Towards ParaCEST MRI Contrast Agents Without Coordinated Water: Lanthanide Complexes of Novel Acetamido-Substituted Diethylenetriamine- and Triethylenetetramine Ligands

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Background. Chemical Exchange-dependent Saturation Transfer (CEST) is a novel technique for MRI contrast enhancement.[1] It is based on altering the signal intensity of the water protons by selectively saturating the resonance frequency of mildly acidic protons of a CEST contrast agent that are in an intermediately fast exchange equilibrium with bulk water protons. The CEST effect depends on pH, and pre-saturation frequency and intensity. It can, therefore, be utilized for pH-mapping, frequency-based multiplexing, as well as (almost) instantaneous "pre- and post-contrast imaging", which, in combination with a suitable molecular amplification strategy, makes this technique very attractive for molecular imaging.

Main factors limiting the sensitivity of this type of contrast agent are (1) the maximum exchange rate attainable without entering a fast exchange situation and (2) the finite T1 of bulk water, which causes incomplete transfer of saturation transfer. The presence of a paramagnetic center potentially increases the sensitivity of CEST agents by allowing higher exchange rates of the paramagnetically shifted exchangeable-proton resonance without exceeding the intermediate exchange limit.[2] However, these so-called ParaCEST agents concomitantly increase T1 relaxation of bulk water, which has a negative effect on the sensitivity.

Aim and Strategy. First generation dotam-based ParaCEST contrast agents of the general formula $[Ln(dotam)(H_2O)]^{3+}$ provide exchangeable, CEST-active amide protons. The dotam ligand and its derivatives only allows for a coordination of up to eight donor groups to suitable paramagnetic lanthanide ions, and a water molecule typically fills the ninth coordination site (Figure 1). The coordinated water molecule limits the achievable CEST-based contrast, due to an undesired lanthanide-induced relaxation enhancement of the pre-saturated water molecules. We have, therefore, synthesized novel ligands, which were designed to occupy all available coordination sites in respective lanthanide complexes, hence to block this first sphere T_1-relaxation effect.

Results. Figure 1 summarizes the formulae of some of the newly synthesized ligands ttham, ttbpam, ttha-tm^{2-}, and ttaham. Their lanthanide complexes were synthesized; in the case of ttham all lanthanides were probed. The crystal structure of $[Eu(ttham)](ClO_4)_3 \bullet 3.25H_2O$ confirms a ten-fold, bicapped square-antiprismatic coordination of the ttham ligand to the europium ion. For the various lanthanide ions, the coordination number in their ttham complexes differs. A switch in coordination number from ten- to nine-coordinated for the late lanthanides results in highly flexible molecules. This causes significant line broadening in their NMR and Z-spectra (Figure 2) and an overall reduced CEST effect of the amide protons, when compared to the complexes of the related nine-coordinating ligand ttbpam. The type and total charge of the directly coordinating ligand groups, and the symmetry of the metal complexes are further important parameters that determine the achievable CEST effect. As expected, the observed relaxivities decreased by a factor of about two when compared to related complexes bearing exchangeable water ligands.

Conclusion. Key parameters for the design of next generation molecular ParaCEST contrast agents for molecular MRI are the exclusion of coordinated water molecules, the ligand denticity, and the complex symmetry and dynamics.

Methods. All ligands and lanthanide complexes were prepared in our laboratories and characterized by LC-mass, FT-IR, fluorescence (Eu complexes), and NMR spectrometry, and elemental analysis. NMR and Z-Spectra were measured at 7 T and 310 K in 20 mM solution (pH 7.40, 20 mM MOPS). Pre-saturation: 2 s CW-RF pulse of ~22 µT.

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References. 1) J. Zhou et al. *Prog. Nucl. Magn. Reson. Spectros.* **2006**, *48*, 109-136. 2) S. Zhang et al., *Angew. Chem. Int. Ed.* **2002**, *41*, 1919-1921; S. Aime et al. *Magn. Reson. Med*. **2002**, *47*, 639-648.

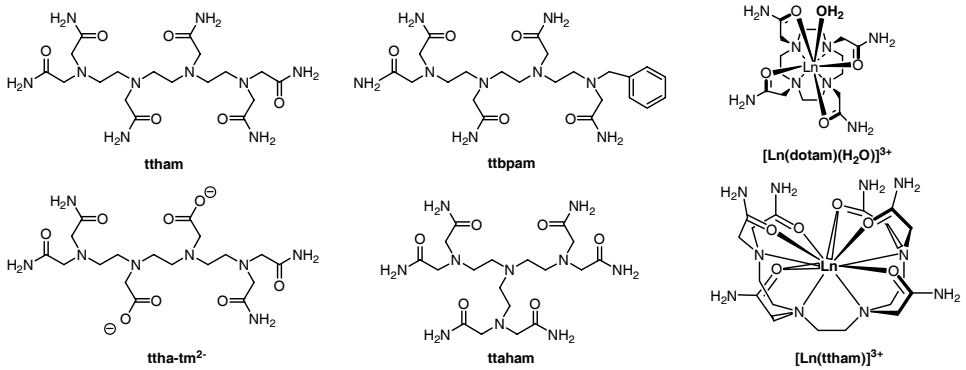

Figure 1. Structural formulae of relevant ligand molecules and lanthanide complexes

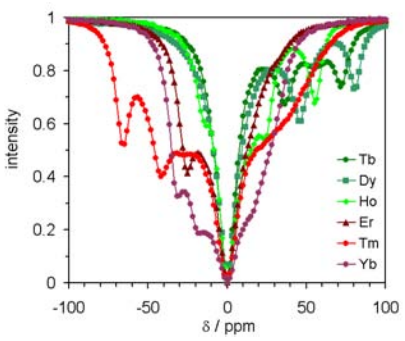

Figure 2. Z-Spectra of ttham complexes $[Ln(ttham)]Cl_3$