Expedited Articles

Synthesis, Structural Characterization, and Antitumor Properties of a Novel Class of Large-Ring Platinum(II) Chelate Complexes Incorporating the *cis*-1,4-Diaminocyclohexane Ligand in a Unique Locked Boat Conformation

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The first two analogs **5a,b** of a new class of neutral large-ring square-planar Pt(II) chelate complexes of the generic structure [Pt(*cis*-1,4-dach)X_2] were synthesized via a refined technique, structurally characterized by NMR (^{1}H, ^{13}C, ^{195}Pt), FAB mass spectrometry, and X-ray crystallography, and evaluated for antitumor activity in vitro and in vivo in sensitive and Pt-resistant murine leukemia cell systems. An X-ray crystal structure analysis confirmed that [Pt(*cis*-1,4-dach)malonate] **5b** is monomeric and that the *cis*-1,4-diaminocyclohexane (dach) ligand is incorporated in a unique and previously unknown locked boat conformation. Complex **5b** crystallized as colorless rectangular plates in the orthorhombic space group *Pcmn* with $Z = 4$ and the lattice parameters $a = 6.239(1)$ Å, $b = 9.965(2)$ Å, and $c = 18.437(4)$ Å. Important structural parameters are Pt–O = 2.024(5) Å, Pt–N = 2.021(6) Å, N–Pt–N = 100°, and N–Pt–O = 85°; $R = 0.0515$, $R_w = 0.0635$. Antitumor results in murine tumor models show that the parent molecule **5a** (X_2 = 2 Cl) (a) is more dose potent than cisplatin against the leukemias and solid tumors examined, (b) possesses significant activity against cisplatin-resistant leukemias, but exhibits partial cross-resistance with cisplatin, and (c) may possess a spectrum of activity different from that of cisplatin. Antitumor test results in vitro indicate that (a) **5a** is at least equivalent to cisplatin in dose potency and effectiveness in the leukemia cell systems studied except in the [Pt(1,2-dach)Cl_2]-resistant L1210 cell line, (b) the cisplatin-resistant leukemia cell systems exhibit partial cross-resistance to **5a**, (c) **5a** possesses either comparable or greater cytotoxicity than the reference complexes, CI-973 (**3**) and bis(platinum) complex **4**, and (d) **5a** is more effective (~18-fold) than [Pt(1*R*,2*R*-dach)Cl_2] **2** in inhibiting growth in the Pt(1,2-dach)-resistant L1210 cell line, suggesting that [Pt(*cis*-1,4-dach)Cl_2] is either not recognized as or is not acting as a "typical" Pt(dach) complex. The encouraging antitumor activity of **5a**, coupled with a 10-fold higher aqueous solubility compared to [Pt(1*R*,2*R*-dach)-Cl_2] **2** warrants the following future studies: synthesis of selected analogs, elucidating the nature of Pt–DNA binding sites, the mechanism of action, and the mechanistic basis for the lack of cross-resistance of [Pt(*cis*-1,4-dach)Cl_2] against the [Pt(1,2-dach)Cl_2]-resistant L1210 cell line.

Introduction

Cisplatin **1** and its close analog, carboplatin, are Pt(II) drugs used worldwide in the clinical treatment of many types of solid tumors. However, like other chemotherapeutic agents, their clinical effectiveness is limited by intrinsic and/or acquired drug resistance. In considering a third generation Pt complex for clinical development, a key selection criterion will be the ability of an agent to overcome cisplatin and/or carboplatin resistance. In this context, platinum(1,2-diaminocyclohexane) ("1,2-dach") complexes, related to the prototype complex [Pt(1*R*,2*R*-dach)Cl_2], have been the focus of considerable research over the past two decades.[1] This is primarily due to reports of good in vitro activity[2] and in vivo efficacy[3] against cisplatin-resistant tumors. As an indication of the level of interest in platinum(1,2-dach) complexes, two agents (*i.e.*, Oxaliplatin and Ormaplatin) are currently in clinical trials and incorporate the 1*R*,2*R*-dach ligand as Pt(II) and Pt(IV) complexes, respectively. With the exception of these two complexes, however, low aqueous solubility and/or molecular instability historically have been major impediments to the clinical development of Pt(dach) analogs. In an attempt to redress these problems, we and others more recently have reported on the development of the platinum agents CI-973 (**3**), an agent that has been evaluated clinically in the United States and Japan[4] and which resembles carboplatin in its antitumor properties, and the bis(platinum) complex **4**, a member of an extensive series of dinuclear Pt(II) complexes in which two Pt(II) centers are linked by a diamine bridge.[5]

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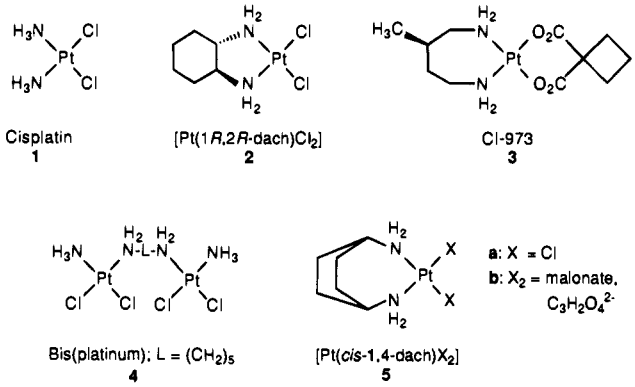


Figure 1. Reference platinum complexes.

In our search for effective newer platinum antitumor complexes that are also capable of overcoming cisplatin resistance in vivo, we report here on the synthesis, X-ray crystal structure, and preliminary in vitro and in vivo antitumor activity of a structurally novel class of large-ring (7-member) chelate complexes of the type $[PtAX_2]$, **5a,b**, wherein A represents the neutral *cis*-1,4-dach ligand and X_2 signifies two chloride (Cl^-) or one malonate ($C_3H_2O_4^{2-}$) anionic group(s).[6,7] These [Pt(*cis*-1,4-dach)X_2] analogs feature a previously unknown mode of metal–ligand chelation in which the *cis*-1,4-dach ligand is bonded to Pt(II) in a locked boat conformation. In evaluating the in vitro antitumor activity of [Pt(*cis*-1,4-dach)Cl_2], four well-known Pt(II) reference complexes **1**–**4** (Figure 1) were also evaluated as positive controls, whereas cisplatin served as the control for the in vivo work.

Chemistry

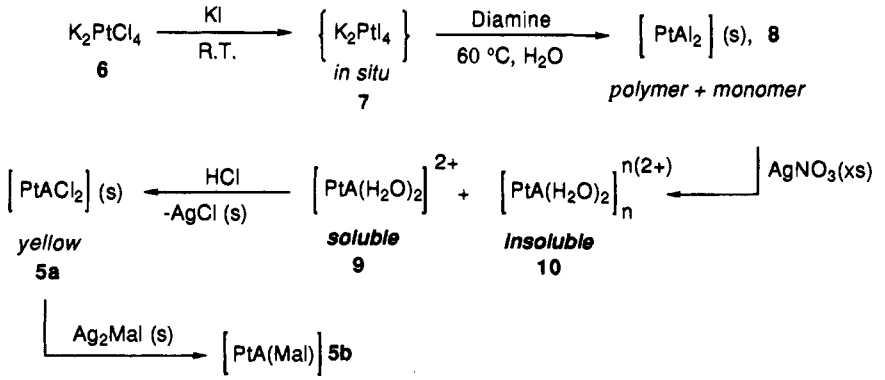
The two [Pt(*cis*-1,4-dach)X_2] complexes **5a,b** described herein were prepared via a generic route of synthesis shown in Scheme 1. The complexes were prepared via a procedure derived from the method of Nowatari et al.[8] In refining this method, yields were optimized as a function of the concentrations and delivery rates (mL/min) of the reactants. The method utilizes the chemistry of the Dhara method[9a] in that the Pt(II) starting material, K_2PtCl_4, **6**, is first converted in situ to the potassium tetraiodoplatinate(II) intermediate **7**. Equimolar solutions of **7** and *cis*-1,4-diaminocyclohexane were then separately and simultaneously delivered via a precision peristaltic pump into a reservoir of H_2O held at 60 °C, producing **8**, a mixture of iododiamine–platinum(II) complexes [*i.e.*, $[PtAI_2]_n$ ($n \geq 1$)] consisting of a discrete monomer and, presumably, various polymers (oligomers). Addition of $AgNO_3$ converted **8** to the corresponding aqua species **9** (soluble) and **10** (insoluble). Subsequent addition of HCl to the soluble diaqua species **9** produced the target complex **5a** which was purified by recrystallization from DMF/HCl. The malonato analog **5b** was prepared by treating **5a** with silver(I) malonate followed by recrystallization from H_2O. The spectroscopic data for both complexes is fully consistent with the proposed structures.

cis-1,4-Diaminocyclohexane (*cis*-1,4-dach) was prepared by purification of a commercially available mixture of 82:18 *cis*:*trans* isomers by a modification of a patent procedure[10] or via hydrolysis of *cis*-1,4-bis-[(ethoxycarbonyl)amino]cyclohexane which is easily derived via a short sequence commencing with a Diels–Alder reaction between cyclohexa-1,3-diene and diethyl azodicarboxylate.[11] Both procedures are detailed in the Experimental Section.

The key step in this synthetic scheme is the initial step, which effectively simulates a condition of "infinite dilution", because (a) the reaction stoichiometry is carefully controlled so as to maintain a Pt(II):diamine ratio of 1.0 at all times, (b) a higher than normal reaction temperature is employed (60 *vs* 45 °C, as specified in the Dhara method), leading to an accelerated reaction rate and to low steady-state concentrations of the reactions during addition, and (c) extraneous reactions with product (that has already formed) are minimized since the insoluble product **8** precipitates from solution immediately on forming. Other factors which contribute to the success of this method are (d) a highly soluble diaqua monomer **9** *vs* an insoluble polymer **10** [presumably $[PtA(H_2O)_2]_n^{2n+}$ ($n > 1$)], which facilitates separation of the two types of aqua species produced and (e) the strong *trans* influence of the iodo groups in PtI_4^{2-} promotes a high degree of (*cis*) stereoselectivity in directing the second incoming NH_2 group *cis* to the NH_2 group initially introduced,[12] thus enhancing the intrinsically low probability of forming the desired large intramolecular ring chelate. The uniqueness and utility of this method are that it facilitates the synthesis of these and other large-ring chelates[13] which *cannot* be prepared via any of the classical methods.[9a-c]

X-ray Crystallography. The crystal structure of [Pt(*cis*-1,4-dach)malonate], **5b**, a seven-membered ring locked boat chelate representative of this generic class of complexes, is illustrated via the ORTEP diagram in Figure 2. Important bond lengths and angles are provided in Table 1, and a comparison of key bond lengths (Pt–O, Pt–N) and angles (N–Pt–N, O–Pt–O)

Scheme 1. General Method of Synthesis of [Pt(*cis*-1,4-dach)X_2]



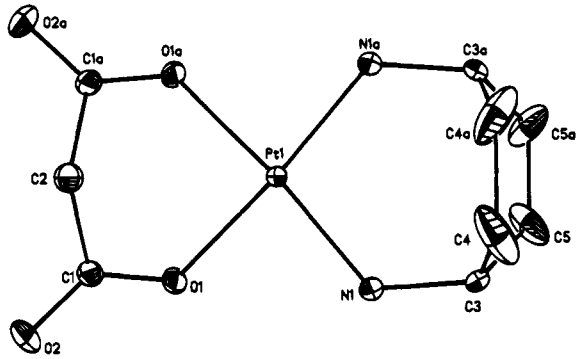


Figure 2. ORTEP representation of the crystal structure of [Pt(*cis*-1,4-dach)mal].

Table 1. Important Bond Lengths (Å) and Bond Angles (deg) for [Pt(*cis*-1,4-dach)malonate] **5b**

	Bond Lengths (Å)		
Pt1–O1	2.024(5)	N1–C3	1.48(1)
Pt1–O1a	2.024(5)	C1–C2	1.521(8)
Pt1–N1	2.021(6)	C1a–C2	1.522(5)
Pt1–N1a	2.021(6)	C3–C4	1.50(2)
O1–C1	1.298(9)	C3–C5	1.51(1)
O1a–C1a	1.299(5)	C4–C4a	1.46(2)
O2–C1	1.214(9)	C5–C5a	1.38(1)
	Bond Angles (deg)		
Pt1–O1–C1	117.8(4)	O1a–C1a–C2	118.4(4)
Pt1–O1a–C1a	117.9(3)	O2–C1–C2	119.8(7)
Pt1–N1–C3	123.2(4)	N1–Pt1–N1a	100.0(2)
O1–Pt1–O1a	90.0(2)	N1–C3–C4	112.1(8)
O1–Pt1–N1	85.0(2)	N1–C3–C5	111.0(8)
O1–Pt1–N1a	174.8(2)	C1–C2–C1a	114.3(6)
O1–C1–O2	121.7(6)	C3–C4–C4a	116.7(9)
O1–C1–C2	118.5(6)	C3–C5–C5a	118.2(8)
O1a–Pt1–N1	174.8(2)	C4–C3–C5	111.3(9)
O1a–Pt1–N1a	85.0(2)		

for [Pt(1,*n*-dach)X_2] complexes (n = 2, 3, and 4; X_2 = 2 Cl, oxalate, or malonate) appears in Table 2. In **5b**, all Pt–N and Pt–O bond lengths are within the range normally observed for complexes of the type, PtO_2N_2 (*i.e.*, 2.03 ± 0.02 and 2.02 ± 0.01 Å, respectively), especially as regards the Pt(1,2-dach)X_2 complexes (*e.g.*, for X_2 = oxalate and malonate).[14a] The PtN_2O_2 core in **5b** lies wholly within a square plane. The structure demonstrates conclusively that the [Pt(*cis*-1,4-dach)-malonate] complex is monomeric and incorporates the *cis*-1,4-dach ligand as a chelate constrained to a unique boat conformation. This conformation is in contrast to the usual chair conformation adopted by the 1,2-dach ligand in [Pt(1,2-dach)X_2] complexes. The N–Pt–N bond angle of 100.0° (*vs* 82.9° in [Pt(*cis*-1,2-dach)Cl_2] and 94.5° in [Pt(*cis*-1,3-dacp)Cl_2], Table 2) is the largest N–Pt–N bond angle ever reported for a PtN_2X_2 complex, underscoring the uniqueness of this previously unknown mode of coordination. In accommodating this mode of coordination, the N–Pt–O bond angles (85°) are compressed to smaller than normal values (*cf.* Table 2), and the thermal ellipsoids are somewhat elongated which seems to indicate slight orientational disorder or torsional flexibility in this bound ligand.

Biological Properties

In Vitro Growth Inhibition for [Pt(*cis*-1,4-dach)-Cl_2]. The effect of [Pt(*cis*-1,4-dach)Cl_2] **5a** on the in vitro growth inhibition of murine leukemia cells sensitive and resistant to cisplatin and [Pt(1*R*,2*R*-dach)Cl_2] **2** is shown in Table 3.. The resistance factors (in parentheses, Table 3) for [Pt(*cis*-1,4-dach)Cl_2] in comparison with those for cisplatin **1**, [Pt(1*R*,2*R*-dach)Cl_2] **2**, CI-973 (**3**), and the bis(platinum) complex **4** are defined by the ratio of the ID_{50} values of the resistant lines divided by the ID_{50} values of the sensitive lines.

The growth inhibition data (on a μg/mL basis) indicate that [Pt(*cis*-1,4-dach)Cl_2] (a) elicits potent activity in sensitive L1210 and P388 cell lines (ID_{50} values of 0.248 and 0.047 μg/mL, respectively), (b) appears more potent than cisplatin with respect to ID_{50} values in all Pt-resistant cell lines tested except for the L1210DACH lines, but the cisplatin-resistant cell lines, L1210PtR4 and L1210DDP5, exhibit partial cross-resistance to **5a**. For example, resistance factors obtained for cisplatin and [Pt(*cis*-1,4-dach)Cl_2] for the L1210DDP5 and L1210PtR4 cell lines are (11 and 2.7) and (21 and 4.9), respectively, and (c) is more cytotoxic than CI-973 and the bis(platinum) complex is cisplatin-resistant cell lines (L1210PtR4 and L1210DDP5), but exhibits higher resistance factors in the L1210DACH and P388Pt4 cell lines than the bis(platinum) complex and (d) is ~18-fold more effective than [Pt(1*R*,2*R*-dach)Cl_2] in the Pt-(1,2-dach)-resistant L1210 leukemia cell line (L1210-DACH). From (d), it would appear that [Pt(*cis*-1,4-dach)Cl_2] is not recognized by the L1210DACH system as a Pt(dach) complex. This strongly suggests that differences in shape/conformation play an important role in the eliciting of the (differential) antitumor activity by Pt(dach) complexes. The notion of selectivity based on differences in ligand (complex) conformation/shape is consistent with (a) the differential activity observed for the individual isomeric [Pt(1,2-dach)Cl_2] complexes[15] as well as with (b) differences in the conformations of the individual isomeric Pt(1,2-dach) complexes recognizable by antibodies specifically developed against the DNA-Pt(II) (1*R*,2*R*-dach) adduct.[16] The precise mechanism(s) by which these putative conformational differences become expressed is of course unknown but of great interest. Obvious possibilities include the processes involving (mono and/or di) adduct formation, recognition/repair, and translation.[17]

In Vivo Antitumor Activity for [Pt(*cis*-1,4-dach)-Cl_2]. [Pt(*cis*-1,4-dach)Cl_2] **5a** is more dose potent (*i.e.*, lower dose producing equivalent or better activity) than cisplatin against the murine leukemias and solid tumors used in these studies (Tables 4 and 5). Against the parental L1210 and P388 leukemias, [Pt(*cis*-1,4-dach)-Cl_2] possesses comparable to or slightly better activity than cisplatin based on %T/C values (Table 4). [Pt(*cis*-1,4-dach)Cl_2] also retained significant activity against the platinum-resistant sublines of L1210 and P388 in contrast to the relative lack of cisplatin activity. However, the reduced activity of [Pt(*cis*-1,4-dach)Cl_2] against these platinum-resistant leukemias compared to the parental lines suggested that these platinum-resistant leukemias were at least partially cross-resistant to this compound.

Against the B16 melanoma and M5076 sarcoma, both [Pt(*cis*-1,4-dach)Cl_2] and cisplatin produced equivalent activity (Table 5). Cisplatin was more active than [Pt-(*cis*-1,4-dach)Cl_2] against colon carcinoma 26 at equitoxic doses, producing a net tumor burden reduction while growth was observed with [Pt(*cis*-1,4-dach)Cl_2] therapy (Table 5). These data indicate that the spec-

Table 2. Bond Angles and Distances for [Pt(1,*n*-dach)X_2] Complexes of the Type PtN_2Cl_2 and PtN_2O_2

complex[a]	PtN_2 chelate ring size	bond angles (deg)			bond distance (Å)	
		N–Pt–N	O–Pt–O	O–Pt–N	Pt–O	Pt–N
[Pt(*trans-l*-1,2-dach)oxalate][14a]	5	83.8	82.5	98.6	2.01	2.05
[Pt(*trans-l*-1,2-dach)malonate][14a]	5	83.8	90.3	93.2	2.02	2.03
[Pt(*cis*-1,2-dach)Cl_2][14b]	5	82.9	–	–	–	–
[Pt(*cis*-1,3-dacp)Cl_2][14c]	6	94.5	–	–	–	2.06
[Pt(*cis*-1,4-dach)malonate]	7	100.0	90.0	85.0	2.02	2.02

[a] dacp = diaminocyclopentane.

Table 3. In Vitro Growth Inhibition (ID_{50}) by [Pt(*cis*-1,4-dach)Cl_2] (**5a**) and Selected Platinum(II) Antitumor Complexes in Murine Leukemia Cell Lines[a,b]

cell lines	cisplatin (1)	[Pt(1*R*,2*R*-dach)Cl_2] (**2**)	[Pt(*cis*-1,4-dach)Cl_2] (**5a**)	CI-973 (**3**)	bis(platinum) (**4**)
L1210S	0.375 ± 0.04	0.059 ± 0.02	0.248	0.76	0.47
	[1.25 ± 0.13]	[0.15 ± 0.05]	[0.652]	[1.73]	[0.70]
L1210PtR4	7.84 (21)	0.161 (2.7)	1.21 (4.9)	2.6 (3.4)	1.3 (2.7)
	[26.1]	[0.423]	[3.18]	[5.9]	[1.95]
L1210DDP5	4.11 (11)	0.164 (2.8)	0.663 (2.7)	3.9 (5.1)	1.1 (2.3)
	[13.7]	[0.431]	[1.74]	[8.9]	[1.65]
L1210DACH	0.52 (1.4)	4.57 (77)	1.08 (4.3)	1.8 (2.4)	0.66 (1.4)
	[1.73]	[12.0]	[2.84]	[4.1]	[0.99]
P388S	0.128	0.077	0.047	0.10	0.22
	[0.427]	[0.20]	[0.12]	[0.23]	[0.33]
P388Pt4	10.0 (78)	0.83 (10.8)	1.43 (30)	1.62 (16)	1.39 (6.3)
	[33.3]	[2.18]	[3.76]	[3.69]	[2.08]

[a] The selected Pt(II) complexes are stable in physiological saline; however, all would be expected to react slowly with the thiol-containing proteins of fetal calf serum (10%) comprising the cell culture medium. No complexes were tested in pure H_2O. [b] ID_{50} values are expressed both in μg/mL and μM placed in brackets immediately below the corresponding μg/mL. Values are averages of at least two separate experiments performed in duplicate. L1210S and P388S are murine leukemia cell lines sensitive to cisplatin. L1210DDP5 and L1210DACH were made resistant to cisplatin and Pt(1,2-dach) complexes, respectively, and were provided by Dr. A. Eastman, Dartmouth College, Hanover, NH.[24] L1210PtR4 and P388PtR4 were made resistant to cisplatin in vivo and were adapted to in vitro culture in our laboratory. Values in parentheses are resistance factors = ID_{50} (resistant, μg/mL)/ID_{50} (sensitive, μg/mL).

Table 4. In Vivo Activity of [Pt(*cis*-1,4-dach)Cl_2] (**5a**) vs Cisplatin (1) against Platinum-Resistant Leukemias

compound	dose[c]	L1210 leukemia[a]				L1210 Pt-resistant leukemia[a]			
		weight change (g)	MLS[d] (days)	%T/C	60-day survivors	weight change (g)	MLS[d] (days)	%T/C	60-day survivors
				Experiment 1					
5a	3.0	−4.8	21.5	203	1/6	−4.3	17.0	167	2/6
	1.5	+0.6	23.0	217	0/6	+1.9	15.7	154	0/6
1	6.0	−1.9	18.5	175	1/6	−3.7	12.0	118	0/6
	4.0	+1.0	19.5	184	1/6		11.1	109	0/6
				Experiment 2					
5a	2.5	−1.9	22.5	242	1/6	−1.6	14.3	139	0/6
	1.6	+0.9	21.3	229	0/6	+1.9	15.0	146	1/6
1	10	−3.3	18.5	199	1/6	−4.8	12.4	120	0/6
	5.0	+0.5	16.0	172	0/6	–	11.0	107	0/6
		P388 leukemia[b]				P388 Pt-resistant leukemia[b]			
5a	3.7	−6.0	26.0	234	0/6	−5.2	25.8	177	0/6
	2.3	−2.2	25.0	225	0/6	−0.1	21.0	144	0/6
1	10	−5.5	9.5	86	3/6	−5.9	18.0	123	0/6
	5.0	+0.2	29.0	261	0/6	+1.0	19.3	132	0/6

[a] Inoculum of 1×10^4 cells IP on day 0. Treatments were administered IP on days 3, 7, and 11. [b] Inoculum of 1×10^6 cells IP on day 0. Treatments were administered IP on days 1, 5, and 9. [c] Dose in mg/kg per injection. [d] Median life span.

trum of activity of [Pt(*cis*-1,4-dach)Cl_2] may be different from that of cisplatin.

Summary and Conclusions

This paper reports on the first synthesis of two members of a novel family of [Pt(*cis*-1,4-dach)X_2] complexes in which the *cis*-1,4-dach ligand is chelated in a unique locked boat conformation as confirmed by X-ray crystallography.

In vitro results indicate that [Pt(*cis*-1,4-dach)Cl_2], the parent member of this series, is a promising agent since it exhibits activity equivalent to or better than cisplatin in most cisplatin- and the single Pt(1,2-dach)-resistant L1210 leukemia cell lines studied.

In vivo results indicate that [Pt(*cis*-1,4-dach)Cl_2] may possess better activity than cisplatin against platinum-resistant murine leukemias but that these tumors will be at least partially resistant to [Pt(*cis*-1,4-dach)Cl_2] therapy. The solid tumor data suggest that its spectrum of activity may be different from that of cisplatin.

Although *complete* lack of cross-resistance was not observed for [Pt(*cis*-1,4-dach)Cl_2] in either Pt-resistant system, a significant level of activity was observed in *both* systems, which supports the notion that [Pt(*cis*-1,4-dach)Cl_2] is *neither* a close cisplatin *nor* a close [Pt(1*R*,2*R*-dach)Cl_2]-type analog but rather a system that incorporates properties of both compounds.

The 10-fold higher aqueous solubility of [Pt(*cis*-1,4-

Table 5. In Vivo Activity of [Pt(*cis*-1,4-dach)Cl_2] (**5a**) vs Cisplatin (1) against Murine Solid Tumors

tumor	compound	dose[c] (mg/kg)	weight change (g)	T − C (days)[d]	net cell kill (log_{10})[e]
B16[a] melanoma	**5a**	4.0	−2.6	4.4	−0.5
	1	9.0	−4.5	5.1	−0.4
colon carcinoma 26[b]	**5a**	2.5	−4.2	3.8 (1/6)	−0.4
	1	5.6	−3.4	16.4 (1/6)	+0.8
M5076 sarcoma[a]	**5a**	4.0	−5.5	13.5	+0.6
	1	9.0	−5.1	15.9	+0.8

[a] Tumor fragments were implanted SC on day 0. Treatments were administered IP on days 1, 5, and 9. [b] Tumor fragments were implanted SC on day 0. Treatments were administered IP on days 3, 7, and 11. [c] The maximum tolerated dose from a complete dose response curve is shown. [d] The difference in days for the treated and control tumors to reach 750 mg. Numbers in parentheses indicate cures over total mice in group. [e] Net tumor burden reduction during therapy.

dach)Cl_2] compared to [Pt(1*R*,2*R*-dach)Cl_2] increases the attractiveness of this therapeutic entity since it can be administered as a homogeneous solution rather than as a slurry as would be required for the latter. On the basis of comparative solubilities, a similar argument can be made regarding the bis(platinum) complex.

The greater in vitro effectiveness of [Pt(*cis*-1,4-dach)-Cl_2] *vs* Pt(1,2-dach)-resistant L1210 leukemia suggests that differences in shape/conformation play an important role in the eliciting of antitumor activity by [Pt-(1,*n*-dach)Cl_2] complexes.

Experimental Section

General Chemical Procedures. The NMR spectrum of [Pt(*cis*-1,4-dach)Cl_2] **5a** was run in DMF-d_7 and the malonato complex **5b** in D_2O (or 33% D_2O in H_2O) at sample concentrations of ~1 mM and 10 mg/mL, respectively. ^1H (300 MHz), ^{13}C (75.43 MHz), and ^{195}Pt (64.40 MHz) NMR spectra were recorded on Varian XL-200 and XL-300 NMR spectrometers. ^{13}C and ^{195}Pt NMR spectra were proton-decoupled. ^{195}Pt spectra were externally referenced to and are reported relative to 0.2 M K_2PtCl_4 in 0.4 M KCl. Referenced to Na_2PtCl_6 in D_2O, σ_{Pt} for K_2PtCl_4 in 0.4 M KCl is −1634 ppm. Samples were run using a pulse width of 15 μs and a sweep width of 30 kHz. A single broad transition was observed for [Pt(*cis*-1,4-dach)-Cl_2] with the chemical shift appearing downfield from K_2PtCl_4 in KCl. Proton NMR spectra were referenced to TMS via the residual solvent protons which were assigned a value of 0.00 ppm. Positive fast-atom bombardment mass spectra (MS-$(FAB)^+$) were acquired using 3-nitrobenzyl alcohol as the FAB^+ matrix. UV−vis spectra were recorded on a Cary 219 spectrophotometer. Purity of [Pt(*cis*-1,4-dach)Cl_2] was assessed by HPLC using a Phenomenex, Ultramex-5 C8 reverse-phase column (0.46 × 25 cm). The mobile phase consisted of 5% (1:1 CH_3CN:MeOH)/95% H_2O with peak monitoring at 205 nm. Elemental analyses were performed via combustion using a Carlo-Erba Model 1106 elemental analyzer. Chloride was determined by silver nitrate titration. Commercial-grade K_2-$PtCl_4$ was purified by recrystallization on adding three volumes of 12 M HCl to a concentrated (and prefiltered) solution of the as-received material in 0.1 M HCl, followed by cooling to 0 °C. The recrystallized product was filtered, washed with absolute EtOH (0 °C), and dried in air (by suction). Conductivity-grade water, obtained via a Millipore system, was used in all operations requiring H_2O. All other chemicals were reagent grade and were used without further purification.

Synthesis of *cis*-1,4-Diaminocyclohexane Dihydrochloride (*cis*-1,4-dach dihydrochloride). Method A. A commercially available source of impure *cis*-1,4-dach was derivitized and reconverted to >98% purity *cis*-1,4-dach as follows by a modification of a literature procedure[10]: A solution of 24.2 g of an 82:18 *cis*:*trans* mixture of 1,4-dach (DuPont) and 200 mL of acetic anhydride was heated at reflux for 2 h, then evaporated to dryness to yield the bisacetamides. The solid product was refluxed in 400 mL of CH_3CN for 15 min, and then filtered hot to collect 19.3 g of a mixture of *cis*- and *trans*-bisacetamides after drying. The filtrate was stored at room temperature overnight and the resultant precipitate was collected by filtration to give 14.6 g of a predominantly *cis*-bisacetamide product. The latter was boiled in 450 mL of CH_3-CN, the hot mixture filtered to remove a small amount of solid material, and the filtrate cooled in an ice−H_2O bath for 2 h. The resulting solids were collected by filtration and dried at 50 °C for 2 h to yield 11.2 g of analytically pure bisacetamide that was shown (by ^1H NMR integration) to be a 93:7 *cis*:*trans* mixture [^1H NMR (D_2O) ppm: 3.77 (*cis*-bisacetamide) and 3.57 (*trans*-bisacetamide); ^{13}C NMR (D_2O) ppm: 49.47 and 30.50 (*cis* isomer), 51.26 and 33.65 (*trans* isomer)].

The predominantly *cis*-bisacetamide (10.5 g) was boiled in 350 mL of CH_3CN, the hot solution filtered, and the filtrate cooled in an ice bath overnight. The solids were collected by filtration, washed (3 times) with cold CH_3CN, and dried in vacuo at 45 °C for 2 h to give 9.0 g of solids, shown by HPLC to be a 94:6 mixture. Finally, 8.8 g of this lot was recrystallized three more times from ~250 mL of CH_3CN (per recrystallization) to yield 7 g (18%) of the *cis*-bisacetamide, shown by HPLC to contain 96.3% of the *cis* isomer. The *cis*:*trans* ratio in this material remained unchanged following two additional recrystallizations.

A mixture of 4 g (20 mmol) of *cis*-1,4-bisacetamide and 80 mL of 12 M HCl was refluxed for 5 h. After concentrating the solution to dryness, the residual solid was triturated in Et_2O and then collected by filtration. The solids were triturated in 40 mL of hot absolute EtOH and, after the suspension had cooled to ~40 °C, were collected by filtration and dried to give 2.9 g (77%) of analytically pure *cis*-1,4-dach·2HCl. Repetitive recrystallization (3 times) from 9 M HCl enriched further the percentage of *cis* in the mixture to 98.6%. This material was used in the synthesis of [Pt(*cis*-1,4-dach)Cl_2]. Anal. ($C_6H_{16}N_2$-Cl_2) C, H, N. ^{13}C NMR (D_2O) ppm: 27.84, 50.02.

Synthesis of [*cis*-1,4-Diaminocyclohexane]dichloroplatinum(II) (5a). A 400-mL solution of K_2Pt_4, prepared in situ by mixing solutions of K_2PtCl_4[5.0 g (12 mmol) in 350 mL of H_2O] and KI [14.62 g (88.1 mmol) in 50 mL of H_2O], and *cis*-1,4-dach [2.25 g (12 mmol) and 24 mmol of NaOH in 400 mL of H_2O] were simultaneously pumped into a 3-L flask containing 250 mL of H_2O maintained at 60 °C. The contents of the flask were stirred vigorously via an external mechanical stirrer as the reagents were delivered over 4.76 h (1.4 mL/min). The reaction mixture was maintained at 60 °C during and for 0.5−1 h after delivery of the reagents. Subsequently, the reaction mixture was cooled to ambient temperature and filtered through a medum-porosity sintered-glass filter to afford 5.7 g of the iodoamine intermediate **8** (Scheme 1). Following the addition of a $AgNO_3$ solution (110% of stoichiometry) to this slurry, the resultant mixture was first heated at 60 °C for 0.5−1 h with stirring, then allowed to stir at ambient temperature overnight, and finally filtered (0.2-μm filter) to afford a colorless filtrate. Excess Ag^+ cation was removed as AgCl(s) via several additions of 1 M HCl, followed by filtration after each addition. The Ag^+-free filtrate (300−400 mL) was cooled to 0 °C and then concentrated *in vacuo* to about 50 mL. Four milliliters (48 mmol) of 12 N HCl (twice the stoichiometrically required amount of Cl^- ion) was added to the concentrate and the mixture warmed at 45 °C for 15 min and then cooled in an ice bath to crystallize the pale yellow $PtACl_2$ compound. The product was filtered, washed with cold (0 °C) 1 N HCl and absolute EtOH, and dried in air (by suction) and then in vacuo at 50 °C for 2 h to afford 0.8 g (18%) of crude product. Recrystallization from DMF/HCl afforded 0.51 g (11%) of pure [Pt(*cis*-1,4-dach)Cl_2], **5a**. Anal. ($C_6H_{14}N_2Cl_2$-Pt) C, H, N, Cl. Recrystallization from DMF/0.6 M HCl repeatedly yielded twinned crystals, precluding an X-ray solution of the structure of [Pt(*cis*-1,4-dach)Cl_2]. UV: λ(max) = 279 nm (ϵ = 113 M^{-1} cm^{-1}). Absorbance ratio (270 nm/247 nm) = 3.19. ^{195}Pt NMR (DMF-d_7): −587 ppm. ^{13}C NMR

Table 6. Summary of Crystallographic Data for [*cis*-1,4-Diaminocyclohexane]malonatoplatinum(II) (**5b**)

formula	$C_9H_{16}N_2O_4Pt$
mol wt	411.32
a, Å	6.239(1)
b, Å	9.965(2)
c, Å	18.437(4)
α, deg	90
β, deg	90
γ, deg	90
V, Å^3	1146.2(4)
crys sys	orthorhombic
space group	*Pcmn* (alt. setting of *Pnma* #62)
Z	4
radiation	Mo Kα (0.7107 Å)
temp, °C	298
R^a	0.0515
$R_w{}^b$	0.0635

$^a R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. $^b R_w = [\Sigma(w|F_o - F_c|)^2/\Sigma w|F_o|^2]^{1/2}$.

(DMF-d_7) ppm: 24.41 (CH_2), 50.38 (CH). MS(FAB)$^+$: M = 380.1 (84%). Purity by HPLC = 99.4–99.9% (t_R = 4.84–4.88 min). TLC = 0.86 R_f (acetone:H_2O, 80:20).

Synthesis of [*cis*-1,4-Diaminocyclohexane]malonatoplatinum(II) (5b). Ten milliliters of H_2O was added to a 20-mL scintillation vial containing a physical admixture of **5a** [0.132 g (0.347 mmol)] and silver malonate, $Ag_2C_3H_2O_4$ [0.110 g (0.347 mmol)], and the stirred reaction mixture was heated to and maintained at 45 °C for 0.5 h. The mixture was cooled to ambient temperature, and AgCl(s) was removed by filtration. The water-white filtrate was evaporated to dryness under a stream of N_2 gas. The residue was redissolved in H_2O at ~80 °C, and this solution was allowed to cool to room temperature overnight in producing well-formed transparent hexagonal plates (used for the single-crystal X-ray structural determination of **5b**). While the actual yield was not determined, yields for a metathetical reaction of this type generally exceed ~90%. MS(FAB)$^+$: M + 1 = 412. Anal. ($C_9H_{16}N_2O_4$Pt) C, H, N.

X-ray Crystallography. A single crystal of **5b** was acquired as described above and mounted in a sealed glass capillary. A small amount of mother liquor was included in the capillary to decrease decomposition due to solvent loss from the crystal lattice. Diffraction data were collected on a Siemens R3m/v diffractometer at ambient temperature. Intensity data were obtained by using Mo Kα radiation (0.7107 Å) monochromatized from a graphite crystal whose diffraction vector was parallel to the diffraction vector of the sample. Three standard reflections were measured every 97 reflections. Random fluctuations of less than 3% were observed for three standard reflections. Lattice parameters were determined from a least-squares refinement of 25 reflection settings obtained via an automatic centering routine. Table 6 contains a summary of the conditions of data collection and results for the structure. The data were reduced and the structure solved by direct methods using the program SHELXTL PLUS[18] mounted on a VAXStation 3500. In subsequent refinement, the function $\Sigma w(|F_o| - |F_c|)^2$ was minimized where $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes. The agreement indices $R = \Sigma||F_o| - |F_c||/|F_o|$ and $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w(|F_o|^2]^{1/2}$ were used to evaluate the results. Atomic scattering factors are from ref 19. Hydrogen atoms were included using a riding model [d_{C-H} 0.96 Å, isotropic U(H) fixed at 1.20(eq)]. Analysis of systematic absences in the data gave *Pcmm* and *Pc*21*n* as possible space group choices. The choice of the higher symmetry space group *Pcmn* was confirmed by the successful solution and refinement of the structure. The molecule is on the site of a mirror plane in the crystal lattice. The mirror plane bisects the dach and the malonate ligands while the platinum atom and methylene carbon of the malonate ligand are positioned on the mirror. The carbon atoms of the dach ligand show somewhat elongated thermal ellipsoids which seem to indicate some slight orientational disorder or torsional flexibility in this ligand. Interestingly, the nitrogen atoms do not show any significant disorder. Attempts to model this disorder by placing partial occupancy atoms were not successful as was refinement in the lower symmetry space group.

Mice and Tumor Passage. Mice used for in vivo testing were purchased from the Charles River Breeding Laboratories and were housed in barrier facilities equipped with automatic flush racks. Food and water were provided ad libitum. Mice were maintained on a 12-h light/dark cycle. Tumors of murine origin were maintained in the inbred strain of tumor origin.

Experimental Chemotherapy. In vitro growth inhibition in platinum-resistant murine leukemia cell lines was carried out using a published procedure.[20] In vivo anticancer activity was evaluated in Balb/c × $DBA/2F_1$ mice ($CD2F_1$) for all leukemias and in C57BL/6 × $C3HF_1$ mice ($B6C3F_1$) for B16 melanoma and M5076 sarcoma. For all tests, mice (18–22 g) were randomized and then inoculated with counted numbers of tumor cells or trocar fragments on day 0. Mice were then randomized again and distributed into treatment cages. In each experiment all compounds were tested over a complete dose range response from toxic to ineffective levels to enable comparisons at equitoxic levels. Treatment was on the basis of average cage weight. Test compounds were dissolved in 0.9% saline.

Host body weight change data are reported as the difference between mean group weight on the last and first days of treatment for life span assays, or as the maximum treatment related weight loss for growth delay assays. In all tests, an assessment of the probable cause of death of each mouse was made at necropsy. The median day of death, %T/C (leukemia models), T − C (solid tumor models), and net log cell kill were calculated as described previously.[21-23] Net kill provides a measure of tumor response over the duration of therapy. A positive net kill indicates that the tumor burden at the end of therapy was less than at the beginning of therapy. A negative net log cell kill indicates that the tumor grew during treatment. In growth delay assays, mice were considered to be cured if tumor-free for a period of time sufficient for a single surviving tumor cell to grow to a mass of 0.5 g (approximately 65 days in these studies). Likewise, in life span assays, mice surviving 60 days following treatment were considered cured. Data for cured mice were not included in the calculation of %T/C, T − C, and net logs of tumor cell kill.

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Supplementary Material Available: A complete structure determination summary for **5b** is found in Table S1, fractional atomic coordinates are listed in Table S2, and anisotropic thermal parameters and hydrogen atom fractional coordinates are provided as Table S3 (6 pages). Ordering information is given on any current masthead page.

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