Ring-Opening Reactions of the Anticancer Drug Carboplatin: NMR Characterization of *cis*-[$Pt(NH_3)_2$(CBDCA-*O*)(5′-GMP-*N7*)] in Solution

Urban Frey, John D. Ranford, and Peter J. Sadler*

Department of Chemistry, Birkbeck College, University of London, Gordon House and Christopher Ingold Laboratory, 29 Gordon Square, London WC1H 0PP, U.K.

Received September 25, 1992

We have studied reactions of the anticancer drug [$Pt(NH_3)_2$(CBDCA-*O*,*O*′)] (carboplatin, "Paraplatin", where H_2CBDCA is cyclobutane-1,1-dicarboxylic acid) with nitrate, phosphate, chloride, and 5′-guanosine monophosphate (5′-GMP) in aqueous solution at 310 K using ^{1}H, ^{15}N, [^{1}H, ^{15}N], and ^{31}P NMR spectroscopy. In each case (except nitrate) a ring-opened species containing monodentate CBDCA was detected during the course of the reaction. A structure for *cis*-[$Pt(NH_3)_2$(CBDCA-*O*)(5′-GMP)] is proposed which accounts for the inequivalence of all six cyclobutane ring protons in this complex. There is a close hydrophobic contact between the cyclobutane ring of monodentate CBDCA and the purine ring of coordinated 5′-GMP. The reaction of carboplatin with 5′-GMP (k_{obs} 4.1×10^{-6} s^{-1}) was faster than that with phosphate (k_{obs} 4.3×10^{-7} s^{-1}), phosphate and chloride (k_{obs} 1.2×10^{-6} s^{-1}), or water alone ($<5 \times 10^{-9}$ s^{-1}), suggesting that direct attack of nucleotides on carboplatin may be of importance in the mechanism of action for this drug.

Introduction

Carboplatin, [$Pt(NH_3)_2$(CBDCA-*O*,*O*′)] (**1**)[1], is a widely used second-generation platinum anticancer drug.[2] It has less side-effects than cisplatin; for example, it does not cause loss of high frequency hearing, or significant renal or neural toxicity. These differences are attributable to the lower reactivity of carboplatin due to the presence of the bidentate-chelated bis(carboxylato) ligand.[3] Some workers have suggested that carboplatin is merely a pro-drug for cisplatin,[4] whereas others have postulated that carboplatin is activated by other means, e.g. by enzymic degradation.[5] Urinary excretion studies have shown that $CBDCA^{2-}$ can be displaced from carboplatin in the body,[6] and it has been suggested that aquation is a rate-limiting step in the reaction of carboplatin with DNA.[7]

There are only a few previous studies of ring-opening reactions of carboplatin. The relative inertness of carboplatin to hydrolysis and chloride substitution was demonstrated early on,[8] and Goodgame and Riley detected intermediates by ^{1}H NMR spectroscopy during reactions with nucleotides.[9] Subsequently Knox et al.[7] reported a rate of hydrolysis of 7.2×10^{-7} s^{-1} (in phosphate buffer, 310 K), whereas Canovese et al.[10] suggested a rate of $<10^{-8}$ s^{-1}. The latter workers detected a ring-opened species in acidic media by ^{1}H NMR spectroscopy. Recently Allsopp et al.[11] have studied reactions of carboplatin with a variety of nucleophiles and have concluded that reactions with chloride ions are too slow to account for the reported half-life of carboplatin in blood plasma. The only X-ray structural characterization of a monodentate CBDCA Pt(II) complex appears to be that of [Pt(CHDA)(Me_2SO)(CBDCA-*O*)], where CHDA is *trans*-(−)-1,2-cyclohexanediamine.[12]

In this work we have sought to detect and characterize ring-opened carboplatin adducts in aqueous solution using a combination of ^{1}H and ^{1}H-detected ^{15}N NMR spectroscopy, as well as to investigate the kinetics of ring-opening and CBDCA release. In particular, we show that observation of ^{1}H NMR resonances for the NH_3 ligands via the use of ^{15}N-edited ^{1}H NMR and [^{1}H,^{15}N] HMQC NMR spectroscopy greatly aids the assignment of resonances for ring-opened CBDCA complexes.

Materials and Methods

K_2PtCl_4 and [$Pt(NH_3)_2$(CBDCA-*O*,*O*′)] were purchased from Johnson Matthey plc. and H_2CBDCA, $^{15}NH_4Cl$, 5′-GMP, KNO_3, NaCl, $NaH_2PO_4 \cdot 2H_2O$, and Na_2HPO_4 were all purchased from Aldrich. [$Pt(^{15}NH_3)_2$(CBDCA-*O*,*O*′)] was prepared according to standard methods.[13,14]

NMR Spectroscopy. NMR spectra were recorded at 310 K, unless otherwise stated, on the following instruments: Bruker AM 500 (^{1}H 500 MHz, ^{15}N 50.7 MHz), JEOL GSX 270 (^{31}P 109.3 MHz, ^{15}N 27.3 MHz), JEOL GSX 500 (^{1}H 500 MHz, ^{15}N 50.7 MHz), and Varian VXR 600 (^{1}H 600 MHz) using 5-mm NMR tubes. The chemical shift references were as follows: ^{1}H (internal), TSP;[15] ^{31}P (external), 85% H_3PO_4; and ^{15}N (external), 1.5 M $^{15}NH_4Cl$ in 1 M HCl containing 10% D_2O.

(1) Abbreviations used: H_2CBDCA, cyclobutane-1,1-dicarboxylic-acid; cisplatin, *cis*-[$Pt(NH_3)_2Cl_2$]; TSP, $Me_3Si(CD_2)_2CO_2Na$; 5′-GMP, guanosine-5′monophosphate (the charge on this compound is ignored); pH*, pH meter reading in D_2O; DQF, double quantum filtered; en, 1,2-diaminoethane; HMQC, heteronuclear multiple quantum coherence spectroscopy.

(2) *Platinum and Other Metal Complexes in Cancer Chemotherapy*; Nicolini, M., Ed.; Martinus Nijhoff: Boston, MA, 1988. *Platinum and Other Metal Complexes in Cancer Chemotherapy*; Howell, S. B., Ed.; Plenum Press: New York, 1991.
(3) Neidle, S.; Ismail, I. M.; Sadler, P. J. *J. Inorg. Biochem.* **1980**, *13*, 205.
(4) Tinker, N. D.; Sharma, H. L.; McAuliffe, C. A. In *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy*; Nicolini, M., Ed.; Martinus Nijhoff: Boston, MA, 1988; pp 144–159.
(5) (a) Cleare, M. J.; Hoeschele, J. D. *Bioinorg. Chem.* **1973**, *2*, 187–210. (b) Cleare, M. J. *Coord. Chem. Rev.* **1974**, *12*, 349–405.
(6) Ranford, J. D.; Sadler, P. J.; Balmanno, K.; Newell, D. R. *Magn. Reson. Chem.* **1991**, *29*, S125–S129.
(7) Knox, R. J.; Friedlos, F.; Lydall, D. A.; Roberts, J. J. *Cancer Res.* **1986**, *46*, 1972–1979.
(8) (a) Cleare, M. J.; Hydes, P. C.; Malerbi, B. W.; Watkins, D. M.; *Biochimie* **1978**, *60*, 835–850. (b) Cleare, M. J.; Hydes, P. C.; Hepburn, D. R.; Malerbi, B. W. *Cisplatin Current Status and New Developments*; Prestayko, A. W., Crooke, S. T., Carter, S. K., Eds.; Academic Press: New York, 1980, pp 149–170.
(9) (a) Goodgame, D.; Riley, R. T. Presented at Conference on the Chemistry of the Platinum Group Metals, Bristol, U.K., 1981; Poster A14. (b) Riley, R. T. Ph.D. Thesis, University of London, 1985.
(10) Canovese, L.; Cattalini, L.; Chessa, G.; Tobe, M. L. *J. Chem. Soc., Dalton Trans.* **1988**, 2135–2140.
(11) Allsopp, M. A.; Sewell, G. J.; Rowland, C. G.; Riley, C. M.; Schowen, R. L. *Int. J. Pharm.* **1991**, *69*, 197–210.
(12) Bitha, P.; Morton, G. O.; Dunne, T. S.; Delos Sanos, E. F.; Lin, Y.; Boone, S. R.; Haltiwanger, R. C.; Pierpont, C. G. *Inorg. Chem.* **1990**, *29*, 645–652.
(13) Boreham, C. J.; Broomhead, J. A.; Fairlie, D. P. *Aust. J. Chem.* **1981**, *34*, 659–664.
(14) Ismail, I. M. Ph.D. Thesis, University of London, 1982.
(15) In some samples it was evident that coordination of TSP was occurring (two peaks for TSP observed). In those cases peaks of the Pt complex **1** itself were used as a secondary reference.

0020-1669/93/1332-1333$04.00/0

Typical acquisition conditions for ^1H NMR 1D spectra were as follows: 45–60° pulses; 16–32 K data points; 2–3 s relaxation delay; collection of 64–256 transients; final digital resolution of 0.2 Hz/pt. Spectra were simulated using Varian software.[16] A standard program was used to acquire phase-sensitive DQF COSY spectra.

$^{31}P\{^1H\}$ NMR spectra were typically the result of 60–70° pulses, 32K data points, 5-s relaxation delay, and 300 transients with a digital resolution of 0.3 Hz/pt.

$^{15}N\{^1H\}$ INEPT and DEPT NMR spectra were obtained using standard programs. Acquisition conditions were as follows: 32K data points, 2-s relaxation delay, 5000 transients, and digital resolution of 0.3 Hz/pt. Delays of $1/(4J)$ (INEPT) and $1/(2J)$ (DEPT) were used with a $^1J(^1H,^{15}N)$ value of 73 Hz.

The 500-MHz $^1H\{^{15}N\}$ NMR spectra were recorded on a Bruker AM-500 spectrometer fitted with a BSV-7 transmitter, a BFX-5 X nucleus decoupler, and a 5-mm inverse probehead, as described previously.[17] Sample spinning was not used. All samples were prepared in 95% H_2O/5% D_2O, and the water signal was preirradiated for 1.5 s by means of a DANTE sequence.[18] One-dimensional ^{15}N-edited ^1H spectra were recorded using a spin–echo difference sequence,[19] optimized for $^1J(^1H,^{15}N)$ = 73 Hz ($\tau = 1/(2J)$).

Two dimensional $[^1H,^{15}N]$ HMQC spectra were recorded using the standard sequence,[20] modified to include a pair of purge pulses for improved suppression of signals from protons not bound to ^{15}N, as proposed by Otting and Wüthrich.[21] The sequence was optimized for $^1J(^1H,^{15}N)$ = 73 Hz, and the lengths of the purge pulses were adjusted for each sample in the range 0–3 ms to maximize solvent suppression, with the two pulses of different duration to avoid refocusing effects. Typically 32–128 t_1 increments were used over a total spectral width of 800–2400 Hz in the F_1 dimension. The spectral width in the F_2 dimension was 2000 Hz, and for each increment, 2 dummy scans and 8–128 scans were recorded. After zero filling, the final digital resolution was 6 and 1.9 Hz in the F_1 and F_2 dimensions respectively. All 2D spectra were acquired using the TPPI method[22] to give absorption mode line-shapes with sign discrimination in F_1. During the acquisition time of the 2D experiments, the ^{15}N spins were decoupled by irradiating with the GARP-1 sequence[23] at a field strength of 1.7 kHz. 2D spectra were processed using sine-bell squared weightings in both dimensions.

Sample Preparation. NMR samples were made up in D_2O solutions, except for $^{15}N\{^1H\}$ INEPT and DEPT, ^{15}N-edited ^1H and $[^1H,^{15}N]$ HMQC spectra for which 95% H_2O/5% D_2O was used. A typical sample preparation was as follows. Complex **1** (4.5 mg, 12 μmol), NaCl (4.9 mg, 84 μmol), and 5′-GMP (11.5 mg, 25 μmol) were weighed directly into an NMR tube and 0.6 ml of 0.1 M D_2O phosphate buffer, pH* = 7.0, was added to give a final concentration of 20 mM **1**, 140 mM Cl^-, and 40 mM 5′-GMP. The sample was ultrasonicated for ca. 30 s, then shielded from light by Al foil, and incubated at 310 K in a water bath. Samples were acidified to pH* = 2.0 using solutions of HNO_3.

pH Measurements. These were made directly in the NMR tube, before and after recording spectra, using a Corning 240 meter equipped with an Aldrich micro combination electrode, calibrated with Aldrich buffer solutions at pH 4, 7, and 10.

Ring Current Calculations and Molecular Modeling. Ring-current induced shifts were calculated using the program provided by Perkins.[24] The ring current for guanine was based on that for the amino acid tryptophan with scaling factors for the 5- and 6-membered rings of 0.725 and 0.288, respectively. Molecular modelling, minimization (CVFF force-field), and molecular dynamics (800 K, 100 ps) were carried out using the program Insight.[25] The initial structure for *cis*-$[Pt(NH_3)_2$(CBDCA-*O*)(5′-GMP)] (**6**) was based on the known crystal structures of $[Pt(NH_3)_2$(CBDCA-*O,O*′)][3,26] and [Pt(en)(5′-GMP)$_2$].[27] The PtN_3O coordination plane of **6** was fixed as a square plane and was not included in the energy minimization.

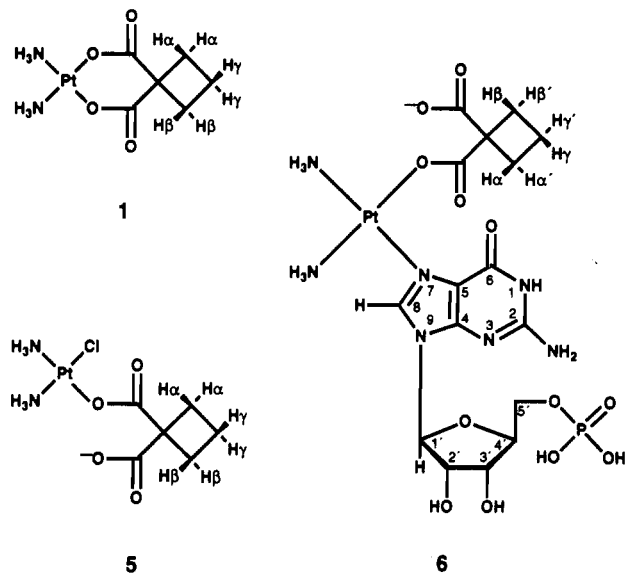

Figure 1. Numbering schemes for the complexes $[Pt(NH_3)_2$(CBDCA-*O,O*′)], **1**, *cis*-$[Pt(NH_3)_2$(CBDCA-*O*)Cl]$^-$, **5**, and *cis*-$[Pt(NH_3)_2$(CBDCA-*O*)(5′-GMP)], **6**.

Kinetics. Data were obtained from NMR spectra recorded at different time intervals from samples at 310 K. The solutions were maintained at 310 K while not in the probe. ^1H NMR peak heights or areas (5′-GMP reactions) were used for the determination of the relative concentrations. When appropriate, natural abundance ^{13}C satellites of **1** were used for comparison of areas with resonances of products. The analysis of the data, using the appropriate equations,[28] was performed using the program KaleidaGraph.[29]

Results

We have used NMR spectroscopy to investigate reactions of carboplatin with various combinations of nitrate, phosphate, chloride, and 5′-GMP in aqueous solutions at pH 7 and 2, 310 K. Both the intermediates and the products have been characterized, and where possible, the kinetics of the reactions have been analyzed.

Carboplatin (1) in Water and Nitrate. The ^1H NMR spectrum of a 20 mM solution of **1** in D_2O after incubation at 310 K for 2 weeks, at pH* 7, showed only an intense triplet at 2.875 ppm (α and β, Figure 1 and Table I) and a quintet at 1.894 ppm (γ). These resonances are assignable to the intact, unreacted drug.[3] As no free CBDCA was detected after 2 weeks, assuming that a 0.5% reaction could be detected, it is possible to calculate that the maximum hydrolysis rate of **1** is $<5 \times 10^{-9}$ s^{-1}, Table II.

When **1** was incubated for 16 days in 1 M KNO_3, pH* 7, at 310 K, signals with the same chemical shifts as $CBDCA^{2-}$,[3] Table I, appeared with an intensity of 1% of those for the intact drug. The estimated rate constant for the release of $CBDCA^{2-}$ is 5×10^{-9} s^{-1}, Table II.

Carboplatin (1) in Phosphate. When **1** was incubated for 12 h in 100 mM phosphate, pH* 7.0, at 310 K, a triplet at 2.340 ppm and a quintet at 1.824 ppm (^3J = 8.0 Hz) were seen in the ^1H NMR spectrum as well as resonances for intact **1**, Table I. The chemical shifts of the new peaks are identical to those of $CBDCA^{2-}$, and the free ligand concentration increases to a steady-state value of 33% of the total integrated intensity after 289 h.

(16) NMR Simulation Program, Varian Software VNMR 3.2b, 1990.
(17) Berners-Price, S. J.; Frenkiel, T. A.; Ranford, J. D.; Sadler, P. J. *J. Chem. Soc., Dalton Trans.* **1992**, 2137–2139.
(18) Morris, G. A.; Freeman, R. *J. Magn. Reson.* **1978**, *29*, 433.
(19) Freeman, R.; Mareci, T. H.; Morris, G. A. *J. Magn. Reson.* 1981, *42*, 341–345.
(20) Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Magn. Reson.* **1983**, *55*, 301–315.
(21) Otting, G.; Wüthrich, K. *J. Magn. Reson.* **1988**, *76*, 569.
(22) Marion, D.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967.
(23) Shaka, A. J.; Barker, P. B.; Freeman, R. *J. Magn. Reson.* **1985**, *64*, 547.
(24) Perkins, S. J. *Biol. Magn. Reson.* **1982**, *4*, 193–336.
(25) (a) InsightII 2.0.0, Biosym Technologies, San Diego, CA, 1991. (b) Discover 2.7.0, Biosym Technologies, San Diego, CA, 1991.
(26) Beagley, B.; Cruickshank, D. W. J.; McAuliffe, C. A.; Pritchard, R. G.; Zaki, A. M.; Beddoes, R. L.; Cernik, R. J.; Mills, O. S. *J. Mol. Struct.* **1985**, *130*, 97–102.
(27) Gellert, R. W. Ph.D. Thesis, University of Southern California, 1980. Bau, R.; Gellert, R. W. *Biochimie* **1978**, *60*, 1040.
(28) Atkins, P. W. *Physical Chemistry*, 2nd ed.; Oxford University Press: Oxford, England, 1982; pp 920–968.
(29) Sinergy Software, Reading, PA, USA.

Table I. ^{1}H NMR Chemical Shifts (δ) and Coupling Constants (Hz) for H_2CBDCA and Pt^{II}CBDCA Complexes at pH* 2.0 and 7.0 and 310 K with the Labeling Scheme Shown in Figure 1

complex	Hα	Hβ	Hγ	H8	H1′
		pH* = 2.0			
H_2CBDCA		2.562	2.013 (8.0)[a]		
[Pt(NH$_3$)$_2$(CBDCA-*O*,*O*′)] (**1**)		2.879	1.898 (8.0)		
cis-[Pt(NH$_3$)$_2$(HCBDCA-*O*)Cl] (**5**)	2.447[b]	2.438[b]	2.033 (8.0)		
cis-[Pt(NH$_3$)$_2$(HCBDCA-*O*)(HPO$_4$)][c] (**3**)	2.473[b]	2.459[b]	2.031 (8.0)		
		pH* = 7.0			
CBDCA^{2-}		2.340	1.824 (8.0)		
[Pt(NH$_3$)$_2$(CBDCA-*O*,*O*′)] (**1**)		2.875	1.894 (8.0)		
cis-[Pt(NH$_3$)$_2$(CBDCA-*O*)Cl]$^-$ (**5**)		2.321	1.824 (8.0)		
cis-[Pt(NH$_3$)$_2$(CBDCA-*O*)(HPO$_4$)][c] (**3**)		*d*	*d*		
5′-GMP				8.180	5.932 (5.9)[e]
cis-[Pt(NH$_3$)$_2$(CBDCA-*O*)(5′-GMP)] (**6**)	2.273 (α) 2.132 (α′)	2.257 (β) 2.086 (β′)	1.756 (γ) 1.606 (γ′)[f]	8.543	5.958 (5.3)
cis-[Pt(NH$_3$)$_2$(5′-GMP)$_2$] (**7**)				8.704	5.886 (4.4)

[a] ^3J(Hα/β, Hγ) in Hz given in parentheses. [b] Assignment to α or β is arbitary. [c] Protonation state of bound phosphate not determined. [d] Overlapped with free CBDCA. [e] ^3J(H1′,H2′) in Hz. [f] ^2J: αα′ = 11.7, ββ′ = −11.5, γγ′ = −11.2. ^3J: αγ = 9.4, αγ′ = 6.7, α′γ = 6.3, α′γ′ = 9.4, βγ = 9.4, βγ′ = 6.7, β′γ = 6.3, β′γ′ = 9.4. ^4J: αβ = 1.8, αβ′ = −0.8, α′β = −0.8, α′β′ = 1.7. All J = ±0.1 Hz, signs of coupling constants according to literature.[41]

Table II. Observed First-Order Rate Constants for the Displacement of CBDCA from [Pt(NH$_3$)$_2$(CBDCA-*O*,*O*′)] (0.02 M) in Aqueous Solution by Different Nucleophiles at 310 K

nucleophile	concn/M	k_{obs}/s^{-1} this work	k_{obs}/s^{-1} others
H_2O (neat)		$<5 \times 10^{-9}$	$<10^{-8}$ [a]
NO_3^-	1.0	$\sim 5 \times 10^{-9}$	5.8×10^{-7} [b]
HPO_4^{2-}	0.1	$(4.3 \pm 0.2) \times 10^{-7}$	7.2×10^{-7} [c]
$Cl^- + HPO_4^{2-}$	0.14 + 0.1	$(1.2 \pm 0.1) \times 10^{-6}$	–
Cl^-	0.14	7.7×10^{-7} [d]	2.1×10^{-7} [b]
5′-GMP (0.1M HPO_4^{2-})	0.04	$(4.1 \pm 0.1) \times 10^{-6}$ [e] $(3.3 \pm 0.4) \times 10^{-5}$ [f]	
5′-GMP (0.14M Cl^- + 0.1M HPO_4^{2-})	0.04	$(4.4 \pm 0.1) \times 10^{-6}$ [e] $(3.2 \pm 0.5) \times 10^{-5}$ [f]	
DNA			1.3×10^{-5} [c,g]

[a] At 298 K.[10] [b] Extrapolated at 310 K from values of ref 11. [c] Reference 7. [d] Difference of rate constants of $k_{obs}(Cl^- + HPO_4^{2-}) - k_{obs}(HPO_4^{2-})$. [e] k_{obs1}; see text. [f] k_{obs2}; see text.

Table III. ^{1}H and ^{15}N Chemical Shifts (δ) of the Ammine Ligands in *cis*-Diammine–Pt(II) Complexes at pH 7.0 and 310 K

complex	$\delta(^1H)$	$\delta(^{15}N)$-(trans ligand)
cis-[Pt(NH$_3$)$_2$Cl$_2$] (**4**)	4.06[a]	−68.1[a] (N)
[Pt(NH$_3$)$_2$(CBDCA-*O*,*O*′)] (**1**)	4.17[b]	−81.3[b] (O)
cis-[Pt(NH$_3$)$_2$(5′-GMP)$_2$] (**7**)	4.67	−66.6[c] (N)
cis-[Pt(NH$_3$)$_2$(HPO$_4$)$_x$][d] (**2**)	4.07	−83.4 (O)
cis-[Pt(NH$_3$)$_2$(CBDCA-*O*)Cl]$^-$ (**5**)	3.93	−84.3 (O)
	4.12	−68.7 (Cl)
cis-[Pt(NH$_3$)$_2$(CBDCA-*O*)(HPO$_4$)][d] (**3**)	3.99	−83.3 (O)
	3.86	−79.2 (O)
cis-[Pt(NH$_3$)$_2$(CBDCA-*O*)(5′-GMP)] (**6**)	4.37	−79.9 (O)
	4.21	−68.1 (N)

[a] $^2J(^1H,^{195}Pt)$ = 64 Hz; $^1J(^{15}N,^{195}Pt)$ = 326 Hz. [b] $^2J(^1H,^{195}Pt)$ = 62 Hz; $^1J(^{15}N,^{195}Pt)$ = 361 Hz. [c] pH = 7.9. [d] Protonation state and number of bound phosphate ligands not determined. ^{31}P peak at 6.67 ppm for bound phosphate.

A 2D [^{1}H,^{15}N] HMQC NMR spectrum of the reaction mixture after 90 h (Figure D1, supplementary material) showed an intense cross-peak at 4.17 ppm (^{1}H)/−81.3 ppm (^{15}N), Table III, with accompanying ^{195}Pt satellites, $^2J(^1H,^{195}Pt)$ = 62 Hz and $^1J(^{15}N,^{195}Pt)$ = 361 Hz, assignable to the NH_3 ligands of **1**. As well, there was a less intense cross-peak at 4.07/−83.4 ppm, assignable to a diammine–Pt(II) phosphato complex such as *cis*-[Pt(NH$_3$)$_2$(HPO$_4$)$_x$] (x = 1 or 2), **2**, and two other weaker cross-peaks of similar intensity at 3.99/−83.3 and 3.86/−79.2 ppm assignable to a ring-opened phosphato complex such as *cis*-[Pt(NH$_3$)$_2$(CBDCA-*O*)(HPO$_4$)]$^{2-}$, **3**, Table III. No ^{195}Pt satellites were observed for the minor species.

The ^{31}P NMR spectrum of the reaction mixture after 100 h showed an intense signal for phosphate at 0.78 ppm and an additional resonance at 6.67 ppm, Table III, assignable to bound phosphate.[30] Integration of the latter peak relative to phosphate suggested that there was ca. 4 mM bound phosphate present (i.e. 20% of the Pt present has a bound phosphate).

After incubation of a similar sample for 48 h, the pH* was lowered to 2.0, and the ^{1}H NMR spectrum was recorded immediately (within minutes). The ^{1}H NMR chemical shifts for **1**, 2.879 (t) and 1.898 (q) ppm, remained almost unchanged, whereas peaks for free CBDCA shifted to 2.562 (t), and 2.013 (q) ppm, consistent with protonation to give H_2CBDCA (pK_a's 2.94 and 5.45; I = 0.1 M).[31] In addition, further resonances were now visible at 2.473 (t), 2.459 (t), and 2.031 (q) ppm. The combined area of these new peaks and that of free H_2CBDCA (relative to those for **1**) was equal to the total area of the peaks previously assigned to free CBDCA^{2-} before the pH* was lowered, suggesting that at pH* 7.0 the peaks for free CBDCA^{2-} are overlapped with those of another species (a ring-opened complex, **3**).

The kinetics of the reaction of carboplatin with 100 mM phosphate at pH* 7.0 was followed by measuring the intensities of the triplets for free and bound CBDCA. A pseudo-first-order rate constant, k_{obs}, was determined for the decrease in concentration of intact **1**, taking into account the steady-state concentrations of the reactant and products, according to eq 1 (Nuc = phosphate). It was assumed that the concentration of *cis*-

$$[Pt(NH_3)_2(CBDCA\text{-}O,O')] + Nuc \rightleftharpoons cis\text{-}[Pt(NH_3)_2(CBDCA\text{-}O)(Nuc)] \rightleftharpoons cis\text{-}[Pt(NH_3)_2(Nuc)_x] + CBDCA^{2-} \quad (1)$$

[Pt(NH$_3$)$_2$(HPO$_4$)$_x$] **2**, was equal to that of free CBDCA^{2-}. The value of k_{obs} was determined to be $(4.3 \pm 0.2) \times 10^{-7}$ s^{-1}, Table II, and the concentration of **1** at equilibrium was 13.5 mM (67.5 ± 3.5% unreacted, see Figure 2). The rate constant for the formation of the *cis*-[Pt(NH$_3$)$_2$(CBDCA-*O*)(HPO$_4$)], **3**, could not be reliably determined from the NMR data available. The resonances for this complex were not resolved from those of CBDCA^{2-} at pH* 7.0 (unlike those of the chloride complex, vide infra).

(30) (a) Appleton, T. G.; Berry, R. D.; Davis, C. A.; Hall, J. R.; Kimlin, H. A. *Inorg. Chem.* **1984**, *23*, 3514–3521. (b) Appleton, T. G.; Hall, J. R.; Ralph, S. F.; Thompson, C. S. M. *Inorg. Chem.* **1984**, *23*, 3521–3525.
(31) *Critical Stability Constants*; Martel, A. E.; Smith, R. M., Eds.; Plenum Press: New York, 1977; Vol. 3.

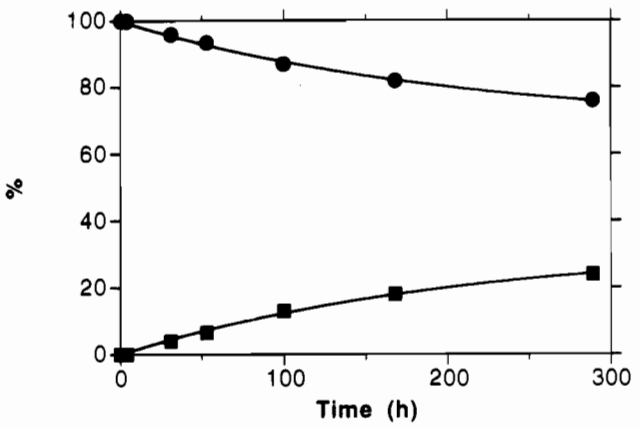

Figure 2. Plot of the concentrations (as a percentage of the total CBDCA) for [Pt(NH$_3$)$_2$(CBDCA-*O*,*O*')], **1** (●), and products (■) vs time for reaction of **1** with 100 mM phosphate, pH* 7.0, at 310 K. Solid lines represent the best fit to the data; see text.

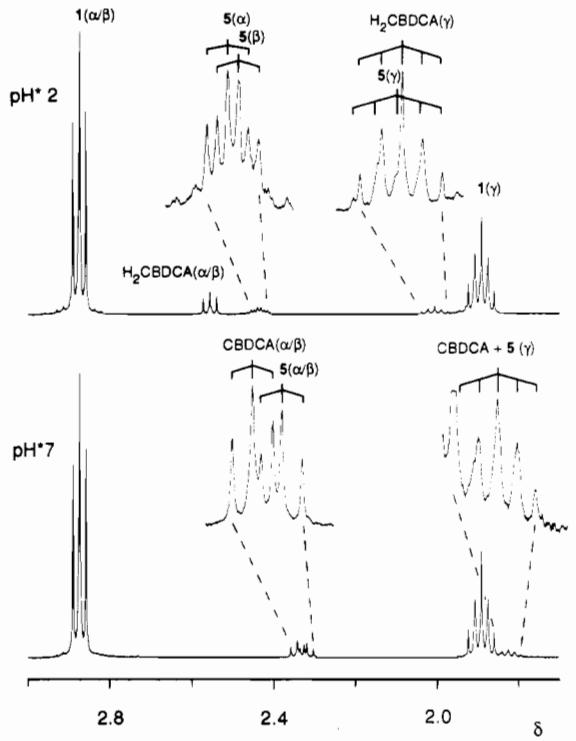

Figure 3. 500 MHz ^{1}H NMR spectrum of a solution containing 20 mM **1** and 140 mM Cl$^-$ in D_2O after incubation at 310 K for 48 h at pH* 7 (bottom). The top spectrum is from the same solution after the pH* had been lowered to 2.0. At pH* 2 it is notable that peaks for **5** are well resolved from those of free ligand H_2CBDCA.

Reactions with Chloride. The ^{1}H NMR spectrum of **1** after incubation with 140 mM Cl$^-$, pH* 7, 310 K, for 48 h consisted of resonances for **1**, CBDCA^{2-}, and an additional triplet at 2.321 ppm. When the pH* of this solution was lowered to 2.0, the latter triplet resolved into two overlapping triplets at 2.447 and 2.438 ppm (Figure 3), and now an additional quintet was just detectable at 2.033 ppm beneath the quintet for H_2CBDCA. The 2D [^{1}H,^{15}N] HMQC spectrum of the reaction mixture after 90 h incubation at 310 K (initial pH = 7.0, final pH = 6.9) shows an intense cross-peak for **1**, a weaker cross-peak at 4.06/−68.1 ppm ($^2J(^1H,^{195}Pt)$ = 64 Hz, $^1J(^{15}N,^{195}Pt)$ = 326 Hz) assignable to *cis*-[Pt(NH$_3$)$_2$Cl$_2$], **4**, and two weaker resonances of approximately the same intensity at 3.93/−84.3 and 4.12/−68.7 ppm assignable to the ring-opened complex *cis*-[Pt(NH$_3$)$_2$(CBDCA-*O*)Cl]$^-$, **5**, Figure 4.

Reactions with Chloride Ions in the Presence of Phosphate. The time-course for the reaction of **1** with 140 mM Cl$^-$ in 100 mM phosphate buffer, pH* 7.0, 310 K, was followed by ^{1}H NMR spectroscopy for 2 weeks. The same resonances were seen as in the absence of phosphate. After 3 h, peaks for complex **5** were seen at 2.321 ppm (t) and 1.824 ppm (q), and, by 30 h, integration showed that complex **5** constituted 4% of the total CBDCA (bound plus free) present in solution, and this remained unchanged for the rest of the observation period (2 weeks). After 2 weeks, 64% of the original **1** remained. Peaks assignable to the free ligand CBDCA^{2-} were observed after 6 h and increased to a steady-state level of 32% of the total CBDCA present in solution.

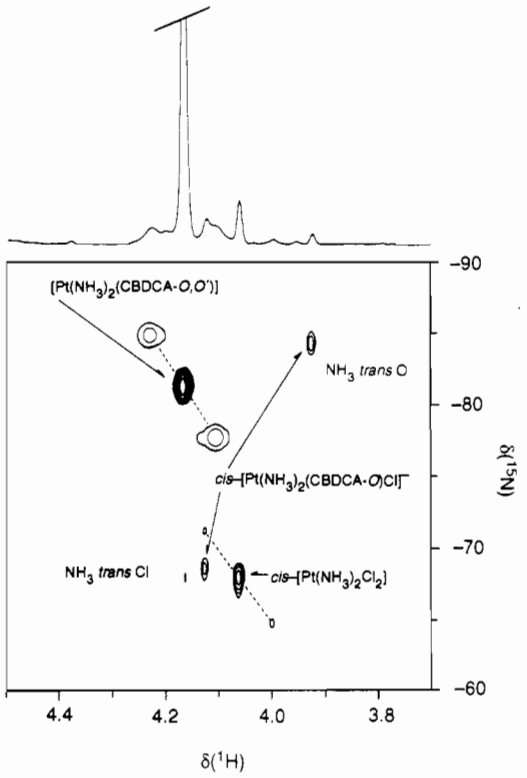

Figure 4. 500-MHz ^{1}H{^{15}N} NMR spectrum and corresponding [^{1}H,^{15}N] 2D NMR spectrum (50.67 MHz ^{15}N) of a solution of 20 mM **1** and 140 mM Cl$^-$ in 95% H_2O/5% D_2O after incubation for 90 h at 310 K, pH 6.9. Dashed lines relate ^{195}Pt satellites to the central peak. Peaks for the ring-opened intermediate **5** can be readily assigned.

The ^{31}P NMR spectrum of the reaction mixture after 100 h showed a resonance at 6.63 ppm assignable to bound phosphate, with an area (measured relative to free phosphate) equivalent to 10% of the total Pt concentration.

The rate constant for the decrease in concentration of **1** was determined (eq 1, Nuc = phosphate or Cl) to be k_{obs} = (1.2 ± 0.1) × 10^{-6} s^{-1}, and the concentration of **1** at equilibrium was 12.8 mM (i.e. 64.1 ± 3.5% of **1** unreacted). It was not feasible to determine the rate constant for the formation of the intermediate **5**.

Reactions with 5'-GMP (1:2) in Phosphate Buffer. The time course for the reaction of **1** with 2 mol equiv of 5'-GMP in 100 mM phosphate buffer, pH* 7, 310 K, was studied.

A new H8 singlet at 8.543 ppm and H1' doublet at 5.958 ppm (Table I) appeared and grew in intensity, peaking at ca. 24 h before decreasing to below detection by 280 h, and was assigned to the intermediate *cis*-[Pt(NH$_3$)$_2$(CBDCA-*O*)(5'-GMP)], **6**. As well, an H8 resonance at 8.704 ppm and accompanying H1' doublet at 5.886 ppm formed and increased in intensity as the reaction proceeded, with the concomitant decrease in free 5'-GMP (H8, 8.180 ppm; H1', 5.932 ppm). In parallel with this, a triplet and quintet for free CBDCA^{2-} appeared and increased in intensity with time, with a concomitant decrease in the intensity of the peaks for **1**. In addition, highly complex multiplets to lower frequency of free CBDCA^{2-} resonances assignable to *cis*-

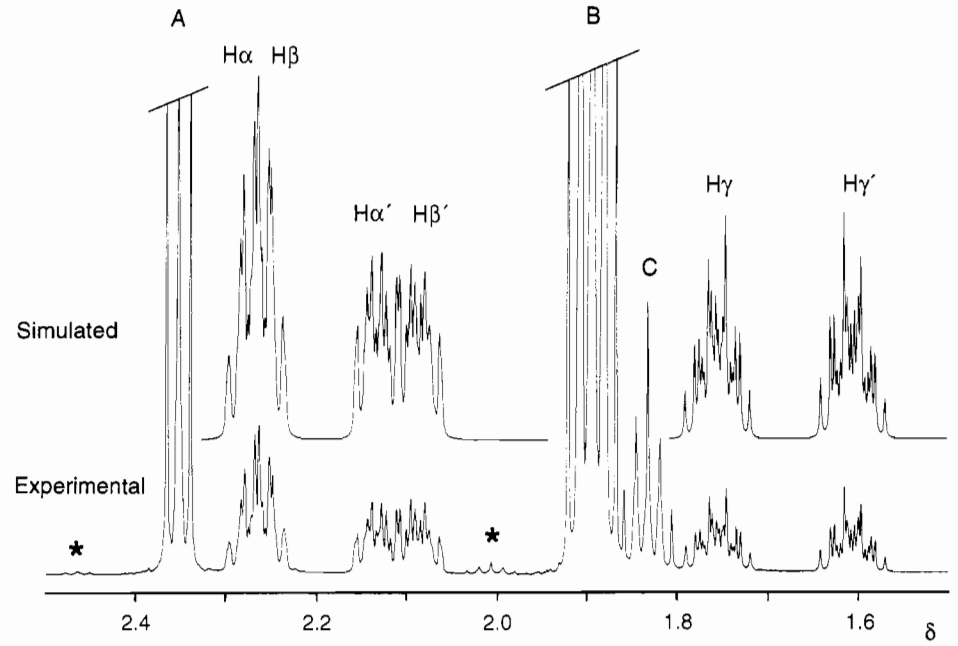

Figure 5. 600-MHz ^{1}H NMR spectrum of a solution of **1** (20 mM) and 5′-GMP (20 mM) in 100 mM D_2O phosphate buffer, pH* 7.0, after 24 h incubation at 310 K, showing the proton resonances for CBDCA in *cis*-[Pt(NH_3)$_2$(CBDCA-*O*)(5′-GMP-*N7*)], **6**. The inserts are simulations using the chemical shifts and coupling constants given in Table I. Assignments: A, Hα and Hβ of $CBDCA^{2-}$; B, Hγ of **1**; C, Hγ of $CBDCA^{2-}$. The remaining resonances are due to the six inequivalent cyclobutane ring protons of **6**. The peaks labeled with an asterisk (*) are ^{13}C satellites from resonances A and B (one of each labelled).

[Pt(NH_3)$_2$(CBDCA-*O*)(5′-GMP)], **6**, were detected, Figure 5. The concentration of the monodentate intermediate increased to a maximum of ca. 10% of the total platinum concentration at 24 h, but by 280 h it had decreased to a level below detection. The concentration of **1** at 280 h was 4% of the total Pt. The data fit shows that it eventually all disappears with the concomitant complete displacement of all the $CBDCA^{2-}$ (vide infra). A phase-sensitive DQF COSY ^{1}H NMR spectrum showed that all six protons on the cyclobutane ring of **6** are now inequivalent, and that each is coupled to every other cyclobutane ring proton. A complete simulation of these resonances was carried out and allowed accurate determination of the chemical shifts and couplings, Figure 5 and Table I. The Hα (Figure 1) resonance is at 2.273 ppm while its neighboring geminal proton, Hα′, is now shifted to 2.132 ppm, a difference of 0.141 ppm. Both Hα and Hα′ are shielded relative to the uncomplexed $CBDCA^{2-}$, by 0.067 and 0.208 ppm, respectively. The β and β′ protons are shielded to a greater extent than the α and α′ signals, 0.083 (Hβ) and 0.254 ppm (Hβ′), relative to free $CBDCA^{2-}$, a shift difference between these two protons of 0.171 ppm. The shift difference between the Hγ and Hγ′ resonances (0.150 ppm) is similar to that of Hα/Hα′ and Hβ/Hβ′, with shieldings relative to $CBDCA^{2-}$ of 0.068 ppm (Hγ) and 0.218 ppm (Hγ′). Protons on one face of the cyclobutane ring (Hα, Hβ and Hγ) are less shielded than those on the opposite face (Hα′, Hβ′ and Hγ′). The ^2J(Hα,Hα′), ^2J(Hβ,Hβ′), and ^2J(Hγ,Hγ′) coupling constants are all approximately the same, −11.2 to −11.7 Hz, Table I. The ^3J(^{1}H,^{1}H) couplings fall into two groups. Those between protons on the same face of the cyclobutane ring (e.g. Hα, Hγ), having values of 9.4 Hz, and those between protons on the opposite sides of the ring (e.g. Hα, Hγ′), having values between 6.3 and 6.7 Hz. The spectrum was well resolved and also allowed determination of ^4J(^{1}H,^{1}H) coupling constants. Again these showed the similar pattern with protons on the same face (Hα, Hβ; Hα′, Hβ′) having larger coupling constants (1.8 and 1.7 Hz) than those on opposite faces (−0.8 Hz).

The time courses for the disappearance of free 5′-GMP and appearance of intermediate **6** and product **7** for reaction of 20 mM **1** with 40 mM 5′-GMP in 100 mM phosphate, pH* 7.0, were determined from integration of 5′-GMP H8 resonances, and are

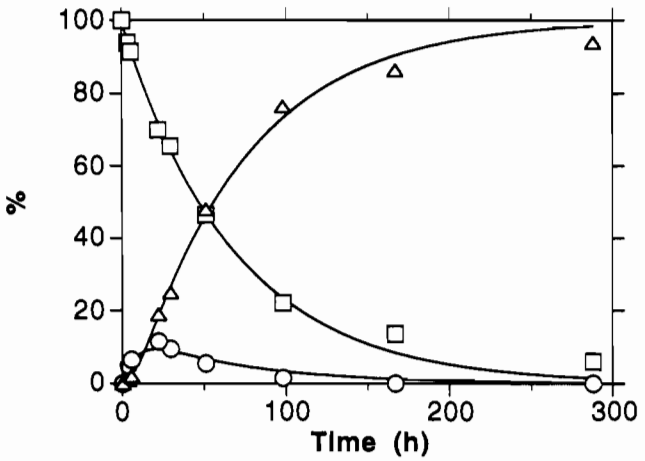

Figure 6. Plot of the concentrations (as a percentage of the total 5′-GMP) for 5′-GMP (□), *cis*-[Pt(NH_3)$_2$(CBDCA-*O*)(5′-GMP)] **4** (○), and *cis*-[Pt(NH_3)$_2$(5′-GMP)$_2$] **5** (△), versus time for the reaction of **1** with 5′-GMP (1:2) in 100 mM phosphate, pH* 7.0, at 310 K. Solid lines represent the best fit to the data; see text.

shown in Figure 6. These data suggest that reaction of **1** with 5′-GMP occurs in two consecutive steps, and therefore two consecutive pseudo-first-order rate constants were fitted to the experimental data according to eq 2. The rate constants obtained were $k_{obs1} = (4.1 \pm 0.1) \times 10^{-6}$ s^{-1} and $k_{obs2} = (3.3 \pm 0.4) \times 10^{-5}$ s^{-1}, Table II (see Figure 6).

$$\mathbf{1} + 5'\text{-GMP} \rightarrow \mathbf{6} \quad k_{obs1} \tag{2a}$$

$$\mathbf{6} + 5'\text{-GMP} \rightarrow \mathbf{7} + CBDCA^{2-} \quad k_{obs2} \tag{2b}$$

Reactions with 5′-GMP (1:2) in Phosphate Buffer in the Presence of Chloride Ions. Reaction of **1** with 2 mol of 5′-GMP in 100 mM phosphate, pH* 7.0, containing 140 mM chloride gave rise to a triplet at 2.321 ppm for complex **5** and a singlet at 8.543 ppm with accompanying CBDCA multiplets between 1.5 and 2.3 ppm for **6**. This shows that both ring-opened monodentate CBDCA intermediates (**5** and **6**) are present during

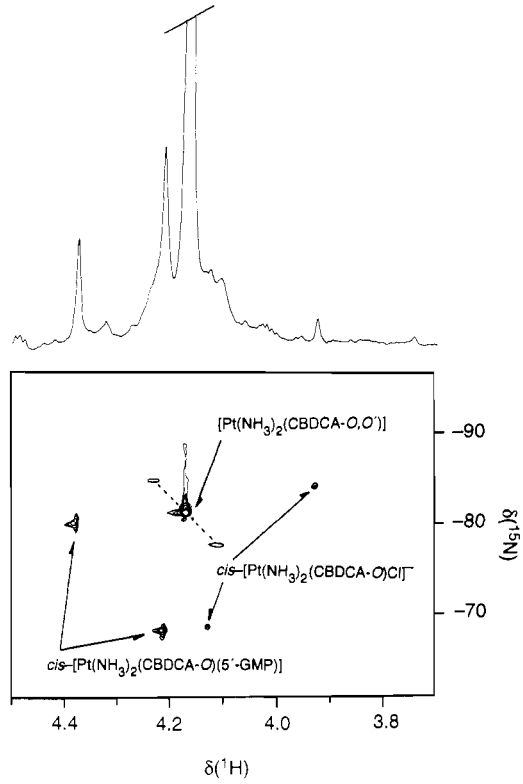

Figure 7. 500-MHz $^1H\{^{15}N\}$ NMR spectrum and corresponding [^1H,^{15}N] 2D NMR spectrum (50.67 MHz ^{15}N) of a solution of 20 mM **1**, 40 mM 5′-GMP, 140 mM Cl^-, and 100 mM phosphate buffer, pH 7.0, in 95% H_2O/5% D_2O after incubation for 24 h at 310 K. Dashed lines relate ^{195}Pt satellites to the central peak. The pairs of cross-peaks for **5** and **6** are readily assignable in the 2D spectrum.

the reaction. The maximum amounts of **5** and **6** were 3% and 12% of the total Pt, respectively. The 2D [^1H,^{15}N] HMQC spectrum confirms the presence of both of these complexes, along with unreacted **1** (Figure 7). The rate constants were determined to be $k_{obs1} = (4.4 \pm 0.1) \times 10^{-6}$ s^{-1} and $k_{obs2} = (3.2 \pm 0.5) \times 10^{-5}$ s^{-1}.

Molecular Modeling and Ring Current Calculations. The observed ring-current shifts of the six CBDCA protons in solution suggests that in *cis*-[$Pt(NH_3)_2$(CBDCA-*O*)(5′-GMP)], **6**, the cyclobutane ring is in close proximity to the purine ring of 5′-GMP. The majority of structures which arose from the molecular dynamics calculations showed this feature, and one such structure was further refined by energy minimization. The resulting structure has the cyclobutane ring inclined at an angle of ca. 10° relative to the plane of the purine ring of 5′-GMP and is centered over the coordinated N7 of the five-membered ring with Hβ' making the closest approach. All six cyclobutane ring protons are within the shielding cone of the π electrons of 5′-GMP, resulting in shifts to lower frequency relative to free $CBDCA^{2-}$. The distances between the cyclobutane protons in the ring-opened complex **6** and the plane of the purine ring range from 3 to 5.5 Å. The calculated (observed in parentheses) shieldings in ppm compared to $CBDCA^{2-}$ are as follows: α, −0.10 (−0.067); α', −0.12 (−0.208); β, −0.30 (−0.083); β', −1.01 (−0.254); γ, −0.19 (−0.068); γ', −0.32 (−0.218). For this model, the calculated ring current shifts for the NH_3 protons cis to 5′-GMP are ca. −0.2 ppm and are +0.05 ppm for the trans NH_3 protons. Since accurate descriptions of the ring currents of a Pt(II)-coordinated purine are not available, no attempt was made to refine this structure further, but the agreement is a reasonable one.

Discussion

In comparison to cisplatin, the second generation anticancer drug carboplatin has reduced, and different side-effects as well as a greater aqueous solubility. There is interest in the possibility that it acts as a pro-drug, reacting with chloride in the plasma via ring-opened forms to produce cisplatin. In this paper we demonstrate that stable ring-opened species are formed in the reactions of carboplatin with nucleophiles and show that these can be readily detected and identified in aqueous solution by 1D and 2D multinuclear NMR techniques. Our kinetic data suggest that carboplatin is not simply a prodrug for cisplatin and that reactions with nucleotides can occur by direct attack on **1**.

The crystal structure[3] of **1** shows C_α and C_β to be inequivalent with Pt···C distances of 3.138 and 4.556 Å, respectively, with possible dynamic puckering of the 4-membered cyclobutane ring.[26] The crystallographic mirror-plane imposes equivalence on the α,α' protons as well as β,β' and γ,γ'. ^1H NMR spectroscopy provides a convenient means of monitoring the reactions of carboplatin, **1**. In solution, the α and β protons in **1**, Table I, are magnetically equivalent due to rapid inversion of the chelate oxygens.[3,10]

No hydrolysis of **1** in D_2O was detectable by NMR even after 2 weeks at 310 K, consistent with previous findings.[10] A trace of hydrolysis was detected in the presence of the very weak nucleophile NO_3^- (1 M), but the rate was essentially the same as that estimated for D_2O alone. Complex **1** reacts faster in the presence of the stronger nucleophile phosphate. At pH* 7.0, the only species seen by ^1H NMR spectroscopy were intact **1** and free $CBDCA^{2-}$. However, when the pH* was lowered to 2.0, additional peaks assignable to the phosphato species **3** were clearly seen. ^{31}P NMR spectra confirmed the presence of a product with bound phosphate. The chemical shift is consistent with monodentate phosphate,[30] perhaps as *cis*-[$Pt(NH_3)_2(HPO_4)_x$], **2**. The importance of phosphate as an attacking nucleophile can be seen from its ability to react with cisplatin[32] and its diaqua cation,[30] as demonstrated previously. Mauldin et al.[33] have shown by HPLC that phosphate can displace malonate from (*d,l-trans*-1,2-diaminocyclohexane)(malonato)platinum(II) at pH 7. The 2D [^1H,^{15}N] HMQC NMR spectrum (Figure D1) showed the presence of two products with ^{15}N shifts consistent with oxygen ligands trans to ammonia,[34,35] one with two different oxygen donors, **3**, and the other a symmetrical species such as **2**.

Two-bond ^1H-^{195}Pt coupling was observable for the NH_3 ligands of **1** but the satellites are broadened by relaxation via chemical shift anisotropy.[34] The pattern of satellite peaks in 2D spectra (Figures 4, 7, and D1) show that $^2J(^1H–^{195}Pt)$ and $^1J(^{15}N–^{195}Pt)$ have opposite signs.

In the ^1H NMR spectrum of the ring-opened intermediate **3** at pH* 2.0, the α and β protons are now inequivalent. The γ signal is, however, still observed as a quintet, $^3J(^1H–^1H)$ 8 Hz, and no $^4J(\alpha$-$\beta)$ couplings are resolved, indicating that the inequivalence is small.

As there is a high concentration of Cl^- in the blood (104 mM)[36] and **1** is administered by intravenous infusion, it is important to determine the kinetics of reaction of **1** with Cl^-. For some cells the nuclear concentration of Cl^- is also reported to be high, e.g. 150 mM in rat-liver cells.[37] Reaction of **1** with Cl^- at pH* 7.0 gave new ^1H NMR peaks (Figure 3) assignable to *cis*-[$Pt(NH_3)_2$(CBDCA-*O*)Cl]$^-$, **5**. In this species the CBDCA ligand is monodentate with the α and β protons magnetically equivalent, as seen for **3**. Further reaction of **5** with Cl^- produces cisplatin, **4**. At high concentrations of Cl^-, cisplatin separates

(32) Green, M.; Garner, M.; Orton, D. M. *Transition Met. Chem. Weinheim (Ger.)* **1992**, *17*, 164–176.
(33) Mauldin, S. K.; Plescia, M.; Richard, F. A.; Wyrick, S. D.; Voyksner, R. D.; Chaney, S. G. *Biochem. Pharm.* **1988**, *37*, 3321–3333.
(34) (a) Pregosin, P. S. *Annu. Rep. NMR Spectrosc.* **1986**, *17*, 287–349. (b) Pregosin, P. S. *Coord. Chem. Rev.* **1982**, *44*, 247–291.
(35) (a) Appleton, T. G.; Hall, J. R.; Ralph, S. F. *Inorg. Chem.* **1985**, *24*, 673–677. (b) Appleton, T. G.; Hall, J. R.; Ralph, S. F. *Inorg. Chem.* **1985**, *24*, 4685–4693.
(36) Lentner, C., Ed. *Geigy Scientific Tables*; 1984; Vol. 3, p 78.
(37) Siebert, G. *Sub-Cell. Biochem.* **1972**, *1*, 277–292.

out of solution[10] due to its lower solubility compared with carboplatin. Cisplatin was readily detected in the 2D HMQC [^{1}H,^{15}N] NMR spectrum, Figure 4, with shifts similar to those reported previously.[35,38] Complex **5** gave two smaller but equally intense cross-peaks in the 2D spectrum, with ^{15}N chemical shifts consistent with NH_3 ligands trans to O and Cl, respectively.[35] The ^{1}H NMR spectrum of **5** at pH* 2.0 is very similar to that of the phosphato complex **3** (vide supra), with the α and β protons now inequivalent but with only one quintet for the γ protons. For species **3** and **5**, which have similar chemical shifts for their monodentate CBDCA ligands and which are present in relatively small amounts (<4% of total CBDCA), the high dispersion and sensitivity of high frequency spectrometers were invaluable for their detection.

Incubation of **1** with both chloride and phosphate produced the same intermediates (**3** and **5**) and products (**2** and **4**) as were seen when these anions were used separately. This is compatible with their similar rates of reaction (Table II). Again it is evident that the ring-opened intermediates are relatively stable species and they persist in solution over the long time course of the reaction. To investigate the possibility that these ring-opened species are reactive towards attack on DNA bases, we studied reactions of **1** with 5′-GMP. This was carried out in phosphate buffer (100 mM, pH* 7.0) in the absence and presence of Cl^- ions (140 mM). As with cisplatin, the final product of the reaction was *cis*-[$Pt(NH_3)_2$(5′-GMP)$_2$], **7** but now additional ^{1}H NMR resonances were observed for the ring-opened CBDCA intermediate *cis*-[$Pt(NH_3)_2$(CBDCA-*O*)(5′-GMP)], **6**. The chemical shift of the H8 proton of **6** (8.543 ppm) was between that of free 5′-GMP (8.180 ppm) and the bis complex **7** (8.704 ppm), and ^3J(H1′-H2′) was only slightly different from that of free 5′-GMP, suggesting only a small change in sugar pucker compared to 5′-GMP.[39]

In contrast to the chloro and phosphato ring-opened intermediates (**3** and **5**), the analogous 5′-GMP species **6**, now exhibits inequivalence for the six CBDCA ring protons, all of which are shifted to low frequency with respect to resonances of the free ligand $CBDCA^{2-}$ at pH* 7. Although previous workers were able to detect H8 and H1′ resonances for such an intermediate, they were unable to resolve resonances for the CBDCA ring protons. They isolated chromatographically an analogous inosine–malonato species.[9] The only known crystal structure of a monodentate CBDCA complex is [Pt(CHDA)(Me_2SO)(CBDCA-*O*)] (where CHDA is *trans*-(–)-1,2-cyclohexanediamine[12]), and in its ^{1}H NMR spectrum, the CBDCA resonances are shifted to higher frequency with respect to free $CBDCA^{2-}$, lending support to our assignments of ring current shift effects due to 5′-GMP.

As far as we are aware this is the first time that the total inequivalence of all six CBDCA protons has been observed. We therefore compared our observed ^{1}H–^{1}H couplings with those in more highly substituted cyclobutane derivatives. The ^3J values for **6** fall into two groups: 9.4 Hz for cofacial protons (e.g. α–γ) and ca. 6.5 Hz for antifacial protons (e.g. α'–γ'). The values of cofacial and antifacial ^3J(^{1}H–^{1}H) couplings reported for substituted organic cyclobutane derivatives are within the range 8–12 Hz.[40] It therefore seems likely that there is significant puckering of the cyclobutane ring in **6**. The $^2J(\alpha\alpha', \beta\beta', \gamma\gamma')$ and $^4J(\alpha\beta, \alpha'\beta', \alpha'\beta, \alpha\beta')$ values show a pattern of cofacial and antifacial couplings similar to those reported for cyclobutane derivatives.[40]

The low frequency shifts of the CBDCA ring protons of **6** are explicable by their proximity to the purine ring of N7-coordinated 5′-GMP in the cis position. A model which is consistent with the observed shifts was obtained from molecular dynamics and energy minimization procedures, Figure 8. From the ^{1}H NMR spectrum, protons on the face of the cyclobutane ring proximal to the purine ring are shielded by –0.19 to –0.24 ppm, and on the distal face by –0.05 to –0.07 ppm. The close hydrophobic contact between the CBDCA and the purine ring (average interplanar distance 4.1 Å) contributes to the stability of this intermediate and may be related to the long half-life of monofunctional carboplatin adducts on DNA (ca. 14.5 h).[7] These hydrophobic forces may directly relate to the efficacy of this anticancer drug and may be a useful parameter in structure–activity relationships.

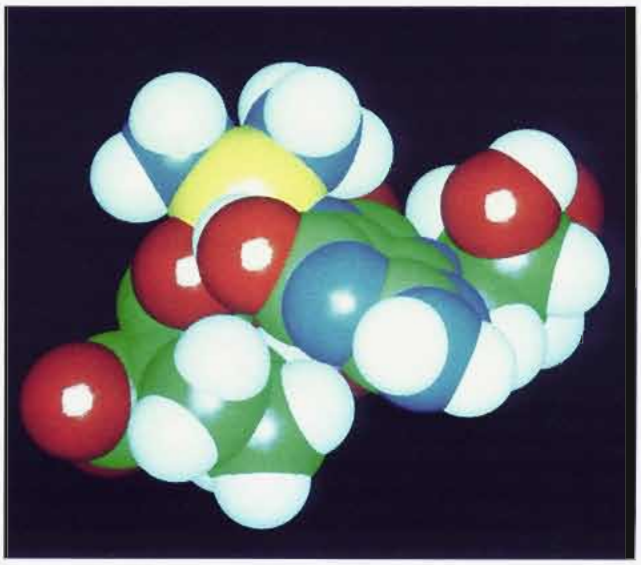

Figure 8. Space-filling model of an energy-minimized structure for *cis*-[$Pt(NH_3)_2$(CBDCA-*O*)(5′-GMP)], **6**, showing close hydrophobic contacts between the cyclobutane ring of the monodentate CBDCA and the 5′-GMP purine ring. The colors for atoms are as follows: Pt, yellow; C, green; H, white; N, blue; O, red; P, purple (almost hidden). It can be seen that the three protons (α', β', γ') on the face of the CBDCA ring nearest the purine are more shielded than those on the opposite face (α, β, γ).

The ^{1}H NMR resonance for the ammonia ligand *cis* to 5′-GMP in **6** is notably deshielded relative to **1**, as was also seen for *cis*-[$Pt(NH_3)_2$(GMP-*N7*)Cl] relative to cisplatin.[41] Our calculations suggest that the ring current of the cis 5′-GMP would cause a shielding of the NH_3 protons. It therefore seems likely that the high frequency shift is caused by H-bonding between NH_3 and the 5′ phosphate of GMP.[17] Such H-bonding is thought to be important in stabilizing *cis*-$Pt(NH_3)_2$–DNA complexes.[42]

When **1** was reacted with 5′-GMP in the presence of Cl^-, the intermediate **5** was present to the same extent as in the absence of 5′-GMP. This suggests that **5** is not an activated intermediate.

Kinetics. Adequate fits to the kinetic data were obtained by assuming pseudo first-order kinetics. Acid catalyzed displacement of CBDCA can be neglected at pH* 7 (k_{obs} <10^{-10} s^{-1}).[10]

Our measured hydrolysis rate for **1** is consistent with that previously reported,[10] and is little influenced by the presence of NO_3^-, Table II. In contrast, other workers[11] have reported a 100-fold higher hydrolysis rate in 1M KNO_3. The discrepancy between these values may arise from the method used in the latter study, which involved HPLC separation on a reverse-phase column.

Reaction with phosphate occurs ca. 100 times faster than hydrolysis, consistent with the HPLC study by Knox et al.[7] from which they determined the rate to be 7.2×10^{-7} s^{-1}. As with

(38) Norman, R. E.; Ranford, J. D.; Sadler, P. J. *Inorg. Chem.* **1992**, *31*, 877–888.
(39) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; Marcelis, A. T. M.; van der Marel, G.; Rinkel, L. J.; Wille-Hazeleger, G.; Reedijk, J. *Eur. J. Biochem.* **1983**, *134*, 485–495.
(40) Weitkamp, H.; Korte, F. *Tetrahedron Suppl.* **1966**, *7*, 75–87.
(41) In the light of further data (Berners-Price, S. J.; Frey, U.; Ranford, J. D.; Sadler, P. J. unpublished) we propose a reversal of our previous assignments[17] for *cis*-[$Pt(NH_3)_2$Cl(5′-GMP-*N7*)]: NH_3 *cis* to 5′-GMP, 4.38 ppm; NH_3 *cis* to Cl, 4.09 ppm.
(42) Reedijk, J. *Inorg. Chim. Acta* **1992**, *198–200*, 873–881.

cisplatin, there are apparently no unreactive buffer systems for carboplatin.[32]

The rate of reaction of **1** with chloride was obtained by difference (see Table II) and was comparable with the value reported by Allsopp et al.[11] and similar to the value for phosphate. However, both chloride and phosphate react only slowly with **1**.

The rate constants for reaction of 5′-GMP with **1** were best fitted as two consecutive first-order reactions, with no back reaction, Figure 6. It is notable that the rate for the first step (ring-opening, Table II) is 10 times faster than the rate for phosphate alone, indicating that buffering can be used here with caution to control the pH. This is important because CBDCA ring-opening and displacement are known to be acid catalyzed ($k_{obs} = 1.61 \times 10^{-4}$ [H$^+$].)[10] The second step of the reaction, formation of the bis 5′-GMP adduct **7**, is seven times faster than the first step, and the observed rate constant is comparable with that reported by Knox et al.[7] (1.3×10^{-5} s^{-1}) for formation of intrastrand cross-links from monofunctional DNA intermediates.

The presence of Cl^- did not affect the rate constant for either step of the reaction of **1** with 5′-GMP. It is evident that neither chloride nor phosphate activate **1** toward reactions with 5′-GMP, and therefore the rate-limiting step appears to involve direct attack of 5′-GMP on **1** under the conditions studied here.

Biological Implications. The second generation anticancer drug carboplatin has reduced, and different side-effects as well as greater aqueous solubility than cisplatin. It has sometimes been considered as a pro-drug for cisplatin, reacting with chloride in the plasma. In patients treated with carboplatin, most of the Pt(65%; cf. 16–35% for cisplatin) is excreted in the urine within the first 24 h, with at least 32% as intact drug.[43] The rate constants which we have determined in vitro for reactions of **1** with Cl^- and HPO_4^{2-} show that these nucleophiles can transform only minor amounts of administered carboplatin. Even if the ring-opened chloro complex **5** can be formed, it appears to be less reactive toward 5′-GMP than intact **1**. Therefore it seems likely that the key step involves direct attack of **1** on DNA or its activation with a stronger nucleophile (e.g. S amino acids). The half-life of reaction between cisplatin and 5′-GMP is ca. 2.5 h compared to 47 h for carboplatin. This suggests that ca. 20 times the dose would need to be administered to achieve the same extent of reaction, correlating well with clinical dosage regimes.

It is interesting to note that the reaction of the ring-opened 5′-GMP intermediate **6** with a second 5′-GMP is seven times faster than with the first 5′-GMP. This suggests that formation of G-G cross-links on DNA will be facile. In contrast, for cisplatin the second step (formation of the bis 5′-GMP adduct, **7**) is 1000 times slower than the first step.[32]

(43) Harland, S. J.; Newell, D. R.; Siddik, Z. H.; Chadwick, R.; Calvert, A. H.; Harrap, K. R. *Cancer Res.* **1984**, *44*, 1693–1697 and references therein.

Conclusions

Reactions between the anticancer drug carboplatin **1** and water, chloride, phosphate, and 5′-GMP can be followed in detail with high-frequency ^{1}H NMR spectroscopy in aqueous solution. The identification of ring-opened intermediates is greatly aided by the use of ^{15}N-labeled carboplatin, together with ^{15}N-edited ^{1}H NMR and 2D [^{1}H,^{15}N] HMQC NMR spectroscopy. cis-Diammino–Pt(II) species with monodentate CBDCA and phosphate, chloride, or 5′-GMP as ligands all appear to be relatively stable and could play a role in the pharmacology of carboplatin. A structure for cis-[Pt(NH$_3$)$_2$(CBDCA-*O*)(5′-GMP-*N7*)], based on a consideration of molecular models, is proposed which is consistent with the observed shifts of CBDCA ring protons induced by the purine ring of 5′-GMP. The cyclobutane ring forms a close hydrophobic contact with the purine ring of 5′-GMP. Such hydrophobic contacts may contribute to the stabilization of drug-DNA complexes.

The reactions of water, chloride, and phosphate with **1** are very slow (half-lives of >4.4 years, 250 h, and 450 h respectively), and reactions with 5′-GMP appear to involve direct attack on intact carboplatin, followed by a second faster step involving displacement of monodentate CBDCA.

Carboplatin does not appear to be simply a pro-drug for cisplatin, and other mechanisms of activation are probably involved.

Acknowledgment. We thank the Medical Research Council, the Royal Society, the Swiss National Science Foundation, the Wolfson Foundation, the Science and Engineering Research Council, and University of London Intercollegiate Research Service for their support for this work. We thank the Biomedical NMR Centres at Birkbeck College (University of London) and Mill Hill (Medical Research Council) for provision of facilities, Dr. Tom Frenkiel (Mill Hill) for assistance with inverse probe work, Dr. Alan Tucker (Birkbeck College) for assistance with computer graphics, and Professor R. Bau (University of Southern California) for providing the X-ray coordinates for [Pt(en)(5′-GMP)$_2$].

Supplementary Material Available: Figure D1 500-MHz ^{1}H{^{15}N} spectrum and corresponding [^{1}H,^{15}N] spectrum (50.67 MHz ^{15}N) of **1** in phosphate buffer showing the unreacted starting material and species **2** and **3** (1 page). Ordering information is given on any current masthead page.