. Such a drop in the value of water residence lifetime for the ***m*** as well as ***M*** isomer can be most likely ascribed to the bulkiness of the phosphinate group, which may cause elongation of the Gd–water bond. Another possible contribution to the reduction of $^{298}\tau_M$ might arise from the overall arrangement of the second hydration sphere. A similar decrease in water residence time was reported for some gadolinium(III) complexes of DO3A (with two water molecules in the first coordination sphere; H_3DO3A = 1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid).[23] This feature was explained by the perturbation of the second hydration sphere due to presence of a distant group able to form hydrogen bonds.

Conclusions

The overall relaxometric properties of $[Gd(H_2O)(DO3AP^{ABn})]^-$ makes this complex a very promising candidate for the preparation of macromolecular systems with very high relaxivities. The optimal water exchange should allow the exploitation of long τ_R values. Moreover $[Gd(H_2O)(DO3AP^{ABn})]^-$ can be easily conjugated to polymeric systems and the presence of the aromatic ring should provide a sufficiently rigid spacer to limit internal motions of the chelate once bound to a macromolecular substrate. The superimposition of an internal rotation of the complex to the overall tumbling of a slowly moving system often represents the source of a "quenching" effect on the attainable relaxivity.[1a]

Experimental

The $H_3DO3A{\cdot}H_2SO_4$ was a kind gift from Bracco SpA (Milano). Hypophosphorus acid (50% aqueous solution), $HC(OEt)_3$, CF_3CO_2H, 4-nitrobenzyl bromide and $HN(SiMe_3)_2$ were obtained from Fluka and 10% Pd on charcoal was from Aldrich. Lanthanide(III) oxides and chlorides were purchased from Aldrich, Strem or Alfa. All commercially available reagents were used as received. Paraformaldehyde was filtered from an aged aqueous formaldehyde solution and dried in a desiccator over P_2O_5. Compound $HP(O)(OEt)[CH(OEt)_2]$ (**1**) was synthesized according to a literature procedure from crystalline H_3PO_2.[14] Synthesis of compounds **1**, **2** and **3** was done under argon atmosphere. D_2O (99.98% D) was received from Chemtrade (Germany). Tlc was performed on silica-coated aluminium sheets (Merck (with UV indicator) or Silufol (Kavalier, Czech Republic)) in ^{i}PrOH–aq. NH_3 (25%)–water 7 : 3 : 3 with detection using ninhydrin or Draggendorf spray, iodine vapours or UV irradiation. Elemental analyses were performed at the Institute of Macromolecular Chemistry of the Czech Academy of Science (Prague). NMR spectra of organic compounds were recorded using Varian ^{UNITY}*INOVA* 400 (see Scheme 1 for labelling of macrocycles). ES/MS spectra were run on the Bruker ESQUIRE 3000 with ion-trap detection in positive or negative modes.

(Ethyloxy)[bis(ethyloxy)methyl](trimethylsilyloxy)phosphine (2)[15]

Crude **1** (28.6 g, 0.146 mol, purity 94%) and $HN(SiMe_3)_2$ (68 ml, 0.32 mol) were mixed and the mixture was heated at 110 °C for 6 h. Excess hexamethyldisilazane was distilled off at rt and 4 kPa pressure into cold finger. Fractional distillation at 0.04 kPa produced 32.2 g (yield 83%, purity 98%) of highly moisture-sensitive phosphite. δ_P (161.9 MHz; $CDCl_3$; ext. 85% H_3PO_4, $^{31}P\{^1H\}$) 146.8; bp 52–55 °C / 0.04 kPa (bp 51 °C / 0.013 kPa; given in ref. 15).

(4-Nitrophenyl)methylphosphinic acid (4)

Solution of 4-nitrobenzyl bromide (26 g, 0.12 mol) in dry CH_2Cl_2 (150 ml) was slowly dropped (3 h) into solution of the phosphite **2** (30 g, 0.11 mol) in dry CH_2Cl_2 (100 ml). The mixture was stirred overnight. Methanol (50 ml) was added, solution was stirred for 1 h and filtered through a fine frit. The filtrate was evaporated to dryness in vacuum. The residue was dissolved in EtOH (150 ml) and aqueous HCl (35%, 150 ml) was added. The mixture was refluxed overnight, cooled and solvents were removed in vacuum. To the residue, water (100 ml) was added and the mixture was slowly neutralized with aqueous KOH (1 mol dm^{-3}) to *ca.* pH 10. The solution was extracted with $CHCl_3$ (3 × 100 ml) and the aqueous phase was acidified with conc. aqueous HCl with stirring. The resulting suspension was stirred for 1 h and filtered to give the first crop of a crude product. The filtrate was evaporated to dryness in vacuum and the residue (mostly KCl) was extracted with EtOH (3 × 50 ml). The ethanol was evaporated, the residue was dissolved in water with a base addition and the second crop of product was obtained upon acidification as above. Both crops of product were recrystallized together from boiling water to get 19 g (86%) of **4** as yellow crystals. δ_H (400 MHz, d_6-dmso, Me_4Si) 3.48 (2 H, d, $^2J_{PH}$ 18.9), 7.42 (1 H, d, $^1J_{PH}$ 541) 7.57 (2 H, m), 8.23 (2 H, m); δ_P (161.9 MHz, d_6-dmso, ext. 85% H_3PO_4) 31.1 (dt, $^1J_{PH}$ 541, $^2J_{PH}$ 19.1)

1,4,7,10-Tetraazacyclododecane-4,7,10-triacetic-1-{methyl [(4-nitrophenyl)methyl)]phosphinic acid} (6)

The $H_3DO3A \cdot H_2SO_4$ (**5**$\cdot H_2SO_4$) (6.4 g, 14.4 mmol) was dissolved in azeotropic HCl (70 ml) and phosphinic acid **4** (11.6 g, 57.6 mmol) was added. The suspension was heated to 80 °C and paraformaldehyde (3.45 g, 115 mmol) was added in small portions over 6 h. The mixture was then heated at 110 °C for 2 d. Subsequently, it was filtered and the filtrate was evaporated to dryness in vacuum and co-distilled with water (3 × 150 ml) to remove excess HCl. The residue was dissolved in water (20 ml) and poured on top of a Dowex 50 column (3 × 15 cm, H^+-form). The column was washed with water (500 ml), 50% aqueous EtOH (1500 ml) and aqueous ammonia (5%, 200 ml). The crude product was eluted with ammonia. The fraction was evaporated to dryness and the residue was dissolved in a small amount of water (5 ml). It was chromatographed on Amberlite CG50 column (5 × 20 cm, H^+-form) with water elution. Fractions containing the pure (^{1}H and ^{31}P NMR) product **6** were combined and evaporated to dryness. The residue was dissolved in water (5 ml) and the solution was dropped into stirred anhydrous EtOH (500 ml). The suspension was stirred overnight, filtered and washed with EtOH (30 ml) and diethylether (30 ml). The light yellow product was dried at rt in air overnight to yield 4.7 g (53%) of **6**$\cdot 3H_2O$. δ_H (400 MHz; 90 °C; *t*BuOH); 3.04–3.19 (20 H, m, ring CH_2 + CH_2–P–CH_2); 3.46 (2 H, br s, *C*H2COOH); 3.63 (4 H, br s, *C*H_2COOH); 7.38 (2 H, m, aryl); 8.08 (2 H, m, aryl); δ_c (100.6 MHz; 90 °C; *t*BuOH) 36.6 (1 C, d, J_{PC} 79.4); 46.1 (2 C,br s); 46.8 (2 C, br s); 47.8 (2 C, br s); 47.9 (2 C, br s); 48.5 (1 C, d, J_{CP} 88.5); 51.3 (1 C, br s); 53.4 (2 C, br s); 120.975 (2 C, s); 127.9 (2 C, d, J_{CP} 4.2); 140.2 (1 C, d, J_{CP} 8); 143.3 (1 C, d, J_{CP} 3) 168.3 (2 C, br s); 170.7 (1 C, br s); δ_P (161.9 MHz; 90 °C; ext. 85% H_3PO_4) 33.8 (br s); *m / z* (ESI/MS) 560.2 $(M + H)^+$, $C_{22}H_{35}N_5O_{10}P$ requires 560.5; 581.1 $(M + Na)^+$, $C_{22}H_{34}N_5NaO_{10}P$ requires 581.5; Found: C, 41.96; H, 7.20; N, 10.76. Calc. for $C_{22}H_{34}N_5O_{10}P \cdot 3H_2O$: C, 43.07; H, 6.57; N, 11.41%

1,4,7,10-Tetraazacyclododecane-4,7,10-triacetic-1-{methyl [(4-aminophenyl)methyl)]phosphinic acid} DO3AP^{ABn}

Macrocycle **6**$\cdot 3H_2O$ (2.5 g, 4.1 mmol) was dissolved in water (150 ml). Several drops of azeotropic HCl and 10% Pd/C (0.5 g) were added. The flask was filled with hydrogen, and the nitroderivative was hydrogenated at rt and 1 atm for 2 d. The catalyst was filtered off and the filtrate evaporated to dryness in vacuum. Chromatography on Amberlite CG50 as for **6** gave fractions of pure ligand which were combined and water was removed in vacuum. The residue was dissolved in water (3 ml) and dropped into stirred anhydrous EtOH (500 ml). The suspension was stirred overnight, filtered and washed with EtOH (30 ml) and diethylether (30 ml). The product was dried in air at rt overnight to yield 2.2 g (91%) of DO3AP$^{ABn}\cdot 3H_2O$. δ_H (400 MHz: D_2O; 90 °C; *t*BuOH) 2.96 (2 H, d, J_{PH} 16, H16), 3.01 (2 H, d, H13, J_{PH} 4), 3.05 (4 H, br s, H6), 3.18 (4 H, br m, H3), 3.20 (4 H, br m, H2), 3.22 (4 H, br m, H5), 3.45 (2 H, br m, H15), 3.65 (4 H, br m, H14), 7.24 (2 H, m, H18), 7.32 (2 H, m, H17); δ_C (100.6 MHz: D_2O; 90 °C; *t*BuOH) 39.1 (1 C, d, C16, J_{CP} 73.4) 49.1 (2 C, s, C6); 49.6 (2 C, s, C2); 51 (1 C, d, C13, J_{CP} 96.6); 51.2 (2 C, s, C3); 51.4 (2 C, s, C5) 54.4 (1 C, s, 15); 56.7 (2 C, s, C14); 123.3 (2 C, d, 19, J_{CP} 2.7); 129.1 (1 C, s, C20); 131.6 (2 C, d, C18, J_{CP} 5.3) 135.6 (1 C, d, C17, J_{CP} 7.2); δ_P (161.9 MHz; D_2O; 90 °C; ext. 85% H_3PO_4) 32.5 (br s); *m / z* (ESI/MS) 552.2 $(M + Na)^+$, $C_{22}H_{36}N_5NaO_8P$ requires 552.0; 530.3 $(M + H)^+$, $C_{22}H_{36}N_5O_8P$ requires 530.5; 359.3 $(M - P(O)(OH)–NO_2Bn)^+$, $C_{15}H_{27}N_4O_6$ requires 359.4; Found: C, 45.70; H, 7.19; N, 11.67. Calc. for $C_{22}H_{36}N_5O_8P \cdot 3H_2O$: C, 45.28; H, 7.25; H, 12.00%

Complex preparation

Lanthanide(III) complexes of DO3AP^{ABn} for ^{17}O and ^{1}H NMR relaxometric measurements were prepared by mixing a 1 : 1.1 molar ratio of $LnCl_3$ and ligand in water followed by addition of KOH to adjust the pH to 7. The reaction mixtures were briefly heated to 70 °C and then stirred at rt overnight. The complexes for ^{1}H and ^{31}P NMR spectroscopy were isolated in the solid state; the slight excess of $LnCl_3 \cdot xH_2O$ was added to the solution of DO3AP^{ABn} followed by pH adjustment to 7. After the pH stabilization the mixture was heated at 50 °C overnight. The complexes were purified on Amberlite CG50 with water elution. All solutions were tested negative in the presence of free lanthanide(III) ions by using xylenol orange as an indicator (in 0.1 M NaAc/HAc buffer solution, pH 5.2). The concentration of lanthanide(III) ions in complex solutions was determined by measuring the ^{1}H NMR shift caused by the change of bulk magnetic susceptibility.[24]

^{1}H relaxometry

The water proton $1/T_1$ longitudinal relaxation rates (10 MHz, 25 and 37 °C) were measured with a Stelar Spinmaster Spectrometer FFC relaxometer (Mede, Pv, Italy; installed at the Laboratorio Integrato di Metodologie Avanzate, Bioindustry Park del Canavese (Colleretto Giacosa, Torino, Italy)) on 0.8–1.2 mM aqueous solution of the complex. The 1H spin–lattice relaxation times T_1 were acquired by the standard inversion recovery method with typical 90° pulse width of 3.5 μs, 16 experiments of 4 scans. The reproducibility of the T_1 data was ±5%. The temperature was controlled with a Stelar VTC-91 airflow heater equipped with a copper–constantan thermocouple (uncertainty of 0.1 ± °C). The $1/T_1$ NMRD profiles of water protons were measured over a continuum of magnetic field strength from 0.00024 to 0.70 T (corresponding to 0.01–30 MHz proton Larmor frequency) on the fast field-cycling relaxometer. The relaxometer operates under complete computer control with an absolute uncertainty in the $1/T_1$ values of ±1%. The concentration of the aqueous solutions of the complexes utilized for the measurements was in the range 1.0–5.0 mM.

^{17}O relaxation measurements

Variable-temperature ^{17}O NMR relaxation measurement were performed at 500 MHz Bruker AM-500 (11.7 T, 67.8 MHz) spectrometer and Bruker VT-1000 temperature control unit was used to stabilize the temperature. The complex solutions were enriched by addition of $H_2{}^{17}O$ (2.6% Yeda, Israel) to overall *ca.* 0.1% ^{17}O concentration. Transversal ^{17}O NMR relaxation rates were measured by standard Carr–Purcell–Meiboom–Gill spin echo pulse sequence; 8–10 increments on *d2* exponentially sampled; $at = 0.2$; $d1 = 0.2$ (*d2* is delay time corresponding to the time of echo; *at* is acquisition time; *d1* is repetition time). These data were compared with those calculated from the line-width at half-height. The deviations were lower that 5%. These NMR spectra were conducted without frequency lock.

Data evaluation

The treatment of the ^{1}H NMR T_1 and ^{17}O NMR T_2 data were performed with Micromath Scientist fitting routines based on SBM equations (refer to supplementary information†).[1,19]

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