Supporting Information

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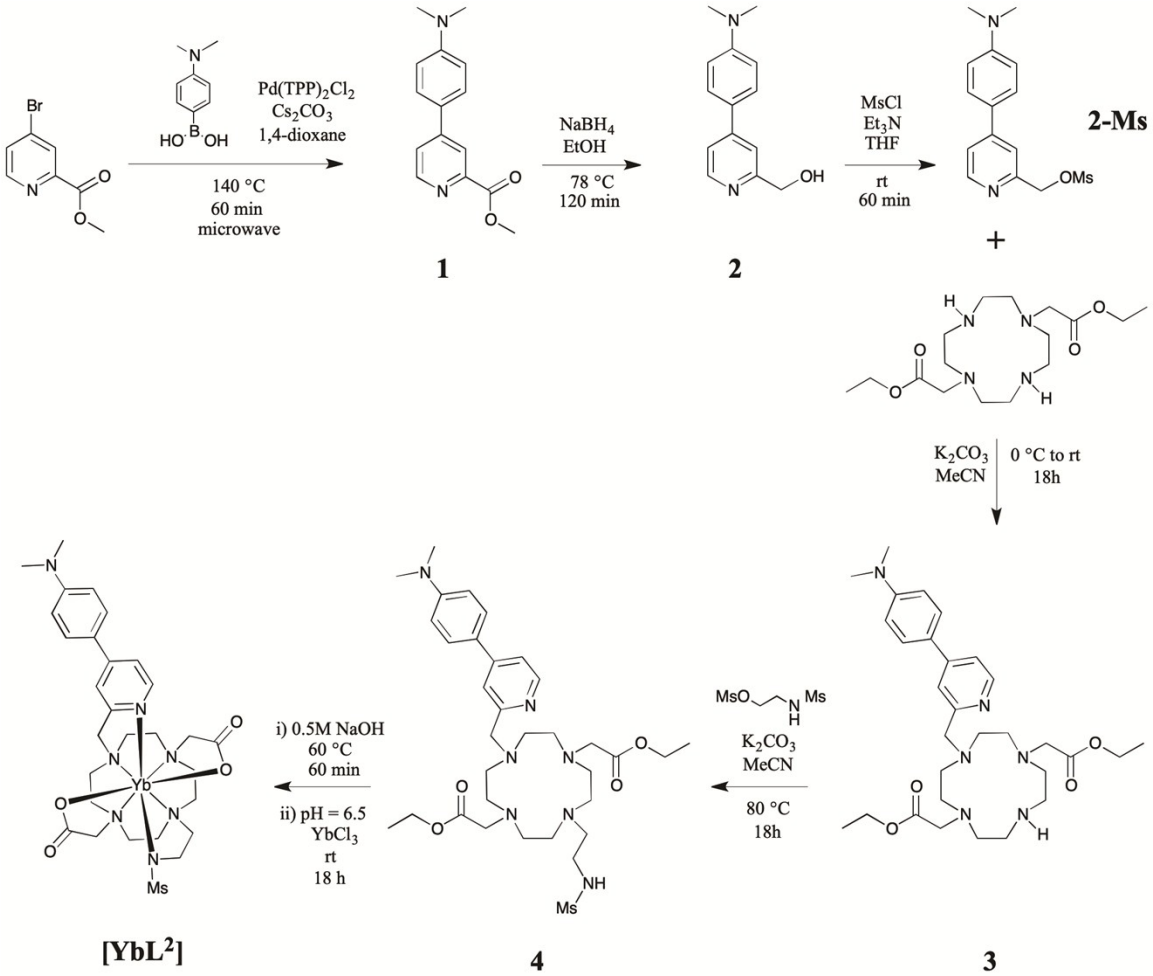
Experimental Procedures

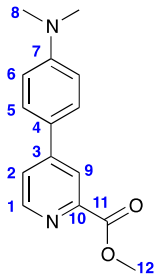
Reagents and solvents were purchased from commercial suppliers (Sigma-Aldrich, Fluorochem, Alfa Aesar, Fisher Scientific, Apollo Scientific) and used as delivered without further purification. Air and/or moisture sensitive reactions were carried out in argon atmosphere using anhydrous solvents. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 400 (^{1}H at 400.06 MHz, ^{13}C at 100.61 MHz), Varian VNMRS-600 (^{1}H at 599.67 MHz, ^{13}C at 150.79 MHz) and Varian VNMRS-700 (^{1}H at 699.73 MHz, ^{13}C at 175.95 MHz) spectrometers. ^{1}H and ^{13}C NMR chemical shifts are referenced to the signals of the solvent ($CDCl_3$). 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., U.K.). Accurate masses were recorded on an LCT Premier XE mass spectrometer or a QToF Premier Mass spectrometer, both equipped with an Acquity UPLC system, a lock-mass electrospray ion source, and an Acquity photodiode array detector (Waters Ltd., U.K.). Methanol or acetonitrile was used as the carrier solvent. Reverse-phase preparative HPLC was performed at 295 K using a Shimadzu system consisting of a Degassing Unit (DGU-20A5R), a Prominence Preparative Liquid Chromatograph (LC- 20AP), a Prominence UV/Vis Detector (SPD-20A) and a Communications Bus Module (CBM-20A). An XBridge C18 OBD 19 x 100 mm, i.d. 5 mM and Merck Chromolith® C-18 columns were used with a flow rate of 2 mL min^{-1} (analytical) or 17 mL min^{-1} (prep). The solvent system was H_2O + 0.1% formic acid/MeOH (MeCN) + 0.1% formic acid (gradient elution). 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 and lifetime values were recorded using a Horiba Jobin-Yvon Spex Fluorolog-3 luminescence spectrometer with a PMT detector using FluorEssence software.

Syntheses



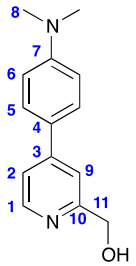
Scheme 1 Synthetic pathway for **[YbL^2]** (Ms = methanesulfonyl: 2-Ms is used to indicate the name of the mesylate ester of 2, i.e. (4-(4-(dimethylamino)phenyl)pyridin-2-yl)methylmethanesulfonate)

Methyl 4-(4-(dimethylamino)phenyl)picolinate, 1



4-Dimethylaminephenyl boronic acid (200 mg, 1.21 mmol), methyl 4-bromopicolinate (200 mg, 0.93 mmol), caesium carbonate (350 mg, 1.07 mmol) and $Pd(TPP)_2Cl_2$ (25 mg, 0.036 mmol) were placed into a microwaveable vial, sealed, evacuated and refilled with argon. Dry 1,4-dioxane (1.3 mL) was added and two freeze-pump-thaw cycles were carried out to degas the solution. The reaction mixture was microwaved at 150 °C for 60 minutes. The crude product was purified on a silica column (0% to 10% of EtOAc in DCM), giving a desired product as a yellow solid (72 mg, 30% yield); ^{1}H NMR (295 K, 700 MHz, $CDCl_3$) δ_H 8.64 (1H, d, $^3J_{H-H}$ = 5.0 Hz, H^1), 8.32 (1H, m, H^9), 7.61 (2H, d, $^3J_{H-H}$ = 8.50 Hz, H^5), 7.60 (1H, m, H^2), 6.76 (2H, s, d, $^3J_{H-H}$ = 8.50 Hz, H^6), 4.00 (3H, s, H^{12}), 3.01 (6H, s, H^8); ^{13}C NMR (295 K, 176 MHz, $CDCl_3$) δ_C 166.1 (C^{11}), 151.3 (C^7), 150.0 (C^1), 149.3 (C^{10}), 148.2 (C^3), 127.7 (C^5), 123.7 (C^4), 123.0 (C^2), 121.6 (C^9), 112.4 (C^6), 52.9 (C^{12}), 40.2 (C^8); *m/z* (HRMS$^+$) 257.1297 $[M+H^+]^+$ ($C_{15}H_{17}N_2O_2$ requires 257.1290).

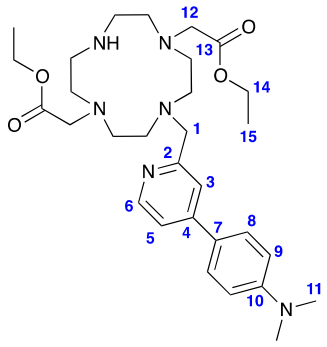
(4-(4-(Dimethylamino)phenyl)pyridin-2-yl)methanol, 2



Methyl 4-(4-(dimethylamino)phenyl)picolinate, **1** (64 mg, 0.25 mmol) was dissolved in ethanol (7 mL, 200 proof) and $NaBH_4$ (122 mg, 3.21 mmol) was added. The reaction mixture was boiled under reflux at 78 °C for 2 h and the reaction mixture was quenched by adding water (35 mL). The solution was washed with DCM (3 x 40 mL), organic fractions were combined and dried over $MgSO_4$ and the solvent was removed under reduced pressure giving a desired product as an off-white solid (44 mg, 77% yield). ^{1}H NMR (295 K, 700 MHz, $CDCl_3$) δ_H 8.49 (1H, m, H^1), 7.57 (2H, d, $^3J_{H-H}$ = 9.0 Hz, H^5), 7.43 (1H, m, H^2), 7.38 (1H, m, H^9), 6.77 (2H, d, $^3J_{H-H}$ = 9.0 Hz, H^6), 4.78 (2H, s, H^{11}), 3.01 (6H, s, H^8); ^{13}C NMR (295 K, 176 MHz, $CDCl_3$) δ_C 159.3 (C^{10}), 151.1 (C^3), 148.9

(C^7), 148.6 (C^1), 127.7 (C^5), 124.9 (C^4), 119.2 (C^9), 116.9 (C^2), 112.4 (C^6), 64.4 (C^{11}), 40.2 (C^8); *m/z* (HRMS$^+$) 229.1340 $[M+H^+]^+$ ($C_{14}H_{17}N_2O$ requires 229.1341).

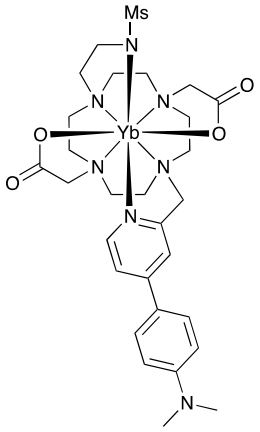
Diethyl 2,2'-(4-((4-(4-(dimethylamino)phenyl)pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate, 3



(4-(4-(Dimethylamino)phenyl)pyridin-2-yl)methanol, **2** (44 mg, 0.19 mmol) was dissolved in 4 mL of dry THF, followed by addition of 0.1 mL of dry triethylamine (0.72 mmol) and 0.025 mL of MsCl (0.32 mmol). The reaction mixture was stirred for 1 h at rt and the solvent was removed under reduced pressure. The crude material was dissolved in DCM (25 mL), washed with water (2 x 25 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure, dried and dissolved in dry acetonitrile (3 mL). DO2A-ethyl ester (68 mg, 0.20 mmol) was dissolved in dry acetonitrile (9 mL) and K_2CO_3 was added (96 mg, 0.70 mmol). The reaction mixture was cooled down using ice bath and stirred for 5 min, followed by dropwise addition of the **2-Ms** within 5 min. The reaction mixture was allowed to reach rt and stirred for 18 h. The reaction mixture was filtered and purified on RP-HPLC (10% to 100% acetonitrile (+0.1% formic acid) in water (+0.1% formic acid) over 10 min). The fractions containing the desired product were combined and neutralised with aqueous ammonia solution. The solvent was removed under reduced pressure, dried and redissolved in acetonitrile. Ammonium formate was filtered off and the solvent was removed under reduced pressure, giving a colourless oil (27 mg, 26% yield); ^{1}H NMR (295 K, 700 MHz, $CDCl_3$) δ_H 8.59 (1H, d,

$^{3}J_{H-H}$ = 5.0 Hz, H^{6}), 7.55 (2H, d, $^{3}J_{H-H}$ = 9.0 Hz, H^{8}), 7.40 (1H, dd, $^{3}J_{H-H}$ = 5.0 Hz, $^{4}J_{H-H}$ = 2.0 Hz, H^{5}), 7.38 (1H, m, H^{3}), 6.77 (2H, d, $^{3}J_{H-H}$ = 9.0 Hz, H^{9}), 4.10 (4H, q, $^{3}J_{H-H}$ = 7.0 Hz, H^{14}), 3.75 (2H, s, H^{1}), 3.25 (4H, s, H^{12}), 3.19-2.60 (16H, m, cyclen), 3.02 (6H, s, H^{11}), 1.22 (6H, t, $^{3}J_{H-H}$ = 7.0 Hz, H^{15}); ^{13}C NMR (295 K, 176 MHz, $CDCl_3$) δ_C 171.3 (C^{13}), 157.5 (C^{2}), 151.2 (C^{10}), 149.8 (C^{6}), 148.8 (C^{4}), 127.6 (C^{8}), 124.4 (C^{7}), 120.2 (C^{3}), 119.2 (C^{5}), 112.4 (C^{9}), 60.5 (C^{14}), 57.6 (C^{1}), 55.6 (C^{12}), 54.7-46.2 (cyclen), 40.2 (C^{11}), 14.2 (C^{15}); *m/z* (HRMS^{+}) 555.3663 $[M+H^{+}]^{+}$ ($C_{30}H_{47}N_6O_4$ requires 555.3659).

[YbL^{2}]



Diethyl 2,2'-(4-((4-(4-(dimethylamino)phenyl)pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate, **3** (27 mg, 0.049 mmol) was dissolved in dry acetonitrile (5mL), followed by addition of K_2CO_3 (11 mg, 0.14 mmol) and 2-(methylsulfonamido)ethyl methanesulfonate (18 mg, 0.049 mmol). The reaction mixture was immersed into an oil bath initially heated to 80 °C and stirred at this temperature for 18 h. The reaction mixture was filtered and on purified on RP-HPLC (0% to 100% acetonitrile (+0.1% formic acid) in water (+0.1% formic acid) over 10 min). The fractions containing the desired product and the compound with two sulfonamide arms were combined and neutralised with aqueous ammonia solution. The solvent was removed under reduced pressure, dried and redissolved in acetonitrile. Ammonium formate was filtered off and the solvent was removed under reduced pressure, giving a transparent oil. The mixture of two products was dissolved in 0.5M NaOH (4 mL) and stirred at 60°C for 2h. The solution was neutralised by addition of 1 M HCl and $YbCl_3$

(0.060 mmol) was added. The reaction mixture was stirred at rt for 1 h and pH was adjusted to 6.5. The reaction mixture was stirred for another hour and excess of Yb^{3+} was precipitated in the form of $Yb(OH)_3$ by adjusting the pH up to 10 with 0.5 M aqueous NaOH solution, followed by neutralization by addition of 0.5 M HCl. The mixture was purified on RP-HPLC (Chromolith® column, 10% to 100% acetonitrile in water). The fractions containing the desired product were combined and freeze-dried to give an off-white solid; *m*/*z* (HRMS$^+$) 753.1941 $[M+H^+]^+$ ($C_{30}H_{44}N_6O_9SYb$ requires 753.1949).

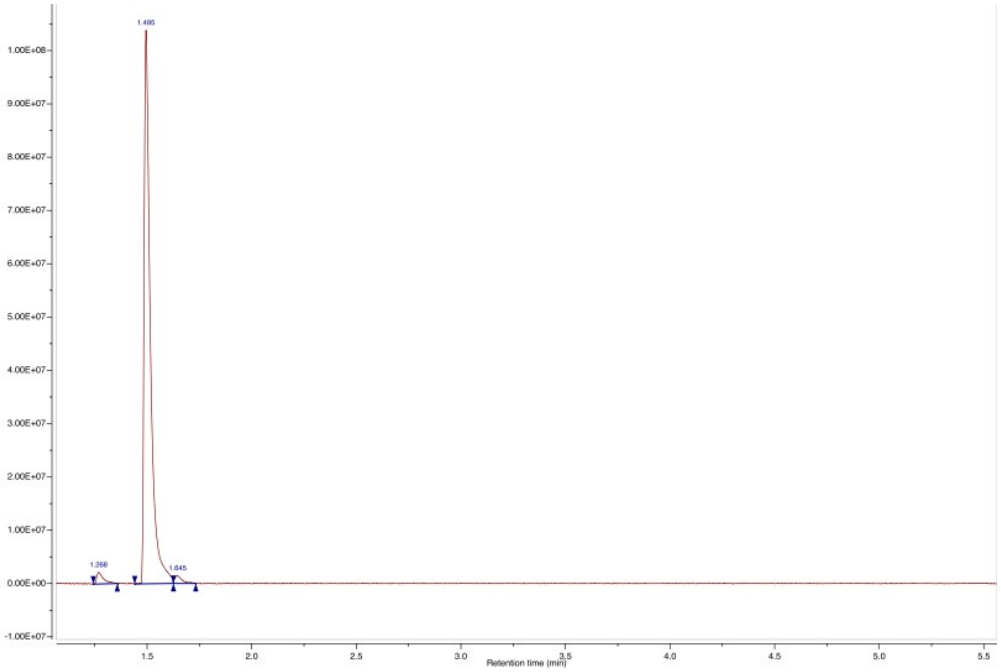
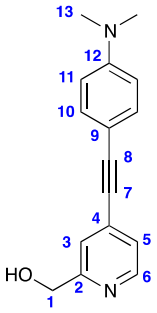


Fig. S1 LC-MS UV trace of **[YbL^2]** (0% H_2O (+0.1% v/v formic acid) in MeOH (+0.1% v/v formic acid) to 99% H_2O (+0.1% v/v formic acid) in MeOH (+0.1% v/v formic acid) over 5.0 min.

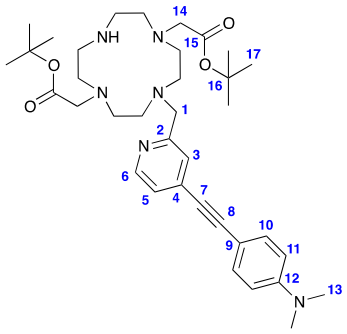
Synthesis of [EuL^1] (for comparison)

(4-((4-(Dimethylamino)phenyl)ethynyl)pyridin-2-yl)methanol



(4-Bromopyridin-2-yl)methanol (0.22 g, 1.18 mmol) and 4-ethynyl-N,N-dimethylaniline (0.20 g, 1.40 mmol) were dissolved in anhydrous THF (10 mL) under argon, followed by addition of pyrrolidine (0.50 mL) and $Pd(TPP)_2Cl_2$ (0.10 g, 0.14 mmol). The reaction mixture was stirred at 50 °C for 24 h and the solvent was removed under reduced pressure. The crude material was purified on a silica column (100% DCM to 50%DCM-50%EtOAc), giving a pale brown oil (0.23 g, 77% yield); ^{1}H NMR (295 K, 400 MHz, $CDCl_3$) δ_H 8.49 (1H, d, $^3J_{H\text{-}H}$ = 6.0 Hz, H^6), 7.44 (2H, d, $^3J_{H\text{-}H}$ = 9.0 Hz, H^{10}), 7.34 (1H, br s, H^3), 7.26 (1H, d, $^3J_{H\text{-}H}$ = 6.0 Hz, H^5), 6.67 (2H, d, $^3J_{H\text{-}H}$ = 9.0 Hz, H^{11}), 4.77 (2H, s, H^1), 3.03 (6H, s, H^{13}); ^{13}C NMR (295 K, 100 MHz, $CDCl_3$) δ_C 159.1 (C^2), 151.0 (C^{12}), 148.3 (C^6), 133.2 (C^{10}, C^4), 123.9 (C^3), 122.0 (C^5), 111.7 (C^9), 108.4 (C^{11}), 96.3 (C^8), 85.3 (C^7), 64.1 (C^1), 40.1 (C^{13}); *m/z* (HRMS$^+$) 253.1344 $[M+H^+]^+$ ($C_{16}H_{17}N_2O$ requires 253.1341).

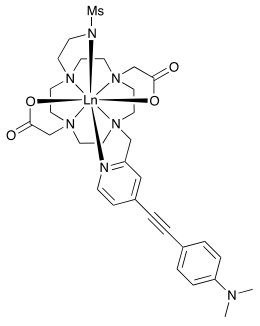
Di-*tert*-butyl 2,2'-(4-((4-((4-(dimethylamino)phenyl)ethynyl)pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate



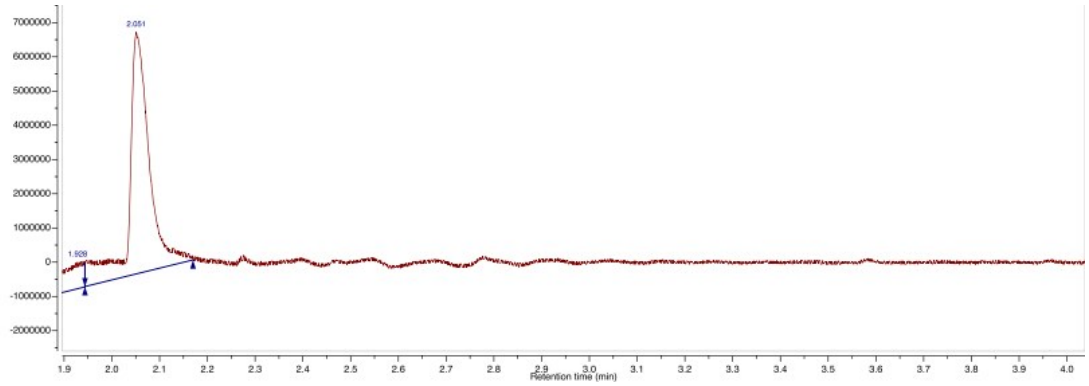
(4-((4-(Dimethylamino)phenyl)ethynyl)pyridin-2-yl)methanol, **2** (64 mg, 0.25 mmol) was dissolved in anhydrous THF (5 mL) under argon, followed by addition of anhydrous

triethylamine (30 µL, 0.21 mmol) and methanesulfonyl chloride (15 µL, 0.19 mmol). The reaction mixture was stirred for 2 h at rt and the solvent was removed under reduced pressure. The crude material was dissolved in dichloromethane (20 mL) and washed with water (2 x 10 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude yellow oil of 4-(4-(dimethylamino)phenyl)pyridin-2-yl)methylmethanesulfonate was dissolved in anhydrous acetonitrile (5 mL) and was slowly added upon cooling (ice/acetone bath) and vigorous stirring to the solution of *bis*-1,7-(t-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclodecane (102 mg, 0.26 mmol) and K_2CO_3 (50 mg, 0.36 mmol) in dry acetonitrile (10 mL). The reaction mixture was stirred for 18 h allowed to reach rt and was purified using reverse phase HPLC (10% water to 100% water in MeOH over 9 min, 0.1% formic acid). Collected fractions were neutralised using aqueous ammonia solution, the solvent was removed under reduced pressure and acetonitrile (20 mL) was added. The insoluble ammonium formate was filtered off and the filtrate was collected. The solvent was removed under reduced pressure to afford a clear oil (60 mg, 37% yield); ^{1}H NMR (295 K, 400 MHz, $CDCl_3$) δ_H 8.62 (1H, d, $^3J_{H-H}$ = 5.0 Hz, H^6), 7.44 (2H, d, $^3J_{H-H}$ = 9.0 Hz, H^{10}), 7.34 (1H, br s, H^3), 7.27 (1H, d, $^3J_{H-H}$ = 5.0 Hz, H^5), 6.68 (2H, d, $^3J_{H-H}$ = 9.0 Hz, H^{11}), 3.73 (2H, s, H^1), 3.04 (6H, s, H^{13}), 3.16-2.62 (20H, m, H^{14}, cyclen), 1.45 (18H, s, H^{17}); ^{13}C NMR (295 K, 100 MHz, $CDCl_3$) δ_C 170.6 (C^{15}), 157.6 (C^2), 150.7 (C^{12}), 149.5 (C^6), 133.2 (C^{10}, C^4), 125.2 (C^3), 123.9 (C^5), 111.7 (C^9), 108.2 (C^{11}), 96.4 (C^8), 85.3 (C^7), 81.5 (C^{16}), 56.6 (C^1), 54.6-46.6 (C^{14}, cyclen), 40.1 (C^{13}), 28.2 (C^{17}); *m*/*z* (HRMS$^+$) 635.4282 $[M+H^+]^+$ ($C_{36}H_{55}N_6O_4$ requires 635.4285).

[LnL^1] (Ln = Eu, Gd)



Di-*tert*-butyl 2,2'-(4-((4-((4-(dimethylamino)phenyl)ethynyl)pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate, (54 mg, 0.09 mmol) was dissolved in dry acetonitrile (10 mL), followed by addition of K_2CO_3 (40 mg, 0.29 mmol) and 2-(methylsulfonamido)ethyl methanesulfonate (17 mg) was added. The oil bath was heated up to 80 °C and the reaction flask was immersed into the bath. The reaction mixture was stirred for 18 h and the formation of the product was confirmed by LC-MS analysis (m/z (HRMS$^+$) 756.4479 $[M+H^+]^+$ ($C_{39}H_{62}N_7O_6S$ requires 756.4482)). The solvent was removed under reduced pressure and dichloromethane (15 mL) was added, filtered and the solvent was removed under reduced pressure. The crude was dissolved in methanol (4 mL) and aqueous NaOH solution (2.5 M, 2 mL) was added. The mixture was stirred at 60 °C for 3 h. The reaction mixture was neutralised by careful addition of concentrated HCl and $LnCl_3$ (0.2 mmol) was added. The reaction mixture was stirred at 60 °C for 3 h and the product was purified using reverse phase HPLC (10% water to 100% water in MeOH over 9 min, NH_4HCO_3 buffer (2 g/L), t_r = 7.6 min), yielding the desired complex as yellow powder (16 mg for **[EuL^1]** and 18 mg for **[GdL^1]**, 24% and 27% yield over 2 steps, respectively); m/z (HRMS$^+$) 792.2199 $[M+H^+]^+$ ($C_{31}H_{43}N_7O_6S^{151}Eu$ requires 792.2194), m/z (HRMS$^+$) 795.2204 $[M+H^+]^+$ ($C_{31}H_{43}N_7O_6S^{154}Gd$ requires 795.2204); τ_{1r} (**[GdL^1]**) = 1.43 $mM^{-1}s^{-1}$ (pH = 7.0); λ_{abs} (**[EuL^1]**) = 365 nm, $\varepsilon(H_2O)$ = 28000 $M^{-1}cm^{-1}$, τ_{H_2O} = 0.30 ms (with HSA).



LC-MS UV trace of **[EuL^1]** (5% H_2O (+0.1% v/v formic acid) in MeOH (+0.1% v/v formic acid) to 95% H_2O (+0.1% v/v formic acid) in MeOH (+0.1% v/v formic acid) over 3.8 min

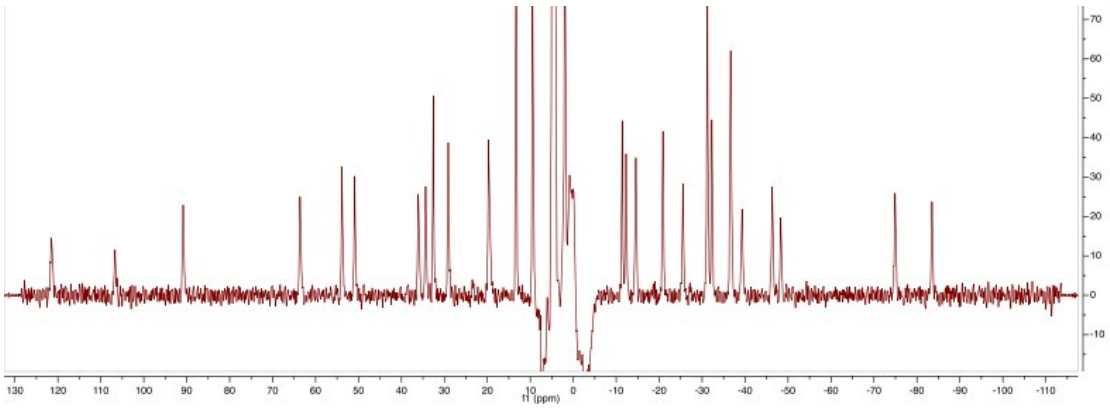


Fig. S2 ^{1}H NMR spectrum of **[YbL^{2}]** (298 K, 9.4 T, pD 8.4). For the PCS analysis of related structural types, see reference 12.

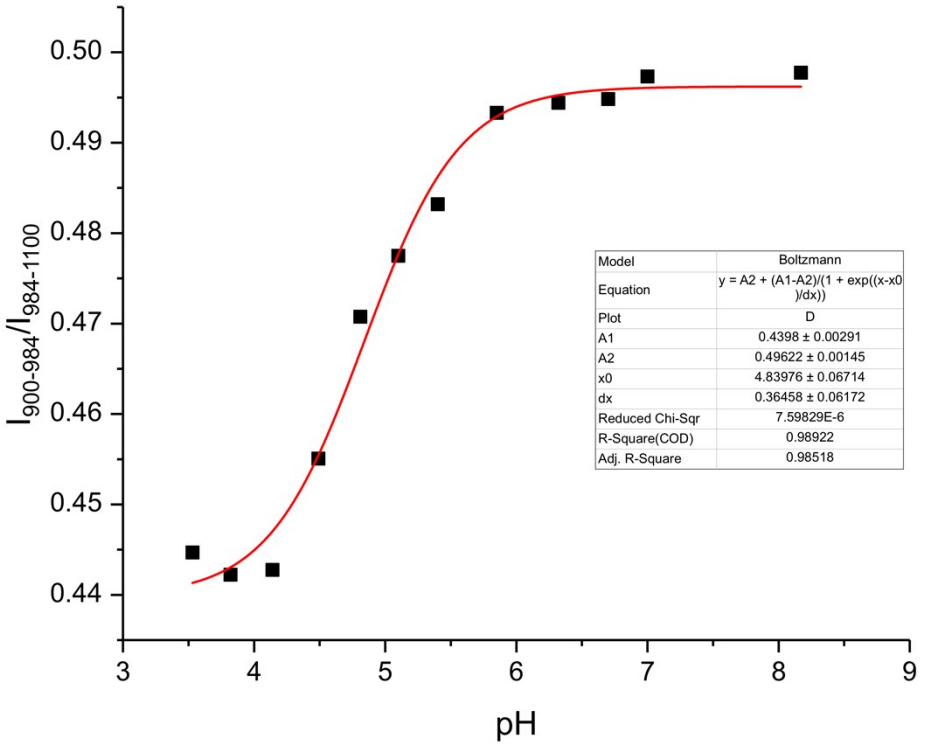


Fig. S3 pH calibration curve of **[YbL^{2}]** with an apparent pK_a = 4.84(05)

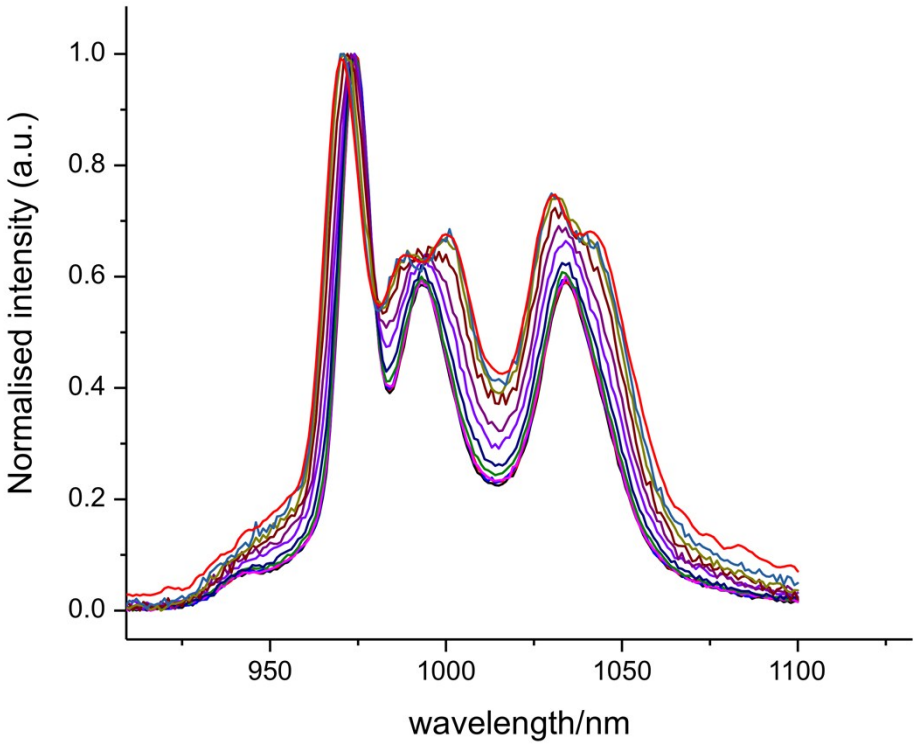


Fig. S4 Changes in the emission spectrum of **[YbL²]** as a function of pH

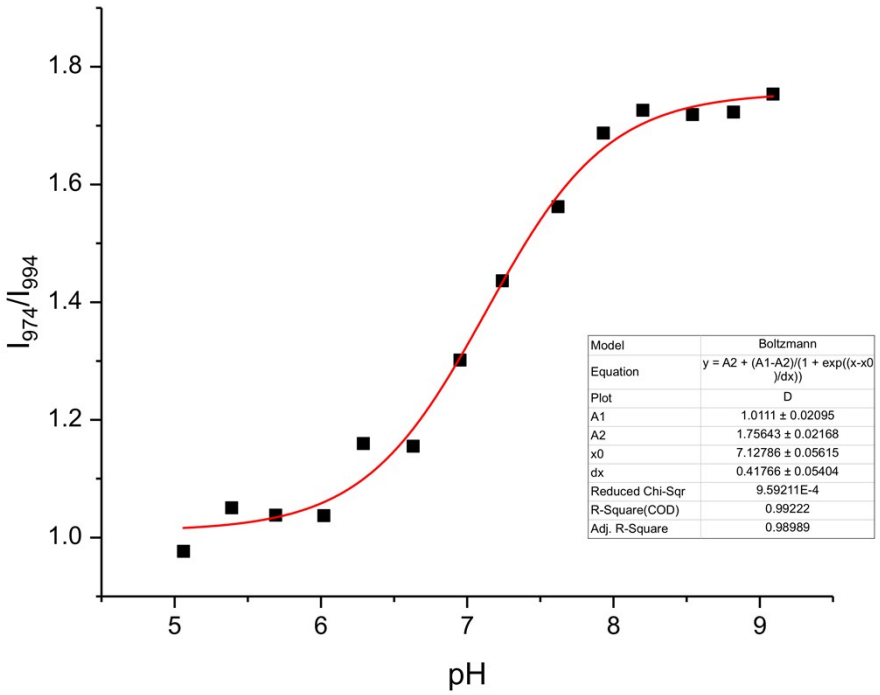


Fig. S5 pH calibration curve of **[YbL]** in the presence of HSA (0.6 mM) with an apparent pK_a = 7.13(04)

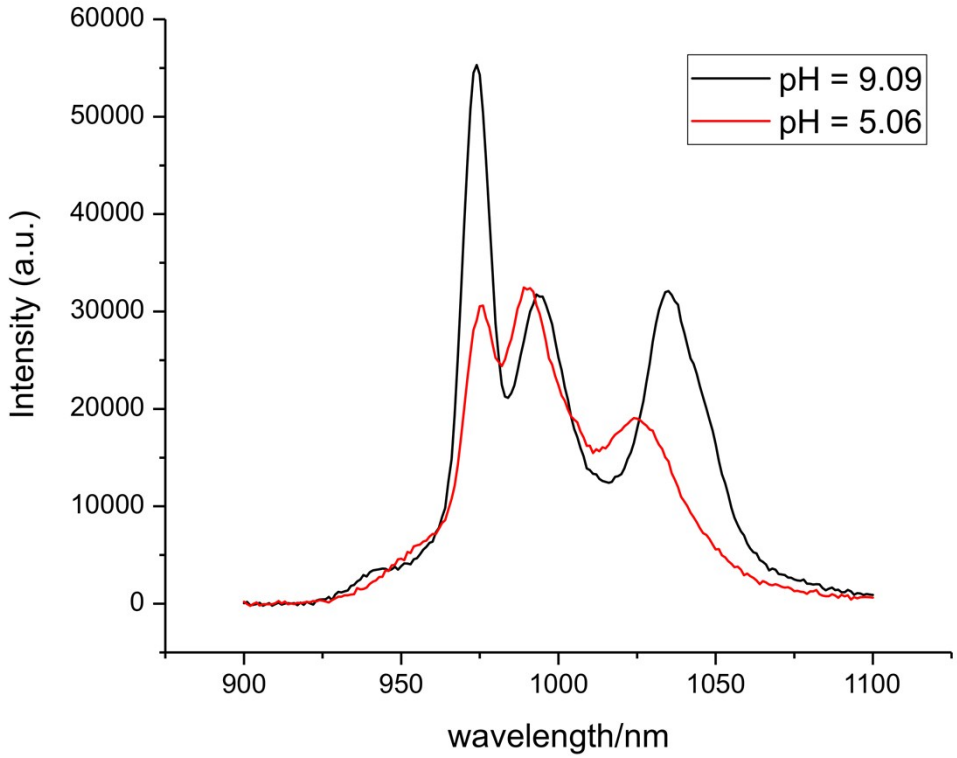


Fig. S6 Emission spectrum of **[YbL²]** in the presence of HSA (0.6 mM) at pH = 9.09 (black) and pH = 5.06 (red)

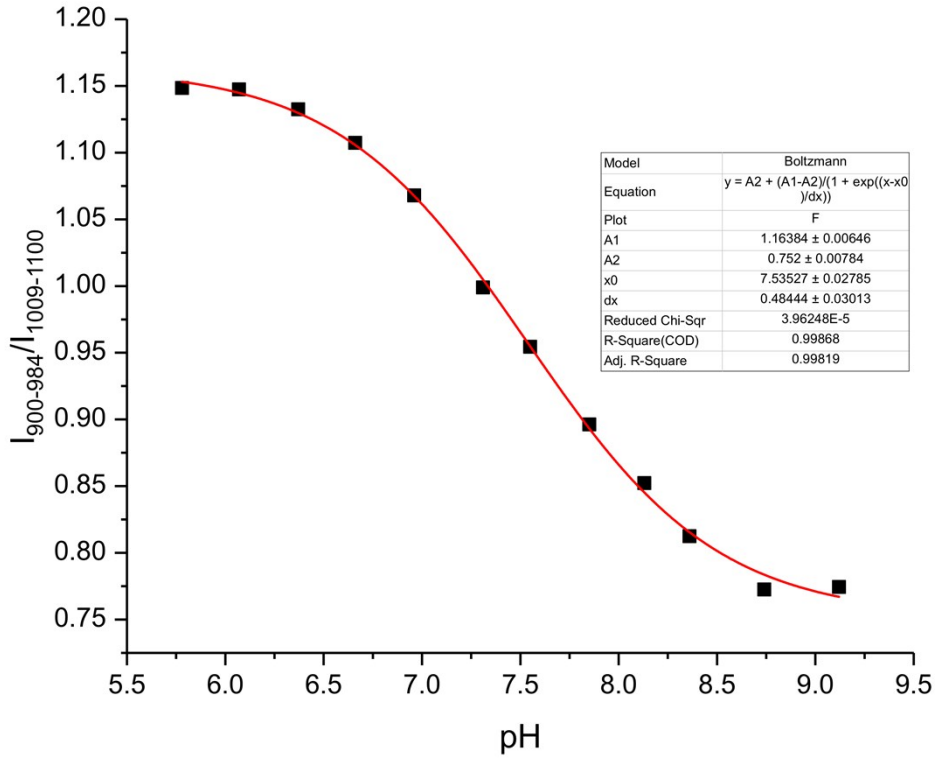


Fig. S7 pH calibration curve of **[YbL²]** in the presence of BSA (0.6 mM) with an apparent pK_a = 7.53(04)

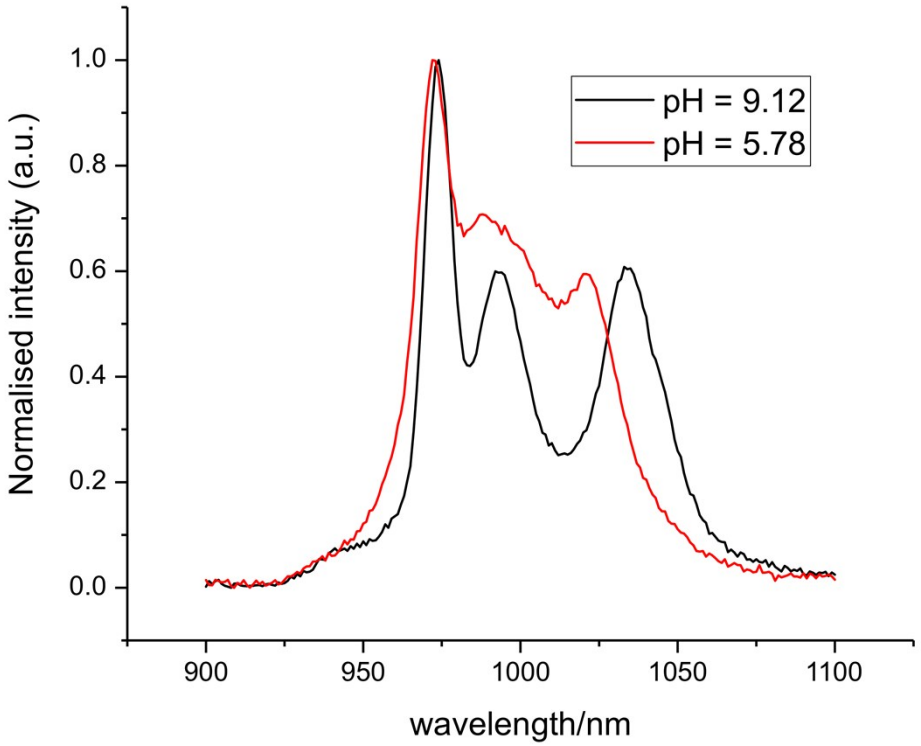


Fig. S8 Emission spectrum of **[YbL²]** in the presence of BSA (0.6 mM) at pH = 9.12 (black) and pH = 5.78 (red)

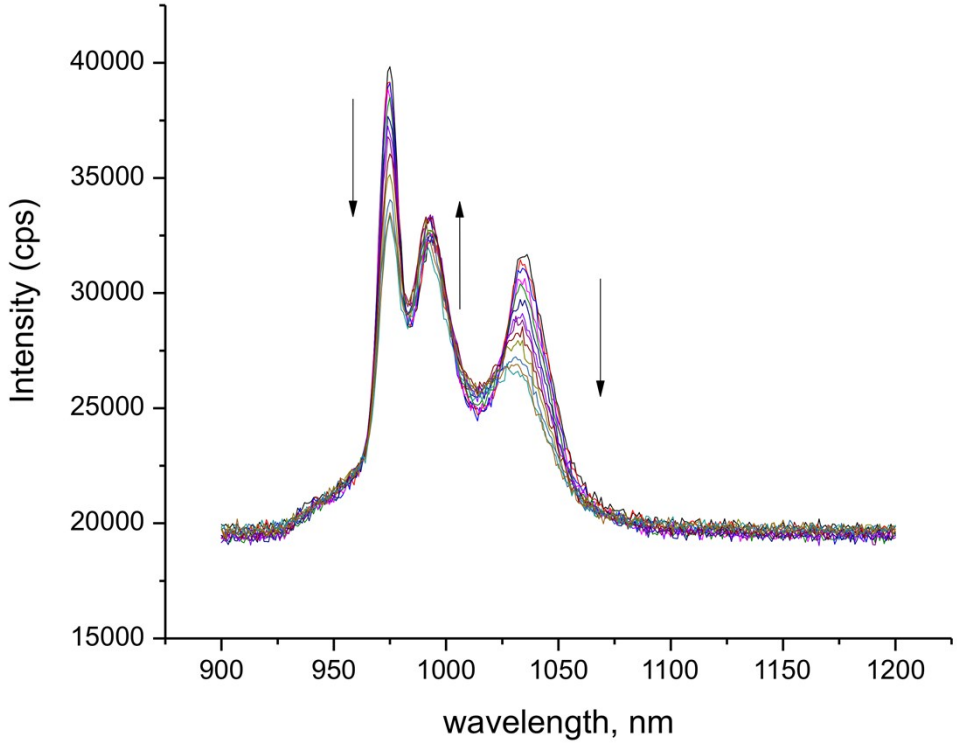


Fig. S9 Changes in the emission spectrum of **[YbL^2]** as a function of added bovine α_1-AGP

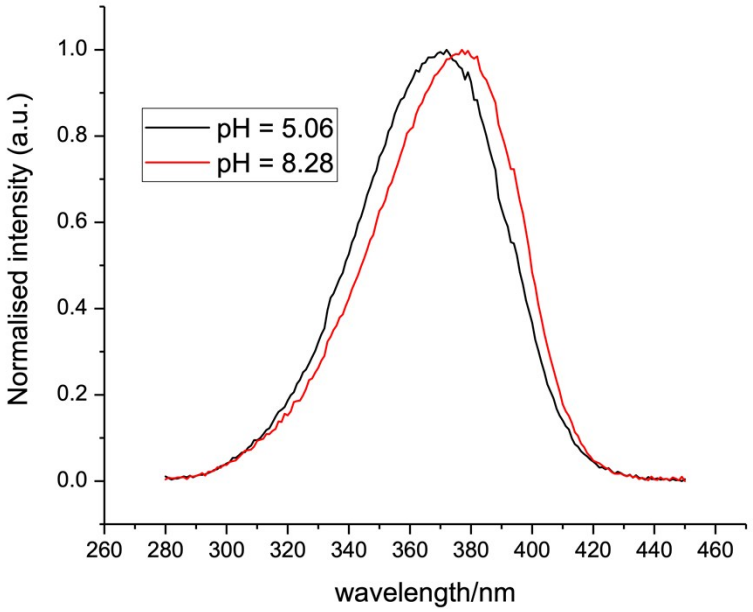


Fig. S10 Excitation spectra of **[YbL^2]** with 0.6 mM HSA added at pH = 5.1 and pH = 8.3