DALTON

Chelate-ring-opened adducts of [Pt(en)(Me-Mal-*O*,*O*′)] (en = ethane-1,2-diamine, Me-Mal = 2-methylmalonate) with methionine derivatives: relevance to the biological activity of platinum anticancer agents‡

Zijian Guo,[a] Trevor W. Hambley,[a] Piedad del Socorro Murdoch,[a] Peter J. Sadler*,†,[a] and Urban Frey[b]

[a] *Department of Chemistry, Birkbeck College, University of London, Gordon House and Christopher Ingold Laboratories, 29 Gordon Square, London WC1H 0PP, UK*
[b] *Institute de Chimie Minérale et Analytique, University of Lausanne, BCH, CH-1015, Lausanne, Switzerland*

The anticancer drug carboplatin [Pt(cbdca-*O*,*O*′)$(NH_3)_2$] which contains the chelated dicarboxylate ligand cbdca, cyclobutane-1,1-dicarboxylate, may be activated *in vivo* by reaction with sulfur ligands. The reactions between the analogue [Pt(en)(Me-Mal-*O*,*O*′)] **1** (en = ethane-1,2-diamine, Me-Mal = 2-methylmalonate) and the methionine derivatives *N*-acetyl-L-methionine (Ac-Met), glycyl-L-methionine (Gly-Met) and L-methionylglycine (Met-Gly) have been studied at pH 7 and 4, 310 K, using ^{1}H and two-dimensional [^{1}H, ^{15}N] heteronuclear single quantum coherence NMR spectroscopy and HPLC. The ring-opened species [Pt(en)(Me-Mal-*O*)(L-*S*)] (L = Ac-Met, Gly-Met or Met-Gly) containing monodentate malonate and S-bound monodentate methionine ligands were predominant in solution after 2 h. The second-order rate constant for the ring-opening reaction of **1** with Ac-Met at pH 6.56 was determined to be $(1.48 \pm 0.03) \times 10^{-1}$ s^{-1} M^{-1}, and was similar for reactions with Gly-Met. Methylmalonate α-CH deuteriation rates were determined to be free Me-Met > ring-opened complex ≫ **1**. Molecular-mechanics modelling suggested that hydrogen bonding between the free carboxylate group of monodentate Me-Mal and the co-ordinated amine groups, and between the two ring-opened ligands may contribute to the stability of the mixed-ligand adducts. However, in the case of Met-Gly, the ring-opening rate [$(5.26 \pm 0.10) \times 10^{-2}$ s^{-1} M^{-1}] was nearly three times slower than that for the reaction of **1** with Ac-Met. In contrast, the ring-closure rate of [Pt(en)(Me-Mal-*O*)(Met-Gly-*S*)] [$k_1 = (1.37 \pm 0.03) \times 10^{-4}$ s^{-1}] to give the *S*,*N*-chelated adduct was faster than that of [Pt(en)(Me-Mal-*O*)(Ac-Met-*S*)]$^-$ **2** [$(2.27 \pm 0.04) \times 10^{-5}$ s^{-1}]. The *S*,*N*-chelated adducts [Pt(en)(Ac-MetH$_{-1}$-*S*,*N*)] **3**, [Pt(en)(Gly-MetH$_{-1}$-*S*,*N*)]$^+$ and [Pt(en)(Met-GlyH$_{-1}$-*S*,*N*)]$^+$ became the predominant products of the reactions after about 24 h. Ring-opened adducts of chelated dicarboxylate platinum anticancer complexes with methionine derivatives could play a significant role in their mechanism of action.

Carboplatin [Pt$(NH_3)_2$(cbdca-*O*,*O*′)], where cbdca is cyclobutane-1,1-dicarboxylate, is a widely used second generation anticancer drug.[1,2] It is less toxic than cisplatin, and lacks significant neurotoxicity and nephrotoxicity. The activation mechanism of this drug is still under investigation.[3–5] Some studies have concluded that the reaction of carboplatin with chloride ions is too slow to account for the reported half-life of the drug in blood plasma, and so it cannot be simply a pro-drug of cisplatin.[3–5] Recent results obtained in our laboratory suggested that reactions of carboplatin with 5′-GMP (guanosine 5′-monophosphate) can occur by direct attack.[5]

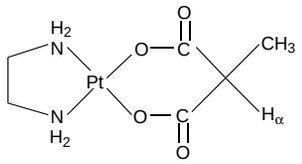
In recent years, there has been an increasing interest in the interactions between the platinum drugs and sulfur-containing molecules.[6,7] Although these interactions are generally considered to have negative (deactivation) effects on the drugs, some experiments have shown that S-bound thioethers can be substituted by guanine bases, the main DNA target for platinum, and potentially this provides a new route for DNA platination.[8,9] The thioether-containing amino acid methionine plays an important role in the metabolism of platinum anticancer drugs. The complex [Pt(Met-*S*,*N*)$_2$] has been isolated from the urine of patients treated with cisplatin,[10] and its two geometrical isomers have been separated and characterised.[11] A remarkably stable ring-opened complex [Pt(cbdca-*O*)$(NH_3)_2$(L-HMet-*S*)] has been detected during the reaction of carboplatin and L-HMet, and moreover, a similar species was found in the urine of animals treated with carboplatin.[12]

A recent *in vivo* study has shown that a mixture of cisplatin and methionine in a molar ratio of *ca*. 1:5 incubated over 24 h at 310 K is significantly cytotoxic, but lacks cisplatin-associated renal toxicity.[13] Under the reported incubation conditions, the predominant platinum(II) species present are likely to be the bis-*S*,*N*-chelated adducts, [Pt(Met-*S*,*N*)$_2$]. These data suggest that methionine adducts are not inert species devoid of cytotoxic activity. Our recent experiments have shown that under mildly acidic conditions *S*,*N*-chelated *N*-acetyl-L-methionine Pt^{II} complexes can be reactive towards the mononucleotide 5′-GMP and the dinucleotide GpG [guanylyl(3′-5′)guanosine] to form mixed-ligand complexes [Pt(en)(Ac-Met-*S*)(GpG-N^7)] (en = ethane-1,2-diamine),[14] and it is of interest to investigate further interactions between methionine-containing ligands and platinum drugs.

We report here reactions between methionine derivatives and the carboplatin analogue, [Pt(en)(Me-Mal-*O*,*O*′)] **1**. Chelated en is less readily displaced from Pt^{II} than monodentate ammine ligands. Ammonia release induced by the high *trans* influence of S can severely complicate the interpretation of reactions of cisplatin and carboplatin.[15]

Our use of 2-methylmalonate (Me-Mal) in the place of cyclobutanedicarboxylate (cbdca) allows convenient monitoring of

† *Present address:* The Department of Chemistry, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JJ, UK.
‡ *Non-SI unit employed:* M = mol dm^{-3}.



1

the biscarboxylate group *via* methyl peaks by ^{1}H NMR and the Me-Mal complex is markedly more soluble than [Pt(en)(cbdca)]. Malonate complexes of Pt–am(m)ine have been reported to exhibit substantial antitumour activity without the nephrotoxic effects of cisplatin.[16] The L-methionine derivatives investigated were *N*-acetyl-L-methionine (Ac-Met), and the dipeptides glycyl-L-methionine (Gly-Met) and L-methionylglycine (Met-Gly). We show that stable ring-opened species are readily detectable by both ^{1}H and two-dimensional [^{1}H, ^{15}N] HSQC† NMR spectroscopy.[17,18] The ring-opening mechanism and the release of the dicarboxylate ligand are also discussed.

Experimental

Materials and methods

The salts $K_2[PtCl_4]$ and $AgNO_3$ were purchased from Johnson Matthey Ltd, 2-methylmalonic acid (Me-H_2Mal), *N*-acetyl-L-methionine (Ac-Met), glycyl-L-methionine (Gly-Met) and L-methionylglycine (Met-Gly) from Sigma, ethane-1,2-diamine, $LiOH·H_2O$ and other chemicals from Aldrich, and used as supplied. The complexes [Pt(en)Cl_2] and [Pt([^{15}N]en)Cl_2] were prepared according to the reported method.[19,20]

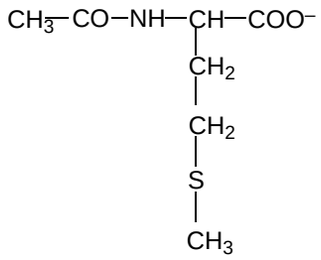
Complex **1** [Pt(en)(Me-Mal-*O*,*O*′)] was prepared according to the literature method[21] (Found: C, 19.55; H, 3.4; N, 7.5. Calc. for $C_6H_{12}N_2O_4Pt$: C, 19.4; H, 3.25; N, 7.55%). ^{13}C NMR (pH 6.60): δ 48.66 (CH_2 of en), 181.11 (CO of Me-Mal), 51.53 (α-CH), 14.38 (CH_3). The ^{1}H NMR shifts of **1** are listed in Table 1.

A 10 mM solution of [Pt([^{15}N]en)(Me-Mal-*O*,*O*′)] **1n** was prepared as follows. A stock solution of [Pt([^{15}N]en)$(H_2O)_2$]$^{2+}$ (10 mM, 1 cm^3) in 90% water–10% D_2O, prepared as reported,[19] was incubated with one mol equivalent of Me-H_2Mal (1.2 mg, 10 mmol) and 2 mol equivalents of $LiOH·H_2O$ (0.8 mg, 20 mmol) for 24 h in the dark at pH *ca.* 6.8. The two-dimensional [^{1}H, ^{15}N] HSQC NMR spectrum of the final solution contained a major cross-peak at δ 5.38/−43.24 which can be assigned to **1n** and accounted for > 85% of the total Pt. A minor cross-peak at δ 5.13/−45.03 (NH_2 *trans* to O) was also observed in the spectrum. The species giving rise to this peak was stable for hours in the presence of S-ligand (see below) and was therefore assigned to the hydroxy-bridged complex [{Pt([^{15}N]en)}$_2$(μ-OH)$_2$]$^{2+}$, which is significantly less reactive towards nucleophiles.[22,23]

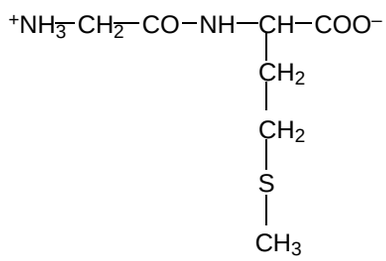
NMR Spectroscopy

The NMR spectra were recorded at 310 K, unless otherwise stated, on the following instruments: JEOL GSX270 (^{1}H 270 MHz, ^{13}C 67.5 MHz), JEOL GSX 500 (^{1}H 500 MHz), Varian Unity 500 and 600 (^{1}H 500 and 600 MHz; ^{15}N 50.7 and 60.8 MHz) using 5 mm NMR tubes. The chemical shift references were as follows (all internal except ^{15}N): ^{1}H, dioxane (δ 3.744); ^{13}C, dioxane (δ 67.3); ^{15}N (external, 1 M $^{15}NH_4Cl$ in 1.5 M HCl).

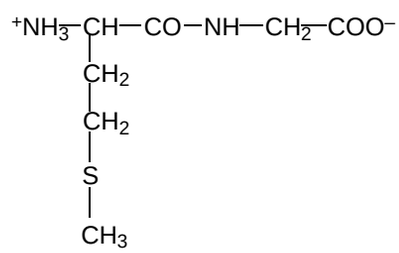
For ^{1}H NMR, typical acquisition conditions for one-dimensional spectra were as follows: 45–60° pulses, 16–32 K data points, 2–3 s relaxation delay, collection of 32–128 transients, final digital resolution of 0.2–1 Hz per point. When necessary, water suppression was achieved by presaturation. Spectra were processed using Varian VNMR software.[24] The ^{13}C-{^{1}H} NMR spectra were typically the result of 12 h acquisitions using 32 K data points, 50° pulses, and relaxation delays of 3 s. Two dimensional, [^{1}H, ^{15}N] HSQC spectra were recorded as previously described[19,25] using standard sequences, optimised for ^1J(NH) = 72 Hz, with ^{15}N decoupling *via* the GARP procedure. Water suppression was achieved by pulsed-field gradients.



Ac-Met



Gly-Met



Met-Gly

HPLC

The following equipment was used: Gilson 305 pumps, Gilson 806 manometric module, LKB 2141 variable wavelength monitor, and Rheodyne sample injector. Analytical separations were carried out on a PLRP-S column (250 × 4.6 mm, 100 Å, 5 μm, Polymer Labs) by injecting aliquots of the mixture at various time intervals with detection at 210 nm, using H_2O as the eluent. The data were analysed using Dynamax Method Manager Software.

pH Measurements

Adjustments of pH were made using NaOD or DNO_3. The measurements were carried out directly in NMR tubes, before recording spectra, using a Corning 240 pH meter equipped with an Aldrich micro combination electrode, calibrated with Aldrich buffer solutions at pH 4, 7 and 10. For D_2O solutions, the value was read directly from the pH meter without correction for deuterium isotope effects and is designated as pH*. The reported pH values are those measured at the beginning of the reactions. No buffers were used in this study in order to avoid buffer co-ordination to platinum, *e.g.* of phosphate.[5,26]

† HSQC: heteronuclear single quantum coherence; this involves inverse (^{1}H detection) of ^{15}N resonances and offers a sensitivity enhancement for ^{15}N by a factor of up to 305. The pulse sequence is described in ref. 17, and some applications to platinum am(m)ines are reviewed in ref. 18.

Table 1 Proton NMR chemical shifts for Me-Mal and its Pt^{II} complexes in D_2O

					Me-Mal*	
Compound	pH*	SCH_3	$COCH_3$	(CH_2) en	CH	CH_3
Me-Mal	3.43				3.408 (7.3)	1.357 (7.1)
	6.10				2.181 (7.5)	1.250 (7.4)
1 [Pt(en)(Me-Mal-*O*,*O*′)]	3.16			2.560	4.103 (7.3)	1.372 (7.2)
	6.50			2.562	4.103 (7.0)	1.373 (7.2)
2 [Pt(en)(Me-Mal-*O*)(Ac-Met-*S*)]$^-$	3.16	2.340	2.048	2.706 2.673	3.454 (7.3)	1.288 (7.1)
	6.56	2.335	2.037	2.697 2.679	3.259 (7.2)	1.221 (7.2)
5 [Pt(en)(Me-Mal-*O*)(Gly-Met-*S*)]	3.78	2.343		2.697 2.677	3.272 (6.5)	1.232 (7.0)
	6.66	2.343		2.700 2.679	3.254 (6.5)	1.228 (7.0)
9 [Pt(en)(Me-Mal-*O*)(Met-Gly-*S*)]	4.48	2.395		2.694	3.293 (6.9)	1.227 (7.3)

* $^3J(CH-CH_3)$ coupling constants (Hz) in parentheses.

Kinetics

The kinetic data were obtained from ^1H NMR spectra recorded at 310 K. The samples were also maintained at the same temperature whilst not in the probe. The relative concentrations were determined from peak integrals and the analysis of the data was performed using the program KALEIDAGRAPH.[27] The rate constants, where applicable, were determined by a non-linear optimisation procedure, using the appropriate equations and integrated numerically.

Molecular-mechanics modelling

Models of a number of conformers of [Pt(en)(Me-Mal-*O*)-(Ac-Met-*S*)]$^-$ **2** were generated using the HYPERCHEM program.[28] The energies of these structures were then minimised using MOMEC[29] and a force field based on others described previously,[30–32] extended using parameters from the AMBER force field[33] to enable the modelling of the *N*-acetylmethionine and methylmalonate ligands.

Sample preparation

The following reactions were investigated in NMR tubes; **1** (5 mM) + Ac-Met (5 mM) at pH* 6.56, **1** (5 mM) + Ac-Met (5 mM) at pH* 3.16, **1** (5 mM) + Gly-Met (5 mM) at pH* 6.66, **1** (5 mM) + Gly-Met (5 mM) at pH* 3.78, **1** (5 mM) + Met-Gly (5 mM) at pH* 4.48.

Samples for the above reactions were prepared as follows. Complex **1** and Ac-Met, Gly-Met or Met-Gly were weighed and dissolved separately in 0.35 cm^3 D_2O. The pH* values of the solutions were adjusted to the desired region before mixing them in the NMR tube. The final pH* of the mixture was measured in the NMR tube after mixing.

Samples for the reactions of **1n** with Ac-Met, Gly-Met or Met-Gly were in 0.6 cm^3 of 90% water–10% D_2O (5 mM) and prepared by mixing aliquots of the stock solution of **1n** (10 mM) with one mol equivalent of the methionine derivative as stated above.

Results

Deuterium exchange on methylmalonate

The CH_3 signal of Me-Mal appeared as a doublet due to coupling with the α-H, but with time this doublet decreased in intensity and became a singlet, overlapped with the low-field peak of the doublet, due to exchange of α-H with deuterium from D_2O. By measuring the variation of peak integrals with time, first-order rate constants for α-H–D exchange of Me-Mal were determined at pH 3.15 and 310 K to be $(2.4 \pm 0.4) \times 10^{-6}$ s^{-1} ($t_{\frac{1}{2}}$, 81 h) for complex **1**, $(2.03 \pm 0.06) \times 10^{-5}$ s^{-1} ($t_{\frac{1}{2}}$, 9.5 h) for the ring-opened complex **2** (see below) and $(6.11 \pm 0.01) \times 10^{-5}$ s^{-1} ($t_{\frac{1}{2}}$, 3.2 h) for free malonate.

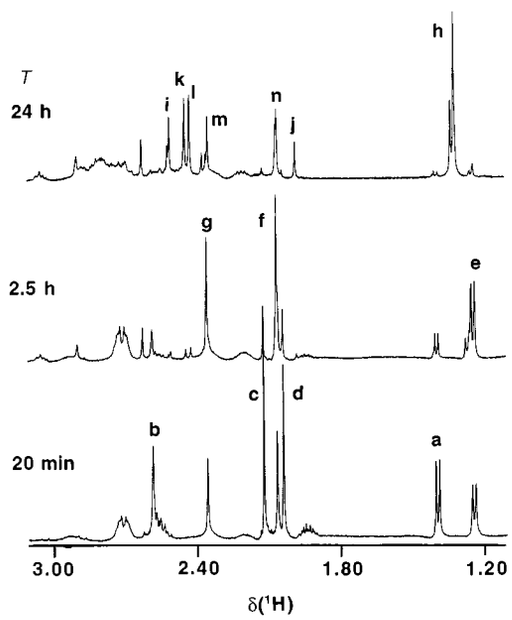


Fig. 1 The 500 MHz ^1H NMR spectra of [Pt(en)(Me-Mal-*O*,*O*′)] **1** with Ac-Met (5 mM, 1:1 molar ratio) after reaction for various times at 310 K, pH* 6.56. Peak assignments: a CH_3 and b CH_2 (en) for **1**; c SCH_3 and d acetyl CH_3 of free Ac-Met; e CH_3, f acetyl CH_3 and g SCH_3 of [Pt(en)(Me-Mal-*O*)(Ac-Met-*S*]$^-$ **2**; h CH_3 for free Me-Mal; i SCH_3 and j acetyl CH_3 of [Pt(en)(Ac-Met-*S*,*O*)]$^+$ **4**; k, l, m, n SCH_3 and acetyl CH_3 of [Pt(en)(Ac-MetH_{-1}-*S*,*N*)] **3**

Reaction of [Pt(en)(Me-Mal-*O*,*O*′)] 1 with Ac-Met (1:1, pH* 6.56)

On reaction of complex **1** with Ac-Met at 310 K for 20 min, the CH_3 doublet for **1** (δ 1.373) decreased in intensity, and a new doublet appeared at δ 1.221. At the same time the SCH_3 signal of free Ac-Met (δ 2.110) decreased in intensity and a new peak at δ 2.335 appeared and increased in intensity. After about 2 h, two new peaks at δ 1.221 (CH_3) and δ 2.335 (SCH_3) reached a maximum intensity (75% of the total integral of CH_3 peaks for different Me-Mal species), followed by a slow decrease in intensity, as shown in Fig. 1. The latter two peaks varied in intensity in the same way during the reaction course, therefore they can be assigned to the same complex. The reaction was repeated with **1n** and studied by two-dimensional [^1H, ^{15}N] HSQC spectroscopy. The cross-peak at δ 5.38/−43.24 for **1n** decreased in intensity, and new cross-peaks appeared

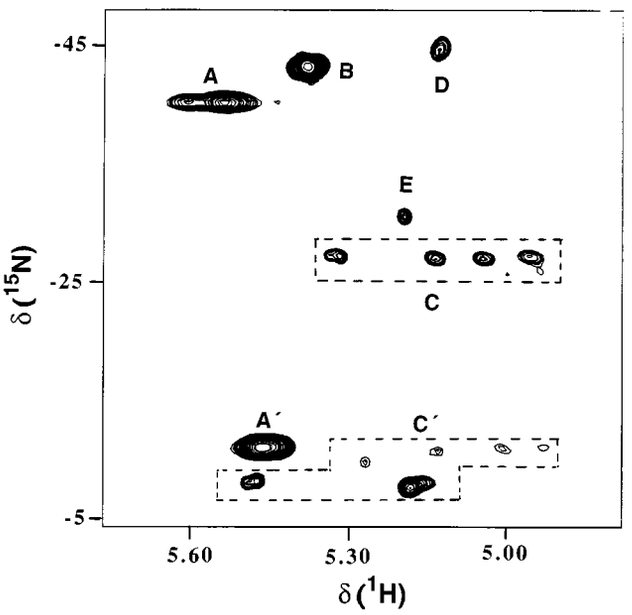
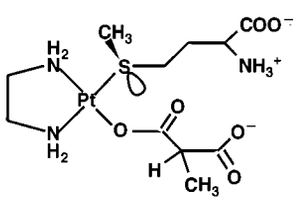


Fig. 2 A two-dimensional [^{1}H, ^{15}N] HSQC NMR spectrum of [Pt([^{15}N]en)(Me-Mal-*O*,*O*′)] with Ac-Met (5 mM, pH* 6.56, 1:1 molar ratio) recorded at 310 K after 2 h incubation. Peaks assignments: A, NH_2 (*trans* to O) and A′, NH_2 (*trans* to S) of [Pt(en)(Me-Mal-*O*)(Ac-Met-*S*)]$^-$ **2**; B, NH_2 (*trans* to O) of [Pt([^{15}N]en)(Me-Mal-*O*,*O*′)] **1**; C, NH_2 (*trans* to N) and C′, NH_2 (*trans* to S) of [Pt(en)(Ac-MetH_{-1}-*S*,*N*)] **3**. Peaks D and E for impurities {hydroxyl bridged [Pt(en)]$^{2+}$ complexes and [Pt([^{15}N]en)Cl_2)]} contained in the stock solution of complex **1**



2

at δ 5.54/−39.94 and 5.46/−10.96 and increased in intensity with time. The former can be assigned to the NH_2 group *trans* to oxygen and the latter to NH_2 *trans* to sulfur. Therefore, the data suggest the formation of the ring-opened species [Pt([^{15}N]en)(Me-Mal-*O*)(Ac-Met-*S*)]$^-$ **2**. These new two-dimensional peaks also reached a maximum intensity after 2 h incubation of the reaction mixture, so they can be correlated with the peaks at δ 1.221 and 2.335 in the ^{1}H NMR spectrum.

A two-dimensional [^{1}H, ^{15}N] HSQC spectrum of the solution that was recorded after 2 h of reaction is shown in Fig. 2. The cross-peaks at δ 5.54/−39.94 and 5.46/−10.96 assignable to complex **2** are the major peaks, together with peaks for unreacted **1**. The two peaks for **2** are very broad in the ^{1}H dimension.

In addition to the cross-peaks for complexes **1** and **2**, a series of cross-peaks is present in the region from δ 5 to 5.5/−25 to −27 (NH_2 *trans* to N) and −8 to −11 (NH_2 *trans* to S). These peaks appeared at the same time and had comparable intensities, suggesting assignment to [Pt(en)(Ac-MetH_{-1}-*S*,*N*)] **3**.

The complexity of the spectrum can be attributed to deprotonation of the amide nitrogen giving rise to *cis* and *trans* isomers, in addition to slow S inversion.[15,34] Inversion at sulfur can lead to changes in conformation of the six-membered chelate ring.[11,35,36] Correspondingly, in the ^{1}H spectrum, new peaks at δ 2.422, 2.401, 2.350 and 2.325 appeared and increased in intensity at a comparable rate to the two-dimensional cross-peaks. Hence these are also assigned to **3**. The chemical shifts of all peaks are listed in Tables 1 and 2.

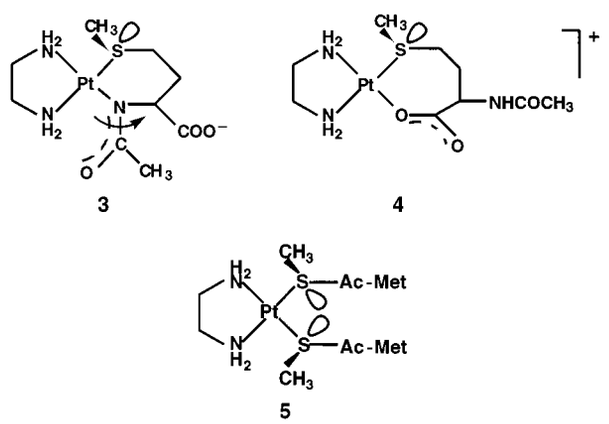
After 24 h, all the Ac-Met had reacted since no peaks for free Ac-Met were observable in the ^{1}H NMR spectrum. As shown in Fig. 1, the peaks at δ 1.221 and 2.335 assigned to complex **2** had nearly disappeared and peaks for complex **3** became dominant. Free Me-Mal accounted for 90% of the total Me-Mal species observed. The CH_3 signal for free Me-Mal became a singlet due to deuterium exchange of the α-H signal with D_2O. At this time, peaks at δ 2.494, 2.486 and 1.960 were also observed in the spectrum. Consequently, in the two-dimensional [^{1}H, ^{15}N] HSQC spectrum recorded after a similar reaction time, cross-peaks at δ 5.95, 5.81, 5.70, 5.60/−43.0 (NH_2 *trans* to O) and δ 5.88, 5.42/−6.2 (NH_2 *trans* to S) had comparable intensity. These data are consistent with the formation of [Pt(en)(Ac-Met-*S*,*O*)]$^+$ **4**.[14] The peaks assigned to complexes **3** and **4** accounted for 90% of the total Pt species. In the two-dimensional [^{1}H, ^{15}N] HSQC NMR spectrum, a minor cross-peak at δ 6.01/−5.39 (NH_2 *trans* to S) was also present and can be assigned to the bis-(Ac-Met) complex [Pt(en)(Ac-Met-*S*)$_2$] **5**.[14]

Kinetic fits to the reaction profile in Scheme 1 are shown in Fig. 3, and the rate constants for each step are listed in Table 3.

Table 2 Proton and ^{15}N NMR Pt–NH_2 chemical shifts for [Pt([^{15}N]en)]$^{2+}$ complexes

Compound	pH	δ(^{1}H)	δ(^{15}N) (*trans* to)
1 [Pt(en)(Me-Mal-*O*,*O*′)]	6.60	5.38	−43.24 (O)
2 [Pt(en)(Me-Mal-*O*)-(Ac-Met-*S*)]$^-$	6.56	5.54	−39.94 (O)
		5.46	−10.96 (S)
3 [Pt(en)(Ac-Met-H_{-1}-*N*,*S*)]	6.56	5.47, 5.17	−8.08 (S)
		5.13	−10.55 (S)
		5.01, 4.93	−11.00 (S)
		5.33, 5.14, 5.05, 4.96	−27.25 (N)
		5.23, 4.86	−26.32 (N)
		5.01, 4.94	−25.85 (N)
4 [Pt(en)(Ac-Met-*O*,*S*)]$^+$	6.56	5.95, 5.81, 5.70, 5.60	−43 (O)
		5.88, 5.42	−6.20 (S)
5 [Pt(en)(Ac-Met-*S*)$_2$]	6.56	6.01	−5.39 (S)
6 [Pt(en)(Me-Mal-*O*)-(Gly-Met-*S*)]	6.66	5.54	−39.93 (O)
		5.45	−10.84 (S)
8 [Pt(en)(Gly-MetH_{-1}-*N*,*S*)]$^+$*	6.66	5.44, 5.29	−8.16 (S)
		5.34, 5.19	−7.83 (S)
		5.43, 5.07	−25.79 (N)
		5.25, 5.19	−25.63 (N)
		5.17, 4.94	−27.54 (N)
		5.12, 5.06, 5.02	−28.09 (N)
7 [Pt(en)(Gly-Met-*S*)$_2$]$^{2+}$	7.40	6.04	−5.50 (S)
10 [Pt(en)(Met-GlyH_{-1}-*N*,*S*)]$^+$	4.48	5.51, 5.46	−7.77 (S)
		5.50, 5.47	−25.75 (N)
		5.56, 5.39	−25.94 (N)

* Tentative assignments.

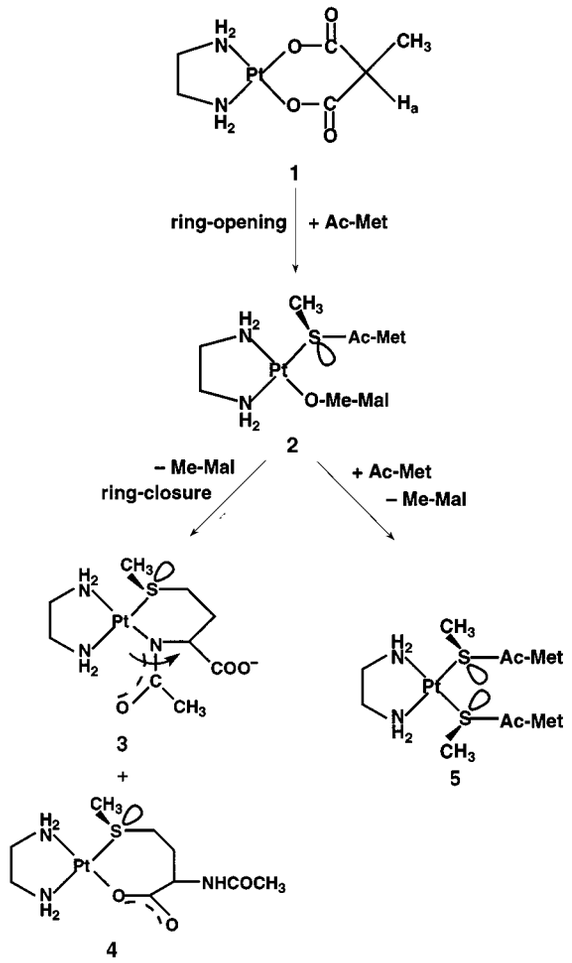


3 **4**

5

Reaction of [Pt(en)(Me-Mal-*O*,*O*′)] 1 with Ac-Met (1:1, pH* 3.16)

The reaction of **1** with Ac-Met at the lower pH* value of 3.16 was also followed by ^{1}H NMR spectroscopy. After 20 min, the



Scheme 1

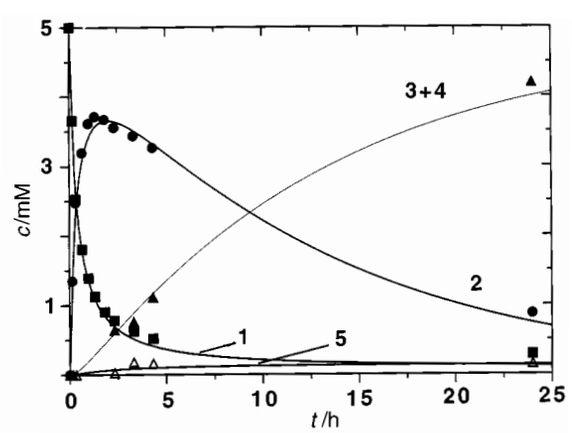


Fig. 3 Kinetic fits for the reaction of [Pt(en)(Me-Mal-*O*,*O*′)] **1** with Ac-Met (5 mM, 1:1 molar ratio) at 310 K and pH 6.56. The curves correspond to the rate constants listed in Table 3

methyl doublet of **1** at δ 1.372 (CH_3) and the SCH_3 signal for free Ac-Met at δ 2.110 had decreased in intensity, whilst peaks at δ 1.288 (doublet) and 2.340 appeared and increased in intensity. The latter peaks can again be assigned to the ring-opened adduct [Pt(en)(Me-Mal-*O*)(Ac-Met-*S*)]$^-$ **2** (the charge on the Ac-Met carboxylate group is still assumed to be −1 although the pK_a was not determined). In parallel with the reaction at pH 6.56, complex **2** became the dominant species after about 2 h (60% of the total Me-Mal). Then, the monodentate Me-Mal ligand was gradually displaced to give *S*,*N* or *S*,*O* chelation of Ac-Met with formation of complexes **3** and **4**. After 22 h these became the dominant products, together with the formation of complex **5** as a minor product. The time-course of

Table 3 Observed rate constants for the reactions of [Pt(en)(Me-Mal-*O*,*O*′)] **1** (5 mM) with Ac-Met (pH 6.56) and Met-Gly (pH 4.48) at 310 K. The rate constants of the reactions of carboplatin with L-HMet, 5′-GMP and chloride are listed for comparison

Nucleophile	Ring-opening reaction (k/M^{-1} s^{-1})	Displacement of Me-Mal or cbdca (k/s^{-1})
Reactions for **1**		
Ac-Met	$(1.48 \pm 0.03) \times 10^{-1}$	$(2.27 \pm 0.04) \times 10^{-5}$ [a]
Met-Gly	$(5.26 \pm 0.10) \times 10^{-2}$	$(1.37 \pm 0.03) \times 10^{-4}$
Reactions for carboplatin		
L-HMet	27×10^{-3}	6.9×10^{-6} [b]
5′-GMP	1.0×10^{-4} [c]	$(3.2 \pm 0.5) \times 10^{-5}$ [d]
Chloride		7.7×10^{-7} [d], 2.1×10^{-7} [e]

[a] The ring-opened adduct **2** (see Scheme 1) reacted with 2 mol equivalents of AC-Met to give [Pt(en)(Ac-Met-*S*)$_2$] as product; the rate was $(2.05 \pm 0.35) \times 10^{-3}$ M^{-1} s^{-1}. [b] Ref. 15. [c] Recalculated from the data of ref. 15. [d] Ref. 5. [e] Extrapolated to 310 K from values of ref. 4 at 298 K.

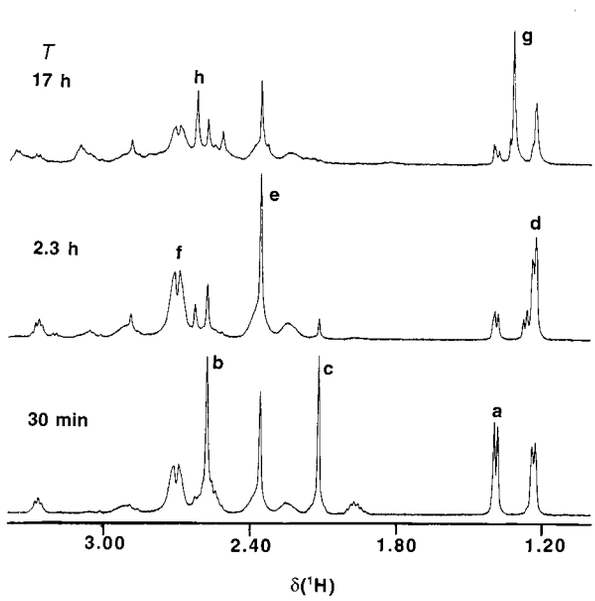


Fig. 4 The 500 MHz ^{1}H NMR spectra of [Pt(en)(Me-Mal-*O*,*O*′)] **1** with Gly-Met (5 mM, 1:1 molar ratio) at 310 K and pH 6.66. Peak assignments: a CH_3 and b CH_2 (en) for **1**; c SCH_3 of free Gly-Met; d CH_3, e SCH_3 and f CH_2 (en) of [Pt(en)(Me-Mal-*O*)(Gly-Met-*S*)] **6**; g CH_3 of free Me-Mal; h SCH_3 of [Pt(en)(Gly-Met-*S*)$_2$]$^{2+}$ **7**

the ring-opening reaction at this pH (data not shown) is quite similar to that observed at pH 6.56. However, the ring-opened adduct **2** was observed for a significantly longer time in solution at pH 3.16.

Reaction of [Pt(en)(Me-Mal-*O*,*O*′)] 1 with Gly-Met (1:1, pH* 6.66)

In order to study the influence of methionine substituents on ring opening, the reaction of **1** with the dipeptide Gly-Met was followed by ^{1}H and two-dimensional [^{1}H, ^{15}N] HSQC spectroscopy. When **1** was incubated with Gly-Met for about 30 min, the Me-Mal CH_3 peak of **1** at δ 1.372 and the SCH_3 peak of free Gly-Met (δ 2.103) decreased in intensity. New peaks at δ 1.228 (doublet) and 2.343 appeared and increased in intensity with time (Fig. 4). These peaks attained maximum intensities after about 2 h (63% of the total integral of the CH_3 signals for Me-Mal species), remained at the same intensity for another few hours and then began to decrease in intensity. At the corresponding times in the reaction of **1n** with Gly-Met, the two-dimensional [^{1}H, ^{15}N] HSQC spectrum showed major cross-peaks at δ 5.54/−39.93 (NH_2 *trans* to O) and 5.45/−10.84

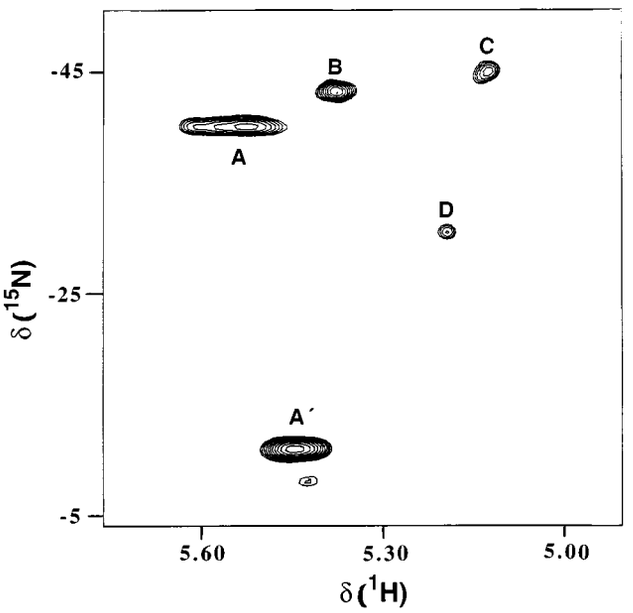
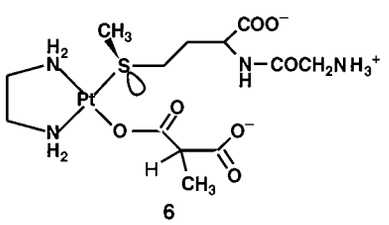
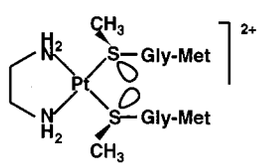


Fig. 5 A two-dimensional [^{1}H, ^{15}N] HSQC NMR spectrum of [Pt([^{15}N]en)(Me-Mal-*O*,*O*′)] with Gly-Met (5 mM, pH 6.66, 1:1 molar ratio) recorded at 310 K after 2 h incubation. Peak assignments: A, NH_2 (*trans* to O) and A′, NH_2 (*trans* to S) of [Pt(en)(Me-Mal-*O*)(Gly-Met-*S*)] **6**; B, NH_2 (*trans* to O) of [Pt([^{15}N]en)(Me-Mal-*O*,*O*′)] **1**; peaks C and D are due to impurities {hydroxo bridged $[Pt(en)]^{2+}$ complexes and [Pt([^{15}N]en)Cl_2)]} contained in the stock solution of complex **1**



6



7

(NH_2 *trans* to S). Therefore, these data suggest the formation of the ring-opened adduct [Pt([^{15}N]en)(Me-Mal-*O*)(Gly-Met-*S*)] **6**. A two-dimensional [^{1}H, ^{15}N] HSQC spectrum recorded after 2.5 h of reaction at 310 K is shown in Fig. 5, and the ring-opened species **6** can be seen to be the major product.

After 17 h of reaction, complex **6** still accounted for about 40% of the total Me-Mal species present in solution, indicative of the high stability of this complex. A two-dimensional [^{1}H, ^{15}N] HSQC spectrum recorded after 17 h was rather complicated. In addition to cross-peaks assignable to **1** and **6**, two series of cross-peaks distributed in the regions for NH_2 *trans* to S and N were observed. A single cross-peak at δ 6.04/−5.50 (NH_2 *trans* to S) was assignable to $[Pt([^{15}N]en)(Gly\text{-}Met\text{-}S)_2]^{2+}$ **7** since it had very similar chemical shifts to those of [Pt([^{15}N]en)(Ac-Met-*S*)$_2$] **5**, whereas, on the basis of their intensities and shifts, the four cross-peaks at δ 5.44, 5.29, 5.34, 5.19/−7.8 to −8.2 (NH_2 *trans* to S) and nine cross-peaks at δ 5.43, 5.07, 5.25, 5.19, 5.17, 4.94, 5.12, 5.06, 5.02/−25.6 to −28.1 (NH_2 *trans* to N) can be tentatively assigned to the *S*,*N*-chelated Gly-Met complex $[Pt([^{15}N]en)(Gly\text{-}MetH_{-1}\text{-}N,S)]^+$ **8**, an analogue of **3**. The complexity of the spectrum of **8** may be due to the presence of diastereoisomers arising from the presence of

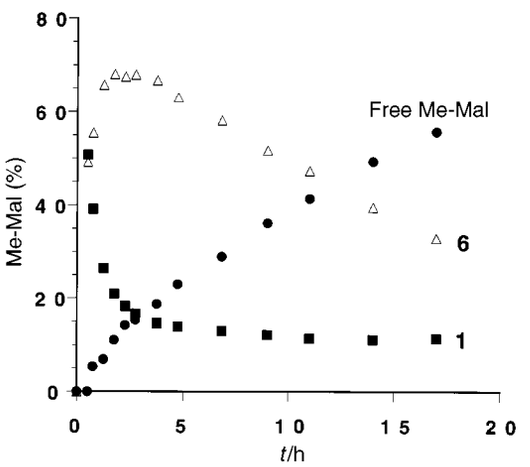
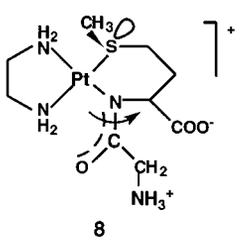


Fig. 6 Plot of relative percentages of Me-Mal species (based on the CH_3 peak integrals) for the reaction of [Pt(en)(Me-Mal-*O*,*O*′)] **1** with Gly-Met (5 mM, 1:1 molar ratio) at 310 K and pH 6.66. The mixed ligand adduct [Pt(en)(Me-Mal-*O*)(Gly-Met-*S*)] **6** accounted for 40% of the Me-Mal adducts after 17 h. A kinetic fit to the data was not possible (see text)



8

chiral co-ordinated S, as well as the *cis*- and *trans*-isomers arising from the peptide bond.[14,34]

There were still five cross-peaks remaining to be assigned at δ 5.03, 4.97, 4.92/−30.45 (NH_2 *trans* to N) and 5.05, 4.93/−45.29 (NH_2 *trans* to O) (see Discussion). The time-course of the reaction is shown in Fig. 6, obtained by plotting the variation in intensity of the resonances for the different Me-Mal species.

The reaction between [Pt(en)(Me-Mal-*O*,*O*′)] **1** and Gly-Met (1:1, 5 mM, pH 6.61, 303 K) was followed also by HPLC (Fig. 7). The dipeptide Gly-Met and **1** have retention times of 2.10 and 2.45 min, respectively. After 30 min of reaction, a new peak with a retention time of 1.65 min appeared in the chromatogram along with peaks for **1** and Gly-Met. Over the next few hours the new peak gradually increased in intensity while the peaks due to **1** and Gly-Met decreased in intensity. By comparing HPLC and NMR peak intensities with time, it is evident that the new HPLC peak can be assigned to the ring-opened complex [Pt(en)(Me-Mal-*O*)(Gly-Met-*S*)] **6**. A very broad peak with retention time of 2.80 min also appeared in the chromatogram after about 4 h incubation. The time of appearance corresponded to the formation of $[Pt(en)(Gly\text{-}MetH_{-1}\text{-}N,S)]^+$ **8** and [Pt([^{15}N]en)(Gly-Met-*S*)$_2$] **7**. After 24 h of reaction, the mixture gave a chromatogram in which peaks for **1** and Gly-Met had nearly disappeared, and the broad peak due to **7** and **8** was dominant. However, the ring-opened species was still present. At the same time, another broad peak appeared with a retention time of 4.4 min. This peak may correspond to the unassigned cross-peaks in the two-dimensional NMR spectrum.

Reaction of [Pt(en)(Me-Mal-*O*,*O*′)] 1 with Gly-Met (1:1, pH* 3.78)

This reaction was followed by ^{1}H NMR spectroscopy. At this pH*, the Me-Mal CH_3 and the Gly-Met SCH_3 signals of the

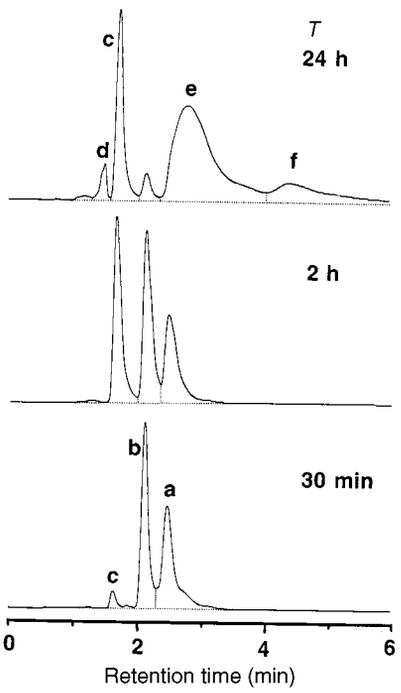
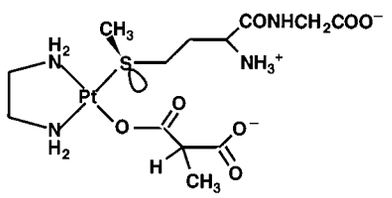


Fig. 7 The HPLC chromatograms of the reaction of [Pt(en)(Me-Mal-*O*,*O*′)] **1** with Gly-Met (5 mM, 1:1 molar ratio) at 303 K and pH 6.61. Peak assignments: a Gly-Met; b [Pt(en)(Me-Mal-*O*,*O*′)] **1**; c [Pt(en)(Me-Mal-*O*)(Gly-Met-*S*)] **6**; d free Me-Mal; e tentatively assigned to [Pt(en)(Gly-MetH$_{-1}$-*N*,*S*)]$^{+}$ **8** and [Pt(en)(Gly-Met-*S*)$_2$]$^{2+}$ **7** (by comparison with the peaks in the two-dimensional [^{1}H, ^{15}N] HSQC NMR spectra at similar incubation time); f unassigned species



9

ring-opened complex **6** were observed at δ 1.232 and 2.343, respectively. These peaks rose to maximum intensity (62% of the total integral of CH_3 signals for Me-Mal species) after 2 h of reaction, followed by a gradual decrease in intensity. Peaks for free Me-Mal were observed after 40 min. After 20 h, complex **6** still accounted for over 20% of the total Me-Mal. The time-course of the reaction was similar to that at pH 6.66.

Reaction of [Pt(en)(Me-Mal-*O*,*O*′)] 1 with Met-Gly (1:1, pH* 4.48)

This reaction was studied by ^{1}H and two-dimensional [^{1}H, ^{15}N] HSQC NMR spectroscopy. The SCH_3 signal of free Met-Gly was observed at δ 2.120. After 30 min reaction of **1** with Met-Gly, new peaks appeared at δ 1.227 (CH_3) and 2.395 (SCH_3). Since these peaks have very similar shifts to those previously assigned to ring-opened complex as **2** and **6**, they can be assigned to the complex [Pt(en)(Me-Mal-*O*)(Met-Gly-*S*)] **9**. In contrast to the reactions of **1** with Ac-Met and Gly-Met, the signal for free Me-Mal appeared concomitantly with that for the ring-opened species. After 1 h, peaks for complex **9** reached a maximum intensity (36% of the total integral of Me-Mal CH_3 signals for different species); however, 20% of the total Me-Mal was present as free Me-Mal. After 12 h incubation, complex **9** had nearly disappeared, which suggested facile *S*,*N*-chelation of Met-Gly.

A two-dimensional [^{1}H, ^{15}N] HSQC NMR spectrum was

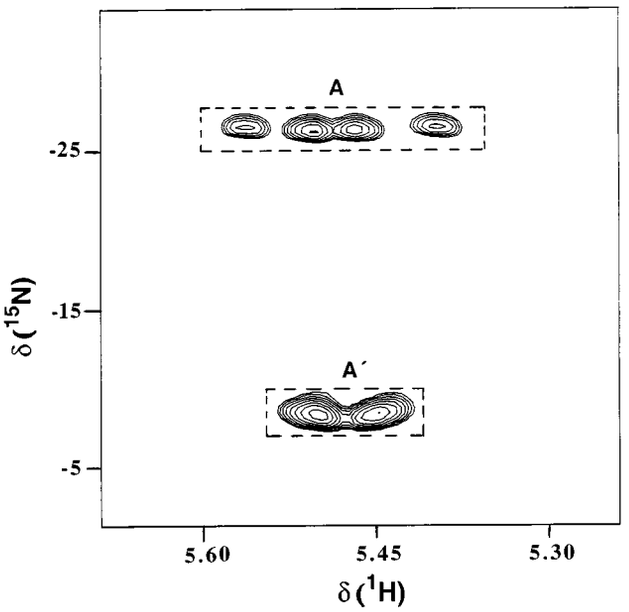
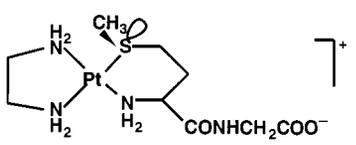


Fig. 8 A two-dimensional [^{1}H, ^{15}N] HSQC NMR spectrum of [Pt([^{15}N]en)(Me-Mal-*O*,*O*′)] **1n** with Gly-Met (5 mM, pH 4.48, 1:1 molar ratio) recorded at 310 K after 24 h incubation. Peak assignments: A, NH_2 (*trans* to N) and A′, NH_2 (*trans* to S) of [Pt(en)(Met-GlyH$_{-1}$-*N*,*S*)]$^{+}$ **10**

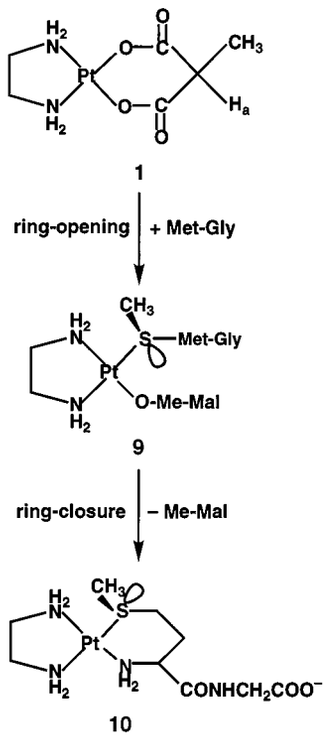


10

recorded at 310 K after 24 h reaction and is shown in Fig. 8. A total of six cross-peaks are observed, among them four peaks at δ 5.50, 5.47/−25.75 and 5.56, 5.39/−25.94 are assignable to the NH_2 *trans* to nitrogen, and two peaks at δ 5.51, 5.46/−7.77 are assignable to the NH_2 *trans* to sulfur. Therefore, the data suggest the formation of [Pt([^{15}N]en)(Met-GlyH$_{-1}$-*N*,*S*)]$^{+}$ **10**. No other peaks were observed in the spectrum. The kinetic fits to the reaction course in Scheme 2 are shown in Fig. 9, and the rate constants for each step are shown in Table 3.

Molecular modelling of [Pt(en)(Me-Mal-*O*)(Ac-Met-*S*)]$^-$ 2

Since the ring-opened mixed-ligand complexes were long-lived, *e.g.* the complex [Pt(en)(Me-Mal-*O*)(Ac-Met-*S*)]$^-$ **2** had a half life of 8.5 h at 310 K, we investigated the potential for hydrogen-bonding networks to contribute towards their stabilities in models of such intermediates. Monodentate co-ordination of both the *N*-acetyl-L-methionine and 2-methylmalonate ligands results in a complex with many degrees of conformational freedom, and very many sensible geometries can be envisaged. Therefore we chose to restrict our attention to those conformations that maximised the possibilities for intracomplex hydrogen-bond formation. Four of these were considered in detail, the two shown in Fig. 10, a third similar to that in Fig. 10(*a*) in which the hydrogen bond between the co-ordinated carboxylate group of the methylmalonate ligand and the ethane-1,2-diamine is missing, and a fourth in which there was a potential hydrogen bond between the free carboxylate groups of the two ligands. This latter model required protonation of the carboxylate groups but was found to result in severe distortion of the ligands and of the co-ordination geometry about the Pt. The other three models all had similar minimised strain energies, as might be expected given the flexibility of the ligands. The structure shown in Fig. 10(*a*) has hydrogen bonds between each of the free carboxylate groups, one from each ligand, and



Scheme 2

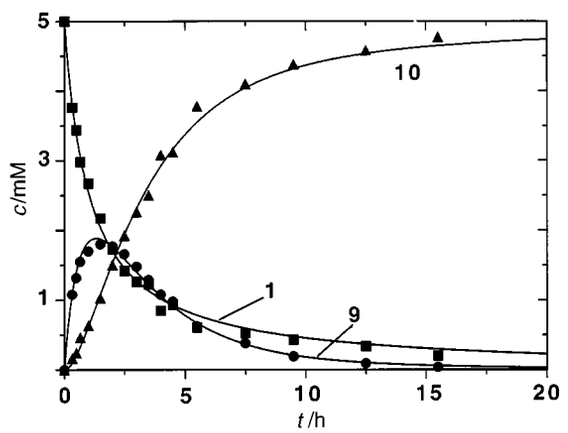
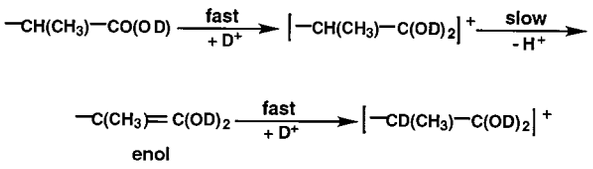


Fig. 9 Kinetic fits for reaction of [Pt(en)(Me-Mal-*O*,*O*′)] **1** with Met-Gly (5 mM, 1:1 molar ratio) at 310 K and pH 4.48. The curves correspond to the rate constants given in Table 3

the two co-ordinated amine groups. That shown in Fig. 10(*b*) has a hydrogen bond between the free carboxylate group of the methylmalonate ligand and the peptide hydrogen atom. There are also close contacts between the co-ordinated carboxylate group and the two hydrogen atoms on the adjacent amine.

Discussion

The stable ring-opened complex [$Pt(NH_3)_2$(cbdca-*O*)(L-HMet-*S*)] has been detected during reactions of carboplatin with L-HMet, and a similar species appears to be present as metabolite in the urine of animals treated with carboplatin.[12] Therefore investigations of reactions between dicarboxylate Pt^{II} complexes and methionine derivatives may lead to a better understanding of activation mechanisms for this class of drug. However, reactions of complexes containing ammine ligands are complicated by the strong *trans* effect of sulfur which promotes the labilisation of the ammine ligands.[15] Therefore the chelating ethane-1,2-diamine ligand was chosen in this work, and in addition the presence of the methyl group in the malonate ligand made it possible to follow the reactions easily by ^{1}H NMR spectroscopy.



Scheme 3

Deuteriation of methylmalonate

The exchange of the α-H of Me-Mal with deuterium has been reported to proceed *via* two steps with the enol form of the acid as an intermediate (Scheme 3).[37,38] The enolisation rate constant has been previously determined by ^{1}H NMR in 1 M D_2SO_4–D_2O to be $(5.7 \pm 0.1) \times 10^{-5}$ s^{-1} for Me-H_2Mal itself at 298 K.[37] This rate is accelerated by increasing the acidity of the medium. Considering the differences in the conditions (pH 3.15, 310 K), the rate constant determined in this work observed for Me-H_2Mal [$(6.11 \pm 0.01) \times 10^{-5}$ s^{-1}] is remarkably similar to the reported value. The exchange rate for [Pt(en)(Me-Mal-*O*)(Ac-Met-*S*)]$^-$ **2** is three times lower than for free Me-H_2Mal, and for [Pt(en)(Me-Mal-*O*,*O*′)] **1** is *ca.* 25 times slower. Protonation of the carboxylate group would be expected to be less favourable for the platinated adducts compared to free Me-H_2Mal and enol formation by the intermediate requires a change in the conformation of the six-membered chelate rings. Both of the factors may play a role in slowing down the rate.

Chelate-ring opening

Only a few kinetic data are available on ring-opening reactions of chelated dicarboxylate complexes: those obtained in the present work, and data for carboplatin.[15] These are collected in Table 3. It can be seen that the rate of ring opening is dependent on the entering ligand suggesting differences in interactions within the expected five-co-ordinate intermediate.

The second step, displacement of the dicarboxylate ligand is again faster for the Met-Gly adduct, [Pt(en)(Me-Mal-*O*)(Met-Gly-*S*)] **9** than for that of Ac-Met. The dipeptide Met-Gly can more readily form a six-membered *S*,*N*-chelate than Ac-Met, although the two reactions were conducted at different pH values.

The first step of the reaction of **1** with Gly-Met is similar to that with Ac-Met, *i.e.* the formation of the ring-opened species [Pt(en)(Me-Mal-*O*)(Gly-Met-*S*)] **6**. However, the kinetic data (not shown) could not be fitted by the same reaction scheme (Scheme 1), presumably because of a back reaction involving re-closure of the dicarboxylate chelate ring. In the case of Gly-Met an *S*,*N*-ring cannot readily form unless the peptide NH is deprotonated. Indeed, the same situation was found for reactions of Ac-Met at pH 3.16, although the chelate of Ac-Met was favoured at high pH (6.56). Thus, it appears that the NH_3^+ group in Gly-Met hinders amide deprotonation and *S*,*N*-chelation. The ring-opened complex species [Pt(en)(Me-Mal-*O*)(Gly-Met-*S*)] **6** appeared to react further by several different pathways to give a mixture of products, perhaps involving polymeric species cross-linked *via* CO_2^- and/or NH_2 groups of Gly-Met giving broad ^{1}H NMR peaks (Fig. 4) and HPLC peaks (Fig. 7). Correspondingly, in the two-dimensional [^{1}H, ^{15}N] HSQC spectrum extra peaks that are assignable to a NH_2 group *trans* to oxygen and *trans* to nitrogen were observed. No signals for free ethane-1,2-diamine were observed which rules out displacement of the diamine ligand.

In general the number of resonances for the NH_2 groups was consistent with rapid S inversion in ring-opened intermediates and slower inversion in *S*,*N*-chelates.[14]

The molecular-mechanics calculations show clearly that a number of intracomplex hydrogen bonds is feasible; these interactions would contribute to the stability of this ring-

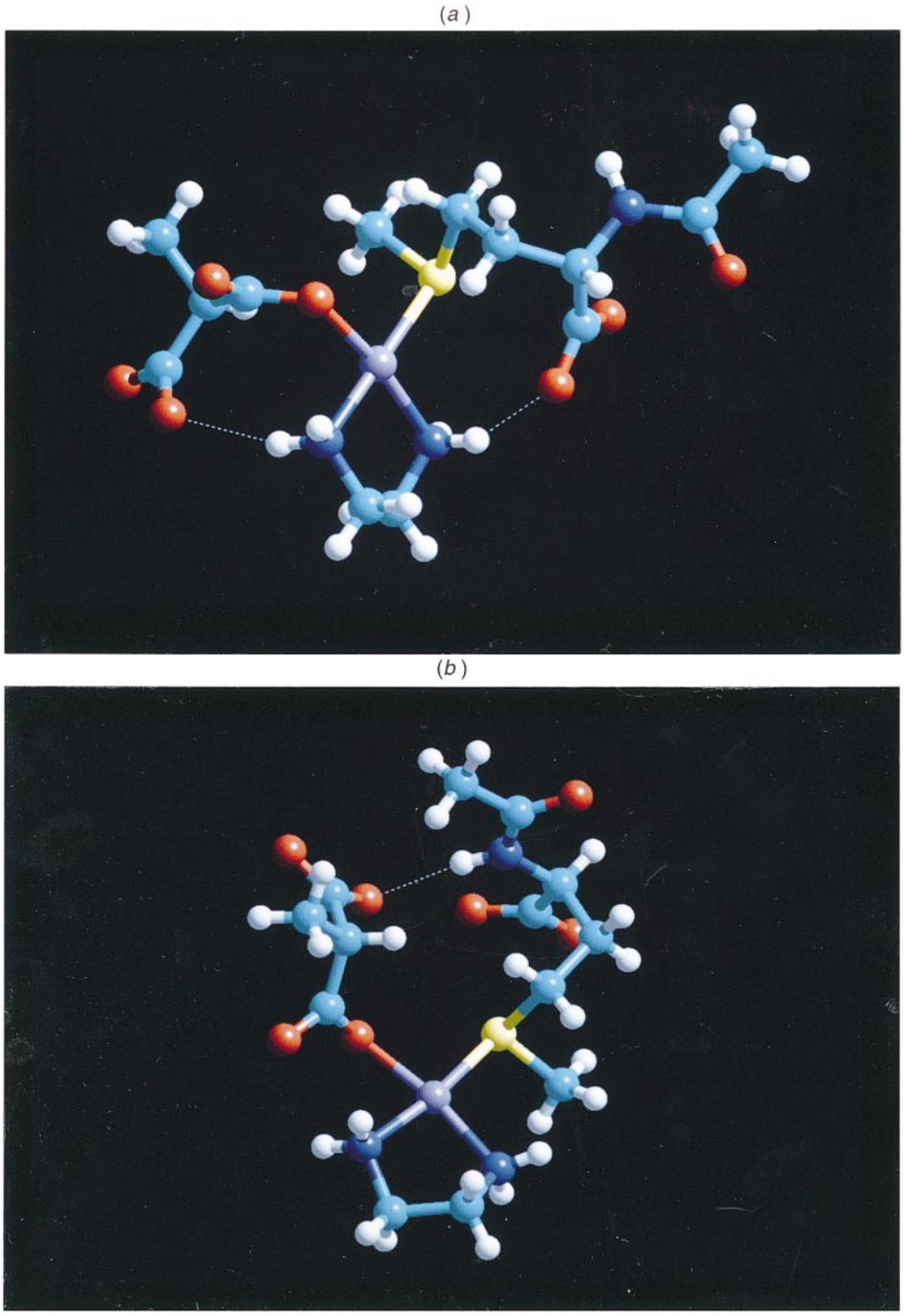


Fig. 10 Models showing the hydrogen bonding which may be involved in the stabilisation of the ring-opened complex [Pt(en)(Me-Mal-*O*)(Ac-Met-*S*)]$^-$: C, cyan; H, white; N, blue; O, red; S, yellow; Pt, violet. Model (*a*) shows the hydrogen bonding between the two amine groups and the carboxylate group of Me-Mal, and model (*b*) shows the hydrogen bonding between the carboxylate group of Me-Mal and the amide group of Ac-Met

opened complex. It is noteworthy in the context of the greater stability of the closely related complex [Pt(cbdca-*O*,*O*′)(HMet-S)(NH_3)$_2$] that similar hydrogen bonding arrangements are possible in the two complexes,[15] but the relatively weak interligand hydrogen bond between the amide group and the free carboxylate as shown in Fig. 10(*b*) would be replaced by two stronger hydrogen bonds between a protonated NH_3^+ group and a free carboxylate group in the methionine complex.

Conclusion

The second generation drug carboplatin is relatively inert towards hydrolysis, and direct attack by DNA bases occurs only very slowly.[5] Chelate-ring opening reactions provide a possible mechanism for *in vivo* activation of carboplatin, especially *via* reactions with methionine derivatives. We have studied chelate-ring-opening of the analogue [Pt(en)(Me-Mal-*O*,*O*′)] in the reactions with *N*-acetyl-L-methionine and dipeptides of L-methionine. These serve as models of potential interactions of platinum drugs with proteins and the formation of DNA–protein cross-links. Platination of Me-Mal dramatically slowed down the rate of α-H exchange with deuterium.

The ring-opened adducts were surprisingly stable and [Pt(en)(Me-Mal-*O*)(Ac-Met-*S*)]$^-$ **2**, for example, had a half-life of 8.5 h at 310 K (body temperature). Intramolecular hydrogen bonding, as suggested by the molecular-mechanics modelling, may contribute to the stability of such ring-opened complexes. The least stable ring-opened intermediates contained poten-

tially *S*,*N*H_2-chelating ligands such as Met-Gly. Hence, methionine residues in the middle of peptide chains may give rise to relatively stable ring-opened adducts of bis(chelated) dicarboxylate Pt^{II} complexes, whereas reactions with N-terminal methionine residues may lead to stable *S*,*N*-chelated species. The latter species may undergo further activation or deactivation with amine release due to the high *trans* influence of sulfur.

Acknowledgements

We thank the Association for International Cancer Research, Biotechnology and Biological Sciences Research Council, and Engineering and Physical Sciences Research Council for their support for this work. We thank the Medical Research Council Biomedical NMR centre, Mill Hill and University of London Intercollegiate Research Service for the provision of NMR facilities and Dr. Kevin J. Barnham for discussion.

References

1 *Platinum and Other Metal Complexes in Cancer Chemotherapy*, ed. M. Nicolini, Martinus Nijhoff, Boston, 1988.
2 *Platinum and Other Metal Complexes in Cancer Chemotherapy*, ed. S. B. Howell, Plenum Press, New York, 1991.
3 M. A. Allsopp, G. J. Sewell, C. G. Rowland, C. M. Riley and R. L. Schowen, *Int. J. Pharm.*, 1991, **69**, 197.
4 L. Canovese, L. Cattalini, G. Chess and M. L. Tobe, *J. Chem. Soc., Dalton Trans.*, 1988, 2135.
5 U. Frey, J. D. Ranford and P. J. Sadler, *Inorg. Chem.*, 1993, **32**, 1333.
6 P. J. Sadler, *Adv. Inorg. Chem.*, 1991, **36**, 1.
7 E. L. M. Lempers and J. Reedijk, *Adv. Inorg. Chem.*, 1991, **37**, 175.
8 K. J. Barnham, M. I. Djuran, P. del S. Murdoch and P. J. Sadler, *J. Chem. Soc., Chem. Commun.*, 1994, 721.
9 S. S. G. E. van Boom and J. Reedijk, *J. Chem. Soc., Chem. Commun.*, 1993, 1397.
10 C. M. Riley, L. A. Sternson, A. J. Repta and S. A. Slyter, *Anal. Biochem.*, 1983, **130**, 203.
11 P. del. S. Murdoch, J. D. Ranford, P. J. Sadler and S. J. Berners-Price, *Inorg. Chem.*, 1993, **32**, 2249.
12 K. J. Barnham, U. Frey, P. del. S. Murdoch, J. D. Ranford, P. J. Sadler and D. R. Newell, *J. Am. Chem. Soc.*, 1994, **116**, 11175.
13 P. M. Deegan, I. S. Pratt and M. P. Ryan, *Toxicology*, 1994, **89**, 1.
14 K. J. Barnham, Z. Guo and P. J. Sadler, *J. Chem. Soc., Dalton Trans.*, 1996, 2867.
15 K. J. Barnham, M. I. Djuran, P. del S. Murdoch, J. D. Ranford and P. J. Sadler, *Inorg. Chem.*, 1996, **35**, 1065.
16 M. J. Cleare, *The Proceedings of the Third International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy*, Leland Fikes Foundation Press, Dallas, 1977, 1; J. M. Hill, E. Loeb, A. S. Pardue, A. Khan, N. O. Hill, J. J. King and R. W. Hill, *The Proceedings of the Third International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy*, Leland Fikes Foundation Press, Dallas, 1977, 681.
17 J. Stonehouse, G. L. Shaw, J. Keeler and E. D. Laue, *J. Magn. Reson., Ser. A*, 1994, **107**, 178.
18 S. J. Berners-Price and P. J. Sadler, *Coord. Chem. Rev.*, 1996, **151**, 1.
19 S. J. Berners-Price, U. Frey, J. D. Ranford and P. J. Sadler, *J. Am. Chem. Soc.*, 1993, **115**, 8649.
20 S. J. S. Kerrison and P. J. Sadler, *J. Chem. Soc., Chem. Commun.*, 1977, 861.
21 A. Pasini and C. Caldirola, *Inorg. Chim. Acta*, 1988, **151**, 19.
22 C. J. Boreham, J. A. Broomhead and D. P. Fairlie, *Aust. J. Chem.*, 1981, **34**, 659.
23 D. P. Fairlie and M. W. Whitehouse, *Biochem. Pharmacol.*, 1982, **31**, 933.
24 VNMR 4.3, Varian Nuclear Magnetic Resonance Instruments, Palo Alto, CA.
25 S. J. Berners-Price, T. A. Frenkiel, U. Frey, J. D. Ranford and P. J. Sadler, *J. Chem. Soc., Dalton Trans.*, 1992, 2137.
26 E. L. M. Lempers, M. J. Bloemink and J. Reedijk, *Inorg. Chem.*, 1991, **30**, 201.
27 KALEIDAGRAPH, Synergy Software, Reading, PA, 1994.
28 HYPERCHEM, release 4.5, Hypercube Inc., Ontario.
29 P. Comba, T. W. Hambley and N. Okon, MOMEC, A Strain Energy Minimization Package adapted to HYPERCHEM, Altenhoff and Schmitz, Dortmund, 1995.
30 T. W. Hambley, *Inorg. Chem.*, 1988, **30**, 1073.
31 T. W. Hambley, *Inorg. Chem.*, 1991, **30**, 937.
32 E. C. L. Ling, G. W. Allen and T. W. Hambley, *J. Chem. Soc., Dalton Trans.*, 1993, 3705.
33 S. J. Weiner, P. A. Kollman, D. A. Case, U. C. Singh, C. Ghio, G. Alagona, S. Profeta, jun. and P. Weiner, *J. Am. Chem. Soc.*, 1984, **106**, 765.
34 T. G. Appleton, P. D. Prenzler and R. A. Webb, 3th International Symposium on Applied Bioinorganic Chemistry, Fremantle, 1994, Abstract, p. A 11; 7th International Symposium on Platinum and other Metal Coordination Compounds in Cancer Chemotherapy, Amsterdam, 1995, Abstract, p. 105.
35 E. W. Abel, M. Booth and K. G. Orrell, *J. Chem. Soc., Dalton Trans.*, 1980, 1582.
36 J. C. Barnes, G. Hunter and M. W. Lown, *J. Chem. Soc., Dalton Trans.*, 1976, 1227.
37 E. W. Hansen and P. Ruoff, *J. Phys. Chem.*, 1988, **92**, 2641.
38 E. W. Hansen and P. Ruoff, *J. Phys. Chem.*, 1989, **93**, 2696.

Received 17*th June* 1996; *Paper* 6/04210D