New PARACEST MRI Contrast Agents Based on the DOTMA Scaffold

Mojmir Suchy[1], Alex X. Li[2], Robert Bartha[2], and Robert H. E. Hudson[1]

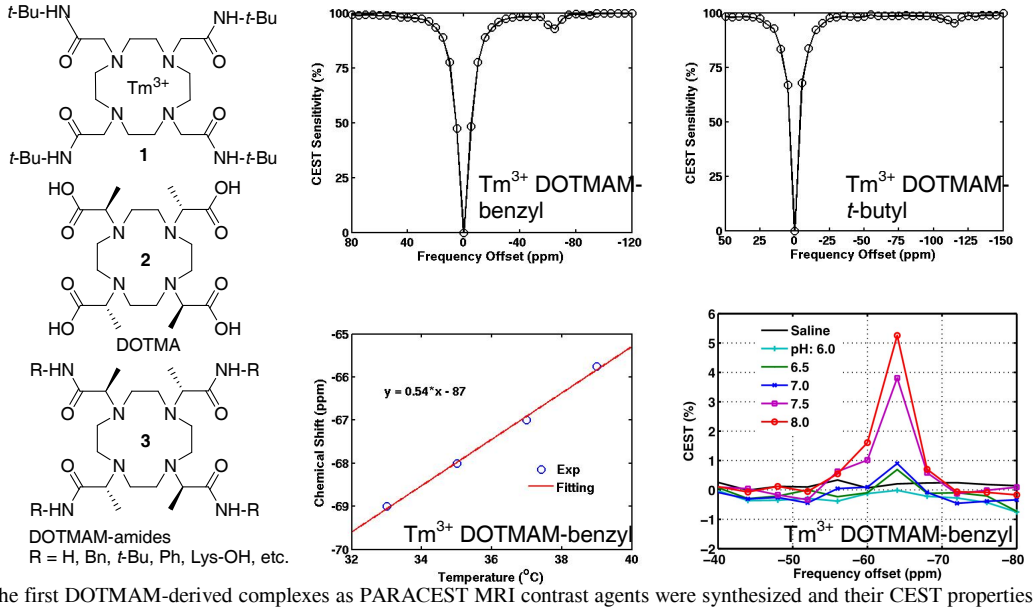
[1]*Department of Chemistry, University of Western Ontario, London, Ontario, Canada,* [2]*Centre for Functional and Metabolic Mapping, University of Western Ontario, London, Ontario, Canada*

Target audience: Researchers involved in the development of new contrast agents for MRI and other imaging modalities (e. g. PET, SPECT).

Purpose: We have recently developed a new type of PARACEST contrast agents for MRI, Tm^{3+} complexes derived from alkyl- or aryl-decorated DOTAM-based ligands.[1-3] This type of complexes is known to adopt two types of structural conformation[4] referred to as square prismatic (SAP) and twisted square antiprismatic (TSAP). Among these contrast agents, Tm^{3+} DOTAM-*t*-butyl (**1**, Figure) was found to exhibit two CEST peaks (6% at -68 ppm and 16% at -102 ppm).[1] The presence of two CEST peaks was attributed to the presence of both SAP and TSAP isomers in the solution. The stronger, highly shifted CEST peak was associated with the more prevalent TSAP isomer.[1] Combining Tm^{3+} DOTAM-*t*-butyl with bovine serum albumin revealed that the highly shifted CEST peak was located beyond the frequency range of macromolecule magnetization transfer. In addition the highly shifted CEST peak was found to possess excellent temperature sensitivity in physiological temperature range at neutral pH.[1] The purpose of this study we set to synthesize and characterize the magnetic properties of related complexes based on the DOTMA (**2**, Figure) scaffold. Ln^{3+} complexes derived from DOTMA are known to occur predominantly as TSAP isomers,[5] therefore we hypothesized, that some of the complexes from this series would exhibit highly shifted CEST peaks similar to that observed for Tm^{3+} DOTAM-*t*-butyl. While the chemical synthesis of DOTAM-type ligands as PARACEST agents is well documented in the literature,[6] the synthesis of the parent DOTMA (**2**, Figure) is less well described[5,7] and no synthetic methodology currently exists for the preparation of related amide ligands (herein abbreviated as DOTMAM) based on DOTMA.

Methods: A small series of DOTMAM ligands (structure **3**, Figure) was prepared by peralkylation of cyclen with corresponding electrophiles. The electrophiles were either secondary halo-derivatives or related secondary pseudohalo-derivatives, in both instances prepared from readily available and inexpensive (*S*)-lactic acid. The Tm^{3+} complexes derived from the above mentioned ligands were prepared by heating the ligands with $TmCl_3 \cdot H_2O$ under neutral (pH ~ 6.5-7.0) conditions. Free ligands were purified by semipreparative HPLC and were characterized by ^1H NMR and HR-ESI-MS, while the complexes (characterized by HR-ESI-MS) were purified by semipreparative HPLC with subsequent neutralization and gel filtration. PARACEST properties of the contrast agents were evaluated as follows: NMR tubes with solutions of the complexes (15 mM, pH 7.5) were imaged at 37 °C [the 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)] 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 = -120 to 80 ppm or -150 to 50 ppm in steps of 1 ppm, saturation time = 3.95 s). CEST spectra were generated using the average signal intensity from each tube. Similar methodology was used to evaluate the temperature (15 mM, pH 7.5, temperature range 33-37 ºC) and pH (15 mM, 37 ºC, pH range 6.0-8.0) sensitivity of selected agents.

Results and Discussion: Although the overall yield of the DOTMAM-derived complexes described in this study was low (ca. 5% over 4 steps), a sufficient amount of each complex was obtained to characterize the CEST properties. Synthesized complexes exhibited a reasonably strong (e. g. 7% at 65 ppm for Tm^{3+} DOTMAM-benzyl and 4% at −115 ppm for Tm^{3+} DOTMAM-*t*-butyl) CEST peak (Figure). As shown in the Figure, the CEST effect associated with Tm^{3+} DOTMAM-benzyl was found to be sensitive to both temperature and pH, it is anticipated, that the highly shifted CEST effect associated with Tm^{3+} DOTMAM-*t*-butyl will exhibit similar sensitivity to temperature and pH.



Conclusion: The first DOTMAM-derived complexes as PARACEST MRI contrast agents were synthesized and their CEST properties evaluated. Future work is aimed at improving the efficiency of the chemical synthesis of these agents. CEST agents with chemical shifts greater than 100 ppm may have significant advantages in-vivo by avoiding the sensitivity loss associated with the endogenous magnetization transfer effect.

References: (1) Stevens TK, Milne M, Elmehriki AAH, Suchý M, Bartha R, Hudson RHE. *Contrast Med. Mol. Imaging* 2013; 8: 287-290. (2) Elmehriki AAH,) Milne M, Suchý M, Bartha R, Hudson RHE. *Can. J. Chem.* 2013; 91: 211-219. (3) Milne M, Lewis M, McVicar N, Suchý M, Bartha R, Hudson RHE. *RSC Adv.* 2014; 4: 1666-1674. (4) Mani T, Tircsó G, Zhao P, Sherry AD, Woods M. *Inorg. Chem.* 2009; 48: 10338-10345. (5) Aime S, Botta M, Garda Z, Kucera BE, Tircsó G, Young VG, Woods M. *Inorg. Chem.* 2011; 50: 7955-7965. (6) Viswanathan S, Kovács Z, Green KN, Ratnakar SJ, Sherry AD. *Chem. Rev.* 2010; 110: 2960-3018. (7) Wiener EC, Abadjian MC, Sengar R, van der Elst L, van Niekerk C, Grotjahn DB, Leung PY, Schulte C, Moore CE, Rheingold AL. *Inorg. Chem.* 2014; 53: 6554-6568.