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Modeling Acid and Cationic Catalysis on the Reactivity of Duocarmycins

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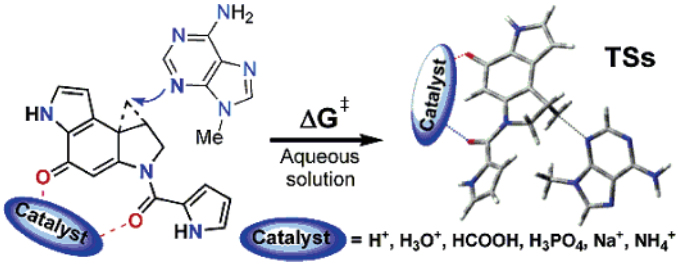

Several catalyzed alkylation reactions of 9-methyladenine by a model [**CPI**, cyclopropa[*c*]pyrrolo-[3,2-*e*]indol-4(5*H*)-one (**1**)] of duocarmycin anticancer drugs have been compared to the uncatalyzed reaction in gas phase and in water solvent bulk, using density functional theory at the B3LYP level with the 6-31+G(d,p) basis set and C-PCM solvation model. The effect on the CPI reactivity induced by water, formic and phosphoric acids (general acid catalysis), H_3O^+ (specific acid catalysis), sodium, and ammonium cation complexation (cationic catalysis) has been investigated. The calculations indicate that the specific acid catalysis and the catalysis induced by sodium cation complexation are strong in the gas phase, but solvation reduces them dramatically by electrostatic effects. The specific acid catalysis is still operative, but strongly reduced in water solution, where the reaction barrier is reduced by 8.6 kcal mol^{-1} in comparison to the uncatalyzed reaction. The general acid catalysis induced by phosphoric acid (−7.3 kcal mol^{-1}) and the catalysis induced by Na^+ and NH_4^+ complexation become competitive, with a catalytic effect of −3.6 and −4.1 kcal mol^{-1} in water, respectively. With the specific acid catalysis, the high acidity (low pK_a value) of the conjugated acid of CPI (**CPIH**$^+$), computed in water solution using both C-PCM (pK_a = +2.6) and PCM-B3LYP/6-31+G(d,p) (pK_a = +2.4) solvation models, suggests that the catalytic effects induced by NH_4^+ complexation could become more important than the specific acid catalysis and the general catalysis by H_3PO_4 under physiological conditions, due to concentration effects of the catalysts.

Introduction

The cyclopropylpyrroloindoles (**CPI**s) have received considerable attention as extremely potent DNA alkylating agents.[1] Among CPIs, of particular notoriety are the (+)-duocarmycin SA (**2**), (+)-CC-1065 (**3**),[2,3] and more recently (+)-yatakemycin (**4**) (Chart 1).[4] These natural products and several synthetic analogues contain the CPI moiety of the model compound 1,2,8,8a-tetrahydrocyclopropa[*c*]pyrrolo[3,2-*e*]indol-4(5*H*)-one (**1**). They are capable of sequence-selective alkylation of DNA at the adenine N-3 center, within the DNA minor groove.[5] The formation of a covalent bond involving the unsubstituted cyclopropane carbon (C_7) is the chemical reaction causing DNA damage, which results in the antitumoral activity of these natural products.

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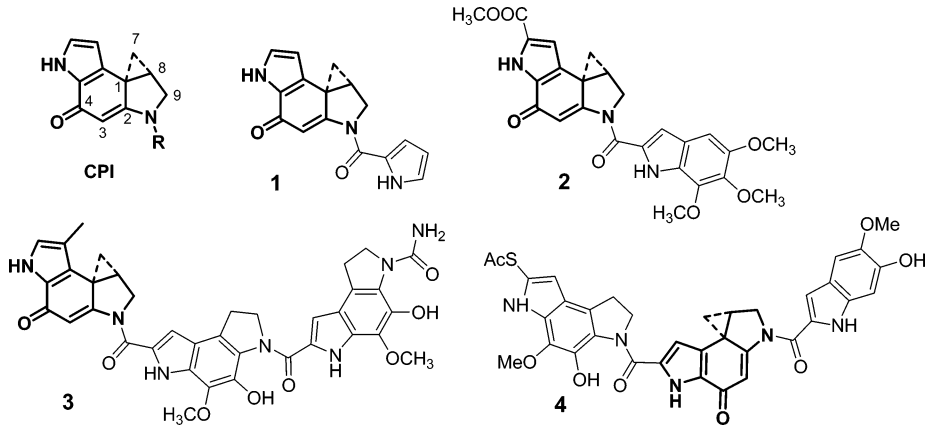
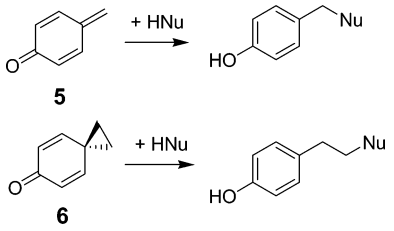
CHART 1



SCHEME 1



The reacting core of these drugs is the cyclopropa[*c*]-pyrrolo[3,2-*e*]indol-4(5*H*)-one moiety (**CPI**), which as pointed out previously by Winstein may be considered a homologue of the *p*-quinone methide (*p*-QM, **5**, in Scheme 1).[6]

Unlike quinone methides[7−11] and other more traditional alkylating agents such as diazonium phenylnitrenium ions,[12] carbocations,[13] and benzyl halides,[14] CPIs are unreactive toward biological nucleophiles in water solution under neutral conditions, but they become highly activated when precomplexed to a DNA duplex, under mild conditions (proceeding in less than 1 h at 4−25 °C).[15]

The origin of the selectivity and the source of the catalytic effect in the alkylation reaction with DNA remains quite controversial. In fact, to date, two distinct models have emerged from all of the investigations focused on this issue. The first one, which was proposed by Hurley and Warpehoski more than a decade ago,[16,17] suggests that the exceptional electrophilicity of CPIs complexed to DNA could be ascribed to an acid catalysis of the environment associated with DNA itself.

The second model has been more recently proposed by Boger's group through an extensive and impressive experimental investigation with both simplified[18] and extended analogues of the drug.[19]

According to Boger, an important activating factor of the DNA catalysis could be the disruption of the vinylogous amide conjugation, which is sufficient to provide activation for a nucleophilic addition independent of pH, under physiological conditions. In fact, the author also established through pH−rate profiles of a few solvolysis reactions that the requirement of acid catalysis for the DNA alkylation in not necessary.[20] Such a unique mode of activation of these alkylating agents has been defined by Boger as "shape-dependent catalysis".[15] Moreover, Boger has suggested that such a "conformational catalysis" can be directly induced by the DNA binding, which activates the CPI drugs for nucleophilic attack.[21]

To our knowledge, the issue conformational vs acid catalysis of the DNA on CPI reactivity has been ad-

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dressed so far only with experimental approaches. Very recently, Barone et al. addressed a few aspects of the mechanism of the acid-catalyzed methanolysis of CPI derivatives,[22a] and the chemoselectivity under neutral condition of the duocarmycin cyclopropane ring opening by methanol, pyridine, and methylamine at PBE0/6-31G-(d) level of theory[22b] in the gas phase and in water solution. These authors conclude that acid catalysis is mandatory for methanol, but physiological conditions could be sufficient for the alkylation of nitrogen nucleophiles such as methylamine and pyridine.

A thorough computational investigation, including solvation effects, could allow a clarification and a quantitative comparison of the two "triggering" modes, with the goal of improving understanding of such important processes. We began to address the issue recently by tackling the "conformational catalysis" in the absence of acid catalysis, displaying that the conformational catalysis may reduce the reaction activation energy by 4.3 kcal mol^{-1}, in aqueous solution.[23] This paper report the follow up investigation aimed at quantifying the role of general and specific acid catalysis by comparing them with other sources of catalytic effect such as sodium and ammonium cation complexation. The latter cations, which balance the negative charge of the DNA phosphate backbone, could play an important role due to their high concentration (unlike the H^+) under physiological conditions.

Methods and Computation Details

All calculations were carried out using the B2 version of Gaussian 2003[24] program package.

All of the geometric structures of the reactants and transition states (**S**) located were fully optimized both in the gas phase and in water solution using the hybrid density functional B3LYP[25] with the 6-31G(d) basis set. It is known that diffuse functions are mandatory for a reliable evaluation of anion energies and in our reactive system a zwitterionic character develops in the TSs, with a partial negative charge on the carbonyl oxygen atom. To assess the best compromise between the best basis set and the computationally demanding real systems, we optimized the free reactant **1**, its complexes with the catalysts (**1**−$\mathbf{H_2O}$, **1**−**HCOOH**, **1**−$\mathbf{H_3PO_4}$, **1**−$\mathbf{H^+}$, **1**−$\mathbf{H_3O^+}$, **1**−$\mathbf{Na^+}$, **1**−$\mathbf{Na^+H_2O}$, **1**−$\mathbf{NH_4^+}$, and **1**−$\mathbf{NH_4^+H_2O}$), and their related TSs (**S1**−$\mathbf{H_2O}$, **S1**−**HCOOH**, **S1**−$\mathbf{H_3PO_4}$, **S1**−$\mathbf{H^+}$, **S1**−$\mathbf{H_3O^+}$, **S1**−$\mathbf{Na^+}$, **S1**−$\mathbf{Na^+H_2O}$, **S1**−$\mathbf{NH_4^+}$, and **S1**−$\mathbf{NH_4^+H_2O}$) for the alkylation reaction of 9-methyladenine at the B3LYP/6-31G(d) level, both in the gas phase and in water solution, then refining the energies by single-point calculation at the B3LYP/6-31+G(d,p) level of theory. Thermal contributions (δG), to activation free energy ($\Delta G^{\ddagger}$), were computed from B3LYP/6-31G(d) structures and harmonic frequencies, by using the harmonic oscillator approximation and the standard expressions for an ideal gas in the canonical ensemble at 298.15 K and 1 atm.

The optimization of the stationary points in the solvent bulk were calculated via the self-consistent reaction field (SCRF) method using the conductor version of PCM (C-PCM)[26] as implemented in the B.02 version of Gaussian 2003. The geometry optimization in water solution for TS has been quite difficult, therefore to solve the problem the "loose" convergence criteria on geometry optimization was adopted. The cavity is composed by interlocking spheres centered on non-hydrogen atoms with radii obtained by the HF parametrization of Barone known as united atom topological model (UAHF).[27] Such a model includes the nonelectrostatic terms (cavitation, dispersion, and repulsion energy) in addition to the classical electrostatic contribution. For all C-PCM-UAHF calculations, the number of initial tesserae per atomic sphere was set to 100.

We remind readers that the energies resulting from C-PCM computations have the status of free energies, since they take implicitly into account thermal and entropic contributions of the solvent, but since they do not include the thermal contributions (δG) of solute molecular motions, to the activation free energy ($\Delta G^{\ddagger}$), we will refer to them as ($\Delta E^{\ddagger}_{sol}$). Gas phase thermal contribution of solute molecular motions were added to the $\Delta E^{\ddagger}_{sol}$ to evaluate the corresponding activation free energy in water ($\Delta G^{\ddagger}_{sol}$).

Results and Discussion

To evaluate the effect of specific, as well as general acid catalysis and to compare them with other potential catalytic effects associated with ions complexation, first we locate the TS of the uncatalyzed alkylation reactions of 9-methyladenine by the cyclopropa[*c*]pyrrolo[3,2-*e*]-indol-4(5*H*)-one (**1**). To date, this is the closest computational model to the real reactive system duocarmycins + DNA adenosine. Second, we explore the effects of the catalyst complexation with the substrate **1**, both at reactant and TSs, in the gas phase and in water solution. The catalysts taken into account can be classified into three classes according to their catalytic effects: (i) general acid catalysis (by H_2O, HCOOH, and H_3PO_4), (ii) specific acid catalysis (H^+ and H_3O^+), and (iii) cationic catalysis which has been simulated for those cations (NH_4^+ and Na^+) which exist at high concentration under physiological condition in the proximity of DNA phosphate backbone. In all cases we have evaluated both the energy barriers ($\Delta E^{\ddagger}$) in the gas phase and in water ($\Delta E^{\ddagger}_{sol}$) and the corresponding activation free energies ($\Delta G^{\ddagger}$, $\Delta G^{\ddagger}_{sol}$) which include the nonpotential energy terms. Table 1 shows that $\Delta E^{\ddagger}$ and $\Delta G^{\ddagger}$ display parallel trends. For sake of simplicity and clarity, discussion on catalytic effects will be based mainly on $\Delta\Delta E^{\ddagger}$ ($\Delta\Delta E^{\ddagger} = \Delta E^{\ddagger}_{cat} - \Delta E^{\ddagger}_{uncat}$), which is a comparison of the energy

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TABLE 1. Activation Energy ($\Delta E^{\ddagger}$ in kcal mol^{-1}),and Activation Free Energy ($\Delta G^{\ddagger}$ in kcal mol^{-1}) in the Gas Phase and in Aqueous Solution at B3LYP/6-31+(d,p)// B3LYP/6-31(d) and CPCM-B3LYP/6-31+(d,p)// CPCM-B3LYP/6-31(d) Levels of Theory, Respectively

	gas phase		aqueous solution	
structure	$\Delta E^{\ddagger}$	$\Delta G^{\ddagger}$	$\Delta E^{\ddagger}_{sol}$ [a]	$\Delta G^{\ddagger}_{sol}$
Uncatalyzed Alkylation				
S1	35.0	44.3	31.7	41.0
General Acid Catalysis				
S1−H_2O	32.8	43.5	30.2	40.9
S1−HCOOH	31.2	41.2	29.1	39.1
S1−H_3PO_4	30.0	41.0	24.4	35.4
Specific Acid Catalysis				
S1−H^+	8.6	17.5	22.1	31.0
S1−H_3O^+	10.0	19.3	23.1	32.4
Na^+ Catalyzed Alkylation				
S1−Na^+	18.3	28.3	29.3	39.2
S1−Na^+H_2O	19.2	29.2	28.1	38.1
NH_4^+ Catalyzed Alkylation				
S1−NH_4^+	-	-	27.9	38.5
S1−$NH_4^+H_2O$	-	-	27.5	39.0

[a] Activation energies resulting from CPCM computations have the status of free energies, since they take implicitly into account thermal and entropic contributions of the solvent, but since they do not include the thermal contributions (δG) to activation free energy ($\Delta G^{\ddagger}$) we will refer to them as ($\Delta E^{\ddagger}_{sol}$).

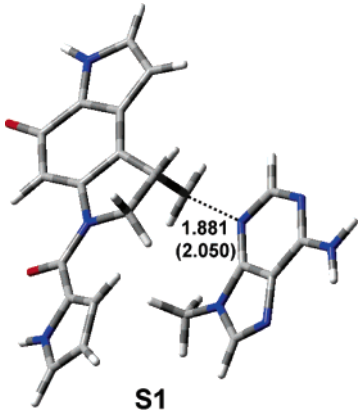


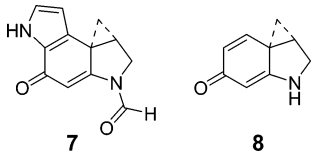
FIGURE 1. Gas-phase-optimized geometry (bond lengths in Å) for the B3LYP/6-31G(d) transition structure **S1** of the uncatalyzed alkylation of 9-methyladenine by **1**. Data in parentheses are for full B3LYP/6-31G(d) optimization in aqueous solution.

barriers of the catalyzed reactions ($\Delta E^{\ddagger}_{cat}$) relative to those of the corresponding uncatalyzed ones ($\Delta E^{\ddagger}_{uncat}$).

1. CPI Reactivity toward 9-Methyadenine: The Uncatalyzed Process. We have chosen the compound **1** and 9-methyladenine to model the reactivity of duocarmycins drugs toward DNA. The choice of this purinic base is suggested by the experimental evidence that only the N3-adenine alkylation adduct has been detected from DNA alkylation experiments by duocarmycins. In addition, we think that 9-methyladenine better reproduces the steric demand of the adenosine in the DNA nucleotide than adenine.

Examination of the TS geometries for the alkylation reaction in the gas phase and in water solution (Figure 1) reveals that the C- -N forming bond length in the gas phase (1.881 Å) is slightly shorter than that in water solution (2.050 Å).

CHART 2



7 **8**

Such a difference in the forming bond length (δC- -N 0.169 Å) is much smaller than that for the alkylation of ammonia by a slightly simpler CPI model (δC- -N 0.438 Å), previously investigated by us (see **7** in Chart 2),[23] where the corresponding forming bond lengths are 1.751 and 2.189 Å in the gas phase and in water, respectively. This could be partially caused by steric encumbrance of the pyrrole ring (which is absent in **7**) and by also a better solvation of the TS involving **7** and ammonia in comparison to **S1**.

In fact, from an energetic point of view the computed activation barrier at B3LYP/6-31+G(d,p) level of theory is very high (35.0 kcal mol^{-1}, Table 1) in the gas phase. Bulk solvent effects of a water solution reduces the barrier down to 31.7 kcal mol^{-1}. The solvent effect on the reaction activation barrier (−3.3 kcal mol^{-1}) is much smaller in comparison to that evaluated for the alkylation reaction of the CPI **7** with ammonia as nucleophile (−14.4 kcal mol^{-1}).[23] This trend confirms that the TS involving **7** is better solvated by the aqueous solution in comparison to **S1**, due to the higher cationic character of the nitrogen atom in the former TS in comparison to the latter.

The reactivity of another simple CPI model **8** (Chart 2) with pyridine has been recently investigated by Barone,[22] and a straightforward comparison is possible on the basis of free activation energy in gas phase and in solution. The reactivity of Barone's model ($\Delta G^{\ddagger}$ = 42.4 kcal mol^{-1}) is similar to that of CPI **1** and 9-methyl adenine in the gas phase ($\Delta G^{\ddagger}$ = 44.3 kcal mol^{-1}), but the former system becomes more reactive ($\Delta G^{\ddagger}$ = 33.4 kcal mol^{-1}) than the latter in water solution ($\Delta G^{\ddagger}$ = 41.0 kcal mol^{-1}). The difference in reactivity between our reactive system (**1** + 9-methyladeniene) and the other two already investigated (**7** + NH_3 and **8** + adenine)[28] suggests that the nucleophilicity of 9-methyladenine (which is a more adeguate substrate than pyridine or ammonia for a computational simulation of DNA−adenosine reactivity) toward CPI **1** is less solvent dependent than that of smaller nucleophiles such as ammonia and pyridine. Such a solvent effect is caused by the dipolar nature of these TSs, which develop a negative charge on the oxygen atom and a positive charge on the nucleophile undergoing addition. If the nucleophile is a small molecule like ammonia, the charge is highly localized and the electrostatic stabilization induced by the solvent on the TS is strong. The addition of a nucleophile with higher molecular weight, such as 9-methyladenine, causes a delocalization of the positive charge on the whole nucleophile structure, reducing the electrostatic stabilization by the solvent. These data suggest that for a balanced evaluation of the duocarmycin reactivity in aqueous solution, the model used for both drug and nucleophilic substrate should not be too simplified.

(28) The differences between the reactive systems is not due to the slightly different solvation method (C-PCM vs PCM) used by us, since PCM calculations on CPI **1** and 9-methyladenine confirms similar data.

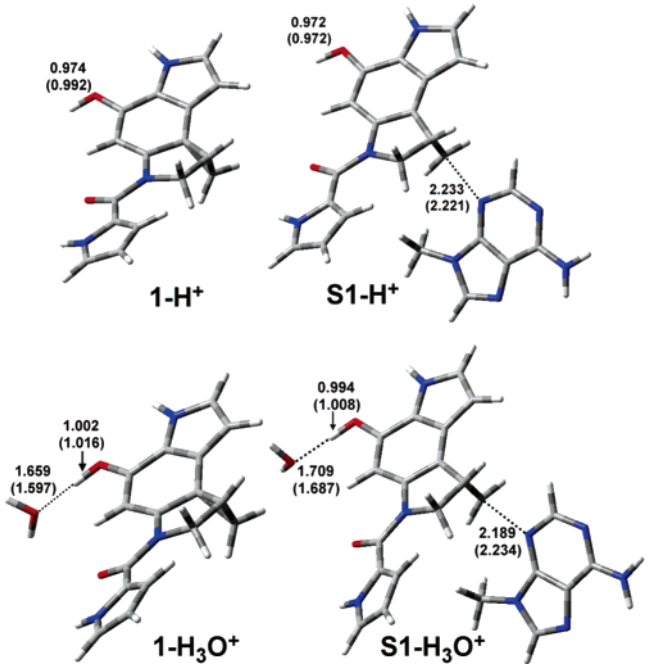


FIGURE 2. Gas-phase-optimized geometries (bond lengths in Å) for reactants and transition structures of the specific acid-catalyzed alkylation of 9-methyladenine by **1**. Data in parentheses are for full B3LYP/6-31G(d) optimization in aqueous solution.

Although a quantitative comparison between computational and experimental data related to the uncatalyzed process is desirable for the validation of the computational model, it is not possible, because experimental data suggest that duocarmycins do not react in solvolitic processes under neutral condition.[18b] In more detail, the simplified analogues of duocarmycins studied by Boger (N-BOC-CPI and N-BOC-DSA) are stable at pH 7, [18b] and no solvolysis rates are available at such a pH.

2. Specific Acid-Catalyzed addition of CPI to 9-Methyladenine. The specific acid catalysis on the addition of CPI **1** to 9-methyladenine has been evaluated using two reacting models, where the proton, free (H^+) or as hydronium ion (H_3O^+) is complexed to the carbonyl oxygen atom, in both the reactants (**1**−**H**$^+$ and **1**−**H$_3$O**$^+$) and TSs (**S1**−**H**$^+$ and **S1**−**H$_3$O**$^+$, in Figure 2).

The two reacting models are very similar, since pre-complexed reactants and TSs display comparable geometries and reaction free activation energies (Table 1), both in the gas phase ($\Delta G^{\ddagger}$ = 17.5 and 19.3 kcal mol^{-1}, respectively) and in water solution ($\Delta G^{\ddagger}$ = 31.0 and 32.4 kcal mol^{-1}, respectively). The effect of the specific catalysis can be measured by the differences between activation barriers ($\Delta\Delta E^{\ddagger}$) or free activation energies ($\Delta\Delta G^{\ddagger}$) of the catalyzed and uncatalyzed alkylation processes (Table 2). The specific catalysis is remarkable in gas phase, where the above-mentioned difference for H_3O^+ is −25.0 kcal mol^{-1} but is strongly reduced to −8.6 kcal mol^{-1} in water solution. The comparison of our results with those obtained by Barone, (who used pyridine as nucleophile model) relative to the H^+-catalyzed addition of pyridine to CPI **8**, where the catalytic effect is −32.5 and −13.3 kcal mol^{-1}, in the gas and in water, respectively, suggest that the importance of specific acid catalysis, as well as of solvation effects, is reduced increasing the molecular size of the nucleophile.

TABLE 2. Catalytic Effects Evaluated Comparing the Energy Barriers ($\Delta E^{\ddagger}$) and Activation Free Energies ($\Delta G^{\ddagger}$) of the Catalyzed Reactions to That of the Uncatalyzed One ($\Delta\Delta E^{\ddagger} = \Delta E^{\ddagger}_{cat} - \Delta E^{\ddagger}_{uncat}$; $\Delta\Delta G^{\ddagger} = \Delta G^{\ddagger}_{cat} - \Delta G^{\ddagger}_{uncat}$), in kcal mol^{-1}, in the Gas Phase and in Water Solution at B3LYP/6-31+(d,p)//B3LYP/6-31(d) and CPCM-B3LYP/6-31+(d,p)//CPCM-B3LYP/6-31(d) Levels of Theory, Respectively

	gas phase		aqueous solution	
catalyst	$-\Delta\Delta E^{\ddagger}$	$-\Delta\Delta G^{\ddagger}$	$-\Delta\Delta E^{\ddagger}_{sol}$ [a]	$-\Delta\Delta G^{\ddagger}_{sol}$
General Acid Catalysis				
H_2O	2.2	0.8	1.4	0.1
HCOOH	3.8	3.1	2.6	2.9
H_3PO_4	4.9	3.3	7.3	5.6
Specific Acid Catalysis				
H^+	26.4	26.8	9.5	9.9
H_3O^+	25.0	25.0	8.6	8.6
Na^+-Catalyzed Alkylation				
Na^+	16.7	16.0	2.4	1.8
Na^+H_2O	15.8	15.0	3.6	2.9
NH_4^+-Catalyzed Alkylation				
NH_4^+	-	-	3.8	2.5
$NH_4^+H_2O$	-	-	4.1	3.0

[a] See Table 1 footnote.

2.1. Specific Acid Catalysis vs General Acid. To explore the extent of the general acid catalysis we also studied the alkylation reaction of 9-methyladenine by CPI **1** complexed with H_2O, HCOOH, and H_3PO_4, optimizing reactants and the corresponding TSs, in the gas phase and in water solution (Figure 3).

Energetically the complexation of CPI **1** by small protic and neutral molecules always reduces the reaction barriers (Table 1). This suggests that general acid catalysis in the alkylation of 9-methyladenine is operative. The extent of such a catalytic effect can be evaluated by the differences in the activation barriers (or similarly in the free activation energies, see Table 2) between uncatalyzed and catalyzed alkylation processes, in gas and in water solution. From the data in Table 2 it is seen that there is a general catalytic effect on the alkylation reaction, but it is quite small in the gas phase (−2.2, −3.8, and −4.9 kcal mol^{-1} for H_2O, HCOOH, and H_3PO_4, respectively), in comparison to that of the specific acid catalysis (−25.0 kcal mol^{-1}). Unlike the specific acid catalysis, which is strongly reduced by solvation effects (down to −8.6 kcal mol^{-1}), general acid catalysis remain similar in water solution, for water, and formic acid with a catalytic effect of −1.4 and −2.6 kcal mol^{-1}, respectively, and it becomes even stronger in the case of phosphoric acid complexation (−7.3 kcal mol^{-1}). However, a balanced evaluation of the general catalysis induced by H_3PO_4 under physiological conditions cannot neglect the effect of its low concentration (<10^{-8} mol/L). Therefore, if we also take into account the concentration effects of the undissociated acid (H_3PO_4) the general catalytic effect of H_3PO_4 is negligible in comparison to the general catalytic effect of H_2O.

2.2. Other Catalytic Effects: Na^+ and NH_4^+ Catalysis. We continued our survey on other catalytic effects exploring also the role of cationic complexation to the substrate **1**. The choice of analyzing the effects of

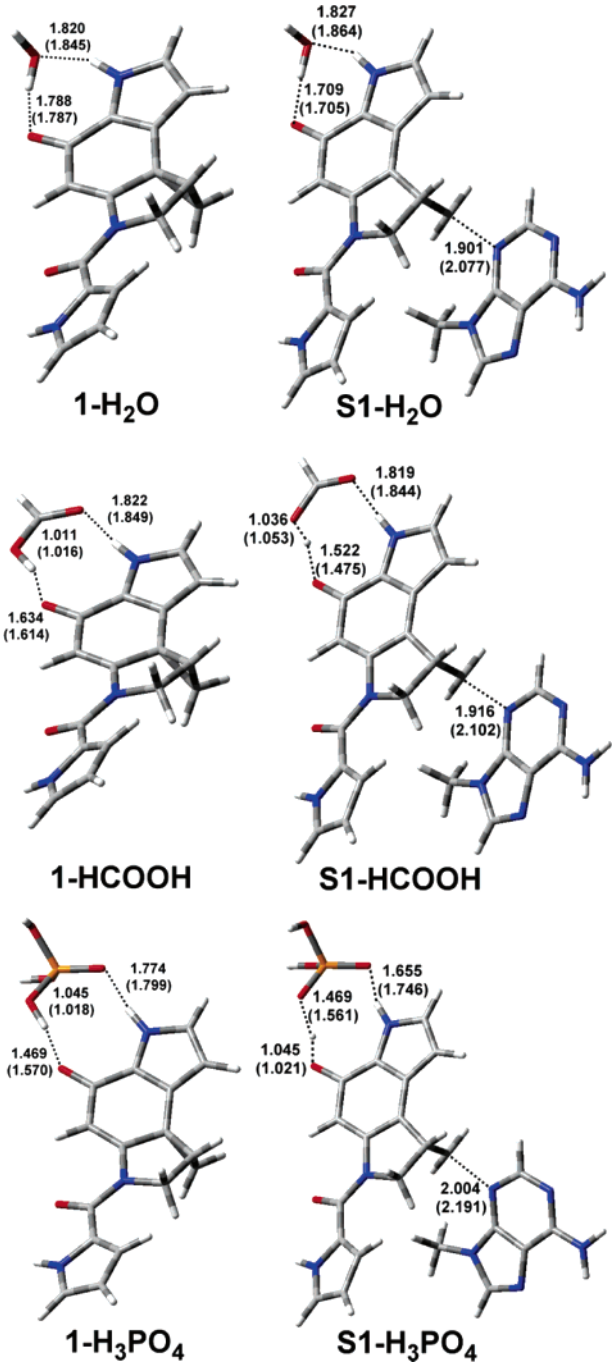


FIGURE 3. Gas-phase-optimized geometries (bond lengths in Å) for reactants and transition structures of the general acid-catalyzed alkylations (with H_2O, HCOOH, and H_3PO_4) of 9-methyladenine by **1**. Data in parentheses are for full B3LYP/6-31G(d) optimization in aqueous solution.

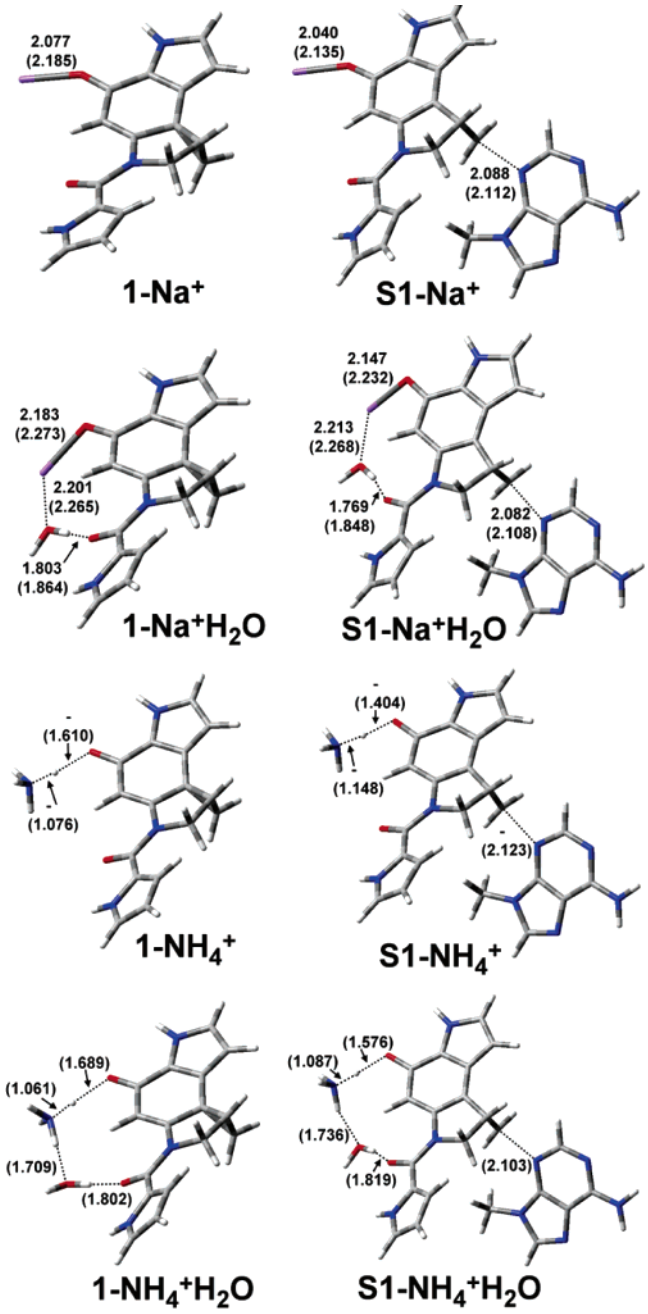


FIGURE 4. Gas-phase-optimized geometries (bond lengths in Å) for reactants and transition structures of the alkylation of 9-methyladenine by **1** catalyzed by sodium and ammonium cation complexation. Data in parentheses are for full B3LYP/6-31G(d) optimization in aqueous solution.

sodium and ammonium cations on the energetics of the alkylation reaction of 9-methyladenine by **1** has been suggested by the fact that approximately 90% of DNA charge must be neutralized by counterions, which are mainly Na^+, NH_4^+, or RNH_3^+. Therefore, there is a high concentration of such cations in the proximity of the phosphate backbone. In addition, from Chazin's work it is seen that the docked CPI into DNA displays a proper orientation with the carbonyl moiety pointing toward the phosphate groups.[29]

The effect of sodium and ammonium complexation on the alkylation of CPI **1** to 9-methyladenine has been evaluated using two reacting models (see Figure 4), where the cations are free (**1-Na**$^+$ and **1-NH**$_4^+$) or coordinated with a water molecule (**1-Na**$^+$**H**$_2$**O** and **1-NH**$_4^+$**H**$_2$**O**).

(29) (a) Schnell, J. R.; Ketchem, R. R.; Boger, D. L.; Chazin, W. J. *J. Am. Chem. Soc.* **1999**, *121*, 5645. (b) Smith, J. A.; Bifulco, G.; Case, D. A.; Boger, D. L.; Gomez-Paloma, L.; Chazin, W. J. *J. Mol. Biol.* **2000**, *300*, 1195. (c) Eis, P. S.; Smith, J. A.; Rydzewski, J. M.; Case, D. A.; Boger, D. L.; Chazin, W. J. *J. Mol. Biol.* **1997**, *272*, 237.

TABLE 3. Reaction Energy (ΔE, in kcal mol^{-1}), Reaction Free Energy (ΔG, in kcal mol^{-1}) for the Alkylation Reaction and Activation Free Energy for the Alkylation Reversal Pathway ($\Delta G^{\ddagger}_{Rev}$, in kcal mol^{-1}), in Gas Phase and in Aqueous Solution, at B3LYP/6-31+G(d,p)//B3LYP/6-31G(d) and CPCM-B3LYP/6-31+G(d,p)//CPCM-B3LYP/6-31G(d) Levels of Theory, Respectively

	gas phase			aqueous solution	
alkylation adduct	ΔE	ΔG	$\Delta G^{\ddagger}_{gas,rev}$	ΔG_{sol}	$\Delta G^{\ddagger}_{sol,rev}$
P	+25.1	+35.9	+8.4	+21.6	+19.3
P−H$^+$	−19.0	−6.2	+23.6	+2.7	+28.2

The second model should be, in principle, slightly better than the first one because it takes into account also specific interaction with the cation by the aqueous solution which is neglected by the any continuum solvation model. Therefore we will take into consideration this latter model for the evaluation of the catalytic effects induced by cations on the alkylation reaction. From an energetic point of view, sodium and ammonium complexation in water solution reduce the activation free energy of the reaction, in comparison to the uncatalyzed process, by −3.6 and −4.1 kcal mol^{-1} in water solution. This evidence suggests that the catalytic effect induced by NH_4^+ on the alkylation reaction of 9-methyladenine by CPI **1** could efficiently compete with specific acid catalysis. In fact, the latter catalytic effect, which has been evaluated in −8.6 kcal mol^{-1}, should be strongly reduced due to the low CPIH$^+$ concentration under neutral condition (pH 7). A quantitative evaluation of CPIH$^+$ concentration effects on the specific acid catalysis can be easily evaluated from the absolute pK_a value of the conjugated acid of CPI **1** (**CPIH**$^+$).

2.3. Effect of the Solvent on the Reactivity. The effect of the water as solvent on the reactivity of **1** in the uncatalyzed and in all the catalyzed processes requires an overall analysis, since it favors the uncatalyzed alkylation and the general catalysis, but it strongly disfavors the specific acid catalysis and the Na^+ catalysis (see data in Table 1). The reasons of such a trend is clearly ascribed to an electrostatic stabilization. In more detail, the dipole moment for uncharged reactants (i.e., μ = 6.3 D for **1** and μ = 2.7 D for 9-MeA in vacuo), is always much lower than that of the related TS (μ = 15.0 D for **S1**), but the opposite is true for charged reactants (i.e., μ = 4.0 D for **1-H**$^+$; μ = 11.5 D for **1-Na**$^+$ in vacuo), which display higher dipole moments that those of the related TSs (i.e., μ = 2.5 D for **S1**−**H**$^+$; μ = 10.6 D for **S1**−**Na**$^+$ in vacuo). These data suggest that uncharged TSs would be better solvated by polar solvents.

3. Alkylation under Thermodynamic Control. Stability of the Resulting Adduct. Given the lack of experimental kinetic rates related to the alkylation of 9-methyladenine by the model CPI (**1**) under neutral conditions, we decided to calculate the reaction free energies for the overall alkylation reaction (ΔG, in Table 3) and the activation free energy for the alkylation reversal pathway ($\Delta G^{\ddagger}_{rev}$, in Table 3), with the aim to achieve a validation of our computational model by comparison with known experimental data. In addition, this approach allowed us to evaluate how the protonation at the oxygen atom of **CPI** and solvent bulk effects may control the adduct stability.

It is known experimentally that covalent DNA adducts of the antitumor antibiotic (+)-CC-1065 and its analogues undergo reverse reaction in aqueous−organic solvents to regenerate the initial **CPI** alkylating structures and unmodified DNA.[30] In more detail, the alkylation reversal pathway is not negligible at 37 °C under neutral conditions (pH 7.4), and it is accelerated by rising the pH up to 8.4.[30]

The alkylation adduct arising from the covalent modification at N3 nitrogen atom of 9-methyladenine by the model compound **1** has been optimized both in the gas phase and in water solution (**P**). Such an adduct displays a well formed C−N bond (1.48 Å in gas phase and 1.50 Å in water solution) and a dipolar character with well developed anionic charge located on the oxygen atom. Due to its basicity (comparable to a phenoxide ion), the anionic oxygen atom is likely to be mainly protonated under neutral condition. Therefore, we have also optimized the protonated form of the alkylated adduct (**P**−**H**$^+$).

The alkylation adduct in the zwitterionic form (**P**) is thermodynamically unstable in gas phase, with a reaction free energy of +35.9 kcal mol^{-1} (relative to free reactants, see Table 3). Although the solvation by the aqueous solution strongly stabilizes the adduct due to its dipolar nature (μ = 16.6 D, 31.9 D in gas phase and in aqueous solution, respectively), **P** still is less stable than free reactants by 21.6 kcal mol^{-1}. This suggests that when the alkylation adduct is mainly in its deprotonated form (**P**) the alkylation reversal pathway, displaying an activation energy of 19.3 kcal mol^{-1} in water solution, becomes an available reacting channel. This computational consideration is in fairly good agreement with the experimental data on adozelesin-DNA adduct stability at pH 8.4,[30] from which is possible to estimate an activation free energy at 55 °C of 25 kcal/mol. The discrepancy between experimental and computational data could be partially due to the fact that at pH 8.4 the adduct is not mainly in its zwitterionic form.

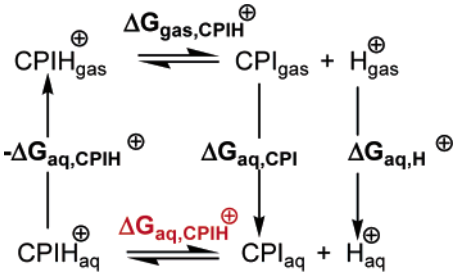
The protonation of **P** at the oxygen atom, which should occur promptly under physiological conditions, owing to its basic character, strongly stabilizes the adduct **P-H**$^+$, which becomes 6.2 kcal mol^{-1} more stable than the free reactants (**1-H**$^+$ and **9-MeA**) in gas phase. The Gibbs free energy of **P-H**$^+$ is very similar to that of the free reactants (**1-H**$^+$ and **9-MeA**), in water solution [−0.3 and +2.7 kcal mol^{-1}, using 6-31G(d) and 6-31+G(d,p) basis sets, respectively]. These data suggest that the alkylation process of **9-MeA** by CPI (**1**) in aqueous solution is a reversible process. Considering the reversibility of the alkylation process involving the free reactants (**9-MeA** and **1**) in aqueous solution, additional stabilizing interactions between DNA-alkylated adduct and DNA itself (i.e.: hydrogen bonding and electrostatic interactions) must play an important role in stabilizing the covalent damage to DNA by CPI.[31]

Our computational data also suggest that when the alkylation adduct is mainly in its protonated form (**P-H**$^+$) the alkylation reversal pathway, disclosing an acti-

(30) Warpehoski, M. A.; Harper, D. E.; Mitchell M. A.; Monroe T. J. *Biochemistry* **1992**, *31*, 2502.

(31) Further investigations on the effect of hydrogen-bonding with the DNA on the alkylation adduct stability is in progress in our group.

SCHEME 2. Thermochemical Cycle Used To Evaluate the Reaction Free Energy of the CPIH$^+$ Dissociation Process ($\Delta G_{aq,CPIH^+}$) in Aqueous Solution



vation energy of 28.2 kcal mol^{-1}, becomes much slower in comparison to the same process starting from the adduct in its dipolar form (**P**). These data are in exceptionally good agreement with the experimental evaluation on adduct stability at pH 7.4,[30] from which it has been possible to estimate an activation free energy at 55 °C of 27 kcal/mol. Such an excellent agreement with the experimental data on adduct stability under neutral conditions represent a validation of our computational model.

In addition, our data represent a quantitative evaluation of the important destabilization of the alkylation adducts induced by the deprotonation of the hydroxyl moiety.

4. Acidity of the Conjugate Acid CPIH$^+$. A computational procedure for the evaluation of absolute pK_a's has been recently accomplished for several carboxylic acids by Barone using a Born−Haber cycle at the PBE0/6-31G(d,p) level using the PCM solvation model with the standard UAHF atomic radii.[32] We have evaluated the CPIH$^+$ acidity applying a similar approach to the thermochemical cycle in Scheme 2.

$$\begin{aligned}\Delta G_{aq,CPIH^+} &= G_{gas,CPI} - G_{gas,CPIH^+} + \Delta G_{aq,CPI} - \\ &\qquad \Delta G_{aq,\,CPIH^+} + G_{gas,H^+} + \Delta G_{aq,H^+} \\ &= G_{gas,CPI} - G_{gas,CPIH^+} + \Delta G_{aq,CPI} - \\ &\qquad \Delta G_{aq,\,CPIH^+} - 270.26 \\ &= G_{aq,CPI} - G_{aq,CPIH^+} - 270.26 \qquad \text{(I)}\end{aligned}$$

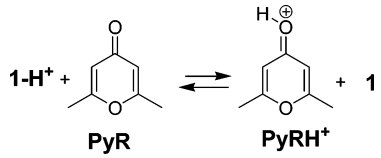
In this thermochemical cycle, the proton energy terms free energy in the gas phase ($G_{gas,H^+} = -6.28$ kcal mol^{-1})[33] and solvation ($\Delta G_{aq,H^+} = -263.98$ kcal mol^{-1}),[34] similarly to Barone's strategy, have been taken from the most recent experimental values. The free energy in the gas phase and the solvation energies for CPI **1** and its conjugated acid (CPIH$^+$) have been computed by polarizable continuum models (C-PCM and the most recent implementation of PCM). We used both the hybrid B3LYP functional with the 6-31+G(d,p) basis set for geometry optimization. The calculation of harmonic frequencies both in gas phase and water solution and the evaluation of the non potential energy contribution to the reaction free energy has been accomplish at B3LYP/6-31G(d) level of theory. The data are all listed in Table 4.

TABLE 4. Aqueous Solution Free Energy Data and Corresponding CPIH$^+$ pK_a at B3LYP/6-31+G(d,p) Level of Theory with C-PCM and PCM Solvation Models

substrate	G_{solv}[a]	ΔG_{solv}[b]	$\Delta\Delta G_{solv}$	$\Delta G_{aq,CPIH^+}$[c]	pK_a
	C-PCM B3LYP/6-31+G(d,p)				
CPI	−932.069043	−18.56	36.01	4.54	3.3
CPIH$^+$	−932.503955	−54.57			
	PCM B3LYP/6-31+G(d,p)				
CPI	−932.069071	−18.57	36.07	4.60	3.4
CPIH$^+$	−932.504074	−54.64			

[a] Gibbs free energies in aqueous solution: ($G_{solv} = E_{solv} + \delta G_{solv}$, hartree). E_{solv} = energy in solution without the thermal correction. −932.289243 (CPI), −932.735186 (CPIH$^+$), [CPCM-B3LYP/6-31+G(d,p)]. −932.289271 (CPI), −932.735305 (CPIH$^+$), [PCM-B3LYP/6-31+G(d,p)]. δG_{solv} = thermal correction to Gibbs free energy in aqueous solution: 0.220200 (CPI), 0.231231 (CPIH$^+$), at C-PCM-B3LYP/6-31G(d) level of theory. [b] Gibbs free solvation energy $\Delta G_{solv} = G_{solv} - G$. Gas-phase Gibbs free energies ($G = E + \delta G$, hartree) −932.0394651 (CPI), −932.4169923 (CPIH$^+$), [B3LYP/6-31+G(d,p)]. Gas-phase potential energies (E_{gas}, hartree): −932.2623321 (CPI), −932.6529003 (CPIH$^+$), [B3LYP/6-31+G(d,p)]; thermal correction to Gibbs free energy in gas phase, (δG, hartree): 0.222867 (CPI), 0.235908 (CPIH$^+$), at B3LYP/6-31G(d) level of theory. [c] $\Delta\Delta G_{solv} = \Delta G_{solv,CPI} - \Delta G_{solv,CPIH+}$

SCHEME 3. Proton-Transfer Reaction from CPIH$^+$ (1-H$^+$) to 2,6-Dimethyl-4-pyrone (PyR)



The computed pK_a value of CPIH$^+$ using UAHF radii and CPCM salvation method, according to eqs I and II, is 3.3.

$$pK_a = \Delta G_{aq,CPIH^+}/2.303RT \qquad \text{(II)}$$

We have also estimated the pK_a of protonated CPI **1** (CPIH$^+$) by an alternative protocol, which has been used recently and successfully to evaluate the pK_a for twisted amide.[35] Considerable cancellation of errors is expected if relative pK_a's are evaluated instead of absolute pK_a's. This requires the choice of a similar reference molecule for which the experimental pK_a is known. Using a thermodynamic cycle with the reference molecule avoids the need to deal with the proton solvation free energy, for which several different values have been reported.[34,36] It is also important, in the choice of the reference molecule, that the number of charged species is conserved on both sides of the proton-transfer chemical reaction (such as in Scheme 3), for which the free energy difference in aqueous solution (ΔG_{aq}) has been computed at B3LYP/6-31+G(d,p) level of theory, using both PCM and C-PCM polarizable continuum model for solvation effects.

In this present work, we used 2,6-dimethyl-4-pyrone (**PyR**) as the reference molecule, evaluating the free

(32) Saracino, G. A. A.; Improta, R.; Barone, V. *Chem. Phys. Lett.* **2003**,, *373*, 411.
(33) Topol, I. A.; Tawa, G. J.; Burt, S. K.; Rashin, A. A. *J. Chem. Phys.* **1999**, *111*, 10998.
(34) Tissandier, M. D.; Cowen, K. A.; Feng, W. Y.; Gundluach, E.; Cohen, M. H.; Earhart, A. D.; Coe, J. V.; Tuttle, T. R. *J. Phys.Chem. A.* **1998**, *102*, 7787.
(35) Mujika, J. I.; Mercero, J. M.; Lopez, X. *J. Phys.Chem. A.* **2003**, *107*, 6099.
(36) Lyne, P.; Karplus, M. *J. Am. Chem. Soc.* **2000**, *122,* 166.

energy change corresponding to the proton-transfer reaction in Scheme 3.

PyR is a carbonyl compound, with structural and electronic features quite similar to those of CPI, for which the experimental pK_a has been experimentally measured (pK_a = +0.4).[37]

The pK_a of the **1-H**$^+$ (CPIH$^+$) has been calculated from the computed ΔG_{aq} value for the proton-transfer reaction in Scheme 3 and the experimental pK_a of **PyR**, according to the equation III.

$$\text{p}K_a\,(\text{CPIH}^+) = \Delta G_{aq}(2.303 \ln RT) + \text{p}K_{a\,exp}(\mathbf{PyRH}^+) \quad \text{(III)}$$

Two slightly different values of ΔG_{aq} (+2.80 and +3.01 kcal mol^{-1}) have been computed at the B3LYP/6-31+G(d,p) level of theory using PCM and C-PCM solvation models, respectively. The above ΔG_{aq} values suggest that CPIH$^+$ displays a comparable acidity (pK_a ~ 2.4) to H_3PO_4 (pK_a = 2.30, corrected by statistical factors).[38]

Therefore, for a balanced evaluation of the catalytic effects of the specific acid catalysis one must take into account CPIH$^+$ concentration effect under physiological condition (pH 7). If we do so, we should add to the catalytic effect of H_3O^+ complexation computed above (−8.6 kcal mol^{-1}) the term $-RT$ ln[H$^+$]/K_a (+6.3 kcal mol^{-1}). This correction reduces significantly the catalytic effect of H_3O^+ complexation on CPI to only −2.3 kcal mol^{-1}, under physiological conditions. Therefore we can state that specific acid catalysis although operative is slightly less effective than the catalysis associated to cationic complexation.

5. Approximate Evaluation of the Entropic Effects on the Reactivity of DNA-Complexed CPI. It is possible to set an upper limit to the catalytic effect associated to the binding of duocarmycins to the DNA. The kinetic advantage resulting from complexation of CPI to DNA may be roughly assumed as corresponding to the differences in the loss of translation and rotational entropy of free reactants relative to the TS **1**. This approach is very approximate (because it has been done for a gas-phase model and it neglects the presence of low frequencies in the TS, resulting in a significant underestimation of TS entropy); however, it allows us to get a rough evaluation of the maximum entropic advantage induced by the approximation of the reactant CPI (**1**) to DNA within the complex. The difference in total loss of translation and rotational entropy (see Table 5) should lower the activation free energy by −17.4 kcal mol^{-1}. This evaluation of the entropic contribution to the catalysis is very approximate, and it should be verified by a molecular dynamic simulation. However, it is important to underline that the favorable contribution to the activation free energy being a maximum limit allows saying that the other catalytic effects computed in this paper, in particular ammonium cation-complexation, are not negligible.

TABLE 5. Translational and Rotational Contribution to the Reaction Entropy for the Alkylation Reaction of 9-Methyladenine by CPI 1 (in cal/M K) in Gas Phase at B3LYP/6-31G(d) Level

	$\Delta S_{translational}$	$\Delta S_{rotational}$
1	42.8	34.0
9MeA	40.9	29.6
S1	44.0	36.6

Conclusion

The present calculations indicate that the general acid catalysis and surprisingly also the specific acid catalysis under physiological conditions (neutral water solution) play a minor role in the DNA alkylation, because the conjugate acid of CPI shows a quite low pK_a value (~2.4). On the other hand, the catalytic effect associated to cations-complexation (Na^+ and NH_4^+) is more important because it causes a decrease in the activation energy by −4 kcal mol^{-1}. This catalytic effect could be even greater due to the high local concentration of Na^+ and NH_4^+ cations on the phosphate backbone. All of these catalytic effects, in particular the cationic catalysis, should not be negligible in comparison to the favorable entropic factors related to precomplexation of the drug.

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Supporting Information Available: Energies and Cartesian coordinates of stationary points in Figures 1−4, in the gas phase and in water solution (C-PCM), optimized at the B3LYP/6-31G(d) level of theory. This material is available free of charge via the Internet at http://pubs.acs.org.

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