

Organometallic Glutathione S-Transferase Inhibitors

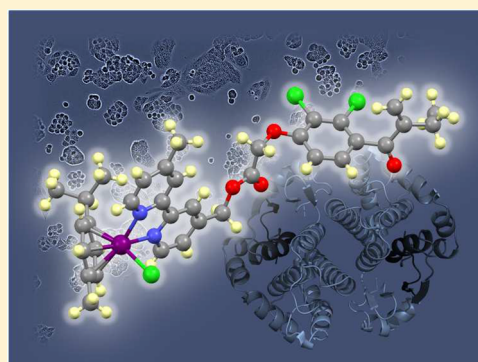
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Supporting Information

ABSTRACT: A new family of organometallic *p*-cymene ruthenium(II) and osmium(II) complexes conjugated to ethacrynic acid, a glutathione transferase (GST) inhibitor, is reported. The ethacrynic acid moiety (either one or two) is tethered to the arene ruthenium(II) and osmium(II) fragments via strongly coordinating modified bipyridine ligands. The solid-state structure of one of the complexes, i.e. [Os(η^6 -*p*-cymene)Cl][(4'-methyl-[2,2'-bipyridin]-4-yl)-methyl-2-(2,3-dichloro-4-(2-methylenebutanoyl)phenoxy)acetate]Cl, was established by single-crystal X-ray diffraction, corroborating the expected structure. The complexes are efficient inhibitors of GST P1-1, an enzyme expressed in cancer cells and implicated in drug resistance, and are cytotoxic to the GST-overexpressing chemoresistant A2780cisR ovarian cancer cell line.



INTRODUCTION

Resistance to chemotherapy, either intrinsic or acquired, remains a serious obstacle in cancer treatment. Drug resistance is a multifactorial process, and several basic mechanisms have been identified, including enhanced drug efflux associated with the P-glycoprotein, enhanced repair of drug-induced injury, and enhanced detoxification of drug intermediates, frequently related to intracellular glutathione (GSH) and GSH-associated enzymes.^{1–4} In particular, the overexpression of glutathione transferases (GSTs) and efflux pumps in cancer cells reduces the efficacy of various anticancer drugs,^{1–4} and the search for compounds capable of inhibiting GSTs and/or efflux pumps is key to overcoming resistance to anticancer drugs.

GSTs are a family of homo- and heterodimeric cytosolic enzymes that catalyze the conjugation of GSH with a variety of exogenous and endogenous electrophiles and, consequently, are involved in the inactivation of various electrophile-producing anticancer agents. The products, in the form of mercapturates, are subsequently eliminated from the cell.⁵ As GSH and the GST superfamily play an important role in the mechanism of anticancer drug resistance,^{2,6–8} including metal-based drugs,^{9–15} efforts have focused on the development of GST inhibitors and GST-activated prodrugs as means of sensitizing drug-resistant cancer cells to treatment.^{16–18} In this context, ethacrynic acid ([2,3-dichloro-4-(2-methylene-1-oxobutyl)phenoxy]acetic acid, EA-H; Figure 1) is a versatile, water-soluble compound that modulates GST activity. EA-H conjugation to GSH may occur in a spontaneous fashion or via the catalytic action of the major classes of GST (α , μ , and π), with the π isozyme being the most active.¹⁹ EA-H potentiates the in vitro cytotoxicity of chemotherapeutic agents, including alkylating agents such as melfalan,^{20–23} carmustine,²⁴

mitomycin C,²⁵ nitrogen mustards,^{17,26} and chlorambucil.^{19,27} EA-H induced sensitization of tumor cells to doxorubicin^{26,28} or cisplatin^{20,29,30} has also been reported. It was suggested that EA-H may modulate the drug resistance of tumor cells not only by inhibiting GST activity but also by inhibiting the export of GSH conjugates of drugs from the cell by the multidrug resistance protein.³¹

Platinum-based cancer drugs, such as cisplatin, are highly effective chemotherapeutic agents used extensively for the treatment of solid tumors. Nevertheless, drug resistance is a major obstacle for platinum-based chemotherapy, frequently attributed to GSH-mediated detoxification. GST enzymes, specifically GST- π isozymes, are overexpressed in cisplatin-resistant cell lines, and the inhibition of these enzymes can lead to the reversal of drug resistance, as the combination of cisplatin with EA-H resulted in improved activity against cisplatin-resistant cells.^{20,29,30} The codelivery of EA-H and DACHPt (a precursor of oxaliplatin) using biodegradable nanoparticles results in an enhancement of the anticancer efficacy.³² In vivo, the nanoparticles demonstrated greater anticancer efficacy and less systemic toxicity, in comparison to a combination therapy involving the free drugs.³² Recently, GST inhibitor–anticancer drug conjugates have been reported, such as ethacraplatin, a platinum(IV) metallodrug with two tethered EA-H moieties.^{33–35} Ethacraplatin is a stronger inhibitor of GST activity in live mammalian cells in comparison to either cisplatin or EA-H alone,³³ and the complex was shown to reverse cisplatin resistance in the microsomal-GST-over-

Received: June 20, 2017

Published: August 24, 2017

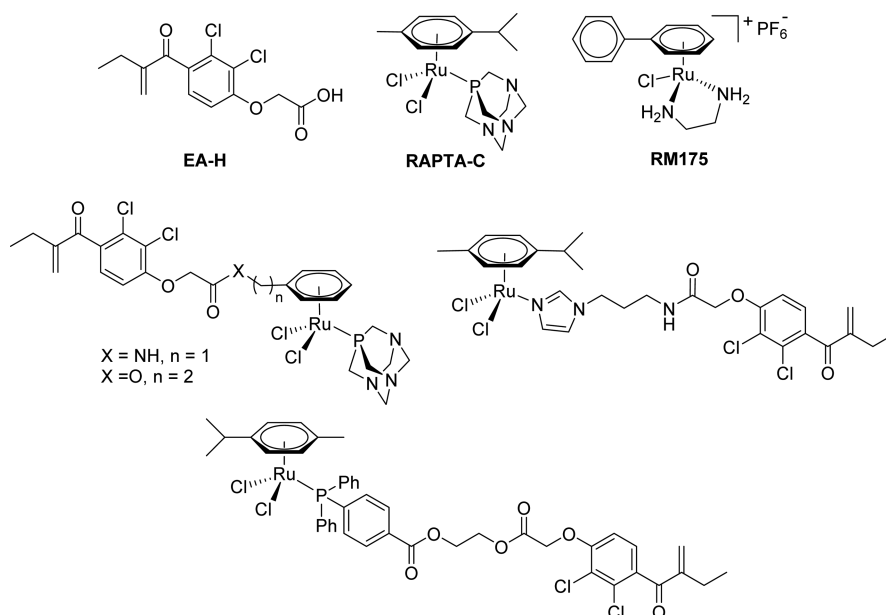


Figure 1. Structure of ethacrynic acid (EA-H), RAPTA-C, RM175, and examples of ethacrynic acid–ruthenium(II) conjugates.

expressing MCF7 cell line.³⁶ Platinum(II)–EA conjugates have been also reported.³⁴

Ruthenium compounds are promising alternatives to classical platinum-based derivatives, and various ruthenium(III) and ruthenium(II) complexes have been reported to exhibit relevant antitumor activity. Two octahedral ruthenium(III) complexes are under clinical development: i.e., *trans*-[HIm]-[Ru^{III}Cl₄(DMSO)(Im)] (Im = imidazole; NAMI-A), which is essentially inactive on primary tumors but exhibits antimetastatic activity,^{37–39} and *trans*-[HIn][Ru^{III}Cl₄(Ind)₂] (Ind = indazole; KP1019), which is active against primary tumors.^{40,41} Organometallic ruthenium(II) arene complexes such as [Ru^{II}(η^6 -*p*-cymene)(PTA)Cl₂] (PTA = 1,3,5-triaza-7-phosphatrycyclo[3.3.1.1]decane; RAPTA-C)^{42–45} and [Ru^{II}(η^6 -*p*-cymene)(en)Cl]⁺ (en = ethylenediamine; RM175)^{46–48} (Figure 1) show considerable promise as anticancer drugs in a number of preclinical models. Osmium(II)-based analogues of these compounds have also been reported and show promising anticancer properties.^{48,49}

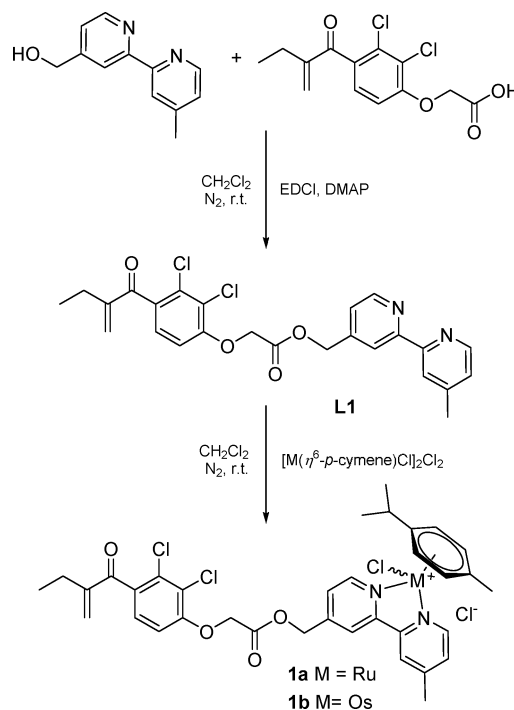
One strategy used to modulate the medicinal properties of ruthenium(II) arene complexes (and related osmium compounds) is to tether a functional bioactive molecule to the metal framework.^{50–57}

Since bifunctional RAPTA-like compounds modified with EA show promise as both GST inhibitors and anticancer agents (Figure 1),^{33,58–62} we decided to prepare and evaluate a new generation of dual-targeting ruthenium(II)– and osmium(II)–EA conjugates related to the [Ru^{II}(η^6 -*p*-cymene)(en)Cl]⁺ structural motif, in which the en ligand is replaced by EA-modified bipyridine ligands.

RESULTS AND DISCUSSION

The compounds described in this study were prepared according to the routes shown in Schemes 1 and 2. Bipyridine derivatives were modified with either one or two EA units (it has been shown that organic compounds containing two EA units are potent inhibitors of GST^{63,64}). The route to the bipyridine ligand with one EA moiety, i.e. L1, involves the direct reaction of EA-H with 4-hydroxymethyl-4'-methyl-2,2'

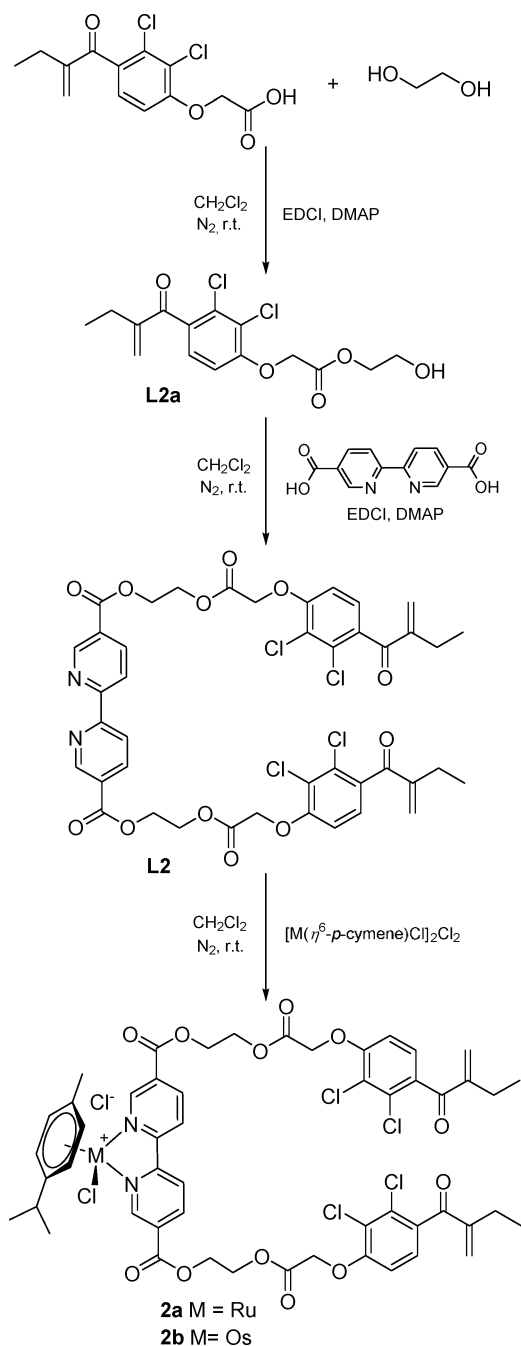
Scheme 1. Synthesis of Ligand L1 and Complexes 1a,b



bipyridyl in the presence of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDCI) employed as a coupling agent and 4-(dimethylamino)pyridine (DMAP) catalyst. Subsequent reaction of L1 with the dimers [M(η^6 -*p*-cymene)Cl]₂Cl₂ (M = Ru, Os) affords complexes 1a,b in good yield (Scheme 1).

The disubstituted EA-bipyridine ligand L2 was obtained following a two-step procedure (Scheme 2) by initially modifying EA-H with ethylene glycol to afford L2a and subsequent coupling to (2,2'-bipyridine)-5,5'-dicarboxylic acid. Both steps employ EDCI and DMAP as the coupling agent and catalyst, respectively. The resulting ligand, L2, reacts smoothly with [M(η^6 -*p*-cymene)Cl]₂Cl₂ (M = Ru, Os) to afford complexes 2a,b.

Scheme 2. Synthesis of Ligand L2 and Complexes 2a,b



The ligands and complexes were fully characterized by ^1H and ^{13}C NMR spectroscopy, mass spectrometry, IR spectroscopy and elemental analysis (see the [Experimental Section](#) for full details). The asymmetric bidentate ligand **L1** induces chirality at the metal center in **1a,b**, resulting in loss of the 2-fold symmetry of the η^6 -*p*-cymene moiety,⁶⁵ and thus, **1a,b** were obtained as a racemic mixture.

The ^1H NMR spectra of **1a,b** contain four separate doublets corresponding to the aromatic protons of the *p*-cymene ring and two doublets attributable to the isopropyl group, one for each methyl group, these resonances revealing the stereogenic and chirotopic nature of the metal center (local C_1 symmetry).⁶⁵ The ^1H NMR spectra of ligand **L1** and the corresponding complexes **1a,b** exhibit peaks which show that the protons α to the N atoms of the bipyridine unit are strongly

influenced by coordination to the metal ion; for **L1** peaks corresponding to the α protons appear at 8.65 and 8.53 ppm and are observed at 9.41 and 9.28 ppm in **1a** and at 9.34 and 9.22 ppm in **1b** (for all of the compounds the signals observed at higher frequencies may be attributed to the EA-modified pyridine ring, whereas the peaks at lower frequencies correspond to the pyridine ring substituted with the methyl group). For the corresponding C atoms a similar shift to higher frequencies is observed upon coordination of **L1** (**L1**, 150.3 and 149.7 ppm; **1a**, 156.7 and 156.1 ppm; **1b**, 156.1 and 156.0 ppm). Coordination of ligand **L2** to the metal centers leads to a shift of all the peaks corresponding to the pyridine rings to higher frequencies.

The structure of the compounds was further confirmed by electrospray ionization mass spectrometry. For the ligands **L1** and **L2** the representative parent peak appears at $[\text{M} + \text{H}]^+$, and for the complexes **1a,b** and **2a,b** the highest mass peak corresponds to the $[\text{M} - \text{Cl}]^+$ ion. Moreover, all of the complexes are stable in DMSO solutions used for the biological tests (see [Figures S5 and S6](#) in the Supporting Information).

The crystal structure of **1b** was established in the solid state by single-crystal X-ray diffraction ([Figure 2](#)), confirming the

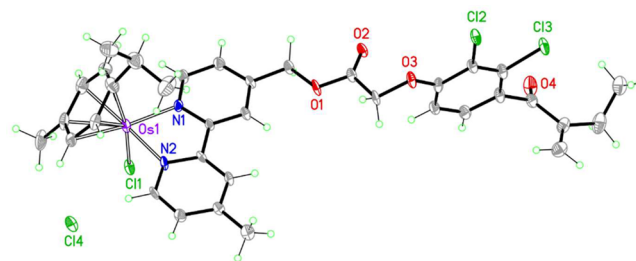


Figure 2. ORTEP representation of complex **1b** (thermal ellipsoids are 40% equiprobability envelopes, and H atoms are spheres of arbitrary diameter).

expected structure. The structure comprises the classical three-legged piano-stool arrangement. The coordination sphere of the Os(II) ion contains an η^6 -*p*-cymene ring, the bidentate ligand, and a chloride ligand. The average Os– η^6 centroid distance is 1.675(4) Å. The bipyridine ligand forms essentially a planar five-membered chelating ring, the two pyridine rings of the bipyridine ligands being almost coplanar (dihedral angle N1–C–C–N2 2.2(11)°) ([Figure S1](#)). The chelate bite angle N1–Os–N2 is 76.6(3)°, and the N1–Os–Cl and N2–Os–Cl angles are 84.6(2) and 86.1(2)°, respectively. The Os–N_{py} bond lengths are 2.090(7) Å (for Os–N1) and 2.103(7) Å (for Os–N2), and the Os–Cl bond length is 2.404(3) Å. The packing in the crystal comprises intermolecular parallel-displaced π – π stacking interactions between the bipyridine ligands of two adjacent molecules (centroid–centroid distance 3.955 Å, [Figure S2](#) in the Supporting Information). Another key interaction in **1b** comprises a three-center hydrogen bond between the chloride counteranion (acting as a bifurcated acceptor) and the hydrogen atoms of CH₃OH and CHCl₃ solvent molecules (see [Figure S4](#) and [Table S1](#) in the Supporting Information for the parameters characterizing the hydrogen bonding interaction).

Human GST P1-1 used for the experiments was expressed in and purified from *E. coli* using a protocol reported earlier.^{66,67} The inhibitory activity of the compounds was investigated by determining the residual catalytic activity of the enzyme using

the CDNB-GSH (1-chloro-2,4-dinitrobenzene–glutathione) assay.⁶⁸ In brief, the enzyme was treated with varying concentrations of complexes **1a,b** and **2a,b** to determine the IC₅₀ value i.e., the concentration at which 50% enzyme activity is inhibited.

Complexes **1a,b** (containing only one pendant ethacrynic acid unit) inhibit GST P1-1 activity slightly more efficiently than EA-H⁵⁸ (see Table 1). Notably, complexes **2a,b**,

Table 1. Inhibition of Human GST P1-1^a

compound	GST P1-1
RAPTA-C	>200 ^b
EA	12.0 ^b
1a	9.06 ± 1.43
1b	9.53 ± 3.32
2a	3.96 ± 0.98
2b	3.98 ± 1.68

^aIC₅₀ values (μM) are given as the mean ± SD. ^bValues taken from ref 58.

containing two EA units, are more potent inhibitors of GST P1-1. Furthermore, treatment with **2b** results in almost complete inhibition of GST P1-1 activity (residual activity <5%) at the highest concentration tested (100 μM), whereas there is more significant residual GST P1-1 activity (~14%) after treatment with **1a,b** at the same concentration (see Figure S7 in the Supporting Information). These findings suggest a role of the organometallic ruthenium fragment in the inhibition of GST P1-1, potentially involving direct coordination of the metal ion to the enzyme as established previously.⁵⁸

The cytotoxicity of ligands **L1** and **L2**, complexes **1a,b** and **2a,b**, and relevant control compounds was assessed in two cancer cell lines (human ovarian carcinoma cisplatin-sensitive A2780 and -resistant A2780cisR) and human embryonic kidney (HEK-293) cells used as a model for normal cells. Cytotoxicity studies were carried out at 37 °C for 72 h (Table 2 and Figure S8 in the Supporting Information) using an MTT test (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) to monitor cell viability. All of the complexes are more cytotoxic than RAPTA-C⁶⁹ and its Os analogue, termed OAPTA-C ([Os^{II}(η⁶-*p*-cymene)(PTA)Cl₂], PTA = 1,3,5-

Table 2. IC₅₀ Values (μM) Determined for RAPTA-C, OAPTA-C, EA-H, Ligands L1 and L2, and Complexes 1a,b and 2a,b towards Human Ovarian Cancer A2780 and A2780cisR (Cisplatin Sensitive and Resistant) and Immortalized Human Embryonic Kidney HEK-293 Cells Using the MTT Assay^a

compound	A2780	A2780cisR	HEK-293
RAPTA-C	>200 ^b	>200 ^b	>1000 ^b
OAPTA-C	>200	>200	>200
EA	40 ± 3	53 ± 5	39 ± 1
L1	14.44 ± 1.97	24.3 ± 0.9	15.9 ± 5.1
1a	26.1 ± 1.6	84 ± 1	67 ± 2
1b	18.81 ± 0.85	59 ± 7	42.6 ± 1.0
L2	73 ± 3	639 ± 7	157 ± 20
2a	7.77 ± 0.01	8.37 ± 1.01	6.16 ± 1.79
2b	9.31 ± 2.62	15.7 ± 1.3	12.80 ± 0.18
cisplatin	0.95	11.14	16.51

^aValues are given as the mean ± SD. ^bValues taken from ref 69.

triaz-7-phosphatricyclo[3.3.1.1]decane). Ligand **L1** as well as the complexes containing two EA units, i.e. **2a,b**, present a higher antiproliferative activity on all the tested cell lines in comparison to EA-H. For complexes **1a,b** with only one EA unit a ca. 3-fold decrease in cytotoxicity against the cisplatin-resistant ovarian carcinoma cells A2780cisR, in comparison to the cisplatin-sensitive cells A2780 cells, is observed. For these compounds the attachment of the organometallic M^{II}-*p*-cymene unit to ligand **L1** leads to a decrease in cytotoxicity to both A2780 and A2780cisR cells, whereas the Os(II) complex is more active than the Ru(II) complex. The IC₅₀ values determined for **1a,b** on the nontumoral HEK-293 cells are ca. 2-fold higher in comparison to those of the A2780 cells.

Interestingly, ligand **L1**, derivatized with one EA unit, has a higher antiproliferative activity in comparison to EA-H on all the tested cell lines, whereas attachment of two EA units in **L2** is accompanied by a significant decrease in cytotoxicity.

Attachment of an organometallic M^{II}-*p*-cymene moiety to **L2** in **2a,b** leads to a significant increase in the antiproliferative activity toward all tested cell lines. The ruthenium complex with two EA moieties, **2a**, displays very similar IC₅₀ values in both the A2780 and A2780cisR cell lines (7.77 and 8.37 μM, respectively), and the Os analogue is slightly less cytotoxic with values of 9.31 and 15.7 μM, respectively. However, **2a,b** are both cytotoxic to the nontumorigenic HEK-293 cells with IC₅₀ values of 6.16 and 12.8 μM, respectively. The relatively potent cytotoxicity of **2a,b** cannot be attributed entirely to the ability of the complexes to inhibit GST activity but is presumably also in part due to the increased hydrophobicity of the complexes relative to RAPTA-C and OAPTA-C, which potentially enhances drug uptake and, consequently, their cytotoxic effects. Overall, complexes **1a,b** are less cytotoxic in all three cell lines than **2a,b**. Since the former pair are chiral but obtained as a racemic mixture, it is possible that the individual optical isomers present different antiproliferative activities. Indeed, it has been previously shown that different optical isomers of chiral-at-metal or chiral-at-ligand ruthenium(II) and Os(II) arene complexes may or may not exhibit similar cytotoxicities.^{70–73} Interestingly, in all three cell lines, for the Ru/Os pair **1a,b** the osmium complex is more cytotoxic, whereas for the pair **2a,b** the ruthenium complex presents higher antiproliferative activity. Such a difference is not entirely unexpected, as there appears to be no clear correlation from the multitude of studies reporting analogues based on these two metals.⁷⁴

CONCLUSIONS

In summary, we have shown that incorporation of organometallic *p*-cymene ruthenium(II) or osmium(II) centers to EA-derived compounds enhances the inhibition of GST P1-1 activity, an enzyme expressed in cancer cells and responsible for drug resistance. The enhanced inhibition is particularly notable for **2a,b**, in which the metal ion is located between two EA moieties. These two compounds are also the most cytotoxic of the series, displaying similar antiproliferative activity to both A2780 and A2780cisR cells, indicative of overcoming GST-type resistance in the latter cell line which is known to overexpress GSTs.¹¹ Interestingly, while there is no difference between the pairs of ruthenium and osmium complexes in terms of GST P1-1 inhibition and only a slight difference with respect to antiproliferative activity, the metal ions appear to play a critical role, i.e. in comparison to the free ligands, implying that direct interactions with the enzyme might be involved.

EXPERIMENTAL SECTION

General Experimental Conditions. RuCl₃·3H₂O was obtained from Precious Metals Online, and all other chemicals were purchased from Aldrich, AlfaAesar, Acros, ABCR, or TCI Chemicals and used without further purification. Reactions were performed under an inert atmosphere of N₂ using Schlenk techniques with solvents dried using drying columns. The dimers [Ru(η^6 -*p*-cymene)Cl]₂Cl₂ and [Os(η^6 -*p*-cymene)Cl]₂Cl₂ were prepared and purified according to literature procedures.⁷⁵ ¹H (400.13 MHz) and ¹³C (100.62 MHz) NMR spectra were recorded on a Bruker Avance II 400 spectrometer at 298 K unless otherwise stated. The chemical shifts are reported in parts per million (ppm) and referenced to residual solvent peaks (CDCl₃, ¹H δ 7.26, ¹³C{¹H} δ 77.16 ppm; MeOD-*d*₄, ¹H δ 3.31, ¹³C{¹H} δ 49.00 ppm),⁷⁶ and coupling constants (*J*) are reported in hertz (Hz). IR spectra were recorded on a PerkinElmer Spectrum One FT-IR spectrometer at room temperature. Electrospray ionization mass spectra (ESI-MS) were obtained on a Thermo-Finnigan LCQ Deca XP Plus quadrupole ion-trap instrument operated in positive-ion mode. Elemental analyses were carried out by the microanalytical laboratory at the Institute of Chemical Sciences and Engineering (EPFL). Melting points were determined using a SMP3 Stuart Melting Point Apparatus and are uncorrected. Reactions were monitored by TLC using Merck TLC silica gel coated aluminum sheets 60 F₂₅₄ and visualized with UV at 254 nm and KMnO₄ stain. Compounds were purified by column flash chromatography on silica gel using the elution systems indicated.

Syntheses of the Ligands. *Synthesis of (4'-Methyl-[2,2'-bipyridin]-4-yl)methyl-2-(2,3-dichloro-4-(2-methylenebutanoyl)-phenoxy)acetate (L1).* To a solution of ethacrynic acid (1.090 g, 1.2 equiv) in CH₂Cl₂ (100 mL) were added EDCI (0.804 g, 1.4 equiv), 4'-methyl-[2,2'-bipyridin]-4-ylmethanol (0.600 g, 1 equiv), and DMAP (0.110 g, 0.3 equiv). The reaction mixture was stirred at room temperature for 24 h, and then the mixture was diluted with CH₂Cl₂ (50 mL) and washed with H₂O (150 mL). The isolated organic phase was further washed with brine (150 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. Purification by flash column chromatography using a CH₂Cl₂/MeOH mixture as eluent afforded the product as a colorless solid (1.276 g, yield 88%): mp (°C): 71–72; R_f (CH₂Cl₂/MeOH 9.5/0.5): 0.543; ¹H NMR (CDCl₃) δ _H (ppm) 8.65 (1H, dd, O-CH₂-(Py)C-CH-CH-N, ³J_{H,H} = 5.0 Hz, ⁴J_{H,H} = 0.4 Hz), 8.53 (1H, d, CH₃-(Py)C-CH-CH-N, ³J_{H,H} = 5.0 Hz), 8.40 (1H, m, O-CH₂-(Py)C-CH-C-N), 8.25 (1H, m, CH₃-(Py)C-CH-C-N), 7.24 (1H, dd, O-CH₂-(Py)C-CH-CH-N, ³J_{H,H} = 5.0 Hz, ⁴J_{H,H} = 1.7 Hz), 7.16 (1H, dd, CH₃-(Py)C-CH-CH-N, ³J_{H,H} = 5.0 Hz, ⁴J_{H,H} = 0.7 Hz), 7.10 (1H, d, (Ar)CH-C-(C=O)), ³J_{H,H} = 8.5 Hz), 6.80 (1H, d, (Ar)CH-C-O-CH₂-(C=O)), ³J_{H,H} = 8.5 Hz), 5.90 (1H, t, cis (CH=C)-CH₂-CH₃, ⁴J_{H,H} = 1.4 Hz), 5.55 (1H, s, trans (CH=C)-CH₂-CH₃), 5.33 (2H, s, O-CH₂-(Py)C-CH-CH-N), 4.88 (2H, s, (Ar)C-O-CH₂-(C=O)), 2.45 (2H, m, (CH₂=C)-CH₂-CH₃, ³J_{H,H} = 7.5 Hz), 2.44 (3H, s, CH₃-(Py)C-CH-CH-N), 1.13 (3H, t, (CH₂=C)-CH₂-CH₃, ⁴J_{H,H} = 7.5 Hz); ¹³C NMR (CDCl₃) δ _C (ppm) 195.8 (1C, (Ar)C-(C=O)-(C=CH₂)), 167.6 (1C, (Ar)C-O-CH₂-(C=O)-O), 156.8 (1C, O-CH₂-(Py)C-CH-C-N), 155.4 (1C, (Ar)CH-C-O-CH₂-(C=O)), 155.3 (1C, CH₃-(Py)C-CH-C-N), 150.3 (1C, O-CH₂-(Py)C-CH-CH-N), 149.7 (1C, CH₃-(Py)C-CH-CH-N), 149.0 (1C, CH₃-CH₂-(C=CH₂)), 148.7 (1C, O-CH₂-(Py)C-CH-CH-N), 144.9 (1C, CH₃-(Py)C-CH-CH-N), 134.2 (1C, Cl-(Ar)C-C-(C=O)), 131.7 (1C, Cl-(Ar)C-C-(C=O)), 128.7 (1C, (CH₂=C)-CH₂-CH₃), 126.9 (1C, (Ar)CH-C-(C=O)), 125.2 (1C, CH₃-(Py)C-CH-CH-N), 123.6 (1C, Cl-(Ar)C-C-O-CH₂-(C=O)), 122.3 (1C, CH₃-(Py)C-CH-C-N), 122.0 (1C, O-CH₂-(Py)C-CH-CH-N), 119.6 (1C, O-CH₂-(Py)C-CH-C-N), 111.0 (1C, (Ar)CH-C-O-CH₂-(C=O)), 66.3 (1C, (Ar)C-O-CH₂-(C=O)), 65.6 (1C, O-CH₂-(Py)C-CH-CH-N), 23.5 (1C, (CH₂=C)-CH₂-CH₃), 21.4 (1C, CH₃-(Py)C-CH-CH-N), 12.5 (1C, (CH₂=C)-CH₂-CH₃); IR (ν , cm⁻¹) 2925 (C-H, CH₂, CH₃), 1760 (C=O, ester), 1664 (C=O, ketone, C=C, alkene), 1584, 1462, 1381 (ring skeleton C=C, C-C, C=N, C-N), 1291, 1258, 1186, 1076 (C-O, ester, ether), 820 (C-H aromatic); ESI-MS(+) *m/z* 485.14 [M]⁺, calcd for C₂₅H₂₂Cl₂N₂O₄ 485.36 (the

isotopic pattern corresponds well to the calculated pattern). Anal. Calcd for C₂₅H₂₂Cl₂N₂O₄: C, 61.87; H, 4.57; N, 5.77. Found: C, 61.77; H, 4.63; N, 5.48.

Synthesis of 2-Hydroxyethyl-2-(2,3-dichloro-4-(2-methylenebutanoyl)phenoxy)acetate (L2a). To a solution of ethacrynic acid (4.000 g, 1 equiv) in CH₂Cl₂ (mL) were added EDCI (3.035 g, 1.2 equiv), ethylene glycol (3 mL, 4 equiv), and DMAP (0.322 g, 0.2 equiv). The reaction mixture was stirred at room temperature for 24 h, and then the mixture was diluted with CH₂Cl₂ (50 mL) and washed with H₂O (150 mL) and the isolated organic phase was further washed with brine (150 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. Purification by flash column chromatography using a Hex/EtOAc mixture as eluent afforded the product as a white solid (3.459 g, yield 76%): Mp (°C) 94.5–95.5; R_f (Hex/AcOEt 4/6) 0.260; ¹H NMR (CDCl₃) δ _H (ppm) 7.10 (1H, d, (Ar)CH-C-(C=O)), ³J_{H,H} = 8.5 Hz), 6.78 (1H, d, (Ar)CH-C-O-CH₂-(C=O)), ³J_{H,H} = 8.5 Hz), 5.92 (1H, t, cis (CH=C)-CH₂-CH₃, ⁴J_{H,H} = 1.4 Hz), 5.57 (1H, s, trans (CH=C)-CH₂-CH₃), 4.78 (2H, s, (Ar)C-O-CH₂-(C=O)-O), 4.30–4.32 (2H, m, (C=O)-O-CH₂-CH₂-OH), 3.80–3.83 (2H, m, (C=O)-O-CH₂-CH₂-OH), 2.42 (2H, m, (CH₂=C)-CH₂-CH₃, ³J_{H,H} = 7.5 Hz), 1.11 (3H, t, (CH₂=C)-CH₂-CH₃, ³J_{H,H} = 7.5 Hz); ¹³C NMR (CDCl₃) δ _C (ppm) 196.0 (1C, (Ar)C-(C=O)-(C=CH₂)), 168.1 (1C, (Ar)C-O-CH₂-(C=O)), 155.5 (1C, (Ar)CH-C-O), 150.2 (1C, (Ar)C-CH₂-(C=CH₂)), 134.0 (1C, Cl-(Ar)C-C-(C=O)), 131.6 (1C, Cl-(Ar)C-C-(C=O)), 128.9 (1C, (CH₂=C)-CH₂-CH₃), 126.9 (1C, (Ar)CH-C-(C=O)), 123.4 (1C, Cl-(Ar)C-C-O-CH₂-(C=O)), 111.0 (1C, (Ar)CH-C-O-CH₂), 67.0 (1C, (Ar)C-O-CH₂-(C=O)-O), 66.3 (1C, (C=O)-O-CH₂-CH₂-OH), 60.8 (1C, (C=O)-O-CH₂-CH₂-OH), 23.5 (1C, (CH₂=C)-CH₂-CH₃), 12.5 (1C, (CH₂=C)-CH₂-CH₃); IR (ν , cm⁻¹): 3509 (O-H); 2975–2880 (C-H, CH₂, CH₃), 1736 (C=O, ester), 1661 (C=O, ketone, C=C, alkene), 1587, 1471, 1383 (ring skeleton C=C, C-C); 1291, 1229, 1204, 1075 (C-O, ester, ether), 891 (C-H aromatic); ESI-MS(+): *m/z* 347.01 [M]⁺, calcd for C₁₅H₁₆Cl₂O₅ 347.19 (the isotopic pattern corresponds well to the calculated pattern). Anal. Calcd for C₁₅H₁₆Cl₂O₅: C, 51.89; H, 4.65. Found: C, 51.95; H, 4.69;

Synthesis of bis(2-(2-(2,3-dichloro-4-(2-methylenebutanoyl)-phenoxy)acetoxylethyl)-(2,2'-bipyridine)-5,5'-dicarboxylate (L2). To a suspension of [2,2'-bipyridine]-5,5'-dicarboxylic acid (0.765 g, 1 equiv) in a mixture of CH₂Cl₂ (20 mL) and (CH₃)₂NC(O)H (40 mL) were added EDCI (1.441 g, 2.4 equiv), L2a (2.500 g, 2.3 equiv), and DMAP (0.230 g, 0.6 equiv). The reaction mixture was stirred at room temperature for 48 h and then the mixture was concentrated to dryness under reduced pressure and the crude product was solubilized in CH₂Cl₂ (150 mL) and washed with H₂O (150 mL). The isolated organic phase was further washed with brine (150 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. Purification by flash column chromatography using a Hex/EtOAc mixture as eluent afforded the product as a white solid (0.875 g, yield 28%): mp (°C) 136–137; R_f (Hex/EtOAc 3/7 (v/v)) 0.729; ¹H NMR (CDCl₃) δ _H (ppm) 9.28 (2H, s, 2xO-(C=O)-(Py)C-CH-N), 8.60 (2H, d, 2 x (Py)N-C-CH-CH-C), ³J_{H,H} = 8.2 Hz), 8.42 (2H, d, 2 x (Py)N-C-CH-CH-C, ³J_{H,H} = 8.2 Hz), 7.10 (2H, d, (Ar)CH-C-(C=O)), ³J_{H,H} = 8.5 Hz), 6.81 (2H, d, (Ar)CH-C-O-CH₂-(C=O)), ³J_{H,H} = 8.5 Hz), 5.90 (2H, s, 2 x cis (CH=C)-CH₂-CH₃), 5.56 (2H, s, 2 x trans (CH=C)-CH₂-CH₃), 4.82 (4H, s, 2 x (Ar)C-O-CH₂-(C=O)), 4.63 (8H, m, 2 x (Ar)C-O-CH₂-(C=O)-O-CH₂-CH₂-(C=O)), 4.63 (8H, m, 2 x (Ar)C-O-CH₂-(C=O)-O-CH₂-CH₂-(C=O)), 2.43 (4H, m, 2 x (CH₂=C)-CH₂-CH₃, ³J_{H,H} = 7.4 Hz), 1.12 (6H, t, 2 x (CH₂=C)-CH₂-CH₃, ⁴J_{H,H} = 7.4 Hz); ¹³C NMR (CDCl₃) δ _C (ppm) 195.8 (2C, 2 x (Ar)C-(C=O)-(C=CH₂)), 167.9 (2C, 2 x (Ar)C-O-CH₂-(C=O)), 164.9 (2C, 2 x (Py)N-CH-C-(C=O)-O), 158.6 (2C, 2 x (Py)N-C-CH-CH-C), 155.5 (2C, 2 x (Ar)CH-C-O), 150.8 (2C, 2 x (Py)N-CH-C-(C=O)), 150.3 (2C, 2xCH₃-CH₂-(C=CH₂)), 138.4 (2C, 2 x (Py)N-C-CH-CH-C), 134.2 (2C, 2 x Cl-(Ar)C-C-(C=O)), 131.7 (2C, 2 x Cl-(Ar)C-C-(C=O)), 128.7 (2C, 2 x (CH₂=C)-CH₂-CH₃), 126.9 (2C, 2 x (Ar)CH-C-(C=O)), 126.0 (2C, 2 x (Py)N-CH-C-(C=O)-O), 123.6 (2C, 2 x Cl-(Ar)C-C-O), 121.6 (2C, 2 x (Py)N-C-CH-CH-C), 111.0 (2C, 2 x (Ar)CH-C-O-CH₂-(C=O)), 66.3 (2C, 2

\times (Ar)C-O-CH₂-(C=O)), 63.2 (2C, $2 \times$ (Ar)C-O-CH₂-(C=O)-O-CH₂-CH₂), 62.9 (2C, $2 \times$ (Ar)C-O-CH₂-(C=O)-O-CH₂-CH₂), 23.5 (2C, $2 \times$ (CH₂=C)-CH₂-CH₃), 12.5 (2C, $2 \times$ (CH₂=C)-CH₂-CH₃); IR (ν , cm⁻¹) 2967 (C-H, CH₂, CH₃), 1762 (C=O, ester), 1720 (C=O, ester), 1663 (C=O, ketone, C=C, alkene), 1585, 1466, 1384 (ring skeleton C=C, C-C, C=N, C-N), 1267, 1190, 1111, 1079 (C-O, ester, ether), 802 (C-H aromatic); ESI-MS(+) m/z 903.11 [M + H]⁺, 925.09 [M + Na]⁺, calcd for C₄₂H₃₆Cl₄N₂O₁₂ 902.55 (the isotopic pattern corresponds well to the calculated pattern). Anal. Calcd for C₄₂H₃₆Cl₄N₂O₁₂: C, 55.89; H, 4.02; N, 3.10. Found: C, 56.00; H, 4.12; N, 3.09.

General Procedure for the Synthesis of the Ru(II)- and Os(II)-Arene Complexes. To a solution of the appropriate dimer, [Ru(η^6 -p-cymene)Cl]₂Cl₂ or [Os(η^6 -p-cymene)Cl]₂Cl₂, in CH₂Cl₂ (10 mL) was added a solution of the appropriate ligand in CH₂Cl₂ (25 mL), and the obtained mixture was stirred at room temperature in the dark for 24 h. The solvent was removed under reduced pressure, the crude product was redissolved in CH₂Cl₂ (1 mL), and the product was precipitated with Et₂O (5 mL) and then successively washed with warm Et₂O (4 \times 50 mL) and hexane (2 \times 25 mL). The resulting solid was dried under high vacuum.

Synthesis of [Ru(η^6 -p-cymene)Cl]([4'-methyl-[2,2'-bipyridin]-4-yl)methyl-2-(2,3-dichloro-4-(2-ethylenebutanoyl)phenoxy)acetate]-Cl (1a). Complex 1a was prepared following the general procedure starting from [Ru(η^6 -p-cymene)Cl]₂Cl₂ (0.222 g, 1 equiv) and ligand L1 (0.352 g, 2 equiv), to afford an orange solid (0.537 g, yield 94%): mp (°C) 149–151 dec; ¹H NMR (MeOD-*d*₄) δ_{H} (ppm) 9.41 (1H, d, O-CH₂-(Py)C-CH-CH-N, ³J_{H,H} = 5.9 Hz), 9.28 (1H, d, CH₃-(Py)C-CH-CH-N, ³J_{H,H} = 5.9 Hz), 8.46 (1H, m, O-CH₂-(Py)C-CH-C-N), 8.39 (1H, m, CH₃-(Py)C-CH-C-N), 7.61 (1H, dd, O-CH₂-(Py)C-CH-CH-N, ³J_{H,H} = 5.9 Hz, ⁴J_{H,H} = 1.1 Hz), 7.59 (1H, dd, CH₃-(Py)C-CH-CH-N, ³J_{H,H} = 5.9 Hz, ⁴J_{H,H} = 1.7 Hz), 7.21 (1H, d, (Ar)CH-C-(C=O), ³J_{H,H} = 8.6 Hz), 7.11 (1H, d, (Ar)CH-C-O-CH₂-(C=O), ³J_{H,H} = 8.6 Hz), 6.09–6.11 (2H, m, 2xCH₃-(Ar)C-CH-CH-C), 6.04 (1H, t, cis (CH=C)-CH₂-CH₃, ⁴J_{H,H} = 1.3 Hz), 5.86 (1H, m, CH₃-(Ar)C-CH-CH-C, ³J_{H,H} = 8.5 Hz), 5.84 (1H, m, CH₃-(Ar)C-CH-CH-C, ³J_{H,H} = 8.5 Hz), 5.59 (1H, s, trans (CH=C)-CH₂-CH₃), 5.51 (2H, s, O-CH₂-(Py)C-CH-CH-N), 5.14 (2H, s, (Ar)C-O-CH₂-(C=O)), 2.63 (3H, s, CH₃-(Py)C-CH-CH-N), 2.62 (1H, sept, (Ar)C-CH-CH-C-CH(CH₃)₂, ³J_{H,H} = 6.9 Hz), 2.45 (2H, m, (CH₂=C)-CH₂-CH₃, ³J_{H,H} = 7.4 Hz), 2.26 (3H, s, CH₃-(Ar)C-CH-CH-C), 1.15 (3H, t, (CH₂=C)-CH₂-CH₃, ⁴J_{H,H} = 7.4 Hz), 1.04 (3H, d, (Ar)C-CH-CH-C-CH-CH₃, ³J_{H,H} = 6.9 Hz), 1.02 (3H, d, (Ar)C-CH-CH-C-CH-CH₃, ³J_{H,H} = 6.9 Hz); ¹³C NMR (MeOD-*d*₄) δ_{C} (ppm) 197.3 (1C, (Ar)C-(C=O)-(C=CH₂)), 169.1 (1C, (Ar)C-O-CH₂-(C=O)), 157.0 (1C, O-CH₂-(Py)C-CH-C-N), 156.7 (1C, O-CH₂-(Py)C-CH-CH-N), 156.5 (1C, (Ar)CH-C-O-CH₂-(C=O)), 156.1 (1C, CH₃-(Py)C-CH-CH-N), 155.5 (1C, CH₃-(Py)C-CH-C-N), 154.2 (1C, CH₃-CH₂-(C=CH₂)), 151.6 (1C, O-CH₂-(Py)C-CH-CH-N), 151.1 (1C, CH₃-(Py)C-CH-CH-N), 134.9 (1C, Cl-(Ar)C-C-(C=O)), 131.9 (1C, Cl-(Ar)C-C-(C=O)), 130.1 (1C, (CH₂=C)-CH₂-CH₃), 129.9 (1C, (Ar)CH-C-(C=O)), 128.4 (1C, CH₃-(Py)C-CH-CH-N), 126.5 (1C, CH₃-(Py)C-CH-C-N), 125.9 (1C, O-CH₂-(Py)C-CH-CH-N), 123.8 (1C, Cl-(Ar)C-C-O), 122.7 (1C, O-CH₂-(Py)C-CH-C-N), 112.6 (1C, (Ar)CH-C-O-CH₂-(C=O)), 105.8 (1C, CH₃-(Ar)C-CH-CH-C), 105.6 (1C, CH₃-(Ar)C-CH-CH-C), 88.2 (1C, CH₃-(Ar)C-CH-CH-C), 88.0 (1C, CH₃-(Ar)C-CH-CH-C), 85.5 (1C, CH₃-(Ar)C-CH-CH-C), 85.3 (1C, CH₃-(Ar)C-CH-CH-C), 67.1 (1C, (Ar)C-O-CH₂-(C=O)), 65.2 (1C, O-CH₂-(Py)C-CH-CH-N), 32.3 (1C, (Ar)CH-CH-C-CH(CH₃)₂), 24.5 (1C, (CH₂=C)-CH₂-CH₃), 22.3 (2C, (Ar)CH-CH-C-CH(CH₃)₂), 21.3 (1C, CH₃-(Py)C-CH-CH-N), 19.0 (1C, CH₃-(Ar)C-CH-CH-C), 13.0 (1C, (CH₂=C)-CH₂-CH₃); IR (ν , cm⁻¹) 3060–2872 (C-H, CH₂, CH₃), 1763 (C=O, ester), 1661, 1620 (C=O, ketone, C=C, alkene), 1580, 1470, 1384 (ring skeleton C=C, C-C, C=N, C-N), 1284, 1210, 1075 (C-O, ester, ether), 800 (C-H aromatic); ESI-MS(+): m/z 756.06 [M - Cl]⁺, calcd for C₃₅H₃₆Cl₃N₂O₄Ru⁺ 756.10 (the isotopic pattern corresponds well to the calculated pattern). Anal. Calcd for C₃₅H₃₆Cl₃N₂O₄Ru: C, 53.11; H, 4.58; N, 3.54. Found: C, 53.16; H, 4.58; N, 3.34.

Synthesis of [Os(η^6 -p-cymene)Cl]([4'-methyl-[2,2'-bipyridin]-4-yl)methyl-2-(2,3-dichloro-4-(2-ethylenebutanoyl)phenoxy)acetate]-Cl (1b). Complex 1b was prepared following the general procedure starting from [Os(η^6 -p-cymene)Cl]₂Cl₂ (0.291 g, 1 equiv) and ligand L1 (0.357 g, 1 equiv), to afford a yellow solid (0.597 g, yield 92%): mp (°C): 159–161 dec; ¹H NMR (MeOD-*d*₄) δ_{H} (ppm) 9.34 (1H, d, O-CH₂-(Py)C-CH-CH-N, ³J_{H,H} = 6.0 Hz), 9.22 (1H, d, CH₃-(Py)C-CH-CH-N, ³J_{H,H} = 5.9 Hz), 8.56 (1H, m, O-CH₂-(Py)C-CH-C-N), 8.48 (1H, m, CH₃-(Py)C-CH-C-N), 7.58 (1H, dd, O-CH₂-(Py)C-CH-CH-N, ³J_{H,H} = 6.0 Hz, ⁴J_{H,H} = 1.4 Hz), 7.56 (1H, dd, CH₃-(Py)C-CH-CH-N, ³J_{H,H} = 5.9 Hz, ⁴J_{H,H} = 1.9 Hz), 7.21 (1H, d, (Ar)CH-C-(C=O), ³J_{H,H} = 8.6 Hz), 7.11 (1H, d, (Ar)CH-C-O-CH₂-(C=O), ³J_{H,H} = 8.6 Hz), 6.32 (1H, m, CH₃-(Ar)C-CH-CH-C), 6.30 (1H, m, CH₃-(Ar)C-CH-CH-C), 6.04 (1H, t, cis (CH=C)-CH₂-CH₃, ⁴J_{H,H} = 0.9 Hz), 6.01 (1H, d, CH₃-(Ar)C-CH-CH-C, ³J_{H,H} = 6.2 Hz), 5.99 (1H, d, CH₃-(Ar)C-CH-CH-C, ³J_{H,H} = 6.2 Hz), 5.59 (1H, s, trans (CH=C)-CH₂-CH₃), 5.56 (2H, s, O-CH₂-(Py)C-CH-CH-N), 5.14 (2H, s, (Ar)C-O-CH₂-(C=O)), 2.70 (3H, s, CH₃-(Py)C-CH-CH-N), 2.48 (1H, sept, (Ar)C-CH-CH-C-CH(CH₃)₂, ³J_{H,H} = 6.9 Hz), 2.45 (2H, m, (CH₂=C)-CH₂-CH₃, ³J_{H,H} = 7.4 Hz), 2.33 (3H, s, CH₃-(Ar)C-CH-CH-C), 1.15 (3H, t, (CH₂=C)-CH₂-CH₃, ⁴J_{H,H} = 7.4 Hz), 0.98 (3H, d, (Ar)C-CH-CH-C-CH-CH₃, ³J_{H,H} = 6.9 Hz), 0.97 (3H, d, (Ar)C-CH-CH-C-CH-CH₃, ³J_{H,H} = 6.9 Hz); ¹³C NMR (MeOD-*d*₄) δ_{C} (ppm) 197.3 (1C, (Ar)C-(C=O)-(C=CH₂)), 169.1 (1C, (Ar)C-O-CH₂-(C=O)), 157.4 (1C, O-CH₂-(Py)C-CH-C-N), 157.0 (1C, CH₃-(Py)C-CH-C-N), 156.6 (1C, O-CH₂-(Py)C-CH-CH-N), 156.3 (1C, (Ar)CH-C-O-CH₂-(C=O)), 156.0 (1C, CH₃-(Py)C-CH-CH-N), 154.3 (1C, CH₃-CH₂-(C=CH₂)), 151.7 (1C, O-CH₂-(Py)C-CH-CH-N), 151.0 (1C, CH₃-(Py)C-CH-CH-N), 134.9 (1C, Cl-(Ar)C-C-(C=O)), 131.9 (1C, Cl-(Ar)C-C-(C=O)), 130.6 (1C, (CH₂=C)-CH₂-CH₃), 130.1 (1C, (Ar)CH-C-(C=O)), 128.4 (1C, CH₃-(Py)C-CH-CH-N), 127.3 (1C, CH₃-(Py)C-CH-C-N), 125.9 (1C, O-CH₂-(Py)C-CH-CH-N), 123.8 (1C, Cl-(Ar)C-C-O), 122.9 (1C, O-CH₂-(Py)C-CH-C-N), 112.6 (1C, (Ar)CH-C-O-CH₂-(C=O)), 98.8 (1C, CH₃-(Ar)C-CH-CH-C), 96.6 (1C, CH₃-(Ar)C-CH-CH-C), 79.6 (1C, CH₃-(Ar)C-CH-CH-C), 79.5 (1C, CH₃-(Ar)C-CH-CH-C), 75.4 (1C, CH₃-(Ar)C-CH-CH-C), 75.2 (1C, CH₃-(Ar)C-CH-CH-C), 67.1 (1C, (Ar)C-O-CH₂-(C=O)), 65.2 (1C, O-CH₂-(Py)C-CH-CH-N), 32.6 (1C, (Ar)CH-CH-C-CH(CH₃)₂), 24.5 (1C, (CH₂=C)-CH₂-CH₃), 22.6 (2C, (Ar)CH-CH-C-CH(CH₃)₂), 21.3 (1C, CH₃-(Py)C-CH-CH-N), 18.9 (1C, CH₃-(Ar)C-CH-CH-C), 13.0 (1C, (CH₂=C)-CH₂-CH₃); IR (ν , cm⁻¹) 2961–2924 (C-H, CH₂, CH₃), 1762 (C=O, ester), 1663, 1621 (C=O, ketone, C=C, alkene), 1580, 1468, 1382 (ring skeleton C=C, C-C, C=N, C-N), 1283, 1195, 1074 (C-O, ester, ether), 801 (C-H aromatic); ESI-MS(+) m/z 845.11 [M - Cl]⁺, calcd for C₃₅H₃₆Cl₃N₂O₄Os⁺ 845.26 (the isotopic pattern corresponds well to the calculated pattern). Anal. Calcd for C₃₅H₃₆Cl₃N₂O₄Os: C, 47.73; H, 4.12; N, 3.18. Found: C, 47.93; H, 4.12; N, 2.99.

Synthesis of [Ru(η^6 -p-cymene)Cl][bis(2-(2-(2,3-dichloro-4-(2-methylenebutanoyl)phenoxy)acetoxylethyl)-(2,2'-bipyridine)-5,5'-dicarboxylate)Cl (2a). Complex 2a was prepared following the general procedure starting from [Ru(η^6 -p-cymene)Cl]₂Cl₂ (0.085 g, 1 equiv) and ligand L2 (0.250 g, 2 equiv), to afford a yellow solid (0.263 g, yield 79%): mp (°C): 97–99; ¹H NMR (CDCl₃) δ_{H} (ppm) 9.75 (2H, s, $2 \times$ O-(C=O)-(Py)C-CH-N), 9.27 (2H, d, $2 \times$ (Py)N-C-CH-CH-C, ³J_{H,H} = 7.8 Hz), 8.59 (2H, d, $2 \times$ (Py)N-C-CH-CH-C, ³J_{H,H} = 7.8 Hz), 7.11 (2H, d, $2 \times$ (Ar)CH-C-(C=O), ³J_{H,H} = 8.4 Hz), 6.93 (2H, d, $2 \times$ (Ar)CH-C-O-CH₂-(C=O), ³J_{H,H} = 8.4 Hz), 6.15 (2H, d, $2 \times$ CH₃-(Ar)C-CH-CH-C, ³J_{H,H} = 5.4 Hz), 6.01 (2H, d, $2 \times$ CH₃-(Ar)C-CH-CH-C, ³J_{H,H} = 5.4 Hz), 5.92 (2H, s, $2 \times$ cis (CH=C)-CH₂-CH₃), 5.55 (2H, s, $2 \times$ trans (CH=C)-CH₂-CH₃), 5.02 (2H, d, $2 \times$ (Ar)C-O-CH-(C=O)-O-(CH₂)₂-O, ¹J_{H,H} = 17.7 Hz), 4.91 (2H, d, $2 \times$ (Ar)C-O-CH-(C=O)-O-(CH₂)₂-O, ¹J_{H,H} = 17.7 Hz), 4.54–4.83 (8H, m, $2 \times$ (Ar)C-O-CH₂-(C=O)-O-CH₂-CH₂, $2 \times$ (Ar)C-O-CH₂-(C=O)-O-CH₂-CH₂), 2.75 (1H, sept, (Ar)C-CH-CH-C-CH(CH₃)₂, ³J_{H,H} = 6.7 Hz), 2.42 (4H, m, $2 \times$ (CH₂=C)-CH₂-CH₃, ³J_{H,H} = 7.4 Hz), 2.21 (3H, s, CH₃-(Ar)C-CH-CH-C), 1.07–1.15 (12H, m, $2 \times$ (CH₂=C)-CH₂-CH₃, (Ar)C-CH-CH-C-CH(CH₃)₂); ¹³C NMR (CDCl₃) δ_{C} (ppm) 195.8 (2C, $2 \times$ (Ar)C-(C=O)-(C=

CH₂), 168.3 (2C, 2 × (Ar)C-O-CH₂-(C=O)), 162.3 (2C, 2 × (Py)N-CH-C-(C=O)-O), 157.1 (2C, 2 × (Py)N-C-CH-CH-C), 156.1 (2C, 2 × (Py)N-CH-C-(C=O)), 155.3 (2C, 2 × (Ar)CH-C-O), 150.2 (2C, 2 × CH₃-CH₂-(C=CH₂)), 140.8 (2C, 2 × (Py)N-C-CH-CH-C), 134.0 (2C, 2 × Cl-(Ar)C-C-(C=O)), 131.4 (2C, 2 × Cl-(Ar)C-C-(C=O)), 129.5 (2C, 2 × (Ar)CH-C-(C=O)), 129.0 (2C, 2 × (CH₂=C)-CH₂-CH₃), 127.1 (2C, 2 × (Py)N-C-CH-CH-C), 126.6 (2C, 2 × (Py)N-CH-C-(C=O)-O), 123.2 (2C, 2 × Cl-(Ar)C-C-O), 111.3 (2C, 2 × (Ar)CH-C-O-CH₂-(C=O)), 106.3 (1C, CH₃-(Ar)C-CH-CH-C), 103.5 (1C, CH₃-(Ar)C-CH-CH-C), 87.2 (2C, 2 × CH₃-(Ar)C-CH-CH-C), 85.1 (2C, 2 × CH₃-(Ar)C-CH-CH-C), 66.5 (2C, 2 × (Ar)C-O-CH₂-(C=O)), 64.7 (2C, 2 × (Ar)C-O-CH₂-(C=O)-O-CH₂-CH₂), 62.8 (2C, 2 × (Ar)C-O-CH₂-(C=O)-O-CH₂-CH₂), 31.4 (1C, (Ar)CH-CH-C-CH(CH₃)₂), 23.5 (2C, 2 × (CH₂=C)-CH₂-CH₃), 22.3 (2C, (Ar)CH-CH-C-CH(CH₃)₂), 18.9 (1C, CH₃-(Ar)C-CH-CH-C), 12.5 (2C, 2 × (CH₂=C)-CH₂-CH₃); IR (ν , cm⁻¹) 2966 (C-H, CH₂, CH₃), 1731 (C=O, ester), 1661 (C=O, ketone, C=C, alkene), 1582, 1468, 1382 (ring skeleton C=C, C-C, C=N, C-N), 1286, 1194, 1119, 1077 (C-O, ester, ether), 804 (C-H aromatic); ESI-MS(+) *m/z* 1173.08 [M - Cl]⁺, calcd for C₅₂H₅₀Cl₃N₂O₁₂Ru⁺ 1173.29 (the isotopic pattern corresponds well to the calculated pattern). Anal. Calcd for C₅₂H₅₀Cl₆N₂O₁₂Ru: C, 51.67; H, 4.17; N, 2.32. Found: C, 51.71; H, 4.19; N, 2.23.

Synthesis of [Os(η^6 -*p*-cymene)Cl][bis(2-(2-(2,3-dichloro-4-(2-methylenebutanoyl)phenoxy)acetoxylethyl)-(2,2'-bipyridine)-5,5'-dicarboxylate)Cl (2b). Complex 2b was prepared following the general procedure starting from [Os(η^6 -*p*-cymene)Cl]₂Cl₂ (0.110 g, 1 equiv) and ligand L2 (0.250 g, 2 equiv), to afford an orange-red solid (0.332 g, yield 93%): mp (°C) 89–91; ¹H NMR (CDCl₃) δ _H (ppm) 9.65 (2H, s, 2 × O-(C=O)-(Py)C-CH-N), 9.55 (2H, d, 2 × (Py)N-C-CH-CH-C, ³J_{H,H} = 5.2 Hz), 8.61 (2H, d, 2 × (Py)N-C-CH-CH-C, ³J_{H,H} = 5.2 Hz), 7.11 (2H, d, 2 × (Ar)CH-C-(C=O)), ³J_{H,H} = 8.1 Hz), 6.28 (2H, m, 2 × CH₃-(Ar)C-CH-CH-C), 6.10 (2H, m, 2 × CH₃-(Ar)C-CH-CH-C), 5.91 (2H, s, 2 × cis (CH=C)-CH₂-CH₃), 5.55 (2H, s, 2 × trans (CH=C)-CH₂-CH₃), 4.97 (2H, d, 2 × (Ar)C-O-CH-(C=O)-O-(CH₂)₂-O, ¹J_{H,H} = 16.4 Hz), 4.87 (2H, d, 2 × (Ar)C-O-CH-(C=O)-O-(CH₂)₂-O, ¹J_{H,H} = 16.4 Hz), 4.57–4.79 (8H, m, 2 × O-CH₂-(C=O)-O-CH₂-CH₂, 2 × O-CH₂-(C=O)-O-CH₂-CH₂), 2.60 (1H, sept, (Ar)C-CH-CH-C-CH(CH₃)₂, ³J_{H,H} = 6.2 Hz), 2.41 (4H, m, 2 × (CH₂=C)-CH₂-CH₃, ³J_{H,H} = 7.4 Hz), 2.27 (3H, s, CH₃-(Ar)C-CH-CH-C), 1.10 (6H, t, 2 × (CH₂=C)-CH₂-CH₃), 1.03 (6H, d, (Ar)C-CH-CH-C-CH(CH₃)₂); ¹³C NMR (CDCl₃) δ _C (ppm) 195.8 (2C, 2 × (Ar)C-(C=O)-(C=CH₂)), 168.2 (2C, 2 × (Ar)C-O-CH₂-(C=O)), 162.1 (2C, 2 × (Py)N-CH-C-(C=O)-O), 158.0 (2C, 2 × (Py)N-C-CH-CH-C), 155.9 (2C, 2 × (Py)N-CH-C-(C=O)), 155.3 (2C, 2 × (Ar)-CH-C-O), 150.1 (2C, 2 × CH₃-CH₂-(C=CH₂)), 140.9 (2C, 2 × (Py)N-C-CH-CH-C), 133.9 (2C, 2 × Cl-(Ar)-C-C-(C=O)), 131.3 (2C, 2 × Cl-(Ar)-C-C-(C=O)), 130.1 (2C, 2 × (Ar)-CH-C-(C=O)), 129.1 (2C, 2 × (CH₂=C)-CH₂-CH₃), 127.1 (2C, 2 × (Py)N-C-CH-CH-C), 126.9 (2C, 2 × (Py)N-CH-C-(C=O)-O), 123.1 (2C, 2 × Cl-(Ar)C-C-O), 111.3 (2C, 2 × (Ar)CH-C-O-CH₂-(C=O)), 97.2 (1C, CH₃-(Ar)C-CH-CH-C), 96.9 (1C, CH₃-(Ar)C-CH-CH-C), 78.9 (2C, 2 × CH₃-(Ar)C-CH-CH-C), 75.4 (2C, 2 × CH₃-(Ar)C-CH-CH-C), 66.4 (2C, 2 × (Ar)C-O-CH₂-(C=O)), 64.7 (2C, 2 × (Ar)C-O-CH₂-(C=O)-O-CH₂-CH₂), 62.8 (2C, 2 × (Ar)C-O-CH₂-(C=O)-O-CH₂-CH₂), 31.4 (1C, (Ar)CH-CH-C-CH(CH₃)₂), 23.4 (2C, 2 × (CH₂=C)-CH₂-CH₃), 22.5 (2C, (Ar)CH-CH-C-CH(CH₃)₂), 18.8 (1C, CH₃-(Ar)C-CH-CH-C), 12.4 (2C, 2 × (CH₂=C)-CH₂-CH₃); IR (ν , cm⁻¹) 2967 (C-H, CH₂, CH₃), 1731 (C=O, ester), 1662 (C=O, ketone, C=C, alkene), 1583, 1469, 1382 (ring skeleton C=C, C-C, C=N, C-N), 1287, 1195, 1120, 1078 (C-O, ester, ether), 805 (C-H aromatic); ESI-MS(+) *m/z* 1262.14 [M-Cl]⁺, calcd for C₅₂H₅₀Cl₃N₂O₁₂Os⁺ 1262.45 (the isotopic pattern corresponds well to the calculated pattern). Anal. Calcd for C₅₂H₅₀Cl₆N₂O₁₂Os: C, 48.12; H, 3.88, N, 2.16. Found: C, 48.13; H, 3.92; N, 2.06.

X-ray Structure Determination for 1b. Diffraction data were recorded at low temperature (100(2) K) using Mo K α radiation on a

Bruker APEX II CCD diffractometer equipped with a κ geometry goniometer.

The data sets were reduced by EvalCCD⁷⁷ and then corrected for absorption.⁷⁸ The solution and refinement were performed by SHELXT and SHELXL2014 (release 7).⁷⁹ 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. Additional electron density found in the difference Fourier map of the compound (due to disordered solvent) was treated by the SQUEEZE algorithm of PLATON⁸⁰ and then treated by the ABIN instruction in SHELXL2014. Crystal data and structure refinement details are given in Table 3.

Table 3. Crystal Data and Structure Refinement Details for 1b

chem formula	C ₃₅ H ₃₆ Cl ₄ N ₂ O ₄ Os·CHCl ₃ ·CH ₃ OH
formula wt	1032.07
temp (K)	100(2)
wavelength (Å)	1.07073
cryst syst	triclinic
space group	P $\bar{1}$
unit cell dimens	
<i>a</i> (Å)	11.7589(16)
<i>b</i> (Å)	12.2253(7)
<i>c</i> (Å)	16.4565(10)
α (deg)	70.951(7)
β (deg)	79.147(8)
γ (deg)	75.472(6)
<i>V</i> (Å ³)	2150.0(4)
<i>Z</i>	2
<i>D</i> _{calcd} (g/cm ³)	1.594
μ (mm ⁻¹)	3.442
<i>F</i> (000)	1024
cryst size (mm)	0.319 × 0.212 × 0.102
θ range for data collection (deg)	1.893–27.498
index ranges	
<i>h</i>	–15 to +15
<i>k</i>	–15 to +15
<i>l</i>	–21 to +21
no. of measd rflns	27952
no. of indep rflns	9668 (<i>R</i> (int) = 0.0690)
completeness to $\theta = 25.242^\circ$ (%)	99.2
abs cor	semiempirical from equivalents
max and min transmission	0.7456 and 0.3830
refinement method	full-matrix least squares on F^2
no. of data/restraints/params	9668/187/543
GOF	1.052
<i>R</i> 1 (<i>I</i> > 2 σ (<i>I</i>))	0.0696
<i>wR</i> 2 (<i>I</i> > 2 σ (<i>I</i>))	0.1595
<i>R</i> 1 (all data)	0.0996
<i>wR</i> 2 (all data)	0.1808
largest diff peak and hole (e Å ⁻³)	2.802 and –1.706

Protein Expression and Purification. Human GST P1-1 wild-type was expressed in *Escherichia coli* and purified as previously described.⁶⁶ The reaction was monitored by spectrophotometry at 340 nm, corresponding to the absorbance maximum of glutathione-2,4-dinitrobenzene conjugate ($\epsilon_{340} = 9.6 \text{ M}^{-1} \text{ cm}^{-1}$).⁸¹ Spectrophotometric measurements were performed using a double-beam Cary UV–vis 4000 spectrophotometer (Kontron Instruments) equipped with a thermostated cuvette compartment.

GST P1-1 Inhibition Assay. The residual enzymatic activity of GST P1-1 was assayed by spectrophotometry in 100 mM phosphate buffer, pH 6.5, in the presence of variable amounts of compound 1a,

1b, **2a**, or **2b** (from 1 to 100 μM), predissolved in DMSO, 1 mM GSH (glutathione), and 1 mM CDNB (1-chloro-2,4-dinitrobenzene), at 25 $^{\circ}\text{C}$. The IC_{50} values for the inhibition of GST P1-1 were determined by fitting a plot of residual GST activity against the inhibitor concentrations with a sigmoidal dose–response function using Graph PAD Prism (Graph PAD Software, San Diego, CA).

Cell Culture. Human A2780 and A2780cisR ovarian carcinoma cells were obtained from the European Centre of Cell Cultures (ECACC, U.K.). The resistance of the A2780cisR cells was maintained by a monthly treatment with cisplatin (2 μM /one 4 day passage). Nontumorigenic HEK-293 cells were provided by the Institute of Pathology, CHUV, Lausanne, Switzerland. A2780 and A2780cisR were grown in RPMI 1640 medium supplemented with GlutaMAX (Gibco), and HEK-293 cells were grown in DMEM medium, all containing heat-inactivated fetal calf serum (FCS, Sigma, USA) (10%) and antibiotics (penicillin (100 U/mL):streptomycin (100 μg /mL), working concentration 1:100, Life Technologies, Gibco) at 37 $^{\circ}\text{C}$ and CO_2 (5%).

Antiproliferative Activity in Vitro. Cytotoxicity was determined using the MTT assay (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide). Cells were seeded in 96-well plates as monolayers with 100 μL of cell solution per well and preincubated for 24 h in the cell medium. Compounds were prepared as DMSO solutions that were rapidly dissolved in the culture medium and serially diluted to the appropriate concentration to give a final DMSO concentration of 0.5%. A 100 μL portion of the drug solution was added to each well, and the plates were incubated for another 72 h. Subsequently, MTT (5 mg/mL solution) was added to the cells and the plates were incubated for a further 4 h. The culture medium was aspirated, and the purple formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in DMSO. The optical density, directly proportional to the number of surviving cells, was quantified at 540 nm using a multiwell plate reader, and the fraction of surviving cells was calculated from the absorbance of untreated control cells. Evaluation was based on means from two independent experiments, each comprising three microcultures per concentration level.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.organomet.7b00468.

Packing diagrams of the crystal structure and relevant bond parameters, stability data for the complexes in DMSO- d_6 , and GST P1-1 inhibition curves (PDF)

Accession Codes

CCDC 1555830 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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■ ACKNOWLEDGMENTS

We thank the Swiss National Science Foundation (200020-157107) and EPFL for financial support and Dr. Euro Solari (EPFL) for the elemental analysis and assistance with the crystal structure determination.

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