4-2012

Chemical Exchange Saturation Transfer is Unaffected by Modest Changes in Pressure

Benjamin Charles Webber
Portland State University, benjamin_webber@hotmail.com

Christiane A. Carney
Portland State University

Mark Woods
Portland State University, mark.woods@pdx.edu

Citation Details

Published as: Webber, B.C., Carney, C.E. and Woods, M. (2012), Chemical Exchange Saturation Transfer Is Unaffected by Modest Changes in Pressure. Eur. J. Inorg. Chem., 2012: 2040-2043. doi:10.1002/ejic.201101291

Published in final edited form as:
Eur J Inorg Chem. 2012 April 1; 2012(12): 2040–2043. doi:10.1002/ejic.201101291.

Chemical Exchange Saturation Transfer is Unaffected by Modest Changes in Pressure

Benjamin C. Webber[a], **Christiane E. Carney**[a], and **Mark Woods**[a,b]

Mark Woods: mark.woods@pdx.edu

[a]Department of Chemistry, Portland State University, 1719 SW 10th, Ave, Portland, OR 97201, USA

[b]Advanced Imaging Research Center, Oregon Health and Science, University, Portland OR 97239, USA

Abstract

ParaCEST (paramagnetic Chemical Exchange Saturation Transfer) agents offer an unparalleled opportunity to perform quantitative molecular imaging by MRI. Agents that can alter the image contrast they generate in response to changes in local environmental parameters such as pH, glucose concentration or lactate concentration can be used ratiometrically to quantitatively describe the local tissue environment. However, when performing such quantitative measurements it is important that the results are not confounded by changes in a second environmental parameter. In vivo pressure varies quite considerably, both through the respiratory cycle and from tissue to tissue (tumors in particular have high interstitial pressures). Since paraCEST agents have positive activation volumes, their exchange kinetics and therefore the CEST effect that they generate are necessarily related to pressure. The purpose of this investigation was to examine whether the relatively small changes in pressure exhibited in vivo could affect CEST sufficiently to confound attempts to quantify other local environmental parameters. The CEST properties of a rigid EuDOTA-tetraamide was examined at temperatures ranging from 288 to 319 K, at applied pressures ranging from 0 to 414 kPa and pre-saturation (B_1) powers ranging from 524 to 935 Hz. At no point was pressure found to affect the CEST generated by this chelate, indicating that changes in in vivo pressure is unlikely to confound the quantitative measurement of physiologically relevant parameters by paraCEST MRI.

Keywords

Europium chelates; Macrocyclic ligands; MRI contrast agents; paraCEST; Variable pressure

Introduction

Exogenous MRI contrast media that generate image contrast through a chemical exchange saturation transfer (CEST) mechanism have attracted considerable interest over the past decade or so.[1] One reason for this interest is the potential of such agents to act as reporters for a variety of important biological markers, such as pH,[2] lactate,[3] glucose,[4] metal ion concentrations[5] and even gene therapy.[6] Although conventional Gd^{3+}-based MRI contrast agents have been proposed that report many of these same parameters, CEST agents offer the advantage that ratiometric methods can be used to quantify the parameter of interest.[1a, 7] Ratiometric detection by MRI cannot be achieved using responsive Gd^{3+}-

Correspondence to: Mark Woods, mark.woods@pdx.eduwoodsmar@ohsu.edu.

based agents and more elaborate methods that establish in vivo agent distributions are required for these agents.[8] Of course if a parameter is to be quantified using a CEST agent it is imperative that its CEST measurements are not confounded by the presence of or changes in a second parameter. For instance, both temperature and pH are understood to intrinsically affect the rate of proton exchange and thus CEST.[2b] As such care must be taken that any deviation from standard physiological conditions in other parameters (pH, temperature, etc) do not give rise to erroneous quantification of the parameter of interest, such as metal ion concentration. For this reason it is important to know how exogenous CEST agents are affected by all parameters that could influence their CEST response.

Paramagnetic CEST agents or paraCEST agents have provoked considerable interest as exogenous CEST agents. These agents, commonly derived from Ln^{3+} DOTA tetraamides (Chart 1) or structurally related systems, have highly shifted protons that eliminate problems of direct off-resonance water saturation and permit more rapid exchange rates to be employed.[1a, 1b] The exchange of water molecules coordinated to the Ln^{3+} ion in these chelates occurs through a dissociative (D) mechanism in which the coordinated water molecule must first dissociate from the chelate before being replaced by a second from the solvent water. In consequence there is a positive activation volume in the range of 5 – 7 cm^3mol^{-1}, associated with the exchange process.[9] Because the volume of the system changes during exchange the rate of the exchange process will vary depending upon the pressure it is under; in this case accelerating as the pressure is decreased, decelerating when the pressure is increased. Since CEST is acutely sensitive to even small changes in exchange rate it is important to know whether a small change in pressure could generate a change in CEST. It is well documented that pressure in vivo can vary substantially beyond the obvious changes in blood pressure during respiration. Conditions such as stroke and cancer for instance are known to produce significant increases in interstitial pressure. The interstitial pressure of a tumor varies considerably depending upon the type of tumor and the stage of development.[10] In vivo pressure is measured relative to the back pressure exerted by the surrounding tissue and membranes. In these terms the interstitial pressure of a tumour may vary from marginally above the back pressure to as much as 5.2 kPa above. Indeed interstitial pressures as high as 2.6 kPa are commonly observed in tumors.[10] If CEST is sensitive to these small changes in pressure then this must be taken into account when quantifying a parameter such as pH in a tumor or a stroke when using exogenous CEST agents. We have accordingly investigated the effect on CEST of modest increases in pressure that extend well beyond those expected in vivo.

Results and Discussion

In attempting to understand the effect of pressure upon paraCEST it is important to consider whether an increase in pressure could affect the structure of the agent. Any changes upon the structure or internal dynamics of a chelate affected by changes pressure would in turn confound our own measurements. We have recently reported that the Eu^{3+} DOTA-tetraamide chelate Eu**1** has an extremely rigid structure and does not undergo the intramolecular exchange processes common to most DOTA-tetraamide chelates.[11] As such this is an ideal chelate for this study as we can eliminate the possibility that this agent will alter its structure in response to an increase in pressure and any changes can be attributed solely to the effect of changing water exchange. Furthermore Eu**1** adopts a structure that is representative of that adopted by most flexible LnDOTA-tetraamide chelates.[11]

Different paraCEST agents exhibit different water exchange kinetics under the same conditions depending upon the structure and electronics of the ligand system.[12] It is conceivable that a change in pressure could affect agents with different exchange characteristics differently. Thus our selected agent may not be representative of the range of

exchange parameters observed within the paraCEST family. We envisioned that this problem could be overcome by performing measurements at different temperatures – affording a range of different exchange kinetics – rather than surveying a range of paraCEST agents; there are currently insufficient conformationally constrained chelates available for such a survey.

CEST spectra of a solution of Eu**1** in 1:1 CD_3CN/H_2O (pH 3) were acquired under atmospheric pressure at 288, 298, 308 and 318 K. The shapes of the resulting CEST spectra (Figure 1) were consistent with the normally observed effect of temperature,[1a] exhibiting a sharp CEST peak for the spectra at lower temperatures which gradually broadens, moves closer to bulk water and reduces in intensity as the temperature increases. Fitting these spectra to the Bloch equations modified for exchange[13] afforded water exchange kinetics for Eu**1** that are much faster than those previously reported.[11] The water proton residence lifetime ($\tau_M{}^H$) at 298 K had been measured at 116 μs in 10% H_2O in CD_3CN. Not surprisingly,[14] when the proportion of water in the solvent was increased to 50% (this work) the exchange rate accelerated considerably affording only a narrow range of $\tau_M{}^H$ values (Table 1). pH can also be used to modulate proton exchange rates, in this case even though the sample pH is comparatively low it lies within a pH range expected to afford the slowest exchange kinetics and these would not be expected to change on raising the value as high as pH 8.[15] Such relatively rapid exchange occurring even at comparatively low temperatures frustrated our plan to span a wide range of exchange kinetics by varying temperature. The final range of atmospheric pressure water proton residence lifetimes achieved was a relatively small increase from 60 μs at 288 K to 20 μs at 318 K (Table 1). The pressure of each sample in the NMR tube was then increased from atmospheric pressure to 139, 276 and eventually 414 kPa. CEST spectra were then acquired at each temperature and pressure. In each case no change in the magnitude or shape of the CEST peak corresponding to bound water was observed (Figure 2).

In Figures 1 & 2 the data shown was acquired using a pre-saturation (B_1) power of 935 Hz. However, it has been shown that the magnitude of the CEST effect obtained with a given B_1 power varies substantially as the water exchange kinetics vary.[1a] To eliminate the possibility that a change in B_1 power increases the susceptibility of CEST to changes in pressure the magnitude of CEST from the coordinated water was measured as a function of B_1 power from 524 Hz to 935 Hz. CEST itself is inherently sensitive to changes in the B_1 power[13] and so there is a considerable increase in CEST as the B_1 power increases. But no difference in is observed in the amount of CEST generated for a given B_1 power as the applied pressure is increased from 0 to 414 kPa (Figure 3).

Conclusions

A good guide to the minimum change in contrast-to-noise (CNR) required for detection comes from fMRI experiments and is generally thought to be about a 3% change in water signal intensity. The physiologically relevant range of pressures probably only extends as far as a high systolic pressure plus the maximum likely interstitial pressure of a tumor; probably about 32 kPa. Within this pressure range any variation in the CEST generated from Eu**1** under the conditions examined herein lies within experimental error and is certainly much smaller than the 3% change needed to be observed during an MR acquisition. It is important to note however, that Eu**1** represents a fairly narrow range of possible exchange kinetics and that these observations may not hold true for systems with radically different water exchange kinetics. Nonetheless, we can conclude that pressure is unlikely to be a confounding parameter for the quantification of biologically relevant parameters by paraCEST MRI.

Experimental Section

The preparation of Eu**1** has been described previously.[11] All CEST spectra were acquired on a Bruker Avance IIa operating at 400.13 MHz and equipped with a 5mm broadband probe and temperature control. The chelate Eu**1** was dissolved in a 1:1: mixture of CD_3CN and H_2O at a concentration of 17.1 mM. The sample was placed in a heavy walled 5 mm NMR tube quipped with a Young valve (Wilmad). CEST spectra were acquired using a pre-saturation power of 935 Hz and a pre-saturation time of 10 s at 288, 298, 308 and 318 K. Pressure was then applied to the sample using an over pressure of helium and the tube sealed. CEST data were then acquired at the same four temperatures. In each case the temperature of the sample was adjusted prior to pressurization to eliminate errors in sample pressure arising from changes in sample temperature. The effect of changing pre-saturation power was determined by applying pre-saturation pulses (of 10 s duration) at a fixed offset to the sample and varying B_1 from 935 to 524 Hz.

Acknowledgments

The authors thank the National institutes of Health (EB-11687), Oregon Nanoscience and Microtechnology Institute (N00014-11-1-0193) and the Oregon Opportunity for Biomedical Research for financial support.

References

1. a) Woods M, Woessner DE, Sherry AD. Chem. Soc. Rev. 2006; 35:500–511. [PubMed: 16729144] b) Sherry AD, Woods M. Ann. Rev. Biomed. Eng. 2008; 10:391–411. [PubMed: 18647117] c) Terreno E, Delli Castelli D, Aime S. Contrast Media & Mol. Imaging. 2010; 5:78–98.d) Viswanathan S, Kovacs Z, Green KN, Ratnakar SJ, Sherry AD. Chem. Rev. 2010; 110:2960–3018. [PubMed: 20397688]
2. a) Ward KM, Balaban RS. Magn. Reson. Med. 2000; 44:799–802. [PubMed: 11064415] b) Terreno E, Castelli Daniela D, Cravotto G, Milone L, Aime S. Invest. Radiol. 2004; 39:235–243. [PubMed: 15021328]
3. Aime S, Delli Castelli D, Fedeli F, Terreno E. J. Am. Chem. Soc. 2002; 124:9364–9365. [PubMed: 12167018]
4. a) Trokowski R, Zhang S, Sherry AD. Bioconjug. Chem. 2004; 15:1431–1440. [PubMed: 15546212] b) Zhang S, Trokowski R, Sherry AD. J. Am. Chem. Soc. 2003; 125:15288–15289. [PubMed: 14664562]
5. Trokowski R, Ren JM, Kalman FK, Sherry AD. Angew. Chem. Int. Ed. 2005; 44:6920–6923.
6. a) Snoussi K, Bulte JWM, Gueron M, van Zijl PCM. Magn. Reson. Med. 2003; 49:998–1005. [PubMed: 12768576] b) Wu Y, Carney CE, Denton M, Hart E, Zhao P, Streblow DN, Sherry AD, Woods M. Org. Biomol. Chem. 2010; 8:5333–5338. [PubMed: 20848030]
7. a) Lamerichs RMJN, Wegh RT, Pikkemaat JA, Gruell H. 2007 WO 2007141767 A2 20071213. b) Ali MM, Liu G, Shah T, Flask CA, Pagel MD. Acc. Chem. Res. 2009; 42:915–924. [PubMed: 19514717]
8. a) Garcia-Martin ML, Martinez GV, Raghunand N, Sherry AD, Zhang S, Gillies RJ. Magn. Reson. Med. 2006; 55:309–315. [PubMed: 16402385] b) Martinez GV, Zhang X, García-Martín ML, Morse DL, Woods M, Sherry AD, Gillies RJ. NMR Biomed. 2011 Early View. c) Raghunand N, Howison C, Sherry AD, Zhang S, Gillies Robert J. Magn. Reson. Med. 2003; 49:249–257. [PubMed: 12541244]
9. Dunand FA, Dickins RS, Parker D, Merbach AE. Chem. Eur. J. 2001; 7:5160–5167. [PubMed: 11775689]
10. Fukumura D, Jain RK. J. Cell. Biochem. 2007; 101:937–949. [PubMed: 17171643]
11. Carney CE, Tran AD, Wang J, Schabel MC, Sherry AD, Woods M. Chem. Eur. J. 2011; 17:10372–10378. [PubMed: 21837722]

12. a) Ratnakar SJ, Woods M, Lubag AJM, Kovacs Z, Sherry AD. J. Am. Chem. Soc. 2008; 130:6–7. [PubMed: 18067296] b) Aime S, Barge A, Batsanov AS, Botta M, Castelli DD, Fedeli F, Mortillaro A, Parker D, Puschmann H. Chem. Commun. 2002:1120–1121.
13. Woessner DE, Zhang S, Merritt ME, Sherry AD. Magn. Reson. Med. 2005; 53:790–799. [PubMed: 15799055]
14. Woods M, Woessner DE, Zhao P, Pasha A, Yang M-Y, Huang C-H, Vasalitiy O, Morrow JR, Sherry AD. J. Am. Chem. Soc. 2006; 128:10155–10162. [PubMed: 16881645]
15. Woods M, Pasha A, Zhao P, Tircso G, Chowdhury S, Kiefer G, Woessner DE, Sherry AD. Dalton Trans. 2011; 40:6759–6764. [PubMed: 21625687]

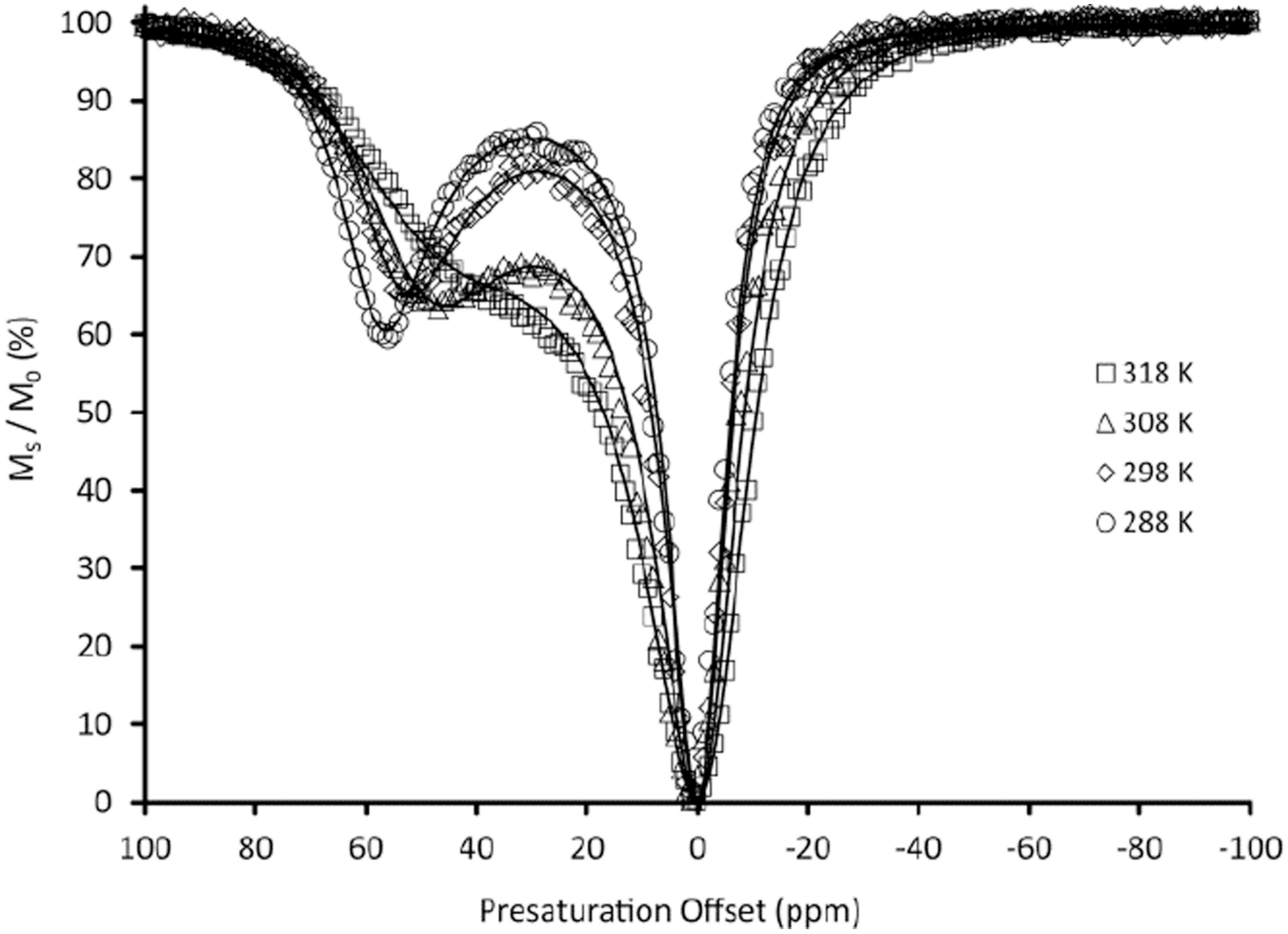

Figure 1.
CEST spectra of Eu**1** in 1:1 CD_3CN/H_2O acquired at different temperatures.

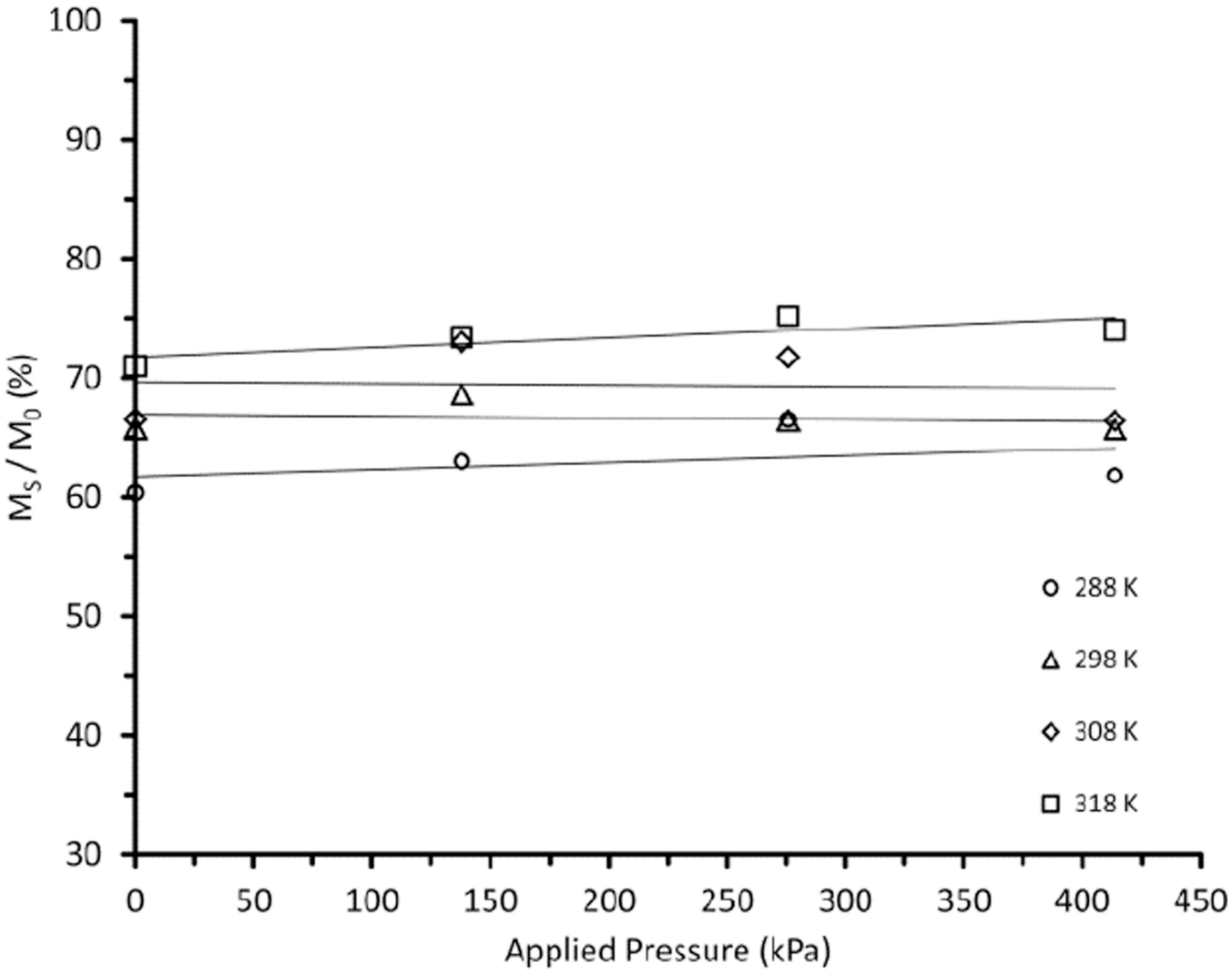

Figure 2.
The maximal CEST effect of Eu**1** at 288 K (58 ppm), 298 K (55 ppm), 308 K (52 ppm) and 318 K (49 ppm) in 1:1 CD_3CN/H_2O as a function of pressure. Solid lines are linear fits to the data.

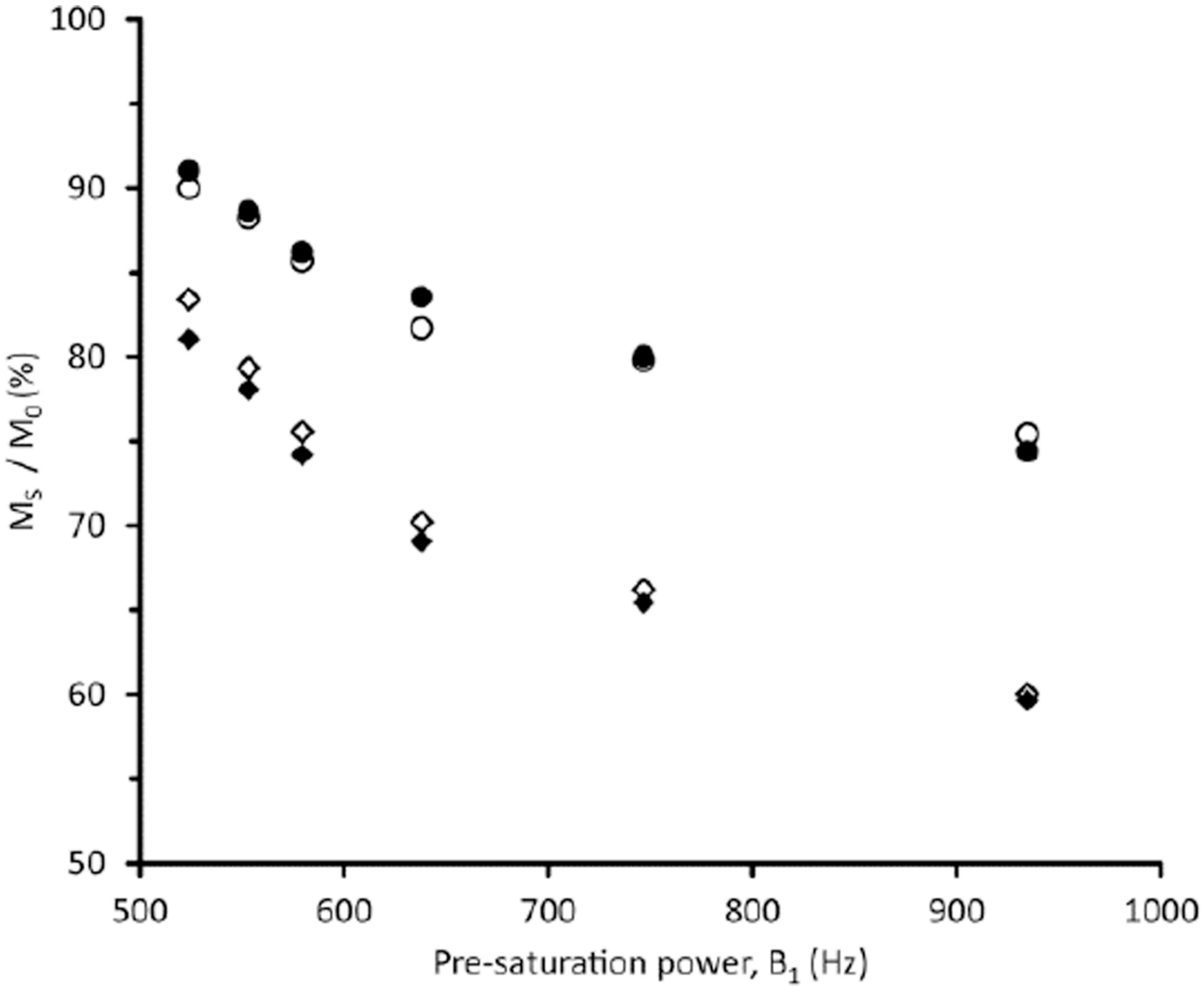

Figure 3.

The magnitude of the CEST effect of Eu**1** in 1:1 CD_3CN/H_2O at 288 K (diamonds) and 318 K (circles) with 0 kPa (open symbols) and 414 kPa (closed symbols) as a function of the pre-saturation power, B_1.

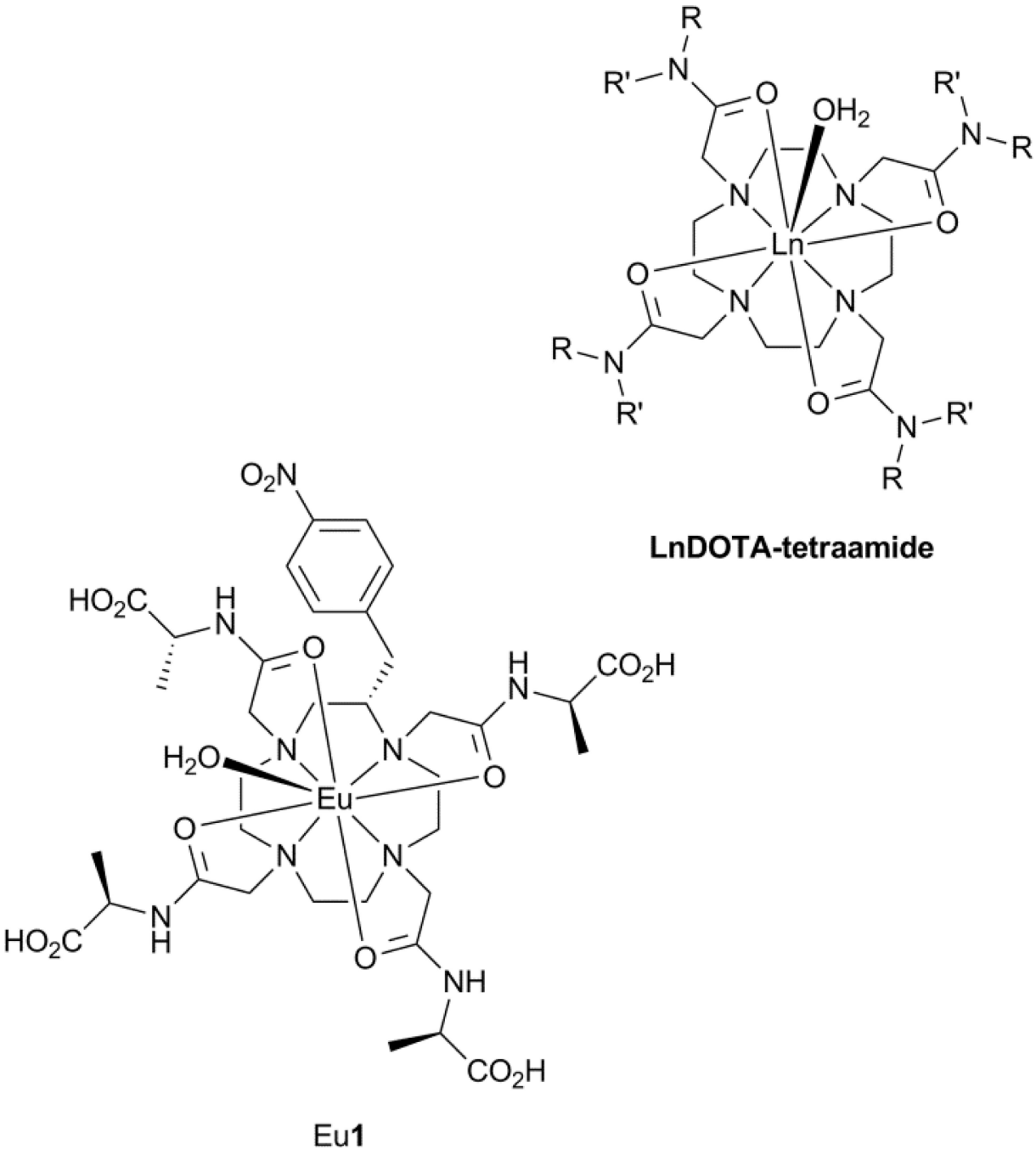

Chart 1.
The structures of LnDOTA-tetraamides and Eu**1**

Table 1

The atmospheric pressure water proton residence lifetimes (τ_M^H) of Eu**1** in 1:1 CD_3CN/H_2O as a function of temperature.

T (K)	τ_M^H (μs)
288	60
298	46
308	31
318	20