

ESI: The mechanism of quenching of the lanthanide excited state for optical probes using sensitised emission

Ga-Lai Law, David Parker, Siobhan L. Richardson and Ka-Leung Wong*

ESI

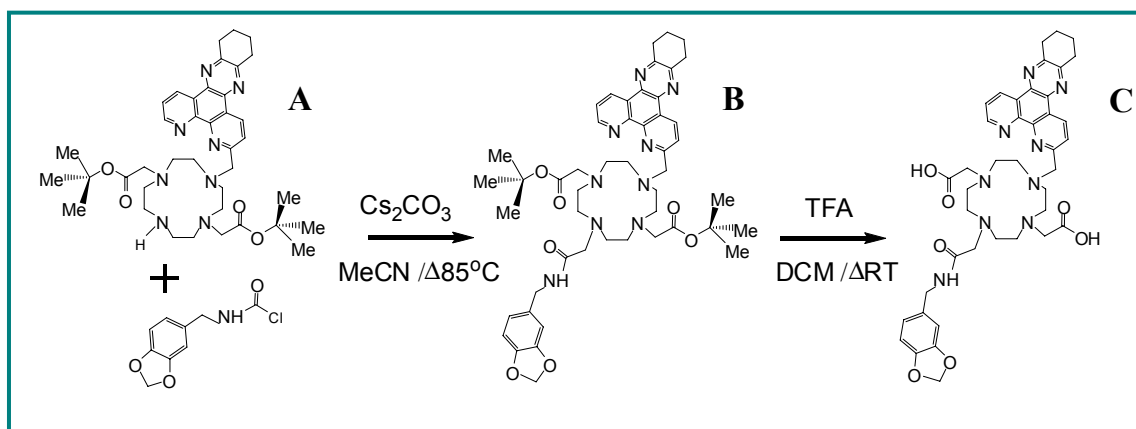
- 1. Ligand and Complex Synthesis**
- 2. Mass spectra for [Tb.L⁶]⁺ showing deuterium incorporation on photolysis in D₂O.**

2. Synthesis of L⁵ and L⁶ and their Eu and Tb complexes

N-Chloroethanoyl-3, 4-methylenedioxybenzylamine

3,4-Methylenedioxybenzylamine (0.13 ml, 1.04 mmol) and 1-(2-chloroacetyl)pyrrolidine-2,5 – dione (200 mg, 1.04 mmol) were dissolved in dry triethylamine (0.18 ml, 1.25 mmol) and THF (5 ml) and the solution stirred at room temperature for 2 h. . The thick white precipitate that formed was filtered and the solid washed with THF. The solvent was removed to yield a yellow oil which was treated with methanol (10 ml) to give a yellow solid. The solid was dissolved in chloroform (20 ml) and successively washed with water (pH 2, 7, 9), dried (K₂CO₃) , filtered and solvent removed to yield a pale yellow solid (170 mg, 0.75 mmol, 72%). R_f = 0.75 (5% MeOH:DCM). M.p: 113 -114 °

δ_H (CDCl₃) 4.09 (2H, s, CH₂Cl), 4.39 (2H, d, *J* 6.0, CH₂NH), 5.95 (2H, s, OCH₂O), 6.77 (3H, m, 3 x benzyl H); δ_C (CDCl₃, 125MHz) 42.7 (CH₂Cl), 43.8 (CH₂NH), 101.3 (OCH₂O); 108.5, 108.6, 121.4, 131.2, 147.4, 148.1 (aryl C), 165.8 (C=O); *m/z* (ESMS⁺) 228 (M + H, 15%), 250 (M + Na, 100%). Calc. For C₁₀H₁₀NO₃Cl : C:52.76; H:4.43 N: 6.15%. Found: C: 52.95, H:4.42, N:6.08%.



1-(3-Methyl-10,11,12,13-tetrahydrodipyrido[3,2-a:2',3'-c]phenazine)-4,10-bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (A)

1,7-Bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (150 mg, 0.37 mmol), NaHCO₃ (31 mg, 0.37 mmol) and 3-chloromethyl-10,11,12,13-tetrahydro-dipyrido[3,2-a:2',3'-c]phenazine (125 mg, 0.37 mmol) were stirred in dry acetonitrile (5 ml) at 70°C under argon monitoring for 100 h, monitoring reaction progress by 1-H NMR. The reaction mixture was filtered and solvent removed under reduced pressure, to yield a residue that was purified by column chromatography on silica, (DCM → 5% MeOH:DCM, R_f=0.20, 5% MeOH:DCM) to yield a pale yellow oil (170 mg, 0.64 mmol, 53%). δ_H (CDCl₃) 1.36 (18H, s, 2 x ^tBu), 2.09 (4H, m, 2 x CH₂), 2.68-3.06 (16H, m br, 8 x cyclen CH₂), 3.24 (4H, m, 2 x CH₂), 3.63 (4H, m, 4 x CH₂CO₂^tBu), 4.08 (2H, s, CH₂dpqC), 7.76 (1H, d, *J* 8.0, ArH), 7.90 (1H, dd, *J* 4.4, 8.0, ArH), 9.43 (1H, d, *J* 8.0, ArH), 9.51 (1H, dd, *J* 1.6, 8.0, ArH), 9.81 (1H, dd, *J* 1.6, 4.4, ArH); *m/z* (ESMS⁺) 669 (M + H, 100%). HRMS (ES⁺): found: 699.4338 (M + H); C₃₉H₅₅N₈O₄ requires 699.4341.

1-(3-Methyl-10,11,12,13-tetrahydrodipyrido[3,2-a:2',3'-c]phenazine)-7-(ethanoyl-3,4-methylenedioxybenzylamine)-4,10-bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (B)

1-(3-Methyl-10,11,12,13-tetrahydrodipyrido[3,2-a:2',3'-c]phenazine)-4,10-bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (25 mg, 0.036 mmol) was dissolved in dry acetonitrile (5 ml) with Cs₂CO₃ (20 mg, 0.057 mmol) and N-chloroethanoyl-3,4-methylenedioxybenzylamine (8 mg, 0.036 mmol) and the mixture was heated at 70°C (bath temperature) under argon for 100 h. The solvent was removed and the crude oil taken into chloroform and washed with water (pH 7, 9), and dried with K₂CO₃; removal of solvent yielded a pale brown oil that was used

directly in the next step (26 mg, 0.029 mmol, 81%). δ_{H} (CDCl_3) 1.43 (18H, s, 2 x ^tBu), 2.09 (4H, m), 2.68-3.06 (16H, m br, 8 x cyclen CH_2), 3.24 (4H, m), 3.63 (4H, m, 2 x $\text{CH}_2\text{CO}_2^t\text{Bu}$), 4.08 (2H, s, CH_2dpqC), 4.38 (2H, d, J 5.6, CH_2NH), 4.59 (2H, s, $\text{O}=\text{C}-\text{CH}_2$), 5.29 (2H, s, OCH_2O), 5.86-5.93 (3H, m, 3 x benzyl H), 7.72 (1H, dd, J 8.4, 4.0, ArH), 8.14 (1H, d, J 8.0, ArH), 9.24 (1H, dd, J 3.2, 0.8, ArH), 9.33 (1H, d, J 8.0, ArH), 9.45 (1H, dd, J 8.4, 0.8 ArH).

1-(3-Methyl-10,11,12,13-tetrahydrodipyrido[3,2-a:2',3'-c]phenazine)-7-(ethanoyl-3,4-methylenedioxybenzylamine)-4,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecane, L⁶

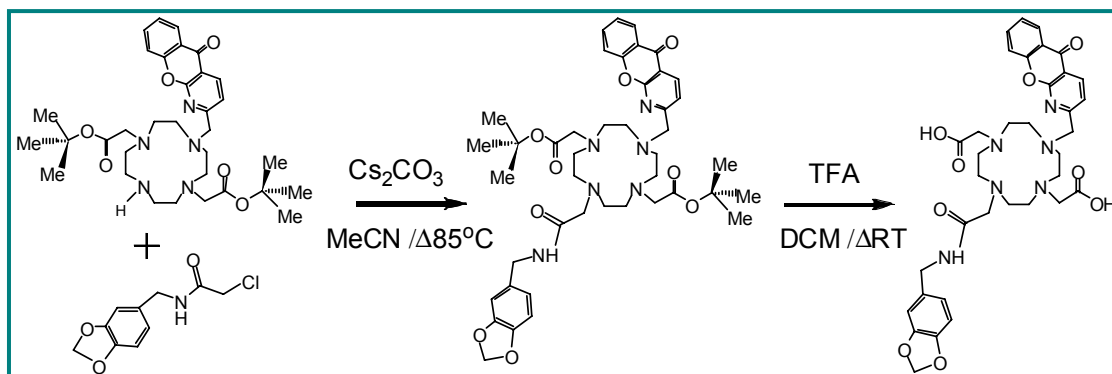
1-(3-Methyl-10,11,12,13-tetrahydrodipyrido[3,2-a:2',3'-c]phenazine)-7-(ethanoyl-3,4-methylenedioxybenzylamine)-4,10-bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (25 mg, 0.028 mmol) was stirred in TFA:DCM, 1:1 for 18 h. The solvents were removed and the crude product washed with DCM (3 x 5 cm^3) to yield the tris-trifluoroacetate salt of the title compound as a brown oil that was used directly in the following complexation reaction (20 mg, 0.026 mmol, 92%). δ_{H} (CDCl_3) 2.14 (4H, m, H10, H13), 3.29-3.75 (24H, m br, 8 x cyclen CH_2 , H11, H12, 2 x $\text{CH}_2\text{CO}_2\text{H}$, CH_2dpqC), 4.40 (2H, d, J 5.6, CH_2NH), 5.49 (2H, s, $\text{O}=\text{C}-\text{CH}_2$), 5.91 (2H, s, OCH_2O), 6.82-6.88 (3H, m, 3 x benzyl H), 8.30 (1H, dd, J 8.0, 5.4, ArH), 8.41 (1H, d, J 8.4, ArH), 9.30 (1H, dd, J 5.4, 0.8, ArH), 9.41 (1H, d, J 8.4, ArH), 9.98 (1H, dd, J 8.0, 0.8, ArH).

[Eu.L⁶]⁺

1-(3-Methyl-10,11,12,13-tetrahydrodipyrido[3,2-a:2',3'-c]phenazine)-7-(ethanoyl-3,4-methylenedioxybenzylamine)-4,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecane (10 mg, 0.013 mmol) and $\text{EuCl}_3 \cdot 3\text{H}_2\text{O}$ (4.7 mg, 0.013 mmol) were dissolved in $\text{MeOH}:\text{H}_2\text{O}$ (1:1, 3 cm^3). The pH of the solution was adjusted to 5.5 by addition of dilute aqueous KOH solution, and the mixture heated to 90°C for 24 h. The pH of the solution was adjusted to 10, by the addition of dilute aqueous KOH solution. The fine precipitate was removed via centrifugation and the water removed under reduced pressure. The pH was re-adjusted to pH 7 and the solution filtered using syringe filtration. Water was again removed under reduced pressure, yielding the crude complex as a white solid (10 mg, 0.011 mmol, 83%). A small sample of the formate salt was purified by reverse phase HPLC (Phenomenex Synergi 4 micron Fusion-RP80 using 100% water/0.1% formic acid to 100% MeCN /0.1% formic acid at 15 mins) m.p. > 250°C. m/z (ESMS⁺) 928 (M^+ , 100%), 926 (M^+ , 40%), 929 (M^+ , 30%). HRMS (ES⁺), found: 928.2646 (M^+); $\text{EuC}_{41}\text{H}_{45}\text{N}_9\text{O}_7^+$ requires 928.2649; λ_{ex} (H_2O): 348 nm; $\tau_{\text{H}_2\text{O}}$: 0.69 ms; $\tau_{\text{D}_2\text{O}}$: 1.01 ms; $\phi_{\text{H}_2\text{O}}$: 0.049; t_{R} (HPLC): 8.35 min

[Tb.L⁶⁺]⁺

This was prepared similarly and purified as the formate salt by reverse phase HPLC, m.p. > 250°C. *m/z* (ESMS⁺) 934 (M⁺, 100%), 935 (M⁺, 45%), 936 (M⁺, 10%), 937 (M⁺, 5%). HRMS (ES⁺), found: 934.2697 (M⁺); TbC₄₁H₄₅N₉O₇⁺ requires 934.2690; λ_{ex} (H₂O): 348 nm; τ_{H₂O}: 0.47 ms; τ_{D₂O}: 0.69 ms; t_R (HPLC): 8.30 min. .



1-(Methyl-1-azaxanthone)-7-(ethanoyl-3,4-methylenedioxybenzylamine)-4,10-bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane

1-(Methyl-1-azaxanthone)-4,10-bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (134 mg, 0.22 mmol) was dissolved in dry acetonitrile (5 ml) and Cs₂CO₃ added (143.4 mg, 0.44 mmol) followed by N-chloroethanoyl-3,4-methylenedioxybenzylamine (50 mg, 0.22 mmol) and the mixture heated at 85° (bath temperature) for 24 h. The mixture was filtered and solvent removed to give a dark brown oil that was purified using column chromatography on silica, (DCM:MeOH, 10:1) to afford a pale brown oil (142.5 mg, 0.178 mmol, 81%).

δ_H (CDCl₃, 200 MHz) 8.84 (1H, d, ArH), 8.60 (1H, d, J = 7.8, ArH), 8.19 (1H, dd, J = 8.0, 1.4 ArH), 7.58 (1H, t, J = 8.0, ArH), 7.43 (1H, d, ArH), 7.37 (1H, t, J = 8, 1.4, ArH), 6.83-6.61 (3H, m, ArH), 5.84 (2H, s, OCH₂O), 4.45 (2H, s, CH₂NH), 4.30 (2H, s, br, CH₂), 3.85 (2H, s, br, CH₂), 2.25-3.36 (20H, m, br, 8 x cyclen CH₂, 2 x CH₂), 1.17 (18H, s, br, CH₃). δ_C (CDCl₃) 177.40 (azaxanthone C=O), 171.87 (^tbut C=O), 164.62 (C=O), 160.29 (2C, ArO-C-N, ArC-O), 155.74 (azaxanthone, ArC-N), 147.71 (ArC), 146.46 (ArC), 138.24 (azaxanthone, ArC), 135.81 (azaxanthone, Ar C), 133.64 (ArC), 126.59 (azaxanthone, ArC-H), 124.95 (azaxanthone, Ar C-H), 121.63 (ArC), 120.90 (azaxanthone, Ar C-H), 118.96 (azaxanthone, Ar C-H), 115.50 (azaxanthone, ArC-H), 108.52 (ArC-H), 108.21 (ArC-H), 100.96 (OCH₂O), 82.27 (2C, ^tbut C),

59.9- 57.1 (3C, CH₂ and 8C, cyclen CH₂), 42.53(CH₂NH), 28.31 (6C, CH₃). *m/z* (ESI-MS⁺) (800), 801.0 [M + H]⁺; HRMS(ESI⁺) 801.4182; calc., C₄₃H₅₇O₉N₆, 801.4182 [M+H]⁺.

1-(Methyl-1-azaxanthone)-7-(ethanoyl-3,4-methylenedioxybenzylamine)-4,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecane, L⁵

1-(Methyl-1-azaxanthone)-7-(ethanoyl-3,4-methylenedioxybenzylamine)-4,10-bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (120 mg, 0.15 mmol) was stirred in TFA:DCM, (1:1) for 12 h.. The solvent was removed under reduced pressure to yield a dark oil. The crude material was repeatedly (x 3) dissolved in CH₂Cl₂ (5 ml) and solvent removed under reduced pressure to facilitate elimination of excess acid and *tert*-butyl alcohol. This yielded a pale brown oil of the tris-trifluoroacetate salt of L⁵ that was used directly in the complexation reaction (103 mg, 0.15 mmol, 100%).

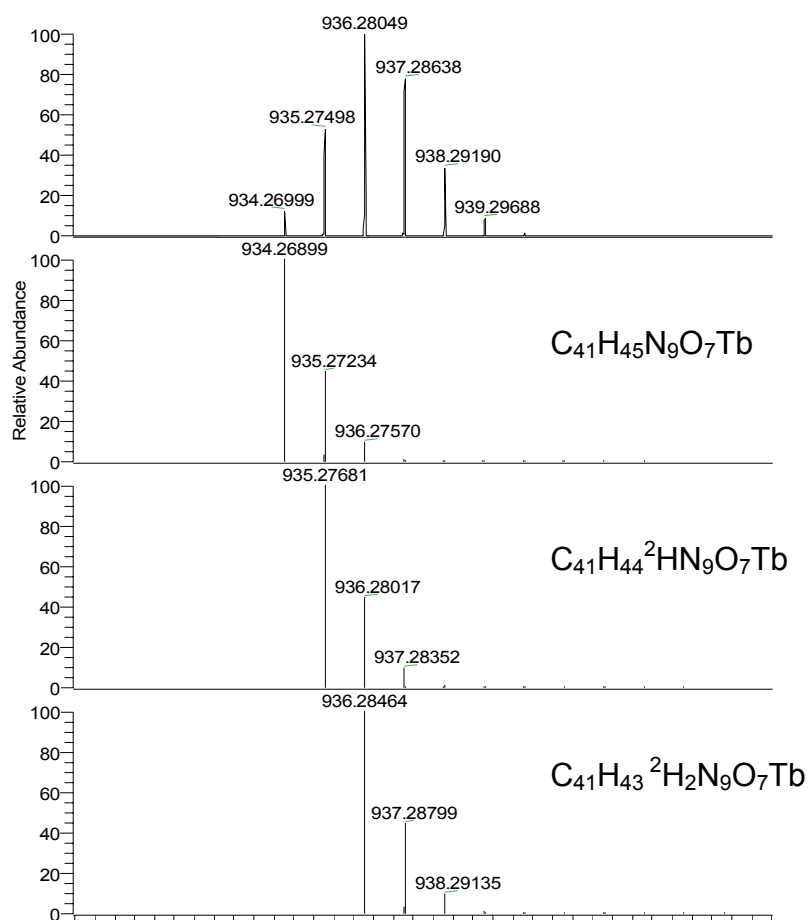
δ_{H} (CD₃OD, 400MHz) 8.56-8.64 (1H, d, br, ArH), 8.17 (1H, dd, J = 1.6, ArH), 7.87 (1H, t, J = 7.2, 1.6 ArH), 7.68 (1H, d, J = 8.0, ArH), 7.57-7.63 (1H, d, br, ArH), 7.45 (1H, t, J = 8, 1.2, ArH), 6.75-6.64 (3H, m, ArH), 5.82 (2H, s, OCH₂O), 4.64-4.84 (1H, s, br, NH), 4.19 (2H, s, CH₂NH), 3.96-4.02 (4H, m, br), 3.70-3.81 (4H, m, br), 3.10-3.66 (14H, m,br, 8 x cyclen CH₂) . δ_{C} (CD₃OD) 177.51(azaxanthone C=O), 160.46 (2C, C=O), 160.07 (NH-C=O), 159.86 (2C, ArO-C-N + ArC-O), 155.78 (ArC-N), 147.98 (ArC-O), 147.13 (Ar C-O), 138.31 (azaxanthone, ArC), 136.11 (azaxanthone, ArC), 131.87 (ArC), 126.09 (azaxanthone, ArC-H), 125.07 (azaxanthone, ArC-H), 121.36 (ArC-H), 121.15 (azaxanthone, ArC-H), 118.74 (azaxanthone, ArC-H), 117.57 (azaxanthone, ArC), 115.73 (azaxanthone, ArC-H), 114.70 (azaxanthone, ArC-H), 108.23(ArC-H), 107.94 (ArC-H), 101.18 (OCH₂O), 57.48 (4C, CH₂), 53.22 (8C, cyclen CH₂), 43.05 (CH₂NH).

[Tb.L⁵]⁺

L⁵ (50 mg, 0.073 mmol) and terbium acetate tri-hydrate (43 mg, 0.11 mmol) were dissolved in MeOH:H₂O (1:3ml). The pH of the solution was adjusted to 5.5 by addition of aqueous KOH solution, and the mixture heated to 90° for 24 h. The pH of the solution was adjusted to 10, by the addition of dilute aqueous KOH solution. The fine precipitate was removed via syringe filtration and the solution was re-adjusted to pH 7. Water was then removed on a freeze dryer, yielding the crude complex as a white solid (39 mg, 0.046 mmol, 64%). A small sample of the formate salt was purified by reverse phase HPLC as described above. *m/z* (ESIMS⁺) (845) 845 [M⁺] HRMS(ESI⁺) 845.1948: C₃₅H₃₈N₆O₉Tb⁺ requires 845.1946 [M⁺]; λ_{ex} (H₂O): 337 nm; τ_{H2O} : 1.65 ms; τ_{D2O} : 2.87 ms; ϕ = 24%; σ^2 = 4.7 GM; t_{R} (HPLC): 11.0 min. .

[Eu.L⁵]⁺

This was prepared and purified as the formate salt in a similar manner. m/z (ESIMS⁺) (839) 839 [M⁺]; λ_{ex} (H₂O): 337 nm; $\tau_{\text{H}_2\text{O}}$: 0.53 ms; $\tau_{\text{D}_2\text{O}}$: 1.78 ms; ϕ = 12%; t_{R} (HPLC): 11.0 min. .



2. ESI Figure 1

Mass spectra for [Tb.L⁶]⁺; *top*: observed isotope pattern after 10 min. irradiation (Xe lamp); *lower three*: calculated isotope patterns for [M]⁺; [(M-H) + ²H]⁺; and [(M-2H) + (²H)₂]⁺ .