A new heptadentate tripodal ligand leading to a gadolinium complex with an improved relaxation efficiency

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The new heptadentate tripodal ligand tpaa containing three pyridinecarboxylate binding units reacts with Gd(III) in water to give a thermodynamically stable complex which displays high relaxivity.

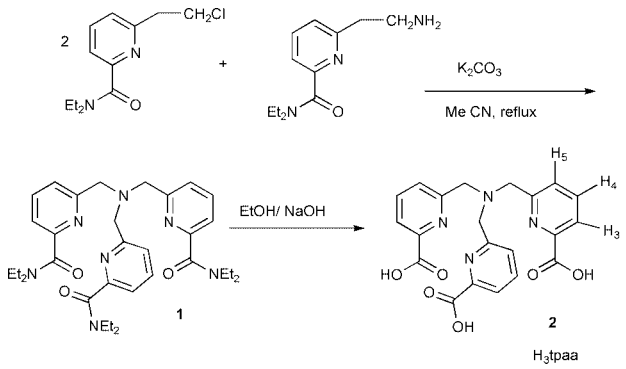
In recent years there has been a rapid growth in the field of coordination chemistry of lanthanides(III) with polydentate ligands.[1,2] This development has been mainly spurred by the application of gadolinium complexes as magnetic resonance imaging (MRI) contrast agents[3–5] and of europium or terbium complexes as luminescent probes in time-resolved fluoroimmunoassays.[6,7] The key property of an efficient contrast agent is its ability to enhance the nuclear magnetic relaxation rate of solvent water protons. This can be achieved by the presence of a high number of inner sphere water molecules in combination with fast water exchange, long rotational correlation times and long electronic relaxation times. The current approach to achieve higher relaxivity consists in increasing the rotational correlation time (τ_R) by increasing the molecular weight of the contrast agent through the formation of macromolecular complexes by covalent or non-covalent linkage of Gd(III) chelates to large biomolecules. However the effective enhancement of the rotational correlation time attainable in these systems is limited by the residence lifetime (τ_M) of the coordinated water molecule. The relatively long τ_M of Gd complexes of octadentate ligands such as the currently approved contrast agents $[Gd(dtpa)(H_2O)]^{2-}$ and $[Gd(dota)(H_2O)]^{-}$† appears to be related to the dissociative exchange mechanism of the water molecule. This prevents dendrimeric Gd(III) derivatives[8] and non-covalent adducts of Gd(III) chelates with large bio-molecules[9] from attaining their potential relaxation enhancement.

Heptadentate ligands allow the coordination of two water molecules in the inner sphere of the metal and consequently yield higher relaxivity with respect to Gd(III) complexes of octadentate ligands. In addition Gd(III) complexes containing heptadentate[10,11] or hexadentate ligands[12] have shown faster water-exchange rates than complexes with octa-coordinating ligands. In spite of this, heptadentate ligands have seldom been used in the design of Gd(III) based contrast agents.[5]

Here we report the synthesis of the new tripodal ligand, tpaa (containing three pyridinecarboxylate arms connected to a nitrogen atom)‡ which acts an an heptadendate chelator thus allowing the coordination of at least two water molecules in its lanthanide(III) complexes. Moreover the ligand tpaa leads to an uncharged Gd(III) complex which should show reduced potential for osmotic cell damage.

As shown in Scheme 1, H_3tpaa **2** was prepared by hydrolysis of the heptadentate ligand tpa(trisamide) **1**, obtained from the condensation of the 6-aminomethyl derivative and the 6-chloromethyl derivative of 2-(*N*,*N*-diethylcarboxamido)pyridine in the presence of K_2CO_3. The protonation constants of H_3tpaa were determined by potentiometry [pK_{a1} = 2.5(2), pK_{a2} = 3.3(1), pK_{a3} = 4.11(6), pK_{a4} = 6.78(4)]. The $[Gd(tpaa)(H_2O)]_2$ complex **3**§ was obtained as colorless crystals suitable for X-ray diffraction¶ by slow evaporation of a 1 : 1 solution of $GdCl_3$ and H_3tpaa in water. Fig. 1 shows the structure of **3** in which two Gd(III) centers are joined by two bridging oxygens from the monodentate carboxylate groups of two different tpaa ligands to form a dimeric complex. The metal ion is nine-coordinated by four nitrogens and three carboxylate oxygens of tpaa, one water molecule and a carboxylate oxygen of the neighboring complexed ligand. The coordination geometry can be described as a distorted tricapped trigonal prism in which O(1), N(2) and N(3) occupy the capping positions in the rectangular faces. The average Gd–N(pyridyl) distance [2.56(4) Å] is shorter than the average Gd–N(tert) distances found in the neutral nonadentate complexes $(Gd(do3ma)(H_2O)]_2$[13]† (2.66 Å) and [Gd(dtpa-bea)(H_2O)][14]† [2.70(3) Å], while the distance Gd–N(apical) [2.7886(19) Å] in **3** is 0.222 Å longer than the sum of the ionic radii of Gd and N, probably due to the oligomerization. The Gd–O(water) distance [2.399(2) Å] is shorter than those found in the dimeric 9-coordinate complex $[Gd(do3ma)(H_2O)]_2$[13]† [2.451(5) and 2.56(4) Å] and in the eight-coordinate complex $[Gd(tren\text{-}Me\text{-}3,2\text{-}hopo)(H_2O)_2]$[15]† [2.446(5) and 2.436(4) Å], but is similar to the Gd–O(water) distances in the monomeric nine-coordinate complex [Gd(dtpa-bea)(H_2O)] [2.42(3) Å].[14] The tendency of the $[Gd(tpaa)(H_2O)_2]$ complex to oligomerize in the solid state, and in the conditions generated in the electrospray analysis arises from the presence of a relatively exposed metal face due to the flexibility of the unhindered tpaa ligand. This property of aminocarboxylate to oligomerize in water has only been observed before in the presence of macrocyclic ligands.

In order to avoid toxicity, Gd(III) complexes are required to be highly thermodynamically (and preferably also kinetically) stable for application as contrast agents. Potentiometric data



Scheme 1

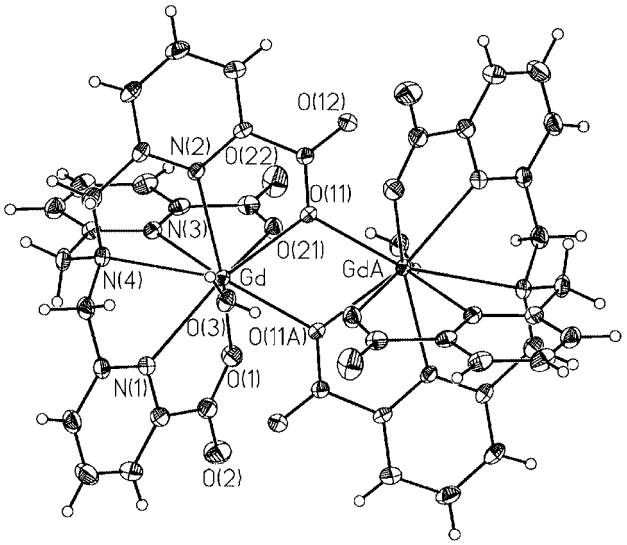


Fig. 1 Crystal structure of $[Gd(tpaa)(H_2O)]_2{\cdot}7H_2O$ **3** with thermal ellipsoids at 30% probability. Selected bond lengths (Å): Gd···GdA 4.125, Gd–O(11) 2.,4726(17), Gd–O(11A) 2.4010(15), Gd–O(1) 2.4414(17), Gd–O(21) 2.3783(17), Gd–O(3) 2.399(2), Gd–N(1) 2.546(2), Gd–N(2) 2.6051(19), Gd–N(3) 2.531(2), Gd–N(4) 2.7886(19) (symmetry transformation used to generate equivalent atoms A: $-x + 1$, $-y$, $-z$).

obtained by titration of 1:1 metal:H_3tpaa mixtures in the pH range 2.8–8 can be satisfactorily fitted to eqn. (1) leading to a

$$Gd^{3+} + tpaa^{3-} \rightleftharpoons [Gd(tpaa)] \quad \log\beta_{GdL} = 10.2(2) \qquad (1)$$

pM value of 11.2 for Gd(III) {$-\log[M]_{free}$ at pH 7.4, $[M]_{total}$ = 1 μM, $[tpaa]_{total}$ = 10 μM} which indicates a reasonable physiological stability of $[Gd(tpaa)(H_2O)]_2$ as compared to the lowest pGd value (15.8) found in commercially used MRI agents.[5] Only a small decrease of the physiological stability is observed for the Gd complex of the less rigid podate tpaa with respect to Gd complexes containing macrocyclic heptadentate ligands such as do3a (14.5).[5] Moreover we expect that the stability and the solubility of the gadolinium complex could be improved by suitable substitution of the pyridine rings.

The relaxivity r_{1p} is a critical property of a potential contrast agent which represents the increase in the water proton nuclear magnetic relaxation rate per millimolar concentration of the paramagnetic compound. At 60 MHz, r_{1p} was measured to be 13.3 $mM^{-1}\ s^{-1}$ at 25 °C (9.37 $mM^{-1}\ s^{-1}$ at 37 °C) for **3**. This value is higher than those found in the mono-aquo complexes $[Gd(dtpa)(H_2O)]^{2-}$ or $[Gd(dota)(H_2O)]^{-}$ (4.3 $mM^{-1}\ s^{-1}$ at 25 °C) and than the values found in bis-aquo complexes containing macrocyclic heptadentate ligands such as $[Gd(do3a)(H_2O)_2]$† (6.1 $mM^{-1}\ s^{-1}$ at 25 °C) and $[Gd(pcta[12])(H_2O)_2]$† (6.9 mM s^{-1} at 25 °C).[10,11] This high value cannot simply be explained by the presence in aqueous solution of two water molecules coordinated to the Gd(III) ion (each molecule makes a contribution to the relaxivity of *ca.* 2.5 $mM^{-1}\ s^{-1}$). A preliminary study of the temperature dependence of the relaxivity indicates that the inner sphere water molecules are involved in a fast exchange with the bulk water, in agreement with what is expected for complexes containing heptadentate ligands. More detailed NMR studies are in progress in order to elucidate the origin of this high relaxivity.

In summary we have described a new type of heptadentate ligand which leads to a gadolinium complex with unusually high relaxivity and fast water exchange.

Moreover the straightforward introduction of substituents on the pyridine rings allows us to envisage ligands with a higher water solubility, containing functional groups capable of non-covalent or covalent interactions with slowly moving substrates such as proteins or polysaccharides. This could lead to contrast agents with longer rotational correlation times and consequently higher relaxivities or to increased specific tissue affinity.

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Notes and references

† H_4dota = 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid, H_5dtpa = diethylenetriaminepentaacetic acid, H_3dtpa-bea = dtpa-bis(ethylamide), H_3pcta[12] = 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid, H_3tren-Me-3,2-hopo = tris[(3-hydroxy-1-methyl-2-oxo-1,2-didehydropyridine-4-carboxamido)ethyl]amine, H_3do3ma = (1*R*,4*R*,7*R*)-α,α′,α″-trimethyl-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, H_3do3a = 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid.

‡ H_3tpaa = α,α′,α″nitrilo(6-methyl-2-pyridinecarboxylic acid). The synthesis of tris[6-(2-*N*,*N*-diethylcarbamoyl)pyridyl)methyl]amine **1** will be described in detail elsewhere. Anal. Calc. for $H_3tpaa{\cdot}1.6\ H_2O$ ($C_{21}H_{15.2}N_4O_{7.6}$): C, 55.9; H, 4.7; N, 12.4; O, 26.9. Found: C, 55.9; H, 4.8; N, 12.25; O, 27.0%. δ_H(400 MHz, DMSO-d_6, 298 K). 7.92 (H4, t, 3H), 7.89 (H3/5, dd, 3H, *J* 7.2, 1.8 ż), 7.82 (H3/5, dd, 3H, *J* 7.2, 1.8 Hz), 3.93 (CH_2, s, 6H); δ_C(400 MHz, DMSO-d_6, 298 K), 165.9 (CO_2), 158.9, 147.6, 137.6, 125.9, 122.8, 59.2 (CH_2). ES^+-MS: *m/z* 423 $[LH_3 + H]^+$, 445 $[LH_3 + Na]^+$, 461 $[LH_3 + K]^+$, 401 $[LH_3 + Na - CO_2]^+$; 417 $[LH_3 + K - CO_2]^+$ ES^-: *m/z* 459 $[LH_3 + K - 2H]^-$; 421 $[LH_3 - H]^-$; 415 $[LH_3 + K - 2H - CO_2]^-$; 322 $[LH_3 + K - C_7H_7NO_2]^-$.

§ *Experimental*: stoichiometric amounts of H_3tpaa (30 mg, 0.07 mmol) and of $GdCl_3{\cdot}6H_2O$ (26.4 mg, 0.07 mmol) were dissolved in boiling water (10 mL). Slow cooling followed by slow evaporation of the resulting solution yielded the gadolinium complex $[Gd(tpaa)(H_2O)]_2{\cdot}7H_2O$ as colorless crystals (35 mg, yield 75%). ES^+-MS (based on the most abundant isotope of GD): *m/z* 578 $[Gd(tpaa) + H]^+$, 590 $[Gd(tpaa - CO_2)OH + K]^+$, 616 $[Gd(tpaa) + K]^+$, 763 $[Gd(tpaa) + KCl + K]^+$, 1154 $\{[Gd(tpaa)]_2 + H\}^+$, 1193 $\{[Gd(tpaa)]_2 + K\}^+$.

¶ *Crystal data*: $[Gd(tpaa)(H_2O)]_2{\cdot}7H_2O$ **3**, $C_{42}H_{48}N_8O_{21}Gd_2$, *M* = 1315.38, orthorhombic, *Pbcn*, *a* = 13.0347(15), *b* = 15.6221(17), *c* = 23.506(4) Å, *V* = 4786.5(12) $Å^3$, *Z* = 4, D_c = 1.825 g cm^{-3}, μ = 2837 mm^{-1}. 5943 independent reflections (θ_{max} = 29.12°) were collected at 298 K. Refinement using the SHELXTL 5.05 package on all data converged at R_1 = 0.0234, *wR*2 = 0.0496. Data were collected using a Bruker SMART CCD area detector three-circle diffractometer (Mo-Kα radiation, λ = 0.71073 Å, graphite monochromator). CCDC 157262. See http://www.rsc.org/suppdata/cc/b1/b100657f/ for crystallographic data in .cif or other electronic format.

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