Very Bright, Enantiopure Europium(III) Complexes Allow Time-Gated Chiral Image Contrast

Andrew T. Frawley, Robert Pal* and David Parker *

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

List of Figures

Figure S1. Λ- and Δ-[Eu.L^2] deposited onto optical-brightener free paper and the sample microscope slide used for imaging (main text Figure 3.) imaged under a 365 nm UV torch.

Figure S2 Absorbance and emission spectra of [Eu.L^1] (295 K, MeOH, λ_{exc} 356 nm).

Figure S3 Absorbance and emission spectra of [Eu.L^2] (295 K, MeOH, λ_{exc} 343 nm).

Figure S4 Absorbance and emission spectra of [Eu.L^3] (295 K, MeOH, λ_{exc} 355 nm).

Figure S5 Absorbance and emission spectra of [Eu.L^4] (295 K, MeOH, λ_{exc} 342 nm).

Figure S6 Expanded CPL spectra of [Eu.L^2] showing less commonly observed transitions.

Figure S7 The CPL spectra for [Eu.L^1] (top) and [Eu.L^4] (bottom) (red – Δ, blue – Λ, 5 μM complex, 5 scan average, 295 K, MeOH).

Figure S8 CPL spectra (sum of 3 scans, excited at 342 nm) of [Eu.L^2] (red: Δ; blue: Λ) impregnated in separate regions on paper, *as used* in the imaging experiments described in Figure 3 (main paper). The *g* value for the emission band at 595 nm is 0.11 under the experimental set-up used.

Figure S9. Time dependent image acquisition and the evolution of the contrast ratio with accumulation number in time-resolved microscopy images of (*top*) Λ - [Eu.L^2] and (*bottom*) Δ - [Eu.L^2], following sample excitation using a 365 nm UV-LED ($t_{acq.}$ = 7.2 ms/frame, t_d = 6 μs, λ_{em} BP-589/20/395DC using the Right (Horizontal polarisation) circularly polarised light channel (A) 50 frame accumulations; contrast ratio, CR $_{\Lambda:\Delta}$ = 1.43 : 1), (B) 100 frame accumulations; contrast ratio, CR $_{\Lambda:\Delta}$ = 1.73 : 1), (C) 150 frame accumulations; contrast ratio, CR $_{\Lambda:\Delta}$ = 1.83 : 1), (D) 200 frame accumulations; contrast ratio, CR $_{\Lambda:\Delta}$ = 2.01 : 1), (E) 300 frame accumulations; contrast ratio, CR $_{\Lambda:\Delta}$ = 2.37 : 1) ; (F) 397 frame accumulations; contrast ratio, CR $_{\Lambda:\Delta}$ = 3.42 : 1),

Figure S10 Chiral HPLC traces showing appearance of the second enantiomer over time.

Final 2 pages: enlarged views of Schemes 1 and 2 (main text)

Experimental Section

General Procedures

Commercially available reagents were used as received. Where appropriate, solvents were degassed using freeze-pump-thaw cycles.

Thin layer chromatography (TLC) was carried out on aluminium-backed silica gel plates with 0.2 mm thick silica gel 60 F_{254} (Merck), and visualised by UV irradiation at 254 nm or 356 nm. Preparative flash column chromatography was performed using flash silica gel 60 (230-400 mesh) from Merck or Fluorochem.

^{1}H, ^{13}C and ^{31}P NMR spectra were recorded in commercially available deuteriated solvents on a Bruker Avance-400 (^{1}H at 400.06 MHz, ^{13}C at 100.61 MHz and ^{31}P at 161.95 MHz), a Mercury 400 (^{1}H at 399.95 MHz), a Varian VNMRS-600 (^{1}H at 599.67 MHz, ^{13}C at 150.79 MHz and ^{31}P at 242.75 MHz), or a Varian VNMRS-700 (^{1}H at 699.73 MHz, ^{13}C at 175.95 MHz and ^{31}P at 283.26 MHz). All chemical shifts are in ppm, coupling constants are in Hz.

Electrospray mass spectra were obtained on a TQD mass spectrometer equipped with an Acquity UPLC system, an electrospray ion source and an Acquity photodiode array detector (Waters Ltd., UK). Accurate masses were recorded on an LCT Premier XE mass spectrometer or a QToF Premier Mass spectrometer, both equipped with an Acquity UPLC, a lock-mass electrospray ion source and an Acquity photodiode array detector (Waters Ltd., UK). Methanol was used as the carrier solvent.

Gas chromatography mass spectra were obtained using a Shimadzu QP2010-Ultra spectrometer equipped with an Rxi-5Sil-MS column, operating in EI mode at 70 eV using helium as the carrier gas (0.41 mL/min).

Melting points were determined using a Sanyo Gallenkamp Melting Point Apparatus at atmospheric pressure and are uncorrected.

HPLC analysis

Reverse phase HPLC was performed at 295 K using a Shimadzu system comprising a Degassing Unit (DGU-20A_{5R}), a Prominence Preparative Liquid Chromatography pump (LC-20AP), a Prominence UV-Vis Detector (SPD-20A) and Communications Bus Module (CBM-20A). For preparative HPLC an XBridge C18 OBD column was used (19 x 100 mm, 5 µm) with

a flow rate of 17 mL/min. For analytical HPLC a Shimadzu Shim-Pack VP-ODS column was used (4.6 x 150 mm, 5 μm) with a flow rate of 2.0 mL/min. Fraction collection was performed manually. A solvent system of H_2O (0.1% HCOOH) / CH_3OH (0.1% HCOOH) was used with gradient elution as follows:

Method A

Time / min	**%H_2O**	**%CH_3OH**
0	90	10
3	90	10
13	0	100
17	0	100
18	90	10

Chiral HPLC analysis was carried out on a Perkin Elmer Series 200 system comprising a Perkin Elmer Series 200 pump, autosampler, and UV/Vis detector, using either Daicel CHIRALPAK-IC or ID columns (4.6 x 250 mm for analytical with a flow rate of 1.0 mL/min, 10 x 250 mm for preparative with a flow rate of 4.4 mL/min, all 5 μm particle size). 100% MeOH was used as the mobile phase. Fraction collection was performed manually. Data was analysed using TotalChrom 6.3.1 software.

Optical measurements

All optical analyses were carried out in quartz cuvettes with a path length of 1 cm. UV/Vis absorbance spectra were measured on an ATI Unicam UV/Vis spectrometer (Model UV2) using Vision software (version 3.33). Emission spectra were recorded using an ISA Jobin-Yvon Spex Fluorolog-3 luminescence spectrometer using DataMax software (version 2.2.10). Lifetime measurements were carried out using a Perkin Elmer LS55 spectrometer using FL Winlab software. Quantum yields were calculated by comparison with known standards.

CPL spectra were recorded on a custom built spectrometer consisting of a laser driven light source (Energetiq EQ-99 LDLS, spectral range 170 to 2100 nm) coupled to an Acton SP2150 monochromator (600 g/nm, 300 nm Blaze) allowing excitation wavelengths to be selected with a 6 nm FWHM band-pass. The collection of the emitted light was facilitated (90° angle set up, 1 cm path length quartz cuvette) by a Lock-In Amplifier (Hinds Instruments Signaloc 2100) and Photoelastic Modulator (Hinds Series II/FS2AA). The differentiated light was focused onto an Acton SP2150 monochromator (1200 g/nm, 500 nm Blaze) equipped with a

high sensitivity cooled Photo Multiplier Tube (Hamamatsu H10723-20 PhotoSensor red corrected). The detection of the CPL signal was achieved using the field modulation lock-in technique. The electronic signal from the PMT was fed into the lock-in amplifier (Hinds Instruments Signaloc 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 with a desktop PC and controlled by Labview code.

Circular Polarised Microscopy (Proof of Concept)

Chiroptical contrast based imaging, i.e. the separation of left and right handed circularly polarised light emitted by the separate *Λ*- and *Δ*-[Eu.L^2] enantiomers has been facilitated via modification of a time-resolved Zeiss Axiovert 200M epifluorescence microscope set-up.[1] Areas of the two enantiopure [Eu.L^2] spots with equal brightness were selected (contrast values: *Λ*-[Eu.L^2] 222/255 and *Δ*-[Eu.L^2] 231/255) and using a dye punch (0.3 x 150 mm), two well-defined line-shaped EuL^2 blotted pieces of paper were mounted parallel to each other onto a SuperFrost microscope slide capped with a 170 μm coverslip for imaging (Fig. S1). The microscope is equipped with a variable pulse sequence generator, which allows both CW and time-resolved operation. Acquisition using an EO-1312M(monochrome, Edmund Optics) 0.7M pixel rolling shutter camera was set at 7.2 ms per frame, and a typical value for time gating was 6-10 μs, after pulsed excitation with a 365 nm UV LED (Nichia, 24 V, 1.2 W, collimated and scrambled to 1” diameter, focused to the back focal plane (BFP) of the Zeiss x10/0.25NA A-Plan air objective). The sequence can be programmed for any number of frame averages or, for low light collection, accumulation, controlled manually or by an average FOV pixel contrast saturation-limiting algorithm.

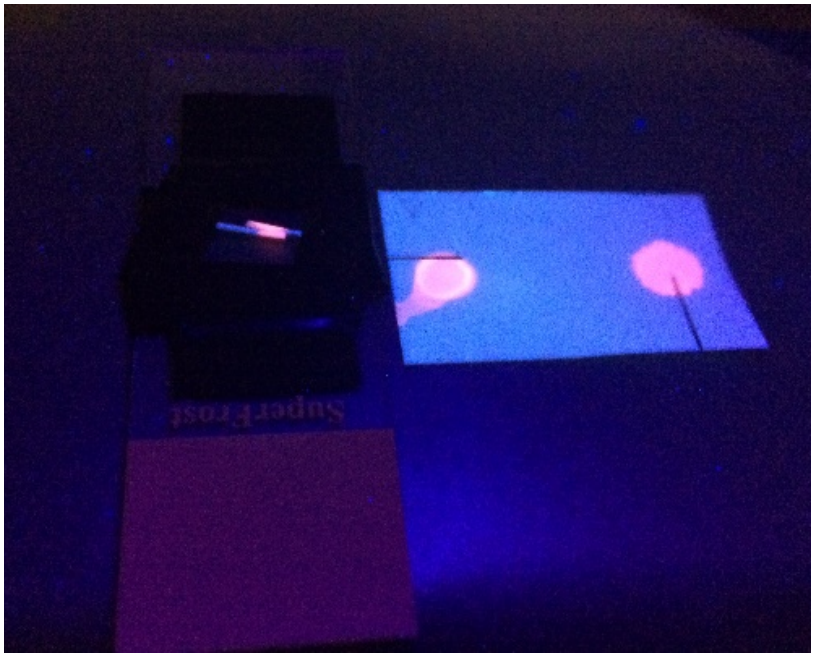


Figure S1. Λ- and Δ-[Eu.L^2] deposited onto optical-brightener free paper and the sample microscope slide used for imaging (main text Figure 3.) imaged under a 365 nm UV torch.

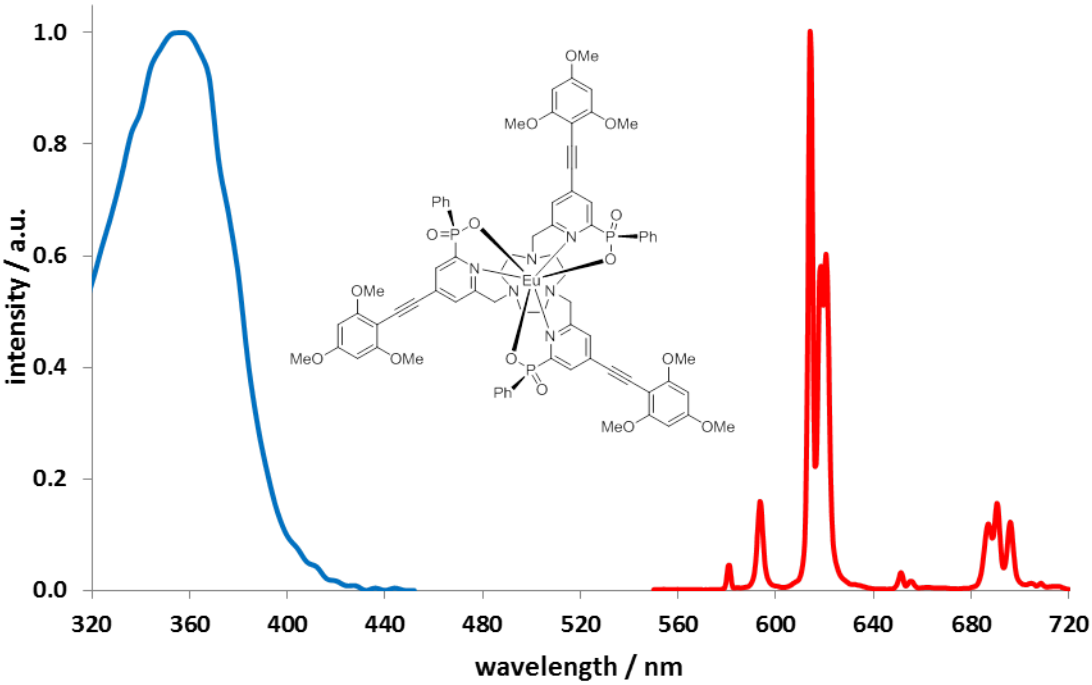


Figure S2 Absorbance and emission spectra of [Eu.L^1] (295 K, MeOH, λ_{exc} 356 nm)

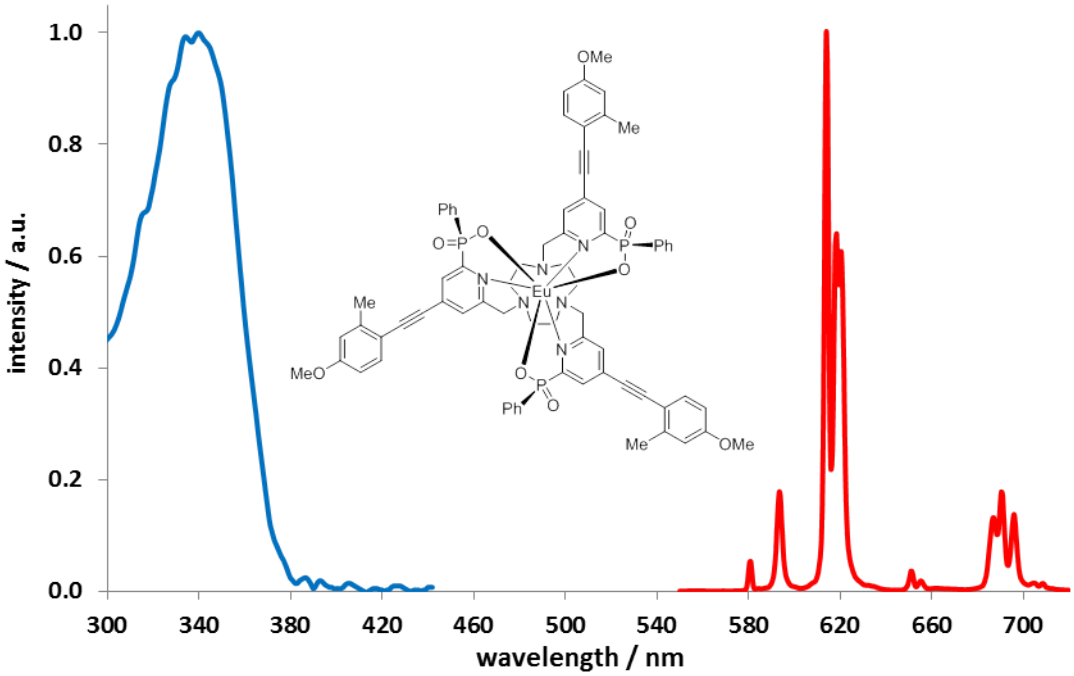


Figure S3 Absorbance and emission spectra of [Eu.L^2] (295 K, MeOH, λ_{exc} 343 nm)

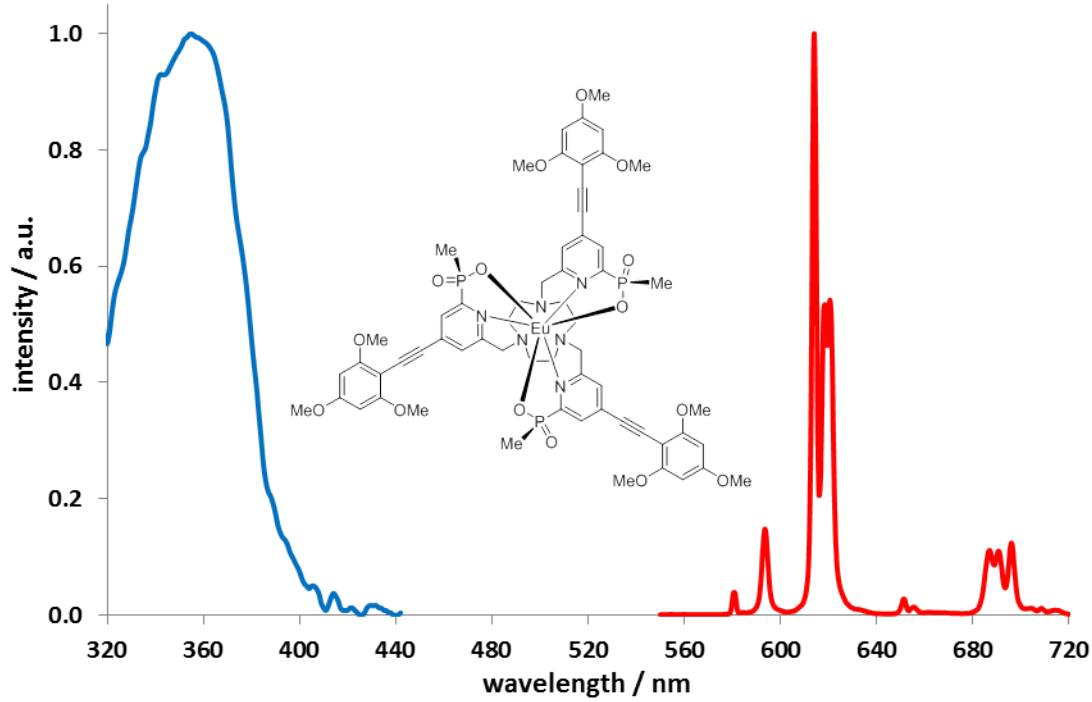


Figure S4 Absorbance and emission spectra of [Eu.L^3] (295 K, MeOH, λ_{exc} 355 nm)

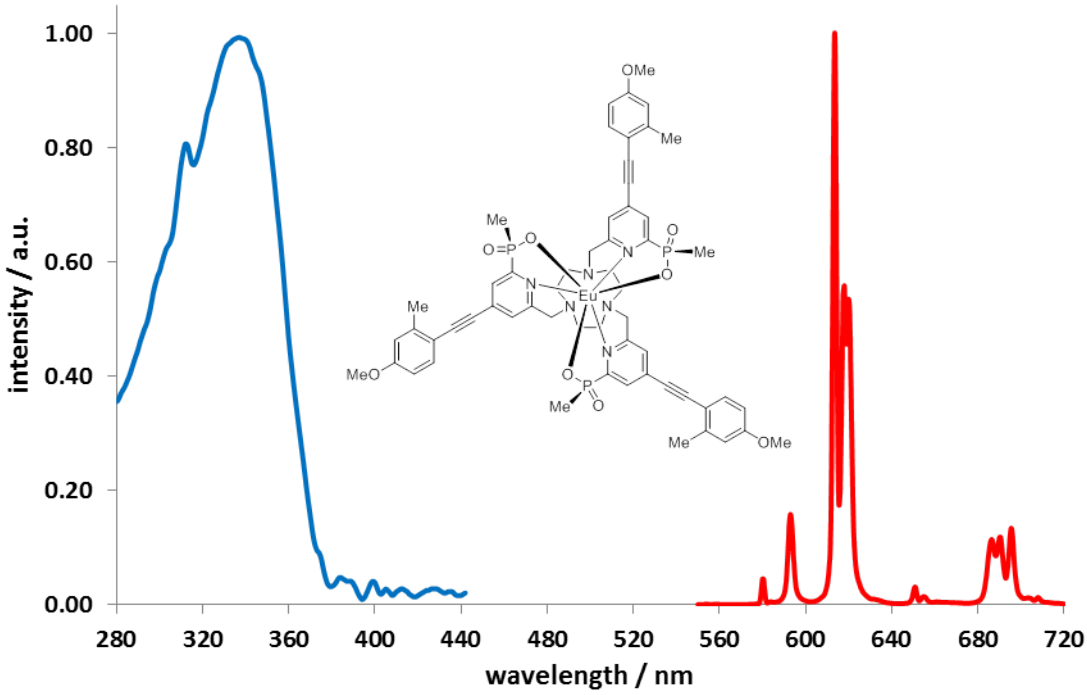
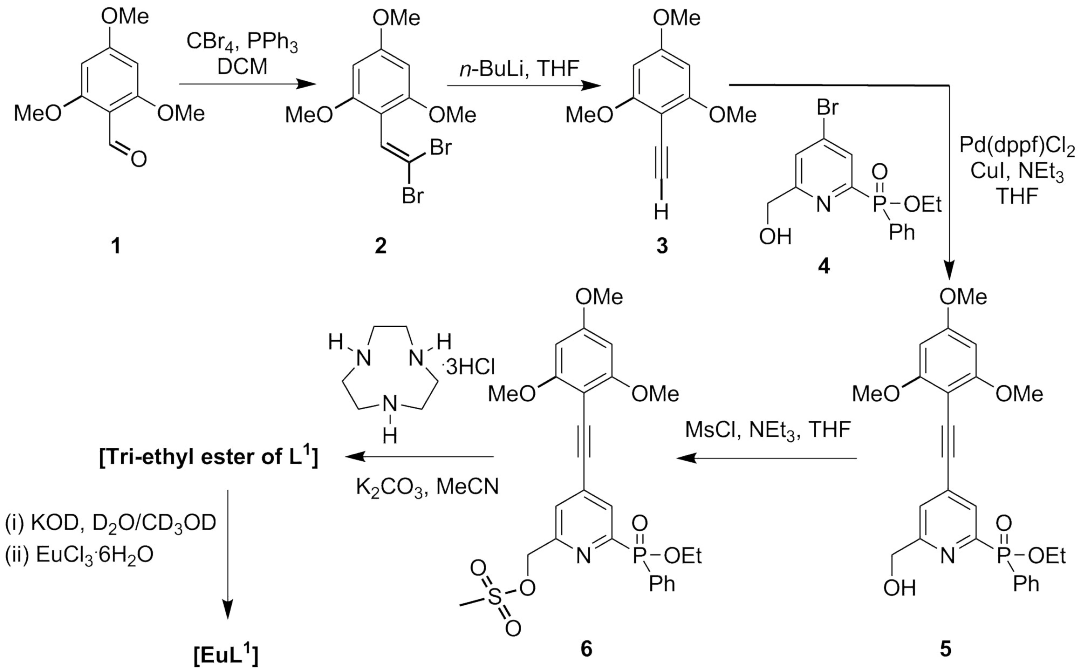


Figure S5 Absorbance and emission spectra of [Eu.L^4] (295 K, MeOH, λ_{exc} 342 nm)

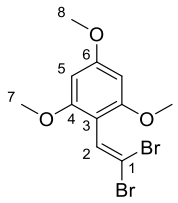
Synthetic procedures

The syntheses and characterisation of the europium complexes of L^3 and L^4 were carried out as reported in references 11 and 14 (main text). Chiral separation of [Eu.L^3] and [Eu.L^4] was achieved using a ChiralPak ID column (10 x 250 mm, 5 µm particle size, isocratic MeOH, 4.4 mL/min). [Eu.L^3] t_R = 13.7 & 32.6 min; [Eu.L^4] t_R = 25.9 & 43.2 min.



Scheme S1 *The synthesis of [Eu.L^1].*

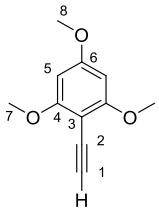
2-(2,2-Dibromovinyl)-1,3,5-trimethoxybenzene (2)



2,4,6-Trimethoxybenzaldehyde **1** (0.50 g, 2.5 mmol) and triphenylphosphine (1.34 g, 5.10 mmol) were dissolved in anhydrous dichloromethane (2 mL) and the solution was cooled in ice. A solution of tetrabromomethane (1.69 g, 5.10 mmol) in anhydrous dichloromethane (3 mL) was added slowly. The reaction was allowed to warm to room temperature and was stirred for 22 h at which time further triphenylphosphine (1.34 g, 5.10 mmol) was added. The reaction was stirred for a further 6 h, before the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (neat hexane to 7:3 v/v hexane:ethyl acetate) to give **2** as a white solid (0.205 g, 23%); m.p. 126-129 °C (lit.[2] 128-

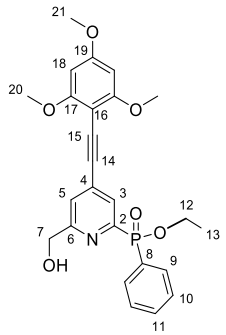
130 °C); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.19 (1 H, s, H^2), 6.11 (2 H, s, H^5), 3.82 (3 H, s, H^8), 3.81 (6 H, s, H^7); ^{13}C NMR (176 MHz, $CDCl_3$) δ 161.9 (C^6), 158.2 (C^4), 131.1 (C^2), 107.0 (C^3), 92.9 (C^1), 90.6 (C^5), 55.8 (C^7), 55.5 (C^8); LCMS (ESI^+) *m/z* 353 $[M+H]^+$; HRMS (ESI^+) *m/z* 350.9220 $[M+H]^+$ ($C_{11}H_{13}O_3{}^{79}Br_2$) requires 350.9231.

2-Ethynyl-1,3,5-trimethoxybenzene (3)



2-(2,2-Dibromovinyl)-1,3,5-trimethoxybenzene **2** (0.300 g, 0.85 mmol) was dissolved in anhydrous tetrahydrofuran (8 mL) and the solution was cooled to -78 °C. *n*-Butyllithium (1.13 mL of a 1.6 M solution in hexane) was added dropwise and the pale yellow solution was stirred for 20 min. Water (3 mL) was added slowly and the solution was stirred for 30 min. The organic solvents were removed under reduced pressure and the remaining aqueous solution was extracted with ethyl acetate (2 x 10 mL). The organic layers were combined and washed with brine (20 mL), then dried with $MgSO_4$, filtered and concentrated under reduced pressure to yield **3** as a yellow solid (0.162 g, 99%) which was used without further purification; m.p. 120-123 °C (lit.[2] 119-122 °C); ^{1}H NMR (700 MHz, $CDCl_3$) δ 6.11 (2 H, s, H^5), 3.88 (6 H, s, H^7), 3.83 (3 H, s, H^8), 3.49 (1 H, s, H^1); ^{13}C NMR (176 MHz, $CDCl_3$) δ 163.2 (C^4), 162.1 (C^6), 93.2 (C^3), 90.5 (C^5), 83.9 (C^1), 76.7 (C^2), 56.2 (C^7), 55.6 (C^8). LCMS (ESI^+) *m/z* 192 $[M+H]^+$; HRMS (ESI^+) *m/z* 193.0853 $[M+H]^+$ ($C_{11}H_{13}O_3$) requires 193.0865.

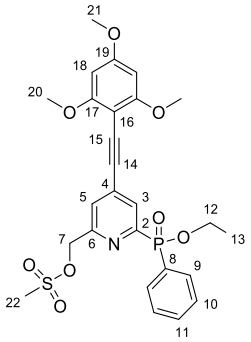
Ethyl-[6-(hydroxymethyl)-4-[2-(2,4,6-trimethoxyphenyl)ethynyl]-pyridin-2 yl](phenyl)phosphinate (5)



2-Ethynyl-1,3,5-trimethoxybenzene **3** (30 mg, 0.15 mmol) and ethyl [4-bromo-6-(hydroxymethyl)pyridine-2-yl](phenyl) phosphinate **4** (50 mg, 0.14 mmol) were dissolved in

anhydrous tetrahydrofuran (1 mL) and the solution was degassed (freeze-pump-thaw cycle x 3). Triethylamine (96 µL, 70 mg, 0.7 mmol) was added and the solution degassed again (freeze-pump-thaw x 1). [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (11.4 mg, 0.0140 mmol) and CuI (5.3 mg, 0.0280 mmol) were added and the solution stirred at 65 °C under argon for 4 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (neat CH_2Cl_2 to 3% CH_3OH in CH_2Cl_2). The product was still contaminated with catalyst residue so the mixture was subjected to a second purification by silica gel column chromatography (1:1 to 4:1 v/v ethyl acetate:hexane) which gave **5** as a yellow oil (41 mg, 62%); ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.12 (1 H, d, $^3J_{H\text{-}P}$ 6.5, H^3), 7.95 (2 H, dd, $^3J_{H\text{-}P}$ 12.0, $^3J_{H\text{-}H}$ 7.0, H^9), 7.53 (1 H, t, $^3J_{H\text{-}H}$ 7.0, H^{11}), 7.45 (2 H, dt, $^3J_{H\text{-}H}$ 7.0, $^4J_{H\text{-}P}$ 3.5, H^{10}), 7.41 (1 H, s, H^5), 6.10 (2 H, s, H^{18}), 4.73 (2 H, d, $^5J_{H\text{-}P}$ 5.0, H^7), 4.19-4.08 (2 H, m, H^{12}), 3.88 (6 H, s, H^{20}), 3.84 (3 H, s, H^{21}), 1.36 (3 H, t, $^3J_{H\text{-}H}$ 7.0, H^{13}); ^{13}C NMR (176 MHz, $CDCl_3$) δ 163.0 (C^{19}), 162.9 (C^{17}), 160.0 (d, $^3J_{C\text{-}P}$ 19, C^6), 152.8 (d, $^1J_{C\text{-}P}$ 167, C^2), 133.9 (d, $^3J_{C\text{-}P}$ 12, C^4), 132.7 (d, $^4J_{C\text{-}P}$ 3, C^{11}), 132.4 (d, $^2J_{C\text{-}P}$ 10, C^9), 129.9 (d, $^1J_{C\text{-}P}$ 140, C^8), 128.8 (d, $^2J_{C\text{-}P}$ 23, C^3), 128.5 (d, $^3J_{C\text{-}P}$ 13, C^{10}), 123.9 (d, $^4J_{C\text{-}P}$ 3, C^5), 93.5 (d, $^4J_{C\text{-}P}$ 2, C^{14}), 93.2 (C^{16}), 90.6 (C^{18}), 89.9 (C^{15}), 63.9 (C^7), 62.0 (d, $^2J_{C\text{-}P}$ 6, C^{12}), 56.2 (C^{20}), 55.6 (C^{21}), 16.6 (d, $^3J_{C\text{-}P}$ 6, C^{13}); ^{31}P NMR (162 MHz, $CDCl_3$) δ +25.8; LCMS (ESI^+) *m/z* 468 $[M+H]^+$; HRMS (ESI^+) *m/z* 468.1568 $[M+H]^+$ ($C_{25}H_{27}NO_6P$) requires 468.1576.

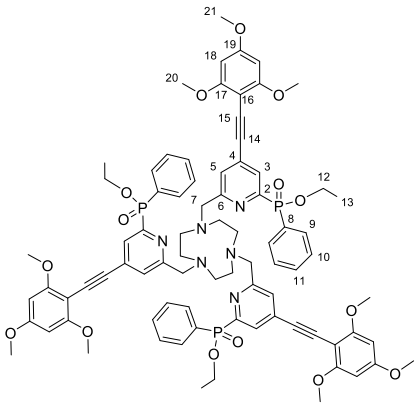
{4-[2-(2,4,6-Trimethoxyphenyl)ethynyl]-6-[ethoxy(phenyl)phosphoryl]pyridin-2-yl}methyl methanesulfonate (6)



Ethyl [6-(hydroxymethyl)-4-[2-(2,4,6-trimethoxyphenyl)ethynyl] pyridin-2-yl](phenyl)phosphinate **5** (41 mg, 0.088 mmol) was dissolved in anhydrous tetrahydrofuran (2 mL) and the solution was cooled in an ice bath. Triethylamine (25 µL, 0.18 mmol) was added followed by dropwise addition of methanesulfonyl chloride (10 µL, 0.13 mmol), which resulted in the formation of a precipitate. The reaction was stirred under argon for 45 min and the solvent was removed under reduced pressure. The residue was redissolved in

dichloromethane (20 mL) and washed with saturated brine solution (20 mL). The aqueous solution was re-extracted with dichloromethane (3 x 20 mL) and the organic layers combined, dried with $MgSO_4$, filtered and concentrated under reduced pressure to give **6** as a pale yellow oil (48 mg, quant.) which was used without further purification; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (1 H, d, $^3J_{H\text{-}P}$ 6.0, H^3), 7.98-7.89 (2 H, m, H^9), 7.55 (1 H, s, H^{11}), 7.45 (2 H, d, $^4J_{H\text{-}P}$ 4.0, H^{10}), 7.43 (1 H, s, H^5), 6.08 (2 H, s, H^{18}), 5.28 (2 H, s, H^7), 4.11 (2 H, q, $^3J_{H\text{-}H}$ 7.0, H^{12}), 3.87 (6 H, s, H^{20}), 3.82 (3 H, s, H^{21}), 2.94 (3 H, s, H^{22}), 1.36 (3 H, t, $^3J_{H\text{-}H}$ 7.0, H^{13}); LCMS (ESI^+) *m/z* 546 $[M+H]^+$; HRMS (ESI^+) *m/z* 546.1345 $[M+H]^+$ ($C_{26}H_{29}NO_8PS$) requires 546.1352.

1,4,7-Tris({4-[2-(2,4,6-trimethoxyphenyl)ethynyl]-6-[ethoxy(phenyl)phosphoryl]pyridin-2-yl}methyl)-1,4,7-triazacyclononane (tri-ethyl ester of L^1)

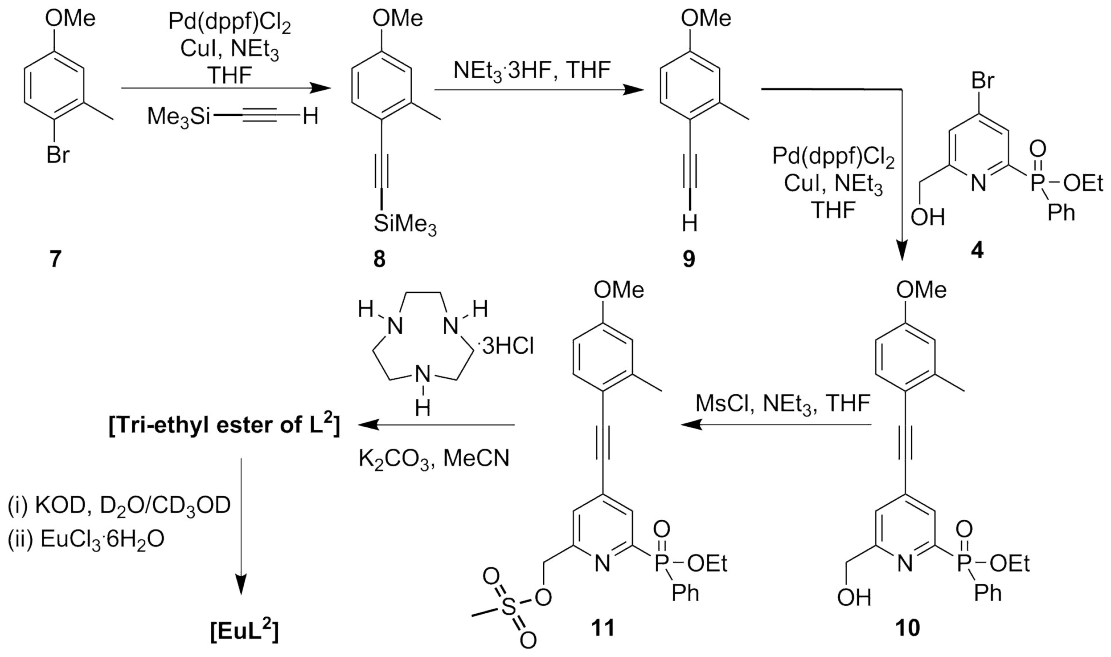


1,4,7-Triazacyclononane trihydrochloride (6.5 mg, 0.027 mmol) and potassium carbonate (15.0 mg, 0.109 mmol) were dissolved in anhydrous acetonitrile (1 mL). {4-[2-(2,4,6-Trimethoxyphenyl)ethynyl]-6-[ethoxy(phenyl)phosphoryl]pyridin-2-yl}methyl methanesulfonate **6** (48 mg, 0.088 mmol) was added as a solution in anhydrous acetonitrile (1 mL). The solution was stirred at 65 °C under argon for 16 h, at which time the solution was cooled to room temperature, and the solution decanted from excess potassium salts and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (neat CH_2Cl_2 to 10% CH_3OH in CH_2Cl_2) to give the ligand as a yellow film (7.1 mg, 18%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.12 (3 H, d, $^3J_{H\text{-}P}$ 6.5, H^3), 7.85 (6 H, dd, $^3J_{H\text{-}P}$ 12.0, $^3J_{H\text{-}H}$ 7.0, H^9), 7.50 (3 H, t, $^3J_{H\text{-}H}$ 7.0, H^{11}), 7.44-7.40 (9 H , m, H^5 + H^{10}, peaks overlap), 6.11 (6 H, s, H^{18}), 4.14-4.09 (6 H, m, H^{12}), 3.90-3.89 (24 H, overlapping s, H^7 + H^{20}), 3.85 (9 H, s, H^{21}), 3.03-2.65 (12 H, m, ring CH_2), 1.34 (9 H, t, $^3J_{H\text{-}H}$ 7.0, H^{13}); ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.2 (C^{19}), 163.1 (C^{17}), 159.9 (C^6), 154.4 (d, $^1J_{C\text{-}P}$ 168, C^2), 134.2 (d, $^3J_{C\text{-}P}$ 15, C^4), 132.8 (C^{11}), 132.4 (d, $^2J_{C\text{-}P}$ 10, C^9), 130.5 (C^8), 129.5 (C^{10}), 128.6 (d, $^2J_{C\text{-}P}$ 12, C^3), 127.3 (C^5), 93.3 (C^{14}), 93.2 (C^{16}), 90.7 (C^{15}), 90.6 (C^{18}), 61.9 (d, $^2J_{C\text{-}P}$ 6, C^{12}), 59.6 (C^7), 56.2 (C^{20}), 55.7 (C^{21}), 51.5-45.4 (ring CH_2), 16.7

(d, $^{3}J_{C\text{-}P}$ 6, C^{13}); ^{31}P NMR (243 MHz, $CDCl_3$) δ +25.7; LCMS (ESI^+) *m/z* 1478 $[M+H]^+$; HRMS (ESI^+) *m/z* 1477.551 $[M+H]^+$ ($C_{81}H_{88}N_6O_{15}P_3$) requires 1477.552.

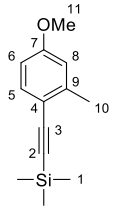
[Eu.L^1]

1,4,7-Tris({4-[2-(2,4,6-trimethoxyphenyl)ethynyl]-6-[ethoxy(phenyl)phosphoryl]pyridin-2-yl}methyl)-1,4,7-triazacyclononane (tri-ethyl ester of L^1) (7 mg, 4.8 µmol) was dissolved in CD_3OD (0.5 mL) and a solution (0.3 mL) of 0.05 M potassium hydroxide in D_2O was added. The solution was stirred at 60 °C under argon for 4 days at which point further potassium hydroxide (1 mg) was added. The solution was stirred for a further 4 days. Hydrolysis of the phosphinate esters was monitored by ^{31}P NMR (starting material: +25.7 ppm, product: +16.5 ppm). After hydrolysis was complete, $EuCl_3{\cdot}6H_2O$ (1.8 mg, 4.9 µmol) was added and the reaction stirred at 60 °C for 18 h. The solution was then neutralised with dilute HCl and the solvent removed under reduced pressure. The crude product was purified by RP-HPLC (Method A, t_R = 13.5 min) to give a colourless solid (2.3 mg, 30%); LCMS (ESI^+) *m/z* 1548 $[M(^2H_5)+H]^+$; λ_{max} (MeOH) = 356 nm; τ_{Eu} (MeOH) = 1.18 ms; Φ^{em}_{MeOH} = 50 (±5)%. Chiral separation was achieved using a ChiralPak-IC column (10 x 250 mm, 5 µm, isocratic MeOH, 4.4 mL/min) t_R = 18.7 & 27.2 min.



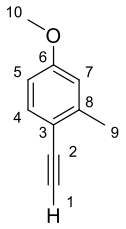
Scheme S2 *The synthesis of [Eu.L^2].*

[2-(4-Methoxyphenyl)ethynyl]trimethylsilane (8)



4-Bromo-3-methylanisole **7** (0.500 g, 2.49 mmol) was dissolved in anhydrous tetrahydrofuran (18 mL) and the solution was degassed (freeze-pump-thaw cycle x 3). Triethylamine (1.73 mL, 12.5 mmol) and ethynyltrimethylsilane (1.05 mL, 7.47 mmol) were added and the solution was degassed again (freeze-pump-thaw x 3). [1,1-Bis(diphenylphosphino)ferrocene] dichloropalladium(II) (108 mg, 0.25 mmol) and copper (I) iodide (48 mg, 0.25 mmol) were added and the solution was stirred at 65 °C under argon for 90 h. The solvent was removed under reduced pressure and the brown residue was purified by silica gel column chromatography (neat hexane to 12% dichloromethane in hexane) to yield the desired product **8** as a yellow oil (0.54 g, quant.); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.36 (1 H, d, $^{3}J_{H\text{-}H}$ 8.5, H^{5}), 6.72 (1 H, d, $^{4}J_{H\text{-}H}$ 2.0, H^{8}), 6.65 (1 H, dd, $^{3}J_{H\text{-}H}$ 8.5, $^{4}J_{H\text{-}H}$ 2.0, H^{6}), 3.79 (3 H, s, H^{11}), 2.41 (3 H, s, H^{10}), 0.25 (9 H, s, H^{1}); ^{13}C NMR (176 MHz, $CDCl_3$) δ 159.8 (C^{7}), 142.6 (C^{9}), 133.6 (C^{5}), 115.4 (C^{4}), 115.1 (C^{8}), 111.2 (C^{6}), 104.3 (C^{3}), 96.5 (C^{2}), 55.3 (C^{11}), 21.1 (C^{10}), 0.3 (C^{1}); GCMS-EI *m/z* 218 $[M]^{+}$.

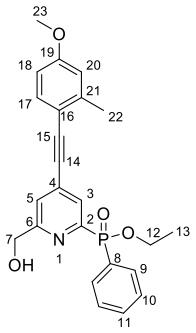
1-Ethynyl-4-methoxy-2-methylbenzene (9)



[2-(4-Methoxyphenyl)ethynyl]trimethylsilane **8** (0.542 g, 2.49 mmol) was dissolved in anhydrous tetrahydrofuran (9 mL) and triethylamine trihydrofluoride (4.1 mL, 25 mmol) was added. The resulting orange solution was stirred at 35 °C for 140 h after which the solvent was removed under reduced pressure. The residue was redissolved in water (20 mL) and this solution was extracted with toluene (3 x 20 mL). The organic layers were combined, dried with $MgSO_4$, filtered, combined and concentrated to give **9** as a red oil (0.260 g, 71%); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.39 (1 H, d, $^{3}J_{H\text{-}H}$ 8.5, H^{4}), 6.74 (1 H, d, $^{4}J_{H\text{-}H}$ 3.0, H^{7}), 6.68 (1 H, dd, $^{3}J_{H\text{-}H}$ 8.5, $^{4}J_{H\text{-}H}$ 3.0, H^{5}), 3.80 (3 H, s, H^{10}), 3.19 (1 H, s, H^{1}), 2.43 (3 H, s, H^{9}); ^{13}C NMR (176 MHz,

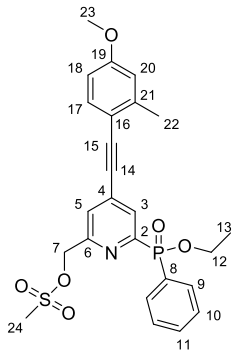
$CDCl_3$) δ 160.0 (C^6), 142.7 (C^8), 134.0 (C^4), 115.2 (C^7), 114.3 (C^3), 111.4 (C^5), 82.7 (C^2), 79.6 (C^1), 53.4 (C^{10}), 21.0 (C^9); GCMS-EI *m/z* 146 $[M]^+$.

Ethyl-[6-(hydroxymethyl)-4-[2-(4-methoxy-2-methylphenyl)ethynyl]-pyridin-2-yl](phenyl)phosphinate (10)



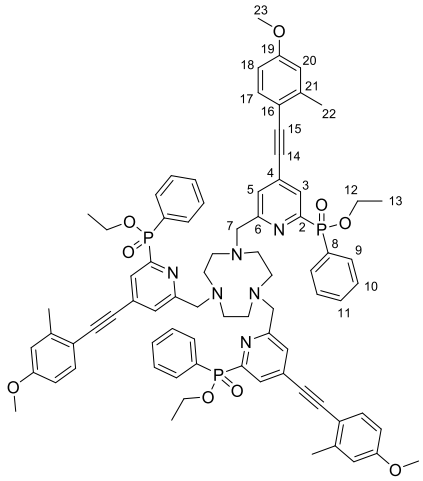
1-Ethynyl-4-methoxy-2-methylbenzene **9** (100 mg, 0.684 mmol) and ethyl [4-bromo-6-(hydroxymethyl)] pyridin-2-yl](phenyl)phosphinate **4** (221 mg, 0.622 mmol) were dissolved in anhydrous tetrahydrofuran (4.5 mL) and the solution was degassed (freeze-pump-thaw cycle x 3). Triethylamine (435 µL, 316 mg, 3.12 mmol) was added and the solution was further degassed (freeze-pump-thaw x 3). [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (51 mg, 0.062 mmol) and copper (I) iodide (24 mg, 0.12 mmol) were added and the solution was stirred at 65 °C under argon for 24 h. The solvent was removed under reduced pressure and the brown residue was purified by silica gel column chromatography (neat CH_2Cl_2 to 3% CH_3OH in CH_2Cl_2) to yield a brown oil. This was further purified by silica gel column chromatography (50:50 ethyl acetate:hexane to 80:20 ethyl acetate:hexane) to yield the desired product **10** as a yellow oil (0.192 g, 73%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07 (1 H, br s, H^3), 7.97 (2 H, dd, $^3J_{H\text{-}P}$ 11.5, $^3J_{H\text{-}H}$ 8.5, H^9), 7.54 (1 H, t, $^3J_{H\text{-}H}$ 7.0, H^{11}), 7.45-7.48 (2 H, m, H^{10}), 7.42 (1 H, d, $^3J_{H\text{-}H}$ 8.5, H^{17}), 7.40 (1 H, s, H^5), 6.77 (1 H, d, $^4J_{H\text{-}H}$ 2.5, H^{20}), 6.73 (1 H, dd, $^3J_{H\text{-}H}$ 8.5, $^4J_{H\text{-}H}$ 2.5, H^{18}), 4.76 (2 H, s, H^7), 4.09-4.19 (2 H, m, H^{12}), 3.81 (3 H, s, H^{23}), 2.47 (3 H, s, H^{22}), 1.38 (3 H, t, $^3J_{H\text{-}H}$ 7.0, H^{13}); ^{13}C NMR (151 MHz, $CDCl_3$) δ 160.7 (C^{19}), 160.4 (d, $^3J_{C\text{-}P}$ 19, C^6), 153.3 (d, $^1J_{C\text{-}P}$ 165, C^2), 143.0 (C^{21}), 134.1 (C^{17}), 133.3 (d, $^3J_{C\text{-}P}$ 12, C^4), 132.7 (C^{11}), 132.4 (d, $^2J_{C\text{-}P}$ 10, C^9), 129.8 (d, $^1J_{C\text{-}P}$ 141, C^8), 128.7 (C^3), 128.6 (d, $^3J_{C\text{-}P}$ 13, C^{10}), 123.9 (C^5), 115.4 (C^{20}), 113.8 (C^{16}), 111.7 (C^{16}), 95.3 (C^{15}), 89.1 (C^{14}), 64.0 (C^7), 62.0 (C^{12}), 55.4 (C^{23}), 21.1 (C^{22}), 16.7 (C^{13}) ; ^{31}P (243 MHz, $CDCl_3$) δ +25.5; LCMS (ESI^+) *m/z* 422 $[M+H]^+$; HRMS (ESI^+) *m/z* 422.1528 $[M+H]^+$ ($C_{24}H_{25}NO_4P$) requires 422.1521.

{4-[2-(4-Methoxy-2-methylphenyl)ethynyl]-6-[ethoxy(phenyl)phosphoryl]pyridin-2-yl}methyl methanesulfonate (11)



Ethyl [6-(hydroxymethyl)-4-[2-(4-methoxy-2-methylphenyl)ethynyl] pyridin-2-yl](phenyl)phosphinate **10** (96 mg, 0.23 mmol) was dissolved in anhydrous tetrahydrofuran (3 mL) and the solution was cooled in an ice bath. Triethylamine (64 µL, 0.46 mmol) was added followed by dropwise addition of methanesulfonyl chloride (27 µL, 0.34 mmol), which resulted in the formation of a precipitate. The reaction was stirred under argon for 45 min and the solvent was then removed under reduced pressure. The residue was redissolved in dichloromethane (20 mL) and washed with saturated brine solution (30 mL). The aqueous solution was re-extracted with dichloromethane (3 x 20 mL) and the organic layers combined, dried with $MgSO_4$, filtered and concentrated under reduced pressure to give the product **11** as a pale yellow oil (115 mg) which was used immediately without further purification; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (1 H, d, $^{3}J_{H\text{-}P}$ 6.0, H^{3}), 7.96 (2 H, dd, $^{3}J_{H\text{-}P}$ 12.0, $^{3}J_{H\text{-}H}$ 7.0, H^{9}), 7.54 (1 H, br s, H^{11}), 7.49-7.46 (2 H, m, H^{10}), 7.45 (1 H, br s, H^{17}), 7.42 (1 H, s, H^{5}), 6.77 (1 H, d, $^{4}J_{H\text{-}H}$ 2.0, H^{20}), 6.73 (1 H, dd, $^{3}J_{H\text{-}H}$ 8.5, $^{4}J_{H\text{-}H}$ 2.0, H^{18}), 5.32 (2 H, s, H^{7}), 4.14 (2 H, q, $^{3}J_{C\text{-}P}$ 7.0, H^{12}), 3.81 (3 H, s, H^{23}), 2.99 (3 H, s, H^{24}), 2.48 (3 H, s, H^{22}), 1.37 (3 H, t, $^{3}J_{H\text{-}H}$ 7.0, H^{13}); ^{31}P NMR (162 MHz, $CDCl_3$) δ +24.9; LCMS (ESI^+) *m/z* 500$[M+H]^+$; HRMS (ESI^+) *m/z* 500.1286 $[M+H]^+$ ($C_{25}H_{27}NO_6PS$) requires 500.1297.

1,4,7-Tris({4-[2-(4-methoxy-2-methylphenyl)ethynyl]-6-[ethoxy(phenyl)phosphoryl]pyridin-2-yl}methyl)-1,4,7-triazacyclononane (tri-ethyl ester of L^2)



{4-[2-(4-Methoxy-2-methylphenyl)ethynyl]-6-[ethoxy(phenyl)phosphoryl]pyridin-2-yl}methyl methanesulfonate **11** (114 mg, 0.228 mmol), 1,4,7-triazacyclononane trihydrochloride (16.5 mg, 0.0691 mmol) and potassium carbonate (189 mg, 1.37 mmol) were dissolved in anhydrous acetonitrile (5 mL). The solution was stirred at 65 °C under argon for 66 h, at which time the solution was cooled to room temperature, and the solution decanted from excess potassium salts and concentrated under reduced pressure. Attempts to find a suitable solvent system for column chromatography were unsuccessful so the crude product (66 mg) that was taken on without further purification; ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.03 (3 H, d, $^3J_{H\text{-}P}$ 5.5, H^3), 7.95 (6 H, dd, $^3J_{H\text{-}P}$ 12.0, $^3J_{H\text{-}H}$ 7.5, H^9), 7.56 (3 H, s, H^5), 7.44-7.41 (3 H , m, H^{11}), 7.39 (3 H, d, $^3J_{H\text{-}H}$ 7.5, H^{17}), 7.37-7.36 (6 H, m, H^{10}), 6.74 (3 H, d, $^4J_{H\text{-}H}$ 2.0, H^{20}), 6.70 (3 H, dd, $^3J_{H\text{-}H}$ 7.5, $^4J_{H\text{-}H}$ 2.0, H^{18}), 4.13-4.06 (6 H, m, H^{12}), 3.83 (6 H, s, H^7), 3.79 (9 H, s, H^{23}), 2.75 (12 H, br s, ring CH_2), 2.43 (9 H, s, H^{22}), 1.32 (9 H, t, $^3J_{H\text{-}H}$ 7.0, H^{13}); ^{13}C NMR (176 MHz, $CDCl_3$) δ 161.5 (d, $^3J_{C\text{-}P}$ 20, C^6), 160.4 (C^{19}), 153.8 (d, $^1J_{C\text{-}P}$ 167, C^2), 142.8 (C^{21}), 133.8 (C^{17}), 132.5 (d, $^3J_{C\text{-}P}$ 12, C^4), 132.4 (d, $^2J_{C\text{-}P}$ 10, C^9), 132.3 (d, $^4J_{C\text{-}P}$ 2, C^{11}), 130.1 (d, $^1J_{C\text{-}P}$ 139, C^8), 128.2 (d, $^3J_{C\text{-}P}$ 13, C^{10}), 127.9 (d, $^2J_{C\text{-}P}$ 24, C^3), 126.3 (C^5), 115.2 (C^{20}), 114.0 (C^{16}), 111.5 (C^{18}), 94.5 (C^{15}), 89.5 (C^{14}), 63.7 (C^7), 61.6 (d, $^2J_{C\text{-}P}$ 6, C^{12}), 55.6 (br s, ring CH_2), 55.3 (C^{23}), 21.0 (C^{22}), 16.5 (d, $^3J_{C\text{-}P}$ 6, C^{13}); ^{31}P NMR (283 MHz, $CDCl_3$) δ +25.5; LCMS (ESI^+) *m/z* 1340 $[M+H]^+$; HRMS (ESI^+) *m/z* 1339.538 $[M+H]^+$ ($C_{78}H_{82}N_6O_9P_3$) requires 1339.536.

[EuL^2]

1,4,7-Tris({4-[2-(4-methoxy-2-methylphenyl)ethynyl]-6-[ethoxy(phenyl)phosphoryl]pyridin-2-yl}methyl)-1,4,7-triazacyclononane (18.4 mg, 0.0137 mmol) and potassium hydroxide (6 mg, 0.1 mmol) were dissolved in a D_2O/CD_3OD mixture (2 mL, v/v 3:1) and stirred at 60 °C under argon for 20 days. Phosphinate ester hydrolysis was confirmed by ^{31}P NMR (starting material: +25.5 ppm, product: +15.5 ppm). $EuCl_3 \cdot 6H_2O$ (6 mg, 0.0164 mmol) was added and the reaction stirred at 60 °C for 19 h. The solution was then neutralised with dilute HCl and the solvent removed under reduced pressure. The crude product was purified by RP-HPLC (Method A, t_R = 14.5 min) to give a white solid (5.2 mg, 29%); LCMS (ESI$^+$) *m/z* 1403 $[M+H]^+$; HRMS (ESI$^+$) *m/z* 1403.341 $[M(^{151}Eu)+H]^+$ ($C_{72}H_{67}N_6O_9P_3{}^{151}Eu$) requires 1403.338; λ_{max} (MeOH) = 343 nm; τ_{Eu} (MeOH) = 1.22 ms; Φ^{em}_{MeOH} = 47 (±5)%. Chiral separation was achieved using a ChiralPak ID column (10 x 250 mm, 5 µm, isocratic MeOH, 4.4 mL/min) t_R = 19.2 & 34.3 min.

Circularly Polarised Luminescence

As discussed in the main text, the less commonly observed $^5D_0 - {}^7F_5$ transition was observed in the CPL of [Eu.L^2], along with transitions from $^5D_1$ to various ^7F_J states. These transitions are expanded in Figure S6.

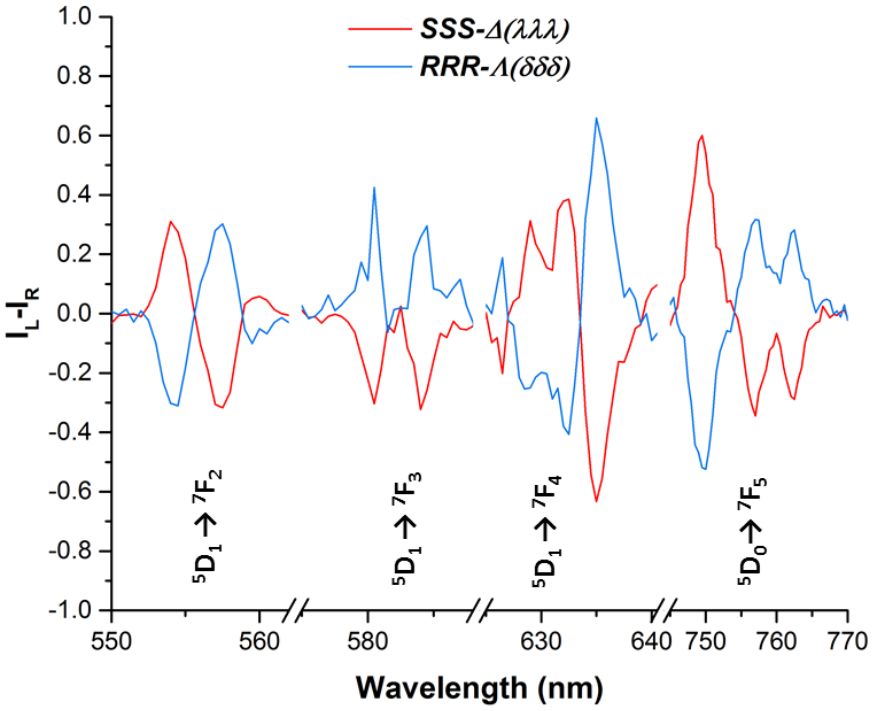


Figure S6 Expanded CPL spectra of [Eu.L^2] showing less commonly observed transitions.

CPL spectra were recorded for [Eu.L^1] and [Eu.L^4] in addition to [Eu.L^2]. Whilst the spectra are similar in many respects, the ΔJ = 1 manifold (585-600 nm) varies considerably between the methyl- and phenyl-phosphinates. In the phenyl phosphinates, all transitions are of the same handedness, while in the methyl phosphinates transitions of opposite handedness are observed.

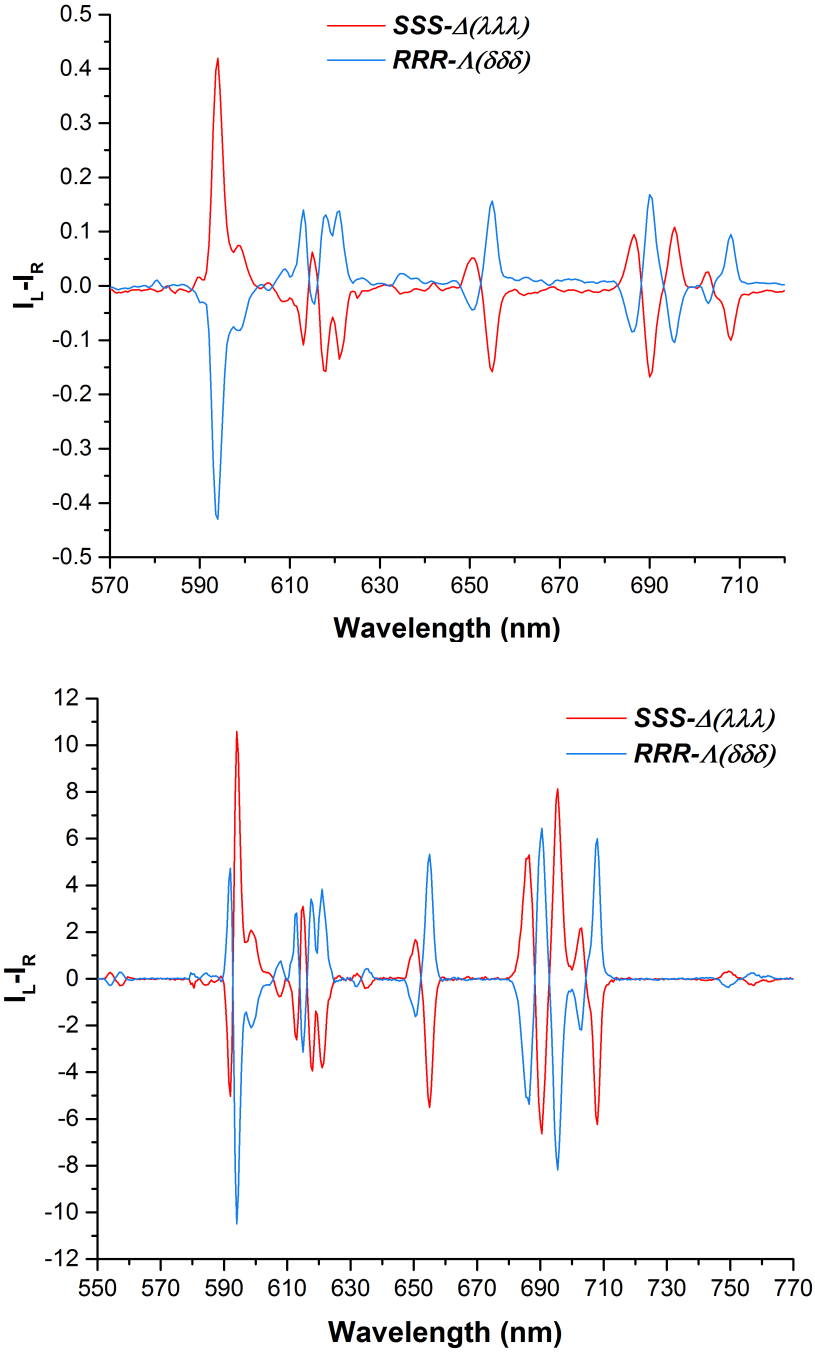


Figure S7 The CPL spectra for [Eu.L^1] (top) and [Eu.L^4] (bottom) (red – Δ, blue – Λ, 5 μM complex, 5 scan average, 295 K, MeOH).

Emission dissymetry factors, g_{em}, for various transitions in [Eu.L^1], [Eu.L^2] and [Eu.L^4] are shown in Table S1.

		Table S1 *Selected g_{em} values for [Eu.L^{1,2,4}]*					
		g_{em} / arb					
λ / nm		**554**	**557**	**599**	**655**	**708**	**757**
[Eu.L^1]	***Δ***	n.d.	n.d.	+0.10	-0.12	-0.23	n.d.
	Λ	n.d.	n.d.	-0.12	+0.14	+0.25	n.d.
[Eu.L^2]	***Δ***	+0.13	-0.19	+0.15	-0.19	-0.32	-0.14
	Λ	-0.10	+0.18	-0.14	+0.18	+0.29	+0.12
[Eu.L^4]	***Δ***	+0.15	-0.22	+0.11	-0.19	-0.31	-0.12
	Λ	-0.13	+0.21	-0.11	+0.18	+0.32	+0.12

CPL Spectra (Figure S9) of the samples of the complex impregnated onto paper (as used for the imaging experiments) were recorded: the paper sample was aligned at 45° to the incident light and the emission collected at 90°.

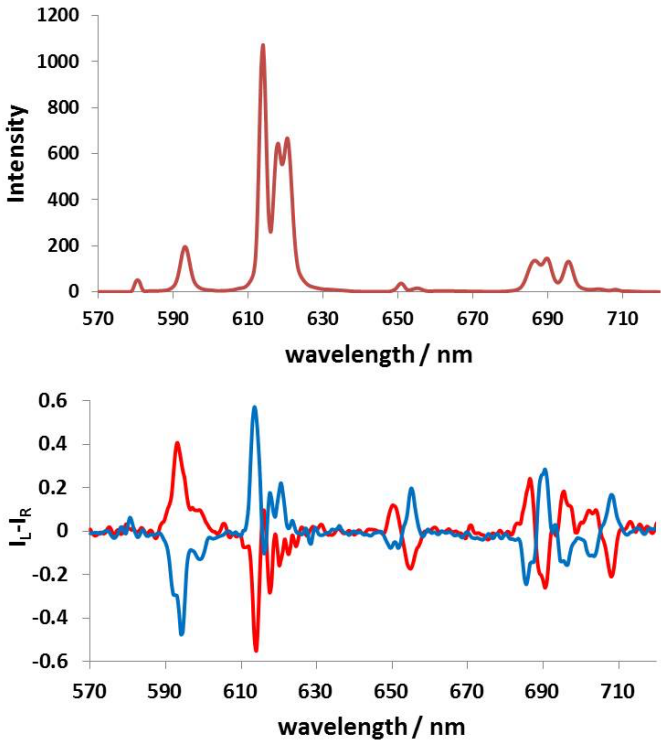


Figure S8 Total emission (upper) and CPL spectra (sum of 3 scans, excited at 342 nm) of [Eu.L^2] (red: Δ; blue: Λ) impregnated in separate regions on paper, *as used* in the imaging experiments described in Figure 3 (main paper). The *g* value for the emission band at 599 nm is 0.11 under the experimental set-up used.

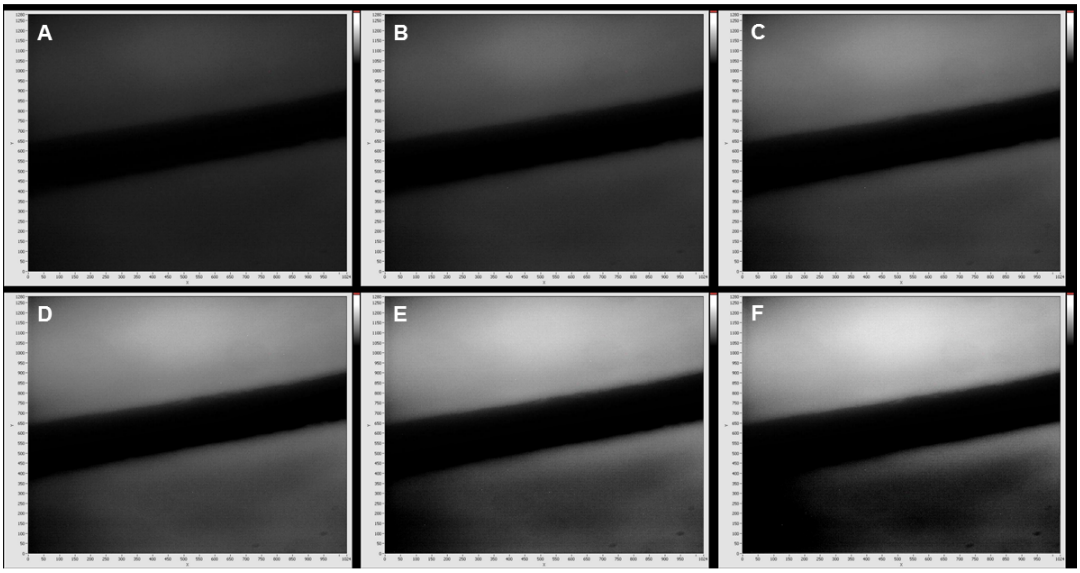


Figure S9. Time dependent image acquisition and the evolution of the contrast ratio with accumulation number in time-resolved microscopy images of (*top*) Λ - [Eu.L^2] and (*bottom*) Δ - [Eu.L^2], following sample excitation using a 365 nm UV-LED ($t_{acq.}$ = 7.2 ms/frame, t_d = 6 µs, λ_{em} BP-589/20/395DC using the Right (Horizontal polarisation) circularly polarised light channel (A) 50 frame accumulations; contrast ratio, CR $_{\Lambda:\Delta}$ = 1.43 : 1), (B) 100 frame accumulations; contrast ratio, CR $_{\Lambda:\Delta}$ = 1.73 : 1), (C) 150 frame accumulations; contrast ratio, CR $_{\Lambda:\Delta}$ = 1.83 : 1), (D) 200 frame accumulations; contrast ratio, CR $_{\Lambda:\Delta}$ = 2.01 : 1), (E) 300 frame accumulations; contrast ratio, CR $_{\Lambda:\Delta}$ = 2.37 : 1) ; (F) 397 frame accumulations; contrast ratio, CR $_{\Lambda:\Delta}$ = 3.42 : 1),

Racemisation Study

In order to assess the stability of the resolved enantiomers to racemisation, the enantiopure complex was stirred in methanol solution at 60 °C. At regular intervals, small aliquots were analysed by analytical chiral HPLC (ChiralPak ID, 4.6 x 250 mm) to assess the relative ratio of the two enantiomers (see Fig. S11).

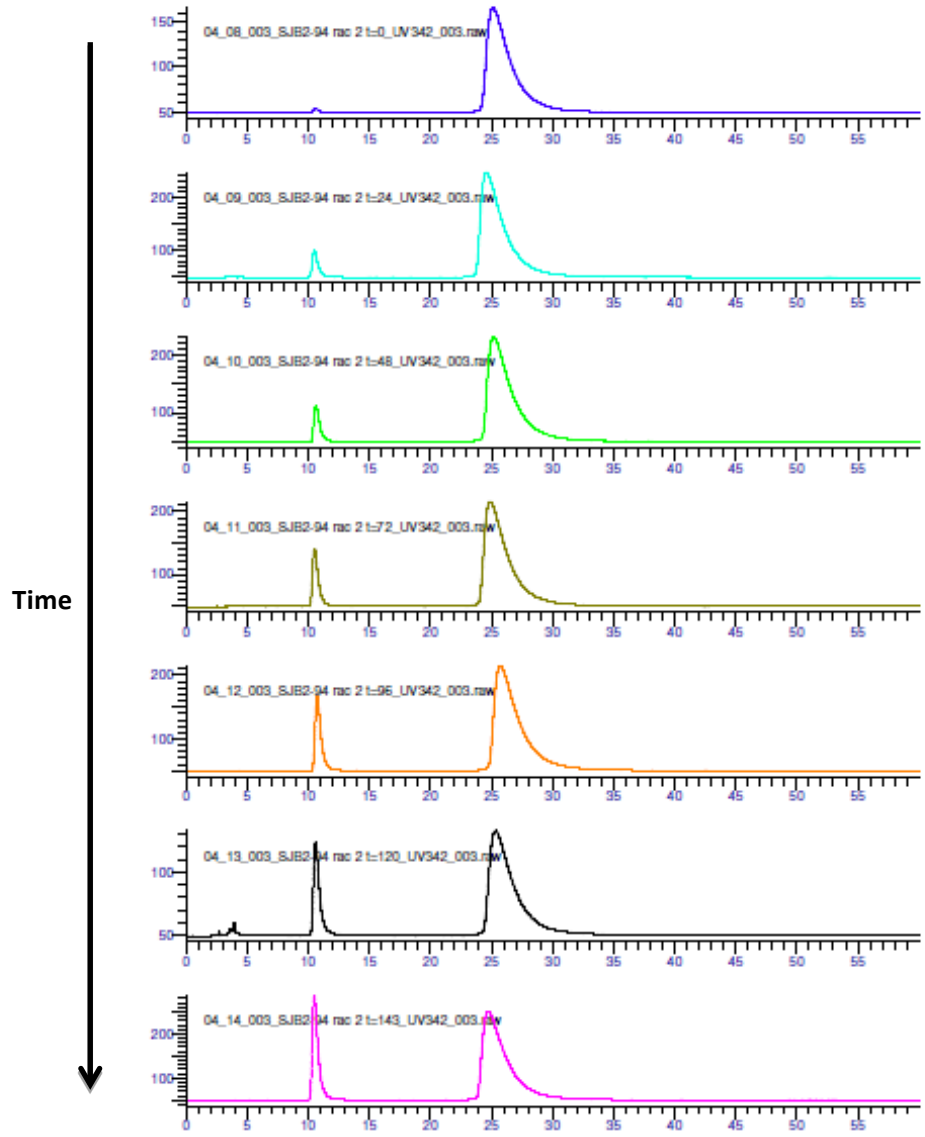
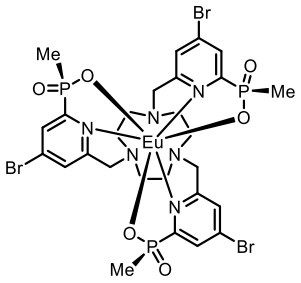


Figure S10 Chiral HPLC traces showing appearance of the second enantiomer over time.

Treating the process as first order with respect to complex concentration allows a half life to be calculated for the process. For [Eu.L^4] this was found to be 410 ± 20 h compared to 185 ± 20 h for the parent complex [Eu.L^7].[3]

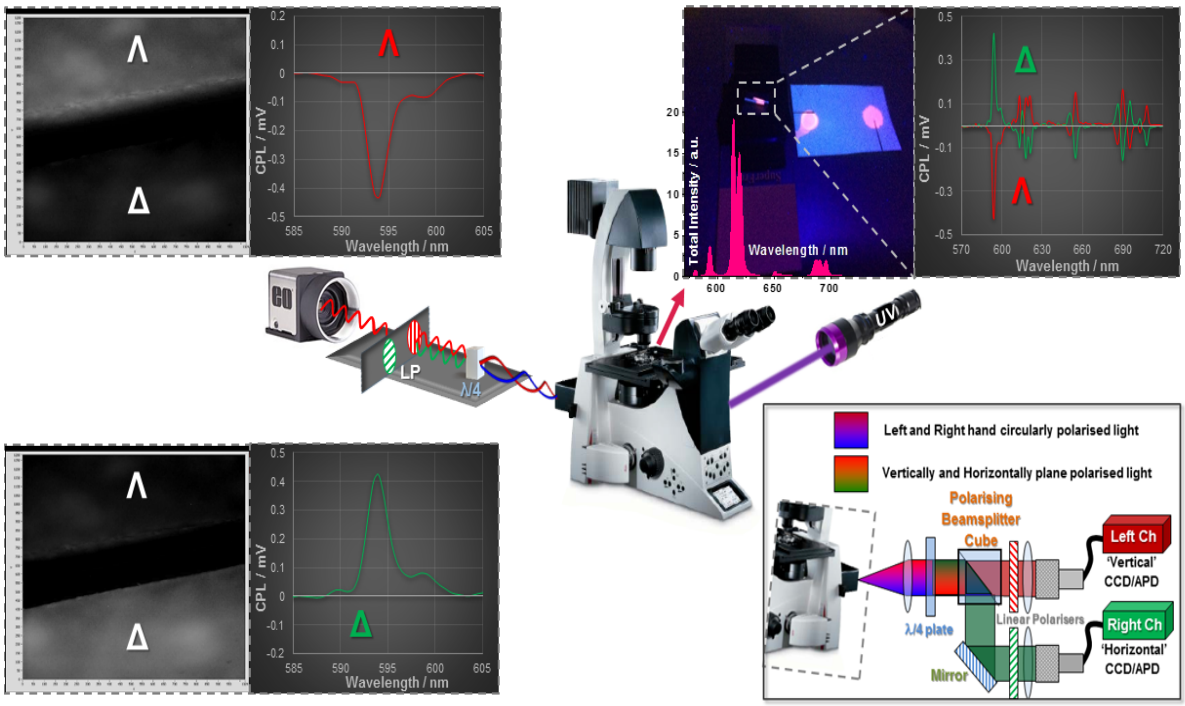


[Eu.L^7]

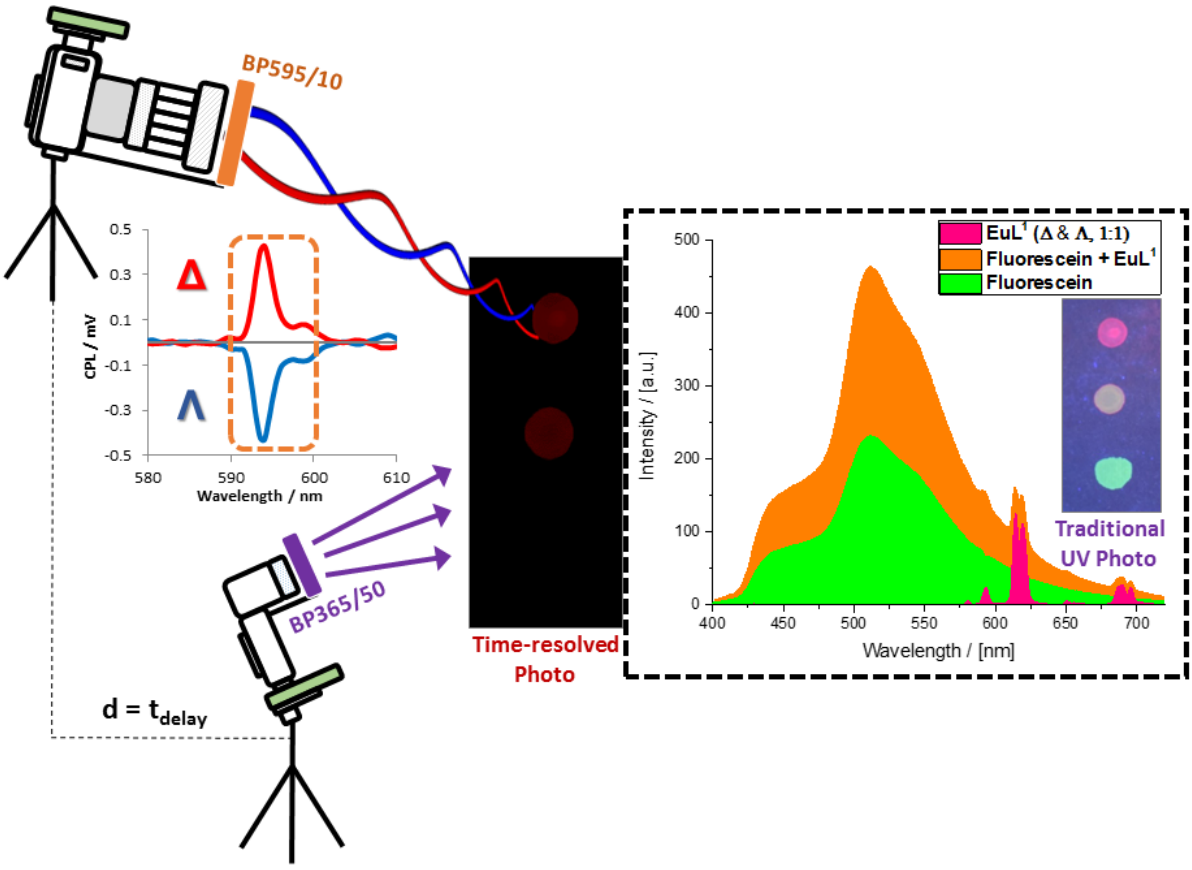
References

1. *GB Patent Appl.,* GB1507296.0, 2015, R. Pal, L.O. Pålsson and A. Beeby.
2. M. V. R. Reddy, P. Venkatapuram, M. R. Mallireddigari, V. R. Pallela, S. C. Cosenza, K. A. Robell, B. Akula, B. S. Hoffman and E. P. Reddy, *J. Med. Chem.*, 2011, **54**, 6254-6276.
3. N. H. Evans, R. Carr, M. Delbianco, R. Pal, D. S. Yufit and D. Parker, *Dalton Trans.*, 2014, **42**, 15610-15616.

Schemes 1 and 2 (main text)



Scheme 1 Schematic set-up and proof-of-concept for time-resolved image separation using off-the shelf DSLR equipment. The three spots in the paper sample are: *L* & *D*-[EuL^1] (1:1) (*top*); *L* & *D*-[EuL^1] (1:1) and fluorescein (*centre*) and fluorescein only (*bottom*). The time resolved image cuts out the fluorescein emission; the applied band-pass filter selects the spectral part of interest, as further separated with respect to sign by the parallel chiroptical image analysis (*in microscopy, see Figure 3, main text*).



Scheme 2 Schematic set-up and proof-of-concept for CPL microscopy consisting of a Zeiss Axiovert 200M epifluorescence setup, coupled to a 365nm UV LED (Nichia, 24V, 1.2W) and EO-1312M (Edmund Optics) camera. Chiroptical signal translation and separation is facilitated using a broad *λ*/4 wave plate with a pair of linear polarisers (40000:1 extinction ratio) that allow differentiation of vertically and horizontally polarised linear light. Here we used a pair of pre-aligned 1" optical polarisers in a 90° orientation. Images shown are: (*top left*) 3E and (*bottom left*) 3F coupled to their corresponding CPL spectra with respect to the 589/20 band pass filter used in the detection arm; *(bottom right)* schematic layout of a dual channel chiroptical separator assembly based on an LSCM setup, to aid optical sectioning and increased optical resolution.