

Synthesis of Polyamines from Ethylenediamine and Their Platinum(II) Complexes, Potential Antitumor Agents

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This work describes the synthesis and characterization of five new amine ligands and also the preparation and characterization of their respective platinum(II) complexes by reaction with K_2PtCl_4 in water. These ligands were obtained by treatment of different halides or epoxides with ethylenediamine. Cytotoxic activity and cellular accumulation of three complexes were investigated in a human small-cell lung carcinoma cell line and its cisplatin resistant subline. The introduction of a spacer (cycle) between the two platinum atoms leads to a significant decrease in cytotoxic activity. At equitoxic doses, the intracellular platinum concentrations found

for compounds **12** and **15** were significantly higher than those found for the reference compounds, cisplatin, carboplatin, or compound **9**. This fact suggests that the formation of adducts between compounds **12** and **15** and the putative pharmacological target, DNA, is less favored. If these compounds bind more slowly to DNA, interaction with other intracellular ligands such as sulfur-containing molecules will become relevant and it may be the reason for the elevated intracellular platinum concentrations.

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Introduction

The polyamines spermidine (1,8-diamino-4-azaoctane) and spermine (1,12-diamino-4,9-diazadodecane), as well as the precursor molecule putrescine (1,4-diaminobutane), are polycation compounds that are found in significant amounts in nearly every prokaryotic and eukaryotic cell type.^[1]

Many of these bases and their derivatives play key roles in a number of biological processes and possess a variety of pharmacological properties.^[2] Polyamines may act as carcinogenesis promoting factors.^[2,3] The connection between polyamines and cancer growth has been established by biological and molecular techniques.^[4] Polyamines have been shown to accumulate in cancer tissues, and the concentration of polyamines and their derivatives increases in cancer patient fluids.^[5–9] These compounds also represent an important target for chemotherapeutic intervention, since de-

pletion of polyamines results in the disruption of a variety of functions and may, in specific cases, result in cytotoxicity.^[10] Inhibitors of the polyamine pathway, therefore, have traditionally been developed as potential antitumor agents.^[11]

Polyamines have multiple cellular functions that can interfere with cell growth and cell death. Therefore, their analogs can play an important role in controlling cancer. Compounds of this class can directly bind to DNA and modulate DNA–protein interactions. The production of hydrogen peroxide from polyamine catabolism might also be responsible for cell death or apoptosis.^[12]

In addition to these facts, neutral ligands derived from amines have been utilized to prepare platinum(II) complexes since the discovery of the anticancer activity of cisplatin by Rosenberg et al.^[13] *cis*-Diaminedichloroplatinum(II) (cisplatin, CDDP)^[14] is one of the most widely used and effective oncological agents against cancers of the testicles, ovaries, bladder, head, and neck.^[14–16] It is also an important adjunct for cancers of the lung, cervix, and breast.^[16] However, its clinical usefulness has frequently been limited by severe side effects,^[17–19] such as nephrotoxicity, ototoxicity, and neurotoxicity, and by the emergence of cancer cells resistant to cisplatin.

Tumor cells having the resistance phenotype exhibit a reduced sensitivity to cytotoxic drugs relative to sensitive cells. The molecular basis for resistance has been the subject of intensive research. Three main events are frequently observed: (i) decreased accumulation of the drug; the intracel-

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lular drug concentration falls below the threshold necessary for cytotoxic activity; (ii) increased levels of sulfur-containing molecules, such as glutathione or metallothionein; these molecules could play a role in metal detoxification; and (iii) enhanced repair of DNA damage caused by cisplatin–DNA adducts.^[20–22]

The need to overcome these problems, as well as to increase the antitumor activity of the platinum complexes, led to the development of structurally different compounds, which might act in different ways against the tumor cells.^[23] The family of dinuclear complexes containing two monofunctional platinum spheres linked by a variable diamine chain has been extensively studied.^[24] The first cytotoxic dinuclear platinum compounds described in the literature received special attention because they have shown a degree of cytotoxicity comparable to that of cisplatin in sensitive cell lines, but a significantly higher activity in acquired cisplatin-resistant cell lines.^[25,26]

Since some substituted ethylenediamine platinum complexes have shown antitumor activity against a variety of cell tumors,^[27,28] we sought to synthesize platinum(II) mono- and dinuclear complexes containing ethylenediamine derivatives as ligands. The novel compounds that were prepared may show a different reactivity towards DNA with the possibility of the formation of new adducts; they therefore may be active in cisplatin-resistant cell lines. In addition, compound **12** could possibly intercalate between the DNA base pairs because of its aromatic ring. Furthermore, we intended to prepare potentially active antitumor compounds in an attempt to combine the activity of the ligand to that of the metal.

Results and Discussion

The intermediate **3** was obtained in 92% yield by the reaction of diol **2** with iodine, imidazole, and triphenylphosphane in toluene at reflux (Scheme 1). The diepoxide **8** (not isolated) was prepared by treatment of ditosylate **7** with sodium methoxide in methanol according to the procedure previously described in the literature.^[29] The ligand **a** and complex **9** were prepared as described in ref.^[26] The ligands **b–g** were prepared in satisfactory yields by treating ethylenediamine with the corresponding intermediate (halide or epoxide) in ethanol at room temperature for 24 h. In the preparation of the ligand *N,N'*-bis(2-aminoethyl)-2-xylylenediamine (**e**), we have also isolated the ligand *N*-(2-aminoethyl)-isoindoline (**f**). In the IR spectra, absorptions corresponding to $\nu_{\text{N-H}}$ at 3300–3100 cm^{-1} can be observed for these ligands. In the ^1H and ^{13}C NMR spectra signals for the ethylenediamine moiety are observed.

The platinum(II) complexes **10–15** were obtained by the reaction of the corresponding ligands with potassium tetrachloroplatinate(II) in water at room temperature for 24 h, and were isolated by simple filtration (Scheme 2). Even though we have used 2 mmol of K_2PtCl_4 we have observed that under these reaction conditions, ligand **c** was transformed into the mononuclear platinum(II) complex **11**. For

these compounds, one may observe IR spectra absorptions corresponding to $\nu_{\text{Pt-N}}$ and $\nu_{\text{Pt-Cl}}$ at 550 and 325 cm^{-1} , respectively, in addition to the absorptions observed for the ligand. In the ^1H NMR spectra there is a downfield shift for the signals corresponding to NH and NH_2 relative to the free ligands. The ^{195}Pt NMR spectra for the complexes show only one signal, and the chemical shift values are expected to be close to those of similar compounds in which the platinum is coordinated to two chlorides and two nitrogens.^[30]

Cell Growth Inhibition

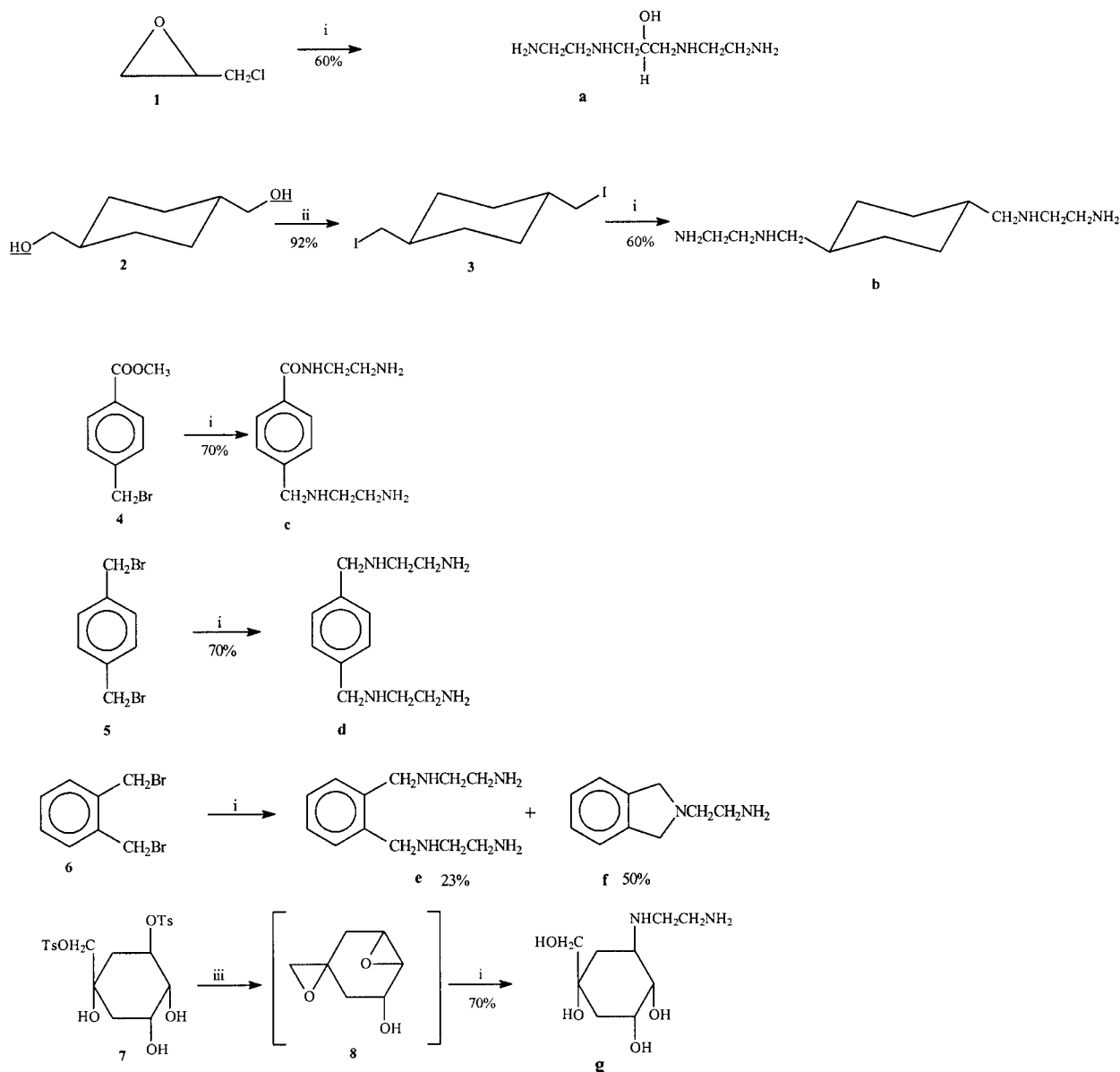
The IC_{50} values obtained for complexes **9**, **12**, and **15** in sensitive and resistant cells, together with the resistant factor (RF) and the intracellular platinum concentration, Ci , are shown in Table 1. For the sake of comparison, cisplatin and carboplatin data are also shown. The GLC4/CDDP subline is 6.3-fold resistant to cisplatin and 1.4- to carboplatin.

The cytotoxic activity of the compounds decreases in the order **9** > **12** > **15**. The sensitivity of the GLC4 cell line to compound **9** is comparable to that of carboplatin, and the GLC4/CDDP subline is not cross-resistant to compound **9**. The cytotoxic activity of compounds **12** and **15** is very low relative to that of the reference compounds. For **12** the RF obtained is 2.9, and for **15** the RF is 1.2 (Figure 1). In other words, the GLC4/CDDP cells are 3- and 1.2 times less sensitive than the GLC4 cells to **12** and **15**, respectively.

Platinum Accumulation

Resistance to cisplatin has been intensively studied in tumor cells repeatedly exposed to the drug in vitro. Among the numerous mechanisms of acquired resistance to cisplatin that have been reported, decreased accumulation is frequently present in resistant cells as a result of reduced drug uptake. In a previous study, we have determined the uptake rate of cisplatin, carboplatin, and aqua-substituted cisplatin in both GLC4 and GLC4/CDDP cell lines.^[31] The sensitive cell line accumulates approximately twice as much cisplatin as the resistant subline, mainly by an energy-dependent mechanism. We have postulated that the uptake of cisplatin is composed of passive diffusion of the $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$ species, which is neutral, and of an active uptake of an aqua-substituted species, probably the $[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})\text{OH}]^+$ or $[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})\text{Cl}]^+$ species. For carboplatin, the rate constant for the first aquation reaction is 100-fold lower than that for cisplatin. Therefore, the concentration of aquated species will be 100 times lower. The uptake rate of carboplatin is similar in both GLC4 and GLC4/CDDP cell lines because the active uptake becomes negligible. Therefore, carboplatin enters the cells mainly by passive diffusion, which is in accordance with the low RF observed for carboplatin (Table 1).

The cytotoxic activities of compounds **12** and **15** were much lower than those exhibited by cisplatin and by com-



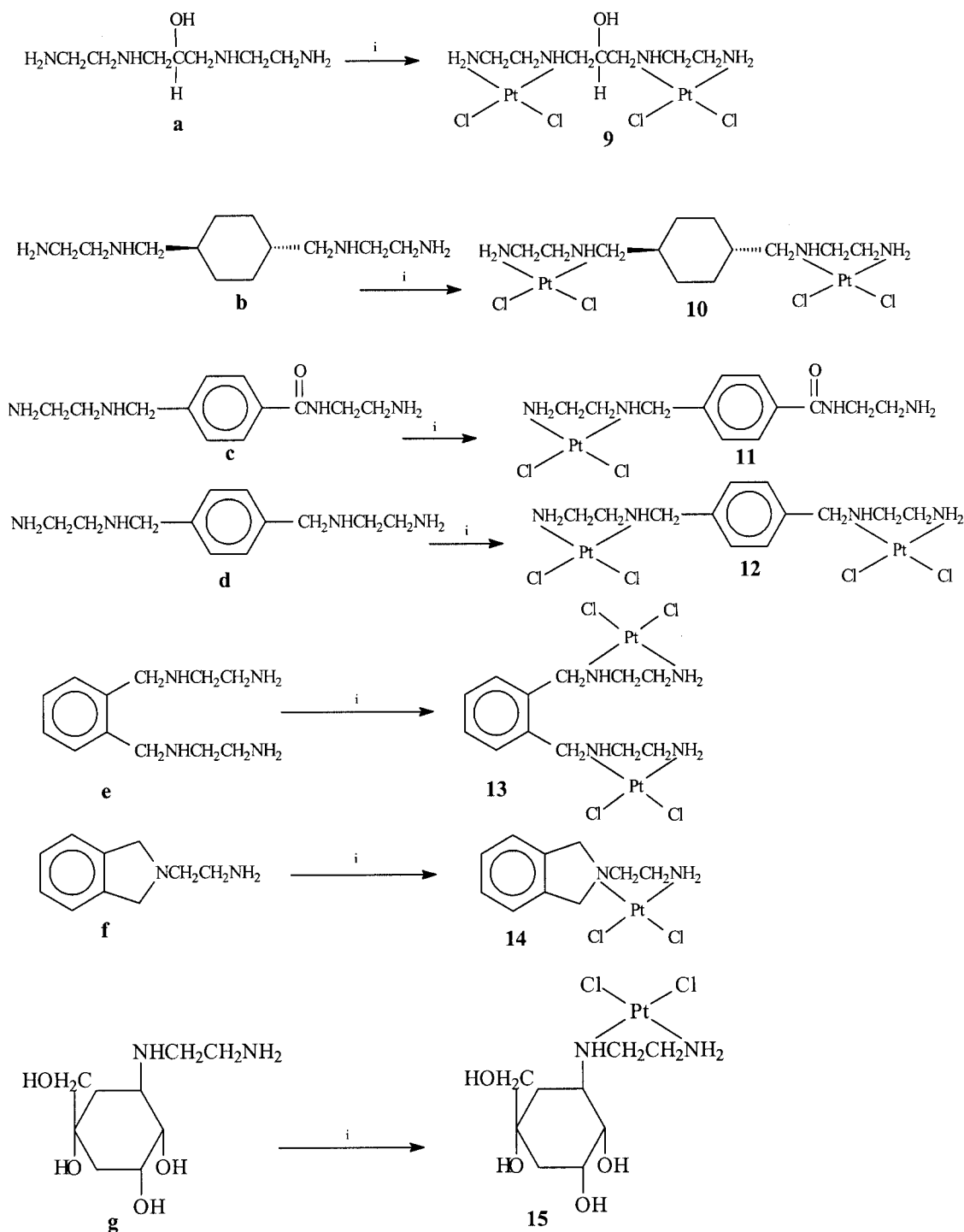
Scheme 1. Reagents and conditions: (i) $\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2/\text{EtOH}$, reflux; (ii) $\text{Ph}_3\text{P}/\text{I}_2/\text{imidazole}$, toluene, reflux, 24 h; (iii) $\text{MeO}^-/\text{Na}^+/\text{MeOH}$, room temp., 30 min.

pound **9**. These results can be explained by two possibilities: (i) the uptake of compounds **12** and **15** is very slow, thus a much higher concentration is needed to force their transport into cells (similar to the case of carboplatin when compared to cisplatin) or (ii) the interaction of the compounds with the pharmacological target, i.e. DNA, is deficient. In order to investigate whether the reason for this low activity is related to a deficient uptake, we have determined the intracellular platinum concentration after 72 h of incubation.

Let us first discuss the results obtained with the sensitive cell line. In a previous study, we found that cytotoxic activity correlates well with the intracellular drug concentration for cisplatin and carboplatin, and at IC_{50} , the intracellular platinum concentration was about 10×10^{-17} mol/cell.^[31] Furthermore, by studying the effect of some dinuclear platinum compounds, we also found that equitoxic

platinum concentrations lead to similar intracellular platinum concentrations.^[26]

Compound **9** exhibits the same behavior, for example, by incubating cells with the IC_{50} dose, the intracellular concentration is approximately 10×10^{-17} mol/cell. The ratio between the IC_{50} of carboplatin and that of cisplatin is approximately 25, i.e. more carboplatin is needed to produce a cytotoxic effect because the former enters cells more slowly. Our results showed that the reason for the low cytotoxic activities of **12** and **15** was not an insufficient intracellular platinum concentration. On the contrary, the intracellular platinum concentrations found for compounds **12** and **15** were significantly higher than those found for the reference compounds, cisplatin and carboplatin, and compound **9**. For the sensitive cell line, at IC_{50} values, the intracellular platinum concentrations were 14.9- and 9.3-fold higher for

Scheme 2. Reagents and conditions: (i) K_2PtCl_4 , H_2O , room temp.

compounds **12** and **15**, respectively, than that attained with cisplatin. This fact suggests that the formation of adducts between compounds **12** and **15** and the putative pharmacological target DNA is less favored. Knox et al.^[32] postulated that the difference in the kinetics of the interaction with DNA is responsible for the different sensitivity of the same cell line to cisplatin and carboplatin. According to these authors, 20- to 40-fold larger doses of carboplatin were required to produce equal binding to DNA and equal cytoto-

xicity. In a review of structure–activity relationships of some dinuclear platinum compounds, Farrell et al.^[33] reported that the cytotoxic activity of a dinuclear compound containing cyclohexane-1,4-diamine as a linker between two platinum atoms was very disappointing, probably because sterically hindered compounds bind more slowly to DNA. Assuming that equal binding to DNA would result in an equal cytotoxic effect, the fact that the intracellular concentrations found for compounds **12** and **15** are much

Table 1. IC₅₀, RF, and intracellular platinum concentration^[a] for complexes **9**, **12**, and **15**.

Compound	Cell Line	IC ₅₀ [μM]	RF	Ci × 10 ¹⁶ [mol/cell]
DDP ^[b]	GLC4	0.40 ± 0.05		0.98 ± 0.09
CDDP ^[b]	GLC4/ CDDP	2.5 ± 0.2	6.3	1.07 ± 0.11
CBDCA ^[b]	GLC4	9.9 ± 1.1		0.79 ± 0.08
CBDCA ^[b]	GLC4/ CDDP	13.5 ± 1.3	1.4	1.02 ± 0.11
9	GLC4	9.9 ± 0.9		0.96 ± 0.10
9	GLC4/ CDDP	8.9 ± 0.9	0.9	1.05 ± 0.10
12	GLC4	28.10 ± 2.80		14.92 ± 1.50
12	GLC4/ CDDP	81.0 ± 8.1	2.88	65.90 ± 6.70
15	GLC4	84.0 ± 8.3		9.28 ± 0.90
15	GLC4/ CDDP	102.5 ± 10.3	1.22	24.10 ± 2.50

[a] IC₅₀ is the complex concentration required to inhibit 50% of cell growth. [b] ref.^[31] RF value was calculated as resistant cell IC₅₀/sensitive cell IC₅₀. The values represent the mean ± SD of triplicate determinations; Ci is the mol number of platinum per cell at IC₅₀ doses after 3 d.

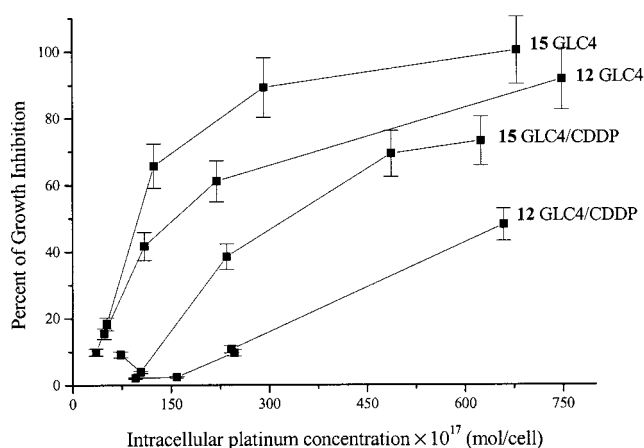


Figure 1. Growth inhibition of GLC4 and GLC4/CDDP cells as a function of the intracellular platinum concentration after 3 d of incubation with equitoxic doses of complexes **12** and **15**. The values are the mean values of two independent experiments. The platinum content was assessed by flameless atomic absorption spectroscopy.

higher than those of the reference compounds indicates that **12** and **15** bind to DNA more slowly or with a lower affinity, probably because of steric hindrance.

If these compounds bind more slowly to DNA, interaction with other intracellular ligands such as sulfur-containing molecules will become relevant and this may be the reason for the elevated intracellular platinum concentrations.

Considering **9** and **12**, these dinuclear platinum compounds are expected to form interstrand cross-links with DNA. The structural difference between them lies in the diamine linker. One can speculate that the insertion of a spacer (cycle) between the two platinum atoms engenders a steric hindrance to form DNA adducts. It is also possible that **12** only forms monoadducts with DNA.

An intriguing point to be considered is that a substantially higher intracellular platinum concentration is required to achieve a comparable cytotoxic effect in resistant cells by compounds **12** and **15** than in sensitive cells. Increased levels of sulfur-containing molecules have been found in various resistant cell lines. Hospers et al.^[34] found that the GLC4/CDDP cell line possesses a significantly higher amount of sulfur-containing molecules than the GLC4 cell line, especially glutathione. Increased levels of sulfhydryl compounds are pointed as a possible mechanism for resistance. These thiols could bind to platinum and prevent it from attaining its target, DNA. If resistant cells possess higher levels of sulfur-containing compounds, one could expect to find a higher platinum concentration within resistant cells, because these compounds could bind to platinum in a detoxification mechanism. This is the case for compounds **12** and **15**. A possible explanation is that the interactions of these compounds with DNA are very slow, thus interactions of other intracellular ligands such as glutathione become determinant. They are able to accumulate in cells but are not very effective in binding to DNA and inhibiting cell growth.

In the cases of cisplatin, carboplatin, and compound **9** we have observed that the equal intracellular platinum amounts are responsible for equal cytotoxicity. Thus, the larger doses required in the case of resistant cells are necessary to force the uptake, and, once inside cells, equal platinum amounts can produce equal binding to DNA and equal cytotoxic effects. Sadowitz et al.^[35] have shown that at low concentrations of cisplatin, endogenous thiols intercept cellular platinum, but this mechanism is not relevant at CDDP concentrations within the therapeutic range.

Conclusion

This work describes the synthesis and characterization of different amine-ligand derivatives of 1,2-ethylenediamine, and also the preparation and characterization of their respective platinum(II) complexes.

Concerning the dinuclear platinum complexes, the introduction of a spacer (cycle) between the two platinum atoms leads to a significant decrease in cytotoxic activity. This finding is very important in guiding further design of dinuclear platinum compounds aimed at treating cancer.

Experimental Section

Reagents: All chemicals were of reagent grade and were used without further purification.

Starting Materials

Compound 3: Imidazole (2.82 g, 41.6 mmol), triphenylphosphane (10.92 g, 41.6 mmol), and iodine (10.6 g, 41.6 mmol) were added to a solution of *trans*-1,4-bis(hydroxymethyl)cyclohexane (**2**) (2.0 g, 14 mmol) in toluene (50 mL). The reaction mixture was stirred at 60 °C in an oil bath for 2 h. The solution was cooled, treated with an aqueous solution of sodium bisulfite and extracted with toluene (3 × 50 mL). The organic phases were combined and concentrated.

The crude product was purified on silica gel using *n*-hexane as the eluent to give **3** in 92% yield (4.65 g, 12.77 mmol).

Compound 8:^[29] Methanolic sodium methoxide (25 mmol of sodium in 10 mL of methanol) was slowly added to a solution of ditosylate **7** (5 g, 10.35 mmol) in methanol (15 mL). The reaction was stirred for 30 min at room temperature, concentrated under reduced pressure and added to chloroform (10 mL). The resulting precipitate was collected and washed, and the filtrate was concentrated under reduced pressure to give **8** in quantitative yield.

Synthesis of Ligands

Ligand a: This ligand was prepared by following the procedure described in ref.^[26]

General procedure for the preparation of ligands **b–g** is shown in Scheme 1.

The corresponding halide or epoxide (10 mmol) was slowly added to a solution of ethylenediamine (6.7 mL, 100 mmol) in ethanol (20 mL) over 5 h. The reaction mixture was stirred for 12–24 h under reflux, evaporated under reduced pressure, and the residue purified on silica gel using dichloromethane/methanol as the eluent. Yields: (**b**) 1.37 g (60%), (**c**) 1.18 g (50%), (**d**) 1.56 g (70%), (**e**) 1.20 g (54%), (**f**) 0.18 g (11%), and (**g**) 1.54 g (70%)

(**b**): IR (KBr): $\tilde{\nu}_{\max}$ = 2925, 2853, 1634, 1401, 1350, 1037, 772 cm⁻¹. ¹H NMR (200 MHz, CD₃OD): δ = 3.25 (m, 8 H, CH₂N), 1.38 (m, 6 H, CH, CH₂ cyclohexyl) ppm. ¹³C NMR (50 MHz, CD₃OD): δ = 54.0 (CH₂NHCH₂CH₂NH₂), 44.7 (NHCH₂), 36.5 (CH₂NH₂), 35.4, 28.6 (cyclohexyl) ppm.

(**c**): IR (KBr): $\tilde{\nu}_{\max}$ = 2933, 1646, 1543, 1506, 1313, 1160, 1108, 1019, 859, 757 cm⁻¹. ¹H NMR (200 MHz, CD₃OD): δ = 7.79 (d, 2 H, H-2, H-6, *J* = 8.0 Hz), 7.55 (d, 2 H, H-3, H-5, *J* = 8.0 Hz) ppm. ¹³C NMR (50 MHz, CD₃OD): δ = 170.6 (C=O), 134.6, 134.0, 130.1, 128.1 (Ph), 51.0 (CH₂Ph), 44.0, 39.3 (CH₂NH), 37.4, 35.7 (CH₂NH₂) ppm.

(**d**): IR (KBr): $\tilde{\nu}_{\max}$ = 2948, 2821, 1594, 1384, 1352, 1247, 1055, 774, 669 cm⁻¹. ¹H NMR (200 MHz, CD₃OD): δ = 7.32 (s, 4 H, CH), 3.74 (s, 4 H, CH), 3.74 (s, 4 H, CH₂Ph), 2.71 (t, 8 H, NHCH₂, CH₂NH₂) ppm. ¹³C NMR (50 MHz, CD₃OD): δ = 139.7, 129.6 (Ph), 54.1 (CH₂Ph), 51.8 (NHCH₂), 41.7 (CH₂NH₂) ppm.

(**e**): IR (KBr): $\tilde{\nu}_{\max}$ = 2950, 2820, 1592, 1386, 1352, 1248, 1055, 772, 669 cm⁻¹. ¹H NMR (200 MHz, CD₃OD): δ = 7.44 (m, 4 H, Ph), 4.17 (s, 4 H, CH₂Ph), 3.11 (m, 8 H, NHCH₂, CH₂NH₂) ppm. ¹³C NMR (50 MHz, CD₃OD): δ = 136.6, 132.9, 130.4 (Ph), 52.2 (CH₂Ph), 46.6 (CH₂NH), 39.5 (CH₂NH₂) ppm.

(**f**): IR (KBr): $\tilde{\nu}_{\max}$ = 2948, 1594, 1383, 1352, 1058, 775, 667 cm⁻¹. ¹H NMR (200 MHz, CD₃OD): δ = 7.21 (m, 4 H, Ph), 4.04 (s, 4 H, CH₂Ph), 3.09 (m, 4 H, NHCH₂, CH₂NH₂) ppm. ¹³C NMR (50 MHz, CD₃OD): δ = 140.4, 130.0, 124.6 (Ph), 59.9 (CH₂Ph), 53.5 (CH₂NH), 39.1 (CH₂NH₂) ppm.

(**g**): IR (KBr): $\tilde{\nu}_{\max}$ = 3403, 2936, 2862, 1648, 1573, 1460, 1343, 1301, 1151, 1052, 958 cm⁻¹. ¹H NMR (200 MHz, CD₃OD): δ = 3.92 (m, 1 H, H-3), 3.70 (t, 1 H, H-4), 3.13 (t, 1 H, H-5), 2.64 (m, 4 H, CH₂NH, CH₂NH₂), 1.80 (m, 4 H, H-2, H-2', H-6, H-6') ppm. ¹³C NMR (50 MHz, CD₃OD): δ = 76.7, 71.1, 68.5, 65.6 (C-1, C-3, C-4, C-7), 63.8, 59.8, 44.0 (C-5, CH₂N), 38.7, 36.7 (C-2, C-6) ppm.

Synthesis of Complexes 9–15

Complex 9: Prepared following the procedure described in ref.^[26]

Complexes 10–15 (Scheme 2): The appropriate ligand (1 mmol), dissolved in water (5 mL), was slowly added to a solution of K₂PtCl₄ (0.830 g, 2 mmol) in water (10 mL). After stirring for 24 h in the

dark at room temperature, the solid that formed was filtered off, washed with water, and dried. In the case of complexes **14** and **15** only 1 mmol of K₂PtCl₄ was used. Yields: (**10**) 0.47 g (62%), (**11**) 0.25 g (50%), (**12**) 0.69 g (92%), (**13**) 0.32 g (42%), (**14**) 0.17 g (40%), (**15**) 0.364 g (75%).

(**10**): IR (KBr): $\tilde{\nu}_{\max}$ = 3270, 2910, 2846, 1628, 1313, 1045, 827, 549, 326 cm⁻¹. ¹⁹⁵Pt NMR (86 MHz, [D₆]DMSO) (DMSO = dimethyl sulfoxide): δ = -2380 ppm. C₁₂H₂₈N₄Pt₂Cl₄ (760.18): calcd. C 18.95, H 3.68, N 7.37; found C 19.15, H 3.86, N 7.35.

(**11**): IR (KBr): $\tilde{\nu}_{\max}$ = 3207, 3114, 2923, 2847, 1639, 1541, 1462, 1306, 1188, 1060, 1014, 861, 759, 569, 317 cm⁻¹. ¹H NMR (400 MHz, [D₆]DMSO): δ = 2.83, 3.59 (m, 4 H, CH₂NH, CH₂NH₂), 4.25 (m, 4 H, CH₂Ph), 5.08, 6.29 (s, 3 H, NH, NH₂), 7.95 (m, 4 H, Ar) ppm. ¹³C NMR (100 MHz, [D₆]DMSO): δ = 54.3–45.6 (CH₂NH, CH₂NH₂), 61.1 (CH₂Ph); 128.0–130.4 (Ar) ppm. ¹⁹⁵Pt NMR (86 MHz, [D₆]DMSO): δ = -2018 ppm. C₁₂H₂₀N₄PtCl₂ (486.21): calcd. C 28.69, H 3.98, N 11.15; found C 29.03, H 3.86, N 11.45.

(**12**): IR (KBr): $\tilde{\nu}_{\max}$ = 3130, 3056, 2984, 2952, 2883, 1643, 1451, 1287, 1189, 1064, 1018, 954, 853, 759, 576, 501, 324 cm⁻¹. ¹H NMR (400 MHz, [D₆]DMSO): δ = 2.53 (m, 8 H, CH₂NH, CH₂NH₂), 3.98 (s, 4 H, CH₂Ph), 5.30, 6.25 (s, 4 H, NH₂), 6.38 (s, 2 H, NH), 7.56 (m, 4 H, Ph) ppm. ¹³C NMR (100 MHz, [D₆]DMSO): δ = 43.8, 44.7 (CH₂NH₂), 45.6, 46.9 (CH₂NH), 54.3, 54.9 (CH₂Ph), 130.4–136.7 (Ar) ppm. C₁₂H₂₂N₄Pt₂Cl₄ (754.14): calcd. C 19.10, H 2.92, N 7.43; found C 19.45, H 2.86, N 7.75.

(**13**): IR (KBr): $\tilde{\nu}_{\max}$ = 3259, 3192, 3109, 2954, 2885, 2858, 1586, 1441, 1191, 1164, 1064, 762, 531, 314 cm⁻¹. ¹H NMR (400 MHz, [D₆]DMSO): δ = 3.40 (m, 4 H, CH₂NH₂); 3.80 (m, 4 H, CH₂NH); 4.30 (s, 4 H, CH₂Ph); 5.52 (s, 4 H, NH₂); 5.80, 6.30 (s, 2 H, NH); 7.50 (m, 4 H, Ar) ppm.

(**14**): IR (KBr): $\tilde{\nu}_{\max}$ = 3200, 3116, 2949, 1622, 1313, 1158, 1049, 978, 926, 894, 823, 749, 609, 409 cm⁻¹. ¹H NMR (400 MHz, [D₆]DMSO): δ = 2.51, 2.84 (2m, 4 H, CH₂NH₂); 4.25, 4.45 (2d, 2 H, CH₂N); 4.25, 4.45, 4.87, 4.97 (4d, 4 H, CH₂Ph), 5.52 (s, 1 H, NH₂), 6.31 (s, 1 H, NH₂), 7.28 (m, 4 H, Ph) ppm. ¹³C NMR (100 MHz, [D₆]DMSO): δ = 44.7 (CH₂NH₂), 46.2 (NCH₂), 64.4, 65.4 (CH₂Ph), 122.9–136.7 (Ar). C₁₀H₁₄N₂PtCl₂ (428.13): calcd. C 28.04, H 3.27, N 6.54; found C 28.40, H 2.96, N 6.75.

(**15**): IR (KBr): $\tilde{\nu}_{\max}$ = 3415, 3269, 3197, 2958, 2928, 1140, 1034, 1018, 716, 549, 326 cm⁻¹. C₉H₂₀N₂O₄PtCl₂ (486.17): calcd. C 22.20, H 4.12, N 5.76; found C 22.15, H 3.86, N 5.75.

Cell Lines and Cultures

The GLC4 cell line was derived from pleural effusion of a patient with small-cell lung carcinoma in the laboratory of Prof. E. G. de Vries (Department of Internal Medicine, University Hospital, Groningen, The Netherlands). The GLC4/CDDP was obtained in the same laboratory by continuous exposure to CDDP. No amplification of the MDR gene or expression of P-glycoprotein was found in the GLC4/CDDP subline.

The cell lines were cultured in an RPMI 1640 (Sigma Chemical Co.) medium supplemented with 10% fetal calf serum (Biomedica Co.) at 37 °C in a humidified 5% CO₂ atmosphere. Cultures grow exponentially from 10⁵ cells/mL to about 10⁶ cells/mL in 3 d. Cell viability was checked by Trypan blue exclusion. The cell number was determined by Coulter counter analysis. For the cytotoxicity assessment, 1 × 10⁵ cells/mL were cultured for 72 h in the absence and the presence of various concentrations of each platinum-based compound. The sensitivity to the drug was evaluated by the drug concentration that inhibits cell growth by 50%, IC₅₀. A resistance

factor (RF) was obtained by dividing the IC₅₀ of resistant cells by the IC₅₀ of sensitive cells. The RF to cisplatin is 6.3.

Stock solutions of compounds **9**, **12**, and **15** were prepared in a mixture of water/DMSO (90:10). In the cytotoxic assays, the final concentration of DMSO was below 1.5%.

Cellular Accumulation

Cells were incubated with different complex concentrations for 3 d. After incubation, an aliquot was removed, washed twice with ice-cold PBS (phosphate-buffered saline), and the pellet mineralized in 65% HNO₃. The platinum concentration was determined by atomic absorption spectroscopy with a Varian model Zeeman 220 spectrophotometer equipped with a graphite tube atomizer and an autosampler. The heating program consisted of sequential drying (85 °C for 5 s, 95 °C for 40 s, and 95 °C for 20 s), an ashing stage (500 °C for 5.0 s followed by 15 s at 1100 °C), and atomizing at 2500 °C for 5 s.

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