Solvent polarity and oxygen sensitivity, rather than viscosity, determine lifetimes of biaryl-sensitised terbium luminescence

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Electronic Supplementary Information

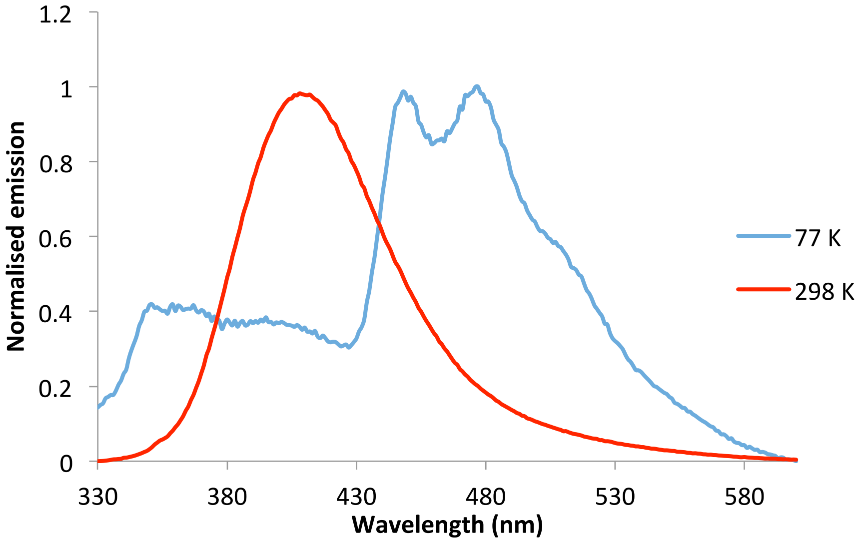


Fig. S1 Emission spectra of **GdL**^{1} in ethanol at room temperature (298 K, red) and 77 K (transparent glass, blue). The shortest-wavelength phosphorescence band was assumed to be the 0-0 transition, λ_{ex}= 320 nm. $\tau_{77\ K}$= 14 ms (λ_{ex}= 320 nm, λ_{em}= 450 nm).

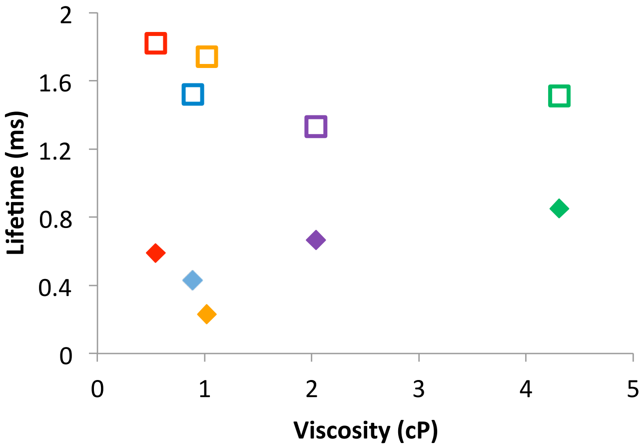


Fig. S2 The excited state lifetime of **TbL**^{1} relative to the solvent viscosity in water (*blue*); MeOH (*red*); EtOH (*orange*) ^{i}PrOH (*purple*); ^{t}BuOH (*green*). Squares represent data in deoxygenated solutions and diamonds in air-equilibrated solutions at 298 K.

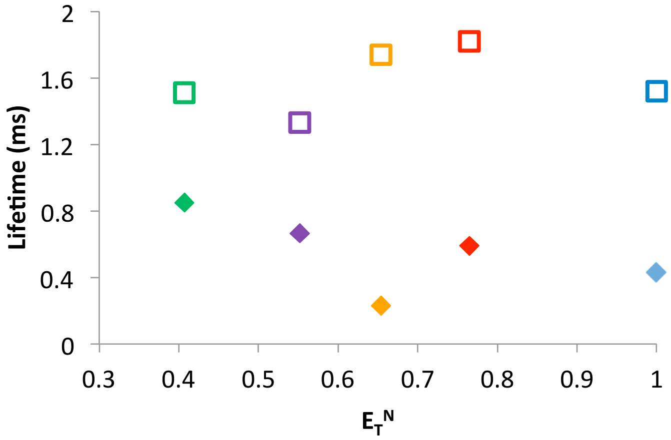


Fig. S3 The excited state lifetime of **TbL^1** relative to the Reichardt's normalised solvent polarity parameter (E_T^N) in H_2O (*blue*); MeOH (*red*); EtOH (*orange*) ^{i}PrOH (*purple*); ^{t}BuOH (*green*). Squares represent data in deoxygenated solutions and diamonds in air-equilibrated solutions at 298 K.

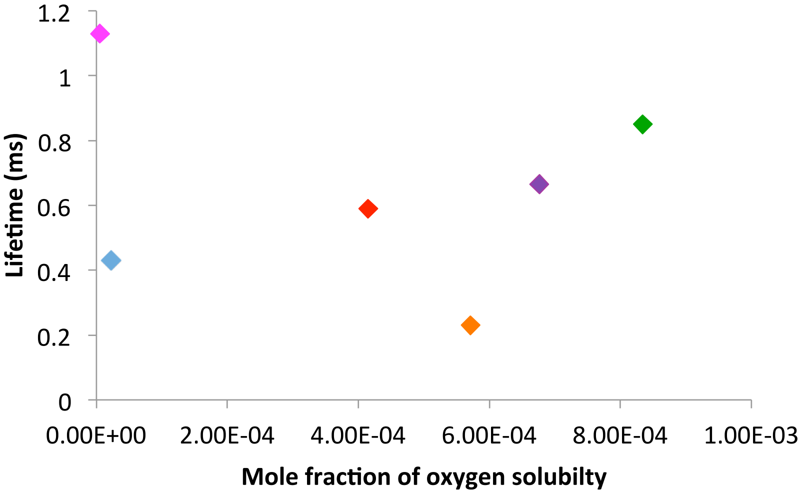


Fig. S4 The excited state lifetime of **TbL^1** relative to the oxygen solubility (**Table 2**) in H_2O (*blue*); MeOH (*red*); EtOH (*orange*); ^{i}ProH (*purple*); ^{t}BuOH (*green,* value for 2-butanol); glycerol (*pink*). Oxygen solubility is expressed as a mole fraction at 298 K and 101.3 kPa.

Experimental

General procedures

Materials and Chromatography

All commercially available reagents were used as received from suppliers without further purification. Solvents used were laboratory grade. Anhydrous solvents were dried over the appropriate drying agent. All moisture-sensitive reactions were carried out by Schlenk-line techniques, under an inert atmosphere of either argon or nitrogen. For air sensitive reactions, solvents were degassed using the freeze-pump-thaw cycle method. Water was purified by the 'Purite$_{\mathrm{STILL}}$plus' system with a conductivity of $\leq 0.04\ \mu\mathrm{S\ cm^{-1}}$.

Thin layer chromatography was performed on neutral alumina (Merck Art 5550) or silica (Merck Art 5554) and visualized under UV irradiation (254 nm) or by staining with either iodine or potassium permanganate. Column chromatography was carried out either manually using neutral alumina or silica (Merck Silica Gel, 230 – 400 mesh) or using a Teledyne Combi-flash instrument equipped with RediSep R_f silica cartridges to perform automated elution. Melting points were measured on a Gallenkamp melting point apparatus, and are uncorrected.

Instrumentation

NMR: Routine ^{1}H (400 MHz) and ^{13}C (101 MHz) and ^{31}P (162 MHz) NMR spectra were acquired on Bruker Avance or Varian Mercury 400 NMR spectrometers. ^{13}C NMR and ^{31}P NMR spectra were run on a proton decoupled experiment. Two-dimensional NMR spectra (COSY, NOESY, HSQC and HSBC) were carried out by the solution state NMR service at Durham University on Varian-600 (600 MHz) or VNMRS-700 (700 MHz) instruments.

Mass spectrometry: Mass spectra of **[LnL^{1}]** were recorded on a Waters Xevo QToF instrument in an acetonitrile and ammonium bicarbonate buffered (25 mM) system.

Absorption spectroscopy

UV-Vis absorption spectra were measured using a UVIKON XS spectrometer operating with LabPower software. The sample was held in a quartz cuvette with a path length of 1 cm. Absorption spectra were recorded against pure solvent in an optically matched cuvette. Extinction coefficients were calculated using a sample dilution method, from a linear plot of absorbance against concentration according to the Beer-Lambert law.

Fluorescence Spectroscopy

Emission and excitation spectra were acquired on a Jobin Yvon Fluoromax-2 spectrometer, in quartz cuvettes with a path length of 1 cm. Excitation and emission wavelengths were selected according to the specific sample. An integration time of 0.5 seconds and increment of 1.0 nm was used. Emission and excitation band passes used were either 2:2 or 3:3 (in nm) depending on the brightness of the sample. Lanthanide emission and excitation spectra were recorded using the appropriate glass filter (435 nm filter for Eu(III) complexes and 395 nm filter for Tb(III) complexes), to remove $2\lambda_{ex}$ light from the emission and $\frac{\lambda_{em}}{2}$ from the excitation spectrum.

Degassed samples were measured in an adapted cuvette which can be attached to a vacuum line and degassed using the freeze-pump-thaw cycle method. Low temperature measurements were carried out at 77 K in a transparent glass of ethanol. In each case, the complex was first dissolved in the minimum volume of water, before ethanol was added.

Luminescence lifetimes

Lifetime measurements were carried out using two instruments. Lifetimes of metal-based emission were measured using a Perkin Elmer LS55 luminescence spectrometer. Lanthanide excited state lifetimes were measured by excitation of the sample by a short pulse of light, followed by the monitoring of the integrated intensity of light emitted during a fixed gate time t_g, after a delay time of t_d. Measurements were made for at least 30 separate decay times over a period of a minimum of three lifetimes. A gate time of 0.1 ms was selected. The decay curves were fitted to **eq. 1** using Origin 8.0 software:

$$I = A_0 + A_1 e^{-kt} \quad (1)$$

where: I is the intensity at time t after the excitation pulse, A_0 is the background intensity after the fluorescence decay, A_1 is the pre-exponential factor and k is the rate constant for the excited state decay.

The inner sphere hydration number (q) of Eu^{3+} and Tb^{3+} complexes are calculated by measuring the luminescence lifetimes in both H_2O and D_2O. The q values for Eu^{3+} and Tb^{3+} complexes were calculated using **equations 2** and **3** respectively.[1]

$$q_{Eu} = 1.2\left(k_{H_2O} - k_{D_2O} - 0.25 - 0.075n\right) \quad (2)$$

$$q_{Tb} = 5.0\ (k_{H_2O} - k_{D_2O} - 0.06 - 0.010n) \quad (3)$$

where q_{Eu} / q_{Tb} is the inner sphere hydration number, k is rate constant for luminescence decay and n is the number of proximal amide –NH oscillators.

Lanthanide luminescence lifetimes as measured above were corroborated through measurements using an Edinburgh Instruments OB 920 Spectrometer operating in multichannel scaling mode, with a xenon flash lamp as the excitation source. This system was also used for the 77K lifetime data in Fig. S1. For both instruments, excitation and emission wavelengths were selected based upon the individual lanthanide complex being measured.

Quantum yields

The luminescence quantum yields of the Tb^{3+} complex were measured using quinine sulfate in 0.5 M H_2SO_4 ($\Phi = 0.546$)[2] as the standard. All measurements were recorded at room temperature under both aerated and deoxygenated conditions. Absorbance and emission spectra of the reference and the sample were run consecutively with identical parameters. The quantum yields for **[Ln.L¹]** were calculated from **eq. 4**:

$$\Phi_a = \left(\frac{I_a}{I_b}\right) x \left(\frac{A_b}{A_a}\right) x \left(\frac{n_a}{n_b}\right)^2 x\ \Phi_b \quad (4)$$

where '*a*' refers to the sample and '*b*' refers to the standard. *Φ* is the quantum yield, *I* is the integrated intensity of the emission spectrum, *A* is the absorbance at the excitation wavelength (λ_{ex} = < 0.1) and *n* is the refractive index of the solvent.

HPLC analysis

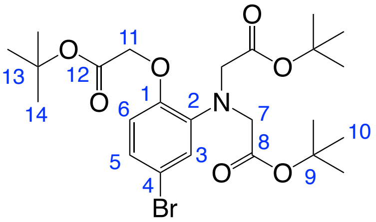
Reverse phase HPLC was performed at 295 K using a Shimadzu system consisting of a Degassing Unit (DGU-20A_{5R}), a Prominence Preparative Liquid Chromatograph (LC-20AP). An XBridge C18 10 x 100 mm, 5 *μ*M column was used to purify the complexes on a preparative scale with a flow rate of 17 mL / minute. Analytical scale purifications were performed on a Shimadzu ShimPacl VP-ODS column, 4.6 x 150 mm, 5 *μ*M column with a flow rate of 2 mL / min. A gradient elution was used with a total run time of 25 minutes for each individual injection. The conditions used for all preparative HPLC purifications of lanthanide complexes can be seen in **Table S.1**.

Table S.1 Preparative HPLC procedure for **[Ln.L^1]**. Solvent A = Ammonium bicarbonate buffer (25 mM) in water, Solvent B = CH_3CN.

Time (min)	Solvent A (%)	Solvent B (%)
0	100	0
3	100	0
13	0	100
16	0	100
25	100	0

Synthetic Procedures

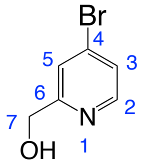
Di-*tert*-butyl2,2'-((5-bromo-2-(2-(*tert*-butoxy)-2-oxoethoxy)phenyl) azanediyl) diacetate, 1 [3]



N,N-Diisopropylethylamine (0.43 g, 0.57 mL, 3.27 mmol) and *tert*-butyl bromoacetate (367 µL, 2.49 mmol) were added to a solution of 2-amino-4-bromophenol (146 mg, 0.78 mmol) and sodium iodide (156 mg, 1.04 mmol) in anhydrous acetonitrile (1.5 mL). The reaction was heated at 85 °C for 5 d under an inert atmosphere of argon, before the reaction mixture was cooled to room temperature and diluted with the addition of ethyl acetate (10 mL). Inorganic impurities were removed by filtration before the filtrate was washed with brine (3 x 10 mL), and water (1 x 10 mL). Organic extracts were combined and dried over $MgSO_4$, and the solvent was removed under reduced pressure to form a brown residue. Purification by silica gel column chromatography (gradient 2:1 CH_2Cl_2 / hexane to 100% DCM) formed the title compound as a pale brown oil (121 mg, 29%). R_f (silica, CH_2Cl_2) = 0.29; ^{1}H NMR (700 MHz, $CDCl_3$) 6.95 – 6.92 (2 H, m, H^3 and H^5), 6.61 (1 H, d, *J* 8, H^3), 4.49 (2 H, s, H^{11}), 4.03 (4 H, s, H^8), 1.44 (9 H, s, H^{14}), 1.43 (18 H, s, H^{10}); ^{13}C NMR (176 MHz, $CDCl_3$) 170.1 (C^{12}), 167.8 (C^8), 148.8 (C^1), 140.9 (C^2), 124.2 (C^3 or C^5), 122.5 (C^3 or C^5), 115.4 (C^4), 114.4 (C^6), 82.3 (C^{13}), 81.3 (C^9), 66.6 (C^{11}), 54.5 (C^7), 28.1 (C^{10}), 28.0 (C^{14}); ASAP-LRMS $[C_{24}H_{36}NO_7{}^{79}Br]^+$ (+) *m/z* 530.2; ASAP-HRMS calcd for $[C_{24}H_{36}NO_7{}^{79}Br]^+$ 530.1695 found 530.1700.

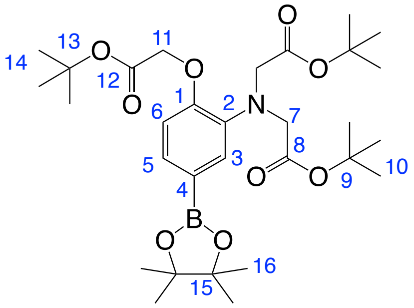
[1, 4, 7- Tris(tert-butylcarbonylmethyl)]-1,4,7,10- tetraazacyclodecane hydrobromide, and 4-bromo-2-methyl pyridine-N-oxide, were made according to the published procedures [4,5]

4-Bromo-2-(hydroxymethyl)pyridine, [5]



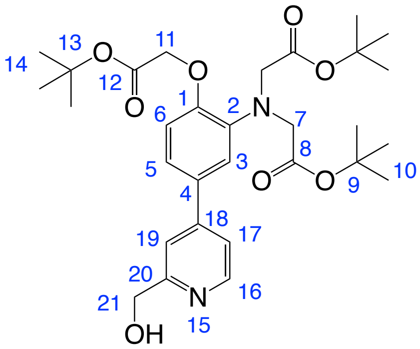
Trifluoroacetic anhydride (16 mL, 115.0 mmol) was added to a solution of 4-bromo-2-methyl pyridine-N-oxide (2.2 g, 11.5 mmol) in anhydrous $CHCl_3$ (32 mL). The solution was heated at 60 C for 20 h under an inert atmosphere of argon. The reaction mixture was then cooled to room temperature, and the solvent was removed under reduced pressure for form a light brown oil. The brown residue was dissolved in CH_2Cl_2 (20 mL) before NaOH (2 M) was added and the reaction mixture was stirred at room temperature under argon for 16 h. The organic layer was extracted with CH_2Cl_2 (3 x 30 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to form a dark brown oil. Purification by silica gel column chromatography (gradient 100 % CH_2Cl_2 to 95% CH_2Cl_2 / 5% CH_3OH) formed the title compound as a pale brown oil, that slowly solidified to a fluffy off-white solid on standing at room temperature (1.4 g, 64%). m.p. 34-36°C; ^{1}H NMR (700 MHz, $CDCl_3$) 8.37 (1 H, d, *J* 5, H^2), 7.49 (1 H, d, *J* 1, H^5), 7.39 (1 H, dd, *J* 5, 1, H^3), 4.75 (2 H, s, H^7), ^{13}C NMR (176 MHz, $CDCl_3$) 160.8 (C^6), 149.1 (C^2), 133.7 (C^4), 125.8 (C^3), 124.0 (C^5), 63.8 (C^7); ESI-LRMS $[C_6H_7NO^{79}Br]^+$ (+) *m/z* 188.1; ESI-HRMS calcd for $[C_6H_7NO^{79}Br]^+$ 187.9711 found 187.9688.

Tert-butyl 2-(2-{bis[2-(tert-butoxy)-2-oxoethyl]amino}-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)acetate, 2 [6]



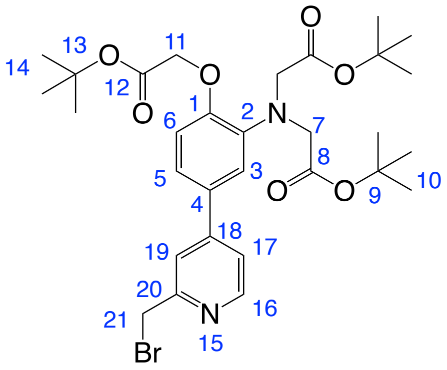
Pd(dppf)Cl_2 (11.8 mg, 0.016 mmol) was added to a solution of **1** (264 mg, 0.5 mmol), bis(pinacolato)diboron (B_2pin_2) (155 mg, 0.61 mmol) and potassium acetate (158 mg, 1.61 mmol) in 1,4-dioxane (1.4 mL). The solution was degassed via four freeze-pump-thaw cycles, and heated in a microwave reactor for 30 mins at 120°C. The reaction mixture was suspended between CH_2Cl_2 (20 mL) and water (10 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The organic extracts were combined, dried over $MgSO_4$, and the solvent was removed under reduced pressure to form a dark brown residue. Purification by silica gel column chromatography (gradient hexane to 80 / 20 (hexane / ethyl acetate)) formed the title compound as a pale yellow solid (203 mg, 70%). R_f (silica, 80 / 20 (hexane / ethyl acetate)) = 0.36; m.p = 136-138 C; ^{1}H ($CDCl_3$, 700 MHz) 7.30 (1 H, dd, *J* 8, 2, H^5), 7.27 (1 H, d, *J* 2, H^3), 6.70 (1 H, d, *J* 8, H^6), 4.53 (2 H, s, H^{11}), 4.07 (4 H, s, H^7), 1.44 (18 H, s, H^{10}), 1.43 (9 H, s, H^{14}), 1.28 (12 H, s, H^{16}); ^{11}B ($CDCl_3$, 128 MHz) + 30.7; ^{13}C ($CDCl_3$, 176 MHz) 170.8 (C^8, C^{12}), 167.9 (d, *J* 1, C^4),152.1 (C^1), 138.8 (C^2), 129.0 (C^5), 125.8 (C^3), 112.4 (C^6), 83.4 (C^{15}), 82.1 (C^{13}), 80.9 (C^9), 66.0 (C^{11}), 54.5 (C^7), 28.1 (C^{10}), 28.0 (C^{14}), 24.9 (C^{16}); ASAP-LRMS $[C_{30}H_{48}BNO_9]^+$ (+) *m/z* 578.3; ASAP-HRMS calcd for $[C_{30}H_{48}BNO_9]^+$ 577.3511 found 577.3517.

Di-*tert*-butyl 2,2'-((2-(2-(*tert*-butoxy)-2-oxoethoxy)-5-(2-(hydroxyl-methyl)pyridin-4-yl)phenyl)azanediyl)diacetate, 3



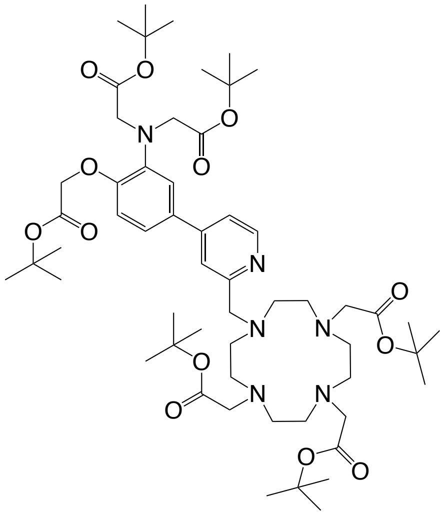
Compound **2** (205 mg, 0.35 mmol), 4-bromo-2-(hydroxymethyl) pyridine (69 mg, 0.37 mmol) and sodium carbonate (151 mg, 1.48 mmol) were dissolved in a mixture of DME (1.4 mL) and water (0.6 mL). The solution was degassed by three freeze-pump-thaw cycles, before the addition of $Pd(PPh_3)_4$ (42 mg, 0.036 mmol). The reaction mixture was stirred at 85 C for 18 h, before the solvent was removed under reduced pressure to form a dark brown / black residue. Purification by column chromatography (gradient hexane to ethyl acetate (with 1% triethylamine)) formed the title compound as a yellow oil (112 mg, 51 %). ^{1}H NMR (600 MHz, $CDCl_3$) 8.53 (1 H, d, *J* 5.22, H^{16}), 7.36 (1 H, br s, H^{19}), 7.34 (1 H, dd, *J* 5, 1, H^{17}), 7.17 (1 H, dd, *J* 8, 2, H^5), 7.12 (1 H, d, *J* 2, H^3), 6.82 (1 H, d, J 8, H^6), 4.78 (2 H, s, H^{21}), 4.58 (2 H, s, H^{11}), 4.12 (4 H, s, H^7), 1.47 (9 H, s, H^{14}), 1.43 (18 H, s, H^{10}); ^{13}C NMR (151 MHz, $CDCl_3$) 170.4 (C^8), 167.8 (C^{12}), 159.3 (C^{20}), 150.7 (C^1), 148.9 (C^{18}), 148.7 (C^{20}), 140.0 (C^2), 131.4 (C^4), 120.4 (C^{17}), 120.0 (C^5), 118.0 (C^3), 117.7 (C^{19}), 113.9 (C^6), 82.4 (C^{13}), 81.2 (C^9), 66.3 (C^7), 64.2 (C^{11}), 54.6 (C^{21}), 28.1 (C^{10} and C^{14}); ESI-LRMS $[C_{30}H_{43}N_2O_8]^+$ (+) *m/z* 599.3; ESI-HRMS calcd for $[C_{30}H_{43}N_2O_8]^+$ 559.3019 found 599.2999.

Di-*tert*-butyl 2,2'-((5-(2-(bromomethyl)pyridin-4-yl)-2-(2-(*tert*-butoxy)-2-oxoethoxy)phenyl)azanediyl)diacetate, 4



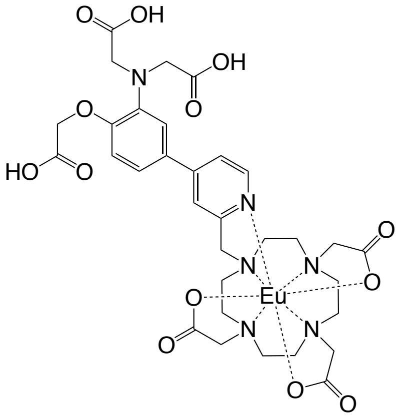
Phosphorus tribromide (PBr_3) (52 μL, 0.55 mmol) was added to an ice cold solution of **3** (252 mg, 0.45 mmol) in anhydrous CH_2Cl_2 (3 mL). The reaction was warmed to room temperature and stirred at room temperature for 1 h, reaction completion was determined by ESI-MS by the disappearance of the starting material peak. The solvent was removed under reduced pressure to form the title compound as a yellow oil which was used in subsequent steps without further purification. ESI-LRMS $[C_{30}H_{42}N_2O_7{}^{79}Br]^+$ (+) *m/z* 621.2; ESI-HRMS calcd for $[C_{30}H_{42}N_2O_7{}^{79}Br]^+$ 621.2175 found 621.2165.

Tri-tert-butyl 2,2',2''-(10-((4-(3-(bis(2-(tert-butoxy)-2-oxoethyl) amino)–4-(3,3-dimethyl-2-oxobutoxy)phenyl)pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate, 5



Caesium carbonate (620 mg, 1.9 mmol) and sodium iodide (120 mg, 0.8 mmol) were added to a solution of (**4**) (201 mg, 0.32 mmol) and [1,4,7-tris(ethoxycarbonylmethyl)]-1,4,7,10-tetraazacyclodecane hydrobromide (82 mg, 0.158 mmol) in anhydrous acetonitrile (2 mL). The reaction mixture was stirred at 60°C for 17 h under argon. After completion, the solution was diluted with ethyl acetate (15 mL) and all inorganic impurities were collected by filtration. The solvent was removed under reduced pressure to form a dark brown residue. Purification by silica gel column chromatography (gradient 100% CH_2Cl_2 to 90% CH_2Cl_2 / 10% MeOH) formed the title compound as a pale yellow oil (118 mg). ESI-LRMS $[C_{56}H_{91}N_6O_{13}]^+$ (+) *m/z* 1056.8; ESI-HRMS calcd for $[C_{56}H_{91}N_6O_{13}]^+$ 1055.6644 found 1055.6678.

[Eu.L^1]



Compound **5** (47 mg, 0.045 mmol) was dissolved in CH_2Cl_2 (1 mL) and TFA (1 mL). The pale brown solution was stirred under argon at room temperature for 20 h, ester hydrolysis was confirmed by ^{1}H NMR spectrometry, and ESI-LRMS. The solvent was lyophilised to form the title compound as a white solid (39 mg), which was used in subsequent steps without further purification ESI-LRMS $[C_{32}H_{42}N_6O_{13}]^+$ (+) *m/z* 719.2; ESI-HRMS calcd for $[C_{32}H_{42}N_6O_{13}]^+$ 719.1949 found 719.1948. The *tert*-butyl ester hydrolysed product (39 mg, 0.029 mmol) was dissolved in H_2O (4 mL). The pH was adjusted to 5.5 using NaOH (aq, 1 M) and $EuCl_3.6H_2O$ (22 mg, 0.069 mmol) was added. The resulting yellow solution was stirred at 65°C for 18 h. The pH was adjusted to pH 8 and $Eu(OH)_3$ was removed by filtration. The solvent was lyophilised to form a pale yellow solid. Purification by reverse phase HPLC (0 % - 100% - 0% CH_3CN in ammonium bicarbonate buffer (25 mM), t_R = 1.06 min) formed the title compound as a pale yellow solid (9 mg); ESI-LRMS $[C_{32}H_{40}N_6O_{13}{}^{151}Eu]^+$ (+) *m/z* 867.1; ESI-HRMS calcd for $[C_{32}H_{40}N_6O_{13}{}^{151}Eu]^+$ 867.1840 found 867.1852; $\Phi_{H_2O} = < 0.03$ %

[Tb. L^1(H$_2$O)]

The complex was synthesised in a procedure analogous to that used for the europium complex, using **5** (30 mg, 0.04 mmol) and $TbCl_3.6H_2O$ (35 mg, 0.09 mmol) in H_2O (3.0 mL). Purification by reverse phase HPLC (0 % - 100 % - 0% CH_3CN in ammonium bicarbonate buffer (25 mM), t_R = 1.63 min) formed the title compound as a white solid (4 mg); ESI-LRMS $[C_{32}H_{40}N_6O_{13}Tb]^+$ (+) *m/z* 875.2; ESI-HRMS calcd for $[C_{32}H_{39}N_6O_{13}Tb]^+$ 875.1908 found 875.1907; Φ_{H_2O}= 3.4% (aerated), Φ_{H_2O}= 5.4% (deoxygenated).

[Gd. L^1(H$_2$O)]

The complex was synthesised in a analogous procedure using **5** (79 mg, 0.11 mmol) and $GdI_3.6H_2O$ (81 mg, 0.22 mmol) in H_2O (5.0 mL). Purification by reverse phase HPLC (0 % - 100 % - 0% CH_3CN in ammonium bicarbonate buffer (25 mM), t_R = 1.48 min) formed the title compound as a white solid (11 mg); ESI-LRMS $[C_{32}H_{40}N_6O_{13}{}^{155}Gd]^+$ (+) *m/z* 871.2; ESI-HRMS calcd for $[C_{32}H_{40}N_6O_{13}{}^{155}Gd]^+$ 871.1908 found 871.1907.

HPLC purity traces:

[Gd. L^1(H$_2$O)]

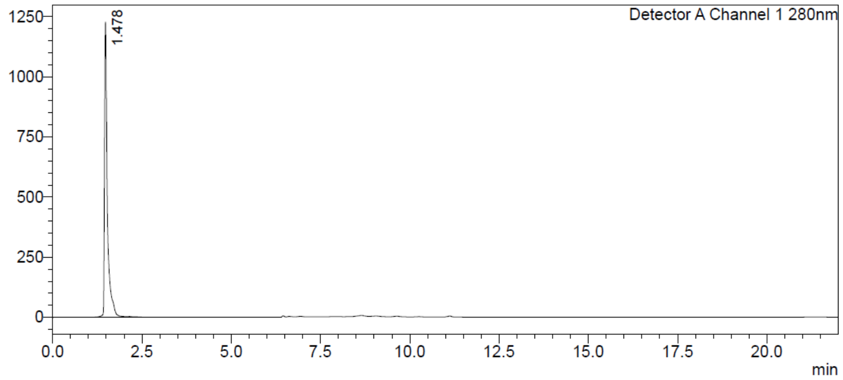


Fig. S.4 The analytical reverse-phase HPLC trace for **[Gd.L^1]** . Conditions are shown in **Table S.1**. λ_{abs} = 280 nm.

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