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Ab initio and DFT studies on tautomerism of 5-methyl cytosine in gaseous phase

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ABSTRACT

Ab initio and DFT methods have been used to study the seven tautomeric forms of 5-methylcytosine molecule. The related tautomer in gas phase have been studied at HF/6-31G, HF/6-31G* and B3LYP/6-31G* levels of theory. The structures,enthalpies,entropies,Gibbs free energies,relative tautomerization energies of tautomers and tautomeric equilibrium constants were compared and analyzed along with full geometry optimization. The calculations showed that the Oxo-amino(6), Oxo-imino(7) and Hydroxy-amino(4) tautomers are the most stable in the gas phase. The results are in a good agreement with the available experimental data. The entropy effect on the Gibbs free energy of the 5-methylcytosine bases is very small and it has a little significance on the tautomeric equilibria of pyrimidine bases.Therefore the enthalpic term is dominant in the determination of the equilibrium constants. ^{13}C-NMR studies have been carried out for these tautomers and the results are discussed.We have also evaluated the hybridation coefficient for bonds and hetero atom LP_S in the aromatic ring for the stable tautomers. Natural Bond Orbital Theory (NBO) calculation showed that the stable tautomers must be considered aromatic.

Keywords: 5-methylcytosine; Tautomerism; Tautomeric equilibrium constant; NBO;^{13}C-NMR; Chemical shift; Abinitio; DFT

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INTRODUCTION

Tautomerism phenomenon studies are valuable in many areas of chemistry and biochemistry as demonstrated by several reviews of experimental and theoretical studies[1-5].Tautomers are structural isomers that are conceptually related by the shift of hydrogen and one or more π bonds.For last two decades, there has been much interest in studying the tautomerism of heterocyclic compounds to identify the influence of tautomerization on chemical and biological properties of considered molecules. The phenomenon of tautomerism in organic compounds is related to aromaticity and lone pair–lone pair repulsion.The experimental studies on tautomerism are still a challenging problem in chemistry and molecular biology. Most of tautomers are not observed in the experimental studies due to their low concentration[6].Tautomeric equilibria of pyrimidine bases are of continuing interest both from the theoretical [7-21] and experimental[22-25] view points, partly due to suggestions that the presence of unusual tautomers may have important biological properties, such as mutagenesis.One hypothesis suggests that the frequency of mispairing in DNA and thus, mutagenesis is correlated with equilibrium constants for the keto-enol or amino-imino tautomerization [26,27].The relative stability of tautomers of the pyrimidine base cytosine is of fundumental importance to the DNA structure[28,29] .The obvious interest in cytosine is due to its biological allure as one of the four fundamental bases constituting the double helix of DNA. For chemists of the structural chemistry, cytosine and 5-methylcytosine are especially very interest due to the existence of various tautomeric forms[30-34] and this process is intimately connected to the energetics of the chemical bonds[35].

5-Methylcytosine is a minor base of DNA. Its percentage with respect to the total content of cytosine varies over a wide range, from 0.03% in insects, to 2-8% in mammals, to 50% in the higher plants. DNA is modified after synthesis by the enzymatic conversion of many cytosine residues to 5-methylcytosine, and the pattern of DNA methylation is then maintained in the consecutive cell divisions[36].A detailed analysis of the structure and changes in geometrical and energetical parameters caused by the migration of hydrogen atom would enable us to understand the different properties of tautomers. A knowledge of the relative stabilities of tautomeric forms of heterocycles as well as the conversion from one tautomeric form to another is important from the point of view of structural chemistry[6].Previous works on pyrimidine bases are in connection to the structures and relative stability of different tautomers of these bases[8-14]. In most of them the effects of entropy on the equilibrium is not considered.

The temperature-dependence of enthalpy and entropy contributions to the Gibbs free energy of tautomerization of DNA bases and their 5-methyl derivatives are known to be important. Analyzing of the entropy effect thus allows for better understanding of the tautomerization process. If several tautomers exist in comparable concentrations, the entropy contribution may be an important part of relative Gibbs free energies due to the fact that the exact equilibrium concentration depends on the Gibbs free energy of each tautomer. Consequently, both entropy and enthalpy in general should be included for a proper comparison of the calculated and experimental tautomeric stability of the bases.

Experimental information about the relative stability of two tautomeric forms of a molecule ($a \rightleftharpoons b$)is obtained from the measurement of the tautomeric equilibrium constant $K_{a,b}(T)$. As a consequence, the standard Gibbs free energy of the tautomerization ,$\Delta G^{\circ}_{a,b}(T)$, can be estimated at the defined temperature T [37]. Previous studies on tautomerization show that the semi-empirical methods are inadequate for predicting the order of stability,physical and chemical properties of tautomers, thus we should consider the appropriate theoretical models, such as ab initio and DFT calculations which are invaluable tools for obtaining sufficient informations about the structure, relative stability and other properties of such

tautomers, in the sense, the physical properties of tautomers can be directly analyzed by the results of the quantum mechanical calculations[6].

In the present paper, we have reported the enthalpy, entropy, Gibbs free energy, relative stability, tautomeric equilibrium constants and dipole moments for the pyrimidine bases at 298.15 K. In addition after having predicted the relative stability of tautomers, we have estimated the tautomeric equilibrium constants with respect to the more stable tautomers in the gas phase. Finally, we have reported ^{13}C-NMR properties and hybridation coefficient of bonds for stable tautomers.

METHOD OF CALCULATION

Full geometry optimization for all seven tautomers related to 5-methylcytosine were carried out using the HF (Hartree-Fock) theory at the 6-31G & 6-31G* levels, and performed at the B3LYP(Becke3-Lee-Yang-Parr) density functional theory(DFT) at the 6-31G* level within the GAUSSIAN 03 suite of programs (revision B.03; Gaussian, Inc.:Pittsburgh, PA, 2003). To assess the performance of this approach the optimized geometrical outputs are used as inputs for thermochemistry , NMR and NBO calculatins.To calculate thermodynamic properties of the tautomers, all vibrational frequencies were scaled by a factor of 0.893. Natural bond orbital (NBO) population analysis was performed with the use of the NBO program, version 3.1 (link 607,Gaussian 03W).

RESULTS AND DISCUSSION

Relative stability

The calculated energies, enthalpies, Gibbs free energies and dipole moments of seven tautomers related to 5-methylcytosine are given in Table 1.The calculated Gibbs free energies of considered tautomers based on the HF/6-31G and B3LYP/6-31G* reveals that the 1H-Oxo amino(6) form is the most stable form in the gas phase. But the results of calculations with HF/6-31G* of Gibbs free energies indicate that the most stable form is the Hydroxy-amino (form 4). However,this results indicate that substantial amount of the Hydroxy-amino (form 4) , 1-H-Oxo-amino(6)(form 6) , and Oxo-imino (form 7) may be present in the gas phase. Infrared spectra of 5-methylcytosine in an argon matrix are reported with **Andrzej Lei and Ludwik Adamowicz**.These experimental results indicate that three tautomeric forms, Hydroxy-amino(4), Oxo-amino(6), and Oxo-imino(7), exist simultaneously in the matrix.The final order of stability from the HF/6-31G method is : 6>7>4>1>3>2>5 and from the HF/6-31G* is : 4>6>7>1>3>2>5 and from the B3LYP/6-31G* is : 6>4>7>1>3>2>5

Our calculated relative stability values (ΔG^0) are very similar to those of calculated relative stability values (ΔH^0) respect to 4→5 , 4→6 and 4→7 stages(Table 2).In fact, the stability trend (relative values of ΔG^0) is almost very similar to the enthalpic trend (Table2).

As we can see from the data of table 2 , the values of ΔS^0 related to the tautomeric interconversion is fairly small and it maybe neglect the $T\Delta S^0$ contribution in calculating the values of considered ΔG^0 .Thus, the enthalpic term is dominant in determinig the equilibrium constant. However, when the concentration ratio of two considered tautomers is very close, it is necessary to consider the entropy contribution effects. This is particularly important when accurate calculations are carried out to estimate relative stabilities of the tautomers.

$$a \underset{}{\overset{K}{\rightleftharpoons}} b$$

The thermodynamic equilibrium constant for each step of tautomerization is :

$$K = \exp(-\Delta G^0 / RT) \qquad (1)$$

where K is the considered tautomeric equilibrium constant of $a \rightleftharpoons b$. The quantity ΔG^0 is the difference between the standard gibbs free energies of a and b tautomers. The pK values of the studied steps were calculated by the following equation:

$$pK = \Delta G^0 / (2.303RT) \qquad (2)$$

The calculated equilibrium constants (by any used method) show that the tautomers 1 , 2 , 3

and 5(See Fig.1) are not present in detectable amount.But the calculations with HF/6-31G indicate that the 1H-Oxo-amino(6) tautomer percent is 78.474, Oxo-imino(7) tautomer percent is 21.173 and Hydroxy-amino(4) tautomer percent is 0.353 in the gas phase. On the other hand, the calculation with HF/6-31G* method show that 40.4265 percent of 1H-Oxo-amino(6) tautomer , 9.6086 percent of Oxo-imino(7) tautomer , and 49.9648 percent of Hydroxy-amino(4) tautomer are present.Finally,the calculated values with B3LYP/6-31G* method show that 85.207 percent of 1H-Oxo-amino(6) tautomer , 2.6 percent of Oxo-imino(7) tautomer and 12.19 percent of Hydroxy-amino(4) tautomer are present in the gas phase.DFT results as well as experimental studies indicate that 1H-Oxo-amino(6) is strongly favoured while Oxo-imino(7) and Hydroxy-amino(4) forms exist simultaneously.

Geometric features of stable tautomers

The structures of stable tautomers of 5-methylcytosine are given in fig.2.The structural parameters for optimized tautomers (bond lengths, bond angles,and dihedral angles)are listed in Tables 4-6 .

The N_1 –C_2 and N_6 –C_2 bond length in Oxo-amino(6) tautomer , N_4 –C_5 and N_1 –C_8 bond length in Oxo-imino(7) tautomer , N_1 –C_3 and N_5 –C_8 bond length in Hydroxy amino(4) tautomer show slight elongation , which suggests a much weaker binding in this regions. The C_9 –C_7 bond length in Oxo-amino(6) tautomer , C_7 –C_5 bond length in Oxo-imino(7) tautomer and C_6 –C_8 bond length in Hydroxy amino(4) tautomer get larger indicating that delocalization of the charge density of the π-system has decreased in this region upon amino and/or methyl groups substitution.This would lead to a much weaker binding in this region.

Generally(in the ring), the C –C –C bond angles in 1H-Oxo-amino(6) tautomer are slightly smaller than the C –N –C or C –C –N bond angles, while the C –N –C bond angles in Oxo-imino(7) tautomer and C –C –N bond angles in Hydroxy amino(4) tautomer are slightly larger than C –C –C andC –C –N or C –C –C and C –N –C bond angles respectively.

1H-Oxo-amino(6),Oxo-imino(7) and Hydroxy-amino(4) tautomers of 5-methylcytosine by definition have a planar cyclic backbone, as illustrated in fig.2.

^{13}C-NMR analysis

^{13}C-NMR chemical shift is one of the important tools in determining the presence or absence of particular atom in a particular position of a molecular system. In a molecule,shielding of atoms like carbon is greatly affected by neighbouring bonded atoms and similar bonded atoms give different shielding values in different environments.The guage-including atomic orbital(GIAO)method is used to study the stable tautomers of 5-methylcytosine in the gas phase.This study involves the calculations by NMR keyword for chemical shift calculation.

In the present study the chemical shift (δ) of carbon atoms in various tautomers has been studied. The relationship between the shielding and chemical shift is:

$$\delta = \text{shielding of carbon atom}(\sigma_{iso}) \text{ in TMS} - \text{shielding value}(\sigma_{iso}) \text{ of carbon} \quad (3)$$

while TMS (Tetra methyl silane) is as a reference and

$$(\sigma_{iso}) = (\sigma_{11} + \sigma_{22} + \sigma_{33}) /3 \quad \& \quad (\sigma_{aniso}) = \sigma_{33} - 1/2(\sigma_{11} + \sigma_{22}) \quad (4)$$

Depending on the local symmetry at the nuclear site, the magnitude of the chemical shift will vary as a function of the orientation of the molecule with respect to the external magnetic field. This orientation dependence of the chemical shift is referred to as chemical shift anisotropy (CSA). Mathematically, the chemical shift anisotropy is described by a second-rank tensor (a 3 by 3 matrix), which in the case of the symmetric part of the chemical shift (CS) tensor consists of six independent components. Generally, one can express the chemical shift tensor in a coordinate frame where all off-diagonal elements vanish. In this principal axis system, the chemical shift tensor is fully described by the three diagonal

elements - the principal components - and the three eigenvectors or Euler angles describing the orientation of the principal axes with respect to an arbitrary frame. In addition, various combinations of the principal components (and their orientations) are used to describe the chemical shift tensor.

The shielding value of carbon atom in TMS are calculated at HF/6-31G,HF/6-31G* and B3LYP/6-31G* levels of theory and are found to be 208.1685, 201.7313 and 189.700275 ppm,respectively. By using this TMS value and shielding values of carbon atom that have been obtained in NMR studies, the chemical shift values (δ) were calculated and are given in Tables 7-9. The presence of an electronegative atom causes the electron cloud of carbon atom to be attracted towards the electronegative atom. This leads to deshielding of carbon atom and the net result is an increase in chemical shift value and hence, the requirement of field for resonance becomes low and large decrease in chemical shift values have been noticed which may be due to the presence of hydrogen atoms or/ methylgroup bonded to that carbon atoms and consequently,requirement of field for resonance becomes high. The chemical shift values obtained for all other carbon atoms in all tautomers show more or less similar range. This is reasonably due to the presence of the same environment surrounding for those carbon atoms.

NBO analysis

NBO analysis provides the charges, bond types, hybrid directions, resonance weights, bond orders and other familiar valence descriptors. NBO is firmly rooted in traditional orbital concepts of Mulliken, Pauling, and Coulson. Unlike methods based on numerically differentiating the charge density (a classical concept), NBO analysis remain closely tied to quantum mechanical wavefunction, phase, and superposition concepts. NBO methods are increasingly recognized as standard in theoretical presentations.

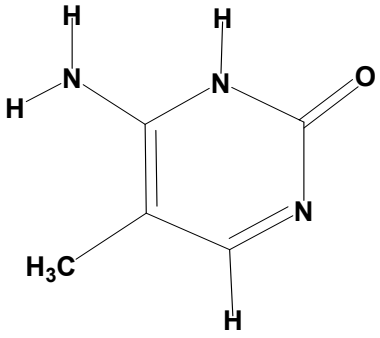
This work involves some NBO calculations based on POP=NBO keyword. The hybrid composition of the atoms of ring and heteroatoms LPs in the ring were determined for the stable tautomers (Table 10-12).Hybridation coefficient is different in various methods and basis sets.

For 1H-Oxo-amino(6) tautomer C_3-C_7 and N_6-C_9 bonds is doublet and electrons of LP(N_1) occupy *p* orbitals. On the other hand, in Oxo-imino(7) tautomer the C_7-C_8 bond is doublet and electrons of LP(N_1)&LP(N_4) occupy *p* orbitals.Finally, we saw three doublet bond (N_1-C_2 , C_3-C_6 , N_5-C_8), in Hydroxy-amino(4) form.On the basis of these properties, the stable tautomers must be considered *aromatic* and the π clouds contain a total of six electrons, the aromatic sextet, and the delocalization of the π electrons stabilizes the ring.

CONCLUSIONS

Several consequences can be made on the basis of the results of the present theoretical study:

1. Calculations with DFT method clearly indicate that 5-methylcytosine exist predominantly in Hydroxy-amino(4), Oxo-amino(6), and Oxo-imino(7) tautomeric forms. These results are in agreement with previous experimental studies[36].
2. The entropy effect on the Gibbs free energy of the pyrimidine bases is very small and it has a little significance for the tautomeric equilibria of pyrimidine bases. So, the enthalpic terms are dominant in the determination of the equilibrium constants.
3. The calculated ^{13}C-NMR chemical shift values indicate that the delocalisation of electron cloud is always more toward to the electronegative atom, which leads to deshielding of carbon atoms.
4. NBO analysis indicate that all stable tautomers in the gas phase , must be considered aromatic.



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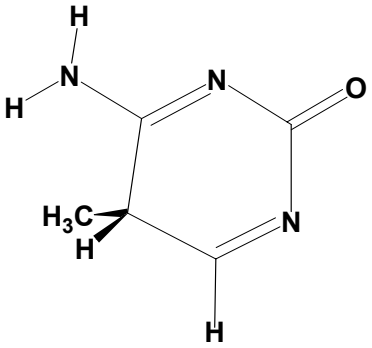

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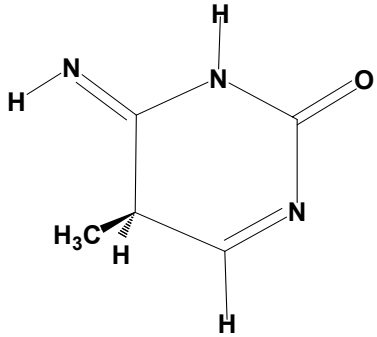

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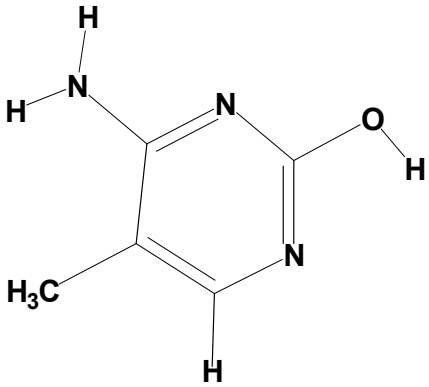

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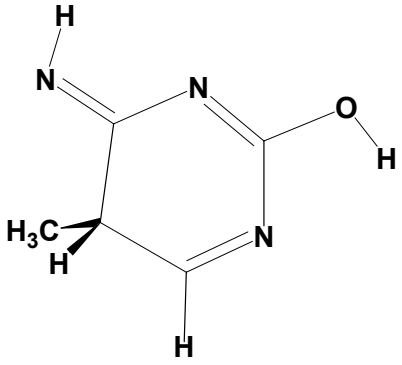

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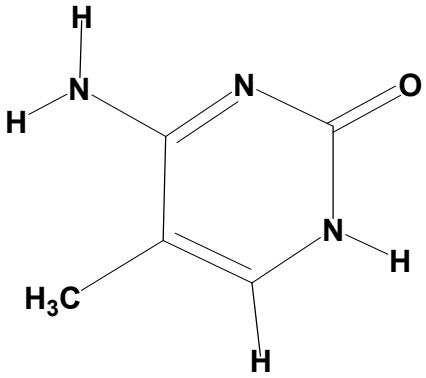

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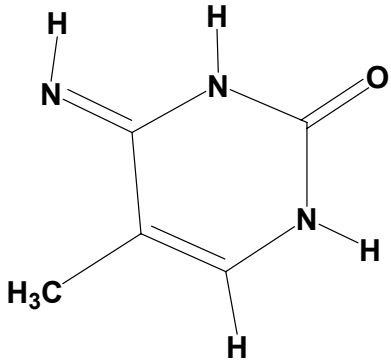

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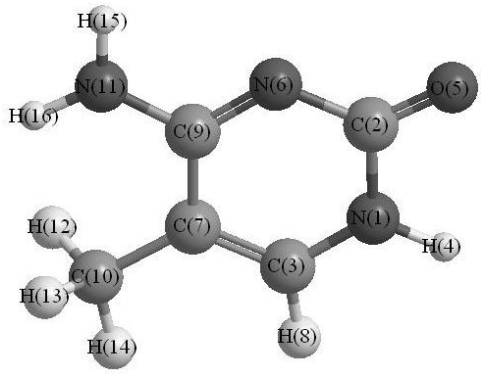
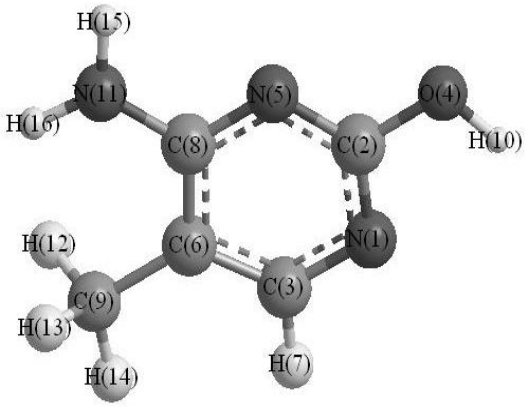
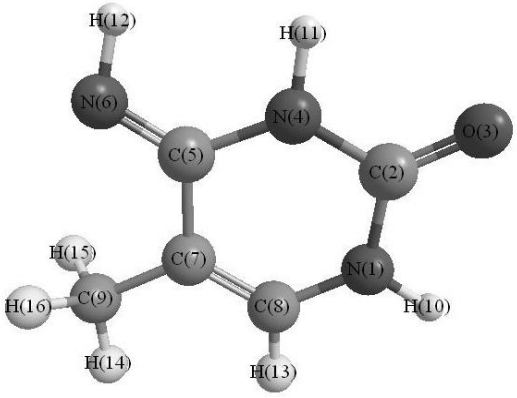
Fig. 1. Considered tautomers of 5-methylcytosine upon the present work.



1H-Oxo-amino(6)



Hydroxy-amino(4)



Oxo-imino(7)

Fig.2. The optimized structures of stable tautomers for 5-methylcytosine molecule.

Table 1. The calculated energies and other thermodynamic properties for seven tautomers related to 5-methylcytosine in the gas phase at 298.15 K

Tautomer	Method	energy (Hartree)	Standard Gibbs free energy (Hartree)	Standard Enthalpy (Hartree)	μ (D)
	HF/6-31G	-431.4441383	-431.354097	-431.312384	9.4836
1	HF/6-31G*	-431.6387817	-431.550646	-431.508894	8.6483
	B3LYP/6-31G*	-434.2345421	-434.145559	-434.101448	8.3563
	HF/6-31G	-431.4076619	-431.318893	-431.276567	9.922
2	HF/6-31G*	-431.620709	-431.532947	-431.489919	8.9431
	B3LYP/6-31G*	-434.2102683	-434.131913	-434.087915	8.2207
	HF/6-31G	-431.413631	-431.324433	-431.282144	7.1206
3	HF/6-31G*	-431.6277349	-431.538866	-431.496281	6.1902
	B3LYP/6-31G*	-434.2163962	-434.137306	-434.09359	5.3788
	HF/6-31G	-431.4523342	-431.361859	-431.32072	4.4773
4	HF/6-31G*	-431.6518899	-431.56259	-431.521698	3.7078
	B3LYP/6-31G*	-434.244359	-434.164103	-434.122211	3.5639
	HF/6-31G	-431.3840393	-431.296068	-431.253332	4.5734
5	HF/6-31G*	-431.6041351	-431.515163	-431.472852	4.4013
	B3LYP/6-31G*	-434.194041	-434.114800	-434.071573	3.8152
	HF/6-31G	-431.45821	-431.366962	-431.325822	8.3674
6	HF/6-31G*	-431.6516004	-431.56239	-431.521214	7.4037
	B3LYP/6-31G*	-434.254868	-434.165939	-434.123669	6.6678
	HF/6-31G	-431.4572948	-431.365725	-431.324594	2.6603
7	HF/6-31G*	-431.6511023	-431.561034	-431.519208	2.4753
	B3LYP/6-31G*	-434.2432367	-434.162645	-434.119784	2.235

Table 2. The theoretical calculated equilibrium constants related to the steps taking part in the 5-methyl- cytosine tautomerism in the gas phase at 298.15K

Tautomeric equilibria	Method	ΔG^0 kcal/mol	ΔH^0 kcal/mol	ΔS^0 cal/mol.K	K	pK
1→2	HF/6-31G	22.0908	22.4755	1.2902	$6.428*10^{-17}$	16.1919
	HF/6-31G*	11.1063	11.9070	2.6855	$7.234*10^{-9}$	8.1406
	B3LYP/6-31G*	8.5630	8.4920	-0.2381	$5.291*10^{-7}$	6.2764
1→3	HF/6-31G	18.6144	18.9758	1.2121	$2.270*10^{-14}$	13.6439
	HF/6-31G*	7.3920	7.9147	1.7531	$3.818*10^{-6}$	5.4181
	B3LYP/6-31G*	5.1788	4.9309	-0.8314	$1.560*10^{-4}$	3.8068
1→4	HF/6-31G	-4.8707	-5.2309	-1.2081	$3.716*10^{3}$	-3.57
	HF/6-31G*	-7.4949	-8.0346	-1.8101	$3.116*10^{5}$	-5.4936
	B3LYP/6-31G*	-11.6365	-13.0289	-4.6701	$3.382*10^{8}$	-8.5291
4→5	HF/6-31G	41.2844	42.2866	3.3614	$5.492*10^{-31}$	30.2602
	HF/6-31G*	29.7609	30.6513	2.9864	$1.535*10^{-22}$	21.8139
	B3LYP/6-31G*	30.9381	31.7758	0.8377	$2.100*10^{-23}$	22.6777
4→6	HF/6-31G	-3.2022	-3.2015	0.0023	$2.224*10^{2}$	-2.3471
	HF/6-31G*	0.1255	0.3037	0.5976	0.80911	0.0920
	B3LYP/6-31G*	-1.1521	-0.9149	0.7955	6.9897	-0.8444
4→7	HF/6-31G	-2.4259	-2.4309	-0.0016	60	-1.7781
	HF/6-31G*	0.9764	1.5625	1.9658	0.1924	0.7158
	B3LYP/6-31G*	0.9149	1.5230	2.0395	0.2135	0.6706

Table3. Distribution of stable tautomers

Method	1H-Oxo-amino(6)	Oxo-imino(7)	Hydroxy-amino(4)
HF/6-31G	78.474%	21.173%	0.353%
HF/6-31G*	40.4265%	9.6086%	49.9648%
B3LYP/6-31G*	85.2070%	2.6000%	12.1930%

Table 4. The molecular geometries of 1H-Oxo-amino(6) form of 5-methylcytosine at HF/6-31G//HF/6-31G*//B3LYP/6-31G* level of theory , respectively

Bond length (A^0)		Angles(degrees)		Dihedral angles (degrees)	
R(1,2)	1.3983//1.3969//1.4259	A(2,1,3)	123.1018//123.2481//123.5183	D(4,1,3,8)	0.00//0.00//-0.01
R(1,3)	1.3606//1.3518//1.3574	A(3,1,4)	121.1525//121.1759//121.3850	D(3,1,2,5)	180.00//180.03//-180.08
R(1,4)	0.9930/ 0.9955//1.0115	A(1,2,5)	119.1199//118.6805//118.4758	D(3,1,2,6)	0.00//-0.02//0.05
R(2,5)	1.2245//1.1975//1.2207	A(1,2,6)	115.7290//116.0933//115.7050	D(2,1,3,7)	0.00//0.01//-0.02
R(2,6)	1.3675//1.3615//1.3718	A(1,3,7)	121.3765//121.4223//120.9758	D(2,1,3,8)	180.00//180.00//-180.01
R(3,7)	1.3443//1.3393//1.3611	A(1,3,8)	116.3015//116.0792//116.6318	D(1,2,6,9)	0.00//0.02//-0.05
R(3,8)	1.0710//1.0743//1.0864	A(2,6,9)	121.7009//120.7126//120.5141	D(1,3,7,10)	180.00//180.00//-180.00
R(6,9)	1.3079//1.2963//1.3196	A(3,7,10)	123.1273//123.3223//122.8857	D(2,6,9,11)	180.00//-180.01//180.02
R(7,9)	1.4518//1.4546//1.4509	A(6,9,11)	117.0206//116.9930//116.4619	D(3,7,10,12)	-119.43//-119.42//-119.62
R(7,10)	1.5036//1.5058//1.5043	A(7,10,12)	111.8373//111.9259//112.0777	D(3,7,10,13)	119.43//119.43//119.60
R(9,11)	1.3461//1.3442//1.3600	A(7,10,13)	111.8362//111.9257//112.0773	D(3,7,10,14)	0.00//0.00//0.00
R(10,12)	1.0860//1.0871//1.0991	A(7,10,14)	110.5871//110.4211//110.8171	D(6,9,11,15)	0.00//0.00//0.00
R(10,13)	1.0860//1.0871//1.0990	A(9,11,15)	117.5229//117.8883//117.7129	D(6,9,11,16)	179.98//179.99//179.97
R(10,14)	1.0827//1.0838//1.0947	A(9,11,16)	123.2111//122.9763//122.7578		
R(11,15)	0.9920//0.9946//1.0092	A(3,7,9)	114.7800//114.0110//114.4263		
R(11,16)	0.9882//0.9908//1.0057	A(7,9,6)	123.3118//124.5128//124.8606		

Table 5. The molecular geometries of Oxo-imino(7) form of 5-methylcytosine at HF/6-31G//HF/6-31G*//B3LYP/6-31G* level of theory , respectively

Bond length (A^0)		Angles(degrees)		Dihedral angles (degrees)	
R(1,2)	1.3631//1.3633//1.3840	A(2,1,10)	116.1008//115.7409//115.2753	D(8,1,2,4)	0.00//0.00//0.00
R(1,8)	1.3905//1.3844//1.3868	A(1,2,3)	123.2303//123.4434//123.5626	D(10,1,2,3)	-0.01//-0.01//0.00
R(1,10)	0.9918//0.9944//1.0098	A(1,2,4)	114.2638//113.7743//112.9196	D(1,2,4,5)	-0.01//0.00//0.00
R(2,3)	1.2263/ 1.1969//1.2196	A(2,4,5)	127.0658//127.5903//128.0706	D(1,2,4,11)	179.99//-180.00//180.00
R(2,4)	1.3717//1.3707//1.3873	A(2,4,11)	114.1291//113.7475//113.4825	D(2,4,5,6)	-179.98//180.00//-180.00
R(4,5)	1.4024//1.3966//1.4110	A(4,5,6)	124.4261//124.4388//124.8026	D(2,4,5,7)	0.02//0.00//0.00
R(4,11)	0.9947//0.9971//1.0129	A(4,5,7)	114.6440//114.4248//114.2626	D(4,5,6,12)	0.00//0.00//0.00
R(5,6)	1.2679//1.2580//1.2836	A(5,6,12)	116.9692//112.9990//111.8002	D(4,5,7,8)	-0.01//0.00//0.00
R(5,7)	1.4684//1.4728//1.4681	A(5,7,8)	118.3556//118.0344//118.4985	D(4,5,7,9)	179.98//179.99//180.00
R(6,12)	1.0047//1.0046//1.0246	A(5,7,9)	118.1392//118.3757//118.3110	D(5,7,8,13)	179.99//-180//180.00
R(7,8)	1.3312//1.3267//1.3487	A(7,8,13)	122.4071//122.3882//122.3158	D(5,7,9,14)	179.99//180.00//-180.00
R(7,9)	1.4995//1.5024//1.5007	A(7,9,14)	110.8372//110.6479//111.1367	D(5,7,9,15)	59.20//59.34//58.88
R(8,13)	1.0695//1.0728//1.0847	A(7,9,15)	110.7587//110.7686//110.8171	D(5,7,9,16)	-59.20//-59.34//-58.89
R(9,14)	1.0831//1.0844//1.0949	A(7,9,16)	110.7600//110.7685//110.8170		
R(9,15)	1.0824//1.0836//1.0956	A(2,1,8)	123.0945//123.2022//123.5453		
R(9,16)	1.0824//1.0836//1.0956	A(7,8,1)	122.5762//122.9740//122.7033		

Table 6. The molecular geometries of Hydroxy-amino(4) form of 5-methylcytosine at HF/6-31G//HF/6-31G*//B3LYP/6-31G* level of theory , respectively

Bond length (A^0)		Angles(degrees)		Dihedral angles (degrees)	
R(1,2)	1.3214//1.3127//1.3355	A(2,1,3)	116.4248//114.9603//114.5548	D(4,1,3,8)	180.00//-180.00//180.02
R(1,3)	1.3424//1.3318//1.3438	A(1,2,4)	117.6274//117.0389//116.6356	D(3,1,2,5)	0.01//0.00//-0.01
R(2,4)	1.3436/ 1.3253//1.3486	A(1,2,5)	125.8461//127.6220//128.0713	D(3,1,2,6)	-0.01//0.00//0.00
R(2,5)	1.3205//1.3149//1.3294	A(1,3,6)	123.4546//124.6140//124.5233	D(2,1,3,7)	180.01//-180.00//180.00
R(3,6)	1.3734//1.3677//1.3846	A(1,3,7)	115.7907//115.5162//115.7970	D(2,1,3,8)	0.00//0.00//-0.01
R(3,7)	1.0715//1.0770//1.0902	A(2,4,10)	111.8654//107.6318//105.3007	D(1,2,6,9)	0.00//0.00//0.01
R(4,10)	0.9523//0.9500//0.9734	A(2,5,8)	117.7635//116.4394//115.8668	D(1,3,7,10)	180.02//-180.00//180.00
R(5,8)	1.3341//1.3215//1.3409	A(3,6,9)	122.8745//123.3467//123.1026	D(2,6,9,11)	179.99//180.00//-180.01
R(6-8)	1.4154//1.4154//1.4219	A(5,8,11)	116.2403//116.2556//116.0594	D(3,7,10,12)	-119.50//-119.47//-119.66
R(6,9)	1.5033//1.5060//1.5043	A(6,9,12)	111.8598//111.9916//112.0898	D(3,7,10,13)	119.47//119.47//119.67
R(8,11)	1.3501//1.3467//1.3606	A(6,9,13)	111.8595//111.9910//112.0895	D(3,7,10,14)	-0.01//0.00//0.00
R(9,12)	1.0865//1.0877//1.0994	A(6,9,14)	110.5304//110.4210//110.7037	D(6,9,11,15)	0.00//0.00//0.03
R(9,13)	1.0865//1.0877//1.0994	A(8,11,15)	118.0209//118.4314//118.2528	D(6,9,11,16)	179.98//179.98//179.95
R(9,14)	1.0824//1.0837//1.0946	A(8,11,16)	122.7211//122.4531//122.2577		
R(11,15)	0.9913//0.9938//1.0086	A(3,6,8)	115.0708//114.0669//114.5049		
R(11,16)	0.9881//0.9909//1.0056	A(6,8,5)	121.4402//122.2974//122.4789		

Table 7. ^{13}C-NMR chemical shielding values (in ppm) calculated for the Tautomers of 5-methylcytosine at HF/6-31G level of theory

Tautomer	Atoms	σ_{11}	σ_{22}	σ_{33}	σ_{iso}	σ_{aniso}	δ
1H-Oxo-amino(6)							
	C(2)	-69.1686	78.2461	125.9113	44.9963	121.3726	163.1722
	C(3)	-48.5664	71.4670	163.7781	62.2262	152.3278	145.9423
	C(7)	20.6711	112.9026	195.9707	109.8481	129.1839	98.32040
	C(9)	-63.5377	15.0745	157.7973	36.4447	182.0290	171.7238
Oxo-imino(7)							
	C(2)	-57.9937	73.1374	128.4514	47.8650	120.8795	160.3035
	C(5)	-63.7668	73.8097	147.8719	52.6383	142.8504	155.5302
	C(7)	1.58400	97.6403	184.9061	94.7101	135.2940	113.4584
	C(8)	-36.6530	102.7441	160.8773	75.6561	127.8318	132.5124
Hydroxy-amino(4)							
	C(2)	-60.7132	42.8126	132.3031	38.1342	141.2534	170.0343
	C(3)	-68.3218	39.3206	162.8991	44.6326	177.3997	163.5359
	C(6)	13.8125	96.8845	200.4119	103.7030	145.0634	104.4655
	C(8)	-56.4627	8.12160	163.5721	38.4103	187.7427	169.7582

Table 8. ^{13}C-NMR chemical shielding values (in ppm) calculated for the Tautomers of 5-methylcytosine at HF/6-31G* level of theory

Tautomer	Atoms	σ_{11}	σ_{22}	σ_{33}	σ_{iso}	σ_{aniso}	δ
1H-Oxo-amino(6)							
	C(2)	-39.2290	85.8024	114.2020	53.5918	90.91530	148.1395
	C(3)	-47.7136	70.6238	155.5304	59.4802	144.0753	142.2511
	C(7)	30.5134	119.5733	187.8108	112.6325	112.7674	89.09880
	C(9)	-53.6576	15.9347	146.5825	36.2865	165.4439	165.4448
Oxo-imino(7)							
	C(2)	-27.1137	82.0347	117.8598	57.5936	90.39930	144.1377
	C(5)	-55.9316	71.7518	137.6305	51.1502	129.7204	150.5811
	C(7)	10.3428	101.559	178.3766	96.7595	122.4257	104.9718
	C(8)	-34.9070	98.5910	153.6309	72.4383	121.7889	129.293
Hydroxy-amino(4)							
	C(2)	-43.0630	45.8556	121.5837	41.4588	120.1875	160.2725
	C(3)	-66.9221	39.2386	152.2552	41.5239	166.0969	160.2074
	C(6)	24.7130	107.8302	191.6924	108.0786	125.4208	93.65270
	C(8)	-49.4874	7.703200	151.2788	36.4982	172.1710	165.2331

Table 9. ^{13}C-NMR chemical shielding values (in ppm) calculated for the Tautomers of 5-methylcytosine at B3LYP/6-31G* level of theory

Tautomer	Atoms	σ_{11}	σ_{22}	σ_{33}	σ_{iso}	σ_{aniso}	δ
1H-Oxo-amino(6)							
	C(2)	-39.6735	90.4871	91.12420	47.3126	65.71750	142.3876
	C(3)	-29.9247	62.0504	138.4035	56.8431	122.3407	132.8571
	C(7)	26.8321	94.1413	173.4801	98.1512	112.9934	91.549
	C(9)	-41.7222	21.2827	130.6656	36.7421	140.8853	152.9581
Oxo-imino(7)							
	C(2)	-27.8074	85.969	98.64310	52.2683	69.56230	137.4319
	C(5)	-45.8905	63.3525	119.3265	45.5961	110.5955	144.1041
	C(7)	9.03950	73.7042	163.6119	82.1186	122.2400	107.5816
	C(8)	-22.2621	84.2480	137.5909	66.5256	106.5980	123.1746
Hydroxy-amino(4)							
	C(2)	-35.3785	33.5591	99.21430	32.4650	100.1239	157.2352
	C(3)	-50.7324	39.0954	131.2879	39.8836	137.1064	149.8166
	C(6)	15.4401	82.1568	176.7776	91.4582	127.9791	98.242
	C(8)	-33.6150	10.3578	133.5140	36.7523	145.1427	152.9479

Table 10. Hybridation coefficient of ring bonds and LPs calculated by NBO method for1H-Oxo-amino(6) tautomer

NBO Type	HF/6-31G	HF/6-31G*	B3LYP/6-31G*
N_1-C_2(σ)	$0.8038(sp^{1.88})_N+0.5949(sp^{2.32})_C$	$0.8083(sp^{1.87})_N+0.5887(sp^{2.33})_C$	$0.8049(sp^{1.91})_N+0.5934(sp^{2.37})_C$
N_1-C_3(σ)	$0.7841(sp^{1.74})_N+0.6206(sp^{2.48})_C$	$0.7847(sp^{1.71})_N+0.6198(sp^{2.43})_C$	$0.7835(sp^{1.66})_N+0.6214(sp^{2.43})_C$
C_2-N_6(σ)	$0.6327(sp^{1.88})_C+0.7744(sp^{1.90})_N$	$0.6282(sp^{1.86})_C+0.7781(sp^{1.94})_N$	$0.6328(sp^{1.82})_C+0.7743(sp^{2.02})_N$
C_3-C_7(σ)	$0.7038(sp^{1.51})_C+0.7104(sp^{1.81})_C$	$0.7038(sp^{1.50})_C+0.7104(sp^{1.8})_C$	$0.7037(sp^{1.52})_C+0.7105(sp^{1.85})_C$
C_3-C_7(π)	$0.6390(p^{1.00})_C+0.7692(p^{1.00})_C$	$0.6310(p^{1.00})_C+0.7757(p^{1.00})_C$	$0.6589(p^{1.00})_C+0.7522(p^{1.00})_C$
N_6-C_9(σ)	$0.7606(sp^{1.58})_N+0.6493(sp^{2.02})_C$	$0.7627(sp^{1.57})_N+0.6467(sp^{1.95})_C$	$0.7600(sp^{1.68})_N+0.6499(sp^{1.99})_C$
N_6-C_9(π)	$0.8418(p^{1.00})_N+0.5399(p^{1.00})_C$	$0.8491(p^{1.00})_N+0.5282(p^{1.00})_C$	$0.8152(p^{1.00})_N+0.5759(p^{1.00})_C$
C_7-C_9(σ)	$0.7082(sp^{2.26})_C+0.7060(sp^{1.73})_C$	$0.7103(sp^{2.27})_C+0.7039(sp^{1.75})_C$	$0.7100(sp^{2.20})_C+0.7042(sp^{1.68})_C$
LP(N_1)	$1.0000(p^{1.00})$	$1.0000(p^{1.00})$	$1.0000(p^{1.00})$
LP(N_6)	$1.0000(sp^{2.73})$	$1.0000(sp^{2.68})$	$1.0000(sp^{2.37})$

Table 11. Hybridation coefficient of ring bonds and LPs calculated by NBO method for Oxo-imino(7) tautomer

NBO Type	HF/6-31G	HF/6-31G*	B3LYP/6-31G*
N_1-C_2(σ)	$0.7910(sp^{1.86})_N+0.6118(sp^{2.07})_C$	$0.7956(sp^{1.85})_N+0.6058(sp^{2.09})_C$	$0.7930(sp^{1.87})_N+0.6092(sp^{2.11})_C$
N_1-C_8(σ)	$0.7895(sp^{1.77})_N+0.6137(sp^{2.64})_C$	$0.7898(sp^{1.75})_N+0.6134(sp^{2.59})_C$	$0.7881(sp^{1.70})_N+0.6156(sp^{2.58})_C$
C_2-N_4(σ)	$0.6099(sp^{2.11})_C+0.7925(sp^{1.82})_N$	$0.6043(sp^{2.12})_C+0.7968(sp^{1.81})_N$	$0.6093(sp^{2.10})_C+0.7929(sp^{1.81})_N$
N_4-C_5(σ)	$0.7928(sp^{1.71})_N+0.6095(sp^{2.50})_C$	$0.7948(sp^{1.69})_N+0.6068(sp^{2.46})_C$	$0.7914(sp^{1.67})_N+0.6113(sp^{2.49})_C$
C_5-C_7(σ)	$0.7072(sp^{1.88})_C+0.7070(sp^{2.34})_C$	$0.7050(sp^{1.93})_C+0.7092(sp^{2.34})_C$	$0.7041(sp^{1.87})_C+0.7101(sp^{2.27})_C$
C_7-C_8(σ)	$0.7097(sp^{1.75})_C+0.7045(sp^{1.45})_C$	$0.7093(sp^{1.74})_C+0.7049(sp^{1.44})_C$	$0.7094(sp^{1.78})_C+0.7048(sp^{1.45})_C$
C_7-C_8(π)	$0.7321(p^{1.00})_C+0.6812(p^{1.00})_C$	$0.7371(p^{1.00})_C+0.6758(p^{1.00})_C$	$0.7232(p^{1.00})_C+0.6907(p^{1.00})_C$
LP(N_1)	$1.0000(p^{1.00})$	$1.0000(p^{1.00})$	$1.0000(p^{1.00})$
LP(N_4)	$1.0000(p^{1.00})$	$1.0000(p^{1.00})$	$1.0000(p^{1.00})$

Table 12. Hybridation coefficient of ring bonds and LPs calculated by NBO method for Hydroxy-amino(4) tautomer

NBO Type	HF/6-31G	HF/6-31G*	B3LYP/6-31G*
N_1-C_2(σ)	$0.7702(sp^{1.84})_N+0.6378(sp^{1.74})_C$	$0.7731(sp^{1.85})_N+0.6343(sp^{1.70})_C$	$0.7699(sp^{2.00})_N+0.6381(sp^{1.69})_C$
N_1-C_2(π)	$0.8354(p^{1.00})_N+0.5496(p^{1.00})_C$	$0.8520(p^{1.00})_N+0.5235(p^{1.00})_C$	$0.8162(p^{1.00})_N+0.5778(p^{1.00})_C$
N_1-C_3(σ)	$0.7698(sp^{1.70})_N+0.6382(sp^{2.29})_C$	$0.7707(sp^{1.70})_N+0.6372(sp^{2.20})_C$	$0.7693(sp^{1.77})_N+0.6389(sp^{2.21})_C$
C_2-N_5(σ)	$0.6355(sp^{1.75})_C+0.7721(sp^{1.73})_N$	$0.6321(sp^{1.75})_C+0.7749(sp^{1.76})_N$	$0.6358(sp^{1.73})_C+0.7719(sp^{1.84})_N$
C_3-C_6(σ)	$0.6955(sp^{1.59})_C+0.7176(sp^{1.86})_C$	$0.6957(sp^{1.57})_C+0.7183(sp^{1.85})_C$	$0.6957(sp^{1.56})_C+0.7183(sp^{1.87})_C$
C_3-C_6(π)	$0.6276(p^{1.00})_C+0.7786(p^{1.00})_C$	$0.6121(p^{1.00})_C+0.7908(p^{1.00})_C$	$0.6515(p^{1.00})_C+0.7586(p^{1.00})_C$
N_5-C_8(σ)	$0.7637(sp^{1.75})_N+0.6456(sp^{2.20})_C$	$0.7656(sp^{1.73})_N+0.6433(sp^{2.12})_C$	$0.7629(sp^{1.86})_N+0.6466(sp^{2.14})_C$
N_5-C_8(π)	$0.8399(p^{1.00})_N+0.5428(p^{1.00})_C$	$0.8535(p^{1.00})_N+0.5210(p^{1.00})_C$	$0.8163(p^{1.00})_N+0.5776(p^{1.00})_C$
C_6-C_8(σ)	$0.7058(sp^{2.15})_C+0.7084(sp^{1.61})_C$	$0.7069(sp^{2.17})_C+0.7073(sp^{1.62})_C$	$0.7071(sp^{2.14})_C+0.7071(sp^{1.58})_C$
LP(N_1)	$1.0000(sp^{2.61})$	$1.0000(sp^{2.56})$	$1.0000(sp^{2.25})$
LP(N_5)	$1.0000(sp^{2.71})$	$1.0000(sp^{2.67})$	$1.0000(sp^{2.35})$

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