Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Pyridine-Based Lanthanide Complexes: Towards Bimodal Probes Operating as Near Infrared Luminescent and MRI Reporters

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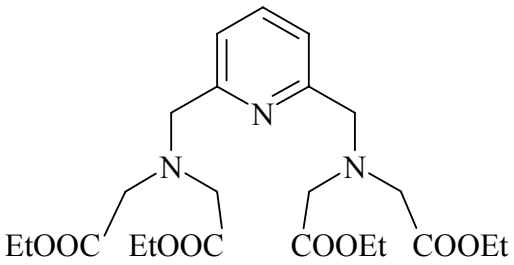
Synthesis

^{1}H NMR and ^{13}C NMR were recorded on a Bruker Avance DPX250 spectrometer (250.19 MHz ^{1}H, 62.89 MHz ^{13}C) and on a *Bruker Advance II Ultrashield plus* NMR spectrometer at 400 MHz (100.6 MHz for ^{13}C) using tetramethylsilane as the internal standard. Multiplicities were determined by the DEPT 135 sequence, chemical shifts were reported in parts par million (ppm, δ units). Coupling constants were reported in units of hertz (Hz) if applicable. Multiplicities are described with abbreviations as follow: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet). Spectra are described as δ (multiplicity, number of protons, attribution, coupling constant). Infrared (IR) spectra were obtained on *Nicolet AVATAR 320 FT-IR* spectrometer (NaCl films or KBr pellets). Low-resolution mass spectra (ionized by ion spray technique) were recorded on a *Perkin-Elmer SCIEX API 3000 spectrometer*. Melting points were determined in open capillary tubes and are uncorrected. Flash chromatography was performed on silica gel 60 (40-63 mesh). Thin layer chromatography (TLC) was carried out on Merck silica gel 60F254 precoated plates. Visualization was made with ultraviolet light and/or potassium permanganate solution. Tetrahydrofuran was freshly distilled from sodium/benzophenone under argon prior to use. All other solvents were used with p.a. quality and without distillation. Chemicals products were obtained from the following sources: Aldrich and Acros.

- **Tetraethyl [2,6-pyridinediylbis(methylene nitrilo)] tetraacetate (1)**



$C_{23}H_{35}N_3O_8$
481.55 g.mol^{-1}

A mixture of the commercially available 2,6-dibromomethylpyridine (300mg, 1.2mmol) , potassium carbonate (630mg, 4.8mmol, 4 eq.) and potassium iodide (375mg, 2.4mmol, 2eq.) was dissolved into acetonitrile (40mL). Iminodiethyl acetate (397µL, 2.4mmol, 2 eq.) was then added. The mixture was refluxed during 12 hours. After cooling to room temperature, the excess of potassium carbonate was dissolved by adding water and acetonitrile was evaporated. The aqueous layer was extracted with methylene chloride and the crude was concentrated under reduced pressure. The compound **(1)** was obtained without purification as a pale yellow solid paste. Yield: 70%.

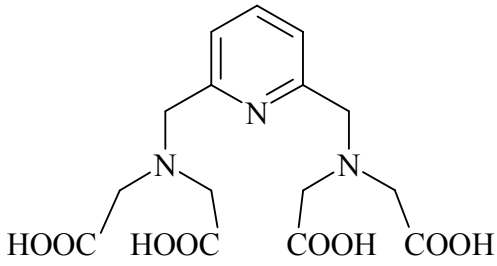
NMR^{1}H ($CDCl_3$) δ (ppm): 7.66 (t, 1H, $H_{pyridine}$, J_3=7.5Hz); 7.48 (d, 2H, $H_{pyridine}$, J_3=7.5Hz); 4.04 (s, 4H, PyrCH_2N); 4.17 (q, 8H, CH_3CH_2O, J_3=4.2Hz) 3.61 (s, 8H, CH_2N); 1.26 (t, 12H, CH_3CH_2O, J_3=4.2Hz).

NMR^{13}C ($CDCl_3$) δ (ppm): 171.1(C9); 158.2 (C2/6); 137.1 (C4); 121.2 (C3/5); 60.4 (C7); 59.9 (C8); 54.8 (C10); 14.1 (C11).

IR: ν (cm^{-1}): 3008, 1786, 1740, 1216.

MS (Ionspray®) m/z: 482.5 $(M+H)^+$.

- **[2,6-pyridinediylbis(methylene nitrilo)] tetraacetic acid (2)[1, 2]**

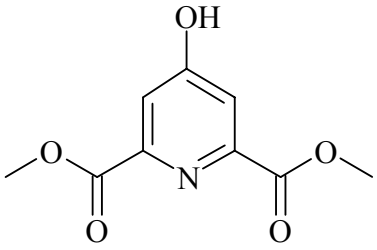


$C_{15}H_{19}N_3O_8$
369.33 g.mol^{-1}

Esters **(1)** (1eq.) was dissolved in THF (10mL) and diluted with water (10mL). The mixture was cooled to 0°C. LiOH (12eq.) was added and the medium was stirred at room temperature during 5 hours. The organic solvent was evaporated and the aqueous mixture was purifying on an anionic exchange resin (DOWEX 1X2-100Cl) (washed with H_2O/MeOH; 99/1 and eluted with formic acid 1M) to obtain the ligand **(2)** as beige solid. Yield: 70%.

NMR^{1}H (D_2O) δ (ppm): 8.08 (t, 1H, $H_{pyridine}$, J_3=7.6Hz); 7.65 (d, 2H, $H_{pyridine}$, J_3=7.6Hz); 4.75 (s, 4H, PyrCH_2N); 4.21 (s, 8H, CH_2N).
NMR^{13}C (D_2O) δ (ppm): 168.6(C); 148.9 (C); 140.0 (CH); 124.6 (CH); 57.3 (CH_2); 54.6 (CH_2).
IR: ν (cm^{-1}): 3418, 2964, 1732, 1417, 1257.
MS (Ionspray®) m/z: 370 $(M+H)^+$.
HRMS calculated for $C_{15}H_{20}N_3O_8$ $(M+H)^+$ 370.1250, found 370.1244
T_f : 233-235°C (Litt[1] 237°C)

- **Dimethyl 4-hydroxypyridine-2,6-dicarboxylate (3)**



$C_9H_9NO_5$
211.18 g.mol^{-1}

Thionyl chloride (5.77mL, 79.5mmol, 8 equiv) was dropped into methanol (20mL) cooled at -10°C. The commercially available chelidamic acid (2g, 9.94mmol) was added. The solution was stirred at room temperature for 24 hours and then heated at reflux for an additional 2 hours. The excess of $SOCl_2$ and the solvent were removed. The crude solid was recrystallized from ethanol to obtain the compound **(3)** (1,84g) as a white solid. Yield: 80%.

NMR^{1}H (MeOD) δ (ppm): 7.60 (s, 2H, $CH_{pyridine}$); 3.97 (s, 6H, OCH_3).

NMR^{13}C (MeOD) δ (ppm): 167.6(C); 166.1(C); 116.9 (C); 101.4 (CH); 53.4 (CH_3).

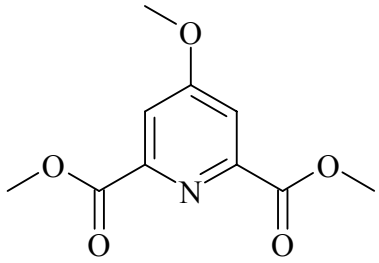
IR: ν (cm^{-1}) 3423, 1730.

MS (Ionspray®) m/z: 212 $(M+H)^+$.

T_f : 169°C (Litt[3] 169-170°C).

R_f: DCM/MeOH 9/1: 0.59

- **Dimethyl 4-methoxypyridine-2,6-dicarboxylate (4)**



$C_{10}H_{11}NO_5$
225.20 g.mol^{-1}

Potassium carbonate (982mg, 7.1 mmol, 1.5 equiv.) was added to a solution of 4-hydroxy-2,6-pyridinedicarboxylate dimethyle **(3)** (1g, 4.7mmol) in acetonitrile (25mL). Methyliodide (1.08mL, 7.1mmol, 1.5 equiv) was added dropwise. The mixture was refluxed and strongly stirred during 18 hours. Water was added to the cooled reaction until total dissolution of potassium carbonate. The solvent was evaporated under reduced pressure and the aqueous layer was extract with CH_2Cl_2. Organics layers were dried over $MgSO_4$ and concentrated. The compound **(4)** (853mg) was obtain by purification on column chromatography (CH_2Cl_2/MeOH; 9:1) as a white solid. Yield: 80%.

NMR^{1}H ($CDCl_3$) δ (ppm): 7.82 (s, 2H, $CH_{pyridine}$); 4.02 (s, 6H, CH_3(ester)); 3.99 (s, 3H, CH_3O).

NMR^{13}C ($CDCl_3$) δ (ppm): 167.6(C); 165.1(C); 149.8(C); 114.1 (CH); 56.0 (CH_3); 53.2 (CH_3).

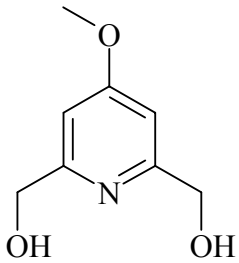
IR: ν (cm^{-1}): 1727, 1715.

MS (Ionspray®) m/z: 226 $(M+H)^+$, 248 $(M+Na)^+$.

T_f : 121-122°C (Litt[4] 125°C)

R_f: DCM/MeOH 9/1 : 0.81

- **-2,6-Dihydroxymethyl-4-methoxypyridine (5)**



$C_8H_{11}NO_3$
169.18 g.mol^{-1}

MeOH (15mL) was added over a period of 1h to a boiling mixture of 4-methoxy-2,6-pyridinedicarboxylatedimethyle **(4)** (1.41g, 6.2mmol) and $NaBH_4$ (1.18g, 9.4mmol, 1.5 eq.)) in distilled THF (55mL). The reaction mixture was refluxed for an additional 2 hours, then cooled to room temperature and slowly diluted with H_2O (30mL). The organic solvent was evaporated and the residual aqueous layer was extracted with AcOEt. The combined organic extracts were evaporated to dryness. The compound **(5)** is obtain by purification on column chromatography (CH_2Cl_2/MeOH - 9:1) as a white solid. Yield: 81%.

NMR^{1}H (MeOD) δ (ppm): 6.98 (s, 2H, $CH_{pyridine}$); 4.62 (s, 4H, CH_2); 3.90 (s, 3H, OCH_3).

NMR^{13}C (MeOD) δ (ppm): 169.5 (C4); 163.7 (C2/6); 106.0 (C3/5); 65.3 (C7); 55.9(C9).

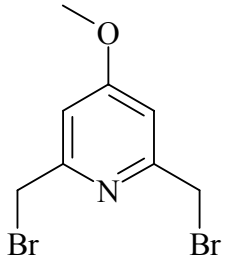
IR: ν (cm^{-1}): 3424, 1636, 1215, 766.

MS (Ionspray®) m/z: 170 $(M+H)^+$, 192 $(M+Na)^+$.

T_f : 119-120°C (Litt[5] 121-122°C)

R_f: DCM/MeOH 9/1: 0.34

- **2,6-Dibromomethyl-4-methoxypyridine (6)**



$C_8H_9Br_2NO$
294.98 g.mol^{-1}

PBr_3 (7.45mL) in $CHCl_3$ (60mL) was added dropwise to a suspension of 4-methoxy-2,6-dihydroxymethylpyridine **(5)** (837mg) in $CHCl_3$ (120mL) at room temperature. The mixture was refluxed during 12 hours. After cooling to room temperature, $NaHCO_3$ (1M, 100mL) was added and the solution was stirred during 1 hour until it became clear. The organic layer was dried and concentrated under reduced pressure. The compound **(6)** was obtained by purification on column chromatography (CH_2Cl_2/MeOH; 99.5:0.5) as a white solid. Yield: 72%.

NMR^{1}H ($CDCl_3$) δ (ppm): 6.88 (s, 2H, *$CH_{pyridine}$*); 4.48 (s, 4H, *CH_2*); 3.87 (s, 3H, O*CH_3*).

NMR^{13}C ($CDCl_3$) δ (ppm): 167.1 (C); 158.1 (C); 108.8 (CH); 55.4 (CH_3); 33.5 (CH_2).

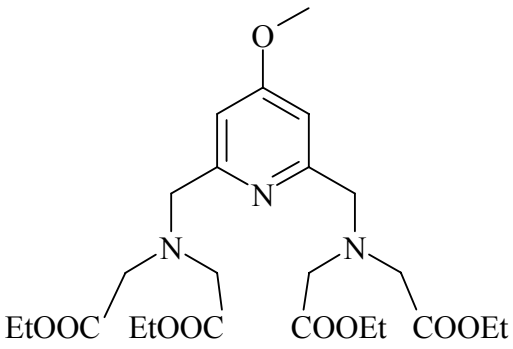
IR: ν (cm^{-1}): 2976, 667.

MS (Ionspray®) m/z: 294, 296, 298 $(M+H)^+$

T_f : 88-89°C (Litt[6] 99°C)

R_f: DCM/MeOH 99.5/0.5: 0.68

- **Tetraethyl [2,6-(4-methoxypyridine)diylbis(methylene nitrilo)] tetraacetate (7)**



$C_{24}H_{37}N_3O_9$
511.58 g.mol^{-1}

A mixture of 4-methoxy-2,6-dibromomethylpyridine **(6)** (365mg, 1.2mmol), potassium carbonate (684mg, 4.8mmol, 4 eq.) and potassium iodide (411mg, 2.4mmol, 2eq.) was dissolved into acetonitrile (30mL). Iminodiethyl acetate (433µL, 2.4mmol, 2 eq.) was then added. The medium was refluxed during 12 hours. After cooling to room temperature, the excess of potassium carbonate was dissolved by adding water and acetonitrile was evaporated. The aqueous layer was extracted with methylene chloride and the crude was concentrated under reduced pressure. The compound **(7)** was obtained without purification as a pale yellow solid paste. Yield: 99%.

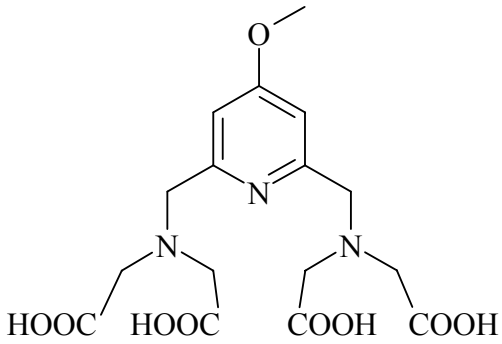
NMR^{1}H ($CDCl_3$) δ (ppm): 7.07 (s, 2H, $H_{pyridine}$); 4.17 (q, 8H, CH_2O, J_3=7.25Hz); 3.99 (s, 4H, PyrCH_2N); 3.86 (s, 3H, CH_3O), 3.61 (s, 8H, CH_2N), 1.27 (t, 12H, CH_3CH_2, J_3=7.25Hz).

NMR^{13}C ($CDCl_3$) δ (ppm): 171.1(C); 167.2 (C); 159.9(C); 107.2 (CH); 60.4 (CH_2); 59.8 (CH_2); 55.1 (CH_3); 54.8 (CH_2); 14.1 (CH_3).

IR: ν (cm^{-1}): 2982, 2939, 2908, 1739, 1598, 1195, 1151.

MS (Ionspray®) m/z: 512.5 $(M+H)^+$, 534.5 $(M+Na)^+$.

- **[2,6-(4-methoxypyridine)diylbis(methylene nitrilo)] tetraacetic acid (8)[1]**



$C_{16}H_{21}N_3O_9$
399.36 g.mol^{-1}

Esters **(7)** (1eq.) was dissolved in THF (10mL) and diluted with water (10mL). The mixture was cooled to 0°C. LiOH (12eq.) was added and the medium was stirred at room temperature during 5 hours. The organic solvent was evaporated and the aqueous mixture was purifying on an anionic exchange resin (DOWEX 1X2-100Cl) (washed with H20/MeOH; 99/1 and eluted with formic acid 1M) to obtain the ligand **(8)** as a white solid. Yield: 100 % (purity : 96% by colorimetry (Xylenol orange)).

NMR^{1}H (MeOD) δ (ppm): 7.27 (s, 2H, $H_{pyridine}$); 4.29 (s, 4H, PyrCH_2N); 4.03 (s, 3H, CH_3O); 3.67 (s, 8H, CH_2N).

NMR^{13}C (MeOD) δ (ppm): 174.4 (CH); 172.4 (C); 157.2 (C); 110.5 (CH); 57.7 (CH_2); 57.5 (CH_2); 57.1 (CH_3)

IR: ν (cm^{-1}): 3428, 2960, 1731, 1635, 1609.

T_f : 150-152 °C (Litt[3] 162°C)

MS (Ionspray®) m/z: 400.5 $(M+H)^+$, 422.5 $(M+Na)^+$.

HRMS calculated for $C_{16}H_{22}N_3O_9$ $(M+H)^+$ 400.1356, found 400.1344

Luminescence Measurements

UV-vis absorption spectra were recorded on a Perkin-Elmer Lambda 9 spectrophotometer. Emission and excitation spectra were measured using a JY Horiba Fluorolog-322 spectrofluorimeter equipped with a Hamamatsu R928 detector for the visible domain and an Electro-Optical Systems, Inc. DSS-IGA020L detector for the NIR domain. The NIR luminescence relative quantum yields were measured by using $KEr(Trop)_4$ complex ($\Phi = 1.7 \times 10^{-4}$ in DMSO[7]) as reference. Excitation wavelengths for these measurements: 267nm for NdL^1 and 249nm for NdL^2. Spectra were corrected for the instrumental function for both excitation and emission. Values were calculated using the following equation:

$$\frac{\Phi_x}{\Phi_r} = \frac{A_{r_{\lambda_x}}}{A_{x_{\lambda_x}}} \frac{I_{\lambda_r}}{I_{\lambda_x}} \frac{\eta_x^2}{\eta_r^2} \frac{D_x}{D_r}$$

where subscript r stands for the reference and x for the sample; A is the absorbance at the excitation wavelength, I is the intensity of the excitation light at the same wavelength, η is the refractive index($\eta = 1.328$ in D_2O, $\eta = 1.333$ in water, $\eta = 1.478$ in DMSO), and D is the measured integrated luminescence intensity.

The luminescence lifetime measurements were performed by excitation of solutions in 1 cm quartz cells using a Nd:YAG Continuum Powerlite 8010 Laser at 266 nm (4^{th} harmonic) as excitation source. Emission was collected at a right angle to the excitation beam, the emission wavelength selected with a Spectral Products CM 110 1/8 meter monochromator. The signal was monitored by a cooled photomultiplier (Hamamatsu R316-2) coupled to a 500 MHz bandpass digital oscilloscope (Tektronix TDS 754D). The signals to be treated (at least 15,000 points resolution for each trace) were averaged from at least 500 individual decay curves. Luminescence decay curves were imported into Origin 7.0 scientific data analysis software. The decay curves were analyzed using the Advanced Fitting Tool module. Reported luminescence lifetimes are averages of at least three independent determinations.

The energies of the triplet states of the coordinated L^1 and L^2 have been recorded on GdL^1 and GdL^2 through time-resolved phosphorescence measurements. Both triplet states are fairly high in energy in comparison to other Nd^{3+} complexes. We have previously demonstrated that lowering the energy of the triplet state is a promising strategy to increase the efficiency of energy transfer from the ligand to NIR emitting lanthanides. We hypothesize that it will be possible to improve the energy conversion by lowering the energy of the triplet state through modification of the pyridine with appropriate substituents.

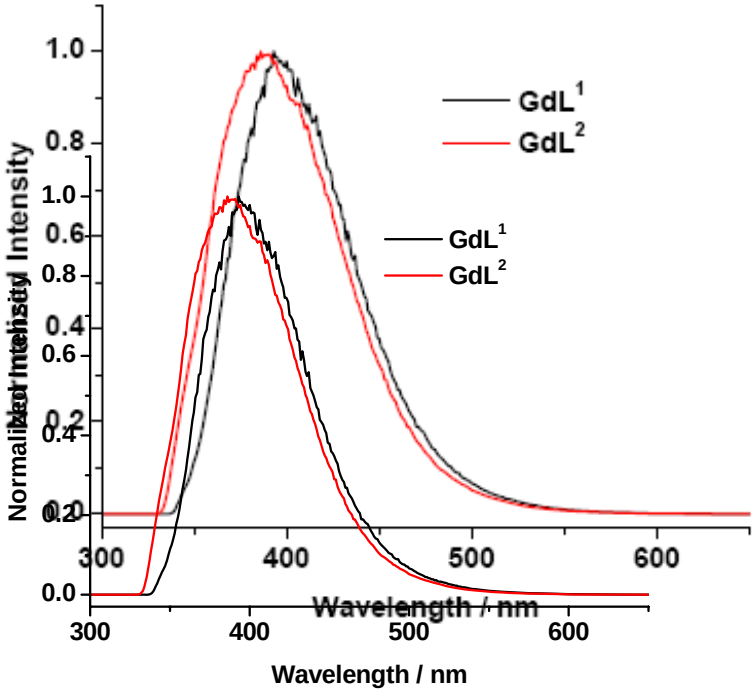


Figure S1. *Phosphorescence spectra of Gd^{3+} complexes formed with L^1 and L^2 in water (10^{-4} M, HEPES buffer 0.01 M, pH = 7.02, ionic strength KCl 0.01M) at 77K, delay time 0.1 ms, window time 20 ms.*

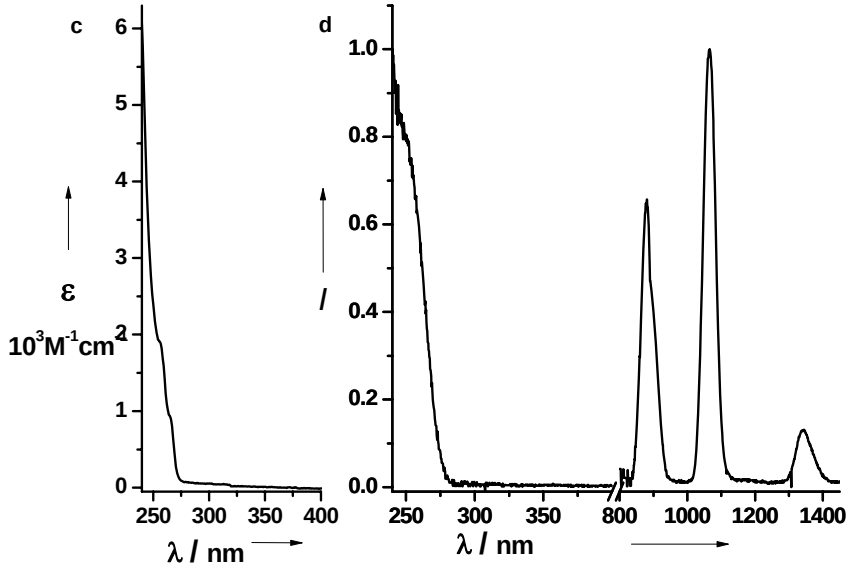


Figure S2. *Absorption (a), normalized emission (λ_{ex} = 267 nm) and excitation (λ_{em} = 1065 nm) spectra (b) of 5.1×10^{-4} M NdL^2. 0.01 M HEPES, pH 7.02, I = 0.01 M.*

Potentiometric studies

The protonation and the stability constant were determined with pH-potentiometry by titrating 3 ml solutions with standardized KOH solution using a Metrohm Dosimat 665 automate burette. A combined glass electrode (LL Biotrode, Metrohm) connected to a Metrohm 692 pH/ion-meter was used to measure pH. The titrated samples were stirred and N_2 was bubbled through the solutions. The ligand concentration was ~3 mM and equimolar metal concentration were used (except for the Zn^{2+} - L^1 and L^2 systems where 2 equivalents of $ZnCl_2$ was also applied). The titrations were carried out at 25 °C, the ionic strength of the solution was maintained constant (0.1 M KCl). The H^+ concentration was obtained from the measured pH values according to the method proposed by Irving *et al.*[8] The protonation and stability constants were calculated with the program PSEQUAD.[9] The errors given correspond to one standard deviation.

Dissociation kinetics

The exchange reaction between GdL^1 and Eu^{3+} has been studied by measuring the longitudinal relaxation rates ($1/T_1$) of water protons by inversion recovery at 500 MHz on a Bruker NMR spectrometer. The Eu^{3+} concentration varied between 5×10^{-3} and 3×10^{-2} M, while the concentration of GdL^1 was 5×10^{-4} M. 0.02 M *N*-methyl-piperazine was used as buffer and the ionic strength was 0.1 M KCl.

The relaxivities, r_1, were calculated from the measured $1/T_{1obs}$ water proton relaxation rates according to Eq. (1), where $1/T_{1w}$ is the relaxation rate of water at the given temperature, and [Gd] is the Gd^{3+} concentration in mM.

$$\frac{1}{T_{1obs}} = \frac{1}{T_{1w}} + \frac{1}{T_{1p}} = \frac{1}{T_{1w}} + r_1 \times [Gd] \quad (1)$$

The pseudo-first-order rate constants (k_{obs}) were calculated by fitting the relaxation rate data to Eq. (2), where r_{1t}, r_{10} and r_{1e} are the relaxivity values at time t, time zero and at equilibrium, respectively.

$$r_{1t} = \left(r_{10} - r_{1e}\right)\exp\left(-k_{obs}t\right) + r_{1e} \quad (2)$$

The observed rate constants, k_{obs}, did not show any significant dependence on the concentration of the exchanging metal ion, Eu^{3+}.

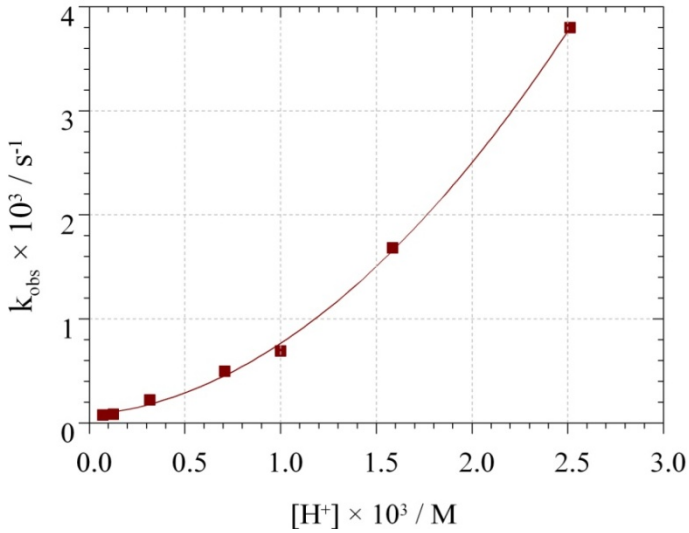


Figure S3. *pH dependency of the observed rate constants.* c_{GdL1} *= 0.5 mM;* c_{Eu} *= 5 mM, I = 0.1 KCl.*

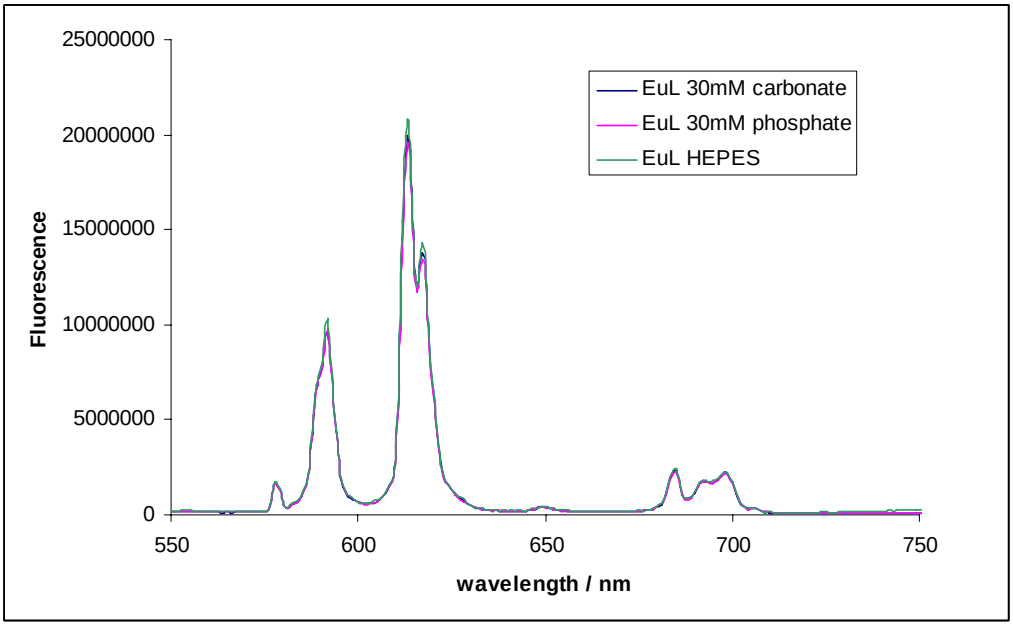


Fig. S4. *Fluorescence emission spectra of 0.05 mM* EuL^1 *in HEPES in the absence and in the presence of 30 mM of carbonate or phosphate, pH 7.4. Fluorescence emission spectra (excitation wavelength 270 nm, 2 nm bandwidth) were recorded at room temperature in silica cells of 1 cm pathlength using a Jobin-Yvon Fluoromax 2 spectrofluorimeter. Emission spectra were corrected with the computer program supplied with the instrument.*

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