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Accelerating water exchange for Gd^{III} chelates by steric compression around the water binding site†

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The water exchange process was accelerated for nine-coordinate, monohydrated macrocyclic Gd^{III} complexes by inducing steric compression around the water binding site; the increased steric crowding was achieved by replacing an ethylene bridge of $DOTA^{4-}$ by a propylene bridge;‡ in addition to the optimal water exchange rate, the stability of $[Gd(TRITA)(H_2O)]^-$ is sufficiently high to ensure safe medical use which makes it a potential synthon for the development of high relaxivity, macromolecular MRI contrast agents.

Some currently emerging applications in magnetic resonance imaging require contrast agents of particularly high efficiency. For instance, in molecular imaging the amount of contrast agent delivered to a target site can be very much limited by biological constraints (low receptor concentration in receptor targeting, *etc.*), therefore only high relaxivity agents can allow visualizeation the specific site.§ The Solomon–Bloembergen–Morgan theory, which relates the observed paramagnetic relaxation rate enhancement to microscopic properties, predicts maximum proton relaxivities for Gd^{III} complexes (>100 *cf.* 4–5 mM^{-1} s^{-1} of commercial agents) when the three most important influencing factors, rotation, electron paramagnetic relaxation and water exchange are simultaneously optimised.[1] Namely, the rotation has to be slow enough, which can be achieved by using macromolecules. The electron spin relaxation, despite the recent theoretical developments, is difficult to modify on a rational basis. The tuning of the water exchange rate to the optimal value of around $k_{ex} = 10^8$ s^{-1} has also been problematic. While it is relatively easy to slow down the water exchange process as compared to the currently used Gd^{III}-based contrast agents ($k_{ex} \approx 10^6$ s^{-1}), it is much more difficult to accelerate, especially if high thermodynamic and kinetic stability has to be retained to ensure non-toxicity. Here we report a Gd^{III} chelate which is the first to be selected on a rational basis to present optimal water exchange rate. In addition, this complex is sufficiently stable to be applied as a diagnostic agent.

Nine-coordinate Gd^{III} poly(amino carboxylates), including all commercial Gd^{III}-based MRI contrast agents, undergo a dissociative, **D**, or dissociative interchange, $\mathbf{I_d}$, water exchange, in contrast to the mechanism, **A**, on $[Gd(H_2O)_8]^{3+}$.[1] The rate of such dissociative exchange processes is primarily determined by the overall charge of the chelate (more negative charge leads to faster exchange) and by the steric crowding around the bound water site. An increased steric compression around the inner sphere water molecule will facilitate its leaving which, in a dissociative process, constitutes the rate determining step. Therefore, our objective was to induce steric crowding around the water binding site in Gd^{III} poly(amino carboxylates) in order to increase the exchange rate without loosing complex stability.

† Electronic supplementary information (ESI) available: synthesis of TRITA and TRITA-bz-NO_2-^{17}O relaxation rates and chemical shifts for Gd^{III} chelates. See http://www.rsc.org/suppdata/cc/b2/b207713b/

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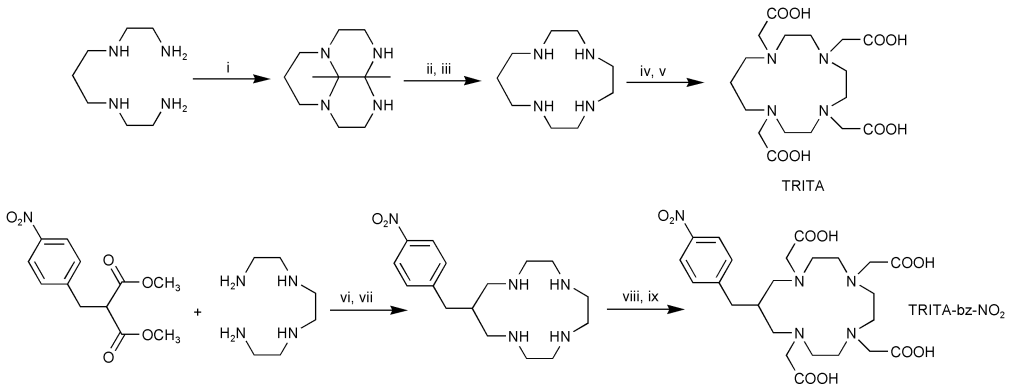
Among macrocyclic poly(amino carboxylates), the 12-membered $DOTA^{4-}$ is known to form a monohydrated Gd^{III} chelate which has the highest thermodynamic and kinetic stability of all MRI contrast agents.§ The 14-membered $TETA^{4-}$ forms a less stable Gd^{III} complex which, due to steric constraints induced by the larger cycle, has no inner sphere water molecule. Consequently, the intermediate 13-membered macrocycle, $TRITA^{4-}$, is likely to form a monohydrated Gd^{III} complex with sufficiently high steric crowding to have fast water exchange. Importantly, the thermodynamic stability of $Gd(TRITA)^-$ is high enough for medical use ($\log \beta = 19.2$).[2] It is more apprioriate to compare pGd values: the slightly lower pGd for $Gd(TRITA)^-$ shows that the stability is not much decreased compared to the commercial agents: pGd = 14.6 ($Gd(TRITA)^-$); 15.8 (Gd(DTPA-BMA); 19.1 ($Gd(DTPA)^{2-}$) or 19.2 ($Gd(DOTA)^-$) at pH 7.4; $c_{Gd} = 1$ μM; $c_{lig} = 10$ μM. The kinetic inertness, as important for non-toxicity as thermodynamic stability, is also expected to be high for this macrocyclic chelate, though it is likely lower than for $[Gd(DOTA)(H_2O)]^-$.

We synthesized TRITA *via* the bis-aminal methodology,[3] and TRITA-bz-NO_2 by modifying the synthesis described by Maecke and coworkers.[4] The bifunctional derivative is intended to be covalently linked to macromolecules to optimise rotation (Scheme 1).

Longitudinal and transverse ^{17}O relaxation rates and chemical shifts were measured as a function of the temperature on aqueous solutions of $[Gd(TRITA)(H_2O)]^-$ and $[Gd(TRITA\text{-}bz\text{-}NO_2)(H_2O)]^-$¶ and on a diamagnetic reference solution ($HClO_4$, pH 4) at $B = 9.4$ T. The experimental data were analysed with the Swift–Connick equations to yield parameters describing water exchange and rotation.[1] In the whole temperature range, the transverse relaxation rates, $1/T_{2r}$, decrease with increasing temperature, indicating that the system is in the fast exchange regime. Under such conditions, the transverse relaxation rate is determined by both the water exchange rate and the longitudinal electron spin relaxation. Hence, information on electronic relaxation is indispensable to calculate the water exchange rate. We have performed variable temperature, multiple field (0.34, 2.7, 5.4 and 8.1 T) EPR measurements on $[Gd(TRITA)(H_2O)]^-$. The EPR linewidths at all magnetic fields are very similar to those for $[Gd(DOTA)(H_2O)]^-$. For this latter, a complete analysis of multiple field EPR data has already been performed with the recently developed Rast–Borel theory.[5] Therefore as a quick but reliable estimation of $1/T_{1e}$ for $[Gd(TRITA)(H_2O)]^-$ and $[Gd(TRITA\text{-}bz\text{-}NO_2)(H_2O)]^-$, we used the value calculated for $[Gd(DOTA)(H_2O)]^-$ at the NMR field $B = 9.4$ T ($1/T_1 = 8 \times 10^7$ s^{-1}). (A rigorous analysis of the EPR spectra by using the recently developed theory has been undertaken and will be reported in due course). The parameters obtained from ^{17}O NMR are given in Table 1 and the fitted data shown in the ESI†).

The value of the scalar coupling constant, $A/\hbar$, is typical of Gd^{III} chelates and proves unambigously the presence of one inner sphere water molecule. The water exchange rate, k_{ex}^{298}, is



Scheme 1 *Reagents and conditions*: (i) butanedione, CH_3CN, (ii) dibromoethane, K_2CO_3, CH_3CN, (iii) $N_2H_4{\cdot}H_2O$ (iv) ethyl bromoacetate, CH_3CN, K_2CO_3, (v) 1. 6 M HCl, 2. ion exchange, (vi) EtOH, (vii) 1. $BH_3{\cdot}THF$, THF, 2. conc. HCl, (viii) *tert*-butyl bromoacetate, DMF, DIEA, KI, (ix) 1. 6 M HCl, 2. ion exchange (for details, see ESI†).

Table 1 Parameters obtained for $[GdL(H_2O)]^-$ complexes from ^{17}O NMR

Ligand	TRITA	TRITA-bz-NO_2	DOTA[a]
$k_{ex}^{298}/10^6$ s^{-1}	270 ± 40	120 ± 20	4.1
$\Delta H^{\ddagger}$/kJ mol^{-1}	17.5 ± 1.8	35.5 ± 1.9	49.8
$\Delta S^{\ddagger}$/J mol^{-1}K^{-1}	−24 ± 9	+21 ± 5	+48.5
$A/\hbar$ 10^6 rad s^{-1}	−3.8 ± 0.2	−3.7 ± 0.1	−3.7
τ_R^{298}[b]/ps	82 ± 4	225 ± 7	77
E_R [b]/kJ mol^{-1}	21.9 ± 1.3	23.3 ± 1	16.1

[a] Ref. 6 simultaneous fit of ^{17}O NMR, EPR and NMRD data. [b] τ_R = rotational correlation time of the Gd–O_{water} vector; E_R: its activation energy.

almost two orders of magnitude higher than on $[Gd(DOTA)(H_2O)]^-$ and falls into the optimal range to attain maximum proton relaxivities, provided the rotation is optimised. The less positive activation entropies and the lower activation enthalpies seem to point to a less dissociative exchange as compared to $[Gd(DOTA)(H_2O)]^-$.

The faster water exchange on $[Gd(TRITA)(H_2O)]^-$ *vs.* $[Gd(DOTA)(H_2O)]^-$ is a consequence of the increased steric compression around the bound water molecule, induced by the enlarged macrocycle. Unfortunately, we failed to establish a complete solid state structure of the chelate as all attempts of crystallisation led to relatively disordered structures. Although the position of the macrocycle carbons is not unambiguous, the position of the metal and the donor atoms is well determined (Fig. 1). In comparison to $[Gd(DOTA)(H_2O)]^-$, the Gd–bound water distance is similar (2.48 ± 0.01 Å for TRITA, and 2.45 Å for DOTA),[7] whereas the distance between the plane of carboxylate oxygens and the metal is remarkably longer in the TRITA complex (0.83 ± 0.01 *vs.* 0.70 Å). As a consequence, the

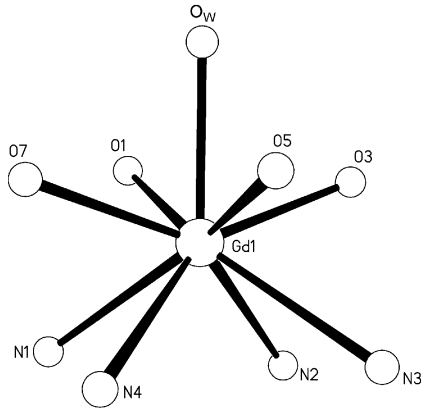


Fig. 1 Coordination polyhedron of Gd^{3+} in $[C(NH_2)_3][Gd(TRITA)(H_2O)]$.

bound water molecule is much closer to the negatively charged carboxylates which will facilitate its leaving in the rate determining step of the exchange. Another proof of the compressed structure around the bound water site is the lower value of the $O_{carboxylate}$–Gd–$O_{carboxylate}$ angles : 136.7 ± 0.7 (O1–Gd–O5) and 142.7 ± 0.6° (O3–Gd–O7) compared to 146° in $[Gd(DOTA)(H_2O)]^-$. As expected, in $[Eu(TETA)]^-$, which has no inner sphere water, these angles are even lower (104, 131°).[8]

In conclusion, based on structural considerations in the inner sphere of macrocyclic Gd^{III} complexes, we succeeded in accelerating the water exchange by inducing steric compression around the water binding site. The increased steric crowding was achieved by replacing an ethylene bridge of $DOTA^{4-}$ by a propylene bridge. Although this modification reduces to a slight extent the thermodynamic stability of the chelate, $[Gd(TRITA)(H_2O)]^-$ is stable enough to be used as an MRI contrast agent. Therefore this chelate is a potential synthon to develop high relaxivity, macromolecular agents.

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Notes and references

‡ $DOTA^{4-}$ = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate; $TRITA^{4-}$ = 1,4,7,10-tetraazacyclotridecane-1,4,7,10-tetraacetate; $TETA^{4-}$ = 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetate.

§ Proton relaxivity directly describes the efficiency of an MRI contrast agent. It is defined as the paramagnetic enhancement of the water proton relaxation rate, referred to 1 mmol concentration of the paramagnetic agent.

¶ The Gd^{III} complexes were prepared by mixing aqueous solutions of the ligand and $Gd(ClO_4)_3$ in equimolar amounts, and adjusting the pH to 6.2. The absence of free Gd^{3+} was proved by the xylenolorange test. The Gd^{III} concentration in the ^{17}O NMR measurements was 30.4 and 21.9 mmol kg^{-1} for TRITA and TRITA-bz-NO_2, respectively.

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