Evidence for the optical signalling of changes in bicarbonate concentration within the mitochondrial region of living cells

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1. **Cell culture: methods and procedures and representative cell images showing complex localization to the mitochondria**
2. **Ligand and complex synthesis and characterization, including HPLC traces for complexes of L^1 , L^2 and L^3**
3. **Examples of spectral titrations and data fitting.**
4. **Table of photophysical data for Tb and Eu complexes.**

1. Cell culture: methods and procedures

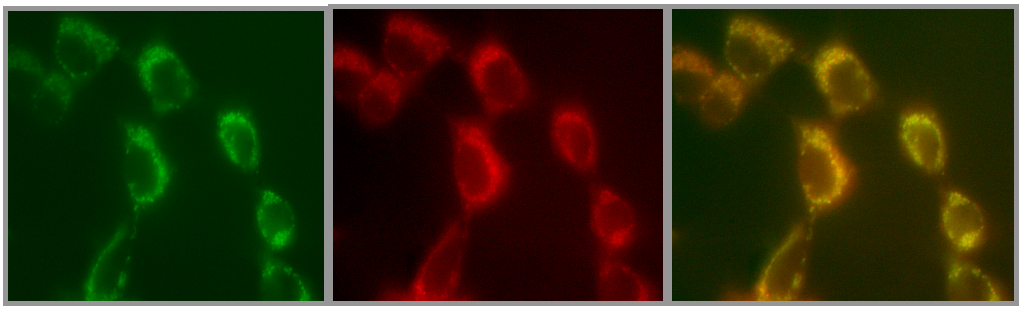
Human lung carcinoma A549 cells were purchased from the American type Culture Collection (ATCC) (#CCL-185, ATCC, Manassas, VA, USA). Cells were cultured in Ham's F12K medium with L-glutamine and phenol red (N3520, Sigma, St. Louis, MO, USA) supplemented with 10% foetal bovine serum at 37°C and 5% CO_2.. Human cervical carcinoma (HeLa) cells were maintained in an RMPI 1640 medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin and streptomycinat 37°Cin 5% CO_2.MCF-7 cells were purchasedfrom the American type Culture Collection (ATCC) (#HTB-22, ATCC, Manassas, VA, USA) and were cultured in DMEM medium with L-glutamine and phenol red, supplemented with 10% neo-natal calf serum containing 1% streptomycin and penicillin. In general,cells were passaged every 3-5 days, maintaining confluence at around 80% for the imaging experiments.

To study the localization behaviour of the europium/terbium complexes, experiments were carried using various microscopes. One setup involved use of aLeica SP5 multi-photon confocal microscope (upright configuration) equipped with a UV lamp, an argon laser and a femtosecond-pulsed Ti:Sapphire laser (Libra II, Coherent) in which light was focused on to coverslip-adherent cells using a 40x oil or 63x water immersion objective. The second set up used a Zeiss 510 LSM (upright configuration) confocal microscope equipped with a femtosecond-pulsed Ti:Sapphire laser (Libra II, Coherent), argon laser, and a UV lamp and was focused on coverslip-adherent cells using a 63 x oil immersion objective.For the in-vitro emission spectra, a lambda scan system was used with a long-pass filter (LP 450 nm) for monitoring terbium emission and a band-pass filter (550 nm - 700 nm) to obtain the emission from europium.

For the evaluation of the variation of lanthanide emission intensity with external carbon dioxide partial pressure, imaging of living cells under the microscope required the maintenance of the cell culture under accurately controlled conditions of temperature and carbon dioxide. The cells were placed in the Zeiss PM S CO_2 incubator. The temperature within the CO_2 chamber enclosure were thermostatically maintained at37°Callowing the observed cells to be kept alive for days. In addition variation of the percentage of carbon dioxide of the heated air was enabled using a

CO_2controller. 10μM concentrations of lanthanide complexes were dosed in the growth medium of the cells and incubated for up to 6 hours in 5% CO_2. The CO_2 content inside the incubator was adjusted from 5 to 4 to 3% and then back to 5%. In each case, the spectral emission data and microscopic images were taken 30 minutes after varying the CO_2 content to allow equilibration.

ESI Figure 1

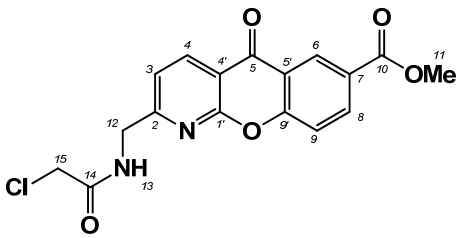


Epifluorescence microscopy images showing the staining of MCF-7 cells with : *left*: Mitotracker Green; *centre*: $[Eu.L^2]^{3+}$ using a 570 nm long pass filter; *right* : the co-localised image showing good correspondence.

2. Ligand and complex synthesis

Synthesis of ligands L^2 and L^3

7-Methoxycarbonyl-2-chloromethylcarbonylaminomethyl-1-azaxanthone

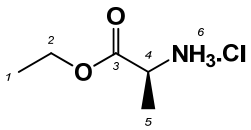


7-Methoxycarbonyl-2-aminomethyl-1-azaxanthone (147 mg, 0.459 mmol), chloroacetic acid 2,5-dioxo-pyrrolidin-1-yl ester (175 mg, 0.917 mmol) and triethylamine were stirred in dry THF (20 ml) under argon for 18h at room temperature. DCM (20 ml) was added and the mixture was washed with H_2O (3 x 20

ml). The solvent was removed under reduced pressure to leave the desired product as a light yellow powder (101 mg, 282 µmol, 61%).

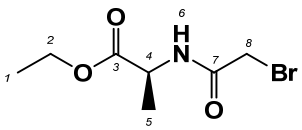
^{1}H-NMR ($CDCl_3$, 700 MHz) δ 8.99 (1H, d, J = 2.1, H^6), 8.70 (1H, d, J = 7.9, H^4), 8.43 (1H, dd, J = 8.7, 2.1, H^8), 7.67 (1H, d, J = 8.7, H^9), 7.66 (1H, br s, H^{13}), 7.45 (1H, d, J = 7.9, H^3), 4.76 (2H, d, J = 5.5, H^{12}), 4.17 (2H, s, H^{15}), 3.98 (3H, s, H^{11}); ^{13}C-NMR ($CDCl_3$, 175 MHz) δ 176.7 (C^5), 166.5 (C^{14}), 165.7 (C^{11}), 162.4 (C^2), 160.0 ($C^{1'}$), 158.3 ($C^{9'}$), 138.6 (C^4), 136.4 (C^8), 129.3 (C^6), 127.2 ($C^{5'}$), 121.5 (C^7), 119.9 (C^3), 119.1 (C^9), 115.8 ($C^{4'}$), 52.7 (C^{12}), 45.0 (C^{10}), 42.7 (C^{15}); MS (ES+) *m/z* 361.1 $[M + H]^+$; HRMS (+*m/z*): $[M+Na]^+$ calculated for $C_{17}H_{13}ClNaN_2O_5$ 383.0419, found 383.0411.

(S)-Alanine ethyl ester hydrochloride



(S)-Alanine (1.00 g, 11.2 mmol) was suspended in dry ethanol (40 ml). Dry HCl (produced by dropping conc. H_2SO_4 onto NaCl and passing through conc. H_2SO_4) was bubbled through the solution for 3 h, while refluxing under argon. The bubbling was stopped and the mixture boiled under reflux for a further 12 h. The solvent was removed under reduced pressure to yield the product as a colourless solid (1.71 g, 11.2 mmol, >99 %).m.p. 82 – 83 ˚C; ^{1}H-NMR (CD_3OD, 400 MHz) δ 4.27 (q, 2H, J = 6.8, H^1), 4.02 (1H, q, J = 7.2, H^4), 1.53 (d, 3H, J = 6.8, H^2), 1.29 (t, 3H, J = 7.2, H^5); ^{13}C-NMR ($CDCl_3$, 175 MHz) δ 170.0 (C^3), 62.4 (C^2), 49.3 (C^4), 16.1 (C^5), 14.0 (C^1); ESI/MS^+ m/z 118.2 $[M+H]^+$; HRMS (+*m/z*): $[M+H]^+$ calculated for $C_5H_{12}O_2N$ 118.0863, found 118.0863.

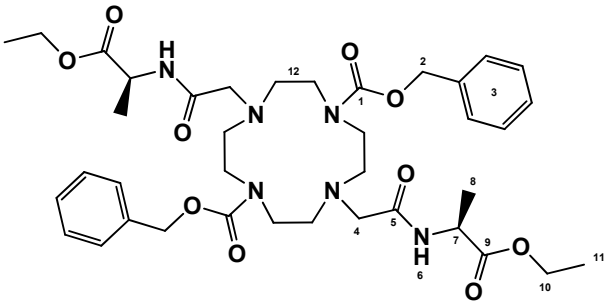
Bromoacetyl-(*S*)-alanine ethyl ester



(S)-Alanine ethyl ester hydrochloride (1.71 g, 11.2 mmol) was stirred under argon at -20 ˚C in dry $CHCl_3$ (10 ml). Triethylamine (2.3 ml, 32.0 mmol) and bromoacetyl bromide (1.6 ml, 17.9 mmol) were added and the mixture stirred at -20 ˚C for 2 h

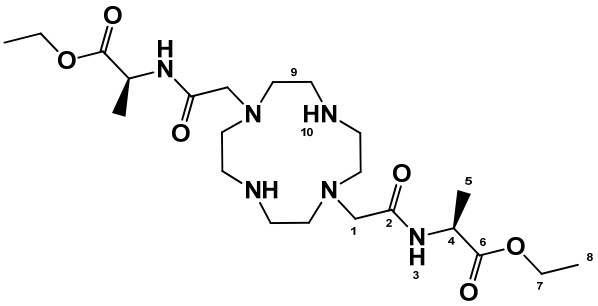
before being allowed to gradually warm to room temperature and stirred for a further 12 h. The reaction was then halted and washed with aq. HCl (6 M, 30 ml) and then H_2O (4 x 25 ml). The organic phase was dried and the product recrystallised from DCM / pet. ether as white crystals (1.88 g, 7.95 mmol, 71 %). mp 33 – 34 ˚C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.03 (s, br, H^6), 4.55 (m, H^4), 4.23 (q, J = 7.2, H^2), 3.88 (s, 2H, H^8), 1.45 (d, 3H, J = 7.0, H^4), 1.28 (t, 3H, J = 7.2, H^1); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 172.5 (C^7), 165.6 (C^3), 62.1 (C^2), 48.8 (C^4), 42.3 (C^8), 18.0 (C^5), 14.0 (C^1); ESI/MS$^+$ m/z 260.1 $[M+Na]^+$; HRMS (+*m/z*): $[M+H]^+$ calculated for $C_7H_{12}NO_3BrNa$ 259.9893, found 259.9895.

(SS)-1,7-Bis(benzyloxycarbonyl)-4,10-bis(ethyl-N-acetyl-S-alanine)-1,4,7,10-tetraazacyclododecane



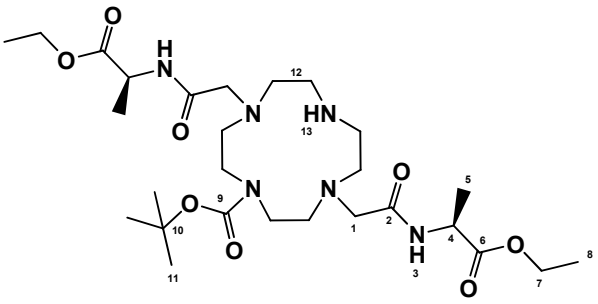
1,7-Bis(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (0.738 g, 1.69 mmol) was dissolved in dry MeCN (30 ml) and bromoacetyl-(*S*)-alanine ethyl ester (0.834 g, 3.52 mmol) was added. K_2CO_3 (1.3 g, 9.4 mmol) was added and the mixture stirred at 60 ˚C under argon for 4 days. The reaction was allowed to cool to room temperature and filtered. Solvent was removed under reduced pressure. The desired product was isolated by column chromatography on silica gel (DCM → 3 % MeOH), as a light orange glassy oil (0.973 g, 1.35 mmol, 80 %).^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.27 (m, 10H, H^3), 5.05 (s, 4H, H^2), 4.43 (s, br, 2H, H^7), 4.09 (q, 4H, J = 7.2, H^{10}), 2.76 – 3.47 (m, br, 16H, H^{12}), 1.34 (m, br, 3H, H^8), 1.21 (t, 6H, J = 7.2, H^{11}); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 172.9 (C^9), 170.9 (C^5), 157.0 (C^1), 136.7 (C^3), 128.8 (C^3), 128.4 (C^3), 67.6 (C^{10}), 61.5 (C^4), 55.9, 54.9 (C^{12}), 48.1 (C^7), 18.1 (C^8), 14.4 (C^{10}); MS (ES+) *m/z* 755.3 $[M + H]^+$; HRMS (+*m/z*): $[M+H]^+$ calculated for $C_{38}H_{55}N_6O_{10}$ 755.3973, found 755.3980.

(SS)-1,7-Bis(ethyl-N-acetyl-S-alanine)-1,4,7,10-tetraazacyclododecane



(SS)-1,7-Bis(benzyloxycarbonyl)-4,10-bis(ethyl-N-acetyl-*S*-alanine)-1,4,7,10-tetraazacyclododecane (0.973 g, 1.35 mmol) was dissolved in MeOH (20 ml) and $Pd(OH)_2/C$ (Pd content 10 %) (75 mg) added. The mixture was shaken in a Parr hydrogenator flask at 40 psi H_2 for 3 days. The resulting mixture was filtered through Celite leaving a clear solution which was evaporated and dried under reduced pressure to yield a yellow viscous oil (0.400 g, 0.823 mmol, 61 %).^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.70 (2H, m, br, H^{10}), 7.53 (2H, m, br, H^3), 4.54 (2H, m, H^4), 4.17 (4H, m, H^7), 3.26 (4H, m, H^1), 2.60-2.85 (16H, m, br, H^9), 1.38 (6H, m, H^8), 1.26 (6H, m, H^7); MS (ES+); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 173.1 (C^6), 171.0 (C^2), 62.1 (C^7), 61.4 (C^1), 53.4, 52.5 (C^9), 48.2 (C^4), 18.5 (C^6), 14.4 (C^8); MS (ES+) *m/z* 487.4 $[M + H]^+$; HRMS (+*m/z*): $[M+H]^+$ calculated for $C_{22}H_{43}O_6N_6$ 487.3239 found, 487.3248.

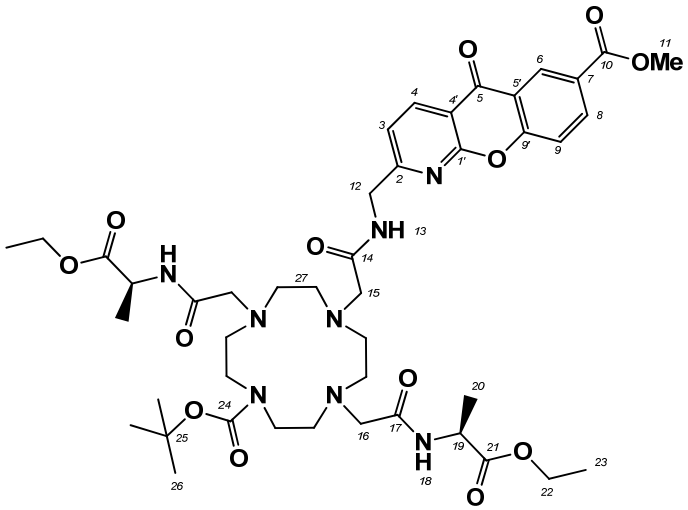
(SS)-1,7-Bis(ethyl-N-acetyl-S-alanine)-4-carboxylic acid-tert-butyl-ester-1,4,7,10-tetraazacyclododecane



(SS)-1,7-Bis(ethyl-N-acetyl-S-alanine)-1,4,7,10-tetraazacyclododecane (0.812 g, 1.67 mmol) and di-tert-butyl dicarbonate (0.363 mg, 1.67 mmol) were stirred, under argon, in CHCl3 at 30 ºC for 18 h. Solvent was then removed under reduced pressure and the product purified by column chromatography on silica gel (DCM → 3 % MeOH) (239 mg, 0.407 mmol, 24%).

R_F = 0.23 (alumina, DCM : 8% MeOH); ^{1}H-NMR ($CDCl_3$, 700 MHz) δ 7.47 (2H, d, br, H^3), 4.58 (2H, q, J = 7.6, H^4), 4.16 (4H, q, J = 7.6, H^7), 3.26 (4H, m, H^1), 2.80-3.40 (16H, m, br, H^9), 1.42 (9H, s, H^{11}), 1.40 (6H, t, J = 7.6, H^5), 1.24 (6H, t, J = 7.6, H^8); ^{13}C-NMR ($CDCl_3$, 126 MHz) δ 173.2 (C^6), 80.8 (C^9), 61.9 (C^7), 61.0 (C^1), 55.3 (C^4), 52.1 (C^9), 42.0 (C^4), 30.8 (C^{10}), 28.7 (C^{11}), 27.0 (C^5), 14.4 (C^1), 14.0 (C^8); MS (ES+) m/z 587.5 $[M + H]^+$; HRMS (+*m/z*): $[M+H]^+$ calculated for $C_{27}H_{51}N_6O_8$ 587.3758, found 587.3768.

Tert-butyl 4,10-bis({[(2S)-1-ethoxy-1-oxopropan-2-yl]carbamoyl}methyl)-7-[({[7-(methoxycarbonyl)-5-oxo-5H-chromeno[2,3-b]pyridin-2-yl]methyl}carbamoyl)methyl]-1,4,7,10-tetraazacyclododecane-1-carboxylate



(SS)-1,7-Bis(ethyl-N-acetyl-S-alanine)-4-carboxylic acid tert-butyl ester-1,4,7,10-tetraazacyclododecane (98.7 mg, 0.168 mmol) was combined with 7-methoxycarbonyl-2-chloromethylcarbonylaminomethyl-1-azaxanthone (16.8 mg, 46.6 µmol) and DIPEA (40 µl, 0.230 mmol) in dry MeCN under argon. The mixture was stirred at 60 ˚C for 24 h. A further addition of 7-methoxycarbonyl-2-chloromethylcarbonylaminomethyl-1-azaxanthone (19.0 mg, 52.7 µmol) and DIPEA (50 µl, 0.287 mmol) was made at this time-point and the mixture stirred for a further 18 h. Solvent was then removed under reduced pressure and the residue purified by column chromatography (silica, DCM → 7 % MeOH) to yield the product as a glassy orange solid (63.9 mg, 70.2 µmol, 71 %).^{1}H-NMR ($CDCl_3$, 500 MHz) δ 11.21 (8.96 (1H, d, J = 2.3, H^6), 8.64 (1H, d, J = 7.8, H^4), 8.41 (1H, dd, J = 8.9, 2.3, H^8), 7.60 (1H, d, J = 8.9, H^9), 7.43 (1H, d, J = 7.8, H^3), 4.71 (2H, d, J = 4.8, H^{12}), 4.50 (2H, p, J = 6.8, H^{19}), 4.12 (4H, q, J = 7.0, H^{22}), 3.96 (3H, s, H^{11}), 3.55 - 2.60 (22H, br. m, $H^{15,}$

$^{16, 27}$), 1.53 (6H, t, J = 7.4, H^{20}), 1.39 (9H, s, H^{26}), 1.37 (6H, t, J = 7.0, H^{23}); ^{13}C-NMR ($CDCl_3$, 126 MHz) δ 176.8 (C^5), 173.1 (C^{14}), 165.7 (C^{17}), 160.0 ($C^{1'}$), 158.2 ($C^{9'}$), 155.8 (C^{24}), 138.2 (C^4), 136.3 (C^8), 129.3 (C^6), 127.0 ($C^{5'}$), 121.5 (C^7), 119.7 (C^3), 118.9 (C^9), 115.4 ($C^{4'}$), 61.6 (C^{22}), 53.7 (C^{27}), 52.7 (C^{11}), 47.4 (C^{16}), 44.8 (C^{12}), 42.0 (C^{19}), 28.6 (C^{25}), 18.8 (C^{26}), 14.3 (C^{23}), 12.2 (C^{20}); HRMS (+*m/z*): $[M+H]^+$ calculated for $C_{44}H_{63}N_8O_{13}$ 911.4515 found, 911.4518.

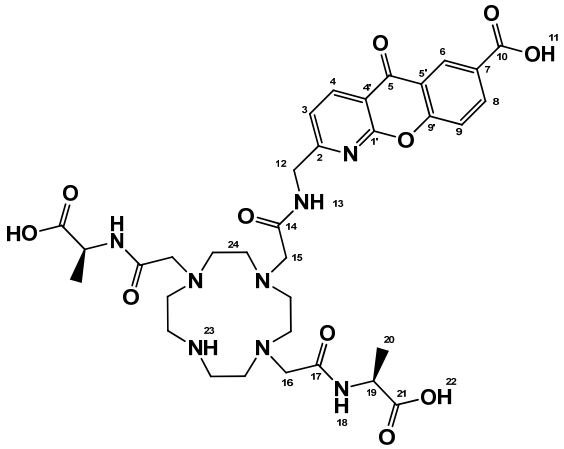
[Eu.L^2]Cl$_3$

Tert-butyl 4,10-bis({[(2S)-1-ethoxy-1-oxopropan-2-yl]carbamoyl}methyl)-7-[({[7-(methoxycarbonyl)-5-oxo-5H-chromeno[2,3-b]pyridin-2 yl]methyl}carbamoyl)methyl]-1,4,7,10-tetraazacyclododecane-1-carboxylate (98.7 mg, 0.168 mmol) in DCM:TFA (50:50, 2 ml) was stirred in a sealed flask for 12 h yielding a yellow solution. Solvent was then removed under reduced pressure to yield the final ligand as a glassy yellow solid in a quantitative yield which was used immediately in the next step after checking that removal of the BOC protecting group was complete by mass spectrometry. {(ES+) *m/z* 811.4 $[M + H]^+$}. This final ligand (22.4 mg, 23.3 μmol) was added to $Eu(OTf)_3$ (19 mg, 31.9 μmol) and dissolved in MeCN (1 ml). The pH was carefully adjusted to 5 by the addition of acetic acid and the reaction stirred for 48 h at 75 ˚C. The reaction was cooled to room temperature the solvents removed under reduced pressure. The remaining residue was dissolved in dry MeCN (0.1 ml) and the mixture dropped onto anhydrous Et_2O (5 ml) which resulted in the precipitation of the title compound as a triflate salt. The precipitate was centrifuged and dissolved in aqueous MeOH:H_2O (50 : 50, 3 ml). 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, which was removed by centrifugation. The pH was adjusted back to neutral and the mixture lyophilised to give a bright yellow solid which was loaded onto a DOWEX 1-X8(Cl) anion exchange resin. The column was eluted with water → 10% NH_4OH. The fractions were combined and lyophilized to yield the chloride salt of the Eu-complex as a light yellow glassy solid (14.0 mg, 14.5 μmol, 62 %).HRMS (+*m/z*): $[M+HCO_2^-]^{2+}$ calculated for $C_{40}H_{57}{}^{153}EuN_8O_{13}$504.1550found, 504.1538; t_R = 9.41 min; τ_{H2O} = 0.26 ms, τ_{D2O} = 0.58 ms; q = 2.0

[Tb.L^2]Cl_3

The complex was prepared using an analogous method as for [Eu.L^2]Cl_3 using L^2 (11.2 mg, 11.7 µmol) and $Tb(OTf)_3$ (8.3 mg, 14.0 µmol) to yield the Tb-complex as its chloride salt (9.0 mg, 9.3 µmol, 79%). HRMS (+*m/z*): $[M+2Na]^{2+}$ calculated for $C_{40}H_{57}TbN_8O_{13}$ 507.1571found, 507.1540; t_R = 9.45 min; τ_{H2O} = 1.11 ms, τ_{D2O} = 2.44 ms; q = 2.15.

2-{[2-(4-{[(1-carboxyethyl)carbamoyl]methyl}-10{[(carboxymethyl)carbamoyl]methyl}-1,4,7,10-tetraazacyclododecan-1-yl)acetamido]methyl}-5-oxo-5H-chromeno[2,3-b]pyridine-7-carboxylic acid , H_3L^3



Aqueous KOD solution (1 ml, 0.1 M) was added to (SS)-1,7-bis(ethyl-N-acetyl-S-alanine)-4-[7-methoxycarbonyl-2-chloromethylcarbonylmethyl-1-azaxanthone]-1,4,7,10-tetraazacyclododecane (30 mg, 40.0 µmol). The reaction mixture was stirred under argon at room temperature and progress monitored by ^{1}H-NMR, observing the formation of ethanol and methanol solvent peaks, with the corresponding elimination of the methyl- and ethyl-ester peaks. After 3 h the pH of the mixture was decreased to pH 6.5 with conc. HCl and the solution loaded onto a DOWEX 50X4-100 strong cation exchange resin. The column was eluted with 10% NH_4OH and the fractions containing the desired product lyophilized to yield the title compound as an orange glassy solid which was immediately used in the complexation reactions. (16.1 mg, 21.6 µmol, 54%); ^{1}H-NMR (D_2O, 400 MHz) δ (signals all quite broad), 8.85 (1H, m, H^6), 8.57 (1H, d, H^4), 8.36 (1H, m, H^8), 7.60 (1H, m, H^9), 7.37 (1H, m, H^3), 4.60 (2H,

m, H^{12}), 4.46 (2H, m, H^{19}), 4.08 (4H, m, H^{22}), 3.89 (3H, s, H^{11}), 3.55 - 2.60 (22H, br. m, $H^{15,16,27}$), 1.53 (6H, t, J = 7.4, H^{20}).

Eu.L^3

2-{[2-(4-{[(1-carboxyethyl)carbamoyl]methyl}-10{[(carboxymethyl)carbamoyl]methyl}-1,4,7,10-tetraazacyclododecan-1-yl)acetamido]methyl}-5-oxo-5H-chromeno[2,3-b]pyridine-7-carboxylic acid (12.1 mg, 16.0 µmol) was added to $Eu(OAc)_3$ (1.2 eq, 19.2 µmol) and dissolved in 3 ml H_2O:MeOH (7:1). The pH was carefully adjusted to 5 by the addition acetic acid and the reaction stirred for 48 h at 75 ˚C. The reaction was cooled to room temperature and the pH carefully adjusted to 10 by the addition of conc. NaOH solution, to precipitate excess europium as $Eu(OH)_3$ which was removed by centrifugation. The pH was adjusted back to 6.5 with acetic acid and the sample lyophilized to yield the product (2.5 mg, 2.8 µmol, 18%). HRMS (+*m/z*): $[M+H]^+$ calculated for $C_{34}H_{42}{}^{151}EuN_8O_{11}$ 891.2185found, 891.2205; t_R = 8.79 min; τ_{H2O} = 0.26 ms, τ_{D2O} = 0.67 ms; q = 2.3.

Tb.L^3

The complex was prepared using an analogous method using L^3 (4.0 mg, 5.4 µmol) and $Tb(OAc)_3$ (2.3 mg, 6.5 µmol) to yield the complex as its chloride salt (1.0 mg, 1.1 µmol, 20%). HRMS (+*m/z*): $[M+H]^+$ calculated for $C_{34}H_{42}TbN_8O_{11}$ 897.2227found, 897.2270; t_R = 8.78 min; τ_{H2O} = 1.11 ms, τ_{D2O} = 2.55 ms; q = 2.25.

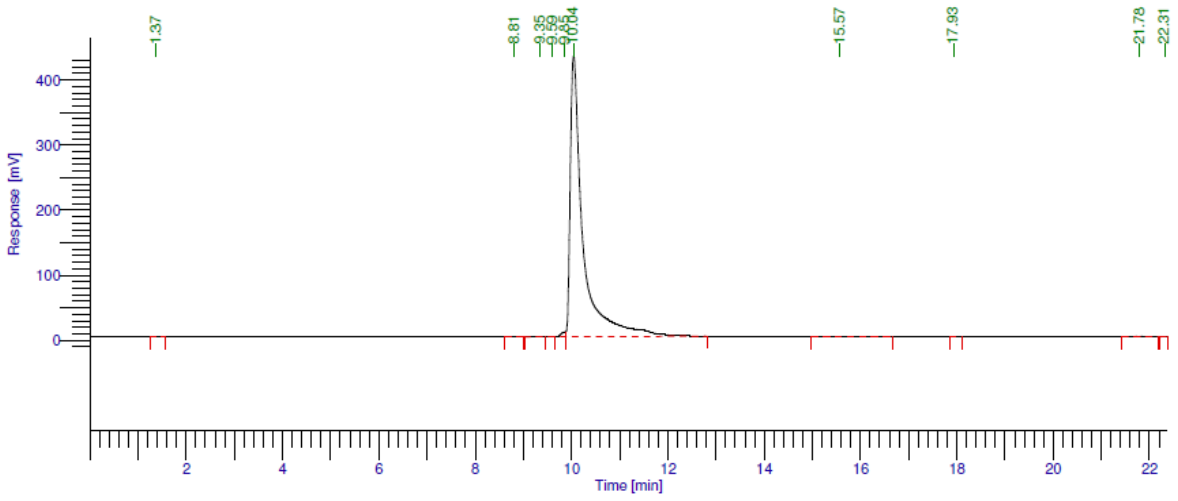
HPLC Traces for Eu and Tb complexes: (λ_{exc} 340 nm, λ_{em} 545 or 620 nm)

Reverse phase HPLC traces were recorded at 298 K using a Perkin Elmer system equipped with a Perkin Elmer Series 200 Pump, a Perkin Elmer Series 200 Autosampler and a Perkin Elmer Series 200 fluorescence detector. A 4.6 x 150 mm 4µm Phenomenex Synergi Fusion RP 80Å analytical column was used. A gradient elution with a solvent system composed of H_2O + 0.1% HCOOH/ MeCN + 0.1% HCOOH was performed for a total run time of 22.4 min.

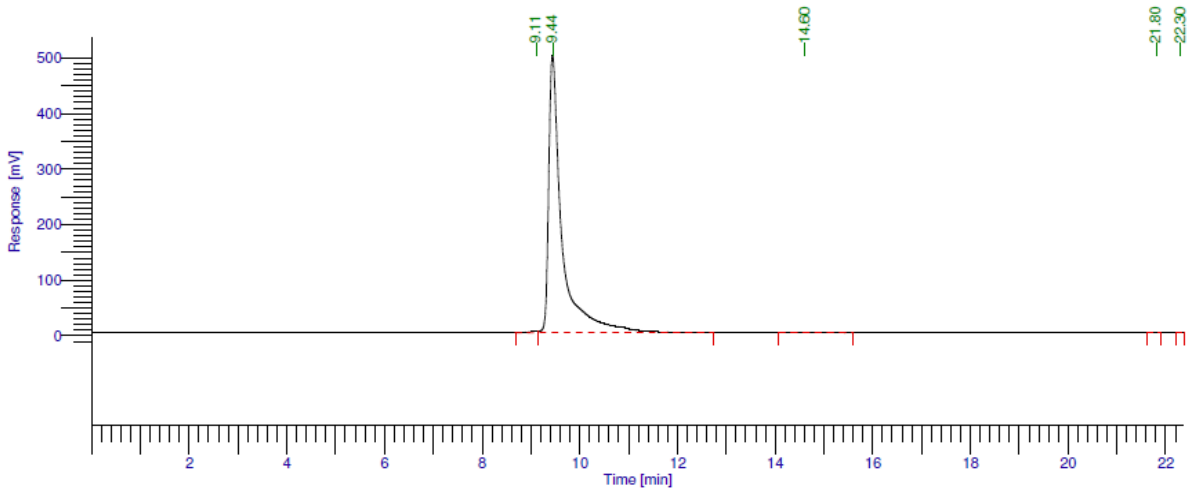
Time (min)	Solvent A (%)	Solvent B (%)	Curvature
0	95	5	0
2.4	95	5	0
15.4	0	100	1
17.4	0	100	0
19.4	95	5	1
22.4	95	5	0

Flow rate = 1ml/min; Solvent A = H_2O + 0.1% HCOOH; Solvent B = MeCN + 0.1% HCOOH.

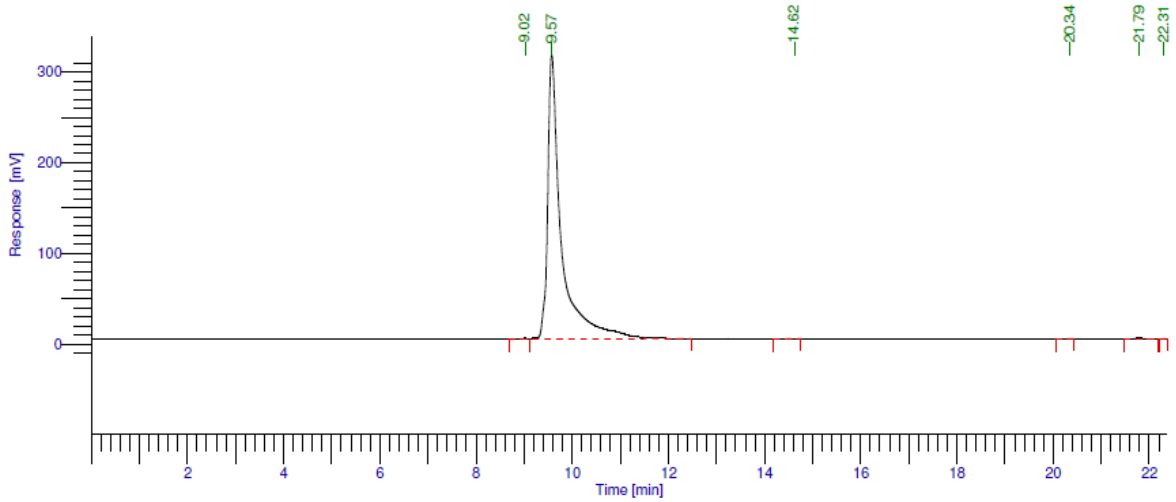
$[Tb.L^1]^{3+}$



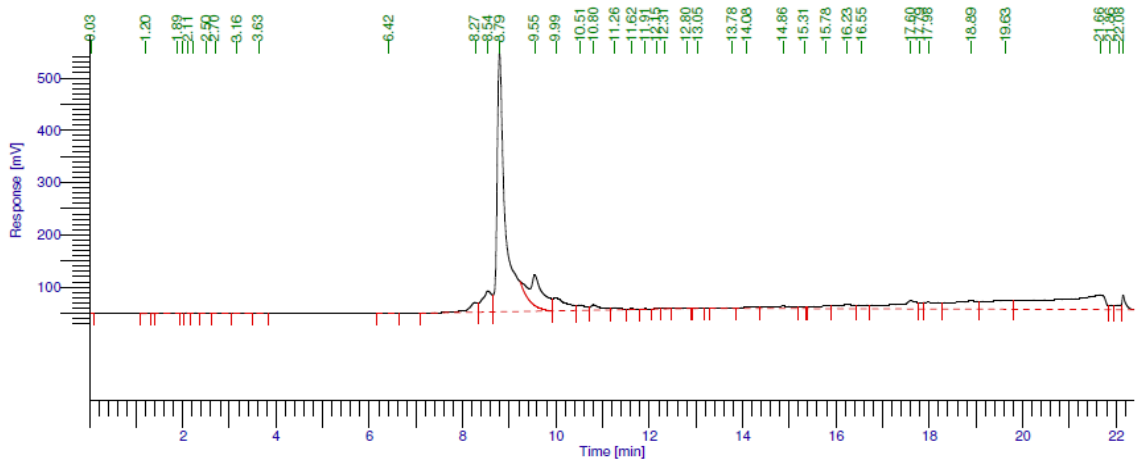
$[Eu.L^2]^{3+}$



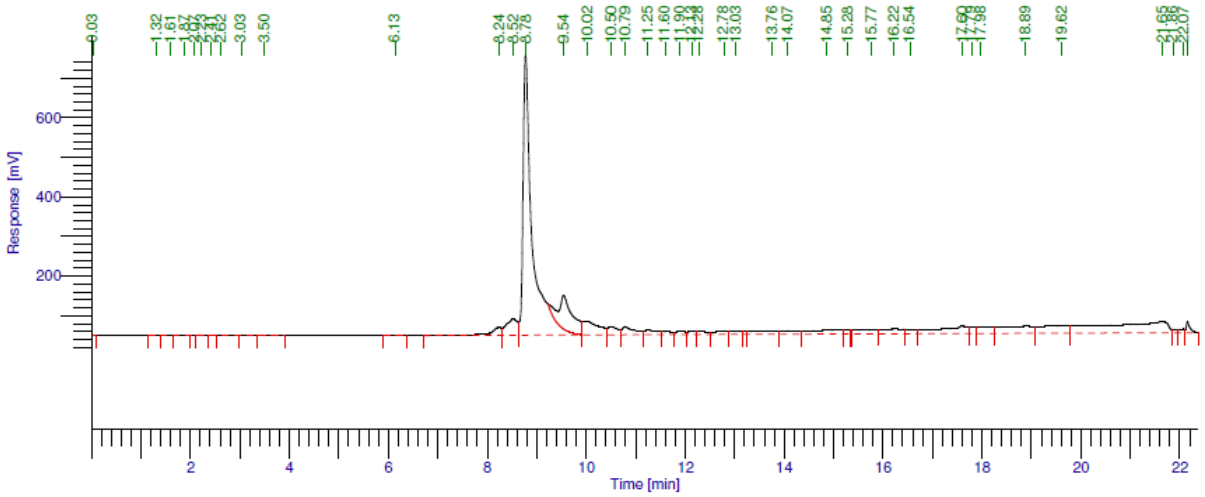
$[Tb.L^2]^{3+}$



$[Eu.L^3]$



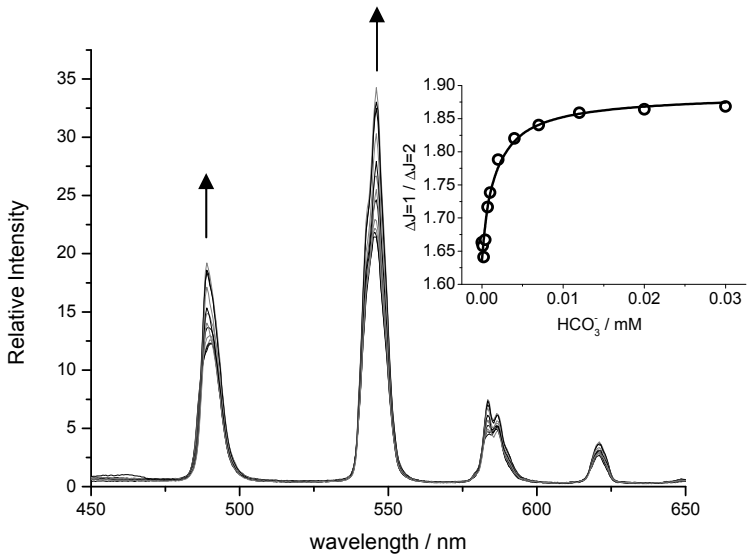
$[Tb.L^3]$

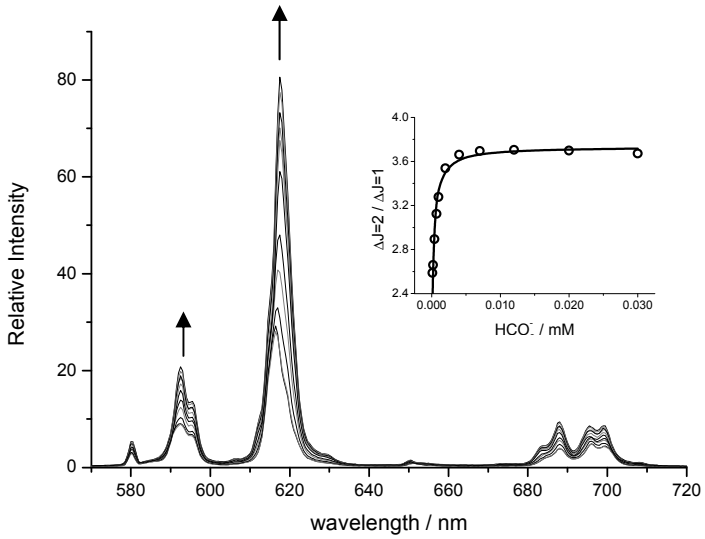


3.Examples of spectral titrations and data fitting.

Three representative spectral titrations (ESI Figures 2, 3 and 4) and associated binding isotherms are shown. Data were obtained and analysed as described in references 8 and 11.

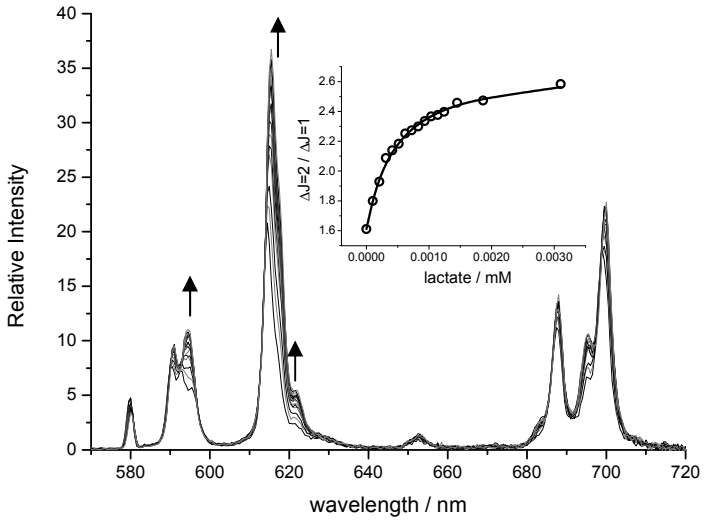
Tb emission spectra for $[Tb.L^1]^{3+}$(pH 7.4, 298K, λ_{ex}=332 nm, [complex] = 20 μM) as a function of sodium bicarbonate concentration displaying (*insert*) the binding isotherm determined using emission area intensity ratios (ΔJ = 1 *vs.* ΔJ = 2 bands) , showing the fit to the data for log K = 2.90(0.03).





Eu emission spectra for $[Eu.L^2]^{3+}$(pH 7.4, 298K, λ_{ex}=332 nm, [complex] = 20 μM) as a function of sodium bicarbonate concentration, displaying (*insert*) the binding isotherm determined using emission area intensity ratios (ΔJ = 2 *vs.* ΔJ = 1 bands, showing the fit to the data for log K = 3.62(0.07)).

Eu emission spectra for $[Eu.L^1]^{3+}$(pH 6, 298K, λ_{ex}=332 nm, [complex] = 20 μM) as a function of sodium lactate concentration, displaying (*insert*) the binding curve obtained by monitoring band intensity ratios (ΔJ = 2 *vs.* ΔJ = 1) as a function of added lactate , showing the fit to the data for log K = 3.35(0.07)).



ESI Table Selected photophysical data[a] for lanthanide (III) complexes of L^1, L^2 and L^3 (H_2O, 298 K, λ_{exc} 332 nm)

Complex	$k_{H_2O}/(ms)^{-1}$	$k_{D_2O}/(ms)^{-1}$	q
$[Eu.L^1]Cl_3$[b]	3.85	1.56	2.18
$[Tb.L^1]Cl_3$	0.87	0.40	2.05
$[Eu.L^2]Cl_3$[c]	3.85	1.73	1.99
$[Tb.L^2]Cl_3$[d]	0.90	0.41	2.15
$[Eu.L^3]$	3.86	1.48	2.30
$[Tb.L^3]$	0.90	0.39	2.25

[a] For each complex, λ_{max} = 332 nm (ε = 4,300 (± 200) M^{-1} cm^{-1}); k values have an error of ±10%, and q values are reported ± 20%.

[b] In the presence of added HSA, k_{H_2O} = 2.63 $(ms)^{-1}$ k_{D_2O} = 1.85 $(ms)^{-1}$ and q = 0.35, consistent with water displacement.

[c] In the presence of added HSA, k_{H_2O} = 2.58 $(ms)^{-1}$ k_{D_2O} = 1.63 $(ms)^{-1}$ and q = 0.59.

[d] In the presence of added HSA, k_{H_2O} = 0.73 $(ms)^{-1}$ k_{D_2O} = 0.55 $(ms)^{-1}$ and q = 0.60