Non-Covalent Self Assembly Controls the Relaxivity of Magnetically Active Guests

Vincent Li, Yoo-Jin Ghang, Richard J. Hooley,* and Travis J. Williams*

Electronic Supplementary Information

Loker Hydrocarbon Research Institute and Department of Chemistry, University of Southern California, 837 Bloom Walk, Los Angeles, CA 90089. Department of Chemistry, University of California - Riverside, Riverside CA 92521.

travisw@usc.edu; richard.hooley@ucr.edu

Table of Contents

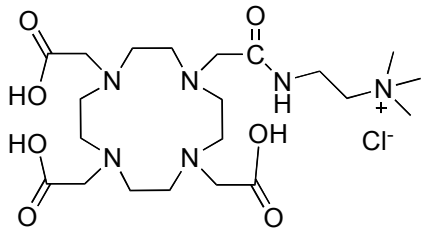
1. Synthetic Details

General Procedures

Deuterated NMR solvents were purchased from Cambridge Isotopes Labs. All NMR spectra were obtained on a Varian 400 MHz spectrometer at 25 ºC. Proton (^{1}H) chemical shifts are reported in parts per million (δ) with respect to tetramethylsilane (TMS, δ = 0), and referenced internally with respect to the protio solvent impurity. DLS were acquired on a Wyatt Dynapro Titan instrument, and each DLS measurement was replicated at least 3 times consecutively. All reversed-phase C18 columns were purchased from Teledyne. Elemental analysis and ICP-MS data were acquired by the University of Illinois at Urbana-Champaign School of Chemical Sciences Microanalysis Laboratory. Reverse-phase chromatography was achieved with a Teledyne Combiflash RF automated chromatography system.

Molecular modeling (semi-empirical calculations) was performed using the AM1 force field using SPARTAN.[1] Cavitand **1** was synthesized according to literature procedures.[2] Distilled water was purchased from Arrowhead. Acetonitrile (CH_3CN) was purchased from EMD. Dicyclohexylcarbodiimide (DCC) was purchased from Lancaster. Methanol (MeOH) was purchased from Macron. 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) was purchased from Strem Chemicals. 2-aminoethyltrimethylammonium chloride hydrochloride was purchased from Sigma Aldrich. Gadolinium (III) hexahydrate, yttrium (III) hydrate, and choline chloride were purchased from Alfa Aesar. All chemicals were used as received.

Synthesis of ligand 4



Aqueous acetonitrile (50%, 10 mL) was added to 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA, 100 mg, 0.25 mmol), and 2-aminoethyltrimethylammonium chloride hydrochloride (43.5 mg, 0.25 mmol) and stirred at room temperature until fully dissolved (15 minutes). DCC (dicyclohexylcarbodiimide, 51 mg, 0.31 mmol) was dissolved in pyridine (2 mL) and added dropwise to the reaction mixture. The reaction was stirred for 2 days in a sealed flask at room temperature. The resulting precipitate was filtered, and the filtrate was purified via reversed phase column chromatography (C18 Silica, H_2O eluent) to provide **4** as a white powder (93 mg, 71%). ^{1}H NMR (500 MHz, D_2O, 298 K) δ: 3.90-3.08 ppm (m, broad 26H), 3.20 ppm (s, 9H). ^{13}C NMR (125 MHz, D_2O, 298 K) δ: 174.56, 172.31, 170.29, 64.06, 56.20, 55.48, 53.58, 51.55, 50.86, 48.99, 48.47, 33.72. MS (MALDI) *m/z* calcd for $C_{21}H_{41}N_6O_7^+$: 489.30 g/mol, found 489.09 g/mol.

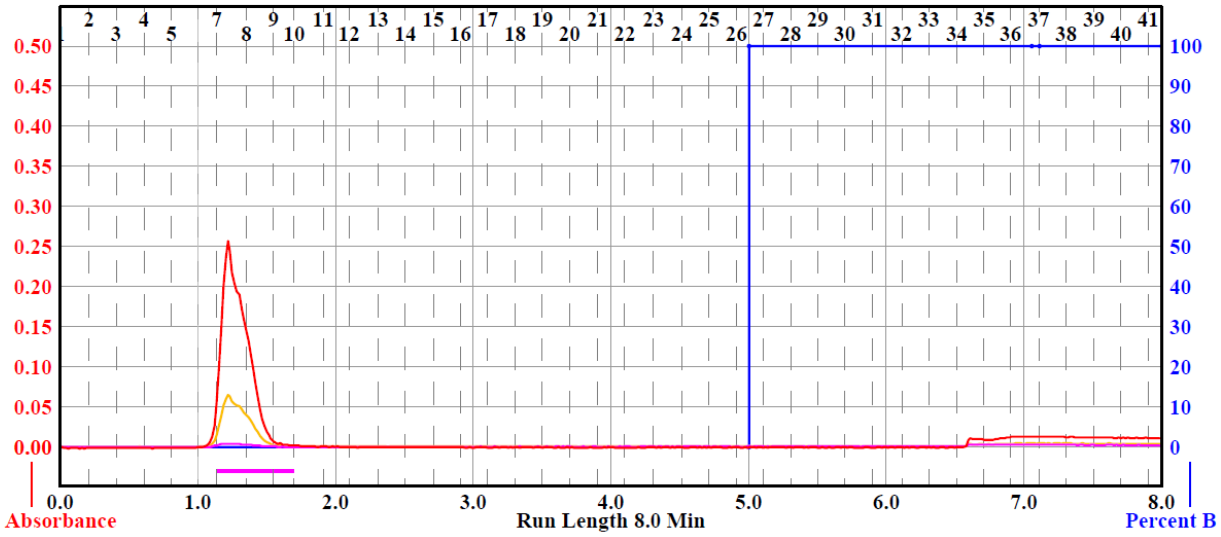


Figure *S*-1. Chromatography trace of **4**.

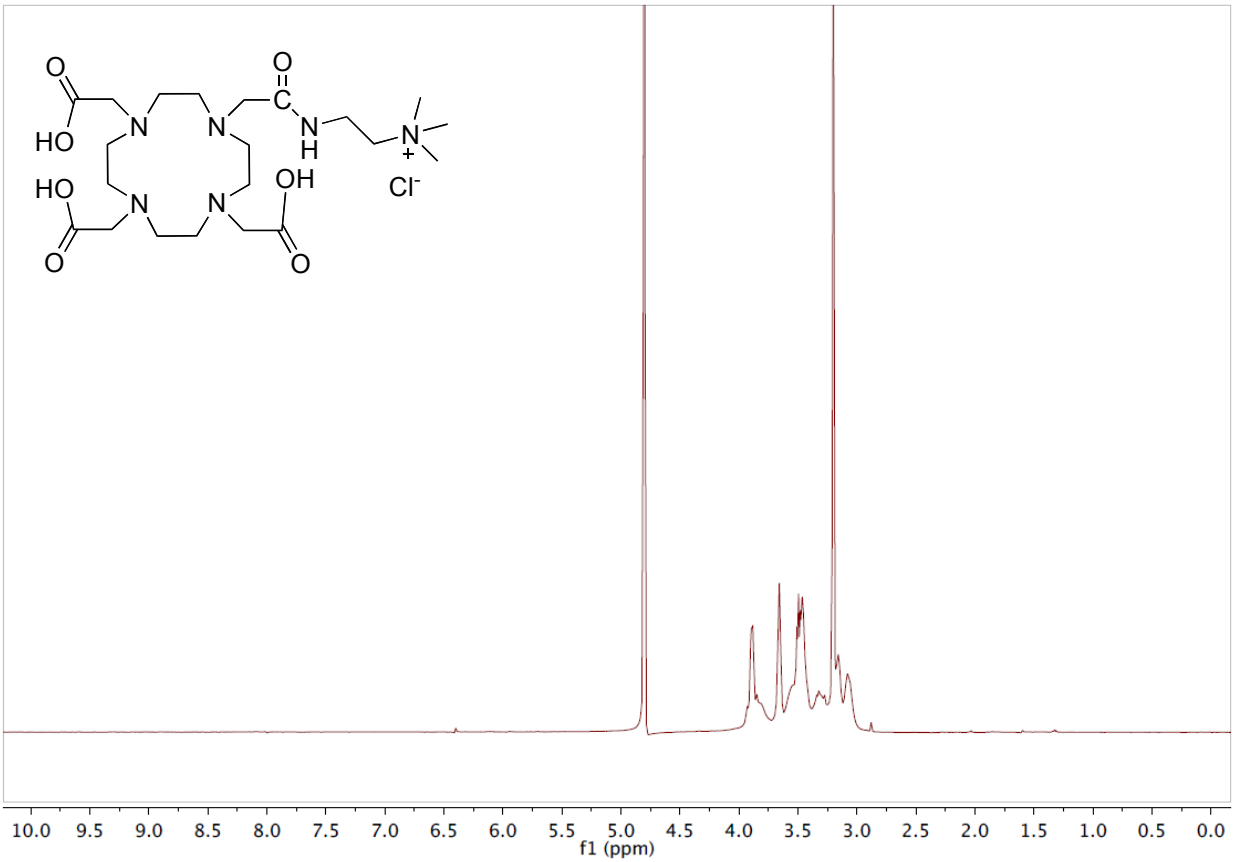


Figure *S*-2. ^{1}H NMR spectrum of **4** (500 MHz, D_2O, 298 K).

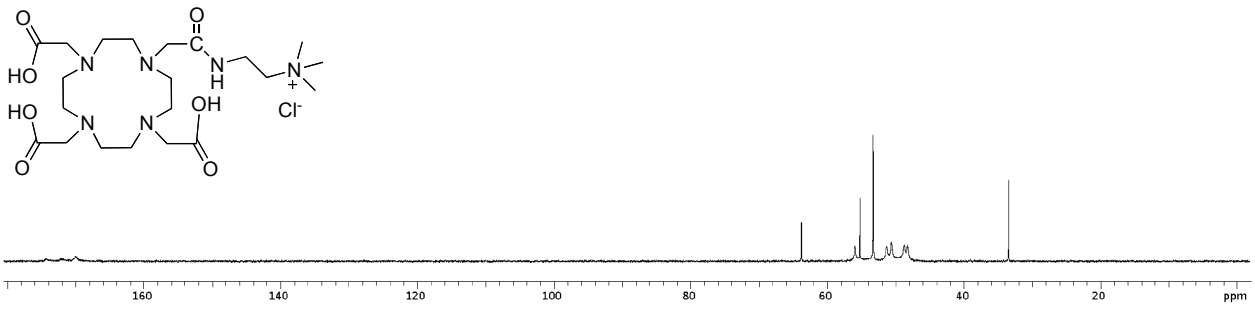
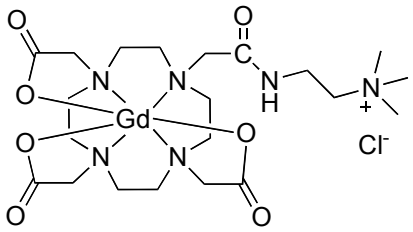


Figure *S*-3. ^{13}C NMR spectrum of **4** (125 MHz, D_2O, 298 K).

Synthesis of Gd-containing guest 2a



To a solution of **6** (6.8 mg, 0.01 mmol) in H_2O (1 mL) was added $GdCl_3 \cdot 6H_2O$ (4.8 mg, 0.01 mmol). The resulting solution was then stirred at 50 ºC for 20 hours. The pH of the solution was neutralized to pH ~ 7 by the addition of NaOH (0.1 M aq) every hour over the first ten hours. The product lyopholized to powder then purified via reversed phase column chromatography (C18 silica, H_2O eluent) to provide **2** as a white powder (4.6 mg, 55%). A single chromatography trace was observed. The product was dissolved in distilled water and the final Gd^{3+} concentration was determined by ICP-MS. No free Gd^{3+} was detected in solution by xylenol orange test.[3] NMR for this compound cannot be recorded because this compound is paramagnetic. MS (MALDI) *m/z* calcd for $C_{21}H_{38}GdN_6O_7$: 644.20 g/mol; found 643.94 g/mol. Measured isotopic distribution matches calculated prediction.

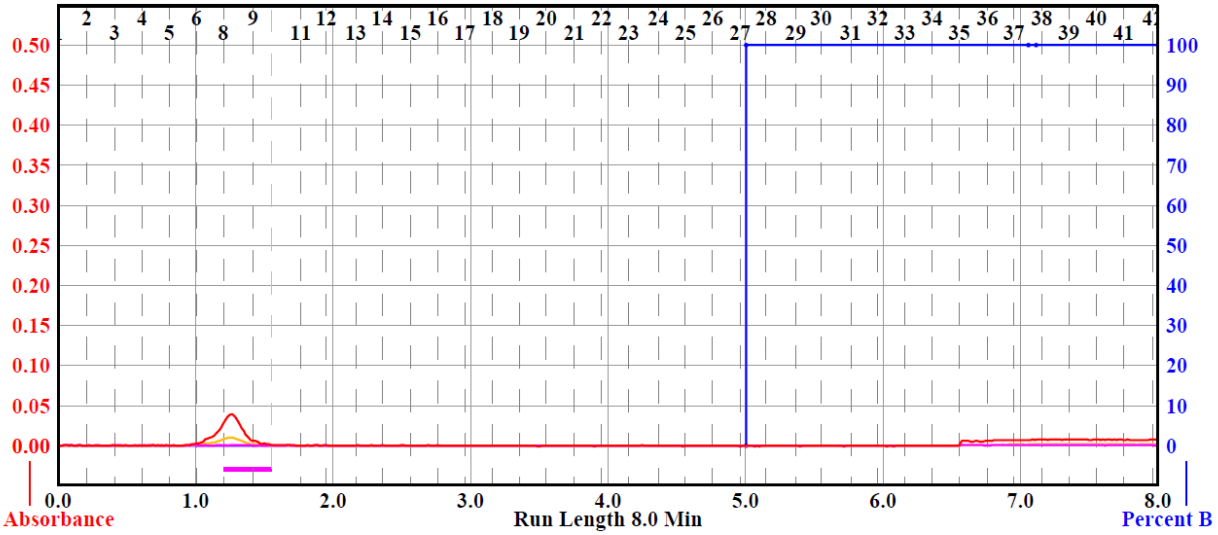
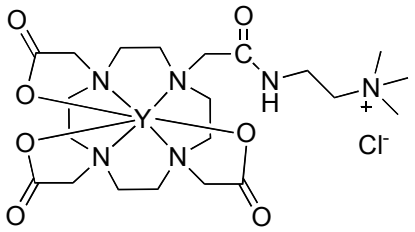


Figure *S*-4. Chromatography trace of **2a**.

Synthesis of Yttrium Complex 2b



Ligand **6** (148 mg, 0.282 mmol) and $YCl_3 \cdot xH_2O$ (64 mg) were dissolved in H_2O (5 mL). The reaction was stirred at room temperature for 12 hours. The solution was neutralized over the course of the reaction to pH ~ 7 by the addition of NaOH (0.1 M aq). The product was purified via reversed phase column chromatography (C18 silica, 0-5% MeOH:H_2O) to yield product as a white solid (63 mg, 36%). ^{1}H NMR (500 MHz, D_2O, 25 ºC) δ 3.96-3.25 (m, broad, 16H) 3.21 (s, 9H) 2.81 (s, broad, 7H) 2.61-2.50 (m, broad, 6H). ^{13}C NMR was not obtained as significant broadening of the peaks (c.f. ^{13}C spectrum of ligand **4**, Fig *S*-3) rendered the signal:noise ratio too low for detection, even after extensive acquisition. MS (MALDI) *m/z* calcd for $C_{21}H_{38}N_6O_7Y$: 575.19 g/mol; found 574.94 g/mol.

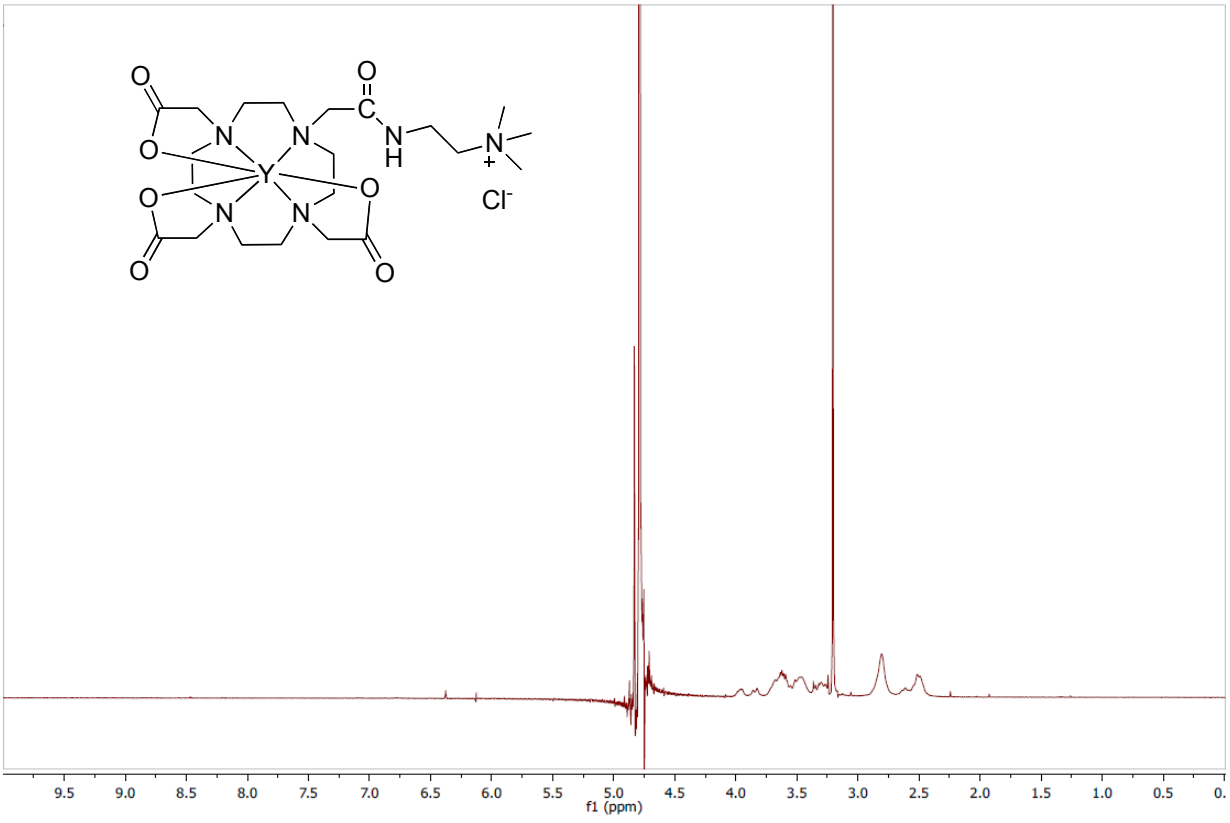
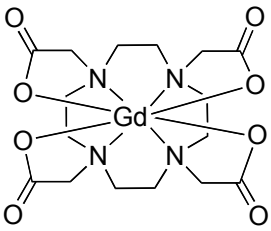


Figure *S*-5. ^{1}H NMR spectrum of **2b** (500 MHz, D_2O, 25 ºC).

Synthesis of Na[Gd(DOTA)] 5



DOTA (24.3 mg, 0.06 mmol) and $GdCl_3 \cdot 6H_2O$ were dissolved in 3 mL H_2O. The solution was neutralized over the course of the reaction to pH ~ 7 by the addition of NaOH (0.1 M aq). The reaction was stirred until the pH was constant for 1 hour (4 hour total reaction time). The solution was then adjusted to pH ~ 11 by the addition of NaOH (0.1 M aq) and the reaction was stirred for 20 minutes more, then filtered through a 0.45 μm syringe filter. The product was lyopholized to powder, then purified via reversed phase column chromatography (C18 silica, 0-5% MeOH:H_2O) to yield Gd·DOTA as a white solid (10.2 mg, 31%). Product was dissolved in distilled water and the final gadolinium concentration was determined via ICP-MS. NMR for this compound cannot be recorded because this compound is paramagnetic.

Effect of cavitand on gadolinium complex 2a

2a (125 μl of a 1 mM solution) was added to six 0.5 dram glass vials, which were lyophilized to dryness. Cavitand **1** (5 mg, 3.6 μmol) was dissolved in 368 μL H_2O. The cavitand solution (12.5 μL per equivalent of Gd) was added to each vial to make vials containing gadolinium complex **2a** with 1, 2, 3, 4, 5, and 10 equivalents cavitand, respectively. An appropriate volume of water was added to each vial to bring the total volume of water to 125 μL. The solutions were then transferred to a 3 mm diameter coaxial NMR tube insert for T_1 measurements. T_1 relaxation rates were then acquired using Varian's inversion recovery sequence, with interpulse from 62.5 ms to 32 s, and T_1 times were tabulated by the native software.

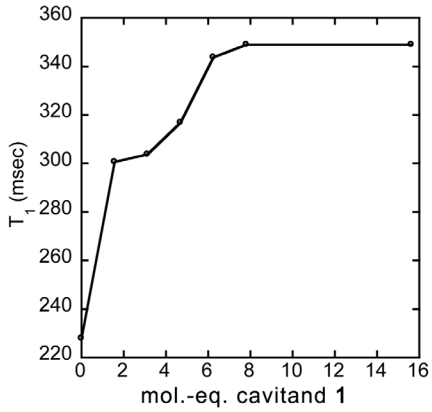


Figure *S*-6. Modulation of the T_1 relaxation rate of **2a** by cavitand **1**. a) T_1 (H_2O) variation upon increasing [**1**] (H_2O, 298 K, [**2a**] = 1 mM).

Effect of choline on masked complex

To 9 glass vials were distributed choline chloride (10 mM aq). These were lyopholized to dryness. A solution of complex **2a** (0.5 mM) along with 4 equivalents of cavitand **1** (125 µL, 0.5 mM [Gd]) was added to the vials, which were then transferred to a 3 mm diameter coaxial NMR tube insert for T_1 measurements. Data were recorded as above.

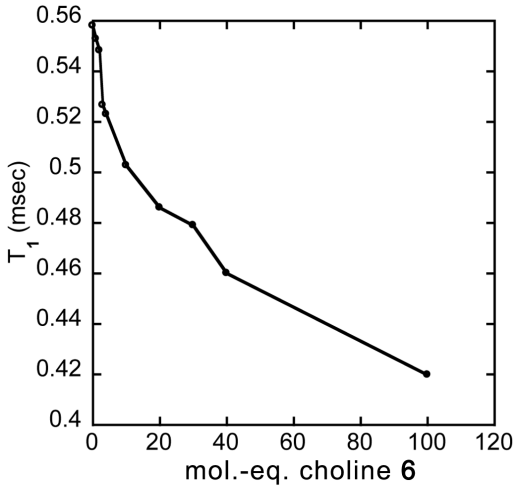


Figure *S*-7. Modulation of T_1 relaxation rate upon addition of a competitive guest. a) $T_1(H_2O)$ variation upon addition of choline **6** to a solution of **1•2a** (H_2O, 298 K, [**2a**] = 0.5 mM, [**1**] = 3 mM)

2. Molar Relaxivity Curves

[Gd] (mM)	T_1 (s)	$1/T_1$ (s^{-1})
0.64	0.228(3)	4.388(57)
0.32	0.352(8)	2.840(61)
0.213	0.473(10)	2.115(45)
0.16	0.500(20)	1.999(80)

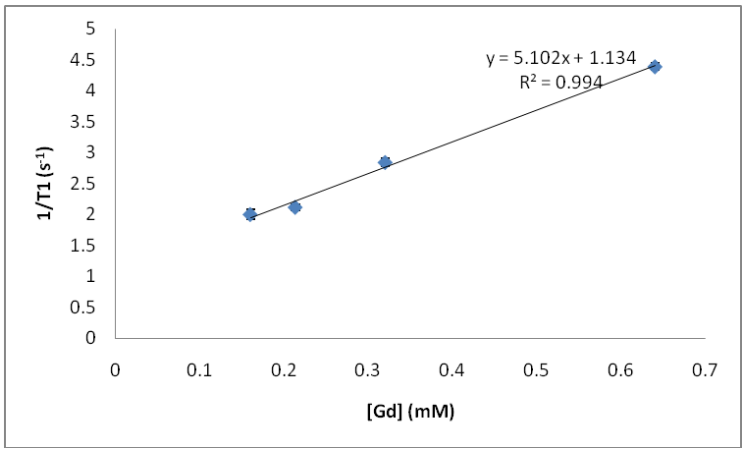


Figure *S*-8. Relaxivity of Gd Complex **2a**

[Gd] (mM)	T_1 (s)	$1/T_1$ (s^{-1})
0.64	0.355(1)	2.817(7)
0.32	0.596(2)	1.678(5)
0.213	0.702(5)	1.425(10)
0.16	0.758(6)	1.319(11)

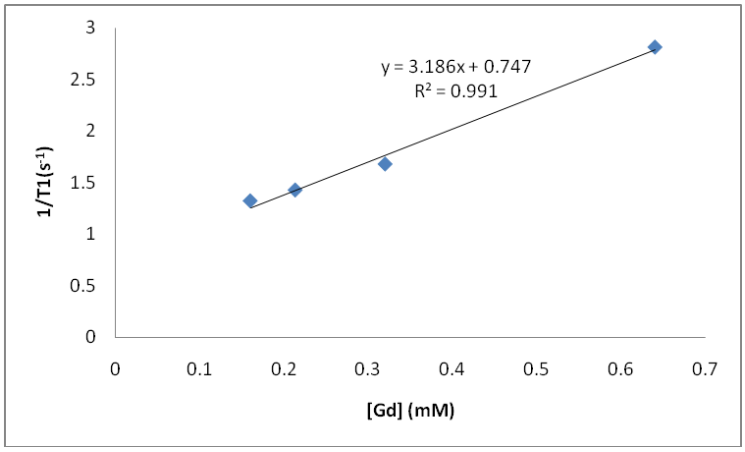


Figure *S*-9. Relaxivity of Gd Complex **2a** with 6 equivalents of **1**

[Gd] (mM)	T_1 (s)	$1/T_1$ (s^{-1})
0.64	0.272(2)	3.682(25)
0.32	0.458(6)	2.183(30)
0.213	0.510(7)	1.962(26)
0.16	0.610(12)	1.640(32)

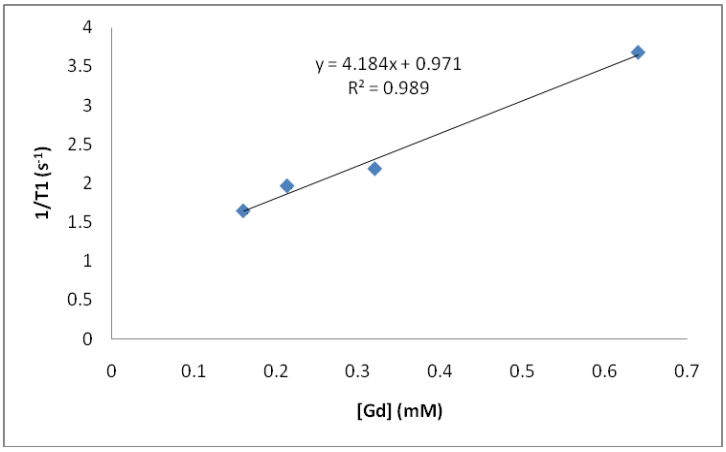


Figure *S*-10. Relaxivity of Gd Complex **2a** with 6 equivalents **1** and excess choline

[Gd] (mM)	T_1 (s)	$1/T_1$ (s^{-1})
1	0.277(6)	3.617(74)
0.5	0.439(16)	2.279(84)
0.33	0.522(23)	1.917(83)
0.25	0.730(28)	1.370(52)

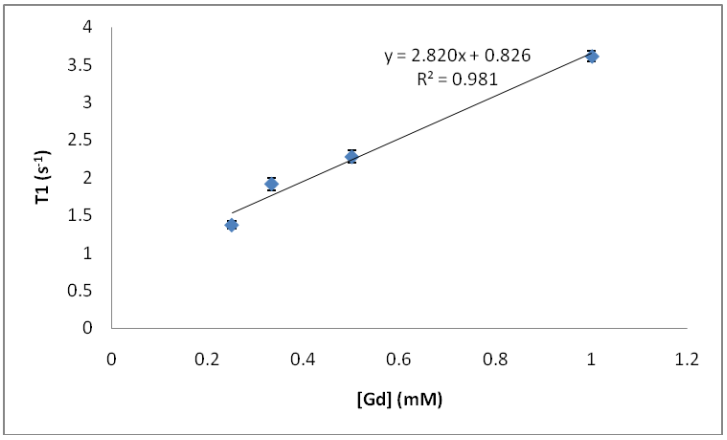


Figure *S*-11. Relaxivity of Gd·DOTA **5**

[Gd] (mM)	T_1 (s)	$1/T_1$ (s^{-1})
1	0.286(4)	3.494(51)
0.5	0.446(12)	2.242(60)
0.33	0.509(22)	1.963(84)
0.25	0.588(43)	1.701(125)

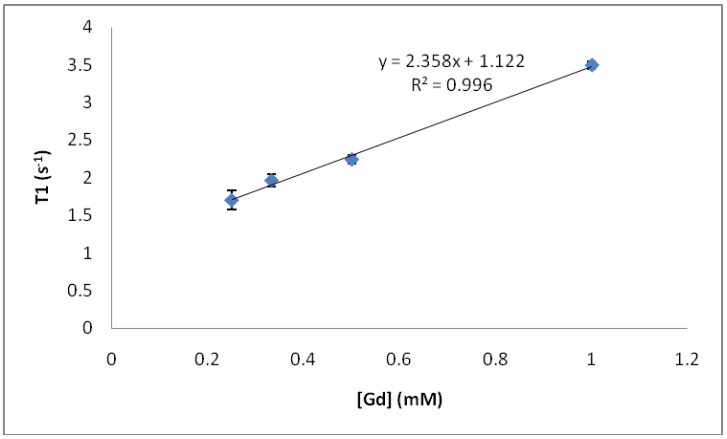


Figure *S*-12. Relaxivity of Gd·DOTA **5** with 4 equivalents of **1**

Relaxivity of Gd·DOTA **5** with 4 equivalents of cavitand 2 upon exposure to excess choline

[Gd] (mM)	T_1 (s)	$1/T_1$ (s^{-1})
1	0.298(3)	3.357(29)
0.5	0.476(7)	2.103(31)
0.33	0.621(14)	1.611(36)
0.25	0.657(26)	1.522(60)

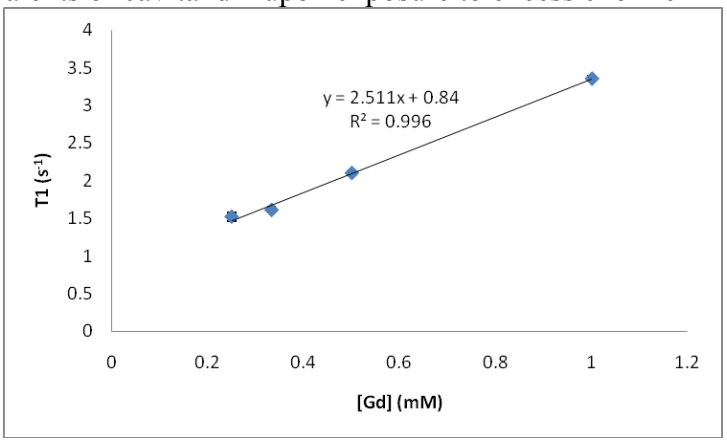


Figure *S*-13. Relaxivity of Gd·DOTA **5** with 4 equivalents of **1** and excess choline

3. ^{1}H NMR spectra of Y-DOTA titration experiments

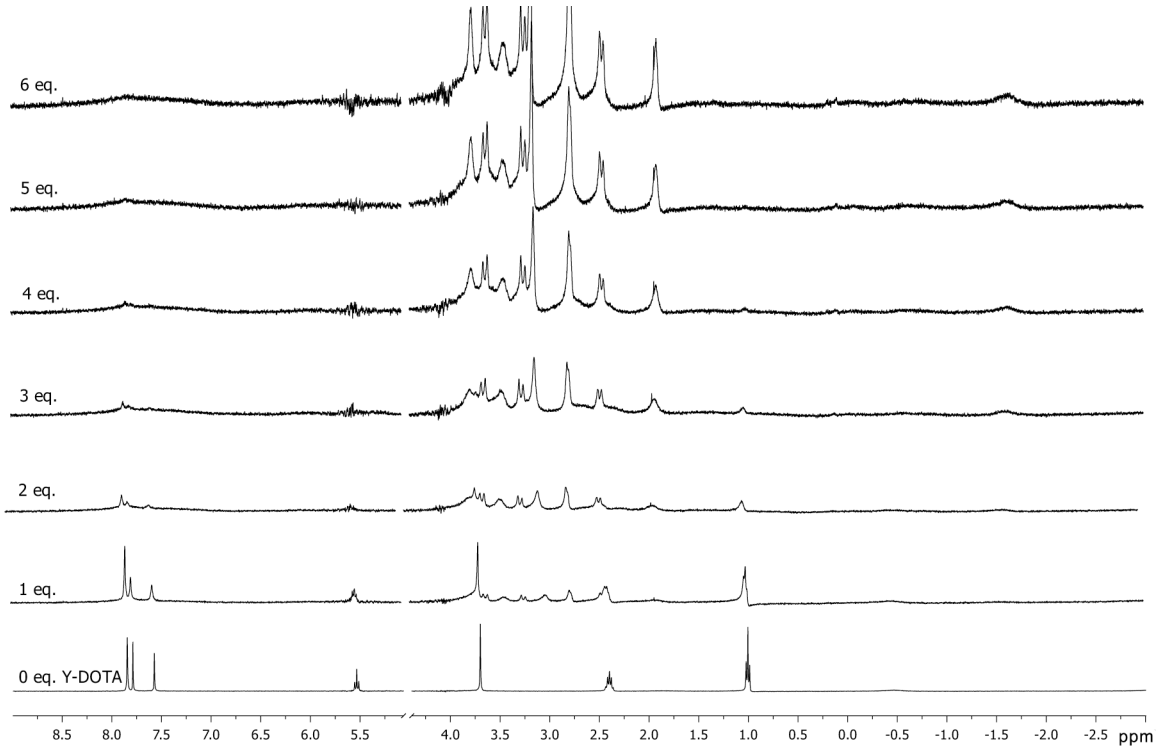


Figure *S*-14. ^{1}H NMR spectra of the titration of Y·DOTA complex **2b** into a solution of cavitand **1** ([**1**] = 2 mM, D_2O, 400 MHz, 298 K).

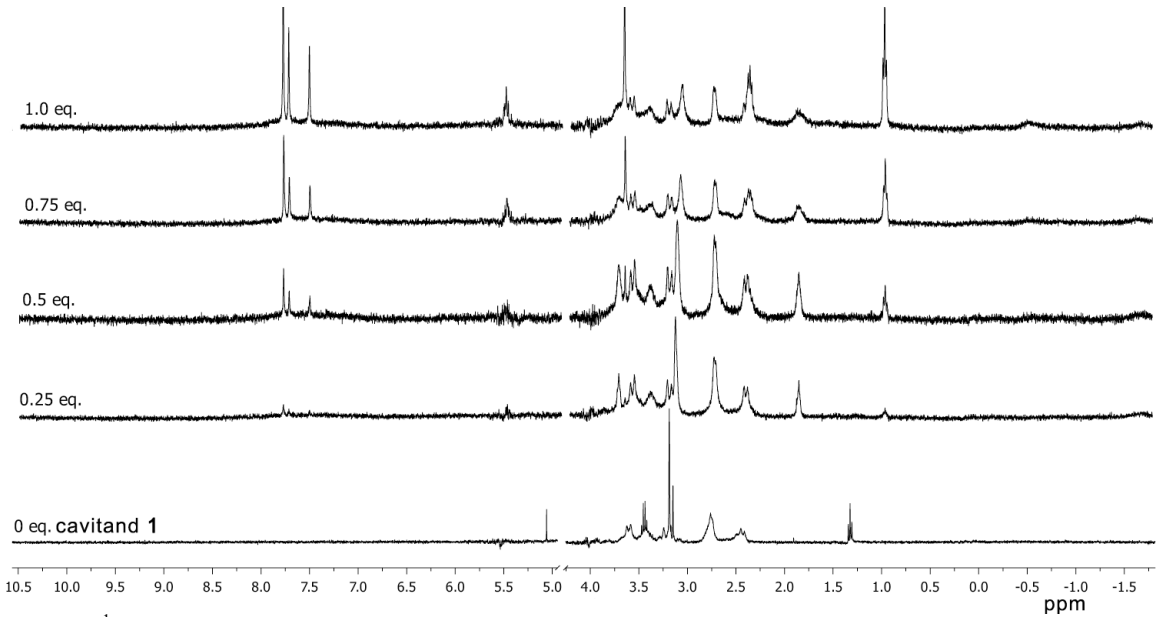


Figure *S*-15. ^{1}H NMR spectra of the titration of cavitand **1** into a solution of Y·DOTA complex **2b** ([**2b**] = 2 mM, D_2O, 400 MHz, 298 K).

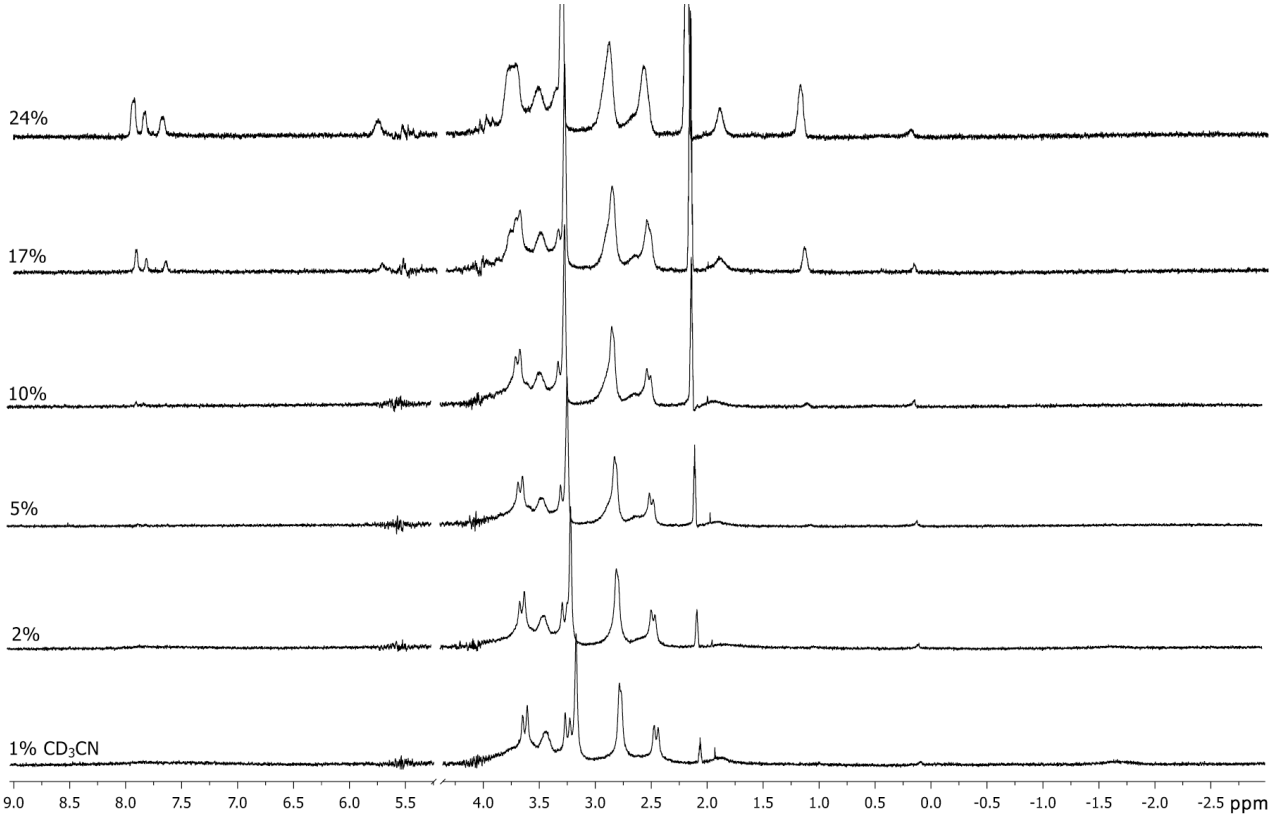


Figure *S-16*. ^{1}H NMR spectra of the titration of acetonitrile-d_3 into a 6:1 mixture of cavitand **1** and Y·DOTA complex **2b** ([**2b**] = 2 mM, D_2O, 400 MHz, 298 K).

4. Graphical DLS Data

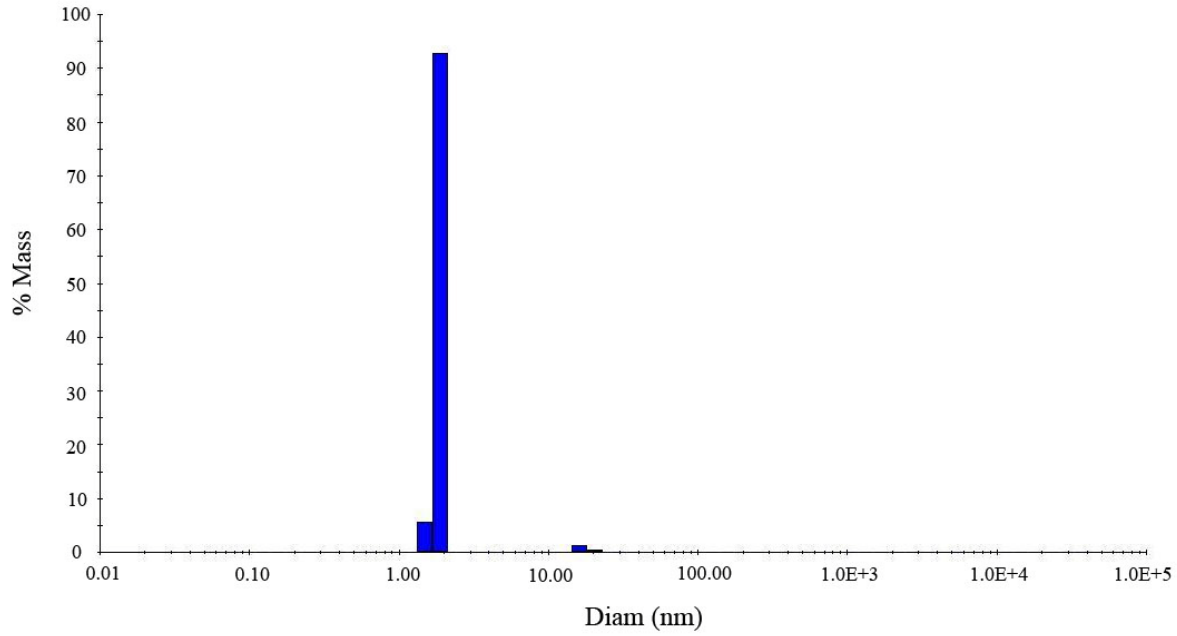


Figure *S*-17. DLS histogram of cavitand **1.**

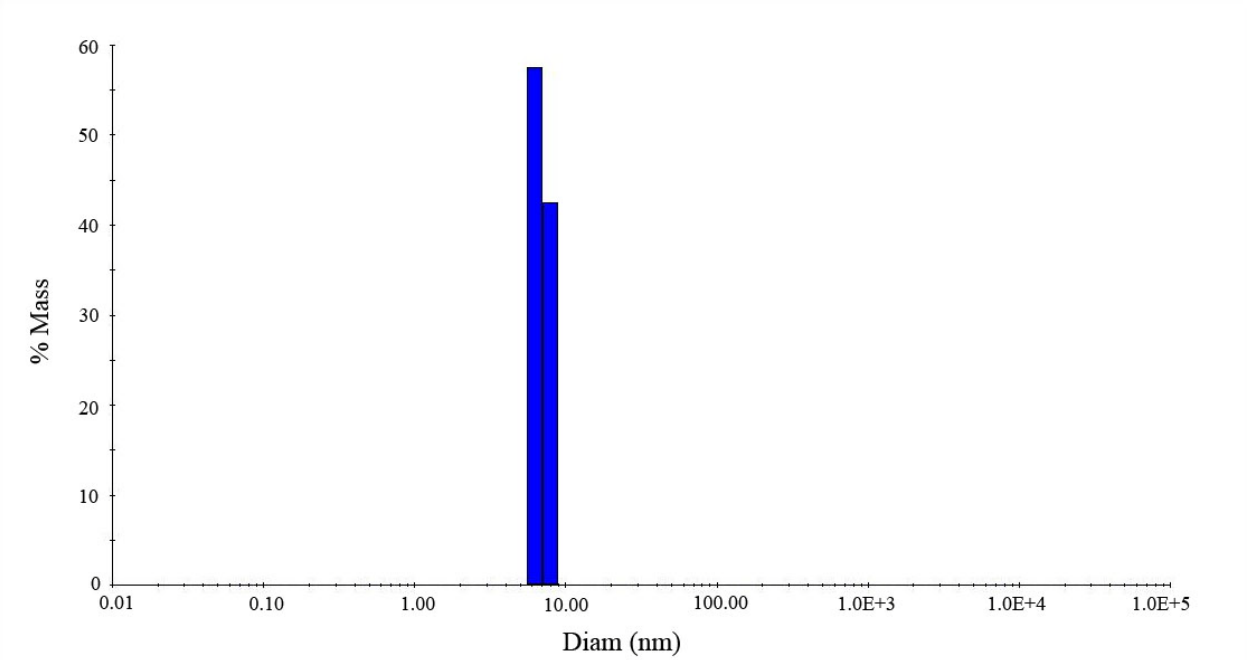


Figure *S*-18. DLS histogram of cavitand **1** with Gd complex **2a.**

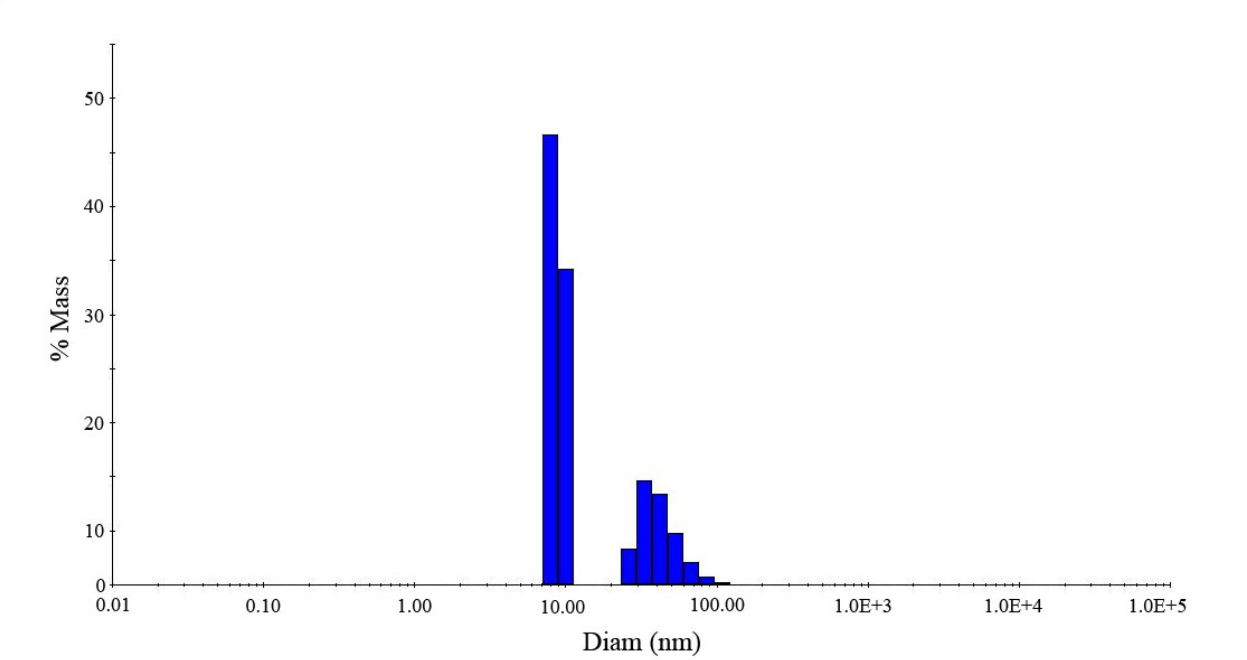


Figure *S*-19. DLS histogram of cavitand **1** with Gd complex **2a** upon exposure to choline.

[1] M. J. S. Dewar, E. G. Zoebisch, E. F. Healy and J. J. P. Stewart, *J. Am. Chem. Soc.* 1985, **107**, 3902-3909; calculations performed on SPARTAN 06, Wavefunction Inc.
[2] S. M. Biros, E. C. Ullrich, F. Hof, L. Trembleau and J. Rebek, Jr. *J. Am. Chem. Soc.* 2004, **126**, 2870-2876.
[3] A. Barge, G. Cravotto, E. Gianolio and F. Fedeli, *Contrast Media Mol. Imaging* 2006, **1**, 184-188.