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ESI

Responsive and reactive terbium complexes with an azaxanthone sensitiser and one naphthyl group: applications in ratiometric oxygen sensing in vitro and in regioselective cell killing

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1. **Confocal microscopy. Time-lapsed experiments and cell culture.**
2. **ESI Figures 1 and 2 showing oxygen and protein sensitivity in aqueous solution at pH 7.4.**
3. **Low temperature phosphorescence emission spectra for Gd complexes with ($[Gd.L^1]^{3+}$)and without ($[Gd.L^2]$) an integral naphthyl group.**
4. **Complex synthesis and characterisation.**

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1. Confocal Microscopy:

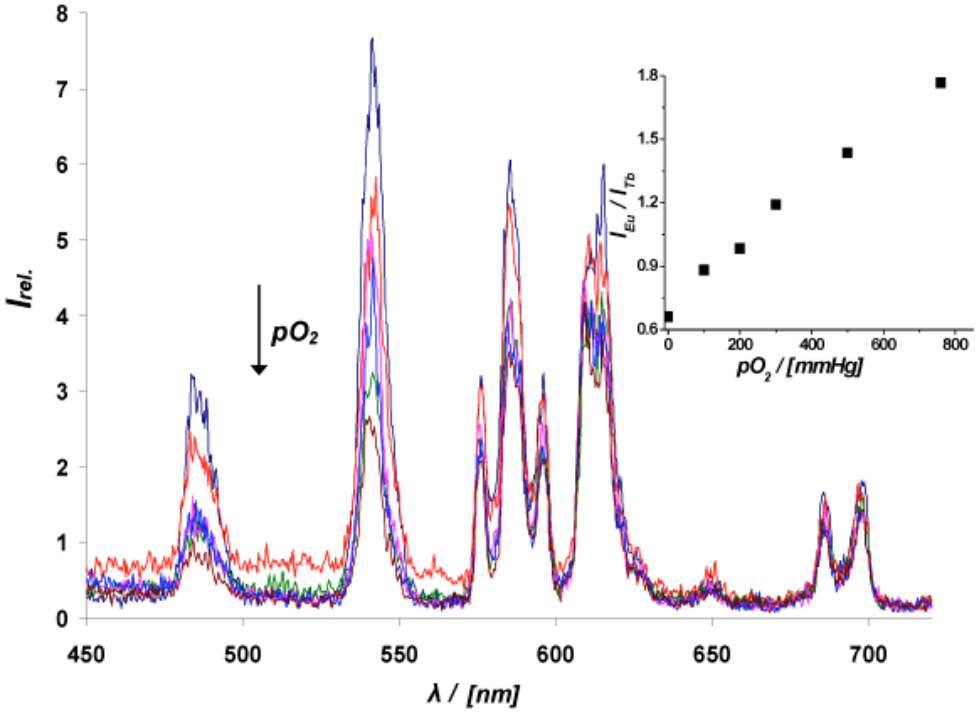
Experiments were carried using a Zeiss LSM 510, (upright configuration) laser scanning confocal microscope. The confocal microscope was equipped with various laser systems (Argon laser, HeNe laser and Ti:Sapphire laser) and a controllable CO_2 content stage-top tissue culture chamber. (2-7% CO_2, 37^oC)

Time lapse experiments: ~A 5 µmol solution of the lanthanide complex was set up in the cell culture dish with HeLa cells (~70% confluence) using 2 mL solution containing the growth medium. After incubation for 1h, direct excitation of the metal ion using an argon laser (λ_{ex} = 488 nm for Tb and λ_{ex} = 457 nm for Eu) was used to confirm the localization of the complexes in the HeLa cells. After SHG using a femtosecond-pulsed Ti:Sapphire laser (Libra II, Coherent, 76MHz) at 710 nm to generate 355 nm light, the sample was subjected to intermittent irradiation for ~30 min. (taking a snapshot each minute, duration time for each shot ~ 10 seconds, filter: 30% transmission/70% reflection). Light emission from the Tb/Eu complexes after UV excitation was monitored with the argon laser at 488 nm (BP filter: 500-565 nm) and for the Eu complexes with a HeNe laser at 400 nm. (LP filter 565 nm). Laser power used was 9.8 mW/cm^2. Control experiments were carried out by similar time-lapse experiments, using the laser lines at 488 nm and 457 nm of the argon laser, instead of the femto-second laser.

Cell culture: Human cervical carcinoma cells (HeLa) were maintained in DMEM medium supplemented with 10% foetal bovine serum and 1% penicillin/ streptomycin in 5% CO_2. Thirty hours prior to imaging, 0.5 x 10^6 cells were seeded onto 60 mm culture dishes (MatTek Corporation, MA, USA). The cells were allowed to attach overnight. The culture medium in each dish was changed prior to exposure to the lanthanide complexes.

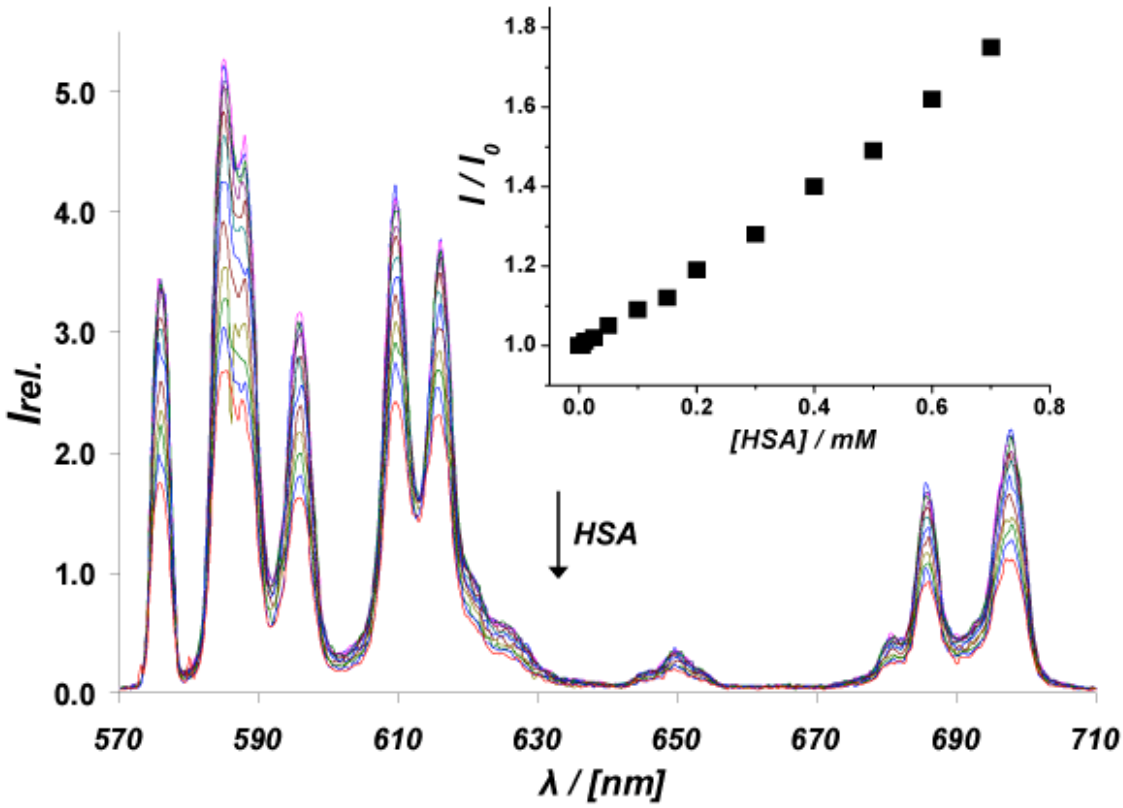
A stock solution of the lanthanide complex was made up (1 mg in 1 ml = ~1m M) and 10 µl of this solution added to 2mL of the growth medium, to give a final concentration of the lanthanide complex in the medium of ~5µM.

ESI Figure1



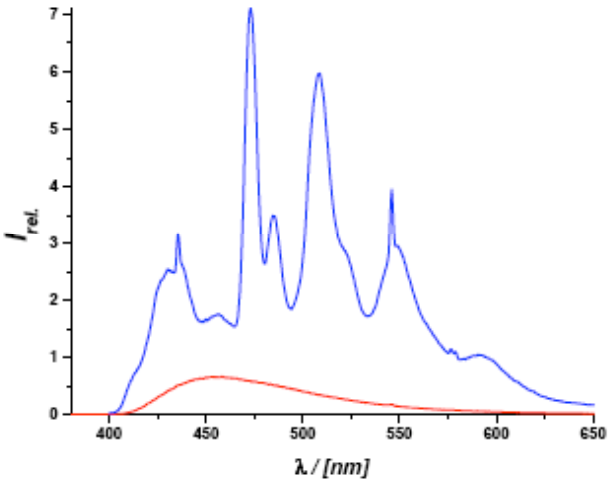
Variation of metal based emission as a function of oxygen partial pressure in solution (2, 55, 100, 160, 260, 360, 460 and 760 mmHg) (λ_{exc} 365 nm, 10µs gate, pH 7.4 (0.1M HEPES, 2: 1 ratio of Tb/Eu total concentration 45µM)

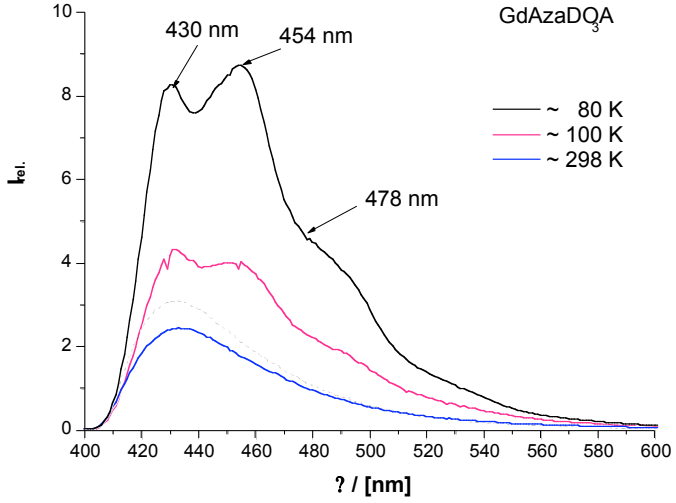
ESI Figure 2



Quenching of europium emission with added human serum albumin ((λ_{exc} 365 nm, 10µs gate, pH 7.4 (0.1M HEPES, [Eu] 25µM). No change in the form of Eu emission is apparent and no change in the metal-based lifetime was found, consistent with quenching of the intermediate sensitiser excited states only.

3. Variable temperature phosphorescence emission spectra for Gd complexes with (*upper*: $[Gd.L^1]]^{3+}$) and without (*lower*: $[Gd.L^2]$) an integral naphthyl group. In the upper Figure, the spectrum at 77K is in blue and the 295K spectrum is in red



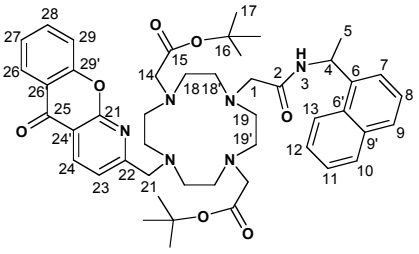


4. Ligand and Complex Synthesis and Characterisation

(*S*)-1,7-Bis(*tert*-butoxycarbonylmethyl)-4-(methylcarbamoylmethylnaphthalene)-1,4,7,10-tetraazacyclododecane

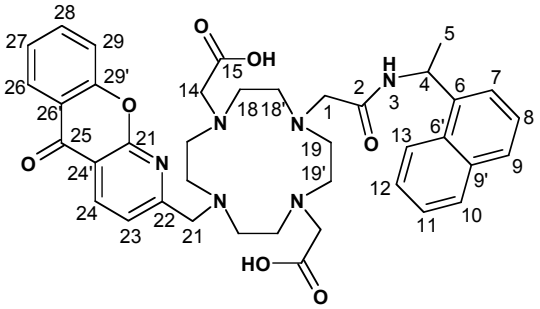
1,7-Bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (400 mg, 1.0 mmol) was combined with (*S*)-1-(chloromethylcarbamoylmethyl)-naphthalene (1 eq., 248 mg) and $NaHCO_3$ (1 eq., 84 mg) and the mixture stirred in dry MeCN (10 mL) and heated at 55 ºC under argon for 16h. The reaction was monitored by TLC (DCM : MeOH, 97 : 3, alumina) and $ESMS^+$ to confirm that the aromatic starting material had been consumed. The solvent was removed under reduced pressure and the resulting solid dissolved in DCM (5 mL) and the inorganic salts filtered out. The crude mixture was purified by column chromatography (DCM→2% MeOH) to yield a colourless oil (330 mg, 54.1 mmol, 54%) δ_H ($CDCl_3$) 9.93 (1H, s, H^{20}), 8.06 (1H, d, *J* 8.0 Hz, H^8), 7.28 (1H, d, *J* 8.0 Hz, H^{13}), 7.70 (1H, d, *J* 8.0 Hz, H^{11}), 7.58 (1H, d, *J* 8.0 Hz, H^{10}), 7.41 (3H, m, $H^{9,12,7}$), 5.86 (1H, p, *J* 6.4 Hz, H^4), 3.30 (1H, d, *J* 4.6 Hz, H^1), 3.06 (4H, br.s, H^{14}), 2.87, 2.71 (16H, br.m, $H^{18,19}$), 1.61 (3H, d, *J* 6.4 Hz, H^5), H^{13}), 1.34 (18H, s, H^{17}); δ_c ($CDCl_3$) 170.7 (C^{15}), 170.3 (C^2), 139.1 (C^6), 134.0 ($C^{9'}$), 131.1 ($C^{6'}$), 129.1 (C^{13}), 128.4 (C^{11}), 126.0 (C^9), 126.7 (C^{12}), 125.6 (C^7), 123.4 (C^8), 123.2 (C^{10}), 81.7 (C^{16}), 57.5 (C^{14}), 54.8, 53.8, 41.8, 49.3 ($C^{18,19}$), 54.2 (C^1), 44.6 (C^4), 28.3 (C^{17}), 21.5 (C^5); R_f 0.63 (DCM – 4% MeOH, alumina); *m/*z ($HRMS^+$) 612.4114 $(M + H)^+$ ($C_{34}H_{54}O_5N_5$ requires 612.4120).

(*S*)-1,7-Bis(*tert*-butoxycarbonylmethyl)-4-(methyl-1-azaxanthone)-7-(methylcarbamoylmethyl-naphthalene)-1,4,7,10-tetraazacyclododecane



1,7-Bis(*tert*-butoxycarbonylmethyl)-4-(methylcarbamoylmethyl-naphthalene)-1,4,7,10-tetraazacyclododecane (220 mg, 360 μmol) was combined with 2-bromomethyl-1-azaxanthone (1 eq., 110 mg) and K_2CO_3 (1 eq., 50 mg) and the mixture stirred in dry MeCN (10 mL) and heated at 80 ºC under argon for 7h. The reaction was monitored by TLC (DCM : MeOH, 97 : 3, alumina) and $ESMS^+$. The solvent was removed under reduced pressure and the resulting solid dissolved in DCM (5 mL) and the salts filtered out. The crude mixture was purified by column chromatography (DCM→2% MeOH) to yield a pale yellow oil (200 mg, 0.24 mol, 68%) δ_H ($CDCl_3$): 8.55 (1H, d, *J* 8.0 Hz, H^{24}), 8.41 (1H, d, *J* 8.0 Hz, H^3), 8.55 (1H, dt, *J* 8.0 Hz, H^{26}), 8.04 (1H, d, *J* 8.0 Hz, H^8), 7.81 (1H, d, *J* 8.0 Hz, H^{29}), 7.77 (1H, d, *J* 8.0 Hz, H^{13}), 7.72 (1H, t, *J* 8.0 Hz, H^{28}), 7.66 (1H, d, *J* 8.0 Hz, H^{23}), 7.54 (2H, dd, *J* 8.0 Hz, $H^{10,11}$), 7.41 (2H, dt, *J* 8.0 Hz, $H^{9,12}$), 7.37 (1H, t, *J* 8.0 Hz, H^7), 5.91 (1H, p, *J* 6.4 Hz, H^4), 3.43 (2H, d, *J* 7 Hz, H^{21}), 3.10 (2H, d, *J* 7 Hz, H^1), 2.60 (20H, br.m, $H^{18,18',19,19',14}$), 1.69 (3H, d, *J* 6.7 Hz, H^5), 1.35 (18H, s, H^{17}); δ_c ($CDCl_3$) 177.4 (C^{25}), 170.8 (C^{15}), 170.3 (C^2), 159.6 ($C^{24'}$), 155.6 ($C^{26'}$), 138.1 (C^6), 137.4 (C^{24}), 135.4 (C^{28}), 133.9 ($C^{9'}$), 131.8 ($C^{6'}$), 128.7 (C^{13}), 128.5 (C^{11}), 126.7 (C^{29}), 126.6 (C^{12}), 125.9 (C^9), 125.0 (C^7), 124.5 (C^{27}), 123.7 (C^8), 122.8 (C^{10}), 121.7 ($C^{22'}$), 120.2 (C^{29}), 115.0 ($C^{29'}$), 80.8 (C^{16}), 62.2 (C^{21}), 58.4 (C^{14}), 55.4 (C^1), 55.1, 53.6, 52.7, 52.1, ($C^{18,18',19,19'}$), 43.6 (C^4), 28.2 (C^{17}), 19.8 (C^5); R_f 0.58 (DCM – 3% MeOH, alumina); *m/*z ($HRMS^+$) 821.4601 $(M + H)^+$ ($C_{47}H_{61}O_7N_6$ requires 821.4596).

1,7-Bis(carboxymethyl)-4-(methyl-1-azaxanthone)-7-(methylcarbamoylmethyl-naphthalene)-1,4,7,10-tetraazacyclododecane, L^1



A mixture of trifluoroacetic acid (1.5 mL) and DCM (0.5 mL) was added to 1,7-bis(*tert*-butoxycarbonylmethyl)-4-(methyl-1-azaxanthone)-7-(methyl-carbamoylmethyl-naphthalene)-1,4,7,10-tetraazacyclododecane (120 mg, 146 μmol) and the mixture stirred under argon at room temperature for 6h. The solvents were removed under reduced pressure and DCM (3 x 3 mL) was successively added and removed under reduced pressure. The crude mixture was dissolved in water (5 mL) and extracted with DCM (3 x 5 mL), and solvent removed to yield a yellow oil (93 mg, 131 μmol, 90%). This trifluoroacetate salt was used for complexation immediately. δ_H (D_2O) 8.29 (1H, d, *J* 8.0 Hz, H^{24}), 7.36 (1H, d, *J* 8.0 Hz, H^{3}), 6.80 (11H, br.m, $H^{7,8,9,10,11,12,13,23,26,28,29}$), 5.21 (1H, m, H^{4}), 3.54 (24H, br.m, $H^{1,14,18,18',19,19'}$), 1.03 (3H, d, *J* 6.7 Hz, H^{5}); *m/*z (ESMS$^-$) 352 $(M - 2H)^-$;

[Eu.L^1]OAc

(*S*)-1,7-Bis(carboxymethyl)-4-(methyl-1-azaxanthone)-7-(methyl-carbamoylmethyl-naphthalene)-1,4,7,10-tetraazacyclododecane (45 mg, 63 μmol) was added to $Eu(CH_3CO_2)_3.3H_2O$ (1.1 eq., 26 mg) dissolved in aqueous methanol (10 : 1, 1mL). The pH was carefully adjusted to 5.8 by addition of acetic acid and the reaction left to stir at 55°C for 24h. Solvents were removed under reduced pressure and the remaining residue was dissolved in H_2O (3 mL). The pH was adjusted carefully to 10 by addition of 35% aqueous ammonia solution (removing excess Eu^{3+} as $Eu(OH)_3$) resulting in a white precipitate that was removed via centrifugation. The pH was adjusted back to neutral with acetic acid and the mixture lyophilised and pumped under vacuum, to give a bright yellow solid (44 mg, 51 μmol). *m/*z (HRMS$^+$) 857.2311 $(M + H)^+$ ($C_{39}H_{42}O_7N_6{}^{151}Eu$ requires 857.2308); $\lambda_{max}(H_2O)$ 336 nm (5010 $dm^3mol^{-1}cm^{-1}$); $\tau^{Eu}_{(H2O,\ pH=6.5)}$: 0.56 ms, $\tau^{Eu}(H_2O,$ pH=6.5, deoxygenated) : 0.54 ms; $\tau^{Eu}(D_2O,$ pD=6.1): 2.05 ms, $\tau^{Eu}(D_2O,$ pD=6.1, deoxygentated) 1.93 ms; q = 1.2; ϕ^{Eu}(pH 6.5) 5 %.

[Tb.L^1]OAc

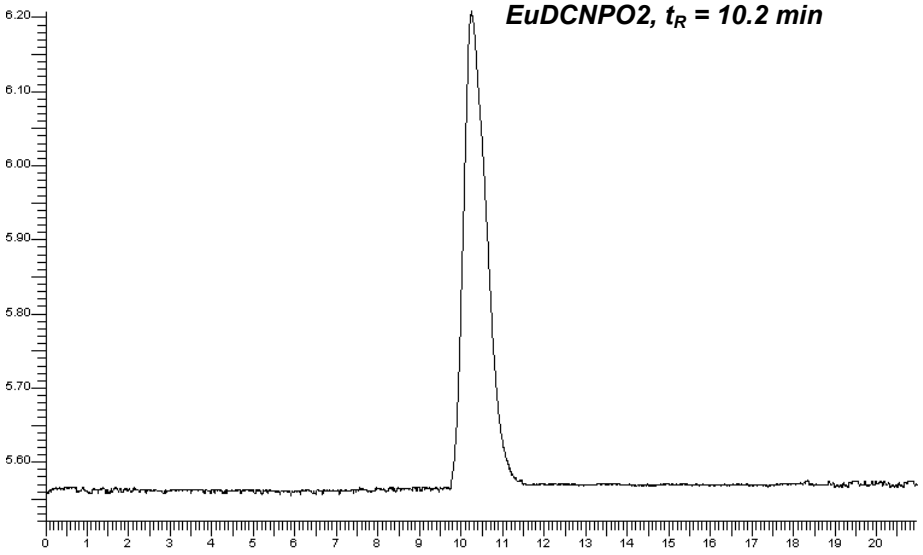
The Tb-complex was prepared as described for the europium analogue. (41 mg, 48 μmol). *m/*z (HRMS$^+$) 865.2360 $(M + H)^+$ ($C_{39}H_{42}O_7N_6Tb$ requires 865.2363); $\lambda_{max}(H_2O)$ 336 nm (5010 $dm^3mol^{-1}cm^{-1}$); $\tau^{Tb}(H_2O$, pH 6.5, deoxygenated): 1.68 ms, $\tau^{Tb}(H_2O$, pH 6.5, aerated): 0.36 ms; $\tau^{Tb}(D_2O$, pD=6.1 deoxygenated): 2.62 ms, τ^{Tb} (D_2O, pD=6.1, aerated): 0.38 ms ; ϕ^{Tb}(pH 6.5) = 2 %

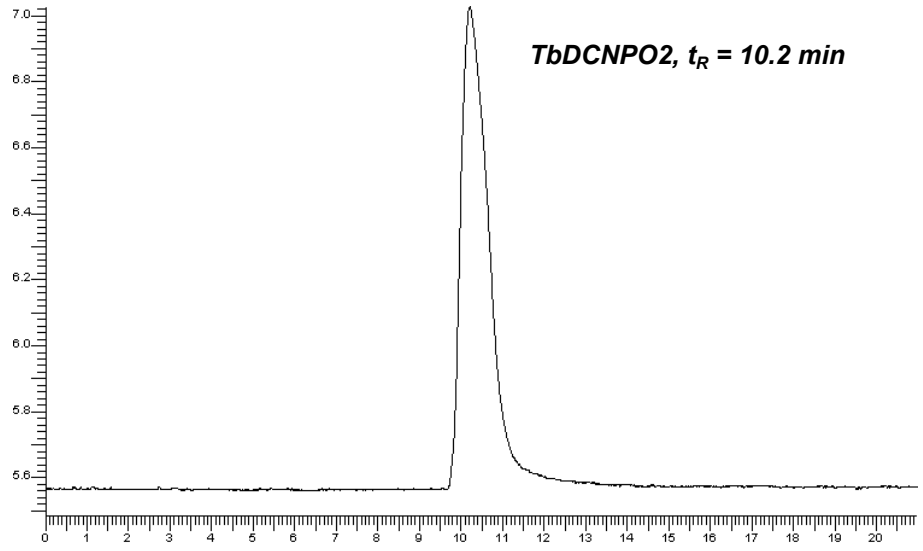
HPLC Analysis and Purification

Reverse phase HPLC analyses were performed at 298 K using a Perkin Elmer System with a 4.6 x 20 mm 4 micron Phenomenex Synergi Fusion RP 80i analytical column. In each case the solvent system used was H_2O + 0.1% HCOOH / MeCN + 0.1% HCOOH using gradient elution with a run time of 20 minutes. In each case, a major product was observed in >98% purity using a diode array UV-Vis detector operating at 340 nm, which corresponds to the absorption band of the appropriate azaxanthone sensitizing moiety (analysis was also undertaken at 280 nm). Such behaviour indicated that each of the species that were eluted bear this chromophore. A fluorescence detector was also usd in parallel, monitoring eluent from the column at a wavelength corresponding to the Eu centred emission (616 nm); again emission was seen for each peak, suggesting that each chromophore containing species was also coordinated to Eu or Tb.

Time (min)	Flow (mL/min)	H_2O (%)	MeCN (%)	Gradient
0.5	1	95	5	0
1.0	1	95	5	0
1.0	1	0	100	1
1.0	1	0	100	0
2.0	1	95	5	2

Gradient elution programme for HPLC analysis.


EuDCNPO2, t_R = 10.2 min
6.20
6.10
6.00
5.90
5.80
5.70
5.60
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20


TbDCNPO2, t_R = 10.2 min
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20