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Bulky *N*(,*N*)-(Di)alkylethane-1,2-diamineplatinum(II) Compounds as Precursors for Generating Unsymmetrically Substituted Platinum(IV) Complexes

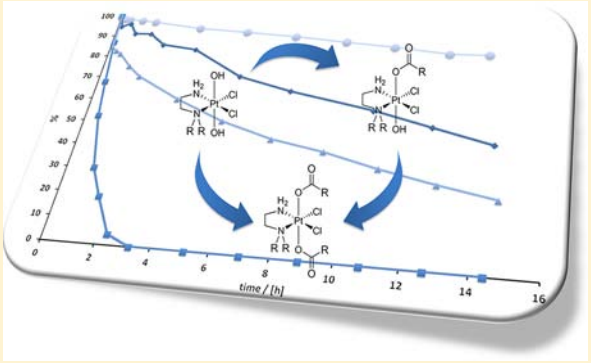
Verena Pichler,[†] Simone Göschl,[†] Samuel M. Meier,[†,‡] Alexander Roller,[†] Michael A. Jakupec,[†,‡] Markus Galanski,*[,†,‡] and Bernhard K. Keppler*[,†,‡]

[†]Institute of Inorganic Chemistry, University of Vienna, Währinger Straße 42, A-1090 Vienna, Austria

[‡]Research Platform "Translational Cancer Therapy Research", University of Vienna, Währinger Straße 42, A-1090 Vienna, Austria

Supporting Information

ABSTRACT: Investigations of the influence of bulky groups in the equatorial ligand sphere of platinum(IV) compounds on the complexes' stability and reaction pattern were performed. Four dihydroxidoplatinum(IV) complexes were reacted with anhydrides, cinnamoyl chloride, and *n*-propyl isocyanate and yielded the symmetric dicarboxylated products or, if steric hindrance was observed, unsymmetrically substituted monocarboxylated analogues. With the aim of raising the steric demand, the following ligands were chosen: *N*-cyclohexylethane-1,2-diamine, *N*,*N*-dimethylethane-1,2-diamine, *N*,*N*-diethylethane-1,2-diamine, and *N*,*N*-diisopropylethane-1,2-diamine. All of the novel complexes were characterized by electrospray ionization mass spectrometry (ESI-MS), one- and two-dimensional NMR spectroscopy, elemental analysis, and reversed-phase HPLC; complexes **B3, C3**, **C6**, and **D4** were also analyzed by X-ray diffraction. Additionally, the cytotoxicities of 10 compounds toward the cisplatin-sensitive cell line CH1 and the intrinsically cisplatin-resistant cell lines A549 and SW480 were investigated, and IC_{50} values down to the nanomolar range were found. To aid in the interpretation of structure−activity relationships, log k_w values as a measure for the lipophilicity were determined for all of the new complexes, and the rates of reduction of **C1**, **C3**, and **C4** relative to satraplatin were determined by means of NMR spectroscopy and ESI-MS.



INTRODUCTION

Platinum(II) compounds, namely, cisplatin, carboplatin, and oxaliplatin, belong to an extremely successful group of chemotherapeutics in worldwide clinical application.[1,2] Although these anticancer platinum(II) complexes are widely used in the treatment of cancer, certain limitations have been encountered, mainly related to their toxicity, low kinetic inertness, application in a limited number of cancer types because of intrinsic drug resistance, and development of acquired resistance.[3−8]

The development of platinum(IV) prodrugs is a promising approach to overcome the mentioned limitations. To date, four platinum(IV) complexes have entered clinical trials (Figure 1), but none of them has been approved for clinical use. (*OC*-6-33)-Dichloridodihydroxidobis(isopropylamine)platinum(IV) (iproplatin, CHIP, JM9) exhibited high solubility coupled with low toxicity but was abandoned because of its lack of activity.[9] Dose-limiting neurotoxicity led to the abandonment of clinical trials of (*OC*-6-22)-tetrachlorido(*trans*-cyclohexane-1,2-diamine)platinum(IV) (tetraplatin, ormaplatin).[10] (*OC*-6-43)-Bis(acetato)amminedichlorido(cyclohexylamine)platinum(IV) (satraplatin, JM216) is currently the most advanced compound in different phase-I and -II trials, but the Satraplatin and Prednisone Against Refractory Cancer (SPARC) phase-III trial showed no significant improvement in overall survival rates compared with standard treatment.[11] (*OC*-6-43)-Bis(acetato)-(adamantylamine)amminedichloridoplatinum(IV) (LA-12) offers a quite similar ligand sphere compared to satraplatin, and

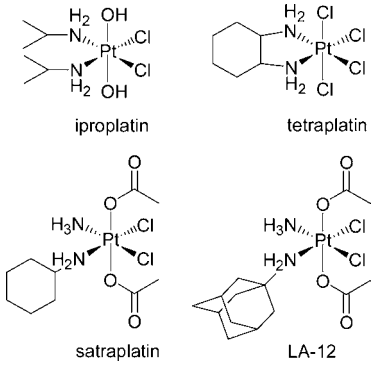


Figure 1. Platinum(IV) complexes that have entered clinical trials.

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clinical trials are currently in progress or have been finished.[12−14] The high kinetic inertness of platinum(IV) complexes is the reason for a range of advantages compared with the more labile platinum(II) complexes. They include (i) an extension of synthetic pathways known from organic chemistry, (ii) possible oral availability, and (iii) reduced side effects.[15] Interactions with biomolecules take place mainly via reduction of the platinum(IV) prodrug to platinum(II) species. These platinum(II) drugs then exhibit the same reactivity as related platinum(II) compounds (i.e., binding to thiol-containing molecules is observed within seconds).[16,17]

The most promising platinum(IV) compounds, satraplatin and LA-12, share a sterically demanding amine ligand at an equatorial position. Another effective platinum complex offering a bulky ligand is picoplatin (ZD0473, JM472, AMD473), which showed less binding to glutathione or methionine compared with cisplatin. This behavior is attributable to the bulky picoline ligand that protects the platinum center from axial attack, thereby reducing side reactions with biomolecules in the human body.[18] As recently reported, steric effects may dictate the synthetic access to platinum(IV) compounds. For example, employing *N*,*N*-dimethylethane-1,2-diamine as a chelating equatorial ligand in (*OC*-6-43)-dichlorido(*N*,*N*-dimethylethane-1,2-diamine)-dihydroxidoplatinum(IV) leads to selective monocarboxylation in the reaction of this complex with succinic anhydride.[19]

In this work, we examined the influence of increasing the size of the alkyl group(s) positioned on one of the equatorial amines on the synthesis, stability, and lipophilicity of symmetrically (X−Pt−X) and unsymmetrically (X−Pt−Y) trans-substituted platinum(IV) complexes. It has to be stated that the unsymmetric trans substitution in all cases generated a chiral platinum center, and therefore, all of the unsymmetrically substituted complexes were asymmetric and present as racemic mixtures. Four platinum(II) precursors **A**−**D** based on the ligands *N*,*N*-diisopropylethane-1,2-diamine, *N*,*N*-diethylethane-1,2-diamine, *N*,*N*-dimethylethane-1,2-diamine, and *N*-cyclohexylethane-1,2-diamine, respectively (Figure 2), were oxidized

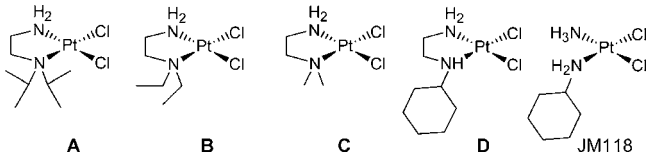

Figure 2. Structures of platinum(II) building blocks **A**−**D**. The structure of JM118 is shown for comparison.

to the *trans*-hydroxidoplatinum(IV) analogues and further reacted with succinic or acetic anhydride,[20,21] cinnamoyl chloride,[22] or *n*-propyl isocyanate[23] in accord with recently published procedures. Furthermore, the mono- and disubstituted complexes were investigated with respect to their cytotoxic potency in three human cancer cell lines, namely, CH1 (ovarian carcinoma), A549 (non-small-cell lung carcinoma), and SW480 (colon adenocarcinoma). Additionally, the rates of reduction of three selected complexes were determined.

RESULTS AND DISCUSSION

Syntheses. All of the chelating diamines were obtained from commercial suppliers except for *N*-cyclohexylethane-1,2-diamine (**S2**), which was synthesized in two steps (see the Supporting Information): after imine formation using cyclohexanone and *N*-Boc-ethane-1,2-diamine, reduction of the imine and cleavage of the *N*-Boc protecting group gave amine **S2** in 82% yield. The starting platinum(II) complexes **A**−**D** (Figure 2; also see the Supporting Information) and the platinum(IV) complexes **C1** and **C2** (Figure 3) were synthesized according to standard literature procedures.[19,24]

All of the platinum(II) precursors were oxidized in aqueous H_2O_2 solution at room temperature, and the dihydroxidoplatinum(IV) complexes **A1**−**D1** (Figure 3) were obtained in good yields (55−99%). Compound **A1** was unstable in solution, most probably because of the bulky isopropyl groups, and therefore, further reactions could not be performed (Figure S1 in the Supporting Information). The stable dihydroxido compounds **B1**, **C1**, and **D1** were investigated with regard to their reactivity toward succinic anhydride, acetic anhydride, cinnamoyl chloride, and *n*-propyl isocyanate, yielding symmetrically and unsymmetrically substituted compounds (see the overview in Figure 3).

The reactions of dihydroxido complexes **B1** and **C1** with a 4-fold excess of succinic anhydride led to the monocarboxylated compounds **B2** and **C2** in excellent yields (Scheme 1). The diacetato complexes **B4**, **C4**, and **D4** were observed when acetic anhydride was used as the solvent (>100 equiv excess) (Scheme 2). When the amount of acetic anhydride was reduced to 2 equiv, monoadducts **B3** and **C3** were isolated in excellent yields of 75−99%. In case of the satraplatin-related educt **D1**, a selective unsymmetric synthesis could not be performed.

Changing the reactant to cinnamoyl chloride (Scheme 3) demonstrated more clearly the influence of steric hindrance of the equatorial ligand. The ethyl groups of *N*,*N*-diethylethane-1,2-diamine blocked one of the axial OH groups most effectively, as a small excess (2.1 equiv) led to the unsymmetrically substituted compound **B5**. A small amount of symmetric byproduct **B6** (<10% yield; see the Supporting Information) was formed by increasing the excess to 4 equiv, but no complete turnover could be accomplished. Nevertheless, the synthesis of a mixed dicarboxylated product was performed by suspending **B5** in excess acetic anhydride, which yielded **B9** in excellent yield (84%). Reaction of the less sterically hindered methyl derivative **C1** and even a small excess of cinammoyl chloride led to the dicarboxylated compound **C6** (∼65% yield). However, when equi- to dimolar amounts of the reagents were used, the corresponding monocarboxylation product **C5** could be obtained, but further separation with column chromatography was necessary to remove minor amounts of the symmetrically substituted byproduct. Once again, only the dicarboxylated derivative **D6** was found for the satraplatin analogue **D1**.

For the most reactive species, *n*-propyl isocyanate, a 2- or 1.2-fold excess led to the monocarboxylated products **B7** and **C7**. Increasing the excess of reagent to >100 equiv yielded dicarboxylated products **B8** (see the Supporting Information) and **C8** (Scheme 4). The latter complex was obtained in good yield, whereas for **B8** a yield of only ∼20% was detected. Furthermore, the compound could not be separated on silica, as it decomposed under normal-phase chromatographic conditions.

In summary, it can be stated that the steric hindrance of the chelating equatorial ligand increases in the following order: *N*-cyclohexylethane-1,2-diamine (series **D**) < *N*,*N*-dimethylethane-1,2-diamine (series **C**) < *N*,*N*-diethylethane-1,2-diamine (series **B**) < *N*,*N*-diisopropylethane-1,2-diamine (series **A**). In

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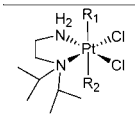
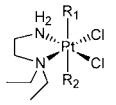
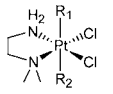
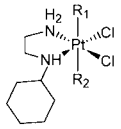
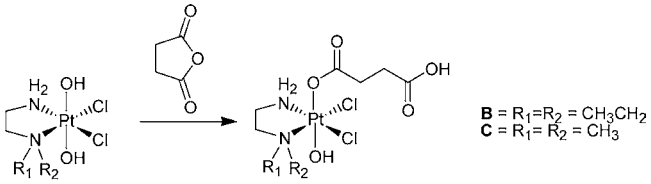
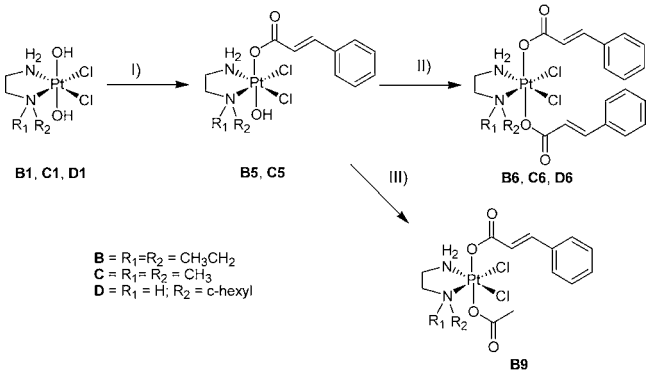
	R_1=R_2=OH	R_1=OH R_2=succ	R_1=OH R_2=oac	R_1=R_2=oac	R_1=OH R_2=cin	R_1=R_2=cin	R_1=OH R_2=carb	R_1=R_2=carb
	89% **A1** unstable, decomposition	–	–	–	–	–	–	–
	83% **B1**	89% **B2**	99% **B3**	95% **B4**	71% **B5**	~ 10% **B6**	85% **B7**	~ 20% **B8**
	99% **C1**	73% **C2**	75% **C3**	81% **C4**	35% **C5**	66% **C6**	45% **C7**	65% **C8**
	55% **D1**	–	–	66% **D4**	–	54% **D6**	–	64% **D8**

Figure 3. Schematic overview of the investigated complexes, including their yields. Abbreviations: succ = 3-carboxypropanoato; oac = acetato; cin = (2*E*)-3-phenylprop-2-enoato; carb = propylcarbamoato.

Scheme 1. Reactivity of Dihydroxidoplatinum(IV) Compounds toward Succinic Anhydride



Scheme 3. Reactivity of Dihydroxidoplatinum(IV) Compounds toward Cinnamoyl Chloride[a]



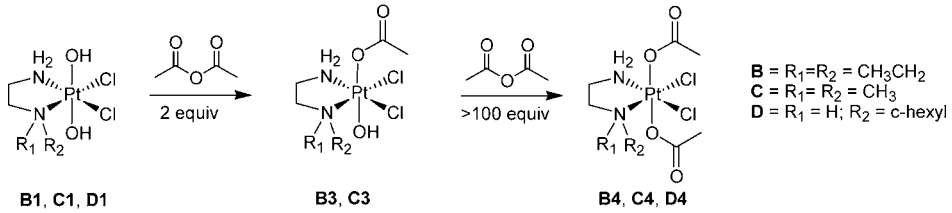
[a]Conditions: (I) 2 to 2.1 equiv of cinnamoyl chloride, pyridine, acetone; (II) 4 equiv of cinnamoyl chloride, pyridine, acetone; (III) acetic anhydride.

the sterically most demanding cases, unstable compounds were obtained, in particular **A1** and **B8**. It seems that increasing the bulkiness at the equatorial position increases the ease with which monocarboxylated products can be synthesized. However, this facilitation of the synthesis of unsymmetrically substituted products is associated with a decrease in compound stability.

Characterization. All of the novel metal complexes were characterized by NMR spectroscopy, electrospray ionization mass spectrometry (ESI-MS), reversed-phase HPLC (RP-HPLC), and elemental analysis, and complexes **B3**, **C3**, **C6**, and **D4** were also analyzed by X-ray crystallography.

The ^{1}H, ^{13}C, ^{15}N, and ^{195}Pt NMR resonances were in the expected ranges; ^{195}Pt, ^{15}N, and selected ^{1}H chemical shifts are listed in Table S1 in the Supporting Information. In the ^{1}H NMR spectrum, the N*H* signals of the equatorially coordinated *N*(,*N*)-(di)alkylethane-1,2-diamine ligands were detected be-

Scheme 2. Reactivity of Dihydroxidoplatinum(IV) Compounds toward Acetic Anhydride



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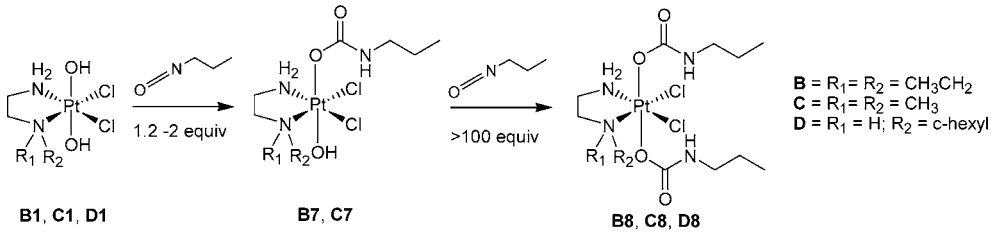
Scheme 4. Reactivity of Dihydroxidoplatinum(IV) Compounds toward *n*-Propyl Isocyanate



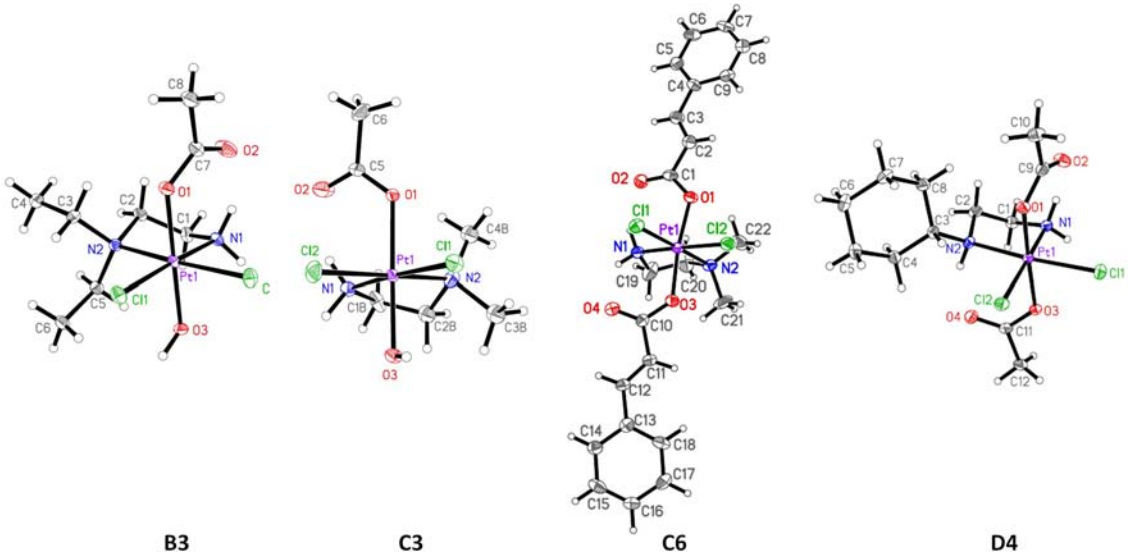


Figure 4. ORTEP views of **B3**, **C3**, **C6**, and **D4** with atom-labeling schemes. Thermal ellipsoids are drawn at the 50% probability level.

Table 1. Crystal Data and Details of Data Collection for B3, C3, C6, and D4

	B3	C3	C6	D4
empirical formula	$C_8H_{20}Cl_2N_2O_3Pt$	$C_6H_{16}Cl_2N_2O_3Pt$	$C_{22}H_{26}Cl_2N_2O_4Pt$	$C_{12}H_{24}Cl_2N_2O_4Pt$
formula weight	458.25	430.20	648.44	526.32
space group	triclinic, $P\bar{1}$	monoclinic, $P2_1/c$	triclinic, $P\bar{1}$	monoclinic, $P2_1/c$
a (Å)	7.3751(4)	7.8017(5)	7.8654(6)	10.3015(3)
b (Å)	7.7180(4)	12.6113(8)	12.8074(11)	22.4208(8)
c (Å)	12.7856(9)	11.9901(7)	13.3055(12)	7.8210(3)
α (deg)	101.004(2)	–	65.149(4)	–
β (deg)	96.950(3)	98.306(2)	72.991(4)	111.494(1)
γ (deg)	109.822(2)	–	85.489(4)	–
V (Å^3)	658.33(7)	1167.33(13)	1161.51(17)	1680.77(10)
Z	2	4	2	4
λ (Å)	0.71073	0.71073	0.71073	0.71073
ρ_{calcd} (g cm^{-3})	2.312	2.448	1.854	2.080
crystal size (mm^3)	0.10 × 0.04 × 0.03	0.15 × 0.04 × 0.02	0.10 × 0.10 × 0.03	0.15 × 0.15 × 0.10
T (K)	100(2)	100(2)	100(2)	100(2)
μ (mm^{-1})	11.057	12.463	6.301	8.681
R_1[a]	0.0155	0.0232	0.0370	0.0249
wR_2[b]	0.0340	0.0629	0.0716	0.0538
GOF[c]	1.008	1.026	0.963	1.036

[a] $R_1 = \sum||F_o| - |F_c||/\sum|F_o|$. [b] $wR_2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$. [c] GOF $= \{\sum[w(F_o^2 - F_c^2)^2]/(n - p)\}^{1/2}$, where n is the number of reflections and p is the total number of parameters refined.

tween 6.9 and 10.7 ppm, strongly depending on the axial ligand sphere. In the case of the dihydroxidoplatinum(IV) compounds **A1**–**C1**, the N*H* signals appeared as broad singlets at ∼7 ppm, whereas the signals of the dicarboxylated compounds **B4**, **B6**, **C4**, and **C6** were shifted to lower field at ∼9.5 ppm. The unsymmetrically substituted compounds **B2**, **B3**, **B5**, **C2**, **C3**, and **C5** featured a splitting of the N*H* signals into two broad singlets as a result of the chiral platinum center, one at ∼7 ppm and the other at ∼9.5 ppm. Changing the carboxylato group to carbamate (complexes **B7**, **B8**, **C7**, and **C8**) led to a downfield shift of the corresponding signals to 10.0–10.6 ppm. Therefore, these signals are suitable for differentiating between mono- and

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Table 2. Cytotoxicities toward Three Human Cancer Cell Lines

	IC_{50} (μM)[a]			IC_{50} ratio	
complex	CH1	A549	SW480	A549/CH1	SW480/CH1
B1	2.5 ± 0.2	105 ± 35	45 ± 12	42	18
B2	9.6 ± 3.2	239 ± 31	150 ± 26	25	16
B3	0.84 ± 0.12	30 ± 9	5.5 ± 1.3	36	7
B4	0.72 ± 0.11	30 ± 3	2.6 ± 0.2	42	4
B7	0.26 ± 0.06	11 ± 1	1.7 ± 0.1	42	7
C3	1.8 ± 0.4	77 ± 13	23 ± 6	43	13
C4	0.87 ± 0.10	65 ± 14	4.4 ± 0.7	75	5
C7	1.0 ± 0.3	26 ± 2	5.8 ± 0.7	26	6
C8	0.12 ± 0.03	9.9 ± 1.4	0.47 ± 0.03	83	4
D4	0.89 ± 0.15	16 ± 2	4.2 ± 0.3	18	5
C1[b]	3.3 ± 1.4	111 ± 15	115 ± 10	34	35
C2[b]	8.4 ± 1.0	145 ± 39	75 ± 13	17	9
cisplatin[b]	0.16 ± 0.03	1.3 ± 0.4	3.5 ± 0.3	8	22

[a]Half-maximal inhibitory concentrations in the MTT assay (96 h exposure). Values are means ± standard deviations obtained from at least three independent experiments. [b]Data taken from ref 19.

bisderivatization. Furthermore, all of the carbamate-containing complexes showed two signals for the CON*H* proton in a ratio of ∼4:1 due to the generation of conformational isomers.[23] This effect could also be studied in the ^{195}Pt NMR spectrum. In the case of **D8**, four platinum signals and two sets of ^{1}H signals were observed, as the combination of the chiral center of the *N*-cyclohexylethane-1,2-diamine ligand and the conformational isomers of the carbamate group led to diastereoisomers. Heating of the sample resulted in the reduction to one set of signals (Figure S2 in the Supporting Information).

All of the measured ^{195}Pt chemical shifts were in the expected range and in accordance with literature values indicating a $Pt^{IV}Cl_2N_2O_2$ geometry.[25,26] Furthermore, the ^{195}Pt NMR spectra displayed differences between the dihydroxido, monocarboxylated, and dicarboxylated products. For the dihydroxido compounds **A1**, **B1**, and **C1**, the ^{195}Pt chemical shifts were 2412, 2493, and 2764 ppm, respectively. Consequently, the platinum shift relocation increased with increasing size of the sterically demanding ligand. In the case of system **B** based on the *N*,*N*-diethyl chelating ligand, the ^{195}Pt shift started in the 2578−2587 ppm range indicating monocarboxylation (**B2**, **B3**, and **B5**), while a shift of 2819−2615 ppm indicated dicarboxylation (compounds **B4** and **B6**). Changing the chelating equatorial ligand to *N*,*N*-dimethylethane-1,2-diamine (system **C**) resulted in an upfield shift of ∼90 ppm for the monohydroxido (**C2** and **C3**, ∼2500 ppm) and dicarboxylato complexes (**B4** and **B9**, ∼2800 ppm). The resonances of **D4**, **D6**, and **D8** were in the range 2742−2812 ppm without an observable influence of the axial ligands.

^{15}N signals were found for the monocarboxylato compounds between −4.0 and −6.8 ppm and for the dicarboxylato compounds between −16.6 and −20.5 ppm. Once more, complexes bearing the chelating ligand including cyclohexylamine did not conform to this behavior, as their ^{15}N shifts appeared at ∼9.0 ppm.

Crystal Structures. Results of the X-ray diffraction studies on single crystals of **B3**, **C3**, **C6**, and **D4** are shown in Figure 4. Crystal data, data collection parameters, and structure refinement details are given in Table 1. Selected bond lengths and angles are listed in Table S2 in the Supporting Information. Complexes **C6**/**B3** and **C3**/**D4** crystallized in the triclinic space group $P\bar{1}$ and the monoclinic space group $P2_1/c$, respectively. The platinum(IV) centers feature an octahedral coordination sphere with the general formula [*cis*,*cis*,*trans*-$Pt^{IV}Cl_2N_2O_2$]. The bond distances are close to 2.3 Å for Pt−Cl and 2.0 Å for Pt−N and Pt−O. These findings are in accordance with similar structures reported in the literature.[26,27] The ethane-1,2-diamine ligands form a five-membered PtN_2C_2 chelate cycle with the Pt(IV) center. The $\Theta_{N1-C1-C2-N2}$ torsion angle, which serves as a measure of the deviation of the chelate ring from planarity, is −56.9(2)° in **B3**, 56(3)° in **C3**, 51.8(9)° in **C6**, and −53.8(3)° in **D4**.

The steric effects of the *N*(,*N*)-(di)alkylethane-1,2-diamine ligands are also reflected in the X-ray structures. Increasing the length of the alkyl chain leads to deformation of the square-planar platinum core. The Cl−Pt−N_{alkyl} angle increases in going from *N*-cyclohexylethane-1,2-diamine [**D4**, 93.58(8)°] and *N*,*N*-dimethylethane-1,2-diamine [**C3**, 92.93(16)°; **C6**, 93.79(15)°] to *N*,*N*-diethylethane-1,2-diamine [**B3**, 95.44(5)°], whereas on the other side, the Cl−Pt−NH_2 angle decreases in going from **D4** [92.11(7)°] to **C3** [90.3(2)°] to **C6** [89.25(14)°] to **B3** [88.60(6)°]. The remaining angles, Cl−Pt−Cl and NH_2−Pt−N_{alkyl}, are barely influenced. The extent of the Cl−Pt−N_{alkyl} angle seems to correlate with the instability of the platinum(IV) complex in this series.

A common observation in the crystal structures is the presence of intramolecular hydrogen bonds between the axial oxygens and the equatorial NH_2 of the ligand. In the symmetrically substituted compound **C6**, the amine nitrogen N1 is involved in intramolecular hydrogen bonding to oxygen atoms O2 and O4 (donor−acceptor contacts of 2.730 and 2.726 Å, respectively), whereas in the case of **D4**, one hydrogen bond is located between N1 and O2 (N1···O2, 2.691 Å) and the second between N2 and O4 (N2···O4, 2.705 Å). In the two unsymmetrically substituted complexes **B3** and **C3**, only one hydrogen bond between N1 and O2 is formed. The axial OH ligand enables further intermolecular hydrogen bonds, which are found in both cases between coordinated hydroxido oxygens and N1.

Partition Coefficients. The partition coefficient (k_w), which is the ratio of the concentrations of a drug in lipophilic and hydrophilic phases, can describe the lipophilicity of a complex and consequently aid in predicting a part of its pharmacokinetic behavior in a living organism. In general, passive diffusion through a membrane bilayer is easier for a compound with increasing lipophilic character. A clear-cut

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relationship between lipophilicity and drug accumulation was also reported for platinum anticancer agents.[28,29] In recent times, RP-HPLC measurements have increasingly been used to determine the lipophilicity of a drug, as the method is highly reproducible, fast, and independent of concentration. As the chromatographic system requires an organic modifier (in our case methanol), extrapolation to 0% methanol is essential to obtain the value of log k_w, which contains information about the lipophilicity of the compound.[27,30,31]

Values of log k_w for the synthesized compounds are shown in Table S3 in the Supporting Information. The value for complex **A1** could not be determined because of the instability of the complex. The investigated set of compounds covers a wide range of log k_w values from 0.05 for the least lipophilic compound (**C3**) to 4.42 for the most lipophilic compound (**C6**). In general, the lipophilic character increases with the alkyl-chain length in the equatorial ligand: *N*,*N*-dimethylethane-1,2-diamine (series **C**) < *N*,*N*-diethylethane-1,2-diamine (series **B**) < *N*-cyclohexylethane-1,2-diamine (series **D**). Furthermore, compounds with log k_w > 2.84 showed insufficient solubility for cell-culture studies.

Cytotoxicities toward Human Cancer Cell Lines. Ten compounds with sufficient aqueous solubility were investigated with respect to their cytotoxicities toward three human cancer cell lines (CH1, A549, and SW480) by means of a colorimetric microculture (MTT) assay, and the results were compared with those for cisplatin and the previously published compounds **C1** and **C2** (Table 2).[19]

All of the tested complexes showed a strong capacity to inhibit growth of the cisplatin-sensitive CH1 cell line, with half-maximal inhibitory concentration (IC_{50}) values in the range 9.6−0.12 μM. In particular, **B7** and **C8** showed antiproliferative activity in nanomolar concentrations and were as potent as cisplatin. In general, the dicarboxylated complexes were more active than their respective monocarboxylated counterparts (e.g., **C8** vs **C7** and **C4** vs **C3**), presumably because of their greater lipophilic character. However, the influence of lipophilicity on the cytotoxicity was not always observable, particularly with respect to increasing the alkyl-chain length of the *N*(,*N*)-(di)alkylethane-1,2-diamine chelating ligand. The IC_{50} values for the diacetato compounds **B4**, **C4**, and **D4** were found in a small range of 0.72−0.89 μM, although their log k_w values varied broadly from 0.7 to 2.22.

A similar activity pattern was seen in SW480 cells. Except for **B1**, **B2**, and **C3**, the tested compounds were equally as potent as or more potent than cisplatin, with IC_{50} values as low as 0.47 μM (**C8**).

The intrinsically cisplatin-resistant A549 cell line seemed to be indifferent to the mono- and dicarboxylated products, as compounds of each matched pair (**B3** vs **B4** and **C3** vs **C4**) showed similarly moderate cytotoxicities with IC_{50} values of ∼30 and ∼70 μM, respectively. An exception to this was the carbamate pair **C7** and **C8**, where the monocarbamate yielded an IC_{50} value ∼2.5 times higher than the dicarbamate. Interestingly, when only the influence of the equatorial chelate ligand was considered, the cytotoxicity toward this cell line decreased in the order *N*-cyclohexylethane-1,2-diamine (series **D**) > *N*,*N*-diethylethane-1,2-diamine (series **B**) > *N*,*N*-dimethylethane-1,2-diamine (series **C**), in accordance with decreasing lipophilicity. However, as expected, the least cytotoxic compound, **B2**, featured a free carboxylic group, which is in accordance with previous findings.[19,26,27] Upon comparison of the ratios of the IC_{50} values for the intrinsically cisplatin-resistant cell lines A549 and SW480 to those for the cisplatin-sensitive cell line CH1, it became obvious that cytotoxicities of the platinum(IV) complexes were affected to different extents relative to cisplatin. A549 cells were more resistant to all of the platinum(IV) complexes (A549/CH1 IC_{50} ratios = 18−83) than to cisplatin (A549/CH1 IC_{50} ratio = 8). Furthermore, A549 cells were more resistant to dicarboxylated platinum(IV) complexes than to monocarboxylated ones (e.g., the A549/CH1 IC_{50} ratios for **C3** and **C4** were 43 and 75, respectively). In contrast, SW480 cells were less resistant to the dicarboxylated complexes than to compounds with a Pt−OH group (e.g., SW480/CH1 IC_{50} ratio = 13 for **C3** vs 5 for **C4**). The investigated complexes had SW480/CH1 IC_{50} ratios of 4−18, indicating that the extent to which SW480 resistance affects the potency of these complexes is generally less than that for cisplatin (SW480/CH1 IC_{50} ratio = 22).

Cytotoxicity versus log k_w. Semilogarithmic plots of log k_w versus IC_{50} (Figure S3 in the Supporting Information) showed a very rough correlation for the tested complexes. As the more lipophilic compounds always showed increased cytotoxicity, we suggest that there is an influence of lipophilicity, although it is neither strong nor strictly linear.[32] Especially compound **B1** constitutes an exception. These results are in good accordance with previously published results showing that the terminal group of the axial ligand has a conspicuous influence on the cytotoxicity of platinum(IV) compounds[33] and that correlation of log k_w with IC_{50} is rather poor.[34]

Rates of Reduction. As Wexselblatt and co-workers recently showed, the rate of reduction of platinum(IV) complexes is dependent on the axial ligands, at least in the case of oxaliplatin derivatives.[15,35] To investigate whether similar behavior exists for platinum(IV) complexes containing an equatorial N_2Cl_2 coordination sphere, the rates of reduction of complexes **C1**, **C3**, and **C4** and the reference compound satraplatin (Figure 5) were measured by ^{1}H NMR spectroscopy and ESI-MS.

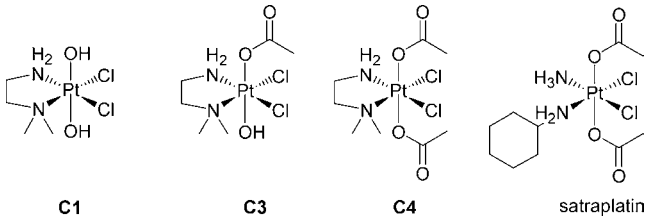

Figure 5. Complexes used to investigate the rate of reduction.

A clear trend in the reduction rates was observed in ^{1}H NMR experiments depending on the axial ligands (Figure 6). During these experiments, the metal complex was reacted with ascorbic acid at a 1:2 molar ratio and a final metal concentration of 0.5 mM. The dihydroxido compound **C1** was reduced comparatively slowly within a few days. The monohydroxido complex **C3** showed an increased rate of reduction with a half-life of ∼14 h. Intriguingly, the diacetato complex **C4** displayed a half-life of ∼30 min and was completely reduced within 4 h. Although satraplatin features an analogous ligand sphere, it showed a half-life of ∼6 h for reduction. Varbanov et al.[34] showed that a compound similar to **C4** and satraplatin has a half-life of ∼5 h, which corresponds to the rate of reduction of satraplatin rather than **C4**. The ∼10-fold increase in the reduction rate for **C4** compared with satraplatin could be a result of diminished

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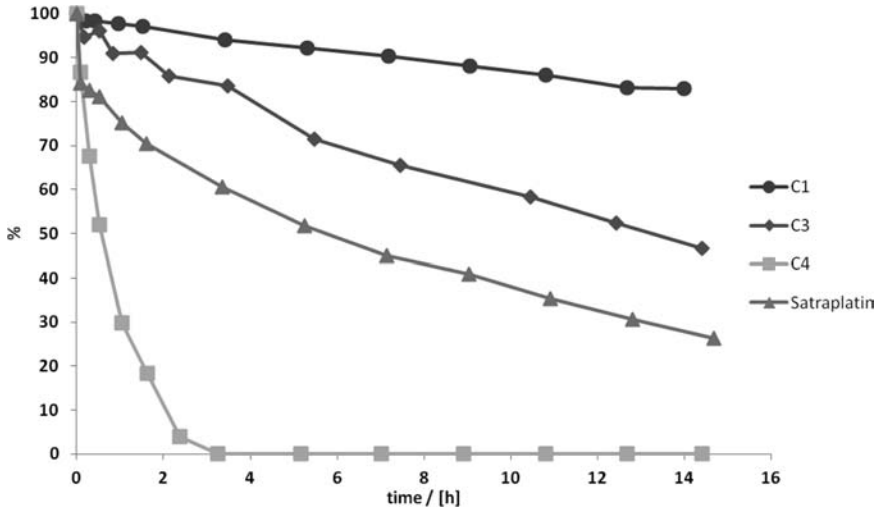

Figure 6. Time courses for the reductions of compounds **C1** (●), **C3** (♦), **C4** (■), and satraplatin (▲) incubated with a 2-fold excess of ascorbic acid in phosphate buffer.

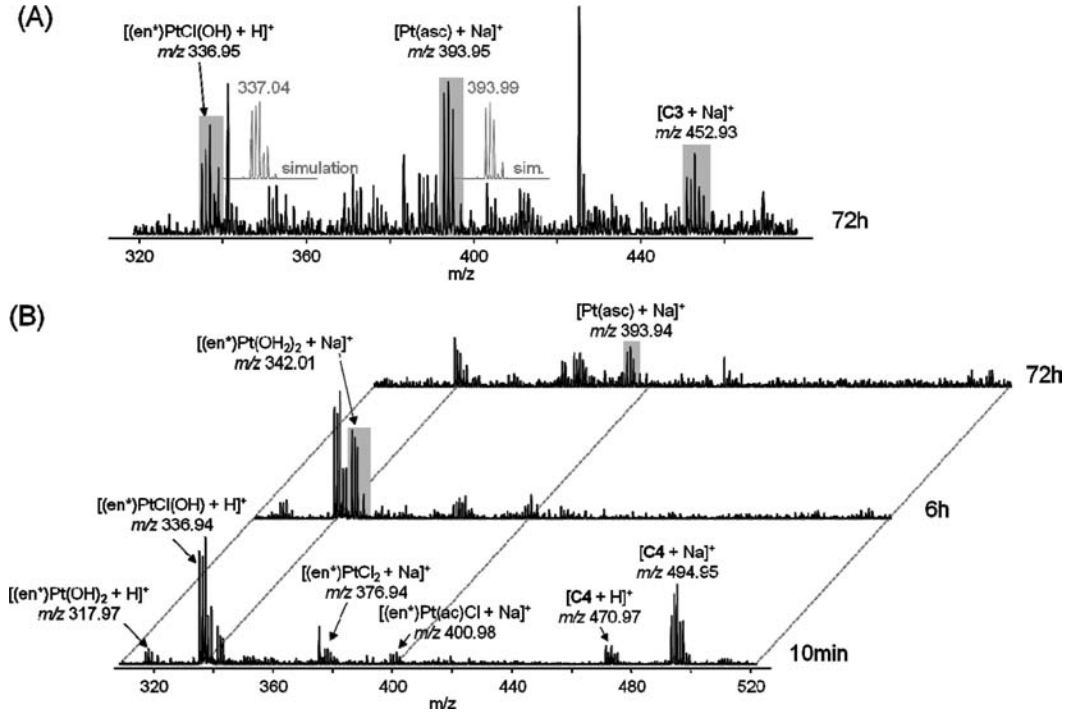

Figure 7. (A) Excerpt of the positive-ion-mode ESI mass spectrum of an incubation solution containing NaAsc and **C3** (molar ratio 2:1) after 72 h. (B) ESI mass spectra showing the time evolution of the incubation solution containing ascorbate and **C4** (molar ratio 2:1). The experimental ESI mass signals include a standard deviation of m/z ±0.05. Simulations of isotopic distributions are shown in gray. Abbreviations: en* = *N,N*-dimethylethane-1,2-diamine; ac = acetate; asc = ascorbate.

stability due to the bulky methyl groups. Consequently, the significantly increased reduction rate of **C4** suggests that N,N-dialkylation of the equatorial amine allows fine-tuning of the reduction rates of diacetatoplatinum(IV) complexes.

The monoacetato complex **C3** has a reduction rate between those of the diacetato and dihydroxido complexes, in agreement with literature data for platinum(IV) complexes featuring chlorido ligands.[15] In summary, the following trend in the rates of reduction was observed: **C4** > **C3** ≈ satraplatin > **C1**.

ESI-MS experiments confirmed the results of the NMR measurements, and a similar trend in the rates of reduction of the platinum(IV) compounds by sodium ascorbate (NaAsc) was observed. In these experiments, the complexes were reacted with NaAsc at an identical molar ratio as in the NMR experiments. The final metal concentration in the reaction mixture was 50 μM. The compounds were stable in aqueous solution in the absence of NaAsc for at least 72 h, and characteristic mass signals were observed, typically corresponding to $[M + H]^+$ or $[M + Na]^+$, where M is **C1**, **C3**, **C4**, or satraplatin. In addition, ESI-MS allowed the identification of some of the species formed upon reduction of the platinum(IV) complexes.

In the ESI-MS experiments, **C1** was not reduced by NaAsc, and the detected mass signals were identical to those observed

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during stability testing (Figure S4 in the Supporting Information). On the other hand, **C3** was reduced to a significant extent by NaAsc, and several distinct mass signals were detected with a characteristic platinum isotopic distribution (Figure 7A). The mass spectrum recorded immediately after preparation of the incubation solution revealed a peak for $[(en^*)PtCl(OH) + H]^+$ (en* = *N*,*N*-dimethylethane-1,2-diamine) at m/z 336.95 ± 0.05 (m_{theor} = 337.04 Da, 19%) (all intensities are given relative to the most abundant signal in the mass spectrum). This is indicative of a rapidly initiated reduction process that is accompanied by the loss of the axial acetato ligands and hydrolysis of one Pt−Cl bond. It must be noted that hydrolysis is generally not observed for kinetically inert platinum(IV) complexes. The latter signal increased to 83% relative to the parent mass signal $[\mathbf{C3} + Na]^+$ after 6 h. After 72 h, the most prominent Pt-containing mass signal corresponded to $[Pt(asc) + Na]^+$ (m/z 393.94 ± 0.05, m_{theor} = 393.99 Da), indicating even the loss of the chelating diamine ligand under the conditions of the ESI-MS measurement.

Finally, **C4** was quickly reduced by NaAsc (Figure 7B) and even after 10 min, the most abundant peak in the mass spectrum corresponded to $[(en^*)PtCl(OH) + H]^+$ (m/z 336.94 ± 0.05, m_{theor} = 337.04, 100%), as similarly observed with **C3**. In the same spectrum, the parent mass signal $[\mathbf{C4} + Na]^+$ was detected at 63% relative abundance. After 72 h, $[(en^*)PtCl(OH) + H]^+$ (100%) and $[Pt(asc) + Na]^+$ (82%) were detected as thermodynamically stable species, as observed in the case of **C3**. The ESI-MS experiments also confirmed that satraplatin was reduced at a lower rate than **C4**, although the two complexes feature similar ligand spheres. However, mass signals assignable to reduction products were observed only during the initial 6 h in minor amounts before all of the signals slowly vanished, indicating decomposition of satraplatin. Finally, glutathione was not able to reduce any of the compounds during a period of 72 h.

CONCLUSION

A series of symmetrically and unsymmetrically substituted platinum(IV) complexes was synthesized in order to investigate the barrier to synthesis caused by *N*(,*N*)-(di)alkylethane-1,2-diamine chelating ligands. The equatorial dimethylamine unit hampered the reaction between reagents and axial Pt−OH groups. This effect was stronger when the methyl substituents were replaced with ethyl substituents, whereas no effect was observed in the case of a cyclohexylamine group. However, two isopropyl substituents led to an unstable compound. The influence on the reaction pattern was highly replicable, and the steric hindrance could be overcome by using more reactive reagents such as acyl halides or isocyanides or an enormous excess of less reactive anhydrides. The set of compounds was furthermore investigated with respect to cytotoxicity, lipophilicity, and rate of reduction. The most active compounds showed promising IC_{50} values in the low micromolar to nanomolar concentration range toward three cancer cell lines (CH1, SW480, and A549), which are comparable to or better than those of cisplatin. A very rough correlation between cytotoxicity and lipophilicity was observed, showing only a minor influence of this parameter. The rate of reduction clearly changed depending on the axial ligands in the case of an N_2Cl_2Pt ground core. The dicarboxylated complex was reduced extremely rapidly relative to the dihydroxido counterpart; the half-life of the monocarboxylated analogues was situated in between. However, to understand the differences in the chemical and biological behavior in more detail, further investigations have to be performed.

EXPERIMENTAL SECTION

Materials and Methods. All of the solvents and reagents were obtained from commercial suppliers and were used as received, except for methanol, which was dried using standard procedures. K_2PtCl_4 was obtained from Johnson Matthey (Switzerland). Reverse-osmosis water was doubly distilled before use. For column chromatography, silica gel 60 (Fluka) was used. The starting platinum(II) compounds (*SP*-4-3)-dichlorido(*N*,*N*-diisopropylethane-1,2-diamine)platinum(II) (**A**), (*SP*-4-3)-dichlorido(*N*,*N*-diethylethane-1,2-diamine)platinum(II) (**B**), (*SP*-4-3)-dichlorido(*N*,*N*-dimethylethane-1,2-diamine)platinum(II) (**C**), and (*SP*-4-3)-dichlorido(*N*-cyclohexylethane-1,2-diamine)-platinum(II) (**D**) were synthesized according to literature procedures.[24,36] The platinum(IV) complexes **C1** and **C2** were also synthesized using previously published procedures.[19]

^1H, ^{13}C, ^{15}N, and ^{195}Pt one- and two-dimensional NMR spectra were recorded with a Bruker Avance III 500 MHz instrument at 500.32 (^1H), 125.81 (^{13}C), 50.70 (^{15}N), and 107.55 (^{195}Pt) MHz or 500.10 (^1H), 125.75 (^{13}C), 50.68 (^{15}N), and 107.51 (^{195}Pt) MHz in perdeuterated dimethyl sulfoxide (DMSO-d_6) at 298 K. The solvent residual peaks for ^1H and ^{13}C were used as internal references, whereas ^{195}Pt chemical shifts were referenced to external K_2PtCl_4 and ^{15}N chemical shifts to external NH_4Cl. ESI-MS was carried out with a Bruker esquire3000 ion trap using MeOH as the solvent. Elemental analyses were performed by the Microanalytical Laboratory of the University of Vienna using a PerkinElmer 2400 CHN elemental analyzer.

Syntheses. *General Procedure I: Oxidation of Pt(II) Complexes.* The platinum(II) complex was suspended in H_2O, and 30% H_2O_2 was added. The reaction mixture was stirred at room temperature (rt). Subsequently, the solvent was removed under reduced pressure and the residue was washed with ice-cold ethanol and diethyl ether.

*(OC-6-43)-Dichlorido(N,N-diisopropylethane-1,2-diamine)-dihydroxidoplatinum(IV) (**A1**).* General procedure I was used with platinum(II) complex **A** (0.05 g, 0.12 mmol), H_2O (15 mL), H_2O_2 (0.5 mL), and stirring for 4 h. **A1** was not stable in solution (Figure S1 in the Supporting Information). Yield: 47 mg (89%). Elemental analysis for $C_8H_{22}Cl_2N_2O_2Pt{\cdot}0.1KCl$: calcd C 21.27, H 4.91, N 6.20; found C 21.09, H 4.54, N 5.84. ^1H NMR (DMSO-d_6): δ 7.06 (bs, 2H, NH_2); 4.15 (m, 2H, CH); 2.97 (m, 2H, CH_2CH_2); 2.72 (bs, 2H, CH_2CH_2); 1.37 (t, 12H, CH_3, J = 7.0 Hz). ^{195}Pt NMR (D_2O): δ 2746.

*(OC-6-43)-Dichlorido(N,N-diethylethane-1,2-diamine)-dihydroxidoplatinum(IV) (**B1**).* General procedure I was used with platinum(II) complex **B** (0.20 g, 0.52 mmol), H_2O (5 mL), H_2O_2 (1 mL), and stirring for 2.5 h. Yield: 0.18 g (83%). Elemental analysis for $C_6H_{18}Cl_2N_2O_2Pt{\cdot}0.5H_2O$: calcd C 16.95, H 4.50, N 6.59; found C 16.61, H 4.29, N 6.34. ^1H NMR (DMSO-d_6): δ 6.95 (bs, 2H, NH_2), 3.65 (m, 2H, $N(CH_2CH_3)_2$), 2.76 (bm, 4H, $N(CH_2CH_3)_2 + NCH_2$), 2.64 (bs, 2H, NH_2CH_2), 1.19 (t, 6H, CH_3, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6): δ 63.6 (NCH_2), 48.3 ($N(CH_2CH_3)_2$), 44.6 (NH_2CH_2), 9.3 ($N(CH_2CH_3)_2$). ^{15}N NMR (DMSO-d_6): δ −1.9. ^{195}Pt NMR (DMSO-d_6): δ 2493.

*(OC-6-43)-Dichlorido(N-cyclohexylethane-1,2-diamine)-dihydroxidoplatinum(IV) (**D1**).* General procedure I was used with platinum(II) complex **D** (0.55 g, 1.35 mmol), H_2O (15 mL), H_2O_2 (5.5 mL), and stirring for 7 h. Yield: 0.32 g (55%). Elemental analysis for $C_8H_{20}Cl_2N_2O_2Pt$: calcd C 21.73, H 4.56, N 6.33; found C 21.39, H 4.17, N 5.97. Insoluble in H_2O, *N*,*N*-dimethylformamide (DMF), MeOH, and DMSO.

*(OC-6-54)-(3-Carboxypropanoato)dichlorido(N,N-diethylethane-1,2-diamine)hydroxidoplatinum(IV) (**B2**).* **B1** (100 mg; 0.24 mmol) and succinic anhydride (96 mg; 0.96 mmol) in DMF (2 mL) were stirred at 40 °C for 18 h. The solvent was removed at 40 °C under reduced pressure to form a yellow oil. The residue was suspended in acetone, and the yellow solid was filtered off, washed with acetone and diethyl ether, and dried in vacuo. The acetone phase was reduced to

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half of the volume, and a second fraction was precipitated using diethyl ether. Yield: 110 mg (89%). Elemental analysis for $C_{10}H_{22}N_2O_5Cl_2Pt\cdot0.5H_2O$: calcd C 22.87, H 4.41, N 5.33; found C 22.79, H 4.16, N 5.18. ^{1}H NMR (DMSO-d_6): δ 12.06 (bs, 1H, COO*H*), 9.36 (bs, 1H, N*H*$_2$), 7.11 (bs, 1H, N*H*$_2$), 3.65 (m, 1H, N(C*H*$_2$CH$_3$)$_2$), 3.44 (m, 1H, N(C*H*$_2$CH$_3$)$_2$), 3.01−2.87 (bm, 2H, NC*H*$_2$CH$_2$N), 2.83 (m, 1H, N(C*H*$_2$CH$_3$)$_2$), 2.78−2.70 (bm, 2H, NCH$_2$C*H*$_2$N), 2.68 (m, 1H, N(C*H*$_2$CH$_3$)$_2$), 2.49−2.37 (bm, 4H, PtOOCC*H*$_2$C*H*$_2$), 1.66 (bs, 1H, O*H*), 1.23 (t, 3H, N(CH$_2$C*H*$_3$)$_2$, J = 7.0 Hz); 1.17 (t, 3H, N(CH$_2$C*H*$_3$)$_2$, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6): δ 181.2 (PtO*O*C), 174.6 (*C*OOH), 64.2 (NCH$_2$CH$_2$N), 50.7 (N(CH$_2$CH$_3$)$_2$), 48.7 (N(*C*H$_2$CH$_3$)$_2$), 44.7 (NCH$_2$CH$_2$N), 32.7 (CH$_2$CH$_2$COOH), 30.4 (CH$_2$CH$_2$COOH), 9.6 (N(CH$_2$CH$_3$)$_2$), 8.7 (N(CH$_2$CH$_3$)$_2$). ^{15}N NMR (DMSO-d_6): δ −6.9. ^{195}Pt NMR (DMSO-d_6): δ 2582. ESI-MS (MeOH) m/z: (pos) 516.8 [M + H]$^+$, 538.7 [M + Na]$^+$; (neg) 514.6 [M − H]$^-$, 1029.0 [2M − H]$^-$.

General Procedure II. **B1** or **C1** was suspended in DMF, and acetic anhydride was added. The solution was stirred at rt and quenched with a small amount of H_2O, and the crude product was precipitated by addition of diethyl ether. The precipitate was filtered, washed with diethyl ether, and dried under reduced pressure.

*(OC-6-54)-Acetatodichlorido(N,N-diethylethane-1,2-diamine)-hydroxidoplatinum(IV) (**B3**).* General procedure II was used with **B1** (100 mg, 0.24 mmol), DMF (4 mL), acetic anhydride (49 mg, 0.48 mmol), and stirring for 4 h. Yield: 109 mg (99%). Elemental analysis for $C_8H_{20}Cl_2N_2O_3Pt\cdot0.8$DMF: calcd C 24.17, H 4.99, N 7.59; found C 24.25, H 4.78, N 7.54. ^{1}H NMR (DMSO-d_6): δ 9.39 (bs, 1H, N*H*$_2$), 7.13 (bs, 1H, N*H*$_2$), 3.65 (m, 1H, N(C*H*$_2$CH$_3$)$_2$), 3.39 (m, 1H, N(C*H*$_2$CH$_3$)$_2$), 3.03−2.94 (bm, 2H, NC*H*$_2$C*H*$_2$N), 2.84 (m, 1H, N(C*H*$_2$CH$_3$)$_2$), 2.75−2.64 (bm, 3H, NC*H*$_2$C*H*$_2$N + N(C*H*$_2$CH$_3$)$_2$), 1.92 (s, 3H, C*H*$_3$), 1.65 (s+d, 1H, O*H*, J = 9.5 Hz), 1.24 (t, 3H, N(CH$_2$C*H*$_3$)$_2$, J = 7.0 Hz), 1.17 (t, 3H, N(CH$_2$C*H*$_3$)$_2$, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6): δ 180.1 (*C*O), 64.3 (*C*H$_2$NR$_2$), 50.9 (N-(*C*H$_2$CH$_3$)$_2$), 48.8 (N(*C*H$_2$CH$_3$)$_2$), 44.6 (*C*H$_2$NH$_2$), 25.1 (CO*C*H$_3$), 9.6 (N(*C*H$_2$CH$_3$)$_2$), 8.6 (N(CH$_2$*C*H$_3$)$_2$). ^{15}N NMR (DMSO-d_6): δ −6.8. ^{195}Pt NMR (DMSO-d_6): δ 2587. ESI-MS (MeOH) m/z: (pos) 481.0 [M + Na]$^+$; (neg) 456.9 [M − H]$^-$, 492.0 [M + Cl]$^-$.

*(OC-6-54)-Acetatodichlorido(N,N-dimethylethane-1,2-diamine)-hydroxidoplatinum(IV) (**C3**).* General procedure II was used with **C1** (0.10 g, 0.26 mmol), DMF (4 mL), acetic anhydride (53 mg, 0.52 mmol), and stirring for 1 h. The crude product was recrystallized in ethanol. Yield: 83 mg (75%). Elemental analysis for $C_6H_{16}Cl_2N_2O_3Pt$: calcd C 16.75, H 3.75, N 6.51; found C 16.72, H 3.44, N 6.44. ^{1}H NMR (DMSO-d_6): δ 9.46 (bs, 1H, N*H*$_2$), 7.10 (bs, 1H, N*H*$_2$), 2.89−2.75 (m, 4H, NC*H*$_2$C*H*$_2$N), 2.66 (s+d, 3H, N(C*H*$_3$)$_2$, J = 12 Hz), 2.59 (s+d, 3H, N(C*H*$_3$)$_2$, J = 13 Hz), 1.92 (s, 3H, COC*H*$_3$), 1.49 (s+d, 1H, O*H*, J = 9.5 Hz). ^{13}C NMR (DMSO-d_6): δ 180.1 (*C*O), 67.9 (*C*NR$_2$), 50.3 (N(*C*H$_3$)$_2$), 48.6 (N(*C*H$_3$)$_2$), 45.1 (*C*NH$_2$), 24.9 (CO*C*H$_3$). ^{15}N NMR (DMSO-d_6): δ −6.0. ^{195}Pt NMR (DMSO-d_6): δ 2503. ESI-MS (MeOH) m/z: (pos) 468.9 [M + K]$^+$.

General Procedure III. **B1**, **C1**, or **D1** was suspended in acetic anhydride and stirred at rt until a clear solution was formed. The product was precipitated by slow addition of diethyl ether. The precipitate was filtered and washed with diethyl ether and dried under reduced pressure.

*(OC-6-43)-Diacetatodichlorido(N,N-diethylethane-1,2-diamine)-platinum(IV) (**B4**).* General procedure III was used with **B1** (100 mg, 0.24 mmol) and acetic anhydride (2 mL). Yield: 105 mg (95%). Elemental analysis for $C_{10}H_{22}Cl_2N_2O_4Pt$: calcd C 24.01, H 4.43, N 5.60; found C 23.62, H 4.13, N 5.38. ^{1}H NMR (DMSO-d_6): δ 9.64 (bs, 2H, N*H*$_2$), 3.56 (m, 2H, N(C*H*$_2$CH$_3$)$_2$), 2.99 (bm, 4H, C*H*$_2$C*H*$_2$), 2.72 (m, 2H, N(C*H*$_2$CH$_3$)$_2$), 1.95 (s, 6H, COC*H*$_3$), 1.24 (t, 6H, N(CH$_2$C*H*$_3$)$_2$, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6): δ 180.1 (*C*O), 65.8 (*C*H$_2$N(CH$_2$CH$_3$)$_2$), 50.9 (N(*C*H$_2$CH$_3$)$_2$), 45.0 (*C*H$_2$NH$_2$), 24.6 (CO*C*H$_3$), 9.0 (N(CH$_2$*C*H$_3$)$_2$). ^{15}N NMR (DMSO-d_6): δ −20.5. ^{195}Pt NMR (DMSO-d_6): δ 2819. ESI-MS (MeOH) m/z: (pos) 522.7 [M + Na]$^+$, 538.6 [M + K]$^+$; (neg) 498.4 [M − H]$^-$, 534.4 [M + Cl]$^-$.

*(OC-6-43)-Diacetatodichlorido(N,N-dimethylethane-1,2-diamine)platinum(IV) (**C4**).* General procedure III was used with **C1** (100 mg, 0.26 mmol) and acetic anhydride (3 mL). Yield: 98 mg (81%). Elemental analysis for $C_8H_{18}Cl_2N_2O_4Pt$: calcd C 20.35, H 3.84, N 5.93; found C 20.16, H 3.89, N 5.66. ^{1}H NMR (DMSO-d_6): δ 9.47 (bs, 2H, N*H*$_2$), 2.96 (s, 4H, NC*H*$_2$C*H*$_2$N), 2.64 (s, 6H, N(C*H*$_3$)$_2$), 1.95 (s, 6H, COC*H*$_3$). ^{13}C NMR (DMSO-d_6): δ 179.8 (*C*O), 68.8 (NH$_2$*C*H$_2$), 50.0 (N(*C*H$_3$)$_2$), 45.7 (CH$_3$N*C*H$_2$), 24.4 (CO*C*H$_3$). ^{15}N NMR (DMSO-d_6): δ −18.1. ^{195}Pt NMR (DMSO-d_6): δ 2717. ESI-MS (MeOH) m/z: (pos) 472.8 [M + H]$^+$, 494.8 [M + Na]$^+$, 510.7 [M + K]$^+$; (neg) 470.3 [M − H]$^-$, 506.6 [M + Cl]$^-$.

*(OC-6-43)-Diacetatodichlorido(N-cyclohexylethane-1,2-diamine)platinum(IV) (**D4**).* General procedure III was used with **D1** (125 mg, 0.28 mmol), acetic anhydride (10 mL), and stirring for 10 h. Yield: 98 mg (66%). Elemental analysis for $C_{12}H_{24}Cl_2N_2O_4Pt\cdot0.5H_2O$: calcd C 26.92, H 4.71, N 5.23; found C 26.57, H 4.34, N 5.05. ^{1}H NMR (DMSO-d_6): δ 10.03 (bs, 1H, N*H*), 9.79 (bs, 1H, N*H*), 8.13 (bs, 1H, N*H*), 3.61 (t, 1H, cyclohexyl C*H*, J = 11 Hz), 2.86−2.63 (m, 4H, C*H*$_2$C*H*$_2$), 2.00 (bs, 2H, cyclohexyl C*H*$_2$), 1.95 (s, 3H, C*H*$_3$), 1.91 (s, 3H, C*H*$_3$), 1.75 (bs, 2H, cyclohexyl C*H*$_2$), 1.67−1.59 (m, 2H, cyclohexyl C*H*$_2$), 1.36−1.28 (m, 2H, cyclohexyl C*H*$_2$), 1.25−1.18 (m, 1H, cyclohexyl C*H*$_2$), 1.13−1.05 (m, 1H, cyclohexyl C*H*$_2$). ^{13}C NMR (DMSO-d_6): δ 181.4 (*C*O), 180.5 (*C*O), 60.5 (cyclohexyl *C*H), 51.1 (*C*H$_2$CH$_2$), 47.2 (CH$_2$*C*H$_2$), 31.5 (cyclohexyl *C*H$_2$), 28.0 (cyclohexyl *C*H$_2$), 25.6 (cyclohexyl *C*H$_2$), 25.6 (cyclohexyl *C*H$_2$), 25.2 (cyclohexyl *C*H$_2$), 24.0 (*C*H$_3$), 23.8 (*C*H$_3$). ^{15}N NMR (DMSO-d_6): δ −9.5 (*N*H$_2$), 21.5 (*N*H). ^{195}Pt NMR (DMSO-d_6): δ 2768. ESI-MS (MeOH) m/z: (pos) 429.2 [C$_8$H$_{17}$N$_2$Cl$_2$Pt + Na]$^+$, 548.2 [M + Na]$^+$; (neg) 526.2 [M − H]$^-$, 561.0 [M + Cl]$^-$.

*(OC-6-54)-Dichlorido(N,N-diethylethane-1,2-diamine)hydroxido-[(2E)-3-phenylprop-2-enoato]platinum(IV) (**B5**).* **B1** (100 mg, 0.24 mmol) was suspended in dry acetone (8 mL), and pyridine (190 mg, 2.50 mmol) was added. The reaction mixture was heated to 50 °C, after which cinnamoyl chloride (84 mg, 0.51 mmol) dissolved in dry acetone (2 mL) was added. After 30 min, the reaction mixture was quenched with H_2O, and acetone was removed under reduced pressure. The aqueous solution was stored in the refrigerator overnight. and the formed precipitate was filtered off and washed with cold ethanol and ether. The crude product was recrystallized in methanol. Yield: 93 mg (71%). Elemental analysis for $C_{15}H_{24}Cl_2N_2O_3Pt\cdot0.5\ H_2O$: calcd C 32.44, H 4.54, N 5.04; found C 32.09, H 4.22, N 4.77. ^{1}H NMR (DMSO-d_6): δ 9.45 (bs, 1H, N*H*$_2$), 7.62 (m, 2H, phenyl C*H*), 7.39 (m, 3H, phenyl C*H*), 7.37 (d, 1H, PtOOCC*H*=CH, J = 16 Hz), 7.22 (bs, 1H, N*H*$_2$), 6.49 (d, 1H, PtOOCCH=C*H*, J = 16 Hz), 3.69 (m, 1H, N(C*H*$_2$CH$_3$)$_2$), 3.39 (m, 1H, N(C*H*$_2$CH$_3$)$_2$), 3.05 (m, 2H, NC*H*$_2$CH$_2$N), 2.91 (m, 1H, N(C*H*$_2$CH$_3$)$_2$), 2.71 (m, 3H, NCH$_2$C*H*$_2$N + N(C*H*$_2$CH$_3$)$_2$), 1.84 (s, 1H, O*H*), 1.28 (t, 3H, N(CH$_2$C*H*$_3$)$_2$, J = 7.0 Hz), 1.19 (t, 3H, N(CH$_2$C*H*$_3$)$_2$, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6): δ 175.3 (PtO*O*C), 141.5 (PtCOOCH=CH), 135.2 (phenyl C), 130.1 (phenyl CH), 129.3 (2 × phenyl CH), 128.3 (2 × phenyl CH), 124.1 (PtCOOCH=*C*H), 64.4 (*C*H$_2$CH$_2$), 51.0 (CH$_2$*C*H$_3$), 48.8 (*C*H$_2$CH$_3$), 44.8 (*C*H$_2$CH$_2$), 9.7 (*C*H$_3$), 8.6 (*C*H$_3$). ^{15}N NMR (DMSO-d_6): δ −5.9. ^{195}Pt NMR (DMSO-d_6): δ 2578. ESI-MS (MeOH) m/z: (pos) 546.8 [M + H]$^+$, 568.8 [M + Na]$^+$, 584.8 [M + K]$^+$; (neg) 544.6 [M − H]$^-$, 579.7 [M + Cl]$^-$.

*(OC-6-54)-Dichlorido(N,N-dimethylethane-1,2-diamine)-hydroxido[(2E)-3-phenylprop-2-enoato]platinum(IV) (**C5**).* **C1** (100 mg, 0.26 mmol) and pyridine (122 mg, 1.55 mmol) were suspended in dry acetone (10 mL), and cinnamoyl chloride (86 mg, 0.52 mmol) in acetone (5 mL) was added. The reaction mixture was stirred at rt for 5 h, after which the solvent was removed under reduced pressure. The residue was dissolved in methanol, and the symmetrically substituted product was separated from the unsymmetrically substituted one by column chromatography (4:1 MeOH/EE). Yield: 47 mg (35%). Elemental analysis for $C_{13}H_{20}Cl_2N_2O_3Pt$: calcd C 30.13, H 3.89, N 5.40; found C 29.85, H 3.58, N 5.30. ^{1}H NMR (DMSO-d_6): δ 9.51 (bs, 1H, N*H*$_2$), 7.63 (m, 2H, phenyl C*H*), 7.39 (m, 4H, 3 × (phenyl C*H* + OOCC*H*=CH)), 7.23 (bs, 1H, N*H*$_2$), 6.54 (d, 1H, OOCCH=C*H*, J = 15.9 Hz), 2.95−2.80 (m, 4H, NC*H*$_2$C*H*$_2$N), 2.71 (bs, 3H, N(C*H*$_3$)$_2$), 2.64 (bs, 3H, N(C*H*$_3$)$_2$), 1.66 (s, 1H, O*H*). ^{13}C NMR (DMSO-d_6): δ 175.4 (PtO*O*C), 141.5 (PtCOOCH=CH), 135.1 (phenyl C), 130.1 (phenyl CH), 129.3 (2 × phenyl CH), 128.3 (2 ×

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phenyl CH), 124.0 (PtCOOCH=CH), 68.0 ($CH_2N(CH_3)_2$), 50.3 ($N(CH_3)_2$), 48.6 ($N(CH_3)_2$), 45.3 (H_2NCH_2). ^{15}N NMR (DMSO-d_6): δ −5.4. ^{195}Pt NMR (DMSO-d_6): δ 2493. ESI-MS (MeOH) m/z: (pos) 518.8 $[M + H]^+$, 540.7 $[M + Na]^+$, 556.7 $[M + K]^+$; (neg) 516.5 $[M - H]^-$, 552.6 $[M + Cl]^-$.

(OC-6-43)-Dichlorido(N,N-dimethylethane-1,2-diamine)bis[(2E)-3-phenylprop-2-enoato]platinum(IV) (**C6**). **C1** (50 mg, 0.13 mmol) and pyridine (102 mg, 1.29 mmol) were suspended in dry acetone (10 mL), and cinnamoyl chloride (107 mg, 0.64 mmol) in acetone (5 mL) was added. The reaction mixture was refluxed for 2 h and subsequently cooled to rt. The solvent was removed under reduced pressure, and the residue was suspended in ethanol (96%). The yellow solid was filtered off, washed with diethyl ether, and dried in vacuo. Yield: 56 mg (66%). Elemental analysis for $C_{22}H_{26}Cl_2N_2O_4Pt$: calcd C 40.75, H 4.04, N 4.32; found C 40.69, H 3.89, N 4.17. ^1H NMR (DMSO-d_6): δ 9.65 (bs, 2H, NH_2), 7.68−7.66 (m, 4H, phenyl C*H*), 7.44 (d, 2H, OOCC*H*=CH, J = 16 Hz), 7.42−7.40 (m, 6H, phenyl C*H*), 6.54 (d, 2H, OOCCH=C*H*, J = 16 Hz), 3.06−3.00 (m, 4H, NCH_2CH_2N), 2.73 (bs, 6H, $N(CH_3)_2$). ^{13}C NMR (DMSO-d_6): δ 174.9 (PtOOC), 142.5 (PtCOOCH=CH), 134.7 (phenyl *C*), 130.4 (phenyl CH), 129.4 (2 × phenyl CH), 128.6 (2 × phenyl CH), 122.1 (PtCOOCH=CH), 68.9 (NCH_2CH_2N), 50.2 ($N(CH_3)_2$), 45.9 (NCH_2CH_2N). ^{15}N NMR (DMSO-d_6): δ −16.6. ^{195}Pt NMR (DMSO-d_6): δ 2693. ESI-MS (MeOH/DMSO) m/z: (pos) 648.9 $[M + H]^+$, 670.9 $[M + Na]^+$, 686.8 $[M + K]^+$.

(OC-6-43)-Dichlorido(N-cyclohexylethane-1,2-diamine)bis[(2E)-3-phenylprop-2-enoato]platinum(IV) (**D6**). **D1** (100 mg, 0.23 mmol) and pyridine (71 mg, 0.91 mmol) were suspended in dry acetone, and solid cinnamoyl chloride (0.15 g, 0.91 mmol) was added. Subsequently, the reaction mixture was stirred at rt until a clear solution was obtained. A small amount of H_2O was added to stop the reaction, and acetone was removed under reduced pressure. The solution was kept in the refrigerator overnight, and the formed solid was filtered off and recrystallized in 8:1 MeOH/H_2O. Yield: 72 mg (45%). Elemental analysis for $C_{26}H_{32}Cl_2N_2O_4Pt$: calcd C 44.45, H 4.59, N 3.99; found C 44.40, H 4.55, N 3.82. ^1H NMR (DMSO-d_6): δ 10.21 (bs, 1H, NH_2), 9.94 (bs, 1H, NH_2), 8.31 (bs, 1H, N*H*), 7.66 (m, 4H, phenyl C*H*), 7.49 (d, 1H, C*H*=CH, J = 16.0 Hz), 7.41 (d, 1H, C*H*=CH, J = 16.0 Hz), 7.40 (m, 6H, phenyl C*H*), 6.55 (d, 1H, CH=C*H*, J = 16.0 Hz), 6.49 (d, 1H, CH=C*H*, J = 16.0 Hz), 3.75 (t, 1H, cyclohexyl C*H*, J = 11.0 Hz), 2.88−2.72 (m, 4H, CH_2CH_2), 2.08 (m, 2H, cyclohexyl CH_2), 1.70 (m, 3H, cyclohexyl CH_2), 1.60 (m, 1H, cyclohexyl CH_2), 1.31 (m, 3H, cyclohexyl CH_2), 1.09 (m, 1H, cyclohexyl CH_2). ^{13}C NMR (DMSO-d_6): δ 176.5 (CO), 175.7 (CO), 142.9 (PtCOOCH=CH), 142.8 (PtCOOCH=CH), 134.7 (phenyl *C*), 134.6 (phenyl *C*), 130.5 (phenyl CH), 129.4 (phenyl CH), 129.4 (phenyl CH), 128.6 (phenyl CH), 128.5 (phenyl CH), 121.4 (PtCOOCH=CH), 60.8 (cyclohexyl CH), 51.38 (CH_2CH_2), 47.6 (CH_2CH_2), 31.7 (cyclohexyl CH_2), 28.3 (cyclohexyl CH_2), 25.6 (cyclohexyl CH_2), 25.6 (cyclohexyl CH_2), 25.22 (cyclohexyl CH_2). ^{15}N NMR (DMSO-d_6): δ −8.2 (NH_2), 22.7 (N*H*). ^{195}Pt NMR (DMSO-d_6): δ 2743. ESI-MS (MeOH) m/z: (pos) 429.1 $[C_8H_{17}N_2Cl_2Pt + Na]^+$, 724.2 $[M + Na]^+$; (neg) 699.9 $[M - H]^-$.

(OC-6-54)-Dichlorido(N,N-diethylethane-1,2-diamine)hydroxidopropylcarbamatoplatinum(IV) (**B7**). **B1** (50 mg, 0.12 mmol) was suspended in dry acetone (5 mL), and *n*-propyl isocyanate (20 mg, 0.24 mmol) was added. The suspension was then stirred at rt for 3 h. The solvent was removed under reduced pressure, and the residue was dissolved in EtOH. Subsequently, the crude product was precipitated using Et_2O, filtered off, and dried in vacuo. Yield: 51 mg (85%). Elemental analysis for $C_{10}H_{25}Cl_2N_3O_3Pt{\cdot}0.5H_2O$: calcd C 23.54, H 5.14, N 8.23; found C 23.24, H 4.83, N 7.85. ^1H NMR (DMSO-d_6): δ 10.58 (bs, 1H, NH_2), 6.95 (bs, 1H, NH_2), 6.31 (major) 5.96 (minor) (bs, 1H, N*H*), 3.64 (m, 1H, CH_2), 3.50 (m, 1H, CH_2), 2.94−2.50 (bm, 8H, CH_2CH_2, CH_2CH_3, $CH_2CH_2CH_3$), 1.38 (m, 2H, CH_2CH_3), 1.20 (m, 6H, CH_3), 0.82 (t, 3H, CH_3, J = 7.5 Hz). ^{13}C NMR (DMSO-d_6): δ 165.79 (CO), 64.45 (NCH_2CH_2N), 50.29 (CH_2CH_3), 48.44 (CH_2CH_3), 44.47 (NCH_2CH_2N), 43.15 ($CH_2CH_2CH_3$), 23.58 ($CH_2CH_2CH_3$), 11.81 ($CH_2CH_2CH_3$), 9.32 (CH_3) 9.12 (CH_3). ^{15}N NMR (DMSO-d_6): δ −4.9. ^{195}Pt NMR (DMSO-d_6): δ 2615 (major), 2600 (minor). ESI-MS (MeOH) m/z: (pos) 501.8 $[M + H]^+$, 523.1 $[M + Na]^+$, 539.7 $[M + K]^+$; (neg) 499.5 $[M - H]^-$.

(OC-6-54)-Dichlorido(N,N-dimethylethane-1,2-diamine)-hydroxidopropylcarbamatoplatinum(IV) (**C7**). **C1** (100 mg, 0.26 mmol) was suspended in DMF (3 mL), and *n*-propyl isocyanate (26 mg, 0.31 mmol) was added. The suspension was then stirred for 10 h at rt. The solvent was removed under reduced pressure. The residue was dissolved in MeOH, and the unsymmetrically substituted product was separated from the symmetrically substituted one by column chromatography (5:1 MeOH/EE). Yield: 41 mg (42%). Elemental analysis for $C_8H_{21}Cl_2N_3O_3Pt$: calcd C 20.30, H 4.47, N 8.88; found C 20.00, H 4.16, N 8.56. ^1H NMR (DMSO-d_6): δ 10.63 (bs, 1H, NH_2), 6.96 (bs, 1H, NH_2), 6.38 (major) 5.96 (minor) (bs, 1H, OOCN*H*), 2.87 (bm, 4H, NCH_2CH_2N), 2.68 (s+d, 3H, $N(CH_3)_2$, J = 12.5 Hz), 2.62 (m, 2H, $NHCH_2$), 2.56 (s+d, 3H, $N(CH_3)_2$, J = 12.5 Hz), 1.37 (m, 2H, $NHCH_2CH_2$), 0.80 (t, 3H, CH_3, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6): δ 165.9 (PtOOC), 67.9 ($CH_2N(CH_3)_2$), 50.2 ($N(CH_3)_2$), 48.0 ($N(CH_3)_2$), 45.0 (NH_2CH_2), 43.2 ($NHCH_2$), 23.6 ($NHCH_2CH_2$), 11.8 (CH_3). ^{15}N NMR (DMSO-d_6): δ −4.0 (NH_2), 66.3 (*NH*). ^{195}Pt NMR (DMSO-d_6): δ 2530 (major), 2519 (minor). ESI-MS (MeOH) m/z: (pos) 511.8 $[M + K]^+$; (neg) 472.4 $[M - H]^-$, 507.7 $[M + Cl]^-$.

(OC-6-43)-Dichlorido(N,N-dimethylethane-1,2-diamine)bis-(propylcarbamato)platinum(IV) (**C8**). **C1** (100 mg, 0.26 mmol) was suspended in dry acetone (5 mL), and *n*-propyl isocyanate (87 mg, 1.03 mmol) was added. The solution was stirred at rt for 24 h until the solid was dissolved completely. The solvent was removed under reduced pressure, and the residue was suspended in acetone (1 mL). The product was precipitated by addition of ether, filtered off, and washed with ether. Yield: 94 mg (65%). Elemental analysis for $C_{12}H_{28}Cl_2N_4O_4Pt$: calcd C 25.81, H 5.05, N 10.03; found C 25.90, H 4.75, N 9.71. ^1H NMR (DMSO-d_6): δ 10.29 (bs, 2H, NH_2), 6.67 + 6.20 (bs, 2H, OOCN*H*), 2.89 (m, 4H, $NHCH_2$), 2.89−2.87 (m, 4H, NCH_2CH_2N), 2.61 (bs, 6H, $N(CH_3)_2$), 1.36 (m, 4H, $NHCH_2CH_2$), 0.80 (t, 6H, CH_3, J = 7.5 Hz). ^{13}C NMR (DMSO-d_6): δ 164.5 (PtOOC), 68.4 ($NHCH_2$), 49.2 ($N(CH_3)_2$), 45.3 (NCH_2CH_2N), 43.2 (NCH_2CH_2N), 23.3 ($NHCH_2CH_2$), 11.8 (CH_3). ^{15}N NMR (DMSO-d_6): δ −9.3 (NH_2), 67.4 (CO*N*H). ^{195}Pt NMR (DMSO-d_6): δ 2720 (major), 2707 (minor). ESI-MS (MeOH) m/z: (pos) 558.9 $[M + H]^+$, 580.8 $[M + Na]^+$.

(OC-6-43)-Dichlorido(N-cyclohexylethane-1,2-diamine)bis-(propylcarbamato)platinum(IV) (**D8**). To a stirred suspension of **D1** (105 mg, 0.24 mmol) in DMF (1 mL) was added *n*-propyl isocyanate (1 mL), and after 3.5 h, the solvent was removed under reduced pressure. The residue was dissolved in MeOH, and a solid was precipitated by EtOAc. For complete precipitation, hexane was added and the suspension was stored overnight at 4 °C. The yellow product was filtered off, washed with Et_2O, and dried in vacuo. Yield: 93 mg (64%). Elemental analysis for $C_{16}H_{34}Cl_2N_4O_4Pt{\cdot}0.6H_2O$: calcd C 30.83, H 5.69, N 8.99; found C 30.46, H 5.28, N 8.65. ^1H NMR (DMSO-d_6): δ 11.58 (s, 1H, N*H*), 11.08 (s, 1H, N*H*), 7.80 (s, 1H, N*H*), 6.76 (major) 6.28 + 6.20 (minor) (s, 2H, CON*H*), 3.66 (m, 1H, cyclohexyl C*H*), 2.92−2.74 (m, 4H, CH_2CH_2), 2.89 (m, 4H, $NHCH_2$), 2.09 (m, 2H, cyclohexyl CH_2), 1.73 (m, 3H, cyclohexyl CH_2), 1.62 (m, 1H, cyclohexyl CH_2), 1.36 (m, 4H, $NHCH_2CH_2$), 1.26 (m, 3H, cyclohexyl CH_2), 1.10 (m, 1H, cyclohexyl CH_2), 0.81 (m, 6H, CH_3). ^{13}C NMR (DMSO-d_6): δ 166.1 (CO), 165.7 (CO), 59.9 (cyclohexyl CH), 50.3 (CH_2CH_2), 46.9 (CH_2CH_2), 43.1 ($CH_2CH_2CH_3$), 43.0 ($CH_2CH_2CH_3$), 31.6 (cyclohexyl CH_2), 28.3 (cyclohexyl CH_2), 25.7 (cyclohexyl CH_2), 25.7 (cyclohexyl CH_2), 25.3 (cyclohexyl CH_2), 23.4 ($CH_2CH_2CH_3$), 11.8 ($CH_2CH_2CH_3$), 11.6 ($CH_2CH_2CH_3$). ^{15}N NMR (DMSO-d_6): δ −3.3 (NH_2), 23.5 (*NH*), 67.3 (CONH). ^{195}Pt NMR (DMSO-d_6): δ 2812.2 (major), 2800.7 (minor); diastereomer 2796.3 (major), 2783.9 (minor). ESI-MS (MeOH) m/z: (pos) 612.0 $[M + H]^+$, 634.0 $[M + Na]^+$.

(OC-6-54)-Acetatodichlorido(N,N-diethylethane-1,2-diamine)-[(2E)-3-phenylprop-2-enoato]platinum(IV) (**B9**). **B5** (61 mg, 0.11 mmol) was stirred in 3 mL of acetic anhydride at rt for 24 h. Subsequently, 1 mL of acetone was added to the reaction mixture, and the product was precipitated by addition of Et_2O. To complete the

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precipitation, the suspension was kept in the refrigerator for 48 h. The solid material was filtered off, washed with Et_2O, and dried under reduced pressure. Yield: 55 mg (84%). Elemental analysis for $C_{17}H_{26}Cl_2N_2O_4Pt \cdot 0.3C_3H_6O$: calcd C 35.49, H 4.63, N 4.62; found C 35.37, H 4.49, N 4.25. ^{1}H NMR (DMSO-d_6): δ 9.74 (bs, 1H, NH_2), 9.12 (bs, 1H, NH_2), 7.65 (m, 2H, phenyl C*H*), 7.42 (d, 1H, PtOOCC*H*=CH, J = 16.5 Hz), 7.40 (m, 3H, phenyl C*H*), 6.49 (d, 1H, PtOOCCH=C*H*, J = 16.5 Hz), 3.56 (m, 2H, $N(CH_2CH_3)_2$), 3.08−2.99 (bm, 4H, NCH_2CH_2N), 2.76 (m, 2H, $N(CH_2CH_3)_2$), 1.98 (s, 3H, CH_3), 1.27 (m, 6H, $N(CH_2CH_3)_2$). ^{13}C NMR (DMSO-d_6): δ 179.6 (PtOO*C*), 174.8 (PtOO*C*), 142.1 (phenyl *C*), 134.2 (phenyl *C*H), 130.0 (2 × phenyl *C*H), 128.9 (CH=*C*H), 128.1 (2 × phenyl *C*H), 121.8 (CH=*C*H), 65.4 (NCH_2CH_2N), 50.59 ($N(CH_2CH_3)_2$), 50.5 ($N(CH_2CH_3)_2$), 44.7 (NCH_2CH_2N), 24.2 (CH_3), 8.7 ($N(CH_2CH_3)_2$), 8.6 ($N(CH_2CH_3)_2$). ^{15}N NMR (DMSO-d_6): δ −19.0. ^{195}Pt NMR (DMSO-d_6): δ 2808. ESI-MS (MeOH) m/z: (pos) 588.5 $[M + H]^+$, 609.7 $[M + Na]^+$, 625.7 $[M + K]^+$; (neg) 586.5 $[M - H]^-$.

Crystallographic Structure Measurements. X-ray diffraction measurements on **C3**, **B3**, **D4**, and **C6** were performed on a Bruker X8 APEX II CCD diffractometer at 100 K. Single crystals were positioned 35 mm from the detector, and 1496, 2844, 631, and 1102 frames were measured for 30, 20, 5, and 50 s, respectively, over 1° scan width. The data were processed using the SAINT software package.[37] Crystal data, data collection parameters, and structure refinement details are given in Table 1. Structures were solved by direct methods and refined by full-matrix least-squares techniques. Non-H atoms were refined with anisotropic displacement parameters. H atoms were inserted at calculated positions and refined with a riding model. Structure solution was achieved with SHELXS-97 and refinement with SHELXL-97,[38] and graphics were produced with ORTEP-3.[39]

Reversed-Phase HPLC Measurements.[27,30,31] Analytical RP-HPLC analysis was performed on a Dionex Summit system controlled by the Dionex Chromeleon 6.60 software. The experimental conditions were as follows: a silica-based C18 gel as stationary phase (Agilent Zorbax Eclipse, 4.6 mm × 250 mm); MeOH/15 mM aqueous formic acid-based mobile phases; UV−vis detection set at 225, 250, 275, and 330 nm; column temperature of 25 °C; flow rate of 1 mL/min; concentration of 0.25 mM for the investigated complexes; 0.1 mM KI solution as the internal standard; injection volume of 25 μL. The capacity factor is defined as $k' = (t_R - t_0)/t_0$, where t_R is the retention time of the investigated compound and t_0 is the retention time of the internal reference KI (dead-volume marker). Values of t_R were determined for at least three different MeOH/HCOOH ratios. Extrapolation of the resulting linear dependence of log k' on the percentage of MeOH in the solvent afforded the value of log k_w: log $k' = \log k_w - S_{MeOH}\varphi$, where k' is the capacity factor at the measured mobile phase composition, k_w is the extrapolated capacity factor in 100% H_2O, S_{MeOH} is a constant for a given substance and the given HPLC system, and φ is the volume percentage of the organic modifier (MeOH) in the mobile phase.

Reduction Behavior Measurements. The reactivities of complexes **C1**, **C3**, and **C4** and the reference compound satraplatin toward cellular reductants was investigated by NMR spectroscopy and ESI-MS. For NMR measurements, stock solutions of the compounds (1 mM) and ascorbic acid (2 mM) in 20 mM phosphate buffer in D_2O (pD 7.51) were prepared. Reaction mixtures were then prepared (compound:reductant molar ratio = 1:2), and spectra were measured at ambient temperature over a period of 72 h. The reductions were monitored by following the decreases in the intensities of the signals at 2.83, 2.12, 2.12, and 2.12 ppm for **C1**, **C3**, **C4**, and satraplatin, respectively. For ESI-MS measurements, stock solutions of the compounds (400 μM), NaAsc (200 μM), and glutathione (200 μM) were prepared. Reaction mixtures were prepared (compound:reductant molar ratio = 1:2) and incubated for 72 h at 37 °C. Mass spectra were recorded in the positive- and negative-ion modes after 0, 3, 6, and 72 h. The samples were diluted to 5 μM using 1:1 water/MeOH and directly infused into an AmaZon SL ESI ion trap (Bruker Daltonics GmbH, Bremen, Germany). The mass spectra were processed using ESI Compass 1.3 and DataAnalysis 4.0 software (Bruker Daltonics).

Cell Lines and Culture Conditions. Human CH1 cells were kindly provided by Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, UK) and SW480 and A549 cells by Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria). Cells were grown in 75 cm^2 culture flasks (Iwaki/Asahi Technoglass, Gyouda, Japan) as adherent monolayer cultures in complete culture medium [minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1 mM sodium pyruvate, 4 mM L-glutamine, and 1% (v/v) nonessential amino acids (from 100× ready-to-use stock solution), all purchased from Sigma-Aldrich, Vienna, Austria]. Cultures were maintained at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2.

Cytotoxicity Tests in Cancer Cell Lines. Cytotoxicity was determined by a colorimetric microculture assay (MTT assay). For this purpose, CH1, A549, and SW480 cells were harvested from culture flasks by trypsinization and seeded into 96-well microculture plates (CytoOne, Starlab) in densities of 1 × 10^3 (CH1), 2 × 10^3 (SW480), 3 × 10^3 (A549) viable cells/well. After a 24 h preincubation, cells were exposed to dilutions of the test compounds in complete culture medium (200 μL/well) for 96 h. At the end of the exposure period, the drug solutions were replaced with RPMI 1640 medium supplemented with 10% heat-inactivated FBS and 4 mM L-glutamine (100 μL/well) and MTT solution (MTT reagent in phosphate-buffered saline; 20 μL/well). After incubation for 4 h, the medium was removed, and the formazan product formed by viable cells was dissolved in DMSO (150 μL/well). Optical densities at 550 nm were measured with a microplate reader (Biotek ELx808) using a reference wavelength of 690 nm to correct for unspecific absorption. The quantity of viable cells was expressed in terms of T/C values by comparison to untreated controls, and IC_{50} values were calculated from concentration−effect curves by interpolation. Evaluations were based on means from at least three independent experiments, each comprising triplicates for each concentration level.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis of ligand **S2**; characterization of platinum compounds **A**, **B**, **B6**, and **B8**; synthesis and characterization of platinum(II) compound **D**; time-dependent decomposition of **A1** in DMSO-d_6; variable-temperature ^{1}H NMR spectra of **D8**; plots of log k_w vs IC_{50}; excerpts of mass spectra of **C1** and satraplatin after 72 h; selected ^{1}H, ^{15}N, and ^{195}Pt NMR shifts; selected bond lengths and angles for **B3**, **C3**, **C6**, and **D4**; log k_w values; elemental analysis results for all of the compounds; and X-ray crystallographic data (CIF) for **B3**, **C3**, **C6**, and **D4**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +43-1-4277-52600. Fax: +43-1-4277-52680. E-mail: markus.galanski@univie.ac.at (M.G.), bernhard.keppler@univie.ac.at (B.K.K).

Notes

The authors declare no competing financial interest.

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