Effects of N7 Platinum Binding on the Hydrogen-Bonding Behavior of 9-Ethylguanine[1]

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Abstract: The ^{1}H NMR spectra of several *cis*-diammineplatinum(II) complexes of the model nucleobases 9-ethylguanine, G, and 1-methylcytosine, C, in Me_2SO are reported: *cis*-$[Pt(NH_3)_2G_2](ClO_4)_2$, *cis*-$[Pt(NH_3)_2GC](ClO_4)_2$, *cis*-$[Pt(NH_3)_2(G\text{-}H)C]ClO_4$, and *cis*-$[\{Pt(NH_3)_2GC\}\{Pt(NH_3)_2(G\text{-}H)C\}](ClO_4)_3$. In all complexes platinum is bound to G via N7 and to C via N3. Deprotonation of the G ligand at N1 is facilitated through Pt coordination at N7 (pK = 8.2 compared to 9.8 for the free G). The hydrogen-bonding behavior of the platinum complexes toward G, C, and 1-methylthymine, T, has been studied in Me_2SO by means of ^{1}H NMR spectroscopy. Downfield shifts of the protons involved in hydrogen bonding were used as a qualitative estimation of the stability of hydrogen bonding and compared with the Watson–Crick G≡C base pair in the same solvent. The G ligand undergoes profound changes in its hydrogen-bonding pattern when coordinated to platinum through N7. Hydrogen bonding with C is reduced and almost completely prevented with the G ligand deprotonated at N1. There is also a complete loss of selectivity for G≡C base pairing as indicated by hydrogen bonding of the neutral G ligand with T and of the anionic G ligand with T and G. In particular the novel hydrogen-bonding scheme between the platinated guanine anion and neutral guanine, which involves three hydrogen bonds, is quite strong. The *cis*-diammine groups contribute to the observed loss of G≡C base pairing selectivity.

The ability of metal ions to either stabilize or destabilize double stranded DNA is well-established.[2] A stabilization usually is achieved through metal binding to the phosphate groups, either electrostatically or covalently,[3] whereas a destabilization is generally associated with irreversible metal binding to hydrogen-bonding sites of the nucleobases. Occasionally a reversible unwinding and rewinding of DNA is observed in the presence of certain metal cations, for example Zn^{2+}, and this is probably due to a reversible cross-link formation between two DNA strands.[4]

Little is known about the effect of metal binding on the DNA structure if the site of coordination is not interfering with hydrogen bonding of the bases, e.g., N7 of guanine or adenine or C8 of these bases. These sites are more accessible for an initial nucleophilic attack than those involved in hydrogen bonding. Therefore, it is feasible that metal binding to these sites may be important for the un- and rewinding process of DNA. Moreover, genetic miscoding through an alteration of the template base specificity, a well-known phenomena for a large number of metals,[5] could conceivably take place through metal binding to such sites.

It was the objective of this study to find out how N7 platination of guanine and deprotonation of guanine at N1 as a consequence of N7 metal binding affect the hydrogen-bonding pattern of this ligand. This question appeared to be of importance because any alteration of the hydrogen-bonding behavior of a nucleobase could influence both the stability of the base pair and the selectivity of the base pairing process. Apart from these effects, others are of course also possible, e.g., those on base stacking or the sterical arrangement of adjacent bases.

The compounds used in this study were model complexes of possible *cis*-diammineplatinum(II)–DNA cross-linking products containing 9-ethylguanine, G, and 1-methylcytosine, C, as model nucleobases. They include *cis*-$[Pt(NH_3)_2G\ C](ClO_4)_2$ and *cis*-$[Pt(NH_3)_2(G\text{-}H)C]ClO_4$, the structures of which have been determined by X-ray diffraction,[6] as well as *cis*-$[Pt(NH_3)_2G_2](ClO_4)_2$. The hydrogen-bonding partners that were investigated were 9-ethylguanine, G, 1-methylcytosine, C, and 1-methylthymine, T.

cis-Diammineplatinum(II) complexes were used because of their interesting biological activities: they not only represent excellent antitumor agents,[7] and they are also mutagenic[8] and weakly carcenogenic.[9] There is substantial evidence pointing to DNA as the primary target for at least the first two modes of action,[10] and to regions rich in guanine and cytosine content as the preferred binding sites.[11] In particular, binding to N7 of guanine has been favored,[12,13] even though N7,O6 chelation of guanine has been proposed as well,[14] and despite controversial results on the preferred binding sites in GC dinucleotides.[15] Thus far platinum binding to guanine residues has unambiguously been confirmed only at the N7 position.[6,16]

Experimental Section

Materials. C and T and (Vega) were recrystallized from water and vacuum sublimed, respectively; G (Vega, Sigma) was recrystallized from water. *cis*-$Pt(NH_3)_2Cl_2$ (Degussa) was recrystallized from DMF + 0.1

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N HCl. Vacuum dried NBu_4ClO_4 (Fluka) was stored under N_2. Dimethyl-d_6 sulfoxide, Me_2SO (Merck, Roth, Merck Sharp and Dohme Canada Ltd.), and dimethylformamide-d_7, Me_2NCHO (DMF) (Roth), were stored over 4 Å molecular sieves under dry N_2. Samples of Me_2SO were distilled over CaH_2 but did not show properties different from those of undistilled Me_2SO (cf. section on G + T). Molecular sieves (Roth) were washed with distilled water and EtOH and dried at 340 °C under high vacuum.

Pt Compounds. ***cis*-[Pt(NH$_3$)$_2$G$_2$](ClO$_4$)$_2$.** *cis*-$Pt(NH_3)_2Cl_2$ and $2AgClO_4 \cdot H_2O$ were stirred in water (1 h, 60 °C), and 2G was added. After 30 h at 40 °C (*c* 0.05 M Pt) the mixture was briefly heated to 100 °C, cooled, and filtered from AgCl. Slow evaporation at 40 °C yielded 80% compound as a white powder. Anal. Calcd (monohydrate): C, 20.90; H, 3.26; N, 20.90. Found: C, 21.28; H, 3.22; N, 20.80.

***cis*-[Pt(NH$_3$)$_2$GC](ClO$_4$)$_2$.** Reaction of *cis*-$Pt(NH_3)_2Cl_2$ with C gave *cis*-$[Pt(NH_3)_2ClC]Cl \cdot H_2O$.[17] Reaction with $2AgClO_4 \cdot H_2O$ and 1G (*c* = 0.03 M Pt, H_2O, 24 h, 40 °C), filtration of AgCl, and slow evaporation gave the desired compound, yield 80–90% with recrystallization from H_2O as colorless crystals. Anal. Calcd (hemihydrate): C, 19.44; H, 3.13; N, 18.90; O, 22.66; Pt, 26.31. Found (air dried): C, 19.70; H, 2.96; N, 18.85; O, 23.06; Pt, 26.0.

***cis*-[Pt(NH$_3$)$_2$(G-H)C]ClO$_4$.** Titration of *cis*-$[Pt(NH_3)_2GC](ClO_4)_2$ with 1NaOH under N_2 and subsequent concentration gave the compound in 80–85% yield as colorless crystals. Anal. Calcd (tetrahydrate): C, 20.45; H, 4.16; N, 19.90. Found (freshly prepared sample): C, 20.79; H, 4.34; N, 19.62. Anal. Calcd (monohydrate): C, 22.17; H, 3.57; N, 21.55; Pt, 30.01. Found (sample 20 h at high vacuum): C, 22.26; H, 3.60; N, 21.82; Pt, 29.6.

***cis*-[{Pt(NH$_3$)$_2$GC}{Pt(NH$_3$)$_2$(G-H)C}](ClO$_4$)$_3$.** Titration of *cis*-$[Pt(NH_3)_2GC](ClO_4)_2$ with 0.5NaOH under N_2 and subsequent concentration of the solution (pH 8.2) gave the compound in 65% yield as colorless crystals. Anal. Calcd (monohydrate): C, 20.87; H, 3.29; N, 20.28; O, 19.69; Pt, 28.24. Found: C, 21.11; H, 3.23; N, 20.16; O, 20.26; Pt, 27.7.

Sample Preparation. Weighted amounts of the respective components were dissolved in the dry solvent and succeedingly diluted by addition of solvent. Spectra were recorded immediately after dissolving the compounds or after 1–3 days of drying over molecular sieves. All procedures were performed under dry N_2. Experiments were carried out at least twice to ensure reproducibility. Agreement was generally very good, and limited by errors in concentration only.

Apparatus. ^1H NMR spectra were recorded on a JEOL JNM-FX 60 Fourier transform spectrometer (200–3000 transients, 8K data points) at 30 ± 1 °C unless otherwise stated. Shifts are quoted in ppm relative to internal Me_4Si (δ scale) and are believed to be accurate ±0.004 ppm for CH signals and ±0.012 ppm for NH signals at concentrations down to 0.1 M. At lower concentrations the accuracy of the NH shifts is somewhat smaller.

Methods and Results

The hydrogen-bonding behavior of the compounds containing N7 platinated G was studied by means of ^1H NMR spectroscoy in Me_2SO-d_6. Hydrogen-bond formation causes a decrease in magnetic shielding of the protons involved and consequently to a downfield shift.[18] By studying both temperature and concentration dependencies of these shifts, it is possible to differentiate between inter- and intramolecular hydrogen bonding. Several studies on the interaction of nucleobases in Me_2SO or solvent mixtures containing Me_2SO have been performed and have demonstrated the usefulness of this method.[19-23]

Self-Association of Nucleobases and the Influence of ClO_4^-. Since this study is based on observed shift differences between the individual components and mixtures of these, the self-association had to be taken into account to avoid misinterpretations. Moreover, the influence of ClO_4^- on the shifts of the base resonances had to be studied in order to compensate for possible hydrogen bonding with the anion(s) of the platinum complexes and/or changes in the solvent structure due to the presence of ClO_4^-. Interactions of nucleobases with Cl^- in Me_2SO have been observed,[24,25] whereas NO_3^- and ClO_4^- have been reported not to cause appreciable shifts of NH resonances. In the present study tetrabutylammonium perchlorate, NBu_4ClO_4, was used to exclude possible interactions between the bases and the cation of the perchlorate salt. The effect of NBu_4ClO_4 on the NH,NH_2 resonances of G and C was such as to slightly shift these resonances upfield. Certainly, this does not reflect a hydrogen-bonding interaction between ClO_4^- and G,C, but some other effect. Since the presence of ClO_4^- does not affect the interaction shifts between two bases (cf. G + C, Supplementary Material), ClO_4^- needs not be considered a potential hydrogen bonding partner for these bases in the case of the platinum complexes discussed below.

No self-association of T was observed in dry Me_2SO and no influnce of NBu_4ClO_4. The NH_2 signal of C shows a very slight concentration dependency, in agreement with earlier findings.[19,21] The same applies to NH, NH_2, and H8 resonances of G.[21] Interaction shifts between G and C are considerably larger than those resulting from self-association, and reflect the high stability of the Watson–Crick G + C base pair. They are in the order of 1 ppm for the NH signal of G and 0.5 ppm for the NH_2 signals of G and C at a concentration of 0.2 M each, and in good agreement with published data.[19-21] Possible explanations for the also observed small downfield shifts of the ring CH protons and the CH_3 signal of C have been given.[21] There is no hydrogen bonding between C and T and between G and T. However, spectra of mixtures of G + T, likewise those of mixtures of the Pt complexes with T and G, respectively, revealed coalescence of the NH resonances of G and T, if the solutions were extensively dried over molecular sieves. The presence of small amounts of water in Me_2SO, or addition of trace amounts of P_4O_{10} to the dry solution, gave separate NH signals for G and T.[26] This phenomena is attributed to a base-catalyzed proton exchange caused by a partial hydrolysis of the zeolite used as drying agent,[27] rather than to a basic impurity in Me_2SO. Distilled Me_2SO showed the same behavior when water plus molecular sieves were added (cf. Supplementary Material).

^1H NMR Spectra of the Platinum Complexes. ***cis*-[Pt(NH$_3$)$_2$G$_2$](ClO$_4$)$_2$.** NH, and NH_2, and H8 resonances of the coordinated G ligands are shifted downfield relative to free G′ (in order to avoid confusion between coordinated and free nucleobases, free bases are denoted as G′, C′, etc.) by about 0.8, 0.5, and 0.4 ppm, respectively, as is to be seen particularly well in the spectrum of a mixture containing the platinum complex and G′ (cf. Supplementary Material). These shifts are smaller than those observed upon protonation of G.[29] ^{195}Pt coupling is observed for NH_3 (58.0 Hz) and H8 (21.0 Hz), thus verifying N7 platinum binding.[16b,30] The monohydrate exhibits separate signals for H_2O, NH, NH_2, and NH_3, thus indicating no proton exchange on the NMR time scale. Some self-association of $Pt(NH_3)_2G_2^{2+}$ is occurring in the anhydrate, involving NH and NH_3 as hydrogen donors, and a considerable one in the monohydrate, involving NH, H_2O, and NH_3. In both cases involvement of the NH_3 groups may be more pronounced than the relatively small shift might

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(26) In the presence of P_4O_{10}, some of the G resonances, either alone or in a mixture with T, undergo downfield shifts (H8,NH_2) and broadening (NH_2) upon dilution. Since this does not occur with N7 platinated complexes, it is tentatively assigned to an interaction of H_3PO_4 at N7. Cf. also ref. 28 and 29.
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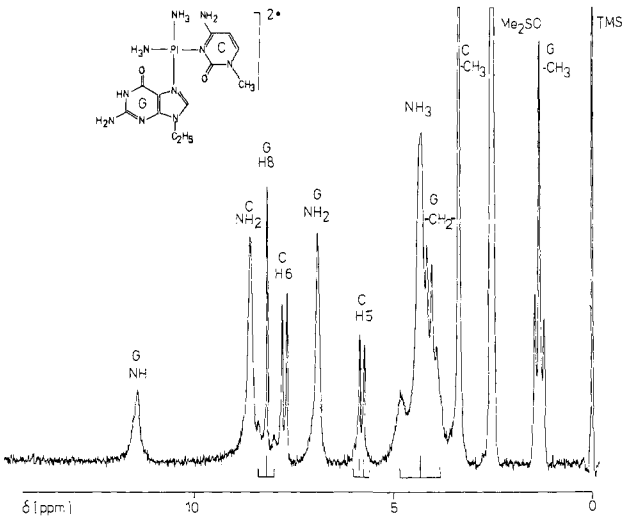

Figure 1. ^{1}H NMR spectrum of *cis*-$[Pt(NH_3)_2GC](ClO_4)_2$ (0.1 M) after drying over molecular sieves.

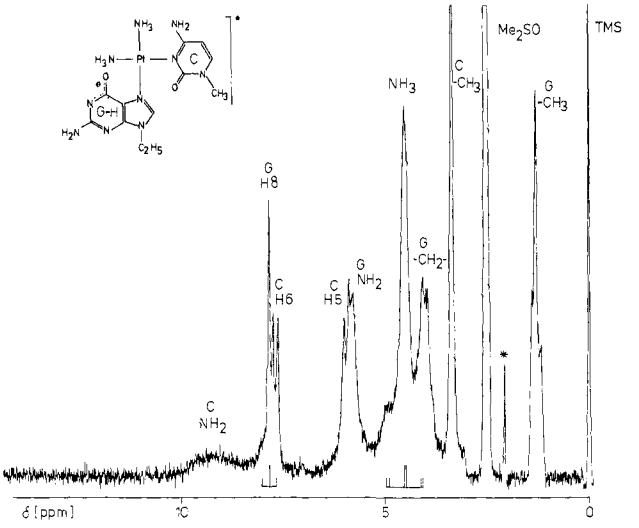

Figure 2. ^{1}H NMR spectrum of *cis*-$[Pt(NH_3)_2(G\text{-}H)C]ClO_4$ (0.2 M) after drying over molecular sieves (the asterisk indicates an acetone impurity).

indicate, since averaging over hydrogen-bonded and nonbonded protons occurs. Intramolecular hydrogen bonding between O6 of G and NH_3[32] cannot be excluded, because it is not expected to show a concentration-dependent shift for the NH_3 protons.

***cis*-$[Pt(NH_3)_2GC](ClO_4)_2$.** Its ^{1}H NMR spectrum after drying over molecular sieves is shown in Figure 1. G resonances occur at positions close to those observed in the bis(G) complex. The H5 signal of C shows ^{195}Pt coupling ($\simeq$14 Hz) as expected when Pt binding is through N3. H5, H6, and CH_3 resonances of C undergo downfield shifts of ca. 0.2 ppm upon platination, and NH_2 of 1.7 ppm. The latter reflects a substantial acidification of the NH_2 protons, believed to contribute to the observed easy deprotonation in the presence of additional Pt.[33] Similar or even larger downfield shifts of NH_2 upon metal coordination at N3[23,34] or protonation[25,35] have been reported before. Self-association of the anhydrate is to be neglected, but there is some weak association between NH of G and H_2O in the hydrous compound.

***cis*-$[Pt(NH_3)_2(G\text{-}H)C]ClO_4$.** As a consequence of Pt coordination at N7 of G, marked increase in the acidity of the N(1)H proton takes place ($\Delta pK = 1.6$).[6] Similar acidifications have been reported before for both Pt and other metals.[15a,32,36] As expected from the method of preparation, and confirmed by X-ray analysis, G-H is bound to Pt via N7, and C through N3. In Figure 2 the ^{1}H NMR spectrum after drying over molecular sieves is given. Compared to the GC compound, the NH_2 signal of G is influenced most on deprotonation, and shifted upfield by 1.1 ppm, followed by H8 (0.34 ppm). Clearly, the effect of the negative charge is more pronounced in the pyrimidic ring than in the imidazolic ring. The C ligand cis to G-H is also affected: NH_2 is shifted downfield and broadened considerably (beyond resolution with hydrous samples), H6 is shifted upfield, and H5 is shifted downfield. Most of the resonances (NH_3; G: NH, NH_2, H8; C: H5, NH_2(?)) are concentration dependent, indicating intermolecular association. As is evident from the X-ray structure,[6] an intramolecular hydrogen bond between O6 of G and NH_2 of C is feasible as well. It might contribute to the observed downfield shift of the C-NH_2 resonance and should not be concentration dependent. With D_2O as solvent, a gradual decrease of the G-H8 signal intensity as a conseqence of isotopic hydrogen exchange is observed. Similar metal-induced activations of H8 of purines have been reported before.[37]

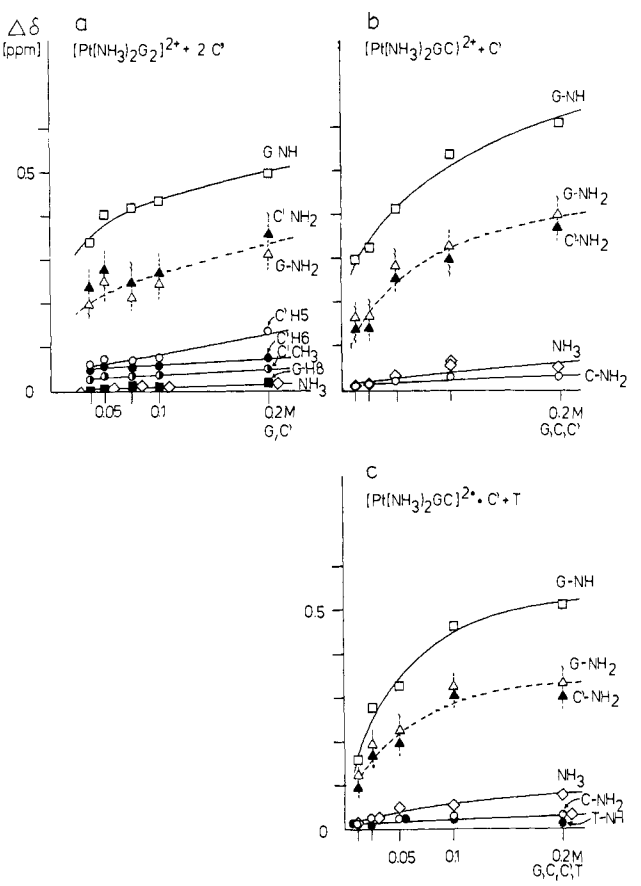

Figure 3. Interaction shifts of: (a) *cis*-$[Pt(NH_3)_2G_2]^{2+}$ + 2C'; (b) *cis*-$[Pt(NH_3)_2GC]^{2+}$ + C'; and (c) *cis*-$[Pt(NH_3)_2GC]^{2+}$ + C' + T. Interaction shifts of C' relative to free base in the presence of $2NBu_4ClO_4$ per base. For clarity, shifts of G-H8 and C'-(H5,H6,-CH_3) are omitted in Figures 3b and 3c. Their values in Figure 3b are close to those in Figure 3a, and in Figure 3c they are slightly smaller. Interaction shifts of C-(H5,H6,-CH_3) are omitted in Figures 3b and 3c, since they are even smaller than the C-NH_2 shift. A similar reduction in G-NH and G-NH_2, C'-NH_2 interaction shifts is observed for *cis*-$[Pt(NH_3)_2G_2]^{2+}$ + 2C' in the presence of 2T (not shown).

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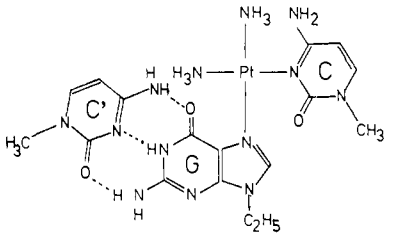

Figure 4. Principal hydrogen bonding scheme between the N7 platinated G ligand and C′.

Interactions between *cis*-$[Pt(NH_3)_2G_2]^{2+}$, $[Pt(NH_3)_2GC]^{2+}$, $[Pt(NH_3)_2(G\text{-}H)C]^+$, and 1-Methylcytosine. Pt Complexes with Neutral G Ligands. In Figure 3 interaction shifts between the GC and G_2 complexes in the presence of 1C′ and 2C′, respectively, at different concentrations are presented. Because of the superposition of the NH_2 signals of G and C′, only approximate interaction shifts for these resonances are given. Small amounts of water (up to 1.5 M per Pt) in Me_2SO do not cause significant shifts in either direction. As can be seen, downfield shifts with increasing concentrations are observed for almost all resonances, with G-NH, NH_2, and C′-NH_2 affected the most. Compared with the Watson–Crick G′ + C′ base pair, however, the G-NH interaction shifts are only 50–60% with the Pt complexes, and NH_2 interaction shifts of G and C′ are reduced by about 30%. Downfield shifts of G-H8 and C′-(H5,H6,CH_3) are of similar magnitudes as those between G′ and C′, and the downfield shifts of C-(H5,H6,CH_3) in the GC complex are too low to be considered a consequence of any substantial hydrogen-bonding interaction. Hydrogen bonding between C′ and NH_3 appears possible in the GC complex, but hardly in the G_2 compound. Thus the principal hydrogen-bonding interaction between N7 platinated G and free C′ occurs in the Watons–Crick fashion, although to a clearly reduced extent (Figure 4). Some additional hydrogen bonding involving the NH_2 group(s) of G and/or C′ has to be postulated to account for these shifts in comparison with the G-NH shift.

Protonation of C′ according to $Pt(G) + C' \rightleftharpoons Pt(G\text{-}H) + HC'$ as an alternative explanation for the observed downfield shifts of C′ resonances is ruled out, since the G-H8 shift is not consistent with deprotonation.

***cis*-$[Pt(NH_3)_2(G\text{-}H)C]^+$ + C′.** Interaction shifts are plotted in Figure 5. No accurate interaction shifts were obtained for C-NH_2 because of the broadness of this signal in both the complex itself and in the mixtures with C′. However, it appears certain that a shift in either direction would be less than 0.1 ppm in the concentration range studied. For some of the resonances negative interaction shifts are obtained (Figure 5a), which probably is a consequence of the change from self-association of the (G-H)C complex to association with C′. If it were due to stacking interactions, no crossing of the curves with the $\Delta\delta = 0$ line should occur. By taking the shifts of the Pt complex at infinite dilution (self-association negligible) as the basis, "corrected" interaction shifts are obtained (Figure 5b), which are of the characteristic of those observed in Figure 3 with no self-association of the Pt complex. The interaction shift of C′-NH_2 clearly tells that hydrogen bonding of C′ with the NH_2 protons acting as hydrogen donors must be weak, weaker than that between platinated G and free C′, and much weaker than that between G′ and C′. The considerable interaction shift of one of the two NH_3 groups demonstrates that C′ acts as a strong hydrogen acceptor through N3 and O2. The same applies to G-NH_2 and, to some extent, also to C-H5 and G-H8. With the many ways of possible hydrogen bonds between C′ and the Pt complex that could account for the observed interaction shifts, no unique hydrogen-bonding model can be proposed at present. However, the important conclusion to be drawn from the above finding is, that hydrogen bonding between deprotonated G and C′ in a Watson–Crick-like fashion is either very much reduced or not occurring at all in Me_2SO. As to the reason for this behavior apart from possible electronic effects within the deprotonated G ligand, two other aspects can be seen: First, there is a reduction in hydrogen bonding

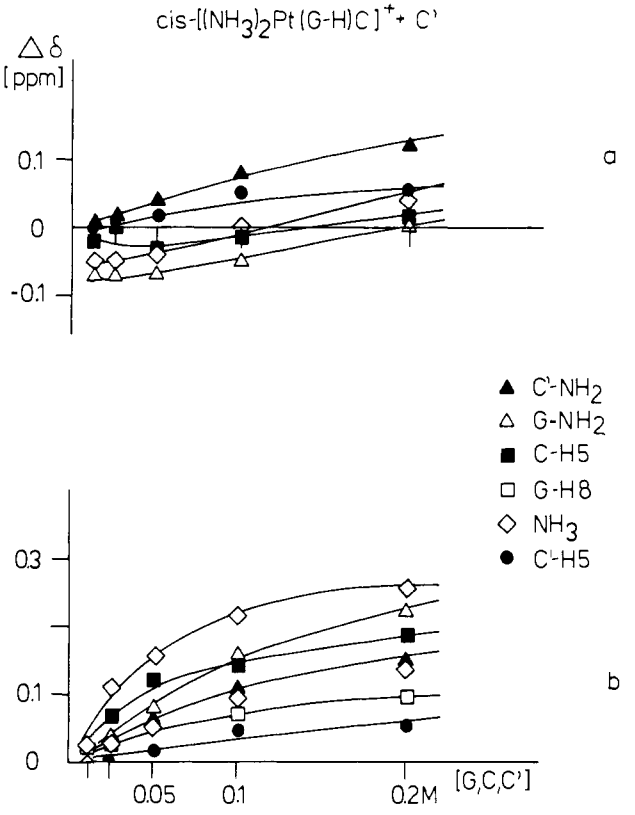

Figure 5. Interaction shifts for 1:1 mixtures of *cis*-$[Pt(NH_3)_2(G\text{-}H)C]^+$ and C′: (a) uncorrected for Pt complex resonances; (b) corrected for Pt complex without self-association. Interaction shifts of C′ are unaffected by "correction". They are relative to C′ in the presence of 2 equiv of NBu_4ClO_4.

sites between (G-H) and C′. Only a maximum of two hydrogen-bonding sites remain as the N1 position becomes deprotonated. This necessarily reduces the stability of such a base pair and permits Me_2SO as a strong hydrogen acceptor to successfully compete for binding sites. Second, the introduction of six protons of the NH_3 groups increases the overall number of available hydrogen donors considerably.[38] It appears that, unless formation of three hydrogen bonds between two partners is possible, there is a good chance of other hydrogen bonds being formed with the free nucleobase primarily acting as a hydrogen acceptor.

Interactions between *cis*-$[Pt(NH_3)_2G_2]^{2+}$, $[Pt(NH_3)_2GC]^{2+}$, $[Pt(NH_3)_2(G\text{-}H)C]^+$, and 1-Methylthymine. *cis*-$[Pt(NH_3)_2G_2]^{2+}$, *cis*-$[Pt(NH_3)_2GC]^{2+}$ + T′. Very slight interaction shifts for G-NH, T′-NH, and NH_3 were measured (cf. Supplementary Material), which do not justify the postulation of a particular hydrogen-bonding scheme. Nevertheless it is possible to confirm hydrogen bonding between the above Pt complexes and T′ by an indirect method: Since there is no hydrogen bonding between C′ and T′, it is possible to use the interaction shift between platinated G and free G′ in the presence of T′ as a probe for an interaction between the platinum complexes and T′. When doing so, one finds that binding between platinated G and C′ is reduced if T′ is present (Figure 3c). The observed reductions between G and C′ interaction shifts are very similar for both Pt complexes. From comparison with the interaction shifts in the absence of T′ it becomes evident that T′ forms hydrogen bonds with the Pt complex in such a way that it interferes with the three hydrogen-bonding sites of G. As to the possible binding modes, it seems highly unlikely that a single hydrogen bond between G and T′ (T′-NH, G-O6 or G-NH, T′-O) could favorably compete with the three hydrogen bonds possible between G and C′. Formation of an additional

(38) G′ + C′: 7 acceptor sites (G′: 06(2),N3,N7; C′: 02(2),N3), 5 donors (G′: NH,NH_2(2); C′: NH_2(2)). $[Pt(NH_3)_2(G\text{-}H)C]^+$ + C′: 9 acceptor sites, 12 donors. Acceptor sites do not include the NH_2 groups. CH protons are not counted as donors.

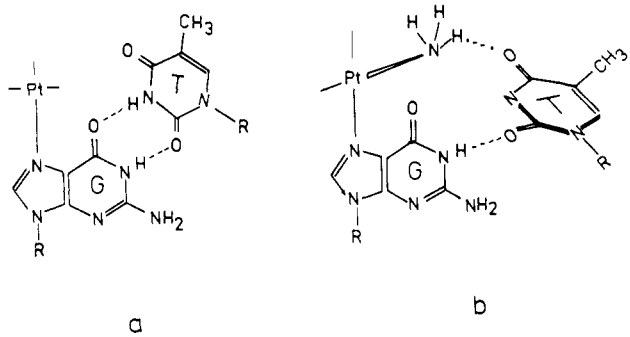

Figure 6. Possible hydrogen-bonding interactions between N7 platinated guanine and T: (a) "wobble" pair with G and T approximately in the same plane; (b) hydrogen bonding between G, T, and NH_3. T is not coplanar with G. In both models O2 and O4 of T could be interchanged.

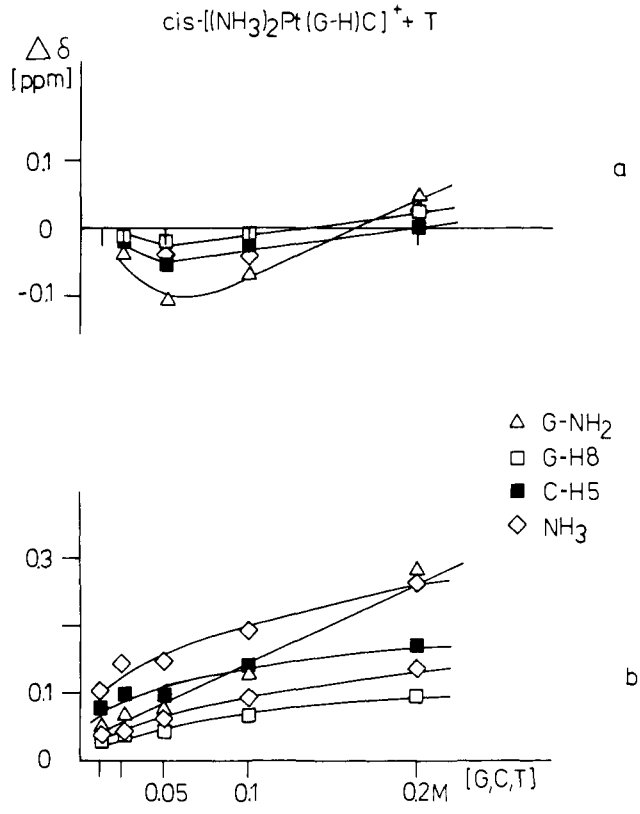

Figure 7. Interaction shifts for a 1:1 mixture of *cis*-$[Pt(NH_3)_2(G\text{-}H)C]^+$ and T: (a) uncorrected for Pt complex resonances; (b) corrected for Pt complex without self-association.

bond between T′ and the Pt complex might permit some competition of T′ and C′ for hydrogen-bonding sites at G. Two models, neither of which by itself fully explains the observed (reduced) interaction shifts, might be considered: a G + T′ interaction of the "wobble" type[39] with two hydrogen bonds between G and T′, or an arrangement allowing hydrogen bonding between G-NH, NH_3, and the two exocyclic oxygens of T′ (Figure 6). In this case, G and T′ are not coplanar, but T′ is twisted out of the G plane, thus minimizing any G-O6, T′-NH interaction.

***cis*-$[Pt(NH_3)_2(G\text{-}H)C]^+$ + T′.** Calculated and corrected interaction shifts are plotted in Figure 7. Because of proton exchange between C-NH_2 and T′-NH, no interaction shifts could be obtained for these resonances. As to the reason for this exchange, no clear answer is possible at present. Plausible explanations would be that it is due to the presence of small amounts of NaOH introduced into the system with the Pt compound,[40] or that OH^- is generated if H_2O is present in Me_2SO according to $Pt(G\text{-}H) + H_2O \rightleftharpoons Pt(G) + OH^-$. The observed interaction shifts must be due to hydrogen bonding, and cannot be explained on the basis of an assumed acid–base reaction $[Pt(NH_3)_2(G\text{-}H)C]^+ + T' \rightleftharpoons [Pt(NH_3)_2GC]^{2+} + (T'\text{-}H)$.

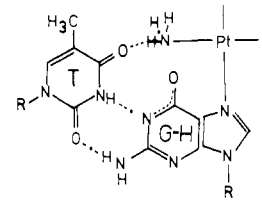

Figure 8. Possible hydrogen bonding interaction between N7 platinated, N1 deprotonated guanine and T. (G-H) and T are not copolanar.

The H6 resonance of T′ remains unchanged as the Pt compound is added, thus ruling out ionization of T′, and the shift of the NH_3 signals occurs in a direction opposite to what is expected if proton transfer were to happen. The relatively large NH_3 shifts clearly demonstrate involvement of these groups in hydrogen bonding. Strong or only moderate binding has to be anticipated for the G-NH_2 protons, depending on whether a single or both protons are involved. The unavailability of T′-NH shifts represents a serious disadvantage with regard to possible hydrogen-bonding models. A similar interaction as proposed for association of T′ with anionic G,[41] 7,9-alkylated G,[42] and O6-methylguanosine[43] could occur between Pt(G-H) and T′, possible reinforced by an additional bond between T′-O4 and NH_3 cis to (G-H). If so, it would require T and (G-H) not to be coplanar, a reasonable assumption with respect to lone electron pair repulsion between T′-O4 and G-O6 (Figure 8). Again, other hydrogen-bonding schemes have to be considered to account for C-H5 and (G-H)-H8 shifts, for example.

Neutral and Anionic G Ligands in the Presence of 9-Ethylguanine. *cis*-$[Pt(NH_3)_2G_2]^{2+}$ + G′. No hydrogen bonding has been observed in mixtures of these two components. Interestingly, however, the G-NH signal is considerably broadened as G′ is added, and the H_2O peak of the Pt complex disappears, indicating proton exchange.

***cis*-$[Pt(NH_3)_2(G\text{-}H)C]^+$ + G′.** In Figure 9 interaction shifts are given. The G′-NH signal was not detected, probably due to exchange with C-NH_2 (cf. discussion on *cis*-$[Pt(NH_3)_2(G\text{-}H)C]^+ + T'$). There is no H transfer from G′ to (G′H), as indicated by the unchanged position of G′-H8. The unequal interaction shifts of the NH_2 signals of G′ and (G-H) suggest that not only a single proton of G′-NH_2 is involved in hydrogen bonding. Provided the (G-H)-NH_2 shift is due to a single hydrogen bond, such a hydrogen-bonding interaction between anionic and neutral G must be stronger than that between G′ and C′. A plausible bonding scheme is shown in Figure 10. Additional hydrogen bonding through G′-NH_2 (possibly to C-O2, (G-H)-N3, O6) may lead to formation of larger aggregates. Despite the lack of information on the G′-NH shift, it seems very likely that only a triple hydrogen bond, with Me_2SO as solvent, causes NH_2 shifts of the magnitude observed.

The ^1H NMR Spectrum of *cis*-[{$Pt(NH_3)_2GC$}{$Pt(NH_3)_2(G\text{-}H)C$}]$(ClO_4)_3$. Titration of *cis*-$[Pt(NH_3)_2GC]^{2+}$ with base to a pH corresponding to the pK of the deprotonation of G ($\simeq$8.2) gives *cis*-[{$Pt(NH_3)_2GC$}{$Pt(NH_3)_2(G\text{-}H)C$}]$^{3+}$. X-ray analysis shows two $Pt(NH_3)_2GC$ units connected through three hydrogen bonds between G and (G-H), involving NH_2, NH, and O6.[6] Only a single set of proton signals for the Pt ligands is observed, thus

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(40) Since $[Pt(NH_3)_2(G\text{-}H)C]^+$ is isolated from an aqueous solution of pH 10–11, such an assumption is reasonable.

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(43) Abbot, P. J.; Saffhill, R. *Biochim. Biophys. Acta* **1979**, *562*, 51 and references therein.

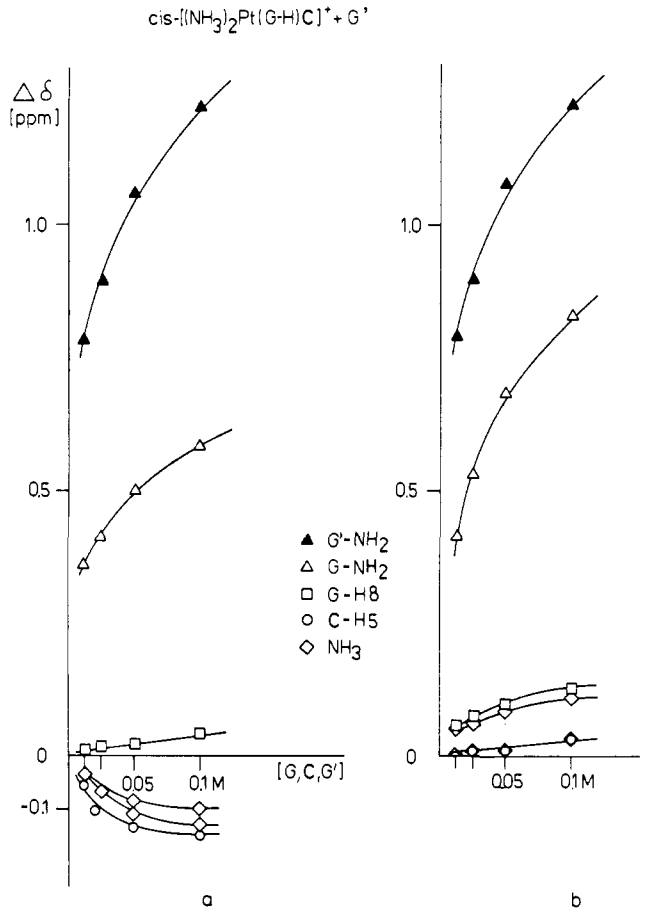

Figure 9. Interaction shifts for 1:1 mixtures of *cis*-$[Pt(NH_3)_2(G\text{-}H)C]^+$ and G': (a) uncorrected for Pt complex resonances; (b) corrected for Pt complex without self-association. Interaction shifts of G' are unaffected by "correction", and are relative to G' in the presence of 2 equiv of NBu_4ClO_4.

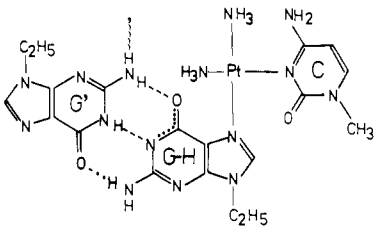

Figure 10. Possible hydrogen-bonding scheme between *cis*-$[Pt(NH_3)_2(G\text{-}H)C]^+$ and G'.

indicating exchange of NH between G and (G-H), with the NH signal broadened beyond resolution. Signals are generally between those of the individual components, except the G-NH_2 signal, which is shifted downfield by about 1 ppm at a concentration of 0.1 M G,(G-H). Cooling (Me_2SO, Me_2NCHO mixture) shifts this resonance further downfield. When *cis*-$[Pt(NH_3)_2(G\text{-}H)C]^+$ is added to a solution of $[Pt(NH_3)_2GC]^{2+}$ in increasing amounts,[44] a downfield shift and a simultaneous broadening of G-NH are observed. The downfield shift is on the order of 0.15 ppm at G:(G-H) = 9 and a total c_{Pt} = 0.25 M. At G:(G-H) = 5, the NH signal is already too broad to be observed, and cooling to −10 °C does not sharpen it again.

The observed downfield shifts of guanine NH,NH_2 resonances must be a consequence of hydrogen bonding involving these groups. If the G-NH_2 shifts were due to an exchange with the G-NH signal, the latter should not be shifted downfield as well, but upfield. Moreover, the integrated intensity of the NH_2 signal clearly rules out such a possibility. In essence, it is suggested that in Me_2SO solution the same triply hydrogen bonded dimer exists as found in the solid state.[6] As to the stability of this associate, it should be even stronger than that between $[Pt(NH_3)_2(G\text{-}H)C]^+$ and G', provided only a single proton of each NH_2 group is involved in hydrogen bonding. The C-NH_2 signal of $[Pt(NH_3)_2GC]^{2+}$ becomes split as $[Pt(NH_3)_2(G\text{-}H)C]^+$ is added, with the high-field component broader than the low-field one. This broadening could be a consequence of proton exchange between G and (G-H), which might be felt by one of the two C-NH_2 protons involved in an intramolecular hydrogen bond with G-O6. There is evidence from X-ray work for such a hydrogen bond, and spectroscopic findings (downfield shift with decreasing temperature, concentration insensitivity) support this interpretation. Splitting of C-NH_2, observed also in related Pt complexes,[33,45] appears not necessarily to be the direct consequence of N3 platination, as can be concluded from the appearance of a single signal in $[Pt(NH_3)_2GC]^{2+}$. As has been made clear by Marzilli et al.,[25] inequivalence of C-NH_2 protons can result from simple hydrogen-bonding interactions, e.g., between C and Cl^-.

The triple hydrogen bonding in the G,(G-H) dimer bears a close ressemblance to the corresponding one observed in cytosine, cytosinium compounds[46] and hemiprotonated polyribocytidilic acid.[47] Surprisingly, little or no hydrogen bonding occurs in Me_2SO solution.[25] There is definite hydrogen bonding in aqueous solution, however, which, in contrast to the situation in the solid state, appears to be symmetrical with respect to the position of the proton.[48]

Summary and Possible Biological Relevance

Three important conclusions concerning the hydrogen-bonding behavior of *cis*-$Pt^{II}(NH_3)_2$ complexes of 9-ethylguanine can be drawn:

(1) Platinum binding to N7 of G markedly reduces hydrogen bonding between G and C. With the G ligand deprotonated, hydrogen bonding with C is almost completely prevented.

(2) The high selectivity for G + C base pairing is greatly reduced when Pt is bound to N7 of G, and completely lost with G deprotonated at N1. Other bases (G,T) form hydrogen bonds as well or even preferentially.

(3) The NH_3 ligands of Pt appear to contribute to the loss of base pairing selectivity.

At present, these results are restricted to perchlorate salts used in this study. One has to be aware that the choice of another counterion, e.g., Cl^-, because of its hydrogen-bond-forming properties with nucleobases, may modify some of the findings.

As has been pointed out, in most cases hydrogen bonding between the Pt complexes and nucleobases is not unambiguous. Several types of hydrogen bonding are usually feasible, some of which may coexist and contribute to the observed interaction shifts.

The role of the NH_3 groups requires further investigation. The discussion on the possible importance of hydrogen bonding between G-O6 and amine protons[16] with regard to the decrease in antitumor activity of *cis*-$Pt(amine)_2$ complexes in the order $NH_3 \sim NH_2R > NHR_2 \gg NR_3$ should be remembered, however.

It has been proposed that a base substitution mutation mechanism invoking the existence of a G-N7,O6 chelate with Pt might explain the differences in antitumor activity between cis and trans complexes of Pt.[49] The present study does not disprove such a possibility. However, it clearly demonstrates that monodentate Pt binding to G-N7 by itself is capable of causing base mispairing.

(44) In order to avoid introduction of a basic impurity, anhydrous *cis*-$[\{Pt(NH_3)_2GC\}\{Pt(NH_3)_2(G\text{-}H)C\}]^{3+}$ was added instead of just *cis*-$[Pt(NH_3)_2(G\text{-}H)C]^+$ (cf. ref 40 and text).

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(46) (a) Kistenmacher, T. J.; Rossi, M.; Caradonna, J. P.; Marzilli, L. G. *Adv. Mol. Relaxation Interact. Processes* **1979**, *15*, 119. (b) Kistenmacher, T. J.; Rossi, M.; Chiang, C. C.; Caradonna, J. P.; Marzilli, L. G. *Ibid.* **1980**, *17*, 113.

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Nevertheless, it is difficult, at present, to assess the biological relevance of some of the reported results, e.g., whether Pt(G-H)≡G pairing is sterically possible in native DNA.

One final aspect should be mentioned. In connection with the mutagenic effects of modified nucleobases, the possibility of base mispairing caused by base ionization has been discussed.[50] Provided such a mechanism works, Pt-G complexes should be regarded likely candidates for it.

Acknowledgment. The author wishes to thank the Deutsche Forschungsgemeinschaft and the Technische Universität München for financial support, and one of the referees for kindly making available some references on relevant work he had not been aware of.

Supplementary Material Available: Tables of chemical shifts, graphs, and ^{1}H NMR spectra (17 pages). Ordering information is given on any current masthead page.

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Binding Site of the Antibiotic Vancomycin for a Cell-Wall Peptide Analogue

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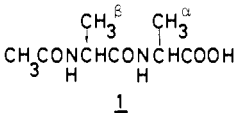
Abstract: The previously proposed binding site of the antibiotic vancomycin for a cell-wall peptide analogue, Ac-D-Ala-D-Ala, while correct in the interactions postulated, omits further important interactions. It is shown that, relative to the conformation of vancomycin inferred from the X-ray structure of CDP-I, bound vancomycin has undergone a major conformational change involving isoasparagine, *N*-methylleucine, and the β-hydroxychlorotyrosine unit located between these two amino acids. As a result of the conformational change, vancomycin can form two further hydrogen bonds, as well as a salt bridge, in binding the cell-wall peptide analogue. The binding pocket for the carboxylate anion of Ac-D-Ala-D-Ala which is so formed closely resembles that found in ristocetin A.

Vancomycin is a clinically important antibiotic whose structure was determined by X-ray analysis in 1978.[1] Due to adverse side effects (tolerable, however, in serious illness), its use has been restricted to the treatment of staphylococcal infections (for example, wound septicaemia and pneumonia) when other antibiotics are ineffective. However, recently it has been reported that vancomycin is effective in the treatment of postoperative diarrhea; it causes the disappearance of *Clostridium difficile*, which may otherwise lead to potentially lethal complications following earlier treatment with other antibiotics.[2] Vancomycin acts by inhibiting the synthesis of cell-wall mucopeptide, which results in the eventual destruction of the cell by lysis. Nieto and Perkins have shown[3] that it forms complexes with cell-wall precursors which terminate with D-alanyl-D-alanine at the carboxyl terminus of a peptide portion. On the basis of the X-ray structure and conformation of vancomycin, the shifts of a limited number of proton resonances in its ^{1}H nuclear magnetic resonance (NMR) spectrum upon addition of the cell-wall peptide analogue acetyl-D-alanyl-D-alanine (**1**)[4,5] and an examination of space-filling (CPK) models, it was possible to propose a structure for the noncovalently bonded complex formed between the antibiotic and cell-wall analogue.[1] We now report that this complex, although correct in the proposed interactions between the antibiotic and cell-wall analogue, omits further important interactions. We show that the complexed form of the antibiotic undergoes a major conformational change (relative to the X-ray structure) and in so doing generates an efficient carboxylate-binding pocket for C-terminal D-alanine.



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The structure of vancomycin, based on the X-ray structure and conformation of the derivative CDP-I[1] (in which the primary amide of isoasparagine in vancomycin is replaced by a carboxyl group), is reproduced in Figure 1. An exploded view of the earlier proposed[1] structure of the complex between Ac-D-Ala-D-Ala and vancomycin is reproduced in Figure 2. It can be seen that since the carboxyl terminus (marked "C" in Figure 2) of the terminal D-Ala is only hydrogen bonded to NH_{C4}, in this model the *N*-methylleucine, "right-hand" β-hydroxychlorotyrosine, and isoasparagine residues of vancomycin are redundant in binding the cell-wall analogue (Figures 1 and 2). Recently, Convert and co-workers[6] have noted that the proposed model (Figure 2) does not offer an understanding for the increased binding of Ac-D-Ala-D-Ala when its terminal carboxylate group is in the anionic state and the vancomycin *N*-methylleucine is in its cationic state.[3,4] This is because the complex shown in Figure 2 places the D-Ala carboxylate anion ~9 Å from the positively charged *N*-methylamino group (Figure 1).[6] To overcome this problem, they have proposed[6] that the positively charged *N*-methylamino group approaches the D-Ala carboxylate anion more closely (~5 Å) in the complex by rotating it about the arrowed bond in Figure 1

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