JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

The Mechanism of Guanine Alkylation by Nitrogen Mustards: A Computational Study

Abhigna Polavarapu,[†] Jacob A. Stillabower,[‡] Skyler G. W. Stubblefield,[‡] William M. Taylor,[‡] and Mu-Hyun Baik*[,†,§]

[†]Department of Chemistry, Indiana University, 800 E. Kirkwood Ave., Bloomington, Indiana 47405, United States

[‡]Southwestern Junior/Senior High School, 3406 W 600 S. Shelbyville Indiana 46176, United States

[§]Department of Chemistry, Korea University, 208 Seochang, Chochiwon, Chung-nam 339-700, South Korea

Supporting Information

ABSTRACT: The thermodynamics and kinetics for the monofunctional binding of nitrogen mustard class of anticancer drugs to purine bases of DNA were studied computationally using guanine and adenine as model substrates. Mechlorethamine and melphalan are used as model systems in order to better understand the difference in antitumor activity of aliphatic and aromatic mustards, respectively. In good agreement with experiments that suggested the accumulation of a reactive intermediate in the case of mechlorethamine, our model predicts a significant preference for the formation of corresponding aziridinium ion for mechlorethamine, while the formation of the aziridinium ion is not computed to be preferred when melphalan is used. Two effects are found that contribute to this difference. First, the ground state of the drug shows a highly delocalized lone pair on the amine nitrogen of the melphalan, which makes the subsequent cyclization more difficult. Second, because of the aromatic substituent connected to the amine nitrogen of melphalan, a large energy penalty has to be paid for solvation. A detailed study of energy profiles for the two-step mechanism for alkylation of guanine and adenine was performed. Alkylation of guanine is ∼6 kcal mol^{-1} preferred over adenine, and the factors contributing to this preference were explained in our previous study of cisplatin binding to purine bases. A detailed analysis of energy profiles of mechlorethamine and melphalan binding to guanine and adenine are presented to provide an insight into rate limiting step and the difference in reactivity and stability of the intermediate in both nitrogen mustards, respectively.

INTRODUCTION

Nitrogen mustards form a class of versatile anticancer drugs used in the treatment of various cancers including Hodgkin's disease, non-Hodgkin's lymphoma, chronic leukemia, and lung, ovarian, and breast cancer.[1−6] Mechlorethamine, *N*-methylbis-(2-chloroethyl)amine (**1**), is the simplest representative of the nitrogen mustard drug family and was the first effective clinical anticancer drug that is still in use today.[7−9] The mode of action of these drugs is relatively well understood with the main cellular target being genomic DNA.[10−15] Nitrogen mustards act as nonspecific DNA alkylating agents with the N7-site of guanine being the preferred site of initial attack.[11,13,16−23] Two subsequent electrophilic attacks on two guanine moieties can afford bifunctional adducts with both inter- and intrastrand cross-links[24,25] in DNA that are reminiscent of the binding mode of cisplatin,[26,27] another potent and widely used anticancer drug.[11,28−41] These cross-links and the resulting distortions of the double helix of DNA are believed to be responsible for the cytotoxic action, as they are expected to block or at least interfere with the passage of polymerases, thereby inhibiting DNA transcription, which ultimately leads to cell apoptosis.[42−53] Mechlorethamine, chlorambucil,[54−59] and melphalan[60−64] (Figure 1) are some of the nitrogen mustards

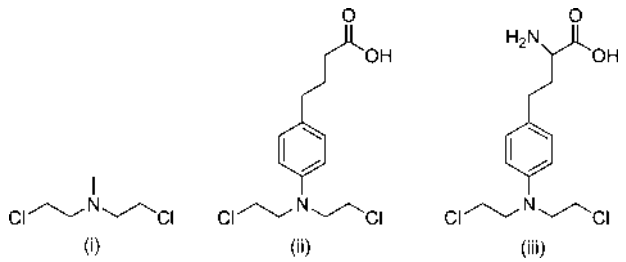


Figure 1. (i) Mechlorethamine, (ii) chlorambucil, and (iii) melphalan.

that are in clinical use. Antitumor activity and cytotoxicity require the presence of two chloroethyl groups per nitrogen mustard molecule. Previous work established that most nitrogen mustards alkylate at the N7 position of guanine.[30,65,66]

Despite significant efforts toward devising second and third generation drugs that exploit the DNA-binding strategy while minimizing side effects,[67−69] such as nephro- and neuro-toxicity[4,70,71] and fighting resistance,[4,70,72−82] our current ability of systematically improving these drug candidates remains

Received: February 17, 2012
Published: June 8, 2012

limited.[83−85] A promising future direction is to base our design efforts on a deeper mechanistic understanding accompanied by a quantitative assessment of the intrinsic reactivity patterns using high-level quantum chemical models.[83,86−89] We have recently demonstrated that significant mechanistic insight can be derived from these models.[90−93] Several mechanistic issues have been discussed in the past that deserve a closer examination.[30,44,52,58,94−99] For example, the difference in anticancer activity of aliphatic and aromatic mustards was suggested to result from differences in the rate of DNA-alkylation, but there is no satisfying explanation of why the rates are so different.[96,100−103] Similarly, it is unclear what controls the activation process and whether or not the aliphatic and aromatic mustards display different reactivity patterns at this early stage of the reaction.[99,101] As the structural characterization of the mustard adducts with DNA has been notoriously difficult because of their instability in solution, computational studies provide a unique alternative for structure assessment while allowing for comparing the energetic consequences of decorating the periphery of the drug molecule.[31,58,104−106] We modeled both the initial activation of mustards involving the aziridinium ion intermediate and the DNA-binding event using guanine and adenine as primary targets.[58,59,107−112] The nature of the rate limiting step of the overall reaction is currently unknown, and it is unclear whether or not meaningful reactivity differences between the aromatic and aliphatic mustards exist. Below, we present common mechanistic features and highlight distinctive differences between these two classes of nitrogen mustards.

COMPUTATIONAL DETAILS

All calculations were carried out using density functional theory as implemented in the Jaguar 7 suite of ab initio quantum chemistry programs.[113] Geometries were optimized by using the B3LYP functional[114−117] with the 6-31G** basis set. The energies were reevaluated by additional single point calculations at each optimized geometry using Dunning's correlation consistent triple-ζ basis set cc−pVTZ(−f)[118] with the standard double set of polarization functions. Vibrational frequency calculations based on analytical second derivatives at the B3LYP/6-31G** level of theory were used to confirm proper convergence to local minima and saddle points for equilibrium and transition state geometries, respectively. Unscaled vibrational frequencies were used to derive the zero-point energy (ZPE) and vibrational entropy corrections at room temperature. Solvation energies were evaluated by a self-consistent reaction field (SCRF) approach based on accurate numerical solutions of the Poisson−Boltzmann equations.[119] These solvation calculations were carried out at the gas phase geometry using the 6-31G** basis and employing a dielectric constant of ε = 80.37 for water. Absolute solvation energies remain challenging to estimate accurately, and we cannot expect simplistic continuum models to deliver highly accurate solvation energies of complex molecular assemblies. However, we expect solvation energy difference to be more meaningful because of significant error cancellation when the same empirical parameters are used. As utilized in many of our previous, unrelated works,[120−123] the energy components have been computed with the following protocol:

$$G(\text{sol}) = G(\text{gas}) + \Delta G(\text{solv}) \quad (1)$$

$$G(\text{gas}) = H(\text{gas}) - TS(\text{gas}) \quad (2)$$

$$H(\text{gas}) = E(\text{SCF}) + \text{ZPE} \quad (3)$$

$$\Delta E(\text{SCF}) = \sum E(\text{SCF}) \text{ for products} - \sum E(\text{SCF}) \text{ for reactants} \quad (4)$$

$$\Delta G(\text{sol}) = \sum G(\text{sol}) \text{ for products} - \sum G(\text{sol}) \text{ for reactants} \quad (5)$$

G(gas) is the free energy in gas phase; ΔG(solv) is the free energy of solvation as computed using the continuum solvation model. For the chloride ion, the free energy of solvation is obtained from experiments (−74.7 kcal mol^{-1});[124] H(gas) is the enthalpy in gas phase; T is the temperature (298.15 K); S(gas) is the entropy in gas phase; E(SCF) is the self-consistent field energy, i.e. "raw" electronic energy as computed from the SCF procedure; and ZPE is the zero-point energy. Note that by entropy here we refer specifically to the vibrational/rotational/translational entropy of the solute(s); the entropy of the solvent is incorporated implicitly in the continuum solvation model. The entropy of chloride ion that is released during the formation of aziridinium ion intermediate was calculated using the Sackur−Tetrode equation. To locate transition states, the potential energy surface was first explored approximately using the linear synchronous transit (LST) method, followed by a quadratic synchronous transit (QST)[125] search using the LST geometry as an initial guess. For computing the pK_a values of the conjugated acids of nitrogen mustards, it is essential to include the free energy of proton in solution. The following formula is used to compute the free energy of a proton in solution, $G(H^+)$:

$$G(H^+) = H_{gas}(H^+) - TS + 5/2RT + G_{solv}(H^+) \quad (6)$$

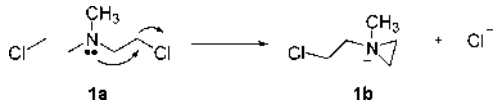
where $H_{gas}(H^+)$, the enthalpy in gas phase is zero, R is the universal gas constant, T is 298.15 K, S is the translational entropy of a free hydrogen atom (7.76 kcal mol^{-1}) calculated using the Sackur−Tetrode equation, and $G_{solv}(H^+)$ is the free energy of solvation (−265.9 kcal mol^{-1} in water).[126] At room temperature (298.15 K), free energy in solution phase $G(H^+)$ is calculated to be −272.11 kcal mol^{-1} in water. pK_a is calculated using the reaction isotherm:

$$\begin{aligned}\Delta G(\text{sol}) &= -RT \ln K_a \\ &= (2.303)RT\text{p}K_a \\ &= 1.36\text{p}K_a \text{ (at 298.15 K)}\end{aligned} \quad (7)$$

RESULTS AND DISCUSSION

The mechanism of drug activation involves the elimination of a chloride anion to afford an aziridinium cation in the first step,[127−130] illustrated in Scheme 1 using mechlorethamine

Scheme 1



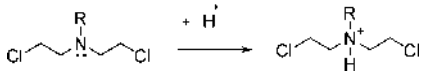
1a 1b

(**1a**) as an example. As shown in Figure 1, the nitrogen atom serves as an important functionalization site that differentiates the various drug molecules from each other, where the bis(2-chloroethyl)amine fragment constitutes the common structural pattern.[128,131] As the charge distribution profiles of the parent molecule and the activated aziridinium ion are significantly different, we expect the energetics of this step to be highly dependent on the inductive properties of the N-substituents. In general, electron-donating groups should increase the rate of activation, as additional electron density is pushed into the aziridinium moiety, likely leading to a stabilization of the product and increasing the driving force of the reaction. Conversely, electron-withdrawing groups should make activation more difficult, if not impossible, by distorting the relative energy in favor of the reactant state. Of particular interest is the addition of a proton to the N-site, as protonation of the

dx.doi.org/10.1021/jo300351g | J. Org. Chem. 2012, 77, 5914−5921

nitrogen should make the activation of nitrogen mustards impossible (Scheme 2).

Scheme 2



To quantify the influence of the *N*-substituents (R) on the basicity of the nitrogen mustards, we calculated the pK_a values of the respective conjugate acids, and they are summarized in Table 1. All aromatic substituents give rise to highly negative

Table 1. Computed pK_a Values of the Conjugate Acids of Various Nitrogen Mustards

substituents R	pK_a
aliphatic R	
$-CH_2OH$	1.2
$-CH_2CH_3$	2.9
$-CH_3$	4.9
$-CH_2NH_2$	6.3
aromatic R	
$-C_6H_4COOH$	−9.1
$-C_6H_4CH_3$	−0.1
$-C_6H_5$	−2.1

pK_a values ranging from 0 to −9, indicating that the protonation equilibrium lies far on the deprotonated side, whereas the aliphatic substituents shift the equilibrium to the protonated side with calculated pK_a values ranging from 1 to 6. Thus, the nitrogen center is much more basic when it carries an aliphatic substituent. This trend is intuitively understandable, if we consider that the nitrogen lone-pair will engage in a π-delocalization into the aromatic ring forcing the nitrogen center to adopt a sp^2 hybridization, whereas such interaction will be absent when an aliphatic group is attached to the nitrogen atom. As a consequence, the nitrogen lone-pair is not as available to act as a proton acceptor when the nitrogen atom carries an aromatic substituent. This intuitive rationale is supported by the trigonal-planar geometry, characteristic of the sp^2 hybridization, obtained for the nitrogen mustards carrying aromatic N-substituents (see Figure 3). The basicity of the nitrogen atom assessed in this intuitive manner has a profound impact on the drug activation profile, as we can conclude that the nitrogen in the aromatic mustards is much less nucleophilic than the aliphatic mustards. Therefore, we expect that the aliphatic mustards will engage much more readily in the chloride elimination reaction, outlined in Scheme 1. In other words, the aliphatic mustards should form the active form of the drug much more readily. In previous experimental work, it was recognized that aliphatic mustards disappeared more rapidly from the solution than aromatic analogues,[100] which may be interpreted as a reflection of the above-mentioned reactivity difference. The orbitals responsible for the π-delocalization in the phenyl mustard **2a** and its analogous molecular orbital in the aliphatic analogue **1a** are shown in Figure 2.

Drug Activation. To better understand the mechanism of drug activation and investigate the reaction pathway explicitly, we carried out a series of calculations using mechlorethamine (**1a**) and melphalan (**3a**) as reactants that serve as representatives of aliphatic and aromatic mustards, respectively.

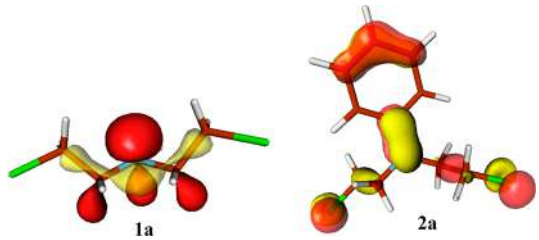


Figure 2. HOMO of (**1a**) mechlorethamine and HOMO-2 of (**2a**) phenyl mustard.

As a convenient reference point, we also studied the simple *N*-phenyl substituted mustard **2a**. The structures of the nitrogen mustards and their respective aziridinium ions are shown in Figure 3, and their energies are listed in Table 2. The π-delocalization present in the aromatic mustards, as discussed above, should also lead to higher activation barrier for the aziridinium forming reaction, and Figure 4 compares the computed reaction energy profiles of **1a**, **2a**, and **3a**. The transition state energy differences between the aromatic and aliphatic mustards are much greater than anticipated: mechlorethamine **1a** forms the associated aziridinium ion **1b** traversing the transition state **1a-TS** at 9.3 kcal mol^{-1}, whereas the same reaction requires an activation barrier of 22.5 kcal mol^{-1} in the case of melphalan (**3a**). The transition state associated with the model compound **2a** is found at 23.0 kcal mol^{-1}, indicating that the aminocarboxylic acid moiety in **3a** has a negligible impact on the reaction profile. The transition state structures shown in Figure 3 characterize the chloride displacement as 1,2-shift from the β to α carbon.

Whereas this reaction energy profile is plausible, there is an alternative imminum intermediate that deserves attention. After the formation of the cyclic intermediate **1b,** a rearrangement can be envisioned to occur involving an opening of the three-membered ring concomitant to a 1,2-hydride shift to afford the imminium ion intermediate **1b′**, as shown in Scheme 3. Interestingly, this reaction is −23.53 kcal mol^{-1} downhill thermodynamically, raising serious questions about the relevance of the proposed mechanism, as **1b′** should be the dominating intermediate in a competitive equilibrium situation with **1b**. Figure 5 shows the reaction energy profile for this equilibrium reaction and reveals that the transition state that connects the two imminium intermediates is extremely high in energy and intermediate **1b** is kinetically protected by a reaction barrier of nearly 46 kcal mol^{-1}, rendering this thermodynamically viable rearrangement practically impossible under standard reaction conditions. Hence, we conclude that the aziridinium ion **1b** is the only relevant reactive intermediate in the DNA adduct formation by nitrogen mustards.

Binding to G and A. Once activated, the aziridinium ion attacks the N7-position of purine bases in an electrophilic fashion to alkylate the purine, as illustrated in Scheme 4. Among the possible attack sites, only the N7 position is biologically relevant, as the other basic nitrogen sites in the nucleobase are structurally not accessible to an incoming alkylating agent.[132] Figure 6 shows the optimized geometries of the mechlorethamine adducts with guanine (G) and adenine (A). Whereas we only found one low energy adduct structure with G labeled $\mathbf{1c_G}$, there are two distinctively different nearly isoenergetic adducts $\mathbf{1c_A}$ and $\mathbf{1c_{A'}}$ with A. In $\mathbf{1c_{A'}}$ a strong hydrogen bond is formed between the C6-NH_2 moiety of adenine and the nitrogen atom of the mustard, as illustrated in

dx.doi.org/10.1021/jo300351g | J. Org. Chem. 2012, 77, 5914−5921

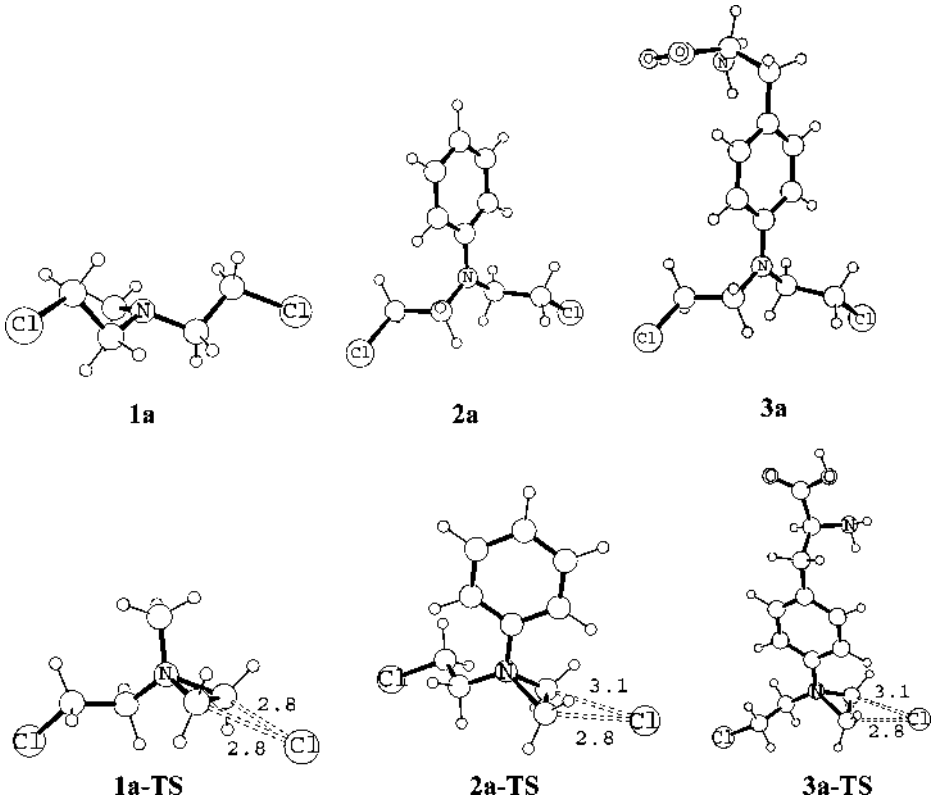


Figure 3. Optimized nitrogen mustard reactants and transition states. Selected atomic distances are given in Å.

Table 2. Computed Energy Components in kcal mol^{-1} for the Aziridinium Ion Forming Reaction, as shown in Scheme 1

	ΔH(gas)	ΔG(gas)	ΔG(solv)	ΔG(sol)
1b	134.22	127.79	−130.31	−2.52
2b	135.56	128.77	−121.75	7.02
3b	136.09	129.23	−122.39	6.84
1a-TS	44.11	43.67	−34.41	9.26
2a-TS	50.82	50.59	−27.59	23.00
3a-TS	54.33	51.86	−29.33	22.53

Figure 6, whereas the mustard fragment is extended in **1c$_A$**. Table 3 summarizes the energies of the three adducts. Interestingly, our calculations predict no thermodynamic driving force for **1b** binding to adenine, with relative free energies of **1c$_A$** and **1c$_{A'}$** being essentially zero, whereas the binding energy to guanine is approximately −6 kcal mol^{-1}. This intrinsic preference for binding of G over A is in good agreement with previous experimental observations[132,133] and is well documented in other purine binding agents. In our previous work,[91] we found that cisplatin prefers G over A by ∼5 kcal mol^{-1}, as the N7 position of G is notably more nucleophilic than that of A. It is interesting to note that the absolute value of the binding energy is significantly smaller for mechlorethamine than what we have found for cisplatin, as we had previously found monofunctional binding energies of −11.5 and −16.0 kcal mol^{-1} for the cisplatin-A and cisplatin-G adducts, respectively.[91]

The energetics of the phenyl mustard and melphalan reaction with guanine and adenine are enumerated in Table 3. With the aromatic mustards, the transition state leading to the guanine adduct is ∼7 kcal mol^{-1} lower in energy than that giving rise to the adenine adduct, which is very comparable to what was observed for mechlorethamine, as discussed above. We observed similar profiles for cisplatin binding to guanine and

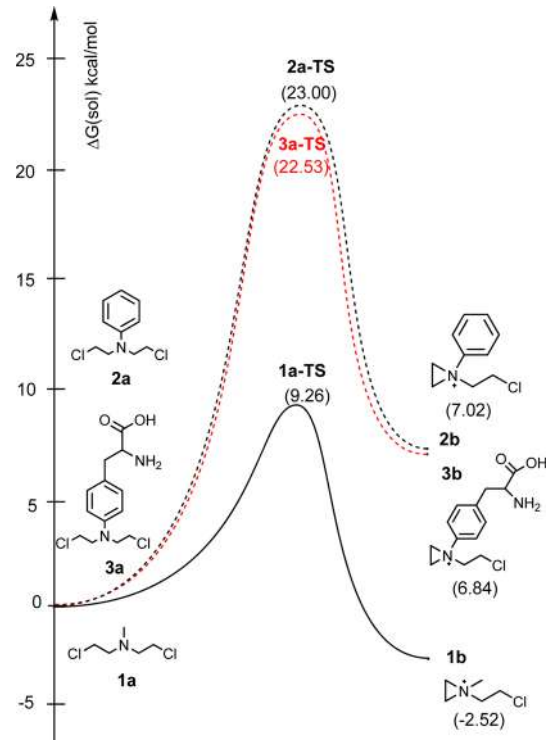
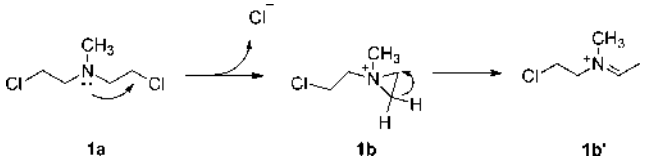


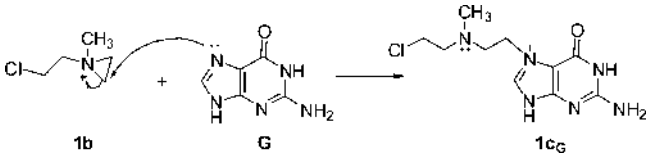
Figure 4. Computed reaction profile for the formation of aziridinium ion from mechlorethamine (**1a**), phenyl mustard (**2a**), and melphalan (**3a**).

dx.doi.org/10.1021/jo300351g | J. Org. Chem. 2012, 77, 5914−5921

Scheme 3



Scheme 4



adenine previously,[91] thus further supporting the notion that *N*-mustard and cisplatin binding to DNA are comparable processes mechanistically. Figures 7, 8, and 9 show the energy profiles for the reaction of mechlorethamine, phenyl mustard, and melphalan, respectively. It is evident from these profiles that the formation of aziridinium ion is thermodynamically downhill for aliphatic mustards, whereas in the case of aromatic mustards, the corresponding aziridinium ions are higher in energy than the reactants. This consequence of the electronic demand of the aliphatic vs aromatic functional groups leads to a slightly different chemical behavior, in that we expect that the aziridinium intermediate should accumulate in a rapid pre-equilibrium type of scenario and should be experimentally detectable, whereas the aromatic mustards will not exhibit such an equilibrium, and the aziridinium intermediate should be truly a transient intermediate with a short lifetime at best. This result is in good agreement with prior experiments,[100] which suggested that aziridinium ion intermediate formation was much faster for mechlorethamine than for L-sarcolysin (melphalan) and accumulation of the reactive intermediate aziridinium ion was indeed observed for mechlorethamine but not for melphalan and other aromatic mustards. To test the aforementioned role of the π-delocalization, we repeated the calculations on the phenyl *N*-mustard with a constraint that the phenyl ring must maintain a dihedral angle of 90° to the N-lone pair, thus effectively destroying the π-delocalization. The electronic energies of this putative system **2a*-TS** are shown in Table 4. As the geometrical constraint renders all of the intermediates not true minima on the potential energy surface, we cannot reliably compute the entropy corrections for these species and only the electronic energies are meaningful. The electronic component of the cyclization barrier of this putative system **2a*-TS** is 43.15 kcal mol^{-1}, which is very similar to that of mechlorethamine. Because of the loss of π-delocalization in **2a***, the transition state **2a*-TS** has acquired an electronic stabilization of ~7 kcal mol^{-1}. Therefore, when there is no π-delocalization, the aromatic phenyl mustard behaves similarly to mechlorethamine. In a recent computational study,[134] it was suggested that the internal cyclization step may be rate limiting for both aliphatic and aromatic mustards, which is not consistent with the above-mentioned experimental observation and also disagrees with our computational results.

CONCLUSIONS

Our detailed study of how nitrogen mustards are activated and how they interact with purine bases confirmed that aliphatic *N*-mustards are easier to activate than their aromatic analogues. This finding is in good agreement with the experimental observation that mechlorethamine activates faster compared to aromatic mustards like melphalan. The formation of aziridinium ion in the case of mechlorethamine is both thermodynamically and kinetically more favorable compared to aromatic mustards. The reason for this preference lies in the greater basicity of the nitrogen in the aliphatic *N*-mustards. The lone pair in the

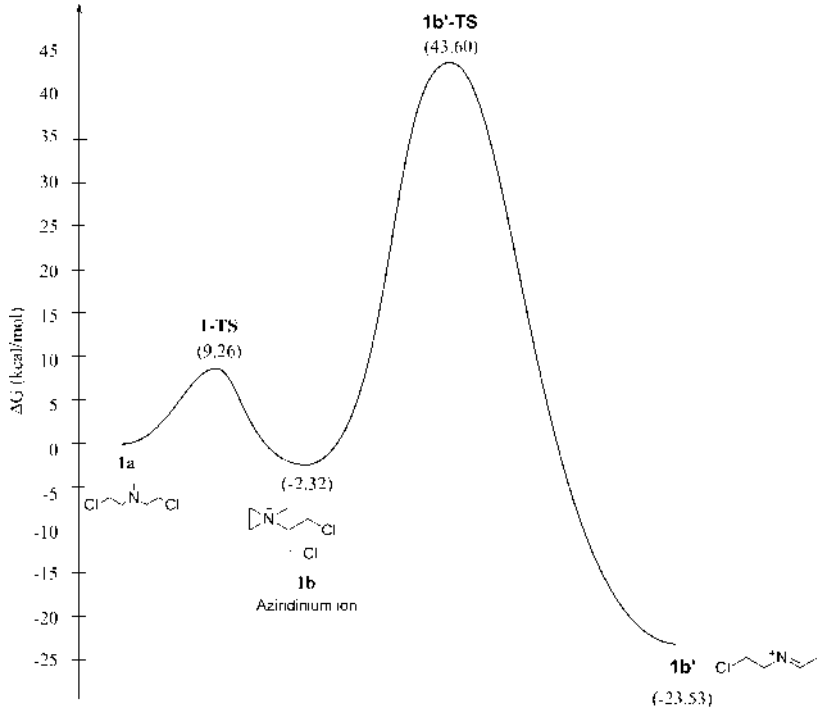


Figure 5. Reaction profile for the formation of imminium ion intermediate **1b′**.

dx.doi.org/10.1021/jo300351g | J. Org. Chem. 2012, 77, 5914−5921

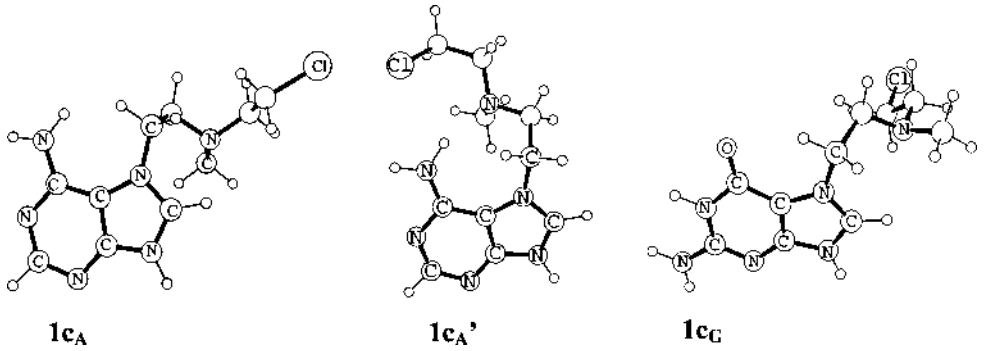


Figure 6. Optimized structures of mechlorethamine adducts with adenine and guanine.

Table 3. Computed Energy Components for the Formation of Guanine and Adenine Adducts

	ΔH(gas)	ΔG(gas)	ΔG(solv)	ΔG(sol)
Mechlorethamine adducts				
1c$_A$	111.23	115.46	−118.08	−2.62
1c$_{A'}$	106.06	112.36	−114.03	−1.67
1c$_G$	94.04	101.33	−109.80	−8.47
1b-TS$_A$	135.40	140.96	−113.37	27.59
1b-TS$_G$	121.66	126.08	−104.84	21.24
Phenyl mustard adducts				
2c$_A$	112.61	116.41	−118.90	−2.49
2c$_G$	99.89	104.52	−114.41	−9.89
2b-TS$_A$	144.00	147.68	−112.35	35.33
2b-TS$_G$	125.45	129.34	−101.60	27.74
Melphalan adducts				
3c$_A$	144.50	122.85	−121.56	1.29
3c$_G$	101.47	107.97	−113.14	−5.17
3b-TS$_A$	145.24	150.24	−117.23	33.01
3b-TS$_G$	126.64	134.78	−105.90	28.88

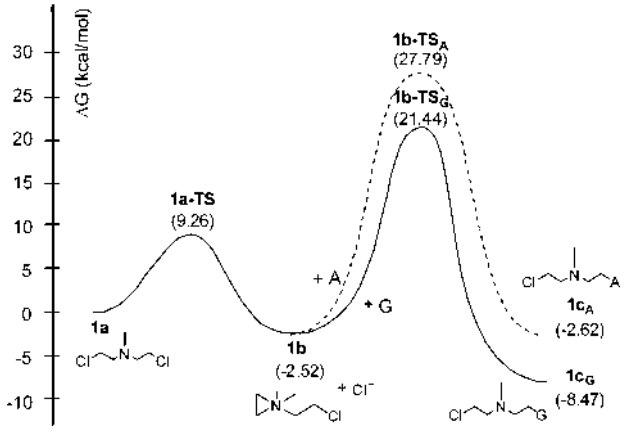


Figure 7. Reaction profile for the addition of mechlorethamine to A and G.

aromatic mustards is delocalized to a greater extent because of conjugation with the π orbitals in the phenyl ring and is not as available for stabilizing the positive charge of the aziridinium ion. In mechlorethamine no such delocalization is possible, and the nitrogen lone pair orbital is fully available to participate in the attack by an electrophile. As a result, mechlorethamine is easily activated.

The energy profiles of the adduct formation with DNA bases show many common features with those of cisplatin reacting with purine bases. Both the transition state and product

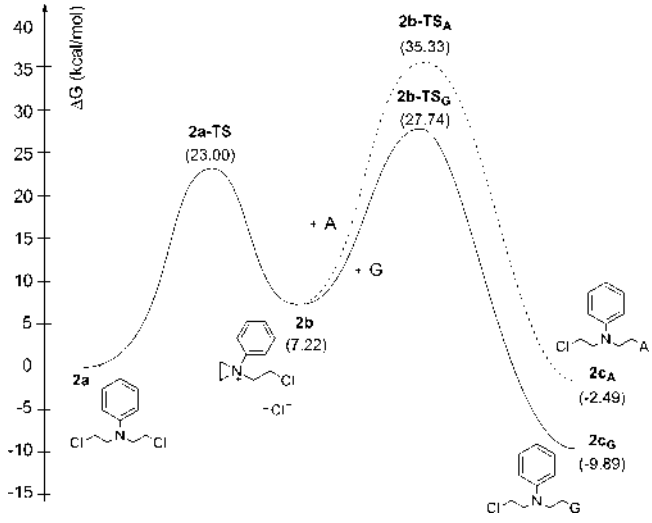


Figure 8. Computed reaction profile for addition of phenyl mustard to A and G.

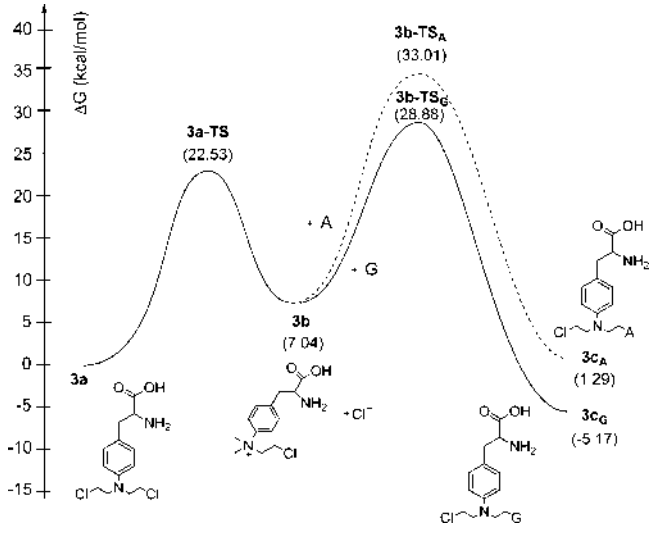


Figure 9. Computed reaction profile for the addition of melphalan to A and G.

structures are also comparable to those located for cisplatin binding to purine bases. The alkylation of guanine and adenine by mechlorethamine has a barrier difference of ~6 kcal mol^{-1}, and the thermodynamic preference is also around 6 kcal mol^{-1}. In the case of phenyl mustard, the thermodynamic and kinetic preference of guanine over adenine is ~7 kcal mol^{-1}. These

dx.doi.org/10.1021/jo300351g | J. Org. Chem. 2012, 77, 5914−5921

Table 4. Computed Cyclization Barrier Energy Components in kcal mol^{-1} for the Aziridinium Ion Forming Reaction

transition states	ΔH(gas)
1a-TS	44.11
2a-TS	50.82
2a*-TS	43.15
3a-TS	54.33

values are again very comparable to those found in the reaction of cisplatin with guanine and adenine.

■ ASSOCIATED CONTENT

Supporting Information

Cartesian coordinates of all complexes and computed energy components. This material is available free of charge via the Internet at http://pubs.acs.org

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: mbaik@indiana.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the NSF (CHE-0645381, CHE-1001589, and 0116050) for financial support. M.-H.B. thanks the Research Corporation for the Cottrell Scholarship and the Scialog Award. M.-H.B. thanks Korea University for a visiting WCU-Professor appointment.

■ REFERENCES

(1) Fisher, B.; Sherman, B.; Rockette, H.; Redmond, C.; Margolese, R.; Fisher, E. R. *Cancer* **1979**, *44*, 847.
(2) Jacobson, L.; Wachowski, T. J. *Bull. North Shore Branch Chicago Med. Soc.* **1947**, 3.
(3) Ben-Asher, S. *Am. J. Med. Sci.* **1949**, *217*, 162.
(4) Bramson, J.; McQuillan, A.; Aubin, R.; Alaouijamali, M.; Batist, G.; Christodoulopoulos, G.; Panasci, L. C. *Mutat. Res.* **1995**, *336*, 269.
(5) Frick, H. C.; Tretter, P.; Tretter, W.; Hyman, G. A. *Cancer* **1968**, *21*, 508.
(6) Weisberger, A. S.; Levine, B.; Storaasli, J. P. *JAMA, J. Am. Med. Assoc.* **1955**, *159*, 1704.
(7) Goodman, L. S.; Wintrobe, M. M. *JAMA, J. Am. Med. Assoc.* **1946**, *132*, 126.
(8) Jacobson, L. O.; Spurr, C. L. *J. Clin. Invest.* **1946**, *25*, 909.
(9) Gilman, A.; Philips, F. S. *Science* **1946**, *103*, 409.
(10) Goldacre, R. J.; Loveless, A.; Ross, W. C. J. *Nature* **1949**, *163*, 667.
(11) Osborne, M. R.; Wilman, D. E. V.; Lawley, P. D. *Chem. Res. Toxicol.* **1995**, *8*, 316.
(12) Warwick, G. P. *Cancer Res.* **1963**, *23*, 1315.
(13) Brookes, P.; Lawley, P. D. *Biochem. J.* **1961**, *80*, 496.
(14) Hemminki, K.; Kallama, S. *Proc. Am. Assoc. Cancer Res.* **1986**, *27*, 83.
(15) Lawley, P. D.; Phillips, D. H. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **1996**, *355*, 13.
(16) Millard, J.; Raucher, S.; Hopkins, P. *J. Am. Chem. Soc.* **1990**, *112*, 2459.
(17) Rink, S.; Hopkins, P. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2845.
(18) Hamza, A.; Broch, H.; Vasilescu, D. *J. Biomol. Struct. Dyn.* **1996**, *13*, 915.
(19) Rink, S.; Solomon, M.; Taylor, M.; Rajur, S.; Mclaughlin, L.; Hopkins, P. *J. Am. Chem. Soc.* **1993**, *115*, 2551.
(20) Mattes, W. B.; Hartley, J. A.; Kohn, K. W. *Nucleic Acids Res.* **1986**, *14*, 2971.
(21) Brookes, P.; Lawley, P. D. *J. Chem. Soc.* **1961**, 3923.
(22) Brookes, P.; Lawley, P. D. *Biochem. J.* **1961**, *78*, P02.
(23) Lawley, P. D.; Brookes, P. *Nature* **1961**, *192*, 1081.
(24) Kohn, K. W.; Spears, C. L.; Doty, P. *J. Mol. Biol.* **1966**, *19*, 266.
(25) Thomas, C. B.; Kohn, K. W.; Bonner, W. M. *Biochemistry* **1978**, *17*, 3954.
(26) Rosenberg, B.; Van Camp, L.; Trosko, J. E.; Mansour, V. H. *Nature* **1969**, *222*, 385.
(27) Rosenberg, B.; Van Camp, L.; Krigas, T. *Nature* **1965**, *205*, 698.
(28) Kohn, K. W.; Hartley, J. A.; Mattes, W. B. *Nucleic Acids Res.* **1987**, *15*, 10531.
(29) Hopkins, P.; Millard, J.; Woo, J.; Weidner, M.; Kirchner, J.; Sigurdsson, S.; Raucher, S. *Tetrahedron Lett.* **1991**, *47*, 2475.
(30) Broch, H.; Hamza, A.; Vasilescu, D. *Int. J. Quantum Chem.* **1996**, *60*, 1745.
(31) Guainazzi, A.; Campbell, A. J.; Angelov, T.; Simmerling, C.; Scharer, O. D. *Chem.—Eur. J.* **2010**, *16*, 12100.
(32) Gelasco, A.; Lippard, S. J. *Biochemistry* **1998**, *37*, 9230.
(33) Hartwig, J. F.; Lippard, S. J. *J. Am. Chem. Soc.* **1992**, *114*, 5646.
(34) Pinto, A. L.; Lippard, S. J. *Biochim. Biophys. Acta* **1984**, *780*, 167.
(35) Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, *87*, 1153.
(36) Sundquist, W. I.; Lippard, S. J. *Coord. Chem. Rev.* **1990**, *100*, 293.
(37) Tullius, T. D.; Ushay, H. M.; Merkel, C. M.; Caradonna, J. P.; Lippard, S. J. *ACS Symp. Ser.* **1983**, *209*, 51.
(38) Teuben, J. M.; Bauer, C.; Wang, A. H. J.; Reedijk, J. *Biochemistry* **1999**, *38*, 12305.
(39) Poklar, N.; Pilch, D. S.; Lippard, S. J.; Redding, E. A.; Dunham, S. U.; Breslauer, K. J. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 7606.
(40) Takahara, P. M.; Frederick, C. A.; Lippard, S. J. *J. Am. Chem. Soc.* **1996**, *118*, 12309.
(41) Hartley, J. A.; Gibson, N. W.; Kohn, K. W.; Mattes, W. B. *Cancer Res.* **1986**, *46*, 1943.
(42) Johnson, J. M.; Ruddon, R. W. *Mol. Pharmacol.* **1967**, *3*, 195.
(43) Ruddon, R. W.; Johnson, J. M. *Fed. Proc.* **1967**, *26*, 685.
(44) Wang, F.; Li, F.; Ganguly, M.; Marky, L. A.; Gold, B.; Egli, M.; Stone, M. P. *Biochemistry* **2008**, *47*, 7147.
(45) Masta, A.; Gray, P. J.; Phillips, D. R. *Nucleic Acids Res.* **1995**, *23*, 3508.
(46) Bruhn, S. L.; Toney, J. H.; Lippard, S. J. *Prog. Inorg. Chem.* **1990**, *38*, 477.
(47) Bellon, S. F.; Coleman, J. H.; Lippard, S. J. *Biochemistry* **1991**, *30*, 8026.
(48) Bellon, S. F.; Lippard, S. J. *Biophys. Chem.* **1990**, *35*, 179.
(49) Caradonna, J. P.; Lippard, S. J. *Inorg. Chem.* **1988**, *27*, 1454.
(50) Cohen, G. L.; Bauer, W. R.; Barton, J. K.; Lippard, S. J. *Science* **1979**, *203*, 1014.
(51) Whitehead, J. P.; Lippard, S. J. In *Metal Ions in Biological Systems*, Vol. *32*; Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, 1996; p 687.
(52) Masta, A.; Gray, P. J.; Phillips, D. R. *Nucleic Acids Res.* **1994**, *22*, 3880.
(53) Gray, P. J.; Cullinane, C.; Phillips, D. R. *Biochemistry* **1991**, *30*, 8036.
(54) Galton, D. A.; Dacie, J. V.; Szur, L.; Wiltshaw, E. *Br. J. Haematol.* **1961**, *7*, 73.
(55) Ezdinli, E. Z.; Stutzman, L. *JAMA, J. Am. Med. Assoc.* **1965**, *191*, 444.
(56) Doan, C. A.; Wiseman, B. K.; Bouronclle, B. A. *Ann. N. Y. Acad. Sci.* **1958**, *68*, 979.
(57) Hillmen, P.; Gribben, J. G.; Follows, G. A.; Milligan, D.; Sayala, H. A.; Moreton, P.; Oscier, D. G.; Dearden, C. E.; Kennedy, D. B.; Pettitt, A. R.; Nathwani, A.; Cohen, D.; Rawstron, A.; Pocock, C. F. *Ann. Oncol.* **2011**, *22*, 123.
(58) Pineda, F. P.; Ortega-Castro, J.; Alvarez-Idaboy, J. R.; Frau, J.; Cabrera, B. M.; Ramirez, J. C.; Donoso, J.; Munoz, F. *J. Phys. Chem. A* **2011**, *115*, 2359.

(59) Everett, J. L.; Roberts, J. J.; Ross, W. C. J. *J. Chem. Soc.* **1953**, 2386−2392.
(60) Altman, S. J.; Haut, A.; Cartwright, G. E.; Wintrobe, M. M. *Cancer* **1956**, *9*, 512.
(61) Larionov, L. F.; Shkodinskaja, E. N.; Troosheikina, V. I.; Khokhlov, A. S.; Vasina, O. S.; Novikova, M. A. *Lancet* **1955**, *2*, 169.
(62) Sarosy, G.; Leylandjones, B.; Soochan, P.; Cheson, B. D. *J. Clin. Oncol.* **1988**, *6*, 1768.
(63) Kapoor, P.; Rajkumar, S. V.; Dispenzieri, A.; Gertz, M. A.; Lacy, M. Q.; Dingli, D.; Mikhael, J. R.; Roy, V.; Kyle, R. A.; Greipp, P. R.; Kumar, S.; Mandrekar, S. J. *Leukemia* **2011**, *25*, 1523.
(64) Bergel, F.; Stock, J. A. *J. Chem. Soc.* **1954**, 2409.
(65) Kohn, K. W.; Hartley, J. A.; Mattes, W. B. *Biochem. Pharmacol.* **1988**, *37*, 1799.
(66) Povirk, L. F.; Shuker, D. E. *Mutat. Res.* **1994**, *318*, 205.
(67) Hofmann, J.; Doppler, W.; Jakob, A.; Maly, K.; Posch, L.; Uberall, F.; Grunicke, H. H. *Int. J. Cancer* **1988**, *42*, 382.
(68) Efthimiou, M.; Andrianopoulos, C.; Stephanou, G.; Demopoulos, N. A.; Nikolaropoulos, S. S. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **2007**, *617*, 125.
(69) Kemp, K.; Morse, R.; Sanders, K.; Hows, J.; Donaldson, C. *Ann. Hematol.* **2011**, *90*, 777.
(70) Andersson, B. S.; Mroue, M.; Britten, R. A.; Murray, D. *Cancer Res.* **1994**, *54*, 5394.
(71) Schweitzer, V. G. *Otolaryngol. Clin. North Am.* **1993**, *26*, 759.
(72) Choi, M. K.; Kim, D. D. *Arch. Pharmacal. Res.* **2006**, *29*, 1067.
(73) Dulik, D. M.; Fenselau, C. *Drug Metab. Dispos.* **1987**, *15*, 195.
(74) Lau, J. K. C.; Deubel, D. V. *Chem.—Eur. J.* **2005**, *11*, 2849.
(75) Fuertes, M. A.; Alonso, C.; Perez, J. M. *Chem. Rev.* **2003**, *103*, 645.
(76) Kelland, L. R. *Drugs* **2000**, *59*, 1.
(77) Giaccone, G. *Drugs* **2000**, *59*, 9.
(78) Buller, A. L.; Clapper, M. L.; Tew, K. D. *Mol. Pharmacol.* **1987**, *31*, 575.
(79) Clapper, M. L.; Hoffman, S. J.; Bomber, A. M.; Tew, K. D. *Proc. Am. Assoc. Cancer Res.* **1987**, *28*, 296.
(80) Spanswick, V. J.; Craddock, C.; Sekhar, M.; Mahendra, P.; Shankaranarayana, P.; Hughes, R. G.; Hochhauser, D.; Hartley, J. A. *Blood* **2002**, *100*, 224.
(81) Fox, M.; Roberts, J. J. *Cancer Metastasis Rev.* **1987**, *6*, 261.
(82) Panasci, L.; Xu, Z. Y.; Bello, V.; Aloyz, R. *Anti-Cancer Drugs* **2002**, *13*, 211.
(83) Neidle, S.; Thurston, D. E. *Nat. Rev. Cancer* **2005**, *5*, 285.
(84) Gupta, R.; Wang, H. Y.; Huang, L. R.; Lown, J. W. *Anti-Cancer Drug Des.* **1995**, *10*, 25.
(85) Alexander, R. P.; Beeley, N. R. A.; Odriscoll, M.; Oneill, F. P.; Millican, T. A.; Pratt, A. J.; Willenbrock, F. W. *Tetrahedron Lett.* **1991**, *32*, 3269.
(86) Colvin, M. E.; Sasaki, J. C.; Tran, N. L. *Curr. Pharm. Des.* **1999**, *5*, 645.
(87) Rosales-Hernandez, M. C.; Bermudez-Lugo, J.; Garcia, J.; Trujillo-Ferrara, J.; Correa-Basurto, J. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 230.
(88) Lau, J. K.-C.; Deubel, D. V. *Chem.—Eur. J.* **2005**, *11*, 2849.
(89) Raber, J.; Zhu, C.; Eriksson, L. A. *J. Phys. Chem. B* **2005**, *109*, 11006.
(90) Mantri, Y.; Lippard, S. J.; Baik, M.-H. *J. Am. Chem. Soc.* **2007**, *129*, 5023.
(91) Baik, M. H.; Friesner, R. A.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 14082.
(92) Baik, M. H.; Friesner, R. A.; Lippard, S. J. *J. Am. Chem. Soc.* **2002**, *124*, 4495.
(93) Baik, M. H.; Friesner, R. A.; Lippard, S. J. *Inorg. Chem.* **2003**, *42*, 8615.
(94) Shukla, P. K.; Mishra, P. C.; Suhai, S. *Chem. Phys. Lett.* **2007**, *449*, 323.
(95) Gresham, G. L.; Groenewold, G. S.; Olson, J. E. *J. Mass Spectrom.* **2000**, *35*, 1460.
(96) Bhattacharyya, P. K.; Medhi, C. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **2005**, *44*, 1319.
(97) Sunters, A.; Springer, C. J.; Bagshawe, K. D.; Souhami, R. L.; Hartley, J. A. *Biochem. Pharmacol.* **1992**, *44*, 59.
(98) Chatterji, D. C.; Yeager, R. L.; Gallelli, J. F. *J. Pharm. Sci.* **1982**, *71*, 50.
(99) Bauer, G. B.; Povirk, L. F. *Nucleic Acids Res.* **1997**, *25*, 1211.
(100) Williams, Ce; Witten, B. *Cancer Res.* **1967**, *27*, 33.
(101) Ross, W. E.; Ewig, R. A. G.; Kohn, K. W. *Cancer Res.* **1978**, *38*, 1502.
(102) Ross, W. E.; Kohn, K. W. *Proc. Am. Assoc. Cancer Res.* **1977**, *18*, 62.
(103) Hansson, J.; Lewensohn, R.; Ringborg, U.; Nilsson, B. *Cancer Res.* **1987**, *47*, 2631.
(104) Remias, M. G.; Lee, C. S.; Haworth, I. S. *J. Biomol. Struct. Dyn.* **1995**, *12*, 911.
(105) Dong, Q.; Barsky, D.; Colvin, M. E.; Melius, C. F.; Ludeman, S. M.; Moravek, J. F.; Colvin, O. M.; Bigner, D. D.; Modrich, P.; Friedman, H. S. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 12170.
(106) Srivastava, H. K.; Chourasia, M.; Kumar, D.; Sastry, G. N. *J. Chem. Inf. Model.* **2011**, *51*, 558.
(107) Gamcsik, M. P.; Millis, K. K.; Hamill, T. G. *Chem.-Biol. Interact.* **1997**, *105*, 35.
(108) Bardos, T. J.; Dattagup., N; Hebborn, P.; Triggle, D. J. *J. Med. Chem.* **1965**, *8*, 167.
(109) Levins, P. L.; Papanastassiou, Z. B. *J. Am. Chem. Soc.* **1965**, *87*, 826.
(110) Ross, S. D. *J. Am. Chem. Soc.* **1947**, *69*, 2982.
(111) Rutman, R. J.; Chun, E. H. L.; Jones, J. *Biochim. Biophys. Acta* **1969**, *174*, 663.
(112) Champman, N. B.; James, J. W. *J. Chem. Soc.* **1954**, 2103−2108.
(113) *Jaguar 7.0*; Schrödinger, Inc.: Portland, OR, 2003.
(114) Vosko, S. H.; Wilk, L.; Nusair, M. *Can. J. Phys.* **1980**, *58*, 1200.
(115) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
(116) Lee, C. T.; Yang, W. T.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
(117) Slater, J. C. *Quantum Theory of Molecules and Solids*; McGraw-Hill: New York, 1974; Vol. *4*.
(118) Dunning, T. H., Jr. *J. Chem. Phys.* **1989**, *90*, 1007.
(119) Marten, B.; Kim, K.; Cortis, C.; Friesner, R. A.; Murphy, R. B.; Ringnalda, M. N.; Sitkoff, D.; Honig, B. *J. Phys. Chem.* **1996**, *100*, 11775.
(120) Baik, M.-H.; Friesner, R. A. *J. Phys. Chem. A* **2002**, *106*, 7407.
(121) Baik, M.-H.; Ziegler, T.; Schauer, C. K. *J. Am. Chem. Soc.* **2000**, *122*, 9143.
(122) Baik, M.-H.; Silverman, J. S.; Yang, I. V.; Ropp, P. A.; Szalai, V. A.; Yang, W.; Thorp, H. H. *J. Phys. Chem. B* **2001**, *105*, 6437.
(123) Baik, M.-H.; Schauer, C. K.; Ziegler, T. *J. Am. Chem. Soc.* **2002**, *124*, 11167.
(124) Kelly, C. P.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem. B* **2006**, *110*, 16066.
(125) Peng, C. Y.; Schlegel, H. B. *Isr. J. Chem.* **1993**, *33*, 449.
(126) Tissandier, M. D.; Cowen, K. A.; Feng, W. Y.; Gundlach, E.; Cohen, M. H.; Earhart, A. D.; Tuttle, T. R.; Coe, J. V. *J. Phys. Chem. A* **1998**, *102*, 9308.
(127) Ringdahl, B.; Mellin, C.; Ehlert, F. J.; Roch, M.; Rice, K. M.; Jenden, D. J. *J. Med. Chem.* **1990**, *33*, 281.
(128) Chong, H. S.; Song, H. A.; Dadwal, M.; Sun, X.; Sin, I.; Chen, Y. W. *J. Org. Chem.* **2010**, *75*, 219.
(129) Jones, G. B.; Mathews, J. E. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 93.
(130) Palmer, B. D.; Wilson, W. R.; Pullen, S. M.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 112.
(131) Chong, H. S.; Song, H. A.; Dadwal, M.; Sun, X. A.; Sin, I.; Chen, Y. W. *J. Org. Chem.* **2010**, *75*, 7966.
(132) Price, C. C.; Gaucher, G. M.; Koneru, P.; Shibakaw., R; Sowa, J. R.; Yamaguch., M *Biochim. Biophys. Acta* **1968**, *166*, 327.
(133) Arpalahti, J.; Lippert, B. *Inorg. Chem.* **1990**, *29*, 104.
(134) Mann, D. J. *J. Phys. Chem. A* **2010**, *114*, 4486.