Cytotoxic Diamine–Platinum(II) Complexes with Methylsulfinyl Carboxylates as the Leaving Groups. Synthesis, Characterization, and Reactivity toward Chloride Ions, 5′-GMP, and 9-Methylguanine

Alessandro Pasini,*[1a] Giuseppe D'Alfonso,*[1a] Carla Manzotti,[1b] Massimo Moret,[1c] Silvano Spinelli,*[1b] and Mariella Valsecchi[1b]

Dipartimento di Chimica Inorganica, Metallorganica e Analitica, Milan University, via Venezian 21, 20133 Milan Italy, Istituto di Chimica Strutturistica Inorganica, Milan University, via Venezian 21, 20133 Milan, Italy, and Boehringer Mannheim Italia S.p.A, Research Laboratories, Viale della Libertá Km 0.750, 20052 Monza, Italy

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Compounds of formula [Pt(diam)(Soa)]NO_3, **3**, and [Pt(diam)(Sob)]NO_3, **4**, (Soa, (methylsulfinyl)acetate; Sob, 2-(methylsulfinyl)benzoate; diam, chelating diamines: 1,2-ethylenediamine, en; (±)-, *R*,*R*-, *S*,*S*-, and meso-1,2-diaminocyclohexane, dach; 1,1-bis(aminomethyl)cyclohexane, damch) have been synthesized. IR, NMR, and FAB-mass spectroscopy suggest that the Soa and Sob anions are chelated to Pt through the S atom and the carboxylate group. Such a mode of coordination was confirmed for compounds **3** by the X-ray crystal structure determination of [Pt(en)(Soa)]NO_3. This compound crystallizes in space group *R*3 (No. 146) with cell constants a = 15.139(3) Å and c = 12.886(2) Å. The structure was refined using 1001 independent reflections with $I > 3\sigma(I)$, giving a final *R* value of 0.018. In the complex cation Pt is in a square planar environment with en (Pt–N, 2.019(8) and 2.055(8) Å) and Soa (Pt–S, 2.184(2) Å, and Pt–O, 2.025(6) Å) chelated to Pt. The reactivities of **3** and **4** toward Cl^-, 5′-GMP, and 9-methylguanine have been investigated by ^1H NMR spectroscopy at 40 °C and complex concentration 10^{-2} mol L^{-1} in D_2O. Compounds **3** react readily with Cl^- by displacement of the carboxylate group, yielding [PtCl(diam)(Soa-*S*)] (with monodentate S-coordinated Soa) which reacts with excess chloride to give [$PtCl_2$(diam)] at a very slow rate (for the en derivative formation of [$PtCl_2$(en)] <20% after 24 h in 0.15 mol L^{-1} NaCl). In the case of compounds **4**, $t_{1/2}$ for complete replacement of Sob to give [$PtCl_2$(diam)] in 0.15 mol L^{-1} NaCl is >24 h, but the concentration of the intermediates with S-coordinated monodentate Sob remains very low throughout the course of the reaction, indicating that the Sob chelate ring is more inert toward ring opening. Reactions of **3** and **4** toward 5′-GMP are rather fast: formation of $[Pt(dach)(GMP)_2]^{2-}$ is complete in 3 and 1 h respectively (Pt/GMP = 1/2). These reactions proceed via the formation of intermediates with one N(7)-bound GMP and one monodentate Soa, coordinated to Pt via the S atom, or Sob probably bound to Pt via the O (sulfinyl) atom. Reactions with 9-methylguanine are slower and occur with a similar mechanism. The first step of the reaction of **5** with 2 mol of GMP is displacement of Cl by GMP. Formation of $[Pt(en)(GMP)_2]^{2-}$ is complete in 75 min. Complexes **3** and **4** are moderately cytotoxic toward L1210 leukemia cells; the dach and damch derivatives are cytotoxic also against the L1210 cisplatin-resistant line. The cytotoxicities of the dach complexes depend not only on the absolute configuration of the diamine, but also on the configuration of the leaving group.

Introduction

Cisplatin[2] and carboplatin are well-established antitumor agents routinely used in the clinical practice for the treatment of certain tumors.[3] The use of these drugs, however, is limited by toxic side effects and lack of activity toward tumors with natural or acquired resistance to cisplatin, cross-resistance, and a narrow spectrum of action. Although some new platinum complexes (including one Pt(IV) derivative[4]), with more favorable biological properties, will probably be introduced in the future,[5] there is still the need of synthesizing and testing for antitumor activity more platinum complexes with novel ligands, in the hope of overcoming the above limitations.[6,7] Substitution of the two ammonia ligands with chelating diamines like 1,2-diaminocyclohexane (dach), or 1,1-bis(aminomethyl)cyclohexane (damch), has produced complexes of formula [Pt(diamine)X_2] (X, labile ligand) which are not cross-resistant with cisplatin, at least toward certain murine tumors,[6,8–10] since this property has not been always confirmed in clinical trials.[4,11]

Both antitumor activity and toxicity of a Pt complex depend, inter alia, on the reactivity of the *cis*-[PtA_2X_2] species[12,13] (A, ammine, or $^1/_2$ diamine; X, leaving ligand) toward the nucleophilic sites of macromolecules (proteins, DNA)[14–16] to give, ultimately, species bound to the macromolecule, of general formula *cis*-[PtA_2Nu_2] (Nu, nucleophilic site of the macromolecule). Much effort has therefore been devoted to finding new leaving ligands of appropriate lability, and possibly selectivity, with the hope of tuning such a reactivity.

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(1) (a) Dipartimento di Chimica Inorganica, Metallorganica e Analitica, Milan University. (b) Boehringer Mannheim Italia. (c) Istituto di Chimica Strutturistica Inorganica, Milan University.

(2) Abbreviations used in this paper: cisplatin, *cis*-diamminodichloroplatinum(II); carboplatin, diammino(cyclobutane-1,1-dicarboxylato)platinum(II); diam, chelating diamine; en, 1,2-ethylenediamine; dach, 1,2-diaminocyclohexane (usually the racemic mixture, if the chirality is not specified); damch, 1,1-bis(aminomethyl)cyclohexane; HSoa, (methylsulfinyl)acetic acid; HSob, 2-(methylsulfinyl)benzoic acid; G, guanine moiety; GMP, 5′-GMP; MeG, 9-methylguanine.

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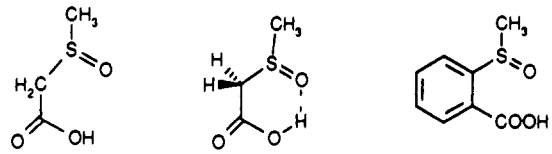
Chart 1



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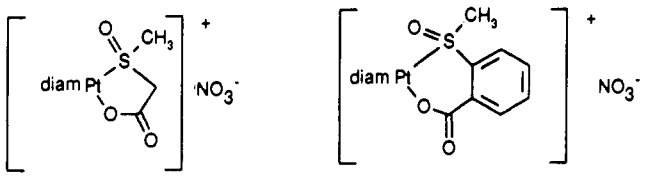

	diam
3a	en
3b	(±)-dach
3c	R,R-dach
3d	S,S-dach

	diam	Sob
4a	en	(±)-Sob
4b	(±)-dach	(±)-Sob
4c	R,R-dach	(±)-Sob
4d	S,S-dach	(±)-Sob
4e	S,S-dach	(+)-Sob
4f	S,S-dach	(-)-Sob
4g	R,R-dach	(+)-Sob
4h	R,R-dach	(-)-Sob
4i	cis-dach	(±)-Sob
4l	damch	(±)-Sob

The use of sulfinyl groups as leaving ligands has been proposed by Farrell some time ago[17] and has led to a series of compounds of formula [Pt(diamine)Cl(R′R″SO)]$^+$ which showed some interesting biological properties.[18,19] In this paper we present a modulation of this approach, in that the sulfinyl moiety is linked to an anionic group, the carboxylate moiety, as in the anions of methylsulfinylacetic acid, **1**, and 2-(methylsulfinyl)benzoic acid, **2**. Such anions can chelate the Pt atom, giving compounds **3** and **4** respectively (Chart 1).[21]

Experimental Section

Analyses (Table 1) are from the Microanalytical Laboratory, Milan University, or Redox SNC, Cologno M., Italy. The following instruments were used: infrared spectra, Jasco FT/IR 5000; ^{1}H, ^{13}C, and ^{195}Pt NMR spectra, Bruker AC 200 and Bruker WP 80 (δ values from external Me_4Si and H_2PtCl_6); circular dichroism spectra, Jasco J-500C. FAB-mass spectra were obtained on a VCA Analytical 7070 EQ, with xenon as the FAB source. Isotope cluster abundance was checked by computer simulations using local programs.

Methylthioacetic acid, thiosalicylic acid, ethylenediamine, the diaminocyclohexane isomers, and Na_2(5′-GMP) were purchased from Fluka. 1,1-bis(aminomethyl)cyclohexane,[18] [$PtCl_2$(diam)], and [Pt(diam)-$(NO_3)_2$][22] were prepared according to published methods.

(Methylsulfinyl)acetic Acid, 1. It was obtained by addition of a slight excess of 35% H_2O_2 (25 mL) to an ethanol solution of CH_3SCH_2COOH (21.23 g in 70 mL) at 5 °C. The solution was heated at 35 °C for 2 h and evaporated to dryness under reduced pressure. The residue was dissolved in methanol and crystallized by addition of acetone. Yield: 90%. Mp: 86 °C (lit.[23] 85–86.5 °C). For analysis see Table 1.

2-(Methylthio)benzoic Acid. 39.22 g (0.254 mmol) of thiosalicylic acid in 200 mL of ethanol and 100 mL of 20% aqueous NaOH were treated dropwise with 36.5 g of CH_3I. The solution was refluxed for 2 h, concentrated under reduced pressure, and acidified with 10% HCl. The precipitate was collected, washed with water, and crystallized from methanol/water. Yield 90%. Mp: 170 °C (lit.[24] 170–171 °C). Anal. Calcd for $C_8H_8O_2S$: C, 57.0; H, 4.3. Found: C, 57.1; H, 4.3. ^{1}H NMR (in $CDCl_3$): 2.44 (CH_3S); 7.05–8.10 (C_6H_4).

2-(Methylsulfinyl)benzoic Acid, 2. Addition of a water solution of $NaIO_4$ (6.30 g, 22.5 mmol, in 35 mL) to a solution of 4.92 g (29.3 mmol) of 2-(methylthio)benzoic acid in 75 mL of methanol gave a reddish solution and a white precipitate. After 5 h this was filtered, and the solution was evaporated to dryness, giving a residue which was crystallized from methanol/water (10/1, v/v). The compound was further purified by extraction with warm ethyl acetate in a Soxlet apparatus. Yield: 92%. Mp: 167 °C (lit.[25] 178–180 °C). Different preparations and/or repeated crystallizations gave materials with correct elemental analyses, but with the same, reproducible, melting point (167 °C), lower than the literature value.

Potassium (methylsulfinyl)acetate was prepared by treating the acid with equimolar amounts of aqueous 0.1 N KOH. Evaporation to dryness under reduced pressure gave gummy residues. ^{13}C NMR (D_2O): 39.2 (CH_3S); 62.8 (CH_2); 173.0 (COO).

Potassium (methylsulfinyl)benzoate was obtained as above. ^{13}C NMR (D_2O): 46.1 (CH_3S); 125.3, 133.1, 133.5, 134.6, 136.9, 147.3 (benzene ring); 174.7 (COO).

Resolution of 2-(methylsulfinyl)benzoic acid was accomplished through the brucine salt, according to ref 24. $[\alpha]_D = \pm 227.5°$ (lit.[24] ± 227.5°).

(Ethylenediamine)((methylsulfinyl)acetato)platinum(II) Nitrate, 3a. A solution of 0.375 g of [Pt(en)$(NO_3)_2$] (0.999 mmol) in 20 mL of water was treated with 0.122 g of HSoa (0.999 mmol) and 9.95 mL of 0.1 N KOH and heated at 60 °C for 3 h. The solution was evaporated to dryness under reduced pressure, and the residue was extracted with 40 mL of a methanol/chloroform mixture (2/1, v/v), which was concentrated to a few drops and treated with diethyl ether, yielding 0.321 g (74%) of a white solid. ^{13}C NMR (D_2O): 45.2, 49.0 (CH_2 of en); 46.3 (J_{Pt-C} = 60 Hz, CH_3SO); 59.1 (J_{Pt-C} = 31 Hz, CH_2 of Soa); 177 (COO).

The other (methylsulfinyl)acetato complexes were prepared by the same method.

((±)-Diaminocyclohexane)((±)-2-(methylsulfinyl)benzoato)platinum(II) Nitrate, 4b. Method A. A solution of 0.253 g (0.584 mmol) of [Pt((±)-dach)$(NO_3)_2$], 0.107 g (0.584 mmol) of HSob, and 5.8 mL of 0.1 N KOH in 10 mL of water was heated at 60 °C for 5 h. The filtered solution was concentrated to 2 mL, giving 0.219 g of the compound (yield 67%).

Method B. An excess of silver carbonate (2.529 g, 9.773 mmol) was suspended in 50 mL of water and 5 mL of methanol together with 0.567 g (3.07 mmol) of HSob and stirred in the dark for 3 h. The filtered

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Table 1. Elemental Analyses and Relevant Spectroscopic Data of Compounds **1–5**

compound	elem anal.: found (calcd)				FAB	IR[a]		NMR[b]	
	C	H	N	Pt		ν_{as}(COO)	ν(SO)	^{195}Pt	^{1}H(J_{HPt})
HSoa (**1**)	29.6 (29.5)	5.0 (4.9)				1715	998		2.80
KSoa						1605	1017		2.81
HSob (**2**)	52.1 (52.2)	4.5 (4.4)				1698	961–948		2.92
KSob						1600	1015		2.82
[Pt(en)(Soa)]NO_3 (**3a**)	13.6 (13.7)	3.0 (3.0)	9.5 (9.6)	44.0 (44.5)	376	1690	1100	−3033	3.77 (25)
[Pt((±)-dach)(Soa)]NO_3 (**3b**)	21.8 (22.0)	4.1 (3.9)	8.4 (8.5)	39.4 (39.6)	430	1680	1120	−2995	3.76–3.79 (25)
[Pt(*R*,*R*-dach)(Soa)]NO_3 (**3c**)	21.9	4.0	8.3	40.0	430	1680	1120	−2995	3.76–3.79 (25)
[Pt(*S*,*S*-dach)(Soa)]NO_3 (**3d**)	21.7	3.9	8.6	39.9	430	1680	1120	−2995	3.76–3.79 (25)
[Pt(en)((±)-Sob)]NO_3 (**4a**)	23.7 (24.0)	3.0 (3.0)	8.6 (8.4)	38.5 (38.9)	438	1650	1139	−3012	3.70 (23)
[Pt((±)-dach)((±)-Sob)]NO_3 (**4b**)	30.3 (30.3)	3.7 (3.8)	7.4 (7.6)	35.4 (35.2)	492	1640	1139	−2995	3.62–3.63 (23)
[Pt(*R*,*R*-dach)((±)-Sob)]NO_3 (**4c**)	30.1	3.5	7.6	35.8	492	1640	1139		3.62–3.63 (23)
[Pt(*S*,*S*-dach)((±)-Sob)]NO_3 (**4d**)	30.3	3.8	7.6	35.0	492	1640	1139	−2994	3.62–3.63 (23)
[Pt(*S*,*S*-dach)((±)-Sob)]NO_3 (**4e**)	30.1	3.5	7.5	35.7	492	1640	1139	−2992	3.62 (23)
[Pt(*S*,*S*-dach)((−)-Sob)]NO_3 (**4f**)	30.5	3.6	7.9	35.5	492	1640	1139	−3004	3.63 (23)
[Pt(*R*,*R*-dach)((+)-Sob)]NO_3 (**4g**)	30.4	3.8	7.5	35.3	492	1640	1139	−3004	3.63 (23)
[Pt(*R*,*R*-dach)((−)-Sob)]NO_3 (**4h**)	30.5	4.0	7.3	35.4	492	1640	1139	−2992	3.62 (23)
[Pt(*cis*-dach)((−)-Sob)]NO_3 (**4i**)	30.5	3.9	7.4	35.3	492	1636	1137	−3000	3.70 (22)
[Pt(damch)((±)-Sob)]NO_3[c] (**41**)	30.1 (30.3)	3.5 (3.7)	7.6 (7.6)	35.5 (35.2)	520	1636	1139	−3027	3.65 (23)
[PtCl(en)(Soa)] (**5a**)	14.3 (14.6)	2.9 (3.2)	6.7 (6.8)	47.6 (47.8)	412	1620	1107	−3268	3.59 (23)

[a] In cm^{-1}; the ν_{sym}(COO) of the complexes could not be determined with certainty, because the region is crowed by other bands. [b] Spectra at room temperature, in D_2O solutions; δ, in ppm, *vs* external Me_4Si (for the CH_3SO groups) or $PtCl_6^{2-}$; J in Hz. [c] Monohydrate.

solution was treated with 1.165 g (3.07 mmol) of [$PtCl_2$((±)-dach)] and 30.8 mL of 0.1 M $AgNO_3$ and stirred at room temperature for 20 h. Filtration and concentration to 3 mL gave 1.443 g (80%) of the product. ^{13}C NMR (D_2O): 24.0, 24.1, 32.3, 32.6 (CH_2 of dach); 47.5 (J_{Pt-C} = 50 Hz, CH_3SO); 60.1, 63.2 (CH–N of dach); 123.8, 130.1, 134.0, 134.2, 134.5, 137.3 (benzene ring); 167.9 (COO).

Chloro(ethylenediamine)((methylsulfinyl)acetate-*S*)platinum(II), 5a. (i) A 0.180-g sample of 3a, in 4 mL of water, was treated with 1 mL (wet) of Amberlyst A 26 (Cl form, Cl/Pt ratio approximately 2.5) and stirred at room temperature for 2 h. The filtered solution was evaporated to a few drops and treated with acetone. Yield: 65% (0.110 g).

(ii) Alternatively, a solution of 0.122 g of HSoa (1 mmol) in 10 mL of 0.1 N KOH was added to 0.326 g of [$PtCl_2$(en)] (1.0 mmol) in 25 mL of DMF. The slurry was heated at 60 °C for 5 h to give a pale yellow solution. This was concentrated under vacuum, cooled, filtered, and treated with ethanol. The precipitate was washed with ethyl acetate. Yield: 40%, 0.614 g. ^{13}C NMR (D_2O solution): 42.2 (J_{Pt-C} = 48 Hz, CH_3SO); 48.0, 48.2 (CH_2 of en); 61.3 (J_{Pt-C} = 42 Hz, CH_2 of Soa); 169.0 (COO).

Disodium (Ethylenediamine)bis(guanosine-5′-phosphato)platinum(II), 8a. It was prepared by reaction of [$PtCl_2$(en)] with two moles of GMP as the disodium salt, in water at 50 °C for 5 h. The solution was concentrated and treated with *tert*-butyl alcohol giving a white precipitate. This was redissolved in the minimum amount of water and treated again with *tert*-butyl alcohol, giving the product as the decahydrate. Yields never exceeded 5%. Anal. Calcd for Na_2[Pt(en)$(GMP)_2$]·$10H_2O$: C, 21.2; H, 4.4; N, 14.1. C, 20.7; H, 4.3; N, 13.7. This method gives compounds free from NaCl, but yields are very low, consequently the bis(guanosine–phosphate) complexes with other diamines were obtained, as a mixture with NaCl, by evaporating to dryness the reaction mixture and were characterized by NMR spectroscopy.

Diaminebis(9-methylguanine)platinum(II) Dichloride, 9 (diamine = en, dach) were obtained according to ref 26.

Reactions with GMP. Either **3** or **4** was treated with GMP (disodium salt), in water at 50 °C for 3 h. The solutions were evaporated to dryness and products **8** were characterized by ^{1}H NMR and FAB-mass spectroscopy.

Reactivity Studies. The course of the reactions of **3** and **4** with Cl^-, GMP, and MeG were followed by ^{1}H NMR spectroscopy with an 80-MHz instrument in order to better detect the Pt satellites. The reactions were carried out in D_2O, at 40 ± 1 °C and complex concentration 10^{-2} mol L^{-1}. No buffer was added to avoid interferences; the pH* was around 6 for the reactions with NaCl, and it varied from 7.2 (at the beginning of the reaction) to 6.9 at the end of the reactions with GMP and about 6.2 for the reaction with MeG. When, as judged by the NMR spectra, the concentration of the intermediates was sufficiently high, aliquots of the solutions were evaporated to dryness and redissolved in H_2O, to remove deuteration of the exchangable protons. This solution was dispersed in glycerol and analyzed by FAB-mass spectroscopy.

Table 2. Summary of Crystallographic Data for **3a**

chem formula	$C_5H_{13}N_3O_6PtS$	space group	*R*3 (No. 146)
fw	438.33	*Z*; ρ_{calcd}, g cm^{-3}	9; 2.56
cryst syst	trigonal (hexagonal axes)	μ(Mo Kα), cm^{-1}	126.65
a, Å	15.139(3)	R^a	0.018
c, Å	12.886(2)	R_w^b	0.022
V, Å^3	2558(1)		

[a] $R = \sum||F_o| - \kappa|F_c||/\sum|F_o|$. [b] $R_w = [\sum w(|F_o| - \kappa|F_c|)^2/\sum w|F_o|^2]^{1/2}$. $w = 4|F_o|^2/\sigma^2(|F_o|^2)$. $\sigma(|F_o|^2) = (\sigma^2(I) + (aI)^2]^{1/2}/Lp$ and $a = 0.04$.

X-ray Crystal Structure Determination of 3a. Slow diffusion of diethyl ether in a water/methanol solution of **3a** gave well shaped crystals. Crystallographic data are listed in Table 2. Diffraction data were collected at room temperature on an Enraf-Nonius CAD-4 diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å). The diffracted intensities were corrected by Lorenz polarization effects and absorption, the latter based on Ψ-scans correction.[27] The structure was solved using heavy-atom methods and refined by full-matrix least-squares techniques using the SDP-Plus package.[28] Anisotropic thermal parameters were assigned to all non-hydrogen atoms excluding the diamine carbon atoms which were found disordered. The disorder has been interpreted as the result of two different (λ/δ) ring conformations having 0.67 and 0.33 occupancy, respectively. Hydrogen atoms were introduced in the final stages of refinement in calculated positions (d_{C-H} and d_{N-H} 0.95 Å, common B_{iso} 5.0 Å^2). Refinement of the enantiomorphic structure gave a higher *R* value (0.028) than that of the adopted chirality. Final values for conventional agreement indexes are listed in Table 2, while final selected atomic coordinates are reported in Table 3. Scattering factors for neutral atoms and anomalous dispersion corrections for scattering factors were taken from refs 29 and 30 respectively.

In Vitro Cytotoxicity Assay. L1210 murine leukemia cells sensitive and resistant to cisplatin (L1210/CDDP) were cultured as a suspension in RPMI 1640 medium supplemented with 20% fetal calf serum, 1% L-glutamine (0.2 mol L^{-1}), 0.5% 2-mercaptoethanol (0.1 mol L^{-1}) and 2% hepes. Both cell lines were grown in a humidified atmosphere (5% CO_2 and 95% air) at 37 °C. For testing purposes 2.5 × 10^4 cells of either cell line were plated in 96 well plates and preincubated for 24 h in complete culture medium. The cells were then exposed to the drugs (dissolved in sterile water immediately before use and diluted in complete culture

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Table 3. Final Selected Atomic Coordinates and Equivalent Isotropic Thermal Parameters for **3a**

atom	*x/a*	*y/b*	*z/c*	B_{eq}
Pt	0.29356(2)	0.33603(2)	0.000	2.442(5)
S	0.3533(1)	0.2315(1)	0.0148(2)	2.68(4)
O(1)	0.4387(4)	0.4425(4)	–0.0371(6)	3.8(1)
O(2)	0.6004(4)	0.4918(5)	–0.0220(7)	5.4(2)
O(3)	0.3198(5)	0.1594(4)	0.1004(5)	4.0(1)
N(1)	0.1464(5)	0.2398(5)	0.0390(7)	3.9(2)
N(2)	0.2433(5)	0.4395(5)	–0.0102(6)	3.4(1)
C(1)	0.4880(5)	0.3227(6)	0.0224(8)	3.8(2)
C(2)	0.5132(6)	0.4269(6)	–0.0141(7)	3.5(2)
C(3)	0.3448(6)	0.1663(6)	–0.1021(7)	3.6(2)
C(4)	0.092(1)	0.296(1)	0.067(1)	4.0(2)*
C(4′)	0.086(2)	0.286(2)	0.006(2)	4.0(2)*
C(5)	0.1291(8)	0.3878(9)	0.002(1)	3.4(2)*
C(5′)	0.145(2)	0.396(2)	0.045(2)	3.4(2)*
O(11)	0.0923(5)	0.0353(7)	–0.0877(7)	6.4(2)
O(12)	0.0693(6)	–0.0160(8)	0.1834(9)	12.2(3)
O(13)	0.0438(5)	–0.0508(5)	0.4553(6)	5.5(2)
N(11)	0.000	0.000	–0.087(1)	3.2(2)
N(12)	0.000	0.000	0.181(1)	4.6(2)
N(13)	0.000	0.000	0.457(1)	4.0(2)

[a] The isotropic equivalent thermal parameter (B_{eq}) is defined as one-third of the trace of the orthogonalized tensor. Starred values denote atoms that were refined isotropically. Atoms C(4)/C(5) and C(4′)/C(5′) have occupancy factors of 0.67 and 0.33, respectively.

medium) for 48 h, and the following MTT essay was performed to evaluate the cytotoxic effects. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Chemicals, St. Louis, MO) was dissolved in PBS at a concentration of 5 mg/mL and filtered. The MTT solution (dilution 1:5) was added (50 μL/well) and incubated for 4 h. Reduction of MTT by cellular dehydrogenasis yielded formazan crystals at the bottom of the plates. The supernatant was removed by centrifugation, and the crystals were solubilized by the addition of 150 μL of dimethyl sulfoxide and gentle stirring. The plates were read at 570 nm with a Dynatech MR 700 reader and the inhibiting concentration 50% of cellular growth (IC_{50}) was calculated. The resistance index for each compound was obtained as the ratio between IC_{50} on L1210/CDDP and IC_{50} on L1210.

Results and Discussion

The Ligands Soa and Sob. The acids **1** and **2** are easily prepared by oxidation of the thioethers. HSob has been resolved into its enantiomers, through the brucine salt.[24] The reported[24] absolute configuration for the (+) isomer is *R*–. Resolution of **1** with brucine gave irreproducible results. HSoa has been reported to have a low stability.[31]

Compounds **1** and **2** are strongly associated both in the solid state and in solution.[24,32] The OH stretching frequency of both compounds (KBr pellets) are at 2500 cm^{-1}, and the S=O stretches are at 997 and 961 cm^{-1} for **1** and **2** respectively. These values are lower than those of their potassium salts (1020 and 1000 cm^{-1} respectively), a lowering that is reminiscent of the shift observed when a sulfoxide group acts as a donor via the oxygen atom.[33] This suggests strong association involving the carboxylic group and the S=O oxygen atom, which is the site of protonation of sulfoxides.[34] Intermolecular associations of this type have indeed been found in the X-ray structure of **2**.[32]

For **1** a strong intramolecular association is present even in D_2O solution. In fact in the ^1H NMR spectrum the methylene protons give rise to an AB pattern centered at 3.95 ppm, implying that rotations around the C–C and C–S bonds are inhibited, as in structure **1′**, in which the methylene protons are diastereotopic. Such a structure is no longer present in the anion: addition of 1 equiv of KOH simplifies the spectrum and only a singlet at δ 3.81 is observed for the methylene protons. Interestingly this singlet is reduced in intensity and eventually disappears (pH* 10), showing the presence of an H–D exchange process. Such a base-catalyzed exchange is accelerated upon chelation to platinum, when it occurs even at pH* 5 (see below).

Synthesis and Characterization of the Complexes. Complexes **3** and **4** were synthesized by reaction, in water solution, of [Pt(diam)$(NO_3)_2$] with KSoa or KSob. Compounds **4** were obtained also by reaction of [$PtCl_2$(diam)] with the silver salt of Sob, prepared in situ, and 1 equiv of $AgNO_3$. This method gave better yields and products of higher purity, but it could not be used for compounds **3** since AgSoa is little stable. No relevant difference was found in the preparations of the various diastereoisomeric derivatives of dach and Sob. The reaction between a Pt complex of resolved dach with racemic Sob (or of racemic dach–Pt derivatives with resolved Sob) gave the diastereoisomeric mixture in a 1:1 ratio, as judged by ^1H NMR spectroscopy (see below and Table 1). Compounds **3** could also be obtained by reaction of [Pt(diam)$(NO_3)_2$] with the methyl ester of Soa, at pH 6. At this pH, but in the absence of the Pt complex, the ester is not hydrolysed. Coordination of the ester therefore activates such a hydrolysis reaction, as in the well-known case of the metal coordinated amino acid esters.[35]

The compounds are 1/1 electrolytes in water and DMF solutions (Λ_M 100–120 and 70–80 Ω^{-1} cm^2 mol^{-1} respectively, at 25 °C).

The presence of ionic nitrate in the solid state is confirmed by a strong band at 1380 cm^{-1} in the IR spectra of Nujol mulls of all compounds. The S=O stretching frequencies, shifted at wavenumbers higher than those of the potassium salts of Soa and Sob, and the position of the $\nu_{C=O}$ (see Table 1) suggest chelation of the sulfinyl carboxylate ligands through coordination of the sulfur atom and the carboxylate group.[33,36–38] In the IR spectra of compounds **3** recorded from KBr disks, the band at 1690 cm^{-1} is replaced by a band at 1620 cm^{-1}, i.e. at a frequency close to that of the potassium salt of Soa, while the stretching frequency of the SO group is only marginally affected. This fact suggests that the carboxylate group is no longer coordinated, probably being replaced by the bromide ion. On the contrary the KBr matrix has little effect on compounds **4**, since the intensity of the 1640-cm^{-1} band is only slightly reduced and only a weak absorption at 1600 cm^{-1} shows up. These observations are in agreement with the behavior of the compounds in saline solutions, as described later. Finally the FAB-mass spectra of complexes **3** and **4** show a cluster of peaks (100% abundance) at the correct *m/z* values for the cations. If Soa and Sob were not chelated, the fourth coordination position of Pt should be occupied by the nitrato group (or by a water molecule), giving rise to peaks at *m/z* = M + 62 (or M + 18), which were never observed.

The structure of compounds **3** was confirmed by the X-ray structure determination of [Pt(en)(Soa)]NO_3 (**3a**).[39]

Crystal Structure of [Pt(en)(Soa)]NO_3, 3a. The crystal structure of **3a** consists of the packing of [Pt(en)(SOA)]$^+$ cations and NO_3^- anions in a 1/1 ratio with normal non bonding interactions. Final bond distances and angles for the complex are collected in Table 4, while Figure 1 shows an ORTEP drawing of the cation.

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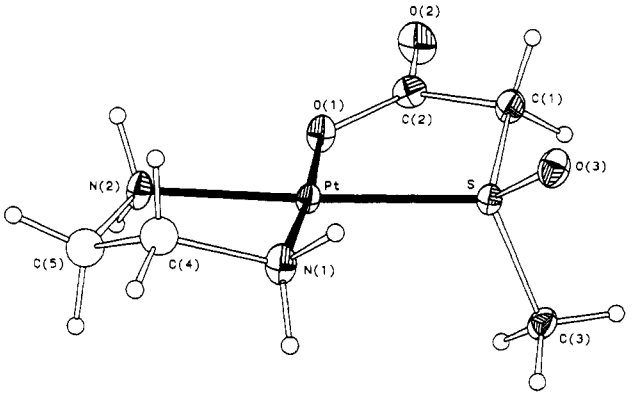

Figure 1. ORTEP drawing of the cation of $[Pt(en)(Soa)]NO_3$, **3a**. Only the λ conformer of the disordered ethylenediamine chain is shown. Thermal ellipsoid are drawn at the 30% probability level.

Table 4. Selected Bond Distances (Å) and Angles (deg) for **3a**

Pt–S	2.184(2)	O(2)–C(2)	1.19(1)
Pt–O(1)	2.025(6)	N(1)–C(4)	1.48(2)
Pt–N(1)	2.019(8)	N(1)–C(4′)	1.46(4)
Pt–N(2)	2.055(8)	N(2)–C(5)	1.51(2)
S–O(3)	1.452(7)	N(2)–C(5′)	1.47(3)
S–C(1)	1.80(1)	C(1)–C(2)	1.50(2)
S–C(3)	1.77(1)	C(4)–C(5)	1.48(2)
O(1)–C(2)	1.29(1)	C(4′)–C(5′)	1.53(5)
S–Pt–O(1)	85.4(2)	O(3)–S–C(1)	111.7(5)
S–Pt–N(1)	99.3(3)	O(3)–S–C(3)	109.0(5)
S–Pt–N(2)	177.3(3)	C(1)–S–C(3)	102.2(5)
O(1)–Pt–N(1)	175.1(3)	Pt–O(1)–C(2)	119.8(6)
O(1)–Pt–N(2)	92.6(3)	S–C(1)–C(2)	112.5(7)
N(1)–Pt–N(2)	82.6(3)	O(1)–C(2)–O(2)	122(1)
Pt–S–O(3)	119.6(3)	O(1)–C(2)–C(1)	118.4(8)
Pt–S–C(1)	99.7(4)	O(2)–C(2)–C(1)	119(1)
Pt–S–C(3)	112.9(4)		

C(1)–C(2)–O(1)–Pt	10.5
C(2)–O(1)–Pt–S	−17.9
O(1)–Pt–S–C(1)	16.5
Pt–S–C(1)–C(2)	−16.7
S–C(1)–C(2)–O(1)	6.1

The Pt atom has an almost perfect square planar coordination defined by the N atoms of the chelating ethylenediamine and the S and O(carboxylate) atoms of Soa. The maximum deviation for the plane defined by N(1), N(2), O(1), and S is 0.007(8) Å, with Pt displaced 0.029 Å off the plane. Pt–N, Pt–S, and Pt–O bond lengths are close to those found in $[Pt(dach)(Me_2SO)(CBDC)]$[40] (in which CBDC, is the anion, 1,1-cyclobutanedicarboxylate, coordinated to Pt via only one carboxylato moiety), i.e. Pt–NH_2R = 2.02–2.10 Å, Pt–O–C(O)–C = 1.98–2.05 Å, and Pt–S(O)RR′ = 2.207–2.230 Å for the four independent molecules, and compare well with the average values reported for the same type of bond interactions.[41] In **3a** the Pt–N bond trans to the sulfur atom is slightly longer than the other (2.055(8) and 2.019(8) Å, respectively).

The ethylenediamine chelate ring, exhibiting a twist conformation, has been found to be disordered: for the particular configuration of the sulfur atom of Figure 1 the λ/δ ratio is 2/1. The sulfinyl–carboxylato chelate ring is perfectly ordered and shows an envelope conformation, with the metal atom 0.5 Å out of the OCCS mean plane. The slight deviations from such a plane (see torsion angles in Table 4) define a chirality λ for this chelate ring.

Conformational Aspects. The circular dichroism (CD) spectra of aqueous solutions of the diastereoisomers $[Pt(R,R\text{-dach})((+)\text{-Sob})]^+$, **4g**, and $[Pt(R,R\text{-dach})((-)\text{-Sob})]^+$, **4h**, display an almost enantiomeric behavior; i.e., they are almost mirror images between 220 and 350 nm, see Figure 2. The published CD spectra of $[Pt(R,R\text{-dach})L_2]$ (L = H_2O, $^1/_2$ oxalate, $^1/_2$ malonate) have very low intensities in the same region,[42,43] as the Cotton effects are mainly due to vicinal effects. This is also the case of complexes **4** with chiral dach and racemic Sob (Figure 2). On the contrary the Cotton effects reported[18] for the diastereoisomeric couple $[PtCl(R,R\text{-dach})(\text{methyl tolyl sulfoxide})]^+$ are not mirror images, as expected for diastereoisomers, and are rather intense, being originated by intraligand and/or charge transfer transitions involving the sulfinyl moiety. The high intensity Cotton effects of **4g** and **4h** must have a similar origin, and their enantiomeric relationship can be explained assuming the presence of distorted S–COO chelate rings whose opposite conformations are dictated by the opposite configurations of the sulfur atoms.[44]

Interestingly the spectra of Figure 2 do not change on standing (>24 h). A slow decrease of the intensities is observed in 0.15 M NaCl, on account of a slow reaction with Cl^- which will be described in a subsequent section.

Solution Studies. The S–COO chelate structure of both series of compounds is maintained also in water solution, for at least 24 h, on account of the following observations.

(i) There is only one species in solution as all the compounds give rise to only one peak in the ^{195}Pt NMR spectra (Table 1).

(ii) In the ^1H NMR spectra (D_2O solutions) the resonance of the CH_3 protons of the methylsulfinyl groups are downfield shifted with respect to those of the Soa^- and Sob^- anions and show Pt–H coupling ($J_{Pt-H} \simeq 25$ Hz). Both facts are typical of sulfoxide groups S-bonded to Pt.[36,37,45,46] These protons give rise to two signals of equal intensities in the case of diastereoisomeric complexes such as $[Pt(R,R\text{-dach})((\pm)\text{-Sob})]^+$. These spectra are indefinitely stable (>24 h) and change only in the presence of nucleophiles, such as Cl^- or guanine (see below).

(iii) In the ^{13}C NMR spectra (D_2O solutions) the resonances of the carboxylic carbon atoms in the complexes (177.0 and 167.9 ppm for **3a** and **4b** respectively) are different from those of the carboxylate anions (173.0 for Soa^- and 174.7 ppm for Sob^-). Moreover in compound **5a** (in which Soa is coordinated to Pt only through the S atom, see below) the C(carboxylate) resonates at 169 ppm). The Pt satellites of the carboxylic carbon atoms of the complexes could not be identified unambiguosly. However the Pt–C (carboxylate) satellites have been seldom observed,[47,48] probably because of small coupling constants and/or the low intensity of the resonance of the metal-bound carboxylate carbon atom.[13,14]

(iv) The CD spectra of the Sob derivatives can be rationalized assuming the presence of such a S–COO chelate ring, as discussed above.

(v) The FAB-mass spectra of water solutions (dispersed in glycerol) of both series of compounds show peaks at m/z values

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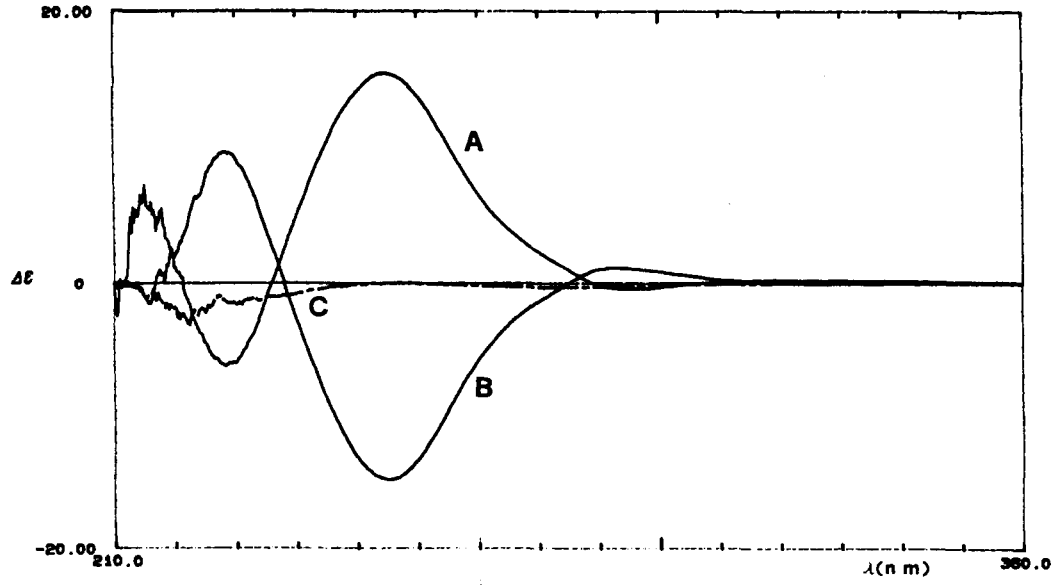


Figure 2. Circular dichroism spectra (water solutions): (A) [Pt(*R*,*R*-dach)((+)-Sob)]NO_3, **4g**; (B) [Pt(*R*,*R*-dach)((−)-Sob)]NO_3, **4h**; (C) [Pt(*S*,*S*-dach)((±)-Sob)]NO_3, **4d**. The spectrum of the *R*,*R*-dach (±)-Sob Pt complex is the mirror image of that of **4d**.

corresponding to the cationic complexes, with 100% abundance. (e.g. for [Pt(en)(Soa)]NO_3, m/z = 376 and for [Pt(dach)(Sob)]-NO_3, m/z = 492, both corresponding to the cation). If the sulfinyl–carboxylate ligands were not chelated, but monodentate, the vacant coordination site of Pt should be occupied by a water molecule, giving rise to M + 18 molecular ions. Only faint peaks (<5% relative abundance) at m/z = M + 18 were observed.

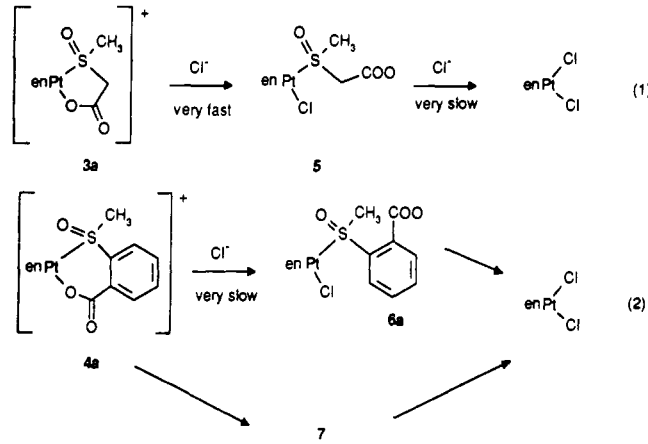
(vi) Finally the reactivity studies described below can be easily explained if one assumes such a chelate structure.

The ^1H and ^{13}C NMR spectra of compounds **3** deserve further discussion, which is presented here for **3a** as an example. The methylene protons of Soa gave no detctable signal in the ^1H NMR spectrum recorded in D_2O solution. This is due both to extensive H/D exchange and partial accidental overlap with the HOD resonance. In fact the *J*-modulated ^1H–^{13}C NMR spectrum shows a signal at 59.1 ppm, due to the CH_2 group, which is a singlet in H_2O solution and a 1:2:3:2:1 quintet in D_2O, in agreement with the presence of a CD_2 group in this solvent. The proton resonance of the CH_2 group was located through a 2D ^{13}C–^1H correlation experiment in H_2O solution which showed a cross-peak between the ^{13}C resonance at 59.1 ppm and a ^1H signal at 4.66 ppm. Such a resonance is in fact partly obscured by the broad resonance of HOD in the "normal" spectrum. These experiments have shown that when Soa is chelated to Pt, the CH_2 group of this ligand is activated toward H/D exchange in D_2O solution, as such reaction occur even at pH* 5 (the "natural" pH of aqueous solutions of these complexes). Again such behavior resembles that of chelated amino acids.[35] In the free ligand such an exchange occurs only under basic conditions.

Reactivity Studies. Behavior in Saline. Due to the presence of chloride ions in many biological fluids, it is important to know the stability of these compounds and/or the species present in saline solution. The course of the reactions performed at 40 °C (complex concentration 10^{-2} mol L^{-1}, for details see Experimental Section) can be easily investigated by ^1H NMR spectroscopy, following the shifts of the resonance of the methyl group of the sulfinylcarboxylate ligands (see Scheme 1 and Table 5). No detailed kinetics investigation was attempted because of the partial overlap of many peaks.

Reaction 1. When compounds **3** are dissolved in D_2O in the presence of 1 equiv of NaCl, the resonance of the methyl group of chelated Soa, at about 3.90 ppm, is gradually replaced by a resonance at 3.80 ppm (with Pt–H satellites) attributable to a new species **5**. The rates of such reactions depend, inter alia, on

Scheme 1



Charges of intermediates are omitted for simplicity

the nature of the spectator diamine,[49] with en, $t_{1/2}$ < 10 min, while with dach $t_{1/2} \simeq 2$ h. Compounds **5** are very stable even in the presence of a large excess (0.15 mol L^{-1}) of NaCl. For the en derivative, at 40 °C, after 24 h only about 20% of Soa is present as the free ion, corresponding to the amount of [$PtCl_2$-(en)] which precipitates at the bottom of the tube.

Compound **5a** is so stable that it could be isolated and characterized either by treating $[Pt(en)(Soa)]^+$ with an ion exchange resin in the Cl^- form, or by reaction of [$PtCl_2$(en)] with KSoa. Elemental analyses, FAB-mass, and IR spectra of this compound (Table 1), as well as the above-discussed ^1H NMR spectra, are in agreement with the formulation [PtCl(en)(Soa)], with the Soa ion coordinated to Pt *via* the sulfur atom. A further confirmation of such a coordination sphere is provided by the ^{195}Pt chemical shift of **5a** (−3268 ppm), close to the values reported for [PtCl(diamine)(R′R″SO)] (around −3300)[18] and [PtCl(en)-(Me_2SO)] (−3307).[51] The shift to higher fields of 325 ppm, displayed by **5a** compared with **3a** (−3033) is what expected when

(49) The influence of the nature and of the configuration of the "spectator" diamine on the the reactivity of [Pt(diam)X_2] type complexes has been noted in various instances.[18,50] For dach an explanation which takes into account the conformational freedom of the diamine chelate ring has been proposed on the basis of kinetic measurements. See: D'Alfonso, G.; Pasini, A. *J. Chem. Soc. Dalton Trans.* **1993**, 1231.

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Table 5. ^{1}H NMR and FAB Data for Reagents, Intermediates, and Products of Reactions 1–7 (40 °C)[a]

compound	^{1}H NMR[b] CH_3SO (J_{HPt})	CH_2en (J_{HPt})	H8-GMP (J_{HPt})	H1′-GMP (J_{HH})	CH_3MeG	FAB m/z
Soa$^-$	2.94					
Sob$^-$	3.08					
[Pt(en)(Soa)]NO_3 (**3a**)	3.90 (25)	2.99 (44)				
[PtCl(en)(Soa)] (**5a**)	3.81 (23)	3.10 (40)				412[c]
[$PtCl_2$(en)]		2.82[d] (49)				
[Pt(en)(Sob)]NO_3 (**4a**)	3.86 (23)	3.02 (44)				
[PtCl(en)(Sob)] (**6**)	4.00 (18)	2.89 (50)				
7	3.12	n.o.				
[Pt((±)-dach)(Soa)]NO_3 (**3b**)	3.88, 3.91 (24)					
[Pt((±)-dach)(Soa)(GMP)]$^-$ (**10b**)	3.55, 3.60 (25)		9.05 (20)	6.18 (4.5)		791[f]
[Pt((±)-dach)$(GMP)_2]^{2-}$ (**8b**)			8.90 (22)	6.13 (4.5)		1031[f]
[Pt((±)-dach)((±)-Sob)]NO_3 (**4b**)	3.82, 3.84 (23)					
[Pt((±)-dach)((±)-Sob)(GMP)]$^-$ (**11b**)	2.85, 2.89[e] (n.o.)		9.05 (20)	6.18 (4.5)		853[f]
[Pt(en)(Soa)(GMP)]$^-$ (**10a**)	3.51 (24)	3.04 (42)	9.05 (d.m.)	6.17 (4.5)		
[Pt(en)$(GMP)_2]^{2-}$ (**8a**)		3.04 (42)	8.89 (23)	6.12 (4.7)		
GMP^{2-}			8.36	6.10 (6.0)		
[Pt((±)-dach)(Soa)(MeG)]$^+$ (**12**)	3.45, 3.51 (24)		8.48 (22)		3.92	595[g]
[Pt((±)-dach)(±)-Sob)(MeG)]$^+$ (**13**)	2.97, 2.98 (n.o.)		8.5 (d.m.)		3.86	657[g]
[Pt((±)-dach)$(MeG)_2]^+$ (**9**)			8.11 (23)		3.80	639[g,h]
MeG			7.85		3.83	

[a] For conditions, see text; n.o., not observed; d.m., difficult to measure, signals buried by other peaks or just above the noise. [b] δ in ppm vs external Me_4Si, J in Hz; the values for the CH_3SO groups are different with respect to Table 1, due to the different temperatures. [c] M + 1. [d] In the presence of NaCl 0.15 M. [e] Only one peak is observed in the same region for reactions performed with the en derivatives. [f] Negative ions. [g] Positive ions. [h] Also a peak at $m/2$, 319.5, was observed.

an O donor ligand is replaced by Cl in this type of complexes.[52] Interestingly, the ^{1}H NMR spectrum of **5a**, prepared in H_2O and dissolved in D_2O, shows also a peak at 4.43 ppm attributable to the CH_2 group of S–coordinated Soa, the H/D exchange process therefore takes place only in the chelate structure.

Compound **5a** is a nonelectrolyte in water solution.

Reaction 2. The carboxylate group of the Sob chelate ring of compounds **4** is more inert toward chloride substitution, but the intermediates with monodentate Sob, **6**, are more reactive than **5** and show up only as faint signals in the NMR spectra. In fact the ^{1}H NMR spectrum of a solution of **4a** in 0.15 M NaCl in D_2O shows, initially, only the resonances of the starting material, and the peaks of the CH_3SO group of intermediate **6a** and of free Sob begin to be detectable after about 20 minutes at 40 °C. $t_{1/2}$ for complete loss of Sob and formation of [$PtCl_2$(en)] is >24 h. As for intermediate **6a**, it has a peak at 4.0 ppm (J_{Pt-H} = 18 Hz), whose intensity remains very low even in 0.15 mol L^{-1} NaCl. This peak is split into two equally intense signals for reactions performed with diastereoisomeric dach derivatives and can therefore be attributed to an S-bonded Sob.[53] Careful observation of the spectra recorded during the course of the reaction, reveals also the presence of a peak at 3.12 ppm (with no Pt–H coupling) of very low intensity, which becomes clearly detectable only in the presence of a large excess of NaCl. Such a signal is a sharp singlet even in the case of reactions performed with diastereoisomeric complexes, it grows up together with the peaks of **6a** and is no longer observable at the end of the reaction. We tentatively attribute such a resonance to an intermediate, **7**, of unknown structure.

In conclusion in the presence of chloride ions, complexes **4** lose the Sob ligand very slowly, whereas compounds **3** transform immediately into species **5**. Therefore any biological activity displayed by **3** is likely to occur through the intermediacy of complexes **5**.

Reaction with the Guanine Moiety. Both series of compounds react with the guanine moiety of either 5′-GMP, or 9-methylguanine. In the presence of 2 mol of G, the Soa or Sob ligands are completely substituted to give species [Pt(diam)$(GMP)_2]^{2-}$, **8**, and [Pt(diam)$(MeG)_2]^{2+}$, **9**, with the guanine residues bound to Pt at the N(7) position.[54] **8** and **9** were characterized according to literature data[26,55] and by comparison with authentic samples. These reactions are rather fast if compared with the stability of [Pt(diam)(Sob)]$^+$ and of species **5** in the presence of chloride. Also **5** reacts with GMP or MeG to give the same final products **8** or **9**. The progress of the reactions has been followed at 40 °C by ^{1}H NMR spectroscopy (see Scheme 2 and Table 5; for details see Experimental Section). We describe here the reactions of the (±)-dach derivatives as examples. Both reactions of **3b** and **4b** proceed through the formation of intermediates **10b** and **11b**, respectively, whose nature was inferred by NMR data. Moreover, when **10b** or **11b** reached the highest concentrations, as judged by the NMR spectra, an aliquot of the solution was analyzed by FAB-mass spectroscopy as described in the Experimental Section. Beside the peaks due to **3**, **4**, **8**, and **9**, these spectra showed also peaks at m/z values corresponding to the structure proposed for **10b** and **11b** (Table 5).

Reaction 3. Intermediate 10b. This has an S-bonded monodentate Soa and one N(7) bound GMP: (i) The resonances at 3.55 and 3.60 ppm due to the diastereoisomeric CH_3SO become a single resonance in the case of reactions performed with the en complex. (ii) The low-field shift of H(8) with respect of both free GMP and **8** compares well with published data for similar [Pt(diam)X(GMP)] complexes.[55,56] Under our conditions **10b** reaches its highest concentration after about 10 min and it remains roughly constant for about 1 h, while the peak due to the methyl group of free Soa$^-$ increases in intensity. After about 3 h, the reaction is complete, and the NMR spectrum shows the presence of only free Soa$^-$ and [Pt(dach)$(GMP)_2]^{2-}$.

Reaction 4. The spectrum of intermediate **11b**, besides the peaks due to N(7)-coordinated GMP, shows two resonances of

(52) For example: Ismail, I. M.; Sadler, P. J. In *Platinum, Gold and Other Metal Chemotherapeutic Agents*; Lippard, S. J., Ed.; ACS Symposium Series 209; American Chemical Society: 1983; Washington, DC; p. 171.

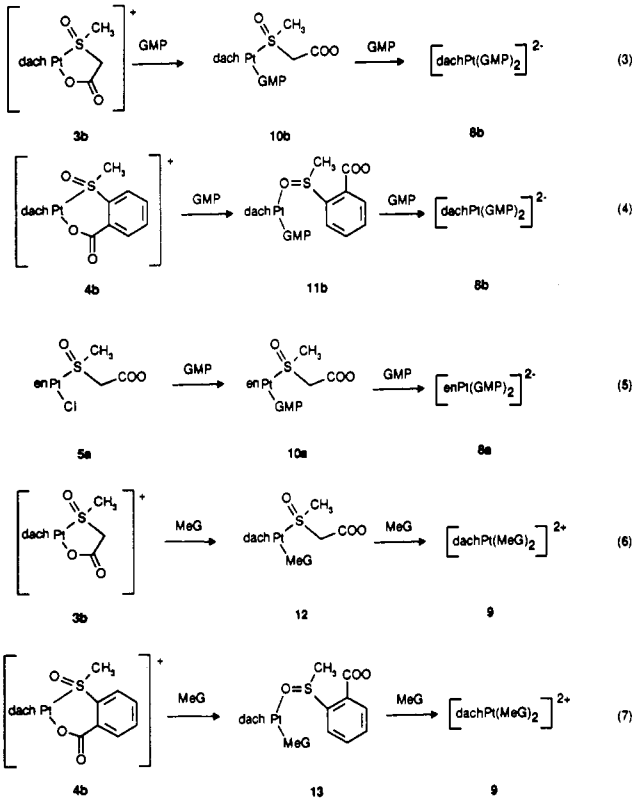
(53) Note that this peak is downfield shifted with respect to the chelated structure, whereas in the Soa case the opposite shift is observed. This may be the result of the different environments of the CH_3SO groups in intermediates **5** and **6**.

(54) See, for example: Lippert, B. *Prog. Inorg. Chem.* **1989**, *37*, 1.

(55) Lempers, E. L. M.; Bloemink, M. J.; Reedijk, J. *Inorg. Chem.* **1991**, *30*, 201.

(56) (a) Marcelis, A. T. M.; van Kralingen, C. G.; Reedijk, J. *J. Inorg. Biochem.* **1980**, *13*, 213. (b) Clore, G. M.; Gronenborn, A. M. *J. Am. Chem. Soc.* **1982**, *104*, 1369. (c) Dijt, F. J.; Canters, G. W.; den Hartog, J. H. J.; Marcelis, A. T. M.; Reedijk, J. *J. Am. Chem. Soc.* **1984**, *106*, 3644. (d) Fanchiang, Y.-T. *J. Chem. Soc., Dalton Trans.* **1986**, 135. (e) Evans, D. J.; Green, M.; Van Eldik, R. *Inorg. Chim. Acta* **1987**, *128*, 27.

Scheme 2



Charges of intermediates are omitted for simplicity

equal intensities, at 2.85 and 2.89 ppm, with no Pt–H coupling, which increases in intensity at the expenses of the peaks of CH_3-SO group of the starting complex. These two peaks become a single resonance if the reaction is carried out with complexes of en or of resolved dach and resolved Sob. These signals reach highest intensity after about 10 min, when the sharp singlet of the CH_3SO of free Sob^- starts to grow. In about 1 h all Sob is present as the free ion, and the spectrum shows the presence of **8** as the only species. The nature of intermediate **11b** is rather speculative; we can tentatively propose that Sob is coordinated to Pt through the sulfinyl oxygen atom. Such a mode of coordination has sometimes been observed also for soft metals, in the presence of steric crowding,[57,58] as might be the case for intermediates **11**. For this mode of coordination the CH_3SO resonance is high-field shifted with respect of the *S*-bonded case[45,46,57] and may not show Pt–H coupling.[45,57] If coordination were through the carboxylate group, the CH_3SO moiety would probably be too far away from Pt to show the observed diastereoisomeric behavior.

Reaction 5. (Reaction of 5a with GMP). Upon treatment of **5a** with 2 mol of GMP we observed a fast increase of peaks attributable to an intermediate **10a**, together with the peaks of the methyl group of free Soa^- and of the final product $[Pt(en)(GMP)_2]^{2-}$. The overall reaction is complete in about 75 min. We could not detect any peak attributable to an intermediate of the type [PtCl(en)(GMP)]. The behavior of **5** may, therefore, be similar to that of $[PtCl(diam)(sulfoxide)]^+$ in the presence of GMP[55] or other nucleophiles.[59]

Reactions 6 and 7. Reactions of compounds **3** and **4** with 9-MeG proceed via similar mechanisms, but are much slower, as they go to completeness in about 12 h, presumably also because of the insolubility of MeG in water.

(57) Davies, J. A. *Adv. Inorg. Chem. Radiochem.* **1981**, *24*, 115.
(58) Elding, L. I.; Oskarsson, A. *Inorg. Chim. Acta* **1987**, *130*, 209.
(59) Alibrandi, G.; Romeo, R.; Monsú Scolaro, L.; Tobe, M. L. *Inorg. Chem.* **1992**, *31*, 5061.

Table 6. Cytotoxicity of Representative Complexes toward L1210 and L1210/CDDP Murine Leukemia Cells

compounds	IC_{50},[a] μg/mL L1210	IC_{50},[a] μg/mL L1210/CDDP	R.I.[b]
cisplatin[c]	1.0 ■ 0.3	8.6 ± 2.2	8.6
carboplatin[d]	29.0 ± 7.0	92.6 ± 27.0	3.7
[Pt(*R*,*R*-dach)((−)-Sob)]NO_3	28.1	18.3	0.6
[Pt(*R*,*R*-dach)((+)-Sob)]NO_3	19.4	17.3	0.8
[Pt((±)-dach)((±)-Sob)]NO_3	38.7	34.8	0.9
[Pt(*S*,*S*-dach)((+)-Sob)]NO_3	64.6	61.6	1.0
[Pt(*S*,*S*-dach)((−)-Sob)]NO_3	77.5	88.5	1.1
[Pt(damch)((−)-Sob)]NO_3	14.4	12.3	0.8
[Pt((±)-dach)(Soa)]NO_3	57.8	60.5	1.0

[a] Concentration inhibiting 50% of the cellular growth. [b] Resistance index: ratio of IC_{50} on L1210/CDDP over IC_{50} on L1210. [c] Mean values and standard deviation of 135 experiments. [d] Mean values and standard deviation of 124 experiments.

Biological Studies. Table 6 reports the cytotoxicities of compounds **3** and **4** toward L1210 and L1210/cisplatin resistant (L1210/CDDP) leukemia cells, together with the values of cisplatin and carboplatin as reference. The compounds here presented are moderately cytotoxic, the cytotoxicity being comparable to that of carboplatin. As expected,[6,8,9,18] the presence of dach and damch as the nonleaving ligands confers cytotoxicity also toward the cisplatin-resistant line. The derivatives of Soa are the least cytotoxic.

An interesting aspect is the relationship between the biological activity of the complexes and the absolute configuration of both the non leaving dach[42,60] and the leaving ligands.[18,61] Our findings confirm the general observations that Pt complexes with *R*,*R*-dach display higher cytotoxicity than the *S*,*S*-dach derivatives.[60] On the other hand there are a few reports also on the influence of the configuration of chiral leaving groups.[18,61] Also in the present case there appears to be some influence of the configuration of the sulfoxide group, although such influence is overshadowed by that of the configuration of the diamine.

The most cytotoxic complexes, i.e. the *R*,*R*-dach and the damch derivatives of **4** were also tested for antitumor activity against the murine reticulum sarcoma M5076 and were found inactive against this tumor model at the highest doses tested.[62] No further biological study was undertaken.

Conclusions

The complexes reported in this paper are rather stable in the presence of a large excess of chloride ions (or transform into a stable product as compound **5**), but react promptly with the guanine moiety. The complexes are only moderately cytotoxic toward L1210 leukemia cells. The reactions with 5′-GMP or 9-methylguanine are clearly too naive models of the mode of action of Pt compounds, and other factors such as biodistribution, transport systems, and probably also enzymatic activation should be taken into account. The damch and dach derivatives of **4** are cytotoxic also against the cisplatin resistant lines, confirming that Pt complexes of these diamines are not cross resistant with cisplatin, independently on the nature of the leaving ligand. We have also confirmed the influence of the absolute configuration of chiral leaving ligands on the biological properties of a Pt complex; in the present case, however, such an influence is overshadowed by that of the diamine.

(60) For example: (a) Kidani, Y.; Inagaki, K.; Iigo, M.; Hoshi, A.; Kuretani, K. *J. Med. Chem.* **1978**, *21*, 1315. (b) Gullotti, M.; Pasini, A.; Ugo, R.; Filippeschi, S.; Marmonti, L.; Spreafico, F. *Inorg. Chim. Acta* **1984**, *91*, 223. (c) Vollano, J. F.; Al-Baker, S.; Dabrowiak, J. C.; Schurig, J. E. *J. Med. Chem.* **1987**, *30*, 716. (d) Fanizzi, F. P.; Maresca, L.; Natile, G.; Quaranta, R.; Coluccia, M.; Di Bari, L.; Giordano, D.; Mariggio', M. A. *Inorg. Chim. Acta* **1987**, *137*, 45.
(61) Miyamoto, T. K.; Okude, K.; Maeda, K.; Ichida, H.; Sasaki, Y.; Tashiro, T. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 3239.
(62) Manzotti, C. Unpublished results.

Two interesting points arise from the reactivity studies. (i) The rates and the mechanisms of the reactions of **3** and **4** toward either Cl^- or G are different and depend on the nature of the chelating ligand, being a balance of basicity of the donor groups and of the type of the chelate ring. (ii) While reaction of cisplatin toward nucleobases proceeds via aquation steps,[14,15] compounds **3** and **4** are stable to hydrolysis, but react promptly toward G, suggesting a direct attack of this nucleophile on the starting complexes, as in the case of carboplatin.[63] It is therefore likely that the reactivity of the compounds reported here can be tuned by changes of the electronic and chelating properties of the sulfinyl carboxylato ligands, in order to increase the discrimination of a Pt complex toward a specific nucleophilic site of the target macromolecule. Work is in progress toward this end.

(63) Frey, U.; Ranford, J. D.; Sadler, P. J. *Inorg. Chem.* **1993**, *32*, 1323.

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Supplementary Material Available: Text giving details of the crystal structure determination of **3a** and crystallographic data, coordinates of the hydrogen atoms, anisotropic thermal parameters, bond distances and angles, and least square planes (Tables S1–S5) (7 pages). Ordering information is given on any current masthead page.