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NMR Investigation of the Spontaneous Thermal- and/or Photoinduced Reduction of trans Dihydroxido Pt(IV) Derivatives

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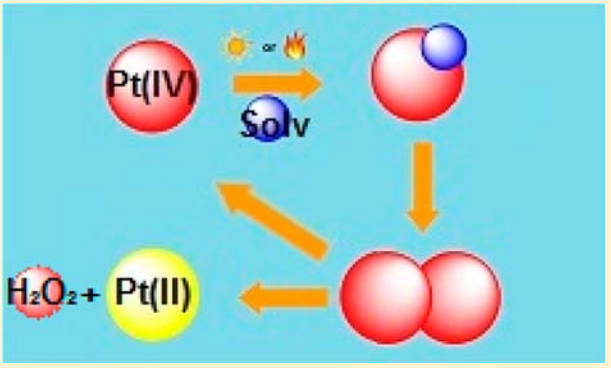
Supporting Information

ABSTRACT: The initial aim of the present work was the synthesis of the axial disuccinato Pt(IV) derivative of [$PtCl_2$(*cis*-1,4-DACH)] (Kiteplatin, **1** in Figure 1) (DACH = diaminocyclohexane), which contains an isomeric form of the diamine ligand present in oxaliplatin (i.e., 1*R*,2*R*-DACH). The interest in this compound stems from its activity on several cisplatin and oxaliplatin-resistant cell lines. Oxidation of **1** with hydrogen peroxide affords *cis,trans,cis*-[$PtCl_2(OH)_2$(*cis*-1,4-DACH)] (**2**) which was treated with succinic anhydride in suitable solvents. To our surprise, in dimethylformamide (DMF) (50−70 °C or under light irradiation) or in dimethylsulfoxide (DMSO) (under light irradiation) the formation of the succinato complex *cis,trans,cis*-[$PtCl_2${$OC(O)CH_2CH_2C(O)OH$}$_2$(*cis*-1,4-DACH)] (**3**) was accompanied by reduction to **1**. It was found that solvolysis of **2** and formation of a μ-oxo dinuclear species (**5**) is the key step. The dinuclear species can then undergo reduction to a 1:1 mixture of **1** and **2** with concomitant elimination of oxygen (1/2 O_2 in the form of H_2O_2). The whole process is fostered by heat and/or light, which could favor solvolysis of **2** as well as decomposition of hydrogen peroxide to water and oxygen so preventing the reoxidation of **1** to **2**. Because of its peculiar behavior, compound **5** could be exploited also for the development of a technology for water splitting.



INTRODUCTION

Since the discovery of the antiproliferative activity of *cis*-diamminedichloridoplatinum(II) (cisplatin, Figure 1) by Rosenberg,[1−3] also the corresponding platinum(IV) species were known to possess similar biological properties. Platinum(IV) complexes appear to act as prodrugs and are activated in vivo by reduction to the Pt(II) species.[4] Up to now, all platinum drugs in clinical use are Pt(II) compounds (cisplatin, carboplatin (*cis*-diammine-1,1-cyclobutanedicarboxylato-platinum(II)), and oxaliplatin (*R,R*-1,2-diaminocyclohexane-oxalato-platinum(II)), and all of them are administered intravenously because of their instability in the gastrointestinal tract. Pt(IV) complexes are, in general, kinetically more inert, and this feature has been exploited for oral administration.[5,6] Moreover, the presence of two additional ligands in axial positions could be exploited to tuning the lipophilicity, the rate of reduction, and the overall pharmacokinetic profile of Pt(IV) substrates.

The first two Pt(IV) complexes which underwent phase I clinical trials (tetraplatin (tetrachlorido-*R,R*-1,2-diaminocyclohexane-platinum(IV)) and iproplatin (*cis*-dichlorido-*trans*-dihydroxido-bis-isopropylamine-platinum(IV)); Figure 1) showed unfavorable pharmacokinetic behaviors. Tetraplatin, having a cathodic potential of −90 mV, was reduced already in the bloodstream causing systemic toxicity. In contrast, iproplatin, having a reduction potential of −730 mV, was found to be inactive because of its not sufficiently fast reduction.[7] The rate of reduction depends

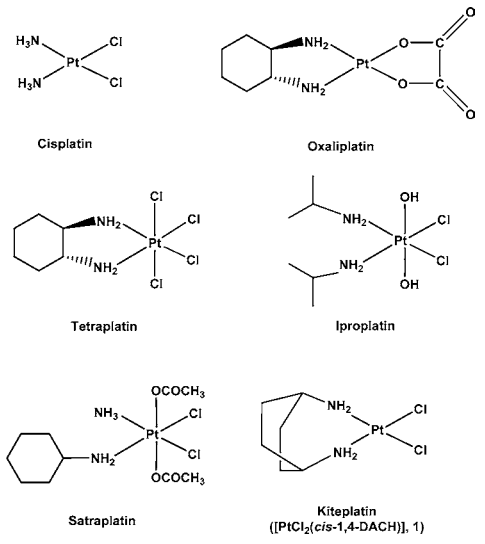


Figure 1. Chemical structures of anticancer platinum complexes.

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strongly upon the nature of the axial ligands and is too fast for axial chlorides, while it is too slow for axial hydroxides. An intermediate behavior is observed for axial carboxylato ligands.[8,9] For instance, satraplatin (*cis*-dichlorido-*trans*-diacetato-*cis*-ammine,cyclohexylamine-platinum(IV), Figure 1) has a reduction potential of −250 mV and is under phase III clinical trials.[7] Interestingly, the electrochemical potentials can be satisfactorily predicted by statistical models based on a combination of surface areas (total and polar), lowest unoccupied molecular orbital (LUMO) energies, and dipole moments.[10]

However, in some instances good results in terms of cytotoxicity have been reported also for axial hydroxido Pt(IV) complexes,[11] in spite of their highly negative reduction potential. Moreover, new interest in this class of Pt(IV) compounds arose from the possibility to obtain complexes activated by visible light.[12]

Pt(IV) complexes with optimal lipophilicity[13] have been prepared by carboxylation of the axial positions. Moreover, by using axial carboxylato ligands carrying also free carboxylic groups, further functionalization of the platinum(IV) substrate can be pursued. The easiest way is to condense the uncoordinated carboxylic group with an aminic or an alcoholic functionality, forming the corresponding amide or ester (the reaction can be favored by the presence of 1,1′-carbonyldiimidazole).[7,13] In this way, biologically active residues can be incorporated in the axial positions of the Pt(IV) complex to act as targeting moiety or to induce an additional pharmacological effect at the active site where reduction to Pt(II) liberates the axial ligands.[14,15] In this context we wish to acknowledge the pioneering work of Lippard, who prepared estrogen-tethered platinum(IV) derivatives with the hope that the estrogen, once liberated, could sensitize the cell to cisplatin.[14] Lippard also tethered several units of platinum(IV) anticancer drugs to soluble, single-walled, carbon nanotubes used as longboat delivery systems for an effective transport of platinum drugs across the cell membrane.[16]

The initial aim of the present work was the synthesis of the axial disuccinato Pt(IV) derivative of [$PtCl_2$(*cis*-1,4-DACH)] (Kiteplatin, **1** in Figure 1) (DACH = diaminocyclohexane), which contains an isomeric form of the diamine ligand present in oxaliplatin (i.e., *1R,2R*-DACH). The interest in compound **1** stems from its activity on several cisplatin and oxaliplatin-resistant cell lines.[17−22] Pt(IV) derivatives of **1**[23−25] have been extensively investigated by Khokhar's group that, among a series of complexes having general formula *cis,trans,cis*-[$Pt^{IV}Cl_2X_2$(*cis*-1,4-DACH)] (X = $CH_3(CH_2)_nCOO$, *n* = 0−8), found that *cis,trans,cis*-[$Pt^{IV}Cl_2$(acetato)$_2$(*cis*-1,4-DACH)] was the most active in the murine L1210 leukemia model.[24] However, to the best of our knowledge, no Pt(IV) derivatives of kiteplatin with free carboxylic functions, suitable for further functionalization, have yet been prepared. Therefore we planned to prepare the desired compound by reaction of *cis,trans,cis*-[$PtCl_2(OH)_2$(*cis*-1,4-DACH)] (**2**), obtained by oxidation of **1** with hydrogen peroxide, with succinic anhydride in suitable solvents. To our surprise, the reaction between **2** and succinic anhydride (performed in the conditions reported in the literature for the preparation of similar compounds),[13,14,16] leads also to reduction of **2** to the parent complex **1**. This prompted us to investigate, in detail, the mechanism of this side reaction.

EXPERIMENTAL SECTION

Materials and Methods. Commercial reagent grade chemicals and solvents were used as received without further purification. ^{1}H NMR and [^{1}H-^{195}Pt] HSQC spectra were recorded on a Bruker Avance DPX 300 MHz instrument. ^{1}H chemical shifts were referenced using the internal residual protic peak of the solvent (2.50 ppm for DMSO-d_6, 8.03 ppm for DMF-d_7, 4.8 ppm for D_2O).

^{195}Pt NMR spectra were referenced to K_2PtCl_4 (external standard placed at −1620 ppm with respect to $Na_2[PtCl_6]$).[27]

ESI-MS spectra were recorded on Agilent 1100 Series LC-MSD-Trap-System VL.

Kiteplatin (**1**) was prepared according to a literature method.[19]

Synthesis of *cis,trans,cis*-[$PtCl_2(OH)_2$(*cis*-1,4-DACH)] (2). Compound **2** was prepared according to an already reported procedure[24] with slight modifications. Briefly, a suspension of [$PtCl_2$(*cis*-1,4-DACH)] (**1**) (208.6 mg, 0.55 mmol) in 28 mL of H_2O was treated with a solution of H_2O_2 in H_2O (30% w/w, 2.0 mL). The mixture was stirred at 70 °C for 2 h in the dark. The resulting yellow solution was filtered and then concentrated under reduced pressure to a minimum volume. Addition of acetone induced the formation of a pale yellow precipitate that was isolated by filtration of the mother liquor, washed with ice cold water, and dried under vacuum. Yield 80% (183 mg, 0.44 mmol).

^{1}H NMR. (DMSO-d_6) 6.33 (4H, N*H*$_2$), 2.89 (2H, C*Ha*, see Figure 2 for the numbering of protons), 2.07 (4H, C*Hb*Hc), 1.45 (4H, CHb*Hc*) ppm; (DMF-d_7) 6.70 (4H, N*H*$_2$), 3.25 (2H, C*Ha*), 2.26 (4H, C*Hb*Hc), 1.67 (4H, CHb*Hc*) ppm. **^{195}Pt NMR:** (DMSO-d_6) 964.6 ppm; (DMF-d_7) 871.7 ppm. **IR:** (KBr pellet) 3421, 3210, 1629, 1574, 547, 396, 322, 215 cm^{-1}. **ESI-MS:** $C_6H_{16}Cl_2N_2O_2PtNa$ [**2**+Na]$^+$ *Calcd*: 437.01. *Found*: *m/z* 436.9. **Anal.:** $C_6H_{18}Cl_2N_2O_3Pt$ (**2**·H_2O) *Calcd*: C, 16.67; H, 4.20; N, 6.48%. *Found:* C, 16.42; H, 4.15; N, 6.29%.

Synthesis of *cis,trans,cis*-[$PtCl_2${$OC(O)CH_2CH_2C(O)OH$}$_2$(*cis*-1,4-DACH)] (3). A suspension of **2** (180.0 mg, 0.44 mmol) in dimethylsulfoxide (DMSO) (2 mL), maintained at 70 °C, was treated with succinic anhydride (350.0 mg, 3.53 mmol). The obtained mixture was kept under magnetic stirring at 70 °C in the dark for 24 h; meanwhile it became a brown solution. The solution was cooled to room temperature, filtered, and frozen at −21 °C to be freeze-dried. The obtained brown oil was treated with diethyl ether and stirred overnight. Diethyl ether was removed, and the resulting yellow oil was treated with methanol. The methanol solution was filtered, concentrated to a minimum volume, and treated with acetone. The obtained solution was concentrated to a minimum volume. The latter procedure (addition of acetone and evaporation) was repeated three times. Finally, addition of diethyl ether induced the precipitation of a sticky pale-yellow solid. Diethyl ether was removed and the solid was dried under vacuum. The obtained solid was suspended in diethyl ether, and the suspension was kept under magnetic stirring for 1 h; then the solid was collected by filtration of the solvent, washed with diethyl ether, and dried under vacuum. Yield 70% (191.0 mg, 0.31 mmol).

^{1}H NMR. (DMSO-d_6) 12.10 (s, 2H, COO*H*), 8.13 (m, 4H, N*H*$_2$), 2.94 (s, 2H, C*Ha*; see Figure 3 for numbering of protons), 2.51 (4H, C*Hd*), 2.38 (m, 4H, C*He*), 1.59 (m, 8H, C*Hb,c*) ppm. **^{195}Pt NMR:** (DMSO-d_6) 1217.0 ppm. **IR:** (KBr pellet) 3434 (s), 1710 (s), 1632 (s), 1374 (m), 1248 (m), 667(m), 340 (m) cm^{-1}. **ESI-MS:** $C_{14}H_{24}Cl_2N_2O_8PtNa$ [**3**+Na]$^+$ *Calcd*: 637.05. *Found: m/z* 636.9. **Anal.:** $C_{14}H_{24}Cl_2N_2O_8Pt$ (**3**) *Calcd*: C, 27.37; H, 3.94; N, 4.56%. *Found:* C, 27.44; H, 4.32; N, 4.99%.

Synthesis of *cis,trans,cis*-[$PtCl_2(OH)(\mu\text{-}O)_{1/2}$(*cis*-1,4-DACH)]$_2$ (5). A solution of **2** (106.0 mg, 0.26 mmol) in H_2O (3.5 mL) was treated with dimethylformamide (DMF, 12.0 mL). The resulting yellow solution was stirred at 50 °C for 30 min in the dark and then allowed to cool to room temperature; meanwhile, a pale-yellow precipitate formed. The solid was isolated by filtration of the solvent, washed with diethyl ether, and dried under vacuum. Yield 50% (51.9 mg).

^{1}H NMR. (DMSO-d_6) 6.28 (8H, N*H*$_2$), 2.87 (4H, C*Ha*), 2.04 (8H, C*Hb*Hc), 1.44 (8H, CHb*Hc*), −0.25 (broad, 2H, O*H*) ppm; (DMF-d_7) 6.42 (8H, N*H*$_2$), 3.23 (4H, C*Ha*), 2.26 (8H, C*Hb*Hc), 1.62 (8H, CHb*Hc*), −0.26 (broad, 2H, O*H*) ppm. **^{195}Pt NMR:** (DMSO-d_6) 970.7 ppm; (DMF-d_7) 873.0 ppm. **IR:** (KBr pellet) 3426, 3213, 1631, 1575, 544, 395, 321, 210 cm^{-1}. **ESI-MS:** $C_{12}H_{30}Cl_4N_4O_3Pt_2$ [**5**-H]$^-$ *Calcd*: 808.03. *Found*: *m/z* 808. **Anal.:** $C_{12}H_{30}Cl_4N_4O_3Pt_2$ (**5**) *Calcd*: C, 17.79, H, 3.73, N, 6.91%. *Found:* C, 17.84, H, 3.96, N, 6.70%.

Synthesis of [$PtCl_4$(*cis*-1,4-DACH)] (6). Compound **6** was prepared according to a procedure reported in the literature[23] with a

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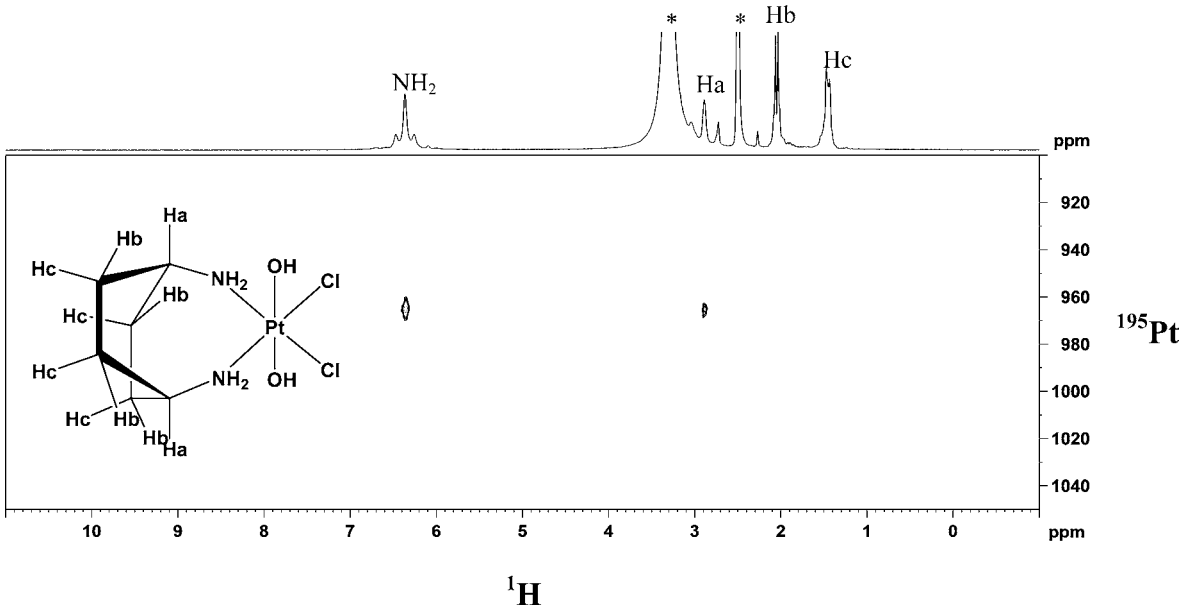

Figure 2. ^{1}H NMR (top) and [^{1}H-^{195}Pt] HSQC 2D (bottom) spectra of **2** in DMSO-d$_6$, the asterisks indicate residual solvent peaks (the resonance at 2.50 ppm showing coupling of residual methyl protons of DMSO-d$_6$ with ^{13}C, $J_{H,13C}$ = 137.4 Hz). The sketch of compound **2** is also reported with the numbering of protons.

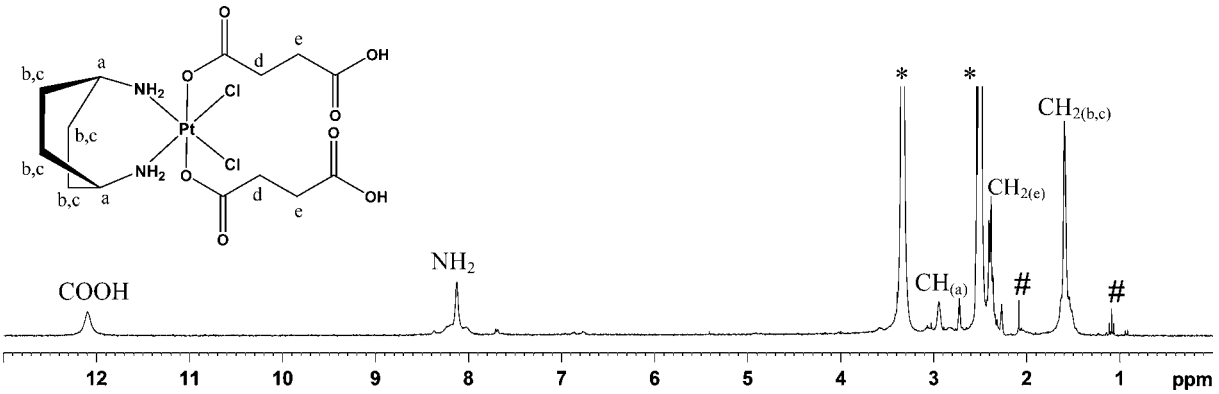

Figure 3. ^{1}H NMR spectrum of **3** in DMSO-d$_6$, the asterisks indicate residual solvent peaks (the resonance at 2.50 ppm showing coupling of residual methyl protons of DMSO-d$_6$ with ^{13}C, $J_{H,13C}$ = 137.4 Hz). # indicate residual traces of acetone and diethylehter used in the synthesis of compound **3.** The sketch of compound **3** and the numbering of protons are also reported.

slight modification. Briefly, a solution of **2** (124.0 mg, 0.30 mmol) in H_2O (25 mL) was treated with concentrated HCl (12.4 mL). The mixture was stirred for 2 days, then the solution was evaporated to dryness under reduced pressure. The resulting solid was washed with ice-cold water and dried under vacuum. Yield 65% (87.0 mg, 0.19 mmol).

^{1}H NMR. (DMSO-d$_6$) 6.78 (4H, N*H*$_2$), 2.83 (2H, C*Ha*), 2.07 (4H, C*Hb*Hc), 1.52 (4H, CHb*Hc*) ppm. **Anal.:** $C_6H_{14}Cl_4N_2Pt$ (**6**) *Calcd*: C, 15.98, H, 3.13, N, 6.21%. *Found:* C, 15.88, H, 3.22, N, 6.13%.

NMR Investigation of the Reduction of *cis,trans,cis*-[PtCl$_2$(OH)$_2$(*cis*-1,4-DACH)] (2) and *cis,trans,cis*-[PtCl$_2$(OH)-(μ-O)$_{1/2}$(*cis*-1,4-DACH)]$_2$ (5). *General Procedure.* Three milligrams of **2** (0.007 mmol) or **5** (0.004 mmol) were dissolved in 750 μL of solvent (DMF-d$_7$ or DMSO-d$_6$) and transferred into an NMR tube. The samples were kept in different light and temperature conditions (Table 1) and monitored by ^{1}H NMR.

Electrochemical Measurements. An Autolab PGSTAT12 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands), interfaced to a personal computer running GPES 4.9 electrochemical software, was used for the electrochemical measurements. A standard three-electrode cell was designed to allow the tip of the reference electrode (Ag/AgCl, saturated KCl) to closely approach the working electrode (a glassy carbon, GC, disk, diameter 0.1 cm, sealed in epoxy resin). The GC working electrode was polished with alumina followed by diamond paste, then rinsed with distilled water and dried. This process yielded an almost completely reproducible surface for all experiments. All measurements were carried out under nitrogen in DMSO, containing 0.1 M [NBu$_4$][PF$_6$] as supporting electrolyte, or aqueous 0.05 M phosphate buffer (PB, pH 7.4), containing 5 mM NaCl. The complex concentration was

Table 1. Summary of the Experimental Conditions Used to Investigate the Reduction of Complexes 2 and 5[a]

solvent	temperature	light	reduction of **2**	reduction of **5**
DMF	70 °C	dark	yes	
DMF	50 °C	dark	yes	yes
DMF	room temperature	dark	no	no
DMF	room temperature	artificial light	yes	yes
DMSO	70 °C	dark	no	yes
DMSO	room temperature	dark	no	no
DMSO	room temperature	artificial light	yes	yes

[a]The behavior of complexes **2** and **5** was investigated also in water in the same experimental conditions, no reduction was observed.

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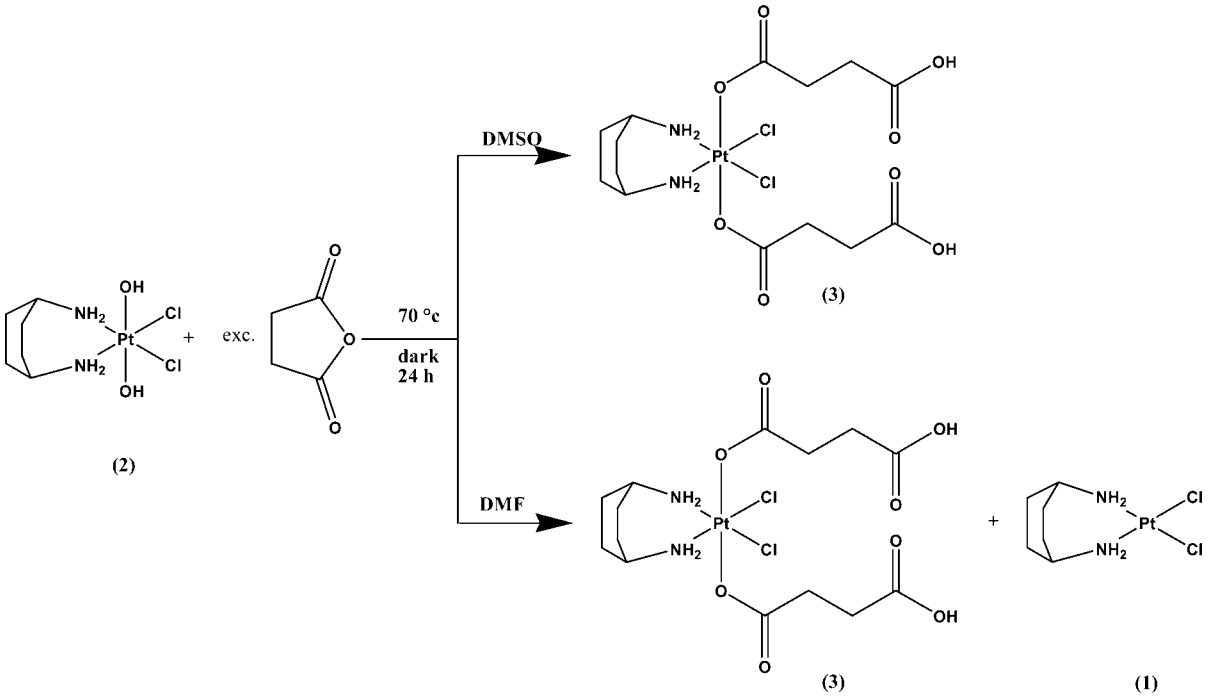
Scheme 1. Different Products of the Carboxylation Reaction Performed in DMSO or DMF



5.0×10^{-4} M. The temperature of the solution was kept constant (25 ± 1 °C) by circulation of a thermostatted water/ethanol mixture through a jacketed cell. The cell was protected from light by wrapping in an aluminum foil. Positive-feedback *iR* compensation was applied routinely. All peak potentials were measured at 0.2 V s^{-1} scan rate and reported vs Ag/AgCl,$KCl_{sat.}$. In the case of experiments performed in DMSO, the stability of the reference electrode was verified frequently by measuring the potential of the reversible ferrocene (0/1+) couple, added as an internal standard, fixed at $E^{\circ\prime}$ = +0.45 V vs SCE (i.e., +0.495 V vs Ag/AgCl,$KCl_{sat.}$).[27]

RESULTS

Synthesis of the Pt Complexes. The synthesis of the platinum(IV) complex *cis,trans,cis*-[$PtCl_2(OH)_2$(*cis*-1,4-DACH)] (**2**) was carried out by treating the corresponding Pt(II) precursor [$PtCl_2$(*cis*-1,4-DACH)] (Kiteplatin, **1**),[18,19] with H_2O_2 (30% w/w water solution) according to an already reported procedure.[23] The reaction time, however, was reduced from 1 day to 2 h. Compound **2** has been characterized by elemental analysis, NMR, IR, and ESI-MS.

The 1D ^{1}H NMR and 2D [^{1}H-^{195}Pt] HSQC spectra of **2** in DMSO-d_6 are reported in Figure 2. The singlets with Pt satellites falling at 6.33 ($^2J_{Pt-H}$= 68.6 Hz) and 2.89 ($^3J_{Pt-H}$= 94.8 Hz) ppm and having cross peaks with the platinum resonance at 964.6 ppm, are assigned to the aminic and to the methynic protons of coordinated *cis*-1,4-DACH (Ha), respectively. Two multiplets centered at 2.07 and 1.45 ppm, each one integrating for 4 protons, are assigned to the methylenic protons Hb and Hc, respectively. The Pt resonance (964.6 ppm) is in the range typical for a Pt(IV) atom in a $N_2Cl_2O_2$ coordination environment.[26,28] A similar spectrum is obtained using DMF-d_7 as solvent (Supporting Information, Figure S1). In the latter case the ^{195}Pt chemical shift falls at 871.7 ppm.

We have been unable to detect the signals of the axial hydroxido ligands, probably because of rapid exchange with the residual water present in our solvents (DMSO-d_6 and DMF-d_7). The same is true for other literature reports on the same compound.[23−25]

The second step was the carboxylation of the dihydroxido platinum(IV) complex by reaction with succinic anhydride. In accord with already reported procedures,[7,13,14] the carboxylation reaction was carried out in DMSO (Scheme 1). The obtained dicarboxylato derivative, *cis,trans,cis*-[$PtCl_2${$OC(O)CH_2CH_2C(O)OH$}$_2$(*cis*-1,4-DACH)] (**3**), was characterized by elemental analysis, NMR, IR, and ESI-MS. The ^{1}H NMR of compound **3** in DMSO-d_6 is reported in Figure 3. The singlet falling at 12.10 ppm is assigned to the proton of the free (uncomplexed) carboxylic groups. The singlets with Pt satellites falling at 8.13 ($^2J_{Pt-H}$ = 62.9 Hz) and 2.94 ($^3J_{Pt-H}$ = 82.6 Hz) ppm are assigned to the aminic and to the methynic protons of coordinated *cis*-1,4-DACH, respectively. The multiplet centered at 2.38 ppm belongs to methylenic protons of monocoordinated succinato ligands. In particular, this signal is assigned to the methylenes which are bound to the uncoordinated carboxylic groups (protons e in Figure 3). The signal of the other methylenic protons (d in Figure 3) overlaps with the residual solvent signal and was assigned with the help of a [^{1}H-^{13}C] HSQC 2D experiment (data not shown). Finally, the multiplet falling at 1.59 ppm and integrating for eight protons was assigned to the four CH_2 groups of coordinated DACH.

The ^{195}Pt NMR of **3** in DMSO-d_6 is reported in Supporting Information, Figure S2 and exhibits a broad singlet falling at 1217.0 ppm. The ^{195}Pt chemical shift is in good agreement with that already reported for similar Pt(IV) dicarboxylato derivatives.[14,23]

Carboxylation Performed in DMF, an Unexpected Result. The carboxylation reaction described in the previous paragraph was also carried out in DMF, a solvent that has already been used for the carboxylation of analogous compounds.[7,13,14] By using this latter solvent, we obtained not only the dicarboxylated Pt(IV) derivative **3**, but also the reduced Pt(II) species Kiteplatin (**1**; Scheme 1) as evidenced by the ^{1}H NMR spectrum recorded on the crude reaction product. In particular,

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Table 2. Cathodic E_p values of 0.5 mM Solutions of Complexes 2, 3, and 6 Measured at 0.2 V s^{-1} Scan Rate by Using Cyclic Voltammetry in DMSO, Containing 0.1 M $[NBu_4][PF_6]$ as Supporting Electrolyte, and in Water, Containing 0.5 mM Phosphate Buffer and 5 mM NaCl[a]

complex	$E_{p,c}$ in DMSO[b] (V)	$E_{p,c}$ in H_2O (V)
cis-$[PtCl_4$(*cis*-1,4-DACH)] (**6**)	−0.547	−0.055[b]
ctc-$[PtCl_2(OH)_2$(*cis*-1,4-DACH)] (**2**)	−1.22	−0.967[b]
ctc-$[PtCl_2\{OC(O)CH_2CH_2C(O)OH\}_2$(*cis*-1,4-DACH)] (**3**)	−1.86	−1.42[b]
ctc-$[PtCl_2(OH)(\mu\text{-}O)_{1/2}$(*cis*-1,4-DACH)$]_2$ (**5**)	−1.25	−0.975
cis-$[PtCl_4(NH_3)_2]$		−0.204[c]
ctc-$[PtCl_2(OH)_2(NH_3)_2]$		−0.815[c]
ctc-$[PtCl_2\{OC(O)CH_2CH_2C(O)OH\}_2(NH_3)_2]$		−0.892[c]

[a]Working electrode: glassy carbon; *E* vs. $Ag/AgCl,KCl_{sat.}$. [b]This work. [c]From ref 9.

a signal at 4.9 ppm with broad Pt satellites was unambiguously assigned to the NH_2 protons of coordinated *cis*-1,4-DACH in the Pt(II) precursor complex **1** (data not shown).

We monitored the reaction course by NMR spectroscopy using DMF-d_7 as solvent and similar experimental conditions (70 °C in the dark). The ^{1}H NMR spectrum revealed the presence of the signals of the reduced product **1** already after 24 h reaction time.

To exclude the involvement of the anhydride in the reduction process, the dihydroxido complex **2** was dissolved in DMF and kept in the dark at 70 °C for one day. The spectrum in DMSO-d_6 of the residue obtained by evaporation to dryness of the above solution shows unambiguously the presence of signals belonging to **1** (data not shown).

In contrast with what has been observed in DMF, the carboxylation reaction performed in analogous conditions (dark, 70 °C) but using DMSO as solvent, does not yield the reduction product **1**.

Investigation of the Mechanism Underlying the Reduction Reaction. A series of experiments was performed to investigate the influence of light, temperature, and solvent on the reduction of complex **2** to complex **1** in the absence of an added reducing agent. Table 1 summarizes the obtained results. Analogous reactions were performed on *cis,trans,cis*-$[PtCl_2(OH)_2$(*R,R*-1,2-DACH)] which contains the isomeric form of DACH present in oxaliplatin; no reduction was observed. Therefore the reduction appears to be a characteristic feature of the derivatives containing *cis*-1,4-DACH, and this also explains why no reduction has been observed by other groups who have carried out the synthesis of *axial* disuccinato Pt(IV) complexes starting from the dihydroxido Pt(IV) derivatives of cisplatin, oxaliplatin, and $[PtCl_2$(en)] (en = ethylendiamine).[7,13]

The influence of the axial ligands was investigated by performing analogous reactions starting from $[PtCl_4$(*cis*-1,4-DACH)] (**6**). Under the same experimental conditions used in the case of **2** and summarized in Table 1, no reduction of **6** to **1** was observed. This finding appears to be in contrast with the electrochemical data reported in the literature, showing that Pt(IV) complexes having two axial chlorido ligands have more positive reduction potentials than those having axial hydroxido ligands and therefore should be more prone to undergo reduction.

Reduction Potentials. We measured the reduction potentials $E_{p,c}$ of the Pt(IV) complexes with *cis*-1,4-DACH investigated in this work (namely: *trans*-dihydroxido, **2**; *trans*-disuccinato, **3**; and *trans*-dichlorido, **6**) by cyclic voltammetry (CV), and the results are reported in Table 2. In phosphate buffer, all complexes showed the usual Pt(IV)-electrochemical behavior, with a chemically irreversible $2e^-$ reduction (E_rC mechanism). The irreversibility is due to the detachment of the two axial ligands upon reduction of the octahedral Pt(IV) complex to the square-planar Pt(II) species. The trend of $E_{p,c}$ values is in accord with that observed for other Pt(IV) complexes. In particular, the *trans*-dichlorido complex **6** has the highest $E_{p,c}$ value, the *trans*-dihydroxido derivative **2** has the second highest value, and the *trans*-disuccinato derivative **3** has the less favorable reduction potential. There are several reports indicating that reduction is most easy for axial chlorido complexes, is intermediate for acetato, and is least favorable for hydroxido species,[5,29] with reduction rates generally correlating with reduction potentials.[30] However, on passing from acetato to succinato, the $E_{p,c}$ decreases and becomes the most negative. Therefore, the data reported in Table 2 for the *cis*-1,4-DACH complexes parallel those reported for the cisplatin derivatives *cis,trans,cis*-$[PtCl_2X_2(NH_3)_2]$ (X = Cl, OH, or succinato)[9] (also reported in Table 2).

In pure organic solvent (DMSO) all peak potentials are shifted to lower values with respect to those obtained in water, but the trend is maintained ($E_{p,c}$ **3** < **2** < **6**).

Since we observed the reduction by the solvent only starting from complex **2** and not from complex **6**, this means that factors other than reduction potential must be at play.

Monitoring of the Reduction Reaction by NMR in DMF-d_7. To gain more information about the reduction reaction, the progress of the reaction of **2** in DMF-d_7 (performed at 50 °C in the dark) was monitored by ^{1}H NMR spectroscopy (Figure 4). The spectrum of the initial yellow solution (Figure 4a) showed only the peaks of the starting substrate (marked with ▲ in Figure 4). After 4 h the solution turned to dark yellow and the ^{1}H NMR spectrum (Figure 4b) showed additional signals. Two broad pseudotriplets falling at 7.43 and 6.95 ppm (♦) are assigned to a Pt(IV) species having unequivalent NH_2 protons, possibly because of the presence of different ligands in the axial positions; formation of a *cis,trans,cis*-$[PtCl_2(OH)$(solvent)(*cis*-1,4-DACH)$]^+$ species (**4a** in Scheme 2), by solvolysis of one axial hydroxido ligand, could be the answer.[31] Our attempts to isolate this intermediate species have been so far unsuccessful, but evidence in favor of the hypothesized Pt(IV) complex with unsymmetrical axial ligands came from the $[^1H\text{-}^{195}Pt]$-HSQC 2D spectrum (Figure 5) showing a correlation between the aminic signals falling at 7.43 and 6.95 ppm and a Pt atom having a ^{195}Pt chemical shift of 925.5 ppm. These two cross-peaks indicate that the two aminic signals belong to the same compound. The same spectrum shows also equivalence of the two methylenic protons of 1,4-DACH indicating that they have the same *cis* ligands (chlorides). Furthermore, the ^{195}Pt chemical shift is in agreement with a Pt^{IV} cation in a $N_2Cl_2O_2$ coordination environment and the electrospray ionization mass spectrometry (ESI-MS) spectrum, recorded on the NMR sample, shows a peak at m/z = 476 corresponding to $[PtCl_2(OD)$(DMF-d_7)(*cis*-1,4-DACH)$]^+$.

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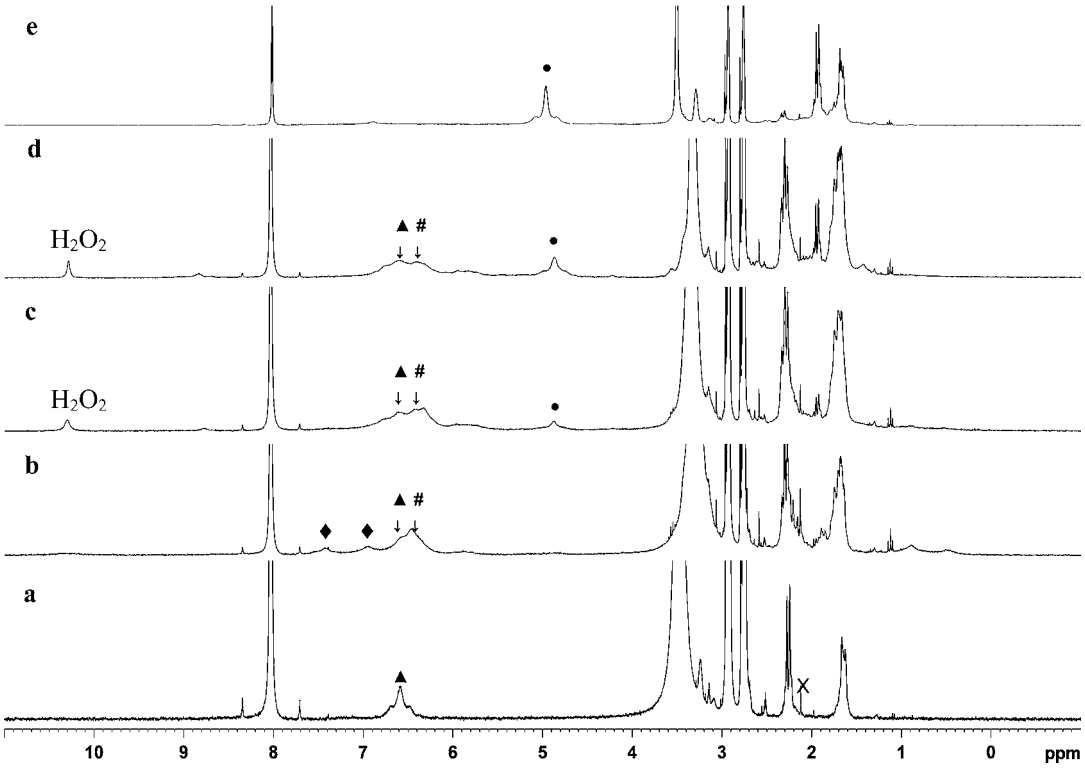
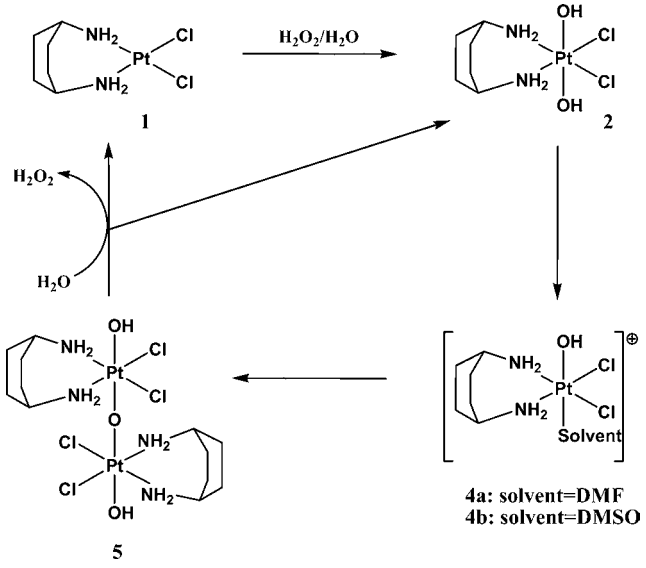


Figure 4. ^{1}H NMR monitoring of the reduction of **2** in DMF-d$_7$ performed at 50 °C in the dark after (a) 0 h; (b) 4 h; (c) 12 h;(d) 1 day; (e) 5 days. The residual methyl protons of DMF-d$_7$ show satellites due to coupling with ^{13}C, $J_{H,13C}$ ca. 140 Hz and × indicates a minor impurity in the sample.

Scheme 2. Oxidation and Reduction Processes for Complexes Bearing *cis*-1,4-DACH Ligand



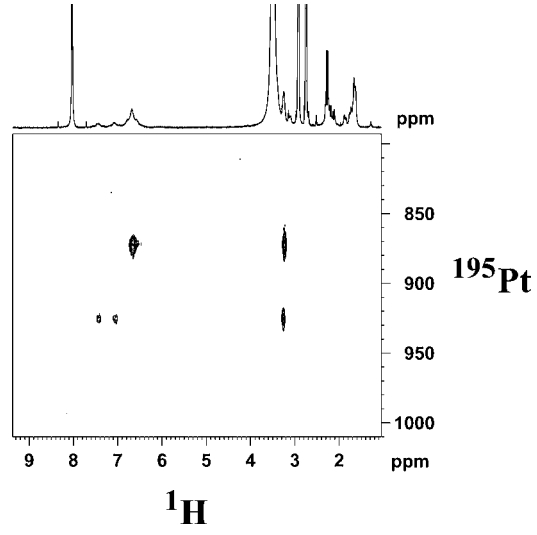


Figure 5. [^{1}H-^{195}Pt]-HSQC 2D spectrum of a mixture of **2** and **4a** in DMF-d$_7$.

Another signal, in the region of the aminic protons (6.42 ppm, # in Figure 4b), indicates the presence of another platinum complex. This species has been shown to be the dinuclear complex **5** that has also been synthesized independently (see following discussion). After 12 h, a signal at 4.90 ppm, which belongs to the aminic protons of the platinum(II) species **1** (● in Figure 4c), and a broad signal at 10.4 ppm, belonging to H_2O_2, are well evident.[32] By this time the aminic signals of the solvato species **4a** (◆ in Figure 4b) have disappeared completely. After 1 day, the solution turned light brown and the signals of complex **1** (●) increased in intensity (Figure 4d). After 5 days, the only species present in solution was the reduced complex **1** (Figure 4e); the absence of the H_2O_2 signal is in accord with its complete disproportionation into O_2 and H_2O fostered by the high temperature and the long waiting time. This experiment demonstrates that (*i*) the reduction of **2** takes place in DMF in the dark already at 50 °C; (*ii*) the reduction mechanism involves intermediate species.

To investigate the role of the temperature in the reduction process, we performed the reaction in the dark at room temperature in DMF-d$_7$: no reduction was observed. Probably, under these experimental conditions, H_2O_2 is not subtracted to the equilibrium or, alternatively, the formation of the intermediate species is not favored.

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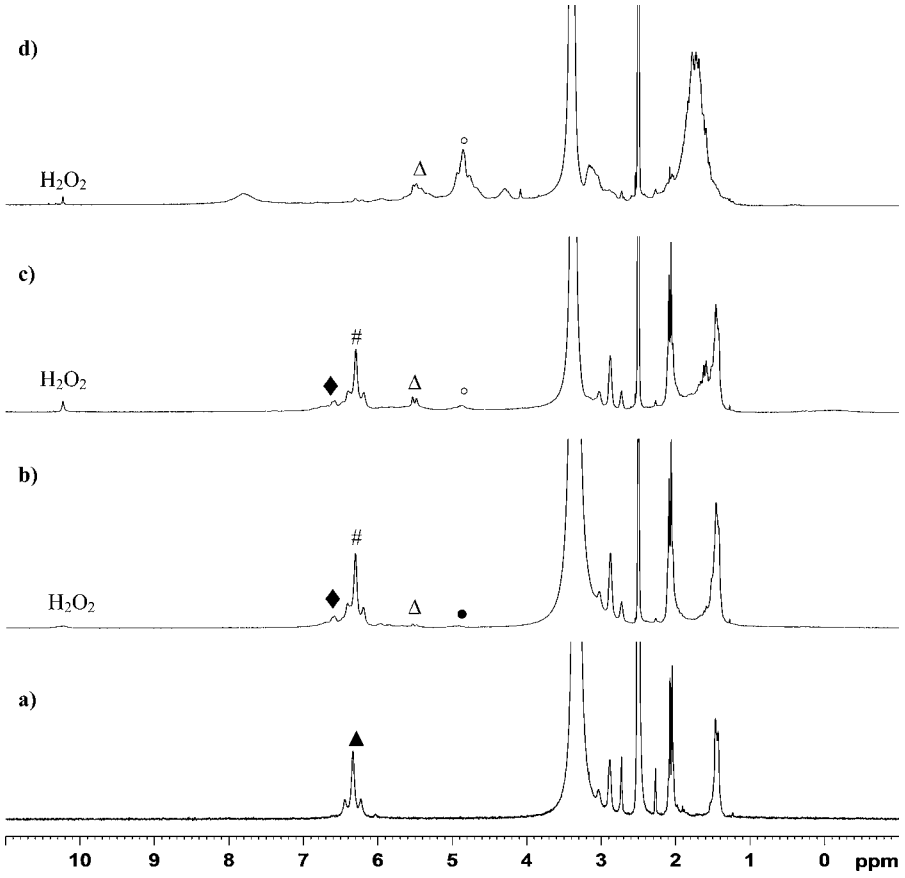

Figure 6. ^{1}H NMR spectra of **2** in DMSO-d_6 exposed to the artificial light at room temperature at zero time (a) and after 3 h (b), 8 h (c), and 4.5 days (d). The solvent peak at 2.50 ppm shows satellites due to coupling of residual methyl protons of DMSO-d_6 with ^{13}C, $J_{H,13C}$ = 137.4 Hz.

Next we investigated the influence of light. The DMF-d_7 solution of **2** was kept at room temperature in artificial light. A reduction of the starting complex **2**, with a trend analogous to that observed in the reaction performed at 50 °C in the dark, was observed (Supporting Information, Figure S3). The formation of H_2O_2 in the reduction process[32] was proven by addition of ethylgallate which acts as a H_2O_2 scavenger; a decrease in the intensity of the signal at 10.4 ppm, assigned to H_2O_2, was observed (Supporting Information, Figure S3).

Monitoring of the Reduction Reaction by NMR in DMSO-d_6. We wanted to see if light could induce reduction of complex **2** in DMSO-d_6 solution. The reaction was monitored by ^{1}H NMR spectroscopy for 4 days, and the results are shown in Figure 6. Starting from a pale yellow solution of **2** in DMSO-d_6 (▲ in Figure 6a), after 3 h exposure to the sunlight the solution turned dark yellow and the ^{1}H NMR spectrum (Figure 6b) showed the appearance of a resonance at 10.4 ppm, assigned to H_2O_2, and a resonance at 6.28 ppm (#), assigned to the aminic protons of the dinuclear Pt(IV) species **5**, which is slightly upfield with respect to the signal of the aminic protons in **2** ($\Delta\delta$ = 0.05 ppm) (see following discussion). Another signal at 6.59 ppm (♦) can be assigned to two of the four aminic protons of the solvato species *cis,trans,cis*-[$PtCl_2$(OH)(DMSO)(*cis*-1,4-DACH)]$^+$ (**4b**). Although this latter species could not be isolated, it was possible to confirm its formation by [^{1}H-^{195}Pt]-HSQC 2D and ESI-MS spectrometry. The [^{1}H-^{195}Pt] HSQC 2D spectrum (Figure 7) showed two (^{1}H-^{195}Pt) cross peaks falling at 6.59/482 and 6.35/482 ppm that can be assigned to the aminic protons of a DACH Pt(IV)-complex with unsymmetrical axial ligands. Another cross peak at 2.84/482 ppm can be assigned to the methynic protons of the DACH ligand in the same complex; their equivalence indicates that solvolysis does not involve the *cis* chlorides. Moreover, the ^{195}Pt chemical shift, which is at lower field with respect to that found for the solvato species containing DMF (complex **4a**, 925.5 ppm), can be taken as an indication that in **4b** the solvent molecule is *S*-coordinated to the metal. Finally, the ESI-MS spectrum recorded on the NMR sample showed the presence of a peak at m/z = 481 corresponding to [$PtCl_2$(OD)(DMSO-d_6)(*cis*-1,4-DACH)]$^+$. The formation of the reduced species was proven by the presence of a signal at 4.9 ppm which is characteristic of the aminic protons of **1** (● in Figure 6b). After 8 h, the solution became dark brown, and the signals of the starting Pt(IV) complex (**2**) almost disappeared. In DMSO, a good coordinating solvent, complex **1** also underwent a solvation process as evidenced by the presence of signals at 5.3 and 4.9 ppm, the latter overlapping with the aminic protons of **1** (Δ and ○ in Figure 6c) assigned to the non equivalent aminic protons of the Pt(II) solvato species *cis*-[PtCl(DMSO)(*cis*-1,4-DACH)]$^+$. A proof in support of the latter assignment came from the [^{1}H-^{195}Pt] HSQC 2D spectrum showing cross peaks in the region 5.3/−3100 and 4.9/−3100 ppm (^{1}H/^{195}Pt; data not shown). The observed value of the ^{195}Pt chemical shift is comparable to literature data reported for *cis*-[PtCl(DMSO)(*cis*-1,4-DACH)]$^+$ species.[33,34]

As already observed in DMF-d_7 (Supporting Information, Figure S3), also the reduction of **2** in DMSO-d_6, carried on at room temperature and in the presence of artificial light, is accompanied by the release of H_2O_2[35] (experiment with ethylgallate, Supporting Information, Figure S4). Therefore, in both solvents the reduction

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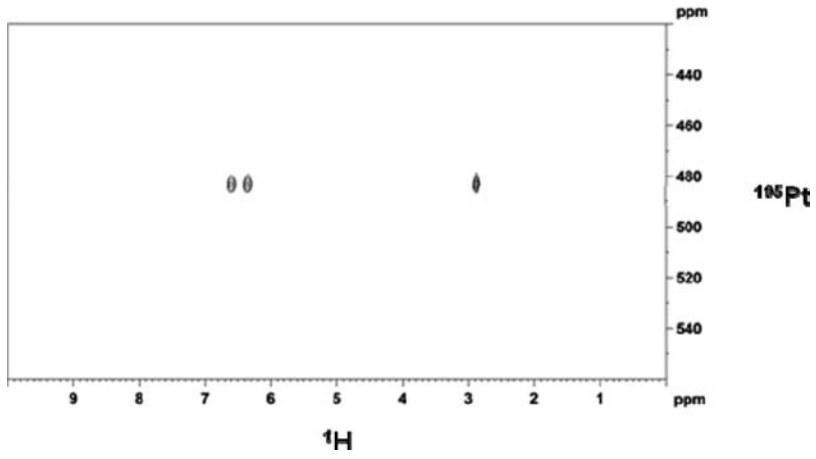

Figure 7. [^{1}H-^{195}Pt] HSQC spectrum of **4b** in DMSO-d$_6$.

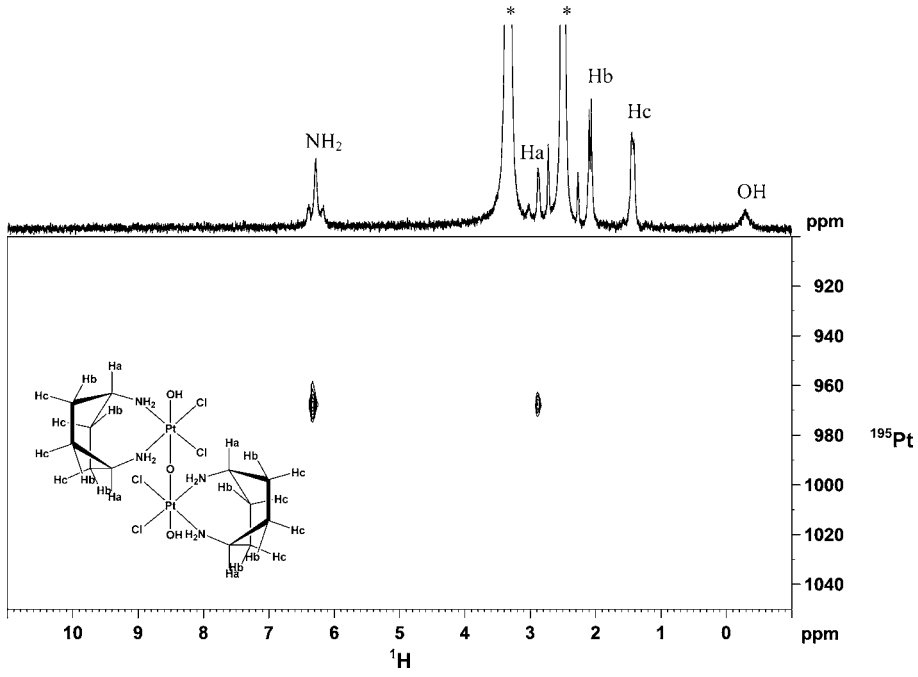

Figure 8. ^{1}H NMR (top) and [^{1}H-^{195}Pt] HSQC 2D (bottom) spectra of **5** in DMSO-d$_6$, the asterisks indicate residual solvent peaks. The solvent peak at 2.50 ppm shows satellites due to coupling of residual methyl protons of DMSO-d$_6$ with ^{13}C, $J_{H,13C}$ = 137.4 Hz.

takes place through a similar mechanism and leads to the same final products.

Synthesis and Reduction of 5 Performed in DMF and DMSO. The second intermediate observed in the reduction of complex **2** in DMF (dinuclear complex **5**) was isolated while attempting to isolate the solvato-species **4a**. **2** was dissolved in the minimum amount of water and treated with DMF. After a while, the temperature was lowered from 50 °C to room temperature causing the precipitation of a compound which proved to be a dinuclear complex (**5**) as evidenced by elemental analysis, ESI-MS, and NMR spectroscopy.

The ESI-MS spectrum of compound **5** exhibits a peak at m/z 808.5 which corresponds to a dinuclear Pt(IV) species having formula $C_{12}H_{29}Cl_4O_3N_4Pt_2$. The ^{1}H NMR spectrum (Figure 8) in DMSO-d$_6$ shows two singlets with Pt satellites at 6.29 ($^{2}J_{Pt-H}$ = 65.0 Hz) and 2.89 ppm ($^{3}J_{Pt-H}$ = 83.3 Hz) assigned to the aminic protons and to the methynic protons of DACH (Ha), respectively. Two multiplets centered at 2.09 and 1.44 ppm can be assigned to the methylenic protons Hb and Hc of DACH, respectively. Finally, the broad signal at −0.25 ppm can be assigned to an hydroxido ligand in axial position. The ^{1}H NMR spectrum of compound **5**, taken in DMF-d$_7$, is reported in Supporting Information, Figure S5.

The [^{1}H-^{195}Pt]-NMR HSQC spectrum of **5** in DMSO-d$_6$ is also reported in Figure 8. It shows two cross peaks correlating the aminic and methynic protons of DACH with a Pt atom falling at 970.7 ppm. The ^{195}Pt chemical shift is in the range typical for a $Cl_2N_2O_2$ coordination environment.

A DMF-d$_7$ solution of **5**, kept in the dark at room temperature, did not show signs of reduction. However, if the sample was warmed to 50 °C, in the dark, reduction to **1** took place (Supporting Information, Figure S6). Analogous reduction took place at room temperature if the sample of **5** in DMF-d$_7$ was exposed to the sun light (Supporting Information, Figure S7).

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Also a DMSO-d_6 solution of **5**, kept at room temperature in the dark, was stable. In contrast, if it was exposed to artificial light reduction took place (Supporting Information, Figure S8).

Our interpretation of the experimental data is that the intermediate dinuclear complex **5** is a key intermediate for the reduction of Pt(IV) to Pt(II), and this takes place with the concomitant formation of H_2O_2.

Unlike complex **2**, complex **5**, dissolved in DMSO-d_6 and kept at 70 °C in the dark, undergoes reduction to complex **1** (Supporting Information, Figure S9), confirming that complex **5** is the key species in the redox process. Moreover, in both solvents **5** did not appear to undergo solvolysis with formation of solvato-species (**4a**,**b**) indicating that, most probably, the solvato-species precedes rather than follows the formation of **5**. Furthermore, the solvato-species (**4a** and **4b**) appear to be crucial for the formation of **5**. While in DMF the formation of **4a** is both light and heat-driven; in contrast, in DMSO the formation of **4b** is only light-driven.

The observation that in DMSO (70 °C, dark) the starting complex **2** is not reduced but **5** is, could mean that complex **5** has an $E_{p,c}$ higher than that of **2** and hence is more easily reduced.

We measured the reduction potential $E_{p,c}$ of complex **5** by cyclic voltammetry and found that the dimetallic complex **5** behaves exactly as its congener **2**, albeit a very small cathodic shift in the value of $E_{p,c}$ is observed ($E_{p,c}$ = −1.25 and −1.22 V for **5** and **2** in DMSO, respectively; Table 2). At equimolar concentration, **5** shows, as expected, a peak current $i_{p,c}$ twice as much as that of **2** (**5** contains two Pt units per molecule), and the situation does not change when maintained in solution for prolonged time. When the CV of **5** was performed in pure water, or when water was added to the DMSO solution, the reduction peak shifted to −0.975 V, a value very similar to that of **2** (−0.967 V; Table 2).

DISCUSSION

Various examples of reductive elimination of H_2O_2 from metal complexes have been reported in the literature. For instance: thermal elimination of hydrogen peroxide from dihydroxytellluranes,[36] photoelimination of H_2O_2 from $Pb^{IV}(OH)_6^{2-}$ with formation of $Pb^{II}(OH)_4^{2-}$,[37] and photoelimination of H_2O_2 from $Tp^*Cu^{II}(\mu{-}OH)_2Cu^{II}Tp^*$ and formation of $Cu^{I}Tp^*$ (Tp* = hydrotris(3,5-dimethyl-1-pyrazolyl)borate).[38]

A recent paper from Milstein and co-workers[39,40] has reported an artificial catalyst for the sunlight-induced splitting of water into O_2 and H_2 with important fallout in the field of renewable energy. The catalyst, the mononuclear ruthenium complex *cis*-$[Ru(OH)_2(PNN)(CO)]$ (PNN stands for the tridentate ligand 2-(di-*tert*-butylphosphino-methyl)-6-diethylaminomethyl)-pyridine), is capable of leading to the stoichiometric liberation of O_2 and H_2 in consecutive thermal- and light-driven steps. In particular, the *cis*-dihydroxido Ru complex was proposed, after irradiation, to liberate H_2O_2 in a reductive elimination step. H_2O_2 would then catalytically disproportionate into O_2 and H_2O, a process which is well-known to be catalyzed by metal complexes. However, only indirect evidence for the formation of H_2O_2 was given. In a theoretical investigation, Fang and co-workers[41] proposed a mechanism for the direct formation of O_2 (without intermediate formation of H_2O_2) involving the formation of a dimeric aggregate of the *cis*-dihydroxido Ru complex.

Another important historical catalyst, capable to oxidize water to O_2, is the μ-oxo blue dimer discovered by Meyer.[42,43] Similarly to complex **5**, Meyer's blue dimer is a μ-oxo species, *cis*-$[(bpy)_2(H_2O)Ru^{III}ORu^{III}(OH_2)(bpy)_2]^{4+}$ (bpy = 2,2′-bipyridine). The mechanism by which Meyer's blue dimer oxidizes water to oxygen has not yet been fully clarified. A possibility is an initial four electron oxidation from Ru^{III}-O-Ru^{III} to Ru^{V}-O-Ru^{V} followed by water oxidation (via a μ-oxo-μ-peroxo intermediate) and regeneration of the blue dimer.

Also Pt(IV) complexes can undergo photoreductive elimination. Unlike the Pt(IV) compound *trans,cis,cis*-$[Pt(OH)_2(MeNH_2)_2(bpy)]^{2+}$, which does not undergo photoreduction to a Pt(II) species in D_2O,[44] the related complexes *mer*-$[PtCl_3(MeNH_2)(bpy)]^{+}$ and *trans,cis,cis*-$[PtCl_2(MeNH_2)_2(bpy)]^{2+}$ undergo reductive elimination of Cl_2 or hypochlorous acid (HOCl) upon irradiation. These results are in line with the more negative reduction potential of the *trans*-dihydroxido Pt(IV) species with respect to the *trans*-dichlorido derivatives.

In the light of the above observation, the formation of a dimeric μ-oxo compound such as **5** could be crucial for reduction of a Pt(IV) *trans*-dihydroxido complex with release of hydrogen peroxide. On this basis we propose for the spontaneous reduction of our compound **2** the mechanism summarized in Scheme 2. A key intermediate, which appears to be involved in the reductive-elimination step, is the μ-oxo dinuclear complex **5**, which has been isolated and fully characterized. Its formation probably requires the preliminary solvation of **2** (displacement of an axial hydroxido ligand by a solvent molecule with formation of **4**). The solvation step, which is only the first step in the overall process leading to reduction with simultaneous elimination of hydrogen peroxide, depends upon the solvent used. It is both light- and heat-driven in DMF while it is only light-driven in DMSO.

The solvato species **4** would then react with **2** forming the dinuclear Pt(IV) complex with an oxo bridge **5**. Complex **5** would spontaneously undergo reduction to a 1:1 mixture of **1** and **2** with simultaneous elimination of oxygen (1/2 O_2 in the form of H_2O_2). Our current hypothesis is that a water molecule could give a nucleophilic attack on an axial hydroxido ligand which leaves a lone-pair of electrons on platinum. This would lead, in one step, to the formation of hydrogen peroxide, compound **2**, and compound **1**. This would correspond to an "inner-sphere" redox mechanism fostered by the presence of the dinuclear μ-oxo species. The reason why such a mechanism would occur in the case of **5**, but not in the case of **2**, could be the simultaneous formation of 1 equiv of **1** and 1 equiv of **2** in the case of **5** as opposed to the formation of only 1 equiv of **1** in the case of **2**.

According to the mechanism outlined in Scheme 2, starting from pure **5**, the first redox process would lead to 1 equiv of **1** and 1 equiv of **2**. Therefore, performing the reaction in DMSO in the dark at high temperature (conditions in which pure **2** does not undergo reduction to **1**), one should expect to end up with an equimolar mixture of **1** and **2**. However, this is not the case and complete reduction of **5** to **1** is observed. Our explanation of this apparently contradictory result is that **1** can catalyze the solvolysis of **2** to **4** and hence the formation of new **5** which can then undergo reduction. The process would go on until the reduction of **2** to **1** is complete. The platinum(II) catalyzed ligand substitution to a Pt(IV) complex is a long known process.[45−47] To check this hypothesis, we added a catalytic amount of **1** to a solution of **2** in DMSO kept in the dark at 70 °C. Compound **2**, which was stable before the addition of **1**, started to undergo reduction which ended up with the complete conversion of **2** to **1**, fully confirming our hypothesis.

CONCLUSION

In this work we have reported an improved procedure for the synthesis of the *cis,trans,cis*-$[PtCl_2(OH)_2(cis\text{-}1,4\text{-DACH})]$

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complex (**2**) and its conversion to the disuccinato complex *cis,trans,cis*-[$PtCl_2${$OC(O)CH_2CH_2C(O)OH$}$_2$(*cis*-1,4-DACH)] (**3**). The presence of two uncoordinated carboxylic groups in the axial positions could allow further functionalization with bioactive molecules.

The serendipitous discovery of a reduction process involving compound **2**, while preparing complex **3**, prompted us to investigate the conditions for such a reaction to occur. It was found that solvolysis of **2**, and formation of a μ-oxo dinuclear species (**5**), is the key step. The dinuclear species can then undergo reduction to 1 equiv of **1** and 1 equiv of **2** with concomitant oxidation of residual water present in the solvent to hydrogen peroxide, as outlined in Scheme 2. The whole process is fostered by heat and/or light, which could favor solvolysis of **2** as well as decomposition of hydrogen peroxide to water and oxygen so preventing the reoxidation of **1** to **2**. The overall process would correspond to a reductive elimination of oxygen (1/2 O_2 in the form of H_2O_2) from a *trans* dihydroxido Pt(IV) complex.

The reduction observed in the present investigation has no precedents in the literature concerning the carboxylation of axial *trans* dihydroxido Pt(IV) complexes derived from clinically used cisplatin or oxaliplatin. Therefore it could be ascribed (but this needs to be proved) to the presence of the *cis*-1,4-DACH ligand.

We believe that complex **5**, with its peculiar behavior, could be further exploited for the development of a technology for water splitting. Moreover, the results of this investigation could serve also as guidelines in planning the carboxylation and other reactions involving complexes of type **2**. Finally this work highlights the role of the solvent in possible side reactions.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures S1−S9: ^{1}H NMR spectra recorded on compounds **2** and **5**, under different experimental conditions, with the intent of unravel their reduction mechanism. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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