

# Total energy of deoxyguanosine bonded to *N*-2-acetylaminofluorene by the semi-empirical modified-neglect of differential diatomic overlap method

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## Abstract

We have computed the total energy surface as a function of two important torsion angles of the carcinogen *N*-2-acetylaminofluorene (AAF) bonded to the carbon C8 of deoxyguanosine using the semi-empirical quantum mechanical method, MNDO. One global minimum and one local minimum are found separated by an appreciable barrier. The equilibrium geometries show the rearrangement of AAF and the base consistent with experimental observations of previous investigators. © 2001 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

The carcinogenic effect of heterocyclic aromatic amines has been the subject of numerous experimental and theoretical studies for over 30 years [1]. Much attention has been given to *N*-2-acetylaminofluorene (AAF) covalently bonded to DNA which has been observed *in vitro* [1–9] and *in vivo* [10–12]. Generally, these interactions produce mutations in the form of transversions and transitions of the base pairs. In about 85% of the cases, the bonding occurs between the carbon C8 of the guanosine and the nitrogen N2 of AAF. In the remaining instances the bond is formed between the nitrogen N2 of the guanosine and the carbon C3 of AAF. It has

been observed that this bonding is accompanied by the insertion of AAF between adjacent bases resulting in base pair rupturing [13–16]. Computational studies using the semi-classical molecular mechanics have been undertaken on AAF-modified guanosine and other modified base polymers [17–22].

It is the purpose of this article to explore the interaction of AAF with deoxyguanosine and to obtain a map of the total energy surface as a function of the two main torsion angles for the principal adduct. This is accomplished by a quantum mechanical semi-empirical molecular orbital approach, the modified-neglect of differential diatomic overlap (MNDO) method. Total energies were obtained for 1200 points in the configuration space of the two important torsion angles. We report the location of the global minimum of this surface and of one other well defined local minimum, and the energetics of the conversion between them.

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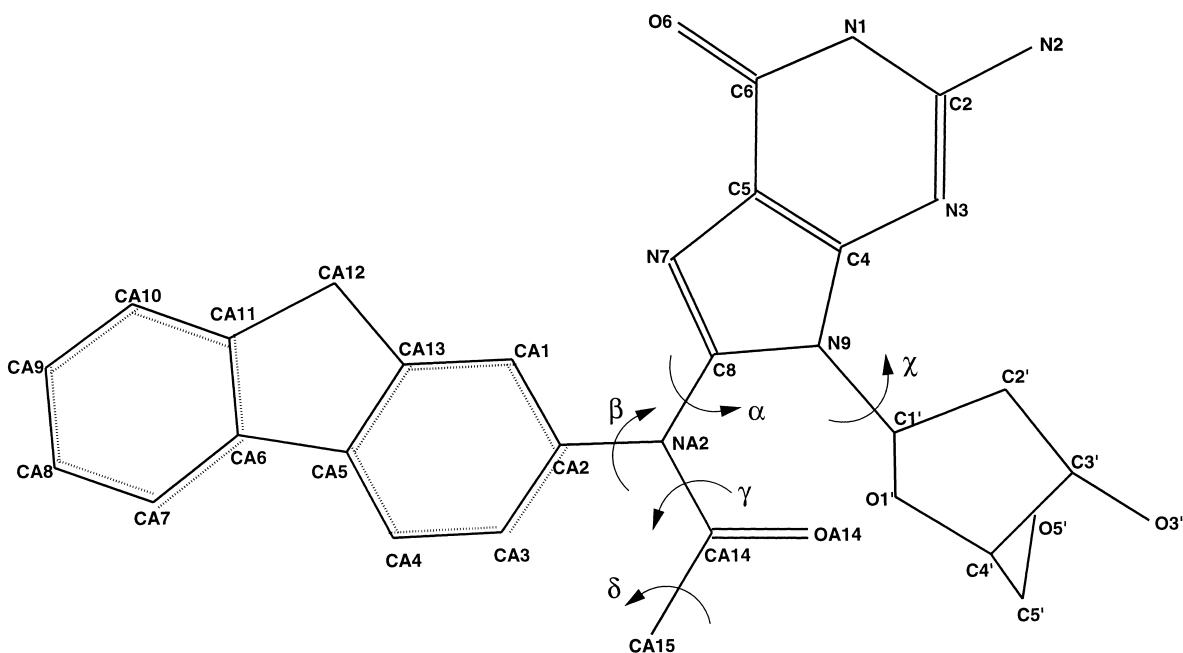


Fig. 1. Representation of the DNA interaction site (hydrogens not shown). The dihedral angles are  $\alpha$ : N9–C8–NA2–CA2;  $\beta$ : C8–NA2–CA2–CA1;  $\gamma$ : C8–NA2–CA14–CA15;  $\delta$ : NA2–CA14–CA15–HA15;  $\chi$ : O1'–C1'–N9–C8.

## 2. Computational method

The environment of the DNA interaction site is simulated by a finite cluster of atoms terminated with hydrogen atoms. The 60-atom cluster is composed of deoxyguanosine bonded at the carbon C8 with the nitrogen N2 of AAF. The initial geometries of the two moieties were obtained from standard published data [23], and from X-ray and NMR studies [24]. The cluster is shown in Fig. 1. Five torsion angles  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ , and  $\chi$  are also shown.

To investigate the energy surface in configuration space of this cluster we have used the MNDO method [25]. This is a semi-empirical self-consistent implementation of the Hartree–Fock–Roothaan equations. The molecular wave function is constructed as a linear combination of atomic orbitals, and the Hamiltonian matrix elements are parameters which have been previously determined from molecular data. Three- and four-center integrals are set to zero, and the remaining one- and two-center terms depend on adjustable parameters. These parameters have been obtained from experimental data on many small molecules such as

bond lengths, heats of formation, and dipole moments. The MNDO method produces reliable total energies as a function of atomic positions and is able to probe the energy surface to find the minimum energy configurations. Although it also determines energy eigenvalues of the molecular orbitals, these cannot be interpreted on an absolute scale as excitation energies. This point has been discussed at length by DeLeo et al. [26].

As an initial check of consistency, the geometry of each of the two moieties was determined by minimizing the total energy separately, allowing all bond lengths and angles to relax. This produced insignificant changes in energy and arrangement from the published data. The final molecule was then assembled by joining the carbon C8 of the deoxyguanosine with the nitrogen N2 of the AAF. A series of starting geometries was used in which the torsion angles  $\alpha$  and  $\chi$  occupied points on a grid of frequency  $15^\circ$  in each direction. The other three torsion angles,  $\beta$ ,  $\gamma$  and  $\delta$  were initialized to arbitrary values. The sugar pucker was fixed in the C2'-endo configuration as is found in B-DNA. All geometry optimization was done with the Davidon–Fletcher–Powell minimization

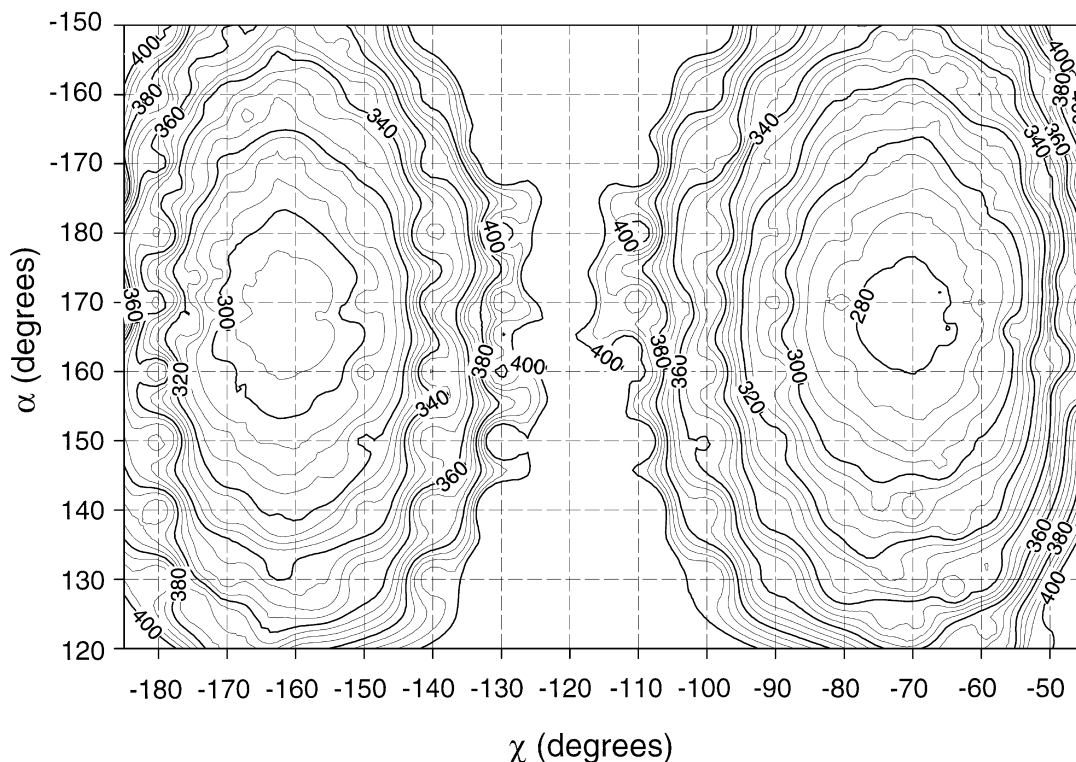


Fig. 2. Total energy surface in the  $\alpha$ - $\chi$  plane for dG-AAF from MNDO. Regions outside the contour lines have energies greater than 400 kcal/mol.

algorithm, and along each path to the final configuration the values of the total energy and the five torsion angles were recorded. Thus, a thorough map of the energy surface in  $\alpha$ - $\chi$  configuration space was obtained.

### 3. Results

Starting from initial values of the torsion angles which covered all of configuration space, we performed a series of MNDO calculations on the dG-AAF entity. In a few cases, steric interactions

prevented the determination of a final equilibrium geometry. Except for these, the system relaxed into one or the other of two minimum energy configurations.

Fig. 2 shows the computed total energy surface for the system. This plot is an interpolated mesh obtained from 1200 data points using an inverse distance method and a distance weight exponent of three. Regions outside the contour lines have energies greater than 400 kcal/mol.

For the global minimum, the computed heat of formation was found to be 277.66 kcal/mol. The other stable minimum was found to have heat of formation

Table 1  
Equilibrium geometries from MNDO<sup>a</sup>

Heat of formation (kcal/mol)	$\alpha$ (degrees)	$\chi$ (degrees)	$\beta$ (degrees)	$\gamma$ (degrees)	$\delta$ (degrees)
277.66	168.2	-72.5	-11.88	180	180
292.34	168.3	-164.3	-11.88	180	180

<sup>a</sup> The torsion angles are defined in Fig. 1.

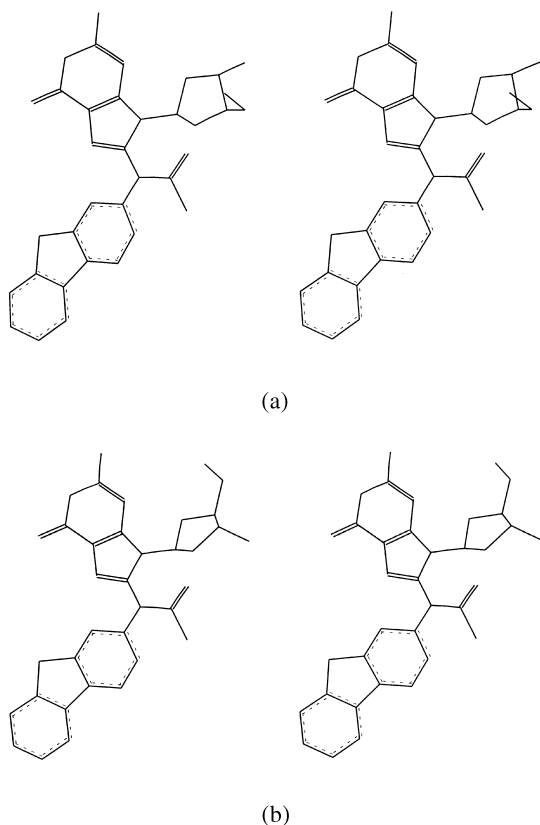


Fig. 3. Stereograms of dG-AAF from MNDO: (a) global minimum; (b) local minimum (hydrogen atoms are not shown).

of 292.34 kcal/mol. The equilibrium values of the torsion angles are shown in Table 1. The spatial geometries for the two minima are shown in Fig. 3.

Initially the torsion angles  $\beta$ ,  $\gamma$  and  $\delta$  were allowed to relax as well, producing small displacements from their equilibrium values. After numerous runs it became evident that these angles had an insignificant effect on the location of the minima. Consequently, we set them to their equilibrium values and did not allow them to vary. The values are listed in Table 1.

#### 4. Discussion

The results of our calculations show two well-defined and localized minima of the total energy surface. In moving away from either well, the energy increases monotonically. There are no other shallow

local minima anywhere on the surface. There is a high energy ridge between the minima located approximately along  $\chi = -120^\circ$ . The minima are somewhat flat through an area about  $15^\circ$  on a side.

These results are markedly different from those of earlier investigators using the molecular mechanics methods. For example, Fritsch and Westhof [21] used the AMBER 3.0 [27] parametrization with a distance-modified dielectric constant; they located 20 minimum energy domains using two sets of force field parameters. Subsequent molecular dynamics runs indicated that the energy barriers were small and that, depending on the parameter set, three or four domains were the most stable. The locations of two of their stable configurations (numbers 2 and 24) are close to our minima.

The most pronounced geometrical consequence is the rotation of the structure around the glycosidic bond and the resulting position of AAF for the global minimum. When  $\chi = -72.5^\circ$  the position of the guanine is anti, but considerably altered from its value of  $84.6^\circ$  which it has in B-DNA. In its lowest energy configuration, the carcinogen is in the position previously occupied by the guanine, as shown in Fig. 4. Within B-DNA this would dramatically alter the sequence of bases both in space (replication) and in chemical activity (protein synthesis). This is consistent with the insertion–denaturation behavior observed by Fuchs et al. [16] in their study of native DNA modified by several fluorene residues. For the local minimum, the guanine is rotated to the *syn*-position, and the AAF again occupies a region corresponding to the interior of the DNA helix.

We note that the minima predicted by MNDO are very deep, i.e. the barrier to reorientation is very high. Examination of Fig. 2 would suggest that a least action path between the two minima would be along  $\alpha \approx 163^\circ$ . An MNDO reaction coordinate calculation along this path yields the results shown in Fig. 5. These calculations were performed for three values of the bond length of the  $\alpha$ -bond. The barrier to reorientation decreases with increasing bond length, as would be expected. For each curve, the Boltzmann factor and the equilibrium probability of occupation of the upper energy state are presented in Table 2 for  $T = 310$  K.

In Fig. 5, the barrier to reorientation is 53 kcal/mol for the experimentally observed [24]  $\alpha$ -bond length of  $1.47 \text{ \AA}$ . The steepness is primarily due to steric

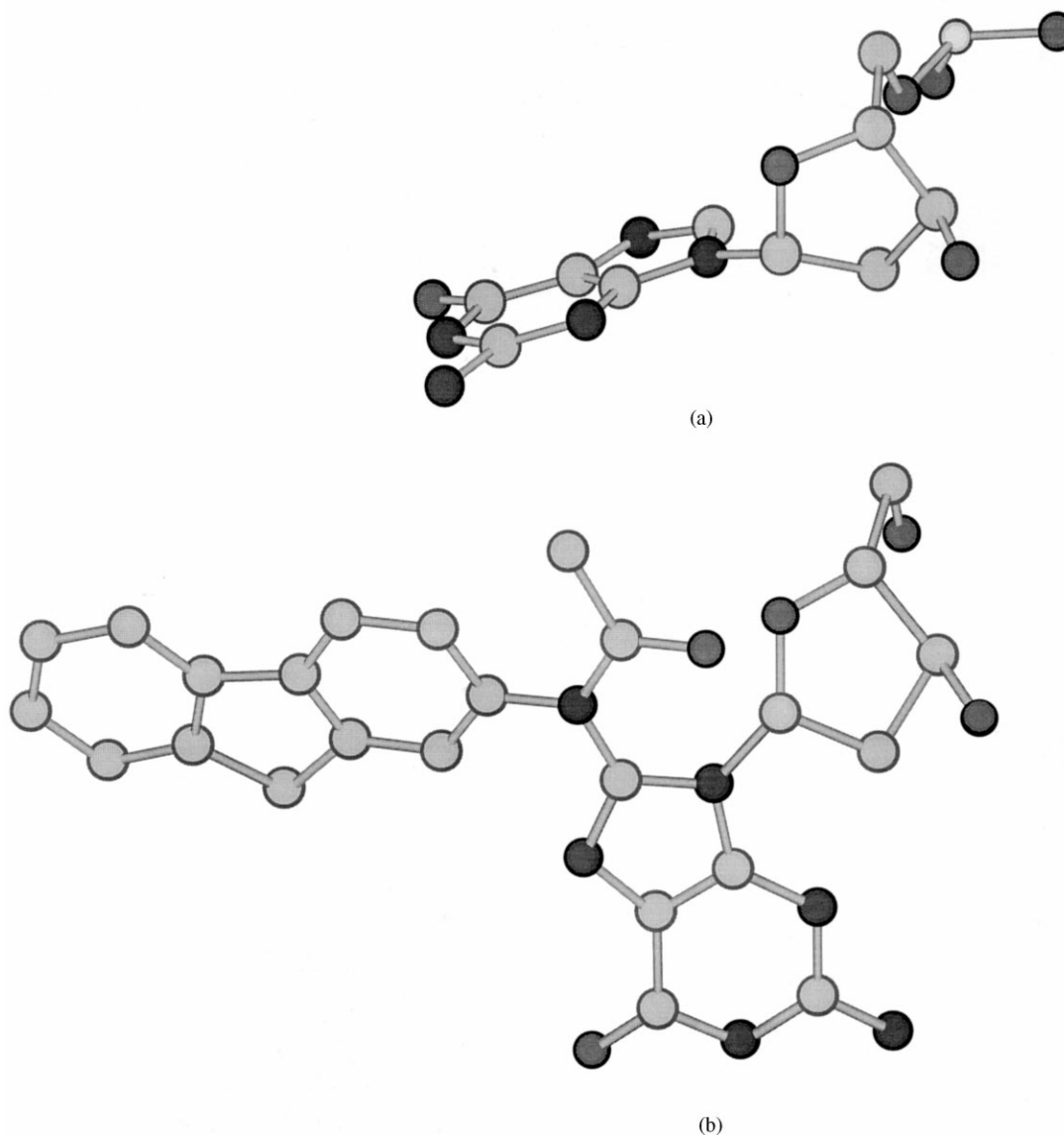


Fig. 4. The geometry of the global minimum from MNDO showing the insertion of AAF: (a) unmodified deoxyguanosine, including the 5' phosphate group; (b) the AAF-modified structure.

interactions along the reaction path. Near  $\chi = -110^\circ$ , the carbonyl oxygen OA14 of the AAF is only  $0.73 \text{ \AA}$  from the hydrogen on the carbon C1' of the sugar. There are other steric interactions for values of  $\chi$  outside the range shown in Fig. 5 which account for very high energies in these regions. For example, near  $\chi = 20^\circ$ , the distance between the AAF oxygen OA14 and

the sugar oxygen O1' is  $0.52 \text{ \AA}$ . For this reason this region has not been shown in the figure.

The actual reaction path in double-stranded DNA would be more complicated due to the presence of other atoms in the chain. However, our calculations give a reasonable estimate of the difference in total energy between the initial and final states for the

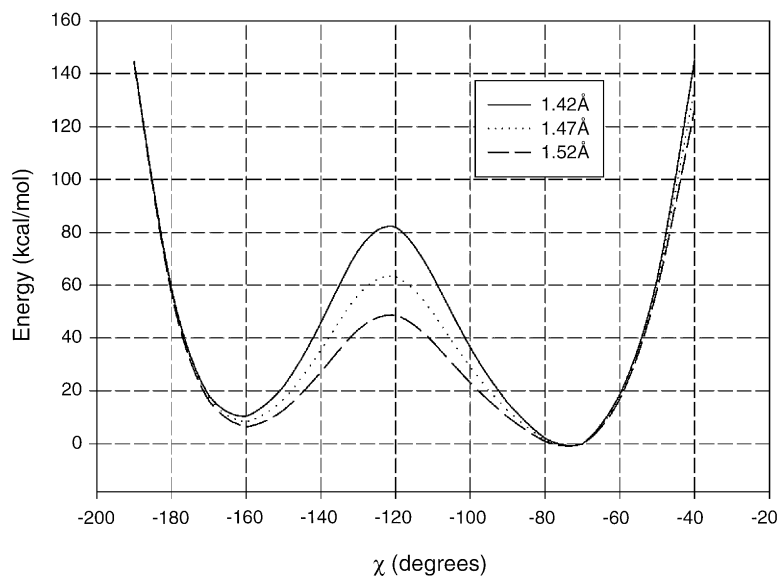


Fig. 5. Reaction coordinate calculation along  $\alpha = 163^\circ$  from MNDO through the global and local minima.

Table 2

Activation energy and relative populations for the two potential wells shown in Fig. 5 for temperature  $T = 310$  K

$\alpha$ -bond length ( $\text{\AA}$ )	$\Delta E$ (kcal/mole)	$\Delta E/kT$	Relative population
1.42	10.35	16.8	$5.05 \times 10^{-8}$
1.47	8.24	13.4	$1.55 \times 10^{-6}$
1.52	6.37	10.3	$3.23 \times 10^{-5}$

reorientation of the carcinogen between the local and global minima.

## 5. Summary

We have investigated the interaction of AAF with the molecule deoxyguanosine to understand the energetics of various conformations as a function of the two torsion angles  $\alpha$  and  $\chi$  using the quantum mechanical semi-empirical molecular orbital method MNDO. We have mapped the total energy surface over the entire  $\alpha$ - $\chi$  plane. We find a global minimum and one local minimum separated by a substantial energy barrier. Both minima predict a geometry in which the carcinogen replaces the base in agreement with published experimental work on viable DNA.

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