Published in final edited form as:

Chem Commun (Camb). 2011 December 28; 47(48): 12858–12860. doi:10.1039/c1cc15219j.

Eu^{II}-containing cryptates as contrast agents for ultra-high field strength magnetic resonance imaging†

Joel Garcia^a, Jaladhar Neelavalli^b, E. Mark Haacke^b, and Matthew J. Allen^{a,*}

^aDepartment of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, MI 48202, USA.

^bDepartment of Radiology, Wayne State University, Detroit, MI 48201, USA

Abstract

The relaxivity (contrast-enhancing ability) of Eu^{II}-containing cryptates was found to be better than a clinically approved Gd^{III}-based agent at 7 T. These cryptates are among a few examples of paramagnetic substances that show an increase in longitudinal relaxivity, r_1 , at ultra-high field strength relative to lower field strengths.

Contrast-agent-enhanced magnetic resonance imaging (MRI) is a widely used, noninvasive imaging technique for clinical and preclinical research as well as diagnostic medicine. Most clinical imaging is performed at high field strengths (1.5 or 3 T); however, a shift to ultra-high field strength MRI (> 7 T) is desirable because of the increased spatial resolution and shorter acquisition times enabled at these fields, which offer the potential to lead to early diagnoses of diseases and more accurate imaging *in vivo*.^{1–4} Consequently, preclinical research relies heavily on ultra-high field strengths. A major limitation is that common T_1 -reducing (positive) contrast agents at lower field strengths, such as Gd^{III}-containing complexes, become inefficient at ultra-high field strengths.^{2,5} Multiple parameters—including the water-exchange rate, the number of inner-sphere water molecules, the rotational correlation time, and the relaxation times of the electron spins of Gd^{III}—are in a complex interplay that is crucial to increasing relaxivity, r_1 , which is a measure of contrast-enhancing ability of a contrast agent.^{2–4} An alternative to Gd^{III} is Eu^{II}, which is isoelectronic with Gd^{III} but has a faster water-exchange rate due to its lower charge density. However, until recently, research on Eu^{II} chemistry in aqueous solution was limited because of its tendency to oxidize to Eu^{III}.^{6–8} We have used modified [2.2.2]cryptands to increase the oxidative stability of Eu^{II},⁶ and here, we report the molecular properties and imaging profiles of a series of Eu^{II}-containing [2.2.2]cryptates (**1–3** in Fig. 1) that are, to the best of our knowledge, among a few reported contrast agents, and the first lanthanide-based contrast agents, that are more effective at ultra-high field strengths than at lower fields.⁹

We hypothesized that Eu^{II}-containing [2.2.2]cryptates in aqueous solution could be used as the basis for efficient positive contrast agents for ultra-high field strength MRI because Eu^{II} and Gd^{III} are isoelectronic but differ in other molecular parameters that influence relaxivity. This hypothesis is supported by studies of Eu^{II}-containing complexes: for example, Eu^{II}-containing [2.2.2]cryptate, **1**, has been reported to have a fast water-exchange rate.⁸ The water-exchange rate of **1** is $3 \times 10^8 \text{ s}^{-1}$, which is faster than most Gd^{III}-based complexes and is very close to the optimal value ($\sim 10^8 \text{ s}^{-1}$ at 7 T) for developing efficient MRI contrast agents at ultra-high fields.^{2,8} Attempts to increase the water-exchange rate of Gd^{III}-

†Electronic supplementary information (ESI) available: Experimental procedures and NMR spectra. See DOI: 10.1039/c1cc15219j

containing complexes have been made by altering the coordination number of ligands for Gd^{III} or by increasing steric bulk at the site where inner-sphere water binds.¹⁰ However, because of the complicated interplay between water-exchange rate and other parameters that affect relaxivity, there is a need to simultaneously optimize all factors to increase the efficacy of contrast agents. In addition to its optimal water-exchange rate, **1** has two inner-sphere water molecules in a ten-coordinate complex, which should enable higher relaxivity than an equivalent complex with only one inner-sphere water molecule according to Solomon–Bloembergen–Morgan (SBM) theory. These favorable molecular properties prompted us to hypothesize that Eu^{II}-containing cryptates would be candidates for contrast agents at ultra-high field MRI.

To test our hypothesis, we investigated the imaging (longitudinal relaxivity, r_1 , at 3 and 7 T) and physical (water-exchange rate, electron-spin relaxation rate, and number of inner-sphere water molecules) properties of a series of Eu^{II}-containing [2.2.2]cryptates (Fig. 1). Relaxivity was determined using multiple flip angles in gradient echo imaging experiments performed between 19 and 20 °C at 3 and 7 T (Table 1) and inversion recovery experiments at either 20 or 37 °C at 1.4 and 11.7 T.¹¹ At all of these field strengths, Eu^{II}-containing cryptates **2** and **3** displayed higher r_1 than clinically approved GdDOTA (up to 46, 71, 92, and 120% higher at 1.4, 3, 7, and 11.7 T, respectively). Furthermore, cryptate **1** displayed a higher relaxivity than GdDOTA at 7 and 11.7 T. We found that the r_1 values of GdDOTA were not different at 3 and 7 T (Student *t* test) but decreased by 29% from 1.4 to 11.7 T. This result was expected based on what is known about fast rotating Gd^{III}-containing complexes.³ However, Eu^{II}-containing complexes **1–3** showed an increase in r_1 values at 7 relative to 3 T.¹² Also, cryptates **1** and **3** displayed an increase in r_1 values at 11.7 relative to 1.4 T. The r_1 value of cryptate **2** was slightly lower at 11.7 T compared to 1.4 T. Additionally, the r_1 values of cryptates **1–3** increased as a function of molecular weight at all field strengths.

To understand our observations of r_1 values, we used variable temperature ¹⁷O NMR spectroscopy to determine the molecular parameters expected to influence relaxivity (Table 2): the residence lifetime of bound water molecules, τ_m ²⁹⁸, where water-exchange rate, k_{ex} ²⁹⁸, is equal to $1/\tau_m$ ²⁹⁸; the longitudinal electronic relaxation time, T_{1e} ²⁹⁸; and the number of inner-sphere water molecules, q . To attain the highest relaxivity at ultra-high field strengths, water-exchange rate must be $\sim 10^8$ s⁻¹.² The water-exchange rates of the Eu^{II} complexes are 6–94 times faster than GdDOTA and, consequently, are closer to being optimal at ultra-high field strengths as described by SBM theory. However, our observed water-exchange rate does not limit the relaxation enhancement for fast rotating complexes even at ultra-high fields. As seen from the data in Tables 1 and 2, r_1 values were larger for cryptates **2** and **3** relative to **1** despite **2** and **3** having slower water-exchange rates than **1**. The relaxivity differences among cryptates **1–3** are likely due to differences in molecular weight because increases in molecular weight correspond to decreases in rotational correlation rates for structurally similar monomeric complexes.¹³

SBM theory also describes the dependence of relaxivity on the electron spin relaxation, which we investigated with ¹⁷O NMR studies. Data acquired from variable temperature ¹⁷O NMR experiments were refitted using 63 unique values of T_{1e} ²⁹⁸ in the range of 10^{-8} – 10^{-3} s, and the T_{1e} ²⁹⁸ values that were associated with the best correlation coefficient are reported in Table 2. The electron-spin relaxation rates of the Eu^{II} ion in cryptates **1–3** were in the range of 10^6 – 10^7 s⁻¹ and were not limiting as evidenced by the change in relaxivity with molecular weight. Another key parameter in increasing relaxivity at ultra-high fields is the number of inner-sphere water molecules. Eu^{II} cryptates have two inner-sphere water molecules, which corresponds to higher efficiency of a contrast agent relative to complexes with fewer inner-sphere water molecules. In general, the superiority of the Eu^{II} cryptates

over GdDOTA in enhancing relaxivity at ultra-high field strengths is likely due to a combination of factors. We have determined k_{ex}^{298} , q , and T_1e^{298} for cryptates **1–3** in an attempt to explain the molecular basis for our observations; however, our results cannot be satisfactorily interpreted using SBM theory.

To demonstrate the consequence of having higher relaxivity, we obtained phantom images (images of solutions) of GdDOTA and Eu^{II}-containing complexes **1–3** using T_1 -weighted imaging at 7 T (Fig. 2). The MR images of Eu^{II} cryptates **1–3** showed higher signal intensity relative to PBS. This observation is indicative that Eu^{II} cryptates are effective at influencing the relaxation of water protons in solution. The signal intensities of the Eu^{II} complexes are different from each other (Student t test) with **3** giving rise to the highest signal intensity. The $1/T_1$ values were between 29 and 89% higher for cryptates **1–3** relative to GdDOTA. An increase in $1/T_1$ corresponds to higher signal intensity and, therefore, is advantageous for imaging. These imaging experiments demonstrate that cryptates **1–3** are effective contrast agents at ultra-high field strengths.

We found that Eu^{II}-based complexes **1–3** are effective contrast agents at ultra-high field strengths likely because of the interplay of water-exchange rate, rotational correlation rate, and the presence of two inner-sphere water molecules. To the best of our knowledge, these complexes are among a few reported examples of contrast agents that display increased r_1 values at ultra-high field strengths relative to lower fields. Further studies on the physical properties of Eu^{II}-containing cryptates are being performed in our laboratory to better understand these results. We expect that our findings will be a step toward addressing the lack of positive contrast agents for ultra-high field strength MRI, and we are currently studying Eu^{II} cryptates in more detail to obtain a more complete understanding of the structure–function relationships as well as the thermodynamic and kinetic stabilities of these complexes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by startup funds from Wayne State University (WSU) and a Pathway to Independence Career Transition Award (R00EB007129) from the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health. J. G. was supported by a Thomas C. Rumble Graduate Fellowship from WSU, and M. J. A. gratefully acknowledges a Schaap Faculty Scholar Award. We thank Prabani Dissanayake for acquiring inductively coupled plasma mass spectrometry data, Jeremiah Moore for helpful discussions, and Latif Zahid and Yimin Shen for performing imaging experiments.

Notes and references

1. (a) Moser E. World J. Radiol. 2010; 2:37. [PubMed: 21160738] (b) Pitt D, Boster A, Pei W, Wohleb E, Jasne A, Zachariah CR, Rammohan K, Knopp MV, Schmalbrock P. Arch. Neurol. 2010; 67:812. [PubMed: 20625086] (c) Blow N. Nature. 2009; 458:925. [PubMed: 19370034]
2. Helm L. Future Med. Chem. 2010; 2:385. [PubMed: 21426173]
3. Caravan P. Chem. Soc. Rev. 2006; 35:512. [PubMed: 16729145]
4. Costa J, Ruloff R, Burai L, Helm L, Merbach AE. J. Am. Chem. Soc. 2005; 127:5147. [PubMed: 15810849]
5. (a) Livramento JB, Weidensteiner C, Prata MIM, Allegrini PR, Geraldies CFGC, Helm L, Kneuer R, Merbach AE, Santos AC, Schmidt P, Tóth É. Contrast MediaMol. Imaging. 2006; 1:30.(b) Livramento JB, Helm L, Sour A, O'Neil C, Merbach AE, Tóth É. Dalton Trans. 2008:1195. [PubMed: 18283380] (c) Moriggi L, Cannizzo C, Prestinari C, Berrière F, Helm L. Inorg. Chem.

- 2008; 47:8357. [PubMed: 18714983] (d) Mamedov I, Táborský P, Lubal P, Laurent S, Elst LV, Mayer HA, Logothetis NK, Angelovski G. Eur. J. Inorg. Chem. 2009:3298.
6. Gamage N-DH, Mei Y, Garcia J, Allen MJ. Angew. Chem., Int. Ed. 2010; 49:8923.
7. (a) Tóth É, Burai L, Merbach AE. Coord. Chem. Rev. 2001:216–217. (b) Caravan P, Tóth É, Rockenbauer A, Merbach AE. J. Am. Chem. Soc. 1999; 121:10403. (c) Caravan P, Merbach AE. Chem. Commun. 1997:2147.
8. Burai L, Scopelliti R, Tóth É. Chem. Commun. 2002:2366.
9. (a) Kowalewski J, Egorov A, Laaksonen A, Nikkhou Aski S, Parigi G, Westlund P-O. J. Magn. Reson. 2008; 195:103. [PubMed: 18809345] (b) Kruk D, Kowalewski J. J. Chem. Phys. 2002; 116:4079. (c) Nilsson T, Parigi G, Kowalewski J. J. Phys. Chem. A. 2002; 18:4476. (d) Svoboda J, Nilsson T, Kowalewski J, Westlund P-O, Larsson PT. J. Magn. Reson., Ser. A. 1996; 121:108.
10. (a) Werner EJ, Avedano S, Botta M, Hay BP, Moore EG, Aime S, Raymond KN. J. Am. Chem. Soc. 2007; 129:1870. [PubMed: 17260995] (b) Torres S, Martins JA, André JP, Pereira GA, Kiraly R, Brücher E, Helm L, Tóth É, Geraldès CFGC. Eur. J. Inorg. Chem. 2007:5489. (c) Pierre VC, Botta M, Raymond KN. J. Am. Chem. Soc. 2005; 127:504. [PubMed: 15643857] (d) Laus S, Ruloff R, Tóth É, Merbach AE. Chem.–Eur. J. 2003; 9:3555. [PubMed: 12898682] (e) Doble DMJ, Botta M, Wang J, Aime S, Barge A, Raymond KN. J. Am. Chem. Soc. 2001; 123:10758. [PubMed: 11674017] (f) Cohen SM, Xu J, Radkov E, Raymond KN. Inorg. Chem. 2000; 39:5747. [PubMed: 11151375] (g) Aime S, Barge A, Borel A, Botta M, Chemerisov S, Merbach AE, Müller U, Pubanz D. Inorg. Chem. 1997; 36:5104.
11. Haacke, EM.; Brown, RW.; Thompson, MR.; Venkatesan, R. Magnetic Resonance Imaging Physical Principles and Sequence Design. New York: John Wiley & Sons, Inc.; 1999. p. 654
12. (a) The temperatures at 3 and 7 T differed by 0.8 °C. To examine the influence of this temperature variation on relaxivity, we measured cryptate **3** at 11.7 T at temperatures of 19 and 19.8 °C. The relaxivity values were 6.82 ± 0.01 and 6.72 ± 0.01 , respectively; Averill DJ, Garcia J, Siriwardena-Mahanama BN, Vithanarachchi SM, Allen MJ. J. Vis. Exp. 2011; 53:e2844.
13. (a) Floyd WC III, Klemm PJ, Smiles DE, Kohlgruber AC, Pierre VC, Mynar JL, Fréchet MJM, Raymond KN. J. Am. Chem. Soc. 2011; 133:2390. [PubMed: 21294571] (b) Kundu A, Peterlik H, Krssak M, Bytzek AK, Pashkunova-Martic I, Arion VB, Helbich TH, Keppler BK. J. Inorg. Biochem. 2011; 105:250. [PubMed: 21194625] (c) Dumas S, Jacques V, Sun W-S, Troughton JS, Welch JT, Chasse JM, Schmitt-Willich H, Caravan P. Invest. Radiol. 2010; 45:600. [PubMed: 20808235] (d) Pierre VC, Botta M, Aime S, Raymond KN. J. Am. Chem. Soc. 2006; 128:9272. [PubMed: 16848429] (e) Chan KW-Y, Wong W-T. Coord. Chem. Rev. 2007; 251:2428. (f) Caravan P, Cloutier NJ, Greenfield MT, McDermid SA, Dunham SU, Bulte JWM, Amedio JC Jr. Looby RJ, Supkowski RM, Horrocks WD Jr. McMurry TJ, Lauffer RB. J. Am. Chem. Soc. 2002; 124:3152. [PubMed: 11902904] (g) Reichert DE, Hancock RD, Welch MJ. Inorg. Chem. 1996; 35:7013. [PubMed: 11666881] (h) Armitage FE, Richardson DE, Li KCP. Bioconjugate Chem. 1990; 1:365.

Fig. 1. Structures of GdDOTA (DOTA = 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetate) and Eu^{II}-containing cryptates **1–3**. Coordinated water molecules have been omitted for clarity.

Fig. 2.

(a) T_1 -weighted MR images of solutions of GdDOTA (1.0 mM in PBS) and **1–3** (1.0 mM in PBS) at 7 T and 19 °C. The diameter of the tubes that were used for imaging was 6 mm.

Imaging parameters were $T_R = 21$ ms; $T_E = 3.26$ ms; and resolution = $0.27 \times 0.27 \times 2$ mm³.

(b) Comparison of $1/T_1$ values of the samples from (a) at 7 T and 19 °C. Error bars represent standard error of the mean.

Table 1

 r_1 values of GdDOTA and Eu^{III}-containing cryptates

	r_1 at 1.4 T (mM ⁻¹ s ⁻¹) $T = 37^\circ\text{C}$	r_1 at 11.7 T (mM ⁻¹ s ⁻¹) $T = 37^\circ\text{C}$	r_1 at 3 T (mM ⁻¹ s ⁻¹) $T = 19.8^\circ\text{C}$	r_1 at 7 T (mM ⁻¹ s ⁻¹) $T = 19^\circ\text{C}$	r_1 at 11.7 T (mM ⁻¹ s ⁻¹) $T = 20^\circ\text{C}$
GdDOTA	3.00 ± 0.06	2.12 ± 0.02	3.69 ± 0.06	3.73 ± 0.01	2.89 ± 0.004
1	2.09 ± 0.02	2.65 ± 0.04	3.94 ± 0.12	5.01 ± 0.02	3.99 ± 0.14
2	3.67 ± 0.09	3.34 ± 0.02	4.84 ± 0.08	6.47 ± 0.14	4.81 ± 0.06
3	4.39 ± 0.10	4.80 ± 0.06	6.31 ± 0.07	7.17 ± 0.03	6.35 ± 0.01

All complexes were in phosphate buffered saline (PBS, pH = 7.4). Results are reported as mean ± standard error.

Table 2Relaxivity parameters (based on ^{17}O NMR data) and molecular weights of GdDOTA and cryptates **1–3**

	GdDOTA	1	2	3
τ_m^{298} (ns)	287 ± 10	3.0 ± 0.4	11.7 ± 0.3	48 ± 4
k_{ex}^{298} (10^8 s^{-1})	0.035	3.3	0.85	0.21
q	1	2	2	2
T_{1e}^{298} (ns)	29	120	41	1100
ΔH (kJ mol $^{-1}$)	70 ± 2	60 ± 8	34 ± 1	49 ± 5
MW (Da)	591	564	657	731