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Platinum(II) complexes with L-methionylglycine and L-methionyl-L-leucine ligands: Synthesis, characterization and *in vitro* antitumoral activity

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Abstract

Four dipeptide complexes of the type $[PtX_2(dipeptide)] \cdot H_2O$ (X = Cl, I, dipeptide = L-methionylglycine, L-methionyl-L-leucine) were prepared. The complexes were characterized by ^{1}H, ^{13}C, ^{195}Pt NMR and infrared spectroscopy, DTG and elemental analysis. From the infrared, ^{1}H and ^{13}C NMR spectroscopy it was concluded that dipeptides coordinate bidentately *via* sulfur and amine nitrogen donor atoms. Confirmed with ^{13}C and ^{195}Pt NMR spectroscopy, each of the complexes exists in two diastereoisomeric forms, which are related by inversion of configuration at the sulfur atom. The ^{1}H NMR spectrum for the platinum(II) complex with L-methionylglycine and chloro ligands exhibited reversible, intramolecular inversion of configuration at the S atom; $\Delta G^{\neq} = 72$ kJ mol^{-1} at coalescence temperature 349 K was calculated. *In vitro* cytotoxicity studies using the human tumor cell lines liposarcoma, lung carcinoma A549 and melanoma 518A2 revealed considerable activity of the platinum(II) complex with L-methionylglycine and chloro ligands. Further *in vitro* cytotoxic evaluation using human testicular germ cell tumor cell lines 1411HP and H12.1 and colon carcinoma cell line DLD-1 showed moderate cytotoxic activity for all platinum(II) complexes only in the cisplatin-sensitive cell line H12.1. Platinum uptake studies using atomic absorption spectroscopy indicated no relationship between uptake and activity. Potential antitumoral activity of this class of platinum(II) complexes is dependent on the kind of ligands as well as on tumor cell type.

Keywords: Platinum(II) complexes; Dipeptide; Antiproliferative activity; Platinum uptake

1. Introduction

Sulfur-containing biomolecules such as L-methionine and glutathione are believed to play an important role in the metabolism and mechanism of platinum based anticancer drugs [1]. The high toxicity of these drugs was ascribed to their interaction with sulfur atoms of cysteinyl or methionyl residues in proteins [2]. In this respect, systematic studies have been carried out to determine the nature of such interactions [3–6].

However, considering the strong affinity of platinum(II) to sulfur donor atoms like those of cysteinyl or methionyl units in dipeptides, it is surprising that such interactions are not studied to the same extent as those with sulfur donor atoms of amino acid residues in proteins. Thus studies on the coordination behaviour of sulfur-containing

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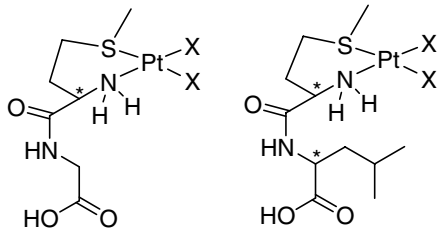
peptides to platinum(II) compounds are scarce even though they are believed to be of key importance in the understanding of the mechanism of interaction of platinum drugs with sulfur atoms in proteins [7].

Complexes of platinum(II) and platinum(IV) with L-methionine, glycylglycine, glycyl-L-methionine and L-alanyl-L-methionine were synthesized and tested for activity against sarcoma 45, Guerin carcinoma, sarcoma 180, adenocarcinoma 755, and Krebs 2 ascites carcinoma in mice and rats, respectively [8]. Many of the complexes showed cytotoxic activity. The reactive group of the peptide to which the platinum was bound also affected the activity as the valent state of platinum did. Platinum(II) and palladium(II) complexes of *S*-(2-aminoethyl)-L-cysteine and *S*-(2-aminoethyl)-D,L-penicillamine were screened against L-1210 lymphoid leukaemia or P388 lymphocytic leukaemia in male or female mice. The platinum(II) complex with *S*-(2-aminoethyl)-D,L-penicillamine showed strong antitumoral activity in female mice by doubling their lifespan [9].

Recently, [(1*R*,2*R*)-*trans*-diaminocyclohexane]platinum-(II) complexes with L-methionine, *S*-methyl-L-cysteine, L-selenomethionine, and *Se*-methylseleno-L-cysteine ligands have been synthesized and characterized. These complexes are of central interest since three out of the four are metabolites in cancer patients treated with oxaliplatin [10].

We report here the preparation of platinum(II) complexes with dipeptides (Fig. 1) comprised of L-methionine, which form six-membered chelating rings. Freeman et al. [11] reported the crystal structure of the [$PtCl_2$(L-met-gly)] complex. We also report similar types of platinum(II) complexes containing halide ligands (chloro or iodo) and L-leucine as second amino acid in dipeptide. Further, these complexes were investigated to establish how the substitution of second amino acid in dipeptides (glycine with L-leucine) and halide ligands (chloro and iodo) affects the *in vitro* antiproliferative activity against a panel of various tumor cell lines from different entities, including liposarcoma, lung carcinoma A549, melanoma 518A2, testicular germ cell tumors H12.1 and 1411HP and colon carcinoma DLD-1. Moreover drug uptake of these platinum complexes was determined in liposarcoma, lung carcinoma A549 and melanoma 518A2 using atomic absorption spectroscopy.



1: X = Cl
2: X = I
3: X = Cl
4: X = I

Fig. 1. [PtX_2(L-met-gly)] (**1**, **2**) and [PtX_2(L-met-L-leu)] (**3**, **4**) complexes.

2. Experimental

2.1. Material and methods

Commercially pure chemicals such as L-methionylglycine, L-methionyl-L-leucine (L-met-gly and L-met-L-leu; Sigma) and $K_2[PtC1_4]$ (Merck) were used as received from distributor.

The elemental analyses (C, H, N, S) were carried out on a CHNS-93 (LECO) elemental analyzer. Infrared spectra were recorded in KBr pellets on a Mattson Galaxy 5000 FT-IR spectrometer, covering the region 4000–300 cm^{-1}. The ^1H, ^{13}C and ^{195}Pt NMR spectra were recorded on a Varian Unity 500 NMR spectrometer in DMF-d_7 at 27 °C. The ^{195}Pt NMR shifts were determined using hexachloroplatinic acid as external standard (δ 0 ppm). From temperature dependent ^1H NMR spectra (27–76 °C) the inversion barrier at coalescence temperature ($\Delta G^{\neq}$) was determined as explained in literature [12–14]. Differential thermogravimetric (DTG) measurements were carried out on a Netzsch STA 409 C under inert atmosphere (Ar) with heating of 10 K/min in the range 25–1000 °C.

2.2. Synthesis

2.2.1. Dichloro(L-methionylglycine)platinum(II) monohydrate [$PtCl_2$(L-met-gly)] · H_2O (**1**)

Complex **1** was prepared by a similar method previously reported [11]. To an aqueous solution (10 ml) of $K_2[PtC1_4]$ (200 mg, 0.482 mmol) a solution (10 ml) of the L-methionylglycine (100 mg, 0.428 mmol) was added. The resulting solution was stirred over 12 h after which the desired product was obtained as a yellow precipitate. After the product was filtered off, it was washed with cold water (3 × 1 ml) and dried on air. Yield: 191 mg, 81% (ratio of diastereoisomers 66:34%). Anal. calcd. for **1**, $C_7H_{16}Cl_2N_2O_4PtS$ (%): C, 17.15; H, 3.29; N, 5.71; S, 6.54. Found (%): C, 17.21; H, 3.55; N, 5.69; S, 6.40. ^1H NMR [δ in ppm, multiplicity (m, multiplet; s, singlet; t, triplet), integral, *J* in Hz][1]: isomer **1A**: δ 2.23 and 2.50 (m, 2H, C^3H_2), 2.65 (s, 3H, C^1H_3), 3.00 and 3.19 (m, 2H, C^2H_2), 3.61 (m, 1H, C^4H), 3.90 and 4.03 (AB part of ABX, $^2J(H^a,H^b) = 17.6$ Hz, $^3J(H^a,H^x) = 5.3$ Hz, $^3J(H^b,H^x) = 4.8$ Hz, 2H, $C^6H^aH^b$), 5.27–5.40 (m, 2H, NH_2), 8.54 ('t', X part of ABX, 1H, $CONH^x$), n.o.[2] COOH; isomer **1B**: δ 2.09 (m, 2H, C^3H_2), 2.58 (s, 3H, C^1H_3), 2.87 (m, 2H, C^2H_2), 3.75 (m, 1H, C^4H), 3.94 (AB part of ABX, 2H, $C^6H^aH^b$), 5.27–5.40 (m, 2H, NH_2), 8.54 ('t', X part of ABX, 1H, $CONH^x$), n.o. COOH. ^{13}C NMR: isomer **1A**: δ 21.4 (C^1), 29.5 (C^3), 32.2 (C^2), 41.5 (C^6), 55.2 (C^4), 171.4 (C^5), 171.8 (C^7); isomer **1B**: δ 21.5 (C^1), 29.6 (C^3), 31.9 (C^2), 41.5 (C^6), 54.2 (C^4), 171.5 (C^5), 171.8 (C^7). ^{195}Pt NMR: isomer **1A**: δ −2933; isomer **1B**: δ −2964. IR [cm^{-1} (m, medium; s, strong; w, weak)]: ν 3326 (m), 2928 (m),

[1] Assignment of atoms as in Fig. 3.
[2] Not observed.

1711 (s), 1640 (s), 1548 (m), 1427 (m), 1341 (m), 1262 (m), 1232 (s), 965 (m), 593 (m), 542 (w), 341 (w), 328 (m). Thermogravimetric analysis (TGA): $1H_2O$ per formula unit, 200 °C, 3.67 (anal. calcd.), 3.55% (found); decomposition to elemental Pt, 1000 °C, 39.8 (anal. calcd.), 40.4% (found).

2.2.2. Diiodo(L-methionylglycine)platinum(II) monohydrate [PtI_2(L-met-gly)] · H_2O (2)

$K_2[PtCl_4]$ (200 mg, 0.482 mmol) was dissolved in 20 ml of water. To this solution 10 ml of an aqueous solution of KI (640 mg, 3.856 mmol) was added in one portion and then stirred and heated to 60 °C for 2 min. An aqueous solution (10 ml) of the L-methionylglycine (100 mg, 0.482 mmol) was mixed with the former solution and under stirring the heating was continued for 2 h. The yellow precipitate was filtered off, washed with cold water (3 × 1 ml) and dried on air. Yield: 256 mg, 79% (ratio of diastereoisomers 79:21%). Anal. calcd. for **2**, $C_7H_{16}I_2N_2O_4PtS$ (%): C, 12.49; H, 2.40; N, 4.16; S, 4.76. Found (%): C, 12.57; H, 2.72; N, 4.28; S, 4.67; ^{1}H NMR [δ in ppm, multiplicity (m, multiplet; s, singlet), integral, J in Hz]: isomer **2A**: δ 2.33 and 2.61 (m, 2H, C^3H_2), 2.73 (s, 3H, C^1H_3), 3.04 (m, 2H, C^2H_2), 3.67 (m, 1H, C^4H), 3.88 and 4.03 (AB part of ABX, $^2J(H^a,H^b) = 17.6$ Hz, $^3J(H^a,H^x) = 5.5$ Hz, $^3J(H^b,H^x) = 6.0$ Hz, 2H, $C^6H^aH^b$), 5.00–5.50 (m, 2H, NH_2), 8.57 (s, X part of ABX, 1H, CONH^x), 13.00 (broad, COOH). ^{13}C NMR: isomer **2A**: δ 21.7 (C^1), 27.9 (C^3), 32.4 (C^2), 41.5 (C^6), 54.4 (C^4), 171.5 (C^5), 172.3 (C^7); isomer **2B**: 23.9 (C^1), 28.5 (C^3), 32.4 (C^2), 41.5 (C^6), 52.4, (C^4), 171.5 (C^5), 172.3 (C^7). ^{195}Pt NMR: isomer **2A**: δ −4063; isomer **2B**: δ −4078. IR [cm^{-1} (m, medium; s, strong; w, weak)]: ν 3326 (m), 2919 (m), 1715 (s), 1648 (s), 1529 (m), 1429 (m), 1382 (m), 1260 (m), 1226 (s), 1143 (m), 970 (m), 584 (m) 546 (w), 340 (w). TGA: $1H_2O$ per formula unit, 200 °C, 2.68 (anal. calcd.), 2.88% (found); decomposition to elemental Pt, 1000 °C, 29.0 (anal. calcd.), 28.7% (found).

2.2.3. Dichloro(L-methionyl-L-leucine)platinum(II) monohydrate [$PtCl_2$(L-met-L-leu)] · H_2O (3)

Compound **3** was prepared as described for [$PtCl_2$(L-met-gly)] · H_2O using L-methionyl-L-leucine (124 mg, 0.482 mmol) instead of L-methionylglycine. Yield: 184 mg, 70% (ratio of diastereoisomers 66:34%). Anal. calcd. for **3**, $C_{11}H_{24}Cl_2N_2O_4PtS$ (%): C, 22.18; H, 4.43; N, 5.13; S, 5.87. Found (%): C, 22.15; H, 4.60; N, 5.17; S, 5.49. ^{1}H NMR [δ in ppm, multiplicity (d, doublet; hp, heptet; m, multiplet; s, singlet, t, triplet; q, quartet), integral]: isomer **3A**: δ 0.87 (d, 3H, $C^{10}H_3$), 0.87 (d, 3H, $C^{11}H_3$), 1.60 (t, 2H, C^8H), 1.75 (hp, 1H, C^9H_2), 2.18 and 2.50 (m, 2H, C^3H_2), 2.65 (s, 3H, C^1H_3), 2.99 and 3.16 (m, 2H, C^2H_2), 3.59 (m, 1H, C^4H), 4.39 (q, 1H, C^6H), 5.27–5.47 (m, 2H, NH_2), 8.44 (m, 1H, CONH^x), n.o. COOH; isomer **3B**: δ 0.87 (d, 3H, $C^{10}H_3$), 0.90 (d, 3H, $C^{11}H_3$), 1.60 (t, 2H, C^8H), 1.75 (hp, 1H, C^9H_2), 2.08 (m, 2H, C^3H_2), 2.58 (s, 3H, C^1H_3), 2.90 (m, 2H, C^2H_2), 3.75 (m, 1H, C^4H), 4.39 (q, 1H, C^6H), 5.27–5.47 (m, 2H, NH_2), 8.44 (m, 1H, CONH^x), n.o. COOH. ^{13}C NMR: isomer **3A**: δ 21.52 (C^1), 21.60 (C^{10}), 23.2 (C^{11}), 25.2 (C^8), 29.6 (C^3), 32.2 (C^2), 41.1 (C^9), 51.7 (C^6), 55.2 (C^4), 171.2 (C^5), 174.6 (C^7); isomer **3B**: δ 21.57 (C^1), 21.60 (C^{10}), 23.2 (C^{11}), 25.2 (C^8), 29.8 (C^3), 31.8 (C^2), 41.1 (C^9), 51.7 (C^6), 54.1 (C^4), 171.5 (C^5), 174.6 (C^7). ^{195}Pt NMR: isomer **3A**: δ −2935; isomer **3B**: δ −2965. IR [cm^{-1} (m, medium; s, strong; w, weak)]: ν 3324 (m), 2956 (m), 1720 (s), 1669 (s), 1543 (s), 1420 (m), 1368 (w), 1248 (m), 975 (m), 542 (w), 341 (w), 330 (m). TGA: $1H_2O$ per formula unit, 180 °C, 3.29 (anal. calcd.), 3.11% (found); decomposition to elemental Pt, 1000 °C, 35.7 (anal. calcd.), 36.5% (found).

2.2.4. Diiodo(L-methionyl-L-leucine)platinum(II) monohydrate [PtI_2(L-met-L-leu)] · H_2O (4)

Compound **4** was prepared as described for [PtI_2(L-met-gly)] · H_2O using L-methionyl-L-leucine (124 mg, 0.482 mmol) instead of L-methionylglycine. Yield: 263 mg, 75% (ratio of diastereoisomers 76:24%). Anal. calcd. for **4**, $C_{11}H_{24}I_2N_2O_4PtS$ (%): C, 18.12; H, 3.32; N, 3.84; S, 4.40. Found (%): C, 17.98; H, 3.35; N, 3.83; S, 4.25. ^{1}H NMR [δ in ppm, multiplicity (d, doublet; m, multiplet; s, singlet; t, triplet), integral]: isomer **4A**: δ 0.69 (d, 3H, $C^{10}H_3$), 0.72 (d, 3H, $C^{11}H_3$), 1.43 (t, 2H, C^8H), 1.56 (m, 1H, C^9H_2), 2.14 and 2.48 (m, 2H, C^3H_2), 2.56 (s, 3H, C^1H_3), 2.72 (m, 2H, C^2H_2), 3.50 (m, 1H, C^4H), 4.24 (m, 1H, C^6H), 5.00–5.20 (m, 2H, NH_2), 8.24 (t, 1H, CONH^x), 12.84 (broad, COOH). ^{13}C NMR: isomer **4A**: δ 21.51 (C^1), 21.9 (C^{10}), 23.2 (C^{11}), 25.3 (C^8), 28.1 (C^3), 32.4 (C^2), 41.0 (C^9), 51.5 (C^6), 54.3 (C^4), 171.3 (C^5), 174.5 (C^7); isomer **4B**: δ 21.55 (C^1), 21.9 (C^{10}), 23.2 (C^{11}), 25.3 (C^8), 29.6 (C^3), 32.4 (C^2), 41.0 (C^9), 51.5 (C^6), 52.3 (C^4), 172.1 (C^5), 174.5 (C^7). ^{195}Pt NMR: isomer **4A**: δ −4066; isomer **4B**: δ −4076. IR [cm^{-1} (m, medium; s, strong; w, weak)]: ν 3324 (m), 2955 (s), 1719 (s), 1665 (s), 1535 (m), 1419 (m), 1387 (m), 1237 (m), 1154 (m), 970 (m), 668 (m) 542 (w), 341 (w). TGA: $1H_2O$ per formula unit, 190 °C, 2.47 (anal. calcd.), 2.25% (found); decomposition to elemental Pt, 1000 °C, 26.8 (anal. calcd.), 28.7% (found).

2.3. In vitro studies

2.3.1. Cell lines and cytotoxicity studies

The human tumor cell lines liposarcoma, A549 (lung carcinoma) and 518A2 (melanoma) were cultivated in the Biozentrum, Martin-Luther-Universität Halle-Wittenberg, and grown in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS) and 0.5% penicillin/streptomycin (everything from Gibco-Invitrogen). The human testicular germ cell tumor cell lines H12.1 and 1411HP and the human colon carcinoma cell line DLD-1 were cultured in the Hämatologisch/Onkologisches Forschungslabor, KIM IV, Martin-Luther-Universität Halle-Wittenberg, maintained in RPMI 1640 (PAA Laboratories), supplemented with 10% FBS and penicillin/streptomycin (PAA Laboratories). All used cell lines were cultivated as monolayer in a fully humidified atmosphere containing 5% CO_2 at 37 °C.

To determine the IC_{50}-values of each synthesized platinum(II) compound, 25,000 cells each liposarcoma, A549 and 518A2 were seeded in Petri dishes (diameter 6 cm) and after 24 h they were exposed to appropriate drug concentration. After 72 h, survival cells were harvested and counted. The drug concentration that reduced the number of living cells by 50% is defined as the IC_{50}-value.

The cytotoxic activity of the platinum compounds in 1411HP, H12.1, and DLD-1 cells was measured by the sulforhodamine-B (SRB) assay [15]. In brief, exponentially growing cells were seeded into 96-well plates and after 24 h they were treated with the platinum drug (0–100 μM) for 96 h. On the fifth day after seeding cells were fixed with 10% trichloric acid and processed according to the published SRB assay protocol. Absorbance was measured at 570 nm using a 96-well plate reader (SpectraFluor Plus Tecan, Germany) and the percentages of surviving cells relative to untreated controls were determined. The IC_{50} values, defined as the drug concentration which inhibits cell growth by 50%, were estimated graphically from semi-logarithmic dose–response plots.

2.3.2. Measurement of platination

For platinum uptake studies, 250,000 cells of liposarcoma, A549 and 518A2 were seeded in Petri dishes (15 cm). On the third day, cells were treated with medium, containing 40 μg/ml of each platinum compound. After 24 h, cells were washed with PBS, collected by trypsinization, lyophilized and the platinum content was measured by flameless atomic absorption spectroscopy with an SS-GFAAS (solid sampling graphite furnace AAS, Germany).

3. Results and discussion

3.1. Synthesis of platinum complexes

The addition of an aqueous solution containing the dipeptides (L-met-gly or L-met-L-leu) to a solution of $K_2[PtCl_4]$ produced yellow precipitates of the chloro complexes **1** and **3** (Eq. (1)). The analogous reaction in the presence of an excess of KI resulted in the formation of the iodo complexes, **2** and **4** that were obtained as yellow precipitates (Eq. 1).

$$K_2[PtX_4] + \text{L-met-NH–C}^*\text{HR–COOH} \xrightarrow{H_2O} [PtX_2(\text{L-met-NH–C}^*\text{HR–COOH})]\cdot H_2O + 2KX \quad (1)$$

1: R = H, X = Cl **3**: R = $CH_2CH(CH_3)_2$, X = Cl

2: R = H, X = I **4**: R$CH_2CH(CH_3)_2$, X = I

Coordination of sulphur gives rise to the formation of two diastereoisomers (see Fig. 2), that were obtained in ratio ca. 2:1 (**1** and **3**, respectively) and 3–4:1 (**2** and **4**, respectively). Complexes precipitated in a analytically pure form with yields between 63% and 81%.

Thermogravimetric analysis of the complexes **1**–**4** shows that the complexes were thermally stable to 180–200 °C.

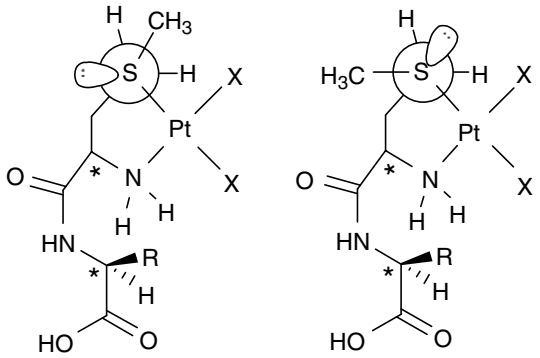


Fig. 2. Diastereoisomers A and B of [PtX_2(dipeptide)] complexes.

The first mass loss indicates that complexes **1**–**4** each contain one molecule of water, as is consistent with the elemental analysis. The complexes decomposed at 1000 °C to elemental platinum.

3.2. Spectroscopic studies

Each of the IR spectra of complexes **1**–**4** shows an antisymmetric and symmetric COOH vibration at around 1720 and 1420 cm^{-1} (Table 1), which is of higher energy than that of free dipeptide (i.e. zwitterion), indicating no bond formation between the metal and the COOH group of ligand that remains to be protonated [16]. Intense bands of the ν(C=O) frequency at around 1650 cm^{-1} in the IR spectra of the compounds **1**–**4** appear upon coordination of ligands suggesting that the amide groups are not involved in the coordination with Pt(II) [17,18]. The band found at around 1540 cm^{-1} [coordinated $\delta(NH_2)$, free ligands 1571–1572 cm^{-1}] indicates metal binding to the NH_2 group [18]. Vibrations at 542 (m) and 341 cm^{-1} (m), assigned to Pt–N and Pt–S vibrations, respectively, indicate coordination of these ligands *via* *S* and *N* donor atoms [16,19].

In the ^{1}H NMR spectrum of L-methionylglycine methylene protons from glycine part at δ 3.88 and 4.03 for H^a and H^b respectively correspond to the AB part of an ABX spin system (CONH^x). In the spectra of the platinum(II) complexes **1** and **2** the protons at δ 3.90 and 4.03 and at δ 3.88 and 4.03, respectively, are the AB protons of the similar spin systems. The $^2J(H^a,H^b)$ coupling constant is 17.6 Hz for the free ligand and also for complexes **1** and **2**. Thus the unchanged splitting pattern and identical ^2J coupling constant indicate that no bond formation with the amide nitrogen atom occurred (Table 1, Fig. 3). In the spectra for complexes **3** and **4** the methyne proton of L-leucine is found at δ 4.39 and 4.24, respectively, (i.e. for the L-met-L-leu at δ 4.37). The acidic proton from the amide nitrogen atom of complexes **1** and **2** is found at δ 8.54 and 8.57, respectively. The ^{13}C NMR spectrum of complex **1** shows shifted signals in comparison to NMR data of the free ligand. The carboxylic and peptide carbon atoms of L-met-gly are shifted upfield 0.9 ppm and 2.4 ppm,

Table 1
Selected IR frequencies (cm^{-1}), ^1H and ^{13}C NMR data (chemical shifts, ppm)[a] of the platinum(II) complexes **1**–**4** and dipeptides L-met-gly and L-met-L-leu

Ligand and complex	IR					^1H		^{13}C		
	$\nu_{as/s}$(COOH)	$\delta(NH_2)$	ν(CON)	ν(Pt–N)	ν(Pt–S)	C^1H_3	C^4H	C^1	C^5	C^7
L-met-gly	1671/1402	1572	1618	–	–	2.09	4.27	14.4	169.1	170.9
1	1711/1427	1548	1640	542	341	2.65[b], 2.58[c]	3.61[b], 3.75[c]	21.4[b], 21.5[c]	171.4[b], 171.5[c]	171.8[d]
2	1715/1429	1529	1649	546	340	2.70[d]	3.67[d]	21.9[b], 23.9[c]	171.5[d]	172.3[d]
L-met-L-leu	1680/1392	1571	1617	–	–	2.07	4.28	14.5	168.5	173.6
3	1720/1420	1543	1660	542	341	2.65[b], 2.58[c]	3.59[b], 3.75[c]	21.5[b], 21.6[c]	171.2[b], 171.5[c]	174.6[d]
4	1719/1419	1535	1665	542	341	2.56[d]	3.50[d]	21.5[b], 21.6[c]	171.3[b], 172.1[c]	174.5[d]

[a] Assignment Fig. 3.
[b] Isomer A.
[c] Isomer B.
[d] Superimposed chemical shift of isomer A and B.

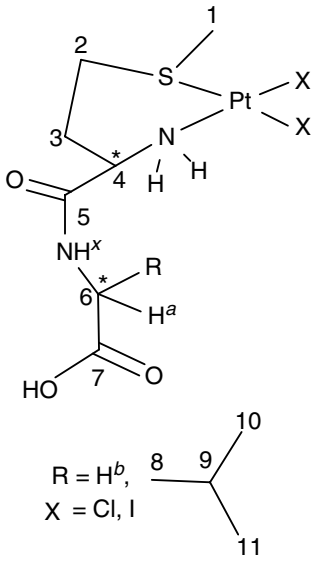


Fig. 3. Labeling of H and C atoms in complexes **1**–**4** (without water molecules).

respectively (Table 1). We conclude that the carboxylic oxygen atoms from glycine and L-leucine and nitrogen atom of peptide bond are not involved in coordination to platinum(II). Chemical shifts which correspond to the methyl group bonded at sulfur atom are shifted downfield, at least 0.5 ppm in ^1H NMR and around 5 ppm in ^{13}C NMR spectra in comparison to free ligands (Table 1). This strongly indicates coordination of sulfur atom to platinum(II) ion. The presence of two resonances at δ 2.58 and 2.65 in ^1H NMR spectra confirms the existence of two isomers, **A** and **B**, in molar ratio 2:1 for complexes **1** and **3** (Fig. 2). Additionally, in the ^{13}C NMR spectrum of **1**, there are two signals for the methyl carbon bonded to sulphur at 21.4 and 21.5 ppm which is consistent with the presence of two diastereoisomers in solution (Fig. 2). NMR spectra of complexes **2**–**4** are similar to those of complex **1**. Although in ^1H NMR spectra for complexes **2** and **4** there seems to be only one isomer, the presence of a second isomer is confirmed by the ^{13}C and ^{195}Pt NMR spectra of these complexes (isomer ratio for **2** and **4: A/B** *ca.* 3–4).

All the complexes **1**–**4** in DMF-d_7 solution give rise to just two ^{195}Pt NMR signals of diastereoisomers (range for: **1** and **3** −2933 to −2965; **2** and **4** −4063 to −4078; see Section 2), with differences in the chemical shifts for chloro complexes of 30 ppm and for iodo complexes of 10–15 ppm. The chemical shifts are in good agreement with those observed for bidentate Pt(II)-methionine complexes such as [$PtCl_2$(L-MetH-κ^2S,N)] which has two signals at −2946 and −2955 ppm [13]. The resonances are at higher field than those of unidentate complexes, e.g. for [$PtCl_3$-(AcMetH-κS)] complex (AcMetH = *N*-acetyl-L-methinone) −2782 and −2788 ppm. This is mainly because of the chelating effect and partly because the donor set [S,N,Cl_2], creating a stronger ligand field than that of the [S,Cl_3] set [9]. By replacement of chloro by iodo ligands in [PtX_2(dipeptide)], the ^{195}Pt NMR shifts follow the expected trend moving to higher field [12,13].

3.3. Inversion of configuration at the sulfur atom

Since the chiral carbon atom in both ligands is stable toward racemization under the conditions of our experiments, the chirality of the sulfur atom give rise to the formation of two diastereoisomers (Fig. 2). To investigate dynamic exchange reactions variable-temperature ^1H NMR of complex **1**, [$PtCl_2$(L-met-gly)] · H_2O, was measured. When the temperature of the sample was raised to 349 K, the two S–CH_3 ^1H NMR singlets (Table 1, Fig. 4) coalesced, giving a $\Delta G^{\neq}$ of 72 kJ mol^{-1}. Separation of the singlets on cooling indicates a reversible process. Diastereoisomers of complex **1** are in thermodynamic equilibrium.

For the complexes of platinum(II) with thioether ligands such as [$PtCl_3$(L-κS)] (L = *N*-formyl-DL-homomethionine, *N*-acetyl-*S*-methyl-DL-cysteine, DL-3-(methylthio)-1,2-propanediol, DL-3-(methylthio)-2-butanone, *S*-methyl-L-cysteine) $\Delta G^{\neq}$ is between 57 and 71 kJ mol^{-1} [12]. The activation barriers for sulfur inversion for the complexes with bidentate κ^2S,N bonded ligands to platinum(II), as was shown for the [$PtCl_2$(L-MetH-κ^2S,N)] and [$PtBr_2$(L-MetH-κ^2S,N)] complexes, are 75 and 72 kJ mol^{-1}, respectively [13]. Thus, the value of $\Delta G^{\neq}$ for complex **1** falls in the expected range.

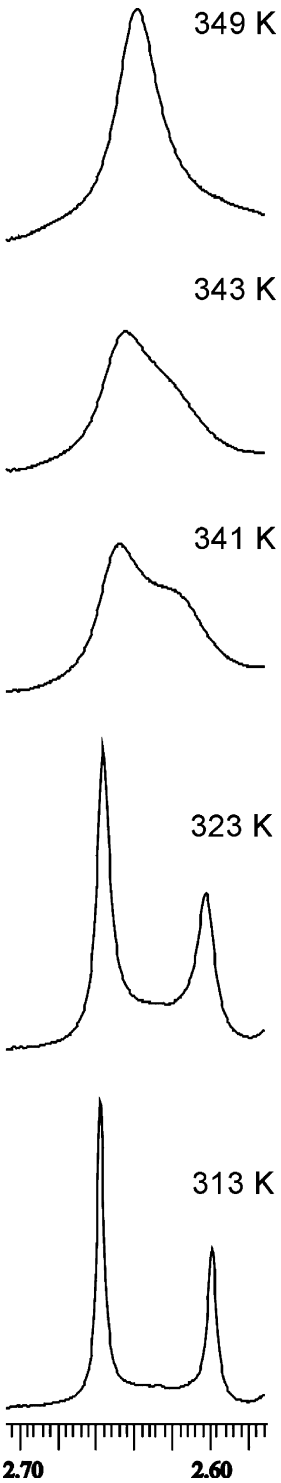


Fig. 4. Variable-temperature ^{1}H NMR spectra of complex **1**.

3.4. Cytotoxicity studies and platinum drug accumulation

The platinum(II) complexes **1**–**4** were tested for cytotoxic activity in the tumor cell lines liposarcoma, A549 and 518A2 (Table 2). Complex **1** showed considerable activity toward liposarcoma, A549 and 518A2 cells (IC_{50} 12.9 μM, 18.2 μM and 17.0 μM, respectively). The replacement of two chloro by two iodo ligands (**1** → **2**) decreased the cytotoxic activity of platinum(II) complex with L-met-gly ligand in these cell lines (IC_{50} 25.1–29.1 μM). The complexes with L-met-L-leu ligand instead of L-met-gly, complexes **3** and **4**, showed weak cytotoxic activity ($IC_{50} > 100$ μM). The efficacy observed for these complexes is probably attributed to the nature of both neutral bidentate (L-met-gly, L-met-L-leu) and anionic monodentate halide ligands.

Further, *in vitro* cytotoxic evaluation using human testicular germ cell tumor cell lines 1411HP and H12.1 and colon carcinoma cell line DLD-1 showed that all platinum(II) complexes described here had moderate cytotoxic activity, but only in the cisplatin-sensitive cell line H12.1 (IC_{50} 55.2–68.0 μM). In this cell line complex **4** presents the platinum compound with the slightly improved efficacy as compared with the other agents. In 1411HP and DLD-1 cells exposed to platinum complexes **1**–**4** only low inhibition of cell growth could be observed (IC_{50} 90.8–96.6 μM).

In order to understand possible reasons for the different cytotoxic activity of complexes **1**–**4**, the uptake of platinum was determined for complexes **1**–**3** using liposarcoma, A549 and 518A2 cells. Complex **4** was excluded because of its similar cytotoxic efficacy with complex **3**. In comparison to literature data [20–22] and as can be seen from Table 3, the amount of platinum uptake for complexes **1**–**3** was very low. This could be a consequence of the acidic carboxylic proton dissociating in the culture media and yielding a charged species that does not easily penetrate the hydrophobic cellular membrane. The platinum uptake in liposarcoma cells was greatest for complex **3**. The highest platinum accumulation in A549 cells could be obtained by treatment with complexes **2** and **3**. Remarkably, despite of the less platinum enrichment in these tumor cells complex **1** showed a greater activity of growth inhibition than complexes **2** and **3**. Only in 518A2 cells the platinum content is highest for complex **1**.

Comparing cytotoxic activity of complexes **1**–**3** and platinum uptake, it may again be concluded that observed cytotoxicity and platinum uptake are attributable to the nature of both the neutral bidentate (L-met-gly, L-met-L-leu) as well as anionic monodentate (Cl, I) ligands. This observation suggests that there is no relationship between drug efficacy and uptake for this class of complexes. This is consistent with observations made for other complexes reported in the literature [23].

4. Conclusions

The present work describes a simple and convenient route for the synthesis of a series of $[PtX_2(dipeptide)] \cdot H_2O$ type complexes (X = Cl, I; dipeptide = L-met-gly, L-met-L-leu). Elemental analyses agree with the proposed formulas. TG analysis indicated that complexes **1**–**4** were precipitated with one water molecule per formula unit. The IR, ^{1}H, ^{13}C and ^{195}Pt NMR spectroscopy approved coordination of these dipeptide ligands *via* sulfur and amine nitrogen donor

Table 2
IC_{50} (μM)[a] values for complexes **1**–**4** and cisplatin

Complex	Liposarcoma	A549	518A2	1411HP	H12.1	DLD-1
1	12.93 ± 1.90	18.19 ± 6.26	16.99 ± 1.08	93.53 ± 3.65	59.42 ± 6.86	94.02 ± 1.20
2	26.92 ± 5.67	25.05 ± 5.72	29.06 ± 4.83	90.83 ± 4.68	60.52 ± 4.69	92.75 ± 2.33
3	>100	>100	>100	96.10 ± 2.19	67.97 ± 5.39	95.73 ± 1.39
4	>100	>100	>100	96.60 ± 2.51	55.18 ± 6.52	96.60 ± 4.34
Ciplatin	0.130	0.163	0.179	3.5	0.6	2.0

[a] Mean value ± SD (standard deviation) from three experiments.

Table 3
Platinum uptake (ppm)[a] of complexes **1**–**3** (40 μg/ml) in liposarcoma, A549 and 518A2 cells after 24 h treatment

Complex	Liposarcoma	A549	518A2
Control	–	0.9 ± 6	–
1	107 ± 15	30 ± 2	65 ± 8
2	62 ± 10	46 ± 9	40 ± 3
3	121 ± 8	45 ± 3	46 ± 7

Values represent means of three independent experiments.
[a] Mean value ± SD from three experiments.

atoms. NMR spectroscopic measurements confirmed presence of two diastereoisomers. The value of activation barriers for sulfur inversion is in the range as found for platinum(II) complexes containing methionine ligand.

In vitro cytotoxicity studies using the human tumor cell lines liposarcoma, lung carcinoma A549 and melanoma 518A2 revealed considerable activity of the platinum(II) complex with L-methionylglycine and chloro ligands (**1**). Substitution of glycine by L-leucine, and Cl with I, resulted in a decrease of cytotoxic activity (complexes **2**–**4**). The platinum uptake for these complexes was relatively low in the selected cell lines, indicating that there is no relationship between uptake and activity. Cytotoxic evaluation using human testicular germ cell tumor cell lines 1411HP and H12.1 and colon carcinoma cell line DLD-1 showed moderate cytotoxic activity for all platinum(II) complexes only in the cisplatin-sensitive cell line H12.1. In conclusion, potential antitumoral activity of platinum(II) complexes of the $[PtX_2(\text{dipeptide})] \cdot H_2O$-type containing *S*,*N* bonded methionine is dependent on the kind of ligands as well as on tumor cell type.

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