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

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**)[1] and (7-tert-butoxycarbonylmethyl-1,4,7,10-tetraazacyclododec-1-yl)-acetic acid tert-butyl ester (DO2A-*tert*-butyl ester).[2]

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 H_2SO_4 as a standard (Φ_{em}= 0.59, lex = 350 nm).[3] 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:[4]

$$q\,(Eu) = 1.2\,(1/\tau_{H2O} - 1/\tau_{D2O} - 0.25 - 0.075n)$$

where t_{H2O} and t_{D2O} are the emission lifetime times in water and D_2O, 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 (ΔJ = 2 / ΔJ = 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:

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

where FB is the fraction bound, calculated by $(I-I_0)/(I_1-I_0)$ where I is the emission intensity at [A], I_0 is the initial emission intensity, and I_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 ($\Delta J = 2$ / $\Delta J = 1$) 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.[5,6] A large-core quasi-relativistic effective core potential (ECP)[7] was used for treating the core along with the $4f^6$ 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.[8]

Additional single-point computations were performed using the SMD solvent model[9] 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.[10] pK_a values were computed using the reaction $H_2PO_4^- \rightarrow HPO_4^{2-} + H^+$ as a reference. All DFT computations were carried out in Q-Chem 5.4.[11]

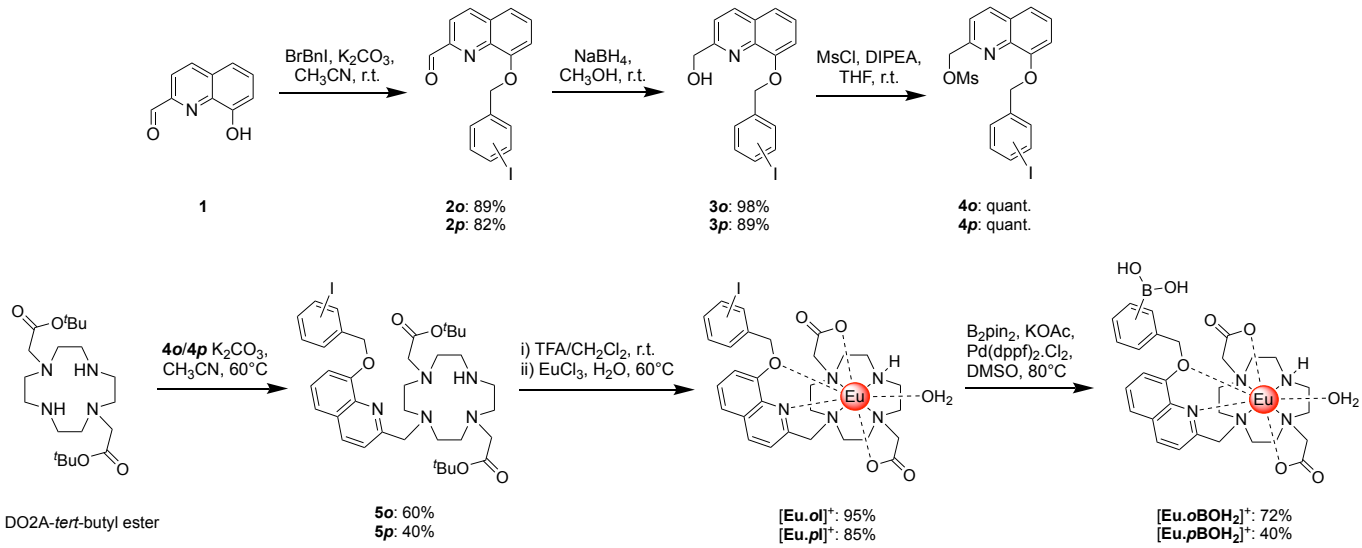
Emission spectra were simulated using the ab initio complete active space self-consistent field (CASSCF) level of theory. These computations employed the ANO-RCC-VTZP basis set for the Eu atom, ANO-RCC-VDZP for heteroatoms, and ANO-RCC-VDZ for C/H atoms.[12] The active space consisted of the 6 f-electrons 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 $^5D_0$ state, i.e. the 50^{th} state in the spin-orbit expansion. Transition strengths for the $\Delta J = 1$ 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 $\Delta J=1$ states were halved. Spectra are shown with a phenomenological broadening of 0.01 eV. CASSCF calculations were carried out in OpenMolcas.[13]

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 NH_4HCO_3) / 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.*o*BOH$_2$**]$^+$ and [**Eu.*p*BOH$_2$**]$^+$.

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

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, d, *J* = 0.8 Hz, H^9), 8.30 (1H, d, *J* = 8.7 Hz, H^4), 8.09 (1H, d, *J* = 8.2 Hz, H^3), 7.89 (1H, d, *J* = 7.8 Hz, H^{13}), 7.70 (1H, d, *J* = 7.8 Hz, H^{16}), 7.57 (1H, t, *J* = 8.0 Hz, H^6), 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^{10}). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.0 (C^9), 154.8 (C^8), 151.7 (C^2), 140.3 (C$^{8'}$), 139.3 (C^{13}), 138.6 (C^{11}), 137.4 (C^4), 131.5 (C$^{4'}$), 129.7 (C^6), 129.6 (C^{14}), 128.6 (C^{15}), 128.3 (C^{16}), 120.4 (C^5), 118.0 (C^3), 111.3 (C^7), 96.4 (C^{12}), 75.1 (C^{10}). R_f = 0.25 (1:4 EtOAc/hexane). HRMS (ESI+) calculated for $[C_{17}H_{12}O_2NINa]^+$ *m/z* 411.9810, found 411.9803. IR: 2840 cm^{-1} (CHO), 1708 (C^{ar}), 1378 cm^{-1} (CHO), 560.8 cm^{-1} (C-I).

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

To a solution of aldehyde **2*o*** (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^4), 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^6, H^5), 7.39 (1H, t, *J* = 7.0 Hz, H^{15}), 7.35

(1H, d, *J* = 8.2 Hz, H^3), 7.15 – 7.11 (1H, m, H^7), 7.04 (1H, td, *J* = 7.6 Hz, 1.5 Hz, H^{14}), 5.32 (2H, s, H^{10}), 4.96 (2H, s, H^9), 4.58 (1H, br s, O-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.1 (C^2), 153.7 ($C^{8'}$), 139.2 (C^{13}), 139.1 (C^8), 139.0 (C^{11}), 136.9 (C^4), 129.5 (C^{14}), 129.0 ($C^{4'}$), 128.6 (C^{15}), 128.4 (C^{16}), 126.5 (C^6), 120.4 (C^5), 119.0 (C^3), 111.3 (C^7), 96.5 (C^{12}), 74.9 (C^{10}), 64.4 (C^9). R_f = 0.35 (1:1 EtOAc/hexane). HRMS (ESI+) calculated for $[C_{17}H_{15}O_2NI]^+$ *m/z* 392.0142, found 392.0140.

*(8-((2-Iodobenzyl)oxy)quinolin-2-yl)methylmethanesulfonate (**4o**)*

To a solution of alcohol **3*o*** (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 ($MgSO_4$), filtered and evaporated under reduced pressure to give the desired mesylate ester **4*o*** (118 mg, 98%) as a yellow oil, which was used immediately in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (1H, d, *J* = 8.2 Hz, H^4), 7.89 (1H, dd, *J* = 7.8 Hz, 0.8 Hz, H^{13}), 7.63 (1H, dd, *J* = 7.6 Hz, 1.4 Hz, H^{16}), 7.59 (1H, d, *J* = 8.7 Hz, H^3), 7.49 – 7.43 (2H, m, H^6, H^5), 7.37 (1H, td, *J* = 7.6 Hz, 1.1 Hz, H^{15}), 7.08 – 7.02 (2H, m, H^7, H^{14}), 5.59 (2H, s, H^9), 5.35 (2H, s, H^{10}), 3.15 (3H, s, H^{17}). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.1 (C^8), 153.1 (C^2), 139.8 ($C^{8'}$), 139.3 (C^{13}), 138.8 (C^{11}), 137.4 (C^4), 129.7 (C^{15}), 129.1 ($C^{4'}$), 128.8 (C^{16}), 128.6 (C^{15}), 127.4 (C^6), 120.3 (C^5), 120.0 (C^3), 110.8 (C^7), 96.9 (C^{12}), 75.0 (C^{10}), 72.4 (C^9), 38.5 (C^{17}). LRMS (ESI+) calculated for $[C_{18}H_{17}INO_4S]^+$ *m/z* 469.9, found 469.8.

*2,2'-(4-((8-((2-Iodobenzyl)oxy)quinolin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (**5o**)*

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 **4*o*** (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 **5*o*** (100 mg, 60%) as a pale-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.98 (1H, br s, N-H), 8.15 (1H, d, *J* = 8.2 Hz, H^4), 7.88 (1H, d, *J* = 7.8 Hz, H^{13}), 7.68 (1H, d, *J* = 9.1 Hz, H^{16}), 7.49 (1H, d, *J* = 8.7 Hz, H^3), 7.44 – 7.41 (3H, m, H^5, H^6, H^{15}), 7.12 (1H, q, *J* = 3.0 Hz, H^7), 7.05 (1H, td, *J* = 7.6 Hz, 1.5 Hz, H^{14}), 5.29 (2H, s, H^{10}), 4.04 (2H, s, H^9), 3.25 (2H, s, H^{17}), 3.05 – 2.89 (16H, m, H^{cyclen}), 1.41 (18H, s, H^{20}). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (C^{18}), 156.9 (C^2), 153.9 (C^8), 140.0 ($C^{8'}$), 139.3 (C^{13}), 138.9 (C^{11}), 136.8 (C^4), 129.8 (C^{14}), 128.9 (C^{16}), 128.7 (C^{15}), 128.5 ($C^{4'}$), 126.7 (C^6), 122.6 (C^3), 120.4 (C^5), 110.7 (C^7), 97.2 (C^{12}), 81.5 (C^{19}), 75.0 (C^{10}), 57.8 (C^{17}), 55.7 (C^9), 53.6 (C^{cyclen}), 51.6 (C^{cyclen}), 49.7 (C^{cyclen}), 47.1 (C^{cyclen}), 28.3 (C^{20}). R_f = 0.25 (95:5 dichloromethane/methanol). HRMS (ESI+) calculated for $[C_{37}H_{53}IN_5O_5]^+$ *m/z* 774.3091, found 774.3075.

***[Eu.oI]*[Cl]**

The deprotected ligand **5*o*** (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. $EuCl_3.6H_2O$ (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.oI**][Cl] (12 mg, 95%) as a yellow solid, which was used in the next step without further purification. HRMS (ESI+) calculated for $[C_{29}H_{34}EuIN_5O_5]^+$ *m/z* 812.0811, found 812.0811.

*[**Eu.oBOH$_2$**][Cl]*

The Eu(III) complex, **[Eu.*o*I]**$^+$ (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 NH_4HCO_3 over 25 minutes, at 20 mL per minute; t_R = 15.2 min] to give [**Eu.*o*BOH$_2$**][Cl] (6.3 mg, 72%) as a white solid. ^{1}H NMR (500 MHz, D_2O) 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 $[C_{29}H_{36}BEuN_5O_7]^+$ *m/z* 730.1915, found 730.1909; $[C_{29}H_{35}BEuN_5O_7Na]^+$ *m/z* 752.1734, found 752.1727. Photophysical data measured in 10 mM HEPES at pH 7.0: λ_{max} = 322 nm, ε = 3800 M^{-1} cm^{-1}, Φ_{em} = 9.0%, τ_{H2O} = 0.420 ms, τ_{D2O} = 0.534 ms, q = 0.3.

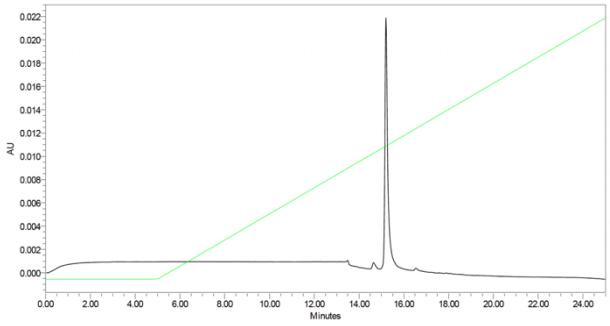


Figure S1. Analytical RP-HPLC trace of **[Eu.*o*BOH$_2$]**$^+$ [gradient: 0 – 100% acetonitrile in 25 mM NH_4HCO_3 over 25 minutes at 1.0 mL per minute].

*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 ($MgSO_4$), 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 **2*p*** as a white solid (1.01 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.30 (1H, s, H^9), 8.28 (1H, d, *J* = 9.1 Hz, H^4), 8.07 (1H, d, *J* = 8.7 Hz, H^3), 7.72 (2H, dt, *J* = 8.5 Hz, 2.1 Hz, H^{13}), 7.55 – 7.46 (2H, m, H^6, H^5), 7.29 (2H, d, *J* = 8.2 Hz, H^{12}), 7.10 (1H, dd, *J* = 7.8 Hz, 1.2 Hz, H^7), 5.42 (2H, s, H^{10}). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.9 (C^9), 155.0 (C^8), 151.7 (C^2), 140.4 (C$^{8'}$), 137.9 (C^{13}), 137.4 (C^4), 136.3 (C^{11}), 131.5 (C$^{4'}$), 129.6 (C^6), 129.0 (C^{12}), 120.3 (C^5), 118.0 (C^3), 111.2 (C^7), 93.6 (C^{14}), 70.6 (C^{10}). R_f = 0.2 (1:4 EtOAc/hexane). HRMS (ESI+) calculated for $[C_{17}H_{13}INO_2]^+$ *m/z* 389.9985, found 389.9974.

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

To a solution of aldehyde **3*p*** (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

mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL) and the organic layers were combined, dried ($MgSO_4$) and concentrated under reduced pressure to give the desired alcohol **7** as a white solid (0.445 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (1H, d, *J* = 8.7 Hz, H^4), 7.72 (2H, d, *J* = 8.2 Hz, H^{13}), 7.44 – 7.38 (2H, m, H^6, H^5), 7.34 (1H, d, *J* = 8.2 Hz, H^3), 7.29 (2H, d, *J* = 7.8 Hz, H^{12}), 7.09 (1H, dd, *J* = 6.8 Hz, 1.9 Hz, H^7), 5.30 (2H, s, H^{10}), 4.94 (2H, s, H^9), 4.50 (1H, br s, O-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.2 (C^2), 153.8 (C^8), 139.0 ($C^{8'}$), 137.8 (C^{13}), 136.9 (C^4), 136.8 (C^{11}), 129.1 (C^{12}), 129.0 ($C^{4'}$), 126.4 (C^6), 120.4 (C^5), 119.1 (C^3), 111.2 (C^7), 93.4 (C^{14}), 70.4 (C^{10}), 64.5 (C^9). R_f = 0.35 (1:1 EtOAc/hexane). HRMS (ESI+) calculated for $[C_{17}H_{15}INO_2]^+$ *m/z* 392.0142, found 392.0135. IR: 3382 cm^{-1} (OH), 1468 cm^{-1} (CH_2), 1366 cm^{-1} (OH), 1260 cm^{-1} (ether), 718 cm^{-1}.

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

To a solution of alcohol **3*p*** (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 ($MgSO_4$) and concentrated under reduced pressure to give mesylate ester **4*p*** as a yellow solid (103 mg, 85%), which was used immediately in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (1H, d, *J* = 8.7 Hz, H^4), 7.72 (2H, d, *J* = 8.2 Hz, H^{13}), 7.60 (1H, d, *J* = 8.2 Hz, H^3), 7.44 – 7.43 (2H, m, H^6, H^5), 7.27 – 7.25 (2H, m, H^{12}), 7.07 (1H, m, H^7), 5.58 (2H, s, H^9), 5.33 (2H, s, H^{10}), 3.15 (1H, s, H^{15}). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.1 (C^8), 153.2 (C^2), 139.7 ($C^{8'}$), 137.8 (C^{13}), 137.5 (C^4), 136.5 (C^{11}),129.3 (C^{12}), 129.1 ($C^{4'}$), 127.3 (C^6), 120.3 (C^5), 120.0 (C^3), 110.9 (C^7), 93.6 (C^{12}), 72.2 (C^9), 70.5 (C^{10}), 38.4 (C^{15}). R_f = 0.25 (1:1 EtOAc/hexane). LRMS (ESI+) calculated for $[C_{18}H_{17}INO_4S]^+$ *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 **4*p*** (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 **5*p*** as a pale-yellow solid (55 mg, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.12 (1H, br s, N-H), 8.12 (1H, d, *J* = 8.7 Hz, H^4), 7.69 (2H, d, *J* = 8.2 Hz, H^{13}), 7.50 (1H, d, *J* = 8.7 Hz, H^3), 7.39 – 7.36 (2H, m, H^6, H^5), 7.23 (2H, d, *J* = 8.2 Hz, H^{12}), 7.04 (1H, q, *J* = 3.0 Hz, H^7), 5.28 (2H, s, H^{10}), 3.98 (2H, s, H^9), 3.19 (4H, s, H^{15}) 3.02 – 2.81 (16H, m, H^{cyclen}), 1.39 (18H, s, H^{18}). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 (C^{16}), 157.2 (C^2), 153.9 (C^8), 139.9 ($C^{8'}$), 137.8 (C^{13}), 136.7 (C^4), 136.7 (C^{11}), 129.1 (C^{12}), 128.5 ($C^{4'}$), 126.6 (C^6), 122.6 (C^3), 120.3 (C^5), 110.6 (C^7), 93.5 (C^{14}), 81.5 (C^{17}), 70.2 (C^{10}), 57.8 (C^{15}), 56.1 (C^9), 53.7 (C^{cyclen}), 51.6 (C^{cyclen}), 49.7 (C^{cyclen}), 47.2 (C^{cyclen}), 28.3 (C^{18}). R_f = 0.25 (95:5 dichloromethane/methanol). HRMS (ESI+) calculated for $[C_{37}H_{53}IN_5O_5]^+$ *m/z* 774.3091, found 774.3084.

[Eu.pl][Cl]

The deprotected ligand **5*p*** (20 mg, 30 μmol) was dissolved in water (5 mL) and the pH adjusted to pH 7.5 using 0.3 M NaOH solution. $EuCl_3.6H_2O$ (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 [**Eu.*p*l**][Cl] (24 mg,

95%) as a yellow solid, which was used in the next step without further purification. HRMS (ESI+) calculated for $[C_{29}H_{34}EuIN_5O_5]^+$ *m/z* 812.0811, found 812.0807.

[Eu.pBOH$_2$][HCO$_2$]

The Eu(III) complex, **[Eu.*p*I]**$^+$ (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^{-1}; t_R = 6.66 min] to give the target complex [**Eu.*p*BOH$_2$**][HCO_2] (20 mg, 40%) as a white solid. ^{1}H NMR (500 MHz, D_2O) 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 $[C_{29}H_{36}BEuN_5O_7]^+$ *m/z* 730.1915, found 730.1908; $[C_{29}H_{35}BEuN_5O_7Na]^+$ *m/z* 752.1734, found 752.1726. Photophysical data measured in 10 mM HEPES at pH 7.0: λ_{max} = 322 nm, ε = 2900 M^{-1} cm^{-1}, Φ_{em} = 2.0%, τ_{H2O} = 0.166 ms, τ_{D2O} = 0.211 ms, *q* = 1.2.

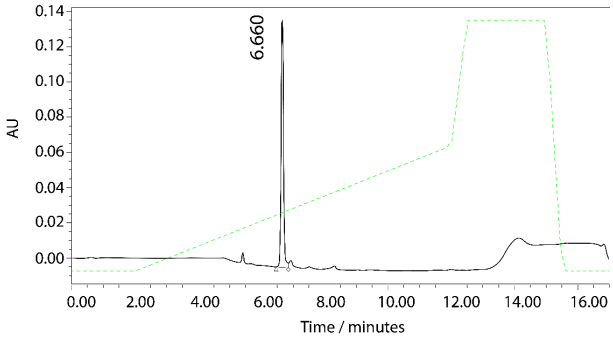


Figure S2. Analytical RP-HPLC trace of [**Eu.*p*BOH$_2$**]$^+$ [gradient: 0 – 100% acetonitrile in 0.05% *v/v* formic acid, over 17 minutes at 0.7 mL per minute].

Photophysical measurements of Eu(III) complexes

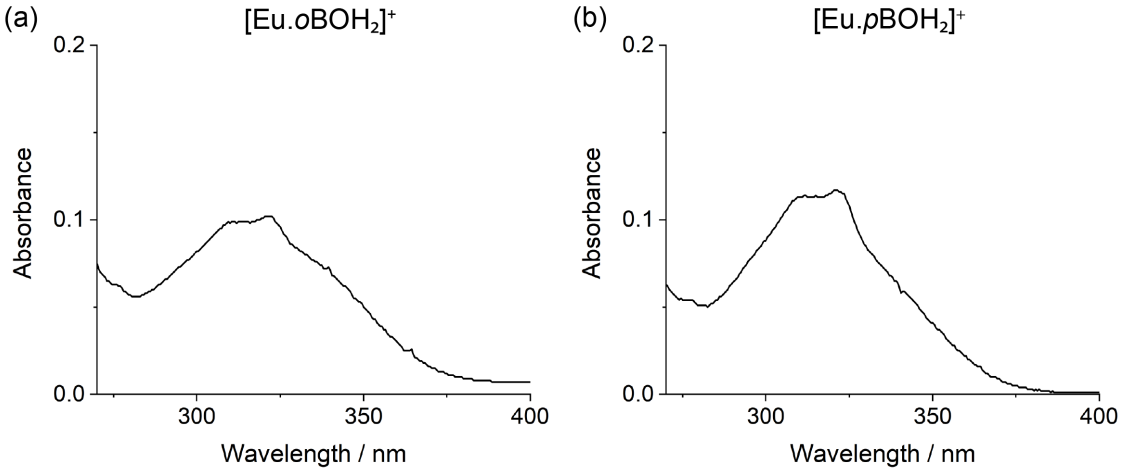


Figure S3. Absorption spectra of (a) [**Eu.*o*BOH$_2$**]$^+$ and (b) [**Eu.*p*BOH$_2$**]$^+$ measured in 10 mM HEPES at pH 7.0 and 295 K.

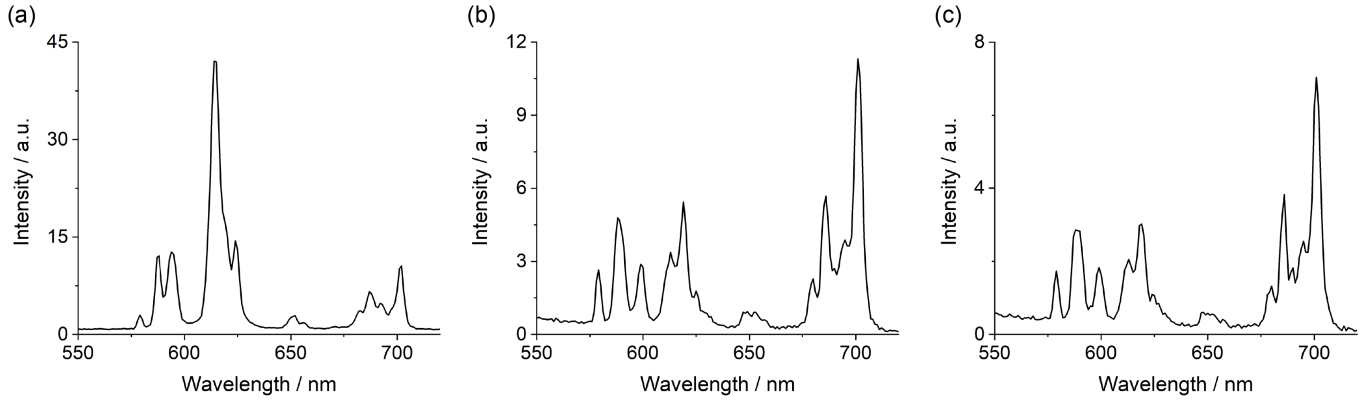


Figure S4. Emission spectra of (a) [**Eu.*o*BOH$_2$**]$^+$, (b) [**Eu.*p*BOH$_2$**]$^+$, and (c) [**Eu.*m*BOH$_2$**]$^{+14}$ measured in 10 mM HEPES at pH 7.0 and 295 K, λ_{exc} = 322 nm.

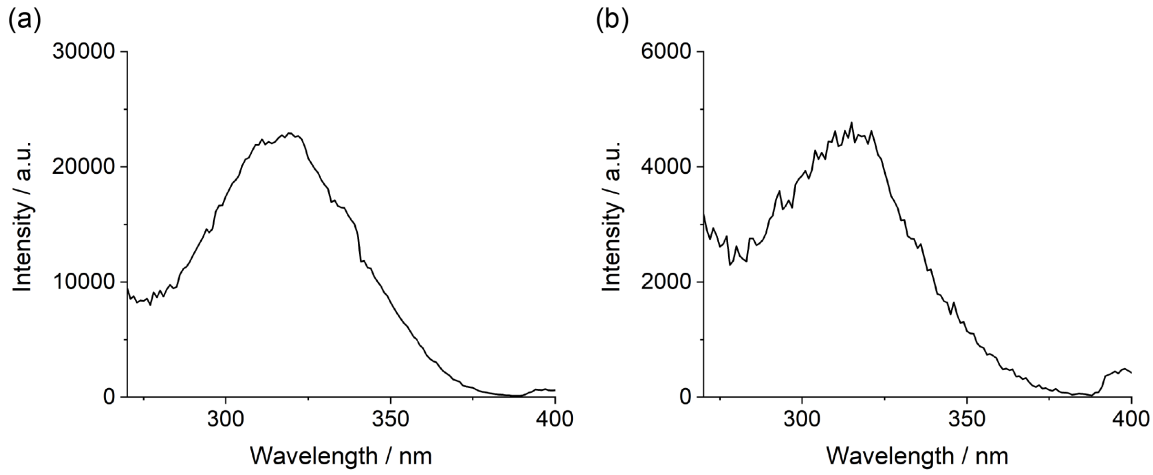


Figure S5. Excitation spectra of (a) [**Eu.*o*BOH$_2$**]$^+$ (5 μM) and (b) [**Eu.*p*BOH$_2$**]$^+$ (5 μM) measured in 10 mM HEPES at pH 7.0 and 295 K, λ_{em} = 615 nm.

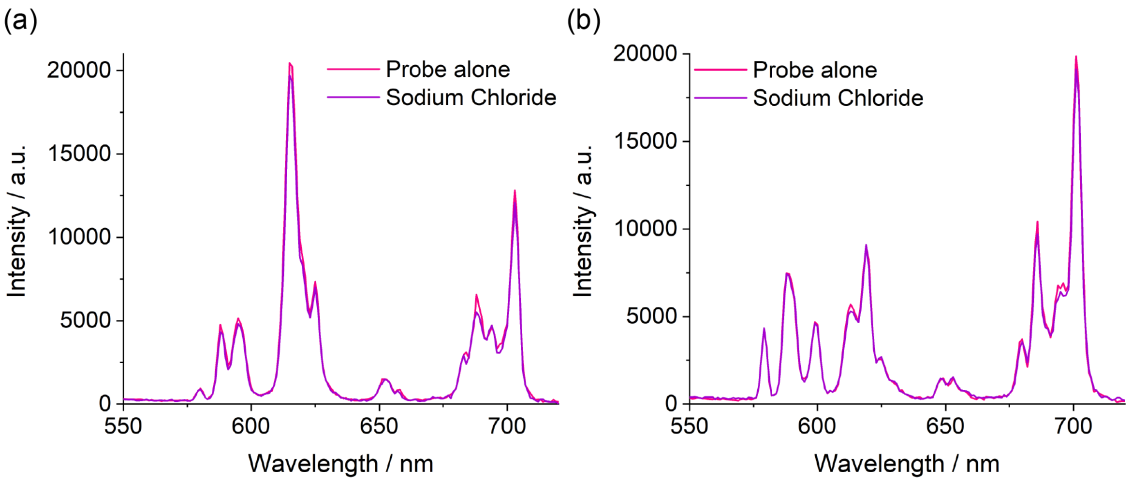


Figure S6. Lack of emission spectral response of (a) [**Eu.*p*BOH$_2$**]$^+$ (5 μM) and (b) [**Eu.*o*BOH$_2$**]$^+$ (5 μM) with added sodium chloride (145 mM). Measured in 10 mM HEPES at pH 7.0 and 295 K, λ_{exc} = 322 nm.

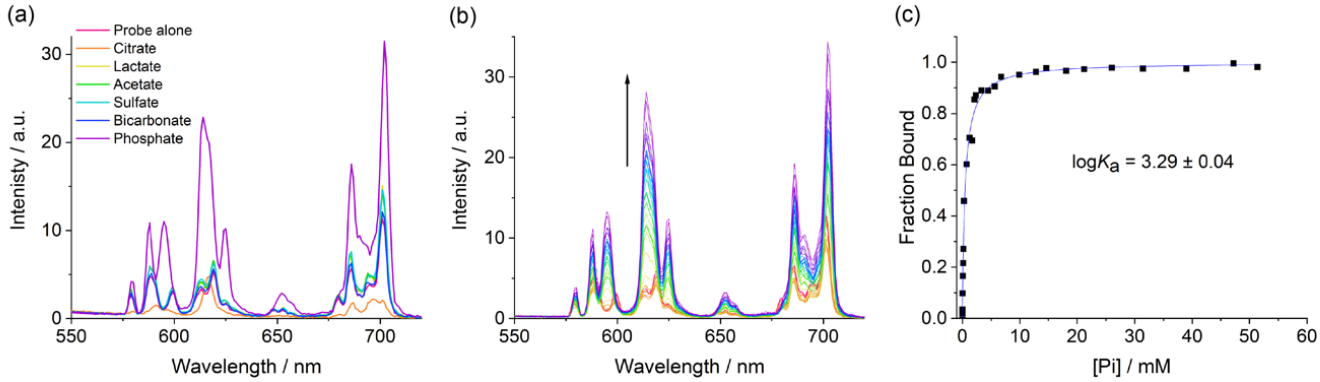


Figure S7. (a) Selective emission spectral response of [**Eu.*p*BOH$_2$**]$^+$ (5 μM) for phosphate (1 mM) over other biologically relevant anions including citrate, lactate, acetate, sulfate, bicarbonate (1 mM each); (b) Variation in emission spectra of [**Eu.*p*BOH$_2$**]$^+$ (5 μM) upon incremental 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_{exc} = 322 nm.

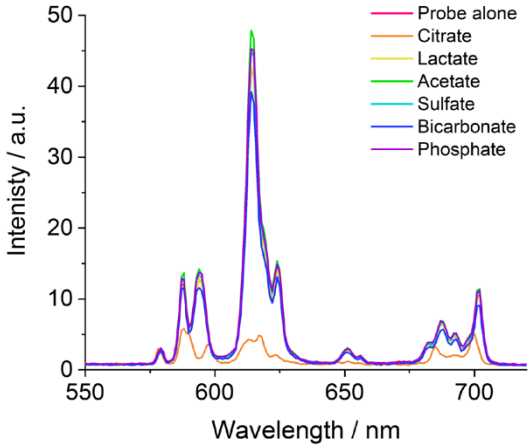


Figure S8. Lack of emission spectral response of [**Eu.*o*BOH$_2$**]$^+$ (5 μM) with added phosphate, lactate, acetate, sulfate and bicarbonate (1 mM each). Citrate (1 mM) caused a decrease in emission intensity and change in spectral form. Measured in 10 mM HEPES at pH 7.0 and 295 K, λ_{exc} = 322 nm.

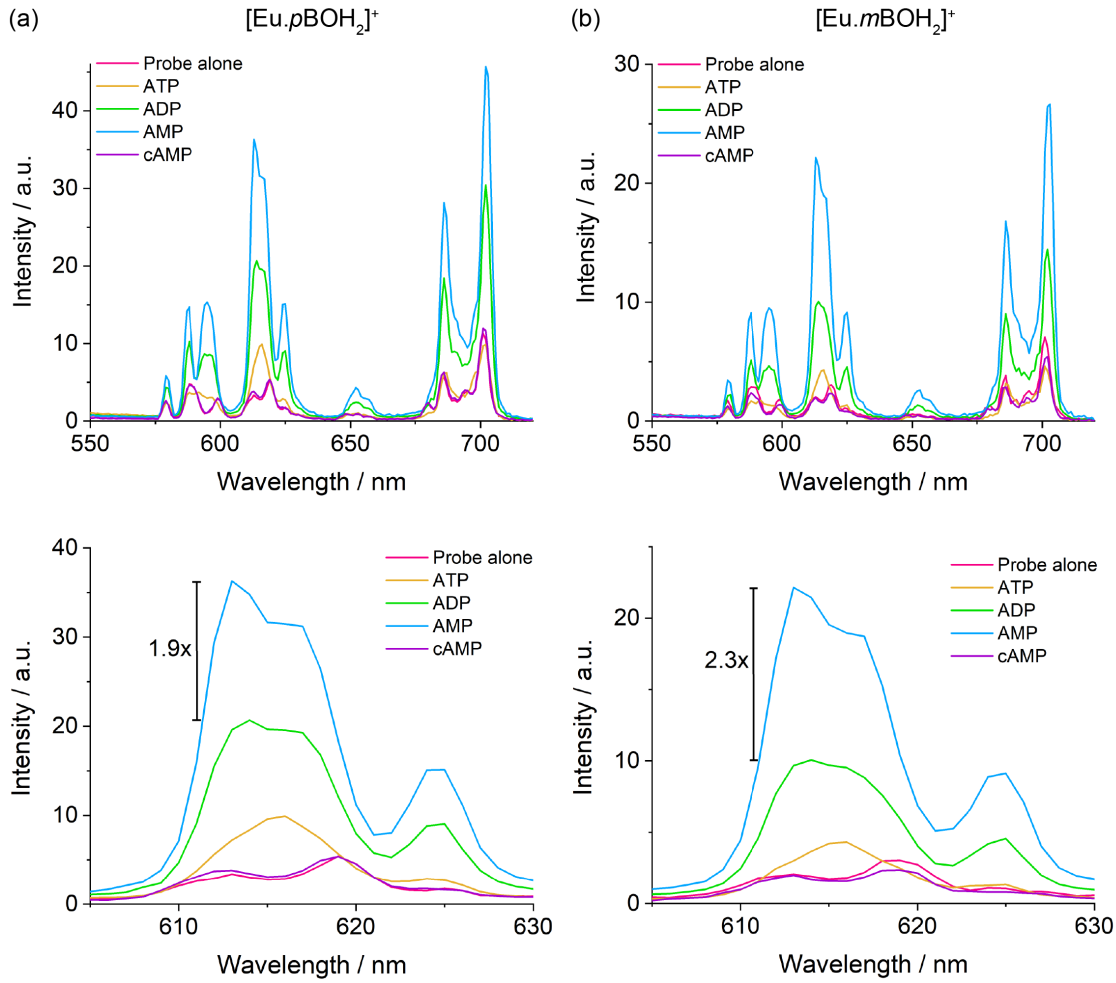


Figure S9. Comparison of the selective emission spectral response of (a) [**Eu.*p*BOH$_2$**]$^+$ (5 μM) and (b) [**Eu.*m*BOH$_2$**]$^+$ (5 μM) with AMP (1 mM) compared with ATP, ADP and cAMP (1 mM each). Spectra measured using 5 μM Eu(III) complex in 10 mM HEPES at pH 7.0 and 295 K, λ_{exc} = 322 nm. Part (a) has been reproduced from Figure 4a of the main text.

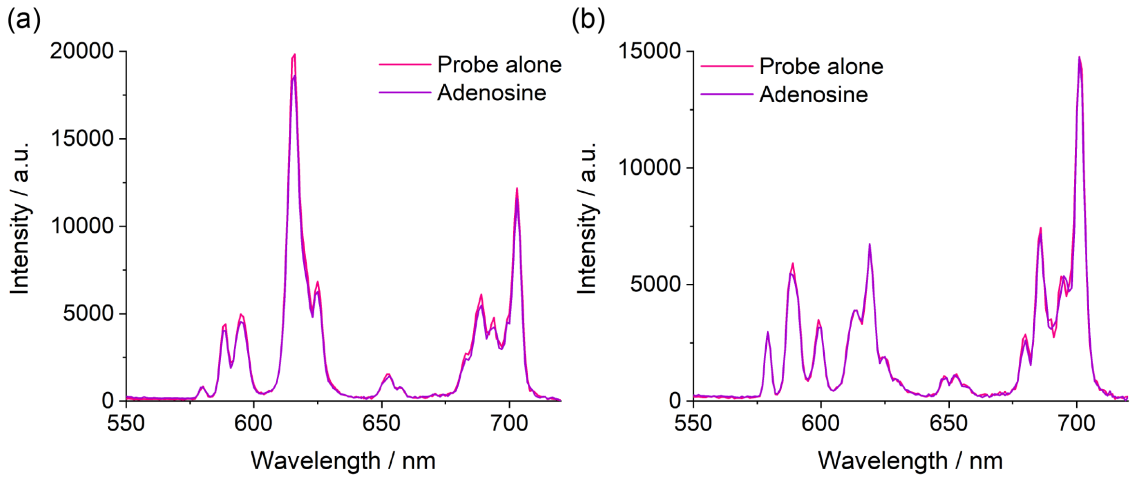


Figure S10. Lack of emission spectral response of (a) [**Eu.*o*BOH$_2$**]$^+$ (5 μM) and (b) [**Eu.*p*BOH$_2$**]$^+$ (5 μM) with added adenosine (1 mM). Measured in 10 mM HEPES at pH 7.0 and 295 K, λ_{exc} = 322 nm.

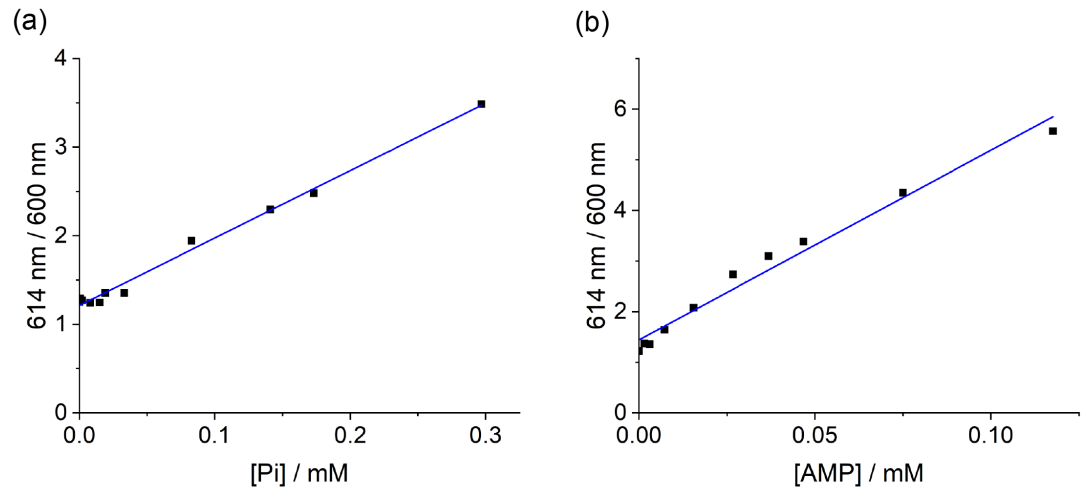


Figure S11. Linear increase in the emission intensity ratio of [**Eu.*p*BOH$_2$**]+ with added (a) phosphate (0 – 300 mM) and (b) AMP (0 – 150 mM or above) measured in 10 mM HEPES buffer at pH 7.0 and 295 K, λ_{exc} = 322 nm.

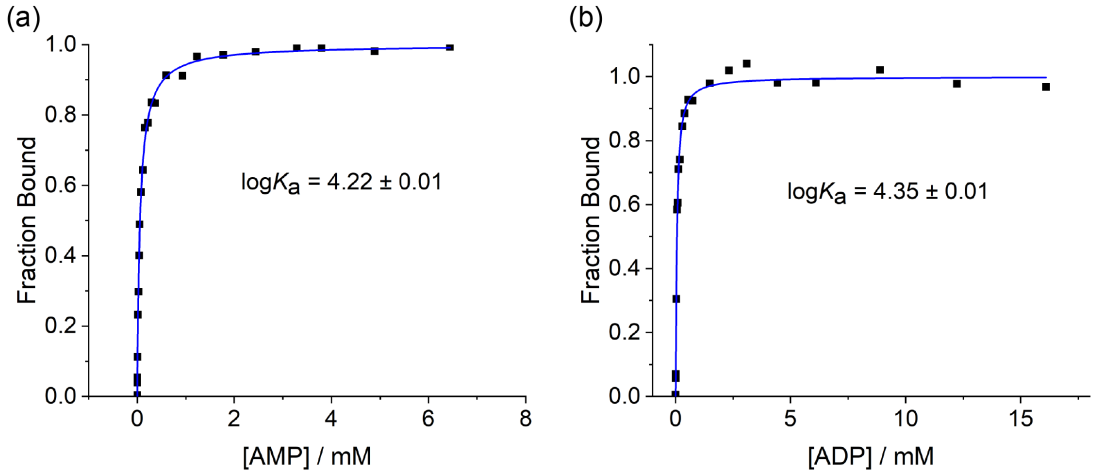


Figure S12. Anion binding isotherms for complex [**Eu.*p*BOH$_2$**]$^+$ with (a) AMP and (b) ADP. Plot of fraction bound (determined from ΔJ = 2 / ΔJ = 1 intensity ratio) versus anion concentration, showing the fit to a 1:1 binding isotherm. Measured in 10 mM HEPES at pH 7.0 and 295 K, λ_{exc} = 322 nm.

Mass Spectrometry analysis of Eu(III) complexes

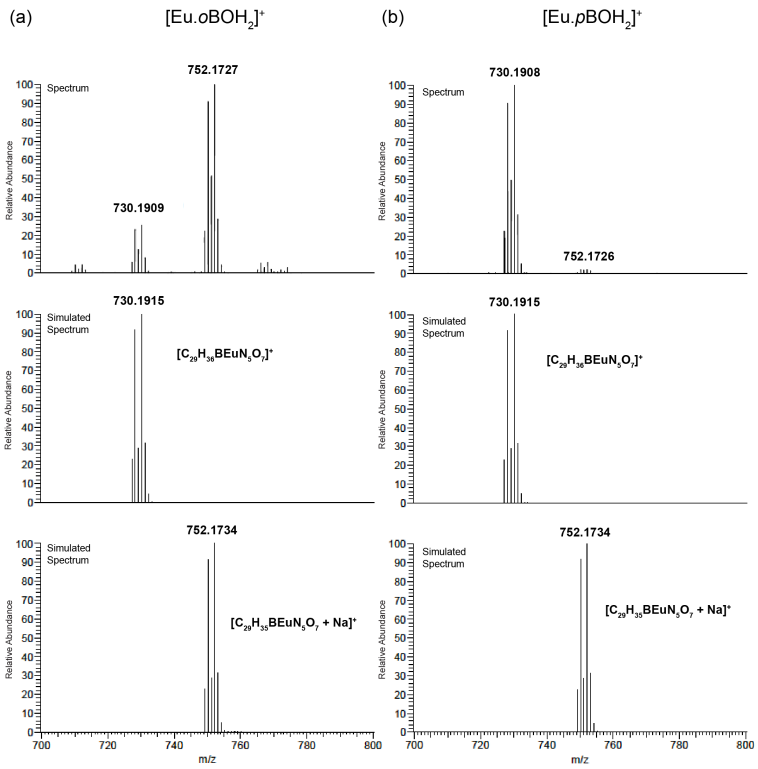


Figure S13. High resolution mass spectra of (a) [**Eu.*o*BOH$_2$**]$^+$ and (b) [**Eu.*p*BOH$_2$**]$^+$.

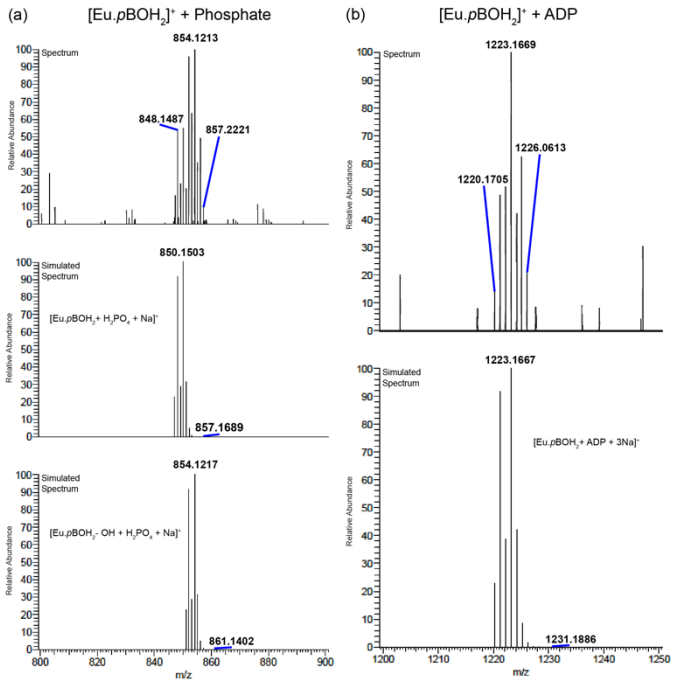


Figure S14. High resolution mass spectra of 1:1 mixtures (250 μM) of (a) [**Eu.*p*BOH$_2$**]$^+$ and phosphate, (b) [**Eu.*p*BOH$_2$**]$^+$ and ADP, showing the formation of 1:1 adducts in all cases.

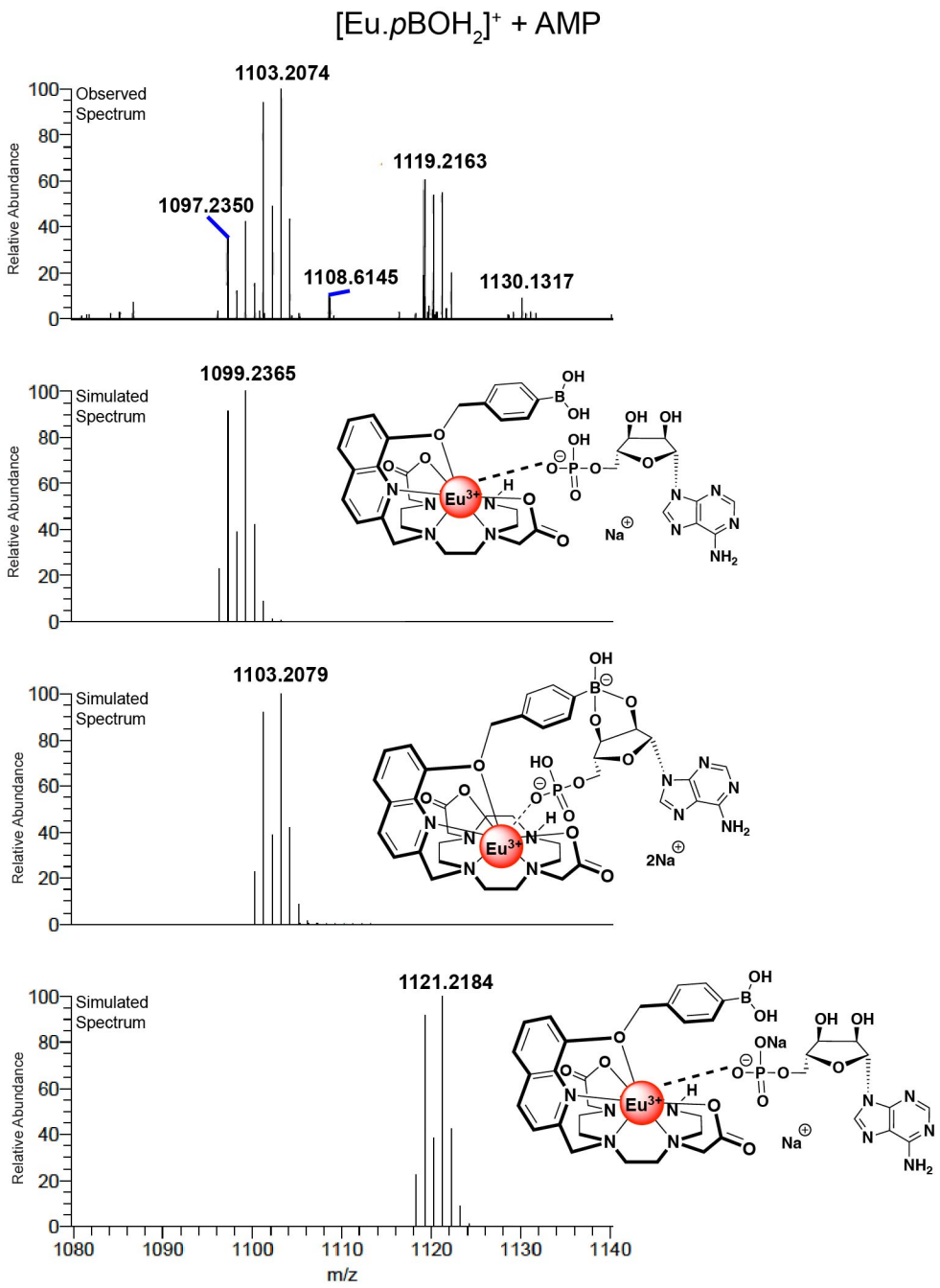


Figure S15. High resolution mass spectra of 1:1 mixtures (250 μM) of [**Eu.*p*BOH$_2$**]$^+$ and AMP, and proposed structural representations of the results presented, showing the formation of 1:1 adducts in all cases.

NMR analysis of Eu(III) complexes

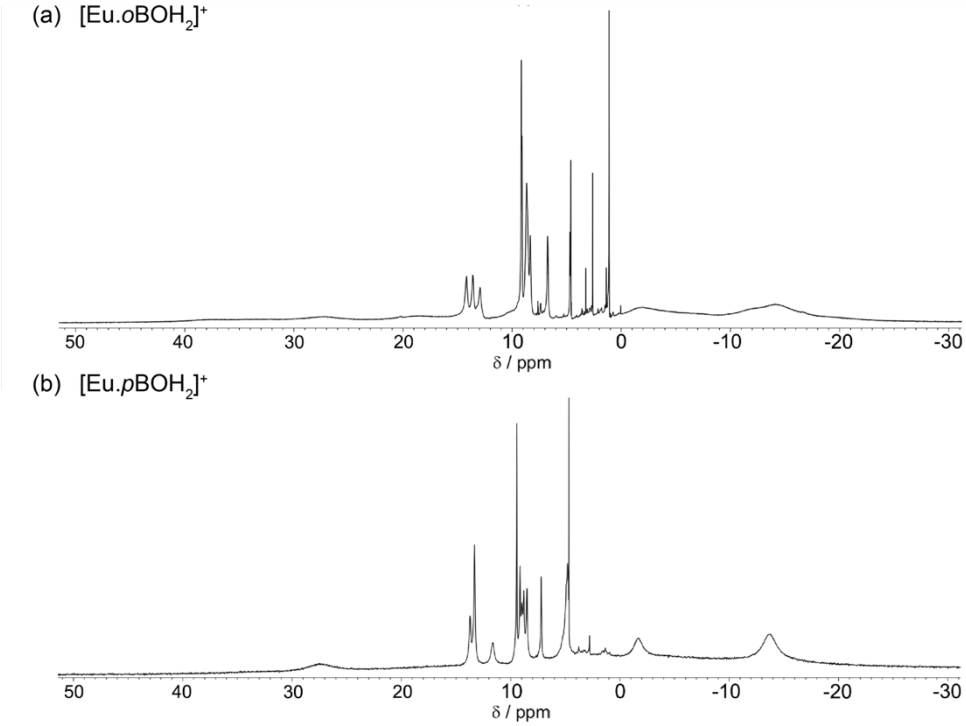


Figure S16. ^{1}H NMR spectra (500 MHz, D_2O) of (a) [**Eu.*o*BOH$_2$**]$^+$ and (b) [**Eu.*p*BOH$_2$**]$^+$ recorded at pD 7.4 and 50 °C.

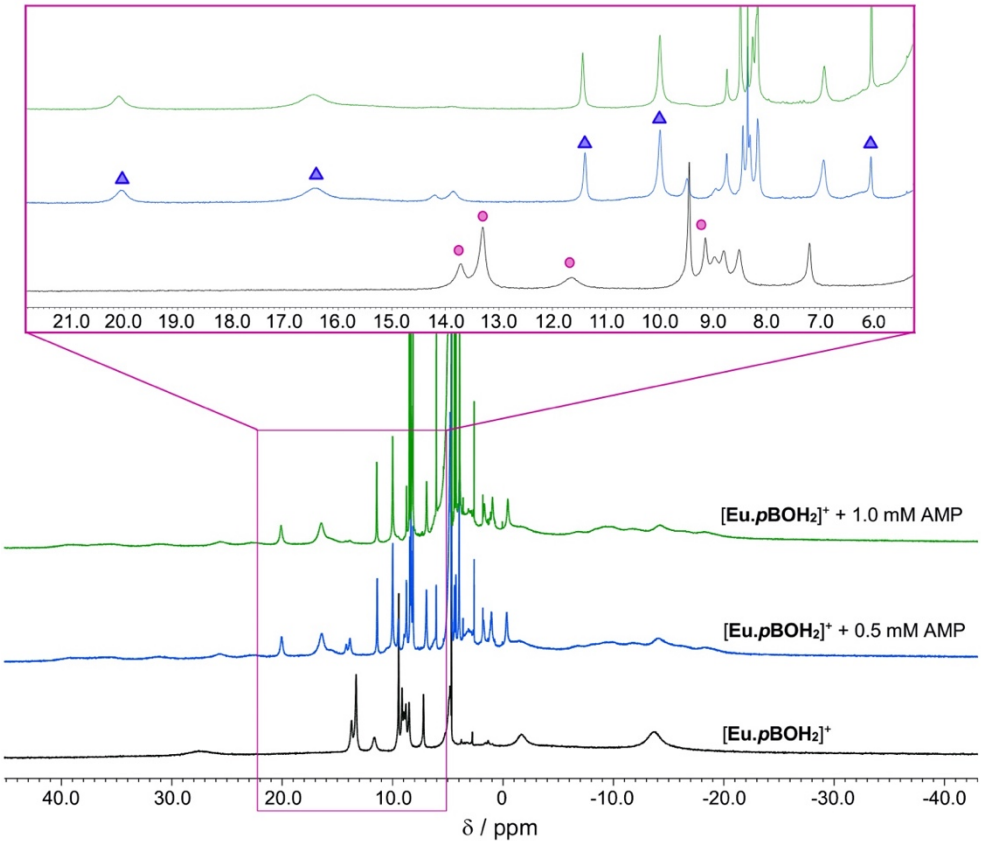


Figure S17. ^{1}H NMR spectra (400 MHz, D_2O, pD 7.4) of [**Eu.*p*BOH$_2$**]$^+$ (1 mM) with increasing amounts of AMP (0, 0.5, 1 mM). Expansion of the region 5.0 – 22.0 ppm showing the emergence of a new set of signals corresponding to the AMP-[**Eu.*p*BOH$_2$**]$^+$ complex (prominent signals highlighted blue) and disappearance of the original signals for the monohydrated [**Eu.*p*BOH$_2$**]$^+$ complex (prominent signals highlighted pink).

DFT-optimised molecular structures

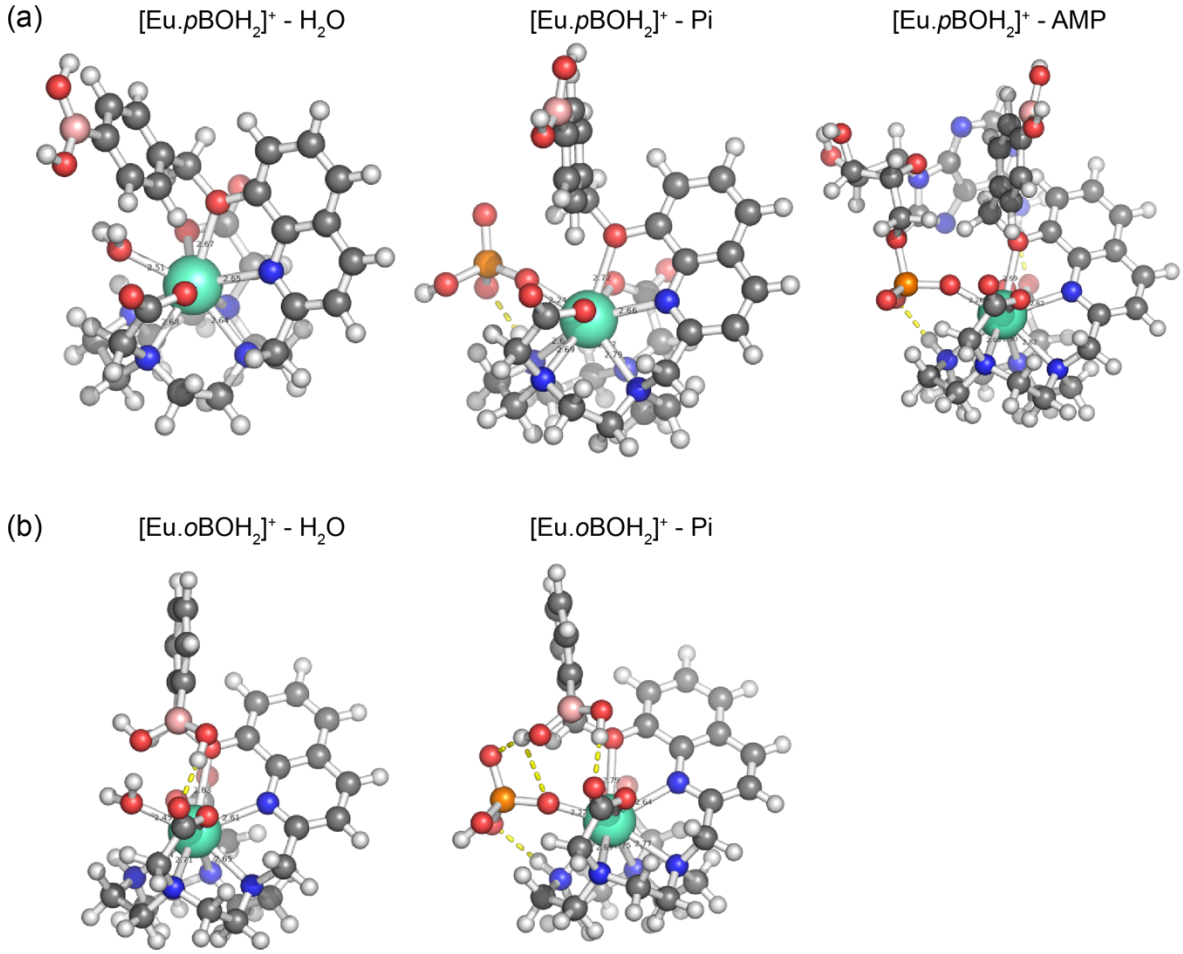


Figure S18. DFT-optimised molecular structures of europium(III) complexes bound to water, phosphate and AMP. Part (a) is reproduced from the main manuscript.

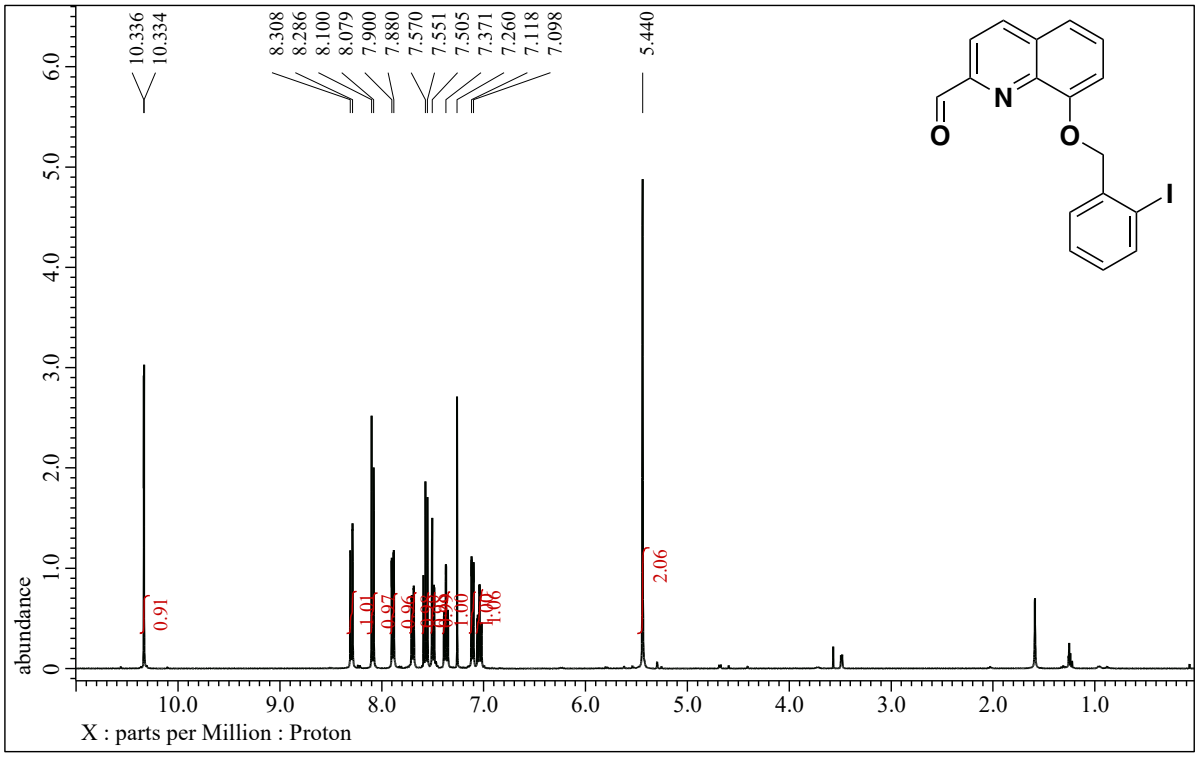
Table S1. Computed binding free energies (kJ/mol) between the europium complexes and various anions. Values given correspond to the free energy difference between water-bound and anion-bound complex.

Anion	**[Eu.*p*BOH$_2$]$^+$**	**[Eu.*m*BOH$_2$]$^+$**	**[Eu.*o*BOH$_2$]$^+$**
OH^-	-	-	-17.3
HPO_4^{2-}	-62.7	-60.9	-
AMP	-85.1	-97.0	-
AMP (cyclic structure)	-55.9	+48.2	-

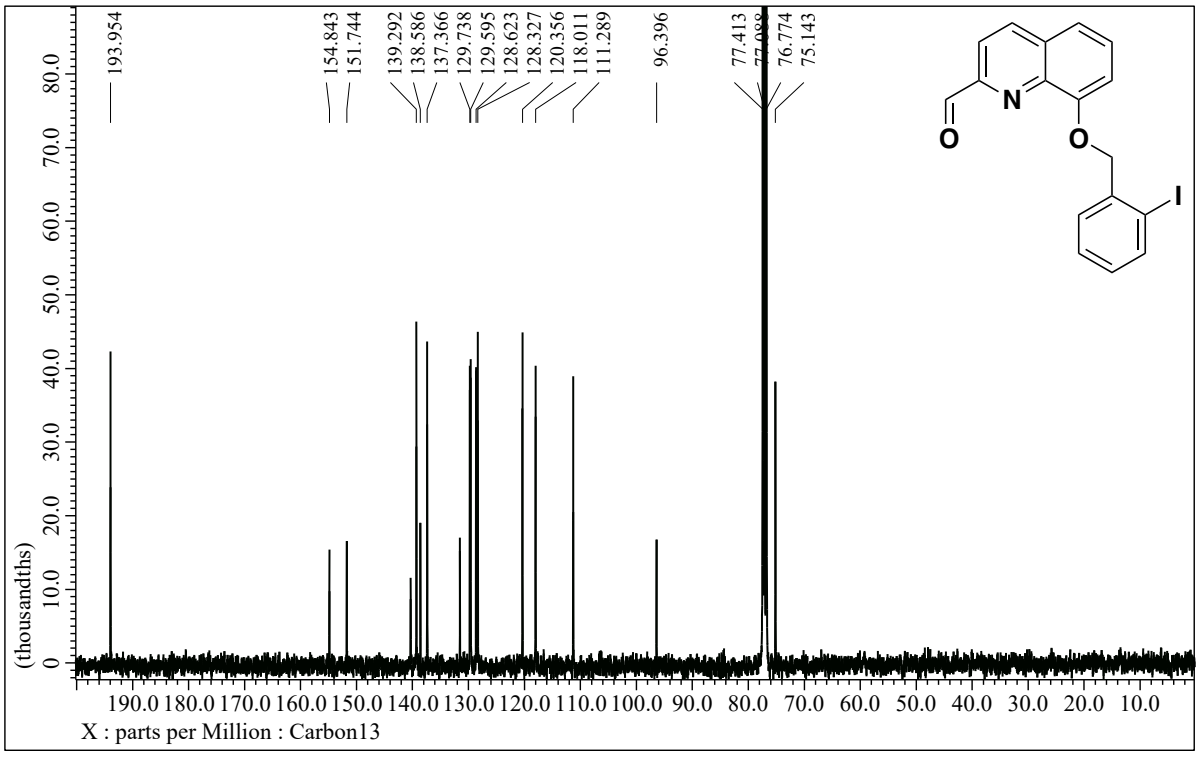
NMR Spectra

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

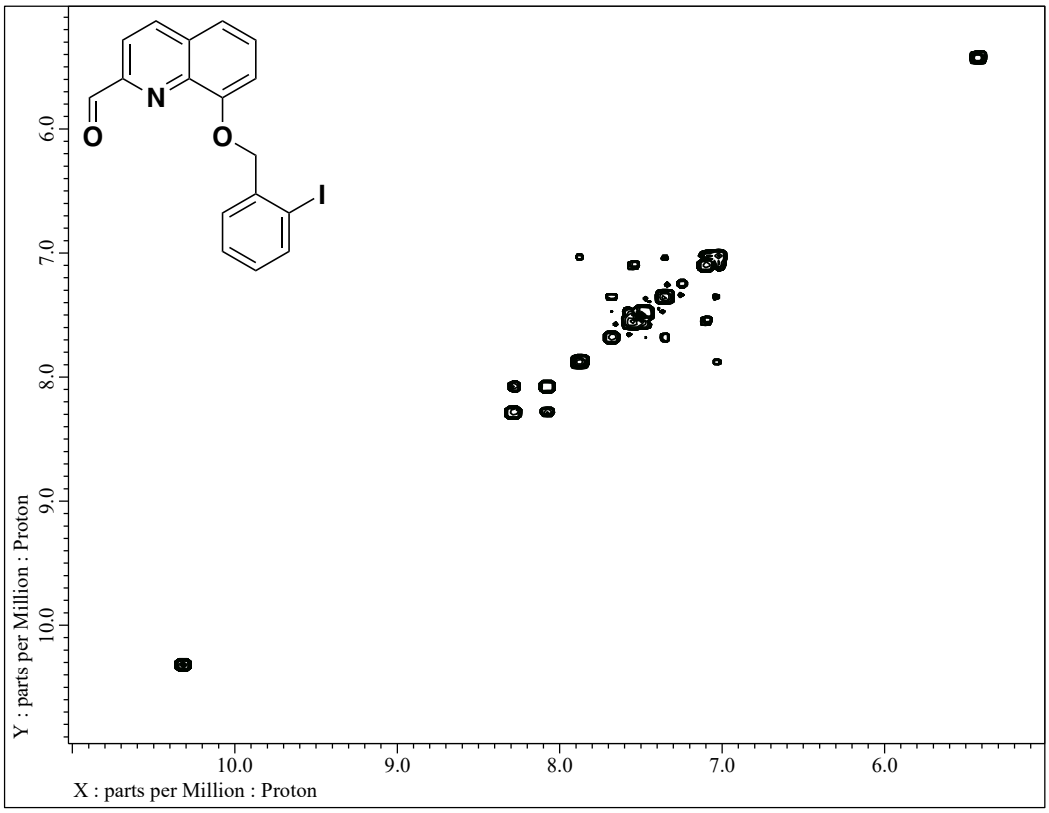
^{1}H NMR (400 MHz, $CDCl_3$, 298 K)



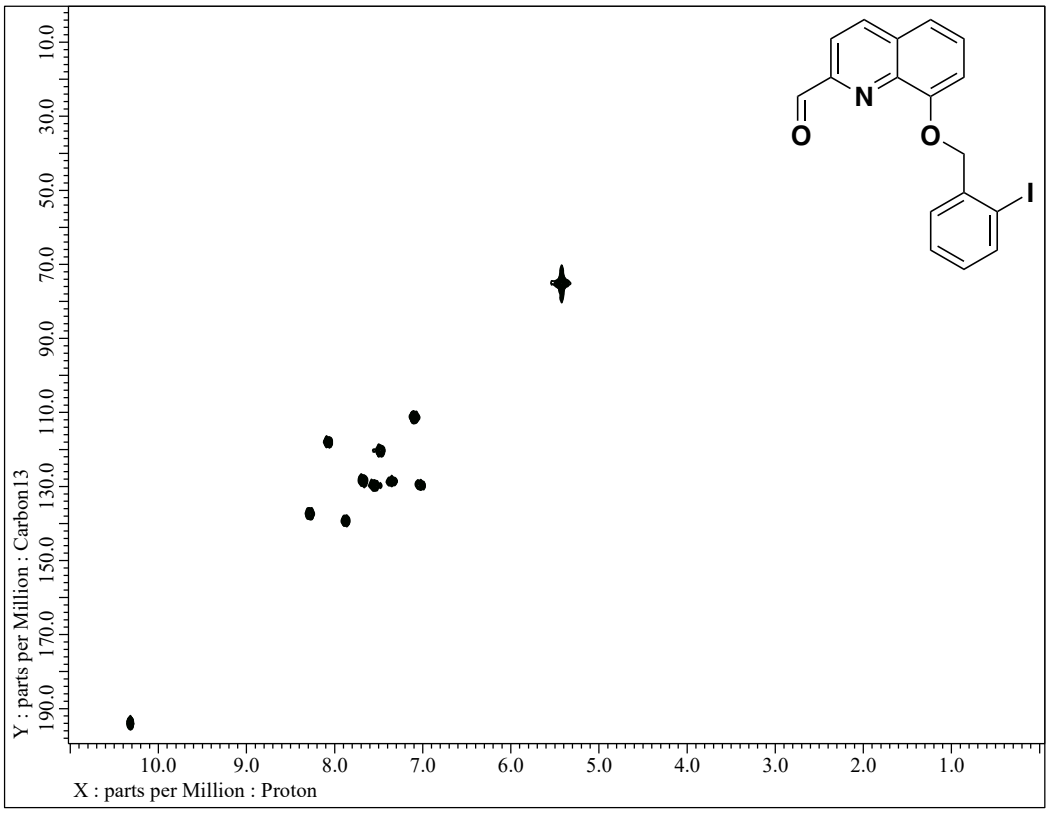
^{13}C NMR (100 MHz, $CDCl_3$, 298 K)



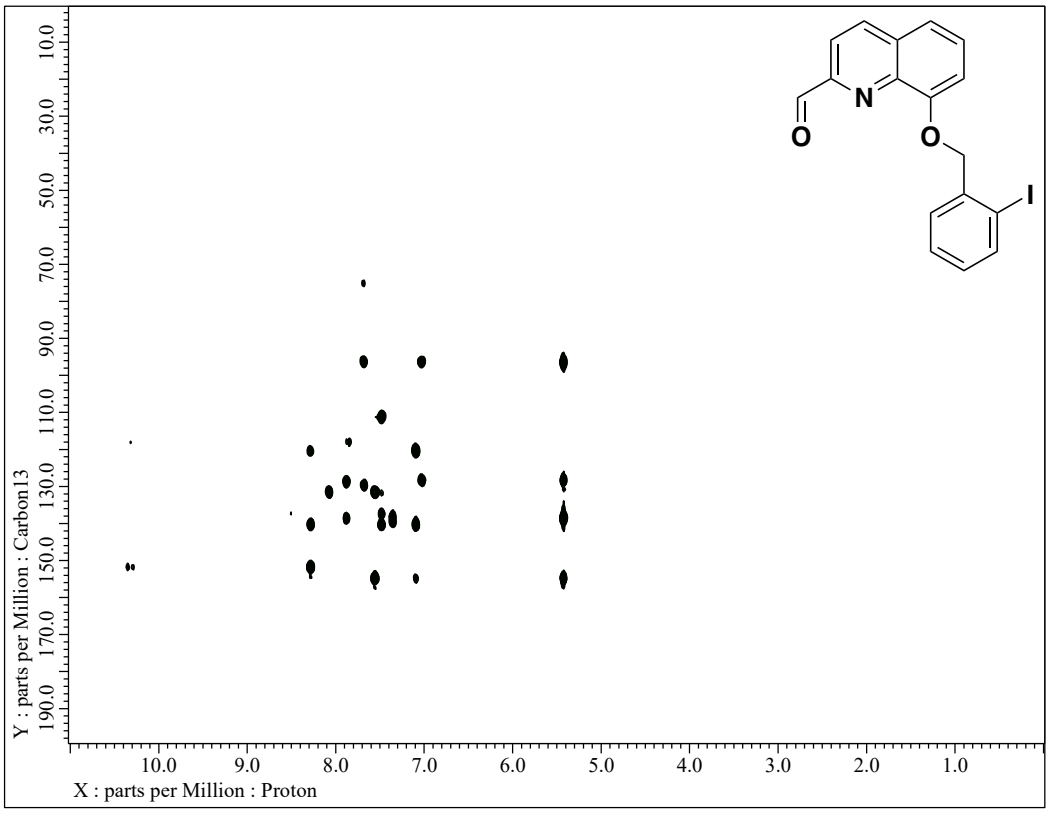
COSY (400 MHz, $CDCl_3$, 298 K) displayed range 5.0 ppm – 11.0 ppm



HSQC (400 MHz, $CDCl_3$, 298 K)

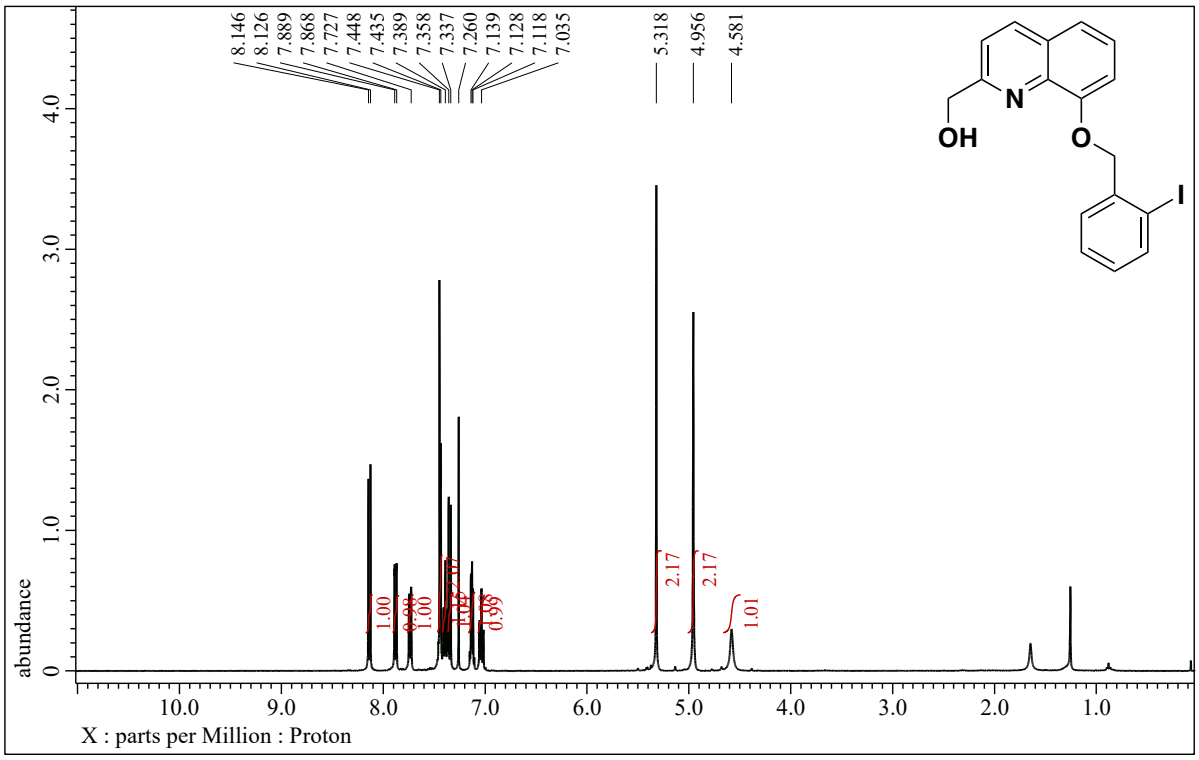


HMBC (400 MHz, $CDCl_3$, 298 K)

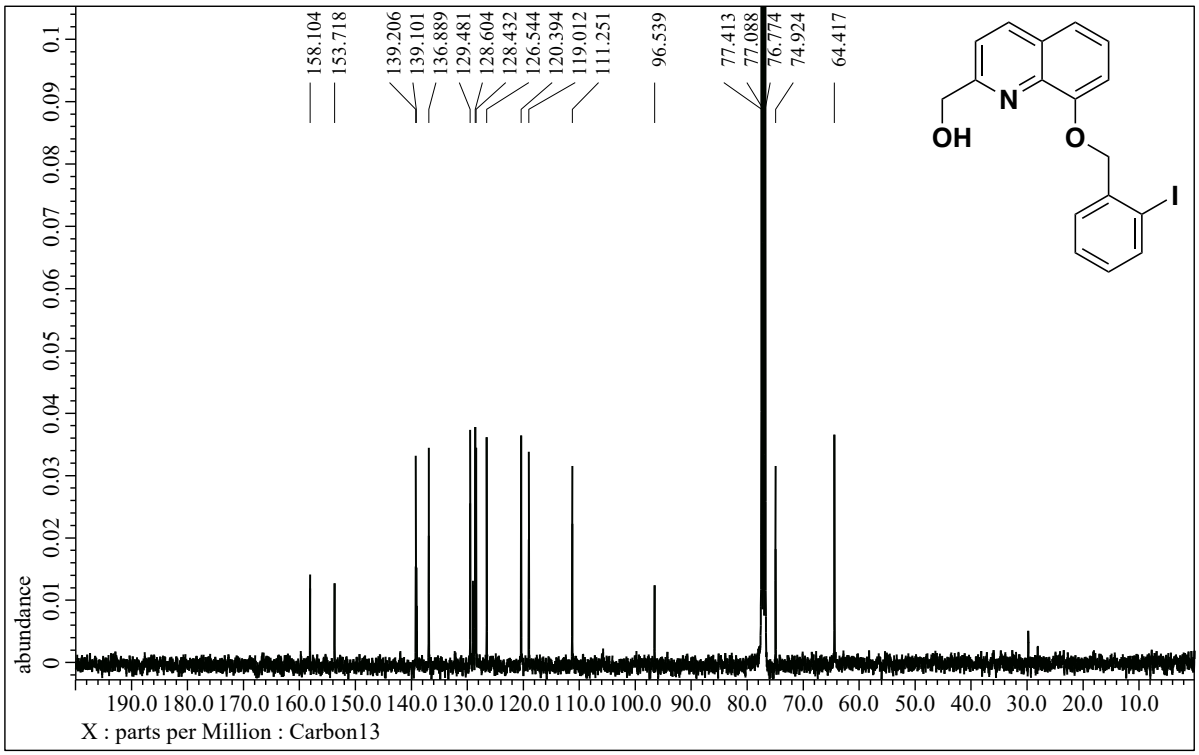


*(8-((2-Iodobenzyl)oxy)quinolin-2-yl)methanol (**3o**)*

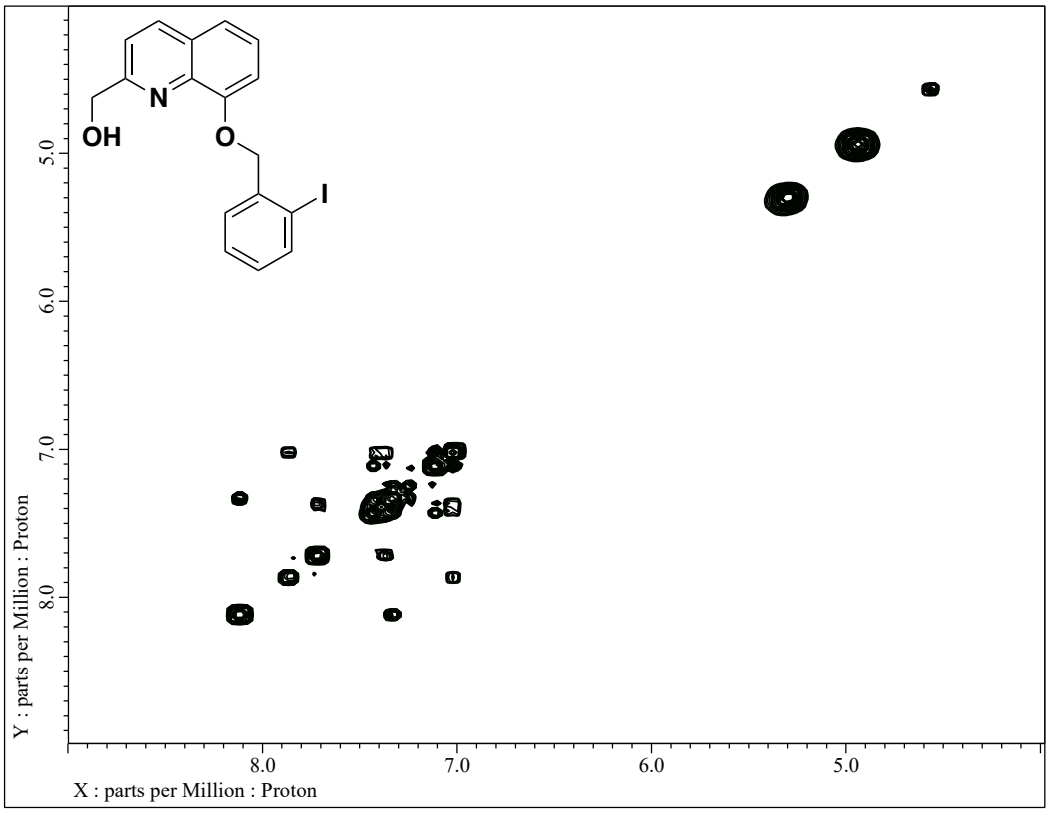
^1H NMR (400 MHz, $CDCl_3$, 298 K)



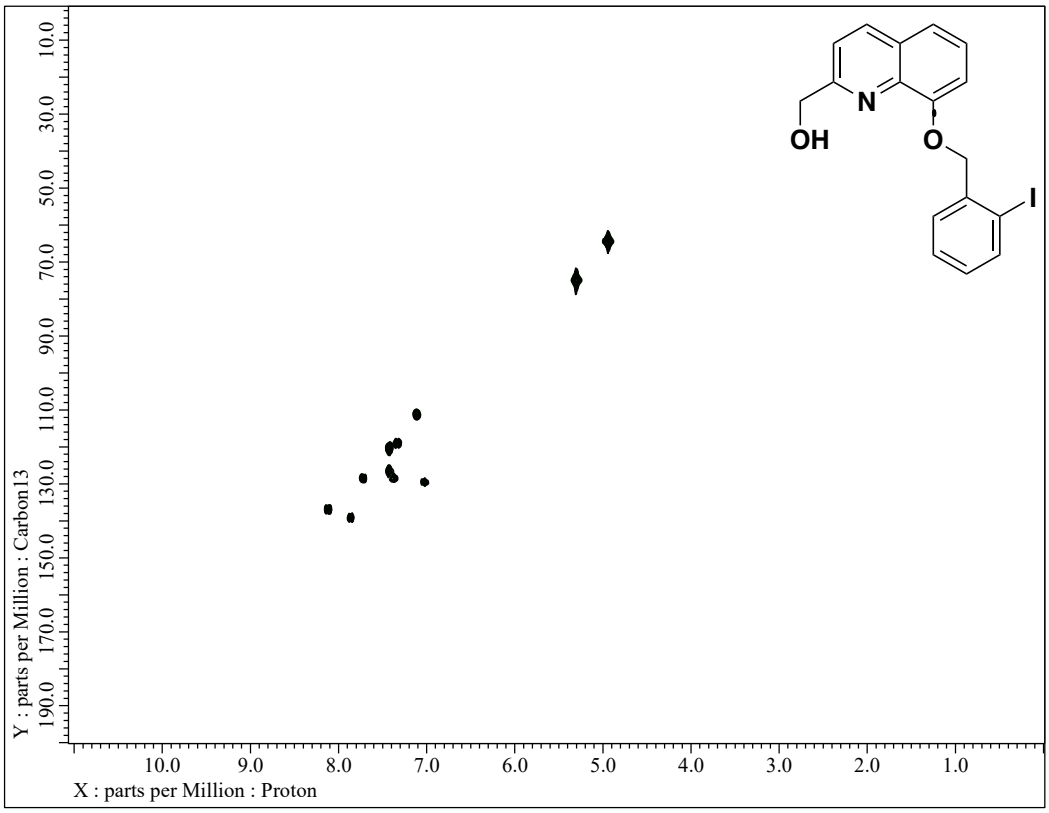
^{13}C NMR (100 MHz, $CDCl_3$, 298 K)



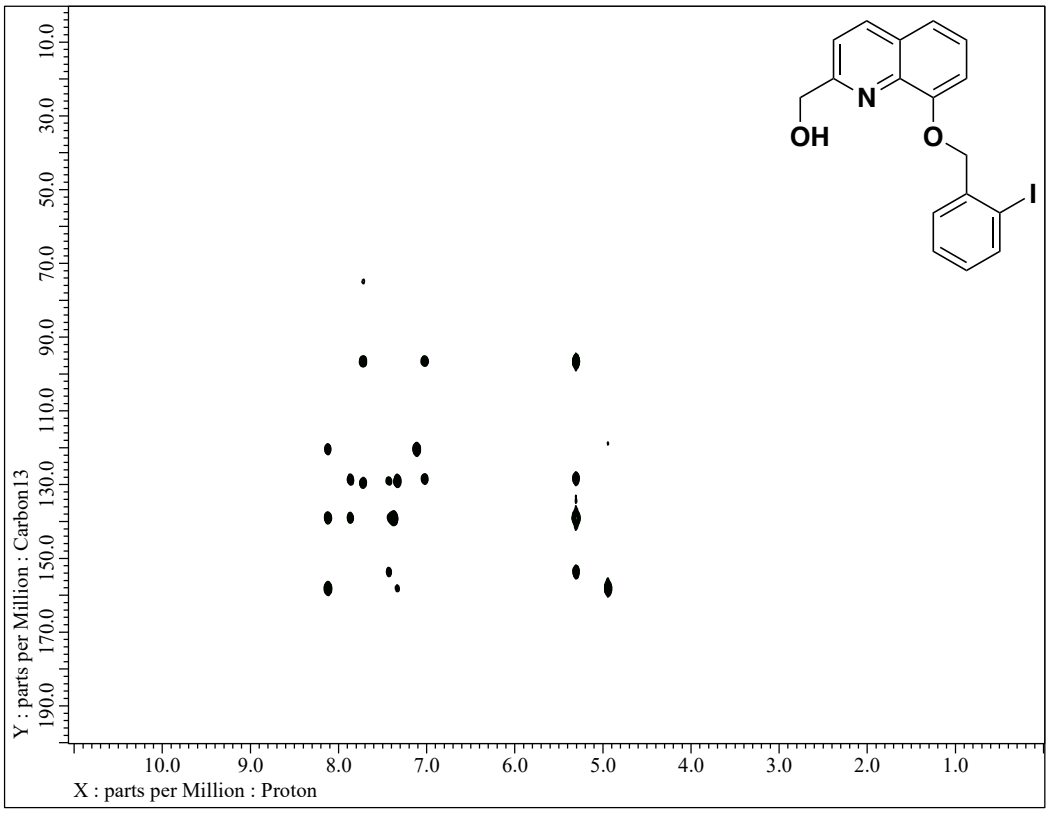
COSY (400 MHz, $CDCl_3$, 298 K) displayed range 4.0 ppm – 9.0 ppm



HMQC (400 MHz, $CDCl_3$, 298 K)

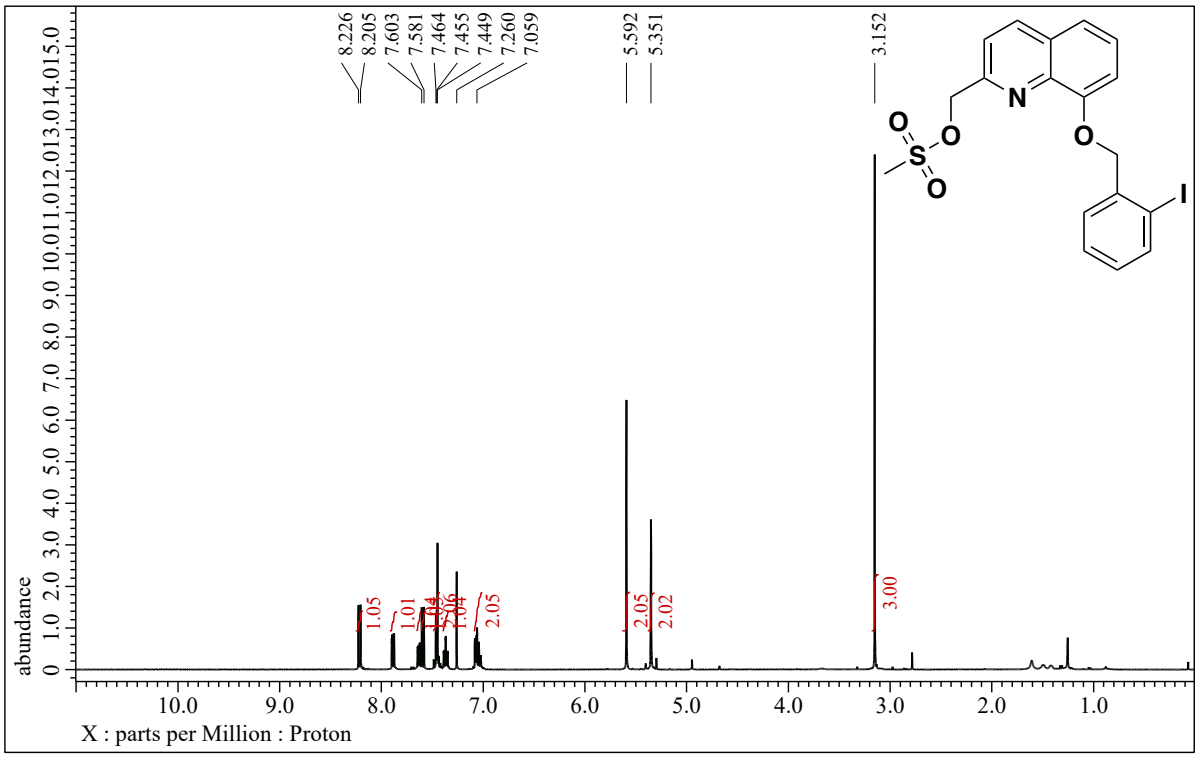


HMBC (400 MHz, $CDCl_3$, 298 K)

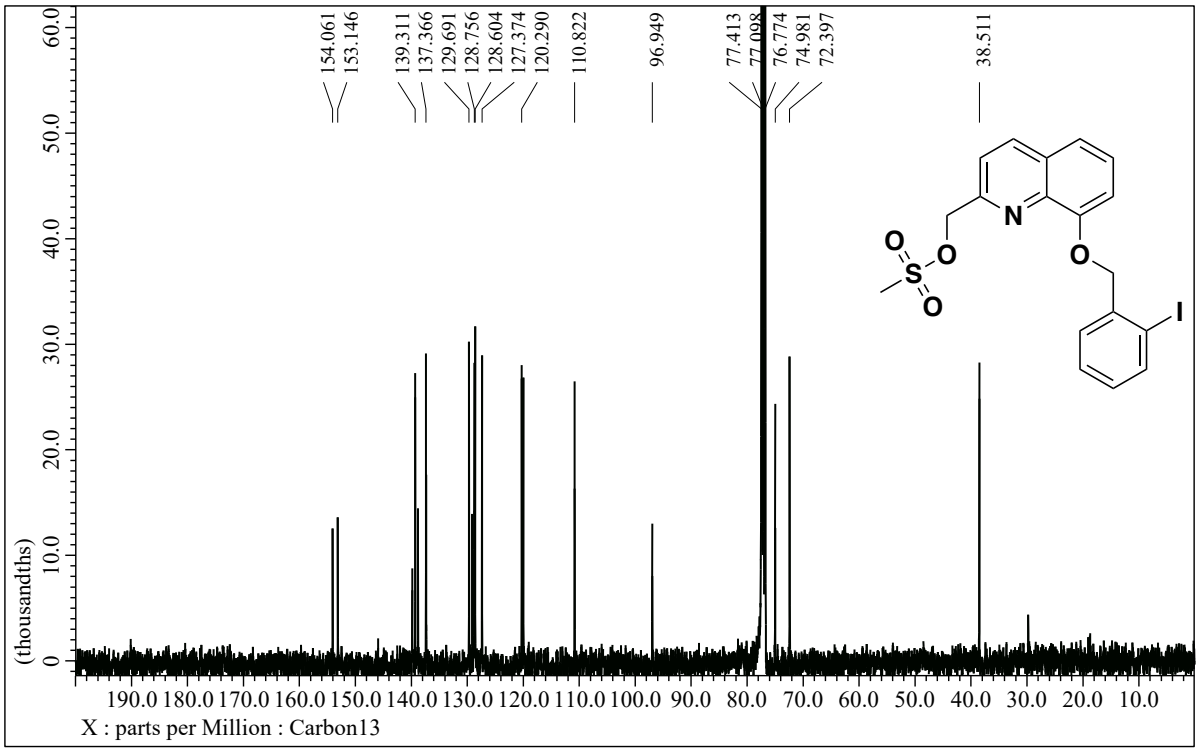


*(8-((2-Iodobenzyl)oxy)quinolin-2-yl)methyl methanesulfonate (**4o**)*

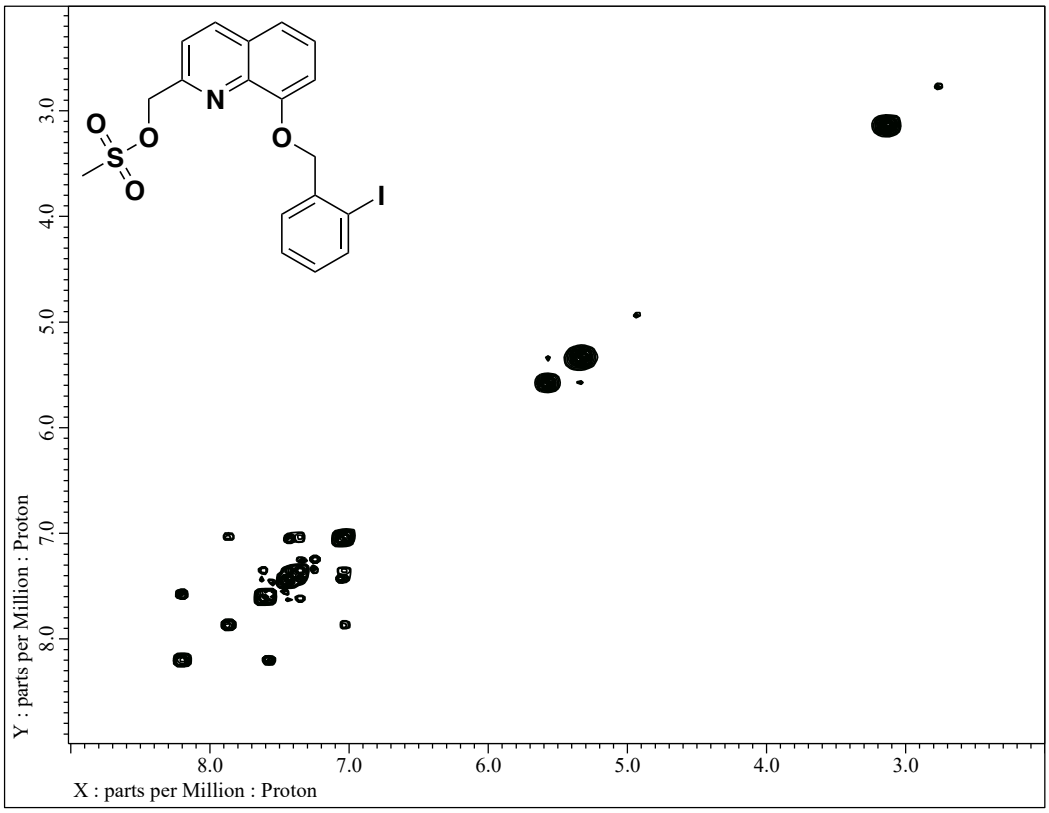
^{1}H NMR (400 MHz, $CDCl_3$, 298 K)



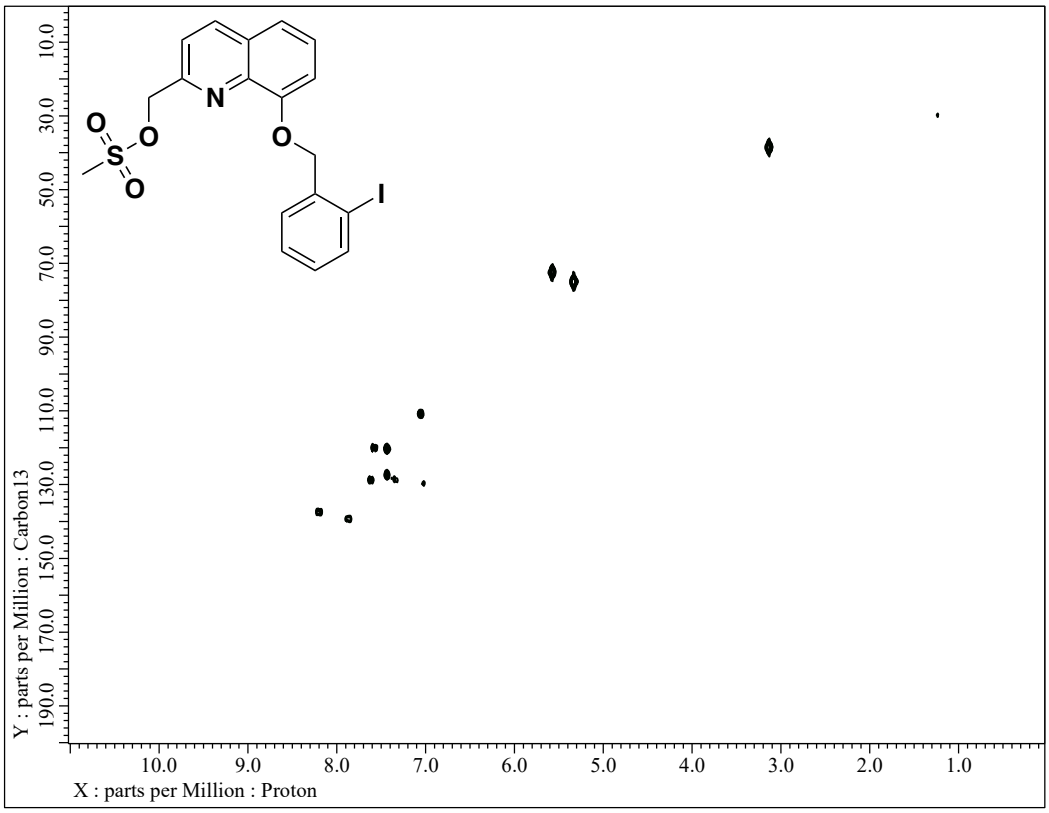
^{13}C NMR (100 MHz, $CDCl_3$, 298 K)



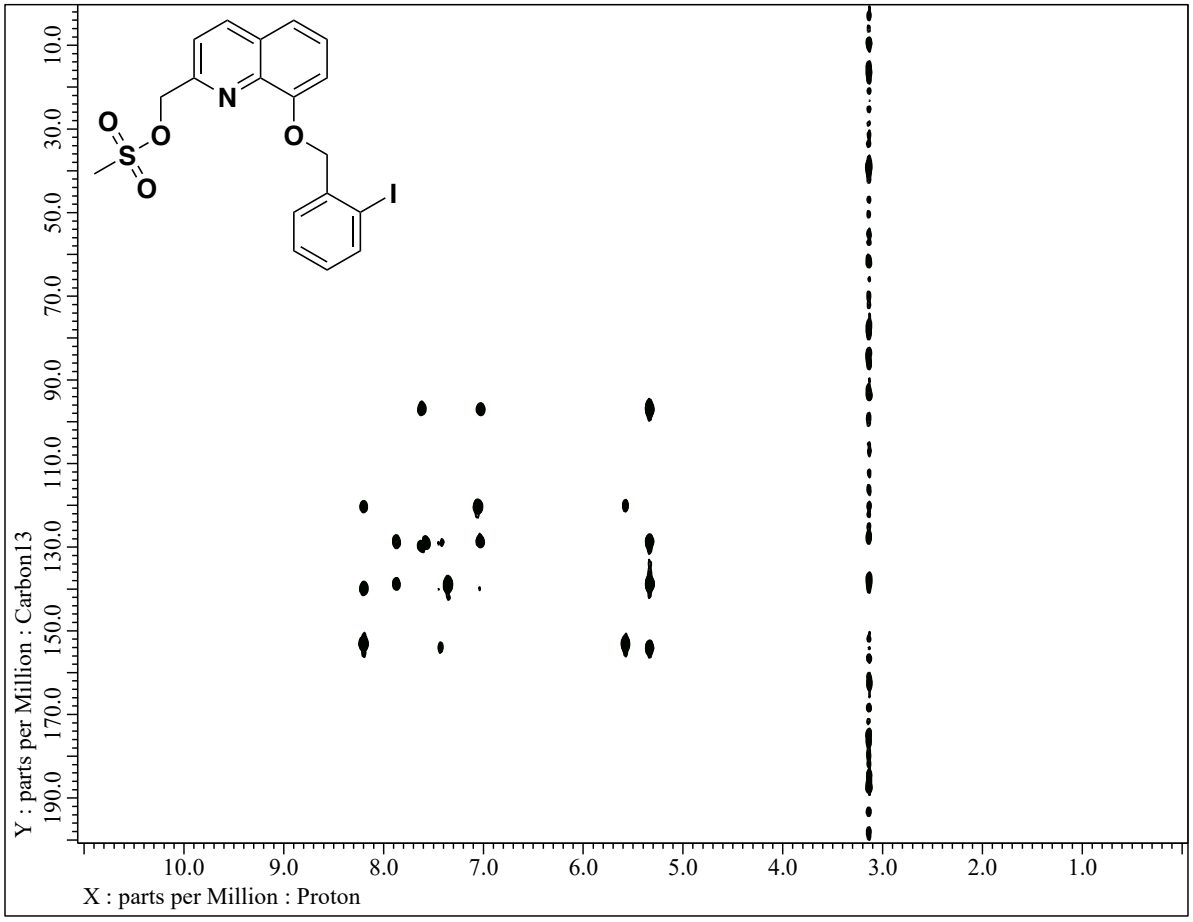
COSY (400 MHz, $CDCl_3$, 298 K) displayed range 2.0 ppm – 9.0 ppm



HMQC (400 MHz, $CDCl_3$, 298 K)

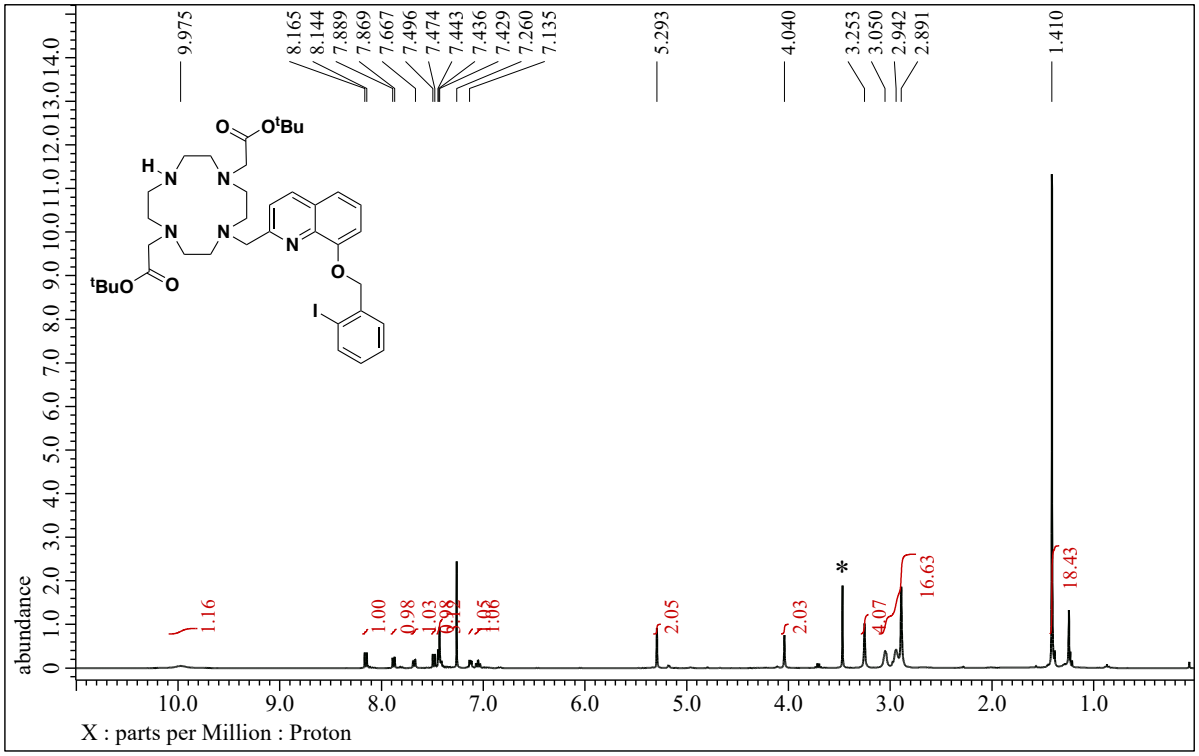


HMBC (400 MHz, $CDCl_3$, 298 K)

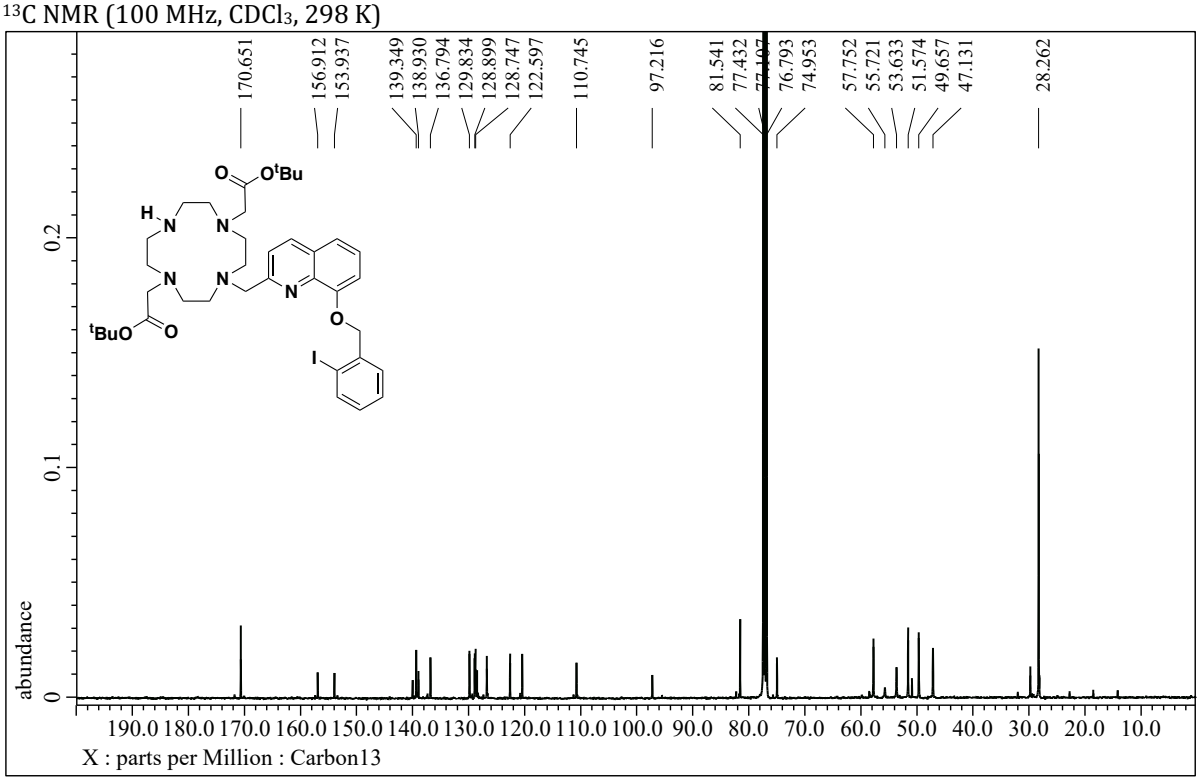


*2,2'-(4-((8-((2-Iodobenzyl)oxy)quinolin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (**5o**)*

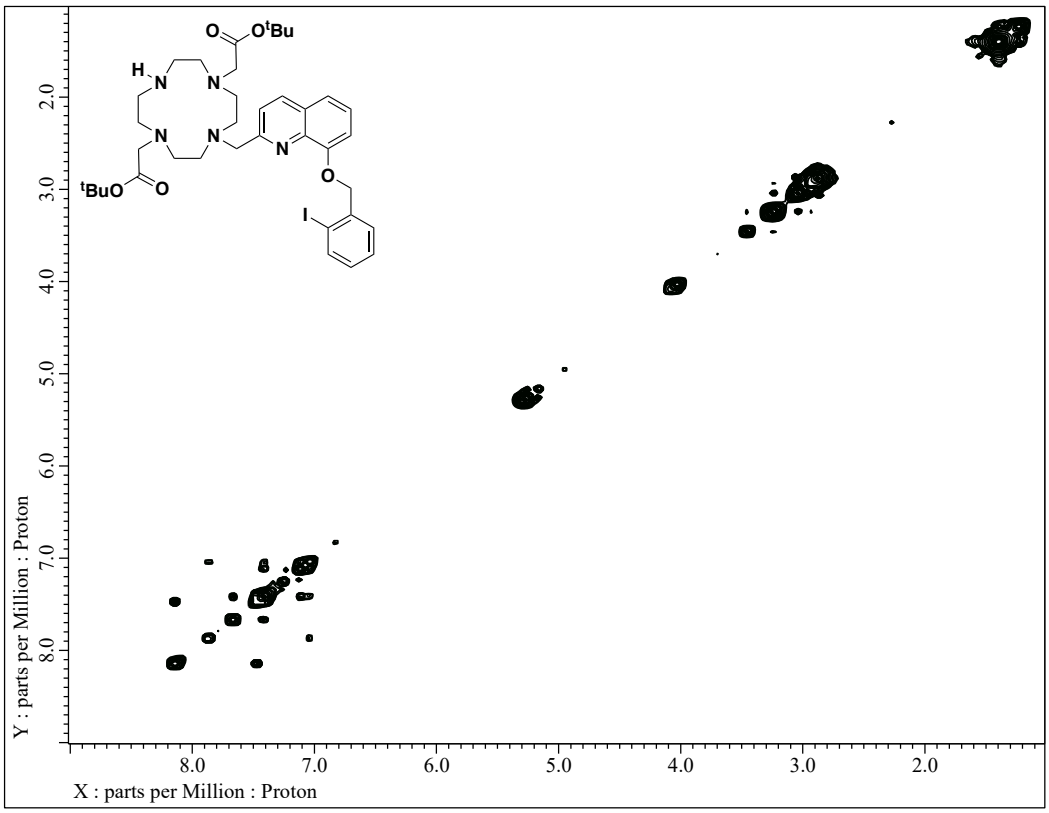
^{1}H NMR (400 MHz, $CDCl_3$, 298 K)



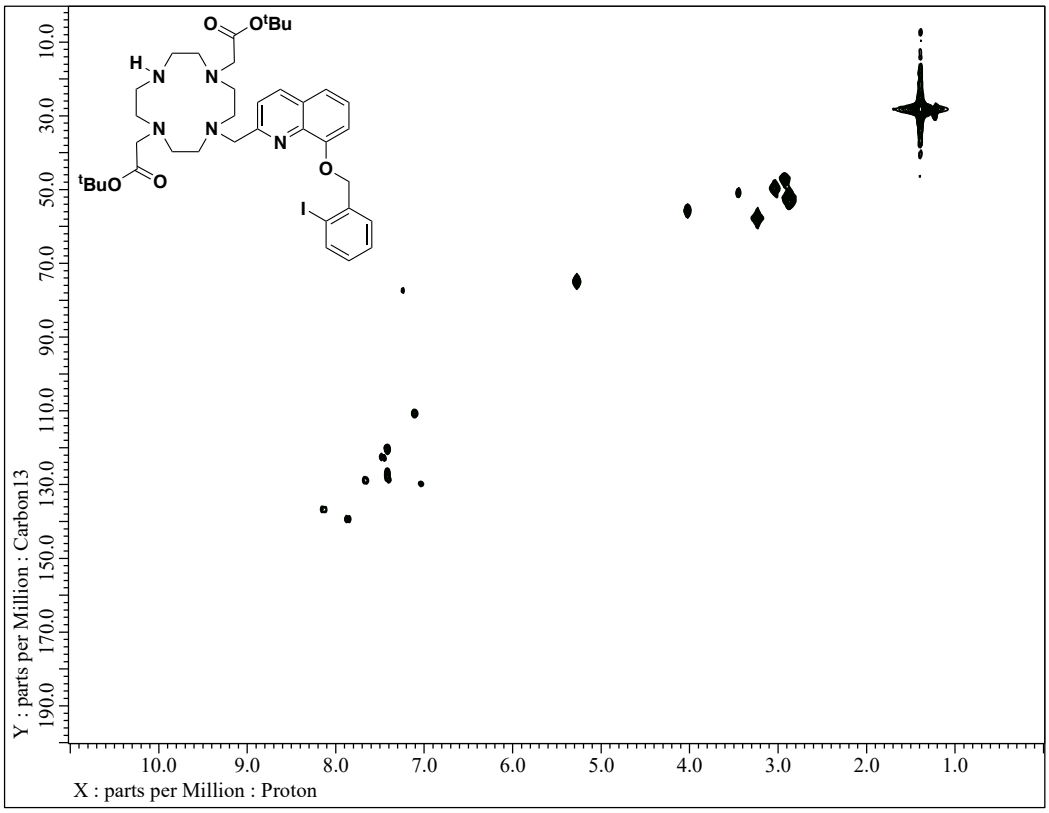
^{13}C NMR (100 MHz, $CDCl_3$, 298 K)



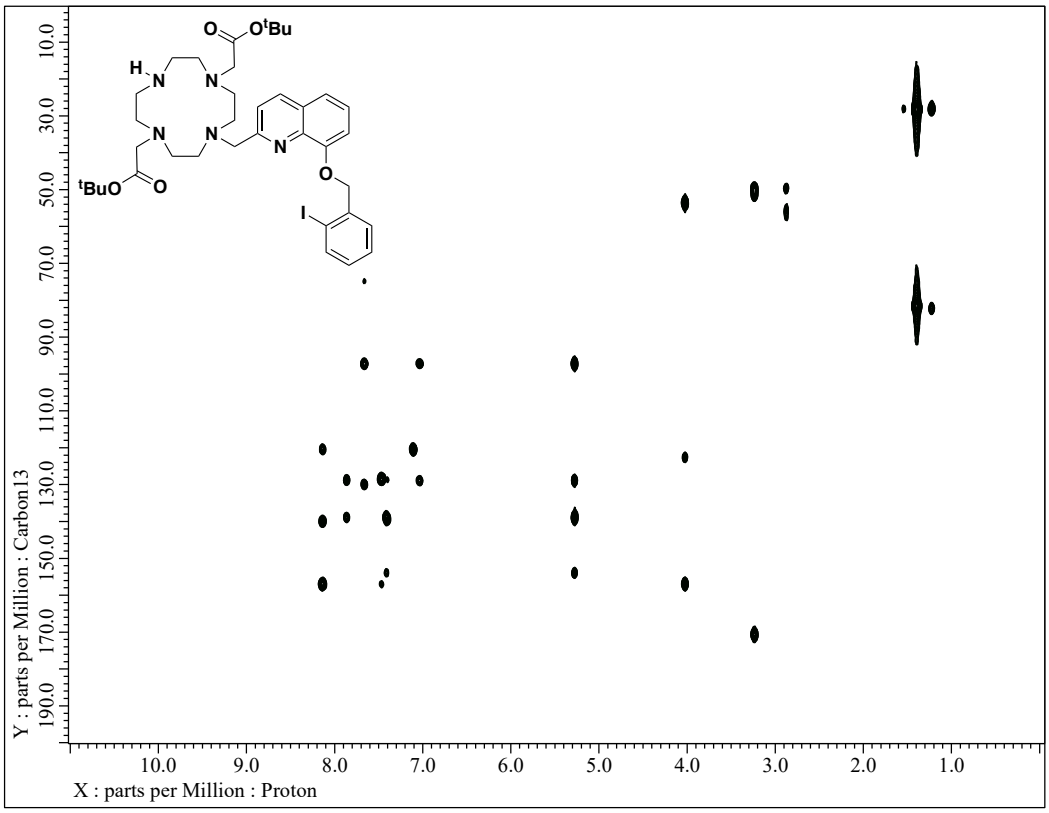
COSY (400 MHz, $CDCl_3$, 298 K) displayed range 1.0 ppm – 9.0 ppm



HMQC (400 MHz, $CDCl_3$, 298 K)

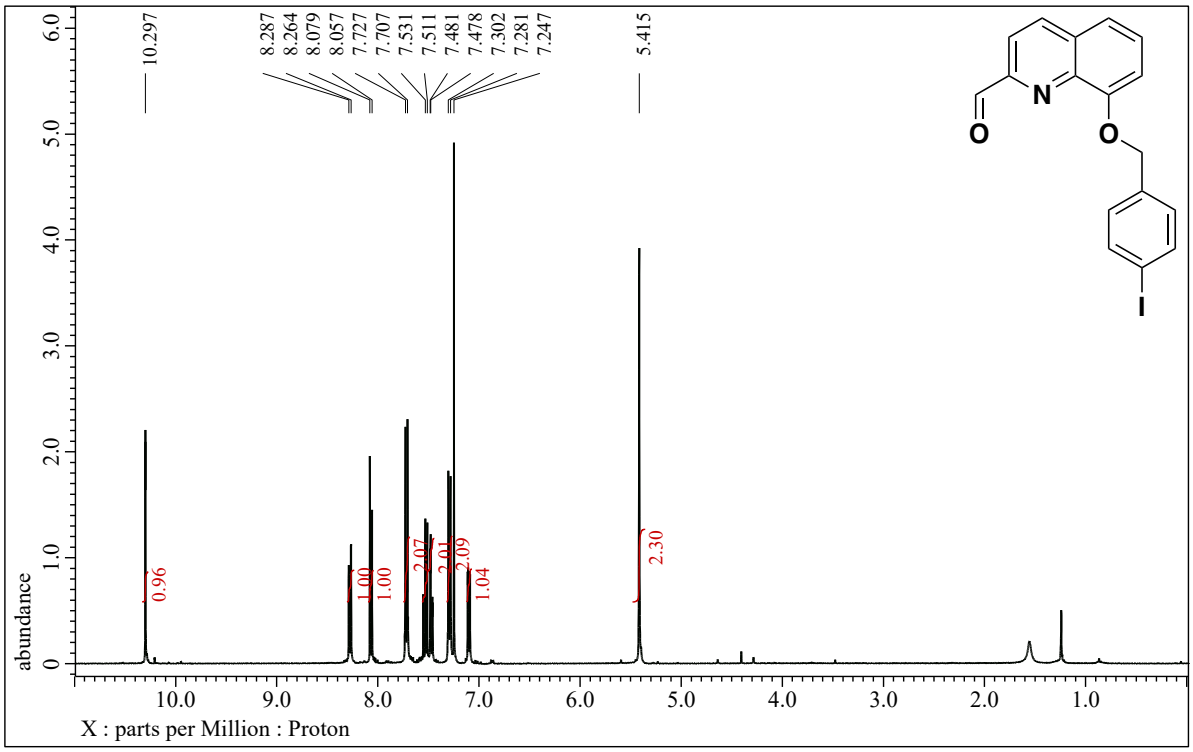


HMBC (400 MHz, $CDCl_3$, 298 K)

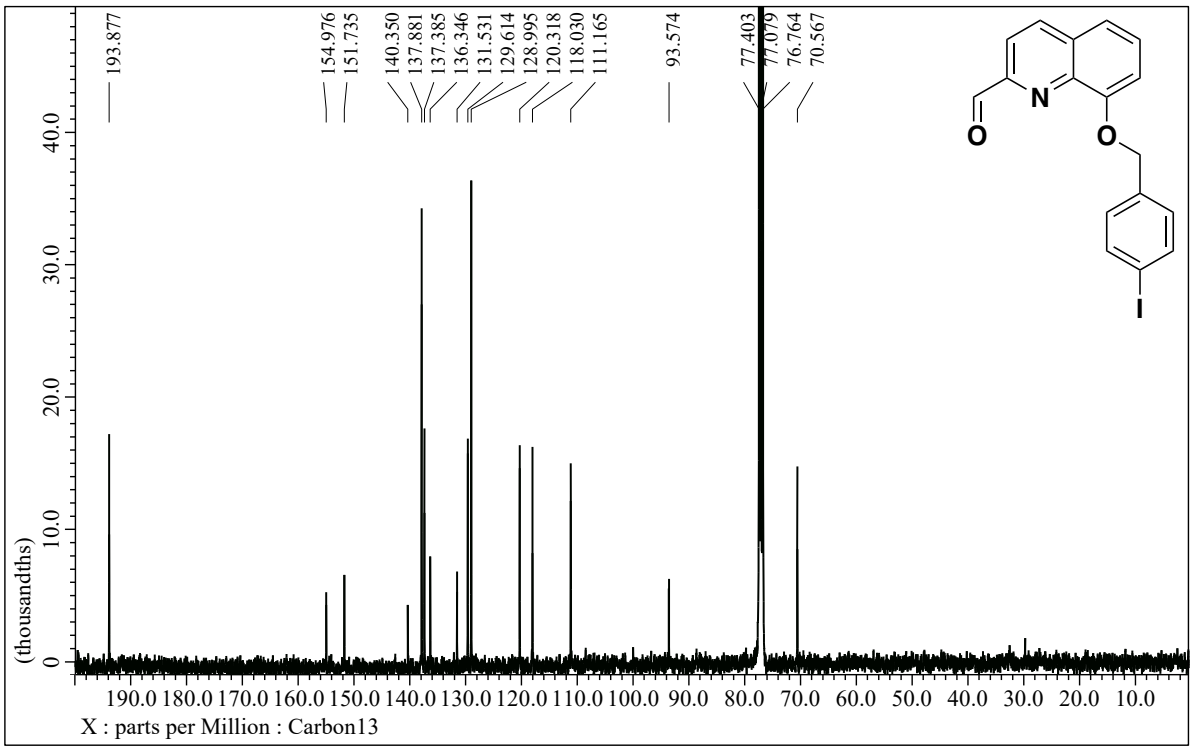


*8-((4-Iodobenzyl)oxy)quinoline-2-carbaldehyde (**2p**)*

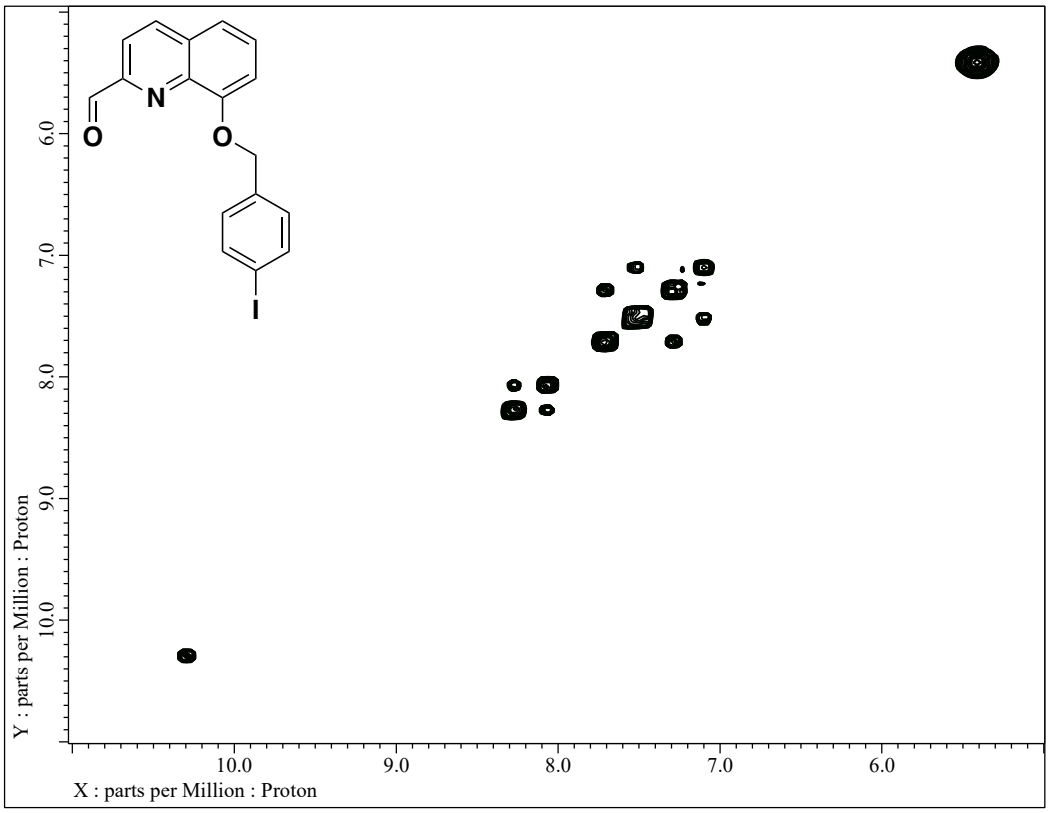
^{1}H NMR (400 MHz, $CDCl_3$, 298 K)



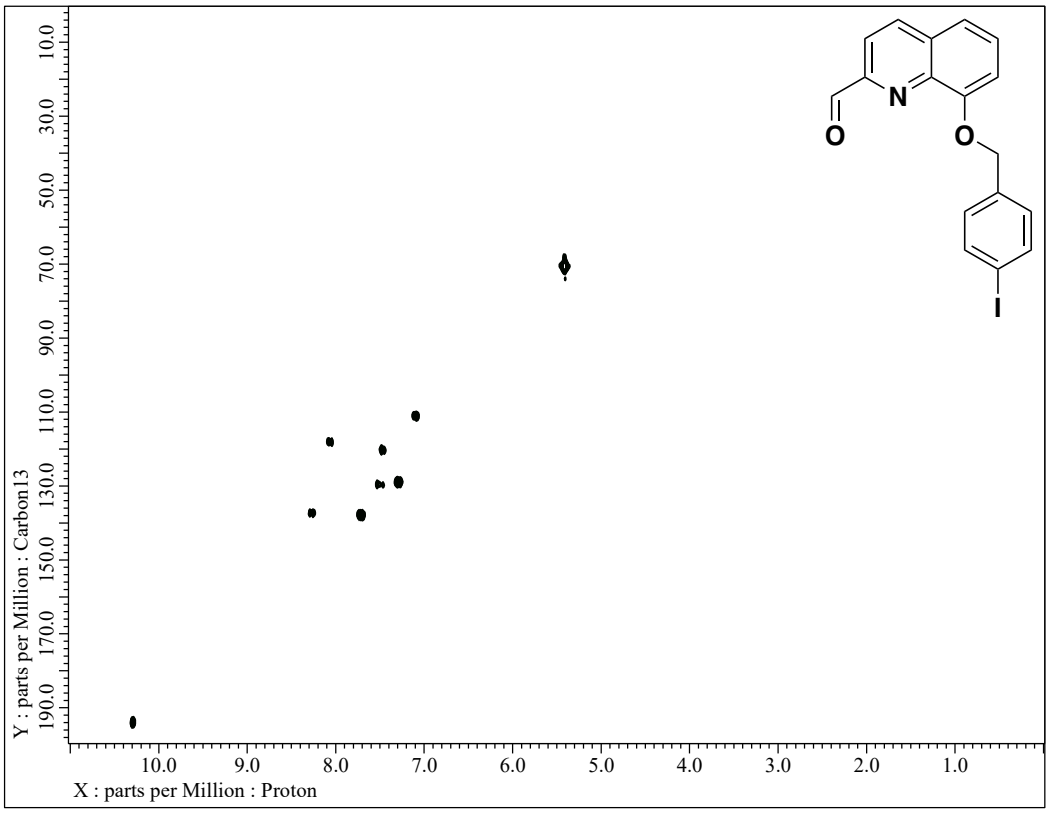
^{13}C NMR (100 MHz, $CDCl_3$, 298 K)



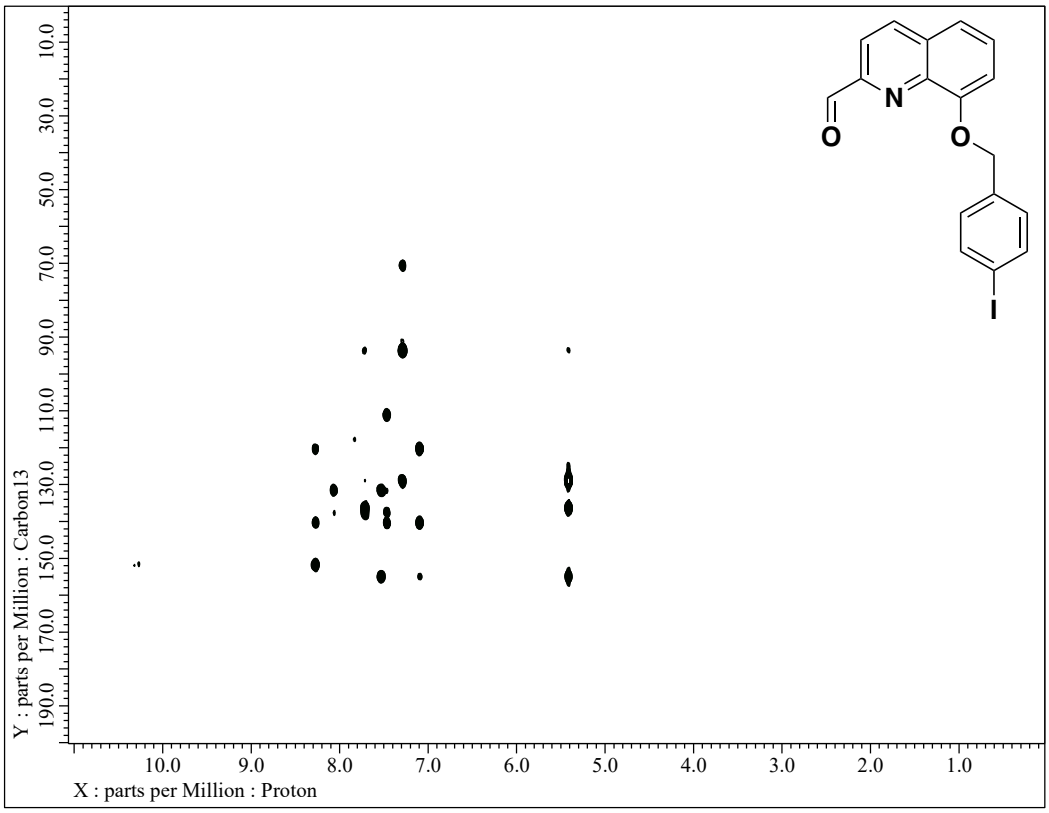
COSY (400 MHz, $CDCl_3$, 298 K) displayed range 5.0 ppm – 11.0 ppm



HMQC (400 MHz, $CDCl_3$, 298 K)

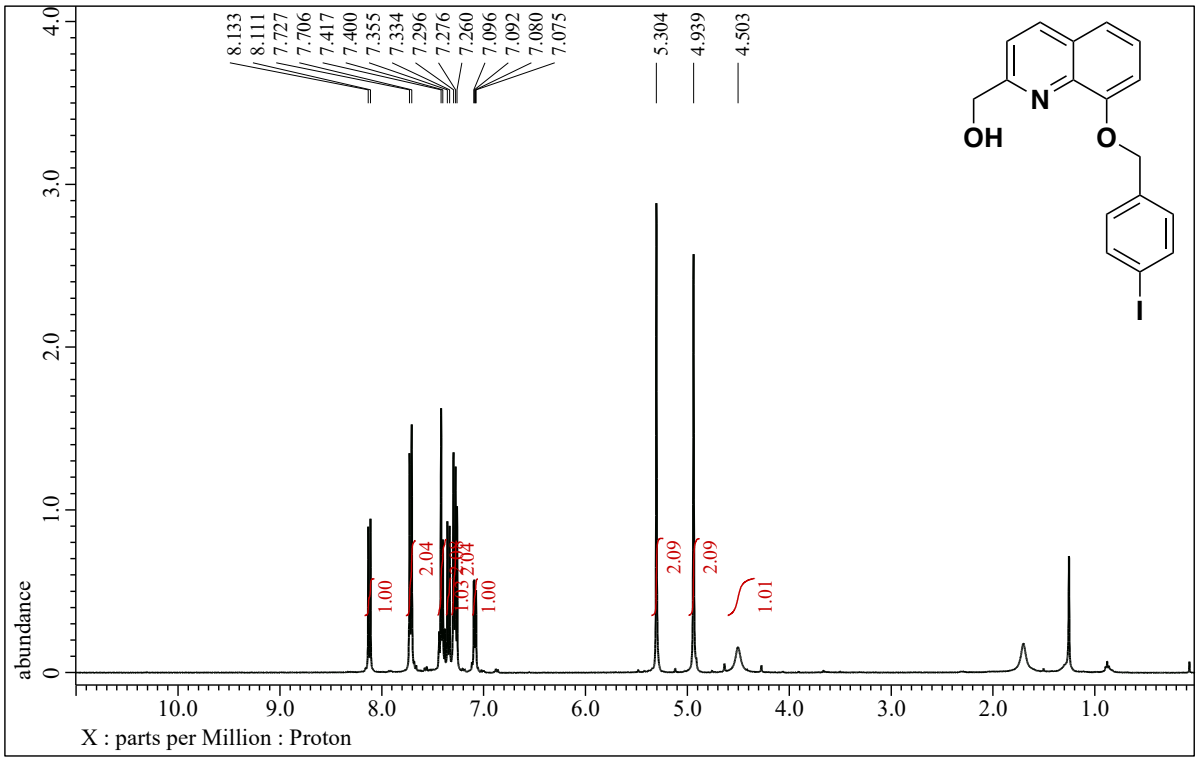


HMBC (400 MHz, $CDCl_3$, 298 K)

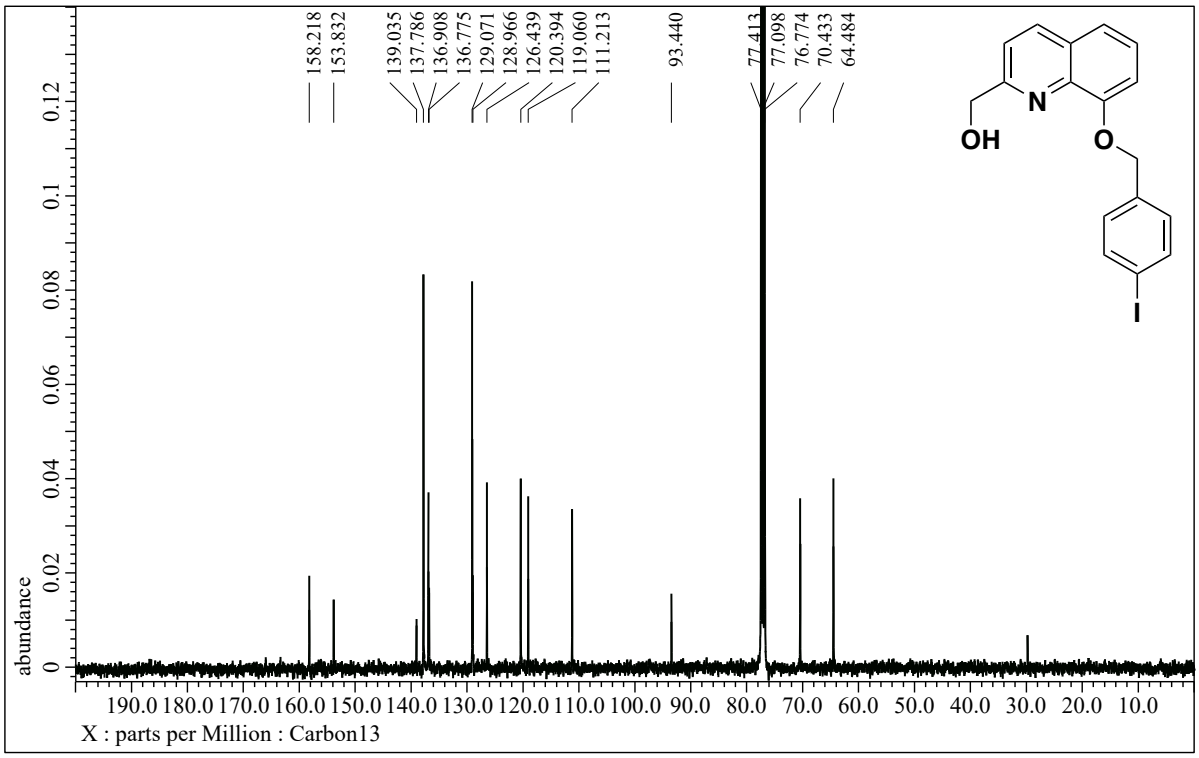


*(8-((4-Iodobenzyl)oxy)quinolin-2-yl)methanol (**3p**)*

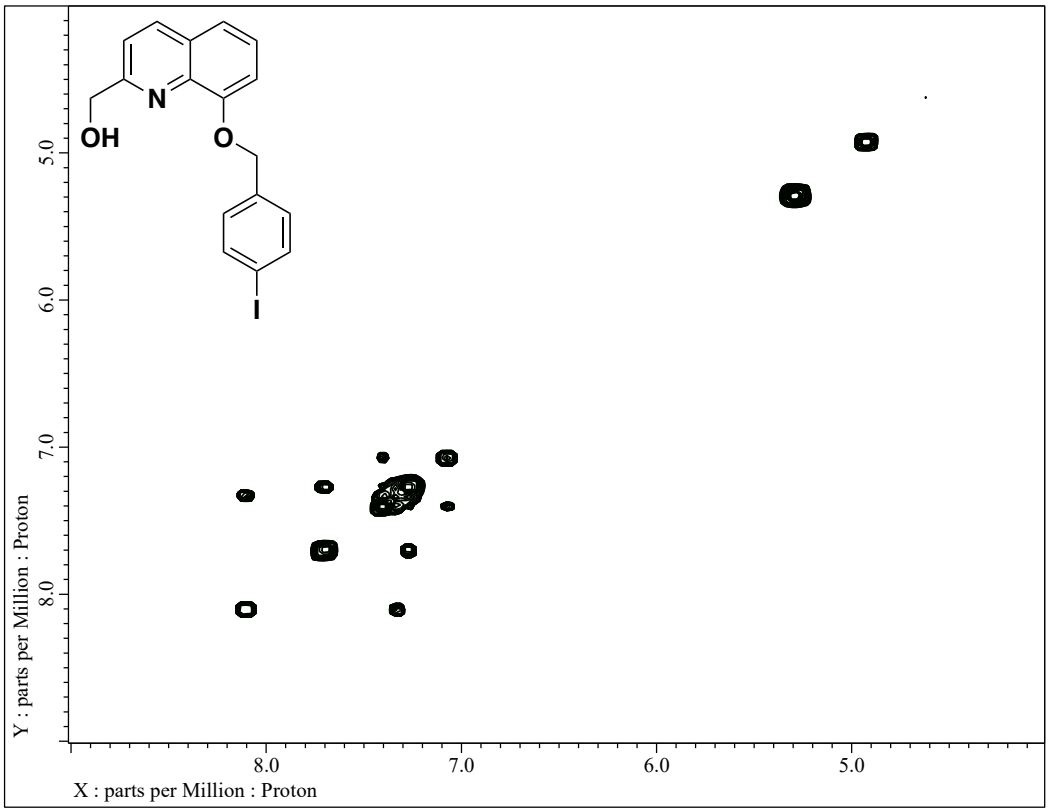
^1H NMR (400 MHz, $CDCl_3$, 298 K)



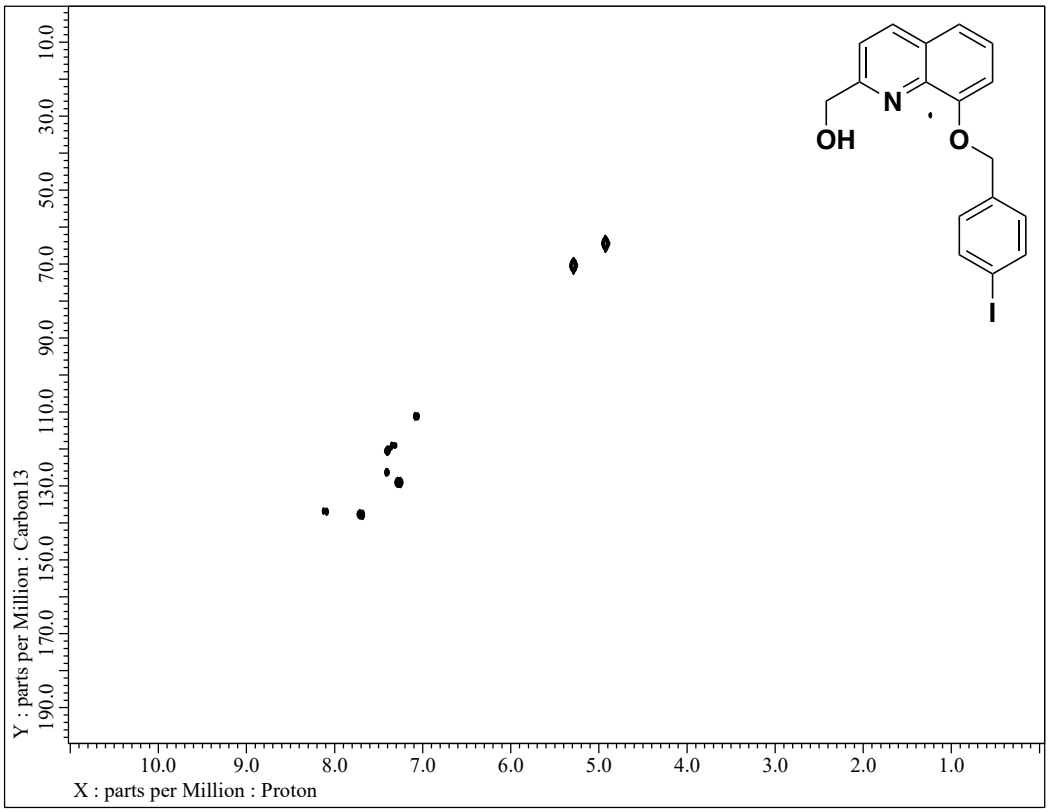
^{13}C NMR (100 MHz, $CDCl_3$, 298 K)



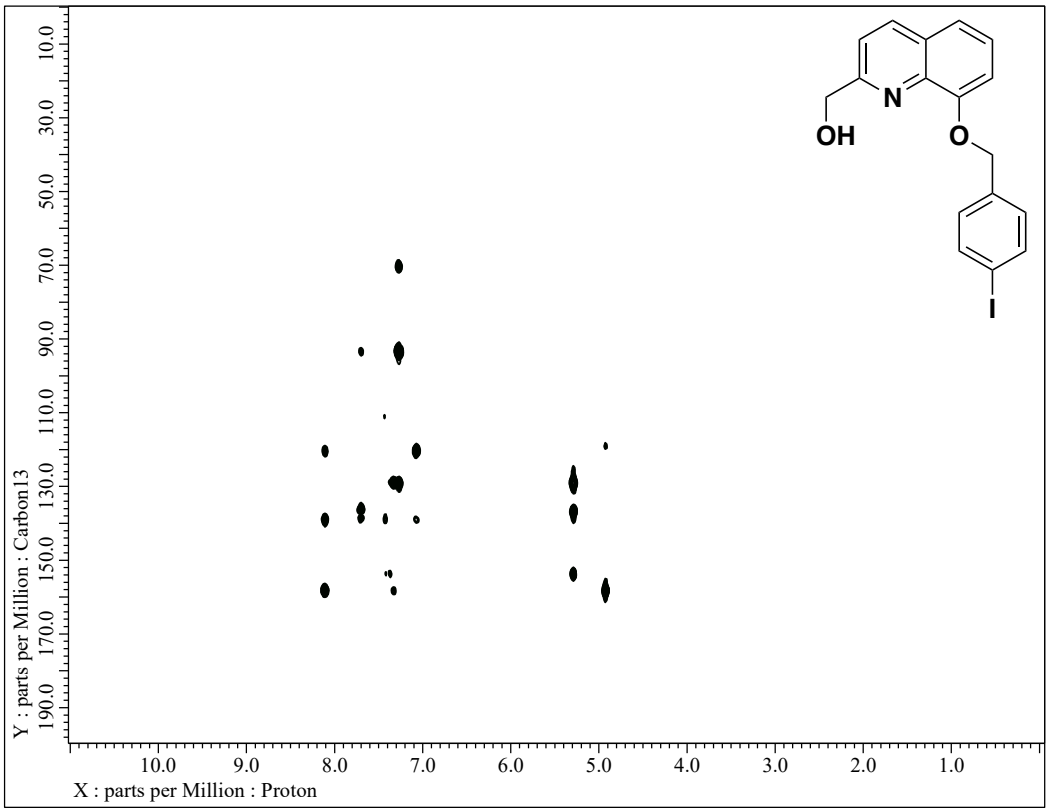
COSY (400 MHz, $CDCl_3$, 298 K) displayed range 4.0 ppm –9.0 ppm



HMQC (400 MHz, $CDCl_3$, 298 K)

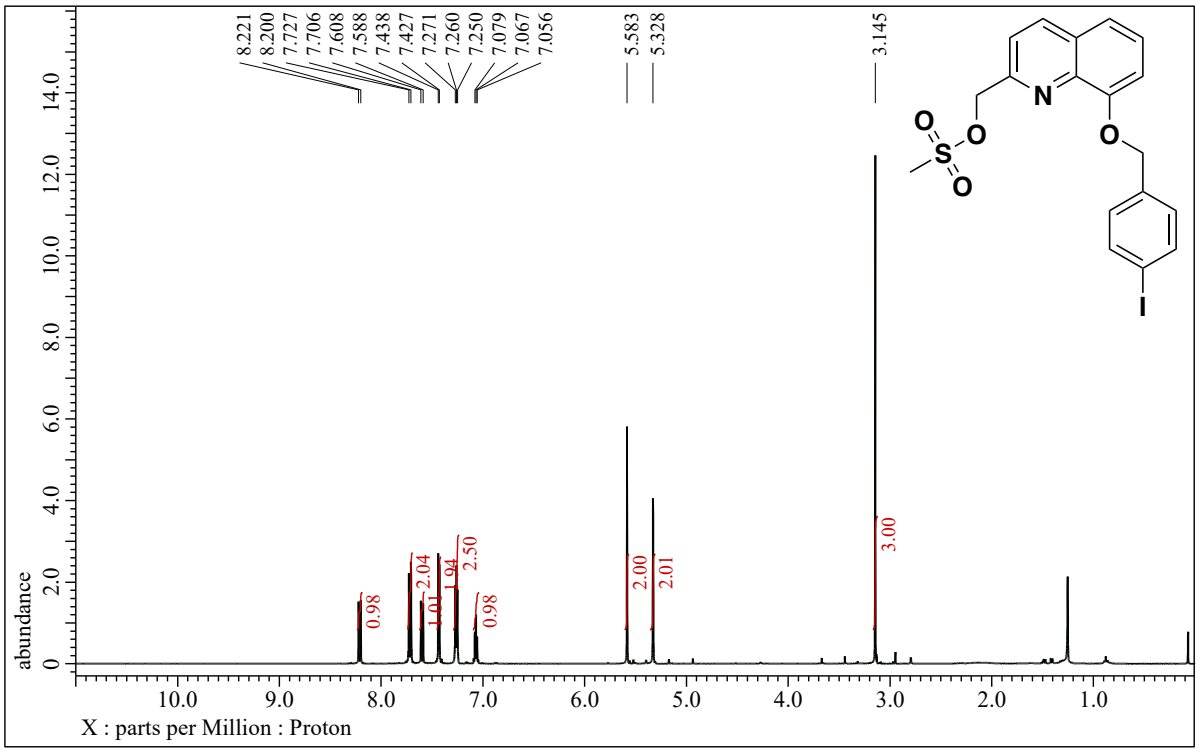


HMBC (400 MHz, $CDCl_3$, 298 K)

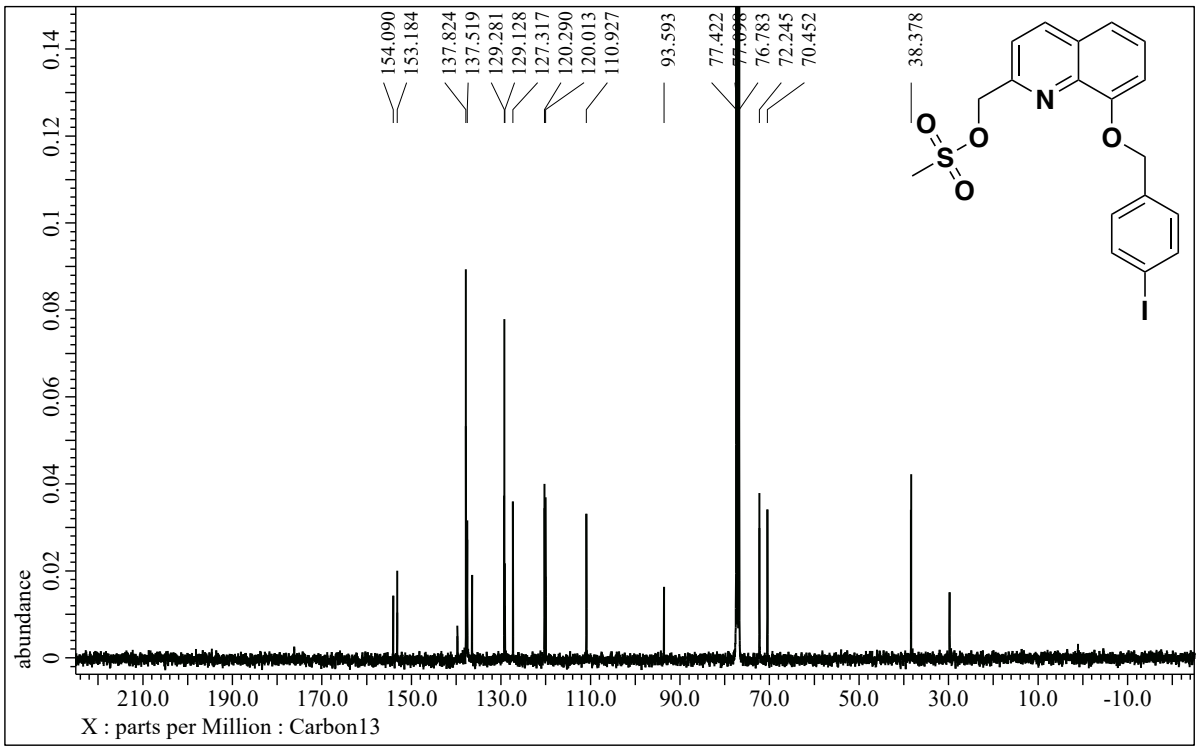


*(8-((4-Iodobenzyl)oxy)quinolin-2-yl)methyl methanesulfonate (**4p**)*

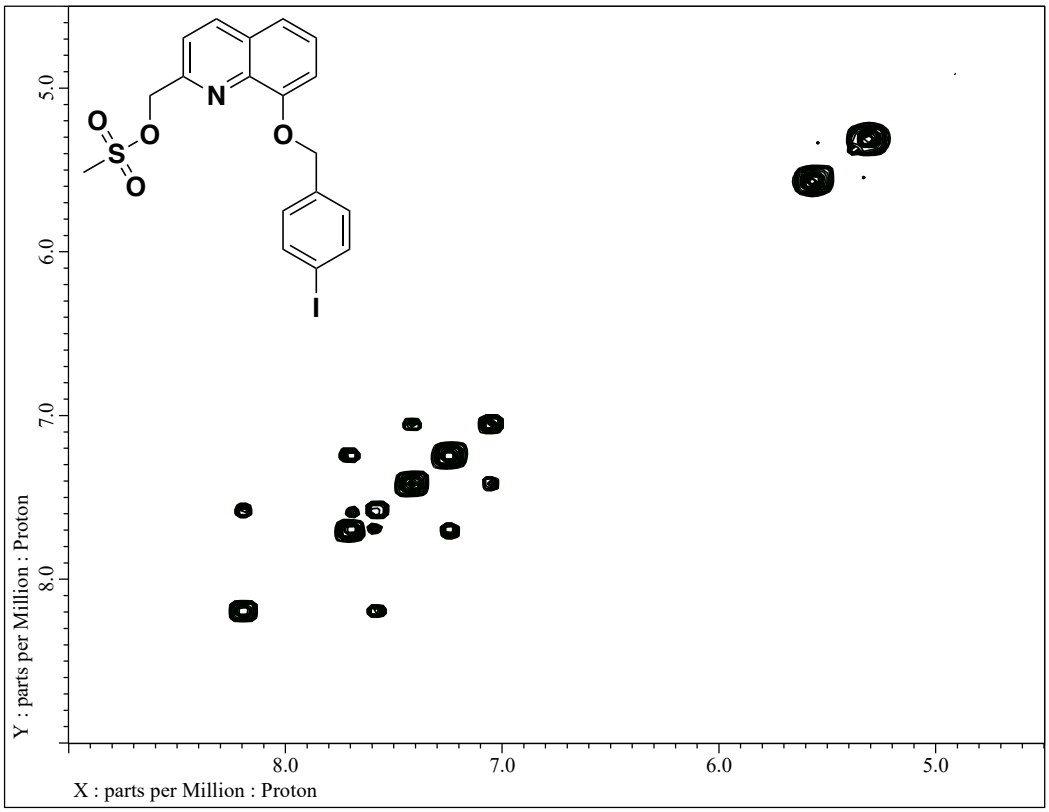
^{1}H NMR (400 MHz, $CDCl_3$, 298 K)



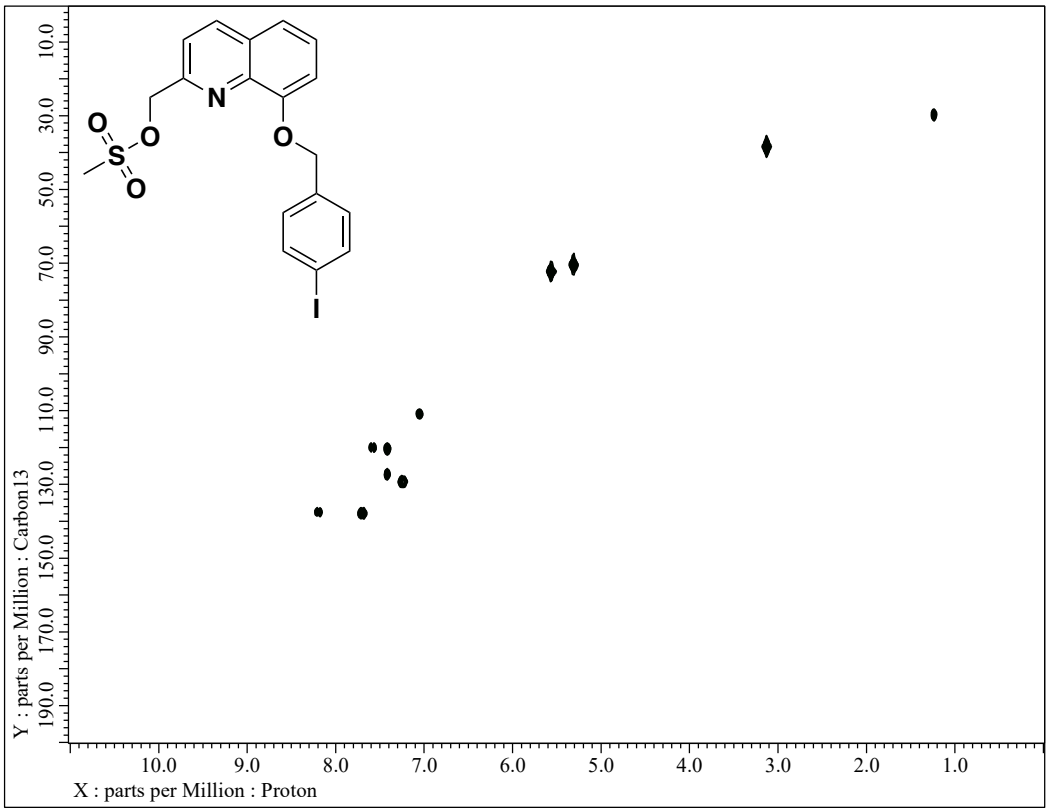
^{13}C NMR (100 MHz, $CDCl_3$, 298 K)



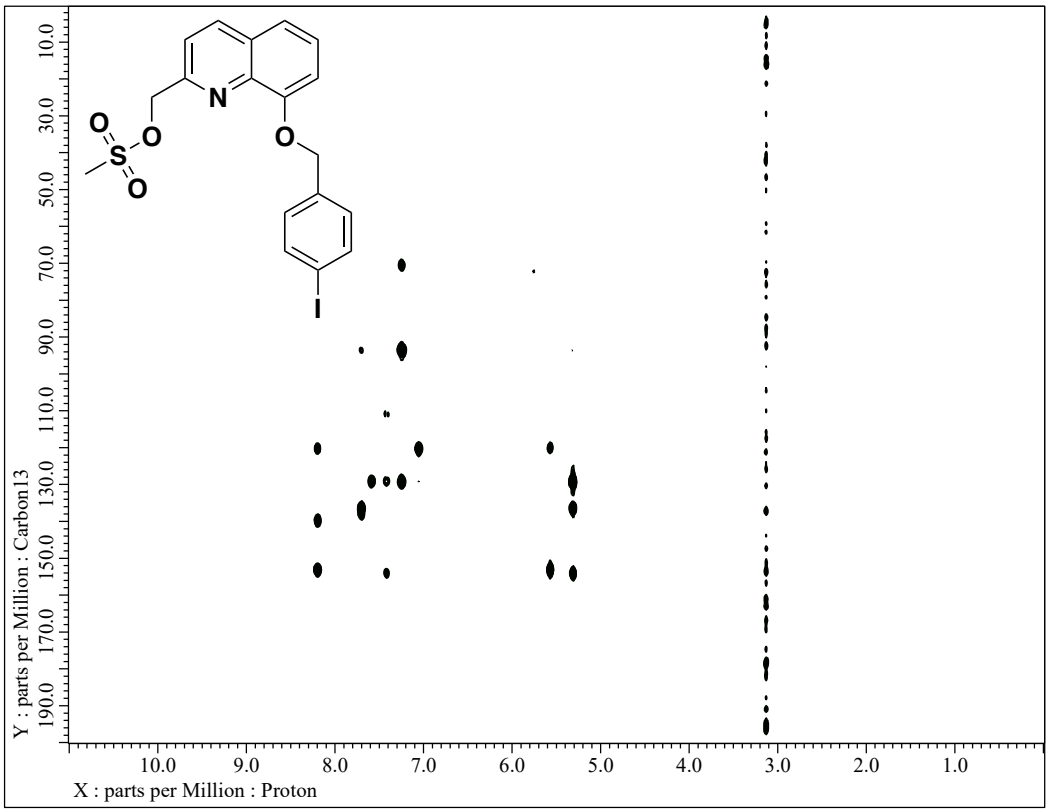
COSY (400 MHz, $CDCl_3$, 298 K) displayed range 4.5 ppm – 9.0 ppm



HMQC (400 MHz, $CDCl_3$, 298 K)

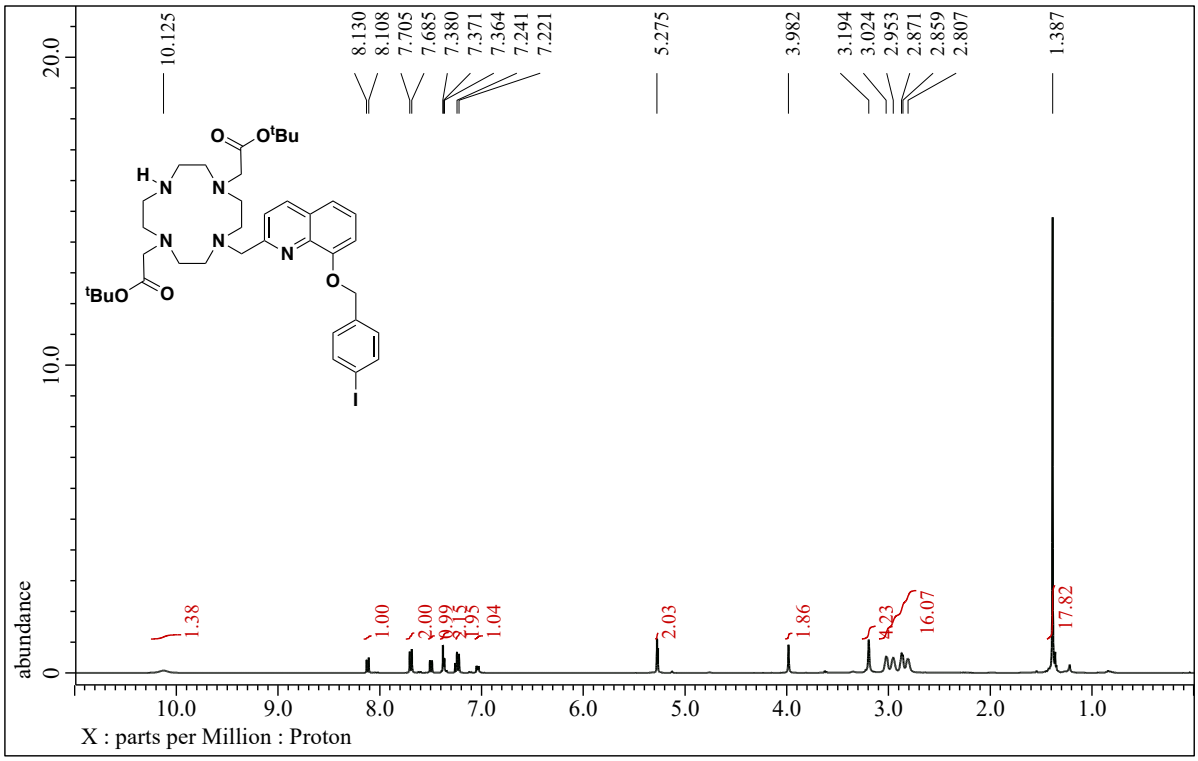


HMBC (400 MHz, $CDCl_3$, 298 K)

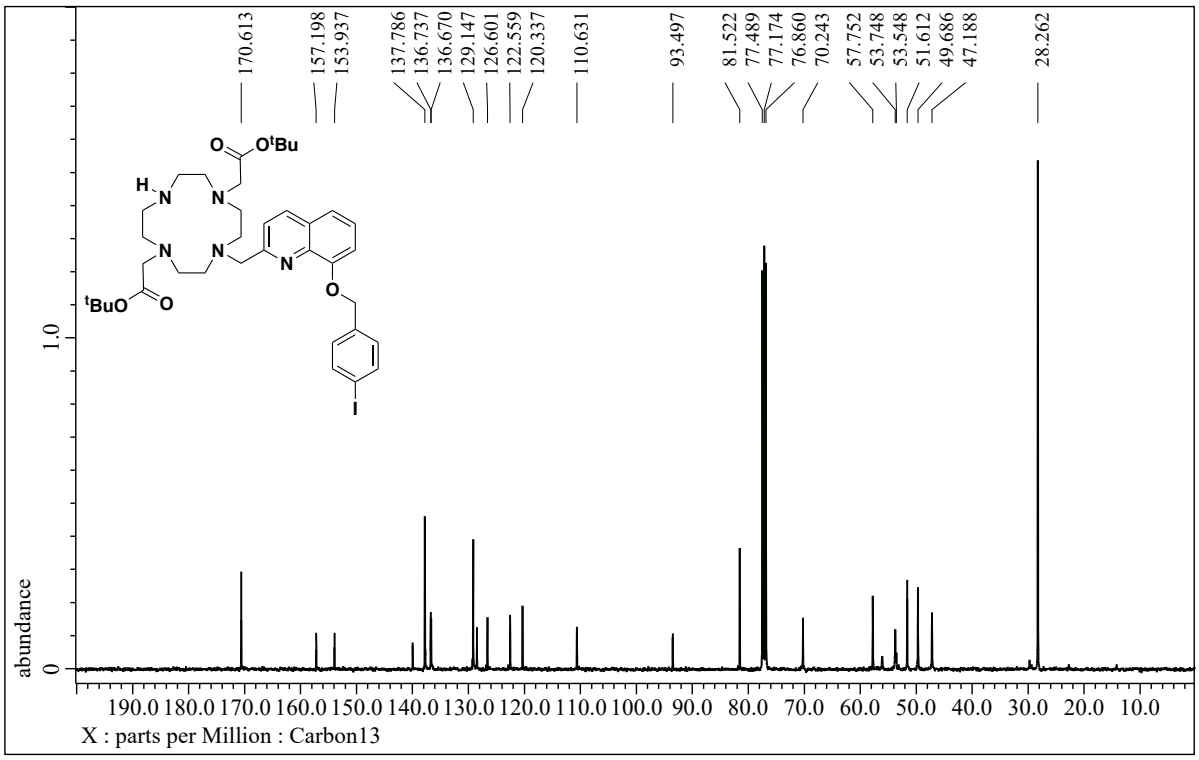


*Di-tert-butyl 2,2'-(4-((8-((4-iodobenzyl)oxy)quinolin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (**5p**)*

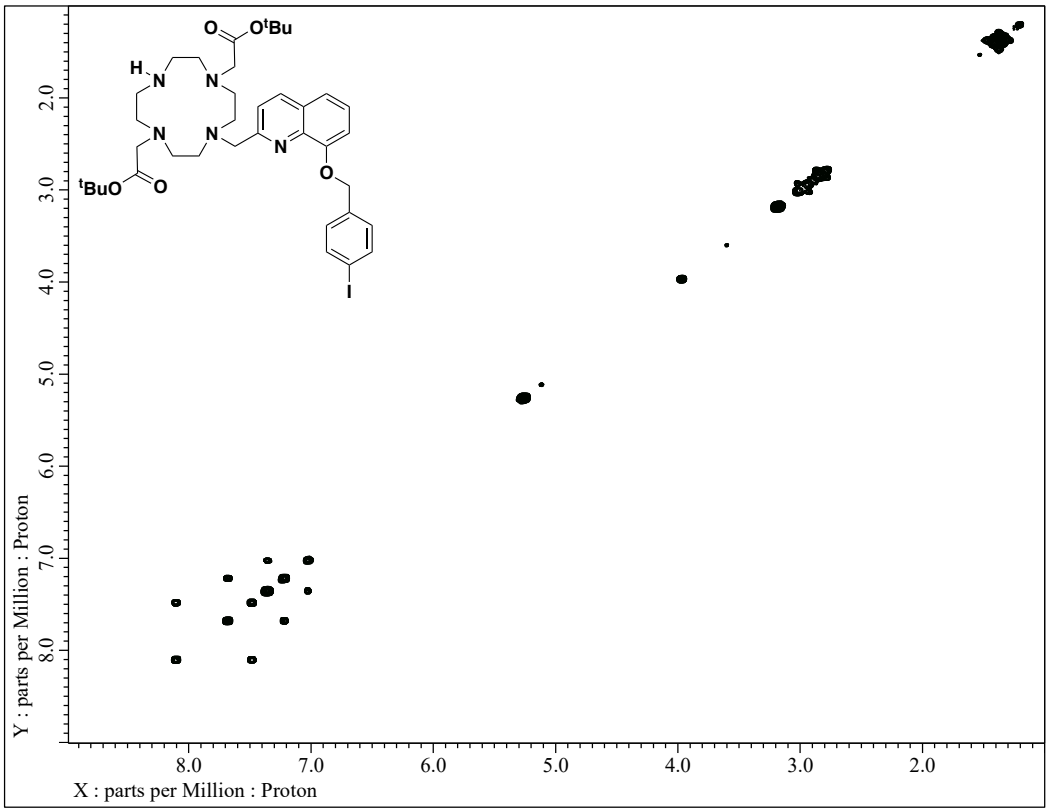
^{1}H NMR (400 MHz, $CDCl_3$, 298 K)



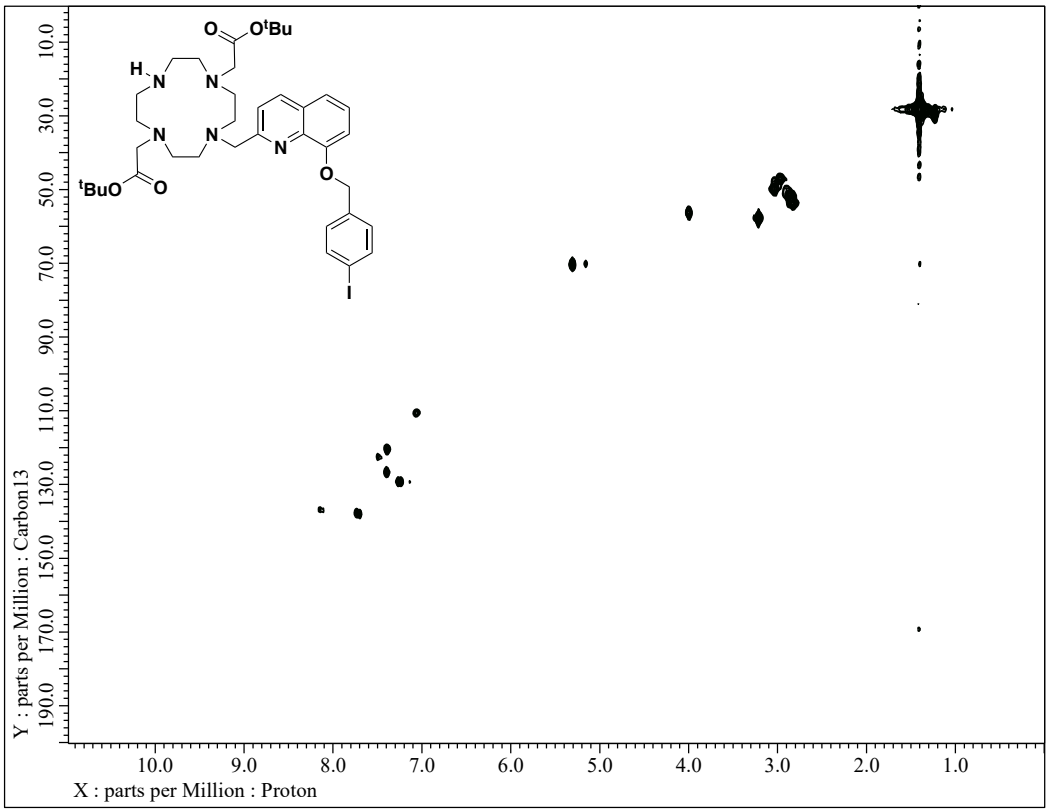
^{13}C NMR (100 MHz, $CDCl_3$, 298 K)



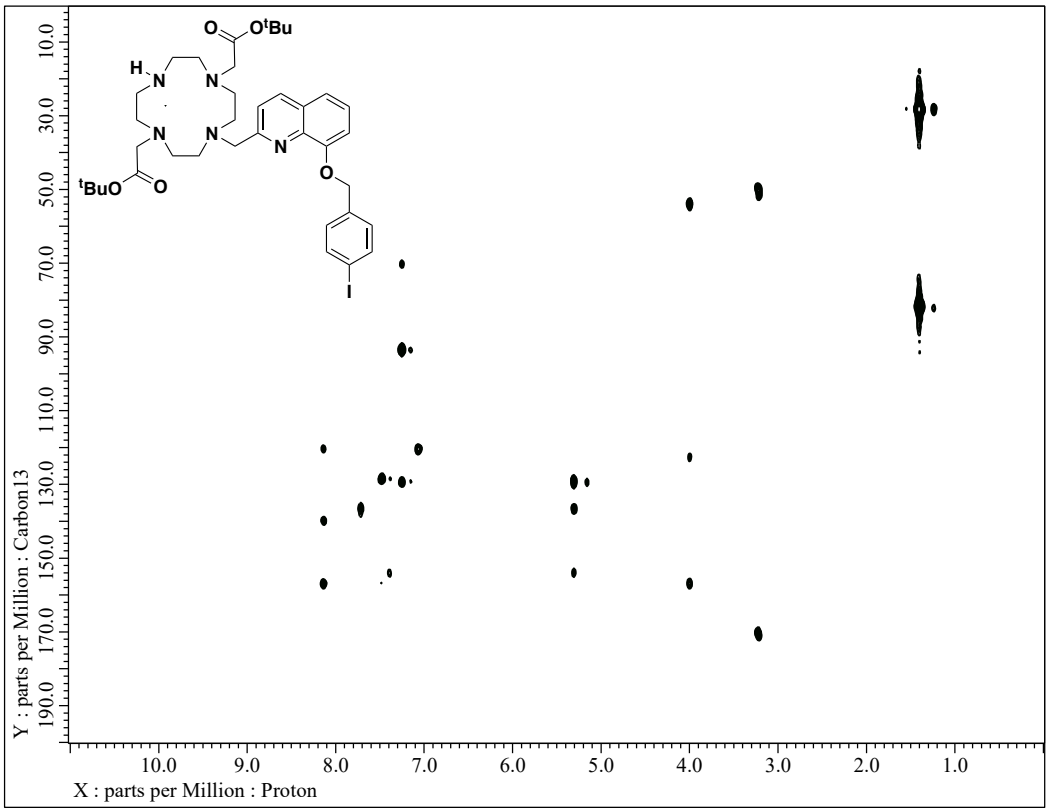
COSY (400 MHz, $CDCl_3$, 298 K) displayed range 1.0 ppm – 9.0 ppm



HMQC (400 MHz, $CDCl_3$, 298 K)



HMBC (400 MHz, $CDCl_3$, 298 K)



References

1 J. Tian, X. Yan, H. Yang and F. Tian, *RSC Adv.*, 2015, **5**, 107012–107019.
2 Z. Kovacs and A. D. Sherry, *J. Chem. Soc., Chem. Commun.*, 1995, **1**, 185–186.
3 K. Suzuki, A. Kobayashi, S. Kaneko, K. Takehira, T. Yoshihara, H. Ishida, Y. Shiina, S. Oishi and S. Tobita, *Phys. Chem. Chem. Phys.*, 2009, **11**, 9850–9860.
4 A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. G. Williams and M. Woods, *J. Chem. Soc., Perkin Trans. 2*, 1999, **2**, 493–503.
5 A. D. Becke, *J. Chem. Phys*, 1993, **98**, 5648–5652.
6 P. C. Hariharan and J. A. Pople, *Theor. Chim. Acta*, 1973, **28**, 213–222.
7 M. Dolg and H. Preuss, *J. Chem. Phys.*, 1989, **90**, 1730–1734.
8 V. Barone and M. Cossi, *J. Phys. Chem. A*, 1998, **102**, 1995–2001.
9 A. v Marenich, C. J. Cramer and D. G. Truhlar, *J. Phys. Chem. B*, 2009, **113**, 6378.
10 S. F. Boys and F. Bernardi, *Mol. Phys.*, 1970, **19**, 553–566.
11 E. Epifanovsky, A. T. B. Gilbert, X. Feng, J. Lee, Y. Mao, N. Mardirossian, P. Pokhilko, A. F. White, M. P. Coons and A. L. Dempwolff, *J. Chem. Phys.*, 2021, **155**, 84801.
12 B. O. Roos, R. Lindh, P.-Å. Malmqvist, V. Veryazov and P.-O. Widmark, *J. Phys. Chem. A*, 2004, **108**, 2851–2858.
13 I. Fdez. Galván, M. Vacher, A. Alavi, C. Angeli, F. Aquilante, J. Autschbach, J. J. Bao, S. I. Bokarev, N. A. Bogdanov and R. K. Carlson, *J. Chem. Theory Comput.*, 2019, **15**, 5925–5964.
14 S. E. Bodman, C. Breen, S. Kirkland, S. Wheeler, E. Robertson, F. Plasser and S. J. Butler, *Chem. Sci.*, 2022, **13**, 3386–3394.