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An *ab initio* Post SCF Study on Stacking Interactions of 8-Oxo-9-methylguanine with Four Canonical DNA Bases

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Stacking properties of 8-oxo-9-methylguanine interacting with all four canonical nucleic acid bases were studied and compared to dimers formed by unmodified guanine. The impact of twist angle and base-base separation distance on the stacked dimers energies were analyzed based on MP2/6-31G*(d=0.25) quantum chemistry and Amber molecular mechanics single point calculations. Besides, solvent affects were taken into account within PCM formalism. Presented data lead to the conclusion that 8-oxo-9-methylguanine has significantly different stacking properties compared to standard guanine. Although the dimers stabilization energies are similar for standard and modified 9-methylguanine structural properties are significantly diverse. The most stable dimers formed by 8-oxo-9-methyl-G are characterized by different conformations compared to canonical 9-methylguanine. This may lead to complete alteration of stacking abilities. For example, 8-oxo-9-methyl-G if paired with 9-methyl-G exhibits strong stacking repulsion in the twist region, for which 9-methylguanine/9-methylguanine dimer has major attraction. The most stable stacking pair is formed by 8-oxo-9-methylguanine with 9-methylguanine, while the least stable one corresponds to 8-oxo-9-methylguanine/1-methylcytosine and 9-methylguanine/1-methylcytosine pairs. Besides, significant changes of stacked complexes polarities are observed, especially in case of pairs containing methylated pyrimidines. Polarities of dimer formed by two 9-methylated purines are much less sensitive to the environment but dipole moments of 9-methylpurine/1-methylpyrimidine stacking pairs are significantly altered by taking into account solvent effects. The observed differences in stacking properties between standard and modified guanine are related mainly to charge redistribution rather than direct interactions of O_8 oxygen. The correlation energies of stacking dimers are very high and are main source of pairs stabilization. Both 9-methylguanine and 8-oxo-9-methylguanine are characterized by similar values of correlation energy.

Key words: stacking, 8-oxo-9-methylguanine, 9-methylguanine, *ab initio*

The three dimensional structure of DNA is strongly affected by base-base interactions. The hydrogen bonding stabilizes multiple strands and is responsible for replication and genetic coding [1]. Base stacking stands for sequence dependence, flexibility and stabilization of polinucleotide chain [2–4]. Both kinds of interactions between canonical bases were subject of many theoretical studies on different levels of theory [5–7]. Molecular mechanics with its empirical potentials [8], semi-empiri-

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cal quantum chemistry as well as *ab initio* post Hartree-Fock quantum chemistry [5–9] calculations were successfully applied for description of nature of stacking forces. The most sophisticated studies on base-base interactions were presented by Hobza *et al*. [5,6]. They performed detailed scan of the potential energy surface with inclusion of electron correlation for characterizing global minima of stacked dimers [9–11]. On the other hand, hydroxyl radical modified canonical bases attracted vast attention of scientific audience. This is related not only to common presence of modified bases in the double polinucleotide strands but also due to observed consequences of such occurrence in cellular DNA. There are evidences that hydroxyl radical modified bases, if present in the nucleic acid, introduce structural and energetical disruptions, leading to mispairing, miscoding [12–14], causing the replication block [12–15] and are potential source of many diseases [16,17]. The 8-oxo-guanine is one of the most abundant derivatives among many possible products of hydroxyl radical DNA degradation [17]. Its promutagenic character was demonstrated in variety of *in vivo* [17] and *in vitro* experiments [18]. The presence of 8-oxoG in DNA may be responsible both for GC=>CG transversion and GC=>TA transition often observed in tumour cells [17–21]. When 8-oxoG is positioned between T and C it may cause the insertion of all four bases with the same frequency. However, if 8-oxoG is present between A and T there were observed mainly addition of C and T. One of the reasons of context dependence coding abilities of 8-oxoG might be related to intermolecular interactions with neighboring bases within the same strand. Thus, the detailed knowledge of stacking interactions of this modified guanine seems to be valuable for deeper insight of 8-oxoG role in cellular processes. It has been shown, that 8-oxo-guanine exists predominantly in amino-diketo tautomer [22,23]. Since modification of guanine at C_8 position lead to significant alteration of coding properties, it is worth to know the impact of such guanine modification on other intermolecular interactions with canonical bases. However, the stacking properties of 8-oxo-guanine were not the subject of detailed studies to date. This paper describes fundamental stacking abilities of 8-oxo-9-methyl-G and compares them to properties of standard 9-methylguanine.

METHOD

The 9-methylated DNA bases were used in this study as a model compound for nucleosides. Methyl group was added to nucleic acid bases for neutralization of the monomer and mimic the deoxyribose backbone. All structures of isolated 8-oxo-9-methylguanine and four canonical DNA bases were optimized by standard post-SCF *ab initio* technique in the MP2/6-31G*(d=0.25) basis set. The restricted minimization was performed for preserving the planar structure. Two sets of stacking pairs were constructed. First one comprises 9-methylguanine stacked with each of four 9-methylated DNA bases, while the second set consisted of dimers formed by 8-oxo-9-methylguanine interacting with each of 9-methylated nucleic acid bases. Thus, there were eight pairs to consider. Stacking dimers were assembled by putting monomers on parallel planes on such way that the line crossing through their center of masses was perpendicular to both planes. The face-to-face orientation was used. The geometry of stacked dimer was declared by twist angle describing mutual orientation of bases and separation distance. The first parameter was defined as a torsion angle formed by N_9 atoms of purines or N_1 atom of pyrimidines and center of mass for both monomers. The zero value of this dihedral angle corresponds to closest position of N-glycosidic bonds. The

twist angle was introduced in right-hand sense. For stacking dimers formed by two purines 0° or 180° value of twist corresponds to maximal overlapping of aromatic rings. The closest position of N-glycosidic bond is in the first case, while the latter conformation corresponds to opposite orientation. The twist equal to 90° or 270° is related to minimal overlapping of aromatic rings. The separation between planes was treated as the distance between monomer center of masses and during *ab initio* calculations was set to 3.4 angstroms. This is consistent with available crystallographic data characterizing DNA double strands [10,11]. Besides, such separation distance is in good accord to results obtained on the basis of Amber force field molecular mechanics calculations. This method was applied in preliminary phase, during which energy of stacked dimers was calculated for broad range of separation distance and twists angle values. Some of these data are presented in Figs. 1–4. Thus, during all *ab initio* calculations base-base separation was fixed to 3.4 Å and twist angle was modified from 0° to 360° with 30° increment. Since correction for electron correlation at least on MP2 level is indispensable for adequate description of the intermolecular potential of stacked aromatic dimers such calculations were performed on post SCF *ab initio* level MP2/6-31G*(d=0.25) calculations. All energies of stacked pairs were corrected for basis set superposition error [25].

Besides, the solvation impact on the dimers energy was estimated by means of PCM method [26]. This approach offers direct analysis of the solute-solvent interactions including electrostatic, non-polar, cavitation, dispersion and non-electrostatic repulsion. Hence, estimated values of free energy of solvation take into consideration both polar and non-polar solute-solvent interactions as well as solute polarization. The free energy of the dimer formation in solvent was estimated as the difference in free energies of solvation of stacked dimer and isolated bases.

For all *ab initio* calculations Gaussian98 [27] program was used, while HyperChem 6.0 [28] program was applied in part related to molecular mechanics level.

RESULTS AND DISCUSSION

Energies and conformations of stacked complexes: The stabilization energies calculated as the difference between pair energy and isolated monomers including correction for BSSE were presented in Figs. 1–4. They contain plots obtained on MP2 level in vapor phase, PCM model accounting for hydration and molecular mechanics level based on Amber force field with charges estimated as ESP fit to MP2/6-31G(d=0.25) potential surface according to standard Merz-Kollman procedure [29]. The last two data were presented only for comparison and further analysis will be related to most reliable MP2 results. However, it is interesting to notice that there is qualitative agreement between shapes of energy plots estimated, based on Amber and MP2 predictions. Bearing in mind discrepancies in costs of such calculations, the correlations is surprisingly good.

From Figs. 1–4 it is evident that there is significant impact of the twist angle on the pair stabilization energy for all methods used in this work. This is common for pairs formed both by canonical 9-methylguanine and 8-oxo-9-methylguanine. Taking into account only one value of base plane separation equal to 3.4 is justified by suitable Amber derived curves. First set of studied dimers contained canonical 9-methyladenine, for which the variations of stacking energies with respects of twist angle were presented in Fig. 1. This purine interacts with stacked 9-methylguanine and forms two stable dimers corresponding to two minima with twist angle values of 60° and 240°. The related values of stacking energies are equal to –9.3 kcal/mol and –12.4 kcal/mol, respectively. To the contrary interactions of 9-methyladenine with 8-oxo-9-methylguanine differ significantly. First of all, there are three minima on the energy

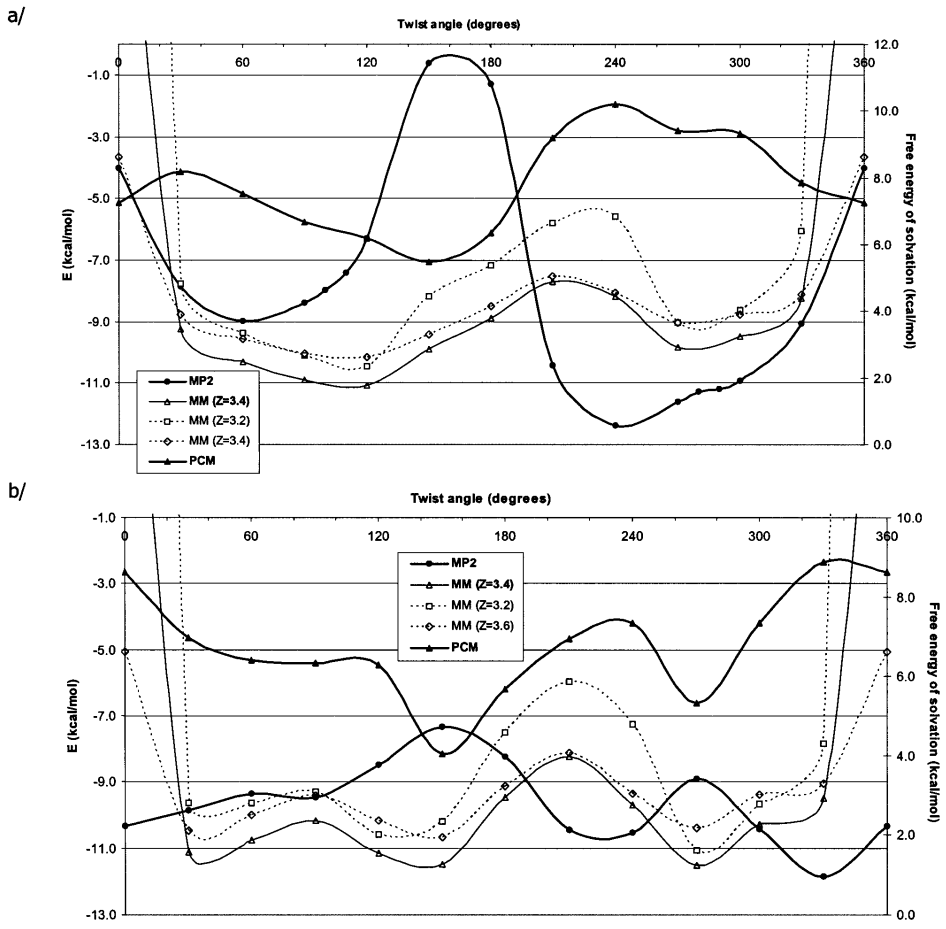


Figure 1. Stacking properties of 9-methyladenine interacting with a/ 9-methylguanine and b/ 8-oxo-9-methylguanine. Plots represent stabilization energies of stacked pair, which was calculated as the difference between pair energy and isolated monomers with correction for BSSE.

plot. Their conformations are characterized by the following values of twist angle 90°, range from 210° to 240° and 330°. Correlated stabilization energies are equal to –9.4 kcal/mol, –10.5 kcal/mol and –11.9 kcal/mol, respectively. These minima are separated by much lower energy barriers compared to pairs comprising 9-methylated adenine and guanine. The most stable pair formed by 9-methyladenine and 8-oxo-9-methylguanine is characterized by much stronger overlapping of the molecular surfaces, compared to stacking dimer formed by adenine with canonical 9-methylguanine.

The second group of studied dimers comprises 9-methylguanine. If this purine is stacked with itself, it may form two stable conformations of the same stacking energy for twist angle equal to 120 and 240 degrees, as it was shown in Fig. 2. Due to the symmetry of this stacked dimer, these minima depict the same pair with stabilization energy equal to –13.0 kcal/mol. The stacking complex comprising 9-methylguanine and

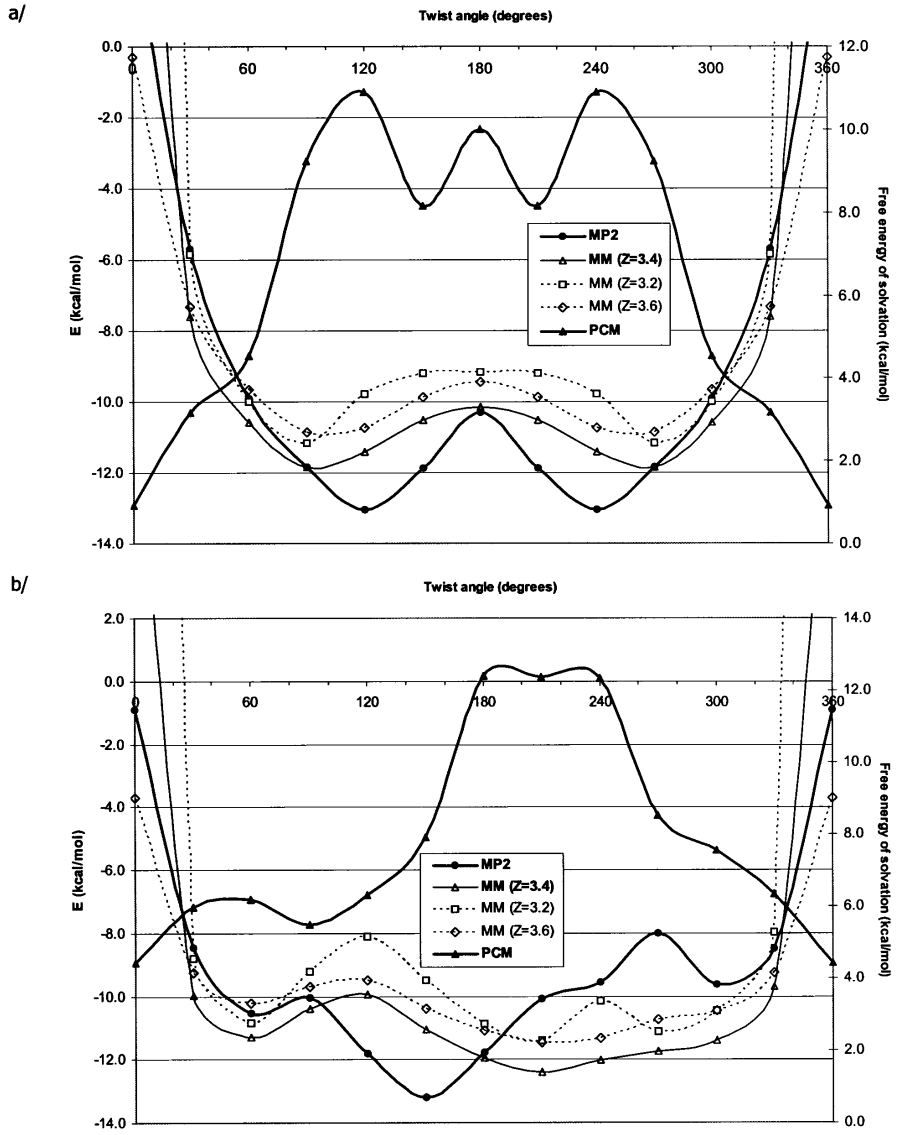


Figure 2. Stacking properties of 9-methylguanine in complexes with a/ 9-methylguanine and b/ 8-oxo-9-methylguanine.

8-oxo-9-methylguanine is characterized by three values of twist angle defining stable dimers separated by relatively shallow energy intervals. The most stable conformation corresponds to twist angle 150° and stabilization energy of –13.2 kcal/mol and represents the most stable dimer among all studied ones. The observed increase in twist angle for this pair compared to dimmer comprising only canonical 9-methylguanine is related to interaction of oxygen atom of C_8 carbonyl group with hydrogen present on amino group of the opposite base. The other minima for 60° and 300° are much more shallow. However, lack of the second minimum related to high values of twist

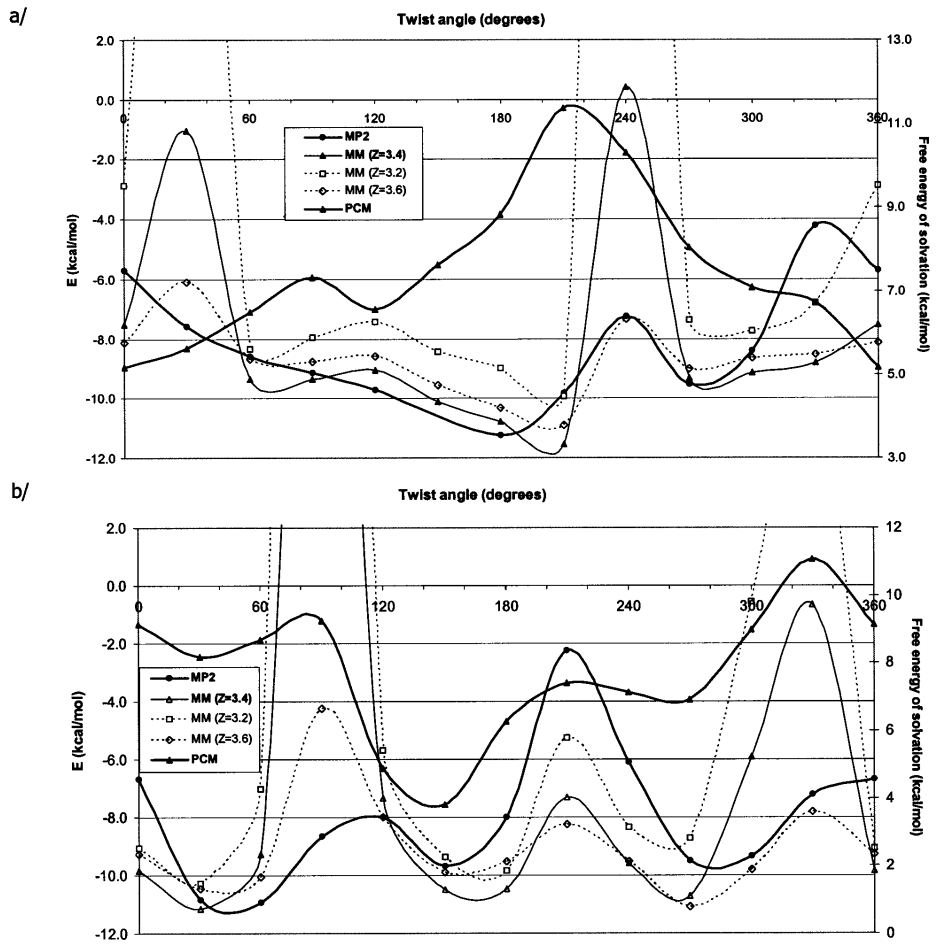


Figure 3. Stacking properties of 1-methylthymine in dimers with a/ 9-methylguanine and b/ 8-oxo-9-methylguanine.

angle makes that stacking interactions of 8-oxo-9-methylguanine with 9-methylguanine are not symmetrical with respect of twist angle. The 8-oxo-9-methylguanine exhibits strong stacking repulsion in the twist region, for which 9-methylguanine has major attraction. This again suggests significant alteration of stacking nature of 8-oxo-9-methylguanine with respect of standard 9-methylguanine.

The third collection of dimers comprises 1-methylthymine stacked to standard and modified 9-methylguanine. Interaction of 1-methylthymine with 9-methylguanine is distorted by methyl groups attached to pyrimidine ring. From Fig. 3 it may be seen that distinct minima located around 180° and 270° are separated by strong repulsion regions. The stacked dimer having conformation corresponding to former twist angle is more stable one and has stabilization energy equal to –11.2 kcal/mol. Such

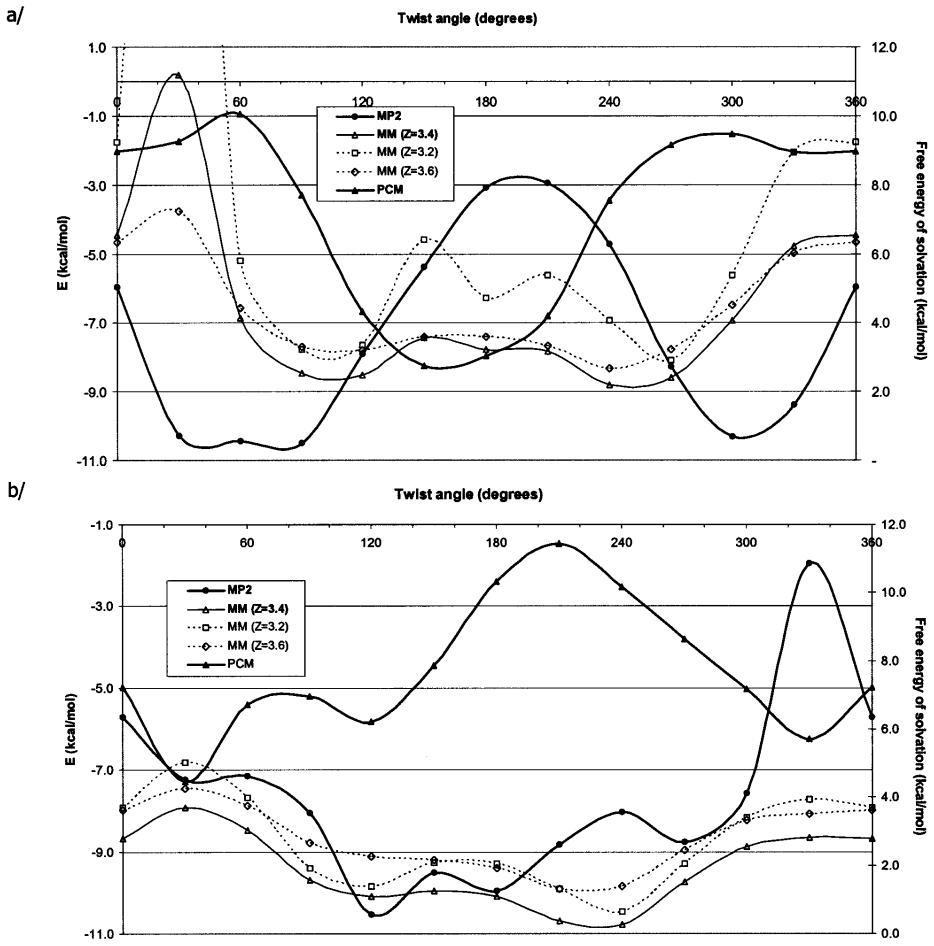


Figure 4. Stacking properties of 1-methylcytosine interacting with a/ 9-methylguanine and b/ 8-oxo-9-methylguanine.

conformation allows for maximal overlapping of purine and pyrimidine surfaces. The 1-methylthymine if stacked with 8-oxo-9-methylguanine is able to form three stable dimers for twist values of 60°, 150° and 270°. However, the most probable intermolecular complex is related to twist angle equal to 60°. Its energy is slightly lower than energy of most stable stacked pair 1-methylthymine/9-methylguanine but conformation is significantly different.

The last studied set of stacked dimers comprises 1-methylcytosine. The 9-methylguanine forms two stable stacked dimers with 1-methylcytosine, which are characterized by twist angle in the range from 30° to 90° and around 300° degrees. The energies of both pairs are almost the same and are equal to –10.5 kcal/mol. To the contrary abilities of 1-methylcytosine stacking with 8-oxo-9-methylguanine are more com-

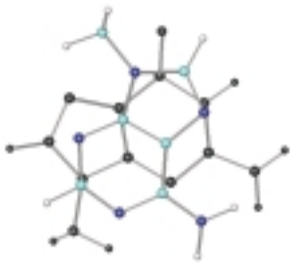
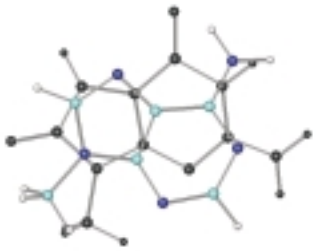
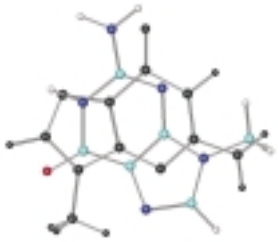
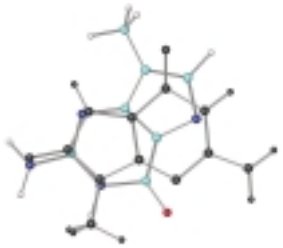
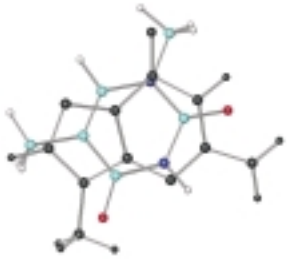
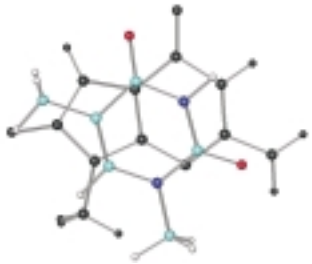
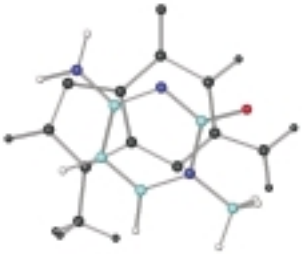
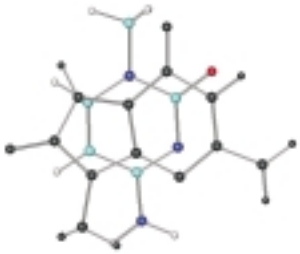
plex. Although, there are three minima, two of them are very shallow and are separated by small barriers. The minimum corresponding to the twist angle equal to 120° represents energetically most preferred structure of 1-methylcytosine stacked to 8-oxo-9-methylguanine. The energy of this dimer has the same value as 9-methylguanine stacked with 1-methylcytosine.

In Table 1 one may find more details about most stable stacked pairs formed by 8-oxo-9-methylguanine and 9-methylguanine. The stabilization energies of dimers are almost the same for both standard and modified 9-methylguanine. However, there are observed significant discrepancies in stacking properties of 9-methylated standard and modified guanine due to altered geometries of analyzed dimers. For all bases, except 1-methylthymine, geometries of most preferred stacked dimers are characterized by higher values of twist angle for 8-oxo-9-methylgunanine compared to canonical 9-methylguanine. Additionally, values of correlation energies, supplied in Table 1, clearly explains the origin of stacking interactions. In accordance with common expectation, dispersion interactions described by values of correlation energies play the most significant role in the overall stabilization of the stacked complexes. The contribution of correlation effect for pairs formed by 8-oxo-9-methylguanine is slightly more significant compared to 9-methylguanine.

Solvation of stacking pairs: The impact of the variation of twist angle on the solvation free energy of stacked dimers is presented in Figs. 1–4. There is strong influence of the mutual orientation of stacked bases on the solvation properties. For 9-methylated analogs of adenine – guanine and guanine – guanine pairs, solvation free energy has the lowest values for twist angle equal to 150°. Interestingly, the same conformation is preferred also for 9-methyladenine stacking with 8-oxo-9-methylguanine. However, there is another favored conformation of these bases for twist equal to 270°. Stacked dimer formed by guanine and 8-oxo-9-methylguanine exhibits the strongest solvation for region corresponding to 0^o of twist angle. Such conformation is related to face-to-face total overlapping of stacking molecule surfaces. It is interesting to notice that 9-methylguanine/1-methylthymine dimer also has the same properties. To the contrary 1-methylthymine/8-oxo-9-methylguanine pair is characterized by highest solvent interaction when its conformation corresponds to 150°. Significant differences in solvation abilities are also observed for pairs containing 1-methylcytosine. When this pyrimidine base is stacked with 9-methylguanine, the minimum on the solvation free energy plot is present around twist angle equal to 0°. On the other hand, the pair comprising 1-methylcytosine interacting with 8-oxo-9-methylguanine has most significant values of free energy for conformation described by twist angle equal to 150°.

Thus, conformations corresponding to lowest values of free energy of solvation differ significantly from those found in non-polar environment. The observed differences may be related to significant charge alteration imposed by polar surrounding.

Table 1. Characteristics of the most stable stacked dimers formed by 8-oxo-9-methylguanine and 9-methylguanine with four canonical 9-methylated DNA bases (A stands for 9-methyladenine, C denotes 1-methylcytosine, G represents 9-methylguanine, T symbolize 1-methylthymine and 8-oxo-G signify 8-oxo-9-methylguanine). The following data were presented: Θ – twist angle [degrees], E – interaction energy [kcal/mol], E^{cor} – correlation energy [in kcal/mol] calculated as difference between correlation energies of dimmer and isolated monomers.

G–A		**8-oxo-G – A**	
Θ = 240° E = –12.4 E^{cor} = –13.9		Θ = 330° E = –11.9 E^{cor} = –14.9	
G–G		**8-oxo-G – G**	
Θ = 120° E = –13.0 E^{cor} = –13.9		Θ = 150° E = –13.2 E^{cor} = –13.5	
G–T		**8-oxo-G – T**	
Θ = 180° E = –11.2 E^{cor} = –12.6		Θ = 60° E = –10.9 E^{cor} = –12.9	
G–C		**8-oxo-G – C**	
Θ = 90° E = –10.5 E^{cor} = –11.2		Θ = 120° E = –10.5 E^{cor} = –12.2	

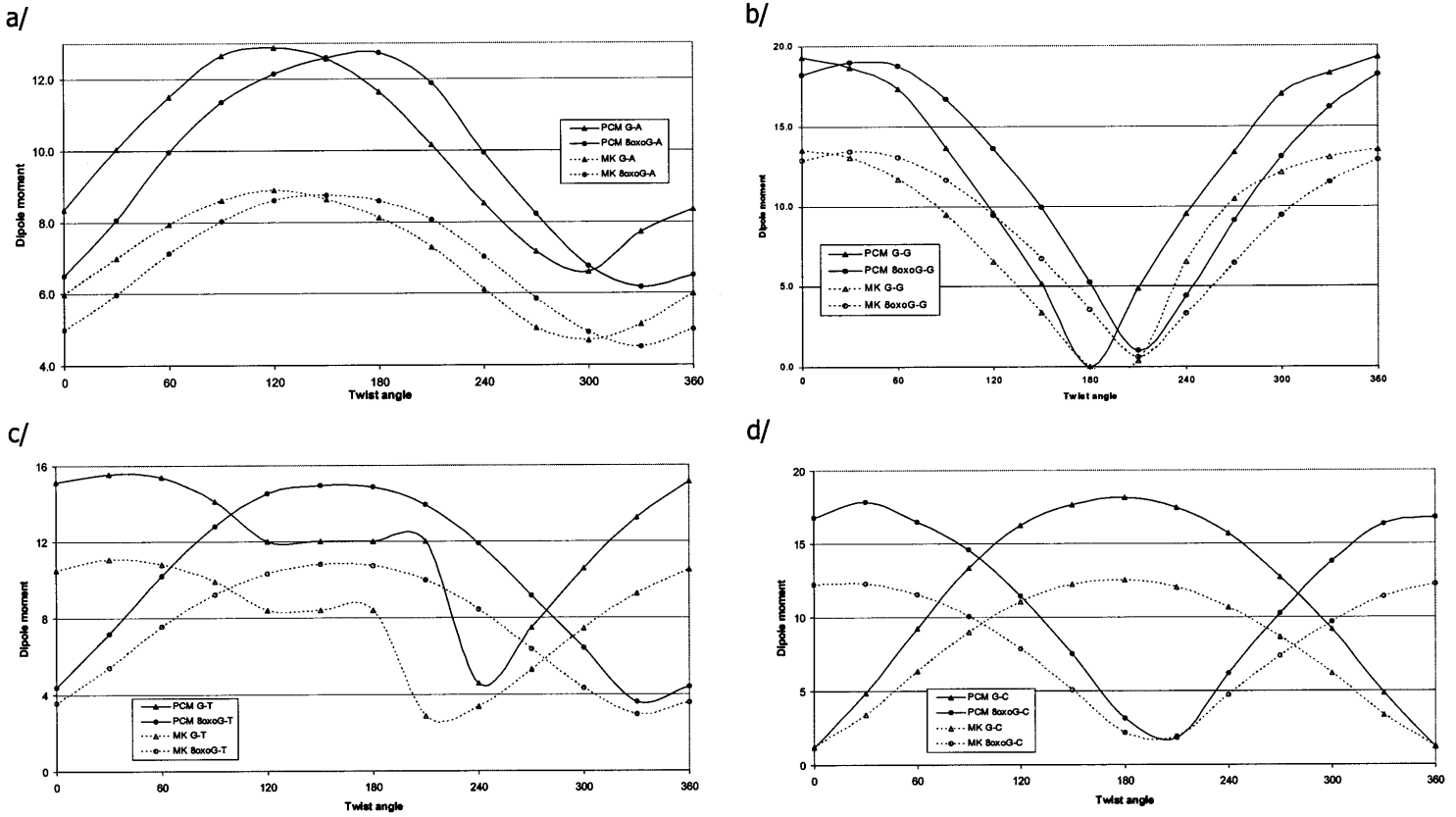


Figure 5. Impact of the twist angle on dipole moment for stacking of 9-methylguanine (G) and 8-oxo-9-methylguanine (8oxoG) with a/ 9-methyladenine (A), b/ 9-methylguanine (G), c/ 1-methylthymine (T) and d/ 1-methylcytosine (C). Presented values of dipole moments were estimated according to Merz-Kollman procedure (MK) both *in vacuo* and after into account solvent effects based on PCM method.

Polarities of stacking pairs: Polarity of stacking pairs may be described by values of dipole moments estimated both for polar and non-polar environment. It is interesting to notice that in both cases significant impact of the mutual orientation of stacked dimers on the dipole moment is observed. Results presented in Fig. 5 lead to the conclusion that the presence of the polar environment usually increases the polarity of pairs formed by native and modified nucleic acids. However, shapes of dipole moment curves estimated for polar and non-polar environment are very similar and show only slight shifting toward higher values of twist angle for pair comprising 8-oxo-9-methylguanine. Besides, the influence of solvation does not change significantly polarities of stacked pairs consisted of canonical or modified purines. To the contrary polarities of dimers formed by pyrimidines and 9-methylguanine differ considerably compared to those formed by 8-oxo-9-methylguanine.

Pair comprising 9-methyladenine and 9-methylguanine has the highest values of dipole moments for twist angle equal to 120°. The dimer formed by 9-methyladenine and 8-oxo-9-methylguanine exhibit the same feature for twist 150°. The region corresponding for minimal polarities is characterized by twist angles 300° and 330°, respectively. Such properties are common for non-polar and polar environment. Stacked dimmers formed by 9-methylguanine with itself and with 8-oxo-9-methylguanine have interesting feature. Their polarities decrease almost to zero for twist angle equal to 180° and 210°, respectively. The highest values of dipole moment may be found for twist angle 0° or 30°, respectively.

The most significant changes in pair polarities are observed for dimers comprising 1-methylcytosine and 1-methylthymine. If these pyrimidines interact with 9-methylguanine the highest polarity corresponds to twist angle equal to 180°. However, for dimers comprising 8-oxo-9-methylguanine this conformation corresponds for minimal values of dipole moment. Again for low values of twist angle 1-methylpyrimidine/9-methylguanine dimers are non-polar, while 1-methylcytosine/8-oxo-9-methylguanine and 1-methylthymine/8-oxo-9-methylguanine dimers are very polar.

Point atomic charges of stacking pairs: It is well known that the dispersion interactions of aromatic rings are responsible for stacking, while inter-strand base-base hydrogen bond formation is strongly dependent on the electrostatic properties of interacting molecules. It seems to be interesting to notice whether mutual orientation of stacking bases has an influence on the point atomic charges. Atoms involved in inter-stand hydrogen bond formation are of special importance. In case of 9-methylguanine or 8-oxo-9-methylguanine the following atoms are to be considered: O_6, H_1, and H_2. Point atomic charges were estimated as ESP fit according to Merz-Kollman scheme both for non-polar and polar environment (PCM model). Stacked dimers charges were related to ones obtained for isolated monomers as a percentage of change calculated according to the follows simple formula: $\left(q_i^{\mathrm{dim}} - q_i^{\mathrm{mon}}\right)/q_i^{\mathrm{mon}}$, (where index *i* denotes atom center and superscripts *dim* or *mon* stands for dimer or monomer, respectively). Positive values indicate increase of point atomic charges for dimer with respect of monomer. To the contrary negative values suggest decrease of electro-

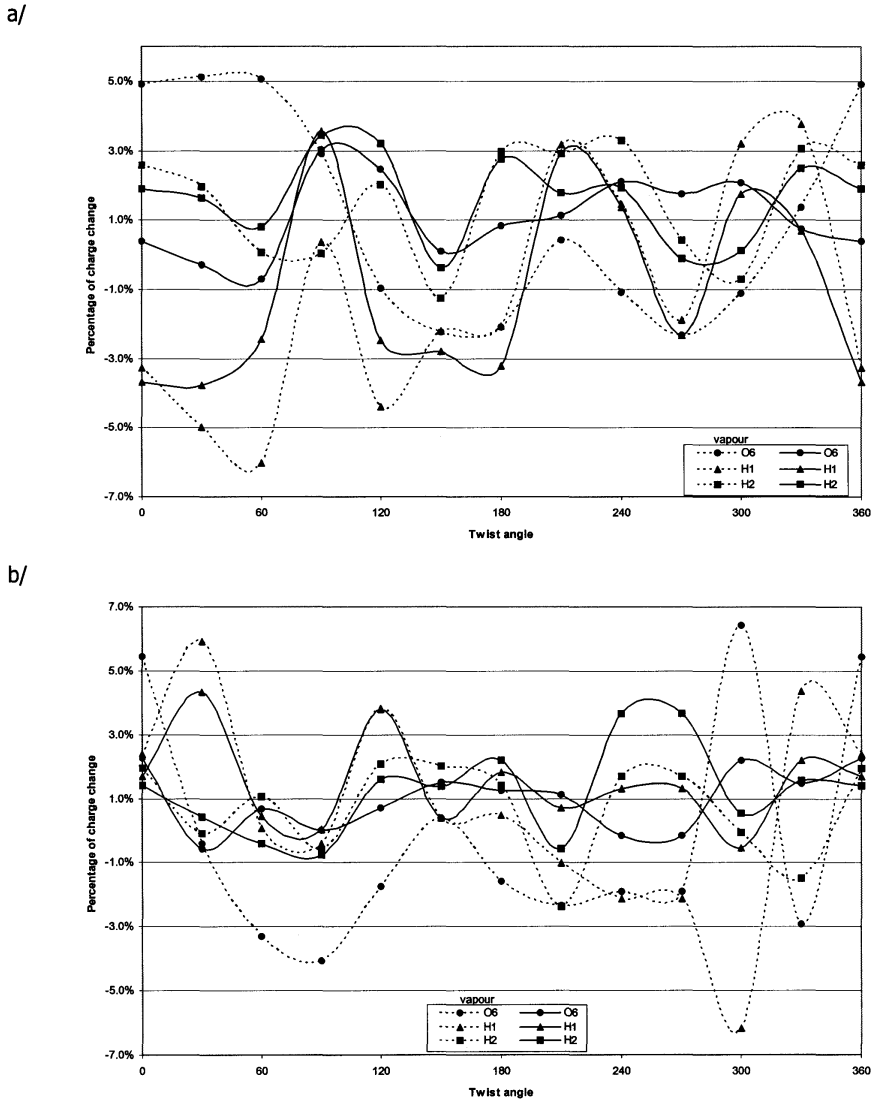


Figure 6. Variation of point atomic charges, which may be potentially involved in intermolecular hydrogen bond formation of a/ 9-methylguanine and b/ 8-oxo-9-methylguanine, as a function of twist angle, for stacked dimers with 9-methylguanine.

static charge. Figure 6 presents variation of such estimated data as a function of twist angle in case of 9-methylguanine/9-methylguanine and 9-methylguanine/8-oxo-9-methylguanine dimers. These dimers were chosen since they represent most stable stacked complexes among all studied in this work. Presented data show significant influence of stacking base conformation on the point atomic charges, what is observed both for non-polar and polar environments. The differences of point atomic charges may have positive or negative values, depending on the twist angle. For example in vapor, the 0_6 center is much less negative if 9-methylguanine/9-methylguanine is cha-

racterized by twist angle equals 60° than 270°. Such an effect may be strengthened by similar changes of atomic charges located on other centers. For example H_1 atom is more polar for twist angle 60° than for other bases orientations. Thus, there is not charge compensation and there might be conformations of stacked bases, for which may occur much stronger hydrogen bonds. To the contrary for some other bases orientations one may expect significant weakening of intermolecular hydrogen complexes. This conclusion may be drawn both for 9-methylguanine/9-methylguanine and 9-methylguanine/8-oxo-9-methylguanine stacking dimers. The influence of molecule electrostatics on hydrogen bond formation is straightforward and is to be considered as one of the most significant factors, describing HB complexes. Even changes within few percent of atomic charges may result in significant contribution to total energy of complex stabilized by hydrogen bonds. Additionally, one may expects a significant impact of charge changes on the geometry of hydrogen bonded complexes. Detailed analysis of this feature is however beyond scope of this paper and will be the subject of the forthcoming study.

CONCLUSIONS

Presented data lead to the conclusion that modification of 9-methylguanine by hydroxyl radical at C_8 position lead to significant changes in the stacking abilities of 8-oxo-9-methylguanine compared to standard 9-methylguanine. Among all studied dimers the most stable is one formed by 8-oxo-9-methylguanine with 9-methylguanine. The least stable is 8-oxo-9-methylguanine/9-methyladenine stacked dimer. It has been noticed significant differences in stacking abilities of 8-oxo-9-methylguanine compared to canonical guanine, which may be summarized as follows:

- Alteration of intermolecular dimer geometry, represented by different values of twist angle corresponding to most stable stacked dimers.
- More complexity of stacking since extra stable stacked dimers may be formed of modified 9-methylguanine.
- Usually much lower energy barriers between minima on energy plots, what indicate much less distorted changes of twist angle of dimers containing 8-oxo-9-methylguanine.
- Significant changes of intermolecular interactions, due to repulsion is present in the regions, where guanine exhibits attraction.
- Polarities of dimers containing pyrimidines differ significantly for 8-oxo-9-methylguanine compared to canonical 9-methylguanine; however, polarities of stacked pair formed only by purines are similar for dimers corresponding to 9-methyl-G and 8-oxo-9-methyl-G.
- Significant differences of solvation effects are observed for dimers containing modified 9-methylguanine, especially if it is stacked with pyrimidines.

Observed differences of 8-oxo-9-methylguanine stacking are related to significant alteration of molecule electrostatics after 9-methylguanine modifications at C_8 position. The presence of O_8 oxygen atom does not lead to noticeable direct interaction

and is rather the source of increase of the overall dispersion interactions. The correlation energies of stacking dimers are very high and are source of pairs stabilization; both 9-methylguanine and 8-oxo-9-methylguanine are characterized by similar values of correlation energy.

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