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Tautomerism of Thioguanine: From Gas Phase to DNA

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High-level *ab initio* calculations were performed on five of the most stable tautomers of 6-thioguanine (6TG) at the second- and fourth-order levels of correlated Møller−Plesset theory using Pople's type of valence double-ζ basis sets with additional sets of polarization and (in some cases) diffusion functions. At the highest applied level of theory (MP4(SDQ)/6-31++G(d,p)//MP2/6-31G(d,p) + 0.9(HF/6-31G(d,p) ZPE)), the order of stability of the tautomers was found to be N9(H)*trans* > N9(H)*cis* > N1,7(H) > N1,9(H) > N7(H). When the ZPE corrections were not employed, all calculations utilizing structures minimized at the correlated MP2 level returned a stability order of N1,7(H) > N9(H)*trans* > N9(H)*cis* > N1,9(H) ≫ N7(H). Calculations carried out using the self-consistent reaction field (SCRF) model to simulate aqueous solution behavior yielded a stability order (with correction for ZPE) of N9(H)*trans* > N1,9(H) > N9(H)*cis* > N1,7(H) ≫ N7(H). CNDO/S calculations on the excited states of 6TG tautomers showed higher oscillator strengths and lower excited-state energies for thiones than for thiols; the latter was confirmed experimentally via UV absorbance and luminescence spectroscopic studies on 6TG and two model compounds for specific tautomers. These experimental spectra confirmed the existence of at least two types of 6TG thione chromophores.

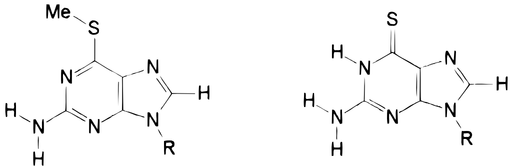
Introduction

Many thio-derivatives of nucleic acid bases play an important role in biological systems.[1,2] The effect of sulfur substitution for oxygen on the molecular structures, properties, and biological activities of pyrimidine-based species has been investigated for a number of compounds.[3−9] However, similar studies are very scarce for derivatives of purine.

The biological relevance of the guanine molecule and its sulfur analog has accelerated theoretical and experimental studies on these systems.[10−17] 6-Thioguanine (6TG) is a known antitumor agent.[18] The uniqueness of its spectroscopic properties and its possible inclusion into primary DNA structure allow its use as a luminescent label for the study of DNA.[16,19] Recent investigation of the IR spectra of 6TG in an argon matrix shows that 6TG occurs mainly (about 85% for an equilibrium established at 500 K) in the N9(H) amino-thiol form.[20] This is in agreement with theoretical *ab initio* calculations.[21] Other available information about the molecular structure of 6TG is derived from X-ray analysis[22] and other IR and Raman solid-state studies.[23] These experiments demonstrate that 6TG exists as the N7(H) amino-thione tautomer **1** (see Figures 5−9 for structures of the studied tautomers) in the crystal structure;[22] this is unlike guanine, which was found to appear as the oxo-N9(H) species.[24] Our experimental investigations[17,19] have shown that in polar solvents 6TG exists as a mixture of two tautomers. At the same time, theoretical calculations by Alhambra *et al.*[25] have shown that in water 6TG must be present as the N7(H) tautomer.

Though 6TG has been studied at the HF/3-21G* level,[15] more recent HF/DZP geometric data[21,26] obtained for guanine (Gua), 6TG, and 6-selenoguanine (6SeG) have shown that the amino groups, which at the 3-21G* approximation are coplanar with the molecular planes, strongly deviate from planarity at higher levels of theory. Even larger nonplanarity has been predicted by MP2 calculations with medium (6-31G**)[27] and large (6-311G(2df,p))[28] basis sets of atomic orbitals. Very recently, we carried out a set of CCSD(T) calculations on the nonplanarity of the amino groups of aniline, aminopyridines, and aminotriazine, using large basis sets of AO.[29] The calculations demonstrated that the MP2 and CCSD(T) methods provide nearly identical results as far as the amino-group pyramidalization is concerned. Flexible and nonplanar amino groups of bases are known to influence the base−base interactions in the DNA double helix.[28,30]

SCHEME 1



10MeTGR 6TGR

We employed *ab initio* molecular theory to calculate the geometries, dipole moments, and energetic stabilities of the five most stable tautomers of 6TG in the gas phase and, using the self-consistent reaction field (SCRF) model, in polar solvent.[15,21] We also used the CNDO/S method[31] to estimate the excited-state energies for the *ab initio*-calculated geometries.

Methods

Experiment. The experiments were carried out on samples of 6TG prepared by the Serva Corporation and on samples of 6-thoiguanosine (6TGR) and 10-methyl-6-thioguanosine (10MeTGR) (Scheme 1) synthesized at the Institute of Organic Synthesis, of the Latvian Academy of Sciences. The above derivatives of 6TG were used to simulate 6TG's tautomeric

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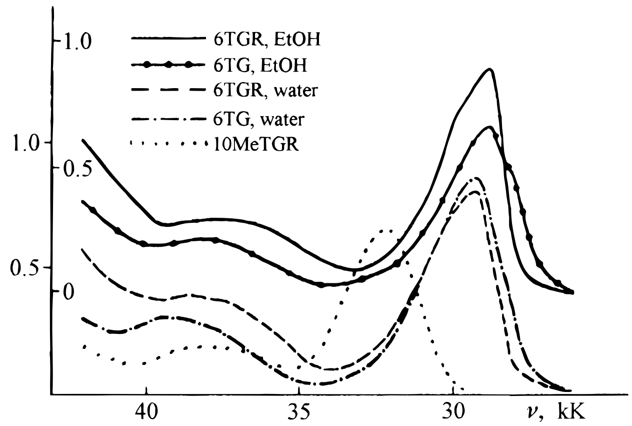

Figure 1. UV absorbance spectra of 6TG and 6TGR in ethanol and water solutions. The absorbance spectrum of 10MeTGR in water solution is also provided for reference. The scale to the right of the *y*-axis refers to the ethanol solutions, and the scale to the left of the axis refers to the water solutions.

forms, with 6TGR and 10MeTGR simulating the thione and thiol forms of the N9(H) tautomer, respectively.

The aqueous solutions were prepared using deionized water. The ethanol used was purified and redistilled but still contained ~5% water. Solutions were acidified to pH = 6.0 by addition of HCl. The concentration of 6TG in solution was in the range 10^{-4}–10^{-5} M.

The luminescence and luminescence excitation spectra were collected with a luminescence apparatus that has been described previously.[9] The preparation of DNA with 6TG has also been described in detail in an earlier work.[19]

Calculations. Geometries, dipole moments, and energetic stabilities of the five most stable tautomers of 6TG were calculated in the gas phase, using the *ab initio* LCAO-MO method.[32] Hartree–Fock (HF) theory was used as the minimum level of theory in this study. Second-order (MP2) and fourth-order (MP4) Møller–Plesset perturbation theory using frozen core (FC) approximation were employed to account for electron correlation. MP4 calculations were carried out using single, double, and quadruple excitations (MP4(SDQ)). A complete listing of the levels of theory and basis sets employed in this study can be found in ref 33. Total energies were corrected for the zero-point energy contributions, scaled by a standard factor of 0.9 (0.9 HF/6-31G(d,p) ZPE). The SCRF model was used (cavity radius 4.6 Å, dielectric constant 40) at the MP2/6-31G(d,p)//HF/6-31G(d,p) level to estimate parameters of the tautomers in polar media. The GAUSSIAN92 set of programs[34] was used to carry out all of these calculations.

The excited-state energies of the 6TG tautomeric forms were calculated by the CNDO/S method, using the programs described in ref 31. As has been done previously,[19,35] the resonance integral of the sulfur atom was set at 14 eV, and the one-center integral of the interelectronic repulsion was set at 7 eV.

Results and Discussion

To gain better insight into the tautomerism of the 6TG neutral molecule, we investigated the UV absorbance and luminescence spectra of aqueous and ethanol solutions of 6TG, 6TGR, and 10MeTGR with pH = 6.0. The 6TG neutral molecule is known to be capable of N7(H)–N9(H) and thione–thiol tautomerism.

The UV absorbance spectra of 6TG (Figure 1), as compared to those of guanine,[9,15] are distinguished by a low-frequency spectral line displacement of 4–8 kK. The UV absorbance spectrum of 10MeTGR is shifted toward higher frequencies, relative to that of 6TG. The absorbance spectra of 6TG and 6TGR in aqueous solution at 293 K are similar, but their spectra in ethanol are somewhat different in shape: in particular, the half-width of the first UV absorbance band is wider for 6TG than for 6TGR.

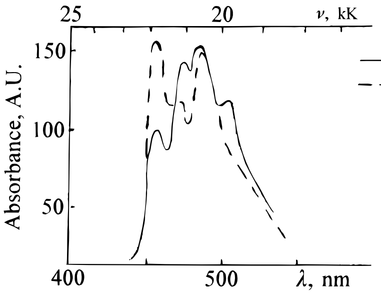

Figure 2. UV emission luminescence spectra of 6TG (solid line) and 6TGR (dashed line).

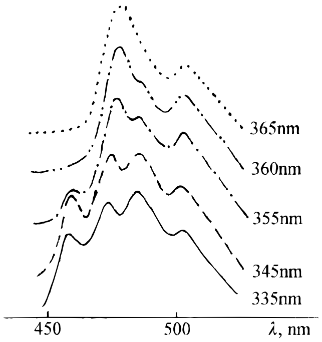

Figure 3. UV emission luminescence spectra of 6TG, collected at the noted excitation wavelengths.

Our study of the UV absorbance spectra of 6TG and 6TGR in ethanol at 77 K reveals the existence of a long-wave absorbance for 6TG within the 370 nm region that is not observed in 6TGR.[19] We have previously shown[19] that the intense UV absorbance band in the 27–32 kK range in the spectra of 6TG and 6TGR is due to absorbance by 6TG thione forms.

The luminescence (phosphorescence) spectra of 6TG and 6TGR, shown in Figure 2, also differ from each other. The luminescence spectrum of 6TGR with λ_{ex} = 350 nm has two intense maxima at 458 and 486 nm, while the 6TG spectrum has both of these and two additional maxima at 477 and 502 nm.

Close examination of the excitation wavelength dependencies of the luminescence spectra of 6TG (Figure 3) reveals that for an excitation wavelength of λ_{ex} = 365 nm the luminescence spectrum of 6TG has a minimum number of peaks (two, at 477 and 502 nm), whereas for λ_{ex} = 355, 345, or 334 nm, new peaks (at 458 and 483 nm) typical of 6-thioguanosine may be observed. It should also be mentioned that the shape and position of the 6TGR spectrum does not vary with λ_{ex} within the first UV absorbance band (27.5–30 kK). Thus, our experiments reveal the existence of at least two types of chromophores in the UV absorbance and luminescence spectra of 6TG.

Finally, from an experimental standpoint, we can consider in what form 6TG exists in native DNA. Figure 4 shows the luminescence spectrum of 6TGR (which models **2**) in ethanol solution and the spectrum of 6TG included in the primary structure of DNA using the biosynthesis method. The high degree of congruence between the spectra confirms that 6TG exists in DNA as **2**.

In order to determine molecular structures of the lowest energy tautomers of 6TG and to characterize their properties, a high-level theoretical study was performed. The *ab initio*-calculated values of the energies and dipole moments of the

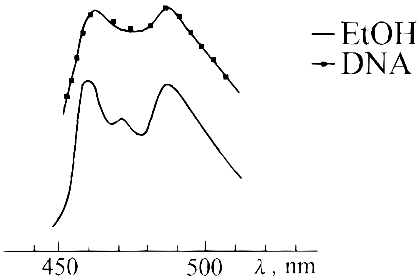

Figure 4. Comparison of UV emission luminescence spectra of 6TGR in ethanol solution and 6TG in native DNA.

TABLE 1: *Ab Initio*-Calculated Energies, Dipole Moments, Relative Energies, and Relative Concentrations of the Five Studied Tautomers of 6TG

tautomer	energy (hartrees/ particle)	dipole moment (D)	relative energy (kJ/mol)	relative concentration
SCRF MP2/6-31G(d,p)//HF/6-31G(d,p) + 0.9(HF/6-31G(d,p) ZPE)				
1	863.514 29	2.25	0.0	0.01
2	863.516 79	9.95	−6.6	0.19
3	863.516 43	4.44	−5.6	0.12
4	863.609 61	4.23	12.3	0.000 04
5	863.518 24	4.89	−10.4	1.0
MP2/6-311+G(d,p)//HF/6-31G(d,p)				
1	863.844 66	1.51	0.0	0.94
2	863.840 97	6.74	9.7	0.01
3	863.844 71	3.30	−0.1	1.0
4	863.836 86	4.03	20.5	0.0001
5	863.839 92	3.80	12.5	0.004
MP4(SDQ)/6-31G(d,p)//HF/6-31G(d,p)				
1	863.633 44	2.04	0.0	1.0
2	863.660 23	8.44	8.4	0.02
3	863.661 08	3.54	6.2	0.06
4	863.652 25	3.67	29.4	0.000 002
5	863.662 24	4.05	3.2	0.25
MP2/6-31G(d,p)//MP2/6-31G(d,p)				
1	863.632 13	1.72	0.0	1.0
2	863.628 06	8.07	10.7	0.009
3	863.628 89	3.50	8.5	0.02
4	863.620 87	4.12	29.6	0.000 002
5	863.630 19	4.24	5.1	0.10
MP4(SDQ)/6-31++G(d,p)//MP2/6-31G(d,p)				
1	863.699 30	1.61	0.0	1.0
2	863.695 98	7.97	8.7	0.02
3	863.697 60	3.44	4.5	0.14
4	863.689 09	4.29	26.8	0.000 007
5	863.698 80	4.22	1.3	0.56
MP4(SDQ)/6-31++G(d,p)//MP2/6-31G(d,p) + 0.9(HF/6-31G(d,p) ZPE)				
1	863.587 31	1.61	0.0	0.03
2	863.584 21	7.97	8.1	0.0008
3	863.589 29	3.44	−5.2	0.28
4	863.581 29	4.29	15.8	0.000 003
5	863.590 39	4.22	−8.1	1.0

five studied tautomers of 6TG at several computational levels are listed in Table 1. Our discussion will center on the MP4(SDQ)/6-31++G(d,p)//MP2/6-31G(d,p) and SCRF MP2/6-31G(d,p)//HF/6-31G(d,p) results. Relative concentrations of the tautomers, calculated at each computational level using the Arrhenius relationship, are also given in Table 1.

As in our previous study of guanine and its thio- and seleno-analogs,[21] the amino groups of all studied 6TG tautomers exhibit noticeable deviation from the plane of the rings. At the MP2/6-31G(d,p) level of theory, the amino nitrogen is from 0.064 Å (tautomer **2**) to 0.087 Å (**4**) below the plane of the rings, and the amino hydrogens are from 0.051 Å (**1**) to 0.230 Å (**3**) (“axial” hydrogen, closer to the sulfur atom) or from 0.241 Å (**5**) to 0.561 Å (**1**) (“equatorial” hydrogen, farther from and pointed away from sulfur) above the plane of the rings.

The change in geometry on going from the HF/6-31G(d,p) to the MP2/6-31G(d,p) level of theory is large, as shown by the bond angle and length measures in Figures 5−9. The largest change in any single bond length is 0.048 Å (N7−C8 in **2** and in **5**; the same bond increases by 0.047 Å in **3**, the other tautomer with an N9 hydrogen), and the largest bond angle change is 4.0° (C2−aminoN−H and H−aminoN−H in **2**). In general, the MP2 structure is more expanded than the HF structure, with most bond lengths increasing by 0.01 Å or more. In particular, the C2−aminoN bond is consistently longer, by 0.022−0.029 Å. The H−aminoN−H bond angle increases in like measure, by 2.8−4.0°.

The relative energies of all tautomers at the examined levels of theory are shown in Table 1. The energy of **1** is used as the baseline value at each level of theory, and the energies of the other tautomers are shown relative to that value. At every tested level of theory, **4** is the least stable, being over 18.1 kJ/mol less stable than the nearest (**2**) and over 26.8 kJ/mol less stable than the lowest energy (**1**) tautomers at the MP4(SDQ)/6-31++G(d,p)//MP2/6-31G(d,p) level of theory. The instability of **4** is possibly due to steric crowding between the thiol and the N7 hydrogen. **3** and **4** have similar changes in relative energy when going from one level of theory to the next, maintaining a stability gap of 19−23 kJ/mol. **3** and **5** have a similar relationship, with **5** being more stable than **3** by approximately 3−5 kJ/mol, except for the MP2/6-311+G(d,p)//HF/6-31G(d,p) level of theory, where **3** is more stable than **5** by 12.6 kJ/mol. **3** and **5** are both stabilized by hydrogen bond-like through-space interaction between the thiol and the adjacent nitrogens. **1** is also more stable than **2** by 8−11 kJ/mol at all levels of gas-phase calculation, but **2** is more stable by 6.6 kJ/mol under SCRF conditions. These groupings of similar trends in energy are to be expected, as **1** and **2** are thione forms and **3**, **4**, and **5** are thiol forms.

We also examined the results of intermediate calculations from our MP4//MP2 series. Figure 10 shows the results of the HF, MP2, and MP4(SDQ) calculations performed during the MP4(SDQ)/6-31++G(d,p)//MP2/6-31G(d,p) calculation, along with the relative total energies (MP4(SDQ) + 0.9(HF/6-31G(d,p) ZPE): the scaling factor of 0.9 is used to account for the lack of electron correlation and the difference between the harmonic vibrational calculation and the anharmonic oscillation of actual bonds). It is readily seen that, in general, the inclusion of higher levels of electron correlation decreases the differences in energy between the tautomers. There is one exception to this rule: the relative energy of **4** *increases* in going from the MP2 to the MP4(SDQ) level. This is the only tautomer with SH and NH groups in close proximity. Inclusion of the ZPE is seen to have a much stronger effect on relative energy than does electron correlation for **3**, **4**, and **5**, drastically decreasing their relative energies (by 9.7, 11.0, and 9.4 kJ/mol, respectively). **2** shows little change in relative energy upon the application of electron correlation or ZPE, with only a 4.0 kJ/mol difference between the HF and total relative energies. On the basis of these results, the thione forms of 6TG appear to be much more destabilized by the ZPE contribution than do the thiol forms.

In addition to the calculations of total energies and geometries of 6TG tautomers, calculations of excited-state energies, oscillator strengths, and dipole moments of these tautomers in the gas phase and polar solvent were carried out using the CNDO/S method. These calculations are useful in particular for identification of 6TG tautomers in polar solvents using their UV absorbance and luminescence spectra. We believe that the CNDO/S method provides a qualitatively correct estimation of the excited-state energies. For a more advanced *ab initio*

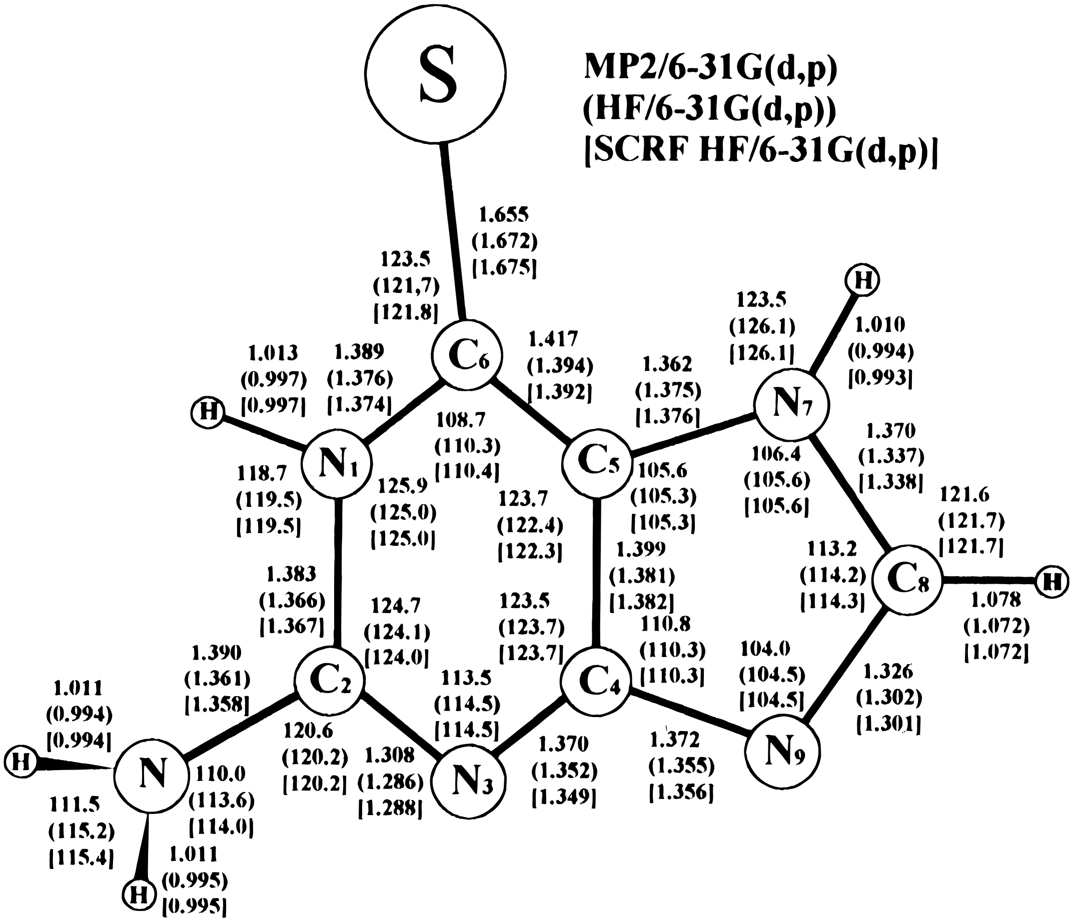

Figure 5. Bond angles and lengths for tautomer **1**, calculated at the MP2/6-31G(d,p), HF/6-31G(d,p), and SCRF HF/6-31G(d,p) levels.

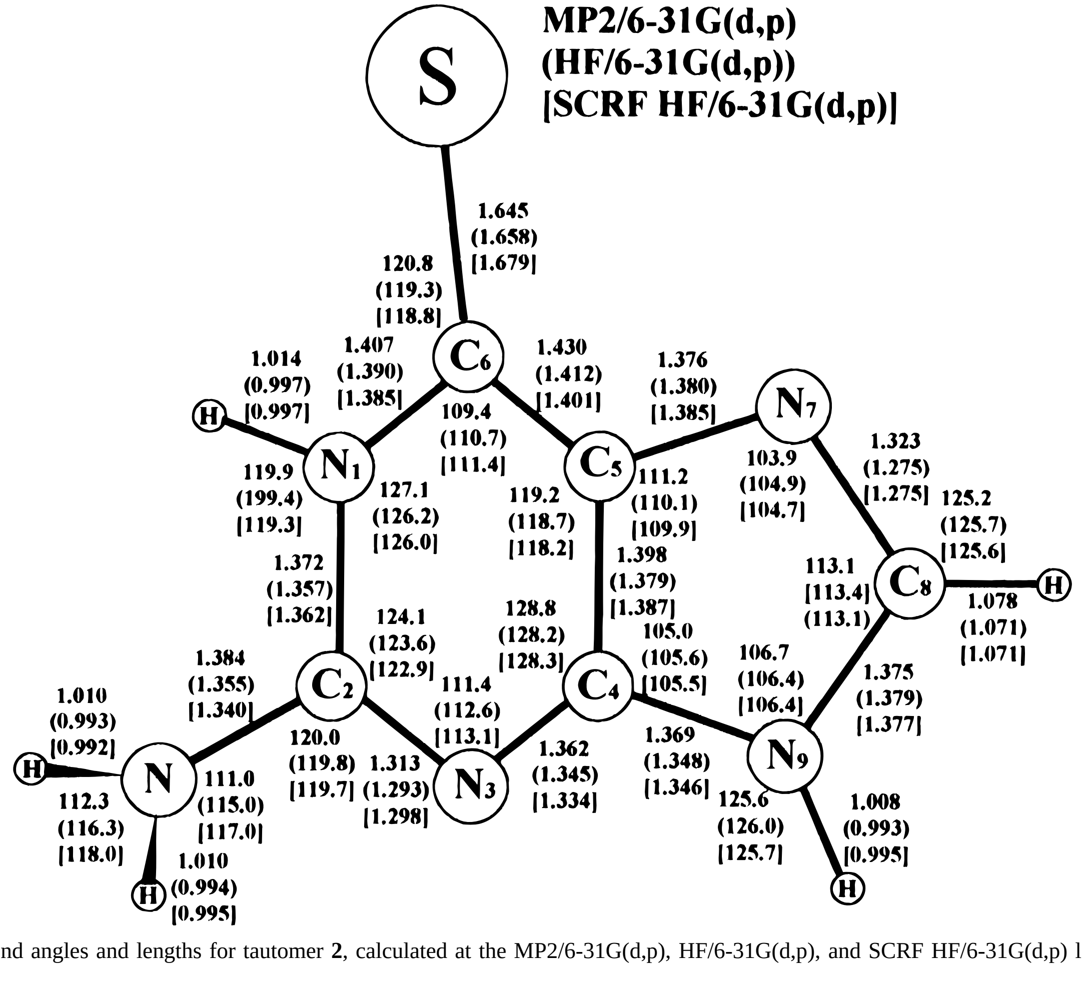

Figure 6. Bond angles and lengths for tautomer **2**, calculated at the MP2/6-31G(d,p), HF/6-31G(d,p), and SCRF HF/6-31G(d,p) levels.

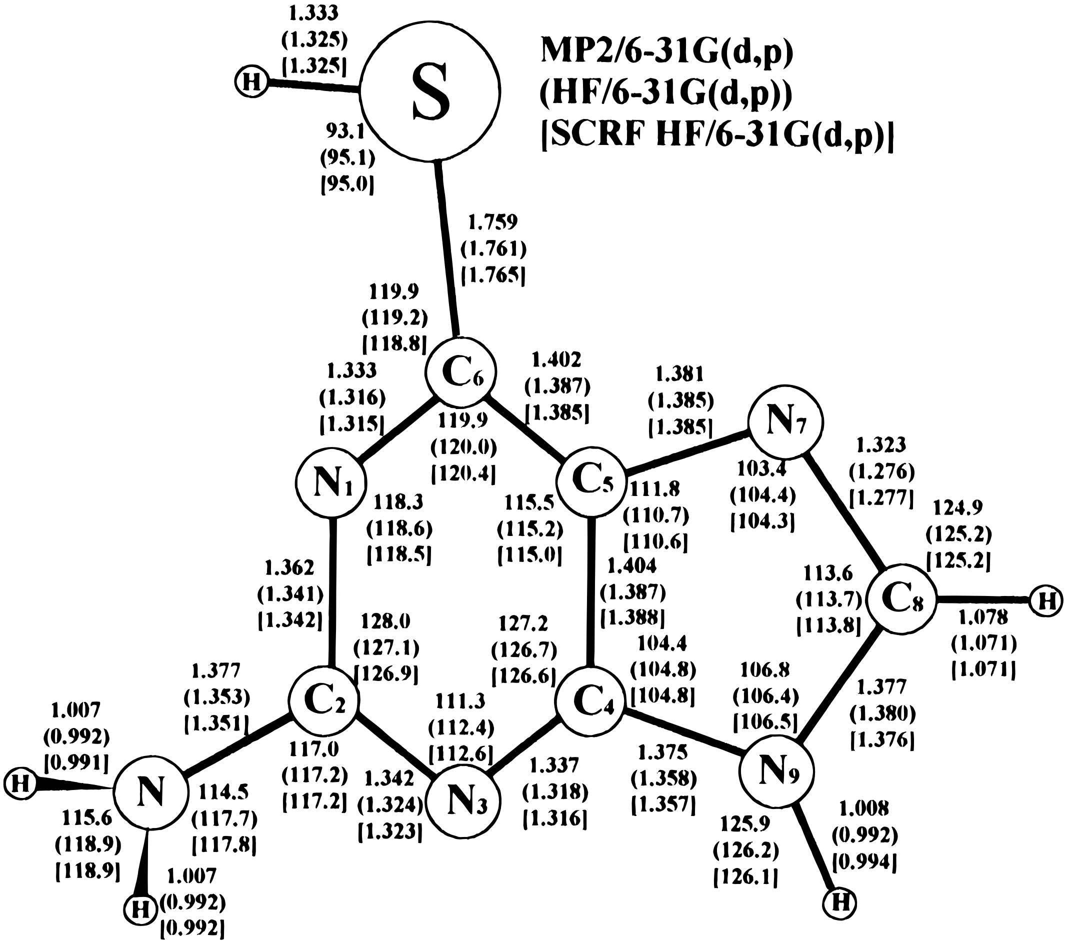

Figure 7. Bond angles and lengths for tautomer **3**, calculated at the MP2/6-31G(d,p), HF/6-31G(d,p), and SCRF HF/6-31G(d,p) levels.

TABLE 2: CNDO/S Calculation Results for the Five Studied Tautomers of 6TG[a]

	tautomer 1	tautomer 2	tautomer 3	tautomer 4	tautomer 5
dipole moment, D	3.36/3.54	9.81/10.33	4.41/4.45	2.69/2.77	4.48/4.57
oscillation strength	0.49	0.49	0.29	0.25	0.30
E(S $\pi \rightarrow \pi^*$)	3.76/3.76	3.87/3.81	4.76/4.77	4.68/4.69	4.77/4.78
E(n$\rightarrow \pi^*$)	3.25/3.25	3.18/3.15	4.82/4.83	4.84/4.83	4.76/4.77
E(T $\pi \rightarrow \pi^*$)	2.80/2.80	2.92/2.87	3.67/3.68	3.65/3.65	3.69/3.69

[a] Values given are for isolated/SCRF-optimized geometries. Energies are given in eV.

CASSCF treatment of the electronic spectra of smaller pyrimidine nucleobases, see refs 36 and 37; such calculations are, however, still far from being available for the purine series modified by heavy elements such as sulfur.

CNDO/S calculation results are presented in Table 2. The analysis of dipole moment values shows that **2** has the largest dipole moment. In polar solvent, all tautomers show an increase in dipole moment values compared to the gas phase. Both of these observations agree with the *ab initio* calculations.

1 and **2**, which are 6TG thione forms, have higher oscillator strengths than the thiol forms (**3**−**5**). The oscillator strengths of **3** and **5**, like their dipole moments, are similar due to their small differences in structure. Likewise, the excited-state energies of these tautomers are similar.

The excited-state energies of **1** and **2** are significantly lower than those of **3**−**5**; this agrees with the experimental data shown above (Figure 1). These calculations show also that the UV absorbance and luminescence spectra of **1** must be red-shifted (bathochromic shift) compared to those of **2**. The largest change in excited-state energy between gas phase and polar solvent is obtained for **2**, which has the largest dipole moment.

Compared to the previous study on 6TG carried out by Alhambra *et al.*,[25] our results show a slight difference in order of stability of the tautomers. At the MP2/6-311++G(d,p)//HF/6-31G(d) + HF/6-31G(d) ZPE level (the highest level reported), Alhambra *et al.* found the order of stability to be **5** > **3** > **1** > **4** > **2** for the five most-stable tautomers in the gas phase. At our highest level of study (MP4(SDQ)/6-31++G(d,p)//MP2/6-31G(d,p) + 0.9(HF/6-31G(d,p) ZPE)), the order of stability is found to be **5** > **3** > **1** > **2** > **4**. The large destabilization of **4** can be attributed to a larger degree of repulsion between the hydrogens from the SH and the NH groups recovered at the correlated level.

At the MP4(SDQ)/6-31G(d,p)//HF/6-31G(d,p) level and all studied levels using MP2-minimized geometries (see Table 1 for a complete listing), the *in vacuo* order of stability of the tautomers (without correction for the ZPE) is found to be **1** > **5** > **3** > **2** ≫ **4**. Inclusion of the ZPE destabilizes thione tautomers **1** and **2** more than thiol tautomers **3**−**5**, decreases the range of energies obtained from 26.8 to 23.9 kJ/mol, and moves **1** below **3** and **5** in stability.

The SCRF model is not sufficient to accurately predict the structures and relative stabilities of molecules in aqueous or other polar solution, but it is a reasonably good and computationally efficient method for the prediction of general structural and stability trends in polar media. Referenced to **1**, **2** becomes

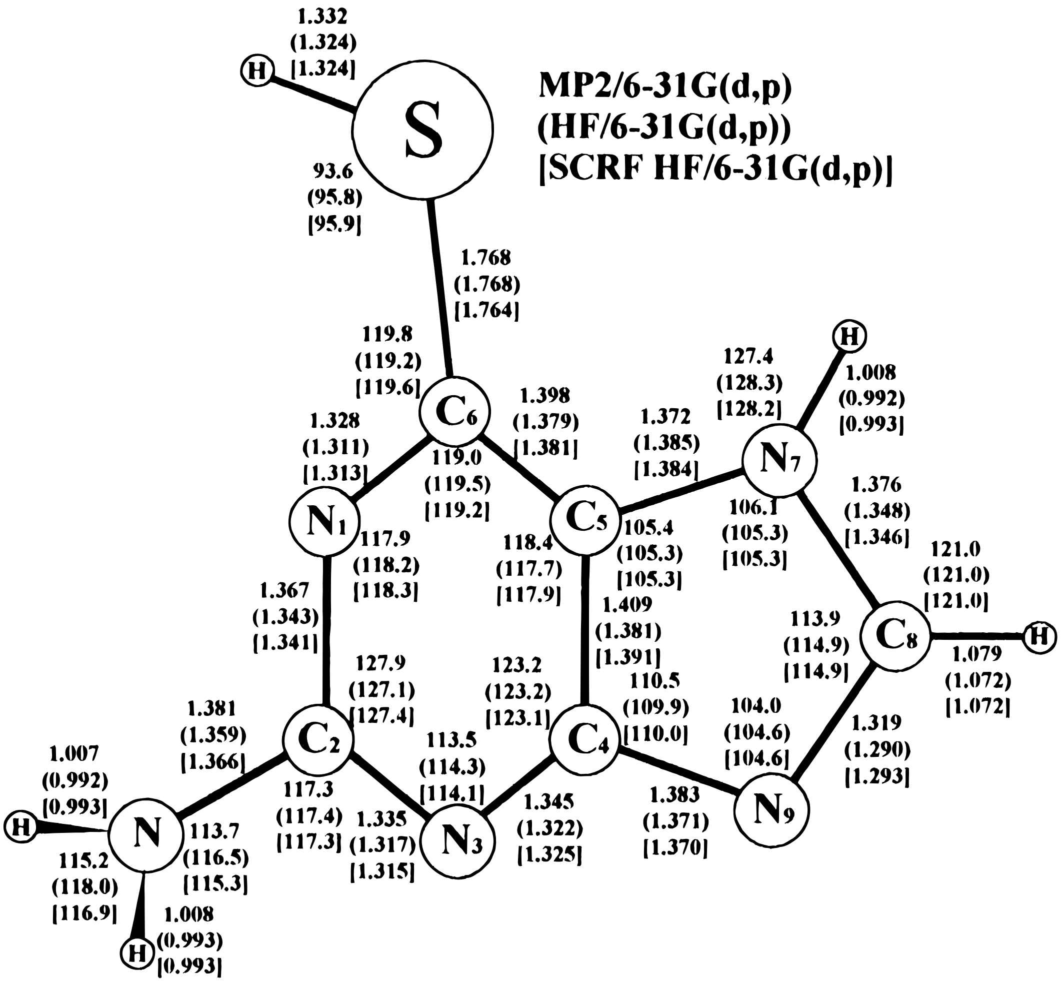

Figure 8. Bond angles and lengths for tautomer **4**, calculated at the MP2/6-31G(d,p), HF/6-31G(d,p), and SCRF HF/6-31G(d,p) levels.

more stable by 17.6 kJ/mol, and **3** and **4** become more stable by 3.9 and 4.7 kJ/mol, respectively, upon application of the SCRF field and minimization at the same level of theory and basis set (MP2/6-31G(d,p)//HF/6-31G(d,p), no ZPE correction). Because these values were taken from previous work,[21] no comparative data were available for **5**. In our SCRF calculations, the order of tautomer stability is found to be **2** > **5** > **1** > **3** ≫ **4** without correction for the ZPE and **5** > **2** > **3** > **1** ≫ **4** with the ZPE correction. Note that this corrected prediction does not exactly correlate with our experimental findings, which indicate that 6TG is present as **2** in DNA and polar solvent. This discrepancy is possibly due to inherent limitations in the accuracy of the SCRF model as discussed above; however, the difference between the stabilities of **5** and **2** in the SCRF prediction is very small (~3.8 kJ/mol), indicating that environmental factors in DNA could easily tip the scales in favor of **2**.

In their previously published study,[25] Alhambra *et al.* calculated a relative concentration of 1.7:1 for **2** and **1**, respectively, in aqueous solution. Their calculations were based on the general cavity SCRF algorithm developed by Miertus, Scrocco, and Tomasi (MST),[38] and free energies of tautomerization were evaluated using a thermodynamic cycle. As shown in Table 1, our SCRF calculations at the MP2/6-31G(d,p)//HF/6-31G(d,p) level predict a relative concentration of 98:18:1 for **5**, **2**, and **1**, respectively, in the aqueous phase; this implies that the inclusion of electron correlation plays a large role in tautomer stability in aqueous media, although the two methods employed may not be strictly comparable due to algorithmic and parametric differences.

At all studied levels of theory, *in vacuo* and SCRF, **2** has the highest dipole moment, and as expected this form is highly stabilized by polar media. There is, however, no direct correlation between dipole moment and relative stability under SCRF conditions: **1**, which has the lowest dipole moment, is the fourth most stable, and **4** has a dipole moment only 0.56 D lower than that of **5** but is nearly 23 kJ/mol less stable.

The dipole moments of all tautomers increase under SCRF conditions, as compared to gas-phase calculations at the same level; except for that of **4**, the dipole moments under SCRF conditions are the highest calculated in this study. The calculated dipole moments at the studied levels of theory are shown in Table 1. It is extremely important to note that only the MP2/6-311+G(d,p)//HF/6-31G(d,p) calculations determine the dipole moment at the correlated MP2 level. All other calculations discussed in this paper involve the calculation of dipole moments at the Hartree−Fock level.

Conclusions

High-level *ab initio* calculations were performed on five stable tautomers of 6-thioguanine at the second- and fourth-order levels of correlated Møller−Plesset theory using large standard basis sets. At the highest studied level of theory (MP4(SDQ)/6-31++G(d,p)//MP2/6-31G(d,p) + 0.9(HF/6-31G(d,p) ZPE), the order of stability is found to be **5** > **3** > **1** > **2** > **4**. Without inclusion of the ZPE, all calculations in which the structure was minimized at the correlated MP2 level yield a stability order of **1** > **5** > **3** > **2** ≫ **4**.

In addition, the self-consistent reaction field (SCRF) model was employed to simulate the behavior of these systems in polar solvent. The order of stability found in this environment (with the inclusion of the ZPE correction) is **5** > **2** > **3** > **1** ≫ **4**.

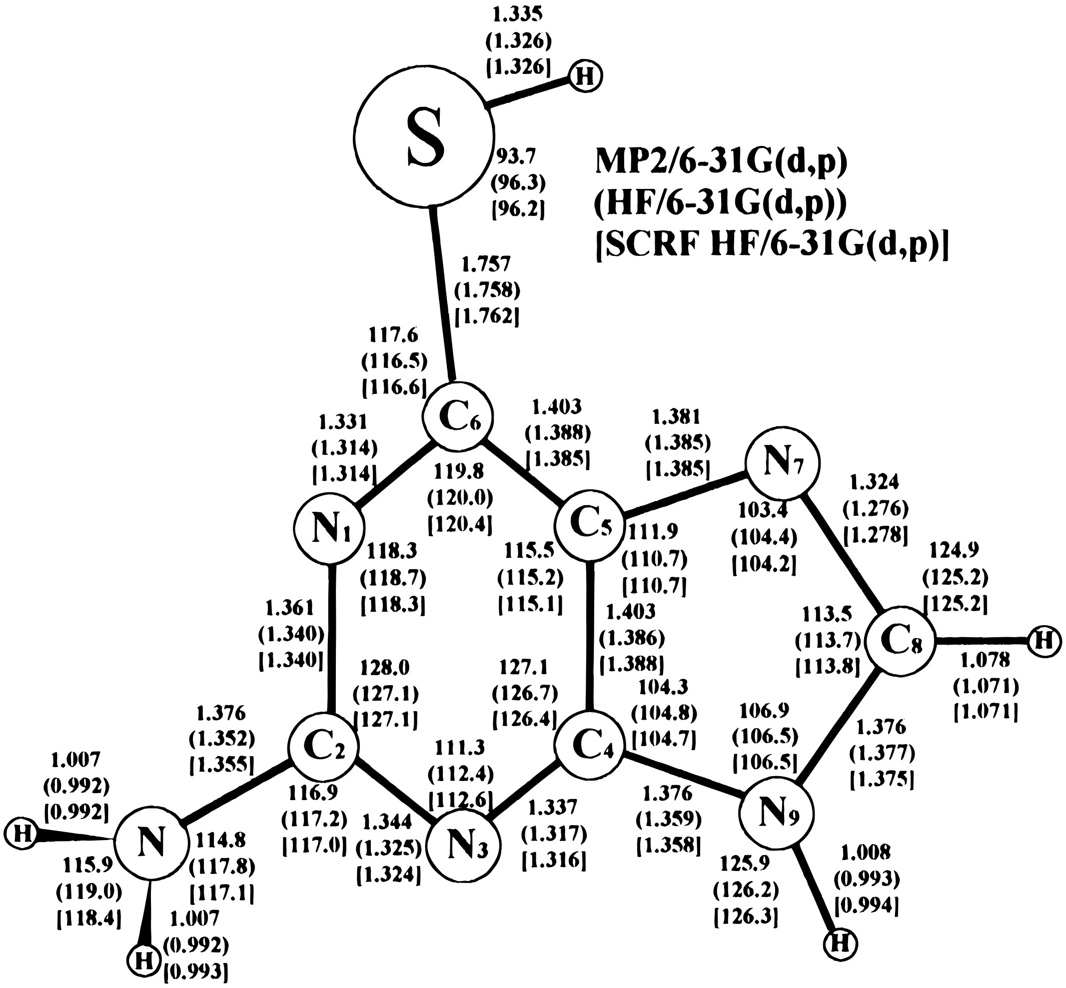

Figure 9. Bond angles and lengths for tautomer **5**, calculated at the MP2/6-31G(d,p), HF/6-31G(d,p), and SCRF HF/6-31G(d,p) levels.

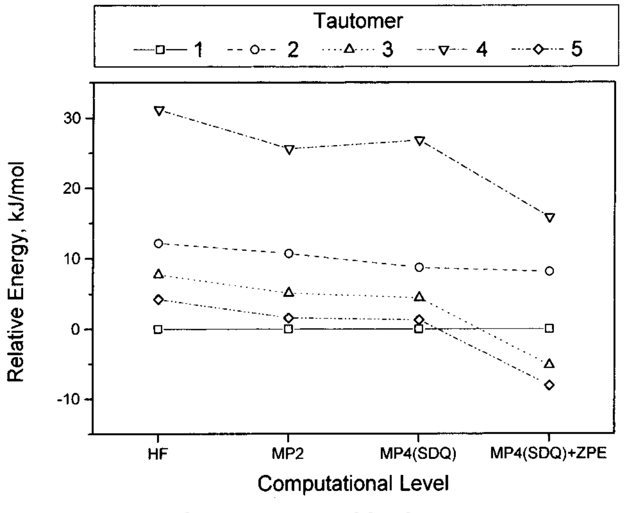

Figure 10. Intermediate computational level comparison (HF, MP2, MP4(SDQ), total) for all studied tautomers, taken from the MP4(SDQ)/6-31G(d,p)//MP2/6-31G(d,p) calculations.

The dipole moment of **2** is roughly twice as large as that of its nearest neighbor (9.95 D, vs 4.89 D for **5**), but no direct correlation is seen between dipole moment and stability under SCRF conditions. The ratio of tautomer concentrations in aqueous solution (98:18:1 for **5**, **2**, and **1**, respectively) is markedly different from that previously predicted by Alhambra *et al.*[25] (1.7:1 for **2** and **1**, respectively).

CNDO/S calculations on the excited states of 6TG tautomers show higher oscillator strengths and lower excited-state energies for thiones than for thiols; the latter was confirmed experimentally via UV absorbance and luminescence spectroscopic studies on 6TG and models for specific tautomers (6TGR and 10Me-TGR). These experimental spectra confirm the existence of at least two types of 6TG thione chromophores and were used to determine that 6TG is present in DNA as **2**.

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