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Low-Potential Cyclometalated Osmium(II) Mediators of Glucose Oxidase

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The osma(II)cycles $[Os(phpy)(LL)_2]PF_6$ (LL = 1,10-phen (**3a**) and 4,4′-Me_2-2,2′-bpy (**3b**)) are made from $[(\eta^6\text{-}C_6H_6)Os(\mu\text{-}Cl)Cl]_2$ (**1**) either via transmetalation using the $[Hg(phpy)_2]$ organomercurial in MeOH or via the sp^2-C–H bond cleavage of 2-phenylpyridine (phpyH) in MeCN to afford $[(\eta^6\text{-}C_6H_6)Os(phpy)Cl]$ or $[(\eta^6\text{-}C_6H_6)Os(phpy)(MeCN)]PF_6$, respectively. The latter two react cleanly with LL to give **3a** and **3b**, the $M^{II/III}$ redox potentials of which equal 30 and −100 mV (vs Ag/AgCl), respectively. The electrochemically made Os^{III} species oxidize rapidly reduced glucose oxidase. The second-order rate constant equals 1.1×10^7 M^{-1} s^{-1} for **3a** at 25 °C, pH 7.

Bioamperometric biosensors based on the mediated electron transfer include multicomponent assemblies of (i) an oxidoreductase as a biocatalyst of a redox reaction (enzyme), (ii) a low-molecular-weight compound that moves electrons between enzyme active sites and an electrode (mediator), and (iii) a polymeric network designed to keep together an enzyme and a mediator at the electrode surface so as to ensure the most facile electron exchange. This should also eliminate leakage of any biosensor component and set up a high operational stability of an enzyme.[1] Components i−iii are equally important, and current research efforts are aimed at improving the performance of enzymes, mediators, and polymeric networks. Many mediators are either inorganic or organometallic derivatives of Fe^{II} (ferrocenes), or complexes of Os^{II} and Ru^{II}.[2] Ferrocenes were the first transition metal based mediators showing the greatest promise.[3] Later, complexes *cis*-$[OsCl_2(LL)_2]$ were introduced,[4] which are more advantageous primarily due to lower redox potentials. The Os^{II}-based mediators are still under intensive investigation.[5] The Ru^{II} analogues compete with the Os^{II} complexes due to affordability and a better developed synthetic chemistry. Benefits of Os mediators compared to Ru ones are in lower, by ca. 300 mV, redox potentials and in higher rates of the electron exchange with enzyme active sites. In particular, the redox potentials of *cis*-$[MCl_2(bpy)_2]$ (M = Os and Ru) equal −36 and 300 mV (vs SCE) at pH 7, but the rate constants for the oxidation of reduced glucose oxidase (GO) from *Aspergillus niger* by the electrochemically generated M^{III} species equal 4.5×10^5 and 1.8×10^4 M^{-1} s^{-1}, respectively.[6] Continuing our efforts in biosensor design,[7] we have recently introduced new cycloruthenated mediators $[Ru^{II}(o\text{-}C_6H_4\text{-}X)(LL)(LL')]PF_6$ (X = 2-pyridinyl or CH_2-NMe_2; LL or LL′ = bpy or phen). Their reactivity is as high as 10^7 M^{-1} s^{-1}, and the redox potentials equal 190−300 mV.[8] The performance of Os mediators is higher, and a challenge

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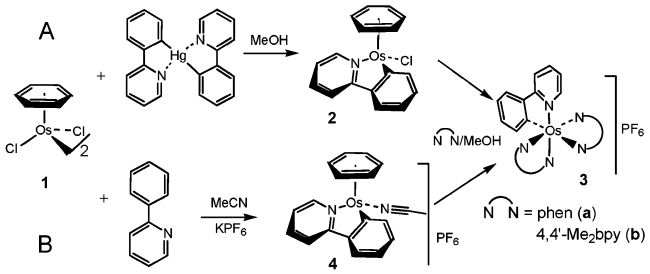
Scheme 1. Two Synthetic Routes to Cyclometalated Osmium(II) Compounds



has been to make and test with GO structurally similar cyclometalated Os^{II} compounds. Reports on osmacycles are limited,[9] and none of these complexes meet the criteria of a good mediator.[2]

Here we describe the synthesis of $[(\eta^6\text{-}C_6H_6)OsCl(phpy)]$ (**2**) and $[Os^{II}(phpy)(LL)_2]PF_6$ (**3**, phpyH = 2-phenylpyridine, LL = 1,10-phen (**a**), 4,4′-Me_2bpy (**b**)), their properties, X-ray structural characterization of **2**, and the reactivity of **3a**,**b** with respect to GO. Two approaches to **3** have been explored using $[(\eta^6\text{-}C_6H_6)Os(\mu\text{-}Cl)Cl]_2$ (**1**)[10] as a starting material (Scheme 1). The transmetalation of ortho-mercurated aryl derivatives onto metal−halide compounds has been a rather useful way to synthesize cyclometalated compounds of Os or Ru.[9h,11] We have found that the transmetalation between **1** and the bis cyclomercurated compound $[Hg(phpy)_2]$[12] affords **2** in a 63% yield (pathway A).[13] We have also tried to react **1** with phpyH directly under the conditions optimal for the preparation of the related ruthena(II)cycles (pathway B).[8,14] But instead of the anticipated product $[Os^{II}(phpy)(MeCN)_4]PF_6$, the reaction in MeCN in the presence of NaOH affords the complex $[(\eta^6\text{-}C_6H_6)Os(phpy)(MeCN)]PF_6$ (**4**) in a low yield (16%).[15]

Significantly higher yield for $[Hg(phpy)_2]$ suggests an electrophilic mechanism for reactions of complex **1** with 2-phenylpyridine and $[Hg(phpy)_2]$. The arylating agent with a better leaving group, i.e., $[Hg(phpy)]^+$ versus H^+, is better for cyclometalation. Complexes **2** and **4** are converted into **3a**,**b** by reacting with 1,10-phen or 4,4′-Me_2bpy in MeOH[16] using essentially the same procedure as in the Ru case.[8] The η^6-bound benzene is substituted by phen in methanol, and therefore **2** is a versatile precursor for a variety of cyclometalated Os^{II} compounds.

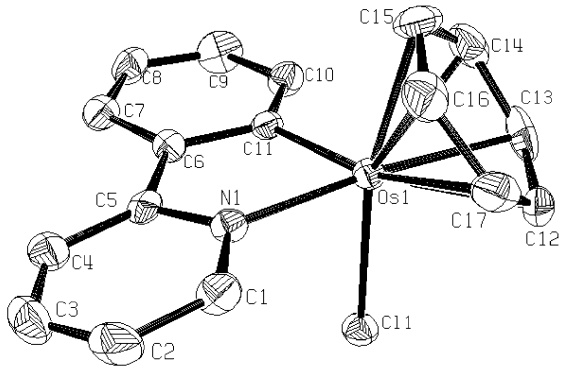


Figure 1. Structure of complex **2**. Selected bond lengths (Å) and angles (deg): Os−N1 2.073(4), Os−C11 2.074(4); Os−C1 2.431(1); N1−Os−C11 77.3(2), Cl1−Os−C11 87.7(1), N1−Os−Cl1 82.1(1).

The composition of **2**−**4** is in accord with the results of the combustion analysis, cyclic voltammetry, ^{1}H NMR, IR, UV−vis, and mass spectroscopy. The structure of **2** was confirmed by an X-ray single crystal study (Figure 1).[17] The osmium metal is in the center of a pseudotetrahedron. The bond distances and angles are typical of such compounds.

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(13) **2**: **1** (0.026 mmol) and $[Hg(phpy)_2]$ (35 mmol) were suspended in 5 mL of MeOH and refluxed for 90 min. MeOH was rotary evaporated, and the residue was dissolved in 1 mL of $CHCl_3$ and brought on a column with neutral Al_2O_3 (5 × 1 cm). The first band was collected and solvent removed. The product was recrystallized from C_6H_6:$CHCl_3$ and air-dried (0.033 mmol, 63%). IR (KBr): 735, 752, 825, 1419, 1475, 1560, 1582, 1603, 2855, 3928, 3046 cm^{-1}. ^{1}H NMR ($(CD_3)_2$CO, δ, J in Hz, atom numbering as in Figure 1): 5.60s (6H, C_6H_6), 6.92 (td, H8, J 7.4 and 1.4), 6.99 (td, H9, J 7.4 and 1.4), 7.11 (td, H2, J 5.8 and 1.6), 7.74 (dd, H4, J 8.0 and 1.4), 7.78 (t, H3, J 7.4), 7.96 (d, H7, J 7.4), 8.07 (d, H10, J 7.4), 9.36 (d, H1, J 5.8). UV−vis (EtOH): λ (ϵ, M^{-1} cm^{-1}) 336sh (3600) and 377sh nm (2600). Anal. Found: C, 46.62; H, 3.10; N, 3.15. Calcd for $C_{17}H_{14}ClNOs\cdot{}^1/_3C_6H_6$: C, 47.15; H, 3.33; N, 2.89.

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(15) **4**: **1** (0.4 mmol), phpyH (0.8 mmol), NaOH (0.8 mmol), and KPF_6 (1.6 mmol) in 20 mL of MeCN were stirred for 24 h at 50 °C under Ar. A dark yellow suspension was filtered over Al_2O_3 and eluted with a 10:1 CH_2Cl_2:MeCN mixture. The first band was collected and concentrated in a vacuum, and a yellow precipitate was formed after addition of hexane (0.1 mmol, 16%). ^{1}H NMR (CD_2Cl_2, δ): 2.30 (s, CH_3), 5.75 (s, C_6H_6), 7.14−7.23 (m, 3H), 7.74 (d, 1H), 7.84 (d, 1H), 7.88 (t, 1H), 8.05 (d, 1H), 9.16 (d, H1). M/z: 424 (M^+ − MeCN), 465 (M^+).

(16) **3a**: **2** (0.023 mmol) and phen·H_2O (0.05 mmol) were refluxed for 1 h in 8 mL MeOH. KPF_6 (0.35 mmol), benzene (15 mL), water (10 mL), and MeCN (5 mL) were added. The organic layer was separated. The aqueous layer was washed with benzene (5 mL) in the presence of 1 mL of MeCN. Organic layers were combined, washed 3 times with water, and dried over Na_2SO_4. The volume was reduced to 2 mL, and 6 mL of Et_2O was slowly added. Black crystals were filtered off and air-dried to afford 8 mg of **3a** (40%). Anal. Found: C, 48.70; H, 3.10; N, 7.63. Calcd for $C_{35}H_{24}F_6N_5OsP\cdot H_2O$: C, 48.44; H, 3.02; N, 8.07. UV−vis (MeOH): λ (ϵ, M^{-1} cm^{-1}) 244 (29160), 290 (26420), 381 (5910), 533 nm (12840). ^{1}H NMR (δ, $(CD_3)_2$CO): phpy, 6.13 (d, H1), 6.63 (t, H2), 6.75 (t, H3). M/z: 706 (M^+), 851 ($M^+PF_6^-$). **3b**: UV−vis (MeOH): λ (ϵ, M^{-1} cm^{-1}) 247 (31590), 290 (35230), 418 (12140), 565 nm (10390). M/z: 714 (M^+), 859 ($M^+PF_6^-$).

(17) Crystal data for **2**: $C_{17}H_{14}ClNOs$, M = 457.96, orange prism, triclinic, $P1$, a = 10.2556(2) Å, b = 12.0349(2) Å, c = 12.5450(2) Å, α = 70.647(5)°, β = 77.229(5)°, γ = 74.994(5)°, V = 1395.44(6) Å^3, Z = 4, ρ_{calcd} = 2.18 g/cm^3, μ(Mo Kα) = 0.71073 Å, T = 173 K, KappaCCD diffractometer, 17211 reflections were collected using program Collect ("Collect" data collection software, Nonius B.V., 1998), R = 0.025, R_w = 0.052.

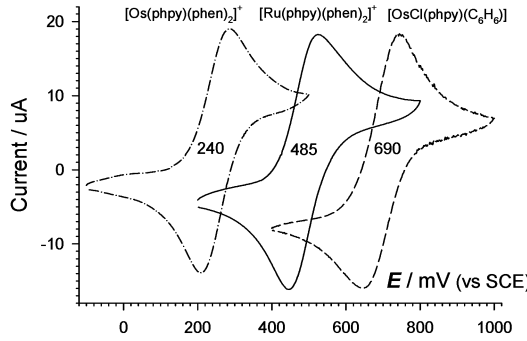

Figure 2. CVs of complexes **3a**, **2**, and **5** in MeCN, 25 °C, 0.1 M (n-Bu)$_4$NPF$_6$, scan rate 0.1 V s^{-1}; IPC BAS 50W electrochemical analyzer; glassy carbon working electrode.

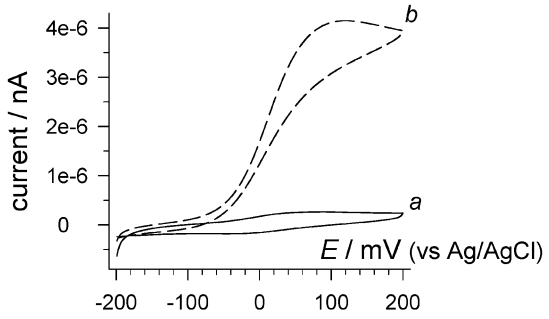

Figure 3. CVs of complex **3a** (1×10^{-4} M) without (a) and with (b) 1×10^{-6} M GO and 0.05 M D-glucose; pH 7 (0.01 M phosphate), scan rate 2 mV s^{-1}.

The mean Os−C$_{benzene}$ distance is 2.212(5) Å. The structure is similar to the related Ru^{II} complex with η^6-coordinated *p*-cymene instead of benzene.[18] The metal−ligand bond distances are by 0.1−0.2 Å longer in the osmium complex, reflecting its larger ionic radius.

Cyclic voltammograms (CVs) of complexes **3a**, **2**, and [Ru(phpy)(phen)$_2$]$^+$ (**5**),[8] i.e., a Ru analogue of **3a**, recorded in MeCN are shown in Figure 2. Osmium complex **2** is more difficult to oxidize into M^{III} due to a strong back-bonding effect from the η^6-bound benzene. Substitution of the latter by diimines results in a significant, by 0.45 V, decrease in the potential. The CV of **5** is just between those for complexes **3a** and **2**. The anticipated difference in redox potentials of the Os and Ru complexes is again 0.245 V. These comparisons suggest a strategy for tuning redox potentials of cyclometalated Os and Ru complexes.

High capability of **3a**,**b** to exchange electrons with the active site of GO, i.e., to be mediators, is demonstrated in Figure 3. Cyclic voltammograms of **3a** with and without GO and D-glucose in water illustrate a significant current increase when GO and D-glucose are present. The current growth is accounted for in terms of eqs 1−3. The second-order rate

$$\text{GO(ox)} + \text{D-glucose} \rightarrow \text{GO(red)} + \delta\text{-D-gluconolactone} + 2\text{H}^+ \quad (1)$$

$$\text{GO(red)} + 2\text{Os}^{III} \xrightarrow{k_3} \text{GO(ox)} + 2\text{Os}^{II} \quad (2)$$

$$2\text{Os}^{II} - 2\text{e} \rightarrow 2\,\text{Os}^{III}\,\text{(electrode)} \quad (3)$$

constants k_3 for step 2 calculated by means of the procedure of Bourdillon et al.[19] are shown in Table 1. Some comments

Table 1. Redox Potentials and the Rate Constants k_3 for Oxidation of GO(red) by Cyclometalated Os and Ru Complexes at pH 7 and 25 °C

complex	$E^{\circ\prime}$ (mV, Ag/AgCl)	$10^{-7} \times k_3$ (M^{-1} s^{-1})	ref
[Os(phpy)(phen)$_2$]$^+$ **3a**	30	1.1 ± 0.1	this work
[Os(phpy)(4,4′-Me$_2$bpy)$_2$]$^+$ **3b**	−100	0.10 ± 0.02	this work
[Ru(phpy)(phen)$_2$]$^+$	280[b]	0.75	8
[Ru(phim)(phen)$_2$]$^+$ [a]	250	0.81	14
[Ru(phim)(4,4′-Me$_2$bpy)$_2$]$^+$	130	0.52	14
[Ru(phpy)(acac)(bpy)$_2$]$^+$	−210[b]	no coupling	*c*

[a] phim = cyclometalated 2-phenylimidazole. [b] Vs SCE. [c] Unpublished.

are appropriate. (i) Resistant to ligand substitution cyclometalated Os^{II} complexes are reactive mediators for GO. Complex **3a** has a low redox potential (30 mV) and a high rate constant for the electron transfer. (ii) The rate constant k_3 for **3a** (1.1×10^7 M^{-1} s^{-1}) is in the range typical of the most reactive mediators of GO.[2] (iii) Further "cathodic" tuning of the redox potential of osma(II)cycles has been achieved by using 4,4′-Me$_2$bpy instead of phen ligand. Mediator **3b** has in fact a very low potential (−100 mV), but this compromises slightly the performance. The rate constant k_3 decreases more than 10-fold on going from **3a** to **3b**. It should be pointed out that a decrease in the mediator potential due to the use of a more electron-rich diimine ligand does not decrease the reactivity of ruthenacyclic mediators with $k_3 \sim 10^7$ M^{-1} s^{-1} (Table 1). However, the redox potential of even [Ru(phim)(4,4′-Me$_2$bpy)$_2$]$^+$ is still appreciably higher than that of flavin adenine dinucleotide (FAD) in GO (estimations for the FADH$_2$/FADH$_2^{\bullet+}$ and FADH$^\bullet$/FADH$^+$ couples are −70 and −90 mV versus SCE, respectively).[19] This suggests that high rates of the electron exchange with GO are difficult to achieve when the redox potential of a mediator becomes close to that of the redox cofactor. (iv) The latter finds support in the fact that ruthenacycles with very low potentials do not exchange electrons with GO at all (Table 1). (v) When adsorbed on a glassy carbon electrode, **3a** does not electrocatalyze the oxidation of ascorbic acid (1.6×10^{-4} M) at 0.1 V vs Ag/AgCl and pH 7.

In summary, the transmetalation reaction and the C−H bond scission of 2-phenylpyridine both afford new cyclometalated Os^{II} derivatives from complex **1**. The transmetalation is synthetically preferred due to a higher yield of the target compound. The reactions of complexes **2** and **4** with diimines give coordinatively saturated, substitutionally inert cyclometalated mediators of glucose oxidase **3**, which have very low redox potentials and high rates of oxidation of the reduced enzyme.

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Supporting Information Available: Tables of X-ray crystallographic data for **2** (PDF); X-ray crystallographic data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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