Electronic Supplementary Information

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Contents

Experimental References

The compounds **1**,[a] **4**,[b] **11**,[c] **16**,[d] and **Eu*L*^{1a}** [a] were prepared using previously reported procedures**.**

a) M. Starck, J. D. Fradgley, R. Pal, J. M. Zwier, L. Lamarque and D. Parker, *Chem. Eur. J.* 2021*,* **27**; 766–777; doi.org/10.1002/chem.202003992.

b) J. W. Walton, R. Carr, N. H. Evans, A. M. Funk, A. M. Kenwright, D. Parker, D. S. Yufit, M. Botta, S. De Pinto and K.-L. Wong, *Inorg. Chem.*, 2012, **51**, 8042–8056; doi.org/10.1021/ic300147p.

c) S. J. Butler, M. Delbianco, L. Lamarque, B. K. McMahon, E. R. Neil, R. Pal, D. Parker, J. W. Walton and J. M. Zwier, *Dalton Trans.* 2015,**44**, 4791–4803; https://doi.org/10.1039/C4DT02785J

d) M. Starck, J. D. Fradgley, S. Di Vita, J. A. Mosely, R. Pal and D. Parker, *Bioconjugate Chem*. 2020, 31, 229–240; 10.1021/acs.bioconjchem.9b00735.

e) K. Nakamaru, *Bull. Chem. Soc. Jpn.,* 1982, **55**, 2697–2705.

Experimental Information

General Procedures

Commercially available reagents were used as received. Solvents were laboratory grade and were dried over appropriate drying agents when required. 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 F254 (Merck), and visualised by UV irradiation at 254 nm or 366 nm. Preparative flash column chromatography was performed using flash silica gel 60 (230-400 mesh) from Merck or Fluorochem.

^{1}H, ^{13}C, ^{19}F, ^{29}Si, and ^{31}P NMR spectra were recorded in commercially available deuterated solvents on a Bruker Avance-400 (^{1}H at 400.06 MHz, ^{13}C at 100.61 MHz, ^{19}F at 376.50 MHz, ^{29}Si at 79.49 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 and 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 or acetonitrile were used as the carrier solvents.

HPLC Analysis

Reverse phase HPLC (RP-HPLC), unless stated otherwise, was performed at 295 K using a Shimadzu system comprising of a Degassing Unit (DGU-20A5R), a Prominence Preparative Liquid Chromatography pump (LC-20AP), a Prominence UV-Vis Detector (SPD-20A) and a Communications Bus Module (CBM-20A). For preparative HPLC, an XBridge C_{18} OBD column was used (19 × 100 mm, 5 µm) with a flow rate of 17 mL min^{-1}. For analytical HPLC, a

Shimadzu Shim-Pack VP-ODS column was used (4.6 × 150 mm, 5 µm) with a flow rate of 2.0 mL min^{-1}. Fraction collection was performed manually. The solvent system used to achieve purification is specified in the text. In general, a solvent system of H_2O / CH_3CN or H_2O / CH_3OH (with or without 0.1% formic acid) was used with gradient elution as follows:

Step	Time / min	% H_2O	% CH_3CN/CH_3OH
0	0	90	10
1	4	90	10
2	14	0	100
3	19	0	100
4	22	90	10

Optical Measurements

All solution state 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 either an ISA Jobin-Yvon Spex Fluorolog-3 luminescence spectrometer using DataMax software (version 2.2.10) or a HORIBA Jobin-Yvon Fluorolog-3 luminescence spectrometer equipped with an iHR320 module, which selects either a HORIBA FL-1073 (Hammatsu R928P) photomultiplier tube or a HORIBA Synapse BIDD CCD for detection of emitted light, using FluorEssence software (based on Origin® software). Quantum yields were recorded using against the reference standard $[Ru(bipy)_3]Cl_2$ as described elsewhere.[d,e]

$$\frac{Q_x}{Q_r} = \left[\frac{A_r(\lambda)}{A_r(\lambda)}\right]\left[\frac{n_x^2}{n_r^2}\right]\left[\frac{I_{lamp}(\lambda)_r}{I_{lamp}(\lambda)_x}\right]$$

where A is the absorbance at the excitation wavelength (λ), n the refractive index, D the integrated luminescence intensity, and I_{lamp} the energy intensity contribution of the excitation lamp at the excitation wavelength (λ). "*r*" and "*x*"

stand for reference and sample. Here, the reference is $[Ru(bipy)_3]Cl_2$ in non-degassed water (Φ = 2.8%).

Molar extinction coefficients were determined at the stated wavelength utilising absorbance measurements at varying complex concentrations (Beer-Lambert law). Brightness values were estimated as the product of the experimentally determined quantum yields and molar extinction coefficients ($B = \Phi \square \varepsilon$). Lifetime measurements were carried out using a Perkin Elmer LS55 spectrometer using FL Winlab software.

Time-gated lanthanide emission measurements were performed using an in house-built time-gated spectrophotometer (www.fscanltd.com). This was designed to maximise europium(III) emission detection sensitivity and reduce physical size. A pulsed LED excitation source (Nichia: 365 nm, 50 s, 200 Hz, 250 mW) was placed in a purpose built sample chamber, designed to accommodate commercially available cuvettes (*e.g.* 12 mm × 12 mm). Luminescence from the sample was collected at 90º to the excitation source and focussed onto the entrance slit of a monochromator (Acton), adjusted to provide a 2 nm band-pass. The emission was detected using a photon counting photomultiplier module (Hamamatsu) and the signal acquired using a PC-based National Instruments data acquisition card. Europium(III) emission spectra were obtained in the range 550–720 nm in 1 nm increments. A 10 s time-delay was applied prior to a 1 ms time-gate for the spectral acquisition, in order to eliminate scattered light, avert auto-fluorescence and avoid any residual short-lived fluorescence.

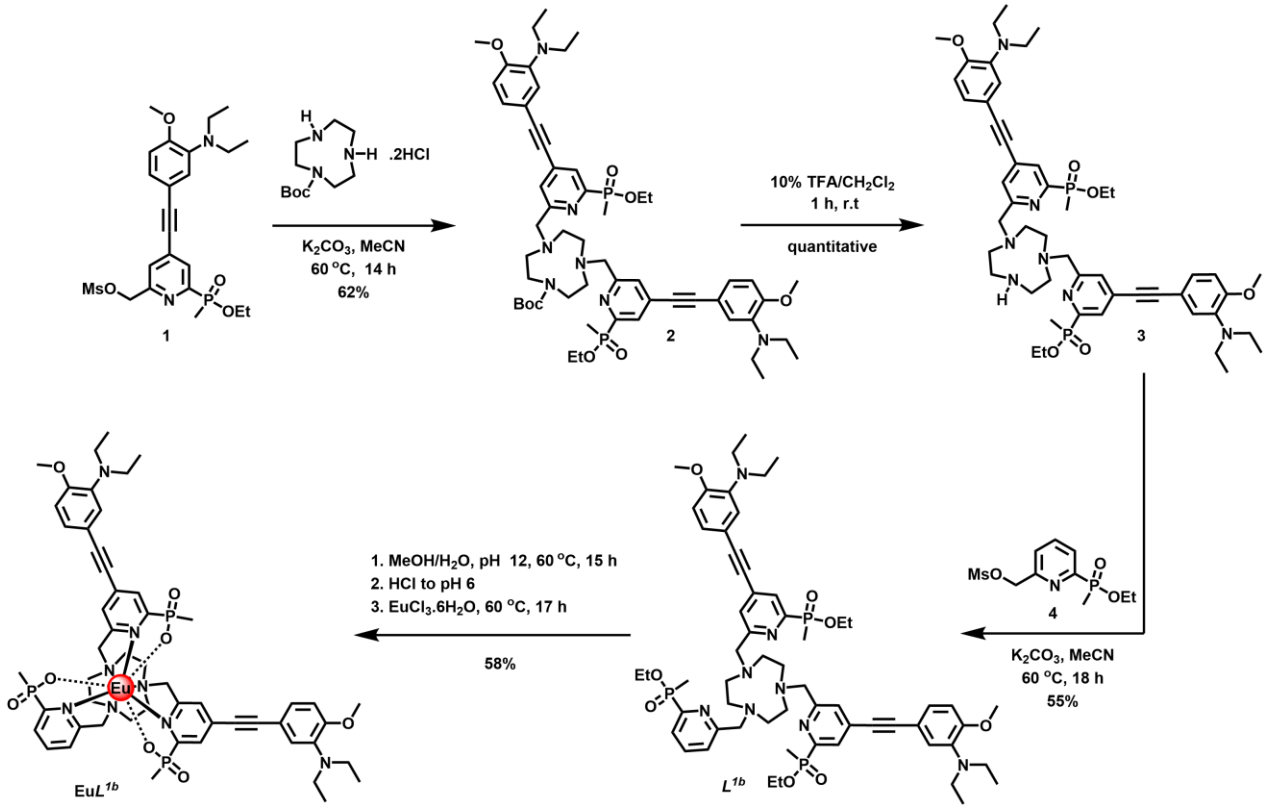
Internalisation Study Protocols

The assays were run in black 96 well cell culture treated plates (GREINER 655086). Each well was precoated with 50 µl of poly-L-ornithine for 30 minutes at 37 °C and were plated at 100,000 cells per well. HEK293 cells stably expressing GLP-1R-ST were obtained from Cisbio Bioassays (HEK293 SNAP-GLP1, #C1SU1GLP1) and non-transfected HEK293 (CLS) cells were plated at 100,000 cells per well in DMEM Glutamax and then placed at 37 °C under 5% CO_2 during 24 h. These adherent cells were used for the following labeling procedure.

After removal of the cell culture medium, a 50 µL solution of 200 nM of the BG derivative in Tag-lite labeling medium (Cisbio Bioassays, Catalog LABMED) was added and incubated for 1.5 h at room temperature. After removal of the buffer, the excess europium complex was removed by three washing steps, using 100 µL of Tag-lite labeling medium. Finally, a solution of 0.1 M of acetate buffer at pH 4.5 (+0.1% BSA) was added to the wells to reveal the luminescence. Each experiment was repeated in triplicate. The plates were read on a PheraStarFS (BMG) plate reader, using the HTRF setting. The 620 nm signal was measured over a 400 µs period, with a 60 µs. delay time (60 – 460 µs time window).

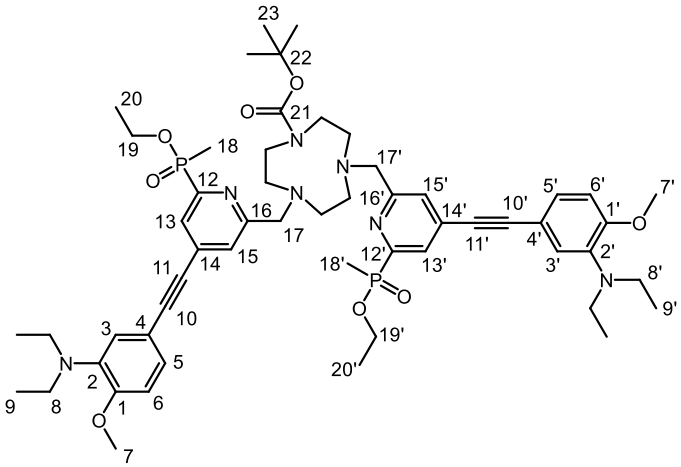
For the internalization study, the same procedure was followed for the SNAP-GLP1 cells. After the three washing steps to remove the excess europium complex, either 50 µL of Tag-lite medium was added or a solution of 100 nM of Exendin-4 (Tocris Cat. No. 1933) in Tag-lite medium. After incubation for 1 h at room temperature the cells were washed once with Tag-Lite medium (100 µL), after which 100 µL of Tag-Lite medium was added to the wells. Each plate was read as described above.

Synthesis of the complex Eu*L*^{1b}



Scheme S1

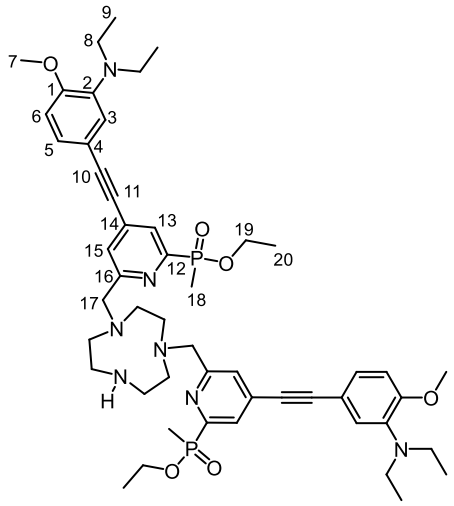
Compound **2**



The dihydrochloride salt of 1-(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane (31 mg, 0.10 mmol), the mesylate **1** (122 mg, 0.26 mmol) and K_2CO_3 (60 mg, 0.43 mmol) were combined in anhydrous CH_3CN (2 mL) under argon. The resulting mixture was heated to 60 °C for 14 h before separation of the crude solution

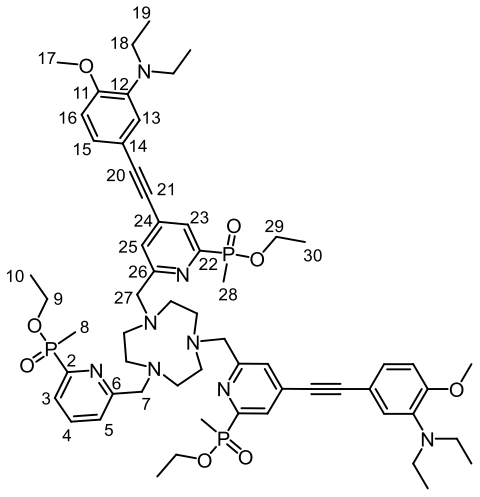
from the inorganic salts by centrifugation. Removal of the solvent under reduced pressure yielded an orange oil that was purified by reverse phase HPLC (10 to 100% CH_3CN in H_2O over 10 min, t_r = 15.6 min) to afford a pale yellow oil (66 mg, 62%); **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 8.03 – 7.96 (m, 2H, H^{13} & $H^{13'}$), 7.66 (s, 1H, H^{15}), 7.58 (s, 1H, $H^{15'}$), 7.17 (d, 2H, H^5 & $H^{5'}$), 7.08 (s, 2H, H^3 & $H^{3'}$), 6.82 (d, 2H, H^6 & $H^{6'}$), 4.15 – 4.02 & 3.91 – 3.78 (m, 10H, H^{19}, $H^{19'}$, H^7 & $H^{7'}$), 3.94 (2 × s, 4H, H^{17} & $H^{17'}$), 3.44 – 3.29 (m, 4H, 9-N_3), 3.20 – 3.04 (m, 12H, H^8, $H^{8'}$ & 9-N_3), 2.76 – 2.61 (m, 4H, 9-N_3), 1.76 (2 × d, 6H, H^{18} & $H^{18'}$), 1.48 (s, 9H, H^{23}), 1.24 (2 × t, 6H, H^{20} & $H^{20'}$), 1.02 (t, 12H, H^9 & $H^{9'}$); **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 161.7 (d, $^3J_{C\text{-}P}$ = 21, C^{16}), 161.5 (d, $^3J_{C\text{-}P}$ = 21, $C^{16'}$), 155.7 (C^{21}), 155.1 (C^1), 155.0 ($C^{1'}$), 153.8 (d, $^1J_{C\text{-}P}$ = 157, C^{12}), 153.6 (d, $^1J_{C\text{-}P}$ = 157, $C^{12'}$), 139.9 (2 × s, C^2 & $C^{2'}$), 132.8 (d, $^3J_{C\text{-}P}$ = 12, C^{14}), 132.7 (d, $^3J_{C\text{-}P}$ = 12, $C^{14'}$), 128.0 (d, $^2J_{C\text{-}P}$ = 22, C^{13}), 127.9 (d, $^2J_{C\text{-}P}$ = 22, $C^{13'}$), 127.1 (C^5 & $C^{5'}$), 126.4 (d, $^4J_{C\text{-}P}$ = 3, C^{15}), 126.1 (d, $^4J_{C\text{-}P}$ = 3, $C^{15'}$), 125.0 (2 × s, C^3 & $C^{3'}$), 113.7 (C^4), 113.6 ($C^{4'}$), 111.4 (2 × s, C^6 & $C^{6'}$), 96.5 (C^{10}), 96.2 ($C^{10'}$), 85.3 (d, $^4J_{C\text{-}P}$ = 2, C^{11}), 85.1 (d, $^4J_{C\text{-}P}$ = 2, $C^{11'}$), 79.5 (C^{22}), 62.9 (C^{17}), 62.6 ($C^{17'}$), 61.1 (2 × d, $^2J_{C\text{-}P}$ = 6, C^{19} & $C^{19'}$), 56.1 (9-N_3 ring), 55.7 (C^7 & $C^{7'}$), 55.0 (9-N_3 ring), 54.6 (9-N_3 ring), 54.0 (9-N_3 ring), 50.0 (9-N_3 ring), 49.7 (9-N_3 ring), 46.0 (C^8 & $C^{8'}$), 28.8 (C^{23}), 16.6 (d, C^{20} & $C^{20'}$), 13.5 (d, $^1J_{C\text{-}P}$ = 1.5, C^{18}), 13.4 (d, $^1J_{C\text{-}P}$ = 1.5, $C^{18'}$), 12.0 (C^9 & $C^{9'}$); **^{31}P{^{1}H}-NMR** (162 MHz, $CDCl_3$) δ + 40.2 (1P), +40.1 (1P); **ESI-LRMS** (+) *m/z* 1027 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{55}H_{78}N_7O_8P_2]^+$ 1026.539, found 1026.543.

Compound **3,** (trifluoroacetate salt)



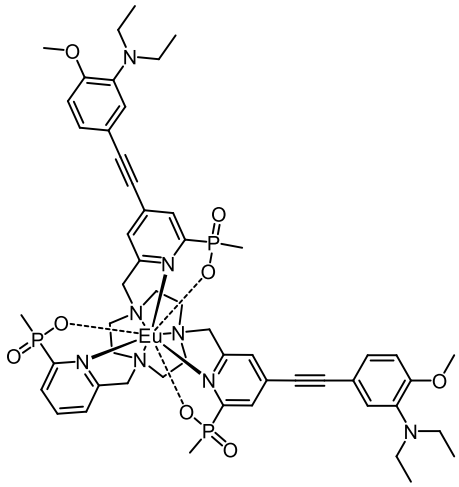
A solution of compound **2** (66 mg, 0.0643 mmol) in trifluoroacetic acid and CH_2Cl_2 (10% v/v, 3 mL total) was prepared. The solution was stirred at room temperature for 1 h before removal of the solvent under reduced pressure to give an orange residue. To this residue was added CH_2Cl_2 (30 mL) before removal of the solvent under reduced pressure once again. This procedure was repeated five times to afford a pale orange oil (67 mg, quant.); **^{1}H-NMR** (600 MHz, CD_3OD) δ 7.97 (s, 2H, H^3), 7.95 (dd, $^3J_{H\text{-}P}$ = 6, $^4J_{H\text{-}H}$ = 1.0, 2H, H^{13}), 7.79 (dd, $^3J_{H\text{-}H}$ = 8.7, $^4J_{H\text{-}H}$ = 1.9, 2H, H^5), 7.72 – 7.70 (m, 2H, H^{15}), 7.41 (d, $^3J_{H\text{-}H}$ = 8.7, 2H, H^6), 4.24 (s, 4H, H^{17}), 4.17 – 4.10 & 4.01 – 3.95 (m, 4H, H^{19}), 4.07 (s, 6H, H^7), 3.70 (q, $^3J_{H\text{-}H}$ = 7.2, 8H, H^8), 3.42 – 3.35 (m, 4H, 9-N_3 ring), 3.29 – 3.23 (m, 4H, 9-N_3 ring), 3.06 – 2.99 (m, 4H, 9-N_3 ring), 1.82 (d, $^2J_{H\text{-}P}$ = 15, 6H, H^{18}), 1.30 (t, $^3J_{H\text{-}H}$ = 7.0, 6H, H^{20}), 1.12 (t, $^3J_{H\text{-}H}$ = 7.2, 12H, H^9); **^{13}C-NMR** (151 MHz, CD_3OD) δ 160.8 (d, $^3J_{C\text{-}P}$ = 20, C^{16}), 155.6 (C^1), 155.2 (d, $^1J_{C\text{-}P}$ = 159, C^{12}), 136.8 (C^5), 133.8 (d, $^3J_{C\text{-}P}$ = 12, C^{14}), 128.6 (C^{15}), 128.5 (d, $^2J_{C\text{-}P}$ = 24, C^{13}), 127.5 (C^3), 126.2 (C^2), 116.9 (C^4), 114.9 (C^6), 94.6 (C^{10}), 87.3 (d, $^4J_{C\text{-}P}$ = 2, C^{11}), 63.1 (d, $^2J_{C\text{-}P}$ = 7, C^{19}), 60.6 (C^{17}), 57.7 (C^7), 55.0 (C^8), 52.3 (9-N_3 ring), 49.9 (9-N_3 ring), 45.5 (9-N_3 ring), 16.8 (d, $^3J_{C\text{-}P}$ = 6.2, C^{20}), 13.6 (d, $^1J_{C\text{-}P}$ = 103, C^{18}), 10.4 (C^9), **^{31}P{^{1}H}-NMR** (162 MHz, CD_3OD) δ +40.8; **ESI-LRMS** (+) *m/z* 926 $[M+H]^+$, 464 $[M+2H]^{2+}$, 309 $[M+3H]^{3+}$; **ESI-HRMS** (+) calcd for $[C_{50}H_{70}N_7O_6P_2]^+$ 926.4863, found 926.4865.

L^{1b}



Compound **3** (39 mg, 0.0375 mmol), the mesylate **4** (36 mg, 0.123 mmol) and K_2CO_3 (30 mg, 0.217 mmol) were combined in anhydrous CH_3CN (2 mL) under argon and heated at 60 ^oC for 18 h. After this time, the crude mixture was separated from the inorganic salts by centrifugation and the resulting solution was subjected directly to reverse phase HPLC (10 to 100% CH_3CN in 25 mM ammonium bicarbonate buffer over 10 min, t_r = 11.8 min) to yield a pale yellow oil (23 mg, 55%); **^{1}H-NMR** (600 MHz, $CDCl_3$) δ 8.00 (dd, $^3J_{H\text{-}P}$ = 6.0, $^4J_{H\text{-}H}$ = 1.3, 2H, H^{23}), 7.93 – 7.88 (m, 1H, H^3), 7.83 – 7.76 (m, 1H, H^4), 7.73 – 7.67 (m, 1H, H^5), 7.65 (s, 2H, H^{25}), 7.18 (dd, $^3J_{H\text{-}H}$ = 8.5, $^4J_{H\text{-}H}$ = 1.8, 2H, H^{15}), 7.09 (d, $^4J_{H\text{-}H}$ = 1.8, 2H, H^{13}), 6.83 (d, $^3J_{H\text{-}H}$ = 8.5, 2H, H^{16}), 4.14 – 4.04 & 3.90 – 3.82 (m, 12H, H^9, H^{17} & H^{29}), 4.01 – 3.91 (m, 4H, H^7 & H^{27}), 3.16 (q, $^3J_{H\text{-}H}$ = 7.1, 8H, H^{18}), 3.07 – 2.83 (m, 12H, 9-N_3 ring), 1.76 (d, $^2J_{H\text{-}P}$ = 15, 6H, H^{28}), 1.75 (d, $^2J_{H\text{-}P}$ = 15, 3H, H^8), 1.25 (t, $^3J_{H\text{-}H}$ = 7.0, 6H, H^{30}), 1.23 (t, $^3J_{H\text{-}H}$ = 7.0, 3H, H^{10}), 1.03 (t, $^3J_{H\text{-}H}$ = 7.1, 12H, H^{19}); **ESI-LRMS** (+) *m/z* 1124 $[M+H]^+$, 563 $[M+2H]^{2+}$, 375 $[M+3H]^{3+}$; **ESI-HRMS** (+) calcd for $[C_{59}H_{82}N_8O_8P_3]^+$ 1123.547, found 1123.548.

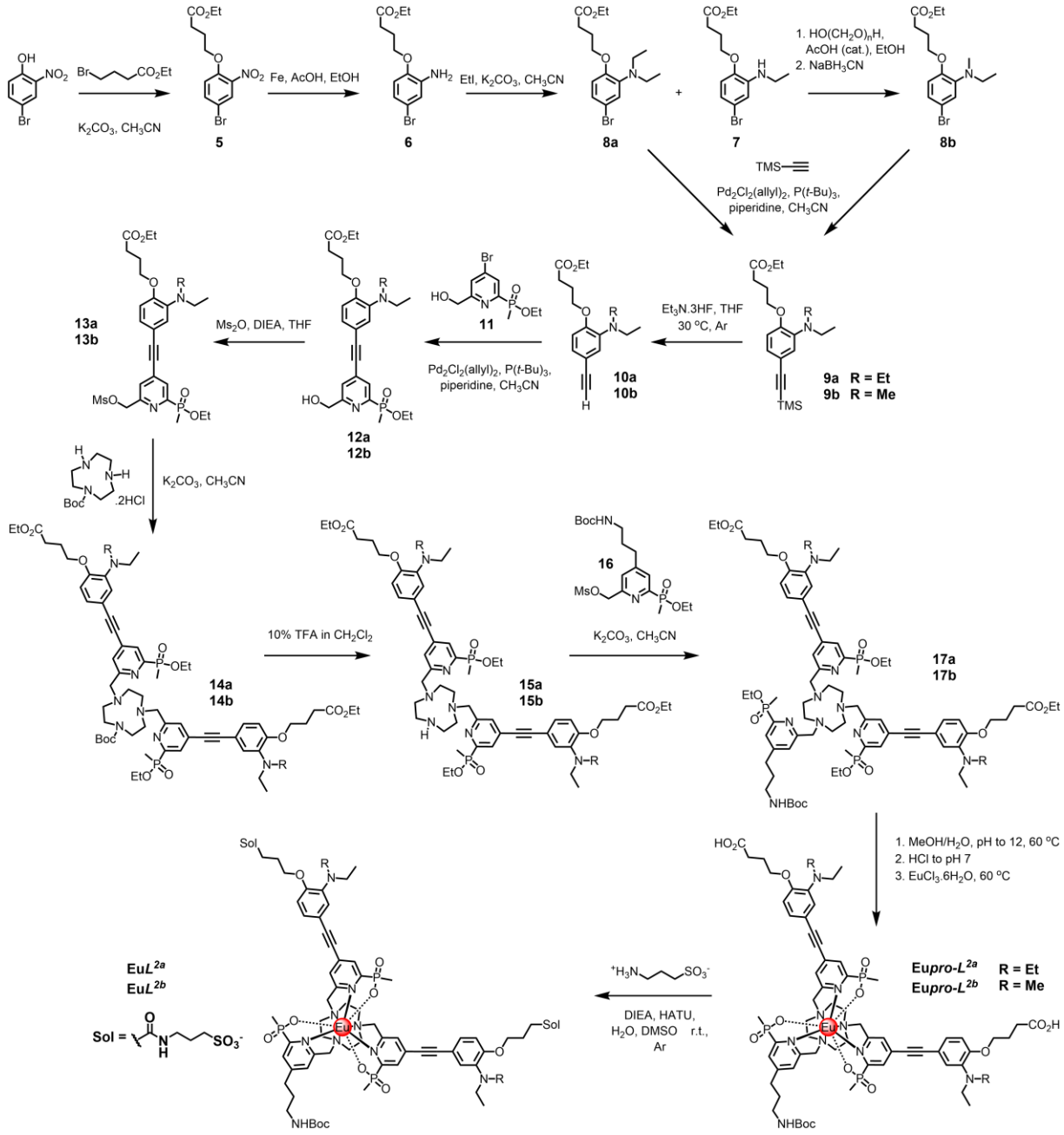
Eu*L*^{1b}



The ligand ***L*^{1b}** (11.5 mg, 0.01024 mmol) was dissolved in a mixture of CH_3OH/H_2O (1:1, 4 mL total) and the pH was adjusted to 12 using aqueous NaOH solution (1M). The solution was heated at 60 ^oC for 15 h. After cooling and adjustment of the pH to 6 using dilute hydrochloric acid (0.1 M), $EuCl_3.6H_2O$ (6 mg, 0.0164 mmol) was added and the reaction mixture was heated to 60 ^oC for 17 h. After this time, the solution was separated from the inorganic salts by

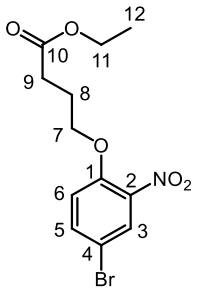
centrifugation and purified by reverse phase HPLC (10 to 100% CH_3OH in H_2O over 10 min, t_r = 13.6 min) to yield a yellow solid (7 mg, 58%); **ESI-LRMS** (+) *m/z* 1189 $[M+H]^+$, 595 $[M+2H]^{2+}$, 397 $[M+3H]^{3+}$; **ESI-HRMS** (+) calcd for $[C_{53}H_{68}N_8O_8P_3{}^{151}Eu]^{2+}$ 595.1796, found 595.1791; $\boldsymbol{\tau_{H2O}}$ (ms) = 0.34 (pH 9), 0.34 (pH 8), 0.47 (pH 7), 0.78 (pH 6), 0.96 (pH 5), 1.00 (pH 4); $\varepsilon_{328\ nm}$ = 35000 M^{-1} cm^{-1}; $\boldsymbol{\Phi}_{pH\ 8}$ = 0.2%, $\boldsymbol{\Phi}_{pH\ 4}$ = 17.6% (λ_{exc} = 328 nm).

Synthesis of the complexes Eu*L*$^{2a/2b}$



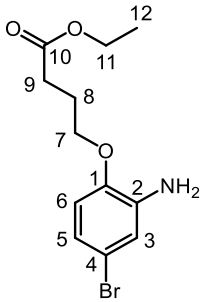
Scheme S2

Ethyl 4-(4-bromo-2-nitrophenoxy)butanoate, **5**



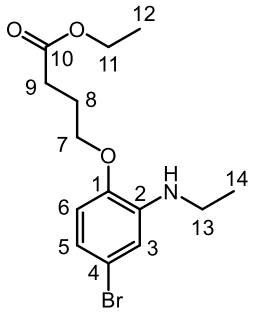
To a mixture of 4-bromo-2-nitrophenol (2.49 g, 11.4 mmol) and K_2CO_3 (2.31 g, 16.7 mmol) in anhydrous CH_3CN (40 mL) under argon was added ethyl 4-bromobutyrate (2.3 mL, 17.2 mmol). The mixture was heated at 70 ^oC for 64 h before removal of solvent under reduced pressure. CH_2Cl_2 (50 mL) was added to the residue and the resulting suspension washed with H_2O (5 × 50 mL). The organic layer was dried over K_2CO_3 before removal of solvent under reduced pressure to yield a crude residue that was purified by column chromatography (SiO_2, 1:1 hexane/CH_2Cl_2 to 100% CH_2Cl_2) to afford a pale yellow oil (3.38 g, 98%); **^{1}H-NMR** (700 MHz, $CDCl_3$) δ 7.95 (d, $^4J_{H\text{-}H}$ = 2.5, 1H, H^3), 7.60 (dd, $^3J_{H\text{-}H}$ = 8.9, $^4J_{H\text{-}H}$ = 2.5, 1H, H^5), 6.97 (d, $^3J_{H\text{-}H}$ = 8.9, 1H, H^6), 4.17 – 4.12 (m, 4H, H^7 & H^{11}), 2.55 (t, $^3J_{H\text{-}H}$ = 7.0, 2H, H^9), 2.18 – 2.12 (m, 2H, H^8), 1.25 (t, $^3J_{H\text{-}H}$ = 7.1, H^{12}); **^{13}C-NMR** (176 MHz, $CDCl_3$) δ 173.1 (C^{10}), 151.6 (C^2), 140.4 (C^1), 137.0 (C^5), 128.5 (C^3), 116.3 (C^6), 112.1 (C^4), 68.9 (C^7), 60.8 (C^{11}), 30.3 (C^9), 24.3 (C^8), 14.4 (C^{12}); **ESI-LRMS** (+) *m/z* 332 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{12}H_{15}NO_5Br]^+$ 332.0134, found 332.0146; **R_f** = 0.47 (SiO_2, 100% CH_2Cl_2).

Ethyl 4-(2-amino-4-bromophenoxy)butanoate, **6**



Compound **5** (4.30 g, 12.95 mmol), iron powder (3.60 g, 64.5 mmol) and glacial acetic acid (3.7 mL, 64.6 mmol) were combined in ethanol (20 mL) under argon and heated at 50 ^{o}C for 6 h. The mixture was allowed to cool before filtering and removal of solvent under reduced pressure. Dichloromethane (50 mL) was added to the resulting mixture and the resulting solution washed with saturated aqueous Na_4EDTA solution (3 × 40 mL) and H_2O (2 × 40 mL). The combined aqueous layers were extracted with CH_2Cl_2 (6 × 40 mL). The combined organic layers were dried over K_2CO_3 and the solvent was removed under reduced pressure to afford a pale golden oil (3.5 g, 90%); **^{1}H-NMR** (700 MHz, $CDCl_3$) δ 6.75 (d, $^{4}J_{H\text{-}H}$ = 2.4, 1H, H^3), 6.70 (dd, $^{3}J_{H\text{-}H}$ = 8.4, $^{4}J_{H\text{-}H}$ = 2.4, 1H, H^5), 6.55 (d, $^{3}J_{H\text{-}H}$ = 8.4, 1H, H^6), 4.11 (q, $^{3}J_{H\text{-}H}$ = 7.2, 2H, H^{11}), 3.93 (t, $^{3}J_{H\text{-}H}$ = 6.1, 2H, H^9), 3.89 (br s, 2H, NH_2), 2.46 (t, $^{3}J_{H\text{-}H}$ = 7.1, 1H, H^7), 2.11 – 2.06 (m, 2H, H^8), 1.22 (t, $^{3}J_{H\text{-}H}$ = 7.2, 3H, H^{12}); **^{13}C-NMR** (176 MHz, $CDCl_3$) δ 173.1 (C^{10}), 145.3 (C^2), 138.0 (C^1), 120.3 (C^5), 117.2 (C^3), 113.3 (C^4), 112.6 (C^6), 67.4 (C^9), 60.5 (C^{11}), 31.0 (C^7), 24.6 (C^8), 14.2 (C^{12}); **ESI-LRMS** (+) *m/z* 302 $[M+H]^+$; **ESI-HRMS** (+) calc for $[C_{12}H_{17}NO_3Br]^+$ 302.0392, found 302.0402.

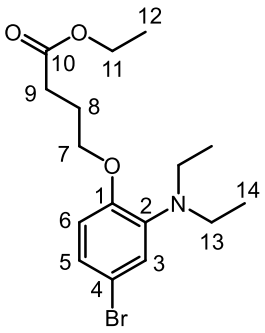
Ethyl 4-(2-aminoethyl-4-bromophenoxy)butanoate, **7**



To compound **6** (3.50 g, 11.6 mmol) and K_2CO_3 (4.8 g, 34.5 mmol) in anhydrous CH_3CN under argon (3 mL) was added iodoethane (1.21 mL, 15.0 mmol). This mixture was heated at 55 ^{o}C for 70 h before filtration to remove the inorganic salts, followed by removal of the solvent under reduced pressure. The resulting oil was purified by column chromatography (SiO_2, 100% CH_2Cl_2) to afford a pale yellow oil (1.65 g, 43%); **^{1}H-NMR** (700 MHz, $CDCl_3$) δ 6.70 (dd, $^{3}J_{H\text{-}H}$ = 8.3, 1H, H^5), 6.67 (s, 1H, H^3), 6.57 (d, $^{3}J_{H\text{-}H}$ = 8.3, 1H, H^6), 4.15 (q, $^{3}J_{H\text{-}H}$ = 7.1, 2H, H^{11}), 4.00 (t, $^{3}J_{H\text{-}H}$ = 6.1, 2H, H^9), 3.13 (q, $^{3}J_{H\text{-}H}$ = 7.2, 2H, H^{13}), 2.49 (t, $^{3}J_{H\text{-}H}$ = 7.1, 2H, H^7), 2.18 – 2.11 (m, 2H, H^8), 1.29 (t, $^{3}J_{H\text{-}H}$ = 7.2, 3H, H^{14}), 1.25 (t, $^{3}J_{H\text{-}}$

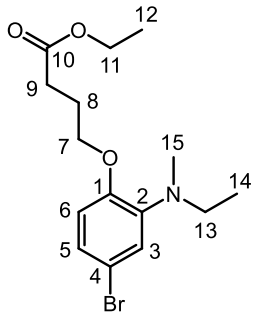
$_H$ = 7.1, 3H, H^{12}); **^{13}C-NMR** (176 MHz, $CDCl_3$) δ 173.3 (C^{10}), 145.0 (C^2), 139.8 (C^1), 118.4 (C^5), 114.3 (C^4), 112.7 (C^3), 111.6 (C^6), 67.6 (C^9), 60.7 (C^{11}), 38.2 (C^{13}), 31.3 (C^7), 24.7 (C^8), 14.7 (C^{14}), 14.4 (C^{12}); **ESI-LRMS** (+) *m/z* 331 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{14}H_{21}NO_3Br]^+$ 330.0705, found 330.0714; **R_f** = 0.4 (SiO_2, neat CH_2Cl_2).

Ethyl 4-(4-bromo-2-(diethylamino)phenoxy)butanoate, **8a**



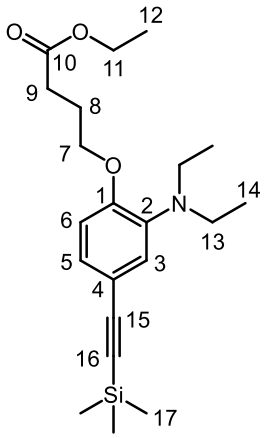
To compound **6** (3.19 g, 10.6 mmol) and K_2CO_3 (3.73 g, 27.0 mmol) in anhydrous CH_3CN (10 mL) under argon was added iodoethane (4 mL, 50 mmol). The mixture was heated at 70 C for 65 h before removal of solvent under reduced pressure. The resulting residue was dissolved in CH_2Cl_2 (50 mL) and washed with H_2O (4 × 50 mL), before drying over K_2CO_3. Removal of solvent under reduced pressure yielded a crude residue that was purified by column chromatography (SiO_2, neat CH_2Cl_2 to 1% CH_3OH in CH_2Cl_2) to afford a light red oil (2.51 g, 66%); **^{1}H-NMR** (700 MHz, $CDCl_3$) δ 6.99 – 6.96 (m, 2H, H^5 & H^3), 6.69 (d, $^3J_{H\text{-}H}$ = 8.4, 1H, H^6), 4.13 (q, $^3J_{H\text{-}H}$ = 7.2, 2H, H^{11}), 3.99 (t, $^3J_{H\text{-}H}$ = 6.3, 2H, H^7), 3.13 (q, $^3J_{H\text{-}H}$ = 7.0, 4H, H^{13}), 2.51 (t, $^3J_{H\text{-}H}$ = 7.4, 2H, H^9), 2.15 – 2.10 (m, 2H, H^8), 1.25 (t, $^3J_{H\text{-}H}$ = 7.0, 3H, H^{12}), 1.04 (t, $^3J_{H\text{-}H}$ = 7.0, 6H, H^{14}); **^{13}C-NMR** (176 MHz, $CDCl_3$) δ 173.2 (C^{10}), 151.8 (C^2), 141.8 (C^1), 124.4 (C^3), 124.1 (C^5), 114.8 (C^6), 113.4 (C^4), 67.8 (C^7), 60.6 (C^{11}), 45.7 (C^{13}), 31.0 (C^9), 24.9 (C^8), 14.4 (C^{12}), 12.5 (C^{14}); **ESI-LRMS** (+) *m/z* 358 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{16}H_{25}NO_3Br]^+$ 358.1018, found 358.1020; **R_f** = 0.04 (SiO_2, neat CH_2Cl_2), 0.3 (1% CH_3OH in CH_2Cl_2).

Ethyl 4-(4-bromo-2-(ethylmethylamino)phenoxy)butanoate, **8b**



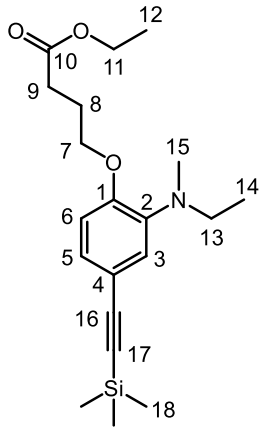
Compound **7** (939 mg, 2.84 mmol), paraformaldehyde (415 mg, 4.61 mmol) and a few drops of acetic acid were combined in anhydrous ethanol (25 mL). The mixture was stirred at room temperature under argon for 20 min, at which point $NaBH_3CN$ was added (640 mg, 10 mmol) and the reaction stirred for a further 48 h. Following the removal of solvent under reduced pressure, the resulting residue was dissolved in CH_2Cl_2 (30 mL) and washed successively with $NaHCO_3$ solution (1 × 30 mL) and water (3 × 30 mL). The organic layer was dried over K_2CO_3 and the solvent removed under reduced pressure to yield a pale yellow oil (833 mg, 85%); **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 7.00 (dd, $^3J_{H\text{-}H}$ = 8.3, $^4J_{H\text{-}H}$ = 2.3, 1H, H^5), 6.98 (d, $^4J_{H\text{-}H}$ = 2.3, 1H, H^3), 6.70 (d, $^3J_{H\text{-}H}$ = 8.3, 1H, H^6), 4.16 (q, $^3J_{H\text{-}H}$ = 7.1, 2H, H^{11}), 4.02 (t, $^3J_{H\text{-}H}$ = 6.3, 2H, H^7), 3.13 (q, $^3J_{H\text{-}H}$ = 7.2, 2H, H^{13}), 2.76 (s, 2H, H^{15}), 2.54 (t, $^3J_{H\text{-}H}$ = 7.3, 2H, H^9), 2.17 – 2.10 (m, 2H, H^8), 1.27 (t, $^3J_{H\text{-}H}$ = 7.1, 3H, H^{12}), 1.12 (t, $^3J_{H\text{-}H}$ = 7.2, 3H, H^{14}); **^{13}C-NMR** (176 MHz, $CDCl_3$) δ 172.9 (C^{10}), 150.6 (C^1), 143.3 (C^2), 123.9 (C^5), 118.0 (C^3), 113.8 (C^6), 113.3 (C^4), 67.4 (C^7), 60.5 (C^{11}), 49.2 (C^{13}), 39.0 (C^{15}), 30.8 (C^9), 24.7 (C^8), 14.2 (C^{12}), 12.2 (C^{14}); **ESI-LRMS** (+) m/z 344 $[M+H]^+$. **ESI-HRMS** (+) calcd for $[C_{15}H_{23}NO_3Br]^+$ 344.0861, found 344.0872.

Ethyl 4-(2-(diethylamino)-4-((trimethylsilyl)ethynyl)phenoxy)butanoate, **9a**



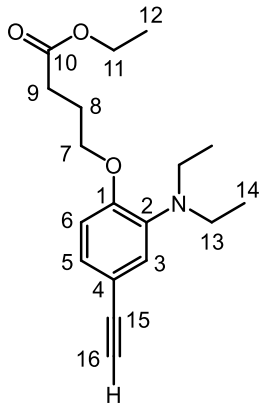
Compound **8a** (3.03 g, 8.46 mmol) and $Pd_2Cl_2(allyl)_2$ (310 mg, 0.85 mmol) were combined and the vessel was degassed then back-filled with argon in three cycles, followed by the addition of anhydrous CH_3CN (15 mL). To this mixture was added P(*t*-Bu)$_3$ (0.31 mL, 1.28 mmol), trimethylsilylacetylene (2.4 mL, 17.3 mmol) and piperidine (2.1 mL, 21.3 mmol) in that order. The reaction mixture was stirred at 35 ^oC for 30 h. The solvent was subsequently removed under reduced pressure and the residue dissolved in CH_2Cl_2 (50 mL). The solution was washed with H_2O (4 × 50 mL) before drying over K_2CO_3. Removal of solvent under reduced pressure yielded a brown oil which was purified by column chromatography (SiO_2, 100% hexane to 6% EtOAc in hexane) to afford a yellow oil (2.29 mg, 75%); **^{1}H-NMR** (600 MHz, $CDCl_3$) δ 7.06 (dd, $^3J_{H\text{-}H}$ = 8.4, $^4J_{H\text{-}H}$ = 2.0, 1H, H^5), 7.01 (d, $^4J_{H\text{-}H}$ = 2.0, 1H, H^3), 6.74 (d, $^3J_{H\text{-}H}$ = 8.4, 1H, H^6), 4.14 (q, $^3J_{H\text{-}H}$ = 7.1, 2H, H^{11}), 4.03 (t, $^3J_{H\text{-}H}$ = 6.1, 2H, H^7), 3.13 (q, $^3J_{H\text{-}H}$ = 7.2, 4H, H^{13}), 2.52 (t, $^3J_{H\text{-}H}$ = 7.3, 2H, H^9), 2.17 – 2.11 (m, 2H, H^8), 1.27 (t, $^3J_{H\text{-}H}$ = 7.1, 3H, H^{12}), 1.03 (t, $^3J_{H\text{-}H}$ = 7.2, 6H, H^{14}), 0.24 (s, 9H, H^{17}); **^{13}C-NMR** (151 MHz, $CDCl_3$) δ 173.1 (C^{10}), 153.3 (C^2), 139.7 (C^1), 126.6 (C^5), 125.0 (C^3), 115.2 (C^4), 112.6 (C^6), 105.8 (C^{15}) 91.7 (C^{16}), 67.3 (C^7), 60.4 (C^{11}), 45.6 (C^{13}), 30.9 (C^9), 24.6 (C^8), 14.2 (C^{12}), 12.3 (C^{14}), 0.10 (C^{17}); **^{29}Si NMR** (139 MHz, $CDCl_3$) δ -16.0; **ESI-LRMS** (+) *m/z* 376 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{21}H_{34}NO_3Si]^+$ 376.2308, found 376.2299; **R_f** = 0.3 (SiO_2, 10% EtOAc in hexane).

Ethyl 4-(2-(ethylmethylamino)-4-((trimethylsilyl)ethynyl)phenoxy)butanoate, **9b**



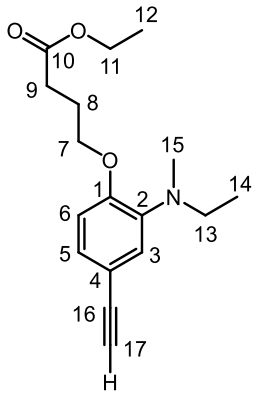
Compound **8b** (1.47 g, 4.27 mmol) and $Pd_2Cl_2(allyl)_2$ (160 mg, 0.44 mmol) were combined and the vessel was degassed then back-filled with argon in three cycles followed by the addition of anhydrous CH_3CN (8 mL). To this mixture was added P(*t*-Bu)$_3$ (0.16 mL, 0.66 mmol), trimethylsilylacetylene (1.2 mL, 8.66 mmol) and piperidine (1.0 mL, 10.1 mmol) in that order. The reaction mixture was stirred at 35 ^oC for 19 h. The solvent was subsequently removed under reduced pressure and the residue dissolved in CH_2Cl_2 (50 mL). The solution was washed with H_2O (2 × 50 mL) before drying over K_2CO_3. Removal of solvent under reduced pressure yielded a brown oil which was purified by column chromatography (SiO_2, 100% hexane to 7% EtOAc in hexane) to afford a pale orange oil (1.19 g, 77%); **^{1}H-NMR** (600 MHz, $CDCl_3$) δ 7.05 (d, $^3J_{H\text{-}H}$ = 8.2, 1H, H^5), 7.00 (s, 1H, H^3), 6.73 (d, $^3J_{H\text{-}H}$ = 8.2, 1H, H^6), 4.14 (q, $^3J_{H\text{-}H}$ = 7.1, 2H, H^{11}), 4.04 (t, $^3J_{H\text{-}H}$ = 6.2, 2H, H^7), 3.09 (d, $^3J_{H\text{-}H}$ = 7.0, 2H, H^{13}), 2.75 (s, 3H, H^{15}), 2.53 (t, $^3J_{H\text{-}H}$ = 7.3, 2H, H^9), 2.20 – 2.13 (m, 2H, H^8), 1.25 (t, $^3J_{H\text{-}H}$ = 7.1, 3H, H^{12}), 1.09 (t, $^3J_{H\text{-}H}$ = 7.0, 3H, H^{14}), 0.24 (s, 9H, H^{18}); **^{13}C-NMR** (151 MHz, $CDCl_3$) δ 173.2 (C^{10}), 152.3 (C^1), 141.7 (C^2), 126.4 (C^5), 122.7 (C^3), 115.4 (C^4), 112.1 (C^6), 105.9 (C^{16}), 92.0 (C^{17}), 67.3 (C^7), 60.6 (C^{11}), 49.5 (C^{13}), 39.3 (C^{15}), 31.0 (C^9), 24.8 (C^8), 14.4 (C^{12}), 12.3 (C^{14}), 0.2 (C^{18}); **^{29}Si-NMR** (139 MHz, $CDCl_3$) δ -18.3; **ESI-LRMS** (+) *m/z* 362 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{20}H_{32}NO_3Si]^+$ 362.2151, found 362.2152; **R_f** = 0.2 (SiO_2, 10% EtOAc in hexane).

Ethyl 4-(2-(diethylamino)-4-ethynylphenoxy)butanoate, **10a**



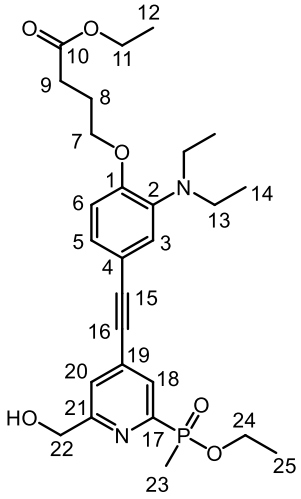
Triethylamine trihydrofluoride (6.25 mL, 38 mmol) was added to compound **9a** (960 mg, 2.56 mmol) in anhydrous tetrahydrofuran (8 mL) under argon. The solution was stirred at 30 ^{o}C for 24 h before removal of solvent under reduced pressure. The residue was subsequently dissolved in CH_2Cl_2 (30 mL) and washed with water (6 × 30 mL). The combined aqueous layers were extracted with CH_2Cl_2 (2 × 30 mL) before drying the combined organic layers over K_2CO_3 to yield a pale yellow oil (749 mg, 97%). The product was used in the next step without further purification; **^{1}H-NMR** (700 MHz, $CDCl_3$) δ 7.07 (dd, $^{3}J_{H\text{-}H}$ = 8.3, $^{4}J_{H\text{-}H}$ = 2.0, 1H, H^5), 7.03 (d, $^{4}J_{H\text{-}H}$ = 2.0, 1H, H^3), 6.75 (d, $^{3}J_{H\text{-}H}$ = 8.3, 1H, H^6), 4.13 (q, $^{3}J_{H\text{-}H}$ = 7.2, 2H, H^{11}), 4.03 (t, $^{3}J_{H\text{-}H}$ = 6.3, 2H, H^7), 3.13 (q, $^{3}J_{H\text{-}H}$ = 7.2, 4H, H^{13}), 2.97 (s, 1H, H^{16}), 2.52 (t, $^{3}J_{H\text{-}H}$ = 7.4, 2H, H^9), 2.17 – 2.12 (m, 2H H^8), 1.25 (t, $^{3}J_{H\text{-}H}$ = 7.2, 3H, H^{12}), 1.03 (t, $^{3}J_{H\text{-}H}$ = 7.1, 6H, H^{14}); **^{13}C-NMR** (176 MHz, $CDCl_3$) δ 173.1 (C^{10}), 153.4 (C^1), 139.8 (C^2), 126.4 (C^5), 125.0 (C^3), 114.0 (C^4), 112.7 (C^6), 84.3 (C^{15}), 75.2 (C^{16}), 67.3 (C^7), 60.4 (C^{11}), 45.6 (C^{13}), 30.9 (C^9), 24.6 (C^8), 14.2 (C^{12}), 12.3 (C^{14}); **ESI-LRMS** (+) *m/z* 304 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{18}H_{26}NO_3]^+$ 304.1913, found 304.1914.

Ethyl 4-(2-(diethylamino)-4-ethynylphenoxy)butanoate, **10b**



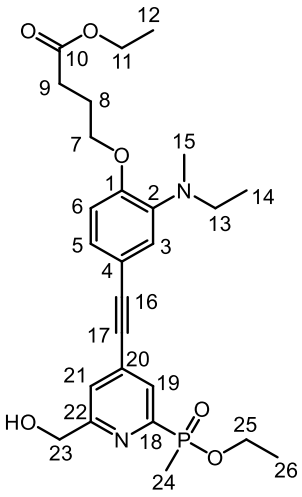
Triethylamine trihydrofluoride (3.6 mL, 22.0 mmol) was added to compound **9b** (530 mg, 1.47 mmol) in anhydrous tetrahydrofuran (5 mL) under argon. The solution was stirred at 30 ^{o}C for 17 h before removal of solvent under reduced pressure. The residue was subsequently dissolved in CH_2Cl_2 (30 mL) and washed with water (3 × 30 mL). The combined aqueous layers were extracted with CH_2Cl_2 (2 × 30 mL) and the combined organic layers dried over K_2CO_3 to yield a pale yellow oil (410 mg, 97%). The product was used in the next step without further purification; **^{1}H-NMR** (600 MHz, $CDCl_3$) δ 7.07 (dd, $^{3}J_{H\text{-}H}$ = 8.3, $^{4}J_{H\text{-}H}$ = 2.0, 1H, H^{5}), 7.02 (d, $^{4}J_{H\text{-}H}$ = 2.0, 1H, H^{3}), 6.75 (d, $^{3}J_{H\text{-}H}$ = 8.3, 1H, H^{6}), 4.14 (q, $^{3}J_{H\text{-}H}$ = 7.1, 2H, H^{11}), 4.04 (t, $^{3}J_{H\text{-}H}$ = 6.3, 2H, H^{7}), 3.10 (q, $^{3}J_{H\text{-}H}$ = 7.2, 2H, H^{13}), 2.97 (s, 1H, H^{17}), 2.75 (s, 3H, H^{15}), 2.53 (t, $^{3}J_{H\text{-}H}$ = 7.3, 2H, H^{9}), 2.21 – 2.14 (m, 2H, H^{8}), 1.25 (t, $^{3}J_{H\text{-}H}$ = 7.1, 3H, H^{12}), 1.10 (t, $^{3}J_{H\text{-}H}$ = 7.2, 3H, H^{14}); **^{13}C-NMR** (151 MHz, $CDCl_3$) δ 173.2 (C^{10}), 152.5 (C^{1}), 141.8 (C^{2}), 126.3 (C^{5}), 122.8 (C^{3}), 114.3 (C^{4}), 112.2 (C^{6}), 84.4 (C^{16}), 75.4 (C^{17}), 67.3 (C^{7}), 60.6 (C^{11}), 49.4 (C^{13}), 39.2 (C^{15}), 31.0 (C^{9}), 24.8 (C^{8}), 14.4 (C^{12}), 12.4 (C^{14}); **ESI-LRMS** (+) *m/z* 290 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{17}H_{24}NO_3]^+$ 290.1756, found 290.1752.

Ethyl 4-(2-(diethylamino)-4-((2-(ethoxy(methyl)phosphoryl)-6-(hydroxymethyl)pyridin-4-yl)ethynyl)phenoxy)butanoate, **12a**



Compound **10a** (528 mg, 1.74 mmol) and ethyl (6-(hydroxymethyl)-4-(bromopyridin-2-yl)(methyl)phosphinate, **11**, (486 mg, 1.65 mmol) were combined in anhydrous CH_3CN (10 mL) under argon. To this solution was added $Pd_2Cl_2(allyl)_2$ (70 mg, 0.191 mmol), $P(^tBu)_3$ (0.06 mL, 0.25 mmol) and piperidine (0.43 mL, 4.70 mmol) in that order. The resulting mixture was stirred at 40 ^oC under argon for 36 h before removal of the solvent under reduced pressure. The residue was dissolved in CH_2Cl_2 (40 mL) and washed with H_2O (3 × 40 mL) before drying over K_2CO_3 followed by removal of solvent under reduced pressure. The crude product was purified by reverse phase HPLC (10 to 100% CH_3CN in H_2O over 10 min, t_r = 12.2 min) to yield a pale orange oil (359 mg, 40%); **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 8.04 (d, $^3J_{H\text{-}P}$ = 6.0, 1H, H^{18}), 7.47 (s, 1H, H^{20}), 7.13 (d, $^3J_{H\text{-}H}$ = 8.4, 1H, H^5), 7.07 (s, 1H, H^3), 6.82 (d, $^3J_{H\text{-}H}$ = 8.4, 1H, H^6), 4.81 (s, 2H, H^{22}), 4.22 – 3.78 (m, 6H, H^7, H^{11} & H^{24}), 3.17 (q, $^3J_{H\text{-}H}$ = 6.9, 4H, H^{13}), 2.53 (t, $^3J_{H\text{-}H}$ = 7.2, 2H, H^9), 2.22 – 2.11 (m, 2H, H^8), 1.78 (d, $^2J_{H\text{-}P}$ = 15, 3H, H^{23}), 1.31 – 1.22 (m, 6H, H^{12} & H^{25}), 1.06 (t, $^3J_{H\text{-}H}$ = 6.9, 6H, H^{14}); **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 173.0 (C^{10}), 160.4 (d, $^3J_{C\text{-}P}$ = 19, C^{21}), 153.9 (C^1), 153.1 (d, $^1J_{C\text{-}P}$ = 155, C^{17}), 140.0 (C^2), 133.2 (d, $^3J_{C\text{-}P}$ = 11, C^{19}), 128.3 (d, $^2J_{C\text{-}P}$ = 22, C^{18}), 126.5 (C^5), 124.8 (C^3), 124.0 (d, $^4J_{C\text{-}P}$ = 3, C^{20}), 113.6 (C^4), 112.8 (C^6), 97.1 (C^{15}), 84.7 (C^{16}), 67.4 (C^7), 64.0 (C^{22}), 61.2 (d, $^2J_{C\text{-}P}$ = 6.2, C^{24}), 60.5 (C^{11}), 45.6 (C^{13}), 30.8 (C^9), 24.6 (C^8), 16.5 (d, $^3J_{C\text{-}P}$ = 6.0, C^{25}), 14.2 (C^{12}), 13.5 (d, $^1J_{C\text{-}P}$ = 105, C^{23}), 12.3 (C^{14}); **^{31}P{^{1}H}-NMR** (162 MHz, $CDCl_3$) δ +39.4; **ESI-LRMS** (+) *m/z* 517 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{27}H_{38}N_2O_6P]^+$ 517.2468, found 517.2451.

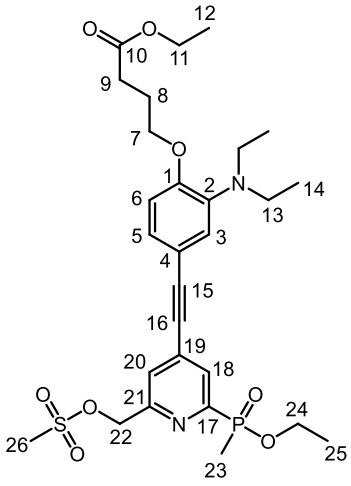
Ethyl 4-(2-(diethylamino)-4-((2-(ethoxy(methyl)phosphoryl)-6-(hydroxymethyl)pyridin-4-yl)ethynyl)phenoxy)butanoate,**12b**



Compound **10b** (396 mg, 1.37 mmol) and ethyl (6-(hydroxymethyl)-4-(bromopyridin-2-yl)(methyl)phosphinate, **11**, (362 mg, 1.23 mmol) were combined in anhydrous CH_3CN (10 mL) under argon. To this solution was added $Pd_2Cl_2(allyl)_2$ (50 mg, 0.137 mmol), $P(^tBu)_3$ (0.05 mL, 0.21 mmol) and piperidine (0.4 mL, 4.37 mmol) in that order. The resulting mixture was stirred at 40 ^oC under argon for 36 h before removal of the solvent under reduced pressure. The residue was dissolved in CH_2Cl_2 (40 mL) and washed with H_2O (3 × 40 mL) before drying over K_2CO_3 and removal of the solvent under reduced pressure to give an orange oil that was purified by reverse phase HPLC (10 to 100% CH_3CN over 10 min, t_r = 13.2 min) to afford a pale orange oil (185 mg, 30%); **^{1}H-NMR** (600 MHz, $CDCl_3$) δ 8.02 (d, $^3J_{H\text{-}P}$ = 6.0, 1H, H^{19}), 7.47 (s, 1H, H^{21}), 7.12 (dd, $^3J_{H\text{-}H}$ = 8.3, $^4J_{H\text{-}H}$ = 2.0, 1H, H^5), 7.05 (d, $^4J_{H\text{-}H}$ = 2.0, 1H, H^3), 6.80 (d, $^3J_{H\text{-}H}$ = 8.3, 1H, H^6), 4.80 (s, 2H, H^{23}), 4.14 (q, $^3J_{H\text{-}H}$ = 7.2, 2H, H^{11}), 4.12 – 4.08 & 3.90 – 3.83 (m, 2H, H^{25}), 4.07 (t, $^3J_{H\text{-}H}$ = 6.4, 2H, H^7), 3.12 (q, $^3J_{H\text{-}H}$ = 7.0, 2H, H^{13}), 2.78 (s, 3H, H^{15}), 2.53 (t, $^3J_{H\text{-}H}$ = 7.3, 2H, H^9), 2.21 – 2.15 (m, 2H, H^8), 1.77 (d, $^2J_{H\text{-}P}$ = 15, 3H, H^{24}), 1.27 (t, $^3J_{H\text{-}H}$ = 7.0, 3H, H^{26}), 1.25 (t, $^3J_{H\text{-}H}$ = 7.2, 3H, H^{12}), 1.12 (t, $^3J_{H\text{-}H}$ = 7.0, 3H, H^{14}); **^{13}C-NMR** (151 MHz, $CDCl_3$) δ 173.1 (C^{10}), 160.7 (d, $^3J_{C\text{-}P}$ = 19, C^{22}), 153.3 (d, $^1J_{C\text{-}P}$ = 155, C^{18}), 153.1 (C^1), 142.1 (C^2), 133.3 (d, $^3J_{C\text{-}P}$ = 11, C^{20}), 128.3 (d, $^2J_{C\text{-}P}$ = 22, C^{19}), 126.4 (C^5), 124.1 (d, $^4J_{C\text{-}P}$ = 3, C^{21}), 122.6 (C^3), 113.9 (C^4), 112.3 (C^6), 97.1 (C^{16}), 84.9 (C^{17}), 67.4 (C^7), 64.2 (C^{23}), 61.3 (d, $^2J_{C\text{-}P}$ = 6, C^{25}), 60.6 (C^{11}), 49.4 (C^{13}), 39.2 (C^{15}), 31.0

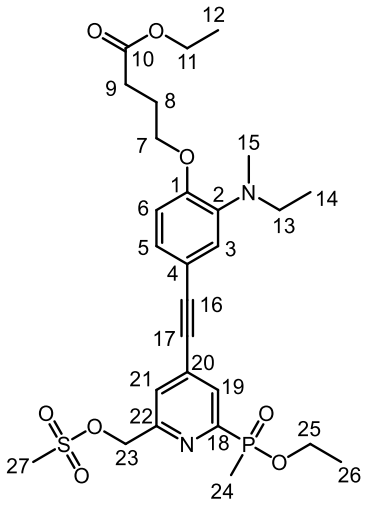
(C^{9}), 24.7 (C^{8}), 16.6 (d, $^{3}J_{C\text{-}P}$ = 6, C^{26}), 14.3 (C^{12}), 13.6 (d, $^{1}J_{C\text{-}P}$ = 104, C^{24}), 12.4 (C^{14}); **$^{31}P\{^{1}H\}$-NMR** (162 MHz, $CDCl_3$) δ +39.5; **ESI-LRMS** (+) *m/z* 503 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{26}H_{36}N_2O_6P]^+$ 503.2311, found 503.2297.

Ethyl-4-(2-(diethylamino)-4-((2-(ethoxy(methyl)phosphoryl)-6-(((methylsulfonyl)oxy)methyl)pyridin-4-yl)ethynyl)phenoxy)butanoate, **13a**



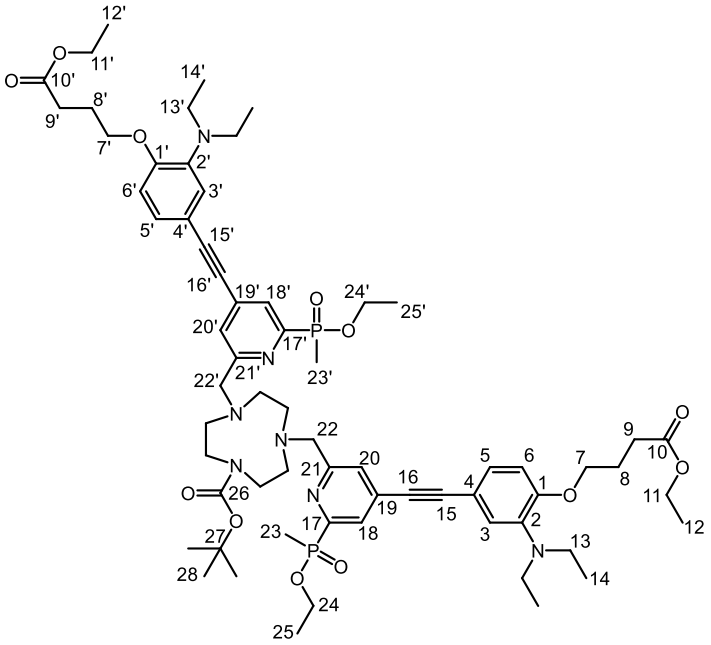
Compound **12a** (250 mg, 0.484 mmol), methanesulfonic anhydride (126 mg, 0.72 mmol) and DIPEA (0.25 mL, 1.44 mmol) were combined in anhydrous THF (2 mL) under argon. The reaction mixture was stirred at room temperature for 1 h, monitoring the progress of the reaction by TLC. Following complete conversion, the solvent was removed under reduced pressure and the resulting crude residue dissolved in CH_2Cl_2 (40 mL), washed with water (3 × 40 mL) and the organic layer dried over K_2CO_3. Removal of solvent under reduced pressure afforded a viscous orange oil (288 mg, quant.); **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 8.10 (d, $^{3}J_{H\text{-}P}$ = 6.0, 1H, H^{18}), 7.64 (s, 1H, H^{20}), 7.15 (d, $^{3}J_{H\text{-}H}$ = 8.4, 1H, H^{5}), 7.09 (s, 1H, H^{3}), 6.83 (d, $^{3}J_{H\text{-}H}$ = 8.4, 1H, H^{6}), 5.37 (s, 2H, H^{22}), 4.19 – 3.82 (m, 6H, H^{7}, H^{11} & H^{24}), 3.18 (q, $^{3}J_{H\text{-}H}$ = 7.0, 4H, H^{13}), 3.14 (s, 3H, H^{26}), 2.54 (t, $^{3}J_{H\text{-}H}$ = 7.2, 2H, H^{9}), 2.22 – 2.13 (m, 2H, H^{8}), 1.78 (d, $^{2}J_{H\text{-}P}$ = 15, 3H, H^{23}), 1.32 – 1.24 (m, 6H, H^{12} & H^{25}), 1.07 (t, $^{3}J_{H\text{-}H}$ = 6.9, 6H, H^{14}); **ESI-LRMS** (+) *m/z* 595 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{28}H_{40}N_2O_8SP]^+$ 595.2242, found 595.2236; $\mathbf{R_f}$ = 0.4 (SiO_2, 5% CH_3OH in CH_2Cl_2).

Ethyl-4-(2-(diethylamino)-4-((2-(ethoxy(methyl)phosphoryl)-6-(((methylsulfonyl)oxy)methyl)pyridin-4-yl)ethynyl)phenoxy)butanoate, **13b**



Compound **12b** (160 mg, 0.318 mmol), methanesulfonic anhydride (111 mg, 0.637 mmol) and DIPEA (0.11 mL, 0.632 mmol) were combined in anhydrous THF (4 mL) under argon. The reaction mixture was stirred at room temperature for 1 h at which point the solvent was removed under reduced pressure. The resulting crude residue was dissolved in CH_2Cl_2 (40 mL), washed with water (3 × 40 mL) and the combined aqueous layers were extracted with CH_2Cl_2 (3 × 40 mL). The combined organic layers were dried over K_2CO_3 and the solvent removed under reduced pressure to afford an orange oil (167 mg, 90%); **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 8.10 (d, $^3J_{H\text{-}P}$ = 6.0, 1H, H^{18}), 7.64 (s, 1H, H^{20}), 7.15 (d, $^3J_{H\text{-}H}$ = 8.4, 1H, H^5), 7.09 (s, 1H, H^3), 6.83 (d, $^3J_{H\text{-}H}$ = 8.4, 1H, H^6), 5.37 (s, 2H, H^{22}), 4.19 – 3.82 (m, 6H, H^7, H^{11} & H^{24}), 3.18 (q, $^3J_{H\text{-}H}$ = 7.0, 4H, H^{13}), 3.14 (s, 3H, H^{26}), 2.54 (t, $^3J_{H\text{-}H}$ = 7.2, 2H, H^9), 2.22 – 2.13 (m, 2H, H^8), 1.78 (d, $^2J_{H\text{-}P}$ = 15, 3H, H^{23}), 1.32 – 1.24 (m, 6H, H^{12} & H^{25}), 1.07 (t, $^3J_{H\text{-}H}$ = 6.9, 6H, H^{14}); **ESI-LRMS** (+) *m/z* 595 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{27}H_{38}N_2O_8SP]^+$ 581.2087, found 581.2099; $\mathbf{R_f}$ = 0.4 (SiO_2, 5% CH_3OH in CH_2Cl_2).

Diethyl 4,4'-((((((7-(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))bis(6-(ethoxy(methyl)phosphoryl)pyridine-2,4-diyl))bis(ethyne-2,1-diyl))bis(2-(diethylamino)-4,1-phenylene))bis(oxy))dibutyrate, **14a**

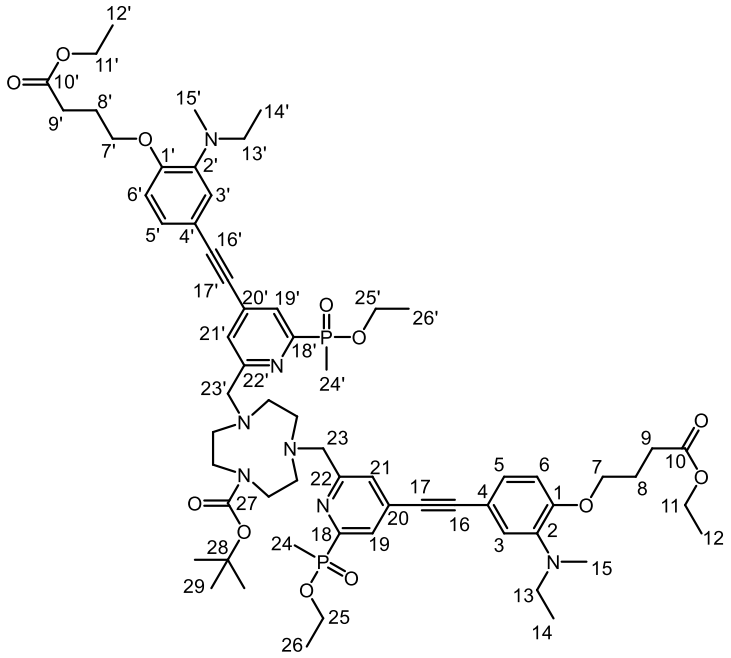


1-*t*-Butoxycarbonyl-1,4,7-triazacyclononane dihydrochloride (66 mg, 0.218 mmol), the mesylate **13a** (288 mg, 0.484 mmol) and K_2CO_3 (122 mg, 0.833 mmol) were combined in anhydrous CH_3CN (2.5 mL) under argon. The resulting mixture was heated to 65 °C for 16 h before cooling to room temperature. Separation from the inorganic salts was achieved by filtration and removal of the solvent under reduced pressure yielded a crude product as a viscous residue that was purified by reverse phase HPLC (10 to 100% CH_3CN in H_2O over 10 min, t_r = 17.5 min) to afford an orange oil (160 mg, 59%); **^{1}H-NMR** (700 MHz, $CDCl_3$) δ 8.01 – 7.97 (m, 2H, H^{18} & $H^{18'}$), 7.65 (s, 1H, H^{20}), 7.56 (s, 1H, $H^{20'}$), 7.11 (d, 2H, H^5 & $H^{5'}$), 7.05 (s, 2H, H^3 & $H^{3'}$), 6.80 (d, 2H, H^6 & $H^{6'}$), 4.13 (q, 4H, H^{11} & $H^{11'}$), 4.11 – 4.06 & 3.88 – 3.80 (m, 4H, H^{24} & $H^{24'}$), 4.05 (t, 4H, H^7 & $H^{7'}$), 3.94 (s, 2H, H^{22}), 3.93 (s, 2H, $H^{22'}$), 3.41 – 3.31 (m, 4H, 9-N_3 ring), 3.15 (q, 8H, H^{13} & $H^{13'}$), 3.12 – 3.04 (m, 4H, 9-N_3 ring), 2.74 – 2.62 (m, 4H, 9-N_3 ring), 2.51 (t, 4H, H^9 & $H^{9'}$), 2.18 – 2.11 (m, 4H, H^8 & $H^{8'}$), 1.76 (d, $^2J_{H\text{-}P}$ = 15, H^{23}), 1.75 (d, $^2J_{H\text{-}P}$ = 15, $H^{23'}$), 1.47 (s, 9H, H^{28}), 1.26 – 1.22 (m, 12H, H^{12}, $H^{12'}$, H^{25} & $H^{25'}$), 1.04 (t, 12H, H^{14} & $H^{14'}$); **^{13}C-NMR** (176 MHz, $CDCl_3$) δ 173.1 (C^{10} & $C^{10'}$), 161.7 (d, $^3J_{C\text{-}P}$ = 20, C^{21}), 161.4 (d, $^3J_{C\text{-}P}$ = 20, $C^{21'}$), 155.7 (C^{26}), 154.1 (C^1 & $C^{1'}$), 153.9 (d, $^1J_{C\text{-}P}$ = 157, $C^{17'}$), 153.6 (d, $^1J_{C\text{-}P}$ = 157, C^{17}), 140.1 (C^2 & $C^{2'}$), 132.8 (app. t, $^3J_{C\text{-}P}$ = 11, C^{19} & $C^{19'}$), 127.9 (app. t, $^2J_{C\text{-}P}$ = 21, C^{18} & $C^{18'}$), 126.5 (C^5 & $C^{5'}$), 126.4 (d, $^4J_{C\text{-}P}$ = 3, C^{20}), 126.2 (d, $^4J_{C\text{-}P}$ = 3, $C^{20'}$), 124.8 (C^3 & $C^{3'}$), 114.0 ($C^{4'}$), 113.8 (C^4), 112.8 (C^6 & $C^{6'}$), 96.5 ($C^{15'}$), 96.3 (C^{15}),

85.2 ($C^{16'}$), 85.1 (C^{16}), 79.5 (C^{27}), 67.5 (C^{7} & $C^{7'}$), 62.9 ($C^{22'}$), 62.6 (C^{22}), 61.1 (d, $^{2}J_{C\text{-}P}$ = 6.4, $C^{24'}$), 61.0 (d, $^{2}J_{C\text{-}P}$ = 6.4, C^{24}), 60.6 (C^{11} & $C^{11'}$), 56.1 (9-N_3 ring), 55.0 (9-N_3 ring), 54.7 (9-N_3 ring), 54.0 (9-N_3 ring), 50.0 (9-N_3 ring), 49.7 (9-N_3 ring), 45.7 (C^{13} & $C^{13'}$), 30.9 (C^{9} & $C^{9'}$), 28.8 (C^{28}), 24.7 (C^{8} & $C^{8'}$), 16.6 (d, C^{25} & $C^{25'}$), 14.3 (C^{12} & $C^{12'}$), 13.4 (d, $^{1}J_{C\text{-}P}$ = 104, C^{23}), 13.3 (d, $^{1}J_{C\text{-}P}$ = 104, $C^{23'}$), 12.4 (C^{14} & $C^{14'}$); **$^{31}P\{^{1}H\}$-NMR** (283 MHz, $CDCl_3$) δ +40.1, +40.0; **ESI-LRMS** (+) *m/z* 1226 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{65}H_{94}N_7O_{12}P_2]^+$ 1226.644, found 1226.646.

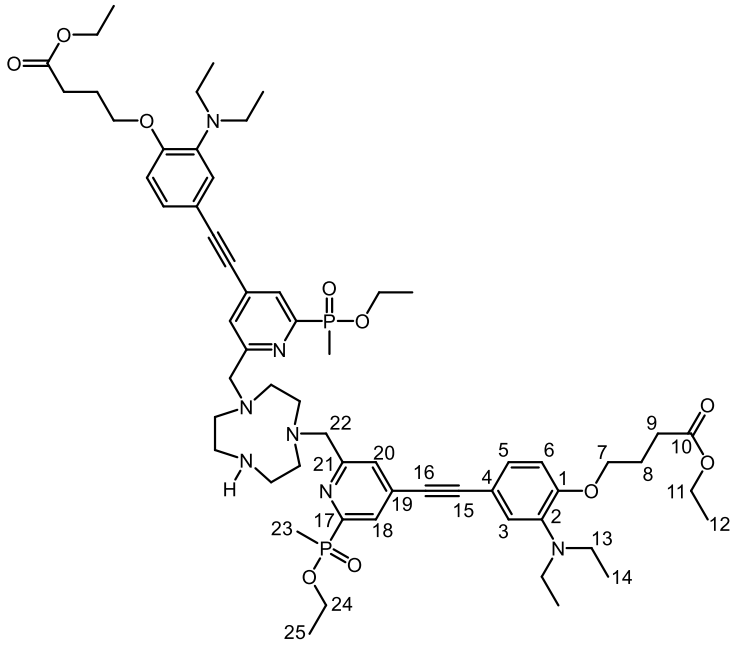
Note: ^{1}H, ^{31}P and ^{13}C NMR analysis each indicate that the chromophore environments are non-equivalent, but are highly similar in several respects. As an illustration, C^{10} and $C^{10'}$ have different corresponding resonances in the ^{13}C spectrum (Δ = 0.01 ppm). It is not prudent to report these environments individually when reporting to an accuracy of 0.1 ppm for ^{13}C NMR. The chemical shift differences for the resonances of the chromophore were observed to decrease as a function of the distance from the macrocyclic ring. Due to the non-equivalent nature of their chemical environments, the *J*-coupling constant values for the resonances corresponding to these multiple environments are not given.

Diethyl-4,4'-((((((7-(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))bis(6-(ethoxy(methyl)phosphoryl)pyridine-2,4-diyl))bis(ethyne-2,1-diyl))bis(2-(ethylmethylamino)-4,1-phenylene))bis(oxy))dibutyrate,**14b**



1-*t*-Butoxycarbonyl-1,4,7-triazacyclononane dihydrochloride (38 mg, 0.126 mmol), the mesylate **13b** (167 mg, 0.288 mmol) and K_2CO_3 (104 mg, 0.753 mmol) were combined in anhydrous CH_3CN (4 mL) under argon. The resulting mixture was heated to 65 ^oC for 48 h before cooling to room temperature. Separation from the inorganic salts was achieved by filtration and removal of the solvent under reduced pressure yielded a crude product that was purified by reverse phase HPLC (10 to 100% CH_3CN in H_2O over 10 min, t_r = 12.2 min) to afford a pale orange oil (110 mg, 73%); **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 8.00 (d, 2H, H^{19} & $H^{19'}$), 7.65 (s, 1H, H^{21}), 7.59 (s, 1H, $H^{21'}$), 7.12 (dd, 2H, H^5 & $H^{5'}$), 7.06 (s, 2H, H^3 & $H^{3'}$), 6.80 (d, 2H, H^6 & $H^{6'}$), 4.18 – 3.78 (m, 16H, H^{11}, $H^{11'}$, H^7, $H^{7'}$, H^{23}, $H^{23'}$, H^{25} & $H^{25'}$), 3.47 – 3.32 (m, 4H, 9-N_3 ring), 3.21 – 3.06 (m, 8H, H^{13}, $H^{13'}$ & 9-N_3 ring), 2.83 – 2.72 (m, 10H, H^{15}, $H^{15'}$ & 9-N_3 ring), 2.53 (t, 4H, H^9 & $H^{9'}$), 2.23 – 2.14 (m, 4H, H^8 & $H^{8'}$), 1.76 (d, 6H, H^{24} & $H^{24'}$), 1.48 (s, 9H, H^{29}), 1.28 – 1.22 (m, 12H, H^{12}, $H^{12'}$, H^{26} & $H^{26'}$), 1.11 (t, 6H, H^{14} & $H^{14'}$); **^{31}P{^{1}H}-NMR** (162 MHz, $CDCl_3$) δ +40.2, +40.1; **ESI-LRMS** (+) *m/z* 400 $[M+3H]^{3+}$, 600 $[M+2H]^{2+}$, 1198 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{63}H_{90}N_7O_{12}P_2]^+$ 1198.612, found 1198.607.

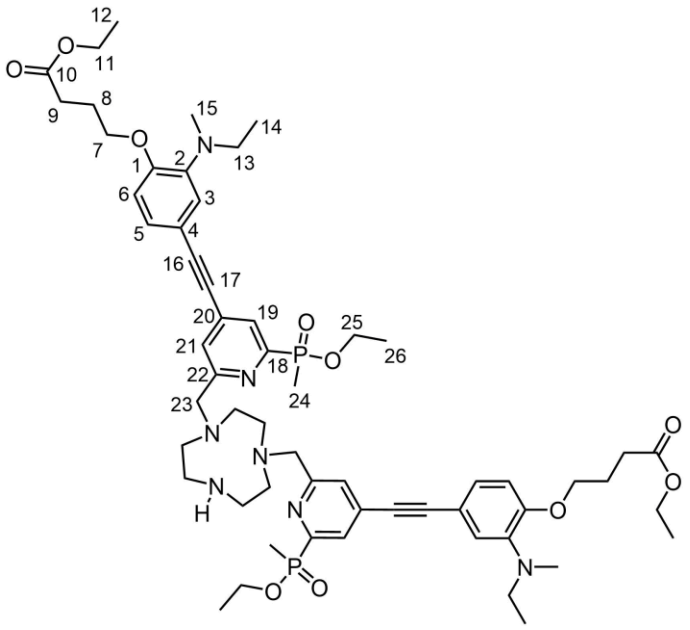
Diethyl-4,4'-((((((1,4,7-triazacyclononane-1,4-diyl)bis(methylene))bis(6-(ethoxy(methyl)phosphoryl)pyridine-2,4-diyl))bis(ethyne-2,1-diyl))bis(2-(diethylamino)-4,1-phenylene))bis(oxy))dibutyrate, **15a**, (trifluoroacetate salt)



A solution of compound **14a** (19 mg, 0.016 mmol) in trifluoroacetic acid and CH_2Cl_2 was prepared (10% v/v, 3 mL total) and stirred under argon for 40 min. Immediately following this, the solvent was removed under reduced pressure. Additional CH_2Cl_2 (~50 mL) was added and the solvent removed under reduced pressure. This procedure was repeated 3 times. The residue was dissolved in CH_2Cl_2 (30 mL), washed with aqueous Na_2CO_3 solution (4%, pH = 11, 3 × 30 mL) and dried over K_2CO_3. Removal of the solvent under reduced pressure yielded an orange oil (17 mg, quant.); **^{1}H-NMR** (400 MHz, $CDCl_3$) δ 7.95 (dd, $^3J_{H\text{-}P}$ = 6.0, $^4J_{H\text{-}H}$ = 1.3, 2H, H^{18}), 7.38 (app. s, 2H, H^{20}), 7.12 (dd, $^3J_{H\text{-}H}$ = 8.4, $^4J_{H\text{-}H}$ = 2.0, 2H, H^5), 7.06 (d, $^4J_{H\text{-}H}$ = 2.0, 2H, H^3), 6.81 (d, $^3J_{H\text{-}H}$ = 8.4, 2H, H^6), 4.23 – 4.10 & 3.96 – 3.86 (m, 8H, H^{11} & H^{24}), 4.05 (t, $^3J_{H\text{-}H}$ = 6.4, 4H, H^7), 3.99 (s, 4H, H^{22}), 3.31 – 3.24 (m, 4H, 9-N_3 ring), 3.16 (q, $^3J_{H\text{-}H}$ = 7.1, 8H, H^{13}), 3.13 – 3.07 (m, 4H, 9-N_3 ring), 2.73 (br s, 4H, 9-N_3 ring), 2.52 (t, $^3J_{H\text{-}H}$ = 7.2, 4H, H^9), 2.20 – 2.11 (m, 4H, H^8), 1.77 (d, $^2J_{H\text{-}P}$ = 15, 6H, H^{23}), 1.31 – 1.22 (m, 12H, H^{12} & H^{25}), 1.05 (t, $^3J_{H\text{-}H}$ = 7.1, 12H, H^{14}); **^{13}C-NMR** (101 MHz, $CDCl_3$) δ 173.2 (C^{10}), 159.4 (d, $^3J_{C\text{-}P}$ = 20, C^{21}), 154.6 (d, $^1J_{C\text{-}P}$ = 157, C^{17}), 154.2 (C^1), 140.2 (C^2), 133.4 (d, $^3J_{C\text{-}P}$ = 12, C^{19}), 127.9 (d, $^2J_{C\text{-}P}$ = 22, C^{18}), 126.7 (C^5), 126.3 (C^{20}), 124.8 (C^3), 113.5 (C^4), 112.8 (C^6), 97.5 (C^{15}), 84.7 (C^{16}), 67.5 (C^7), 61.3 (d, $^2J_{C\text{-}P}$ = 6, C^{21}), 60.7 (C^{11}), 60.0 (C^{22}), 53.9 (9-N_3 ring), 51.6 (9-N_3 ring), 50.1 (9-N_3 ring), 49.4 (9-N_3 ring), 49.2 (9-N_3 ring), 45.7 (C^{13}), 44.8 (9-N_3 ring), 31.0 (C^9), 24.7 (C^8), 16.7 (d, $^3J_{C\text{-}P}$ = 6, C^{25}), 14.4 (C^{12}), 13.8 (d, $^1J_{C\text{-}P}$ = 103, C^{23}), 12.5

(C^{14}); **^{31}P{^{1}H}-NMR** (162 MHz, $CDCl_3$) δ +39.2; **ESI-LRMS** (+) *m/z* 1127 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{60}H_{86}N_7O_{10}P_2]^+$ 1126.592, found 1126.592.

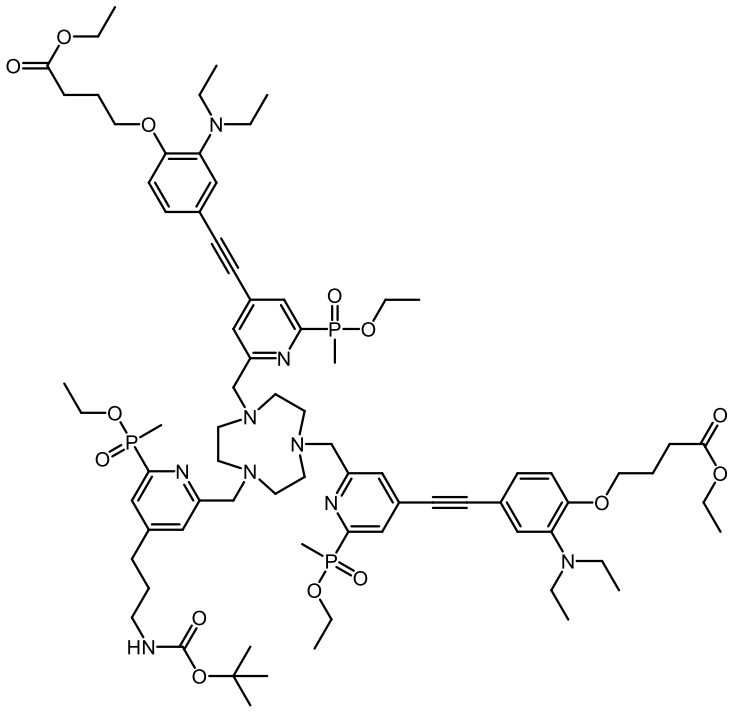
Diethyl-4,4'-((((((1,4,7-triazacyclononane-1,4-diyl)bis(methylene))bis(6-(ethoxy(methyl)phosphoryl)pyridine-2,4-diyl))bis(ethyne-2,1-diyl))bis(2-(ethylmethylamino)-4,1-phenylene))bis(oxy))dibutyrate **15b**, (trifluoroacetate salt)



A solution of compound **14b** (110 mg, 0.092 mmol) in trifluoroacetic acid and CH_2Cl_2 was prepared (10% v/v, 3 mL total) and stirred under argon for 60 min. The solvent was removed under reduced pressure, additional CH_2Cl_2 (~50 mL) was added and the solvent removed under reduced pressure. This process was repeated 4 times. The residue was dissolved in CH_2Cl_2 (30 mL), washed with aqueous Na_2CO_3 solution (4%, pH = 11, 3 × 30 mL) and dried over K_2CO_3. Removal of the solvent under reduced pressure yielded an orange oil (111 mg, quant.); **^{1}H-NMR** (700 MHz, $CDCl_3$) δ 7.89 (d, $^{3}J_{H\text{-}P}$ = 6.0, 2H, H^{19}), 7.77 (s, 2H, H^{21}), 7.64 (dd, $^{3}J_{H\text{-}H}$ = 8.5, $^{4}J_{H\text{-}H}$ = 1.3, 2H, H^{5}), 7.57 (s, 2H, H^{3}), 7.13 (d, $^{3}J_{H\text{-}H}$ = 8.5, 2H, H^{6}), 4.39 (s, 4H, H^{23}), 4.22 (t, $^{3}J_{H\text{-}H}$ = 6.5, 4H, H^{7}), 4.15 – 4.07 & 3.97 – 3.90 (m, 8H, H^{11} & H^{25}), 3.68 (q, $^{3}J_{H\text{-}H}$ = 7.2, 4H, H^{13}), 3.64 – 3.51 (m, 8H, 9-N_3 ring), 3.43 – 3.35 (m, 4H, 9-N_3 ring), 3.29 (s, 6H, H^{15}), 2.51 (t, $^{3}J_{H\text{-}H}$ = 6.8, 4H, H^{9}), 2.21 – 2.16 (m, 4H, H^{8}), 1.75 (d, $^{2}J_{H\text{-}P}$ = 15, 6H, H^{24}), 1.27 (td, , $^{3}J_{H\text{-}H}$ = 7.1, $^{4}J_{H\text{-}P}$ = 3.0, 6H, H^{26}), 1.23 (t, $^{3}J_{H\text{-}P}$ = 7.1, 6H, H^{12}), 1.18 (t, $^{3}J_{H\text{-}H}$ = 7.2, 6H,

H^{14}); **^{13}C-NMR** (176 MHz, $CDCl_3$) δ 173.1 (C^{10}), 156.2 (d, $^3J_{C\text{-}P}$ = 21, C^{22}), 153.3 (d, $^1J_{C\text{-}P}$ = 160, C^{18}), 152.5 (C^1), 135.6 (C^5), 128.2 (C^3), 128.1 (d, $^2J_{C\text{-}P}$ = 20, C^{19}), 127.7 (C^2), 127.1 (d, $^4J_{C\text{-}P}$ = 4, C^{21}), 115.4 (C^4), 114.2 (C^6), 94.6 (C^{16}), 86.2 (d, $^4J_{C\text{-}P}$ = 2.6, C^{17}), 69.1 (C^7), 62.6 (d, $^2J_{C\text{-}P}$ = 7.0, C^{25}), 61.0 (C^{11}), 59.7 (C^{23}), 54.1 (C^{13}), 53.6 (9-N_3 ring), 51.5 (9-N_3 ring), 50.0 (9-N_3 ring), 44.2 (C^{15}), 30.5 (C^9), 24.0 (C^8), 16.3 (d, $^3J_{C\text{-}P}$ = 6.2, C^{26}), 14.2 (C^{12}), 13.4 (d, $^1J_{C\text{-}P}$ = 102, C^{24}), 10.3 (C^{14}); **^{31}P-NMR** (162 MHz, $CDCl_3$) δ +40.8; **ESI-LRMS** (+) *m/z* 1098 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{58}H_{82}N_7O_{10}P_2]^+$ 1098.556, found 1098.557.

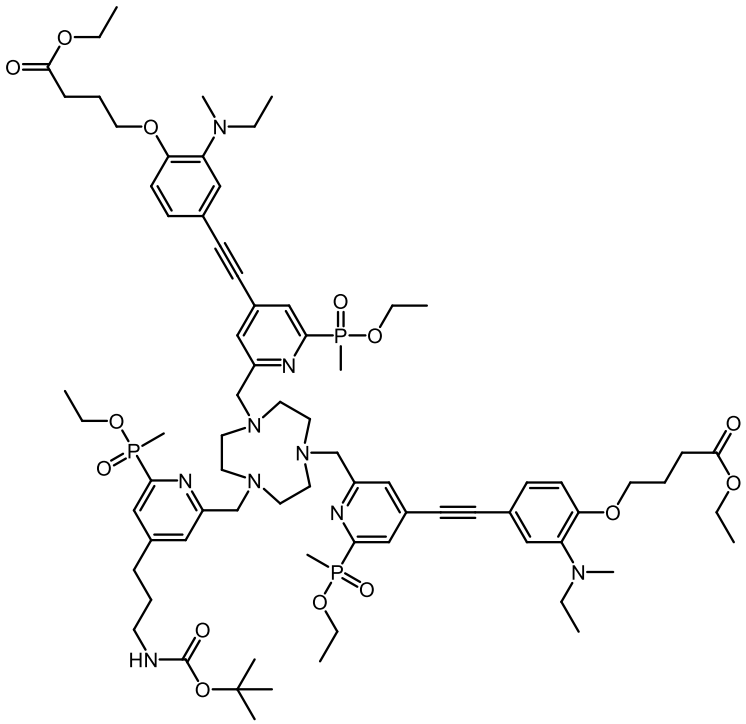
Diethyl 4,4'-((((((7-((4-(3-((tert-butoxycarbonyl)amino)propyl)-6-(ethoxy(methyl)phosphoryl)pyridin-2-yl)methyl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))bis(6-(ethoxy(methyl)phosphoryl)pyridine-2,4-diyl))bis(ethyne-2,1-diyl))bis(2-(diethylamino)-4,1-phenylene))bis(oxy))dibutyrate, **17a**



Compounds **15a** (43 mg, 0.038 mmol), **16** (45 mg, 0.10 mmol) and K_2CO_3 (30 mg, 0.22 mmol) were combined in anhydrous acetonitrile (2 mL) under argon and heated at 65 C for 18 h. After this time, LCMS analysis confirmed the complete transformation of starting material. The reaction mixture was allowed to cool before separation from the inorganic salts by filtration. Removal of the

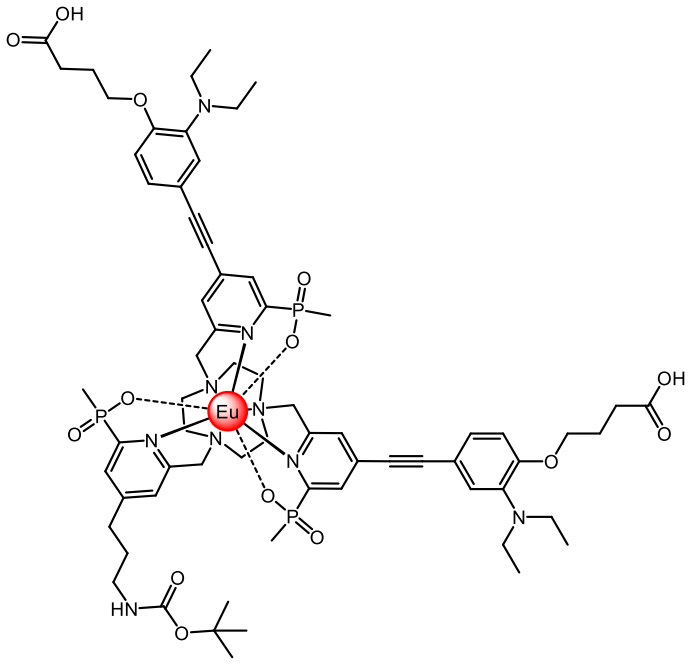
solvent under reduced pressure afforded an orange solid (88 mg) that was used directly in the next step without further purification; **ESI-LRMS** (+) *m/z* 1481 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{77}H_{113}N_9O_{14}P_3]^+$ 1480.762, found 1480.760.

Diethyl 4,4'-((((((7-((4-(3-((tert-butoxycarbonyl)amino)propyl)-6-(ethoxy(methyl)phosphoryl)pyridin-2-yl)methyl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))bis(6-(ethoxy(methyl)phosphoryl)pyridine-2,4-diyl))bis(ethyne-2,1-diyl))bis(2-(ethylmethylamino)-4,1-phenylene))bis(oxy))dibutyrate, **17b**



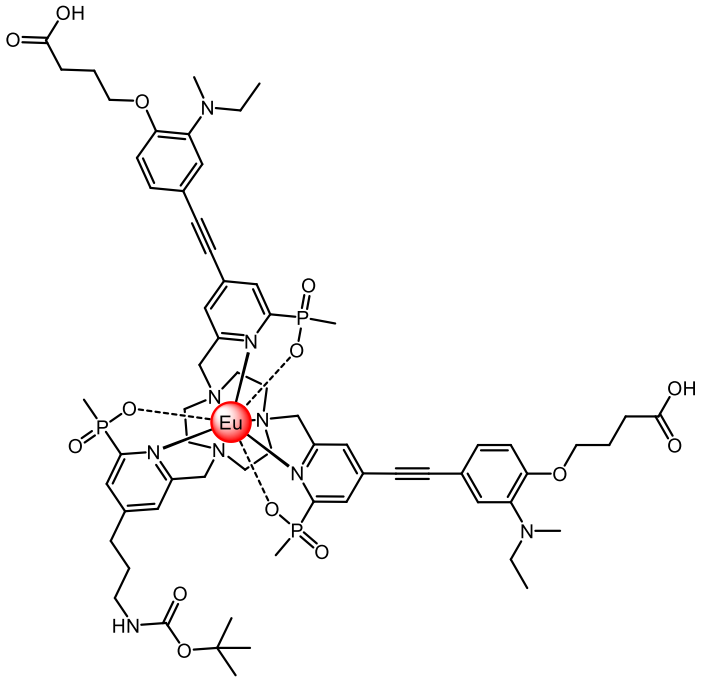
Compounds **15b** (111 mg, 0.0919 mmol), **16** (59 mg, 0.130 mmol) and K_2CO_3 (45 mg, 0.33 mmol) were combined in anhydrous acetonitrile (3 mL) under argon and heated at 65 C for 16 h. After this time, LCMS analysis confirmed the complete transformation of the macrocyclic amine. The reaction mixture was allowed to cool before separation from the inorganic salts by filtration. Removal of the solvent under reduced pressure afforded an orange oil (165 mg) that was used directly in the next step without further purification; **ESI-LRMS** (+) *m/z* 1453 $[M+H]^+$; **ESI-HRMS** (+) calcd for $[C_{75}H_{109}N_9O_{14}P_3]^+$ 1452.731, found 1452.729.

Europium complex of diethyl-4,4'-((((((7-((4-(3-((tert-butoxycarbonyl)amino)propyl)-6-(ethoxy(methyl)phosphoryl)pyridin-2-yl)methyl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))bis(6-(ethoxy(methyl)phosphoryl)pyridine-2,4-diyl))bis(ethyne-2,1-diyl))bis(2-(diethylamino)-4,1-phenylene))bis(oxy))dibutyrate, **Eu*pro-L*^{2a}**



The crude ligand **17a** (88 mg) from the previous step was dissolved in a mixture of CH_3OH/H_2O (4:1, 2.5 mL total) and the pH was adjusted to 12 using aqueous NaOH solution. The solution was heated at 60 C for 1.5 h. After this time, LCMS analysis confirmed complete hydrolysis of the phosphinate and carboxylic acid ester groups. After cooling and adjustment of the pH to 7 using hydrochloric acid (0.1 M), $EuCl_3.6H_2O$ (42 mg, 0.115 mmol) was added and the reaction mixture was heated to 60 ^oC for 18 h. The reaction mixture was purified by reverse phase HPLC (10 to 100% CH_3CN in H_2O over 10 min, t_r = 8.5 min) to yield a yellow solid (22 mg, 39% over three steps); **ESI-LRMS** (+) *m/z* 1490 $[M+H]^+$, 746 $[M+2H]^{2+}$; **ESI-HRMS** (+) calcd for $[C_{67}H_{91}{}^{151}EuN_9O_{14}P_3]^{2+}$ 745.7561, found 745.7562; τ_{H2O} (ms) = 0.27 (pH = 8), 0.39 (pH = 7), 0.53 (pH = 6), 0.95 (pH = 5), 0.97 (pH = 4).

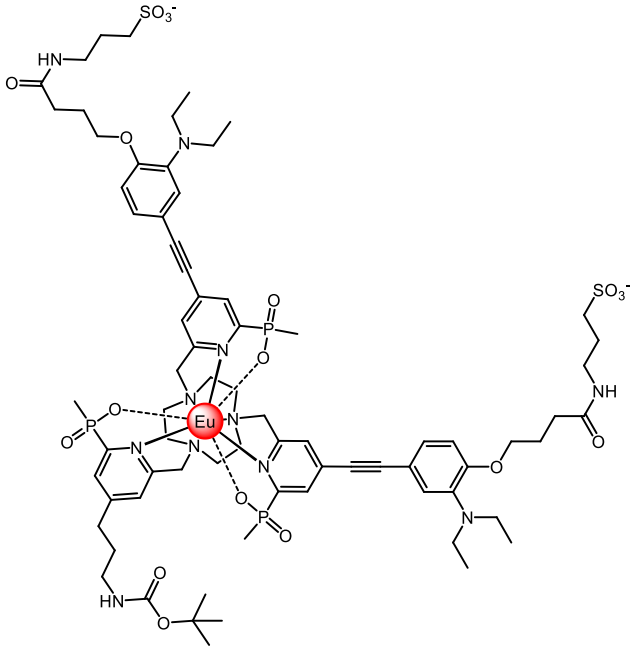
Europium complex of diethyl-4,4'-((((((7-((4-(3-((tert-butoxycarbonyl)amino)propyl)-6-(ethoxy(methyl)phosphoryl)pyridin-2-yl)methyl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))bis(6-(ethoxy(methyl)phosphoryl)pyridine-2,4-diyl))bis(ethyne-2,1-diyl))bis(2-(ethylmethylamino)-4,1-phenylene))bis(oxy))dibutyrate, **Eu*pro-L*^{2b}**



The crude ligand **17b** (165 mg) from the previous step was dissolved in a mixture of CH_3OH/H_2O (1:1, 2 mL total) and the pH was adjusted to 12 using aqueous NaOH solution. The solution was heated at 60 C for 1.5 h. After this time, LCMS analysis confirmed complete hydrolysis of the phosphinate and carboxylic acid ester groups. After cooling and adjustment of the pH to 7 using hydrochloric acid (0.1 M), $EuCl_3.6H_2O$ (34 mg, 0.093 mmol) was added and the reaction mixture was heated to 60 ^oC for 17 h. The reaction mixture was purified by reverse phase HPLC (10 to 100% CH_3CN in H_2O over 10 min, t_r = 8.7 min) to yield a yellow solid (50 mg, 37% over three steps); **ESI-LRMS** (+) *m/z* 1462 $[M+H]^+$, 732 $[M+2H]^{2+}$; **ESI-HRMS** (+) calcd for $[C_{65}H_{86}{}^{151}EuN_9O_{14}P_3]^+$ 1462.473, found 1462.468.

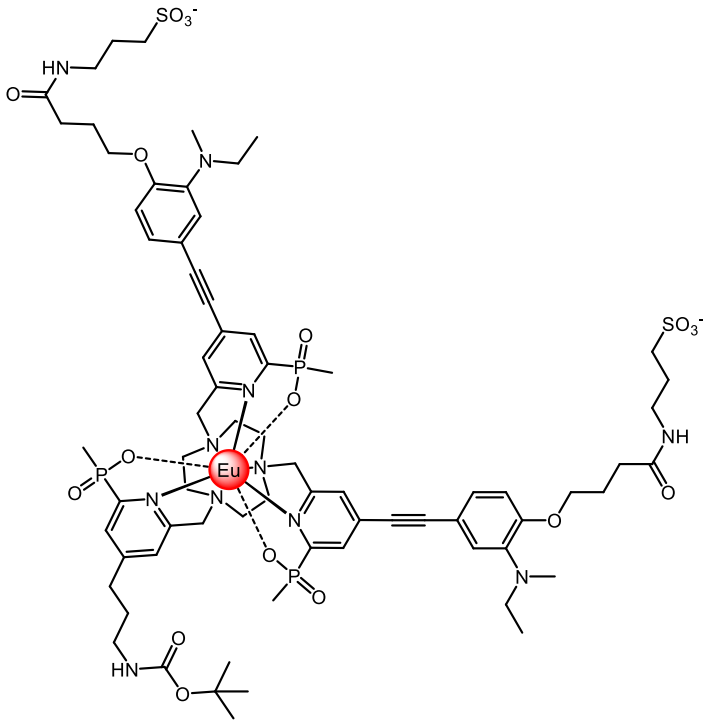
Europium complex of 3,3'-((4,4'-((((((7-((4-(3-((*tert*-butoxycarbonyl)amino)propyl)-6-(hydroxy(methyl)phosphoryl)pyridin-2-yl)methyl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))bis(6-(hydroxy(methyl)phosphoryl)pyridine-2,4-diyl))bis(ethyne-2,1-diyl))bis(2-(diethylamino)-4,1-

phenylene))bis(oxy))bis(butanoyl))bis(azanediyl))bis(propane-1-sulfonic acid), **Eu*L*^{2a}**



The complex prepared as above, **Eu*pro-L*^{2a}** (3.9 mg, 2.6 µmol) and DIPEA (6 µL, 34 µmol) were combined in anhydrous DMSO (0.4 mL) under argon. To this solution was added HATU (5 mg, 13 µmol), homotaurine (2 mg, 14 µmol) and water (40 µL). The mixture was stirred at room temperature for 19 h. Following dilution with water, the reaction mixture was purified by reverse phase HPLC (10 to 100% CH_3OH in H_2O over 10 min, t_r = 11.6 min) yielding a yellow solid (4 mg, 89%); **ESI-LRMS** (-) *m/z* 1730 $[M-H]^-$, 865 $[M-2H]^{2-}$; **ESI-HRMS** (+) calcd for $[C_{73}H_{101}{}^{151}EuN_{11}O_{18}P_3S_2]^{2-}$ 864.7601, found 864.7610; $\boldsymbol{\tau_{H2O}}$ (ms) = 0.25 (pH 8), 0.34 (pH 7), 0.70 (pH 6), 0.98 (pH 5), 1.04 (pH 4); $\boldsymbol{\varepsilon}_{332\ nm}$ = 39000 M^{-1} cm^{-1}; $\boldsymbol{\Phi}_{pH\ 8}$ = 0.3%, $\boldsymbol{\Phi}_{pH\ 4}$ = 16% (λ_{exc} = 330 nm).

Europium complex of 3,3'-((4,4'-((((((7-((4-(3-((*tert*-butoxycarbonyl)amino)propyl)-6-(hydroxy(methyl)phosphoryl)pyridin-2-yl)methyl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))bis(6-(hydroxy(methyl)phosphoryl)pyridine-2,4-diyl))bis(ethyne-2,1-diyl))bis(2-(ethylmethylamino)-4,1-phenylene))bis(oxy))bis(butanoyl))bis(azanediyl))bis(propane-1-sulfonic acid), **Eu*L*^{2b}**



Eu*pro-L*^{2b} (20 mg, 13.7 µmol) and DIPEA (15 µL, 85 µmol) were combined in anhydrous DMSO (0.5 mL) under argon. To this solution was added HATU (12 mg, 31 µmol), homotaurine (4.5 mg, 31 µmol) and water (50 µL), and the mixture was stirred at room temperature for 16 h. Following dilution with water, the reaction mixture was purified by reverse phase HPLC (10 to 100% CH_3OH in H_2O over 10 min, t_r = 10.6 min) to yield a yellow solid (9 mg, 38%); **ESI-LRMS** (-) *m/z* 1701 $[M-H]^-$, 850 $[M-2H]^{2-}$; **ESI-HRMS** (+) calcd for $[C_{71}H_{99}{}^{151}EuN_{11}O_{18}P_3S_2]^{2+}$ 852.7530 found 852.7541; $\boldsymbol{\tau_{H2O}}$ (ms) = 0.30 (pH 8), 0.29 (pH 7), 0.42 (pH 6), 0.77 (pH 5), 1.05 (pH 4); $\boldsymbol{\varepsilon_{332\ nm}}$ = 39000 M^{-1} cm^{-1}; $\boldsymbol{\Phi_{pH\ 8}}$ = 0.01%, $\boldsymbol{\Phi_{pH\ 4}}$ = 14.5% (λ_{exc} = 330 nm).

pH Dependence of Absorption, Emission and Excitation Spectra for Eu(III) Complexes: S1-4, Eu*L*^{1b}; S5-9, Eu*L*^{2a}; S10-13, Eu*L*^{2b}

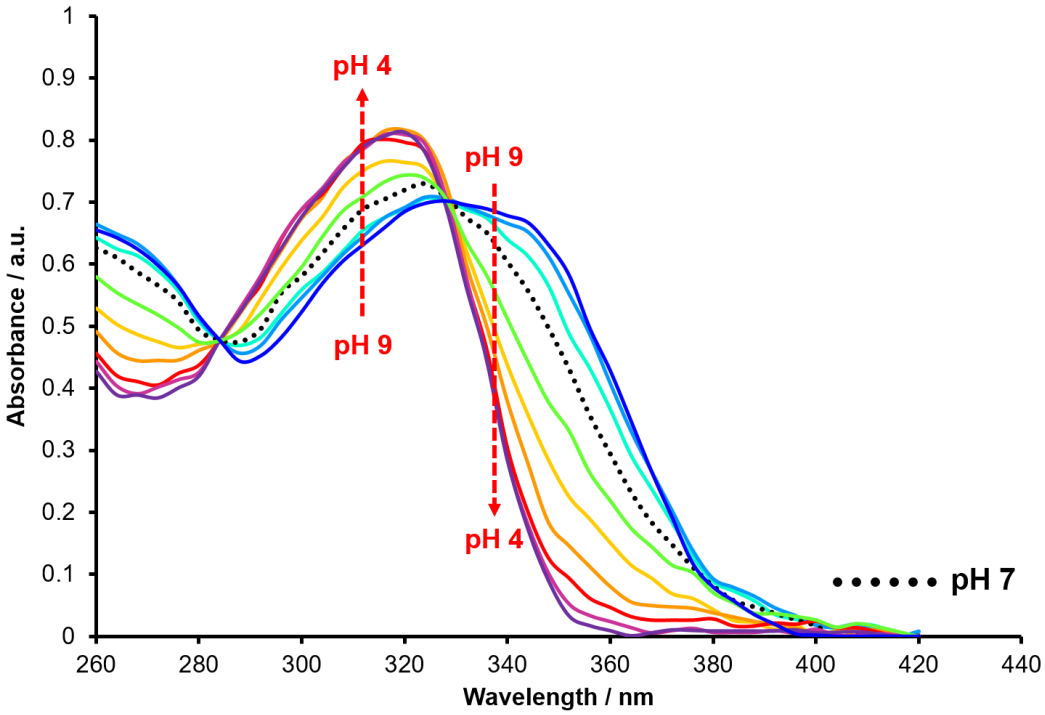


Figure S1 Variation of the absorbance spectrum with pH for Eu*L*^{1b} (295 K, c = 15 µM, 0.1 M NaCl). Isosbestic points are observed at 284 and 328 nm.

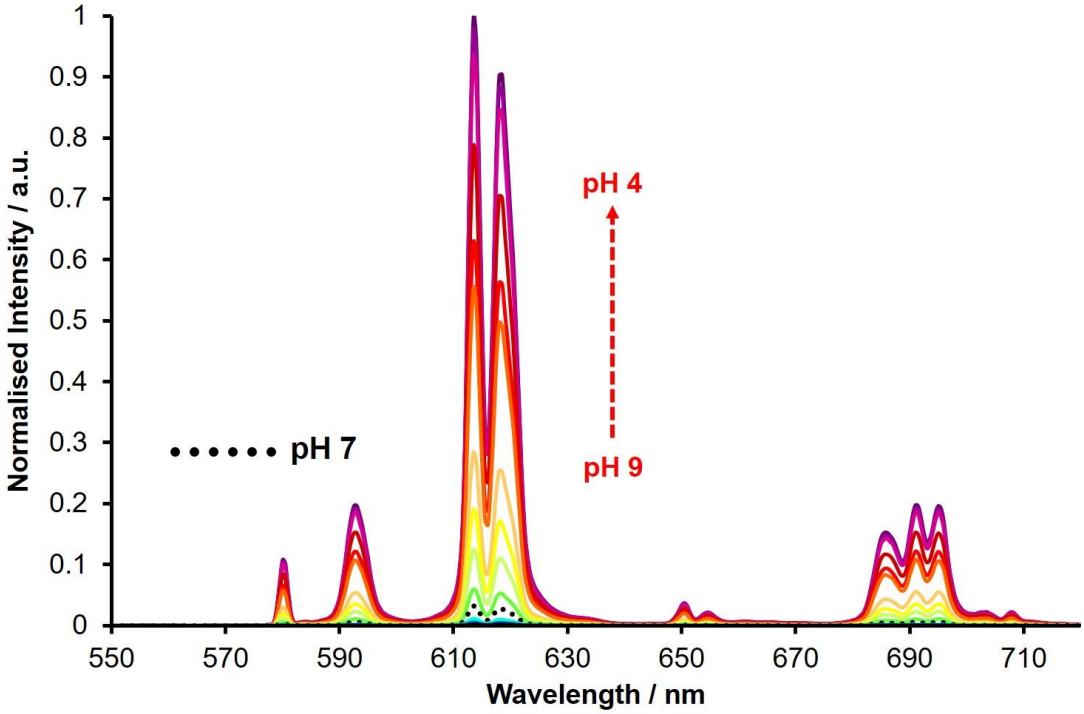


Figure S2 Variation of the europium emission spectrum with pH for Eu*L*^{1b} (λ_{exc} 328 nm, 295 K, c = 15 µM, 0.1 M NaCl)

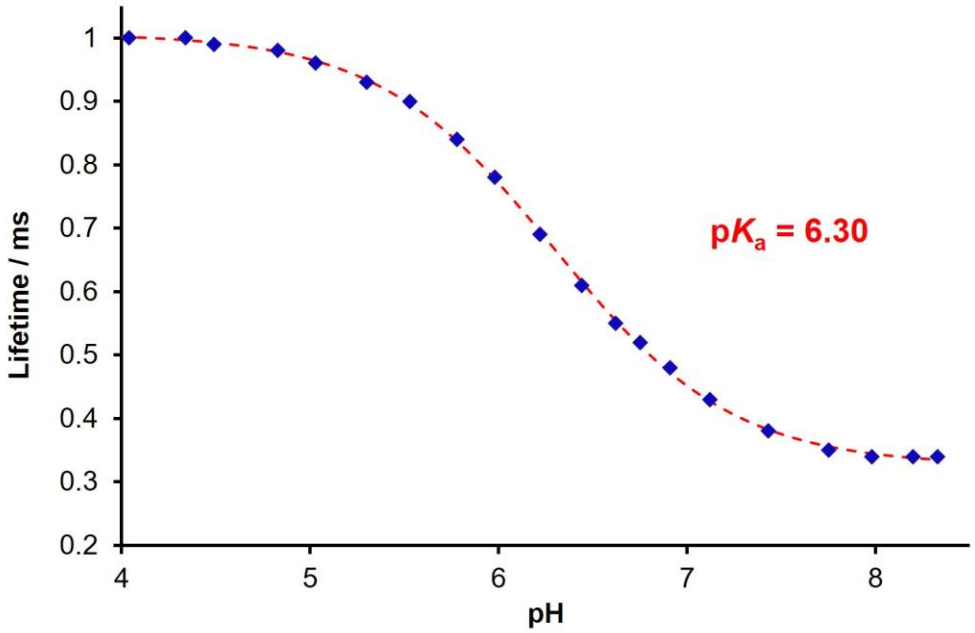


Figure S3 Variation of the europium emission lifetime with pH for **Eu*L*^{1b}** (λ_{exc} 328 nm, λ_{em} 613 nm, 295 K, 0.1 M NaCl). The pK_a value is estimated by non-linear least squares regression analysis.

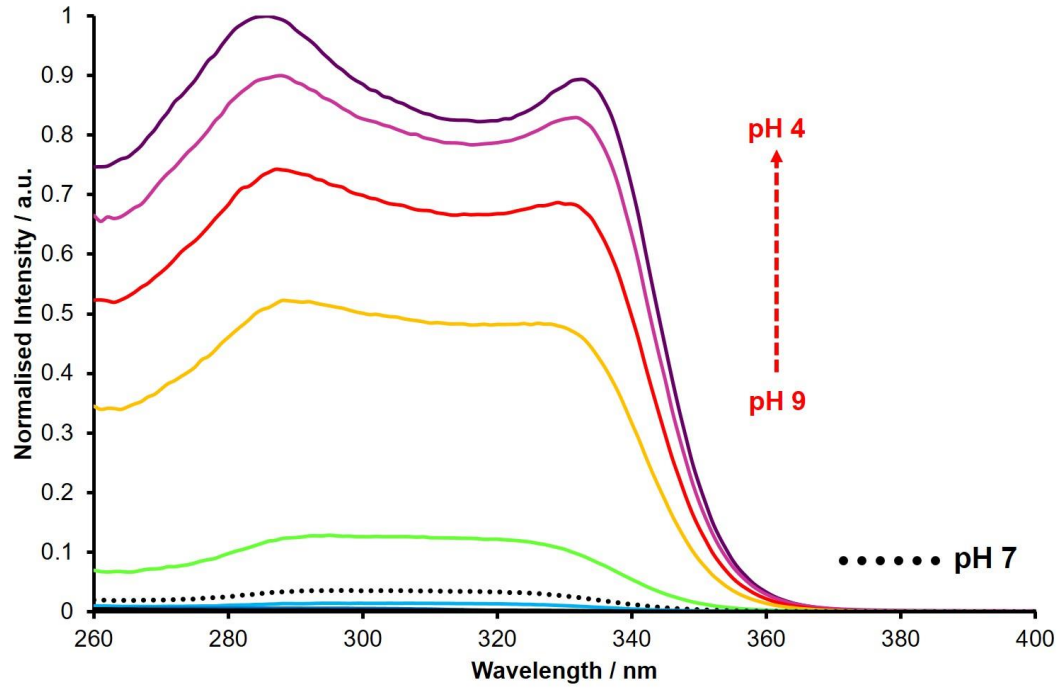


Figure S4 Variation of the europium excitation spectral profile with pH for **Eu*L*^{1b}** (λ_{em} 613 nm, 295 K, c = 15 μM, 0.1 M NaCl).

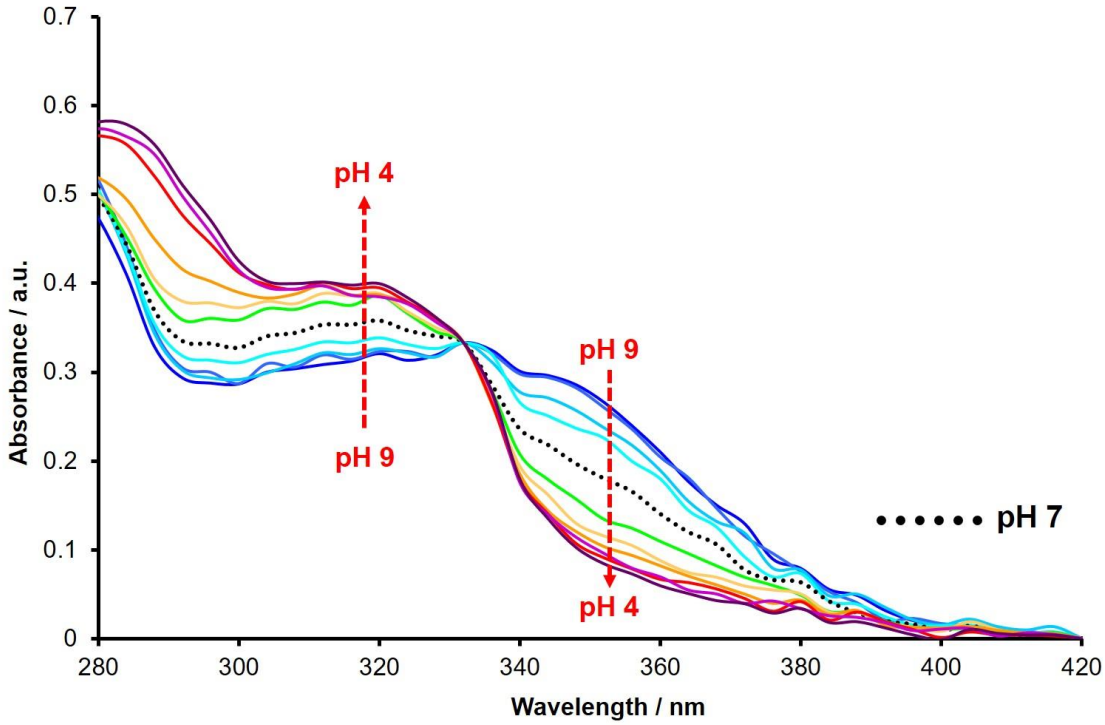


Figure S5 Variation of the absorbance spectrum with pH for **Eu*L*^{2a}** (295 K, 0.1 M NaCl). An isosbestic point is observed at 332 nm.

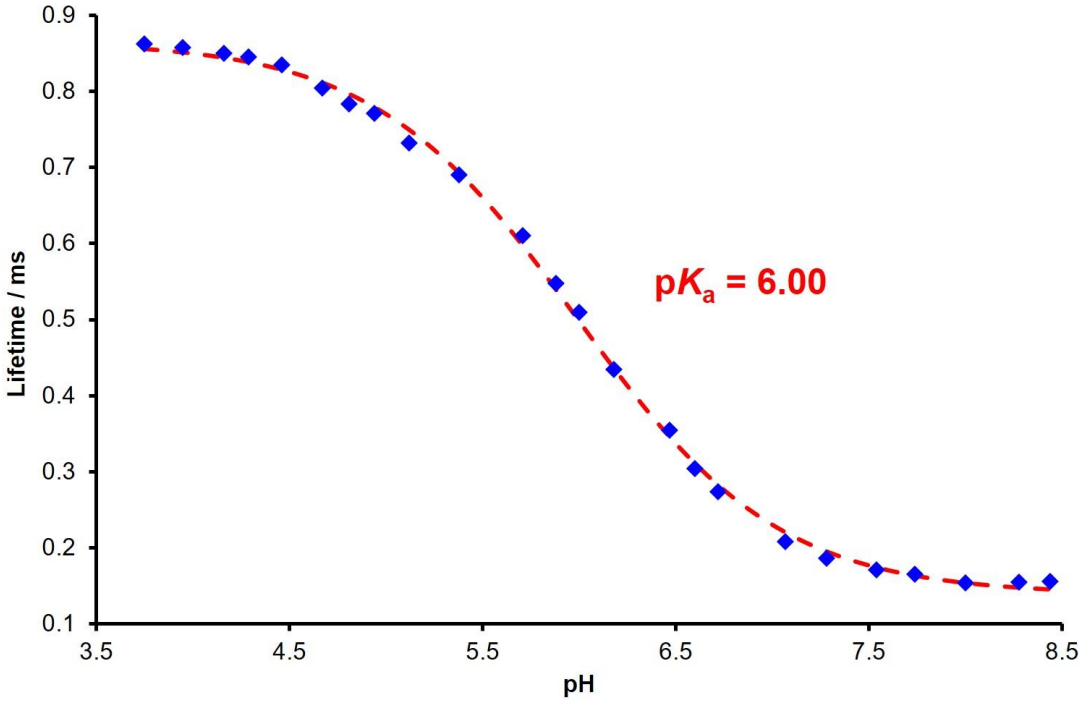


Figure S6 Variation of the europium emission lifetime with pH for **Eu*L*^{2a}** (λ_{exc} 332 nm, λ_{em} 613 nm, 295 K, NIH-3T3 cell lysate). The pK_a value is estimated by non-linear least squares regression analysis.

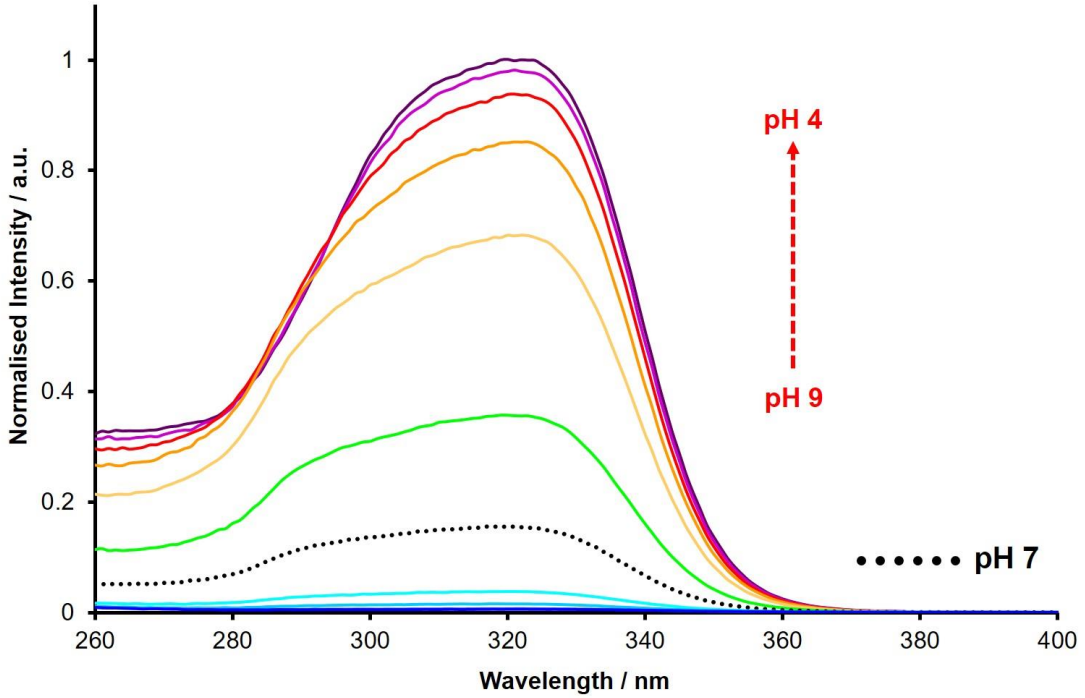


Figure S7 Variation of the europium excitation spectral profile with pH for **Eu*L*^{2a}** (λ_{em} 613 nm, 295 K, c = 20 µM, 0.1 M NaCl).

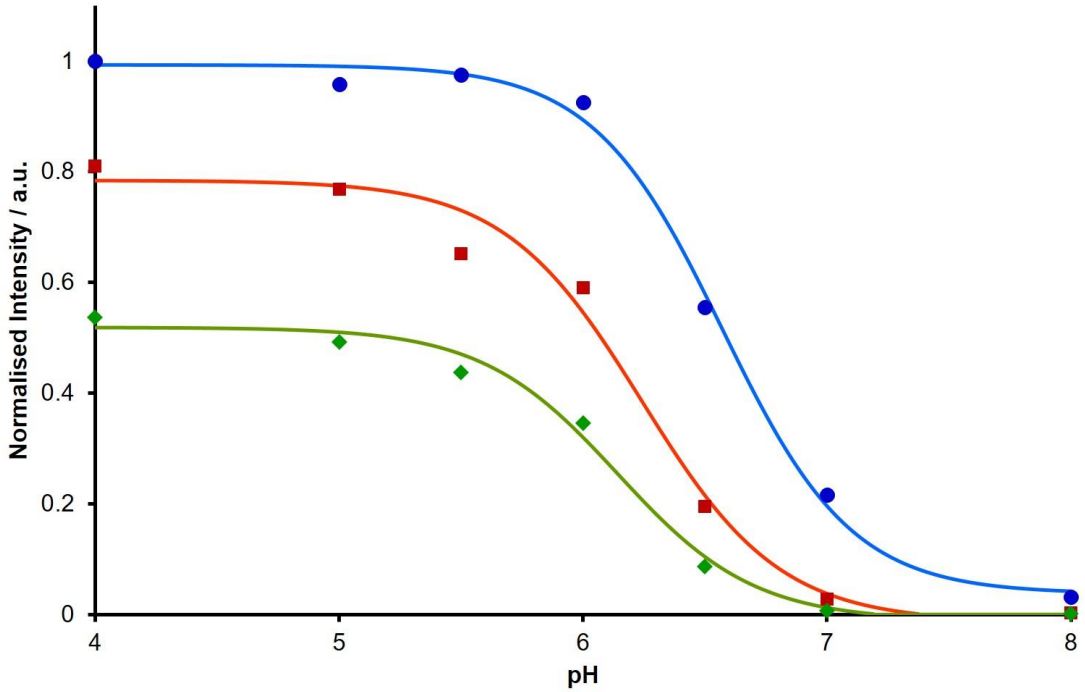


Figure S8 Emission intensity of **Eu*L*^{2a}** (λ_{em} 613 nm) as a function of pH with different time periods of acquisition (*blue* = 60 – 460 µs, *red* = 1000 – 2000 µs, *green* = 1500 – 2500 µs). Data are normalised to a 60 – 460 µs time window at pH 4. Measurements were taken in aqueous solutions of NH_4OAc (pH 4 and 5), MES (pH 5.5, 6 and 6.5), HEPES (pH 7) and NH_4HCO_3 (pH 8) buffers (c = 20 µM, 0.1 M buffer in 0.1 M NaCl).

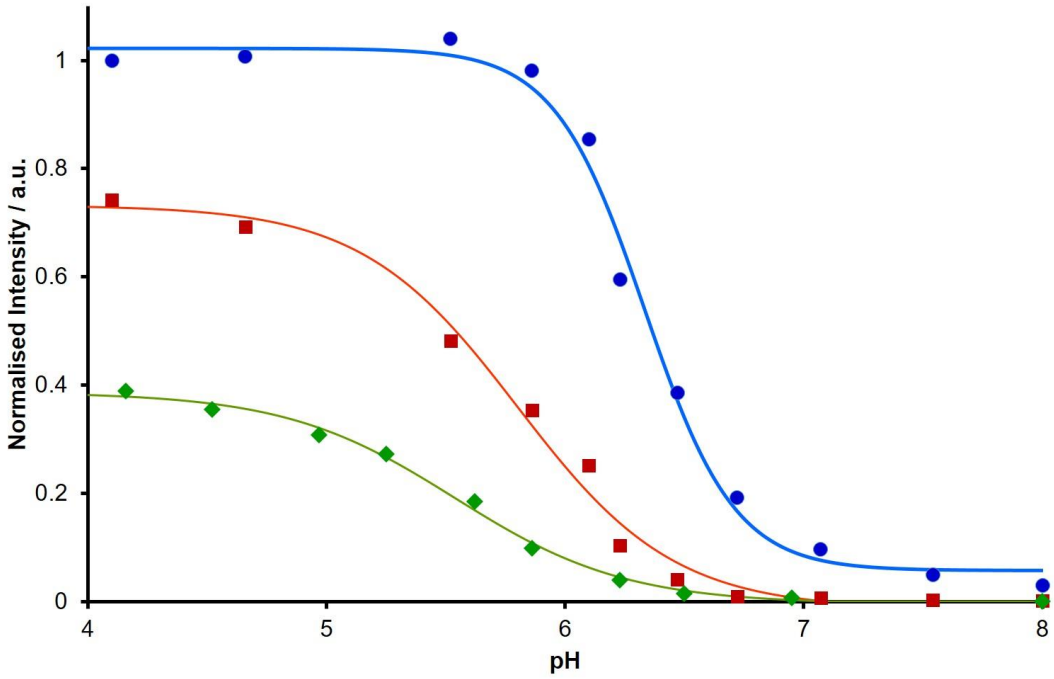


Figure S9 Emission intensity of **Eu*L*^{2a}** (λ_{em} 613 nm) as a function of pH in a cell lysate medium with different time periods of acquisition (*blue* = 60 – 460 µs, *red* = 1000 – 2000 µs, *green* = 1500 – 2500 µs). Data are normalised to a 60 – 460 µs time window at pH 4.

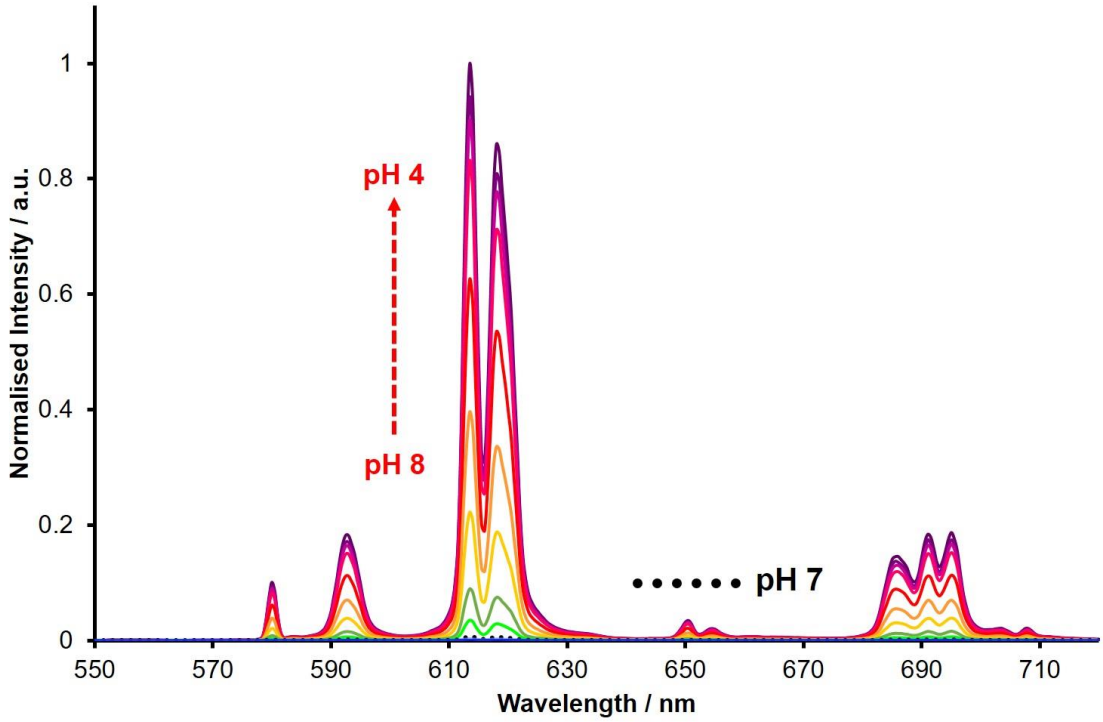


Figure S10 Variation of the europium emission spectrum with pH for **Eu*L*^{2b}** (λ_{exc} 332 nm, 295 K, c = 20 µM, 0.1 M NaCl)

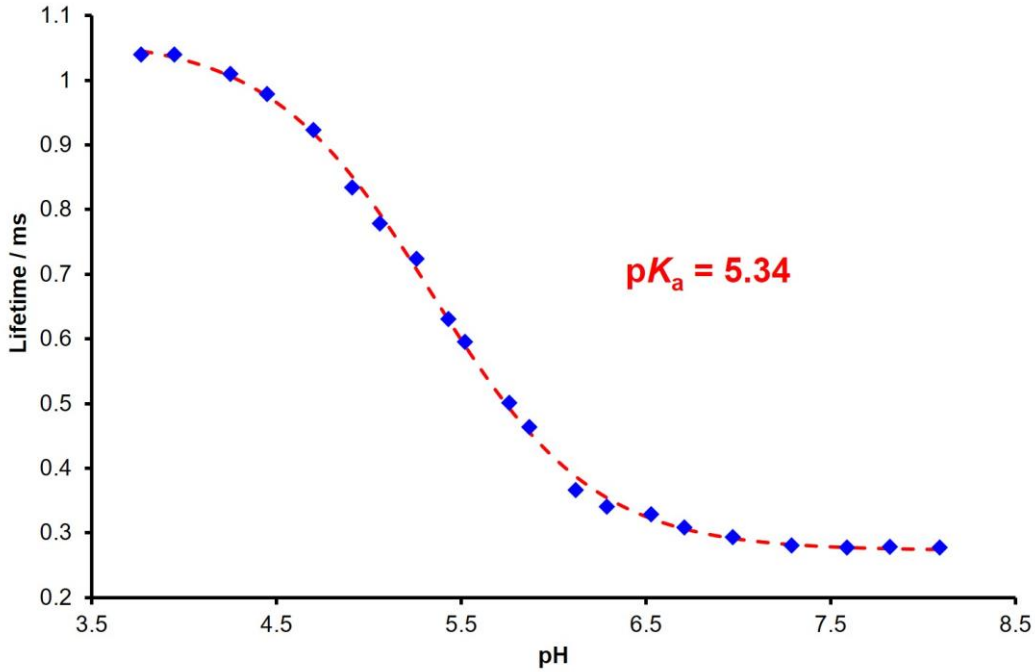


Figure S11 Variation of the europium emission lifetime with pH for **Eu*L*^{2b}** (λ_{exc} 332 nm, λ_{em} 613 nm, 295 K, 0.1 M NaCl). The pK_a value is estimated by non-linear least squares regression analysis.

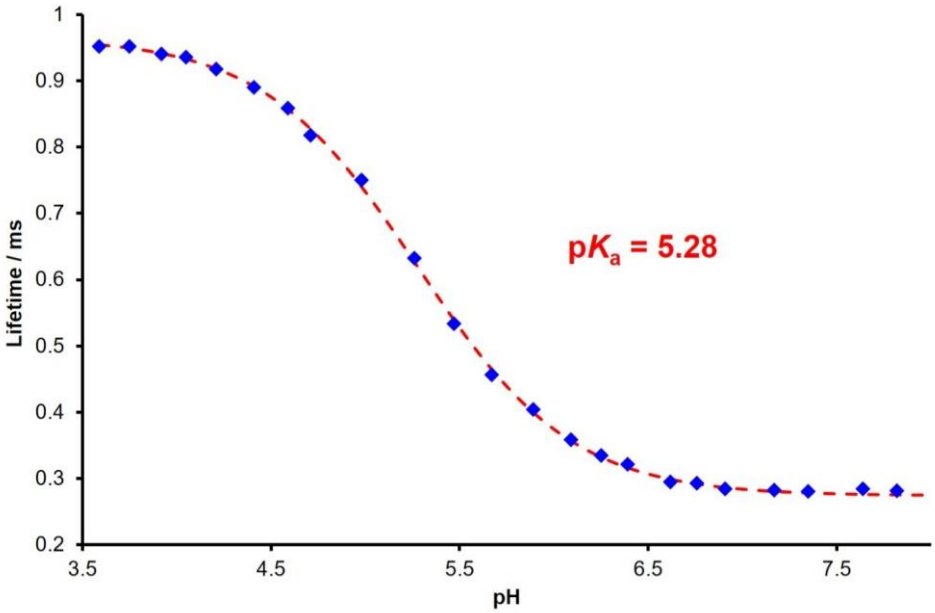


Figure S12 Variation of the europium emission lifetime with pH for **Eu*L*^{2b}** (λ_{exc} 332 nm, λ_{em} 613 nm, 295 K, NIH-3T3 cell lysate). The pK_a value is estimated by non-linear least squares regression analysis.

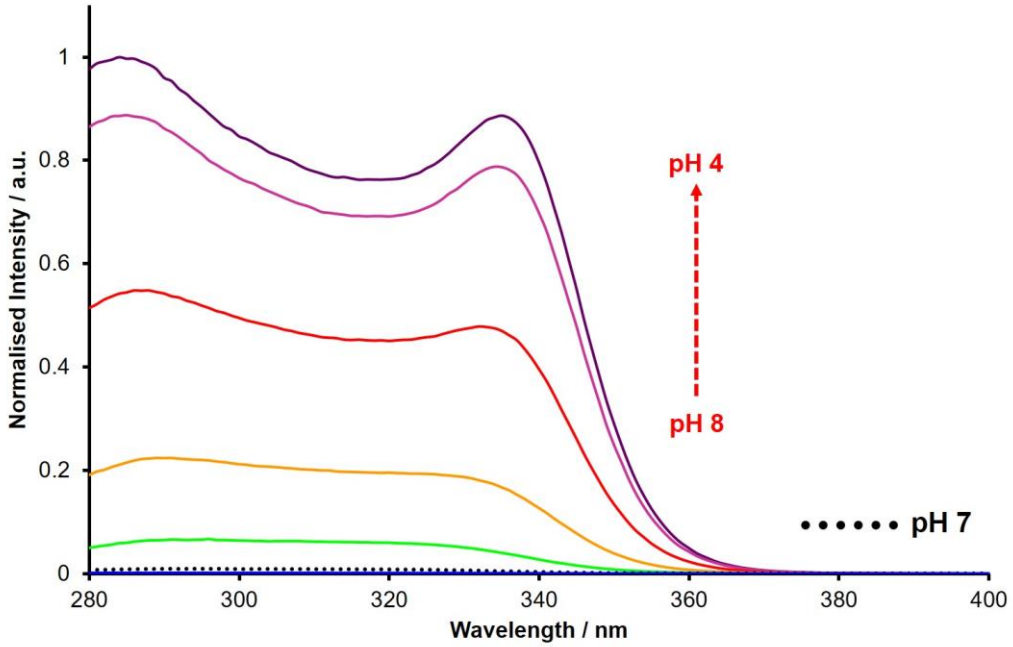


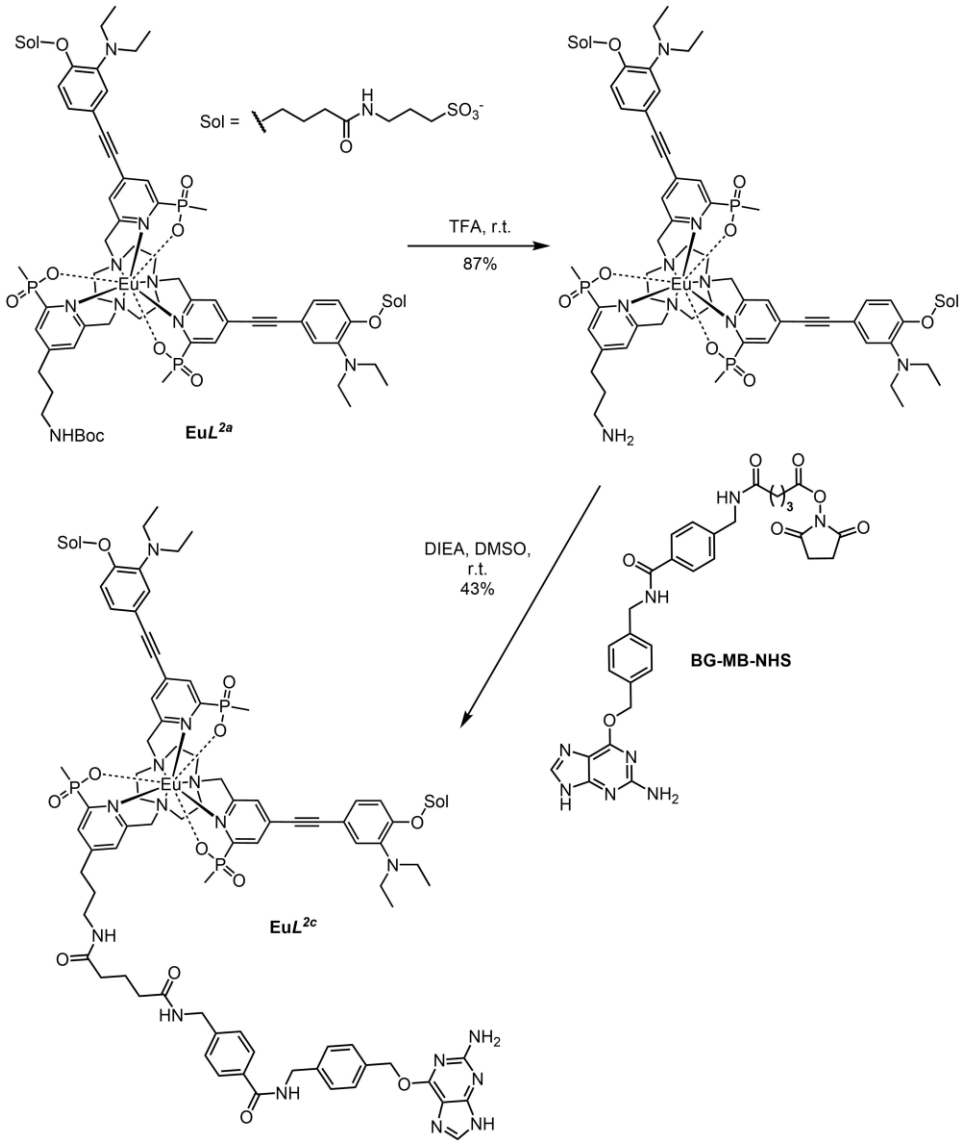
Figure S13 Variation of the europium excitation spectral profile with pH for **Eu*L*^{2b}** (λ_{em} 613 nm, 295 K, c = 20 µM, 0.1 M NaCl).

Table S1 Ratios of emission intensities (I_{rel} = 'switch-on' factors) for **Eu*L*^{2b}** for differing time gate periods, showing the effect on the apparent pK_a values (295 K, c = 20 µM, cell lysate).

	60 – 460 µs	1000 – 2000 µs	1500 – 2500 µs
I_{rel} : pH 4 / pH 8	34	311	651
Apparent pK_a	6.33	5.80	5.54

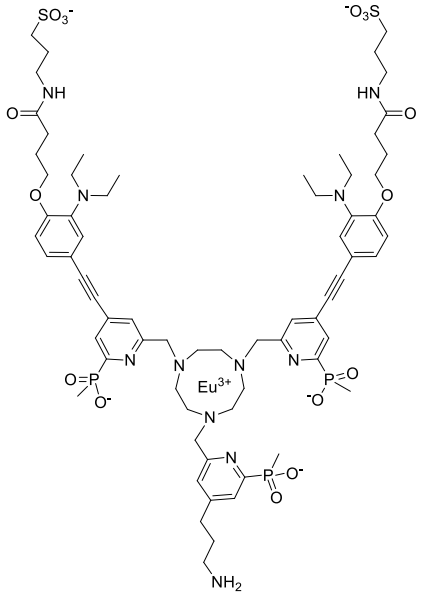
Synthesis of the Benzyl Guanine (BG) Derivative, Eu*L*^{2c}

The compound **BG-MB-NHS** was prepared as reported elsewhere (*Inorg. Chem.* 2014, 53, 4, 1854–1866).



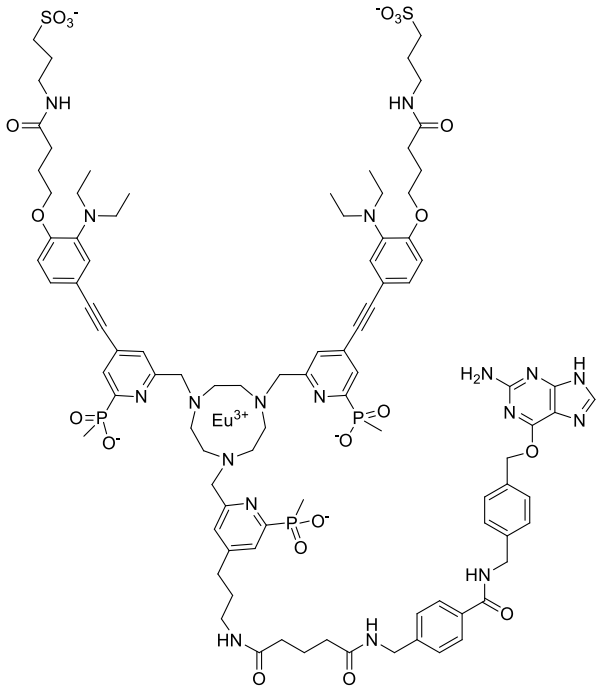
Scheme S3

Deprotection of Eu*L*^{2a}



To complex **Eu*L*^{2a}** (6.93 mg, 4 µmol) was added trifluoroacetic acid (200 µL). The mixture was stirred at RT for 1 h and then purified by preparative HPLC (Column Waters Xbridge C_{18}, 5 µm, 20 × 100 mm – A / H_2O 25 mM TEAAc pH 7 B / CH_3CN t = 0 min 2% B – t = 18 min 40% B – 20 mL.min^{-1}) to afford a yellow solid (3.5 µmol, 87%). ESI-LRMS (-) *m/z* 814.70 $[M-2H]^{2-}$.

Eu*L*^{2c}



A solution of **BG-MB-NHS** (0.615 mg, 1 µmol) in dry DMSO (100 µL) was added to deprotected **Eu*L*^{2a}** (1.403 mg, 860 nmol). To the resulting solution was added DIPEA (0.5 µl, 2862 nmol) and the mixture was stirred at RT for 1 h and purified by preparative HPLC (Column Waters Xbridge C_{18}, 5 µm, 20 × 100 mm – A / H_2O 25 mM TEAAc pH 7 B / CH_3CN; t = 0 min 2% B – t = 18 min 40% B – 20 mL.min^{-1}) to give a white powder (370 nmol, 43%). ESI-LRMS (-) *m/z* 1064.89 $[M-2H]^{2-}$.

Analysis of Deprotected Intermediate and Eu*L*^{2c}

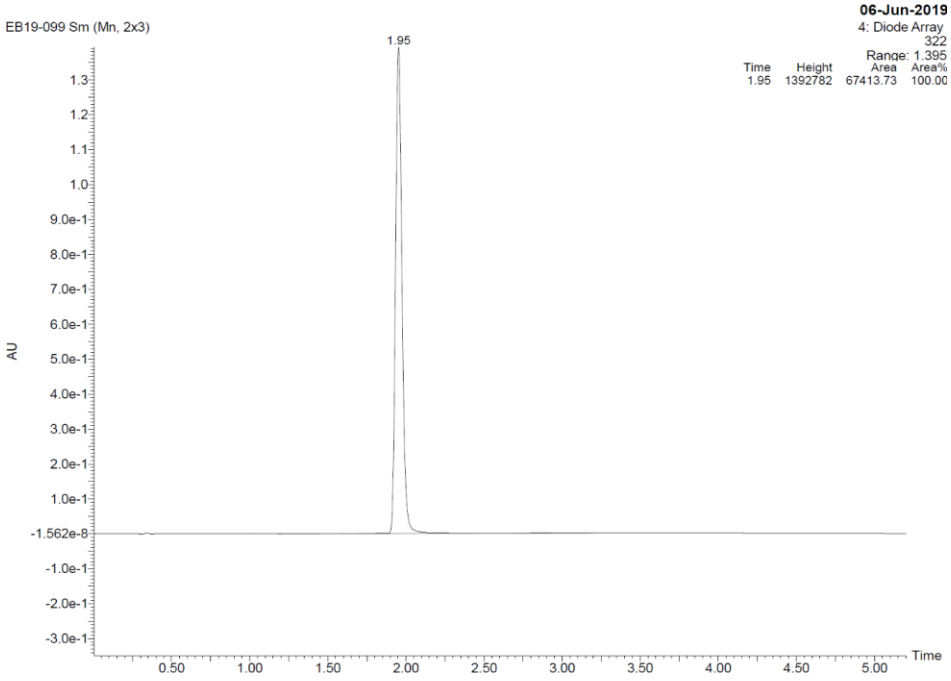


Figure S14 HPLC trace of the pure deprotected **Eu*L*^{2a}** (Acquity C_{18} Column, 300 Å, 1.7 µm, 2.1 × 50 mm; λ_{exc} 322 nm; 0.6 mL min^{-1}). The solvents (A) 5 mM $NH_4OAc_{(aq)}$ pH 5.5, and (B) MeCN were used with the following gradient: 5% B *t* 0 – 0.2 min then 5% to 100% B over 4.8 min.

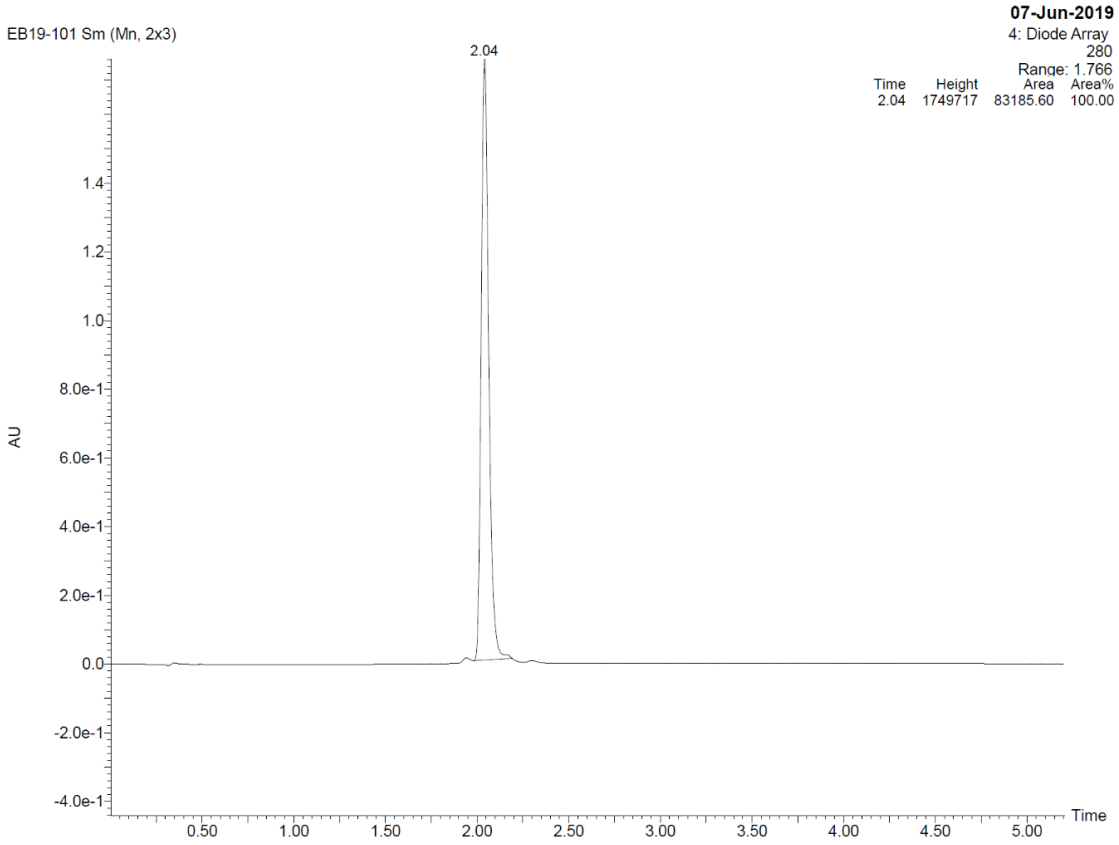


Figure S15 HPLC trace of the pure BG derivative, **Eu*L*^{2c}** (Acquity C_{18} Column, 300 Å, 1.7 µm, 2.1 × 50 mm; λ_{exc} 280 nm; 0.6 mL min^{-1}). The solvents (A) 5 mM $NH_4OAc_{(aq)}$ pH 5.5, and (B) MeCN were used with the following gradient: 5% B *t* 0 – 0.2 min then 5% to 100% B over 4.8 min.

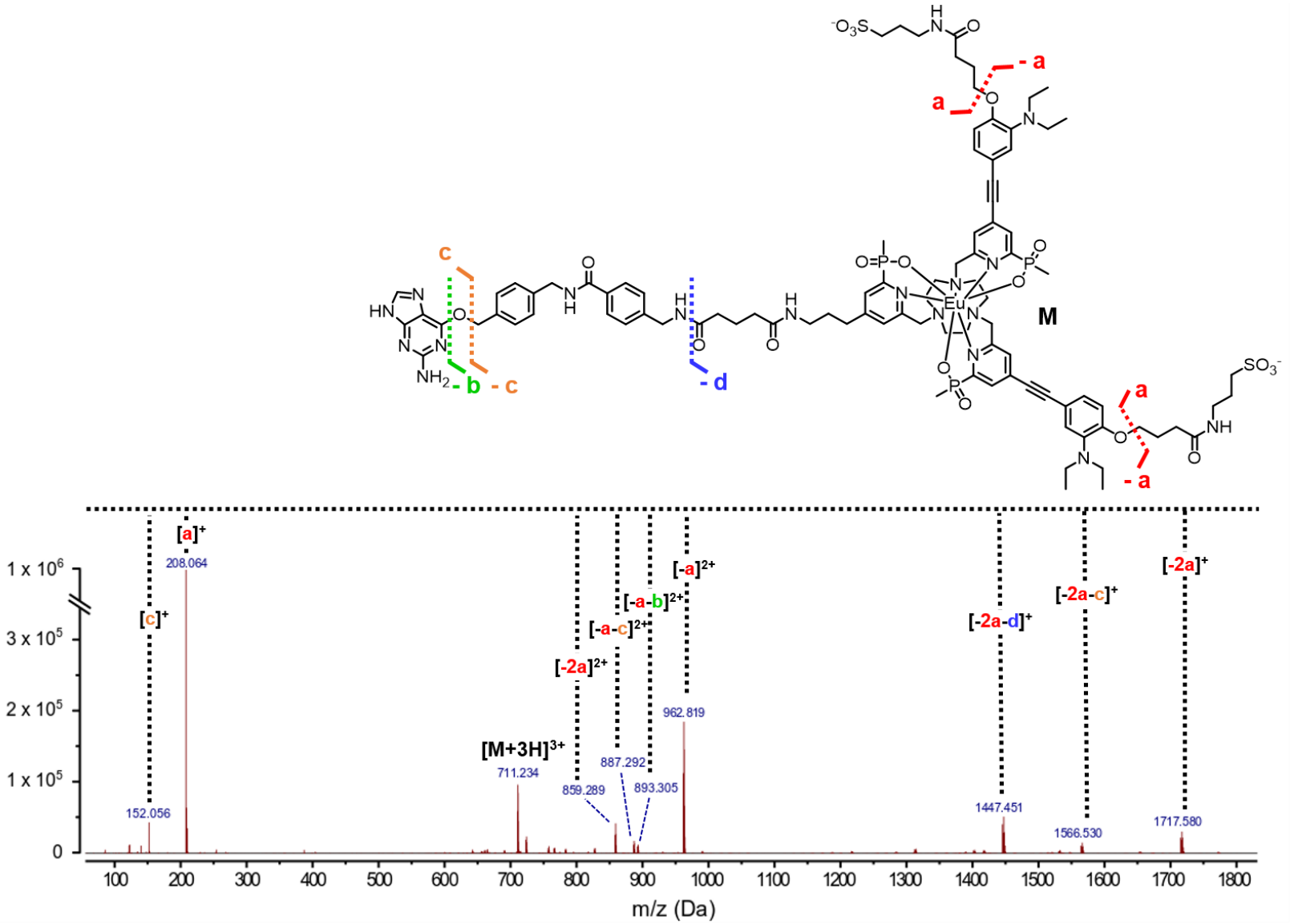


Figure S16 MS-MS analysis of the BG-derivative **Eu*L*^{2c}**. Fragmentation patterns enabling proof of constitution of the BG conjugate. See the following description for more information.

LC MS-MS Description

The LC MS-MS spectrum for **Eu*L*^{2c}** is shown below the structure of the BG derivative, where the key observed fragmentations are indicated. The significant m/z peaks observed in the MS-MS spectrum have been assigned with the identity of the relevant fragmentation(s). For example, cleavage of the long alkyl chain at the periphery of the chromophore at the O-CH_2 bond (-a, *red*) is a frequently observed fragment. The corresponding positively charged alkyl fragment is also observed ($[a]^+$). Evidence for the conjugation of the benzyl guanine to the complex was confirmed through the observation of the b and c fragments.