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Interconversion between S- and N-bound L-methionine adducts of $Pt(dien)^{2+}$ (dien = diethylenetriamine) *via* dien ring-opened intermediates

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The reaction between $[Pt(^{15}N\text{-dien})Cl]^+$ **1** and L-methionine (L-MetH) has been studied using $[^1H,^{15}N]$ 2D NMR spectroscopy and HPLC, together with ^{15}N-labelled amine ligands. The complex $[Pt(dien)(L\text{-MetH-}S)]^{2+}$ **2** was dominant at neutral pH and converted partially and reversibly into $[Pt(dien)(L\text{-Met-}N)]^+$ **3** at pH >8. When the pH was lowered from 8 to 3, the dien ring-opened intermediate $[Pt(dienH\text{-}N,N')(L\text{-Met-}S,N)]^{2+}$ **4** was formed which converted slowly into complex **2**. Complex **4** was separated by HPLC and characterized by NMR spectroscopy as a mixture of four diastereomers (due to chiral centres at S and NH) present in a 2 : 2 : 1 : 1 molar ratio. This isolated intermediate had a surprisingly long lifetime (days) in 0.55 M $NH_4H_2PO_4$ (pH 4.0).

Methionine (L-MetH) is an important amino acid which is involved in the metabolism of platinum drugs, and platinum bis(methionine) complexes have been identified as metabolites in the urine of patients undergoing cisplatin *cis*-$[Pt(NH_3)_2Cl_2]$ therapy.[1] The complex $[Pt(L\text{-Met-}S,N)_2]$ exists in aqueous solution as a mixture of *cis* and *trans* isomers which undergo interconversion,[2] but these are unreactive species which can be considered to be a detoxified form of Pt. However, a recent *in vivo* experiment has demonstrated that cisplatin incubated with methionine has reduced nephrotoxicity while the cytotoxicity against cancer cells is maintained.[3] Methionine and its derivatives can form stable ring-opened complexes with carboplatin and its analogues.[4] Since carboplatin $[Pt(cbdca\text{-}O,O')(NH_3)_2]$, cbdca = 1,1-dicarboxycyclobutane, itself reacts with nucleobases very slowly, it is conceivable that a methionine-containing peptide or protein may play an important role in transport or activation of carboplatin and in the transfer of Pt onto DNA. S-Bound methionine can be displaced by N7 of the DNA base guanine.[5,6]

Methionine is able to co-ordinate to Pt^{II} in a very versatile manner in aqueous solution. A simple system of L-methionine and $K_2[PtCl_4]$ in molar ratio 2 : 1 generates over 10 platinum species.[7] Thioether sulfur, amino nitrogen and carboxylate oxygen[8] are all capable of binding to Pt^{II}. The favored binding site is sulfur, and S,N-chelation is a common binding mode. In methionine-containing peptides, co-ordination of the amide N is also possible.[9,10]

Platinum–dien chelate rings are considered to be stable, and reactions of $[Pt(dien)Cl]^+$ are often studied as models of the first binding step of cisplatin. It has been shown that $[Pt(dien)Cl]^+$ binds to homocysteine or methionine through the thioether sulfur,[11] and S-bound thioethers can be displayed from Pt^{II} intra-[6,11] or inter-molecularly[5] by nitrogen (N7) from guanine at physiological pH. Guanine is known to be a major target for DNA platination by anticancer drugs.[12] An intramolecular migration of $Pt(dien)^{2+}$ from sulfur to imidazole N1 in the dipeptide histidylmethionine at pH >6 has also been reported recently.[13] In this work we have sought to gain further insight into sulfur–nitrogen migration by 2D $[^1H,^{15}N]$ heteronuclear single quantum coherence (HSQC) NMR spectroscopic and HPLC studies of reactions of $[Pt(dien)Cl]^+$ with L-methionine *via* the use of ^{15}N-labelled dien and L-MetH.

Experimental

Materials and methods

L-Methionine (L-MetH) and ^{15}N labelled L-MetH (99% ^{15}N) were purchased from Sigma. ^{15}N-Labelled diethylenetriamine (^{15}N-dien) and $[Pt(^{15}N\text{-dien})Cl]Cl$ were prepared according to the reported procedures.[14,15] All other chemicals were purchased from Aldrich.

NMR spectroscopy

The NMR spectra were recorded at 298 K on a Bruker DMX500 spectrometer (^1H 500.13 MHz, ^{15}N 50.7 MHz), using 5-mm NMR tubes. The chemical shift references were as follows: ^1H (1,4-dioxane, internal, δ 3.743), ^{15}N (external, 1 M $^{15}NH_4Cl$ in 1.5 M HCl). For ^1H NMR typical acquisition conditions for 1D 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. The 2D $[^1H,^{15}N]$ HSQC NMR spectra [optimized for $^1J(N, H) = 72$ Hz] were recorded using the sequence of Stonehouse *et al.*[16] The ^{15}N spins were decoupled by irradiation with the GARP-1 sequence during acquisition. Water suppression was achieved by pulsed-field gradients. Typically, 8 scans were acquired for each of 128 increments of t_1 and the final resolution was 4 Hz per point for the F2 dimension and 8 Hz per point for the F1 dimension. For the HPLC-separated sample, 256 acquisitions were used per t_1 period and the final resolution was 2 Hz per point for the F2 dimension and 1 Hz per point for the F1 dimension.

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 at 298 K on a Nucleosil (100-5SA) cation-exchange column by injecting aliquots of the reaction mixture at various pH and time intervals with detection at 210 nm. A solution of $NH_4H_2PO_4$ solution (0.55 M, pH 4.0) was used as eluent. The data were analysed using Dynamax Method Manager Software.

pH Measurements

The pH values of the solutions were adjusted with $HClO_4$ (1 M) or NaOH (1 M) and determined using a Corning 240 pH meter equipped with an Aldrich micro combination electrode, calibrated with Aldrich buffer solutions at pH 4, 7 and 10.

NMR sample preparation

All the NMR samples (except those separated by HPLC) were in 90% water–10% D_2O (0.6 ml), and contained 0.1 M $NaClO_4$ to

Table 1 Proton and ^{15}N chemical shifts of $Pt(dien)^{2+}$ and $Pt(dienH\text{-}N,N')^{3+}$ complexes. The spectra were recorded during the reaction of ^{15}N-labelled complex **1** with ^{15}N-labelled L-methionine at 298 K

		^{15}N-dien ($^1H/^{15}N$)[a]		^{15}N-L-Met ($^1H/^{15}N$)[a]
Compound	pH	NH (*trans* to)	NH_2 (*trans* to)	(NH_2 *trans* to N)
1 $[Pt(^{15}N\text{-dien})Cl]^+$	6.0	6.69/7.68 (Cl)	5.29, 5.08/−34.20 (N)	
2 $[Pt(^{15}N\text{-dien})(^{15}N\text{-L-MetH-}S)]^{2+}$	6.0	6.97/26.36 (S)	5.75, 5.58/−30.29 (N)	
3 $[Pt(^{15}N\text{-dien})(^{15}N\text{-L-Met-}N)]^+$	8.5	6.46/6.76 (N)	5.42/−32.40 (N) 5.02/−32.40 (N) 5.25/−32.83 (N)	4.89/−36.76
4 $[Pt(^{15}N\text{-dienH-}N,N')(^{15}N\text{-L-Met-}S,N)]^{2+}$[b]	3.3			
R,*R*-		6.71/−13.18 (N)	5.39/−10.17; 5.74/−10.17 (S)	5.00/−46.01; 5.81/−46.01[c]
R,*S*-		6.59/−12.48 (N)	5.33/−9.32; 5.62/−9.32[d] (S)	4.94/−46.37; 5.85/−46.37[c]
S,*R*-		6.66/−14.06 (N)	5.48/−9.82; 5.78/−9.82 (S)	5.18/−46.59; 5.82/−46.59[c]
S,*S*-		6.57/−12.80 (N)	5.59/−10.10[d] (S)	5.11/−46.77; 5.97/−46.77[c]

[a] Some cross-peaks are multiplets but no detailed analysis was attempted due to the resolution limitation of the 2D spectra (4 Hz per data point in ^1H dimension). [b] The assignment of peaks to individual diastereoisomers is arbitrary, and the pairing of the cross-peaks is based on the peak intensities. [c] These cross-peaks are better resolved for the HPLC-purified complex **4**, as shown in Fig. 5, and the minor differences in $\delta(^1H)$ (*ca.* 0.1 ppm) and $\delta(^{15}N)$ (*ca.* 0.3 ppm) compared to the values in Table 2 may be due to the different ionic strengths and pH values of the samples. [d] Overlapped.

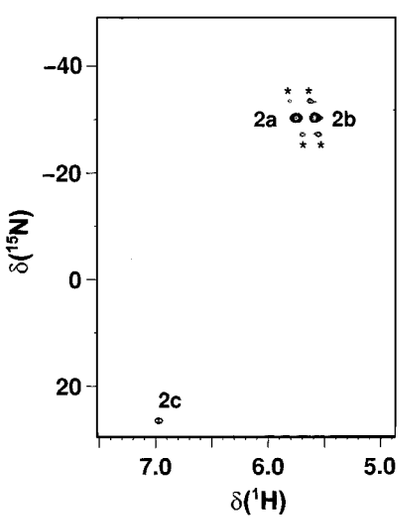


Fig. 1 Formation of $[Pt(^{15}N\text{-dien})(^{15}N\text{-L-MetH-}S)]^{2+}$ **2** by reaction of $[Pt(^{15}N\text{-dien})Cl]^+$ **1** (5 mM) with ^{15}N-L-MetH in 1:1 molar ratio at 298 K, pH 6.0. The 2D [^1H,^{15}N] HSQC NMR spectrum was recorded after 12 h of reaction. The peaks labelled **2a** and **2b** are assigned to protons on each of the two NH_2 groups, and **2c** to the NH group; ^{195}Pt satellites are labelled with *

maintain a constant ionic strength. Buffer was not used in order to avoid possible interference with the reactions. The reactions of $[Pt(^{15}N\text{-dien})Cl]Cl$ (5 mM) with L-MetH (^{15}N or ^{14}N) were conducted at a 1:1 molar ratio at constant temperature.

Results

First we studied the substitution of the chloride ligand of $[Pt(^{15}N\text{-dien})Cl]^+$ **1** by the S of L-MetH at pH 6. The effect on the product of increasing the pH to 8 was then investigated, followed by lowering the pH to 3. All of the reactions were studied by both NMR and HPLC. For 2D [^1H,^{15}N] NMR both ^{15}N-labelled dien and L-MetH were used, and some HPLC-separated fractions of ^{15}N-labelled L-MetH were examined by 2D [^1H,^{15}N] NMR. As we have summarized recently, the ^{15}N chemical shifts of Pt–NH of Pt–^{15}N-dien complexes are usually diagnostic of the *trans* ligands.[17] For a Pt–NH *trans* to an oxygen donor the ^{15}N chemical shift is *ca.* δ −10, for an Pt–NH *trans* to nitrogen or chloride the ^{15}N shift is *ca.* δ 10, and *trans* to sulfur about δ 30. For Pt–NH_2 of amine the ^{15}N chemical shifts are as follows: *trans* to O, δ −40 to −50; *trans* to N, Cl, δ −25 to −35; *trans* to S, δ −5 to −15. In this study we have used the above information to assign the ligands *trans* to the amine groups.

NMR spectroscopy

Reaction of $[Pt(dien)Cl]^+$ with L-MetH at pH 6 followed by pH adjustment to 8.5. The reaction of ^{15}N-labelled complex **1** (5 mM) with ^{15}N-L-MetH in a 1:1 molar ratio at pH 6.0 and 298 K was followed by 2D [^1H,^{15}N] NMR for a period of 12 h. After 30 min of incubation, three new cross-peaks at δ 5.75, 5.58/−30.29 (peaks **2a** and **2b**, Fig. 1) and 6.97/26.36 (peak **2c**, Fig. 1) were present in the spectrum in addition to three cross-peaks of the starting complex **1**. The new cross-peaks increased in intensity with time, while the cross-peaks for complex **1** decreased in intensity. After 10 h only peaks **2a**, **2b** and **2c** were observable. Fig. 1 shows a 2D [^1H,^{15}N] NMR spectrum recorded 12 h after incubation. Peaks **2a** and **2b** have ^{15}N chemical shifts similar to those for Pt–NH_2 of complex **1** for which two pairs of non-equivalent ^1H are observed, and can be assigned to the protons of the two *trans* Pt–NH_2 groups. Peak **2c** has a ^{15}N chemical shift in the region expected for Pt–NH *trans* to a sulfur. Therefore the cross-peaks **2a**, **2b** and **2c** can be assigned to the S-bound L-MetH adduct $[Pt(^{15}N\text{-dien})(^{15}N\text{-L-MetH-}S)]^{2+}$ **2**. The 2D NMR spectrum of the solution was recorded again after 7 d at 298 K, and peaks for complex **2** were unchanged.

The pH of the above solution was raised from 6.0 to 8.5, and the time dependence of the resultant [^1H,^{15}N] 2D NMR spectrum investigated for a period of 24 h. The cross-peaks assigned to complex **2** gradually decreased in intensity, whilst five new cross-peaks appeared with ^1H, ^{15}N chemical shifts of δ 5.42/−32.40 (peak **3a** in Fig. 2A), 5.25/−32.83 (**3b**), 5.02/−32.40 (**3a′**), 4.89/−36.76 (**3d**) and 6.46/6.76 (**3c**). Fig. 2A shows the spectrum recorded 12 h after the pH adjustment, by which time equilibrium had been reached. Each peak contained fine structure which can be assigned to NH_AH_B and NH–CH_2 coupling (7 to 11 Hz) but this was not further analysed. Cross-peaks **3a**, **3a′** and **3b** have ^{15}N chemical shifts in the region expected for Pt–NH_2 groups *trans* to N or Cl, and peak **3c** has a ^{15}N chemical shift characteristic of Pt–NH *trans* to N or Cl. The new peaks have ^1H, ^{15}N chemical shifts different from those of complex **1**, Table 1, and Cl can be ruled out as the ligand *trans* to Pt–NH. Peaks **3a** and **3a′** have the same ^{15}N chemical shifts, and the relative areas of peaks **3a**:**3b**:**3a′** are 1:2:1, therefore **3a** and **3a′** can reasonably be assigned to two non-equivalent protons on one NH_2 group of dien, and peak **3b** to the other NH_2 group with coincident ^1H shifts.

Peak **3d** also has a ^{15}N chemical shift in the region of Pt–NH_2 groups *trans* to N or Cl, and when the above reaction was repeated using ^{15}N-labelled complex **1**, but unlabelled L-MetH, peak **3d** disappeared (Fig. 2B) showing that this arises from a co-ordinated NH_2 group of L-Met. Therefore peaks **3a**–**3c** can be assigned to the Pt–NH_2 and Pt–NH groups of a dien ligand

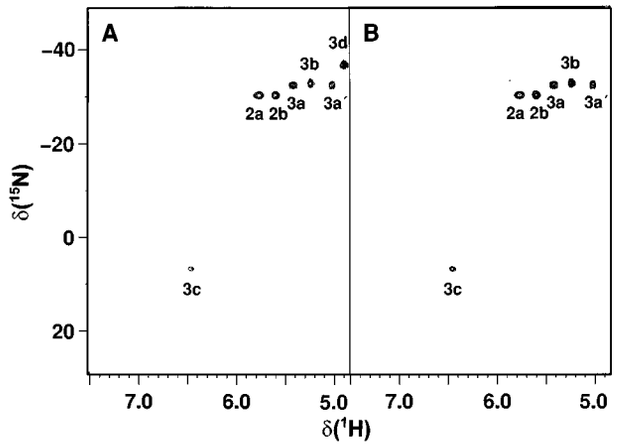


Fig. 2 A 2D [^{1}H,^{15}N] HSQC NMR spectrum recorded 12 h after increasing the pH of a solution containing complex **2** as [Pt(^{15}N-dien)(^{15}N-L-MetH-*S*)]$^{2+}$ (Fig. 2A) or [Pt(^{15}N-dien)(^{14}N-L-MetH-*S*)]$^{2+}$ (Fig. 2B) from 6.0 to 8.5. Peak assignments: **2a** and **2b**, NH_2 (*trans* to N) of complex **2**; **3a**, **3b** and **3a′**, NH_2 (*trans* to N) and **3c**, NH (*trans* to N) of [Pt(^{15}N-dien)(L-Met-*N*)]$^+$ **3**; **3d**, NH_2 (*trans* to N) of N-bound ^{15}N-L-Met in complex **3**. The cross-peak for NH (*trans* to S) of complex **2** is not observed because of fast exchange with solvent protons on the NMR timescale at this pH. Cross-peaks **3a**–**3d** exhibit further coupling in the ^{1}H dimension

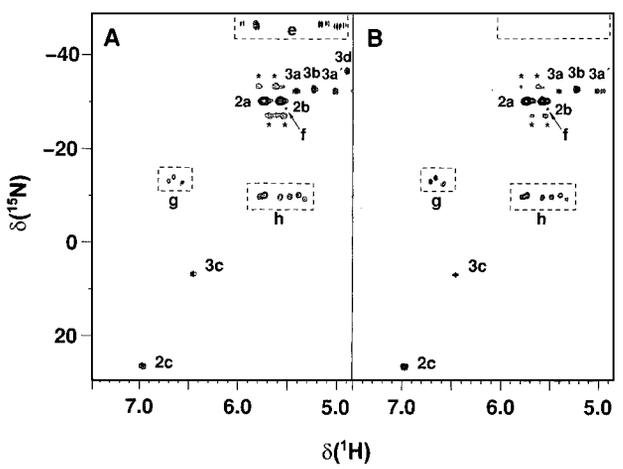


Fig. 3 The 2D [^{1}H,^{15}N] HSQC NMR spectra recorded 1 h after lowering the pH of solutions containing complexes **2** and **3** (see Fig. 2) containing ^{15}N-labelled L-Met (Fig. 3A) or unlabelled L-Met (Fig. 3B) from 8.5 to 3.3. The boxed cross-peaks are assignable to the dien-ring-opened intermediate [Pt(^{15}N-dienH-*N*,*N′*)(L-Met-*S*,*N*)]$^{2+}$ **4**. Note the absence of the peaks in box **e** and peak **3d** in Fig. 3B (^{14}N-L-MetH used). Peak assignments: **2a** and **2b**, NH_2 *trans* to N with ^{195}Pt satellites (*); **2c**, NH *trans* to S of complex **2**; **3a**, **3b** and **3a′**, NH_2 (*trans* to N); **3c**, NH (*trans* to N) of complex **3**; **3d**, NH_2 (*trans* to N) of N-bound ^{15}N-L-Met in complex **3**; boxes **g** and **h** can be tentatively assigned to Pt–NH (*trans* to N) and Pt–NH_2 (*trans* to S) groups of dien in ring-opened intermediate **4**; box **e**, NH_2 (*trans* to N) of N-bound ^{15}N-L-Met in intermediate **4**; **f**, unassigned

and the observed peaks are consistent with the formation of [Pt(^{15}N-dien)(^{15}N-L-Met-*N*)]$^+$ **3** at pH 8.5, Table 1. Curiously cross-peak **2c** (Fig. 1), assigned to the Pt–NH group of the S-bound L-MetH complex **2**, became broader with time and disappeared from the 2D spectrum 5 h after pH adjustment to 8.5.

Effect of pH decrease from 8.5 to 3.3. The pH of the above solution containing an equilibrium mixture of complexes **2** and **3** (labelled with both ^{15}N-dien and ^{15}N-L-MetH) was lowered from 8.5 to 3.3 and the time dependence of the 2D spectrum was followed for 24 h at 298 K. During the first hour peak **2c** reappeared (Pt–NH of complex **2**, slowing down of NH exchange rate at low pH) and new cross-peaks appeared in the ^{1}H/^{15}N regions from δ 5.99 to 4.91/−46.01 to −46.77, 6.71 to 6.57/−13.18 to −12.80, and 5.80 to 5.32/−10.22 to −9.32 (boxes **e**, **g** and **h**, respectively, in Fig. 3A), and peaks for complexes **2** and **3** (**2a**, **2b** and **3a** to **3d**) were still observable, with the same ^{1}H and ^{15}N chemical shifts as at the higher pH (Fig. 2A). The cross-peaks in boxes **e**, **g** and **h** each corresponded to a total of four different ^{15}N chemical shifts (Table 1) with intensity ratio of *ca.* 2:2:1:1. All these new cross-peaks decreased in intensity with time and eventually disappeared after 10 h, whereas the cross-peaks for complex **2** increased in intensity with time and those for **3** decreased in intensity but were still just visible after 24 h. A cross-peak at δ 7.81/17.68, which has similar ^{1}H and ^{15}N chemical shifts to those of the NH_3^+ group for free ^{15}N-L-MetH, was initially observed, but disappeared within 1 h after the pH adjustment.

This reaction was reinvestigated using ^{15}N-labelled complex **1** but unlabelled L-MetH. In this case cross-peak **3d** and those in box **e** of Fig. 3A were not observed, Fig. 3B. Therefore these cross-peaks must be due to the co-ordination of $^{15}NH_2$ of L-methionine.

No new 2D cross-peaks were observed when the pH of a solution containing complex **2** (^{15}N-dien and ^{15}N-labelled L-Met) was adjusted directly from 6 to 3, without initially being raised to pH 8.5.

HPLC

Reactions of unlabelled [Pt(dien)Cl]$^+$ **1** with ^{15}N-L-MetH were also followed by HPLC at different pH values. Several eluents were tested and 0.55 M $NH_4H_2PO_4$, pH 4.0 was found to provide the optimum separation of reactants and products present in the solutions and was used in all the HPLC work.

The HPLC chromatogram from the reaction of complex **1** with ^{15}N-L-MetH (5 mM, 1:1) at pH 6.0, 298 K for 24 h showed only one major peak with retention time of *ca.* 12.0 min. After the pH of the solution was raised to 8.5 and left for 12 h the HPLC chromatogram showed a new broad peak with elution time of *ca.* 20.0 min (peak **b**) as well as a strong peak with retention time of *ca.* 12.0 min (peak **a**, Fig. 4A). The pH of the solution was then lowered from 8.5 to 3.3 and the HPLC chromatogram was followed with time for a period of 24 h. Within the first hour a new peak (**c**) with retention time of 8.2 min appeared and reached its maximum intensity (Fig. 4B), and then decreased in intensity along with peak **b** (Fig. 4C). After 24 h peaks **b** and **c** had almost disappeared (Fig. 4D).

The fraction corresponding to peak **b** (Fig. 4) from the reaction at pH 8.5 was collected. When this was rechromatographed after standing for 3 d at 298 K the chromatogram showed only peaks **c** and **a** (3:1 ratio). This change can be explained by the decrease in the pH of the collected fraction to 4.0 (that of the 0.55 M $NH_4H_2PO_4$ eluent). Under these conditions peak **c** was very long-lived, and even detectable after several weeks.

Characterization of intermediate detected during pH adjustment from 8.5 to 3.3

Attempts were made to separate the intermediate and characterize it further by NMR methods. Unlabelled complex **1** and ^{15}N-L-MetH (10 mM, 1:1) was incubated for 24 h at pH 6 to allow formation of complex **2**. The pH of the solution was then adjusted to 8.5, and after 12 h were lowered to 3.3. The fraction corresponding to the intermediate of peak **c** in the HPLC chromatogram of Fig. 4 was collected 30 min after pH adjustment and lyophilized. The lyophilized sample was redissolved in 0.6 ml of 90% water–10% D_2O, which gave rise to a solution of pH *ca.* 4.0 because of the presence of $NH_4H_2PO_4$. The resulting 2D [^{1}H,^{15}N] HSQC NMR spectrum is shown in Fig. 5, and appears to contain six doublets (**a′**, **d′**, **c**, **d** and **b′** overlapped with **c′**), and two triplets (**a** and **b**) corresponding to four ^{15}N chemical shifts, Table 2. The splittings (13–14 Hz) can be assigned to $^2J(NH_a,NH_b)$ and $^3J(\alpha CH,NH)$ couplings involving co-ordinated ^{15}N-L-Met. The relative intensities of cross-

Table 2 Proton and ^{15}N chemical shifts of HPLC-separated complex **4** [Pt(dienH-*N*,*N*′)(^{15}N-L-Met-*S*,*N*)]$^{2+}$ (pH 4.0, 298 K)

Diastereomer*	δ (^{1}H) SCH_3	Peak	δ (^{1}H/^{15}N) (NH_2 *trans* to N)
R,*R*	2.641	**a**	5.08/−46.32 (t)
		a′	5.86/−46.32 (d)
R,*S*	2.579	**b**	5.03/−46.70 (t)
		b′	5.86/−46.70 (d)
S,*R*	2.635	**c**	5.31/−46.68 (d)
		c′	5.84/−46.68 (d)
S,*S*	2.567	**d**	5.26/−46.92 (d)
		d′	5.97/−46.92 (d)

* The assignment of peaks to individual diastereomers is arbitrary, and the pairing of the SCH_3 signals with $^{15}NH_2$ cross-peaks is based on the peak intensities. The upfield cross-peaks for *R*,*R* and *R*,*S* diastereomers appeared as triplets due to ^3J(αCH,NH) (*ca.* 13 Hz) as well as $^2J(NH_a,NH_b)$ (*ca.* 12 Hz), whilst only $^2J(NH_a,NH_b)$ splittings (*ca.* 12 Hz) were observable for all other signals.

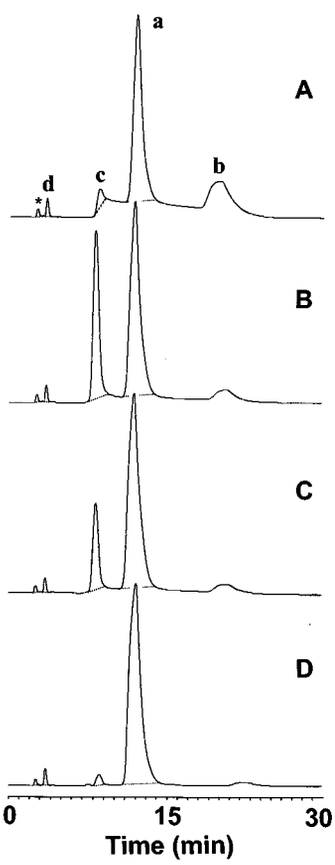


Fig. 4 The HPLC chromatograms for the reaction of [Pt(dien)Cl]$^+$ **1** with L-MetH (5 mM, 1:1, 298 K, pH 6.0, 24 h): (A) 12 h after pH adjustment from 6.0 to 8.5; (B) 0.5 h after lowering the pH from 8.5 to 3.3; (C) 2 h after pH adjustment from 8.5 to 3.3; (D) 24 h after pH adjustment from 8.5 to 3.3. Peak assignments: **a**, [Pt(dien)(L-MetH-*S*)]$^{2+}$ **2**; **b**, [Pt(dien)(L-Met-*N*)]$^+$ **3**; **c**, dien ring-opened intermediate [Pt(dienH-*N*,*N*′)(L-Met-*S*,*N*)]$^{2+}$ **4**; **d** and * are from free L-MetH and the solvent front, respectively

peaks **c**:**d** and **a**:**b** is *ca.* 2:1. The chemical shifts and relative intensities of these cross-peaks in Fig. 5 are nearly the same as those in box **e** of Fig. 3 (δ ^{15}N between −46 and −47, Table 2). The peaks in boxes **g** and **h** (Fig. 3A) for complex **4** are not observed in this experiment because the dien ligand is not ^{15}N-labelled. The ^{1}H NMR spectrum of this sample contained four singlets with shifts of δ 2.641, 2.635, 2.579, 2.567 assignable to co-ordinated SCH_3 groups of L-Met ligands (Fig. 6). These had relative areas of 2:2:1:1. The ^{1}H NMR spectrum

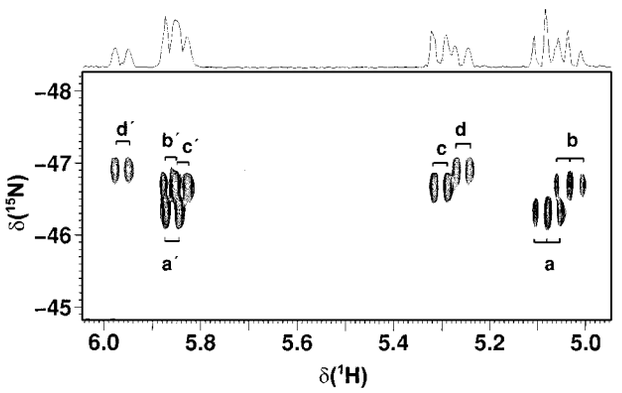


Fig. 5 The 2D [^{1}H,^{15}N] HSQC NMR spectrum of the HPLC-isolated dien ring-opened complex **4** (peak **c** in Fig. 4B) at pH 4.0. Only the NH_2 group of L-MetH was ^{15}N-labelled, and the four sets of cross-peaks (peaks **a**, **a**′ to **d**, **d**′) can be assigned to the non-equivalent Pt–NH_2 groups in the four diastereomers of [Pt(dienH-*N*,*N*′)(^{15}N-L-Met-*S*,*N*)]$^{2+}$. All peaks have $^2J(NH_a,NH_b)$ of *ca.* 12 Hz, while only peaks **a** and **b** have an additional ^3J(αCH,NH) of *ca.* 13 Hz

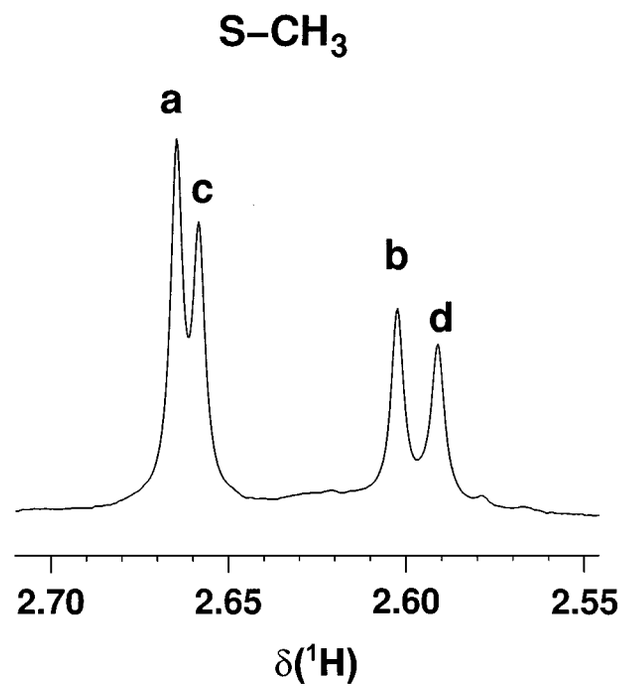


Fig. 6 Part of the 500 MHz ^{1}H NMR spectrum of HPLC-isolated dien ring-opened complex **4** at pH 4.0 (peak **c**, Fig. 4B), showing four singlets from SCH_3 of diastereomers containing *S*,*N*-chelated L-Met

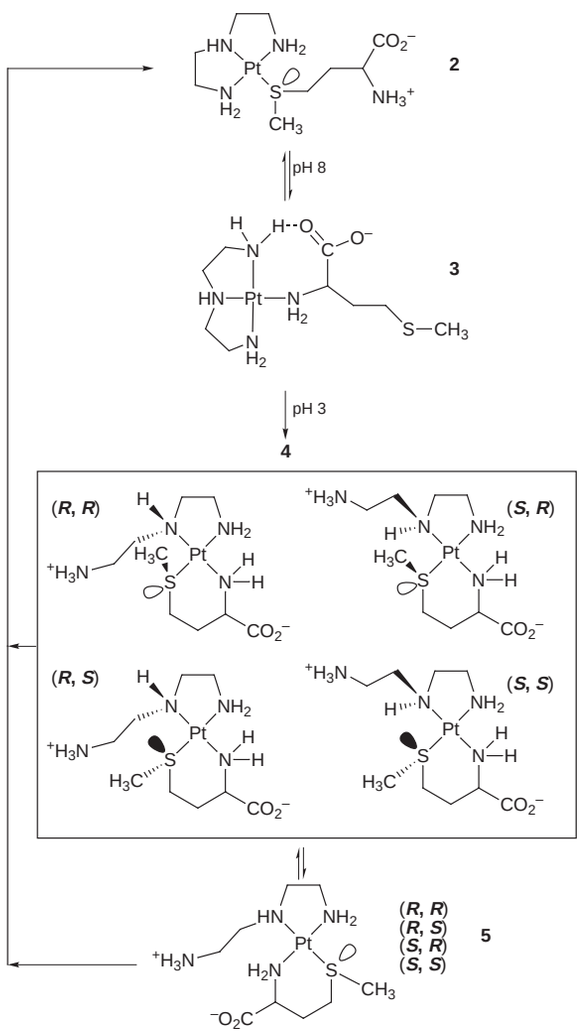
of the solution was recorded again after 3 d at 298 K, and no changes were observed.

Discussion

Competition between sulfur and nitrogen ligands in co-ordination to platinum(II) is currently attracting attention because this could provide new routes for DNA platination *via* a protein fragment. Thioether S can bind reversibly to Pt^{II}, and at physiological pH it can be replaced by a N donor from N7 of guanine.[5,6,11] It can also be replaced by N1 of imidazole from His at pH >6.[13] In this work we have investigated the mode of binding of L-MetH to Pt(dien)$^{2+}$ at a variety of pH values.

S- and N-bound [Pt(dien)(L-Met)] complexes

The NMR data show unambiguously that at pH 6 initial binding of L-MetH to [Pt(dien)Cl]$^+$ **1** takes place *via* Cl^- displacement with S which has a high affinity for Pt^{II}. Correspondingly, in the HPLC experiment at this pH only one major peak



Scheme 1 Migration of $Pt(dien)^{2+}$ from S- to N-bound L-Met and dien ring opening *via* S,N-chelation of L-Met

(peak **a**, Fig. 4) was observed for the product. When the pH of $[Pt(dien)(L\text{-}MetH\text{-}S)]^{2+}$ **2** was adjusted to 8.5 the deprotonated amino group (pK_a 9.28 for free L-MetH[18]) from L-Met slowly displaces S to form the thermodynamically favored product $[Pt(dien)(L\text{-}Met\text{-}N)]^{+}$ **3**.

The two $Pt–NH_2$ groups of complex **3** have different ^{15}N chemical shifts and the two ^1H NMR peaks for one of the two $Pt–NH_2$ groups are well separated (*ca*. 0.4 ppm), which may be a consequence of hydrogen bonding with the carboxylate group of N-bound L-methionine at both pH 8.5 and 3.3 (Scheme 1).

Identification of intermediate (complex 4)

Lowering the pH of a solution containing the N-bound complex **3** from pH 8.5 to 3.3 gave rise to peaks for intermediates both in 2D [^1H,^{15}N] NMR spectra and in HPLC chromatograms. By using ^{15}N-labelled L-MetH in comparison with unlabelled L-MetH (Fig. 3A, B), the co-ordination of NH_2 of L-Met in the intermediate was confirmed and the ^{15}N chemical shifts of the cross-peaks (box **e**, Fig. 3A) are comparable to those of *trans*-[Pt(^{15}N-L-Met-*S*,*N*)$_2$] (for which the NH_2–Pt–NH_2 groups gave rise to cross-peaks at δ −41 to −43).[2] Cross-peaks in box **h** (NH_2 *trans* to S) in Fig. 3 can be assigned to $Pt–NH_2$ (^{15}N-dien) groups of the intermediate. Therefore, it seems likely that a major intermediate observed after pH adjustment from 8.5 to 3.3 is a complex with S,N-chelated L-Met and a chelate-ring-opened dien ligand, [Pt(^{15}N-dienH-*N*,*N*′)(^{15}N-L-Met-*S*,*N*)]$^{2+}$. Cross peaks in box **g** (Fig. 3) can be tentatively assigned to NH *trans* to a nitrogen ligand for the Pt–NH of [Pt(^{15}N-dienH-*N*,*N*′)(^{15}N-L-Met-*S*,*N*)]$^{2+}$. For a bis-chelated dien ligand a Pt–NH group *trans* to N would be expected to have an ^{15}N chemical shift of *ca*. δ 10;[17] in the present case the ^{15}NH resonances of [Pt(^{15}N-dienH-*N*,*N*′)(^{15}N-L-Met-*S*,*N*)]$^{2+}$ appear to be shifted to high field by 20 ppm, an effect which is presumably caused by the ring opening. The appearance and disappearance of peak **c** observed in the HPLC chromatogram after pH adjustment from 8.5 to 3.3 are consistent with those cross-peaks observed in the NMR experiments (Fig. 3), and therefore can be assigned to the same ring-opened species.

Due to the presence of chiral NH and S centers in [Pt(dienH-*N*,*N*′)(L-Met-*S*,*N*)]$^{2+}$ **4**, four diastereomers would be expected: *R*,*R*; *R*,*S*; *S*,*R*; *S*,*S* (omitting the *S* center at αC for L-methionine). The *R*,*S* inversion at the chiral SCH_3 center in *S*,*N*-chelated L-Met is usually slow on the NMR timescale; for example the energy barrier to such inversion in [Pt(L-Met-*S*,*N*)Cl_2] has been estimated to be 74.6 kJ mol^{-1}.[7] Therefore, as shown in Fig. 3, the four sets of cross-peaks in boxes **e**, **g** and **h** can be assigned to the four diastereomers. Similarly, four SCH_3 singlets were observed in the ^1H NMR spectrum of the HPLC-purified intermediate (Fig. 6). The relative area of the four sets of cross-peaks in boxes **e**, **g** and **h**, and the four SCH_3 singlets, is about 2 : 2 : 1 : 1. This can be attributed to the predominance of one of the two configurations at chiral S. A 2 : 1 ratio has been observed previously for *R*,*S* diastereomers of *N*,*S*-chelated methionine[8] and methionine-containing dipeptide[10] complexes of Pt^{II}.

The four different ^{15}N chemical shifts of cross-peaks in the 2D [^1H,^{15}N] spectrum of the HPLC-purified intermediate (Fig. 5) are also consistent with the presence of four diastereomers. Each pair of cross-peaks has the same ^{15}N chemical shift, but very different ^1H chemical shifts (Δδ 0.5–0.8 ppm). This suggests that one of the two NH_2 protons of chelated ^{15}N-L-Met in complex **4** is strongly involved in hydrogen bonding, perhaps with the deprotonated carboxylate group, which forms a strained five-membered ring. Alternatively, the ring conformation may allow the dien NH_3^+ group to interact with the CO_2^- group of co-ordinated L-Met, and, in so doing, may influence the ^1H chemical shifts of the NH_2 groups. For comparison the ^1H chemical shift differences for the resonances of NH_2 in *trans*-[Pt(^{15}N-L-Met-*S*,*N*)$_2$] are only *ca*. 0.3–0.4 ppm.[2]

The $^2J(NH_a,NH_b)$ values for cross-peaks observed in the 2D [^1H,^{15}N] HSQC NMR spectrum (Fig. 5) are comparable to those values reported for *trans*-[Pt(^{15}N-L-Met-*S*,*N*)$_2$] (*ca*. 12 Hz);[2] ^3J(αCH,NH) splittings (*ca*. 13 Hz) were also observed for cross-peaks (**a** and **b**), which implies that envelope conformations may be adopted for the six-membered ring in two of the four isomers. The other two isomers may adopt flattened boat conformations for which ^3J(αCH,NH) tends to be small, and therefore perhaps not observable. This is the case for *trans*-[Pt(^{15}N-L-Met-*S*,*N*)$_2$]: several $Pt–NH_2$ peaks have ^3J(αCH,NH) values of 9–14 Hz, whilst other peaks have no detectable couplings.[2]

After pH adjustment from 8.5 to 3.3 the free nucleophilic S atom of complex **3** could attack the apical position of platinum, leading to labilization of the $Pt–NH_2$ bond and the formation of dien ring-opened S,N-chelate [Pt(dienH-*N*,*N*′)(L-Met-*S*,*N*)]$^{2+}$ **4** with S *trans* to NH_2 of dien. Complex **4** may convert into **2** *via* isomerization to **5** (S *trans* to NH of dien). The unassigned cross-peaks which are in very low intensity in Fig. 3 could possibly be due to complex **5**. Proton transfer from the free NH_3^+ group of ring-opened dien in **5** to the more basic methionine amino group could rapidly give complex **2**. The fact that no dien ring-opened intermediate was observed when the pH of **2** was adjusted directly from 6 to 3 shows that dien chelate ring opening requires displacement of dien NH_2 by S.

Very few examples of chelate ring-opened dien platinum complexes have been reported. The only crystal structure of such a complex is that for [Pt(dienH-*N*,*N*′)Cl]Cl_2, obtained from a strongly acidic solution (1 M HCl).[19] Ring opening of a

Pt–dien ring has been reported in the presence of an excess of diethyldithiocarbamate and thiourea,[20,21] and the complex [Pt(dienH-*N*,*N*′)(Guo-N7){SC(NH_2)$_2$}]$^{2+}$ has been isolated by HPLC at pH <2.0.[21] Ring-opened ethylenediamine platinum complexes with the di- and tri-peptides Gly-HMet and Gly-Met-HGly have been detected at pH 2.4, and such a ring-opened complex containing Met-HHis has also been identified at pH 9.6.[10,22]

The present work appears to provide the first example of an HPLC-isolated and NMR-characterized Pt–dien ring-opened complex. Complex **4** is very long-lived (weeks) in the presence of high concentrations of $NH_4H_2PO_4$, which suggests that the phosphate group may form strong hydrogen bonds with the dangling NH_3^+ of dien and stabilize the dien ring-opened form.

Lempers and Reedijk[23] have observed a pH-dependent platinum migration from S to N while studying the reaction between complex **1** and *S*-adenosyl-L-homocysteine. When they adjusted the pH from 11 to 4 the N-bound complex gave rise to *ca*. 20% of 'side products' which could not be characterized. These could also be due to the formation of dien ring-opened adducts.

Taken together, these results show that significant migration of Pt(dien) from S- to N-bound L-Met occurs at pH values above 8, while N-bound L-Met transfers to S-bound L-Met at pH 3 *via* dien ring-opened intermediates, long-lived isomers in which L-Met S is *trans* to co-ordinated dien NH_2. The process is summarized in Scheme 1.

Conclusion

This work has shown that at neutral pH [Pt(dien)Cl]$^+$ **1** forms predominantly an S-bound L-Met complex **2**. Complex **2** reversibly converted into the N-bound complex **3** at pH 8.5. The adjustment of pH from 8.5 to 3.3 gave rise to formation of the dien ring-opened intermediate [Pt(dienH-*N*,*N*′)(L-Met-*S*,*N*)]$^{2+}$ **4** with NH *trans* to L-Met NH_2, which converted slowly into complex **2**, the only stable adduct at pH 3.3. Complex **4** exists as a mixture of four diastereomers with molar ratio 2:2:1:1 due to the chiral sulfur centre of L-Met and chiral NH of dien. The two NH_2 protons of L-Met in each of the four diastereomers of complex **4** exhibit remarkably different ^{1}H NMR shifts.

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