cis-[Pt(NH$_3$)$_2$(9-MeA-*N7*)(9-EtGH-*N7*)](PF$_6$)$_2$·1.5H$_2$O (9-MeA = 9-Methyladenine; 9-EtGH = 9-Ethylguanine): A Right-Handed Helicoidal Model Compound for the Intrastrand A,G Cross-Link in Duplex DNA

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Introduction

Very recently we described the synthesis, crystal structure, and conformational analysis of a model nucleobase complex, *cis*-[Pt(NH$_3$)$_2$(9-MeA-*N7*)(9-EtGH-*N7*)](NO$_3$)$_2$·2H$_2$O (9-MeA = 9-methyladenine; 9-EtGH = 9-ethylguanine), which we considered a model compound for an intrastrand adduct of the antitumor compound cisplatin between adjacent adenine and guanine nucleobases in DNA.[2] Cross-linking between these two bases, in the 5′ApG sequence, is the second most abundant mode of cisplatin binding to DNA and accounts for ca. 20−30% of all Pt adducts.[3] A detailed conformational analysis led to the conclusion that the orientation of the two purine bases in this model compound corresponds to a situation encountered in the minor rotamer form of *cis*-[(NH$_3$)$_2$Pt{d(ApG)}]$^+$ and probably relevant to this adduct in single-stranded DNA.[2] Being aware of the fact that, in model nucleobase complexes, counterions sometimes are crucial in stabilizing different conformers[4] or rotamers,[5] we have successfully crystallized the above model compound, as the PF$_6^-$ salt, in which the nucleobases assume the right-handed orientation R2 relevant to cisplatin−d(ApG) adducts of double-stranded DNA. Because the crystal system is centrosymmetric, the enantiomeric conformation L2 is present in the crystal as well. As it turned out, the PF$_6^-$ salt crystallizes with two independent cations which differ slightly as far as dihedral and torsional angles are concerned.

Experimental Section

Synthesis of *cis*-[Pt(NH$_3$)$_2$(9-MeA-*N7*)(9-EtGH-*N7*)](PF$_6$)$_2$·1.5H$_2$O (1). Compound **1** was obtained upon anion exchange (Merck, type II) from *cis*-[Pt(NH$_3$)$_2$(9-MeA-*N7*)(9-EtGH-*N7*)](NO$_3$)$_2$·2H$_2$O, which was prepared as described previously.[2] A 281 mg sample (0.392 mmol) of *cis*-[Pt(NH$_3$)$_2$(9-MeA-*N7*)(9-EtGH-*N7*)](NO$_3$)$_2$·2H$_2$O was dissolved in 3 mL of water, and the solution was placed on the column. Elution of **1** was carried out with 150 mL of water. The resulting solution was reduced to a volume of 4 mL by rotary evaporation and stored at 4 °C.

Table 1. Crystallographic Data for *cis*-[Pt(NH$_3$)$_2$(9-MeA-*N7*)(9-EtGH-*N7*)](PF$_6$)$_2$·1.5H$_2$O (**1**)

empirical formula	$C_{13}H_{25}N_{12}O_{2.5}P_2F_{12}Pt$
fw	874.44
crystal color and habit	colorless plate
crystal dimensions	0.28 × 0.15 × 0.34 mm
crystal system	triclinic
lattice parameters	a = 14.758(4) Å b = 17.454(7) Å c = 11.559(4) Å α = 108.75(3)° β = 100.82(3)° γ = 96.86(3)° V = 2717(4) Å^3
space group	$P\bar{1}$ (No. 2)
Z	4
D_{calc}	2.14 g cm^{-3}
radiation	Mo Kα (λ = 0.710 69 Å)
μ(Mo Kα)	54.53 cm^{-1}
temperature	−100 °C
$2\theta_{max}$	46°
no. of reflns measd	total: 7940 unique: 7575 (R_{int} = 0.038)
no. of reflns with $I > 3\sigma(I)$	4246
no. of variables	491
residuals R, R_w	0.056, 0.067
goodness of fit	1.62
max peak in final diff map	2.11 e/Å^3 (1.1 Å from Pt2)

1 was obtained in 42% yield. Anal. Calcd for $C_{13}H_{25}N_{12}O_{2.5}P_2F_{12}Pt$: C, 17.9; H, 2.9; N, 19.2. Found: C, 17.8; H, 3.1; N, 19.9.

Synthesis of *cis*-[Pt(NH$_3$)$_2$(9-MeA-*N7*)(9-EtG-*N7*)]NO$_3$·0.5H$_2$O (2). A 100 mg sample of *cis*-[Pt(NH$_3$)$_2$(9-MaA-*N7*)(9-EtGH-*N7*)](NO$_3$)$_2$·2H$_2$O added to 10 mL of a 0.029 M NaOH solution was heated to 90 °C during 30 min. The reaction mixture was then filtered, and the filtrate was kept in a closed flask at 4 °C. The precipitated product was filtered off and dried at 40 °C. The yield was 54%. Anal. Calcd for $C_{13}H_{22}N_{13}O_{4.5}Pt$: C, 24.9; H, 3.5; N, 29.0. Found: C, 24.8; H, 3.3; N, 28.7.

^{1}H NMR Spectroscopy. ^{1}H NMR spectra were recorded on a Bruker AC200 spectrometer (200 MHz) in DMSO-d_6 using the signal of nondeuterated DMSO as internal reference (δ = 2.50 ppm relative to TMS).

X-ray Crystallography. X-ray measurements were carried out on a Rigaku AFC6S diffractometer using Mo Kα radiation (λ = 0.710 69 Å). Calculations were performed on a VAXstation 3500 computer using TEXSAN 5.0 software[6] and in the later stage on a Silicon Graphics Personal Iris 4D35 computer with the teXsan 1.7 package.[7]

Relevant crystallographic data are listed in Table 1. Unit cell dimensions were determined by applying the setting angles of 25 high-angle reflections. Three standard reflections monitored during the data collection showed no significant variance. The intensities were corrected for absorption by applying the DIFABS program[8] with the transmission factors in the range 0.77−1.32.

The structure was solved by direct methods in SIRR88.[9] Full-matrix least-squares refinement was carried out with anisotropic thermal displacement parameters for the Pt, P, and F atoms. The final difference Fourier map revealed a 2.11 e/Å^3 peak located in the vicinity of the Pt2 atom.

Results and Discussion

One of the two crystallographically independent cations of *cis*-[Pt(NH$_3$)$_2$(9-MeA-*N7*)(9-EtGH-*N7*)](PF$_6$)$_2$·1.5H$_2$O (**1**) is shown in Figure 1. The second cation is very similar (Supporting Information). In both cations the purine nucleobases

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(2) Schröder, G.; Kozelka, J.; Sabat, M.; Fouchet, M.-H.; Beyerle-Pfnür, R.; Lippert, B. *Inorg. Chem.* **1996**, *35*, 1647.
(3) (a) Fichtinger-Schepman, A. M. J.; van der Veer, J. L.; den Hartog, J. H. J.; Lohman, P. H. M.; Reedijk, J. *Biochemistry* **1985**, *24*, 707. (b) Eastman, A. *Pharmacol. Ther.* **1987**, *34*, 155 and references cited.
(4) Schöllhorn, H.; Raudaschl-Sieber, G.; Müller, G.; Thewalt, U.; Lippert, B. *J. Am. Chem. Soc.* **1985**, *107*, 5932.
(5) Holthenrich, D.; Sóvágó, I.; Fusch, G.; Erxleben, A.; Fusch, E. C.; Rombeck, I.; Lippert, B. *Z. Naturforsch.* **1995**, *50B*, 1767.
(6) *TEXSAN 5.0: Single Crystal Structure Analysis Software*; Molecular Structure Corp.: The Woodlands, TX 77381, 1989.
(7) *teXsan 1.7: Single Crystal Structure Analysis Software*, Molecular Structure Corp.: The Woodlands, TX 77381, 1995.
(8) Walker, N.; Stuart, D. *Acta Crystallogr., Sect. A* **1983**, *39*, 158.
(9) Burla, M. G.; Camalli, M.; Cascarano, G.; Giacovazzo, C.; Polidori, G.; Spagna, R.; Viterbo, D. *J. Appl. Crystallogr.* **1989**, *22*, 389.

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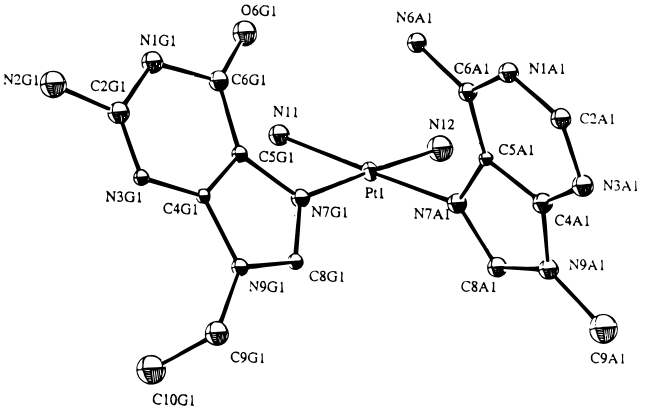

Figure 1. View of one of two crystallographically independent cations of **1** with its atom labeling scheme. Thermal ellipsoids are 30% equiprobability envelopes.

Table 2. Conformational Angles α and β, Dihedral Angles, and Intracomplex H-Bond Data for Enantiomers of *cis*-$[Pt(NH_3)_2$(9-MeA-*N7*)(9-EtGH-*N7*)$]^{2+}$ (Head−Head)[a]

α, deg	β, deg	conformnl domain	9-MeA/9-EtGH, deg	O6G···N6A, Å	ref
105	−45	HH1 (R2)	87.5	3.37	*b*
−105	+45	HH2 (L2)			
115	−48	HH1 (R2)	81.8	3.23	*b*
−115	+48	HH2 (L2)			
+66	−88	HH1 (L1)	92.1	3.11	2
−66	+88	HH2 (R1)			

[a] See ref 10 for the definition of α and β and for the designation of the conformational domains. [b] This work.

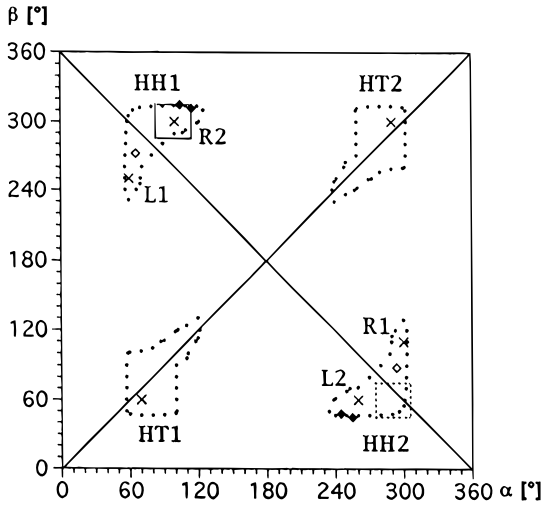

Figure 2. Calculated energy map for *cis*-$[Pt(NH_3)_2$(9-MeA-*N7*)(9-EtGH-*N7*)$]^{2+}$. ◇ = α, β coordinates of the NO_3^- salt; ◆ = α, β coordinates of the two cations of the PF_6^- salt. For other details see Figure 5 in ref 2.

are platinated at their respective N7 positions, consistent with the method of preparation, and the two bases adopt a head−head orientation. Except for torsional angles α and β,[10] dihedral base/base angles, and intracomplex O6G···N6A hydrogen bonds (Table 2), other geometrical parameters (nucleobase geometries; Pt coordination spheres) of NO_3^- and PF_6^- salts are rather similar. Both chiral cations have the respective enantiomers present in the crystal. The principal difference between the cations of the NO_3^- salt and the two variants of the PF_6^- salt orignates from the combination of α and β angles (Figure 2) and the resulting helicities of the cations (Table 2). The values of α and β for the cations of the PF_6^- salt are in the low-energy zones calculated by molecular mechanics methods for *cis*-$[Pt(NH_3)_2$(Ade)(Gua)$]^{2+}$.[2] As we have argued previously,[2] the enantiomer of *cis*-$[Pt(NH_3)_2$(9-MeA-*N7*)(9-EtGH-*N7*)$](NO_3)_2$ having negative α and positive β values (i.e., belonging to the HH2 zone) is close to the conformational domain of the minor rotamer of *cis*-$[Pt(NH_3)_2${d(ApG)}$]^+$ (solid-line square in Figure 2) and can therefore be considered a model for this rotamer. On the other hand, the two HH1 enantiomers of the PF_6^- salt with positive α and negative β values are right-handed helicity are in the domain of the major rotamer of *cis*-$[Pt(NH_3)_2$-{d(ApG)}$]^+$ (dotted square in Figure 2) and thus represent models thereof. Since the conformational domain around the "R2" energy minimum in the HH1 domain is also expected to be favored by duplex Pt−d(ApG) adducts,[10] the two HH1 enantiomers of the present crystal structure analysis should be considered models of the d(ApG) adducts of cisplatin in double-stranded DNA.

As compared to the guanine/guanine intrastrand cross-link, for which a number of model compounds have been studied both in the solid state[11a−c,11f−h,12] and in solution[11d,e,13a−c] little is known about the effect of the adenine/guanine adduct on DNA geometry and H bonding with the complementary strand. Available knowledge on this adduct is primarily based on biochemical[14] and mutagenicity[15] studies. Base substitution reactions observed in mutagenicity studies with *Escherchia coli*[15] suggest that distortion of the DNA helix is larger at the 5′-side, consistent with the T,A pair being inherently weaker than the G,C pair. The degree by which base platination affects the strengths of these pairs is unknown, however.

In order to obtain some qualitative insight into this question, we decided to study the H-bonding properties of our model compound with regard to 1-methylcytosine (1-MeC) by recording ^{1}H NMR spectra of 1:1 mixtures of *cis*-$[Pt(NH_3)_2$(9-MeA-*N7*)(9-EtGH-*N7*)$]^{2+}$ and 1-MeC in DMSO-d_6. Due to the general weakness of A,T pairing in this solvent, analogous work with 1-methylthymine proved inconclusive. A comparison with H bonding properties of free 9-EtGH and 1-MeC in the same solvent reveals an interesting difference: While, for 9-EtGH/1-MeC, interaction shifts[16,17] of guanine N1H and $N2H_2$ as well as cytosine $N4H_2$ are fully consistent with Watson−Crick pairing (viz., the guanine N1H resonance undergoes a concen-

(10) Kozelka, J.; Fouchet, M.-H.; Chottard, J.-C. *Eur. J. Biochem.* **1992**, *205*, 895.
(11) (a) Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *Science* **1985**, *230*, 415. (b) Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 7368. (c) Coll, M.; Sherman, S. E.; Gibson, D.; Lippard, S. J.; Wang, A. H.-J. *J. Biomol. Struct. Dyn.* **1990**, *8*, 315. (d) Girault, J. P.; Chottard, G.; Lallemand, J. Y.; Chottard, J. C. *Biochemistry* **1982**, *21*, 1352. (e) den Hartog, J. H. J.; Altona, C.; Chottard, J. C.; Girault, J. P.; Lallemand, J. Y.; de Leeuw, F. A. A. M.; Marcelis, A. T. M.; Reedijk, J. *Nucleic Acids Res.* **1982**, *10*, 4715. (f) Admiraal, G.; van der Veer, J. L.; de Graff, R. A. G.; den Hartog, J. H. J.; Reedijk, J. *J. Am. Chem. Soc.* **1987**, *109*, 592. (g) Lippert, B.; Raudaschl, G.; Lock, C. J. L.; Pilon, P. *Inorg. Chim. Acta* **1984**, *93*, 43. (h) Schöllhorn, H.; Raudaschl-Sieber, G.; Müller, G.; Thewalt, U.; Lippert, B. *J. Am. Chem. Soc.* **1985**, *107*, 5932.
(12) Takahara, P. M.; Rosenzweig, A. C.; Frederick, C. A.; Lippard, S. J. *Nature* **1995**, *377*, 649.
(13) (a) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; van der Marel, G. A.; Haasnoot, C. A. G.; Reedijk, J. *J. Biomol. Struct. Dyn.* **1985**, *2*, 1137. (b) Herman, F.; Kozelka, J.; Stoven, V.; Guittet, E.; Girault, J. P.; Huynh-Dinh, T.; Igolen, J.; Lallemand, J. Y.; Chottard, J. C. *Eur. J. Biochem.* **1990**, *194*, 119. (c) Yang, D.; van Boom, S. S. G. E.; Reedijk, J.; van Boom, J. H.; Wang, A. H. J. *Biochemistry* **1995**, *34*, 12912.
(14) See, e.g.: (a) Corda, Y.; Anin, M.-F.; Leng, M.; Job, D. *Biochemistry* **1992**, *31*, 1904. (b) Corda, Y.; Anin, M.-F.; Leng, M.; Job, D. *Biochemistry* **1991**, *30*, 222. (c) Schwartz, A.; Marrot, L.; Leng, M. *Biochemistry* **1989**, *28*, 7975.
(15) (a) Burnouf, D.; Daune, M.; Fuchs, R. P. P. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 3758. (b) Burnouf, D.; Gauthier, C.; Chottard, J. C.; Fuchs, R. P. P. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6087.

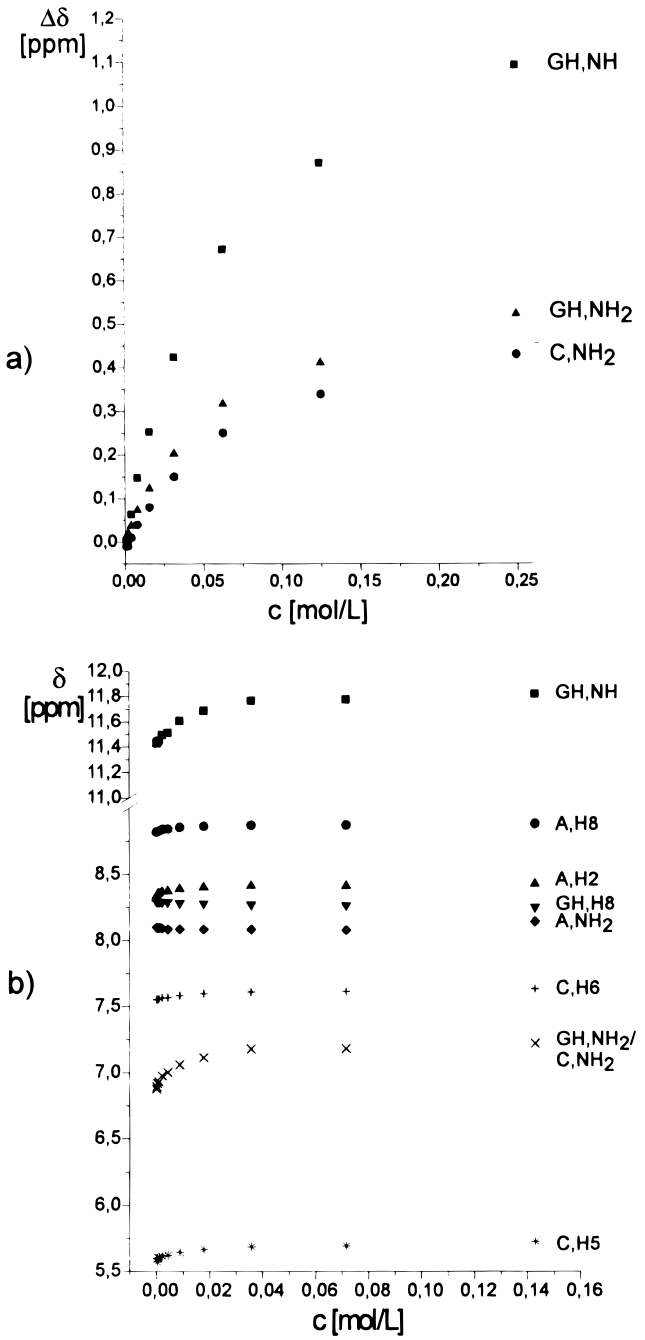

Figure 3. Concentration dependent interaction shifts of selected ^{1}H NMR resonances (DMSO-d_6, 20 °C) of (a) equimolar mixtures of 9-EtGH (GH) and 1-MeC (C) and (b) equimolar mixtures of **1** (A = 9-methyladenine, GH = 9-ethylguanine) and 1-MeC (C). $\Delta\delta$ values in (a) account for self-association of GH. There is no detectable self-association of **1** in the absence of 1-MeC. Note the differences in scales in (a) and (b).

tration-dependent downfield shift that is roughly twice that of the NH_2 resonances),[18] in the case of equimolar mixtures of *cis*-[Pt(NH_3)$_2$(9-MeA-*N7*)(9-EtGH-*N7*)]$^{2+}$ and 1-MeC, this is not observed (Figure 3). Absolute interaction shifts of the N1H of guanine are significantly reduced in the Pt complex, and both H5 and H6 resonances of the cytosine base are likewise shifted

(16) Downfield shifts $\Delta\delta$ relative to indefinite dilution of resonance of one component in the presence of the other, with self-association at the same concentration taken into account. Cf. ref 17.
(17) Lippert, B. *J. Am. Chem. Soc.* **1981**, *103*, 5691.
(18) (a) Shoup, R. R.; Miles, H. T.; Becker, L. D. *Biochem. Biophys. Res. Commun.* **1966**, *23*, 194. (b) Katz, L.; Pennan, S. *J. Mol. Biol.* **1966**, *15*, 220. (c) Newmark, R. A.; Cantor, C. R. *J. Am. Chem. Soc.* **1968**, *90*, 5010.

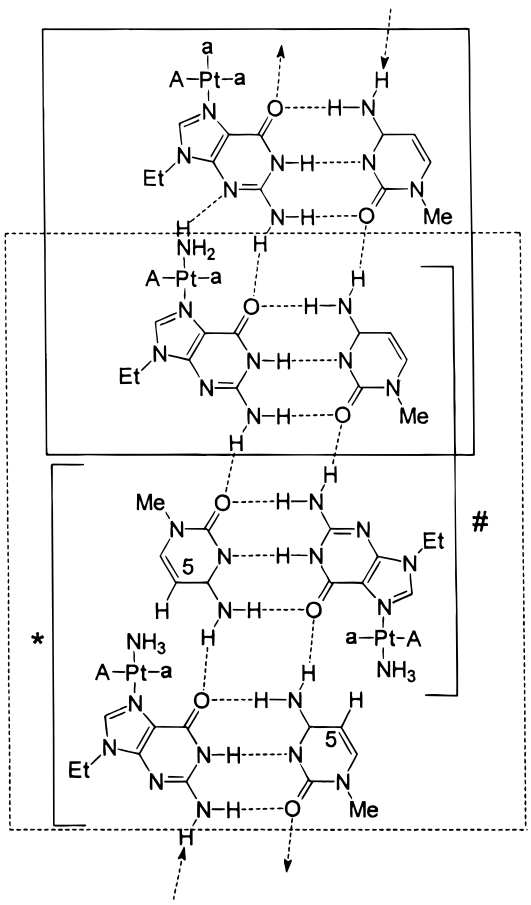

Figure 4. Schematic representation of four feasible ways (square, dotted square, number sign, asterisk) of association of Watson−Crick **1**·1-MeC entities that could account for additional H-bond formation between the NH_2 groups of guanine and cytosine.

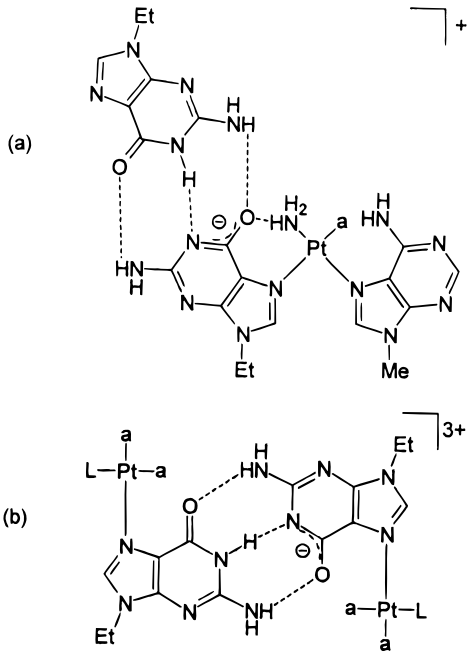

Figure 5. Guanine−guanine H bonding (a) between **2** and 9-EtGH and (b) between **1** and **2**.

downfield. The adenine N6H$_2$ resonance is not affected by the presence of 1-MeC. While these findings appear to be inconsistent with a Watson−Crick pairing scheme between platinated guanine and free cytosine at first glance, we feel that they do not really rule it out, considering the following points:

First, H bonding between a platinated guanine and a cytosine has been established to be still possible, both in model compounds[19] and in a platinated DNA double-stranded dodecamer.[12,13a−c] Second, with the *trans* isomer of the title complex, *trans*-[Pt(NH_3)$_2$(9-MeA-*N7*)(9-MeGH-*N7*)]$^{2+}$, H bonding with 1-MeC in DMSO solution is still largely consistent with the Watson−Crick scheme.[20] Third, with *cis*-[Pt(NH_3)$_2$(9-EtGH-*N7*)(1-MeC-*N3*)]$^{2+}$ H bonding with free 1-MeC again is largely according to Watson and Crick, despite some loss in selectivity.[17] Two features could account for the observed interaction shifts (Figure 3b): (i) The *cis* orientation of the adenine base may, through stacking with guanine, affect the shift of the N1H of guanine, thereby counteracting the downfield shift of H bonding. The virtually identical chemical shifts of the guanine N1H proton in the two isomers (11.45 and 11.49 ppm, DMSO-d_6) in the absence of 1-MeC do not support this assumption. (ii) An aggregation of platinated guanine and free cytosine beyond the level of pair formation might explain why guanine NH_2 and cytosine NH_2 interaction shifts increase relative to that of the N1H of guanine. It is well established that, in the solid state, Watson−Crick pairs between guanine and 8-bromocytosine associate via the still available second NH protons of the amino groups.[21] We tentatively consider this explanation to be more likely in our case. Accordingly, intermolecular H bonding of N7-platinated guanine increases at the expense of the normal Watson−Crick pair with cytosine. Concerning possible ways of association of *cis*-[Pt(NH_3)$_2$(9-MeA-*N7*)(9-EtGH-*N7*)](NO_3)$_2$·1-MeC entities, a number of possible combinations are feasible (Figure 4), some of which could account for the effect of cytosine H5, for example (proximity of H5 and Pt with slightly propeller-twisted entities). No particular scheme can be favored at this point, however.

Deprotonation of **1** gives *cis*-[Pt(NH_3)$_2$Pt(9-MeA-*N7*)(9-EtG-*N7*)]$^+$ (**2**), which was isolated as its NO_3^- salt. As compared to those of **1**, all ^{1}H NMR resonances of **2** are shifted upfield (DMSO-d_6), as expected, with the N2H_2 of 9-EtG affected most strongly (+1.4 ppm). By application of ^{1}H NMR spectroscopy, H bonding between **1** and **2** as well as between **2** and free 9-EtGH has been studied in solution. Strong H-bond formation between platinated, N1-deprotonated guanine and neutral guanine, platinated or unplatinated, is observed (Supporting Information), similar to the situation with *cis*-[Pt(NH_3)$_2$(1-MeC-*N3*)(9-EtG-*N7*)]$^+$.[17] Interaction shifts indicate 3-fold guanine/guanine H bonding as previously established by us using X-ray crystallography[22,23] and ^{1}H NMR spectroscopy.[17]

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Supporting Information Available: Tables of positional parameters, thermal displacement parameters, and interatomic distances and angles, a view of the second cation, a stereoview of the unit cell, ^{1}H NMR spectra of **1**·1-MeC, **2**, and **1·2**, and a plot of the concentration dependency of the interaction shifts of **2** + 9-EtGH (20 pages). Ordering information is given on any current masthead page.

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(19) Dieter-Wurm, I.; Sabat, M.; Lippert, B. *J. Am. Chem. Soc.* **1992**, *114*, 357.
(20) Schreiber, A.; Lüth, M. S.; Erxleben, A.; Fusch, E. C.; Lippert, B. *J. Am. Chem. Soc.* **1996**, *118*, 4124.
(21) (a) Sobell, H. M.; Tomita, K.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *49*, 885. (b) Haschemeyer, A. E. V.; Sobell, H. M. *Acta Crystallogr.* **1965**, *19*, 125.
(22) (a) Faggiani, R.; Lock, C. J. L.; Lippert, B. *J. Am. Chem. Soc.* **1980**, *102*, 5418. (b) Faggiani, R.; Lippert, B.; Lock, C. J. L.; Speranzini, R. A. *Inorg. Chem.* **1982**, *21*, 3216.
(23) Schröder, G.; Lippert, B.; Sabat, M.; Lock, C. J. L.; Faggiani, R.; Song, B.; Sigel, H. *J. Chem. Soc., Dalton Trans.* **1995**, 3767.