Supplementary Information

Impact of Varying the Phenylboronic Acid Position within Macrocyclic Eu(III) Complexes on the Recognition of Adenosine Monophosphate

Samantha E. Bodman, Colum Breen, Felix Plasser and Stephen J. Butler*

Department of Chemistry, Loughborough University, Epinal Way, Loughborough, LE11 3TU, UK.

*Correspondence: S.J.Butler@lboro.ac.uk

Contents

Materials and Methods S2
Synthesis and characterisation of ligands and corresponding Eu(III) complexes S4
Photophysical measurements of Eu(III) complexes S9
Mass spectrometry analysis of Eu(III) complexes S12
NMR analysis of Eu(III) complexes S14
DFT molecular structures S15
Appendix: NMR spectra of compounds S16
References S36
Materials and Methods

General Considerations
Reagent grade chemicals, including the anhydrous solvents, were purchased from Sigma Aldrich and Fluorochem and used without further purification. The following compounds were synthesised using literature procedures: 8-hydroxyquinoline-2-carbaldehyde (1) and (7-tert-butoxycarbonylmethyl-1,4,7,10-tetraazacyclododec-1-yl)-acetic acid tert-butyl ester (DO2A-tert-butyl ester).

Luminescence experiments
Luminescence spectra were recorded on a Camlin Photonics luminescence spectrometer with FluoroSENS version 3.4.7.2024 software. Emission spectra were obtained using a 40 µL or 100 µL Hellma Analytics quartz cuvette (Art no. 111-10-K-40). Excitation light was set at 322 nm and emission recorded in the range 400 – 720 nm using an integration time of 0.5 seconds, increment of 1.0 nm, excitation slit of 0.2 nm and emission slit of 0.5 nm. Quantum yields were measured using quinine sulfate in 0.05 M H2SO4 as a standard (Φem = 0.59, lex = 350 nm). Emission lifetime measurements were performed on the FluoroSENS instrument. Measurements were taken of 1 mL of 0.1 absorbance samples of Eu(III) complexes in 10 mM HEPES at pH 7.0, unless stated otherwise. Measurements were obtained by indirect excitation of the Eu(III) ion via the quinoline antennae using a short pulse of light at 322 nm followed by monitoring the integrated intensity of the light emitted at 615 nm, with 500 data points collected over a 10 millisecond time period. The decay curves were plotted in Origin Labs 2019 version 9.6.0.172, and fitted to the equation:

\[ I = A_0 + A_1 e^{-kt} \]

where I is the intensity at time, t, following excitation, \( A_0 \) is the intensity when decay has ceased, \( A_1 \) is the pre-exponential factor and k is the rate constant for the depopulation of the excited state.

The hydration state, q, of the Eu(III) complexes was determined using the modified Horrocks equation:

\[ q(\text{Eu}) = 1.2 \left( \frac{1}{\tau_{\text{H2O}}} - \frac{1}{\tau_{\text{D2O}}} - 0.25 - 0.075n \right) \]

where \( \tau_{\text{H2O}} \) and \( \tau_{\text{D2O}} \) are the emission lifetime times in water and D2O, respectively, and n is the number of carbonyl-bound amide NH groups.

Anion binding titrations
Anion binding titrations were carried out in degassed 10 mM HEPES buffer at pH 7.0. Stock solutions of anions (e.g. hydrogen phosphate, AMP) containing Eu(III) complex (5 µM) were made up at 0.4, 4 and 40 mM anion. The appropriate anion stock solution was added incrementally to 100 µL of Eu(III) complex (5 µM) and the emission spectrum was recorded after each addition. The ratio of emission bands 605 – 630 nm/ 585 – 600 nm (\( \Delta I / \Delta I = 2 / \Delta I = 1 \)) was plotted as a function of anion concentration. The data was analysed using a nonlinear least-squares curve fitting procedure, based on a 1:1 binding model described by the equation:

\[ FB = \frac{1}{K_a} + [A] + [\text{Eu}] - \frac{\sqrt{(1/[K_a] + [A] + [\text{Eu}])^2 + 4[A][\text{Eu}]}}{2[\text{Eu}]} \]

where FB is the fraction bound, calculated by \((l-l_0)/(l_1-l_0)\) where l is the emission intensity at [A], \( l_0 \) is the initial emission intensity, and \( l_1 \) is the final emission intensity. [A] is the total concentration of anion in solution, [Eu] is the total concentration of Eu(III) complex, \( K_a \) is the apparent binding constant.
**pH titrations**

A solution of Eu(III) complex (5 μM) in water was adjusted to pH 3.5 by the addition of 1 M HCl and an emission spectrum was recorded. The pH was increased slowly by 0.2 – 0.5 units by the addition of 1 M or 0.1 M NaOH solution, and an emission spectrum recorded at each pH. The ratio of emission bands 605 – 630 nm/ 585 – 600 nm (ΔJ = 2 / ΔJ = 1) was plotted as a function of pH and fitted to a sigmoidal curve using OriginLab 2019 to determine the pKa value.

**Computational details**

Geometry optimisations were performed at the DFT level using the B3LYP functional along with the 6-31G* basis set. A large-core quasi-relativistic effective core potential (ECP) was used for treating the core along with the 4f6 shell of Eu(III) and the associated (7s6p5d)/[5s4p3d] basis set was used for the valence electrons. All computations were performed using spin-restricted orbitals using a pseudo-singlet configuration. Solvation in water was modelled using a conductor-like polarisable continuum model.

Additional single-point computations were performed using the SMD solvent model along with an enhanced basis set (6-311+G*) for O and P atoms to reduce basis superposition error and 6-31G* for the remaining atoms. Thermal corrections were carried out following a standard free-particle/rigid-rotor/harmonic-oscillator model. Corrections for basis-set superposition error were carried out using the counterpoise correction. pKa values were computed using the reaction H2PO4− → HPO42− + H+ as a reference. All DFT computations were carried out in Q-Chem 5.4.

Emission spectra were simulated using the ab initio complete active space self-consistent field (CASSCF) level of theory. These computations employed the ANO-RCC-VTZP basis set for the Eu atom, ANO-RCC-VDZP for heteroatoms, and ANO-RCC-VDZ for C/H atoms. The active space consisted of the 6 f-electrons distributed over the 7 f-orbitals. CASSCF orbital optimizations were performed by averaging over 7 states (making up the full CAS(6,7) heptet configuration space). Using these orbitals, CASCI computations on the quintet states were performed considering 5 roots. Heptets and quintets were combined in a spin-orbit state interaction procedure (SO-RASSI) to produce the final spin-orbit coupled states. Emission spectra were computed with respect to the 5D0 state, i.e. the 50th state in the spin-orbit expansion. Transition strengths for the ΔJ = 1 states were computed from the second-order expansion of the wave vector whereas all other transition strengths were computed via standard transition dipole moments in the length representation. To match the results closer to the experimental spectra, energies were shifted down by 0.67 eV and the quadrupole intensities of the ΔJ=1 states were halved. Spectra are shown with a phenomenological broadening of 0.01 eV. CASSCF calculations were carried out in OpenMolcas.

The underlying computational research data (molecular structures and input/output files of Q-Chem and OpenMolcas) are provided via separate repository, DOI: 10.17028/rd.lboro.20214002.

**High Performance Liquid Chromatography**

Preparative RP-HPLC was performed using a Waters 2489 UV/Visible detector performed at 254 nm, a Waters 1525 Binary HPLC pump controlled by the Waters Breeze 2 HPLC system software. Separation was achieved using a semi-preparative XBridge C18 (5 μm OBD 19 × 100 mm) column at a flow rate maintained at 17 mL min⁻¹. A solvent system composed of either water (0.05% formic acid) / acetonitrile (0.05% formic acid) or water (25 mM NH₄HCO₃) / acetonitrile was used over the stated linear gradient (usually 0 to 100% organic solvent over 10 min). Analytical RP-HPLC was performed using a XBridge C18 (5 μm 4.6 × 100 mm) column at a flow rate maintained at 2.0 mL min⁻¹ using the stated gradient and solvents.
Synthesis and characterisation of ligands and corresponding Eu(III) complexes

Scheme 1. Synthesis of boronic acid-functionalised Eu(III) complexes [Eu.\textit{o}BOH$_2$]$^+$ and [Eu.\textit{p}BOH$_2$]$^+$.

8-\{(2-iodobenzyl)oxy\}quinoline-2-carbaldehyde (20)  
To a solution of aldehyde 1 (0.500 g, 2.89 mmol) in acetonitrile (25 mL) was added potassium carbonate (1.20 g, 8.65 mmol) and 2-iodobenzyl bromide (0.88 g, 2.95 mmol) and the yellow solution was stirred at room temperature for 24 hours. The resulting orange solution was centrifugated at 120 rpm for 3 minutes. The solution was decanted, and the solid pellet was washed with dichloromethane (3 × 15 mL) and the solvent was evaporated under reduced pressure. The resulting residue was partitioned between dichloromethane (10 mL) and saturated aqueous sodium chloride (10 mL). The aqueous phase was extracted with dichloromethane (3 × 10 mL) and the combined organic phases were dried (MgSO$_4$), filtered, and concentrated under reduced pressure. The crude material was purified by column chromatography (silica gel; 20 ~ 60% EtOAc/petroleum ether) to give the desired aldehyde 2 (1.00 g, 89%) as an off-white solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.33 (1H, s, H$_8$), 8.30 (1H, d, J = 8.7 Hz, H$_9$), 8.09 (1H, d, J = 8.2 Hz, H$_5$), 7.89 (1H, d, J = 7.8 Hz, H$_{13}$), 7.70 (1H, d, J = 7.8 Hz, H$_{14}$), 7.57 (1H, t, J = 8.0 Hz, H$_9$), 7.49 (1H, d, J = 8.2 Hz, H$_5$), 7.37 (1H, t, J = 8.0 Hz, H$_{15}$), 7.11 (1H, d, J = 7.8 Hz, H$_7$), 7.04 (1H, t, J = 8.4 Hz, H$_{14}$), 5.44 (2H, s, H$_{19}$). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 194.0 (C$_5$), 154.8 (C$_9$), 151.7 (C$_2$), 140.3 (C$_{10}$), 139.3 (C$_{13}$), 138.6 (C$_{11}$), 137.4 (C$_4$), 131.5 (C$_6$), 129.7 (C$_{14}$), 129.6 (C$_{15}$), 128.6 (C$_{19}$), 128.3 (C$_{16}$), 120.4 (C$_3$), 118.0 (C$_7$), 111.3 (C$_9$), 96.4 (C$_{12}$), 75.1 (C$_{18}$). R$_f$ = 0.25 (1:4 EtOAc/hexane). HRMS (ESI$^+$) calculated for [C$_{17}$H$_{15}$O$_2$NiNa]$^+$ m/z 411.9810, found 411.9803. IR: 2840 cm$^{-1}$ (CHO), 1708 (C=0), 1378 cm$^{-1}$ (CHO), 560.8 cm$^{-1}$ (C-I).

(8-\{(2-iodobenzyl)oxy\}quinolin-2-yl)methanol (30)  
To a solution of aldehyde 20 (0.500 g, 1.28 mmol) in methanol (20 mL), was added sodium borohydride (0.150 g, 3.85 mmol) and the reaction mixture was stirred at 40 °C for 24 hours. The reaction was quenched with deionised water (3 mL) and the solvent was removed under reduced pressure. The crude material was partitioned between dichloromethane (20 mL) and saturated sodium chloride solution (10 mL). The aqueous layer was extracted with dichloromethane (3 × 15 mL) and the organic layers were combined, dried (MgSO$_4$) and concentrated under reduced pressure to give the desired alcohol 5 (0.490 g, 98%) as a white solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.14 (1H, d, J = 8.2 Hz, H$_5$), 7.88 (1H, d, J = 8.2 Hz, H$_{13}$), 7.74 (1H, d, J = 7.0 Hz, H$_{16}$), 7.46 ~ 7.44 (2H, m, H$_5$, H$_7$), 7.39 (1H, t, J = 7.0 Hz, H$_{15}$), 7.35
(1H, d, J = 8.2 Hz, H') 7.15 – 7.11 (1H, m, H'), 7.04 (1H, td, J = 7.6 Hz, 1.5 Hz, H'), 5.32 (2H, s, H'), 4.96 (2H, s, H'), 4.58 (1H, br s, O-H). 13C NMR (100 MHz, CDCl3) δ 158.1 (C'), 153.7 (C'), 139.2 (C'), 139.1 (C'), 139.0 (C'), 136.9 (C'), 129.5 (C'), 129.0 (C'), 128.6 (C'), 128.4 (C'), 126.5 (C'), 120.4 (C'), 119.0 (C'), 111.3 (C'), 96.5 (C'), 74.9 (C'), 64.4 (C'). Rf = 0.35 (1:1 EtOAc/hexane). HRMS (ESI+) calculated for [C17H15O2Ni]⁺ m/z 392.0142, found 392.0140.

(8-[(2-Iodobenzyl)oxy]quinolin-2-yl)methylmethanesulfonate (40)
To a solution of alcohol 3o (100 mg, 256 µmol) and triethylamine (54 mL, 384 µmol) in anhydrous acetonitrile (5 mL) was added methanesulfonyl chloride (22 mL, 282 mmol). The resulting orange reaction mixture was stirred at room temperature for 3 hours. The solvent was removed under reduced pressure and the residue was partitioned between dichloromethane (10 mL) and saturated sodium chloride solution (10 mL). The aqueous layer was extracted with dichloromethane (3 × 15 mL) and the organic layers were combined, dried (MgSO₄), filtered and evaporated under reduced pressure to give the desired mesylate ester 40 (118 mg, 98%) as a yellow oil, which was used immediately in the next step. 1H NMR (400 MHz, CDCl3) δ 8.22 (1H, d, J = 8.2 Hz, H'), 7.89 (1H, dd, J = 7.8 Hz, 0.8 Hz, H'), 7.63 (1H, dd, J = 7.6 Hz, 1.4 Hz, H'), 7.59 (1H, d, J = 8.7 Hz, H'), 7.49 – 7.43 (2H, m, H', H'), 7.37 (1H, td, J = 7.6 Hz, 1.1 Hz, H'), 7.08 – 7.02 (2H, m, H', H'), 5.59 (2H, s, H'), 5.35 (2H, s, H'), 3.15 (3H, s, H'). 13C NMR (100 MHz, CDCl3) δ 154.1 (C'), 153.1 (C'), 139.8 (C'), 139.3 (C'), 138.8 (C'), 137.4 (C'), 129.7 (C'), 129.1 (C'), 128.8 (C'), 128.6 (C'), 127.4 (C'), 120.3 (C'), 120.0 (C'), 110.8 (C'), 96.9 (C'), 75.0 (C'), 72.4 (C'), 38.5 (C'). LRMS (ESI+) calculated for [C18H12NO5S]⁺ m/z 469.9, found 469.8.

2,2′-[(8-[(2-Iodobenzyl)oxy]quinolin-2-yl)methyl]-1,4,7,10-tetraazacyclododecane-1,7-diyldiacetic acid (50)
To a solution of tert-butyl protected DO2A (78 mg, 195 µmol) and potassium carbonate (54 mg, 390 µmol) in anhydrous acetonitrile (10 mL) was added mesylate ester 40 (100 mg, 214 µmol). The pale-yellow solution was stirred at 60 °C for 24 hours, then the solution was centrifuged at 1500 rpm for 3 minutes. The organic layer was removed, and the pellet was washed with dichloromethane (2 × 10 mL). The organic layers were combined and the solvent was removed under reduced pressure. The crude material was purified by column chromatography (silica gel; 9:1 dichloromethane/methanol) to give the desired protected ligand 50 (100 mg, 60%) as a pale-yellow solid. 1H NMR (500 MHz, CDCl3) δ 9.98 (1H, br s, N-H), 8.15 (1H, d, J = 8.2 Hz, H'), 7.88 (1H, d, J = 7.8 Hz, H'), 7.68 (1H, d, J = 9.1 Hz, H'), 7.49 (1H, d, J = 8.7 Hz, H'), 7.44 – 7.41 (3H, m, H', H', H'), 7.12 (1H, q, J = 3.0 Hz, H'), 7.05 (1H, td, J = 7.6 Hz, 1.5 Hz, H'), 5.29 (2H, s, H'), 4.04 (2H, s, H'), 3.25 (2H, s, H'), 3.05 – 2.89 (16H, m, Hcyclen), 1.41 (18H, s, H20). 13C NMR (125 MHz, CDCl3) δ 170.7 (C'), 156.9 (C'), 153.9 (C'), 140.0 (C'), 139.3 (C'), 138.9 (C'), 136.8 (C'), 129.8 (C'), 128.9 (C'), 128.7 (C'), 128.5 (C'), 126.7 (C'), 122.6 (C'), 120.4 (C'), 110.7 (C'), 97.2 (C'), 81.5 (C'), 75.0 (C'), 57.8 (C'), 55.7 (C'), 53.6 (Cyclen), 51.6 (Cyclen), 49.7 (Cyclen), 47.1 (Cyclen), 28.3 (C20). Rf = 0.25 (95:5 dichloromethane/methanol). HRMS (ESI+) calculated for [C27H36EuN8O3]⁺ m/z 774.3091, found 774.3075.

[Eu(OH)][Cl]
The deprotected ligand 50 (10 mg, 15 µmol) was dissolved in deionised water (3 mL) and the pH adjusted to pH 7.5 using 0.3 M NaOH solution. EuCl₃·6H₂O (1.5 mg, 15 µmol) was added and the pH was readjusted to 7.5, before stirring the solution at 60 °C for 24 hours. The solvent was removed by freeze drying to give the desired Eu(III) complex [Eu(OH)][Cl] (12 mg, 95%) as a yellow solid, which was used in the next step without further purification. HRMS (ESI+) calculated for [C25H34EuN₈O₃]⁺ m/z 812.0811, found 812.0811.
The Eu(III) complex, [Eu.oBOH₂][Cl] (10 mg, 12 µmol) was dissolved in DMSO (2 mL) and potassium acetate (16 mg, 240 mmol) and bis(pinacolato)diboron (13 mg, 51 mmol) were added. The mixture was degassed by freeze-pump-thaw cycle prior to the addition of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (2 mg, 2.7 mmol). The reaction mixture was stirred at 80 °C for 2 hours under an atmosphere of argon. The solution was diluted with deionised water (50 mL) and the solvent was removed by freeze drying. The resulting yellow solid was purified by preparative RP-HPLC [gradient: 0 – 100% acetonitrile in 25 mM NH₄HCO₃ over 25 minutes, at 20 mL per minute; tᵣ = 15.2 min] to give [Eu.oBOH₂][Cl] (6.3 mg, 72%) as a white solid. ¹H NMR (500 MHz, D₂O) δ 27.42, 14.18, 13.60, 12.94, 9.16, 9.11, 8.67, 8.33, 7.65, 7.41, 6.75, 4.70, 4.63, 3.25, 2.62, 1.37, 1.12, 0.06, -1.90, -11.91, -14.12; several signals obscured or overlapping. HRMS (ESI+) calculated for [C₂₀H₃₂BEuN₃O₂]+ m/z 730.1915, found 730.1909; [C₂₀H₃₂BEuN₃O₂Na]+ m/z 752.1734, found 752.1727.

Photophysical data measured in 10 mM HEPES at pH 7.0: λₓₐₓₐ₇ = 322 nm, ε = 3800 M⁻¹ cm⁻¹, Φₑₑₑₑ = 9.0%, τ₂₀₂₀ = 0.420 ms, τD₂₀ = 0.534 ms, q = 0.3.

**Figure S1.** Analytical RP-HPLC trace of [Eu.oBOH₂]+ [gradient: 0 – 100% acetonitrile in 25 mM NH₄HCO₃ over 25 minutes at 1.0 mL per minute].

**8-((4-Iodobenzyl)oxy)quinoline-2-carbaldehyde (2p)**

To a solution of 1 (0.500 g, 2.89 mmol) and potassium carbonate (0.599 g, 4.33 mmol) in anhydrous acetonitrile (15 mL) was added 4-iodobenzyl bromide (1.03 g, 2.95 mmol). The reaction mixture was stirred at room temperature for 24 hours. The resulting orange solution was centrifugated at 120 rpm for 3 minutes. The solution was decanted, and the solid pellet was washed twice with dichloromethane (3 × 15 mL). The solvent was evaporated under reduced pressure and the resulting residue was partitioned between dichloromethane and saturated aqueous sodium chloride. The aqueous phase was extracted with dichloromethane (3 × 10 mL) and the combined organic layers were dried (MgSO₄), filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel; 25 – 60% EtOAc/petroleum ether) to give the desired compound 2p as a white solid (1.01 g, 82%). ¹H NMR (400 MHz, CDCl₃) δ 10.30 (1H, s, H⁴), 8.28 (1H, d, J = 9.1 Hz, H⁸), 8.07 (1H, d, J = 8.7 Hz, H³), 7.72 (2H, dt, J = 8.5 Hz, 2.1 Hz, H¹³), 7.55 – 7.46 (2H, m, H⁶, H⁹), 7.29 (2H, d, J = 8.2 Hz, H¹²), 7.10 (1H, dd, J = 7.8 Hz, 1.2 Hz, H⁷), 5.42 (2H, s, H¹⁰). ¹³C NMR (100 MHz, CDCl₃) δ 193.9 (C⁵), 155.0 (C⁶), 151.7 (C²), 140.4 (C⁸), 137.9 (C¹³), 137.4 (C⁹), 136.3 (C¹¹), 131.5 (C⁴), 129.6 (C⁸), 129.0 (C¹²), 120.3 (C⁷), 118.0 (C³), 111.2 (C⁹), 93.6 (C¹⁴), 70.6 (C¹⁰). Rₜ = 0.2 (1:4 EtOAc/hexane). HRMS (ESI+) calculated for [C₁₇H₁₃INO₂]+ m/z 389.9985, found 389.9974.

**8-((4-Iodobenzyl)oxy)quinolin-2-yl)methanol (3p)**

To a solution of aldehyde 3p (0.500 g, 1.28 mmol) in methanol (20 mL), was added sodium borohydride (60 mg, 1.60 mmol) and the reaction mixture was stirred at 30 °C for 24 hours. The solvent was removed under reduced pressure and the resulting residue was partitioned between dichloromethane (20 mL) and saturated aqueous sodium chloride (10
The deprotected ligand was extracted with dichloromethane (3 × 20 mL) and the organic layers were combined, dried (MgSO₄) and concentrated under reduced pressure to give the desired alcohol 7 as a white solid (0.445 g, 89%). ¹H NMR (400 MHz, CDCl₃) δ 8.12 (1H, d, J = 8.7 Hz, H°), 7.72 (2H, d, J = 8.2 Hz, H¹), 7.44 – 7.38 (2H, m, H⁶, H⁷), 7.34 (1H, d, J = 8.2 Hz, H⁸), 7.29 (2H, d, J = 7.8 Hz, H¹³), 7.09 (1H, dd, J = 6.8 Hz, 1.9 Hz, H¹⁵), 5.30 (2H, s, H¹⁰), 4.94 (2H, s, H⁹), 4.50 (1H, br s, O-H). ¹³C NMR (100 MHz, CDCl₃) δ 158.2 (C°), 153.8 (C⁹), 139.0 (C⁸), 137.8 (C¹⁵), 136.9 (C⁶), 136.8 (C¹¹), 129.1 (C¹²), 129.0 (C⁴), 126.4 (C³), 120.4 (C⁸), 119.1 (C⁵), 111.2 (C⁷), 93.4 (C⁴), 70.4 (C¹⁰), 64.5 (C³). Rf = 0.35 (1:1 EtOAc/hexane). HRMS (EI+) calculated for [C₁₇H₁₅INO₂⁺ m/z 392.0142, found 392.0135. IR: 3382 cm⁻¹.

To a solution of alcohol 3p (100 mg, 0.26 mmol) and triethylamine (55 mL, 0.38 mmol) in anhydrous acetonitrile (5 mL) was added methanesulfonyl chloride (22 mL, 0.28 mmol) and the orange mixture was stirred at room temperature for 3 hours. The solvent was removed under reduced pressure and the residue was partitioned between dichloromethane (15 mL) and saturated aqueous sodium chloride (10 mL). The aqueous layer was extracted with dichloromethane (3 × 10 mL) and the organic layers were combined, dried (MgSO₄) and concentrated under reduced pressure to give mesylate ester 4p as a yellow solid (103 mg, 85%), which was used immediately in the next step. ¹H NMR (400 MHz, CDCl₃) δ 8.21 (1H, d, J = 8.7 Hz, H°), 7.72 (2H, d, J = 8.2 Hz, H¹³), 7.60 (1H, d, J = 8.2 Hz, H°), 7.44 – 7.43 (2H, m, H⁶, H⁷), 7.27 – 7.25 (2H, m, H¹²), 7.07 (1H, m, H⁷), 5.58 (2H, s, H²), 5.33 (2H, s, H¹⁰), 3.15 (1H, s, H¹⁵). ¹³C NMR (100 MHz, CDCl₃) δ 154.1 (C⁹), 153.2 (C³), 139.7 (C⁸), 137.8 (C¹³), 137.5 (C⁴), 136.5 (C¹¹), 129.3 (C¹²), 129.1 (C⁴), 127.3 (C⁶), 127.0 (C³), 120.3 (C⁸), 110.9 (C⁷), 93.6 (C¹²), 72.2 (C⁹), 70.5 (C¹⁰), 38.4 (C¹⁵). Rf = 0.25 (1:1 EtOAc/hexane). LRMS (EI+) calculated for [C₁₉H₁₇INO₃S⁺ m/z 469.9, found 469.8.

Di-tert-butyl 2,2′-(4-((8-(4-iodobenzyl)oxy)quinolin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (5p)

To a solution of tert-butyl protected DO2A (50 mg, 125 mmol) and potassium carbonate (52 mg, 375 µmol) in anhydrous acetonitrile (8 mL) was added mesylate ester 4p (70 mg, 150 µmol). The pale-yellow solution was stirred at 60 °C for 24 hours, and the solution was centrifuged at 1500 rpm for 3 minutes. The organic layer was removed, and the pellet was washed with dichloromethane (2 × 10 mL). The organic layers were combined, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (silica gel; 9:1 dichloromethane/methanol) to give the desired protected ligand 5p as a pale-yellow solid (55 mg, 57%). ¹H NMR (400 MHz, CDCl₃) δ 10.12 (1H, br s, N-H), 8.12 (1H, d, J = 8.7 Hz, H°), 7.69 (2H, d, J = 8.2 Hz, H¹³), 7.50 (1H, d, J = 8.7 Hz, H³), 7.39 – 7.36 (2H, m, H⁶, H⁷), 7.23 (2H, d, J = 8.2 Hz, H¹²), 7.04 (1H, q, J = 3.0 Hz, H¹⁵), 5.28 (2H, s, H¹⁰), 3.98 (2H, s, H⁹), 3.19 (4H, s, H¹⁵) 3.02 – 2.81 (16H, m, H⁸,cyclen), 1.39 (18H, s, H¹⁸,cyclen). ¹³C NMR (100 MHz, CDCl₃) δ 170.6 (C¹⁶), 157.2 (C²), 153.9 (C⁹), 139.9 (C³), 137.8 (C¹³), 136.7 (C⁴), 136.7 (C¹⁴), 129.1 (C¹²), 128.5 (C⁶), 126.6 (C⁸), 122.6 (C⁷), 120.3 (C³), 110.6 (C⁷), 93.5 (C¹⁴), 81.5 (C¹⁷), 70.2 (C¹⁰), 57.8 (C¹⁵), 56.1 (C⁹), 53.7 (C⁸,cyclen), 51.6 (C¹⁰,cyclen), 49.7 (C⁴,cyclen), 47.2 (C⁹,cyclen), 28.3 (C¹⁴). Rf = 0.25 (95:5 dichloromethane/methanol). HRMS (ESI+) calculated for [C₄₀H₄₁IN₃O₁₃⁺ m/z 774.3091, found 774.3084.

[Eu₂⁺][Cl]⁺

The deprotected ligand 5p (20 mg, 30 µmol) was dissolved in water (5 mL) and the pH adjusted to pH 7.5 using 0.3 M NaOH solution. EuCl₃.6H₂O (11 mg, 30 µmol) was added and the pH was readjusted to 7.5. The solution was stirred at 70 °C for 24 hours and then the solvent was removed by freeze drying to give the Eu(III) complex [Eu₂⁺][Cl]⁺ (24 mg,
95%) as a yellow solid, which was used in the next step without further purification. HRMS (ESI+) calculated for [C\textsubscript{29}H\textsubscript{34}EuIN\textsubscript{5}O\textsubscript{5}]\textsuperscript{+} m/z 812.0811, found 812.0807.

\textbf{[Eu.pBOH\textsubscript{2}][HCO\textsubscript{2}]} \\
The Eu(III) complex, [Eu.pI]\textsuperscript{+} (50 mg, 58 mmol) was dissolved in DMSO (2 mL) and potassium acetate (17 mg, 173 mmol) and bis(pinacolato)diboron (44 mg, 173 mmol) were added. The mixture was degassed by freeze-pump-thaw cycle prior to the addition of [1,1′-bis(diphenylphosphino) ferrocene]dichloropalladium(II) (2 mg, 2.7 mmol). The reaction mixture was stirred at 80 °C for 2 hours under an atmosphere of argon. The solution was diluted with 100 mL of deionised water and the solvent was removed by freeze drying. The resulting yellow solid was purified by preparative RP-HPLC [gradient: 0 − 100% acetonitrile in 0.05% v/v formic acid, over 10 minutes at 17 mL per min\textsuperscript{−1}; t\textsubscript{R} = 6.66 min] to give the target complex [Eu.pBOH\textsubscript{2}][HCO\textsubscript{2}] (20 mg, 40%) as a white solid. \textsuperscript{1}H NMR (500 MHz, D\textsubscript{2}O) δ 27.52, 13.71, 13.30, 11.64, 9.44, 9.14, 8.80, 8.51, 7.20, 4.67, 3.79, 2.78, 1.34, -1.65, -13.32. Several signals obscured or overlapping. HRMS (ESI+) calculated for [C\textsubscript{29}H\textsubscript{36}BEuN\textsubscript{5}O\textsubscript{7}]\textsuperscript{+} m/z 730.1915, found 730.1908; [C\textsubscript{29}H\textsubscript{36}BEuN\textsubscript{5}O\textsubscript{7}Na]\textsuperscript{+} m/z 752.1734, found 752.1726. Photophysical data measured in 10 mM HEPES at pH 7.0: \(\lambda_{\text{max}} = 322\ \text{nm}\), \(\varepsilon = 2900\ \text{M}^{-1}\ \text{cm}^{-1}\), \(\Phi_{\text{em}} = 2.0\%\), \(\tau_{\text{H2O}} = 0.166\ \text{ms}\), \(\tau_{\text{D2O}} = 0.211\ \text{ms}\), \(q = 1.2\).

\textbf{Figure S2.} Analytical RP-HPLC trace of [Eu.pBOH\textsubscript{2}]\textsuperscript{+} [gradient: 0 − 100% acetonitrile in 0.05% v/v formic acid, over 17 minutes at 0.7 mL per minute].
Photophysical measurements of Eu(III) complexes

Figure S3. Absorption spectra of (a) [Eu.oBOH₂]⁺ and (b) [Eu.pBOH₂]⁺ measured in 10 mM HEPES at pH 7.0 and 295 K.

Figure S4. Emission spectra of (a) [Eu.oBOH₂]⁺, (b) [Eu.pBOH₂]⁺, and (c) [Eu.mBOH₂]⁺⁺⁺ measured in 10 mM HEPES at pH 7.0 and 295 K, λ_{exc} = 322 nm.

Figure S5. Excitation spectra of (a) [Eu.oBOH₂]⁺ (5 µM) and (b) [Eu.pBOH₂]⁺ (5 µM) measured in 10 mM HEPES at pH 7.0 and 295 K, λ_{em} = 615 nm.
Figure S6. Lack of emission spectral response of (a) [Eu,pBOH₂]⁺ (5 µM) and (b) [Eu,oBOH₂]⁺ (5 µM) with added sodium chloride (145 mM). Measured in 10 mM HEPES at pH 7.0 and 295 K, λ<sub>exc</sub> = 322 nm.

Figure S7. (a) Selective emission spectral response of [Eu,pBOH₂]⁺ (5 µM) for phosphate (1 mM) over other biologically relevant anions including citrate, lactate, acetate, sulfate, bicarbonate (1 mM each); (b) Variation in emission spectra of [Eu,pBOH₂]⁺ (5 µM) upon incremental addition of phosphate (0–60 mM); (c) Plot of fraction bound (determined from ΔJ = 2 / ΔJ = 1 intensity ratio) versus phosphate concentration, showing the fit to a 1:1 binding isotherm. Measured in 10 mM HEPES at pH 7.0 and 295 K, λ<sub>exc</sub> = 322 nm.

Figure S8. Lack of emission spectral response of [Eu,oBOH₂]⁺ (5 µM) with added phosphate, lactate, acetate, sulfate and bicarbonate (1 mM each). Citrate (1 mM) caused a decrease in emission intensity and change in spectral form. Measured in 10 mM HEPES at pH 7.0 and 295 K, λ<sub>exc</sub> = 322 nm.
Figure S9. Comparison of the selective emission spectral response of (a) [Eu.pBOH$_2$]$^+$ (5 µM) and (b) [Eu.mBOH$_2$]$^+$ (5 µM) with AMP (1 mM) compared with ATP, ADP and cAMP (1 mM each). Spectra measured using 5 µM Eu(III) complex in 10 mM HEPES at pH 7.0 and 295 K, $\lambda_{ex} = 322$ nm. Part (a) has been reproduced from Figure 4a of the main text.

Figure S10. Lack of emission spectral response of (a) [Eu.oBOH$_2$]$^+$ (5 µM) and (b) [Eu.pBOH$_2$]$^+$ (5 µM) with added adenosine (1 mM). Measured in 10 mM HEPES at pH 7.0 and 295 K, $\lambda_{ex} = 322$ nm.
**Figure S11.** Linear increase in the emission intensity ratio of $[{\text{Eu}}_{2}{\text{pBOH}}_{2}]^{+}$ with added (a) phosphate (0 – 300 mM) and (b) AMP (0 – 150 mM or above) measured in 10 mM HEPES buffer at pH 7.0 and 295 K, $\lambda_{\text{exc}} = 322$ nm.

**Figure S12.** Anion binding isotherms for complex $[{\text{Eu}}_{2}{\text{pBOH}}_{2}]^{+}$ with (a) AMP and (b) ADP. Plot of fraction bound (determined from $\Delta J = 2 / \Delta J = 1$ intensity ratio) versus anion concentration, showing the fit to a 1:1 binding isotherm. Measured in 10 mM HEPES at pH 7.0 and 295 K, $\lambda_{\text{exc}} = 322$ nm.
Mass Spectrometry analysis of Eu(III) complexes

Figure S13. High resolution mass spectra of (a) [Eu,oBOH₂]+ and (b) [Eu,pBOH₂]+.

Figure S14. High resolution mass spectra of 1:1 mixtures (250 µM) of (a) [Eu,pBOH₂]+ and phosphate, (b) [Eu,pBOH₂]+ and ADP, showing the formation of 1:1 adducts in all cases.
Figure S15. High resolution mass spectra of 1:1 mixtures (250 µM) of [Eu.pBOH₂]⁺ and AMP, and proposed structural representations of the results presented, showing the formation of 1:1 adducts in all cases.
NMR analysis of Eu(III) complexes

Figure S16. $^1$H NMR spectra (500 MHz, D$_2$O) of (a) [Eu,oBOH$_2$]$^+$ and (b) [Eu,pBOH$_2$]$^+$ recorded at pD 7.4 and 50 °C.

Figure S17. $^1$H NMR spectra (400 MHz, D$_2$O, pD 7.4) of [Eu,pBOH$_2$]$^+$ (1 mM) with increasing amounts of AMP (0, 0.5, 1 mM). Expansion of the region 5.0 – 22.0 ppm showing the emergence of a new set of signals corresponding to the AMP-[Eu,pBOH$_2$]$^+$ complex (prominent signals highlighted blue) and disappearance of the original signals for the monohydrated [Eu,pBOH$_2$]$^+$ complex (prominent signals highlighted pink).
DFT-optimised molecular structures

**Figure S18.** DFT-optimised molecular structures of europium(III) complexes bound to water, phosphate and AMP. Part (a) is reproduced from the main manuscript.

<table>
<thead>
<tr>
<th>Anion</th>
<th>[EuₖBOH₂]⁺</th>
<th>[EuₘBOH₂]⁺</th>
<th>[EuₒBOH₂]⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH⁻</td>
<td>-</td>
<td>-</td>
<td>-17.3</td>
</tr>
<tr>
<td>HPO₄²⁻</td>
<td>-62.7</td>
<td>-60.9</td>
<td>-</td>
</tr>
<tr>
<td>AMP</td>
<td>-85.1</td>
<td>-97.0</td>
<td>-</td>
</tr>
<tr>
<td>AMP (cyclic structure)</td>
<td>-55.9</td>
<td>+48.2</td>
<td>-</td>
</tr>
</tbody>
</table>

*Table S1.* Computed binding free energies (kJ/mol) between the europium complexes and various anions. Values given correspond to the free energy difference between water-bound and anion-bound complex.
NMR Spectra

8-((2-Iodobenzyl)oxy)quinoline-2-carbaldehyde (2o)

$^1$H NMR (400 MHz, CDCl$_3$, 298 K)

$^{13}$C NMR (100 MHz, CDCl$_3$, 298 K)
COSY (400 MHz, CDCl₃, 298 K) displayed range 5.0 ppm – 11.0 ppm

HSQC (400 MHz, CDCl₃, 298 K)
HMBC (400 MHz, CDCl₃, 298 K)

$\text{(B-((2-Iodobenzyl)oxy)quinolin-2-yl)methanol (3o)}$

$^1\text{H NMR (400 MHz, CDCl₃, 298 K)}$
$^{13}$C NMR (100 MHz, CDCl$_3$, 298 K)

COSY (400 MHz, CDCl$_3$, 298 K) displayed range 4.0 ppm – 9.0 ppm
HMQC (400 MHz, CDCl₃, 298 K)

HMBC (400 MHz, CDCl₃, 298 K)
(8-((2-Iodobenzyl)oxy)quinolin-2-yl)methyl methanesulfonate (4o)

$^1$H NMR (400 MHz, CDCl$_3$, 298 K)

$^{13}$C NMR (100 MHz, CDCl$_3$, 298 K)
COSY (400 MHz, CDCl₃, 298 K) displayed range 2.0 ppm – 9.0 ppm

HMOC (400 MHz, CDCl₃, 298 K)
2,2’-(4-((8-((2-iodobenzyl)oxy)quinolin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (5o)

$^1$H NMR (400 MHz, CDCl$_3$, 298 K)

HMBC (400 MHz, CDCl$_3$, 298 K)
$^{13}$C NMR (100 MHz, CDCl$_3$, 298 K)

COSY (400 MHz, CDCl$_3$, 298 K) displayed range 1.0 ppm – 9.0 ppm
HMBC (400 MHz, CDCl₃, 298 K)

HMRC (400 MHz, CDCl₃, 298 K)
8-((4-iodobenzyl)oxy)quinoline-2-carbaldehyde (2p)

$^1$H NMR (400 MHz, CDCl$_3$, 298 K)

$^{13}$C NMR (100 MHz, CDCl$_3$, 298 K)
COSY (400 MHz, CDCl₃, 298 K) displayed range 5.0 ppm – 11.0 ppm

HMOC (400 MHz, CDCl₃, 298 K)
HMBC (400 MHz, CDCl₃, 298 K)

(8-((4-Iodobenzyl)oxy)quinolin-2-yl)methanol (3p)

¹H NMR (400 MHz, CDCl₃, 298 K)
$^{13}$C NMR (100 MHz, CDCl$_3$, 298 K)

COSY (400 MHz, CDCl$_3$, 298 K) displayed range 4.0 ppm – 9.0 ppm
HMOC (400 MHz, CDCl₃, 298 K)

HMBC (400 MHz, CDCl₃, 298 K)
(8-((4-Iodobenzyl)oxy)quinolin-2-yl)methyl methanesulfonate (4p)  

$^1$H NMR (400 MHz, CDCl$_3$, 298 K)  

$^{13}$C NMR (100 MHz, CDCl$_3$, 298 K)
COSY (400 MHz, CDCl₃, 298 K) displayed range 4.5 ppm – 9.0 ppm

HMOC (400 MHz, CDCl₃, 298 K)
Di-tert-butyl 2,2′-(4-((8-((4-iodobenzyl)oxy)quinolin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (5p)

$^1$H NMR (400 MHz, CDCl$_3$, 298 K)
$^{13}$C NMR (100 MHz, CDCl$_3$, 298 K)

COSY (400 MHz, CDCl$_3$, 298 K) displayed range 1.0 ppm – 9.0 ppm
References