Supporting Information

Dougan *et al.* 10.1073/pnas.0800076105

SI Text

Synthesis. ***[(η⁶-p-cymene)RuI₂]₂.*** The dimer $[(\eta^6\text{-}p\text{-cymene})RuCl_2]_2$ (0.65 g, 1 mmol) was heated to reflux in water (250 ml) for 1 h. The solution was hot-filtered and KI (4.45 g, 27 mmol) was added to the filtrate. A brown/red precipitate immediately formed. This was filtered off and washed with ethanol and ether. Yield: 841 mg (86.0%). (Found C, 24.70; H, 2.50. Calcd for $Ru_2C_{20}H_{28}I_4$ C, 24.56; H, 2.59%). ^{1}H NMR (DMSO-d_6): δ 5.87 (d of d, 4H), 3.16 (septet, 1H), 2.40 (s, 3H), 1.22 (d, 6H).

[(η⁶-biphenyl)RuI₂]₂. The dimer $[(\eta^6\text{-biphenyl})RuCl_2]_2$ (0.3 g, 0.46 mmol) was stirred at ambient temperature in water (250 ml) for 30 min. The solution was filtered and KI (2.12 g, 13 mmol) was added to the filtrate. A brown/red precipitate immediately formed. This was filtered off and washed with ethanol and ether. Yield: 388 mg (82.9%). (Found C, 28.38; H, 1.98%. Calcd for $Ru_2C_{24}H_{20}I_4$ C, 28.31; H, 1.61%). ^{1}H NMR (DMSO-d_6): δ 7.84 (d, 2H), 7.54–7.46 (m, 3H), 6.66 (d, 2H), 6.38 (t, 1H), 6.12 (d, 2H).

[(η⁶-p-cymene)Ru(azpy-NMe₂)I]PF₆ (1). The dimer $[(\eta^6\text{-}p\text{-cymene})RuI_2]_2$ (54.8 mg, 0.051 mmol) in methanol (20 ml) was heated to ≈313 K until the solution turned clear. Azpy-NMe_2 (23 mg, 0.102 mmol) dissolved in methanol (10 ml) was added dropwise, and the solution immediately turned from brown to dark blue. The solution was stirred at room temperature for 3 h. The volume of solvent was reduced to ≈10 ml by removal of methanol on a rotary evaporator. NH_4PF_6 (83 mg, 0.51 mmol) was then added and the solution was placed in the freezer overnight. A black microcrystalline product precipitated out and this was filtered off and washed with diethyl ether. The product was dried overnight *in vacuo*. Yield: 40.9 mg (68.2%) (Found C, 37.74; H, 3.25; N, 7.40. Calcd for $RuC_{23}H_{27}N_4IPF_6$: C, 37.66; H, 3.85; N, 7.64). ^{1}H NMR ($CDCl_3$): δ 9.17 (d, 1H), 8.22 (d, 1H), 8.15 (d, 2H), 8.03 (t, 1H), 7.54 (t, 1H), 6.77 (d, 2H), 6.05 (d, 1H), 5.81 (t, 2H), 5.68 (d, 1H), 3.29 (s, 6H), 2.70–2.54 (m, 4H), 1.04 (d of d, 6H). ESI MS: calcd for $RuC_{23}H_{27}N_4I^+$ $[M^+]$ *m/z* 589.04, found 589.2 $[M^+]$.

[(η⁶-p-cymene)Ru(azpy-OH)I]PF₆ (2). The dimer $[(\eta^6\text{-}p\text{-cymene})RuI_2]_2$ (73 mg, 0.07 mmol) in methanol (70 ml) was stirred at ambient temperature until the solution turned clear. Azpy-OH (30 mg, 0.14 mmol) dissolved in methanol (10 ml) was added dropwise and the solution immediately turned from brown to intense brown/yellow. Six drops of 1 M HCl were added to the solution and the mixture was stirred for 4 h. The volume of solvent was reduced slightly and a black precipitate was filtered off. The resulting mixture was passed down a Sephadex LH20 column (Amersham Biosciences) with methanol as the eluent. The intense brown/yellow fraction was collected and excess NH_4PF_6 (160 mg, 0.1 mmol) was added. The solvent was removed and the product was redissolved in dichloromethane, from which a white precipitate was filtered off. The solvent was removed to give a dark brown solid. The product was dried overnight *in vacuo*. Yield 33 mg (46.6%). (Found C, 34.48; H, 3.24; N, 5.74. Calcd for $RuC_{21}H_{23}N_3OIPF_6$: C, 35.65; H, 3.28; N, 5.94%). ^{1}H NMR (DMSO-d_6): δ 9.87 (d, 1H), 8.92 (d, 1H), 8.78–8.65 (m, 3H), 8.07 (t, 1H), 7.37 (d, 2H), 6.83 (d, 1H), 6.64–6.51 (m, 3H), 3.18 (septet, 1H), 3.11 (s, 3H), 1.53 (dd, 6H). ESI-MS: calcd for $RuC_{21}H_{23}N_3OI^+$ $[M^+]$ *m/z* 561.99, found 561.7 $[M^+]$.

[(η⁶-p-cymene)Ru(azpy)I]PF₆ (3). The dimer $[(\eta^6\text{-}p\text{-cymene})RuI_2]_2$ (100 mg, 0.1 mmol) was dissolved in methanol (50 ml) and heated to ≈313 K until the solution turned clear. Azpy (38 mg, 0.2 mmol) dissolved in methanol (10 ml) was added dropwise and the solution gradually turned from brown to brown/purple. The solution was allowed to cool to ambient temperature and stirred for 3 h. The volume of solvent was reduced to ≈10 ml by removal of methanol on a rotary evaporator. NH_4PF_6 (160 mg, 1 mmol) was then added and the solution was placed in a freezer at 253 K overnight. A black microcrystalline product precipitated out and this was filtered off and washed with ether. The product was dried overnight *in vacuo*. Yield 110 mg (79.6%). (Found C, 36.90; H, 3.23; N, 5.96. Calcd for $RuC_{21}H_{23}N_3IPF_6$: C, 36.54; H, 3.36; N, 6.09). ^{1}H NMR (DMSO-d_6): δ 9.67 (d, 1H), 8.93 (d, 1H), 8.52 (t, 2H), 8.28 (d, 1H), 7.95–7.86 (m, 2H), 7.77 (t, 2H), 6.55 (d, 1H), 6.23 (t, 2H), 6.15 (d, 1H), 2.76 (septet, 1H), 2.62 (s, 3H), 1.08 (dd, 6H). ESI MS: calcd for $RuC_{21}H_{23}N_3I^+$ $[M^+]$ *m/z* 545.98, found 546.1 $[M^+]$.

[(η⁶-biphenyl)Ru(azpy-NMe₂)I]PF₆, (4). The dimer $[(\eta^6\text{-biphenyl})RuI_2]_2$: (100 mg, 0.1 mmol) was dissolved in methanol (60 ml) and water (20 ml) and heated to reflux for 2 h. Azpy-NMe_2 (44.4 mg, 0.2 mmol) dissolved in methanol (20 ml) was added dropwise and the solution immediately turned from brown to dark blue. The solution was refluxed for a further hour, hot-filtered, and then the volume of solvent was reduced to about 15 ml by removal of methanol on a rotary evaporator. NH_4PF_6 (160 mg, 1 mmol) was then added and the solution was placed in a fridge at 277 K for 1 h. A black powdery product precipitated out and this was filtered off and washed with cold ethanol then diethyl ether. The product was dried overnight *in vacuo*. Yield 121 mg (80.2%). (Found C, 38.52; H, 2.67; N, 7.03. Calcd for $RuC_{25}H_{24}N_4IPF_6$: C, 39,85; H,3.21; N,7.74%). ^{1}H NMR (DMSO-d_6): δ9.35 (d, 1H), 8.37 (d, 1H), 8.15 (t, 1H), 8.07 (d, 2H), 7.51–7.34 (m, 5H), 6.86–6.78 (m, 3H), 6.68–6.48 (m, 4H), 3.27 (s, 6H). ESI MS: calcd for $RuC_{25}H_{24}N_4I^+$ $[M^+]$ *m/z* 609.03, found 609.2 $[M^+]$. Crystals suitable for x-ray diffraction were obtained by slow diffusion of diethyl ether into a methanol solution of the complex at ambient temperature.

[(η⁶-biphenyl)Ru(azpy-OH)I]PF₆ (5). The dimer $[(\eta^6\text{-biphenyl})RuI_2]_2$ (100 mg, 0.1 mmol) was dissolved in methanol (60 ml) and water (20 ml) and heated to reflux for 2 h. Azpy-OH (37.5 mg, 0.2 mmol) dissolved in methanol (20 ml) was added dropwise and the solution gradually turned from brown to intense brown/yellow. The solution was refluxed for a further 2 h, hot-filtered, and then the volume reduced to ≈15 ml by removal of methanol on a rotary evaporator. NH_4PF_6 (160 mg, 1 mmol) was added and the solution was placed in a fridge at 277 K for 2 h. A brown powdery product precipitated out and this was filtered off and washed with cold ethanol then diethyl ether. The product was dried overnight *in vacuo*. Yield 94.1 mg (66.1%). (Found C, 38.03; H, 2.14; N, 5.35. Calcd for $RuC_{23}H_{19}N_3OIPF_6$: C, 37.21; H, 2.14; N, 5.79). ^{1}H NMR (DMSO-d_6): δ 9.45 (d, 1H), 8.93 (d, 1H), 8.41 (t, 1H), 7.94 (d, 2H), 7.64–7.56 (m, 3H), 7.60–7.45 (m, 5H), 7.43–7.33 (m, 2H), 6.89 (d, 1H), 6.75 (t, 1H), 6.70–6.54 (m, 3H). ESI-MS: calcd for $RuC_{23}H_{19}N_3OI^+$ $[M^+]$ *m/z* 581.65, found 582.1 $[M^+]$.

[(η⁶-biphenyl)Ru(azpy)I]PF₆ (6). The dimer $[(\eta^6\text{-biphenyl})RuI_2]_2$ (100 mg, 0.1 mmol) was dissolved in methanol (60 ml)/water (20 ml) and heated to reflux for 2 h. Azpy (37.5 mg, 0.2 mmol) dissolved in methanol (20 ml) was added dropwise and the solution gradually turned from brown to brown/purple. The solution was refluxed for a further 2 h, hot-filtered, and then the volume of solvent was reduced to about 15 ml by removal of methanol on a rotary evaporator. NH_4PF_6 (160 mg, 1 mmol) was then added and the solution was placed in a fridge at 277 K for 2h. A black powdery product precipitated out and this was filtered off and washed with cold ethanol then diethyl ether. The product was

dried overnight *in vacuo*. Yield 94.1 mg (66.1%). (Found C, 38.86; H, 2.68; N, 5.74. Calcd for $RuC_{23}H_{19}N_3IPF_6$: C, 38.89; H, 2.70; N, 5.92). ^{1}H NMR (DMSO-d_6): δ 9.45 (d, 1H), 8.93 (d, 1H), 8.41 (t, 1H), 7.94 (d, 2H), 7.64–7.56 (m, 3H), 7.60–7.45 (m, 5H), 7.43–7.33 (m, 2H), 6.89 (d, 1H), 6.75 (t, 1H), 6.70–6.54 (m, 3H). ESI MS: calcd for $RuC_{23}H_{19}N_3I^+$ [M^+] *m/z* 565.66, found 566.1 [M^+].

Crystal data for 4·MeOH. The sample was a dark purple chip of dimensions 0.29 × 0.13 × 0.10 mm^3: monoclinic, space group $P2_1/c$; a = 13.5491 (3), b = 16.5798 (4), c = 13.3329 (3) Å, β = 110.813 (2); V = 2799.67 (11) Å^3; Z = 4; D_{calc} = 1.863 mg·m^{-3}; μ = 1.788 mm^{-1}; F (000) = 1544. The final conventional R factor [$R1$, based on F and 4,634 data with $F > 4\sigma(F)$] was 0.0490, and w$R2$ (based on F^2 and all 8088 unique data from θ = 1.61–30.49°) was 0.1197. The final ΔF synthesis extremes were +1.160 and −0.748 e Å^{-3}.

Detection of ROS. A vial of DCFH-DA was opened under a blanket of argon, aliquoted in DMSO, and stored frozen. When used, care was taken to ensure minimum exposure to light. A549 cancer cells were plated out at a density of 20 × 10^3 cells per well into black 96-well plates and were incubated at 310 K, 5% CO_2, high humidity for 24 h. Cells were loaded with DCFH-DA [10 μM, 0.5% DMSO (vol/vol)] and were incubated at 310 K, 5% CO_2, high humidity for 30 min. The probe was removed and the cells were washed twice with PBS (200 μl). The cells were then kept in Hanks' Balanced Salt Solution (HBSS) and the ruthenium compounds were diluted with HBSS and added to the wells [25 μM, 0.5% DMSO (vol/vol)]. Hydrogen peroxide (25 μM) was added as a positive control and the fluorescence was recorded every 200 s over a period of 6 h at 310 K by excitation at 480 ± 10 nm and emission at 538 ± 15 nm on a BMG Fluostar plate reader.

Detection of H_2. Gas chromatography.

Solutions containing complex **4** [(η^6-biphenyl)Ru(azpy-NMe_2)I]PF_6 and GSH (Ru 100 μM, GSH, 10 mM, 100 mM phosphate buffer, pH 7, 95% H_2O), 5% acetone were prepared from stock solutions of the reactants which had been thoroughly purged with N_2 before mixing, and were incubated at 310 K for 18 h before sampling. Aliquots of the head space (50 μl) were removed by using a gas-tight syringe and analyzed on an Agilent GC 7890A instrument equipped with a thermal conductivity detector, a 5-Å molecular sieve column, using N_2 as the carrier gas. Under these conditions H_2 had a retention time of 0.73 min and O_2 a retention time of 1.73 min.

Additional experiments were performed in which samples were purged with Ar or degassed by using freeze-pump-thaw cycles, and the head space was sampled immediately, after 30 min, 2 h, and 18 h of incubation at 310 K.

Colorimetric assays. These assays involved detection of a change in color of a dye on reaction with H_2 by UV-vis spectroscopy, in the presence of a catalyst (1). The azo dye disperse red 13 was used together with the well known hydrogenation catalyst Pd/C (2, 3). Saturating the Pd/C disperse red 13 solution (25 μM in MeOH) with H_2 led to complete loss of color (i.e., loss of absorption at 501 nm). However, no such loss of absorption was observed for reaction mixtures of GSH and **4**. Better detection sensitivity was achieved with the azopyridine dye Azpy-NMe_2 (50 μM in EtOH), but again no H_2 was detected (no reduction in absorption at 430 nm) for reaction mixtures of GSH and **4**.

NMR. The detection of H_2 (δ 4.24 ppm) at levels expected for the reactions of GSH and these Ru arene complexes was readily possible using toluene-d_8 (in which H_2 has a reasonable solubility) as solvent (4, 5), but no H_2 was detected for the GSH/ **4** system by using various transfer methods (flushing with N_2, and glass bridge connection). Attempts to detect an H_2 peak for aqueous reaction mixtures (90% H_2O/10% D_2O) were unsuccessful even after lowering the pH (6).

1. Katsuda T, Ooshima H, Azuma M, Kato J (2006) New detection method for hydrogen gas for screening hydrogen-producing microorganisms using water-soluble Wilkinson's catalyst derivative. *J Biosci Bioeng* 102: 220–226.
2. Abiraj K, Srinivasa GR, Gowda DC (2005) Palladium-catalyzed simple and efficient hydrogenative cleavage of azo compounds using recyclable polymer-supported formate. *Can J Chem* 83:517–520.
3. Borovik VP, Sedova VF, Shukurko OP (1993) Catalytic hydrogenation of 2,5-bis(p-nitrophenyl)pyrimidine. *Chem Heterocycl Compd* 29:1323–1327.
4. Nishijima W, Ochi Y, Tsai T-Y, Nakano Y, Okada M (2004) Catalytic hydrodechlorination of chlorinated ethylenes in organic solvents at room temperature and atmospheric pressure. *Appl Catal B Environ* 51:135–140.
5. Waters JA, Mortimer GA, Clements HE (1970) Solubility of some light hydrocarbons and hydrogen in some organic solvents. *J Chem Eng Data* 15:174–176.
6. Gilboa H, Bogdan E, Chapman BE, Kuchel PW (1996) Spin-lattice relaxation times of H_2 and D_2 in aqueous solutions. *J Magn Reson Ser A* 119:1–5.

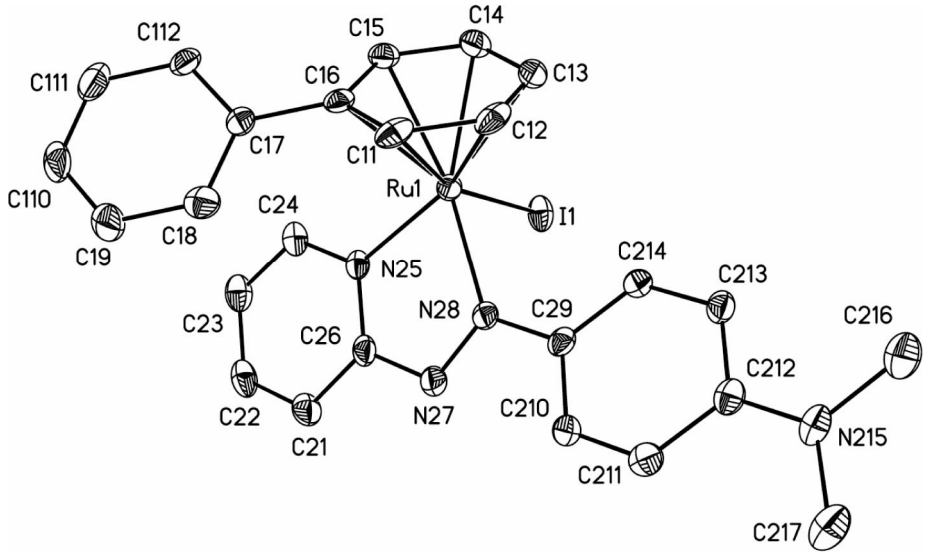

Fig. S1. X-ray structure of the cation in [(η^6-bip)Ru(azpy-NMe_2)I]PF_6·MeOH (4·MeOH). Thermal ellipsoids show 30% probability. The hydrogen atoms and molecule of methanol of crystallization have been omitted for clarity.

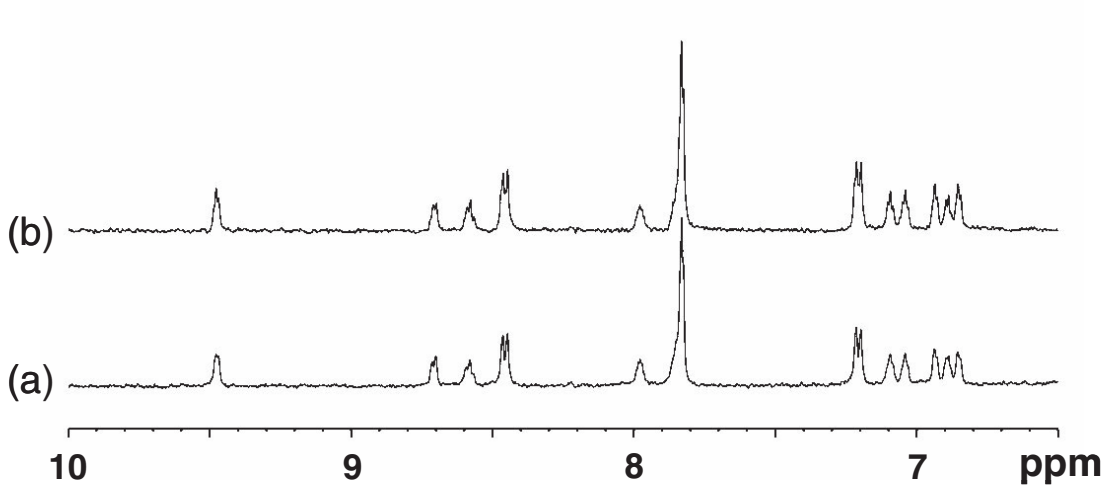

Fig. S2. High-frequency region of ^{1}H NMR spectrum of [(η^6-bip)Ru(azpy-NMe_2)I]PF_6, **4**, (100 μM in 95% D_2O, 5% MeOD, 10 mM phosphate buffer, 310 K) 28 min (*a*) and 24 h after dissolution (*b*). The absence of spectral changes suggests that no hydrolysis is occurring.

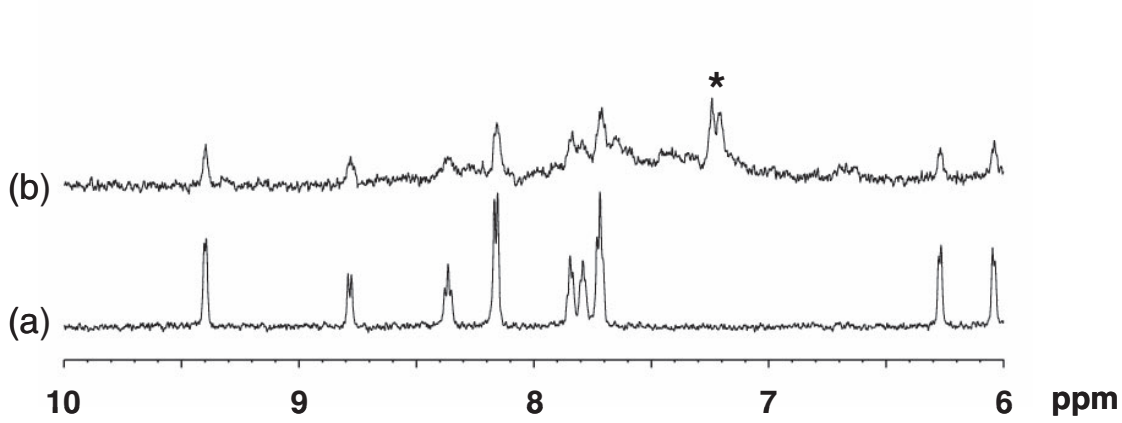

Fig. S3. High-frequency region of ^{1}H NMR spectrum of [(η^6-*p*-cymene)Ru(azpy)I]PF_6, **3**, (100 μM, 95% D_2O, 5% MeOD, 10 mM phosphate buffer, 310 K) 10 min (*a*) and 24 h (*b*) after dissolution. Peaks corresponding to the intact cation are reduced in intensity, and the only new resolved peak appearing is assignable to free *p*-cymene (labeled *).

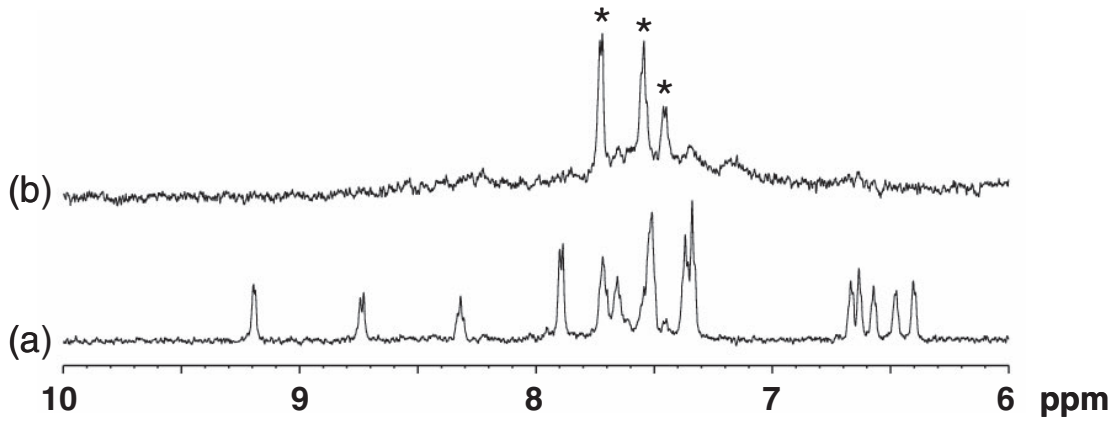

Fig. S4. High-frequency region of ^{1}H NMR spectrum of [(η^6-bip)Ru(azpy)I]PF_6, **6**, (100 μM, 95% D_2O, 5% MeOD, 10 mM phosphate buffer, 310 K) 11 min (*a*) and 24 h (*b*) after dissolution. Peaks corresponding to the intact cation have disappeared, and the only sharp peaks are those assignable to free biphenyl (labeled *).

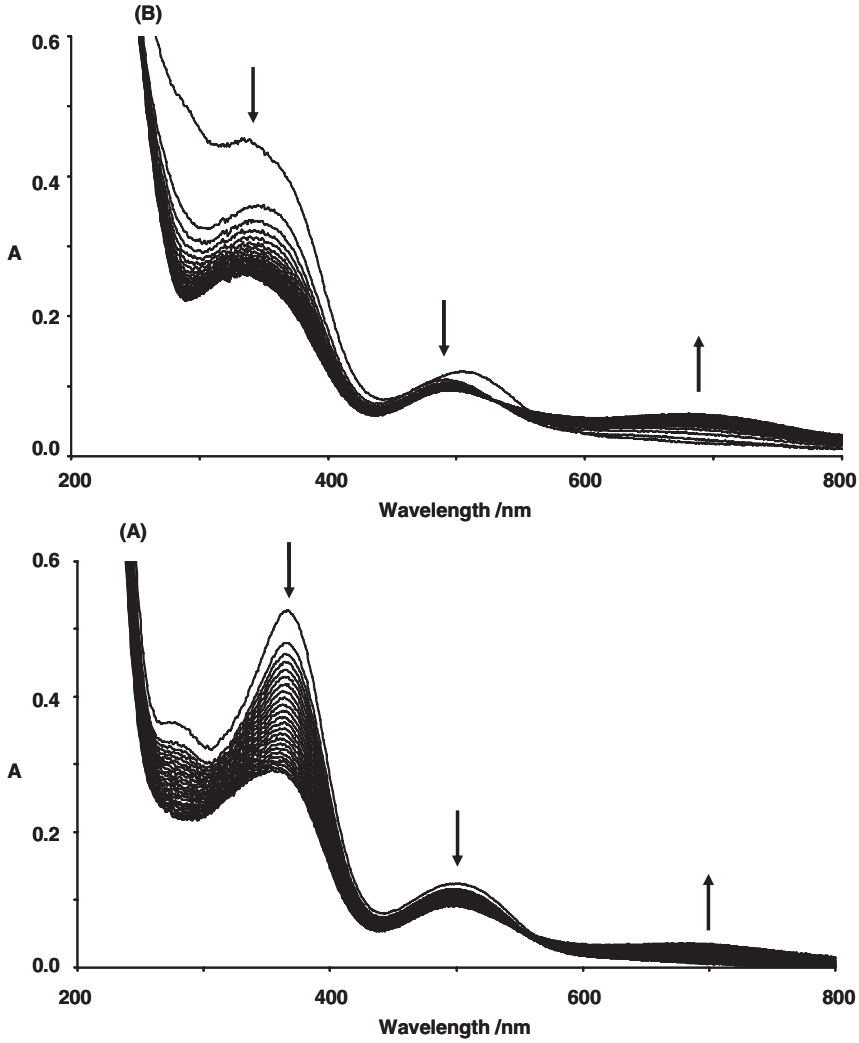

Fig. S5. UV-Vis spectra of **3** (*A*) and **6** (*B*) (50 μM, 10 mM phosphate buffer, pH 7.3, 95% H_2O, 5% MeOH) recorded every hour at 310 K for 24 h.

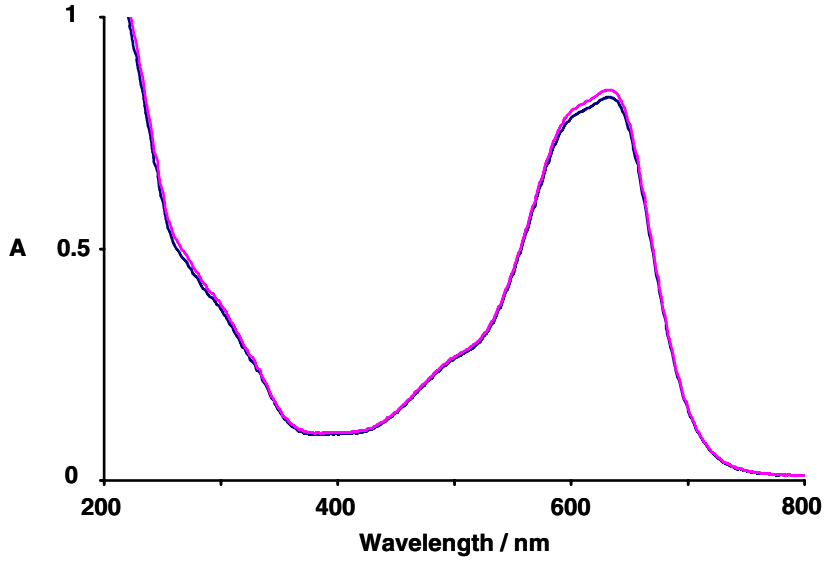

Fig. S6. UV-Vis spectra for [(η^6-bip)Ru(azpy-NMe_2)I]PF_6, **4** (30 μM) in 104 mM NaCl solution. The blue line is the initial spectrum and the pink line the specturm after 24 h incubation at 310 K. The lack of spectral change indicates that iodide is not readily displaced by chloride.

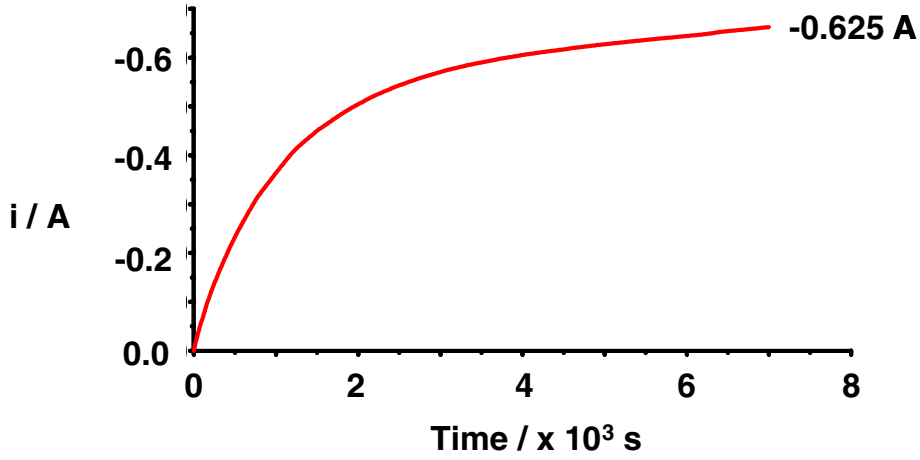

Fig. S7. Constant potential (−0.40 V) coulometry verifying a one-electron reduction process for complex [(η^6-*p*-cymene)Ru(azpy)I]PF_6, **3**. Current versus time plot for the reduction of 6.6 μmol **3**, $n = 0.98$; theoretically for $n = 1$, $i = 0.636$ A.

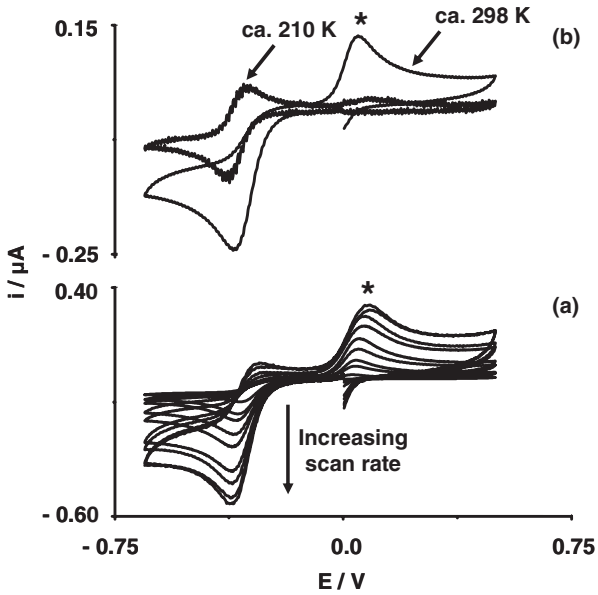

Fig. S8. Cyclic voltammograms for [(η^6-*p*-cymene)Ru(azpy-NMe_2)I]PF_6, **1** (in 0.1 M tetrabutylammonium BF_4 in DMF, sweep from 0 to ≈ -0.75 to $\approx +0.5$ to ≈ 0 V). (*a*) Variation of scan rate from 0.01 to 1 Vs^{-1} at $\approx$298 K. The reaction becomes slightly more reversible at higher scan rates, but in all cases the main product on the return sweep is marked *. (*b*) Effect of decreasing temperature from $\approx$298 K to 210 K. At the lower temperature the reduction becomes more reversible and the product marked * is significantly reduced in intensity. The complex undergoes an electrochemical-chemical-type reduction; the electrochemical reduction product is not stable. Reduction is followed by a chemical reaction/transformation. The major peak that appears on the return oxidative sweep (marked * in *a*) arises from oxidation of the product formed from the reduced species, and is not due to reversible reoxidation. At lower temperatures where the first reduction peak becomes more reversible, the new oxidation peak either disappeared or was significantly reduced in intensity (*b*). Similarly, faster scan rates increased the reversibility slightly with a corresponding decrease in the new oxidation product (*a*).

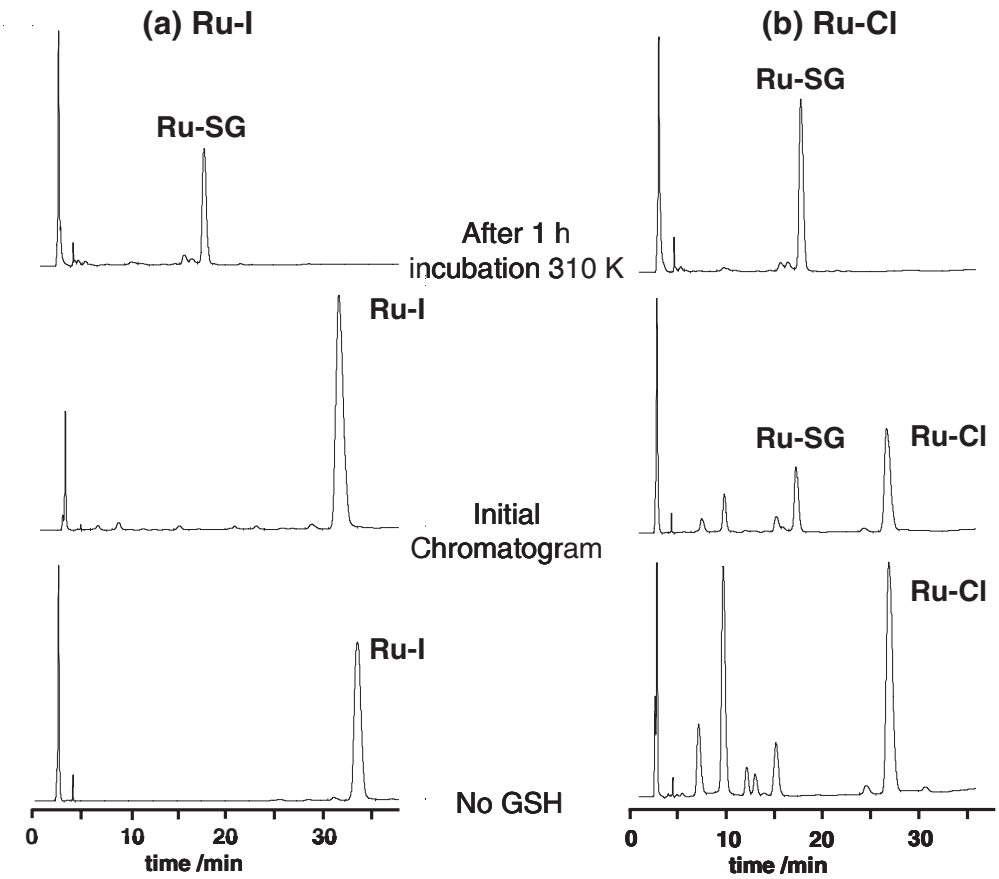

Fig. S9. HPLC chromatograms (detection at 286 nm) for the reaction of GSH (5 mM) with [(η^6-biphenyl)Ru(azpy-NMe_2)I]PF_6, complex **4** (Ru-I) (*a*) and the corresponding chlorido complex [(η^6-biphenyl)Ru(azpy-NMe_2)Cl]PF_6 (50 μM) (Ru-Cl) at pH 7.9 (10 mM phosphate buffer, 95% H_2O, 5% MeOH) (*b*). After 1 h incubation at 310 K, the major peak corresponds to the GS^- adduct [(η^6-biphenyl)Ru(azpy-NMe_2)GS]$^+$ and the peaks corresponding to the starting material have disappeared. For the chlorido complex, several peaks were present initially, probably a mixture of phosphate, TFA, and aqua adducts, as well as a peak for the GS^- adduct.

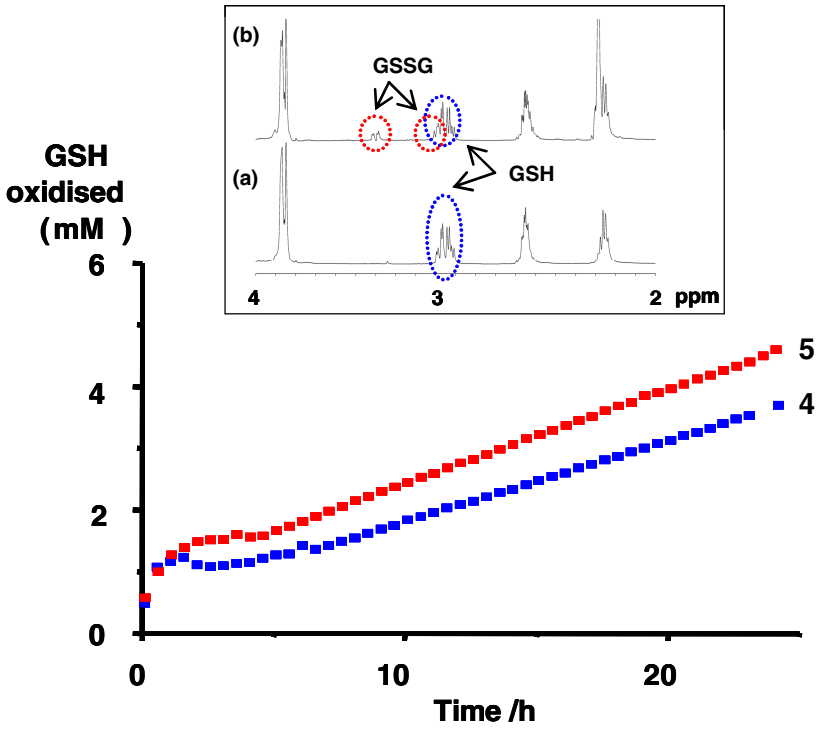

Fig. S10. Catalytic oxidation of GSH (10 mM) to GSSG in the presence of complex **4** (100 μM; blue) or 5 (100 μM; red) for 24 h at 310 K. (*Inset*) ^{1}H NMR spectra of GSH after incubation with complex **4** for 30 min (*a*) and 24 h (*b*) , when peaks assignable to GSSG can be identified. The chemical shifts of the β-CH_2 peaks for GSSG are consistent with literature values [Nakayama T, Isobe T, Nakamiya K, Edmonds JS, Shibata Y, Morita M (2005) Complexes of diphenylarsinic acid and phenylarsonic acid with thiols: A ^{1}H and ^{13}C NMR study. *Magn Reson Chem* 43:543–550].

Table S1. Selected bond lengths (Å) and angles (°) for [(η^6-bip)Ru(azpy-NMe_2)I]PF_6·MeOH (4·MeOH)

Bond/angle	Bond length, Å/angle,°
Ru(1)–N(28)	2.064(3)
Ru(1)–N(25)	2.046(3)
Ru(1)–I(1)	2.6984(5)
Ru(1)–C(11)	2.219(5)
Ru(1)–C(12)	2.208(4)
Ru(1)–C(13)	2.224(4)
Ru(1)–C(14)	2.215(4)
Ru(1)–C(15)	2.225(4)
Ru(1)–C(16)	2.233(4)
Ru(1)–η^6-arene centroid	1.713(2)
N(28)–Ru(1)–N(25)	75.82(13)