ORGANOMETALLICS

Arene Osmium Complexes with Ethacrynic Acid-Modified Ligands: Synthesis, Characterization, and Evaluation of Intracellular Glutathione S-Transferase Inhibition and Antiproliferative Activity

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S Supporting Information

ABSTRACT: Four arene osmium complexes were prepared containing derivatives of ethacrynic acid, a potent inhibitor of glutathione *S*-transferases, either by direct coordination or via N- or P-donor ligands. The complexes were characterized by spectroscopic and analytical methods and, for $Os(\eta^6$ -*p*-cymene)(acetylacetonato)(2-(2,3-dichloro-4-(2-ethylenebutanoyl)phenoxy)acetato) and $Os(\eta^6$ -*p*-cymene)Cl₂(2-(2, (2,3-dichloro-4-(2-methylenebutanoyl)phenoxy)acetoxy)ethyl nicotina-to), by single-crystal X-ray diffraction. The cytotoxicity of the complexes toward human ovarian cancer cells and nontumorous human embryonic kidney cells was investigated, and two of the complexes, for which ruthenium analogues are known, helped to delineate the influence of the metal ion. Inhibition studies of intracellular glutathione *S*-transferases (GSTs, detoxification enzymes implicated in drug resistance) indicate



that the observed cytotoxicity of the complexes involves GST inhibition, as well as other targets following dissociation of the ethacrynic acid group from the osmium(II) ion.

INTRODUCTION

Arene ruthenium(II) complexes have been intensively investigated as possible anticancer agents,¹ and especially those complexes containing 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane (RAPTA complexes) and ethylene-1,2-diamine as ligands show promising antitumor properties.² On account of the proximity of ruthenium and osmium in the same group of the periodic table, a variety of osmium(II) derivatives have been considered as a natural extension of this research.³ In particular, arene osmium complexes usually exhibit slower rates for ligand exchange than the ruthenium analogues, a desirable property providing increased drug stability under physiological conditions.⁴

One of the common approaches aimed at improving the efficacy of metal drugs consists of incorporating an organic fragment with a complementary biological function.⁵ This strategy has already been applied also to various anticancer arene ruthenium(II) and osmium(II) complexes, which have been functionalized with biologically active moieties such as 3-hydroxyflavones,⁶ lapachol,⁷ paullones,⁸ and lonidamine.⁹

It has previously been shown that ethacrynic acid, originally developed as an inhibitor of glutathione S-transferases (GSTs, a family of cytosolic detoxification enzymes associated with drug resistance), potentiates the cytotoxic effect of several chemotherapeutic agents, including cisplatin.¹⁰ Consequently, in an attempt to overcome drug resistance, ethacrynic acid has been tethered to the ruthenium(II)-arene framework via two distinct synthetic approaches, i.e., the modification of the arene ligand¹¹ and the introduction via an appropriate monodentate ligand. In this respect, ethacrynic acid-functionalized imidazole,¹² pyr-idine, and triphenylphosphine-type¹³ ligands have been used (Figure 1). Also ruthenium(III), platinum(II), and platinum(IV) complexes with ethacrynic acid-derived appendages have been prepared and tested as anticancer drugs.¹⁴

All the ruthenium and platinum complexes modified with ethacrynic acid manifest increased ability to inactivate GSTs and induce apoptosis, even in cisplatin-resistant cell lines.¹⁵ To the best of our knowledge, the inclusion of ethacrynic acid in osmium complexes has not been reported heretofore. In the present paper, the synthesis, characterization, and biological activity of a series of four osmium complexes containing ethacrynic acid-modified ligands (including two complexes for which Ru analogues are known) are described.

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Figure 1. Structures of ethacrynic acid and of arene ruthenium(II) complexes containing ethacrynic acid-modified ligands.

Scheme 1. Synthesis of Osmium(II)-p-Cymene Ethacrynato Complex 1



RESULTS AND DISCUSSION

The direct reaction of the bis-acetylacetonato complex $Os(\eta^6$ -p-cymene)($\kappa(C)$ -acac)($\kappa(O,O')$ -acac)¹⁶ with ethacrynic acid proceeded at room temperature to give complex 1, which was isolated as a bright yellow solid in 75% yield (Scheme 1).

The ethacrynic acid-functionalized ligands 2-((4-(diphenylphosphanyl)benzyl)oxy)ethyl-2-(2,3-dichloro-4-(2methylenebutanoyl)phenoxy)acetato (LP)¹³ and 2-(2-(2,3dichloro-4-(2-methylenebutanoyl)phenoxy)acetoxy)ethylnicotinato (LN)¹³ and 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane (PTA)¹⁷ were prepared according to literature procedures. Then the ligands were allowed to react with the dimer $[OsCl_2(\eta^6-p-cymene)]_2$ to give the corresponding mononuclear complexes 2 and 3 in 90% and 75% yield, respectively, and $[OsCl_2(\eta^6-p-cymene)(PTA)]^{18}$ (Scheme 2). $[OsCl_2(\eta^6-p-cymene)(PTA)]$ was further treated with LN, in the presence of silver tetrafluoroborate, to afford 4 in an overall yield of 81%.

Complexes 1-4 are air-stable in the solid state and when dissolved in chlorinated solvents. Complex 4 is a racemate, due to the presence of a stereogenic osmium center. Complexes 1-3 in CH₂Cl₂ exhibit molar conductance values in the range 0.12-0.41 S·cm²·mol⁻¹, while the value found for 4 is 9.32 S· cm²·mol⁻¹, in agreement with the neutral character of 1-3 and the ionic character of 4. The IR spectra of 1-4 (solid state)

display a series of absorptions in the range 1759-1518 cm⁻¹ attributable to the stretching vibrations of different carbonyl moieties. The NMR spectra of 1-4 (in CD_2Cl_2) contain single sets of resonances. The carbonyl carbon atom of the ethacrynato moiety in 1 exhibits a resonance at 172.5 ppm in the ¹³C NMR spectrum, i.e., at comparable frequency to that in ethacrynic acid itself (172.8 ppm). The ¹H and ¹³C NMR spectra of 2 and 3 display resonances due to the LP and LN ligands close in value to those observed for the same ligands coordinated in analogous ruthenium complexes.¹³ As a consequence of the formal substitution of a chloride with PTA on going from the neutral complex 3 to the cationic 4, the resonances of some pyridyl nuclei undergo a significant shift to higher frequency ($\Delta \delta_{C20-H} = 0.27$ in the ¹H spectra; $\Delta \delta_{C19} =$ 3.1, $\Delta\delta_{C17} = 1.6$, and $\Delta\delta_{C20} = 2.6$ in the ¹³C spectra; see Chart 4 in Experimental Section). The ³¹P NMR resonance in 2 (at -12.1 ppm) appears at lower frequency with respect to the free ligand, LP (-4.9 ppm),¹³ whereas the ³¹P NMR resonance due to the PTA ligand in 4 (-77.4 ppm) resembles the values previously reported for related arene osmium(II) complexes.¹⁹ The expected structures of 1-4 were further corroborated by mass spectrometry and elemental analysis (see Experimental Section).

Crystals of 1 and 3 suitable for X-ray diffraction analysis were obtained from $CH_2Cl_2/pentane$ and $CH_2Cl_2/hexane$ mixtures, respectively. A small amount of hydroquinone was added in

Scheme 2. Synthesis of the Complexes 2-4 Containing Ethacrynic Acid-Modified Ligands



order to facilitate the crystallization of 3^{20} The structures of 1 and 3 are shown, respectively, in Figures 2 and 3, while selected bond distances and angles are given in Tables 1 and 2. Complexes 1 and 3 adopt the characteristic three-leg pianostool geometry, comprising the *p*-cymene "seat".^{3b,7,18,21} The three "leg" positions are occupied by the bidentate acac ligand [O(5)-Os-O(6) bite angle 88.14(9)°] and the monodentate carboxylate [O(1)-Os-O(5) and O(1)-Os-O(6) angles are



Figure 2. Molecular structure of 1. Displacement ellipsoids are at the 50% probability level. Hydrogen atoms are omitted for clarity.

80.59(10)° and 77.35(10)°, respectively] in 1 and by LN and two chlorides in 3 [N(1)–Os–Cl(1), N(1)–Os–Cl(2), and Cl(1)–Os–Cl(2) angles measure 82.20(10)°, 86.62(10)°, and 85.08(4)°, respectively]. The bonding parameters of the ethacrynic acid (EA) units are not significantly different from those found in other reported structures.^{10a,12,13,22} Compound 1 represents the first example of a crystallographically characterized osmium *p*-cymene complex with a monodentate carboxylate ligand: the Os–O(1) bond length [2.081(3) Å] is reminiscent of the situation found in other Os(II)-monodentate carboxylate systems.²³ The Os–O(5) and Os–O(6) bond lengths [2.072(2) and 2.081(2) Å, respectively] in 1 resemble those previously reported for other Os(II)-acac complexes.^{21b,24}

NMR and UV–vis spectroscopy and conductivity measurements were used to assess the stability of the complexes 1-4 in dmso/water solutions at 37 °C as a function of time. Parallel NMR and UV–vis analyses were carried out also on 1-4 in 0.1 M sodium chloride dmso/water solutions (this chloride concentration being similar to that present in blood). The compounds identified from these studies are depicted in Scheme 3.

A complicated mixture of compounds form from 1 in dmso/ water, and two sets of resonances have been tentatively assigned to 1a and 1b; the ethacrynato moiety behaves as a leaving group, and the NaCl environment accelerates this dissociation. EA-CO₂K was prepared from EA-CO₂H/KN-(SiMe₃)₂ and characterized in order to identify EA-CO₂⁻ in the studied solutions (see Experimental Section).

Conductivity data suggest that 2–4 undergo rapid solvolysis in dmso/water, presumably via the release of a chloride ligand.



Figure 3. Molecular structure of 3. Displacement ellipsoids are at the 50% probability level. Hydrogen atoms are omitted for clarity.

Table 1. Selected E	ond Distanc	ces (Å) and Angles	(deg) for 1		
Os-C(23)	2.147(3)	Os-C(24)	2.179(4)		
Os-C(25)	2.195(4)	Os-C(27)	2.176(3)		
Os-C(28)	2.150(3)	Os-C(19)	2.188(3)		
Os-O(5)	2.072(2)	Os-O(6)	2.081(2)		
Os-O(1)	2.081(3)	O(1) - C(1)	1.286(4)		
O(2) - C(1)	1.224(4)	C(1) - C(2)	1.521(5)		
O(5) - Os - O(6)	88.14(9)	O(5) - Os - O(1)	80.59(10)		
O(1)-Os-O(6)	77.35(10)	Os - O(1) - C(1)	126.5(2)		
O(1)-C(1)-O(2)	128.1(3)	O(1) - C(1) - C(2)	114.0(3)		
Table 2. Selected Bond Distances (Å) and Angles (deg) for 3					

Os-C(22)	2.193(4)	Os-C(26)	2.162(4)
Os-C(27)	2.182(5)	Os-C(28)	2.192(4)
Os-C(30)	2.180(5)	Os-C(31)	2.149(4)
Os-Cl(1)	2.4231(11)	Os-Cl(2)	2.4155(11)
Os-N(1)	2.119(3)	N(1)-Os-Cl(2)	86.62(10)
N(1)-Os-Cl(1)	82.20(10)	Cl(2)-Os-Cl(1)	85.08(4)

According to NMR and UV-vis spectroscopy, this phenomenon does not appear to be inhibited in the presence of 0.1 M NaCl.

It should be noted that the ligands LP and LN in 2 and 3 are less prone to dissociation than that previously observed for the analogous ruthenium complexes.¹³ Indeed, LP in 2 is substantially inert toward dissociation (5% released after 72 h in dmso/water and 12% released after 72 h in the chloridecontaining solution). Spectroscopic and conductivity studies indicate that only minor amounts of 3 and 4 remain unchanged in the dmso/water and dmso/water/NaCl solutions for 62–72 h and that the LN ligand and chloride ligand compete for the exchange with the solvent in both cases. Interestingly, the dissociation of the LN ligand from 3 and to a greater extent from 4 is partially suppressed in the respective NaCl solutions.

Cytotoxicity Studies. The ability of complexes 1–4 to inhibit cell growth was evaluated against the cisplatin-sensitive A2780 and the cisplatin-resistant A2780cisR human ovarian carcinoma cell lines and nontumoral immortalized human embryonic kidney HEK-293 cells (Table 3). All the complexes are considerably more cytotoxic than RAPTA-C on the two cancer cell lines.

Since both EA-CO₂H and EA-(C=O)O(CH₂)₂OH might be generated from the ligands/complexes, their antiproliferative activities were also evaluated. The ester EA-(C=O)O-(CH₂)₂OH shows a 15-20-fold higher cytotoxicity than the carboxylic acid EA-CO₂H, most probably derived from the better uptake of the former into cells, due to increased solubility in the cell culture medium.^{10b} Complex 1 has IC_{50} values similar to those determined for EA-CO₂H, whereas 2-4 are significantly more cytotoxic, albeit not too dissimilar from their corresponding ethacrynic acid-derived ligands LP and LN. Complex 3 is more cytotoxic than 4, both containing the same LN ligand, but the latter has a PTA ligand in place of a chloride ligand. The IC_{50} values of 2 and 3 are comparable to those previously described for ruthenium(II)-arene complexes modified with an ethacrynic acid unit.^{10a,12,13} Nonetheless, 2 and 3 display a slightly higher selectivity toward cancer cells with respect to nontumorigenic HEK-293 cells than the respective Ru analogues (Table 3).13

Intracellular GST Inhibition. Due to the potential of 1–4 to inhibit GSTs inside cells, residual intracellular GST activity after incubation with the complexes and the ligands at the respective IC₅₀ concentrations was determined by fluorescence spectroscopy in A2780 and A2780cisR cells (Figure 4). Incubation for 18 h led to a modest decrease in GST activity in both A2780 and A2780cisR cells with all the compounds, with 1 being the least effective GST inhibitor. This appears to be in accordance with the low cytotoxicity exhibited by 1, which is prone to quickly release the ethacrynato anion in aqueous conditions (see above).

In principle, both the EA-CO₂H and EA-(C=O)O- $(CH_2)_2OH$ fragments might be generated inside the cells following dissociation of LN and LP from 2–4.^{15,26} The most pronounced effects on GST activity were observed for 2 (20% inhibition) and 4 (30% inhibition) in A2780 cells and to a lesser extent in A2780cisR cells, which indicates that the GSH/GST detoxification system in A2780cisR cells is more active, reflected by 2-fold higher IC₅₀ values, compared to A2780 cells (Table 3).

The ligand LN inhibits GST in A2780cisR cells slightly more than in A2780 cells, which might contribute to the comparable cytotoxicity in A2780 cells and A2780cisR cells, respectively (Table 3). The intracellular GST inhibition values of 2-4 do not correlate directly with the IC₅₀ values (compare Table 3 and Figure 4), thus suggesting that the cytotoxicity of the

Scheme 3. Compounds Detected in dmso/H₂O and dmso/H₂O/NaCl Solutions of 1-4 Maintained at 37 °C^b



"Not soluble under the experimental conditions. ^b% values are based on ¹H NMR spectroscopy and refer to the identified compounds only.

Table 3. IC₅₀ Values (μ M) Determined for RAPTA-C,²⁵ Ethacrynic Acid (EA-CO₂H),¹³ EA-(C=O)O(CH₂)₂OH, Cisplatin, LP,¹³ LN,¹³ and 1–4 on Human Ovarian (A2780 and A2780cisR) Cancer Cells and Human Embryonic Kidney (HEK-293) Cells after Incubation for 72 h^a

	A2780	A2780cisR	HEK-293
RAPTA-C	230	270	>1000
EA-CO ₂ H	40 ± 3	53 ± 5	
$EA-(C=O)O(CH_2)_2OH$	2.0 ± 0.1	3.8 ± 0.7	3.3 ± 0.5
LP	9 ± 1	40 ± 4	38 ± 9
LN	13 ± 2	11 ± 1	7 ± 1
1	43 ± 6	69 ± 2	40 ± 4
2	$13 \pm 2 (11 \pm 2)$	$20 \pm 5 (15 \pm 4)$	$21 \pm 2 (13 \pm 4)$
3	$8 \pm 1 \ (13 \pm 3)$	$26 \pm 8 \ (26 \pm 2)$	$13 \pm 1 \ (16 \pm 3)$
4	17 ± 3	34 ± 1	25 ± 2
cisplatin	0.9 ± 0.1	25 ± 3	9 ± 2

compounds may only be partially attributed to GST inhibition. Indeed, these results are consistent with a previous mechanistic study on a bifunctional arene ruthenium(II)–ethacrynic acid compound, indicating that the EA unit inhibits GSTs, releasing the organometallic fragment, which leads to a surge of cell death.^{15a}

CONCLUSIONS

The first osmium complexes containing ethacrynic acid, a broad-acting inhibitor of GSTs, have been synthesized and characterized. For two of the complexes, 2 and 3, ruthenium

analogues are known, whereas 1 and 4 are unique in that no ruthenium congeners have been reported. Compounds 2 and 3 are, as expected, more stable than the ruthenium analogues, in dmso/water and dmso/water/NaCl solutions. To within experimental error, there is no significant trend indicating that complexes of one metal (Ru or Os) are substantially more cytotoxic than the analogues of the other one, as previously noted.²⁷ The complex with the most stable Os–EA ligand bond, i.e., 2, in which the ethacrynic acid entity is connected to the osmium(II) ion via a P-donor ligand, is not the most cytotoxic compound, indicating that dissociation of the



Figure 4. Residual GST activity in A2780 and A2780cisR cells, expressed as % activity of control, after incubation at IC_{50} concentrations for 18 h. The changes in fluorescence over a 20 min period were used to calculate GST activities.

bioactive EA group from the metal ion could be important in the mechanism of drug action. Indeed, the antiproliferative activity of **2** may be attributed predominantly to the ethacrynic acid-derived ligand, as their IC_{50} values are similar. This could explain why there is not a direct correlation between cytotoxicity and inhibition of intracellular GSTs, as the data suggest that GSTs are relevant targets, but not exclusive targets. Finally, incorporation of the PTA ligand in **3** to afford **4** does not improve the cytotoxicity or selectivity of the compounds, presumably due to the major role of the ethacrynic acid-derived ligand in the overall biological activity observed.

EXPERIMENTAL SECTION

OsCl₃·3H₂O (~54% purity) was purchased from Heraeus, while the organic reactants were purchased from Acros Organics and were of the highest purity available. Solvents were purchased from Scharlau and then obtained oxygen- and water-free with an MBraun solvent purification apparatus. The ligands PTA,¹⁷ LP,¹³ and LN¹³ and the complexes $[OsCl_2(\eta^6-p-cymene)]_2^{28} Os(\eta^6-p-cymene)(\kappa(C)-acac)(\kappa-16)^{16} Oscilation (\kappa-16)^{16} Oscilat$ (O,O')-acac),¹⁶ and OsCl₂ $(\eta^{6}$ -p-cymene)(PTA)¹⁸ were prepared according to the respective literature methods. NMR spectra were recorded on either a Bruker ARX 300 MHz or a Bruker Avance 300 MHz instrument. Chemical shifts (expressed in parts per million) are referenced to the residual solvent peaks $({}^{1}H, {}^{13}C{}^{1}H)$ or to external standard ($^{31}P\{^1H\}$ to 85% $H_3PO_4\bar{)}.$ Coupling constants are given in Hz. NMR assignments refer to Charts 1-5 for the numbering of carbon atoms. Conductivity measurements were carried out using a Eutech Con 700 instrument (cell constant = 1.0 cm^{-1}).²⁹ UV-vis spectra were recorded with an Ultraspec 2100 Pro spectrophotometer. Attenuated total reflection infrared spectra (ATR-IR) of solid samples were recorded on a PerkinElmer Spectrum 100 FT-IR spectrometer. C, H, and N analyses were carried out with a PerkinElmer 2400 CHNS/O analyzer. High-resolution electrospray mass spectra (HRMS) were acquired using a MicroTOF-Q hybrid quadrupole time-of-flight spectrometer (Bruker Daltonics, Bremen, Germany).

Synthesis of $Os(\eta^6$ -*p*-cymene)(acetylacetonato)(2-(2,3-dichloro-4-(2-ethylenebutanoyl)phenoxy)acetato), 1. A solution of $Os(\eta^6$ -*p*-cymene)($\kappa(C)$ -acac)($\kappa(O,O')$ -acac) (0.097 g, 0.19 mmol) in CH₂Cl₂ (5 mL) was treated with EA-CO₂H (0.069 g, 0.23 mmol) and then left stirring at room temperature for 24 h. The solvent was removed under vacuum to obtain a red, oily residue. Repeated washings with Et₂O (3 × 10 mL) afforded 1 as a bright yellow powder. Yield: 0.103 g, 75%. Crystals suitable for X-ray analysis were obtained by slow diffusion of pentane into a CH₂Cl₂ solution of 1. Anal. Calcd for C28H32Cl2O6Os: C, 46.34; H, 4.44. Found: C, 46.34; H, 4.28. ESI- $MS(+): \tilde{m/z}$ found 749.106 [M + Na]⁺, calcd for $C_{28}H_{32}Cl_2NaO_6Os^+$ 749.108; the isotopic pattern fits well the calculated one. IR (solid state) v: 2966w, 2936w, 2912w, 2876w, 1660m, 1644s, 1587m, 1575m, 1556m, 1518s, 1469m, 1434w, 1383m, 1358vs, 1343w, 1285m, 1270m, 1265s, 1244w, 1219w, 1197w, 1146w, 1120w, 1083w, 1060s, 1031w, 1006m, 962w, 938w, 925w, 891w, 864m, 823w, 803s, 785m, 757w, 729s, 684w, 659w, 633m, 610m cm⁻¹. UV-vis (CH₂Cl₂): λ_{max} nm $(\varepsilon/M^{-1}cm^{-1}) = 263 (7.4 \times 10^3), 327 (1.9 \times 10^3). \Lambda_M (CH_2Cl_2) =$ 0.12 S·cm²·mol⁻¹. ¹H NMR (CD₂Cl₂): δ = 7.01 (d, 1H, ³J_{HH} = 8.6 Hz, C11–H); 6.59 (d, 1H, ${}^{3}J_{HH}$ = 8.6 Hz, C10–H); 6.06, 5.82 (d, 4H, ${}^{3}J_{HH}$ = 5.6 Hz, arom CH_{cymene}); 5.91, 5.58 (m, 2H, C4-H); 5.04 (s, 1H, CH_{acac} ; 4.55 (s, 2H, C12-H); 2.64 (sept, 1H, ${}^{3}J_{HH} = 6.9$ Hz, CHMe₂); 2.43 (q, 2H, ${}^{3}J_{HH} = 7.4$ Hz, C2–H); 2.17 (s, 3H, $MeC_{6}H_{4}$); 1.80 (s, 6H, Me_{acac}); 1.24 (d, 6H, ${}^{3}J_{HH} = 6.9$ Hz, CHMe₂); 1.12 (t, 3H, ${}^{3}J_{\text{HH}} = 7.4 \text{ Hz}, \text{ C1-H}$). ${}^{13}\text{C}\{{}^{1}\text{H}\}$ NMR (CD₂Cl₂): $\delta = 196.3$ (C5); 185.8 (CO_{acac}); 172.5 (C13); 157.0 (C9); 150.8 (C3); 132.5 (C8); 131.0 (C7); 128.3 (C4); 127.3 (C11); 122.3 (C6); 110.6 (C10); 99.2 (CH_{acac}); 88.6 (CCHMe₂); 88.0 (CMe_{cymene}); 73.2, 68.5 (arom CH_{cvmene} ; 67.7 (C12); 32.0 (CHMe₂); 27.0 (Me_{acac}); 24.1 (C2); 22.9 (CHMe₂); 18.4 (MeC₆H₄); 12.8 (C1).

Chart 1. $Os(\eta^6$ -p-cymene)($\kappa(0,0')$ -acac)($\kappa(0)$ -O₂C-EA), 1^a



^aNumbering refers to carbon atoms.

Synthesis of Os (η^6 -p-cymene) Cl₂(2-((4-(diphenylphosphanyl)benzyl)oxy)ethyl-2-(2,3-dichloro-4-(2methylenebutanoyl)phenoxy)acetato), 2. Compound $[Os(\eta^6-p)]$ cymene) Cl_2]₂ (0.137 g, 0.173 mmol) was added to a solution of LP (0.265 g, 0.417 mmol) in CH₂Cl₂ (10 mL). The resulting orange solution was left stirring for 3 h at room temperature, then the volatile substances were removed under vacuum to afford an orange, foamy residue. This solid was dissolved into a small volume of CH₂Cl₂ and loaded on top of a silica column (h 4.5 cm, d 2.5 cm). Impurities were eluted with CH2Cl2; then an orange band was eluted with CH2Cl2/ THF (1:1 v/v). Complex 2 was finally obtained as an orange powder after solvent removal under vacuum (50 °C). Yield: 0.319 g (90%). Anal. Calcd for C44H43Cl4O6OsP: C, 51.27; H, 4.20. Found: C, 51.08; H, 4.33. ESI-MS(+): m/z found 1053.097 [M + Na]⁺, calcd for $C_{44}H_{43}Cl_4NaO_6OsP^+$ 1053.105; the isotopic pattern fits well the calculated one. IR (solid state) v: 3058w, 2963w, 2957w, 1759m, 1720s, 1662m, 1584m, 1568w, 1468m, 1435m, 1391w, 1384m, 1339w, 1263s, 1187s, 1111m, 1079vs, 1030m, 1017m, 1000m, 948w, 895w, 858w, 800m, 762m, 749m, 721m, 696vs, 632w, 565w, 541s, 519vs cm⁻¹. UV–vis (CH₂Cl₂): $\lambda_{max}/nm \ (\epsilon/M^{-1}cm^{-1}) = 250 \ (2.3 \times 10^4)$, 322 (1.9 × 10³). $\Lambda_{\rm M}$ (CH₂Cl₂) = 0.25 S·cm²·mol⁻¹. ¹H NMR $(CD_2Cl_2): \delta = 7.91-7.73, 7.44 (14H, C18-H + C18'-H + C19-H + C19-H$ C19'-H + Ph); 7.13 (d, 1H, ${}^{3}J_{HH} = 8.5$ Hz, C11-H), 6.84 (d, 1H, ${}^{3}J_{HH} = 8.5 \text{ Hz}, \text{C10-H}$; 5.95, 5.58 (m, 2H, C4-H); 5.43, 5.19 (d, 4H, ${}^{3}J_{\text{HH}} = 5.6 \text{ Hz}$, arom CH_{cymene}); 4.80 (s, 2H, C12–H); 4.54 (s, 4H, C14–H + C15–H); 2.69 (sept, 1H, ${}^{3}J_{HH}$ = 6.9 Hz, CHMe₂); 2.45 (q, 2H, ${}^{3}J_{HH}$ = 7.4 Hz, C2–H); 1.98 (s, 3H, MeC₆H₄); 1.17 (d, 6H, ${}^{3}J_{HH}$ = 6.8 Hz, CHMe₂); 1.14 (t, 3H, ${}^{3}J_{HH} = 7.2$ Hz, C1–H). ${}^{13}C{}^{1}H$ NMR $(CD_2Cl_2): \delta = 196.3 (C5); 168.4 (C13); 166.4 (C16); 156.1 (C9);$ 151.0 (C3); 140.7, 140.1 (m, C20 + P-C); 135.5-135.3 (m, C19 + C19' + arom CH_{Ph}); 134.0 (C8); 133.4 (C7); 131.4 (C17); 131.3 (arom CH_{Ph}); 129.4 (C4); 129.2, 128.9 (m, C18 + C18' + arom CH_{Ph} ; 127.7 (C11); 123.7 (C6); 111.7 (C10); 103.9 (CCHMe₂);

90.0 (arom CMe); 81.4 (d, ${}^{2}J_{PC}$ = 2.8 Hz, arom CH_{cymene}); 81.1 (d, ${}^{2}J_{PC}$ = 4.6 Hz, arom CH_{cymene}); 66.9 (C12); 64.1, 63.3 (C14 + C15); 31.0 (CHMe₂); 24.2 (C2); 22.8 (CHMe₂); 18.4 (MeC₆H₄); 13.0 (C1). ³¹P NMR (CD₂Cl₂): δ = -12.1.

Chart 2. $Os(\eta^6$ -p-cymene)Cl₂(LP), 2^{*a*}



^{*a*}Numbering refers to carbon atoms.

Synthesis of $Os(\eta^6-p$ -cymene) $Cl_2(2-(2-2), 3-dichloro-4-(2-2))$ methylenebutanoyl)phenoxy)acetoxy)ethylnicotinato), 3. This compound was prepared by the same procedure described for the synthesis of 2, from $[OsCl_2(\eta^6-p-cymene)]_2$ (0.100 g, 0.126 mmol) and LN (0.139 g, 0.307 mmol). Yellow solid, yield: 0.153 g (75%). Crystals of 3·CH₂Cl₂·1/2[1,4-C₆H₄(OH)₂] suitable for X-ray analysis were obtained by slow diffusion of hexane into a CH₂Cl₂ solution of 3, with a few grains of hydroquinone added. Anal. Calcd for C31H33Cl4NO6Os: C, 43.93; H, 3.92; N, 1.65. Found: C, 44.25; H, 4.10; N, 1.62. ESI-MS(+): m/z found 812.096 [M - Cl]⁺, calcd for C₃₁H₃₃Cl₃NO₆Os⁺ 812.099; the isotopic pattern fits well the calculated one. IR (solid state) v: 2964w, 2940w, 2893w, 1751m, 1728s, 1662m, 1584m, 1467w, 1432w, 1383w, 1339w, 1286vs, 1194vs, 1137s, 1116s, 1079vs, 1062s, 1025m, 1000m, 941w, 891w, 876w, 820w, 803m, 767w, 745s, 702w, 689m, 637w cm⁻¹. UV-vis (CH₂Cl₂): λ_{max}/nm ($\varepsilon/$ C18-H); 9.13 (m, 1H, C19-H); 8.29 (m, 1H, C21-H); 7.47 (m, 1H, C20–H); 7.18 (d, 1H, ${}^{3}J_{HH}$ = 8.6 Hz, C11–H); 6.95 (d, 1H, ${}^{3}J_{HH}$ = 8.6 Hz, C10–H); 5.99, 5.61 (m, 2H, C4–H); 5.86, 5.64 (d, 4H, ${}^{3}J_{HH}$ = 5.7 Hz, arom CH_{cymene}); 4.94 (s, 2H, C12–H); 4.62 (s, 4H, C14 + C15); 2.80 (sept, 1H, ${}^{3}J_{HH}$ = 6.9 Hz, $CHMe_{2}$); 2.47 (q, 2H, ${}^{3}J_{HH}$ = 7.4 Hz, C2–H); 2.03 (s, 3H, MeC_6H_4); 1.31 (d, 6H, ${}^{3}J_{HH} = 6.9$ Hz, CHMe₂); 1.16 (t, 3H, ${}^{3}J_{HH} = 7.4$ Hz, C1–H). ${}^{13}C{}^{1}H{}$ NMR $(CD_2Cl_2): \delta = 196.0 (C5); 168.3 (C13); 163.7 (C16); 158.1 (C19);$ 155.9 (C18); 155.8 (C9); 150.7 (C3); 139.1 (C21); 134.3 (C8); 131.6 (C7); 129.1 (C4); 127.6 (C11); 127.4 (C17); 125.0 (C20); 123.3 (C6); 111.5 (C10); 94.1 (CCHMe2); 89.4 (arom CMe); 75.9, 73.4 (arom CH_{cymene}); 66.6 (C12); 63.9, 63.3 (C14, C15); 31.5 (CHMe₂); 23.9 (C2); 22.8 (CHMe₂); 18.4 (MeC₆H₄); 12.8 (C1).

Synthesis of Os(η^6 -*p*-cymene)Cl(2-(2-(2,3-dichloro-4-(2-methylenebutanoyl)phenoxy)acetoxy)ethylnicotinato)(1,3,5-triaza-7-phosphaadamantane)tetrafluoroborato, 4. A stirred solution of OsCl₂(η^6 -*p*-cymene)(PTA) (0.100 g, 0.181 mmol) in CH₂Cl₂ (6 mL) was treated with LN (0.098 g, 0.216 mmol) and then

Chart 3. (η^6 -p-Cymene)OsCl₂(LN), 3^{*a*}



^aNumbering refers to carbon atoms.

with $AgBF_4$ (0.048 g, 0.25 mmol). The mixture was stirred for 72 h at room temperature under protection from light. The final mixture was filtered through Celite; then the volatiles substances were removed under vacuum. The residue was precipitated with Et₂O (30 mL) and then washed with hexane (20 mL). Complex 4 was obtained as a pale yellow solid. Yield: 0.156 g (81%). Anal. Calcd for C₃₇H₄₅BCl₃F₄N₄O₆OsP: C, 42.08; H, 4.29; N, 5.30. Found: C, 42.70; H, 4.36; N, 5.47. ESI-MS(+): m/z found 969.177 [M]⁺, calcd for $C_{37}H_{45}Cl_3N_4O_6OsP^+$ 969.175; the isotopic pattern fits well the calculated one. IR (solid state) v: 2967w, 2936w, 2880w, 1757m-sh, 1733s, 1662m, 1585m, 1469m, 1444w-m, 1385w-m, 1288s, 1242m, 1197s, 1138m, 1117s-sh, 1054vs-br, 973s, 947s, 896m, 803m, 766w-m, 744s, 695w-m, 606w, 577s cm⁻¹. UV-vis (CH₂Cl₂): λ_{max} /nm (ϵ / $M^{-1}cm^{-1}$) = 258 (1.4 × 10⁴), 317 (5.2 × 10³). Λ_{M} (CH₂Cl₂) = 9.32 S· cm²·mol⁻¹. ¹H NMR (CD₂Cl₂): δ = 9.55 (s, 1H, C18–H); 9.09 (d, 1H, ${}^{3}J_{HH} = 5.5$ Hz, C19–H); 8.43 (d, 1H, ${}^{3}J_{HH} = 8.0$ Hz, C21–H); 7.74 (m, 1H, C20–H); 7.15 (d, 1H, ${}^{3}J_{HH}$ = 8.6 Hz, C11–H); 6.89 (d, 1H, ${}^{3}J_{HH}$ = 8.6 Hz, C10–H); 6.03, 5.79 (d, 4H, ${}^{3}J_{HH}$ = 5.7 Hz, arom CH_{cvmene}); 5.96, 5.58 (m, 2H, C4–H); 4.88 (d, 2H, ² J_{HH} = 1.5 Hz C12-H; 4.71-4.58 (m, 6H, NCH₂N); 4.62 (s, 4H, C14 + C15); 4.19 (q, 6H, ${}^{2}J_{HP}$ = 15.4 Hz, NCH₂P); 2.54 (sept, 1H, ${}^{3}J_{HH}$ = 6.6 Hz, CHMe₂); 2.44 (q, 2H, ${}^{3}J_{HH} = 7.3 \text{ Hz}$, C2–H); 2.05 (s, 3H, $MeC_{6}H_{4}$); 1.26 (d, 6H, ${}^{3}J_{HH} = 6.8 \text{ Hz}$, CHMe₂); 1.13 (t, 3H, ${}^{3}J_{HH} = 7.4 \text{ Hz}$, C1– H). ${}^{13}C{}^{1}H{}$ NMR (CD₂Cl₂): δ = 196.1 (C5); 168.4 (C13); 163.2 (C16); 161.2 (C19); 156.7 (C18); 155.9 (C9); 150.8 (C3); 140.8 (C21); 134.6 (C8); 131.8 (C7); 129.2 (C4); 129.0 (C17); 127.6 (C11); 127.6 (C20); 123.3 (C6); 111.7 (C10); 104.3 (CCHMe₂); 95.0 (arom CMe); 75.9, 73.4 (arom CH_{cymene}); 72.9 (NCH₂N); 66.8 (C12); 64.5, 63.5 (C14, C15); 49.5 (d, ${}^{1}J_{CP} = 21.1 \text{ Hz}, \text{NCH}_{2}\text{P}$); 31.6 (CHMe₂); 24.0 (C2); 23.0, 22.4 (CHMe₂); 18.4 (MeC₆H₄); 12.8 (C1). ³¹P NMR (CD₂Cl₂): $\delta = -77.4$.





^aNumbering refers to carbon atoms.

Synthesis of EA-CO₂K. KN(SiMe₃)₂ (0.36 mL, 0.33 mmol) was added to a solution of EA-CO₂H (0.100 g, 0.330 mmol) in Et₂O (10 mL), under a nitrogen atmosphere. The mixture was left stirring for an additional 24 h. The resulting colorless solid was separated, washed with pentane (20 mL), and dried under vacuum. Yield: 0.075 g, 67%. Anal. Calcd for C13H11Cl2KO4: C, 45.76; H, 3.25; Cl, 20.78. Found: C, 45.71; H, 3.18; Cl, 20.82. IR (solid state): $\nu = 2965$ w, 2938w, 2922w, 2878w, 2550w, 1657m (C=O), 1598vs (COO), 1586vs, 1554m, 1471m-s, 1417s, 1385m, 1339m, 1267m-s, 1202m, 1120w, 1054vs, 1002m, 941m, 908w, 824w, 799s, 760m, 737m, 724m, 698w, 680w, 627m, 590w, 570w, 531w cm⁻¹. ¹H NMR (D₂O): δ = 7.26 (d, ³J_{HH} = 8.80 Hz, 1H, C10–H); 6.92 (d, ${}^{3}J_{HH}$ = 8.80 Hz, 1H, C11–H); 6.08, 5.69 (s, 2 H, C4-H); 4.58 (s, 2 H, C12-H); 2.36 (m, 2 H, C2-H); 1.06 (m, 3 H, C1–H). ¹³C NMR (D₂O): δ = 199.2 (C5); 174.9 (C13); 156.1 (C9); 150.1 (C6); 131.8 (C7), 131.6 (C8); 130.3 (C3); 128.0 (C11); 122.0 (C4); 111.1 (C10); 67.8 (C12); 23.2 (C2); 12.0 (C1).

Stability Studies. General Procedure. Complexes 1–4 were dissolved in d_6 -dmso/D₂O mixture (2.0 mL) ([Os] = 2.1×10^{-2} mol L⁻¹). An aliquot of the resulting solution (0.40 mL) was transferred into a NMR tube, maintained at 37 °C, and analyzed by NMR spectroscopy as a function of time. The remaining solution was diluted up to 4.2 mL with the appropriate dmso/water mixture (final [Os] =

Chart 5. EA-CO₂K^a



7.8 × 10⁻³ mol L⁻¹), maintained at 37 °C, and analyzed by conductivity measurements and UV−vis spectroscopy as a function of time (*t*). Parallel analyses were carried out on dmso/water mixtures prepared by the same procedure described above and with the addition of NaCl (ca. 0.1 M). CH₃OD (2.5 × 10⁻² mol L⁻¹) was used as a reference for the ¹H NMR spectra. For NMR assignments refer to Scheme 3. The NMR spectra of LP, LN, EA-CO₂H, EA-C(=O)O(CH₂)₂OH, **PTA**, and acacH were recorded in *d*₆-dmso/D₂O (9:1 v/v) and used for comparison for NMR assignments. FeCp₂ was selected as an organometallic standard for conductivity data ($\Lambda_{\rm M} = 0.69 \ {\rm S} \cdot {\rm cm}^2 \cdot {\rm mol}^{-1}$ in 9:1 dmso/H₂O at 37 °C).

Compound 1. $\Lambda_{\rm M}$ (dmso/m20 erg 9:1) = 3.4 S·cm²·mol⁻¹ (t = 5 min); 5.3 S·cm²·mol⁻¹ (t = 50 min); 4.6 S·cm²·mol⁻¹ (t = 6 h); 5.3 S·cm²·mol⁻¹ (t = 24 h); 3.8 S·cm²·mol⁻¹ (t = 114 h). UV–vis (dmso/water, 9:1), $\lambda_{\rm max}/{\rm nm}$ ($e/{\rm M}^{-1}{\rm cm}^{-1}$) = 327 (2.6 × 10³) (t = 2–114 h). UV–vis (dmso/water, 9:1, + NaCl 0.1 M) = 330 (2.7 × 10³) (t = 2 h); 330 (2.1 × 10³) (t = 72 h). ¹H NMR (d_6 -dmso/D₂O, 9:1): $\delta = 7.23$ (d, EACO₂⁻), 7.16 (d, 1), 6.91 (d, EACO₂⁻), 6.67 (d, 1), 6.24 (s, 1a), 6.13 (s, 1a), 6.03 (s, 1 + EACO₂⁻), 5.86 (s, 1a), 5.55 (s, EACO₂⁻), 5.54 (s, 1), 4.97 (s, 1), 4.52 (s, 1), 4.47 (s, EACO₂⁻), 2.33 (q, 1 + EACO₂⁻). Ratio 1:EACO₂⁻:1a = 1:1:1 (t = 1–72 h). ¹H NMR (d_6 -dmso/D₂O, 9:1, + NaCl 0.1 M): 1, EACO₂⁻, and 1b [$\delta = 6.00$ (d), 5.78 (d), 5.31 (s), 2.08 (s), 1.80 (s)], ratio 2:8:7 (t = 5 min), 0:5:3 (t = 2–72 h).

Compound 2. $\Lambda_{\rm M}$ (dmso/water, 9:1) = 8.9 S·cm²·mol⁻¹ (t = 5 min to 72 h). UV–vis (dmso/water, 9:1), $\lambda_{max}/nm (\epsilon/M^{-1} \cdot cm^{-1}) = 322$ (2.8×10^3) , 423 (4.3×10^2) (t = 5 min to 72 h). UV-vis (dmso/ water, 9:1, + NaCl 0.1 M) = 320 (2.8×10^3), 423 (4.0×10^2) (t = 5min); 320 (2.4 × 10³), 423 (4.0 × 10²) (t = 72 h). ¹H NMR (d_{6} dmso/D₂O, 9:1, or d_6 -dmso/D₂O, 9:1, + NaCl 0.1 M): δ = 7.85 (m, 2H), 7.79 (m, 2H), 7.64 (m, 4H), 7.43 (m, 6H), 7.18, 7.11 (d, 2H), 6.00 (s, 1H), 5.50, 5.37 (d, 4H), 5.47 (s, 1H), 5.01 (s, 2H), 4.45 (s, 4H), 2.41 (m, 1H), 2.31 (q, 2H), 1.83 (s, 3H), 1.02 (t, 3H), 0.98 (d, 6H) (t = 5 min to 72 h). ${}^{13}C{}^{1}H{}$ NMR (d₆-dmso/D₂O, 9:1, or d₆dmso/D₂O, 9:1, + NaCl 0.1 M): δ = 195.7, 168.4, 165.7, 155.5, 149.7, 134.8-134.5, 133.3, 133.0, 132.9, 131.1, 130.1, 128.6-128.4, 127.8, 112.1, 88.9, 81.3, 80.3, 66.0, 63.4, 63.2, 30.1, 23.3, 22.1, 17.5, 12.7 (*t* = 5 min to 72 h). ³¹P NMR (d_6 -dmso/D₂O, 9:1): $\delta = -12.8$ (t = 5 min to 22 h); $\delta = -12.8$ (major, 2a), 26.6 (ca. 6%, LP=O) (t = 51-72 h). ³¹P NMR (d_6 -dmso/D₂O, 9:1, + NaCl 0.1 M): $\delta = -12.8$ (major, 2a), 26.6 (ca. 9%, LP=O) (t = 5 min to 23 h); -12.8 (major, 2a), -6.3 (LP, ca. 7%), 26.6 (ca. 5%, LP=O) (t = 72 h).

Compound 3. $\Lambda_{\rm M}$ (dmso/water, 9:1) = 2.2 S·cm²·mol⁻¹ (t = 5 min); 3.2 S·cm²·mol⁻¹ (t = 50 min); 3.4 S·cm²·mol⁻¹ (t = 6 h); 3.3 S· $cm^2 mol^{-1}$ (t = 24 h); 3.9 S·cm²·mol⁻¹ (t = 114 h). UV-vis (dmso/ water, 9:1), $\lambda_{\text{max}}/\text{nm} (\varepsilon/\text{M}^{-1} \cdot \text{cm}^{-1}) = 322 (2.2 \times 10^3), 418 (3.6 \times 10^2)$ (t = 5 min to 72 h). UV-vis (dmso/water, 7:3, + NaCl 0.1 M) = 322 (1.6×10^3) , 418 (2.4×10^2) (t = 5 min); 322 (1.8×10^3) , 418 (2.4×10^3) 10²) (t = 72 h). ¹H NMR (d_6 -dmso/D₂O, 9:1): δ = 9.30 (s, 3 + 3a), 9.04 (s, LN), 8.99 (t, 3a), 8.98 (d, 3), 8.78 (d, LN), 8.47 (d, 3a), 8.30 (d, 3), 8.24 (d, LN), 7.77 (m, 3a), 7.59 (dd, 3), 7.54 (dd, LN), 7.23 (d, 3 + 3a), 7.16 (d, LN), 7.14 (m, 3a), 7.10 (d, LN), 6.38 (d, 3a), 6.25 (d, 3a), 6.17 (d, 3a), 6.08 (d, 3a), 6.04 (d, 3b), 6.03 (s, LN), 5.94 (d, 3b), 5.92 (d, 3), 5.74 (d, 3), 5.52 (s, 3a), 5.49 (s, LN), 5.04 (s, LN), 4.50 (s, LN), 2.70 (m, 3b), 2.33 (q, LN), 2.14 (s, 3a), 2.11 (s, 3b), 1.89 (s, 3), 1.17 (d, 3b), 1.04 (t, LN). Ratio 3:3a:3b:LN = 13:0:87:88 $(t = 5 \text{ min}), 10:7:79:82 \ (t = 1 \text{ h}), 10:5:83:87 \ (t = 3 \text{ h}), 11:6:82:77 \ (t = 3 \text{ h}), 12:6:82:77 \ (t = 3 \text{ h}), 12:6:82:77$ 48 h), 11:6:83:81 (t = 114 h). ¹H NMR (d_6 -dmso/D₂O, 7:3, + NaCl 0.1 M): 3, 3a, 3b, and LN, ratio 25:10:70:60 (t = 5 min to 72 h). ¹³C{¹H} NMR (d_6 -dmso/D₂O, 9:1): δ = 195.7 (LN), 168.4 (LN), 165.1 (LN), 155.5 (LN), 154.1 (LN), 150.4 (LN), 149.7 (LN), 137.5 (LN), 133.0 (LN), 130.1 (LN), 127.8 (LN), 125.8 (LN), 124.4 (LN),

Table 4. Crystal Data and Measurement Details for 1 and 3·CH₂Cl₂·1/2[1,4-C₆H₄(OH)₂]

	1	$3 \cdot CH_2 Cl_2 \cdot 1/2 [1, 4 - C_6 H_4 (OH)_2]$
formula	$C_{28}H_{32}Cl_2O_6Os$	C ₃₅ H ₃₈ Cl ₆ NO ₇ Os
fw	725.64	987.56
Т, К	100(2)	100(2)
λ, Å	0.71073	0.71073
cryst syst	orthorhombic	monoclinic
space group	Pbca	P21/n
<i>a,</i> Å	19.6723(19)	17.0370(17)
<i>b,</i> Å	11.5289(11)	13.3798(13)
<i>c,</i> Å	24.095(2)	17.2905(17)
<i>β</i> , °		103.3800(10)
cell volume, Å ³	5464.8(9)	3834.4(7)
Z	8	4
$D_{c'} \text{ g} \cdot \text{cm}^{-3}$	1.764	1.711
μ , mm ⁻¹	4.903	3.791
F(000)	2864	1956
Crystal size, mm	$0.10 \times 0.09 \times 0.06$	$0.19\times0.10\times0.06$
heta limits, deg	1.7-29.6	1.9-28.6
reflns collected	57 473	45 640
independent reflns	7332	9208
data/restraints/params	7332/0/340	9208/4/457
goodness of fit on F^2	1.011	1.001
$R_1 \ (I > 2\sigma(I))$	0.0299	0.0389
wR_2 (all data)	0.0621	0.0821
largest diff peak and hole, e·Å ⁻³	1.501, -1.111	1.146, -1.313

121.5 (LN), 112.0 (LN), 98.4 (3b), 93.6 (3b), 78.5 (3b), 78.4 (3b), 65.9 (LN), 63.5 (LN), 63.1 (LN), 30.3 (3b), 23.3 (LN),): 22.1 (3b), 18.1 (3b), 12.7 (LN) (t = 72 h).

Compound 4. $\Lambda_{\rm M}$ (dmso/water, 9:1) = 67 S·cm²·mol⁻¹ (t = 5 min); 60 S·cm²·mol⁻¹ (t = 2 h); 65 S·cm²·mol⁻¹ (t = 24 h); 71 S·cm²· mol⁻¹ (t = 62 h). UV-vis (dmso/water, 9:1), $\lambda_{max}/nm (\epsilon/M^{-1} \cdot cm^{-1})$ = 317 (8.2 × 10³) (t = 5 min); 317 (4.1 × 10³) (t = 62 h). UV-vis $(dmso/water, 1:1, + NaCl 0.1 M) = 317 (8.3 \times 10^3) (t = 5 min); 317$ (6.9×10^3) (t = 62 h). ¹H NMR (d_6 -dmso/D₂O, 9:1): δ = 9.31 (s, 4a), 9.04 (m, LN), 8.92 (d, 4a), 8.75 (m, LN), 8.41 (d, 4a), 8.24 (m, LN), 7.62 (m, 4a), 7.54 (m, LN), 7.24 (d, 4a), 7.16 (d, LN), 7.11 (d, 4a), 7.09 (d, LN), 6.57 (d, 4b), 6.39 (d, 4b), 6.27 (d, 4b), 6.15 (m, 4a), 6.07 (d, 4b), 6.06 (m, 4a), 6.03 (s, LN), 5.96 (d, 4a), 5.51 (s, 4a), 5.48 (s, LN), 5.02 (s, 4a), 4.50 (m-br, 4a + LN), 4.26 (q, 4b), 4.06 (q, 4a), 2.43 (m, 4a), 2.33 (q, 4a), 2.25 (s, 4b), 1.93 (s, 4a), 1.15 (d, 4a), 1.04 (t, 4a). Ratio 4a:4b:LN = 100:0:0 (t = 5 min), 45:50:55 (t = 24 h), 23:60:63 (t = 46 h), 17:65:67 (t = 62 h). ¹H NMR (d_6 -dmso/D₂O, 1:1, + NaCl 0.1 M): 4a, 4b, and 4c [δ = 8.92 (m), 8.64 (d), 8.21 (d), 7.49 (m), 7.01 (d), 6.92 (d), 6.97, 6.02, 5.98, 5.90, 5.41 (s), 4.91 (s), 2.01 (s)]. Ratio 100:0:0 (t = 5 min), 100:31:11 (t = 24 h), 100:70:20 $(t = 46 \text{ h}), 100:100:20 \ (t = 62 \text{ h}).$ LN is not soluble in these conditions. ¹³C{¹H} NMR $(d_6$ -dmso/D₂O, 9:1): $\delta =$ LN and 4a [$\delta =$ 86.6, 83.8, 71.8, 51.3, 30.8, 22.2, 22.0, 18.6] (t = 62 h). ³¹P NMR (d_6 dmso/D₂O, 9:1): $\delta = -76.5$ (4a), -74.8 (4b). Ratio 100:0 (t = 2 h), 42:58 (t = 24 h), 23:76 (t = 46 h), 17:82 (t = 62 h). ³¹P NMR (d_{6} dmso/D₂O, 1:1, + NaCl 0.1 M): 4a, 4b, and 4c [$\delta = -72.0$]. Ratio 100:0:0 (t = 2 h), 64:35:0 (t = 24 h), 46:46:7 (t = 46 h), 35:47:12 (t = 46 h)62 h).

X-ray Crystallography. Crystal data and collection details for 1 and 3·CH₂Cl₂·1/2[1,4-C₆H₄(OH)₂] are reported in Table 4. X-ray data were collected on Bruker Smart APEX CCD (3) and Smart Apex CCD DUO (1) diffractometers equipped with a normal focus, 2.4 kW sealed tube source (molybdenum radiation, $\lambda = 0.71073$ Å), operating at 50 kV and 30 (1) or 40 (3) mA. Data were collected over the complete sphere by a combination of four sets. Each frame exposure time was 30 s (1) or 10 s (3), covering 0.3° in ω . Data were corrected for absorption by using a multiscan method applied with the Sadabs program.³⁰ The structures were solved by the Patterson method or by direct methods. Refinement, by full-matrix least-squares on F^2 with SHELXL97,³¹ was similar for the two complexes, including isotropic and subsequently anisotropic displacement parameters. The hydrogen atoms were observed or calculated and refined freely or refined using a restricted riding model. In the case of 3, a half-molecule of hydroquinone (added to facilitate the crystallization, vide infra) and one molecule of dichloromethane were observed in the asymmetric unit. All the highest electronic residuals were observed in close proximity to the Os centers and do not hold any chemical significance.

Cell Culture. Human A2780 and A2780cisR ovarian carcinoma cells were obtained from the European Centre of Cell Cultures (ECACC, UK). Nontumorigenic HEK-293 cells were obtained from ATCC (Sigma, Switzerland). A2780 and A2780cisR were routinely grown in RPMI 1640 GlutaMAX (Lifetechnologies, Switzerland), while HEK-293 cells were grown in DMEM medium, both containing 10% heat-inactivated fetal bovine serum (FBS, Pan Biotech, Germany) and 1% antibiotics (penicillin/streptomycin), at 37 °C and CO₂ (5%).

Determination of Antiproliferative Activity. 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 culture 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 amount 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 590 nm using a multiwell plate reader

(Molecular Devices), and the fraction of surviving cells was calculated from the absorbance of untreated control cells. Evaluation is based on means from three independent experiments, each comprising four testings per concentration level.

GST Activity Assay. A2780 and A2780cisR cells were plated in sixwell plates $(2 \times 10^6 \text{ cells/well})$ and incubated for 24 h in a CO₂ incubator at 37 °C. The cells were then exposed to compounds LN, LP, EA-(C=O)O(CH₂)₂OH, and 1-4 for 18 h, at final concentrations according to the IC₅₀ concentration for each respective cell line. The cells were then washed twice with PBS, harvested, lysed by repetitive freeze-thaw cycles, and centrifuged at 10000g for 15 min at 4 °C. The supernatants were used for the analysis of GST activity according to a fluorometric GST detection kit (Abnova). The changes in fluorescent intensities at Ex/Em 380/460 nm were recorded in a kinetic mode, every 5 min over 60 min, on a microplate reader (Molecular Devices). Two independent experiments were performed in which the GST activities were measured in duplicate. The GST activity expressed as U/min/mL per mg protein was converted to % activity of control (nontreated cells). The GST activity of the test samples was calculated by applying the equation $\Delta RFU = RFU_2 -$ RFU₁ to the GST standard curve to get B [mU] during the reaction time ($\Delta T = T_2 - T_1$). The sample GST activity is calculated by the following formula: sample GST activity = $B/(\Delta T \times V) \times dilution$ factor [mU/min/mL], where B is sample GST activity from the GST standard curve [mU], ΔT is the reaction time (min), and V is the sample volume added into the reaction well [mL].

Protein Determination. The protein concentration was determined by a Bradford assay (Bio-Rad) using BSA as a standard.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.organomet.6b00197. CCDC reference numbers 1421240 (1) and 1421241 (3) contain the supplementary crystallographic data for the X-ray studies reported in this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/ retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (internat.) + 44-1223/336-033; e-mail: deposit@ccdc.cam.ac.uk).

Crystallographic data for 1 (CIF) Crystallographic data for 3 (CIF)

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Notes

The authors declare no competing financial interest.

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