Improved Efficacy of Synthesizing *M^{III}-labeled DOTA Complexes in Binary Mixtures of Water and Organic Solvents. A Combined Radio- and Physico-Chemical Study

Marylaine Pérez-Malo,[†,‡] Gergely Szabó,[⊥,‡] Elisabeth Eppard,[†,‡] Adrienn Vagner,[⊥] Ernő Brücher,[⊥] Imre Tóth,[⊥] Alessandro Maiocchi,[§] Eul Hyun Suh,[‰] Zoltán Kovács,[‰] Zsolt Baranyai,[*,⊥,§] and Frank Rösch[*,†]

[†] Institute of Nuclear Chemistry, Johannes Gutenberg-University of Mainz, Fritz-Strassmann-Weg 2, D-55128 Mainz, Germany

[⊥] Department of Inorganic and Analytical Chemistry, Faculty of Science and Technology, University of Debrecen, Debrecen, Egyetem tér 1, H-4032, Hungary

[§] Bracco Imaging spa, Bracco Research Centre, Via Ribes 5, 10010 Colleretto Giacosa (TO), Italy

‰Advanced Imaging Research Center, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390 (US)

*Corresponding Authors:
Frank Rösch
Institute of Nuclear Chemistry, University of Mainz, Fritz-Strassmann-Weg 2, 55128 Mainz, Germany
tel. 06131-392 5302
fax 06131-392 4692
E-mail: frank.roesch@uni-mainz.de

Zsolt Baranyai
Department of Inorganic and Analytical Chemistry, University of Debrecen, H-4010, Debrecen, Egyetem tér 1., Hungary
Present address: Bracco Imaging – CRB/Trieste, Area Science Park. Ed. Q, SS 14, km 163.5, I-34149, Basovizza Trieste, Italy
tel. 040-3757842
fax 040-3757831
E-mail: zsolt.baranyai@bracco.com

EXPERIMENTAL PROCEDURES

Chemicals & Equipment

The macrocyclic chelator DOTA was obtained from ABX (Radeberg, Germany) and aqueous stock solutions of 1 mg/mL was prepared. All other chemicals including the non-aqueous solvents ethanol (EtOH), isopropanol (iPrOH) and acetonitrile (MeCN) were pure reagent grade (Sigma-Aldrich, Germany). TraceSelect water (Sigma-Aldrich, Germany) was used for all radiolabeling solutions.

Alumina-backed silica gel (silica-gel 60 F254; Merck, Darmstadt, Germany) was used for radio-thin-layer chromatography (TLC). Complex formation analyses were performed using an electronic autoradiography system and associated software (Instant Imager, Packard Canberra, Schwadorf, Austria). Additionally, radiolabeling yields were analysed using high-performance liquid chromatography (HPLC) with LiChrosphere 100 RP EC column (5µm, 250 x 4 mm). HPLC was performed using Hitachi L-7100 pump system coupled with UV (Hitachi L-7400) and radiometric (Gaby Star, Elysia-Raytest, Straubenhardt, Germany) detectors. Solvents for HPLC were obtained as HPLC grade and degassed by ultra-sonication for 15-20 min directly before use.

Radiometals *M^{III} and mixtures of initial aqueous solution and non-aqueous solvents

Gallium-68. A commercial 1,100 MBq generator with ^{68}Ge^{IV} adsorbed on a TiO_2 matrix by Cyclotron Co. Ltd. (Obninsk, Russian Federation) purchased from EZAG, Berlin, Germany was used. Gallium-68 was eluted with 5 mL 0.1 N HCl. For purification, the eluate was trapped online on a micro chromatography column containing a cation exchange resin (Bio-Rad; AG 50W-X8, -400 mesh) and post-processed using acetone-based post-processing.[1] The purified eluate in 400 µL comprised of 97.56 % acetone and 0.05 M HCl (60-100 MBq/of gallium-68) was used for further radiolabeling studies.

For the initial radiolabeling in pure aqueous solution 3 mL water was used. To this solution chelator in water and gallium-68 in 400 µL 97.56 % acetone/0.05 N HCl were added (Figure S1a). To evaluate the effect of organic solvents, 10-40 vol% of the water in the initial solution was substituted by EtOH, iPrOH or MeCN (Figure S1b).

Scandium-44. Scandium-44 was obtained from a prototype 180 MBq ^{44}Ti/^{44}Sc-generator and was eluted with 20 mL of a 0.005 M $H_2C_2O_4$/0.07 M HCl solution.[2] Scandium-44 was trapped online on a cation exchange resin (Bio-Rad, AG 50W-X8, 200-400 mesh) and post-processed following the previously reported procedure.[3] Purified scandium-44 was obtained in 3 mL ammonium acetate buffer (0.25 M, pH 4) in batch radioactivities of 120-160 MBq. To 300 µL of this aqueous buffer solution containing scandium-44 the chelator, dissolved in water, was added. To evaluate the effect of organic solvents on scandium-44 radiolabeling performances, 10-40 vol% buffer in the initial solution was substituted by EtOH, iPrOH and meCN.

Lutetium-177. No-carrier-added lutetium-177 (n.c.a. in 0.05 mol/L HCl), produced indirectly via the ^{176}Yb(n,γ)^{177}Yb→^{177}Lu reaction,[4] was purchased from Isotope Technologies Garching (ITG, Munich, Germany) with a specific radioactivity of > 3000 GBq/mg in 0.05 N HCl. For each experiment 100 µL of 0.2 MBq/µL ^{177}Lu-solution in 0.05 N HCl were used. For the initial radiolabeling in pure aqueous solution 400 µL sodium acetate buffer (0.1 M, pH 8) was used. To this solution the chelator dissolved in water was added. The effect of the organic solvents was determined as described for gallium-68 and scandium-44.

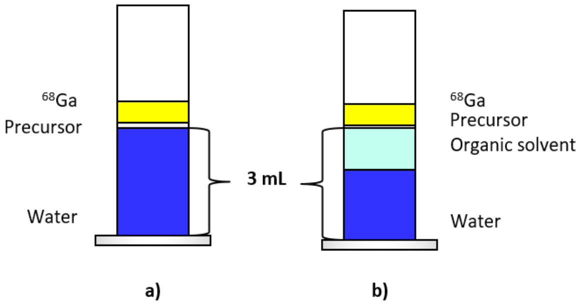

Figure S1: Composition of the initial aqueous radiolabeling solution (a) and the mixtures with additional organic solvents (b). Blue: water; grey: chelator in water; yellow: ^{68}Ga-eluate; turquoise: organic solvent.

Synthesis of *M^{III}-radiolabeled complexes

[^{68}Ga]Ga-DOTA. Different amounts (0-40 vol%) of non-aqueous solvents were investigated containing 10 nmol DOTA in all mixtures. The post-processed ^{68}Ga-eluate (100 µL) was added to the mixture (3 mL) in the open glass vial followed by agitation in a heating block for 15 min at 70°C and as a reference at 95°C (referred to as “standard” condition).

The effect of DOTA content on the radiolabeling process was also investigated at constant amount of EtOH (30 vol%) varying the DOTA content (0.1 – 20 µM).

[^{44}Sc]Sc-DOTA. Radiolabeling with scandium-44 was performed by mixing 10 nmol DOTA with 3 mL post-processed ^{44}Sc-eluate containing non-aqueous solvent (0-40 vol%), followed by agitation in an open glass vial in a heating block at 70°C for 20 min.

[^{177}Lu]Lu-DOTA. Lutetium-177 (100 µL) was added to 0.5 nmol DOTA in 0.1 M sodium acetate buffer (400 µL, pH 8). Non-aqueous solvents (0-40 vol%) were added to the reaction mixture in an open 2 mL Eppendorf vial, which was stirred in a heating block at 70°C for 30 min. The stoichiometric ratio of chelator to lutetium-177 was 10:1.

Quality control

Aliquots were taken at different time points to analyze radiochemical yields. RadioTLC was performed using aliquots of each reaction spotted on TLC plates and developed in 0.1 M sodium citrate buffer (pH°4). Analyses was performed using a flatbed scanner.

RadioHPLC was performed using a gradient elution system comprising mobile phase A (deionized H_2O + 0.01% TFA) and mobile phase B (100 % MeCN). At a flow rate of 0.8 ml/min starting with 82% A/18% B for 2 min; the gradient was increased to 30% B over the next 25 min and then held at 30% B for 6 min. Afterwards gradient parameters returned to the initial conditions during 2 min.

Materials of the equilibrium and kinetic studies

The chemicals used for the experiments were of the highest analytical grade. The $LnCl_3$ solutions were prepared from $LnCl_3{\cdot}xH_2O$ (x=5-7) (Aldrich; 99.9%). $ScCl_3$ stock solution was prepared by dissolving Sc_2O_3 (99.9%, Fluka) in 6 M HCl and evaporating the acid excess. The concentration of the $LnCl_3$ and $ScCl_3$ solutions were determined by complexometric titration with standardized Na_2H_2edta and xylenol orange as indicator. $Ga(NO_3)_3$ solution was prepared by dissolving Ga_2O_3 (99.9%, Fluka) in 6 M HNO_3 and evaporating of the excess acid. The solid $Ga(NO_3)_3$ was dissolved in 0.1 M HNO_3 solution. The concentration of the $Ga(NO_3)_3$ solution was determined by using the standardized Na_2H_2EDTA in excess. The excess of the Na_2H_2EDTA was measured with standardized $ZnCl_2$ solution and xylenol orange as indicator. The H^+ concentration of the $Ga(NO_3)_3$ solution was determined by pH potentiometric titration in the presence of Na_2H_2EDTA excess. The concentration of the H_4DOTA solution was determined by pH-potentiometric titration in the presence and absence of a large (40-fold) excess of $CaCl_2$.

Equilibrium measurements

For determining the protonation constants of DOTA chelator three parallel pH-potentiometric titrations were made with 0.2 M NaOH in 0.002 M chelator solutions. The pH-potentiometric titrations were carried out with a Metrohm 785 DMP Titrino titration workstation with the use of a Metrohm-6.0233.100 combined electrode. The titrated solution (8 mL) was thermostated at 25°C. The samples were stirred with a magnetic stirrer and to avoid the effect of CO_2, N_2 gas was bubbled through the solutions. The titrations were made in the pH range 1.7-12.2. For the calibration of the pH meter, 0.05 M KH-phthalate (pH=4.005) and 0.01 M borax (pH=9.177) buffers were used. For the calculation of the H^+ concentration from the measured pH values, the method proposed by Irving et al. was used.[5] A 0.01 M HCl solution was titrated with 0.2 M NaOH at each water/ethanol mixtures in the presence of 0.15 M NaCl to maintain a constant ionic strength. The preparation of the water/ethanol solutions was made by considering the density and the m/m% concentration of mixtures and by measuring the weight of ethanol (96 vol%). The composition (vol% and m/m %) and density of the ethanol/water mixtures are shown in Table S1. The differences (A) between the measured (pH_{read}) and calculated pH (-log[H^+]) values were used to obtain the equilibrium H^+

concentration from the pH values, measured in the titration experiments. The ionic product of water (pK_w) was also determined at each water/ethanol mixtures in the presence of 0.15 M NaCl. The pA and pK_w values obtained in the different water/ethanol mixtures are presented in Table S1. For the calculation of the protonation constants, the PSEQUAD program was used.[6]

Kinetic studies

Formation rates of $[Ce(DOTA)]^-$ and $[Eu(DOTA)]^-$ were studied by spectrophotometry at 320 and 255 nm, respectively. The formation of $[Ga(DOTA)]^-$, $[Y(DOTA)]^-$ and $[Lu(DOTA)]^-$ was monitored via the release of H^+ ions from the chelator by spectrophotometry with the use of 2,4-dinitrophenol (pH=2.5–2.7, λ=410 nm), methylorange (pH=2.9–3.5, λ=508 nm), bromophenol blue (pH=3.5–4.1, λ=592 nm) and bromocresolgreen (pH=4.4–7.0, λ=616 nm) indicators.[7] The formation reactions were studied with a Cary 1E spectrophotometer (pH=2,5–4,7) and an Applied Photophisics DX-17MV stopped-flow instrument (pH=5.0–7.0) at 25°C in the presence of 0.15 M NaCl. In the presence of 0.01 M buffers (Dichloroacetic acid (DCA), Chloroacetic acid (CA), N-methyl-piperazine (NMP), N,N-dimethyl-piperazine (DMP), MES) in the required concentration ([DCA]=[CA]=[NMP]=[DMP]=[MES]=0.01 M , 0.15 M NaCl) , the decrease of pH was about 0.07–0.1 pH unit. The formation of Ce^{III}- and Eu^{III}-complexes were studied in the presence of a 5 to 40 fold metal ion excess in order to assure the pseudo-first-order conditions ([DOTA]= 2×10^{-4} M). The kinetic studies of Ga^{3+}-DOTA, Y^{3+}-DOTA and Lu^{3+}-DOTA systems have been performed in the presence of DOTA excess, when the solution concentrations meet the requirements for the pseudo-first-order conditions. The concentrations of DOTA were 5–30 times higher than those of Ga^{3+}, Y^{3+} and Lu^{3+} ($[Ga^{3+}]=[Y^{3+}]=[Lu^{3+}]=2.0\times10^{-4}$ M). The pseudo-first-order rate constants (k_{obs}) were calculated by fitting the absorbance values to the equation (1):

$$A_t = (A_0 - A_e)e^{(-k_{obs}t)} + A_e \quad (1)$$

where A_0, A_e and A_t are the absorbance values at the start (t=0 s) , at equilibrium and at the t time of the reaction, respectively. The calculations were performed by using the computer program Micromath Scientist, version 2.0 (Salt Lake City, UT, USA).

NMR experiments

^{1}H-, ^{45}Sc- and ^{71}Ga-NMR measurement were performed with a Bruker DRX 400 (9.4 T) spectrometer equipped with a Bruker VT-1000 thermocontroller and a BB inverse z gradient probe (5 mm). The formation of the [Ga(DOTA)]$^-$, [Y(DOTA)]$^-$ and [Lu(DOTA)]$^-$ complexes was followed by ^{1}H-NMR spectroscopy. Ga(NO$_3$)$_3$, YCl$_3$, LuCl$_3$ and DOTA solutions (3 mL) were prepared in D$_2$O ([Ga^{3+}]=[Y^{3+}]=[Lu^{3+}]=0.03 M, [DOTA]=0.015 M). For keeping the pH values constant, dichloroacetic acid (Ga^{3+}-DOTA system) and sodium-acetate (Y^{3+}-DOTA and Lu^{3+}-DOTA systems) buffers were used ([buffer]=0.1 M). The temperature was maintained at 298 K and the ionic strength of the solutions was kept constant (0.15 M NaCl). The longitudinal relaxation time of ^{45}Sc and ^{71}Ga nuclei was measured with the “inversion recovery” method (180° - τ - 90°) by using 10 different τ values. The measurements were performed with 20 mM Ga(NO$_3$)$_3$ and ScCl$_3$ solution prepared in water and 10, 40 and 60 vol% EtOH-water mixtures. In order to prevent the hydrolysis of Ga^{3+} and Sc^{3+} ions the solutions were of 1.4 M and 1.0 M for HNO$_3$, respectively. Due to the extremely long longitudinal relaxation time of the ^{89}Y nucleus, the ^{89}Y T$_1$ relaxation was measured using hyperpolarized YCl$_3$ samples. ^{89}Y dynamic nuclear polarization (DNP) was performed using the HyperSense commercial polarizer as described in the literature.[8-9] The DNP samples were made up by dissolving YCl$_3$ 6 H$_2$O in water glycerol (1:1) matrix and adding the polarizing agent trityl OX063 radical (15 mM in the matrix). The samples were polarized for 2 hours in the HyperSense polarizer and then dissolved with superheated water/ethanol mixtures. The solutions were acidified by adding concentrated HNO$_3$ to the samples after dissolution (to a final concentration of 0.5 M). The final concentration of YCl$_3$ in the dissolution liquid was approximately 30 mM). NMR spectra were recorded on a Varian 9.4 T spectrometer using a 10 degree flip angle. The temperature of the sample holder was maintained at 298 K with a thermostated air stream. The ^{89}Y T_1 value was determined from the decay of hyperpolarized ^{89}Y magnetization as described in the literature.[8-9] The processing of the NMR spectra was performed by using the Bruker WIN-NMR software package.

Table S1. Differences (p*A*) between the measured (pH_{read}) and calculated pH ($-\log[H^+]$) and the ionic product of water (pK_w) in ethanol/water mixtures (0.15 M NaCl, 25°C)

vol %	m/m%	ρ (g/ml)	p*A*	p*K*w
0	0	0.99708	0.014	13.834
10.6	7.83	0.98727	0.042	13.961
20.6	15.8	0.97537	0.056	14.106
31.4	25.0	0.95968	0.102	14.192
41.5	34.1	0.94247	0.189	14.271
52.4	44.7	0.92091	0.272	14.346
65.1	57.8	0.89185	0.336	14.511
73.4	66.8	0.87072	0.365	14.644

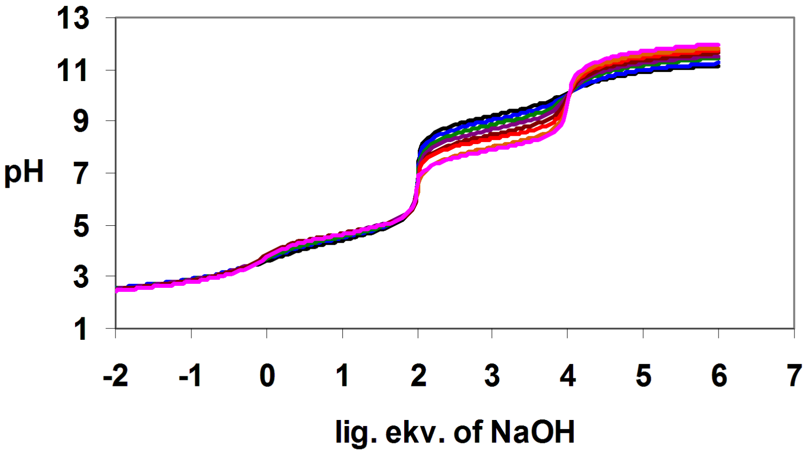

Figure S2. pH-potentiometric titration data of H_4DOTA in ethanol/water mixtures ($[H_4DOTA]$=2.00 mM, [HCl]=4.00 mM, **0**; **10.6**, **20.6**, **31.4**, **41.3**, **52.4**, **65.1** and **73.4** vol% EtOH, 0.15 M NaCl, 25 °C)

Table S2. Protonation constant of H_4DOTA chelator in ethanol/water mixtures (0.15 M NaCl, 25°C)

EtOH (vol%)	0	10.6	20.6	31.4	41.3	52.4	65.1	73.4
$\log K_1^H$	9.23 (1)	9.03 (2)	8.85 (1)	8.66 (1)	8.42 (1)	8.08 (2)	7.90 (2)	7.67 (1)
$\log K_2^H$	9.21 (2)	9.07 (1)	8.88 (1)	8.73 (1)	8.53 (2)	8.47 (2)	8.09 (2)	8.13 (2)
$\log K_3^H$	4.51 (1)	4.54 (2)	4.53 (2)	4.52 (2)	4.36 (1)	4.16 (2)		
$\log K_4^H$	4.26 (3)	4.43 (2)	4.54 (2)	4.77 (1)	4.95 (2)	5.36 (2)		
$\log K_5^H$	2.36 (2)	2.46 (1)	2.25 (2)	2.24 (3)	1.98 (1)			
$\log K_6^H$	1.49 (1)	1.41 (3)	1.34 (3)	1.48 (3)	1.53 (3)			

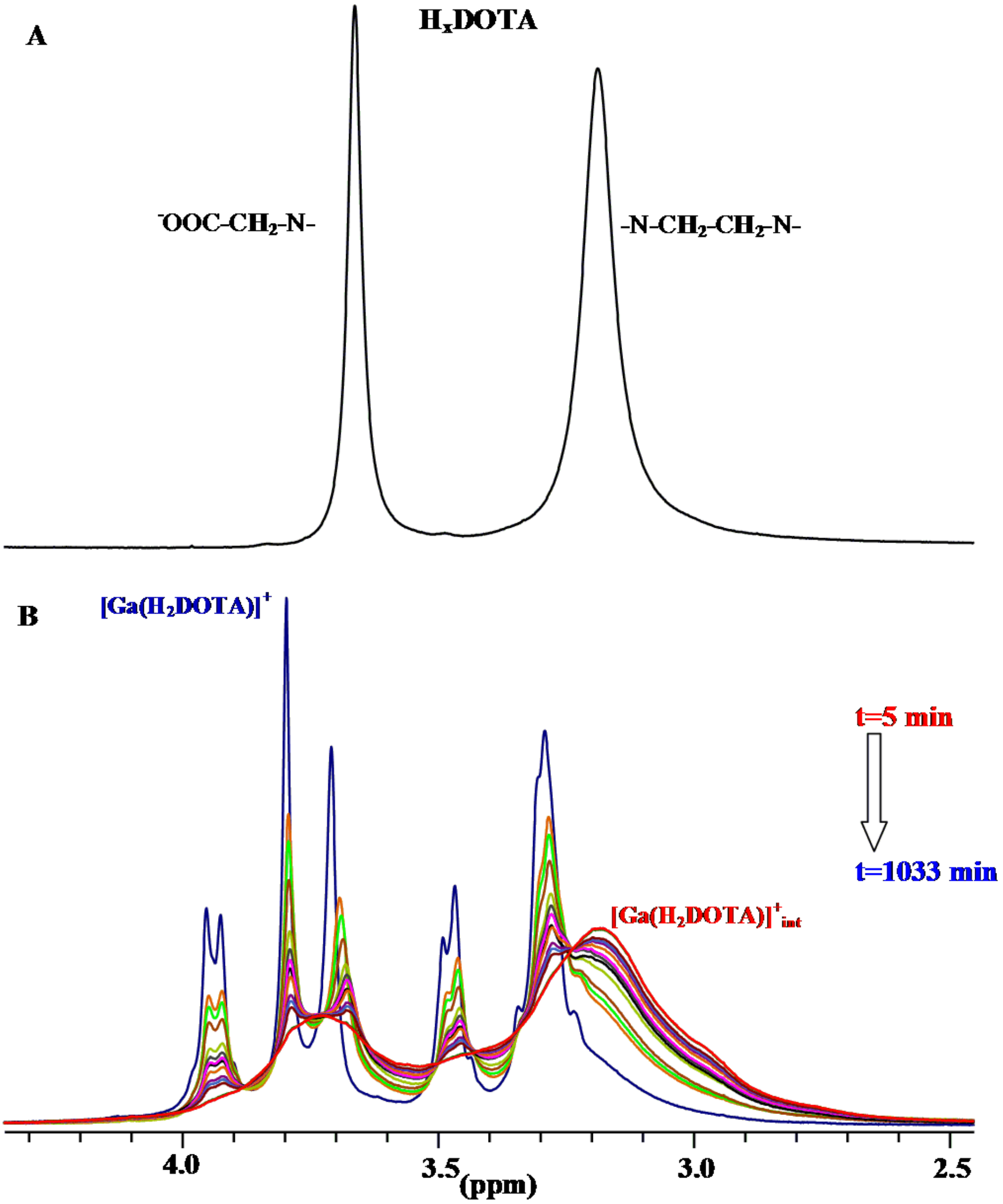

Figure S3. 400 MHz ^1H-NMR spectra of H_xDOTA (**A**) and Ga^{3+}-DOTA systems (**B**) [H_xDOTA]=0.015 M; **B**: [Ga^{3+}]= 0.03 M, [H_xDOTA]=0.015 M, x=5, 6; pH=2.0; [DCA]=0.1 M, 0.15 M NaCl, 298 K)

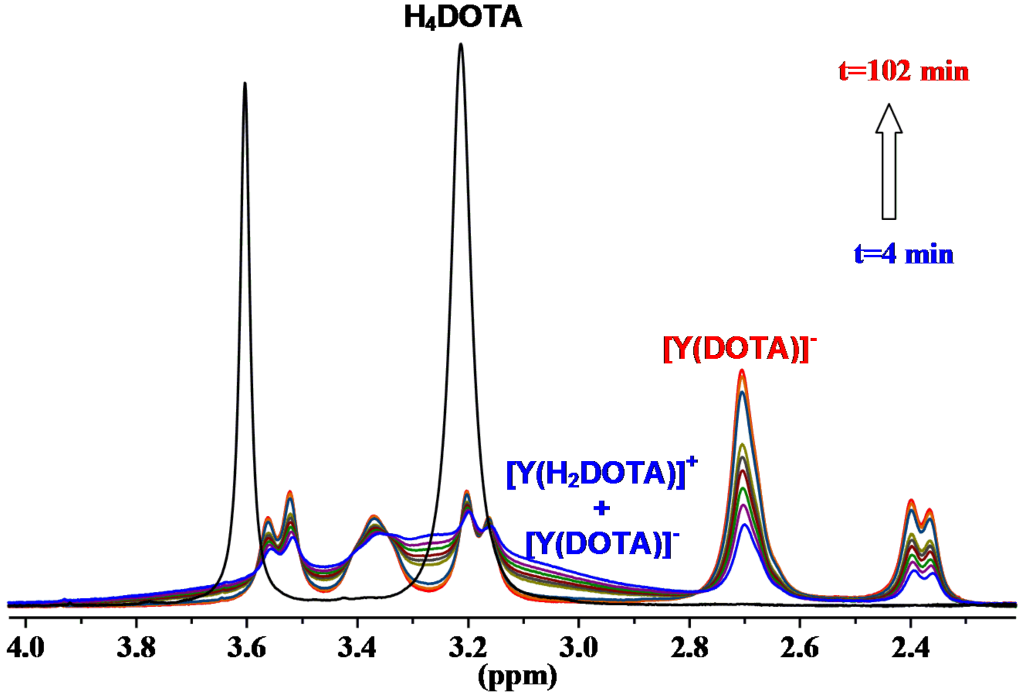

Figure S4. 400 MHz ^{1}H-NMR spectra of Y^{3+}-DOTA systems ($[Y^{3+}]$= 0.03 M, $[H_xDOTA]$=0.015 M, x=3, 4; [NaAc]=0.1 M, pH=4.50, 0.15 M NaCl, 298 K)

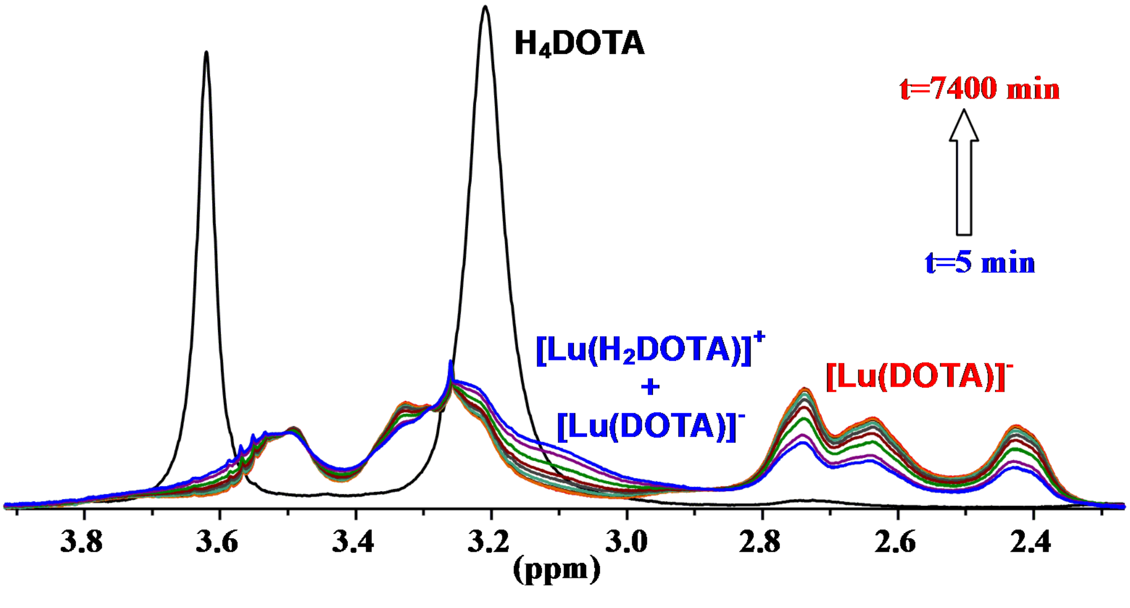

Figure S5. 400 MHz ^{1}H-NMR spectra of Lu^{3+}-DOTA systems ($[Lu^{3+}]$= 0.03 M, $[H_xDOTA]$=0.015 M, x=4, 5; [NaAc]=0.1 M, pH=3.60, 0,15 M NaCl, 298 K)

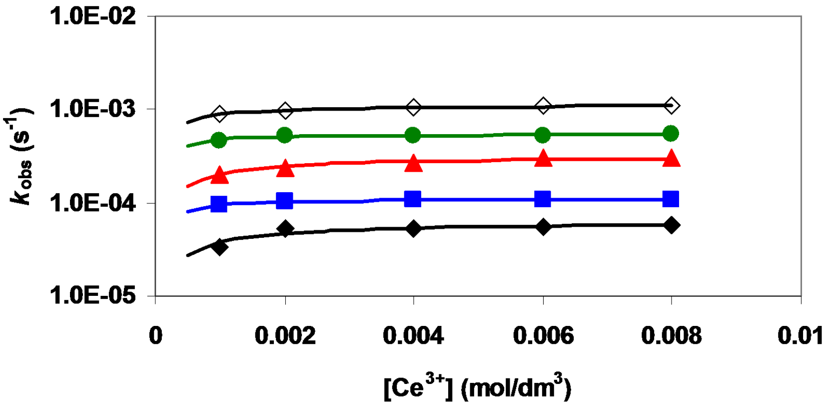

Figure S6. k_{obs} values obtained for the formation reaction of [Ce(**DOTA**)]$^-$ in 10 vol% EtOH solution. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (5). (pH=3.50 (◆), 3.75 (■), 4.11 (▲), 4.43 (●) and 4.71 (◇), [DOTA]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

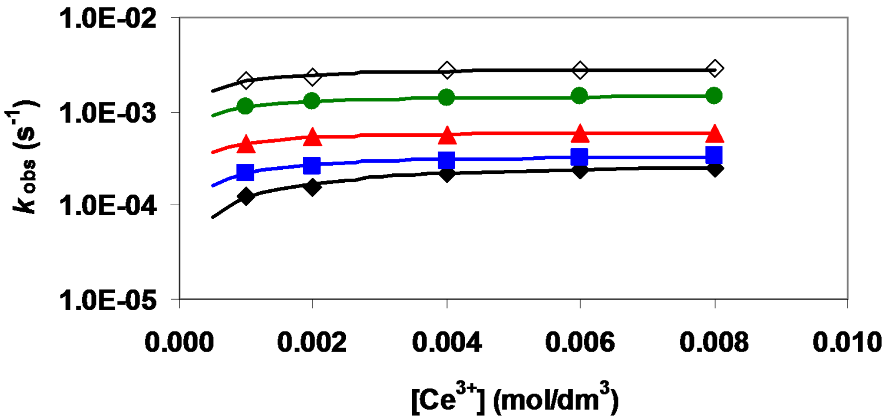

Figure S7. k_{obs} values obtained for the formation reaction of [Ce(**DOTA**)]$^-$ in 40 vol% EtOH solution. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (5). (pH=3.60 (◆), 3.90 (■), 4.22 (▲), 4.51 (●) and 4.75 (◇), [DOTA]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

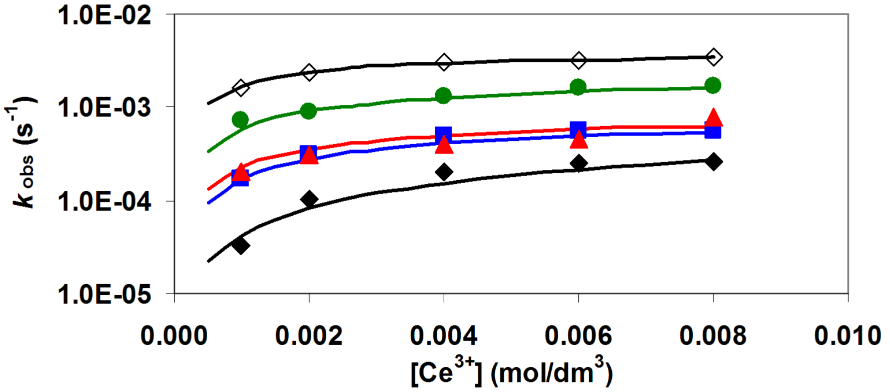

Figure S8. k_{obs} values obtained for the formation reaction of [Ce(**DOTA**)]$^-$ in 70 vol% EtOH solution. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (5). (pH=3.47 (◆), 3.84 (■), 4.19 (▲), 4.46 (●) and 4.77 (◇), [DOTA]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

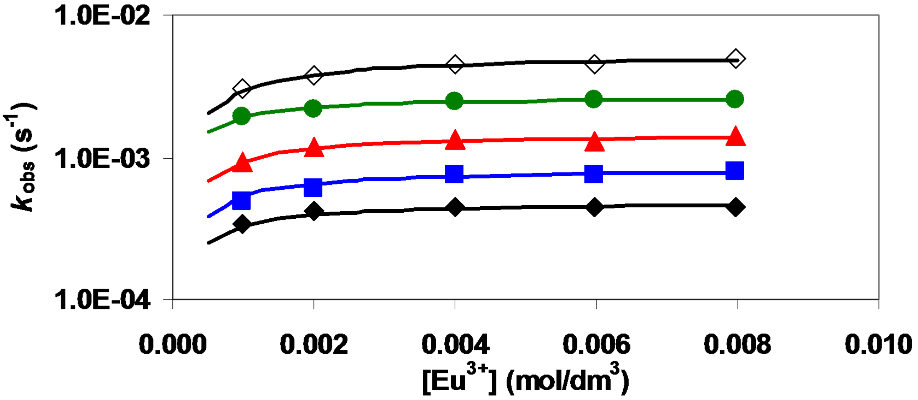

Figure S9. k_{obs} values obtained for the formation reaction of [Eu(**DOTA**)]$^-$ in 10 vol% EtOH solution. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (5). (pH=3.25 (◆), 3.48 (■), 3.81 (▲), 4.12 (●) and 4.39 (◇), [DOTA]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

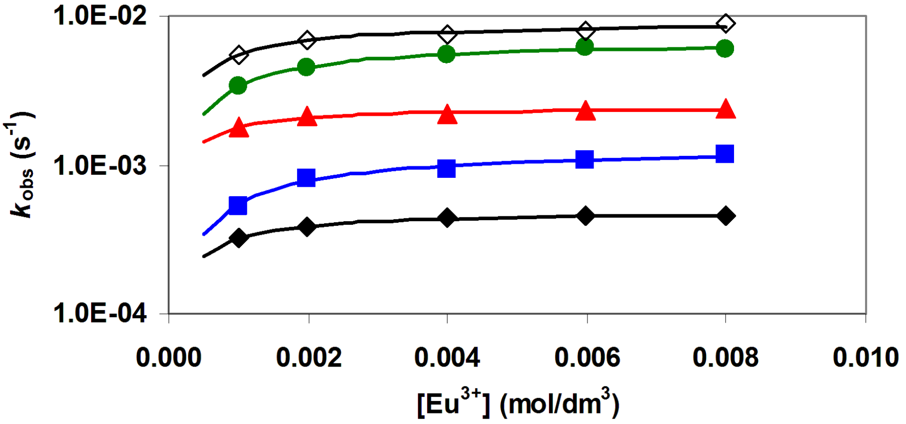

Figure S10. k_{obs} values obtained for the formation reaction of [Eu(**DOTA**)]$^-$ in 40 vol% EtOH solution. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (5). (pH=3.15 (◆), 3.55 (■), 3.93 (▲), 4.22 (●) and 4.51 (◇), [DOTA]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

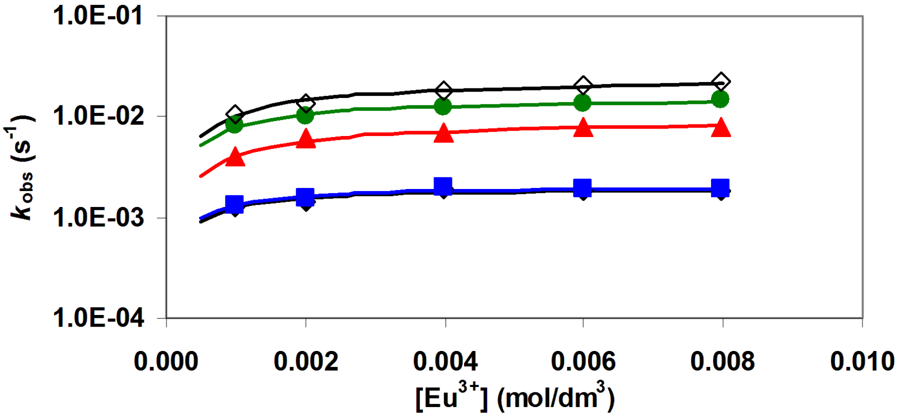

Figure S11. k_{obs} values obtained for the formation reaction of [Eu(**DOTA**)]$^-$ in 70 vol% EtOH solution. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (5). (pH=3.82 (◆), 3.91 (■), 4.31 (▲), 4.54 (●) and 4.68 (◇), [DOTA]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

Table S3. Stability constants ($\log K_{\mathrm{M(H_2L)}}$) of [M($H_2$DOTA)] intermediates ($M^{3+}$=$Ga^{3+}$, Ce^{3+}, Eu^{3+}, Y^{3+} and Lu^{3+}) in water and in 10, 40 and 70 vol% EtOH solutions 0.15 M NaCl, 25°C)

	H_2O	10 vol% EtOH	40 vol% EtOH	70 vol% EtOH
[Ga(H_2DOTA)]$^+$	6.32 (6)	6.50 (3)	7.11 (4)	–
[Ce(H_2DOTA)]$^+$	4.4 [a]	4.60 (5)	4.40 (2)	3.60 (4)
[Eu(H_2DOTA)]$^+$	4.3 [a]	4.80 (1)	4.80 (1)	4.01 (2)
[Y(H_2DOTA)]$^+$	4.60 (3)	4.28 (4)	4.20 (2)	–
[Lu(H_2DOTA)]$^+$	4.54 (7)	4.48 (3)	4.28 (5)	–

[a] Ref. 10

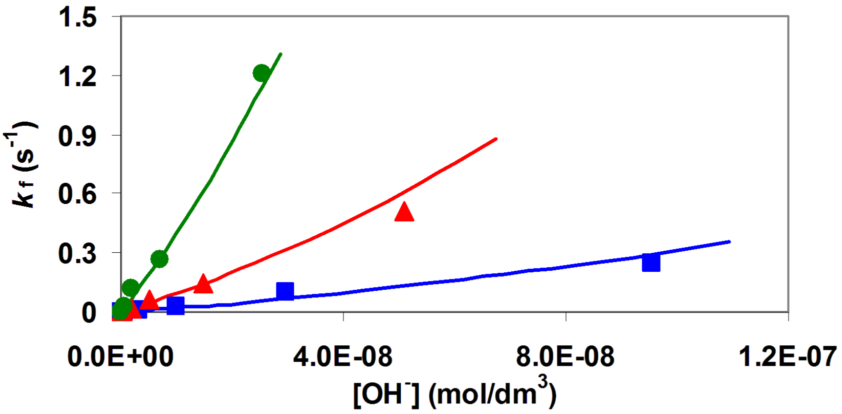

Figure S12. k_f values as a function of [OH$^-$] for the formation of [Ce(**DOTA**)]$^-$ in **10**, **40** and **70** vol% EtOH solutions. Symbols and solid lines represent experimental and calculated k_f values, respectively. Calculations have been performed by the use of Eq. (13). (0.15 M NaCl, 25°C)

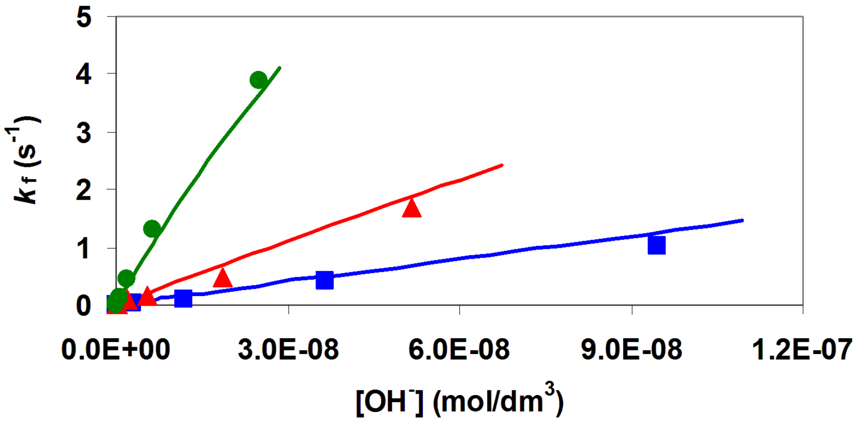

Figure S13. k_f values as a function of [OH$^-$] for the formation of [Eu(**DOTA**)]$^-$ in **10**, **40** and **70** vol% EtOH solutions. Symbols and solid lines represent experimental and calculated k_f values, respectively. Calculations have been performed by the use of Eq. (13). (0.15 M NaCl, 25°C)

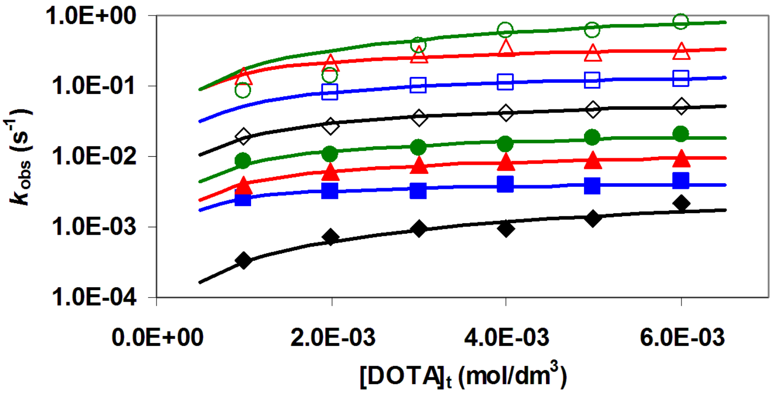

Figure S14. k_{obs} values obtained for the formation reaction of [Ga(**DOTA**)]$^-$ in water. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (10). (pH=2.65 (◆), 2.95 (■), 3.17 (▲), 3.48 (●), 3.69 (◇), 4.00 (□), 4.27 (△) and 4.65 (○), [Ga^{3+}]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

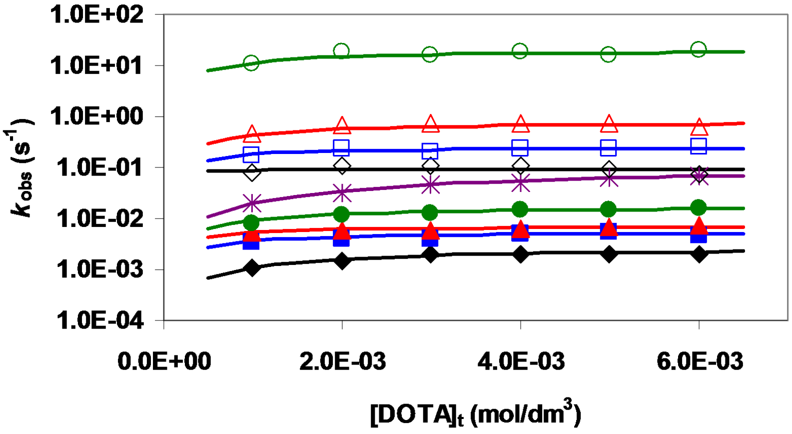

Figure S15. k_{obs} values obtained for the formation reaction of [Ga(**DOTA**)]$^-$ in 10 vol% EtOH. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (10). (pH=2.65 (◆), 2.95 (■), 3.17 (▲), 3.48 (●), 3.70 (✳), 4.01 (◇), 4.27 (□), 4.65 (△) and 4.94 (○), [Ga^{3+}]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

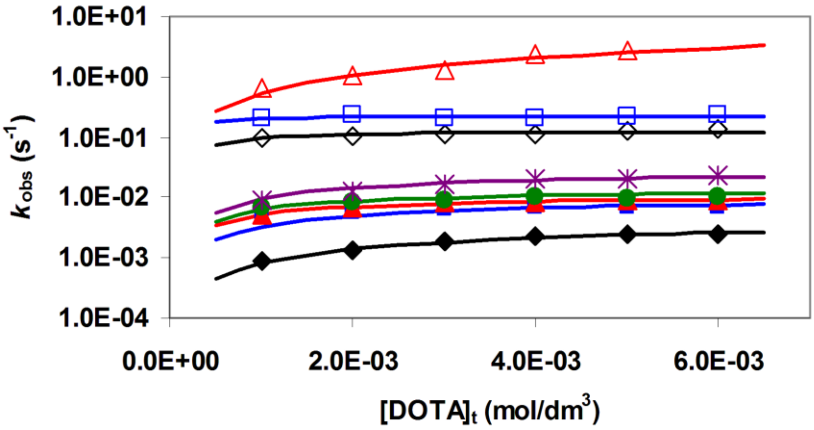

Figure S16. k_{obs} values obtained for the formation reaction of [Ga(**DOTA**)]$^-$ in 40 vol% EtOH. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (10). (pH=2.69 (◆), 2.93 (■), 3.13 (▲), 3.35 (●), 3.60 (✳), 3.94 (◇), 4.21 (□) and 4.68 (△), [Ga^{3+}]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

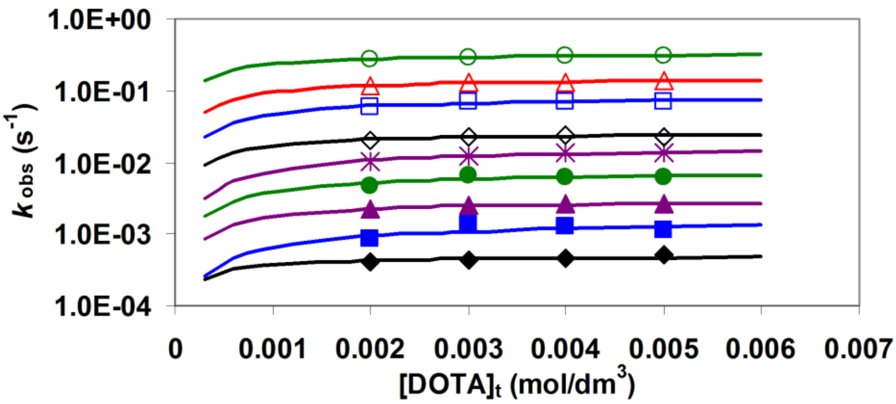

Figure S17. k_{obs} values obtained for the formation reaction of [Y(**DOTA**)]$^-$ in water. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (10). (pH=3.55 (◆), 3.85 (■), 4.13 (▲), 4.49 (●), 4.82 (✳), 5.08 (◇), 5.41 (□), 5.68 (△) and 6.04 (○), [Y^{3+}]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

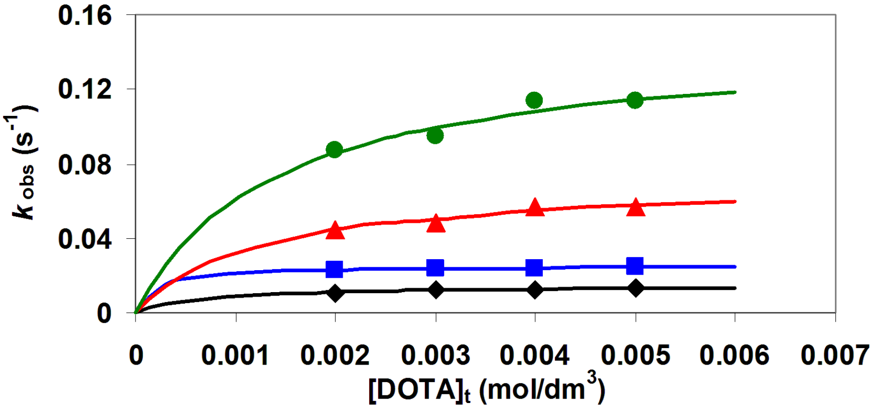

Figure S18. k_{obs} values obtained for the formation reaction of [Y(**DOTA**)]$^-$ in 10 vol% EtOH solution. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (10). (pH=4.72 (◆), 5.02 (■), 5.40 (▲) and 5.66 (●), [Y^{3+}]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

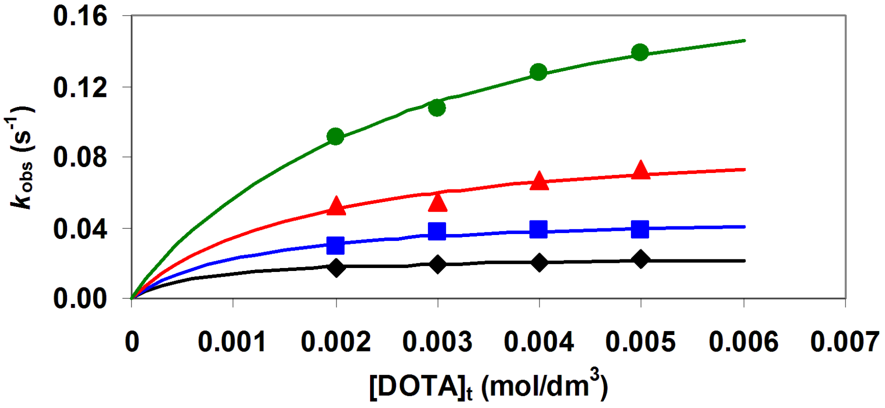

Figure S19. k_{obs} values obtained for the formation reaction of [Y(**DOTA**)]$^-$ in 40 vol% EtOH solution. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (10). (pH=4.83 (◆), 5.12 (■), 5.43 (▲) and 5.69 (●), [Y^{3+}]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

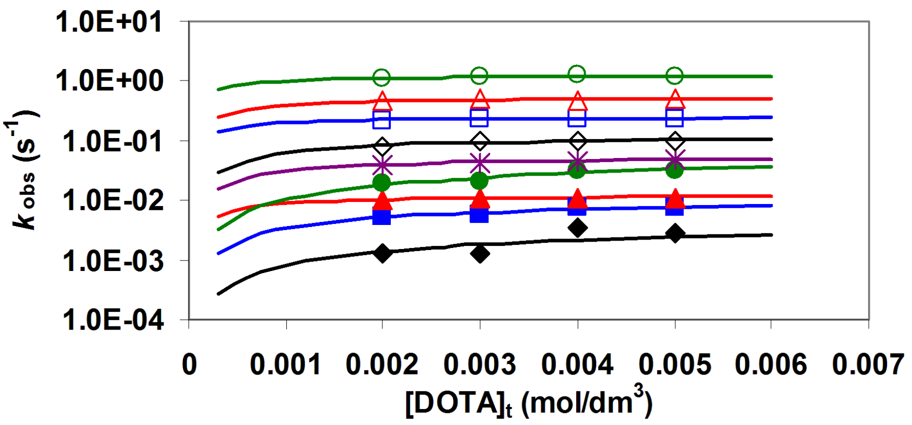

Figure S20. k_{obs} values obtained for the formation reaction of [Lu(**DOTA**)]$^-$ in water. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (10). (pH=3.57 (◆), 3.88 (■), 4.17 (▲), 4.53 (●), 4.74 (✳), 5.08 (◇), 5.41 (□), 5.67 (△) and 6.04 (○), [Lu^{3+}]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

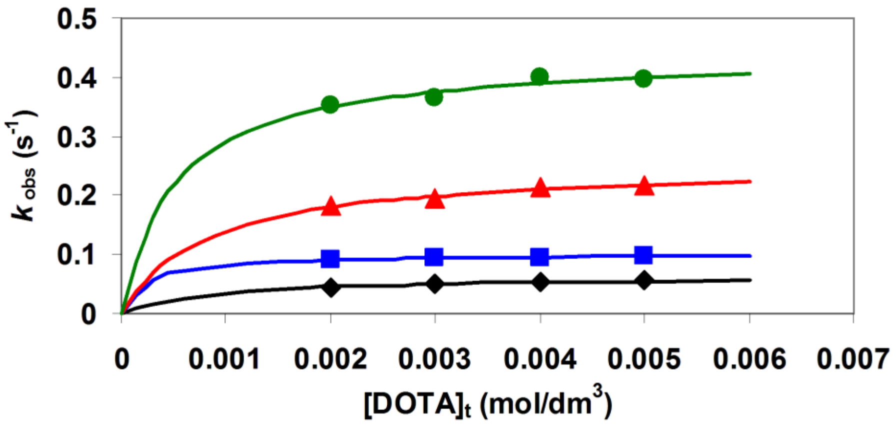

Figure S21. k_{obs} values obtained for the formation reaction of [Lu(**DOTA**)]$^-$ in 10 vol% EtOH solution. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (10). (pH=4.73 (◆), 5.04 (■), 5.40 (▲) and 5.65 (●), [Y^{3+}]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

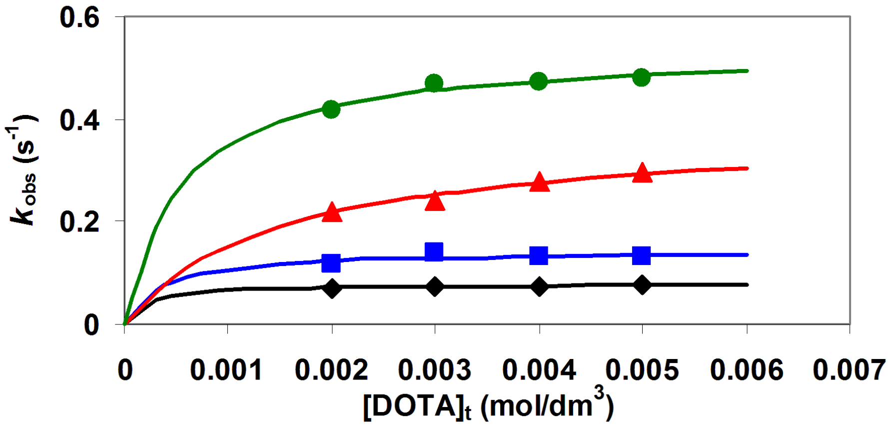

Figure S22. k_{obs} values obtained for the formation reaction of [Lu(**DOTA**)]$^-$ in 40 vol% EtOH solution. Symbols and solid lines represent experimental and calculated k_{obs} values, respectively. Calculations have been performed by the use of Eq. (10). (pH=4.84 (◆), 5.13 (■), 5.44 (▲) and 5.70 (●), [Y^{3+}]$_t$=0.2 mM, 0.15 M NaCl, 25°C)

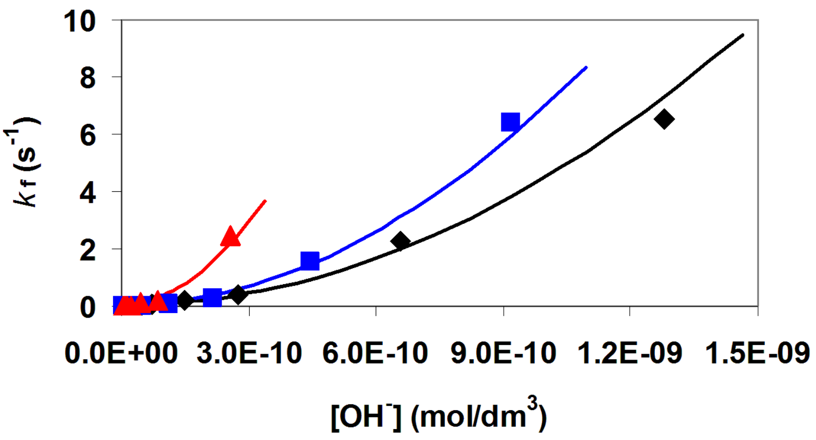

Figure S23. k_f values as a function of [OH$^-$] for the formation of [Ga(**DOTA**)]$^-$ in **water**, **10** and **40** vol% EtOH solutions. Symbols and solid lines represent experimental and calculated k_f values, respectively. Calculations have been performed by the use of Eq. (13). (0.15 M NaCl, 25°C)

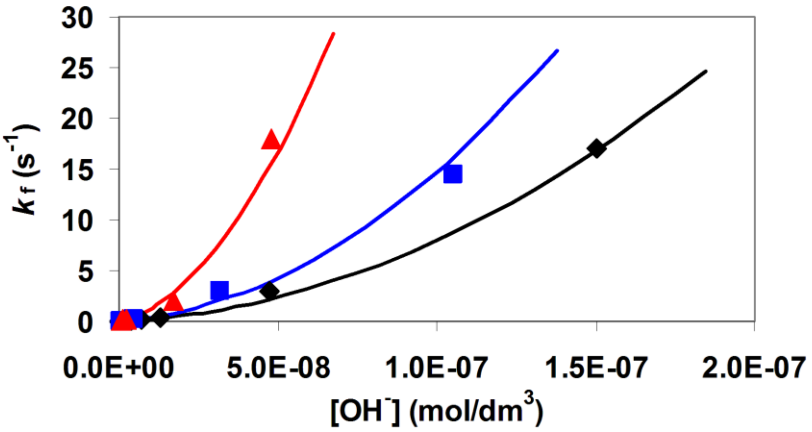

Figure S24. k_f values as a function of [OH$^-$] for the formation of [Y(**DOTA**)]$^-$ in **water**, **10** and **40** vol% EtOH solutions. Symbols and solid lines represent experimental and calculated k_f values, respectively. Calculations have been performed by the use of Eq. (13). (0.15 M NaCl, 25°C)

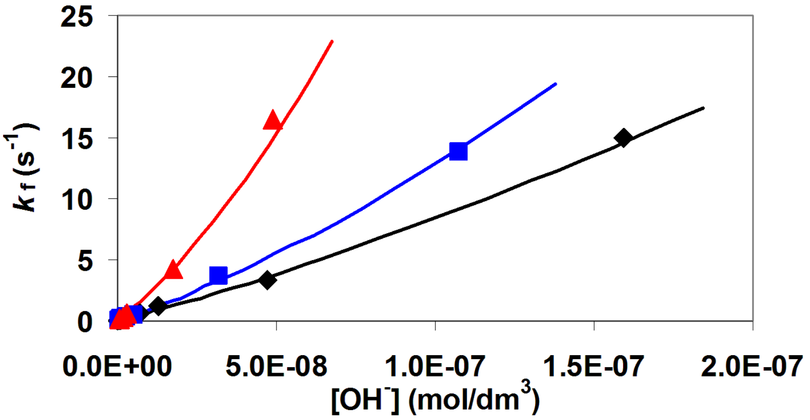

Figure S25. k_f values as a function of [OH$^-$] for the formation of [Lu(**DOTA**)]$^-$ in **water**, **10** and **40** vol% EtOH solutions. Symbols and solid lines represent experimental and calculated k_f values, respectively. Calculations have been performed by the use of Eq. (13). (0.15 M NaCl, 25°C)

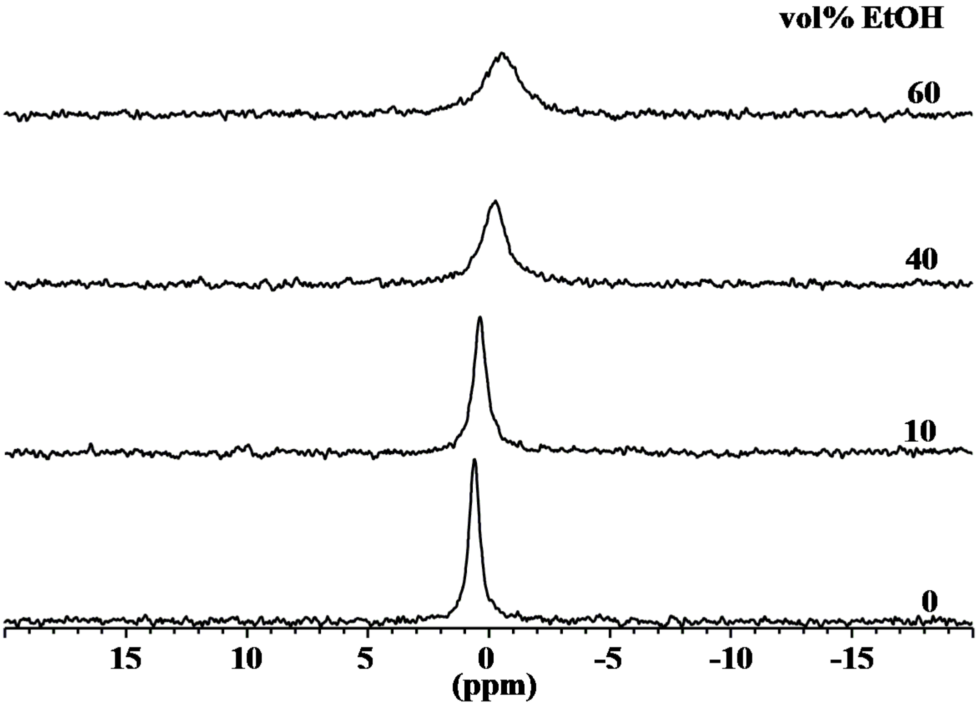

Figure S26. 122MHz ^{71}Ga-NMR spectra of Ga(NO$_3$)$_3$ solution in water and in 10, 40 and 60 vol% EtOH solutions ([Ga^{3+}]=0.020 M, [HNO$_3$]=1.4 M, 298 K)

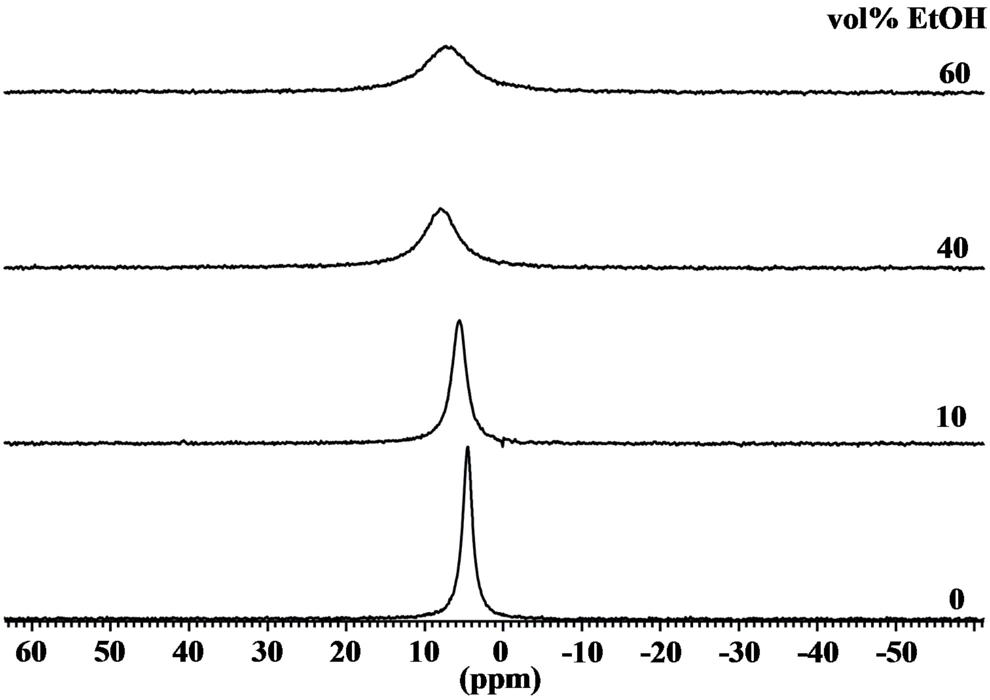

Figure S27. 97.2 MHz ^{45}Sc-NMR spectra of ScCl$_3$ solution in water and in 10, 40 and 60 vol% EtOH solutions ([Sc^{3+}]=0.020 M, [HNO$_3$]=1.0 M, 298 K)

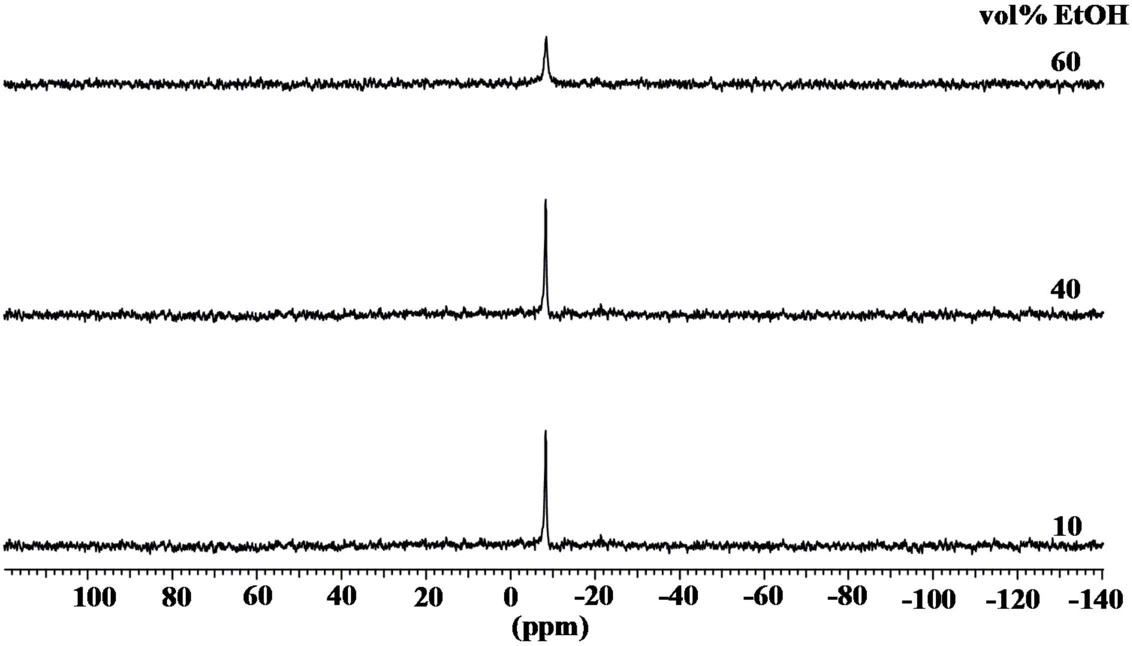

Figure S28. 19.6 MHz ^{89}Y-NMR spectra of YCl_3 solution in 10, 40 and 60 vol% EtOH solutions ($[Y^{3+}]$=0.030 M, $[HNO_3]$=0.5 M, 298 K)

References

(1) Zhernosekov, K. P.; Filosofov, D. V.; Baum, R. P.; Aschoff, P.; Bihl, H.; Razbash, A. A.; Jahn, M.; Jennewein, M.; Rosch, F., Processing of generator-produced 68Ga for medical application. *J. Nucl. Med.* **2007,** *48* (10), 1741-1748.

(2) Filosofov, D. V.; Loktionova, N. S.; Rösch, F., A 44Ti/44Sc radionuclide generator for potential application of 44Sc-based PET-radiopharmaceuticals. *Radiochim Acta* **2010,** *98* (3), 149-156.

(3) Pruszynski, M.; Majkowska-Pilip, A.; Loktionova, N. S.; Eppard, E.; Roesch, F., Radiolabeling of DOTATOC with the long-lived positron emitter 44Sc. *Appl. Radiat. Isot.* **2012,** *70* (6), 974-979.

(4) Lebedev, N. A.; Novgorodov, A. F.; Misiak, R.; Brockmann, J.; Rosch, F., Radiochemical separation of no-carrier-added 177Lu as produced via the 176Yb(n,gamma)177Yb-->177Lu process. *Appl. Radiat. Isot.* **2000,** *53* (3), 421-425.

(5) Irving, H. M.; Miles, M. G.; Pettit, L. D., A study of some problems in determining the stoicheiometric proton dissociation constants of complexes by potentiometric titrations using a glass electrode. *Anal. Chim. Acta* **1967,** *38* (0), 475-488.

(6) Zekany, L.; Nagypal, I., PSEQUAD. In *Computational Methods for the Determination of Formation Constants*, Leggett, D., Ed. Springer US: **1985**; pp 291-353.

(7) Cassatt, J. C.; Wilkins, R. G., The kinetics of reaction of nickel(II) ion with a variety of amino acids and pyridinecarboxylates. *J. Am. Chem. Soc.* **1968,** *90* (22), 6045-6050.

(8) Lumata, L.; Jindal, A. K.; Merritt, M. E.; Malloy, C. R.; Sherry, A. D.; Kovacs, Z., DNP by thermal mixing under optimized conditions yields >60,000-fold enhancement of 89Y NMR signal. *J. Am. Chem. Soc.* **2011,** *133* (22), 8673-8680.

(9) Merritt, M. E.; Harrison, C.; Kovacs, Z.; Kshirsagar, P.; Malloy, C. R.; Sherry, A. D., Hyperpolarized (89)Y offers the potential of direct imaging of metal ions in biological systems by magnetic resonance. *J. Am. Chem. Soc.* **2007,** *129* (43), 12942-12943.

(10) Tóth, É.; Brücher, E.; Lázár, I.; Tóth, I., Kinetics of Formation and Dissociation of Lanthanide(Iii)-Dota Complexes. *Inorg. Chem.* **1994,** *33* (18), 4070-4076.