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Catalytic organometallic anticancer complexes
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Catalytic organometallic complexes offer chemistry that is not accessible to purely organic molecules and, hence, potentially new mechanisms of drug action. We show here that the presence of both an iodo
donor ligand and a o-donor/ o-acceptor phenylazopyridine ligand confers remarkable inertness toward ligand substitution on the half-sand
wich “piano-stool” ruthenium arene complexes ([η6-arene]Ru(az-
pyr)I) where arene is p-cymene or biphenyl, and azpy = N,N-
dimethylphenyl- or hydroxophenyl-azopyridine) in aqueous solution. Surprisingly, despite this inertness, these complexes are
are highly cytotoxic to human ovarian A2780 and human lung A549
cancer cells. Fluorescence-trapping experiments in A549 cells sug-

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Azpy-OH (5) for the intact cation decreased in intensity and peaks for free MeOD, pH 7.3) and at 310 K over 24 h by 1H NMR. For iodido complexes 1, 2, 4, and 5 containing phenylazopyridine ligands with OH or NMe2 substituents, no changes in the spectra occurred (e.g., Fig. S5 for complex 4) suggesting that no hydrolysis took place. This was confirmed by ESI-MS studies of the NMR sample where only the intact cation was detected [Ref. 14.].

Importantly, with 104 mM NaCl present (close to extracellular [Cl−]) no changes in the UV-Vis spectrum of complex 4 were observed over 24 h at 310 K (see Fig. S6), suggesting that no substitution of iodide by chloride takes place.

Table 1. IC50 values (µM) for [(η6-arene)Ru(azpy-R)]PF6 complexes, their corresponding chloride analogues [(η6-arene)Ru(azpy-R)]Cl|PF6 and free phenylazopyridine ligands for A2780 ovarian and A549 lung cancer cell lines, and the first and second (where observed) electrochemical reduction potentials (DMF vs. Ag/AgCl)

<table>
<thead>
<tr>
<th>Complex*</th>
<th>IC50, µM</th>
<th>A2780</th>
<th>A549</th>
<th>Ered, V</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1-C)</td>
<td>4 (&gt;100)</td>
<td>3 (&gt;100)</td>
<td>–0.40, –1.00</td>
<td></td>
</tr>
<tr>
<td>4 (4-C)</td>
<td>3 (44)</td>
<td>2 (49)</td>
<td>–0.36</td>
<td></td>
</tr>
<tr>
<td>Azpy-NMe2</td>
<td>&gt;100</td>
<td>14</td>
<td>–1.28</td>
<td></td>
</tr>
<tr>
<td>2 (2-C)</td>
<td>4 (58)</td>
<td>4 (&gt;100)</td>
<td>–0.33, –0.77</td>
<td></td>
</tr>
<tr>
<td>5 (5-C)</td>
<td>5 (18)</td>
<td>6 (56)</td>
<td>–0.26, –0.72</td>
<td></td>
</tr>
<tr>
<td>Azpy-OH</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>nd†</td>
<td></td>
</tr>
<tr>
<td>3 (3-C)</td>
<td>&gt;100 (&gt;100)</td>
<td>&gt;100 (&gt;100)</td>
<td>–0.22, –0.74</td>
<td></td>
</tr>
<tr>
<td>6 (6-C)</td>
<td>39 (&gt;100)</td>
<td>51 (&gt;100)</td>
<td>–0.18, –0.67</td>
<td></td>
</tr>
<tr>
<td>Azpy</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>–1.31 (vs. SCE)</td>
<td></td>
</tr>
</tbody>
</table>

*Azpy-NMe2 and Azpy-OH refer to azpy-NMe2 and azpy-OH as ligands.
†nd, not determined.

Electrochemical Reductions. In general, the complexes exhibited two electrochemical reductions (in dimethylformamide), the first at ~−0.2 to −0.4 V and a second at ~−0.7 to −1 V (Table 1). Reductions were also observed near to the solvent cutoff (~−2 V), but were not considered further.

In all cases the first reduction (confirmed as a one-electron reduction for complex 3, n = 0.98. Fig. S7) was essentially irreversible (no reverse scan peak at 0.1 V s−1, Fig. S8). The reduction potential becomes more positive as the arenne is changed from p-cymene to biphenyl and also as the chelating azo ligand is changed from azpy-NMe2 to azpy-OH to azpy (Table 1).

Based on literature assignments for other ruthenium–phenylazopyridine complexes (14, 15), this reduction can be assigned to addition of an electron into the π* orbital centered on the azo group of the phenylazopyridine ligands to form the azo anion radical (−N=N− + e− → (−N=N−)−). The relative order therefore reflects the decreasing π-acceptor capability of the substituted azo ligands with the addition of electron-donating groups onto the phenyl ring (16). The irreversible second reduction step is assigned to conversion to the dianionic species (−N=N−)2−. Azo groups usually give rise to two separate electrochemical reductions in polar aprotic solvents (17). In aqueous media the two-electron reduction of azo groups is accompanied by proton transfer to give hydroazo groups (NH−NH) in a single step (17).

Reactions with Glutathione. In cells, the major reducing agent is the tripeptide glutathione (GSH), a thiol present at millimolar concentrations (18). We were thus interested to follow the reactions of the ruthenium complexes with GSH. HPLC and electrospray ionization (ESI)-MS studies showed that the initial step in the reaction of excess GSH with [(η6-biphenyl)Ru(azpy-NMe2)]PF6, complex 4, and the corresponding chlorido complex involves complete substitution of halide by GS− to give [(η6-biphenyl)Ru(azpy-NMe2)GS]− (m/z obs 788.0, calcd 789.2; Fig. S9). Half-lives for this substitution were determined as 1.3 min (kobs = 8.73 × 10−3 s−1) for the chlorido complex 4-C1, and 17.5 min for the iodido complex 4-C2 (kobs = 6.58 × 10−4 s−1, 298 K by UV-Vis spectroscopy). Thus iodide substitution by GS− occurs 14 times more slowly than chloride substitution. During 1H NMR studies of reactions of [(η6-biphenyl)Ru(azpy-NMe2)]PF6, complexes 4, and [(η6-biphenyl)Ru(azpy-OH)]PF6, complexes 5, with glutathione (carried out in deoxygenated solutions under N2 or Ar to avoid autoxidation), it became apparent that some of these complexes can act as catalysts for the oxidation of GSH to GSSG.

The NMR spectra showed that incubation of 10 mM GSH with 100 µM 4 or 5 (in 10 mM phosphate, pH 7.2, 85% H2O, 10% D2O, 5% acetone-d6, 310 K, followed over 24 h) led to a steady oxidation of 3.7 mM (turnover frequency = 0.30 h−1, R2 = 0.98) and 4.6 mM, (turnover frequency = 0.37 h−1, R2 = 0.99), respectively, of GSH to GSSG (Fig. S10), as evidenced by the appearance of new peaks at δ 3.30 ppm corresponding to the β-CH2 of GSSG (Fig. S10 Inset). The formation of GSSG was further confirmed by ESI-MS spectra of these solutions after 24 h incubation, which showed peaks consistent with the presence of glutathione (m/z obs 305.2, calcd for GSH− 305.1) and glutathione disulfide (m/z obs 611.1, calcd for GSSG 611.2).

LC-MS attempts to detect intermediates in the catalytic cycle were unsuccessful, probably because they are transient. Inerting the samples formed in the reaction during the course of the reaction, suggesting that a gas had been produced. A variety of methods were used in attempts to detect H2 during the course of the reaction (colorimetric assays, NMR, and gas chromatography). However, none of these led to H2 detection (see SI Text). The GC analysis showed that small amounts of O2 were present in the head space of reactions despite thorough initial purging with N2 or Ar. No H2O2 was detected in the reaction mixtures by using peroxide test sticks.
Under similar conditions, neither of the free ligands Azpy-NMe2 nor Azpy-OH caused any oxidation of GSH to GSSG. Hence, the redox activity of 2-phenylazopyridine ligands depends on being coordinated to ruthenium.

**Detection of Reactive Oxygen Species (ROS) in A549 Cancer Cells.** The molecular probe 2′,7′-dichlorodihydrofluorescein-diacetate (DCFH-DA) is taken up by live cells, is hydrolyzed to 2′,7′-dichlorodihydrofluorescein (DCFH), and in the presence of ROS is oxidized to highly fluorescent 2′,7′-dichlorofluorescein (DCF) (19). This probe measures general oxidative stress (20). The thiol levels in A549 cells were increased by preincubation with 5 mM N-acetyl-L-cysteine (NAC). The cells were washed to remove excess NAC before treatment with the complexes. Fig. 3 shows that after treatment with complexes 1, 2, 4, or 5 for 24 h incubation followed by 96 h recovery time, there is greater cell survival for the cells that have increased thiol levels, whereas increased thiol levels had little effect on survival of cells treated with cisplatin.

**Discussion**

**Cytotoxicity Without Hydrolysis.** Many examples are known of metal anticancer complexes containing metal–halide bonds that are activated in cells by hydrolysis and subsequently bind to DNA as a target, for example, PtII diam(m)ine complexes, including the drug cisplatin (21), and diamino RuII arene complexes (22). In contrast, the organometallic iodido RuII arene azopyridine complexes studied in this article are inert toward hydrolysis and yet are still cytotoxic.

For example, complexes 1, 2, 4, and 5 are all resistant to hydrolysis over 24 h at 310 K and at micromolar concentrations and physiological pH. Complexes containing unsubstituted azpy (3 and 6) were not as cytotoxic toward the two cancer cell lines. This low activity can be correlated with their instability in phosphate-buffered solutions (Figs. S3–S5). High cytotoxicity is therefore associated with iodido azopyridine complexes that are highly stable in aqueous media. The corresponding chlorido azpy complexes are much less stable in water, with slow hydrolysis of both Ru–Cl and Ru–arene bonds occurring [τ1/2 9–21 h (13)], and these complexes are much less cytotoxic (Table 1). Iodide is less electronegative than chloride, behaves more like a simple two-electron donor, and forms stronger bonds to RuII. The increased electron density at the ruthenium center is therefore available for backdonation onto the arene, which also strengthens the RuII–arene bonds. Hydrolysis rates of RuIV arenes decrease when a π-acceptor-chelating ligand is incorporated into the complex, as we have discussed elsewhere (13).

The dramatic increase in cytotoxicity of iodido complexes compared with chlorido complexes observed here is not seen for RuII arene complexes containing the σ-donor ethylenediamine as the chelating ligand; compare, for example, [([η⁴-benzene]Ru(en)Cl)PF₆, IC₅₀ = 17 μM, ([η⁴-benzene]Ru(phen)Cl)PF₆, IC₅₀ = 20 μM (12). In the case of ethylenediamine complexes, the Ru–I bonds are converted to Ru–Cl bonds during the cytotoxicity assays because of the large excess of NaCl in the cell culture medium. In contrast, the iodido phenylazopyridine complexes do not appear to react with excess NaCl (see Fig. S5 for 4).

The iodido complexes also react more slowly with GSH than the chlorido complexes. In cells, GSH exerts a major protective role as a detoxifier of electrophilic substances (E), such as metals, forming thioether conjugates GS-E (18). These conjugates are eliminated via an ATP-dependent GS-E pump (23). GS-conjugation is the major deactivation mechanism for platinum complexes such as cisplatin (24). The greater resistance of the Ru–I bond to conjugation may further enhance their potency compared with chlorido complexes.

**Ligand-Based Redox Reactions.** The coordination of phenylazopyridine ligands to arene-ruthenium centers shifts their reduction potentials to biologically accessible values and results in cytotoxic complexes. Phenylazopyridine ligands can accept two electrons in one electrochemically accessible, lowest unoccupied molecular orbital, which is primarily azo in character (25). Coordination of these ligands to the dipositive ruthenium center allows ligand-based reduction to occur at a much more positive potential; the first electrochemical reduction for the free ligand azpy-NMe2, for example, occurs at −1.28 V, compared with −0.40 V for complex 1 and −0.36 V for complex 4 (Table 1).

**Catalytic Activity Toward Glutathione.** Ruthenium complexes 4 and 5 oxidize GSH to GSSG catalytically, unlike the free ligands azpy-NMe2 and azpy-OH. Here we have shown that under physiologically relevant conditions micromolar concentrations of organometallic azpy complexes can deplete millimolar amounts of GSH over 24 h by catalytic oxidation to GSSG.
A possible catalytic cycle for the conversion of GSH into GSSG is shown in Fig. 4. The initial step involves the conjugation of GSH across the azo double bond and subsequent reduction of the azo group to an hydrazo group with GSH oxidation. Such a process is reported to occur for the azo ester C₆H₅N=NCOOCH₃ (26) and for the diamide (CH₃)₂NCON=NCON(CH₃)₂ (27). However for these reported reactions, a stoichiometric amount of complex is required, that is, the compounds are not catalytic. Because no H₂ was detected during the course of the reaction, it seems likely that the second step in which the azo N=N group is regenerated, and the catalytic cycle is completed, proceeds via concomitant hydrogenation of dissolved O₂ to generate H₂O₂. Given that no H₂O₂ was detected in the reaction mixture, it is reasonable to propose that iodide (displaced from Ru by the initial substitution reaction with GSH) causes its decomposition (I⁻/H₂O₂/H₂O → I₂O₃⁻; I₂O₃⁻ + H₂O₂ → I₂ + H₂O + O₂). Such catalytic behavior of iodide toward H₂O₂ is well known (28) and explains the observed gas bubbles (O₂).

Examples in the literature of azo groups facilitating the oxidation of GSH include the Hodgkins and non-Hodgkins lymphoma drug procarbazine [CH₃NH–NHCH₂C₆H₄CONHCH₂(CH₃)₂], an hydrazo compound that is easily oxidized to the corresponding azo compound and re-reduced via GSH oxidation to GSSG (29). The reoxidation of this hydrazo group is facilitated by O₂ inside cells, which is hydrogenated to form H₂O₂.

Oxidation of GSH to GSSG was not induced by the free ligands. This is perhaps not surprising because electron-donating groups attached to azo groups (here OH or NMe₂) decrease their ability to oxidize GSH (17). Coordination to Ru¹І is necessary to make the azo nitrogen atoms positive enough for the redox reaction to occur.

**Detection of Reactive Oxygen Species.** The dichlorofluorescein fluorescence experiments showed that the iodido Ru¹І arene N,N-dimethyl- and hydroxy-azopyridine complexes 1, 2, 4, and 5 cause a buildup of reactive oxygen species in A549 lung cancer cells (Fig. 2). Reactive Oxygen Species (ROS) present a higher reactivity than molecular oxygen (30), and encompass not only free radicals such as the superoxide (O₂⁻), hydroperoxyl radical (HO₂⁻) and the hydroxyl radical (HO⁻), but also hydrogen peroxide (H₂O₂) and singlet oxygen (¹O₂). ROS are continuously generated in small quantities during normal cellular processes and are involved in several biological functions, such as cell growth and signaling (30). Excessive ROS generation inside cells is harmful, causing oxidation of biomolecules such as lipids, cell membranes, proteins, and DNA, and giving rise to oxidative stress (30). Importantly, no DCF fluorescence was detected for ruthenium arene complexes containing ethylenediamine (en) as chelating ligand, illustrating a fundamental difference in the mechanism of action of these two classes (azpy/en) of cytotoxic ruthenium arene complexes.

To assess the impact of ROS inside A549 lung cancer cells, intracellular thiol levels were increased by adding the antioxidant N-acetyl-L-cysteine, which is believed to act by raising intracellular concentrations of cysteine and hence GSH, and also by direct reaction of its thiol group with ROS (31). Treatment of cells with N-acetyl-l-cysteine increased the survival of cells treated with Ru¹І arene phenylazopyridine compounds (Fig. 3) providing evidence that ROS are implicated in cell death.

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**Fig. 4.** Proposed cycle for the catalytic oxidation of GSH to its corresponding disulfide GSSG by ruthenium(II) arene phenylazopyridine complexes. Initially X is iodide, which is displaced by G5⁻ during the early stages. Released iodide can catalyze the decomposition of H₂O₂. Bound GSH at pH 7 has deprotonated thiol and carboxyl groups, and a protonated amino group.
Oxidative Stress Causes Cell Death. Oxidative stress is caused by a serious imbalance between the levels of ROS in a cell and its antioxidant defenses in favor of the former (20). On the basis that GSH is the primary cellular antioxidant, it seems likely that the depletion of antioxidants is the primary mechanism by which ROS can cause cell death and by which oxidative stress occurs in the systems studied here. Because cancer cells (mainly multi-drug-resistant cells) are more sensitive to the additional exposure to ROS than normal cells (32), the proposal has been made that ROS can be used therapeutically to treat cancer (27, 31). Several established anticancer drugs are known to generate ROS or other radicals, including adriamycin (33), arsenic trioxide (34), and cisplatin (35).

Conclusions
Organometallic complexes present versatile platforms for drug design. Appropriate choice of the metal, its oxidation state, and the ligands can allow fine-tuning of the steric and electronic properties of the complex and hence its biological activity and mechanism of action. In the present case we have assembled Ru\textsuperscript{II} complexes containing a \pi-bonded arene ligand together with a strongly chelated \sigma-donor/\pi-acceptor azopyridine ligand and iodide as a strong monodentate ligand to produce complexes that are relatively inert toward activation by hydrolysis and yet potently cytotoxic toward cancer cells. These azopyridine complexes undergo activation by reduction. Whereas azopyridine ligands alone are difficult to reduce, the reduction potentials are biologically accessible when the azopyridine is coordinated to Ru\textsuperscript{II}. The complexes can induce redox reactions inside cancer cells leading to an increase in the accumulation of reactive oxygen species. Intriguingly iodido Ru\textsuperscript{II} arene azopyridine complexes can act as catalysts for the oxidation of the major intracellular reducing agent glutathione to glutathione disulfide, in a cycle that appears to involve ligand-centered reduction of the azo bond by glutathione forming an hydrazo intermediate, followed by formation of glutathione disulfide and regeneration of the azo bond. Catalytic drugs with ligand-centered mechanisms might have the advantage of not being so readily poisoned as metal-centered catalysts and therefore have greater potential for biological activity.

Materials and Methods

Materials.
4-(2-Pyridylazo)-N,N-dimethylaniline (azpy-NMe\textsubscript{2}), KI, NH\textsubscript{4}PF\textsubscript{6}, dichlorodihydroflourescein diacetate, glutathione, and N-acetyl-L-cysteine were purchased from Sigma-Aldrich and ascorbic acid from Alfa Aesar. Methanol was anhydrous (Sigma-Aldrich) or dried over Mg\textsubscript{2}O, and anhydrous DMF (Aldrich) was used for electrochemical experiments. The starting materials [(\textsuperscript{11}C-arene)RuCl\textsubscript{3}]\textsubscript{2} (arene = p-cymene, biphenyl) were prepared by reaction of RuCl\textsubscript{3}•2H\textsubscript{2}O with the reduced form of the arene (11), and the corresponding iodo dimers [(\textsuperscript{19}I-arene)RuI\textsubscript{2}]\textsubscript{2} from the reaction of [(\textsuperscript{11}C-arene)RuCl\textsubscript{3}]\textsubscript{2} with excess KI. The syntheses of 2-phenylazopyridine (azpy) and 4-(2-phenylazo)phenol (azpy-OH) have been reported previously (13). Full details of the synthesis and characterization of complexes are in the SI Text.

NMR Spectroscopy.
NMR spectra were recorded on either a Bruker Avance 600 MHz equipped with either a TXI \textsuperscript{1}H, \textsuperscript{13}C, \textsuperscript{15}N, x,y,z-gradient probe or a TXI \textsuperscript{1}H, \textsuperscript{13}C, \textsuperscript{15}N, x-gradient cryoprobe, a Bruker Avance 800 MHz equipped with a TCI \textsuperscript{1}H, \textsuperscript{13}C, \textsuperscript{15}N, x-gradient cryoprobe, or a Bruker DPX 360 MHz spectrometer equipped with a QNP \textsuperscript{1}H, \textsuperscript{13}C, \textsuperscript{15}N probe. 1H NMR signals were referenced to the residual solvent, δ\textsubscript{H} 7.27 (CDCl\textsubscript{3}), 2.52 (DMSO-d\textsubscript{6}), and for aqueous solutions (containing D\textsubscript{2}O or H\textsubscript{2}O/D\textsubscript{2}O mixtures) dioxane was added as an internal reference (δ\textsubscript{H} 3.75). The water resonance was suppressed by using a 1D double-pulsed field gradient spin echo (DPFGSE) experiment (36) or presaturation method. All spectra were recorded at 298 or 310 K and data were processed by using XWINNMR (Version 3.6, Bruker).

X-Ray Crystallography.
Diffraction data were collected at 150 K by using Mo Ka radiation (λ = 0.7073 Å) on a Bruker Smart Apex CCD diffractometer. The crystal structure of 4 was solved by using Patterson methods (DIRDIF) (37) and refined against F\textsuperscript{2} by using SHELXL-97 (38). The H atoms that are part of methyl and hydroxyl groups were located in a difference map, and the groups treated as rotating rigid bodies; other H atoms were placed in calculated positions.

Electrospray Mass Spectrometry. ESI-MS was obtained on a Micromas Platform II mass spectrometer and solutions were infused directly. The capillary voltage was 3.5 V and the cone voltage was typically varied between 10 and 45 V. The source temperature depended on the solvent used. Both positive and negative ion modes were used (as appropriate).

UV-Vis Spectroscopy. A Perkin-Elmer Lambda-16 UV/Vis spectrophotometer was used with 1-cm path length quartz cuvettes (0.5 ml) and a PTP1 Peltier temperature controller. Spectra were recorded at 310 K and were processed by using UV-Winlab software for Windows 95.

pH Measurements. The pH values of aqueous solutions were measured at −298 K by using a Corning pH meter 240 equipped with a Thermo micro combination KCl or KNO\textsubscript{3} electrode calibrated with pH 4, pH 7, and pH 10 buffer solutions (Sigma-Aldrich). The pH meter readings for D\textsubscript{2}O solutions were recorded without correction for the effect of deuterium on the glass electrode, and are termed pH*.

Cyclic Voltametry and Coulometry.
Electrochemical studies were performed with General Purpose Electrochemical System (GPES) Version 4.5 software connected to an Autolab system containing a PSTAT20 potentiostat. All of the electrochemical techniques used a three-electrode configuration. The reference electrode was Ag/AgCl in a solution of 0.1 M [TBA][BF\textsubscript{4}] in DMF against which E\textsubscript{Cu} for the ferrocinium/ferrocene couple was measured to be 0.55 V.

High Performance Liquid Chromatography (HPLC). A Hewlett-Packard 1100 quaternary pump and a Rheodyne sample injector with a 1.0-ml loop, an HP 1100 series UV/Vis detector, and HP 1100 series Chemstation with an HP 1100 series UV/Vis spectrophotometer. A 5-Å molecular sieve column on an Agilent 7890A GC system equipped with a thermal conductivity detector was used in attempts to detect H\textsubscript{2} with N\textsubscript{2} as a carrier gas (for details and other methods see SI Text).

Gas Chromatography (GC). A 5-Å molecular sieve column on an Agilent 7890A GC system equipped with a thermal conductivity detector was used in attempts to detect H\textsubscript{2} with N\textsubscript{2} as a carrier gas (for details and other methods see SI Text).

Detection of Reactive Oxygen Species (ROS) in AS49 Cancer Cells. The detection of ROS was based on earlier experimental protocols by Wang and Joseph (19) with some modifications. The full procedure is reported in the SI Text.

Reactivity of Complexes 1–6 in Buffered Solutions. Solutions of ruthenium complexes in MeOD were diluted in 10 mM phosphate buffer/D\textsubscript{2}O to give a final concentration of 100 μM Ru (95% D\textsubscript{2}O, 5% MeOD) and NMR spectra were recorded at 310 K after −15 min and after 24 h. The pH values of the samples were 7.35 (1), 7.38 (2), 7.38 (3), 7.40 (4), 7.3 (5), and 7.32 (6), and the samples were kept in the water bath at 310 K between NMR acquisitions. After 24 h, ESIMS was obtained on samples of 1, 2, 4, and 5.

Substitution of Iodide by Chloride. Complex 4 was dissolved in water, sonicated for 15 min to ensure dissolution, filtered, and then diluted to ∼30 μM. NaCl was added from a concentrated stock solution to give 100 mM NaCl and the
UV-Vis Spectroscopy and HPLC of Reactions of Complexes 4 and 6 with GSH. Buffers were filtered through Chelex-100 to remove iron and degassed by using N2/Ar before dissolving GSH. Solutions of GSH were made fresh for each experiment and the pH adjusted to ~7 with dilute NaOH/HCl, and the solutions degassed again. Aliquots of complex 4 or 4-Cl in MeOH were added to the solution (final concentrations: 50 μM Ru, 5 mM GSH, 10 mM phosphate buffer; 55% H2O, 5% MeOH); the pH values were 7.06 (4) and 7.03 (4-Cl) for UV-Vis experiments and 7.88 (4) and 7.89 (4-Cl) for HPLC separations.

The rate of substitution of Ru-Cl/I by GS/H11015 was determined by UV-Vis spectroscopy, by fitting the change in absorbance at 597 nm with time (298 K) for UV-Vis experiments and 7.57 (4-Cl) for HPLC separations. For details of chemicals and biochemicals, synthesis and characterization of complexes, and methods and techniques used, see SI Text.

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