\(^1\)H NMR relaxivity of aqueous suspensions of titanium dioxide nanoparticles coated with a gadolinium(III) chelate of a DOTA-monoamide with a phenylphosphonate pendant arm†

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A new efficient and easy method to anchor Gd(III) chelates onto a TiO\(_2\) surface in order to prepare a nanoparticulate T\(_1\) MRI contrast agent is reported. For this purpose, a new ligand, DOTAPP, a DOTA-monoamide derivative containing a phenylphosphonic acid function in the amide side chain, was synthesized. As expected, its Ln(III) complexes exhibit structure analogy to the Ln(III) complexes of DOTA with one water molecule bound in the apical position. The Gd(III)-DOTAPP complex shows similar relaxometric properties as Gd(III) complexes of other DOTA-monoamides. Gd(III)-DOTAPP was anchored onto the surface of nanocrystalline TiO\(_2\) via its phosphonate moiety. The oxide surface is completely covered by the complexes and a strong interaction between the TiO\(_2\) surface and the phosphonate group results in stable chemisorption. The millimolar relaxivity (r\(_T\)) of the Gd(III)-DOTAPP complex adsorbed on the surface is substantially higher than that of the free (non-anchored) complex.

Introduction

Magnetic resonance imaging (MRI) is one of the most widely applied diagnostic tools in medicine. The contrast in an MRI image is dependent on differences in the water content among tissues and on the \(^1\)H NMR relaxation rate of water, which is influenced by pH, viscosity, concentration of salts, proteins and other components in the body fluids, etc. To improve the capability of MRI, contrast agents (CAs) are often applied; about 40% of all MRI examinations are made after administration of a CA. For this purpose, complexes of paramagnetic ions or magnetic nanoparticles are utilized as they accelerate the NMR relaxation of water protons and, thus, enhance the contrast among different types of tissues. Nowadays, the majority of CAs are based on Gd(III) chelates which are used to enhance contrast mainly in T\(_1\)-weighted MR images.\(^1,2\) During recent decades, the rapid progress in biochemistry has provided detailed insight into molecular recognition processes. This opened the way to the use of MRI CAs for molecular imaging, the visualization of cellular and molecular events in normal and pathological processes. However, due to the relatively low sensitivity of the method, this requires high local concentrations of CAs, which can only be achieved if a targeting vector delivers a high payload of Gd(III) chelates, each with optimal relaxation enhancing capabilities, to a site of interest.

The longitudinal relaxation rate enhancement of the water protons (the efficiency of a T\(_1\) contrast agent) is commonly expressed as the millimolar relaxivity, r\(_T\). One of the most important parameters determining the value of the relaxivity of a Gd(III) complex is its rotational correlation time (\(\tau_R\)),\(^1\) which is determined mainly by its molecular volume (proportional to the molecular weight). In general, increasing the molecular weight leads to slower rotation and, consequently, to higher relaxivity.

Various approaches have been used to increase the molecular weight of CAs; they involve binding of the Gd(III) chelates to macromolecules such as inulins,\(^3,4\) dendrimers,\(^3,6\) or proteins (e.g. HSA\(^7\)). Unfortunately, the relaxivity enhancement of these conjugates was significantly lower than expected from simulations. This has been ascribed to local motions caused by flexibility of the macromolecular carrier and/or flexibility of the linker anchoring the complex moiety to the carrier.

An alternative way to slow down the tumbling of CA molecules is by anchoring the Gd(III) complexes to the surface of oxide nanoparticles. Several studies dealing with T\(_1\) contrast agents based on nanoparticles bearing Gd(III) chelates have recently been published. Dye-doped silica particles were covered with Gd(III) complexes of DTPA-/DOTA-amide.\(^8,9\) A Gd(III)-DOTA complex was anchored onto silica-coated quantum dots and on gold nanoparticles.\(^10\) The conjugates obtained were

proposed as dual modality probes for cell labeling. Similarly, cells have been efficiently labeled with silica particles coated with the Gd(III) complex of a DTPA-monoamide.11 A Gd(III) complex of DO3A, with two water molecules in the first coordination sphere of Gd(III), was attached to the surface of boehmite through a silane pendant arm.12 In all cases mentioned above, the Gd(III) chelates were bound to the surface of the nanoparticles via hydrolysis of –Si(OR)3 groups linked to the chelate. Furthermore, a methanethiosulfonate group was used for covalent anchoring of the Gd(III) complex of a DOTA derivative on a –SH modified silica surface through an S–S bridge.13 A sulfide bond was also used for modification of gold nanoparticles with Ln(III) complexes to prepare dual MRI/CT CAs14 or luminescence probes.15

Titanium dioxide may be a suitable carrier for Gd(III) chelates since it is a biocompatible material, which is stable towards chemical and biological degradation and which can be prepared in a well-defined nanocrystalline form. During the preparation of this manuscript, a paper on a Gd(III) complex of a DOTA-monoamide derivative bound to a TiO2 surface was published; the pyrocatechol group was used to anchor the complex.16

The phosphonic acid group has been found to be very effective in anchoring different molecules to metal oxide surfaces.17–19 The surface of titanium dioxide has been modified with a well-ordered monolayer of phosphonic acids.20,21 In particular, reaction of phenylphosphonic acid and its derivatives with TiO2 particles has led to the formation of very stable covalent Ti–O–P bonds on the surface even under mild conditions.22 Therefore, we decided to study the Gd(III) complex of a DOTA-monoamide containing a phenylphosphonate group in a pendant arm (DOTAPP) (Scheme 1) and to use it for the surface modification of TiO2 nanoparticles. The phenylphosphonate-amide was selected as a linker, because it is expected that its rigidity will limit the local motions of the chelate. In that way optimal benefit may be obtained from the slow global tumbling of the TiO2-DOTA conjugate and thus a significant T1 relaxivity increase may be expected upon the conjugation of the chelate to the TiO2 nanoparticle.

**Experimental**

**Materials**

Commercially available chemicals were used as received. MeCN was dried by distillation from P2O5. t-Bu3DO3A-HBr23 and tetraethyl 4-acetamidophenylphosphonate24 were synthesized according to modified published procedures. TiO2 was purchased from Degussa (type P25, primary particle diameter 30 nm, specific surface area ~50 m2/g).

**Methods**

**NMR spectroscopy.** 1H (399.95 or 300.16 MHz), 13C (100.58 or 75.48 MHz), 19F (161.9 or 121.5 MHz) and 17O (54.22 or 40.69 MHz) NMR spectra were acquired at 25 °C (unless stated otherwise) with Varian Unity Inova-400 and 300 spectrometers, using 5 mm sample tubes. For the 1H and 13C measurements in D2O, the methyl signal of t-BuOH was used as an internal standard (δ = 1.2 and 31.2 ppm, respectively). The 31P chemical shifts were measured with respect to 1% H3PO4 in D2O as an external reference. The pH values of the samples were measured at ambient temperature using a Corning 125 pH-meter with an NMR electrode (Aldrich) calibrated with standard buffers. For the 17O NMR measurements, 10 μl of 17O-enriched water (10% H217O) was added to the samples. 1H NMR spectra of the samples containing H217O were acquired using water suppression by means of presaturation of the water resonance. Variable-temperature 17O NMR measurements of the Gd(III)-DOTAPP complex (92 mM, pH 7.5) were performed without frequency lock in the temperature range 7–94 °C. Concentrations of Ln(III) in the samples were determined with bulk magnetic susceptibility (BMS) measurements.25 The 17O NMR chemical shifts were corrected for the BMS as described previously.26 The diamagnetic contributions to the 17O NMR shifts and relaxation rates were determined with a sample of water at pH 5.9 in the absence of paramagnetic complexes. The longitudinal (R1) and transversal (R2) relaxation rates were obtained by the inversion recovery method,27 and the Carr–Purcell–Meiboom–Gil pulse sequence,28 respectively.

The 31P NMR MAS spectrum was obtained on a Varian Unity Inova-400 spectrometer using a 7 mm VT CP–MAS probe head, spinning frequency 7 kHz. Ca3(PO4)2 was used as an external standard.

**Proton NMRD profiles.** The 1H NMRD profiles (NMRD = nuclear magnetic relaxation dispersion) of an aqueous solution of the Gd(III)-DOTAPP complex (4.6 mM, pH = 7.5) and of the TiO2 suspension with the adsorbed Gd(III)-DOTAPP complex (0.52 mM, pH = 3.5) were measured in the magnetic field range 2.34 × 10−4–0.35 T using a Stelar SpinMaster FFC-2000.
relaxometer. Measurements at 0.47 and 1.42 T were performed with a Minispec mq-20 and a Minispec mq60 (Bruker), respectively.

**Data evaluation.** The experimental $^1$H NMRD data were fitted simultaneously with $^{17}$O NMR data by means of a least-square fitting procedure using the Micromath Scientist program version 2.0 (Salt Lake City, UT).

**Mass spectrometry.** Mass spectra were acquired on a Bruker Esquire 3000 spectrometer equipped with an electrospray ion source.

**ICP-AES.** The concentrations of Gd, La, and Ti were 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 sample was dissolved in H$_2$SO$_4$ (5.5% v/v) and 1% HNO$_3$. The resulting solution was washed with H$_2$O (100 mL) and measured after dilution as solutions in 5.5% H$_2$SO$_4$.

**TEM.** Images were obtained with a Philips EM 201 transmission electron microscope, using an accelerating voltage of 80 kV.

**Luminescence.** The Eu(III) luminescence measurements were performed on an Edinburgh Instruments FS900 spectrofluorimeter, equipped with a 450 W xenon arc lamp, a microsecond flash lamp and a red-sensitive photomultiplier (300–850 nm). The luminescence spectra were obtained after excitation of the Eu(III) $^3$L$_6$ ← $^5$F$_{0}$ band (394 nm).

**Molecular modeling.** Molecular modeling was performed with the computer program HyperChem for Windows, release 7.5 (Hypercube Inc, Gainsville FL, USA).

**Synthesis**

Diethyl 4-aminophenylphosphonate (2). Sodium (0.24 g, 10 mmol) was dissolved in dry EtOH (34 mL, 0.58 mol) under Ar. A solution of 1 (1.00 g, 3.6 mmol) in dry EtOH (20 mL) was added in one portion and the reaction mixture was refluxed under an argon atmosphere for 12 h. Then, the volatiles were removed with a rotary evaporator. The oily residue was dissolved in CHCl$_3$ (100 mL), the obtained solution was washed with H$_2$O (100 mL) and dried with Na$_2$SO$_4$. The CHCl$_3$ was removed by rotary evaporation. The crude product was purified by column chromatography (silica, EtOH, $R_f = 0.8$). Amine 2 was obtained as a white powder (0.70 g, 82%). $\delta_{^1}$(400 MHz, CD$_2$OD) 1.29 (6H, t, $^1J_{CH} = 6.8$ Hz, $2 \times$ CH$_3$), 4.02 (4H, m, $2 \times$ O–CH$_2$), 6.71 (2H, m, CH$_2$ arom.), 7.44 (2H, m, $2 \times$ CH arom.). $\delta_{^3}$ (121 MHz, CD$_2$OD) 27.4 (s).

Diethyl 4-(chloroacetamido)phenylphosphonate (3). A suspension of dried K$_2$CO$_3$ (1.26 g, 9.1 mmol) in a solution of chloroacetyl chloride (0.75 mL, 9.25 mmol) in dry MeCN (10 mL) was cooled to −30 °C and then a solution of 2 (0.70 g, 3.04 mmol) in dry MeCN (10 mL) was added dropwise. The reaction mixture was warmed to room temperature over 3 h and then stirred overnight. The solids were filtered off and volatiles were removed by evaporation. The resulting oil was three times evaporated with toluene (10 mL) to give the crude product as yellowish crystals (0.90 g, 97%); it was used without further purification in the next step. $\delta_{^1}$(400 MHz, CD$_2$OD) 1.32 (6H, t, $^1J_{HH} = 7.2$ Hz, $2 \times$ CH$_2$–CH$_3$), 4.09 (4H, m, $2 \times$ O–CH$_2$), 4.21 (4H, s, Cl–CH$_2$Br), 7.77 (4H, m, CH$_2$ arom.). $\delta_{^3}$ (121 MHz, CD$_2$OD) 19.3 (s).

**DOTAPP 10-([N-[4-(dihydroxyphosphoryl)phenyl][carbamoyl]methyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid)**. Dried K$_2$CO$_3$ (0.79 g, 5.72 mmol), $\gamma$Bu$_3$DO$_3$H·Br (0.682 g, 1.15 mmol), crude 3 (0.367 g, 1.20 mmol) and dry MeCN (30 mL) were mixed in a 50 ml flask. The reaction mixture was stirred at room temperature for 16 h. The solids were removed by filtration and the volatiles were removed by evaporation. The resulting yellow oil was dissolved in dry MeCN (20 mL) and trimethylsilyl bromide (3.2 g, 21 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 16 h in the dark. Volatiles were removed by evaporation and the crude product was purified on a cation exchange resin (Dowex 50, H$^+$-form, elution with 5% pyridine in water : ethanol = 1 : 1). The eluate was concentrated by evaporation to ~5 ml and kept at 4 °C for 16 h. The precipitate was filtered off and purified on an anion exchange resin (Dowex 1, OH$^-$-form, elution with 5%aq. HCl). The volatiles were removed by evaporation and the resulting white powder was dried under vacuum. The product was obtained as DOTAPP·0.75HCl·4.5H$_2$O (0.48 g, 70%). (Found: C, 39.54; H, 6.46; N, 10.35; Cl, 4.08. Calcd. for: C$_{22}$H$_{43.75}$Cl$_{0.75}$N$_{5}$O$_{14.5}$P: C, 39.56; H, 6.60; N, 10.49; Cl, 3.98%). $\delta_{^1}$(400 MHz, D$_2$O, 90 °C) 3.52 (8H, bs, C–H$_2$ cyclen), 3.61 (8H, bs, C–H$_2$ cyclen), 3.86 (2H, s, N–C–H$_2$–CO), 3.99 (4H, s, N–CH$_2$–CO), 4.11 (2H, s, N–C–H$_2$–CO), 7.98 (2H, m, NH–C–CH arom.), 7.25 (2H, m, P–C–CH arom.). $\delta_{^3}$ (100 MHz, D$_2$O, 70 °C) 7.2 (bm), 64.4 (bs), 127.7 (bs, N–C–CH arom.), 128.0 (d, $^1J_{CP}$ = 9.1, P–C–CH arom.), 134.8 (d, $^1J_{CP}$ = 11.8, P–C arom.), 145.6 (s, NH–C arom.), 178.5 (s), 181.5 (bs), 184.4 (s); $\delta_{^3}$ (121 MHz, D$_2$O) 12.0 (t, $^1J_{HH}$ = 11.1 Hz).

**Preparation of Ln(III) complexes of DOTAPP (general procedure).** Ligand 4 (62.5 mg, 0.11 mmol) was dissolved in hot H$_2$O (0.5 mL). The pH of the solution was adjusted to 7.5 with 30%aq. NaOH and then a solution of LnCl$_3$·nH$_2$O (0.10 mmol) in H$_2$O (0.2 mL) was added dropwise. The resulting suspension of the Ln(III) phosphate precipitate was heated at 70 °C for 12 h yielding a clear solution. The pH of this solution was adjusted to 6.5, after which the solution was heated again at 70 °C for 12 h. Then the pH was adjusted to 8.5 and the solution was heated at 70 °C overnight.

**Adsorption of the Ln(III) DOTAPP complexes on the surface of TiO$_2$ (general procedure).** TiO$_2$ (0.1 g, 1.25 mmol) was suspended in H$_2$O (50 mL) and the suspension was sonicated in an ultrasonic bath for 20 min. Then, a solution of a Ln(III) DOTAPP complex (22 µmol) in H$_2$O (1.5 mL) was added. The pH of the obtained suspension was adjusted to 3.5 and then it was stirred at 70 °C for 3 d followed by 4 h of cooling to room temperature. The resulting suspension was washed with water six times. In each washing step, the suspension was concentrated from 50 to
10 ml in an ultrafiltration cell (membrane cutoff: 50 kDa) and 40 ml of H₂O was added. The final volume of the suspension was 10 mL and the pH was adjusted to 3.5.

Results and discussion

Synthesis

The ligand DOTAPP was synthesized in good yield by a multi-step procedure starting from 4-bromoacetanilide (Scheme 2). Reaction with diethylphosphite using Pd(PPh₃)₄ as the catalyst gave ester ¹ for the optimized reaction conditions see ESI†. The acetyl group was cleaved off with NaOEt and then the free amine ² was treated with chloroacetyl chloride to give ³. Treatment with t-Bu₃DO3A afforded the target ligand in the ester form. After full de-esterification with an excess of Me₃SiBr, the ligand DOTAPP obtained was purified on a strong cation exchange resin and crystallized as the pyridinium salt.

Finally, pyridine was removed on a strong anion exchange resin and the ligand was obtained as a non-stoichiometric hydrochloride hydrate.

The preparation of the Ln(III) complexes of DOTAPP proceeded in a three-step process. Immediately upon mixing of the ligand and Ln(III) chloride, a precipitate was formed while the pH of the resulting suspension dropped below 2. The precipitate may be ascribed to the formation of a Ln(III)-phosphonate complex as Ln(III) phosphonates are known to be mostly polymeric and insoluble.³⁰,³¹ This precipitate dissolved during heating at pH ~ 5. Analogously to complexes of other DOTA-like ligands, the intermediate formation of an “out-of-cage” complex with coordination of phosphonic acid and carboxylic groups is expected.³² The formation of the “in-cage” DOTA-like complex upon final heating of the solution at pH 8–9 was confirmed by the characteristic ¹H NMR spectrum of the Eu(III) complex (for comparison of ¹H NMR of Eu(III)-DOTAPP with spectra of the Eu(III) complexes of other DOTA-like ligands, see ESI†). Increasing of the pH in two steps is necessary as a one-step adjustment led to precipitation of the Ln(III) hydroxide.

Coordination properties

The ¹H NMR spectra of the Ln(III)-DOTAPP (Ln = Nd, Eu, Dy, Yb) complexes (see ESI†) are very similar to those of the corresponding spectra of Ln(III) complexes of other DOTA-like ligands. Therefore, it may be concluded that the Ln(III) ions are coordinated with four nitrogen atoms and four oxygen atoms of carboxylic/amide groups forming two planes above and below the central ion, and a water oxygen atom. The mutual orientation of the “nitrogen” and “oxygen” planes in the coordination polyhedron leads to two diastereomeric forms, a square-antiprism (SA, also called M) and a twisted square-antiprism (TSA, also called m).² These two diastereomers can be recognized as two sets of resonances in the ¹H NMR spectrum. The chemical shifts and intensities of signals of four protons of the cyclen ring³³ were used to determine the diastereoisomeric ratio of the complexes in solution. The TSA/SA ratio changes along the lanthanide series (see Table 1); for the light lanthanides the TSA is predominant whereas towards the end of the series the SA isomer is preferred. The trend in the TSA/SA ratios is very similar to that generally observed in Ln(III) complexes of DOTA and its derivatives.²⁹

The number of water molecules directly coordinated to the Eu(III) ion (q) was confirmed to be 1 by the values of luminescence lifetimes measured for the complex in H₂O (t = 580 μs) and in D₂O (t = 1830 μs). These values are in good agreement with those observed previously for complexes containing one water molecule in the inner coordination sphere (q = 1).³⁴–³⁶

Relaxometric study of the Gd(III)-DOTAPP complex in solution

Variable-temperature ¹⁷O relaxation rates (measured at pH 7.5, sample concentration 92 mM) and ¹H NMRD profiles (measured at 5 mM sample at pH 7.5) were measured to evaluate parameters governing relaxivity of the Gd(III)-DOTAPP complex (Fig. 1). The data were fitted simultaneously with a set of equations governing relaxivity of the Gd(III)-DOTAPP complex (Fig. 1). 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⁻¹.³⁸ The distance of a water proton to the Gd(III) nucleus (r_GdH) was fixed

<table>
<thead>
<tr>
<th>Lanthanide ion</th>
<th>DOTAPP</th>
<th>DOTA²⁹</th>
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<tbody>
<tr>
<td>Nd</td>
<td>56:44</td>
<td>55:45</td>
</tr>
<tr>
<td>Eu</td>
<td>30:70</td>
<td>20:80</td>
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<tr>
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<tr>
<td>Yb</td>
<td>9:91</td>
<td>8:92</td>
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Table 1 TSA/SA ratio in the Ln(III)-DOTAPP complexes
at 3.10 Å and the distance of closest approach of a proton of an outer sphere water molecule ($\sigma_{\text{GdH}}$) to 3.50 Å, the same values as in most of the previous studies. The ratio of the the Gd inner-sphere water O and Gd inner-sphere water H rotational vector ($t_{\text{RO}}$ and $t_{\text{RH}}$) was fixed at 1.5, the value determined by Merbach et al. for Gd-DOTA. The calculated relaxivity data are presented as curves in Fig. 1, and a selection of the best-fit parameters are compared in Table 2 with values obtained previously for related complexes (for a full set of best-fit parameters and a detailed discussion, see ESI†). The rotational correlation time ($t_{\text{RH}}$) was found to be 135 ps, which is a factor of 1.8 higher than the value for the Gd(III)-DOTA complex. With molecular models, it can be estimated that the molecular volume increases by a factor of 1.4 upon attaching the phenylphosphonate group. For spherical molecules, the molecular volume, according to the Stokes–Debye–Einstein relation, is linearly proportional to the rotational correlation time. The relatively large $t_{\text{RH}}$ value for Gd(III)-DOTAPP obtained can be attributed to the shape of the molecule, which is far from spherical. The same value for $t_{\text{RH}}$ has been reported for Gd-(DOTA-pBn-NH$_2$). The water residence time ($t_{\text{M}}$ = 1.00 ms) is significantly longer than that reported for the Gd(III)-DOTA complex, which is typical for complexes of DOTA-amides. The electronic parameters governing the relaxivity, the mean-square zero-field splitting energy, $D^2$, and the correlation time for the modulation of the zero-field splitting interaction, $t_{\nu}$, are also very similar to related DOTA complexes with an amide pendant arm. The relaxivity (6.17 s/M/M$^\circ$/0.1 mM/20 MHz, 298 K, pH 7.5) was found to be higher than the value reported for the Gd(III)-DOTA complex, which can mainly be attributed to the slower tumbling of the Gd-DOTAPP complex.

**Nanocrystalline TiO$_2$ modified with the Ln(III)-DOTAPP complexes—synthesis and relaxometry**

The Ln(III)-DOTAPP complexes were adsorbed on the surface of nanocrystalline TiO$_2$ under mild conditions (70 °C, pH 3.5) using a five fold excess of the adsorbate compared to the amount needed for full surface coverage. The theoretical amount of adsorbate needed for full surface coverage was estimated from the specific surface area of the material (50 m$^2$/g) and the area of surface occupied by one complex molecule (200 Å$^2$ as estimated from molecular modeling).

The pH appeared to be crucial for the stability of the colloidal suspension. The optimal pH for the studied TiO$_2$-complex systems was found to be 3.5. At that pH, the colloidal suspensions are stable for at least several days. A lower pH led to dissociation of the Ln(III)-DOTAPP complexes whereas, at higher pH, the colloid was not stable in the long term. Comparison of the TEM images of the TiO$_2$ nanoparticles before and after sorption (Fig. 2) shows that the diameter and the shape of the nanoparticles (30–80 nm) remain unchanged. Electron diffraction patterns of both materials correspond to that of anatase.

The $^{31}$P MAS NMR spectrum of a freeze-dried suspension of the La(III)-DOTAPP modified material shows a broad peak with a center at 14 ppm and a half-width of 15 ppm, indicating surface sorption of the complex. The broadening of the peak originates from different modes of adsorption. The spectrum also indicates a negligible recrystallisation of the material as the recrystallized material, i.e. the titanium(IV)-phenylphosphonate phase, usually exhibits a $^{31}$P resonance at ~4 ppm with a smaller half-width. Furthermore, recrystallisation would lead to
a change of size and shape of the particles as well, which was not observed in the TEM images (Fig. 2).

The concentration of TiO$_2$ in the colloid and the adsorbed amount of the Ln(III)-DOTAPP complex were quantified by ICP-AES. The content of TiO$_2$ was 10.0 g/L and the adsorbed amount of the Ln(III)-DOTAPP complex was 52 $\text{mol/g TiO}_2$. This value is a factor of 1.2 higher than the value calculated for a surface covered by a monolayer, suggesting that the TiO$_2$ surface is fully covered by the Ln(III)-DOTAPP complex. The last filtrate obtained after ultrafiltration of the suspension contained a negligible amount of Gd(III) (<3% of the adsorbed amount), which indicates that the material is highly stable under the conditions applied. This suggests that stable Ti–O–P bonds have been formed (i.e. chemisorption), since any physico-sorbed complex would have been removed during the washings applied during the synthesis of the material.$^{21}$

NMRD profiles of a suspension of the Gd(III)-DOTAPP modified TiO$_2$ (Fig. 1) were measured at 25 and 37 °C (Gd(III) concentration 0.52 mM, pH 3.5). The diamagnetic contribution to the relaxation originating from the oxidic material, was subtracted (see ESI†). The profiles show a local maximum at 20 MHz, which is typical for slowly tumbling CAs.

The resulting NMRD profile has an uncommon shape in the region 0.1–1 MHz, which may be due to the inadequacy of the correction for the diamagnetic contribution to the relaxation. Due to the uncommon shape of the NMRD profile an evaluation of the parameters governing the relaxivity by fitting the profile with the theoretical equations was not possible. The value of $\tau_{\text{RH}}$ for a nanoparticle with a diameter of 30 nm, as estimated with the Debye–Stokes–Einstein relation, is about 3 ms. For such slowly tumbling systems, the relaxivity is independent of variations in the value of $\tau_{\text{RH}}$. An NMRD profile, simulated using a $\tau_{\text{RH}}$ value of 3 ms and with the other parameters the same as those of free Gd(III)-DOTAPP (see ESI†), has a maximum which is of about the same magnitude as that observed for Gd(III)-DOTAPP + TiO$_2$. This suggests that the Gd(III) chelates are effectively immobilized on the nanoparticles. The low field part of the simulated profile, which is mainly determined by the electronic relaxation parameters, is higher than that of the experimental one. Probably, the electronic relaxation times for Gd(III) on the particles are somewhat higher than in free Gd(III)-DOTAPP.

As can be seen in Fig. 1(c), the relaxivity of the suspension increases upon increasing the temperature. This confirms that the relaxivity is no longer governed by the rotational correlation time, since that would be reflected in a decrease of relaxivity upon increasing the temperature. The observed increase can be attributed to the increase in exchange rate of water between the Gd(III) chelate and the bulk at higher temperature.

For a particle with a diameter of 30 nm with a surface fully covered with Gd-DOTAPP chelates, the number of Gd(III) ions per particle can be estimated to be 3300. Therefore, the relaxivity per particle is about 75 000 mM$^{-1}$s$^{-1}$(20 MHz, 25 °C). It may be concluded that such particles, when stabilised at neutral pH, will be suitable for application in molecular imaging.

**Conclusion**

A new DOTA-monoamido derivative containing a phenylphosphonic acid group in the side chain (DOTAPP) was synthesized. The Gd(III)-DOTAPP complex shows relaxometric properties similar to other DOTA-monoamides. The rigid
phenylphosphonate linker is very efficient for anchoring the Ln(III) complexes of the ligand onto the surface of TiO\textsubscript{2} nanoparticles. The surface of the particles is completely covered and a strong interaction between TiO\textsubscript{2} surface and phosphate results in stable chemisorption. The approach represents an effective and simple approach to nanoparticle-Gd(III) chelate based CAs. The resulting particles will be able to carry a payload of Gd(III) chelates which is sufficient for application in molecular imaging.

Acknowledgements

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