**Electronic Supplementary Information for**

# An aryl-phosphonate appended macrocyclic platform for lanthanide based bimodal imaging agents

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## 1. General methods

All chemicals were purchased from commercial sources and used without further purification, with the exception of THF, which was distilled prior to use. Column chromatography was performed using silica

gel 60 (70-230 mesh ASTM) or aluminium oxide 90 active basic from Merck (Germany). Reversed-phase high-performance liquid chromatography was performed on a Varian PrepStar Instrument (Australia), equipped with PrepStar SD-1 pump heads. Analytical RP-HPLC was performed on an Atlantis C18 column 4.6 mm x 150 mm, particle size 5 $\mu$m, using method A (Table S1). Preparative RP-HPLC was performed on an Atlantis C18 column 19 mm x 150 mm, particle size 5 $\mu$m, Waters corporation (USA) using method B (Table S2).

$^{1}$H, $^{13}$C {$^{1}$H}, $^{31}$P {$^{1}$H} NMR spectra and all relaxometric experiments were recorded on a Bruker Avance III 300 MHz 'Microbay' spectrometer at 25 °C Bruker (Germany). The relaxivities of the complexes **GdL$^{1-4}$** are an average of three measurements at concentrations ranging from 2-5 mM, in MOPS buffered solution (0.1 M, pH 7.4). For each measurement the exact concentration of the $Gd^{3+}$ complex was determined using the bulk magnetic susceptibility shift.[1] ESI-LRMS were performed on an ion trap SL 1100 system Agilent (Germany). FT-ICR-MS were performed on a Bruker FT-ICR Apex II spectrometer Agilent (Germany). HR-EI-MS were performed on a MAT Sektorfeld mass spectrometer Finnigan (Germany).

UV/visible absorption spectra were recorded using a Perkin-Elmer lambda 25 spectrometer (USA). Luminescence steady-state and time resolved measurements were performed on QuantaMasterTM 3 PH fluorescence spectrometer from Photon Technology International, Inc. (USA). The steady state measurements were performed in $H_2O$ (25 °C, pH 7.4, MOPS) at a concentration of 5 mM for **EuL$^{1-4}$** and 25 µM for **TbL$^{1-4}$**. Excitation and emission slits were set to 2 nm and 1 nm for **EuL$^{1-4}$** and **TbL$^{1-4}$** respectively. Datasets are an average of 10 scans. The time resolved measurements were performed in $H_2O$ and $D_2O$ (25 °C, pH 7.4, MOPS) at a concentration of 25 µM for **TbL$^{1-4}$**. Excitation and emission slits were set to 15 and 5 nm bandpass respectively, with 10 µs resolution. Datasets are recorded with a 100 µs delay and are an average of 10 scans. Each reported value is the mean of three independent measurements and obtained curves are fitted to the first order exponential decay with $R^2 > 0.99$.

The quantum yield of luminescence of the complexes **TbL$^{1-4}$** were measured using Perkin-Elmer LS55 and LS50 spectrometers (USA) operating in phosphorescence mode, relative to a terbium standard with a known quantum yield; trisodium terbium(tris-2,6-dipicolinate) ($Na_3$·[Tb(dpa)$_3$]) in 0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer ($\phi$ = 26.5 %),[2-4] using solutions of the sample and standard with the same absorbance value (between 0.18 and 0.22). Concentrations higher or lower than this gave erroneous results due to solution speciation of the $Na_3$·[Tb(dpa)$_3$] complex. The details of these approaches are described in the quoted references.

The model extracellular medium used in the relaxometric and luminescence measurements consisted of Dulbecco´s Modified Eagle Medium (DMEM), supplemented with 10% fetal calf serum (FCS), 4mM L-

glutamine, 100 µg/mL streptomycin and 100 U/mL penicillin. DMEM and FCS were obtained from Biochrom AG (Germany). For the relaxometric measurements in the presence of cells, NIH 3T3 mouse embryonic fibroblasts were grown in 175 $cm^2$ tissue culture flasks to reach about 80% confluency. Cells were washed with Hank´s Balanced Salt Solution (HBSS), trypsinized, centrifuged and re-suspended in 0.6 mL Eppendorf tubes at $1\times10^7$ cells in 500 µL DMEM containing 20 – 80 µM **GdL$^{1\text{-}4}$**. Tubes containing only medium, cells without **GdL$^{1\text{-}4}$**, and cells re-suspended in medium containing the extracellular contrast agent Dotarem® Guerbert (France) at 20 – 80 µM served as controls. For the cell binding/interaction studies 3T3 cells growing at an exponential rate were labeled with 40 and 80 µM of **GdL$^{1\text{-}4}$** in 175 $cm^2$ tissue culture flasks for 18 h. After repeated washes with HBSS, cells were trypsinized, centrifuged and re-suspended at a cell density of $1\times10^7$ cells/500 µL in fresh culture medium without contrast agent. Cells incubated in the absence of CA served as control. The cells were allowed to settle before starting the MR measurements.

MR imaging of the cell phantoms was performed at 3T (128 MHz, 21°C) on a clinical human MR scanner (MAGNETOM Tim Trio, Siemens Healthcare, Germany). One axial slice of interest was positioned through the cell pellet, a second one above the cell pellet through the supernatant. Longitudinal relaxation times ($T_1$) were measured using an inversion recovery sequence to obtain images from a 1 mm thick slice through the samples. The inversion time (*Ti*) was varied from 23 ms to 3000 ms in about 12 steps. Images were read out with a turbo spin echo technique, acquiring 5 echoes per scan. The repetition time (TR) was 10,000 ms to ensure complete relaxation. A matrix of 256 x 256 voxels was used over a field-of-view of 110 x 110 $mm^2$. Six averages per *Ti* were possible within 18 min.

Fitting to relaxivity curves was achieved using self-written routines in MATLAB 6.5 R13 (The Mathworks Inc., USA). $T_1$ relaxation data with varying $t$ = TR were fitted to $S=S_0(1-a \times exp(-t/T_1))$. Nonlinear least-squares fitting of three parameters $S_0$ (initial signal at $t$=0), $T_1$ and $a$ was done for each voxel with the Gauss-Newton method (MATLAB function nlinfit). For each fitted parameter, the 95% confidence intervals were calculated (MATLAB functions nlparci, nlpreci) and used as an error estimate of the fitted relaxation times $T_1$ and $S_0$. The fit procedure resulted in parameter maps of $T_1$, $S_0$ and corresponding error maps $\sigma_{T1}$, $\sigma_{S0}$.

Circular image-regions in the tubes were defined as Regions Of Interest (ROIs), and the means and distribution width of the relaxation times of voxels in these regions were calculated. An iterative Gaussian fit was used to determine mean and standard deviation (SD) of a distribution with outliers correction. For this purpose a distribution histogram was first fitted to a Gaussian to estimate mean and SD. The tails of the distribution were then discarded by using a threshold of three SDs. A repeated fit proved to be robust and converged to the 'true' Gaussian mean and width of the distribution barring the outliers, observed as a

result of the non-linear fit of noisy voxels. The processing of the relaxation data thus resulted in specific $R_1 = 1/T_1$ values for each tube sample including the standard deviation in the selected ROI ensemble. The ensemble error matched closely the errors of a single-voxel fit, which showed that no further systematic errors were introduced by the image encoding.

**Table S1.** Elution conditions for analytical HPLC (method A)

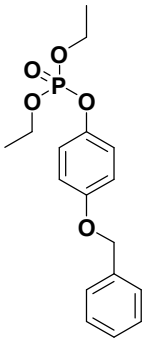
| Time (s) | %$H_2O$ | % MeCN |
|---|---|---|
| 0:00 | 90 | 10 |
| 6:00 | 70 | 30 |
| 11:00 | 70 | 30 |
| 13:00 | 10 | 90 |
| 15:00 | 10 | 90 |
| 18:00 | 90 | 10 |
| 20:00 | 90 | 10 |

**Table S2.** Elution conditions for preparative HPLC (method B)

| Time (s) | %$H_2O$ | % MeCN |
|---|---|---|
| 0:00 | 90 | 10 |
| 3:00 | 80 | 20 |
| 11:00 | 80 | 20 |
| 13:00 | 30 | 70 |
| 15:00 | 10 | 90 |
| 18:00 | 90 | 10 |
| 25:00 | 90 | 10 |

## 2. Synthesis and characterisation of the ligands and complexes

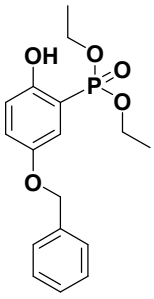
**4-(benzyloxy)phenyl diethyl phosphate (2)**



4-(benzyloxy)phenol **1** (4.70 g, 23.5 mmol) and triethylamine (4.43 mL, 31.8 mmol) were mixed together in carbon tetrachloride (60 mL). The mixture was cooled in an ice bath to 0 ºC and diethyl phosphite (4.09 mL, 31.8 mmol) was added drop wise; upon warming to room temperature the precipitate dissolved and the solution changed to an orange colour. After 24 hours, dichloromethane was added and the organic phase was washed with 1 M HCl, saturated NaCl solution and water. This was then dried over $Na_2SO_4$ and the solvent was removed under reduced pressure. The crude product was purified using column chromatography (silica gel, from 9:1 hexane in ethyl acetate to 100% ethyl acetate). Removal of the solvent yielded **4** as a yellow waxy solid (7.00 g, 93 %).

TLC (silica, ethyl acetate:hexane 1:1) $R_f$ = 0.38. $^1$H NMR (300 MHz, $CD_3OD$) δ (ppm) 1.31 (td, $^3J_{HH}$ = 7.0, 1.1 Hz, 3H, $POCH_2\mathit{CH_3}$), 4.12-4.24 (m, 4H, $PO\mathit{CH_2}CH_3$), 5.02 (s, 2H, $BnC\mathit{H_2}$), 6.97 (dd, 2H, Ar*H*), 7.12 (dd, 2H, Ar*H*), 7.25-7.42 (m, 5H, Bn*H*). $^{13}$C{$^1$H} NMR (75 MHz, $CDCl_3$) δ (ppm) 15.23 (d, $^4J_{CP}$ = 6.8 Hz, $POCH_2\mathit{CH_3}$), 63.61 (d, $^3J_{CP}$ = 6.7 Hz, $PO\mathit{CH_2}CH_3$), 69.42 ($BnCH_2$), 114.83 (ArCH), 120.11 (BnCH), 126.61 (BnCH), 127.68 (BnCH), 136.07 (Bn*C*), 143.73 (d, $^3J_{CP}$ = 6.8 Hz, Ph*C*OP) , 155.06 (Bn*C*O). $^{31}$P{$^1$H} NMR (122 MHz, $CDCl_3$) δ (ppm) 5.78. HR-EI-MS for $C_{17}H_{21}O_5P$ calcd. 336.11266, found 336.11225.

**Diethyl 5-(benzyloxy)-2-hydroxyphenylphosphonate (3)**

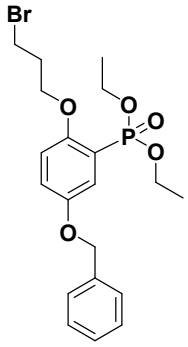


A flask containing the phosphate ester **2** (7.00 g, 20.81 mmol) was purged and kept under dinitrogen. Dry tetrahydrofuran (30 mL) was added and the solution cooled to -78 ºC. A solution of lithium

diisopropylamine was prepared over 30 minutes by mixing diisopropylamine (14.83 mL, 104.1 mmol) with *n*-butyl lithium (39.02 mL, 104.1 mmol, 1.6 M in hexane) in tetrahyrofuran (20 mL) under a dinitrogen atmosphere at -78 °C. This was then transferred via cannula to the phosphate containing solution, changing the colour to bright red. After five hours, saturated $NH_4Cl$ solution was added to quench the reaction and the mixture was warmed to room temperature. Water was added and the product was extracted into diethyl ether. The organic phase was dried over $Na_2SO_4$ and the solvent was then removed under reduced pressure. The crude product was purified using column chromatography (silica, from 9:1 to 3:7 hexane: ethyl acetate). Following removal of the solvent, **3** was isolated as an orange oil that on cooling to 0 °C becomes a yellow waxy solid (5.93 g, 85 %).

TLC (silica, Ethyl acetate:hexane 1:1) $R_f$ = 0.70. $^1$H NMR (300 MHz, $CDCl_3$,) δ (ppm) 1.31 (t, $^3J_{HH}$ = 7.0 Hz, 6H, $POCH_2\mathit{CH_3}$), 4.08 (m, 4H, $PO\mathit{CH_2}CH_3$), 5.02 (s, 2H, $BnC\mathit{H_2}$), 6.97 (m, 2H, Ar*H*), 7.14 (m, 1H, Ar*H*), 7.38 (m, 5H, Bn*H*), 9.88 (s, 1H, PhO*H*). $^{13}$C{$^1$H} NMR (75 MHz, $CDCl_3$) δ (ppm) 15.88 (d, $^4J_{CP}$ = 5.2 Hz, $POCH_2\mathit{CH_3}$), 62.45 (d, $^3J_{CP}$ = 5.0 Hz, $PO\mathit{CH_2}CH_3$), 70.53 ($BnCH_2$),107.02 (Ar*C*), 109.39 (Ar*C*), 115.39 (Ar*C*H), 123.51 (Ar*C*), 127.22 (Bn*C*H), 127.74 (Ar*C*H) 128.30 (Bn*C*H), 136.51 (Bn*C*), 151.14 (d, $^3J_{CP}$ = 17.1 Hz, Ph*C*OP) , 156.26 (Bn*C*O). $^{31}$P{$^1$H} NMR (122 MHz, $CDCl_3$) δ (ppm) 21.73. HR-EI-MS for $C_{17}H_{21}O_5P$ calcd. 336.11266, found 336.11176.

**Diethyl 5-(benzyloxy)-2-(3-bromopropoxy)phenylphosphonate (4)**

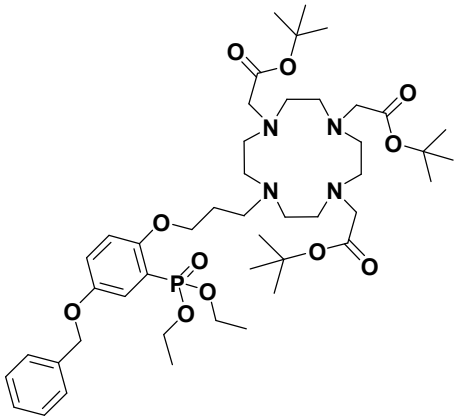



The phosphonate **3** (2.50 g, 7.43 mmol) was dissolved in anhydrous dimethylformamide (120 mL), $K_2CO_3$ (2.05 g, 14.87 mmol) was added and the mixture was heated to 60 °C for 1 hour under a dinitrogen atmosphere. 1,3-dibromopropane (2.26 mL, 22.29 mmol) in dimethylformamide (20 mL) was added drop wise and the mixture was heated for an additional 15 hours. After cooling to room temperature, the inorganic salts were removed through filtration and dichloromethane was added. The organic phase was washed with water, saturated NaCl solution and dried over $Na_2SO_4$; removal of the solvent resulted in a brown oil. The compound was purified using column chromatography (silica from 9:1 hexane in ethyl acetate to 100% ethyl acetate). Removal of the solvents yielded **4** as an orange oil (2.42 g, 71 %).

TLC (silica, Ethyl acetate:hexane 1:1) $R_f$ = 0.32. $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.27 (t, $^3J_{HH}$ = 5.7 Hz, 2H, $POCH_2\mathit{CH_3}$), 2.27 (m, 2H, $BrCH_2C\mathit{H_2}$), 3.65–3.71 (m, 2H, $\mathit{CH_2}CH_2CH_2Br$), 3.96–4.19 (m, 4H, $PO\mathit{CH_2}CH_3$, $\mathit{CH_2}CH_2Br$ ), 5.00 (s, 2H, $BnC\mathit{H_2}$), 6.81–6.87 (m, 1H, Ar*H*), 7.03–7.08 (m 1H Ar*H*), 7.21–7.47 (m 6H Ar*H*, Bn*H*). $^{13}$C{$^1$H} NMR (75 MHz, $CDCl_3$) δ (ppm) 16.37, 30.21, 32.30, 61.99, 66.07,

70.77, 113.48, 116.01, 120.79, 121.00, 127.47, 128.52, 136.73, 152.36, 154.59. $^{31}P\{^{1}H\}$ NMR (122 MHz, $CDCl_3$) δ (ppm) 16.85. HR-EI-MS for $C_{20}H_{26}O_5PBr$ calcd. 456.070123, found 456.06890.

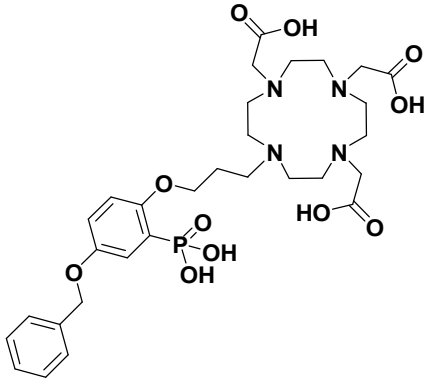
**tri-tert-butyl-2,2',2''-(10-(3-(4-(benzyloxy)-2-(diethoxyphosphoryl)phenoxy)propyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (6)**



$Cs_2CO_3$ (0.51 g, 6.02 mmol) and KI (0.03g, 0.18 mmol) were added to a solution of 1,4,7-tri(*t*-butoxycarbonylmethyl)cyclen (1.04 g, 1.75 mmol) in anhydrous acetonitrile (10 mL). The phosphonate arm **4** (1.20 g, 2.62 mmol) was dissolved in anhydrous acetonitrile (10 mL) and added to the solution; this was then heated to 70 °C under a dinitrogen atmosphere. After 24 hours the solution was cooled to room temperature, the inorganic salts were removed by filtration and the solvent was evaporated under reduced pressure. The resulting yellow oil was dissolved in dichloromethane and washed with saturated NaCl solution. The organic phase was then dried over $Na_2SO_4$ and the solvent evaporated. The crude product was first purified by dissolving in hot toluene and slowly cooling the solution to 0 °C where the majority of the triester starting material was removed by filtration. Then, through column chromatography (alumina, from 100% dichloromethane to 96:4 dichloromethane : methanol), evaporation of the solvent yielded **6** as a yellow solid (0.98 g, 62 %).

TLC (silica, 9:1 dichloromethane : methanol) $R_f$ = 0.29. $^{1}H$ NMR (300 MHz, $CDCl_3$) δ (ppm) 1.32 (t, $^{3}J_{HH}$ = 7.0 Hz, 6H, $POCH_2CH_3$), 1.45 (br, 27H, $CCH_3$), 1.93–4.17 (br, 34H, $CH_2$ ring $NCH_2COO$, $NCH_2CH_2CH_2O$, $POCH_2CH_3$), 5.04 (s, 2H, $BnCH_2$), 6.90 (m, 1H, Ar*H*), 7.08–7.12 (m, 1H, Ar*H*), 7.29–7.51 (m, 6H, Bn*H*, Ar*H*). $^{13}C\{^{1}H\}$ NMR (75 MHz, $CDCl_3$) δ (ppm) 16.22, 25.30, 27.76, 50.60, 51.66, 51.85, 52.43, 55.47, 56.31, 61.71, 67.29, 70.53, 80.36, 82.14, 82.45, 113.24, 113.40, 115.56, 118.03, 118.35, 120.46, 120.55, 120.66, 120.88, 127.23, 128.27, 128.31, 136.48, 136.64, 151.68, 151.92, 152.00, 154.90, 170.78, 170.85, 172.39, 173.40. $^{31}P\{^{1}H\}$ NMR (122 MHz, $CDCl_3$) δ (ppm) 16.35, 16.91. FT-ICR-MS for $C_{46}H_{75}N_4O_{11}P$ calcd. 891.522217 $[M + H]^+$, found 891.523087.

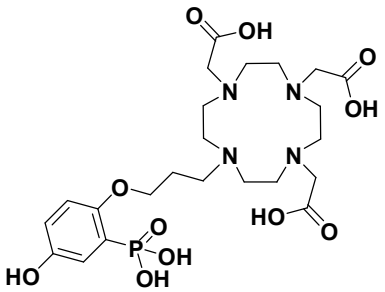
**2,2',2''-(10-(3-(4-(benzyloxy)-2-phosphonophenoxy)propyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid ($L^1$)**



The ligand precursor **6** (0.800 g, 0.90 mmol) was dissolved in dichloromethane (3 mL) and cooled in an ice bath to 0 °C under a dinitrogen atmosphere. Bromotrimethylsilane (0.59 mL, 4.49 mmol) was added slowly and the solution was stirred at room temperature for 3 hours. The excess reagent and solvent were removed under reduced pressure and the cleavage of the ethyl esters was confirmed through $^{1}$H and $^{31}$P NMR spectroscopy. Trifluoroacetic acid in dichloromethane (4 mL, 1:1 v:v) was added to the crude material and the mixture was stirred at room temperature for 4 hours. The solvent and excess volatiles were removed under reduced pressure to produce an orange solid. Upon analysis by ESI mass spectrometry, partial cleavage of the benzyl ether protecting group had occurred. The products were purified by RP-HPLC. Following lyophilisation, a white powder was obtained. This rapidly became a yellow oil on exposure to air due to the hygroscopic nature of the product (0.162 g, 27 %).

$^{1}$H NMR (300 MHz, $D_2O$) δ (ppm) 1.98–4.58 (br, 30H, *CH*$_2$ ring NC*H*$_2$COO, NC*H*$_2$C*H*$_2$C*H*$_2$O, BnC*H*$_2$), 6.46–6.57 (m, 2H, Ar*H*), 7.09 (m, 6H, Ar*H*). $^{13}$C{$^{1}$H} NMR (75 MHz, $D_2O$) δ (ppm) 20.31, 48.53 – 56.19, 66.55, 70.10, 113.48, 118.10, 119.64, 123.00, 125.35, 127.70, 128.46, 137.06, 151.51, 151.69, 153.82, 170.03, 174.47, $^{31}$P{$^{1}$H} NMR (122 MHz, $D_2O$) δ (ppm) 9.58. FT-ICR-MS for $C_{30}H_{43}N_4O_{11}P_1$ calcd. 665.25932 $[M - H]^-$, found 665.259523.

**2,2',2''-(10-(3-(4-hydroxy-2-phosphonophenoxy)propyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid ($L^2$)**

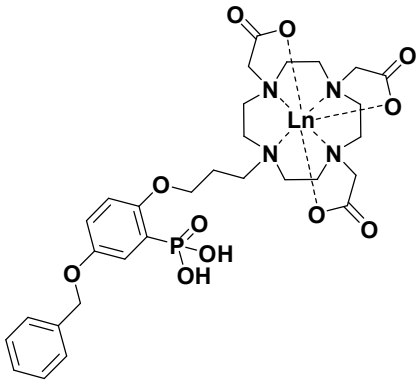


**6** was dissolved in ethanol (25 mL) and 10% palladium on charcoal was added. The solution was heated to 60 °C and 60 psi of $H_2$ gas was introduced using Parr hydrogenator apparatus. Then the solution was

left to shake overnight. The Pd/C was removed by filtration through celite and the ethanol was evaporated under reduced pressure. The same procedure was then followed as for **L[1]** (0.114 g, 31 %).

$^{1}$H NMR (300 MHz, $D_2O$) δ (ppm) 2.06–3.89 (br, 28H, *CH*$_2$ ring NC*H*$_2$COO, NC*H*$_2$C*H*$_2$C*H*$_2$O), 6.81–7.10 (m, 3H, Ar*H*). $^{13}$C{$^{1}$H} NMR (75 MHz, $D_2O$, 25°C) δ (ppm) 21.01, 46.32, 46.74, 48.01, 49.85, 50.12, 50.79, 51.26, 53.66, 63.85, 108.78, 112.65, 112.76, 116.51, 117.78, 118.17, 118.90, 120.38, 121.27, 147.27, 147.50, 151.41, 160.30, 160.77, 161.24, 161.71, 167.39, 171.42, 172.62. $^{31}$P{$^{1}$H} NMR (122 MHz, $D_2O$) δ (ppm) 11.52. FT-ICR-MS for $C_{23}H_{37}N_4O_{11}P$ calcd. 575.21237 [M - H]$^-$, found 575.212361.

**2,2',2''-(10-(3-(4-(benzyloxy)-2-phosphonophenoxy)propyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid lanthanide complex (LnL[1])**



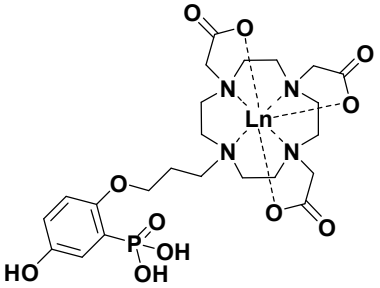

All the complexes were prepared by the following procedure: The ligand and 1.2 equivalents of the corresponding lanthanide chloride salt were dissolved in water and heated to 60 °C for 16 hours at pH 5.3–5.8, (adjusted using 1 M NaOH). The excess lanthanide salts were removed by precipitation from solution by raising the pH to 10, and by treating the supernatant with chelex 100. The xylenol orange test was performed to confirm the absence of any free lanthanide ions in solution. Following lyophilisation a white powder was obtained.

**EuL[1]**: UV/Vis ($H_2O$) $\lambda_{max}$ (ε ($M^{-1}$ $cm^{-1}$)) = 291 nm (ε = 3,300). ESI-MS (pos.) m/z: 815.0 ([M - H]$^-$), 836.9 ([M + Na - H]$^-$). $^{31}$P{$^{1}$H} NMR (122 MHz, $D_2O$) δ (ppm) 9.61, -82.79.

**GdL[1]**: ESI-MS (neg.) m/z: 820.0 ([M - H]$^-$). $r_1$ 7.20 $mM^{-1}s^{-1}$ (pH 7.4, MOPS, 300 MHz).

**TbL[1]**: UV/Vis ($H_2O$) $\lambda_{max}$ (ε ($M^{-1}$ $cm^{-1}$)) ($\pi \rightarrow \pi^*$) = 291 nm (ε = 3,300). ESI-MS (neg.) m/z: 821.2 ([M - H]$^-$).

**2,2',2''-(10-(3-(4-hydroxy-2-phosphonophenoxy)propyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid lanthanide complex ($LnL^2$)**



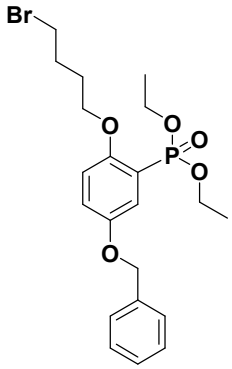

The complexes were prepared in the same manner as described for **$LnL^1$**.

**$EuL^2$**: /Vis ($H_2O$) $\lambda_{max}$ ($\varepsilon$ ($M^{-1}$ $cm^{-1}$)) = 291 nm ($\varepsilon$ = 2,900). ESI-MS (neg.) m/z: 723.1 ([M - H]$^-$). $^{31}P\{^1H\}$ NMR (122 MHz, $D_2O$) $\delta$ (ppm) -75.47.

**$GdL^2$**: ESI-MS (neg.) m/z: 730.1 ([M - H]$^-$). $r_1$ 7.33 $mM^{-1}s^{-1}$ (pH 7.4, MOPS, 300 MHz).

**$TbL^2$**: UV/Vis ($H_2O$) $\lambda_{max}$ ($\varepsilon$ ($M^{-1}$ $cm^{-1}$)) = 291 nm ($\varepsilon$ = 2,500). ESI-MS (neg.) m/z: 731.1 ([M - H]$^-$).

**Diethyl 5-(benzyloxy)-2-(4-bromobutoxy)phenylphosphonate (5)**

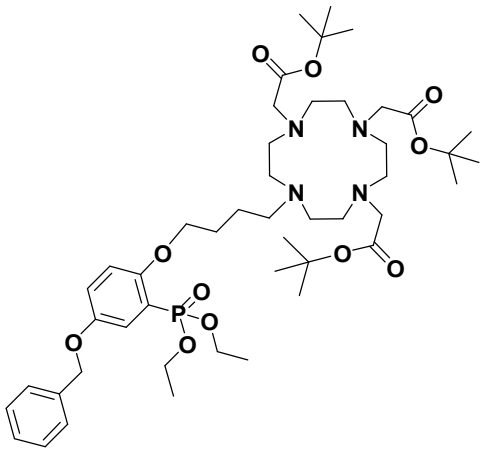



**3** (3.00 g, 8.92 mmol) was dissolved in anhydrous dimethylformamide (120 mL), potassium carbonate (2.47 g, 17.84 mmol) was added and the mixture was heated to 60 °C for 1 hour under a dinitrogen atmosphere. 1,4-Dibromobutane (3.20 mL, 26.76 mmol) in dimethylformamide (20 mL) was added drop-wise and the mixture was heated for an additional 15 hours. After cooling to room temperature, the inorganic salts were removed through filtration and dichloromethane was added. The organic phase was washed with water, saturated NaCl solution and dried over $Na_2SO_4$; removal of the solvent resulted in a brown oil. The compound was purified using column chromatography (silica from 100% dichloromethane to 93:7 methanol in dichloromethane). Removal of the solvents yielded **5** as an orange oil (3.10 g, 74 %).

TLC (silica, Ethyl acetate:hexane 6:4) $R_f$ = 0.44. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ (ppm) 1.30 (t, $^3J_{HH}$ = 7.0 Hz, 3H, $POCH_2\mathit{CH_3}$), 1.90–2.13 (m, 4H, $BrCH_2C\mathit{H_2}C\mathit{H_2}$), 3.47 (t, $^3J_{HH}$ = 6.6 Hz, 2H, $\mathit{CH_2}OC$), 3.96–4.20 (m, 6H, $PO\mathit{CH_2}CH_3$, $\mathit{CH_2}CH_2Br$), 5.00 (s, 2H, $BnC\mathit{H_2}$), 6.79–6.84 (m, 1H, Ar*H*), 7.04-7.08 (m, 1H, Ar*H*), 7.26–7.49 (m, 6H, Ar*H*, Bn*H*). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$) $\delta$ (ppm) 16.17, 27.61, 29.01, 33.41,

61.74, 67.70 70.50, 113.25, 115.86, 118.34, 120.64, 127.68, 136.54, 151.98, 154.61. $^{31}P\{^{1}H\}$ NMR (122 MHz, $CDCl_3$) δ (ppm) 16.74. HR-EI-MS for $C_{21}H_{28}O_5PBr$ calcd. 470.085773, found 470.08499.

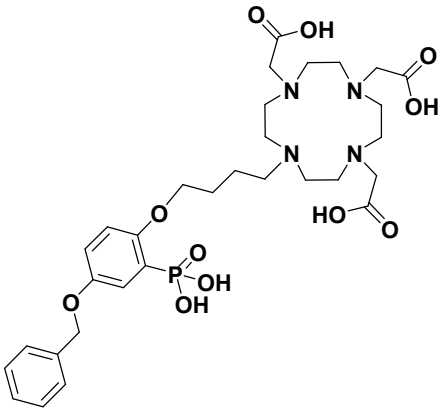
**tri-tert-butyl-2,2',2''-(10-(4-(4-(benzyloxy)-2-(diethoxyphosphoryl)phenoxy)butyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (7)**



$Cs_2CO_3$ (3.28 g, 10.18 mmol) was added to a solution of 1,4,7-tri(*t*-butoxycarbonylmethyl)cyclen (2.00 g, 3.36 mmol) in anhydrous acetonitrile (20 mL). The phosphonate arm **5** (1.98 g, 4.20 mmol) was dissolved in anhydrous acetonitrile (10 mL) and added to the solution; this was then heated to 70 °C under a dinitrogen atmosphere. After 72 hours, the solution was cooled to room temperature, the inorganic salts removed by filtration and the solvent evaporated under reduced pressure. The resulting yellow oil was dissolved in dichloromethane and washed with saturated NaCl solution. The organic phase was then dried over $Na_2SO_4$ and the solvent evaporated. The crude product was first purified by dissolving in hot toluene and slowly cooling the solution to 0 °C where the majority of the triester starting material was removed by filtration. Then, through column chromatography (silica, from 9:1 to 1:1 dichloromethane : ethyl acetate and then at 97:3 dichloromethane : methanol); evaporation of the solvent yielded **7** as a yellow oil (2.51 g, 83 %).

TLC (silica, 9:1 dichloromethane:methanol) $R_f$ = 0.27. $^{1}H$ NMR (300 MHz, $CDCl_3$) δ (ppm) 1.19–1.41(m, 33H, $POCH_2CH_3$, $CCH_3$), 1.73–4.14 (br, 34H, $CH_2$ ring $NCH_2COO$, N $CH_2CH_2CH_2CH_2O$, $POCH_2CH_3$), 5.00 (s, 2H, $BnCH_2$), 6.73–6.94 (m, 1H, Ar*H*), 7.04–7.08 (m, 1H, Ar*H*), 7.26–7.39 (m, 6H, Bn*H*, Ar*H*). $^{13}C\{^{1}H\}$ NMR (75 MHz, $CDCl_3$) δ (ppm) 16.08, 19.09, 25.19, 26.08, 27.80, 30.60, 47.43, 49.90, 51.82, 52.83, 56.53, 61.77, 67.81, 68.84, 70.45, 81.22, 113.51, 115.73, 118.20, 120.31, 127.14, 127.68, 128.24, 136.47, 152.00, 154.43, 169.74, 170.20. $^{31}P\{^{1}H\}$ NMR (122 MHz, $CDCl_3$) δ = 15.84, 15.92, 16.74. FT-ICR-MS for $C_{47}H_{77}N_4O_{11}P$ calcd. 905.53992 $[M + H]^+$, found 905.539946.

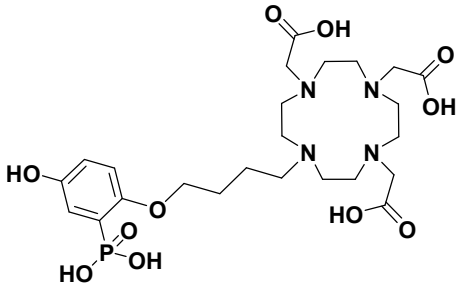
**2,2',2''-(10-(4-(4-(benzyloxy)-2-phosphonophenoxy)butyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid ($L^3$)**



The ligand precursor **7** (0.35 g, 0.38 mmol) was dissolved in dichloromethane (3 mL) and cooled in an ice bath to 0 °C under a dinitrogen atmosphere. Bromotrimethylsilane (0.26 mL, 1.93 mmol) was added slowly and the solution was stirred at room temperature for 3 hours. The excess reagent and solvent were removed under reduced pressure and the cleavage of the ethyl esters was confirmed through $^{1}$H and $^{31}$P NMR spectroscopy. Trifluoroacetic acid in dichloromethane (4 mL, 1:1 v:v) was added to the crude material and the mixture was stirred at room temperature for 4 hours. The solvent and excess volatiles were removed under reduced pressure to produce an orange solid. Upon analysis by ESI mass spectrometry, partial cleavage of the benzyl ether protecting group had occurred. The products were purified by RP-HPLC. Following lyophilisation, a white powder was obtained. This rapidly became a yellow oil on exposure to air due to the hygroscopic nature of the product (0.09 g, 32 %).

$^{1}$H NMR (300 MHz, $D_2O$) δ (ppm) 1.51–4.41 (br, 32H, *CH*$_2$ ring, NC*H*$_2$COO, NC*H*$_2$C*H*$_2$C*H*$_2$C*H*$_2$O, BnC*H*$_2$), 6.35–6.98 (m, 8H, Ar*H*). $^{13}$C{$^{1}$H} NMR (75 MHz, $D_2O$) δ (ppm) 14.12, 20.11, 25.66, 48.20, 49.85, 51.67, 54.17, 55.79, 65.94, 67.63, 70.13, 65.94, 67.63, 70.13, 110.61, 114.48, 118.36, 119.53, 123.59, 127.63, 128.40, 136.94, 148.39, 151.48, 154.14, 161.95, 162.40, 162.87, 163.34, 169.23, 174.41. $^{31}$P{$^{1}$H} NMR (122 MHz, $D_2O$) δ (ppm) 10.41. FT-ICR-MS for $C_{31}H_{45}N_4O_{11}P$ calcd. 681.28952 [M + H]$^{+}$, found 681.290135.

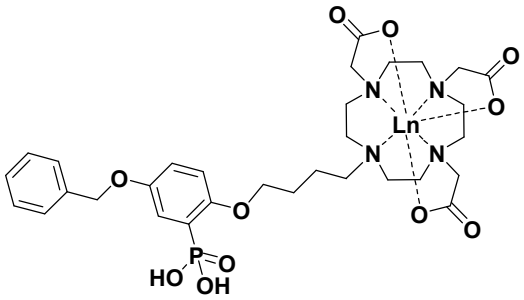
**2,2',2''-(10-(4-(4-hydroxy-2-phosphonophenoxy)butyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid ($L^4$)**



See the procedure for **L**$^{2}$ (0.240 g, 37 %).

$^{1}$H NMR (300 MHz, $D_2O$) δ (ppm) 1.67–1.80 (br, 4H $NCH_2CH_2CH_2CH_2O$), 2.57–3.88 (br, 26H *CH*$_2$ ring, $NCH_2COO$, $NCH_2CH_2CH_2CH_2O$), 6.80–7.09 (m, 3H, Ar*H*). $^{13}$C{$^{1}$H} NMR (75 MHz, $D_2O$) δ (ppm) 20.08, 25.69, 31.38, 48.21, 49.85, 51.58, 53.10, 54.12, 55.84, 67.93, 114.20, 119.54, 121.35, 123.17, 148.76, 153.51, 164.85, 169.27, 174.47. $^{31}$P{$^{1}$H} NMR (122 MHz, $D_2O$) δ (ppm) 11.36. FT-ICR-MS for $C_{24}H_{39}N_4O_{11}P$ calcd. 591.24257 $[M + H]^+$, found 591.242455.

**2,2',2''-(10-(4-(4-(benzyloxy)-2-phosphonophenoxy)butyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid lanthanide complex (LnL$^3$)**



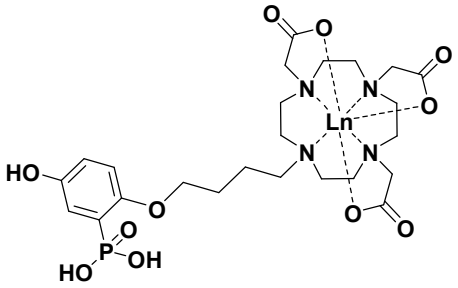

The complexes were prepared in the same manner as described for **LnL$^1$**.

**EuL$^3$**: UV/Vis ($H_2O$) $\lambda_{max}$ (ε ($M^{-1}$ $cm^{-1}$)) = 299 nm (ε = 5,200). ESI-MS (neg.) m/z: 829.0 ($[M - H]^-$). $^{31}$P{$^{1}$H} NMR (122 MHz, $D_2O$) δ (ppm) -118.29, 9.34.

**GdL$^3$**: ESI-MS (neg.) m/z: 834.2 ($[M - H]^-$). $r_1$ 6.65 $mM^{-1}s^{-1}$ (pH 7.4, MOPS, 300 MHz).

**TbL$^3$**: UV/Vis ($H_2O$) $\lambda_{max}$ (ε ($M^{-1}$ $cm^{-1}$)) = 299 nm (ε = 5,200). ESI-MS (neg.) m/z: 835.2 ($[M - H]^-$).

**2,2',2''-(10-(4-(4-hydroxy-2-phosphonophenoxy)butyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid lanthanide complex (LnL$^4$)**




The complexes were prepared in the same manner as described for **LnL$^1$**.

**EuL$^4$**: UV/Vis ($H_2O$) $\lambda_{max}$ (ε ($M^{-1}$ $cm^{-1}$)) = 299 nm (ε = 5,300). ESI-MS (neg.) m/z: 737.0 ($[M - H]^-$). $^{31}$P{$^{1}$H} NMR (122 MHz, $D_2O$) δ = -116.54, 9.13.

**GdL$^4$**: ESI-MS (neg.) m/z: 743.9 ($[M - H]^-$). $r_1$ 5.23 $mM^{-1}s^{-1}$ (pH 7.4, MOPS, 300 MHz).

**TbL$^4$**: UV/Vis ($H_2O$) $\lambda_{max}$ (ε ($M^{-1}$ $cm^{-1}$)) ($\pi \rightarrow \pi^*$) = 299 nm (ε = 4,900). ESI-MS (neg.) m/z: 745.1 ($[M - H]^-$).

## 3. Absorbance, excitation and emission spectra

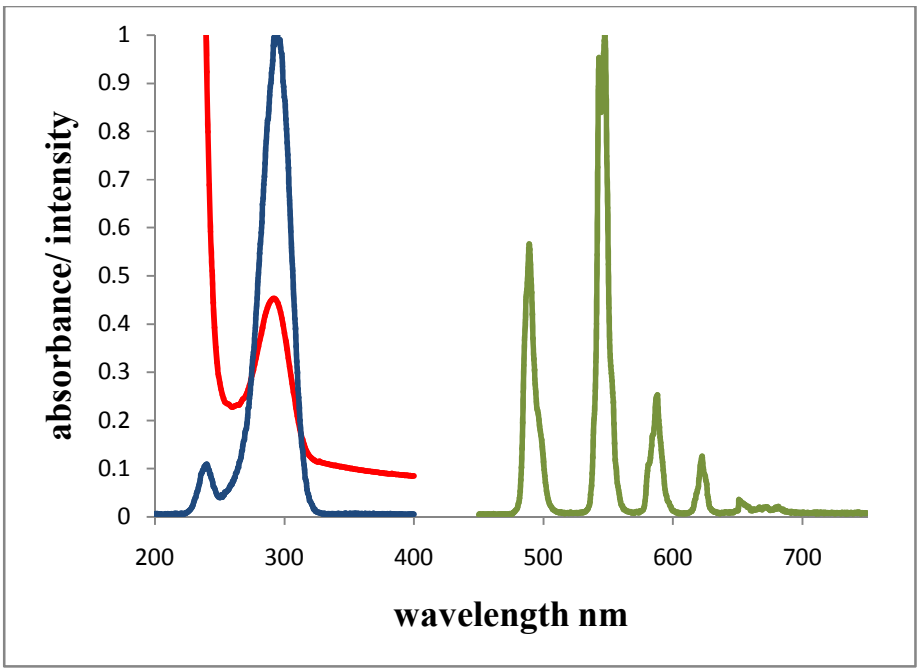



**Figure S1**. Absorption (red) and normalized excitation (blue) and emission (green) spectra of **TbL**[1] ($H_2O$, MOPS, pH 7.4).

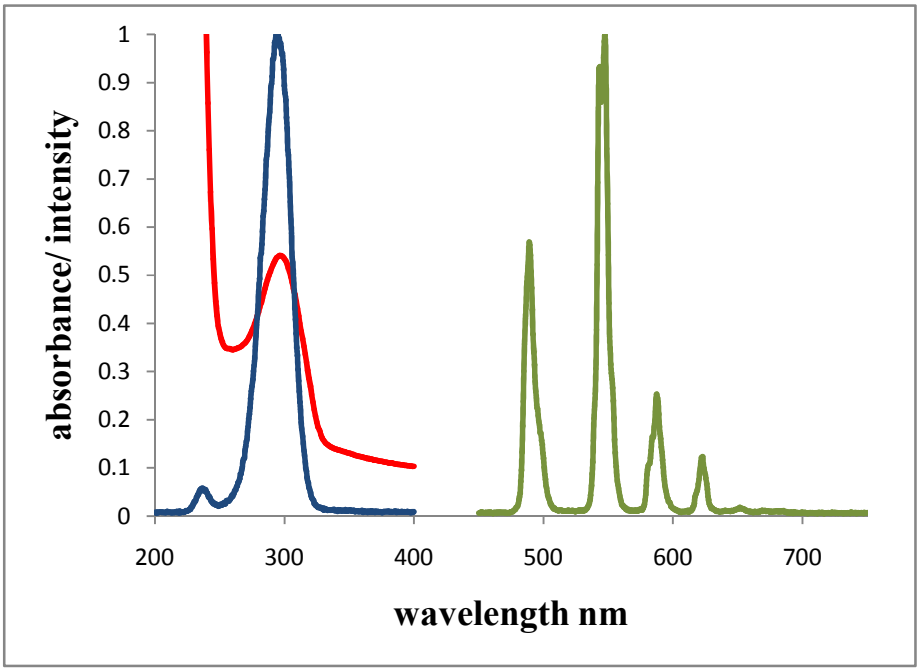



**Figure S2.** Absorption (red) and normalized excitation (blue) and emission (green) spectra of **TbL**[2] ($H_2O$, MOPS, pH 7.4).

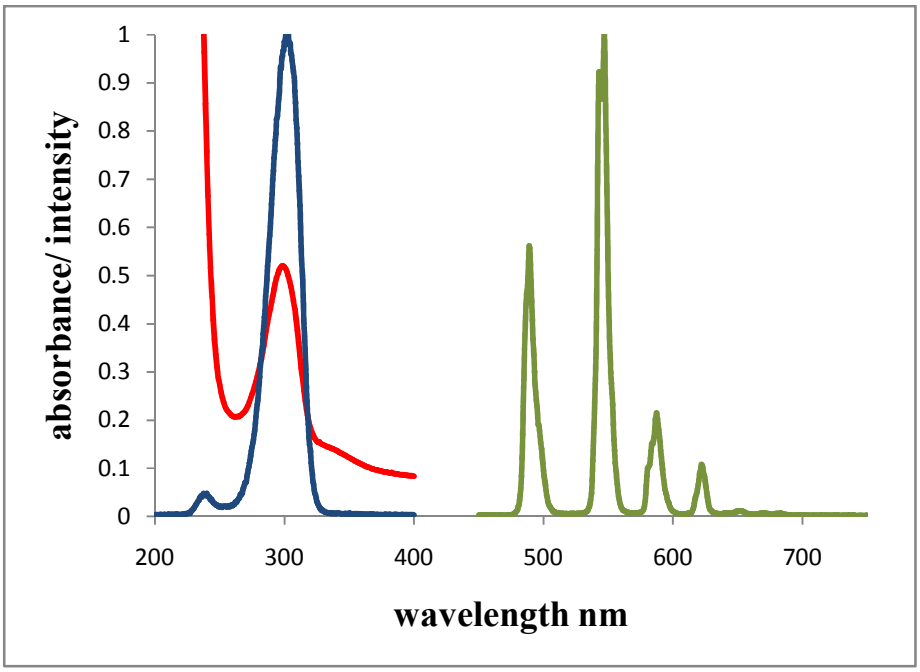



**Figure S3.** Absorption (red) and normalized excitation (blue) and emission (green) spectra of **TbL**$^{3}$ ($H_2O$, MOPS, pH 7.4).

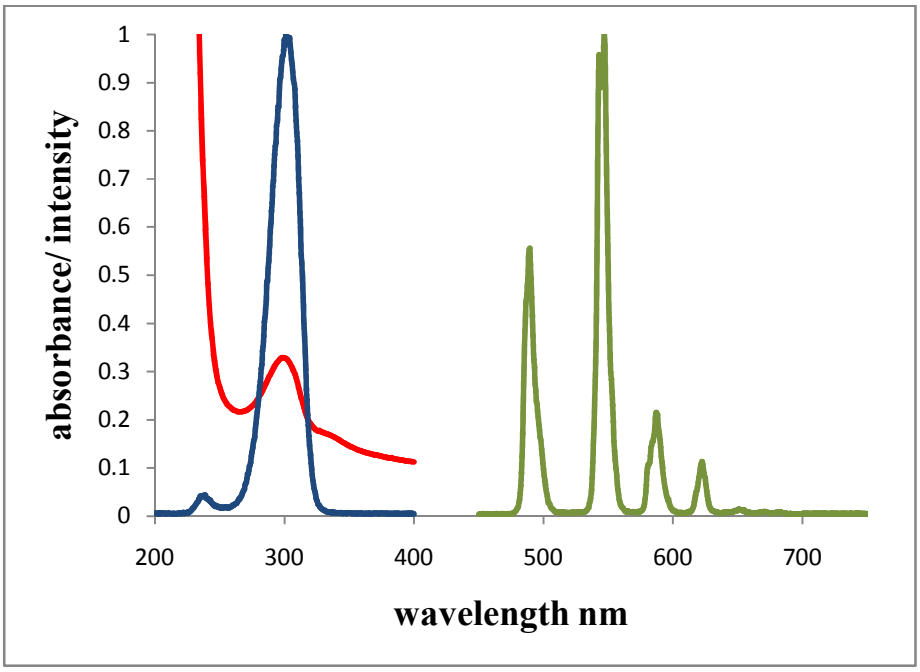



**Figure S4.** Absorption (red) and normalized excitation (blue) and emission (green) spectra of **TbL**$^{4}$ ($H_2O$, MOPS, pH 7.4).

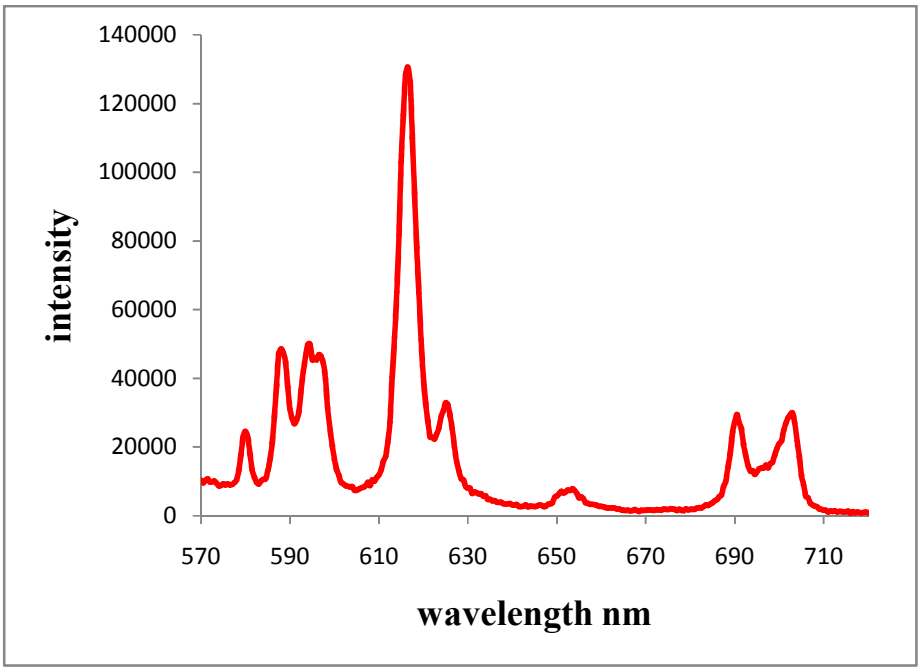



**Figure S5.** Emission spectrum of **EuL**$^{1}$ ($H_2O$, MOPS, pH 7.4).

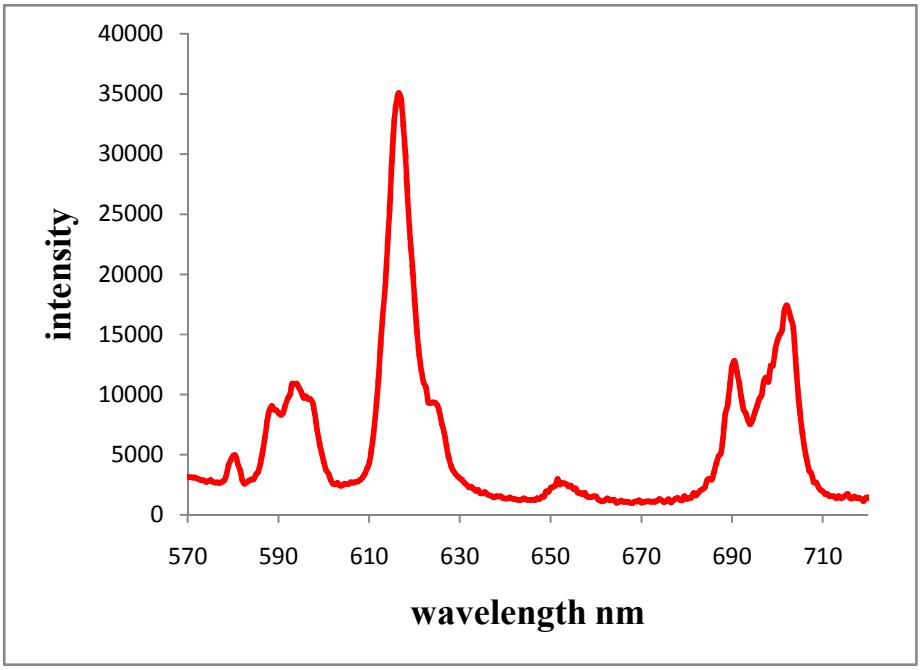



**Figure S6.** Emission spectrum of **EuL**$^{2}$ ($H_2O$, MOPS, pH 7.4).

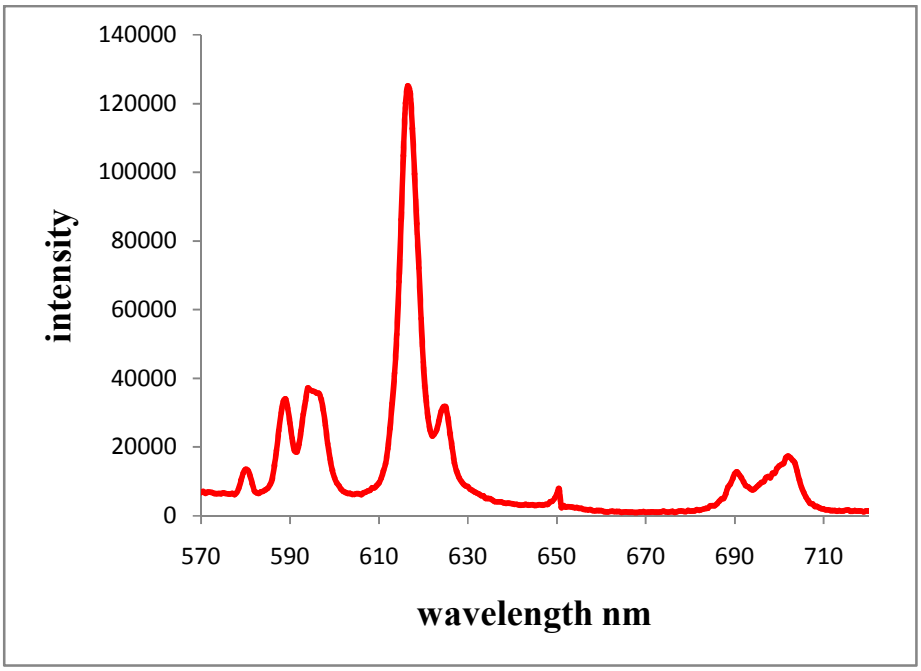



**Figure S7.** Emission spectrum of **EuL$^3$** ($H_2O$, MOPS, pH 7.4)

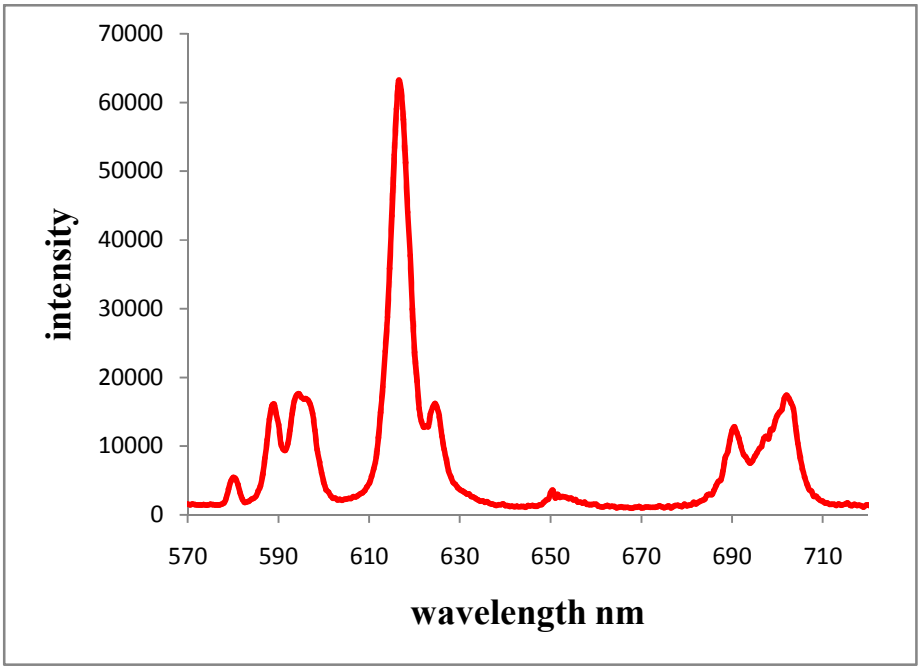



**Figure S8.** Emission spectrum of **EuL$^4$** ($H_2O$, MOPS, pH 7.4)

## 4. $^{31}$P NMR spectra

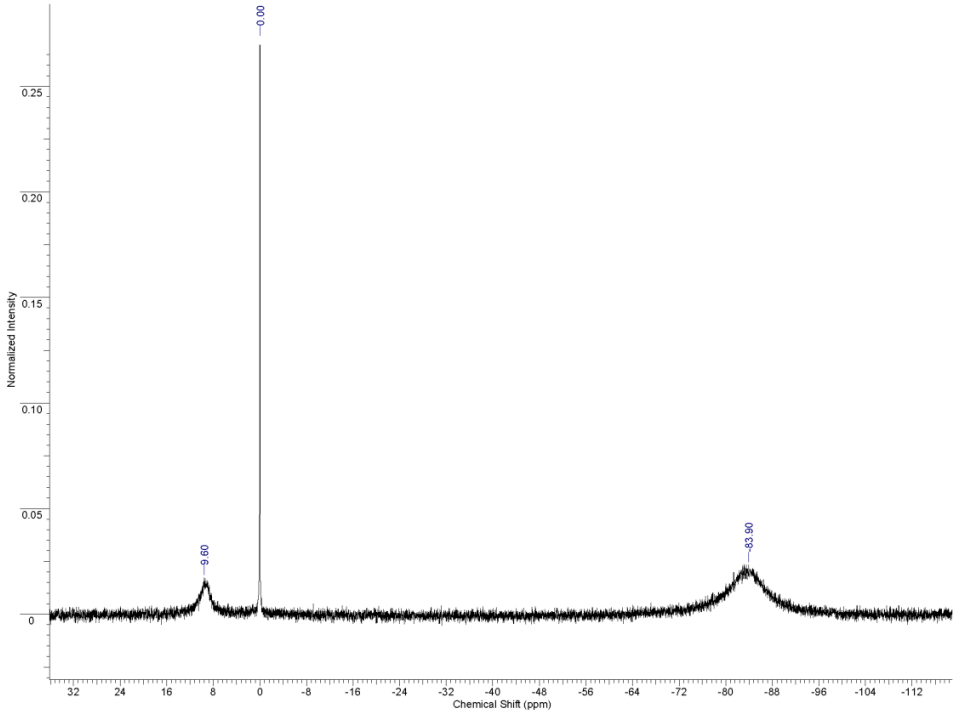



**Figure S9.** $^{31}$P NMR spectrum of **EuL$^1$** ($D_2O$, external reference 1% $H_3PO_4$).

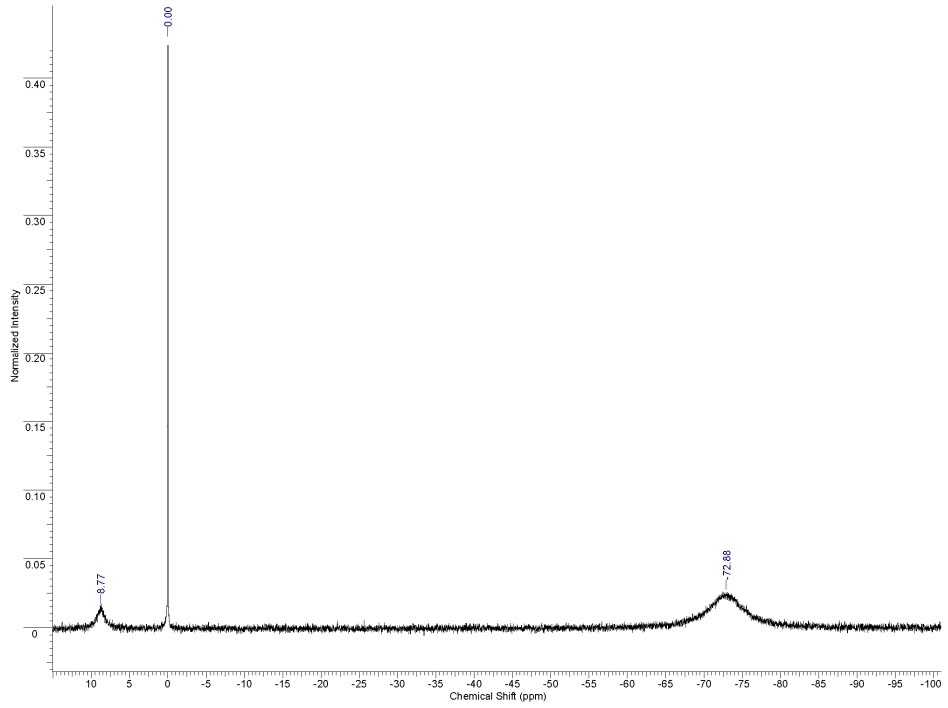



**Figure S10.** $^{31}$P NMR spectrum of **EuL$^2$** ($D_2O$, external reference 1% $H_3PO_4$).

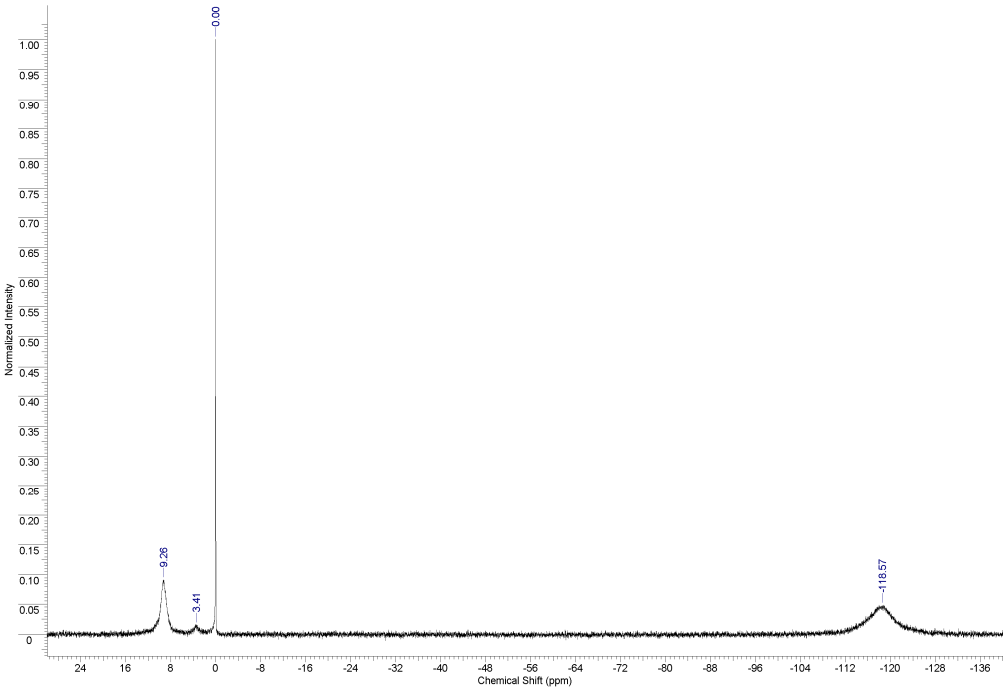



**Figure S11.** $^{31}$P NMR spectrum of **EuL$^{3}$**, ($D_2O$, external reference 1% $H_3PO_4$).

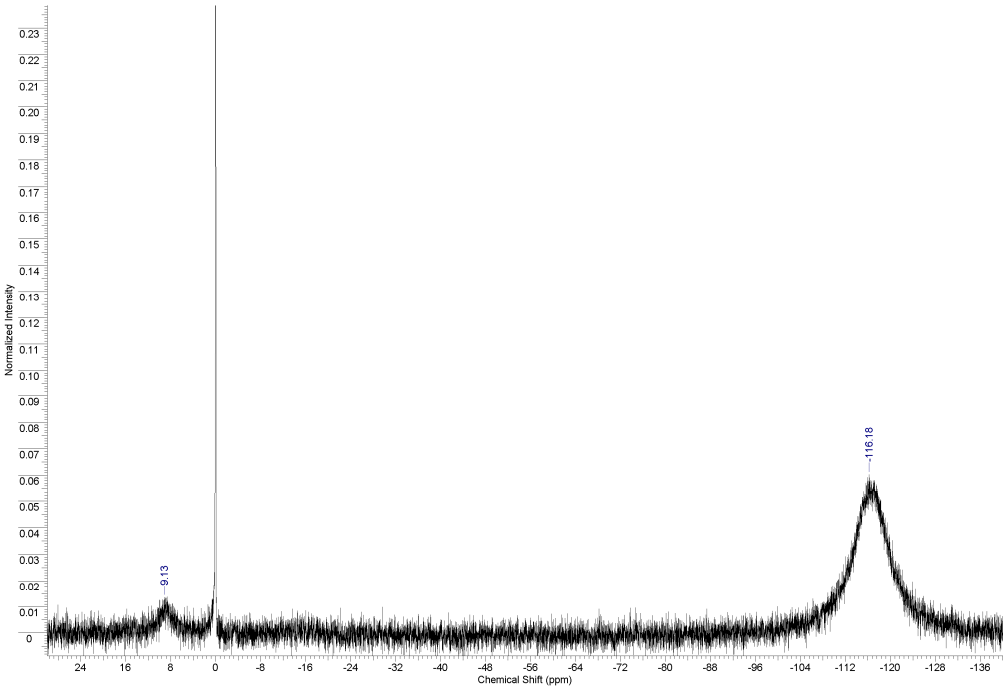



**Figure S12.** $^{31}$P NMR spectrum of **EuL$^{4}$**, ($D_2O$, external reference 1% $H_3PO_4$).

## 5. Relaxometric and luminescent properties in model extracellular medium and in the presence of selected anions

**Table S3.** Longitudinal relaxivities of **GdL$^{1-4}$** MOPS and DMEM with FCS (7 T).

| Complex | $r_1$ (MOPS)/ $mM^{-1}s^{-1}$ | $r_1$ (DMEM)/ $mM^{-1}s^{-1}$ |
|---|---|---|
| **GdL$^1$** | 7.20 | 5.31 |
| **GdL$^2$** | 7.33 | 5.25 |
| **GdL$^3$** | 6.65 | 6.15 |
| **GdL$^4$** | 5.23 | 4.65 |

**Table S4.** Luminescence lifetimes of **TbL$^{1-4}$** in MOPS, anionic solutions and DMEM with FCS.

| Complex | $\tau H_2O$ (MOPS)/ ms | $\tau D_2O$ (MOPS)/ ms | $\tau H_2O$ (DMEM)/ ms | $\tau H_2O$ ($HCO_3^-$)/ ms | $\tau D_2O$ ($HCO_3^-$)/ ms | $\tau H_2O$ ($HPO_4^{2-}$)/ ms | $\tau D_2O$ ($HPO_4^{2-}$)/ ms |
|---|---|---|---|---|---|---|---|
| **TbL$^1$** | 2.01 | 3.40 | 2.18 | 1.99 | 3.06 | 2.03 | 3.30 |
| **TbL$^2$** | 1.97 | 3.23 | 2.05 | 1.80 | 2.86 | 2.03 | 3.22 |
| **TbL$^3$** | 1.90 | 3.10 | 2.17 | 1.90 | 2.96 | 2.06 | 3.14 |
| **TbL$^4$** | 2.20 | 3.04 | 2.17 | 1.82 | 2.80 | 2.16 | 3.16 |

Lifetimes quoted are subject to ± 10 % error.

## 6. References:

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[2] I.M. Clarkson, A. Beeby, I.J. Bruce, L.J. Govenlock, M.P. Lowe, C.E. Mathieu, D. Parker, K. Senanayake, *New. J. Chem.,* 2000, **24**, 377.

[3] R.A. Poole, G. Bobba, M.J. Cann, J.-C. Frias, D. Parker, R. Peacock, *Org. Biomol. Chem.,* 2005, **3**, 1013.

[4] A.S. Chauvin, F. Gumy, D. Imbert, J.-C. G. Bünzli, J.-C. G. *Spectrosc. Lett.* 2004, **37**, 517.