Cytotoxicity and Antitumor Activity of Bis(platinum) Complexes. A Novel Class of Platinum Complexes Active in Cell Lines Resistant to Both Cisplatin and 1,2-Diaminocyclohexane Complexes

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The in vitro cytotoxicity and in vivo antitumor activity of bis(platinum) complexes of general formula [{PtX$_2$-(L)}$_2$H$_2$N(CH$_2$)$_n$NH$_2$] (L = NH$_3$, X = Cl or X$_2$ = malonato or where L = py, X = Cl) is reported. Chloride complexes [{PtCl$_2$(NH$_3$)}$_2$H$_2$N(CH$_2$)$_n$NH$_2$] may exist as three possible isomers: those containing both coordination units in the cis configuration (2,2/c,c), both coordination units in the trans configuration (2,2/t,t), and the mixed cis,trans species (2,2/c,t), whose synthesis is reported here. The preparation of further complexes with sterically hindered diamine backbones, such as 2,5-dimethyl-2,5-hexanediamine, is exemplified. The biological activity of all complexes were compared. The 2,2/c,c complexes are particularly active in tissue culture in cells resistant both to cisplatin and its 1,2-diaminocyclohexane (dach) analogue. The inhibition of DNA synthesis in L1210/0 cells by the 2,2/c,c complexes is equivalent to that of cisplatin. The presence of at least one cis-[Pt(amine)$_2$] unit appears necessary for activity in cell lines sensitive to cisplatin.

The molecular mechanism of action of the anticancer drug cis-[PtCl$_2$(NH$_3$)$_2$] (cisplatin, cisDDP) is accepted to be by interaction with DNA.[1] The principal target of cisplatin on DNA is the intrastrand link between two adjacent guanine or adjacent guanine/adenine base pairs, and these adducts constitute a block to both replication and transcription. It is important to recognize that it is not the intrastrand link per se but rather the overall conformational change this binding causes which eventually leads to the observed biological effects. This understanding raises questions as to whether greater conformational distortion by structurally different Pt complexes or greater DNA affinity can be reflected in increased antitumor activity. Enhanced DNA-binding affinity and conformational changes may also be associated with more difficult repair of the drug–DNA combination (lesion).

To address some of these points we have recently prepared bis(platinum) complexes which contain two platinum–amine units linked by a variable length diamine chain. We have now reported on the synthesis and characterization of complexes containing both platinum atoms in cis-[PtCl$_2$(amine)$_2$], trans-[PtCl$_2$(amine)$_2$], and Pt–tetraamine coordination spheres.[2,3] DNA-binding studies on the complexes containing two cis-[Pt(amine)$_2$] moieties have shown that they produce unique interstrand crosslinks through binding of each Pt atom to opposite strands of DNA.[4] Complexes capable of molecular interactions not accessible to monomeric complexes or acting by different mechanisms might also display a broader spectrum of clinical activity. This paper presents our initial studies on the cytotoxicity and antitumor activity of bis(platinum) complexes.[5]

Results and Discussion

Characterization of New Bis(platinum) Complexes. Neutral bis(platinum) complexes are in fact described by the general structural formula [{PtX$_2$(L)}$_2$H$_2$N(CH$_2$)$_n$NH$_2$], and there is considerable scope for variation in the leaving group X, the unique amine L (usually NH$_3$), and the diamine backbone. In this paper we consider principally the simplest diamine, straight-chain, and aliphatic complex. Considerable variation is possible in the diamine backbone, and one example is given. Chloride complexes of formula [{PtCl$_2$(NH$_3$)}$_2$H$_2$N(CH$_2$)$_n$NH$_2$] may exist as three possible isomers: those containing both coordination units in the

Scheme I
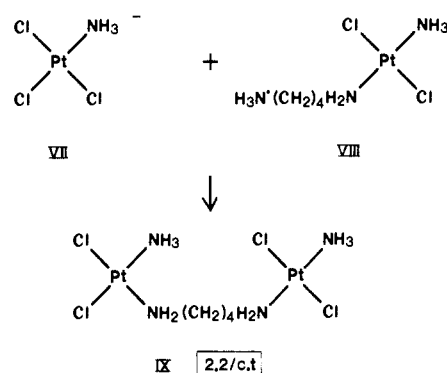
cis configuration, both coordination units in the trans configuration, and the mixed cis,trans species (Figure 1). For a convenient abbreviation we denote these possibilities as 2,2/c,c, 2,2/t,t, and 2,2/c,t, respectively, where the numbers refer to the number of chlorides (or anionic leaving groups) on each platinum and the lettering specifies the geometries.

The 2,2/c,c (I, II, III; n = 4, 5, 6, respectively) and 2,2/t,t (IV, V, VI; n = 4, 5, 6, respectively) complexes were prepared as described previously, and their characterization will not be detailed here. The 2,2/c,t complex (n = 4) was prepared by the reaction shown in Scheme I.

This scheme was developed from our observation that trans-[PtCl$_2$(NH$_3$)(H$_2$N(CH$_2$)$_4$NH$_3$)]Cl (VIII), a monomeric complex with one end of the diamine coordinated to platinum and the other end protonated, was a side product of the formation of the 2,2/t,t complex, which is formed by reaction of HCl with a doubly bridged tetraamine species.[3] The presence of the free, uncoordinated amine suggested to us that this potential coordination site

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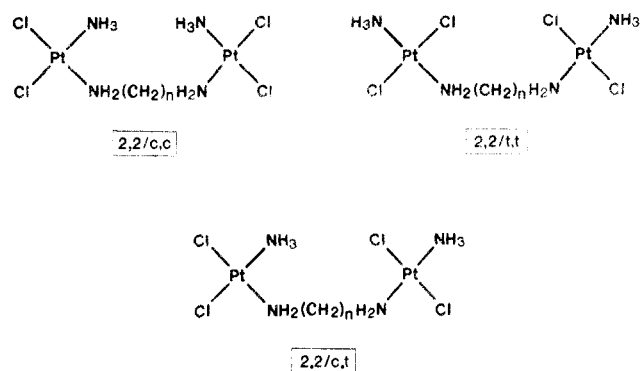
Figure 1. Structures of the three possible isomers of bis(platinum) complexes [{PtCl₂(NH₃)}₂H₂N(CH₂)ₙNH₂].
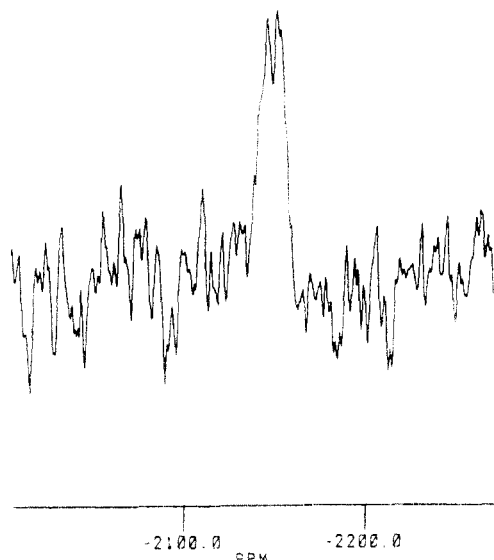
Figure 2. ¹⁹⁵Pt NMR spectrum of complex IX, [{cis-PtCl₂-(NH₃)}H₂N(CH₂)₄NH₂{trans-PtCl₂(NH₃)}] showing the separation of the peaks due to the two different coordination spheres.

could be used as a starting point for further bis(platinum) complexes. Thus, reaction can occur with any monoanion K[PtCl₃L], giving a bis(platinum) complex. The actual geometry of the product will be dictated by the nature of L and its trans influence relative to chloride. In the case of L = NH₃ (VII), substitution must occur cis to the amine, and the product that precipitates upon reaction in MeOH/Et₃N is IX, which now must contain one Pt coordination sphere in the trans geometry (derived from VIII) and the other in the cis geometry (derived from VII). The 2,2/c,t complex was characterized by elemental analysis, IR, and ¹H and ¹⁹⁵Pt NMR spectroscopy (Table I). The IR and ¹H NMR spectra were consistent with those we have measured previously for bridging diamine.[2,3] The ¹⁹⁵Pt NMR spectrum (Figure 2) is particularly instructive as it clearly shows the presence of two peaks at −2165 and −2171 ppm corresponding to the two coordination geometries. The complexes [{PtCl₂(NH₃)}₂H₂N(C-H₂)₄NH₂] show single peaks at −2167 ppm (trans) and −2163 ppm (cis), respectively.[3] The peaks are broad, however, due to the ¹⁴N amines, and isotopic substitution with ¹⁵N is in progress to further refine the spectrum. The use of a dangling amine of a metal complex is a unique way to produce functionalized bis(platinum) complexes containing two different coordination spheres and opens the interesting possibility for synthesis of further highly specific complexes.

Bis(platinum) complexes containing chloride as leaving group are, as might be expected, only very sparingly water

Table I. Spectral Data for New Bis(platinum) Complexes

no.	complex	NMR δ, ppm[a]	
		¹H	¹⁹⁵Pt
IX	[{PtCl₂(NH₃)}₂H₂N(CH₂)₄NH₂]	1.62, 2.68	−2165, 2171
X	[{Pt(mal)(NH₃)}₂H₂N(CH₂)₄NH₂][b]	1.63, 2.42, 3.23	−1810
XI	[{Pt(mal)(NH₃)}₂H₂N(CH₂)₅NH₂]	1.37, 1.61, 2.42, 3.22	−1811
XII	[{Pt(mal)(NH₃)}₂H₂N(CH₂)₆NH₂]	1.30, 1.60, 2.41, 3.21	−1811
XIII	[{cis-PtCl₂(py)}₂H₂N(CH₂)₄NH₂]	1.75, 2.60, 7.58, 8.10, 9.00	−2107
XIV	[{cis-PtCl₂(NH₃)}₂(DMHD)][c]	1.35, 1.80	−2175
XV	[{trans-PtCl₂(NH₃)}₂(DMHD)	1.35, 1.78	−2165

[a] Chemical shifts relative to TMS (¹H) and PtCl₆²⁻ (¹⁹⁵Pt). IX and XIII–XV are in DMF-d₇, and X–XII in DMSO-d₆. Integrations are as expected. [b] Complexes X–XII all showed two intense IR bands at 1610 and 1485 cm⁻¹ attributed respectively to νₐₛᵧₘ(CO) and νₛᵧₘ(CO) of the carboxylate ligands. [c] DMHD = 2,5-dimethyl-2,5-hexanediamine (H₂-NC(CH₃)₂(CH₂)₂C(CH₃)₂NH₂).

soluble. Substitution of chloride by dicarboxylates has been very successful in producing water-soluble monomeric platinum complexes and so bis(platinum) malonate derivatives (Complexes X, XI, XII; n = 4, 5, 6, respectively)
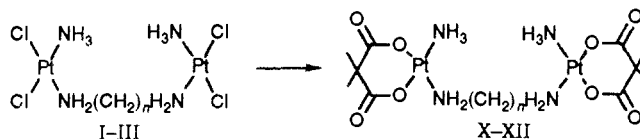
were prepared by action of silver malonate on the parent dichlorides. The characterizing data are also given in Table I. The ¹⁹⁵Pt chemical shifts were as expected for this type of coordination (cf. carboplatin, CBDCA at −1723 ppm).[6] In D₂O we did not observe the malonate protons, but these appeared at the expected values in dry DMSO-d₆, along with the two distinct amine signals. All three signals disappeared upon addition of H₂O, indicating that the malonate protons are also quite acidic.

The general structure of bis(platinum) complexes allows for considerable variation in both the diamine bridge and in the unique amine group, derived from the [PtCl₃-(amine)] precursor. In an extension of our original series, we have studied the complex derived from K[PtCl₃(py)] (XIII) (characterization data in Table I). The trans influence of pyridine is similar to NH₃ and so substitution also occurs cis to the pyridine ligand:
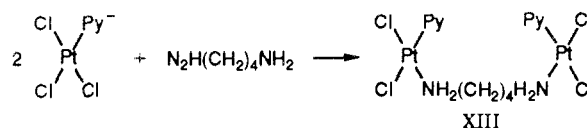
Biological Properties

In Vitro Cytotoxicity. The cytotoxicities of all complexes in tissue cultures were examined in L1210 cell lines which are sensitive and resistant to cisplatin and in a cell line rendered resistant to [Pt(R,R-dach)SO₄] (Table II). With the exception of the malonate complexes, which were dissolved in H₂O, all complexes were originally dissolved in DMF and then immediately diluted in saline to a maximum concentration of 0.5% DMF.

The results show that all 2,2/c,c complexes I–III and X–XIII represent a unique class of complexes non-cross-resistant to both cisplatin and Pt–dach complexes. Most monomeric complexes with different amine ligands show some lack of cross-resistance to cisplatin but few have been shown to retain that property in the Pt–dach system.[7]

Table II. In Vitro Cytotoxicity of Bis(platinum) Complexes [{PtX$_2$(am)}$_2$H$_2$N(CH$_2$)$_n$NH$_2$] in L1210 Leukemia[a]

no.	X	geometry	n	ID$_{50}$, μM[b]		
				L1210/0	L1210/DDP	L1210/dach
I	Cl	2,2/c,c	4	0.28 ± 0.08	1.88 ± 0.46 (6.5)	0.38 ± 0.07 (1.4)
II	Cl	2,2/c,c	5	0.37 ± 0.12	1.04 ± 0.07 (2.8)	0.43 ± 0.08 (1.2)
III	Cl	2,2/c,c	6	0.54 ± 0.2	1.14 ± 0.2 (2.1)	0.303 ± 0.04 (0.6)
IV	Cl	2,2/t,t	4	10–15	10–15	10–15
V	Cl	2,2/t,t	5	10–15	10–15	10–15
VI	Cl	2,2/t,t	6	10–15	10–15	10–15
IX	Cl	2,2/c,t	4	0.98	~10.0 (10)	1.50 (1.53)
				0.76	3.36 (4.4)	2.60 (3.4)
X	mal	2,2/c,c	4	1.59 ± 0.59	6.7 ± 2.1 (4.2)	2.42 (1.7)
XI	mal	2,2/c,c	5	1.16	2.33 (2.0)	
XII	mal	2,2/c,c	6	1.51	2.36 (2.21)	
XIII	Cl	2,2/c,c	4	4.71	~15.0	4.1–8.5
cis-[PtCl$_2$(NH$_3$)$_2$]	–	–		0.23 ± 0.05	5.7 ± 2.0 (25)	0.85 ± 0.14 (3.5)
trans-[PtCl$_2$(NH$_3$)$_2$]	–	–		13.6	24.6 (1.8)	18.0 (1.33)
[Pt(R,R-dach)SO$_4$]	–	–		0.23 ± 0.07	0.75 ± 0.15 (3.3)	5.65 ± 0.28 (25)

[a] am = NH$_3$, except for Complex XIII where am = py. Other abbreviations as in text. All complexes in 0.5% DMF, except X–XII in H$_2$O. Standards in saline except trans-DDP in 0.5% DMF. Chain length of diamine is designated by n. [b] L1210/0 is sensitive to cisplatin. L1210/DDP is resistant to cisplatin. L1210/dach is rendered resistant to [Pt(R,R-dach)(SO$_4$)]. Resistance factor defined as ID$_{50}$ (sensitive)/ID$_{50}$ (resistant) in parentheses. Standard deviations are given when at least three separate experiments were run. All other values quoted are averages of two independent tests. [c] In 10% DMSO.

Table III. Comparison of the Effect of Diamine on in Vitro Cytotoxicity of Bis(platinum) Complexes [{PtCl$_2$(NH$_3$)}$_2$(diamine)] in L1210 Leukemia

no.	diamine	geometry	ID$_{50}$, μM		
			L1210/0	L1210/DDP	L1210/dach
I	H$_2$N(CH$_2$)$_4$NH$_2$	2,2/c,c	0.28 ± 0.08[a]	1.88 ± 0.46 (6.5)	0.38 ± 0.07 (1.4)
IV	H$_2$N(CH$_2$)$_4$NH$_2$	2,2/t,t	10–15[b]	10–15	10–15
XIV	H$_2$NC(CH$_3$)$_2$(CH$_2$)$_2$C(CH$_3$)$_2$NH$_2$	2,2/c,c	0.98 ± 0.1	5.53 ± 0.8 (5.6)	1.23 ± 0.25 (1.25)
XV	H$_2$NC(CH$_3$)$_2$(CH$_2$)$_2$C(CH$_3$)$_2$NH$_2$	2,2/t,t	6.2 ± 0.24	9.09 ± 1.9 (1.5)	14.4 ± 1.2 (2.3)

[a] Complex I was run independently of experiments in Table II but values given are averages of both sets of experiments. Standard deviations as per Table II. [b] From Table II.

Bis(platinum) complexes are not significantly more active than cisplatin in cisplatin-sensitive cells but display significantly higher activity in the resistant line, with the resistance factor varying with chain length. Thus, whereas the n = 5 and n = 6 complexes show resistance factors of 2–3, resistance factors rise to 6–8 for the n = 4 derivative. This order is also maintained in the malonate series. The differences in resistance factors are probably not that significant between the n = 5 and n = 6 compounds, but there is a clear superiority over n = 4. The malonate complexes are themselves less active than the parent chlorides, as has been found in the extensive studies on monomeric species. Presumably the same explanation applies here, and the decreased potency is a reflection of the more inert nature of the dicarboxylate ligand.[8,9] In the case of the pyridine complex, it is of interest that while substitution of py for NH$_3$ affects potency the resistance factor is also ca. 3. The complex cis-[PtCl$_2$(NH$_3$)(py)] is also an active antitumor compound in its own right.[10] The use of pyridine as ligand may have interesting effects on structure–activity relationships in platinum complexes, and it is worthy of note that cationic triamine complexes cis-[PtCl(NH$_3$)$_2$(py)]$^+$ are antitumor active[11] and that

trans-[PtCl$_2$(py)$_2$] is also as cytotoxic in vitro as cisplatin.[12] This feature and the extension to bis(platinum) complexes is under further study.

The 2,2/t,t complexes IV–VI with both platinum coordination spheres in the trans configuration do not show significantly improved cytotoxicity over their monomeric analogue, trans-DDP. During these assays we have observed variable results for these trans complexes but averaging of the various experiments gives ID$_{50}$ values of 10–15 μM in all systems, and thus the activity of the straight-chain 2,2/t,t complexes in sensitive and resistant cell lines appears approximately equivalent, although no quantitative conclusions can be made (see also the results for sterically hindered diamines.) Note that trans-DDP itself displays some lack of cross-resistance with cis-DDP.

On the other hand, a noteworthy result is that the mixed 2,2/c,t complex IX does show reasonable cytotoxicity. The complex begins to precipitate out of DMF/saline solution at the highest concentrations used and an accurate resistance factor is difficult to obtain. In 10% DMSO, in which IX is readily soluble, the resistance factor is similar to that of I. However, values from DMSO should be treated with caution,[13] especially considering the reactivity of bis(platinum) complexes in this solvent.[3]

Sterically Hindered Diamines. A principal feature of bis(platinum) complexes is that there is considerable scope for structural variation of the diamine backbone, which can affect both chemical reactivity (aqueous solubility, reactivity with entering nucleophiles etc.) and DNA-binding affinity (steric effects, H-bonding, possible

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Table IV. Summary of Antitumor Activity of [{cis-PtCl$_2$(NH$_3$)}$_2$H$_2$N(CH$_2$)$_n$NH$_2$]^a in L1210 and P388 Leukemias

| no.	n	cell line	dose, mg/kg	schedule[b]	% T/C	elemental analysis
I	4	L1210	12.5 × 3	1, 5, 9	Toxic	C,H,N,Cl(C$_4$H$_{18}$N$_4$Cl$_4$Pt$_2$)
			6.25 × 3	1, 5, 9	159, 163	
II	5	L1210	25.0 × 2	1, 5	206 (1/6)	C,H,N,Cl(C$_5$H$_{20}$N$_4$Cl$_4$Pt$_2$)
			12.5 × 2	1, 5	163	
			5.0 × 3	1, 5, 9	197	
		P388	6.25 × 3	1, 5, 9	157	
III	6	L1210	25.0 × 2	1, 5	156	C,H,N,Cl(C$_6$H$_{22}$N$_4$Cl$_4$Pt$_2$)
			12.5 × 2	1, 5	200	
			12.5 × 3	1, 5, 9	175	
			5.0 × 3	1, 5, 9	160	
		P388	25.0 × 3	1, 5, 9	168 (1/6)	
			12.5 × 3	1, 5, 9	159	
cis-[PtCl$_2$(NH$_3$)$_2$]		L1210	5.0 × 3	1, 5, 9	210	
		P388	5.0 × 3	1, 5, 9	170	

^{a} Tests conducted as per Experimental Section. Where only two doses are employed, over 50% of treated animals had died by day 9 presumably due to drug toxicity, and no further drug was administered. ^{b} The appropriate dose is administered on day 1, one day after tumor inoculation, and second and third doses on days 5 and 9, respectively.

sequence specificity). As an example of a sterically hindered diamine, we have examined complexes of the diamine 2,5-dimethyl-2,5-hexanediamine (DMHD):

$$H_2NCCH_2CH_2CNH_2$$

(with CH$_3$ groups)
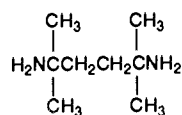
For our purposes this ligand is 1,4-butanediamine with all C1 and C4 positions occupied by methyl groups, and the chemical and biological activity can then be compared with the straight-chain 1,4-butanediamine. The 2,2/c,c XIV and 2,2/t,t XV complexes were prepared by the published method[3] (characterization data, Table I). The in vitro results are collected separately for clarity in Table III. The 2,2/c,c complex XIV is somewhat less active in the sensitive line but retains activity in L1210/DDP with a similar resistance factor to the straight-chain analogue (see footnote to Table III). The consistent values for the 2,2/t,t derivative XV allow us to confirm that there is no really significant increase in activity in comparison to *trans*-DDP, although the pattern of cross-resistance is similar.

The explanation of the differences in cytotoxicity and antitumor activity between *cis*- and *trans*-DDP has been a major feature of the mechanistic studies on platinum complexes. The inactivity of trans compounds may be due to the fact that their DNA adducts inhibit DNA replication to a lesser extent than those of *cis*-DDP[14,15] or, alternatively, that DNA adducts formed by trans compounds are repaired more rapidly.[16] Upon reaction with DNA, the tetrafunctional 2,2/t,t complexes would be expected to give a complex array of lesions including possibly intrastrand crosslinks, by monodentate binding of both Pt atoms to the same strand (see also below). In view of this and the possible effects of placing two *trans*-DDP lesions in close proximity to each other on DNA, the low cytotoxicity of this set of complexes is somewhat surprising. Trans complexes are kinetically more reactive than their cis counterparts, and it is possible that the inconsistency of the 2,2/t,t results for straight-chain diamines is a reflection of deactivating chemistry occurring even in dilute DMF solutions, or upon introduction of these solutions into
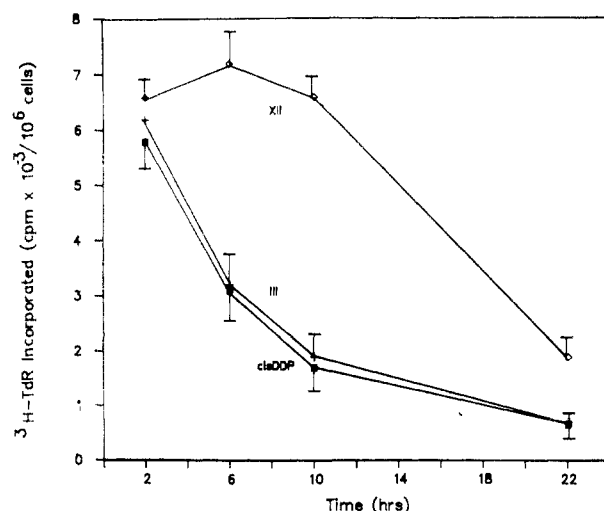
Figure 3. The inhibition of L1210/0 DNA synthesis following exposure to platinum antitumor drugs, *cis*-[PtCl$_2$(NH$_3$)$_2$] (cisDDP), [{*cis*-PtCl$_2$(NH$_3$)}$_2$H$_2$N(CH$_2$)$_6$NH$_2$] (III), and [{Pt(mal)(NH$_3$)}$_2$H$_2$N(CH$_2$)$_6$NH$_2$] (XII). L1210/0 cells were exposed to 5 × ID$_{50}$ of each drug for 2 h, removed from drug, and incubated for various time in the absence of drug. The effect of drug treatment on DNA synthesis was determined by measuring the amount of [^{3}H]TdR incorporated into acid-insoluble material of treated cells.

medium and into cells, especially since these experiments are performed at the margins of drug solubility. We are actively exploring the differences in the chemistry of the straight-chain and substituted diamine complexes to aid in full interpretation of the biological results.

Inhibition of DNA Synthesis. The vast majority of evidence has confirmed the importance of DNA interaction in the mechanism of cytotoxicity of cisplatin.[1] An early indication of this importance was the selective inhibition of DNA synthesis by platinum complexes.[17] It is reasonable to assume that the principal mode of action of bis(platinum) complexes will also be at the level of DNA. The 2,2/c,c complexes inhibit DNA synthesis in cultured L1210 cells, as assayed by incorporation of labeled thymidine. Results for one set of complexes (n = 6, III and XII) are shown in Figure 3, and the n = 4 and 5 complexes behave in a very similar manner. The chloride complexes I–III all inhibit at approximately the rate of *cis*-DDP and at equivalent concentrations. The malonate derivatives X–XII are somewhat slower with a clear lag time before

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inhibition. This phenomenon has been observed previously and may reflect the lesser reactivity of malonate complexes.[9] Complex V shows only slight inhibition under identical conditions.

Antitumor Activity. The antitumor activity has been initially studied in L1210 and P388 leukemia (Table IV). The objective was to ascertain that the complexes do show in vivo activity in standard murine screens. At this stage of new platinum complex development, an important goal must also be to show activity for new analogues in vivo in cisplatin-resistant lines and also in solid tumors or tumors inherently resistant to cisplatin. A larger series of complexes are being extensively tested from these aspects and will be the subject of a more detailed manuscript confirming activity in cisplatin-sensitive and resistant lines in vitro and in vivo.[18] The present preliminary studies confirm that the complexes do indeed exhibit antitumor activity, with the $n = 5$ derivative showing approximately equivalent activity to cisplatin itself (selected data shown for comparison) under similar conditions. Again, there is variation of toxicity with chain length, with the $n = 4$ compound being the most toxic in our hands.

Summary and Conclusions

The fact that the complexes do inhibit DNA synthesis (independent of the differences between individual species) and their reactivity with DNA in general allows us to begin to address the fundamental question of what DNA interactions are responsible for the cytotoxicity in cisplatin-resistant cell lines and whether they differ from those in sensitive cells. Differential repair appears responsible for some of the resistance in the L1210 cell lines used in tissue culture studies,[19] and thus the differential activity between sensitive and resistant lines may lie at the DNA level. Bis(platinum) complexes are tetrafunctional and their possible DNA adducts will be significantly different from those of monomers and may include novel structures such as intra- and interstrand cross-links bridging several base pairs and complex intra-/interstrand or intra-/interstrand–protein cross-links. A principal difference we have recently delineated is that of interstrand cross-linking through binding of each Pt atom to opposite strands of DNA.[4] The cytotoxicity results presented here indicate that the presence of at least one cis-[Pt(amine)$_2$] unit is a requisite for good activity in cisplatin-sensitive lines. One of the most interesting questions with these compounds is also the delineation of the minimum structural features necessary for activity in cisplatin-resistant systems. The results with the mixed 2,2/c,t complex IX are therefore of significance because the complex is non-cross-resistant but contains only one cis coordination unit. Interstrand crosslinking would still be accessible to complex IX, which may be considered as the cis isomer "carrying" a less reactive moiety, i.e. the trans portion, to the target site. The results imply that the nature of the second platinum coordination sphere may not be critical as long as it is still possible to form this interstrand crosslink. Further studies on these compounds will address this question, and in particular the relationship of interstrand crosslinking to activity in the cisplatin-resistant systems.

In conclusion, the 2,2/c,c bis(platinum) complexes display high activity in vitro and in vivo and are unique in being non-cross-resistant with cell lines resistant to cisplatin and its dach analogue. There is no significant increase in cytotoxicity over cisplatin in cisplatin sensitive

cells. Similarly, the 2,2/t,t bis trans complexes do not show significantly increased cytotoxicity over *trans*-DDP nor do they show enhanced cytotoxicity in the cisplatin-sensitive line. The clinical use of platinum complexes in cancer chemotherapy is now well established. A critical problem that compromises the use of these agents is the acquisition of resistance by tumor cells to the cytotoxic effects of cisplatin. Much emphasis has been placed on 1,2-diaminocyclohexane complexes as possible clinical candidates because of their non-cross-resistance in murine tumors such as L1210.[20] The mode of action of dach complexes is, however, likely to be very similar to that of cisplatin. The development of bis(platinum) agents with the potential for unique molecular interactions not available to monomeric complexes and non-cross-resistant with both cisplatin and Pt–dach renders this series of considerable clinical potential and mechanistic interest. The property of interstrand crosslinking suggests that bis(platinum) complexes are now more analogous to the bifunctional alkylating agents and raises the possibility that these new agents may display a different or broader spectrum of clinical activity in comparison to cisplatin.

Experimental Section

Materials and Methods. IR spectra were obtained as KBr disks on Nicolet FT6000 series and Perkin-Elmer 1430 spectrophotometers. NMR spectra were run on Bruker 250- and 270-MHz spectrometers. ^{1}H NMR spectra are referenced to TMS. [195]Pt NMR spectra (250 MHz) were run in D$_2$O (malonate complexes) or DMF-d_7 (chlorides) with reference to a 0.1 M Na$_2$PtCl$_6$ solution in D$_2$O as external reference. Samples were run with a pulse width of 15 μs. Usually a sweep width of 30 KHz was used and 5000–10000 scans were adequate. All shifts are positive to lower shielding. Elemental analyses were by Robertson Laboratories, NJ.

Preparation of Complexes. The 2,2/c,c and 2,2/t,t complexes I–VI were prepared by the previously published methods.[3] The complex [{cis-PtCl$_2$(py)}$_2$H$_2$N(CH$_2$)$_4$NH$_2$] was also prepared by this procedure with the monoanion starting material K[PtCl$_3$-(py)].[21]

Preparation of [{*trans*-PtCl$_2$(NH$_3$)}H$_2$N(CH$_2$)$_4$NH$_2${*cis*-PtCl$_2$(NH$_3$)}]. To the precursor, *trans*-[PtCl$_2$(NH$_3$)(H$_2$N-(CH$_2$)$_4$NH$_3$)]Cl dissolved in MeOH, was added 1 equiv of K-[PtCl$_3$(NH$_3$)] as a suspension in MeOH in the presence of triethylamine. The complex of {cis-PtCl$_2$(NH$_3$)}H$_2$N(CH$_2$)$_4$NH$_2$-{*trans*-PtCl$_2$(NH$_3$)} was precipitated out of solution upon stirring overnight, filtered, washed with water and acetone, and dried. The complex was recrystallized from DMF/0.1 N HCl.

Preparation of Malonate Derivatives. The bis(platinum) complex and silver malonate in stoichiometric proportions were suspended in a 1:1 by volume mixture of H$_2$O and acetone and stirred continuously for 2 days at room temperature. The precipitated AgCl was then filtered off, and the clear filtrate was evaporated to 1 mL in volume. Upon cooling, or addition of acetone, the product precipitated out and was washed with ice-cold water and acetone. The complexes were recrystallized from H$_2$O/acetone or H$_2$O/EtOH.

Biological Assays. The in vitro and in vivo biological activities in L1210 cell lines were assessed with use of standard assays.[22] The malonate complexes were assayed as water solutions, all other complexes were dissolved initially in DMF followed by immediate serial dilution in saline to a maximum concentration of 0.5% DMF.

In Vitro Cytotoxicity Assay. L1210 murine leukemia cells sensitive to the cytotoxicity of DDP (L1210/0) were cultured as a suspension in McCoy's 5A medium supplemented with 5% donor horse serum and glutamine. L1210 cells 40–60-fold resistant to DDP (L1210/DDP) or 30-fold resistant to [Pt(R,R-dach)SO$_4$]

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(L1210/dach) were cultured in McCoy's 5A medium supplemented with 10% fetal bovine serum and glutamine. Both cell lines were grown in a humidified atmosphere to 5% CO_2/95% air at 37 °C. For testing purposes, cells were diluted to 5×10^4 cells/mL and 1 mL of cell suspension was aliquoted to disposable tissue culture tubes. Test compound was then added to the appropriate tubes (40 μL/tube) to attain final concentrations of 0.01, 0.1, 1.0, and 10 μg/mL. After 72 h of drug exposure, the cell concentrations of all tubes were determined by using a Coulter counter. The percent growth inhibition for each drug concentration was then calculated and the ID_{50} (concentration of drug required to inhibit cell growth by 50%) was derived. The resistance factor for each compound was obtained by dividing the ID_{50} (L1210/DDP) by the ID_{50} (L1210/0).

In Vivo Efficacy Studies. Male BDF_1 mice weighing 18–20 g were purchased from the National Cancer Institute and housed in an environment having controlled humidity, temperature, and photoperiods. The animals had food and water available ad libitum, and wood chip bedding was changed daily. The L1210/0 and P388 murine leukemias were maintained as ascites tumors by weekly intraperitoneal (ip) inoculations of 10^6 cells. For testing purposes, 10^6 tumor cells were inoculated ip (day 0) and mice were administered test compound ip on days 1, 5, and 9. Animals were observed daily for signs of toxicity, and deaths and the day of death were recorded for each animal that died during the 60-day observation period. The efficacy of each dose of compound tested was evaluated by calculating the percent increased life span de-termined by dividing the mean survival time of treated mice (using the day of death of only those animals that died during the 60-day period) by the mean survival time of nontreated tumor-bearing control animals (%T/C). Compounds exhibiting a %T/C > 140 are considered to have significant antitumor activity. An additional index of antitumor activity is the number of long term survivors defined as treated animals alive at the end of the study.

DNA Synthesis Studies. L1210 cells (5×10^4 mL^{-1}) were treated for 2 h with the $5 \times ID_{50}$ dose calculated for each drug by the method outlined above. The cells were then centrifuged, washed twice with ice-cold phosphate buffered saline (pH 7.4), and resuspended in fresh, drug-free medium at a concentration of 5×10^4 cells/mL. At appropriate times (2, 4, 6, 22 h) 10 mL of cells was exposed to ^{3}H-thymidine (0.25 μCi/mL) for 2 h and the cells were harvested on glass fiber filters. After washing twice each with ice-cold PBS, 5% trichloroacetic acid, and 95% ethanol, the filters were air-dried, and the radioactivity was measured by using a Beckman LS7000 liquid scintillation counter.

Antitumor and DNA-Binding Properties of a Group of Oligomeric Complexes of Pt(II) and Pt(IV)

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The antitumor and DNA-binding properties of a group of oligomeric platinum(II) and platinum(IV) complexes are described. The compounds, having the stoichiometry $[cis\text{-}Pt^{II}(X)_2(\mu\text{-}OH)]_2(NO_3)_2$, where X is NH_3, $NH_2CH_2CH_3$, and $NH_2CH(CH_3)_2$, were found to be inactive or only weakly active against L-1210 leukemia. In vitro studies involving PM2-DNA show that these compounds bind to and unwind closed circular DNA in a manner similar to $cis\text{-}Pt^{II}\text{-}(NH_3)_2Cl_2$. The Pt(IV) complexes produced by hydrogen peroxide oxidation of the Pt(II) dimers are inactive as antitumor agents and are incapable of unwinding PM2-DNA. The cyclotrimer $[cis\text{-}Pt^{II}(RR\text{-}DACH)(\mu\text{-}OH)]_3(NO_3)_3$, where RR-DACH is (R,R)-1,2 diaminocyclohexane, exhibits potent antitumor activity against L-1210 leukemia and modest activities with B-16 and M5076 tumor lines. This compound platinates DNA, causing DNA unwinding and mobility shifts.

Since the initial report of the anticancer properties of *cis*-diamminedichloroplatinum(II) (1,[1] cisplatin) and the subsequent introduction of the compound into the clinic, a large number of Pt(II) as well as Pt(IV) compounds have been examined for their antitumor effects.[2,3] Studies focusing on the mechanism of action of the compound have strongly suggested that the cytotoxicity of the agent is related to its ability to bind to cellular DNA.[4–7] In examining the aqueous solution chemistry of 1, it was discovered that the compound readily undergoes oligomerization to yield a μ-oxo bridged dimer $[cis\text{-}Pt^{II}(NH_3)_2(\mu\text{-}OH)]_2^{2+}$ (**2**) and trimer $[cis\text{-}Pt^{II}(NH_3)_2(\mu\text{-}OH)]_3^{3-}$ (**3**), both of which have been characterized via ^{195}Pt NMR and X-ray structural analysis.[8–11] Although no detailed in vivo antitumor test data for oligomeric platinum complexes have appeared, it has been reported that both **2** and **3** are more

toxic than 1, but that they are devoid of antitumor effects.[12,13] On the other hand, the dimer and trimer

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