Conformational control of anticancer activity: the application of arene-linked dinuclear ruthenium(II) organometallics

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Experimental

Materials

All commercially purchased materials were used as received. Ruthenium trichloride hydrate was purchased from Precious Metals Online, guanosine 5'-monophosphate disodium salt hydrate and L-histidine were purchased from Sigma, 5'-ATACATCGTACAT-3' was purchased from Microsynth and H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-OH as its trifluoroacetate salt from Bachem. (1S,2S)-(-)-1,2-diphenylethylenediamine (min. 97%) was obtained from Strem Chemicals, (1R,2R)-(+)-1,2-diphenylethylenediamine (98+%) was obtained from Alfa Aesar and *meso*-1,2-diphenylethylenediamine (98%) was purchased from Aldrich. Dichloromethane and diethyl ether were purified and degassed prior to use using a PureSolv solvent purification system (Innovative Technology INC). *N'N*-dimethylformamide (99.8%, Extra Dry, Acroseal®) and acetone (99.8%, Extra Dry, Acroseal®) were obtained from Acros Organics and methanol (anhydrous, 99.8%) was purchased from Sigma-Aldrich. H_2O was obtained from a Milli-Q Integral 5 purification system.

Thin-layer chromatography was carried out on silica plates (Merck 5554), visualised under UV irradiation (254 nm), with iodine staining or using potassium permanganate dip. Where required, compounds were purified using either manual chromatography using silica gel (SiliCycle R12030B) or a Varian 971-FP flash chromatography system using pre-packaged silica gel columns (Luknova).

Instrumentation and methods

NMR

^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker Avance II 400 spectrometer (^{1}H at 400 MHz, ^{13}C at 101 MHz and ^{31}P at 162 MHz). Spectra are referenced internally to residual solvent peaks (D_2O: ^{1}H δ 4.79, ^{13}C δ unreferenced; DMSO-d_6: ^{1}H δ 2.50, ^{13}C δ 39.52); ^{31}P NMR spectra are reported relative to an 85% H_3PO_4 external reference.

Mass Spectrometry

Electrospray-Ionisation MS (ESI-MS) data were acquired on a Q-Tof Ultima mass spectrometer (Waters) operated in the positive ionization mode and fitted with a standard Z-spray ion source equipped with the Lock-Spray™ interface. The samples were diluted in $CH_3CN/H_2O/HCOOH$ (50:49.9:0.1) (~10^{-5}M) and 5 µl was introduced into the mass spectrometer by infusion at a flow rate of 20 µl/min with a solution of $CH_3CN/H_2O/HCOOH$ (50:49.9:0.1). Experimental parameters were set as follows: capillary voltage: 3.5 kV, sample cone: 35 V, source temperature: 80°C, desolvation temperature: 200°C, acquisition window: m/z 300-1500 in 1 s. External calibration was carried out with a solution of phosphoric acid at 0.01% introduced through an orthogonal ESI probe. Data from

the Lock-Spray were used to calculate a correction factor for the mass scale and provide accurate mass information of the analyte. Data were processed using the MassLynx 4.1 software.

Electron-Transfer Dissociation peptide (ETD) fragmentation studies were performed on an ETD-enabled hybrid linear ion trap (LTQ) Orbitrap Elite mass spectrometer (Thermo Scientific, Bremen, Germany) coupled to a Triversa Nanomate (Advion) chip-based electrospray system. The samples were diluted at a final concentration of 10 µM in a solution of $CH_3CN/H_2O/HCOOH$ (50:49.9:0.1) and infused using a spray voltage of 1.6 kV. The automatic gain control (AGC) target was set to $1x10^6$ for full scans in the Orbitrap mass analyzer. ETD experiments used fluoranthene as the reagent anion and the target for fluoranthene anions was set to $5x10^5$. Precursor ions for MS/MS were detected in the Orbitrap mass analyzer at a resolving power of 30,000 (at 400 *m/z*) with an isolation width of 8, and product ions were transferred to the FTMS operated with an AGC of $5x10^4$ over a *m/z* range of 200-3000. The reaction time with the fluoranthene radical anions into the LTQ was set from 50 to 100 ms. A total of 100 scans were averaged for each ETD fragmentation spectra. The Orbitrap FTMS was calibrated for the high mass range, keeping a mass accuracy in the 1-3 ppm level. Data were analyzed manually, with tools available at http://www.chemcalc.org and using ProSightPC 2.0 (Thermo).

Ion Mobility-Mass Spectra (IM-MS) were recorded in ion mobility positive ion resolution mode with a Synapt G2-S quadrupole-time-of-flight HDMS mass spectrometer (Waters MS Technologies, Manchester, UK). A nanoflow source was used with a PicoTip sprayer. The capillary, sampling cone and source offset voltages were set to 1.8 kV, 20 V and 80 V, respectively. The source temperature was set to 60°C. Mass spectral data were acquired over the range m/z 50-1200. The trap, IMS and transfer travelling wave device were operated with travelling wave amplitudes and velocities of 4 V and 311 m/s and 40 V and 550 m/s and 3 V and 191 m/s, respectively. The trap/transfer cells were operated with argon at a pressure of 2.25e-2 mbar. A gas flow of 90 mL/min of nitrogen was introduced as drift gas into the IMS cell to maintain a pressure of 3.0 mbar. Samples diluted at a concentration of 50 µM in H_2O were infused into the mass spectrometer at 0.5 µL/min. The TOF mass analyser was calibrated using the fragment ions of Glu-1 Fibrinopeptide B (EGVNDNEEGFFSAR) (m/z 785.8426). Data were processed using DriftScope v2.6 and MassLynx 4.1 (SCN 901)

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) measurements were performed on an Elan DRC II ICP-MS instrument (Perkin Elmer) equipped with a Meinhard nebulizer and a cyclonic spray chamber. The instrument was tuned using a solution (containing 1 ppb Mg, In, Ce, Ba, Pb, and U) provided by the manufacturer. External Ru calibration solutions were prepared from a single element Ru standard solution (CPI international) at 0.5, 1, 5, 10, 50 and 100 µg/L, utilising identical HNO_3 and H_2O as for the samples.

X-ray crystallography

The data collection of compound **1** was collected at low temperature [140(2) K] using Mo K_α radiation on a mar345dtb system in combination with a Genix Hi-Flux small focus generator (*marµX* system). The data reduction was carried out by automar.[1] The data collections of compounds **2a**, **4a** and **5a** were performed at room temperature using Cu (**2a**, **5a**) or Mo (**4a**) K_α radiation on an Agilent Technologies SuperNova dual system in combination with an Atlas CCD detector. The data reduction was carried out by Crysalis PRO.[2]

The solutions and refinements were performed by SHELX.[3] The crystal structures were refined using full-matrix least-squares based on F^2 with all non hydrogen atoms anisotropically defined. Hydrogen atoms were placed in calculated positions by means of the "riding" model. Disorder problems were encountered during the refinement of complex **2a** and **5a** and treated by the split model. Additional electron density (due to very disordered water molecules) was found in the difference Fourier map of compound **5a** and treated by the SQUEEZE algorithm of PLATON.[4]

Miscellaneous

UV-Vis absorbance spectra were recorded on a JASCO V-550 UV/VIS spectrophotometer (using Spectra Manager software (version 1.53.01)).

Melting points were measured using a Stuart Scientific SMP3 apparatus and are uncorrected.

Elemental analysis was carried out at the EPFL by the microanalytical laboratory using a Thermo Scientific Flash 2000 Organic Elemental Analyzer.

A SpectroMax M5e multi-mode microplate reader was used to record the absorbance of solutions contained in 96-well plates (using SoftMax Pro software (version 6.2.2)).

Synthesis

3-(4-methylcyclohexa-1,4-dien-1-yl)propanoic acid,[5] PTA (1,3,5-triaza-7-phosphaadamantane)[6] and silver oxalate[7] were synthesized according to literature procedures.

Compound 1: $RuCl_3 \bullet xH_2O$ (2.82 g, 13.6 mmol, anhydrous basis) was dissolved in MeOH (10 ml) and the deep red solution allowed to boil dry. This process was repeated then the dark solid was dried under reduced pressure to remove residual traces of MeOH. To the solid was added 3-(4-methylcyclohexa-1,4-dien-1-yl)propanoic acid (5.08 g, 30.6 mmol), followed by the addition of acetone:H_2O (60 ml, 5:1). The resulting dark solution was heated at reflux for 5 h, concentrated to 50 ml under reduced pressure then allowed to cool and stand at room temperature for 18 h. The resulting precipitate was isolated by filtration, washed with diethyl ether (10 ml) then dried under reduced pressure to yield the chlorido-bridged half-sandwich precursor to **1** as a dark red powder (3.65 g, 5.4 mmol, 79%). The product was used in the next step of the reaction without further purification.

The dark red powder was added to a suspension of silver oxalate (4.12 g, 13.6 mmol) in H_2O (300 ml) and the mixture was stirred for 3 h. The resulting suspension was filtered through celite and the yellow filtrate dried under reduced pressure. The residue was then suspended in MeOH (200 ml) followed by the addition of PTA (2.13 g, 13.6 mmol). The yellow suspension was briefly sonicated then stirred at room temperature for 12 h. The yellow solid was then isolated by filtration, washed with cold MeOH (0°C, 50 ml), acetone (20 ml) and ether (20 ml) then dried under reduced pressure to afford **1** as a yellow powder (4.0 g, 7.8 mmol, 72%). For X-ray diffraction studies a quantity of the product was recrystallized by vapour diffusion of acetone into a H_2O/acetone solution of **1** to yield yellow crystals. ^{1}H NMR (D_2O, 400 MHz): δ = 6.02 (d, 2H, J = 6.5 Hz, Ar), 5.92 (d, 2H, J = 6.5 Hz, Ar), 4.64 (s, 6H, PTA), 4.20 (s, 6H, PTA), 3.34 (s, residual MeOH), 2.60-2.73 (m, 4H, 2 x CH_2), 2.07 (s, 3H, -CH_3); ^{31}P{^{1}H} NMR (D_2O, 162 MHz): δ = -32.2; ^{13}C{^{1}H} NMR (D_2O, 101 MHz): δ = 177.1 (1C, -COOH), 166.1 (2C, oxalate C=O), 98.8 (1C, $Ar_{(q)}$), 97.2 (1C, $Ar_{(q)}$), 88.7 (d, 2C, J = 3.5 Hz, Ar), 87.7 (d, 2C, J = 4.0 Hz, Ar), 70.7 (d, 3C, J = 6.5 Hz, PTA), 48.9 (residual MeOH), 48.2 (d, 3C, J = 15 Hz, PTA), 34.1, 27.3 (2C, -CH_2CH_2COOH), 17.3 (1C, -CH_3); HRMS (ES^+) m/z found 512.0546 [M +

H]$^+$ $C_{18}H_{25}N_3O_6PRu$ requires 512.0530; $C_{18}H_{24}N_3O_6PRu$•$\frac{1}{10}CH_3OH$ (%): calcd C 42.32 H 4.79 N 8.18; found C 41.76 H 4.63 N 8.21; mp > 250°C.

Compound 2a: **1** (178 mg, 0.349 mmol) and TBTU (160 mg, 0.498 mmol) were suspended in DMF (0.5 ml) and DIPEA (121 µl, 0.695 mmol) added. The suspension was stirred for 5 min then propylamine (29 µl, 0.353 mmol) was added. The suspension was stirred for 4 h and the reaction mixture was then dried under reduced pressure. To isolate the crude product from the residue the solid was suspended in DCM (5 ml) then passed through a plug of silica gel (DCM to DCM:MeOH (80:20)). Fractions containing **2a** (R_F = 0.38 (TLC), MeOH:H_2O (50:50)) were combined and dried under reduced pressure followed by recrystallization of the solid from DCM and MeOH. **2a** was obtained as a crystalline yellow solid (80 mg, 0.145 mmol, 42%). Crystals suitable for X-ray diffraction were obtained by the slow diffusion of diethyl ether into a methanolic solution of **2a**. ^{1}H NMR (D_2O, 400 MHz): δ = 5.95 (m, 4H, Ar), 4.59 (s, 6H, PTA), 4.18 (s, 6H, PTA), 3.09 (t, 2H, *J* = 7.0 Hz, -C*H*$_2$-NH-CO-), 2.61 (s, 4H, -C*H*$_2$-C*H*$_2$-CO-), 2.08 (s, 3H, Ar-CH_3), 1.42 (m, 2H, -CH_2-C*H*$_2$-CH_3), 0.79 (t, 3H, *J* = 8.0 Hz, -CH_2-CH_2-C*H*$_3$); ^{31}P{^{1}H} NMR (D_2O, 162 MHz): δ = -33.5; ^{13}C{^{1}H} NMR (D_2O, 101 MHz): δ = 173.8 (1C, amide C=O), 166.1 (2C, oxalate C=O), 99.1 (1C, -*C*$_{(q)}$-CH_3), 96.4 (1C, -*C*$_{(q)}$-CH_2-), 88.4 (d, 2C, *J* = 3.5 Hz, 2 x -*C*H$_{(Ar)}$-C-CH_2-), 87.6 (d, 2C, *J* = 4.0 Hz, 2 x -*C*H$_{(Ar)}$-C-CH_3), 70.7 (d, 3C, *J* = 7.0 Hz, PTA), 48.5 (d, 3C, *J* = 15.5 Hz, PTA), 41.2, 35.7, 28.3, 21.7 (4C, 4 x -*C*H_2-), 17.3 (1C, Ar-CH_3), 10.6 (1C, -CH_2*C*H_3); HRMS (ES$^+$) *m*/*z* found 553.1160 [M + H]$^+$ $C_{21}H_{32}N_4O_5PRu$ requires 553.1164; $C_{21}H_{31}N_4O_5PRu$ (%): calcd C 45.73 H 5.67 N 10.16; found C 45.70 H 5.86 N 10.08; mp = 173-176°C (dec.).

Compound 2b: Acetyl chloride (270 µl, 3.8 mmol) was added to anhydrous MeOH (3 ml) at 0°C under an atmosphere of N_2. **2a** (30 mg, 0.054 mmol) was added as a yellow solution in MeOH (0.5 ml) to immediately form a red solution that was stirred for a further 18 h. Diethyl ether was then added to the solution until a solid precipitated which was isolated by centrifugation then dried under reduced pressure to yield the hydrochloride salt of **2b** as a red solid (20 mg, 0.033 mmol, 61%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.90 (t, 1H, *J* = 6.0 Hz, NH), 5.91 (d, 2H, *J* = 6.0 Hz, Ar), 5.84 (d, 2H, *J* = 6.0 Hz, Ar), 4.81 (m, 6H, PTA), 4.28 (s, 6H, PTA), 2.97 (q, 2H, *J* = 6.5 Hz, -CONHC*H*$_2$-), 2.37 (s, 4H, -C*H*$_2$-C*H*$_2$-CO), 1.91 (s, 3H, Ar-CH_3), 1.36 (sextet, 2H, *J* = 7.5 Hz, -CH_2-C*H*$_2$-CH_3), 0.79 (t, 3H, *J* = 7.5 Hz, -CH_2-CH_2-C*H*$_3$); ^{31}P{^{1}H} NMR (DMSO-d_6, 162 MHz): δ = -26.6; ^{13}C{^{1}H} NMR (DMSO-d_6, 101 MHz): δ = 170.9 (1C, -CONH-), 98.3 (2C, Ar$_{(q)}$), 88.9 (d, 2C, *J* = 5.0 Hz, Ar), 88.2 (d, 2C, *J* = 5.0 Hz, Ar), 70.7 (3C, PTA), 48.7 (3C, PTA), 35.7, 28.7, 22.8, 18.4 (4C, 4 x -CH_2-), 11.9 (1C, -CH_2*C*H_3); HRMS (ES$^+$) *m*/*z* found 535.0749 [M + H]$^+$ $C_{19}H_{32}Cl_2N_4OPRu$ requires 535.0734; $C_{19}H_{31}Cl_2N_4OPRu$•1.7 HCl (%): calcd C 38.26 H 5.53 N 9.39; found C 38.24 H 5.65 N 9.18; mp = 164-167°C.

Compound 3a: **1** (200 mg, 0.392 mmol) and TBTU (126 mg, 0.392 mmol) were suspended in DMF (22 ml) and DIPEA (68 µl, 0.390 mmol) added. The resulting yellow suspension was stirred for 5 min and then ethylene diamine (11.8 µl, 0.177 mmol) was added and the mixture was stirred for a further 12 h under an atmosphere of N_2. The resulting solid was collected by filtration, washed with DCM (20 ml), then dried under high vacuum to leave a crude yellow solid (183 mg, 0.175 mmol, 99%). A portion of this solid (120 mg, 0.115 mmol) was further purified by dissolution in the minimum volume of boiling MeOH (250 ml), then filtered, allowed to cool to room temperature to yield a precipitate (38 mg) which was removed by filtration and washed with cold MeOH (2 ml). The filtrate was further concentrated under reduced pressure at 60°C to a volume of 100 ml then allowed to stand at 4°C for 12 h to yield further yellow solid (18 mg), that was isolated by filtration and washed with cold MeOH (2 ml). The combined solid was dried under reduced pressure to yield **3a** as

a fine yellow powder (56 mg, 0.054 mmol, 47%). ^{1}H NMR (D_2O, 400 MHz): δ = 5.95 (m, 8H, Ar), 4.59 (s, 12H, PTA), 4.18 (s, 12H, PTA), 3.26 (s, 4H, -NCH_2CH_2N-), 2.60 (s, 8H, 4 x CH_2), 2.10 (s, 6H, CH_3); $^{31}P\{^{1}H\}$ NMR (D_2O, 162 MHz): δ = -33.5; $^{13}C\{^{1}H\}$ NMR (D_2O, 101 MHz): δ = 174.2 (2C, amide C=O), 166.1 (4C, oxalate C=O), 98.3 (2C, $Ar_{(q)}$), 97.0 (2C, $Ar_{(q)}$), 88.2 (d, 4C, *J* = 3.5 Hz, Ar), 87.7 (d, 4C, *J* = 4.0 Hz, Ar), 70.7 (d, 6C, *J* = 7.0 Hz, PTA), 48.6 (d, 6C, *J* = 15.5 Hz, PTA), 38.6, 35.3, 28.0 (6C, 6 x CH_2), 17.3 (2C, 2 x CH_3); HRMS (ES^+) *m*/*z* found 524.0779 $[M + 2H]^{2+}$ $C_{38}H_{54}N_8O_{10}P_2Ru_2$ requires 524.0773; $C_{38}H_{52}N_8O_{10}P_2Ru_2$•$3H_2O$ (%): calcd C 41.53 H 5.32 N 10.20; found C 41.48 H 5.39 N 10.43; mp = 195-197°C (dec.).

Compound 3b: Acetyl chloride (350 µl, 4.92 mmol) was added to stirring MeOH (3 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred for 20 min followed by the addition of a filtered solution of **3a** (74 mg, 0.071 mmol in MeOH:DCM, 5:1, 3 ml). The orange-yellow solution turned orange-red and a red precipitate formed. The suspension was stirred for 12 h, the precipitated solid was collected by filtration, washed with MeOH (5 ml) then dried under reduced pressure to yield the hydrochloride salt of **3b** as a red powder (52 mg, 0.046 mmol, 65%). ^{1}H NMR (D_2O (100 mM NaCl), 400 MHz): δ = 5.94 (m, 8H, Ar), 4.93 (s, 12H, PTA), 4.48 (s, 12H, PTA), 3.30 (s, 4H, -NCH_2CH_2N-), 2.59 (m, 8H, 4 x CH_2), 2.07 (s, 6H, 2 x CH_3); $^{31}P\{^{1}H\}$ NMR (D_2O (100 mM NaCl), 162 MHz): δ = -28.6; $^{13}C\{^{1}H\}$ NMR (D_2O (100 mM NaCl), 101 MHz): δ = 174.4 (2C, amide C=O), 99.2 (4C, 4 x $Ar_{(q)}$), 88.9 (d, 4C, *J* = 5.0 Hz, Ar), 88.5 (d, 4C, *J* = 5.0 Hz, Ar), 71.1 (d, 6C, *J* = 5.5 Hz, PTA), 48.7 (d, 6C, *J* = 18.0 Hz, PTA), 38.6, 35.5, 28.7 (6C, 6 x CH_2), 17.9 (2C, 2 x CH_3); HRMS (ES^+) *m*/*z* found 1013.0564 $[M + H]^+$ $C_{34}H_{52}N_8O_2P_2Ru_2$ requires 1013.0601; $C_{34}H_{52}Cl_4N_8O_2P_2Ru_2$•3HCl (%): calcd C 36.46 H 4.95 N 10.00; found C 36.34 H 4.97 N 9.88; mp = 210-213°C (dec.).

Compound 4a: **1** (100 mg, 0.196 mmol) and TBTU (63 mg, 0.196 mmol) were suspended in DMF (20 ml) and DIPEA (68 µl, 0.390 mmol) was added. The yellow suspension was stirred for 5 min and meso-1,2-diphenylethylenediamine (21 mg, 0.099 mmol) was added. The reaction mixture was stirred for a further 3 h under an atmosphere of N_2 then filtered to isolate a yellow solid, which was washed with DMF (5 ml) then acetone (10 ml), and then dried under high vacuum. The solid was suspended in boiling MeOH (50 ml) then filtered. The yellow solution was diluted by the addition of diethyl ether (20 ml) then allowed to stand at 4°C for 12 h. The resulting semi-crystalline material was collected by filtration then dried under reduced pressure to yield **4a** as a yellow powder (35 mg, 0.029 mmol, 29%). For X-ray diffraction studies a quantity of the product was recrystallized by vapour diffusion of acetone into a H_2O/acetone solution of **4a** to yield yellow crystals. ^{1}H NMR (D_2O, 400 MHz): δ = 7.36-7.48 (m, 10H, linker Ar), 5.70 (d, 2H, *J* = 6.0 Hz, Ru-Ar), 5.56 (d, 2H, *J* = 6.0 Hz, Ru-Ar), 5.53 (d, 2H, *J* = 6.0 Hz, Ru-Ar), 5.16 (s, 2H, linker CH), 5.08 (d, 2H, *J* = 6.5 Hz, Ru-Ar), 4.50–4.58 (m, 12H, PTA), 4.06 (s, 12H, PTA), 2.37-2.15 (m, 8H, 2 x -CH_2-CH_2-CO-), 1.98 (s, 6H, 2 x CH_3); $^{31}P\{^{1}H\}$ NMR (D_2O, 162 MHz): δ = -33.4; $^{13}C\{^{1}H\}$ NMR (D_2O, 101 MHz): δ = 172.5 (2C, amide CO), 165.9 (4C, oxalate CO), 139.1 (2C, $Ar_{(q)}$), 129.0, 128.4, 127.5 (10C, linker Ar), 97.5 (2C, $Ar_{(q)}$), 95.5 (2C, $Ar_{(q)}$), 89.2 (m, 4C, Ru-Ar), 86.6 (d, 2C, *J* = 3.0 Hz, arene CH), 86.5 (d, 2C, *J* = 2.0 Hz, arene CH), 70.6 (d, 6C, *J* = 7.0 Hz, PTA), 56.4 (2C, linker CH), 48.5 (d, 6C, *J* = 15.5 Hz, PTA), 35.4 (2C, 2 x Ar-CH_2-*C*H_2-), 28.2 (2C, 2 x Ar-*C*H_2-CH_2-), 17.3 (2C, 2 x CH_3); HRMS (ES^+) *m*/*z* found 600.1094 $[M + 2H]^{2+}$ $C_{50}H_{62}N_8O_{10}P_2Ru_2$ requires 600.1089; $C_{50}H_{60}N_8O_{10}P_2Ru_2$•$3H_2O$ (%): calcd C 48.00 H 5.32 N 8.96; found C 48.14 H 4.94 N 8.91; mp = 242-245°C (dec.).

Compound 4b: Acetyl chloride (1 ml, 14.06 mmol) was added to anhydrous MeOH (15 ml) at 0°C under an atmosphere of N_2. **4a** (70 mg, 0.058 mmol) was then added as a solution in MeOH (10 ml) and DCM (10 ml), the solution rapidly turned red followed by the slow precipitation of a red solid. The mixture was stirred for 18 h then the solid collected by centrifugation and washed with MeOH

(10 ml) to yield the hydrochloride salt of **4b** as a red powder (60 mg, 0.046 mmol, 79%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.39 (d, 2H, J = 7.5 Hz, 2 x NH), 7.33-7.38 and 7.18-7.28 (m, 10H, linker Ar), 5.68-5.76 and 5.59-5.63 (m, 8H, Ru-Ar), 5.13 (m, 2H, 2 x CH), 4.66-4.88 (m, 12H, PTA), 4.22 (s, 12H, PTA), 2.03-2.22 (m, 8H, 2 x -CH_2-CH_2-CO-), 1.88 (s, 6H, 2 x CH_3); ^{31}P{^{1}H} NMR (DMSO-d_6, 162 MHz): δ = -27.1; ^{13}C{^{1}H} NMR (DMSO-d_6, 101 MHz): δ = 169.2 (2C, amide C=O), 141.0 (2C, $Ar_{(q)}$), 127.8, 127.6, 126.9 (10C, linker Ar), 97.7 (2C, Ru-$Ar_{(q)}$), 97.3 (2C, Ru-$Ar_{(q)}$), 88.3 (d, 2C, J = 5.0 Hz, Ru-Ar), 88.0 (d, 2C, J = 4.0 Hz, Ru-Ar), 87.6 (d, 2C, J = 6.0 Hz, Ru-Ar), 87.4 (d, 2C, J = 4.0 Hz, Ru-Ar), 70.4 (6C, PTA), 55.4 (2C, 2 x CH), 48.4 (br, 6C, PTA), 35.1, 27.9 (4C, 4 x -CH_2-), 17.9 (2C, 2 x CH_3); HRMS (ES$^+$) m/z found 1165.1213 $[M + H]^+$ $C_{46}H_{61}Cl_4N_8O_2P_2Ru_2$ requires 1165.1232; $C_{46}H_{60}Cl_4N_8O_2P_2Ru_2$•4HCl (%): calcd C 42.21 H 4.93 N 8.56; found C 42.11 H 4.84 N 8.54; mp = 223-226°C (dec.).

Compound 5a: **1** (200 mg, 0.392 mmol) and TBTU (126 mg, 0.392 mmol) were suspended in DMF (1 ml) and DIPEA (68 µl, 0.390 mmol) was added. The yellow suspension was stirred for 5 min followed by the addition of (1*S*,2*S*)-(-)-1,2-diphenylethylenediamine (37 mg, 0.174 mmol). The reaction mixture was stirred for a further 1.5 h under an atmosphere of N_2, filtered to remove unreacted **1**, then washed with cold DMF (2 ml). Acetone (20 ml) was added to the combined filtrates to yield a precipitate which was diluted by the addition of MeCN (5 ml), heated to 70°C then filtered to isolate a yellow solid which was washed with acetone (5 ml) then dried under high vacuum. The yellow solid was dissolved in boiling MeOH (100 ml), filtered and then concentrated to a volume of 5 ml, followed by the addition of acetone until precipitation began (approx. 0.5 ml). The suspension was left to stand at 4°C to produce a semi-crystalline precipitate which was isolated by decanting the solvent then washed with acetone (3 ml) and dried under reduced pressure to yield **5a** as a yellow-orange powder (40 mg, 0.033 mmol, 19%). For X-ray diffraction studies a quantity of the product was recrystallized by vapour diffusion of acetone into a H_2O/acetone solution of **5a** to yield yellow crystals. ^{1}H NMR (D_2O, 400 MHz): δ = 7.28-7.10 (m, 10H, linker Ar), 5.74 (d, 2H, J = 6.5 Hz, Ru-Ar), 5.64 (d, 2H, J = 6.0 Hz, Ru-Ar), 5.44 (d, 2H, J = 6.0 Hz, Ru-Ar), 5.32 (d, 2H, J = 6.0 Hz, Ru-Ar), 5.22 (s, 2H, 2 x linker CH), 4.58–4.45 (m, 12H, PTA), 4.07 (s, 12H, PTA), 2.62-2.35 (m, 8H, 2 x -CH_2-CH_2-CO-), 1.95 (s, 6H, 2 x CH_3); ^{31}P{^{1}H} NMR (D_2O, 162 MHz): δ = -33.5; ^{13}C{^{1}H} NMR (D_2O, 101 MHz): δ = 173.5 (2C, amide CO), 166.0 (4C, oxalate CO), 138.3 (2C, $Ar_{(q)}$), 128.7, 127.9, 127.3 (10C, linker Ar), 98.2 (2C, $arene_{(q)}$), 95.3 (2C, $arene_{(q)}$), 88.3 (d, 2C, J = 3.5 Hz, Ru-arene *C*H), 88.2 (d, 2C, J = 4.5 Hz, Ru-arene *C*H), 88.1 (d, 2C, J = 3.5 Hz, Ru-arene *C*H), 87.2 (d, 2C, J = 4.0 Hz, arene *C*H), 70.6 (d, 6C, J = 7.0 Hz, PTA), 57.1 (2C, linker CH), 48.5 (d, 6C, J = 15.5 Hz, PTA), 35.7 (2C, 2 x arene-CH_2-*C*H_2-), 28.4 (2C, arene-*C*H_2-CH_2-), 17.3 (2C, Ar-CH_3); HRMS (ES$^+$) m/z found 600.1064 $[M + 2H]^{2+}$ $C_{50}H_{62}N_8O_{10}P_2Ru_2$ requires 600.1089; $C_{50}H_{60}N_8O_{10}P_2Ru_2$•3$H_2O$ (%): calcd C 48.00 H 5.32 N 8.96; found C 48.04 H 5.14 N 9.05; mp = 230-234°C (dec.).

Compound 5b: Acetyl chloride (478 µl, 6.72 mmol) was added to anhydrous MeOH (5 ml) at 0°C under an atmosphere of N_2. **5a** (85 mg, 0.084 mmol) was then added as a solution in MeOH:DCM (3:2, 5 ml), the resultant yellow solution rapidly turned a red colour with the formation of a precipitate. The suspension was stirred for 12 h then the precipitate collected by filtration, washed with further MeOH (20 ml), then dried under reduced pressure to yield the hydrochloride salt of **5b** as a red powder (50 mg, 0.038 mmol, 42%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.16 (d, 2H, J = 9.0 Hz, amide NH), 7.36-7.28 (m, 4H, linker Ar), 7.26-7.11 (m, 6H, linker Ar), 5.90 (d, 2H, J = 6.0 Hz, Ru-Ar), 5.79 (d, 4H, J = 6.0 Hz , Ru-Ar), 5.69 (d, 2H, J = 6.0 Hz, Ru-Ar), 5.20 (d, 2H, J = 8.5 Hz, 2 x CH), 4.74-4.89 (m br, 12H, PTA), 4.28 (s, 12H, PTA), 2.19-2.35 (m, 8H, 2 x -CH_2-CH_2-CO-), 1.90 (s, 6H, 2 x CH_3); ^{31}P{^{1}H} NMR (DMSO-d_6, 162 MHz): δ = -27.0; ^{13}C{^{1}H} NMR (DMSO-d_6, 101 MHz): δ = 170.1 (2C, amide CO), 140.9 (2C, $Ar_{(q)}$), 127.8, 127.1, 126.6 (10C, linker Ar), 98.1 (2C, Ru-

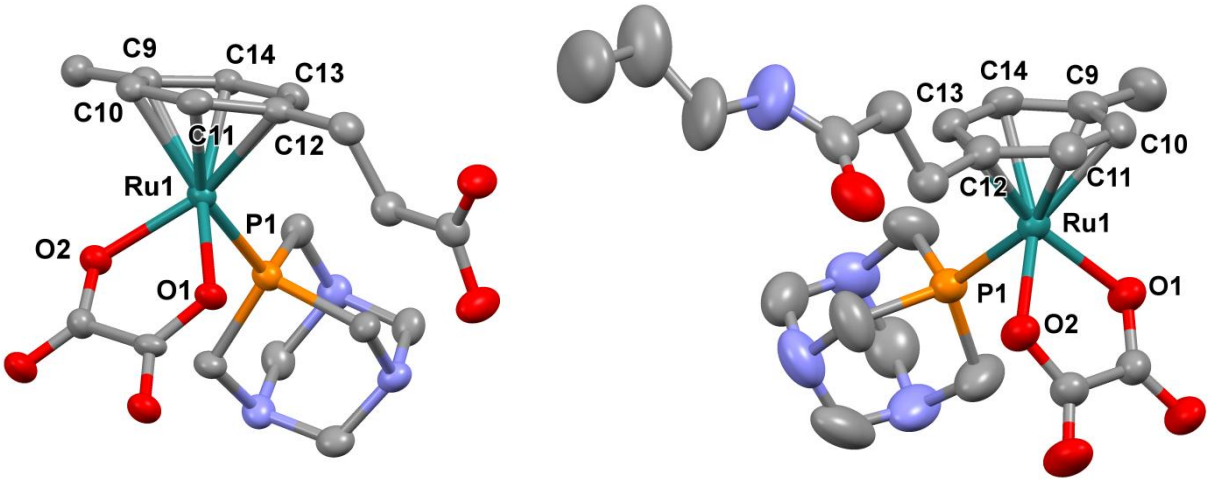
$Ar_{(q)}$), 97.1 (2C, Ru-$Ar_{(q)}$), 88.6 (d, 2C, *J* = 4.0 Hz, Ru-arene CH), 88.4 (d, 2C, *J* = 4.0 Hz, arene CH), 87.6 (d, 2C, *J* = 6.0 Hz, arene CH), 87.4 (d, 2C, *J* = 5.0 Hz, arene CH), 70.3 (m, 6C, PTA), 56.9 (2C, linker CH), 48.3 (d, 6C, *J* = 17.5 Hz, PTA), 35.2 (2C, 2 x arene-CH_2-*C*H_2-), 28.2 (2C, 2 x arene-*C*H_2-CH_2-), 18.0 (2C, 2 x arene-CH_3); HRMS (ES^+) *m/z* found 1165.1246 $[M + H]^+$ $C_{46}H_{61}Cl_4N_8O_2P_2Ru_2$ requires 1165.1232; $C_{46}H_{60}Cl_4N_8O_2P_2Ru_2$•4HCl (%): calcd C 42.21 H 4.93 N 8.56; found C 42.36 H 5.17 N 8.65; mp = 212 - 214°C (dec.).

Compound 6a: **1** (209 mg, 0.409 mmol) and TBTU (126 mg, 0.392 mmol) were suspended in DMF (1 ml) and DIPEA (137 µl, 0.787 mmol) was added. The yellow suspension was stirred for 5 min, followed by the addition of (1*R*,2*R*)-(+)-1,2-diphenylethylenediamine (42 mg, 0.198 mmol). The reaction mixture was stirred for a further 2 h under an atmosphere of N_2, filtered to remove unreacted **1**, then washed with cold DMF (2 ml). Acetone (20 ml) was added to the combined filtrates to yield a precipitate which was collected by centrifugation then resuspended in MeCN (15 ml) and sonicated for 5 min. The solid was collected by centrifugation, dried under reduced pressure then recrystallized from EtOH/DCM to yield **6a** as a crystalline yellow solid (46 mg, 0.038 mmol, 19%). For X-ray diffraction studies a quantity of the product was recrystallized by vapour diffusion of acetone into a H_2O/acetone solution of **6a** to yield yellow crystals. ^1H NMR (D_2O, 400 MHz): δ = 7.28-7.10 (m, 10H, linker Ar), 5.77 (d, 2H, *J* = 6.0 Hz, Ru-Ar), 5.66 (d, 2H, *J* = 6.0 Hz, Ru-Ar), 5.46 (d, 2H, *J* = 6.0 Hz, Ru-Ar), 5.35 (d, 2H, *J* = 6.0 Hz, Ru-Ar), 5.22 (s, 2H, 2 x linker CH), 4.59–4.49 (m, 12H, PTA), 4.08 (s, 12H, PTA), 2.68-2.38 (m, 8H, 2 x -CH_2-CH_2-CO-), 1.97 (s, 6H, 2 x CH_3); $^{31}P\{^1H\}$ NMR (D_2O, 162 MHz): δ = -33.5; $^{13}C\{^1H\}$ NMR (D_2O, 101 MHz): δ = 173.5 (2C, amide CO), 166.0 (4C, oxalate CO), 138.3 (2C, $Ar_{(q)}$), 128.7, 127.9, 127.3 (10C, linker Ar), 98.2 (2C, Ru-$arene_{(q)}$), 95.3 (2C, Ru-$arene_{(q)}$), 88.3 (d, 2C, *J* = 3.5 Hz, Ru-arene *C*H), 88.2 (d, 2C, *J* = 4.5 Hz, Ru-arene *C*H), 88.1 (d, 2C, *J* = 4.5 Hz, Ru-arene *C*H), 87.2 (d, 2C, *J* = 4.0 Hz, Ru-arene *C*H), 70.7 (d, 6C, *J* = 7.0 Hz, PTA), 57.1 (2C, 2 x linker CH), 48.5 (d, 6C, *J* = 15.0 Hz, PTA), 35.7 (2C, 2 x arene-CH_2-*C*H_2-), 28.5 (2C, 2 x arene-*C*H_2-CH_2-), 17.3 ((2C, Ar-*C*H_3); HRMS (ES^+) *m/z* found 600.1089 $[M + 2H]^{2+}$ $C_{50}H_{62}N_8O_{10}P_2Ru_2$ requires 600.1089; $C_{50}H_{60}N_8O_{10}P_2Ru_2$•4$H_2O$ (%): calcd C 47.32 H 5.40 N 8.83; found C 46.94 H 5.02 N 8.83; mp = 229-232°C (dec.).

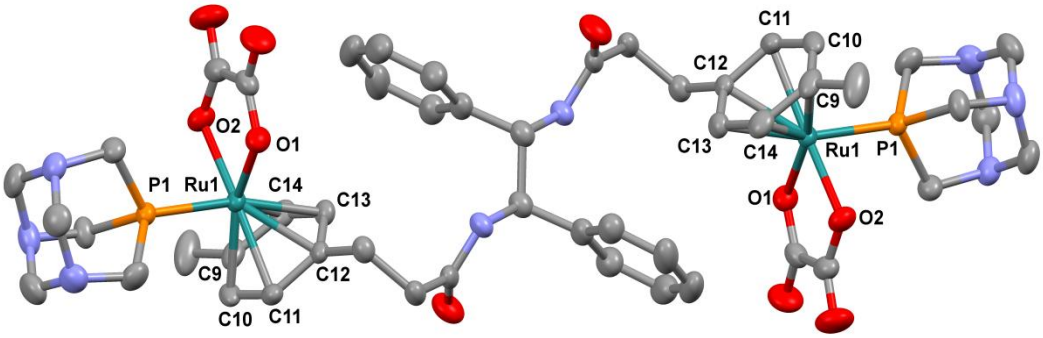
Compound 6b: Acetyl chloride (121 µl, 1.70 mmol) was added to anhydrous MeOH (3 ml) at 0°C under an atmosphere of N_2. **6a** (29 mg, 0.024 mmol) was then added as a suspension in MeOH (0.5 ml) and the resultant suspension rapidly turned red with the formation of a red solid. The suspension was stirred for 5 h then the precipitate collected by centrifugation. Further solid was obtained by cooling the filtrate at 4°C for 28 h. The combined solids were washed with cold MeOH (2 ml) then dried under reduced pressure to yield the hydrochloride salt of **6b** as a red powder (16 mg, 0.012 mmol, 50%). ^1H NMR (DMSO-d_6, 400 MHz): δ = 9.18 (d, 2H, *J* = 9.0 Hz, amide NH), 7.33 and 7.12-7.26 (m, 10H, linker Ar), 5.90 (d, 2H, *J* = 6.0 Hz, Ru-Ar), 5.79 (d, 4H, *J* = 7.5 Hz, Ru-Ar), 5.69 (d, 2H, *J* = 6.0 Hz, Ru-Ar), 5.21 (d, 2H, *J* = 8.5 Hz, 2 x CH), 4.76-4.88 (m, 12H, PTA), 4.27 (s, 12H, PTA), 2.17-2.52 (m, 8H, 2 x -CH_2-CH_2-CO-), 1.90 (s, 6H, 2 x CH_3); $^{31}P\{^1H\}$ NMR (DMSO-d_6, 162 MHz): δ = -27.0; $^{13}C\{^1H\}$ NMR (DMSO-d_6, 101 MHz): δ = 170.2 (2C, amide CO), 140.9 (2C, $Ar_{(q)}$), 127.8, 127.1, 126.6 (10C, linker Ar), 98.0 (2C, Ru-$Ar_{(q)}$), 97.1 (2C, Ru-$Ar_{(q)}$), 88.5 (m, 4C, Ru-arene CH), 87.6 (d, 2C, *J* = 6.0 Hz, Ru-arene CH), 87.4 (d, 2C, *J* = 5.0 Hz, Ru-arene CH), 70.3 (6C, PTA), 56.9 (2C, linker CH), 48.3 (d, 6C, *J* = 17.0 Hz, PTA), 35.2 (2C, 2 x arene-CH_2-*C*H_2-), 28.2 (2C, 2 x arene-*C*H_2-CH_2-), 18.0 (2C, 2 x CH_3); HRMS (ES^+) *m/z* found 1165.1244 $[M + H]^+$ $C_{46}H_{61}Cl_4N_8O_2P_2Ru_2$ requires 1165.1232; $C_{46}H_{60}Cl_4N_8O_2P_2Ru_2$•4HCl (%): calcd C 42.21 H 4.93 N 8.56; found C 42.48 H 5.09 N 8.63; mp = 214-216°C (dec.).

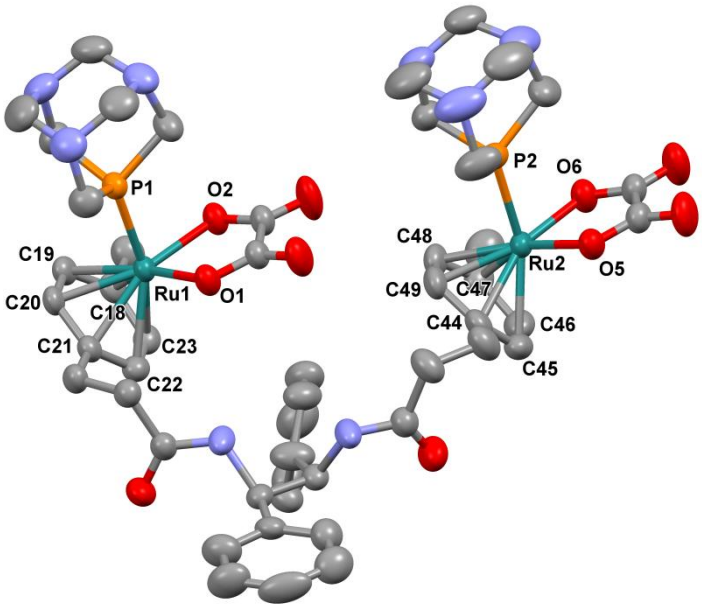
X-ray crystallography

Figure S1: Molecular structures of **1**, **2a**, **4a** and **5a/6a**. Hydrogen atoms and solvent molecules have been omitted for clarity. Thermal ellipsoids are drawn at the 50% probability level. Selected bond lengths (Å) and angles (°) are given in the tables.



Interatomic distances (Å)	1	2a
Ru1-O1	2.097(3)	2.080(2)
Ru1-O2	2.083(3)	2.086(2)
Ru1-P1	2.311(1)	2.3166(5)
Ru1-C9	2.182(5)	2.201(2)
Ru1-C10	2.234(5)	2.233(2)
Ru1-C11	2.248(4)	2.231(2)
Ru1-C12	2.202(4)	2.173(2)
Ru1-C13	2.200(4)	2.182(2)
Ru1-C14	2.198(4)	2.188(2)
Angles (°)		
O1-Ru1-O2	77.8(1)	78.39(6)
O1-Ru1-P1	86.14(9)	88.03(5)
O2-Ru1-P1	87.2(1)	84.46(5)





Interatomic distances (Å)	4a
Ru1-O1	2.088(2)
Ru1-O2	2.086(2)
Ru1-P1	2.320(1)
Ru1-C9	2.188(3)
Ru1-C10	2.166(3)
Ru1-C11	2.195(3)
Ru1-C12	2.211(4)
Ru1-C13	2.238(5)
Ru1-C14	2.205(4)
Angles (°)	
O1-Ru1-O2	78.5(1)
O1-Ru1-P1	87.95(8)
O2-Ru1-P1	85.46(8)

Interatomic distances (Å)	5a/6a
Ru1-O1	2.100(2)
Ru1-O2	2.096(3)
Ru1-P1	2.3013(6)
Ru1-C18	2.202(2)
Ru1-C19	2.199(3)
Ru1-C20	2.174(3)
Ru1-C21	2.201(4)
Ru1-C22	2.244(3)
Ru1-C23	2.256(2)
Ru2-O5	2.091(2)
Ru2-O6	2.085(3)
Ru2-P2	2.3022(7)
Ru2-C44	2.170(5)
Ru2-C45	2.246(3)
Ru2-C46	2.258(2)
Ru2-C47	2.195(3)
Ru2-C48	2.179(3)
Ru2-C49	2.158(5)
Angles (°)	
O1-Ru1-O2	77.67(8)
O1-Ru1-P1	84.99(6)
O2-Ru1-P1	89.36(6)
O5-Ru2-O6	78.24(9)
O5-Ru2-P2	84.49(7)
O6-Ru2-P2	88.69(7)

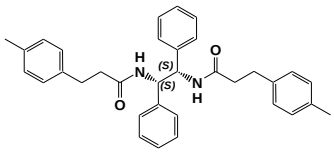
Table S1. X-ray diffraction parameters for the measurement of single crystals grown from samples of **1**, **2a**, **4a** and **5a**.

Compound	**1**	**2a**
CCDC deposition number	978401	978402
Empirical formula	$C_{18}H_{24}N_3O_6PRu$	$C_{21}H_{31}N_4O_5PRu$
Formula weight	510.44	551.54
Temperature	140(2) K	293(2) K
Wavelength	0.71073 Å	1.54178 Å
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/c$
Unit cell dimensions	a = 14.595(5) Å α= 90° b = 9.371(3) Å β= 114.510(16)° c = 15.859(4) Å γ = 90°	a = 11.58624(12) Å α= 90° b = 15.39855(14) Å β= 98.3744(10)° c = 13.23391(14) Å γ = 90°
Volume	1973.6(11) $Å^3$	2335.90(4) $Å^3$
Z	4	4
Density (calculated)	1.718 Mg/m^3	1.568 Mg/m^3
Absorption coefficient	0.917 mm^{-1}	6.422 mm^{-1}
F(000)	1040	1136
Crystal size	0.30 x 0.26 x 0.18 mm^3	0.35 x 0.30 x 0.26 mm^3
Theta range for data collection	2.48 to 30.06°.	4.43 to 73.30°.
Index ranges	-20<=h<=20, -13<=k<=11, -22<=l<=22	-13<=h<=14, -19<=k<=13, -16<=l<=15
Reflections collected	9044	16480
Independent reflections	5604 [R(int) = 0.0427]	4620 [R(int) = 0.0175]
Completeness to theta = 25.00°	98.5%	98.4%
Absorption correction	None	Semi-empirical from equivalents
Max. and min. transmission		1.00000 and 0.46622
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	5604 / 0 / 264	4620 / 153 / 320
Goodness-of-fit on F^2	1.033	1.069
Final R indices [I>2sigma(I)]	R1 = 0.0715, wR2 = 0.1986	R1 = 0.0254, wR2 = 0.0690
R indices (all data)	R1 = 0.0868, wR2 = 0.2253	R1 = 0.0261, wR2 = 0.0695
Largest diff. peak and hole	1.380 and -2.303 e.$Å^{-3}$	0.640 and -0.491 e.$Å^{-3}$

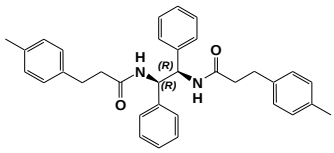
Compound	**4a.4H_2O**	**5a.H_2O**
CCDC deposition number	978403	978404
Empirical formula	$C_{50}H_{68}N_8O_{14}P_2Ru_2$	$C_{50}H_{62}N_8O_{11}P_2Ru_2$
Formula weight	1269.20	1215.16
Temperature	293(2) K	293(2) K
Wavelength	0.71073 Å	1.54178 Å
Crystal system	Triclinic	Triclinic
Space group	*P*-1	*P*-1
Unit cell dimensions	a = 9.8881(8) Å α= 72.028(7)°. b = 12.5811(9) Å β= 67.580(7)°. c = 12.6829(10) Å γ = 67.069(7)°.	a = 13.7555(4) Å α= 70.736(3)°. b = 14.6826(5) Å β= 76.491(2)°. c = 16.8467(4) Å γ = 64.286(3)°.
Volume	1319.36(17) $Å^3$	2877.44(14) $Å^3$
Z	1	2
Density (calculated)	1.597 Mg/m^3	1.403 Mg/m^3
Absorption coefficient	0.707 mm^{-1}	5.284 mm^{-1}
F(000)	654	1248
Crystal size	0.30 x 0.23 x 0.04 mm^3	0.51 x 0.21 x 0.18 mm^3
Theta range for data collection	2.74 to 29.38°.	3.46 to 73.41°.
Index ranges	-13<=h<=13, -16<=k<=16, -16<=l<=17	-17<=h<=17, -18<=k<=18, -20<=l<=20
Reflections collected	23414	49397
Independent reflections	6516 [R(int) = 0.0714]	11408 [R(int) = 0.0319]
Completeness to theta = 25.00°	98.5%	98.6%
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents
Max. and min. transmission	1.00000 and 0.70076	1.00000 and 0.26653
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	6516 / 6 / 360	11408 / 1 / 715
Goodness-of-fit on F^2	1.075	1.127
Final R indices [I>2sigma(I)]	R1 = 0.0491, wR2 = 0.1154	R1 = 0.0323, wR2 = 0.0821
R indices (all data)	R1 = 0.0639, wR2 = 0.1246	R1 = 0.0344, wR2 = 0.0830
Extinction coefficient		0.00021(2)
Largest diff. peak and hole	1.336 and -0.654 e.$Å^{-3}$	0.350 and -0.521 e.$Å^{-3}$

HPLC analysis of 5a and 6a

A suspension of complex **5a** or **6a** (3 mg) in DMF (50 µl) was heated at 50°C for 72 h resulting in a black suspension. The suspension was diluted with the addition of H_2O (100 µl), extracted with DCM (2 x 100 µl) then the organic phase was dried under reduced pressure. The product was redissolved in MeOH (500 µl) and filtered through a disposable syringe filter (CHROMAFIL® Xtra H-PTFE-20/25) then dried under reduced pressure to yield the cleaved arene ligand, **5a-ligand** or **6a-ligand** (below).



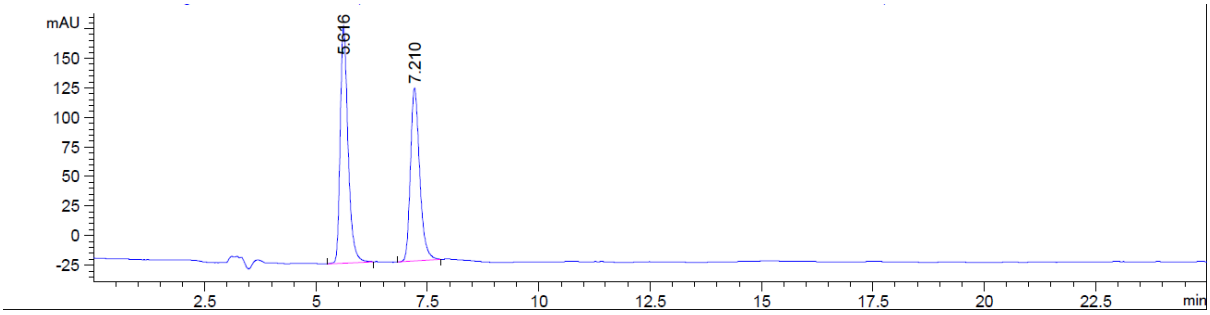
5a-ligand



6a-ligand

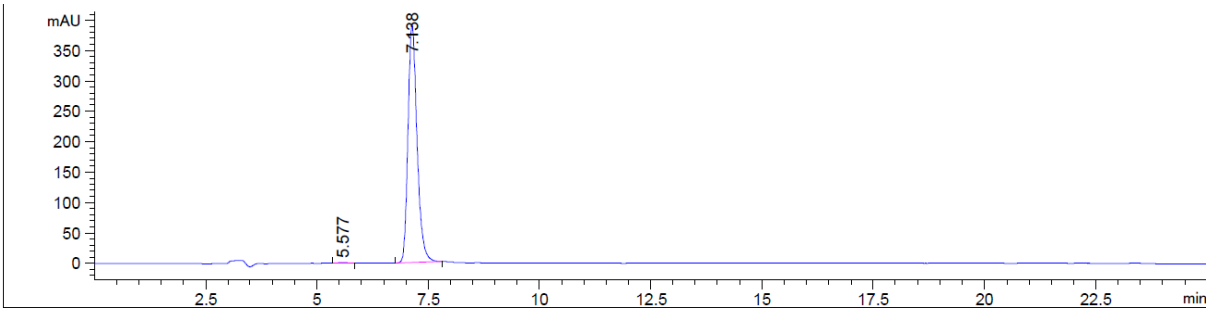
The samples were then analysed by HPLC (Chiralpak IA, 4.6 x 250 mm; *i*-PrOH:hexane 20:80, 1.0 ml/min, 210 nm).

Figure S2: HPLC trace of the separation of an approximately 50:50 mix of arene ligands cleaved from **5a** and **6a**. The peak at 7.21 min corresponds to **5a-ligand** whilst the peak at 5.62 min corresponds to **6a-ligand** (assignments made based on results shown in Figs S3 and S4).



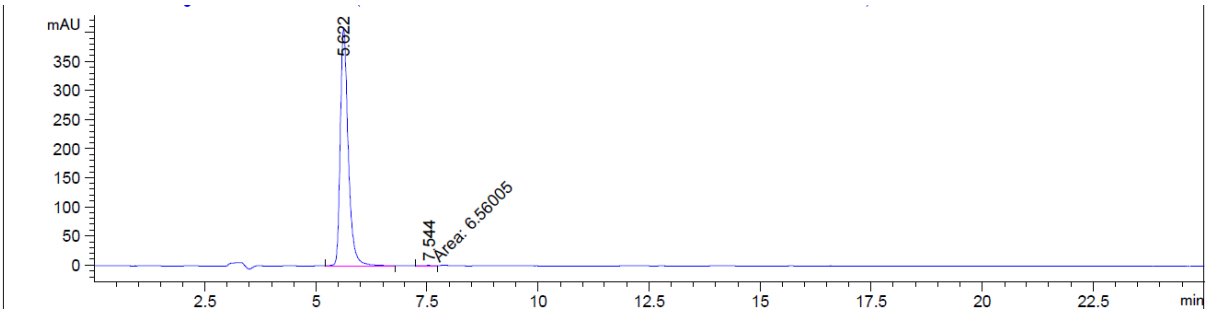
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.616	BB	0.1833	2432.58643	200.81104	53.5939
2	7.210	BB	0.2199	2106.33643	146.38919	46.4061

Figure S3: HPLC trace of the arene ligand cleaved from sample **5a**. t_r (**5a-ligand**) = 7.1 min, t_r (**6a-ligand**) = 5.6 min, enantiomeric ratio SS:RR = 99.7:0.3.



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.577	BB	0.1830	15.65968	1.33356	0.2811
2	7.138	BB	0.2151	5554.52344	392.47653	99.7189

Figure S4: HPLC trace of the arene ligand cleaved from sample **6a**. t_r (**6a-ligand**) = 5.6 min, t_r (**5a-ligand**) = 7.5 min, enantiomeric ratio SS:RR = 0.1:99.9.



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.622	BB	0.1865	5067.62646	409.02371	99.8707
2	7.544	MM	0.1851	6.56005	4.38432e-1	0.1293

UV-Vis spectroscopy

Figure S5: UV-Vis spectra of **2b** in phosphate buffer (10 mM, pH 7.2, 5mM NaCl) at 298 K, recorded every 150 s for approximately 8000 s (inset shows the plot of 310 nm/388 nm vs. time (s) and a fit of the data by a single exponential growth function).

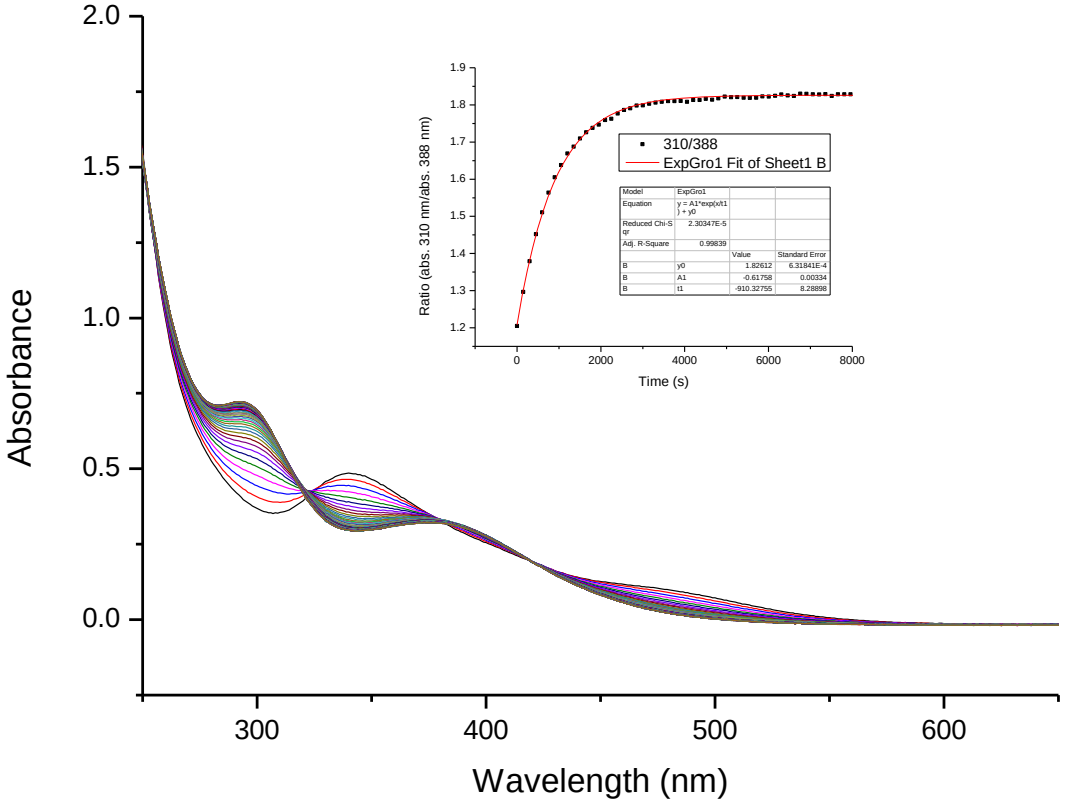


Figure S6: UV-Vis spectra of **3b** in phosphate buffer (10 mM, pH 7.2, 5mM NaCl) at 298 K, recorded every 150 s for approximately 8000 s (inset shows the plot of 302 nm/382 nm vs. time (s) and a fit of the data by a single exponential growth function).

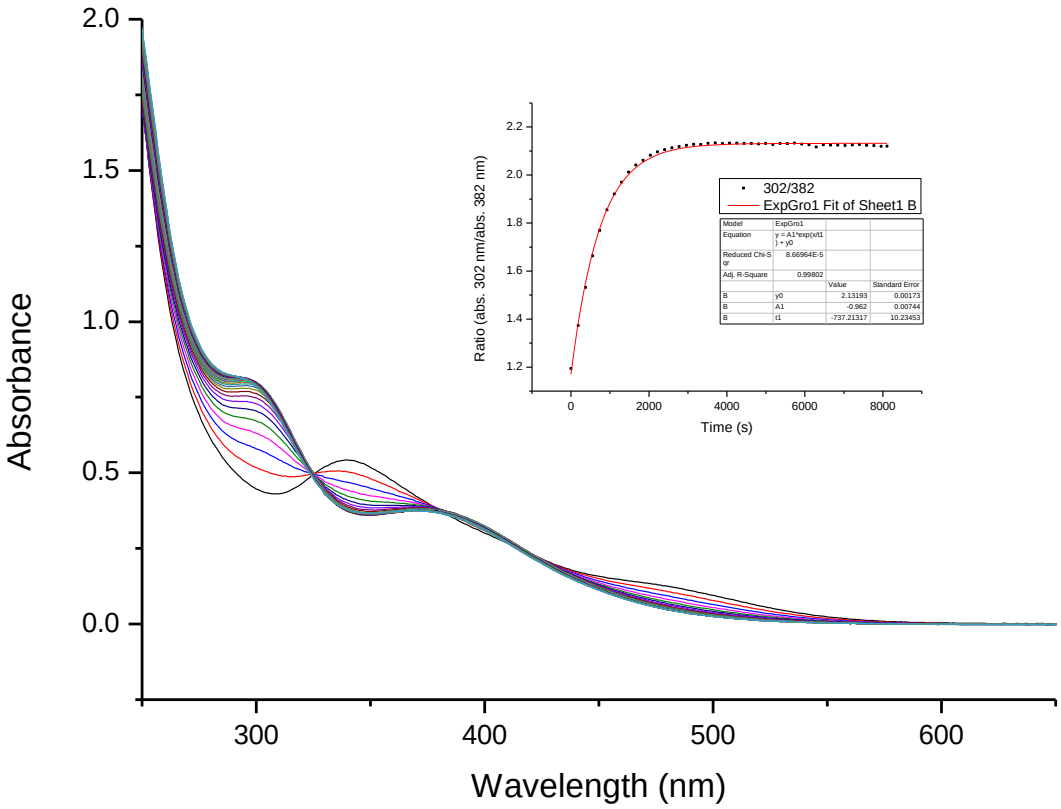


Figure S7: UV-Vis spectra of **4b** in phosphate buffer (10 mM, pH 7.2, 5mM NaCl) at 298 K, recorded every 150 s for approximately 8000 s (inset shows the plot of 302 nm/382 nm vs. time (s) and a fit of the data by a single exponential growth function).

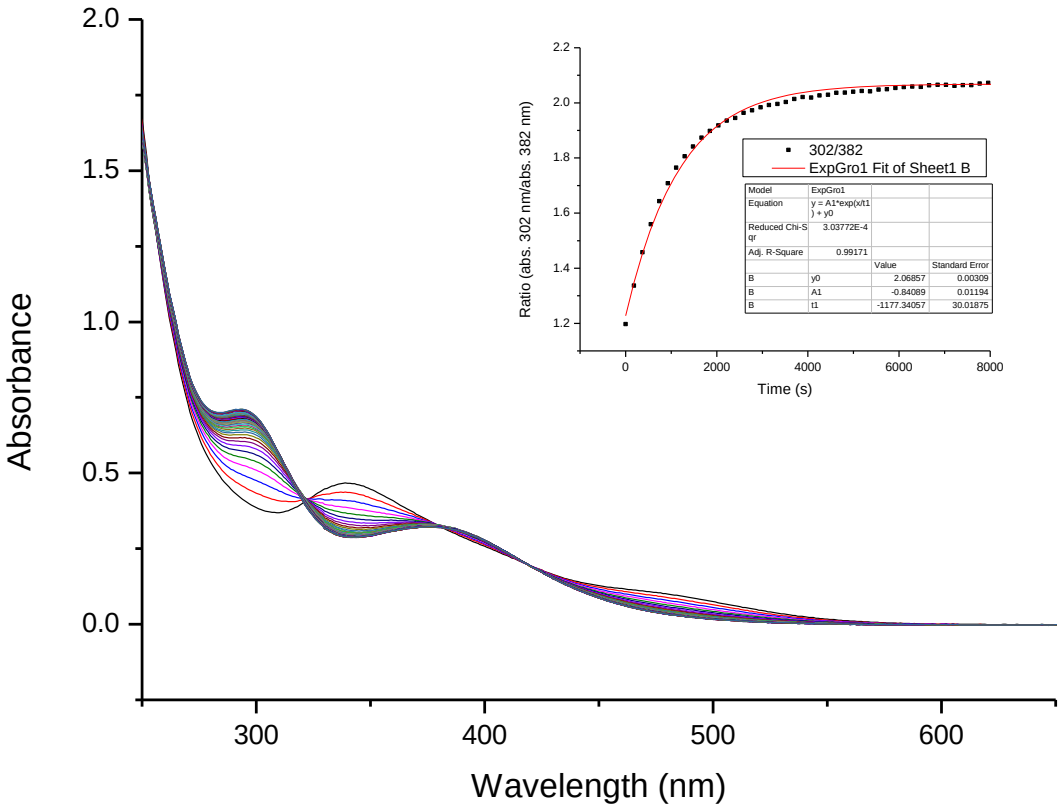


Figure S8: UV-Vis spectra of **5b** in phosphate buffer (10 mM, pH 7.2, 5mM NaCl) at 298 K, recorded every 150 s for approximately 8000 s (inset shows the plot of 302 nm/382 nm vs. time (s) and a fit of the data by a single exponential growth function).

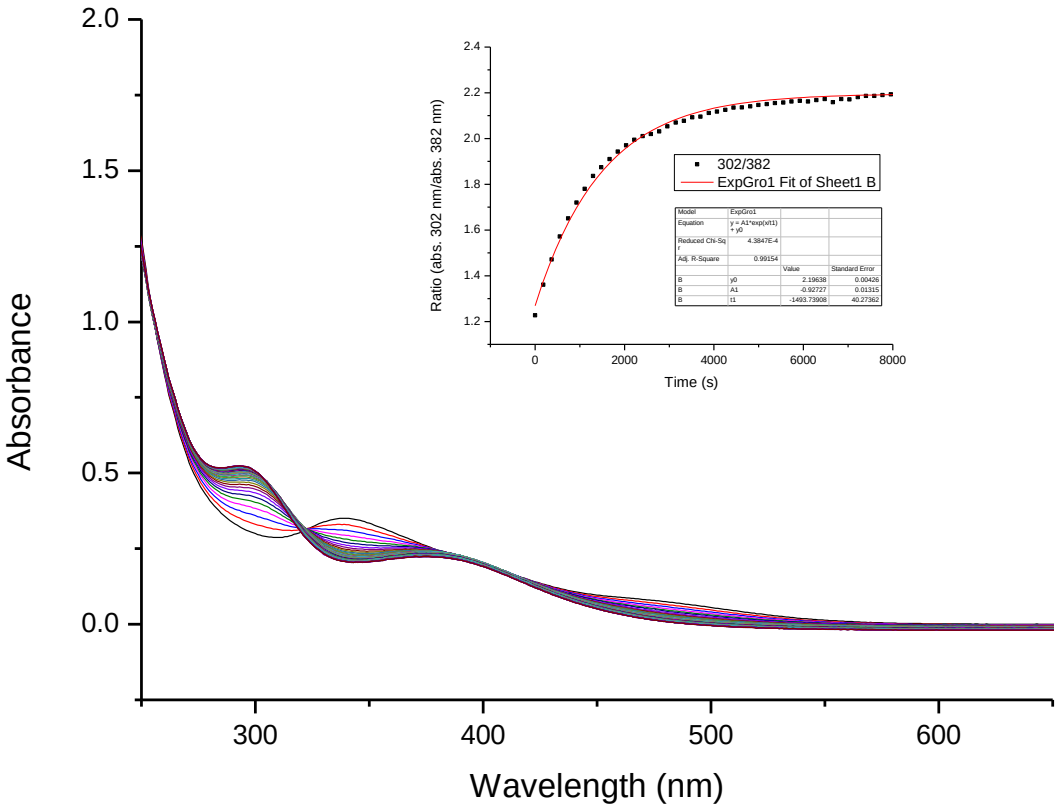
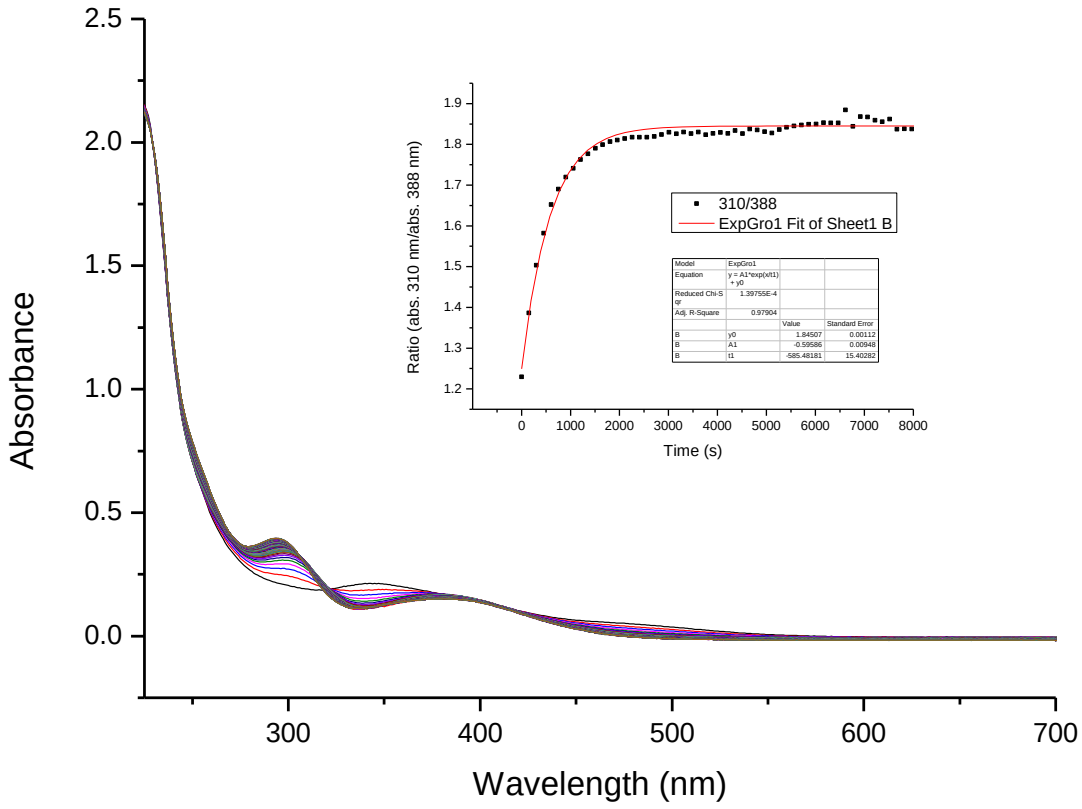


Figure S9: UV-Vis spectra of **RAPTA-C** in phosphate buffer (10 mM, pH 7.2, 5mM NaCl) at 298 K. Spectra recorded every 150 s for approximately 8000 s (left, inset shows the plot of 310 nm/388 nm vs. time (s) and a fit of the data by a single exponential growth function).



^{31}P NMR stability studies

Figure S10: $^{31}P\{^{1}H\}$ NMR spectra of **2a** (18 mM) and BSA (0.35 mM) (D_2O, pD 7.65) after 15 minutes (bottom) and 72 h (top) at 310 K.

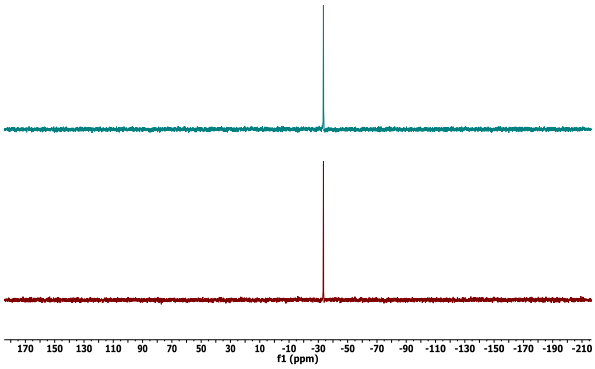


Figure S11: $^{31}P\{^{1}H\}$ NMR spectra of **2a** (18 mM) in HEPES buffer (200 mM, D_2O, pD 7.65) after 15 minutes (bottom) and 72 h (top) at 310 K.

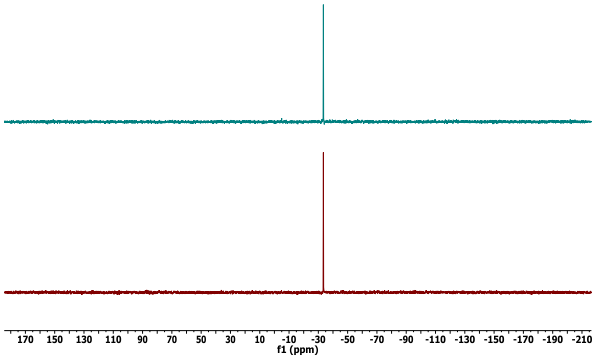


Figure S12: $^{31}P\{^{1}H\}$ NMR spectra of **2a** (18 mM) and CT-DNA (11.5 mM base pairs) (D_2O, HEPES 200 mM, pD 7.65) after 15 minutes (bottom) and 72 h (top) at 310 K.

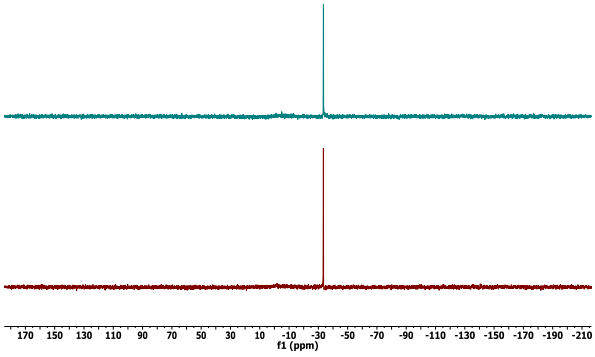


Figure S13: $^{31}P\{^{1}H\}$ NMR spectra of **2a** (18 mM) and glutathione (4.5 mM) (D_2O, HEPES 200 mM, pD 7.65) after 15 minutes (bottom) and 72 h (top).

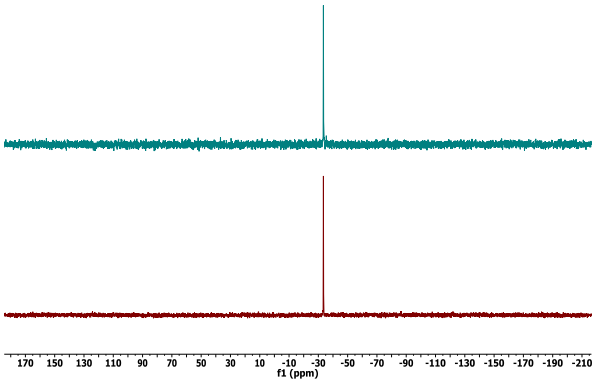


Figure S14: $^{31}P\{^{1}H\}$ NMR spectra of **2a** (18 mM) in phosphate buffer (25 mM, D_2O, pD 7.6) after 15 minutes (bottom) and 72 h (top).

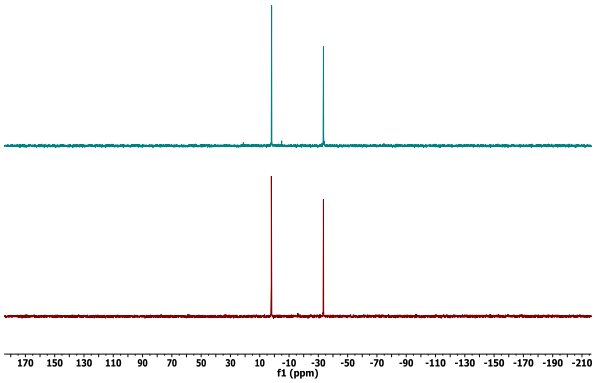


Figure S15: $^{31}P\{^{1}H\}$ NMR spectra of **2a** (18 mM) in RPMI media containing 10% D_2O after 10 minutes (bottom) and 72 h (top) at 37°C.

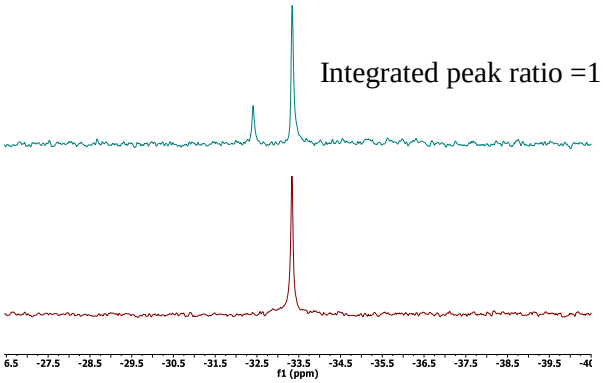
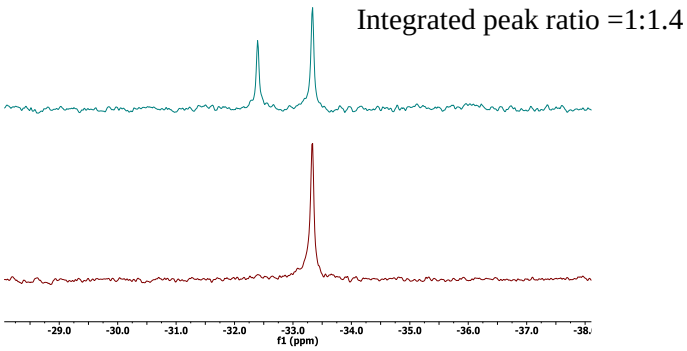


Figure S16: $^{31}P\{^{1}H\}$ NMR spectra of **2a** (18 mM) in DMEM media containing 10% D_2O after 10 minutes (bottom) and 72 h (top) at 37°C.



Binding studies with 5'-GMP, L-histidine and H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-OH

Figure S17: Electrospray-ionization mass spectra of **4b** (top), **6b** (middle) and **5b** (bottom) following incubation with 5'-GMP. Incubation conditions: **4b**/**5b**/**6b** (0.85 mM) and 5'-GMP (2 eq.) in H_2O (72 h, pH 4.5) at 310 K. Peak assignments are given using X = **4b**/**5b**/**6b.**

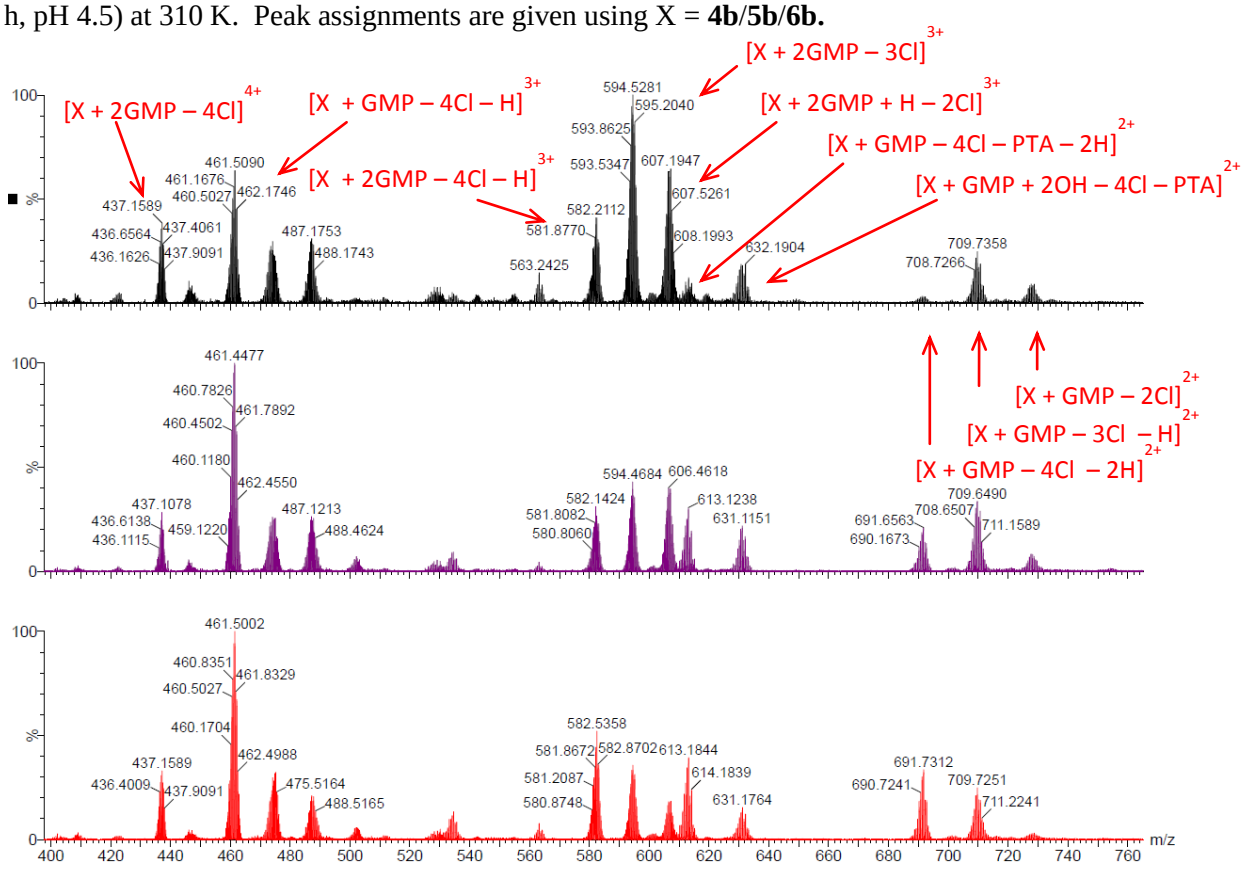


Figure S18: ^{1}H NMR (left) and ^{31}P{^{1}H} NMR (right) spectra of **3b** (14 mM) with 5'-GMP (2 eq.) in HEPES buffer in D_2O (200 mM, pD 7.5) at time intervals between 15 min to 24 h. 1 = 15 min, 2 = 2 h, 3 = 3h, 4 = 5h, 5 = 8.5 h, 6 = 24 h after incubation at 310K.

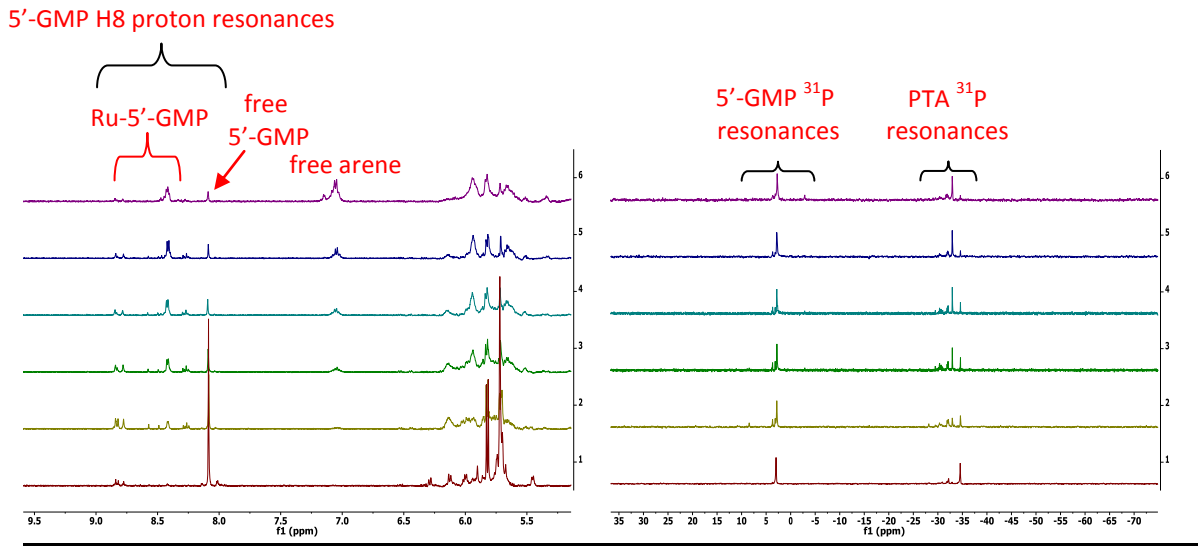


Figure S19: Electrospray-ionization mass spectra of **4b** (top), **5b** (middle) and **6b** (bottom) following incubation with L-histidine. Incubation conditions: **4b**/**5b**/**6b** (0.85 mM), L-histidine (2 eq.) in H_2O (72 h, pH 7.1) at 310 K. Peak assignments are given using X = **4b**/**5b**/**6b.**

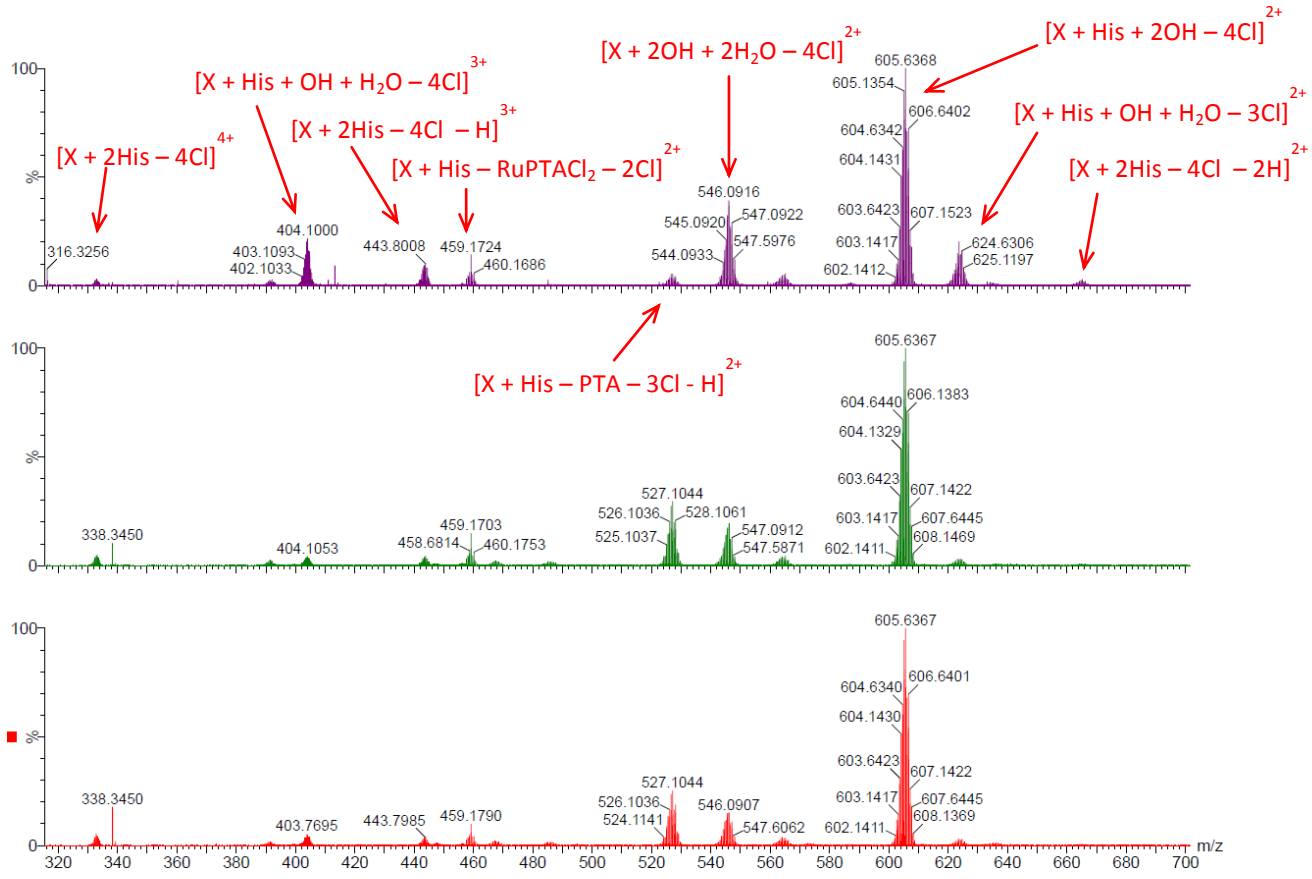
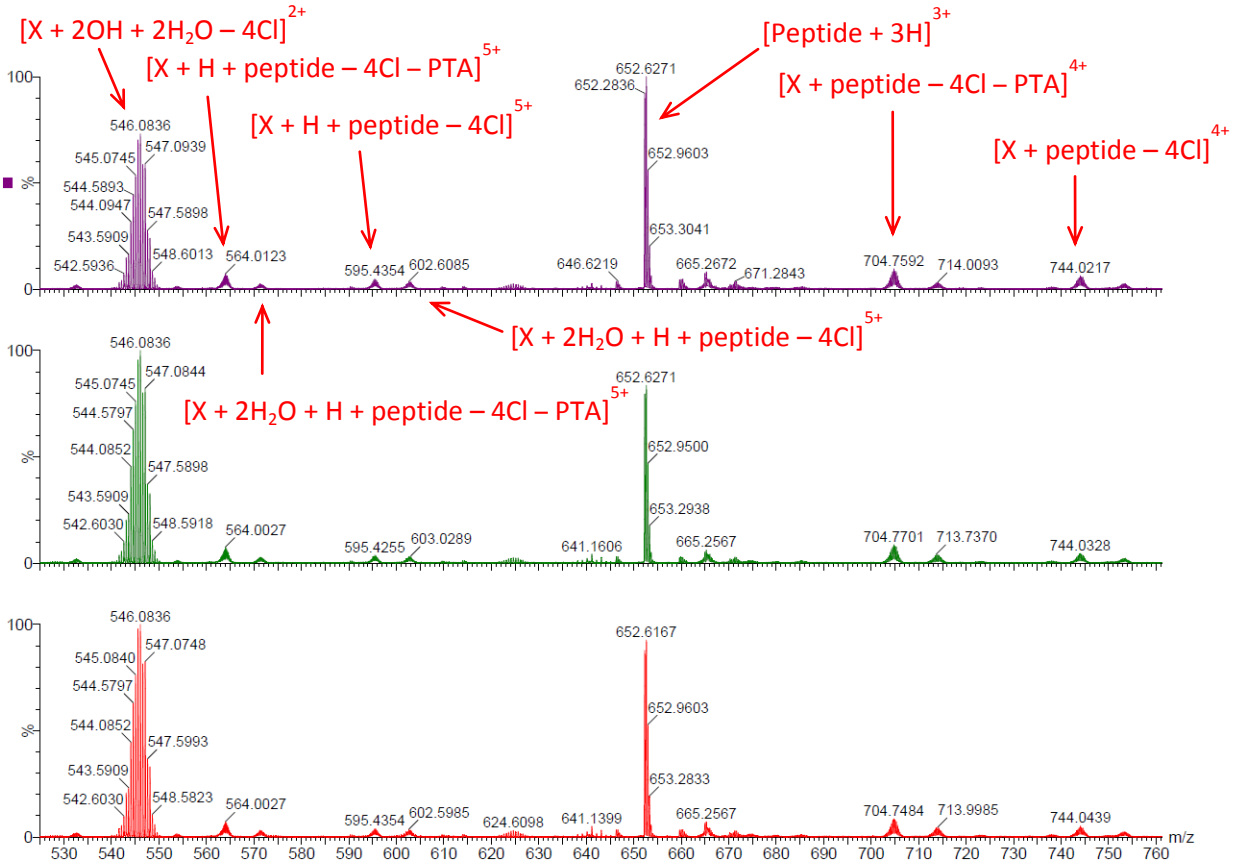
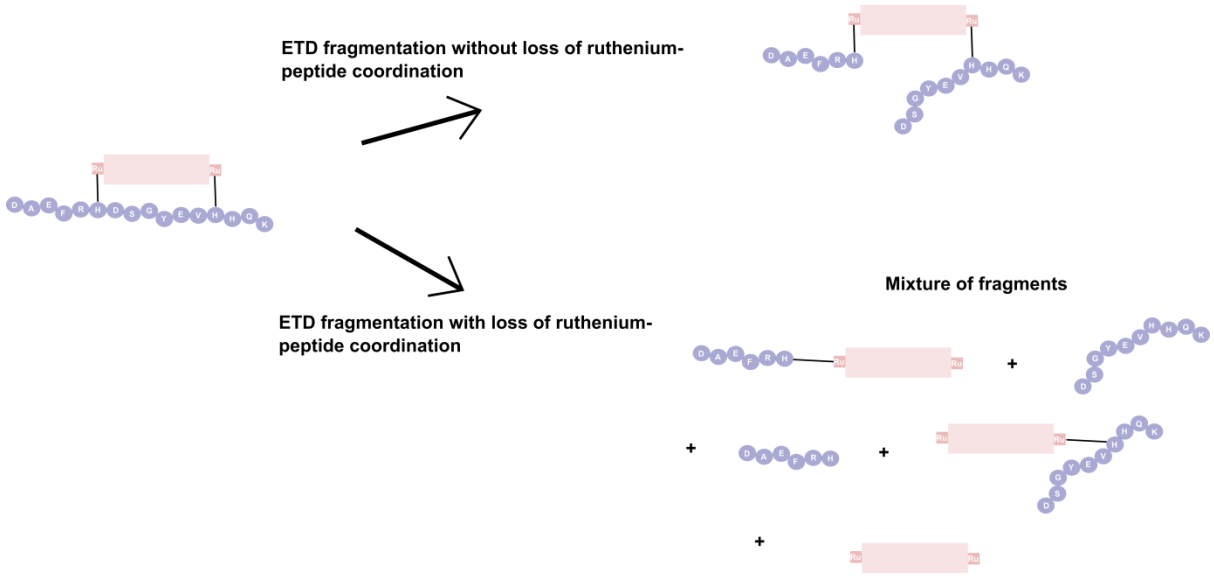


Figure S20: Electrospray-ionization mass spectra of **4b** (top), **5b** (middle) and **6b** (bottom) after incubation with H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-OH. Incubation conditions: **4b**/**5b**/**6b** (0.6 mM), peptide (1 eq.) in H_2O (72 h) at 310 K. Peak assignments are given using X = **4b**/**5b**/**6b.**



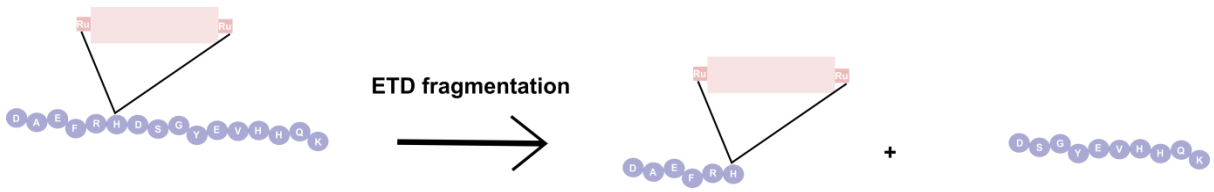
ETD fragmentation studies

For 1:1 peptide (H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-OH) adducts with dinuclear complexes **3b** – **6b**, if crosslinking were to occur between His^{6} and $His^{13/14}$, ETD fragmentation between amino acid residues Asp^{7}-Val^{12} would not be detected if each ruthenium-peptide bond remained intact, as the resulting ETD product would have the same mass and overall charge as the parent ion due to it being a 'ring-opened' analogue (see cartoon representation below). However, in ETD spectra of dinuclear 1:1 adducts, fragments such as metallated C_6^{+}-C_{12}^{+} and $Z_5^{+\bullet}$-$Z_{10}^{+\bullet}$ are observed. We believe the most plausible explanation for the presence of these fragments is due to loss of coordination of one of the ruthenum ions to the peptide during the ETD fragmentation process, resulting in metallated C^{+} and $Z^{+\bullet}$ ions where only one ruthenium center is coordinated to the fragment and the other, due to loss of coordination, is free. This explanation is supported by the observation of the reduced complex fragments alone in the corresponding ETD spectra (e.g. [**3b** + H - 4Cl]$^{+}$, [**4b** + H - 4Cl]$^{+}$ and [**4b** - 4Cl - PTA]$^{+}$) where ETD fragmentation has resulted in the complete loss of ruthenium coordination to the peptide fragments.



Cartoon representation of a 1:1 peptide:dinuclear complex adduct crosslinking His^{6} and His^{13} showing postulated products from fragmentation between His^{6} and Asp^{7}. Amino acids are represented by circles containing their 1-letter code and ruthenium atoms are represented by squares linked by a representation of the organic linker. For reasons of simplicity the cartoon does not include charges or further ruthenium coordination bonds to the peptide.

An alternative explanation for the presence of the metallated C_6^{+}-C_{12}^{+} and $Z_5^{+\bullet}$-$Z_{10}^{+\bullet}$ fragments is that both ruthenium centers are simultaneously coordinating to the same amino acid residue (see cartoon representation below).



Cartoon representation of a 1:1 peptide:dinuclear complex adduct where both ruthenium ions are coordinated to His^{6}. Amino acids are represented by circles containing their 1-letter code and ruthenium atoms are represented by squares linked by a representation of the organic linker. For reasons of simplicity the cartoon does not include charges or further ruthenium coordination bonds to the peptide.

If we consider this second explanation in the case of the [H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-OH + **3b** + H - 4Cl]$^{5+}$ and the [H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-OH + **4b/5b/6b** + H - 4Cl - PTA]$^{5+}$ adducts, we observe metallated C_6^+ fragments where the site of metallation is solely at His^6 (as elucidated from other fragments identified within the corresponding ETD spectra). We judge it to be very unlikely that both ruthenium centers in these adducts would be simultaneously coordinated to same His^6 amino acid residue based on the steric bulk of the complex ligands, the ability of the histidine residues to coordinate to two ruthenium centers and the rigidity of the complexes inhibiting such binding.

Figure S21: ETD LTQ Orbitrap FTMS of the [H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-OH + **X** + H – 4Cl]$^{5+}$ (**X** = **4b**,**5b**,**6b**) adducts (**4b**-top; **5b**-middle; **6b**-bottom) highlighting the similarity between the three spectra.

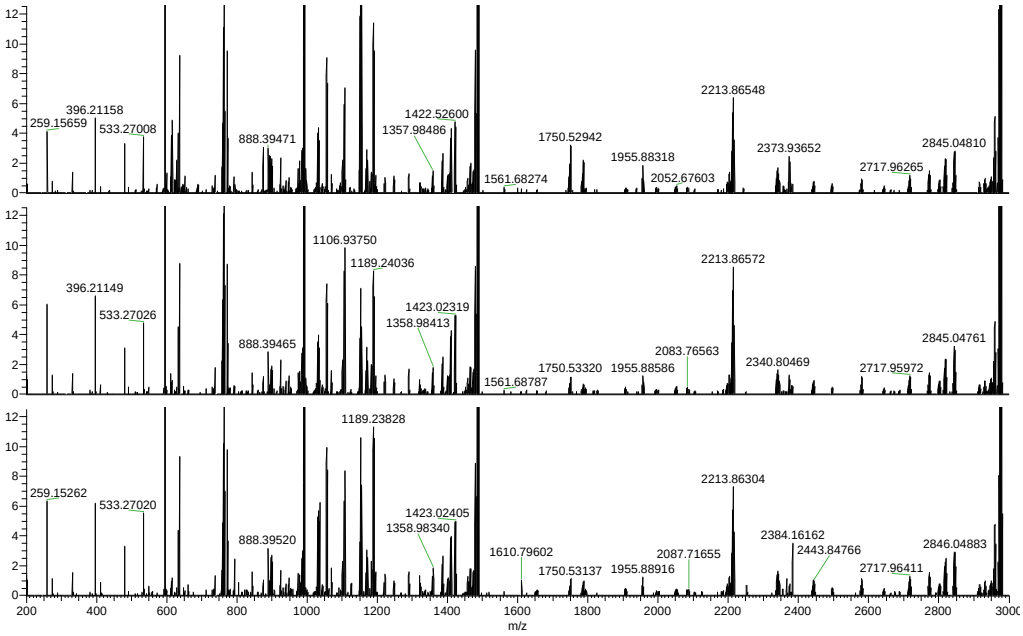
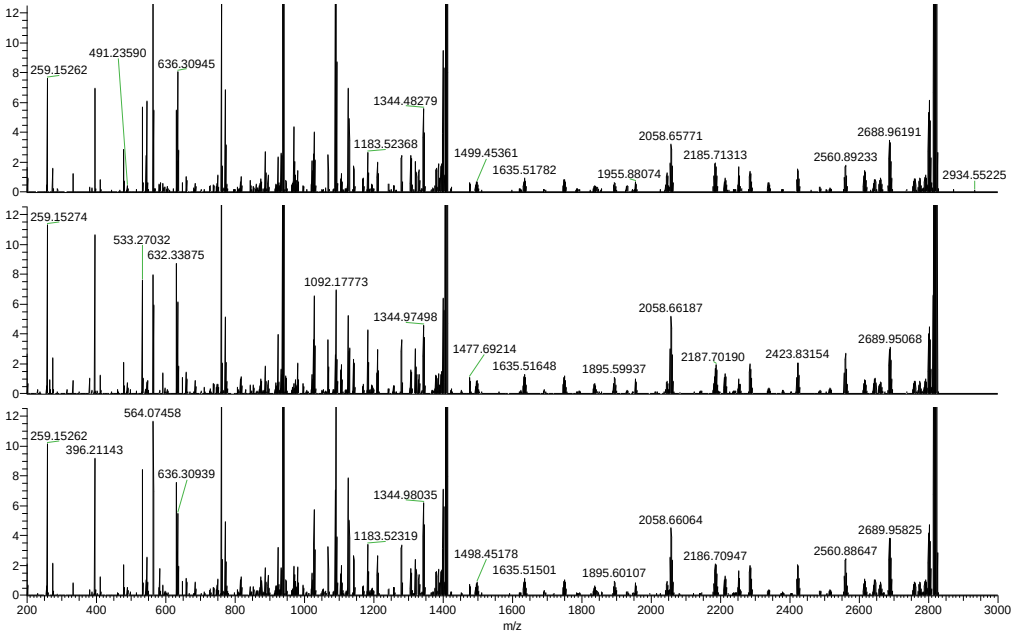


Figure S22: ETD LTQ Orbitrap FTMS of the [H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-OH + **X** + H – 4Cl - PTA]$^{5+}$ (**X** = **4b**,**5b**,**6b**) adducts (**4b**-top; **5b**-middle; **6b**-bottom) highlighting the similarity between the three spectra.



Ion Mobility-Mass Spectrometry studies

Figure S23: Comparison of the arrival time distributions (ATDs) of the [peptide + **X** – 4Cl – PTA]$^{4+}$ (**X** = **4b**, **5b**, **6b**) adducts (**4b** – bottom, **5b** – middle, **6b** – top).

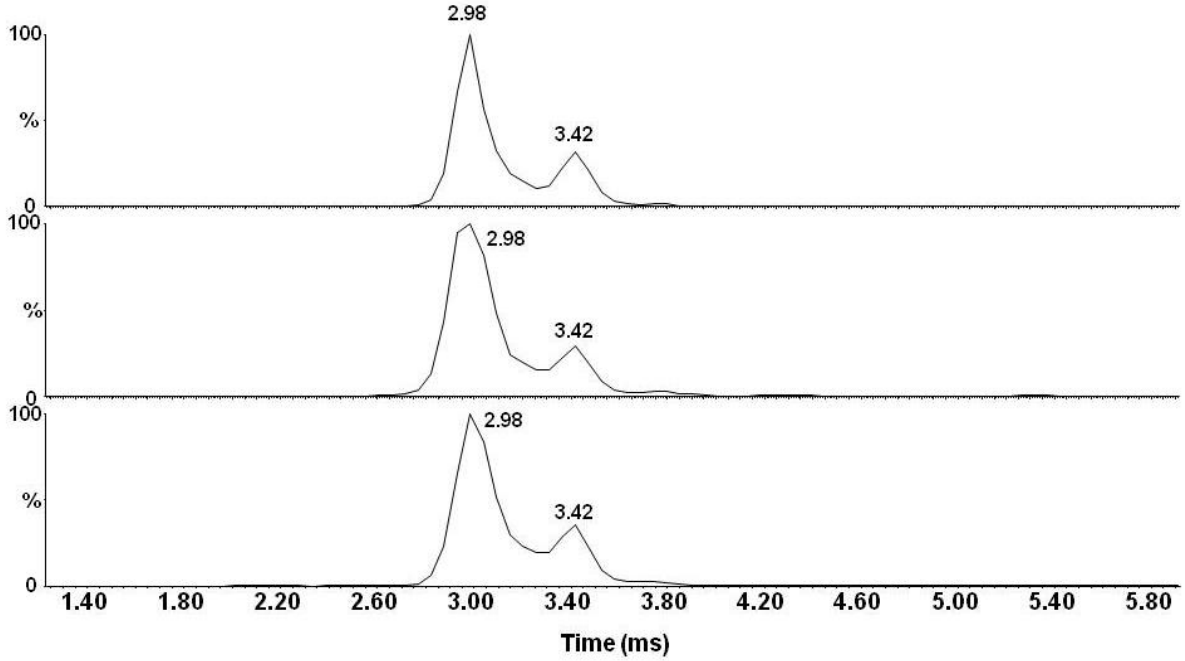


Figure S24: Comparison of the ATDs of the [peptide + **X** – 4Cl]$^{4+}$ (**X** = **4b**, **5b**, **6b**) adducts (**4b** – bottom, **5b** – middle, **6b** – top).

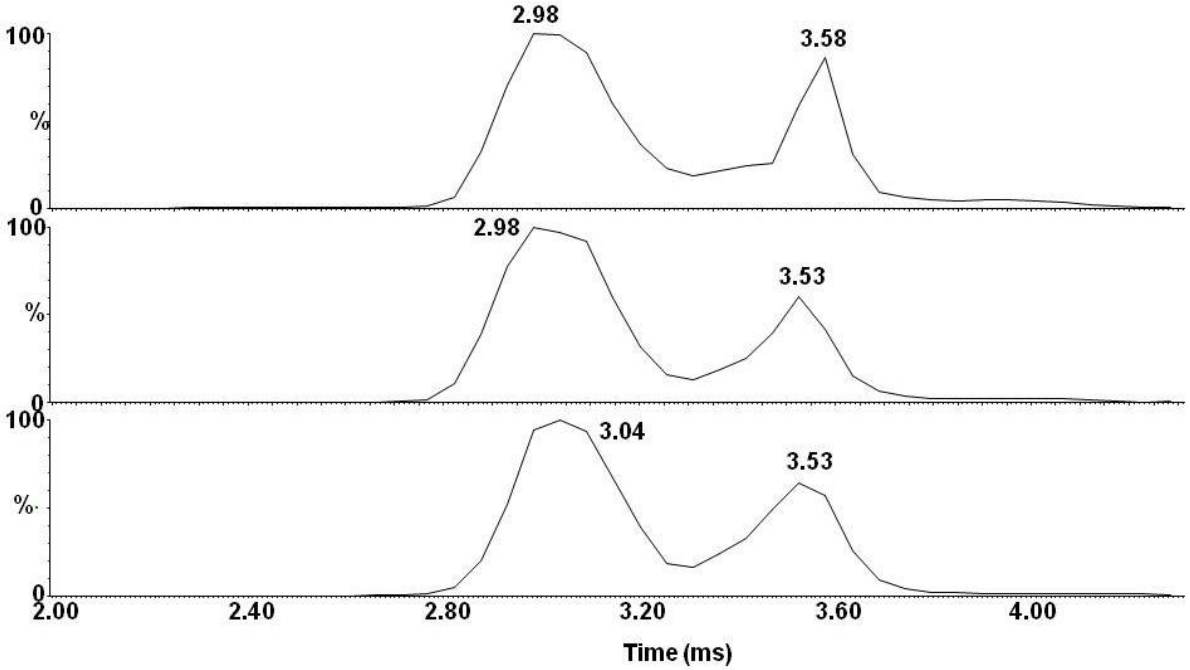


Figure S25: Comparison of the ATDs of the [**X** – 4Cl + 2OH + 2H_2O]$^{2+}$ (**X** = **4b**, **5b**, **6b**) ions (**4b** – bottom, **5b** – middle, **6b** – top) with the inset showing the corresponding mass spectral isotope patterns (**4b** – bottom, **5b** – middle, **6b** – top).

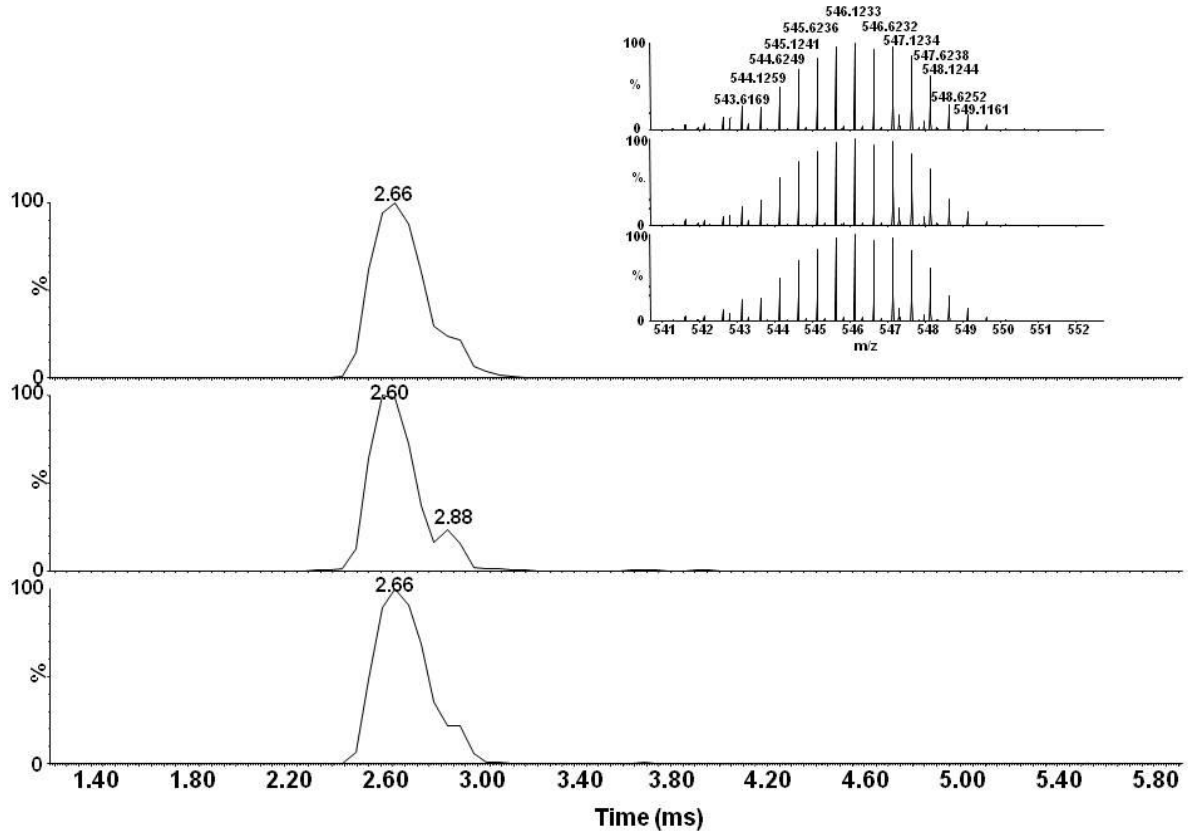
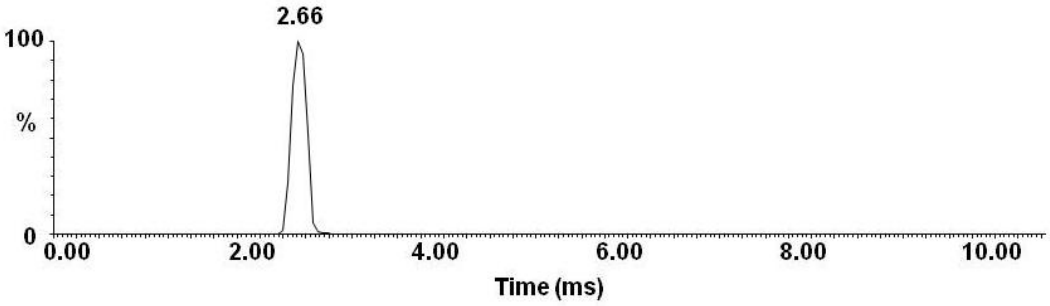


Figure S26: Representative ATD of the [peptide + 4H]$^{4+}$ ion.



Cell culture

A2780, A2780cisR and HEK-293 cell lines were obtained from the European Collection of Cell Cultures (ECACC) (Salisbury, UK). RPMI-1640, DMEM high glucose with GlutaMAX media and penicillin streptomycin solution were obtained from Life Technologies, fetal bovine serum was obtained from Sigma and cisplatin was obtained from TCI.

Cells were cultured in RPMI-1640 with GlutaMAX (A2780, A2780cisR) or DMEM high glucose with GlutaMAX (HEK 293) medium containing 10% fetal bovine serum (FBS) and penicillin at 37ºC and 5% CO_2. A2780cisR cells were cultured in media containing cisplatin (2 μM) every second passage.

Cytotoxicity was determined using the MTT assay.[8] Cells were seeded in flat-bottomed 96-well plates by the addition of cells as a suspension in their respective media containing 10% FBS (100 μl per well, approximately 4300 cells) and pre-incubated for 24 h. Compound stock solutions were prepared in H_2O immediately prior to use then diluted by the addition to the culture medium. These stock solutions were then sequentially diluted to yield compound solutions of the required concentrations. Aliquots (100 μl) of these stock solutions were added to plate wells to yield final compound concentrations in the range 0 μM to 300 μM/400 μM. For each cell line the cisplatin control was run at 0 μM to 75 μM. The 96-well plates were then incubated for a further 72 h followed by the addition of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) solution (20 μl, 5 mg/mL in H_2O) to each well and the plates incubated for a further 2 h. The culture medium was then aspirated and the formazan precipitate produced by mitochondrial dehydrogenases of living cells was dissolved by the addition of DMSO (100 μl) to each well. The absorbance of the resultant solutions at 590 nm, which is directly proportional to the number of surviving cells, was recorded using a microplate reader. The percentage of surviving cells was determined by measurement of the absorbance of wells corresponding to untreated control cells. The reported IC_{50} values are based on the mean values from two independent experiments; each concentration level per experiment was evaluated in triplicate, and those values are reported in Table 2.

Cellular uptake studies

A2780 cells were seeded in 6 well plates in RPMI media (3 ml per well, approximately 240000 cells) and pre-incubated for 24 h. Media was then removed and replaced with fresh media containing either **2a**, **3a**, **4a**, **5a** or **6a** (1 ml, 300 μM). The plates were incubated for 5 h followed by removal of media and the cells were washed with phosphate buffered saline (2 x 1 ml). Cells were then detached by the addition of enzyme-free cell dissociation solution (1 ml) then incubation at 37°C for 5 mins. An aliquot (800 μl) of the resulting cell suspension was then taken and the number of cells estimated

using a hemocytometer. The cell suspension was dried under reduced pressure followed by the addition of HNO_3 (70%, 0.5 ml), briefly sonicated then allowed to stand for 18 h followed by the addition of H_2O (4.5 ml). The solutions were filtered through syringe filters (0.20 μm) then kept frozen until analysis. The concentration of Ru (and therefore complex concentration per 10^6 cells) in each solution was determined by ICP-MS analysis, each reported value is the mean of three independent measurements less the background concentration of Ru found in untreated cells.

Measurement of complex lipophilicity

The lipophilicity of **2a**-**6a** was compared by determining log *P* values using the shake-flask method as described in a literature procedure.[9]

^{1}H, ^{13}C and ^{31}P NMR spectra

Figure S27: ^{1}H (top), $^{13}C\{^{1}H\}$ (middle) and $^{31}P\{^{1}H\}$ (bottom) NMR spectra (D_2O) of **1**

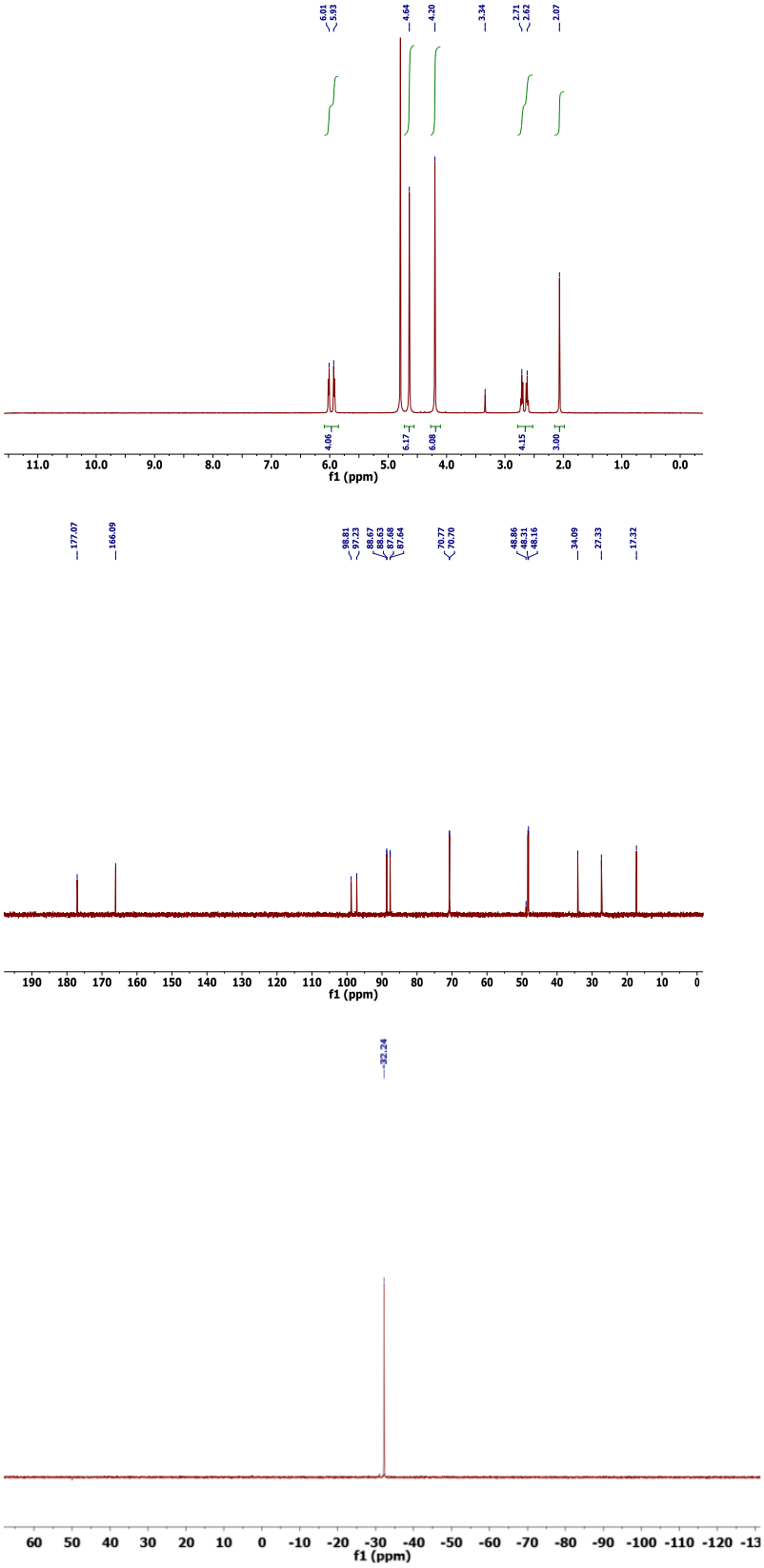


Figure S28: ^{1}H (top), $^{13}C\{^{1}H\}$ (middle) and $^{31}P\{^{1}H\}$ (bottom) NMR spectra (D_2O) of **2a**

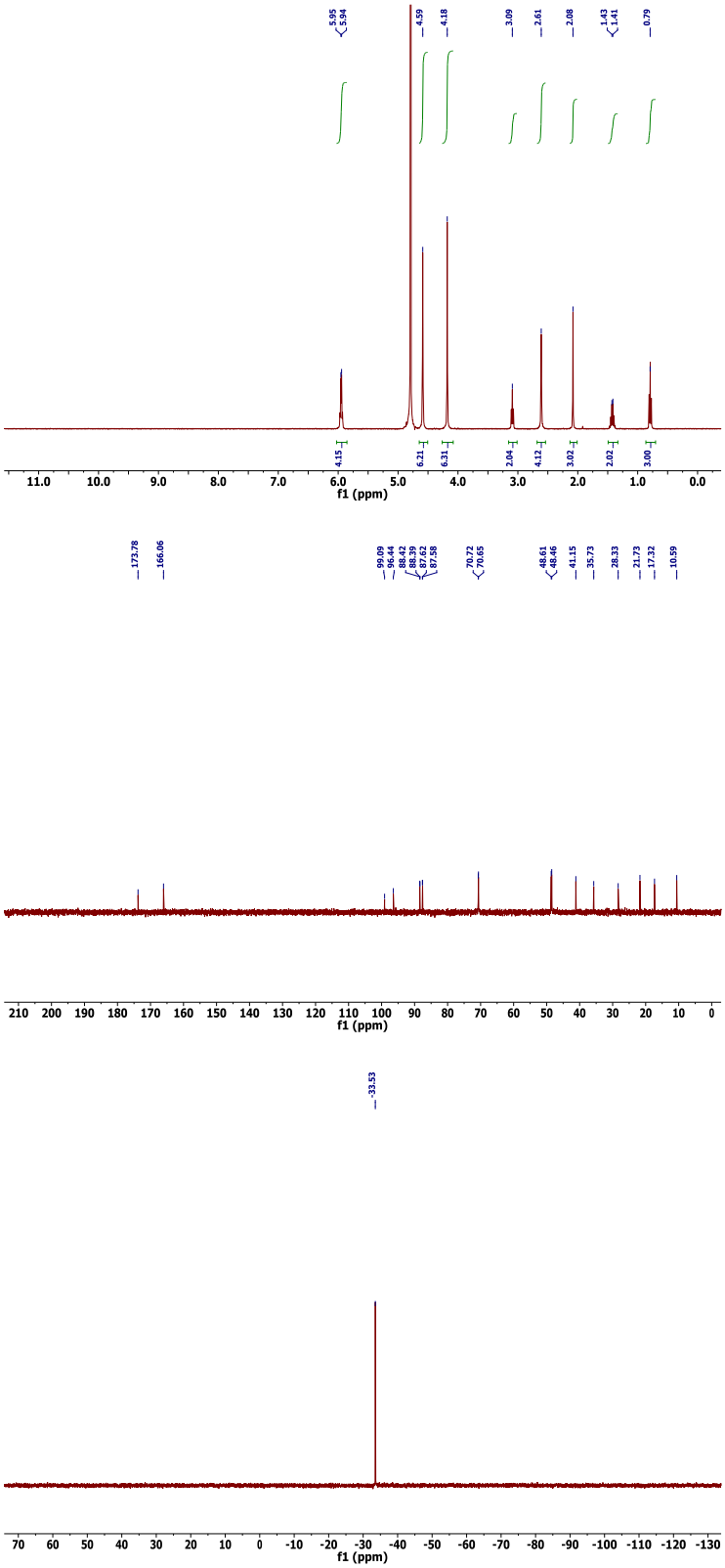


Figure S29: ^{1}H (top), ^{13}C{^{1}H} (middle) and ^{31}P{^{1}H} (bottom) NMR spectra (DMSO-d_6) of **2b**

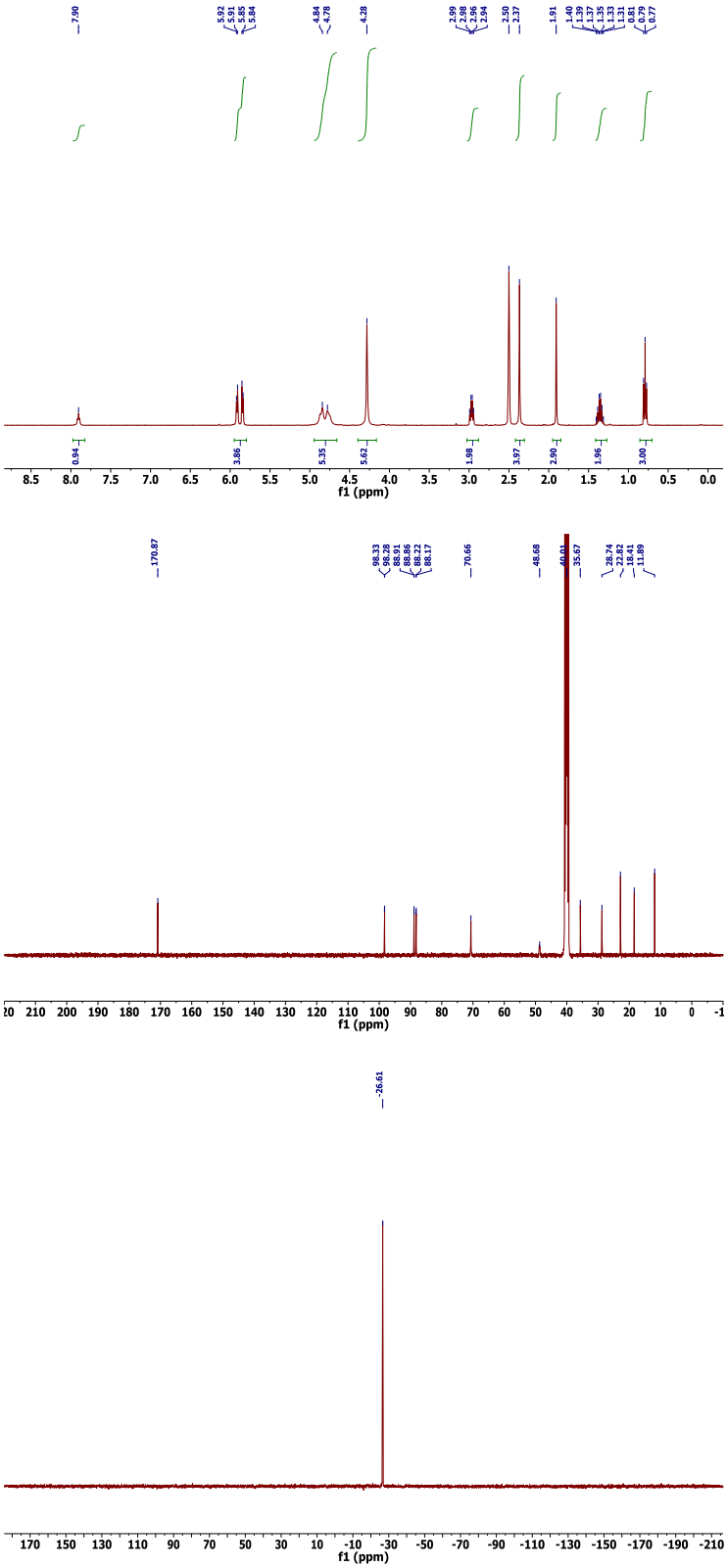


Figure S30: ^{1}H (top), $^{13}C\{^{1}H\}$ (middle) and $^{31}P\{^{1}H\}$ (bottom) NMR spectra (D_2O) of **3a**

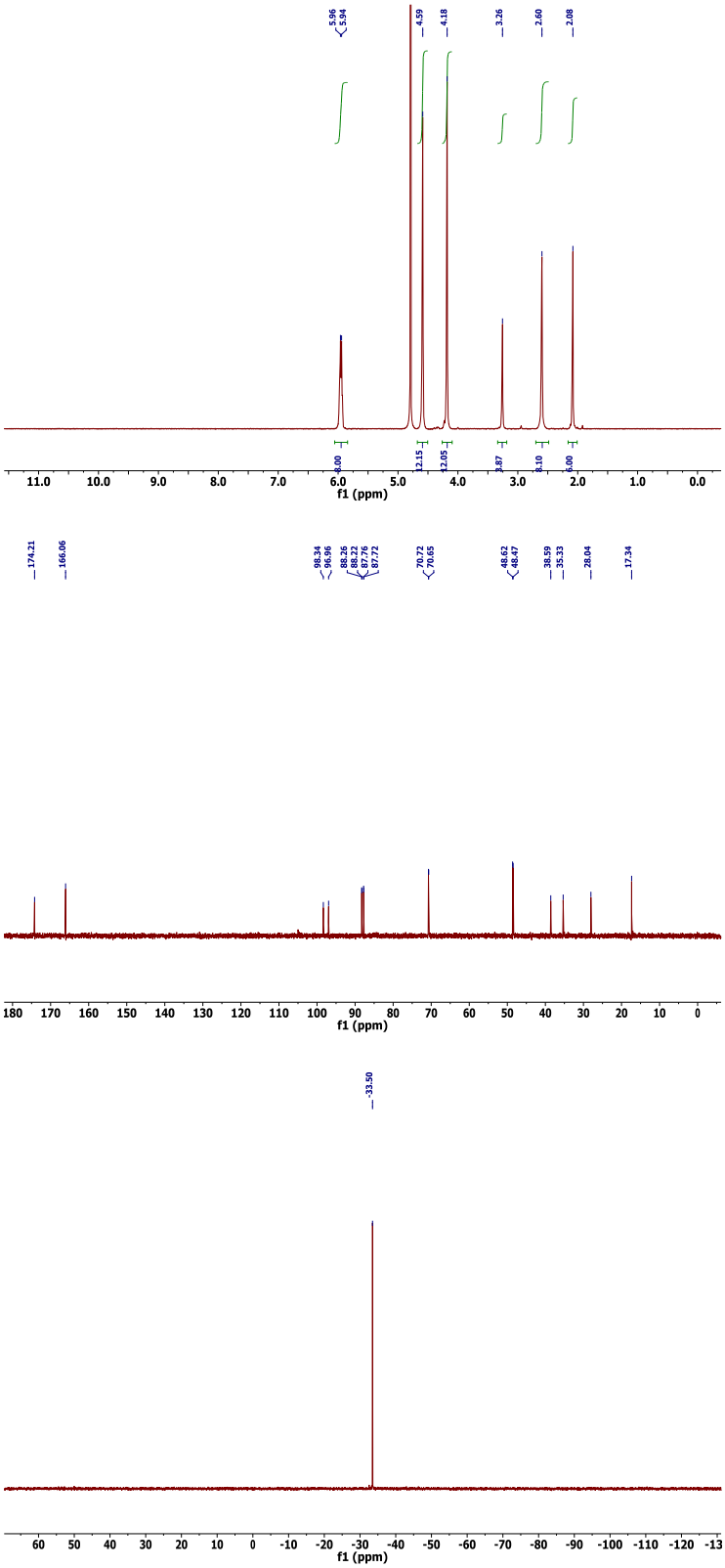


Figure S31: ^{1}H (top), $^{13}C\{^{1}H\}$ (middle) and $^{31}P\{^{1}H\}$ (bottom) NMR spectra (D_2O + 100 mM NaCl) of **3b**

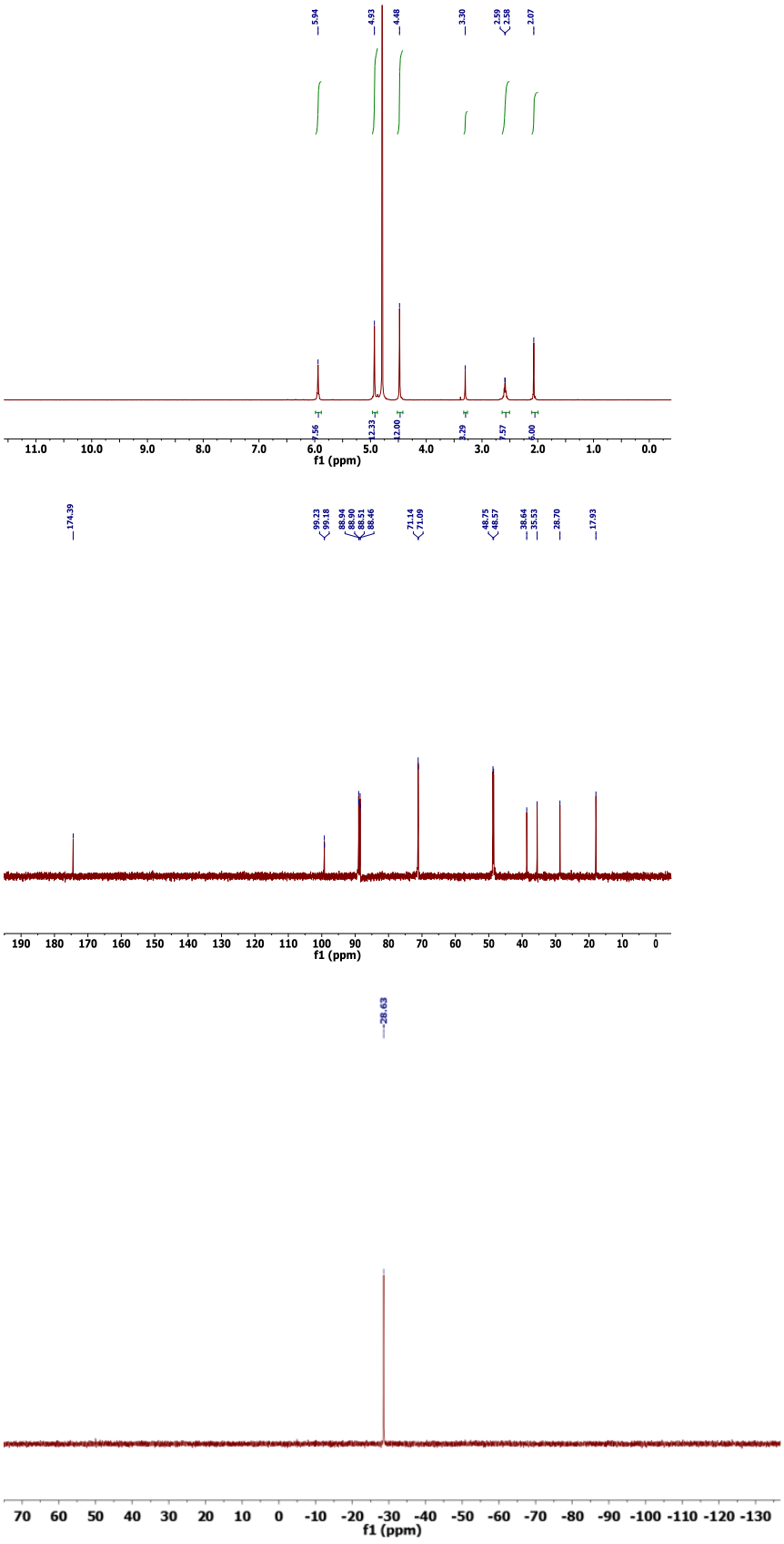


Figure S32: ^{1}H (top), $^{13}C\{^{1}H\}$ (middle) and $^{31}P\{^{1}H\}$ (bottom) NMR spectra (D_2O) of **4a**

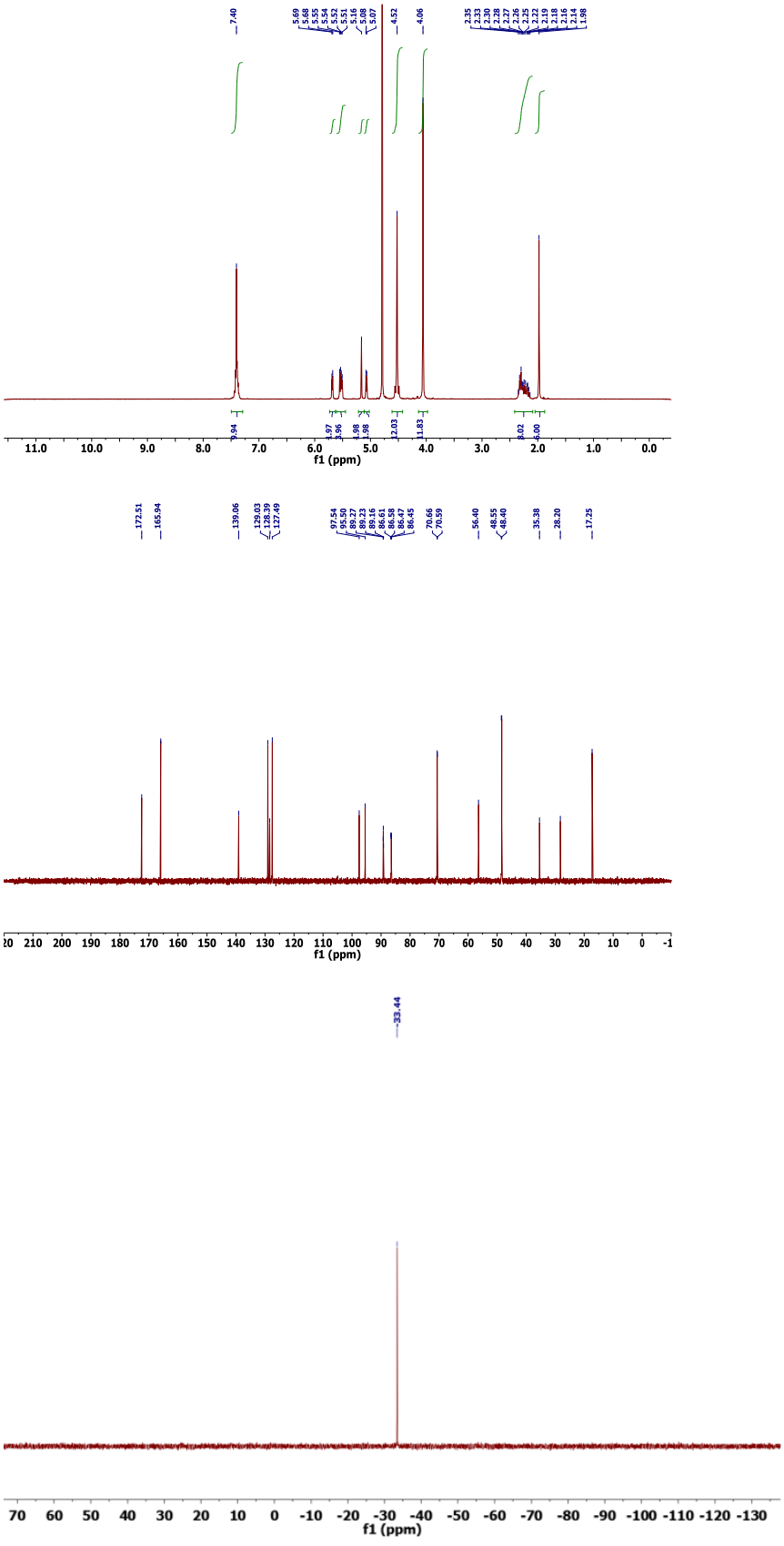


Figure S33: ^{1}H-^{1}H COSY NMR spectrum (D_2O) of **4a**

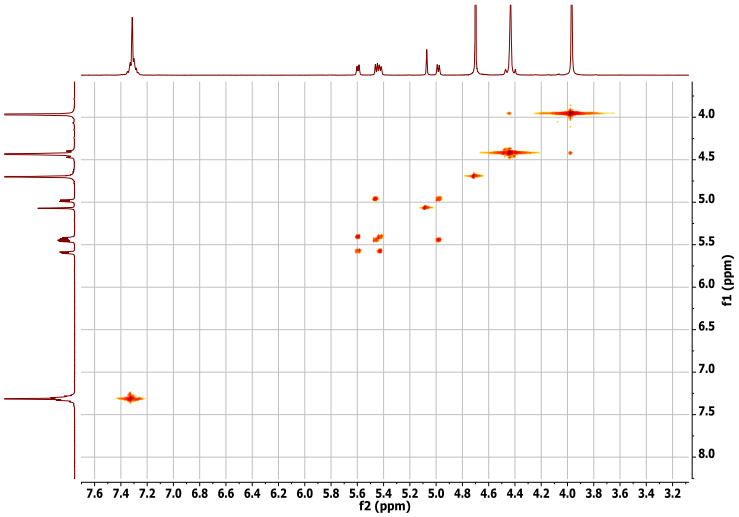


Figure S34: ^{1}H-^{13}C HMBC NMR spectrum (D_2O) of **4a**

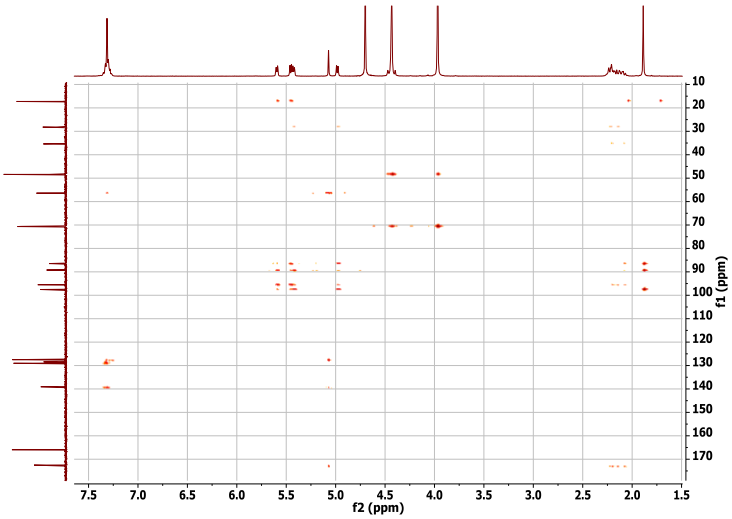


Figure S35: ^{1}H (top), ^{13}C{^{1}H} (middle) and ^{31}P{^{1}H} (bottom) NMR spectra (DMSO-d_6) of **4b**

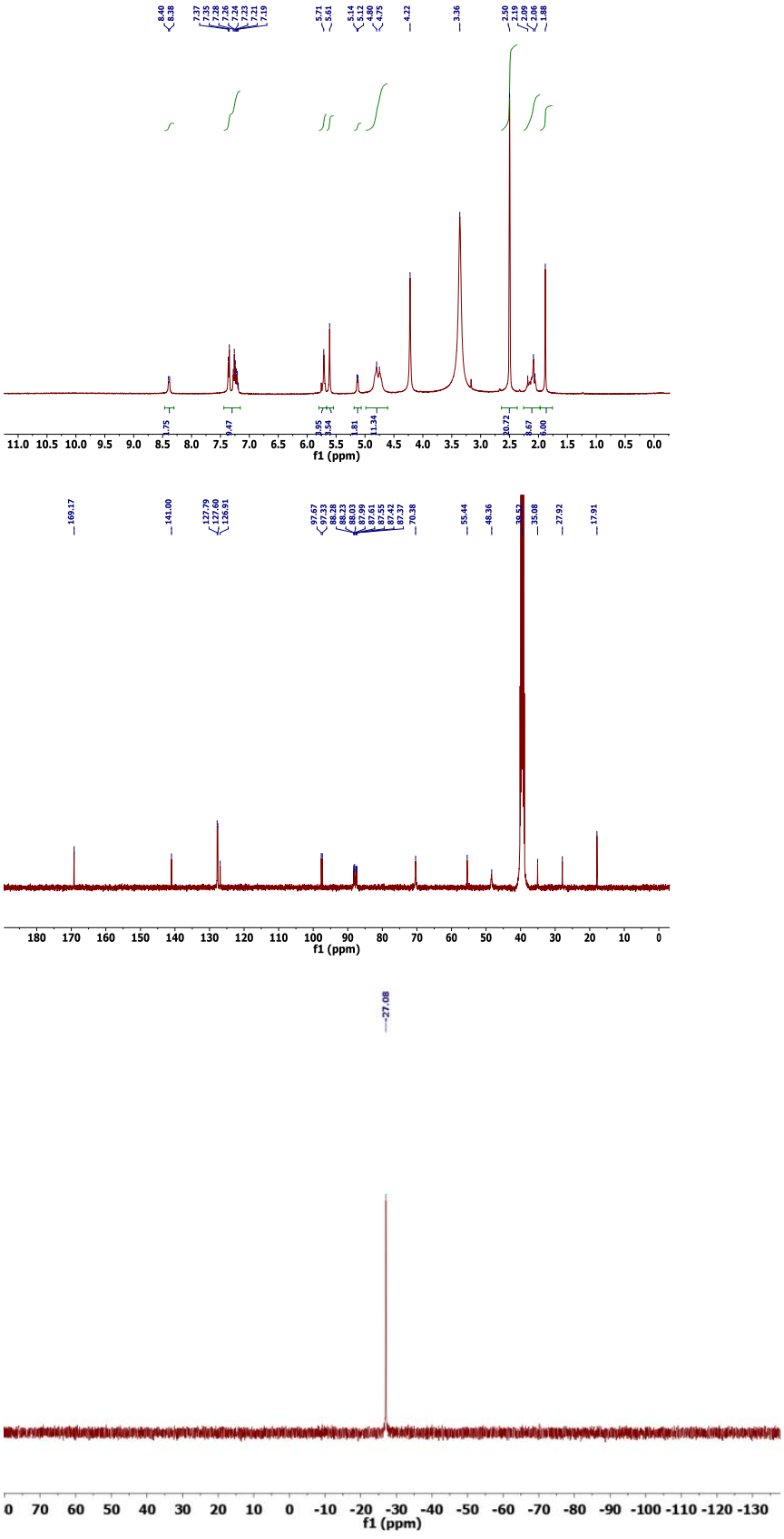


Figure S36: ^{1}H (top), $^{13}C\{^{1}H\}$ (middle) and $^{31}P\{^{1}H\}$ (bottom) NMR spectra (D_2O) of **5a**

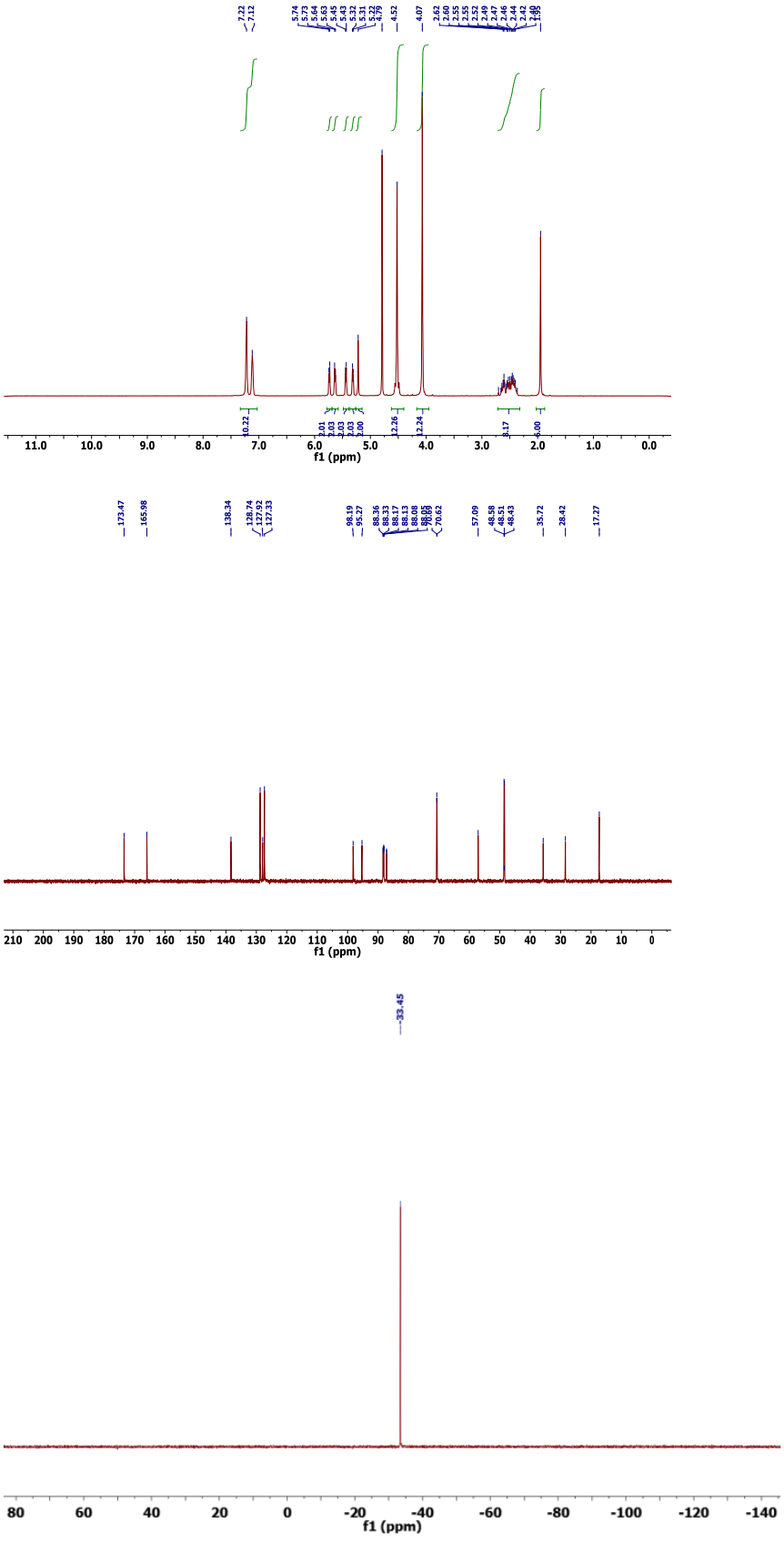


Figure S37: ^{1}H-^{1}H COSY NMR spectrum (D_2O) of **5a**

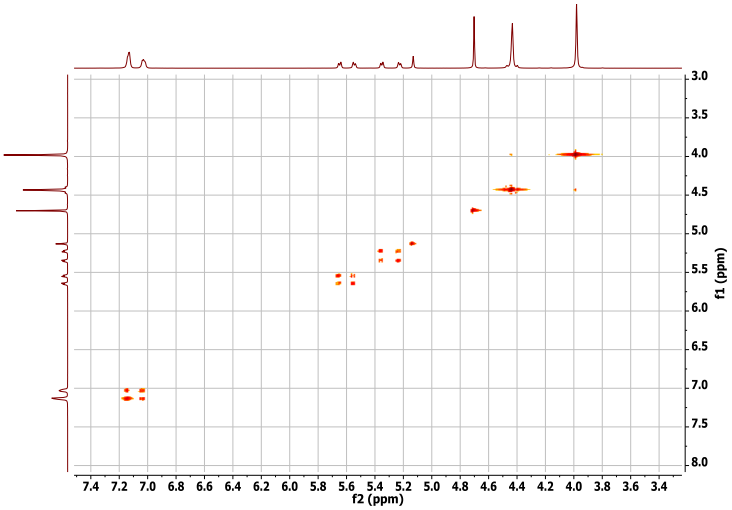


Figure S38: ^{1}H-^{13}C HMBC NMR spectrum (D_2O) of **5a**

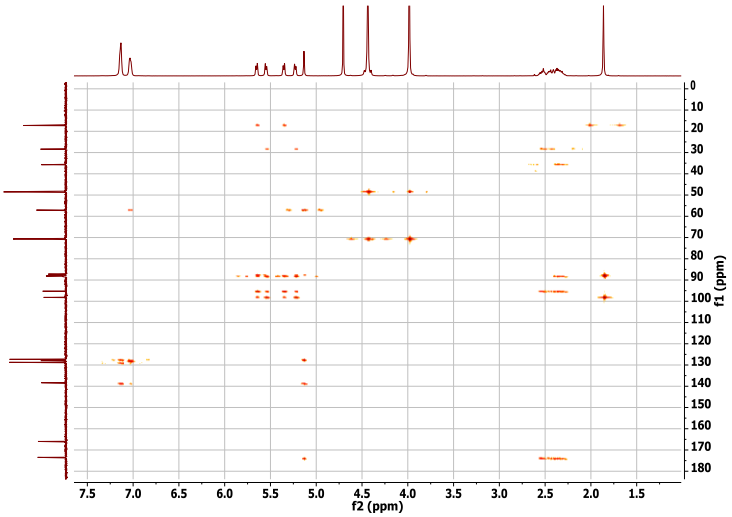


Figure S39: ^{1}H (top), ^{13}C{^{1}H} (middle) and ^{31}P{^{1}H} (bottom) NMR spectra (DMSO-d_6) of **5b**

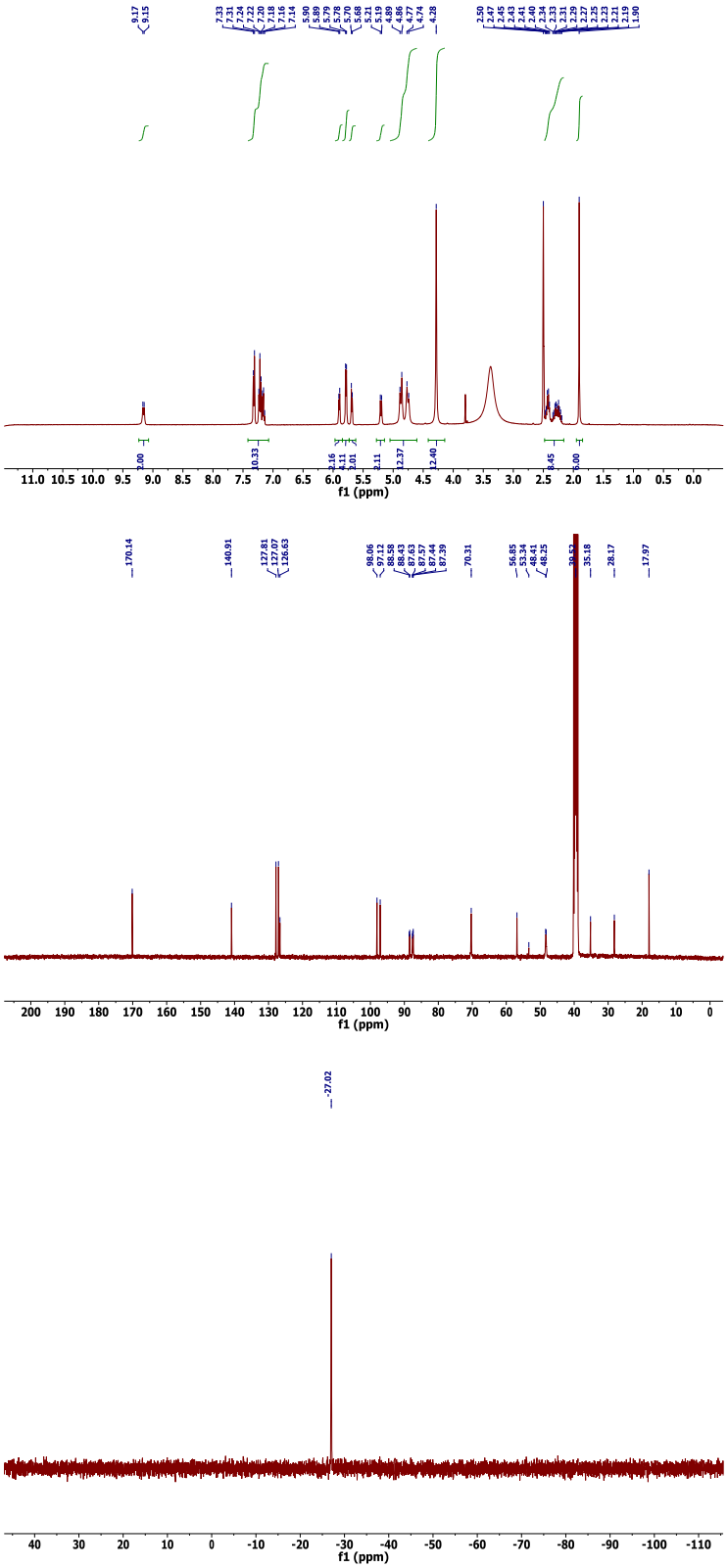


Figure S40: ^{1}H (top), $^{13}C\{^{1}H\}$ (middle) and $^{31}P\{^{1}H\}$ (bottom) NMR spectra (D_2O) of **6a**

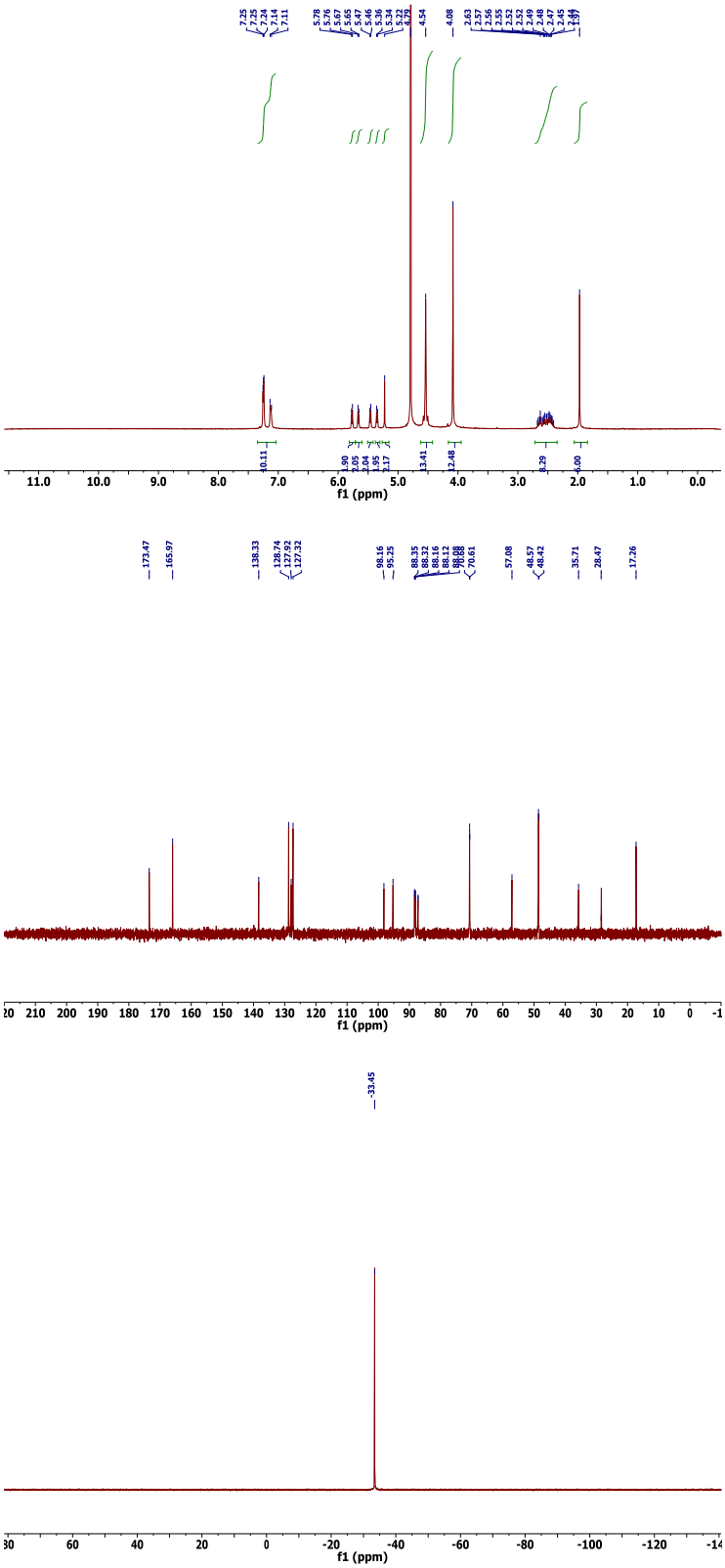
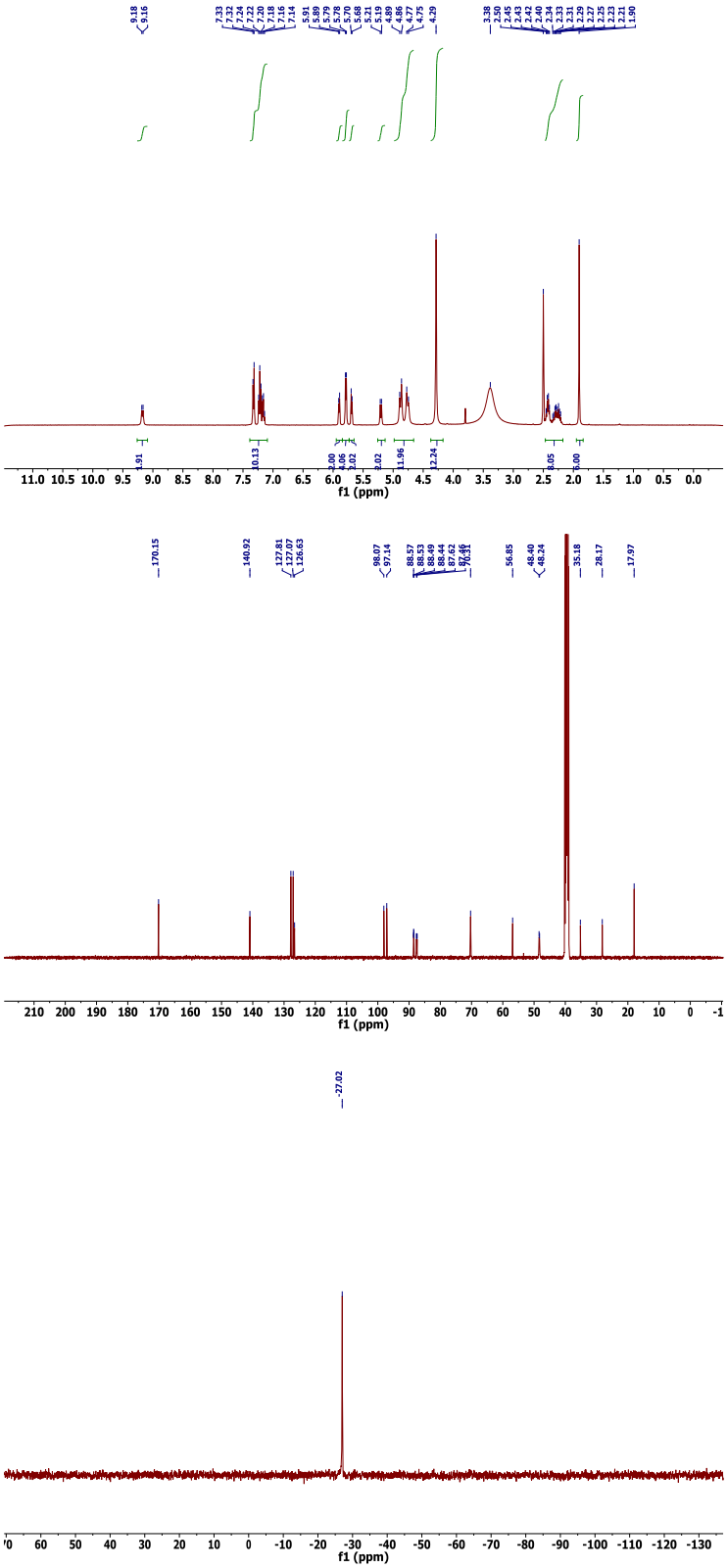


Figure S41: ^{1}H (top), $^{13}C\{^{1}H\}$ (middle) and $^{31}P\{^{1}H\}$ (bottom) NMR spectra (DMSO-d_6) of **6b**



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