Supporting Text

Synthesis of Complexes. The starting materials $[(C_{12}H_{18})RuCl_2]_2$ and $[(C_9H_{10})RuCl_2]_2$ were prepared according to methods described in refs. 1-3. The preparations of $[(\eta^6$-hmb)Ru(en)Cl$][PF_6]$ (**1**) (hmb, hexamethylbenzene; en, ethylenediamine) and $[(\eta^6$-ind)Ru(en)Cl$][PF_6]$ (**3**) (ind, indan) were based on a published synthesis (4), and the same general procedure was followed for the chloride complexes $[(\eta^6$-bip)Ru(en)Cl$][PF_6]$ (**2**) (bip, biphenyl) and $[(\eta^6$-bz)Ru(en)Cl$][PF_6]$ (**4**) (bz, benzene).

$[(\eta^6$-hmb)Ru(en)Br$][PF_6]$ (5). This complex was prepared by refluxing $[(\eta^6$-hmb)Ru(en)Cl$][PF_6]$ (**1**, 25.0 mg, 0.0496 mmol) and $AgNO_3$ (8.4 mg, 0.0494 mmol) in 2.5 ml of a 1:1 mixture of MeOH and H_2O for 1 h. AgCl was removed by filtration. LiBr (434 mg, 5.00 mmol) was added, and the solution was stirred for a day. After rotary evaporation to dryness, 3 ml of H_2O, 250 mg of NH_4PF_6, and CH_3CN were added to produce a clear solution. Further rotary evaporation resulted in a microcrystalline product, recrystallization of which from H_2O/acetone with excess NH_4PF_6 yielded crystals suitable for x-ray analysis. Yield: 21.3 mg (78%). Anal. (percent) Calc. for $BrC_{14}F_6H_{26}N_2PRu$: C 30.67, H 4.78, N 5.11. Found: C 30.81, H 4.64, N 4.85.

$[(\eta^6$-bip)Ru(en)Br$][PF_6]$ (6), $[(\eta^6$-indan)Ru(en)Br$][PF_6]$ (7) and $[(\eta^6$-bz)Ru(en)Br$][PF_6]$ (8). These were synthesized as for **5**. Anal. Calc. for **6**: $BrC_{14}F_6H_{18}N_2PRu$: C 31.12, H 3.36, N 5.19. Found: C 31.69, H 3.19, N 5.23. Anal. Calc. for **7**: $BrC_{11}F_6H_{18}N_2PRu$: C 26.20, H 3.60, N 5.56. Found: C 26.12, H 3.39, N 5.95. Anal. Calc. for **8**: $BrC_8F_6H_{14}N_2PRu$: C 21.97, H 4.03, N 5.12. Found: C 21.43, H 4.16, N 5.21.

$[(\eta^6$-hmb)Ru(en)I$][PF_6]$ (9). This complex was prepared by refluxing $[(\eta^6$-hmb)Ru(en)Cl$][PF_6]$ (**1**, 50.0 mg, 0.0992 mmol) and $AgNO_3$ (16.8 mg, 0.0988 mmol) in 5 ml of a 1:1 mixture of MeOH and H_2O for 1 h. AgCl was removed by filtration. LiI (939 mg, 5.00 mmol) was added, and the solution was stirred for a day. After rotary evaporation to dryness, 6 ml of H_2O, 250 mg of NH_4PF_6, and CH_3CN were added to produce a clear solution. Further rotary evaporation resulted in a microcrystalline

product. Crystals suitable for x-ray analysis were produced by slow evaporation from H_2O/acetone containing excess NH_4PF_6. Yield: 50.2 mg (85%). Anal. Calc. for $C_{14}F_6H_{26}IN_2PRu$: C 28.24, H 4.40, N 4.71. Found: C 27.84, H 4.22, N 4.38.

[(η^6-bip)Ru(en)I][PF_6] (10), [(η^6-indan)Ru(en)I][PF_6] (11) and [(η^6-bz)Ru(en)I][PF_6] (12). These were synthesized as for **9**. Anal. Calc. for **10**: $C_{14}F_6H_{18}IN_2PRu$: C 28.63, H 3.09, N 4.77. Found: C 28.75, H 3.02, N 4.61. **11**: Anal. Calc. for $C_{11}F_6H_{18}IN_2PRu$: C 23.97, H 3.29, N 5.08. Found: C 24.34, H 3.27, N 4.94. **12**: Anal. Calc. for $C_8F_6H_{14}IN_2PRu$: C 18.80, H 2.75, N 5.47. Found: C 19.16, H 3.00, N 5.30.

[(η^6-hmb)Ru(en)N_3][PF_6] (14). This pseudohalide complex was prepared by refluxing complex **1** (25.0 mg, 0.0496 mmol) and $AgNO_3$ (8.4 mg, 0.0494 mmol) in 2.5 ml of a 1:1 mixture of MeOH and H_2O for 1 h. AgCl was removed by filtration. NaN_3 was added (163 mg, 2.51 mmol), which dissolved with heating, and the solution was left overnight. NH_4PF_6 (250 mg) was added, leading to a microcrystalline, yellow precipitate. Recrystallization of the precipitate from acetone gave rise to a yellow crystalline product. Yield of **14**: 16.4 mg (65%). Anal. Calc. for $C_{14}F_6H_{26}N_5PRu$: C 32.94, H 5.13, N 13.72. Found: C 32.32, H 4.89, N 13.09. **[(η^6-bip)Ru(en)N_3][PF_6]** (**15**) was prepared analogously. **15**: Anal. Calc. for $C_{14}F_6H_{18}N_5PRu$: C 33.47, H 3.61, N 13.94. Found: C 33.37, H 3.46, N 13.68.

[(η^6-hmb)Ru(en)(py)][PF_6]$_2$ (16) (py, pyridine) was prepared by refluxing complex **1** (25.0 mg, 0.0496 mmol) and $AgNO_3$ (8.4 mg, 0.0494 mmol) in 2.5 ml of a 1:1 mixture of MeOH and H_2O for 1 h. AgCl was removed by filtration. Pyridine (101 μl, 1.25 mmol) was added, and the mixture was left overnight. The volume was reduced to ≈1.5 ml by rotary evaporation, and 100 mg of NH_4PF_6 was added. The yellow precipitate that formed was dissolved in acetone. The solution was filtered and allowed to evaporate slowly, resulting in a microcrystalline, yellow product. Yield of **16**: 19.3 mg (56%). Anal. Calc. for $C_{19}F_{12}H_{31}N_3P_2Ru$: C 32.96, H 4.51, N 6.07. Found: C 33.47, H 4.50, N 6.24.

[(η^6-hmb)Ru(en)(dcp)][PF_6]$_2$ (17) (dcp, 3,5-dichloropyridine), [(η^6-hmb)Ru(en)(dfp)][PF_6]$_2$ (18) (dfp, 3,5-difluoropyridine), [(η^6-hmb)Ru(en)(pcp)][PF_6]$_2$ (19) (pcp, *p*-cyanopyridine), and [(η^6-hmb)Ru(en)(pic)][PF_6]$_2$ (20) (pic, 3-picoline) were synthesised analogously. MS: *m/z* 616.0 for [**17** - PF_6]$^+$ (Calc. 616.0), *m/z* 583.9 for [**18** - PF_6]$^+$ (Calc. 584.1), *m/z* 572.9 for [**19** - PF_6]$^+$ (Calc. 573.1), *m/z* 562.1 for [**20** - PF_6]$^+$ (Calc. 562.1). Crystals of **19** suitable for x-ray analysis were obtained by slow evaporation of a methanol/acetone solution.

[(η^6-hmb)Ru(en)(SPh)][PF_6] (21). This complex was prepared by refluxing complex **1** (25.0 mg, 0.0496 mmol) and $AgNO_3$ (8.4 mg, 0.0494 mmol) in 2.5 ml of a 1:1 mixture of MeOH and H_2O for 1 h. AgCl was removed by filtration. NaSPh was added (7.9 mg, 0.0595 mmol), and the solution was left overnight. NH_4PF_6 (250 mg) was added, and this gave rise to an orange precipitate. Slow evaporation of an H_2O/acetone solution of the precipitate led to a crystalline orange product and a yellow powder, both of which, by mass spectrometry, were the desired compound. Yield: 10.2 mg (36%). MS: *m/z* 433.0 for [M – PF_6]$^+$ (Calc. 433.1).

HPLC. A Hewlett-Packard 1100 series quaternary pump and a Rheodyne sample injector with 100-μl loop, a Hewlett-Packard 1100 series UV-Vis detector, and a Hewlett-Packard 1100 series Chemstation with a Hewlett-Packard enhanced integrator were used. Separations were carried out on a PLRP-S reversed-phase column (250 × 4.6 mm, 100 Å, 5 μm, Polymer Labs) with detection at selected wavelengths. The mobile phases were A, water (purified by using a Millipore Elix 5 system); B, acetonitrile (for HPLC application, Fisher Chemical, Fair Lawn, NJ), both which contained 0.1% TFAH as the ion-pairing reagent, with a flow rate of 1.0 ml•min^{-1}. Gradient elution was achieved as follows (B%): 0–5 min (Ru complexes alone) or 0–8 min (reaction mixtures of Ru complexes with Cl and GMP) 20%; 11–15 min, 80%, followed by 20% during 16–21 min.

ESI-MS. Positive-ion ESI mass spectra were obtained with a Platform II mass spectrometer (Micromass, Manchester, U.K.). A Waters 2690 HPLC system was

interfaced with the mass spectrometer, using the same column and gradients as described above for the HPLC assays, flow rate of 1.0 ml•min^{-1}, splitting ratio 1/5. The spray voltage was 3.50–3.68 kV. The cone voltage was varied from 15 to 30 V. The capillary temperature was 413 K, with a 450 liter•h^{-1} flow of N_2 drying gas. The quadrupole analyzer, operated at a background pressure of 2×10^{-5} Torr, was scanned at 750 Da•s^{-1}. Data were collected and analyzed on a MASS LYNX V3.5 Windows NT PC data system using the MAX ENT ELECTROSPRAY software algorithm and calibrated versus an NaI calibration file. The mass accuracy of all measurements was within 0.1 *m/z* unit.

X-Ray Crystallography. All data were collected at 150 K on a Bruker Smart Apex charge-coupled device diffractometer equipped with an Oxford Cryosystems low-temperature device. After application of a multiscan absorption correction (SADABS) (5), the structures were all solved by direct methods (SHELXS or SIR92) (6, 7) and refined against F^2 using all data (SHELXL, **3** and **19**, or CRYSTALS, **1**, **5**, **9** and **11**) (8). Complexes **1**, **5**, and **9** are isostructural, and all contain the PF_6^- anion disordered about an .m. special position. Part of the PF_6, comprising 42.4% of the total fluorine occupancy, is disordered about the mirror, with one FPF axis (F31-P1-F41) lying in the mirror plane. The remaining electron density appears to be consistent with a PF_6 oriented with a PF_4 unit in the mirror plane. Successive cycles of refinement and difference maps showed that this PF_4 unit was completely disordered, and so the four part-weight F atoms were modeled with a torus of electron density centered on the P site with a radius restrained to 1.58(2) Å. The atom labeled F2 therefore represents a torus of electron density located in the mirror plane. The en ligand is also disordered over the mirror plane; explicit restraints were applied to the distances and angles involving the C and N atoms of this ligand.

Refinement of the crystal structure of **3** appeared to converge at $R(F > 4\sigma(F)) = 12.6\%$. Inspection of the poorly fitting data showed that all had $|h| = 3n$, and the ROTAX (9) procedure suggested that the crystal was twinned via a two-fold rotation about [100]. This operation is described by the matrix

$$\begin{pmatrix} 1 & 0 & 0 \\ -0.354 & -1 & 0 \\ -0.638 & 0 & -1 \end{pmatrix}.$$

Although the off-diagonal terms in this matrix are near -1/3 and -2/3, they are sufficiently far away from these ideal values that a straightforward nonmerohedral twin refinement did not appear to improve the refinement statistics very significantly. After some experimentation, we have followed Young and colleagues (10) in refining different twin scale factors for the $|h| = 0$, 3, and 6 layers only, whilst leaving other -16 layers with $|h| = 3n$ unsplit. These scales factors refined to 0.387(3), 0.199(2), and 0.042(2), respectively. In addition to the twinning, the structure also exhibits disorder in the PF_6 anion, which lies over two orientations of weights 0.603(13) and 0.397(13), related by an approximate 45° rotation about one FPF axis. No special problems were encountered in the refinement of **11**. A disordered solvent region in **19** was treated by using the "squeeze" procedure (11). Phenyl groups were, in addition, treated as rigid hexagons.

Computation. Basis set I comprised a triple-ζ plus 5p orbital set (TZP) on Ru with double-ζ (DZ) on all H atoms and the two carbon centers of ethylenediamine; all other bases were of DZP quality. Basis set II is as per Basis I except all carbon and H atoms were described with DZP bases. Default convergence criteria were applied for SCF and geometry optimization except the angle threshold, which was set to 1.5° for transition state searches and 2.5° otherwise. The criteria were relaxed due to the long bond lengths at the transition states, which make it harder to define torsional terms accurately. The same problem occurs for reactant and product species because the respective entering and leaving groups are included in the calculation, and their relatively weak interaction with the rest of the complex again leads to less well defined torsional terms. However, the energetic consequences of relaxing the angle constraints are negligible. The Amsterdam Density Functional (ADF) reported a single negative Eigenvalue in the Hessian matrix for all transition state optimizations. Transition states were not confirmed with frequency calculations. The conductor-like screening model (COSMO) as implemented in ADF was used to simulate the aqueous environment with ε = 80, probe radius = 1.4 Å, and the ND

parameter set to 5 (default 3). The atomic radii (Å) used were Ru = 2.120, O = 1.349, C = 1.462, *n* = 1.392, H = 1.135, Cl = 1.912, Br = 2.037, and I = 2.264. The arene ligand was bz or hmb as described.

Kinetics. Aliquots (37–15 μl) of stock solutions of complexes **1**–**21** (4–10 mM) in methanol were diluted to 500 μl with deionized water, and the absorbance at selected wavelengths was recorded at 6- to 20-s intervals at 298 K. The hydrolysis rate constant k_{H2O} for each complex was determined by computer fit of the absorbance/time data to the first-order rate equation

$$A = C_0 + C_1 e^{-kt} \qquad [\mathbf{1}]$$

where C_0 and C_1 are computer-fitted constants, and A is the absorbance corresponding to time t. For the reversible hydrolysis of Ru arene complexes, the forward aquation is pseudo first order, and the backward anation is second order. When the initial concentration of parent compound is small, the relation between the forward (k_{aq}) and backward (k_x) rate constants can be described by Eq. **2** (12):

$$k_{H2Oobs} = k_{aq} + k_x ([Ru(H_2O)]_e + [X]_e), \qquad [\mathbf{2}]$$

where $[Ru(H_2O)]_e$ and $[X]_e$ are the equilibrium concentrations of aqua species and leaving group, respectively. Furthermore, if $k_{aq} >> k_x ([Ru(H_2O)]_e + [X]_e)$, the overall hydrolysis rate constant $k_{H2Oobs} \approx k_{aq}$.

The pseudo-first-order rate constants for substitution of X in **15**, **16**, **17**, and **21** by Cl and GMP were determined analogously by adding aliquots of stock solutions of the respective complexes in methanol to a 104 mM NaCl solution or an appropriate GMP solution.

Partition Coefficients of Complexes 1 and 21. Stock solutions of complexes **1** and **21** (1 mM) were prepared by dissolving the compounds in octanol (spectroscopic grade, Sigma)-saturated water (HPLC grade, Fisher). Aliquots (0.2 ml) of these stock solutions

were transferred to 15-ml test tubes and diluted with octanol-saturated water to 1 ml to give 0.2 mM solutions. Then, 1 ml of water-saturated octanol was added. The mixture was shaken in an IKA Vibrax shaker for 1 h at 500 *g*/min and then centrifuged at 2,000 *g*/min for 10 min. The ruthenium content of samples from the aqueous layer and from the octanol layer was then determined using a UNICAM M series graphite furnace (GF95) atomic absorption spectrometer (Thermo Electron, San Jose, CA). The partition coefficients of complexes **1** and **21** were calculated by using Eq. **3** and represent the mean ± SD of six replicates,

$$\log P_{oct} = \log ([Ru]_{oct}/[Ru]_{aq}), \qquad [3]$$

where $[Ru]_{oct}$ and $[Ru]_{aq}$ are the concentrations of Ru in the octanol and aqueous layers, respectively.

IC_{50} Values. For complexes **1–4**, **12**, **13**, **22**, and **23**, these were determined by using cell counts as described in ref. 13. For all other complexes, the sulforhodamine B assay (14) was used with a 72-h incubation of the plates, except for complexes **16**, **19**, **20**, and **21** for which a 96-h incubation was used.

Cell/DNA Uptake of Complexes 1 and 21. Human ovarian cancer cells (A2780) were plated at a density of 5×10^6 cells in 100 mm Petri dishes containing 9 ml of culture medium (RPMI medium 1640). After 24 h, cells were exposed to a 20 μM concentration of complexes **1** or **21**. Control cells were exposed to DMSO at a similar dilution (0.1% vol/vol) as compounds **1** and **21**. After 24 h of exposure, cells were harvested and counted. For determination of cellular ruthenium, cell pellets were stored until analysis. For determination of DNA-bound ruthenium in the cells, DNA was extracted by using a Nucleon DNA extraction kit (Tepnel Life Sciences, Manchester, U.K.) and dissolved in water. The concentrations of Ru in nitric acid-digested solutions of cell pellets and DNA extracts were determined using an ELAN 600 ICP mass spectrometer (PerkinElmer). Results are presented as pmol of Ru per million cells and are the mean ± SEM of triplicate samples.

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