Ca, Cd, Zn, and Their Ions Interacting with Cytosine: A Theoretical Study

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Metal atoms play a major role in the chemical behavior of biological systems. In this work, known issues of the metal−base interactions, such as the stabilization of different tautomers of cytosine that could be incompatible with the DNA double helix, are researched using DFT methods. Ca−, Zn−, and Cd−cytosine in neutral and ionic forms were studied at the B3LYP/LANL2DZ level. Several neutral and ionic isomers were found within an interval of 10 kcal/mol of relative stability, with the most stable isomer in each group being a compound derived from the canonical isomer of cytosine, except for the dications where two isoenergetic isomers were found. Interatomic lengths from each metal atom to the nearest atoms in cytosine's ring were larger than 2 Å, discouraging the possibility of a covalent interaction, as supported by additional evidence from molecular orbitals. The interaction between metal and cytosine, electrostatic in nature, is reinforced with the increase of the metal's nuclear charge. Additionally, the ionization energies of the metal−cytosine compounds exhibit a significant reduction (below 6 eV) compared with that of plain cytosine (8.7 eV), posing an interesting possibility with respect to the experimental determination of the photoelectron spectra of these compounds. Analyses of the energetics of the global reactions to form cationic species show that metal cations bind more strongly to neutral cytosine than to neutral metals. Metal dications form the most stable compounds with neutral cytosine, and the stabilities of these systems decrease as $(Zn-cyt)^{2+} > (Cd-cyt)^{2+} > (Ca-cyt)^{2+}$. Aromaticities computed via the HOMA indexes also support the observation regarding the greater affinity of cytosine for metal cations.

Introduction

The Earth's crust and the sea afford us abundant sources of a variety of minerals containing metal compounds. In biological systems, metals perform a major role in the biochemical processes that sustain life; however, they also represent causal factors in a number of pathologies. Lately, the study of the interactions of metals with biological molecules, such as DNA and proteins, has attracted a renewed interest, due to the relation of metals and their ionic species in the development of certain pathologic processes, such as carcinogenesis,[1−3] some metabolic imbalances, and other neuro-degenerative pathologies. It is a well-known fact that metallic ions, such as Zn(II), Fe(II), Cu(II), and Al(III), have a role in the pathogenesis of Alzheimer's disease,[4] possibly influencing the enzyme-specificity by means of binding to specific positions in the DNA molecule.[5−7] On the other hand, under different circumstances a number of tautomers of the DNA bases can be stabilized,[8−11] leading to the formation of anomalous base-pairs[12,13] that are incompatible with the DNA double-helix structure.[14]

Weak interactions such as hydrogen-bonds (H-bonds), and shorter range interactions, such as van der Waals interactions, are responsible for the patterns and motifs that stabilize and shape the three-dimensional structure of biologically active macromolecules, such as structural proteins, enzymes, and nucleic acids. Metal centers in biologically active systems are fundamental constituents that give functionality to the molecular aggregate. Removal of those centers often leads to inactive compounds. Moreover, where it is possible to retain some minimal structural features around the metal centers, some biological activity is also kept.[15] Thereby, due to the close relationship between the structure and biological function of biomolecules such as DNA,[14,16] the factors that stabilize those structures,[17] as well as those factors capable of disrupting the structures, receive a great deal of attention. The theoretical study of the tautomerism of nucleobases is important due to the effects on base pairing, base stacking, and formation of H-bonded complexes.[18−21]

Recently, in materials science and engineering, people have shown an interest in double and single stranded DNA, owing to their possible use as molecular wires, and in the design of novel opto-electronical devices at the nano scale.[22] The π-orbital overlap between stacking nucleobases is known to promote, to some extent, charge transfer (CT) processes along DNA double-helix.[23] Single stranded DNA also exhibits capability to CT.[24] The involved mechanism is electronic in nature, but there is no unified model to explain short-range and long-range behavior,[22,24] or its dependency on DNA composition, temperature, and other experimental factors.[25−28] For these new materials, the interaction with metal atoms could also be significant, because the CT will be different when metal atoms are interacting with the DNA bases. All these factors make the study of the reaction between metal atoms and the DNA molecule very important.

To carry out the study of macromolecules, such as nucleic acids and their interactions with metals, nitrogen bases can be taken as minimum models for the reactions.[17] It is possible to obtain good results with these models because, despite the larger size of the molecule that is intended to be modeled, the main interactions that take place between nucleic acids and other biologically relevant molecules occur only in a small portion of each of those molecules.[15,17,29] This small section of the molecule is used in this work as a model for the theoretical

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study concerning the reactivity of these systems with metal atoms. In particular, cadmium and zinc are two metals that share features, such as chemical behavior. Their valence shells also have a similar electronic configuration. Those facts notwithstanding, the chemical behavior of cadmium and zinc is different when they are included in biological systems. Cadmium is a heavy metal well-known for its toxicity,[3] whereas zinc is an essential nutrient for living organisms, required for cellular division.[30] Why do the biological roles of cadmium and zinc differ so greatly? One explanation may involve the local distortions of the molecular structure, induced by the differences in the atomic size of Zn and Cd.[30,31] Alternatively, a novel mechanism to explain carcinogenesis recently presented by Witkiewicz-Kucharczyk et al., involves the inhibition of DNA repair systems due to cadmium toxicity.[32] In this work, we ask the question: Are the differences between the electronic structures of these metals, or between the properties of their complexes with relevant biomolecules, such as DNA, enough to explain the dissimilarities in biological systems?

To answer that question, we use a theoretical approach based on the density functional theory[33,34] (DFT) and a model with one nitrogen basis (cytosine) and three metal atoms (Ca, Cd and Zn), searching for stable complexes of cytosine and Ca, Zn, and Cd, in their neutral and ionic states. Calcium, another biologically important metal, is included in this study as an additional system to elucidate the effect of the electronic structure of the metal atom on the stability and physical properties of M−cytosine (M = Ca, Cd, Zn), as calcium also presents a closed shell electronic configuration, with two s-type electrons that determine its chemical behavior in a fashion that could be similar to that of Zn and Cd.

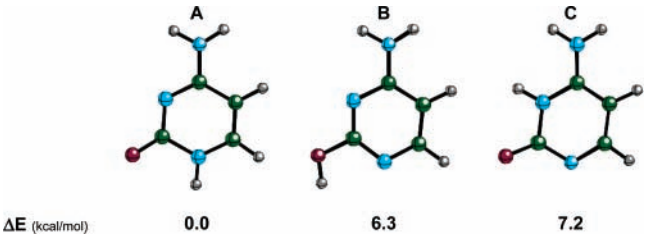
Computational Details

Density functional theory,[34−36] as implemented in the suite of programs *Gaussian 03*,[37] has been used to carry out all calculations. The hybrid three-parameter B3LYP[38−40] functional and the LANL2DZ[41−43] basis set were used to perform complete optimizations of molecular geometries, without symmetry constraints for the several M−cytosine isomers (M = Ca, Zn, Cd) included in this study. Harmonic frequencies analyses[44,45] allowed us to verify optimized minima.

Previous studies show that DFT reproduces equilibrium geometries and relative stabilities with hybrid functionals, which partially include the Hartree−Fock exchange energy. The results are in good agreement with those obtained using Møller−Plesset perturbational theory at second order and basis sets of medium quality, such as 6-31G(d,p), and cc-pVDZ.[15,29,46−48]

The number of isomers used in the initial stage of the study provided several initial geometries, which, in turn, allow us to widely explore the potential energy surface, in search of the global minimum. Notwithstanding the difficulties associated with the localization of ground states, we cannot exclude the possibility that the global minimum could be missed. Nonetheless, the number of initial geometries examined is large enough to reliably identify the global minima in each system. To compute vertical electron detachment energies (VEDE) of anionic species, further single-point calculations were required. Formation energies for neutral and cationic species were calculated using zero-point corrected energies. The M−cytosine compounds were considered as being in their lowest electronic states[49] (singlets and doublets). Density surfaces were also obtained for the qualitative analysis of the frontier molecular orbitals, in search of bonding patterns and other electronic effects.

TABLE 1: Optimized Structures of Cytosine Isomers[a]



[a] The most stable is the Watson−Crick (**A**) isomer, although the other two isomers (**B** and **C**) show similar stability. The energy differences between each species and the most stable one, in kcal/mol, along with adiabatic ionization energies (IE) and electron affinities, in eV, are also shown,

Although there is no universally accepted method of assigning electrostatic charges to atoms, and no experimental technique is actually available to measure them directly, in a former study, de Oliveira et al.,[50] reported the testing of the quality of charges obtained via the Mulliken and Bader population analysis methods. They found a good agreement between both of them, taking into account the qualitative description of the atomic charges. Thus, in this paper, Mulliken atomic charges are used to discuss the qualitative behavior of the charge-transfer process.

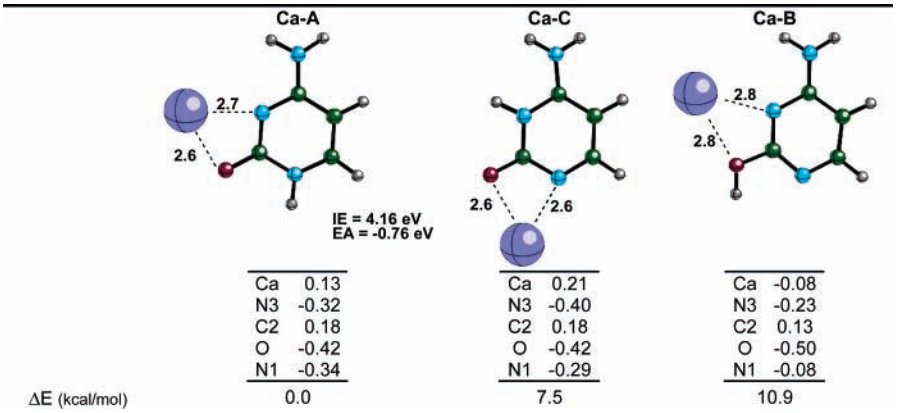
The HOMA (harmonic oscillator model of aromaticity) method[51] was used as reported by Krygowski.[52−55] The bond lengths of the optimized structures were employed for the study of aromaticity with this model.

Visualization of the results was carried out using the Cerius2,[56] the Molekel,[57,58] and the Ball&Stick[59] packages.

Results and Discussion

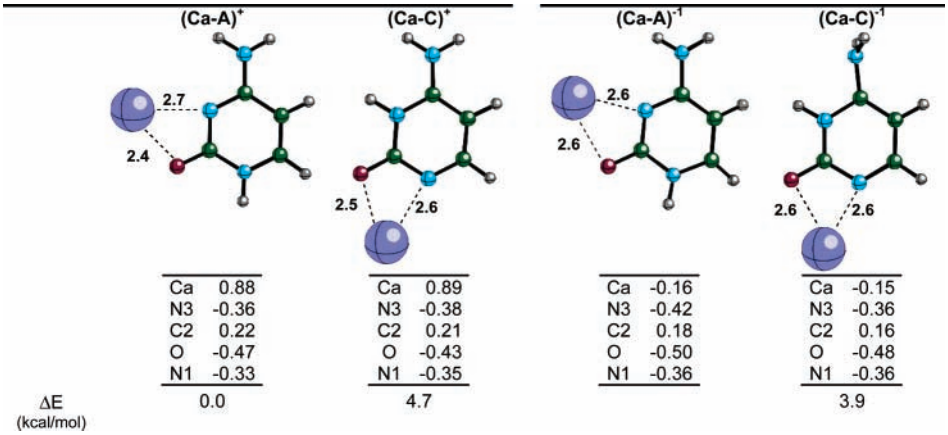
1. Cytosine. The Watson−Crick isomer of cytosine is a pyrimidine base with minimal formula $C_4H_5N_2O$. In the DNA-double helix, cytosine is complementary bonded to a guanine base via three H-bonds, involving the O, N3 and N (of the amino group) of cytosine. Optimized geometries presented in Table 1 show that cytosine is a planar molecule. The amino group lacks pyramidal symmetry. Instead, all three atoms lie in the plane of the ring. Our optimizations are in good agreement with others found in the literature.[60,61] The isomer labeled **B** is 6.3 kcal/mol higher in energy than ground-state **A**. The isomer labeled **C** is 7.2 kcal/mol less stable than **A**. Other optimized isomers, not shown in Table 1, are more than 37.5 kcal/mol higher in energy than isomer **A**, and for this reason they are not considered further in our analysis. The only difference between the three most stable isomers reported in Table 1 is the position of one hydrogen atom, so the stabilization energy can be taken as the energetic cost of moving one hydrogen atom, i.e., tautomerization energy. The calculated energetic cost to tautomerize **B** to **C** is less than 1 kcal/mol, and although this value is below the known uncertainty of the method, it could be considered for a reaction in equilibrium. Each isomer has two available positions (N or O atoms that are partially negative) for the interaction with a metal atom. The electronic ground state of the cytosine neutral isomers **A**, **B**, and **C** are closed-shell singlets, where the first ionization potentials were calculated to 8.7, 8.6, and 8.5 eV, respectively (the first two values are in agreement with those calculated by Dolgounitcheva et al.,[61] 8.79 and 8.93 eV, respectively). Per se, cytosine has low electron affinity (−0.4, −0.5, and −0.2 eV, for **A**, **B**, and **C**, correspondingly). The electron propagator theory[60] gives us a good insight on both the monoelectronic nature of the process and the nature of the anions.[62] Then, the energetically unfavorable process of taking

TABLE 2: Optimized Structures of Ca−Cytosine Neutral Isomers[a]



[a] Energy differences are in kcal/mol. Electrostatic charges, in au, on selected atoms, and adiabatic ionization energies (IE) and electron affinities (EA), in eV, are shown, along with the most significant interatomic lengths, in Å, to the metal atom.

TABLE 3: Optimized Structures of the Most Stable Ca−Cytosine Cationic and Anionic Isomers[a]



[a] Energy differences are in kcal/mol. Electrostatic charges, in au, on selected atoms are shown along with the most significant interatomic lengths, in Å, to the metal atom.

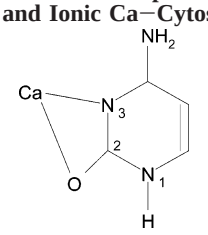
an extra electron is accompanied by an exchange in the stability order between **B** and **C**.

Once we know there is a stabilizing effect on the tautomerization of the cytosine isomers, we can conjecture whether there is an additional stabilization due to the interaction with a metal atom, which could change the order of the relative stability of the M−cytosine compounds (differential stabilization), or maintain the same order established among the cytosine isomers. It is also important to know whether the stabilization order of cytosine isomers with different metal atoms remains the same, and whether this is an indication of a poor, or even a null, interaction between the base and the metal atom.

2. Ca−Cytosine. The optimized neutral Ca−cytosine geometries presented in Table 2 show three isomers within an interval of 10.9 kcal/mol, where the most stable compound (**Ca-A**) is derived from the Watson−Crick isomer (**A** in Table 1). In all compounds, the calcium atom is placed in such a position that the interatomic lengths lie farther away than 2 Å, this length being greater than calcium's covalent radius. Therefore, it is not feasible to expect the formation of any covalent bond, as further analyses will confirm. The second compound in stability (**Ca-C**) is derived from the cytosine isomer **C** and is 7.5 kcal/mol less stable than **Ca-A**. It is worthy of consideration that the energy gap between both Ca−cytosine compounds is comparable to the gap between the plain cytosine isomers from which they were derived ($\Delta E_{C-A} = 7.2$ kcal/mol). As pointed out previously, this fact is an indication of a weak interaction between calcium and cytosine. Actually, this was expected, because the calcium atom is a closed shell species with poor electron affinity (−0.2 eV, calculated at the B3LYP/LANL2DZ level). The expected interaction between the electron-rich cytosine ring and the calcium atom is the promotion of some relevant charge-transfer process from cytosine to calcium. Due to the low electron affinity of calcium, the charge transfer is not very strong and, as a consequence, the interaction is weak. The remaining compound (**Ca-B**) was derived from the cytosine isomer **B**, and the interatomic lengths to the calcium atom appear to be slightly larger (by approximately 0.2 Å). This feature will be explained by the charge-transfer process analyses found further on in this work. Compared to the relative stability among plain cytosine isomers, where isomer **B** is 0.9 kcal/mol more stable than isomer **C**, the interaction with calcium produces an order exchange between **Ca-B** and **Ca-C**, at the same time increasing their energy separation to 3.4 kcal/mol.

The optimized cationic species of the Ca−cytosine compounds reported in Table 3 exhibit the same stability order as the neutral species. After the removal of one electron, it is possible to observe that the structural features are nearly maintained intact, except for the observed reduction in the interatomic lengths to calcium. This was expected, due to an increased electrostatic attraction, as a result of the change in the pattern of atomic charges. The energy gap between the two most stable species, **(Ca-A)**$^+$ and **(Ca-C)**$^+$, is reduced to 4.7 kcal/mol. According to this tendency, the energy difference

TABLE 4: Comparison between Optimized Species of the Most Stable Neutral and Ionic Ca−Cytosine Isomers[a]



anion		neutral		cation 1+		cation 2+	
Ca−N3	2.6	Ca−N3	2.7	Ca−N3	2.7	Ca−N3	2.6
Ca−O	2.6	Ca−O	2.6	Ca−O	2.4	Ca−O	2.3
Ca	−0.16	Ca	0.13	Ca	0.88	Ca	1.80
N3	−0.42	N3	−0.32	N3	−0.36	N3	−0.44
C2	0.18	C2	0.18	C2	0.22	C2	0.22
O	−0.50	O	−0.42	O	−0.47	O	−0.44
N1	−0.36	N1	−0.34	N1	−0.33	N1	−0.34

[a] The interatomic lengths are shown in Å, and electrostatic charges in au. Cartesian coordinates for all of the structures are provided as Supporting Information.

between the two isomers is smaller for the Ca−cytosine dication (not shown) than for the cation and the neutral Ca−cytosine. In this case, there are isoenergetic isomers that are related to two cytosine tautomers (**A** and **C** in Table 1). The energy difference between $\mathbf{(Ca\text{-}A)}^{2+}$ and $\mathbf{(Ca\text{-}C)}^{2+}$ is 0.7 kcal/mol, approximately. This fact does not affect the discussion and conclusions, because the energetics and the stabilizing effect will be the same. Our analysis continues with Ca−cytosine related to the Watson−Crick tautomer of cytosine, **A**, but it is important to remember the presence of $\mathbf{(Ca\text{-}C)}^{2+}$, because this tautomer could be responsible for some kind of destabilization of the DNA molecule that could change the experimental results.

The addition of an extra electron does not change the order of stability as seen for the neutral and cationic species, but does induce some structural changes. The energy gap between the two most stable anionic species, $\mathbf{(Ca\text{-}A)}^{-1}$ and $\mathbf{(Ca\text{-}C)}^{-1}$, in Table 3, was shortened to 3.9 kcal/mol. The amino group of the second compound has the hydrogen atoms out of the plane. Compared with the cations, here there is no reinforcement of electrostatic attractions, although there is a noticeable distortion of the cytosine ring, which allows for a reduction in energy. This geometrical shift on the optimized molecular structure of the Ca−cytosine anion is an outcome when a system with low electron affinity is trying to reach stability after the introduction of an additional electron. Two other anionic isomers are respectively 18.8 and 28.0 kcal/mol less stable than the ground-state anion. Examining the atomic charge distribution in Table 4, from left to right, the tendency of the calcium atom to have a positive charge is noticeable. For the anion, the metal atom shows a poor capacity to accommodate a negative charge. This is related to the electron affinity and the ionization energies of the calcium compared with cytosine. The electron affinity of both calcium and cytosine is very low, and the anion's charge is spread all over the molecule. The first ionization energy of calcium (6.44 eV) is more than 2 eV lower than the first ionization energy of cytosine (8.74 eV calculated in this work, in good agreement with the experimental value[63]). When one electron is removed, it is energetically easier to remove the electron from calcium than from cytosine. For the second electron, the situation is similar. It is also removed from the metal atom. These facts are reflected in the atomic charges distribution.

In Figure 1, a comparison is shown of the molecular orbitals for the most stable Ca−cytosine compounds (neutral and anionic). The distribution of electronic density in the Kohn−Sham orbitals, obtained trough DFT calculations, can be rationalized and correlated with the expected behavior along common one-electron processes, such as electron detachment (raising the calculation of ionization energies) and electron attachment (related to electron affinities).

The HOMO of the neutral is a doubly occupied MO. The main participation comes from an atomic orbital centered on the Ca atom. Nevertheless, this orbital is widely spread over the metal atom and the nearest cytosine's ring. It is also involved in the electron detachment process as an electron donor. After the removal of the first electron, the neutral's HOMO becomes singly occupied, and the distribution of charge density in the orbital is essentially preserved. Then, after the second electron removal, the HOMO in the cation corresponds to the charge density distribution of the LUMO in the dication. On the other hand, when an electron is attached, the spreading pattern of density for the last singly occupied molecular orbital (SOMO), in the anion corresponds to the LUMO structure of the neutral system. As expected, the additional electron of the anion is located in the lowest unoccupied orbital of the neutral.

After performing the analysis of the electronic density distribution over molecular orbitals (see Figure 1) of the species related to the most stable Ca−cytosine compound, it is also noticeable that orbital contribution from calcium is from a 4s orbital, in opposite phase with the orbital contributions of the nearest atoms. In the progression $\mathbf{Ca\text{-}A} \rightarrow \mathbf{(Ca\text{-}A)}^{+} \rightarrow \mathbf{(Ca\text{-}A1)}^{2+}$, a decrease is observed in the Ca−O distance (9.4% and 5.4%, respectively; see Table 4), due chiefly to electrostatic attraction between the Ca atom, positively charged, and the O atom, which bears the major negative charge. This latter interaction overrides the no stabilization effect of electron localization of the **Ca-A**-HOMO. On the other hand, when an electron is attached to the neutral species, $\mathbf{Ca\text{-}A} \rightarrow \mathbf{(Ca\text{-}A)}^{-1}$, Ca acquires a small amount of negative charge. Therefore, electrostatic interactions with adjacent N3 and O atoms are repulsive, although their magnitude is very small. The main contributions to the anion's HOMO show a more extended delocalization over the cytosine's ring.

Up to this point, it is clear that calcium does not form any covalent interaction with cytosine. Moreover, the calcium atom is attached to the cytosine ring via weak electrostatic attractive interactions. This observation is reinforced by the analysis of the interatomic lengths described above, which, for the most stable species, produce values greater than the atomic radius of the metal (1.94 Å), in proportions from 24.7%, for the shortest distance, to 45.9%, for the longest one. The computed dissociation energies for the neutral Ca−cytosine compounds, on the other hand, show that the two most stable compounds, **Ca-A** and **Ca-C**, in Table 2, take about the same amount of energy (11.7 kcal/mol for **Ca-A**, 11.6 kcal/mol for **Ca-C**) to dissociate each into its constituents (cytosine plus a metal atom). For the less stable **Ca-B**, we observe a reduced value of the dissociation energy, 7.1 kcal/mol, as would be expected for a weak interaction between calcium and cytosine.

3. Zn− and Cd−Cytosine. The optimized structures for the Zn− and Cd−cytosine isomers show a close resemblance to the Ca−cytosine discussed in detail earlier. Zinc and cadmium are both iso-electronic with regard to their valence shells, so it is expected that their chemical behaviors will be similar. In fact, as with calcium in the Ca−cytosine compounds, Zn and Cd do not form any covalent bond with cytosine in their respective compounds. Bond distances are larger (2.6 Å for neutral Zn−cytosine, 2.8 Å for neutral Cd−cytosine) than their covalent

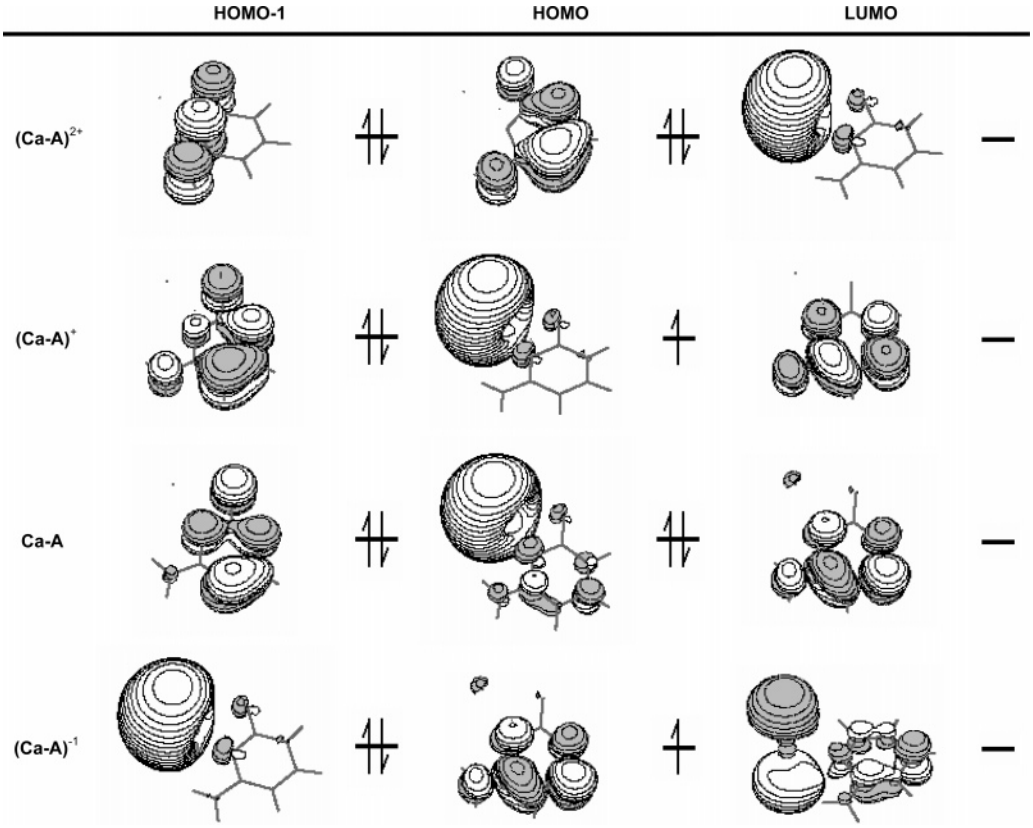


Figure 1. Frontier molecular orbitals corresponding to the most stable Ca−cytosine isomer, in neutral and ionic states. The ionic species are shown, along with the neutral isomer, to allow the inspection of the changes in the charge density distribution in the MO as one electron is successively detached from bottom to top. (MO surfaces were obtained with the MOLDEN[69] package.)

radii (1.31 Å for Zn, 1.48 Å for Cd). Additionally, although all ground-state isomers of M−cytosine, neutral and ionic, are derived from the canonical tautomer of cytosine (**A** in Table 1), for the Zn− and Cd−cytosine dications there are isomers related to another, almost isoenergetic (the energy difference is approximately 0.5 kcal/mol), cytosine tautomer (**C** in Table 1). This finding does not affect the previous discussion, or any further calculations based on those energies, and thus, our analysis will continue with those M−cytosine isomers that are related to the Watson−Crick tautomer of cytosine, **A**, these being the most stable compounds. However, the existence of distinct isomers having similar stability may have a role in future experimental determinations, such as the photoelectron spectroscopy (PES).[64]

Upon making a comparison of interatomic lengths from the nearest atoms in cytosine's ring to the metal atom in Table 5, the same observed pattern that has already been explained for calcium was found in both groups of zinc and cadmium compounds with cytosine. According to the Mulliken atomic charges, also in Table 5, from left to right, the metal atom is bearing an increasingly positive charge, and the positive charge of the cytosine molecule is also increasing. In harmony with this behavior, ionization potentials (IP) seem to have a great deal of influence on binding energies for our metal−base compounds and, therefore, on their stabilities also. Of primary concern is the accuracy in the calculation of the IP with the methodology used in this work. The results for the first and second ionization potentials (IP) are reported in Table 6. Theoretical values obtained for first IP are in good agreement with experimental data for Ca, Zn, Cd, and cytosine, within an error of 0.2% to 5.4%. For the second IP, the error is maintained almost at the same level for Ca (−0.84%), Zn (−4.18%), and Cd (−3.5%). To the best of our knowledge, there are no reported values for cytosine. Therefore, in general, our method slightly underestimates the first IP for Zn and Cd, whereas for Ca, it is overestimated. The second IP is underestimated for Ca, Zn, and Cd. Thus, we can use those values to represent significant tendencies, as we will see later in this work.

The computed ionization energies of zinc (9.17 eV) and cadmium (8.81 eV) are similar to the ionization energy of cytosine (8.74 eV), and for this reason, in their cationic compounds with cytosine, the metal atom bears almost half of the positive charge due to the loss of one electron, and the other half is distributed over the base molecule. In contrast, for the Ca−cytosine cation, where the calcium atom has a lower ionization energy (6.44 eV), the greater part of the positive charge is distributed over the metal. The same tendency is observed for the M−cytosine^{2+} system; calcium has the smaller second ionization energy (11.77 eV) and accommodates almost all the positive charge in the metal atom due to the loss of a second electron, whereas for the Zn− and Cd−cytosine dications, with higher second ionization energies (17.21 and 16.32 eV, respectively), the cytosine's ring accommodates a major part of the positive charge, correspondingly reducing the net charge over the metal atom. The anions show that both metals, Zn and Cd, gain a small amount of negative charge, again as expected for species with low electron affinities.

The analysis of frontier molecular orbitals for Zn− and Cd−cytosine compounds (not shown) produces results similar to those described for Ca−cytosine. The **Zn-A** and **Cd-A** HOMO exhibit a major participation of a 4s and 5s shell centered on the metal atom, respectively. This MO is the electron donor in both ionization processes, which lead successively to the **(M-A)**$^{+}$ and **(M-A)**$^{2+}$ species (where M = Zn, Cd). Further, from the neutral **M-A** to the **(M-A)**$^{-1}$ species, the attached electron is received in the neutral's LUMO. In general, the **(M-A)**$^{-1}$

TABLE 5: Comparison of Interatomic Lengths, in Å, and Atomic Charges, in au, of the Optimized Neutral and Ionic Species of the Most Stable Zn− and Cd−Cytosine Isomers, Respectively

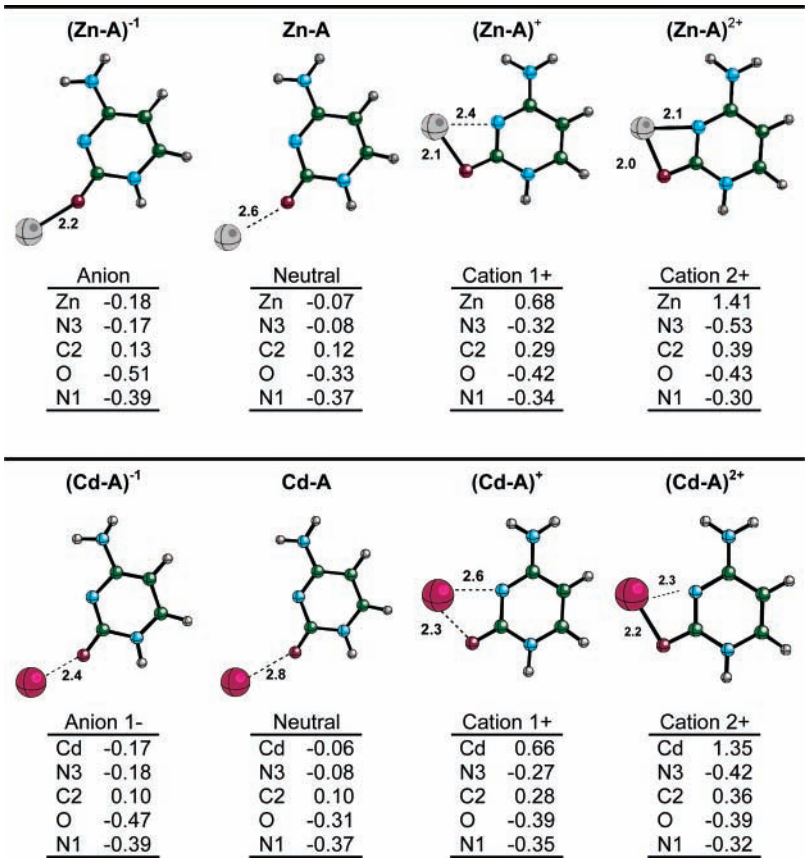


TABLE 6: Ionization Potentials, in eV, for Metals, Plain Cytosine, and Ca−, Zn−, and Cd−Cytosine Compounds

	ionization potentials				EA
	1st		2nd		
	theo	exp	theo	exp	theo
metal					
Ca	6.44	6.11[a]	11.77	11.87[a]	−0.22
Zn	9.17	9.39[b]	17.21	17.96[b]	−0.92
Cd	8.81	8.99[c]	16.32	16.91[d]	−0.96
base					
cyt	8.74	8.7−8.9[e]	nc[f]	nd[f]	−0.42
metal−base compounds					
Ca−cyt	4.16	nd	9.08	nd	−0.76
Zn−cyt	5.73	nd	11.39	nd	0.20
Cd−cyt	5.85	nd	11.39	nd	0.14

[a] Sugar and Corliss.[65] [b] Sugar and Musgrove.[66] [c] Brown et al.[67] [d] Shenstone and Pittenger.[68] [e] Crespo-Hernández et al.[63] Theoretical values computed from energies obtained at the B3LYP/LANL2DZ level for ground-state geometries. [f] nc = not calculated. nd = no available data.

HOMO shows an electronic density from cytosine, which increases the electronic delocalization, favoring a small additional stabilization to counteract the repulsive electrostatic interactions between the metal and adjacent atoms.

The diffuse nature of the HOMO in neutral Zn− and Cd−cytosine compounds is related to observed low ionization energies (compare the IE values computed for the most stable neutral isomer of each group, **Ca-A**, 4.16 eV, **Zn-A**, 5.73 eV, and **Cd-A**, 5.85 eV, with the IP computed for cytosine, **A**, 8.74 eV), which could be of interest in the charge-transfer process along a single DNA strand, where metalated cytosine can be ionized with a lower energetic cost than plain guanine[63] (7.77 eV). The electrons in diffuse orbitals are loosely attached and some vibrational broadening could be observed in the photoelectron spectra of these compounds. The low values computed for ionization potentials also suggest that experimental determination is feasible.

4. Stability of M−Cytosine Compounds and the Electronic Nature of the Metal. To date, it has not been possible to explain the different biological behavior of zinc and cadmium. The optimization procedure usually leads to similar isomers with similar energy differences. Molecular orbitals and atomic charges are also comparable for these three metal atoms (neutral and ionic). In an effort to find an explanation, the binding energies for all the systems presented in this work were calculated.

Several reaction schemes were examined to find distinctive patterns among the metal−cytosine compounds. Two reaction schemes for the formation of the M−cytosine cation were considered, each with a different charge distribution of the reactants: a metal cation and neutral cytosine, and another with cationic cytosine and neutral metal. Two reaction schemes for the formation of an M−cytosine dication were also analyzed. The reaction to produce the neutral M−cytosine compounds is also shown, to allow further comparisons. Formation energies (ΔE_f), reported in Table 7, are all negative, and thus, the reactions presented favor the formation of the compounds over the separated atoms and molecules.

The energetic differences computed between ground-state geometries have shown that the energy required to carry out the presented reactions, toward the formation of the compounds, favor neutral Ca−cytosine formation over Zn− and Cd−cytosine. With Zn and Cd, the formation energies are very similar. For the cationic species, if the neutral cytosine is one

TABLE 7: Energy Differences, in kcal/mol, Computed for Several Reaction Models for the Formation of Ground-State M−Cytosine (M−cyt) Neutral and Cationic Species

		ΔE_f		
key	reaction model	Ca	Zn	Cd
a	$M + cyt \rightarrow M-cyt$	−11.7	−2.9	−2.3
b	$M + cyt^+ \rightarrow (M-cyt)^+$	−116.0	−71.3	−68.2
c	$M^+ + cyt \rightarrow (M-cyt)^+$	−62.5	−80.7	−69.4
d	$M^{2+} + cyt \rightarrow (M-cyt)^{2+}$	−123.9	−214.3	−182.8
e	$M^+ + cyt^+ \rightarrow (M-cyt)^{2+}$	−54.6	−19.6	−8.5

TABLE 8: Energy Differences for the Exchange of a Metal Atom in M−Cytosine Compounds (ΔE_M, kcal/mol)

reaction model	ΔE
$Cd-cyt + Ca \rightarrow Ca-cyt + Cd$	−9.3
$Ca-cyt^+ + Cd^+ \rightarrow Cd-cyt^+ + Ca^+$	−7.0
$Ca-cyt^{2+} + Cd^{2+} \rightarrow Cd-cyt^{2+} + Ca^{2+}$	−58.9
$Zn-cyt + Ca \rightarrow Ca-cyt + Zn$	−8.7
$Ca-cyt^+ + Zn^+ \rightarrow Zn-cyt^+ + Ca^+$	−18.2
$Ca-cyt^{2+} + Zn^{2+} \rightarrow Zn-cyt^{2+} + Ca^{2+}$	−90.5
$Cd-cyt + Zn \rightarrow Zn-cyt + Cd$	−0.6
$Cd-cyt^+ + Zn^+ \rightarrow Zn-cyt^+ + Cd^+$	−11.3
$Cd-cyt^{2+} + Zn^{2+} \rightarrow Zn-cyt^{2+} + Cd^{2+}$	−31.6

of the reactants, the reaction with Zn^+ is more favored than the respective reactions with Cd^+ and Ca^+, in that order. This is due to the low affinity of Ca^+ for neutral cytosine. Further, if cationic cytosine is one of the reactants, the reaction with neutral Ca is more favored than the reactions with neutral Zn and Cd. For the dication formation, when a metal cation is one of the reactants and the other one is the cationic cytosine, the most favorable reaction is the formation of Ca−cytosine^{2+}. The explanation is related to the great affinity of Ca^+ for cytosine$^+$, compared with the other two metal cations. In the presence of neutral cytosine, the formation energy of the (M−cytosine)$^{2+}$ is larger for Zn^{+2} than for Cd^{+2} and Ca^{+2}. This is as expected, due to the lower affinity of Ca^{2+} for neutral cytosine.

This discussion on the affinity between neutral and cationic cytosine and metals, leads us to another question: How likely is a M−cytosine compound to experience the replacement of its metal atom by another metal? The energetic changes associated with the metal replacement (ΔE_M) in M−cytosine compounds can be computed for several model reactions, as may be observed in Table 8. For the neutral species, calcium is able to remove Zn and Cd from the compounds they form with cytosine (releasing 9.3 and 8.7 kcal/mol, respectively), because of the great stability of the Ca−cytosine compound. Additionally, the ΔE_M for the replacement of Cd by Zn has a small value (−0.6 kcal/mol), thus both directions of this reaction are close to the equilibrium, as would be expected of two compounds of similar stability. The results indicate that for the cationic species, the favored reactions are those where Zn^+ removes Ca^+ ($\Delta E_M = -18.2$ kcal/mol) and Cd^+ ($\Delta E_M = -11.3$ kcal/mol), as well as the reaction where Cd^+ replaces Ca^+ ($\Delta E_M = -7.0$ kcal/mol). In the reactions with the dicationic species, it is Zn^{2+} that removes Ca^{2+} ($\Delta E_M = -90.5$ kcal/mol) and Cd^{2+} ($\Delta E_M = -31.6$ kcal/mol) from their compounds, although the replacing of Ca^{2+} by Cd^{2+} ($\Delta E_M = -58.9$ kcal/mol) is also well favored.

It is worth noting that the order in which these replacements are favored for the cationic species, as well as the order of their energy differences, agrees with the formation-energies differences for the reaction model **c** in Table 7. By way of illustration, the reaction for the replacement of Ca^+ with Cd^+, may be considered as the sum of two reaction steps: the dissociation of Ca−cytosine$^+$ (62.5 kcal/mol), followed by the formation of Cd−cytosine$^+$ (−69.4 kcal/mol). The total energy difference for the concerted reaction (−7.1 kcal/mol) is almost the same as that shown in Table 8 for the replacement reaction. The concordance between those values obtained for the same global reaction (the formation of M−cytosine cation), suggests that there is only one preferred reaction path for producing the M−cytosine cationic species, viz.

$$M^+ + cyt \rightarrow M-cyt^+$$

Similarly, for the dicationic species there is a preferred reaction path for the formation of M−cytosine^{2+}, the reaction labeled **d** in Table 7:

$$M^{2+} + cyt \rightarrow M-cyt^{2+}$$

As discussed earlier, the metal *n*s valence-shell orbitals are of a very diffuse nature compared to the *n*d orbitals. As such, the low IP shown by the M−cytosine compounds can be explained by the electrostatic repulsion between those valence-shell electrons and the electron-rich ring of cytosine. The IP of the M−cytosine compounds is related to the IP of the metal atoms, such that, when the IP of the atoms increases, the IP of the correspondent M−cytosine compound also increases. Thus, it would be expected that the metal with the smallest ionic radius, Zn, and also the highest first and second IP (9.17 and 17.21 eV, respectively), would form a M−cytosine compound with the highest IP also. Nonetheless, the next atom in ionic radius, Cd, has IP values (8.81 and 16.32 eV) close to those of Zn, and therefore, the predicted IP values for Zn−cytosine (5.73 and 11.39 eV for first and second IP) and Cd−cytosine (5.85 and 11.39 eV for first and second IP) would be close too, as is observed to be the case. Moreover, increasing the interaction will likely remove those loosely bound valence-shell electrons in the metal. As a consequence, there is an increment of the effective nuclear charge that maximizes the electrostatic attraction between the metal (now positively charged) and the cytosine. If this is the case, the affinity for a metal dication must be greater than the affinity for a cation, and also much higher than for a neutral metal. This was shown from the energetics of neutral and cationic species recently discussed.

TABLE 9: Harmonic Oscillator Models of Aromaticity (HOMA) for Ground-State Geometries of M−Cytosine Compounds and Cytosine Canonical Tautomer

compound	anion	neutral	cation	cation 2+
Ca−cyt	0.70	0.88	0.83	0.82
Zn−cyt	0.67	0.74	0.87	0.80
Cd−cyt	0.66	0.77	0.84	0.82
cytosine	0.62	0.70	0.85	nc[a]

[a] nc = not calculated.

For a complementary comparison on stabilities, the harmonic oscillator model of aromaticity (HOMA) was also computed. The results are presented in Table 9. The HOMA index shows that among the neutral compounds, Ca−cytosine is more aromatic, and thus more stable, than Cd− and Zn−cytosine (which have almost the same aromaticity), a finding that is consistent with previous results. Those values also show a general tendency toward an increase in the aromaticity, and hence the stability, of the Zn− and Cd−cytosine compounds going to the cationic species. For Ca−cytosine, the HOMA values show a reduction in aromaticity from the neutral to the cation, but the cationic value is slightly larger than that for the

dication. The aromaticity, according to the HOMA, decreases as follows for Zn and Cd:

$$(M-cyt)^{+} > (M-cyt)^{2+} > M-cyt > (M-cyt)^{-1}$$

When compared with the HOMA of neutral cytosine (0.70), the interaction with metals increases the aromaticity of the six-membered ring, leading to more stable compounds. In this case, the stability decreases in the following order:

$$\text{Ca}-\text{cytosine}\ (0.88) > \text{Cd}-\text{cytosine}\ (0.77) \sim \text{Zn}-\text{cytosine}\ (0.74)$$

Further, the cytosine cation has a HOMA (0.85) close to the values of the Cd− and Ca−cytosine cations, but slightly lower than that of the Zn−cytosine^{+}. The aromaticity of cationic species decreases as follows:

$$\text{Zn}-\text{cytosine}^{+}\ (0.87) > \text{cytosine}^{+}\ (0.85) \sim \text{Cd}-\text{cytosine}^{+}\ (0.84) \sim \text{Ca}-\text{cytosine}^{+}\ (0.83)$$

where the Zn−cytosine cation seems to be more stable. It is notable that the similar stability of the cytosine and the Cd− and Ca−cytosine cations makes the reaction of the metal cations with neutral cytosine more stable than with cationic cytosine.

These observations are in agreement with the previous discussion regarding the energetics of those species. Nonetheless, the HOMA values of cationic and dicationic compounds are similar and, thus, do not allow us to make further comparisons of their stabilities.

Conclusions

A number of isomers of Ca-, Zn−, and Cd−cytosine that involve three cytosine tautomers were found within an interval of 10 kcal/mol for each group. The calcium group includes three isomers, and the zinc and cadmium groups have five isomers each. The most stable one in each group, for the neutral and ionic species, is derived from the Watson−Crick tautomer of cytosine. Nevertheless, for the Zn− and Cd−cytosine dications, there are other isoenergetic isomers, which are related to a distinct cytosine tautomer that could be important, particularly with regard to experimental determinations such as the photoelectron spectroscopy. Evidence supported on molecular orbitals shows that neither Ca, nor Zn, nor Cd form any covalent interaction with cytosine. However, these metals promote the significant stabilization of a number of additional isomers, chiefly by means of electrostatic interactions. The electrostatic interaction is stronger when the nuclear charge of the metal atom increases, thus the strongest interaction is for the cytosine compounds with Ca(II), Zn(II) and Cd(II). HOMA values computed for the cytosine ring support these observations.

The analyses of the energetics of neutral and cationic compounds show that neutral Ca−cytosine is more stable than the Zn and Cd compounds, but that the different affinities between the three metals and cytosine lead to a different ordering of the stabilities of cationic species, where the Zn−cytosine species is more stable than the Cd and Ca species, in that order. According to the energetics for metal replacements and the formation of the M−cytosine^{+} and M−cytosine^{2+}, the preferred reaction path for producing the M−cytosine cationic species involves neutral cytosine and the respective metal cation or dication. Therefore, the ionization potentials of the metals have a role in the stabilization of the compounds with cytosine. In general, Ca, Zn, and Cd cations effectively stabilize other tautomers of cytosine that are incompatible with the DNA-double helix formation, binding more strongly to them as the ionization potential of the metal increases.

As reported in the literature, Zn^{2+} has a role in the development of neurodegenerative diseases, such as Alzheimer's disease, and the underlying mechanism could involve changes in enzyme-specificity, through DNA-binding in specific sites. Through our research, we found that Zn^{2+} binds neutral cytosine more strongly than does Cd^{2+} and that the metal dications can stabilize distinct tautomers of cytosine. Thus, the metal binding DNA, through electrostatic interaction with cytosine in particular sites of the double-helix, could interfere with the molecular recognition needed for the performance of fundamental tasks by the living cell, such as DNA repair and transcription.

Finally, with this model, the comparison between Zn and Cd reveals more similarities than differences. Therefore, based solely on the study of this metal−base interaction, there is not enough information to allow us to render an explanation of the different roles of these metals in biological systems.

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Supporting Information Available: A summary of *Gaussian 03* outputs are provided as Supporting Information for the principal compounds treated in this work. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Liang, R.; Senturker, S.; Shi, X.; Bal, W.; Dizdarogluand, M.; Kasprzak, K. S. *Carcinogenesis* **1999**, *20*, 893.
(2) Hartwig, A. *Pure Appl. Chem.* **2000**, *72*, 1007.
(3) Müller, J.; Sigel, R. K. O.; Lippert, B. *J. Inorg. Biochem.* **2000**, *79*, 261.
(4) Bidlack, W. R. *J. Am. College Nutrition* **1999**, *18*, 368.
(5) Clark, P.; Eichhorn, G. L. *Biochemistry* **1974**, *13*, 5098.
(6) Arakawa, H.; Neault, J. F.; Tajmir-Riahi, H. A. *Biophys. J.* **2001**, *81*, 1580.
(7) Polyanichko *Nucleic Acids Res.* **2004**, *32*, 989.
(8) Barsky, D.; Colvin, M. E. *J. Phys. Chem. A* **2000**, *104*, 8570.
(9) Šponer, J.; V., B. J.; Sabat, M.; Leszczynski, J.; Hobza, P. *J. Phys. Chem. A* **1998**, *102*, 5951.
(10) Dolgounitcheva, O.; Zakrzewski, V. G.; Ortiz, J. V. *J. Am. Chem. Soc.* **2000**, *122*, 12304.
(11) Alemán, C. *Chem. Phys.* **2000**, *253*, 13.
(12) Šponer, J.; Šponer, J. E.; Gorb, L.; Leszczynski, J.; Lippert, B. *J. Phys. Chem. A* **1999**, *103*, 11406.
(13) Pedersen, D. B.; Simard, B.; Moussatova, A.; Martínez, A. *J. Phys. Chem.* **2003**, *107*, 6464.
(14) Watson, J. D.; Crick, F. H. C. *Nature* **1953**, *171*, 964.
(15) Siegbahn, P. E. M.; Blomberg, M. R. A. *Annu. Rev. Phys. Chem.* **1999**, *50*, 221.
(16) Watson, J. D.; Crick, F. H. C. *Nature* **1953**, *171*, 737.
(17) Desfrançois, C.; Carles, S.; Schermann, J. P. *Chem. Rev.* **2000**, *100*, 3943.
(18) Colominas, C.; Luque, F. J.; Orozco, M. *J. Am. Chem. Soc.* **1996**, *118*, 6811.
(19) Fonseca Guerra, C.; Bickelhaupt, F. M.; Snijders, J. G.; Baerends, E. J. *J. Am. Chem. Soc.* **2000**, *122*, 4117.
(20) Šponer, J. E.; Špackova, N.; Kulhánek, P.; Leszczynski, J.; Šponer, J. *J. Phys. Chem. A* **2005**, *109*, 2292.
(21) Müller, A.; Frey, J. A.; Leutwyler, S. *J. Phys. Chem. A* **2005**, *109*, 5055.
(22) Heath, J. R.; Ratner, M. A. *Phys. Today* **2003**, 43.
(23) Eley, D. D.; Spivey, D. I. *Faraday Soc. Trans.* **1962**, *58*, 411.
(24) Berlin, Y. A.; Burin, A. L.; Ratner, M. A. *Superlattices Microstruct.* **2000**, *28*, 241.
(25) Murphy, C. J.; Arkin, M. R.; Jenkins, Y.; Ghatlia, N. D.; Bossman, S. H.; Turro, N. J.; Barton, J. K. *Science* **1993**, *262*, 1025.

(26) Murphy, C. J.; Arkin, M. R.; Ghatlia, N. D.; Bossman, S. H.; Turro, N. J.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5315.
(27) Arkin, M. R.; Stemp, E. D. A.; Holmlin, R. E.; Barton, J. K.; Hörmann, A.; Olson, E. J. C.; Barbara, P. F. *Science* **1996**, *273*, 475.
(28) Stemp, E. D. A.; Holmlin, R. E.; Barton, J. K. *Inorg. Chim. Acta* **2000**, *297*, 88.
(29) Piacenza, M.; Grimme, S. *J. Comput. Chem.* **2004**, *25*, 83.
(30) Ramalho, T. C.; Figueroa-Villar, J. D. *J. Mol. Struct. (THEOCHEM)* **2002**, *580*, 217.
(31) Giaginis, C.; Gatzidou, E.; Theocharis, S. *Toxicol. Appl. Pharmacol.* **2006**, *213*, 282.
(32) Witkiewicz-Kucharczyk, A.; Bal, W. *Toxicol. Lett.* **2006**, *162*, 29.
(33) Geerlings, P.; De Proft, F.; Langenaeker, W. *Chem. Rev.* **2003**, *103*, 1793.
(34) Kohn, W.; Becke, A. D.; Parr, R. G. *J. Phys. Chem.* **1996**, *100*, 12974.
(35) Hohenberg, P.; Kohn, W. *Phys. Rev.* **1964**, *136*, B864.
(36) Kohn, W.; Sham, L. J. *Phys. Rev.* **1965**, *140*, A1133.
(37) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian03*; Gaussian, Inc.: Wallingford, CT, 2004.
(38) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098.
(39) Mielich, B.; Savin, A.; Stoll, H.; Preuss, H. *Chem. Phys. Lett.* **1989**, *157*, 200.
(40) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
(41) Hay, P. J.; Wadt, W. R. *J. Chem. Phys.* **1985**, *82*, 270.
(42) Hay, P. J.; Wadt, W. R. *J. Chem. Phys.* **1985**, *82*, 299.
(43) Wadt, W. R. *J. Chem. Phys.* **1985**, *82*, 284.
(44) Foresman, J. B.; Frisch, Æ. *Exploring Chemistry with Electronic Structure Methods*, 2nd ed.; Gaussian, Inc.: Pittsburgh, PA, 1996.
(45) Ochterski, J. W. Vibrational Analisys. In *Gaussian*; Gaussian Inc.: Pittsburgh, PA, 1999 (on line, http://www.gaussian.com/g_whitepap/vib/vib.pdf).
(46) Shishkin, O. V.; Gorb, L.; Luzanov, A. V.; Elstner, M.; Suhai, S.; Leszczynski, J. *J. Mol. Struct. (THEOCHEM)* **2003**, *625*, 295.
(47) Møller, C.; Plesset, M. S. *Phys. Rev.* **1934**, *46*, 1618.
(48) Saebo, S.; Almlof, J. *Chem. Phys. Lett.* **1989**, *154*, 83.
(49) Lippert, B. *Coord. Chem. Rev.* **2000**, *200*−*202*, 487.
(50) de Oliveira, A. E.; Guadagnini, P. H.; Haiduke, R. L. A.; Bruns, R. E. *J. Phys. Chem. A* **1999**, *103*, 4918.
(51) Kruszewski, J.; Krygowski, T. M. *Tetrahedron Lett.* **1972**, *13*, 3839.
(52) Krygowski, T. M. *J. Chem. Inf. Comput. Sci.* **1993**, *33*, 70.
(53) Krygowski, T. M.; Anulewicz, R.; Kruszewski, J. *Acta Crystallogr.* **1983**, *B39*, 732.
(54) Krygowski, T. M.; Cyranski, M. K. *Tetrahedron* **1996**, *52*, 10255.
(55) Krygowski, T. M.; Cyranski, M. K. *Chem. Rev.* **2001**, *101*, 1385.
(56) SGI-Software; Molecular Simulations Inc.: San Diego, 1997.
(57) Flükiger, P.; Lüthi, H. P.; Portmann, S.; Weber, J. Molekel, 4.3 ed.; Swiss Center for Scientific Computing: Manno, Switzerland, 2000−2002.
(58) Portmann, S.; Lüthi, H. P. *Chimia* **2000**, *54*, 776.
(59) Müller, N.; Falk, A. Ball&Stick, 3.75 ed.; Johannes Kepler University: Linz, 2000.
(60) Dolgounitcheva, O.; Zakrzewski, V. G.; Ortiz, J. V. In *Fundamental World of Quantum Chemistry*; Brändas, E. J., Kryachko, E. S., Eds.; Kluwer Academic Publishers: The Netherlands, 2003; Vol. 2.
(61) Dolgounitcheva, O.; Zakrzewski, V. G.; Ortiz, J. V. *J. Phys. Chem. A* **2003**, *107*, 822.
(62) (a) Dolgounitcheva, O.; Zakrzewski, V. G.; Ortiz, J. V. *J. Phys. Chem. A* **2001**, *105*, 8782. (b) Wesolowski, S. S.; Leininger, M. L.; Pentchev, P. N.; Schaefer, H. F., III. *J. Am. Chem. Soc.* **2001**, *123*, 4023. (c) Richardson, N. A.; Wesolowski, S. S.; Schaefer, H. F., III. *J. Am. Chem. Soc.* **2002**, *124*, 10163.
(63) Crespo-Hernandez, C. E.; Arce, R.; Ishikawa, Y.; Gorb, L.; Leszczynski, J.; Close, D. M. *J. Phys. Chem. A* **2004**, *108*, 6373.
(64) Vazquez, M.-V.; Martínez, A.; Dolgounitcheva, O.; Ortiz, J. V. *J. Phys. Chem. A* **2006**, *110*, 11174.
(65) Sugar, J.; Corliss, C. *J. Phys. Chem. Ref. Data* **1985**, *14*, Suppl. 2.
(66) Sugar, J.; Musgrove *J. Phys. Chem. Ref. Data* **1995**, *24*, 1803.
(67) Brown, C. M.; Tilford, S. G.; Ginter, M. L. *J. Opt. Soc. Am.* **1975**, *65*, 1404.
(68) Shenstone, A. G.; Pittenger, J. T. *J. Opt. Soc. Am.* **1949**, *39*, 219.
(69) Schaftenaar, G.; Noordik, J. H. *J. Comput.-Aided Mol. Design* **2000**, *14*, 123.