

# REACTIONS OF PLATINUM(II) COMPLEXES WITH SULFUR- AND NITROGEN- CONTAINING BIOMOLECULES: SELECTIVE INTERMOLECULAR MIGRATION OF THE THIOETHER-BOUND PLATINUM(II) COMPLEX TO THE N7 NITROGEN ATOM OF GUANOSINE-5'-MONOPHOSPHATE

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## REAKCIJE KOMPLEKSA PLATINE(II) SA SUMPOR- I AZOT-DONORSKIM BIOMOLEKULIMA: SELEKTIVNA INTERMOLEKULSKA MIGRACIJA PLATINA(II) KOMPLEKSA SA TIOETARSKOG SUMPORA NA N7 ATOM AZOTA 5'-GUANOZIN-MONOFOSFATA

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### ABSTRACT

The interactions of platinum(II) complexes with nitrogen- and sulfur-containing biomolecules are responsible for antitumor activity due to attack on DNA or a variety of toxic side effects. Therefore, the monofunctional Pt(II) complexes, [Pt(Gly-Gly-N,N,O)]<sup>+</sup> (Gly-Gly is the dipeptide glycyl-glycine coordinated through the oxygen and two nitrogen atoms) and [Pt(Gly-L-Met-S,N,N)Cl] (Gly-L-Met is the dipeptide glycyl-L-methionine coordinated through the sulfur and two nitrogen atoms) have been used to study their interactions with S-methylglutathione (GS-Me) and guanosine-5'-monophosphate (5'-GMP). All reactions have been studied by <sup>1</sup>H NMR spectroscopy and at room temperature in 50 mM phosphate buffer at pD 7.4. The investigation of the competitive binding of 5'-GMP and GS-Me to the Pt(II) complexes (1:1:1 molar ratio) has shown that in the initial stages of the reaction the corresponding Pt(II) complex only reacts with GS-Me, and second step of the reaction is very slow intermolecular displacement of the S-bound thioether ligand with N7 atom of the guanine base of 5'-GMP. The obtained results have been analyzed in relation to the antitumor activity and toxicity of Pt(II) complexes.

**Keywords.** Platinum(II) complexes; S-methylglutathione (GS-Me); guanosine-5'-monophosphate (5'-GMP); Intermolecular migration.

### SAŽETAK

Interakcije kompleksa platine(II) sa biomolekulima koji sadrže sumpor i azot donorske atome su veoma značajne kako za antitumorsku aktivnost usled reakcija Pt(II) kompleksa sa DNA tako i za toksično delovanje ovih kompleksa. U ovom radu su ispitivane reakcije monofunkcionalnih kompleksa platine(II), [Pt(Gly-Gly-N,N,O)]<sup>+</sup> (Gly-Gly je tridentatno koordinovani glicil-glicin preko atoma kiseonika i dva azotova atoma) i [Pt(Gly-L-Met-S,N,N)Cl] (Gly-L-Met je tridentatno koordinovani glicil-L-metionin preko atoma sumpora i dva azotova atoma) sa 5'-guanozin-monofosfatom (5'-GMP) i S-metil-glutationom (GS-Me) primenom <sup>1</sup>H NMR spektroskopije, na sobnoj temperaturi u 50 mM fosfatnom puferu na pD = 7,4. Konkurentne reakcije 5'-GMP i GS-Me sa Pt(II) kompleksom (molski odnos 1:1:1) pokazuju da u prvoj fazi Pt(II) kompleks reaguje samo sa GS-Me, dok u sledećem koraku dolazi do veoma spore intermolekulske migracije sa S-koordinovanog tioetarskog liganda na N7 atomom guanina iz 5'-GMP. Dobijeni rezultati ovih ispitivanja su diskutovani u smislu antitumorskog i toksičnog delovanja kompleksa platine.

**Ključne reči:** Platina(II) kompleksi; S-metil-glutation (GS-Me); 5'-guanozin-monofosfat (5'-GMP); intermolekulska migracija.



### ABBREVIATIONS

DNA - deoxyribonucleic acid	KI - potassium iodide
D <sub>2</sub> O - deuterium oxide	KHCO <sub>3</sub> - potassium bicarbonate
HCl - hydrochloric acid	KOH - potassium hydroxide
K <sub>2</sub> [PtCl <sub>4</sub> ] - potassium-tetrachloridoplatinate(II)	NMR - nuclear magnetic resonance



## INTRODUCTION

Due to the clinical success of cisplatin, carboplatin, and more recently oxaliplatin, platinum(II) complexes represents important class of inorganic compounds as well as their mechanism of action during their antitumor activity (1,2). These successes have provided an increasing interest in the interactions of Pt(II) complexes with nitrogen- and sulfur-containing biomolecules which has revealed new and interesting findings, both thermodynamic and kinetic. The interactions of these platinum(II) complexes with sulfur-containing biomolecules are responsible for a variety of biological effects, such as inactivation of platinum(II) antitumor complexes, development of cellular resistance to platinum drugs, and toxic side effects such as nephrotoxicity (3). Additionally, it was found that the thioether-containing amino acid methionine plays an important role in the metabolism of platinum anticancer drugs. Not only does interaction with sulfur-containing protein appear to be important in cell entry of platinum drugs, also targeting of nucleic acids processing with platination of DNA lesions play important roles in their antitumor activity (4-6).

Discovering that *S*-bound thioether ligand can be selectively displaced by one of the nitrogen atoms of the imidazole ring in the histidine side chain opens a chance for designing a new Pt(II) complex for selective covalent modification of proteins. The monofunctional  $[\text{PtCl}(\text{dien})]^+$  complex, with the dien (dien is 1,5-diamino-3-azapentane) acting as a non-removable tridentate ligand, has been shown to be a very useful model for the study of the kinetics and mechanism of the interactions of Pt(II) antitumor complexes with nitrogen- and sulfur-containing biomolecules. Results obtained by the NMR investigation of the competitive binding of the L-methionine (L-Met), *N*-acetylated dipeptide glycyl-L-methionine (Ac-Gly-L-Met) and guanosine-5'-monophosphate (5'-GMP) to the monofunctional  $[\text{PtCl}(\text{dien})]^+$  complex have shown that 5'-GMP selectively displaces Pt-S-Met bound (7, 8). Slow intramolecular displacement of a  $[\text{Pt}(\text{L})]^{2+}$  unit (L is dien, Gly-Gly, or Gly-Met) from the sulfur to the nitrogen atom of imidazole ring in *N*-acetylated dipeptide L-methionyl-L-histidine (Ac-L-Met-His) has been observed (9). In the reaction with  $[\text{PtCl}(\text{dien})]^+$  complex this migration reaction is strongly selective to the N1 atom of the imidazole ring, while with  $[\text{Pt}(\text{Gly-Gly-N,N,O})\text{I}]^+$  complex this migration reaction occurs to the N3 atom of imidazole ring of the histidine side chain. No migration reaction from the sulfur to the either N1 or N3 nitrogen atom of the imidazole was observed for the reaction of Ac-L-Met-His peptide with  $[\text{Pt}(\text{Gly-L-Met-S,N,N})\text{Cl}]$  complex.

In this work we have sought to gain further insight into sulfur-nitrogen intermolecular migration. Thus,  $^1\text{H}$  NMR spectroscopy is applied for investigation of the reaction of the monofunctional Pt(II) complexes,  $[\text{Pt}(\text{Gly-L-Met-S,N,N})\text{Cl}]$  and  $[\text{Pt}(\text{Gly-Gly-N,N,O})\text{I}]^+$ , in which Gly-L-Met is glycyl-L-methionine coordinated through the sulfur and two nitrogen atoms and Gly-Gly is glycylglycine coordinat-

ed through two nitrogen and oxygen atoms, with *S*-methylglutathione (GS-Me) and guanosine 5'-monophosphate (5'-GMP).

## EXPERIMENTAL

### 2.1. Synthesis of Platinum(II) Complex

The complex  $\text{K}[\text{Pt}(\text{Gly-Gly-N,N',O})\text{I}]$  was prepared by a modification of a literature method (9, 10). To  $\text{K}_2[\text{PtCl}_4]$  (0.2076 g,  $5.00 \times 10^{-4}$  mol) dissolved in  $5 \text{ cm}^3$  of water was added 0.3320 g ( $2.00 \times 10^{-4}$  mol) of KI and the mixture was heated at  $60^\circ\text{C}$  for 5 min. Subsequently, an aqueous solution ( $5 \text{ cm}^3$ ) of the peptide glycyl-glycine (0.0660 g, 0.5 mM) was added to the obtained reaction mixture and the heating ( $60^\circ\text{C}$ ) with stirring was continued for 30 min. During this time, the pH of the reaction mixture was controlled every 5 min and adjusted to about 6.5 with 1 M  $\text{KHCO}_3$  solution. The obtained solution was concentrated to  $5 \text{ cm}^3$  under vacuum and then left at room temperature over night. The obtained yellow crystals were filtered off, washed with ethanol and air dried. Yield 0.098 g (40%). Calculated for  $\text{K}[\text{Pt}(\text{Gly-Gly-N,N',O})\text{I}] = \text{C}_4\text{H}_6\text{N}_2\text{O}_3\text{IKPt}$  (FW = 491.18): C, 9.78; N, 5.70; H, 1.23%; found: C, 9.63; N, 5.81; H, 1.34%.  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 200 MHz);  $\delta = 3.99$  (s, 2H,  $\text{CH}_2$ ) and  $\delta = 3.62$  (s, 2H,  $\text{CH}_2$ ).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , 200 MHz);  $\delta = 51.72$  ( $\text{CH}_2$ );  $\delta = 53.37$  ( $\text{CH}_2$ );  $\delta = 182.55$  (C=O) and  $\delta = 194.00$  (COO).

Complex  $[\text{Pt}(\text{Gly-L-Met-S,N,N})\text{Cl}]\cdot\text{H}_2\text{O}$  was prepared by a modification of the method of Freeman et. al (11).  $\text{K}_2[\text{PtCl}_4]$  (207.6 mg, 0.5 mmol) was dissolved in 3 ml of water and to this an aqueous solution of glycyl-L-methionine (103.2 mg, 0.5 mmol) was added. The pH of the solution was adjusted to c. 3.5 by the addition of 1 M KOH and mixture was stirred at  $50^\circ\text{C}$  for 3 h. The yellow solution obtained was cooled to room temperature and then the pH was reduced to c. 2 by the additional of 1 M HCl. The solution was left overnight at room temperature; crystals of  $[\text{Pt}(\text{Gly-L-Met-S,N,N})\text{Cl}]\cdot\text{H}_2\text{O}$  were removed by filtration, washed with a small amount of ethanol, and air-dried. The yield was 136 mg (60%). The pure complex was obtained by recrystallization from a small amount of water and cooling. Calculated for  $[\text{Pt}(\text{Gly-L-Met-S,N,N})\text{Cl}] \cdot \text{H}_2\text{O} = \text{C}_7\text{H}_{15}\text{ClN}_2\text{O}_4\text{PtS}$  (FW = 348.52): C, 18.5; H, 3.3; N, 6.1%; found: C, 18.4; H, 3.3; N, 6.1%.  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 200 MHz);  $\delta = 3.99$  (s, 2H,  $\text{CH}_2$ ),  $\delta = 2.66$  (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , 200 MHz);  $\delta = 51.72$  ( $\text{CH}_2$ );  $\delta = 182.55$  (C=O) and  $\delta = 194.00$  (COO).

### 2.2. pH measurements

All pH measurements were made at room temperature. The pH meter (Mettler Toledo Seven Compact S220-U) was calibrated with Mettler Toledo certified buffer solutions of pH 4.00 and 7.00. The results were converted into pD by the standard formula:  $\text{pD} = \text{pH} + 0.41$  (12). However, in conceptual references to acidity



and basicity, the common symbol pH is used. Elemental microanalyses for carbon, hydrogen, and nitrogen were performed at the Faculty of Chemistry of the University of Belgrade.

### 2.3. $^1\text{H}$ NMR Measurements

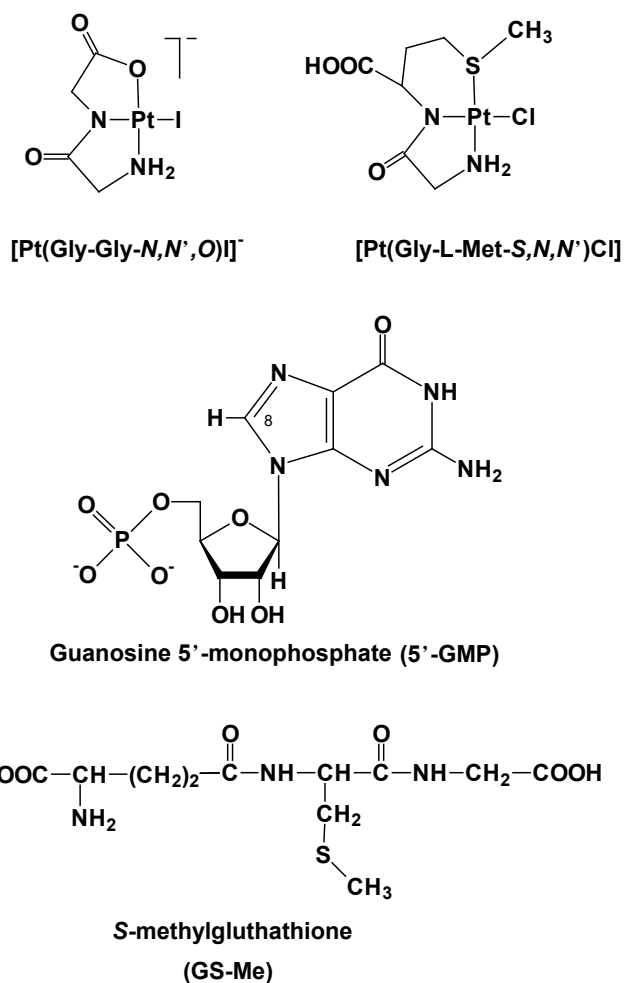
Reactions of peptides with Pt(II) complexes were followed by  $^1\text{H}$  NMR spectroscopy using a Varian Gemini 2000 spectrometer (200 MHz for  $^1\text{H}$  and 500 MHz for  $^{13}\text{C}$ ). Equimolar amounts of the Pt(II) complex and ligands (GS-Me and 5'-GMP) were mixed in an NMR tube. The final solution was 20 mM in each reactant. All reactions were carried out at 298 K and at pD 7.4 in 50 mM phosphate buffer solution prepared in  $\text{D}_2\text{O}$  solvent. The internal reference was TSP (sodium trimethylsilylpropane-3-sulfonate). The kinetic data for the reactions of Pt(II) complexes with thioether-containing ligand (see Table 1) were obtained from  $^1\text{H}$  NMR measurements at 298 K. The values of the rate constants for these reactions were determined when the data from the early part of the reaction (up to 2 h) were fitted to a second-order process (13) by plotting  $x/a_0(a_0-x)$  against  $t$  ( $a_0$  is the initial concentration of the thioether ligand and  $x$  is the concentration of the Pt(II) complex with S-bound thioether ligand at time  $t$ ).

## RESULTS AND DISCUSSION

### Intermolecular Migration of Pt(II) Complexes

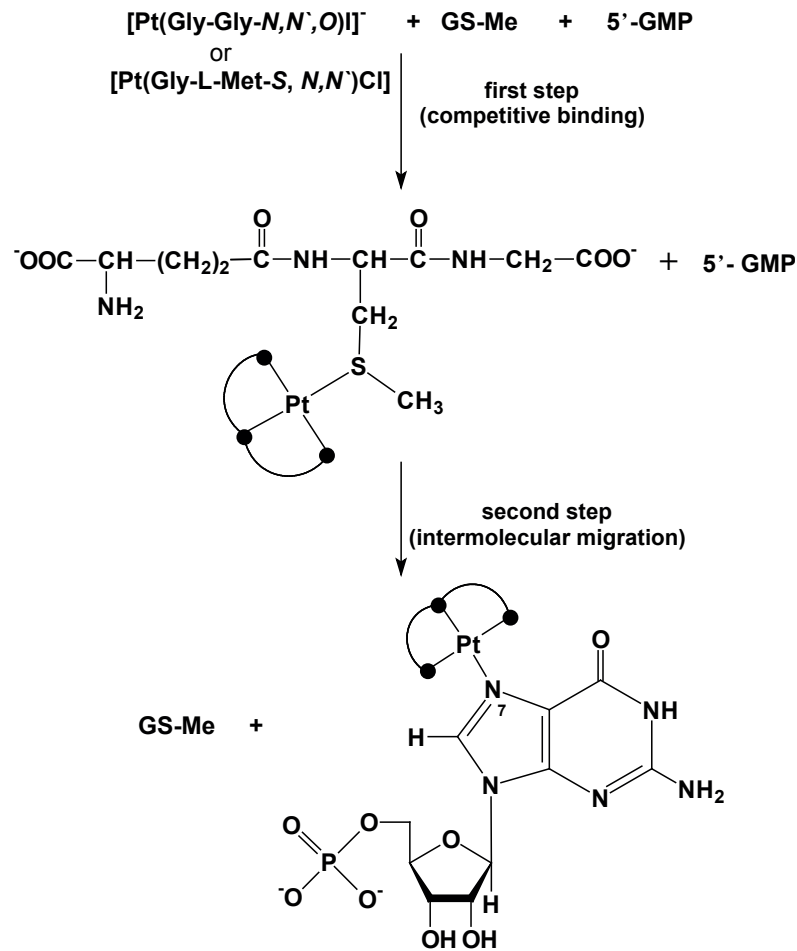
Reactions of monofunctional Pt(II) complexes,  $[\text{Pt}(\text{Gly-L-Met-S},N,N')\text{Cl}]$  and  $[\text{Pt}(\text{Gly-Gly-N},N',\text{O})\text{I}]$ , in which Gly-L-Met is glycyl-L-methionine coordinated through the sulfur and two nitrogen atoms and Gly-Gly is glycylglycine coordinated through two nitrogen and oxygen atoms, with *S*-methylglutathione (GS-Me) and guanosine 5'-monophosphate (5'-GMP) have been studied by  $^1\text{H}$  NMR spectroscopy (Figure 1). All reactions were carried out in equimolar amounts of the reactants in 50 mM phosphate buffer at pD 7.4 and at 25 °C. The formation of the products in these reactions was followed by  $^1\text{H}$  NMR spectroscopic measurements of the chemical shifts of *S*-methyl protons in *S*-methylglutathione and those for H8 proton in guanosine-5'-monophosphate.

The platinum(II) complex was stable under the above mentioned experimental conditions and Gly-Gly and Gly-L-Met ligands stay tridentate coordinated to the Pt(II) during the reaction time. Release of the coordinated dipeptide from the Pt(II) was observed after 34 days. The detachment of the dipeptides from the Pt(II) was observed in the  $^1\text{H}$  NMR spectrum by appearance of two new signals at 3.82 and 3.86 ppm due to methylene protons of the free Gly-Gly and at 2.11 and 3.82 ppm for *S*-CH<sub>3</sub> protons and CH<sub>2</sub> protons of the free Gly-L-Met, respectively. Addition of the free dipeptide to the reaction mixture caused an increase of these two signals in both cases.

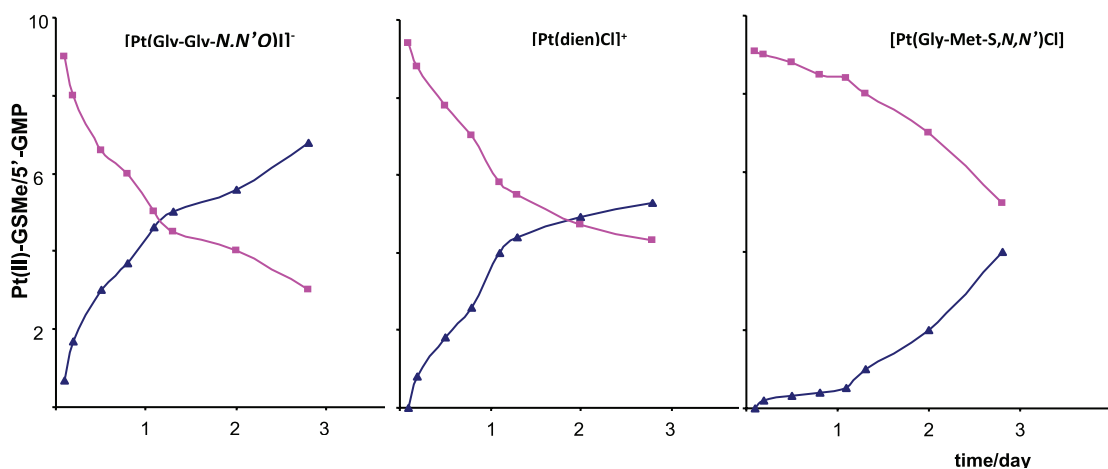


**Figure 1.** The platinum(II) complexes,  $[\text{Pt}(\text{Gly-Gly-N},N',\text{O})\text{I}]$  and  $[\text{Pt}(\text{Gly-L-Met-S},N,N')\text{Cl}]$ , *S*-methylglutathione (GS-Me) and guanosine 5'-monophosphate (5'-GMP) employed in this study.

In the initial stage of the reaction Pt(II) complexes forms a kinetically favored Pt(II)-GS-Me complex with unidentate coordination of GS-Me through the sulfur atom of the *S*-methylglutathione residue (Figure 2 and 3). The singlet for the *S*-methyl protons of the free *S*-methylglutathione at 2.11 ppm was shifted downfield and new resonance at 2.54 ppm for the *S*-bound *S*-methylglutathione (Figure 1 and 4) appeared in the spectrum. This Pt(II)-GS-Me-*S* complex is an intermediate kinetically favored product, which is at the same time thermodynamically labile. The platinum(II)-thioether bond can be cleaved in the presence of a strong nucleophile at physiological pH values. In the second step of the reaction intermolecular displacement of the *S*-bound thioether ligand with the N7 nitrogen atom of the guanine base of 5'-GMP has been observed (Figure 2 and 3). This migration reaction is very slow and strongly selective to the N7 nitrogen atom of the 5'-GMP. The monodentate binding of the platinum(II) to the N7 nitrogen atom of the 5'-GMP was registered from the simultaneous decline of the resonance at 8.17 ppm due



**Figure 2.** Competitive binding of the methionine sulfur atom in *S*-methylgluthathione (GS-Me) and guanosine *N*7 atom in guanosine 5'-monophosphate (5'-GMP) in the reaction with  $[\text{Pt}(\text{Gly-Gly-}N,N',O)]^-$  and  $[\text{Pt}(\text{Gly-L-Met-S},N,N')\text{Cl}]$  complexes. The components were reacted in 1:1:1 molar ratio at pD 7.4 in 50 mM phosphate buffer and at 25 °C.



**Figure 3.** Time dependence of product formation in the reaction of  $[\text{Pt}(\text{Gly-Gly-}N,N',O)]^-$ ,  $[\text{Pt}(\text{dien})\text{Cl}]^+$  and  $[\text{Pt}(\text{Gly-L-Met-S},N,N')\text{Cl}]$  complexes with GS-Me and 5'-GMP (1 : 1 : 1 mol ratio) at pD 7.4 in 50 mM phosphate buffer and at 25 °C: (■) Pt(peptide-S) complex; (▲) Pt(5'-GMP-N7) complex.



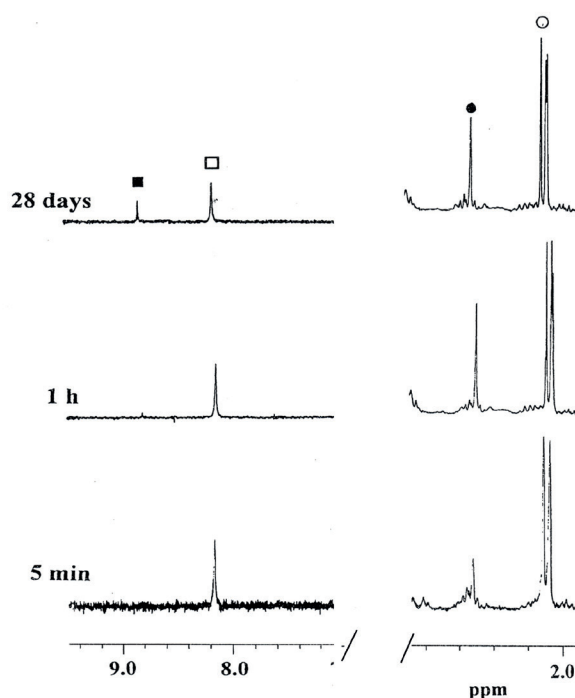


to the H8 proton of the free 5'-GMP and the growth of a resonance at 8.85 ppm, corresponding to the same protons of the 5'-GMP coordinated to platinum(II) (**Figure 4**). These chemical shifts are in accordance with those previously reported for the reactions of platinum(II) complexes with 5'-GMP (14).

The above-mentioned findings are in accordance to those previously reported for the selective intramolecular migration of  $[\text{Pt}(\text{dien})\text{Cl}]^+$  complex (dien is diethylenetriamine) from the methionine sulfur to the imidazole N1 atom of the *N*-acetylated L-methionyl-L-histidine (Ac-L-Met-His) (8), as well as to the N7 nitrogen atom of guanosine-5'-monophosphate (14). Also, it was found that this migration reaction is very slow and strongly selective to the N1 atom of the imidazole ring of the histidine side chain. On the other hand, no migration of the Pt(II) complex was observed in the reaction between  $[\text{Pt}(\text{Gly-L-Met-S,N,N}')\text{Cl}]$  and Ac-L-Met-His dipeptide (8). It was explained by the fact that this complex, with a more sterically hindered Gly-L-Met ligand dipeptide, reacts more slowly with thioether-containing molecules than other two Pt(II) complexes and forms a more stable Pt(II)-sulfur bond (**Table 1**) (7, 8, 15).

The present investigation shows that  $[\text{Pt}(\text{Gly-Gly-N,N',O})\text{I}]^-$  complex is more reactive with the methionine sulfur atom from the *S*-methylglutathione and undergo intramolecular migration to the N7 nitrogen atom in guanosine-5'-monophosphate than the  $[\text{Pt}(\text{dien})\text{Cl}]^+$  complex (**Table 1** and **Figure 3**). The reaction of  $[\text{Pt}(\text{Gly-Gly-N,N',O})\text{I}]^-$  with *S*-methylglutathione was much faster than with the corresponding  $[\text{Pt}(\text{Gly-L-Met-S,N,N}')\text{Cl}]$  complex. Time dependence of the product formation in these reactions shows that, after 30 days of migration reaction, about 70% of  $[\text{Pt}(\text{Gly-Gly-N,N',O})(5'\text{-GMP-N7})]$  complex is present in the reaction mixture, while with  $[\text{Pt}(\text{Gly-L-Met-S,N,N}')\text{Cl}]$ , approximately 40% of the  $[\text{Pt}(\text{Gly-L-Met-S,N,N'})(5'\text{-GMP-N7})]$  complex was formed (**Table 1** and **Figure 3**).

The highest rate constant for  $[\text{Pt}(\text{Gly-Gly-N,N',O})\text{I}]^-$  in comparison with those of other platinum(II) complexes observed for reactions with sulfur-containing donors (**Table 1**) and the very rapid intramolecular migration of this complex in the reaction with guanosine-5'-monophosphate can be attributed to the *trans*-effect of the deprotonated peptide nitrogen, as well as to the electronegative oxygen atom in the coordination sphere of the investigated Pt(II) complex. These factors contribute to



**Figure 4.** Part of the  $^1\text{H}$  NMR spectra for the reaction of the  $[\text{Pt}(\text{Gly-Gly-N,N',O})\text{I}]^-$  complex with GS-Me and 5'-GMP (1 : 1 : 1 molar ratio) as a function of time. Chemical shifts are labeled for 5'-GMP bound (■) and free (□) as well as for GS-Me bound (●) and free (○).

the weakness of the Pt-I or Pt-S-CH<sub>3</sub> bond and together with the large size of the iodido ligand could be considered for explanation of the fast reactivity of  $[\text{Pt}(\text{Gly-Gly-N,N',O})\text{I}]^-$  with the 5'-GMP.

## CONCLUSION

The present study related the competitive binding of the sulfur- and nitrogen-containing biomolecules, *S*-methylglutathione and guanosine-5'-monophosphate, to the  $[\text{Pt}(\text{Gly-Gly-N,N',O})\text{I}]^-$  and  $[\text{Pt}(\text{Gly-L-Met-S,N,N}')\text{Cl}]$  complexes shows that in the initial stages of the reaction the Pt(II) complex only reacts with *S*-methylglutathione forming kinetically favored product. In the second step

**Table 1.** Kinetic data for the reactions of Pt(II) complexes with thioether-containing ligands at room temperature, where  $k_2$  is the second-order rate constant

Reactants [complex + ligand]	pD value	$10^3 k_2 / \text{M}^{-1}\text{s}^{-1}$	Ref.
$[\text{Pt}(\text{dien})\text{Cl}]^+ + \text{L-Met}$	4.31	14	7
$[\text{Pt}(\text{dien})\text{Cl}]^+ + \text{S-methylglutathione}$	5.41	33	15
$[\text{Pt}(\text{dien})\text{Cl}]^+ + \text{Ac-L-Met-His}$	4.40	44	8
$[\text{Pt}(\text{Gly-Met-S,N,N}')\text{Cl}] + \text{L-Met}$	4.31	4.5	8
$[\text{Pt}(\text{Gly-Met-S,N,N}')\text{Cl}] + \text{S-methylglutathione}$	4.34	0.3	This work
$[\text{Pt}(\text{Gly-Met-S,N,N}')\text{Cl}] + \text{Ac-L-Met-His}$	4.40	8	9
$[\text{Pt}(\text{Gly-Gly-N,N',O})\text{I}]^- + \text{S-methylglutathione}$	4.21	40	This work
$[\text{Pt}(\text{Gly-Gly-N,N',O})\text{I}]^- + \text{Ac-L-Met-His}$	4.11	70	9



of the reaction very slow intermolecular displacement of the *S*-bound thioether ligand with the N7 atom of the guanine base of guanosine-5'-monophosphate is observed. Thiols, such as glutathione, are abundant sulfur containing ligands in cells and are responsible for inactivation of Pt(II) complexes, as well as numerous toxic side effects. The obtained results for intermolecular migration of the thioether-bound platinum(II) complex to the N7 nitrogen atom of guanosine-5'-monophosphate could support the hypothesis that Pt(II) initially bound to a protein side chain may further react with nitrogen atom in DNA and therefore act as some kind of drug reservoir.

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