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Effective and efficient sensitisation of terbium luminescence at 355 nm with cell permeable pyrazoyl-1-azaxanthone macrocyclic complexes

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1. *Ligand and Complex Synthesis*

Analytical reverse phase HPLC analyses were performed at 295K on a Perkin Elmer system using a 150 x 4.66 mm 4 micron Phenomenex Synergi 4m Fusion-RP 80i column operating at 1 ml min^{-1} . . Each analysis was performed using a water (+0.1% formic acid)/MeCN (+0.1% formic acid) solvent system going from 100% aqueous elutant at t = 0 to 100% MeCN at t = 16 mins.

2-4'-*tert*-Butylphenoxynicotinic acid

Sodium (1.02 g, 44.37 mmol) was added in small pieces to *dry* MeOH (25 cm^3). Once hydrogen evolution had ceased, 2-chloronicotinic acid (3.31 g, 21.01 mmol) and 4-*tert*-butylphenol (15.20 g, 101.18 mmol) were added to form a thick cream coloured solution. The MeOH was removed under reduced pressure to leave a cream residue which was heated for 20 h at 190 °C with stirring. After cooling, the coloured gum was treated with H_2O (200 cm^3) and washed successively with Et_2O (2 x 150 cm^3). The aqueous solution was acidified to pH 5 by the addition of acetic acid to afford a fine precipitate. The precipitate was filtered, washed with water and dried under vacuum to yield the *title compound* as a white fine crystalline solid (4.89 g, 18.02 mmol, 86%), m.p. 185-186*C. δ_H ($CDCl_3$, 500 MHz) 1.37 (9H, s, ^{t}Bu), 7.14 (2H, d, J 8.5, $H^{2'}$), 7.20 (1H, dd, J 7.5; 5, H^2), 7.49 (2H, d, J 9, $H^{3'}$), 8.35 (1H, dd, J 4.5; 2, H^1), 8.55 (1H, dd, J 8; 2, H^3). δ_C ($CDCl_3$, 125 MHz) 31.7 ($C^{6'}$), 34.8 ($C^{5'}$), 113.5 (C^4), 119.7 (C^2), 121.4 ($C^{2'}$), 127.1 ($C^{3'}$), 143.8 (C^3), 149.3 ($C^{4'}$), 149.8 ($C^{1'}$), 152.4 (C^1),

161.5 (C^5), 164.9 ($C{=}O_{(acid)}$). *m/z* (ES^-) 270.1 (100%, M – H). Found: C, 70.54; H, 6.20; N, 4.91%; $C_{16}H_{17}NO_3$ requires C, 70.83; H, 6.32; N, 5.16%.

7-*tert*-Butyl-1-azaxanthone, 2a

Polyphosphoric acid (90 g) was added to 2-4'-*tert*–butylphenoxynicotinic acid (2.15 g, 7.93 mmol) and the mixture heated at 120 °C for 16 h. The light brown mixture was allowed to cool slightly before being poured onto ice water (400 cm^3) to afford a pale yellow solution. The pH of the solution was then adjusted to neutral pH 7 by the careful addition of CONC. $NaOH_{(aq)}$. The solution was extracted with Et_2O (3 x 300 cm^3), the organic phases combined, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to afford 7-*tert*-butyl-1-azaxanthone as a cream coloured solid (1.79 g, 7.08 mmol, 89%), m.p. 106-107°C. δ_H ($CDCl_3$, 500 MHz) 1.42 (9H, s, ^{t}Bu), 7.45 (1H, dd, J 7.5; 4.5, H^2), 7.58 (1H, d, J 8.5, H^{10}), 7.86 (1H, dd, J 9; 3, H^9), 8.30 (1H, d, J 2.5, H^7), 8.73-8.76 (2H, H^1/H^3). δ_C ($CDCl_3$, 125 MHz) 31.6 (C^{14}), 35.1 (C^{13}), 117.0 (C^4), 118.4 (C^{10}), 121.1 (C^2), 121.1, 122.7 (C^7), 133.9 (C^9), 137.6, 148.2 (C^6), 154.1, 154.3(C^{12}), 160.6 (C^{11}), 178.1 (C^5). *m/z* (ES^+) 529.5 (100%, 2M + Na), 782.3 (70%, 3M + Na), 275.8 (25%, M + Na). Found: C, 75.80; H, 5.91; N, 5.61%; $C_{16}H_{15}NO_2$ requires C, 75.87; H, 5.97; N, 5.53%.

7-*tert*-Butyl-N-methyl-1-azaxanthonium trifluoromethylsulfonate

7-*tert*-Butyl-1-azaxanthone (1.00 g, 3.95 mmol) was dissolved in *dry* toluene (20 cm^3) under an atmosphere of argon. The resultant yellow solution was then cooled in an ice bath to approximately 0 ^oC. An excess of methyl trifluoromethanesulfonate (6 cm^3, 8.70 g, 53.02 mmol) was then carefully added to the cooled solution in a dropwise fashion. Almost instantaneously a pale cream precipitate formed in a faint yellow coloured solute. The precipitate was filtered and dried under vacuum to afford the *title compound* as a white solid (1.49 g, 3.58 mmol, 91%). δ_H (CD_3OD, 400 MHz) 1.43 (9H, s, ^{t}Bu), 4.51 (3H, s, Me), 7.84 (1H, d, J 8.8, H^{10}), 7.99 (1H, dd, J 8; 6, H^2), 8.15 (1H, dd, J 8.8; 2.4, H^9), 8.33 (1H, d, J 2.4, H^7), 9.14 (1H, dd, J 6; 2, H^1), 9.30 (1H, dd, J 8; 2, H^3). δ_C (CD_3OD, 100 MHz) 30.3 (C^{14}), 34.8 (C^{13}), 41.7 (CH_3), 118.2 (C^{10}), 120.4 (C^4), 120.8 (C^6), 121.2 (C^2), 122.6 (C^7), 135.4 (C^9), 145.9 (C^3), 149.1 (C^1),

151.1 (C^8), 152.4 (C^{11}), 156.3 (C^{12}), 173.8 (C^5). δ_F (CD_3OD, 188 MHz) – 80.5 (CF_3). *m/z* (ES^+) 268.2 (100%, M).

7-*tert*-Butyl-N-methyl-1-azaxanthonium chloride

δ_H (CD_3OD, 500 MHz) 1.46 (9H, s, ^tBu), 4.55 (3H, s, Me), 7.88 (1H, d, J 9, H^{10}), 8.03 (1H, t, J 6.5, H^2), 8.18 (1H, dd, J 9; 2, H^9), 8.36 (1H, d, J 2, H^7), 9.22 (1H, d, J 6.5, H^1), 9.33 (1H, d, J 7.5, H^3). δ_C (CD_3OD, 125 MHz) 30.4 (C^{14}), 34.8 (C^{13}), 41.8 (CH_3), 118.2 (C^{10}), 120.5 (C^4), 120.8 (C^6), 121.2 (C^2), 122.6 (C^7), 135.4 (C^9), 145.9 (C^3), 149.1 (C^1), 151.1 (C^8), 152.4 (C^{11}), 156.3 (C^{12}), 173.8 (C^5).

6-*tert*-Butyl-1-methyl-1H-9-oxa-1-aza-anthracene-2,10-dione

7-*tert*-Butyl-N-methyl-1-azaxanthonium chloride (0.36 g, 1.18 mmol) dissolved in H_2O (10 cm^3) was added dropwise to a solution of potassium hexacyanoferrate (III) (1.16 g, 3.54 mmol) in H_2O (6 cm^3). The solution was cooled to approximately 0 ^oC and a solution of NaOH (0.85 g, 21.24 mmol) in H_2O (10 cm^3) added to the reaction mixture over a period of 20 min. The solution was stirred at approximately 0 ^oC for 24 h. The solution was acidified to pH 3 by the addition of sulphuric acid to afford a green precipitate. The material was filtered, dissolved in $CHCl_3$ (50 cm^3) and partitioned with H_2O (2 x 50 cm^3). The organic phases were separated, dried over $MgSO_4$ and the solvent removed under reduced pressure to yield the *title compound* as a red solid (0.25 g, 0.87 mmol, 74%), m.p. 237-238 °C. δ_H ($CDCl_3$, 500 MHz) 1.41 (9H, s, ^tBu), 3.76 (3H, s, Me), 6.54 (1H, d, J 9.5, H^2), 7.47 (1H, d, J 8.5, H^{10}), 7.79 (1H, dd, J 9; 2, H^9), 8.21 (1H, d, J 9.5, H^3), 8.29 (1H, d, J 2, H^7). δ_C ($CDCl_3$, 125 MHz) 28.5 (CH_3), 31.6 (C^{14}), 35.2 (C^{13}), 102.8 (C^4), 116.0 (C^2), 117.3 (C^{10}), 121.6 (C^6), 122.9 (C^7), 132.3 (C^9), 135.7 (C^3), 149.7 (C^8), 152.0 (C^{11}), 156.5 (C^{12}), 162.3 (C^1), 174.2 (C^5). *m/z* (ES^+) 284.3 (100%, M + H). HRMS (ES^+) 284.1281; $C_{17}H_{18}O_3N_1$ requires 284.1281, $[M + H]^+$. Found: C, 71.82; H, 5.91; N, 4.90%; $C_{17}H_{17}NO_3$ requires C, 72.07; H, 6.05; N, 4.94%.

7-*tert*-Butyl-2-chloro-1-azaxanthone, 2b

N,*N*-Dimethylaniline (0.3 cm^3) was added to a solution of 6-*tert*-butyl-1-methyl-1H-9-oxa-1-aza-anthracene-2,10-dione (0.18 g, 0.63 mmol) in $POCl_3$ (10 cm^3) and the solution heated at reflux for 24 h. The solvent was removed under reduced pressure to yield a dark green residual solid. The residue was treated with H_2O (100 cm^3) and the aqueous phase extracted with CH_2Cl_2 (2 x 50 cm^3). The combined organic phases were washed with aqueous K_2CO_3 (0.1 M, 100 cm^3), dried over K_2CO_3, filtered and the filtrate concentrated under reduced pressure. The residue purified by chromatography on silica (gradient elution: Hexane to 10% EtOAc/Hexane, R_F = 0.33, 10% EtOAc/Hexane) to yield the *title compound* as a pink solid (0.09g, 0.31 mmol, 49%), m.p. 127-129°C. δ_H ($CDCl_3$, 500 MHz) 1.41 (9H, s, ^{t}Bu), 7.43 (1H, d, J 8, H^2), 7.54 (1H, d, J 9, H^{10}), 7.86 (1H, dd, J 9; 2.5, H^9), 8.27 (1H, d, J 2.5, H^7), 8.65 (1H, d, J 8, H^3). δ_C ($CDCl_3$, 125 MHz) 31.5 (C^{14}), 35.1 (C^{13}), 115.6 (C^4), 118.4 (C^{10}), 121.1 (C^6), 121.9 (C^2), 122.7 (C^7), 134.1 (C^9), 139.9 (C^3), 148.8 (C^8), 153.8 (C^{11}), 155.6 (C^{12}), 159.7 (C^1), 177.2 (C^5).

7-*tert*-Butyl-2-(1'-3'-methylpyrazole)-1-azaxanthone, 1a

Sodium hydride (30 mg, 1.25 mmol) was added to a solution of 3-methylpyrazole (88 mg, 1.07 mmol) in *dry* THF (5 cm^3) under an atmosphere of argon. A solution of 7-*tert*-butyl-2-chloro-1-azaxanthone (280 mg, 0.97 mmol) in *dry* THF (5 cm^3) was then added to the reaction mixture, which was stirred at 65 ^{o}C for 16 h. The reaction mixture was allowed to cool to room temperature before water (~10 cm^3) was added to the reaction mixture. The precipitate was collected via centrifugation and the resultant solid triturated with a minimum volume of Et_2O. The solvent was decanted to yield the title compound as a white solid (290 mg, 0.87 mmol, 90%). δ_H ($CDCl_3$, 400 MHz) 1.39 (9H, s, ^{t}Bu), 2.37 (1H, s, Me), 6.31 (1H, d, J 2.8, $H^{2'}$), 7.51 (1H, d, J 8.8, H^{10}), 7.80 (1H, dd, J 8.8; 2.8, H^9), 7.99 (1H, d, J 8.4, H^2), 8.27 (1H, d, J 2.8, H^7), 8.51 (1H, d, J 2.8, $H^{1'}$), 8.72 (1H, d, J 8.4, H^3). δ_C ($CDCl_3$, 100 MHz) 14.2 (Me), 31.6 (C^{14}), 35.1 (C^{13}), 109.7 (C^2), 110.1 ($C^{2'}$), 113.9 (C^4), 118.1 (C^{10}), 121.4 (C^6), 122.7 (C^7), 129.0 ($C^{1'}$), 133.4 (C^9), 140.1 (C^3), 148.3 (C^8), 153.7 (C^{11}), 153.9 (C^{12}), 154.0 ($C^{3'}$), 160.0 (C^1), 177.0 (C^5). *m/z* (ES^+) 688.9 (100%, 2M + H), 334.3 (50%, M + H). HRMS (ES^+) 334.1551; $C_{20}H_{20}O_2N_3$ requires 334.1550, $[M + H]^+$.

2-(3'-Bromomethylpyrazole)-7-*tert*-butyl-1-azaxanthone, 1b

N-Bromosuccinimide (NBS) (113 mg, 0.64 mmol) and dibenzoyl peroxide (10 mg, 0.04 mmol) were added to a solution of 7-*tert*-butyl-2-(1'-3'-methylpyrazole)-1-azaxanthone (212 mg, 0.64 mmol) in CCl_4 (15 cm^3). The reaction mixture was heated at reflux under argon for 16 h. The reaction mixture was allowed to cool to room temperature, filtered and the solvent removed under reduced pressure to yield a yellow residue. The crude material was purified by chromatography on silica (100% CH_2Cl_2, RF = 0.28, 100% CH_2Cl_2) to yield the *title compound* as a white solid (148 mg, 0.36 mmol, 56%). δ_H ($CDCl_3$, 500 MHz) 1.42 (9H, s, ^{t}Bu), 4.56 (2H, s, CH_2Br), 6.62 (1H, d, J 2.5, $H^{2'}$), 7.56 (1H, d, J 9, H^{10}), 7.85 (1H, dd, J 9; 2.5, H^9), 8.06 (1H, d, J 8, H^2), 8.31 (1H, d, J 2.5, H^7), 8.63 (1H, d, J 2.5, $H^{1'}$), 8.80 (1H, d, J 8.5, H^3). δ_C ($CDCl_3$, 125 MHz) 24.6 (CH_2Br), 31.6 (C^{14}), 35.1 (C^{13}), 109.7 ($C^{2'}$), 110.0 (C^2), 114.6 (C^4), 118.1 (C^{10}), 121.4 (C^6), 122.8 (C^7), 129.8 ($C^{1'}$), 133.6 (C^9), 140.5 (C^3), 148.6 (C^8), 153.3 (C^{11}), 153.7 ($C^{3'}$), 153.9 (C^{12}), 159.9 (C^1), 177.0 (C^5). *m/z* (ES^+) 409.3 (100%, M + H), 846.6 (75%, 2M + H). HRMS (ES^+) 434.0475; $C_{20}H_{18}O_2N_3{}^{79}Br_1{}^{23}Na_1$ requires 434.0475, $[M + Na]^+$.

1-(7-*tert*-Butyl-2-(pyrazoylmethyl)-1-azaxanthone)-4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane

Caesium carbonate (50 mg, 0.153 mmol) was added to a solution of 1,4,7,10-tetraazacyclododecane1,4,7-triacetic acid (75 mg, 0.146 mmol) and 2-(1'-3'-bromomethyl pyrazole)-7-*tert*-butyl-1-azaxanthone (60 mg, 0.146 mmol) in *dry* MeCN (5 cm^3). The reaction mixture was heated at reflux under argon for 16 h. The solvent was removed under reduced pressure and CH_2Cl_2 (10 cm^3) added to the solid. The insoluble inorganic solid was removed by filtration and the filtrate concentrated under reduced pressure to yield a yellow oil. The crude material was purified by column chromatography on silica (gradient elution; CH_2Cl_2 to 8% MeOH – 92% CH_2Cl_2) to yield the title compound as a yellow oil (60 mg, 0.071 mmol, 49%). δ_H ($CDCl_3$, 500 MHz) 1.40 (H, s, tBu), 1.51 (H, s, tBu), 2.48-3.12 (22H, br m, Cyclen CH_2; 3 x CH_2), 6.57 (1H, d, J 2.5, $H^{2'}$), 7.56 (1H, d, J 8.5, H^{10}), 7.86 (1H, dd, J 9; 2.5, H^9), 8.23 (1H, d, J 8.5, H^2), 8.27 (1H, d, J 2.5, H^7), 8.57 (1H, d, J 2.5, $H^{1'}$), 8.62 (1H, d, J 8.5, H^3). δ_C ($CDCl_3$, 125 MHz) 28.3 (^{t}Bu), 31.5 (^{t}Bu), 35.1 (**C**^{t}Bu), 50.6, 51.8, 56.1 (CH_2), 56.7 (CH_2), 82.4 (**C**^{t}Bu), 110.8 (C^2), 110.9 ($C^{2'}$), 114.3, 118.2 (C^{10}), 121.1, 122.7 (C^7), 129.6 ($C^{1'}$), 133.9 (C^9), 140.1 (C^3), 148.7 (C^8), 153.6(C^{11}), 153.9

($C^{1'}$), 155.3 (C^{12}), 159.8 (C^{1}), 172.9 ($C{=}O_{ester}$), 176.9 (C^{5}) *m/z* (ES^{+}) 846.5 (100%, M + H).

1-(7-*tert*-Butyl-2-(pyrazoylmethyl)-1-azaxanthone)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane

A solution of 1-(7-*tert*-butyl-2-(1'-3'-dibromomethylpyrazole)-1-azaxanthone)-4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (45 mg, 0.053 mmol) in TFA (2 cm^3) and CH_2Cl_2 (2 cm^3) was stirred under argon at room temperature for 24 h. The solvents were removed under reduced pressure. The residue was repeatedly (3x) dissolved in CH_2Cl_2 (5 cm^3) and the solvent removed under reduced pressure to facilitate elimination of excess acid and *tert*-butyl alcohol. This procedure yielded the hydrolysed ligand as a pale yellow solid that was used directly for complexation with lanthanide ions.

TbDO3AAzaPyrazole7^{t}Bu: [TbL^{1}]

$TbCl_3.6H_2O$ (10 mg, 0.0276 mmol) was added to a solution of 1-(7-*tert*-butyl-2-(1'-3'-dibromomethylpyrazole)-1-azaxanthone)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (17 mg, 0.0251 mmol) in water (1 cm^3) and MeOH (1 cm^3). The pH of the solution was adjusted to 5.4 by the addition of 1M KOH solution. The reaction mixture was stirred under argon at 80^{o}C for 5 h. The pH dropped to 3.4 during this period and was consequently adjusted back to pH 5 by addition of 1M KOH. The reaction mixture was stirred at 80^{o}C for a further 16 h. The reaction mixture was allowed to cool to room temperature and the MeOH removed under reduced pressure. The pH of the remaining aqueous solution was raised to 10.0 using dilute KOH solution. The suspension was centrifuged before removing the solid precipitate by filtration. The pH of the aqueous solution was reduced to pH 5.5 by the addition of HCl and the solution freeze-dried to yield the terbium complex as a white solid. λ_{max} (H_2O) = 348 nm, τ(H_2O) 2.24 ms

Synthesis of L^{2a} and [TbL^{2a}]$^{3+}$

1-(7-*tert*-Butyl-2-(pyrazoylmethyl)-1-azaxanthone)-4,7,10-tetraazacyclododecane

1,4,7-tris-*tert*-butoxycarbonyl-1,4,7,10-tetraazacyclododecane (45 mg, 0.109 mmol), 2-(1'-3'-bromomethylpyrazole)-7-*tert*-butyl-1-azaxanthone (57 mg, 0.119 mmol), potassium carbonate (19 mg, 0.131 mmol) and a catalytic amount (2mgs) of KI were dissolved in a mixture of CH_3CN and DCM (5 cm^3, 1:1). The reaction mixture was heated at reflux under argon for 16 h. The mixture was allowed to cool to room temperature before the insoluble inorganic salts were removed by filtration. The filtrate was concentrated under reduced pressure to afford a residual oil, which was purified by chromatography on silica gel (gradient elution: CH_2Cl_2 to 3 % CH_3OH/CH_2Cl_2) to yield the title compound as a pale yellow solid (75 mg, 0.093 mmol, 85%). δ_H ($CDCl_3$, 500 MHz) 1.44 (36H, br s, 3 x ^{t}Bu; ^{t}Bu), 2.78 (4H, br s, Cyclen 2 x CH_2), 3.39 (8H, br, Cyclen 4 x CH_2), 3.59 (4H, s, Cyclen 2 x CH_2), 3.89 (2H, s, CH_2-PyAza), 6.44 (1H, d, J 3, $H^{2'}$), 7.56 (1H, d, J 8.5, H^{10}), 7.86 (1H, dd, J 9; 2.5, H^9), 8.04 (1H, d, J 8.5, H^2), 8.30 (1H, d, J 2.5, H^7), 8.60 (1H, d, J 2, $H^{1'}$), 8.78 (1H, d, J 8.5, H^3). δ_C ($CDCl_3$, 125 MHz) 28.7 (3 x ^{t}Bu), 31.5 (^{t}Bu), 35.1 (**C**^{t}Bu), 47.5, 47.9, 48.4, 50.1, 53.6, 54.0, 55.3 (All cyclen CH_2, CH_2-PyAza), 79.5, 109.9 (C^2), 110.8 ($C^{2'}$), 114.2, 118.1 (C^{10}), 121.4, 122.8 (C^7), 129.0 ($C^{1'}$), 133.6 (C^9), 140.3 (C^3), 148.5, 152.7, 153.6, 153.9, 155.6, 155.9, 159.9, 177.0 (C^5).

1-(7-*tert*-Butyl-2-(pyrazoylmethyl)-1-azaxanthone)-1,4,7,10-tetraazacyclododecane

A solution of 1-(7-*tert*-butyl-2-(pyrazoylmethyl)-1-azaxanthone)-4,7,10-tetraazacyclododecane (75 mg, 0.093 mmol) in TFA (2 cm^3) and CH_2Cl_2 (2 cm^3) was stirred at room temperature for 24 h. The solvents were removed under reduced pressure and the resulting residue repeatedly (3x) dissolved in CH_2Cl_2 (5 cm^3) and concentrated under vacuum to facilitate elimination of excess acid and *tert*-butyl alcohol. The residue was finally taken into a 1 M KOH solution (5 cm^3) and extracted with CH_2Cl_2 (3 x 5 cm^3). The organic layer was dried over K_2CO_3, filtered and the filtrate concentrated under reduced pressure to yield the de-protected ligand as a pale orange solid (40 mg, 0.079 mmol, 85%).

1-(7-*tert*-Butyl-2-(pyrazoylmethyl)-1-azaxanthone)-4,7,10-tris[(S)-1-(1-phenyl)ethylcarbamoylmethyl]-1,4,7,10-tetraazacyclododecane, L^{2a}

1-(7-*tert*-Butyl-2-(1'-3'-dibromomethylpyrazole)-1-azaxanthone)-1,4,7,10-tetraazacyclo dodecane (29 mg, 0.057 mmol), 2-chloro-*N*-[(*S*)-

methylbenzyl]ethanamide (32 mg, 0.181 mmol), potassium carbonate (27 mg, 0.192 mmol) a catalytic amount of KI were dissolved in *dry* MeCN (5 cm^3) and heated at reflux under argon for 16 h. The reaction mixture was allowed to cool to room temperature before removing the inorganic salts by filtration. The filtrate was concentrated under reduced pressure to yield a residual oil. The residue was purified by chromatography on neutral alumina (gradient elution: CH_2Cl_2 to 1 % CH_3OH/CH_2Cl_2) to yield the title compound as a pale yellow solid (30 mg, 0.030 mmol, 53%). δ_H ($CDCl_3$, 500 MHz) 1.41 (9H, s, ^{t}Bu), 1.52 (9H, br, 3 x Me), 2.48-3.12 (20H, br m, Cyclen CH_2, 2 x CH_2), 3.79 (2H, s, CH_2), 4.01 (2H, s, CH_2-PyAza), 5.01 (2H, m, 2 x CH), 5.07 (H, m, CH), .78 (4H, br s, Cyclen 2 x CH_2), 3.39 (8H, br, Cyclen 4 x CH_2), 3.59 (4H, s, Cyclen 2 x CH_2), 3.89 (2H, s, CH_2-PyAza), 6.38 (1H, d, J 3, $H^{2'}$), 7.10-7.40 (15H, br m, 3 x Ph), 7.58 (1H, d, J 8.5, H^{10}), 7.86 (1H, dd, J 9; 2.5, H^9), 7.98 (1H, d, J 8.5, H^2), 8.11 (1H, d, J 2.5, H^7), 8.60 (1H, d, J 2, $H^{1'}$), 8.78 (1H, d, J 8.5, H^3). *m/z* (ES^+) 988.4 (100%, M + H).

$[TbL^{2a}](CF_3SO_3)_3$

A solution of 1-(7-*tert*-butyl-2-(1'-3'-dibromomethylpyrazole)-1-azaxanthone)-4,7,10-tris[(S)-1-(1-phenyl)ethylcarbamoylmethyl]-1,4,7,10-tetraazacyclododecane (16 mg, 0.016 mmol) and $Tb(OTf)_3$ (11.7 mg, 0.019 mmol) in *dry* CH_3CN (1 cm^3) was heated at reflux under argon for 16 h. The solution was then dropped onto Et_2O (~ 20 cm^3) to yield a solid precipitate. The solid was isolated by centrifugation and the solvent decanted. The solid was re-dissolved in CH_3CN and the process repeated to yield an off-white solid product (13 mg, 0.011 mmol, 69 %). λ_{max} (H_2O) = 348 nm, τ (H_2O) 2.00 ms

This complex was converted to the more water soluble chloride salt by ion exchange chromatography in water using a DOWEX 1X8 200-400 MESH Cl resin.

L^{2b} and $[TbL^{2b}]^{3+}$: a complex suitable for conjugation

(SS)-1-(7-*tert*-Butyl-2-(pyrazoylmethyl)-1-azaxanthone)-4,10-(1-(1-phenyl)ethylcarbamoylmethyl)-1,4,7,10-tetrazacyclododecane

N-((*S*)-1-Phenyl-ethyl)-2-(7-[((*S*)-1-phenyl-ethylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl)-acetamide (104 mg, 0.211 mmol), 2-(bromomethylpyrazole)-7-*tert*-butyl-1-azaxanthone (87 mg, 0.211 mmol) and $NaHCO_3$ (20 mg, 0.231 mmol) were dissolved in *dry* MeCN (5 ml) and heated at 60 ^oC for 18 h.

The reaction mixture was allowed to cool to room temperature before removing the inorganic salts by syringe filtration. The filtrate was concentrated under reduced pressure to afford a crude solid. The crude material was purified by chromatography on neutral alumina (gradient elution: CH_2Cl_2 to 0.5 % CH_3OH/CH_2Cl_2) to yield the *title compound* as a pale cream coloured solid (124 mg, 0.150 mmol, 71 %). m.p. 152-154 ^oC. δ_H ($CDCl_3$, 500 MHz) 1.40 (9H, s, ^{t}Bu), 1.47 (6H, d, J 7, 2 x Me), 2.58 (4H, br s, cyclen 2 x CH_2), 2.79 (4H, br s, cyclen 2 x CH_2), 2.91 (8H, br s, cyclen 4 x CH_2), 3.36 (4H, s, **CH_2**C(O)), 3.71 (2H, s, CH_2-PyAza), 5.07 (2H, q, J 14; 7.5, 2 x CH), 6.31 (1H, d, J 2.5, $H^{2'}$), 7.13 (2H, t, J 7.5, Ph), 7.21 (4H, t, J 7.5, Ph), 7.35 (4H, d, J 7.5, Ph), 7.54 (1H, d, J 9, H^{10}), 7.84 (1H, dd, J 8.5; 2.5, H^9), 7.91 (2H, br s, 2 x NH), 7.98 (1H, d, J 8.5, H^2), 8.29 (1H, d, J 2.5, H^7), 8.55 (1H, d, J 2.5, $H^{1'}$), 8.76 (1H, d, J 8.5, H^3). δ_C ($CDCl_3$, 125 MHz) 15.5, 22.2 (2C, Me), 31.6 (3C, ^{t}Bu), 35.1, 46.5, 49.4 (2C, CH), 50.9 (cyclen CH_2), 52.3 (cyclen CH_2), 52.7 (Cyclen CH_2), 60.6 (2C, **CH_2**CO), 66.1, 110.0, 111.0, 114.4, 118.1, 121.3, 122.8, 126.0, 126.8, 127.0, 127.3, 128.5, 128.7, 128.8, 129.1, 133.7, 140.5, 144.0, 148.6, 153.3, 153.9, 160.0, 170.2, 177.0. MS (ES^+) *m/z* 826.0 (100 %, $[M + H]^+$).

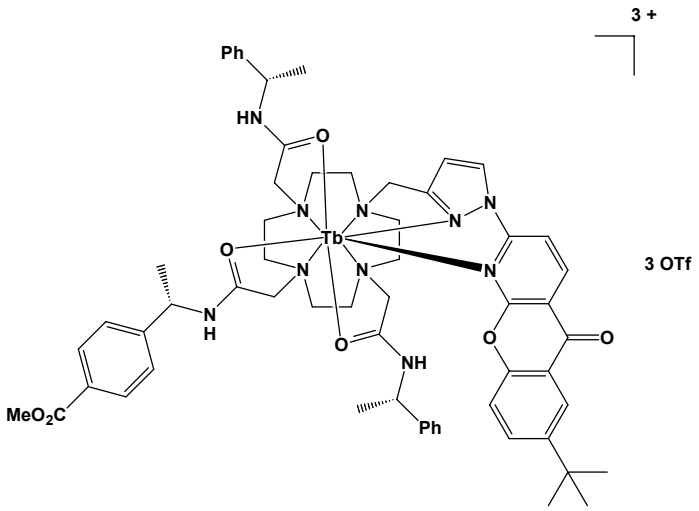
Methyl 4-[(S)-1-(2-{7-[1-(6-*tert*-Butyl-10-oxo-10*H*-9-oxa-1-aza-anthracen-2-yl)-1*H*-pyrazol-3-ylmethyl]-4,10-bis-[((S)-1-phenyl-ethylcarbamoyl)-methyl]-1,4,7,10tetraaza-cyclododec-1-yl}-acetylamino)-ethyl]-benzoate

1-(7-*tert*-Butyl-2-(1'-3'-dibromomethylpyrazole)-1-azaxanthone)-4,10-(1-(1-phenyl) ethylcarbamoylmethyl)-1,4,7,10-tetrazacyclododecane (97 mg, 0.118 mmol), methyl [*N*-2-(chloroethanoyl)-4-(*S*)-(1-aminoethyl)]benzoate (33 mg, 0.130 mmol) and Cs_2CO_3 (48 mg, 0.147 mmol) were dissolved in *dry* MeCN (5 cm^3) and heated at reflux under argon for 16 h. The reaction mixture was allowed to cool to room temperature and the solvent removed under reduced pressure. The crude material was triturated with Et_2O and the solvent decanted. This procedure was repeated three

times to yield the *title compound* as a pale yellow semi-crystalline solid (97 mg, 0.094 mmol, 80%). (*S,S*)-1-(7-*tert*-Butyl-2-(pyrazoylmethyl)-1-azaxanthone)-4,10-(1-(1-phenyl)ethyl carbamoylmethyl)-1,4,7,10-tetraazacyclododecane (60 mg, 0.073 mmol), methyl [*N*-2-(chloroethanoyl)-4-(*S*)-(1-aminoethyl)]benzoate (21.3 mg, 0.084 mmol) and Cs_2CO_3 (31 mg, 0.095 mmol) were dissolved in *dry* MeCN (3 ml) and heated at reflux under argon for 16 h.

The reaction mixture was allowed to cool to room temperature before removing the inorganic salts by syringe filtration. The filtrate was concentrated under reduced pressure and dried under vacuum to afford a glassy solid. The crude material was sonicated in Et_2O (10 ml) to yield a fine pale yellow precipitate which was isolated via centrifugation. The material was sonicated in Et_2O and centrifuged twice more to yield the *title compound* as a free flowing cream coloured solid (60 mg, 0.058 mmol, 79 %). m.p. 187-189 ^oC. δ_H ($CDCl_3$, 200 MHz) 1.41 (12H. s, ^{t}Bu; 1 x Me), 1.45 (6H, d, J 7, 2 x Me), 2.57 (16H, br, cyclen 8 x CH_2), 2.86 (2H, br s, CH_2CO), 2.99 (4H, br s, 2 x CH_2CO), 3.62-3.84 (5H, br, CH_2-PyAza; CO_2Me), 5.14 (3H, q, J 7, 3 x CH), 6.30 (1H, d, J 2.5, $H^{2'}$), 6.21-7.32 (12H, br, 2 x Ph; 2 x Ar), 7.47 (2H, d, J 8, 2 x Ar), 7.55 (1H, d, J 9, H^{10}), 7.82 (1H, dd, J 8; 2.5, H^9), 7.98 (1H, d, J 8, H^2), 8.31 (1H, d, J 2.5, H^7), 8.78 (1H, d, J 8, H^3). MS (ES^+) *m/z* 1067.7 (100 %, $[M + Na]^+$); HRMS (ES^+) *m/z* found 1045.5706 $[M + H]^+$ $C_{60}H_{73}N_{10}O_7$ requires 1045.5698 HPLC (t_R = 10.44 min)

$[TbL^{2b}]^{3+}$



4-[(S)-1-(2-{7-[1-(6-*tert*-Butyl-10-oxo-10*H*-9-oxa-1-aza-anthracen-2-yl)-1*H*-pyrazol-3-ylmethyl]-4,10-bis-[((S)-1-phenyl-ethylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl}-acetylamino)-ethyl]-benzoic acid methyl ester (10 mg, 0.010 mmol) and $Tb(OTf)_3$ (6.7 mg, 0.011 mol) were dissolved in dry MeCN (1 cm^3) and stirred at reflux under argon for 16 h. The solution was allowed to cool before being dropped onto Et_2O (~ 25 cm^3) to yield a solid precipitate. The solid was isolated by centrifugation and the solvent decanted. The solid was re-dissolved in CH_3CN and the process repeated to yield an off-white solid product (11.6 mg, 0.007 mmol, 74 %). λ_{max} (H_2O) = 348 nm; τ(H_2O) 2.24 ms. HPLC (t_R = 9.81 min).

This complex was converted to the more water soluble chloride salt by ion exchange chromatography in water using a DOWEX 1X8 200-400 MESH Cl resin.

2. ESI *Figure 1*

Circularly polarised luminescence spectra (I_L-I_R) for the Δ(*SSS*) and Λ(*RRR*) terbium(III) complexes of ligand $\mathbf{L^{2a}}$ (295K, pH 5.5) were measured using a home-built CPL spectrometer (Glasgow University) based on a Spex-2 spectrofluorimeter. Emission dissymmetry factors (g values) for the (*SSS*)-isomer are +0.02 (489nm), -0.13 (540nm), +0.01 (583nm), with the strongest CPL observed for the magnetic-dipole allowed ΔJ = -1 transitions around 540 nm. The sign and sequence of the

observed CPL is consistent with the (*SSS*)-[Tb.**L**^{2a}] complex possessing a Λ(δδδδ) configuration.