Supporting information

Contents

Supporting information

1. General

All reagents and solvents were purchased from commercial sources and used as received without other purification unless stated in the work. Proton and carbon NMR have been recorded on a Bruker AVANCE III 400 NMR spectrometer operating at 400 MHz and 100 MHz. Preparative HPLC was performed on a Waters Delta prep HPLC system equipped with a Water 2996 photodiode array detector and a Phenomenex Luna C18 column (5 mm, 30 mm × 250 mm). The CEST, T1 and T2 measurements were performed with aqueous solutions with mili-Q water and pH was adjusted by slowly adding concentrated solutions of 1N NaOH and 1N HCl. pH potentiometric titrations were performed with 0.15 M NaCl as ionic strength to mimic the ionic background present in common biofluids.

2. Methods

2.1. CEST experiment in vitro

All CEST NMR studies were recorded on a Bruker AVANCE III 400 NMR (9.4 T) spectrometer. Saturation power range was from 2.35~23.5 μT. Temperature unit controller Model # 2416 was used to control the temperature in the range of 288~310 K. CEST spectra were acquired by applying a long, frequency selective pre-saturation pulse over the range of ±100 ppm to cover all potentially exchanging species, including the Eu-bound water molecule and amide proton. The chemical shift of bulk water proton was set to 0 ppm.

2.2. Fitting the CEST spectra into Bloch equations modified for exchange

The proton exchange rates of Eu complex were calculated fitting the experimental spectra data in MATLAB (The MathWorks, Natick, MA, USA) with the Bloch equations (3-pool). T_1 of the solvent water was measured using a standard inversion recovery sequence. T_2 of the solvent water was measured by the Carr-Purcell Meiboom-Gill (CPMG). The agreement factor (AF= $\sqrt{\frac{\sum(CEST_{exp}-CEST_{cal})^2}{\sum CEST_{exp}^2}}$) of fitting results were all lower than 3%.

2.3 Relaxivity Measurement

The T_1 and T_2 of the samples were measured at 9.4 T at 298 and 310 K using a Bruker AVANCE III 400 MHz vertical bore spectrometer. Transverse relaxivities and longitudinal relaxivities were determined for each Dy^{3+} complex by linear fitting of the relaxation rates at four different concentrations (1, 2, 3, 4 mM) as shown in following figures.

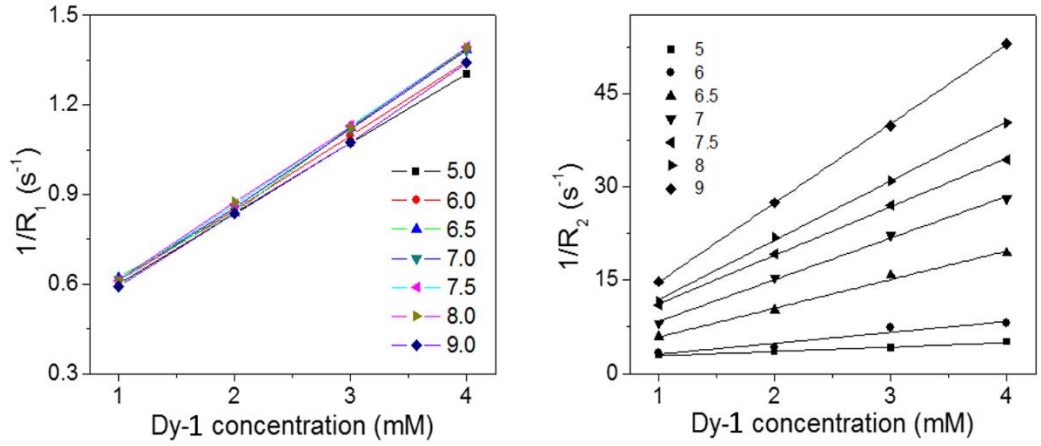


Figure S1: Determination of the r_1 and r_2 values for Dy-1 at different pH, B_0= 9.4 T, T= 298 K.

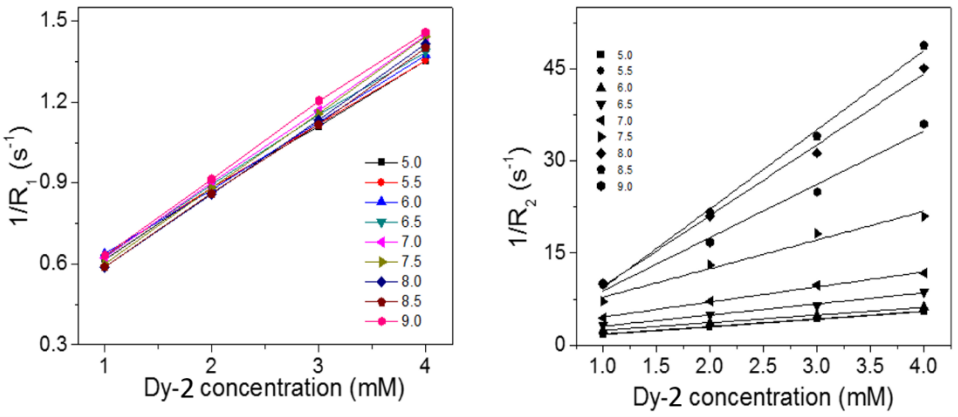


Figure S2: Determination of the r_1 ($mM^{-1}s^{-1}$) and r_2 ($mM^{-1}s^{-1}$) values for Dy-2 at different pH, B_0= 9.4 T, T= 298 K.

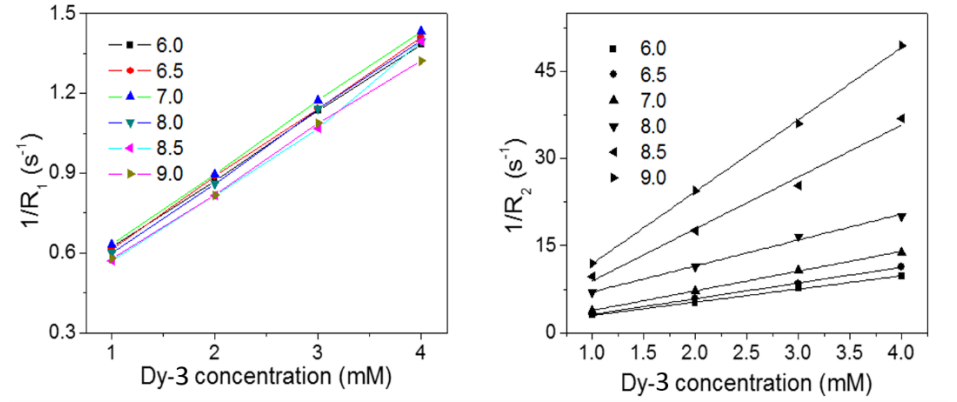
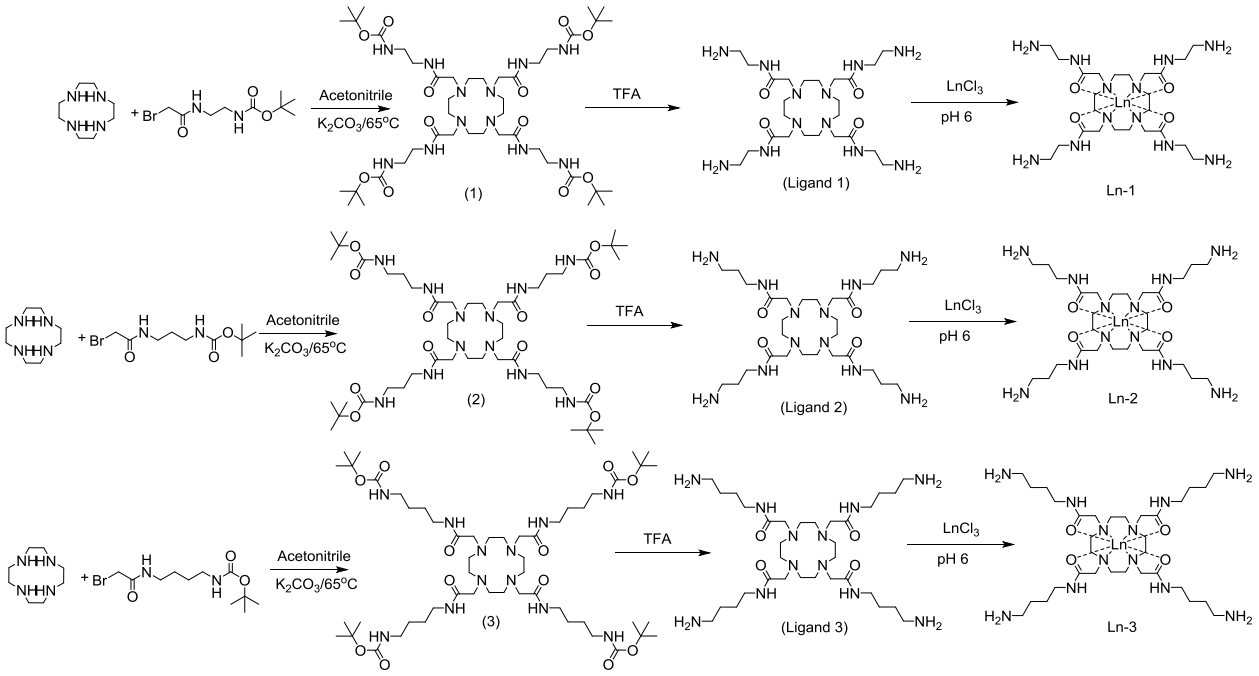


Figure S3: Determination of the r_1 ($mM^{-1}s^{-1}$) and r_2 ($mM^{-1}s^{-1}$) values for Dy-3 at different pH, B_0= 9.4 T, T= 298 K.

3. Synthesis and characterization



Scheme S1: synthetic pathways for three final ligands and complexes.

1,4,7,10- tetraazacyclododecane- 1,4,7,10-tetrakis(N-ethylene (tert-butoxycarbonyl) amino acetamide) (1): 1,4,7,10- tetraazacyclododecane (1 g, 5.8 mmol) and 2-Methyl-2-propanyl {2-[(bromoacetyl)amino]ethyl} carbamate (6.61 g, 23.5 mmol) were dissolved in anhydrous CH_3CN in the presence of K_2CO_3 (6.4 g, 46.4 mmol). The resulting solution was stirred at 65°C for 24 hours under N_2 and then the solids were filtered. The solvents were removed under vacuum and the residue was purified by chromatography using a Al_2O_3 column eluted with 2%methanol/98% dichloromethane to afford the title compound as a colorless oil (3.5 g, 61%).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.38 (CH_3, s, 36H), 2.46 (CH_2 ring, s, 16H), 3.29 (NCH_2CO, s, 8H), 3.42 (CH_2CH_2NH, m, 8H), 3.66 (CH_2CH_2NH, m, 8H), 8.03 (NH, m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 28.4 (CH_3), 37.6 (CH_2CH_2NH), 40.6 (CH_2CH_2NH), 55.1 (CH_2 ring), 59.5 (NCH_2CO), 79.5 ($C(CH_3)_3$), 155.9 ($NHCOO$), 170.7 (CH_2CONH).

1,4,7,10- tetraazacyclododecane- 1,4,7,10-tetrakis(N-propyl (tert-butoxycarbonyl) amino acetamide) (2): 1,4,7,10- tetraazacyclododecane (1 g, 5.8 mmol) and 2-Methyl-2-propanyl {3-[(bromoacetyl)amino]propyl} carbamate (6.93 g, 23.5 mmol) were dissolved in anhydrous CH_3CN in the presence of K_2CO_3(6.4 g, 46.4 mmol). The resulting solution was stirred at 65°C for 24 hours under N_2 and then the solids were filtered. The solvents were removed under vacuum and the residue was purified by chromatography using a Al_2O_3 column eluted with 2%methanol/98% dichloromethane to afford the title compound as a colorless oil (4.3 g, 71%).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.38 (CH3, s, 36H), 1.86 ($CH_2CH_2CH_2$, m, 8H), 2.46 (CH2 on ring, s, 16H), 3.18 (CH_2CH_2NH, m, 8H), 3.29 (NCH_2CO, s, 8H), 3.42 ($NHCH_2CH_2$, m, 8H), 8.03 (NH, m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 28.4 (CH_3), 28.6 ($CH_2CH_2CH_2$), 37.1 ($CH_2CH_2CH_2$), 55.1 (CH_2 ring), 59.5 ($NCH2CO$), 79.5 ($C(CH_3)_3$), 155.9 ($NHCOO$), 170.7 (CH_2CONH).

1,4,7,10- tetraazacyclododecane- 1,4,7,10-tetrakis(N-butylidene (tert-butoxycarbonyl) amino acetamide) (3): 1,4,7,10- tetraazacyclododecane (1 g, 5.8 mmol) and tert butyl N-aminopropyl carbamic acid tert-butyl ester bromoacetamide (7.26 g, 23.5 mmol) were dissolved in anhydrous CH_3CN in the presence of K_2CO_3(6.4 g, 46.4 mmol). The resulting solution was stirred at 65°C for 24 hours under N_2 and then the solids were filtered. The

solvents were removed under vacuum and the residue was purified by chromatography using a Al_2O_3 column eluted with 2%methanol/98% dichloromethane to afford the title compound as a colorless oil (4.5 g, 71%).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.38 (CH_3, s, 36H), 1.52 ($CH_2C\underline{H_2}C\underline{H_2}CH_2$, m, 16H), 2.46 (CH2 on ring, s, 16H), 3.02 ($NHC\underline{H_2}CH_2$, m, 8H), 3.18 ($CH_2C\underline{H_2}NH$, m, 8H), 3.29 ($NC\underline{H_2}CO$, s, 8H), 8.03 (NH, m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 27.0 ($CH_2\underline{C}H_2\underline{C}H_2CH_2$), 28.4 ($CH_3$), 35.8 ($CH_2\underline{C}H_2NH$), 38.6 ($NH\underline{C}H_2CH_2$), 55.1 (CH2 on ring), 59.5 ($N\underline{C}H2CO$), 79.5 ($\underline{C}(CH_3)_3$), 155.9 ($NH\underline{C}OO$), 170.7 ($CH_2\underline{C}ONH$).

1,4,7,10- tetraazacyclododecane- 1,4,7,10-tetrakis(N-ethyleneamine acetamide) (ligand 1): compound 1 (2 g, 2 mmol) was reacted directly with 4 mL TFA for 16 hours. Solvents were removed under vacuum and the final compound was obtained as yellow oil (1.1 g, 92.5%).

^{1}H NMR (400 MHz, D_2O): δ (ppm) 3.03 ($CH_2C\underline{H_2}NH_2$, t, 8H), 3.18 (CH_2 on ring, s, 16H), 3.41 ($NHC\underline{H_2}CH_2$, m, 8H), 3.72 ($NC\underline{H_2}CO$, s, 8H). ^{13}C NMR (100 MHz, D_2O): δ (ppm) 36.6 ($CH_2\underline{C}H_2NH_2$), 38.8 ($NH\underline{C}H_2CH_2$), 50.0 (CH_2 on ring), 54.4 ($N\underline{C}H_2CO$), 169.3 ($NH\underline{C}OCH_2$).

1,4,7,10- tetraazacyclododecane- 1,4,7,10-tetrakis(N-propylamine acetamide) (ligand 2): ligand 2 was synthesized by same method as previous compound with the yield of 95%.

^{1}H NMR (400 MHz, D_2O): δ (ppm) 1.86 ($CH_2C\underline{H_2}CH_2$, m, 8H), 2.46 (CH_2 on ring, s, 16H), 2.65 ($CH_2C\underline{H_2}NH_2$, m, 8H), 3.29 ($NC\underline{H_2}CO$, s, 8H), 3.42 ($NHC\underline{H_2}CH_2$, m, 8H). ^{13}C NMR (100 MHz, D_2O): δ (ppm) 33.4 ($CH_2\underline{C}H_2CH_2$), 37.1 ($NH\underline{C}H_2CH_2$), 39.1 ($CH_2\underline{C}H_2NH$), 55.1 (CH_2 on ring), 59.6 ($N\underline{C}H_2CO$), 170.1 ($NH\underline{C}OCH_2$).

1,4,7,10- tetraazacyclododecane- 1,4,7,10-tetrakis(N-butylideneamine acetamide) (ligand 3): ligand 3 was synthesized by same method as previous compound with the yield of 94%.

^{1}H NMR (400 MHz, D_2O): δ (ppm) 1.34 ($CH_2CH_2C\underline{H_2}CH_2NH_2$, m, 8H), 1.42 ($CH_2C\underline{H_2}CH_2CH_2NH_2$, m, 8H), 2.75 ($CH_2CH_2CH_2C\underline{H_2}NH_2$, t, 8H), 2.99 ($C\underline{H_2}CH_2CH_2CH_2NH_2$, t, 8H), 3.07 ($CH_2$ on ring, s, 16H), 33.5 ($NC\underline{H_2}CO$, s, 8H). ^{13}C NMR (100 MHz, D_2O): δ (ppm) 24.0 ($CH_2CH_2\underline{C}H_2CH_2NH_2$), 25.2 ($CH_2\underline{C}H_2CH_2CH_2NH_2$), 38.5 ($CH_2CH_2CH_2\underline{C}H_2NH_2$), 38.9 ($\underline{C}H_2CH_2CH_2CH_2NH_2$), 49.8 ($CH_2$ on ring), 54.6 ($N\underline{C}H_2CO$), 170.1 ($NH\underline{C}OCH_2$).

General procedure for the preparation of Ln^{3+} complexes. The ligands were dissolved in either H_2O (ligand 1,2 and 3) or H_2O/acetonitrile (CBz-protected ligand 2) followed by adding 1 equivalent of $LnCl_3$. pH was changed using concentrated solutions of 1N NaOH and 1N HCl. The presence of free metal in solution was tested by competition with Xylenol Orange at pH 5.8. No free metal was detected.

4. pH dependence of CEST and T_{2ex} profile

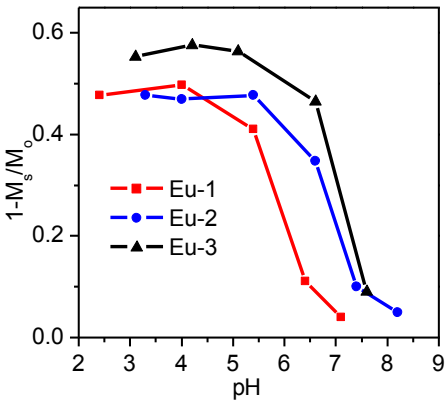


Figure S4. CEST pH dependence of Eu(III) complexes. [Eu_{1-3}] = 20 mM, saturation power= 1000 Hz, saturation delay= 2 s and T= 298 K.

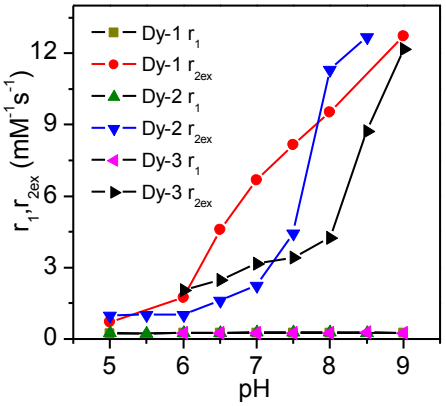


Figure S5. r_1 and r_{2ex} pH dependence of Dy(III) complexes. All points were determined using different concentrations of Dy-complexes (1, 2, 3, 4 mM).

5. PH-potentiometric titrations.

5.1. Protonation and stability constants of the Eu^{3+} complexes formed with ligand1 1-3.

The protonation and stability constants of the Eu^{3+} complexes were determined by using pH-potentiometry in the samples containing equal molar amounts of the metal ion and the ligand. [1-2]

Table S1. Protonation and stability constants of Eu-1, Eu-2 and Eu-3 complexes.

	[Eu(L¹)]	[Eu(L²)]	[Eu(L³)]
log K_{ML}	-	-	-
log K_{MHL}	**9.22(3)**	**9.78(2)**	**10.68(2)**
log K_{MH_2L}	**8.56(3)**	**9.57(2)**	**9.85(3)**
log K_{MH_3L}	**8.05(3)**	**9.03(2)**	**9.86(2)**
log K_{MH_4L}	**7.22(2)**	**8.50(2)**	**8.82(2)**
log $K_{MH_{-1}}$	**11.35(1)**	**11.63(1)**	**12.06(2)**
Fitting (mL)	**6.65×10^{-3}**	**2.13×10^{-3}**	**6.76×10^{-3}**

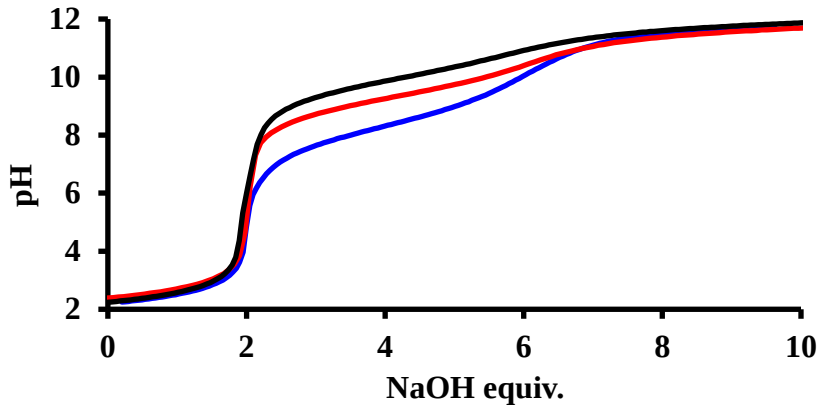
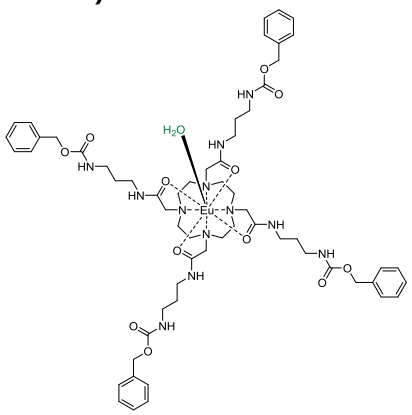


Figure S6. Titration curve of the Eu-1 (black curve), Eu-2 (red curve) and Eu-3 (blue curve) complexes. The titrated Eu-complexes started with the addition of 0.8 equivalents of acid right before the titration took place.

5.3. Experimental.

The pH-potentiometric titrations were carried out by using carbonate-free 0.1374 mol/L NaOH prepared from Fisher Chemicals. Potentiometric titrations were performed in 0.15 mol/L aqueous NaCl under nitrogen atmosphere and the temperature was controlled to 25±0.1 °C with a circulating water bath. The p[H] (p[H] = -log[H^+]) was measured in each titration with a combined pH glass electrode (Metrohm 6.0224.100) filled with 3 M KCl and the titrant addition was automated by use of a Metrohm 785 DMP titrator system. The electrode was calibrated in hydrogen ion concentration by titration of HCl with NaOH in 0.15 mol/L NaCl electrolyte solution. Continuous potentiometric titrations with NaOH 0.1374 mol/L were conducted on aqueous solutions containing 6.00 mL of ligand at 1.8 and 2.2 mM in 0.15 M NaCl with 1 minute and 15 minutes waiting time between successive points for the Eu-complexes. Fast (on-line) titration was also performed for the Eu-complexes prepared at 70-80 ºC by gradual addition of NaOH base to keep the pH near 6.5 for 24 hours (the absence of free Eu^{3+} in the samples was than confirmed by using Xylenol Orange test[3]). The protonation constants (log K_i^H) were evaluated from the potentiometric data by using the PSEQUAD computer program.[3]

6. pH dependence of CEST study of Eu-(1,4,7,10- tetraazacyclododecane- 1,4,7,10-tetrakis(N-propyl (benzyl ester carbonyl) amino acetamide).



Scheme S2. Structure of Eu-4 complex.

Eu-4 was synthesized adding 0.98 equivalents of $EuCl_3$ in 50% acetonitrile/water (v/v) to CBz-protected ligand 2. The solution pH was adjusted to 5.5, at 323 K and leave the reaction for overnight until no free metal was detected by the xylenol orange test. Sample was freeze-dried and dissolved in water.

Table S2. Longitudinal and transverse relaxivities of Dy 1-3 measured at 9.4 T NMR, T = 298 K and 310 K, pH= 7.

	Dy-1	Dy-2	Dy-3
r_1 ($mM^{-1}s^{-1}$) 298/310 K	0.2/0.2	0.3/0.2	0.3/0.3
r_2 ($mM^{-1}s^{-1}$) 298/310 K	6.7/6.9	2.3/5.3	3.2/7.2

7. Fitting methodology.

Data were fitted using MATLAB (The MathWorks, Natick, MA, USA) for the Bloch equations and Scientist 3.0 software (Micromath®) to determine the catalytic parameter represented by equation 1 and 2 in the main text.

7.1. Fitting proton exchange rates over pH for the Eu^{3+}-complexes

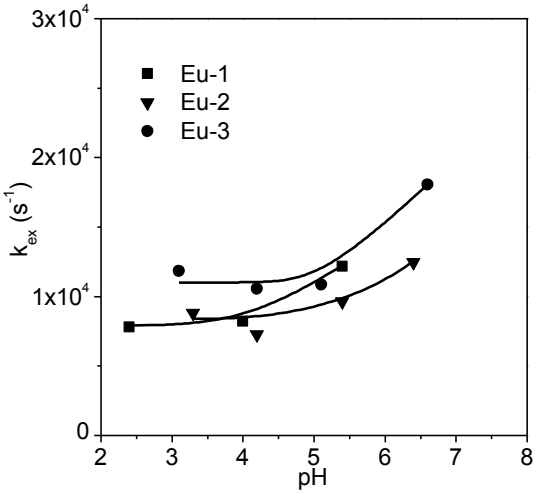


Figure S7. Proton exchange rates vs pH fitted by Eqn.1. The fitting parameters were shown in Table 2 from the main text.

Table S3. pH dependence of proton exchange rates in the three Eu^{3+} complexes as determined by fitting the CEST spectra to Bloch theory.

Eu-1		Eu-2		Eu-3	
pH	k_{ex}(x10^3 s^{-1})*	pH	k_{ex}(x10^3 s^{-1})	pH	k_{ex}(x10^3 s^{-1})
2.4	7.8	3.3	8.8	3.1	12
4.0	8.2	4.2	7.3	4.2	11
5.4	12.0	5.4	9.6	5.1	11
6.4	-	6.4	12	6.6	18
7.1	-	7.4	-	7.6	-

*The agreement factors (AF=$\sqrt{\frac{\sum(CEST_{exp}-CEST_{cal})^2}{\sum CEST_{exp}^2}}$) were <3%.

Table S4. Rate constants derived from Eqn. 1 for an amine-catalyzed exchange mechanism

Complexes	c (x10^3 s^{-1})	k_{ci}(x10^7 s^{-1})
Eu-1	7.9±0.1	17.0±1
Eu-2	8.4±0.6	1.7±0.1
Eu-3	11.0±0.4	1.6±0.1

7.2. Fitting of the T_2 exchange over pH for the Dy^{3+}- complexes.

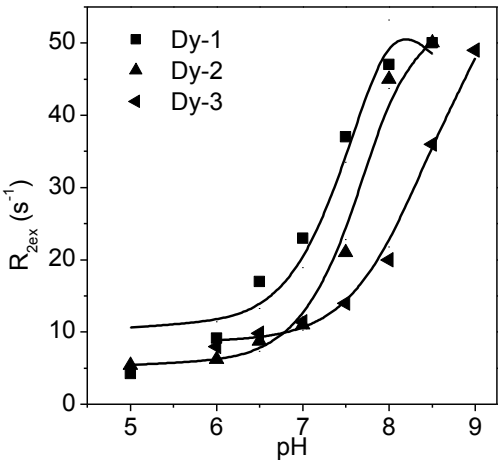


Figure S8. Data fitted by the amine-catalyzed exchange model described in Eqn 1 and 3.

Table S5. Rate constants for proton exchange in the Dy^{3+} complexes as estimated by Swift-Connick theory.

Complex	c (x10^4 s^{-1})	k_{ci} (×10^8 s^{-1})
Dy-1	17.7±6.0	7.5±1.3
Dy-2	9.6±1.0	5.6±0.3
Dy-3	16.0±0.1	1.5±0.1

8. Literature references

[1] F. K. Kálmán, M. Woods, P. Caravan, P. Jurek, M. Spiller, G. Tircsó, R. Király, E. Brücher, A. D. Sherry, *Inorg. Chem.* **2007**, *46*, 5260–5270.
[2] A. Pasha, G. Tircsó, E. T. Benyó, E. Brücher, A. D. Sherry, *Eur. J. Inorg. Chem.* **2007**, *2007*, 4340–4349.
[3] A. Barge, G. Cravotto, E. Gianolio, F. Fedeli, *Contrast Media Mol. Imaging* **2006**, *1*, 184–188.