Cisplatin Analogues with Sulfur Donor Ligands. (Ethylenediamine)platinum(II) Complexes with Ligands possessing a Sulfinyl or Sulfanyl Group linked to an Anionic Oxygen Donor Atom. Reactivity and Cytotoxicity

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Complexes of formula $[Pt(en)L]NO_3$, where en = ethylenediamine and L is a ligand in which a methylsulfinyl or methylsulfanyl group is linked to a carboxylate or phenolate moiety, have been synthesized and characterized by spectroscopic techniques. The ligand L is chelated to Pt *via* the sulfur and anionic oxygen atoms. The reactivities of these complexes towards nucleophiles [Nu = Cl^- or guanosine 5'-monophosphate(2−), GMP] have been investigated by ^1H NMR spectroscopy; GMP is more reactive than Cl^-. The Pt–O bonds react more readily than the Pt–S bonds and, depending on the nature of L and Nu, the reactions either stop at the monosubstituted complex [Pt(en)L(Nu)] (with L monodentate and co-ordinated to Pt *via* the sulfur atom), or yield $[Pt(en)(Nu)_2]$. The complex with L = 2-(methylsulfanyl)phenolate is unreactive. The stable monosubstituted products have been characterized. There is little correlation between the rates and the modes of reactivity of complexes $[Pt(en)L]NO_3$ towards GMP and their cytotoxicities against the human ovarian carcinoma A2780 cell line.

The antitumour activity and toxicity of a cisplatin analogue of formula *cis*-$[PtA_2X_2]$ (A = ammonia, amine or $\frac{1}{2}$ chelating diamine; X = leaving ligand) depend on the nature of A and X,[1,2] but the reactivity of the Pt–X bonds is considered relevant to toxicity[1–4] (see, however, ref. 5). Complexes with highly labile leaving ligands X, can react with the nucleophilic sites of any macromolecule in the body fluids before reaching the target. Sulfur nucleophiles are particularly appropriate, as for instance it appears that certain forms of toxicity arise from the reaction of the PtX_2 group with the nucleophilic sulfur centres of some proteins.[6–9] *In vivo* reaction of Pt with sulfur centres has, however, other important consequences for the biological properties of a cisplatin analogue. An example is its inactivation by intracellular glutathione [*N*-(*N*-L-γ-glutamyl-L-cysteinyl)-glycine] which may be responsible, at least in part, for the phenomenon of resistance to cisplatin.[8] Finally certain sulfur ligands have been proposed as protective and rescue agents towards cisplatin toxicity.[7,10] Toxicity, rescue and resistance may therefore arise from a balance of the relative stabilities and/or labilities of different Pt–S bonds. Consequently a sulfur ligand whose Pt–S bond can react (perhaps specifically) with a nucleobase could be a good candidate for the leaving group of a low-toxicity cisplatin analogue.

The use of Me_2SO as a leaving group for antitumour platinum complexes was first proposed by Farrell[11] who has recently described[12] a series of low-toxicity antitumour complexes of formula $[Pt(diamine)(R'R''SO)X]^+$. Following his suggestion we synthesized some compounds of formula $[Pt(diamine)L]^+$ where L is the chelating anion (methylsulfinyl)acetate or 2-(methylsulfinyl) benzoate.[13] These compounds were found to be marginally toxic, but their low antitumour activities discouraged further pharmacological investigation. However some interesting aspects of their chemistry has led us to investigate more compounds of this family and to extend such an investigation to the analogous methylsulfanyl derivatives, with the hope of gaining a deeper insight into the behaviour of Pt–S bonds in the presence of biologically relevant nucleophiles. In this paper we therefore report the synthesis, characterization, reactivity towards Cl^- and guanosine 5'-monophosphate(2−) (GMP) and cytotoxicity against A2780 human ovarian carcinoma of the compounds $[Pt(en)L]^+$ **1–8**, where en is ethylenediamine and L are the anions of a series of chelating ligands in which the donor methylsulfinyl or methylsulfanyl groups are linked to the anionic side-arms of carboxylates or phenolates. Complexes $[Pt(en)L^1]NO_3$ **1** and $[Pt(en)L^5]NO_3$ **5** have been described previously.[13]

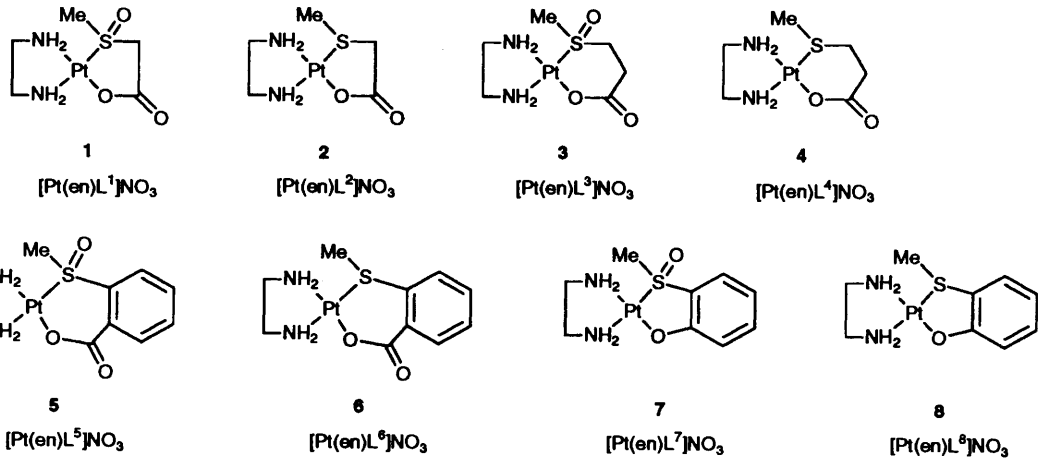
Table 1 Analytical data for the isolated platinum complexes*

Compound	Analysis (%) C	H	N
2 $[Pt(en)L^2]NO_3$	14.4 (14.2)	3.0 (3.1)	9.9 (10.0)
3 $[Pt(en)L^3]NO_3$	15.5 (15.9)	3.2 (3.3)	9.0 (9.3)
4 $[Pt(en)L^4]NO_3$	16.3 (16.5)	3.3 (3.4)	9.4 (9.6)
6 $[Pt(en)L^6]NO_3$	24.8 (24.8)	2.9 (3.1)	8.6 (8.7)
7 $[Pt(en)L^7]NO_3$	25.1 (25.4)	3.7 (3.8)	8.7 (8.9)
8 $[Pt(en)L^8]NO_3$	23.5 (23.7)	3.4 (3.3)	9.1 (9.2)
17 $[Pt(en)(L^4)Cl]$	17.5 (17.6)	3.5 (3.7)	7.0 (6.8)
21 $[Pt(en)(L^6)Cl]$	22.3 (22.2)	3.5 (3.3)	5.9 (6.1)

* Required values are given in parentheses. Analyses of complexes **1**, **5** and **9** are reported in ref. 13.

Experimental

Analyses (see Table 1) were from the microanalytical laboratory at this Department. Spectroscopic data were obtained with the following instruments: IR, JASCO FT/IR 500; ^1H, ^{13}C and ^{195}Pt NMR, Bruker AC200 and Bruker WP80 (δ values from external



$SiMe_4$ and H_2PtCl_6, assignments of the ^{13}C resonances confirmed by J-mode experiments); FAB mass spectra, VCA Analytical 7070 EQ (xenon as the FAB source, isotope cluster abundance checked by computer simulations using local programs).

All chemicals were reagent grade. (Methylsulfanyl)acetic acid (HL^2) (Fluka) and 2-(methylsulfanyl)phenol (HL^8) (Aldrich) were used as received.

Preparation of the Ligands.—The preparations of (methylsulfinyl)acetic (HL^1), 2-(methylsulfanyl)benzoic (HL^6) and 2-(methylsulfinyl)benzoic (HL^5) acids have already been described.[13]

3-(*Methylsulfanyl*)*propanoic acid* (HL^4).[14] Methyl iodide (17.02 g, 120 mmol) was added dropwise to a solution of 3-sulfanylpropanoic acid (Merck) (10.59 g, 100 mmol) and KOH (12.31 g, 220 mmol) in methanol (15 cm^3). The solution was refluxed for 2 h under a nitrogen atmosphere. After removal of the solvent under vacuum, the residue was treated with water (15 cm^3) and brought to pH 2 with 15% H_2SO_4. The product was extracted with diethyl ether and isolated as an oil by evaporation (4.830 g, 40%) [Found (calc.): C, 39.9 (40.0); H, 6.7 (6.7)%]. $\delta_H(D_2O$, 25 °C) 2.12 (3 H, s, CH_3S) and 2.73 (4 H, m, CH_2CH_2).

3-(*Methylsulfinyl*)*propanoic acid* (HL^3). A solution of 3-(methylsulfanyl)propanoic acid (3.20 g, 26.6 mmol) in ethanol (15 cm^3) was cooled on an ice-bath. A stoichiometric amount of H_2O_2 (3.00 g of a 30% solution) was added dropwise, the solution allowed to warm to room temperature and left aside for 24 h. It was concentrated under vacuum to 5 cm^3 and cooled to give a waxy, hygroscopic product in 64% yield (2.320 g) [Found (calc.): C, 35.3 (35.3); H, 5.8 (5.9)%). The compound was further characterized by comparison with published spectral data:[14] ν(SO) 1015 cm^{-1}; $\delta_H(CDCl_3)$ 2.73 (3 H, s, CH_3SO). This compound is unstable especially in solution.

2-(*Methylsulfinyl*)*phenol* (HL^7).[15] 2-(Methylsulfanyl)phenol (2-hydroxythioanisole, Merck) (1.044 g, 7.45 mmol) was dissolved in acetic acid (5 cm^3) with acetic anhydride (1 cm^3). Hydrogen peroxide (0.845 g of a 30% solution, 7.45 mmol) was added to the ice-cooled solution which was then left aside at room temperature for 24 h. The product was precipitated by the addition of cold water (30 cm^3). Extraction with chloroform and evaporation of this solvent to dryness gave a second crop. The combined solids were crystallized from methanol to give a white product in 85% yield (0.989 g). M.p. 127 °C (lit.[15] 127–128 °C) [Found (calc.): C, 53.7 (53.8); H, 5.0 (5.1)%]. $\delta_H(CDCl_3)$ 2.94 (3 H, s, CH_3SO), 6.8–7.5 (4 H, m, C_6H_4) and 10.1 (1 H, s, OH).

The potassium or sodium salts of the acids and phenols were obtained as gummy materials by neutralization with 0.1 mol dm^{-3} KOH or NaOH and taking the solutions to dryness.

Preparation of the Complexes.—The compounds $[Pt(en)Cl_2]$ and $[Pt(en)(NO_3)_2]$ were obtained by standard procedures.[16] The preparations of compounds **1** and **5** have already been described.[13] The other complexes **2**, **3**, **4**, **6**, **7** and **8** were obtained by similar methods and three representative examples are given below.

(*Ethylenediamine*)[3-(*methylsulfanyl*)*propanoato*]*platinum*(II) *nitrate* **4**. A suspension of $[Pt(en)Cl_2]$ (0.4010 g, 1.227 mmol) in water (20 cm^3) was treated with a two-fold excess of Ag_2CO_3 (0.6702 g), 3-(methylsulfanyl)propanoic acid (0.1473 g, 1.227 mmol) and 0.1 mol dm^{-3} $AgNO_3$ (12.2 cm^3). The slurry was stirred at 50 °C for 12 h in the dark, cooled and filtered. The solution was evaporated to dryness under vacuum and the oily residue crystallized from acetone giving 0.4817 g (90%) of a white solid.

(*Ethylenediamine*)[2-(*methylsulfanyl*)*benzoato*]*platinum*(II) *nitrate* **6**. A solution of 2-(methylsulfanyl)benzoic acid (0.1003 g, 0.596 mmol) in 0.1 mol dm^{-3} KOH (5.9 cm^3) was added to an aqueous solution of $[Pt(en)(NO_3)_2]$ (0.2253 g, 0.594 mmol in 10 cm^3). The mixture was heated at 60 °C for 10 h, cooled and filtered. The solution was rotovaporated to dryness and the residue extracted at 50 °C with methanol–chloroform [1 : 1 v/v, 50 cm^3], which upon concentration gave the white product in 80% yield (0.2301 g).

(*Ethylenediamine*)[2-(*methylsulfinyl*)*phenolato*]*platinum*(II) *nitrate* **7**. This was prepared as above but, due to its low solubility in methanol, three crystallizations from dimethylformamide (dmf) were necessary to obtain the compound, free from KNO_3, in 50% yield.

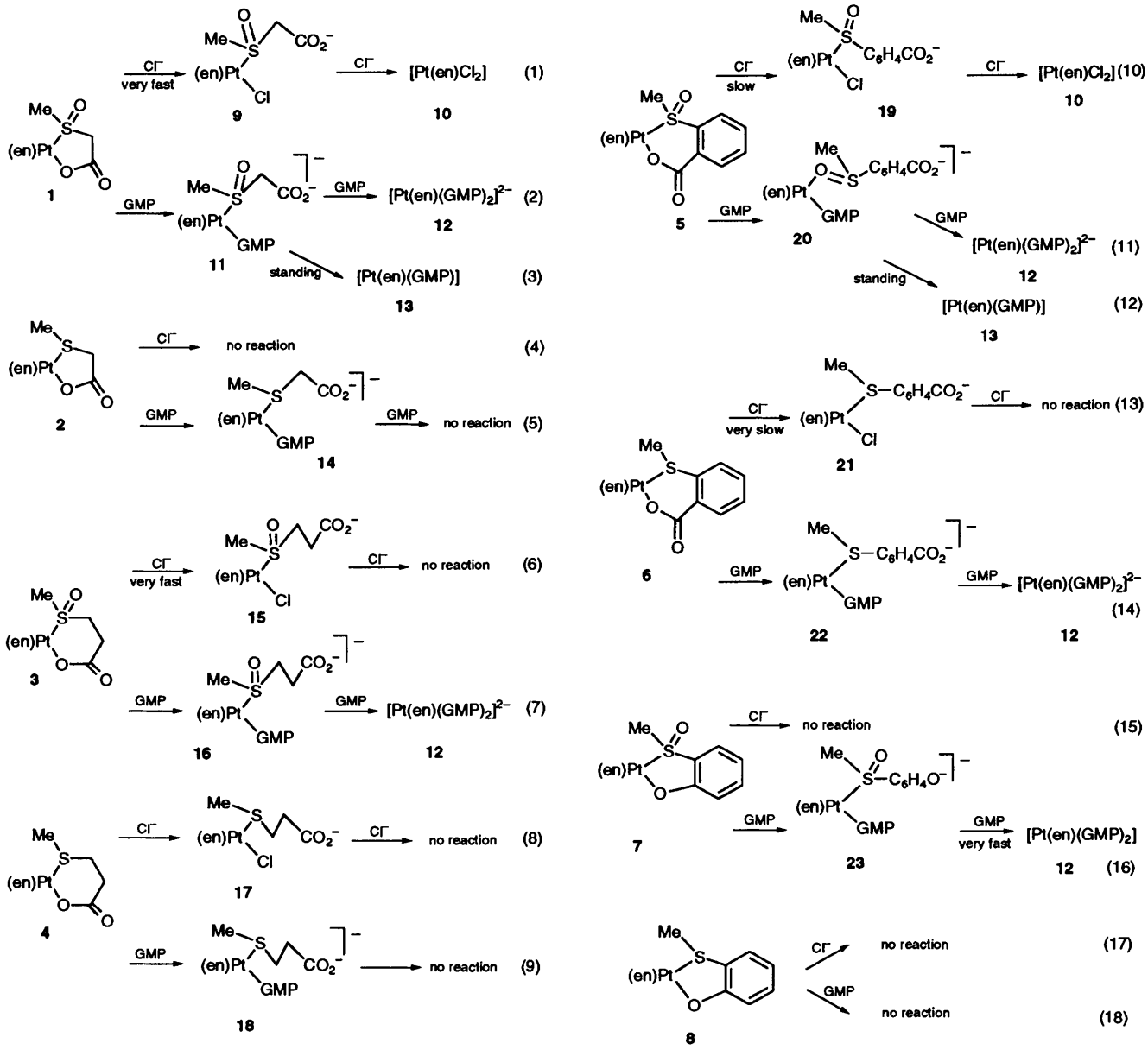
Chloro(*ethylenediamine*)[(*methylsulfinyl*)*acetato*-S]*platinum*(II) **9**. This compound was obtained according to ref. 13.

Chloro(*ethylenediamine*)[3-(*methylsulfinyl*)*propanoato*-S]-*platinum*(II) **15**. This compound was prepared by treating an aqueous solution of **3** with a stoichiometric amount of sodium chloride and evaporating the solution to dryness. It could not be obtained as a pure material.

Chloro(*ethylenediamine*)[3-(*methylsulfanyl*)*propanoato*-S]-*platinum*(II) **17**. This compound was obtained in almost quantitative yield by treating an aqueous solution of **4** with a four-fold excess of an ion-exchange resin (Amberlyst A26, Cl^- form). The filtered solution was evaporated to dryness.

Chloro(*ethylenediamine*)[2-(*methylsulfanyl*)*benzoato*-S]*platinum*(II) **21**. Equimolar amounts of $[Pt(en)Cl_2]$, 2-(methylsulfanyl)benzoic acid and KOH (0.687 mmol: 0.224 g, 0.116 g and 6.8 cm^3 of 0.1 mol dm^{-3} aqueous KOH, respectively) in dmf (25 cm^3) were heated at 60 °C for 5 h. The solution was concentrated to 1 cm^3, cooled, filtered (KCl) and treated with ethanol. The pale yellow precipitate was crystallized twice from dmf (0.310 g, 45%).

Disodium (*ethylenediamine*)*bis*(*guanosine 5'-phosphato*)*platinate*(2−) **12**. This compound was obtained as in ref. 13.



Scheme 1

Sodium (ethylenediamine)(guanosine-5'-phosphato)[(methylsulfanyl)acetato-S)]platinate(1−) **14**. Equimolar amounts of GMP (disodium salt, dihydrate) and $[Pt(en)L^2]NO_3$ **2** (0.710 mmol, 0.3147 and 0.3000 g, respectively) in water (10 cm^3) were heated at 60 °C for 4 h. The filtered solution was evaporated to dryness under reduced pressure. Since we were not able to separate the product from $NaNO_3$ by solvent extraction, nor to crystallize it as the salt of other cations, the compound was characterized by spectroscopic methods (see text).

Sodium (ethylenediamine)(guanosine 5'-phosphato)[3-(methylsulfanyl)propanoato-*S*]platinate(1−) **18** was obtained in a similar way.

Reactivity Studies.—The reactions of Scheme 1 were followed by ^1H NMR spectroscopy with an 80 MHz instrument to detect better the Pt satellites. The reactions were performed at 40 ± 1 °C. Appropriate amounts of the mother solutions (D_2O) of the reactants were mixed directly in the NMR tube so that the concentration of the complexes was 10^{-2} mol dm^{-3}. The reaction with Cl^- was carried out with 0.15 mol dm^{-3} NaCl, whereas varying complex:GMP ratios were used for the reactions with this nucleophile.

Cytotoxicity Evaluation.—The human ovarian carcinoma cell line A2780 (kindly supplied by Dr. R. F. Ozols, National Cancer Institute, Bethesda, MD) was cultured in RPMI-1640 containing 10% (v/v) fetal calf serum and 1% glutamine. Cytotoxicity was assessed by sulforhodamine assay.[17] Briefly, exponentially growing cells (0.4 × 10^4 per well), seeded in microtitre plates, were allowed to attach for 24 h and then exposed to the drugs for 24 h. The medium was removed and each well was washed twice with the medium. The cells were incubated in fresh medium for 48 h and the sulforhodamine assay performed. IC_{50} is defined as the inhibitory drug concentration causing a 50% reduction of absorbance compared with an untreated control.

Results and Discussion

Complexes **1–8** were prepared[13] either by reaction of $[Pt(en)Cl_2]$ with AgL (prepared *in situ*) and 1 equivalent of

Table 2 Relevant IR, ^{195}Pt NMR and FAB mass spectroscopic data for the ligands and platinum complexes[a]

Compound	IR (cm^{-1}) $\nu_{asym}(CO_2)$	IR (cm^{-1}) $\nu(SO)$	^{195}Pt NMR (δ)	FAB mass spectrum *m/z*
HL^1	1715	998		
L^1	1605	1017		
1	1690	1100	−3033	376 $[M - NO_3]^+$
9			−3268	412 $[M + H]^+$
12[b]			−2669	1024 $[M + H]^+$, 1000 $[M - Na]^-$ 980 $[M - 2Na + 3H]^+$
HL^2	1711			
L^2	1578			
2	1618		−2882	360 $[M - NO_3]^+$
14	1580		−3178	721 $[M - Na]^-$, 742 $[M - H^+]^-$
HL^3	1725	1011		
L^3	1600	1017		
3	1655	1118	−3016, −3094[c]	390 $[M - NO_3]^+$
15	1584	1110	−3194	—
HL^4	1711			
L^4	1570			
4	1636		−2863, −2921[d]	
17[b]	1576		−3199	410 $[M + H]^+$
18	1580		−3185	735 $[M - Na]^-$, 757 $[M - H^+]^-$
HL^5	1698	961, 948		
L^5	1600	1015		
5	1636	1114	−3012	438 $[M - NO_3]^+$
HL^6	1676			
L^6	1543			
6	1624		−2872	422 $[M - NO_3]^+$
21	1580		−3148	458 $[M + H]^+$
22	1600		−3127	783 $[M - Na]^-$
HL^7		965		
L^7		1008		
7		1131	−3256	410 $[M - NO_3]^+$
8			−2983	394 $[M - NO_3]^+$

[a] The data for complexes **1**, **5** and **9** are from ref. 13. IR spectra from Nujol mulls, $\nu_{sym}(CO_2)$, could not be identified unambiguously. ^{195}Pt NMR spectra in D_2O solutions. FAB mass spectra from glycerol mulls, positive or negative ions as indicated; the peaks reported have 100% relative abundance, the other peaks in the spectra have intensities lower than 30%. [b] FAB mass spectrum from 3-nitrobenzyl alcohol. [c] Approximate ratio 1:1. [d] Minor component, approximate ratio 3:1.

$AgNO_3$, or by treating aqueous solutions of $[Pt(en)(NO_3)_2]$ with KL. The former method gives purer products, but could be used only for L^4 and L^5, since the other ligands are only slightly stable in the presence of silver ion. Analytical and relevant spectroscopic data are reported in Tables 1–4.

The compounds are 1:1 electrolytes in aqueous and dmf solutions (Λ_M *ca.* 120 and 80 ohm^{-1} cm^2 mol^{-1} at 25 °C, respectively). In the solid state the presence of ionic nitrate is shown by a sharp IR band at 1380 cm^{-1} (Nujol mull).

X-Ray investigations[13,19] on $[Pt(en)L^1]NO_3$ **1** and $[Pt\{(S,S)\text{-dach}\}L^5]NO_3$ (dach = 1,2-diaminocyclohexane) have shown that L^1 and L^5 are chelated to Pt through the S atom and carboxylate group. Evidence that the other ligands are also chelated to Pt and that, with the exception of **3** and **4**, such a chelate structure is maintained in aqueous solution is as follows.

(*i*) The FAB mass spectra show only peaks corresponding to the cation $[M - NO_3]^+$ and no peak corresponding to M^+ (Table 2).

(*ii*) The values of $\nu_{asym}(CO_2)$ for compounds **1**–**6**, and for the sulfinyl complexes, the high-frequency shifts of ν(SO), compared to the values of KL,* are in agreement with co-ordination of the carboxylate group and the S atom of the sulfinyl moiety.[21–23]

(*iii*) In the NMR spectra (Tables 3 and 4, D_2O solutions) the downfield shifts of the ^{1}H (with Pt–H coupling) and ^{13}C resonances of the MeS and MeSO groups, compared to those of the free anions, are diagnostic of their co-ordination to Pt *via* the S atom.[22–29] In the ^{13}C NMR spectra the resonances of the CO_2 groups are also shifted with respect of those of L^-; Pt–C(carboxylato) couplings are usually difficult to detect[26,27] and were observed only for compounds **2** and **4**.

(*iv*) For the phenolato complexes **7** and **8** there is a downfield shift of the ^{13}C resonances attributed[18] to C^1(phenol) compared to the values of the free ligands. The peaks of C^2–S are coupled to Pt. Interestingly, the Pt–C coupling of two more peaks confirms their assignments[18] to C^3 and C^6. See Table 4.

(*v*) The Pt–O bonds of L^3 and L^4 in complexes **3** and **4** are only slightly stable in aqueous solution. The ^{1}H NMR spectra (D_2O solution) change with ageing (>4 h at room temperature), however even after 10 h at 40 °C there is no trace of free L. The ^{13}C NMR spectrum of **3** (10 h accumulation time) is difficult to interpret, while that of the L^4 derivative **4** shows the presence of both co-ordinated (Pt–C satellites) and free carboxylate and two peaks which we assign to the MeS group of chelated and *S*-co-ordinated monodentate L^4. The ^{195}Pt NMR spectra of both complexes (obtained in *ca.* 4 h) display two resonances attributable to the starting complex and to the semi-hydrolysed species. According to the intensities of these peaks, the ratios between the chelated complexes and the monodentate species are 1:1 for **3** and 3:1 for **4**. Due to the low stability, the reactivities of complexes **3** and **4**, discussed below, must be considered with care.

Reactivity Studies.—We have investigated the reaction of compounds **1**–**8** with Cl^- and GMP as models of some of the

* Comparison must be made with the values of the anions, since, with the probable exception of HL^3, the acids are strongly associated in the solid state[13,15,18,20] and ν(SO) occurs at a lower frequency than that of the free anion, see Table 2.

Table 3 Proton NMR data for reagents, intermediate and products of reactions (1)–(14)[a]

Compound	MeS	CH_2(en)	GMP H^8	GMP $H^{1'}$
L^1	2.94			
1 $[Pt(en)L^1]^+$	3.92	2.99		
	(25)	(44)		
9 $[Pt(en)(L^1)Cl]$	3.67	3.04		
	(22)	(43)		
10 $[Pt(en)Cl_2]$		2.84		
		(49)		
Na_2(GMP)			8.36	6.10
				(6.0)
11 $[Pt(en)L^1(GMP)]^-$	3.52	3.04	9.05	6.17
	(24)	(42)	(25)	(4.7)
12 $[Pt(en)(GMP)_2]^{2-}$		3.04	8.86	6.10
		(42)	(23)	(4.7)
13 $[Pt(en)(GMP)]$[b]		3.02	8.75	6.11
		(40)	(25)	(4.5)
L^2	2.24			
2 $[Pt(en)L^2]^+$	2.80	2.89		
	(49)	(45)		
14 $[Pt(en)L^2(GMP)]^-$	2.49	3.00	8.96	6.20
	(42)	(46)	(24)	(4.5)
L^3	2.94			
3 $[Pt(en)L^3]^+$	3.86	2.98		
	(n.o.)	(42)		
15 $[Pt(en)(L^3)Cl]$	3.69	3.06		
	(23)	(42)		
16 $[Pt(en)L^3(GMP)]^-$	3.53	3.11	9.12	6.21
	(22)	(30)	(23)	(6.0)
L^4	2.34			
4 $[Pt(en)L^4]^+$	2.58	2.90		
	(n.o.)	(n.o.)		
17 $[Pt(en)(L^4)Cl]$	2.64	2.96		
	(n.o.)	(n.o.)		
18 $[Pt(en)L^4(GMP)]^-$	2.51	3.02	9.03	6.22
	(43)	(47)	(23)	(4.7)
L^5	3.08			
5 $[Pt(en)L^5]^+$	3.86	3.02		
	(23)	(44)		
19 $[Pt(en)(L^5)Cl]$	4.01	2.78		
	(20)	(44)		
20 $[Pt(en)L^5(GMP)]^-$	2.84	2.90	9.00	6.18
		(44)	(20)	(5.0)
L^6	2.41			
6 $[Pt(en)L^6]^+$	2.90	2.90		
	(45)	(45)		
21 $[Pt(en)(L^6)Cl]$	2.83	2.82		
	(49)	(45)		
22 $[Pt(en)L^6(GMP)]^-$	3.01	2.90	8.48	5.94
	(42)	(45)	(21)	(4.0)
L^7	2.97			
7 $[Pt(en)L^7]^+$	3.94	3.07		
	(24)	(43)		
23 $[Pt(en)L^7(GMP)]^-$			9.03[c]	
8 $[Pt(en)L^8]^+$	3.00	3.00[d]		
	(50)	(42)		

[a] In D_2O at 40 °C; δ values in ppm, Pt–H (and H–H for $H^{1'}$) coupling constants in Hz. n.o. = Not observed. For other details see text. Data for compounds **1**, **5**, **9**, **19** and **20** taken from ref. 13. [b] Gives rise to rather broad signals and the coupling constants are approximate. [c] At 25 °C, see text. [d] The MeS and the en protons display the same chemical shift but different Pt–H coupling constants.

reactions which may occur in biological fluids (high Cl^- concentrations) or with the target guanine bases of DNA.[28] The time course of the reaction has been followed by ^1H NMR spectroscopy, with an 80 MHz instrument (to detect better the Pt–H satellites) observing the rise and decrease of the peaks of the reagents, intermediates and products reported in Table 3. The reactions were performed at 40 °C, the concentrations of the complexes were 10^{-2} mol dm^{-3} in D_2O, and either an excess of chloride ions (0.15 mol dm^{-3}) or various molar ratios of GMP were used. No buffer was added in order to avoid possible interferences. The pH* was around 6–6.5 for the reactions with Cl^- and 7–7.5 for the reactions with GMP. The latter were therefore performed at nearly physiological pH values.

The results of the studies are summarized in Scheme 1, while Table 5 reports the $t_{\frac{1}{2}}$ values for the reactions of complexes **1**–**8** with 0.15 mol dm^{-3} NaCl or a ten-fold excess of GMP. Such an excess was the upper limit for a reliable evaluation of the integration of the NMR spectra. Compound **8** is unreactive, while **2** and **7** are unaffected by Cl^-. For the other reactions, either nucleophile first displaces the anionic oxygen atom of L giving the monosubstituted species [Pt(en)L(Nu)] (Nu = Cl or GMP), with L monodentate *S*-co-ordinate. Depending on the nature of both L and Nu, in the presence of an excess of Nu, these species may either be stable, or react further to give the disubstitution products $[Pt(en)(Nu)_2]$. The $t_{\frac{1}{2}}$ values of Table 5 are those for the formation of the final (*i.e.* mono- or di-substituted) product as described in Scheme 1.

Characterization of Intermediates and Products.—The compounds $[Pt(en)Cl_2]$, **10**, and $[Pt(en)(GMP)_2]^{2-}$ **12** were characterized by comparison of their spectra with those of authentic samples. The nature of [Pt(en)L(Nu)] was inferred from the spectral changes, *i.e.* from the shifts of the MeS resonances of L and of H^8 of GMP and from the presence of Pt–H couplings of these signals.

The monochloro species **9**, **15**, **17** and **21** are rather inert in 0.15 mol dm^{-3} NaCl. Complex **9** has already been described.[13] The other derivatives were obtained in a similar way, *i.e.* by treating the parent complex with a Cl source (NaCl or ion-exchange resin) or, interestingly, by the reaction of $[Pt(en)Cl_2]$ with the potassium salt of L. For their characterization data see Tables 1–4. Note that the *m/z* values are correct, the ^1H and ^{13}C NMR data are in agreement with *S*-co-ordination of the various L and the IR spectra are consistent with ionic CO_2 groups. The ^{195}Pt chemical shifts (Table 2) are 250–270 ppm lower than those of the parent complexes with chelated L, as expected when an O donor atom is replaced by Cl.[29] The ^{195}Pt δ values of **9** and **15** are slightly higher than those reported for other [Pt(diamine)-(SOR′R″)Cl_2]$^+$ complexes.[12,30]

For the mono-GMP species **11**, **14**, **16**, **18**, **20**, **22** and **23**, the ^1H NMR spectra are in accordance with co-ordination of both the MeS of L and N^7 of GMP to Pt. The low-field shift of H^8, compared to both free GMP and $[Pt(en)(GMP)_2]^{2-}$ **12**, is similar to that reported[31–35] for other $[PtA_2(GMP)Y]$ complexes (Y = H_2O, Cl and, more appropriate,[36] also Me_2SO). The nature of the intermediate **20** has been discussed previously.[13] Briefly, the high-field, sharp ^1H resonance, with no Pt–H coupling, of the MeSO group was tentatively attributed to an *O*-bonded sulfinyl group, a mode of bonding rare for Pt, but observed in the presence of severe steric crowding.[37]

The mono-GMP species **14** and **18** are stable compounds and do not react with an excess of GMP. They were prepared by reaction of equimolar amounts of **2**, or **4**, and Na_2(GMP), but because of their high solubilities in water, **14** and **18** could not be obtained free from $NaNO_3$, and were thus characterized spectroscopically (Tables 2–4). Attempts to prepare **16**, **22** and **23** in a similar way gave mixtures of mono- and di-substituted products together with some unreacted starting complex.

The behaviour of the mono-GMP species **11** and **20** is unexpected as they are unstable even in the absence of an excess of nucleobase [reactions (3) and (12)]. In fact treatment of **1**, or **5**, with an equimolar amount of GMP produces **11**, or **20**, quickly, but, after about 1 h, their resonances start to decrease and in about 4 and 6 h, respectively, the ^1H NMR spectra show that L^1, or L^5, are completely detached and present as the free anions. The other resonances are, in either case, consistent with an unknown compound, **13**, with both en and GMP co-ordinated to Pt in the ratio 1:1:1. Attempts to crystallize **13**

Table 4 Carbon-13 NMR data for ligands and compounds[a]

Compound	MeS	Other C atoms	CO_2	CH_2(en)
L^1	39.2	62.8	173.0	
1	46.3 (60)	59.1	177.0	45.2, 49.0
9	42.2 (48)	61.3 (42)	169.0	48.0, 48.2
12[b,c]				50.6
L^2	18.1	41.8	180.7	
2	25.4 (20)	40.3	187.7 (49)	47.6, 50.0
14[d]	22.7	45.2	174.6	48.7, 50.9
L^3	39.4	32.5, 51.9	181.2	
3	41.2	—	172–175	—
15	42.0	—	177.0	—
HL^4[e]	16.3	25.6, 35.1	178.8	
L^4	16.9	32.4, 39.6	183.3	
4	23.5	32.7, 36.6	181.2 (57)	47.2, 52.0
4[f]	21.0		181.6	48.4, 49.8
17	22.0	37.6, 38.1	181.5	49.5, 49.7
18[g]	21.0	37.1, 37.6	181.3	49.2, 51.4
L^5	46.1	125.3, 133.1, 133.5, 134.6, 136.9, 147.3	174.7	
5	49.9 (49)	126.3, 136.5, 136.7, 132.5, 139.7 (30)	170.2	48.2, 51.2 (48)
L^6	17.8	127.6, 127.9, 142.8, 151.2, 139.3, 138.4	178.5	
6	27.9 (21)	134.2, 134.8, 135.7, 136.5, 129.7 (35), 138.5 (31)	173.0	48.2, 52.3
21	22.4	126–140	176.0	50.3, 51.7
22[h]	23.6	116–140	176.2	49.5, 51.5
HL^7[e,i]	41.3	157.1, 125.0, 124.7, 120.3, 132.9, 118.3		
L^7[i]	40.3	167.4, 131.6, 126.6, 123.3, 136.2, 117.6		
7[i]	49.3	172.1, 131.4 (80), 128.0 (19), 122.6, 140.4, 121.3 (45)		47.8, 50.9
HL^8[e,i]	18.8	155.2, 120.0, 129.0, 119.4, 133.8, 114.3		
L^8[i]	16.8	165.3, 128.8, 129.7, 120.0, 129.2, 118.4		
8[i]	31.3	172.1, 120.6 (77), 137.7 (39), 120.0, 134.6, 120.3 (50)		47.9, 50.9

[a] Recorded at room temperature in D_2O solutions, unless otherwise stated; δ in ppm from $SiMe_4$. J(Pt–H) in Hz are given in parentheses. n.o. = Not observed. Data for compounds **1**, **5** and **9** are from ref. 13. [b] Relevant GMP resonance: δ 143.0 (n.o.), C^8; due to partial H–D exchange this signal is partly inverted in the J-mode experiment. [c] For Na_2(GMP): δ 134.4, C^8; 118.9, C^5. [d] Relevant GMP resonances: δ 141.9 (83), C^8; 116.1 (279), C^5. [e] In $CDCl_3$. [f] This species is a minor component of the solution, approximate ratio 3:1, see text. [g] Relevant GMP resonance: δ 141.5 (n.o.), C^8. [h] This species was obtained as a mixture with **6** and **12**. Relevant GMP resonance: δ 142.2 (72), C^8. [i] For the 2-(methylsulfanyl)- and 2-(methylsulfinyl)-phenol derivatives, the chemical shifts of the aromatic carbon atoms are given in the order C^1–C^6. Assignments are based on ref. 18, on J-mode experiments and, when observed, on Pt–C couplings in complexes.

Table 5 Half-lives $t_{\frac{1}{2}}$ for the reactions with Cl^- and GMP and cytotoxicities of compounds [Pt(en)L]NO_3 **1**–**8**[a,b]

	Reaction with Cl^-		Reaction with GMP		Cytotoxicity IC_{50}[c]	
Compound, L	Cl	Cl_2	mono-GMP	bis-GMP	μg cm^{-3}	nmol cm^{-3}
1, L^1	v.f.	> 24 h		40 min	0.19	0.43
2, L^2	n.r		27 m	n.r.	> 30	> 71
3, L^3	2 min[d]	> 24 h		345 min	5.0	11.1
4, L^4	15 min[d]	n.r.	48 m	n.r.	2.5	5.7
5, L^5		> 24 h		23 min	0.44	0.9
6, L^6	24 h	days		135 min	3.7	7.6
7, L^7	n.r			93 min	2.6	5.5
8, L^8	n.r.		n.r.		3.7	7.8
Cisplatin					0.02	0.07

[a] Reaction conditions: 40 °C, D_2O solutions. Concentrations: Pt complexes, 10^{-2} mol dm^{-3}; Cl^-, 0.15 mol dm^{-3}; GMP, 0.1 mol dm^{-3}. [b] v.f. = Very fast, reaction occurs upon mixing the reagents; n.r. = no reaction. [c] On A2780 ovarian carcinoma. IC_{50}, concentration inhibiting 50% of cellular growth. [d] This figure refers to the formation of the resonances of the products and does not take into account the aquation step, see text.

were unsuccessful, but the FAB mass spectra of these solutions (treated with H_2O to remove deuteriation of the exchangeable protons and dispersed in 3-nitrobenzyl alcohol) show a peak at an m/z = 617 (100% relative abundance), corresponding to $[Pt(en)(GMP) + H]^+$. Species **13** may therefore be a macrochelate, with N^7 and the phosphate group co-ordinated to Pt, similar to that proposed[38,39] to form by reaction of $[PtA_2X_2]$ (X = Cl or H_2O) with GMP in a 1:1 ratio. Equimolar amounts of $[Pt(en)X_2]$ and GMP do yield a solution with the same spectral properties as **13**, but further work is necessary to characterize this species fully. Addition of GMP to a solution of **13** produces $[Pt(en)(GMP)_2]^{2-}$ **12**.

Comments on the Modes and Rates of Substitution of the Various L *in Compounds* **1**–**8**.—(*i*) Pt–O bonds are more reactive than Pt–S bonds, the Pt–S(sulfanyl) bonds being the least reactive. (*ii*) GMP is more reactive than Cl and gives disubstituted products more easily. It appears that, with the exception of L^2 and L^4, co-ordinated GMP labilises the Pt–S bonds of $[Pt(en)(GMP)L]^-$ (see also the behaviour of **11** and **20**). A *cis*-labilising effect of co-ordinated GMP has already been observed[36,40] in other compounds of the type $[PtA_2(GMP)X]$. (*iii*) The Pt–S bond of L^5 is very labile, intermediates **19** and **20** are present at very low concentrations and the linkage isomerism (Pt–S to Pt–O) in intermediate **20**, described above, is in agreement with this observation. (*iv*) The Pt–S bond of monodentate L^7 is very reactive in the presence of GMP. In fact we could not detect intermediate **23** in the ^1H NMR spectra recorded during the course of reaction (16) at 40 °C. Only by performing the experiment at 25 °C and with a 200 MHz

instrument we could observe a faint signal attributable to H^8 of **23** (Table 3). Surprisingly, however, reaction (16) does not go to completion: even with a Pt : GMP molar ratio of 1 : 10, about 20% of unreacted **7** is still present after 6 d at 40 °C. The reaction is, however, irreversible: $[Pt(en)(GMP)_2]^{2-}$ does not react with a ten-fold excess of L^7. (*v*) The chelate ring of L^8 is unreactive, probably due to kinetic inertness: $[Pt(en)(GMP)_2]^{2-}$ does not react with a ten-fold excess of L^8 under the same conditions (pH 7, 40 °C).

Cytotoxicity Studies.—The cytotoxicities of compounds **1**–**8** against the human ovarian carcinoma cell line A2780 are also reported in Table 5, to allow a comparison with the reactivity data. The L^2 complex is inactive, while the other compounds are one to two orders of magnitude less cytotoxic than cisplatin. There is a rough inverse correlation between IC_{50} and $t_{\frac{1}{2}}$ of the reaction with GMP for complexes **1**, **3**, **5**, **6** and **7**, which yield $[Pt(en)(GMP)_2]^{2-}$ (*i.e.* the most reactive compounds, **1** and **5**, are the most cytotoxic). Cytotoxicity and reactivity of the other complexes are difficult to correlate: both the L^2 and L^4 derivatives give monosubstituted products with GMP, but only the latter is cytotoxic and the inert complex of L^8 displays a low, but significant, cytotoxicity.

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