Supplementary material (ESI) for Journal of Materials Chemistry

ESI

Critical evaluation of five emissive europium (III) complexes as optical probes: correlation of cytotoxicity, anion and protein affinity with complex structure, stability and intracellular localisation profile

Benjamin S. Murray, Elizabeth J. New, Robert Pal and David Parker*

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

1. General experimental details

All commercially available reagents were used as received, from their respective suppliers. Solvents were dried using an appropriate drying agent when required (CH_3CN over CaH_2, CH_3OH over $Mg(OMe)_2$ and THF over sodium/benzophenone). Water and air sensitive reactions were carried out under argon atmosphere. Water and H_2O refer to high purity water with conductivity ≤0.04 μS cm^{-1} obtained from the 'PuriteSTILL Plus' purification system. Thin-layer chromatography was carried out on neutral aluminium oxide plates (Merck Art 5550) or silica plates (Merck 5554), both visualised under UV irradiation (254 nm) or iodine staining. Preparative column chromatography was carried out using neutral aluminium oxide (Merck Aluminium Oxide 90, activity II-III, 70-230 mesh), pre-soaked in ethyl acetate, or silica (Merck Silica Gel 60, 230-400 mesh). Melting points were measured using a Reichart-Kofler block and are uncorrected.

^1H and ^{13}C NMR spectra were recorded on a Varian Mercury 200 (^1H at 199.97 MHz, ^{13}C at 50.29 MHz), Varian Unity 300 (^1H at 299.91 MHz, ^{13}C at 75.41 MHz), Varian VXR 400 (^1H at 399.97 MHz, ^{13}C at 100.57 MHz), on a Bruker Avance spectrometer (^1H at 400.13 MHz, ^{13}C at 100.61 MHz), on a Varian Inova-500 (^1H at 499.78 MHz,

^{13}C at 125.67 MHz). All spectra were referenced internally to the solvent residual proton signals, except for complexes in D_2O, where *tert*-butanol was added as an internal reference (δ = 0 ppm).

Electrospray mass spectra were recorded on a VG Platform II (Fisons Instrument), operating in positive or negative ion mode as stated, with methanol as the carrier solvent. Accurate masses were measured on a Thermo Finnigan LQT. UV/Vis absorbance spectra were recorded either on a Perkin Elmer Lambda 900 UV/Vis/NIR spectrometer (using FL Winlab software) or a Unicam UV/Vis UV2. Emission spectra were measured on a ISA Joblin-Yvon Spex Fluorolog-3 luminescent spectrometer (using DataMax v2.20 software), while lifetimes were measured on a Perkin Elmer LS55 luminescence spectrometer (using FL Winlab software). All samples were contained in quartz cuvettes with a path length of 1 cm and polished base. Measurements were obtained relative to a reference of pure solvent contained in a matched cell. Luminescent titrations were carried out by normalising the emission spectra with the absorption spectra in each point in order to revise the decrease in the sample concentration caused by pH adjustment or addition of an anion/cation stock solution where appropriate. All measurements were carried out using I = 0.1 M NaCl ionic background.

Relaxivity measurements were carried out at 37 °C and 60 MHz on a Bruker Minispec mq60 instrument. The mean value of three separate measurements was recorded and the mean value reported. pH measurements used a Jenway 3020 or a Jenway 3320 pH meter attached to an Aldrich Chemical Company micro-pH combination electrode (three point calibration using pH = 4.0 ±0.02, pH = 7.00 ±0.02 and pH = 10.00 ±0.02 (T = 20 °C) buffer solution supplied by Aldrich. The adjustment of pH was carried out using conc. NaOH and conc. HCl (or NaOD and DCl if required) solution to avoid any significant increase in sample volume. For measurements carried out in D_2O the pD was calculated using the actual pH meter reading and the equation : pD = pH (meter reading) + 0.41.

Lifetimes of europium complexes were measured by excitation of the sample using a short pulse of light (340, 372 or 384 nm depending on the nature of the complex) followed by monitoring the integrated intensity of light (for europium 612-618 nm depending on the measured species and the pH) emitted during a fixed gate time, t_g,

after a delay time, t_d. At least 20 delay times were used covering 3 or more lifetimes. A gate time of 0.1 ms was used, and the excitation and emission slits were set to 10 nm and 2.5 nm band-pass respectively. The obtained exponential decay curves were fitted to the equation below, using Origin 6.0 software (Data Analysis & Technical Graphics):

$$I = A_0 + A_1 \exp(-kt)$$

To examine the influence of some biologically common anions on Eu complexes, luminescent titrations were carried out examining separate solutions containing either Na_2CO_3, sodium lactate, NaH_2PO_4 or trisodium citrate. Each measurement was carried out using a constant I = 0.1 M NaCl ionic strength. Titrations with pH variation were carried out from basic solutions with acidification in order to avoid the undesirable evolution of carbon dioxide. All of these measurements were carried out by adding the selected anion as liquid concentrated stock solution where the addition at each point was approx. 0.1-0.5% in volume of the original solution observed to avoid significant increase in sample volume. HSA was added as a solid. Each Eu emission spectrum was corrected for dilution. The apparent binding constant of the selected anion was calculated as described in reference 23.

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

Microscopy

Epifluorescence images were taken on a Zeiss Axiovert 200M epifluorescence microscope with objectives 63x/1.40 oil DIC and 40x/1.40 oil DIC respectively, equipped with an Axiocam CCD camera. For excitation a 340-390 nm (90% transmission) band-pass(BP) filter was used. Ligand fluorescence were observed using a BP 445-465 nm filter (80% transmission), while Eu emission was observed using a 570 nm long-pass (LP) filter (85% transmission). Confocal images were taken on a Zeiss LSM 500 META confocal microscope with a BIORad 405 diode laser excitation and an LP 590 nm emission filter were used for europium luminescence and a BP 505-550 filter for study of ligand fluorescence.

Cell Culture and Toxicity

Two cell lines were selected for cell cultural studies CHO (Chinese Hamster Ovary)

cells and NIH 3T3, mouse skin fibroblast (connective tissue) cells. Each line is transformed, and compromise adherent cells, which grow in a monolayer. These cell lines were cultured in a copper jacket incubator at 37°C, average 20% humidity and 5% (v/v) CO_2 in 50 mL volume plastic grow plates. Cells for microscopy were grown in a 24 well-plate using d = 13mm glass cover slips (average thickness l = 0.1 mm). DMEM, (Dulbecco's Modified Eagle Media), and F-12(Ham) medias were used for NIH 3T3 and CHO cells respectively, each containing 10% (v/v) NCS (Newborn Calf Serum) and 1 % (v/v) penicillin-streptomycin. Complexes were loaded onto cells using the appropriate growth medium. For flow cytometry measurements, cells were detached from the glass surface using 1% (v/v) trypsin solution at 37°C for 5 min. The solutions and washings were analysed in separate ICP-MS measurements to measure any possible europium complex egress.

IC_{50} values were determined using the MTT assay, as described by Carmichael *et al*[31] which makes use of the conversion of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to a purple formazan product by the mitochondrial dehydrogenase of viable cells. This insoluble formazan was quantified spectrophotometrically upon dissolution in DMSO. Approximately 5 x 10^3 NIH 3T3 cells in 100 μL DMEM were seeded into each well of flat-bottomed 96-well plates and allowed to attach overnight. Complex solutions were added to triplicate wells to give final concentrations over a 2-log range. Following 24 h incubation, MTT (1.0 mM) was added to each well, and the plates incubated for a further 4 h. The culture medium was removed, and DMSO (150 μL) was added. The plates were shaken for 20 seconds and the absorbance measured immediately at 540 nm in a microplate reader. IC_{50} values were determined as the drug concentration required to reduce the absorbance to 50% of that in the untreated, control wells, and represent the mean for data from three independent experiments.

Inductively Coupled Plasma Mass Spectrometry

ICP-MS determination of europium concentrations from cells and growth media were carried out by Dr. Chris Ottley in the Department of Earth Sciences at Durham University using a Thermo Finningan ELEMENT2 High Resolution Select Field ICP-MS.

HPLC Analysis/Purification

Reverse phase HPLC analysis were performed at 298 K on a Perkin Elmer system using a 4.6 x 20 mm 4μ Phenomenex Synergi Fusion RP 80i analytical column. In each case an H_2O + 0.1 % HCOOH / MeCN + 0.1 % HCOOH solvent system was used (gradient elution) with a run time of 20 minutes. In each case, a single major product was observed in >95% purity using a diode array UV-Vis detector operating at 380 mm (analysis was also undertaken at 280 nm). This corresponds to the absorption band of the appropriate azathioxanthone used as sensitizing moiety for each Eu-complex and is to longer wavelength of any organic contaminant. Such behaviour indicated that each of the species that was eluted bore this chromophore. A fluorescence detector was also connected to the HPLC, monitoring eluent from the column at a wavelength corresponding to the Eu centred emission (616 nm); again emission was seen for each of these peaks, suggesting that each peak corresponding to a chromophore bound species also was coordinated to Eu in such way that it was efficiently sensitized. For each Gd complex, a UV-Vis detector was used operating with a LP 250 nm detection filter.

2. Synthesis of ligands L^{3a} and L^{3b} and their lanthanide complexes

The precursor **3** i.e. 4,10-bis-[((S)-1-phenyl-ethylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododecane-1-carboxylic acid *tert*-butyl ester was synthesised as described reference 20. 2-Bromomethyl-7-methoxycarbonyl-1-azathioxanthone, **4**, was prepared as described in reference 21.

2-(7-*tert*-Butoxycarbonyl-4,10-bis-[((*S*)-1-phenyl-ethylcarbamoyl)-methyl]-1-ylmethyl)-5-oxo-5*H*-[1]benzothiopyrano[2,3-*b*]pyridine-7-carboxylic acid methyl ester

4,10-Bis-[((*S*)-1-phenyl-ethylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododecane-1-carboxylic acid *tert*-butyl ester (77 mg, 0.130 mmol), 2-bromomethyl-7-methoxycarbonyl-1-azathioxanthone, **4**, (47 mg, 0.129 mmol) and K_2CO_3 (18 mg, 0.130 mmol) in dry MeCN (5 ml) were boiled under reflux under argon for 12 h. The resultant yellow solution was dried, solvent removed under reduced pressure, the residue re-dissolved in DCM (5 ml) and extracted with $HCl_{(aq)}$ (0.05 M, 10 ml) then

H_2O (2 x 10 ml). The organic phase was dried under reduced pressure followed by isolation of the desired product from the yellow residue by column chromatography (alumina using a DCM/MeOH solvent system starting from 100 % DCM then increasing the volume of MeOH by 0.1 % every 100 ml thereafter) to yield the desired product as a clear yellow glassy solid (100 mg, 0.114 mmol, 88 %); m.p. 56-58 ^{o}C; R_F (SiO_2, DCM-MeOH, 98.5:1.5) : 0.33; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.24 (d, 1H, *J*=1.5 Hz, H_O), 8.66 (d, 1H, *J*=8.5 Hz, H_K), 8.32 (dd, 1H, *J*=8.0, 1.5 Hz, H_D), 7.73 (d, 1H, *J*=8.5 Hz, H_E), 7.64 (br, 1H, amide arm NH), 7.36 (br, 1H, amide arm NH), 7.31 (d, 1H, *J*=8.5 Hz, H_J), 7.19-7.28 (m, 10H, amide arms Ar), 5.17 (m, 2H, amide arms CH), 4.02 (s, 3H, H_A), 3.58 (m, 2H, H_I), 2.41-3.48 (br m, 20H, cyclen and amide arms CH_2), 1.48 (m, 15H, amide arms and ^{t}Bu CH_3); ^{13}C NMR ($CDCl_3$) δ 179.8 (1C, C_M), 170.4, 169.9 (2C, amide arms C=O), 165.9 (1C, C_B), 163.8 (1C, C_H), 157.7 (1C, C_G), 155.9 (1C, ^{t}Bu C=O), 143.5, 142.6 (2C, amide arms $Ar_{(q)}$), 142.1 (1C, C_F), 138.1 (1C, C_K), 132.9 (1C, C_D), 131.5 (1C, C_O), 128.9, 128.8 (2C, $C_{N,C}$), 126.8 (1C, C_E), 128.5, 127.2, 126.5, 126.3 (10C, amide arms Ar), 125.0 (1C, C_L), 121.7 (1C, C_J), 80.0 (1C, ^{t}Bu$_{(q)}$), 60.2 (2C, amide arms CH_2), 59.2 (1C, C_I), 54.1-52.9 (8C, cyclen CH_2), 52.6 (1C, C_A), 48.1, 47.9, 47.6 (2C, amide arms CH), 28.6 (3C, ^{t}Bu CH_3), 21.6, 20.9 (2C, amide arms CH_3); MS (ES^+) *m/z* 877.9 $[M + H]^+$ 100 %, 899.9 $[M + Na]^+$ 40 %. HRMS (ES^+) *m/z* found 878.4266 $[M + H]^+$ $C_{48}H_{60}O_7N_7{}^{32}S$ requires 878.4270.

2-(4,10-Bis-[((*S*)-1-phenyl-ethylcarbamoyl)-methyl]-1-ylmethyl)-5-oxo-5*H*-[1]benzothiopyrano[2,3-*b*]pyridine-7-carboxylic acid methyl ester, L^{3a}

2-(7-*tert*-Butoxycarbonyl-4,10-bis-[((*S*)-1-phenyl-ethylcarbamoyl)-methyl]-1-ylmethyl)-5-oxo-5*H*-[1]benzothiopyrano[2,3-*b*]pyridine-7-carboxylic acid methyl ester (0.100 g, 0.114 mmol) was dissolved in DCM/TFA (1:1, 2 ml) then stirred overnight to yield a bright yellow solution. The solution was dried under reduced pressure to yield the desired product, as the *bis*(trifluoroacetate salt), as a glassy yellow solid, in quantitative yield; ^{1}H NMR (MeCN, 500 MHz) δ 8.81 (d, 1H, *J*=2.0 Hz, H_O), 8.50 (d, 1H, *J*=8.5 Hz, H_K), 8.06 (dd, 1H, *J*=8.5, 2.0 Hz, H_D), 7.51 (d, 1H, *J*=8.5 Hz, H_E), 7.24 (d, 1H, *J*=8.0 Hz, H_J), 6.92-7.08 (m, 10H, amide arms Ar), 4.71 (m, 1H, amide arm CH), 4.63 (m, 1H, amide arm CH), 4.24 (m, 2H, H_I), 3.80 (s, 3H, H_A), 2.77-3.60 (br m, 20H, cyclen and amide arms CH_2), 1.16 (m, 6H, amide arms

CH_3); ^{13}C NMR (MeCN, 125 MHz) δ 180.2 (1C, C_M), 171.5, 170.4 (2C, amide arms C=O), 166.6 (1C, C_B), 160.4 (q, 1C, TFA C=O), 158.3 (1C, C_H), 156.3 (1C, C_G), 144.5, 144.4 (2C, amide arms $Ar_{(q)}$), 142.5 (1C, C_F), 139.7 (1C, C_K), 133.9 (1C, C_D), 131.4 (1C, C_O), 130.1 (2C, $C_{N,C}$), 128.2 (1C, C_E), 129.4, 128.0, 126.7 (10C, amide arms Ar), 127.0 (1C, C_L), 123.7 (1C, C_J), 116.9 (q, 1C, TFA CF_3), 57.8 (2C, amide arms CH_2), 55.6 (1C, C_I), 43.9, 50.7, 51.1, 52.8, 53.6 (8C, cyclen CH_2), 53.2 (1C, C_A), 50.2, 49.7 (2C, amide arms CH), 22.5, 22.4 (2C, amide arms CH_3); MS (ES^+) *m/z* 778.7 $[M + H]^+$ 100 %, 800.7 $[M + Na]^+$ 15 %. HRMS (ES^+) *m/z* found 778.3740 $[M + H]^+$ $C_{43}H_{52}O_5N_7{}^{32}S$ requires 778.3745.

$[Eu.L^{3a}(H_2O)]Cl_3$

A solution of 2-(4,10-bis-[((*S*)-1-phenyl-ethylcarbamoyl)-methyl]-1-ylmethyl)-5-oxo-5*H*-[1]benzothiopyrano[2,3-*b*]pyridine-7-carboxylic acid methyl ester (15.4 mg, 0.020 mmol) and $Eu(OTf)_3.6H_2O$ (13.4 mg, 0.019 mmol) in MeCN was heated at 70°C for 72 h. The resultant orange solution was dried under reduced pressure to yield a glassy orange solid that was sonicated in DCM (5 ml) followed by decanting of the solvent. The remaining solid was further sonicated in DCM (5 ml) followed by decanting of the solvent to yield an orange solid that was dried under reduced pressure. The solid was made water soluble by the exchange of triflate anions for chloride anions using DOWEX 1x8 200-400 mesh ion exchange resin (chloride form). The resin was prepared by boiling it under reflux in MeOH overnight followed by sequential washing with H_2O (500 ml), 0.1 M HCl (200 ml) then H_2O (500 ml) at which point washings were neutral pH. The procedure involved dissolving of the solid in MeOH (1 ml) followed by the addition of H_2O (1 ml); this complex solution was added to a mixture of the resin (0.2 g) in H_2O:MeOH (50:50, 5 ml) then stirred for 2 h. The resin was then removed by filtration followed by the drying of the solution under reduced pressure to yield the complex as a pale orange solid (15.5 mg, 0.015 mmol, 79 %); ^1H NMR (as the trichloride salt, D_2O, 700 MHz) δ broadened resonances between +43 and -21 ppm; MS (ES+) *m/z* 1048.3 $[M + 2CH_3CO_2]^+$ 100 %; HRMS (ES^+) *m/z* found 1048.3126 $[M + 2CH_3CO_2]^+$ $C_{47}H_{57}O_9N_7{}^{153}Eu$ ^{32}S requires 1048.3145; $\lambda_{abs}(H_2O)$: 375 nm; $\tau_{(H2O)}$: 0.32 ms, $\tau_{(D2O)}$: 0.62 ms; q = 1.33.

2-(7-*tert*-Butoxycarbonyl-4,10-bis-[((S)-1-phenyl-ethylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-ylmethyl)-10-oxo-10*H*-9-oxa-1-aza-anthracene-6-carboxylic acid methyl ester

4,10-Bis-[((S)-1-phenyl-ethylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododecane-1-carboxylic acid tert-butyl ester (56 mg, 0.094 mmol), 7-methoxycarbonyl-2-bromomethyl-1-azaxanthone (33 mg, 0.095 mmol) and K_2CO_3 (13 mg, 0.094 mmol) in dry MeCN (2 ml) were heated under reflux under argon, for 12 h. The resultant orange solution was dried under reduced pressure, the residue re-dissolved in DCM (10 ml) then extracted with H_2O (2 x 10 ml). The organic phase was dried under reduced pressure followed by isolation of the desired product from the yellow residue by column chromatography (alumina using a DCM/MeOH solvent system starting from 100 % DCM then increasing the volume of MeOH by 0.1 % every 100 ml thereafter) to yield the desired product as a clear yellow glassy solid (62 mg, 0.072 mmol, 77 %); m.p. 84-86 ^{o}C; R_F (alumina, DCM-MeOH, 97.5:2.5): 0.51; ^{1}H NMR ($CDCl_3$, 700 Mhz) δ 8.99 (d, 1H, J=2.0 Hz, H_O), 8.48 (d, 1H, J=7.5 Hz, H_K), 8.42 (dd, 1H, J=8.5, 2.0 Hz, H_D), 7.64 (d, 1H, J=8.5 Hz, H_E), 7.64 (br, 1H, amide arm NH), 7.31 (br, 1H, amide arm NH), 7.14-7.27 (m, 11H, amide arms Ar and H_J), 5.16 (m, 2H, amide arms CH), 3.98 (s, 3H, H_A), 3.57 (m, 2H, H_I), 3.41 (br, 4H, amide arms CH_2), 2.40-3.13 (br m, 16H, cyclen CH_2), 1.43-1.48 (m, 15H, amide arms and ^{t}Bu CH_3); ^{13}C NMR ($CDCl_3$, 175 MHz, ^{1}H decoupled 700 MHz) δ 176.9 (1C, C_M), 170.4, 170.1 (2C, amide arms C=O), 165.9 (1C, C_B), 165.4 (1C, C_H), 160.0 (1C, C_G), 158.5 (1C, C_F), 156.3 (1C, ^{t}Bu C=O), 142.8, 143.9 (2C, amide arms $Ar_{(q)}$), 137.9 (1C, C_K), 136.5 (1C, C_D), 129.5 (1C, C_O), 127.3 (1C, C_C), 129.0, 128.8, 126.8, 126.5 (10C, amide arms Ar), 121.7 (1C, C_N), 121.1 (1C, C_J), 119.2 (1C, C_E), 115.6 (1C, C_L), 80.3 (1C, $^{t}Bu_{(q)}$), 60.6 (2C, amide arms CH_2), 59.7 (1C, C_I), 54.6-53.4 (8C, cyclen CH_2), 52.9 (1C, C_A), 48.5 (2C, amide arms CH), 28.9 (3C, ^{t}Bu CH_3), 22.0, 21.3 (2C, amide arms CH_3); MS (ES^+) m/z 862.4[M + H]$^+$ 100 %. HRMS (ES^+) m/z found 862.4503 [M + H]$^+$ $C_{48}H_{59}O_8N_7$ requires 862.4498.

2-(4,10-Bis-[((S)-1-phenyl-ethylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-ylmethyl)-10-oxo-10*H*-9-oxa-1-aza-anthracene-6-carboxylic acid methyl ester, L^{3b}

2-(7-*tert*-Butoxycarbonyl-4,10-bis-[((S)-1-phenyl-ethylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-ylmethyl)-10-oxo-10*H*-9-oxa-1-aza-anthracene-6-carboxylic acid methyl ester (25 mg, 0.029 mmol) was dissolved in DCM/TFA (1:1, 2 ml) then stirred for 12 h. in a stoppered flask to yield a bright yellow solution. The solution was dried under reduced pressure to yield the desired product, as a glassy yellow solid, in quantitative yield; ^{1}H NMR (MeCN, 700 MHz) δ 8.74 (d, 1H, J=2.0 Hz, H_O), 8.58 (d, 1H, J=8.5 Hz, H_K), 8.28 (dd, 1H, J=8.5, 2.0 Hz, H_D), 7.52 (d, 1H, J=9.0 Hz, H_E), 7.31 (d, 1H, J=7.5 Hz, H_J), 7.03-7.27 (m, 12H, amide arms Ar and NH), 4.81 (m, 1H, amide arm CH), 4.75 (m, 1H, amide arm CH), 4.35 (m, 2H, H_I), 3.94 (s, 3H, H_A), 2.94-3.80 (br m, 20H, cyclen and amide arms CH_2), 1.26 (d, 6H, J=6.5 Hz, amide arms CH_3); ^{13}C NMR (MeCN, 175 MHz, ^{1}H decoupled 700 MHz) δ 177.2 (1C, C_M), 171.5, 170.5 (2C, amide arms C=O), 166.2 (1C, C_B), 160.3 (q, 1C, TFA C=O), 158.8 (1C, C_G), 156.8 (2C, $C_{H,F}$), 144.6, 144.3 (2C, amide arms $Ar_{(q)}$), 139.4 (1C, C_K), 136.9 (1C, C_D), 128.9 (1C, C_O), 128.1 (1C, C_C), 129.4, 129.2, 127.8, 126.9, 126.7 (10C, amide arms Ar), 122.8 (1C, C_J), 122.2 (1C, C_N), 120.1 (1C, C_E), 117.3 (1C, C_L), 116.9 (q, 1C, TFA CF_3), 57.4 (1C, C_I), 56.5, 55.6 (2C, amide arms CH_2), 43.9, 50.5, 51.2, 53.5 (8C, cyclen CH_2), 53.1 (1C, C_A), 50.1, (2C, amide arms CH), 22.5, 22.3 (2C, amide arms CH_3); MS (ES^+) m/z 762.3 $[M + H]^+$ 100 %. HRMS (ES^+) m/z found 762.3972 $[M + H]^+$ $C_{43}H_{52}O_6N_7$ requires 762.3974.

$[Eu.L^{3b}(H_2O)_2]Cl_3$

A solution of 2-(4,10-bis-[((S)-1-phenyl-ethylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-ylmethyl)-10-oxo-10*H* -9-oxa-1-aza-anthracene-6-carboxylic acid methyl ester (16 mg, 0.021 mmol) and $Eu(OTf)_3.6H_2O$ (11 mg, 0.018 mmol) in MeCN was heated at 90°C for 48 h. The resultant yellow solution was dried under reduced pressure to yield a glassy orange solid that was sonicated in $CHCl_3$ (5 ml) followed by decanting of the solvent. The remaining solid was further sonicated in $CHCl_3$ (5 ml) followed by decanting of the solvent to yield a orange solid that was dried under reduced pressure. The solid was made water soluble by the exchange of triflate anions for chloride anions using DOWEX 1x8 200-400 mesh Cl ion exchange resin. The resin was prepared by reflux in MeOH overnight followed by washing with H_2O (500 ml), 0.1 M HCl (200 ml) then H_2O (500 ml) at which point washings were neutral pH. The procedure involved the dissolving of the solid in MeOH (1 ml)

followed by the addition of H_2O (1 ml), this complex solution was added to a mixture of the resin (0.2 g) in H_2O:MeOH (50:50, 5 ml) then stirred for 2 h. The resin was then removed by filtration followed by the drying of the solution under reduced pressure to yield the complex as a pale yellow powder (15 mg, 0.014 mmol, 78 %); ^{1}H NMR (as tri-chloride salt, D_2O, 700 MHz) δ Broadened resonances between +51 and -21 ppm; MS (ES+) *m/z* 479.9 $[M + HCO_2]^{2+}$ 100 %, 958.3 $[M + HCO_2 - H]^+$ 70 % ; HRMS (ES^+) *m/z* found 956.3007 $[M + HCO_2 - H]^+$ $C_{44}H_{51}O_8N_7{}^{151}Eu$ requires 956.2992; $\lambda_{abs}(H_2O)$: 333 nm; $\tau_{(H2O)}$: 0.26 ms, $\tau_{(D2O)}$: 0.60 ms.