

DFT studies on the favored and rare tautomers of neutral and redox cytosine

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Abstract The complete tautomeric mixture consisting of nine prototropic tautomers has been studied in the gas phase at the DFT(B3LYP)/6-311+G(d,p) level for neutral, oxidized, and reduced cytosine. Rotational isomerism of the exo –OH group and geometrical isomerism of the exo =NH group have also been considered. Tautomeric conversions possible for cytosine have been compared with those for its structural models, 4-amino- and 2-hydroxypyrimidine. Effects of intramolecular interactions between neighboring groups for cytosine are analogous to those observed for model compounds. Although they are not very strong, they are sufficient to influence tautomeric equilibria and relative stabilities of individual tautomers. One-electron oxidation and one-electron reduction change tautomeric preferences. Tautomers that are rare forms for neutral cytosine become favored ones for oxidized and reduced cytosine. Aromaticity is not the main factor that dictates the tautomeric preferences. Stability of functional groups seems to be more important than full electron delocalization.

Keywords Cytosine · Favored and rare tautomers · Redox forms · Electron delocalization · DFT calculations

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Introduction

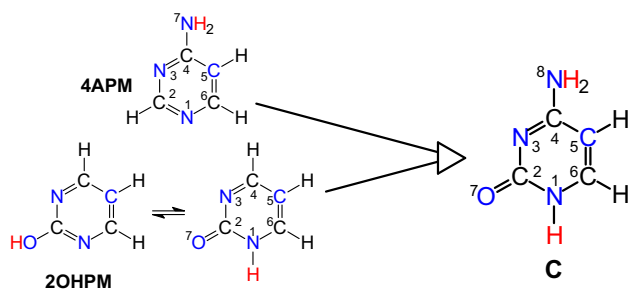
It is well recognized that prototropy in nucleobases influences the structure of nucleic acids and their replication, mutation, and degradation processes [1–13]. Labile hydrogens can move as protons between conjugated sites and change properties of nucleobases, particularly their ability to H-bonding. This phenomenon and the hypothesis of rare tautomers, suggested by Watson and Crick [2] in 1953 for DNA mutations, and later developed by Löwdin [3, 4] and advanced by Topal and Fresco [5], encouraged many chemists to theoretical and experimental studies on the structure of nucleobases in different environments. After six decades of research, although one can find in the literature hundred articles on tautomerism of nucleobases, this phenomenon attracts permanently the attention of scientists.

In normal DNA, cytosine (C) forms the H-bonded pair with guanine (G), and adenine (A) forms the other one with thymine (T) [1]. When the tautomeric equilibrium for one base is disturbed, the pairing may be mismatched, and mutations of single nucleotide (point mutation) may appear [2, 9–13]. When the point mutation in some genes is followed by changes in the sequence of amino acids, the changes may lead to serious diseases, e.g., cystic fibrosis, mucosal melanoma, colorectal cancer, lung cancer, and pancreatic cancer. Fortunately, the DNA mismatch repair system recognizes and repairs errors in DNA that appear during DNA replication, and only in exceptional cases this system fails to detect errors and a serious disease appears [14]. The reasons of failing are not yet well recognized, and the hypothesis of rare tautomers is continually verified by experimental and theoretical methods [9–13, 15–20].

One should mention here that experimental investigations of tautomeric conversions require exceptional efforts,

because prototropy is a very fast and reversible process [6, 7, 21]. Prototropy is also very sensitive to experimental conditions. Individual tautomers are very difficult to separate and to study. Very frequently, experimental techniques cannot give complete information on all possible tautomeric forms and on all possible tautomeric equilibria. The principal reason is as follows. An experiment gives the possibility to identify major tautomers, signals of which have significant intensities. In some cases, minor tautomers can also be detected. Rare tautomers are usually undetectable, probably because their amounts are too small and their signals cannot be distinguished from the background. Moreover, the experimental techniques, such as ultraviolet (UV), infrared (IR), Raman, microwave (MV), nuclear magnetic resonance (NMR), and mass spectrometry (MS), have their own limits of detection, and thus, the number of detected tautomers may be different for different methods. It may also be smaller than that of possible tautomeric forms. Quantum chemical methods have this advantage that they give the possibility to study all possible individual tautomers and all intramolecular and intermolecular interactions [6, 7, 21–23]. One can also model all possible tautomeric conversions and predict all possible microscopic parameters for isolated, microsolvated and macrosolvated systems. Effects of different factors that influence stabilities of individual tautomers and their associates can also be examined. Such kind of investigations helps to understand the properties of tautomeric systems and the mechanisms of important chemical and biochemical transformations.

For our quantum chemical studies on the favored and rare tautomers of nucleic bases, we chose cytosine (Scheme 1). The reasons are as follows. Its rare tautomer may cause mutations [2]. It may form the H-bonded pair with adenine. Consequently, cytosine may be replaced by thymine during replication. The GC → AT transition seems to be the most frequent DNA mutations [2, 10–13, 24, 25]. Hence, it is very important to study the favored and rare tautomers of cytosine in various environments.



Scheme 1 Canonical form of cytosine (C) and the structures of its convenient models, 4-amino- (4APM) and 2-hydroxypyrimidine (2OHPM). Labile protons marked in red and conjugated sites in blue (Color figure online)

Cytosine contains two labile protons and five conjugated tautomeric sites. The two protons can move according to 1,3, 1,5 and/or 1,7 proton shifts between the O7, N8, N1, N3, and/or C5 atoms. The proton transfers in tautomeric conversions are accompanied by migrations of one, two, and/or three double bonds, respectively, and no separation of the charge takes place [6, 21, 26]. Some proton transfers for cytosine are analogous to those for its structural models, 4-aminopyrimidine (4APM) and 2-pyrimidone—tautomeric form of 2-hydroxypyrimidine (2OHPM). Combinations of various types of tautomeric conversions, such as amide-iminol, amine-imine, and enamine-imine, lead to the complete tautomeric mixture for cytosine consisting of nine tautomers (Table 1). It should be noted here that the number of tautomers is a property of tautomeric systems. It depends on the number of labile protons and on the number of conjugated tautomeric sites [26]. However, relative stabilities of individual tautomers depend on various internal and external factors, which strongly influence tautomeric preferences [6, 7, 21]. For cytosine, the C2 (N1N8) isomer was called the ‘canonical tautomer’ [1]. This tautomeric form is the most frequently present in nucleic acids [1, 8, 24, 25]. The C8 (N1N3) isomer, probably responsible for the point mutations of DNA, was called the rare tautomer [2].

Numerous interesting experimental and theoretical reports can be found in the literature on prototropic conversions for neutral cytosine in the gas phase. There are also some documents on microsolvated and macrosolvated cytosine. In the solid state, the canonical form C2 has been found for cytosine [27]. In the gas phase, depending on the experimental method applied (matrix isolation IR, MW, REMPI, IR laser in helium nanodroplets, MS, core-level X-ray photoemission, and near-edge X-ray absorption), two (C1 and C2) or three (C1, C2, and C8) tautomers have been detected for cytosine [28–35]. Recently, five isomers of gaseous cytosine (two rotational isomers **a** and **b** of C1, one isomer of C2, and two geometrical isomers **a** and **b** of

Table 1 Positions of labile protons for tautomers of cytosine (C), 4-amino- (4APM), and 2-hydroxypyrimidine (2OHPM)

Tautomer	Position of H	Tautomer	Position of H
C1	O7N8	4APM1	N7
C2	N1N8	4APM2	N1
C3	N3N8	4APM3	N3
C4	C5N8	4APM4	C5
C5	O7N1	2OHPM1	O7
C6	O7N3	2OHPM2	N1
C7	O7C5	2OHPM3	N3
C8	N1N3	2OHPM4	C5
C9	N3C5		

C8) have been characterized by Alonso et al. [36], who applied a laser-ablation molecular-beam Fourier-transform microwave (LA-MB-FT-MW) spectroscopy. In aqueous solution, two tautomeric forms of cytosine (**C2** and **C3**) seem to dominate [37].

Unfortunately, the complete tautomeric mixture of cytosine has not been investigated by quantum chemical methods [38–50]. Maximum six tautomers have been studied for neutral cytosine (**C1–C3**, **C5**, **C6**, and **C8**), and the amide-iminol and amine-imine conversions analyzed [40–42]. In some papers, even rotational isomerism of the exo –OH group and geometrical isomerism of the exo =NH group have not been considered. The enamine-imine conversions and the CH tautomers (**C4**, **C7**, and **C9**) have usually been neglected. Favored and rare tautomers have solely been studied for adiabatically bound valence anions of cytosine, and the importance of the **C9** tautomer discussed [51]. The CH tautomer has also been found to be favored for negatively ionized **4APM** [52, 53].

The hypothesis of rare tautomers and also the variability of tautomeric preferences for the adiabatically bound valence anions of cytosine and for the ionized forms of **4APM** encouraged us to undertake the studies for the complete tautomeric mixture of cytosine at various oxidation states, the neutral (**C**), oxidized ($\text{C} - e \rightarrow \text{C}^+$), and reduced ($\text{C} + e \rightarrow \text{C}^-$) states in the gas phase. In the literature, one-electron oxidation and one-electron reduction are also called positive and negative ionization in mass spectrometry, or electron detachment and electron attachment in photoelectron spectroscopy. In this work, tautomeric conversions and various internal effects such as substituent effects and intramolecular interactions between neighboring groups have been discussed for the neutral and redox forms of cytosine. Geometric and energetic consequences of prototropy have also been examined and compared to those observed earlier for **4APM** and **2OHPM**. For investigations, the DFT method [54] has been employed with the hybrid functional of Becke [55] and gradient correction of Lee et al. (B3LYP) [56] and the 6-311+G(d,p) basis set [57] as previously described for adenine and its models [52, 53, 58, 59]. The B3LYP functional has been recommended for charged radicals [60] and applied for the anionic states of nucleic bases [51]. It has also been used for geometry optimization in the G3B3 theory [61].

Methods

Geometries of the neutral and charged forms of all possible cytosine isomers in their ground states (Fig. 1) were fully optimized in the gas phase without symmetry constraints

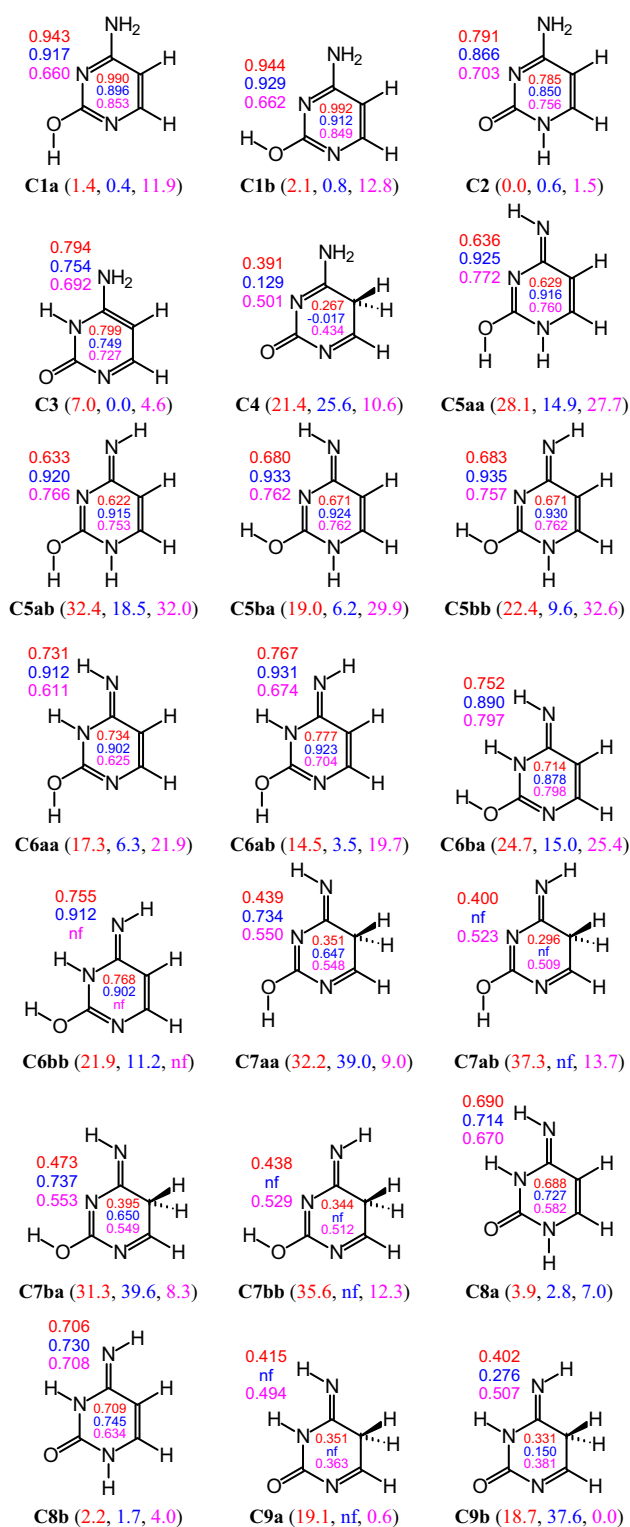


Fig. 1 All possible isomers of cytosine. The relative Gibbs energies (in kcal mol⁻¹ at 298.15 K) and the HOMED indices estimated for neutral (red), oxidized (blue), and reduced (rose) cytosine (nf—structure not found at the DFT level). ΔG given in parentheses. HOMED6 and HOMED8 placed inside and outside of the ring, respectively (Color figure online)

employing the DFT(B3LYP) method [54–56] and the 6-311+G(d,p) basis set [57]. The restricted B3LYP functional was used for neutral isomers, and the unrestricted B3LYP functional was applied for charged radicals. For all structures, frequencies were calculated, first to prove that the structures are minima and next to estimate the corresponding zero-point energies. Thermodynamic parameters such as the energy (E), enthalpy ($H = E + pV$), entropy (S), and Gibbs energy ($G = H - TS$ for $T = 298.15$ K) were calculated using the same level of theory. For tautomeric conversions, the relative thermodynamic parameters (ΔE , ΔH , $T\Delta S$, and ΔG), the tautomeric equilibrium constants (as $pK = \Delta G/2.303RT$), and the percentage contents of individual forms $\{x = K/(1 + K)\}$ were estimated. The ΔG values include changes in the electronic energy, zero-point energy (ZPE), and thermal corrections to the energy and entropy (vibrational, rotational, and translational). The theoretical adiabatic ionization potential $\{IP = E(\text{optimized radical cation}) - E(\text{optimized neutral})\}$ and the theoretical adiabatic electron affinity $\{EA = E(\text{optimized neutral}) - E(\text{optimized radical anion})\}$ were calculated for the tautomeric mixture, taking the total energies of the neutral and charged forms at their respective equilibrium nuclear configurations, and their percentage contents in the tautomeric mixture were also considered. All calculations were performed according to the procedures included in the Gaussian-03 series of programs [62].

To properly determine the distribution of π - and n -electrons for all tautomers/rotamers of cytosine and to well describe the variations of electron delocalization, the geometry-based HOMED (harmonic oscillator model of electron delocalization) procedure [63, 64] was applied to the geometries optimized at the DFT(B3LYP)/6-311+G(d,p) level. The abbreviation HOMED was proposed in 2006 for the modified index [63], but it may also be abbreviated as moHOMA (modified original HOMA) or simply HOMA. The HOMA (harmonic oscillator model of aromaticity) index [65, 66], reformulated by Krygowski [67], and the HOMHED (harmonic oscillator model of heterocyclic electron delocalization) index, proposed by Frizzo and Martins in 2012 [68] and based on hypotheses of the HOMED index [64], were not applied here for cytosine. The reasons were discussed previously [53, 69]. Since the same resonance phenomenon takes place for neutral molecules, ions, and radicals [26], the HOMED indices were estimated for neutral and redox forms of cytosine using the following equation: $\text{HOMED} = 1 - \{\alpha(\text{CC}) \times \Sigma[R_o(\text{CC}) - R_i(\text{CC})]^2 + \alpha(\text{CX}) \times \Sigma[R_o(\text{CX}) - R_i(\text{CX})]^2\}/n$. In this equation, α are the normalization constants, R_o are the optimum bond lengths (assumed to be realized for fully delocalized systems), R_i are the running bond lengths in the tautomeric system, and n is the number of bonds taken into

account. In the case of cytosine isomers, six bonds for the pyrimidine ring and eight bonds for the whole molecule, including the exo -OH/=O and $\text{-NH}_2\text{/=NH}$ groups, were taken into account. The normalization α constants for the even number of bonds were calculated from the following equation: $\alpha = 2 \times [(R_o - R_s)^2 + (R_o - R_d)^2]^{-1}$, where R_s and R_d are the reference single and double bonds, respectively. The following R_s , R_d , and R_o values (in Å), calculated at the B3LYP/6-311+G(d,p) level for the reference molecules, were taken here [64]: 1.530 (ethane), 1.329 (ethene), and 1.394 (benzene) for the CC bonds; 1.466 (methylamine), 1.267 (methylimine), and 1.334 (1,3,5-triazine) for CN bonds; and 1.424 (methanol), 1.202 (formaldehyde), and 1.281 (protonated carbonic acid) for the CO bonds. On the basis of these R values, the normalization α constants equal to 88.09, 91.60, and 75.0 were used for the CC, CN, and CO bonds, respectively [64].

Results and discussion

For the hydroxy forms of cytosine (**C1** and **C5–C7**), two rotational isomers can be considered, one with the hydroxy H atom synperiplanar to the endo N1 atom (**a**) and the other one with this atom synperiplanar to the endo N3 atom (**b**). Due to geometrical isomerism of the exo =NH group, two isomers are also possible for the imino forms of cytosine (**C5–C9**), one with the imino H atom synperiplanar to the endo N3 atom (**a**) and the other one with this atom synperiplanar to the endo C5 atom (**b**). Taking into account all type of isomerism possible for cytosine (tautomerism, rotational, and geometrical isomerism), the structures of twenty-one neutral isomers were optimized at the DFT(B3LYP)/6-311+G** level (Fig. 1). The same number of isomers was considered for the charged radicals, and their structures optimized at the DFT level. For the thermodynamically stable structures, the relative Gibbs energies (ΔG) were calculated, and the HOMED indices estimated for the ring (six bonds—HOMED6) and for the whole tautomeric system, including the $\text{exo -NH}_2\text{/=NH}$ and -OH/=O groups (eight bonds—HOMED8). All calculated ΔG and HOMED values are given in Fig. 1.

The minima with real frequencies were found for all possible twenty-one isomers of neutral cytosine. For the amino tautomers, the exo NH_2 group is not in the ring plane [43]. It has a pyramidal conformation. For the imino tautomers, transfer of labile proton(s) to the endo N atom(s) in **C2**, **C3**, **C5**, **C6**, and **C8** does not destroy the planarity of the ring. Solely the CH amino (**C4**) and imino tautomers (**C7** and **C9**) lose the planarity of the ring due to the presence of the C5-sp^3 atom. For positively charged forms (radical cations), three isomers **C7ab**⁺, **C7bb**⁺, and **C9a**⁺ were not found. The exo =NH group in the

thermodynamically stable **C7aa**⁺, **C7ba**⁺, and **C9b**⁺ isomers is out of the ring plane by the dihedral angle equal to 34, 37, and 62°, respectively. The exo =NH group is also twisted for **C5ab**⁺ and **C5bb**⁺ by 51 and 24°, respectively. The exo NH₂ group is planar for all positively charged amino forms. For radical anions, this group takes the pyramidal conformation. Solely one isomer **C6bb**[−] was not found for negatively charged cytosine. The exo =NH group in all stable negatively charged isomers is almost in the ring plane. The dihedral angle is not larger than 5°.

Positions of labile protons and oxidation states strongly influence the CC, CN, and CO bond lengths for the cytosine isomers. The effects are not parallel for neutral and redox forms. This suggests that there is no common mechanism for one-electron oxidation and separately for one-electron reduction for all cytosine isomers. An analysis of the total atomic spin densities confirms the differences. Various exo and endo heteroatoms and/or π -bonds lose one electron in cytosine tautomers. One electron is also gained by different sites. The differences have already been observed for the hydroxy and amino derivatives of azines [53]. Consequently, the HOMED indices estimated for the six-membered ring (HOMED6) as well as for the whole molecule including the exo groups (HOMED8) vary in a different way for the neutral and ionized amino-hydroxy, amino-oxo, imino-hydroxy, and imino-oxo isomers, and there is no linear relation between the HOMED indices of the neutral and ionized forms (Fig. 2).

The HOMED8 values for neutral isomers of cytosine vary from 0.39 to 0.94. The **C1a** and **C1b** isomers have the largest HOMED values (close to unity), and they are aromatic. The **C4** isomer has the lowest HOMED value, and it is non-aromatic due to the presence of the C5-sp³ atom in the ring. The HOMED indices of the other CH isomers with the C5-sp³ atom (**C7aa**, **C7ab**, **C7ba**, **C7bb**, **C9a**, and **C9b**) are only slightly larger (HOMED8 0.40–0.47) than

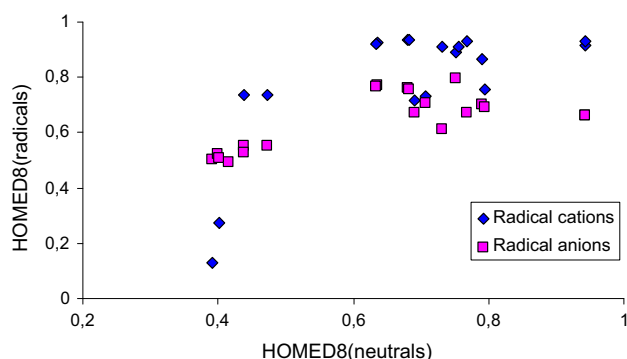


Fig. 2 Plots between the HOMED8 indices of neutral and ionized forms of cytosine

that of **C4**. The canonical tautomer **C2** is less delocalized (HOMED8 0.79) than the **C1a** and **C1b** isomers, but it is still aromatic. Similar π -electron delocalization has been previously reported for the canonical tautomer using various measures of aromaticity [70–73]. The N1 atom in **C2** taking the labile proton retains its planarity. The n - π conjugation in the six-membered ring of **C2** is similar to that for the five-membered ring in pyrrole, imidazole, etc. [64].

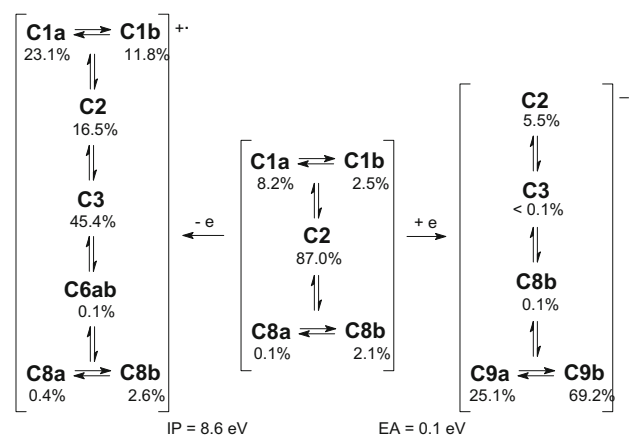
One-electron oxidation dramatically changes bond lengths and electron delocalization for all cytosine isomers. Consequently, the geometry-based indices (HOMED6 and HOMED8) also change. They decrease for the CH–NH isomers (**C4**⁺ and **C9b**⁺) even to zero, while they increase for the CH–OH ones (**C7aa**⁺ and **C7ba**⁺) to 0.73–0.74. They also increase for the imine NH–OH isomers (**C5aa**⁺, **C5ab**⁺, **C5ba**⁺, **C5bb**⁺, **C6aa**⁺, **C6ab**⁺, **C6ba**⁺, and **C6bb**⁺, HOMED8 \geq 0.89) and for the canonical NH–NH form **C2**⁺ (HOMED8 0.87). However, they slightly decrease for the NH–OH isomers **C1a**⁺ and **C1b**⁺ (HOMED8 0.92 and 0.93, respectively). One-electron reduction causes also different effects. For negatively ionized cytosine isomers, the HOMED8 values vary solely from 0.49 to 0.80. For the canonical form **C2**[−], the HOMED8 value slightly decreases (to 0.70) when compared to the neutral form. In higher degree, the HOMED8 values decrease for the NH–OH isomers **C1a**[−] and **C1b**[−] (0.66). Interestingly, the HOMED8 values increase for the CH tautomers (0.49–0.55). Similar tendencies for the HOMED indices have been observed for model compounds [52, 53].

The relative entropy term ($T\Delta S$) values are not very large (± 1 kcal mol^{−1}) for neutral and charged isomers of cytosine, similar to other tautomeric systems [21, 52, 53, 58, 59]. This indicates that all tautomeric conversions for cytosine (Scheme S1, Supplementary Material) are isoentropic processes in the gas phase. There are no large structural changes during tautomerization. Some exceptions are those for the amine and imine CH tautomers. They are a consequence of the ring planarity loss. The relative thermal corrections for all tautomeric conversions are also close to zero, and $\Delta E \cong \Delta H \cong \Delta G$. These observations suggest that the relative thermodynamic parameters depend very little on temperature. When proceeding from 0 to 298.15 K, orders of the relative energies for major isomers do not change for neutral and charged cytosine. Generally, proton transfer reactions are isoentropic for almost all organic acids and bases [74, 75]. The relative entropies and the relative thermal corrections are close to zero.

First, perusal of the relative Gibbs energies calculated for neutral isomers of cytosine gives the possibility to distinguish the following characteristic properties for

major, minor, and rare tautomers. All CH isomers (**C4**, **C7**, and **C9**) can be considered as very rare forms for neutral cytosine ($\Delta G \gg 10 \text{ kcal mol}^{-1}$). Indeed, the proton transfers from the N1, N3, O7, or N8 atom to the C5 atom are very unfavorable processes, and all CH isomers can be neglected for neutral cytosine. Analogous conclusions have been derived for model compounds, **4APM** [52] and **2OHPM** [53], using the same level of theory. The CH tautomers (**4APM4** and **2OHPM4**) can be neglected for neutral **4APM** and **2OHPM** ($\Delta G \gg 10 \text{ kcal mol}^{-1}$). The imino OH–NH forms ($\Delta G \gg 10 \text{ kcal mol}^{-1}$) containing one labile proton at the O7 and the other one at the N1 (**C5**) or N3 atom (**C6**) can also be considered as very rare forms, and thus, they can also be neglected for neutral cytosine. They are analogous to the imino NH forms of **4APM** (**4APM2** and **4APM3**) [52]. One exception is the imino NH–NH form with labile protons at the N1 and N3 atoms (**C8**), typical for the pyrimidine bases (uracil, thymine, and cytosine). Its two rotamers **a** and **b** ($\Delta G 2\text{--}4 \text{ kcal mol}^{-1}$) can be considered as minor forms rather than rare isomers for neutral cytosine (Scheme 2). The amino **C2** and **C3** (NH–NH) tautomers with one labile proton at the N1 and N3 atoms, respectively, are analogous to **2OHPM2** and **2OHPM3**. For model compound, **2OHPM2** and **2OHPM3** are identical. They have identical thermodynamic stabilities and the same energies [53]. However, the presence of the exo NH_2 group at the 4-position in cytosine strongly differentiates relative stabilities of **C2** and **C3**. The **C3** tautomer can be considered as rare amino form ($\Delta G 7 \text{ kcal mol}^{-1}$), whereas the **C2** tautomer is the favored ‘canonical’ form for neutral cytosine at the B3LYP level ($\Delta G 0 \text{ kcal mol}^{-1}$). The two rotamers **a** and **b** of the amino **C1** (NH–OH) tautomer with labile proton at the O7 atom are major amino forms ($\Delta G \leq 2 \text{ kcal mol}^{-1}$).

The presence of all five major and minor isomers (**C1a**, **C1b**, **C2**, **C8a**, and **C8b**) in the tautomeric mixture of



Scheme 2 Favored redox processes for cytosine estimated at the DFT level

neutral cytosine has been experimentally proven [36]. Our DFT calculations are in good agreement with those reported in the literature [39–42, 47, 49]. However, it should be mentioned here that the percentage contents of major and minor forms depend on the level of calculations [38–50] as well as on the experimental method applied [30–36]. For example, the DFT methods predict the canonical tautomer **C2** as the favored form for neutral cytosine, whereas the HF, MPn and CC methods indicate the **C1a** isomer. Table S1 (Supplementary Material) lists some selected DFT, HF, MPn, and CC data for the five major and minor tautomers of cytosine. Differences in the predicted ΔE values are not larger than 2 kcal mol^{-1} . On the other hand, Brown et al. [31], using the MW spectroscopy to jet-cooled cytosine with a nozzle temperature of 568 K, estimated the populations of **C2** and **C1a** to be in the approximate ratio 1:1 in the gas phase. Alonso et al. [36], who applied the LA-MB-FT-MW spectroscopy, found recently a slight predominance of **C1a** and concluded that ab initio calculations did not reproduce well the experimental observations. When compared to thymine and uracil, cytosine is a very complex molecular system. The common conclusion on its major tautomers (**C2** and **C1a**) in the gas phase has not yet been formulated. The microhydration by one, two, or three water molecules [42, 45, 46] and the macrohydration by using the continuum models [40, 41, 45, 48, 49] favor the ‘canonical’ tautomer **C2**.

One-electron oxidation changes the relative energies of all individual isomers (Fig. 1), and consequently, it changes the composition of the tautomeric mixture (Scheme 2). The tautomeric mixture of oxidized cytosine consists of four major forms (**C1a**⁺, **C1b**⁺, **C2**⁺, and **C3**⁺, $\Delta G < 1 \text{ kcal mol}^{-1}$), three minor forms (**C6ab**⁺, **C8a**⁺, and **C8b**⁺, $\Delta G 2\text{--}4 \text{ kcal mol}^{-1}$), and three rare forms (**C5ba**⁺, **C5bb**⁺, and **C6aa**⁺, $\Delta G 6\text{--}10 \text{ kcal mol}^{-1}$). The other isomers have ΔG larger than 10 kcal mol^{-1} . They may be neglected in the tautomeric mixture of positively ionized cytosine. Most dramatical changes in the tautomeric mixture of cytosine are caused by one-electron reduction. The CH isomers **C9a**[−] and **C9b**[−], very rare forms for neutral and oxidized cytosine, become the favored structures for reduced cytosine. The Gibbs energy of the reduced canonical form **C2**[−] is larger by ca. 1 kcal mol^{-1} than that of **C9b**[−], and those of **C1a**[−] and **C1b**[−] are larger by more than 10 kcal mol^{-1} than that of **C9b**[−]. The tautomeric mixture of reduced cytosine consists of three major forms (**C2**[−], **C9a**[−], and **C9b**[−], $\Delta G < 2 \text{ kcal mol}^{-1}$), two minor forms (**C3**[−] and **C8b**[−], $\Delta G 4\text{--}5 \text{ kcal mol}^{-1}$), and three rare forms (**C7aa**[−], **C7ba**[−], and **C8a**[−], $\Delta G 7\text{--}9 \text{ kcal mol}^{-1}$). The other isomers have ΔG values larger than 10 kcal mol^{-1} . They may be neglected for negatively ionized cytosine.

The variations of the composition of the tautomeric mixture when proceeding from neutral to redox cytosine (Scheme 2) seem to originate from a combination of the analogous variations observed for model compounds, **4APM** (Scheme S2) and **2OHPM** (Scheme S3) estimated at the same level of theory. The aromatic amine tautomer predominates in the gas phase for neutral and oxidized **4APM**, whereas the non-aromatic CH imino isomer is favored for reduced **4APM** [52]. For 2-hydroxypyrimidine, the oxo tautomers dominate in the tautomeric mixture for neutral, oxidized, and reduced forms [53]. Moreover, direct comparison of the DFT calculated thermodynamic parameters for the oxidized and neutral isomers of 4-aminopyrimidine, 2-hydroxypyrimidine, and cytosine indicates that one-electron oxidation is very endothermic process and requires ca. 200 kcal mol⁻¹. One-electron reduction is more profitable process than one-electron oxidation and requires considerably lower energy. Both model compounds and cytosine may take spontaneously one electron from a reducing agent.

There are no experimental data in the literature for the ionization potential (IP) and for the electron affinity (EA) of model compounds for comparison [74, 75]. However, it should be mentioned here that the literature IPs for 4-aminopyridine (8.8 eV [76]), 2-aminopyridine (8.5 eV [76]), and unsubstituted pyrimidine (9.3 eV [75]) are of the same order of magnitude as the DFT estimated adiabatic IPs for 4-aminopyrimidine (8.8 eV) and 2-hydroxypyrimidine (9.1 eV). On the other hand, the literature EA for unsubstituted pyrimidine is not very large: EA (electron transmission spectroscopy) -0.25 eV [77] and EA (G2MP2B3) -0.17 eV [74]. Taking the composition of the tautomeric mixture for neutral and ionized cytosine into account, one can estimate the IP (8.6 eV) and EA (0.1 eV) values in the gas phase for the favored ionization processes at the DFT level (Scheme 2). They are close to those for model compounds (Schemes S2 and S3). The experimental IP value for cytosine (8.45 eV [78]) is not very different from the DFT one. Schiedt et al. [79] found two EA values for the cytosine dipole bound state, one for the amino-oxo tautomer (0.23 eV) and the other one for the amino-hydroxy tautomer (0.08 eV). For the valence state of the rare CH tautomer, Li et al. [51] estimated larger EA value (2.34 eV).

Comparison of the relative Gibbs energies estimated at the DFT level for cytosine isomers with those estimated at the same level of theory for analogous isomers of **2OHPM** and **4APM** gives the possibility to estimate the total energetic effects (δG) of the exo NH₂ and OH groups. These effects, $\delta G(\text{NH}_2)$ and $\delta G(\text{OH})$, include the classical inductive and resonance electronic substituent effects of the exo NH₂ and OH groups and also the additional internal effects being a consequence of specific favorable and

unfavorable interactions of the exo with endo neighboring groups. The $\delta G(\text{NH}_2)$ and $\delta G(\text{OH})$ values can be estimated for neutral and redox forms of cytosine for selected tautomeric conversions, which are analogous to those for model compounds.

The δG values of the exo NH₂ group were found (Table 2), when cytosine was considered as the amino derivative of **2OHPM**. Proceeding from **2OHPM** to cytosine, the total energetic effects of the exo NH₂ group (δG) on the iminol-amide and iminol-iminon conversions were estimated as differences between the relative Gibbs energies of analogous tautomeric conversions for cytosine and **2OHPM** { $\delta G(\text{NH}_2) = \Delta G(\text{cytosine}) - \Delta G(\text{2OHPM})$ }. For neutral forms, larger effects (4–5 kcal mol⁻¹) occur for the **C1** → **C3** and **C1** → **C4** conversions than for the **C1** → **C2** one ($\delta G \leq 3$ kcal mol⁻¹). The reasons are as follows. For **C4**, the NH₂ group can interact unfavorably with the C5H₂ group. For **C3**, the NH₂ group can interact unfavorably with the N3H and C5H groups, whereas for **C2** an interaction between the NH₂ group and the N3 atom can be favorable. For **C1a** and **C1b**, interactions between the OH group and the N1 or N3 atom are favorable. A similar tendency occurs for the reduced forms, for which one excess electron is taken by the ring for **2OHPM** and cytosine. The $\delta G(\text{NH}_2)$ values are larger for the **C1** → **C3** and **C1** → **C4** conversions ($\delta G > 2$ kcal mol⁻¹) than for the **C1** → **C2** one ($\delta G \leq 0.1$ kcal mol⁻¹). However, one-electron oxidation strongly increases the total effects of the exo NH₂ group ($\delta G \geq 7$ kcal mol⁻¹). This group is favored for one electron loss in cytosine.

When cytosine was considered as the hydroxy derivative of **4APM**, the total energetic effects of the exo OH group (δG) were calculated for the amine-imine and enamine-imine conversions. Table 3 summarizes the $\delta G(\text{OH})$ values for the conformations **a** and **b** of the OH group. They were estimated as differences between the relative Gibbs energies of analogous tautomeric conversions for cytosine and **4APM** { $\delta G(\text{OH}) = \Delta G(\text{cytosine}) - \Delta G(\text{4APM})$ }. The largest effects ($\delta G > 5$ kcal mol⁻¹) occur for tautomeric

Table 2 Total energetic effects of the exo NH₂ group (δG in kcal mol⁻¹) on selected tautomeric conversions for cytosine analogous to those for **2OHPM**

Conversion	$\delta G(\text{NH}_2)$		
	Neutral	Radical cation	Radical anion
C1a → C2	-2.2	8.1	-0.1
C1b → C2	-3.0	7.7	-0.1
C1a → C3	4.7	7.5	3.0
C1b → C3	4.0	7.1	2.1
C1a → C4	-4.7	7.3	-4.3
C1b → C4	-5.4	6.9	-5.2

conversions of neutral isomers, for which the OH group interacts unfavorably with the N1H (**C5aa** and **C5ab**) or N3H group (**C6ba** and **C6bb**). When intramolecular interactions between neighboring groups are favorable, the total effects of the exo OH group are considerably lower, and the δG values do not exceed 4 kcal mol^{-1} . A similar tendency and slightly larger δG values are found for oxidized cytosine. For reduced cytosine, variations of the δG values are not parallel to those for neutral and oxidized cytosine. However, the δG values are not larger than 8 kcal mol^{-1} .

A relation between prototropy and electron delocalization has been signaled more than 50 years ago by Pauling [26]. This relation has been recently discussed for some simple tautomeric systems [21]. Good linear relationships have been found for the neutral aromatic NH and non-aromatic CH tautomers of imidazole and purine, which have no substituent, and which possess solely the endo functional groups [52, 53, 80–83]. Prototropy is also well related to electron delocalization for the neutral NH and CH tautomers of aminoazines and for the NH–NH and NH–CH tautomers of adenine [52, 53, 81]. Aminoazines and adenine contain not only the endo aza group(s) but also the exo $-\text{NH}_2=\text{NH}$ group. For particular isomers, the exo group can interact intramolecularly with the endo functional groups. Since these internal effects are less important factors than aromaticity, they only slightly perturb the relation between prototropy and electron delocalization for neutral aminoazines and adenine.

Quite a different situation takes place for hydroxyazines and uracyl, which contain the exo $-\text{OH}=\text{O}$ functional group(s) [53]. The relation between prototropy and electron delocalization seems to be more complex. The energetic

Table 3 Total energetic effects of the exo OH group (δG in kcal mol^{-1}) on selected tautomeric conversions for cytosine analogous to those for **4APM**

Conversion	$\delta G(\text{OH})$		
	Neutral	Radical cation	Radical anion
C1a → C5aa	8.5	12.7	1.5
C1a → C5ab	8.8	11.9	2.1
C1b → C5ba	−1.3	3.6	2.8
C1b → C5bb	−1.9	2.6	1.8
C1a → C6aa	−0.3	1.1	5.4
C1a → C6ab	−0.2	1.2	5.4
C1b → C6ba	6.4	9.4	8.0
C1b → C6bb	5.9	8.9	–
C1a → C7aa	−1.4	−3.8	2.0
C1a → C7ab	−0.9	–	2.1
C1b → C7ba	−3.0	−3.6	0.4
C1b → C7bb	−3.3	–	−0.2

parameters (ΔG), which measure prototropy, are not parallel to the geometric ones (HOMED), which measure electron delocalization. We observe similar tendency for cytosine, which possesses one exo $-\text{OH}=\text{O}$ group (Fig. 3). Stability of functionalities seems to be more important factor than aromaticity and seems to dictate the tautomeric preferences for neutral cytosine. Intramolecular interactions between the exo and endo groups influence also the conformational and configurational preferences for the hydroxy and imino tautomers. For ionized cytosine, the HOMED8/ ΔG plots are more complex. There is no common HOMED8/ ΔG relationship for radical cations nor for radical anions.

However, when cytosine is considered as the 4-amino derivative of 2-hydroxypyrimidine (**2OHPM**), variations of the HOMED indices estimated for the whole tautomeric system of neutral cytosine isomers and those of their relative Gibbs energies (ΔG) are almost analogous to those for neutral **2OHPM** (Fig. 4). The iminol-amide and iminol–iminon conversions for cytosine cause geometric changes similar to those for **2OHPM** [53]. Some differences take place for energetic parameters, particularly for the iminol-amide conversions, i.e., between the **C2** and **C3** isomers of cytosine and **2OHPM2/2OHPM3**.

Interestingly, when cytosine is considered as the 2-hydroxy derivative of 4-aminopyrimidine, a plot between the HOMED indices estimated for the whole tautomeric system (eight bonds) and the relative Gibbs energies calculated for the neutral **C1a**, **C1b**, **C5aa**, **C5ab**, **C5ba**, **C5bb**, **C6aa**, **C6ab**, **C6ba**, **C6bb**, **C7aa**, **C7ab**, **C7ba**, and **C7bb** isomers is similar to that found previously [52] for the corresponding neutral **4AMP** isomers (Fig. 5). The amine-imine and enamine-imine conversions for neutral cytosine cause almost parallel changes of the geometric (HOMED) and energetic (ΔG) parameters. Some stronger deviations of points for cytosine than those for **4APM** may result from intramolecular interactions (favorable or unfavorable) between the exo OH and endo N1/N1H or N3/N3H group in two different conformations (**a** and **b**) of the OH group. These interactions influence stronger the relative Gibbs

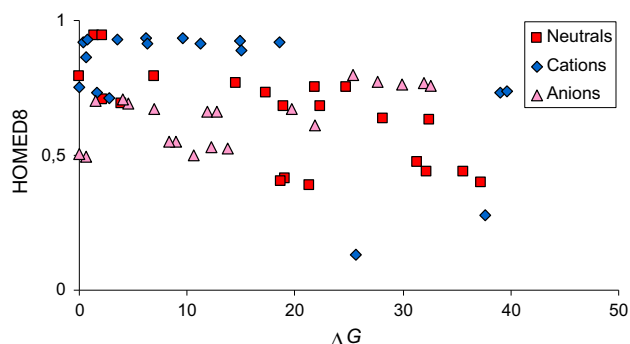


Fig. 3 HOMED8/ ΔG plots for neutral (red points), oxidized (blue points), and reduced (rose points) cytosine (Color figure online)

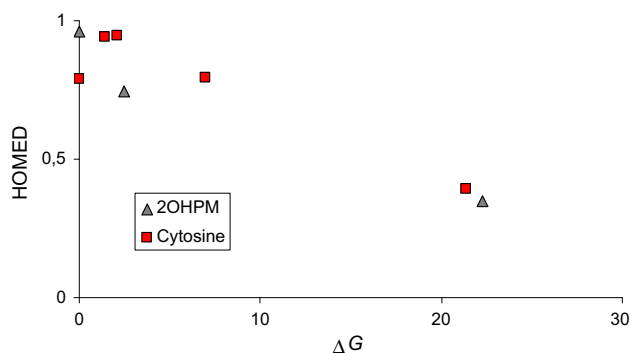


Fig. 4 Plots between the HOMED indices and the ΔG values (in kcal mol⁻¹) estimated for neutral 2-hydroxypyrimidine (**2OHPM**) isomers (gray points) and for analogous neutral isomers in cytosine (red points) considered as the 4-amino derivative of **2OHPM** (Color figure online)

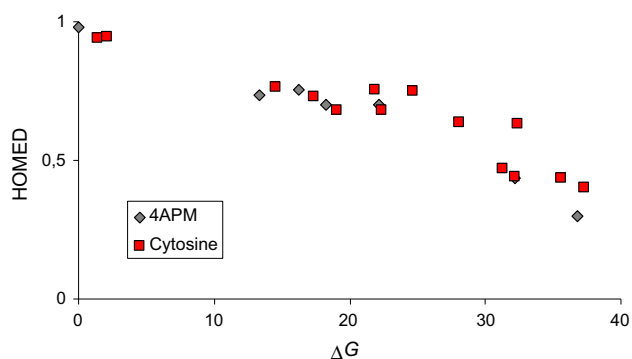


Fig. 5 Plots between the HOMED indices and the ΔG values (in kcal mol⁻¹) estimated for neutral 4-aminopyrimidine (**4APM**) isomers (gray points) and for analogous neutral cytosine isomers (red points), considered as the 2-hydroxy derivative of **4APM** (Color figure online)

energies than the HOMED indices. For example, differences between the ΔG values for the rotational isomers **C5aa** and **C5ba**, **C5ab** and **C5bb**, **C6aa** and **C6ba**, and **C6ab** and **C6bb** are close to 7–10 kcal mol⁻¹, whereas those between the HOMED8 values are considerably lower and close to 0.01–0.05. Rotation of the exo =NH group and consequently intramolecular interactions (favorable or unfavorable) between this group and the endo N3/N3H or C5H_{*i*} (*i* = 1 or 2) group cause lower energetic effects (≤ 5 kcal mol⁻¹) than rotation of the OH group. Geometric effects (0.00–0.04 HOMED units) are not very large for the exo =NH group. They are similar to those for the exo OH group.

Conclusions

The DFT calculations performed for the complete tautomeric mixture of neutral and redox cytosine consisting of all possible twenty-one isomers show clearly that

prototropy affects weaker the geometric parameters than the energetic ones. When proceeding from the neutral to oxidized (positively ionized) or reduced (negatively ionized) form of cytosine, the composition of the tautomeric mixture changes significantly (Scheme 2). The rare tautomers for neutral cytosine become the favored ones for oxidized and reduced cytosine. These variations seem to originate from those observed earlier for models, 4-amino- and 2-hydroxypyrimidine [52, 53].

There is no good relation between the geometric (HOMED) and energetic (ΔG) parameters estimated for individual isomers of cytosine (Fig. 3). This indicates that aromaticity is not the main factor that influences the tautomeric preferences. High stability of the amide form for cytosine is similar to that for 2-pyrimidone [53]. It destroys the relation between the geometric and energetic parameters for the amide-iminol conversions (Fig. 4). A good relation exists solely for the amine-imine and enamine-imine conversions (Fig. 5). It is similar to that found previously for 4-aminopyrimidine [52]. Deviations of some points result from intramolecular interactions between neighboring groups.

For cytosine included in the DNA acid, solely seven isomers may be considered (**C2**, **C5aa**, **C5ab**, **C5ba**, **C5bb**, **C8a**, and **C8b**). The DFT calculations clearly show that the **C8** tautomer, proposed by Watson and Crick as the rare form [2], is present in the tautomeric mixture of cytosine at each oxidation state. The **C8** tautomer may be responsible for the point mutation of DNA. Four isomers of **C5** may be neglected in the tautomeric mixture. Further studies for the 1-alkyl derivative of cytosine may give some quantitative estimation of the contribution of **C2** and **C8** in the DNA nucleotide when oxidizing or reducing agents appear in living organisms.

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References

1. Watson JD, Crick FHC (1953) Nature 171:737–738
2. Watson JD, Crick FHC (1953) Nature 171:964–967
3. Löwdin P-O (1963) Rev Mod Phys 35:724–732
4. Löwdin P-O (1965) Adv Quantum Chem 2:213–360
5. Topal MD, Fresco JR (1976) Nature 263:285–289
6. Elguero J, Marzin C, Katritzky AR, Linda P (1976) The tautomerism of heterocycles. Academic Press, New York
7. Pozharskii AF, Soldatenkov AT, Katritzky AR (1997) Heterocycles in life and society. Wiley, New York

8. Saenger W (1994) Principles of nucleic acids structure. Springer, New York
9. Florián J, Goodman M, Warshel A (2002) *J Phys Chem B* 106:5739–5753
10. Grebneva HA (2003) *J Mol Struct* 645:133–143
11. Grebneva HA (2006) *Environ Mol Mutagen* 47:733–745
12. Danilov VI, Stewart JJP, Leš A, Alderfer JL (2000) *Chem Phys Lett* 328:75–82
13. Danilov VI, Anisimov VM, Kurita N, Hovorun D (2005) *Chem Phys Lett* 412:285–293
14. Peña-Díaz J, Jiricny J (2012) *Trends Biochem Sci* 37:206–214
15. Leszczynski J (ed) (1999) Computational molecular biology: theoretical and computational chemistry, vol 8. Elsevier, New York
16. Hobza P, Šponer J (1999) *Chem Rev* 99:3247–3276
17. Lippert B, Gupta D (2009) *Dalton Trans* 4619–4634
18. Serrano-Andrés L, Merchán M (2009) *J Photochem Photobiol C Photochem Rev* 10:21–32
19. Brovarets' OO, Kolomiets IM, Hovorun DM (2012) Quantum-chemistry models for innovations. In: Toda T (ed) Tech. <http://cdn.intechopen.com/pdfs-wm/33305.pdf>
20. Singh V, Fedeles BI, Essigmann JM (2015) *RNA* 21:1–13
21. Raczynska ED, Kosińska W, Ośmiatowski B, Gawinecki R (2005) *Chem Rev* 105:3561–3612
22. Kwiatkowski JS, Pullman B (1975) *Adv Heterocycl Chem* 18:199–335
23. Kwiatkowski JS, Person WB (1990) In: Beveridge DL, Lavery R (eds) Theoretical biochemistry and molecular biology. Academic Press, New York, pp 153–171
24. Griffiths AJF, Gelbart WM, Miller JH, Lewontin RC (1999) Modern genetic analysis. Freeman WH, New York
25. Berg JM, Tymoczko JL, Stryer L (2002) Biochemistry, 5th edn. New York, Freeman WH
26. Pauling L (1960) The nature of chemical bond, 3rd edn. Cornell University Press, New York
27. McClure RJ, Craven BM (1973) *Acta Crystallogr Sect B* 29:1234–1238
28. Radchenko ED, Sheina GG, Smorygo NA, Blagoi YP (1984) *J Mol Struct* 116:387–396
29. Kuczera K, Szczesniak M, Szczepaniak K (1988) *J Mol Struct* 172:101–112
30. Szczesniak M, Szczepaniak K, Kwiatkowski JS, Kubulat K, Person WB (1988) *J Am Chem Soc* 110:8319–8330
31. Brown RD, Godfrey PD, Mc Naughton D, Pierlot AP (1989) *J Am Chem Soc* 111:2308–2310
32. Nir E, Müller M, Grace LI, de Vries MS (2002) *Chem Phys Lett* 355:59–64
33. Choi MY, Dong F, Miller RE (2005) *Phil Trans R Soc A* 363:393–413
34. Wolken JK, Chunxiang Y, Tureček F, Polce MJ, Wesdemiotis C (2007) *Int J Mass Spectrom* 267:30–42
35. Feyer V, Plekan O, Richter R, Coreno M, Vall-Ilosera G, Prince KC, Trofimov AB, Zaytseva IL, Moskovskaya TE, Gromov EV, Schirmer J (2009) *J Phys Chem A* 113:5736–5742
36. Alonso JL, Vaquero V, Peña I, López JC, Mata S, Caminati W (2013) *Ang Chem Int Ed* 52:2331–2334
37. Dreyfus M, Bensaude O, Dodin G, Dubois JE (1976) *J Am Chem Soc* 98:6338–6349
38. Kwiatkowski JS, Bartlett RJ, Person WB (1988) *J Am Chem Soc* 110:2353–2358
39. Kobayashi R (1998) *J Phys Chem A* 102:10813–10817
40. Sambrano JR, de Souza AR, Queralt JJ, Andrés J (2000) *Chem Phys Lett* 317:437–443
41. Fazaeli R, Monajjemi M, Ataherian F, Zare K (2002) *J Mol Struct (Theochem)* 581:51–58
42. Mazzuca D, Marino T, Russo N, Toscano M (2007) *J Mol Struct (Theochem)* 811:161–167
43. Fogarasi G (2002) *J Phys Chem A* 106:1381–1390
44. Fogarasi G (2008) *Chem Phys* 349:204–209
45. Trygubenko SA, Bogdan TV, Rueda M, Orozco M, Luque FJ, Šponer J, Slavíček P, Hobza P (2002) *Phys Chem Chem Phys* 4:4192–4203
46. Shukla MK, Leszczyński J (2002) *J Phys Chem A* 106:11338–11346
47. Chandra AK, Michalska D, Wysokinsky R, Zeegers-Huyskens T (2004) *J Phys Chem A* 108:9593–9600
48. Friedman H, Nguyen HN, Truong TN (2004) *J Phys Chem B* 108:19043–19048
49. Sathyabama V, Anandan K, Kanagaraju R (2009) *J Mol Struct (Theochem)* 897:106–110
50. Zheng HT, Zhao DX, Yang ZZ (2011) *Chin J Chem* 29:2243–2248
51. Li X, Bowen KH, Haranczyk M, Bachorz RA, Mazurkiewicz K, Rak J, Gutowski M (2007) *J Chem Phys* 127:174309
52. Raczynska ED, Kolczyńska K, Stępniewski TM (2012) *J Mol Model* 18:3523–3533
53. Raczynska ED (2014) *Comput Theor Chem* 1042:8–15
54. Parr RG, Yang W (1998) Density functional theory of atoms and molecular orbital theory. Oxford University Press, New York
55. Becke AD (1993) *J Chem Phys* 98:5648–5652
56. Lee C, Yang W, Parr RG (1988) *Phys Rev B* 37:785–789
57. Hehre WJ, Radom L, Pvr Schleyer, Pople JA (1986) Ab initio molecular theory. Wiley, New York
58. Raczynska ED, Makowski M, Zientara-Rytter K, Kolczyńska K, Stępniewski TM, Hallmann M (2013) *J Phys Chem A* 117:1548–1559
59. Raczynska ED, Stępniewski TM, Kolczyńska K (2011) *J Mol Model* 17:3229–3239
60. Riestra-Kiracofe JC, Tschumper GS, Schaefer HF III, Nandi S, Ellison GB (2002) *Chem Rev* 102:231–282
61. Baboul AG, Curtiss LA, Redfern PC, Raghavachari K (1999) *J Chem Phys* 110:7650–7657
62. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA Jr, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson R, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Bakken V, Adamo C, Jaramillo R, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW., Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Cheng, Wong MW, Gonzalez C, Pople JA (2004) Gaussian-03, Revision E.01, Gaussian, Inc., Wallingford
63. Raczynska ED, Krygowski TM, Duczmal K, Hallmann M (2006) XVIII International Conference on Physical Organic Chemistry, Warsaw (Book of abstracts, p. 31)
64. Raczynska ED, Hallmann M, Kolczyńska K, Stępniewski TM (2010) *Symmetry* 2:1485–1509
65. Kruszewski J, Krygowski TM (1972) *Tetrahedron Lett* 13:3839–3842
66. Krygowski TM, Kruszewski J (1974) *Bull Acad Pol Sci. Sér Sci Chim* 22:871–876
67. Krygowski TM (1993) *J Chem Inf Comput Sci* 33:70–78
68. Frizzo CP, Martin MP (2012) *Struct Chem* 23:375–380

69. Raczyńska ED, Kolczyńska K, Stępniewski TM, Kamińska B (2013) *Comput Theor Chem* 1022:35–44
70. Cyrański MK, Gilski M, Jaskólski M, Krygowski TM (2003) *J Org Chem* 68:8607–8613
71. Cysewski P (2005) *J Mol Struct (Theochem)* 714:29–34
72. Huertas O, Poater J, Fuentes-Cabrera M, Orozco M, Sola M, Luque FJ (2006) *J Phys Chem A* 110:12249–12258
73. Sun GY, Nicklaus MC (2007) *Theoret Chem Acc* 117:323–332
74. Bartmess JE NIST Chemistry WebBook, NIST Standard Reference Database Number 69. Linstrom PJ, Mallard WG (Eds), National Institute of Standards and Technology, Gaithersburg MD, 20899. <http://webbook.nist.gov>
75. Lias SG in NIST Chemistry WebBook, NIST Standard Reference Database Number 69. Linstrom PJ, Mallard WG (Eds), National Institute of Standards and Technology, Gaithersburg MD, 20899. <http://webbook.nist.gov>
76. Stefanovic D, Grützmacher HF (1974) *Org Mass Spectrom* 9:1052–1054
77. Nenner I, Schultz GJ (1975) *J Chem Phys* 62:1747–1758
78. Dougherty D, Younathan ES, Voll R, Abdulnur S, McGlynn SP (1978) *J Electron Spectrosc Relat Phenom* 13:379–393
79. Schiedt J, Weinkauff R, Neumark DM, Schlag EW (1998) *Chem Phys* 239:511–524
80. Raczyńska ED, Kamińska B (2013) *J Mol Model* 19:3947–3960
81. Raczyńska ED, Kolczyńska K, Stępniewski TM, Kamińska B (2013) *Comput Theor Chem* 1022:35–44
82. Raczyńska ED, Makowski M (2014) *J Mol Model* 20:2234
83. Raczyńska ED, Makowski M, Hallmann M, Kamińska B (2015) *RSC Adv* 5:36587–36604