A Metalated Guanine,Cytosine Base Quartet with a Novel GC Pairing Pattern Involving H(5) of C

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Four-stranded DNA structures, formed upon H-bonding interactions between two DNA duplexes, have been proposed for more than two decades and have, among others, been considered possible models for DNA strand exchange processes.[1−6] H-bonding patterns exceeding the known ones between two nucleobases have been observed occasionally, e.g. in the solid state structures of guanine,5-bromocytosine adducts,[7,8] in guanine quartets,[9,10] and in conjunction with the latter, even for thymine[11] or, in RNA, uracil quartets.[12]

Our interest in systems in which a suitable metal ion replaces a proton in a H bond between nucleobases ("metal modified base pairs") has led us to synthesize and characterize a number of model nucleobase complexes containing essentially coplanar nucleobases, linked by metal entities.[13−18] In many cases, in particular in Hoogsteen-modified pairs between purine and pyrimidine bases, coplanarity of the nucleobases is supported by additional H bonding between exocyclic groups, directly or via a water molecule. Among others, we have isolated *trans*-$[(NH_3)_2Pt(9\text{-EtGH-}N7)(1\text{-MeC-}N3)]^{2+}$ (**1**, with 9-EtGH-*N7* = 9-ethylguanine platinated at N(7) and 1-MeC-*N3* = 1-methylcytosine platinated at N(3)),[19] which is to be considered a model for the interstrand DNA cross-link of *trans*-$(NH_3)_2PtCl_2$ with guanine and its complementary nucleobase cytosine.[20] As a consequence of Pt(II) coordination to N(7) of guanine, the proton at N(1) becomes more acidic by ca. 1.5 log units,[21] thereby facilitating formation of the deprotonated species *trans*-$[(NH_3)_2Pt(9\text{-EtG-}N7)(1\text{-MeC-}N3)]^+$ (**2**) at alkaline pH.

Here we report on our findings of the dimerization of **2** in solution which gives a metalated G_2C_2 base quartet with an unprecedented H-bonding pattern between deprotonated guanine and neutral cytosine which involves the cytosine H(5) and guanine N(1) (Figure 1).

Established H-bonding patterns between G and C include the Watson−Crick pair between the neutral bases, the Hoogsteen scheme between neutral guanine and protonated cytosine, the combination of both in C,G,C triplets,[22] as well as a recently reported C,G,G triplet,[23] which again is between neutral bases. As stated in the introduction, aggregation beyond the Watson−Crick pair is occasionally seen with 5-bromocytosine,guanine combinations.[7,8]

The title compound *trans*-$[(NH_3)_2Pt(9\text{-EtG})(1\text{-MeC})]ClO_4$ (**2**) was obtained from *trans*-$[(NH_3)_2Pt(9\text{-EtGH-}N7)(1\text{-MeC-}N3)](ClO_4)_2$ (**1**) following addition of NaOH and crystallization.[24] The identity of **2** was established by use of conventional techniques, including electrospray ionization mass spectrometry (ESI-MS).[25] The concentration dependence of two of the ^{1}H NMR resonances of **2** in DMSO-d_6 provided clear evidence for an associative process (Figure 2). Specifically, the large downfield shift of cytosine H(5) and of one of the two cytosine NH_2(4) protons with increasing concentration is consistent with the proposed dimerization (Supporting Information). All the other ^{1}H NMR resonances remain essentially unaffected. From the NMR data, an association constant of 59.1 ± 1.0 l mol^{-1} was estimated,[26] which markedly exceeds that of the Watson−Crick pair between guanosine and cytidine in the same solvent (3.7 ± 0.6 l mol^{-1}; 32 °C).[27] The involvement of an aromatic proton in H bonding with a N acceptor site is still quite rare,[28] in contrast to CH···O bonds, the significance of which in biological systems[29] is increasingly recognized and lately has been verified in a UU pair in the RNA fragment 5′-r(UUCGCG)-3′.[30] The existence of the H-bonding scheme given in Figure 1 is further substantiated by a NOESY experiment which indicates a close proximity between cytosine H(5) and guanine NH_2(2) (Supporting Information).[31]

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(24) Preparation of *trans*-$[(NH_3)_2Pt(9\text{-EtG-}N7)(1\text{-MeC-}N3)](ClO_4)_2$ (**1**): A mixture of 0.5 mmol (226 mg) of *trans*-$[(NH_3)_2Pt(1\text{-MeC})Cl]NO_3$, 0.5 mmol (90 mg) of 9-EtGH, and 0.99 equiv of $AgNO_3$ (84 mg) in 40 mL of H_2O is stirred at 40 °C for 48 h in the dark and AgCl is filtered off. Upon addition of a twofold excess of $NaClO_4$, **1** precipitates as white microcrystals; yield 78% (285 mg). *trans*-$[(NH_3)_2Pt(9\text{-EtG-}N7)(1\text{-MeC-}N3)]ClO_4\cdot 1.5H_2O$ (**2**) is prepared by adjusting the pH of a saturated solution of **1** (0.5 mmol (366 mg) in 10 mL of H_2O) to 10.7. Upon cooling to 4 °C for 48 h, a white powder precipitates which is filtered off, washed with H_2O, and dried at 40 °C; yield 75% (247 mg). Anal. Calcd (found) for $C_{12}H_{21}N_{10}O_6PtCl\cdot 1.5H_2O$ (**2**): C, 21.9 (21.8); H, 3.7 (3.6); N, 21.3 (21.5).
(25) MS: *m*/*z* 1063, dimer of **2**; m/z 1085, Na adduct of the dimer; *m*/*z* 1163, ClO_4^- adduct of the dimer. Mass spectra were recorded on a Finnigan MAT90 spectrometer using an electrospray source (Finnigan) at an acceleration potential of +5000 V and an infusion pump E540101 (TSE) (Harvard Apparatus). The solution of **2** in methanol was infused through a 75 μm fused silica capillary at a flow rate of 0.5 μL/min. 89 scans were recorded in the range of *m*/z 400−1200 (scan speed 8.27 s/scan).
(26) From a dilution experiment, K_a (which is a function of complex concentration, observed chemical shift, and the shifts of the isolated monomeric and dimeric species, δ_{mono} and δ_{dimer}) was obtained by linear regression. δ_{mono} and δ_{dimer}, which could not be measured directly, were refined iteratively in steps of 0.001 ppm each and the resulting K_a was used to simulate a theoretical dilution curve. A minimum for the sum of the squared deviations of observed and calculated δ values was found for $\delta_{mono} = 5.879$ ppm and $\delta_{dimer} = 7.121$ ppm to give a final $K_a = 59.1 \pm 1.0$ L mol^{-1}. DMSO-d_6 samples in all cases contained H_2O introduced by the complex. Removal of water, e.g. by molecular sieves, represents a problem. See: Lippert, B. *J. Am Chem. Soc.* **1981**, *103*, 5691.
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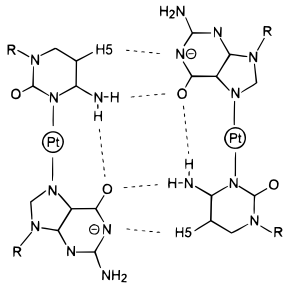

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Figure 1. Schematic view of the proposed structure of the dimerized complex *trans*-[$(NH_3)_2$Pt(9-EtG-*N7*)(1-MeC-*N3*)]ClO_4 (**2**). NH_3 ligands are omitted for clarity.

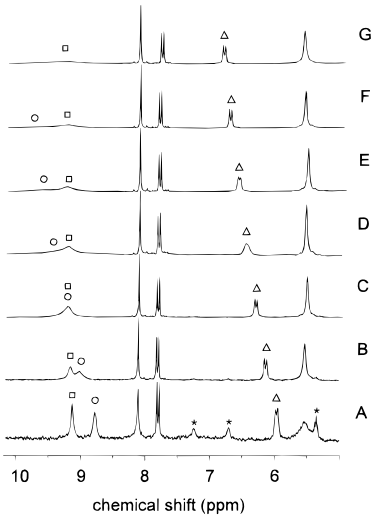

Figure 2. Downfield part of the ^{1}H NMR spectra of **2** at different concentrations. Spectra were taken in deuterated DMSO without presaturation of solvent resonances using the signal of the non-deuterated DMSO as internal reference (δ 2.53 ppm relative to TMS). Concentrations were 0.5 (A), 3.2 (B), 7.5 (C), 13.6 (D), 27.8 (E), 49.7 (F), and 169.9 mM (G). The two resonances influenced most by intermolacular hydrogen bonding are marked by Δ (H(5) of cytosine) and $\circ$ (NH_2(4) of cytosine). At very low concentrations of **2**, some minor impurities of the solvent (*) become visible.

The H-bonding pattern realized in **2** (Figure 3, iv) is one of four cases now established to occur when a guanine nucleobase becomes metalated at the N(7) position. Three of these have previously been demonstrated using X-ray crystallography: (i) normal Watson−Crick pairing of N(7)-platinated guanine with cytosine,[13] (ii) guanine−guanine pairing upon hemideprotonation of the purine nucleobase,[32] and (iii) guanine−guanine pairing between a metalated, deprotonated guanine and a neutral, free guanine.[21] These patterns (Figure 3) are also seen with **1** and **2**, respectively, according to ^{1}H NMR spectroscopy in DMSO-d_6.[33]

The isolation of the various products from aqueous solution requires successively higher pH values, e.g. pH ≈ 6−7 for (i), pH ≈ 8 for (ii), pH ≈ 9 for (iii), and pH ≈ 10 for (iv). In discussing the existence of (ii)−(iv) under biologically relevant conditions, the pH argument should not be overemphasized, however.[34]

Findings in our model system suggest that an interference of metal ions of suitable geometry with DNA strand exchange processes is feasible, in principle. Depending on the nature of the metal ion (kinetically inert or labile), both an inhibition of

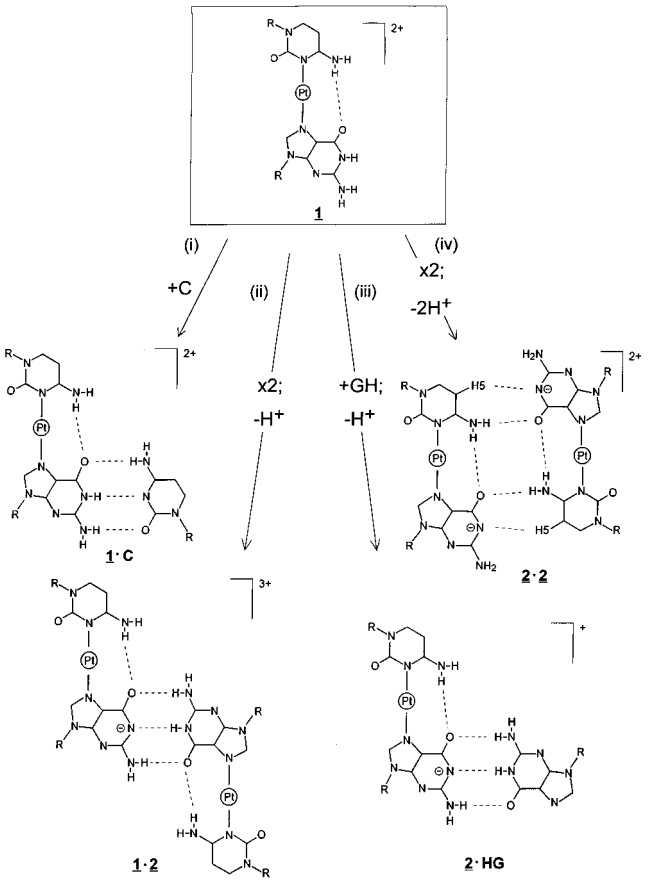

Figure 3. H bonding involving N(7) platinated guanine. Patterns (i)−(iii) have been verified in related Pt complexes using X-ray crystallography[13,21,32] and ^{1}H NMR spectroscopy.[21,33]

such processes and an acceleration seem possible. From a geometrical point of view, metalated adenine/uracil and adenine/thymine pairs might behave similar to **2** without requiring high pH for purine deprotonation.

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Supporting Information Available: Concentration dependence of selected ^{1}H NMR resonances of **2** in DMSO-d_6, section of NOESY spectum of **2**, and ESI-MS of **2** (4 pages). See any current masthead page for ordering and Internet access instructions.

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(31) An alternative model, proposed by one of the reviewers, with H bonding between N(1) of guanine and N(4)H_2 of cytosine is ruled out. It would lead to only two H bonds between the two halves plus two repulsive interactions between H(5) of cytosine and a proton of guanine N(2)H_2. Such a model could explain neither the concentration dependence of H(5) nor the apparently unaffected guanine−NH_2 resonance, and would be sterically unlikely. It would require an intermolecular separation between cytosine C(5) and guanine N(2) of ca. 3 Å.

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(33) Interaction shifts in DMSO-d_6 are similar to those reported previously, e.g. refs 21 and 26. Details will be reported elsewhere.

(34) We note that from the point of view of pK_a, protonated cytosine should not exist at physiological pH. As to nucleobase deprotonation, despite the negative charge of the nucleotides in DNA and RNA, it appears not to be impossible. See, e.g.: Yu, H.; Eritja, R.; Bloom, L. B.; Goodman, M. F. *J. Biol. Chem.* **1993**, *268*, 15935.