Luminescent chemosensors for pH, halide and hydroxide ions based on kinetically stable, macrocyclic europium–phenanthridinium conjugates†

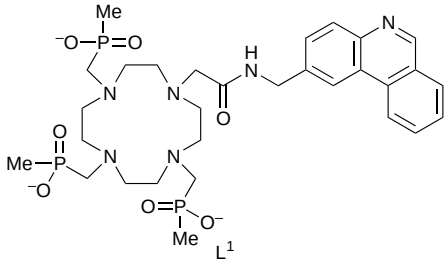
David Parker,* Kanthi Senanayake and J. A. Gareth Williams

Department of Chemistry, University of Durham, South Road, Durham, UK DH1 3LE

The neutral europium complex [EuL^1] shows a pH dependent luminescence enhancement of over 500, while in the *N*-methylated analogue the metal-based luminescence is selectively quenched by chloride and (above pH 10) by hydroxide anions.

Chemically robust, single-component luminescent sensors are needed not only for the analysis of the ionic composition of cells[1] but also in monitoring aqueous systems in the environment or during industrial processes. Fluorescent sensors have been devised for determining intracellular concentrations of species such as Ca^{2+},[2] Zn^{2+},[3] H^+ [4] and Cl^-.[5] Recently, the long-lived (ms range) luminescence of lanthanide complexes has been exploited in various bioassays with time-gating, permitting easy distinction from the shorter-lived (sub-μs) background fluorescence present in most biological systems.[6] Several of these sensors operate by ion-promoted suppression of photo-induced electron transfer (PET) from the ion receptor to the proximate lumophore. Examples include pH sensors incorporating a $Ru(bpy)_3^{2+}$ reporter (in H_2O–MeOH)[7] and a pentadentate terpyridyl ligand bearing aminomethyl substituents which in water forms a weak 1 : 1 complex with Tb.[8] The lanthanide luminescence is 'switched on' following amine protonation and luminescence enhancements of up to 16 were reported. The PET sensor principle is not the only means of allowing changes in ionic concentrations to be signalled: modulation of the spectral properties of the chromophore (ε, λ_{abs}) can be equally effective.

Following our recent work on the synthesis,[9] stereochemical analysis[10] and physicochemical properties[11] of kinetically stable macrocyclic lanthanide complexes in aqueous media, we have now prepared the charge-neutral europium complex of ligand L^1. Monoamide triphosphinate derivatives of



L^1

1,4,7,10-tetraazacyclododecane (cyclen) have been shown to form one predominant diastereomer in water following lanthanide binding[10] with partial hydration of the firmly bound lanthanide[12] ($\beta_{ML} \geqslant 10^{19}$). The phenanthridine chromophore was chosen because of its favourable absorption characteristics for excitation in biological media, its ease of N-alkylation allowing the study of halide induced quenching[5] of the derived phenanthridinium ion and because in its protonated or alkylated forms the phenanthridine moiety should serve as an effective 'antenna' for europium sensitisation minimising the ligand-to-metal charge-transfer process that often limits the overall luminescence efficiency of europium complexes in solution.[10]

Cyanation of 2-bromophenanthridine[13] (CuCN, dmf, 170 °C) followed by reduction with BH_3·thf afforded the aminomethyl derivative in 79% overall yield. Coupling with chloroacetic acid (EDC, HOBt, CH_2Cl_2) gave the corresponding α-haloamide (96%) which was used to monoalkylate the molybdenum-tricarbonyl complex of cyclen using established methodology.[9] Subsequent phosphinoxymethylation [$MeP(OEt)_2$, thf, $(CH_2O)_n$, 4 Å series] followed by base hydrolysis gave the octadentate ligand L^1 [δ_P (pD 6): 40.3] and reaction with $Eu(NO_3)_3$ in water followed by purification by alumina chromatography gave the neutral complex [EuL^1] [δ_P (pD 5.5): 101.1, 88.9, 70.3 with ⩾90% of this diastereoisomer]. Quaternisation of the phenanthridine N in [EuL^1] proceeded cleanly (MeI, MeCN, 40 °C), with the europium ion serving as a suitable protecting group for the ligand heteroatoms.

At pH 1.5 and 7, [EuL^1] possessed one bound water molecule (τ_{H_2O} = 0.71 ms, τ_{D_2O} = 1.92 ms). The phenanthridyl absorption and fluorescence excitation spectra were pH dependent and shifted to longer wavelength upon protonation with CF_3CO_2H. With excitation of the phenanthridyl antenna at 370 nm, luminescence from the Eu ion was 'switched on' following protonation (Figs. 1 and 2). The overall luminescence enhancement factor was at least 500 as can be seen by comparing the metal emission intensity at 594 nm (ΔJ = 1) as a function of pH (Fig. 2). Indeed excitation at 380 nm (pH 7.4) gave rise to no measurable europium emission and acidification 'turns on' the red Eu europium luminescence, thereby creating a genuine pH switch. The pK_a of [EuL^1] was calculated to be 4.45(8), and the same value was obtained from an analysis of the pH dependence of phenanthridyl fluorescence intensity (λ_{em} = 405 nm, λ_{exc} = 320 nm). Absolute quantum yields [relative to $Ru(bpy)_3^{2+}$, ϕ = 0.028][18] were measured for [EuL^1] (0.011 in H_2O, 0.034 in D_2O) and [Eu(HL^1)]$^+$ (0.030 in H_2O), and were independent of excitation wavelength above 300 nm. The PET process operative in this system evidently contributes little to the measured metal-based luminescence enhancement factors which are ascribed to the pH dependence (λ_{abs}, ε) of the sensitising antenna chromophore, (Fig. 1). Consistent with this analysis was the luminescence enhancement factor measured

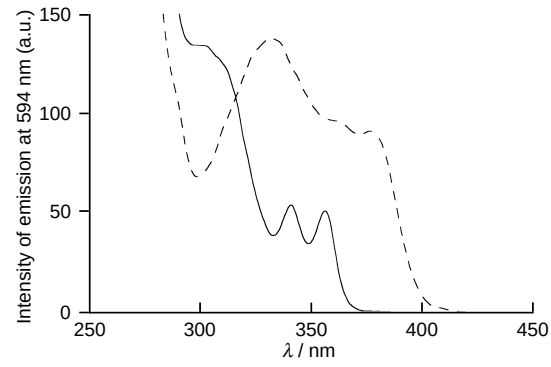


Fig. 1 Metal luminescence excitation spectra (λ_{em} = 594 nm, delay 0.1 ms) at pH 6.8 (solid line) and 1.5 (dashed)

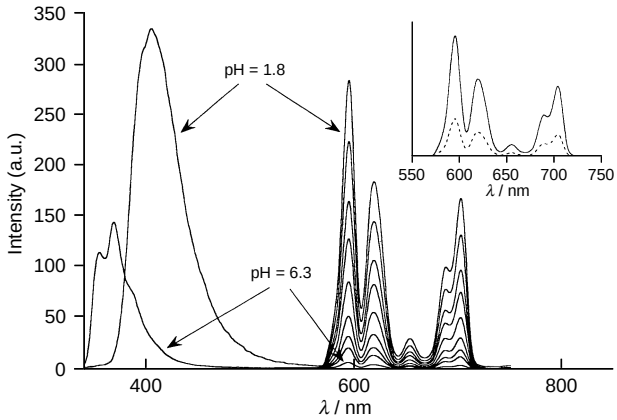


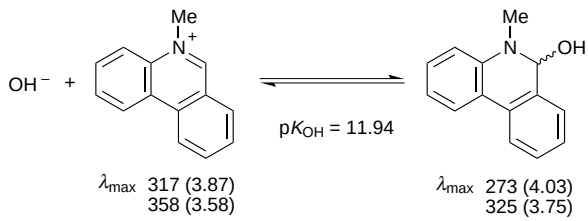
Fig. 2 Effect of pH on phenanthridinium fluorescence (excitation at 320 nm) and on the intensity of europium luminescence (λ_{exc} 370 nm, delay 0.1 ms, pH 1.88 to 6.33, I = 0.1 M NMe_4ClO_4; spectra were acquired using a Perkin-Elmer LS 50B spectrofluorimeter). Insert shows the effect following excitation at the isosbestic point (solid line pH 1.8, dotted 6.3), where the difference in intensity reflects differences in the excited state behaviour only.

following excitation at the isosbestic point (315 nm): an enhancement of 3 was also seen (Fig. 2, insert).

N-Alkylated quinolinium and phenanthridinium ions possess a fluorescence which is quenched selectively by halide ions.[5] In complex [Eu(NMe)L1] [at pH 6.8, τ_{H_2O} = 0.73 ms, ϕ = 0.014 (as iodide salt with λ_{exc} = 320 or 360 nm)], the europium luminescence was also quenched by addition of Cl^-, Br^- or I^- ions and an effective dissociation constant for Cl^- binding of 45 mM was calculated. The observed luminescence quenching by Cl^- was independent of pH (in the range 1.5–9), and was unaffected by the presence of added phosphate (5 mM), citrate (1 mM), lactate (5 mM) or bicarbonate (30 mM) auguring well for the development of such a sensory system for intracellular Cl^- (typically 55–80 mM).

N-Methylphenanthridinium ions reversibly add hydroxide at the 6-position forming a pseudo-base (Scheme 1) and the equilibrium constant for the parent system is 11.94.[14] The concomitant change in the spectral characteristics of the aromatic chromophore (*e.g.* band at 358 nm disappears on adduct formation) suggested that the europium luminescence would be sensitive to the presence of OH^- ions above pH 10. Addition of KOH to **1** caused a steep decrease in the sensitised europium luminescence, (Fig. 3), (λ_{exc} = 360 nm, quenching factor ⩾200) and an effective binding constant of 12.2 (0.1) (I = 0.1 M NMe_4ClO_4) was calculated in reasonable agreement with that measured for the parent system. Such behaviour, involving reversible C–O bond formation, constitutes an unusual method of assaying anionic species in solution, and has been observed previously in the quenching of N-methylacridinium fluorescence by OH^-, following attack at the 9-position.[14]

In summary, the europium complexes described constitute attractive new sensory systems for pH and for halide and hydroxide anions. Given the ease of modification of the heteroaromatic chromophore (and parallel quinolinium systems with p*K*as of *ca*. 5.8 have been recently prepared)[15] the facility of changing the Ln ion to Yb for near IR emission,[16] and the good selectivity of the halide sensor, practicable sensors for intracellular signalling based on such lanthanide probes (*e.g.* measuring the time-resolved emission intensity at 594 or 616 nm) may be expected. Furthermore the *N*-alkylphenanthridyl moieties are known to form relatively stable adducts with DNA and given that emissive enantiopure tetraamide complexes of Eu and Tb have been defined recently,[17,18] luminescent chiral lanthanide probes for DNA may also be feasible.



Scheme 1

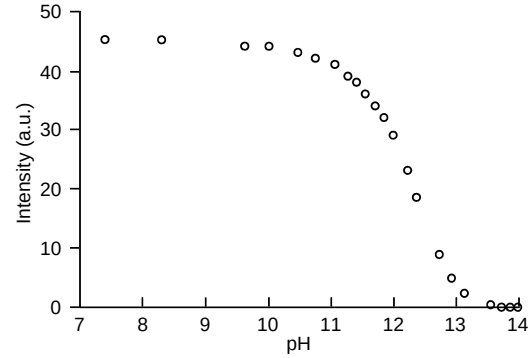


Fig. 3 Effect of hydroxide ions on the intensity of the europium luminescence (λ_{exc} = 380 nm, λ_{em} = 594 nm, delay 0.1 ms; I = 0.1 M NMe_4ClO_4)

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Footnotes and References

* E-mail: david.parker@durham.ac.uk

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