ESI

Observation of the selective staining of chromosomal DNA in dividing cells using a luminescent terbium(III) complex

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A **Figures**: *Figure 1* Emission spectra for [Eu.L^1]Cl and [Eu.L^2]Cl (λ_{exc} 337 nm, pH 7, 0.1 M NaCl)

Figure 2 Variation of europium emission spectrum for [Eu.L^1]Cl (20 μM) with added human serum albumin (λ_{exc} 340 nm, 298 K, pH 7.4, 10 mM HEPES, 10 μM NaCl). The inset shows the concomitant decrease in the Eu emission lifetime, showing the fit (*line*) to the observed data for logK = 3.55(±0.03).

B **Cell culture and microscopy**

C **Ligand and complex synthesis**

Figure 1

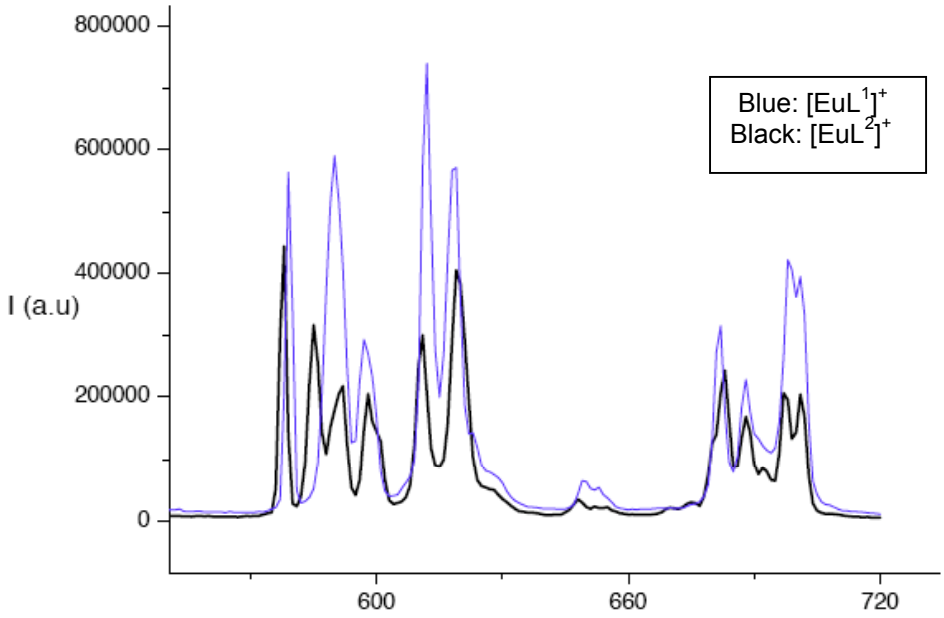
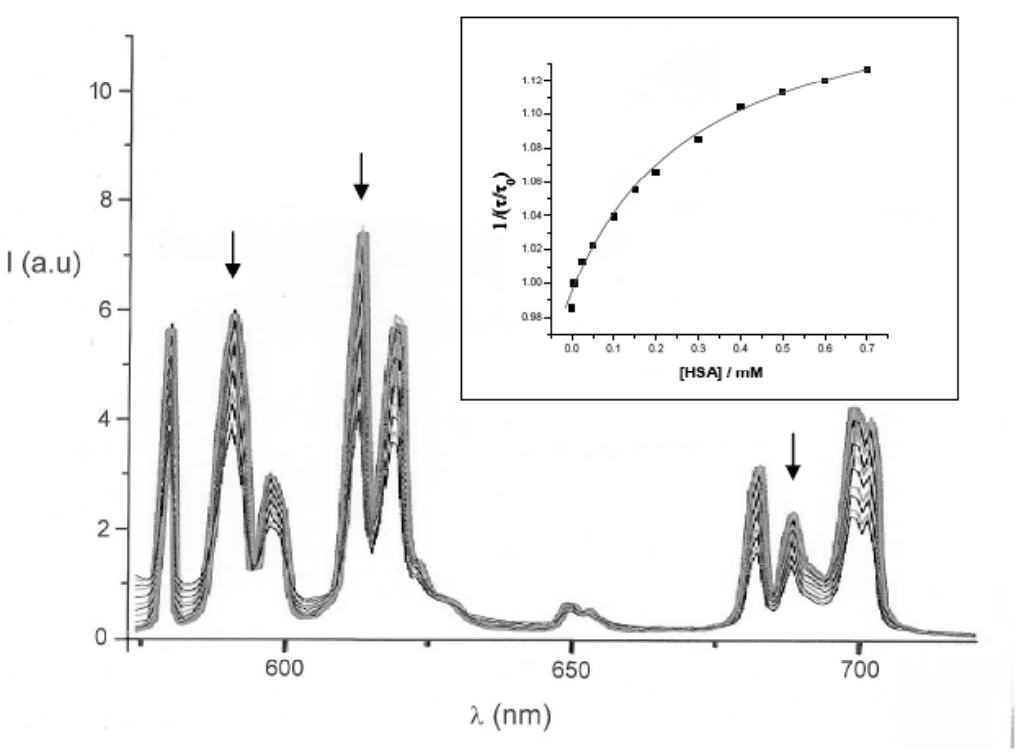


Figure 2



B Cell culture and microscopy

HeLa cells (American Type Culture Collection, ATCC #CCL-2) were grown on 2 chambered glass slides (Lab-Tek) in DMEM with 10% FBS in 5% CO_2 at 37ºC. Cells were grown to 80% confluence and culture medium was changed immediately prior to exposure to the Tb52 complex. The cells were incubated with the complex for 30 minutes at a final concentration of $0.5x10^{-7}$M. Live cell images were collected using 63X objective using a Zeiss Axiovert 200M inverted microscope equipped with a stage heater, air curtain, and a Hamamatsu ORCA-ER camera. The excitation wavelength was at 300 nm, and emission wavelength at 520 nm (UV light source). Z-stack images were collected at an exposure time of 100 ms per plane (10 planes per stack). The images were analyzed and de-convolved with the MetaMorph software (MDS Analytical Technologies).

C Ligand and complex synthesis and characterisation

General Experimental

All reagents were used as received from their respective suppliers. Acetonitrile was dried over calcium hydride when required. Air sensitive reactions were carried out under an atmosphere of argon. Thin-layer chromatography was carried out on silica plates (Merck 5554) or neutral alumina oxide plates (Merck Art 5550) and visualised under irradiation at 254 nm or iodine staining. Preparative column chromatography was carried out using silica (Merck Silica Gel 60, 230 – 400 mesh) or neutral aluminium oxide (Merck Aluminium Oxide 90, activity II – III, 70 – 230 mesh), soaked in ethyl acetate prior to use. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Mercury 400 (^{1}H at 399.97 MHz, ^{13}C at 100.61 MHz). Spectra were recorded in commercially available deuterated solvents. All chemical shifts are given in ppm and coupling constants in Hz. Electrospray mass spectroscopy was carried out on a Thermo Finnigan LTQ and accurate masses recorded on a Thermo Finnigan LTQ-FT.

UV/Vis absorbance spectra were recorded on a Perkin Elmer Lambda 900 UV/Vis/NIR spectrometer. Emission spectra were recorded on a ISA Jobin-Yvon Spex Fluorolog-3 luminescence spectrometer. Lifetime measurements were carried out using Perkin Elmer LS55 spectrometer using FL Winlab software. Analytical reverse phase HPLC analysis was performed at 298 K on a Perkin Elmer system using an XBridge C18 10 cm 3.5 _m column at a flow rate of 1 mL / min using the following method.

Time (min)	H_2O + 0.1 % HCO_2H	CH_3CN + 0.1 % HCO_2H
0	95	5
2	95	5
17	0	100
19	0	100
23	95	5
28	95	5

3-(Dimethylaminomethylene)-4-oxo-6-methyl-2-pyrone

To a suspension of 4-hydroxy-6-methyl-2-pyrone (25 g, 0.2 mol) in *p*-dioxane (100 mL) was added N,N-dimethylformamide dimethyl acetal (44 g, 0.37 mol). The resulting solution was stirred at room temperature for 1 h to give a deep red solution containing a yellow precipitate. The mixture was left in the fridge overnight before the precipitate was collected by filtration to yield the *title compound* (25 g, 69 %), m.p. 145 - 147 °C (Lit. *152 – 154 °C), δ_H ($CDCl_3$) 8.22 (1H, s, NCH), 5.64 (1H, s, CH), 3.45 (3H, s, *Me*N), 3.36 (3H, s, *Me*N), 2.12 (3H, s, *Me*C), *m/z* ($HRMS^+$) 182.0821 $[M + H]^+$ ($C_9H_{12}NO_3$ requires 182.0817).

4-Hydroxy-6-methylnicotinic acid

To a solution of 3-(dimethylaminomethylene)-4-oxo-6-methyl-2-pyrone (6.5 g, 36 mmol) in distilled water (45 mL) was added 35 % ammonia solution (17.8 g, 15.7 mL, 0.37 mol) causing a yellow precipitate to form. After stirring at room temperature for 30 min, 40 % dimethylamine solution was added (4.05 g, 4.55 mL, 36 mmol). The resulting yellow solution was heated at 50 °C for 30 mins before being cooled to room temperature. Upon acidification to pH 5 by addition of sulphuric acid a yellow precipitate formed. The mixture was stirred in an ice bath for 1 h before the precipitate was collected by filtration to yield the *title compound* (4.0 g, 72 %), m.p. 241 - 243 °C (Lit.* 267 – 268 °C) , δ_H (D_2O) 8.44 (1H, s, H^1), 6.71 (1H, s, H^5), 2.37 (3H, s, Me), δ_C (D_2O) 176.5 (CO_2H), 168.9 (C-OH), 154.5 (*C*-CO_2H), 143.0 (C-2), 115.6 (C-5), 114.2 (*C*-Me), 18.5 (Me), *m/z* ($HRMS^-$) 152.0352 $[M - H]^-$ ($C_7H_6NO_3$ requires 152.0353).

(* E. E. Kilbourn and M. C. Siedel, *J. Org. Chem.*, 1972, **37,** 1145)

4-Chloro-6-methylnicotinic acid

4-Hydroxy-6-methylnicotinic acid (6.49 g, 38 mmol) was stirred in neat phosphorus oxychloride (50 mL) at 100 °C for 14 h under argon. Excess phosphorus oxychloride was removed by distillation to leave a brown residue. Addition of water (50 mL) afforded a yellow solution containing a light brown precipitate which was collected by filtration to yield the *title compound* (3.65 g, 56%), m.p. 215 - 217 °C, δ_H (D_2O) 8.85 (1H, s, H-2), 7.91 (1H, s, H-5), 2.63 (3H, s, Me), δ_C (D_2O) 165.3 (CO_2H), 156.2 (C-OH), 152.9 (*C*-CO_2H), 142.8 (C-2), 130.2 (C-5), 129.7 (*C*-Me), 18.9 (Me), *m/z* ($HRMS^-$) 170.0014 $[M - H]^-$ ($C_7H_6{}^{35}ClNO_2$ requires 170.0014).

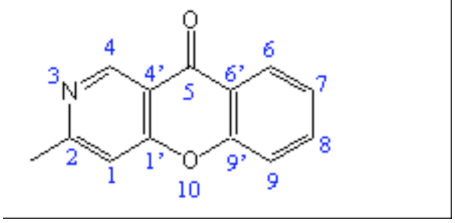
4-Phenoxy-6-methylnicotinic acid

A solution of sodium methoxide was prepared by dissolving sodium metal (1.38 g, 60 mmol) in dry methanol (30 mL). To this solution were added phenol (9.60 g, 100 mmol) and 4-chloro-6-methylnicotinic acid (3.50 g, 20 mmol). The brown solution was stirred at 65 °C for 1 h under argon before the methanol was removed under reduced pressure. The resulting brown residue was stirred at 180 °C for 3 h under argon. The residue was allowed to cool to room temperature before being dissolved in water (20 mL). Excess phenol was removed by extraction with diethyl ether (3 _ 25 mL). Acidification of the aqueous layer by addition of acetic acid generated a white

precipitate which was collected by filtration to yield the *title compound* (1.5 g, 30 %), m.p. 123-126 °C, δ_H ($CDCl_3$) 8.41 (1H, s, H-2), 7.37 (2H, t, *J* 8.0 Hz, *meta*-CH), 7.22 (1H, t, *J* 8.0 Hz, *para*-CH), 7.04 (2H, d, *J* 8.0 Hz, *ortho*-CH), 6.61 (1H, s, H-5), 2.28 (3H, s, Me), δ_C ($CDCl_3$) 170.2 (CO_2H), 163.7 (C-O), 160.3 (*C*-CO_2H), 154.4 (C-2), 149.7 (C-O-Ph), 130.2 (C-5), 125.4 (*C*-Me)123.2 (*ortho*-CH-Ph), 120.9 (*meta*-CH-Ph), 110.5 (*para*-CHPh), 20.1 (Me), *m/z* (HRMS$^-$) 228.0665 $[M - H]^-$ ($C_{13}H_{10}NO_3$ requires 228.0666).

2-Methyl-3-azaxanthone

4-Phenoxy-6-methylnicotinic acid (820 mg, 3.6 mmol) and polyphosphoric acid (50 g) were heated to 120 °C for 14 h under argon. The resulting brown solution was cooled to room temperature and poured into crushed ice (250g). The pH of the yellow solution was adjusted to 7 by addition of KOH pellets. The aqueous solution was extracted with CH_2Cl_2 (3 _ 100 mL) and the organic layers combined, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to yield the *title compound* as a pale brown solid (600 mg, 79 %), m.p. 156 - 157 °C, δ_H ($CDCl_3$) 9.32 (1H, s, H^4), 8.24 (1H, d, *J* 8.4 Hz, H^6), 7.69 (1H, dd, *J* 7.0 Hz, 8.4 Hz, H^8), 7.41 (1H, d, *J* 8.4 Hz, H^9), 7.36 (1H, dd, *J* 7.0 Hz, 8.4 Hz, H^7), 7.13 (1H, s, H^1), 2.64 (3H, s, C*H*$_3$), δ_C ($CDCl_3$) 176.3 (CO), 164.5 (N*C*Me), 161.6 (*C*CO-pyridyl), 156.0 (C-O), 150.3 (NCH), 135.6 (CH), 126.8 (OCC*C*H-phenyl), 124.9 (CH-phenyl), 123.1 (OC*C*-phenyl), 118.3 (OC*C*H-phenyl), 115.8 (OC-pyridyl), 111.2 C-1), 25.2 (*C*H$_3$), *m/z* (HRMS$^+$) 212.0705 $[M + H]^+$ ($C_{13}H_{10}NO_2$ requires 212.0706).



2-Bromomethyl-3-azaxanthone

To a solution of 2-methyl-3-azaxanthone (1.48 g, 7.0 mmol) in carbon tetrachloride (50 mL) was added N-bromosuccinimde (1.87 g, 10.5 mmol) and dibenzoyl peroxide (40 mg, 0.17 mmol). The mixture was stirred and irradiated by a 100 W lamp for 14 h under argon. The reaction was monitored by ^{1}H-NMR and stopped after 14 h. The solvent was removed under reduced pressure and the crude product dissolved in CH_2Cl_2 (30 mL) and washed with dilute aqueous K_2CO_3 solution (30 mL) to remove excess succinimide. The organic layer was dried over $MgSO_4$, filtered and solvent removed under reduced pressure. Purification by column chromatography on silica (elution: toluene-CH_2Cl_2 1 : 1, MeOH 0 – 2 % using 0.1 % increments) yielded the *title compound* as a yellow solid (620 mg, 30 %), m.p. 177 - 179 °C, δ_H ($CDCl_3$) 9.44 (1H, s, H^4), 8.31 (1H, d, *J* 8.4 Hz, H^6), 7.77 (1H, dd, *J* 7.0 Hz, 8.4 Hz, H^8), 7.53 (1H, s, H^1), 7.52 (1H, d, *J* 8.4 Hz, H^9), 7.44 (1H, dd, *J* 7.0 Hz, 8.4 Hz, H^7), 4.63 (2H, s, C*H*$_2$Br), δ_C ($CDCl_3$) 175.9 (C^5), 162.1 (C^2), 161.9 ($C^{4'}$), 156.1 ($C^{9'}$), 150.9 (C^4), 135.9 (C^8), 127.0 (C^6), 125.4 (C^7), 123.2 ($C^{6'}$), 118.4 (C^9), 117.0 ($C^{1'}$), 112.1 (C^1), 33.0 (*C*H$_2$Br), *m/z* (HRMS$^+$) 291.9785 $[M + H]^+$ ($C_{13}H_9\,^{81}BrNO_2$ requires 291.9791), R_f = 0.57 (silica, toluene-CH_2Cl_2 1 : 1 – MeOH 5 %).

1,7-Bis(tert-butoxycarbonylmethyl)-4,10-bis[2-methyl-3-azaxanthone]-1,4,7,10-tetraazacyclododecane

1,7-Bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (75 mg, 0.19 mmol), 2-bromomethyl-3-azaxanthone (108 mg, 0.37 mmol) and K_2CO_3 (26 mg, 0.19 mmol) were stirred in dry CH_3CN (3 mL) at 78 °C for 48 h under argon. The reaction was monitored by TLC to confirm that all the brominated starting material had been consumed. The solvent was removed under reduced pressure and the resultant solid was dissolved in CH_2Cl_2 (25 mL) and extracted with water (3 _ 25 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give a glassy pale yellow solid which was purified by column chromatography on neutral alumina (CH_2Cl_2/0-4% MeOH) to afford an off-white solid (61 mg, 40 %), δ_H ($CDCl_3$) 9.42 (2H, s, H^4), 8.30 (2H, s, H^1), 7.98 (2H, d, *J* 8.4 Hz, H^6), 7.41 (2H, dd, *J* 7.0 Hz, 8.4 Hz, H^8), 7.10 (2H, dd, *J* 7.0 Hz, 8.4 Hz, H^7), 6.62 (2H, d, *J* 8.4 Hz, H^9), 3.94 (4H, s, H^a), 3.25 (4H, s, H^b), 3.00 – 2.80 (16H, m, *cyclen Hs*), 1.40 (18H, s, H^e), δ_C ($CDCl_3$) 176.3 (C^5), 171.1 (C^c), 167.6 (C^2), 162.0 ($C^{4'}$), 155.4 ($C^{9'}$), 150.1 (C^4), 135.2 (C^8), 126.4 (C^6), 124.6 (C^7), 122.6 ($C^{6'}$), 117.7 (C^9), 116.3 ($C^{1'}$), 111.1 (C^1), 81.2 (C^d), 60.5 (C^b), 56.9 (C^a), 55.0 – 52.0 (*cyclen Cs*), 28.4 (C^e), *m*/*z* (HRMS$^+$) 819.4080 $[M + H]^+$ ($C_{46}H_{55}N_6O_8$ requires 819.4076).

[EuL^1]Cl

1,7-Bis(*tert*-butoxycarbonylmethyl)-4,10-bis[2-methyl-3-azaxanthone]-1,4,7,10-tetraazacyclododecane (22 mg, 0.031 mmol) was dissolved in CH_2Cl_2 (2 mL) and CF_3CO_2H (2 mL) was added. The mixture was stirred at 22 °C, under argon, for 12 h. Excess CF_3CO_2H and CH_2Cl_2 were removed under reduced pressure and the yellow residue was re-dissolved in CH_2Cl_2. The solvent was, again, removed under reduced pressure to ensure the removal of all CF_3CO_2H. Deprotection of the O^{t}Bu groups was confirmed by ^{1}H-NMR before the residue was dissolved in H_2O – CH_3OH (4 : 1 v/v, 4 mL).

Addition of $Eu(OAc)_3$ (15 mg, 0.037 mmol) was followed by adjustment of the pH to 5.8, by the addition of aqueous ammonia, and the mixture was stirred at 60 °C for 48 h. After allowing the solution to cool to room temperature, the pH was raised to 10 by the addition of aqueous ammonia. The solution was stirred for 1 h causing excess Eu^{3+} to precipitate as $Eu(OH)_3$; this was removed by syringe filtration. Adjustment of the pH to 5.8 by the addition of CH_3CO_2H, followed by lyophilisation of the solvent, gave the product as the acetate salt. The solid was converted to the chloride salt by stirring in H_2O for 1 h with Dowex 1 _ 8 200-400 mesh Cl^-, which had been copiously washed with 1 M HCl and then with water. The Dowex was removed by filtration and the solvent lyophilised to yield the *title compound* as a yellow solid (18 mg, 66 %), *m*/*z* (HRMS$^+$) 855.1798 $[M - Cl]^+$ ($C_{38}H_{36}O_8N_6{}^{151}Eu$ requires 855.1788), t_R = 11.0 min, τ_{H_2O} = 0.56 ms, τ_{D_2O} = 1.54 ms, $\Phi^{em}_{H_2O}$ = 10 %.

[TbL^1]Cl

An analogous procedure to that described for the synthesis of $[Eu.L^2]^+$ was followed using 1,7-bis(*tert*-butoxycarbonylmethyl)-4,10-bis[2-methyl-3-azaxanthone]-1,4,7,10-tetraazacyclododecane (22 mg, 0.031 mmol) and $Tb(OAc)_3$ (15 mg, 0.037 mmol). The *title compound* was isolated as a yellow solid (19 mg, 69 %), *m*/*z* (HRMS$^+$) 863.1853

$[M]^+$ ($C_{38}H_{36}O_8N_6{}^{159}Tb$ requires 863.1843), t_R = 10.9 min, τ_{H_2O} = 1.52 ms, τ_{D_2O} = 2.75 ms, $\Phi^{em}_{H_2O}$ = 34 %.

Ligand L^2 was prepared analogously to L^1, from the literature compound 2-bromomethyl-1-azaxanthone.
δ_H (CD_3OD) 8.73 (2H, d, *J* 8.0 , H-4), 7.82 (2H, d, *J* 8.0 Hz), 7.63 (2H, d, *J* 8.0 Hz), 7.47 (2H, dd, *J* 8.0 Hz), 7.24 (2H, dd, *J* 8.0 Hz), 6.84 (2H, d, *J* 8.0 Hz), 5.12 (4H, s) 3.80 (4H, s, CH_2CO), 3.78, 3.43 – 3.30 (16H,br. m, *cyclen* CH_2)
δ_C (CD_3OD) 176.89 (C=O), 159.74 (Ar-C=O), 154.88 (Ar-O-C-N), 138.36 (Ar-C-N), 135.74 (Ar-C-O), 125.86 (Ar-C=C), 124.88 (Ar-C=C), 120.84 (Ar-C-H), 119.43 (Ar-C-H), 117.99 (Ar-C-H), 116.05(Ar-C-H), 110.43 (Ar-C-H), 105 (Ar-C-H), 57.54 (NCH_2), 53.83 , 51.91 (*cyclen* CH_2).

[Tb.L^2]
This was isolated as an off-white solid (23 mg, 82 %), *m/z* ($HRMS^+$) 863.1845 $[M]^+$ ($C_{38}H_{36}O_8N_6{}^{159}Tb$ requires 863.1843), t_R = 11.3 min, τ_{H_2O} = 1.93ms, τ_{D_2O} = 2.25 ms, $\Phi^{em}_{H_2O}$ = 36 %.

[Eu.L^2]
This was isolated as a off-white solid (28 mg, 86 %) *m/z* ($HRMS^+$) 855.1786 $[M]^+$ ($C_{38}H_{36}O_8N_6{}^{151}Eu$ requires 855.1788), τ_{H_2O} = 0.67 ms, τ_{D_2O} = 1.05 ms, $\Phi^{em}_{H_2O}$ = 18 %.