ESI

Selective Signalling of Glyphosate in Water Using Europium Luminescence

Laura B. Jennings, Sergey Shuvaev, Mark A. Fox, Robert Pal and David Parker*

Department of Chemistry, Durham University, South Road, Durham. DH1 3LE, UK

Figure S1 Variation of europium emission intensity ratio with pH in the given pH range (7 µM [Eu.L^3], 0.1 M NaCl, 295 K); pK_a = 6.99(±0.05).

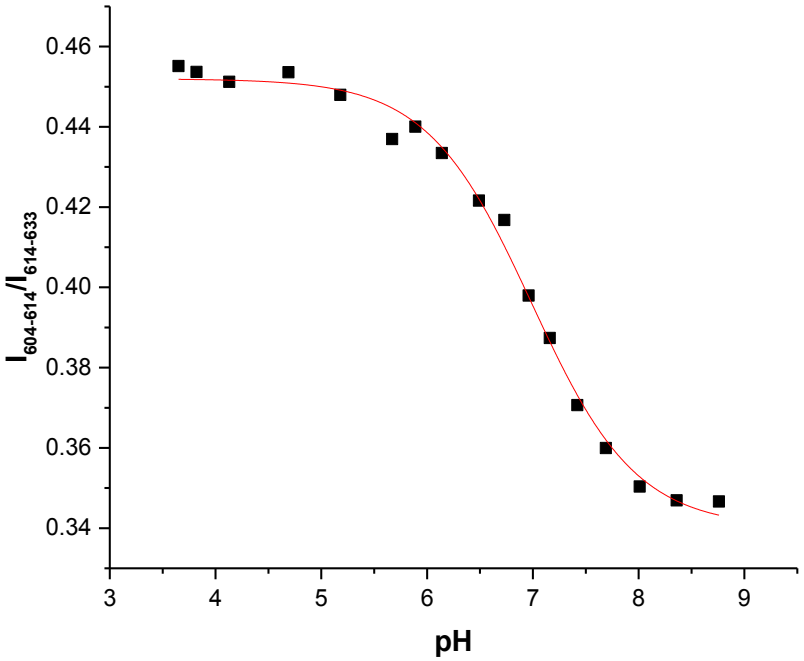


Figure S2 Variation of the europium emission spectrum with pH in the stated pH range (7 µM [Eu.**L**[1]], 0.1 M NaCl, 295 K)

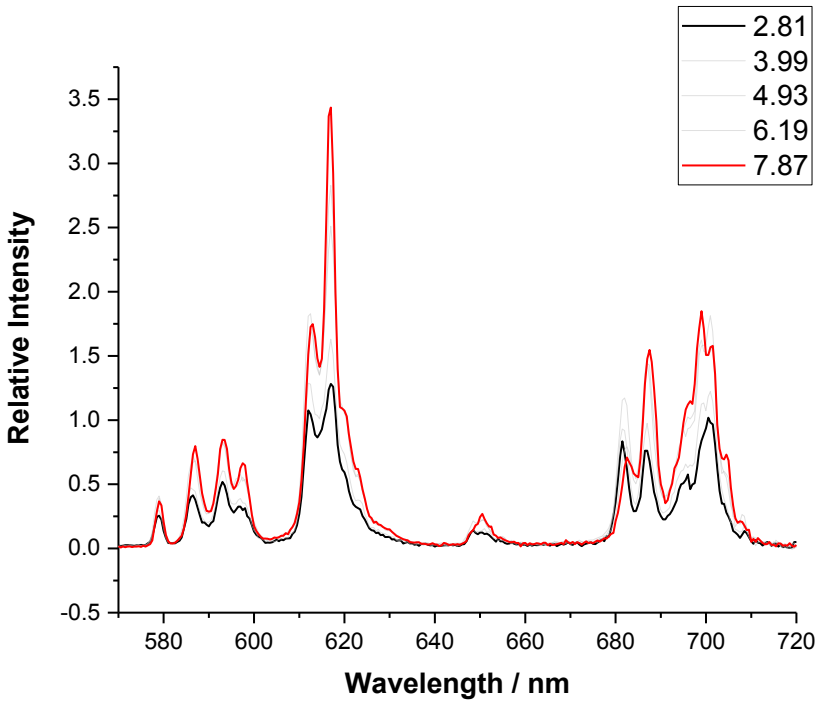
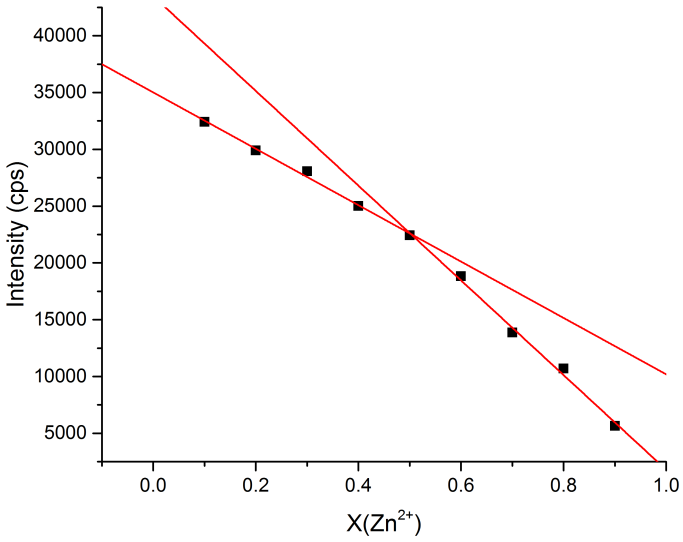


Figure S3

Job plot for [EuL[2]] following addition of $ZnCl_2$ ([EuL[2]] 2.5 µM, 0.1 M HEPES, pH = 7.40, 298 K, λ_{ex} = 335 nm).



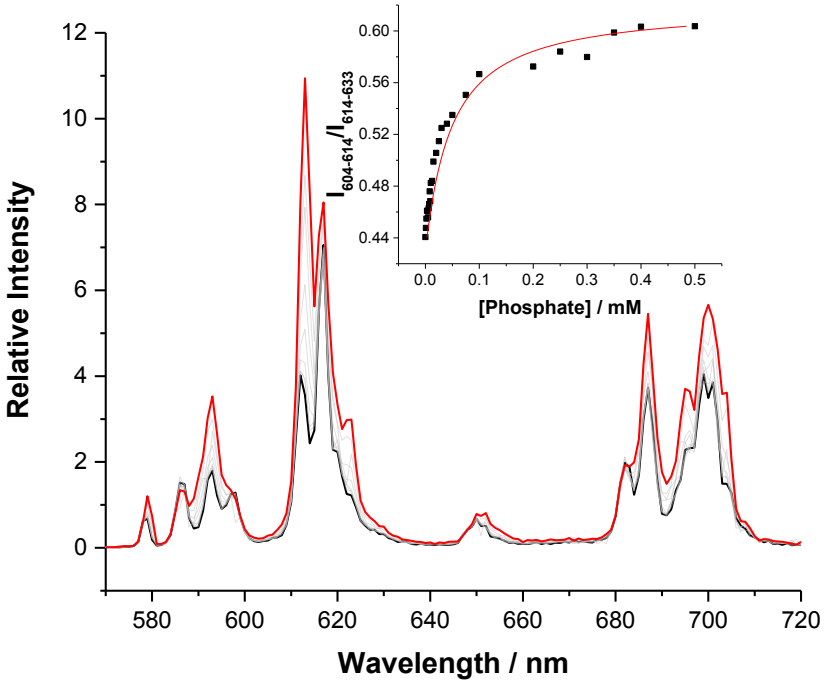


Figure S4 Variation of the emission intensity ratio for [Eu.**L**^{1}] (black) (6 µM, 0.1 M NaCl, 0.1 M MES, pH 5.9, 295 K) with incremental additions of phosphate, up to 0.5 mM (red). The inset shows the intensity ratio of 604-614 nm / 614-633 nm as a function of increasing phosphate concentration.

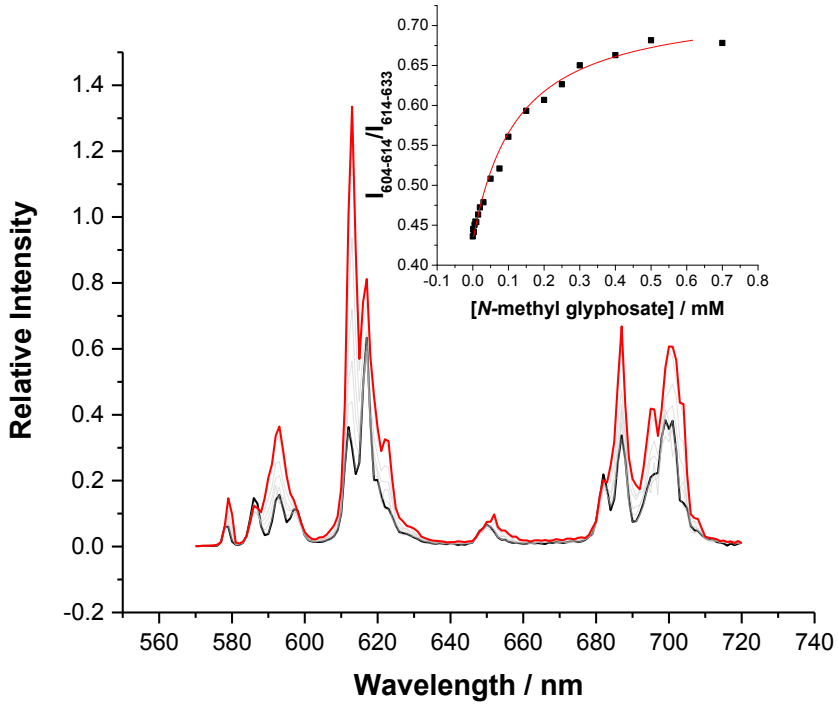


Figure S5 Variation of the emission intensity ratio for [Eu.**L**^{1}] (7 µM, 0.1 M NaCl, 0.1 M MES, pH 5.9, 295 K) with increasing concentration of N-methyl glyphosate, up to 0.7 mM (red). The inset shows the intensity ratio 604-614 nm / 614-633 nm as a function of increasing N-methyl glyphosate concentration.

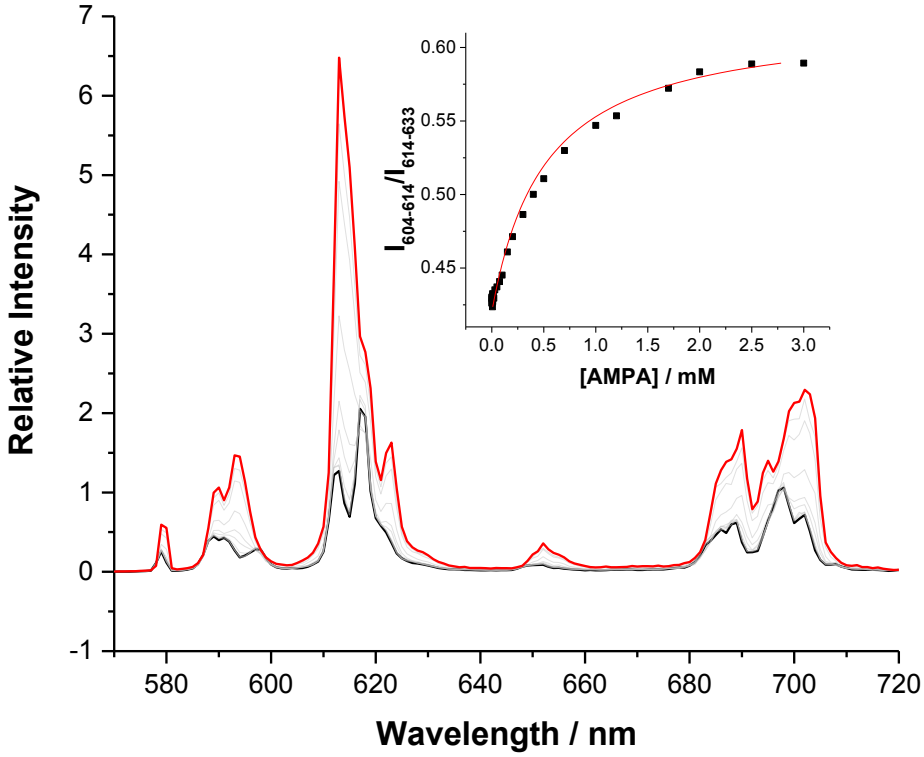


Figure S6 Variation of the emission intensity ratio for [Eu.**L**[1]] (7 µM, 0.1 M NaCl, 0.1 M MES, pH 5.9, 295 K) with increasing concentration of AMPA, up to 3 mM (red). The inset shows the intensity ratio 604-614 nm / 614-633 nm, as a function of increasing AMPA, **2**, concentration.

General Experimental

All solvents used were laboratory grade and anhydrous solvents, when required, were freshly distilled over the appropriate drying agent. Water was purified by the 'PuriteSTILLplus' system, with conductivity of ≤4 µS cm^{-1}. All reagents used were purchased from commercial suppliers (Aldrich, Fisher Scientific, Fluorochem, Apollo Scientific) and were used without further purification unless otherwise stated. Reactions requiring anhydrous conditions were carried out using Schlenk-line techniques under an atmosphere of argon.

Absorption and Emission Spectroscopy

UV/Vis absorption measurements were recorded using a Perkin-Elmer Lambda 900 absorption spectrophotometer, using matched quartz cells.

Emission spectra were measured using a Horiba-Jobin Yvon Fluorolog-3® and Horiba-Jobin Yvon Fluoromax-3®. The steady-state luminescence was excited by unpolarised light from a 450W xenon CW lamp and detected at an angle of 90° for diluted solution measurements (10 mm quartz cell) by a red-sensitive Hamamatsu R928 photomultiplier tube. Spectra were reference corrected for both the excitation source light intensity variation (lamp and grating) and the emission spectral response (detector and grating). Phosphorescence lifetimes (> 30 µs) were obtained by pulsed excitation using a FL-1040 UP Xenon Lamp. Luminescence decay curves were fitted by least-squares analysis using

Origin®. Luminescence quantum yields ϕ were measured in diluted aqueous solution with an absorbance lower than 0.3, using an integrating sphere.

The apparent binding constant of each selected anion was calculated according to the equation below, using Origin2015™ software and non-linear iterative least squares regression.

$$[X] = \frac{\frac{(F-F_0)/(F_1-F_0)}{K} + [Eu]*(F-F_0)/(F_1-F_0) - [Eu]*\left((F-F_0)/(F_1-F_0)\right)^2}{1-(F-F_0)/(F_1-F_0)}$$

$$\text{Eu} + \text{X} \leftrightarrow \text{EuX} \qquad K = \frac{[EuX]}{[X_f][Eu_f]}$$

[X]: the total concentration of protein in the solution

[Eu]: the total concentration of the complex

K: the binding constant

F: the ratio of selected peaks

F_0: the ratio at the beginning

F_1: the final ratio

[EuX]: the concentration of the appropriate SA or drug -coordinated complex

[X_f]: the concentration of free SA or drug in the mixture

[Eu_f]: the concentration of the free complex

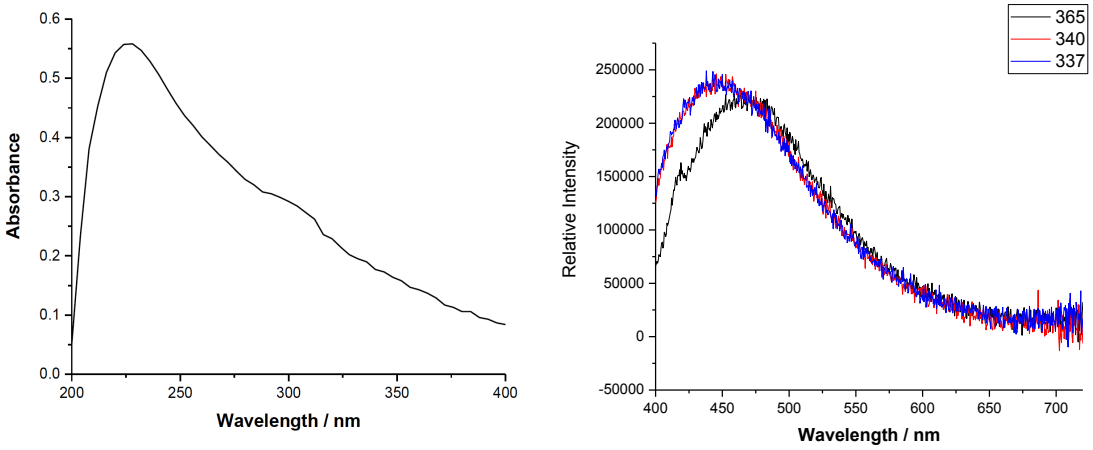
Time gated experiments were carried out using a custom built time-gated spectrophotometer. It comprises a 365 nm 330 mW pulsed LED with 2 ms per pulse in a 10 Hz cycle sequence. This was connected to a cuvette holder and an Acton 2155 scanning monochromator with a 1200 blaze grating, tuned between 400 and 750 nm, and a Hamamatsu 7155 red corrected PMT. The instrument s was operated using custom written Labview2013 software.

HPLC Analysis

HPLC analysis and purification were performed at 295 K using a Shimadzu system (degassing unit DGU-20A5R, Prominence semi-preparative liquid chromatograph LC-20AP, Prominence UV/Vis detector SPD-20A and communications bus module CBM-20A). The solvent system used was ammonium bicarbonate buffer (25 mM, pH = 7) / methanol [isocratic 10 % methanol in buffer (3

min), linear gradient to 100% methanol (10 min), isocratic 100 % methanol (5 min)] or formic acid buffer (0.1%) / Acetonitrile [isocratic 10 % acetonitrile in buffer (3 min), linear gradient to 100% Acetonitrile (10 min), isocratic 100 % acetonitrile (5 min)] flow: 2 ml / min for analytical mode on XBridge C18 column, 4.6 x 100 mm, i.d. 5 µm, and 17 ml / min for preparative mode on XBridge C18 column, 19 x 100 mm, i.d. 5 µm.

Figure S6 Absorption and emission spectra of the river Wear water sample



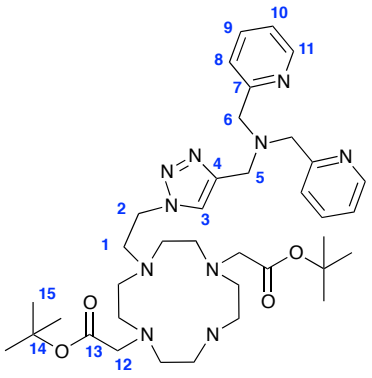
The UV absorption spectrum of the river water alone (*left*) was taken to see if anything in the sample absorbs at, or close to, the complex excitation wavelength. There is a strong maximum absorbance at 228 nm (A_{228} = 0.56). The absorbance decreases slowly at longer wavelengths and at 365 nm (A_{365} = 0.14), where the complex is excited on the time-gated instrument, the absorbance is still significant. It is also clear that the use of a time-gated method is prudent as some background luminescence from fluorescent species occur in the water sample when excited in this range. The emission spectra (*right*) show the relatively weak emission from unknown aromatic components of the water sample, at the stated excitation wavelengths.

Grain/Oat Extract Handling

Ten wheat grains were selected and soaked in 2 mL of Purite water for 24 hr. The water was then filtered and lyophilised. The residue was taken up in 2 mL of water with the [Eu.L^1] complex (7 µM) in 0.1 M NaCl, 0.1 M MES at pH 5.9.

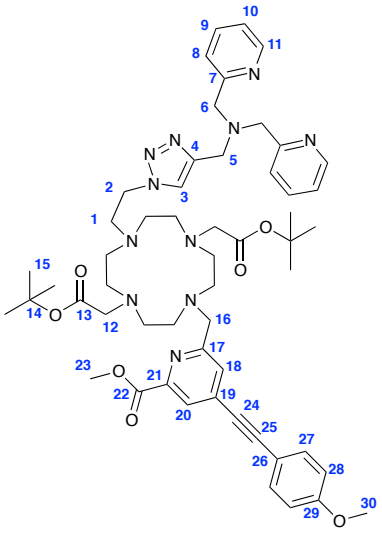
To spike the grains, ten cordiale grains were taken and an 0.01 mL aliquot of a glyphosate solution (0.15-1.2 mM) was added and the container sealed for 24 h, to allow the grains to soak up the glyphosate. After lyophilisation to remove water, the grains were soaked for 24 h in 2 mL of water. The solution was filtered and lyophilised again, before the residue was taken up in 2 mL of the complex (7 µM) solution containing 0.1 M NaCl, 0.1 M MES at pH 5.9.

Synthesis of [Eu.L^4]



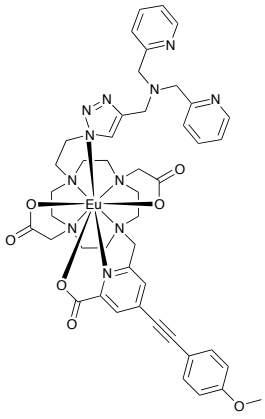
2-Azidoethanol (65 mg, 0.75 mmol) was dissolved in dry THF (3 mL), followed by addition of dry triethylamine (0.1 mL, 0.72 mmol) and MsCl (15 mL, 0.19 mmol). The reaction mixture was stirred at rt for 1h and the solvent was removed under reduced pressure. The crude material was redissolved in 15 mL of DCM and was washed with water (2 x 15 mL). The organic fraction was dried over $MgSO_4$ and the solvent was removed under reduced pressure, dried and redissolved in 3 mL of dry acetonitrile. 1,7-Bis(t-butoxycarbonylmethyl)-1,4,7,10-tetraaza-cyclododecane (300 mg, 0.75 mmol) was dissolved in dry acetonitrile (10 mL) and K_2CO_3 was added (300 mg, 2.17 mmol) was added. The reaction mixture was cooled down with an ice bath and stirred for 5 min. The mesylate solution was added dropwise within 5 min and the reaction mixture was allowed to reach rt and stirred for 18 h. The solution was filtered, concentrated down to 4 mL, followed by addition of *N*,*N*-*bis*(pyridin-2-ylmethyl)prop-2-yn-1-amine (178 mg, 0.75 mmol), piperidine (1 mL) and CuI (10 mg, 0.05 mmol) were added. The reaction mixture was placed into a microwaveable vial, sealed and microwaved at 100 °C for 30 min. The reaction mixture was filtered and purified using RP-HPLC (10% to 100% of acetonitrile (+0.1% formic acid) in water (+0.1% formic acid) over 10 min). The fractions containing the desired product were combined and neutralised with aqueous ammonia solution. The solvent was removed under reduced pressure, dried and re-dissolved in acetonitrile. Ammonium formate was filtered off and the solvent was removed under reduced pressure, giving brown oil, containing the desired product and tetra-alkylated side-product. The components were separated using alumina column (0.5%→1% of MeOH in DCM), giving the desired product as a yellow oil (51 mg, 10% yield over two steps); ^{1}H NMR (295 K, 400 MHz, $CDCl_3$) δ_H 8.54 (2H, d, $^3J_{H\text{-}H}$ = 5.0 Hz, H^{11}), 7.89 (1H, s, H^3), 7.73-7.62 (4H, m, H^8, H^9), 7.21-7.18 (2H, m, H^{10}), 4.51 (2H, m, H^1), 3.86 (2H, s, H^6), 3.85 (4H, s, H^6), 3.38 (4H, s, H^{12}), 3.11 (2H, m, H^2), 3.08-2.77 (16H, m, cyclen), 1.43 (18H, s, H^{15}); ^{13}C NMR (295 K, 100 MHz, $CDCl_3$) δ_C 170.4 (C^{13}), 158.6 (C^7), 148.5 (C^{11}), 143.9 (C^4), 137.1 (C^9), 124.0 (C^3), 123.5 (C^8), 122.3 (C^{10}), 81.7 (C^{14}), 59.1 (C^6), 57.8 (C^{12}), 52.7-45.8 (C^1, C^2, C^5, cyclen), 28.2 (C^{15}); *m/z* (HRMS$^+$) 707.4736 [M+H$^+$]$^+$ ($C_{37}H_{59}N_{10}O_4$ requires 707.4721).

Di-*tert*-butyl 2,2'-(4-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-10-((6-(methoxycarbonyl)-4-((4-methoxyphenyl) ethynyl) pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate

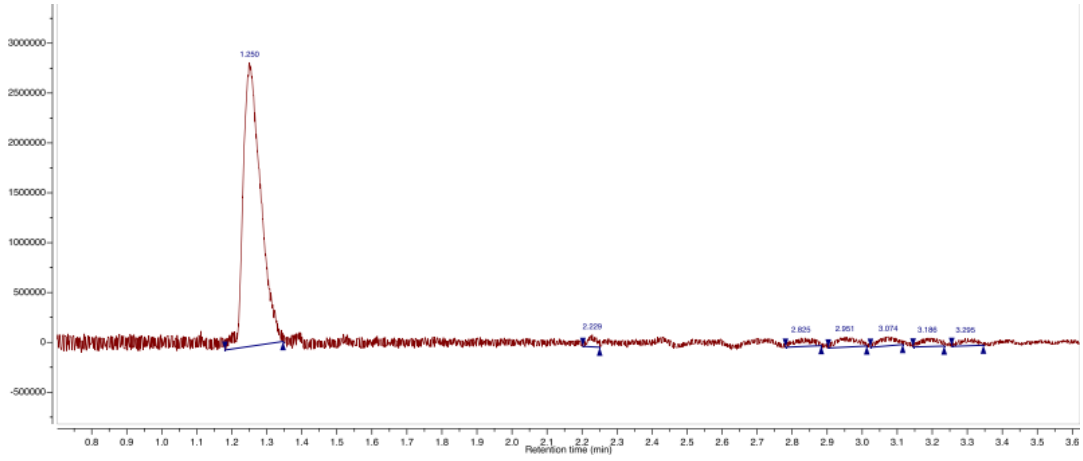


Methyl 6-(hydroxymethyl)-4-((4-methoxyphenyl)ethynyl)picolinate (30 mg, 0.10 mmol) was dissolved in dry THF (4 mL), followed by addition of dry triethylamine (0.1 mL, 0.72 mmol) and methanesulfonyl chloride (15 µL, 0.19 mmol). The reaction mixture was stirred at rt for 1 h and the solvent was removed under reduced pressure. The crude material was redissolved in 15 mL of DCM and was washed with water (2 x 15 mL). The organic fraction was dried over $MgSO_4$ and the solvent was removed under reduced pressure, dried and redissolved in dry acetonitrile (3 mL). 1-(1-(2-Ethyl)-1H-1,2,3-triazol-4-yl)-N,N-bis(pyridin-2-ylmethyl)methanamine-4,10-(bis)-t-butoxycarbonylmethyl)-1,4,7,10-tetraaza-cyclododecane, (51 mg, 0.072 mmol) was dissolved in dry acetonitrile (8 mL) and K_2CO_3 (100 mg, 0.72 mmol) was added. The mesylate solution was added to the solution and the reaction mixture stirred at 60 °C for 18 h. The reaction mixture was filtered and purified on RP-HPLC (10% to 100% acetonitrile (+0.1% formic acid) in water (+0.1% formic acid) over 9 min). The fractions containing the desired product were combined and neutralised with aqueous ammonia solution. The solvent was removed under reduced pressure, dried and redissolved in acetonitrile. Ammonium formate was filtered off and the solvent was removed under reduced pressure, giving a colourless oil (53 mg, 75% yield); ^{1}H NMR (295 K, 600 MHz, $CDCl_3$) δ_H 8.50 (2H, m, H^{11}), 8.06 (1H, $^4J_{H\text{-}H}$ = 1.5 Hz, H^{20}), 7.95 (1H, s, H^3), 7.69-7.66 (2H, m, H^9), 7.64 (1H, m, H^{18}), 7.61-7.60 (2H, m, H^8), 7.47 (2H, d, $^3J_{H\text{-}H}$ = 9.0 Hz, H^{27}), 7.15-7.13 (2H, m, H^{10}), 6.89 (2H, d, $^3J_{H\text{-}H}$ = 9.0 Hz, H^{28}), 4.64 (2H, t, $^3J_{H\text{-}H}$ = 7.0 Hz, H^1), 4.38 (2H, m, H^{16}), 3.95 (3H, s, H^{30}), 3.83 (4H, s, H^6), 3.82 (2H, s, H^5), 3.80 (3H, s, H^{23}), 3.52 (2H, t, $^3J_{H\text{-}H}$ = 7.0 Hz, H^2), 3.41 (4H, s, H^{12}), 3.12-2.98 (16H, m, cyclen), 1.39 (18H, s, H^{15}); ^{13}C NMR (295 K, 151 MHz, $CDCl_3$) δ_C 169.4 (C^{13}), 164.7 (C^{22}), 160.7 (C^{17}), 159.0 (C^7), 148.6 (C^{11}), 147.8 (C^{21}), 144.6 (C^4), 136.9 (C^9), 134.5 (C^{19}), 133.7 (C^{27}), 128.9 (C^{18}), 126.2 (C^{20}), 124.1 (C^3), 123.2 (C^8), 122.2 (C^{10}), 114.2 (C^{28}), 113.3 (C^{26}), 97.0 (C^{25}), 84.7 (C^{24}), 82.3 (C^{14}), 59.3 (C^{23}), 58.1 (C^{16}), 56.4 (C^{12}), 55.4 (C^6), 53.8 (C^2), 53.0 (C^{30}), 52.0-50.2 (cyclen), 48.7 (C^5), 45.9 (C^1), 28.0 (C^{15}); *m/z* (HRMS$^+$) 986.5619 $[M+H^+]^+$ ($C_{54}H_{72}N_{11}O_7$ requires 986.5616).

[EuL^4]



The diester (53 mg, 0.054 mmol) was dissolved in aqueous NaOH (0.5M, 4 mL) and stirred at 60 °C for 2 h. The solution was neutralised by addition of 1 M HCl and $EuCl_3$ (0.090 mmol) was added. The reaction mixture was stirred at rt for 1 h, pH was adjusted to 6.5 and the reaction mixture was stirred for another hour. Aqueous ammonia solution was added dropwise to pH 10, in order to precipitate excess europium as $Eu(OH)_3$, which was separated by centrifugation. The solution was neutralised again and was left in the fridge, causing precipitation of the desired complex as an off-white solid, which was separated by decanting the solution (20 mg, 37% yield); *m/z* (HRMS$^+$) 1008.320 $[M+H^+]^+$ ($C_{45}H_{51}N_{11}O_7{}^{151}Eu$ requires 1008.319), $\varepsilon(H_2O)$ = 20,000 $M^{-1}cm^{-1}$; $\tau(H_2O)$ = 0.51 ms (pH = 7.4), $\tau(D_2O)$ = 1.12 ms (pD = 7.8).



LC-MS UV trace of **[**EuL^4] (5% H_2O (+0.1% v/v formic acid) in MeOH (+0.1% v/v formic acid) to 95% H_2O (+0.1% v/v formic acid) in MeOH (+0.1% v/v formic acid) over 3.8 min