Guanine Alkylation by Ethylene Oxide: Calculation of Chemical Reactivity

Agata Kranjc and Janez Mavri*

National Institute of Chemistry, Hajdrihova 19, P.O. Box 660, SI-1001 Ljubljana, Slovenia, and International School for Advanced Studies (SISSA/ISAS), Via Beirut 2−4, 34014 Trieste, Italy

Received: September 9, 2005; In Final Form: March 10, 2006

In this paper we report on calculations of the activation free energy for a chemical reaction between ethylene oxide and guanine. Ethylene oxide is biologically relevant per se and is also a model compound for numerous ultimate carcinogens. Calculations were performed on the medium-high ab initio, DFT, and semiempirical MO levels. Effects of solvation were considered using the Langevine dipole method and solvent reaction field method of Tomasi and co-workers. The calculated activation free energies are in reasonable agreement with the experimental value.

1. Introduction

Carcinogenesis is a complex pathological process where normal cells become neoplastic. In most of the cases the process is associated with chemical modification of DNA. Chemical modification of DNA can be associated with viruses, photochemical reactions, and reactions induced by chemicals.[1−4] If the chemicals are hormones or their metabolites, then they are referred to as endogenous carcinogens.[5,6] If they come from the environment, they are referred to as exogenous carcinogens. Polyaromatic hydrocarbons (PAHs) are exogenous carcinogens that are typically highly carcinogenic. To be precise, they are not carcinogenic per se, but are metabolized to carcinogenic substances that react with DNA, typically with guanine at position N7.[7−9] A typical metabolite of PAH is in its epoxidized form. The former substances are called procarcinogens, while the metabolites are called ultimate carcinogens. This transformation is catalyzed by cytochrome P450. The ultimate carcinogen alkylates DNA, typically guanine at position N7, although other alkylation sites are reported.[7] Alkylation is followed by other reactions, depurination being a typical example.

Ethylene oxide (ETO) is the smallest model for the PAH ultimate carcinogens.[10−12] ETO is used for medical equipment sterilization and hospital disinfection and as a fumigant for spices and pharmaceutical products. It is also an intermediate in the synthesis of ethylene glycol, glycol ether, and nonionic surface-active agents.[13] Ethylene oxide is a direct alkylating agent which can react with nucleophilic sites in DNA and proteins and is therefore mutagenic, cytotoxic, and carcinogenic.[7] As previously mentioned, the N7 of guanine is the major site of ETO alkylation[7−9,14] (see Scheme 1). It is well established that the rate-limiting step for reaction of the ultimate carcinogens of the epoxy type with the nucleophilic sites of DNA and proteins is the epoxide ring opening.[15] The intermediate picks up the proton from the aqueous environment, which is believed to be a fast step.[16]

The kinetics of guanine alkylation was studied experimentally,[17] and the determined free energy of activation was 24.66 kcal/mol, which corresponds to a rate constant of 4.44×10^{-6} s^{-1}. Pauwels and Veulemans[17] performed a kinetic study of DNA alkylation at the guanine N7 site by ethylene oxide. The reaction rate constant has been determined in a whole blood solution. HPLC in conjunction with UV spectroscopy was used to measure the time-dependent concentrations of the adducts. Only the N7 adduct of Gua and ethylene oxide was detected. Moreover, they demonstrated that alkylation of the N-terminal valine of hemoglobin by ethylene oxide is about 3 orders of magnitude faster, but this reaction is irrelevant in the context of carcinogenesis. The second-order rate constant was determined, from which the activation free energy was calculated. Realistic simulation of the chemical reactivity of nucleic acids in aqueous solution is a challenge for computational chemistry.

In this study, we calculated the activation free energy for alkylation of guanine by ethylene oxide and compared it to the experimental free energy of activation. We applied the ab initio, DFT, and semiempirical MO methods. The effects of solvation were included by using the solvent reaction field level of Tomasi and co-workers[18] and Langevin dipole method of Florian and Warshel.[19] Moreover, the solution effects in conjuction with the semiempirical MO methods were studied on the AM1-SM1 and PM3-SM3 levels.

The organization of this paper is as follows. In section 2 the applied computational methods are described, while in section 3 the results are collected, and the discussion is in section 4.

2. Computational Methods

For calculation of the Born−Oppenheimer hypersurface and consequently the rate constant for the reaction between ethylene oxide and guanine, we performed DFT, ab initio Hartree−Fock, and semiempirical MO calculations. The distance between the carbon atom of the ethylene oxide linked to N7 of guanine was chosen to be the reaction coordinate. We optimized all degrees of freedom except the fixed value of the reaction coordinate for each calculation; the highest energy point on this path represents the approximation of the transition state. For the reactants a full geometry optimization was performed. The transition state was refined by the methodology built in Gaussian 03.[22] The difference between the energy of the transition state and the reactants is the activation energy. For the reactants and transition state we performed vibrational analysis in the harmonic approximation. For reactants all frequencies were real, while the transition state had one imaginary frequency predicted by all levels of theory.

* To whom correspondence should be addressed. E-mail: janez@kihp2.ki.si.

10.1021/jp055092z CCC: $33.50
Published on Web 04/07/2006

SCHEME 1: Guanine Alkylation by Ethylene Oxide

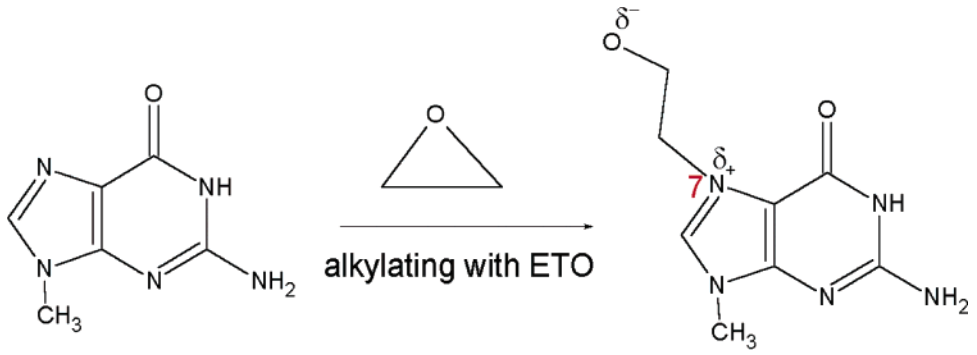

TABLE 1: Calculated Energies of Activation for Reaction between Ethylene Oxide and Guanine Using Different Methods

method	$E^{\ddagger}$ [a] (kcal/mol)	ZPE(TS)[b] (kcal/mol)	ZPE(R)[c] (kcal/mol)	ΔZPE[d] (kcal/mol)	ω_i[e] (cm^{-1})	CN distance[f] (Å)
HF/6-31G(d)	53.29	137.35	137.89	−0.54	555	1.85
HF/6-31+G(d,p)	49.72	136.39	136.99	−0.6	545	1.89
HF/6-311++G(d,p)	49.63	135.79	136.32	−0.53	542	1.9
HF/6-311++G(2d,2p)	49.13	136.12	136.57	−0.45	543	1.89
B3LYP/6-31G(d)	33.89	127.17	127	0.17	500	1.79
B3LYP/6-31+G(d,p)	36.46	126.51	127	−0.49	475	1.83
B3LYP/6-311++G(d,p)	36.22	126.02	126.47	−0.45	470	1.84
B3LYP/6-311++G(2d,2p)	36.24	126.25	126.75	−0.5	470	1.83
PM3	47.43	122.58	123.1	−0.52	710	1.84
AM1	53.39	128.16	129.18	−1.02	778	1.82

[a] Classical activation energy. [b] Zero-point vibrational energy for the transition state. [c] Zero-point vibrational energy for the reactants. [d] Zero-point energy of the transition state minus zero-point energy of the reactants. [e] Imaginary frequency value corresponding to the transition state. [f] Reaction coordinate values (distances between carbon in the ethylene oxide and nitrogen N7 in the guanine) corresponding to the transition state.

As suggested by a reviewer we considered also the product structure and energy. The same procedure was used as for the reactants except that we performed the calculations only on the selected levels of theory.

Calculation of the Born−Oppenheimer surface for chemical reactions is not a trivial task. It is generally believed that one needs relatively flexible basis sets and inclusion of electron correlation. The ab initio calculations were performed on the Hartree−Fock level in conjunction with the following basis sets: 6-31G(d), 6-31+G(d,p), 6-311++G(d,p), and 6-311++G-(2d,2p). Calculations beyond the Hartree−Fock level (e.g., MP2) were not possible because of the large size of the system. Therefore, we considered the DFT method B3LYP that has the exchange functional introduced by Becke[20] and the correlation functional introduced by Lee, Yang, and Parr.[21] Basis sets 6-31G(d), 6-31+G(d,p), 6-311++G(d,p), and 6-311++G(2d,-2p) were used. In addition, we applied the semiempirical MO methods AM1 and PM3. The latter two methods we applied because of their low CPU cost, which allows for QM/MM applications and thermal averaging. We are aware that DFT methods also have significant empirical character; nevertheless, they include to some extent electron correlation.

The free energy of hydration for the reactants and transition state was calculated with two methods, the solvent reaction field (SCRF) of Tomasi and co-workers[18] and the Langevin dipole (LD) method parametrized by Flórian and Warshel.[19] The SCRF method was applied on all DFT and ab initio levels. In LD calculations, Merz−Kollman atomic charges were determined for each level of theory.

DFT, ab initio, and semiempirical MO calculations and Tomasi's free energies of hydration were performed and determined by the Gaussian 03 suite of programs,[22] while the LD calculations were performed using the LD program ChemSol 2.1.[19] The AM1-SM1 and PM3-SM3 calculations were performed by the program AMSOL-5.4.1.[34] All calculations were performed on a cluster of Linux-based PCs running AMD Athlon processors at 700 MHz. We estimated that about 47 days of single-processor CPU time was used.

3. Results

The calculated activation energies, zero point energies, and data for the imaginary frequencies are collected in Table 1. The free energies of hydration calculated by the solvent reaction field are represented in Table 2. The Langevin-dipole-calculated free energies of hydration are collected in Table 3. The AM1-SM1- and PM3-SM3-calculated free energies of hydration are shown in Table 4. Tables 2−4 also include the calculated free energies of activation.

From Table 1 it is evident that for ab initio HF calculations of the barrier height we achieved convergence in terms of the basis set size. It looks like the addition of a diffuse function on heavy atoms and polarization functions on both heavy atoms and hydrogens is crucial for prediction of the reaction barrier. The predicted Hartree−Fock level barrier is between 49 and 53 kcal/mol.

Application of DFT drastically reduces the barrier. Again the barrier does not change anymore with the addition of basis functions when polarization functions are used on heavy atoms and hydrogens, while diffuse functions are only on heavy atoms.

Semiempirical MO methods AM1 and PM3 yield barriers comparable to those of the HF level. It was demonstrated that the PM3 method performs well for energetics associated with the reaction catalyzed by xylose isomerase.[23] Table 1 also shows that the zero-point vibrational energy correction of the reaction barrier is almost negligible. In addition, one can see from Table 1 that the DFT-calculated BO surfaces are shallower than the HF-calculated surfaces, which is reflected in the absolute values of the zero-point vibrational energies.

TABLE 2: Free Energies of Hydration Calculated by the SCRF Method[a]

method	$\Delta G_{hydr}^{SCRF}(TS)$[b] (kcal/mol)	$\Delta G_{hydr}^{SCRF}(R)$[c] (kcal/mol)	$\Delta G_{hydr}^{SCRF}(TS - R)$[d] (kcal/mol)	$\Delta G^{\ddagger}_{SCRF}$[e] (kcal/mol)
HF/6-31G(d)	−41.51	−15.89	−25.62	27.13
HF/6-31+G(d,p)	−47.04	−18.45	−28.59	20.53
HF/6-311++G(d,p)	−45.66	−17.56	−28.1	21
HF/6-311++G(2d,2p)	−44.34	−16.71	−27.63	21.05
B3LYP/6-31G(d)	−33.86	−13	−20.86	13.2
B3LYP/6-31+G(d,p)	−42.44	−16.56	−25.88	10.09
B3LYP/6-311++G(d,p)	−41.9	−16.02	−25.88	9.89
B3LYP/6-311++G(2d,2p)	−41.16	−15.17	−25.99	9.75

[a] $\Delta G^{\ddagger}$(exptl) = 24.66 kcal/mol. $k_r = 4.44 \times 10^{-6}$ s^{-1}. [b] Free energy of hydration for the transition state. [c] Free energy of hydration for the reactants. [d] Free energy of hydration of the transition state minus free energy of hydration of the reactants. [e] Free energy of activation calculated by the SCRF method.

TABLE 3: Calculated Free Energies of Hydration Using the Langevin Dipole Method[a]

method	$\Delta G_{hydr}^{LD}(TS)$[b] (kcal/mol)	$\Delta G_{hydr}^{LD}(R)$[c] (kcal/mol)	$\Delta G_{hydr}^{LD}(TS - R)$[d] (kcal/mol)	$\Delta G^{\ddagger}_{LD}$[e] (kcal/mol)
HF/6-31G(d)	−45.91	−22.16	−23.75	29
HF/6-31+G(d,p)	−48.25	−23.47	−24.78	24.34
HF/6-311++G(d,p)	−47.18	−22.7	−24.48	24.62
HF/6-311++G(2d,2p)	−45.95	−21.99	−23.96	24.72
B3LYP/6-31G(d)	−34.89	−19.87	−15.02	19.04
B3LYP/6-31+G(d,p)	−39.69	−21.2	−18.49	17.48
B3LYP/6-311++G(d,p)	−39.32	−20.85	−18.47	17.3
B3LYP/6-311++G(2d,2p)	−39.58	−20.15	−19.43	16.31

[a] $\Delta G^{\ddagger}$(exptl) = 24.66 kcal/mol. $k_r = 4.44 \times 10^{-6}$ s^{-1} [b] Free energy of hydration for the transition state. [c] Free energy of hydration for the reactants. [d] Free energy of hydration of the transition state minus free energy of hydration of the reactants. [e] Free energy of activation obtained by the Langevin dipole method.

TABLE 4: Calculated Free Energies of Hydration by the PM3-SM3 and AM1-SM1 Methods

method	$\Delta G_{hydr}(TS)$[a] (kcal/mol)	$\Delta G_{hydr}(R)$[b] (kcal/mol)	$\Delta G_{hydr}(TS - R)$[c] (kcal/mol)	$\Delta G^{\ddagger}$ [d] (kcal/mol)
PM3-SM3	−41.57	−25.3	−16.27	30.64
AM1-SM1	−33.12	−20.76	−12.36	40.01

[a] Free energy of hydration for the transition state. [b] Free energy of hydration for the reactants. [c] Free energy of hydration for the transition state minus free energy of hydration for the reactants. [d] Free energy of activation calculated by the PM3-SM3 and AM1-SM1 methods.

It is interesting to compare the activation energy calculated as described above with the activation energy obtained by scanning the CN distance and optimizing all other degrees of freedom. The "properly" calculated activation energy of 53.29 kcal/mol compares nicely with the activation energy of 53.30 kcal/mol obtained by scanning the CN distance with a series of constrained minimizations using the HF/6-31G(d) level. The result gives strong evidence that the reaction can be adequately described by varying the CN distance.

In Table 2 are collected the free energies of hydration calculated by the solvent reaction field method of Tomasi and co-workers. The transition state has a lower free energy of hydration than the reactants, and therefore, the solvent accelerates the reaction. Reduction of the barrier is between 26 and 29 kcal/mol if one omits the calculations performed by basis set 6-31G(d), which seems to be not fully converged in terms of the basis set size. The DFT-calculated reduction of the barrier in terms of the hydration free energies is smaller than the corresponding HF values. All in all, agreement with the experimental activation energy of 24.66 kcal/mol is poor. The DFT/SCRF-calculated activation energy is systematically too low at about 14 kcal/mol.

In Table 3 are collected the hydration free energies calculated with Langevin dipoles. Reduction of the barrier relative to the corresponding SCRF values is small but significant. The HF-calculated activation free energies together with more flexible basis sets in conjunction with the LD free energies of hydration are in almost perfect agreement with the experiment. The corresponding B3LYP barriers are too low.

The AM1-SM1 and PM3-SM3 results are shown in Table 4. The transition-state hydration free energies seem to be not favorable enough, and the result is too high an activation free energy.

The calculated energies and geometric parameters for the products are collected in Table 5. Due to the fact that the species are subject to further reactions, comparison with the experiment is not possible. We emphasize at this point that the so-obtained minimum corresponds to the reaction intermediate rather than the true product. Direct comparison with the experimental data is therefore not possible.

4. Discussion

In this work we studied a chemical reaction between the smallest ultimate carcinogen, ethylene oxide, and guanine. The structures of the transition state, reactants, and products, calculated on the B3LYP/6-311++G(2d,2p) level, are shown in Figure 1.

Ethylene oxide is biologically relevant per se and is also a model compound for numerous ultimate carcinogens. DNA was truncated to guanine, the chemically relevant part that enters the reaction. The reaction is a prototype for chemical modifications of the DNA induced by the ultimate carcinogens that originate from polyaromatic hydrocarbons.

To our best knowledge, this is the first quantum-chemical calculation of the activation energy for a chemical reaction between a part of DNA and an ultimate carcinogen of the epoxy type. Effects of solvation were calculated using the Langevin dipole and solvent reaction field methods.

We demonstrated that the Hartree−Fock-calculated barrier when combined with the Langevin dipole method for calculation of hydration free energies gives very good agreement with the experimental free energy of activation. On the other hand, DFT

TABLE 5

method	ΔE_r[a] (kcal/mol)	$\Delta G_{hydr}^{LD}(P)$[b] (kcal/mol)	$\Delta G_{hydr}^{SCRF}(P)$[c] (kcal/mol)	$\Delta G_r^{LD}(P)$[d] (kcal/mol)	$\Delta G_r^{SCRF}(P)$[e] (kcal/mol)	CN distance[f] (Å)
HF/6-31+G(d,p)	40.33	−55.94	−61.24	7.86	−2.46	1.51
HF/6-311++G(d,p)	39.85	−55.25	−60.08	7.3	−2.67	1.51
HF/6-311++G(2d,2p)	39.28	−53.76	−58.62	7.51	−2.63	1.51
B3LYP/6-311++G(2d,2p)	26.98	−38.22	−50.96	8.91	−8.81	1.46

[a] Energy of the products minus energy of the reactants. [b] Langevin-dipole-calculated free energy of hydration for the products. [c] Solvent-reaction-field-calculated free energy of hydration for the products. [d] Calculated reaction free energy by considering the Langevine dipole hydration free energy. [e] Calculated reaction free energy by considering the solvent reaction field hydration free energy. [f] Reaction coordinate values corresponding to the products.

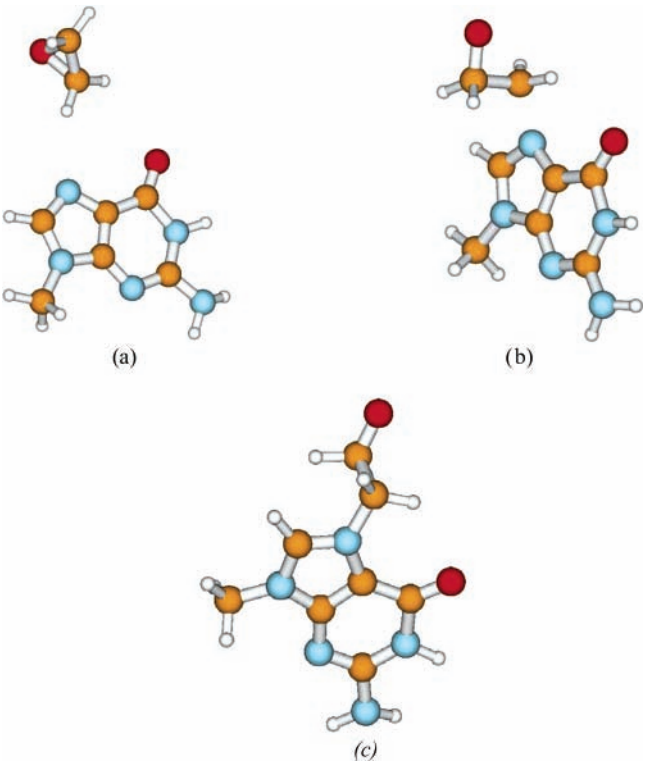

Figure 1. Structures of the reactants (a), the transition state (b), and the product (c), calculated on the B3LYP/6-311++G(2d,2p) level for alkylation of guanine with ethylene oxide.

calculations predict too low an activation free energy for the LD and SCRF solvation models. Disagreement between the experimental and calculated activation free energies can also be explained by considering only part of the DNA (guanine) and not treating water and counterions in atomic detail.

Carcinogenesis is a complex biomolecular process involving many chemical reactions.[1] It is therefore a major challenge to understand and model those reactions. We applied some of the available methods, and we are aware that there is still room to apply QM/MM methodology with all-atom representation of the polar environment and application of thermal averaging.[24−30] We have the impression that the B3LYP functional systematically underestimates the reaction barrier. A possible explanation is that there were no epoxy species in the parametrization set. We did not manage to perform the MP2 calculations for this system, and this is obviously associated with an error in the Gaussian 03 code. Calculation of the activation energy for this system beyond the HF level remains a challenge. In addition, it would be a challenge to test novel DFT functionals[32] or to reparametrize the semiempirical methods as reported by Truhlar and co-workers.[33] Despite the fact that there are no experimental data available for hydration free energies for the species being studied, we feel that the LD method outperforms the SCRF method in calculation of hydration energies. In contrast to the SCRF method, the LD method involves to a certain extent specific interactions between the solute and solvent and thermal averaging.

All in all, we found very good agreement between the experimental and calculated free energy of activation for alkylation of guanine by ethylene oxide by a combination of Hartree−Fock calculation using flexible basis sets and Langevin dipole calculation of hydration free energies. We are sure that a calculation of this type will give an important contribution toward the understanding, prevention, and treatment of cancer.[1,31]

Acknowledgment. We thank Drs. Arieh Warshel, University of Southern California, and Danijel Kikelj, Faculty of Pharmacy, University of Ljubljana, for many stimulating discussions and Dr. Sonja Braun-Sand, University of Southern California, for critical reading of the manuscript. J.M. thanks J. William Fulbright Scholarship Board for the award of a research scholarship and Dr. Arieh Warshel for his hospitality during the stay in his laboratory.

References and Notes

(1) Underwood, J. C. E., Ed. *General and Systematic Pathology*, 3rd ed.; Churchill Livingstone: Edinburgh, U.K., 2000.
(2) Brookes, P.; Lawley, P. D. *Nature* **1964**, *202*, 781.
(3) Volk, D. E.; Rice, J. S.; Luxon, B. A.; Yeh, H. J. C.; Liang, C.; Xie, G.; Sayer, J. M.; Jerina, D. M.; Gorenstein, D. G. *Biochemistry* **2000**, *39*, 14040.
(4) Smela, M. E.; Hamm, M. L.; Henderson, P. T.; Harris, C. M.; Harris, T. M.; Essigmann, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6655.
(5) Stack, D. E.; Byun, J.; Gross, M. L.; Rogan, E. G.; Cavalieri, E. L. *Chem. Res. Toxicol.* **1996**, *9*, 851.
(6) Huetz, P.; Kamarulzaman, E. E.; Wahab, H. A.; Mavri, J. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 310.
(7) Kolman, A.; Chovanec, M.; Osterman Golkar, S. *Mutat. Res./Rev. Mutat. Res.* **2002**, *512*, 173.
(8) Their, R.; Bolt, H. M. *Crit. Rev. Toxicol.* **2000**, *30*, 595.
(9) Farmer, P. B.; Shuker, D. E. G. *Mutat. Res./Fundam. Mol. Mech. Mutagen.* **1999**, *424*, 275.
(10) Murray, J. S.; Politzer, P. The Use of the Molecular Electrostatic Potential in Medicinal Chemistry. In *Quantum Medicinal Chemistry*; Carloni, P., Alber, F., Eds.; John Wiley: Weinheim, Germany, 2003.
(11) Politzer, P.; Laurence, P. R. *Carcinogenesis* **1984**, *5*, 845.
(12) Politzer, P. *Toxicol. Lett.* **1988**, *43*, 257.
(13) *IARC Monogr. Eval. Carcinog. Risk Chem. Humans* **1994**, *60*, 73 (http://www.iarc.fr/).
(14) Blackburn, G. M.; Gait, M. J. *Nucleic Acids in Chemistry and Biology*, 2nd ed.; Oxford University Press: Oxford, 1997.
(15) Borosky, G. L. *J. Org. Chem.* **1999**, *64*, 7738. Hulbert, P. B. *Nature* **1975**, *256*, 146. Yang, S. K.; McCourt, D. W.; Gelboin H. V. *J. Am. Chem. Soc.* **1977**, *99*, 5130. Bruice, T. C.; Bruice P. Y. *Acc. Chem. Res.* **1976**, *9*, 378.
(16) Lensink, M. F.; Mavri, J.; Berendsen H. J. C. *J. Comput. Chem.* **1999**, *20*, 886.
(17) Pauwels, W.; Veulemans, H. *Mutat. Res.* **1998**, *418*, 21.

(18) Miertus, S.; Scrocco, E.; Tomasi, J. *Chem. Phys.* **1981**, *55*, 117.
(19) Flórian, J.; Warshel, A. *J. Phys. Chem.* **1997**, *101*, 5583.
(20) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
(21) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
(22) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; 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.; Hona, Y.; Kitao, K.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; 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.; 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. *Gaussian 03*, revision B.04; Gaussian, Inc.: Pittsburgh, PA, 2003.
(23) Garcia-Viloca, M.; Alhambra, C.; Truhlar, D. G.; Gao, J. *J. Comput. Chem.* **2003**, *24*, 177.
(24) Warshel, A. *Computer Modeling of Chemical Reactions in Enzymes and Solutions*; John Wiley & Sons: New York, 1991.
(25) Vayner, G.; Houk, K. N.; Jorgensen, W. L.; Brauman, J. I. *J. Am. Chem. Soc.* **2004**, *126*, 9054.
(26) Carloni, P.; Alber, F.; Eds. *Quantum Medicinal Chemistry*; John Wiley: Weinheim, Germany, 2003.
(27) Spiegel, K.; Rothlisberger, U.; Carloni, P. *J. Phys. Chem. B* **2004**, *108*, 2699.
(28) Warshel, A.; Strajbl, M.; Villa, J.; Florian, J. *Biochemistry* **2000**, *39*, 14728.
(29) (a) Florian, J.; Goodman, M. F.; Warshel, A. *J. Phys. Chem. B* **2000**, *104*, 10092. (b) Bren, U.; Hodoscek, M.; Koller, J. *J. Chem. Inf. Model.* **2005**, *45*, 1546.
(30) Braun-Sand, S.; Burykin, A.; Chu, Z. T.; Warshel, A. *J. Phys. Chem. B* **2005,** 109, 583.
(31) Huetz, P.; Mavaddat, N.; Mavri, J. *J. Chem. Inf. Model.* **2005**, *45*, 1564.
(32) Zhao, Y.; Truhlar, D. G. *J. Phys. Chem. A* **2005**, *109*, 6624.
(33) Pu, J.; Ma, S.; Gao, J.; Truhlar, D. G. *J. Phys. Chem. B* **2005**, *109*, 8551.
(34) AMSOL version 5.4.1: G. D. Hawkins, G. C. Lynch, D. J. Giesen, I. Rossi, J. W. Storer, D. A. Liotard, C. J. Cramer, and D. G. Truhlar, University of Minnesota, Minneapolis.