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Stabilization of monofunctional platinum–nucleotide adducts: reactions of *N*-acetyl-L-methionine complexes with guanosine 5′-monophosphate and guanylyl(3′-5′)guanosine †

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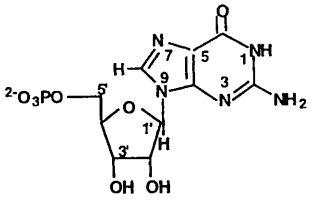
The complexes [Pt(en)(MeCO-Met-*S*)Cl]NO_3 **1**, [Pt(en)(MeCO-Met-*S*)$_2$][NO_3]$_2$ **2** (en = ethane-1,2-diamine, MeCO-Met = *N*-acetyl-L-methionine) and their ^{15}N analogues (**1n** and **2n**) have been prepared and characterized by ^1H, ^{13}C and two-dimensional [^1H,^{15}N] spectroscopy. Complex **1** (half-life 3.9 h at 310 K) hydrolysed more slowly than [Pt(en)Cl_2], whereas **2** was stable in water. The reaction of **1n** with guanosine 5′-monophosphate (5′-GMP) gave a stable mixed-ligand complex [Pt([^{15}N]en){MeCO-Met(1−)-*S*}(5′-GMP-N^7)]$^+$, and the reaction with guanylyl(3′-5′)guanosine (GpG) gave two different monofunctional adducts [Pt([^{15}N]en){MeCO-Met(1−)-*S*}(GpG-N^7)]$^+$, due to platination of either 3′- or 5′-G, with a preferential formation of one over the other (ratio 60:40). During the initial stages of the reaction the chelated complex [Pt([^{15}N]en){MeCO-Met(2−)-*S*,*N*}] **3** was also observed, which subsequently reacted with 5′-GMP or GpG *via* ring opening to give monofunctional adducts. Reactions of complex **2** with 5′-GMP and GpG also lead to such adducts, with release of MeCO-Met. Little conversion of monofunctional adducts into bifunctional adducts was observed. Methionine and its derivatives could play a role in the trapping of monofunctional adducts of platinum anticancer drugs with DNA *in vivo*.

The anticancer drug cisplatin, *cis*-[$PtCl_2(NH_3)_2$], is thought to exert its cytotoxic effects largely *via* the formation of GpG intrastrand cross-links on DNA.[1,2] Crystal structures have been determined for *cis*-[$Pt(NH_3)_2$]$^{2+}$ adducts of pGpG, CpGpG and the double-stranded deoxyoligonucleotide d(CCTCTG*G*TCTCC)·d(GGAGACCAGAGG),[3–5] and an NMR structure of the adduct with d(CCTG*G*TCC)·d(GGACCAGG), where G* denotes the platination site.[6] In these complexes the N^7-bound guanine bases are oriented head-to-head, and intramolecular hydrogen bonding between a 5′-phosphate oxygen and a proton of the ammine ligand occurs. Such hydrogen bonding has also been detected in solution.[7,8] It is apparent that a GG intrastrand cross-link can occur in an oligonucleotide with little distortion of the interstrand hydrogen-bonding pattern, although the DNA becomes bent (*ca.* 45° towards the major groove) and slightly unwound.

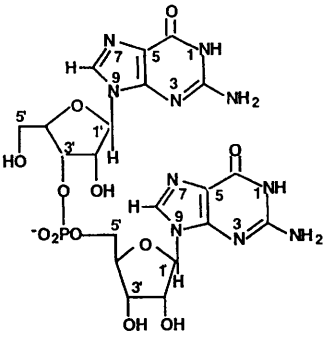
There is increasing interest in the modification to DNA structure caused by other types of platination, in particular monofunctional adducts which are precursors of bifunctional cross-links.[2,9–11] For example, it has been suggested that monofunctional adducts can cause conformational distortions and destabilize DNA in a sequence-dependent manner.[12–14]

There is increasing interest too in interactions between platinum drugs and sulfur-containing molecules.[15,16] Recently it has been shown that S-bound thioethers in platinum(II) complexes can be displaced inter- or intra-molecularly by N^7 of guanine ligands.[17,18] These observations have highlighted a possible role for thioethers, such as the amino acid methionine, in the mechanism of DNA platination.

Two-dimensional [^1H,^{15}N] heteronuclear multiple-quantum coherence (HMQC) and heteronuclear single-quantum coherence (HSQC) NMR spectroscopy are powerful methods for the study of ligand substitution reactions in ^{15}N-labelled platinum ammine and amine complexes in aqueous solution at concentrations approaching those of physiological relevance.[8,19–22] In this work we have synthesized S-bound *N*-acetyl-L-methionine complexes [Pt([^{15}N]en)(MeCO-Met-*S*)Cl]NO_3 **1n** and [Pt([^{15}N]en)(MeCO-Met-*S*)$_2$][NO_3]$_2$ **2n** (MeCO-Met = *N*-acetyl-L-methionine) and investigated their reactivity towards 5′-GMP (guanosine 5′-monophosphate) and GpG [guanylyl(3′-5′)guanosine] using ^1H and two-dimensional [^1H,^{15}N] HSQC spectroscopy. Some novel reaction intermediates and products are identified, and the formation of the monofunctional adducts stabilized by the presence of S-bound *N*-acetyl-L-methionine is discussed.



5′-GMP



GpG

† The charges on the nucleotides are ignored in formulae.
Non-SI unit employed: M = mol dm^{-3}.

Experimental

Materials and methods

The sodium salt of 5′-GMP, GpG (triethylammonium salt) and *N*-acetyl-L-methionine were obtained from Sigma, $K_2[PtCl_4]$ and $AgNO_3$ from Johnson Matthey, ethane-1,2-diamine (en) and all other chemicals from Aldrich, and were used as supplied. The complexes $[Pt(en)Cl_2]$ and $[Pt([^{15}N]en)Cl_2]$ were prepared by the reported methods.[8,23]

NMR spectroscopy

The NMR spectra were recorded at 310 K, unless otherwise stated, on the following instruments: JEOL GSX270 (^1H, 270; ^{13}C, 67.5 MHz), GSX500 (^1H, 500 MHz), Varian Unity 500 and 600 (^1H, 500 and 600; ^{15}N, 50.7 and 60.8 MHz), respectively, using 5 mm NMR tubes. The chemical shift references (all internal except ^{15}N) were as follows: ^1H, dioxane (δ 3.744), ^{13}C, dioxane (δ 67.3), ^{15}N (external, 1 M $^{15}NH_4Cl$ in 1.5 M HCl). For ^1H NMR, typical acquisition conditions for one-dimensional spectra were as follows: 45–60° pulses, 16–32 K data points, 2–3 s relaxation delay, 32–128 transients collected, final digital resolution 0.2 Hz per point. When necessary, the water resonance was suppressed by pre-saturation. Spectra were processed using Varian VNMR software.[24] The ^{13}C NMR spectra were typically the result of a 12 h acquisition collected into 32 K data points using 50° pulses and relaxation delays of 3 s, with broad-band ^1H decoupling. Two-dimensional $[^1H,^{15}N]$ HSQC spectra were recorded as previously described[20,21] using the standard sequence, optimized for $^1J(NH) = 72$ Hz. The acquisition parameters used were as described previously with ^{15}N decoupling.[20] Water suppression was achieved by pulsed-field gradients.

pH Measurements

The pH values of the solutions were adjusted with 1 M HNO_3 or NaOH 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. For D_2O solutions (adjusted with 1 M DNO_3 or NaOD) the value was read directly from the pH meter without correction for deuterium isotope effects and designated as pH*. The reported pH values are those measured at the beginning of the reactions.

Kinetics

The kinetic data were obtained from ^1H NMR spectra recorded at 310 K. The samples were also maintained at 310 K whilst not in the probe. The relative concentrations were determined by peak integration and the analysis of the data was performed using the program KALEIDAGRAPH.[25]

Preparations

$[Pt(en)(MeCO\text{-}Met\text{-}S)Cl]NO_3$ 1 and $[Pt([^{15}N]en)(MeCO\text{-}Met\text{-}S)Cl]NO_3$ 1n. The complex $[Pt(en)Cl_2]$ (163.0 mg, 0.5 mmol) and equimolar $AgNO_3$ (84.9 mg, 0.5 mmol) were stirred in dimethylformamide (dmf) (4 cm^3) for 24 h at room temperature in the dark. The AgCl was filtered off and *N*-acetyl-L-methionine (95.7 mg, 0.5 mmol) added to the filtrate, which was stirred for 4 h at room temperature. By adding CH_2Cl_2 (20 cm^3) to the solution a pale yellow precipitate was obtained, which was then repeatedly washed with CH_2Cl_2. The resulting solid was dried *in vacuo* (Found: C, 20.30; H, 4.15; N, 9.85. Calc. for $C_9H_{21}ClN_4O_6PtS\cdot 3\%$ dmf: C, 20.00; H, 3.90; N, 10.25%). The ^1H and ^{13}C NMR data for complex **1** are listed in Table 1. The complex $[Pt([^{15}N]en)(MeCO\text{-}Met\text{-}S)Cl]NO_3$ **1n** was prepared using a similar procedure starting from $[Pt([^{15}N]en)Cl_2]$.

$[Pt(en)(MeCO\text{-}Met\text{-}S)_2][NO_3]_2$ 2 and $[Pt([^{15}N]en)(MeCO\text{-}Met\text{-}S)_2][NO_3]_2$ 2n. A suspension of $[Pt(en)Cl_2]$ (163.0 mg, 0.5 mmol) and $AgNO_3$ (169.9 mg, 1.0 mmol) in dmf (4 cm^3) was stirred for 24 h at ambient temperature. After removal of the AgCl precipitate, *N*-acetyl-L-methionine (191.3 mg, 1.0 mmol) was added to the filtrate, which was stirred for 4 h at ambient temperature. By adding CH_2Cl_2 (20 cm^3) to the solution a grey-white solid was obtained which was washed repeatedly with CH_2Cl_2, then dried *in vacuo* (Found: C, 25.15; H, 5.05; N, 10.55. Calc. for $C_{16}H_{34}N_6O_{12}PtS_2\cdot 5\%$ dmf: C, 25.35; H, 4.50; N, 11.05%). The ^1H and ^{13}C NMR data for complex **2** are listed in Table 1. Complex **2n** was prepared similarly using $[Pt([^{15}N]en)Cl_2]$ as starting material.

NMR samples and reaction conditions

No buffers were used in the reactions, in order to avoid buffer co-ordination to platinum, *e.g.* phosphate,[26,27] which may interfere with the present studies. All the reactions were carried out in the region pH 3.3–4.5.

The following reactions were carried out in 90% water–10% D_2O (0.6 cm^3) in NMR tubes at 310 K: **1n** (5 mM) + 5′-GMP (10 mM), pH 3.70; **1n** (5 mM) + GpG (5 mM), pH 3.99; **2n** (5 mM) + 5′-GMP (10 mM), pH 3.70; and **2n** (5 mM) + GpG (5 mM), pH 3.37. These samples were prepared as follows: complexes **1n** or **2n** and 5′-GMP or GpG were weighed and dissolved separately in 90% water–10% D_2O (0.3 cm^3). The pH of each solution was adjusted separately to the desired value using 0.1 M HNO_3 or NaOH. After mixing the reactants in an NMR tube the final pH of the solution was measured.

The sample for the reaction of the chelated MeCO-Met complexes $[Pt([^{15}N]en)\{MeCO\text{-}Met\text{-}(2-)\text{-}S,N\}]$ **3** and $[Pt([^{15}N]en)\{MeCO\text{-}Met\text{-}(1-)\text{-}S,O\}]^+$ **4** with 5′-GMP was prepared as follows: an aliquot of a stock solution of $[Pt([^{15}N]en)(H_2O)_2]^{2+}$, prepared according to previous work,[8] was incubated with 1 mol equivalent of MeCO-Met (5 mM) in 90% water–10% D_2O for 24 h at pH 7.0. Two mol equivalents of 5′-GMP (10 mM) were added to the solution, and then the pH was lowered to 3.70.

The sample for the reaction of $[Pt(en)(MeCO\text{-}Met\text{-}S)Cl]NO_3$ **1** (5 mM) + 5′-GMP (5 mM) at pH* 4.42 was prepared by mixing separately dissolved **1** and 5′-GMP in D_2O (0.35 cm^3).

Results

Characterization of complexes 1 and 2

The elemental analyses of complexes $[Pt(en)(MeCO\text{-}Met\text{-}S)Cl]NO_3$ **1** and $[Pt(en)(MeCO\text{-}Met\text{-}S)_2][NO_3]_2$ **2** and their ^1H NMR spectra are in accordance with their proposed formulae. The ^1H NMR spectra showed the presence of minor amounts of dmf [$\delta(CH_3)$ 3.001, 2.845].

The signals due to the *S*-methyl and acetyl-methyl groups provided convenient probes to monitor the co-ordination of *N*-acetyl-L-methionine. The SCH_3 and $COCH_3$ signals of free MeCO-Met were observed at δ 2.110 and 2.040, respectively (pH 3.70). In the spectra of complexes **1** and **2** the SCH_3 signals are shifted downfield by 0.33 and 0.50 ppm, respectively, but the shift of the $COCH_3$ signal was nearly unchanged. In the ^{13}C NMR spectrum the SCH_3 and γ-CH_2 signals of free MeCO-Met were observed at δ 14.83 and 30.67, which on co-ordination were shifted downfield by 6.40 and 5.10 ppm for complexes **1** and **2**, respectively, whereas the shifts of the two ^{13}C peaks of the acetyl group were almost unchanged. Very similar ^{13}C NMR data have been reported previously for the S-bound MeCO-Met platinum complex $[PtCl_3(MeCO\text{-}Met\text{-}S)]^-$.[28,29] The chemical shifts of the complexes are listed in Table 1.

The two-dimensional $[^1H,^{15}N]$ HSQC spectrum of $[Pt([^{15}N]en)(MeCO\text{-}Met\text{-}S)Cl]^+$ contained two major cross-peaks at δ 5.42/−8.7 and 5.71/−24.4, and two minor cross-

Table 1 Proton and ^{13}C NMR chemical shifts for mono and bis MeCO-Met complexes of Pt^{II}, **1** and **2**

		δ(^{1}H)					
Compound	pH	α-CH	β-CH_2	γ-CH_2	SCH_3	$COCH_3$	CH_2 (en)
MeCO-Met	3.16	4.487	2.020 2.200	2.630 2.620	2.110	2.040	
1 [Pt(en)(MeCO-Met-*S*)Cl]NO_3	2.32	*	*	*	2.448	2.055	2.748
	4.00	*	*	*	2.432	2.041	2.754
2 [Pt(en)(MeCO-Met-*S*)$_2$][NO_3]$_2$	3.10	4.50	2.339 2.173	3.060	2.610	2.045	2.873

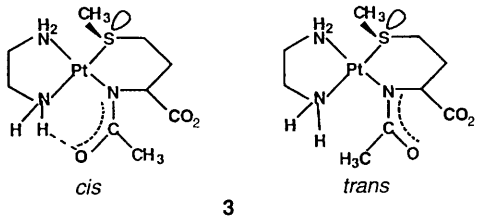
		δ(^{13}C)							
		CO_2H	α-C	β-C	γ-C	SCH_3	$COCH_3$	$COCH_3$	C (en)
MeCO-Met	2.73	176.24	52.46	30.15	30.67	14.83	22.37	175.02	
1	2.32	175.31	52.17	29.27	34.52	20.25	22.54	175.02	49.65 47.89
2	2.20	174.96	51.99	29.27	35.63	20.09	22.51	174.92	48.06
	7.40	177.73	54.19	30.53	35.87	20.22	22.73	174.47	48.06

* Very broad signal.

peaks which accounted for less than 5% of the total peak volume at δ 6.11/−5.9 and 5.38/−30.8. The peak at δ 5.42/−8.7 is assigned to the amine group *trans* to sulfur and that at δ 5.71/−24.4 is in the ^{15}N chemical shift region for amine *trans* to chloride or nitrogen.[8,30,32] The only nitrogen donor would be the amide group of MeCO-Met, but its co-ordination to platinum can be ruled out from the ^1H and ^{13}C NMR data discussed above and therefore the *trans* ligand must be chloride. As a result the major peaks are assigned to **1n**. The minor peak at δ 5.38/−30.8 can be assigned to unreacted [Pt([^{15}N]en)Cl_2],[8] and the other minor peak at δ 6.11/−5.9 (NH_2 *trans* to sulfur) has the same shift as that of [Pt([^{15}N]en)(MeCO-Met-*S*)$_2$]$^{2+}$. The spectrum of **2n** consisted of only one cross-peak at δ 6.11/−5.9, consistent with NH_2 *trans* to sulfur.

Stability of complexes 1 and 2 in water

The stabilities of complex **1** or **1n** at pH 3.5 and **2** or **2n** at pH 3.6 were studied using ^1H and two-dimensional [^{1}H,^{15}N] HSQC spectroscopy. For **1**, upon dissolution in D_2O at 310 K (Scheme 1) the SCH_3 signal at δ 2.432 and the $COCH_3$ signal at 2.041 gradually decreased in intensity with time. Correspondingly, peaks at δ 2.519, 2.496, 2.448, 2.429 and 2.356 appeared after about 2 h and then increased in intensity with time. A two-dimensional [^{1}H,^{15}N] HSQC spectrum of complex **1n** recorded 6 h after dissolution in 90% water–10% D_2O is shown in Fig. 1. In this spectrum, apart from the two cross-peaks belonging to complex **1n** (δ 5.71/−24.4 and 5.42/−8.7) and the minor peaks for the dichloride and bis(*N*-acetyl-L-methionine) complexes present as impurities in **1n**, a series of new cross-peaks is observed. Among them, seven (δ 5.60, 5.56, 5.53, 5.35, 5.25, 5.13/−26.9 and 5.46/−25.4) have ^{15}N shifts which are compatible with amine groups *trans* to nitrogen or chloride. The only nitrogen donor available is the amide group of MeCO-Met, the co-ordination of which would give a six-membered *S,N*-chelate. This could give rise to four different isomers, due to the chiral sulfur centre and the *cis* and *trans* arrangement about the C–N bond of the acetyl group.[33] The three cross-peaks at δ 5.39, 5.13/−8.2 and 5.25/−10.9 (NH_2 *trans* to sulfur) appeared at the same time together with peaks in the region for NH_2 *trans* to nitrogen, and had comparable intensity to them. Therefore, these peaks can be assigned to the chelated complex [Pt([^{15}N]en){MeCO-Met(2−)-*S,N*}] **3**. Fewer peaks are observed for it than expected, which may be due to the fact that some of the isomers are not favoured or due to the fast exchange rate at this temperature. By comparing the two-dimensional data with the ^1H NMR spectrum, the singlet peaks



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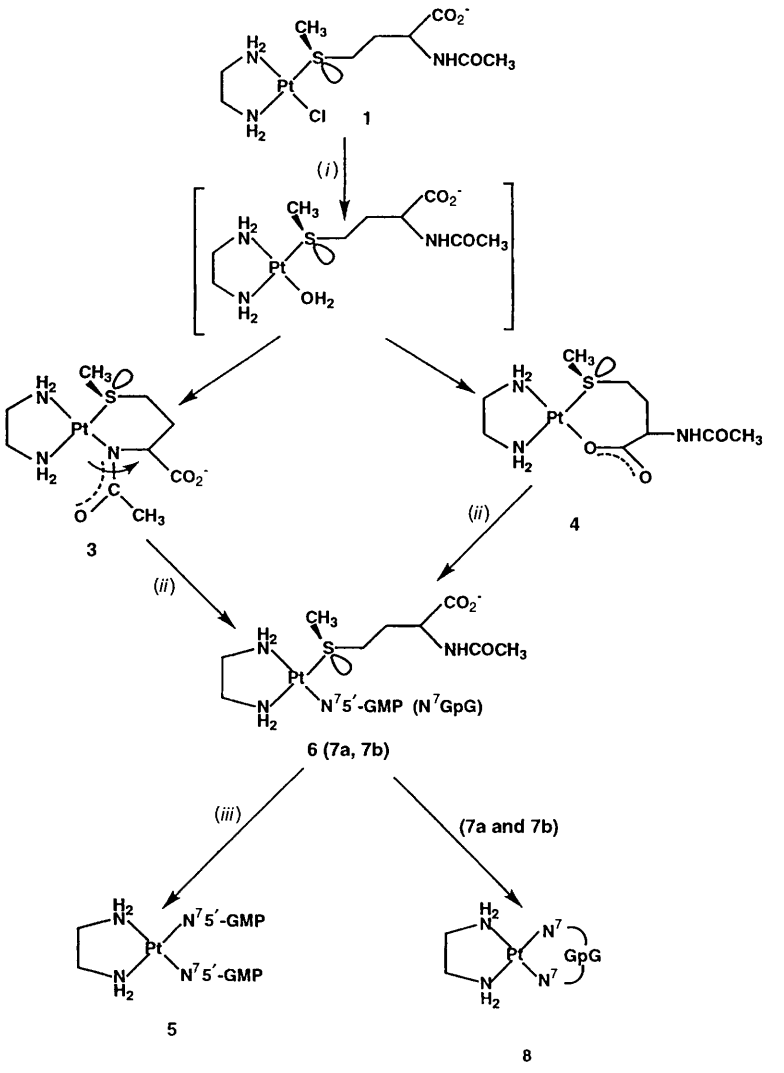
observed at δ 2.448, 2.429 and 2.356 can be related to the formation of complex **3** by their intensities.

Of the other six remaining cross-peaks in the two-dimensional spectrum, two broad ones (5.95, 5.59/−6.7) are assignable to NH_2 groups *trans* to S, and the other four to NH_2 *trans* to O (δ 6.04, 5.88, 5.76, 5.66/−43.0). There are three possible O donors in this system: water, carbonyl and carboxylate groups of the MeCO-Met. The co-ordination of carbonyl can be excluded because it would lead to an unstable eight-membered ring; that of water can also be ruled out, because four cross-peaks for amine groups *trans* to oxygen are observed (Fig. 1) and this is unlikely to arise with only monodentate sulfur since inversion is usually very fast.[28,29] The most likely O donor is the carboxylate group, to form [Pt([^{15}N]en){MeCO-Met(1−)-*S,O*}]$^+$ **4** with a seven-membered *S,O*-chelate ring, and slow sulfur inversion. The only reported *S,O*-chelated methionine complex is [Pt(NH_3)$_2$){Met(1−)-*S,O*}]$^+$, which was characterized by NMR spectroscopy at very low pH.[34] There is an apparent intensity difference between peaks due to NH_2 *trans* to O and those due to NH_2 *trans* to S, which may be due to differences in relaxation rates. A large ^1H chemical shift difference between peaks for the two protons of the NH_2 group *trans* to sulfur (*cis* to O) is also observed (Table 2).

Therefore, it can be concluded that complex **1** and **1n** in water at this pH gradually form the chelated complexes **3** and **4**. By plotting the natural log of the decrease in intensities of the two methyl signals (SCH_3 and $COCH_3$) of **1n** *versus* time a pseudo-first-order rate constant, k_{obs} of (4.9 ± 0.2) × 10^{-5} s^{-1} ($t_{\frac{1}{2}}$, 3.9 h) was obtained. For comparison, the rate constants for hydrolysis of [Pt(en)Cl_2] or its (1*R*,2*R*,4*S*)-*exo*-2-(aminomethyl)-2-amino-7-oxabicyclo[2.2.1]heptane analogue at similar temperature are between 12 × 10^{-5} and 15 × 10^{-5} s^{-1} for the first chloride and 15 × 10^{-5} to 17 × 10^{-5} s^{-1} for the second chloride.[35–38]

In contrast, complex **2n** was quite stable in water at pH 3.6 for several days. A ^1H NMR spectrum in D_2O recorded after 72 h at 310 K showed only about 5% of free MeCO-Met (SCH_3, δ 2.102). The appearance of peaks at δ 2.448, 2.429 and 2.356



Scheme 1 (*i*) Water; (*ii*) 5′-GMP or GpG; (*iii*) 5′-GMP

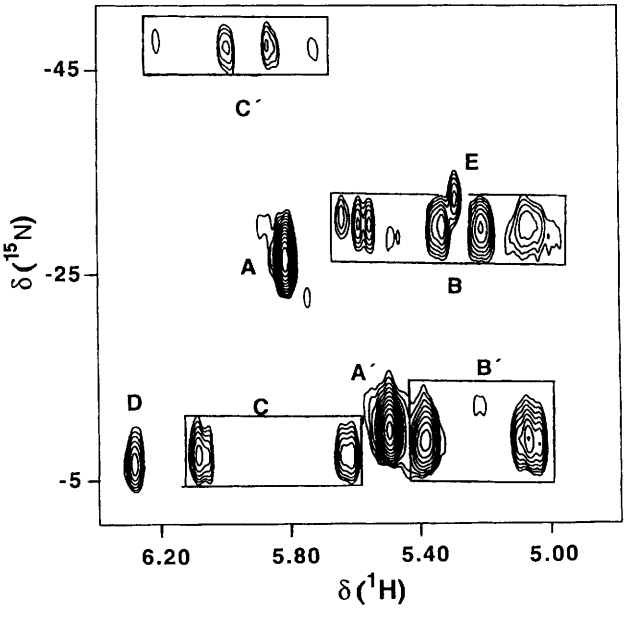


Fig. 1 A 600 MHz two-dimensional [^{1}H,^{15}N] HSQC spectrum at 310 K of a solution of complex **1n** (5 mM) in 90% water–10% D_2O at pH 3.5 after 6 h at 310 K. Peak assignments: A, NH_2 (*trans* to Cl) and A′, NH_2 (*trans* to S) for **1n**; B, NH_2 (*trans* to N) and B′, NH_2 (*trans* to S) for **3**; C, NH_2 (*trans* to S) and C′, NH_2 (*trans* to O) for **4**; D, NH_2 (*trans* to S) for **2n**; E, NH_2 (*trans* to Cl) for [Pt([^{15}N]en)Cl_2]

showed that a small amount of the chelated complex **3** had formed. Therefore, the hydrolysis of **2n** in water was slow enough to be ignored in the reactions studied below.

Reaction of complexes 1 and 1n with 5′-GMP

The reactions between complex **1** or **1n** and 5′-GMP in a 1:1 molar ratio (5 mM, pH* 4.42, 310 K) were monitored by ^{1}H and two-dimensional [^{1}H,^{15}N] NMR spectroscopy. In the 5′-GMP H^8 region of the ^{1}H NMR spectrum a new peak at δ 8.706 appeared and increased in intensity with time, while the H^8 peak of free 5′-GMP at δ 8.130 decreased in intensity. In the region for *S*-methyl signals a new peak appeared at δ 2.384, whilst the SCH_3 peak of complex **1** at δ 2.432 decreased in intensity. Simultaneously, peaks appeared at δ 2.448, 2.429 and 2.356, which are assigned to complex **3** (see above). When the reaction was repeated with **1n** two-dimensional [^{1}H,^{15}N] cross-peaks assignable to complex **3** appeared after 3 h together with new cross-peaks at δ 5.92/−27.9, 5.77/−8.3 and 5.79/−28.7, and peaks for **1n** at δ 5.71/−24.4 and 5.42/−8.7 were also observed.

The peak at δ 8.501 in the ^{1}H NMR spectrum and the cross-peak at δ 5.79/−28.7 in the two-dimensional spectrum are assignable to the bis complex [Pt([^{15}N]en)(5′-GMP-N^7)$_2$]$^{2+}$ **5**, which has been characterized previously both in solution and in the solid state.[8,32] The peaks at δ 8.706 (H^8 signal of N^7-co-ordinated 5′-GMP) and 2.384 (*S*-methyl signal of MeCO-Met co-ordinated through sulfur) in the ^{1}H NMR spectrum

Table 2 Proton and ^{15}N NMR chemical shifts for complexes **1n**, **2n** and chelated MeCO-Met products

Compound	pH	$\delta(^1H)$	$\delta(^{15}N)$ (*trans* to)
1n [Pt([^{15}N]en)(MeCO-Met-*S*)Cl]NO_3	3.99	5.71	−24.4 (Cl)
		5.42	−8.7 (S)
2n [Pt([^{15}N]en)(MeCO-Met-*S*)$_2$][NO_3]$_2$	3.70	6.11	−5.9 (S)
3 [Pt([^{15}N]en){MeCO-Met(2−)-*S*,N}]	3.50	5.60, 5.56, 5.53, 5.35, 5.25, 5.13	−26.9 (N)
		5.46	−25.4 (N)
		5.39, 5.13	−8.2 (S)
		5.25	−10.9 (S)
4 [Pt([^{15}N]en){MeCO-Met(1−)-*S*,O}]$^+$	3.50	6.04, 5.88, 5.76, 5.66	−43.0 (O)
		5.95, 5.59	−6.7 (S)

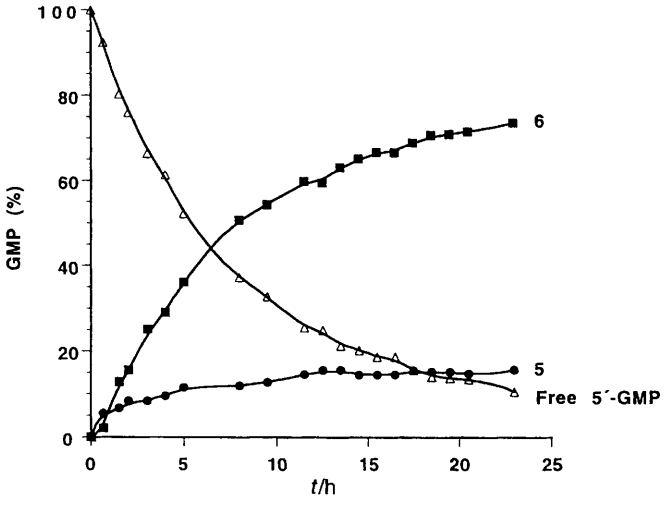


Fig. 2 Plot of the intensity variation of the H^8 signal of 5′-GMP with time, for the reaction of complex **1** (5 mM) with 5′-GMP (5 mM) at pH 4.42 at 310 K

increased in intensity at a comparable rate. At the corresponding time, the cross-peaks at δ 5.92/−27.9 (amine *trans* to nitrogen) and 5.77/−8.3 (amine *trans* to sulfur) increased in intensity in the two-dimensional [^{1}H,^{15}N] NMR spectrum. The latter corresponds to the amine ligand *trans* to sulfur of MeCO-Met, while the most likely nitrogen donors in the reaction are N^7 of 5′-GMP and the deprotonated amide nitrogen of MeCO-Met. The latter can be ruled out since peaks for complex **3** have been assigned above, and therefore the nitrogen donor is assigned to N^7 of 5′-GMP and the data are consistent with the formation of the mixed-ligand complex [Pt(en){MeCO-Met(1−)-*S*}(5′-GMP-N^7)]$^+$ **6**.

The reaction course was followed by plotting the areas of the H^8 signals for different adducts *versus* time (Fig. 2). After 24 h complex **6** was the main product (75%), with **5** and free 5′-GMP accounting for 15 and 10%, respectively, of the total 5′-GMP.

A pseudo-first-order rate, k_{obs}, of (3.1 ± 0.2) × 10^{-5} s^{-1} ($t_{\frac{1}{2}}$ 6.2 h, 310 K) was determined from a plot of the log of the decrease in intensity of the H^8 signal of free 5′-GMP *versus* time. It is only slightly smaller than that for hydrolysis of **1n** at a similar pH. Surprisingly, peaks at δ 2.448, 2.429 and 2.356 first appeared and then by 24 h had almost disappeared, suggesting that the *S*,*N*-chelated MeCO-Met complex **3** is an intermediate which reacts with 5′-GMP to give **6**. That such a reaction is possible was confirmed as described below.

Reaction of complexes 3 and 4 with 5′-GMP

In order to confirm that chelated MeCO-Met complexes react with 5′-GMP the following reaction was studied by ^{1}H and two-dimensional [^{1}H,^{15}H] NMR spectroscopy. A solution of [Pt([^{15}N]en)$(H_2O)_2$]$^{2+}$ (5 mM) was incubated at pH 7.0 with 1 equivalent of MeCO-Met for 24 h to give complexes **3** and **4** in 10:1 ratio as determined by ^{1}H and two-dimensional [^{1}H,^{15}N] NMR spectroscopy. To this solution 5′-GMP (2 mol equivalents, total 10 mM) was added and incubated at pH 3.70. After 24 h all the peaks belonging to **3** and **4** had nearly disappeared, and an H^8 signal for bound 5′-GMP at δ 8.680 and SCH_3 signal for S-bound MeCO-Met at δ 2.384 had appeared (Fig. 3). In the two-dimensional [^{1}H,^{15}N] HSQC spectrum, cross-peaks at δ 5.71/−24.4 and 5.42/−8.7 were dominant. This experiment confirmed that at pH *ca.* 4 complexes **3** and **4** do react with 5′-GMP to give the mixed-ligand complex **6**. This appears to be the first example of an *S*,*N*-chelated methionine derivative reacting with a nucleotide *via* ring opening.

Reaction of complex 1n with 5′-GMP (1:2)

The reaction of complex **1n** (5 mM) and 2 mol equivalents of 5′-GMP (10 mM) at pH 3.7 was monitored by ^{1}H and two-dimensional [^{1}H,^{15}N] HSQC spectroscopy. It followed a similar course as that for the 1:1 reaction. After 3 h incubation the formation of both *S*,*N*-chelated **3** and **6** was observed. After 24 h all the cross-peaks in the two-dimensional [^{1}H,^{15}N] spectrum, belonging to **3** had disappeared, **6** accounted for about 90% of the total peak volume, and **5** for about 5%. The time course of the reaction is shown in Fig. 4. After 4 d incubation about 10% of the MeCO-Met was free in solution, which was related to the relative increase in intensity of peaks for complex **5**, the bis 5′-GMP adduct. By plotting the natural log of the decrease in intensity of the SCH_3 and $COCH_3$ signals of complex **1n** *versus* time for the initial stage of the reaction (first 24 h), a pseudo-first-order rate constant k_{obs} of (3.3 ± 0.2) × 10^{-5} s^{-1} ($t_{\frac{1}{2}}$ 5.9 h) was determined, a value similar to that obtained for the 1:1 reaction.

A typical two-dimensional [^{1}H,^{15}N] HSQC spectrum is shown in Fig. 5, recorded after 72 h of reaction at 310 K. The cross-peak at δ 5.79/−28.7 is assigned to complex **5** and the remaining peaks at δ 5.92/−28.0 and 5.47, 5.32/−8.5 to **6**.

Reaction of complex 1n with GpG

The 1:1 reaction between complex **1n** (5 mM) and GpG (5 mM) in 90% water–10% D_2O was investigated at 310 K, pH 3.99. In the H^8 region of the ^{1}H NMR spectrum free GpG gives two singlets at δ 7.997 and 7.935, assignable to 3′-G and 5′-G, respectively.[39,40] Upon reaction with **1n** a pair of signals at δ 8.423 and 8.140 appeared together with two other pairs at δ 8.602, 7.980 and 8.553, 7.918. In the methyl region the peaks for the starting complex **1n** (SCH_3, δ 2.432; $COCH_3$, 2.041) decreased in intensity with time and a broad signal at δ 2.311 (SCH_3) and two singlets at δ 1.972 and 1.953 appeared. In addition, peaks appeared at δ 2.448, 2.429 and 2.356 which were assigned as above to **3**. The two-dimensional [^{1}H,^{15}N] HSQC spectrum recorded after 4 h incubation was complicated. There were cross-peaks for **1n** and for **3**, together with a series of new cross-peaks. Two cross-peaks at δ 5.78, 5.70/−28.8, assignable to amine groups *trans* to N, were correlated with two ^{1}H signals at δ 8.423 and 8.140 according to the time of their appearance and intensities. Therefore, the data suggested the formation of

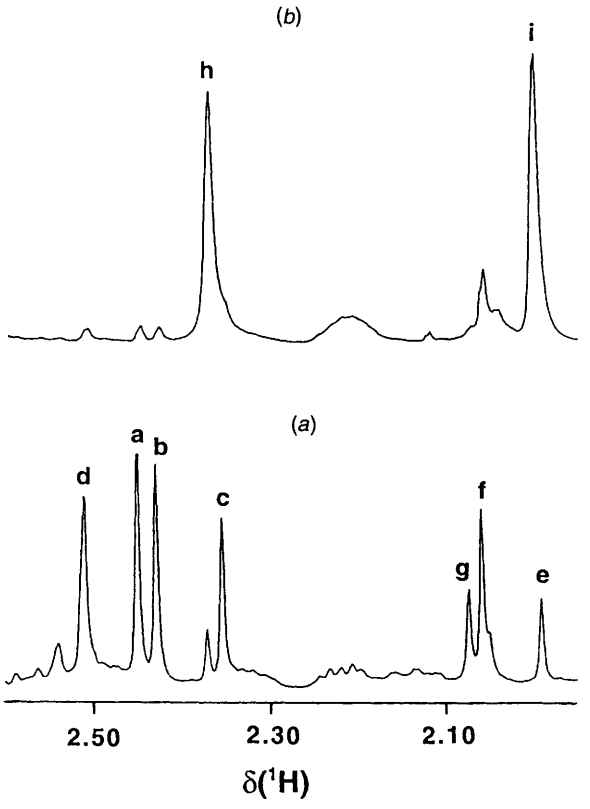


Fig. 3 The 600 MHz ^{1}H NMR spectra for reaction of complexes **3** and **4** (5 mM) with 5′-GMP (10 mM) at pH 3.70 at 310 K in 90% water–10% D_2O. (*a*) A mixture of **3** and **4** before incubation with 5′-GMP; (*b*) 24 h after the incubation with 5′-GMP. Peak assignments: a–c, f and g (SCH_3 and acetyl CH_3) for **3**; d (SCH_3) and e (acetyl CH_3) for **4**; h (SCH_3) and i (acetyl CH_3) for [Pt(en){MeCO-Met(1−)-*S*}(5′-GMP-N^7)]$^+$ **6**

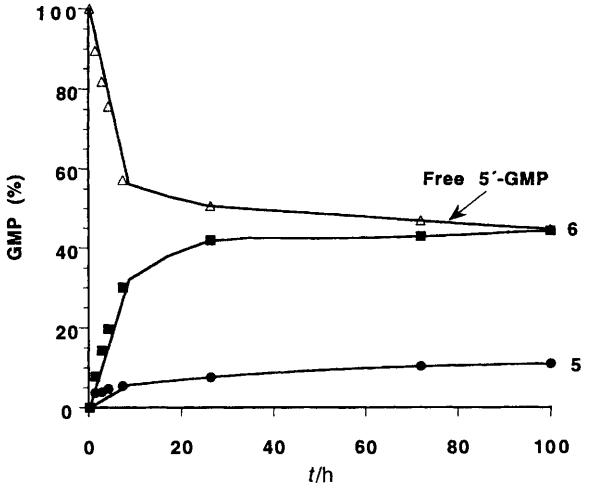


Fig. 4 Plot of the intensity variation of the H^8 signal of 5′-GMP with time, for the reaction of complex **1n** (5 mM) with 5′-GMP (10 mM) at pH 3.85 at 310 K

complex **8** [Pt([^{15}N]en)(GpG-N^7,N^7)]$^{2+}$, where both guanine bases are co-ordinated through N^7. The assignments of the ^{1}H signals of 5′- and 3′-G are based on the reported data for the related complex [Pt(NH_3)$_2$(GpG-N^7,N^7)]$^{2+}$: H^8 (5′-G), δ 8.54, (3′-G) 8.30.[41,42] The remaining cross-peaks at δ 5.99, 5.77/−7.4 and 5.94/−27.7 are assignable to amine groups *trans* to S and N, respectively. The sulfur donor can be assigned to MeCO-Met and the N donor to N^7 of GpG. By correlation with the two-dimensional [^{1}H,^{15}N] NMR data, the ^{1}H signals at δ 8.602, 7.980 and 8.553, 7.918 (H^8), 2.311 (SCH_3) and 1.972 and

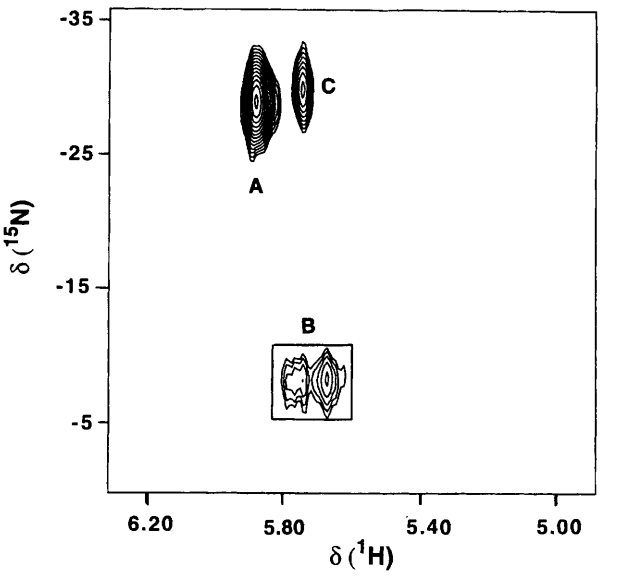


Fig. 5 The 600 MHz two-dimensional [^{1}H,^{15}N] HSQC spectrum at 283 K of a solution of complex **1n** (5 mM) with 5′-GMP (10 mM) at pH 3.85 in 90% water–10% D_2O after 72 h incubation at 310 K. Peak assignments: A, NH_2 (*trans* to N) and B, NH_2 (*trans* to S) for complex **6** (slow inversion at S); C, NH_2 (*trans* to N) for **5**

1.953 ($COCH_3$) can be assigned to [Pt([^{15}N]en){MeCO-Met(1−)-*S*}(GpG-$N^{7(1)}$)]$^+$ **7a** and [Pt([^{15}N]en){MeCO-Met(1−)-*S*}(GpG-$N^{7(2)}$)]$^+$ **7b**, as major and minor species, respectively (Table 3).

A two-dimensional [^{1}H,^{15}N] HSQC spectrum of the reaction mixture recorded at 283 K after 30 h incubation at 310 K is shown in Fig. 6. Four cross-peaks at δ 5.47, 5.58/−29.6 and 5.41, 5.51/−28.7 can be assigned to the bifunctional adduct **8**. At this temperature, cross-peaks which were assignable to complexes **7a** and **7b** were now well resolved. On the basis of their intensities, those at δ 5.71/−27.7 (NH_2 *trans* to N), 5.58, 5.66/−6.9 (NH_2 *trans* to S) are tentatively assigned to **7a**, and other peaks at δ 5.77/−27.7 (NH_2 *trans* to N) and 5.79, 5.90/−6.9 (NH_2 *trans* to S) to **7b**. Interestingly, the ^{1}H signals for NH_2 *trans* to S (*cis* to monoco-ordinated GpG) in **7a** (δ 5.58, 5.66) and **7b** (δ 5.79, 5.90) are widely separated, which may be due to the presence of differences in hydrogen bonding in 5′- and 3′-G co-ordinated adducts.

Both the SCH_3 and acetyl CH_3 ^{1}H NMR signals of the monofunctional adducts (**7a** and **7b**) are shifted upfield with respect to the starting complex **1n** (SCH_3, by 0.14 ppm for both **7a** and **7b**; $COCH_3$, by 0.10 ppm for **7a** and 0.08 ppm for **7b**), an effect which may arise from ring-current shifts induced by the guanine bases.

In the ^{1}H NMR spectrum, another pair of H^8 signals at δ 8.641 and 8.368 was observed after 3 h, and increased in intensity in a similar way to peaks for the bifunctional adduct. These may correspond to the two unassigned cross-peaks at δ 5.51, 5.49/−7.9 in the two-dimensional [^{1}H,^{15}N] spectrum. Although no definite assignment of the signals can be made, the ^{1}H shifts suggest that both guanine bases of GpG are co-ordinated to platinum in this adduct.

A plot of the variation in concentrations of the different species (based on integration of H^8 signals) *versus* time is shown in Fig. 7(*a*), where the preference for formation of the monofunctional adduct **7a** over **7b**, by 60:40, can be clearly seen. The concentrations of the two monofunctional adducts increased with time in a similar way, and their ratio (60:40) was constant during the reaction course. The concentrations of the bifunctional adduct and the two unassigned peaks (δ 8.641 and 8.368) increased in a similar way, but both very slowly such that they each accounted for less than 10% of the total GpG in solution at equilibrium. Complexes **7a** and **7b** were very stable,

Table 3 Proton and ^{15}N NMR chemical shifts for the products from the reactions of complexes **1** and **2** with nucleotides at 310 K unless otherwise stated

Compound[a]	pH	δ(^{1}H) SCH_3	$COCH_3$	H^8 of 5′-G	H^8 of 3′-G	CH_2 (en)	en NH_2	δ(^{15}N) (*trans* to)
6 [Pt(en){MeCO-Met(1−)-*S*}(5′-GMP-N^7)]$^+$	4.42	2.384	2.002	8.706				
[Pt([^{15}N]en){MeCO-Met(1−)-*S*}(5′-GMP-N^7)]$^+$	3.85	2.370	1.988	8.683		2.839	5.92	−27.9 (N)
							5.77	−8.3 (S)
6[b]							5.92	−28.0 (N)
							5.47, 5.32	−8.5 (S)
5 [Pt(en)(5′-GMP-N^7)$_2$]$^{2+}$	4.42			8.501				
[Pt([^{15}N]en)(5′-GMP-N^7)$_2$]$^{2+}$	3.85			8.469		2.806	5.79	−28.7 (N)
7a [Pt([^{15}N]en){MeCO-Met(1−)*S*}(GpG-N^7)]$^+$	3.99		1.953	7.918	8.553		5.94	−27.7 (N)
		2.311				2.984	5.77	−7.4 (S)
						2.780		
7b			1.972	8.602	7.980		5.94	−27.7 (N)
							5.99	−7.4 (S)
7a[b]							5.71	−27.7 (N)
							5.58, 5.66[c]	−6.9 (S)
7b[b]							5.77	−27.7 (N)
							5.79, 5.90[c]	−6.9 (S)
8 [Pt([^{15}N]en)(GpG-N^7,N^7)]$^{2+}$	3.99			8.423	8.140	2.780	5.78, 5.70	−28.8 (N)
8[b]							5.47, 5.58	−29.6 (N)
							5.41, 5.51	−28.7 (N)

[a] Charge on nucleotides is ignored. [b] Spectrum recorded at 283 K; two different forms of monofunctional GpG were observed. [c] Tentative assignment (see Fig. 6).

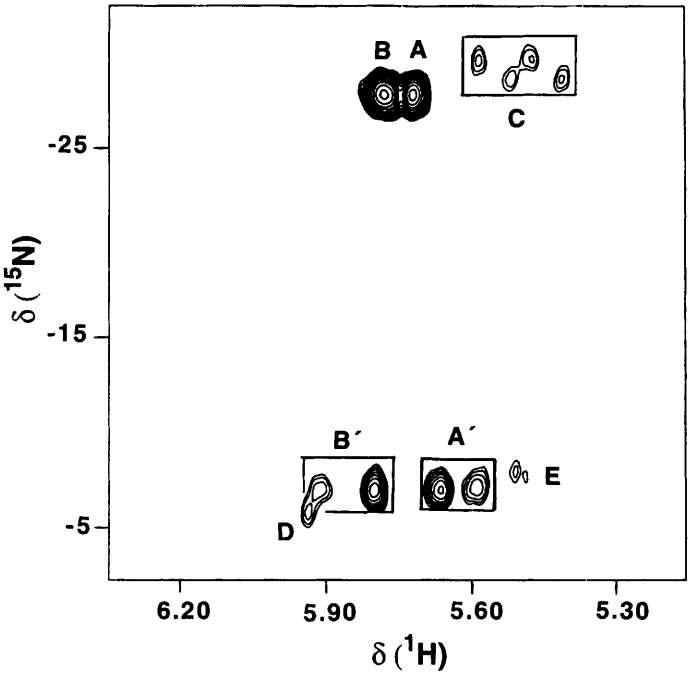


Fig. 6 A 600 MHz two-dimensional [^{1}H,^{15}N] HSQC spectrum at 283 K of a solution of complex **1n** (5 mM) with GpG (5 mM) at pH 3.99 at 310 K in 90% water–10% D_2O after 30 h incubation at 310 K. Peak assignments: A, NH_2 (*trans* to N) and A′, NH_2 (*trans* to S) for complex **7a**; B, NH_2 (*trans* to N) and B′, NH_2 (*trans* to S) for **7b**; C, NH_2 (*trans* to N) for **8**, D, NH_2 (*trans* to S) for **2n**; E, NH_2 (*trans* to S) for unassigned species. The pairings of the four peaks A′ and B′ are tentative and based on intensities only

there being no increase in the concentration of the bifunctional adduct **8** even after several weeks.

A plot of the variation in intensities of the two methyl peaks of MeCO-Met with time is shown in Fig. 7(*b*). After 50 h only about 5% of complex **1n** remained, the two monofunctional adducts accounted for about 80% of the MeCO-Met, free MeCO-Met about 5%, and 10% was in other species, such as complex **3**. It is evident from Fig. 7 that there is a relatively rapid formation of monofunctional adducts during the first 20 h, followed by a very slow conversion into the bifunctional adduct.

Reaction of complex 2 with 5′-GMP

The reaction of [Pt([^{15}N]en)(MeCO-Met-*S*)$_2$][NO_3]$_2$ **2** (5 mM) with 2 mol equivalents 5′-GMP was carried out at pH 3.70 (Scheme 2). The displacement of MeCO-Met by 5′-GMP was readily monitored by the SCH_3 ^{1}H NMR signal at δ 2.610. As this decreased in intensity a new signal appeared at δ 2.110 assignable to free MeCO-Met. Concomitantly, a new peak at δ 2.370 increased in intensity. This was previously assigned to **6** (Fig. 8). The two-dimensional [^{1}H,^{15}N] spectrum which was recorded after 3 d of reaction appeared to be identical to Fig. 5 (reaction of **1n** with 5′-GMP), and confirmed the formation of **6**. The next step in the reaction, *i.e.* conversion of **6** into the bis(5′-GMP) adduct **5**, was not observed even after several days.

Reaction of complex 2 with GpG

The reaction of GpG (5 mM) with complex **2** (5 mM) gave rise to the two monofunctional adducts **7a**, **7b** and free MeCO-Met, again with the preferential formation of **7a** over **7b** (ratio, 60:40). After 24 h free MeCO-Met accounted for about 30% of the total MeCO-Met, in line with formation of **7a** and **7b**. The bifunctional adduct **8** was not observed even after several days. A typical two-dimensional [^{1}H,^{15}N] HSQC spectrum recorded after 70 h incubation is shown in Fig. 9.

Discussion

Previous studies have shown unexpectedly that it is possible to displace a Pt^{II}-co-ordinated thioether by N^7 of guanine.[17,18] However, these were carried out with [Pt(dien)]$^{2+}$ (dien = diethylenetriamine) complexes and therefore it was of interest to extend the work to *cis*-diam(m)ine platinum(II) anticancer complexes. Reactions between cisplatin and L-methionine are complicated by the loss of ammine ligands due to the strong *trans* influence of sulfur,[43] and therefore, in the present work, we have used the chelating ligand ethane-1,2-diamine since this is less readily displaced.

We prepared both the mono and bis S-bound *N*-acetyl-L-

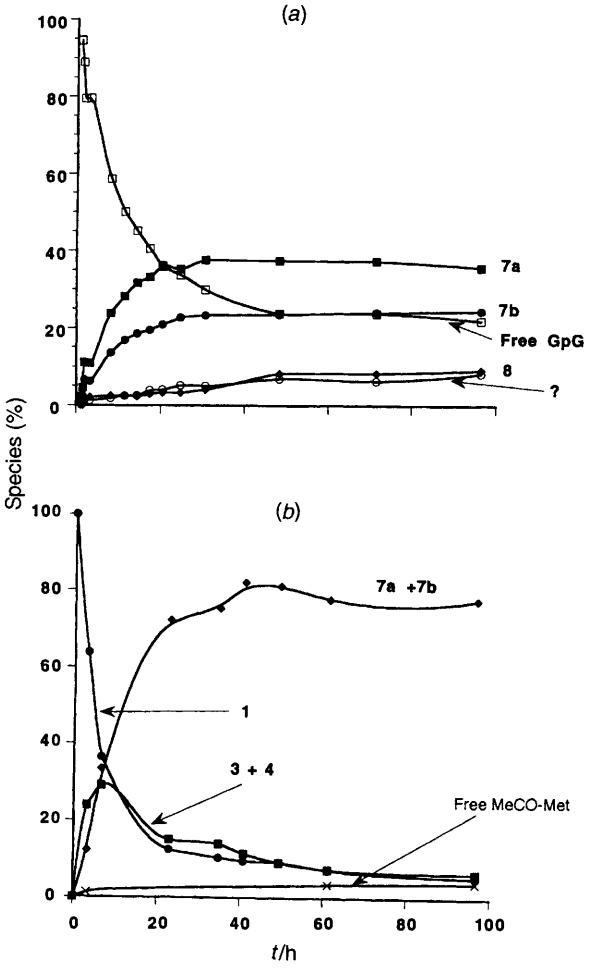
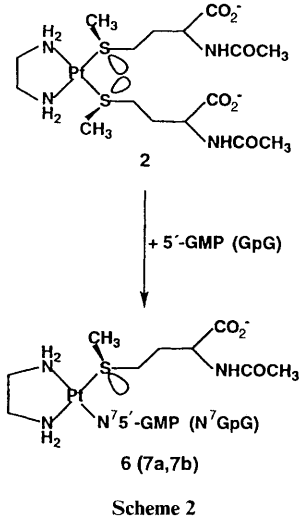


Fig. 7 Plots of the intensity variation of (*a*) H^8 ^1H NMR signals of GpG with time, for the reaction of complex **1n** (5 mM) with GpG (10 mM) at pH 3.99 at 310 K (? indicates an unassigned species), (*b*) two methyl ^1H NMR signals (SCH_3 and $COCH_3$) during the same reaction. The curves are drawn only to connect the points and illustrate the time course of the reaction



Scheme 2

methionine complexes [Pt(en)(MeCO-Met-*S*)Cl]NO_3 **1** and [Pt(en)(MeCO-Met-*S*)$_2$][NO_3]$_2$ **2** and investigated their reactions with 5′-GMP and GpG. Complex **1** had an acceptable elemental analysis, although the two-dimensional [^1H, ^{15}N] HSQC NMR spectra of **1n** showed that it contained minor amounts of [Pt([^{15}N]en)Cl_2] and **2n** as impurities. Provided the preparation was carried out with care it was

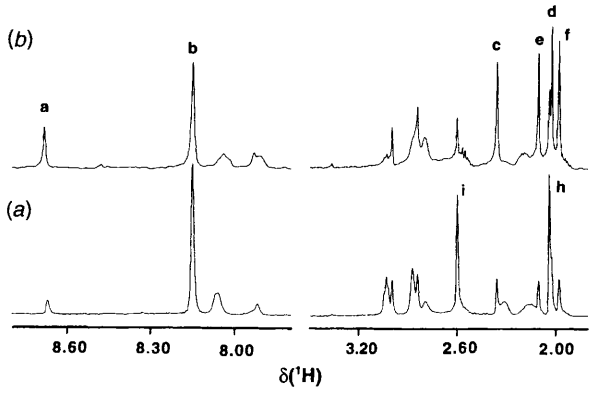


Fig. 8 The 600 MHz ^1H NMR spectra at 310 K of a solution of complex **2n** (5 mM) with 5′-GMP (10 mM) at pH 3.70 in 90% water–10% D_2O after 24 h incubation (*a*) and after 5 d (*b*). Peak assignments: a (H^8), c (SCH_3) and d (acetyl CH_3) for complex **6**; b (H^8) for free 5′-GMP; e (SCH_3) and f (acetyl CH_3) for free MeCO-Met; h (acetyl CH_3) and i (SCH_3) for unreacted **2n**

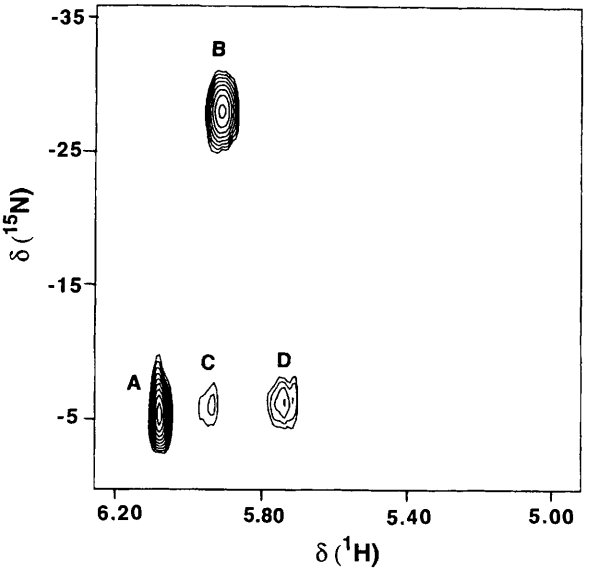


Fig. 9 The 500 MHz two-dimensional [^1H,^{15}N] HSQC spectrum at 310 K of a solution of complex **2n** (5 mM) with GpG (5 mM) at pH 3.40 in 90% water–10% D_2O after 70 h incubation at 310 K. Peak assignments: A, unreacted **2n**; B, NH_2 (*trans* to N) for **7a** and **7b**; C, NH_2 (*trans* to S) for **7b**; D, NH_2 (*trans* to S) for **7a**

possible to obtain complexes **2** and **2n** relatively free from impurities.

In water, complex **1** reacted to give predominantly the *S*,*N*-chelated complex **3**, [Pt(en){MeCO-Met(2−)-*S*,*N*}], together with a minor amount of a complex tentatively assigned to the *S*,*O*-chelated complex **4**, [Pt(en){MeCO-Met(1−)-*S*,*O*}]$^+$. No peaks assignable to the aqua complex [Pt(en){MeCO-Met(1−)*S*}(H_2O)]$^+$ were detectable and therefore it seems likely that this is short-lived due to rapid ring closure giving **3** and **4**. The rate of reaction of **1** ($t_{\frac{1}{2}}$ 3.9 h at 310 K) in water is markedly slower than the hydrolysis rate of [Pt(en)Cl_2] ($t_{\frac{1}{2}}$ 1.6 h for the first Cl and 1.2 h for the second Cl at 309 K).[35,37] The introduction of bulky groups on the ethylenediamine backbone does not influence dramatically the hydrolysis rate of the Pt–Cl bond in these complexes, as illustrated by recent papers on [PtLCl_2] [L = (1*R*,2*R*,4*S*)-*exo*-2-(aminomethyl)-2-amino-7-oxabicyclo[2.2.1]heptane, $t_{\frac{1}{2}}$ 1.4 h for the first Cl and 1.1 h for the second at 311 K; or L = *meso*-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethane-1,2-diamine, $t_{\frac{1}{2}}$ 2.4 h].[38,44] Therefore it appears that the Pt–Cl bond in **1** is

stabilized by the *cis* MeCO-Met ligand and approach of H_2O is hindered, perhaps by axial interactions between MeCO-Met and Pt. Appleton *et al.*[34] have reported detection of the *S,O* chelate $[Pt(NH_3)_2\{Met(1-)\text{-}S,O\}]^+$ at low pH (0.5) and only *S,N* chelation at high pH (7). Recently the same workers[33] have reported the detection of rotational isomers of [Pt(en){MeCO-Met(2−)-*S,N*}].

The complicated nature of the two-dimensional [^1H,^{15}N] NMR spectrum of complex **3** can be attributed to the slow inversion of co-ordinated S and the *cis*/*trans* isomerization about the amide bond. The four possible isomers should give rise to four sets of four ^1H,^{15}N cross-peaks. Less peaks are observed than expected, which may be due to the fact that some of the isomers are not favoured or to the fast exchange rate at 310 K. As previously suggested, the isomers with the *cis* configuration about the peptide bond may be stabilized by $PtNH_2 \cdots OC$ hydrogen bonding.[43]

In contrast, the bis MeCO-Met complex **2** was found to be relatively stable in water, with only a minor amount of MeCO-Met displacement occurring *via* intramolecular substitution and *S,N* chelation.

The rate of reaction of complex **1** with 5′-GMP was independent of the 5′-GMP concentration and appeared to be determined by the rate of hydrolysis, with rapid displacement of bound H_2O by N^7 of 5′-GMP being a faster step. The rate of *S,N*-chelate-ring closure appeared to be competitive with 5′-GMP binding since the formation of **3** was observed during the course of the reaction of **1** with 5′-GMP. However, complex **3** itself reacted with 5′-GMP to give the product $[Pt(en)\{MeCO\text{-}Met(1-)\text{-}S\}(5'\text{-}GMP\text{-}N^7)]^+$ **6**. This appears to be the first report of a ring-opening reaction of an *S,N*-chelated methionine derivative with a nucleotide and could be of significance to the mechanism of action of platinum drugs. However, this is a proton-assisted reaction, and at neutral pH no reaction between 5′-GMP and **3** was observed, as was also reported recently by Appleton *et al.*[33]

The monofunctional adduct **6** was remarkably stable with little displacement of the co-ordinated S even in the presence of an excess of 5′-GMP after 1 week at 310 K. This contrasts with previous observations of the intra- and inter-molecular displacement of thioether S by N^7 of guanine,[17,18] suggesting that **6** is stabilized by interactions between the nucleotide and the amino acid side chain. Such interactions could be important if mixed-ligand complexes are formed between platinated DNA and peptides or proteins *in vivo* and account for stabilization of the monofunctional adduct.

Similarly, the monofunctional adducts of complex **1** with GpG, **7a** and **7b**, were also exceptionally stable. This reaction followed a similar course to that observed with 5′-GMP, notably again one pathway at pH 4 can involve ring opening of the chelated intermediate [Pt(en){MeCO-Met(2−)-*S,N*}] **3**. One of the adducts, **7a**, was preferentially stabilized over the other, **7b**. On the basis of the literature reports[45,46] of the guanosine H^8 ^1H NMR peaks in $[Pt(dien)]^{2+}$ and $[Pt(NH_3)_2(mpy)]^{2+}$ (mpy = 4-methylpyridine) monoadducts of GpG and d(GpG), in comparison to our data, it was possible to assign the major adduct **7a** to $[Pt(en)\{MeCO\text{-}Met(1-)\text{-}S\}(GpG\text{-}N^{7(2)})]^+$, *i.e.* 3′-G co-ordinated to Pt. Indeed the preference for **7a** or **7b** (60:40) is similar to that reported previously for $[Pt(dien)]^{2+}$ and $[Pt(NH_3)_2(mpy)]^{2+}$ adducts.[45,46] It is commonly found that there is enhanced platination of a guanosine base which has a 5′-phosphate group.[47,48] Therefore it can be concluded that the *cis*-co-ordinated amino acid has little affect on the selective platination of guanosine in a simple GpG sequence, and the site of platination may be determined primarily by Pt–NH–phosphate hydrogen-bonding. On the other hand, it is possible that the binding of MeCO-Met to Pt(en) is stabilized by the presence of the *cis*-co-ordinated GpG since little ring closure to form the GG chelate *via* displacement of MeCO-Met was observed. The small upfield shift of the MeCO-Met $COCH_3$ group (Tables 1 and 3) may be indicative of such an interaction.

Finally we can consider the pattern of the NH chemical shifts in the complexes studied here in relation to possible structural information. Previously we have noted that large low-field shifts of Pt–NH ^1H NMR resonances occur in situations where strong hydrogen bonding is likely, *e.g.* when there is a *cis* nucleotide containing a 5′-phosphate.[8,22] In complexes **6** and **7a** it is notable that the Pt–NH_2 *cis* to the S of *N*-acetyl-L-methionine has the largest low-field shift. This suggests that hydrogen bonding between Pt–NH and the amino acid carboxylate group may play a role in stabilizing these monofunctional adducts. Indeed for complex **2**, which has two S-co-ordinated *N*-acetyl-L-methionine ligands, there is an even larger low-field shift to δ 6.11 (Table 2).

We anticipated that intramolecular displacement of MeCO-Met by guanosine-N^7 in complexes **7a** and **7b** to give the bifunctional GG chelate might be more facile than for the mononucleotide adducts of $[Pt(dien)]^{2+}$ studied previously.[17,18] However, this was not the case, and chelate-ring formation is apparently not a driving force under the conditions used here. However, in a DNA duplex where the guanosine bases are more rigidly held, sulfur displacement could be more favourable. We are currently investigating this possibility.

Conclusion

Reactions of [Pt(en)(MeCO-Met-*S*)Cl]NO_3 **1** and **1n** with GMP and GpG lead to the formation of very stable monofunctional adducts **6** and **7a** or **7b**, respectively, in which chloride is substituted by N^7. The reaction probably proceeds *via* an aqua intermediate as it does for diam(m)ine chloroplatinum(II) complexes. However, the rate of hydrolysis of **1** is at least twice as slow as that of the dichloro complex $[Pt(en)Cl_2]$, and during the initial stages of the reactions of **1** with nucleotides, *S,N*-chelation of MeCO-Met to give **3** is competitive with N^7 binding. The disappearance of **3** during the later stages of the reaction provided evidence that the MeCO-Met chelate ring can be opened by guanine bases at pH *ca.* 4.

The reaction of complex **1** with GpG apparently led to the preferred formation of the 3′-G monofunctional adduct **7a** over the 5′-G monofunctional adduct **7b**, with a ratio of 60:40. Both were stable for weeks. The displacement of MeCO-Met by nucleotides to give bifunctional adducts was very slow.

In contrast, S-bound MeCO-Met is more readily displaced from the bis complex **2** by 5′-GMP or GpG, again giving rise to the formation of the related stable monofunctional adducts. The high stability of the monofunctional adducts may be due to the presence of a hydrogen-bond network and such adducts could play an important role in the mechanism of action of platinum anticancer complexes.

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