

## Magnetic resonance thermometry using a Gd<sup>III</sup>-based contrast agent

S. A. Amali S. Subasinghe,<sup>a</sup> Jonathan Romero,<sup>b</sup> Cassandra L. Ward,<sup>c</sup> Matthew D. Bailey,<sup>a</sup> Donna R. Zehner,<sup>b</sup> Prakrut J. Mehta,<sup>b</sup> Fabio Carniato,<sup>d</sup> Mauro Botta,<sup>d</sup> Jason T. Yustein,<sup>c</sup> Robia G. Pautler,<sup>b</sup> and Matthew J. Allen<sup>\*a</sup>

<sup>a</sup>Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, Michigan 48202, United States

<sup>b</sup>Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas 77030, United States

<sup>c</sup>Lumigen Instrument Center, Wayne State University, 5101 Cass Avenue, Detroit, Michigan 48202, United States

<sup>d</sup>Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale “Amedeo Avogadro”, Viale T. Michel 11, 15121 Alessandria, Italy

<sup>e</sup>Integrative Molecular and Biomedical Sciences and the Department of Pediatrics in the Texas Children’s Cancer and Hematology Centers and The Faris D. Virani Ewing Sarcoma Center, Baylor College of Medicine, Houston, Texas 77030, United States

\*Email: mallen@chem.wayne.edu

Page	Contents
S1	Table of contents
S2	Experimental procedures
S4	Synthesis and characterization
S5	Crystallographic data
S6	Thermal gravimetric analysis data
S7	Luminescence-decay measurements
S9	ANOVA test results
S10	Parameters from the analysis of NMRD and variable-temperature NMR data
S11	Plots of $T_{1m}$ and $\tau_m$ of <b>1</b> and <b>2</b> as a function of temperature at 9.4 T.
S11	References

## Experimental procedures

Commercially available chemicals were of reagent-grade purity or better and were used without further purification. Water was purified using a PURELAB Ultra Mk2 water purification system (ELGA). 2,2,2,2-(1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetrayl)tetrakis(*N*-(4-(trifluoromethyl)benzyl)acetamide), 2,2,2,2-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetrakis(*N*-(4-methyl)benzyl)acetamide, and Eu<sup>III</sup>-containing complexes of both ligands were prepared following published procedures.<sup>1</sup> Gd<sup>III</sup>-containing complexes of both 2,2,2,2-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetrakis(*N*-(4-(trifluoromethyl)benzyl)acetamide) and 2,2,2,2-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetrakis(*N*-(4-methyl)benzyl)acetamide were synthesized as shown in Scheme S1.

Elemental analysis (C, H, and N) was performed by Midwest Microlab (Indianapolis). Thermal gravimetric analysis (TGA) was performed at 10 °C/min under flowing nitrogen using an SDT-2960 thermal gravimetric analysis and differential thermal analysis analyzer. Concentrations of Gd were determined using energy-dispersive X-ray fluorescence (EDXF) spectroscopy at the Lumigen Instrument Center in the Department of Chemistry at Wayne State University. All dilutions were made with purified water, that was also used for blank samples during calibration. Calibration curves were created using the Gd isotope ion count for a 15–1000 ppm concentration range (diluted from Sigma ICP standard solution). High-resolution electrospray ionization mass spectrometry (HRESIMS) was performed using an electrospray time-of-flight high-resolution Waters Micromass LCT Premier XE mass spectrometer.

Luminescence decay measurements were acquired using a Horiba Scientific Fluorolog 3-222 spectrofluorometer in decay-by-delay scan mode using the phosphorescence lifetime setting. For luminescence-decay measurements of Eu<sup>III</sup>-containing analogs of **1** and **2**, excitation and emission wavelengths of 395 and 594 nm, respectively, were used. Other parameters were kept constant for both samples included excitation and emission slit widths (5 nm), flash count (10), initial delay (0.001 ms), maximum delay (15 ms), and delay increment (0.02 ms). The samples were kept at least 10 minutes in temperature-controlled water baths to reach thermal equilibrium when performing experiments at 288 and 313 K. This procedure was performed in H<sub>2</sub>O and D<sub>2</sub>O. Measurements were performed in triplicate with three independently prepared solutions. For each complex in either H<sub>2</sub>O ( $\tau_{H_2O}$ ) or D<sub>2</sub>O ( $\tau_{D_2O}$ ), the decay rates were used in **Equation S1** to determine the number of water molecules,  $q$ , coordinated to the Eu<sup>III</sup> ion, which is also an approximation of the number for Gd<sup>III</sup>.<sup>2</sup>

$$\text{Eq S1: } q = 1.11[|\tau_{H_2O}^{-1} - \tau_{D_2O}^{-1}| - 0.31 - 0.075n_{(O=CNH)}]$$

$T_1$  values were acquired with inversion-recovery experiments using an Agilent VNMRJ-400 (400 MHz, 9.4 T) from 293 to 318 K. Relaxivity was obtained from the slope of  $1/T_1$  (s<sup>-1</sup>) versus the concentration of either **1** (0.71 mM) or **2** (0.66 mM) in 3-(*N*-morpholino)propanesulfonic acid (20 mM, pH 7.4) using a linear regression curve. All solutions were prepared in a wet (water allowed but not molecular oxygen) glove box under an atmosphere of N<sub>2</sub>.

Nuclear magnetic relaxation dispersion (NMRD) profiles were measured at the Università del Piemonte Orientale “Amedeo Avogadro” using two different instruments. Low-field data (0.00024–0.25 T, corresponding to 0.01–10 MHz proton Larmor frequency) were collected using a fast-field cycling Stellar SMARtracer relaxometer (Stelar s.r.l., Mede, PV, Italy) equipped with a silver magnet. The relaxometer operates under complete computer control with an absolute uncertainty in the  $1/T_1$  values of  $\pm 1\%$ . Additional points at high field strengths (0.5–3T, corresponding to 20–120 MHz proton Larmor frequency) were collected with a High Field Relaxometer (Stelar) equipped with the HTS-110 3T Metrology Cryogen-free Superconducting Magnet. The measurements were performed using the standard inversion recovery sequence (20 experiments, 2 scans) with a typical  $90^\circ$  pulse width of 3.5  $\mu\text{s}$ , and the reproducibility of the data was within  $\pm 0.5\%$ . The temperature was controlled with a Stelar VTC-91 heater airflow equipped with a copper-constantan thermocouple (uncertainty of  $\pm 0.1$  K). Samples ( $\sim 350$   $\mu\text{L}$ ) of **1** or **2** in 3-(N-morpholino)propanesulfonic acid (20 mM, pH 7.4) were prepared with concentrations of 0.75 and 0.8 mM, respectively, for **1** and **2**.  $T_{1m}$  values of **1** and **2** were calculated using equations S2 and S3:

$$\text{Eq S2: } \frac{1}{T_{1m}} = \frac{1}{T_1^{DD}} = \frac{2}{15} \frac{\gamma_I^2 g^2 \mu_B^2}{r_{IS}^6} S(S+1) \left(\frac{\mu_0}{4\pi}\right)^2 \left[ 7 \frac{\tau_{c2}}{1+\omega_S^2 \tau_{c2}^2} + 3 \frac{\tau_{c1}}{1+\omega_I^2 \tau_{c1}^2} \right]$$

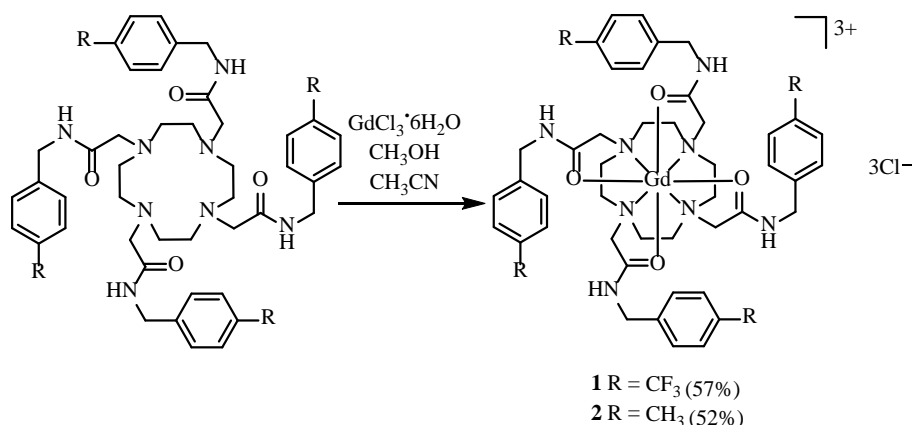
$$\text{Eq S3: } \frac{1}{\tau_{ci}} = \frac{1}{\tau_R} + \frac{1}{\tau_m} + \frac{1}{T_{ie}}$$

with  $i = 1, 2$

In equations S2 and S3,  $1/T_{1m}$  is the proton longitudinal relaxation rate of a bound water molecule;  $1/T_1^{DD}$  is the relaxation rate due to dipole-dipole relaxation;  $\gamma_I$  is the nuclear gyromagnetic ratio;  $g$  is the electron  $g$  factor;  $\mu_B$  is the Bohr magneton;  $r_{IS}$  is the nuclear-spin-electron-spin distance;  $S$  is the electron spin;  $\mu_0$  is permeability;  $\tau_{ci}$  is the total correlation time;  $\omega_S$  and  $\omega_I$  are the nuclear and electron Larmor frequencies, respectively;  $\tau_R$  is the rotational correlation time;  $\tau_m$  is the residence lifetime of a bound water molecule; and  $T_{ie}$  is the electronic relaxation time.

Single crystal X-ray crystallographic data were collected on a Bruker APEX-II CCD diffractometer using Mo  $K\alpha$  radiation. The temperature of the crystals was maintained at 100.1 K during data collection. Using Apex 3 v2018/7-2, the intensities were integrated using SAINT V8.38a and were corrected for absorption by multi-scan methods using SADABS-2012. Using Olex2,<sup>5</sup> the structure was solved by Intrinsic Phasing using the ShelXT structure solution program<sup>6</sup> and refined with the ShelXL<sup>7</sup> refinement package on all  $F^2$  data. All nonhydrogen atoms were refined anisotropically. Hydrogen atoms were positioned with idealized geometry and refined isotropically. Additionally, structure **1** exhibited whole-molecule disorder over two positions and was refined with site occupational factors of 0.53 and 0.47, and restraints and constraints were required. Structure **1** was also a pseudo-merohedral twin and refined with a batch scale factor (BASF) parameter of 0.090(3).

### Synthesis and characterization



### Scheme S1. Synthesis of 1 and 2

**Gadolinium(III) 2,2',2'',2'''-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetrakis-(N-(4-(trifluoromethyl)benzyl)acetamide) trichloride (1):** To a flask containing the fluorinated tetraamide ligand (0.5243 g, 0.5076 mmol) and  $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$  (0.1716 g, 0.4614 mmol),  $\text{CH}_3\text{CN}$  (100 mL) and  $\text{CH}_3\text{OH}$  (1 mL) were added. The resulting colorless solution was heated at 85 °C. After 24 h, a white solid had formed. The solid was collected by filtration using a fine glass frit while the solution was hot. The collected solid was washed ( $3 \times 10$  mL) with boiling acetonitrile. The washed material was dried under reduced pressure to yield the product as a white solid (0.2630 g, 57%). HRMS (ESI):  $m/z$  calcd for  $[\text{C}_{48}\text{H}_{52}\text{O}_4\text{N}_8\text{F}_{12}\text{Gd}]^{3+}$ : 396.7733  $[\text{M}]^{3+}$ ; found, 396.7704, with expected isotope pattern; elemental analysis: Found: C, 44.05; H, 4.1; N, 8.3. Calc. for  $\text{C}_{48}\text{H}_{52}\text{N}_8\text{O}_4\text{F}_{12}\text{GdCl}_3$ : C, 44.5; H, 4.0; N, 8.6%.

**Gadolinium(III) 2,2',2'',2'''-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetrakis-(N-(4-methylbenzyl)acetamide) trichloride (2):** To a flask containing the methylated tetraamide ligand (0.7346 g, 0.8990 mmol) and  $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$  (0.3038 g, 0.8173 mmol),  $\text{CH}_3\text{CN}$  (100 mL) and  $\text{CH}_3\text{OH}$  (1 mL) were added. The resultant colorless solution was heated at 85 °C. After 24 h, a white solid had formed. The solid was collected by filtration using a fine glass frit while the solution is hot. The collected solid was washed ( $3 \times 10$  mL) with boiling acetonitrile. The washed material was dried under reduced pressure to yield the product as a white solid (0.6219 g, 52%). HRMS (ESI):  $m/z$  calcd for  $[\text{C}_{48}\text{H}_{64}\text{O}_4\text{N}_8\text{Gd}]^{3+}$ : 324.8092  $[\text{M}]^{3+}$ ; found, 324.8093, with expected isotope pattern; HRMS (ESI):  $m/z$  calcd for  $[\text{C}_{48}\text{H}_{64}\text{O}_4\text{N}_8\text{GdI}]^{2+}$ : 1136.3021  $[\text{M}]^{2+}$ ; found, 1136.2988, with expected isotope pattern (iodide counterions were from the ligand synthesis); elemental analysis: Found: C, 40.4; H, 5.05; N, 7.8. Calc. for  $\text{C}_{48}\text{H}_{52}\text{N}_8\text{O}_4\text{F}_{12}\text{GdI}_3 \cdot 4\text{H}_2\text{O}$ : C, 40.4; H, 5.1; N, 7.85%; Thermal gravimetric analysis (Supplementary Figure S1) shows a 4.4% mass loss corresponding to 4  $\text{H}_2\text{O}$ , which is consistent with the elemental analysis.

**Crystallographic data**

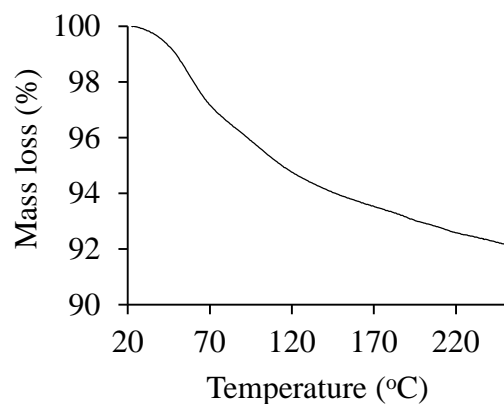
A solution of **1** or **2** (10 mg) in methanol (1 mL) was filtered through a 0.2  $\mu\text{m}$  hydrophilic filter into an NMR tube. The tube was covered with parafilm, and a small hole was formed in the parafilm using a syringe needle. After two weeks, clear, colorless crystals were collected.

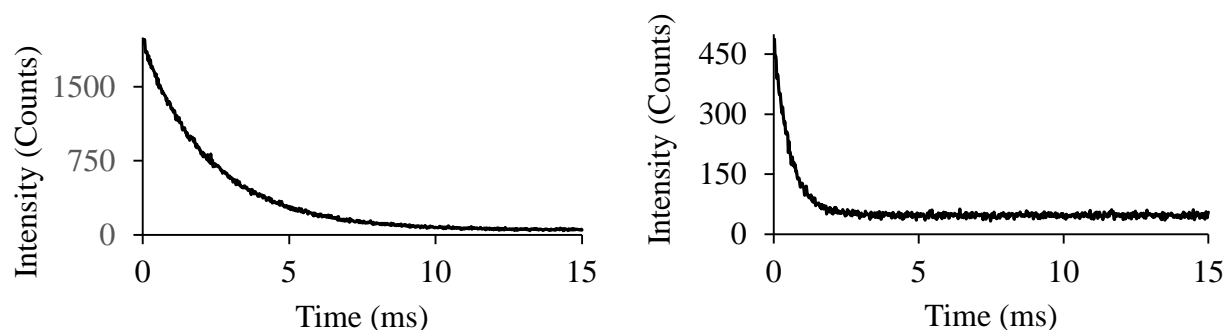
**Table S1. Crystallographic properties of 1**

Chemical formula	$\text{C}_{48}\text{H}_{66}\text{O}_{11}\text{N}_8\text{F}_{12}\text{Cl}_3\text{Gd}$	
Formula weight	1422.68	
$R_1$	10.00%	
$wR_2$	27.88%	
Goodness of Fit	1.026	
Largest residual peak/hole	2.49/−1.36	
Temperature	100.1 K	
Wavelength	0.71073 Å	
Crystal system	monoclinic	
Space group	$P2/n$	
Unit cell dimensions	$a = 10.8408(6)$ Å $b = 26.2799(13)$ Å $c = 11.5932(5)$ Å	$\alpha = 90^\circ$ $\beta = 117.247(3)^\circ$ $\gamma = 90^\circ$
Volume	$2936.4(3)$ Å <sup>3</sup>	
Z	2	
Density (calculated)	$1.609$ g cm <sup>−3</sup>	
Absorption coefficient	$1.366$ mm <sup>−1</sup>	
F(000)	1442.0	
2 $\theta$ range for data collection	1.55 to 51.02°	
Reflections collected	73882	
Data/restraints/parameters	5522/1729/687	
Independent reflections	5522	
$R_{\text{int}}$	0.2448	
Crystal size	0.2 mm × 0.15 mm × 0.15 mm	
CCDC no.	1991782	

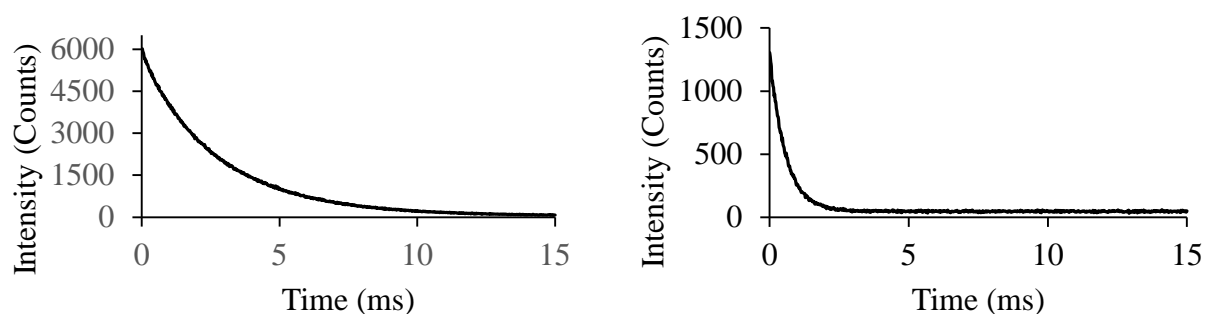
**Table S2. Crystallographic properties of 2**

Chemical formula	C <sub>48</sub> H <sub>70</sub> O <sub>7</sub> N <sub>8</sub> I <sub>3</sub> Gd	
Formula weight	1409.07	
R <sub>1</sub>	3.97%	
wR <sub>2</sub>	7.29%	
Goodness of Fit	1.034	
Largest residual peak/hole	1.01/-1.03	
Flack parameter	-0.009(6)	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	monoclinic	
Space group	P2 <sub>1</sub>	
Unit cell dimensions	a = 15.2967(7) Å b = 11.2692(5) Å c = 15.7712(7) Å	α = 90° β = 90.312(2)° γ = 90°
Volume	2718.6(2) Å <sup>3</sup>	
Z	2	
Density (calculated)	1.721 g cm <sup>-3</sup>	
Absorption coefficient	2.977 mm <sup>-1</sup>	
F(000)	1386.0	
2θ range for data collection	3.72 to 57.57°	
Reflections collected	60168	
Data/restraints/parameters	13946/10/638	
Independent reflections	13946	
R <sub>int</sub>	0.0674	
Crystal size	0.26 mm × 0.21 mm × 0.18 mm	
CCDC no.	1991783	

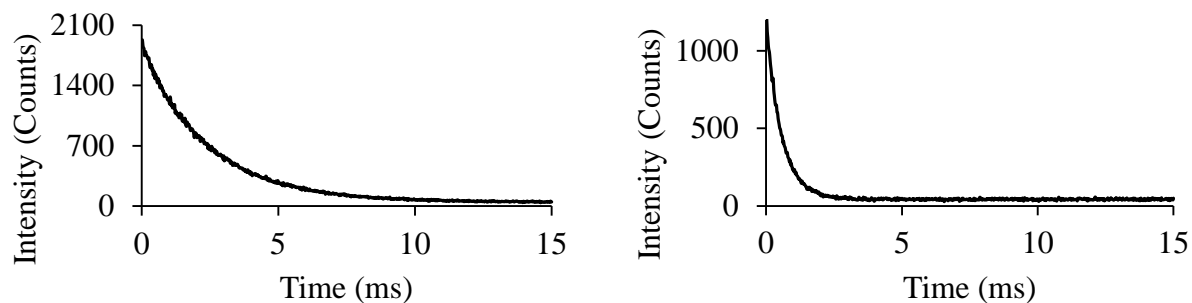
**Thermal gravimetric analysis data****Figure S1.** Thermal gravimetric analysis of **2** indicating the presence of four water molecules per complex.

**Luminescence-decay measurements**

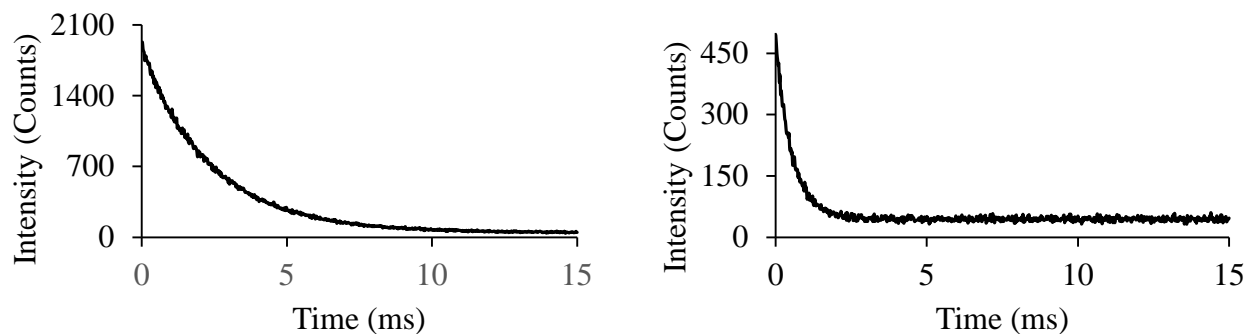
**Figure S2.** Plot of a representative luminescence-decay curve of the Eu<sup>III</sup>-containing analog of **1** in (left) D<sub>2</sub>O and (right) H<sub>2</sub>O at 288 K.



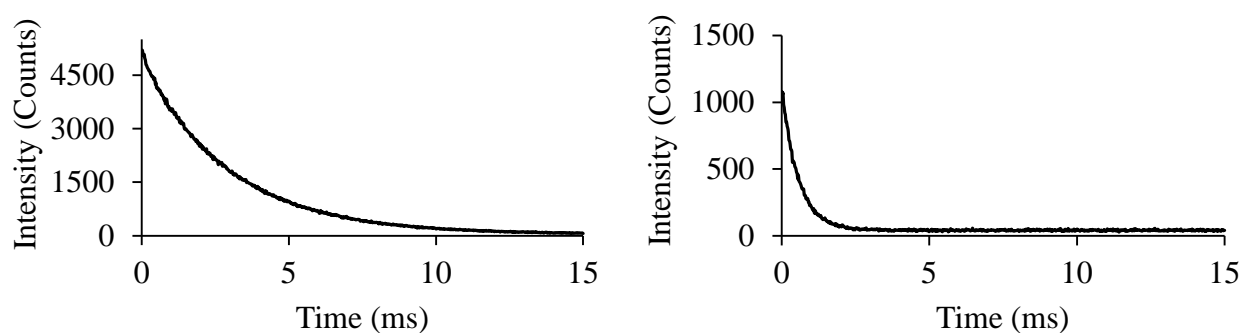
**Figure S3.** Plot of a representative luminescence-decay curve of the Eu<sup>III</sup>-containing analog of **2** in (left) D<sub>2</sub>O and (right) H<sub>2</sub>O at 288 K.



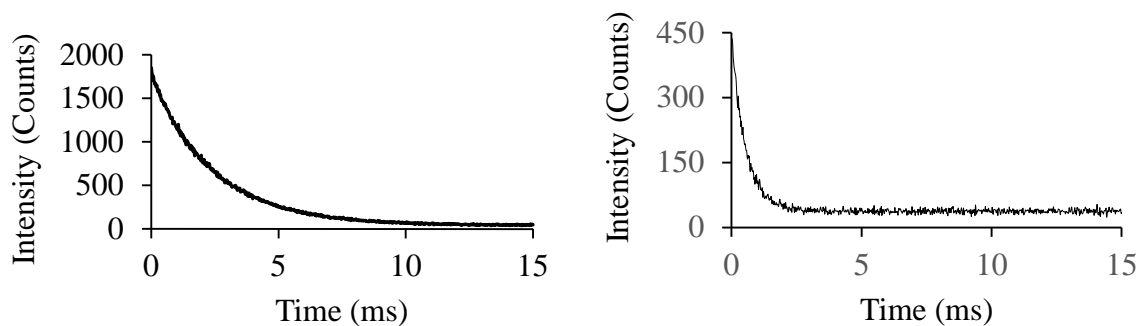
**Figure S4.** Plot of a representative luminescence-decay curve of the Eu<sup>III</sup>-containing analog of **1** in (left) D<sub>2</sub>O and (right) H<sub>2</sub>O at 298 K.



**Figure S5.** Plot of a representative luminescence-decay curve of the Eu<sup>III</sup>-containing analog of **2** in (left) D<sub>2</sub>O and (right) H<sub>2</sub>O at 298 K.



**Figure S6.** Plot of a representative luminescence-decay curve of the Eu<sup>III</sup>-containing analog of **1** in (left) D<sub>2</sub>O and (right) H<sub>2</sub>O at 313 K.



**Figure S7.** Plot of a representative luminescence-decay curve of the Eu<sup>III</sup>-containing analog of **2** in (left) D<sub>2</sub>O and (right) H<sub>2</sub>O at 313 K.



**Tables S3. ANOVA test results:**  $F < F_{crit}$  and P-value  $>$  Alpha (0.05). Therefore, there is no significant difference between the relaxivities of **1** from 293 to 318 K at 9.4 T.

Anova: Single Factor

SUMMARY

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
Column 1	2	3.915315	1.957657	0.012775
Column 2	2	3.744766	1.872383	0.004701
Column 3	2	3.805497	1.902749	0.002434

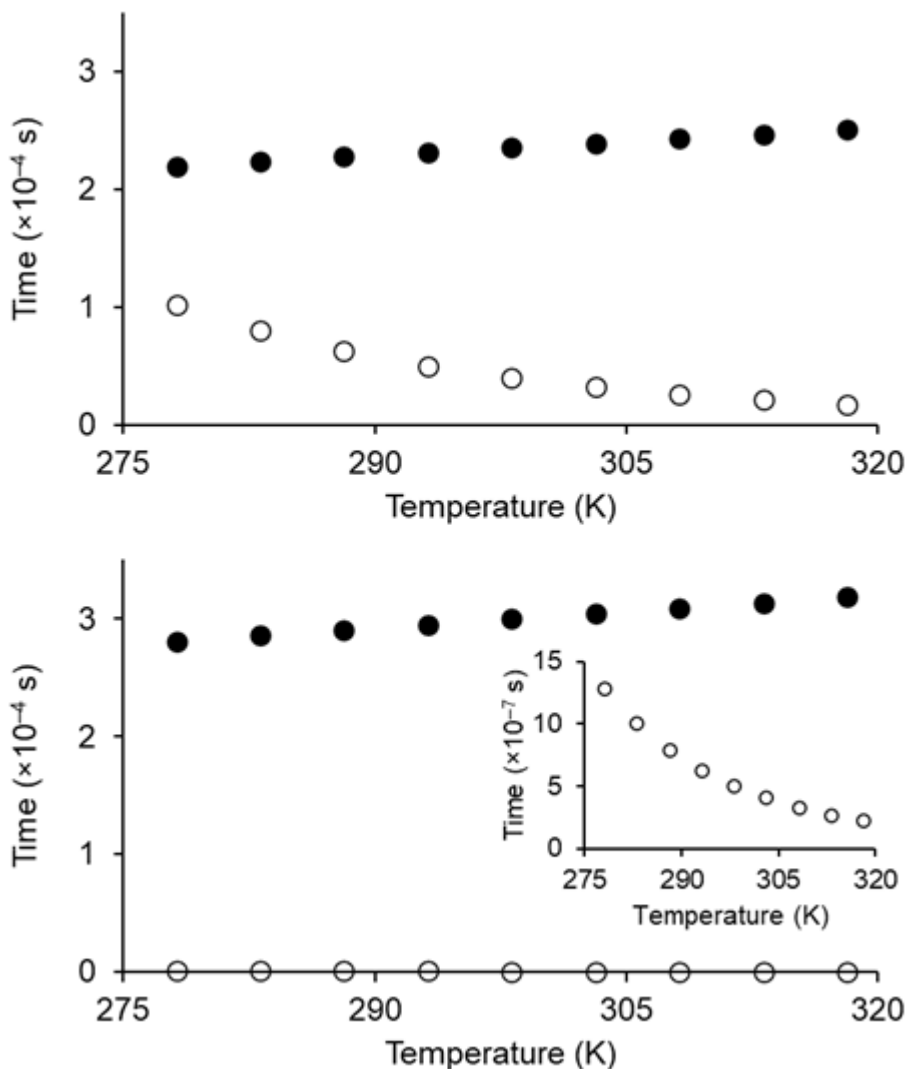
ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	0.007472	2	0.003736	0.562986	0.620001	9.552094
Within Groups	0.019909	3	0.006636			
Total	0.027382	5				

**Table S4. Parameters from the analysis of NMRD and variable-temperature NMR data**

Parameters	1	2
$\Delta^2$ ( $10^{19} \text{ s}^{-2}$ )	3.0±0.2	5.3±0.3
$\tau_V$ (ps)	6±1	8±1
$E_V$ (kJ mol <sup>-1</sup> )	2 <sup>a</sup>	2 <sup>a</sup>
$\tau_m$ (μs)	40±1	0.5±0.1
$\Delta H_M$ (kJ mol <sup>-1</sup> )	52±3	30±3
$\tau_R$ (ps)	100±5	81±2
$E_R$ (kJ mol <sup>-1</sup> )	20±1	22±2
$Q$	1 <sup>a</sup>	1 <sup>a</sup>
$r$ (Å)	3.1 <sup>a</sup>	3.1 <sup>a</sup>
$a$ (Å)	4.3 <sup>a</sup>	4.3 <sup>a</sup>
$^{298}D$ ( $10^5 \text{ cm}^2 \text{ s}^{-1}$ )	2.24 <sup>a</sup>	2.24 <sup>a</sup>
$E_D$ (kJ mol <sup>-1</sup> )	20.0 <sup>a</sup>	20.0 <sup>a</sup>

[a] parameters fixed during the best-fitting procedure.



**Figure S8.** Plots of  $T_{1m}$  (●) and  $\tau_m$  (○) of **1** (top graph) and **2** (bottom graph) as a function of temperature at 9.4 T. The inset on the bottom plot shows the behavior of  $\tau_m$  for **2**.

## References

1. L. A. Basal, M. D. Bailey, J. Romero, M. M. Ali, L. Kurenbekova, J. Yustein, R. G. Pautler and M. J. Allen, *Chem. Sci.*, 2017, **8**, 8345.
2. R. M. Supkowski and W. D. Horrocks, Jr., *Inorg. Chim. Acta*, 2002, **340**, 44.
3. L. M. Urbanczyk-Pearson, F. J. Femia, J. Smith, G. Parigi, J. A. Duimstra, A. L. Eckermann, C. Luchinat, T. J. Meade, *Inorg. Chem.*, 2008, **47**, 56.
4. Z. Lin, M. L. Shelby, D. Hayes, K. A. Fransted, L. X. Chen and M. J. Allen, *Dalton Trans.*, 2014, **43**, 16156.
5. O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, *J. Appl. Cryst.*, 2009, **42**, 339.
6. G. M. Sheldrick, *Acta Cryst.*, 2015, **A71**, 3.
7. G. M. Sheldrick, *Acta Cryst.*, 2015, **C71**, 3.