ELECTRONIC SUPPLEMENTARY INFORMATION

A Single Component Ratiometric pH Probe with Long Wavelength Excitation of Europium Emission

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- Table of affinity constants for hydrogenearbonate and lactate adducts of Eu complexes.
- 2. Thirteen figures showing emission and relaxivity variation under the stated conditions.
- 3. Experimental section for ligand and complex synthesis and characterisation.
- TableApparent affinity constants^a for complexation with lactate (pH 6.0) and
hydrogencarbonate (pH 7.4) [298K, 0.1M NaCl]

Complex	HCO ₃ -	Lactate
EuL ¹	1.97	3.50 ^b
EuL^{L}	2.15	3.86
EuL ³	2.75	4.11

^a) binding constants (log K ± 0.05) refer to a 1:1 stoichiometry, validated by Job plots and observation of a 1:1 adduct by negative ion ESMS; ^b) affinity for citrate under these conditions was log K = 4.59.

ESI : Figure Legends

- <u>ES Fig 1</u> Absorption (left), chromophore fluorescence (centre) and europium emission spectrum for $[EuL^3]$ (298K, I = 0.1M NaCl, 5mM carbonate, 2.3mM lactate, 0.9mM HPO₄¹⁻, 0.13mM citrate, pH8).
- ES Fig 2 Variation of the europium emission spectrum of $[EuL^3]$ with pH in the pH range 3 to 9 (0.1M NaCl, 298K, λ_{exc} 384nm).
- <u>ES Fig 3</u> Variation of the europium emission spectrum of $[EuL^1]$ with pH over the range 3 to 9 (λ_{exc} 384nm, 298K, I = 0.1M NaCl).
- ES Fig 4 Variation of the europium emission spectrum of $[EuL^2]$ with pH in the range 3 to 9 (λ_{exc} 384nm, 298K, I = 0.1M NaCl) showing the impact of intramolecular carboxylate ligation over the range 4 to 7.
- <u>ES Fig 5</u> upper Variation of the relaxivity of $[GdL^3]$ with pH in the absence (squares) and presence of 0.7mM human serum albumin (diamonds); *lower* plot of the change in relaxivity of $[GdL^3]$ with added protein concentration showing a fit for a 1:1 binding model (K = 1.7 (±0.6) × 10⁴ M⁻¹, 298K, pH 7.4).
- <u>ES Fig 6</u> Change in the form of the europium emission spectrum with pH for [EuL³] (pH 3.3 to 8.7) in a simulated extracellular ionic background (0.1 M NaCl, 30mM KHCO₃, 2.3mM Na lactate, 0.9mM Na₂HPO₄, 0.13mM sodium citrate, 298K).
- ES Fig 7 Effect of pH on the form of the europium emission spectrum for [EuL³] in a background of 30mM Na₂CO₃ going from pH 9 (upper) to 4 (lower); note the signalling of carbonate binding at 612nm and the competition between carbonate binding and sulfonamide ligation indicated by the relative intensity of 680/687nm bands

ES Fig 8 Change in the form of the europium emission spectrum for [EuL³] with added sodium hydrogencarbonate (pH 7.4, 298K), showing the least square fit (line)

to the data for $K = 560 (\pm 40) M^{-1}$, assuming a 1:1 binding model.

- ES Fig 9 *upper* Variation of the europium emission spectrum of [EuL³] with pH in the range 3.0 to 8.7 in a background of added sodium lactate (2.3mM); *lower* change in the intensity ratio at 626.5/618nm with pH, illustrating the effect of sulfonamide ligation (increase in 627 and 680nm bands)
- <u>ES Fig 10</u> Change in the form of the europium emission spectrum for $[EuL^3]$ as a function of added sodium lactate (298K, pH6 0.1M NaCl), showing the least squares fit (line) to the data (K = 1.3 (±0.2) × 10⁴ M⁻¹).
- ES Fig 11 Variation of the europium emission spectrum of {EuL³] with pH in a simulated extracellular ionic background plus 0.7mM human serum albumin; *lower* : showing the pH effect on the change in the intensity ratio of the 680/684 nm bands
- ES Fig 12 The change in the emission spectral form for [EuL³] with pH in reconstituted human serum solution; *lower*: showing variations in various emission intensity ratios (680/684; 615/618; 615/622) with pH.
- ES Fig 13 The change in the selected Eu emission bands versus pH in human serum solution showing a 100% change between pH 6.3 and 7.5.
- ES Fig 14 Confocal fluorescent microscopy images of NIH 3T3 cells loaded with $[EuL^3]$ (6h incubation, 100 μ M complex concentration in the DMEM growth medium, 40x magnification) $\lambda_{exc.}$ 405 nm, observing Eu emission with 550 nm with long band-pass filter (red), and ligand fluorescence at 445-465 nm (green).



Absorption (left), chromophore fluorescence (centre) and europium emission spectrum for $[EuL^3]$ (298K, I = 0.1M NaCl, 5mM carbonate, 2.3mM lactate, 0.9mM HPO₄¹⁻, 0.13mM citrate, pH8).



Variation of the europium emission spectrum of $[EuL^3]$ with pH in the pH range 3 to 9 (0.1M NaCl, 298K, λ_{exc} 384nm).

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Variation of the europium emission spectrum of $[EuL^1]$ with pH over the range 3 to 9 (λ_{exc} 384nm, 298K, I = 0.1M NaCl).





Variation of the europium emission spectrum of $[EuL^2]$ with pH in the range 3 to 9 (λ_{exc} 384nm, 298K, I = 0.1M NaCl) showing the impact of intramolecular carboxylate ligation over the range 4 to 7.



FIG. 5 upper

upper Variation of the relaxivity of [GdL³] with pH in the absence (squares) and presence of 0.7mM human serum albumin (diamonds);

lower plot of the change in relaxivity of $[GdL^3]$ with added protein concentration showing a fit for a 1:1 binding model (K = 1.7 (±0.6) × 10^4 M⁻¹, 298K, pH 7.4).



ESI FIG. 5 lower



Change in the form of the europium emission spectrum with pH for $[EuL^3]$ (pH 3.3 to 8.7) in a simulated extracellular ionic background (0.1 M NaCl, 30mM KHCO₃, 2.3mM Na lactate, 0.9mM Na₂HPO₄, 0.13mM sodium citrate, 298K).



Effect of pH on the form of the europium emission spectrum for $[EuL^3]$ in a background of 30mM Na₂CO₃ going from pH 9 (upper) to 4 (lower); note the signalling of carbonate binding at 612nm and the competition between carbonate binding and sulfonamide ligation indicated by the relative intensity of 680/687nm bands



Effect of pH on the form of the europium emission spectrum for $[EuL^3]$ in a background of 30mM Na₂CO₃ going from pH 9 (upper) to 4 (lower); note the signalling of carbonate binding at 612nm and the competition between carbonate binding and sulfonamide ligation indicated by the relative intensity of 680/687nm bands



Change in the form of the europium emission spectrum for $[EuL^3]$ with added sodium hydrogenearbonate (pH 7.4, 298K), showing the least square fit (line) to the data for K = 560 (±40) M⁻¹, assuming a 1:1 binding model.



upper Variation of the europium emission spectrum of [EuL³] with pH in the range 3.0 to 8.7 in a background of added sodium lactate (2.3mM);

lower change in the intensity ratio at 626.5/618nm with pH, illustrating the effect of sulfonamide ligation (increase in 627 and 680nm bands)



ESI FIG. 9 (lower)

ESI



ESI FIG. 10 upper

Change in the form of the europium emission spectrum for [EuL³] as a function of added sodium lactate (298K, pH6 0.1M NaCl);

lower: showing the least squares fit (line) to the data (K = 1.3 ± 0.2) × 10^4 M^{-1}).



ESI FIG. 10 lower



ESI

ESI FIG. 11 upper

Variation of the europium emission spectrum of {EuL³] with pH in a simulated extracellular ionic background plus 0.7mM human serum albumin;

lower : showing the pH effect on the change in the intensity ratio of the 680/684 nm bands

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ESI FIG. 11 lower



ESI FIG. 12 upper

The change in the emission spectral form for [EuL³] with pH in reconstituted human serum solution;

lower: showing variations in various emission intensity ratios (680/684; 615/618; 615/622) with pH.



ESI FIG. 12 :lower: 680/684 nm change



ESI FIG. 12 lower: 615/618 nm change



ESI FIG. 12 lower: 615/622 nm change



The change in the selected Eu emission bands versus pH in human serum solution showing a 100% change between pH 6.3 and 7.5.



Fluorescenct images was obtained with a BIORAD Radiance 2000 Confocal microscope (objectives: Plan-Apochromat, 63x/1.40 oil DIC or 40x/1.30 oil DIC) by the following settings:

Exitation at 405 nm Eu emission observed 550 nm long band-pass filter (85% transmission) Ligand fluorescence observed 445-465 filter (80% transmission)

Similar images were obtained using a Zeiss Axiovert 200M epifluorescence microscope (objectives: Plan-Apochromat, 63x/1.40 oil DIC or 40x/1.30 oil DIC), observing the overall Eu emission, ligand fluorescence or the $\Delta J2$ -band of europium by the following settings:

Eu emission Exitation filter: 365 nm (± 50 nm) (60% transmission) Emission filter: 570 nm long band-pass (95% transmission)

Ligand emission Exitation filter: 325 nm (± 75 nm) (85% transmission) Emission filter: 450 nm (± 25 nm) (80% transmission)

 $\Delta J2$ -band emission: Exitation filter: 400 nm (± 100nm) (80% transmission) Emission filter: 600 nm (± 50 nm) (90% transmission)

Experimental

General Experimental Aspects and Instrumentation

All reagents were used as supplied by commercial sources unless otherwise stated. Solvents were dried over the appropriate drying agents when required. Water and H2O refer to high purity water with conductivity $\leq 0.04 \ \mu$ Scm-1, obtained from the "PuriteSTILL Plus" purification system.

Thin layer chromatography was carried out using silica plates (Merck Art 5554) or neutral aluminium oxide plates (Merck Art 5550), both of which are fluorescent under UV irradiation (254 nm). Preparative column chromatography was performed using neutral aluminium oxide (Merck Aluminium Oxide 90, activity II–III, 70–230 mesh), washed in ethyl acetate, or silica (Merck Silica Gel 60, 230–400 mesh).

¹H NMR spectra were recorded at 199.99 MHz on a Varian Mercury–200, 299.91 MHz on a Varian Unity–300, 400.13 MHz on a Bruker Avance spectrometer, 499.78 MHz or on a Varian Inova–500. All spectra were referenced to solvent residual proton signals, except for complexes in D₂O, where tert–butanol was added as an internal reference ($\delta = 0$ ppm).

Electrospray mass spectra were recorded on a VG Platform II instrument (Fisons) with methanol as the carrier solvent. Accurate masses were measured on a Thermo Finnigan LTQ or by the ESPRC Mass Spectroscopy Service at the University of Wales in Swansea. Melting points were measured using a Reichart–Köfler block and are uncorrected. All pH measurements were performed using a Jenway 3320 pH meter attached to an Aldrich Chemical Company micro–pH combination electrode, calibrated using pH 4, 7 and 10 buffer solutions. UV/vis absorption spectra were recorded using a Perkin Elmer Lamda 900 UV/vis/IR spectrometer. Emission spectra were recorded at 295 K using an Instruments SA Fluorolog 3–11 spectrometer and DataMax v2.1 for Windows. Luminescence spectra of the lanthanide(III) complexes were recorded following indirect excitation of the lanthanide(III) ion via the azaxanthone chromophore, at a wavelength of 384 nm. Phosphorescence emission spectra were recorded at 77 K using an Oxford Instruments optical cryostat and LS 55B spectrometer, with EPA (diethyl ether, isopentane and ethanol, 5:5:2) as solvent.

Lifetime measurements were measured by excitation of the sample by a short pulse of light (348 nm) followed by monitoring the integrated intensity of light (545 nm for terbium, 620 nm for europium) emitted during a fixed gate time, t_g , a delay time, t_d , later. 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 and 2.5 nm bandpass respectively. The obtained decay curves were fitted to the equation below using Microsoft Excel.

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

where I = intensity at time t after the flash;

 A_0 = intensity after the decay has finished;

 A_1 = pre-exponential factor;

k = rate constant for decay of the excited state.

The quantum yields were determined as reported in recent articles form this laboratory. Epifluorescence images were taken on a Zeiss Axiovert 200M epifluorescence microscope with a digital camera; filters used were as stated; confocal images were taken on a Zeiss LSM 500 META confocal microscope with 405 nm diode laser excitation and an LP 505 emission filter for europium complex.

Ligand and Complex Synthesis

4-[(1-Azathioxanthone)-2-methyl]-1,7-bis(tert-butoxycarbonylmethyl)-1,4,7,10tetraazacyclododecane



1,7-Bis(tert-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (250 mg, 0.62 mmol) was combined with 2-bromomethyl-1-azathiaxanthone (1.1 eq., 190 mg) and K₂CO₃ (1 eq., 86 mg) and the mixture stirred in dry MeCN (12 mL) at reflux under argon for 18 h. The reaction was monitored by TLC (DCM : MeOH, 97 : 3) and ESMS⁺ to confirm that the brominated starting material had been consumed. The solvent was removed under reduced pressure. The resulting solid was dissolved in a small volume of DCM (5 mL) and the KBr/K₂CO₃ was filtered out. The crude mixture was purified by column chromatography (DCM \rightarrow 2% MeOH) to yield the title compound as a yellow oil (161 mg, 0.26 mmol, 42%) $\delta_{\rm H}$ (CDCl₃) 8.71 (1H, H⁴, d, *J* 8.1 Hz), 8.60 (1H, H⁶, d, *J* 8.0 Hz), 7.68 (2H, H^{8,9}, m), 7.49 (1H, H⁷, m), 7.30 (1H, H³, d, *J* 8.1 Hz), 3.87 (2H, H¹⁰, s), 3.13-2.78 (4H, CH₂CO₂ + 16H, NCH₂CH₂N, m), 1.42 (18H, ^tBu, s) $\delta_{\rm c}$ (CDCl₃) 180.7 (C⁵), 170.2 (CO₂tBu), 161.3 (C²), 158.6 (C^{1'}), 139.2 (C^{4'}), 137.5 (C^{6'}), 133.3 (C⁸), 130.2 (C⁶), 129.3 (C^{9'}), 127.5 (C⁷), 127.1 (C⁹), 124.8 (C⁴), 122.2 (C³), 80.9 (CMe₃), 52.4 (C¹⁰), 57.9 (CH₂CO₂), 50.1, 47.8 (NCH₂ CH₂N), 27.8 (CH₃), *m*/z (ESMS⁺) 626 (M + 1); R_f 0.18 (DCM - 3%MeOH, alumina).

4-[(1-Azathioxanthone)-2-methyl]-10-[methylsulfonylamino)ethyl]-1,7-bis(tertbutoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane



4-[(1-Azathioxanthone)-2-methyl]-1,7-bis(tert-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (87 mg, 0.14 mmol) was combined with N-methanesulfonylaziridine (1.1 eq., 17.3 mg) and K_2CO_3 (1 eq., 19 mg) stirred in dry MeCN (8 mL) at reflux under argon for 24 h. The reaction was monitored by TLC (DCM : MeOH, 97 :

3) and ESMS⁺ to confirm that the starting secondary amine had been consumed. The solvent was removed under reduced pressure. The resulting solid was dissolved in a small volume of DCM (3 mL) and the K₂CO₃ was filtered out. The crude mixture was purified by column chromatography on neutral alumina (DCM \rightarrow 2% MeOH) to yield the title compound as a light brown oil (72 mg, 97 µmol, 69%). $\delta_{\rm H}$ (CDCl₃) 8.71 (1H, H⁴, d, *J* 8.0 Hz), 8.60 (1H, H⁶, d, *J* 8.0 Hz), 7.68 (2H, H^{8,9}, m), 7.49 (1H, H⁷, m), 7.31 (1H, H³, d, *J* 8.0 Hz), 3.90 (2H, H¹⁰, s), 3.13-2.78 (4H, CH₂CO₂ + 16H, NCH₂CH₂N + 4H, SO₂NHCH₂CH₂N m),), 2.01 (3H, SO₂CH₃), 1.41 (18H, ¹Bu, s); $\delta_{\rm c}$ (CDCl₃) 180.7 (C⁵), 170.2 (CO₂tBu), 161.3 (C²), 158.6 (C^{1°}), 139.2 (C^{4°}), 137.5 (C^{6°}), 133.3 (C⁸), 130.2 (C⁶), 129.3 (C^{9°}), 127.5 (C⁷), 127.1 (C⁹), 124.8 (C⁴), 122.2 (C³), 81.1 (CMe₃), 67.0, 67.8 (SO₂NHCH₂CH₂N), 52.4 (C¹⁰), 57.9 (CH₂CO₂), 50.1, 47.8 (NCH₂CH₂N), 38.5 (SO₂CH₃), 27.8 (C(CH₃)₃), *m*/z (ESMS⁺) 746 [M + 1], 768 [M+Na]; R_f 0.44 (DCM : MeOH, 97 : 3; alumina)

4-[(1-Azathioxanthone)-2-methyl]-10-[methylsulfonylamino)ethyl]-1,7-bis (carboxymethyl)-1,4,7,10-tetraazacyclododecane



A mixture of trifluoroacetic acid (1.5 mL) and DCM (0.5 mL) was added to 4-[(1-azathioxanthone)-2-methyl]-10-[methylsulfonylamino)ethyl]-1,7-bis(tertbutoxy-carbonylmethyl)-1,4,7,10-tetraazacyclododecane (72 mg (97 μ mol) and the mixture stirred under argon at room temperature for 28 h. The solvents were removed under reduced pressure and a small volume of DCM (3 x 3 mL) was added and removed again under reduced pressure. The crude mixture was dissolved in water (5 mL) and extracted with DCM (5 mL) thrice, and lyophilised to yield the *title compound* as a dark orange oil which slowly crystallised (55 mg, 87 μ mol, 90%). This material was used for complexation immediately, *m*/z (ESMS⁺) 658 [M-H+2Na], m.p. 120-1 °C

EuL¹

4-[(1-Azathioxanthone)-2-methyl]-10-[methylsulfonylamino)ethyl]-1,7-bis (carboxymethyl)-1,4,7,10-tetraazacyclododecane (28 mg, 44 µmol) was added to Eu(CF₃SO₃)₃ (1.1 eq., 26 mg) and the solids dissolved in a MeCN (2 mL) and the reaction left stirred at reflux temperature for 30 hrs. After the reaction was cooled to room temperature the solvents were removed under reduced pressure, the remaining residue was dissolved in 5 mL water : MeOH (5 :1). The pH was then adjusted carefully to 10 by addition of conc. NaOH solution (in order to get rid of the Euexcess as Eu(OH)₃) resulting in a white precipitates removed via a fine syringe filter. The pH was adjusted back to neutral and lyophilised to give a light brown solid, which was loaded onto a DOWEX 1-X8(Cl) anion exchange resin. The column was eluted with water \rightarrow 10% NH₄OH and the fractions were analysed by ESMS⁺. The fractions were combined and lyophilised to yield the Eu-complex as a light brown powder. *m*/z (HRMS⁺) 819.0914 (C₂₈H₃₅O₇N₆S₂EuCl requires 819.0915)

1,7-Bis(α-dimethylglutarate)-1,4,7,10-tetraazacyclododecane



Tetraazacyclododecane (2.00 g, 11.61 mmol), dimethyl-2-bromoglutarate (6.10 g, 25.54 mmol) was dissolved in dry MeCN (20 mL) followed by addition of NaHCO₃ (2.14 g, 2.2 eq.). The mixture was stirred at 55 °C under argon. The reaction was monitored by TLC (DCM : MeOH : NH₄OH, 89 : 10 : 1) and ESMS⁺. After 7 days dimethyl-2-bromoglutarate had been consumed, and the solvent was removed under reduced pressure. The remaining residue was dissolved in DCM (20 mL), the organic layer was washed with HCl (pH 3), dried over K₂CO₃ and the solvents removed under

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reduced pressure. The residue was purified by column chromatography over silica (DCM : THF : MeOH : NH₄OH, 25 : 65 : 5 : 5). The fractions containing the *title product* were combined and the solvents were removed under reduced pressure to yield a pale brown oil (1.23 g, 2.52 mmol, 21%) $\delta_{\rm H}$ (CDCl₃) 7.68 (2H, br.s, NH), 3.63 (6H, s, H⁷), 3.57 (6H, s, H⁶), 3.26 (2H, m, H³), 2.78 (16H, m, H^{1,2}), 2.36 (4H, m, H⁵), 1.92 (4H, m, H⁴) $\delta_{\rm c}$ (CDCl₃) 173.4 (C⁷), 172.9 (C⁶), 64.1 (C³), 51.9 (CH₃⁶), 51.8 (CH₃⁷), 48.7, 46.5 (C^{1,2}), 30.0 (C⁵), 22.6 (C⁴), *m*/z (ESMS⁺) 489 [M + 1], 490 [M+2], R_f 0.32 (DCM : MeOH : NH₄OH, 89 : 10 : 1, silica)

4-[(1-Azathioxanthone)-2-methyl]-1,7-bis(α-dimethylglutarate)-1,4,7,10tetraazacyclododecane



1,7-Bis(α-dimethylglutarate)-1,4,7,10-tetraazacyclododecane (320 mg, 660 µmol) was combined with 2-bromomethyl-1-azathiaxanthone (1 eq., 200 mg) and K₂CO₃ (1 eq., 91 mg) and the mixture stirred in dry MeCN (10 mL) at reflux temperature (85 °C) under argon for 30 h. The reaction was monitored by TLC (DCM : MeOH, 97 : 3) and ESMS⁺ to confirm that the brominated starting material had been consumed. The solvent was removed under reduced pressure and the resulting solid was dissolved in a small volume of DCM (5 mL) and the KBr/K₂CO₃ filtered out. The crude mixture was purified by column chromatography (DCM→2% MeOH) to yield the *title compound* as a pale brown oil (120 mg, 168 µmol, 26%) $\delta_{\rm H}$ (CDCl₃) 8.68 (1H, d, *J* 8.0 Hz, H⁴), 8.43 (1H, m, H⁶), 7.59 (2H, m , H^{8,9}), 7.42 (1H, m, H⁷), 7.24 (1H, d, *J* 8.0 Hz, H³), 3.83 (2H, s, H¹⁰), 3.63 (6H, s, H¹⁶), 3.57 (6H, s, H¹⁷), 3.26 (2H, m, H¹³), 2.97 (16H, m, H^{11,11',12,12'}), 2.36 (4H, m, H¹⁵), 1.92 (4H, m, H¹⁴) $\delta_{\rm c}$ (CDCl₃) 180.5 (C⁵) 173.4 (C^{16'}), 172.9 (C^{17'}), 161.4 (C²), 158.6 (C^{1'}), 138.4 (C^{4'}), 137.5 (C^{6'}), 133.3 (C⁸), 130.0 (C⁶),

129.0 (C^{9'}), 127.1 (C⁷), 126.6 (C⁹), 125.3 (C⁴), 122.2 (C³), 65.1 (C¹³), 51.9 (C¹⁷), 51.8 (C¹⁶), 51.3, 50.4, 49.2, 46.5 (C^{11,11',12,12'}), 46.1 (C¹⁰), 30.9 (C¹⁵), 25.7 (C¹⁴), R_f 0.16 (DCM : MeOH, 97 : 3; alumina) m/z (HRMS⁺) 714.3163 (C₃₅H₄₈O₉N₅S requires 714.3173).

4-[(1-Azathioxanthone)-2-methyl]-10-[methylsulfonylamino)ethyl]-1,7-bis(αdimethylglutarate)-1,4,7,10-tetraazacyclododecane



4-[(1-Azathioxanthone)-2-methyl]-1,7-bis(α-dimethylglutarate)-1,4,7,10-tetraaza-

cyclododecane (110 mg, 150 µmol) was combined with N-methanesulfonyl-aziridine (1.1 eq., 19.4 mg) and K₂CO₃ (1 eq., 22 mg) stirred in dry MeCN (5 mL) at reflux temperature (85 °C) under argon for 46 hrs. The reaction was monitored by TLC (DCM : MeOH. 97 : 3) and $ESMS^+$ to confirm that the starting material had been consumed. The solvent was removed under reduced pressure and the resulting solid was dissolved in a small volume of DCM (3 mL) and the K₂CO₃ removed by filtration. The crude mixture was purified by column chromatography (DCM \rightarrow 2%) MeOH) to yield the title compound as a light brown oil which slowly recrystallised (50 mg, 60 μmol, 41%). δ_H (CDCl₃) 8.70 (1H, d, J 8.0 Hz, H⁴), 8.48 (1H, m, H⁶), 7.63 (2H, m, H^{8,9}), 7.44 (2H, d+dd, H^{3,7}), 3.83 (2H, s, H¹⁰), 3.63 (6H, s, H¹⁶), 3.60 (6H, s, H¹⁷), 3.26 (2H, m, H¹³), 3.02 (3H, s, H²⁰), 2.93 (16H, m, H^{11,11',12,12'}), 2.50 (6H, m, H^{15,18}), 1.90 (4H, m, H¹⁴), 1.56 (2H, t, J 7.8 Hz, H¹⁹) δ_c (CDCl₃) 180.5 (C⁵) 173.3 (C^{16'}), 173.0 (C^{17'}), 162.1 (C²), 158.8 (C^{1'}), 138.5 (C^{4'}), 137.5 (C^{6'}), 133.7 (C⁸), 130.0 (C⁶), 128.9 (C⁹), 127.2 (C⁷), 126.6 (C⁹), 125.5 (C⁴), 122.7 (C³), 65.9 (C¹³), 51.7 (C¹⁷), 51.4 (C¹⁶), 54.2, 51.3, 49.2, 46.6 (C^{11,11',12,12'}), 46.1 (C¹⁰), 38.1 (C²⁰), 33.4 (C¹⁸), 30.9 (C¹⁵), 25.7 (C¹⁴), 22.9 (C¹⁹), R_f 0.39 (DCM : MeOH, 97 : 3, alumina), m.p. 137-9 °C, m/z (HRMS⁺) 835.3358 (C₃₈H₅₅O₁₁N₆S₂ requires 835.3370).

4-[(1-Azathioxanthone)-2-methyl]-10-[methylsulfonylamino)ethyl]-1,7-bis(α-glutarate)-1,4,7,10-tetraazacyclododecane



Freshly made KOD solution (2.5 mL, 0.1 M) was added to 4-[(1-azathioxanthone)-2-methyl]-10-[methylsulfonylamino)ethyl]-1,7-bis(α -dimethylglutarate)-1,4,7,10-tetraaza-cyclododecane (50 mg, 60 µmol). The reaction mixture was kept under argon at room temperature and progress was monitored by NMR. After 3 h no protecting methyl group signals were observed in the ¹H-NMR spectrum, the pH of the mixture was increased (pH \approx 6) with conc. HCl and the solution loaded onto a DOWEX 50X4-100 strong cation exchange resin. The column was eluted with water \rightarrow 10% NH₄OH and the fractions were analysed by ESMS⁺. The fractions were combined and lyophilised to yield the *title compound* as a dark orange oil (26 mg, 33 µmol, 55%), which was used for complexation reaction immediately. $\delta_{\rm H}$ (D₂O): mainly broad overlapping signals, but no Me groups in ¹H-NMR, $\delta_{\rm H}$ (CDCl₃) 8.46 (1H, d, *J* 8.0 Hz, H⁴), 8.18 (1H, m, H⁶), 7.44 (4H, m, H^{8,9,3,7}), 3.83 (2H, s, H¹⁰), 3.26 (2H, m, H¹³), 3.02 (3H, s, H²⁰), 3.09 (22H, br.m, H^{11,11',12,12',15,18}), 2.05 (6H, m, H^{14,19}), *m*/z (ESMS⁻) 779 [M – H].

 $[H_2EuL^2]$



Azathioxanthone)-2-methyl]-10-[methylsulfonylamino)ethyl]-1,7-bis(α -glutarate)-1,4,7,10-tetraazacyclododecane (25 mg, 32 µmol) was added to Eu(CH₃CO₂)₃ (1.1 eq., 15 mg) and the solids dissolved in a H₂O (2 mL). The pH was carefully adjusted to 5 by addition of acetic acid and the reaction left to stir at 70 ° C for 72 hrs. After the reaction was cooled to room temperature, the solvents were removed under reduced pressure and the remaining residue was dissolved in 5 mL H₂O. The pH was then adjusted carefully to 10 by addition of conc. NaOH solution (in order to remove the excess Eu as Eu(OH)₃) resulting in a white precipitate, removed via a fine syringe filter. The pH was adjusted back to neutral and the mixture lyophilised to give a bright yellow solid (26 mg, 28 µmol). *m*/z (HRMS⁻) 927.1562 (C₃₄H₄₂O₁₁N₆S₂Eu requires 927.1565); $\lambda_{max}(H_2O)$ 380 (4070 dm³mol⁻¹cm⁻¹); $\tau^{Eu}_{(H2O, pH=3.0)}$: 588 ms, $\tau^{Eu}_{(H2O, pH=5.5)}$: 709 ms, $\tau^{Eu}_{(H2O, pH=8.0)}$: 469 ms; $\tau^{Eu}_{(D2O, pD=2.6)}$: 1086 ms, $\tau^{Eu}_{(D2O, pD=5.1)}$: 1000 ms, $\tau^{Eu}_{(D2O, pD=7.6)}$: 534 ms; $\phi^{Eu}_{(pH=3.0)}$ = 1.8 %, $\phi^{Eu}_{(pH=5.5)}$ = 2.0 %, $\phi^{Eu}_{(pH=8.0)}$ = 1.7 %

1,7-Bis(α-dimethyladipate)-1,4,7,10-tetraazacyclododecane



Tetraazacyclododecane (1.20 g, 6.98 mmol), dimethyl-2-bromoadipate (3.88 g, 15.35 mmol) was dissolved in dry MeCN (20 mL) followed by addition of NaHCO₃ (1.29 g, 2.2 eq.). The mixture was stirred at 55 °C under argon. The reaction was monitored by TLC (DCM : THF : MeOH, 50 : 50 : 5) and ESMS⁺. After 4 days of reaction all dimethyl-2-bromoadipate had been consumed, and the solvent was removed under reduced pressure. The remaining residue was dissolved in DCM (10 mL). The organic layer was washed with HCl (pH 3), dried over K₂CO₃ and the solvents removed under reduced pressure. The residue was purified by column chromatography over silica (DCM : THF : MeOH, 50 : 50 : 3). The fractions containing the *title product* were

combined and the solvents were removed under reduced pressure to yield a transparent viscous oil which slowly crystallised to reveal a white solid (760 mg, 1.47 mmol, 21%) $\delta_{\rm H}$ (CDCl₃) 4.18 (1H,m, H³) 3.77 (6H, s, H⁷), 3.73 (6H, s, H⁸), 3.29 (16H, m, H^{1,2}), 2.36 (4H, m, H⁶), 1.78 (8H, m, H^{4,5}) $\delta_{\rm c}$ (CDCl₃) 174.0 (C⁷), 173.9 (C⁸), 70.3 (C³), 53.0 (C⁸), 52.7 (C⁷), 52.6, 52.1, 51.8, 46.6 (C^{1,2}), 33.7 (C⁴), 33.6 (C⁶), 31.0 (C⁵), *m*/z (ESMS⁺) 517 [M + H], 530 [M + Na]; R_f 0.42 (THF : DCM : MeOH, 50 : 50 : 5, silica).

4-[(1-Azathioxanthone)-2-methyl]-1,7-bis(α-dimethyladipate)-1,4,7,10tetraazacyclododecane



1,7-Bis(α -dimethyladipate)-1,4,7,10-tetraazacyclododecane (310 mg, 600 µmol) was combined with 2-bromomethyl-1-azathiaxanthone (1 eq., 183 mg) and NaHCO₃ (1 eq., 50 mg) and the mixture stirred in dry MeCN (10 mL), was heated initially at 60 °C for 4 h followed by 70 °C for 20 h under argon. The reaction was monitored by TLC (DCM : THF : MeOH : Et₃N, 80 : 20 : 3.5 : 3.5, silica) and ESMS⁺ to confirm that the brominated starting material had been consumed. The solvent was removed under reduced pressure and the resulting solid was dissolved in a small volume of DCM (5 mL) and the sodium salts removec by filtration. The crude mixture was purified by column chromatography (DCM : THF 80 : 20 \rightarrow 3% MeOH : Et₃N (1 : 1)); fractions containing clean product were combined and the solvents were removed under reduced pressure. The remaining residue was dissolved in DCM (5 mL) and washed with water (3x15 mL). The organic layer was evaporated dry to yield the *title compound* as a pale yellow oil (165 mg, 170 µmol, 28%) $\delta_{\rm H}$ (CDCl₃) 8.80 (1H, d, *J* 8.0 Hz, H⁴), 8.60 (1H, br.d, *J* 7.9 Hz, H⁶), 7.67 (2H, m , H^{8,9}), 7.55 (1H, dt, *J* 7.9 Hz, H⁷), 7,34 (1H, d, *J* 8.0 Hz, H³), 4.19 (1H,m, H¹³), 3.81 (2H, s, H¹⁰), 3.73 (6H, s, H¹⁷), 3.69 (6H, s, H¹⁸), 3.17 (16H, m, H^{11,11',12,12'}), 2.38 (4H, m, H¹⁶), 1.78 (8H, m, H^{14,15}) δ_c (CDCl₃) 181.2 (C⁵) 173.4 (C^{16'}), 172.9 (C^{17'}), 162.1 (C²), 158.8 (C^{1'}), 138.4 (C^{4'}), 137.5 (C^{6'}), 133.4 (C⁸), 130.2 (C⁶), 129.1 (C^{9'}), 127.1 (C⁷), 126.7 (C⁹), 125.8 (C⁴), 122.5 (C³), 70.3 (C¹³), 52.0 (C¹⁸), 51.8 (C¹⁷), 51.9, 51.8, 51.7, 49.2 (C^{11,11',12,12'}), 48.1 (C¹⁰), 33.8 (C¹⁴), 33.7 (C¹⁶), 29.9 (C¹⁵), R_f 0.33 (DCM : THF : MeOH : Et₃N, 80 : 20 : 3.5 : 3.5, silica plate), *m*/z (HRMS⁺) 742.3489 (C₃₇H₅₂O₉N₅S requires 742.3486).

4-[(1-Azathioxanthone)-2-methyl]-10-[methylsulfonylamino)ethyl]-1,7-bis(αdimethyladipate)-1,4,7,10-tetraazacyclododecane



 $4-[(1-Azathioxanthone)-2-methyl]-1,7-bis(\alpha-dimethyladipate)-1,4,7,10-tetraaza-$

cyclododecane (190 mg, 256 μ mol) was combined with 2-Methanesulfonato-Nmethanesulfonylethylamine (1.2 eq., 67.2 mg) and Na₂CO₃ (1.5 eq., 42 mg) and the mixture stirred in dry MeCN (10 mL) was reflux for 30 h under argon. The reaction was monitored by TLC (DCM : THF : MeOH : Et₃N, 80 : 20 : 2.5 : 2.5, silica) and ESMS⁺ to confirm that the starting material had been consumed. The solvent was removed under reduced pressure. The resulting solid was dissolved in a small volume of DCM (5 mL) and the sodium salts were filtered out. The crude mixture was purified by column chromatography (DCM : THF 80 : 20 \rightarrow 3% MeOH : Et₃N (1 : 1)). The fractions containing product only were combined and the solvents were removed under reduced pressure. The remaining residue was dissolved in DCM (5 mL) and washed with water (3x10 mL). The organic layer was evaporated to yield the *title compound* as a pale brown oil (170 mg, 197 µmol, 77%) $\delta_{\rm H}$ (CDCl₃) 8.80 (1H, d, J 8.0 Hz, H⁴), 8.66 (1H, br.d, J 7.9 Hz, H⁶), 7.76 (2H, dd, J 7.9 Hz, H^{8,9}), 7.65 (1H, dt, J 7.9 Hz, H⁷), 7,53 (1H, d, J 8.0 Hz, H³), 4.19 (1H,m, H¹³), 3.84 (2H, s, H¹⁰), 3.66 (6H, s, H¹⁷), 3.64 (6H, s, H¹⁸), 3.06 (16H, m, H^{11,11',12,12'}), 2.98 (3H, dd, H²¹), 2.33 (6H, m, H^{16,19}), 1.68 (10H, m, H^{14,15,20}), $\delta_{\rm c}$ (CDCl₃) 180.8 (C⁵) 174.9 (C^{18'}), 173.5 (C^{17'}), 165.4 (C²), 158.1 (C^{1'}), 138.1 (C^{4'}), 137.7 (C^{6'}), 133.1 (C⁸), 130.1 (C⁶), 129.2 (C^{9'}), 126.9 (C⁷), 126.7 (C⁹), 125.8 (C⁴), 121.7 (C³), 65.7 (C¹³), 51.8 (C¹⁸), 51.4 (C¹⁷), 54.3, 54.1, 50.2, 50.1 (C^{11,11',12,12'}), 50.0 (C¹⁰), 40.1 (C²¹), 34.4 (C¹⁴), 33.8 (C¹⁶), 30.6 (C¹⁹) 29.6 (C¹⁵), 22.3 (C²⁰), R_f 0.61 (DCM : THF : MeOH : Et₃N, 80 : 20 : 2.5 : 2.5, silica), *m*/z (HRESMS⁺) 863.3686 (C₄₀H₅₈O₁₁N₆S₂ requires 863.3679).

4-[(1-Azathioxanthone)-2-methyl]-10-[methylsulfonylamino)ethyl]-1,7-bis(αadipate)-1,4,7,10-tetraazacyclododecane, H₅L₃



Freshly made KOD solution (5 mL, 0.1 M) was added to 4-[(1-azathioxanthone)-2methyl]-10-[methylsulfonylamino)ethyl]-1,7-bis(α -dimethyladipate)-1,4,7,10tetraaza-cyclododecane (57 mg, 66 µmol) and the mixture was stirred under argon at room temperature; the reaction was monitored by ¹H-NMR. After 3 h no methyl group signals were observed in the ¹H-NMR spectrum; the pH of the mixture was adjusted (pH \approx 6) with conc. HCl, washed with DCM (3x5 mL) and loaded onto a DOWEX 50X4-100 strong cation exchange resin. The column was eluted with water \rightarrow 10% NH₄OH and the fractions were analysed by ESMS⁺. The appropriate fractions were combined and lyophilised to yield the *title compound* as a dark orange oil (25 mg, 33 µmol, 47%), which was used in a complexation reaction immediately. $\delta_{\rm H}$ (D₂O): mainly broad overlapping signals, but no sing of proecting Me groups in ¹H-NMR, $\delta_{\rm H}$ (CDCl₃) 8.55 (1H, d, *J* 8.0 Hz, H⁴), 8.24 (1H, m, H⁶), 7.49 (4H, m, H^{8,9,3,7}), 3.16 (27H, br.m, H^{10,11,11',12,12',16,19.21}), 1.67 (10H, m, H^{14,15,20}); *m*/z (ESMS⁻) 805 [M – H].

$[H_3EuL^3]$



4-[(1-Azathioxanthone)-2-methyl]-10-[methylsulfonylamino)ethyl]-1,7-bis(α -

adipate)-1,4,7,10-tetraazacyclododecane (25 mg, 31 µmol) was added to Eu(OAc)₃ (1.1 eq., 13 mg) and the solids dissolved in 2.5 mL H₂O : MeOH (5 : 1). The pH was carefully adjusted to 5 by addition of acetic acid and the reaction left to stir at 75 ° C for 72 h. After the reaction was cooled to room temperature, The pH was then adjusted carefully to 10 by addition of conc. NaOH solution (in order to remove excess Eu as Eu(OH)₃) resulting in a white precipitate removed via centrifugation. The pH was adjusted back to neutral and the sample lyophilised to give a bright yellow solid (30 mg, 30 µmol). *m*/z (HRMS⁺) 983.9305 (C₃₆H₅₁O₁₁N₆S₂EuNa requires 983.9289); λ_{max} (H₂O) 380 (4070 dm³mol⁻¹cm⁻¹); $\tau^{Eu}_{(H2O, pH=4.5)}$: 830 ms, $\tau^{Eu}_{(H2O, pH=8.0)}$: 430 ms; $\tau^{Eu}_{(D2O, pD=4.1)}$: 825 ms, $\tau^{Eu}_{(D2O, pD=7.6)}$: 470 ms; $\phi^{Eu}_{(pH=4.5)} = 6.1$ %, $\phi^{Eu}_{(pH=8.0)} = 5.3$ %

[H₃GdL³]



The Gd-complex was prepared as described above $[H_3EuL^3] m/z$ (HRMS⁺) z (HRMS⁻ in MeOH) 1001.2299 (C₃₆H₄₉O₁₁N₆S₂GdNa mono Me-ester requires: 1001.2273) $r_{1p}^{(pH=4.5)}$: 3.12 mM⁻¹s⁻¹ $r_{1p}^{(pH=8.5)}$: 1.87 mM⁻¹s⁻¹.