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Kinetic Studies on the Reactions of Different Bifunctional Platinum(II) Complexes with Selected Nucleophiles

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Substitution reactions of the complexes *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(SMC)Cl_2]^-$, $[Pt(en)Cl_2]$, and $[Pt(dach)Cl_2]$, where SMC = *S*-methyl-L-cysteine, en = ethylenediamine and dach = 1,2-diaminocyclohexane, with selected biologically important ligands, viz. guanosine-5′-monophosphate (5′-GMP), L-histidine and 1,2,4-triazole, were studied. All reactions were studied in aqueous 25 mM Hepes buffer in the presence of 5 mM NaCl at pH = 7.2 under pseudo-first-order conditions as a function of concentration at 310 K by using UV/Vis spectrophotometry. Two consecutive reaction steps, which both depend on the nucleophile concentration, were observed in all cases. The second-order rate constants for both reaction steps indicate a decrease in the order $[Pt(SMC)Cl_2]^- > cis$-$[Pt(NH_3)_2Cl_2] > [Pt(en)Cl_2] > [Pt(dach)Cl_2]$. DFT calculations (B3LYP/LANL2DZp) showed that the Pt–N7(Guo) adduct is more stable than the Pt–S(thioether) adduct for the studied complexes *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(SMC)Cl_2]^-$, and $[Pt(en)Cl_2]$. The calculations collectively support the experimentally observed substitution of thioethers bound to Pt^{II} complexes by N7(5′-GMP). Finally, this result could be the first to clearly show how much the Pt–N7(Gua) adduct is more stable than the Pt–S(thioether).

Introduction

Transition metals and their reactions are in general important in the environment, in technical processes (catalysis, extraction and purification of metal complexes) and in biology and medicine (biological electron transfer, toxicology and use of metal complexes as drugs). Moreover, nonessential metal ions are very often used in biological systems either for therapeutic application or as diagnostic aids. For instance, metal complexes have been used for the treatment of many diseases (cancer, arthritis, diabetes, Alzheimer's, etc.), but with little understanding of their mechanism of action in biological systems.[1,2] Biochemical studies have not clearly established the molecular basis for the activity and mechanism of action. The growing field of bioinorganic chemistry is presently dealing with the clarification of the mechanisms of action of metal complexes in biological systems.[1–3]

The discovery of the antitumor complex cisplatin in the late 1960s initiated extensive investigations of platinum compounds.[4] The success of cisplatin has aroused much interest in the development of new Pt^{II} complexes, such that today carboplatin and oxaliplatin are extensively used as anticancer drugs. However, many other platinum drugs have been developed to improve on cisplatin.[5–7]

Today it is generally accepted that the antitumor activity of platinum drugs can be ascribed to interactions between the metal complex and DNA.[5–9] There are many other potential biomolecules that can also react with the Pt^{II} complexes, such as small molecules, proteins and enzymes.[9] Sulfur-containing biomolecules have a high affinity for platinum. However, these interactions have been associated with negative phenomena such as nephrotoxicity, gastrointestinal toxicity and neurotoxicity.[5–9]

At present it is not clear how the Pt^{II} species reach the DNA, because Pt^{II} has a high affinity for binding to sulfur donors that compete with nitrogen donor ligands such as DNA bases.[5,10] Intracellular concentrations of mercapto groups could be as high as 10 mM. A conventional hypothesis is that sulfur-containing nucleophiles initially bind to the platinum center and then convert to platinum–DNA complexes, thermodynamically more stable products. Model studies under physiologically relevant conditions have conclusively shown that the kinetic preference of Pt^{II} is for biorelevant thiols (cysteine, glutathione) rather than for 5′-GMP.[10,15] Methionine bound to platinum may be replaced by thiols or nucleobases,[14–18] whereas the Pt–cysteine bond is considered to be kinetically more inert.[10,16] Pt–sulfur adducts have been postulated to be a drug reservoir for platinum at DNA and may act as intermediates of platinum compounds and transform them into Pt–DNA adducts.[11,12] However, platinum drugs could be deactivated

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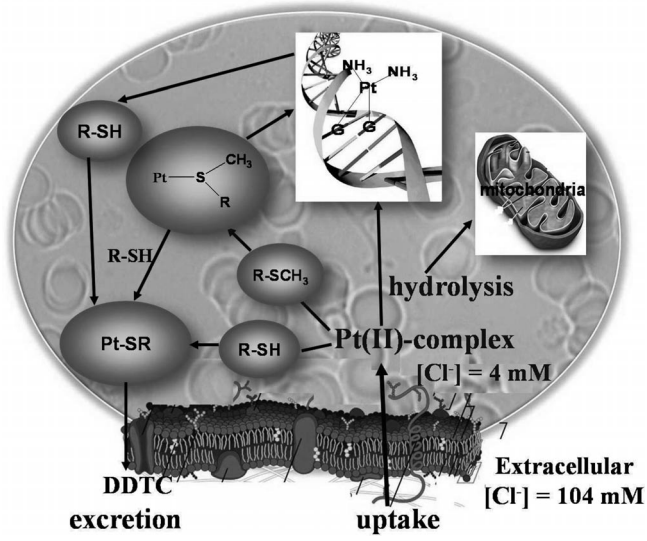
in reactions with cysteine-rich proteins like glutathione.[12] Moreover, these proteins could substitute coordinated bases of DNA from Pt–N7(DNA) adducts and also thioethers from Pt–S(thioether) products to form very stable Pt–S-(thiolate) bonds.[11–14] Therefore, several sulfur-containing compounds, such as diethyldithiocarbamate (ddtc), sodium thiosulfate (sts), thiourea (tu), and glutathione (GSH), considered as so-called rescue or protective agents, have been developed for use in co-administration with the aim to modulate the formation of very stable Pt–S(thiolate) bonds (see Scheme 1).[10]



Scheme 1. Schematic presentation of the levels of action of cisplatin and possible biological consequences.

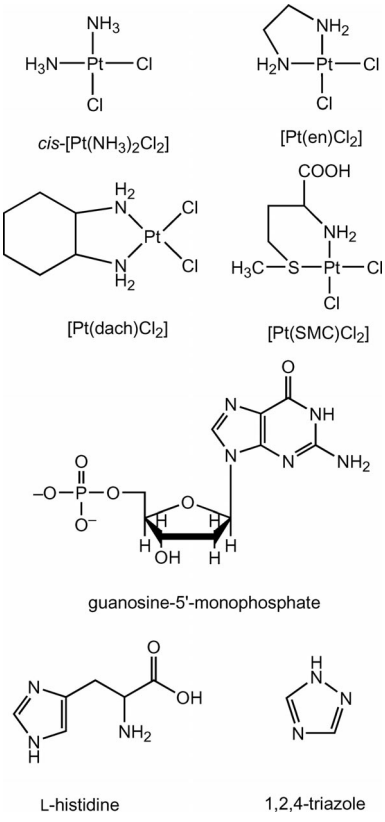


Figure 1. Structures of the studied complexes and ligands.

With the aim to extend our earlier work of the interactions of Pt^{II} complexes with N-bonding ligands,[16,18,19] we investigated the complex-formation kinetics of *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(SMC)Cl_2]^-$, $[Pt(en)Cl_2]$, and $[Pt(dach)Cl_2]$ with selected biologically important ligands such as guanosine-5′-monophosphate (5′-GMP), L-histidine and 1,2,4-triazole. This set of nucleophiles was selected because of their difference in nucleophilicity, steric hindrance, binding properties and biological relevance (structures are shown in Figure 1).

Results and Discussion

Reactions with Nitrogen Donor Nucleophiles

The kinetics of the substitution reactions of four different mononuclear Pt^{II} complexes, viz. *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(SMC)Cl_2]^-$ (in the deprotonated form), $[Pt(en)Cl_2]$, and $[Pt(dach)Cl_2]$, were investigated under physiological conditions at 310 K and pH = 7.2 in Hepes buffer. Hepes and Tris, tris(hydroxymethyl)aminomethane, buffers are usually used in cell tests and DNA binding studies of Pt^{II} drugs. Hepes buffer was selected because it is sterically more crowded then Tris and does not coordinate to Pt^{II} as Tris does.[20] The observed time trace at suitable wavelengths was fitted to a two-exponential function in which the amplitudes have opposite signs as shown in Figures 2 and S1 (Supporting Information). The substitution reactions we studied in the presence of 5 mM chloride to be close to the conditions in the cell where the concentration is ca. 4 mM.

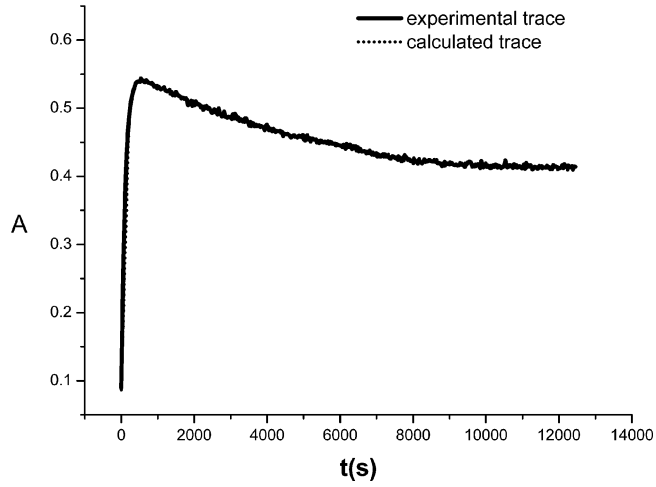


Figure 2. Absorbance-time trace recorded for the reaction of $[Pt(en)Cl_2]$ with 1,2,4-triazole (1.6×10^{-2} M) at 222 nm, T = 310 K, 25 mM Hepes buffer, pH = 7.2 and 5 mM NaCl.

The reactions of the studied complexes with the selected nucleophiles occur in two subsequent steps. Both substitution reactions are reversible and proceed according to the reactions given in Equations (1) and (2). The observed rate

constants for two reactions can be expressed as given in Equation (3). Direct nucleophilic attack is characterized by the rate constants k_2 and k_4, and the reverse reactions are presented by the rate constants k_1 and k_3.

$$[Pt(L)Cl_2] + Nu \underset{k_1}{\overset{k_2}{\rightleftharpoons}} [Pt(L)(Nu)Cl]^+ + Cl^- \quad (1)$$

$$[Pt(L)(Nu)(Cl)]^+ + Nu \underset{k_3}{\overset{k_4}{\rightleftharpoons}} [Pt(L)(Nu)_2]^{2+} + Cl^- \quad (2)$$

$$k_{obsd1} = k_1 + k_2[Nu]; \; k_{obsd2} = k_3 + k_4[Nu] \quad (3)$$

L = dach, en, SMC, $(NH_3)_2$; Nu = 5′-GMP, L-histidine, 1,2,4-triazole

The plots in Figures 3 and S2–S4 (Supporting Information) indicate that both reaction steps exhibit a linear dependence of k_{obsd} on the nucleophile concentration and that meaningful intercepts are observed in all cases.

Nitrogen donor ligands such as 5′-GMP, L-histidine, and 1,2,4-triazole were used in this investigation. The reactions with all nucleophiles revealed two substitution steps. In the first step the nucleophile substitutes one chloride ion, and in the second step the other chloride ion is substituted as shown in Equations (1) and (2). It is known that 5′-GMP can coordinate to metal ions through the N1 and N7 positions, but binding through the N7 position in a neutral or weakly acidic medium has been verified.[21] The pK_a value for 1,2,4-triazole is 2.30,[22] so at a pH of 7.2 it is deprotonated and can act as a good nucleophile. 1,2,4-Triazole can coordinate to the Pt^{II} ion through N1, N2 or N4.[23] The heterocyclic imidazole system in L-histidine forms a bidentate ligand with two competitive donor atoms N1 and N3. In biological systems there are numerous metallo-proteins in which a metal ion is bound to a histidine imidazole through N1 or N3.[24,25]

On the basis of the data reported in Table 1, the most reactive N-donor nucleophile is 1,2,4-triazole. L-Histidine has the same order of reactivity as 5′-GMP and is only slightly faster than 5′-GMP. The difference in the reactivity

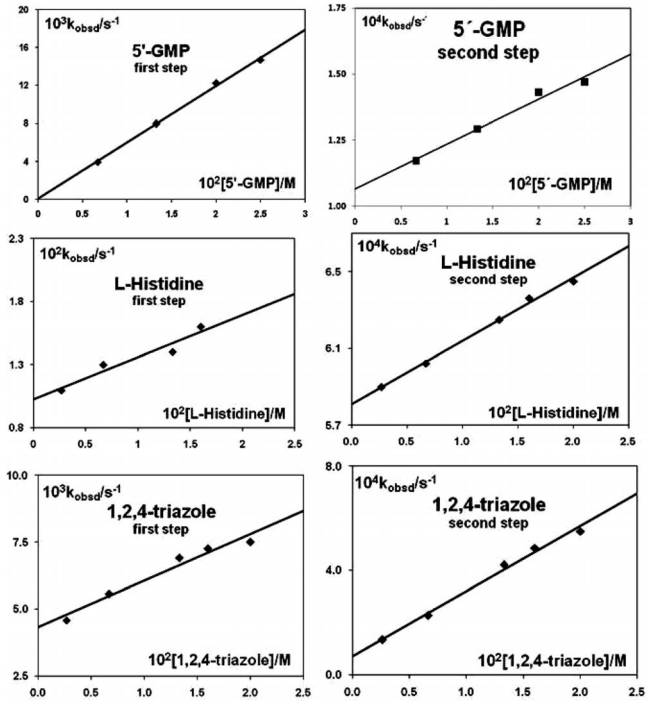


Figure 3. Pseudo-first-order rate constants as a function of nucleophile concentration for the first and second steps of the substitution reactions of $[Pt(SMC)Cl_2]^-$ at T = 310 K, 25 mM Hepes buffer, pH = 7.2 and 5 mM NaCl.

of these nucleophiles can be accounted for in terms of electronic and steric effects. 5′-GMP is sterically more crowded than L-histidine, and that can be the reason why the reactions with 5′-GMP are a bit slower.

The kinetic data in Table 1 clearly show that these nitrogen donor nucleophiles are very good entering ligands for Pt^{II} complexes. From a comparison of the values of the second-order rate constants for the first reaction step, k_2, it can be concluded that the order of reactivity is $[Pt(SMC)Cl_2]^- > cis\text{-}[Pt(NH_3)_2Cl_2] > [Pt(en)Cl_2] > [Pt(dach)Cl_2]$. The high reactivity of $[Pt(SMC)Cl_2]^-$ can be attributed to the strong *trans*-labilization effect of the coordinated sulfur

Table 1. Rate constants for the studied substitution reactions at T = 310 K, 25 mM Hepes buffer, pH = 7.2 and 5 mM NaCl.

	First step 5′-GMP $10^3 k_2$ $M^{-1} s^{-1}$	$10^4 k_1$ s^{-1}	L-Histidine $10^3 k_2$ $M^{-1} s^{-1}$	$10^4 k_1$ s^{-1}	1,2,4-Triazole $10^2 k_2$ $M^{-1} s^{-1}$	$10^3 k_1$ s^{-1}
$[Pt(dach)Cl_2]$	2.2 ± 0.1	3.3 ± 1	6.4 ± 0.2	2.6 ± 0.1	5.9 ± 0.1	1.2 ± 0.1
$[Pt(en)Cl_2]$	4.4 ± 0.3	0.30 ± 0.04	7.9 ± 0.7	1.8 ± 0.1	9.9 ± 0.1	7.3 ± 0.1
$[Pt(SMC)Cl_2]^-$	593 ± 4	0.70 ± 0.08	352 ± 6	99 ± 1	454 ± 2	3.0 ± 0.2
cis-$[Pt(NH_3)_2Cl_2]$	/	/	8.0 ± 0.3	4.5 ± 0.4	12.0 ± 0.4	7.4 ± 0.5
	Second step 5′-GMP $10^4 k_4$ $M^{-1} s^{-1}$	$10^6 k_3$ s^{-1}	L-Histidine $10^4 k_4$ $M^{-1} s^{-1}$	$10^6 k_3$ s^{-1}	1,2,4-Triazole $10^3 k_4$ $M^{-1} s^{-1}$	$10^4 k_3$ s^{-1}
$[Pt(dach)Cl_2]$	2.0 ± 0.1	0.8 ± 0.1	4.8 ± 0.4	2.2 ± 0.6	6.4 ± 0.5	1.0 ± 0.6
$[Pt(en)Cl_2]$	3.0 ± 0.2	35 ± 3	11 ± 1	76 ± 1	11 ± 1	1.1 ± 0.1
$[Pt(SMC)Cl_2]^-$	17 ± 2	11 ± 3	33 ± 1	58 ± 2	24 ± 1	0.7 ± 0.01
cis-$[Pt(NH_3)_2Cl_2]$	/	/	11 ± 1	20 ± 1	12.8 ± 0.2	8.1 ± 0.2

atom from the *S*-methyl-L-cysteine chelate. Such labilization has clearly been illustrated by an earlier study.[26] The reactivity of the complexes *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(en)Cl_2]$ and $[Pt(dach)Cl_2]$, depends on steric effects. The $[Pt(dach)Cl_2]$ complex is the sterically most crowded one, and the reactions are found to be slower than those with $[Pt(en)Cl_2]$ and *cis*-$[Pt(NH_3)_2Cl_2]$. The reactions with $[Pt(dach)Cl_2]$ were expected to be slower than those with $[Pt(en)Cl_2]$, as the Pt^{II} center should be less electrophilic because of the positive inductive effect of the cyclohexane ring.[27]

The reactions for the second step are significantly slower than for the first step in all cases (see Table 1). The order of reactivity for the second step is: $[Pt(SMC)Cl_2]^- >$ *cis*-$[Pt(NH_3)_2Cl_2] > [Pt(en)Cl_2] > [Pt(dach)Cl_2]$. The difference in reactivity between $[Pt(SMC)Cl_2]^-$ and the other complexes is less than in the case of the first reaction step. The slower reactions of $[Pt(SMC)Cl_2]^-$ are assigned to the displacement of the second Cl^- ion *trans* to the nitrogen donor of coordinated *S*-methyl-L-cysteine and *cis* to the sulfur atom.

In an earlier study,[18] the kinetics and mechanism of ligand-substitution reactions of $[PtCl_2(SMC)]$ with biologically relevant ligands were studied as a function of chloride and nucleophile concentration at pH = 2.5 and 7.2. It was observed that the slope and intercept obtained from the linear dependence of the observed rate constant on the nucleophile concentration strongly depended on $[Cl^-]$ for all studied substitution reactions. At higher $[Cl^-]$, the rate constant for the forward reaction is almost zero, indicating that addition of excess chloride almost completely suppresses the displacement of chloride by the entering nucleophile. The obtained rate constant for the reaction with 5′-GMP agreed well with the previously published value.[18] In a recent study[28] of the substitution reactions of $[Pt(dach)Cl_2]$ and $[Pt(en)Cl_2]$ with 5′-GMP and L-His in the presence of 10 mM NaCl, the obtained values of the second-order rate constants for the reaction with L-His are almost identical $\{10.5 \times 10^{-3}\ M^{-1}\,s^{-1}$ for $[Pt(en)Cl_2]$ and $6.4 \times 10^{-3}\ M^{-1}\,s^{-1}$ for $[Pt(dach)Cl_2]\}$, and for 5′-GMP the constants are even less than in the present study. From these trends we conclude that a concentration of 5 mM NaCl should be sufficient to prevent the solvolysis step.

The aqua complex, $[Pt(SMC)(H_2O)_2]^+$, also reacts with 5′-GMP in two reactions steps. The second-order rate constant for the first reaction step 22.44 $M^{-1}\,s^{-1}$, and for the second step 0.24 $M^{-1}\,s^{-1}$.[26] Also the complex-formation reactions of the aqua complexes, $[Pt(en)(H_2O)_2]^{2+}$ and $[Pt(dach)(H_2O)_2]^{2+}$, were studied for 5′-GMP. The second-order rate constant for the first reaction step for $[Pt(en)(H_2O)_2]^{2+}$ is 3.9 $M^{-1}\,s^{-1}$ and for $[Pt(dach)(H_2O)_2]^{2+}$ is 5.8 $M^{-1}\,s^{-1}$.[27] In all cases the aqua complexes were found to be more reactive than the chlorido complexes.

The effect of the leaving group on the reactivity of [Pt(dach)(CBDCA)], [Pt(dach)(*N,S*-methionine)], and [Pt(dach)(gly)] was studied for different nucleophiles including 5′-GMP.[19] On comparing the values for the second-order rate constants for the first reaction step for the reaction with 5′-GMP it is clear that $[Pt(dach)Cl_2]$ is the most reactive $(2.2 \times 10^{-3}\ M^{-1}\,s^{-1})$ compared to [Pt(dach)(CBDCA)] $(0.352 \times 10^{-3}\ M^{-1}\,s^{-1})$ and [Pt(dach)(gly)] (1.77 $10^{-3}\ M^{-1}\,s^{-1}$). It follows that the nature of the chelate, viz. O–O (CBDCA), N–O (glycine) or S–N (l-Met) plays an important role in tuning the kinetic behavior of the Pt^{II} complexes.

DFT Calculations for Gua/SMe_2 Exchange

Transformation from Pt–S(thioether) to Pt–N7(GMP) coordination seems to be common in biological processes.[10–15] We performed quantum chemical calculations to gain more insight into this process. The applied level (B3LYP/LANL2DZp) shows, as expected, a good geometrical correlation between calculated and published experimental structures, as exemplarily shown for the calculated $[Pt(dach)(N7\text{-}Gua)_2]^{2+}$ structure (Figure 4) and related published X-ray data. Whereas the Pt–N bonds and angles show only small deviations throughout Table 2, the experi-

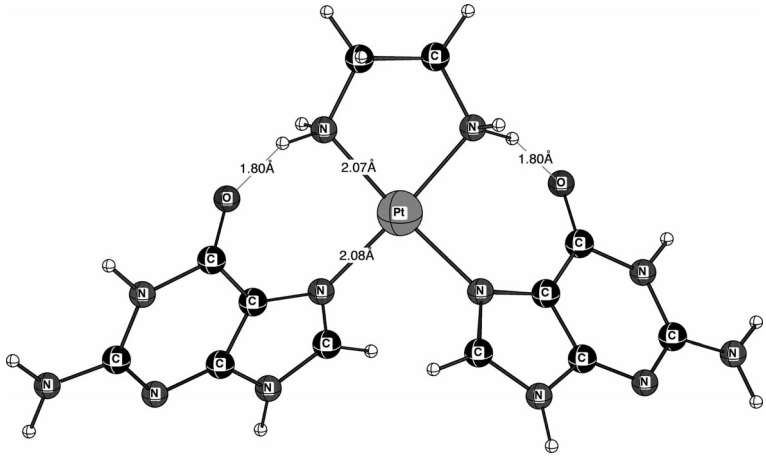


Figure 4. Calculated (B3LYP/LANL2DZp) C_2-symmetrical structure of $[Pt(en)(Gua)_2]^{2+}$ with antiparallel Gua moieties.

Table 2. Selected structural data from calculated (B3LYP/LANL2DZp) geometries and X-ray data for Pt(en) complexes.

	Pt–N_L [Å]	Pt–$N_{GMP\text{-}like}$ [Å]	N_L–Pt–$N_{GMP\text{-}like}$ [°]	N_L–Pt–N_L [°]	$N_{GMP\text{-}like}$–Pt–$N_{GMP\text{-}like}$ [°]
$[Pt(dach)(N7\text{-}Gua)_2]^{2+}$	2.07, 2.07	2.08, 2.08	93.9, 93.4	82.7	90,7
$[Pt(en)(N7\text{-}Gua)_2]^{2+}$	2.07, 2.07	2.08, 2.08	93.3, 93.3	82.8	90.8
$[Pt(en)(N7\text{-}Gua)_2]^{2+}$[29]	2.04, 2.04	1.97, 1.97	94.6, 94.6	83.9	87.0
$[Pt(en)(5'GMP\text{-}N7)_2]$[30]	2.04, 2.04	2.05, 2.05	94.4, 94.4	82.3	89.1
$[Pt(en)(1,3,9\text{-}TMX)]^{2+}$[31]	2.03, 2.03	2.02, 2.02	95.5, 91.7	83.8	89.1
$[Pt(en)(acv)_2]^{2+}$[32]	2.05, 2.00	2.01, 2.03	94.8, 92.6	82.7	90.1
$[Pt(en)(N7\text{-}acv)_2]^{2+}$[33]	2.03, 2.02	2.04, 2.02	95.4	83.4	89.4

mental Pt–N(GMP) bond is 0.1 Å too short compared to the other X-ray data and the calculations. We therefore suggest that this difference could be due to methodic difficulties in X-ray structure determinations 35 years ago.

The Gua moiety in $[Pt(L)(Gua)_2]^{2+}$ can be arranged either parallel or antiparallel with C_2 symmetry. The parallel arrangement in $[Pt(NH_3)_2(Gua)_2]^{2+}$ is only a bit more stable in the gas phase (2.7 kcal/mol) and in CPCM (0.6 kcal/mol) and is best addressed as thermoneutral. In contrast, all the X-ray structures show the Gua moieties throughout to be arranged antiparallel, which can be clearly attributed to the bulky side chains of the GMP-like ligands. The data in Table 2 are for the antiparallel arranged conformer, to be congruent with the experimental values.

To obtain more quantitative data for the difference in stability between Pt–DNA and Pt–S(thioether) adducts, DFT calculations were performed on a model reaction [Equation (4)] [L: $(NH_3)_2$, ethylenediamine, (1*R*,2*R*)-*trans*-1,2-diaminocyclohexane], where guanine approximates the guanosine base interactions and $S(CH_3)_2$ represents a generic thioether. The calculations were performed for both substitution steps. Coordinates of the calculated structures are reported in Table S23 (Supporting Information).

$$[Pt(L)(N7\text{-}Gua)_2]^{2+} + SR_2 \rightarrow [Pt(L)(SR_2)(N7\text{-}Gua)]^{2+} + Gua$$
$$[Pt(L)(SR_2)(N7\text{-}Gua)]^{2+} + SR_2 \rightarrow [Pt(L)(SR_2)_2]^{2+} + Gua \quad (4)$$

In all cases guanine coordination to the fragments $Pt(NH_3)_2$, Pt(en) and Pt(dach) is much more favored than thioether coordination. For the first step in the gas phase Pt–N7(Gua) is more stable than Pt–S(thioether) by ca. 31–33 kcal/mol, and for the second step by 32–34 kcal/mol. The complex $[Pt(NH_3)_2(Gua)_2]^{2+}$ has two H-bonds between Gua C=O and NH_3 of 1.87 Å, $[Pt(dach)(Gua)_2]^{2+}$ has two H-bonds between Gua C=O and dach of 1.83 Å, $[Pt(dach)(Gua)(SMe_2)]^{2+}$ has one H-bond between Gua C=O and dach of 1.90 Å, and $[Pt(en)(Gua)_2]^{2+}$ has two H-bonds between Gua C=O and dach of 1.80 Å.

To evaluate the bulk solvent effects, single-point CPCM calculations [B3LYP(CPCM)/LANL2DZp//B3LYP/LANL2DZp] were performed. As shown in Table 3, the DFT-calculated energy is lowered to less than 50% although guanine coordination is still clearly favored. Finally, this result could be the first to clearly show how much the Pt–N7(Gua) adduct is more stable than the Pt–S(thioether) adduct. This is important since Pt–S(thioether) adducts have been postulated to be a drug reservoir for the binding

Table 3. Energy calculations in kcal/mol for Gua/SMe_2 exchange on $[Pt(NH_3)_2(Gua)_2]^{2+}$, $[Pt(en)(Gua)_2]^{2+}$ and $[Pt(dach)(Gua)_2]^{2+}$ according to Equation (4).

Method			
	$[Pt(NH_3)_2(Gua)_2]^{2+} + SMe_2$	→	$[Pt(NH_3)_2(Gua)(SMe_2)]^{2+} + Gua$
Gas phase	0.0		+31.6
CPCM	0.0		+10.5
	$[Pt(NH_3)_2(Gua)(SMe_2)]^{2+} + SMe_2$	→	$[Pt(NH_3)_2(SMe_2)_2]^{2+} + Gua$
Gas phase	0.0		+33.5
CPCM	0.0		+13.9
	$[Pt(dach)(Gua)_2]^{2+} + SMe_2$	→	$[Pt(dach)(Gua)(SMe_2)]^{2+} + Gua$
Gas phase	0.0		+31.2
CPCM	0.0		+12.2
	$[Pt(dach)(Gua)(SMe_2)]^{2+} + SMe_2$	→	$[Pt(dach)(SMe_2)^2]^{2+} + Gua$
Gas phase	0.0		+32.2
CPCM	0.0		+11.9
	$[Pt(en)(Gua)_2]^{2+} + SMe_2$	→	$[Pt(en)(Gua)(SMe_2)]^{2+} + Gua$
Gas phase	0.0		+32.8
CPCM	0.0		+13.0
	$[Pt(en)(Gua)(SMe_2)]^{2+} + SMe_2$	→	$[Pt(en)(SMe_2)_2]^{2+} + Gua$
Gas phase	0.0		+34.0
CPCM	0.0		+12.5

of platinum to DNA, which may act as intermediates and then be transformed into Pt–N7(Gua) adduct.[8–12]

Conclusions

On the basis of the data presented in this report, we conclude that the studied Pt^{II} complexes have a high affinity for the studied N-bonding nucleophiles of which 1,2,4-triazole is a better nucleophile than 5′-GMP and L-histidine. The reactivity of the complexes is in the order: $[Pt(SMC)Cl_2]^-$ > *cis*-$[Pt(NH_3)_2Cl_2]$ > $[Pt(en)Cl_2]$ > $[Pt(dach)Cl_2]$. DFT calculations (B3LYP/LANL2DZp) for the complexes *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(en)Cl_2]$ and $[Pt(dach)Cl_2]$ showed that the Pt–N7(Gua) adduct is more stable than the Pt–S(thioether) adduct. For the first step in the gas phase Pt–N7(Gua) is more stable than Pt–S(thioether) by ca. 31–33 kcal/mol, and for the second step by 32–34 kcal/mol. The calculations collectively support the experimentally observed substitution of thioethers bound to Pt^{II} complexes by N7(5′-GMP).

Experimental Section

Chemicals and Solutions: The ligands, guanosine-5′-monophosphate sodium salt, L-histidine, 1,2,4-triazole, *S*-methyl-L-cysteine, ethylenediamine and 1,2-diaminocyclohexane were obtained from Fluka, Acros Organics or Sigma and were used without further purification. Potassium tetrachloridoplatinate (K_2PtCl_4) was purchased from Strem Chemicals. Hepes buffer {2-[4-(2-hydroxyethyl)-piperazin-1-yl]ethanesulfonic acid} was obtained from Aldrich, D_2O (Deutero GmbH, 99.9%) is commercially available, and both were used as received. All the other chemicals were of the highest purity commercially available and were used without further purification. Ultrapure water was used in all experiments. Nucleophile stock solutions were prepared shortly before use by dissolving the chemicals. The complexes $[Pt(SMC)Cl_2]$ (isolated in the protonated form from 0.1 M HCl), $[Pt(en)Cl_2]$ and $[Pt(dach)Cl_2]$ were prepared according to published procedures.[34–36] Elemental analysis, ^1H NMR and UV/Vis spectra of these complexes were in good agreement with the previously obtained data. Cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, was purchased from Aldrich.

Instrumentation and Measurements: ^1H NMR measurements were performed with a Varian Gemini 200 MHz NMR spectrometer. UV/Vis spectra and kinetic traces were recorded with a Perkin–Elmer Lamda 35 double-beam spectrophotometer in thermostatted 1.00 cm quartz Suprasil cells. The temperature was controlled to ± 0.1 °C. The pH of the solution was measured by using a Mettler Delta 350 digital pH meter with a combined glass electrode. This electrode was calibrated by using standard buffer solutions of pH = 4, 7 and 9 obtained from Sigma.

Kinetics Measurements: The kinetics of the substitution of coordinated chloride or water was followed spectrophotometrically by monitoring the change in absorbance at suitable wavelengths as a function of time. The working wavelengths were determined by recording spectra of the reaction mixture over the wavelength range 220 to 450 nm. All kinetic experiments were performed under pseudo-first-order conditions, for which the concentration of the nucleophile was always in at least a 20-fold excess. The reactions were initiated by mixing 0.5 mL of Pt^{II} complex solution with 2.5 mL of thermostatted nucleophile solution in the UV/Vis cuvette, and reactions were monitored for at least 8 half-lives. The observed pseudo-first-order rate constants, k_{obsd}, represent an average value of two to four independent kinetic runs for each experimental condition. All reactions were studied at 310 K and pH = 7.2 in the presence of 5 mM NaCl. The experimental data are summarized and reported in the Supporting Information (Tables S1–S22).

Quantum Chemical Methods: B3LYP/LANL2DZp hybrid density functional calculations, i.e., with pseudo-potentials on the heavy elements and the valence basis set augmented with polarization functions, were performed.[37,38] During the optimization of the structures no other constrains than symmetry were applied. In addition, the resulting structures were characterized as minima by computation of vibrational frequencies. The relative energies were corrected for zero-point vibrational energies (ZPE) throughout. The GAUSSIAN suite of programs was used.[39] The influence of the bulk solvent was evaluated by single-point calculations using the CPCM formalism,[40] i.e., B3LYP(CPCM)/LANL2DZp//B3LYP/LANL2DZp and water as solvent.

Supporting Information (see footnote on the first page of this article): Tables S1–S22 report the observed pseudo-first-order rate constants for the first and the second reaction steps as a function of nucleophile concentration for the reactions between the studied complexes and 5′-GMP, L-histidine and 1,2,4-triazole in 25 mM Hepes buffer, pH = 7.2 and $[Cl^-]$ = 5 mM. Table S23 contains the coordinates of the calculated structures. Figure S1 presents the absorbance-time trace recorded for the reaction of cisplatin with 1,2,4-triazole (1.6×10^{-2} M) at 224 nm, T = 310 K, 25 mM Hepes buffer and pH = 7.2. Figures S2–S4 report plots of the pseudo-first-order rate constants as a function of nucleophile concentration for the first and the second steps of the substitution reactions of *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(en)Cl_2]$ and $[Pt(dach)Cl_2]$, at T = 310 K, 25 mM Hepes buffer, pH = 7.2 and 5 mM NaCl.

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