Increasing Anti-Cancer Activity with Longer Tether Lengths of Group 9 Cp* Complexes

Stephanie J. Lucas,[a] Rianne M. Lord,[a] Aida M. Basri,[a] Simon J. Allison,[b] Roger M. Phillips,[b] A. John Blacker[a] and Patrick C. McGowan*[a]

[a] School of Chemistry, University of Leeds, Woodhouse Lane, Leeds, LS2 9JT, p.c.mcgowan@leeds.ac.uk

[b] Department of Pharmacy, School of Applied Sciences, University of Huddersfield, Huddersfield, HD1 3DH

Contents

1 General

1b, **1d**, **2b**, **2d**, **3a** and **3b** were prepared as previously described.[1-3] All manipulations involving the synthesis of **1a-d**, **2a-d** and **3a-b** were conducted using standard Schlenk line techniques under an inert atmosphere of dry dinitrogen using a dual vacuum/dinitrogen line or in a Braun Labmaster100 glove box. Dry dinitrogen was obtained by passing dinitrogen gas through a double column of self-indicating phosphorus pentoxide and activated 4Å molecular sieves. Dichloromethane, diethyl ether and methanol were dried using a Pure Solvent MD Solvent Purification System, with solvents purified by copper catalysts and activated alumina columns. All solvents were subsequently stored in ampoules under dinitrogen.

^{1}H and ^{13}C-NMR spectra were recorded on Bruker DPX 300 spectrometer. Microanalyses were obtained by Mr. Ian Blakeley at the University of Leeds Microanalytical Service. X-ray data was collected by Stephanie Lucas. A suitable single crystal was selected and immersed in an inert oil. The crystal was then mounted onto a glass capillary and attached to a goniometer head on a Bruker X8 Apex diffractor using graphite monochromated Mo-Kα radiation (λ= 0.71073 Å) and 1.0° ϕ-rotation frames. The crystal was then cooled to 150K by an Oxford cryostream low temperature device.[4] The full data set was recorded and the images processed using DENZO and SCALEPACK programs.[5]. Structure solution by direct methods was achieved through the use of SHELXS86[6], SIR92[7] or SIR97[8] programs, and the structural model defined by full matrix least squares on F^2 using SHELX97.[6] Molecular graphics were plotted using POV-Ray[9] *via* the XSeed program. Editing of Crystallographic Information files and construction of tables of bond lengths and angles was achieved using WC[10] and PLATON.[11] Hydrogen atoms were placed using idealised geometric positions (with free rotation for methyl groups), allowed to move in a "riding model" along with the atoms to which they were attached, and refined isotropically.

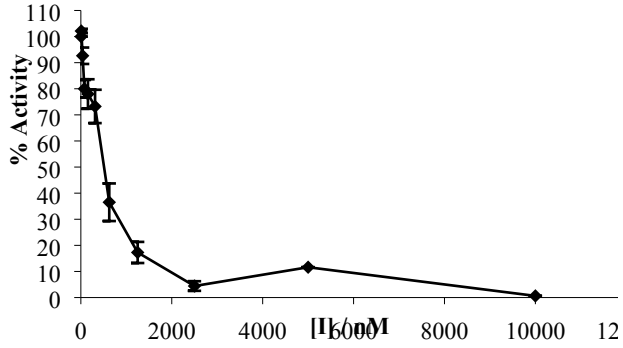
2 Cell Line Chemosensitivity Testing

Cancer cell lines tested for chemosensitivity were HT-29 (human colon adenocarcinoma), A2780 (human ovarian carcinoma) and A2780cis which is a cisplatin-resistant variant of the A2780 cancer cell line. *In vitro* chemosensitivity tests were performed using the MTT assay as previously described.[12] Cells were plated in 96-well plates at a concentration of 2000 cells per well and left to adhere for 24h prior to drug exposure.. All compounds were dissolved in dimethylsulphoxide (DMSO) at a concentration of 25 mM and diluted further in fresh cell culture medium to obtain drug concentrations ranging from 250-0.49 μM with a final DMSO concentration of 0.1% (v/v). Cells were incubated in media containing the indicated range of drug concentrations for 5 days at 37 °C in an atmosphere of 5% CO_2. 20 μL of MTT (5 $mgml^{-1}$) was added to each well and plates incubated for a further 4 hours at 37 °C in an atmosphere of 5% CO_2. Following careful media removal, 150 μL DMSO was added to each well to dissolve any purple formazan crystals formed. A Thermo Scientific Multiskan EX microplate spectrophotometer was used to measure the absorbance at 540 nm. Lanes containing medium only and cells in medium (no drug, DMSO control) were used as blanks for the spectrophotometer and 100% cell survival respectively. Cell survival was determined as the absorbance of treated cells divided by the absorbance of no drug control and expressed as a percentage. The IC_{50} values were determined from plots of % survival against drug concentration. Each experiment was repeated a minimum of three times and a mean value obtained.

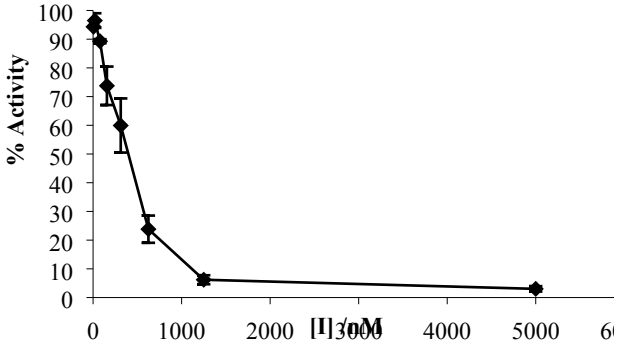
3 Inhibition of thioredoxin reductase activity

Thioredoxin reductase (TrxR) activity and quantification of any inhibition of activity by complexes 2b-2d, 3a and 3b was determined as previously described.[12-13] In brief, activity was assayed by the specific reduction of the substrate 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) by recombinant TrxR (Sigma Aldrich) to TNB. Formation of TNB was quantified in real-time by kinetic spectrophotometric measurement at 412nm on a Cary60 UV/Vis spectrophotometer over a 1 min linear period immediately following addition of substrate to start the reaction. To determine any potential inhibitory effect of the compounds on TrxR activity, a range of known concentrations of the compounds were incubated with TrxR for 30 seconds prior to the

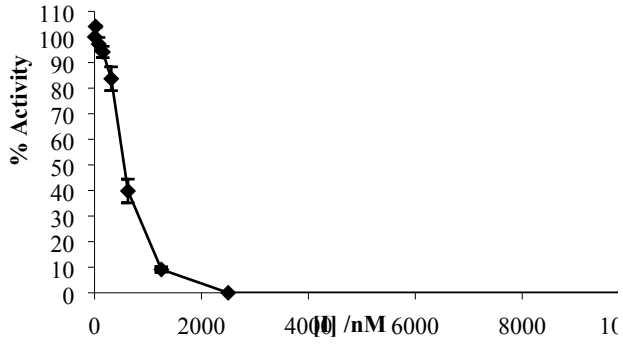
addition of substrate to start the reaction and measurement of the change in absorbance at 412nm. The experiment was performed with TrxR enzyme and no compound (solvent control) to determine the rate of reaction with no TrxR inhibition. The reaction rates in the presence of different concentrations of compounds were compared to controls and used to calculate % TrxR enzyme activity and generate plots of % TrxR activity versus compound concentration to obtain IC_{50} values (concentration that inhibited 50% of enzyme activity) (**Figure S1**)



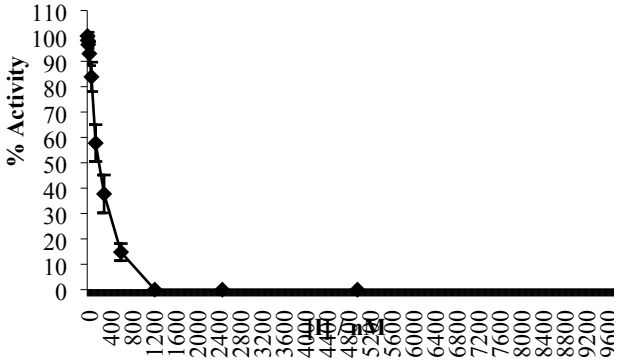
Compound **2b**, IC_{50} = 510.2 nM



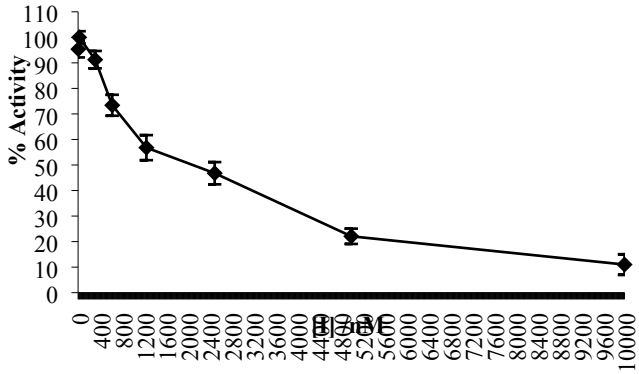
Compound **2c**, IC_{50} = 398.4 nM



Compound **2d**, IC_{50} = 552.3 nM



Compound **3a**, IC_{50} = 216.9 nM



Compound **3b**, IC_{50} = 2098 nM

Figure S1 Inhibition of thioredoxin reductase 1 (Trx-R) activity by compounds **2b-d** and **3a-b**

4 Experimental

Synthesis of $C_5(CH_3)_4C_3H_6OH$ (1a)

Under a nitrogen atmosphere, lithium wire (8 g, 1.15 mol, washed with hexane) was added to anhydrous diethyl ether (250 ml) and the lithium suspension was vigorously stirred. 2-bromo-2-butene (40 g, 0.30 mol, mixture of cis and trans isomers) was added to a dropping funnel and a small portion added to the reaction mixture to initiate the reaction. Diethyl ether (70 ml) was also added to the remaining 2-bromo-2-butene, which was then added at a rate that maintained a gentle reflux. After complete addition of 2-bromo-2-butene the reaction was stirred at r.t. for 2 hours. γ-Butyrolactone (22 g, 0.26 mol) in diethyl ether (50 ml) was then added dropwise and the mixture stirred for 1 hour. The resulting mixture was poured into saturated NH_4Cl (aq) (600 ml) and after separating the ether layer, the aqueous layer was extracted with *tert*-butyl methyl ether (3 x 100 ml). The combined ether layers were washed with brine, dried over magnesium sulfate and concentrated to *ca*. 100 ml. 10% aqueous HCl (300 ml) was added to the resulting concentrate and the biphasic mixture was stirred for 3h at r.t. After separating the ether layer, the aqueous layer was extracted with *tert*-butyl methyl ether (3 x 50 ml). The combined organic layers were washed with water (2 x 100 ml), dried over Na_2SO_4, and the solvent evaporated to leave a brown oil, which was purified through a large plug of silica (hexane/EtOAc 10:1 as eluent) gave a pale yellow oil containing 3 regioisomers of **1a** (19.4 g, 0.11 mmol, 41.4%). **1a** was used without further purification. ES-MS (CH_2Cl_2, m/z): 181.2 [M+H].

^{1}H NMR (300 MHz, $CDCl_3$, 300 K) 3.63 (t, $^3J(^1H\text{-}^1H)$ = 7.5 Hz, 7.5 Hz, 2H, C$\underline{H}_2$OH), 2.13-2.50 (m, 2H, C$\underline{H}_2$CH$_2$CH$_2$OH), 1.57-1.66 (m, 2H, C$\underline{H}_2$CH$_2$OH), 1.55 (s, 6H, 2 × CH_3), 1.51 (s, 3H, CH_3), 0.93 (2 × d, $^3J(^1H\text{-}^1H)$ = 7.0 Hz, 3H, CH_3). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$, 300 K) 144.1, 139.2, 135.4 and 134.7 (alkene C of Cp ring), 68.7, 51.4 and 49.4 (allyl CH of Cp ring), 63.1 (CH_2OH), 31.8 ($\underline{C}H_2CH_2OH$), 26.9 ($\underline{C}H_2CH_2CH_2OH$), 17.4, 15.9, 15.1, 13.4 and 11.8 (CH_3 groups of Cp ring).

Synthesis of $C_5(CH_3)_4C_9H_{18}OH$ (1c)

Triethylamine (9.4 g, 0.09 mol) was added to methyl 10-hydroxydecanoate (9.0 g, 0.05 mmol) in dichloromethane (150 ml). Trimethylsilyl chloride (5.3 g, 0.05 mol) was added dropwise. The solution was stirred at ambient temperature overnight. The resulting solution was washed with saturated ammonium chloride solution (50 ml). The water layer was extracted with diethyl ether (2 × 30 ml) and the ether and dichloromethane layers were combined, washed with brine (30 ml), dried over magnesium sulfate, evaporated to dryness and purified by column chromatography (hexane:ethyl acetate 4:1 as eluent) to afford a yellow oil **A** (8.7 g, 0.03 mmol, 63%).

Under a nitrogen atmosphere, lithium wire (0.9 g, 0.13 mol, washed with hexane) was added to anhydrous diethyl ether (100 ml) and the lithium suspension was vigorously stirred. 2-bromo-2-butene (9.4 g, 0.07 mol, mixture of *cis* and *trans* isomers) was added in small portions. The reaction was initiated through gentle heating, then left at ambient temperature for two hours. **A** (8.7 g, 0.03 mol) in diethyl ether (100 ml) was then added dropwise and the mixture stirred for 1 hour. The resulting mixture was poured into sat NH_4Cl (aq) (100 ml) and after separating the ether layer, the aqueous layer was extracted with *tert*-butyl methyl ether (3 x 50 ml). The combined ether layers were washed with brine (50 ml), dried over magnesium sulfate and concentrated to *ca*. 50 ml. 10% aqueous HCl (100 ml) was added to the resulting concentrate and the biphasic mixture was stirred for 3h at r.t. After separating the ether layer, the aqueous layer was extracted with *tert*-butyl methyl ether (3 x 50 ml). The combined organic layers were washed with water (2 x 100 ml), dried over magnesium sulfate, and the solvent evaporated to leave a brown oil, which was purified through a large plug of silica (hexane/ethyl acetate 5:1 as eluent) to give **1c** as a pale yellow oil with 3 regioisomers (3.5 g, 0.01 mmol, 42%). **1c** was used without further purification. **ES-MS** (CH_2Cl_2, m/z): 285.1 [M-2H+Na].

^{1}H NMR (300 MHz, $CDCl_3$, 300 K) 3.64 (t, $^3J(^1H\text{-}^1H)$ = 6.6 Hz, 2H, C$\underline{H}_2$OH), 2.23-2.65 (m, 2H, CH_2), 1.81 (br. s, 3H, CH_3), 1.77 (br. s, 6H, 2 × CH_3), 1.65-1.75 (m, 2H, CH_2), 1.49-1.65 (m, 3H, CH_2 and allyl CH), 1.30 (br. s, 10H, 10 × CH_2), 1.00 (2 × d, $^3J(^1H\text{-}^1H)$ = 7.6 Hz, 3H, CH_3). **$^{13}C\{^1H\}$ NMR** (75 MHz, $CDCl_3$, 300 K) 142.8, 142.8, 138.8, 138.1, 138.0, 135.5, 135.2, 134.2, 134.0, 133.7 and 130.1 (alkene C of Cp ring), 64.4, 51.4 and 49.3 (allyl CH of Cp ring), 63.0 (CH_2OH), 32.7 (CH_2), 29.5 (br. s,

CH_2), 29.3 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 25.7 (CH_2), 25.0 (CH_2), 23.5, 22.6, 14.0, 11.6 and 11.0 (CH_3 groups of Cp ring).

Synthesis of [Rh{η5-$C_5(CH_3)_4C_3H_6OH$}Cl_2]$_2$ (2a)

Under a nitrogen atmosphere, rhodium trichloride hydrate (0.10 g, 0.38 mmol) was added to 1-(3-hydroxypropyl)-2,3,4,5-tetramethylcyclopentadiene (0.14 g, 0.78 mmol) in MeOH (30 ml) and the mixture was heated under reflux for 15 h. After evaporation of the solvent, the powder was dissolved in dichloromethane and the product precipitated using hexane, and collected by filtration to yield **2a** as red crystals suitable for X-ray crystallography (0.03 g, 0.04 mmol, 22%). **ES-MS** (CH_2Cl_2, m/z): 671.0 [M-Cl]. **Anal. Found: C:** 39.2, H: 5.2, Cl: 22.2% **Anal. Calculated for $C_{24}H_{38}Cl_4O_2Rh_2 \cdot 0.35CH_2Cl_2$:** C: 39.7, H: 5.3, Cl: 22.6%. **^{1}H NMR** (300 MHz, $CDCl_3$, 300 K) 3.66 (br. s, 4H, 2 × C$\underline{H}_2$OH), 2.37 (t, ^1J(^{1}H-^{1}H) = 7.9 Hz, 4H, 2 × CH_2Cp), 1.67-1.80 (m, 4H, 2 × C$\underline{H}_2$C$\underline{H}_2$OH), 1.65 (s, 12H, 4 × CH_3), 1.63 (s, 12H, 4 × CH_3). **^{13}C{^{1}H} NMR** (125 MHz, $CDCl_3$, 300 K) 96.1 (d, ^1J(^{13}C-^{103}Rh) = 8.5 Hz, $\underline{C}CH_3$), 95.4 (d, ^1J(^{13}C-^{103}Rh) = 8.7 Hz, $\underline{C}CH_3$), 94.5 (d, ^1J(^{13}C-^{103}Rh) = 8.9 Hz, $\underline{C}CH_3$), 62.2 (CH_2OH), 29.7 ($\underline{C}H_2CH_2OH$), 20.8 ($\underline{C}H_2$Cp), 9.4 (s, 2 × CH_3), 9.4 (s, 2 × CH_3).

Synthesis of [Rh{η5-$C_5(CH_3)_4C_9H_{18}OH$}Cl_2]$_2$ (2c)

Under a nitrogen atmosphere, rhodium trichloride hydrate (0.20 g, 0.76 mmol) was added to 1-(9-hydroxynonyl)-2,3,4,5-tetramethylcyclopentadiene (**2.3**) (0.40 g, 1.51 mmol) in MeOH (30 ml) and the mixture was heated under reflux for 15 h. After evaporation of the solvent, the powder was dissolved in a minimum of dichloromethane and the product precipitated using hexane, collected by filtration. The precipitation step was repeated and the powder dried *in vacuo* to give **2c** as a red powder (0.34 g, 0.39 mmol, 51%). **ES-MS** (CH_2Cl_2, m/z): 837.2 [M-Cl]. **Anal. Found:** C: 49.7, H: 7.2, Cl: 15.9% **Anal. Calculated for $C_{36}H_{62}Cl_4O_2Rh_2$:** C: 49.4, H: 7.2, Cl: 16.2%. **^{1}H NMR** (300 MHz, $CDCl_3$, 300 K) 3.63 (t, ^3J(^{1}H-^{1}H) = 6.5 Hz, 4H, 2 × C$\underline{H}_2$OH), 2.25 (m, ^1J(^{1}H-^{1}H) = 6.7 Hz, 4H, 2 × CH_2Cp), 1.75-2.00 (m, 4H, C$\underline{H}_2CH_2OH$), 1.63 (s, 12H, 4 × CH_3), 1.61 (s, 12H, 4 × CH_3), 1.50-1.59 (m, 4H, 2 × CH_2), 1.30 (br. s, 20H, 10 × CH_2). **^{13}C{^{1}H} NMR** (75 MHz, $CDCl_3$, 300 K) 96.2 (d, ^1J (^{13}C-^{103}Rh) = 9.4 Hz, $\underline{C}CH_3$), 94.5 (d, ^1J (^{13}C-^{103}Rh) = 9.4 Hz, $\underline{C}CH_3$), 94.2 (d, ^1J (^{13}C-^{103}Rh) = 9.3 Hz, $\underline{C}CH_3$), 63.0 (s, CH_2OH), 32.7 (s, CH_2), 29.6 (s, CH_2), 29.3 (s, CH_2), 29.1 (s, CH_2), 27.5 (s, CH_2), 25.6 (s, CH_2), 24.0 (s, $\underline{C}H_2$Cp), 9.4 (s, 2 × CH_3), 9.4 (s, 2 × CH_3).

Synthesis of Rh{η5-$C_5(CH_3)_4C_9H_{18}OH$}Cl_2Py (4a)

[Rh{η^5-$C_5(CH_3)_4C_5H_{10}OH$}Cl_2]$_2$ (0.30 g, 0.39 mmol) was dissolved in an excess of pyridine (25 ml) and the solution was stirred for 20 hours. After evaporation of the solvent, the red powder was dissolved in a minimum of dichloromethane and the product precipitated using hexane, then collected by filtration. The precipitation process was repeated and the product was dried *in vacuo* to give **4a** as a red powder (0.32 g, 0.70 mmol, 88%). Red crystals suitable for single crystal X-ray diffraction were obtained using vapour diffusion (chloroform/pentane). **Anal. Found:** C: 49.9, H: 6.4, N: 2.8, Cl: 15.8%. **Anal. Calculated for $C_{19}H_{28}NCl_2ORh$:** C 49.6, H 6.1, N: 3.0, Cl: 15.4%. **^{1}H NMR** (300 MHz, $CDCl_3$, 300 K) 8.99 (br. s, 2H, 2 × CH *ortho* to N of pyridine), 7.78 (br. s, 1H, CH *para* to N of pyridine), 7.38 (br. s, 2H, 2 × CH *meta* to N of pyridine), 3.64 (br. s, 2H, C$\underline{H}_2$OH), 2.14 (br. s, 2H, C$\underline{H}_2(CH_2)_4OH$), 1.67 (br. s, 2H, C$\underline{H}_2CH_2OH$), 1.61 (s, 6H, 2 × CH_3 *meta* to alkyl chain on Cp‡), 1.60 (s, 6H, 2 × CH_3 *ortho* to alkyl chain on Cp‡), 1.44 (br. s, 4H, C$\underline{H}_2$C$\underline{H}_2CH_2OH$). **^{13}C{^{1}H} NMR** (75 MHz, $CDCl_3$, 300 K) 153.6 (2 × C *ortho* to N on pyridine ring), 137.8 (C *para* to N on pyridine ring), 125.3 (2 × C *meta* to N on pyridine ring), 96.0 (d, ^1J(^{13}C-^{103}Rh) = 9.0 Hz, quaternary C of Cp‡), 94.4 (d, ^1J (^{13}C-^{103}Rh) = 8.3 Hz, 2C, quaternary C of Cp‡), 94.1 (d, ^1J (^{13}C-^{103}Rh) = 8.7 Hz, 4 C, quaternary C of Cp‡), 62.4 (CH_2OH), 32.3 ($\underline{C}H_2CH_2OH$), 27.9 ($\underline{C}H_2CH_2CH_2OH$), 26.0 ($\underline{C}H_2CH_2CH_2CH_2OH$), 23.7 ($\underline{C}H_2CH_2CH_2CH_2CH_2OH$), 9.0 (2 × CH_3), 8.9 (2 × CH_3).

Synthesis of Ir{η5-$C_5(CH_3)_4C_3H_6OH$}Cl_2Py (4b)

$[Ir\{\eta^5\text{-}C_5(CH_3)_4C_5H_{10}OH\}Cl_2]_2$ (0.50 g, 0.66 mmol) was dissolved in an excess of pyridine (25 ml) and the solution was stirred for 20 hours. After evaporation of the solvent, the residue was dissolved in a minimum of dichloromethane and the product precipitated using hexane, then collected by filtration and dried in vacuo to give **4b** as a yellow powder (0.57 g, 1.13 mmol, 86%). Yellow crystals suitable for single crystal X-ray diffraction were obtained via vapour diffusion (chloroform/diisopropylether). **Anal. Found:** C: 41.8, H: 5.2, N: 2.4, Cl: 12.5%. **Anal. Calculated fo r$C_{19}H_{28}NCl_2OIr$:** C: 41.5, H: 5.1, N: 2.6, Cl: 12.9%. **^{1}H NMR** (300 MHz, $CDCl_3$, 300 K) 8.97 (br. dd, $^3J(^1H\text{-}^1H)$ = 6.9 Hz, $^4J(^1H\text{-}^1H)$ = 1.4 Hz, 2H, 2 × CH *ortho* to N of pyridine), 7.74 (tt, $^3J(^1H\text{-}^1H)$ = 7.6 Hz, $^4J(^1H\text{-}^1H)$ = 1.4 Hz, 1H, CH *para* to N of pyridine), 7.35 (ddd, $^3J(^1H\text{-}^1H)$ = 7.6 Hz, $^3J(^1H\text{-}^1H)$ = 6.7 Hz, $^4J(^1H\text{-}^1H)$ = 1.4 Hz, 2H, 2 × CH *meta* to N of pyridine), 3.64 (m, 2H, C$\underline{H}_2$OH), 2.03 (m, 2H, C$\underline{H}_2(CH_2)_4OH$), 1.58 (m, 2H, C$\underline{H}_2CH_2OH$), 1.56 (s, 6H, 2 × CH_3 *meta* to alkyl chain on Cp‡), 1.55 (s, 6H, 2 × CH_3 *ortho* to alkyl chain on Cp‡), 1.34-1.50 (m, 4H, C$\underline{H}_2$C$\underline{H}_2CH_2OH$). **^{13}C{^{1}H} NMR** (75 MHz, $CDCl_3$, 300 K) 153.5 (2 × C *ortho* to N on pyridine ring), 137.7 (C *para* to N on pyridine ring), 125.4 (2 × C *meta* to N on pyridine ring), 87.2 (quaternary C of Cp‡), 86.2 (quaternary C of Cp‡), 85.9 (quarternary C of Cp‡), 62.6 (CH_2OH), 32.4 (C$\underline{H}_2CH_2OH$), 27.8 (C$\underline{H}_2CH_2CH_2OH$), 26.0 (C$\underline{H}_2CH_2CH_2CH_2OH$), 23.6 (C$\underline{H}_2CH_2CH_2CH_2CH_2OH$), 8.6 (2 × CH_3), 8.5 (2 × CH_3).

Synthesis of Rh{η5-$C_5(CH_3)_4C_3H_6OH$}Cl($C_{12}H_8ClN_2O$) (5a)

Pyridine-2-carboxylic acid (3-chloro-phenyl) amide (0.06 g, 0.26 mmol) and sodium bicarbonate (0.02 g, 0.26 mmol) were added to a stirred suspension of $[Rh\{\eta^5\text{-}C_5(CH_3)_4C_5H_{10}OH\}Cl_2]_2$ (0.10 g, 0.13 mmol) in water (25 ml). The mixture was stirred at 80 °C for 18 hours. The resulting suspension was filtered and the orange powder dried *in vacuo* to give **5a** (0.12 g, 0.21 mmol, 79 %). **ES-MS** (CH_2Cl_2, m/z): 541.1 [M-Cl]. **Anal. Found:** C: 53.3, H: 5.5, N: 4.8, Cl: 12.1%. **Anal. Calculated for $C_{26}H_{31}N_2Cl_2O_2Rh\cdot0.5H_2O$:** C: 53.7, H: 5.4, N: 4.6, Cl: 11.8%. **^{1}H NMR** (300 MHz, $CDCl_3$, 300 K) 8.63 (br. d, ^3J (^{1}H-^{1}H) = 5.7 Hz, 1H, CH of pyridyl *ortho* to N), 8.16 (br. d, ^3J (^{1}H-^{1}H) = 8.0 Hz, 1H, CH of pyridyl *meta* to N and *ortho* to CON), 7.96 (vtd (ddd), ^3J (^{1}H-^{1}H) = 7.7 Hz, ^3J (^{1}H-^{1}H) = 7.7 Hz, ^4J (^{1}H-^{1}H) = 1.4 Hz, 1H, CH of pyridyl *para* to N), 7.84 (vt (dd), ^4J (^{1}H-^{1}H) = 1.9 Hz, ^4J (^{1}H-^{1}H) = 1.9 Hz, 1H, CH *ortho* to NCO and Cl), 7.72 (br. d, ^3J (^{1}H-^{1}H) = 8.1 Hz, 1H, CH of phenyl *para* to NCO), 7.53 (ddd, ^3J (^{1}H-^{1}H) = 9.5 Hz, ^3J (^{1}H-^{1}H) = 5.7 Hz, ^4J (^{1}H-^{1}H) = 1.4 Hz, 1H, CH of pyridyl *meta* to N, *para* to CON), 7.21 (m, 1H, CH of phenyl *meta* to NCO and Cl), 7.07 (br. d, ^3J (^{1}H-^{1}H) = 7.9 Hz, 1H, CH *para* to Cl), 3.62 (br. s, 2H, C$\underline{H}_2$OH), 1.88 (t, ^3J (^{1}H-^{1}H) = 7.0 Hz, 1H, C$\underline{H}_2(CH_2)_4OH$), 1.50-1.60 (br. m, 2H, C$\underline{H}_2CH_2OH$), 1.46 (s, 3H, CH_3 *meta* to alkyl chain on Cp‡), 1.46 (s, 3H, CH_3 *meta* to alkyl chain on Cp‡), 1.45 (s, 3H, CH_3 *ortho* to alkyl chain on Cp‡), 1.41 (s, 3H, CH_3 *ortho* to alkyl chain on Cp‡), 1.33-1.39 (m, 4H, C$\underline{H}_2$C$\underline{H}_2CH_2OH$). **^{13}C{^{1}H} NMR** (125 MHz, CD_2Cl_2, 300 K) 167.0 (NCO), 156.2 (C$\underline{C}$ON), 150.6 (C$\underline{N}$CO), 150.5 (CH *ortho* to N on pyridyl ring), 139.4 (C *para* to N on pyridyl ring), 133.6 (CCl), 129.4 (CH *meta* to Cl and NCO), 127.7 (CH *para* to CO and *meta* to N on pyridyl ring and CH *ortho* to NCO and Cl), 126.2 (CH *ortho* to CO and *meta* to N on pyridyl ring), 126.2 (CH *para* to NCO), 124.1 (CH *para* to Cl), 97.0 (d, ^1J (^{13}C-^{103}Rh) = 8.4 Hz, quaternary C of Cp‡), 95.2-95.7 (m, quarternary C of Cp‡), 62.8 (CH_2OH), 32.8 (2C, 2 × C$\underline{H}_2CH_2OH$), 28.2 (C$\underline{H}_2CH_2CH_2OH$), 26.3 (C$\underline{H}_2CH_2CH_2CH_2OH$), 23.9 (C$\underline{H}_2CH_2CH_2CH_2CH_2OH$), 9.1 ($CH_3$), 9.0 (2 × CH_3), 9.0 (CH_3).

Synthesis of Ir{η5-$C_5(CH_3)_4C_3H_6OH$}Cl($C_{12}H_8ClN_2O$) (5b)

Triethylamine (0.04 ml, 0.28 mmol) was added.to a solution of $[Ir\{\eta^5\text{-}C_5(CH_3)_4C_5H_{10}OH\}Cl_2]_2$ (0.10 g, 0.11 mmol) and Pyridine-2-carboxylic acid (3-chloro-phenyl) amide (0.05 g, 0.22 mmol) in dichloromethane (25 ml). After 19 hours, the resulting yellow solution was washed with water (2 × 10 ml) and brine (10 ml), dried using sodium sulfate, filtered and the solvent evaporated. **5b** was recrystallised using vapour diffusion (dichloromethane/pentane solvent system) (0.07 g, 0.10 mmol, 49%). Single crystals suitable for X-ray crystallography were obtained from hot methanol. **ES-MS** (CH_2Cl_2, m/z): 631.2 [M-Cl]. **Anal. Found:** C: 44.4, H: 4.6, N: 3.8%. **Anal. Calculated for $C_{26}H_{31}N_2Cl_2O_2Ir\cdot0.66CH_2Cl_2$:** C: 44.3, H: 4.5, N: 3.9%. **^{1}H NMR** (300 MHz, $CDCl_3$, 300 K) 8.57 (br. d, ^3J (^{1}H-^{1}H) = 5.5 Hz, 1H, CH of pyridyl *ortho* to N), 8.14 (br. dd, $^3J(^1H\text{-}^1H)$ = 8.0

Hz, ^{4}J (^{1}H-^{1}H) = 1.5 Hz, 1H, CH of pyridyl *meta* to N and *ortho* to CON), 7.93 (vtd (ddd), ^{3}J (^{1}H-^{1}H) = 7.7 Hz, ^{4}J (^{1}H-^{1}H) = 1.4 Hz, 1H, CH of pyridyl *para* to N), 7.72 (vt (dd), ^{4}J (^{1}H-^{1}H) = 2.0 Hz, 1H, CH *ortho* to NCO and Cl), 7.61 (ddd, ^{3}J (^{1}H-^{1}H) = 8.0 Hz, ^{4}J (^{1}H-^{1}H) = 1.9 Hz, ^{4}J (^{1}H-^{1}H) = 1.1 Hz, 1H, CH of phenyl *para* to NCO), 7.50 (ddd, ^{3}J (^{1}H-^{1}H) = 6.6 Hz, ^{3}J (^{1}H-^{1}H) = 5.6 Hz, ^{4}J (^{1}H-^{1}H) = 1.7 Hz, 1H, CH of pyridyl *meta* to N and *para* to CON), 7.23 (masked vt (dd), ^{3}J (^{1}H-^{1}H) = 8.0 Hz, 1H, CH of phenyl *meta* to NCO and Cl), 7.07 (ddd, ^{3}J (^{1}H-^{1}H) = 8.0 Hz, ^{4}J (^{1}H-^{1}H) = 2.1 Hz, ^{4}J (^{1}H-^{1}H) = 1.0 Hz, 1H, CH *para* to Cl), 3.62 (t, ^{3}J (^{1}H-^{1}H) = 6.3 Hz, 2H, CH_2OH), 1.75-1.85 (m, 2H, $C\underline{H}_2(CH_2)_4OH$), 1.47-1.62 (m, 2H, $C\underline{H}_2CH_2OH$), 1.47 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.32-1.39 (m, 4H, $C\underline{H}_2C\underline{H}_2CH_2OH$). **^{13}C{^{1}H} NMR** (75 MHz, $CDCl_3$, 300 K) 168.4 (NCO), 155.4 ($\underline{C}$CON), 149.7 (CH *ortho* to N on pyridyl ring), 149.5 ($\underline{C}$NCO), 138.7 (C *para* to N on pyridyl ring), 133.5 (CCl), 129.0 (CH *meta* to Cl and NCO), 127.5 (CH *para* to CO and *meta* to N on pyridyl ring), 127.3 (CH *ortho* to NCO and Cl), 126.6 (CH *ortho* to CO and *meta* to N on pyridyl ring), 125.3 (CH *ortho* to Cl and *meta* to NCO), 124.3 (CH *para* to Cl), 88.1 (quarternary C of Cp‡), 87.5 (quarternary C of Cp‡), 87.2 (quarternary C of Cp‡), 86.8 (quarternary C of Cp‡), 86.6 (quarternary C of Cp‡), 62.5 (CH_2OH), 32.3 ($\underline{C}H_2CH_2OH$), 27.9 ($\underline{C}H_2CH_2CH_2OH$), 26.0 ($\underline{C}H_2CH_2CH_2CH_2OH$), 23.5 ($\underline{C}H_2CH_2CH_2CH_2CH_2OH$), 8.6 ($CH_3$), 8.5 ($CH_3$), 8.5 ($CH_3$), 8.5 ($CH_3$).

5 X-ray Crystallographic Data

Table S1 X-ray crystallographic data for compounds **2a**, **2c**, **4a**, **5a** and **5b**

Compound	**2a**	**2c**	**4a**	**5a**	**5b**
Chemical formula	$C_{24}H_{38}Cl_4O_2Rh_2$	$C_{36}H_{58}Cl_4O_2Rh_2$, $2(CH_2Cl_2)$	$C_{19}H_{28}Cl_2NORh$	$C_{26}H_{31}Cl_2IrN_2O_2.CH_2Cl_2$	$C_{26}H_{31}Cl_2N_2O_2Rh$
M_r	706.16	1040.30	460.23	751.55	577.34
Crystal system, space group	Monoclinic $P2_1/n$	Monoclinic, $C2/c$	Triclinic, P-1	Monoclinic, $P2_1/c$	Orthorhombic, $Pca2_1$
Temperature (K)	150.15	150.15	150.15	150.15	150.15
a, b, c (Å)	8.5285(11), 14.716(2), 11.1401(16)	32.386(6), 8.8826(14), 20.365(4)	7.4947(8), 8.6089(9), 17.0185(19)	24.353(3), 16.197(2), 14.631(2)	14.686(2), 9.2437(12), 19.177(2)
α, β, γ (°)	90.00, 96.481(6), 90.00	90.00, 125.685(12), 90.00	90.00, 90.00, 90.00	90.00, 93.344(7), 90.00	90.00, 90.00, 90.00
V (Å^3)	1388.9(3)	4758.4(15)	1022.80(19)	5761.3(14)	2603.4(6)
Z	2	4	2	8	4
μ (mm^{-1})	1.689	1.458	1.494	1.733	1.473
Absorption correction	1.59	1.17	1.10	5.03	0.89
No. of measured, independent and observed {$I > 2\sigma(I)$} reflections	41729, 4144	124636, 7067	26416, 4882	285232, 17410	38505, 7525
R_{int}					
$(\sin\theta/\lambda)_{max}$ (Å^{-1})	0.71073	0.71073	0.71073	0.71073	0.71073
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.028, 0.073	0.037, 0.102	0.023, 0.053	0.028, 0.076	0.068, 0.138
GOOF	1.07	1.06	1.05	1.08	1.17
CCDC Number	927162	927163	947905	947906	947907

Table S2 a) Selected bond lengths and **b)** Selected bond angles for compounds **2a**, **2c**, **4a**, **5a** and **5b**

S2 a)	M(1)-Cl(1)	M(1)-X	M(1)-Y	M(1)-Cg(1)
2a X = Cl(2), Y = Cl(2')	2.4341(7)	2.4857(6)	2.4775(6)	1.774
2c X = Cl(2), X = N(1)	2.4353(8)	2.4693(8)	2.4776(9)	1.769
4a X = Cl(2), Y = N(1)	2.4502(5)	2.4393(5)	2.150(1)	1.789
5a X = N(1), Y = N(2)	2.4416(8)/2.4452(8)	2.121(3)/2.127(3)	2.114(2)/2.111(2)	1.809/1.812
5b X = N(1), Y = N(2)	2.451(2)	2.136(5)	2.125(4)	1.823

S2 b)	Cl(1)-M(1)-X	Cl(1)-M(1)-Y	X-M(1)-Y	M(1)-Y-M(1')	Cg(1)-M(1)-Cl(1)	Cg(1)-M(1)-X	Cg(1)-M(1)-Y
2a X = Cl(2), Y = Cl(2')	89.70(2)	90.51(2)	83.20(2)	96.80(2)	127.35	126.92	125.87
2c X = Cl(2), Y = Cl(2')	90.15(2)	89.88(2)	83.48(2)	96.52(2)	127.21	126.80	126.10
4a X = Cl(2), Y = N(1)	90.53(2)	86.91(4)	89.08(4)	-	125.96	126.16	126.08 X = N(1)
5a X = N(1), Y = N(2)	84.91(7)/86.82(7)	84.56(7)/86.49(7)	76.6(1)/76.5(1)	-	125.94/125.95	133.22/133.33	132.14/131.60
5b X = N(1), Y =N(2)	88.5(1)	88.5(1)	77.02(2)	-	125.23	130.74	130.89

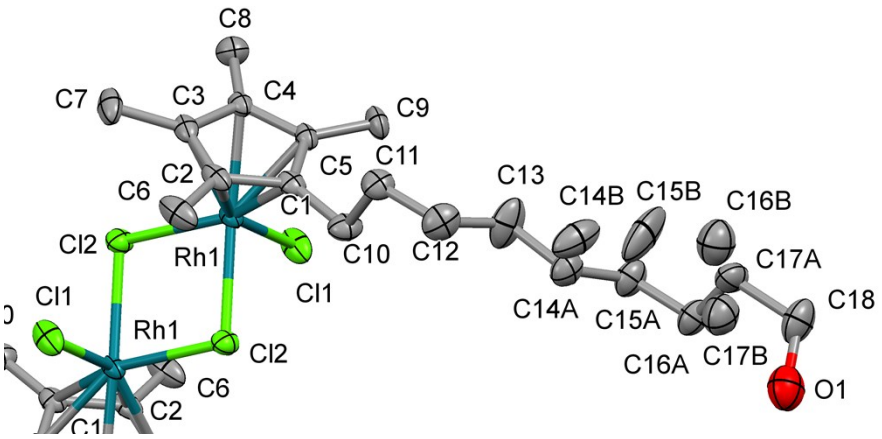


Figure S2 Molecular diagram showing the disorder within the tether of complex 2c. Hydrogen atoms omitted for clarity and ellipsoids are at the 50% probability level.

6 References

1. A. J. Blacker, S. Brown, B. Clique, B. Gourlay, C. E. Headley, S. Ingham, D. Ritson, T. Screen, M. J. Stirling, D. Taylor and G. Thompson, *Org. Process Res. Dev.*, 2009, **13**, 1370-1378.
2. J. Blacker, K. Treacher and T. Screen, WO 2009/093059 A2, 2009.
3. S. J. Lucas, B. D. Crossley, A. Pettman, A. D. Vassileiou, T. E. O. Screen, A. J. Blacker and P. C. McGowan, *Chem. Commun.*, 2013, **49**, 5562-5564
4. J. Cosier and A. M. Glazer, *J. Appl. Crystallogr.*, 1986, **19**, 105-107.
5. Z. Otwinowski and W. Minor, *Methods Enzymol.*, 1997, **276**, 307-326.
6. G. M. Sheldrick and T. R. Schneider, *Methods Enzymol.*, 1997, **277**, 319-343.
7. A. Altomare, G. Cascarano, C. Giacovazzo and A. Guagliardi, *J. Appl. Crystallogr.*, 1993, **26**, 343-350.
8. A. Altomare, M. C. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori and R. Spagna, *J. Appl. Crystallogr.*, 1999, **32**, 115-119.
9. L. J. Barbour, *XSeed*, (1999).
10. M. Thornton-Pett, *WC - A Windows CIF Processor*, (2000).
11. A. L. Spek, *J. Appl. Crystallogr.*, 2003, **36**, 7-13.
12 R. M. Lord, A. J. Hebden, C. M. Pask, I. R. Henderson, S. J. Allison, S. J. Shepherd, R. M. Phillips and P. C. McGowan, *J. Med. Chem*, 2015, **58**, 4940-4953
13 W. –H. Chew, J. Lu, T. D. Bradshaw and A. Holmgren, *FASEB J.*, 2008, **22**, 2072-2083