Effects of Asymmetry and Bulky Substituents in Amine Ligands of *cis*-Di(amine)dichloroplatinum Complexes on the Structure of Adducts formed with Adjacent Guanines in Nucleic Acids

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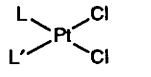
Platinum adducts obtained from the reaction of 3′-*O*,5′-*O*-phosphodiguanosine, r(GpG), with the asymmetric complexes *cis*-[$PtCl_2L(L')$] (L = NH_3; L′ = alkylamine such as NH_2Me, NH_2Et, $NHMe_2$ and $NHEt_2$; LL′ = *N*,*N*-dimethylethylenediamine) have been structurally characterized by ^1H NMR techniques. Two different adducts, in which platinum co-ordinates to the N^7 sites of both guanines, were obtained as geometrical isomers, one with the NH_3 group *cis* to the 5′ base and the other with the alkylamine *cis* to it. Apparently, the structures of these platinum adducts, *cis*-[$PtL(L')(GpG\text{-}N^7,N^7)]^+$, are similar to that of the known *cis*-[$Pt(NH_3)_2(GpG\text{-}N^7,N^7)]^+$, *i.e.* with an *anti-anti* configuration and with the N conformer of the sugar as the 5′ residue. However, the asymmetric character of the non-leaving group induces a slight but significant deviation in the relative orientation of the two guanines which increases with increasing bulkiness of the alkylamine group.

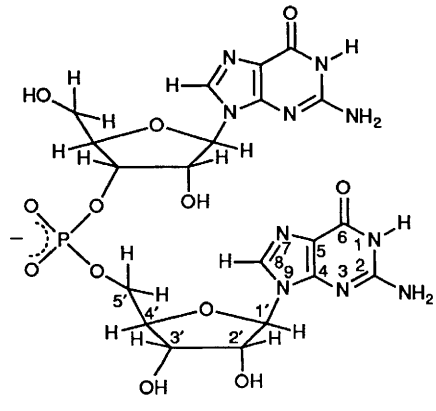
The mode of action of *cis*-[$PtCl_2(NH_3)_2$] at the molecular level is conceived to be its cross-linking between adjacent guanine bases on the same strand of DNA.[1] To form such a cross-linked adduct the leaving groups of antitumour-active platinum complexes are required to have the *cis* configuration. This appears to be the first requisite for antitumour activity. It is known that *cis*-[$PtCl_2(NH_3)_2$] binds to –GpG– (G = guanosine residue, p = 3′ ⟶ 5′ phosphate bridge) sequences on DNA[2] and that this results in a kink on the DNA strands.[3] Such a structural change of the DNA could well be related to the antitumour activity.[1]

The purpose of the present study is to explore the structural change induced by co-ordination of *cis*-[$PtCl_2(NH_3)_2$] analogues to adjacent guanines. The antitumour platinum complexes,[4] synthesised to date, usually have a structure like *cis*-[$PtCl_2(amine)_2$]. They have a C_2 axis through Pt and the centre of the two amine ligands. We have prepared a set of asymmetric platinum analogues *cis*-[$PtCl_2L(L')$] **a**–**g** which do not possess a C_2 symmetry element and therefore geometrical isomers of adducts formed with GpG could be expected. The adducts isolated from the reaction of these complexes with 3′-*O*,5′-*O*-phosphodiguanosine, r(GpG), have been characterized by NMR spectral analysis and the effect of the asymmetry and the bulky substituents on the structure are described.



	L	L′
a	NH_3	NH_2Me
b	NH_3	NH_2Et
c	NH_3	$NHMe_2$
d	NH_3	$NHEt_2$
e	dmen	
f	NH_3	NH_3
g	NH_2Me	NH_2Me

dmen = *N*, *N*-dimethylethylenediamine



r(GpG)

Experimental

The asymmetric platinum complexes *cis*-[$PtCl_2L(L')$] were synthesised as described by Rochon and Kong.[5] The dinucleotide r(GpG) was commercially available from Sigma Chemicals. The complexes were allowed to react with a stoichiometric amount of r(GpG) in water at 37 °C for 3 d and the reaction products were separated and purified using a reversed phase HPLC column (Cosmosil $5C_{18}$). For the separation, a linear gradient from 0 to 25% methanol at 1% min^{-1} against 0.05 mol dm^{-3} phosphate buffer (pH 4.5) was used.

Proton NMR spectra were recorded at 400 MHz on a JEOL GX-400 spectrometer. NMR samples were lyophilized three times from 99.7% D_2O after adjustment of pH to 6.5 and finally dissolved in 99.95% D_2O (0.6 cm^3). A trace amount of the sodium salt of 3-(trimethylsilyl)propionic acid was added to the

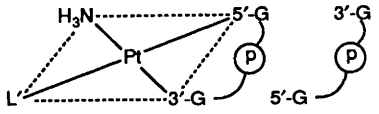


Fig. 1 *Schematic* structure of the two adducts (geometrical isomers) obtained from the reaction of the asymmetric platinum complexes with r(GpG)

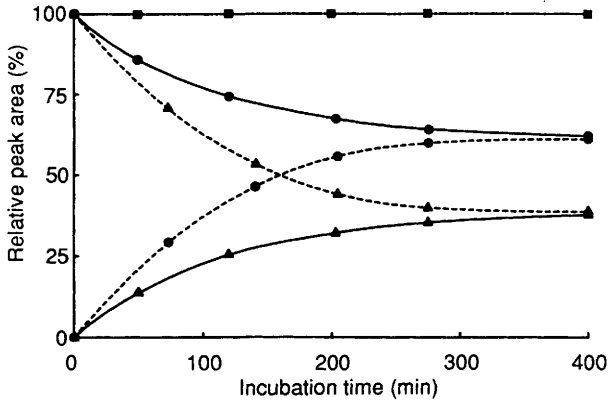


Fig. 2 Isomerization of adducts **1e** (▲) and **1'e** (●) as a function of time. ——, Conversion from **1'e** into **1e**; – – –, conversion from **1e** into **1'e**. The adduct **2e** (■) did not change at all as a function of time

sample as an internal reference. The pH dependence of the chemical shifts of the H^8 protons of guanine was monitored by adding small quantities of 1 mol dm^{-3} $DClO_4$ and/or 1 mol dm^{-3} NaOD. The pH values were not corrected for the deuterium isotope effect, *i.e.* being uncorrected meter readings. Typical conditions for recording the NMR spectrum include 300 scans, 45° pulse width, 32 K data points and 27 °C. The nuclear Overhauser effect (NOE) was measured by the difference spectral mode, the samples used being carefully degassed. For this measurement a pre-irradiation time of 5 s was used for complete saturation. The two-dimensional correlation spectroscopy (COSY) experiments were performed by using the sequence (90° pulse–t_1–90° pulse–acquisition)$_n$, where t_1 = evolution period and n = repeat. The data were collected with an accumulated matrix of 1024 × 512 points which was zero-filled to a final matrix of 1024 × 1024 points.

Results and Discussion

Reaction Products.—The reaction of *cis*-$[PtCl_2(NH_3)_2]$ with the dinucleotides d(GpG) (d = deoxy) and r(GpG) (r = ribo) is known to result in a single product, *cis*-$[Pt(NH_3)_2(GpG\text{-}N^7,N^7)]^{+}$.[6] The asymmetric platinum complexes react with r(GpG) to give at least two adducts in which *cis*-$[PtL(L')]^{2+}$ cross-links between the N^7 sites of the two guanines as does *cis*-$[Pt(NH_3)_2]^{2+}$. Formation of the two adducts is easily predicted by a lack of C_2 symmetry in the platinum complexes, *i.e.* one has the NH_3 group *cis* to the 5' guanosine residue and the other has the alkylamine group *cis* to it. As is illustrated in Fig. 1, these products are geometrical isomers. The presence of two such isomers has been observed before in the reaction products of (1*R*),(2*S*)-cyclohexanediamineplatinum(II) with d(GpG).[7]

The two geometrical isomers have been separated by preparative HPLC and are denoted **1** and **2**, according to their elution order, *e.g.* **1a** and **2a** for the reaction products of platinum complex **a** with r(GpG). Each product was characterized by data from a UV–pH titration[8] (not shown) and an NMR–pH titration (see below) as being an adduct in which the platinum is attached to the N^7 atom of both guanine groups. The HPLC study showed that the reaction of r(GpG) with complexes **a**–**d** yields two adducts in each case. The ratio of the two adducts was 1:1, suggesting that the binding selectivity for either the *cis* or the *trans* side to the alkylamine group is quite low. In the reaction with complex **d** some minor by-products were detected in yields less than 5% of all the products.

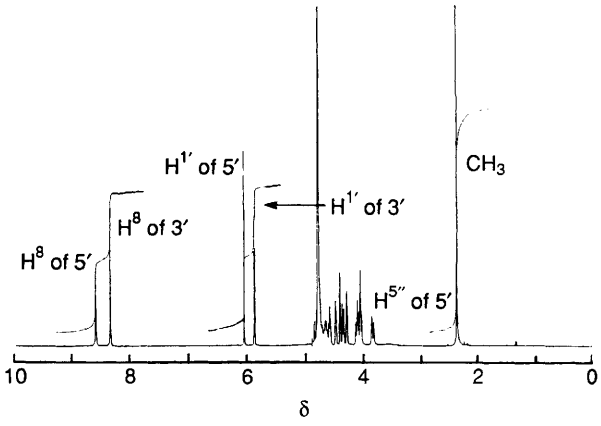


Fig. 3 NMR spectrum of adduct **2a**, obtained at 400 MHz, 27 °C and pH 6

The reaction with $[PtCl_2(dmen)]$ **e** however proceeds somewhat differently. Three adducts result (ratio: **1e**:**1'e**:**2e** = 18:30:52), and two (*i.e.* **1e** and **1'e**) were found to be in equilibrium (see below). After the separation of these adducts, each was monitored by HPLC as a function of time (Fig. 2). One of the adducts, **2e**, did not show any change upon incubation at 37 °C, whereas the incubation of pure **1e** (or **1'e**) results in **1'e** (or **1e**) with decreasing **1e** (**1'e**), strongly suggesting that both **1e** and **1'e** are involved in an isomerization equilibrium. This isomerization reaction was also detected from the NMR spectra of **1e** and **1'e** and continued until the ratio of **1e**:**1'e** = 38:62 was approached. Since both products are platinum adducts with an interbase cross-link *via* the N^7 sites of the adjacent guanines, the isomerization must be due to a rotational process about the Pt–N^7 bond. The presence of such rotamers is known in *cis*-$[Pt(NH_3)_2(GpA\text{-}N^7,N^7)]^{+}$ (A = adenosine residue),[9] and was proposed to be related to a slow rotation of the 3'-purine base. The adducts **1e** and **1'e** have quite bulky substituents *cis* to this base (see below) and therefore this rotation may become so slow that both isomers can be isolated using HPLC.

Assignment of NMR Spectra.—Fig. 3 shows a typical example of an NMR spectrum of a reaction product (in this case **2a**) obtained from the reaction of the asymmetric complex $[PtCl_2(NH_3)(NH_2Me)]$ **a** with r(GpG). The resonances of the non-exchangeable base protons appear in the range δ 7–9, those of the $H^{1'}$ protons in the range δ 5.5–6.5 and those due to the co-ordinated alkylamine group appear in all cases more than 3 ppm upfield and do not overlap with the signals originating from the r(GpG) moiety. The binding ratio of complex **a** to r(GpG) is 1:1. This is clear from the integration of the $H^{1'}$ protons (2 H) and the methylamine group (3 H). The same binding ratio was observed for the adducts involving the other asymmetric platinum complexes. Since only two H^8 signals are observed in all the spectra the adducts should be monomeric compounds.

Table 1 lists the NMR spectral data for the co-ordinated alkylamine group(s). A sharp singlet is observed for the methyl group of adducts **1a** and **2a**. The ethylamine group of **1b** and **2b** shows a quartet and triplet signals. These data indicate that the rotation about the Pt–N bond (alkylamine group) is fast on the NMR time-scale. On the other hand, the dimethylamine group of **1c** and **2c** shows two split peaks with the same intensity, indicating a relatively slow rotation around the Pt–N bond on the NMR time-scale. For the adducts with $[PtCl_2(dmen)]$, rotation around the Pt–N bond is not possible because of the ethylenediamine bridge. Two signals were observed for each of **1e**, **1'e** and **2e**. In this case, one cannot conclude whether or not

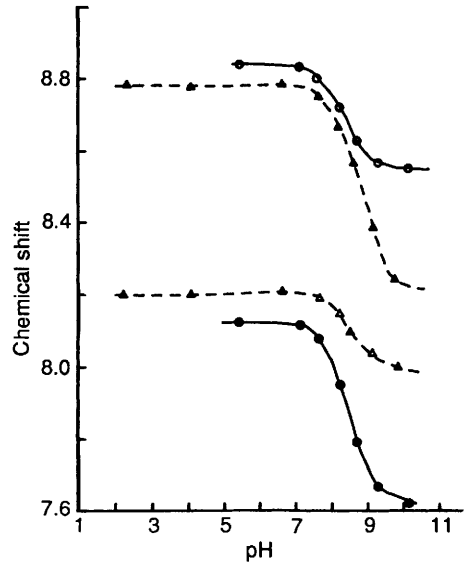


Fig. 4 pH Dependence of the H^8 resonances of the adducts **1c** (○, ●) and **2c** (△, ▲)

Table 1 NMR spectral data for the alkylamine protons of the platinum adducts

Adduct	δ
1a	2.36(s)
2a	2.36(s)
1b	2.65(q), 1.24(t)
2b	2.64(q), 1.25(t)
1c	2.67(s), 2.64(s)
2c	2.66(s), 2.58(s)
1d	2.92(m), 2.76(m), 1.55(t), 1.54(t)
2d	2.92(m), 2.72(m), 1.64(t), 1.58(t)
1e	2.86(s), 2.76(s)
1'e	2.84(s), 2.81(s)
2e	2.87(s), 2.77(s)

Conditions: pH 6, 27 °C. s = Singlet, d = doublet, t = triplet, q = quartet and m = multiplet.

the puckering of the ethylene ring is fast because of the difference in environments of the two methyl substituents. Since the co-ordinated GpG molecule results in a different environment for the up and down sides of the platinum co-ordination plane, the two methyl groups will be inequivalent even in the case of fast puckering between the λ and δ conformations of the ethylene ring.[10]

Fig. 4 shows pH–NMR titration curves for the adducts **1c** and **2c**. A shift of the H^8 signals toward higher field is observed under alkaline conditions, due to deprotonation at N^1. Protonation at the N^7 site of the guanines was not observed, indicating that these sites are co-ordinated by platinum. The pK_a values (at N^1) agree well with those of *cis*-$[Pt(NH_3)_2(GpG\text{-}N^7,N^7)]^+$.[6] From these results, **1c** and **2c** can definitely be assigned to *cis*-$[Pt(NH_3)(NHMe_2)(GpG\text{-}N^7,N^7)]^+$, *i.e.* involving cross-linking between the two guanines. No difference was observed for the pK_a values (at N^1) for 3′ and 5′ guanosine residues. In the pH–NMR titration curve the chemical shift variation of the H^8 proton of the 5′ residue is however more sensitive than that of the 3′, *i.e.* the former is sensitive to the conformational change induced upon deprotonation at N^1. It is likely that the chemical shift of the H^8 protons is mainly influenced by the relative orientation of the two guanines. On the basis of only the chemical shift data, it is therefore difficult to discriminate between H^8 of the 3′ and 5′ guanosine residues. Chottard and co-workers[6c] made a tentative assignment of the two H^8 protons of *cis*-$[Pt(NH_3)_2(GpG)]^+$ by studying the deuterium-exchange rate of these protons and the NMR–pH titration curve. They assigned the H^8 signal with the large chemical shift variation (in NMR–pH titration) to the more slowly exchanged proton (of the 5′ residue). In isomers **1c** and **2c**, if this empirical rule were true, the downfield signals in Fig. 4 can be assigned to H^8 of the 3′ guanosine residue of **1c** and to H^8 of the 5′ residue of **2c** respectively.

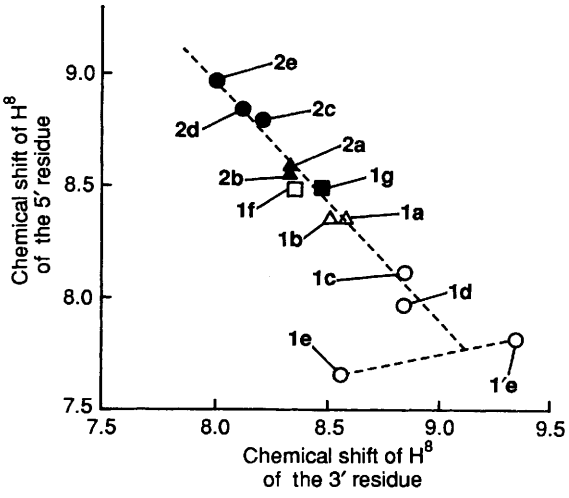


Fig. 5 Plot of the chemical shifts of H^8 of the 3′ and 5′ guanosine residues

To get further information about the assignment of the H^8 protons the two-dimensional homonuclear chemical shift correlation (COSY) and one-dimensional NOE were measured. First, the peaks due to $H^{5'}/H^{5''}$ of the 5′ residue were assigned. The $H^{5''}$ proton is expected to exhibit a doublet due to vicinal coupling with the $H^{4'}$ (^3J = *ca.* 3 Hz), which splits into a doublet of doublets due to an additional geminal coupling between the $H^{5'}$ and $H^{5''}$ (^2J = *ca.* −13 Hz). On the other hand, the $H^{5'}/H^{5''}$ protons of the 3′ residue are expected to show an additional splitting due to the ^{31}P coupling in addition to the doublet of doublets. Therefore, the peaks due to $H^{5'}/H^{5''}$ of the 3′ and 5′ residues could easily be discriminated from the NMR spectral pattern. In the case of adduct **2a** (see Fig. 1), the resonance at δ 3.84 (Table 2) is assigned to $H^{5''}$ of the 5′ residue. Investigation of connectivities among the ribose protons in the COSY spectrum clearly indicates that a singlet at δ 6.05 is assigned to $H^{1'}$ of the same residue. Since a doublet at δ 5.88 is assigned to $H^{1'}$ of the 3′ residue, the other ribose protons are also assigned unambiguously by following the cross-peaks. The H^8 resonances were assigned by a NOE experiment between the H^8 and $H^{1'}$ protons. For adduct **2a**, irradiation of the signal at δ 8.58 resulted in a NOE enhancement of 3.5% for the $H^{1'}$ signal of the 5′ residue. Therefore, this resonance is assigned to the H^8 proton. For adduct **1a**, irradiations of the signals at δ 8.54 and 8.34 resulted in NOE enhancement for $H^{1'}$ of the 3′ (5.0%) and 5′ residue (2.0%), respectively, indicating that these resonances should be assigned to H^8 of the 3′ and 5′ residues respectively. That is, the downfield signal is assigned to H^8 of the 5′ residue in the case of **2a** and to H^8 of the 3′ residue in the case of **1a**. The same relation holds for the chemical shifts of the H^8 protons of adducts **1c** and **2c**. The assignments are also consistent with the tentative assignment from the NMR–pH titration (see above). The results are summarized in Table 2.

Structural Consideration of the Adducts.—When the chemical shift of H^8 of the 5′ residue was plotted against that of H^8 of the 3′ residue for the compounds a very interesting phenomenon was observed. As shown in Fig. 5, the plot is found to be linear with a slope of −1. The weighted mean of the chemical shift of adduct **1e**, weighted by the formation ratio of **1e** and **1'e**, is also located on this straight line. The chemical shift of the H^8

Table 2 NMR spectral data for the GpG moiety of the platinum adducts, *cis*-[PtL(L')(GpG-N^7,N^7)]$^+$

	H^8			$H^{1'}$		
Adduct	5′	3′	Δ^a	5′(s)	3′(d)^b	$H^{5''}$ of 5′
1f	8.47	8.35	0.12	6.03	5.88 (6.6)	3.80
1g	8.48	8.48	0	6.03	5.88 (6.6)	3.81
1a	8.34	8.54	−0.20	6.01	5.89 (6.6)	3.77
2a	8.58	8.33	0.25	6.05	5.88 (6.6)	3.84
1b	8.34	8.51	−0.17	6.01	5.88 (6.6)	3.80
2b	8.55	8.33	0.22	6.04	5.87 (6.6)	3.82
1c	8.10	8.85	−0.75	5.94	5.90 (7.0)	3.64
2c	8.78	8.21	0.57	6.08	5.86 (6.3)	3.89
1d	7.95	8.84	−0.89	5.92	5.88 (6.6)	3.53
2d	8.85	8.12	0.73	6.13	5.88 (7.0)	3.94
1e	7.65	8.55	−0.90	5.93	5.85 (3.6)	3.73
1′e	7.81	9.34	−1.53	5.86	5.96 (8.1)	3.70
2e	8.97	8.00	0.97	6.14	5.87 (5.5)	4.00

Conditions: pH 6, 27 °C. ^{a} Δ = $\delta(H^8$ of 5′) − $\delta(H^8$ of 3′). ^{b} J/Hz in parentheses.

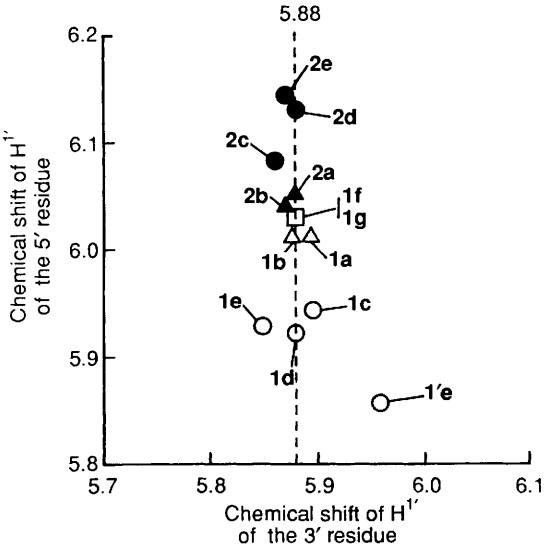


Fig. 6 Plot of the chemical shifts of $H^{1'}$ of the 3′ and 5′ sugar residues

protons due to adducts **f** and **g** (both involving C_2 symmetric complexes) lies at the midpoint of the line. The points due to the adducts **2** are distributed on the line at the upper left-hand side, and those of the adducts **1** on the line at the lower right-hand side. The deviation from the midpoint becomes larger with increasing bulkiness of the alkylamine group. These results strongly suggest that the alkylamine group in the asymmetric platinum complexes induces a gradual structural change from the conformation of the GpG moiety which varies with the bulk of the substituent(s).

For adduct **2a**, irradiation of H^8 of the 5′ residue caused a NOE enhancement (5.0%) for the NH_2Me group, indicating that the latter group is located *cis* to the 5′ base. The relative orientation of the two guanines is head-to-head, because the NOE (6.7%) clearly correlates with the H^8 signals of the 3′ and 5′ residues. The orientations of the guanine bases to the sugar are found to be in *anti-anti* configurations, because of the NOE enhancement at the $H^{3'}$ (10.5%) and the $H^{2'}$ (5.0%) of the 5′ sugar. Irradiation of H^8 of the 3′ residue in adduct **1a** resulted in a NOE enhancement (2.6%) for the NH_2Me group, but no NOE signal for NH_2Me was observed upon irradiation at H^8 of the 5′ residue. Irradiation at H^8 of the 5′ residue results in NOE enhancements for $H^{1'}$ (1.9), $H^{3'}$ (7.0) and $H^{2'}$ (5.5%) of the 5′ sugar. Irradiation at H^8 of the 3′ residue resulted in NOE

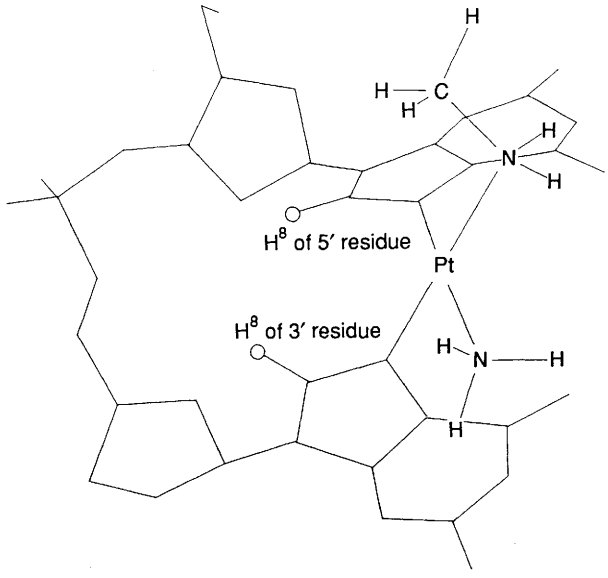


Fig. 7 Schematic representation of the most likely structure of adduct **2a**

enhancements for $H^{1'}$ (4.9) and $H^{2'}$ (7.0%) of the 3′ sugar. These results indicate that, in adduct **1a**, the NH_2Me group is located *cis* to the 3′ base and that the orientation of the two guanines is *anti-anti*.

The conformation of the sugar ring is generally described by a rapid pseudo-rotational equilibrium between the S- ($C^{2'}$-*endo*) and the N-type ($C^{3'}$-*endo*) conformers.[11] It is well known that the behaviour of the coupling constants provides insight into the conformation of the sugar ring.[11] As indicated in Table 2, the $H^{1'}$ of the 5′ sugar shows a sharp singlet in all cases, indicating that the 5′ sugar has a pure N-type conformer. On the other hand, the $H^{1'}$ of the 3′ sugar shows a doublet with a coupling constant of 3–8 Hz. The value of $J(H^{1'}$–$H^{2'})$ is the same (6.6 Hz) for the 3′ sugar of adducts **1a**, **2a**, **1b**, **2b** and **f**, suggesting that there is no difference in the sugar conformation of these adducts. On the other hand, for the adducts involving co-ordinated secondary and tertiary amino groups (**c**–**e**) the coupling constant of one adduct (**2c**, **1d**, **1e**, **2e**) tends to become smaller (<6.6 Hz) and that of the other (**1c**, **2d**, **1′e**) becomes larger (>6.6 Hz). That is, the conformational populations (N and S) of the 3′ sugar would be affected significantly by an increase in the bulkiness of the co-ordinated alkylamine group. For example, the 3′ sugar of **1e** [$J(H^{1'}$–$H^{2'})$ = 3.6 Hz] contains an increased proportion of the N-type conformer.

Fig. 6 clearly shows an effect of the substituents on the chemical shifts of the $H^{1'}$ protons. The shift of $H^{1'}$ of the 3′ sugar is not influenced at all, whereas that of the 5′ sugar is quite sensitive to the alkylamine substituents. The $H^{1'}$ signal of the 5′ sugar tends to shift upfield for adducts **1** and downfield for adducts **2**. This is similar to the behaviour of the chemical shift of H^8 of the 5′ residue described above (see Fig. 5).

It is supposed that the chemical shifts of the H^8 protons are mainly influenced by a ring current due to the adjacent guanine base. Therefore, the behaviour of these chemical shifts is expected to provide information about the geometry and structure of the adducts. The difference in the chemical shifts of H^8 of the 5′ and 3′ residues (see Table 2) becomes larger with increasing bulkiness of the substituents: **a** < **b** < **c** < **d** = **e**. An interesting observation is that the mean value of the chemical shifts of both H^8 protons is approximately the same for all the adducts (including those of **f** and **g**), 8.4–8.5 ppm. This implies that, *e.g.*, an upfield shift of H^8 of the 5′ residue simultaneously results in a downfield shift of H^8 of the 3′ residue. Proton H^8 of the 5′ residue appears at lower field in the case of adduct **2** and at higher field in the case of adduct **1**, *i.e.* the direction of the chemical shift change is opposite for the two

adducts. These results lead to the following conclusion. In the case of adducts **1** the substituent is located *cis* to the 3′ base which makes a small counter-clockwise turn (viewed from Pt toward N^7) to reduce the steric repulsion from the substituent and the O^6. Molecular model inspection suggests that such a structural change leads to a downfield shift of H^8 of the 3′ residue and an upfield shift of H^8 of the 5′ residue. In the case of adducts **2** the alkylamine group is located *cis* to the 5′ base which turns in a clockwise direction. Such a structural change leads to a reduction of the steric repulsion between the alkylamine group and the 5′ base and in a downfield shift of H^8 of the 5′ residue and an upfield shift of H^8 of the 3′ residue. This is depicted in Fig. 7.

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