Unprecedented Migration of [Pt(dien)]$^{2+}$ (dien = 1,5-diamino-3-azapentane) from Sulfur to Guanosine-N^7 in *S*-Guanosyl-L-homocysteine (sgh)

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The species [Pt(dien)(sgh-*S*)]$^{2+}$ **1** formed upon reaction of sgh with one equivalent of [PtCl(dien)Cl at $2 < pH < 6.5$ is found to isomerize intramolecularly into [Pt(dien)(sgh-*N*^{7})]$^{2+}$ **2** with Pt coordination at N^7 of guanosine; upon addition of a second equivalent of [PtCl(dien)]Cl dinuclear [{Pt(dien)}$_2$(sgh-*N*^{7},*S*)]$^{4+}$ **3** is formed.

It is generally accepted that sulfur-containing molecules are responsible for the inactivation of Cisplatin[1] and the observed nephrotoxicity.[2] Therefore the chemical reactivity of Pt-antitumour drugs to these sulfur-containing molecules, like proteins and peptides such as glutathione, has been the subject of increasing research efforts.[3,4] So far, no participation in coordination of a nucleobase could be observed when the reactivity of sulfur-containing molecules was investigated, at least when using *S*-adenosyl-L-homocysteine as a model compound for such an intramolecular competition.[5] Therefore synthetic *S*-guanosyl-L-homocysteine (sgh, Fig. 1) was selected for reaction with Pt compounds, allowing a direct, intramolecular, comparison of the reactivity of the sulfur atom with the reactivity of the N^7 of the very reactive guanine. Monofunctional [PtCl(dien)]Cl (dien = 1,5-diamino-3-azapentane) was taken as the first choice, to avoid possible amine release;[6] this compound has been used before to mimic the first binding step of Cisplatin to biomolecules.[7]

S-Guanosyl-L-homocysteine was synthesized by using sodium in liquid ammonia for the reduction of cystine, according to a modified literature procedure.[8] Reactions (5 mmol l^{-1} concentrations of sgh) with [PtCl(dien)]Cl in D_2O were carried out in an NMR tube over the pH range 2–6.5 and were followed by ^{1}H NMR spectroscopy as a function of time at 295 K. To monitor the pH-dependent chemical shift behaviour of the ^{1}H signals for the various products, the pH was adjusted with 0.1–1 mol l^{-1} solutions of NaOD and DCl.

Three complexes are formed between [PtCl(dien)]Cl and sgh under different conditions: two mononuclear complexes **1**

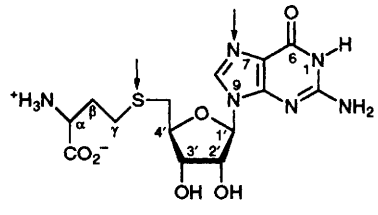

Fig. 1 Schematic structure of *S*-guanosyl-L-homocysteine (sgh). The arrows show the Pt binding sites.

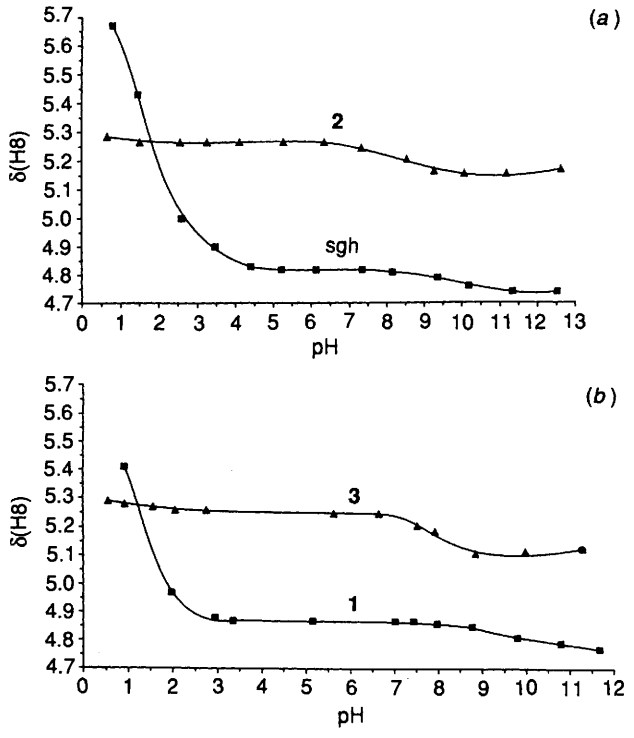

Fig. 2 H^8 guanine proton signals as a function of pH (*a*) for sgh (■) and **2** (▲), and (*b*) for **1** (■) and **3** (▲)

and **2** and one dinuclear complex **3**. The complexes were characterized by ^1H NMR spectroscopy and by their pH titration behaviour of their H^8 and H^β proton signals. Coordination of Pt(dien)2^+ at N^7, as in complex **2**† and complex **3**,† produces a downfield shift of *ca.* δ 0.6 of the H^8 proton.[9] In addition, the chemical shift of the H^8 proton is pH independent at low pH (Fig. 2); protonation of N^7 is not possible at low pH because of the Pt(dien)$^{2+}$ coordination at N^7. Another consequence of Pt(dien)$^{2+}$ coordination at N^7 is the decrease of the pK_a of N^1 by 1.1 and 1.7 log units‡ for complex **2** and **3**, respectively. This increase in acidity is caused by the electron-withdrawing effect of the platinum electrophile at the N^7 atom. When coordination at the sulfur atom occurs, as in complex **1**† and **3**, the protons nearest to the sulfur show the largest downfield shifts upon platination and exhibit broadening of their signal.§ Coordination at the sulfur atom also results in an increased acidity of the amino and the carboxy group.‡

Complexes **1**, **2** and **3** are formed in the range $2 < pH < 6.5$. When $pH > 6.5$ the deprotonated amino group is also capable of Pt(dien)$^{2+}$ coordination. This extra N-donor nucleophile gives rise to four complexes with NH_2 coordination. The identification and formation of these complexes will be discussed elsewhere.

The formation of the complexes **1**, **2** and **3** can simply be represented as: sgh → **1** → **2** → **3**. In the range $2 < pH < 6.5$, 2 equiv. of [PtCl(dien)]Cl are needed to complete the reaction to yield the final product **3**, characterized as dinuclear [{Pt(dien)}$_2$(sgh-N^7,S)]$^{4+}$ **3**. When sgh is reacted with 1 equiv. of [PtCl(dien)]Cl, the major, initially formed product is [Pt(dien)(sgh-S)]$^{2+}$ **1**. Complexes **2** and **3** are formed only as side products in small amounts. Formation of **1** as major product confirms the kinetic preference of Pt compounds for a sulfide linkage.[10] Upon standing, the initially formed complex **1** isomerizes intramolecularly into complex **2** with coordination of Pt(dien)$^{2+}$ at N^7 of guanine. This migration of Pt(dien)$^{2+}$ is an illustration of the thermodynamic lability of the Pt–methionine bond in the presence of a strong nucleophile.[11] Compared to the initial formation of complex **1** ($t_{\frac{1}{2}}$ 2 h)¶ the intramolecular isomerisation into complex **2** is slow ($t_{\frac{1}{2}}$ 10 h).¶ A direct reaction of sgh leading to complex **2** hardly, if at all occurs. The formation of **1** and isomerisation into **2** have been followed as a function of time by the isolated chemical shift value of the H^8 proton. In [Pt(dien)(sgh-N^7)]$^{2+}$ **2**, sulfur is again available for coordination and addition of a second equivalent of [PtCl(dien)]Cl yields the dinuclear [{Pt(dien)}$_2$(sgh-N^7,S)]$^{4+}$ **3**.

Reaction of Pt(dien)$^{2+}$ with the sulfur atom of sgh in the presence of a reactive G-N^7 site in fact would not be unexpected, because of the known high kinetic affinity of Pt for sulfur.[10] Our observations are also in agreement with the observation that Pt antitumour compounds react both *in vitro* and *in vivo* with sulfur-containing molecules. However, no participation of a reactive nucleobase in the bond breaking of a platinum sulfur adduct could be detected[5] until now. To the best of our knowledge these results show for the first time that a N^7 donor atom can intramolecularly replace a sulphur donor atom in a platinum–sulfur adduct. This observation could have important consequences, because it supports the hypothesis of a drug reservoir mechanism in which Pt (initially) bound to a protein may react further to yield Pt bound to DNA.

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† ^1H NMR data with chemical shifts (δ) in ppm relative to TMA at pH 7.0 and 295 K. sgh: 4.82 (H^8), 2.72 ($H^{1'}$), 1.23 ($H^{3'}$), 1.12 ($H^{4'}$), 0.62 (H^α), −0.17 ($H^{5'}/H^{5''}$), −0.49 (H^γ), −1.07 (H^β), $H^{2'}$ under HDO signal.

[Pt(dien)(sgh-S)]$^{2+}$ **1**: 4.87 (H^8), 2.80 ($H^{1'}$), 1.42 ($H^{4'}$), 1.33 ($H^{3'}$), 0.70 (H^α), 0.30 ($H^{5'}/H^{5''}$), −0.81 (H^β), δ$H^{2'}$ under HDO signal, δH^γ under dien signal.

[Pt(dien)(sgh-N^7)]$^{2+}$ **2**: 5.25 (H^8), 2.74 ($H^{1'}$), 1.22 ($H^{3'}$), 1.12 ($H^{4'}$), 0.64 (H^α), −0.49 (H^γ), −1.07 (H^β), δ$H^{2'}$ under HDO signal, $H^{5'}/H^{5''}$ under dien signal.

[{Pt(dien)}$_2$(sgh-N^7,S)]$^{4+}$ **3**: 5.21 (H^8), 2.79 $H^{1'}$), 1.42 ($H^{4'}$), 1.34 ($H^{3'}$), 0.59 (H^α), 0.29 ($H^{5'}/H^{5''}$), −0.88 (H^β), δ$H^{2'}$ under HDO signal, δH^γ under dien signal.

‡ The pK_a value of N^1 can be derived from the titration curve of the H^8 signal (Fig. 2); the pK_a values of the carboxy and amino group can be derived from the titration curve of the H^β signal (not shown).

§ Broadening is the effect of the occurrence of a pair of diastereoisomers owing to different configurations about the sulfur and an intermediate rate of conversion at room temperature on the NMR time scale.

¶ $t_{\frac{1}{2}}$ values were determined by ^1H NMR spectroscopy at pH 4.0. Estimated error is 50% owing to the occurrence of side reactions.