New Lanthanide Agents for BIRDS and CEST Imaging

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INTRODUCTION Recent studies show that LnDOTA-based chelates are very responsive to temperature and have great translational potential [1-2]. Temperature as well as other physiological parameters (such as pH) can be mapped with ultrafast 3D chemical shift imaging technique, called biosensor imaging redundant deviation in shifts (BIRDS) [3-5]. The BIRDS method detects intrinsic signals from the agent (i.e., nonexchangeable protons that are shifted far from water resonances by the lanthanide ion). Considering excellent sensitivity achieved for the methyl protons in the TmDOTMA$^-$, we are interested in designing and characterizing the next generation BIRDS agents by adding methyl groups in different positions of the cyclen chelate to improve BIRDS sensitivity and specificity. An important goal in BIRDS characterization of all new agents will be to achieve SNR enhancements for high spatial resolution with 3D CSI. In the present work, we report temperature sensitivities for the new agents. Since these new agents contain exchangeable amide protons and are variants of the most popular DOTA-tetraamide PARACEST agents, the new agents show capabilities in molecular imaging for BIRDS and PARACEST as well.

MATERIALS AND METHODS BIRDS agents Tm-**1-3** have been synthesized by alkylation of DOTA(N[E]$_4$ to contain methyl groups on different positions of the chelate. All ligands were treated with Tm^{3+} and the complexation process was followed by HPLC until a small excess of the ligand. The final products were characterized and confirmed by ^{1}H NMR, ESI-MS for molecular mass, and ICP-MS for metal content. BIRDS properties of Tm-**1-3** were assessed by collecting series of ^{1}H spectra with phantoms of 4 mM samples as a function of temperature (25 – 40 °C) at different pH values (6.6-8.0). The ^{1}H MR spectra were acquired on an 11.7-T Bruker vertical bore spectrometer. Longitudinal (T_1) and transverse (T_2) relaxation times of the resonances were measured using inversion-recovery and spin echo methods with selective pulses at 35 °C, respectively. CEST spectra for the same phantoms were acquired with continuous wave saturation at 50 μT for 1 sec in the frequency range from -150 to 150 ppm (250 Hz per step).

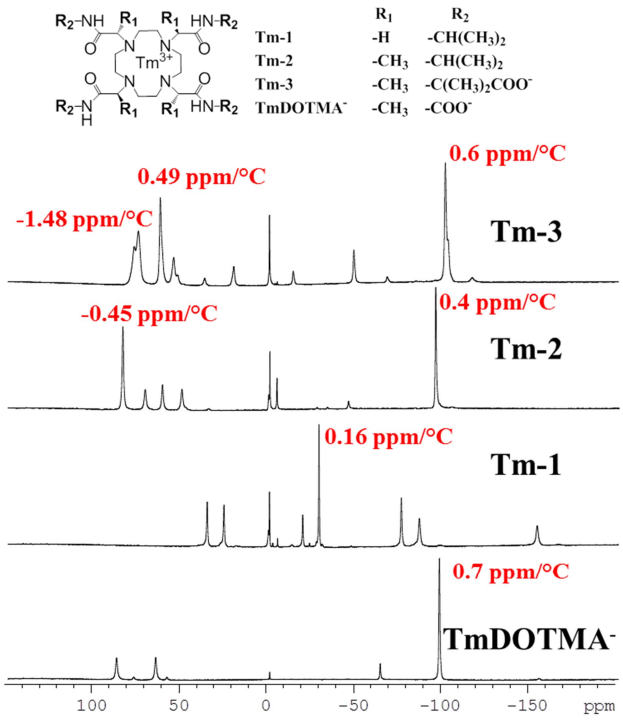

Figure 1. Chemical structures and corresponding NMR spectra (35 °C and pH 7.4) for the new BIRDS agents. Temperature sensitivities are indicated in red for the resonances of interest.

RESULTS The design and the temperature sensitivity of the new agents Tm-**1-3** are directly compared with that of the well-known TmDOTMA$^-$ [Fig. 1]. Tm-**1** is designed to have two methyl protons (~28 ppm) on the R_2 position of the structure and they have smaller sensitivity than that of TmDOTMA$^-$. By introducing an additional methyl group on the R_1 position, as illustrated in Tm-**2**, the sensitivities of the methyl protons are -0.45 and 0.4 ppm/°C. Because Tm-**1** and Tm-**2** are lipophlic, their water solubilities are low relative to Tm-**3**, whose structure is similar to Tm-**2** but has carbonyl groups on the ends. Temperature sensitivities of methyl proton resonances in Tm-**3** are 0.6, 0.49, and -1.48 ppm/°C. T_1 and T_2 values for all methyl protons in Tm-**1-3** are similar to those in TmDOTMA$^-$. In addition, no pH sensitivity is observed for all the resonances in Tm-**1-3**. Interestingly, unlike TmDOTMA$^-$ all three Tm-**1-3** agents show CEST properties [Fig. 2]. Tm-**3** has the most shifted CEST peaks (~-100 ppm) while Tm-**1** has the largest CEST effect.

DISCUSSION All three new Tm-**1-3** agents show good temperature sensitivities, similar to that of TmDOTMA$^-$. In addition, these methyl protons retain high SNR enhancements since the T_2/T_1 ratio is close to unity. The combined temperature sensitivities for two resonances (0.85 ppm/°C for Tm-**2** and 1.97 or 2.08 ppm/°C for Tm-**3**) are much higher than that in TmDOTMA$^-$. The CEST properties observed in Tm-**1-3** can be calibrated with BIRDS for quantitative PARACEST analysis.

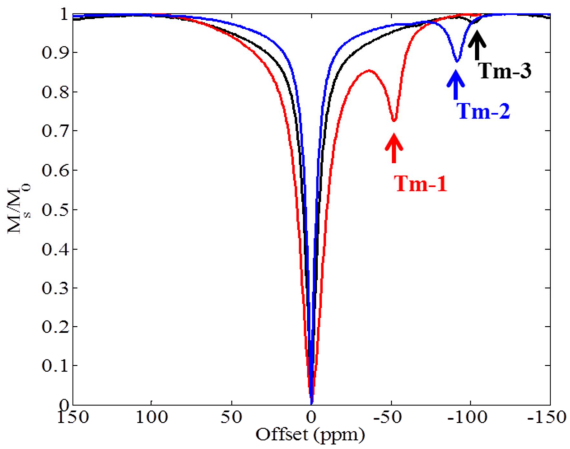

Figure 2. CEST spectra for 4 mM BIRDS agents Tm-**1** (red), Tm-**2** (blue) and Tm-**3** (black) at 35 °C and pH 7.4.

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