Synthesis, Characterization, and in Vitro Antitumor Activity of Osteotropic Diam(m)ineplatinum(II) Complexes Bearing a *N,N*-Bis(phosphonomethyl)glycine Ligand†

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A series of osteotropic (bone-seeking) [(bis(phosphonomethyl)amino-κN)acetato-κO(2-)]platinum(II) complexes attached to diammine, ethane-1,2-diamine, *cis-R,S*-cyclohexane-1,2-diamine, *trans-S,S*-cyclohexane-1,2-diamine, or *trans-R,R*-cyclohexane-1,2-diamine has been synthesized in accord with the concept of drug targeting and characterized by elemental analysis, ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy. The in vitro antitumor activity in ovarian cancer cells (CH1) has been determined by means of the MTT assay. In this cisplatin-sensitive cell line the complexes containing cyclohexane-1,2-diamine (chxn) displayed a high activity in comparison to the diammine and ethane-1,2-diamine counterparts. In agreement with structure−activity relationships of other chxn-containing platinum(II) complexes both [(bis(phosphonomethyl)amino-κN)-acetato-κO(2-)](*trans*-cyclohexane-1,2-diamine)platinum(II) complexes show superior potency than the corresponding *cis*-congener whereas the *trans-R,R* isomer displays the highest activity. Within the series of complexes under investigation, potency decreases depending on the coordinated amine ligand in the following order: *trans-R,R*-chxn > *trans-S,S*-chxn > NH_3 ≥ *cis-R,S*-chxn > en.

Introduction

Despite the worldwide success of cisplatin,[1−4] *cis*-diamminedichloroplatinum(II) (Figure 1), as one of the best agents in cancer chemotherapy in the clinic, there is still a need for other platinum-based antitumor agents in order to overcome the severe side-effects, to improve clinical effectiveness, and to broaden the spectrum of activity. Besides cisplatin, carboplatin, *cis*-diammine-(1,1-cyclobutanedicarboxylato)platinum(II), and oxaliplatin, (*trans-R,R*-cyclohexane-1,2-diamine)oxalatoplatinum(II) (Figure 1), are in clinical use today.[5−7]

Drug interactions with the target, DNA, and the biological transformations which occur in the body are a function of the oxidation state of the platinum center[8] and especially the nature of the attached ligands.[9] By choosing an appropriate ligand sphere activity (potency) of the drugs should be maximized, whereas it is desirable to minimize their general toxicity.

In order to synthesize platinum complexes with selective activity in primary and secondary bone malignancies (osteosarcoma and bone metastases from tumors with other primary sites), bis(phosphonomethyl)-aminoacetic acid, which has a high affinity for the mineral bone matrix (hydroxylapatite), has been used as a ligand for platinum(II) in accord with the concept of drug targeting (Figure 2). The use of phosphonic acid groups follows a principle, which is well-established in the development of osteotropic pharmaceuticals. In particular, bisphosphonates have, due to their inhibitory activity on osteoclast functions, become important drugs for the treatment of bone disorders such as M. Paget and postmenopausal osteoporosis as well as tumor-induced osteolysis and hypercalcemia. Apart from these therapeutic effects, the potential role of bisphosphonates in the prevention of bone metastases in breast cancer patients is currently investigated with promising results, probably reflecting an interruption of the vicious cycle of metastasis-facilitating osteolysis and paracrine stimulation of osteoclasts by the malignant cells rather than a direct cytostatic effect.[10,11]

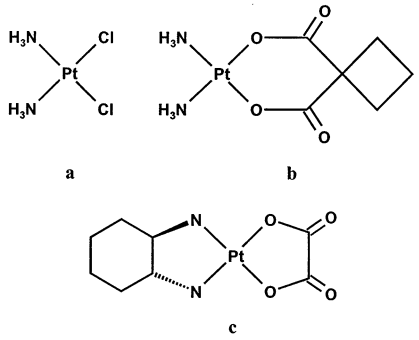

Figure 1. Platinum(II) complexes in clinical use: cisplatin (a), carboplatin (b), and oxaliplatin (c).

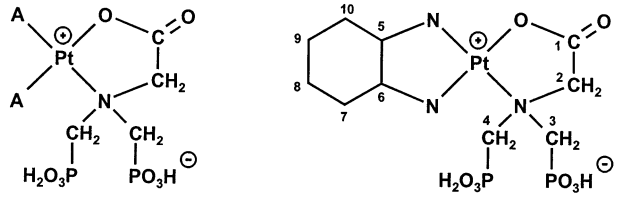

Figure 2. [(Bis(phosphonomethyl)amino-κN)acetato-κO(2-)]-platinum(II) complexes under investigation (left): A_2 = diammine, ethane-1,2-diamine, *cis-R,S*-cyclohexane-1,2-diamine, *trans-S,S*-cyclohexane-1,2-diamine, and *trans-R,R*-cyclohexane-1,2-diamine. Numbering scheme for NMR analysis (right).

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† Dedicated to Prof. Jan Reedijk on the occasion of his 60th birthday.

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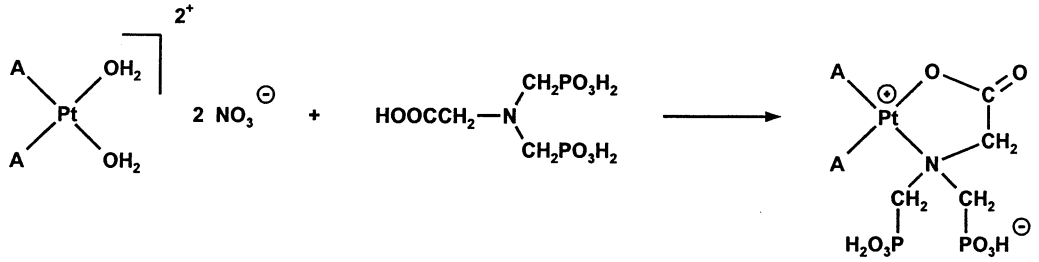
Scheme 1. Synthesis of [(Bis(phosphonomethyl)amino-κN)acetato-κO(2-)]platinum(II) Complexes



Coupling of antineoplastic diammineplatinum(II) moieties to phosphonate-containing ligands has been encouraged by prior experience with bisphosphonate-coupled chlorambucil.[12] In both cases, antineoplastic and osteotropic properties are maintained when these moieties are combined in one molecule. With prototypic cisplatin-analogous aminobis/trismethylene-phosphonate complexes, accumulation in bone tissue has previously been confirmed by autoradiography in rats.[13] In this context, it is worth mentioning that accumulation is more likely the result of a retention of the complexes in the bone rather than of a carrier-mediated transport to the bone. A therapeutic activity superior to cisplatin has been demonstrated in an orthotopically transplanted rat osteosarcoma model, which disseminates to the lung, producing lethal ossifying metastases. In this model, which closely resembles osteosarcoma in humans in terms of histology, pattern of metastases formation, and reduced chemosensitivity, growth of the primary tumor was strongly inhibited and survival was more markedly prolonged than by treatment with either cisplatin or the antimetastatic agent razoxane, probably as a result of a delay of lung metastases growth.[14−16] Cytostatic activity in cell clones obtained from such lung metastases was subsequently confirmed in vitro.[17]

Current attempts to optimize the pharmacological effects within this class of compounds involve modifications of both the leaving phosphonate-containing group, which is responsible for the osteotropic properties, and the nonleaving amine group, which is decisive for the cellular processing of DNA adducts.[18] Bis(phosphonomethyl)aminoacetate (BPMAA) has been favored instead of aminotris(methylenephosphonate) (ATMP) as the osteotropic ligand, because complexes containing the former display a lower acute toxicity, lower renal toxicity and a higher therapeutic index and are well tolerated even in high doses by test animals.[16,19] With respect to the nonleaving ligand, special efforts have been devoted to the development of oxaliplatin-analogous complexes, since this third-generation platinum drug is known to induce DNA adducts which are on average more cytotoxic than those induced by cisplatin[20] and to retain its activity in tumors, which are resistant to cisplatin as a result of increased cisplatin−DNA adduct tolerance.[21] As these properties have to be ascribed to the presence of the bulky cyclohexane-1,2-diamine ligand, we have prepared BPMAA−platinum(II) complexes with this ligand, expecting that this structural modification translates into favorably altered pharmacological properties such as those described for oxaliplatin. We have evaluated the antitumor activity of these new complexes in vitro in comparison to their diammine and ethane-1,2-diamine analogues, but our investigations into the structure−activity relationships also include the stereoisomers resulting from the two stereocenters in cyclohexane-1,2-diamine.

Results and Discussion

Synthesis and Characterization of [(Bis(phosphonomethyl)amino-κN)acetato-κO(2-)]platinum(II) Complexes. The synthesis of the [(bis(phosphonomethyl)amino-κN)acetato-κO(2-)]platinum(II) complexes under investigation is accomplished in three steps. Starting from K_2PtCl_4, the halogenoplatinum(II) compounds [Pt(NH_3)$_2$$I_2$], [Pt(en)$Cl_2$], [Pt(*cis*-*R*,*S*-chxn)Cl_2], [Pt(*trans*-*S*,*S*-chxn)Cl_2], and [Pt(*trans*-*R*,*R*-chxn)Cl_2] can be obtained in one step with yields ranging from 56 to 91%. The activated diaquaplatinum(II) species are prepared by reaction of the before mentioned diam(m)inedihalogeno complexes with silver nitrate. After removing the silver halide, the phosphonic acid is added and the [(bis(phosphonomethyl)amino-κN)acetato-κO(2-)]platinum(II) complexes are formed (Scheme 1).

In case of the *cis*-*R*,*S*-cyclohexane-1,2-diamine ligand, two isomers, (*SP*-4-3)-[Pt(*cis*-*R*,*S*-chxn)BPMAA] and (*SP*-4-4)-[Pt(*cis*-*R*,*S*-chxn)BPMAA], are formed with either NH_2-(C^R) or NH_2-(C^S) in trans position to the coordinated carboxylato group.

Coordination of the phosphonic acid ligand and purity of the complexes can best be judged by use of ^{31}P NMR spectroscopy. In the phosphorus spectra, the free ligand resonates at 7.9 ppm. After coordination of the bis(phosphonomethyl)-functionalized amino acid signals between 11 and 13 ppm can be found. Coordination of phosphonate instead of carboxylate would result in a significant downfield shift with signals around 43 ppm. Furthermore, the ^{13}C signal of the coordinated carboxylato group is found in the range of 184.5−184.9 ppm in contrast to the free ligand with a chemical shift of 168.2 ppm also proving the coordination sphere around the metal center as shown in Figure 2.

When cyclohexane-1,2-diamine is coordinated to platinum(II) two resonances in the ^{31}P NMR spectra can be detected reflecting the nonequivalence of the phosphonomethyl groups. Moreover, the protons of the methylene group, (H(2), NCH_2COO), are diastereotopic and therefore two doublets with a geminal coupling of 16 Hz are observed.

Structure−Activity Relationships. The antiproliferative activity of the [(bis(phosphonomethyl)amino-κN)acetato-κO(2-)]platinum(II) complexes under investigation has been compared in the human ovarian cancer cell line CH1, which is highly sensitive to cisplatin, by means of a colorimetric microculture assay technique (MTT assay). The concentration−response curves obtained after exposure for 96 h are shown in Figure 3 and the corresponding IC_{50} values are reported in Table 1.

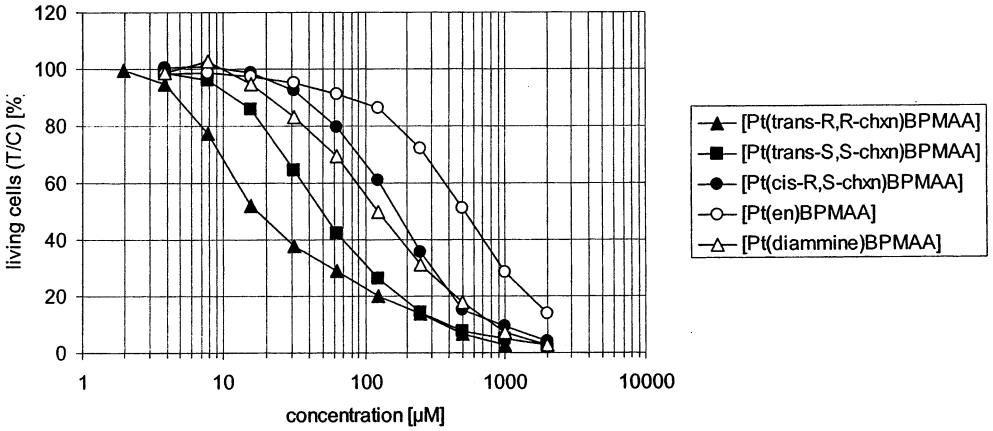


Figure 3. Concentration−response curves of [(bis(phosphonomethyl)amino-κN)acetato-κO(2-)]platinum(II) complexes in ovarian cancer cells (CH1) after exposure for 96 h.

Table 1. Antiproliferative Activity of [(Bis(phosphonomethyl)amino-κN)acetato-κO(2-)]platinum(II) Complexes in Comparison to Established Anticancer Platinum-Based Drugs in the Ovarian Cancer Cell Line CH1[a]

[Pt(NH_3)$_2$BPMAA]	128 ± 22
[Pt(en)BPMAA]	532 ± 154
[Pt(*cis*-*R*,*S*-chxn)BPMAA]	169 ± 56
[Pt(*trans*-*S*,*S*-chxn)BPMAA]	52.4 ± 10.9
[Pt(*trans*-*R*,*R*-chxn)BPMAA]	20.6 ± 1.1
cisplatin	0.15 ± 0.01
carboplatin	2.5 ± 0.4
oxaliplatin	0.27 ± 0.07

[a] IC_{50} values (μM, means ± SD) of [Pt(A_2)BPMAA] complexes in ovarian cancer cells (CH1) after exposure for 96 h, determined by MTT assay.

Both [Pt(*trans*-chxn)BPMAA] compounds show a higher potency than the corresponding [Pt(*cis*-*R*,*S*-chxn)BPMAA] counterpart, with [Pt(*trans*-*R*,*R*-chxn)-BPMAA] being the most active among the three isomers. This is in accord with published structure−activity relationships of oxaliplatin isomers and those of other chxn-containing platinum(II) as well as platinum(IV) complexes in vitro[24,25] and in vivo,[26,27] to which only a few exceptions have been reported.[23,28] Moreover, both [Pt(*trans*-chxn)BPMAA] complexes display a high activity in comparison to [Pt(NH_3)$_2$BPMAA] and [Pt(en)-BPMAA], while [Pt(*cis*-*R*,*S*-chxn)BPMAA] is similarly or even slightly less active than [Pt(NH_3)$_2$BPMAA] and only superior to [Pt(en)BPMAA]. Within the complexes under investigation, potency thus decreases depending on the coordinated amine ligand in the following order:

$$\textit{trans}\text{-}R,R\text{-chxn} > \textit{trans}\text{-}S,S\text{-chxn} > NH_3 \geq \textit{cis}\text{-}R,S\text{-chxn} > \text{en}$$

The superiority of [Pt(*trans*-*R*,*R*-chxn)BPMAA] compared to [Pt(NH_3)$_2$BPMAA] (DBP, KP735), which had previously been favored as a candidate drug for clinical studies, is all the more remarkable, as it is more pronounced than it would be expected from published comparisons of the in vitro activity of the corresponding dichloro analogues [Pt(*trans*-*R*,*R*-chxn)Cl_2] and [Pt-(NH_3)$_2Cl_2$] (cisplatin). The cyclohexane-1,2-diamine-containing platinum compounds [Pt(*trans*-*R*,*R*-chxn)Cl_2] and [Pt(*trans*-*R*,*R*-chxn)(oxalate)] (oxaliplatin) are known to produce DNA adducts, which inhibit replication more effectively than cisplatin−DNA adducts,[20,29] and from comparison of DNA platination levels and cytotoxicity, it has been concluded that these adducts are on average more detrimental to the cell than those induced by cisplatin.[20] However, in cisplatin-sensitive cells this does not necessarily translate into a substantially higher cytotoxic potency of these drugs compared to cisplatin.[20,30] In fact, our own experiments in the CH1 cells used in this study indicate that oxaliplatin is only approximately half as active as cisplatin in terms of IC_{50} values (0.27 μM and 0.15 μM, respectively). In contrast, [Pt(*trans*-*R*,*R*-chxn)BPMAA] displays a 6-fold higher antiproliferative activity than its diammine congener [Pt(NH_3)$_2$BPMAA], based on comparison of IC_{50} values in this cell line.

The superiority of [Pt(*trans*-*R*,*R*-chxn)BPMAA] and the reported structure−activity relationships in general are confirmed by preliminary results in L1210 murine leukemia in terms of therapeutic efficacy in vivo (data not shown). In this tumor model, survival of the test animals is markedly extended by administration of [Pt-(*trans*-*R*,*R*-chxn)BPMAA], as reflected in T/C values of > 250% at optimal dosage. As various cisplatin-resistant tumor cell lines, in particular those with resistance due to increased cisplatin−DNA adduct tolerance, consistently lack cross-resistance to (cyclohexane-1,2-diamine)platinum(II) complexes,[21] we expect the activity of the chxn-containing BPMAA complexes to be retained also in these cisplatin-resistant cells and corresponding in vivo tumor models.

It has previously been demonstrated that aminobis/trismethylenephosphonate ligands endow platinum with a strong affinity for bone tissue[13] and with an improved therapeutic activity in a rat osteosarcoma model, despite decreasing the cytotoxic potency as compared to the parent compound cisplatin.[14−16] It seems reasonable to assume by analogy that the lower cytotoxicity of [Pt-(*trans*-*R*,*R*-chxn)BPMAA] as compared to oxaliplatin should likewise be compensated by the osteotropic properties mediated by the *N*,*N*-bis(phosphonomethyl)-glycine ligand, resulting in an increased selectivity for bone tumors.

Conclusions

In accord with the concept of drug targeting, a series of osteotropic (bone-seeking) [(bis(phosphonomethyl)-amino-κN)acetato-κO(2-)]platinum(II) complexes was prepared. The in vitro antitumor activity in the human ovarian cancer cell line CH1 was determined by means

of the MTT assay. To maximize potency of the drugs and to set up a structure−activity relationship, different kinds of diam(m)ine ligands have been used, whereas the bis(phosphonomethyl)-substituted amino acid ligand, which is responsible for the carrier-mediated transport to the mineral bone matrix, was left unchanged. Special efforts have been expended to use (cyclohexane-1,2-diamine)platinum(II) units, especially the corresponding *trans-R,R* isomer, for the development of oxaliplatin-analogous bis(phosphonomethyl)amino-κN)acetato-κO(2-)]platinum(II) complexes.

As expected, both [Pt(*trans*-chxn)BPMAA] compounds show superior potency compared to the corresponding [Pt(*cis-R,S*-chxn)BPMAA] compound, which is in agreement with structure−activity relationships of other chxn-containing platinum complexes. The activity of the [Pt(*trans-R,R*-chxn)BPMAA] complex was found to be 6-fold higher than that of its diammine counterpart.

Besides variation in the nonleaving diam(m)ine ligand, which is decisive for the cellular processing of DNA adducts, the bis(phosphonomethyl)-containing residue is further modified to optimize the pharmacological effects within the class of these osteotropic compounds.

Materials and Methods

The compounds reported in this study are illustrated in Figure 2. We will refer to these compounds as complexes [Pt-$(NH_3)_2$BPMAA], [Pt(en)BPMAA], [Pt(*cis-R,S*-chxn)BPMAA], [Pt(*trans-S,S*-chxn)BPMAA], and [Pt(*trans-R,R*-chxn)BPMAA].

Chemicals and Supplies for Synthesis. Potassium tetrachloroplatinate was obtained from Degussa. The phosphonic acids were kindly provided by Henkel KGa Düsseldorf. All other chemicals obtained from commercial suppliers were used as received and were of analytical grade. Water was used bidistilled. The synthetic procedures were carried out in a light-protected environment.

NMR Measurements. ^{1}H, ^{13}C{^{1}H}, ^{31}P{^{1}H }, ^{1}H,^{1}H-COSY, and ^{13}C,^{1}H-COSY spectra were recorded in D_2O or H_2O/D_2O (9:1, 2D spectra in a gradient enhanced mode) at 298 K using a Bruker Avance DPX 400 instrument (UltraShield Magnet) and standard pulse programs at 400.13 (^{1}H), 100.62 (^{13}C), and 162.0 MHz (^{31}P). Chemical shifts were measured relative to the solvent peak (4.71 ppm) or to external 85% H_3PO_4. Elemental analyses were performed by the microanalytical laboratory at the University of Vienna.

Syntheses. (*SP*-4-2)-Diamminediiodoplatinum(II) and the dichloroplatinum(II) complexes (*SP*-4-2)-dichloro(ethane-1,2-diamine)platinum(II), (*SP*-4-2)-dichloro(*cis-R,S*-cyclohexane-1,2-diamine)platinum(II), (*SP*-4-2)-dichloro(*trans-S,S*-cyclohexane-1,2-diamine)platinum(II), and (*SP*-4-2)-dichloro(*trans-R,R*-cyclohexane-1,2-diamine)platinum(II) have been synthesized according to standard literature procedures starting from K_2PtCl_4:[22,23]

(*SP*-4-2)-Diamminediiodoplatinum(II), yield 91%. Anal. ($H_6I_2N_2Pt$) H, N. **(***SP*-4-2)-Dichloro(ethane-1,2-diamine)platinum(II), yield 56%. Anal. ($C_2H_8Cl_2N_2Pt$) C, H, N. (*SP*-4-2)-Dichloro(*cis-R,S*-cyclohexane-1,2-diamine)platinum(II), yield 62%. Anal. ($C_6H_{14}Cl_2N_2Pt$) C, H, N. (*SP*-4-2)-Dichloro(*trans-S,S*-cyclohexane-1,2-diamine)platinum(II), yield 64%. Anal. ($C_6H_{14}Cl_2N_2Pt$) C, H, N. (*SP*-4-2)-Dichloro(*trans-R,R*-cyclohexane-1,2-diamine)platinum(II), yield 61%. Anal. ($C_6H_{14}Cl_2N_2$-Pt) C, H, N.

(S*P*-4-3)-Diammine[(bis(phosphonomethyl)amino-κN)-acetato-κO(2-)]platinum(II). (*SP*-4-2)-Diamminediiodoplatinum(II) (3.358 g, 6.95 mmol) was suspended in 60 mL of water. After addition of silver nitrate (2.242 g, 13.20 mmol), the mixture was stirred overnight at room temperature. Silver iodide precipitated and was filtered off. Bis(phosphonomethyl)-aminoacetic acid (1.738 g, 6.61 mmol) was added to the yellow solution in one portion. After the mixture was stirred for 30 min at 50 °C and 3 days at room temperature, the white solid was collected by filtration, washed with small amounts of water, and dried under reduced pressure over P_2O_5 to obtain 2.563 g of [Pt$(NH_3)_2$BPMAA]; yield 79% (based on the amount of silver nitrate used). ^{1}H NMR in D_2O: δ = 3.25 [m, 2H, H(3), H(4)], 3.50 [m, 2H, H(3), H(4)], 4.07 [H(N)], 4.16 [s, 2H, H(2)], 4.51 [H(N)]. ^{13}C NMR in D_2O: δ = 61.6 [dd, 2C, $^1J_{C,P}$ = 141 Hz, $^3J_{C,P}$ = 7 Hz, C(3), C(4)], 67.1 [dd, $^3J_{C,P}$ = 7 Hz, C(2)], 184.9 [C(1)]. ^{31}P NMR in D_2O: δ = 11.8. Anal. ($C_4H_{15}N_3O_8P_2Pt$) C, H, N.

(*SP*-4-3)-[(Bis(phosphonomethyl)amino-κN)acetato-κO(2-)](ethane-1,2-diamine)platinum(II). (*SP*-4-2)-Dichloro(ethane-1,2-diamine)platinum(II) (946 mg, 2.90 mmol, was suspended in 40 mL of water. Silver nitrate (937 mg, 5.52 mmol) was added, and the mixture was stirred for 1 day at room temperature. The silver chloride formed was removed by filtration, and the volume of the remaining yellow solution was reduced to 10 mL. Thereafter, bis(phosphonomethyl)-aminoacetic acid (750 mg, 2.85 mmol) was added, and the solution was stirred for 1 h at 50 °C and subsequently for 1 day at room temperature. [Pt(en)BPMAA] was precipitated with acetone, filtered, and dried over P_2O_5 under reduced pressure to obtain 1.101 g of a white crystalline solid; yield 77% (based on the amount of silver nitrate used). ^{1}H NMR in D_2O: δ = 2.44 [sbr, 4H, H(5), H(6)], 3.31 [m, 2H, H(3), H(4)], 3.50 [m, 2H, H(3), H(4)], 4.05 [s, 2H, H(2)], 5.30 [s, 2H, H(N)], 5.71 [s, 2H, H(N)]. ^{13}C NMR in D_2O: δ = 47.1 [C(5) or C(6)], 47.8 [C(6) or C(5)], 61.7 [d, $^1J_{C,P}$ = 149 Hz, C(3), C(4)], 66.2 [C(2)], 184.5 [C(1)]. ^{31}P NMR in D_2O: δ = 11.7. Anal. ($C_6H_{17}N_3O_8P_2Pt\cdot H_2O$) C, H, N.

[(Bis(phosphonomethyl)amino-κN)acetato-κO(2-)](cis-*R,S*-cyclohexane-1,2-diamine)platinum(II). (*SP*-4-2)-Dichloro(*cis-R,S*-cyclohexane-1,2-diamine)platinum(II) (800 mg, 2.11 mmol) was suspended in 30 mL of water. After addition of silver nitrate (679 mg, 4.0 mmol) the mixture was stirred overnight at room temperature. Silver chloride precipitated and was filtered off. Bis(phosphonomethyl)aminoacetic acid (526 mg, 2.0 mmol) was added to the yellow solution. After the mixture was stirred for 60 min at 50 °C and overnight at room temperature the solvent was removed under reduced pressure. The resulting solid was dissolved in water. [Pt(*cis-R,S*-chxn)BPMAA] was precipitated with acetone, filtered and dried over P_2O_5 under reduced pressure to obtain 829 mg of a white solid; yield 73%. ^{1}H NMR in D_2O: δ = 1.18 [m, 1H, H(8) or H(9)], 1.32 [m, 2H, H(8) or H(9)], 1.56 [m, 1H, H(8) or H(9)], 1.67 [m, 4H, H(7), H(10)], 2.72 [m, 1H, H(5) or H(6)], 2.90 [m, 1H, H(5) or H(6)], 3.20 [m, 1H, H(3) or H(4)], 3.35 [m, 2H, H(3) or H(4)], 3.57 [m, 1H, H(3) or H(4)], 3.86 [d($^2J_{H,H}$ = 16.0 Hz), 1H, H(2)], 4.30 [d($^2J_{H,H}$ = 16.0 Hz), 1H, H(2)], 5.09 [1H, H(N)], 5.44 [1H, H(N)], 5.75 [1H, H(N)], 5.97 [1H, H(N)]. ^{13}C NMR in D_2O: δ = 19.5 [C(8) or C(9)], 21.8 [C(8) or C(9)], 25.8 [C(7) or C(10)], 26.1 [C(7) or C(10)], 57.3 [C(5) or C(6)], 58.3 [C(5) or C(6)], 61.2 [dd, $^1J_{C,P}$ = 142 Hz, $^3J_{C,P}$ = 5 Hz, C(3) or C(4)], 62.9 [dd, $^1J_{C,P}$ = 141 Hz, $^3J_{C,P}$ = 11 Hz, C(3) or C(4)], 66.1 [C(2)], 184.8 [C(1)]. ^{31}P NMR in D_2O: δ = 12.7, 13.0. Anal. ($C_{10}H_{23}N_3O_8P_2Pt$) C, H, N.

(*SP*-4-3)-[(Bis(phosphonomethyl)amino-κN)acetato-κO(2-)](trans-*S,S*-cyclohexane-1,2-diamine)platinum-(II). The synthetic procedure is the same as that for [Pt(*cis-R,S*-chxn)BPMAA]. Yield 36%. ^{1}H NMR in D_2O: δ = 1.03 [m, 2H, H(8) or H(9)], 1.15 [m, 2H, H(7), H(10)], 1.44 [m, 2H, H(8) or H(9)], 1.93 [m, 2H, H(7), H(10)], 2.27 [2H, H(5), H(6)], 3.30 [m, 2H, H(3) or H(4)], 3.50 [m, 2H, H(3) or H(4)], 3.89 [d($^2J_{H,H}$ = 16.1 Hz),1H, H(2)], 4.22 [d($^2J_{H,H}$ = 16.1 Hz), 1H, H(2)], 4.89 [1H, H(N)], 5.28 [1H, H(N)], 5.61 [1H, H(N)], 6.16 [1H, H(N)]. ^{13}C NMR in D_2O: δ = 24.2 [C(8), C(9)], 32.2 [C(7) or C(10)], 32.6 [C(7) or C(10)], 61.4 [dd, $^1J_{C,P}$ = 140 Hz, $^3J_{C,P}$ = 10 Hz, C(3) or C(4)], 61.6 [C(1) or C(2)], 62.0 [C(1) or C(2)], 62.3 [dd, $^1J_{C,P}$ = 141 Hz, $^3J_{C,P}$ = 7 Hz,C(3) or C(4)], 66.1 [C(2)], 184.9 [C(1)]. ^{31}P NMR in D_2O: δ = 12.6, 13.0. Anal. ($C_{10}H_{23}N_3O_8P_2$-$Pt\cdot H_2O$) C, H, N.

(*SP*-4-3)-[(Bis(phosphonomethyl)amino-κN)acetato-κO(2-)](trans-*R,R*-cyclohexane-1,2-diamine)platinum-(II). The synthetic procedure is the same as that for [Pt(*cis-*

R,S-chxn)BPMAA]. Yield 76%. ^{1}H NMR in D_2O: $\delta = 1.03$ [m, 2H, H(8) or H(9)], 1.15 [m, 2H, H(7), H(10)], 1.44 [m, 2H, H(8) or H(9)], 1.93 [m, 2H, H(7), H(10)], 2.27 [2H, H(5), H(6)], 3.30 [m, 2H, H(3) or H(4)], 3.50 [m, 2H, H(3) or H(4)], 3.89 [d($^2J_{H,H}$ = 16.1 Hz),1H, H(2)], 4.22 [d($^2J_{H,H}$ = 16.1 Hz), 1H, H(2)], 4.89 [1H, H(N)], 5.29 [1H, H(N)], 5.61 [1H, H(N)], 6.16 [1H, H(N)]. ^{13}C NMR in D_2O: $\delta = 24.2$ [C(8), C(9)], 32.1 [C(7) or C(10)], 32.5 [C(7) or C(10)], 61.4 [dd, $^1J_{C,P} = 147$ Hz, $^3J_{C,P} = 5$ Hz, C(3) or C(4)], 61.6 [C(5) or C(6)], 62.0 [C(5) or C(6)], 62.3 [dd, $^1J_{C,P} = 145$ Hz, $^3J_{C,P} = 8$ Hz, C(3) or C(4)], 66.1 [C(2)], 184.9 [C(1)]. ^{31}P NMR in D_2O: $\delta = 12.6$, 12.9. Anal. ($C_{10}H_{23}N_3O_8P_2$-Pt) C, H, N.

Cell Culture and Cytotoxicity Test Conditions. The ovarian carcinoma cell line CH1, which has been established from an ascites sample of a patient with a papillary cystadenocarcinoma of the ovary, was kindly provided by Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, UK). Cells were grown as adherent monolayer cultures in culture medium consisting of a standard Minimal Essential Medium (MEM), supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin (all purchased from Gibco). Cultures were maintained at 37 °C in humidified atmosphere containing 5% CO_2.

Cytotoxicity was determined by means of a colorimetric microculture assay (MTT assay, MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide). CH1 cells were harvested from adherent cultures by trypsinization and suspensions were adjusted to cell densities of 1.25×10^4 cells/mL in order to ensure exponential growth throughout drug exposure. Aliquots of 200 μL/well of these suspensions were used to seed microcultures in 96-well plates. After incubation for 24 h cells were exposed to the test compounds, dissolved and serially diluted in complete culture medium. Each concentration was given to eight microcultures in parallel. After incubation for 4 days drug solutions were removed and replaced by 150 μL/well complete culture medium and 20 μL of aqueous MTT solution (5 mg/mL). After incubation for a further 4 h, the medium/MTT mixtures were removed and formazan crystals were dissolved in 150 μL DMSO/well. Optical densities at 550 nm were measured with a microplate spectrophotometer (Tecan Spectra Classic) and quantity of living cells was expressed as T/C values by comparison to untreated control microcultures. The concentrations of complexes that decreased absorption by 50% were calculated by interpolation and taken as the IC_{50} values. Evaluation is based on means of values obtained from three independent experiments.

Stability of [Pt(NH$_3$)$_2$BPMAA] in the Cell Culture Medium. Stability of [Pt(NH$_3$)$_2$BPMAA] as representative for the class of phosphonatoplatinum complexes in the cell culture medium has been investigated using ^{31}P NMR spectroscopy. These measurements were performed at a concentration which is very close to the IC_{50} of the drug (136 μM versus IC_{50}: 128 μM). During drug incubation over 4 days (37 °C), the platinum complex was found to be very stable. Reactions at the platinum(II) center would result in release of the bisphosphonate ligand and appearance of a new resonance in the ^{31}P NMR spectra, which could not be detected.

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