Synthesis, Characterization, and Cytotoxicity of Trifunctional Dinuclear Platinum Complexes: Comparison of Effects of Geometry and Polyfunctionality on Biological Activity

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Received October 25, 1999

The synthesis of two new isomeric trifunctional dinuclear platinum complexes of formula [{PtCl-$(NH_3)_2$}μ-$NH_2(CH_2)_6NH_2$-{$PtCl_2(NH_3)$}]$^+$ (1,2/*c*,*c* and 1,2/*t*,*c*) is reported. Their biological activity in selected human tumor cell lines sensitive and resistant to CDDP (cisplatin, *cis*-[$Pt(NH_3)_2Cl_2$]) is described and compared with the profile for their bifunctional analogues, [{*cis*/*trans*-$PtCl(NH_3)_2$}$_2\mu$-$NH_2(CH_2)_6NH_2$]$^{2+}$. The trifunctional dinuclear platinum complexes showed a unique profile of cytotoxicity against human cancer cell lines, with low resistance factors in A2780, CH1, and 41M cell lines. The resistance factor is dependent on the geometry of the Pt coordination spheres − suggesting that these may be associated with DNA-binding modes. Retention of activity against CDDP-resistant cell lines and a different spectrum of activity compared to CDDP and also within different classes of polynuclear platinum complexes suggest that not only are they mechanistically different from mononuclear platinum complexes but also each individual class of polynuclear platinum structure may have its own unique character.

Introduction

Polynuclear platinum complexes represent a new class of antitumor agents of potential clinical significance.[1] In the case of dinuclear platinum complexes the general structure where two Pt coordination units are linked by a bridging diamine is best represented by [{$PtCl_m(NH_3)_{3-m}$}$_2\mu$-NH_2-R-NH_2]$^{2(2-m)+}$ (m = 0−3 and R is a linear aliphatic linker of variable chain length).[2] We have previously reported the properties of the tetrafunctional DNA-binding agents where m = 2 in the above formula (2,2/*c*,*c*, [{*cis*-$PtCl_2(NH_3)$}$_2\mu$-NH_2-R-NH_2]).[3,4] Likewise the bifunctional DNA-binding agents where m = 1 (1,1/*t*,*t* or 1,1/*c*,*c*, [{*cis*/*trans*-$PtCl(NH_3)_2$}$_2\mu$-NH_2-R-NH_2]$^{2+}$) have been synthesized, and their biological activity has been described.[5,6] Structure−activity relationships within this latter series leading to the choice of a trinuclear but bifunctional DNA-binding agent as the first clinical drug from this work have also been summarized.[7] The polynuclear platinum complexes produce an array of structurally distinct Pt-DNA lesions in comparison to those produced by CDDP.[8−11] Interestingly, it is now clear that within the extensive family of dinuclear structures possible, the individual complexes (2,2/*c*,*c*, 1,1/*t*,*t*, and 1,1/*c*,*c*) show variations in patterns of DNA-binding modes and biological activity dependent on factors such as number of leaving groups and their geometry relative to the bridging diamine.[4,12] Thus, further specific functionalization of DNA-binding modes is, in principle, possible leading to compounds with unique biological activity.

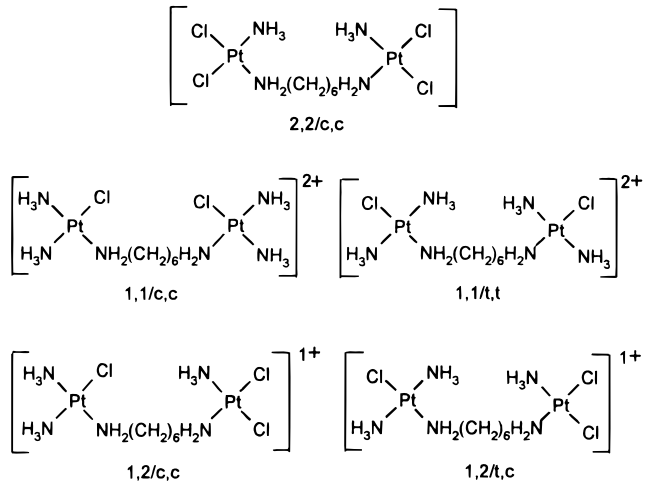

Figure 1. Chemical structures of dinuclear platinum complexes. The system of abbreviation allows the numbers to represent the number of chloride leaving groups on each platinum atom, while the lettering refers to the geometry of the leaving group with respect to the nitrogen of the bridging diamine.

The intermediate case between bifunctional and tetrafunctional DNA-binding agents is represented by a trifunctional agent such as [{$PtCl(NH_3)_2$}μ-NH_2-R-NH_2-{$PtCl_2(NH_3)$}]$^+$, Figure 1. These potential agents thus contain two inequivalent Pt coordination moieties: one monofunctional, {Pt**Cl**$(NH_3)_2$}, and one bifunctional, {Pt**Cl**$_2(NH_3)$}, unit. In this paper, we complete the description of the basic dinuclear structures possible by presenting the preparation and characterization of the two isomeric trifunctional complexes, 1,2/*t*,*c* and 1,2/*c*,*c* (Schemes 1 and 2), that differ only in the geometry of the second platinum coordination sphere. A comparison

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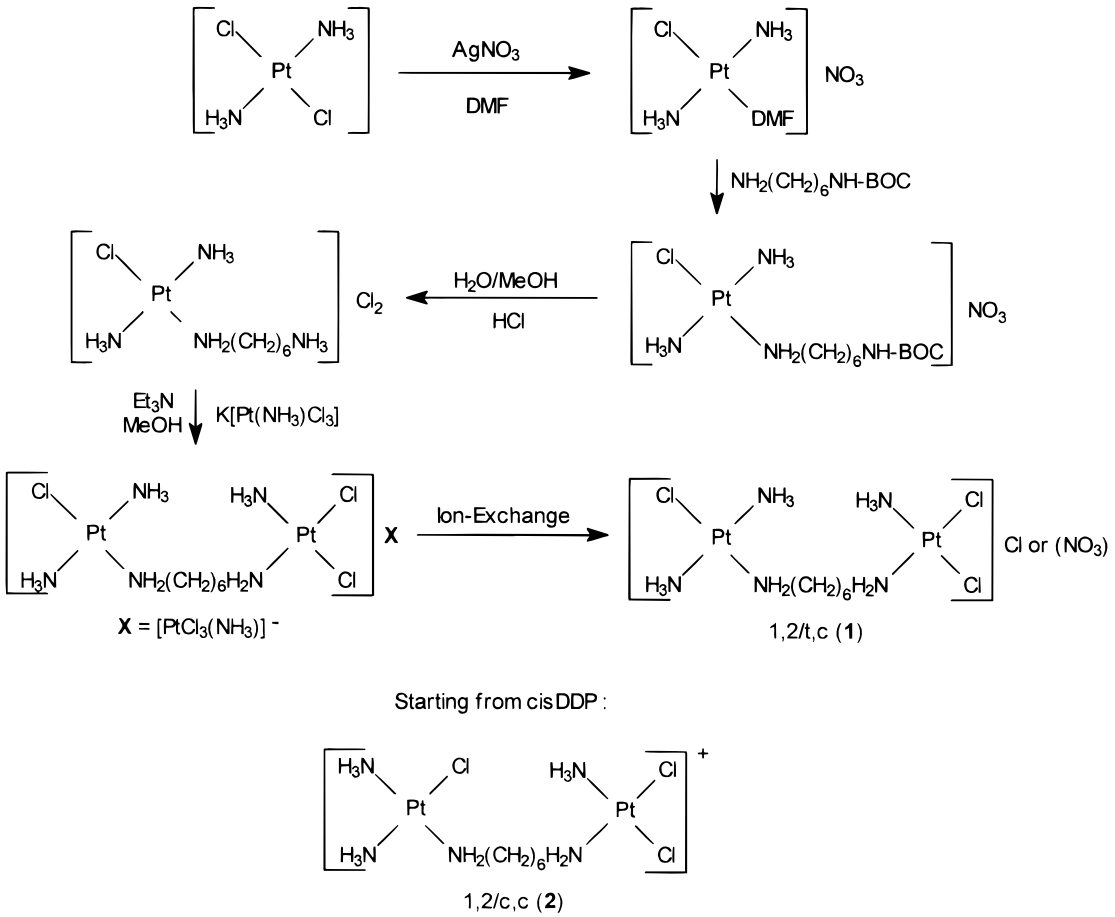
10.1021/jm990536z CCC: $19.00
Published on Web 07/20/2000

Scheme 1



of cytotoxicity in human ovarian tumors is made with their bifunctional dinuclear analogues, showing the variation in patterns of cytotoxicity among the various classes.

Chemistry

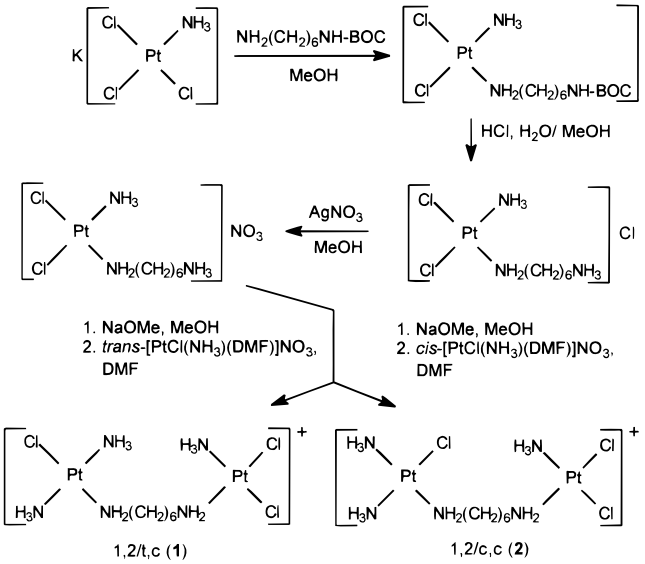
The syntheses of the complexes 1,2/*t*,*c* (**1**) and 1,2/*c*,*c* (**2**) involve a sequence of reactions in order to fuse the monofunctional [**PtCl**$(NH_3)_2$] and bifunctional [**PtCl**$_2$-(NH_3)] units via the linking hexanediamine entity. The complexes were characterized by ^{1}H and ^{195}Pt NMR, ESI-MS, HPLC, and elemental analysis.

Preparation of dinuclear metal complexes with two inequivalent coordination spheres requires first the synthesis of a precursor mononuclear complex containing a "dangling" amine.[5,13] This precursor is then linked to a suitable target molecule:

$$\text{Pt(a)-H}_2\text{N-R-NH}_3 + \text{Pt(b)} \rightarrow \text{Pt(a)-H}_2\text{N-R-NH}_2\text{-Pt(b)}$$

Specifically, there are two routes outlined in Schemes 1 and 2 to produce the desired trifunctional compounds. The hexanediamine linker was used in both cases. The syntheses have been achieved using both methods, but the linking of two inequivalent coordination spheres was best achieved by placing the dangling amine on the unit containing the monofunctional coordination sphere giving first the triamine *cis*/*trans*-[$PtCl(NH_3)_2(H_2N(CH_2)_6$-$NH_3)]^{2+}$ which was isolated and characterized by standard methods. The target molecule was then the monoanion K[$PtCl_3(NH_3)$], which displaced one Cl group

Scheme 2



selectively, Scheme 1. In this case, the desired cations precipitated initially with some $[PtCl_3(NH_3)]^-$ counteranion, as has been observed previously.[14] Ion-exchange chromatography was then used to afford the compounds as their pale yellow Cl or NO_3 salts.

Biological Activity

A comparison of the cytotoxicities of the new complexes with their bifunctional analogues on human

Table 1. Cytotoxicity Data of Dinuclear Platinum Complexes in a Panel of Human Ovarian Tumor Cell Lines after 96 h of Drug Exposure

	cell line (IC_{50}, μM)										
complex	SKOV-3	HX/62	A2780	A2780/CDDP	RF[a]	CH1	CH1/CDDP	RF[a]	41M	41M/CDDP	RF[a]
1,1/*c*,*c*	15.3	11	0.066	1.2	(18)	0.056	0.19	(3.3)	1.9	0.41	(0.21)
1,1/*t*,*t*	87	14	0.25	4.9	(19.6)	1.6	3.2	(2)	7.4	1.8	(0.24)
1,2/*c*,*c*	17.7	12.5	0.066	0.34	(5.2)	0.22	0.98	(4.4)	2.4	1.3	(0.53)
1,2/*t*,*c*	31	24	0.28	0.89	(3.2)	0.3	0.97	(3.2)	2.5	0.67	(0.27)
CDDP	8	25	0.53	6.2	(11.7)	0.18	1.45	(8)	0.54	1.7	(3.1)

[a] Resistance factor, RF = IC_{50} resistant/IC_{50} parent line.

ovarian tumor cell lines and CDDP-resistant cell lines is shown in Table 1. A number of points are of interest. The enhanced cytotoxicity of the 1,1/*c*,*c* in comparison to the isomeric 1,1/*t*,*t* geometry previously observed in murine leukemia L1210 cells is confirmed.[6] Interestingly, resistance factors are similar for both isomers in the human panel, in contrast to the L1210 case.[6] Overall, the trifunctional 1,2/*c*,*c* and 1,2/*t*,*c* are similar in potency to the 1,1/*t*,*t* and 1,1/*c*,*c* complexes and clearly present a further unique profile in comparison to that of cisplatin. However, the differences in geometry between the 1,2/*c*,*c* and 1,2/*t*,*c* isomers are not as marked as in the bifunctional case. The resistance factor for each dinuclear platinum complex in comparison with those for CDDP is defined by the IC_{50} of the resistant line divided by the IC_{50} of the sensitive line, Table 1. In general, all charged complexes retain activity in cisplatin-resistant cell lines, and this is distinctly marked for the transport-deficient 41M/CDDP line, where resistance factors < 1 are seen for all charged species. Cellular pharmacology studies suggest enhanced uptake as one unique feature of charged polynuclear platinum complexes.[16] The ability to consistently overcome resistance in the transport-deficient cell line could be a consequence of this effect. Resistance to the CH1/CDDP line is predominantly due to enhanced DNA repair/tolerance, likely to be of the major intrastrand GG adduct. The low resistance factors for the trifunctional complexes studied in comparison to cisplatin suggest that cisplatin-like binding modes do not dominate for the 1,2 series. The large differences in resistance factors in the A2780 pair between the 1,1/*t*,*t* and 1,1/*c*,*c* (bifunctional) complexes on the one hand and the 1,2/*t*,*c* and 1,2/*c*,*c* (trifunctional) complexes on the other hand suggest that the factors affecting resistance are different for the two types of DNA-binding agents. In vivo, we observe that all the charged complexes are active at doses of 1−4.5 mg/kg, equivalent to the mononuclear drug.[15]

Summary and Conclusions

Whereas the DNA binding of bifunctional dinuclear platinum complexes is restricted to long-range (Pt,Pt) intrastrand or interstrand cross-linking, trifunctional (and indeed also tetrafunctional) binding modes are more complex because of additional possibilities such as ternary DNA−protein cross-linking[4,17] as well as the issue of which coordination sphere will be attacked preferentially. Initial studies show the 1,2 compounds behave similarly to the bifunctional agents producing high percentages of (Pt,Pt) interstrand cross-links, but they are also more effective ternary DNA−protein cross-linking agents because of the extra functionality.[18] Thus, as might be expected from the structure, the DNA-binding profile of trifunctional dinuclear platinum complexes appears to be intermediate between the previously studied cases. In summary, the synthesis of trifunctional DNA-binding complexes completes the formal series of dinuclear structures possible as drugs. The results so far suggest that the 1,2 series is a useful one for studying how to control DNA-binding modes in a polyfunctional complex. The biological activity of dinuclear platinum agents may then be systematically altered within the class itself as well as with respect to mononuclear *cis*-DDP analogues.

Experimental Section

Starting Materials. *trans*-$[PtCl_2(NH_3)_2]$ (TDDP) and *cis*-$[PtCl_2(NH_3)_2]$ (CDDP) were purchased from Johnson Matthey. N-BOC-1,6-diaminohexane was obtained starting from the corresponding hydrochloride salt (Fluka) by treatment with aqueous NaOH and extraction of the free base to dichloromethane followed by removal of the solvent.

Physical Measurements. ^{1}H NMR spectra were recorded on a Varian UNITYplus 500-MHz spectrometer. Chemical shifts (δ, ppm) are referenced to DSS (4,4-dimethyl-4-silapentanesulfonic acid). Proton-decoupled ^{195}Pt NMR spectra were recorded on a GE 300-MHz spectrometer at 64 MHz at 291 K using $K_2[PtCl_4]$ in D_2O as external standard. Chemical shifts are reported vs $[PtCl_6]^{2-}$ standard (δ = 0). Elemental analyses were performed by Robertson Microlit Laboratories, Madison, NJ. The HPLC analyses were performed on an ISCO high-performance liquid chromatography with a Lichrosphere PR8 column, using 2.5 mg/mL octanesulfonate in H_2O/CH_3CN (62/38 and 70/30, pH 2.7 adjusted with H_3PO_4) as eluant. ESI-MS was done by University of Arizona, Tucson, AZ.

Synthesis of *trans*-[{$PtCl(NH_3)_2$}-μ-{$NH_2(CH_2)_6NH_2$}-*cis*-{$PtCl_2(NH_3)$}]Cl (1). A solution of *trans*-DDP (0.940 g, 3.13 mmol) in 60 mL of DMF was prepared at room temperature in the dark. $AgNO_3$ (0.517 g, 3.04 mmol) was added and the mixture stirred for 18 h. The precipitate of AgCl was filtered off, the filtrate cooled to −20 °C and a solution of N-BOC-1,6-diaminohexane (0.658 g, 3.04 mmol) in 13 mL of DMF added within 30 min. The mixture was stirred for 2 h at −20 °C, then the temperature was kept at 20 °C for another hour. The solvent was removed under reduced pressure and the residue stirred in 80 mL of diethyl ether overnight. After filtration the solid was treated for 1 h in 100 mL of methanol and filtered and the filtrate stirred with charcoal for 30 min and filtered again. The solvent was removed under reduced pressure and then stirred for 2 h in 25 mL of acetone, filtered and dried in vacuum giving 1.2 g of *trans*-$[PtCl(NH_3)_2$-$\{NH_2(CH_2)_6NH$-BOC$\}]NO_3$.

trans-$[PtCl(NH_3)_2\{NH_2(CH_2)_6NH$-BOC$\}]NO_3$ was stirred for 3 h in a mixture of methanol (50 mL), H_2O (50 mL), and concentrated HCl (39 mL). The solvent was evaporated under vacuum and the residue stirred for 30 min in 25 mL of acetone, filtered off and dried in vacuum (*trans*-$[PtCl(NH_3)_2\{NH_2(CH_2)_6$-$NH_3\}]Cl_2$, 0.95 g, 2.1 mmol). Purity: 96% by HPLC. Anal. Calcd for $C_6H_{23}N_4Cl_3Pt$: C, 15.92; H, 5.13; N, 12.38; Cl, 23.49. Found: C, 16.52; H, 5.25; N, 12.38; Cl, 23.50. ^{1}H NMR (D_2O): δ 1.39 (4 H, m), 1.67 (4 H, m), 2.67 (2 H, m), 2.98 (2 H, m). ^{195}Pt NMR (D_2O): δ −2423.

A sample of *trans*-$[PtCl(NH_3)_2\{NH_2(CH_2)_6NH_3\}]Cl_2$ (0.90 g, 2.0 mmol) was dissolved in 300 mL of methanol and K[$PtCl_3$-

(NH_3)] (1.80 g, 5.03 mmol) added. While stirring, triethylamine (2.3 mL, 16.3 mmol) in 20 mL of methanol was added within 20 h at room temperature. Stirring was continued for another 28 h, the precipitate filtered off and dried in vacuum. This crude $[PtCl_3(NH_3)]^-$ salt of the product was converted by using an ion-exchange column (DEAE Sephadex A25, Cl^- form) into the corresponding Cl^- salt. The obtained oily material was first stirred in acetone/diethyl ether (100 mL) and then in methanol to give 0.397 g of **1** (0.549 mmol, 18% yield based on *trans*-DDP). Purity: after repeated recrystallization, 96% by HPLC. Anal. Calcd for $C_6H_{25}N_5Cl_4Pt_2 \cdot 0.5CH_3OH$: C, 10.91; H, 3.80; N, 9.79; Cl, 19.82; Pt, 54.54. Found: C, 11.33; H, 3.74; N, 9.89; Cl, 19.90; Pt, 53.43. ^{1}H NMR (D_2O): δ 1.20 (4 H, m), 1.51 (4 H, m), 2.46 (4 H, m). ^{195}Pt NMR (D_2O): δ −2422, −2203.

Synthesis of *cis*-[{$PtCl(NH_3)_2$}-μ-{$NH_2(CH_2)_6NH_2$}-*cis*-{$PtCl_2(NH_3)$}]Cl (2). The complex was prepared in a corresponding way to **1** starting from 0.636 g (2.12 mmol) of *cis*-DDP, giving 0.260 g of **2** (0.359 mmol, 17% yield based on *cis*-DDP). Purity: after repeated recrystallization, 95% by HPLC. Anal. Calcd for $C_6H_{25}N_5Cl_4Pt_2 \cdot 0.5CH_3OH$: C, 10.91; H, 3.80; N, 9.79; Cl, 19.82; Pt, 54.54. Found: C, 11.29; H, 3.66; N, 9.66; Cl, 19.26; Pt, 53.05. ^{1}H NMR (D_2O): δ 1.19 (4 H, m), 1.53 (4 H, m), 2.45 (4 H, m). ^{195}Pt NMR (D_2O): δ −2416, −2202.

The molecular weight was determined by ESI mass spectroscopy (University of Arizona) to be 663(±1) amu (calcd 663.8 amu) for the positive ion $[\{PtCl(NH_3)_2\}\text{-}\mu\{NH_2(CH_2)_6NH_2\}\text{-}\{PtCl_2(NH_3)\}]^+$.

Biological Assays. Cell culture: SKOV-3, HX/62, A2780, A2780/CDDP, CH1, CH1/CDDP, 41M, and 41/CDDP cells were used in this study and maintained according to published procedures.[19,20]

Growth inhibition assay: The sulforhodamine B (SRB) assay was used to determine growth inhibition potency of platinum drugs.[21] The cells were seeded in 96-well microtiter plates at (3−8) × 10^3 cells/well in 160 μL of growth medium and allowed to attach overnight. Platinum agents were then added after serial dilution in quadruplicate wells and exposed to cells for 2 or 96 h. After 2 h drug incubations were complete; plates were washed free of drug with phosphate-buffered saline (PBS) and then refed with normal growth medium for a further 94 h. Quantitation of cell growth in treated and control wells was then assessed using 0.4% SRB dissolved in 1% acetic acid. IC_{50} values were determined graphically.

Acknowledgment. This work was supported by the National Science Foundation (CHE-9615727), the National Institutes of Health (RO1-CA78754), and the American Cancer Society (RPG89-002-11-CDD). N.F. also acknowledges support from Boehringer Mannheim Italy. A.P.S.F. acknowledges a Fulbright Fellowship. The work of A.P.S.F. and R.B. was performed at The University of Vermont.

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JM990536Z