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Improving the relaxivity by dimerizing Gd-AAZTA: Insights for enhancing the sensitivity of MRI contrast agents

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ABSTRACT

The synthesis of a dimeric derivative of Gd-AAZTA is reported. It retains the basic properties of the parent complex ($q=2$, high stability) and displays a relaxivity of 14.0 $mM^{-1}s^{-1}$ at 20 MHz and 298 K. The outstanding relaxivity is the result of the increased molecular reorientational time and of the contribution arising from 4 to 5 water molecules in the second coordination sphere of the Gd(III) ions.

Paramagnetic Gd(III) complexes are by far the most used contrast agents (CA) in Magnetic Resonance Imaging. All the commercially available systems are represented by highly stable octacoordinated complexes containing one inner-sphere water molecule whose exchange with the bulk water causes an overall increase in relaxation rate of the water protons in the regions where they distribute. The ability to act as a relaxation agent is represented by the relaxivity value (r_{1p}) that is defined as the relaxation enhancement brought about by the presence of the paramagnetic complex in 1 mM concentration. The clinically used CA display r_{1p} of 3.7–3.8 $mM^{-1}s^{-1}$ (0.47 T and 315 K). The search for higher relaxivities continues to be very active as the availability of systems (with similar biodistribution/excretion characteristics of the currently used agents) that yield analogous relaxation enhancements at significantly lower doses is, of course, an interesting possibility. Along this line, in 2007, it was reported [1] an heptacoordinated Gd(III) complex, Gd-AAZTA, that displays, at 298 K and 0.47 T, a r_{1p} value of 7.1 $mM^{-1}s^{-1}$. The increase of r_{1p} has to be ascribed to the presence of two coordinated water molecules in the inner coordination sphere of the Gd(III) ion. Furthermore, it has been shown that Gd-AAZTA displays a very good stability (log $K_f=20.2$) [2] and very limited aptitude to transmetallation in the presence of Cu^{2+} and Zn^{2+} ions. The easy preparation of AAZTA ligand is another attractive property and several derivatives have been reported [3–6].

In this communication we report the synthesis, stability and relaxometric characterization of a new dimeric derivative of the Gd(III)-chelate with the heptadentate ligand AAZTA (Scheme 1).

This new Gd(III)-based system contains all the desirable features of the parent Gd-AAZTA complex [1], namely two inner-sphere water molecules in fast exchange with the bulk water, high thermodynamic stability, no tendency to form $q=0$ ternary adducts with endogenous carboxyanions, and, moreover, thanks to its dimeric nature, is characterized by an higher relaxivity as a consequence of the increase in the molecular reorientational time.

As for the parent AAZTA ligand, the synthesis of the dimer was carried out through the four step reaction shown in Scheme 1. The three component Mannich addition product **1** was obtained by the reaction of 1,5-dinitropentane, *N*,*N*-dibenzylethylenediamine, and paraformaldehyde in ethanol using acetic acid. Simultaneous reduction of the nitro groups and debenzylation of **1** was carried out using Pd-C 10% in methanol to afford the amine, in 97% yield. Exhaustive alkyation of the amine with benzyl 2-bromoacetate (K_2CO_3, CH_3CN) furnished the proligand **3**. Deprotection of the benzyl ester (Pd-C 10%) afforded the target ligand **4** in 75% yield (complete synthesis in the Appendix). Lanthanide(III) complexes were obtained by instantaneous reaction of stoichiometric amounts of ligand 4 and Ln(III)Cl_3 (further details in the Appendix).

The relaxivity of $(Gd\text{-}AAZTA)_2$, measured at 25 °C and 37 °C at the proton Larmor Frequency of 20 MHz, is 14.0 and 10.8 $mM^{-1}s^{-1}$ respectively. The relaxivity found for $(Gd\text{-}AAZTA)_2$ is doubled with respect to that of the parent monomeric Gd-AAZTA, sensibly higher than that of other reported mono-aquo dimers [7–11] and almost comparable to that of related di-acquo dimers [12]. A detailed relaxometric characterization of the Gd-complex has been obtained by the measurement of the ^{1}H-relaxivity field dependence (NMRD—Nuclear Magnetic Resonance Dispersion profiles) at two different temperatures and from the ^{17}O transverse relaxation rate profile as a function of temperature (Fig. 1). The parameters reported in Fig. 1 have been obtained by the simultaneous fitting of the three sets of

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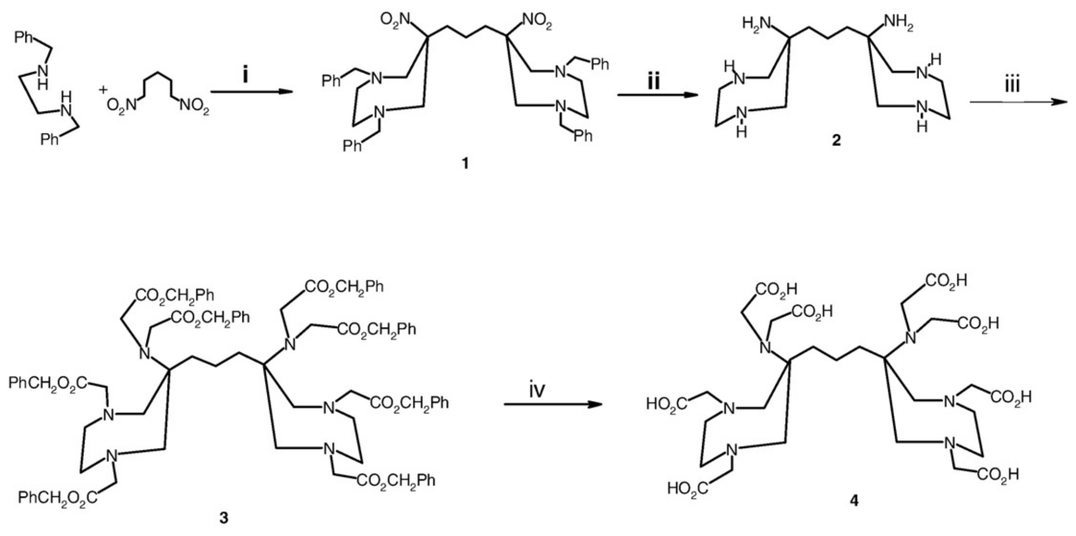


Scheme 1. Synthesis of $(AAZTA)_2$ ligand. Reagents and conditions: (i) acetic acid, $(CH_2O)_n$, ethanol, 80 °C, 5 h; (ii) Pd-C 10%, CH_3OH, 50 psi, 24 h; (iii) Benzyl-2-bromoacetate, K_2CO_3, CH_3CN, 80 °C, 24 h; and (iv) Pd-C 10%, CH_3OH, 50 psi, 24 h.

experimental data, using the well established Solomon-Bolembergen–Morgan equations [13,14] and Freed's model [15] for inner and outer sphere contributions to the ^1H-relaxivity respectively and the Swift and Connick equations for ^{17}O-data [16].

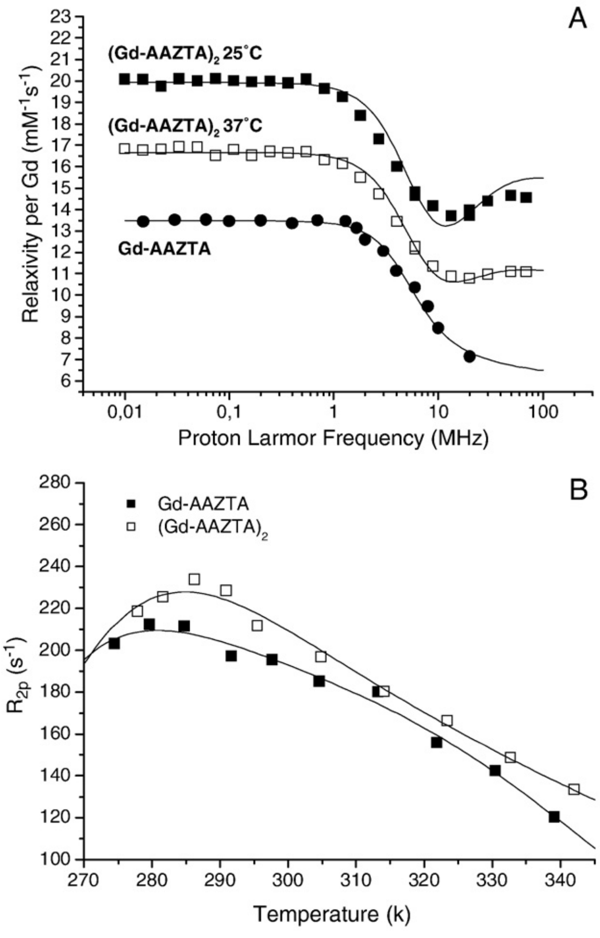
If compared with the parent Gd-AAZTA complex, the main differences may be found in the values of the exchange lifetime of the coordinated water molecules and the reorientational correlation time, both τ_M^{298} and τ_R^{298} values being markedly longer with respect to the monomeric system. Whereas lengthening of τ_R is expected on the basis of the increase of the MW, the slow exchange of the coordinated water does not appear immediately understandable. It is a matter of fact that some of the derivatives of Gd-AAZTA (whether the structural modification involves the arm [5] or the exocyclic carbon) display longer τ_M values. However, this lengthening of the water exchange rate is not a limiting factor on the attainable relaxivity as increasing the temperature from 25 to 37 °C results in lower relaxivity values over the entire range of investigated frequencies.

On the other hand, the obtained τ_R^{298} value exactly reflects the two-fold increase in molecular weight passing from the parent monomeric to the dimeric species. Interestingly, the experimental NMRD profile of $(Gd\text{-}AAZTA)_2$ can be satisfactory fitted only by including the contribution of 4–5 second sphere water molecules. This finding suggests that water molecules may lie between the two halves of the ligand involved in tight H-bonding with carboxylate oxygens from the two sets of acetic arms.

The protonation constants of the ligand and the stability constants of the $(Gd\text{-}AAZTA)_2$ complex were determined by pH-potentiometric titration in 0.15 M NaCl solution at 25 °C. The pH-potentiometric titration allowed identification of 10 p*K*a values (Appendix). The stability constants of the $(AAZTA)_2$ formed with Gd(III) ion ($\log K_{GdL} = 21.25$, $\log K_{Gd2L} = 18.27$) were determined at 1:1 and 2:1 metal to ligand ratio in NaCl 0.15 M. The obtained values are in agreement with the earlier published stability constants of the Gd-AAZTA complex measured in KCl 0.15 M[2]. To get a better basis for the latter comparison the stability constants of the Gd-AAZTA complex has been re-determined in this work using NaCl 0.15 M as ionic strength (Appendix). In fact it has been found that $\log K_f$ is higher when measured in KCl, indicating that the AAZTA coordination cage displays a non-negligible ability to coordinate Na^+ ions.

The kinetic stability determined for the parent monomeric Gd-AAZTA complex seems to be maintained in the dimeric system as in vitro relaxometric assays did not show any competition effects when $(Gd\text{-}AAZTA)_2$ was left for several hours in the presence of up to 10-fold excess of $ZnCl_2$, $CaCl_2$ or $CuCl_2$.

Replacement of the fast relaxing Gd(III) ion with the shift inducing Yb(III) allowed us to record the high resolution ^1H NMR spectrum of



q	τ_M^{298} (ns)	ΔH_M (KJ mol^{-1})	τ_R^{298} (ps)	Δ^2 (10^{19} s^{-2})	τ_v^{298} (ps)	A/h (10^6 rad s^{-1})	$r_{Gd\text{-}H}$ (Å)	$r_{Gd\text{-}O}$ (Å)
2 +5 2ndsph	**193 ±7.5**	**37.8± 1.5**	**147 ± 3.3**	**6.71 ± 2.2**	**17.4 ± 0.8**	**-3.6 ± 0.1**	**2.99 ± 0.01**	**2.40 ± 0.1**

Fig. 1. A — ^1H NMRD profiles of 1 mM Gd-AAZTA at 25 °C (filled circles) and $(Gd\text{-}AAZTA)_2$ at 25 (filled squares) and 37 °C (open squares); B — $^{17}O\text{-}R_{2p}$ profiles as a function of temperature for 12.8 mM Gd-AAZTA (filled squares) and $(Gd\text{-}AAZTA)_2$ (open squares) recorded at 2.1 T. The principal parameters obtained from the simultaneous fitting of the NMRD and $^{17}O\text{-}R_{2p}$ profiles are also reported.

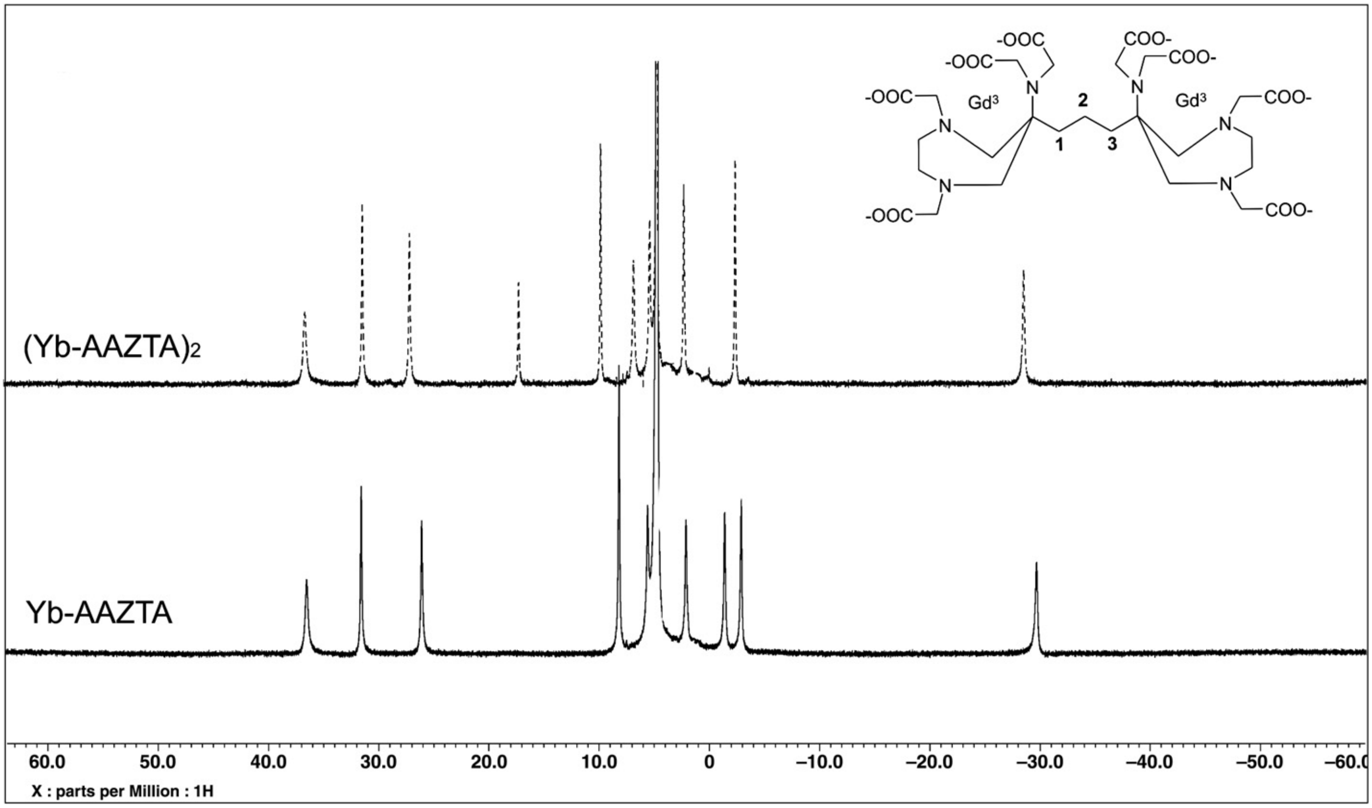


Fig. 2. ^{1}H NMR spectra of Gd-AAZTA and $(Gd\text{-}AAZTA)_2$ registered at 400 MHz and 298 K.

the corresponding $(Yb\text{-}AAZTA)_2$ system (Fig. 2). As expected, the ^{1}H NMR spectrum of $(Yb\text{-}AAZTA)_2$ is quite similar to that of Yb-AAZTA: eight equally intense resonances (integration 4) corresponding to the eight methylenic groups of the two coordination cages and two resonances (integration 2 and 4) corresponding to the methylenic groups of the linker. The observed pattern is fully consistent with a highly flexible solution structure either at the level of the acetate arms or of the macrocyclic ring. Interestingly, the resonances of H1/H3 are less shifted than H2 to indicate a closer spatial proximity between the latter carbon and the Lanthanide centers. Likely, as already surmised from the occurrence of water molecules in the second coordination sphere, the two halves of the complex are not pushed out by the residual negative charges. Rather the bridging water molecules appear to keep them closely associated. To minimize the space between the two halves the propylenic chain adopts a "slice-like" configuration thus yielding H2 at a shorter distance than H1 and H3.

The herein reported results on $(Gd\text{-}AAZTA)_2$ suggest that further higher relaxivities could be obtained by designing multimeric Gd-AAZTA-based systems as the relaxation enhancement properties of each unit are additive and likely such multimeric derivatives may benefit from additional second sphere contributions. The straighforward procedure applied for the synthesis of the herein reported dimeric ligand may be easily extended to design such multimeric derivatives.

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Appendix A. Supplementary data

Supporting information available: full details on the synthesis of AAZTA dimer and on the pH-potentiometric titrations on $(Gd\text{-}AAZTA)_2$ and Gd-AAZTA and on their respective ligands. Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.inoche.2010.03.014.

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