Synthesis and Evaluation of PARACEST MRI Contrast Agents Containing an Amino Acid Arginine

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Introduction: Considering their excellent sensitivity to environmental conditions (e.g. temperature, pH), there is growing interest in the design and synthesis of paramagnetic chemical exchange saturation transfer (PARACEST) MRI contrast agents (CAs) (1). We have recently established a wide scope synthetic methodology for the preparation of mono-, di- and tripeptide decorated cyclens and their lanthanide(III) complexes (2,3) for use as PARACEST MRI CAs. Among them, Tm^{3+} DOTAM-Gly-Lys-OH (Tm-**5**) was found to posses a strong CEST effect (15-20% at -47 ppm; 37 ºC, 10 mM); associated with the exchangeable amide protons. Tm-**5** has an overall positive charge and therefore reasonable pharmacokinetic properties are expected. The detection of Tm-**5** in mouse kidneys (4) and mouse brain tumours (5) has been described recently. Interestingly, our previously reported synthetic methodology (2) failed, when the synthesis of ligands containing an amino acid arginine (Arg) was attempted. Arginine rich peptides are known for their ability to penetrate the cell wall (6), therefore CAs containing Arg in their ligand framework will likely be taken up more easily into cells. Low yielding preparation of ligand DOTAM-Gly-Arg(NO_2)-OMe has been described by Sherry's group (7), however no lanthanide(III) complexes were prepared. In the current study, we describe an improved synthetic methodology for the preparation of both symmetrically and non-symmetrically functionalized ligands containing Arg. Their Tm^{3+} complexes Tm-**1-4** have been prepared and their PARACEST effects have been evaluated.

Methods: CAs Tm-**1-3** have been synthesized by alkylation of cyclen with haloacetyl mono- and dipeptides. Selective trialkylation of cyclen followed by alkylation with *N*-bromoacetyl propargylamine afforded Tm-**4**. Protecting groups (NO_2) in Tm-**1** were removed by catalytic hydrogenation. All ligands were metallated by treatment with $TmCl_3 \cdot H_2O$. Free ligands were purified by HPLC and characterized by ^1H NMR and HR-ESI-MS. CAs Tm-**1-4** were purified by size exclusion chromatography and were characterized by HR-ESI-MS. The absence of unchelated Tm^{3+} was verified using the Xylenol Orange test (8). The PARACEST effect for CAs Tm-**1-4** were evaluated as follows: A phantom composed of five NMR tubes with Tm-**1-5** solutions (10 mM, pH 7) was imaged at 37 ºC using a fast spin echo pulse sequence (FOV: 12.8 × 12.8 mm^2, matrix: 32 × 32, TR = 4000 ms, 4 echoes, and TE = 10 ms), preceded by a frequency selective saturation pulse (B_1 = 14 μT, saturation range = -100 to 100 ppm in steps of 1 ppm, saturation time = 3.95 s). Temperature was monitored and controlled by blowing hot air using a Model 1025 Small Animal Monitoring and Gating System (SA Instruments, Inc., Stony Brook, NY). CEST spectra were generated using the average signal intensity from each tube.

Results and Discussion: CAs Tm-**1-4** (30-50 mg) were prepared in 40-50% overall yields. The CEST effect (Figure 1) associated with Tm-**1** (15% at -47 ppm) compares well with previously described Tm-**5** (2). The CEST effect associated with Tm-**2** is slightly weaker (12% at -46 ppm), while Tm-**4** was found to be the most sensitive (17% at -46%). The absence of glycine (Gly) in Tm-**3** resulted in only a 7% CEST effect and a larger chemical shift (-51 ppm). It appears; that neither the presence of NO_2 protecting groups nor the replacement of one of the arms impacts the CEST effect significantly. The presence of Gly in the ligand framework seemed to increase CEST sensitivity (Figure 1).

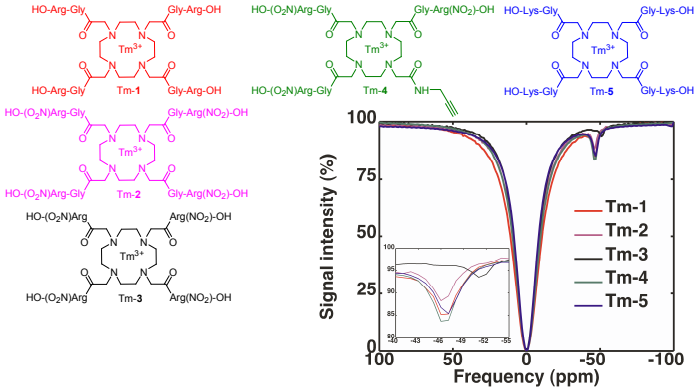

Figure 1: Structures of CAs Tm-**1-5**. CEST spectra associated with CAs Tm-**1-5**.

Conclusion: A general synthetic methodology for the preparation of Arg containing PARACEST MRI CAs has been developed. CAs Tm-**1**, **2** and Tm-**4** were found to have similar detection sensitivity to previously in vivo detected Tm-**5**. Considering the favourable pharmacokinetic properties expected with peptides containing multiple Arg residues, these CAs may be advantageous for in-vivo applications that require agents to cross cell membranes. It is also important to point out, that non-symmetrically functionalized CA Tm-**4** can be subjected to conjugation chemistry (9) using the propargyl moiety.

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