Tautomerism of Neutral and Protonated 6-Thioguanine in the Gas Phase and in Aqueous Solution. An *ab Initio* Study

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Received September 26, 1994[⊗]

The tautomerism of 6-thioguanine in its neutral and protonated forms was studied in the gas phase using high-level *ab initio* methods. The effect of the aqueous environment on the reactive properties and on the tautomer population of neutral and protonated 6-thioguanine was examined using an optimized *ab initio* SCRF method. The results predict that the N9(H) thiol is the most stable tautomer for the neutral form in the gas phase, while the N1,7(H) thione is preferred in aqueous solution. For the protonated molecule the N1,7,9(H) thione and two thiol forms exhibit a close stability in the gas phase, but only the thione form N1,7,9(H) is expected to be found in aqueous solution.

Introduction

Tautomerism involves a large modification in the reactive characteristics of molecules. Among these, the modification of the hydrogen bond pattern is of particular relevance. Thus, it is well known that the biological and pharmacological properties of purines and pyrimidines are dramatically affected by an alteration of the tautomeric preference. Experimental and theoretical studies have demonstrated that the tautomer population can be modified by the presence of substituents and also by the influence of the environment.[1] This raises a wide range of possibilities in the design of molecules that exhibit a change in the tautomeric population upon transfer between different physiological environments. Assuming that, typically, only one of the tautomeric forms is the bioactive species, the tautomerism can be considered *a priori* as a possible "switching" mechanism to control the activity of purine and pyrimidine derivatives with pharmacological potential. The therapeutic possibilities of this "switching" process have not yet been fully explored.

Since the early 1950s a large effort has been focused on the study of thionucleobases, such as 2-thiouridine, 2-thiocytidine, 4-thiouridine, and 6-thioguanosine, which have been examined for antitumoral, antimetabolic, and antibiotic activities.[2] Thiated nucleobases are analogues of nucleic bases in which one of the oxygen atoms has been replaced by a sulfur. The close structural similarity with the parent oxopurines and oxopyrimidines permits the recognition of these thio derivatives by proteins, their incorporation in nucleic acids, or other interactions with oxonucleobases.[3] However, the replacement of the oxygen by the sulfur atom may induce changes in the properties of the nucleobases, which may affect, in turn, the recognition with proteins or with other nucleobases, as well as the ability to stabilize the DNA double helix.[3] Furthermore, it has been suggested that the presence of the thio group may enhance the probability that mutations occur in the DNA.[4]

6-Mercaptopurine derivatives, among them 2-amino-6-mercaptopurine (6-thioguanosine), are known to have interesting chemotherapeutic effects, including powerful antitumoral activity.[2,5] Thioguanine is a chemotherapeutic drug used for the treatment of systemic connective tissue diseases, leukemia, and lymphomas.[2,5] Catalyzed by the hypoxanthine guanine phosphoribosyl transferase, it is converted inside the cell to ribonucleotides, which have multiple metabolic effects resulting in a sequential blockade of the synthesis and utilization of purine nucleotides, since thioguanine ribonucleotides are incorporated into the DNA and RNA of bone narrow cells.[5] In addition, thioguanine inhibits the *de novo* purine synthesis, thus enhancing the incorporation of thioguanine nucleotides through the purine salvage pathway.[2d] The major adverse effect of thioguanine is hematologic toxicity, which is dose related and manifested by leukopenia, thrombocytopenia, and anemia.[6] Most of the therapeutic activities of 6-thioguanosine are expected to depend on the tautomeric equlibrium of the molecule. Accordingly, a deeper knowledge of the tautomerism of 6-thioguanine derivatives in different environments is essential to an understanding of the pharmacological properties of these molecules and to the design of new derivatives with improved activity.

Several experimental and theoretical studies on the tautomerism of 6-thioguanosine are reported in the

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⊗ Abstract published in *Advance ACS Abstracts,* January 15, 1995.

(1) (a) Beak, P.; Fry, F. S.; Lee, J.; Steele, F. *J. Am. Chem. Soc.* **1976**, *98*, 171. (b) Kwiatkowski, J. S.; Zielinski, T. J.; Rein, R. *Adv. Quantum Chem.* **1986**, *18*, 85. (c) Scanlan, M. J.; Hillier, I. H. *J. Am. Chem. Soc.* **1984**, *106*, 3737. (d) Karelson, M. M.; Katrizky, A. R.; Szafran, M.; Zerner, M. C. *J. Chem. Soc., Perkin Trans. 2* **1990**, 195. (e) Cieplak, P.; Bash, P.; Singh, U. C.; Kollman, P. A. *J. Am. Chem. Soc.* **1987**, *109*, 6283. (f) Orozco, M.; Bachs, M.; Luque, F. J. *J. Comput. Chem.*, in press. (g) Karelson, M. M.; Katritzky, A. R.; Szafran M.; Zerner, M. C. *J. Org. Chem.* **1989**, *54*, 6030.

(2) (a) Bennett, L. L.; Montgomery, J. A. In *Methods in Cancer Research;* Busch, H., Ed.; Academic Press: New York, 1967; Vol. III. (b) Calabresi, P.; Parks, R. E. In *The Pharmacological Basis of Therapeutics*; Goodman, L. S., Gilman, A., Eds.; Macmillan: New York, 1970. (c) Astwood, E. B.; Bissel, A.; Hugles, A. M. *Endocrinology* **1945**, 37, 456. (d) Tidd, D. M. Paterson, A. R. P. *Cancer Res.* **1974**, *34*, 738. (e) Clarke, D. A.; Phillips, F. S.; Sternberg, S. S. and Stock, C. C. *Ann. N. Y. Acad. Sci.* **1954**, *60*, 235.

(3) (a) Yu, M. W.; Sedlack, J.; Lindsay, R. H. *Arch. Biochem. Biophys.* **1973**, *111*, 1955. (b) Ono, M.; Kawakami, M. *J. Biochem.* **1977**, *81*, 1247. (c) Carbon, J.; David, H.; Studier, M. H. *Science* **1968**, *161*, 1146.

(4) Leszcynski, J. *J. Phys. Chem.* **1993**, *97*, 3520.

(5) Nerstrom, V. M.; Henriksen, U.; Nielsen, P. E.; Buchardt.; Schmiefelow, K.; Koch, C. *Bioconjugate Chem.* **1994**, *5*, 357 and references therein.

(6) McCormack, J. J.; Johns, D. G. In *Cancer Chemotherapy: Principles and Practice*; Chabner, B. A., Collins, J. M., Eds.; J. B. Lippincott Co.: Philadelphia, pp 234–252.

0022-3263/95/1960-0969$09.00/0

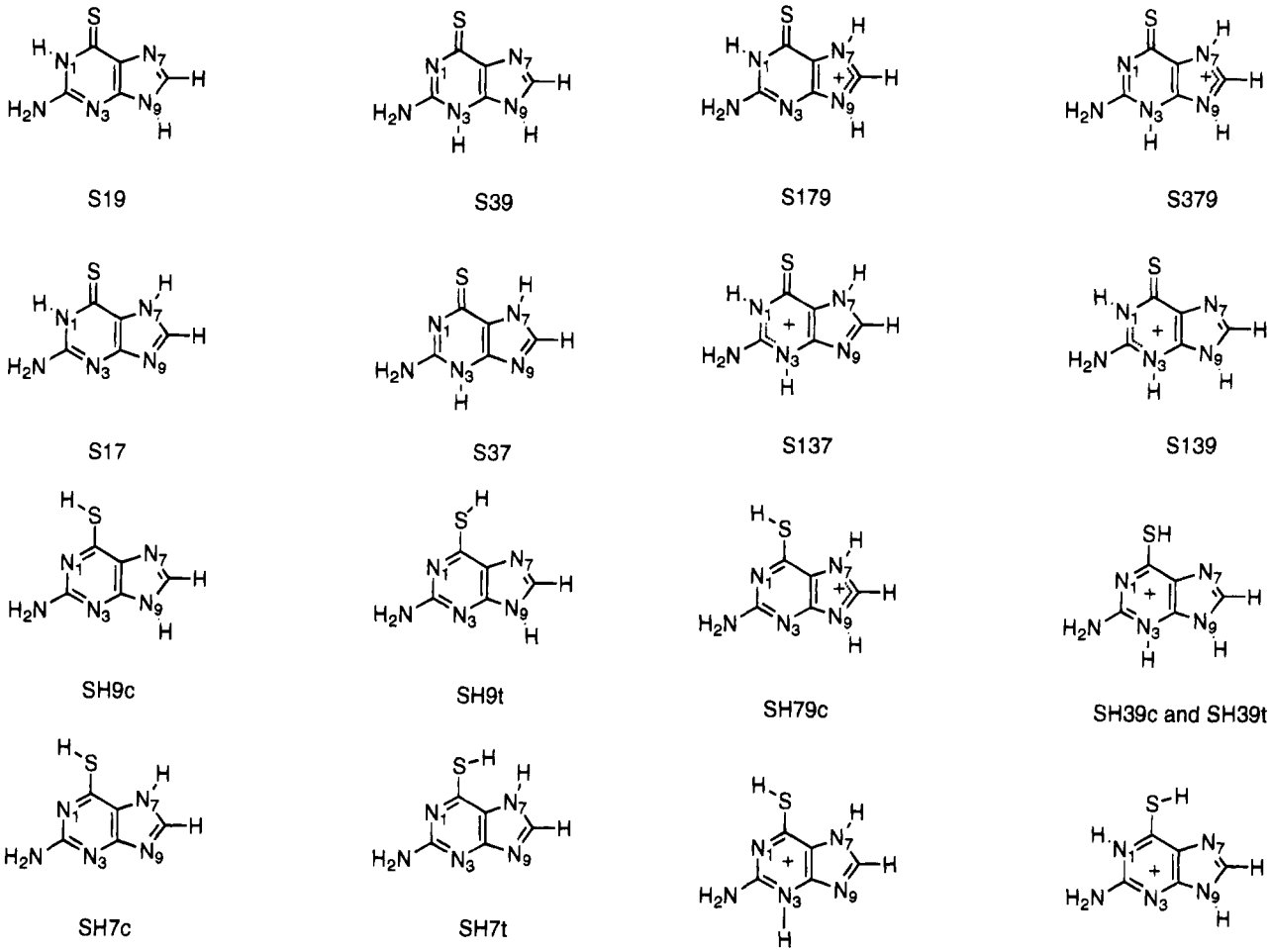

Figure 1. Structure of tautomeric forms of neutral 6-thioguanine.

Figure 2. Structure of tautomeric forms of protonated 6-thioguanine.

literature.[4,7] The highest level calculations[4] published to date (MP2/6-31G(d) including zero point energy correction (ZPE)) suggest a similar stability for the N7(H) thione and N(9)H thiol species in the gas phase. On the other hand, Raman, IR, and X-ray experiments show that the 6-thioguanine is found mainly in the N7(H) thione tautomeric form in the solid state, in contrast to guanine, for which the N9(H) oxo tautomer is detected in crystals.[8] To our knowledge, neither experimental nor theoretical data have been reported on the tautomeric preference of 6-thioguanine in aqueous solution. Furthermore, the influence of the ionization state on the tautomeric preference has not been explored either in the gas phase or in aqueous solution.

Here we present an extensive theoretical study of the tautomerism of 6-thioguanine. *Ab initio* calculations were performed at different levels ranging from the standard SCF-RHF 6-31G(d)//6-31G(d) to large extended MP2/6-311++G(d,p)//HF/6-31G(d) levels. Thermal and entropic corrections were determined at the 6-31G(d) level. The effect of water on the tautomeric equilibrium was explored using a high level *ab initio* 6-31G(d) self-consistent reaction field (SCRF) method developed by Prof. Tomasi's group[9] and recently optimized in our laboratory.[10] Finally, following previous studies,[11] which demonstrated the dependence of the tautomeric preference on the ionization state of nucleic bases, the effect of the protonation on the tautomerism of 6-thioguanine in both the gas phase and aqueous solution was examined.

Methods

Eight tautomers of the neutral 6-thioguanine were considered (Figure 1), which correspond to all the possible tautomers which do not involve tautomerism of the 2-amino group, since these imino tautomers are expected to be highly unstable. The number of possible tautomers of the protonated 6-thioguanine is even larger, but a detailed inspection of the structures suggested that some of them are clearly unfavored. Accordingly, and following the preliminary results derived from force field calculations for all the possible tautomers, we focused our attention on the nine tautomeric forms shown in Figure 2. This study deals with the largest number of tautomers of neutral 6-thioguanine carried out to date at the *ab initio* level. Furthermore, it is also the first study of the tautomeric equilibrium of the protonated 6-thioguanine.

(7) (a) Elion, G. B.; Goodman, I.; Lange, W.; Hitchings, G. H. *J. Am. Chem. Soc.* **1959**, *81*, 1898. (b) Bugg, C. E.; Thewalt, U. *J. Am. Chem. Soc.* **1970**, *92*, 7441. (c) Singh, K.; Yadav, R. A.; Yadav, J. S. *Spectrochim. Acta* **1991**, *47 A*, 819.

(8) Bugg, C. E.; Thewalt, U.; Marsh, R. E. *Biochem. Biophys. Res. Commun.* **1968**, *33*, 436.

(9) (a) Miertus, S.; Scrocco, E.; Tomasi, J. *Chem. Phys.* **1981**, *55*, 117. (b) Miertus, S.; Tomasi, J. *Chem. Phys.* **1982**, *65*, 239.

(10) (a) Bachs, M.; Luque, F. J.; Orozco, M. *J. Comput. Chem.* **1994**, *15*, 446. (b) Orozco, M.; Luque, F. J. *Chem. Phys.* **1994**, *182*, 237. (c) Luque, F. J.; Bachs, M.; Orozco, M. *J. Comput. Chem.* **1994**, *15*, 847.

(11) (a) Orozco, M.; Lluis, C.; Mallol, J.; Canela, E. I.; Franco, R. *J. Org. Chem.* **1190**, *55*, 753. (b) Orozco, M.; Canela, E. I.; Franco, R. *Mol. Pharmacol.* **1989**, *35*, 257. (c) Orozco, M.; Luque, F. J. *J. Am. Chem. Soc.*, in press.

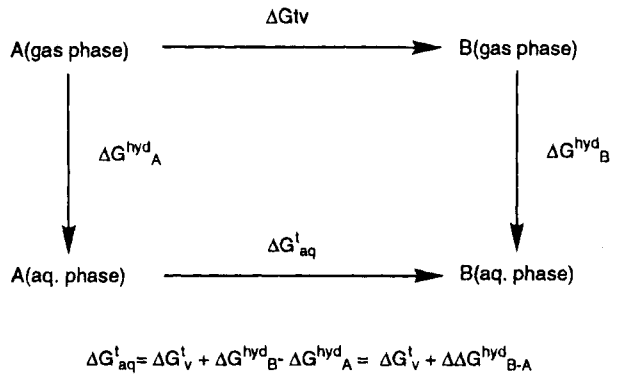

Figure 3. Thermodynamic cycle used to compute tautomerization free energies in aqueous solution.

Gas Phase Calculations. The geometries of the tautomers were fully optimized at the *ab initio* level using the 6-31G(d) basis set.[12] Frequency analyses were performed to verify the nature of the minimum state of the stationary points located during the geometry optimization, as well as to obtain ZPE, thermal, and entropic corrections. Single point SCF-RHF calculations were carried out for all the tautomers using the large, extended 6-311++G(d,p) basis set.[12,13] Electron correlation effects were introduced at the MP2 level[14] using the 6-311++G(d,p) basis and the 6-31G(d) geometry. The use of higher level methods to account for correlation effects is not expected to introduce major modifications in the results.[11c,15] Furthermore, the 6-311++G(d,p) basis set is large enough to guarantee that only small changes can be expected due to the finite size of the basis set.

Tautomerization enthalpies were computed from the energies calculated at the SCF and Moller–Plesset levels corrected for ZPE and thermal effects (T = 298K). Free energies of tautomerization (ΔG_v^t) at 298 K were determined from the addition of enthalpic (ΔH) and entropic ($-T\Delta S$) terms. The vibrational frequencies needed to compute enthalpic and entropic terms were determined at the 6-31G(d) level using the standard procedure in Gaussian 90.[16]

Aqueous Phase Calculations. Calculation of the free energies of tautomerization in aqueous solution was performed, taking advantage of the thermodynamic cycle shown in Figure 3. The free energies of hydration were determined using a 6-31G(d), optimized version of the SCRF algorithm developed by Miertus, Scrocco, and Tomasi (MST). According to this method (see refs 9 and 10 for details), the free energy of hydration was determined as the addition of electrostatic and steric contributions (eq 1). The steric component was

$$\Delta G_{hyd} = \Delta G_{elc} + \Delta G_{cav} + \Delta G_{vw} \quad (1)$$

$$\Delta G_{vw} = \sum_i \xi_i S_i \quad (2)$$

ξ_i: the hardness of atom i

S_i: the portion of the molecular surface area belonging to atom i

computed as the sum of the cavitation and a "van der Waals" contribution, which accounts for dispersion–repulsion plus volume effects. The cavitation term was determined using Pierotti's scaled particle theory,[17] while the "van der Waals" term was evaluated (eq 2) by means of a linear relation with the molecular surface area.[1f,10a,c] Parameters (ξ; in kcal/mol) defining the "hardness" of the different atom types were determined from a previous parametrization:[1f] C = −0.109, H (bound to heteroatoms) = −0.174, H (bound to carbon atoms) = −0.088, S = −0.070, N = −0.052.

The electrostatic interaction between the solute and the water was computed using the MST SCRF approach, in which the solvent is represented as a continuous dielectric, which reacts against the solute charge distribution, generating a reaction field (V_R). The effect of the solvent reaction field on the solute is introduced as a perturbation operator in the solute Hamiltonian (eq 3). The self-consistent nature of the method

$$(\hat{H}^0 + \hat{V}_R)\Psi = E\psi \quad (3)$$

stems from the mutual dependence between the solute charge distribution and the reaction field. The convergence in the SCRF process for the molecules considered here was usually achieved after three to four SCF cycles.

The reaction field operator was described in terms of a set of point charges located at the solute/solvent interface, i.e., the solute cavity (eq 4). Such imaginary charges were determined

$$\hat{V}_R = \sum_{i=1}^{M} \frac{\sigma(s_i)S_i}{|r_0 - r|} = \sum_{i=1}^{M} \frac{q_i}{|r_0 - r|} \quad (4)$$

$$\sigma(s_i) = -\frac{\epsilon - 1}{4\pi\epsilon}\left(\frac{\partial V_T}{\partial n}\right)_n \quad (5)$$

$$V_T(r) = V_\varrho(r) + V_\sigma(r) \quad (6)$$

by solving the Laplace equation (eq 5) at the solute/solvent interface. In eq 5 both the solute, V_ϱ, and the solvent, V_σ, contributions to the electrostatic potential are taken into account (eq 6). It should also be noted that the solute electrostatic potential, V_ϱ, was rigorously computed from the SCF molecular electrostatic potential (MEP).[18]

The electrostatic contribution to the free energy of hydration (ΔG_{ele}) was determined following linear free energy response theory (LFER[9,19]) according to eq 7.

$$\Delta G_{ele} = \langle\psi|\hat{H}|\psi\rangle - \langle\psi^0|\hat{H}^0|\psi^0\rangle - 1/2(\langle\psi|\hat{V}|\psi\rangle + \int \varrho_{nuc} V_\sigma(s)ds) \quad (7)$$

In all cases the solute/solvent interface was determined using a molecular shape algorithm.[9,10] Standard van der Waals radii (C = 1.5 Å, H (bound to polar atom) = 0.9 Å, H (bound to carbon atoms) = 1.2 Å, N = 1.5 Å, S = 1.75 Å) were used.[10a,c] A scaling factor of 1.25 was used to build the solute cavity for neutral tautomers,[10a] while a factor of 1.15 was considered for protonated forms.[10b] For all the tautomers the molecular geometry optimized in gas phase was kept fixed in SCRF calculations.

Calculations were performed using Gaussian 90[16] and a modified version of MonsterGausss.[20] Molecular electrostatic potentials were evaluated using the MOPETE program.[21] All

(12) Hariharan, P. C.; Pople, J. A. *Theor. Chim. Acta* **1973**, *28*, 213.
(13) Frisch, M. J.; Pople, J. A.; Binkley, J. S. *J. Chem. Phys.* **1984**, *80*, 3265.
(14) Moller, C.; Plesset, M. S. *Phys. Rev.* **1934**, *46*, 618.
(15) (a) Krishnan, R.; Binkley, J. S.; Seeger, R.; Pople, J. A. *J. Chem. Phys.* 1980, *72*, 650. (b) De Frees, D. J.; McLean, A. D. *J. Comput. Chem.* **1986**, *7*, 321.
(16) Gaussian 90, Revision F.: Frisch, M. J.; Head-Gordon, M.; Trucks, G. W.; Foresman, J. B.; Schelgel, H. B.; Raghavachari, K.; Robb, M.; Binkley, J. S.; Gonzalez, C.; Defrees, D. J.; Fox, D. J.; Whiteside, R. A.; Seeger, R.; Melius, C. F.; Baker, J.; Martin, R. L.; Kahn, L. R.; Stewart, J. J. P.; Topiol, S.; Pople J. A. Gaussian Inc., Pittsburgh, PA, 1990.
(17) Pierotti, R. A. *Chem. Rev.* **1976**, *76*, 717.
(18) Scrocco, E.; Tomasi, J. *Top. Curr. Chem.* **1973**, *42*, 95.
(19) Boetcher, C. J. F. *Theory of Electric Polarization*; Elsevier: Amsterdam, 1973.
(20) Peterson, M.; Poirier, R. MonsterGauss, University of Toronto. Modified by R. Cammi, R. Bonnaccorsi and J. Tomasi, University of Pisa, 1987. Modified by F. J. Luque amd M. Orozco, University of Barcelona. 1994.
(21) MOPETE: Luque, F. J.; Orozco, M. University of Barcelona, 1993.

Table 1. Energy, Enthalpy, Entropic Term, and Free Energy of Tautomerization Relative to the S19 Tautomer of 6-Thiolguanine in the Gas Phase. (All the Values Are in kcal/mol; See Text for Details)

magnitude[a]	S39	S17	S37	SH9c	SH9t	SH7c	SH7t
6-31G(d)							
ΔE(QM)	22.9	−2.6	6.2	−0.2	−0.9	5.9	8.4
ΔH(298K)	22.4	−2.5	6.1	−2.4	−3.0	3.5	6.9
$-T\Delta S$(298K)	−0.4	0.0	−0.1	−0.2	−0.2	−0.6	−0.9
ΔG(298K)	22.0	−2.5	6.0	−2.6	−3.2	2.9	5.1
6-311++G(d,p)//6-31G(d)							
ΔE(QM)	22.4	−2.7	5.9	−1.1	−2.1	4.7	7.0
ΔH(298K)	21.9	−2.5	5.8	−3.3	−4.2	2.3	4.6
$-T\Delta S$(298K)	−0.4	0.0	−0.1	−0.2	−0.2	−0.6	−0.9
ΔG(298K)	21.5	−2.5	5.7	−3.5	−4.4	1.7	3.7
MP2/6-311++G(d,p)//HF/6-31G(d)							
ΔE(QM)	19.6	−2.7	4.7	−2.6	−3.5	2.1	4.5
ΔH(298K)	19.1	−2.5	4.6	−4.7	−5.7	−0.3	2.0
$-T\Delta S$(298K)	−0.4	0.0	−0.1	−0.2	−0.2	−0.6	−0.9
ΔG(298K)	18.7	−2.5	4.5	−5.0	−5.9	−0.9	1.2

[a] Energy of tautomer S19: −862.031 020 4 au (HF/6-31G(d)); −862.174 038 2 au (HF/6-311++G(d,p)); −863.835 264 5 au (MP2/6-311++G(d,p)). Zero point + thermal corrections: 0.132 375 3 au (HF/6-31G*). Entropy: 89.1 cal K^{-1} mol^{-1}.

the calculations were carried out on a Cray YMP at the Centre de Supercomputació de Catalunya (CESCA) and on SUN and HP workstations in our laboratories.

Results and Discussion

Neutral Form. Gas Phase. The energies, enthalpies, entropies, and free energies of the tautomers of 6-thioguanine relative to the S19 tautomer (N1,9(H) thione; see Figure 1 for nomenclature) are shown in Table 1. Relative energies were computed using three different levels: SCF-RHF 6-31G(d)//6-31G(d) and 6-311++G(d,p)//6-31G(d), and MP2/6-311++G(d,p)//HF/6-31G(d). Zero point energy, thermal, and entropic corrections were computed at the 6-31G(d) level (see Methods for details) and added to the energies to obtain the enthalpies and free energies.

The most surprising finding is the high stability of the thiol tautomers in the gas phase, which is especially remarkable according to results derived at the highest computational level. Thus, the thiol tautomer SH9t is 3.4 and 5.9 kcal/mol, more stable than the thione tautomers S17 and S19, which are the most stable thione species. There are two reasons for this stability of thiol forms in gas phase: (a) the electronic stabilization, as noted in the ΔE values, which indicate that the SH9t tautomer is 0.8 and 3.5 kcal/mol more favored than S17 and S19, respectively, and (b) the ZPE and thermal corrections. These latter terms, which are sometimes neglected in quantum mechanical calculations, are important in the thione/thiol tautomerism (their magnitude is even greater than 2 kcal/mol) and always favor the thiol tautomers. The entropic contribution again stabilizes the thiol tautomers, but the magnitude of this effect is rather small. Inspection of the relative stability of the thione tautomers reveals that the S17 (N7,1(H) thione) form is more stable (around 2.5−2.7 kcal/mol) than the S19 (N9,1(H) thione) tautomer. All the N3(H) thione tautomers are quite unstable, especially when the N−H bond of the five-membered ring occurs at the nitrogen N9 (S39), which can be realized from the unfavorable repulsions arising from 1−4 and 1−5 interactions between hydrogen atoms (see Figure 1).

Comparison of the results derived at the different computational levels shows that in some cases the extension of the basis set from the 6-31G(d) to the very large 6-311++G(d,p) and the introduction of correlation effects *via* Moller−Plesset perturbation theory[14] are not strictly necessary to obtain qualitatively correct results. In contrast, in other cases they must be taken into account to provide a precise description. Thus, extension of the basis or introduction of correlation effects does not modify the results when the thione−thione or thiol−thiol tautomerisms are studied. However, such effects cannot be neglected if reliable results are required when the thiol−thione tautomerism is dealt with. This behavior can be understood by considering that no major changes in the chemical bonds occur in the tautomerism between thione−thione, or thiol−thiol, species, whereas important differences in the nature of the chemical bonds are found in the thione−thiol tautomerism.

Due to the lack of experimental data in the gas phase, it is not possible to establish a direct assessment of the results previously discussed. Nevertheless, indirect information derived from X-ray diffraction confirms that the N7H tautomer could be preferred over the N9H form for thiones.[7h] Comparison with the *ab initio* results provided by Leszcynski[4] (only S19, S17, SH9c, and SH7c tautomers were considered in his study) is of interest, since to our knowledge they were determined at the highest computational level published to date on this molecule. Unfortunately, the comparison should be limited to energies, since enthalpies and entropies were not reported. In general, the agreement between the values reported by Leszcynski and the present results is quite remarkable. Thus, the two studies emphasize the great importance of the ZPE contribution in the calculation of thione−thiol tautomerism. The importance of the basis set and of the correlation effects in the thione−thiol tautomerism, and their scarce relevance in thione−thione and thione−thiol processes, were also demonstrated. However, some intriguing discrepancies are found in the stability of the different tautomers, which merits a more detailed discussion.

Leszcynski's results derived at the MP2/6-31G(d) level plus inclusion of ZPE corrections suggest the following ordering of stability: S17 > S19 > SH9c > SH7c. This ordering does not fully agree with that found here: SH9t > SH9c > S17 > SH7c > S19 > SH7t > S37 ≫ S39. Thiol tautomers are predicted to be more stable than thiones in the gas phase according to the present results, while the reverse order of stabilities is suggested from the values given by Leszcynski. However, the most stable thiol tautomer (SH9t) was not considered in his study. According to our calculations, this tautomer is 1.0 kcal/mol more stable than the SH9c. When this is taken in conjuction with the difference of 0.5 kcal/mol between the S17 and SH9c tautomers cited in Leszcynski's work, both studies qualitatively agree that the thiol (SH9t) is preferred over the thione (S17) in the gas phase. Despite this qualitative agreement, previous results predict, in general, a higher stability of the thione tautomers even when the calculations are carried out with the same basis set, which demonstrates that the origin of this discrepancy lies in the use of different geometries. The geometry of the tautomers was optimized in Leszcynski's study using a mixed basis set, in which a 3-21G basis was employed for all the atoms but the sulfur, which was represented at the 3-21G* level. Comparison[4] of single point calculations performed with this basis set and with 6-31G(d), and comparison of 6-31G(d)//3-21G(d) and 6-31G(d)//6-31G(d) dipole moments, suggests that the

Table 2. Differences in Free Energies of Hydration ($\Delta\Delta G_{hyd}$) and Free Energies of Tautomerization in Water (ΔG^t_{aq}) for Tautomers of Neutral 6-Thioguanine[a]

tautomer	$\Delta\Delta G_{hyd}$	ΔG^t_{aq}	tautomer	$\Delta\Delta G_{hyd}$	ΔG^t_{aq}
S39	−8.2	10.5	SH9t	10.6	4.8
S17	2.2	−0.3	SH7c	6.9	6.0
S37	−0.5	4.0	SH7t	5.2	6.4
SH9c	10.1	5.1			

[a] Values (in kcal/mol) are referred to the S19 tautomer. Gas phase values used to compute ΔG^t_{aq} were taken from results computed at the MP2/6-311++G(d,p)// HF/6-31G* level.

mixed basis set is not flexible enough to provide suitable molecular geometries for this kind of study.

Comparison of the tautomeric characteristics of 6-thioguanine with other nucleobases is also of interest. Once again, such a comparison is difficult due to the lack of the experimental data. Nevertheless, some indirect support is obtained from UV data for the tautomerism of 2-mercaptopyridine,[1a] which state that the 2-thiol form is more than 1.4 kcal/mol more stable than the corresponding thione. The preference for the thiol form in vacuum is also expected from the behavior of 2-pyridones, which are shown to be predominantly in the enol form in gas phase (around 0.4−0.6 kcal/mol in ΔG^{1a}). Comparison with theoretical studies on related molecules confirms the importance of correlation effects, which are necessary for the correct reproduction of the enol preference of 2-oxopyridine in gas phase.[1e,22] Finally, a theoretical study of guanine,[23] in which the highest calculational level was MP2/6-31G(d)//HF/3-21G, indicates that the oxo form was preferred in the gas phase with regard to the enol by around 1.9 kcal/mol.

Solvent Effect. A polar solvent like water may exercise a large influence on the tautomerism of heterocycles. Therefore, we decided to explore the role of water in the tautomeric equilibrium of 6-thioguanine using an optimized 6-31G(d)-SCRF algorithm .[9,10] Differences in free energies of hydration (computed relative to the S19 tautomer), and the tautomerization free energies in aqueous solution (see Figure 3) are shown in Table 2. A detailed analysis of the results clearly reveals the dramatic effect of the solvent on the tautomer population.

Water largely stabilizes all the thione species, especially the N3(H), with regard to the thiol tautomers. This finding is explained by the large polarizability of thiones. In particular, the stabilization of the S39 tautomer is notably large. This is due to the combined effect of (a) the large dipole and (b) the intrinsic polarizability of thiones. On the other hand, the large destabilization of SH9 (relative to S17) by water can be due to the smaller polarizability of the thiol group and to the small dipole moment of these thiol tautomers (see Table 3). It is interesting to note that the large stabilization of thiones compared with thiol tautomers by water completely reverts the preference found in gas phase, which suggests that the N1(H) thione tautomers (S17, S19) are the major species in aqueous solution, the population of thiol tautomers being negligible. In addition, water stabilizes by around 2.2 kcal/mol the S19 tautomer compared with the S17. This result, which can be rationalized in terms of the dipole moment (Table 3), implies that the two species S19 and S17 are likely to be found in aqueous solution, the S19 tautomer being only 0.3 kcal/mol less stable, which corresponds to a predicted ratio (S17/S19) of 1.7.

Table 3. Gas Phase and Water-Induced Dipoles (in Debye) for the Tautomers of Neutral 6-Thioguanine[a]

tautomer	μ_{vac}	$\Delta\mu$	tautomer	μ_{vac}	$\Delta\mu$
S19	8.4	+3.0	SH9c	3.5	+0.7
S39	12.6	+5.5	SH9t	4.1	+1.1
S17	2.0	+0.5	SH7c	3.8	+1.8
S37	6.9	+1.9	SH7t	4.6	+2.2

[a] The values have been determined from 6-31G(d)//6-31G(d) calculations in vacuum and in aqueous solution.

Water not only has a large influence on the ratio between tautomers of the neutral 6-thioguanine, but it greatly modulates the electronic charge distribution. In general, water increases the polarity of the molecule, as noted in the water-induced dipole moments given in Table 3. As expected from previous studies,[10a,24] the larger the dipole moment, the greater the polarizing effect of water. The relative magnitude of the induced dipole moment ranges between 20% (SH9c) and 47% (SH7) of the value of the gas phase dipole. The water-induced dipole of the different tautomers of 6-thioguanine is, on average, around 34%, which is somewhat greater than the average $\Delta\mu/\mu$ ratio reported for a series of small organic molecules,[10a,24] which demonstrates the larger polarizability of heterocycles.[25]

Inspection of the changes in the MEP upon solvation provides further insight into the charge redistribution induced by the polarizing effect of water. The results for the two most significant tautomers, S17 and SH9t, are shown in Figure 4, and the variation of the MEP minima on the molecular plane for these two tautomers is given in Table 4. As expected, water induces marked changes in the electron distribution, mainly in the π system, which enhances the electron density in those regions near the sulfur and the pyridine-type nitrogens, whereas an electron shift from regions around the amino group and the C8−H bond is clearly evident. This electron redistribution, which agrees with that found for similar molecules,[25] leads to a general increase in the depth of MEP minima. Nevertheless, the relative ordering of the MEP minima observed in the gas phase is not altered by the polarization generated by the aqueous environment. The deepest MEP minimum for the tautomers S17 and S19 is associated with the nitrogens N9 (−83.1 and −68.3 kcal/mol in solution and in gas phase) and N7 (−96.1 and −76.3 kcal/mol), respectively, whereas for the tautomer SH9t corresponds to the nitrogen N1 (−71.8 and −60.9 kcal/mol).

Comparison of our theoretical data with experimental data is not possible, but indirect data again allowed us to be confident on the reliability of our simulations. First, the large stabilization of thiones with regard to thiol tautomers has been demonstrated experimentally in 2-mercaptopyridine, which is detected in the thiol form in gas phase, but in the thione tautomer in water (the $\Delta G_{aq}{}^t \approx 6.6$ kcal/mol, similar to our results). Furthermore, the results are also in qualitative agreement with the experimental behavior reported for pyridone derivatives, as well as with theoretical calculations performed

(22) Kwiatkowski, J. S.; Barlett, R. J.; Person, W. B. *J. Am. Chem. Soc.* **1988**, *110*, 2353.
(23) Sabio, M.; Topiol, S.; Lumma, W. C. *J. Phys. Chem.* **1990**, *94*, 1366.
(24) (a) Luque, F. J.; Negre, M.; Orozco, M. *J. Phys. Chem.* **1993**, *87*, 4386. (b) Gao, J.; Luque, F. J.; Orozco, M. *J. Chem.Phys.* **1993**, *98*, 2975. (c) Luque, F. J.; Orozco, M.; Bhadane, P. K., Gadre, S. R. *J. Phys. Chem.* **1993**, *97*, 9380.
(25) Orozco, M.; Luque, F. J. *Biopolymers* **1993**, *33*, 1851.

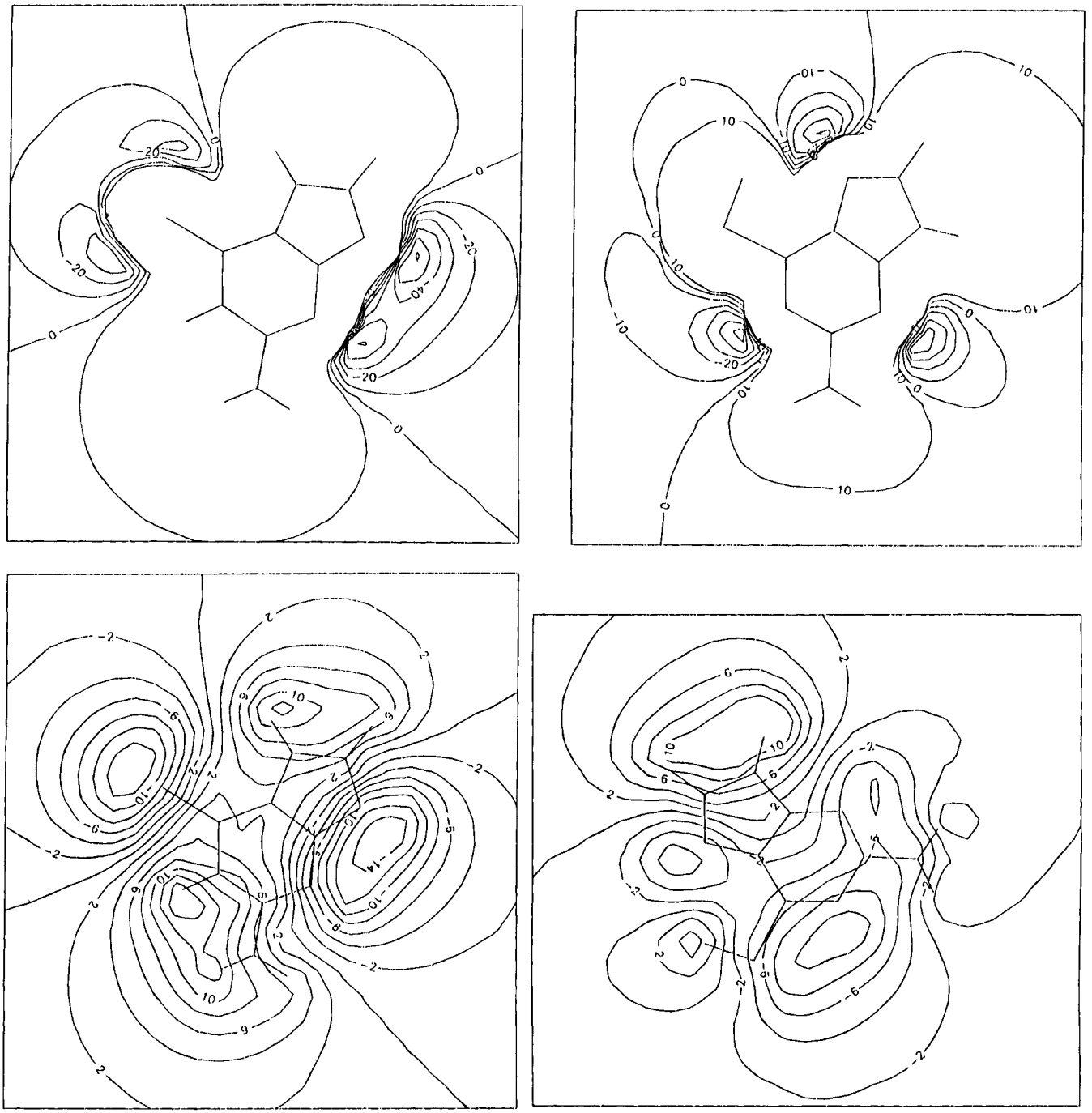

Figure 4. (A (Top left), A bis (top right)) molecular electrostatic potential (MEP) maps in the molecular plane of the S17 and SH9t tautomers of 6-thioguanine computed from 6-31G(d) gas phase wavefunctions. (B (Bottom left), B bis (bottom right)) MEP difference maps ($MEP_{aq} - MEP_{vac}$) in the molecular plane for the same tautomers. All the values are in kcal/mol.

for these compounds,[1e,f,g] for which water largely stabilizes the oxo tautomers with regard to the enol species.

Protonated Forms. Gas Phase. Results concerning the tautomerism of the protonated 6-thioguanine in the gas phase are given in Table 5. As noted before (see Methods), tautomers with unfavorable 1−4 and 1−5 electrostatic interactions were not considered (see Figure 2). In an analogous way to the study of the neutral molecule, calculations were carried out at the SCF-RHF 6-31G(d)//6-31G(d), 6-311++G(d,p)//6-31G(d), and MP2/6-311G(d,p)//HF/6-31G(d) levels.

The results obtained at the highest level (MP2/6-311G-(d,p)//HF/6-31G(d)) suggest that the S179 is the most stable protonated form. However, two thiol tautomers (SH37c and SH19t) are quite stable, and the presence of thiol forms in gas phase cannot be ruled out. Inspection of the free energy components in Table 5 suggests that the energy differences always stabilize the thione form (S179), while (as found for the neutral form) the ZPE, thermal, and entropic contributions favor the thiol tautomers. If attention is focused on the relative stability of the four thione structures, it is clear that S379 and S139 are extremely unstable due to strong 1−4 and 1−5 repulsive interactions and also that these interactions can be responsible for the higher stability of S179 compared with S137. These considerations can also be used to understand the relative stability of the protonated thiol tautomers.

Table 4. MEP Minima (in kcal/mol) in the Gas Phase and in Aqueous Solution of the Tautomers S17, S19, and SH9t

	gas phase	aq soln
tautomer S17		
S	−37.3	-44.7
	−40.1	−47.7
N3	−57.7	−69.0
N9	−68.3	−83.1
tautomer S19		
S	−43.5	−55.5
	−63.5	−85.6
N3	−43.0	−39.8
N7	−76.3	−96.1
tautomer SH9t		
N1	−60.9	−71.8
N3	−55.7	−61.3
N7	−58.8	−65.9

Table 5. Energy, Enthalpy, Entropic Term and Free Energy of Tautomerization Relative to the S179 Tautomer of Protonated 6-Thiolguanine in the Gas Phase. (All the Values are in kcal/mol; See Text for Details)

magnitude[a]	S379	S137	S139	SH79c	SH39c	SH39t	SH37c	SH19t
6-31G(d)								
ΔE(QM)	24.6	5.5	20.2	5.7	9.2	8.5	2.2	2.6
ΔH(298K)	24.0	5.3	19.6	3.5	6.7	6.1	−0.1	0.3
$-T\Delta S$(298K)	−0.5	−0.1	−0.5	−0.3	−0.3	−0.3	−0.4	−0.4
ΔG(298K)	23.5	5.2	19.2	3.2	6.4	5.8	−0.5	−0.1
6-311++G(d,p)//6-31G(d)								
ΔE(QM)	23.9	5.5	19.9	5.0	8.4	7.4	1.4	1.5
ΔH(298K)	23.4	5.3	19.3	2.8	5.9	4.9	−0.9	−0.8
$-T\Delta S$(298K)	−0.5	−0.1	−0.5	−0.3	−0.3	−0.3	−0.4	−0.4
ΔG(298K)	22.9	5.1	18.8	2.5	5.6	4.7	−1.3	−1.2
MP2/6-311++G(d,p)//HF/6-31G(d)								
ΔE(QM)	20.7	4.6	17.6	4.5	9.1	8.1	2.9	3.7
ΔH(298K)	20.2	4.4	17.0	2.3	6.6	5.7	0.6	1.4
$-T\Delta S$(298K)	−0.5	−0.1	−0.5	−0.3	−0.3	−0.3	−0.4	−0.4
ΔG(298K)	19.6	4.3	16.5	2.0	6.3	5.4	0.3	0.9

[a] Energy of tautomer S179: −862.411 955 5 au (HF/6-31G(d)); −862.554 288 5 au (HF/6-311++G(d,p)); −864.207 730 2 au (MP2/6-311++G(d,p)). Zero-point + thermal corrections: 0.146 597 6 au (HF/6-31G*). Entropy: 89.8 cal K^{-1} mol^{-1}.

Combination of the results for neutral and protonated 6-thioguanine and inspection of the MEP maps and MEP minima energies for neutral forms in Figure 4 and Table 4 suggest that in gas phase the 6-thioguanine is predominantly in the SH9t tautomeric form. *A priori* protonation of this form could occur at N1, N3, or N7, but the MEP and the energy results suggest that protonation at N1 is preferred. However, it is expected that the resulting form (SH19t) will change to the most stable S179 form.

Solvent Effect. The stability of the tautomers of the protonated 6-thioguanine is greatly affected by water. As expected from the behavior of the neutral molecule, the thione tautomers are clearly better solvated than the thiol forms (see Table 6). According to our present results, the thione form should be the only species detected in water. Particularly, inspection of Table 6 suggests that the N1,7,9(H) tautomer (S179) is the major form in aqueous solution, followed by another thione species (S137), which is less favored by 2.4 kcal/mol. The thiol tautomers, which are quite stable in gas phase, are around 8−10 kcal/mol less stable in aqueous solution.

A detailed inspection of Table 6 shows that the S179 tautomer is indeed the least well hydrated structure, while the thione tautomers with the worst 1−4 and 1−5 contacts (S139 and S379) exhibit the most negative free

Table 6. Differences in Free Energies of Hydration ($\Delta\Delta G_{hyd}$) and Free Energies of Tautomerization in Water (ΔG^t_{aq}) for Tautomers of Protonated 6-Thioguanine[a]

tautomer	$\Delta\Delta G_{hyd}$	ΔG^t_{aq}	tautomer	$\Delta\Delta G_{hyd}$	ΔG^t_{aq}
S379	−12.5	7.1	SH39c	3.2	9.5
S137	−1.9	2.4	SH39t	4.2	9.6
S139	−10.8	5.7	SH37c	8.1	8.4
SH79c	5.7	7.7	SH19t	7.2	8.2

[a] Values (in kcal/mol) are referred to the S179 tautomer. Gas phase values used to compute ΔG^t_{aq} were taken from results computed at the MP2/6-311++G(d,p)//HF/6-31G* level.

energies of hydration. These results are in good agreement with the trends observed for the neutral molecule and can be easily explained by the large field generated from the charge distribution of these tautomers. Surprisingly, the thiol tautomers achieved similar stability upon solvation. Thus, the SH39 tautomer, which is markedly less stable than the SH37c or SH19t species in the gas phase, is only around 1 kcal/mol less favored in water.

Analysis of the results for neutral and charged 6-thioguanine allows us to obtain a picture of the protonation process in aqueous solution. The results strongly suggest that the neutral molecule exists as the N1,7(H) and N1,9(H) thione forms (S17 and S19), the N1,7(H) being slightly more abundant. The protonation of these thione forms occur at the pyridine-type nitrogen of the five-membered ring (N9 or N7), as expected from the MEP maps and particularly from the MEP minimum values shown in Figure 4 and Table 4. Therefore, the resulting protonated form is the N1,7,9(H) tautomer (S179), which is clearly the most stable species in aqueous solution.

All the present results, as well as the findings discussed on the tautomerism and the protonation of 6-thioguanine, cannot be compared with experimental data or with theoretical results, since no experimental or theoretical study of protonated 6-thioguanines has been published, to our knowledge. However, once again some indirect support can be derived from experimental data on the protonation of guanine. Thus, it has been demonstrated that guanine exists in oxo forms in aqueous solution and that protonation takes place at the free nitrogen of the five-membered ring.[26] The qualitative agreement between our theoretical results for 6-thioguanine and the experimentally demonstrated behavior of guanine is very encouraging and strongly supports the reliability of our calculations.

Conclusions

High level *ab initio* calculations have provided a picture of the tautomerism of neutral and protonated 6-thioguanine in the gas phase and in aqueous solution at room temperature. From a quantitative point of view caution must be taken due to the existence of different source of errors, mainly related to the computation of the hydration effects, such as the neglect of geometry relaxation upon solvation, or the incomplete representation of specific solute−solvent interactions, i.e., hydrogen bonds. However, the agreement between MST and experimental free energies of hydration (RMS in the range of 0.7 kcal/mol in a series of 22 prototypical molecules) and between MST and mixed QM−MM strat-

(26) Izatt, R. M.; Christensen, J. J.; Rytting, H. *Chem. Rev.* **1971**, *71*, 439.

egies[24b,27] gives confidence to present simulations. Therefore, from these calculations several conclusions can be reached:

Large basis sets and correlation corrections are not absolutely necessary to describe the thione–thione and thiol–thiol tautomerisms, at least from a qualitative point of view, but they are essential to describe the thione–thiol tautomerism properly.

In the gas phase 6-thioguanine is found predominantly in a thiol tautomeric form (SH9), the S17 thione being around 2.5 kcal/mol more stable than the S19 thione. Water has a dramatic effect on the charge distribution of 6-thioguanine and leads to important changes in the tautomer population. Thus, SCRF calculations suggest that in aqueous solution only thione tautomers (S17 and S19 in a ratio 1/1.7) should be found.

Protonation of 6-thioguanine does not occur at N3. When protonation takes place in the gas phase, the S179 thione is found to be the most stable form, but two thiol tautomers (SH37c and SH19t) have a close stability compared with the S179 species. Protonation in aqueous solution, where the N1(H) thiones (S17, S19) are the main tautomers, occurs at the free imidazole nitrogen, leading to a protonated tautomer (S179), which is the most stable protonated form of the 6-thioguanine.

Acknowledgment. This work has been supported by the Centre de Supercomputació de Catalunya (CESCA) and by the Dirección General de Investigación Científica y Técnica (DGICYT PB93-0779). We thank Sun Corp. for donation of a workstation and R. Rycroft for helpful assistance in the preparation of the manuscript.

JO941867G

(27) Orozco, M.; Luque, F. J.; Habibollahzadeh, D.; Gao, J. *J. Chem. Phys.*, in press.