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A New Entry to Asymmetric Platinum(IV) Complexes via Oxidative Chlorination

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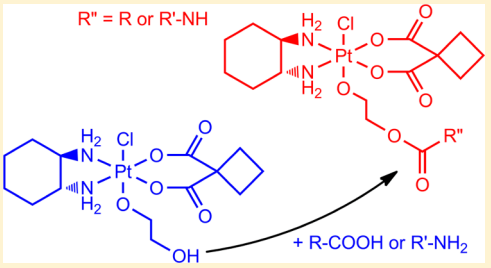
Supporting Information

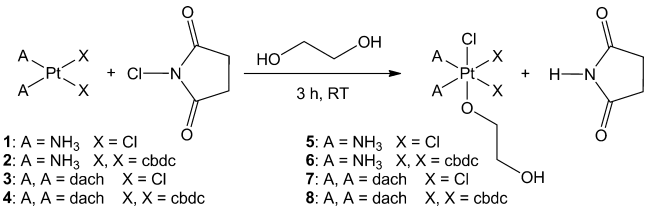
ABSTRACT: Pt(IV) complexes are usually prepared by oxidation of the corresponding Pt(II) counterparts, typically using hydrogen peroxide or chlorine. A different way to synthesize asymmetrical Pt(IV) compounds is the oxidative chlorination of Pt(II) counterparts with *N*-chlorosuccinimide. The reaction between cisplatin *cis*-[$PtCl_2(NH_3)_2$], carboplatin, *cis*-[$PtCl_2$(dach)] and *cis*-[Pt(cbdc)(dach)] (cbdc = cyclobutane-1,1′-dicarboxylato; dach = cyclohexane-1*R*,2*R*-diamine) with *N*-chlorosuccinimide in ethane-1,2-diol was optimized to produce the asymmetric Pt(IV) octahedral complexes [PtA_2Cl(glyc)X_2] (A_2 = 2 NH_3 or dach; glyc = 2-hydroxyethanolato; X_2 = 2 Cl or cbdc) in high yield and purity. The X-ray crystal structure of the [Pt(cbdc)Cl(dach)(glyc)] complex is also reported. Moreover, the oxidation method proved to be versatile enough to produce other mixed Pt(IV) derivatives varying the reaction medium. The two trichlorido complexes easily undergo a pH-dependent hydrolysis reaction, whereas the dicarboxylato compounds are stable enough to allow further coupling reactions for drug targeting and delivery via the glyc reactive pendant. Therefore, the coupling reaction between the [Pt(cbdc)Cl(dach)(glyc)] and a model carboxylic acid, a model amine, and selectively protected amino acids is reported.



INTRODUCTION

Platinum-based drugs, and in particular the prototype cisplatin (i.e., *cis*-diamminedichloridoplatinum(II), **1**, Scheme 1), are used for the treatment of a wide array of solid tumors, including bladder, cervical, ovarian, testicular, colorectal, nonsmall cell lung cancers, squamous cell carcinoma of the head and neck, and malignant mesothelioma. Despite encouraging initial responses, treatment with platinum-based drugs may result in the development of chemoresistance, leading to therapeutic failure. Moreover, the nonspecific mechanism of action of Pt drugs leads to severe side-effects, such as nausea, bone marrow suppression, and kidney toxicity.[1]

Scheme 1. Synthesis of Complexes 5−8 through Oxidative Chlorination of the Corresponding Pt(II) Complexes 1−4 with *N*-Chlorosuccinimide in Ethane-1,2-diol[a]



1: A = NH_3 X = Cl
2: A = NH_3 X, X = cbdc
3: A, A = dach X = Cl
4: A, A = dach X, X = cbdc

5: A = NH_3 X = Cl
6: A = NH_3 X, X = cbdc
7: A, A = dach X = Cl
8: A, A = dach X, X = cbdc

[a]dach = cyclohexane-1*R*,2*R*-diamine, cbdc = cyclobutane-1,1′-dicarboxylato.

The drug targeting and delivery (DTD) approach has been developed in an attempt to reduce chemotherapy-related systemic side effects by using vectors that selectively deliver the cytotoxic agent to tumor cells, thus sparing healthy cells. These vectors include bioactive substances, such as nutrients, that easily enter highly metabolically active tumor cells, or other molecules that are selectively conveyed by receptors/transporters often over/expressed in cancer cells (active targeting). Alternatively, macromolecular vectors, exploiting the so-called enhanced permeability and retention (EPR) effect (i.e., the increased vascular permeability and the inefficient drainage of macromolecules by the lymphatic system in the tumor tissue), can be used (passive targeting). In the case of Pt(II)-drugs, the bioactive or macromolecular carrier must contain a coordinating arm capable of binding the cytotoxic Pt(II) core, acting either as carrier or leaving group. In both cases, the Pt-vector conjugate should be promptly cleaved to generate the active species. The stability of the conjugate versus the release of active drug from the vector is crucial for fine-tuning of the overall cytotoxic properties.[2]

In search for a compromise between stability in blood circuit and release of active Pt(II) metabolites in tumor tissue, octahedral Pt(IV) compounds are extensively studied. Platinum(IV) complexes undergo ligand substitution reactions

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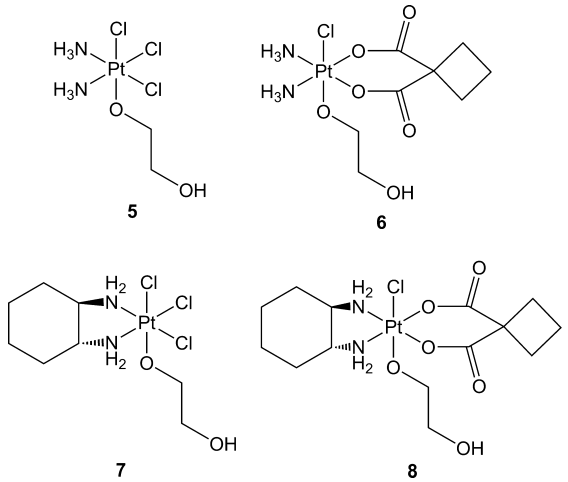
much more slowly than their Pt(II) counterparts, and are therefore considered as prodrugs: the Pt(IV) species can be activated by reduction to their cytotoxic Pt(II) metabolites in the hypoxic (reducing) tumor milieu.[3] Rational choice of axial ligands modulates lipophilicity (and thus ability to enter the tumor cells by passive diffusion) and redox properties (ability to be reduced in tumor tissues).[4,5] Moreover, axial ligands also represent a possible link for biovectors able to improve the selectivity toward tumor, without altering the overall activity since these modified ligands are released upon reduction and generation of the active Pt(II) metabolite.[6]

So far, few Pt(IV)-conjugates containing an active moiety specifically designed for targeting tumor cell have been reported in the literature.[7−11] In the field of passive DTD, both polymeric and inorganic nanoparticles (NPs) have been used to increase the therapeutic index of the corresponding Pt(IV) conjugates.[2,6,12,13]

Succinic acid is the most used ligand for this purpose: one carboxylic group is axially linked to the Pt atom, while the second is available for further reactions with the designed biovector, through amide or ester bond. However, the disuccinato Pt(IV) complexes so far employed react with one or two biovectors, often giving mixtures of molecules difficult to be separated and/or large aggregates of uncontrolled dimensions in the case of reaction with NPs. Thus, monofunctional (asymmetric) Pt(IV) derivatives are highly desired for such a purpose.[14,15]

In this search, we came across a French patent (Applicant: Sanofi Aventis) claiming the synthesis of complexes having a diaminodichlorido or a diaminodicarboxylato square-planar arrangement and two different axial ligands to complete the octahedral scaffold (i.e., a halogen atom and a glycole residue, Chart 1 and Scheme 1) through the oxidative chlorination of

Chart 1. Sketch of the Asymmetric Pt(IV) Complexes 5−8 Containing a Chlorido and an Ethane-1,2-diol Moiety as Axial Ligand



the Pt(II) counterparts with *N*-chlorosuccinimide (NCS).[16] The pendant OH group was then reacted with carboxylic group of a bile or polyunsaturated acid for DTD. An intermediate of general formula $[PtA_2Cl(glyc)X_2]$ (A_2 = 2 NH_3 or dach = cyclohexane-1*R*,2*R*-diamine; glyc =2-hydroxyethanolato; X_2 = 2 Cl or cbdc = cyclobutane-1,1′-dicarboxylato) is particularly interesting for many reasons: (i) it contains a single reactive group (the terminal hydroxyl moiety of glyc), thus preventing the formation of mixtures or large aggregates in the conjugation with biovectors or NPs; (ii) the presence of an axial chlorido makes less negative the reduction potential ("easier" reduction from a thermodynamic point of view) and increases the reduction kinetics.[3,17,18] The reduction Pt(IV) → Pt(II) is totally irreversible likely because slow heterogeneous reduction kinetics and ligand dissociation on passing from octahedral to square-planar geometry. Gibson et al. found that the expected correlation between the electrochemical reduction potentials and rates of reduction of Pt(IV) complexes is sometimes not observed.[19] Trans chlorido ligands can promote an inner sphere pathway for electron transfer, which is much faster than the outer sphere electron transfer generally occurring for the reduction of carboxylato complexes.[20,21]

Surprisingly, to the best of our knowledge, the French patent and the resulting compounds were not cited or used further. To deeply study this reaction and to find promising asymmetric Pt(IV) prodrug candidates, a series of Pt(IV) compounds with cisplatin-like (i.e., $Pt(amine)_2Cl_2$ or carboplatin-like (i.e., $Pt(amine)_2$(dicarboxylato) equatorial arrangement and two different axial ligands (**5**−**15**, Schemes 1−3) were synthesized, characterized, and reported on in the present work.

RESULTS AND DISCUSSION

Synthesis of the Asymmetric Pt(IV) Complexes Containing Ethane-1,2-diol. The Pt(IV) chemistry has been largely based on chlorido, hydroxido, and carboxylato axial ligands[22] via oxidation of predesigned Pt(II) compound with chlorine,[23−25] or hydrogen peroxide.[26] The axial hydroxido Pt(IV) complexes may be further carboxylated with anhydrides[24] or acyl/aryl chlorides.[27]

On the contrary, Pt(IV) complexes **5**−**8** were synthesized through the oxidative chlorination of the corresponding Pt(II) complexes **1**−**4** with *N*-chlorosuccinimide (NCS), a source of "positive chlorine," in ethane-1,2-diol (Scheme 1). This is the first example of use of NCS to oxidize Pt(II) coordination compounds: the only precedent application of such a method concerned the cyclometalated, organometallic $Pt^{II}(phpy)_2$ (phpy = 2-phenylpyridine).[28]

Oxidative addition on Pt(II) complexes is generally assisted by the solvent employed, that is coordinated as second axial ligand. For instance, in the case of oxidation with H_2O_2 in water, one of the two OH groups entering the molecule comes from hydrogen peroxide while the second derives from H_2O.[29] The involvement of the solvent in the oxidation mechanism was also demonstrated in the reaction of Pt(II) complexes with dihalides (X_2).[30,31] In the actual case, being the reaction performed in ethane-1,2-diol, this solvent will be inserted in the coordination sphere, as in the case of the oxidation of (2,2-dimethyl-1,3-propanediamine)(9-fluorenylidenemalonato)-platinum(II) with hydrogen peroxide in glycols.[32] The proposed procedure gives the asymmetric Pt(IV) compounds **5**−**8** (easy to be purified from reaction mixture) in a one-pot synthesis and in high yields (>70−80%, Scheme 1).

It is worth noting that the same synthesis can be used to introduce into the Pt(IV) scaffold a different axial ligand, along with the chlorido. In fact, if the reaction is performed in a coordinating solvent such as water or acetic acid, the corresponding hydroxido- (**9**) or acetato-complex (**10**) is obtained (Scheme 2).

Moreover, when the chlorination of a given Pt(II) complex is carried out in the presence of a nucleophile dissolved in a noncoordinating solvent, the axially asymmetric *trans*-[(Cl)Pt-

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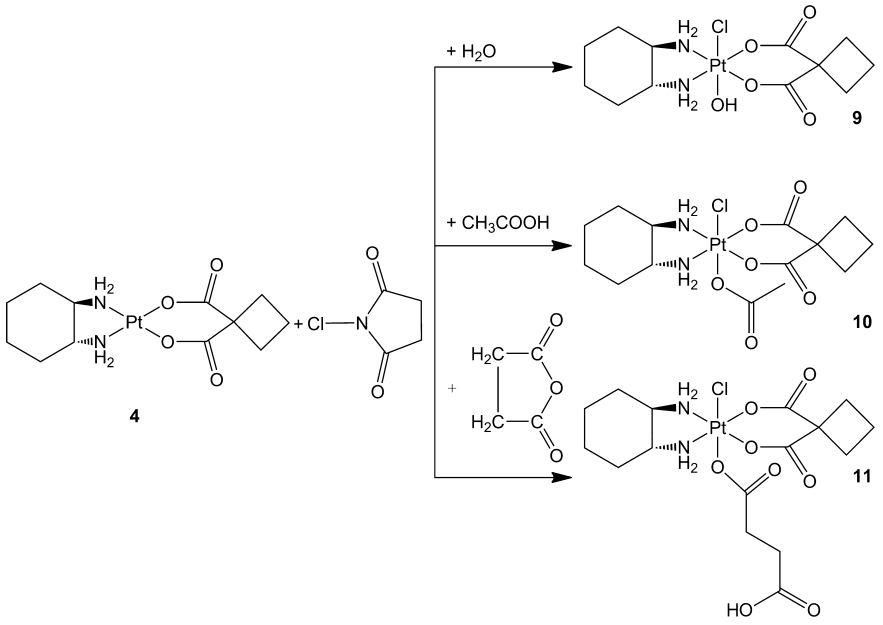
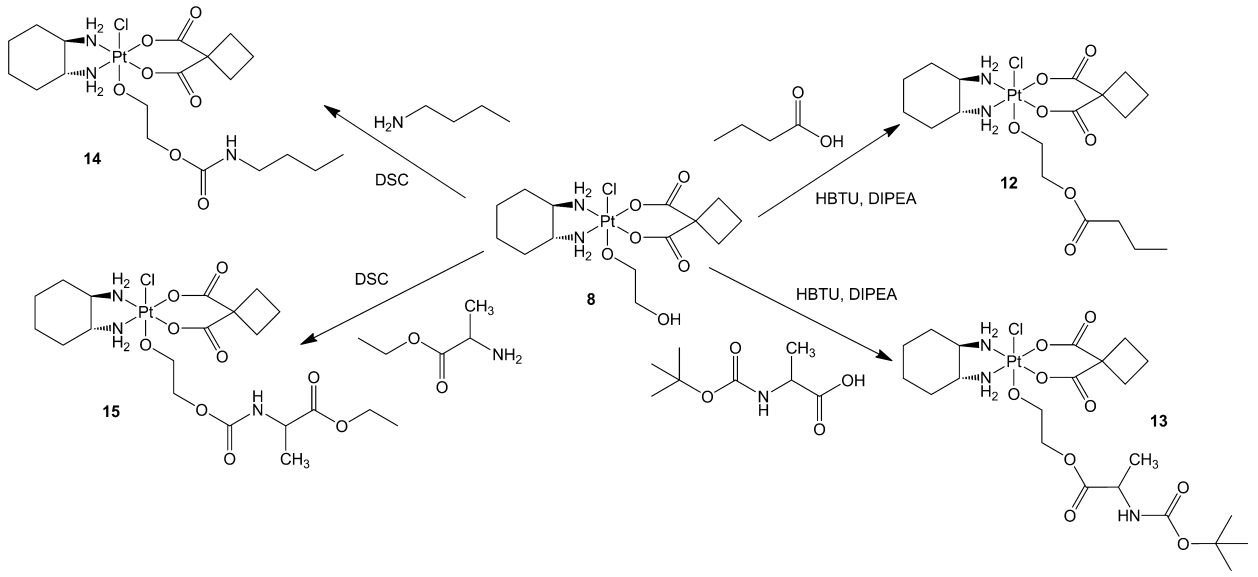
Scheme 2. General Scheme of Synthesis for the Asymmetric Pt(IV) Complexes 9−11



Scheme 3. Scheme of the Reaction between Complex 8 and Acids, Amines, or Amino Acids[a]



[a]HBTU = *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, DIPEA = diisopropylethylamine, DSC = *N*,*N*′-disuccinimidylcarbonate.

(IV)(nucleophile)] complex can be obtained. Actually, by using an anhydride as reactant and reagent grade acetone as solvent, the reaction gives complex **11** in high purity. The presence of traces of water (humidity) in the solvent produces in any case the hydroxido complex **9** as byproduct, but water has proven to be important for the reaction with anhydrides, because **9** seems to acts as an intermediate to give the final carboxylato Pt(IV) complex.[33] In fact, in "wet" acetone this reaction is largely favored, whereas in anhydrous *N*,*N*-dimethylformamide (DMF) the carboxylato product is obtained in small amount together with several byproducts, including a Pt(IV) derivative containing DMF itself in the coordination sphere (albeit obtained in traces; see electrospray ionization−mass spectrometry (ESI-MS) in Supporting Information, Figure S38).

All the complexes here reported were characterized by reversed-phase high performance liquid chromatography (RP-HPLC)-ESI-MS and multinuclear (^{1}H, ^{13}C and ^{195}Pt) NMR spectroscopy using both mono- and bidimensional techniques, such as correlation spectroscopy (COSY), heteronuclear multiple bond correlation (HMBC), and heteronuclear single quantum coherence spectroscopy (HSQC), to assign the whole set of signals (see the NMR spectroscopy section and the Supporting Information for further details).

X-ray Structure of Complex 8. An ORTEP plot with a thermal ellipsoid diagram for complex **8** is shown in Figure 1. The structure is characterized by the presence in the asymmetric unit of two independent molecules of the complex and by six molecules of water. Most of the hydrogen atoms have been calculated in riding positions. On the contrary, the

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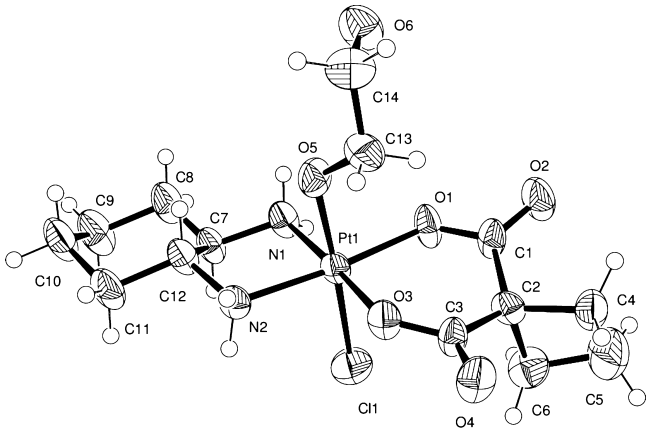

Figure 1. ORTEP plot of compound **8** with thermal ellipsoids at 50% probability.

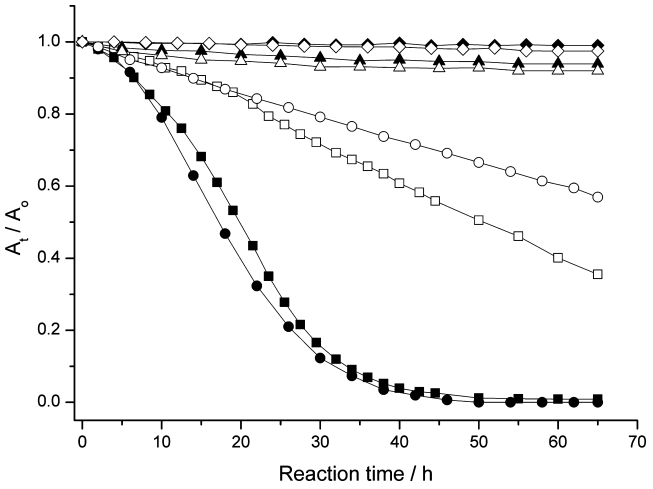

Figure 2. Normalized area (A_t/A_0) (A_t = area of an HPLC peak measured at aging time t; A_0 = area of an HPLC peak measured at aging time $t = 0$) of the HPLC chromatographic peaks of complexes **5** (■), **6** (△), **7** (●), and **8** (◇) vs aging time (t) in water (pH = 6.4, solid symbols) and HEPES buffer (pH = 7.5, empty symbols). Each data point is the mean of three independent experiments; error bars are omitted for clarity.

hydrogen atoms of the water molecules and that of the glyc hydroxyl could neither be found in the electron density map nor be geometrically calculated. The packing is governed by an extensive network of hydrogen bonds involving the glyc terminal OH, the carboxylates, and the water molecules. The coordination geometry around the Pt(IV) ion is octahedral: the equatorial plane is occupied by the two bidentate ligands (cbdc and dach), whereas the axial positions are occupied by a monodeprotonated glycol and a chloride ion. In both independent molecules this octahedral geometry is fairly regular with the angles involving the chloride ion very close to the ideal 90° (in one molecule the range is 90.1(5) to 93.5(4)°, while in the other 91.64(4) to 92.0(4)°). Also the angles in the equatorial plane fall in the range 83.8(6)−92.7(5)°, where the lowest value is due to the restrained bite of the dach ligand. The coordination distances are similar to those found in the literature for this kind of Pt complexes, and range from 2.317(5) to 2.351(5) Å for the Pt−Cl distance, 1.96(1) to 2.04(1) Å for the Pt−O distances and 2.01(2) to 2.03(4) Å for the Pt−N distances.

Solution Behavior of Complexes 5−8. The stability of complexes **5**−**8** was studied both in carbonated water (pH = 6.4) and in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH = 7.5) by monitoring the decrease of the area of the Pt(IV) HPLC peaks as a function of time. Chromatographic data (Figure 2 and Supporting Information, Figures S39 and S41) were elaborated to obtain the pseudo-first-order rate constant k and the corresponding half-life times $t_{1/2}$. The chlorido complexes **5** and **7** undergo fast hydrolysis, while the cbdc complexes **6** and **8** are 100% recovered after 3 d. The stability of **5** and **7** is higher at neutral pH (**5** $t_{1/2}$ = 50 h, **7** $t_{1/2}$ = 70 h) than at slightly acidic pH ($t_{1/2}$ ca. 21 h for both complexes) (Figure 2). ESI-MS spectra (positive ion mode) of the aged solutions show that the first hydrolysis product contains water instead of glyc; this reaction is favored at lower pH, probably through the protonation of the glycol. This intermediate further loses chlorides that are replaced by water/hydroxyl groups (verified by ESI-MS; see Supporting Information, Figures S40 and S42).

In the design of Pt(IV) prodrugs, the assumption is the absence of reaction with nucleophiles in the blood, so that the Pt(IV) complexes can reach intact the tumor site and then release the cytotoxic Pt(II) metabolite by reductive elimination. However, in the several biotransformation products of *cis,trans,cis*-$[PtCl_2(OOCCH_3)_2(NH_3)(cyclohexylamine)]$, the chlorido ligands have been replaced by OH, while some aquation (<5%) was observed for *cis,trans,cis*-$[PtCl_2(OOCCH_3)_2(NH_3)_2]$.[34−36] Finally, Pt(IV) complexes with haloacetato ligands (including Lippard's mitaplatin) hydrolyze very rapidly under biological conditions.[37]

Reduction of Complexes 6 and 8. The stable complexes **6** and **8** were challenged with the two most common biological reductants, that is, ascorbic acid (AsA, present in blood plasma and in the cytosol at 50−150 μM and ca. 1 mM concentrations, respectively[38,39]) and glutathione (γ-glutamylcysteinylglycine, GSH, having concentration up to 8 mM in the cytosol[40] and ca. 850 μM in blood[41,42]). All kinetic measurements were performed with a 10-fold excess of AsA or GSH in HEPES buffer by monitoring the decrease of the area of the Pt(IV) chromatographic peaks (Figure 3 and Supporting Information, Figures S43−S45). HPLC data were elaborated to obtain a

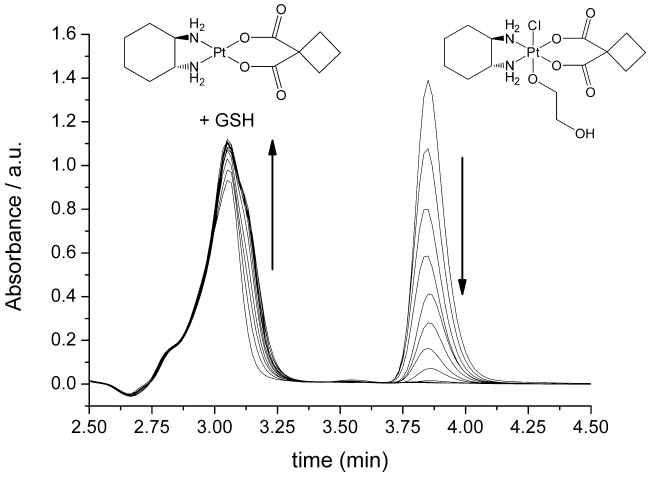

Figure 3. HPLC chromatograms at different time points (from 0 to 160 min) of the reduction of complex **8** with glutathione. The chromatographic peak of the Pt(II) species is overlapped with the peak of glutathione.

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pseudo-first-order rate constant k and the corresponding $t_{1/2}$, as previously reported for several Pt(IV) complexes.[43,44]

Both AsA and GSH proved to be efficient reductants for such complexes. The reduction with AsA is very fast with a $t_{1/2}$ of 1.6 min for **6** and 2.4 min for **8**. On the contrary the reduction time with GSH is longer: $t_{1/2}$ for **6** is 13 and 46 min for **8**. As expected, in both cases and both reductants, the expected Pt(II) parent compounds are formed.

The results confirm that the presence of chloridos trans to good leaving groups in the coordination sphere of Pt(IV) increases the reduction rate.[45] In fact, many researchers proposed that Pt(IV) reduction by AsA and GSH proceeds via the formation of a bridge between the metal and the reducing agent.[20]

Coupling Reaction of Complex 8 with Acids and Amines. The low propensity of **8** toward hydrolysis and the good cytotoxic activity of its Pt(II) metabolite **4**[46] prompted us to assess the chemical reactivity of **8**. To optimize the reaction conditions for esterification of **8**, butanoic acid was used as the model of carboxylic acids. The best results were obtained with the uronium salts as coupling agents and, in particular, with *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and diisopropylethylamine (DIPEA) in dry DMF to give complex **12** (Scheme 3). Importantly, these experimental conditions were among those generally used in the solid-phase synthesis of peptides, and, hence, suitable for the coupling of Pt(IV) complexes to amino acids.[47] The coupling reaction was then performed using *t*-butoxycarbonyl (BOC)-protected L-alanine (Ala) as a model for the coupling with α-COOH of peptides to give complex **13** (Scheme 3).

Interestingly, the coupling reaction with butanoic acid performed on the unstable complex **5** gave almost quantitatively the product containing part of the coupling agent coordinated instead of glyc (see Supporting Information, Figures S46−S49).

Hydroxyl groups may also react with modification agents able to temporarily activate it for coupling with a secondary functional group. For instance, *N*,*N*′-disuccinimidylcarbonate (DSC) can transform a hydroxyl into a succinimidyl carbonate group, that can be further reacted with an amine to form a stable carbamate bond. Also in this case, a simple model amine (i.e., an *n*-butylamine) and a protected amino acid (i.e., an esterified alanine) were used to test the reactivity of **8** with two kinds of $-NH_2$ groups (complexes **14** and **15**, respectively, Scheme 3).

In both kind of coupling reactions the desired products were obtained, albeit in moderate yields, as a consequence of the multiple purification steps. However, the described procedures represent a proof of concept for the use of complex **8** as a building block in the drug targeting and delivery strategy. In solid-state peptide synthesis or conjugation with NPs the elimination of undesired by/products of the reactions (low MW molecules) can be obtained by simple washing.

NMR Spectroscopy. In general the ^{1}H and ^{13}C NMR spectra of the Pt complexes show a shift of the signals of the atoms nearer to the metal center with respect to the free ligands, pointing out the occurred coordination (Supporting Information contains the most relevant mono- and bidimensional NMR spectra). For the glyc moiety, the ^{13}C NMR spectra of complexes **5**−**8** show high frequency shift of the methylene near to the platinum core, whereas the signal of the $-CH_2OH$ is slightly shifted toward low frequencies, with respect to the free glycol. Moreover, both the ^{13}C signals of glyc show satellites bands due to the coupling with ^{195}Pt (I = 1/2, 34% natural abundance). It is interesting to note that $^2J_{Pt-C}$ is smaller (ca. 10−15 Hz) than $^3J_{Pt-C}$ (ca. 30−35 Hz) (see Supporting Information, e.g. Figure S2). The ^{1}H chemical shift of the methylene near to platinum is at lower frequency that the second one. The assignment of the latter signal is corroborated by a further shift of the corresponding proton and carbon signals observed after the coupling of the free OH functionality (complexes **12**−**15**).

For the dach ligand it is possible to observe the coupling between carbon and platinum nuclei in the ^{13}C NMR spectra for the methylene in the ring positions 3 and 6 ($^3J_{Pt-C}$ ca. 30−35 Hz), whereas no $^2J_{Pt-C}$ is observed. Since the dach is in a chair configuration (as it can be observed in the structure of **8** and in other similar structures[48]) and, furthermore, the axial ligands in the complexes are different, the couple of carbon atoms 1−2, 3−6, 4−5 are no longer equivalent and, then, resonate at different frequencies, and separate signals are observed in the ^{1}H NMR spectra for axial and equatorial protons (see Supporting Information, e.g., Figures S11−S16). Since the cbdc ring is almost perpendicular to the equatorial coordination plane (as found in the precursor compound **8**, see Figure 1) its ^{1}H and ^{13}C signals for the methylene groups are split up (see Supporting Information, e.g. Figures S18−S22).

As far as the amine groups are concerned, in the ^{1}H spectra in deuterated dimethyl sulfoxide (DMSO-d_6) of **7**−**15** it is possible to observe the presence of four different signals for the NH_2 groups of coordinated dach: their intrinsical difference is reinforced by the asymmetry of the axial ligands. The relative position of the four signals varies with the change of the other ligands (see Supporting Information, Figures S11, S18, S24, S29, and S33).

In the case of the cisplatin- and carboplatin-based derivatives **5** and **6**, as already observed for other Pt(IV) complexes,[49] the ammine proton resonance around 5.6 ppm appears as a multiplet (see Supporting Information, Figures S1 and S5) due to the coupling to the quadrupolar ^{14}N nuclei (I = 1; $^1J_{N-H}$ = ca. 52 Hz) and to the coupling to the ^{195}Pt nucleus (I = 1/2; $^2J_{Pt-H}$ = ca. 51 Hz).

The assignments of the ^{1}H and ^{13}C signals have been checked by exploiting bidimensional NMR techniques (see Supporting Information, NMR characterization of **5**−**15**) such as COSY, HSQC, HMBC, and/or nuclear Overhauser effect spectroscopy (NOESY) and by comparing the spectra of the complexes. It is worth noting that NOESY spectra (see Supporting Information, Figure S16) show the spatial proximity of the protons of glyc and the axial protons of dach. This data are in agreement with the proposed structures in Chart 1.

^{195}Pt NMR spectroscopy provides information on the oxidation state of the metal and on the nature of ligands. A lot of factors affect the shielding of a heavy atom like ^{195}Pt: the bound donor atoms, the spatial arrangement of the ligands, the concentration of the sample, the temperature, the solvent used, the counterions, and the solution pH value. Moreover, it is generally observed a roughly additive effect of the ligand substitution on ^{195}Pt chemical shift.[50−52] In particular, by comparing complexes **5**−**8** the substitution of chlorides with cbdc causes a high frequency shift of the ^{195}Pt signal of about 810 ppm, whereas the substitution of ammonia with dach causes a low frequency shift of ca. 130 ppm. According to this, the ^{195}Pt NMR signals of the investigated complexes **5**−**15** exhibit chemical shifts (1110−1130 ppm for **8**, its coupled derivatives and **9**; 1230−1260 ppm for the axial carboxylato

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derivatives of **4** and the carboplatin-based complex **6**; 290−430 ppm for the trichlorido complexes) well-suited with an octahedral geometry containing the "$PtClN_2O_3$" and "$PtCl_3N_2O$" core, respectively.[23,49,53−57]

CONCLUSIONS

Four Pt(IV) complexes **5**−**8** containing ethane-1,2-diol as linking arm were successfully prepared by using oxidative chlorination with *N*-chlorosuccinimide of the corresponding Pt(II) complex in a one-pot reaction.

While the *fac*-trichlorido complexes are rapidly hydrolyzed, compounds containing cbdc were stable enough to be handled for couplings with carboxylic acids or amines. Moreover, these complexes, containing axial chlorides and good *trans*- leaving groups are easily reduced by bioreductants such as glutathione and ascorbic acid. Thus, these new Pt(IV) prodrug candidates could be exploited in drug targeting and delivery strategies.

EXPERIMENTAL SECTION

$K_2[PtCl_4]$ (Johnson Matthey and Co.) and all other chemicals (Aldrich) were used without further purification. (*SP*-4−2)-diamminedichloridoplatinum(II) (cisplatin, **1**), (*SP*-4−2)-diammine(cyclobutane-1,1′-dicarboxylato)platinum(II) (carboplatin, **2**), (*SP*-4−2)-dichlorido(cyclohexane-1*R*,2*R*-diamine)platinum(II), **3**, and (*SP*-4−2)-(cyclobutane-1,1′-dicarboxylato)(cyclohexane-1*R*,2*R*-diamine)-platinum(II), **4**, were synthesized according to the Dhara's method.[58−60] Complexes **5**−**8** were synthesized by using a procedure similar to that reported in ref[16] for the oxidative chlorination of oxaliplatin (Scheme 1).

Purity of compounds was assessed by analytical RP-HPLC, elemental analysis and determination of Pt content by inductively coupled plasma-optical emission spectroscopy (ICP-OES). Elemental analyses were carried out with a EA3000 CHN Elemental Analyzer (EuroVector, Milano, Italy). Platinum was quantified by means of a Spectro Genesis ICP-OES spectrometer (Spectro Analytical Instruments, Kleve, Germany) equipped with a crossflow nebulizer. To quantify the platinum concentration the Pt 299.797 nm line was selected. A platinum standard stock solution of 1000 mg L^{-1} was diluted in 1.0% v/v nitric acid to prepare calibration standards.

The multinuclear NMR spectra were measured on a Bruker Advance III NMR spectrometer operating at 500 MHz (^{1}H), 125.7 MHz (^{13}C) and 107.2 MHz (^{195}Pt), respectively. ^{1}H and ^{13}C NMR chemical shifts were reported in parts per million (ppm) referenced to solvent resonances. ^{195}Pt NMR spectra were recorded using a solution of $K_2[PtCl_4]$ in saturated aqueous KCl as the external reference. The shift for $K_2[PtCl_4]$ was adjusted to −1628 ppm from $Na_2[PtCl_6]$ (δ = 0 ppm).

RP-HPLC and mass analysis were performed using a Waters HPLC-MS instrument equipped with Alliance 2695 separations module, 2487 dual lambda absorbance detector, and 3100 mass detector. Electrospray ionization mass spectra (ESI-MS) were obtained setting the source and desolvation temperatures to 150 and 250 °C, respectively, with nitrogen used both as a drying and a nebulizing gas. The cone and the capillary voltages were usually +30 V and 2.70 kV, respectively. Quasi-molecular ion peaks $[M + H]^+$ were assigned on the basis of the *m*/*z* values and of the simulated isotope distribution patterns.

Synthesis of Complexes 5−8. The Pt(II) complex (0.70 mmol, that is, 210 mg of **1**, 260 mg of **2**, 266 mg of **3**, or 316 mg of **4**) was suspended in ethane-1,2-diol (3 mL) and a slight excess of *N*-chlorosuccinimide (0.71 mmol, 95 mg) was added after 30 min (Scheme 1). The reaction mixture was stirred at room temperature for 3 h in the dark. To the resulting clear yellow solution, acetone/diethyl ether 1:3 was added to induce the precipitation of the final complexes **5**−**8**, that were centrifuged and washed with diethyl ether.

(OC-6−33)-Diamminetrichlorido(2-hydroxyethanolato)-platinum(IV) (5). Yield: 208 mg (75%). Anal. Calcd for: $C_2H_{11}Cl_3N_2O_2Pt$ (396.56): C, 6.06; H, 2.80; N, 7.06; Pt, 49.19. Found: C, 5.90; H, 2.85; N, 7.29; Pt, 48.99%. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.39−3.42 (m, 4H, Pt−O−CH_2−CH_2), 4.11 (t, 1H, O*H*, ^3J = 5.5 Hz), 5.58 (m, 6H, NH_3, $^2J_{Pt-H}$ = 51.5 Hz, $^1J_{N-H}$= 52.6 Hz) ppm. ^{13}C{^{1}H} NMR (DMSO-d_6, 125.7 MHz): δ 61.5 (Pt−O−CH_2−CH_2, $^3J_{Pt-C}$ = 35.0 Hz), 71.8 (Pt−O−CH_2−CH_2, $^2J_{Pt-C}$ = 9.48 Hz) ppm. ^{195}Pt NMR (DMSO-d_6, 107.2 MHz): δ 426 ppm. ESI-MS (positive ion mode): 397.2 *m*/*z* $[M + H]^+$; calcd for $C_2H_{12}Cl_3N_2O_2Pt$ 396.96 *m*/*z* $[M + H]^+$.

(OC-6−34)-Diamminechlorido(cyclobutane-1,1′-dicarboxylate)(2-hydroxyethanolato)platinum(IV) (6). Yield: 232 mg (71%). Anal. Calcd for: $C_8H_{17}ClN_2O_6Pt$ (467.76): C, 20.54; H, 3.66; N, 5.99; Pt, 41.70. Found: C, 20.21; H, 3.48; N, 6.11; Pt, 41.52%. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.81 (quint, 2H, OCO−C−CH_2−CH_2, ^3J = 8.1 Hz), 2.46 (t, 2H, OCO−C−CH_2−CH_2, ^3J = 8.1 Hz), 2.56 (t, 2H, OCO−C−CH_2−CH_2, ^3J = 7.8 Hz), 2.94 (m, 2H, Pt−O−CH_2−CH_2), 3.38 (m, 2H, Pt−O−CH_2−CH_2), 5.67 (m, 6H, NH_3, $^2J_{Pt-H}$ = 50.8 Hz, $^1J_{N-H}$ = 51.5 Hz) ppm. ^{13}C{^{1}H} NMR (DMSO-d_6, 125.7 MHz): δ 15.7 (OCO−C−CH_2−CH_2), 28.9 (OCO−C−CH_2−CH_2), 34.4 (OCO−C−CH_2−CH_2), 55.6 (OCO−C−CH_2−CH_2), 60.8 (Pt−O−CH_2−CH_2, $^3J_{Pt-C}$ = 32.4 Hz), 69,6 (Pt−O−CH_2−CH_2, $^2J_{Pt-C}$ = 15.0 Hz), 176.7 (OCO−C−CH_2−CH_2) ppm. ^{195}Pt NMR (DMSO-d_6, 107.2 MHz): δ 1232 ppm. ESI-MS (positive ion mode): 468.4 *m*/*z* $[M + H]^+$; calcd for $C_8H_{18}ClN_2O_6Pt$ 468.7 *m*/*z* $[M + H]^+$.

(OC-6−33)-Trichlorido(cyclohexane-1*R*,2*R*-diamine)(2-hydroxyethanolato)platinum(IV) (7). Yield: 270 mg (81%). Anal. Calcd for: $C_8H_{19}Cl_3N_2O_2Pt$ (476.69): C, 20.16; H, 4.02; N, 5.88; Pt, 40.92. Found: C, 20.09; H, 4.34; N, 5.65; Pt, 40.71%. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.03 (m, 2H, NH_2−CH−CH_2−$CH_{2(ax)}$), 1.41−1.47 (m, 4H, NH_2−CH−$CH_{2(ax)}$−CH_2 and NH_2−CH−CH_2−$CH_{2(eq)}$), 2.02−2.10 (m, 2H, NH_2−CH−$CH_{2(eq)}$−CH_2), 2.58−2.69 (m, 2H, NH_2−*C*H−CH_2−CH_2), 3.38−3.45 (m, 4H, Pt−O−CH_2−CH_2), 4.19 (t, 1H, O*H*, ^3J = 6.0 Hz), 6.48−7.58 (m, 4H, NH_2−CH−CH_2−CH_2) ppm. ^{13}C{^{1}H} NMR (DMSO-d_6, 125.7 MHz): δ 23.7 (NH_2−CH−CH_2−CH_2), 23.8 (NH_2−CH−CH_2−CH_2), 30.1 (NH_2−CH−CH_2−CH_2, $^3J_{Pt-C}$ = 33.5 Hz), 30.7 (NH_2−CH−CH_2−CH_2, $^3J_{Pt-C}$ = 34.1 Hz), 60.4 (NH_2−*C*H−CH_2−CH_2), 61.6 (Pt−O−CH_2−CH_2, $^3J_{Pt-C}$ = 34.3 Hz), 62,7 (NH_2−*C*H−CH_2−CH_2), 71.5 (Pt−O−CH_2−CH_2) ppm. ^{195}Pt NMR (DMSO-d_6, 107.2 MHz): δ 292 ppm. ESI-MS (positive ion mode): 477.4 *m*/*z* $[M + H]^+$; calcd for $C_8H_{20}Cl_3N_2O_2Pt$ 477.02 *m*/*z* $[M + H]^+$.

(OC-6−34)-Chlorido(cyclobutane-1,1′-dicarboxylate)-(cyclohexane-1*R*,2*R*-diamine)(2-hydroxyethanolato)platinum-(IV) (8). Yield: 326 mg (85%). Anal. Calcd for: $C_{14}H_{25}ClN_2O_6Pt$ (547.89): C, 30.69; H, 4.60; N, 5.11; Pt, 35.61. Found: C, 30.88; H, 4.38; N, 5.19; Pt, 35.42% (*Please note that the sample for elemental analysis was obtained by treatment and precipitation in organic solvents; therefore, H_2O molecules, as found in the X-ray crystals growth in water, are not present. See below*). ^{1}H NMR (DMSO-d_6, 500 MHz): 1.05 (m, 2H, NH_2−CH−CH_2−$CH_{2(ax)}$), 1.41−1.48 (m, 4H, NH_2−CH−$CH_{2(ax)}$−CH_2 and NH_2−CH−CH_2−$CH_{2(eq)}$), 1.79−1.83 (m, 2H, OCO−C−CH_2−CH_2), 1.96−2.02 (m, 2H, NH_2−CH−$CH_{2(eq)}$−CH_2), 2.47 (t, 2H, OCO−C−CH_2−CH_2, ^3J = 6.9 Hz), 2.53−2.57 (m, 2H, OCO−C−CH_2−CH_2), 2.59 (m, 2H, NH_2−*C*H−CH_2−CH_2), 2.95−2.99 (m, 2H, Pt−O−CH_2−CH_2), 3.31−3.45 (m, 2H, Pt−O−CH_2−CH_2), 4.15 (br. s, 1H, O*H*), 6.86−7.73 (m, 4H, NH_2−CH−CH_2−CH_2) ppm. ^{13}C{^{1}H} NMR (DMSO-d_6, 125.7 MHz): δ 15.7 (OCO−C−CH_2−CH_2), 23.6 (NH_2−CH−CH_2−CH_2), 23.7 (NH_2−CH−CH_2−CH_2), 29.1 (OCO−C−CH_2−CH_2), 30.1 (NH_2−CH−CH_2−CH_2, $^3J_{Pt-C}$ = 32,7 Hz), 30.7 (NH_2−CH−CH_2−CH_2, $^3J_{Pt-C}$ = 36.0 Hz), 34.4 (OCO−C−CH_2−CH_2), 55.7 (OCO−C−CH_2−CH_2), 59.6 (NH_2−CH−CH_2−CH_2), 60.9 (Pt−O−CH_2−CH_2, $^3J_{Pt-C}$ = 29.2 Hz), 61.7 (NH_2−CH−CH_2−CH_2), 69.2 (Pt−O−CH_2−CH_2, $^2J_{Pt-C}$ = 13.8 Hz), 177.3 (OCO−C−CH_2−CH_2), 177.4 (OCO−C−CH_2−CH_2) ppm. ^{195}Pt NMR (DMSO-d_6, 107.2 MHz): δ 1102 ppm. ESI-MS (positive ion mode): 549.5 *m*/*z* $[M + H]^+$; calcd for $C_{14}H_{26}ClN_2O_6Pt$ 549.11 *m*/*z* $[M + H]^+$.

Synthesis of (OC-6−34)-Chlorido(cyclobutane-1,1′-dicarboxylate)(cyclohexane-1*R*,2*R*-diamine)-hydroxidoplatinum(IV) (9). *N*-chlorosuccinimide (0.0296 g, 0.22

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mmol) dissolved in 14 mL of water was added to a suspension of **4** (100 mg, 0.22 mmol) in 6 mL of water (Scheme 2). The reaction was stirred at room temperature for 3 h in the dark. The solid residue was removed by centrifugation and the solution was evaporated to dryness to get a pale yellow solid that was washed with ethanol and diethyl ether. Yield: 91 mg (82%). Anal. Calcd for: $C_{12}H_{21}ClN_2O_5Pt$ (503.84): C, 28.61; H, 4.20; N, 5.56; Pt, 38.72. Found: C, 28.43; H, 4.03; N, 5.80; Pt, 38.44%. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.05 (m, 2H, $NH_2-CH-CH_2-CH_{2(ax)}$), 1.30–1.55 (m, 4H, 2H $NH_2-CH-CH_{2(ax)}-CH_2$ and 2H $NH_2-CH-CH_2-CH_{2(eq)}$), 1.79 (quint, 2H, $OCO-C-CH_2-CH_2$, ^3J = 8.0 Hz), 1.95 (m, 1H, $NH_2-CH-CH_{2(eq)}-CH_2$), 2.01 (m, 1H, $NH_2-CH-CH_{2(eq)}-CH_2$), 2.40–2.65 (m, 6H, $OCO-C-CH_2-CH_2$ and $NH_2-CH-CH_2-CH_2$), 6.80–7.80 (m, 4H, $NH_2-CH-CH_2-CH_2$) ppm. ^{13}C{^{1}H} NMR (DMSO-d_6, 125.7 MHz): δ 15.7 ($OCO-C-CH_2-CH_2$), 23.6 ($NH_2-CH-CH_2-CH_2$), 23.7 ($NH_2-CH-CH_2-CH_2$), 30.3 ($NH_2-CH-CH_2-CH_2$, $^3J_{Pt-C}$ = 35.2 Hz), 30.7 ($NH_2-CH-CH_2-CH_2$, $^3J_{Pt-C}$ = 36.5 Hz), 31.3 ($OCO-C-CH_2-CH_2$), 32.3 ($OCO-C-CH_2-CH_2$), 56.0 ($OCO-C-CH_2-CH_2$), 60.7 ($NH_2-CH-CH_2-CH_2$), 61.4 ($NH_2-CH-CH_2-CH_2$), 176.7 ($OCO-C-CH_2-CH_2$), 176.8 ($OCO-C-CH_2-CH_2$) ppm. ^{195}Pt NMR (DMSO-d_6, 107.2 MHz): δ 1130 ppm. ESI-MS (positive ion mode): 505.5 m/z $[M + H]^+$; calcd for $C_{12}H_{22}ClN_2O_5Pt$ 504.84 m/z $[M + H]^+$.

Synthesis of (*OC*-6–34)-Acetatochlorido(cyclobutane-1,1′-dicarboxylate)(cyclohexane-1*R*,2*R*-diamine)platinum(IV) (10). *N*-chlorosuccinimide (0.0445 g, 0.33 mmol) dissolved in 2 mL of acetic acid was added to a solution of **4** (150 mg, 0.33 mmol) in 2 mL of acetic acid (Scheme 2). The reaction was allowed to stir at room temperature for 4 h in the dark. The solvent was removed under reduced pressure and the product was washed several times with diethyl ether to give a yellow powder. Yield: 128 mg (71%). Anal. Calcd for: $C_{14}H_{23}ClN_2O_6Pt$ (545.87): C, 30.80; H, 4.25; N, 5.13; Pt, 35.74. Found: C, 30.86; H, 4.03; N, 5.35; Pt, 35.48%. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.09 (m, 2H, $NH_2-CH-CH_2-CH_{2(ax)}$), 1.32 (m, 1H, $NH_2-CH-CH_2-CH_{2(eq)}$), 1.45–1.55 (m, 3H, $NH_2-CH-CH_{2(ax)}-CH_2$ and $NH_2-CH-CH_2-CH_{2(eq)}$), 1.81 (quint, 2H, $OCOCCH_2CH_2$, ^3J = 8.0 Hz), 1.96 (s, 3H, $OCOCH_3$), 1.99 (m, 1H, $NH_2-CH-CH_{2(eq)}-CH_2$), 2.08 (m, 1H, $NH_2-CH-CH_{2(eq)}-CH_2$), 2.40–2.55 (m, 5H, $OCO-C-CH_2$ and $NH_2-CH-CH_2-CH_2$), 2.64 (m, 1H, $NH_2-CH-CH_2-CH_2$), 7.40–8.90 (m, 4H, $NH_2-CH-CH_2-CH_2$) ppm. ^{13}C{^{1}H} NMR (DMSO-d_6, 125.7 MHz): δ 16.1 ($OCO-C-CH_2-CH_2$), 23.4 ($NH_2-CH-CH_2-CH_2$), 23.5 ($NH_2-CH-CH_2-CH_2$), 24.0 ($OCO-CH_3$, $^3J_{Pt-C}$ = 36.5 Hz), 30.6 ($NH_2-CH-CH_2-CH_2$, $^3J_{Pt-C}$ = 28.9 Hz), 30.9 ($NH_2-CH-CH_2-CH_2$, $^3J_{Pt-C}$ = 32.7 Hz), 31.1 ($OCO-C-CH_2-CH_2$), 31.6 ($OCO-C-CH_2-CH_2$), 55.8 ($OCO-C-CH_2-CH_2$), 61.4 ($NH_2-CH-CH_2-CH_2$), 61.6 ($NH_2-CH-CH_2-CH_2$), 176.3 ($OCO-C-CH_2-CH_2$), 178.9 ($OCO-CH_3$) ppm. ^{195}Pt NMR (DMSO-d_6, 107.2 MHz): δ 1261 ppm. ESI-MS (positive ion mode): 546.5 m/z $[M + H]^+$; calcd for $C_{14}H_{24}ClN_2O_6Pt$ 546.88 m/z $[M + H]^+$.

Synthesis of (*OC*-6–34)-Chlorido(cyclobutane-1,1′-dicarboxylate)(cyclohexane-1*R*,2*R*-diamine)-succinatoplatinum(IV) (11). Compound **4** (100 mg, 0.22 mmol) was added to a solution of succinic anhydride (440 mg, 4.40 mmol) in 12 mL of acetone (Scheme 2). After 5 min *N*-chlorosuccinimide (0.0296 g, 0.22 mmol) dissolved in 2 mL of acetone was added and the reaction was allowed to stir at room temperature for 4 h in the dark. The solid residue was removed by centrifugation and the solution was evaporated to dryness to get a pale yellow solid that was washed several times with diethyl ether. Yield: 102 mg (77%). Anal. Calcd for: $C_{16}H_{25}ClN_2O_8Pt$ (603.91): C, 31.82; H, 4.17; N, 4.64; Pt, 32.30. Found: C, 31.74; H, 4.35; N, 4.37; Pt, 32.53%. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.05 (m, 2H, $NH_2-CH-CH_2-CH_{2(ax)}$), 1.29 (m, 1H, $NH_2-CH-CH_{2(ax)}-CH_2$), 1.40–1.55 (m, 3H, $NH_2-CH-CH_{2(ax)}-CH_2$ and $NH_2-CH-CH_2-CH_{2(eq)}$), 1.80 (quint, 2H, $OCO-C-CH_2-CH_2$, ^{3}J = 8.0 Hz), 1.98 (m, 1H, $NH_2-CH-CH_{2(eq)}-CH_2$), 2.07 (m, 1H, $NH_2-CH-CH_{2(eq)}-CH_2$), 2.65–2.30 (m, 10H, 4H $OCO-C-CH_2$, 2H $NH_2-CH-CH_2-CH_2$, $OCO-CH_2-CH_2-COOH$ and $OCO-CH_2-CH_2-COOH$), 7.40–8.70 (m, 4H, $NH_2-CH-CH_2-CH_2$), 12.16 (s, 1H, $OCO-CH_2-CH_2-COOH$) ppm. ^{13}C{^{1}H} NMR (DMSO-d_6, 125.7 MHz): δ 15.5 ($OCO-C-CH_2-CH_2$), 23.4 ($NH_2-CH-CH_2-CH_2$), 23.5 ($NH_2-CH-CH_2-CH_2$), 29.6 ($OCO-CH_2-CH_2-COOH$), 30.6 ($NH_2-CH-CH_2-CH_2$), 30.7 ($OCO-C-CH_2-CH_2$), 30.9 ($NH_2-CH-CH_2-CH_2$), 31.6 ($OCO-C-CH_2-CH_2$), 31.8 ($OCO-CH_2-CH_2-COOH$), 55.7 ($OCO-C-CH_2-CH_2$), 61.3 ($NH_2-CH-CH_2-CH_2$), 61.4 ($NH_2-CH-CH_2-CH_2$), 173.7 ($OCO-CH_2-CH_2-COOH$), 176.2 ($OCO-C-CH_2-CH_2$), 176.3 ($OCO-C-CH_2-CH_2$), 180.0 ($OCO-CH_2-CH_2-COOH$) ppm. ^{195}Pt NMR (DMSO-d_6, 107.2 MHz): δ 1260 ppm. ESI-MS (positive ion mode): 605.4 m/z $[M + H]^+$; calcd for $C_{16}H_{26}ClN_2O_8Pt$ 604.92 m/z $[M + H]^+$.

Single-Crystal X-ray Structure Determination of 8. Pale yellow crystals of **8** were obtained by slow evaporation of the solvent (ca. 1 month) from an aqueous solution at 4 °C. The crystals were badly twinned, but the data are of reasonable quality, albeit affected by this problem. X-ray diffraction analysis was carried out using a SMART BREEZE diffractometer equipped with CCD detector, Mo Kα radiation (λ = 0.710 69). Data were corrected for absorption effects following the *SADABS* procedure.[61] The phase problem was solved by direct methods and the structures were refined by full-matrix least-squares on all F^2 using *SHELXL97*,[62] as implemented in the *WINGX*.[63] Analytical expressions of neutral atom scattering factors were taken from the International Tables for X-ray Crystallography.[64] The structure drawing was obtained using *ORTEPIII*.[65] Crystal data: formula $C_{14}H_{25}N_2O_8ClPt{\cdot}3H_2O$, monoclinic, $P2_1$, a = 9.781(2) Å, b = 21.416(3) Å, c = 11.228(2) Å, β = 115.298(3)°, V = 2126.4(6) Å^3; Z = 4; d_{calc} = 1.88 g cm^{-3}, $F(000)$ = 1184, μ = 67.7 cm^{-1}, total reflections = 15481, hkl range = $-12 < h < 11$, $-26 < k < 20$, $-14 < l < 14$; Θ_{max} = 26.7°, unique reflections = 8191, number of parameters = 487, goodness-of-fit on F^2 = 0.98, R = 0.0507, $wR2$ = 0.1182, Flack parameter = 0.01(2). Additional crystallographic information for this compound is available in the Supporting Information.

Solution Behavior of Complexes 5–8. The solution behavior of complexes **5**–**8** (1 mM) was studied in water or HEPES (2 mM, pH 7.5) at 37 °C. All these reactions were followed by monitoring the decrease of the area of the chromatographic peaks of Pt complexes in HPLC-UV-MS. Stationary phase: 5 μm Phenomenex Gemini C18 column, 250 × 3 mm ID. Mobile phase: flow rate = 0.5 mL min^{-1}, isocratic elution, eluent H_2O 15 mM HCOOH/CH_3OH 70:30 (except for **5** 80:20). UV–visible detector set at 210 nm.

Reduction Reactions. The reduction of complexes **6** and **8** (0.5 mM) with ascorbic acid (5 mM) or glutathione (5 mM) was studied in HEPES (2 mM, pH 7.5) at 37 °C. All these reactions were followed by monitoring the decrease of the area of the chromatographic peaks of Pt complexes in HPLC-UV-MS. For the general chromatographic conditions, see previous section. The mobile phase was H_2O 15 mM HCOOH/CH_3OH 70:30 for **6** and 50:50 for **8**.

Reaction of 8 with Butanoic Acid. A solution of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, 114 mg, 0.30 mmol) and butanoic acid (18 μL, 0.20 mmol) in 3 mL of dry DMF was stirred at room temperature under N_2 atmosphere (Scheme 3). After 10 min, diisopropylethylamine (DIPEA, 73 μL, 0.42 mmol) was added and the reaction mixture was stirred for 15 min. Complex **8** (110 mg, 0.2 mmol) was added and the reaction mixture was stirred overnight at room temperature. The solvent was then removed by evaporation under reduced pressure. The product **12** was isolated by direct-phase chromatography using silica as stationary phase and a solution of 90:10 ethyl acetate/ethanol as eluent. Yield: 59 mg (48%). Anal. Calcd for: $C_{18}H_{31}ClN_2O_7Pt$ (617.98): C, 34.98; H, 5.06; N, 4.53; Pt, 31.57. Found: C, 35.29; H, 4.86; N, 4.16; Pt, 31.10%. ^{1}H NMR (CD_3OD): 0.95 (t, 3H, $OCO-CH_2-CH_2-CH_3$, ^3J = 7.4 Hz), 1.21–1.24 (m, 2H, $NH_2-CH-CH_2-CH_{2(ax)}$), 1.51–1.56 (m, 2H, $NH-CH-CH_{2(ax)}-CH_2$), 1.61–1.66 (m, 4H, $OCO-CH_2-CH_2-CH_3$ and $NH_2-CH-CH_2-CH_{2(eq)}$), 1.99–2.04 (m, 2H, $OCO-C-CH_2-CH_2$), 2.16–2.19 (m, 2H, $NH_2-CH-CH_{2(eq)}-CH_2$), 2.32 (t, 2H, $OCO-CH_2-CH_2-CH_3$, ^3J = 7.4 Hz), 2.63–2.67 (m, 2H, $OCO-C-CH_2-CH_2$), 2.72 (m, 2H, $OCO-C-CH_2-CH_2$), 2.73–2.87 (m, 2H, $NH_2-CH-CH_2-CH_2$), 3.19–3.21 (m, 2H, $Pt-O-CH_2-CH_2$), 4.13–4.24 (m, 2H, $Pt-O-CH_2-CH_2$) ppm. ^{13}C{^{1}H} NMR (CD_3OD, 125.7 MHz): 12.6 ($OCO-CH_2-CH_2-$

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CH_3), 15.5 (OCO−C−CH_2−CH_2), 18.1 (OCO−CH_2−CH_2−CH_3), 23.7 (NH_2−CH−CH_2−CH_2), 23.8 (NH_2−CH−CH_2−CH_2), 29.0 (OCO−C−CH_2−CH_2), 30.4 (NH_2−CH−CH_2−CH_2), 31.1 (NH_2−CH−CH_2−CH_2), 32.4 (OCO−C−CH_2−CH_2), 35.7 (OCO−CH_2−CH_2−CH_3), 56.0 (OCO−*C*−CH_2−CH_2), 60.3 (NH_2−*C*H−CH_2−CH_2), 62.2 (NH_2−*C*H−CH_2−CH_2), 64.8 (Pt−O−CH_2−CH_2, $^3J_{Pt-C}$ = 29.2), 66.5 (Pt−O−CH_2−CH_2, $^2J_{Pt-C}$ = 14.6 Hz), 174.6 (OCO−CH_2−CH_2−CH_3), 179.9 (OCO−C−CH_2−CH_2), 180.0 (OCO−C−CH_2−CH_2) ppm. ^{195}Pt NMR (CD_3OD, 107.2 MHz): δ 1013 ppm. ESI-MS (positive ion mode): 619.6 m/z $[M + H]^+$; calcd for $C_{18}H_{32}ClN_2O_7Pt$ 618.99 m/z $[M + H]^+$.

Reaction of 8 with *t*-Butoxycarbonyl-L-alanine. A solution of HBTU (114 mg, 0.30 mmol) and *t*-butoxycarbonyl-L-alanine (37 mg, 0.20 mmol) in 3 mL of DMF dry was stirred at room temperature under N_2 atmosphere (Scheme 3). After 10 min, DIPEA (71 μL, 0.42 mmol) was added and reaction was stirred for 15 min. Complex **8** (110 mg, 0.20 mmol) was then added and the reaction mixture was stirred at room temperature for 5 h. The solvent was then removed by evaporation under reduced pressure. The product **13** was isolated by direct-phase chromatography using silica as stationary phase and a solution of 90:10 ethyl acetate/ethanol as eluent. Yield: 33 mg (23%). Anal. Calcd for: $C_{22}H_{38}ClN_3O_9Pt$ (719.08): C, 36.75; H, 5.33; N, 5.84; Pt, 27.13. Found: C, 36.29; H, 5.50; N, 6.11; Pt, 27.07%. ^{1}H NMR (DMSO-d_6): 1.04 (m, 2H, NH_2−CH−CH_2−$CH_{2(ax)}$), 1.22 (m, 3H, OCO−NH−CH(CH_3)−COO), 1.37 (s, 9H, $CH_{3(BOC)}$), 1.47 (m, 4H, NH_2−CH−$CH_{2(ax)}$−CH_2 and NH_2−CH−CH_2−$CH_{2(eq)}$), 1.83 (m, 2H, OCO−C−CH_2−CH_2), 1.99 (m, 2H, NH_2−CH−$CH_{2(eq)}$−CH_2), 2.40−2.70 (m, 6H, OCO−C−CH_2−CH_2 and NH_2−*C*H−CH_2−CH_2), 3.07 (m, 2H, Pt−O−CH_2−CH_2), 4.00 (m, 1H, OCO−NH−*C*H(CH_3)−COO), 4.05−4.20 (m, 2H, Pt−O−CH_2−CH_2), 6.50−7.58 (m, 4H, NH_2−CH−CH_2−CH_2), 7.21 (m, 1H, OCO−N*H*−CH−(CH_3)−COO) ppm. ^{13}C{^{1}H} NMR (DMSO-d_6, 125.7 MHz): δ 15.7 (OCO−C−CH_2−CH_2), 17.0 (OCO−NH−CH(CH_3)−COO), 23.7 (NH_2−CH−CH_2−CH_2), 28.2 ($CH_{3(BOC)}$), 28.8 (OCO−C−CH_2−CH_2), 30.1 (NH_2−CH−*C*H_2−CH_2), 30.7 (NH_2−CH−CH_2−CH_2), 35.0 (OCO−C−CH_2−CH_2), 49.0 (OCO−NH−*C*H(CH_3)−COO), 55.6 (OCO−*C*−CH_2−CH_2), 58.9 (NH_2−*C*H−CH_2−CH_2), 61.4 (NH_2−*C*H−CH_2−CH_2), 64.5 (Pt−O−CH_2*C*H_2), 66.0 (Pt−O−CH_2−CH_2), 78.1 ($C_{(BOC)}$), 155.3 ($OCO_{(BOC)}$), 173.4 (OCO−NH−CH(CH_3)−COO), 176.8 (OCO−C−CH_2−CH_2), 176.9 (OCO−C−CH_2−CH_2) ppm. ^{195}Pt NMR (DMSO-d_6, 107.2 MHz): δ 1112 ppm. ESI-MS (positive ion mode): 720.6 m/z $[M + H]^+$; calcd for $C_{22}H_{39}ClN_3O_9Pt$ 720.09 m/z $[M + H]^+$.

Reaction of 8 with *n*-Butylamine. Complex **8** (50 mg, 0.091 mmol) was dissolved in 2 mL of dry DMF; at this solution *N*,*N*′-disuccinimidylcarbonate (DSC, 38 mg, 0.14 mmol) and DIPEA (25 μL, 0.14 mmol) were added (Scheme 3). The solution was left in the dark, under stirring at room temperature for 8 h. After this time *n*-butylamine (45 μL, 0.45 mmol) was added and the solution was left reacting overnight. The solvent was then removed by evaporation under reduced pressure and the residue was dissolved in the minimum quantity of acetone and then precipitated with diethyl ether. The precipitate was washed three times with diethyl ether. Finally, compound **14** was isolated by direct-phase chromatography using a solution of 90/10 ethyl acetate/ethanol as eluent. Yield: 32 mg (54%). Anal. Calcd for: $C_{19}H_{34}ClN_3O_7Pt$ (647.02): C, 35.27; H, 5.30; N, 6.49; Pt, 30.15. Found: C, 34.92; H, 5.64; N, 6.75; Pt, 30.09%. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, 3H, OCO−NH−CH_2−CH_2−CH_2−CH_3, ^3J = 8.5 Hz), 0.98−1.06 (m, 2H, NH_2−CH−CH_2−$CH_{2(ax)}$), 1.25 (m, 2H, OCO−NH−CH_2−CH_2−CH_2−CH_3), 1.37 (quint, 2H, OCO−NH−CH_2−CH_2−CH_2−CH_3, ^3J = 7.4 Hz), 1.45−1.49 (m, 4H, NH_2−CH−CH_2−$CH_{2(eq)}$ and NH_2−CH−$CH_{2(ax)}$−CH_2), 1.83 (quint, 2H, OCO−C−CH_2−CH_2, ^3J = 8.0 Hz), 1.96−2.02 (m, 2H, NH_2−CH−$CH_{2(eq)}$−CH_2), 2.44 (t, 2H, OCO−C−CH_2−CH_2, ^3J = 8.0 Hz), 2.48−2.59 (m, 4H, OCO−C−CH_2−CH_2, NH_2−*C*H−CH_2−CH_2), 2.74−2.77 (m, 2H, Pt−O−CH_2−CH_2), 2.96 (q, 2H, OCO−NH−CH_2−CH_2−CH_2−CH_3, ^3J = 6.1 Hz), 4.03−4.10 (m, 2H, Pt−O−CH_2−CH_2), 6.78−7.75 (m, 4H, NH_2−CH−CH_2−CH_2), 7.15 (t, 1H, OCO−N*H*−CH_2−CH_2−CH_2−CH_3, ^3J = 5.5 Hz). ^{13}C{^{1}H} NMR (DMSO-d_6, 125.7 MHz): δ 13.6 (NH−CH_2−CH_2−CH_2−CH_3), 15.7 (OCO−C−CH_2−CH_2), 19.4 (OCO−NH−CH_2−CH_2−CH_2−CH_3), 23.6 (NH_2−CH−CH_2−CH_2), 23.7 (NH_2−CH−CH_2−CH_2), 28.6 (OCO−C−CH_2−CH_2), 30.1 (NH_2−CH−*C*H_2−CH_2, $^3J_{Pt-C}$ = 26.3 Hz), 30.5 (NH_2−CH−CH_2−CH_2, $^3J_{Pt-C}$ = 33.9 Hz), 31.6 (OCO−NH−CH_2−*C*H_2−CH_2−CH_3), 35.3 (OCO−C−CH_2−CH_2), 39.9 (OCO−NH−*C*H_2−CH_2−CH_2−CH_3), 55.6 (OCO−C−CH_2−CH_2), 59.8 (NH_2−CH−CH_2−CH_2), 61.6 (NH_2−CH−CH_2−CH_2), 64.8 (Pt−O−CH_2−CH_2, $^3J_{Pt-C}$ = 22.3 Hz), 67.3 (Pt−O−*C*H_2−CH_2), 157.0 (OCO−NH−CH_2−CH_2−CH_2−CH_3), 176.7 (OCO−C−CH_2−CH_2), 176.8 (OCO−C−CH_2−CH_2). ^{195}Pt NMR (DMSO-d_6, 107.2 MHz): δ 1114 ppm. ESI-MS (positive ion mode): 648.5 m/z $[M + H]^+$; calcd for $C_{19}H_{35}ClN_3O_7Pt$ 648.03 m/z $[M + H]^+$.

Reaction of 8 with L-Alanine Ethyl Ester. Complex **8** (50 mg, 0.091 mmol) was dissolved in 2 mL of dry DMF; at this solution *N*,*N*′-disuccinimidylcarbonate (DSC, 38 mg, 0.14 mmol) and DIPEA (25 μL, 0.14 mmol) were added (Scheme 3). The solution was left in the dark, under stirring at room temperature for 8 h. After this time L-alanine ethyl ester (69 mg, 0.45 mmol) was added and the solution was left reacting overnight. The solvent was then removed by evaporation under reduced pressure and the residue was dissolved in the minimum quantity of acetone and then precipitated with diethyl ether. The precipitate was washed three times with diethyl ether. Finally, compound **15** was isolated by direct-phase chromatography using a solution of 90/10 ethyl acetate/ethanol as eluent. Yield: 35 mg (56%). Anal. Calcd for: $C_{20}H_{34}ClN_3O_9Pt$ (691.03): C, 34.76; H, 4.96; N, 6.08; Pt, 28.23. Found: C, 34.40; H, 5.26; N, 6.34; Pt, 28.55%. ^{1}H NMR (DMSO-d_6): 1.03−1.05 (m, 2H, NH_2−CH−CH_2−$CH_{2(ax)}$), 1.17 (t, 3H, OCO−CH_2−CH_3, ^3J = 14.1 Hz), 1.25 (d, 3H, OCO−NH−CH(CH_3)COO, ^3J = 7.3 Hz), 1.45−1.48 (m, 4H, NH_2−CH−CH_2−$CH_{2(eq)}$ and NH_2−CH−$CH_{2(ax)}$−CH_2), 1.82 (quint, 2H, OCO−C−CH_2−CH_2, ^3J = 8.7 Hz), 1.93−2.01 (m, 2H, NH_2−CH−$CH_{2(eq)}$−CH_2), 2.45 (t, 2H, OCO−C−CH_2−CH_2, ^3J = 8.0 Hz), 2.54−2.58 (m, 4H, NH_2−*C*H−CH_2−CH_2 and OCO−C−*C*H_2−CH_2), 2.83−2.89 (m, 2H, Pt−O−*C*H_2−CH_2), 4.01−4.12 (m, 5H, Pt−O−CH_2−*C*H_2 and OCO−NH−*C*H(CH_3)−COO and OCO−*C*H_2−CH_3), 6.66−7.76 (m, 4H, N*H*$_2$−CH−CH_2−CH_2), 7.61 (d, 1H, OCO−N*H*−CH(CH_3)−COO, ^3J = 7.3 Hz). ^{13}C{^{1}H} NMR (DMSO-d_6, 125.7 MHz): 14.1 (OCO−CH_2−*C*H_3), 15.7 (OCO−C−CH_2−*C*H_2), 17.0 (OCO−NH−CH(*C*H_3)COO), 23.6 (NH_2−CH−CH_2−*C*H_2), 23.7 (NH_2−CH−CH_2−CH_2), 28.6 (OCO−C−*C*H_2−CH_2), 30.1 (NH_2−CH−*C*H_2−CH_2, $^3J_{Pt-C}$ = 13.1 Hz), 30.6 (NH_2−CH−CH_2−*C*H_2, $^3J_{Pt-C}$ = 17.2 Hz), 35.2 (OCO−C−*C*H_2−CH_2), 49.3 (OCO−NH−*C*H(CH_3)COO), 55.6 (OCO−*C*−CH_2−CH_2), 59.8 (NH_2−CH−CH_2−CH_2), 60.4 (OCO−*C*H_2−CH_3), 61.6 (NH_2−*C*H−CH_2−CH_2), 64.9 (Pt−O−CH_2−*C*H_2, $^3J_{Pt-C}$ = 29.3 Hz), 66.8 (Pt−O−CH_2−CH_2, $^2J_{Pt-C}$ =20.2 Hz), 156.5 (OCO−NH), 172.8 (OCO−CH_2−CH_3), 176.7 (OCO−C−CH_2−CH_2), 176.9 (OCO−C−CH_2−CH_2) ppm. ^{195}Pt NMR (DMSO-d_6, 107.2 MHz): δ 1113 ppm. ESI-MS (positive ion mode): 692.5 m/z $[M + H]^+$; calcd for $C_{20}H_{35}ClN_3O_9Pt$ 692.04 m/z $[M + H]^+$.

■ ASSOCIATED CONTENT

Supporting Information

Crystallographic data of complex **8** in CIF format; NMR and ESI-MS spectra of complexes **5**−**15**; ESI-MS of Pt(IV) complex containing coordinated DMF; HPLC chromatograms of complexes **5** and **7** versus aging time in water and ESI-MS spectra of the hydrolysis products; HPLC chromatograms at different time points of the reduction of complexes **6** and **8** with glutathione and ascorbic acid; NMR and ESI-MS characterization of the coupling byproduct of **5**. This material is available free of charge via the Internet at http://pubs.acs.org. CCDC 1007075 contains the supplementary crystallographic data for this compound. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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The manuscript was written through the contributions of all the Authors. All the Authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Skeel, R. T. Antineoplastic Drugs and Biologic Response Modifiers: Classification, Use, and Toxicity of Clinically Useful Agents. In *Handbook of Cancer Chemotherapy;* 7th ed.; Skeel, R. T., Ed.; Lippincott Williams & Wilkins: Philadelphia, PA, 2007; Section II - Chemotherapeutic and Biotherapeutic Agents and Their Use, Ch 4.

(2) Gabano, E.; Ravera, M.; Osella, D. *Curr. Med. Chem.* **2009**, *16*, 4544−4580.

(3) Hall, M. D.; Hambley, T. W. *Coord. Chem. Rev.* **2002**, *232*, 49−67.

(4) Hall, M. D.; Alderden, R. A.; Zhang, M.; Beale, P. J.; Cai, Z.; Lai, B.; Stamp, A. P. J.; Hambley, T. W. *J. Struct. Biol.* **2006**, *155*, 38−44.

(5) Graf, N.; Lippard, S. J. *Adv. Drug Delivery Rev.* **2012**, *64*, 993−1004.

(6) Wang, X.; Guo, Z. *Chem. Soc. Rev.* **2013**, *42*, 202−224.

(7) Gaviglio, L.; Gross, A.; Metzler-Nolte, N.; Ravera, M. *Metallomics* **2012**, *4*, 260−266.

(8) Graf, N.; Mokhtari, T. E.; Papayannopoulos, I. A.; Lippard, S. J. *J. Inorg. Biochem* **2012**, *110*, 58−63.

(9) Abramkin, S.; Valiahdi, S. M.; Jakupec, M. A.; Galanski, M.; Metzler-Nolte, N.; Keppler, B. K. *Dalton Trans.* **2012**, *41*, 3001−3005.

(10) Mukhopadhyay, S.; Barnes, C. M.; Haskel, A.; Short, S. M.; Barnes, K. R.; Lippard, S. J. *Bioconjugate Chem.* **2008**, *19*, 39−49.

(11) Barnes, K. R.; Kutikov, A.; Lippard, S. J. *Chem. Biol. (Oxford, U. K.)* **2004**, *11*, 557−564.

(12) Barry, N. P. E.; Sadler, P. J. *ACS Nano* **2013**, *7*, 5654−5659.

(13) Butler, J. S.; Sadler, P. J. *Curr. Opin. Chem. Biol.* **2013**, *17*, 175−188.

(14) Zhang, J. Z.; Bonnitcha, P.; Wexselblatt, E.; Klein, A. V.; Najajreh, Y.; Gibson, D.; Hambley, T. W. *Chem.—Eur. J.* **2013**, *19*, 1672−1676.

(15) Chin, C. F.; Tian, Q.; Setyawati, M. I.; Fang, W.; Qing Tan, E. S.; Leong, D. T.; Ang, W. H. *J. Med. Chem.* **2012**, *55*, 7571−7582.

(16) Mailliet, P.; Bourrie, B.; Normand, A. French Patent FR 2 954 321 A1, July 15, 2010.

(17) Choi, S.; Filotto, C.; Bisanzo, M.; Delaney, S.; Lagasee, D.; Whitworth, J. L.; Jusko, A.; Li, C. R.; Wood, N. A.; Willingham, J.; Schwenker, A.; Spaulding, K. *Inorg. Chem.* **1998**, *37*, 2500−2504.

(18) Chen, L.; Lee, P. F.; Ranford, J. D.; Vittal, J. J.; Wong, S. Y. *J. Chem. Soc., Dalton Trans.* **1999**, 1209−1212.

(19) Zhang, J. Z.; Wexselblatt, E.; Hambley, T. W.; Gibson, D. *Chem. Commun.* **2012**, *48*, 847−849.

(20) Wexselblatt, E.; Gibson, D. *J. Inorg. Biochem.* **2012**, *117*, 220−229.

(21) Sinisi, M.; Intini, F. P.; Natile, G. *Inorg. Chem.* **2012**, *51*, 9694−9704.

(22) Wilson, J. J.; Lippard, S. J. *Chem. Rev.* **2014**, *114*, 4470−4495.

(23) Rendina, L. M.; Puddephatt, R. J. *Chem. Rev.* **1997**, *97*, 1735−1754.

(24) Gossage, R. A.; Ryabov, A. D.; Spek, A. L.; Stufkens, D. J.; van Beek, J. A. M.; van Eldik, R.; van Koten, G. *J. Am. Chem. Soc.* **1999**, *121*, 2488−2497.

(25) Margiotta, N.; Ranaldo, R.; Intini, F. P.; Natile, G. *Dalton Trans.* **2012**, *41*, 9689−9699.

(26) Giandomenico, C. M.; Abrams, M. J.; Murrer, B. A.; Vollano, J. F.; Rheinheimer, M. I.; Wyer, S. B.; Bossard, G. E.; Higgins, J. D., III *Inorg. Chem.* **1995**, *34*, 1015−1021.

(27) Galanski, M.; Keppler, B. K. *Inorg. Chem.* **1996**, *35*, 1709−1711.

(28) Whitfield, S. R.; Sanford, M. S. *Organometallics* **2008**, *27*, 1683−1689.

(29) Dunham, S. O.; Larsen, R. D.; Abbott, E. H. *Inorg. Chem.* **1993**, *32*, 2049−2055.

(30) Margiotta, N.; Ranaldo, R.; Intini, F. P.; Natile, G. *Dalton Trans.* **2011**, *40*, 12877−12885.

(31) Tamasi, G.; Cini, R.; Intini, F. P.; Sivo, M. F.; Natile, G. *Angew. Chem.* **2004**, *116*, 5191−5194.

(32) Lee, Y.; Yoo, K. H.; Jung, O. *Inorg. Chem. Commun.* **2003**, *6*, 249−251.

(33) Hall, M. D.; Dolman, R. C.; Hambley, T. W. *Met. Ions Biol. Syst.* **2004**, *42*, 297−322.

(34) Poon, G. K.; Raynaud, F. I.; Mistry, P.; Odell, D. E.; Kelland, L. R.; Harrap, K. R.; Barnard, C. F.; Murrer, B. A. *J. Chromatogr., A* **1995**, *712*, 61−66.

(35) Raynaud, F. I.; Odell, D. E.; Kelland, L. R. *Br. J. Cancer* **1996**, *74*, 380−386.

(36) Davies, M. S.; Hall, M. D.; Berners-Price, S. J.; Hambley, T. W. *Inorg. Chem.* **2008**, *47*, 7673−7680.

(37) Wexselblatt, E.; Yavin, E.; Gibson, D. *Angew. Chem., Int. Ed.* **2013**, *52*, 6059−6062.

(38) Washko, P.; Rotrosen, D.; Levine, M. *Am. J. Clin. Nutr.* **1991**, *54*, 1221S−1227S.

(39) Reiber, H.; Ruff, M.; Uhr, M. *Clin. Chim. Acta* **1993**, *217*, 163−173.

(40) Chen, Y.; Guo, Z.; Parsons, S.; Sadler, P. J. *Chem.—Eur. J.* **1998**, *4*, 672−676.

(41) Michelet, F.; Gueguen, R.; Leroy, P.; Wellman, M.; Nicolas, A.; Siest, G. *Clin. Chem.* **1995**, *41*, 1509−1517.

(42) Dabrowiak, J. C. *Metals in Medicine;* Wiley: Chichester, U.K., 2009.

(43) Žák, F.; Turánek, J.; Kroutil, A.; Sova, P.; Mistr, A.; Poulová, A.; Mikolin, P.; Žák, Z.; Kašná, A.; Záluská, D.; Neča, J.; Šindlerová, L.; Kozubík, A. *J. Med. Chem.* **2004**, *47*, 761−763.

(44) Ravera, M.; Gabano, E.; Zanellato, I.; Bonarrigo, I.; Escribano, E.; Moreno, V.; Font-Bardia, M.; Calvet, T.; Osella, D. *Dalton Trans.* **2012**, *41*, 3313−3320.

(45) Lemma, K.; House, D. A.; Retta, N.; Elding, L. I. *Inorg. Chim. Acta* **2002**, *331*, 98−108.

(46) Monti, E.; Gariboldi, M.; Maiocchi, A.; Marengo, E.; Cassino, C.; Gabano, E.; Osella, D. *J. Med. Chem.* **2005**, *48*, 857−866.

(47) Amblard, M.; Fehrentz, J.-A.; Martinez, J.; Subra, G. In *Fundamentals of Modern Peptide Synthesis in Peptide Synthesis, Applications (Methods in Molecular Biology Vol. 298);* Howl, J., Ed.; Humana Press: Totowa, NJ, 2005, Ch 1, pp 3−24.

(48) Gandin, V.; Marzano, C.; Pelosi, G.; Ravera, M.; Gabano, E.; Osella, D. *ChemMedChem* **2014**, *9*, 1299−1305.

(49) Ang, W. H.; Pilet, S.; Scopelliti, R.; Bussy, F.; Juillerat-Jeanneret, L.; Dyson, P. J. *J. Med. Chem.* **2005**, *48*, 8060−8069.

(50) Gabano, E.; Marengo, E.; Bobba, M.; Robotti, E.; Cassino, C.; Botta, M.; Osella, D. *Coord. Chem. Rev.* **2006**, *250*, 2158−2174.

(51) Freeman, W.; Pregosin, P. S. *J. Magn. Reson.* **1976**, *22*, 473−478.

(52) Appleton, T. G.; Hall, J. R.; Ralph, S. F. *Inorg. Chem.* **1985**, *24*, 4685−4693.

(53) Gramatica, P.; Papa, E.; Luini, M.; Monti, E.; Gariboldi, M. B.; Ravera, M.; Gabano, E.; Gaviglio, L.; Osella, D. *J. Biol. Inorg. Chem.* **2010**, *15*, 1157−1169.

(54) Ermondi, G.; Caron, G.; Ravera, M.; Gabano, E.; Bianco, S.; Platts, J. A.; Osella, D. *Dalton Trans.* **2013**, *42*, 3482−3489.
(55) Pichler, V.; Göschl, S.; Meier, S. M.; Roller, A.; Jakupec, M. A.; Galanski, M.; Keppler, B. K. *Inorg. Chem.* **2013**, *52*, 8151−8162.
(56) Pichler, V.; Heffeter, P.; Valiahdi, S. M.; Kowol, C. R.; Egger, A.; Berger, W.; Jakupec, M. A.; Galanski, M.; Keppler, B. K. *J. Med. Chem.* **2012**, *55*, 11052−11061.
(57) Tsipis, A. C.; Karapetsas, I. N. *Dalton Trans.* **2014**, *43*, 5409−5426.
(58) Dhara, S. C. *Indian J. Chem.* **1970**, *8*, 193−194.
(59) Harrison, R. C.; McAuliffe, C. A. *Inorg. Chim. Acta* **1980**, *46*, L15−L16.
(60) Rochon, F. D.; Gruia, L. M. *Inorg. Chim. Acta* **2000**, *306*, 193−204.
(61) Sheldrick, G. *SADABS: Siemens Area Detector Absorption Correction Software;* University of Goettingen: Goettingen, Germany, 1996.
(62) Sheldrick, G. *SHELXL97, Program for Structure Refinement;* University of Goettingen: Goettingen, Germany, 1997.
(63) Farrugia, L. J. *J. Appl. Crystallogr.* **1999**, *A32*, 837−838.
(64) *International Tables for X-Ray Crystallography;* Kynoch Press: Birmingham, U.K., 1974, Vol. *4*.
(65) Johnson, C. K.; Burnett, M. N. *ORTEPIII;* Technical Report ORNL-6895 for the Oak Ridge National Laboratory: Oak Ridge, TN, 1996.

dx.doi.org/10.1021/ic501446b | *Inorg. Chem.* 2014, 53, 9326−9335