

Utility of tris(4-bromopyridyl) europium complexes as versatile intermediates in the divergent synthesis of emissive chiral probes

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1. General experimental.
2. Data for [Eu.L²].
3. Synthesis and characterisation of [Eu.L⁴] and [Eu.L⁵].
4. HPLC Data for [Eu.L³] and [Eu.L⁶].

1. General experimental: chromatography, optical and CPL spectroscopy

Chromatography

Flash column chromatography was performed using flash silica gel 60 (230 - 400 mesh) from Merck. Thin layer chromatography (TLC) was performed on aluminium sheet silica gel plates with 0.2 mm thick silica gel 60 F₂₅₄ (E. Merck) using different mobile phase. The compounds were visualized by UV irradiation (254 nm) or Dragendorff reagent staining.

Reverse phase HPLC traces were recorded at 298 K using a Perkin Elmer system equipped with a Perkin Elmer Series 200 Pump, a Perkin Elmer Series 200 Autosampler and a Perkin Elmer Series 200 Diode array detector (operated at 254 nm). Separation was achieved using a semi-preparative Waters XBridge RP-C₁₈ column (5 μm, 10 × 100 mm) at a flow rate maintained at 4.4 mL/min Analytical RP-HPLC was performed using a Waters XBridge RP-C₁₈ column (3.5 μm, 4.6 × 100 mm) at a flow rate maintained at 1.0 mL/min over the stated linear gradient.

- *Method A*: (XBridge C₁₈ column, 19 x 100 mm, i.d. 5 μm) flow rate of 17 mL / min with H₂O (0.1% formic acid) – 10 % MeOH (0.1% formic acid) as eluents [linear gradient to 100 % MeOH (0.1% formic acid) (15 min)].

- *Method B:* (XBridge C₁₈ column, 4.6 x 100 mm, i.d. 5 μm) flow rate of 1 mL / min with H₂O (0.1% formic acid) – 10 % MeOH (0.1% formic acid) as eluents [linear gradient to 100 % MeOH (0.1% formic acid) (15 min)].
- Chiral HPLC separations were carried out on a CHIRALPAK-ID 100 mm x 250 mm semi-preparative column, eluting with MeOH at 2.5 mL/min (λ = 320 nm, 295 K), or using the corresponding analytical column 4.6 x 250 mm operating at 1mL/minute, or as stated

Optical Spectroscopy

Emission spectra were recorded using an ISA Jobin-Yvon Spex Fluorolog-3 luminescence spectrometer. Lifetime measurements were carried out with a Perkin-Elmer LS55 spectrometer using FL Winlab software. Quantum yield measurements were calculated by comparison with two standards. For the standards and each of the unknowns, five solutions with absorbance values between 0.05 and 0.1 were used. The quantum yield was calculated according to equation 1:

$$\Phi_x = \Phi_r \cdot \frac{A_r}{A_x} \cdot \frac{E_x}{E_r} \cdot \frac{I_r}{I_x} \cdot \frac{\eta_x^2}{\eta_r^2} \quad (1)$$

where *r* and *x* refer to reference and unknown respectively; *A* is the absorbance at λ_{ex}; *E* is the corrected integrated emission intensity; *I* is the corrected intensity of excitation light; *h* is the refractive index of solution.

CPL Spectroscopy

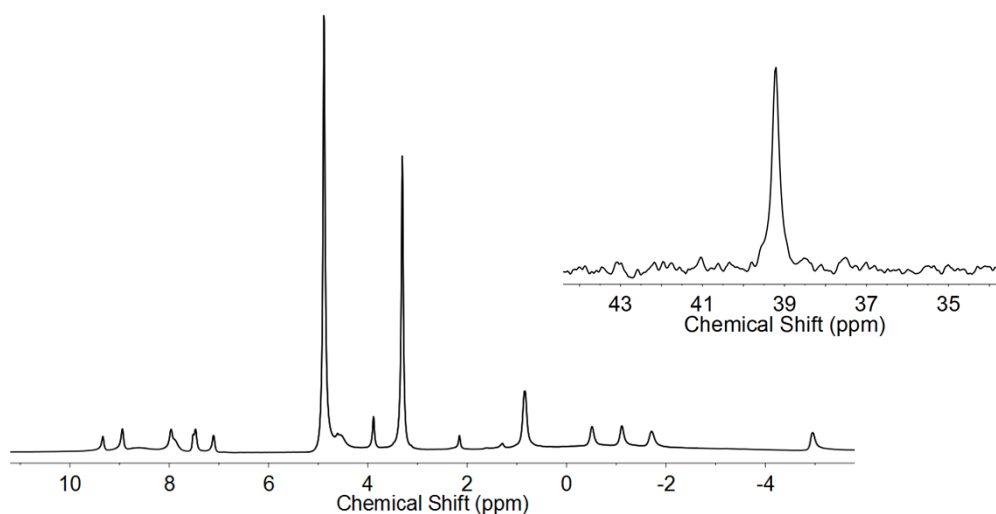
CPL was measured with a home-built (modular) spectrometer. The excitation source was a broad band (200 – 1000 nm) laser- driven light source EQ 99 (Elliot Scientific). The excitation wavelength was selected by feeding the broadband light into an Acton SP-2155 monochromator (Princeton Instruments); the collimated light was focused into the sample cell (1 cm Quarts cuvette). Sample PL emission was collected perpendicular to the excitation direction with a lens (*f* = 150 mm). The emission was fed through a photoelastic modulator (PEM) (Hinds Series II/FS42AA) and through a linear sheet polariser (Comar). The light was then focused into a second scanning monochromator (Acton SP-2155) and subsequently on to a photomultiplier tube (PMT) (Hamamatsu H10723 series). The detection of the CPL signal was achieved using the field modulation lock-in technique. The electronic signal from the PMT was fed into a lock-in amplifier (Hinds Instruments Signaloc Model 2100). The reference signal for the lock-in detection was provided by the PEM control unit. The

monochromators, PEM control unit and lock-in amplifier were interfaced to a desktop PC and controlled by a Labview code. The lock-in amplifier provided two signals, an *AC* signal corresponding to $(I_L - I_R)$ and a *DC* signal corresponding to $(I_L + I_R)$ after background subtraction. The emission dissymmetry factor was therefore readily obtained from the experimental data, as $2 AC/DC$.

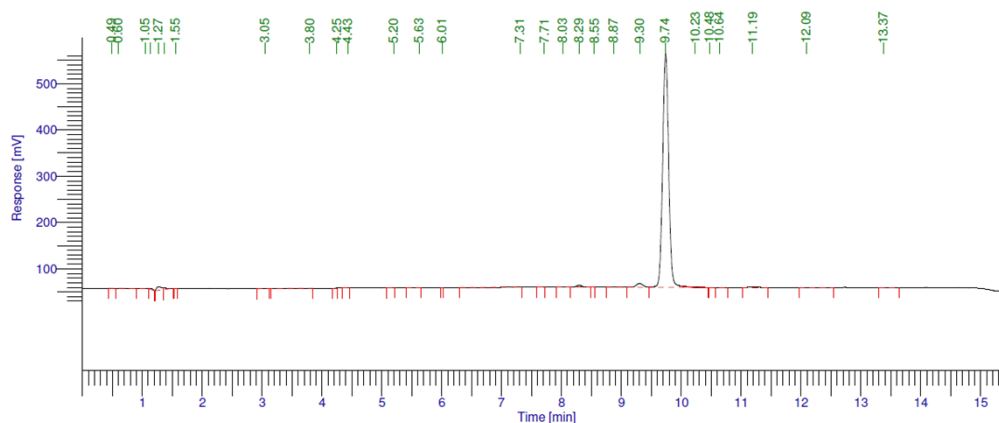
Spectral calibration of the scanning monochromator was performed using a Hg-Ar calibration lamp (Ocean Optics). A correction factor for the wavelength dependence of the detection system was constructed using a calibrated lamp (Ocean Optics). The measured raw data was subsequently corrected using this correction factor. The validation of the CPL detection systems was achieved using light emitting diodes (LEDs) at various emission wavelengths. The LED was mounted in the sample

holder and the light from the LED was fed through a broad band polarising filter and $\lambda/4$ plate (Ocean Optics) to generate circularly polarised light. Prior to all measurements, the $\lambda/4$ plate and a LED were used to set the phase of the lock-in amplifier correctly. The emission spectra were recorded with 0.5 nm step size and the slits of the detection monochromator were set to a slit width corresponding to a spectral resolution of 0.25 nm. CPL spectra (as well as total emission spectra) were obtained through an averaging procedure of several scans. The CPL spectra have been smoothed using Savitzky-Golay smoothing (polynomial order 5, window size 9 with reflection at the boundaries) to enhance visual appearance; all calculations were carried out using raw spectral data. Analysis of smoothed vs raw data was used to help to estimate the uncertainty in the stated g_{em} factors, which was typically $\pm 10\%$.

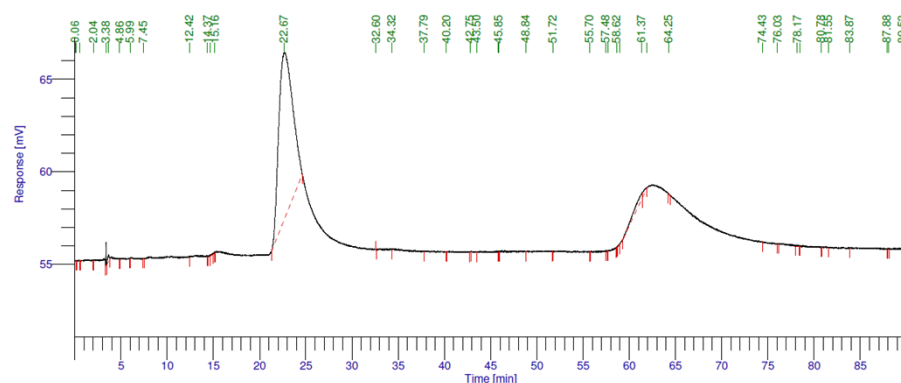
2. Data for [Eu.L²]



¹H and ³¹P NMR Spectra of [Eu.L²] (9.4 T, CD₃OD, 295 K)



Reverse phase HPLC analysis of $[Eu.L^2]$ (XBridge C18 10 cm 3.5 μm , H_2O / CH_3OH with 0.1 % $HCOOH$ [gradient elution], 1 mL/min, $\lambda = 320 \text{ nm}$, 293 K)



Chiral HPLC analysis of $[Eu.L^2]$ (CHIRALPAK-ID 4.0 mm \times 250 mm, $MeOH$, 1 mL/min, $\lambda = 320 \text{ nm}$, 295 K).

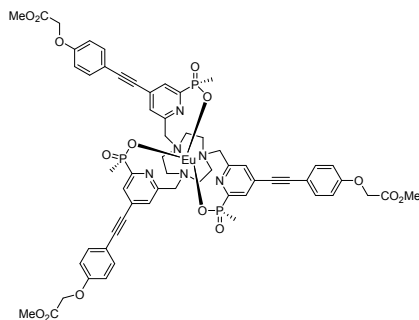
3. Synthesis of $[Eu.L^4]$ and $[Eu.L^5]$

$[Eu.L^4]$

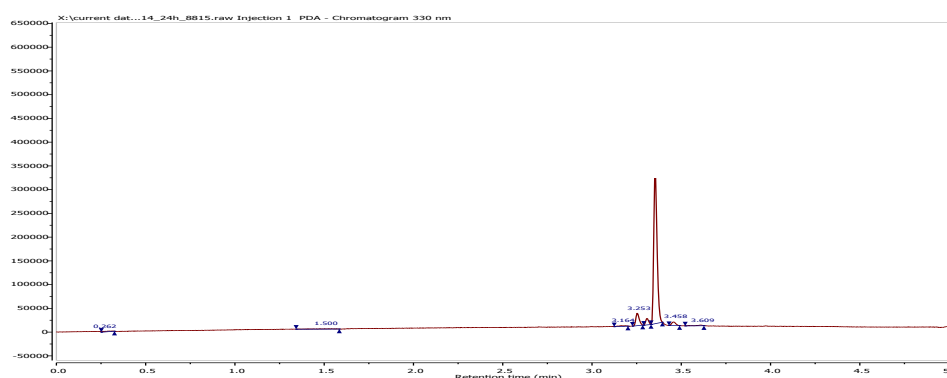
To a stirred and degassed (3 cycles of freeze--thaw) solution of tris *p*-bromo Eu(III) complex (10 mg, 9.8 μmol) and *tert*-butyl 2-(4-ethynyl-3,5-dimethoxyphenoxy)acetate (11.5 mg, 39 μmol) in a mixture of anhydrous THF/DMF (1:1.2 v/v, 1.1 mL) was added triethylamine (81 μL , 0.59 mmol). The solution was degassed (freeze -thaw cycle) once more. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (16 mg, 20 μmol) and CuI (5 mg, 26 μmol) were added and the resulting red solution was stirred at 60 $^\circ\text{C}$ under argon for 24 h, by which time the solution had turned dark brown. The solvent was removed under reduced pressure and the brown residue was purified by semi-preparative RP-HPLC [gradient: 30 - 100% methanol in water (0.1% formic acid) over 15 min; $t_R = 16.4 \text{ min}$]. Trace inorganic salts were removed using a short silica column (eluent 0 – 5% MeOH in DCM) to give an off-white solid (7.8 mg, 48%); LRMS (ESI) m/z 1657 $[M + H]^+$, 829 $[M + 2H]^{2+}$; (HRMS $^+$) m/z

1655.457 [M(¹⁵¹Eu) + H]⁺ (C₇₅H₉₁N₆O₂₁P₃¹⁵¹Eu) requires 1655.465; τ_{Eu} (MeOH) = 1.13 ms; ϕ_{em} (MeOH) = 43(± 5)%; ϵ_{MeOH} (360 nm) 55,000 M⁻¹ cm⁻¹.

Europium (III) complex of 6,6',6''-(1,4,7-triazacyclononane-1,4,7-triyl)tris(methylene)tris(4-((4-(2-methoxy-2-oxoethoxy)phenyl)ethynyl)pyridine-6,2-diyl)tris(methylphosphinate), [Eu.L⁵]

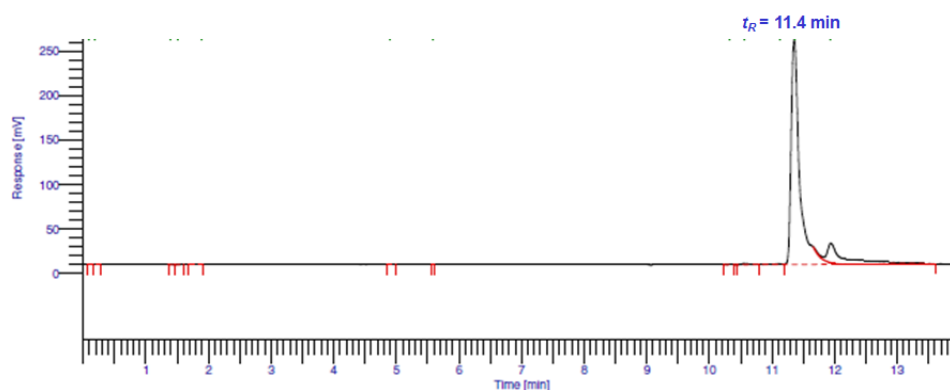


To a stirred degassed solution of [Eu.L¹] (5 mg, 4.9 μ mol) in anhydrous DMF (0.4 mL) was added methyl 2-(4-ethynylphenoxy)acetate (3 mg, 16.2 μ mol) and triethylamine (10 mL, 74 μ mol), and the solution was degassed (freeze-thaw cycle) three times. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (1.2 mg, 1.5 μ mol) and CuI (0.2 mg, 1.5 μ mol) were added and the resulting brown solution was stirred at 65 °C under argon for 18 h. The solvent was removed under reduced pressure and the brown residue was purified by column chromatography (silica, CH₂Cl₂: 0 – 10 % CH₃OH with 10 % aq. ammonia) to afford a white solid (2 mg, 30 %); m/z (HRMS⁺) 676.1351 [M + 2H]²⁺ (C₆₀H₆₂¹⁵³EuN₆O₁₅P₃ requires 676.1354); R_f = 0.67 (silica; CH₂Cl₂ : 10% CH₃OH : 1% aq. ammonia).

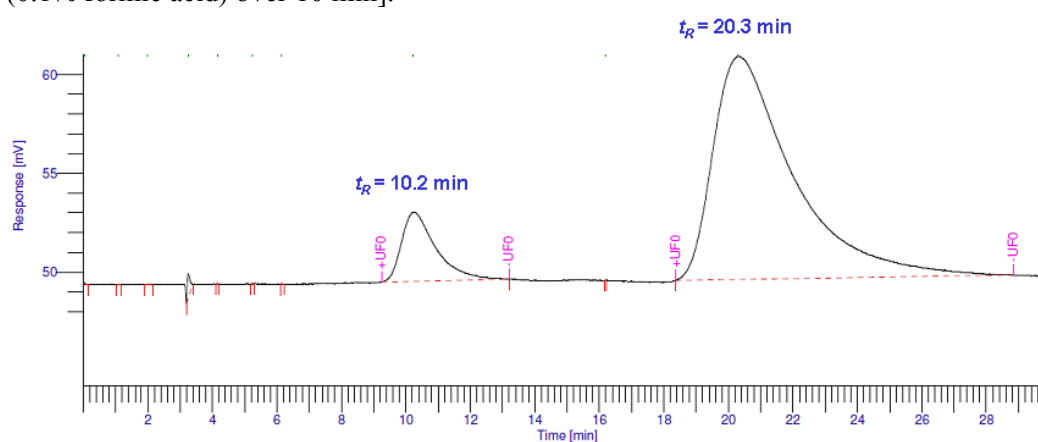


HPLC analysis of [Eu.L⁵] Method B: 10 to 100 % MeOH (0.1% formic acid.) over 5 min, t_R = 3.4 min.

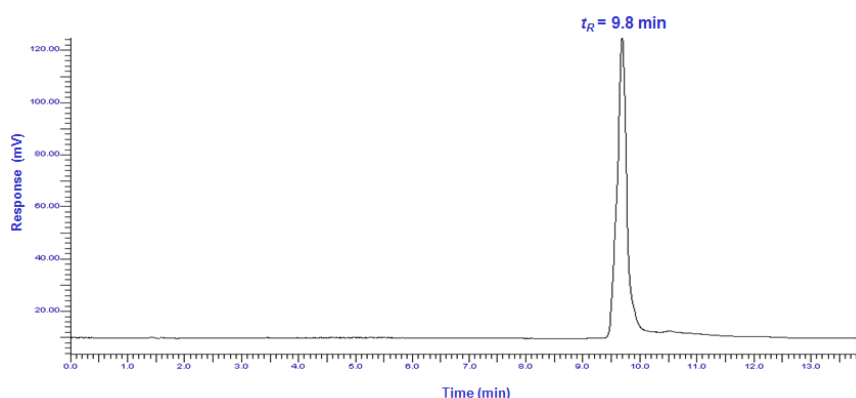
4. Data for [Eu.L³] and [Eu.L⁶]



Analytical RP-HPLC trace of [Eu.L⁶]: $t_R = 11.4$ min [Gradient: 50 to 100% methanol in water (0.1% formic acid) over 10 min].



Chiral HPLC trace of [Eu.L⁶]: Peak 1 $t_R = 10.2$ min; Peak 2 $t_R = 20.3$ min (CHIRALPAK-ID 4.6 mm \times 250 mm, MeOH, 1 mL/min, $\lambda = 355$ nm, 295 K). Enantiomeric purity of the sample above was 76% (88:12), and was traced back to a small degree of racemisation at C-2 in the reaction of the precursor *S*-lysine ethyl ester with neat ethylenediamine. The isomers were separated by chiral HPLC, and the pure enantiomers were used in the acquisition of the CPL data shown in the main text (Figure 6).



Analytical RP-HPLC trace of [Eu.L³]: $t_R = 9.8$ min [Gradient: 50 to 100% methanol in water (0.1% formic acid) over 10 min].