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Selectivity of Purine Alkylation by a Quinone Methide. Kinetic or Thermodynamic Control?

Mauro Freccero,* Remo Gandolfi, and Mirko Sarzi-Amadè.

Dipartimento di Chimica Organica, Università di Pavia, Viale Taramelli 10, 27100 Pavia, Italy

mauro.freccero@unipv.it

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The alkylation reaction of 9-methyladenine and 9-methylguanine (as prototype substrates of deoxyadenosine and -guanosine), by the parent *o*-quinone methide (*o*-QM), has been investigated in the gas phase and in aqueous solution, using density functional theory at the B3LYP/6-311+G(d,p) level. The effect of the medium on the reactivity, and on the stability of the resulting adducts, has been investigated by using the C-PCM solvation model to assess which adduct arises from the kinetically favorable path, or from an equilibrating process. The calculations indicate that the most nucleophilic site of the methyl-substituted nucleobases in the gas phase is the guanine oxygen atom (O^6) ($\Delta G^{\ddagger}_{gas} = 5.6$ kcal mol^{-1}), followed by the adenine N1 ($\Delta G^{\ddagger}_{gas} = 10.3$ kcal mol^{-1}), while other centers exhibit a substantially lower nucleophilicity. The bulk effect of water as a solvent is the dramatic reduction of the nucleophilicity of both 9-methyladenine N1 ($\Delta G^{\ddagger}_{solv} = 14.5$ kcal mol^{-1}) and 9-methylguanine O^6 ($\Delta G^{\ddagger}_{solv} = 17.0$ kcal mol^{-1}). As a result there is a reversal of the nucleophilicity order of the purine bases. While O^6 and N7 nucleophilic centers of 9-methylguanine compete almost on the same footing, the reactivity gap between N1 and N7 of 9-methyladenine in solution is highly reduced. Regarding product stability, calculations predict that only two of the adducts of *o*-QM with 9-methyladenine, those at NH_2 and N1 positions, are lower in energy than reactants, both in the gas phase and in water. However, the adduct at N1 can easily dissociate in water. The adducts arising from the covalent modification of 9-methylguanine are largely more stable than reactants in the gas phase, but their stability is markedly reduced in water. In particular, the oxygen alkylation adduct becomes slightly unstable in water ($\Delta G_{solv} = +1.4$ kcal mol^{-1}), and the N7 alkylation product remains only moderately more stable than free reactants ($\Delta G_{solv} = -2.8$ kcal mol^{-1}). Our data show that site alkylations at the adenine N1 and the guanine O^6 and N7 in water are the result of kinetically controlled processes and that the selective modification of the *exo*-amino groups of guanine N2 and adenine N6 are generated by thermodynamic equilibrations. The ability of *o*-QM to form several metastable adducts with purine nucleobases (at guanine N7 and O^2, and adenine N1) in water suggests that the above adducts may act as *o*-QM carriers.

Introduction

Quinone methides (QMs)[1] are reactive intermediates involved in the biochemistry of several antitumor compounds and antibiotic drugs,[2] where they form covalent linkages with DNA bases. DNA cross-linking,[3] which is often responsible for cytotoxic effects, is the result of two consecutive alkylating steps, both involving QMs.[4]

The bioactivity of QMs has been attributed to their electrophilic nature, which is comparable to that of stabilized carbocations.[5] In fact, QMs react as Michael acceptors, adding nucleophiles at the exocyclic methylene group to form benzylic adducts (Scheme 1). Alkylation of simple sulfur-,[5b,c] nitrogen-, and oxygen-centered nucleophiles by quinone methides has been experimentally[6] and computationally investigated.[7] Their reactivity has also been experimentally studied with biological nucleo-

(1) (a) Peter, M. G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 555 and references therein. (b) McDonald, I. A.; Nyce, P. L.; Jung, M. J.; Sabol, J. S. *Tetrahedron Lett.* **1991**, *32*, 887. (c) Wang, Q.; Dechert, U.; Jirik, F.; Withers, S. G. *Biochem. Biophys. Res. Commun.* **1994**, *200*, 577. (d) Cabaret, D.; Adediran, S. A.; Garcia Gonzales, M. J.; Pratt, R. F.; Wakselman, M. *J. Org. Chem.* **1999**, *64*, 713. (e) Myers, J. K.; Cohen, J. D.; Widlanski, T. S. *J. Am. Chem. Soc.* **1995**, *117*, 11049. (f) Stowell, J. K.; Widlanski, T. S.; Kutateladze, T. G.; Raines, R. T. *J. Org. Chem.* **1995**, *60*, 6930.

(2) (a) Tomasz, M.; Das, A.; Tang, K. S.; Ford, M. G. J.; Minnock, A.; Musser, S.; Waring, M. J. *J. Am. Chem. Soc.* **1998**, *120*, 11581. (b) Han, I.; Russel, J.; Kohn, H. *J. Org. Chem.* **1992**, *57*, 1799. (c) Angle, S. R.; Rainier, J. D.; Woytowicz, C. *J. Org. Chem.* **1997**, *62*, 5884. (d) Gaudiano, G.; Frigerio, M.; Bravo, P.; Koch, T. H. *J. Am. Chem. Soc.* **1990**, *112*, 6704.

(3) (a) Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723. (b) Wolkenbergand, S. E.; Boger, D. L. *Chem. Rev.* **2002**, *102*, 2477. (c) Thuong, N. T.; Hélène, C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 666. (d) Chang, A. Y.; Dervan, P. B. *J. Am. Chem. Soc.* **2000**, *122*, 4856. (e) Zhou, A.; Duan, W.; Simmons, D.; Shayo, Y.; Raymond, M. A.; Dorr, R. T.; Hurley, L. H. *J. Am. Chem. Soc.* **2001**, *123*, 4856. (f) Bando, T.; Iida, H.; Saito, I.; Sugiyama, H. *J. Am. Chem. Soc.* **2001**, *123*, 5158. (g) Zhou, Q.; Pande, P.; Johnson, A. E.; Rokita, S. E. *Bioorg. Med. Chem.* **2001**, *9*, 2347.

(4) (a) Zeng, Q.; Rokita, S. E. *J. Org. Chem.* **1996**, *61*, 9080. (b) Nakatani, K.; Higashida, N.; Saito, I. *Tetrahedron Lett.* **1997**, *38*, 5005. (c) Wang, P.; Liu, R.; Wu, X.; Ma, H.; Cao, X.; Zhou, P.; Zhang, J.; Weng, X.; Zhang, X.-L.; Qi, J.; Zhou, X.; Weng L. *J. Am. Chem. Soc.* **2003**, *125*, 1115.

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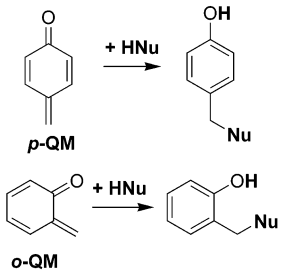
SCHEME 1

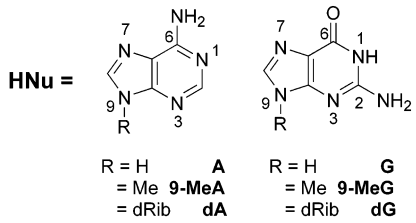


philes such as free amino acids,[6] oligopeptides,[6,8] and DNA nucleobases.[4c,9−11]

Site specificity in the alkylation processes of DNA bases is still a matter of intensive investigation. In fact, it has been experimentally studied by numerous researchers, using a wide variety of reagents, such as diazonium and phenylnitrenium ions,[12] carbocations,[13] benzyl halides,[14] epoxides,[15] and both *p*-QMs[10,16] and *o*-QMs.[9,11]

Intrinsic nucleophilicity[17] is certainly an important factor in governing competition among the various nucleophilic centers in DNA bases. It correlates with the electrostatic potential,[18−20] and it is independent of the reaction partners. However, the nucleophilicity as directly measured by alkylation rates is a different entity and it seldom appears to be transferable among different alkylating reactants. The origin of such a discrepancy is likely due to specific interactions between the substrate and the alkylating agent and to solvent effects. Among such interactions, H-bonding should play a key role in the chemoselectivity control. From this point of view *o*-QM is an interesting model of a polarizable alkylating agent with H-bonding acceptor properties, since its reactivity is highly enhanced by protic solvent[6,7] and by acid catalysis.[6,21]

SCHEME 2



The experimental selectivity of QM-like structures obtained from product distribution analysis appears to be different in comparison to other alkylating agents without H-bonding properties. In fact, QMs are likely to selectively attack the *exo*-amino groups of guanine (N^2) and adenine (N^6), rather than guanine N7 or adenine N1,[9b,11,22−24] which are generally recognized as the most intrinsic nucleophilic sites (see Scheme 2 for numbering). Actually, the 2-amino group of guanine is the most reactive site toward activated Mitomycin C (or other *o*-QM derivatives) among the nucleophilic centers present in the DNA bases.[25] Recently, Rokita and co-workers showed that the "most nucleophilic site of **dA** (deoxyadenosine)**-N1** preferentially, but reversibly, conjugates to a model *ortho*-quinone methide". Such a result clearly suggests that "thermodynamic rather than kinetic" aspects should play an additional and important role in the control of selectivity.[11a] Although the same author suggested that "attention to kinetic and thermodynamic selectivity will no doubt enhance our ability to predict modification of DNA",[11a] thermodynamic aspects [namely the stability of the alkylation adduct] have seldom been analyzed[10,11a,16,26] and thoroughly evaluated besides kinetic parameters (activation free energies for each possible reaction pathway). The potential value of quantum chemical calculations in looking at all the possible reaction pathways and adduct stability was anticipated by Scribner 30 year ago.[27] Since that time many theoretical papers have successfully tried to model physical and chemical properties of nucleobases (natural and modi-

(5) (a) Richard, J. P.; Amyes, T. L.; Toteva, M. M. *Acc. Chem. Res.* **2001**, *34*, 981. (b) Richard, J. P.; Crugeiras, J. *J. Am. Chem. Soc.* **2000**, *122*, 1664. (c) Richard, J. P.; Amyes, T. L.; Bei, L.; Stubblefield, V. *J. Am. Chem. Soc.* **1990**, *112*, 9513. (d) McClelland, R. A.; Moriarty, M. M.; Chevalier, J. M. *J. Chem Soc., Perkin Trans. 2* **2001**, 2235.

(6) (a) Modica, E.; Zanaletti, R.; Freccero, M.; Mella, M. *J. Org. Chem.* **2001**, *66*, 41. (b) Zanaletti, R.; Freccero, M. *Chem. Commun.* **2002**, 1908.

(7) Di Valentin, C.; Freccero, M.; Zanaletti, R.; Sarzi-Amadè, M. *J. Am. Chem. Soc.* **2001**, *123*, 8366.

(8) (a) McCracken, P. G.; Bolton, J. L.; Thatcher, G. R. J. *J. Org. Chem.* **1997**, *62*, 1820. (b) Bolton, J. L.; Turnipseed, S. B.; Thompson, J. A. *Chem.-Biol. Interact.* **1997**, *107*, 185. (c) Bolton, J. L.; Valerio, L. G.; Thompson, J. A. *Chem. Res. Toxicol.* **1992**, *5*, 816. (d) Thompson, D. C.; Perera, K.; Krol, E. S.; Bolton, J. L. *Chem. Res. Toxicol.* **1995**, *8*, 323.

(9) (a) Rokita, S. E.; Yang, J.; Pande, P.; Shearer, J.; Greenberg, W. A. *J. Org. Chem.* **1997**, *62*, 3010. (b) Pande, P.; Shearer, J.; Yang, J.; Greenberg, W. A.; Rokita, S. E. *J. Am. Chem. Soc.* **1999**, *121*, 6773.

(10) Lewis, M. A.; Graff Yoerg, D.; Bolton, J. L.; Thompson, J. A. *Chem. Res. Toxicol.* **1996**, *9*, 1368.

(11) (a) Veldhuyzen, W. F.; Shallop, A. J.; Jones, R. A.; Rokita, S. E. *J. Am. Chem. Soc.* **2001**, *123*, 11126. (b) Veldhuyzen, Lam, Y.-F.; Rokita, S. E. *Chem. Res. Toxicol.* **2001**, *14*, 1345.

(12) Parks, J. M.; Ford, G. P.; Cramer, C. J. *J. Org. Chem.* **2001**, *66*, 8997.

(13) Blans, P.; Fishbein, J. C. *Chem. Res. Toxicol.* **2000**, *13*, 431.

(14) (a) Moschel, R. C.; Hudgins, R. W.; Dipple, A. *J. Org. Chem.* **1986**, *51*, 4180. (b) Moon, K.-Y.; Moschel, R. C. *Chem. Res. Toxicol.* **1998**, *11*, 696.

(15) (a) Nakatani, K.; Okamoto, A.; Matsuno, T.; Saito, I. *J. Am. Chem. Soc.* **1998**, *120*, 11219. (b) Barlow, T.; Dipple, A. *Chem. Res. Toxicol.* **1998**, *11*, 44.

(16) (a) Zhou, Q.; Turnbull, K. D. *J. Org. Chem.* **2001**, *66*, 7072. (b) Zhou, Q.; Turnbull, K. D. *J. Org. Chem.* **2000**, *65*, 2022. (c) Zhou, Q.; Turnbull, K. D. *J. Org. Chem.* **1999**, *64*, 2847.

(17) Lewis, E. D.; Douglas, T. A.; McLaughlin, M. L. A Quantitative Measure of Nucleophilic Character. In *Nucleophilicity*; Harris, J. M., McManus, S. P., Eds.; American Chemical Society: Washington, DC, 1987; Chapter 3, pp 35−49.

(18) (a) Bagdassarian, C. K.; Schramm, V. L.; Schwartz, S. D. *J. Am. Chem. Soc.* **1996**, *118*, 8825. (b) Pullman, A.; Pullman, B. *Q. Rev. Biophys.* **1981**, *14*, 289.

(19) For a comprehensive review of the use of molecular electrostatic potentials for the comparison and description of stable molecules see ref 18.

(20) Tasi, G.; Palinko, I. *Top. Curr. Chem.* **1995**, *174*, 45.

(21) (a) Chiang, Y. A.; Kresge, J.; Zhu, Y. *J. Am. Chem. Soc.* **2001**, *123*, 8089. (b) Chiang, Y. A.; Kresge, J.; Zhu, Y. *J. Am. Chem. Soc.* **2000**, *122*, 9854. (c) Chiang, Y. A.; Kresge, J.; Zhu, Y. *J. Am. Chem. Soc.* **2002**, *124*, 717. (d) Wan, P.; Barker, B.; Diao, L.; Fisher, M.; Shi, Y.; Yang, C. *Can. J. Chem.* **1996**, *74*, 465. (e) Brousmiche, D.; Wan, P. *Chem. Commun.* **1998**, 491.

(22) (a) Ouyang, A.; Skibo, E. B. *Biochemistry* **2000**, *39*, 5817. (b) Ouyang, A.; Skibo, E. B. *J. Org. Chem.* **1998**, *63*, 1893.

(23) Egholm, M.; Koch, T. H. *J. Am. Chem. Soc.* **1989**, *111*, 8291.

(24) Woo, J.; Sigurdsson, S. T.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 3407.

(25) Williams, R. M.; Herberich, B. *J. Am. Chem. Soc.* **1998**, *120*, 10272.

(26) (a) Tomasz, M.; Lipman, R.; Verdine, G.; Nakanishi, K. *Biochemistry* **1986**, *25*, 4337. (b) Zewail-Foote, M.; Hurley, L. H. *J. Am. Chem. Soc.* **2001**, *123*, 6485.

(27) Ford, G. P.; Scribner, J. D. *J. Am. Chem. Soc.* **1983**, *105*, 349.

fied)[28,29] and base pairs,[30] but the evaluation of models for DNA modification by alkylating agents has been to our knowledge sporadic,[31,32] or limited to a few simple alkylating agents, using semiempirical molecular orbital calculation in the gas phase.[33] The computational approach at the ab initio level has been used to establish a correlation between site specificity of the DNA alkylation reactions and the ionization potential of the nucleotide components.[34] Recently, we began to address the role of H-bonding in the alkylation of NH_3, water, cytosine, and **1-MeC** by the parent *o*-QM, both in the gas phase and in water bulk.[7,35] Therefore, we believe we have a model to asses the role of specific interactions and solvent effects in the control of the complex regiochemistry involved in the interaction of electrophiles and DNA bases. The present study is a follow up of the previous work and it is focused on alkylation reactions of adenine and guanine, and their related methyl analogues. To clarify the controlling factors of the *o*-QM regiochemistry toward purine bases, 9-methyladenine (**9-MeA**) and 9-methylguanine (**9-MeG**) have been chosen as prototype substrates of deoxyadenosine (**dA**) and deoxyguanosine (**dG**). In this work, we computationally investigate (i) the nucleophilicity (i.e., a kinetic property) of the purines bases, measured by the activation energies of the competing alkylation pathways, in reactions involving the H-acceptor alkylating *o*-QM, and (ii) a thermodynamic parameter such as the alkylation adduct stability, in the gas phase and water solution. The aim is to examine whether there is a correlation between the most prevalent experimentally observed *o*-QM-adducts and the above parameters, to asses the QM's mode of action as a DNA alkylating agent.

Computational Methods

The B3LYP method is now well-established as a method that can compute potential energy surfaces (PES) for organic reactions described by a single electronic configuration. The suitability of DFT theory for reliably describing hydrogen-bonded systems has been the subject of many investigations,[36] and such calculations have proved quite useful for studying hydrogen-bonded complexes.[37] The B3LYP functional in particular has proven highly effective, at least as long as an appropriate basis set is used.[38] Basis set extensions with polarization function also for hydrogen [i.e., B3LYP/6-311G(d,p)] as well as introduction of diffuse functions [i.e., B3LYP/6-311+G(d,p)] are certainly useful to properly describe lone pairs and hydrogen-bonding interactions,[7] which are very important in controlling the reactivity and selectivity of *o*-QM in the alkylation reactions of nucleobases. The usual dilemma between high computational cost and low-level calculations can be satisfactorily solved for the systems under study by carrying out geometry optimization at the B3LYP/6-31G(d) level and improving the energy description by single-point calculations with more extended basis sets. In fact, we have shown (by studying the *o*-QM alkylation reaction of ammonia, water, and hydrogen sulfide) that optimized TS geometries do not change on going from B3LYP/6-31G(d) to B3LYP/6-311+G(d,p) methods.[7] However, not only the absolute but also the relative energies of stationary points can change appreciably.[7] In particular, a significant variation in absolute and relative energies takes place when the diffuse and polarization functions are introduced [i.e., on going from the B3LYP/6-31G(d) to the B3LYP/6-311+G(d,p) method]. Fortunately, probably as a result of the remarkable geometry constancy when basis set is changed, higher level single-point calculations on B3LYP/6-31G(d) optimized geometries very closely reproduce the relative energies obtained by the corresponding higher level full optimization procedures (as clearly documented by our previous investigation on *o*-QM and three prototype nucleophiles NH_3, H_2O, and H_2S).[7] Thus, we report the B3LYP/6-31G(d) fully optimized geometry of stationary points and related energies as well as the B3LYP/6-311+G(d,p)//B3LYP/6-31G(d) energies. All calculations were carried out with the Gaussian 94[39] and Gaussian 98[40] program packages. To confirm the nature of the stationary points and to produce theoretical activation parameters, vibrational frequencies (in the harmonic approximation) were calculated for all the optimized structures and used, unscaled, to compute the zero-point energies, their thermal corrections, the vibrational entropies, and their contributions to activation enthalpies, entropies, and activation Gibbs free energies (simply called in the paper activation free energies). The computed relative (to reactants) electronic energies for transition structures with the thermodynamic activation parameters [at the B3LYP/6-31G(d) level], obtained from gas-phase vibrational frequencies, are listed in Tables 1 and 3 respectively for adenine and guanine alkylation processes.

The computed enthalpy, entropy, and free energy were converted from the 1 atm standard state into the standard state of molar concentration (ideal mixture at 1 mol L^{-1} and 1

(28) (a) Stewart, E. L.; Foley, C. K.; Allinger, N. L.; Bowen, J. P. *J. Am. Chem. Soc.* **1994**, *116*, 7282. (b) Del Bene, J. E. *J. Phys. Chem.* **1983**, *87*, 367. (c) Sponer, J.; Hobza, P. *J. Phys. Chem.* **1983**, *87*, 367. (d) Roberts, C.; Bandaru, R.; Switzer, C. *J. Am. Chem. Soc.* **1997**, *119*, 4640.

(29) Nowak, M. J.; Lapinski, L.; Kwiatkowski, J. S.; Leszczynsky, J. *Computational Chemistry: Reviews of Current Trends*; Leszczynsky, J., Ed.; World Scientific Publishing: Singapore, 1997; Vol. 2, Chapter 4, and references therein.

(30) (a) Fonseca Guerra, C.; Bickelhaupt, F. M.; Snijders, J. G.; Baerends, E. J. *J. Am. Chem. Soc.* **2000**, *122*, 4117. (b) Gould, I. R.; Kollman, P. A. *J. Am. Chem. Soc.* **1994**, *116*, 2493. (c) Floriàn, J.; Leszczynski, J. *J. Am. Chem. Soc.* **1996**, *118*, 3010.

(31) Ford, G. P.; Scribner, J. D. *Chem. Res. Toxicol.* **1990**, *3*, 219.

(32) Dannenberg, J. J.; Tomasz, M. *J. Am. Chem. Soc.* **2000**, *122*, 2062.

(33) Ford, G. P.; Scribner, J. D. *J. Am. Chem. Soc.* **1983**, *105*, 349.

(34) Zhu, Q.; LeBreton, P. R. *J. Am. Chem. Soc.* **2000**, *122*, 12824 and references therein.

(35) Freccero, M.; Di Valentin, C.; Sarzi-Amadè, M. *J. Am. Chem. Soc.* **2003**, *125*, 3544.

(36) Guo, H.; Sirois, S.; Proynov, E. I.; Salaub, D. R. In *Theoretical Treatment of Hydrogen Bonding*; Hadzy, D., Ed.; Wiley: New York, 1997.

(37) Rablen, P. R.; Lockman, J. W.; Jorgensen, W. L. *J. Phys. Chem.* **1998**, *102*, 3782.

(38) (a) Del Bene, J. E.; Person, W. B.; Szczepaniak, K. *J. Phys. Chem.* **1995**, *99*, 10705. (b) Kim, K.; Jordan, K. D. *J. Phys. Chem.* **1994**, *98*, 10089.

(39) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94*, Revision E.3; Gaussian, Inc.: Pittsburgh, PA, 1995.

(40) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, Revision A.7; Gaussian, Inc.: Pittsburgh, PA, 1998.

atm)[41] to allow a direct comparison with the experimental result in water solution.[6]

The contributions of bulk solvent effects to the activation free energy of the reactions under study were calculated via the self-consistent reaction field (SCRF) method, using the conductor version of PCM (C-PCM)[42] employing the HF parametrization of Barone's united atom topological model (UAHF),[43] as implemented in Gaussian 98. Such a model includes the nonelectrostatic terms (cavitation, dispersion, and repulsion energy) in addition to the classical electrostatic contribution. For all PCM-UAHF calculations, the number of initial tesserae per atomic sphere was set to 60 as in the default. The solvation effect has been evaluated in water solution by single-point calculation (i.e., with unrelaxed gas-phase reactant and TS geometries) at the B3LYP/6-31G(d) level and used to evaluate both the B3LYP/6-31G(d) and B3LYP/6-311+G(d,p)//B3LYP/6-31G(d) free energies in aqueous solution.

We here report only bulk solvent effects (with the C-PCM model) for the alkylation processes at adenine and guanine nitrogen atoms (and their 9-methyl derivatives), neglecting the role of the water-assisted mechanism. The reasons for such a choice reside on the results reported in our previous papers,[7,34] where we demonstrated that *o*-QM alkylation of nitrogen nucleophiles in water solution can be well described with a simple reaction mechanism, where no water molecule is directly involved in the process. However, we have also shown in the same papers that for both nucleophilic addition of water[7] and cytosine oxygen atom[34] onto *o*-QM in water, a mechanism specifically assisted by a water molecule enters a balanced competition with the unassisted process. Consequently, the importance of specific solvent effects (through a water-assisted mechanism) has been explored for the *o*-QM alkylation at the guanine oxygen atom.

Results and Discussion

1. Alkylation of Adenine and 9-Methyladenine by *o*-QM.

1.1. Alkylation Under Thermodynamic Control. Purine base adducts exhibit prototropic tautomerism that can be generally classified into two types. The first one is connected to a hydrogen atom transfer of the ring substituent (the NH_2- group of adenine and the NH_2- or HO− group of guanine). The second type of tautomerism of purine adducts is connected to the mobility of the proton attached at the imidazole ring nitrogen (N9). The first type of prototropic equilibrium, unlike the second one, generates similar tautomers for purine adducts and their 9-methyl derivatives. Obviously, only the first type of tautomers can be assumed as a model to evaluate nucleoside adduct stability.

Exploring the potential energy surface (PES) of the alkylation reaction of adenine and *o*-QM, we located two pre-reaction complexes (**IA** and **IA′**) and several adducts, in the gas phase (Scheme 3). Both **IA** and **IA′** are slightly less stable than reactants. Inclusion of the bulk solvent effect strongly destabilizes them by more than +9 kcal mol^{-1}. Since **IA** and **IA′** are in fast equilibration with free reactants, we will neglect them, and throughout we will consider free activation energy data relative to free reactants.

Among the adenine alkylation adducts classified into the first type of prototropic tautomerism (**P1**−**P4**), **P1** and **P2** (arising respectively from NH_2 and N1 alkylation processes) are thermodynamically stable, both in the gas phase and in solution.

The zwitterionic adduct **P3** and the product **P4** (which is the result of an alkylation process allowed by a formal proton-transfer process from the NH_2 group to the *o*-QM oxygen atom, likely mediated by the protic solvent) are much less stable than reactants both in the gas phase and in water solution.

Methyl substitution at the N9 position of adenine increases the stability of all the resulting adducts. **P1Me** is more stable than **P2Me** in the gas phase and it becomes even more stable in water solution. Therefore, under thermodynamic equilibration in water the NH_2 adduct should prevail. **P2Me** is slightly more stable than reactants in water (by −5.2 kcal mol^{-1}), and being able to revert to reactants, it may act as a carrier of *o*-QM. Such a result is consistent with Rokita's experimental work[11a] previously mentioned in the Introduction. **P3Me** and **P4Me**, deriving from N7 and N3 alkylation processes, are markedly unstable in the gas phase in comparison to free reactants by +8.1 and +15.5 kcal mol^{-1}, respectively. Although **P3Me** and **P4Me** are both stabilized by the solvent (−4.7, and −3.2 kcal mol^{-1}, respectively), such a bulk effect is not sufficiently strong to overcome their instability (relative to the reactants).

The additional tautomeric adducts arising from the second type of tautomerism are numbered for clarity with a suffix "i" (i.e., **P3i** and **P4i**). They are much more stable than the related **P3** (by ∼14 kcal mol^{-1}) and **P4** (by ∼28 kcal mol^{-1}) adducts and than free reactants both in the gas phase and in water.

1.2. Alkylation Under Kinetic Control. To describe kinetic aspects related to **A** and **9-MeA** alkylation processes by QMs, we have performed an investigation on the activation barriers leading to the alkylation adducts, locating seven different transition structures on the PES of adenine + *o*-QM reactive system (**S1**, **S2**, **S2′**, **S3**, **S3′**, **S4**, and **S4′**, see Figure 1). Among these TSs, a few are just conformers (those numbered as prime structures, i.e., **S1′**, etc.), but they will not be neglected in the discussion since they allow an evaluation of the H-bonding stabilization on reaction pathways. This approach allows a quantitative evaluation of the nucleophilicity for each nitrogen atom contained in **A**. Adenine is not a good model substrate to describe the reactivity of **dA** at the N3 position, since the hydrogen atom at the 9 position of adenine forms a strong H-bonding with the *o*-QM oxygen atom (see **S4′** in Figure 1).

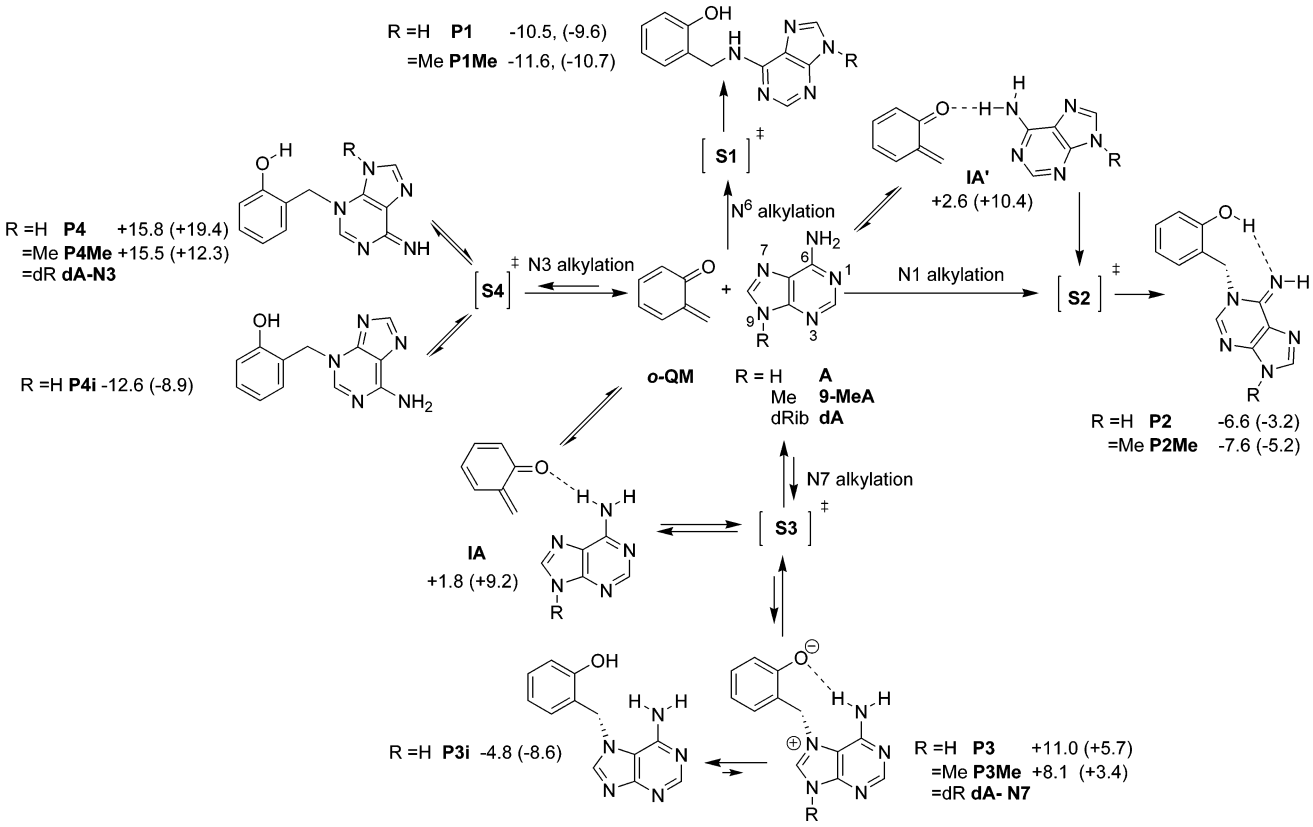
Therefore, we studied the alkylation reaction of **9-MeA** by *o*-QM at the N3 atom considering **S4Me** TS, which bears an N9-methyl group (Figure 1). For the sake of comparisons we also located TSs **S1Me**, **S2Me**,

(41) For conversion from 1 atm standard state to 1 mol/L standard state, the following contribution needs to be added to the standard enthalpy, entropy, and free energy: $-RT$, $-R - R\ln R'T$, and $RT\ln R'T$, where R' is the value of R in L·atm/mol·K (ref 52). For a reaction with A + B = C stoichiometry (such as the unassisted alkylation mechanism), the corrections for $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$ are RT, $R + R\ln R'T$, and $RT\ln R'T$. At 298 K the corrections amount to 0.59 and −1.90 kcal mol^{-1} for $\Delta H^{\ddagger}$ and $\Delta G^{\ddagger}$ and +8.34 eu for $\Delta S^{\ddagger}$ (ref 53). For a reaction with A + B + C = D stoichiometry (such as the water-assisted alkylation mechanism), the corrections for $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$ are $2RT$, $2(R + R\ln R'T)$, and $2RT\ln R'T$. At 298 K the corrections amount to 1.18 and −3.79 kcal mol^{-1} for $\Delta H^{\ddagger}$ and $\Delta G^{\ddagger}$ and +16.68 eu for $\Delta S^{\ddagger}$.

(42) (a) Barone, V.; Cossi, M. *J. Phys. Chem. A* **1998**, *102*, 1995. For the original COSMO model see: Klamt, A.; Schüürmann *J. Chem. Soc., Perkins Trans.* **1993**, 2. Klamt, A.; Jonas, V.; Bürger, T.; Lohrenz, J. C. W. *J. Phys. Chem. A* **1998**, *102*, 5074. (b) For a more comprehensive treatment of solvation models see: (c) Cramer, C. J.; Truhlar, D. G. *Chem Rev*. **1999**, *99*, 2161.

(43) Barone, V.; Cossi, M.; Tomasi, J. *J. Chem. Phys.* **1997**, *107*, 3210.

SCHEME 3. Reaction Pathways of Adenine and 9-Methyladenine Alkylation by *o*-QM[a]



[a] Adduct free energies (kcal mol^{-1}) relative to reactants in the gas phase and in water (in parentheses) are given.

and **S3Me** on the alkylation pathways at the NH_2, N1, and N7 centers of 9-methyladenine, respectively (see Table 1).

1.2.1. Alkylation at NH_2 (N^6). S1 TS led to the alkylation adduct at the NH_2 group (N^6) (**P1**). Methyl substitution at the N9 nitrogen atom of adenine and solvent bulk have negligible effects (ca. +1 kcal mol^{-1}) on the activation free energy (see Table 1).

1.2.2. Alkylation at N1. S2 and **S2′** are TSs leading to N1 alkylation by *o*-QM (Figure 1). **S2** shows collinear hydrogen bonding, involving the QM oxygen atom and the NH_2 hydrogen atom of adenine (H- - -O distance, 1.66 Å; planar angle O−H- -N, 173.8°). Such strong interaction, which is absent in **S2′**, accounts for the higher stability of **S2** in comparison to **S2′** (by +10.2 kcal mol^{-1}). Methyl substitution on the N9 nitrogen atom has a minor effect on the alkylation reaction, reducing the activation free energy from +11.6 to +10.3 kcal mol^{-1} (see **S2Me** TS in Table 1). Among all the TSs located by us for the *o*-QM alkylation reaction of 9-methyladenine, **S2Me** is the most stable one in the gas phase, featuring the lowest activation free energy (+10.3 kcal mol^{-1}). The bulk effect of water induces a strong destabilization (>4.2 kcal mol^{-1}) on both **S2** and its methyl derivative (**S2Me**).

1.2.3. Alkylation at N7. **S3** and **S3′** are TSs on the alkylation pathway at the N7 nucleophilic center of adenine (Figure 1). **S3**, similar to **S2**, benefits from a H-bonding interaction between the approaching reactants [involving the QM oxygen atom and the NH_2 hydrogen atom of adenine, with an H- - -O distance of 1.70 Å, and a favorable O−H- -N planar angle (169.2°)]. Such an interaction, is lacking in **S3′**, and it accounts for the higher stability of **S3** in comparison to **S3′** (by +5.6 kcal mol^{-1}). Methyl substitution on the N9 nitrogen atom of adenine has a small effect on the alkylation reaction, reducing the activation free energy from +16.4 (**S3**) to +14.9 kcal mol^{-1} (**S3Me**). **S3** (or **S3Me**, for 9-methyladenine alkylation processes) resides significantly higher in energy (+4.8 kcal mol^{-1}) than **S2**, in the gas phase. However, it should enter a balanced competition with the latter in water solution, due to the concomitant stabilization of **S3** (−0.8 kcal mol^{-1}) and destabilization of **S2** (+4.1 kcal mol^{-1}) induced by the electrostatic contribution of the solvent bulk. In other words N1 and N7 alkylation processes of adenine in water compete on the same footing, under kinetic control. Interestingly, the methyl derivatives show a slightly different reactivity not only in the gas phase but also in water. In fact, the energy gap between **S2Me** and **S3Me** in favor of the former in water (2.2 kcal mol^{-1}) is small but not negligible, thus suggesting that the N1 alkylation process is still favored over N7, for **9-MeA** under kinetic control.

1.2.4. Alkylation at N3. S4 and **S4′** TSs lead to N3 alkylation of adenine by *o*-QM (Figure 1). **S4′** shows collinear hydrogen bonding, involving the QM oxygen atom and the hydrogen atom at the N9 of adenine (H- - -O distance, 1.62 Å; planar angle O−H- -N, 168.2°). Such a strong interaction, which is absent in **S4**, strongly stabilizes **S4′** in comparison to **S4** (by +11.3 kcal mol^{-1}). **S4′** is by far the most stable TS featuring the lowest activation free energy (+7.5 and 14.5 kcal mol^{-1}, in the gas phase and in water solution, respectively).

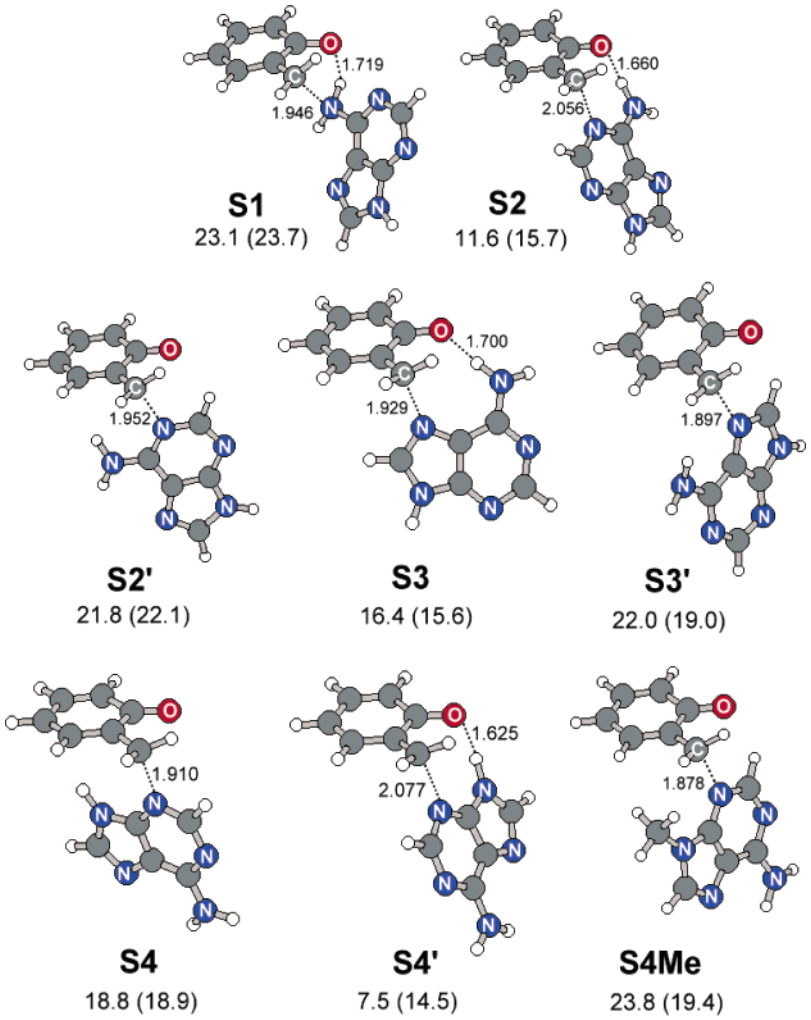


FIGURE 1. Optimized TS geometries of the adenine (**S1**−**S4**) and 9-methyladenine (**S4Me**) alkylation reaction by *o*-QM. Bond lengths (in Å) and activation free energies (in kcal mol^{-1}) in the gas phase and water solution (in parentheses) are given.

Methyl substitution on the N9 nitrogen atom of adenine has a major effect on the N3 alkylation reaction. A **S4′Me** TS is obviously not a viable pathway and also the activation free energy for N3 alkylation of 9-methyladenine through **S4Me** rises significantly (up to 23.8 kcal mol^{-1}, in the gas phase), with respect to **S4**, as a result of two synergistic effects: (i) the higher steric hindrance of the methyl group in comparison to the H atom and (ii) the absence of a weak H-bonding interaction (H-π system), which is operative in **S4** TS (Figure 2). These data definitely demonstrate that adenine is not a good model substrate to describe the reactivity at the N3 position of 9-substituted adenines such as **dA** with QM-like alkylating agents, and **9-MeA** is the more appropriate choice. The relatively lower reactivity of the N3 of **9-MeA**, in comparison to the other nucleophilic centers, is primarily explained by the absence of any N−H- -O hydrogen bonding in **S4Me** in comparison to **S2Me** and **S3Me** TSs (where such an interaction is operative), and secondarily by the lower intrinsic nucleophilicity of N3 in comparison to N1 and N7 centers. The low nucleophilicity of the **9-MeA** N3 center is mainly due to steric effects of the methyl substituent and it is not the result of electronic effects. In fact, the electrostatic potential surface for adenine is very similar in the region of the nitrogen N1 and N3 lone pairs.[18−20] Furthermore, we can reasonably assume that an effective descriptor of the intrinsic nucleophilicity of the 9-methyladenine nitrogen atoms (N1 vs N7 and N3) is the computed activation free energy for **S2′Me**, **S3′Me**, and **S4Me** TSs, in the gas phase (+21.8, +22.0, and +23.8 kcal mol^{-1}, respectively), where no hydrogen-bonding interaction with the alkylating agent is operative. Activation energies for **S2′Me** and **S3′Me** have been assumed identical to that of **S2′** and **S3′**, since methyl substituent effects are negligible, when the 9-methyl substituent is remote to the alkylated site. The similar values strongly suggest that the intrinsic nucleophilicity of nitrogen centers N1, N7, and N3 in 9-methyladenine and by analogy in **dA** should be of comparable strength, particularly that of N1 and N7 atoms.

1.3. Nucleophilicity of Adenine and 9-Methyladenine in the Gas Phase and in Water. A quantitative evaluation of the nucleophilicity of **A** and **9-MeA** toward *o*-QM in the gas phase and in water can be obtained from direct comparison of the activation free energies of the alkylation pathways through the lowest TSs, i.e., **S1**, **S2**, **S3**, and **S4′** for **A** and their methyl analogues TSs (**S1Me**, **S2Me**, **S3Me**, and **S4Me**) for **9-MeA** (Figure 1). Adenine and its 9-methyl analogue display different nucleophi-

TABLE 1. Activation Energy ($\Delta E^{\ddagger}$, in kcal mol^{-1}), Enthalpy ($\Delta H^{\ddagger}$), Entropy ($\Delta S^{\ddagger}$), Free Energy in the Gas Phase ($\Delta G^{\ddagger}_{gas}$), Solvation Free Energy (δG_{sol}), Solvent Effect on Free Energy ($\delta\Delta G_{sol}$), and Activation Free Energy in Water ($\Delta G^{\ddagger}_{sol}$) for the Alkylation of Adenine (A) and 9-Methyladenine (MeA) by *o*-QM[a]

structure	$\Delta E^{\ddagger}$	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	$\Delta G^{\ddagger}_{gas}$	δG_{sol}[b]	$\delta\Delta G_{sol}$[c]	$\Delta G^{\ddagger}_{solv}$[d]	μ_{gas}
				$NH_2(N^6)$ alkylation TSs				
S1	7.2[e]	8.9	−35.1	19.3	−16.2	+0.7	20.0	7.3
	11.0[f]			23.1			23.7	
S1Me	7.0[e]	9.3	−31.0	18.1	−13.1	+0.7	18.8	7.8
	10.6[f]			21.7			22.4	
				N1 alkylation TSs				
S2	−3.4[e]	−1.8	−35.9	8.8	−12.7	+4.1	13.0	5.8
	−0.6[f]			11.6			15.7	
S2Me	−3.5[e]	−1.4	−30.1	7.6	−9.6	+4.2	11.8	6.4
	−0.8[f]			10.3			14.5	
S2′	8.6[e]	10.4	−33.2	20.2	−16.6	+0.2	20.5	7.2
	10.2[f]			21.8			22.1	
				N7 alkylation TSs				
S3	1.3[e]	3.0	−36.1	13.8	−17.7	−0.8	13.0	6.3
	3.9[f]			16.4			15.6	
S3Me	0.7[e]	3.0	−31.4	12.3	−12.0	+1.8	14.2	7.0
	3.2[f]			14.9			16.7	
S3′	8.2[e]	9.8	−34.0	19.9	−19.8	−2.9	17.0	4.9
	10.2[f]			22.0			19.0	
				N3 alkylation TSs				
S4	6.2[e]	7.8	−33.9	17.9	−16.8	+0.1	18.0	6.3
	7.1[f]			18.8			18.9	
S4Me	11.4[e]	13.6	−28.9	22.2	−18.2	−4.4	17.8	9.0
	13.0[f]			23.8			19.4	
S4′	−6.5[e]	−5.3	−35.4	5.2	−9.8	+7.0	12.2	3.4
	−4.2[f]			7.5			14.5	

[a] With respect to reactants. Symmetry numbers used to calculate entropy are $\sigma = 1$ for **1** and **C**, $\sigma = 2$ for H_2O. A correction of $R\ln 2$ to ΔS has been added for the alkylation reactions, as the nucleophile attack to *o*-QM faces is not experimentally distinguishable. For conversion from 1 atm standard state to 1 mol/L standard state, see ref 41. [b] Solvent effect (δG_{sol}) on stationary points by C-PCM single-point calculations on gas-phase geometries B3LYP-C-PCM/6-31G(d)//B3LYP/6-31G(d). [c] Solvent effect on reaction Gibbs free energy, calculated as $\delta\Delta G_{sol} = \delta G_{sol} - \delta G_{reactants}$ ($\delta G_{reactants}$ = sum of the solvent effect on each reactant). [d] Gibbs free energy in water solution calculated as $\Delta G_{solv} = \Delta G_{gas} + \delta\Delta G_{sol}$. [e] B3LYP/6-31G(d). [f] B3LYP/6-311G+(d,p)//B3LYP/6-31G(d).

licity. In fact, the most nucleophilic center is N3 for **A** and N1 for **9-MeA**, both in the gas phase and in solution. The nucleophilicity scale of the N7 and NH_2 in **A** parallels that of **9-MeA** in the gas phase. The key factor of such a scale is the H-bonding involving the QM oxygen atom. In fact, the most stable TS both in the gas phase and in solution (**S4′**) displays the shortest H-bonding distance (N−H- - -O, 1.625 Å).

The bulk effect of water as solvent strongly reduces the nucleophilicity of the most reactive centers of **A** and **9-MeA** (N3 and N1, respectively), reverting the nucleophilicity order between N3 and NH_2 for **9-MeA** in comparison to the gas phase. That is because H-bonding becomes weaker in a solvent bulk with high dielectric constant. The computed nucleophilicity scale of **9-MeA** is noteworthy, because it cannot be experimentally obtained, since the adducts at positions N7 (**P3Me**) and N3 (**P4Me**) are unstable toward the dissociation process into free reactants.

o-QM appears to be a selective alkylating agent of 9-methyladenine in water under kinetic control, favoring the attack at the N1 nucleophilic center over the alkylation at the NH_2 group (by more than 7 kcal mol^{-1}). Our data also show that the **9-MeA-N1** adduct is a source of *o*-QM since the activation energy for its dissociation into *o*-QM and **9-MeA** (at 298 K, in water bulk) is only +19.8 kcal mol^{-1}.

The above findings on the reactivity of 9-methyladenine and the reversibility of the addition process under mild conditions are both consistent with the experimental work by Rokita and co-workers,[11a] who have been able to isolate **dA-N1** as the major adduct from **dA** alkylation by *o*-QM, and to show that **dA-N1** is actually a "transient nucleoside adduct" that decomposes in a DMF−water mixture at 37 °C within 12 h (the activation energy for its dissociation in water−acetonitrile solution is ∼23 kcal mol^{-1}).

2. Alkylation of Guanine by *o*-QM.

2.1. Alkylation Under Thermodynamic Control. Two pre-reaction complexes have been located on the PES for the alkylation reaction of guanine by *o*-QM in the gas phase, corresponding to **IG** and **IG′** and several alkylation adducts (Scheme 4). The former shows a chelate H-bonding,[44] where the *o*-QM oxygen atom interacts simultaneously with the acidic N1 hydrogen atom and with a hydrogen atom of the NH_2 group. **IG** proves more stable than free reactants by −2.5 kcal mol^{-1}, but **IG′** showing only one H-bonding interaction is less stable in the gas phase (by +1.5 kcal mol^{-1}). Inclusion of solvent effects strongly destabilizes both pre-reaction complexes at least by +8.8 kcal mol^{-1}. Both complexes are in fast equilibration with free reactants, therefore dealing with a Curtin−Hammet system[45] we will neglect these complexes and throughout we will evaluate activation energy data relative to free reactants.

The adducts generated by guanine alkylation not involving prototropic N9 hydrogen transfer are **P5**−**P10** (Scheme 4). With the exception of **P8**, they are more stable than reactants, in the gas phase. **P6**, being the most stable, should be dominant under thermodynamic control in the gas phase. Water as solvent has a negligible effect on **P5** and **P9**, but it induces a strong destabilization on the other alkylation adducts **P6**, **P7**, and **P10**, leaving only **P5** and **P6** more stable than reactants.

The stability order between **P5** and **P6** is reversed by solvent effects from the gas phase. Similar considerations also hold for their methyl derivatives. In fact, **P5Me** becomes the adduct of a thermodynamically controlled alkylation of **9-MeG** in solution (water, DMSO, and acetonitrile). Although **P5Me** is always more stable than **P6Me** in solution, the energy gap is considerably reduced in acetonitrile (1.6 kcal mol^{-1}) in comparison to that in water (4.3 kcal mol^{-1}). Such a result is consistent with the experimental **dG-NH$_2$**/**dG-N1** product ratio,[11b] which is dependent on the concentration of organic solvent in water. In particular, the formation of **dG-NH$_2$** adduct (modeled by **P5Me**) in a DMF:water mixture is suppressed reducing the amount of organic solvent.

The oxo-isomer **P8** is less stable than the hydroxy-tautomer **P9** both in the gas phase (by −13.4 kcal mol^{-1}) and in water solution (by −6.3 kcal mol^{-1}). An intramolecular H-bonding interaction is operative in **P9**, and it accounts for its strong stabilization in comparison to **P8**. The strength of such an interaction may be evaluated by the decrease in **P9** O−H frequency (3144.2 cm^{-1}) in

(44) Jeffrey G. A. *An Introduction to Hydrogen Bonding*; Oxford University Press: New York, 1997; Chapter 2, pp 11−32.
(45) Seeman, J. I. *Chem. Rev.* **1983**, *83*, 83.

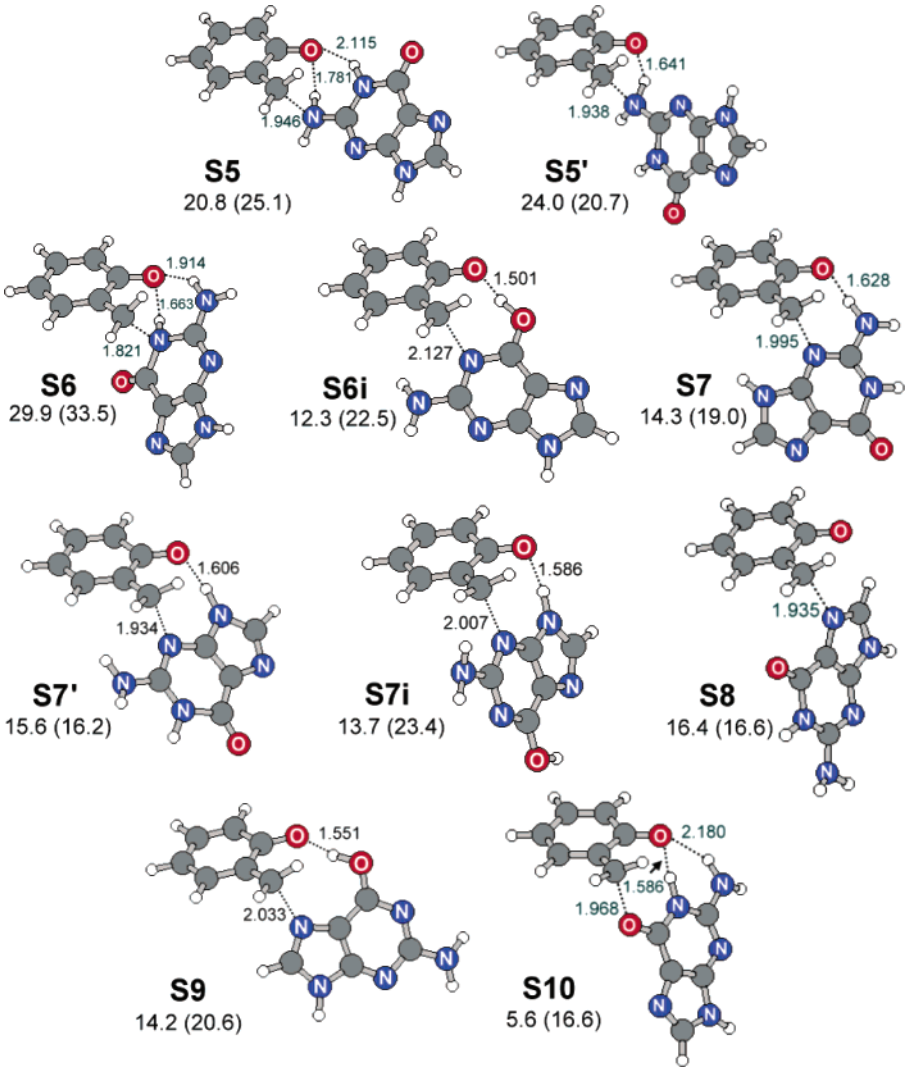


FIGURE 2. Optimized TS geometries (**S5**−**S10**) of the guanine alkylation reaction by *o*-QM. Bond lengths (in Å) and activation Gibbs free energies (referred to *o*-QM and the most stable guanine tautomer **G**, in kcal mol^{-1}) in the gas phase and water solution (in parentheses) are given.

TABLE 2. Adenine and 9-Methyladenine Nucleophilicity Scale in the Gas Phase and in Water Solution[a]

purine	gas phase	water solution
A	N3 (7.5) ≫ N1 (11.6) ≫ N7 (16.4) ≫ NH_2 (23.1)	N3 (14.5) > N7 (15.6) ≈ N1 (15.7) ≫ NH_2 (23.7)
9-MeA	N1 (10.3) ≫ N7 (14.9) ≫ NH_2 (21.7) ≥ N3 (23.8)	N1 (14.5) > N7 (16.7) > N3 (19.4) > NH_2 (22.4)

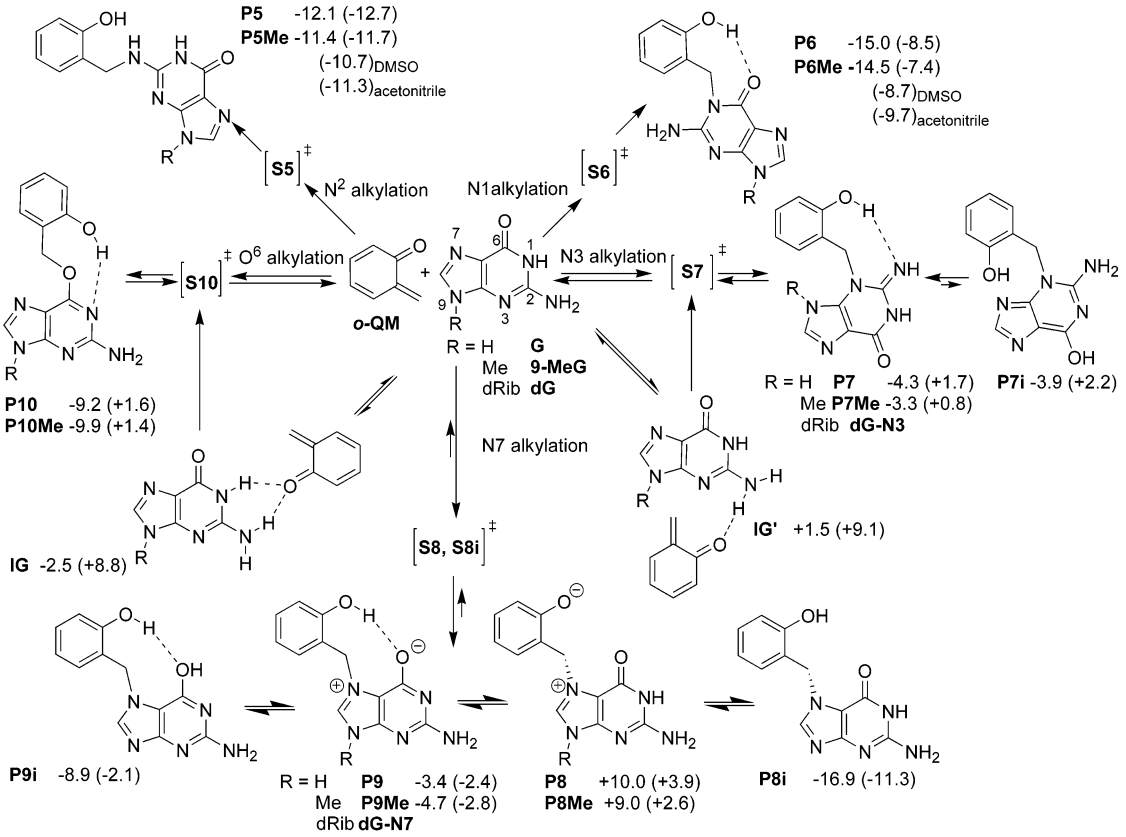
[a] As judged by comparison of the computed activation free energy (in parentheses, in kcal mol^{-1}) for the alkylation processes involving *o*-QM, through the lowest TSs.

comparison to that of phenol (3750.2 cm^{-1}). In fact, **P9** lying −3.4 kcal mol^{-1} in the gas phase and −2.4 kcal mol^{-1} in water below the reactants is a fairly stable adduct (Scheme 4). N9-methyl substitution introduces an additional stabilization and as a result **P9Me** is more stable than free reactants in the gas phase and water (by −4.7 and −2.8 kcal mol^{-1}, respectively). **P9Me**, unlike **P8Me**, is destabilized by the solvent bulk; however, it remains more stable than both **P8Me** and reactants in water solution. The above findings on the stability of **P9Me** conform to the experimental results by Rokita and co-workers,[11b] who have been able to isolate the adduct **G-N7** as a minor product from the **dG** alkylation process. Moreover, the adduct **P9Me**, being only slightly more stable than reactants, shows very similar thermodynamic behavior to that of the **9-MeA-N1** adduct (**P2Me**), and like the latter, being able to easily revert to reactants, it acts as a carrier of *o*-QM.

The adducts generated by guanine alkylation involving prototropic N9 hydrogen transfer are **P7i**, **P8i**, and **P9i** (Scheme 4). **P7i** and **P9i** display comparable stability to their related tautomers **P7** and **P9** in water, but **P8i** is strongly stabilized in comparisons to **P8** by −15.2 kcal mol^{-1}. This suggests that the **G** adduct with *o*-QM at the N7 center is much more stable than **9-MeG** or **dG-N7** alkylation adducts.

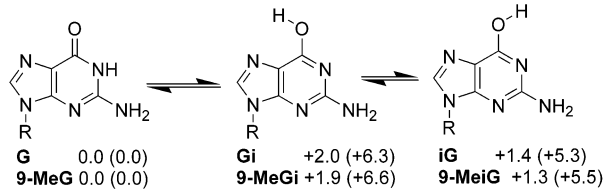
2.2. Alkylation Under Kinetic Control. Among all the possible tautomeric forms[28d,30c,46] of guanine (and **9-MeG**), we have chosen for the present study three of the lowest energy forms related by the oxo-hydroxy

SCHEME 4. Reaction Pathways of Guanine and 9-Methylguanne Alkylation by *o*-QM[a]



[a] Adduct free energies (kcal mol^{-1}) relative to reactants in the gas phase and in water (in parentheses) are given.

SCHEME 5. Tautomers of Guanine and 9-Methylguanine[a]



[a] Free energies in the gas phase and in water (in parentheses) relative to the most stable tautomers **G** and **9-MeG** are in kcal mol^{-1}.

equilibrium according to Scheme 5 (**G**, **Gi**, and **iG**).[47] The choice has been suggested not only by stability reasoning, but also because they offer the possibility of stabilizing specific interactions in TSs involving alkylating agents with H-bonding acceptor properties such as *o*-QM.

On the PES of the *o*-QM + guanine reactive system, we have been able to locate 10 different transition structures (**S5**−**S10** in Figure 2) leading to the products. Among these TSs, a few are just conformers (numbered as primed structures: i.e., **S5′**), others are tautomers (numbered with a suffix "i": i.e., **S6i**). None of them will be neglected in the discussion since they allow important evaluations of the stabilization induced by H-bonding involving tautomers different from the most stable one. Direct comparison of their free energies should allow an evaluation of the nucleophilicity for each nucleophilic center (oxygen and nitrogen atoms) of guanine and 9-methylguanine.

2.2.1. Alkylation at NH_2 (N^2). S5 and **S5′** are TSs describing the chemical pathway toward the alkylation adduct at the *exo*-amino group (N^2) **P5**. **S5** and **S5′** are conformational TSs achievable by rotation around the C_2-NH_2 bond (Figure 2 and Table 3). The presence of chelate H-bonding interactions[44] is the cause of the higher stability of **S5** in comparison to **S5′** (−3.2 kcal mol^{-1}), in the gas phase. The lowering of both N^6-H and N1−H stretching frequencies in **S5** (by 606.9 and 163.4 cm^{-1}, respectively) in comparison to the stretching frequencies of the corresponding bonds in **G** is the spectroscopic evidence that both N−H bonds are involved as hydrogen bond donors with the QM oxygen atom.

Water bulk has a strong effect on the relative activation free energies (see Table 3), mainly due to the higher dipole moment of **S5′** in comparison to **S5**. In fact, the combined stabilization of **S5′** (by −3.2 kcal mol^{-1}) and the destabilization of **S5** (+4.3 kcal mol^{-1}) revert the energy order of the two TSs, dropping **S5′** −4.4 kcal mol^{-1} below that of **S5** (Table 3). The effect of methyl substitution on the reactivity of *o*-QM toward the NH_2 center of 9-methylguanine has been assessed by locating **S5Me** and **S5′Me** TSs, but it is negligible both in the gas phase and in water.

2.2.2. Alkylation at N1. S6 is the TS on the alkylation pathway at the N1 nucleophilic center of the guanine oxo-

(46) Russo, N.; Toscano, M.; Grand, A. *J. Am. Chem. Soc.* **2001**, *123*, 10272.

(47) We have neglected those tautomers of G and G-adduct,involving N9 hydrogen atom because in a prototype model of dG that proton is replaced by an alkyl substituent.

TABLE 3. Activation Energy ($\Delta E^{\ddagger}$, in kcal mol^{-1}), Enthalpy ($\Delta H^{\ddagger}$), Entropy ($\Delta S^{\ddagger}$), Free Energy in the Gas Phase ($\Delta G^{\ddagger}_{sas}$), Solvation Free Energy (δG_{sol}), Solvent Effect on Free Energy ($\Delta\Delta G_{sol}$), and Activation Free Energy in Water ($\Delta G^{\ddagger}_{sol}$) for the Alkylation of Guanine (G) and 9-Methylguanine (MeG) by *o*-QM[a]

structure	$\Delta E^{\ddagger}$	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	$\Delta G^{\ddagger}_{gas}$	δG_{sol}[b]	$\delta\Delta G_{sol}$[c]	$\Delta G^{\ddagger}_{solv}$[d]	μ_{gas}
				$NH_2(N^2)$ alkylation TSs				
S5	3.9[e]	5.8	−36.4	16.7	−19.6	+4.3	21.0	2.8
	8.0[f]			20.8			25.1	
S5Me	3.8[e]	5.5	−37.5	16.5	−17.2	+4.3	20.9	2.3
	7.7[f]			20.5			24.8	
S5′	9.0[e]	10.3	−34.5	20.6	−27.2	−3.3	17.3	8.6
	12.4[f]			24.0			20.7	
S5′Me	8.10[e]	9.4	−36.4	19.8	−24.9	−3.4	16.5	9.1
	11.5[f]			23.2			19.8	
				N1 alkylation TSs				
S6	13.6[e]	14.6	−37.7	25.9	−20.4	+3.5	29.4	6.9
	17.7[f]			29.9			33.5	
S6i	−2.2[e]	−1.5	−38.6	9.6	−13.8	+10.1	19.7	6.4
	0.5[f]			12.3			22.5	
S6iMe	-2.3[e]	-1.6	-39.1	9.6	-10.9	+10.6	20.2	6.9
	0.3[f]			12.3			22.9	
				N3 alkylation TS				
S7	0.0[e]	1.1	−35.1	11.6	−19.2	+4.7	16.3	2.9
	2.7[f]			14.3			19.0	
S7Me	3.0[e]	4.8	−39.8	15.6	−17.8	+3.6	19.3	3.0
	5.7[f]			18.3			21.9	
S7′	1.5[e]	2.5	-36.4	12.9	-23.3	+0.6	13.6	8.0
	4.2			15.6			16.2	
S7i	-0.1[e]	1.2	-37.2	11.8	-14.2	+9.7	21.6	3.0
	1.7[f]			13.7			23.4	
				N7 alkylation TSs				
S8	3.0[e]	4.7	−32.9	14.5	−23.7	+0.2	14.8	11.1
	4.8[f]			16.4			16.6	
S8Me	2.4[e]	4.1	−32.2	13.7	−19.0	+2.5	16.2	11.3
	3.9[f]			15.2			17.7	
S9	1.0[e]	1.9	−37.1	12.5	−17.5	+6.4	19.0	7.3
	2.6[f]			14.2			20.6	
S9Me	0.4[e]	1.4	−35.1	11.5	−14.3	+7.2	18.7	7.8
	1.9[f]			12.9			20.1	
				O alkylation TS				
S10	−11.2[e]	−9.7	−37.3	1.4	−12.9	+11.0	12.4	3.5
	−7.0[f]			5.6			16.6	
S10Me	−11.3[e]	−9.8	−37.8	1.0	−10.0	+11.4	12.5	4.0
	−7.1[f]			5.2			16.6	

[a−f] See Table 1 footnotes.

tautomer **G**. It shows strong chelate hydrogen bonding, involving the QM oxygen atom and both N(1)H (H- - -O distance, 1.66 Å; O−H- -N planar angle, 144.7°) and a hydrogen atom of the guanine NH_2 group (N^2) (H- - -O distance, 1.91 Å; O−H- -N planar angle, 144.0°). Despite this, **S6** shows the highest activation free energy both in the gas phase (+29.9 kcal mol^{-1}) and in water solution (+33.5 kcal mol^{-1}) among all the TSs located by us for the alkylation reaction of guanine by *o*-QM. This finding can be ascribed to the very low intrinsic nucleophilicity of the N1 center in comparison to the other N nucleophilic sites of the most stable tautomer **G**, as judged by evaluation of the electrostatic potential surface in the region of the nitrogen lone pairs,[18b] which cannot be overcome by any specific interaction between reactants. We have been able to locate another TS (**S6i**) relative to the alkylation reaction at the N1 atom, involving the less stable O^6-H tautomer **iG** (Scheme 5).[28d] Such a TS proves to be much more stable than **S6** by more than −17 kcal mol^{-1} (Table 3). The reason of such a massive effect is due to (i) the higher intrinsic nucleophilicity of the N1 atom in **iG** in comparison to the same center in **G** (as evaluated from the electrostatic potential at the van der Waals surface[18a] of the molecule)[18b] and to (ii) the presence of a strong collinear hydrogen bonding (O−H- -O, 1.501 Å, 171.7°) between the OH moiety of the hydroxy-tautomer (**iG**) and the *o*-QM oxygen atom.

The solvent effect induces destabilization (relative to reactants) on both **S6** and **S6i** TSs (by +3.5 and +10.1 kcal mol^{-1}, respectively), but the latter remains more stable, with an activation free energy in water of +22.5 kcal mol^{-1} (Table 3). The activation free energy of **S6iMe** (+22.9 kcal mol^{-1}, relative to 9-methylguanine and *o*-QM) is only slightly higher than that of **S6i**.

Although the *o*-QM alkylation process at guanine N1 could appear to be the less available alkylation pathway under kinetic control as judge by **S6** activation free energy (+33.5 kcal mol^{-1}), a proper evaluation of guanine reactivity, taking into account the contribution to the reactivity of a less stable but much more reactive **iG** tautomer, suggests that guanine covalent modification at N1 is kinetically competitive with other processes only in the gas phase ($\Delta G^{\ddagger}_{gas}$ = +12.3 kcal mol^{-1} for both **S6i** and **S6iMe**) or in solvents much less polar than water.[48] Such a result is a warning about the evaluation of purine base reactivity toward alkylating agents taking into account only the most stable tautomers.

2.2.3. Alkylation at N3. S7, **S7′**, and **S7i** TSs lead to N3 alkylation of guanine by *o*-QM (Figure 2). **S7** and **S7′** involve the oxo-tautomer (**G**) and **S7i** the hydroxy one (**Gi**). Both **S7′** and **S7i** show hydrogen bonding involving the QM oxygen atom and the N9 hydrogen atom of adenine. Another H-bonding involving the QM oxygen atom and a hydrogen atom of the NH_2 group of adenine is present in **S7**. These TSs show similar stability (within 2 kcal mol^{-1}) in the gas phase, but **S7′** becomes the most stable in water solution. The evaluation of N3 nucleophilicity of **dG** requires 9-methylguanine as a model, rather than guanine. The presence of a methyl group in **S7Me** strongly enhances its activation energy (+18.3 kcal mol^{-1}) in comparison to the unsubstituted **S7** TS (+14.3 kcal mol^{-1}). The difference in activation free energy (+4.0 kcal mol^{-1}) is mainly due to steric hindrance of the alkyl substituent. Thus, we always refer to **S7Me** TS to describe the energetic profile of the alkylation at the **dG** N3 center. The bulk solvent effect of water further rises its free activation energy from +18.3 to +21.9 kcal mol^{-1} (Table 3). Therefore, covalent N3 modification of 9-methylguanine (and by analogy of **dG**) by *o*-QM is not kinetically competitive with the NH_2 alkylation process in water.

2.2.4. Alkylation at N7. S8 is the TS on the alkylation pathway at guanine N7. **S8** is a quite stable TS although it does not exhibit any hydrogen-bonding interaction involving the QM oxygen atom and an acidic hydrogen atom of guanine. This is likely due to the intrinsic high nucleophilicity of the N7 guanine center (as inferred by the most negative electrostatic potential, among the nitrogen guanine atoms).[18b] Methyl substitution on the N9 nitrogen atom of guanine has a small effect on the alkylation reaction, reducing the activation energy from

(48) We are currently investigating the role of the bulk effect of acetonitrile and DMSO on the barriers and on adduct stability, because they show dielectric constants very similar to that assumed for the interior of a nucleic acid double helix (ref 28d).

+16.4 to +15.2 kcal mol^{-1}, in the gas phase. We have been able to locate another TS along the reaction pathway at the N7 atom (**S9**), involving the less stable guanine isomer **Gi**. The latter TS (and its methyl derivative) shows a slightly lower activation free energy (+14.6 kcal mol^{-1}) than **S8**, in the gas phase. Nevertheless, the bulk solvent effect strongly destabilizes **S9** in favor of **S8** (by +6.2 kcal mol^{-1}, see Table 3), as the result of the zwitterionic character of **S8**, suggesting that the latter is the only important TS on the alkylation pathway of guanine at the N7 atom, in water.

2.2.5. Alkylation at Oxygen (O^6). **S10** is the TS on the oxygen alkylation pathway (leading to **P10**). It benefits from a chelate hydrogen-bonding interaction involving the QM oxygen atom and both N(1)H [(H- - -O distance of 1.59 Å, with a O−H- - -N planar angle of 163.7°] and the NH_2 (N^2) hydrogen atom of guanine (H- - -O distance of 2.18 Å, and a O−H- - -N planar angle of 137.4°). Methyl substitution on the N9 nitrogen atom of guanine has a small effect on the activation free energy of the alkylation reaction (−0.4 kcal mol^{-1}). Among all the TSs located by us for the alkylation reaction of guanine by *o*-QM, **S10** has the lowest activation free energy in the gas phase (+5.6 kcal mol^{-1}), in comparison with all the alkylation processes involving **A** and **G**. Such a unique stability has to be ascribed to H-bonding. The activation free energy in water solution rises up to +16.6 kcal mol^{-1} (identical value for the methyl analogue **S10Me** TS), as a consequence of bulk solvent effect, which induces a massive destabilization on **S10** (+11.0 kcal mol^{-1}). The origin of such an effect is likely due to (i) the low polarity of **S10** (or **S10Me**), which shows low dipole moment (3.5 D), (ii) and the fact that TSs such as **S10** and **S5**, characterized by strong specific interaction, i.e., chelate H-bonding, are much more destabilized by solvent bulk effects.

Interestingly, **S8** and its 9-methyl analogue **S8Me** that reside at considerably higher energy (+10.8 kcal mol^{-1}) than **S10** in the gas phase become highly competitive in water solution, due to its negligible destabilization (+0.25 kcal mol^{-1}) in comparison to that of **S10**. In other words from a kinetic point of view the guanine alkylations at N7 and O^6 positions in water are the dominant processes and compete with one another on the same level, both being characterized by the very same activation free energy (+16.6 kcal mol^{-1}). Activation free energy comparison for the methyl analogues **S8Me** (+17.7 kcal mol^{-1}) and **S10Me** (+16.6 kcal mol^{-1}) suggests a low selectivity of the alkylation process (O^6 vs N7) in water solution, under kinetic control. Although the energy difference is very small (1.1 kcal mol^{-1}), the O^6 alkylation process is the favored path in water under kinetic control.

2.3. Water-Assisted Alkylation Process at Guanine O^6. We have so far discussed the bulk solvent effect, but what about the specific involvement of water molecules in *o*-QM alkylation? We have previously demonstrated that the latter process cannot compete with its uncatalyzed counterpart in the case of nitrogen nucleophiles, but also that the water-assisted mechanism can be competitive in the case of oxygen nucleophile (as in water and cytosine alkylation by *o*-QM).[7,35]

Thus, to evaluate the competing water-assisted reaction at guanine O^6 two additional TSs (**S10**+**H_2O** and **S10**+**H_2O_{act}**), containing a specific water molecule, have been located (Figure 3).

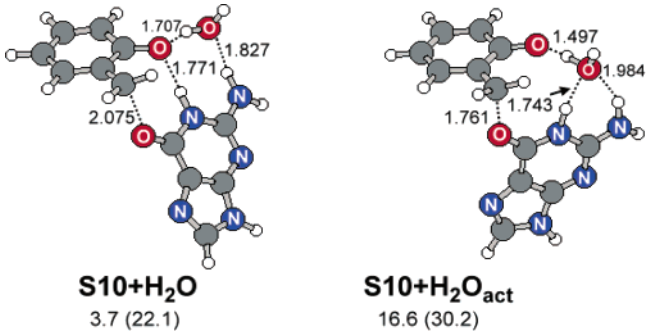


FIGURE 3. Optimized TSs of the guanine oxygen alkylation reaction by *o*-QM, with an explicit water molecule. In **S10**+**H_2O_{act}** TS the water molecule is actively involved in the reaction process. In **S10**+**H_2O** TS the water molecule is complexed to the *o*-QM oxygen atom without taking part in the proton-transfer process. Bond lengths are in Å, and activation Gibbs free energies (at B3LYP/6-311+G(d,p)//B3LYP/6-31G(d) level) in the gas phase and water solution (in parentheses) are in kcal mol^{-1}.

S10+**H_2O** describes the specific effects on reactivity of a water molecule complexed to *o*-QM, which does not take part in the proton-transfer process (passive process). **S10**+**H_2O** shows similar geometric features to **S10**, which describes an uncatalyzed reaction model. **S10**+**H_2O_{act}** allows instead an evaluation of the change introduced by an ancillary water molecule directly involved in the proton transfer (active process) from the N1 atom to the QM oxygen atom [verified by IRC calculations at the B3LYP/6-31G(d) level of theory]. **S10**+**H_2O_{act}** displays a highly different geometry, with the approaching reactants folded over each other. This is probably due to the attempt to accommodate the water molecule between the reactants. Such a distortion from the optimal array, which characterizes **S10**+**H_2O**, accounts for the higher energy of **S10**+**H_2O_{act}** (by +8.5 kcal mol^{-1}). This energy gap rules out the water-assisted reaction model from the mechanism of the alkylation at the guanine oxygen atom.

Direct comparison between **S10**+**H_2O** and **S10** can be done on the basis of activation free energy evaluation (relative to free reactants, which are **G** + *o*-QM and **G** + *o*-QM + water for **S10** and **S10**+**H_2O**, respectively). **S10**+**H_2O** is slightly more stable than **S10**, in the gas phase. Inclusion of bulk solvent effects introduces a strong stabilization in favor of the latter. The retarding effect of the solvent bulk on the oxygen (O^6) alkylation reaction of guanine by *o*-QM is much more important than the acceleration induced by specific hydrogen-bonding interaction with the protic solvent, and guanine O^6 alkylation by *o*-QM is better described by an uncatalyzed mechanism. This statement may appear in contrast with the fact that cytosine alkylation at the oxygen atom (O^2) in water is a process catalyzed by an ancillary water molecule.[35] Actually, a rationalization of such an apparent contradiction is easily found in the presence of the acidic guanine N−H(1) (pK_a = 9.2),[49] which provides very efficient intramolecular protic assistance to the alkylation

(49) Bloomfield, V. A.; Crothers, M. D.; Tinoco, I., Eds. *Nucleic Acids. Structure, properties and functions*; University Science Books: Sausalito, CA, 2000; Chapter 2, pp 28−29.

TABLE 4. Guanine and 9-Methylguanine Nucleophilicity Scales in the Gas Phase and in Water Solution[a]

purine	gas phase	water solution
G	O^6(5.6) ≫ N1 (12.3) > N3 (13.7) ≥ N7 (14.2) ≫ NH_2 (20.8)	N3 (16.2) ≥ O^6 (16.6) = N7 (16.6) ≫ NH_2 (20.7) > N1 (22.5)
9-MeG	O^6 (5.2) ≫ N1 (12.3) ≥ N7 (12.9) ≫ N3 (18.3) > NH_2 (20.5)	O^6 (16.6) > N7 (17.7) > NH_2 (19.8) > N3 (21.9) > N1 (22.9)

[a] As judged by comparisons of the computed activation free energy (in parentheses, in kcal mol^{-1}) for the alkylation processes involving *o*-QM, **G**, and **9-MeG** through the lowest TSs.

TABLE 5. Nucleophilicity and Adduct Stability Scales in the Gas Phase and in Water Solution[a]

	gas phase	water solution
nucleophilicity[a]	**9-MeG-O6** (5.2) ≫ **9-MeA-N1** (10.3)	**9-MeA-N1** (14.5) > **9-MeG-O6** (16.6)
adduct stability[b]	**9-MeG-N1** (−14.5) > **9-MeA-NH_2** (−11.7)	**9-MeG-NH_2** (−11.7) > **9-MeA-NH_2** (−10.7)
exptl[c] rel reactivity		**dA-NH_2** = 1.3 **dG-NH_2**

[a] As judged by comparison of the computed activation free energy (in parentheses, in kcal mol^{-1}) for the alkylation processes involving *o*-QM with **9-MeA** and **9-MeG** as a prototype of **dA** and **dG**, respectively. [b] As judged by comparison of the relative free energy (in parentheses, in kcal mol^{-1}) for the *o*-QM alkylation adducts of **9-MeA** and **9-MeG** (as a prototype of **dA** and **dG** adduct). [c] Data from ref 11a.

process by *o*-QM that is not significantly increased by interaction with an additional water molecule.

2.4. Nucleophilicity of Guanine and 9-Methylguanine in the Gas Phase and in Water. A nucleophilicity scale of guanine and 9-methylguanine toward *o*-QM in the gas phase and in water can be compiled from direct comparison of the activation free energies for each alkylation pathway through the lowest TS. O^6 guanine nucleophilicity represents the most unexpected result. In fact, in the gas phase, and most likely in a lower polar solvent than water,[48] O^6 is by far the most nucleophilic site of both guanine and 9-methylguanine. The nucleophilicity of the N1, N3, and N7 centers in the guanine and N1 and N7 in the 9-methylguanine is comparable. The NH_2 group is the least nucleophilic for both bases (Table 4).

The bulk effect of water as a solvent strongly reduces the nucleophilicity of O^6 and N1 centers of both guanine and 9-methylguanine, by more than 10 kcal mol^{-1}, in comparison to the gas phase and it levels the nucleophilicity order of O^6, N7, and N3 for **G** and O^6 and N7 for **9-MeG**. The above nucleophilicity scales cannot be experimentally obtained from any product distribution analysis in water since the resulting **G** and **9-MeG** adducts at the O^6 atom are both less stable than reactants in water (by +1.2 and +1.4 kcal mol^{-1}). The result is that (i) the oxygen alkylation pathway of 9-methylguanine in water, affording an unstable adduct (**P10Me**),[50] is not a chemically productive process, and that (ii) the alkylation of 9-methylguanine at N7 should be experimentally detectable in water, since the resulting tautomeric adduct (**P9Me**) is slightly more stable than free reactants (by −2.8 kcal mol^{-1}).

3. Nucleophilicity or QM-Adduct Stability. Which Controls the Experimental Selectivity and Relative Reactivity of Purine Bases? On the basis of our data on the methyl analogues, we can compare the nucleophilicity of 9-methylpurines and the stability of the adduct arising from the reaction with *o*-QM, in water solution (Table 5), with the experimental **dA** and **dG** product distribution and the relative reactivity (**dA** vs **dG**) measured by Rokita.[9b,11] From such a comparison we should gain a clear idea of the QM's mechanism of action as a purine alkylating agent.

In the gas phase the **9-MeG**-O^6 atom is the most nucleophilic center among the two bases considered, as a result of a strong chelate H-bonding. Therefore, concerning kinetic selectivity, we may conclude that *o*-QM is highly sensitive to H-bonding with the DNA bases undergoing alkylation. This easily explains why the nucleophilicity scale in Table 5 does not parallel the hierarchy of dimethyl sulfate-dependent alkylation (**dG-N7** > **dA-N1**).[51] The nucleophilicity of the purine bases reverses in water (see Table 5), where **9-MeA-N1** becomes the most nucleophilic center as a result of the reduced H-bonding stabilization due to the solvent bulk effect. This result suggests that the bulk effect of a polar solvent such as water is another important factor in the control of the purine base nucleophilicity, and this has often been overlooked.

Our data clearly show that the adducts obtained under kinetic control (**9-MeG-O^6** and **9-MeA-N1**) should be different from those generated by a thermodynamic equilibration (**9-MeA-NH_2** and **9-MeG-NH_2**). Since our computational adduct stability and not the nucleophilicity of the purine bases agrees with both the product distributions found for **dA**[11a] and **dG**[11b] and with **dA** vs **dG** relative reactivity (Table 5), we may conclude that experimentally (reaction in a mixture of DMF−water, at 37 °C, for hours) the alkylation reactions involving *o*-QM must be for both purines under thermodynamic equilibration.

Although the intramolecular thermodynamic selectivity (between centers of the same purine) of **9-MeA** is not affected by the solvent effect since **9-MeA-NH_2** is the most stable adduct both in the gas phase and in solvent, the intramolecular thermodynamic selectivity of **9-MeG** is reverted by the solvent effect. In the gas phase **9-MeG-N1** is more stable than **9-MeG-NH_2** and the opposite is true in solvents such as acetonitrile, DMSO, and water, although in acetonitrile the selectivity is very small (1.6 kcal mol^{-1}).

(50) Actually, in a solvent less polar than water such as acetonitrile **P10** and **P10Me** are slightly more stable than reactants by −2.4 and −2.5 kcal mol^{-1}, respectively.

(51) Singer, B.; Grunberger, D. In *Molecular Biology of Mutagens and Carcinogens*; Plenum: New York, 1983; Chapter 4, pp 45−141.
(52) Benson, S. *Thermochemical Kinetics*; Wiley: New York 1968; p 8.
(53) Rastelli, A.; Bagatti, M.; Gandolfi, R. *J. Am. Chem. Soc.* **1995**, *117*, 4965.

Conclusion

Our study computing the stability order of QM-DNA base adducts and their related TSs in the gas phase and in water solution clarifies several important aspects of QM reactivity as an alkylating agent: (i) it quantitatively distinguishes the role of thermodynamic and kinetic conditions in the control of selectivity in nucleobase alkylation, (ii) it unravels the importance of solvent effects and H-bonding in controlling the nucleophilicity of DNA bases toward *o*-QMs, and at the same time (iii) it underlines the importance of tautomeric forms of nucleobases and covalent modified adducts.

The presence of several metastable covalent modified DNA bases, arising from alkylation reactions under kinetic conditions in water, such as those arising from the alkylation at N7 of **dG**, and N1 of **dA**, reminds the readers that *o*-QM acts as a reversible alkylating agent. Such a general reversibility of the addition process of *o*-QM to nucleosides is certainly interesting, and it could fuel future experiments. Furthermore, from this point of view, our data on the **dG-N7** adduct, which shows (i) a low activation energy for the alkylation process (in water) and (ii) a slightly lower energy with respect to the reactants, suggest that even DNA (where guanine N7 centers are not involved in H-bonding within the base pairs and therefore remain accessible within the duplex) could be the most significant initial target and carrier of QMs like alkylating agents.

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Supporting Information Available: Electronic energies and Cartesian coordinates of stationary points in Figures 1−3 and Schemes 2−5 in the gas phase, 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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