Metal-Stabilized Rare Tautomers and Mispairs of DNA Bases: N6-Metalated Adenine and N4-Metalated Cytosine, Theoretical and Experimental Views

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Crystal structure studies indicate that metalation of the exocyclic amino group of cytosine and adenine nucleobases by Pt^{II} and Hg^{II} entities, respectively, induces protonation of a nucleobase ring nitrogen atom, and hence, causes a proton shift from an exocyclic to an endocyclic N atom. This metal-assisted process thus leads to the generation of rare nucleobase tautomers. In principle, such processes can lead to the stabilization of mispairs. The present study reports the first quantum chemical analysis of the metal-assisted tautomerization. The calculations clearly demonstrate that metalation of the exocyclic amino group of nucleobases significantly increases the protonation energy of the aromatic rings of nucleobases by about 30−34 kcal/mol for the Pt^{II} adduct and by about 10−14 kcal/mol for the Hg^{II} adduct. The calculations suggest that this kind of metalation could, besides the structural changes of DNA, significantly enhance the probability of formation of mispairs in DNA. In the course of the study, we have realized a substantial difference in terminology, which is used in computational chemistry and in bioinorganic chemistry to characterize the tautomerism of nucleobases. The difference arises since nucleobases are studied in very different environments by quantum chemical and experimental bioinorganic methods. This point is clarified and discussed in detail because it is essential for future studies of metal-assisted tautomerism of nucleobases.

1. Introduction

The structures and properties of nucleic acids are influenced by the interactions of nucleic acid bases with metal cations.[1,2] Metalation can change the probability for the formation of rare (minor) tautomers of bases and could influence the ability of nucleobases to be protonated or deprotonated. Such modifications of nucleobases could further propagate into the formation of mispairs.

At first sight, the major contribution inducing proton shifts in metalated bases should be the ionic electrostatic contribution when the metal entity bears a nonzero charge. It has been noticed that when a divalent cation with a charge of +2 interacts with an H-bonded imidazole pair (a model system for base pairing) a proton would be transferred from one imidazole ring to another, leading to the ion pair imidazole dimer.[3] Similarly, metalation of the N7 position of guanine by a divalent cation could induce proton transfer from the guanine N1 position to the cytosine N3 position in the Watson−Crick base pair. However, this rationalization is valid primarily in the gas phase while trends observed in a condensed phase, e.g., water, are different. The reason is that polar solvents provide very efficient screening of the ionic groups.[4] The free energy gain due to the reorganization of the water structure around the ionic groups can even stabilize close contacts of two groups bearing the same charge.[4] Still, there is a marked increase in the acidity of the proton at the N1 position as a consequence of N7 purine metal binding which is on the order of 1.5−2 log units.[5] However, only a moderate effect of the charge of the metal entity is seen,[5,6] contrary to the gas-phase trends. If the situation in the solid state is considered, such a tendency is even less pronounced, since crystals are strictly neutral and counterions are compensating for the charged groups. Closely spaced cations are often observed even in a crystal lattice of oligonucleotide crystals.[7] Substantial screening of electrostatic interactions occurs also in nucleic acids where the negatively charged phosphate groups play a significant compensatory role.[8] The DNA molecule can contain regions where the charge is not neutralized as strictly as in the small compound crystals[9,10] and the expression of the ionic effects depends substantially on the particular DNA architecture.[11]

Base pairs involving N7-metalated, N1-deprotonated guanine (hemideprotonation) have been observed in crystal structures of metalated bases,[13] which leads to the self-complementarity of two N7-metalated guanines.[14] On the other hand, protonation of metalated nucleobases is also possible. For example, adenine carrying metal entities at N7 is capable of accepting a proton at N1 *irrespective of the charge* of the metal entity, e.g., $PtCl_3^-$ [15] or $ZnCl_3^-$ [16] on one hand or *cis*-$(NH_3)_2Pt^{2+}$, $(dien)Pt^{2+}$,[17] and $(OH)_2(NH_3)_3Pt^{2+}$ [18] on the other. This clearly shows *that the protonation is not driven by the long-range electrostatic forces* and the metal-induced proton shifts are rather assumed to be a consequence of direct substantial changes in the electronic structure of bases upon metalation. Another observed

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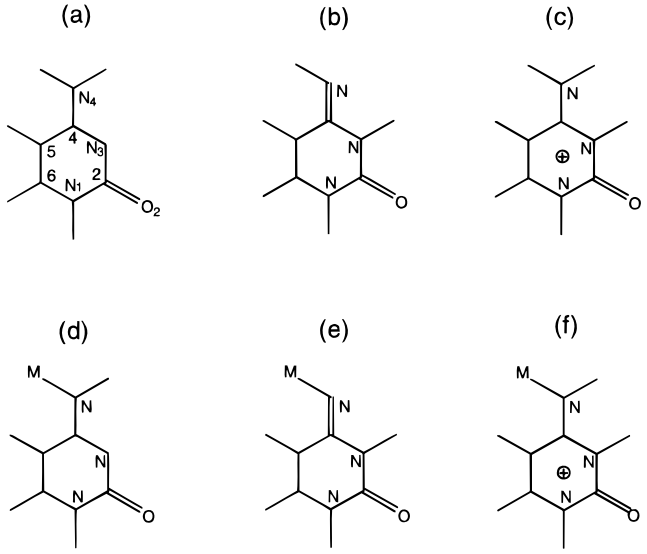

Figure 1. Structures of cytosine: (a) cytosine major (amino−oxo) form; (b) neutral rare imino form; (c) N3-protonated cytosine. Structures d−f represent the corresponding forms of the N4-metalated cytosine. See the text for terminology.

proton shift is formation of rare nucleobase tautomers under the influence of a metal entity. Upon replacement of a hydrogen atom of the N4 amino group of cytosine by a metal entity,[19−21] the N3 position is protonated to produce a metalated form of the rare iminooxo tautomer of this base. Similarly, a proton of the exocylic N6 amino group of adenine can be replaced by a metal entity, e.g., Hg^{2+} [22] or (dien)Pt^{2+},[23,24] and the N1 position protonated to give the rare imino tautomer of adenine in a metal-complexed form.

Two of the above-mentioned amino-metalated complexes appear to be particularly suitable for theoretical calculations since they are neutral: *trans*-(am)$_2$Pt(1-MeC$^-$-*N4*)$_2$ (am = NH_3 or CH_3NH_2, 1-MeC$^-$ = 1-methylcytosine anion, with metal entity at N4)[21a] and Hg(1,3-DimeU$^-$-*C5*)(9-MeA$^-$-*N6*) (1,3-DimeU$^-$ = 1,3-dimethyluracilyl anion with Hg at C5; 9-MeA$^-$ = 9-methyladenine anion with metal entity at N6).[22] The electrostatic (ionic) effects should not play a role in the two cases mentioned and a direct comparison between the calculated and experimental data is justified. Therefore, in this paper we complement the experimental studies of nucleobases metalated at the exocyclic amino group, and by using the ab initio quantum chemical approach we analyze the energetics of the formation of metal-assisted tautomers upon metalation of the amino groups of bases.

2. Method

2.1. Terminology. The present computational study is aimed at achieving a rationalization of selected proton-shifting processes observed in solvent and solid-state experiments on metalated nucleobases. In the course of this study, we have recognized that there is a substantial difference in terminology used in computational chemistry and bioinorganic chemistry. In this paragraph, we explain the difference.

Figure 1 (parts a−c) shows three structures of a cytosine molecule: major (amino−oxo) tautomer (a), minor imino−oxo tautomer (b), and finally N3-protonated cytosine (c). Molecule a is the prevailing form in a polar solvent; however, the neutral structure b is almost isoenergetical with (a) in the gas phase,[25] while protonated cytosine c is rather frequent in nucleic acids.[10−12]

Figure 2. View of (1,3-dimethyl uracilyl-*C5*)Hg^+ attached to the N6 atom of adenine. The N1 protonated form is shown.

Let us now assume that one of the hydrogens of the amino group of cytosine is replaced by a metal cation entity with a charge of +1; that is, the total charge does not change (Figure 1, structures d, e, and f). From a computational chemistry point of view, it is straightforward to consider M as an integral part of the cytosine molecule and structures d, e, and f can be considered an N4-metalated major tautomer of cytosine, an N4-metalated imino tautomer of cytosine, and an N4-metalated N3-protonated cytosine, respectively. In contrast, bioinorganic chemists view the observed structure f as the result of a metal-induced shift of an amino proton to the N3 position. The metal is not considered as a part of the cytosine molecule. Thus, structure f is known as a metal-stabilized imino−oxo tautomer of cytosine, while structure e has never been observed. One of the reasons for the difference is that bioinorganic chemists observe similar proton switches also for metal species carrying a different charge than +1, which is hardly possible in the gas phase.

In the present quantum chemical paper, we follow the nomenclature used in computational chemistry. It allows us to distinguish between structure e and f and to easily compare metalated and nonmetalated bases. In addition, this terminology better reflects the actual electronic structure of the studied species. Finally, the base-pairing energetics of molecule f closely resemble the properties of protonated cytosine c while base pairing of (e) is equivalent to (b).

2.2. Model Systems used in the Calculations. The first system considered was the crystal structure of N6-mercurated adenine where the metal cation entity is represented by (1, 3-dimethyl uracil-C5)Hg^+ attached to the N6 atom of adenine.[22] The mercury atom replaces the adenine amino group hydrogen which is anti with respect to N1 of adenine. The adenine is protonated at the N1 position. The whole structure is depicted in Figure 2. To simplify the calculations we have replaced the (1,3-dimethyl uracil-C5)Hg^+ fragment by $Hg(CH_3)^+$ (note that mercury is attached to a carbon ring atom of the uracil in the crystal). Then we studied four structures of the metalated adenine (Figure 3): the canonical neutral structure (major tautomer, Figure 3a); the neutral imino tautomer of adenine where the remaining amino hydrogen atom is shifted to the N1 position (Figure 3b); and finally protonated structures with a proton attached to either N1 or N3 (Figure 3, parts c and d). The N1-protonated adenine (Figure 3c) corresponds to the structure which has been crystallized, and this structure is referred to as the "imino tautomer" in the experimental paper.[22] This structure is called a protonated base or protonated imino tautomer throughout this paper (see above for explanation).

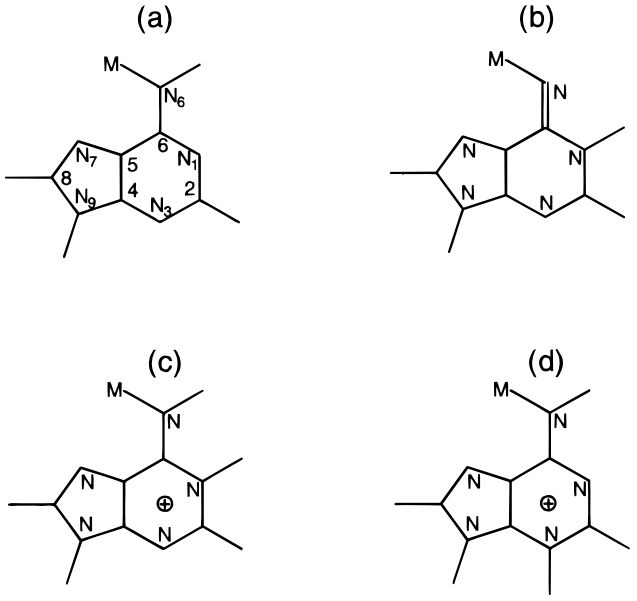

Figure 3. Structures of N6-metalated adenine: (a) major form; (b) rare imino form; (c) N1-protonated adenine (observed in the crystal); (d) N3-protonated adenine.

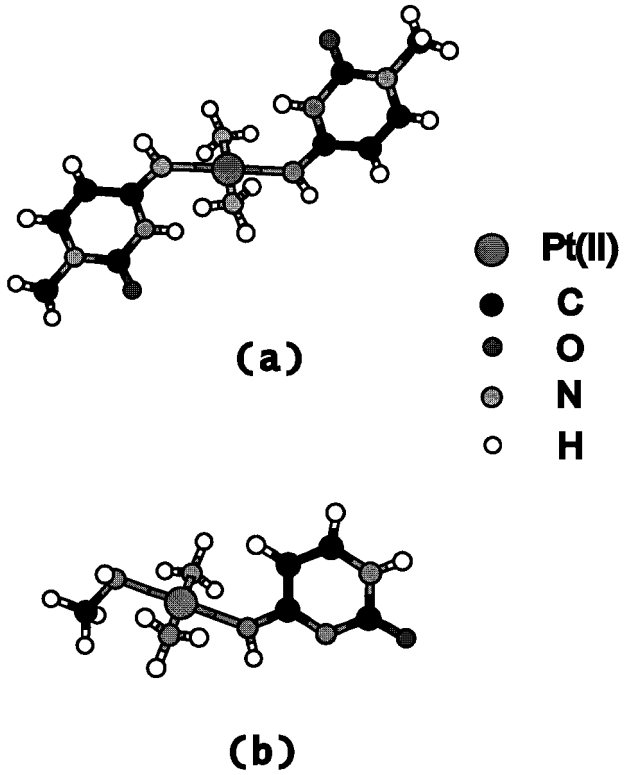

Figure 4. (a) View of *trans*-$[Pt(NH_3)_2(1\text{-MeCyt-}N4)_2]^{2+}$. (b) The model molecule used in the calculations in its major tautomeric form, cf. also Figure 1.

We have also studied metalated cytosine with the $HgCH_3^+$ group replacing the amino hydrogen proton anti relative to N by considering three molecules (Figure 1): canonical metalated cytosine (Figure 1d), its N3 imino tautomer (Figure 1e), and the N3-protonated base (Figure 1f).

The second model system studied was based on the crystal structure of cation *trans*-$[Pt(NH_3)_2(1\text{-MeCyt-N4})_2]^{2+}$ (Figure 4a).[21a] In this cation, the platinum(II) adopts a low-spin state d^8 electronic configuration. Its coordination sphere is made up of two methylated cytosines protonated at the N3 position and two ammonia molecules creating a square planar ligand field of S_2 symmetry. We have ignored the methyl group connected to the cytosine ring and $-NH_2-$Me group substituted for one of the cytosine molecules (Figure 4b). The internal parameters of the $-NH_2-$Me fragment were frozen based on the optimized structure of the methylamine molecule. The centrosymmetry of the original complex was taken into consideration by imposing a geometrical constraint on the mutual positions of the ammonia ligands and of the $-N<^{C}$ groups attached directly to the platinum. Both syn and anti rotamers were studied. The influence of platination on the basicity and tautomeric equilibrium of cytosine was characterized by comparing the stability of platinated cytosine in its amino (Figure 1d), imino (Figure 1e), and N3-protonated (Figure 1f) forms. The last structure corresponds to that observed in the crystal.

TABLE 1: Relative Energies (kcal/mol) of Neutral Adenine Imino Tautomer and N1-Protonated and N3-Protonated Adenine with Respect to the Major Forms of Adenine and Metalated ($HgCH_3^+$) Adenine[e]

	adenine	Ade−$HgCH_3$
major tautomer[a]	0	0
imino tautomer[b]	+12.3	+16.9
N1-protonated base[c]	−237.1	−249.4
N3-protonated base[d]	−234.8	−243.6

[a] Figure 3a. [b] Figure 3b. [c] Figure 3c, experimentally observed. [d] Figure 3d. [e] Data obtained at the MP2/6-31G** level.

Finally, we have studied the base pairing properties of the metalated bases by evaluating the structures and energies of several base pairs as specified in the Result and Discussion.

2.3. Methods of Calculation. The calculations of Hg^{II}-containing systems have been carried out in the following way. The first and second-row elements have been described by the standard 6-31G** basis set of atomic orbitals. The mercury atom has been described via Christiansen's relativistic pseudopotential.[26] All structures have been optimized by means of the second-order Møller−Plesset perturbational method (MP2).

Complexes of platinum with cytosine have been computed using the Becke3LYP nonlocal density functional theory (DFT) method. All atoms but platinum were described with the 6-31G** basis set while the pseudopotentials were used for platinum. We have used two pseudopotentials: Christiansen's relativistic pseudopotential[26] and the Lanl2DZ pseudopotential.[27]

The structures of the base pairs have been optimized at the Hartree−Fock (HF) level for Hg^{II}-containing structures, and at the Becke3LYP level for Pt^{II} structures. Coplanarity of the two bases has been assumed. Christiansen and Lanl2DZ pseudopotentials were used for mercury and platinum, respectively. The other atoms were described with the 6-31G* basis set. The interaction energies (difference in energy of the base pair and the two bases separated into infinity) were corrected for the basis set superposition error. We did not include the deformation energies of the monomers since they are rather small and similar for metalated and nonmetalated structures.[28]

All calculations have been repeated for nonmetaled bases and base pairs to evaluate the net effect of the metalation on the tautomerism, protonation, and base pairing.

The calculations have been performed using the Gaussian 94 suite of programs.[27]

3. Results and Discussion

3.1. Protonation Energies and Tautomeric Equilibria of N6-Mercurated Adenine and N4-Mercurated Cytosine. The results for N6-metalated adenine (Figure 3) and N4-metalated cytosine (Figure 1) are summarized in Tables 1 and 2, respectively.

TABLE 2: Relative Energies (kcal/mol) of Cytosine Imino Tautomer and N3-Protonated Cytosine with Respect to the Major Forms of Cytosine and N4-Metalated ($HgCH_3^+$) Cytosine[d]

	cytosine	Cyt−$HgCH_3$
major tautomer[a]	0	0
imino tautomer[b]	+0.7	+2.9
N3-protonated base[c]	−241.7	−255.7

[a] Figure 1, structures a and d. [b] Figure 1, structures b and e. [c] Figure 1, structures c and f. [d] Data obtained at the MP2/6-31G** level.

The neutral imino tautomer of nonmetalated adenine is ca. 12 kcal/mol less stable than the major amino form which is in agreement with the previous ab initio calculations.[29] Therefore, the formation of the imino tautomer of adenine is unlikely. Metalation further increases the difference between the major (Figure 3a) and rare (Figure 3b) forms of adenine tautomers to 17 kcal/mol.

On the other hand, N6-metalation of adenine by the mercury(II) adduct increases (in absolute value) the protonation energy of the N1-position of adenine (Figure 3c) by ca. 12 kcal/mol. Similarly, the N3-protonation of N6-metalated adenine (Figure 3d) is favored by 10 kcal/mol over protonation of the nonmetalated base. An increase in stabilization energies for protonated bases to about 10−12 kcal/mol due to metalation is rather significant, and it rationalizes the structure, which is observed in the crystal.

The results indicate that metalation of the amino group is associated with a certain positive charge withdrawing effect from the base toward the metal group. This slightly hinders the shift of the remaining amino group hydrogen atom to the N1 ring position (neutral imino tautomer). However, the protonation energy of the N1 ring nitrogen position of the base is enhanced quite significantly (Figure 3c).

We have also evaluated the molecular electrostatic potential (MEP) and carried out molecular orbital (MO) analysis. The MO analysis revealed that the molecular orbitals centered on the mercury contribute to the uppermost five occupied orbitals of adenine, but the effect is rathet moderate. MEP calculations show that upon metalation the genuine minimum of the negative potential around the N1 position of adenine is almost unaffected while the negative minimum at N3 becomes slightly weaker (Figure 5). Therefore, the changes in the protonation energies cannot be rationalized based on MEP and MEP effects caused by mercury are considerably less pronounced than the effects we will report below for the Pt^{II}-containing structures. This explains why the effect of mercury is smaller compared to platination (see below).

Table 2 shows that cytosine responded to the amino group metalation in a similar way as adenine. The isolated imino tautomer of the nonmetalated cytosine is almost isoenergetical with the major form of this base which is in agreement with literature data.[25] As for adenine, this neutral imino tautomer is weakly destabilized by the metalation (Figure 1e) with the energy difference between major and rare forms being 2.9 kcal/mol in favor of the major tautomeric form. Similarly as for adenine, the metalation of the amino group of cytosine improves the protonation energy of the N3 ring position of cytosine (Figure 1f), in this case by 14 kcal/mol.

3.2. Protonation Energies and Tautomeric Equilibria of N4-Platinated Cytosine. Table 3 compares the computed energies of the three forms (Figure 1) of nonmetalated and platinated cytosine. Their relative stability is markedly influenced by the platination.

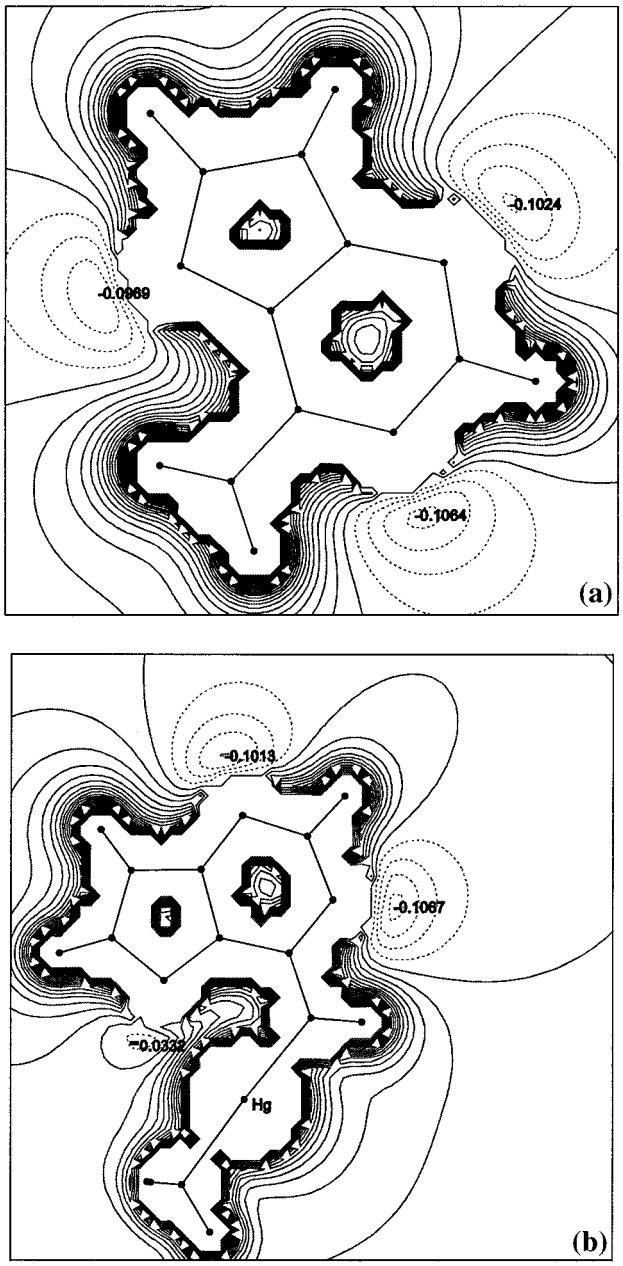

Figure 5. Molecular electrostatic potential (au) of adenine (a) and mercurated adenine (b).

TABLE 3: Relative Energies (kcal/mol) of Cytosine Imino Tautomer and N3-Protonated Cytosine with Respect to the Major Forms of Cytosine and N4-Metalated (Pt^{II}) Cytosine[h]

	cytosine[a]	Cyt−Pt^{II} [b]	Cyt−Pt^{II} [c]	Cyt−Pt^{II}(syn)[d]
neutral tautomer[e]	0	0	0	0
Imino tautomer[f]	+1.3	+11.1	+10.8	+9.7
N3-protonated base[g]	−243.1	−273.4	−273.2	−276.8

[a] 6-31G** basis set. [b] Pt: pseudopotential from Christiansen, anti tautomer. [c] Pt: Lanl2DZ pseudopotential, anti tautomer. [d] syn rotamer, corresponding to the crystal structure. Pt: pseudopotential from Christiansen. [e] Figure 1, structures a and d. [f] Figure 1, structures b and e. [g] Figure 1, structures c and f. [h] Data were obtained from DFT/Becke3LYP + pseudopotential calculations.

The first major difference can be found in the relative stability of the neutral amino and imino tautomers of free and platinated cytosine. While the neutral imino tautomer of nonmetalated cytosine is destabilized by only 1 kcal/mol (with the Becke3LYP method) with respect to the major form, this energy difference increases to 11 kcal/mol upon platination. On the other hand,

platination of cytosine markedly enhances its basicity since the protonated form has been found to be 30 kcal/mol more stable in the platinated complex than in the nonmetalated molecule. Therefore, the calculations clearly suggest that the proton shift observed in the experiment is a direct result of the metalation. A comparative calculation on the rotamers corresponding to the syn crystal orientation of platinum has revealed that the basicity of the N3 nitrogen of cytosine is even slightly more pronounced with the difference with respect to the nonmetalated base being 34 kcal/mol. It can be concluded that the effects of platination of the amino group of the bases on the tautomerism and protonation processes are almost 3 times larger compared to the Hg^{II} adduct. Table 3 also shows that the results are independent of the choice of pseudopotential imposed on the platinum atom.

A molecular orbital analysis of free cytosine forms and their platinated counterparts has shown that the changes in relative stability of the amino, imino, and protonated forms upon platination can be attributed to the influence of the d_{-2} orbitals of platinum on the electronic structure of the cytosine ring. While N3 protonation localizes the π electron density on the cytosine ring, in the amino and especially in the neutral imino cytosine forms, a strong mixing has been observed between the d_{-2} orbital of platinum and the p_y atomic orbitals centered on the heteroatoms of cytosine. Since both orbitals represent electronic density in the plane perpendicular to the cytosine ring, a strong interaction can be expected between the π electron density of cytosine and the vacant d orbital of platinum. The situation can be illustrated using the highest occupied molecular orbitals (HOMO). The HOMO of the protonated platinated cytosine is formed exclusively by the d_{-2} orbital of platinum and is the deepest in energy among the three platinated complexes. In the major amino form, the HOMO is made up of four components: the d_{-2} orbitals of platinum; the p_y orbital centered on N3; and the p_y orbitals of N4 and O2 (both with opposite signs). Only two orbitals participate in the HOMO of the imino form: the d_{-2} orbital of platinum and the p_y orbital centered on N4 with an opposite sign. Let us note that in both the amino and neutral imino tautomeric forms, the HOMO orbital is antibonding and, therefore, higher in energy than its components. It means that the platination of cytosine destabilizes the cytosine ring of the neutral amino and imino tautomers; however, it has practically no impact on the protonated cytosine moiety.

The increased basicity of the N3 position of cytosine upon the platination is accompanied by a considerable strengthening (from -0.105 to ca -0.136 au) and broadening of the minimum of the MEP around the N3 nitrogen atom, as illustrated in Figure 6. Thus, both MO effects and changes of MEP are considerably larger than for the mercury adduct.

From an analysis of the atomic charges evaluated by the natural bonding orbital (NBO) method, we have found that among the cytosine complexes the neutral imino tautomer undergoes the largest loss of π electrons upon platination (0.388 au compared to 0.317 au for the amino form). Therefore, it is not surprising that the neutral imino tautomer is further destabilized in the platinum complex compared to the nonmetalated base. Similarly, the results of the molecular orbital analysis and NBO analysis explain the increased N3 basicity of the platinated cytosine complexes. As shown above, platination of the amino tautomer strongly influences the electronic structure of the amino tautomer, and this leads to energetic destabilization of the structure. On the contrary, we have found that the protonated cytosine ring is not influenced by platination.

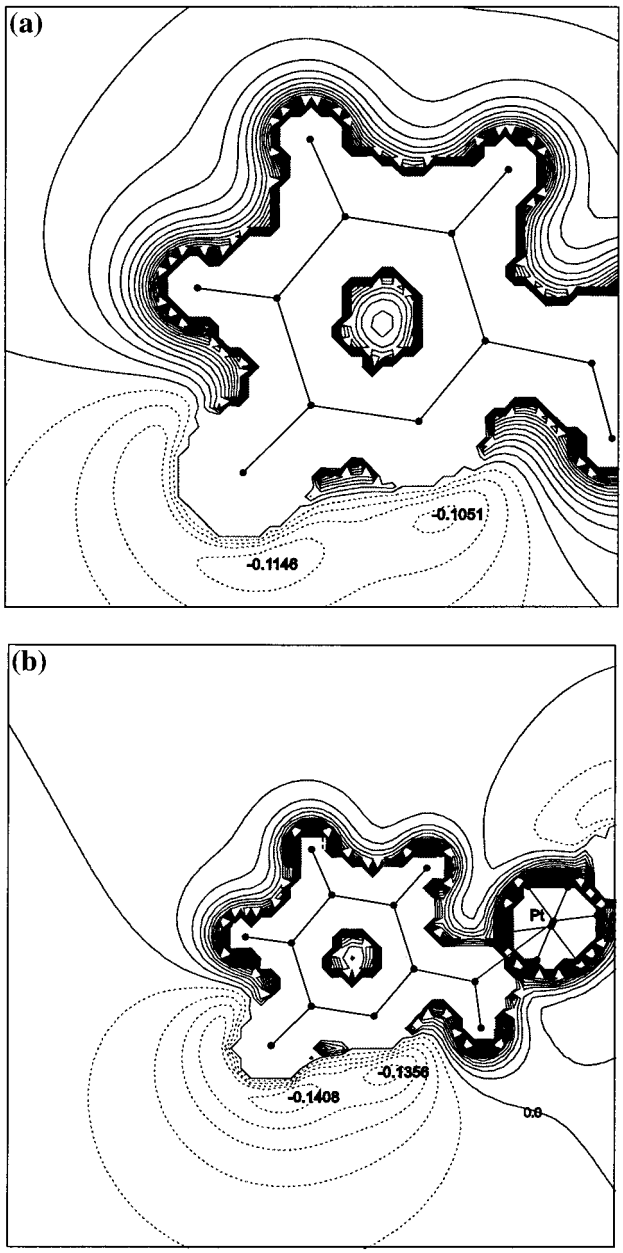

Figure 6. Molecular electrostatic potential (au) of cytosine (a) and platinated cytosine (b).

Therefore, the increased N3 basicity of the platinated cytosine can be attributed to the relative destabilization of the neutral amino tautomer rather than to the increased stability of the protonated form. The computed charges are in accordance with the above considerations. While the cytosine ring loses electrons in the platinated amino complex, a small increase in the total electron density (-0.263 au) is observed on the cytosine part of the protonated form.

A comparison of the Pt−N4 distances shows that the Pt−N bond in the protonated form is much weaker than in the other two structures.[30] This is because the bond has mainly σ-character due to the lack of an interaction with the π electron system of the cytosine ring. On the contrary, three and one p_y orbitals of cytosine participate in the HOMO of the amino and imino forms of platinated cytosine, respectively. In the imino form, a two-centered "classical" π type chemical bond contributes to the binding between the Pt and N4 atoms which, not surprisingly, creates a stronger bond between the platinum and N4 atoms than the four-centered bond in the amino tautomer. This is

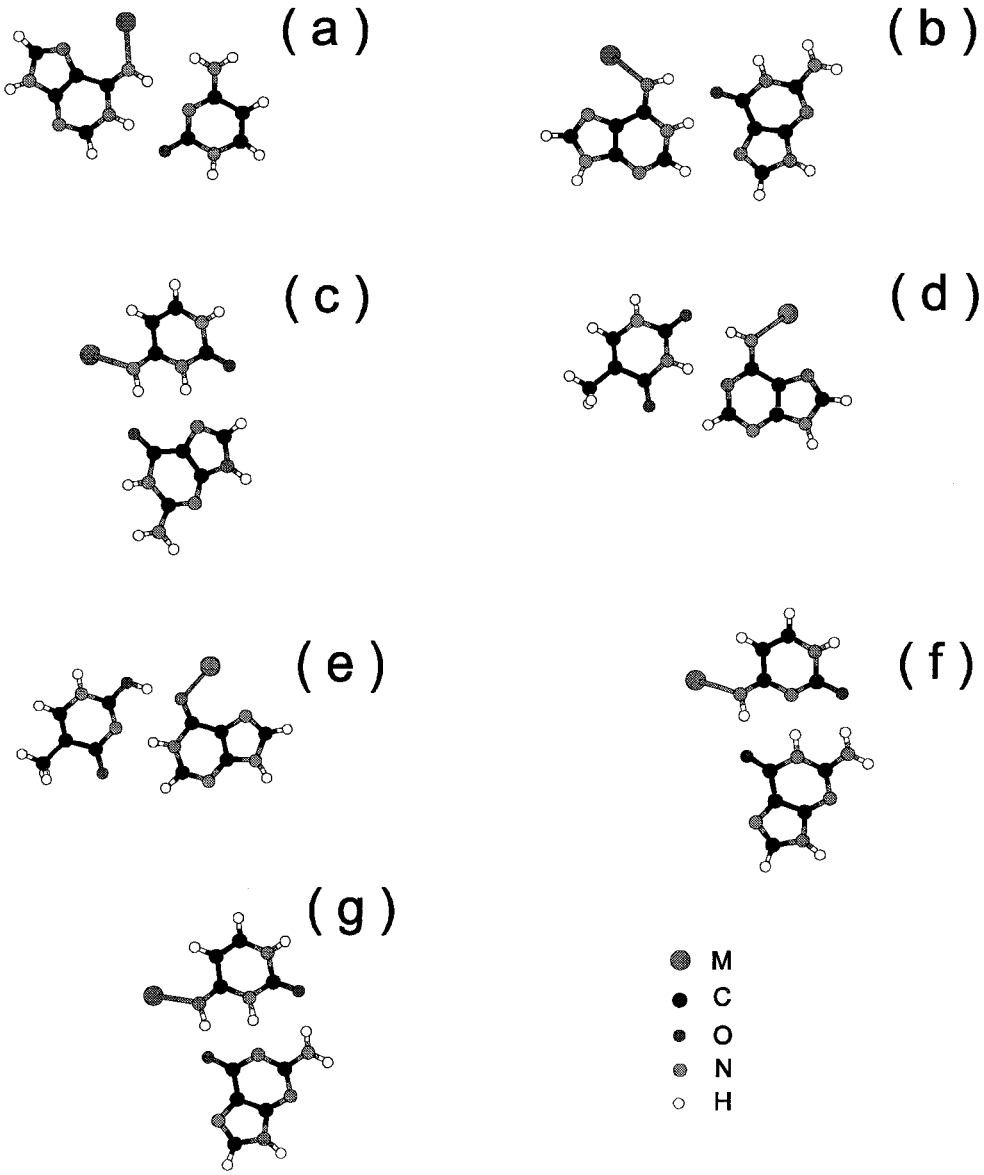

Figure 7. Metalated forms of (a) AH^+·C base pair, (b) AH^+·G base pair, (c) CH^+·G Hoogsteen base pair, (d) A·T Watson−Crick base pair, (e) A*·T* minor tautomeric form base pair, (f) G·C Watson Crick base pair, and (g) G^-·CH^+ ion pair.

reflected by the slightly shorter Pt−N4 bond observed in the imino form relative to the amino tautomer.

The extent of delocalization is clearly revealed by the N4−C4 and C4−N3 distances. Because of the formation of a four-centered HOMO, the N4−C4 and C4−N3 distances are almost the same in the amino tautomer (1.343 and 1.342 Å, respectively). In the protonated form, the N4−C4 distance decreases while the C4−N3 distance increases. A further decrease of the N4−C4 distance in the imino tautomer results in a surprisingly short N4−C4 bond (1.269 Å), whereas the C4−N3 distance adopts an unusually high value, 1.446 Å. Therefore, we conclude that the double bond character of the N4−C4 bond and the single bond character of the C4−N3 bond increase in the following order: amino < protonated < imino.

3.3. Hydrogen Bonding and Proton-Transfer Properties of the Amino-Metalated Bases. In the above paragraphs, we have shown that metals attached to the amino nitrogen of bases enhance the protonation energy of the ring position of bases, and this prediction is in agreement with the experimentally observed proton shifts. Formation of these rare tautomers would be very important if it increases the probability of the formation of mispairs. The stability of metal-induced mispairs compared to nonmetalated mispairs is roughly determined by the sum of two contributions: the difference in protonation energy reported above and the difference of intermolecular interaction (stabilization) energies of the metalated and nonmetalated base pairs.[31] The interaction energy is the difference in electronic energy of the base pair and electronic energies of the bases separated into infinity.[32,33] Thus, to predict the effect of metalation on mispair formation, we have to evaluate, besides the protonation energies, the influence of metalation on the base pairing interaction energy. We have carried out these calculations for a representative set of base pairs.

First, we have considered Hg^{II}−N6 metalated adenine protonated at the N1 position, and we have calculated its pairing energies with cytosine (Figure 7a) and guanine (Figure 7b) assuming planarity of the base pairs. The interaction energies for these two base pairs were −32.4 and −33.3 kcal/mol. The same energies evaluated for nonmetaleted protonated adenine are −39.2 for AH^+·C and −39.7 for AH^+·G. Therefore, the metalation reduces the base pairing interaction roughly by 7 kcal/mol. This can be again related to the positive charge withdrawing effect from the base toward the metal group. It makes the dominating molecular ion−molecular dipole inter-

action[10c] somewhat weaker since the positive charge on adenine is shifted away from the other nucleobase forming the pair. Considering both the protonation and interaction energies, the metalated mispairs are stabilized by ca. 5 kcal/mol compared to the nonmetalated ones, and the probability for a formation of both protonated mispairs (Figure 7, parts a and b) is likely to be enhanced by the amino group metalation.

We have made similar calculations also for N4-platinated cytosine and have evaluated its Hoogsteen (H) pairing with guanine (Figure 7c). This base pairing occurs in pyrimidine−purine−pyrimidine triplexes. The interaction energy in the metalated base pair is −31.1 kcal/mol, while the interaction energy of the nonmetalated CH^+·G H base pair is lower (more stable) by 10 kcal/mol.[28d] However, to obtain the overall effect of metalation on the stability of the mispair compared to the nonmetalated mispair, one should add together the change in the interaction and protonation energies. After considering the protonation energy of platinated cytosine (see the values in Table 3), we conclude that N4-platination increases the relative stability of this mispair by 20 kcal/mol, i.e., quite significantly.

The formation of mispairs and point mutations can be initiated by interbase proton transfer within the Watson−Crick base pairs.[34] Thus, we have estimated the effect of metalation on the proton transfer processes. First, we have investigated the base-pairing properties of the canonical AT Watson−Crick base pair (Figure 7d). The MP2/6-31G*//HF/6-31G* interaction energy of the metalated (Hg^{II}) AT WC base pair is −11.8 kcal/mol. It is identical to the published results for the nonmetalated AT WC base pair at a similar level of theory.[33] Thus, the energetics of the Watson−Crick base pairing is unaffected by metalation. Then, we have considered the double proton transfer that can occur in the Watson−Crick AT base pair, leading to a base pair abbreviated in the literature as A*T* (Figure 6e).[34,35] The A*T* pair is at the HF/6-31G* level destabilized by 21.6 kcal/mol (considering total electronic energy) with respect to the canonical AT WC pair, while this energy difference increases to 27 kcal/mol after metalation. This is because the destabilization of the neutral imino tautomer by metalation (see above) destabilizes also the A*T* base pair. Therefore, the metalation of the N6 position of adenine destabilizes the A*T* pair. (It should be noted that in order to gain full insight into the proton transfer process, a search also for the transition state[34,35,36] would be required and it is not attempted in this study.)

We have also considered proton transfer processes in the G·C base pair (Figure 7f).[34] The calculations have been done with platinated cytosine since the effect of the platinatum adduct is larger. We did not investigate the most favorable double proton transfer mechanism in the G·C base pair (the structure designated as GC2 by Florian and Leszczynski)[34] since it involves the formation of a neutral imino tautomer of cytosine which is destabilized by metalation. However, Florian and Leszczynski demonstrated that there is another possible route to a mispair in the GC base pair, the single proton transfer of the H1 guanine hydrogen to the N3 position of cytosine which leads to the formation of the ion pair GC structure (Figure 7g). The energy difference between these two structures in the nonmetalated pair is ca. 24 kcal/mol in favor of the canonical base pair.[34] Since the ion pair involves protonated cytosine, it should be stabilized by metalation. Indeed, our calculations show that platination of the amino group of cytosine decreases the energy gap between the ion pair and the canonical structures of the GC base pair quite substantially; it is ca. 12 kcal/mol after metalation.

4. Concluding Remarks

Experimental studies provide excellent structural data via X-ray crystallography and allow for the determination of the respective pK_a values and for an evaluation of free energy changes of the protonation processes. However, it is difficult for experiments to separate the effects of environment (solvent, counterions, crystal lattice) and "net" energetics and the physical origin of proton shift processes in metalated bases and base pairs. Similarly, it is difficult to separate long-range electrostatic effects and the "nonelectrostatic" contributions.

High-level quantum chemical calculations of nucleobases and their complexes are comparable in accuracy with the gas-phase experiments.[32] A major limitation of the ab initio method is the consideration of isolated "gas-phase" systems while reliable inclusion of solvent effects within the framework of quantum chemical theory is very difficult.[35] The limitation is important especially for ionic systems since the ionic systems are frequently dominated by the electrostatic effects in the gas phase while the electrostatics are off in condensed phase and in crystals. The crystallographic and ab initio studies of metalated bases complement each other in terms of the structural and energetical information and show the studied systems in two outer situations regarding the expression of the effects associated with the net charge residing on the metal cation entity. The role of the electrostatic interactions in DNA will differ from both crystal and gas-phase situations.

In the present study, we investigated the effect of metalation of the exocyclic amino groups of bases by Hg^{II} and Pt^{II} adducts. Becuase this kind of metalation does not change the electroneutrality of the nucleobase, purely ionic effects do not dominate in these systems. Therefore, direct comparison between the ab initio and experimental data is justified and the observed effects can be attributed to the influence of the metalation on the electronic structure of the nucleobases. The calculations show that the metalation destabilizes the neutral imino tautomers of bases with a fully deprotonated amino group by 2−10 kcal/mol. However, the metalation of the amino group of nucleobases significantly improves the protonation energy of the nucleobase aromatic ring, by about 30−34 kcal/mol for the Pt^{II} adduct and 10−14 kcal/mol for the Hg^{II} adduct. Exactly this proton shift has been observed in condensed phase experiments and crystallographic studies on model complexes with nucleobases. The effect is known in the bioinorganic literature as a formation of metalated forms of the rare imino tautomers of nucleobases, since bioinorganic chemists use different terminology than computational chemists (see section 2.1 above).

During the review process, the referees raised a question, whether the reduced computational models used to mimic the coordinated cations are not too small, possibly leading to exaggeration of the calculated effects. In fact, in both crystal structures considered in this study, the cation simultaneously binds to two nucleobases. However, also the experimental studies represent model complexes (that can be crystallized), and it is not suggested that binding in DNA would necessarily include two DNA bases. Thus, our model systems might be closer to the actual binding of the metals to amino groups in DNA. Further, despite that our models are slightly smaller, they are still closely related to the molecules characterized in the experiments. Nevertheless, we have reinvestigated the effect of the mercury binding to adenine N6 by considering (uracil-C5)-Hg^+ as the metal group. The calculations were done at the HF/6-31G* level, and the following protonation energies were obtained for the N1 position of adenine: 252.5 kcal/mol (metalated base) and 240.2 kcal/mol (nonmetalated base). Thus,

the metalation enhances the protonation energy by 12.3 kcal/mol, and this value is identical to the difference obtained above for the smaller complex.

Although our calculations and the preceding experimental studies are based on simplified models, they indicate that metalation of amino groups of bases could promote formation of those mispairs in DNA which require protonation of the aromatic rings: AH^+·G, AH^+·C, and CH^+·G, and the ion pair structure of the GC base pair. Formation and stabilization of mispairs can have quite significant biological consequences such as the increased probability of point mutations.[34] This could be one of the reasons why many metals are mutagenic.

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References and Notes

(1) *Metal Ions in Biological Systems*; Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, 1996; Vol. 32.

(2) Saenger, W. *Principles of Nucleic Acids Structure*; Springer-Verlag: New York, 1984.

(3) Basch, H.; Krauss, M.; Stevens, J. *J. Am. Chem. Soc*. **1985**, *107*, 7267.

(4) (a) Pettitt, B. M.; Rossky, P. J. *J. Chem. Phys.* **1986**, *84*, 5836. (b) Warshel, A.; Russell, S. T. *Quart. Rev. Biol.* **1984**, *17*, 283. (c) Pratt, L. R.; Hummer, G.; Garcia, A. E. *Biophys. Chem.* **1994**, *51*, 147 (d) No, K. T.; Nam, K. Y.; Scheraga, H. A. *J. Am. Chem. Soc.* **1997,** *119*, 12917.

(5) Song, B.; Zhao, J.; Griesser, R.; Meiser, C.; Sigel, H.; Lippert, B. *Chem. Eur. J*. **1999**, *5*, 2374.

(6) Lippert, B.; Schöllhorn, H.; Thewalt, U. *Inorg. Chim. Acta* **1992**, *198*, 723.

(7) Harper, A.; Brannigan, J. A.; Buck, M.; Hewitt, L.; Lewis, R. J.; Moore, M. H.; Schneider, B. *Acta Crystallogr. D* **1998**, *54*, 1273.

(8) Potaman, V. N.; Soyfer, V. N. *J. Biomol. Struct. Dyn*. **1994**, *11*, 1035.

(9) (a) Phillips, K.; Dauter, Z.; Murchie, A. I. H.; Lilley, D. M. J.; Luisi, B. *J. Mol. Biol.* **1997**, *273*, 171. (b) Špačková, N.; Berger, I.; Šponer, J. *J. Am. Chem. Soc*. **1999**, 121, 5519.

(10) (a) Špačková, N.; Berger, I.; Egli, M.; Šponer, J. *J. Am. Chem. Soc*. **1998**, *120*, 6147. (b) Gallego, J.; Golden, E. B.; Stanley, D. E.; Reid, B. R. *J. Mol. Biol.* **1999**, 285, 1039. (c) Šponer, J.; Leszczynski, J.; Vetterl, V.; Hobza, P. *J. Biomol. Struct. Dyn*. **1996**, 13, 695.

(11) The most striking example is the four stranded intercalated i-DNA characterized by stacking of closely spaced hemiprotonated base pairs with very repulsive gas-phase base stacking interactions.[10] However, the same interaction, stacking of consecutive protonated cytosines, destabilizes the DNA triple-helices.[12] For more details about molecular interactions in protonated base pairs see ref 10c.

(12) Soliva, R.; Laughton, C. A.; Luque, F. J.; Orozco, M. *J. Am. Chem. Soc.* **1998**, 120, 11226.

(13) Schröder, G.; Lippert, B.; Sabat, M.; Lock, C. J. L.; Faggiani, R.; Song, B.; Sigel, H. *J. Chem. Soc., Dalton Trans.* **1995**, 3767.

(14) (a) Meiser, C.; Freisinger, E.; Lippert, B. *J. Chem. Soc., Dalton Trans*. **1998**, 2059. (b) Faggiani, R.; Lippert, B.; Lock, C. J. L.; Speranzini, R. A. *Inorg. Chem.* **1982**, *21*, 3216. (c) Faggiani, R.; Lock, C. J. L.; Lippert, B. *J. Am. Chem. Soc.* **1980**, *102*, 5418.

(15) (a) Terzis, A.; Hadjiliadis, N.; Rivest, R.; Theophanides, T. *Inorg. Chim. Acta* **1975**, *12*, L5. (b) Terzis, A. *Inorg. Chem*. **1976**, *15*, 793.

(16) Cunane, L. M.; Taylor, M. R. *Acta Crystallogr. D* **1997**, *53*, 765.

(17) den Hartog, J. H. J.; van den Elst, H.; Reedijk, J. *J. Inorg. Biochem*. **1984**, *21*, 83.

(18) Beyerle-Pfnür, R.; Jaworski, S.; Lippert, B.; Schöllhorn, H.; Thewalt, U. *Inorg. Chim. Acta* **1985**, *107*, 217.

(19) Clarke, M. J. *J. Am. Chem. Soc*. **1978**, *100*, 5068 and references therein.

(20) Lippert, B.; Schöllhorn, H.; Thewalt, U. *J. Am. Chem. So*c. **1986**, *108*, 6616.

(21) (a) Pichierri, F.; Holthenrich, D.; Zangrando, E.; Lippert, B.; Randaccio, L. *J. Biol. Inorg. Chem.* **1996**, *1*, 439. (b) Müller, J.; Zangrando, E.; Pahlke, N.; Freisinger, E.; Randaccio, L.; Lippert, B. *Chem.—Eur. J.* **1998**, *4*, 397. (c) Müller, J.; Glahé, F.; Freisinger, E.; Lippert, B. *Inorg. Chem.* **1999**, *38*, 3160.

(22) Zamora, F.; Kunsman, M.; Sabat, M.; Lippert, B. *Inorg. Chem*. **1997**, *36*, 1583.

(23) Arpalahti, J.; Klika, K. D. *Eur. J. Inorg. Chem*. **1999**, 1199.

(24) The latter can be produced also in species carrying two metal ions at N7 and N6; see the following: Day, E. F.; Crawford, C. A.; Folting, K.; Dunbar, K. R.; Christon, G. *J. Am. Chem. Soc*. **1994**, *116*, 9449.

(25) (a) Fogarasi, G. *J. Mol. Struct.* **1997**, *413*, 271. (b) Morpugo, S.; Bossa, M.; Morpugo, G. O. *Chem. Phys. Lett*. **1997**, *280*, 233. (c) Kobayshi, R. *J. Phys. Chem. A* **1998**, *102*, 10813. (d) Gorb, L.; Leszczynski, J. *J. Am. Chem. Soc*. **1998**, *70*, 855. (e) Gould, I. R.; Burton, N. A.; Hall, R. J.; Hillier, I. J. *J. Mol. Struct. (THEOCHEM)* **1995**, *331*, 147. (f) Ha, T.-K.; Gunthard, H. H. *J. Mol. Struct. (THEOCHEM)* **1992**, *276*, 209. (g) Les, A.; Adamowicz, L.; Bartlett, R. J. *J. Phys. Chem*. **1989**, *93*, 4001. (h) Kwiatkowski, J. S.; Leszczynski, J. *J. Phys. Chem.* **1996**, *100*, 941. (i) Estrin, D. E.; Paglieri, G.; Corongiu, G. *J. Phys. Chem*. **1994**, *98*, 5653. For theoretical studies of protonation of DNA bases, see: (j) Del Bene, J. E. *J. Phys. Chem*. **1983**, *87*, 367. (k) Florian, J.; Baumruk, V.; Leszczynski, J. *J. Phys. Chem*. **1996**, *100*, 5578.

(26) Roos, R. B.; Powers, J. M.; Atashroo, T.; Ermler, W. C.; LaJohn, L. A.; Christiansen, P. A. *J. Chem. Phys*. **1990**, *93*, 6654.

(27) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V.; Ortiz, J. V.; Foresman, J. B.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94*; Gaussian, Inc.: Pittsburgh, PA, 1995.

(28) (a) Burda, J. V.; Šponer, J.; Hobza, P. *J. Phys. Chem.* **1996**, *100*, 7250. (b) Stewart, G. M.; Tiekink, E. R. T.; Buntine, M. A. *J. Phys. Chem. A* **1997**, *101*, 5368. (c) Burda, J. V.; Šponer, J.; Leszczynski, J.; Hobza, P. *J. Phys. Chem. B* **1997**, *101*, 9670. (d) Šponer, J.; Burda, J. V.; Mejzlik, P.; Leszczynski, J.; Hobza, P. *J. Biomol. Struct. Dyn.* **1997**, *14*, 613. (e) Šponer, J.; Burda, J. V.; Sabat, M.; Leszczynski, J.; Hobza, P. *J. Biomol. Struct. Dyn.* **1998**, *16*, 139. (f) Šponer, J.; Burda, J. V.; Sabat, M.; Leszczynski, J.; Hobza, P. *J. Phys. Chem. A* **1998**, *102*, 5951. (g) Šponer, J.; Burda, J. V.; Leszczynski, J.; Hobza, P. *J. Biomol. Struct. Dyn.* **1999**, *17*, 61.

(29) (a) Broo, A.; Holmen, A. *J. Phys. Chem. A* **1997**, *101*, 3589. (b) Schoone, K.; Houben, L.; Smets, J.; Adamowics, L.; Maes, G. *Spectrochim. Acta A* **1996**, *52*, 383. (c) Kwiatkowski, J.; Leszczynski, J. *J. Mol. Struct. (THEOCHEM)* **1990,** *208*, 35.

(30) Pt—N distances: amino tautomer, 2.085 and 2.096 Å; imino one, 2.064 and 2.076 Å; protonated base, 2.148 and 2.169 Å. Data obtained with Christiansen and Lanl2dz pseudopotential, respectively.

(31) In DNA, the base pairing will be affected by solvent effects. Polar solvents are known to reduce the absolute strength of the pairing of all H-bonded base pairs. However, once again, since metalation in our case does not change the total charge of the system, the solvent effects should not reverse any of the relative trends obtained by the calculations. Another contribution which obviously could influence the stability of the mispairs in DNA (and could not be included in the present study) is the bases stacking.

(32) (a) Šponer, J.; Leszczynski, J.; Hobza, P. *J. Biomol. Struct. Dyn*. **1996**, *14*, 117. (b) Šponer, J.; Hobza, P. In *Encyclopedia of Computational Chemistry*; Schleyer, P. v R., Allinger, N. L., Clark, T., Gasteiger, J., Kollman, P. A., Schaefer, H. F., III, Schreiner, P. R., Eds.; John Wiley and Sons: Chichester, 1998; p 777.

(33) Šponer, J.; Leszczynski, J.; Hobza, P. *J. Phys. Chem*. **1996**, *100*, 1965.

(34) Florian, J.; Leszczynski, J. *J. Am. Chem. Soc*. **1996**, *118*, 3010 and references therein.

(35) Florian, J.; Hrouda, V. Hobza, P. *J. Am. Chem. Soc.* **1994**, *116*, 1457.

(36) Bertran, J.; Oliva, A.; Rodriquez-Santiago, L.; Sodupe, M. *J. Am. Chem. Soc*. **1998**, *120*, 8159.

(37) (a) Luque, F. J.; Lopez-Bes, J.-M.; Cemeli, J.; Aroztequi, M. M.; Orozco, M. *Theor. Chem. Acc.* **1997**, *96*, 105. (b) Florián, J.; Sponer, J.; Warshel, A. *J. Phys. Chem. B* **1999**, *103*, 884. (c) Colominas, C.; Luque, F. J.; Orozco, M. *J. Am. Chem. Soc.* **1996**, *118*, 6811.