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Platinum(II) complexes catalyze reactions between platinum(IV) complexes and 9-methylxanthine‡

Rosette M. Roat,*,†,[a] Maria J. Jerardi,[a] Catherine B. Kopay,[a] Danica C. Heath,[a] Jessica A. Clark,[a] Jessa A. DeMars,[a] John M. Weaver,[a] Ernst Bezemer[b] and Jan Reedijk[b]

[a] *Chemistry Department, Washington College, 300 Washington Ave., Chestertown, MD 21620-1197, USA*
[b] *Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, PO Box 9502, 2300 RA Leiden, the Netherlands*

Reactions between 9-methylxanthine (9-mxan) and platinum(IV) complexes having ammine, organic amine and chloro ligands are speeded up dramatically by additions of small amounts of the analogous platinum(II) complex. Complexes studied include *cis*-dichlorodiammineplatinum(II) {*cis*-$[Pt(NH_3)_2Cl_2]$ **1a**}, *cis*-tetrachlorodiammineplatinum(IV) {*cis*-$[Pt(NH_3)_2Cl_4]$ **1b**}, *cis*-dichloroammine(cyclohexylamine)platinum(II) {*cis*-$[Pt(NH_3)(cha)Cl_2]$ **2a**}, *cis*-tetrachloroammine(cyclohexylamine)platinum(IV) {*cis*-$[Pt(NH_3)(cha)Cl_4]$ **2b**}, chloro(diethylenetriamine)-platinum(II) chloride {[Pt(dien)Cl]Cl **3a**} and *mer*-trichloro(diethylenetriamine)platinum(IV) chloride {*mer*-$[Pt(dien)Cl_3]$Cl **3b**}. For a 10:90 molar ratio of **1a**:**1b**, the half-life for platinum(IV)–9-mxan product formation is approximately 3 h whereas $t_{\frac{1}{2}}$ for pure **1b**–9-mxan product formation is greater than 90 h, indicating a 30 times rate enhancement upon addition of 10% of the platinum(II) complex. For the **2a**:**2b** pair, an eight-fold rate enhancement is observed over that for pure **2b**–9-mxan. This behavior may be related to the chloride bridging mechanism first elucidated for platinum-(II) and -(IV) complexes. Since known platinum-(II) and -(IV) antitumor drugs target nucleobases of DNA to effect cell death in malignant cells, the reactions discussed may shed light on platinum antitumor compound reaction mechanisms.

The compounds *cis*- and *trans*-dichlorodiammineplatinum(II) [see Fig. 1(*a*)] were known to chemists even before the geometric characterization work of Alfred Werner in the nineteenth century.[1] However, in the mid 1960's Rosenberg *et al.*[2a] serendipitously discovered that *cis*-dichlorodiammineplatinum(II) indicated variously by *cis*-$[Pt(NH_3)_2Cl_2]$, cisDDP, or cisplatin, exhibited antitumor activity.[2b] Since then, many other platinum complexes have been investigated for activity and it is now known that platinum(IV) compounds,[3] platinum(II) compounds of *trans* geometry,[4] and dinuclear platinum(II) complexes[5] show antitumor activity.

The solution chemistry of cisplatin has been studied extensively[6] as has its reaction kinetics with nucleobases,[7] as well as single- and double-stranded oligonucleotides.[8] Sadler and co-workers[9] have studied reactions of the anticancer drug $[Pt(NH_3)_2$(CBDCA-*O*,*O*′)], carboplatin, with guanosine-5′ monophosphate (5′-GMP) using ^{1}H, ^{15}N and ^{31}P NMR spectroscopy. Non-antitumor active model compounds such as [Pt(dien)Cl]Cl[10] and *mer*-$[Pt(dien)Cl_3]$Cl have been studied in reactions with 5′-GMP, and other nucleobases.[11]

Renewed interest in the reaction kinetics of platinum complexes having ammine, organic amine and chloro ligands[12] has arisen because of discoveries in platinum compound antitumor activity, although the seminal kinetic work was completed by Pearson and co-workers in the 1950s and 1960s.[13–15] These researchers found that platinum(II) complexes such as cisDDP catalyzed the exchange of chloride ligand with their platinum(IV) analogs such as *cis*-tetrachlorodiammineplatinum(IV), through a 'chloride bridging' mechanism following the third-order rate law [equation (1)].[14] Platinum(IV)–

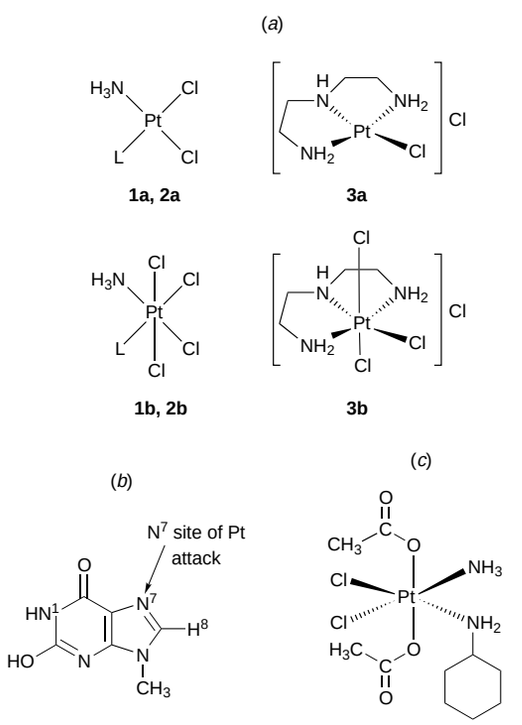

Fig. 1 (*a*) Platinum(II) and platinum(IV) complexes: **1a**, L = NH_3; **2a**, L = cyclohexylamine; **3a**, [Pt(dien)Cl]Cl; **1b**, L = NH_3; **2b**, L = cyclohexylamine; **3b**, *mer*-$[Pt(dien)Cl_3]$Cl. (*b*) Site of attack of platinum complexes on nucleobase 9-methylxanthine. (*c*) *cis*,*cis*,*trans*-Dichloroammine(cyclohexylamine)bis(acetato)platinum(IV), JM216

$$v = k[Pt^{II}][Pt^{IV}][Cl^-] \quad (1)$$

platinum(II) systems having platinum complexes with organic amine ligands such as ethylenediamine (en) were found to behave similarly.[14] For systems in which a bulky ligand such as

† E-Mail: rosette.roat@washcoll.edu

‡ Abbreviations used: cisDDP or cisplatin, *cis*-dichlorodiammineplatinum(II); CBDCA-*O*,*O*′, cyclobutane-1,1-dicarboxylato ligand; dien, diethylenetriamine; 5′-GMP, guanosine-5′ monophosphate, disodium salt; cha, cyclohexanamine, cyclohexylamine; 9-mxan, 9-methylxanthine.

Table 1 Proton resonances for *cis*-$[Pt(NH_3)_2Cl_2]$ **1a**, *cis*-$[Pt(NH_3)_2Cl_4]$ **1b** and 10% **1a**:90% **1b** mixture with 9-methylxanthine

Compound	*T*/°C	*t*/h	δH^8 of Pt^{II} product (ppm)*	δH^8 of Pt^{IV} product (ppm) [J/Hz]
1a	28	3	8.18	—
	50	3	8.11	—
1b	28	48	n.o.	n.o.
		96	n.o.	8.61 [16]
	50	48	n.o.	8.55
		72	n.o.	8.55
10% **1a**:90% **1b**	28	3	n.o.	8.63 [18]
		6	8.18	8.54 [18]
	50	3	8.11	8.54 [18]
		6	8.18	8.54 [18]

* n.o. = not observed.

N,*N*,*N*′,*N*′-tetramethylenediamine (tet) in $[Pt(tet)]^{2+}$ and $[Pt(tet)Cl_2]^{2+}$ were used, chloride exchange was not seen (bridging did not occur due to steric hindrance), however slower reduction to $[Pt(tet)]^{2+}$ with release of chloride ion was observed *via* an unknown mechanism.[15] In the 1970's, Poë and Vaughan[16] reported that the reaction of *trans*-$[Pt(en)(tet)X_2]^{2+}$ (X = Cl or Br) in the presence of iodide ion was independent of $[Pt(en)(tet)]^{2+}$ and proposed that direct attack of I^- on co-ordinated Cl or Br produced ClI or BrI and additional $[Pt(en)(tet)]^{2+}$.

We observe that a variation on the 'chloride bridging' mechanism appears to operate in reactions of platinum compounds with nucleobases and we believe that our results may be relevant to understanding the biological activity of platinum(IV) complexes now undergoing drug trials. The platinum(IV) complex *cis*, *cis*, *trans*-dichloroammine(cyclohexylamine)bis(acetato)platinum(IV), JM216 [Fig. 1(*c*)], now being clinically evaluated as an orally delivered platinum anticancer agent, probably is reduced to its platinum(II) analog before becoming active in a similar manner to that of cisDDP. However both platinum(IV) and platinum(II) metabolites of the drug are found after administration.[17] Studies are ongoing to understand the mechanism of this compound's antitumor activity.[18] The effect of excess chloride ion on the 'chloride bridging' mechanism should be to accelerate the rate of reaction if third-order kinetics are obeyed. The situation is more complex for reactions with nucleobases as it is believed that hydrolysis of chloride ligands is a necessary slow step that takes place before replacement of aqua ligands by the nitrogens of nucleobases.[19]

In the present work, catalysis is seen for the following platinum(II)–platinum(IV) pairs [shown in Fig. 1(*a*)] in their reactions with 9-mxan at pH below 7: *cis*-$[Pt(NH_3)_2Cl_2]$ **1a** and *cis*-$[Pt(NH_3)_2Cl_4]$ **1b**, *cis*-$[Pt(NH_3)(cha)Cl_2]$ **2a** and *cis*-$[Pt(NH_3)(cha)Cl_4]$ **2b**, [Pt(dien)Cl]Cl **3a** and *mer*-$[Pt(dien)Cl_3]Cl$ **3b**, but has not been observed in our experiments for the pair $[Pt(NH_3)_4]Cl_2$ **4a** and [*trans*-$Pt(NH_3)_4Cl_2]Cl_2$ **4b** although catalysis was seen in this system in earlier studies under basic conditions.[20]

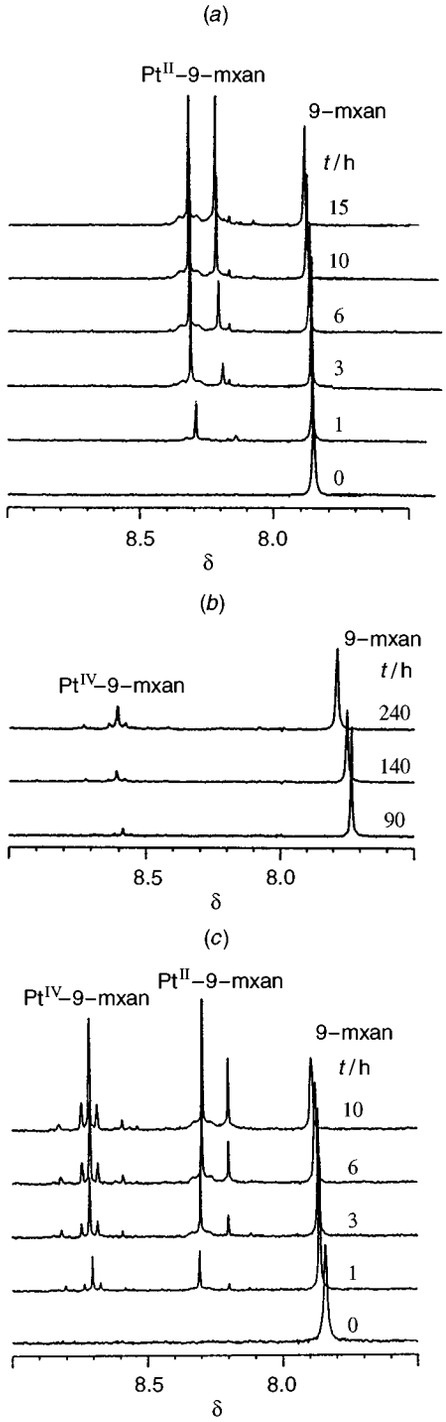

Fig. 2 9-Methylxanthine H^8 proton resonances for (*a*) *cis*-$[Pt(NH_3)_2Cl_2]$ **1a** products, (*b*) *cis*-$[Pt(NH_3)_2Cl_4]$ products **1b**, (*c*) 10% **1a**:90% **1b** products. All reactions contain excess 9-methylxanthine (marked 9-mxan)

Results

Proton NMR provides an excellent detection method for the expected co-ordination of platinum complexes at the N^7 position of nucleobases since the electron-rich platinum metal center causes downfield movement of the nucleobase H^8 proton. This study uses 9-methylxanthine, 9-mxan, [see Fig. 1(*b*)] as the model nucleobase. Platinum(II) complexation causes nucleobase H^8 downfield shifts of up to 0.5 ppm whereas platinum(IV) complexation causes greater shifts of up to 0.8 ppm (see Tables 1–3, Fig. 2).

Additionally and very characteristically, platinum(IV) metal center co-ordination to the nucleobase N^7 position can be detected by three-bond coupling constants [$^3J(^{195}Pt–^1H)$] of 14–20 Hz [see Tables 1–3, Fig. 2(*b*) and (*c*)]. In cases where a very small amount of platinum(IV) product is formed, these satellites may not be detectable [Fig. 2(*c*)]. In contrast, platinum(II)–nucleobase adducts do not usually exhibit coupling in high-field NMR spectra because of a field-dependent chemical shift anisotropy relaxation effect, an effect more pronounced in square-planar platinum(II) complexes.[21] If observed, the platinum(II)–nucleobase three-bond coupling values would be larger and these are observed in Fig. 2(*a*) as shoulders on the platinum(II)–nucleobase resonances.

cis-$[Pt(NH_3)_2Cl_2]$ 1a and *cis*-$[Pt(NH_3)_2Cl_4]$ 1b pair

As can be seen in Table 1, cisplatin reacts much more quickly

(products detected within 3 h at 28 °C) than its kinetically inert platinum(IV) tetrachloro analog (products detected only after 96 h at 28 °C). However, and most surprisingly, for the 10% *cis*-$[Pt(NH_3)_2Cl_2]$:90% *cis*-$[Pt(NH_3)_2Cl_4]$–nucleobase reaction, platinum(IV) products are formed in dramatically shorter times (3 h at 28 °C) than for platinum(IV)–nucleobase mixtures with no added platinum(II) complex (96 h at 28 °C). This is also true for the platinum(II) catalyzed reaction at 50 °C, *i.e.* platinum(IV)–nucleobase products are detected within 3 h, this being the time of the first look for product formation. Acceleration of the reaction rate for the platinum(IV)–nucleobase pair might be expected if a type of 'chloride-bridging' catalysis mechanism[14] was operational in this system (see Discussion).

Additional data on reactions of the *cis*-$[Pt(NH_3)_2Cl_2]$ **1a** and *cis*-$[Pt(NH_3)_2Cl_4]$ **1b** pair with nucleobase 9-mxan have been gathered at 37 °C under reaction conditions described in the Experimental section. Spectra displayed in Fig. 2 show similar behavior to those observed at other temperatures, *i.e.* platinum(II) complexes catalyze the reaction of the platinum(IV) complex with 9-mxan. Owing to differences in following the progress of the reactions from those experiments conducted at 28 and 50 °C (see Experimental section), we observe: (1) the first platinum(II)–nucleobase product forms within 1 h for both platinum(II)–nucleobase and 10% platinum(II):90% platinum(IV)–nucleobase reactions [Fig. 2(*a*) and 2(*c*)] and (2) the first platinum(IV)–nucleobase products are seen at 90 h for platinum(IV)–nucleobase [Fig. 2(*b*)] whereas the first platinum(IV)–nucleobase products appear at 1 h in the platinum(II) catalyzed reaction [Fig. 2(*c*)].

In Fig. 2(*a*), two major platinum(II) products are seen corresponding to substitution of one or both chlorides by nucleobase. For the downfield platinum(II)–nucleobase product, ^{195}Pt–^{1}H satellites are just detectable. In Fig. 2(*c*), the same platinum(II)–nucleobase resonances are noted as well as one major platinum(IV)–nucleobase product, presumably having one chloride substituted by 9-mxan *trans* to a better *trans* directing chloride ligand. Minor platinum(IV)–nucleobase products may have two nucleobases replacing chlorides, these are expected to remain minor due to steric hindrance inherent in adding two nucleobase entities to the platinum(IV) coordination sphere. In all parts of Fig. 2, one notes the downfield movement of the 9-mxan H^8 proton of the uncomplexed nucleobase with time. This behavior is noted for all nucleobases as pH decreases, protonation of the N^7 proton of the nucleobase deshields the H^8 proton. This effect is not noted for platinum-complexed 9-mxan, as now N^7 cannot be protonated. The downfield movement of the 9-mxan H^8 resonance corresponds to a decrease from pH 5.0–5.5 to pH 2.5–3.0 that is observed for all platinum complex–9-mxan reactions studied here. Where observed, reaction of platinum complex with nucleobase begins only after the reaction mixture reaches pH 4.0 or below.

Integration of the H^8 peaks for the products (referenced to the integration of the 9-mxan methyl peak) indicates the following [see Fig. 3(*a*)]: formation of platinum(II)–nucleobase product increases initially then levels off [**1a.1** (H^8 δ = 8.20), **1a.2** (H^8 δ = 8.08)]; formation of platinum(IV)–nucleobase product does not take place over the 15 h for which data are shown. For reaction mixtures of 10% platinum(II) **1a**:90% platinum(IV) **1b** with nucleobase, platinum(II)–nucleobase and platinum(IV)–nucleobase products level off after approximately 10 h. The most striking feature relates to the one to one platinum(II):platinum(IV)–nucleobase product ratio observed when reactants were mixed in a 10% platinum(II):90% platinum(IV) ratio (see Discussion).

Table 2 The H^8 proton resonances for *cis*-$[Pt(NH_3)(cha)Cl_2]$ **2a**, *cis*-$[Pt(NH_3)(cha)Cl_4]$ **2b** and for 10% **2a**:90% **2b** reaction mixtures with 9-methylxanthine

Compound	*T*/°C	*t*/h	δH^8 of Pt^{II} product (ppm)[a]	δH^8 of Pt^{IV} product (ppm) [*J*/Hz]
2a	28	6	8.01	—
		12	8.04	—
		24	7.91, 8.00, 8.05	—
		96	7.94, 8.04, 8.06	—
	50	24	7.95, 8.07	—
2b	28	24	n.o.	n.o.
		48	n.o.	8.58
		72	n.o.	8.59 [20]
	50	24	8.09[b]	n.o.
		72	8.09[b]	8.60, 8.77
10% **2a**:90% **2b**	28	6	n.o.	8.59, 8.72
		12	n.o.	8.59 [18], 8.75
	50	24	7.97, 8.08	8.59, 8.75

[a] n.o. = not observed. [b] Platinum(II) product observed in the reaction of a platinum(IV) complex (see text).

cis-$[Pt(NH_3)(cha)Cl_2]$ 2a and *cis*-$[Pt(NH_3)(cha)Cl_4]$ 2b pair

The *cis*-$[Pt(NH_3)(cha)Cl_2]$–*cis*-$[Pt(NH_3)(cha)Cl_4]$ pair [see Fig. 1(*a*) and Table 2] was chosen to compare its reactivity to the *cis*-$[Pt(NH_3)_2Cl_2]$–*cis*-$[Pt(NH_3)_2Cl_4]$ systems and also because of the success of the first orally active platinum(IV)

Table 3 Proton resonances for [Pt(dien)Cl]Cl **3a**, *mer*-$[Pt(dien)Cl_3]Cl$ **3b** and for a 10% **3a**:90% **3b** mixture with 9-methylxanthine

Compound	*T*/°C	*t*/h	δH^8 of Pt^{II} product (ppm)[a]	δH^8 of Pt^{IV} product (ppm) [*J*/Hz]
3a	28	6	8.13	—
		72	8.13	—
	50	3	8.17	—
		68	8.17	—
3b	28	72	n.o.	n.o.
		222	n.o.	8.37
	50	24	8.16[b]	n.o.[1]
		48	8.17[b]	8.30
		72	8.09[b]	8.30 [19]
10% **3a**:90% **3b**	28	6	8.12	n.o.
		24	8.12	8.52
		48	8.12	8.40, 8.53 [16]
		96	8.11	8.39 [14], 8.51 [16]
	50	2.5	8.17	n.o.
		12	8.17	8.30
		48	8.16	8.30, 8.44 [18]
		60	8.16	8.45 [18]

[a] n.o. = not observed. [b] Platinum(II) product observed in the reaction of a platinum(IV) complex (see text).

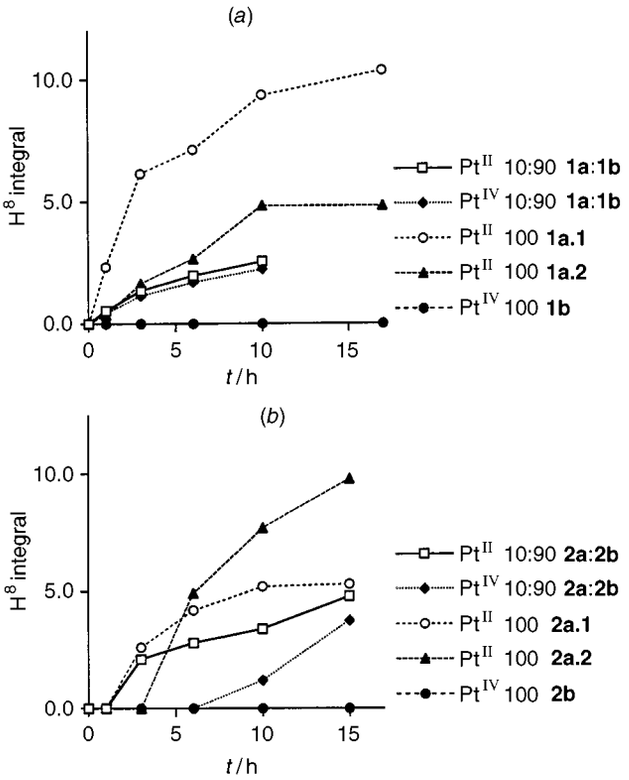

Fig. 3 9-Methylxanthine H^8 proton integrals for (*a*) **1a**, **1b**, and 10% **1a**:90% **1b** mixtures; (*b*) **2a**, **2b**, and 10% **2a**:90% **2b** mixtures

complex *cis*, *cis*, *trans*-dichloroammine(cyclohexylamine)bis(acetato)platinum(IV), JM216 [see Fig. 1(*c*)] now being clinically evaluated as an antitumor agent. For platinum(II) complexes, *cis*-$[Pt(NH_3)(cha)Cl_2]$ reacts with 9-mxan more slowly than *cis*-$[Pt(NH_3)_2Cl_2]$, *i.e.* products are detectable by NMR spectroscopy within 6 h rather than 3 h at 28 °C (see Tables 1 and 2). This effect may be one of water solubility, *cis*-$[Pt(NH_3)(cha)Cl_2]$ being less soluble than *cis*-$[Pt(NH_3)_2Cl_2]$. As it is possible to substitute nucleobase for chlorides at positions which are either *cis* or *trans* to ammine or cyclohexylamine ligands, two products with slightly different H^8 proton resonances are detected in *cis*-$[Pt(NH_3)(cha)Cl_2]$–nucleobase reactions (Table 2). The third product formed results from substitution of both chloride ligands. For the *cis*-$[Pt(NH_3)(cha)Cl_4]$–nucleobase reactions, small amounts of the major product appear within 48 h at 28 °C. This product, which forms twice as quickly as the *cis*-$[Pt(NH_3)_2Cl_4]$–nucleobase product, is expected to have the nucleobase attached *trans* to the better *trans* directing chloride ligand. Another platinum(IV)–nucleobase resonance is observed at δ 8.77, possibly having nucleobase *trans* to ammine or to cyclohexylamine (see Table 2). It should be noted that in reactions at 50 °C, some platinum(II)–nucleobase product is observed although no platinum(II) reactant or reducing agent is added. Reasons for this behavior include platinum(II) impurities resulting from the platinum(IV) compound preparation and purification procedures, or oxidation of the nucleobase, a possibility under active investigation at this time. Since the same analytically pure platinum(IV) compound does not show formation of platinum(II)–nucleobase products when reactions are conducted at lower temperatures, we believe that trace amounts of impurities acting as reducing agents may cause this behavior at 50 °C. For 10% *cis*-$[Pt(NH_3)(cha)Cl_2]$:90% *cis*-$[Pt(NH_3)(cha)Cl_4]$, acceleration of the reaction rate is seen with a behavior similar to that of the 10% *cis*-$[Pt(NH_3)_2Cl_2]$:90% *cis*-$[Pt(NH_3)_2Cl_4]$ couple. Platinum(IV) products are observed at 6 h at 28 °C for the catalyzed reaction whereas platinum(IV)–nucleobase reaction mixtures require 48 h at 28 °C to show the first product.

For reactions at 37 °C, integration of the H^8 peaks for the products (referenced to integration of the 9-mxan methyl peak) indicates the following [Fig. 3(*b*)]: formation of platinum(II)–nucleobase product **2a.2** (H^8 δ = 7.97) increases up to 15 h whereas platinum(II)–nucleobase product **2a.1** (H^8 δ = 8.05) levels off at approximately 10 h; formation of platinum(IV)–nucleobase product does not take place over the 15 h for which data are shown; three times the amount of platinum(II)–nucleobase over platinum(IV)–nucleobase products are observed at 10 h even though the ratio of starting materials is again 10% *cis*-$[Pt(NH_3)(cha)Cl_2]$:90% *cis*-$[Pt(NH_3)(cha)Cl_4]$ (see Discussion).

[Pt(dien)Cl]Cl 3a and *mer*-[Pt(dien)Cl_3]Cl 3b pair

Finally, the [Pt(dien)Cl]Cl–*mer*-[Pt(dien)Cl_3]Cl pair was included, as many studies have been carried out on reactions between nucleobases and this platinum(II) complex having one leaving group. These serve as a model for the first hydrolysis step of the platinum complex followed by DNA nucleobase attachment believed to operate for all platinum-containing antitumor agents.[11,12] Again as expected, the platinum(II) complex reacts much faster (products detected with 6 h at 28 °C) than the platinum(IV) complex (222 h at 28 °C). Also note that platinum(II)–nucleobase product is seen in 50 °C reactions in the absence of any added platinum(II) reactant or added reducing agent. For the 10% [Pt(dien)Cl]Cl:90% *mer*-[Pt(dien)Cl_3]Cl–nucleobase reactions, it was found that platinum(IV) products are seen much more quickly (products detected in 12 h at 50 °C) than for the platinum(IV)–nucleobase mixtures with no added platinum(II) complex (48 h at 50 °C) (see Table 3).

Other reactions

If proportions of platinum complexes are reversed; *i.e.* 90% platinum(II):10% platinum(IV), platinum(II)–nucleobase complexes form at expected times, however platinum(IV)–nucleobase products are not detectable. Other platinum(II)–platinum(IV) proportions are now under study to quantitate this catalysis effect.

For the systems $[Pt(NH_3)_5Cl]Cl_3$–, $[Pt(NH_3)_4]Cl_2$–, *trans*-$[Pt(NH_3)_4Cl_2]Cl_2$– and 10% $[Pt(NH_3)_4]Cl_2$:90% *trans*-$[Pt(NH_3)_4Cl_2]Cl_2$–nucleobase no products were detected even after prolonged reaction (>150 h at 50 °C). The finding for the 10% $[Pt(NH_3)_4]Cl_2$:90% *trans*-$[Pt(NH_3)_4Cl_2]Cl_2$ system seems to contradict the results of earlier work with platinum-(II) and -(IV) tetrammine complexes.[22] However the catalytic reaction reported earlier took place in basic solution whereas our reactions all take place under acidic conditions.

Discussion

Dramatic acceleration in rates of reaction for platinum(IV) complexes with nucleobases are seen upon the addition of small amounts (10% or less) of platinum(II) complexes. Platinum complexes used in this study were selected to compare the reactivity of a known platinum(II) antitumor agent, *i.e.* cisplatin **1a**, and a derivative compound related to a known antitumor agent, JM216 [see Fig. 1(*c*)], *i.e.* *cis*-$[Pt(NH_3)(cha)Cl_4]$ **2b** with other non-antitumor active platinum complexes having fewer leaving ligands, *i.e.* [Pt(dien)Cl]Cl **3a** and *mer*-[Pt(dien)Cl_3]Cl **3b**. The *cis*-$[Pt(NH_3)_2Cl_2]$ **1a**–*cis*-$[Pt(NH_3)_2Cl_4]$ **1b** couple was chosen as the control species since the platinum(II) compound (cisplatin) is currently the clinically most used antitumor agent.[8]

Half-life values for *cis*-$[Pt(NH_3)(cha)Cl_2]$ **2a**, *cis*-$[Pt(NH_3)_2Cl_2]$ **1a**, *cis*-$[Pt(NH_3)(cha)Cl_4]$ **2b**, *cis*-$[Pt(NH_3)_2Cl_4]$ **1b** and 10:90 platinum(II):platinum(IV) mixtures treated with 9-mxan at 37 °C are reported in Table 4. For the platinum(II) compounds, *cis*-$[Pt(NH_3)(cha)Cl_2]$ **2a** nucleobase products form more slowly ($t_{\frac{1}{2}}$ = 3 and 6.5 h) than for *cis*-$[Pt(NH_3)_2Cl_2]$ **1a** ($t_{\frac{1}{2}}$ = 2.5 and 4 h), and this is true for the 10:90 platinum(II):platinum(IV)

Table 4 Values for platinum compound reactions with 9-methylxanthine ($t_{\frac{1}{2}}$/h at 37 °C)

	$t_{\frac{1}{2}}$/h	
Compound	Pt^{II} product	Pt^{IV} product
1a[a]	2.5	
1a[b]	4	
1b		>90
10% **1a**:90% **1b**	3	3
2a[c]	3	
2a[d]	6.5	
2b		>48
10% **2a**:90% **2b**	6	11

[a] 9-mxan $H^8 = \delta$ 8.20. [b] 9-mxan $H^8 = \delta$ 8.08. [c] 9-mxan $H^8 = \delta$ 8.05. [d] 9-mxan $H^8 = \delta$ 7.97.

mixtures also. These are important conclusions for the pharmokinetic activity of cisplatin and JM216 since it is believed that the hydrolyzed platinum(II) species for either drug potentiates cytotoxic activity by crosslinking DNA.[18] For platinum(II)–nucleobase reactions studied at 28 and 50 °C, half-life values have not been calculated as yet. However, trends appear to be *cis*-$[Pt(NH_3)_2Cl_2]$ **1a** > *cis*-$[Pt(NH_3)(cha)Cl_2]$ **2a** ≅ [Pt(dien)Cl]Cl **3a**; whereas for platinum(II) catalyzed platinum(IV)–nucleobase reactions 10% *cis*-$[Pt(NH_3)_2Cl_2]$ **1a**:90% *cis*-$[Pt(NH_3)_2Cl_4]$ **1b** > 10% *cis*-$[Pt(NH_3)(cha)Cl_2]$ **2a**:90% *cis*-$[Pt(NH_3)(cha)Cl_4]$ **2b** ≅ 10% [Pt(dien)Cl]Cl **3a**:90% *mer*-$[Pt(dien)Cl_3]$Cl **3b** (Tables 1–3). For platinum(IV)–nucleobase reactions the trend in speed of reaction appears to be *cis*-$[Pt(NH_3)(cha)Cl_4]$ **2b** > *cis*-$[Pt(NH_3)_2Cl_4]$ **1b** > *mer*-[Pt(dien)Cl_3]Cl **3b** (see Tables 1–3). It is noted in this case and in other experiments (data not shown) that the cyclohexylamine substituted platinum(IV) compound **2b** reacts faster with nucleobase than **1b**, in contrast to behavior with platinum(II) complexes **1a**, **2a** or platinum(II) catalyzed platinum(IV)–nucleobase reactions. Also comparison of the reactivity of JM216 and **2b** have shown recently (data to be reported subsequently) that JM216 does not react with nucleobase within 48 h under identical conditions. Explanations for this behavior are unknown at this time as the acetato ligand is considered to be a better leaving group than the chloro species.

Further work at this time is concentrating on kinetic studies of platinum(II) and platinum(IV) complexes **1a**, **1b**, **2a**, **2b** and JM216 in reactions with the nucleobase 9-methylxanthine as followed by NMR spectroscopy. Additionally other ratios of platinum(II):platinum(IV) are being tested for catalytic effects. We hope to show that a variation on the 'chloride bridging' mechanism facilitates reaction of platinum(IV) complexes with nucleobases. For instance it is possible to explain our observations if one postulates that nucleobase substitution of a chloro ligand precedes or is concurrent with the formation of the chloride bridge.

Fig. 4 schematically indicates some possibilities for platinum(IV) activation and product formation. Explanations for the observed behavior include: (1) attack by nucleobase may be concurrent with the formation of platinum(II)–platinum(IV) chloride bridges; (2) platinum(IV) complexes are not necessarily converted into platinum(II) complexes but are instead activated for reaction with nucleobase; (3) once formed platinum(II) complexes react with nucleobase at faster rates, explaining the increasing concentration of platinum(II)–nucleobase products over the starting material ratio in 10% platinum(II):90% platinum(IV)–nucleobase reactions; (4) platinum(II)–nucleobase products may also participate in chloride bridging activating other platinum(IV) complexes for substitution.

A study of the effect of chloride ion concentration *vs.* rate of reaction is necessary and planned for the future. In the past it has been found that the addition of chloride ion depresses the reaction rate of platinum(II) complexes proving the hypothesis

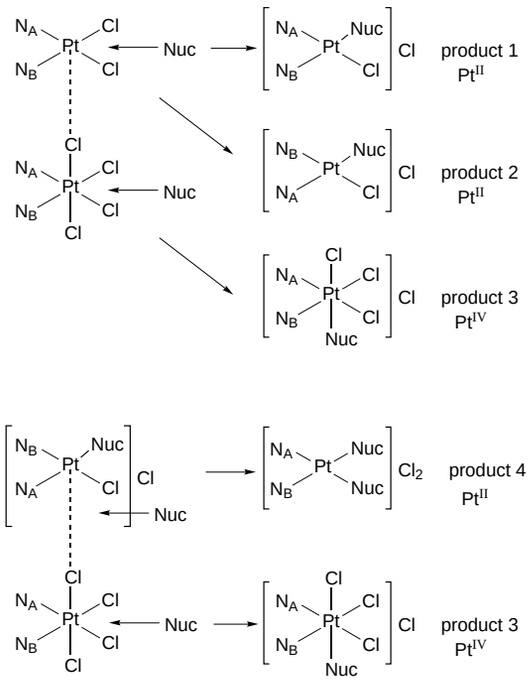

Fig. 4 Proposed products for **2a** catalysis of **2b** in reactions with nucleobases *via* the 'chloride bridging' mechanism. Nuc = 9-methylxanthine; N_A = ammine; N_B = cyclohexylamine

that chloro ligand replacement by aqua ligands precedes substitution by nucleobase.[19]

Conclusion

Platinum(II) complexes catalyze the reactions of their platinum(IV) analogs with the nucleobase 9-methylxanthine through a variant of the 'chloride bridging' mechanism often used to explain the behavior of similar pairs of co-ordination complexes. This behavior has been observed for: (1) complexes having two or four chloro ligands in *cis* conformation and ammine or organic amine ligands; (2) one or three chloro ligands and the chelating amine ligand diethylenetriamine.

For platinum(II) and platinum(IV) complexes, bulkiness of the organic amine ligand decreases the rate of reaction of the platinum complexes with 9-methylxanthine except in the case of substitution of the cyclohexylamine ligand (compound **2b**) for NH_3 (compound **1b**) in platinum(IV) complexes. Both platinum(II) and platinum(IV) species complex with 9-methylxanthine in an identical manner to that for antitumor-active platinum co-ordination complexes reacting with the nucleobases of DNA *in vitro* and *in vivo*. In continuing work, the proposed variation on the 'chloride bridging' mechanism is being applied to the behavior of JM216 [Fig. 1(*c*)] since this platinum(IV) antitumor-active compound is believed to be reduced to its platinum(II) analog through loss of the acetato ligands before drug activity is potentiated.

Experimental

Chemicals

The nucleobase 9-methylxanthine (9-mxan) was obtained from Professor Pfleiderer at Konstanz University and used without further purification. The salt K_2PtCl_4 was obtained from Aldrich Chemicals or as a donation from Colonial Metals, Inc. and used without further purification. The salt $[Pt(NH_3)_4]Cl_2$ **4a** was donated by Colonial Metals, Inc. *cis*-Dichlorodiammineplatinum(II) **1a** was obtained from Aldrich Chemicals and used without further purification. Prepared compounds have satisfactory elemental analyses when compared to calculated and

their spectral IR and NMR behavior agreed with published data.

Preparation of *cis*-[Pt(NH$_3$)$_2$Cl$_4$] 1b

In a round-bottom flask fitted with a water condenser, **1a** (0.5066 g, 1.7 mmol) was dissolved in 2 M HCl (50 cm^3) at room temperature. The solution was filtered to remove undissolved solid. Chlorine gas was bubbled into this solution for 6 min (yellow solid formed in the solution after 5 min), followed by 30 min stirring, followed by 10 min of chlorine gas addition, finally by 30 min stirring. After overnight refrigeration of the solution/solid, 0.5536 g, 88%, of bright yellow solid was collected (Found: H, 1.6; N, 7.6; Cl, 38.2. Calc. for $Cl_4H_6N_2Pt$: H, 1.55; N, 7.6; Cl, 38.5%).

Preparation of *cis*-[Pt(NH$_3$)(cha)Cl$_2$] 2a and *cis*-[Pt(NH$_3$)(cha)Cl$_4$] 2b

The compound *cis*-[Pt(NH$_3$)(cha)Cl$_2$] **2a** was prepared by literature methods.[23]

Method 1 for *cis*-[Pt(NH$_3$)(cha)Cl$_4$]. Compound **2a** (0.116 g, 0.30 mmol) was dissolved in 2 M HCl (200 cm^3) held at 70 °C. After filtration, chlorine gas was bubbled into this solution for six min as the solution became darker yellow in color. The solution was allowed to cool with stirring, then evaporated slowly to 20 cm^3 with heat. After cooling, 0.052 g, 33% of bright yellow solid, **2b** was isolated. NMR [300 MHz, DCON(CD$_3$)$_2$, standard SiMe$_4$]: δ_H 1.10, 1.18, 1.23, 1.31, 1.43, 1.47, 1.56, 1.59, 1.69, 1.72 (8 H, m); 2.35, 2.39 (2 H, d); 3.07 (1 H, m, br); 5.82–6.17 (5 H, spt) (Found: C, 15.8; H, 3.5; Cl, 31.4; N, 6.1. Calc. for $C_6H_{16}Cl_4N_2Pt$: C, 15.9; H, 3.6; Cl, 31.3; N, 6.2%).

Method 2 for *cis*-[Pt(NH$_3$)(cha)Cl$_4$]. The complex *cis*-[Pt(NH$_3$)(cha)Cl$_2$(OH)$_2$][3] (0.2435 g, 0.58 mmol) was dissolved in 1 M HCl (3 cm^3). Within 1 min, a yellow waxy precipitate of **2b** formed. After stirring at room temperature for 6 h, 0.2253 g, 85% yield, of **2b** was removed by filtration, washed with ethanol and diethyl ether, then dried under vacuum.

Preparation of [Pt(dien)Cl]Cl 3a and *mer*-[Pt(dien)Cl$_3$]Cl 3b

The salts [Pt(dien)Cl]Cl **3a** and *mer*-[Pt(dien)Cl$_3$]Cl **3b** were prepared using literature methods.[11] Compound **3b** was also prepared by dissolving **3a** (0.150 g) in dichloromethane (300 cm^3), then bubbling chlorine gas into this solution for 20 min. The yellow precipitate was collected after storage at 40 °C overnight, additional precipitate being collected upon solvent reduction to <15 cm^3.

Preparation of *trans*-[Pt(NH$_3$)$_4$Cl$_2$]Cl$_2$ 4b

After dissolving [Pt(NH$_3$)$_4$]Cl$_2$ (0.500 g, 1.24 mmol) in 2 M HCl (20 cm^3) at 70 °C, 30% H_2O_2 (3.0 cm^3) was added dropwise. The solution was refluxed overnight, then additional 30% H_2O_2 (3 cm^3) was added and reflux continued for 6 h. A light yellow solid was collected and used without further purification.

Platinum complex–nucleobase reaction conditions

Saturated solutions of reactants were prepared by mixing platinum complex (≈10 mg, 0.025 mmol) with 9-methylxanthine (5 mg, 0.025 mmol) in D_2O (0.5 cm^3). In most cases this resulted in incomplete dissolution of the platinum complexes. However, platinum complex slowly dissolves during the reaction. In cases where 9-methylxanthine is more soluble than the platinum compounds, this procedure results in an excess of nucleobase. Only in the case of [Pt(dien)Cl]Cl did the platinum complex completely dissolve. For reactions run at 28 and 50 °C, reaction mixtures were incubated in NMR tubes at the temperature specified and spectra taken at various time intervals. Products are reported at the first time at which they were observed although conceivably product could have been present at an earlier time. The reaction conditions for the set of **1a**, **2a**–9mxan, **1b**, **2b**–9mxan, 10% **1a**:90% **1b**, 10% **2a**:90% **2b**–9mxan experiments at 37 °C is different from the others reported in that the reaction is followeed *in situ* in the thermostatted NMR probe. Reactions were repeated in duplicate or triplicate.

Proton NMR measurements

The NMR measurements were performed using a Bruker 250 MHz spectrometer located in the Blue Hen NMR Complex at University of Delaware, Newark, DE, on a GE 300 MHz spectrometer located at Virginia Commonwealth University, Richmond, VA, and on a Bruker WM 300 spectrometer at Leiden University, the Netherlands.

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