

# A New Generation of High Relaxivity $q=2$ MRI Contrast Agents based on the eptadentate AAZTA ligand

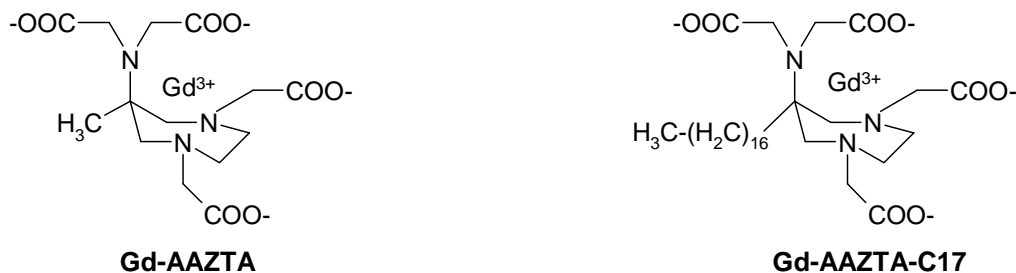
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## Introduction

The attainment of high relaxivity is of fundamental importance for pursuing novel applications of Gd(III) chelates as MR Imaging Probes. The most straightforward route to high relaxivities is provided by systems containing two water molecules in the inner coordination sphere of the paramagnetic ion. However, the increased hydration must not have negative consequences on the overall stability of the complex neither it has to favor the formation of ternary complexes with endogenous negative ions. Few systems have shown to possess these requisites. Gd-AAZTA owns these properties and, moreover, it displays a relatively fast exchange of the coordinated water molecules. On the basis of these favorable features, a derivative of Gd-AAZTA containing a long aliphatic chain has been synthesized and its relaxometric properties investigated in detail.



## Methods

The synthesis of AAZTA ((6-amino-6-methylperhydro-1,4-diazepinetetraacetic acid)) and of its hydrophobic derivative is simple, and relies on available and cheap chemicals. The key step is the reaction of RCH<sub>2</sub>CH<sub>2</sub>NO<sub>2</sub> with formaldehyde and N,N'-dibenzylethylenediamine, followed by hydrogenation and insertion of the four carboxymethyl moieties. The Gd(III) complexes were prepared by reacting stoichiometric amounts of the ligands with Gd(III) trichloride. The ligand pK<sub>a</sub> values and the stability constant of the complex were determined by potentiometric titration at 298K in KCl 0.1 M. Relaxometric measurements were carried out on a Stellar Spinmaster Relaxometer operating at variable frequencies between 20 and 80 MHz and on a Stellar Field Cycling Relaxometer in the frequency range 0.01-40 MHz. The binding to HSA was investigated by the Proton Relaxation Enhancement (PRE) method. The exchange lifetime of the coordinated water molecules was evaluated by measuring the temperature dependence of the water <sup>17</sup>O transverse relaxation rate of 20 mM solutions on a JEOL Spectrometer operating at 90 MHz.

## Results

The complex [Gd(AAZTA-C17)]<sup>-</sup> showed to have the outstanding properties of the parent complex, namely: i) two inner sphere water molecules in fast exchange with the bulk, ii) high thermodynamic stability in aqueous solution and iii) a nearly complete inertness towards the influence of bidentate endogenous anions. The functionalization with the hydrophobic chain induces the formation of micelles already at sub-millimolar concentrations (cmc 0.1 mM). The relaxivity of the self-assembled complex is 30 mM<sup>-1</sup>s<sup>-1</sup> at 20 MHz and 298K. Moreover Gd-AATZA-C17 displays a high affinity binding to Human Serum Albumin (2.4×10<sup>4</sup>M<sup>-1</sup>). The relaxivity shown by the Gd-AATZA-C17/HSA adduct is ca. 80 mM<sup>-1</sup>s<sup>-1</sup> at 20 MHz and 298K.

## Conclusions

A novel Gd(III) chelate based on the unprecedented and easily obtained AAZTA ligand has been reported as a prototype of a new class of highly sensitive agents. The simple and straightforward synthesis of the AATZA ligands, together with the good stability and excellent relaxation enhancement capabilities of their Gd(III) chelates suggest that these systems may have a huge potential in the development of high performance MRI contrast agents.