

# Molecular Energetics of Cytosine Revisited: A Joint Computational and Experimental Study

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A static bomb calorimeter has been used to measure the standard molar energy of combustion, in oxygen, at  $T = 298.15$  K, of a commercial sample of cytosine. From this energy, the standard ( $p^\circ = 0.1$  MPa) molar enthalpy of formation in the crystalline state was derived as  $-(221.9 \pm 1.7)$  kJ·mol<sup>-1</sup>. This value confirms one experimental value already published in the literature but differs from another literature value by 13.5 kJ·mol<sup>-1</sup>. Using the present standard molar enthalpy of formation in the condensed phase and the enthalpy of sublimation due to Burkinshaw and Mortimer [*J. Chem. Soc., Dalton Trans.* **1984**, 75], ( $155.0 \pm 3.0$ ) kJ·mol<sup>-1</sup>, results in a value for the gas-phase standard molar enthalpy of formation for cytosine of  $-66.9$  kJ·mol<sup>-1</sup>. A similar value,  $-65.1$  kJ·mol<sup>-1</sup>, has been estimated after G3MP2B3 calculations combined with the reaction of atomization on three different tautomers of cytosine. In agreement with experimental evidence, the hydroxy-amino tautomer is the most stable form of cytosine in the gas phase. The enthalpies of formation of the other two tautomers were also estimated as  $-60.7$  kJ·mol<sup>-1</sup> and  $-57.2$  kJ·mol<sup>-1</sup> for the oxo-amino and oxo-imino tautomers, respectively. The same composite approach was also used to compute other thermochemical data, which is difficult to be measured experimentally, such as C–H, N–H, and O–H bond dissociation enthalpies, gas-phase acidities, and ionization enthalpies.

## Introduction

The cytosine species (Figure 1) is a nucleobase, and, therefore, it can be found as part of DNA, RNA, or of a nucleotide. Due to its biological significance, thermochemical data for cytosine are important for the understanding of its function on the stability and reactivity of the compounds where this molecular scaffold is present. Therefore, it is not surprising that several very recent works are found in the literature devoted to the gas- or condensed-phase thermochemistry of cytosine and derivatives.<sup>1–9</sup>

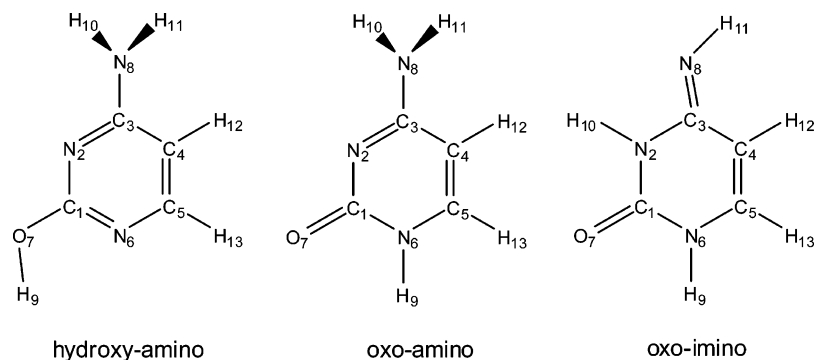
Despite the strong efforts done in the latest years for the fulfillment of gaps on the thermodynamic properties of cytosine, controversy still exists about the gas-phase enthalpy of formation of cytosine. In fact, the uncertainty about its value is rapidly understood after a visit to the NIST's WebBook<sup>10</sup> where the standard molar enthalpy of formation for cytosine appears as  $(59 \pm 10)$  kJ·mol<sup>-1</sup>. This value is a result of an analysis of the experimental data determined by several authors both for the combustion and sublimation of cytosine. In the case of the combustion experiments, static and rotating bomb calorimetric techniques due to Wilson et al.<sup>11</sup> and Sabbah and co-workers,<sup>12</sup> respectively, provided enthalpies of combustion that differ by  $\sim 14$  kJ·mol<sup>-1</sup>, which resulted in a similar difference in the derived standard molar enthalpies of formation in the crystalline state; the result coming from the former apparatus is  $\Delta_f H_m^0(\text{cr}) = -(221.3 \pm 2.3)$  kJ·mol<sup>-1</sup>,<sup>11</sup> whereas that from the latter technique is  $\Delta_f H_m^0(\text{cr}) = -(235.4 \pm 0.5)$  kJ·mol<sup>-1</sup>.<sup>12</sup> The picture is even worse in the case of the enthalpies of phase transition for which the experimental values vary in an interval of more than 25 kJ·mol<sup>-1</sup>.<sup>10,13,14</sup> For the enthalpy of sublimation,

there are several values measured by different techniques, at different temperatures, such as the ones by Yanson et al.,  $\Delta_{\text{cr}}^g H_m^0(T = 458 \text{ K}) = 150.6$  kJ·mol<sup>-1</sup>,<sup>15,16</sup> by Zielenkiewicz et al.,  $\Delta_{\text{cr}}^g H_m^0(T = 515 \text{ K}) = (151.7 \pm 0.7)$  kJ·mol<sup>-1</sup>,<sup>17</sup> and by Burkinshaw and Mortimer who used an apparatus based on mass Knudsen effusion technique and have obtained  $\Delta_{\text{cr}}^g H_m^0(T = 298.15 \text{ K}) = (155.0 \pm 3.0)$  kJ·mol<sup>-1</sup>,<sup>18</sup> as well as much larger values that have been determined by Ferro et al.,  $\Delta_{\text{cr}}^g H_m^0(T = 298.15 \text{ K}) = (167 \pm 10)$  kJ·mol<sup>-1</sup>,<sup>19</sup> and by Sabbah,  $\Delta_{\text{cr}}^g H_m^0(T = 298.15 \text{ K}) = (176 \pm 10)$  kJ·mol<sup>-1</sup>,<sup>20</sup> after torsion effusion and calorimetric experiments, respectively.

From what is exposed above, the cytosine compound seems a good target for the application of modern computational approaches regarding the estimation of its enthalpy of formation in the gas phase. In truth, it is known that state-of-the-art methods provide rather good estimates for molecules with a number of atoms similar to that present in cytosine and that these approaches can be used as tools for the selection of what may be the best experimental values. Herewith, the composite approaches, which use cheap theoretical methods for the determination of both geometries and thermal corrections and that use single-point calculations performed with much more accurate methods, aiming the correction of the energy computed at the lowest level, will be used to estimate the gas-phase standard molar enthalpy of formation for cytosine from the reaction of atomization and corresponding experimental enthalpies of formation for the atoms.

Furthermore, the computational methods will be employed also in the determination of the enthalpies of tautomerization of the hydroxy-amino, oxo-amino, and oxo-imino forms of cytosine, Figure 1. For these low-energy tautomers, the ho-

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**Figure 1.** Atom numbering for the tautomers of cytosine.

molytic C–H, O–H, and N–H bond dissociation enthalpies associated with the formation of radicals and the gas-phase acidities for each of the protons in those compounds are also computed.

### Materials

Cytosine (4-amino-2-hydroxypyrimidine) [CAS 71-30-7] was commercially obtained from Fluka, grade puriss, with purity (HPLC) with mass fraction  $\geq 0.99$ . The analysis of the combustion products showed that the compound was pure enough to be used without any further purification. The average ratio of the mass of carbon dioxide recovered after combustion, to that calculated from the mass of sample in all the six combustion experiments ( $99.999 \pm 0.002\%$ ), with the uncertainty being the overall standard deviation of the mean, confirmed the purity of the sample which was used for the experimental work. The specific density of the sample was estimated to be  $\rho = 1.2 \text{ g cm}^{-3}$ , from measurements of the masses and of the volumes of pellets of this compound.

### Static Bomb Calorimetry

The combustion experiments were performed with a static-bomb calorimeter originally assembled at the National Physical Laboratory, Teddington, U.K.<sup>21</sup> and at the University of Manchester<sup>22</sup> and now installed in the Thermochemistry Laboratory in the University of Porto, Portugal.<sup>23</sup> Both the apparatus and the operating technique have been described already in the literature,<sup>21–23</sup> so only a brief description will be given here. The twin valve bomb, with an internal volume of  $0.290 \text{ dm}^3$  and a wall thickness of 1 cm, is made of stainless steel.

Combustion of certificated benzoic acid NBS Standard Reference Material, Sample 39j was used for calibration of the bomb; its massic energy of combustion is  $-(26434 \pm 3) \text{ J}\cdot\text{g}^{-1}$ , under certificate conditions. The calibration results were corrected to give the energy equivalent  $\epsilon(\text{calor})$  corresponding to the average mass of water added to the calorimeter:  $2900.0 \text{ g}$ . From 11 calibration experiments,  $\epsilon(\text{calor}) = (15553.27 \pm 0.91) \text{ J}\cdot\text{K}^{-1}$ , where the uncertainty quoted (0.0059%) is the standard deviation of the mean.

In all combustion experiments, made under oxygen at  $p = 3.04 \text{ MPa}$ ,  $1.00 \text{ cm}^3$  of water was introduced into the bomb. For all experiments, the calorimeter temperatures were measured to  $\pm(1\cdot 10^{-4}) \text{ K}$ , with an S10 four wire calibrated ultrastable thermistor probe (Thermometrics, standard serial No. 1030) and recorded by a high sensitivity nanovolt/microohm meter (Agilent 34420A) interfaced to a microcomputer programmed to compute the adiabatic temperature change. The ignition of the samples was made at  $T = (298.150 \pm 0.001) \text{ K}$ , at least 100 readings at time intervals of 10 s after the start of the experiment, by the discharge of a  $1400 \mu\text{F}$  capacitor through the platinum ignition

wire. After ignition, 100 readings were taken for the main and after periods.

The crystalline samples were ignited in the pellet form, contained in sealed polyester bags made of Melinex ( $0.025 \text{ mm}$  of thickness) with massic energy of combustion  $\Delta_c u^0 = -(22\,902 \pm 5) \text{ J}\cdot\text{g}^{-1}$ , measured by Skinner and Snelson.<sup>24</sup> The mass of Melinex used in each experiment was corrected for the mass fraction of water ( $w = 0.0032$ ), and the mass of carbon dioxide produced from its combustion was calculated using the factor previously reported.<sup>24</sup> For the cotton thread fuse of empirical formula  $\text{CH}_{1.686}\text{O}_{0.843}$ , the massic energy of combustion was assigned to  $-\Delta_c u^0 = 16\,250 \text{ J}\cdot\text{g}^{-1}$ .<sup>25</sup> The massic energies of combustion of Melinex and of the cotton thread fuse have been confirmed in our laboratory.

The electrical energy for ignition was determined from the change in potential difference across a capacitor when discharged through the platinum ignition wire. The corrections for nitric acid formation were based on  $-59.7 \text{ kJ}\cdot\text{mol}^{-1}$ ,<sup>26</sup> for the molar energy of formation of  $0.1 \text{ mol}\cdot\text{dm}^{-3} \text{ HNO}_3(\text{aq})$  from  $\text{N}_2(\text{g})$ ,  $\text{O}_2(\text{g})$ , and  $\text{H}_2\text{O}(\text{l})$ . No carbon residue soot formation was observed in any combustion. All the necessary weighing was made in a Mettler Toledo AT 201 balance, sensitivity  $\pm(1\cdot 10^{-5}) \text{ g}$ , and corrections from apparent mass to true mass were made. An estimated pressure coefficient of specific energy,  $(\partial u/\partial p)_T = -0.2 \text{ J}\cdot\text{g}^{-1}\cdot\text{MPa}^{-1}$ ,<sup>27</sup> at  $T = 298.15 \text{ K}$ , a typical value for most organic compounds, was assumed. For each compound, the massic energy of combustion,  $\Delta_c u^0$ , was calculated by the procedure given by Hubbard et al.<sup>28</sup> The amounts of cytosine used in each experiment were determined from the total mass of carbon dioxide produced (Mettler Toledo AT 201 balance, sensitivity  $\pm(1\cdot 10^{-4}) \text{ g}$ ) during the experiments taking into account that formed from the combustion of the cotton-thread fuse and of the Melinex and that lost due to carbon formation.

The relative atomic masses used were those recommended by the IUPAC Commission in 2005,<sup>29</sup> yielding  $111.103 \text{ g}\cdot\text{mol}^{-1}$  as the molar mass of cytosine.

### Computational Details

The G3MP2 composite approach based on B3LYP geometries also known as the G3MP2B3 method<sup>30</sup> has been used to compute the enthalpies of all compounds considered. This composite approach combines a series of separately performed standard ab initio calculations that are aimed to successively introduce corrections to the enthalpy initially calculated at a less expensive computational approach. This is possible since HF, MP2, and DFT methods combined with a reasonable basis set are known to be capable to predict molecular geometries, which often are in good agreement with experiment. In fact, it was recently reported that the DFT calculations (BLYP, B3LYP, and B3PW91) performed very well on the optimization of the

TABLE 1: Standard ( $p^\circ = 0.1$  MPa) Massic Energies of Combustion at  $T = 298.15$  K for Cytosine<sup>a</sup>

	1	2	3	4	5	6
$m(\text{CO}_2, \text{total})/\text{g}$	1.46154	0.95693	0.92761	1.03276	1.16557	1.09970
$m'(\text{cpd})/\text{g}$	0.81957	0.50836	0.50173	0.57607	0.66423	0.61267
$m''(\text{Melinex})/\text{g}$	0.06976	0.06469	0.05637	0.05097	0.04794	0.05493
$m'''(\text{fuse})/\text{g}$	0.00198	0.00204	0.00219	0.00202	0.00206	0.00194
$\Delta T_{\text{ad}}/\text{K}$	1.09120	0.70857	0.68878	0.77108	0.87232	0.82142
$\epsilon_f/\text{J K}^{-1}$	14.42	13.87	13.86	13.99	14.07	14.00
$\Delta m(\text{H}_2\text{O})/\text{g}$	-0.2	0	+1.7	-1.2	-2.2	-0.4
$-\Delta U(\text{IBP})^b/\text{J}$	16985.57	11029.57	10726.36	11999.13	13570.75	12784.84
$\Delta U(\text{HNO}_3)/\text{J}$	72.97	50.64	49.10	53.07	66.39	62.09
$\Delta U(\text{ign})/\text{J}$	0.98	0.84	0.87	0.60	0.92	1.05
$\Delta U_\Sigma/\text{J}$	18.13	11.37	11.08	12.54	14.23	13.29
$-m''\Delta_c u^\circ(\text{Melinex})/\text{J}$	1597.53	1481.48	1290.96	1167.37	1097.92	1258.06
$-m''' \Delta_c u^\circ(\text{fuse})/\text{J}$	32.16	33.13	35.57	32.80	33.45	31.51
$-\Delta_c u^\circ(\text{cpd})/\text{J}\cdot\text{g}^{-1}$	18625.35	18594.99	18614.89	18632.02	18606.15	18639.54
			$\langle \Delta_c u^\circ(\text{cpd}) \rangle = (-18\ 618.8 \pm 6.8) \text{ J}\cdot\text{g}^{-1}$			

<sup>a</sup>  $m(\text{CO}_2, \text{total})$  is the total mass of carbon dioxide recovered in each experiment;  $m'(\text{cpd})$  is the mass of compound burnt in each experiment;  $m''(\text{Melinex})$  is the mass of Melinex used in each experiment;  $m'''(\text{fuse})$  is the mass of the fuse (cotton) used in each experiment;  $\Delta T_{\text{ad}}$  is the corrected temperature rise;  $\epsilon_f$  is the energy equivalent of the contents in the final state;  $\epsilon(\text{calor})$  is the energy equivalent of the calorimeter corrected for the deviation of mass of water added to the calorimeter;  $\Delta m(\text{H}_2\text{O})$  is the deviation of mass of water added to the calorimeter from 2900.0 g;  $\Delta U(\text{IBP})$  is the energy change for the isothermal combustion reaction under actual bomb conditions;  $\Delta U(\text{HNO}_3)$  is the energy correction for the nitric acid formation;  $\Delta U(\text{ign})$  is the electric energy for the ignition;  $\Delta U_\Sigma$  is the standard state correction;  $\Delta_c u^\circ(\text{fuse})$  is the massic energy of combustion of the fuse (cotton);  $\Delta U(\text{Melinex})$  is the energy of combustion of the Melinex;  $\Delta_c u^\circ$  is the standard massic energy of combustion. <sup>b</sup>  $\Delta U(\text{IBP})$  includes  $\Delta U(\text{ignition})$ .

geometry of cytosine.<sup>31</sup> Then, for these frozen, but previously optimized geometries, several calculations are performed at higher levels of theory (QCISD(T), CCSD(T), second- and fourth-order Moller–Plesset calculations, etc.) or by using more complete basis sets. In the case of the G3MP2B3 method, the energy computed at  $T = 0$  K is corrected for  $T = 298.15$  K by introducing the vibrational, translational, rotational, and the  $pV$  terms computed at the B3LYP/6-31G(d) level of theory. All calculations were performed by means of the GAUSSIAN 03 suite of programs.<sup>32</sup>

## Results

**Calorimetry.** Detailed results for the combustion experiments are given in Table 1, where  $\Delta m(\text{H}_2\text{O})$  is the deviation of the mass of water added to the calorimeter from 2900.0 g, the mass assigned to  $\epsilon(\text{calor})$ , and  $\Delta U_\Sigma$  is the energy correction to the standard state. The remaining quantities are as previously defined.<sup>28</sup> The samples were ignited at  $T = (298.150 \pm 0.001)$  K, with

$$\Delta U(\text{IPB}) = -\{\epsilon_{\text{cal}} + \Delta m(\text{H}_2\text{O})c_p(\text{H}_2\text{O}, l) + \epsilon_f\} \Delta T_{\text{ad}} + \Delta U(\text{ign}) \quad (1)$$

where  $\Delta T_{\text{ad}}$  is the adiabatic temperature raise.

The individual values of the massic energies of combustion,  $-\Delta_c u^\circ$ , yield the mean value,  $\langle \Delta_c u^\circ \rangle = -(18618.8 \pm 6.8) \text{ J}\cdot\text{g}^{-1}$ , with the associated uncertainty being the standard deviation, and refer to the idealized combustion reaction yielding  $\text{CO}_2(\text{g})$  and  $\text{H}_2\text{O}(l)$ . From this value, the standard molar energy of combustion of cytosine, in the crystalline phase,  $(\text{cr}) = -(2068.6 \pm 1.6) \text{ kJ}\cdot\text{mol}^{-1}$  and its standard molar enthalpy of combustion,  $(\text{cr}) = -(2066.7 \pm 1.6) \text{ kJ}\cdot\text{mol}^{-1}$ , at  $T = 298.15$  K, were derived. In accordance with normal thermochemical practice,<sup>33,34</sup> the uncertainties assigned to the standard molar enthalpies of combustion are, in each case, twice the overall standard deviation of the mean and include the uncertainties in calibration and in the values of auxiliary quantities used. To derive, at  $T = 298.15$  K,  $\Delta_f H_m^\circ(\text{cr})$  from  $\Delta_c H_m^\circ(\text{cr})$ , the standard molar enthalpies of formation of  $\text{CO}_2(\text{g})$  and  $\text{H}_2\text{O}(l)$ , at  $T = 298.15$  K,  $-(393.51 \pm 0.13) \text{ kJ}\cdot\text{mol}^{-1}$ <sup>35</sup> and  $-(285.830 \pm 0.004)$

$\text{kJ}\cdot\text{mol}^{-1}$ ,<sup>35</sup> respectively, were used, yielding  $\Delta_f H_m^\circ(\text{cytosine, cr}) = -(221.9 \pm 1.7) \text{ kJ}\cdot\text{mol}^{-1}$ .

Using the value of the standard molar enthalpy of sublimation of cytosine obtained by Burkinshaw and Mortimer,  $\Delta_{\text{cr}}^{\text{g}} H_m^\circ(T = 298.15 \text{ K}) = (155.0 \pm 3.0) \text{ kJ}\cdot\text{mol}^{-1}$ ,<sup>18</sup> since this value was obtained for the reference temperature of  $T = 298.15$  K from Knudsen effusion measurements, a method that is known to be accurate and reliable,<sup>36</sup> the standard molar enthalpy of formation of cytosine in the gaseous state, at the reference temperature, was calculated as  $\Delta_f H_m^\circ(\text{g}) = -(66.9 \pm 3.0) \text{ kJ}\cdot\text{mol}^{-1}$ .

**Molecular Structures.** The optimized geometries of the different tautomers of cytosine depicted in Figure 1 are reported in Table 2. The values included there almost match those reported recently by Chandra et al.<sup>1</sup> and Fogarasi,<sup>31</sup> which is not a surprise since the computational approach used for structural optimization within the G3MP2B3 composite method is the same used by those authors, i.e., the three-parameter hybrid method due to Becke,<sup>37</sup> even despite a slightly smaller basis set is used here. The composite method uses the 6-31G(d) basis set for geometry optimization, while Chandra et al.<sup>1</sup> used the 6-311++G(d,p) basis set, and Fogarasi<sup>31</sup> used the same basis set used by the former authors but with 2d and 2p polarization functions, cf. 6-311++G(2d,2p) basis set. The comparison of the geometric values listed in Table 2 with those computed using larger basis sets resulted in differences on bond lengths smaller than 0.007 Å and on bond angles smaller than 0.5° except in one single case, this is the  $\text{H}_{10}\text{--N}_8\text{--C}_3$  bond angle in the oxo-amino species. The B3LYP/6-31G(d) angle is 116.0, while the B3LYP/6-311++G(d,p) value<sup>1</sup> is 117.4°. Since in the work due to Fogarasi,<sup>31</sup> the comparison between CCSD/TZP and B3LYP/6-311++G(2d,2p) approaches resulted in small differences, the less-expensive B3LYP/6-31G(d) seems adequate for our purposes; this is the estimation of accurate thermodynamic data concerning the three different tautomers of cytosine.

**Gas-Phase Energetics of Tautomerization.** Two other rotamers, which are not displayed in Figure 1, are possible for the hydroxy-amino and oxo-imino tautomers since they are less stable. In the former tautomer of cytosine, the other possible conformation has the hydrogen atom of the hydroxyl group ( $\text{H}_9$ )

**TABLE 2: G3MP2B3 (B3LYP/6-31G(d)) Bonds in Å and Angles in deg for the Tautomers of Cytosine Shown in Figure 1**

geometrical parameter	hydroxy-amino	oxo-amino	oxo-imino	geometrical parameter	hydroxy-amino	oxo-amino	oxo-imino
C <sub>1</sub> –N <sub>2</sub>	1.330	1.373	1.378	C <sub>1</sub> –N <sub>2</sub> –C <sub>3</sub>	115.8	120.3	128.2
N <sub>2</sub> –C <sub>3</sub>	1.342	1.319	1.409	N <sub>2</sub> –C <sub>3</sub> –C <sub>4</sub>	121.7	124.2	113.5
C <sub>3</sub> –C <sub>4</sub>	1.413	1.441	1.460	C <sub>3</sub> –C <sub>4</sub> –C <sub>5</sub>	116.0	116.0	120.1
C <sub>4</sub> –C <sub>5</sub>	1.382	1.359	1.349	C <sub>4</sub> –C <sub>5</sub> –N <sub>6</sub>	123.5	119.9	121.7
C <sub>5</sub> –N <sub>6</sub>	1.343	1.355	1.378	C <sub>5</sub> –N <sub>6</sub> –C <sub>1</sub>	114.6	123.5	123.3
N <sub>6</sub> –C <sub>1</sub>	1.338	1.430	1.397	N <sub>6</sub> –C <sub>1</sub> –N <sub>2</sub>	128.3	116.0	113.2
C <sub>1</sub> –O <sub>7</sub>	1.347	1.220	1.218	C <sub>1</sub> –O <sub>7</sub> –H <sub>9</sub>	116.4		
C <sub>3</sub> –N <sub>8</sub>	1.367	1.366	1.283	N <sub>8</sub> –C <sub>3</sub> –C <sub>4</sub>	122.0	118.2	129.4
N <sub>2</sub> –H <sub>10</sub>			1.013	O <sub>7</sub> –C <sub>1</sub> –N <sub>6</sub>	116.4	118.2	122.4
C <sub>4</sub> –H <sub>12</sub>	1.085	1.083	1.083	H <sub>9</sub> –O <sub>7</sub> –C <sub>1</sub>	105.4		
C <sub>5</sub> –H <sub>13</sub>	1.089	1.086	1.085	H <sub>9</sub> –N <sub>6</sub> –C <sub>5</sub>		121.6	121.6
N <sub>6</sub> –H <sub>9</sub>		1.012	1.010	H <sub>10</sub> –N <sub>2</sub> –C <sub>1</sub>			115.7
O <sub>7</sub> –H <sub>9</sub>	0.974			H <sub>10</sub> –N <sub>8</sub> –C <sub>3</sub>	115.9	116.0	
N <sub>8</sub> –H <sub>10</sub>	1.011	1.011		H <sub>11</sub> –N <sub>8</sub> –C <sub>3</sub>	119.1	120.0	110.1
N <sub>8</sub> –H <sub>11</sub>	1.009	1.009	1.021	H <sub>10</sub> –N <sub>8</sub> –H <sub>11</sub>	116.7	117.3	
				H <sub>12</sub> –C <sub>4</sub> –C <sub>3</sub>	122.0	122.3	119.1
				H <sub>13</sub> –C <sub>5</sub> –C <sub>4</sub>	120.6	123.2	122.8

pointing to the opposite direction of the rotamer displayed in Figure 1. Similarly, the other rotamer of the oxo-imino tautomer of cytosine has the imino's H<sub>11</sub> atom in position cis relative to H<sub>10</sub>. At the G3MP2B3 level, the differences between the most and the least stable rotamers are  $-2.9 \text{ kJ}\cdot\text{mol}^{-1}$  and  $-6.4 \text{ kJ}\cdot\text{mol}^{-1}$ , for the hydroxy-amino and oxo-imino tautomers, respectively.

The most stable tautomer of cytosine in the gas phase is the hydroxy-amino species. This tautomer is  $4.4 \text{ kJ}\cdot\text{mol}^{-1}$  more stable than the oxo-amino tautomer and  $7.9 \text{ kJ}\cdot\text{mol}^{-1}$  more stable than the oxo-imino. The small enthalpic differences are in agreement with the fact that the oxo-amino is the molecular scaffold present in DNA. The present G3MP2B3 ordering of the enthalpies for the three tautomers of cytosine is in agreement with the experimental (matrix isolation infrared and molecular beam microwave spectroscopy) findings.<sup>38–40</sup> We have also used another composite approach, namely the CBS-4M method, for the computation of the relative stability of the tautomers of cytosine and found that the hydroxy-amino is the most stable of the three species, but the relative enthalpic differences between the three tautomers are smaller than those computed with the G3MP2B3 method. The oxo-amino and the oxo-imino species are less stable than the hydroxy-amino tautomer by  $2.2 \text{ kJ}\cdot\text{mol}^{-1}$  and  $5.6 \text{ kJ}\cdot\text{mol}^{-1}$ , respectively.

Previous coupled cluster electron correlation calculations predicted that the hydroxy-amino form is clearly the most stable tautomer of cytosine (enthalpic differences with respect to the other two tautomers larger than  $6 \text{ kJ}\cdot\text{mol}^{-1}$ ) and that the oxo-tautomers are almost equally stable. Three different DFT exchange-correlation functionals—BLYP, B3LYP, or B3PW91—combined with two different basis sets—6-31G(d,p) or 6-311++G-(2d,2p)—were used by Fogarasi in the study of the relative stabilities of the three low-energy tautomers of cytosine yielding a different picture when compared with the results discussed above. The B3LYP functional yields the oxo-amino form as the most stable tautomer, while the oxo-imino is the least stable one; the enthalpic difference between the oxo-amino and the hydroxy-amino is of  $2.3 \text{ kJ}\cdot\text{mol}^{-1}$ . The same ordering is obtained when the B3PW91 functional is used, while the BLYP functional predicts the hydroxy-amino tautomer as the least stable and overestimates even more the stability of the oxo-amino form.

**Gas-Phase Enthalpies of Formation.** The gas-phase standard molar enthalpies of formation of the three tautomers of cytosine have been estimated by considering their G3MP2B3 computed enthalpies for the reaction of atomization at  $T = 298.15 \text{ K}$ . The

**TABLE 3: N–H, C–H, and O–H Homolytic Bond Dissociation Enthalpies in  $\text{kJ}\cdot\text{mol}^{-1}$  for the Tautomers of Cytosine Shown in Figure 1**

bond label	hydroxy-amino	oxo-amino	oxo-imino
N <sub>2</sub> –H <sub>10</sub>			439.0
C <sub>4</sub> –H <sub>12</sub>	482.9	488.6	494.8
C <sub>5</sub> –H <sub>13</sub>	453.1	469.4	473.0
N <sub>6</sub> –H <sub>9</sub>		435.5	406.8
O <sub>7</sub> –H <sub>9</sub>	440.1		
N <sub>8</sub> –H <sub>10</sub>	447.9	463.8	
N <sub>8</sub> –H <sub>11</sub>	430.3	442.2	413.1

following atomic experimental gas-phase enthalpies of formation were used: hydrogen,  $218.00 \text{ kJ}\cdot\text{mol}^{-1}$ ; carbon,  $716.67 \text{ kJ}\cdot\text{mol}^{-1}$ ; nitrogen,  $472.68 \text{ kJ}\cdot\text{mol}^{-1}$ ; and oxygen,  $249.17 \text{ kJ}\cdot\text{mol}^{-1}$ .<sup>41</sup> The estimated enthalpies of formation are  $-65.1 \text{ kJ}\cdot\text{mol}^{-1}$ ,  $-60.7 \text{ kJ}\cdot\text{mol}^{-1}$ , and  $-57.2 \text{ kJ}\cdot\text{mol}^{-1}$  for the hydroxy-amino, oxo-amino, and oxo-imino tautomers, respectively. The value estimated for the hydroxy-amino form of cytosine is in excellent agreement with the experimental result reported here,  $\Delta_f H_m^\circ(\text{g}) = -(66.9 \pm 3.0) \text{ kJ}\cdot\text{mol}^{-1}$ , supporting both the new combustion experiments and the standard molar enthalpy of sublimation reported by Burkinshaw and Mortimer.<sup>18</sup>

**Bond Dissociation Enthalpies.** The computed enthalpies required to cleave X–H (X = C, N, and O) bonds in the three different forms of cytosine considered are reported in Table 3. In disagreement with previous DFT calculations, these X–H homolytic bond dissociation enthalpies (BDEs) have been calculated using the G3MP2B3 computed enthalpy for the hydrogen atom at  $T = 298.15 \text{ K}$  ( $H = -0.49978 \text{ au}$ ) and not the enthalpy based on the exact energy ( $E = -0.50000 \text{ au}$ ) of atomic hydrogen at  $0 \text{ K}$  corrected for  $T = 298.15 \text{ K}$  ( $H = -0.49764 \text{ au}$ ).<sup>42–45</sup> The values listed in Table 3 may be easily converted in BDEs anchored on the exact energy by consideration of the difference between the two enthalpies, at  $T = 298.15 \text{ K}$ , for the hydrogen atom given above. The analysis of the results compiled in that table shows that the most stable X–H bond is the C<sub>4</sub>–H<sub>12</sub> bond in the oxo-imino compound. Further, it is found for all tautomers that the cleavage of the C<sub>4</sub>–H<sub>12</sub> bond produces the least stable radicals of each form of cytosine. The bond that is easier to be cleaved is the N<sub>6</sub>–H<sub>9</sub> bond in the oxo-imino. The difference between the most and least stable bonds in the oxo-imino species is noteworthy being of about  $90 \text{ kJ}\cdot\text{mol}^{-1}$ . That bond is also the one easier to be cleaved in the oxo-amino form.

The N–H BDEs for the amino group are significantly larger than the G3MP2B3 N–H value,  $396.7 \text{ kJ}\cdot\text{mol}^{-1}$ , reported

**TABLE 4: Gas-Phase Acidities in  $\text{kJ}\cdot\text{mol}^{-1}$  for the Tautomers of Cytosine Shown in Figure 1<sup>a</sup>**

proton label	hydroxy-amino	oxo-amino	oxo-imino
[N <sub>2</sub> ] H <sub>10</sub> <sup>+</sup>			1450.9 (1474.8)
[C <sub>4</sub> ] H <sub>12</sub> <sup>+</sup>	1604.6	1576.8	1578.7
[C <sub>5</sub> ] H <sub>13</sub> <sup>+</sup>	1626.9	1543.2	1544.6
[N <sub>6</sub> ] H <sub>9</sub> <sup>+</sup>		1443.4 (1446.5)	1419.2 (1414.2)
[O <sub>7</sub> ] H <sub>9</sub> <sup>+</sup>	1447.8 (1442.2)		
[N <sub>8</sub> ] H <sub>10</sub> <sup>+</sup>	1482.5 (1488.1)	1476.2 (1482.7)	
[N <sub>8</sub> ] H <sub>11</sub> <sup>+</sup>	1467.4 (1471.1)	1454.4 (1458.4)	1521.6

<sup>a</sup> Values in parentheses are B3LYP/6-311++G(d,p) values and were taken from ref 1.

previously for aniline.<sup>46</sup> This suggests either that the N radicals of cytosine are much more unstable than that for aniline or that the N–H bonds are very stable in the parent cytosine species. A possible explanation is based on the B3LYP/6-31G(d) angles (G3MP2B3 calculations) of the NH<sub>2</sub> group in aniline and in hydroxy-amino and oxo-amino tautomers of cytosine; the H<sub>11</sub>–N<sub>8</sub>–C<sub>3</sub>–H<sub>10</sub> dihedral angle in aniline is 130.1°, while in the tautomers the angles are 147.5° and 150.4°, respectively. These dihedral angles show that the volume occupied by the nitrogen lone-pair is much larger in aniline than in the cytosine tautomers. Therefore, that increased volume leads to a larger destabilization of the N–H bonds and, consequently, to lower BDEs values. The increase of the H<sub>11</sub>–N<sub>8</sub>–C<sub>3</sub>–H<sub>10</sub> dihedral angle on going from aniline to the oxo-amino form of cytosine is accompanied by the increasing of the N–H BDEs. This reasoning is also in agreement with the lower BDE computed for the N<sub>8</sub>–H<sub>11</sub> bond in the oxo-imino tautomer.

**Gas-Phase Acidities.** The gas-phase acidities of each of the X–H bonds in the title compound were computed as  $H[\text{C}_4\text{H}_4\text{N}_3\text{O}]^- - H[\text{C}_4\text{H}_5\text{N}_3\text{O}] + 5RT/2$  and are given in Table 4. The acidities range over a large interval, from 1419.2  $\text{kJ}\cdot\text{mol}^{-1}$  to 1626.9  $\text{kJ}\cdot\text{mol}^{-1}$ , and it is found that the most acidic proton is the H<sub>9</sub> one in the oxo-imino species, while the less acidic is H<sub>13</sub> in the hydroxy-amino tautomer. The same proton is the most acidic one in the oxo-amino tautomer, while in the hydroxy-amino form, the proton that is removed much easier is that attached to the oxygen atom. The G3MP2B3 computed values are close to those reported in a previous theoretical work,<sup>1</sup> where this thermodynamic property has been evaluated at the B3LYP/6-311++G(d,p) level of theory. The differences are all smaller than 6  $\text{kJ}\cdot\text{mol}^{-1}$  except for the acidity of the H<sub>10</sub> proton attached to the N<sub>2</sub> atom in the oxo-imino tautomer where a much larger difference is found (~14  $\text{kJ}\cdot\text{mol}^{-1}$ ). This is most probably due to the fact that the most stable oxo-imino anion has the H<sub>11</sub> atom pointing in the opposite direction than that represented in Figure 1 for the neutral species, that is, pointing toward the H<sub>10</sub> atom instead of pointing toward the H<sub>12</sub> one. In fact, the G3MP2B3 enthalpic difference between the anion with the H<sub>11</sub> pointing toward the H<sub>10</sub> or the H<sub>12</sub> atoms is 20.7  $\text{kJ}\cdot\text{mol}^{-1}$  supporting our hypothesis that the energy of the anion used to compute the gas-phase acidity by Chandra et al.<sup>1</sup> was the least stable one, i.e., with the H<sub>11</sub> atom pointing toward the H<sub>12</sub> atom. Despite the small differences between the G3MP2B3 and the DFT acidities previously reported in the literature,<sup>1</sup> it is proposed that the present values should be used in future compilations of data instead of values derived from DFT calculations since the DFT approaches were found to predict erroneously the relative stability of the three tautomers considered here.<sup>31</sup>

**Adiabatic Ionization Enthalpies.** The enthalpy required to remove a single electron from each of the tautomers considered shows that the most easily ionizable tautomer is the hydroxy-amino form. The largest energy required to extract an electron

is computed for the oxo-amino tautomer. At the G3MP2B3 level of theory, the ionization enthalpies are 843.7  $\text{kJ}\cdot\text{mol}^{-1}$ , 854.2  $\text{kJ}\cdot\text{mol}^{-1}$ , and 847.6  $\text{kJ}\cdot\text{mol}^{-1}$ , for the hydroxy-amino, oxo-imino, and oxo-amino tautomers of cytosine, respectively. The most stable cations for the hydroxy-amino and oxo-imino tautomers have a structure similar to that depicted in Figure 1. The cation derived from the hydroxy-amino tautomer but with the H<sub>9</sub> atom pointing in the opposite direction of the structure displayed in Figure 1 is less stable by 1.7  $\text{kJ}\cdot\text{mol}^{-1}$ . In the case of the oxo-imino, the cation with the H<sub>11</sub> atom in the cis position with the H<sub>10</sub> atom is less stable than the preferred geometry with the H<sub>11</sub> and H<sub>10</sub> atoms in trans-configuration by 4.4  $\text{kJ}\cdot\text{mol}^{-1}$ .

The computed values are in close agreement with the range of experimental results, 815  $\text{kJ}\cdot\text{mol}^{-1}$  to 838  $\text{kJ}\cdot\text{mol}^{-1}$  (vertical values vary in the interval covered between 849  $\text{kJ}\cdot\text{mol}^{-1}$  and 868  $\text{kJ}\cdot\text{mol}^{-1}$ ) and also with the multiconfigurational perturbation (CASPT2) computations,<sup>47</sup> which lie in the range delimited by 810  $\text{kJ}\cdot\text{mol}^{-1}$  and 826  $\text{kJ}\cdot\text{mol}^{-1}$  (vertical values vary in the interval 823  $\text{kJ}\cdot\text{mol}^{-1}$  and 845  $\text{kJ}\cdot\text{mol}^{-1}$ ). Finally, the difference between the lowest G3MP2B3 ionization enthalpy and the largest experimental value is only ~6  $\text{kJ}\cdot\text{mol}^{-1}$ .

## Conclusions

A revised value for the gas-phase standard molar enthalpy of formation of cytosine is suggested after new combustion calorimetry experiments and state-of-the-art computations at the G3MP2//B3LYP level of theory. The experimental and computational values differ by only 1.8  $\text{kJ}\cdot\text{mol}^{-1}$ , and it is strongly suggested that the revised value,  $\Delta_f H_m^0(\text{g}) = -(66.9 \pm 3.0)$   $\text{kJ}\cdot\text{mol}^{-1}$ , should be included in future revisions of tables with thermochemical data. The computational approach was also used to compute the enthalpies of tautomerization, and, as suggested by previous experimental works, the hydroxy-amino tautomer is the most stable species in the gas phase. The oxo-amino tautomer, which is the form present in biological materials, is less stable in the gas phase than the hydroxy-amino tautomer by 4.4  $\text{kJ}\cdot\text{mol}^{-1}$ . The least stable tautomer is the oxo-imino tautomer with an enthalpic difference to the hydroxy-amino form of 7.9  $\text{kJ}\cdot\text{mol}^{-1}$ .

The computation of C–H, N–H, and O–H bond dissociation enthalpies showed that the two most stable radicals obtained after removal of a hydrogen atom are derived from the imino species. Those radicals are formed after N<sub>ring</sub>–H or N<sub>imino</sub>–H bond cleavage. The most acidic proton is that linked to the nitrogen atom in the opposite side of the ring with respect to the amino group, showing why this is the bond that is cleaved when the cytosine fragment is bonded to other chemical materials.

Finally, the adiabatic ionization enthalpy computed for the most stable hydroxy-amino tautomer is in very good agreement with the experimental data and also close to recent computed results based on much more CPU demanding computations.

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