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Loss of Hoogsteen Pairing Ability upon N1 Adenine Platinum Binding

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Chloroform- and Freon-soluble mixed thymine, adenine complexes *trans*-[Pt(MeNH$_2$)$_2$(ChmT-N3)(ChmA-N1)]NO$_3$ (**2**) and *trans*-[Pt(MeNH$_2$)$_2$(ChmT-N3)(TBDMS-ado-N1)]BF$_4$ (**3**) (ChmT = anion of 1-cyclohexylmethylthymine ChmTH, ChmA = 9-cyclohexylmethyladenine, TBDMS-ado = 2′,3′,5′-tri-*tert*-butyldimethylsilyladenosine) have been prepared and characterized to study their propensity to undergo Hoogsteen and/or reversed Hoogsteen pairing in solution with free ChmTH and free 3′,5′-diacetyl-2′-deoxyuridine, respectively. No Hoogsteen or reversed Hoogsteen pairing between **2** and ChmT takes place in CDCl$_3$. In Freon, partial H bonding between N1 platinated TBDMS-ado and 3′,5′-diacetyl-2′-deoxyuridine as well as its [3-^{15}N] labeled analogue is unambiguously observed only below 150 K. Comparison of ^1J (^{15}N–^{1}H) coupling constants of 3′,5′-diacetyl-2′-deoxyuridine involved in Hoogsteen pairing with free and N1 platinated adenine suggests that the interaction is inherently weaker in the case of platinated adenine. To better understand the complete absence of hydrogen bonding between the ChmA ligand in **2** and free ChmTH, ab initio calculations (gas phase, 0 K) have been carried out for Hoogsteen pairs involving adenine (A) and thymine (T), as well as simplified analogues of **2** and T, both in the presence and absence of counteranions. The data strongly suggest that reduction of the effective positive charge of the heavy metal ion Pt^{2+} by counterions diminishes interaction energies. With regard to mixtures of **2** and ChmTH in chloroform, this implies that ion pair formation between the cation of **2** and NO$_3^-$ may be responsible for the lack of any measurable Hoogsteen pairing in this solvent.

Introduction

The complementary nucleobases adenine (A) and thymine (T) can associate through pairs of H bonds in a number of ways, viz. in Watson−Crick, reversed Watson−Crick, Hoogsteen, and reversed Hoogsteen fashion.[1] With isolated bases and unlike in DNA, Hoogsteen and reversed Hoogsteen pairing appears to be more common than Watson−Crick pairing, at least in the solid state.[2] Interestingly, gas-phase calculations provide similar results.[3] Only at very low temperatures (113 K; CDClF$_2$/CDF$_3$ solvent mixture) the predominance of the Watson−Crick pair for suitably substituted A and T model bases has recently been established.[4] Our interest in the effects of metal ion coordination on the H bonding pattern of nucleobases[5] has recently led us to demonstrate that Pt(II) binding to N7 of guanine (G)

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reinforces the Watson−Crick pair with cytosine (C) in DMSO solution.[6] Theoretical calculations (0 K, gas phase) had predicted this feature of metal cations bonded at the G−N7 site as a consequence of polarization effects.[7] Interestingly, no such effects were found with adenine-N7 complexes of divalent cations (Zn^{2+}, Cd^{2+}, Hg^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}) applying high-level quantum mechanical methods.[8] Whether or not this result also holds up in solution has not been studied as yet. This finding is noteworthy in the context of A−N7 being the second most important site of DNA bases for binding of the antitumor agent cisplatin[9] and because of the considerable mutagenic potential of the 5′-ApG adduct of cisplatin with DNA.[10,11]

Of the other possible metal binding sites of adenine, that is, N1,[12] N3,[13] and N6,[14] the first one is of comparable significance as N7, at least if the base is not involved in Watson−Crick base pair formation (vide infra); hence, it is free, or it may adopt Hoogsteen pairing. The other cases are not considered here. Here, we report on our studies concerning the H bonding interactions of a chloroform-soluble platinum complex, with the metal entity fixed at N1, toward thymine and uracil model nucleobases. Studies of this kind, employing Pt(II) species, have proved impossible in the past because of the insolubility of Pt(II) complexes in nonpolar solvents on one hand, and the absence of H bonding between A and T in polar solvents such as H_2O, DMF, or DMSO on the other. By using derivatives, carrying large organic entities attached to the individual components to achieve sufficient solubility,[15] we have been able to circumvent these problems.

Experimental Section

Materials. 9-Cyclohexylmethyladenine (ChmA),[16] 1-cyclohexylmethylthymine (ChmTH),[17] 9-methyladenine (9-MeA),[18] 9-ethyladenine (9-EtA),[16] 1-methylthymine (1-MeTH),[19] 2′,3′,5′-tri-*tert*-butyldimethylsilyladenosine (TBDMS-ado),[20] 3′,5′-diacetyl-2′-deoxyuridine (and the [3-^{15}N]-labeled compound),[21] as well as *trans*-Pt(MeNH$_2$)$_2$Cl$_2$ [22] were prepared according to literature procedures.

***trans*-Pt(MeNH$_2$)$_2$(ChmT-*N3*)Cl (1).** The compound was prepared in a slightly modified version of that used for the corresponding 1-MeT complex:[23] To 0.502 g (1.531 mmol) of *trans*-[Pt(MeNH$_2$)$_2$Cl$_2$] in 30 mL of DMF was added 0.247 g (1.454 mmol) of $AgNO_3$ and the reaction mixture stirred overnight. AgCl was removed by filtration, and 0.4402 g (1.691 mmol) of the potassium salt of 1-*N*-cyclohexylmethylthyminate was added to the filtrate. After stirring at RT for 3 d, DMF was largely removed in vacuo. Addition of diethyl ether led to isolation of 0.555 g (71%) of analytically pure *trans*-Pt(MeNH$_2$)$_2$(ChmT-*N3*)Cl (1). Anal. Calcd for $C_{14}H_{27}N_4O_2ClPt$: C, 32.7; H, 5.3; N, 10.9. Found: C, 32.9; H, 5.1; N, 11.0. ESI-MS: *m*/*z* 515 (M^+). IR (cm^{-1}): 3447.9 (b), 3271.9 (w), 3192.7 (m), 3106.5 (s), 2928.2 (s), 2851.8 (w), 1664.3 (vs), 1633.1 (vs), 1570.5 (vs), 1461.4 (s), 1438.1 (s), 1096.8 (m), 774.1 (m), 585.8 (w), 463.9 (w), 336.5 (w).

***trans*-[Pt(MeNH$_2$)$_2$(ChmT-*N3*)(ChmA-*N1*)]NO$_3$ (2).** It was prepared by adding *trans*-[Pt(MeNH$_2$)$_2$(ChmT)(DMF)]NO$_3$, obtained in situ by reacting **1** with 0.95 equiv of $AgNO_3$ in DMF overnight at room temperature, to a solution of ChmA in DMF and stirring the mixture for 24 h at room temperature. After rotary evaporation of DMF, the crude product was recrystallized from methanol/ether (20/1, v/v) to give analytically pure **2** in 30% yield. Anal. Calcd for $C_{26}H_{44}N_{10}O_5Pt$: C, 40.5; H, 5.8; N, 18.2. Found: C, 40.3; H, 5.6; N, 18.0. ESI-MS: *m*/*z* 709 (M^+). IR (cm^{-1}): 3379.1 (b), 3112.0 (s), 2925.9 (s), 2852.5 (m), 1660.3 (vs), 1634.2 (vs), 1557.8 (vs), 1539.3 (vs), 1534.9 (vs), 1307.4 (vs), 1104.8 (s), 827.3 (w), 776.9 (m), 719.0 (m), 652 (w).

***trans*-[Pt(MeNH$_2$)$_2$(ChmT-*N3*)(TBDMS-ado-*N1*)]BF$_4$ (3).** This compound was prepared by adding *trans*-[Pt(MeNH$_2$)$_2$(ChmT-*N3*)(DMF)]BF$_4$, obtained from **1** and $AgBF_4$ in DMF, to a solution of TBDMS-ado in DMF and stirring the mixture for 24 h at room temperature. The workup of the solution was analogous to that of **2**. The yield was 55%. Anal. Calcd for $C_{42}H_{82}N_9O_6Si_3BF_4$: C, 42.9; H, 7.0; N, 10.7. Found: C, 42.3; H, 6.8; N, 10.7. ESI-MS: *m*/*z* 1088 (M^+). IR (cm^{-1}): 3273.8 (b), 2930.1 (s), 2857.5 (m), 1652.0 (s), 1567.8 (s), 1463.8 (m), 1253.4 (m), 1069.2 (vs), 836.1 (vs), 777.5 (vs), 670.1 (w), 648.8 (w).

Other reactions mentioned in the text were carried out on a NMR scale only. N1 and N7 linkage isomers were assigned by means of pH* dependence (pH* is uncorrected pH meter reading) of the aromatic protons, and yields were estimated by integration of the respective ^{1}H NMR resonances.

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Mass Spectrometry. LC MS analysis was carried out on a PE Sciex API 165 mass unit connected to a Jasco HPLC system. HPLC analysis was performed using a Merck LiChrosphere 100 RP18 column (pore size 5 μm, diameter 4 mm, length 250 mm) and 10 mM aqueous ammonium acetate/acetonitrile (gradient: 0−50% acetonitrile) buffer. Compound **2** (*m*/*z* 709 [M^+], t_r 22 min) was the only observed product. ESI-MS spectra were recorded on a Finnegan MAT 900 double-focusing instrument with an electrospray interface.

NMR Spectroscopy. All NMR spectra except for the ^{1}H−^{195}Pt HMQC experiment, which was performed on a Bruker 200 MHz spectrometer, were recorded on a Bruker DPX-300 MHz spectrometer using a 5 mm multinucleus probe. A variable temperature unit was used to keep the temperature stable. ^{1}H NMR spectra were acquired with normal single-pulse excitation, 45° flip angle, pulse-recycle time of 6 s, and spectral width of 3.9 kHz consisting of 24K data points. For Fourier transformation, a size of 16K was used. ^{1}H NMR chemical shifts in ppm were referenced to the solvent peak. One-dimensional carbon spectra were acquired with normal single-pulse excitation, 45° flip angle, pulse-recycle time of 3 s, and spectral widths of 18.8 kHz consisting of 32K data points. For Fourier transformation, a size of 16K was used. One-dimensional platinum spectra were acquired with normal single-pulse excitation, 70° flip angle, pulse-recycle time of 0.03 s, and spectral width of 148 kHz consisting of 4K data points. For Fourier transformation, a size of 16K was used. ^{195}Pt NMR chemical shifts were calibrated using K_2PtCl_4 as an external reference at −1614 ppm relative to K_2PtCl_6. A sweep width of 148 kHz was used. The NOESY, the long-range COSY, and the ^{1}H−^{195}Pt HMQC spectra were all measured in CD_3OD.

Low-temperature NMR experiments in a Freon solvent were performed on a Bruker AMX500 spectrometer. The deuterated Freon mixture $CDClF_2/CDF_3$ was prepared as earlier described[24] and handled on a vacuum line which was also used for the sample preparation. Temperatures were adjusted by a Eurotherm variable temperature unit to an accuracy of ±1.0 °C. ^{1}H chemical shifts in the Freon mixture were referenced relative to $CHClF_2$ ($\delta_H = 7.13$ ppm). A 2D NOE experiment at 128K was recorded with a mixing time of 60 ms and a pulse-recycle time of 1.2 s. A total of 900 FIDs of 2K complex data points were collected, and the t_1 and t_2 FIDs zero-filled to give a 2K·1K data set prior to Fourier transformation.

Computational Part. Computations have been carried out at DFT (density functional theory) level of theory as implemented in the Gaussian 98 computer code.[25] Similarly to our previous contributions[14e,26] published on platinated nucleobase complexes, we have chosen a combination of Becke's three-parameter hybrid functional[27] with Lee−Yang−Parr's exchange functional.[28] The metal has been described with the LANL2DZ pseudopotential and basis set,[29] while the 6-31G* valence basis set was employed on the C, N, O, and H atoms.[25] Geometry optimizations were carried out with relaxation of all inter- and intramolecular parameters. Interaction energies were computed on the optimized structures and corrected for the basis set superposition error. Pairwise interaction energy terms as well as the three body term have been derived using the procedure given in refs 7a and 30.

Results and Discussion

Metal Binding Sites at Adenine. Established metal coordination patterns of adenine nucleobases have been reviewed.[31] In the following, only metal binding to two (N1, N7) of the three (N1, N7, N3) kinetically favored endocyclic sites of the adenine ring will be discussed. Metal binding to the deprotonated N6 position, either alone[14] or in conjunction with N1 or N7,[31] will not be considered here, although it is the thermodynamically preferred site, and migration from N7 to N6,[14f] as well as from N1 to N6,[32] has been observed. From model chemistry, it is known that the binding behavior of Pt(II) is influenced by several factors. Thus, at acidic pH, the most basic site N1 ($pK_a \sim 4$) is protonated, and consequently, the metal is directed to N7. In the pH range, where both N1 and N7 sites are unprotonated (pH > 6), usually a mixture of linkage isomers is formed. Reasons for the distribution patterns between the two sites are poorly understood. The amino group exercises a steric hindrance for incoming metal electrophiles, which is larger for metals approaching N1 as compared to N7.[33] Similarly, ancillary ligands already bonded to the metal have an effect on the distribution between N1 and N7 sites. For example, *cis*-[Pt(NH$_3$)$_2$(1-MeC-*N3*)(H$_2$O)]$^{2+}$,[34] when reacted with the model adenine base 9-MeA[34] in water, preferentially leads to the N7 cross-linking product, whereas the corresponding trans isomer prefers N1 over N7.[35] A preference of N1 over N7 has also been reported for reactions of *trans*-[Pt(NH$_3$)$_2$(1-MeT-*N3*)(H$_2$O)]$^{2+}$ in water,[23] and for the dipeptide complex [Pd(glyhis)(H$_2$O)]$^{+}$[34] in the same solvent.[36] On the other hand, [Pt(dien)(H$_2$O)]$^{2+}$, although still favoring N1 in the case of adenosine, distributes evenly between N1 and N7 of 5′-AMP at pH 7−9.[37] As demonstrated by Arpalahti et al.,[12b]

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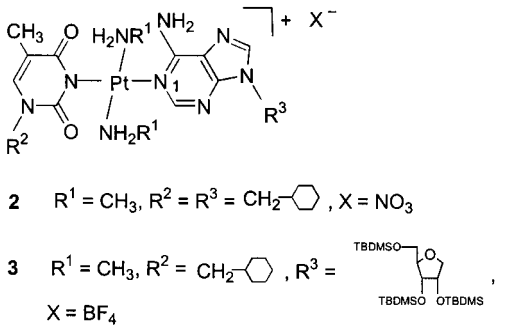
Chart 1



high temperature likewise has an effect on the species distribution, thereby favoring N1.

Formation of Mixed A,T Complexes of Pt(II) in DMF. Reactions of *trans*-[Pta$_2$(1-MeT-*N3*)(H$_2$O)]$^+$ (a = NH_3 or $MeNH_2$) with 9-MeA in water have previously been described.[23] They lead to mixtures of 9-MeA-*N1* and 9-MeA-*N7* linkage isomers. Representative species have been characterized by X-ray crystallography.[23]

Analogous reactions carried out in DMF, hence between *trans*-[Pta$_2$(1-MeT-*N3*)(DMF)]$^+$ and 9-MeA, are hampered by the insufficient solubility of 9-MeA in this solvent, yielding mainly the dinuclear μ-9-MeA complex [a$_2$(1-MeT-*N3*)Pt(*N1*-9-MeA-*N7*)Pta$_2$(1-MeT-*N3*)]$^{2+}$. However, reaction of *trans*-[Pt(NH$_3$)$_2$(1-MeT-*N3*)(DMF)]$^+$ with 1 equiv of 9-EtA and 1 equiv of 1-ChmA, respectively, gives the N1 linkage isomer in 80% and 82% yield. The second species formed is the corresponding N7 linkage isomer in both instances (20% and 18% yield), respectively.

The most regioselective platination reactions in DMF are observed between *trans*-[Pt(MeNH$_2$)(ChmT-*N3*)(DMF)]$^+$ and ChmA as well as TBDMS-ado, giving the N1 product in over 90% yield. Formation of *trans*-[Pt(MeNH$_2$)$_2$(ChmT-*N3*)(DMF)]$^+$ from the corresponding chloro complex **1** by Cl^- abstraction with Ag^+ is confirmed by ^{195}Pt NMR spectroscopy, which reveals a single peak at −2100 ppm, consistent with an N_3O coordination sphere.[38] Reactions with the two adenine derivatives are complete within 25 h at room temperature. Elemental analysis and ESI mass spectrometry reveal the compounds to be of 1:1 stoichiometry, and NMR spectroscopy proves N1 binding (vide infra), indicating a composition of *trans*-[Pt(MeNH$_2$)$_2$(ChmT-*N3*)(ChmA-*N1*)][NO$_3$] (**2**) and *trans*-[Pt(MeNH$_2$)(ChmT-*N3*)(TBDMS-ado-*N1*)][BF$_4$] (**3**), respectively (Chart 1). Minor components of these reactions are the 2:1 complexes with [Pt(MeNH$_2$)$_2$-(ChmT)]$^+$ entities bound to N1 and N7 as confirmed by ESI MS and ^{1}H NMR. Compound **2** is not found to isomerize within several days at 70 °C in a MeOH/H$_2$O mixture (1/1, v/v).

NMR Characterization of 2 and 3. ^{1}H, ^{13}C, and ^{195}Pt NMR chemical shifts of **2** and **3** as well as of the free adenine ligands ChmA and TBDMS-ado in different solvents are provided in the Supporting Information. The assignment of A-H2 and A-H8 protons and the identification of the metal binding sites are accomplished by a combination of NMR experiments which included ^{195}Pt−^{1}H coupling, 2D NMR spectra, and pH*-dependent spectra. For example, A-H8 in **2** is identified by its cross-peak with the 9-CH$_2$ resonance of the cyclohexylmethyl substituent in the ^{1}H−^{1}H NOESY spectrum (Figure 1), while in a ^{1}H−^{195}Pt HMQC experiment, A-H2 is unambiguously identified by a ^3J coupling of ∼20 Hz with the ^{195}Pt resonance (−2584 ppm). This assignment is also confirmed by pH*-dependent spectra of the aromatic protons of **2** in D$_2$O (Supporting Information). The pK_a ∼ 1 for protonation of **2** is consistent with a N1 linkage isomer being present.[23] Finally, long-range ^{1}H−^{13}C COSY experiments reveal the expected coupling of A-H2 with A-C2 (^1J), A-C4 (^3J), and A-C6 (^3J), as well as of A-H8 with A-C8 (^1J), A-C4(^3J), A-C5 (^3J), and A-C9 (^3J). The assignment of the ^{13}C NMR resonances of ChmA is based on literature data for 9-MeA,[39] as well as for [Pt(dien))(ado-*N1*)]$^{2+}$.[12b]

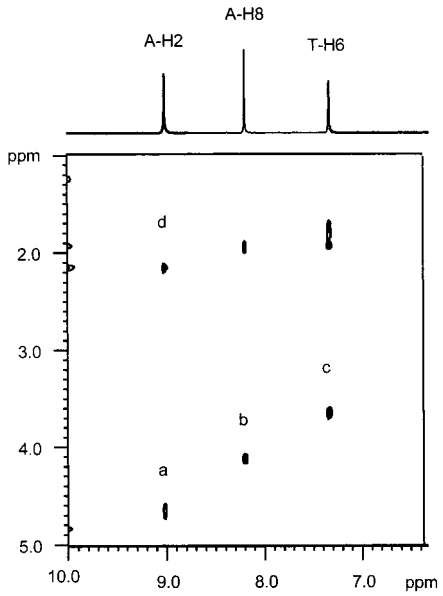


Figure 1. NOESY spectrum (MeOD) of **2**. Cross-peaks are as follows: a, A-H2/Pt-N*H*$_2$CH$_3$; b, T-H6/N1-CH$_2$; c, A-H2/Pt-NH$_2$CH$_3$; d: A-H8/N9-CH$_2$.

The ^{1}H NMR chemical shifts (D$_2$O, 3 < pH* < 11) of H2 and H8 of the ChmA ligand in **2** agree well with those of 9-MeA in *trans*-[Pt(MeNH$_2$)$_2$(1-MeT-*N3*)(9-MeA-*N1*)]$^+$ in the same solvent.[23] As compared to neutral 9-MeA, these resonances are shifted downfield by 0.29 ppm (H8) and 0.95 ppm (H2), respectively. Downfield shifts of these protons in **2**, relative to the free adenine ligand, are also observed in DMF-d_7 ($\Delta\delta$ H2, 0.66; H8, 0.29) and CD$_3$OD ($\Delta\delta$ H2, 0.81; H8, 0.11). In contrast, in CDCl$_3$, the H8 resonance of **2** is surprisingly shifted upfield, by δ −0.11, but H2 is again shifted to lower field (δ 0.32).

A-H8 in **3** is identified by its cross-peak with the H1′ proton of the ribose moiety in the ^{1}H−^{1}H NOESY spectrum. A long-range ^{1}H−^{13}C COSY experiment reveals the expected coupling of A-H2 with A-C2 (^1J), A-C4 (^3J), and A-C6 (^3J),

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(38) Pregosin, P. S. In *Annual Reports on NMR spectroscopy*; Webb, G. A., Ed.; Academic Press: San Diego, 1986; Vol. 17, p 285.
(39) Chenon, M.-T.; Pugmire, R. J.; Grant, D. M.; Panzica, R. P.; Townsend, L. B. *J. Am. Chem. Soc.* **1975**, *97*, 4627.

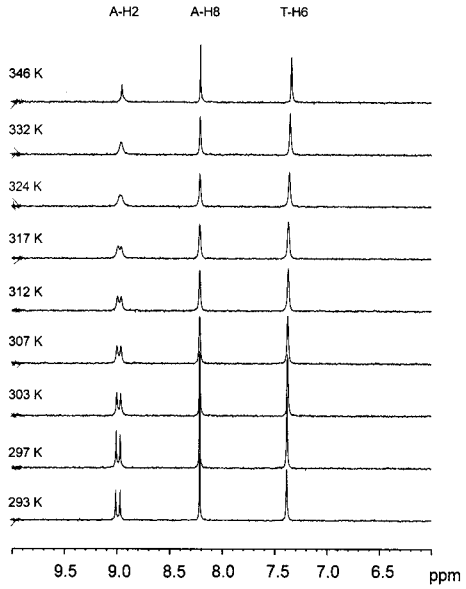

Figure 2. Temperature-dependent ^{1}H NMR spectra of **2** in D_2O.

as well as of A-H8 with A-C8 (^1J), A-C4(^3J), A-C5 (^3J), and A-C9 (^3J). The assignment of the ^{13}C NMR resonances of the TBDMS-ado ligand is again based on literature data for 9-MeA, as well as for [Pt(dien)(ado-*N1*)]$^{2+}$.[12b]

No measurable self-association of **2** is observed in $CDCl_3$, as evidenced by the concentration independence of its ^{1}H NMR resonances in this solvent.

Temperature Dependence of ^{1}H NMR Spectra. Temperature-dependent ^{1}H NMR spectra of **2** were recorded in D_2O (Figure 2), $CDCl_3$, and DMF-d_7 (Figure 3). In D_2O, A-H2 occurs as two resonances (1:1) up to ∼318 K, irrespective of pH*. Above this temperature, coalescence takes place (Figure 2). This process is fully reversible. A similar splitting of A-H2 has been reported by some of us[23] for *trans*-[Pt(MeNH$_2$)$_2$(1-MeT-*N3*)(9-MeA-*N1*)]$^+$ in D_2O. As in this case, we assign this behavior to hindered rotation of the nucleobases about the Pt−N bonds. It appears that hindered rotation depends on the presence of methylamine ligands at the Pt (the NH_3 analogue does not display this phenomenon[23]) *and* the solvent water. The role of water could be to bridge exocyclic groups of the two trans positioned bases, as seen in the X-ray crystal structure[23] of *trans*-[Pt(MeNH$_2$)$_2$(1-MeT-*N3*)(9-MeA-*N1*)]ClO_4·3.25 H_2O. While direct H bonding between exocyclic groups of the two bases is excluded on steric grounds (distance ∼4 Å), the dual function of a water molecule to act as a H bond acceptor for N(6)H_2 and a H donor for T-O4 and T-O2 is ideal for this purpose. The rate of rotation at the coalescence point can be estimated[40] as $k_c = 2.22\Delta\nu = 27.3\ s^{-1}$.

Consistent with this interpretation, hindered nucleobase rotation as evident from A-H2 splitting is seen in DMF-d_7 at lower temperature only, namely below ∼241 K (vide infra, Figure 3). In DMF-d_7, altogether three dynamic processes are observed, which involve the N(6)H_2 resonance. (i) A single resonance at ambient temperature ($\delta \simeq 8.7$), this signal

(40) Friebolin, H. *Basic One and Two-dimensional NMR Spectroscopy*; VCH: Weinheim, 1993.

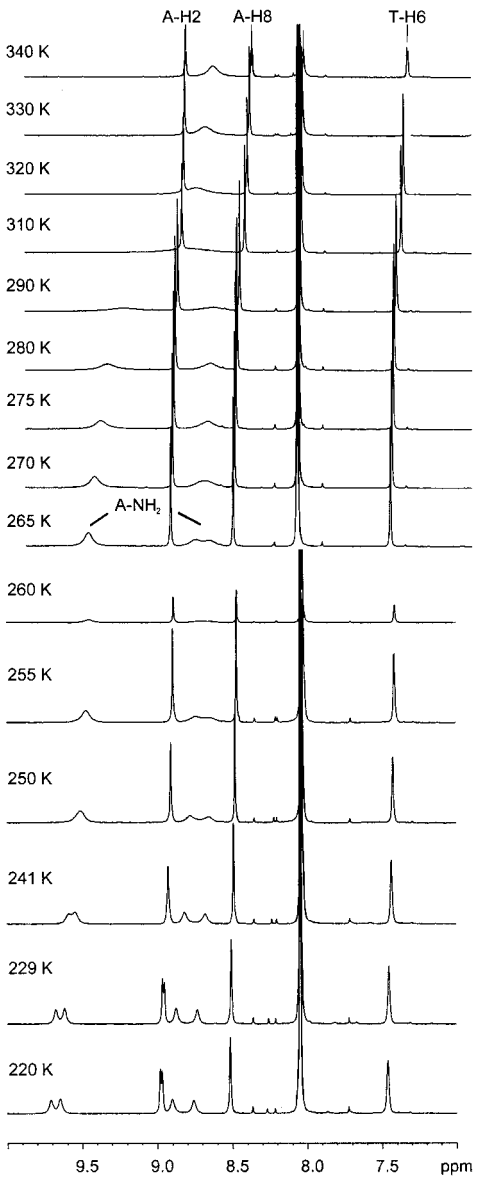

Figure 3. Temperature-dependent ^{1}H NMR spectra of **2** in DMF-d_7.

displays coalescence at ∼310 K before splitting into two components at ∼8.6 and ∼9.2 ppm (280 K). Both resonances undergo further downfield shifts with decreasing temperature, but the effect on the lowfield component is more pronounced. We assign the signal splitting to a freezing of the exocyclic amino group rotation. (ii) Below 270 K, the highfield component starts to split further (1:1), while the lowfield component does so at lower temperature only, namely in the temperature range <241 K, when the A-H2 signal starts to split. At 220 K, the A-NH_2 resonance consists of four components of equal intensities, occurring at $\delta = 9.72$, 9.65, 8.90, and 8.75 (Figure 3). Because splitting of the lowfield A-NH_2 resonance coincides with splitting of A-H2, we assign this process (below 241 K) to a freezing of the nucleobase rotation. Consequently, we attribute the A-NH_2 components above δ 9 to the amino proton which is syn to N(1) in the two possible rotamers, with O4 and O2 of the thymine base across this proton. (iii) It is not immediately evident why

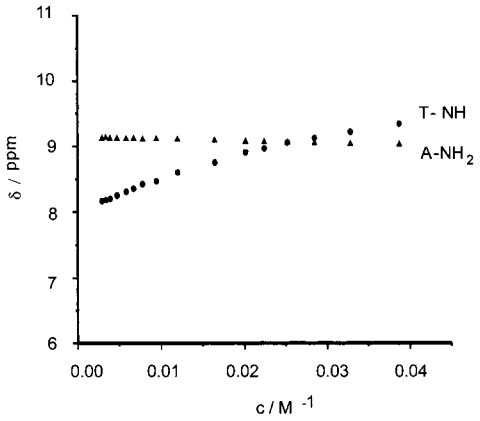

Figure 4. Concentration-dependent ^{1}H NMR shifts (T-NH, A-NH$_2$) of an equimolar mixture of **2** and ChmT in $CDCl_3$.

the upfield component of the A-N(6)H$_2$ resonance, observed in the δ 8.5−9 range, is split already below 270 K.

Concentration-Dependent ^{1}H NMR Spectra of Mixtures of 2 and ChmT in $CDCl_3$. To study the extent of hydrogen bonding between **2** and ChmT, concentration-dependent ^{1}H NMR measurements of equimolar mixtures of the two components in $CDCl_3$ at ambient temperature were carried out (Figure 4). Downfield shifts of other protons with increasing concentration are not observed, except for the N(3)H proton resonance of the free ChmT. Specifically, the shift of the amino resonance of the ChmA ligand of **2** (upfield shift of 0.09 ppm) is in fact opposite to what would be expected for intermolecular H bond formation. From the concentration dependence of the imino proton of the thymine base, an association constant has been estimated,[41] which lies in the same order of magnitude as for the self-association of ChmT in this solvent (4.72 ± 0.23 M^{-1}).[42] This implies that the concentration dependence of the N(3)H resonance of ChmT is primarily due to self-association, rather than to Hoogsteen pairing with the adenine ligand in **2**. This conclusion was further substantiated by a NOESY experiment (data not shown), which did not reveal any cross-peak between H8 of ChmA and N(3)H of ChmT. In contrast, the NOESY spectrum of a 1:1 mixture of ChmA and ChmT in $CDCl_3$ at 20 °C displays cross-peaks between A-H8 and T-NH, as well as between A-H2 and T-NH, indicative of Watson−Crick and Hoogsteen pairing coexisting under these conditions. The association constant (macro constant for all possible H bonding patterns) between A and T has previously been determined by us to be $K = 56.5 \pm 9$ M^{-1} using the same method.[42] This value is in good agreement with literature data obtained from ^{13}C NMR spectroscopy ($K = 60 \pm 5$ and 73 ± 4 M^{-1})[43] and IR spectroscopy ($K = 130$ M^{-1}),[44] respectively, for slightly different nucleobase derivatives.

Theoretical Calculations. Ab initio calculations were carried out in order to understand the rather unexpected observation from the solution NMR studies, that there is virtually no hydrogen bonding of the Hoogsteen type between N1 platinated A and free thymine. Thus, we have investigated molecular interactions in several complexes. Their molecular structures were first optimized using gradient optimization, and for the final structures, we have evaluated the interaction energies characterizing the intrinsic binding strength of the base pairs in a complete isolation (in vacuo) at 0 K. Interaction energy of a dimer is defined as the energy difference between the dimer and the two monomers when they are separated; negative values of interaction energy mean attraction.

Interaction (base pairing) energies were first calculated for a series of models with the following results: (i) adenine, thymine Hoogsteen pair, −14.1 kcal/mol; (ii) thymine, thymine self-pair, −11.0 kcal/mol; (iii) Hoogsteen pair between *trans*-[a$_2$Pt(A−N1)T]$^+$ and thymine (hereafter referred to as complex I), −18.8 kcal/mol. These results suggest that N1 platination of adenine should, in principle, enhance Hoogsteen pairing with thymine. Metal-induced strengthening of this base pairing, however, is caused primarily by long-range electrostatic attraction between the metal cation and thymine. This can be highlighted by calculating the so-called three body term. The thymine···Aa$_2$PtT base pairing energy was decomposed into two pairwise terms (Thymine···A and Thymine···a$_2$PtT) and the three-body term. The three-body term shows how A and a$_2$-PtT subsystems mutually influence their interaction with the free thymine; thus, the three-body term primarily includes the polarization effects. For the present complex I, the calculated polarization (three-body) term is negligible, around −1.3 kcal/mol.

To further understand the molecular interaction in the complex studied, we have evaluated the strength of the base pairing by reduction of the effective charge of the metal entity by the "addition" of anions. This strongly reduces the absolute values of interaction energies of thymine with the rest of the system. Thus, artificial addition of a hydroxide ion in a nearly axial position to the Pt(II) center (not shown) leads to an interaction energy of −14.2 kcal/mol. Replacement of an ammonia ligand at the Pt by a hydroxide (which results in formation of an uncharged complex) further reduces this value to −12.1 kcal/mol. The largest decrease is observed, however, if a nitrate counterion is placed in the vicinity of the exocyclic amino group of adenine (Figure 5). In the fully optimized structure of this complex, the nitrate cation displays a Pt···N interatomic distance of 3.56 Å. The nitrate anion forms a close contact with the exocyclic amino group of adenine as well as with one of the ammonia ligands of Pt(II) (interatomic H bonding distances are 2.72 and 2.79 Å, respectively). The interaction energy between thymine and the rest of the complex (i.e., the platinated adenine plus the nitrate ion) is only −11.6 kcal/mol, close to the value for thymine, thymine self-pairing and well below the stability of the nonmetalated Hoogsteen adenine, thymine base pair. The close contact of the nitrate anion with complex I overcorrects the base pairing energy gains that would be caused by the metal cation itself. It is to be noted that the

(41) Sigel, R. K. O. Unpublished results.
(42) Schmidt, K. S.; Sigel, R. K. O.; Filippov, D. V.; van der Marel, G. A.; Lippert, B.; Reedijk, J. *New J. Chem.* **2000**, *24*, 195.
(43) Iwahashi, H.; Kyogoku, Y. *J. Am. Chem. Soc.* **1977**, *99*, 7761.
(44) Kyogoku, Y.; Lord, R. C.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 250.

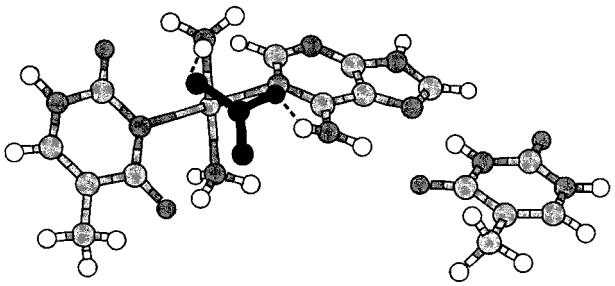

Figure 5. Becke 3LYP optimized structure of the Hoogsteen pair between *trans*-[Pt(NH_3)$_2$(T-*N3*)(A-*N1*)]$^+$T (T = thyminate anion, A = adenine) and neutral thymine in the presence of NO_3^-. The NO_3^- is hydrogen bonded simultaneously to an ammonia ligand of Pt and the NH_2 group of adenine. Although Hoogsteen pairing is possible on steric grounds, the interaction energy is very much reduced as a consequence of charge neutralization by the nitrate anion.

position of the nitrate anion does *not* impede Hoogsteen pairing with thymine for steric reasons (see Figure 5): The 6-amino group proton syn to N7 is, in principle, still available for base pairing. The optimized interatomic distance measured between N6 of adenine and O4 of thymine is the same (2.98 Å) as in the isolated adenine, thymine Hoogsteen pair.

These results tentatively suggest that ion pair formation between cationic complex I and the nitrate anion, hence charge neutralization, is primarily responsible for the nonexistence of Hoogsteen-type H bonds between T and platinated A in chloroform solution. To further support this assumption, we have compared the electron densities in the plane of the N6−H6···O4 H-bond of the (i) AT Hoogsteen pair, (ii) complex I, and (iii) complex I with a nitrate coanion. It has been found that N1 platination of the adenine results in an extensive electron density expansion along the N6−H6···O4 H bond (cf. Figure 6a,b), thereby enhancing the stability of the (Pt−A)T base pair. On the other hand, addition of a nitrate anion to the platinated complex I leads to a partial segregation of the electron density on the N6−H6 and C4−O4 bonds and therefore to a similar profile as for the nonmetalated adenine, thymine pair (cf. Figure 6a,c).

Low-Temperature ^{1}H NMR Spectra in $CDClF_2/CDF_3$ Solvent Mixtures. An alternative method to study hydrogen bonding interactions is low-temperature NMR spectroscopy. In a series of studies, the usefulness of deuterated Freon mixtures as solvents for H bonded associates between nucleobases has been demonstrated.[4,21] Such low-melting solvents allow measurements in the liquid state down to 90 K and thus permit the observation and characterization of individual nucleobase associates in slow exchange on the NMR time scale. Employing specifically ^{15}N-labeled nucleobases, additional information such as ^1J scalar couplings between ^{15}N and the proton in a hydrogen bridge can be obtained and related to the geometry of the hydrogen bonds.

In the present study, low-temperature ^{1}H NMR spectra of a 2:1 mixture of 3′,5′-diacetyl-2′-deoxyuridine or its [3-^{15}N] labeled analogue with the well soluble complex **3** were recorded in a Freon solvent. Because uridine binding to **3** is not expected to significantly differ from the thymine base with a methyl group at the 5-position, the uridine nucleoside was employed to allow a more rigorous comparison with low-temperature data on nonplatinated adenosine−uridine

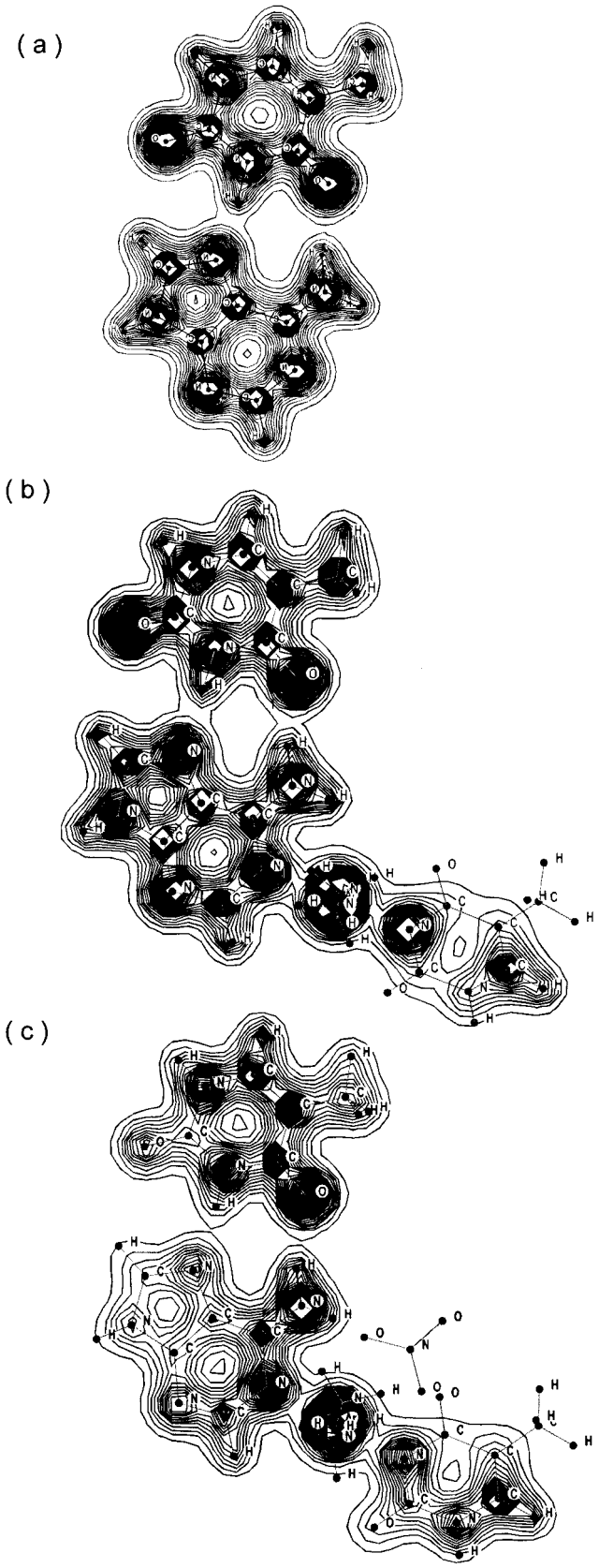

Figure 6. Comparison of calculated in-plane electron densities of (a) adenine, thymine Hoogsteen pair, (b) complex I, and (c) complex I with NO_3^- anion included.

association.[4] Moreover, as a result of a relatively high dielectric constant of the medium at these low temperatures,

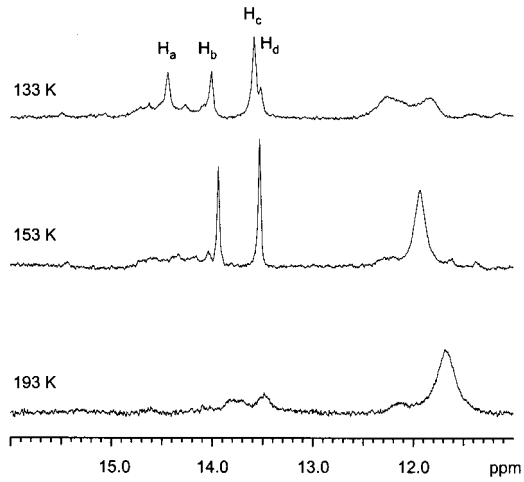

Figure 7. Sections of temperature-dependent ^{1}H NMR spectra in Freon showing the imino proton resonances of a 1:2 mixture of **3** and 3′,5′-diacetyl-2′-deoxyuridine.

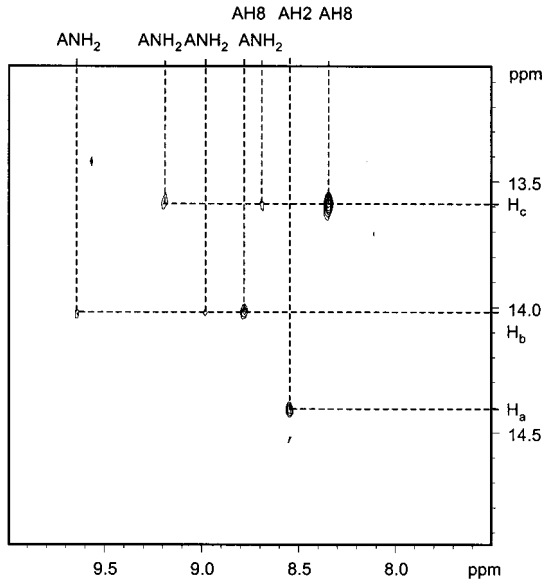

Figure 8. Portion of a 2D NOE spectrum of a mixture of **3** and 3′,5′-diacetyl-2′-deoxyuridine (1:2) in Freon at 128 K showing imino proton cross-peaks.

a good solvation of the BF_4^- counterion is expected.[45] Thus, hydrogen-bonding interactions between **3** and 3′,5′-diacetyl-2′-deoxyuridine are not expected to be influenced by the presence of the counterion. As seen from Figure 7, various uridine N(3)H imino resonances in slow exchange appear in the lowfield portion of the spectrum on lowering the temperature. The broad upfield shifted signals centered at about 12 ppm at 133 K correspond to uridine homodimers,[21] whereas the downfield shifted resonances H_{a-d} at 14.41, 14.01, 13.58, and 13.51 ppm must arise from adducts of the platinated adenosine **3** and uridine. Clearly, and in contrast to H_a and H_d which broaden out above 133 K, the H_b and H_c resonances at 14.01 and 13.58 ppm remain in slow exchange up to 153 K, indicating a higher kinetic stability of the latter. Integration of the various resonances suggests that approximately 40% of the uridine is associated with **3**, whereas the remaining 60% are bound in self-associates. With regard to the fact that the mixture contains two equiv of 3′,5′-diacetyl-2′-deoxyuridine, this implies that approximately only 80% of **3** are associated with **3** and that also free, non-hydrogen-bonded 3′,5′-diacetyl-2′-deoxyuridine is present.

To further characterize the various coexisting species, a 2D NOE spectrum of the mixture was recorded at 128 K. As shown in Figure 8, both H_b and H_c imino protons exhibit NOE contacts to adenine amino and H8 protons, which are identified by their mutual cross-peaks and cross-peaks to adenosine sugar protons, respectively (not shown). This points to their presence in two different Hoogsteen geometries. Whereas no cross-peak to H_d is detected, the H_a imino proton only exhibits an NOE contact to another signal which, based on the absence of cross-peaks to sugar protons, is tentatively assigned to an adenine H2 resonance.

The two heterocomplexes with Hoogsteen bound imino protons H_b and H_c are likely to arise from normal and reverse Hoogsteen geometries or from the two rotameric structures, as a result of hindered rotation about the N−Pt bonds, and may also be associated with the splitting of the A amino resonance anti to N(1) occurring below 270 K in DMF (vide supra). The kinetically more labile H_a and H_d imino protons only observed at very low temperatures probably represent complexes with only one hydrogen bond involving adenine N3, or the 2- or 4-carbonyl group of the ChmT ligand. Whereas participation of the adenine N3 position as proton acceptor in a hydrogen bond with the uridine base is restricted for steric reasons, the hydrogen-bond acceptor capability of T-O2 and T-O4 is expected to increase upon platination at N3 and would thus account for the low-field-shifted imino resonances when compared to the uridine homodimers.

Employing [3-^{15}N] labeled uridine, a $^1J(^{15}N\text{-}^1H)$ coupling constant of 87.6 Hz for both of the two predominant Hoogsteen complexes has been determined at low temperatures. Recently, in a nonplatinated A−U mixture, a value of 84 Hz has been measured for the A−U Hoogsteen pair in the same solvent mixture.[4] Obviously, in the platinated system, the imino proton is located closer to the uridine N3 donor in a weaker hydrogen bond. Accordingly, it is also interesting to note that a H8 resonance in the low-temperature 2D NOE spectrum shows no cross-peak to a U imino proton and demonstrates the presence of **3** with unoccupied Hoogsteen sites even with an excess of uridine.

Relevance and Conclusions

Hydrogen bonding interactions between two nucleobases can be affected by a metal entity attached to the periphery of one of the two bases, causing a strengthening or weakening of H bonding or an alternation of the specificity of base pairing. Biological consequences may range from DNA stabilization to DNA destabilization and mispair formation. Among others, these questions are also relevant to DNA adducts of cisplatin, for example, to distinguish steric effects (e.g., DNA distortion) from electrostatic and polariza-

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tion ones (e.g., effect on base pair formation). The general problem of assessing these questions stems from a frequent incompatibiliy of complex solubility and solvent allowing the observation of hydrogen bond formation. For example, the large majority of synthetic Pt(II) nucleobase complexes is water-soluble, yet base pairing in water is generally poor because of competition with the solvent and therefore cannot be studied experimentally. Only with cationic Pt(II) guanine-N7 complexes has the Watson−Crick pairing with cytosine been studied in Me_2SO.[6] In the present paper, an obvious way out of this dilemma has been presented, namely the use of large hydrophobic residues at the nucleobases to achieve solubility in chloroform and Freon. As examples, adenine complexes carrying *trans*-a_2Pt(II) entities at the N1 positions (as well as an anionic thymine base at the fourth coordination site) have been prepared and their H bonding interactions with thymine and uridine studied.[46] Surprisingly, no H-bond formation whatsoever is observed between N1-platinated adenine (**2**) and free thymine in $CDCl_3$. In Freon, H bonding between **3** and a uridine derivative is observed at very low temperature only. No conclusions concerning its existence at higher temperatures can be made. $^1J(^{15}N-^1H)$ coupling of [3-^{15}N] labeled uridine in the adducts with **3** is indicative of a weaker association as compared to the A−U Hoogsteen pair in the absence of coordinated Pt(II).

In summary, the findings in Freon strongly suggest that Pt(II) coordination to N1 of A leads to an inherently weaker Hoogsteen/reversed Hoogsteen pair. Whether the complete absence of heterobase pairing between the A ligand in **2** and T in $CDCl_3$ is to be solely explained on this basis remains questionable. Ab initio calculations suggest that, at least in the gas phase, Pt(II) coordination to A-N1 enhances pairing, essentially on the basis of favorable long-range electrostatic attraction between the Pt(II) cation and T. At the same time, these calculations reveal that any charge neutralization of the metal entity drastically reduces interbase association. In other words, ion pairing, as expected in poorly solvating chloroform, could effectively suppress interbase hydrogen bond formation under the present experimental conditions, despite the fact that the platination itself would rather stabilize H bonding, as suggested by the gas-phase QM data.

Acknowledgment. This work was supported by a "DAAD Doktorandenstipendium im Rahmen des gemeinsamen Hochschulsonderprogramms III von Bund und Ländern" and performed in the frame of the BIOMED project BMHA-CT97-2485 and COST D20. Furthermore, the study has been supported by the following grants: LN00A016, MSMT CR (J.S.), VW-Stiftung I/74657 (J.E.S., B.L., J.S.), A4040903, IGA AS CR (J.E.S.), DFG, and FCI (B.L.) The calculations were carried out in the Supercomputer Center, Brno.

Supporting Information Available: ^{1}H and ^{13}C NMR data (5 tables) and pH* dependence of H2 and H8 protons of adenine in **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(46) We do not wish to claim that the model compounds are relevant to the biological chemistry of *trans*-a_2PtCl_2, even though A, T Hoogsteen pairing is possible in (distorted) DNA (See, e.g.: Quigley, G. J.; Ughetto, G.; van der Marel, G. A.; van Boom, J. H.; Wang, A. H.-J.; Rich, A. *Science* **1986**, *232*, 1255.), and in principle, the N1 site of A could become metalated.