# Mono- and Bis-Guanosine Adducts of Platinum Complexes with Carrier Ligands Having In-Plane Steric Bulk: The Case of 1,10-Phenanthroline and 2,9-Dimethyl-1,10-phenanthroline

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**Keywords:** Platinum / Nucleobases / Antitumour agents / Atropisomerism / NMR spectroscopy

The steric hindrance generated by carrier ligands, in particular in *cis*-Pt$\mathbf{A}_2\mathbf{G}_2$ adducts ($\mathbf{A}_2$ = diamine, **G** = guanine derivative), has often been used to slow down rotation about the platinum-guanine bonds, thus allowing the characterisation of different conformers and the analysis of the interactions that are involved in the stabilisation of cisplatin adducts with DNA. In a previous study concerning 1,10-phenanthroline and 2,9-dimethyl-1,10-phenanthroline (phen and $Me_2$phen, respectively), the $Me_2$phen ligand was reported to form an unusual bis-guanosine derivative with a *trans* disposition of the nucleobases and monodentate $Me_2$phen. The present investigation has demonstrated that phen and $Me_2$phen, like other bidentate ligands, allow formation of both mono- and bis-guanosine derivatives, the latter having the usual *cis* configuration. In both the phen and $Me_2$phen carrier ligands the rigidity of the phenanthroline skeleton is able to hinder rotation of the guanosine base(s) about the Pt–N(7) bond(s) and render the interconversion between rotamers slow on the NMR timescale even at 350 K (the highest temperature explored). However there are noticeable differences between the two ligands. As a consequence of the increased in-plane steric bulk of $Me_2$phen: (i) the H(8) chemical shifts within the *HH* (*Head-to-Head*) conformer and between the *HT* (*Head-to-Tail*) conformers become closer, (ii) the average H(8) chemical shift of the *HT* conformers is at higher field and that of the *HH* form at lower field, and (iii) the *HT* rotamers become even more favoured than the *HH* rotamer. Observation (ii) is in agreement with the *HT* rotamers preferring the "6-in" conformation, which brings the six-membered rings of the guanines closer to one another and is also in agreement with the phen ligand allowing a greater canting of the guanine bases than $Me_2$phen. Moreover, free chloride ion readily displaces the coordinated guanosines, particularly in solvents of lower dielectric constants than water.
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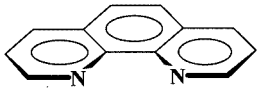
## Introduction

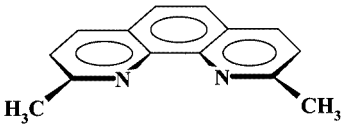
Despite undesirable side effects, cisplatin {*cis*-$[PtCl_2(NH_3)_2]$} remains one of the most widely used drugs in the treatment of several tumours.[1,2] There is general consensus that nuclear DNA is the main target of cisplatin and that 1,2-intrastrand cross-links between adjacent purines are responsible for the anticancer activity of the drug.[3]

A huge number of models of the 1,2-intrastrand cross-link have been investigated.[4,5] In particular, carrier ligands able to introduce steric hindrance in the coordination sphere of platinum and to slow down dynamic motions about the platinum-ligand bonds have proven to be very informative.[6−12] By slowing down the rotation of the nucleobases about the Pt−N(7) bonds different rotamers, with *HT* (*Head-to-Tail*) and *HH* (*Head-to-Head*) conformations, have been identified.[13,14]

In principle 1,10-phenanthroline (phen) and, even better, 2,9-$Me_2$-1,10-phenanthroline ($Me_2$phen, neocuproine) could be used to introduce steric bulk in the coordination plane (Figure 1). 1,10-Phenanthrolines, differently from




phen




Me2phen

Figure 1. Schematic representation of 1,10-phenanthroline (phen) and 2,9-dimethyl-1,10-phenanthroline ($Me_2$phen) ligands

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other chelating diamines, have a very rigid structure and the lone pairs on the nitrogen atoms are convergent and hence ideally oriented for complexation to a metal centre.[15] Because of these distinctive properties, 1,10-phenanthrolines have found biological application as complexing agents of metal ions (removal of essential metal can lead to inactivation of cellular proteins and enzymes) and as bacteriostatic, fungistatic, antihelmintic, tuberculostatic, antispermatozoal, and antifibrilatory agents.[16,17] Moreover, some phenanthrolines are endowed with antitumour activity against sarcomas in mice and inhibit formation of carcinomas in rats.[18,19]

In a recent study the reaction of guanosine with phen-$Pt^{II}$ and $Me_2$phen-$Pt^{II}$ substrates was investigated.[20] The results were interpreted as being due to formation of bis-guanosine adducts of formula *cis*-[Pt(*N*,*N*-phen)$(Guo)_2]^{2+}$ and *trans*-[PtCl(*N*-$Me_2$phen)$(Guo)_2]^{+}$. The presence of two H(8) resonances in *cis*-[Pt(*N*,*N*-phen)$(Guo)_2]^{2+}$ was interpreted as formation of two rotamers: one *HH* and one *HT*. However the *HH* rotamer should have inequivalent G moieties and therefore should give two H(8) signals of equal intensities. Moreover, two *HT* rotamers are possible (Λ*HT* and Δ*HT*), each of which should have equivalent G's, but the H(8) chemical shifts should be different for the two *HT* rotamers. In the second case examined ($Me_2$phen complex) the structure was essentially deduced from the observation of two signals in the methyl region with an intensity ratio of ca. 1:1. This was interpreted as being due to the presence of a *trans*-[PtCl(*N*-$Me_2$phen$)(Guo)_2]^{+}$ complex, with monocoordinated $Me_2$phen undergoing slow exchange of the donor atoms. The latter result was rather unusual since, in all complexes previously investigated by us and containing monocoordinated $Me_2$phen, the diamine undergoes fast exchange between the two nitrogens at room temperature.[21−23] The discrepancy between the newly reported data and those reported in the literature for analogous systems prompted us to reinvestigate the reaction of guanosine with platinum substrates containing phen and $Me_2$phen as carrier ligands.

## Results and Discussion

### Stereochemistry of *cis*-$PtA_2G_2$ Adducts

Previous reports have demonstrated that the 1,2-intrastrand cross-links formed by cisplatin with double-strand oligonucleotides have the guanines in a *Head-to-Head* (*HH*) conformation with both H(8)'s on the same side of the platinum coordination plane (Figure 2).[24−26] The phosphodiester linkage and the GC Watson−Crick base pairing of the vicinal guanosines are supposed to constrain such adducts in the *HH* conformation. In the case of cisplatin adducts with two guanine bases untethered by a phosphodiester linkage, the *Head-to-Tail* (*HT*) conformation is by far the most favoured at equilibrium, although the *HH* conformer should account for 50% on a statistical basis.[27]

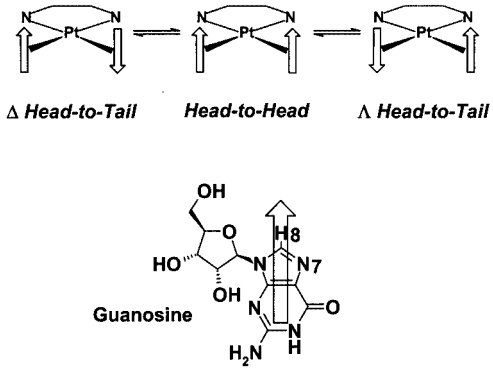


Figure 2. Schematic representation of *HH* and *HT* rotamers; arrows represent guanosines having H(8) coincident with the pointed end

Rotation about the Pt−**G**(N7) bonds in *cis*-$PtA_2G_2$ adducts [$\mathbf{A_2}$ = two monodentate or one bidentate amine ligand, **G** = N(7)-bound guanine base], interconverts *HH* and *HT* rotamers (Figure 2). When $A_2$ is a nonbulky ligand, the rotation about the Pt−**G**(N7) bonds is fast on the NMR timescale and only one set of NMR signals, which is an average of several conformations, is observed. In contrast, when $A_2$ is bulky, the rotation is restricted by steric interactions between the rotating guanines and the $A_2$ ligand(s) and different sets of signals are observed in the NMR spectra. In this respect the signals of the **G**(H8) protons are particularly informative. The number of H(8) signals detectable in solution for each *cis*-$PtA_2G_2$ conformer depends upon the symmetry of the $A_2$ carrier ligand and of the substituents at N(9) of the **G** residues. For carrier ligands of $C_2$, or higher, symmetry and asymmetric N(9) substituents the two **G**'s are equivalent within each *HT* conformer (Δ-*HT* and Λ-*HT*), and inequivalent within the *HH* conformer.

### Complexes with $Me_2$phen (1 and 2)

#### *[Pt(Me₂phen)(Guo)₂]Cl₂ (1)*

The procedure used for the preparation of [Pt($Me_2$phen)-$(Guo)_2]Cl_2$ (**1** in Figure 3) was different from that reported by Clement et al.[20] Instead of performing the reaction in water at high temperature (80 °C, 24 h) we carried out the reaction at room temperature (so reducing the risk of decomposition) in chloroform/methanol since the starting substrate [$PtCl_2$($Me_2$phen)] is soluble in chloroform and guanosine is soluble in methanol. The desired product was formed in a shorter time (2 h) and with higher yield (90%) than reported in reference 20 (60% yield).

The $^1$H NMR spectrum of complex **1**, in $D_2O$ and pH* = 2.90 (the asterisk denotes pH values uncorrected for the effect of deuterium on the glass electrode), is reported in Figure 4 (a small amount of free guanosine was added to the sample for comparison). Two strong signals of unequal intensity (at δ = 8.51 and 8.52 ppm, intensity ratio 1:1.25) and two much weaker signals of equal intensities (at δ = 9.00 and 9.01 ppm) are observed in the region of H(8). The

downfield shift of the H(8) resonances relative to that for free guanosine ($\delta$ = 7.87 ppm at pH* = 2.90) is a clear indication that the nucleobases are coordinated to platinum through N(7).[28] This was further confirmed by pH titration experiments (Figure S1 in the Supporting Information, see also the footnote on the first page of this article). The two strong and more-shielded signals of unequal intensities are assigned to the *HT* rotamers ($\Delta$-*HT* and $\Lambda$-*HT*), while the two weak and less-shielded signals of equal intensities

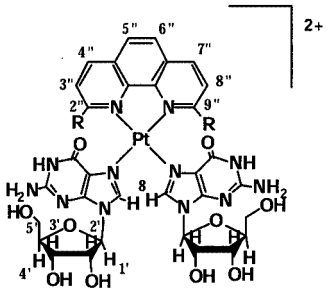


Figure 3. Schematic drawing and numbering schemes for compounds **1**–**4**

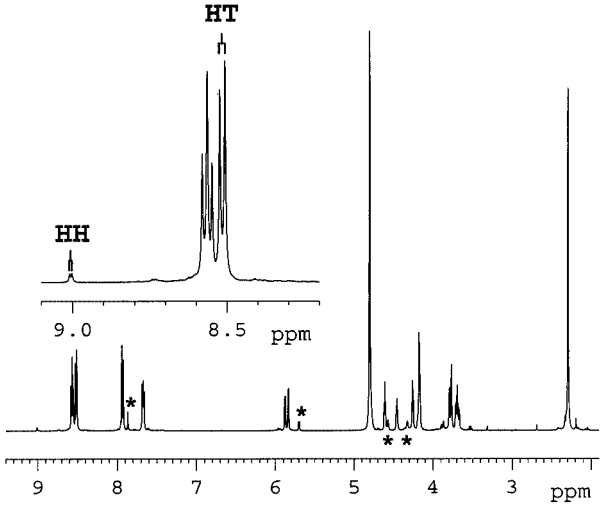


Figure 4. $^1$H NMR spectrum of complex **1** in $D_2O$ (pH* = 2.90, $T$ = 298 K); the asterisks mark the signals of free guanosine added to the sample for comparison; the H(8) signals of the *HH* and *HT* rotamers are also labelled

are assigned to the *HH* conformer. Literature data indicate that the *HT* orientation can be stabilised with respect to the *HH* orientation in *cis*-Pt$\mathbf{A}_2\mathbf{G}_2$ adducts because of a better dipole-dipole interaction between the two guanine residues.

Three singlets at $\delta$ = 2.29, 2.32, and 2.33 ppm (intensity ratios 36:1:1) are observed for the methyl protons. Therefore, a large upfield shift (ca. 1 ppm) is experienced by these methyls upon substitution of the two chlorides by two guanosine molecules (for [$PtCl_2$($Me_2$phen)] the Me chemical shift is at $\delta$ = 3.12 ppm in $CD_3OD$). The most intense signal, at $\delta$ = 2.29 ppm, is assigned to the two *HT* conformers, which therefore give coincident signals for the methyls in positions 2′′ and 9′′ of the phenanthroline moiety (see Figure 3 for the numbering scheme). The less-intense signals at $\delta$ = 2.32 and 2.33 ppm of 1:1 intensities are assigned to the *HH* conformer (for which the two methyls of $Me_2$phen are expected to be inequivalent). The chemical shifts of the phenanthroline Me's are influenced mostly by the shielding effect of the *cis*-coordinated nucleobase, while the effect of the chiral ribose, which should be responsible for the difference in chemical shift between *HT* rotamers and within the *HH* rotamer, is practically negligible.

The pattern of the methyl protons (1 ppm upfield shift) clearly indicates that $Me_2$phen is biscoordinated to platinum and the other two coordination positions are occupied by guanosine molecules. This complex composition is also supported by the absence of the typical Pt−Cl stretching frequency,[29] which is found in the IR spectrum of the precursor complex [$PtCl_2$($Me_2$phen)] at 341 $cm^{-1}$.

The complete $^1$H NMR analysis of the [Pt($Me_2$phen)($Guo)_2$]$^{2+}$ complex with the assignments of all proton signals pertinent to the various rotamers is given in Table 1. The aliphatic region of a gradient-enhanced COSY spectrum showing the sequential assignment of the resonance peaks of the ribose protons is reported in Figure S2 (Supporting Information).

An interesting observation from the NOESY experiment is the presence of strong cross-peaks between H(8) and the sugar proton H(1′) for both *HT* rotamers (Figure S3). The presence of a cross-peak between H(8) of the guanine and the anomeric proton H(1′) is an indication that there is a *syn* contribution to the conformation of the nucleoside (the conformation is *anti* in natural B-DNA).[30−33] This result was also confirmed by a ROESY experiment (Figure S4) showing cross-peaks between the H(8) proton of the guanine and the sugar protons H(1′), H(2′) and H(3′). The ratio between the volumes of cross-peaks H(8)/H(1′) and H(8)/H(2′) is 2.1 and 1.8 for the two *HT* conformers, thus confirming the partial *syn* character of the nucleosides in these adducts. The 4.8 Hz value of the $^3J_{H1',H2'}$ coupling constant observed for the *HT* rotamers suggests a higher percentage of *S* puckering of the ribose rings (S/N sugar pucker ratio of ca. 54:46). None of the above NOE's was observed for the *HH* rotamer because of its low concentration in solution.

$^1$H NMR spectra were also recorded at higher temperatures in order to detect possible interconversion between the *HH* and the *HT* conformers. Under the experimental

Table 1. $^1$H NMR chemical shifts (ppm) of the complexes (numbering of the protons as indicated in Figure 3); $^3J_{H,H}$ values (in Hz) are given in parentheses; s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet

| | | | Phenanthroline | | | | | | | Guanosine | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Comp. | Conform. | Solv. | Me/H(2") | H(3") | H(4") | H(5"/6") | H(7") | H(8") | Me/H(9") | H(8) | H(1′) | H(2′) | H(3′) | H(4′) | H(5′) |
| **1** | *HT*$_M$ | $D_2O$[a] | 2.29 s | 7.68d (8.3) | 8.57 d (8.3) | 7.94 s | 8.57 d (8.3) | 7.68 d (8.3) | 2.29 s | 8.51 s | 5.84 d (4.9) | 4.60 t | 4.26 t | 4.17 m | 3.80−3.67 m |
| | *HT*$_m$ | $D_2O$ | 2.29 s | 7.66 d (8.3) | 8.55 d (8.3) | 7.92 s | 8.55 d (8.3) | 7.66 d (8.3) | 2.29 s | 8.52 s | 5.88 d (4.9) | 4.46 t | 4.17 t | 4.17 m | 3.80−3.67 m |
| | HH | $D_2O$ | 2.32 s | 7.60 dd (8.7) | [b] | 7.95 s | [b] | 7.60 dd (8.7) | 2.33 s | 9.01 s, 9.00 s | 5.96 d, 5.94 d (5.0) | 4.59 t, 4.68 t | 4.19 t | 4.34 | 4.00−3.92 m |
| **2** | M | $CD_3OD$ | 3.27 s | 7.79 d (8.2) | 8.65 d (8.2) | 8.05 s | 8.64 d (8.2) | 7.67 d (8.2) | 2.27 s | 8.79 s | 5.99 d (4.2) | 4.50 t | 4.32 t | 4.14 m | 3.94−3.74 m |
| | m | | 3.27 s | 7.79 d (8.2) | 8.66 d (8.2) | 8.05 s | 8.64 d (8.2) | 7.67 d (8.2) | 2.26 s | 8.73 s | 5.92 d (4.4) | 4.58 t | 4.30 t | 4.14 m | 3.94−3.74 m |
| **3** | *HT*$_M$ | $D_2O$[c] | 8.27 | 7.90 dd (8.3) | 8.94 | 8.21 s | 8.94 | 7.90 dd (8.3) | 8.27 | 8.80 s | 6.05 d (4.9) | 4.65 t | 4.31 t | 4.25 | 3.95−3.60 m |
| | *HT*$_m$ | $D_2O$ | 8.27 | 7.90 dd (8.3) | 8.94 | 8.20 s | 8.94 | 7.90 dd (8.3) | 8.27 | 8.75 s | 6.03 | 4.85 t | 4.42 | 4.25 | 3.95−3.60 m |
| | HH | $D_2O$ | 8.36 | 7.90 d (8.3) | 8.94 | 8.22 s | 8.94 | 7.90 | 8.36 | 8.91 s, 8.86 s | 6.05 d, 6.03 d (4.9) | 4.67 t | 4.43 t | 4.25 | 3.90−3.70 m |
| **4** | M | $CD_3OD$ | 9.72 | 8.09 | 8.93 | 8.20 s | 8.50 | 7.88 | 8.93 | 8.86 s | 6.06 d (4.3) | 4.68 t | 4.37 m | 4.14 m | 3.94−3.72 m |
| | m | | 9.72 | 8.09 | 8.93 | 8.20 s | 8.50 | 7.88 | 8.93 | 8.81 s | 6.00 d (4.3) | 4.57 t | 4.37 m | 4.14 m | 3.94−3.72 m |

[a] pH* = 2.90. [b] Obscured by overlapping signal of the *HT* conformers. [c] pH* = 3.00.

conditions used (solvent $D_2O$, pH* = 3.5), only a slight broadening of the resonance peaks was observed upon raising the temperature to 353 K (Figure S5). Complex **1** proved to be stable at high temperature for quite a long time as indicated by the similarity between the initial spectrum and that recorded at 298 K after a long stay at 353 K. The slow interconversion between rotamers, on the NMR timescale, was also confirmed by the absence of EXSY cross-peaks between the H(8) signals of the three rotamers in the NOESY-EXSY experiment (Figure S3).

### *Effect of Chloride Ions on the Ligand Exchange in Complex 1: Formation of [PtCl(Me$_2$phen)(Guo)]Cl (2)*

It is well documented that in *cis*-Pt**A$_2$G$_2$** species the nucleobases are strongly bound to the platinum centre[34] and their displacement requires the use of strong nucleophiles such as $CN^-$ or sulfur donor ligands. For example, the displacement of guanine from *cis*-$[Pt(NH_3)_2(Guanine)_2]^{2+}$ by thiourea has been calculated from $^{13}$C NMR spectroscopic data to have a rate constant of $1.5 \times 10^{-5}$ M$^{-1}$ s$^{-1}$ at 316 K.[35,36] Only in the case of strong *trans*-labilizing ligands, such as phosphanes, has the ready displacement of a coordinated guanosine by chloride been observed.[37]

The situation appears to be different when a solvent with a lower dielectric constant than water is used.[38] For instance $[Pt(Me_2phen)(Guo)_2]Cl_2$ dissolved in $CD_3OD$ gave rise to a dissociation equilibrium in which two new species were formed, one of which was easily recognised as free guanosine. The second species shows two signals in the region of H(8) (δ = 8.79 and 8.73 ppm) and three singlets (δ = 3.27, 2.27, and 2.26 ppm, relative intensities 2:1:1) in the region of the methyl protons. Moreover, integration of the aromatic protons shows that only one molecule of guanosine is present per molecule of neocuproine. Therefore the new complex can be formulated as [PtCl(Me$_2$phen)(Guo)]Cl with a bidentate Me$_2$phen, one chloride, and one guanosine in the coordination sphere of platinum. Such a complex is expected to have inequivalent Me's with one Me (the one *cis* to the guanosine) more shielded than the other. The presence of two signals for H(8) and two signals at high field for the Me's (δ = 2.27 and 2.26 ppm) is indicative of the presence of two conformers stemming from restricted rotation of the guanosine about the Pt−N(7) bond (as observed for compound **1**). The two conformers are made diastereomeric by the presence of the asymmetric ribose. A noteworthy observation is that the average H(8) chemical shifts of the mono-guanosine derivative (δ = 8.76 ppm), which is not influenced by the magnetic anisotropy of a *cis*-**G**, falls right in between the average H(8) chemical shifts of the *HT* and *HH* rotamers of the bis-guanosine derivative. (δ = 8.51 and 9.01 ppm, respectively). Although some caution is needed since the overall charge of the complex (+1 and +2 for compounds **2** and **1**, respectively) and the set of donor atoms are different in the two cases, it nevertheless appears that, with respect to the monoadduct, in the bis-guanosine derivative the H(8)'s are shielded by the *cis* **G** in the *HT* rotamer and are deshielded by the *cis* **G** in the *HH* rotamer.

The same complex, [PtCl(Me$_2$phen)(Guo)]Cl (**2**), was prepared independently and fully characterised by ele-

mental analysis and $^1$H NMR spectroscopy (the $^1$H NMR spectrum in $CD_3OD$ is reported in Figure S6). The complete assignment of the $^1$H NMR chemical shifts for the two conformers of compound **2** is reported in Table 1. NOESY cross-peaks were observed (Figure S7) between the guanosine H(8)'s and the sugar protons H(1′) and H(2′). The H(8)−H(1′) cross-peaks indicate that the nucleoside conformation has a partial *syn* character, while the value of 4.4 Hz for the $^3J_{H1',H2'}$ coupling indicates a higher percentage of N-pucker for the ribose (S/N of ca. 49:51).

$^1$H NMR spectra were recorded at different temperatures in order to investigate the effect of the temperature upon the rate of interconversion between conformers in compound **2**. Unfortunately the monosubstituted guanosine derivative started to decompose at temperatures slightly above 50 °C. This shows that the attempt by Clement and co-workers[20] to prepare the bis-guanosine derivative of $Me_2$phen from a reaction in $H_2O$ at 80 °C is probably not the most appropriate.

The dependence of the dissociation equilibrium of **1** in methanol [Equation (1)] upon the $Cl^-$ concentration was confirmed by addition of KCl (three times the stoichiometric amount) to a solution of **1** in $CD_3OD$ (NMR tube).

$$[Pt(Me_2phen)(Guo)_2]^{2+} + Cl^- \rightleftharpoons [PtCl(Me_2Phen)(Guo)_2]^+ + Guo \quad (1)$$

The molar ratio **1**:**2** changed from 3:1 to 2:1, supporting the involvement of the chloride ion in the equilibrium of displacement of one guanosine from complex **1**.

The observation of the displacement of a guanosine derivative by a chloride ion in a different solution environment (the phenomenon was not observed in water) could be relevant to the biochemistry of this type of substrates in lipophilic environments.

### Complexes with phen (3 and 4)

#### *[Pt(phen)(Guo)$_2$](NO$_3$)$_2$ (3)*

Because of the low solubility of the starting dichloro complex [$PtCl_2$(phen)], this substrate was first converted into the solvato species $[Pt(phen)(DMF)_2]^{2+}$ by reaction with a stoichiometric amount of $AgNO_3$ in DMF and then allowed to react, at room temperature, with a stoichiometric amount of guanosine (ratio 1:2) to give [Pt(phen)-$(Guo)_2](NO_3)_2$. Once more our experimental conditions were different from those used by Clement and co-workers (starting material [$PtCl_2$(phen)] and reaction performed in water at 65 °C for three days).

The $^1$H NMR spectrum of complex **3** in $D_2O$ at pH* 3.0 is reported in Figure 5 (a small amount of free guanosine was added to the sample for comparison) and the complete assignment of the NMR spectroscopic data is reported in Table 1. Four peaks are observed in the H(8) region: two signals are much more intense and rather shielded (δ = 8.80 and 8.75 ppm, relative intensities 1.1:1) and two signals are very weak, rather deshielded, and of equal intensities (δ = 8.91 and 8.86 ppm). The two strong signals with different intensities were assigned to the two *HT* rotamers, each one having equivalent H(8) protons, while the two weaker signals were assigned to the *HH* conformer with inequivalent H(8)'s.

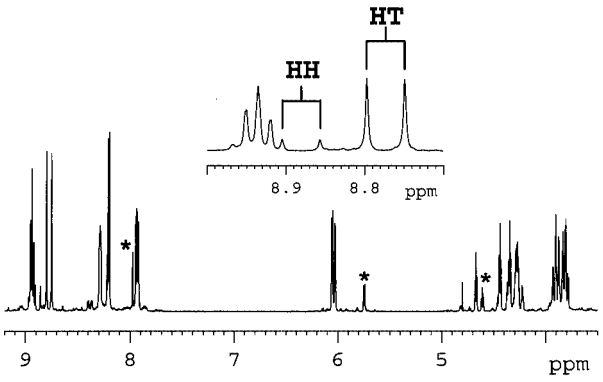


Figure 5. $^1$H NMR spectrum of complex **3** in $D_2O$ (pH* = 3.00, 298 K); the asterisks mark the peaks of free guanosine added to the sample for comparison; the H(8) peaks of the *HH* and *HT* rotamers are also labelled

The chemical shifts of the H(8) protons are indicative of N(7) coordination of the guanosine ligands. This type of coordination was also confirmed by a pH titration experiment.

A COSY experiment (Figure S8) allowed the assignment of all resonances (Table 1). A comparison of the 2D maps obtained in the $^1$H-$^{13}$C HSQC experiments performed on complexes **3** and **1** (Figure 6) indicates that, differently from Clement's assignment,[20] the less-shielded signal (δ = 8.94 ppm) belongs to protons 4"/7′′ and the more-shielded signal (δ = 8.27 ppm) to protons 2"/9′′. It is likely that the aromatic ring current of the *cis*-coordinated guanosines is responsible for the shielding of protons 2"/9′′ (a similar effect has already been observed for methyls 2"/9′′ in the analogous complex **1**). Further support for our assignment of the aromatic protons comes from the presence of a ROESY cross-peak (C in Figure 7) between protons 5"/6′′ (δ = 8.21 ppm) and the less-shielded signal here assigned to protons 4"/7′′ (δ = 8.94 ppm). Significant ROESY cross-peaks are also observed between H(8) and H(2"/9") and between H(8) and H(1′) for the two *HT* rotamers (cross-peaks **B** and E, respectively). Similarly to the case of complexes **1** and **2**, the latter cross-peak gives an indication that the base/sugar conformation has some *syn* character. The ratios between the integrals of the ROESY cross-peaks H(8)−H(1′) and H(8)−H(2′) are equal to 2.17 and 1.63 for the two *HT* rotamers, respectively, suggesting a high percentage of S-pucker for the ribose. Such a pucker was also confirmed by the value of the $^3J_{H1'-H2'}$ coupling constant (4.9 Hz, which corresponds to an S/N ratio of ca. 55:45).

The effect of temperature on the rate of interconversion between *HT* and *HH* conformers was also investigated. The $^1$H NMR spectrum of a sample of **3** in $D_2O$ at pH* 3.50 and 355 K shows only a slight broadening of the peaks of the aromatic protons (Figure S9) with respect to the spectrum at 295 K, indicating that, even at the highest temperature explored, the rate of interconversion between rota-

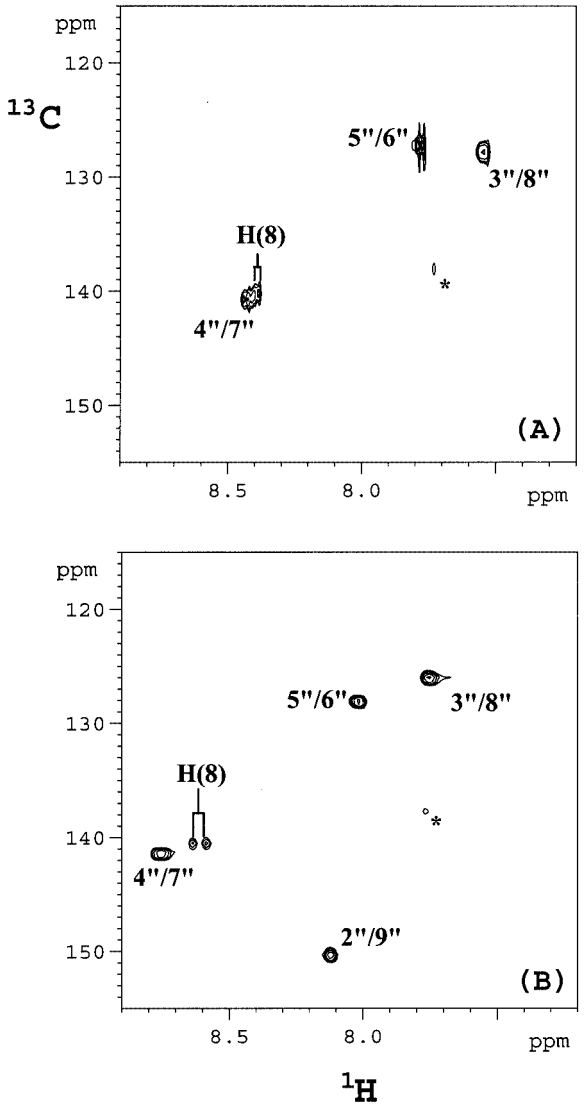


Figure 6. $^{1}$H-$^{13}$C HSQC contour map of complex **1** (A) and complex **3** (B) in $D_2O$ (pH* 2.90 and 3.00 for **1** and **3**, respectively; $T$ = 298 K for both experiments); the asterisks mark the peaks of free guanosine added to the samples for comparison

mers is slow on the NMR timescale. Therefore the steric hindrance of the protons in positions 2′′ and 9′′ of phen is sufficient to prevent the free rotation of the guanosines about the Pt−N(7) bonds.

The analogy between the behaviour of **3** and that of **1** was quite unexpected since protons 2"/9′′ were not believed to inhibit the rotation around the Pt−N(7) bond to the same extent as the methyls 2"/9′′ of $Me_2$phen.

### *[PtCl(phen)(Guo)](NO$_3$) (4)*

For the preparation of [PtCl(phen)(Guo)]($NO_3$) (**4**) the starting [$PtCl_2$(phen)] was first treated with one equivalent of $AgNO_3$ in DMF and then with one equivalent of guanosine. A small amount of complex **3** was always formed as a by-product in this reaction.

Similarly to the case of complex **2**, two sharp H(8) signals (at δ = 8.86 and 8.81 ppm, ratio 1.2:1) indicate the presence

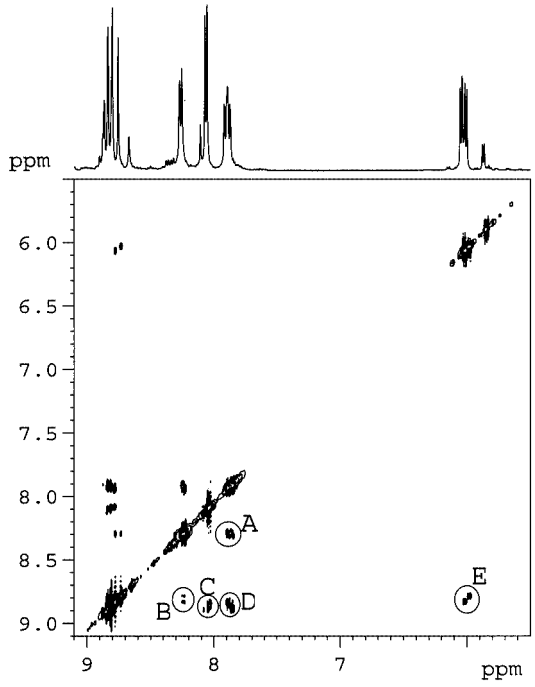


Figure 7. 2D-ROESY contour map of complex **3** in $D_2O$; the labelled cross-peaks are in antiphase with respect to the diagonal and represent intraresidual spatial couplings between protons of the *HT* rotamers: H(3"/8")−H(2"/9"), A; H(2"/9")−H(8), B; H(5"/6")−H(4"/7"), C; H(3"/8")−H(4"/7"), D; H(8)−H(1′), E

of two conformers in slow interconversion on the NMR timescale (Figure S10). The assignment of the $^{1}$H NMR signals was facilitated by a COSY experiment (Figure 8) and is reported in Table 1. One *ortho* proton of phen is less shielded (δ = 9.72 ppm) and is assigned to the proton *cis* to the chlorine ligand (2′′ in Figure 3), since a similar chemical shift was observed for the *ortho* protons in the starting [$PtCl_2$(phen)] complex (δ = 9.80 ppm in [$D_7$]DMF). In con-

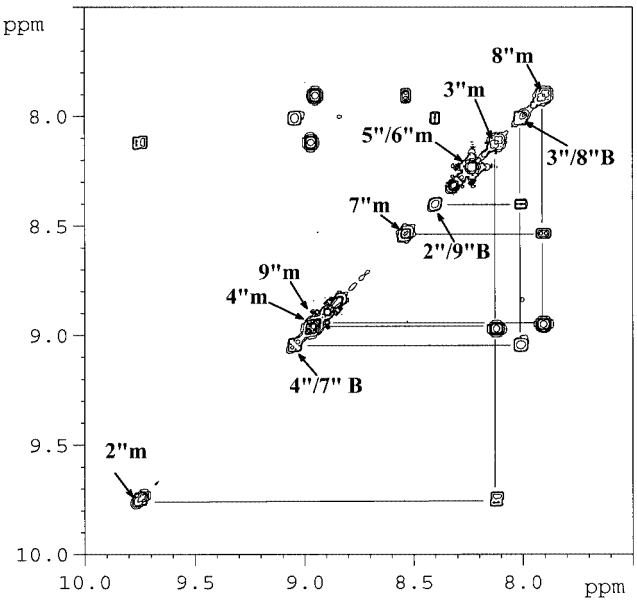


Figure 8. 2D-COSY contour map of complex **4** (resonances labelled m); resonances labelled B belong to complex **3**

trast, the *ortho* proton 9′′ undergoes an upfield shift of ca. 1 ppm which appears to be typical for an *ortho* proton *cis* to a guanine base. A similar upfield shift was observed for the *ortho* methyl(s) of $Me_2$phen *cis* to a guanine base.

The presence of two conformers indicates that there is also restricted rotation about the Pt−N(7) bond in the case of the monoadduct, and that the in-plane steric hindrance of the *ortho* protons of phen is sufficient to inhibit the free rotation of a *cis* guanosine.

The partial *syn* character of the guanosine conformation was also revealed by a ROESY experiment for compound **4**. The value of the $^3J_{H1',H2'}$ coupling (4.3 Hz) corresponds to an S/N pucker ratio of ca. 48:52.

As for $Me_2$phen and for phen the monoadduct has a slightly higher percentage of N pucker than the bis adduct and the average chemical shift of the H(8) protons falls in between the average H(8) chemical shifts of the *HH* and *HT* rotamers of the bis adduct.

### Comparison between $Me_2$phen and Phen Derivatives

The differences in chemical shift between the H(8) signals of the two *HT* rotamers and between the two H(8) signals of the single *HH* rotamer observed in compounds **1** and **3** are very small ($\leq$ 0.05 ppm) when compared to those observed in similar compounds with $C_2$-symmetrical ligands having a strong asymmetry with respect to the coordination plane. These latter ligands were named Chirality Controlling Chelates, **CCC**, since they are able to favour one *HT* rotamer over the other and to induce a difference in the H(8) chemical shift between *HT* rotamers of the order of 0.2−0.4 ppm and a difference in chemical shift between the two guanines of the *HH* rotamer of the order of 0.8−1.1 ppm.[39−41]

The large dispersion of the H(8) signals within the *HH* conformer and between the two *HT* conformers observed in the platinum compounds with **CCC** ligands is mostly determined by the ability of these ligands to induce a preferential canting (either right- or left-handed) to the nucleobases. For a given direction of canting one *HT* rotamer will have the H(8) protons leaning towards the *cis* amine and deshielded while the second *HT* rotamer will have the H(8) protons leaning towards the *cis*-**G** and shielded. The two forms have been termed "6-in" and "6-out" since the former has the six-membered rings of the guanines closer to one another and the latter further apart. The *HH* rotamer will have one "6-in" and one "6-out" guanine, the former guanine will have a deshielded signal and the latter a shielded H(8) signal. Differently from **CCC** ligands, the phenanthroline ligands are perfectly symmetrical with respect to the coordination plane and cannot induce preferential canting of the nucleobases. Therefore the average canting could either be zero for all rotamers or, in the case of *HT* rotamers, be driven by the rotamer chirality. We will see (following section) that the latter is the case here. However there is no question that in **1** and **3** the differences between *HT* rotamers and between the two guanines of the *HH* conformer are determined entirely by the chiral ribose substituent, and are small.

There are some significant differences in chemical shifts between **3** and **1**. First, the chemical shift difference between the H(8) signals of the two *HT* rotamers and between the two H(8) signals of the single *HH* form is ca. 0.05 ppm in **3** and ca. 0.01 ppm in **1**. Second, the average H(8) chemical shift of the *HT* rotamers is at lower field in compound **3** than in compound **1** ($\delta$ = 0.26 ppm). Finally, the average H(8) chemical shift of the *HH* rotamer is at higher field in compound **3** than in **1** ($\delta$ = 0.12 ppm). All these features can be explained by assuming that, because of the smaller steric hindrance of the *ortho* substituents, the two guanosines have significantly greater wagging freedom in the case of phen than in the case of $Me_2$phen.

Therefore, with reference to the first point, the effect of the ribose in rendering the guanine bases within the *HH* rotamer and between the two *HT* rotamers magnetically inequivalent will be greater in **3** than in **1**.

With reference to the second point, the smaller shielding of the H(8) protons in the HT rotamers of **3** than in **1** is in agreement with the *HT* rotamers preferring the "6-in" conformation and the phen ligand allowing a greater canting of the guanine bases. The "6-in" conformation moves the six-membered ring of each guanine towards the *cis* **G** while the H(8) proton is moved towards the *cis* amine; it follows, therefore, that the more canted the bases are, the more deshielded the H(8) protons. The latter observation can be taken as proof that in the absence of other factors, such as the preferential canting imposed by **CCC** ligands, the canting of the bases is driven by the chirality of the *HT* conformer. The Δ-*HT* conformer induces an R canting and the Λ-*HT* conformer an L canting so that in both cases the six-membered rings move closer to each other and give a better internucleoside interaction. The canting direction [right-handed (R) or left-handed (L)] is defined by the chirality of two screw lines, one passing through the N(7) atoms of the two coordinated guanines and the other passing through H(8) and bisecting the given guanine.

With reference to the third point, we have to consider that in the *HH* rotamer the guanine with the "6-out" orientation is generally more canted and has a more-shielded H(8) signal than the guanine with the "6-in" orientation. Moreover, in the case of canting neutral phen ligands each guanine oscillates between the "6-in" and "6-out" conformations leading to an average H(8) signal which is similar for the two guanines. However, since the phen ligand allows for a more canted "6-out" conformation, the average H(8) chemical shift of the *HH* rotamer will be at higher field in compound **3** ($\delta$ = 0.12 ppm) than in compound **1**, in which the guanosines are more constrained to an orthogonal position.

## Conclusions

This investigation has shown that phen and $Me_2$phen behave like other bidentate ligands, allowing the formation of platinum complexes in which one or both of the remaining two coordination positions can host guanosine ligands.

The features of the mono- and bis-guanosine adducts are strongly influenced by the special stereochemistry of the phen and $Me_2$phen ligands. First, both the $Me_2$phen and the phen carrier ligands are able to hinder rotation of the guanosine bases about the Pt−N(7) bonds so that the interconversion between rotamers becomes slow on the NMR timescale. Such a result was rather unexpected for phen. It is likely that the rigidity of the phenanthroline skeleton amplifies the steric impediment created by the small *ortho*-hydrogen atoms and restricts the free rotation of the *cis* guanosine ligand.

An interesting result of this investigation is that, by increasing the in-plane steric bulk of the carrier ligand (Me's instead of H's in positions 2/9 of the phenanthroline), the degree of wagging of the guanosine ligands is reduced and, in the case of $Me_2$phen, these ligands are forced to be more strictly orthogonal to the coordination plane. As a consequence: (i) the H(8) chemical shifts within the *HH* conformer and between *HT* conformers become more similar ($\Delta\delta$ of 0.01 ppm in the $Me_2$phen system as compared to 0.05 ppm in the case of phen), (ii) the H(8) protons of the *HT* conformers are more shielded ($\Delta\delta$ of 0.26 ppm), (iii) the H(8) protons of the *HH* rotamer are less shielded ($\Delta\delta$ of 0.12 ppm), and (iv) the *HT*/*HH* ratio is greater (ca. 18 and 8 in $Me_2$phen and phen, respectively).

Another result of this investigation is that in all the reported compounds the guanosine conformation appears to have some *syn* character.

Finally, the different behaviour of compound **1** in water and methanol solutions indicates that the solvent can play an important role in the stabilisation of cationic platinum adducts with purine bases when coordinating anions, such as $Cl^-$, are also present in solution.

## Experimental Section

**Physical Measurements:** Elemental analyses were performed using a Carlo Erba Elemental Analyzer mod. 1106 instrument. IR Spectra were recorded on a Perkin−Elmer Spectrum One spectrometer using KBr as solid support for pellets. $^1$H NMR spectra were recorded on a DPX 300 MHz WB Avance Bruker instrument. Chemical shifts are referenced to TMS using the residual protic peak of the solvent as internal reference ($\delta$ = 4.80 ppm for $D_2O$ and $\delta$ = 3.30 ppm for $CD_3OD$). $^1$H NMR experiments at different temperatures were performed using the heating control unit of the spectrometer. 2D $^1$H NMR experiments were performed on a DRX 500 MHz WB Avance Bruker Instrument.

A Crison Micro-pH meter Model 2002 equipped with a Crison micro-combination electrode (9 mm diameter) and calibrated with Crison standard buffer solutions at pH 4.00 and 7.02 was used for all pH measurements. The pH readings for $D_2O$ solutions are indicated as pH* values and are uncorrected for the effect of deuterium on glass electrodes.

**Calculations of p$K_a$ Values:** The pH titration curves were fitted to the Henderson−Hasselbach equation using the program Kaleidagraph 3.0 for Macintosh.[42]

**Starting Materials:** Commercial reagent-grade chemicals, 2,9-$Me_2$-1,10-phenanthroline ($Me_2$phen, neocuproine), 1,10-phenanthroline (phen), guanosine (Guo), dimethyl sulfoxide (DMSO) and dimethylformamide (DMF) were used without further purification. $[PtCl_2(DMSO)_2]$ was prepared from an aqueous solution of $K_2PtCl_4$ and DMSO.[43] $[PtCl_2(Me_2phen)]$ was prepared by previously reported procedures.[21]

**Preparation of Complexes**

**$[PtCl_2(phen)]$:** A suspension of $[PtCl_2(DMSO)_2]$ (359 mg, 0.85 mmol) in methanol (100 mL) was treated with 1,10-phenanthroline (170 mg, 0.85 mmol). After stirring for one day, the newly formed precipitate was collected by filtration of the reaction mixture. The filtrate was concentrated to half its volume by evaporation of the solvent under reduced pressure and kept at room temperature for a few more days while another crop of yellow precipitate was formed. The combined solid fractions were washed with methanol and dried under vacuum to give 367 mg of product (97% yield based on starting platinum complex). $C_{12}H_8Cl_2N_2Pt$ (446.2): calcd. C 22.9, H 1.3, N 4.5; found C 22.6, H 1.3, N 4.4.

**$[Pt(Me_2phen)(Guo)_2]Cl_2$ (1):** $[PtCl_2(Me_2phen)]$ (250 mg, 0.53 mmol) was dissolved in methanol/chloroform (150 mL; 2:1, v/v) and a solution of guanosine in methanol (299 mg, 1.06 mmol, in 100 mL of solvent) was added dropwise to the resulting solution. The reaction mixture was stirred for two hours at room temperature and then taken to dryness by evaporation of the solvents under vacuum. The solid residue was treated with water (10 mL) and the mixture stirred overnight. The water solution was then filtered and taken to dryness by evaporation of the solvent under vacuum. The yellow residue proved to be pure $[Pt(Me_2phen)(Guo)_2]Cl_2$ (496 mg, 90% yield). $C_{34}H_{38}Cl_2N_{12}O_{10}Pt$ (1040.7): calcd. C 39.2, H 3.7, N 16.2; found C 39.8, H 3.5, N 16.8.

**$[PtCl(Me_2phen)(Guo)]Cl$ (2):** $[PtCl_2(Me_2phen)]$ (100 mg, 0.21 mmol) was dissolved in methanol/chloroform (80 mL; 2:1, v/v) and a stoichiometric amount (1:1 molar ratio) of guanosine dissolved in methanol (119 mg, 0.1 mmol, in 50 mL of solvent) was added dropwise to the resulting solution. The reaction mixture was stirred for two hours at room temperature and then concentrated under vacuum to 50 mL. Addition of diethyl ether induced the precipitation of a yellow product which was separated by filtration of the reaction mixture, washed with diethyl ether, and dried under vacuum to give 135 mg of product (85% yield). $C_{24}H_{25}Cl_2N_7O_5Pt$ (757.5): calcd. C 38.1, H 3.3, N 12.9; found C 37.8, H 3.1, N 12.7.

**$[Pt(phen)(Guo)_2](NO_3)_2$ (3):** $[PtCl_2(phen)]$ (200 mg, 0.56 mmol) was dissolved in DMF (70 mL) and treated with $AgNO_3$ (190 mg, 1.12 mmol) previously dissolved in the minimum amount of water. The solution was left stirring in the dark for 12 hours and a white precipitate of AgCl formed. The reaction mixture containing the solvato species $[Pt(DMF)_2(phen)]^{2+}$ was filtered through celite and treated with guanosine (317 mg, 1.12 mmol) previously dissolved in the minimum amount of DMF. This mixture was stirred for 5 hours at room temperature and then treated with diethyl ether to induce the formation of a yellow precipitate. The solid product was separated by filtration of the mother liquor, washed with several aliquots of diethyl ether and dried under vacuum to give 477 mg of product (80% yield). $C_{32}H_{34}N_{14}O_{16}Pt$ (1065.8): calcd. C 36.1, H 3.2, N 18.4; found C 36.6, H 3.3, N 18.2.

**$[PtCl(phen)(Guo)](NO_3)$ (4):** The procedure used for the preparation of $[PtCl(phen)(Guo)](NO_3)$ was similar to that used for compound **3** with the only difference being that only one equivalent of $AgNO_3$ and guanosine were added per mol of platinum substrate. In addition to compound **4** some compound **3** was also formed as

side-product, this prevented the characterisation of **4** by elemental analysis.

## Acknowledgments

The authors thank the University of Bari, the "Ministero dell'Istruzione Università e Ricerca (MIUR)" (Cofin. N. 2001053898 and 2000 N. MMO3027791), and the EC (COST Chemistry projects D20/0001/2000 and D20/0003/01) for financial support. The authors are also grateful to Vincenzo Alicino for the help in the synthesis of the complexes.

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Received May 21, 2002
[I02262]