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[Gd-AAZTA]$^{-}$: A New Structural Entry for an Improved Generation of MRI Contrast Agents

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An innovative MRI contrast agent based on the unprecedented and easily obtained ligand AAZTA is described. The simple and straightforward synthesis of the ligand, together with the potentiometric and relaxometric behavior of the corresponding Gd(III) chelate, is reported. The complex [Gd(AAZTA)]$^{-}$ shows outstanding magnetic properties connected with high thermodynamic stability in aqueous solution and a nearly complete inertness toward the influence of bidentate endogenous anions, placing this compound as one of the most promising candidates for the development of high performance MRI contrast agents.

Currently, about one-third of MRI clinical scans are carried out in the presence of Gd-agents because they add relevant physiological information to the superb anatomical resolution attainable with this technique.

To be considered a potential MRI agent, a Gd-chelate must fulfill several requirements, such as good solubility in water, high thermodynamic (and possibly kinetic) stability to ensure against the in vivo release of toxic Gd^{3+} ions and free ligand molecules, and high relaxivity.[1,2] The latter property reports about the ability of a given paramagnetic agent to enhance the relaxation rate of solvent water protons, and it is commonly referred to 1 mM concentration of the relaxation enhancer at the field of 0.5 T and 298 K. The observed relaxivity receives contributions from the exchange of water molecule(s) directly coordinated to the Gd(III) ion (inner sphere term) as well as from water molecules diffusing in its proximity (outer sphere term). In general, the attainment of higher relaxivity can be pursued by proper control of the parameters which determine the inner sphere term, i.e., the number (q) and the exchange lifetime (τ_M) of the water molecules directly coordinated to the paramagnetic metal center, the electronic relaxation time (τ_S), and the molecular reorientational time (τ_R).[3] Whereas the lengthening of τ_R is tackled by designing slow moving macromolecular systems, the other parameters have to be optimized on the basis of the characteristic features of the chelate itself. In principle, doubling the inner-sphere relaxivity can be obtained on going from $q = 1$ to $q = 2$ complexes. The four commercially available MRI contrast agents are Gd(III) complexes with octadentate ligands, thus allowing only one water molecule to enter the inner coordination sphere ($q = 1$). The straightforward route to increase q deals with a decrease of the overall denticity of the coordinating ligand. However, the simple shift from an octadentate to a heptadentate ligand may interfere with toxicological problems associated either with a decrease of the thermodynamic stability or with the replacement of the two water molecules by endogenous anions[4,5] or by coordinating groups of tissue proteins.[6,7]

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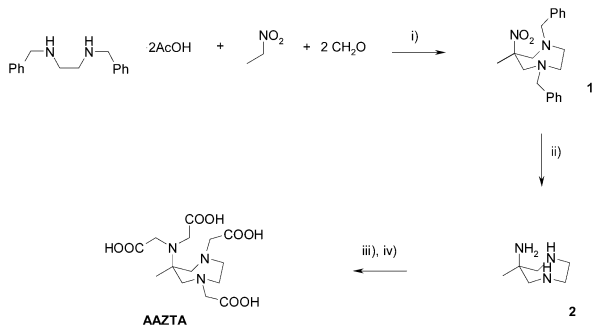
Scheme 1. Synthesis of AAZTA[a]



[a] (i) Δ, EtOH; (ii) H_2, Pd/C, MeOH/H_2O; (iii) $BrCH_2COOtBu$, K_2CO_3, CH_3CN; (iv) CF_3COOH.

Few Gd(III) chelates with heptacoordinating ligand did appear to overcome these drawbacks. Gd-PCP2A[8] has been a particularly well-behaving system, but its further functionalization proved difficult. Another interesting class of systems with $q = 2$ currently under intense scrutiny is represented by Gd-HOPO derivatives.[9−11]

In this Communication, we report on the synthesis and relaxometric properties of a novel Gd(III) chelate with the heptadentate ligand AAZTA (6-amino-6-methylperhydro-1,4-diazepinetetraacetic acid). AAZTA is readily obtained in high yields, and its Gd(III) complex displays excellent relaxation enhancement properties to be considered the prototype of a new class of enhanced MRI agents.

The synthesis of AAZTA is simple and uses readily available and cheap chemicals (Scheme 1).[12]

The key step is the formation of the seven-membered ring. This has been simply achieved through a nitro-Mannich protocol. Thus, heating a mixture of nitroethane, formaldehyde, and *N*,*N*′-dibenzylethylenediamine diacetate in ethyl acetate leads to the formation of compound **1** in almost quantitative yields. Reduction of the nitro group and concomitant bis-N-debenzylation was accomplished by catalytic hydrogenation of **1**, leading to the strongly basic triamine **2**. Exhaustive alkylation of the nitrogen atoms with *tert*-butyl bromoacetate in the presence of potassium carbonate followed by treatment with trifluoroacetic acid in order to remove the tertiary esters yielded AAZTA ligand.

The preparations of lanthanide(III) complexes were straightforward as they consisted of the instantaneous reaction of stoichiometric amounts of H_4AAZTA with the corresponding Ln(III) trichlorides. A preliminary determination of the ligand pK_a values was needed to evaluate the stability constants of the corresponding complexes. The potentiometric titration at 298 K in KCl 0.1 M of H_4AAZTA yielded the following pK_{ai} values: pK_{a1} = 2.40 ± 0.017, pK_{a2} = 3.79 ± 0.011, pK_{a3} = 6.55 ± 0.009, pK_{a4} = 11.16 ± 0.008. The stability constant of $[Gd\text{-}AAZTA]^-$ was determined by potentiometric titration of 1:1 metal/H_4AAZTA mixtures in the pH range 2−8. A log β_{Gd-L} value of 19.26 ± 0.10 was obtained. Such a value is slightly smaller than $[Gd\text{-}DTPA]^{2-}$, by far the most used MRI agent, and significantly higher than that of Gd-DTPA-BMA, another agent currently used in clinical diagnosis. In vitro relaxometric assays did not show any transmetalation effects when $[Gd\text{-}AAZTA]^-$ was left in the presence of 10-fold excess of $ZnCl_2$, or $MnCl_2$, or $CaCl_2$.

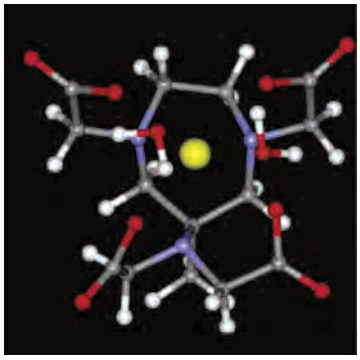


Figure 1. Ab initio derived three-dimensional structure of Gd-AAZTA.

The ^1H NMR spectra of $[Ln\text{-}AAZTA]^-$ (Ln = Yb, Eu) complexes in D_2O (Supporting Information), in the temperature range from 278 to 353 K, showed nine resonances corresponding to eight equally intense methylenic and one methyl group, respectively. This finding is consistent with the occurrence of stereochemical nonrigid behavior based on the exchange of coordination positions of the acetate arms coupled to a wagging motion in the macrocyclic ring.

Unfortunately, no good crystal for X-ray solid state structure determination has yet been obtained. Preliminary modeling studies suggest that the AAZTA ligand wraps around the lanthanide(III) ion to yield a structure intermediate between those found for Gd-DTPA and Gd-DOTA, respectively, with two water molecules coordinated to the Gd(III) ion (Figure 1). The occurrence of $q = 2$ has been assessed by measuring Dy(III) induced ^{17}O NMR water shift.[13] The dis measurements were performed on $DyCl_3$ and $[Dy\text{-}AAZTA]^-$ solutions by variation of the concentration over the range from 20 to 100 mM on JEOL EX-90 spectrometer at 303 K using the ^{17}O shift of water as external reference (Supporting Information).

The relaxivity of $[Gd\text{-}AAZTA]^-$ is 7.1 mM^{-1} s^{-1} at 20 MHz and 298 K, and it is constant when the pH of the solution is changed in the range from 2 to 11. The observed behavior indicates that (i) no deprotonation of the coordinated water molecules takes place at basic pH and (ii) $[Gd\text{-}AAZTA]^-$ is noticeably kinetically inert. In fact, on the basis of the thermodynamic stability of the complex, one could expect, at pH 2, a partial release of Gd(III) ions with a consequent increase of the proton relaxation rate. Actually, when the solution is left at pH 2 for longer time, a steady increase of the relaxation rate is observed to reach a constant

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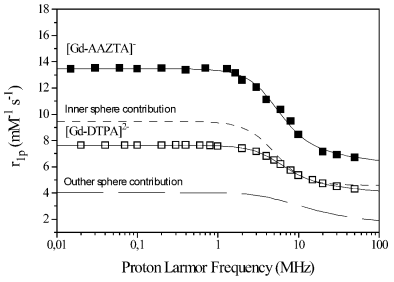

Figure 2. $1/T_1$ NMRD profile of a 1 mM aqueous solution of Gd-AAZTA (■) and Gd-DTPA (Magnevist) (□) at pH = 7 and 25 °C. The solid curves through the data points were calculated according to the Solomon−Bloembergen theory.

value (corresponding to a solution containing 75% of $[Gd\text{-}AAZTA]^-$ and 25% of free Gd(III) ions) after 20 min. At pH 7, the relaxivity decreases exponentially with temperature to indicate that it is not limited by a slow exchange of the coordinated water molecules. An accurate determination of the water exchange rate was pursued by measuring ^{17}O-T_2 as a function of temperature. Fitting the experimental data (Supporting Information) to the Swift−Connick equations[14] led to a τ_M^{298} value of 90 ns. $[Gd\text{-}AAZTA]^-$ displays an exchange rate of the coordinated waters significantly faster than the one shown by $[Gd\text{-}DTPA]^{2-}$ (300 ns) and $[Gd\text{-}DOTA]^-$ (240 ns).[15] Thus, it approaches the optimal value required for the attainment of very high relaxivities once the chelate would have been bound to a macromolecular structure. More information on the determinants of the observed relaxivity was obtained by recording the $1/T_1$ NMRD profile over an extended range of magnetic field strengths. The obtained curve (Figure 2) is well fitted with the following parameters: $q = 2$, $\tau_R = 74$ ps, $\tau_M = 90$ ns, $\Delta^2 = 2.15 \times 10^{19}$ s^{-2}, and $\tau_V = 31$ ps. Interestingly, a relaxivity value of ca. 100 mM^{-1} s^{-1} has been calculated at 20 MHz and 298 K by simulating the NMRD profile of a macromolecular $[Gd\text{-}AAZTA]^-$ system using the same set of parameters as those obtained from the fitting of the profile of Figure 2 but increasing τ_R to 30 ns. This finding supports the view that the electronic relaxation time will not be responsible for a quenching of the attainable relaxivity.

As it was anticipated above, a potential drawback for Gd(III) chelates containing two coordinated water molecules is related to their partial or complete replacement by endogenous anions or donor groups (e.g., aspartate or glutammate residues) from proteins. The occurrence of such replacement can be easily assessed in vitro by titrating a solution of the complex with carboxylate or phosphate containing substrates and measuring the relaxivity changes. When $[Gd\text{-}AAZTA]^-$ was titrated with lactate or phosphate, no change in its relaxivity was detected also at concentrations of the added substrate 200 times higher than the paramagnetic chelate (Supporting Information). Thus, one may conclude that the solution structure of $[Gd\text{-}AAZTA]^-$, coupled with the occurrence of a residual negative charge, does not allow the replacement of the coordinated water molecules with other substrates. This behavior makes $[Gd\text{-}AAZTA]^-$ analogous to $[Gd\text{-}HOPO]^-$ and $[Gd\text{-}PCP2A]^-$.

In summary, the easy synthesis of the AAZTA ligand coupled with the properties of its Gd(III) chelate makes this system an outstanding candidate for the development of a new class of MRI agents.

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Supporting Information Available: Additional synthesis and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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