

Water as a medium and catalyst in tautomeric reactions

Habilitation thesis

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The present habilitation thesis is based on five original research papers from publication list “B” (7, 11, 17, 21 and 29). These articles are presented in the thesis by numbers 78, 33, 39, 50 and 64, respectively. The publication list “I” involves thirteen papers – 6, 9, 10, 12-15, 18, 19, 22, 23, 30, 31.

Introduction

Prototropic tautomerism is one of the most important processes involved in chemical reactions as well as in living systems[1]. The tautomerism in organic compounds has been studied extensively for the past three decades due to its biological importance and highly solvent-dependent nature. Studies of tautomeric equilibrium in structural components of DNA and RNA are of great interest today [2-4]. Tautomerism plays a key role in biological systems [5], from DNA base-pairing [6, 7] to the regulation of the function and activity of various enzymes [8].

It has long been postulated that the presence of rare tautomeric forms might be involved in base mispairing formation during polymerase-mediated DNA replication, resulting in genetic mutations, whereas in RNA, minor tautomeric forms have been proposed to enhance the structural and functional diversity of RNA enzymes and aptamers [9]. According to Watson and Crick [10], “Spontaneous mutation may be due to.... less likely tautomeric forms”. This idea has been advanced by Topal and Fresco[11] and it has also been estimated that these unpreferred tautomeric forms might be present, under physiological conditions, at a low frequency of 10^{-6} to 10^{-5} .

Among the physical and chemical factors that are responsible for tautomeric equilibrium, solvation is very important because most biochemical reactions occur in solution. A number of methods have been proposed in the literature for modeling solute-solvent interactions by computations[12-15]. Solvent effects are usually modeled by quantum chemical approaches based on the Onsager reaction field theory [16]. They are incorporated into the ab initio schemes as self-consistent reaction field models. The simplest reaction field model uses a spherical cavity. The polarizable continuum model (PCM) developed by Tomasi and co-workers [17] and its modified versions isodensity PCM (IPCM) and self-consistent isodensity PCM (SCIPCM) use the solute cavity defined by a set of overlapping spherical atoms having an approximate radii (e.g., 20% greater than the standard van der Waals radii). The surface potential can be calculated by numerical or analytical differentiation, and solute-solvent interactions take a mutual polarization of solute and solvent into account in a self-consistent way. All these methods model the solvent as a dielectric continuum.

The most popular of these, the PCM method, has been successfully applied for tautomeric systems [18-23]. With regard to the relative stability of tautomers in aqueous solution this method provides results which, in spite of some discrepancies, are in agreement with experimental data. The method allows a quantum chemical description of the solute in the solvent continuum at a computational cost slightly higher than that required in the gas phase calculation. However, important effects associated with specific solute-solvent interactions are neglected by the continuum approximation, especially in the description of the proton transfer mechanism in hydrogen-bonded systems. The solvent can control the dynamics of a proton transfer reaction via two distinct types of solute-solvent interactions. The first are long-range solvent polarization interactions and the second - specific short-range hydrogen bonding interactions. In the latter case protic solvents, like water, can accept a proton from the donor site of the solute molecule and transfer a different proton to the acceptor site on the solute. In this way the solvent influences the whole reaction path by lowering the energy barrier due to the direct participation of solvent molecule(s) in the proton transfer. In this case the solvent molecules act as catalyst.

The first theoretical paper describing catalysis of a tautomeric process by a single water molecule was published 38 years ago [24]. Since then, theoretical chemists have paid much attention to the role of solvent molecules in tautomeric and solvolytic processes. Nowadays in the literature water is shown to be important as a catalyst mediating proton transfer. In many proteins hydrogen atoms are transferred linearly for distances of about 10-50 Å between different amino acid residues along so-called “proton wires” [25]. The function of the water molecules is to both accept and donate a proton. Such transfer mechanism along proton wires is commonly referred as Grøtthus mechanism. Another possibility for the protons to be transferred is in a cyclic manner. Such mechanisms, referred to as “water-mediated proton transfer”, “bifunctional water catalysis”, “water-assisted hydration” or “relay proton transfer” were demonstrated to be of relevance in many organic and inorganic reactions.

Water-assisted proton transfer mechanism studies show that the assistance of a water molecule significantly lowers the energy barriers in proton-transfer reactions [26-29]. On the other hand, the electrostatic interaction of a solute molecule with a solvent represented by a continuum model influences only slightly the activation barriers of non-water assisted proton transfer reactions [30]. A reasonable approach to the solvation problem has been proposed by Adamo and Barone [31, 32]. An appropriate number of solvent molecules directly interacting with specific part(s) of the solute are explicitly treated by quantum chemical methods, while a large number of solvent molecules are approximated as a continuum. In this combined discrete/SCRF model both short-range and long-range solvent polarization interactions are included.

Present thesis summarized the scientific contributions of the theoretical investigation of thermodynamic and kinetic aspects of non-water-assisted and water-assisted proton transfer reactions in structural components of DNA and RNA and its derivatives as well as Schiff bases with intramolecular H-bonds. Bulk solvent effects are also considered at high levels of theory. The attention is focused on the influence of hydrogen bonding and bulk solvent effects on the relative stability of tautomers and the mechanism of proton transfer reactions.

In this regard, the main scientific contributions are related to:

- Mechanism of organic reactions, particularly proton transfer processes
- Structure elucidation of organic compounds using theoretical methods

To evaluate the influence of the water on tautomeric conversion three main groups of compounds were investigated and the mechanism of proton transfer reactions was clarified:

I. Nucleic bases derivatives

1. 5-fluorouracil and its anions
2. 5-azauracil and 6-azauracil

Water-assisted proton transfer

II. Nucleosides

1. Guanosine and its analogue acyclovir
2. Inosine

III. Salicylideneanilines

Direct proton transfer

I. Tautomeric Equilibria of Nucleic Bases Derivatives

1. 5-Fluorouracil and its Anions in Water [33]

The 5-substituted pyrimidines comprise a biologically important class of base analogues. The replacement in uracil of the hydrogen in position 5 with fluorine, to give 5-fluorouracil (5FU), an antineoplastic agent, introduces an additional heteroatom, which is a potential H-bond acceptor, and significantly alters the electronic properties of the molecule [34, 35]. In aqueous solution, this can induce significant changes in its physical and biological properties. The ionization constants (pK_a values) of the normal DNA bases are roughly 2-3 pH units away from physiological pH, predicting that the ionized forms might exist several orders of magnitude more frequently than the rare tautomeric forms. The 5FU behavior in water solution at different pH values gives important information about the role of pH in controlling the uptake of the anticancer drug.

Tautomeric equilibrium in 5FU anions (AN1 and AN3) and their tautomeric forms in water was investigated and was compared it to the tautomeric conversion in the neutral molecule. The influence of the water molecules on the tautomeric reactions between different forms was considered by a multiple proton transfer mechanism. Therefore, to identify and investigate for the first time the 5FU species present at different pH values in water solution, ab initio quantum chemical calculations and NMR spectroscopy were employed. Due to their high sensitivity, normal Raman and SERS spectroscopy were used to investigate the conversion between two tautomeric forms related by a keto-enolic equilibrium (diketo and dienolic) and two anions of the diketo form, N1 and N3, respectively [36, 37]. To correctly discriminate this species in water solution at different pH values, ab initio quantum chemical calculations of vibrational wavenumbers in gas phase and in a four water cluster were performed using the GAMESS (US) quantum chemistry package [38].

We consider the solvent effect on the tautomeric conversion of 5-fluorouracil and its anions through a supermolecule model and a multiple proton transfer mechanism. According to the free energies values (ΔG_{298}) from our calculations at CCSD(T)/6-31+G(d,p)//MP2/6-31+G(d,p) level the most stable is the complex of the 2,4-dioxo form A followed by the hydrated hydroxy forms B ($7.34 \text{ kcal mol}^{-1}$) and D ($7.51 \text{ kcal mol}^{-1}$). Due to these differences the populations of the species B and D are $4.2 \times 10^{-4} \%$ and $3.1 \times 10^{-4} \%$, respectively, and these species should coexist with tautomer A in water solution at neutral pH. The reaction barrier of $A \rightarrow B$ calculated at CCSD(T)/6-31+G(d,p)//MP2/6-31+G(d,p) level of theory amounts to $17.63 \text{ kcal mol}^{-1}$, which is by $0.54 \text{ kcal mol}^{-1}$ lower than that of the $A \rightarrow D$ proton transfer reaction. According to the rate constant values obtained the forward assisted proton transfer reaction can occur ($k=10^{-1}$ - 10^1).

The deprotonation of the most stable 2,4-dioxo tautomeric form, A, of 5FU can occur in two possible sites by formation of two anions: AN1 and AN3. Anion AN1 can theoretically exist in two tautomeric forms (AN1 and BN1) while AN3 in three tautomeric forms (AN3, CN3, and DN3) shown in Figure 1.

According to the free energies calculated at CCSD(T)/6-31+G(d,p)//MP2/6-31+G(d,p) level, anion AN3 is more stable than AN1 in gas phase and in water solution by 10.41 and $5.31 \text{ kcal mol}^{-1}$, respectively. The relative stabilities calculated at CCSD(T) level show that the populations of the species CN3 and DN3 are $9.2 \times 10^{-4} \%$ and $1.0 \times 10^{-4} \%$, respectively. These values are comparable to the calculated ones for 5FU tautomers, B (4.2×10^{-4}) and D (3.1×10^{-4}). Tautomer BN1 is not considered because it could be produced from the energetically unfavorable AN1. Thus, we studied the tautomeric equilibria of monoanion AN3 only: $AN3 \rightarrow DN3$ and $AN3 \rightarrow CN3$.

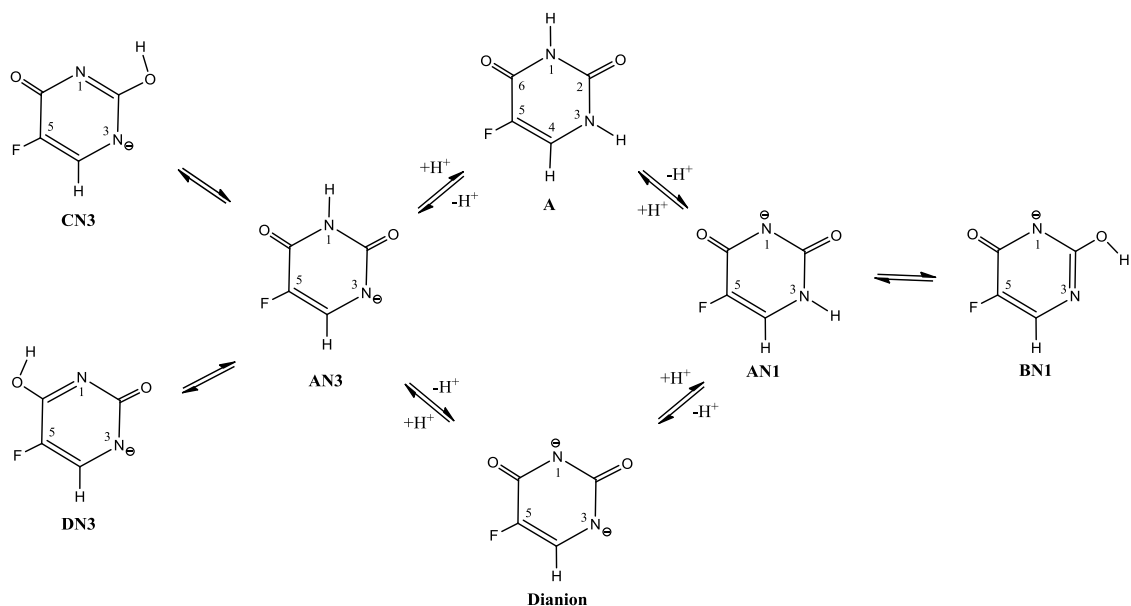


Figure 1. Two possible 5-fluorouracil anions (AN1 and AN3), their tautomeric forms and dianion.

There exists the possibility for two parallel reactions because both barriers are close in energy (Fig. 2). As in 5FU, the calculated rate constants of the $\text{AN3} \rightarrow \text{CN3}$ ($k = 5.16 \text{ s}^{-1}$) and $\text{AN3} \rightarrow \text{DN3}$ ($k = 5.5 \times 10^{-2} \text{ s}^{-1}$) are sufficiently large to be able to generate a concentration of rare hydroxyl anionic tautomeric forms enough to reproduce the frequency of point mutations. Both investigated proton transfer reactions involve concerted atomic movement.

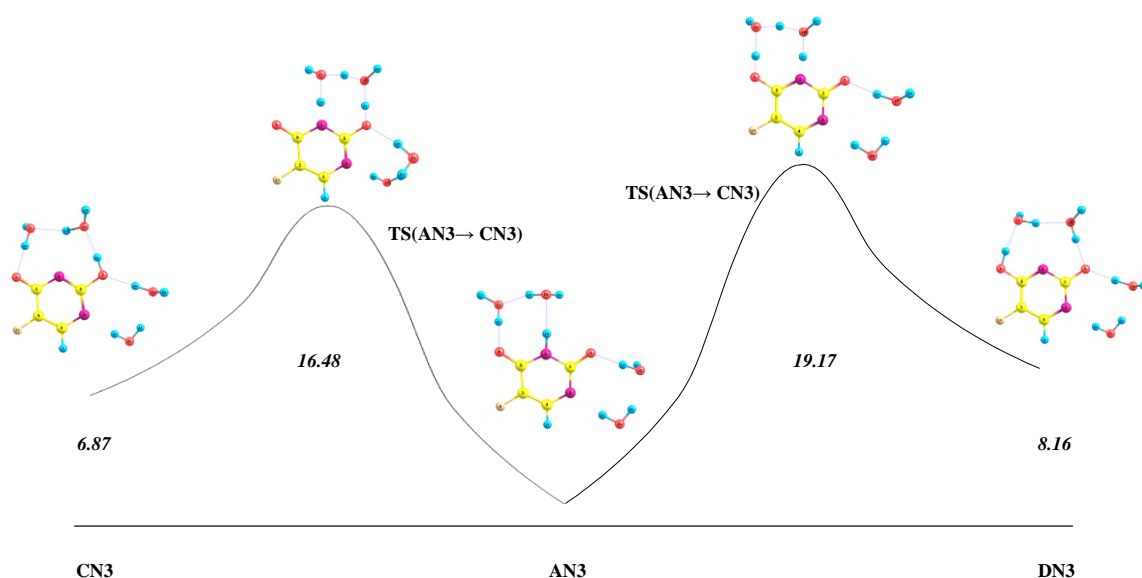


Figure 2. Energy profile of the tautomerization reactions of 5FU monoanion AN3 ($\text{DN3} \leftarrow \text{AN3} \rightarrow \text{CN3}$), calculated at the CCSD(T)/6-31+G(d,p)//MP2/6-31+G(d,p) level. The relative free energies and activation barriers are given in kcal mol^{-1} .

When tautomeric conversion proceeds, more than one proton is transferred along the classical reaction coordinate and the interatomic distances in proton transfer region vary at the same time, i.e., the process becomes highly cooperative. From a mechanistic point of view, this means that the proton transfers happen in a single step without any intermediates. However, the concerted triple proton transfer occurs asynchronously.

In order to establish the pH-induced structural transformation in the molecule of 5FU, NBO analysis, theoretical results for Raman wavenumbers and NMR chemical shifts as well as ^1H , ^{19}F , and ^{13}C NMR spectra in water solution for pH = 6.9-13.8 were performed. On the basis of NMR spectroscopic data obtained for 5FU in aqueous solution at alkaline pH, we suggest the existence of a mixture of the anionic tautomeric forms predicted by our theoretical calculations. Theoretical and experimental data of neutral and AN1 and AN3 deprotonated ions of 5-FU allow us to deduce that the deprotonation at alkaline pH should occur at the N3-H bond. Therefore, the coexistence of different tautomeric forms of AN3 in water solution at pH = 7.8-10 is probable.

2. 5-Azaauracil and 6-Azaauracil in Water Solution [39]

To study the tautomeric equilibria and the mechanism of the proton transfer reaction in a solution, the hybrid chemical model was evaluated on the base of combination of molecular-dynamic and quantum-chemical methods. A new knowledge about the mechanism of the solvent-assisted proton transfer processes can receive using this approach.

The following procedure applies:

- **First stage** – The geometries of the possible tautomeric and rotameric forms were optimized at *ab initio* quantum-chemical level.
- **Second stage** - The lowest energy tautomeric and rotameric forms are used as starting structures in Monte Carlo (MC) simulations to determine the molecule centers which can bond to solvent molecules by hydrogen bonds. In order to generate surroundings of the tautomer or rotamer structure in solution, a series of MC simulations were performed. In each MC simulation, the tautomer molecule (rotamer) is considered to be surrounded by several hundred solvent molecules in a cube with a length of 25 Å on each side. In this way, the first solvate shell can be constructed - a cluster of the solute molecule and several solvent molecules.
- **Stage Three** - The clusters structure obtained using MC simulations next was optimized at the *ab initio* quantum-chemical level. At the same time, a transition structure was also localized and the energy barrier of the proton transfer reaction was determined. The short-range interactions between the molecules of the solute and the solvent via intermolecular hydrogen bonds were accounted. The solvent acts as a catalyst in the reaction of intramolecular proton transfer in this model.
- **Fourth stage** - a more sophisticated model that considers the long-range interactions, i.e. the electrostatic effect of the solvent is also taken into account. Therefore, the clusters obtained were optimized again and the electrostatic field of the solvent is taken also into account in non-empirical quantum-chemical calculations. In this way, a more complete picture of the process is obtained, since both the near and far interactions of the solvent with the molecules of the solute are taken into account.

The approach described above was first applied to the theoretical study of the tautomerism of 5-azauracil and 6-azauracil in aqueous solution.

Aza analogues of nucleobases are of special interest due to their biological and pharmacological activities. Among possible structural modifications of uracil, one may consider the ring alternations via the substitution of the CH groups by nitrogen atom. In this way, one may obtain the 5- and 6-azauracils (Fig. 1). The biological activities of aza analogues of uracil have been intensively investigated. The 5- and 6-azauracils have been described to possess anticancer, antidepressant, hypnotic, antiallergic, antiasthmatic, anxiolytic, antidepressant, and anticoccidial properties [40].

5-azauracil (1,3,5-triazine-2,4(1H,3H)-dione, 5-AU) is considered to be a potential anticancer agent. Experimental investigations [41-44] performed in gas phase, solid state, or in solution indicate that the amino-oxo tautomer of 5-azauracil is the most stable one. The interpretation of IR, UV and NMR spectra of 5-AU are ambiguous and the results were not conclusive.

6-azauracil (1,2,4-triazine-3,5(2H,4H)-dione, 6-AU) was formerly used as an antitumor drug and has been studied extensively. Many experimental and theoretical studies have revealed its structural, chemical, and spectroscopic properties [45-47]. However, there are not studies on tautomeric conversions in solution (particularly, water) of neutral azauracils. As there are findings that 6-azauracil derivatives of 4,5 didehydro-5,6-dideoxy-L-ascorbic acid possess pronounced cytostatic activity against some malignant tumour cell lines, the idea of tautomeric conversion in such compounds attains significant importance [48].

At this point of view, the subject of this study is the tautomerization of 5- and 6-azauracils in the presence of binding water molecules. We consider the effect of hydration using the supermolecule approach in which four-water molecules forming two clusters are attached to the azauracil tautomers. To avoid the inherent arbitrariness, or the necessity of relying on “classical chemical intuition” in the way in which the solvent influence on the tautomerism is accounted for, we implement a two-phase hybrid statistical physics—quantum-chemical approach to this phenomenon. The two-phase approach is based on sequential Metropolis Monte Carlo (MC) simulation followed by analysis of solute–solvent interaction network patterns and subsequent finite cluster quantum mechanical (QM) study of the protontransfer process influenced by water as solvent. The hydration by bulk water is covered by the self-consistent reaction field (SCRF) method using the conductor-polarizable continuum model (C-PCM) approach [49].

To investigate the tautomerism in 5-AU and 6-AU the relative Gibbs free energies of the dioxo A and different hydroxy tautomers B–E (Fig. 3) of the azauracils in gas phase and in aqueous solutions were calculated. The computed energies of the five possible tautomers studied reveal that in gas phase at MP2/6–31+G(d,p) level of theory the dioxo form A of 5-AU and 6-AU is the most stable one, followed by the hydroxy form D. The following forms of 5-AU are E and B, while the stability sequence of 6-AU is A>D>C>B>E. The influence of water as medium was accounted using C-PCM at MP2/6–31+G(d,p) level. The energy sequences are not changed for 6-AU, whereas tautomer B became more stable than E in the relative stability order of the tautomers of 5-AU.

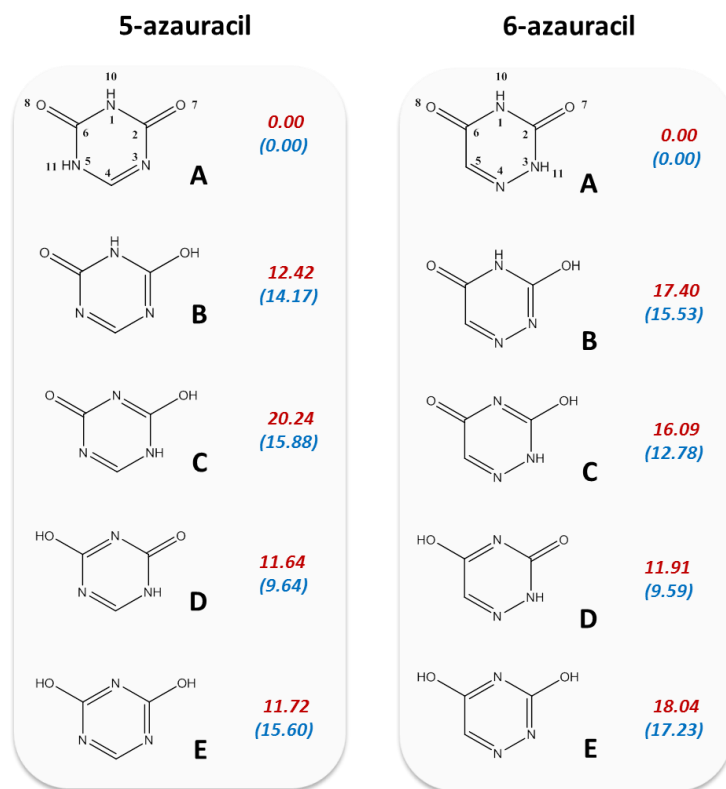


Figure 3. Possible tautomeric forms of 5-azauracil and 6-azauracil, and their calculated MP2/6-31+G(d,p) relative energies (ΔG_{298}) in kcal mol⁻¹ in gas phase and in solution (in brackets).

A hybrid statistical physics—quantum-chemical methodology was implemented to study the water-assisted intermolecular proton-transfer processes in 5- and 6-azauracils in aqueous solutions. The solvent effects were included in the model by explicit inclusion of two pairs of water molecules, which model the relevant part of the first hydration shell around the solute. The position of these water molecules was initially estimated by carrying out a classical Metropolis of dilute water solutions of the title compounds and subsequently analyzing solute–solvent intermolecular interactions in the Monte Carlo-generated configurations. Sequentially to the statistical physics simulation, ab initio quantum mechanical (QM) level of theory was implemented. The effects of the water as solvent (at ab initio QM level) were introduced at two different levels—using solute–solvent clusters (four-water molecules) and using the same clusters embedded in an external continuum. Full geometry optimizations of these complexes were carried out at MP2/6–31+G(d,p) and conductor-polarizable continuum model C-PCM/MP2/6–31+G(d,p). Single point calculations were performed at CCSD(T)/6–31+G(d,p)//MP2/6–31+G(d,p) computational level to obtain more accurate energies.

According to our calculations hydrated azauracils should exist in three forms: mainly dioxo form and two hydroxy forms. The calculated proton transfer activation energies for tautomeric reactions of 5-azauracil (Fig. 4) and 6-azauracil (Fig. 5) show different pictures for these two compounds.

According to C-PCM/MP2/6–31 1G(d, p) data, water-assisted proton transfer in 5-azauracil realizes through two parallel reactions: 1,3,5-triazine-2,4(1H,3H)-dione → 6-hydroxy-1,3,5-triazin-2(1H)-one and 1,3,5-triazine-2,4(1H,3H)-dione → 4-hydroxy-1,3,5-triazin-2(1H)-one.

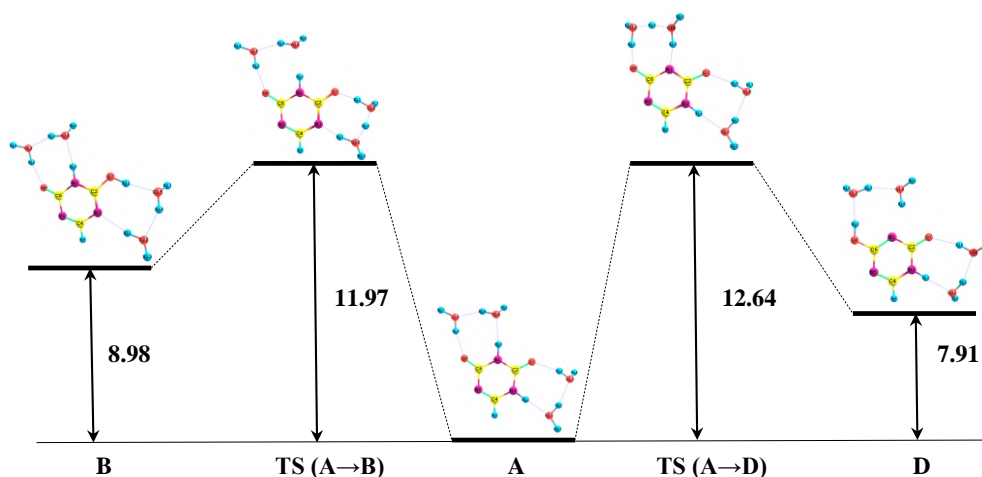


Figure 4. C-PCM/MP2/6-31+G(d,p) calculated relative energies (ΔG_{298}) and energy barriers (ΔG^\ddagger_{298}) (kcal mol⁻¹) for the tautomeric conversions in 5-azauracil.

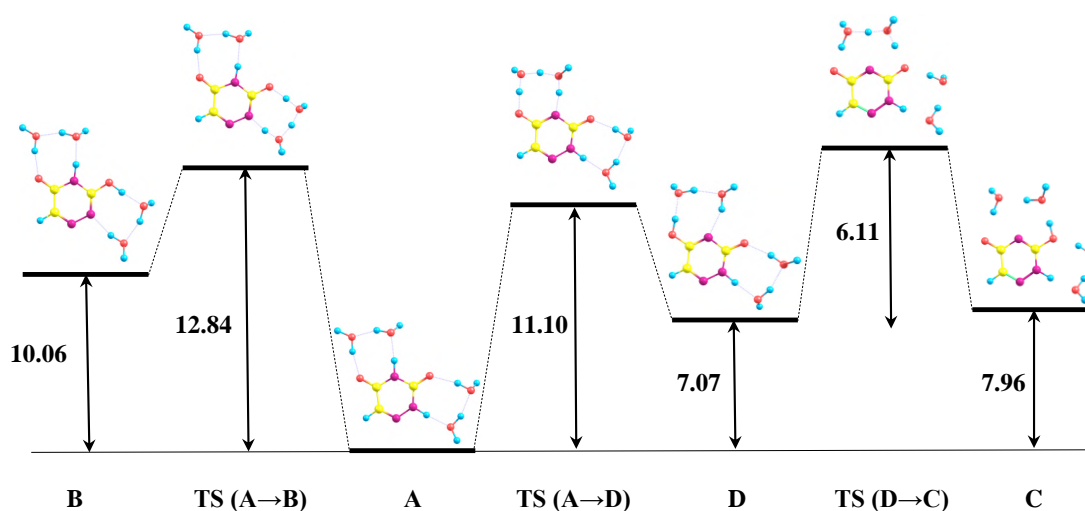


Figure 5. C-PCM/MP2/6-31+G(d,p) calculated relative energies (ΔG_{298}) and energy barriers (ΔG^\ddagger_{298}) (kcal mol⁻¹) for the tautomeric conversions in 6-azauracil.

The solvent-assisted proton transfer activation energies for the two parallel reactions $A \rightarrow B$ and $A \rightarrow D$ of 6-AU (Fig. 5) are close, but the situation is different from that for 5-AU. The $A \rightarrow D$ reaction is kinetically and thermodynamically preferred as compared to the $A \rightarrow B$ one. The rate constant k of the $A \rightarrow D$ reaction is $4.53 \times 10^4 \text{ s}^{-1}$ and it is an order of magnitude higher than that of the $A \rightarrow B$ one ($2.40 \times 10^3 \text{ s}^{-1}$). At the same time, tautomer D is by $2.99 \text{ kcal mol}^{-1}$ more stable than tautomer B. As the activation barrier of the reaction $D \rightarrow C$ is only $6.11 \text{ kcal mol}^{-1}$ (Fig. 5) and the respective rate constant k is $2.06 \times 10^8 \text{ s}^{-1}$, it seems more probably that the two contiguous reactions, 1,2,4-triazine-3,5(2H,4H)-dione, $A \rightarrow$ 5-hydroxy-1,2,4-triazin-3(2H)-one, D and 5-hydroxy-1,2,4-triazin-3(2H)-one, $D \rightarrow$ 3-hydroxy-1,2,4-triazin-5(2H)-one, C could occur in water solution and the hydroxyl tautomers D and C should be coexisting with tautomer A.

The proton transfer investigated reactions in 5- and 6-azauracils involve concerted atomic movement.

II. Tautomeric Equilibria of Nucleosides

1. Guanosine and its analog acyclovir in water solution [50]

The supermolecule + continuum approach was applied for the second time to more complete investigation of nucleosides tautomerism - guanosine and its analogues acyclovir.

It must be emphasized that in all the nucleoside and nucleotide crystal structures published thus far, only the canonic tautomeric forms of bases have been observed. However, several tautomeric forms of the purine ring can be theoretically formulated for both guanine [51, 52] and guanosine [53, 54]. The possibility of tautomeric conversion in 2'-deoxyguanosine has been investigated [55] but not in guanosine.

Guanosine (Gs), the guanine nucleoside is a major component of most types of RNA. From the IR spectra of the mono- and dihydrated clusters of Gs [56], it has been found that multiple structural isomers exist in both mono- and dihydrates of Gs and that the internal hydrogen-bonding structure of the Gs monomer is retained in all hydrates.

2-Amino-1,9-dihydro-9-((2-hydroxyethoxymethyl)-6H-purin-6-one or acyclovir, (ACV), is a guanosine analog antiviral drug [57, 58]. ACV has been extensively studied from the pharmaceutical and medical point of view. However, only a few studies related to its molecular structure have been published. The tautomeric equilibrium in ACV involving the keto and enol forms has been observed from the UV/Vis spectra [59]. This equilibrium depends on the polarity of the solvent, and therefore, in water solution, the keto form prevails, while in methylene chlorides it is the enol one [59, 60].

The effect of water as solvent in the tautomeric conversion of ACV attains significant importance. Water solubility is an important molecular property for successful drug development as it is a key factor governing drug access to biological membranes. According to Fallor and Ertl [61], the main reason for inaccurate calculation of hydrophobicity parameter (logP) is the use of incorrect tautomeric forms of structures in the calculations. Because of the 'tautomeric problem,' some of drug-like structures as ACV may exist in dozen of tautomeric forms, energetically quite close. In some cases, the form present in the database (and therefore used for calculation of properties) does not correspond to the form that is actually present in the test tube.

1.1. Guanosine

The computed energies of the guanosine tautomers (Fig. 6) in gas phase reveal that the 2-amino-6-(sZ)-hydroxy tautomer Gs-B1 and the 1H-2-amino-6-oxo tautomer Gs-A are almost isoenergetic but the Gs-B1 tautomer is more stable by 0.26 kcal mol⁻¹. The energy difference between the rotamers 2-amino-6-(sZ)-hydroxy (Gs-B1) and 2-amino-6-(sE)-hydroxy (Gs-B2) is the same as the difference between Gs-B1 and Gs-A. The quantities of these three species, according to their calculated relative stabilities, amount to 43.26% for Gs-B1 and 27.89 and 28.85% for Gs-A and Gs-B2, respectively. However, in water solution, where the solvent is represented as a structureless polarizable continuum, the tautomer Gs-A is predicted to become most stable and the energy difference between Gs-A and Gs-B1 is calculated to be 4.42 kcal mol⁻¹. The calculated fraction of Gs-A increases to 99.93%, while the fraction of Gs-B1 decreases to 0.06%.

A different conformations of the ribose ring in guanosine tautomer Gs-A have been investigated in [62]. The conformation of the sugar residue considered is closer to the experimental observed in native RNA. In the solid state, guanosine exists in 1H-2-amino-6-

oxo tautomeric form Gs-A [54]. Our MP2/6-31+G(d,p) calculations predict guanosine structure close to the experimentally found.

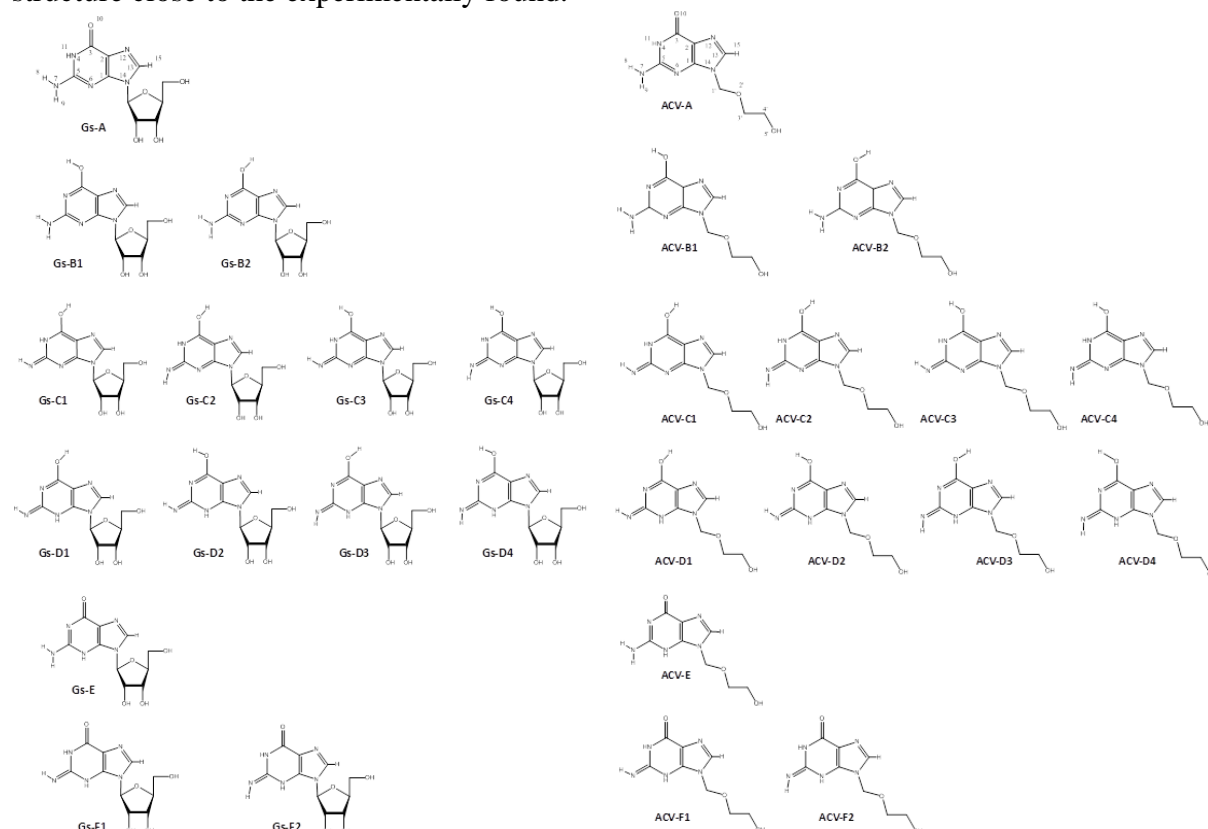


Figure 6. Guanosine and acyclovir tautomers and rotamers, and the label of the guanine fragment.

1.2.Acyclovir

According to theoretical study of ACV [60] the most stable structure corresponds to amino-oxo tautomer ACV-A. The second population is due to rotamer of amino-hydroxy tautomer ACV-B1 (37.7%) and the third one is to rotamer ACV-B2 (14.3%). The rest tautomers have very little population, less than 0.05%. Our results are similar and show that in the gas phase and in water solution, the most stable is the 1H-2-amino-6-oxo form ACV-A followed by the 2-amino-6-(sZ)-hydroxy form, ACV-B1. When isolated molecules are considered the energy difference between ACV-A and ACV-B1 is small (0.36 kcal mol⁻¹), similarly to the case of energy difference between the two tautomers of guanosine. According to calculated populations, the amount of the amino-oxo tautomer ACV-A is 55.83% and the 2-amino-6-(sZ)-hydroxy form ACV-B1 (30.41%) prevails over ACV-B2 (13.76%).

Taking into account the effect of water only as dielectric medium by the C-PCM model at MP2 level, the energy difference between the 1H-2-amino-6-oxo and 2-amino-6-(sZ)-hydroxy forms increases substantially. The energy difference between ACV-A and ACV-B1 increases 15 times and become 5.43 kcal mol⁻¹. The amount of the amino-oxo tautomer prevails over both of the amino-hydroxy forms and the ratio become ACV-A : ACV-B1 : ACV-B2 = 99.98: 0.01: 9.8 × 10⁻³%.

According to crystallographic data [63] and conformational analysis [60] in solid state only one tautomeric form of acyclovir, ACV-A is observed. Three types of structures appear for acyclovir in relation to the side chain that is attached to N14. While in the first two molecules, the bonds are in the preferred gauche conformation in the third one the bonds in

the side chain are all in the transconformation, giving rise to an almost planar, zig-zag arrangement. Our results for geometric parameters of tautomeric form ACV-A calculated at MP2/6-31+G(d,p) level are closer to the zig-zag molecule conformation (Fig. 7).

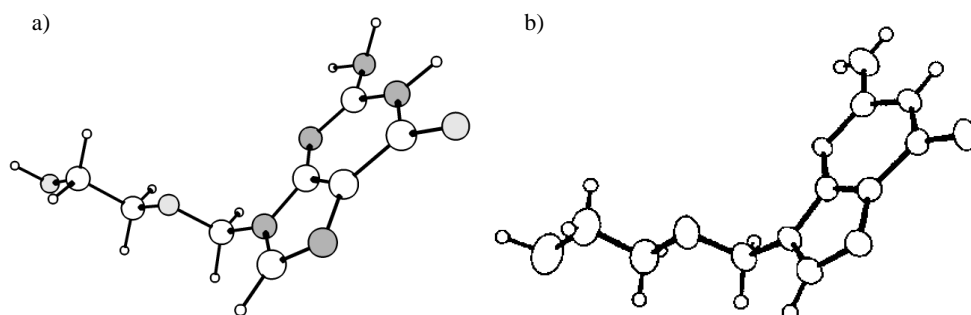


Figure 7. Structure of the tautomeric form A of acyclovir obtained by optimization at MP2 level (a) and X-ray diffraction [63] (b).

The solvent effects were simulated by the procedure described above where explicit inclusion of water molecules that model the relevant part of the first hydration shell around the solute. The position of these water molecules was estimated by carrying out a classical Metropolis Monte Carlo simulation of dilute water solutions of the guanosine (Gs) and acyclovir (ACV) and subsequently analyzing solute–solvent intermolecular interactions in the statistically-independent MC generated configurations. The solvent-assisted proton transfer processes were further investigated using two different *ab initio* MP2 quantum chemical approaches. In the first one, potential energy surfaces of the ‘bare’ finite solute–solvent clusters containing Gs/ACV and four water molecules (MP2/6-31+G(d,p) level) were explored (Fig. 8), while within the second approach, these clusters were embedded in ‘bulk’ solvent treated as polarizable continuum (C-PCM/MP2/6-31+G(d,p) level of theory).

It was found that in the gas phase and in water solution, the most stable tautomer for guanosine and acyclovir is the 1H-2-amino-6-oxo form followed by the 2-amino-6-(sZ)-hydroxy form. The energy barriers of the water-assisted proton transfer reaction in guanosine and in acyclovir are found to be very similar – 11.74 kcal mol⁻¹ for guanosine and 11.16 kcal mol⁻¹ for acyclovir, and the respective rate constants ($k = 1.5 \times 10^1$ s⁻¹, guanosine and $k = 4.09 \times 10^1$ s⁻¹, acyclovir), are sufficiently large to generate the 2-amino-6-(sZ)-hydroxy tautomer. The time necessary to reach 99.9% of the equilibrium concentration between the tautomeric forms 1H-2-amino-6-oxo A and 2-amino-6-(sZ)-hydroxy B1 was calculated to be $\tau_{99.9\%} = 3.53 \times 10^{-5}$ s in Gs and $\tau_{99.9\%} = 1.2 \times 10^{-6}$ s in ACV.

Since the stationary points were located, the reaction pathway was established by following the IRC in the forward and reverse directions from TS (Fig. 9). These calculations ensure that the proper reaction pathway, connecting the reactant and product on each side of the TS, has been found. When only the short-range interactions of the solvent were accounted at MP2/6-31+G(d,p) level (curve A, Fig. 9), the process is cooperative and from the mechanistic point of view this means that the proton transfers happen in a single step without any intermediates. The reaction profile is different when we consider the second, more sophisticated computational model – optimization of the cluster with four water molecules using the conductor-like polarizable continuum model, i.e. C-PCM/MP2/6-31+G(d,p) (curve B, Fig. 9). The analysis of the reaction profiles in both compounds shows that the proton transfer processes occur through the asynchronous concerted mechanism.

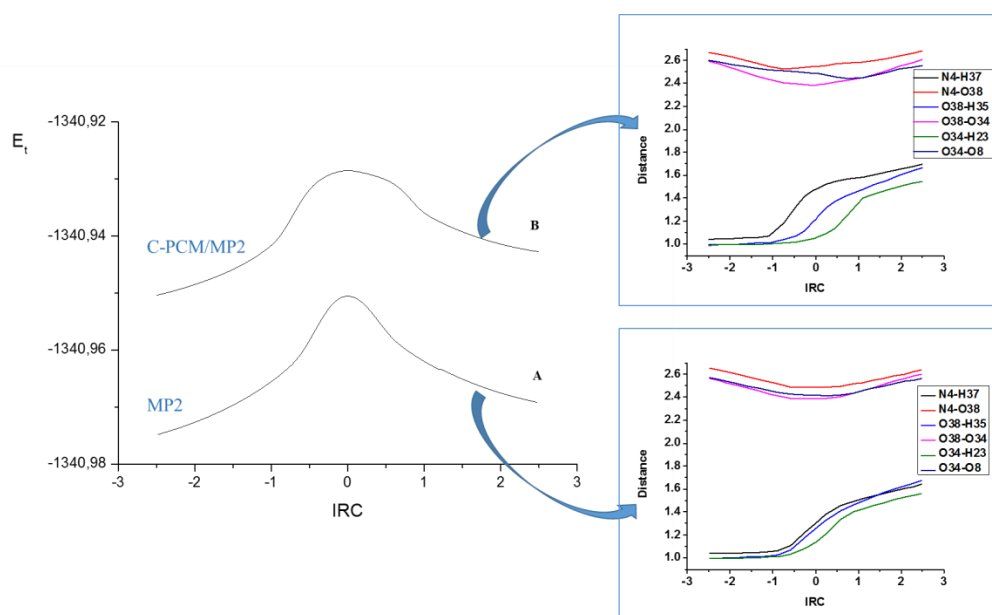


Figure 9. IRC profile of the water-assisted proton transfer reaction of guanosine Gs-A→Gs-B1 calculated at MP2/6-31+G(d,p) (profile A) and C-PCM/MP2/6-31+G(d,p) (profile B) levels. Insert: Distances between two selected atoms along the IRC profile of the four-hydrated guanosine as calculated at MP2 and C-PCM/MP2 levels. Total energies are in a.u., distances are in Å and IRC in $\text{amu}^{1/2} \text{ bohr}$.

2. Inosine in water solution [64]

Inosine, 9-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-3H-purin-6-one is a purine nucleoside that has hypoxanthine linked by the N9 nitrogen to the C1 carbon (β -N₉-glycosidic bond) of ribose. It is an intermediate in the degradation of purines and purine nucleosides to uric acid. Inosine is commonly found in tRNAs and is essential for proper translation of the genetic code in wobble base pairs.

Tautomerism of inosine is considered in several papers [65-67]. Spectra of aqueous solutions of inosine has been obtained in the region $2000\text{-}200 \text{ cm}^{-1}$ using argon laser excitation, and indicate that inosine exists predominantly in the keto form. In the solid state inosine crystallized in three different crystal forms [68]. Two of them occur in the monoclinic system and one in the orthorhombic form. The location in the crystal of a proton on N1 confirms the tautomeric form as amino-oxo tautomer A (Fig. 1) or so called 6-keto form for both molecules.

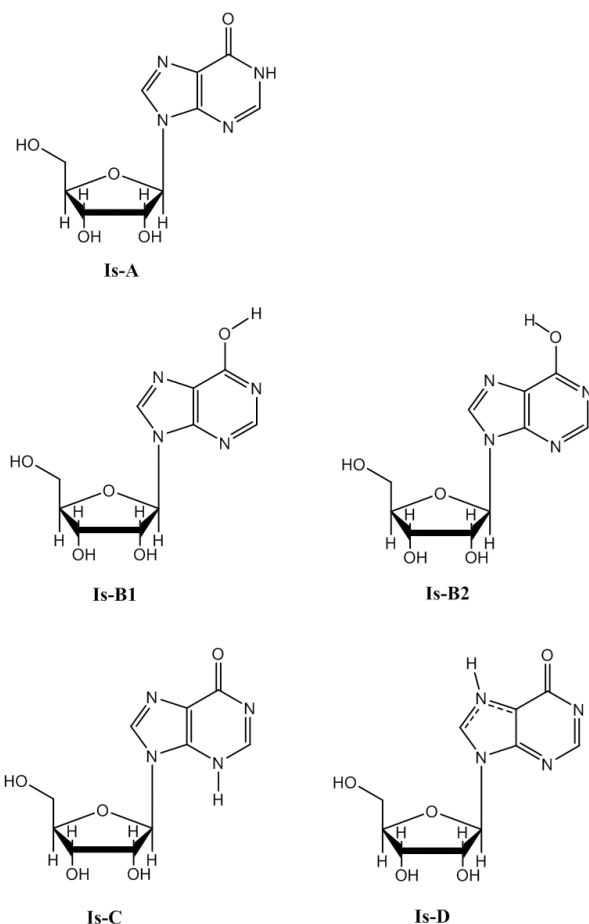


Figure 10. Inosine tautomers and rotamers

Many theoretical papers have been devoted to the conformational isomerism of DNA and RNA structural components [69-71] but few studies appear on inosine. The prototropic tautomerism and basic molecular principles of hypoxanthine mutagenicity were investigated to understanding elementary molecular mechanisms of mutagenic action of hypoxanthine as a product of the adenine deamination in DNA [69]. Five tautomers of inosine were determined and optimized at the MP2 and B3LYP levels of theory and a comprehensive conformational analysis was carried out on the most stable keto tautomer [72]. It has been found that the least stable tautomer (the H-atom is positioned to nitrogen from five membered ring of purine moiety) in the isolated state is the most favored in a polarizable environment with water because of heist dipole moment. While tautomerism of nucleobases and nucleosides has been studied in gas phase, aprotic solvents or excited state, the conclusions are less relevant for DNA and RNA structural components. Under these conditions tautomeric equilibria are significantly altered and the relative proportion of major and minor tautomeric forms is changed, even for the canonical nucleobases [73]. To study tautomerism in aqueous solutions is more difficult because tautomerization is mediated by water molecules. This process leads to fast rates of the tautomeric equilibria and low abundance of minor tautomeric species [50]. Since the inosine tautomerism in water is not studied enough, the investigation on the possibility for water-assisted proton transfer in the title compound gets essential. In realistic media, especially relevant to biomedical sciences, such process occurs in water solutions, with a substantial unavoidable influence of the solvent.

Similar to other nucleosides (guanosine, adenosine) several tautomeric forms are possible in inosine. Four tautomeric forms, three amino-oxo (A, C and D) and one imino-

hydroxy (B), are shown in Fig. 10. The tautomer B has two rotamers: B1 and B2. Five structural parameters should be taken into account for a complete description of the conformation of inosine. Following the Saenger's notation the atomic description of tautomer A is defined in Fig. 11. The main parameter is the glycosidic torsional angle, $\chi(\text{C4-N9-C1'-O1'})$, that determines the position of the plane of the base with respect to the ribose.

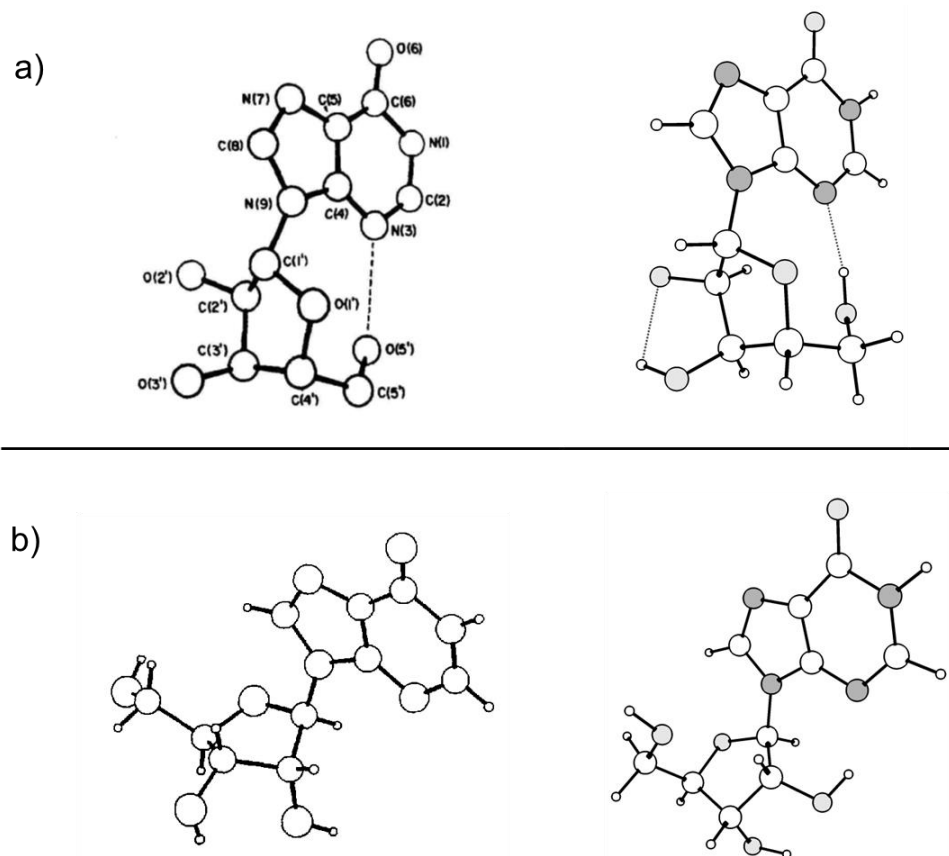


Figure 11. Structure of the tautomeric form **A** of inosine: (a) *syn*-conformer and (b) *anti*-conformer. X-ray diffraction structure of *syn*-form, adapted by Ref. [74] and *anti*-conformer, adapted by Ref. [75] are presented in the left. MP2 optimized structures are shown in the right. The dotted lines depict the intramolecular H-bonds.

The two main conformations generated by this angle are *anti* and *syn*. The exocyclic torsional angles $\gamma(\text{C3'-C4'-C5'-O5'})$ and $\beta(\text{C4'-C5'-O5'-H5'})$ describe the orientation of the C5'-O5' and O5'-H5' groups with respect to the furanose ring, respectively. Tautomers A, C and D, and rotamers B1 and B2 have two conformers. Since inosine can have a different number of conformations, our starting geometries were selected based on the conformers observed in crystal structures.

The relative Gibbs free energies of *syn*- and *anti*-conformers of the inosine tautomers were computed at MP2/6-31+G(d,p) level. Additional single-point calculations at SCS-MP2/6-31+G(d,p) level were carried out.

The computed energies of the inosine tautomers in the gas phase reveal that the *syn*-conformer of tautomer A is most stable followed by its *anti*-conformer as the energy difference between both conformers is 1.11 kcal mol⁻¹. Tautomer *syn*-B1 is higher in energy by 2.07 kcal mol⁻¹. The energy difference between rotamers *syn*-B1 and *syn*-B2 is 1.12 kcal mol⁻¹. The quantity of these species, according to their calculated relative stabilities amount to

83.97 % for *syn*-A, 12.86% *anti*-A, 2.54% *syn*-B1, 0.38% *syn*-B2, 0.35% *anti*-B1 and 0.08% *anti*-B2. The calculated energy barrier of the rotation of the furanose ring around the N9-C1' glycosidic bond is low: 4.89 kcal mol⁻¹. The probably parallel reaction, intramolecular proton transfer *syn*-A → *syn*-B1 should not occur because its calculated energy barrier is too high: 36.08 kcal mol⁻¹. The calculations at SCS-MP2/6-31+G(d,p) level show similar qualitative results.

Syn- and *anti*-coformers of the amino-oxo tautomer A have been isolated in crystal form from water solution [74, 75]. The calculated bond distances are in agreement with available experimental data for both conformers of A.

The relative Gibbs free energies of the tautomers of inosine in water solution where the solvent is considered as a structureless polarizable continuum characterized by its macroscopic dielectric permittivity were calculated. The energy difference between both conformers of tautomer A increases to 5.75 kcal mol⁻¹ and the difference between the *syn*-conformers of tautomers A and B1 is calculated to be 6.37 kcal mol⁻¹. The quantities of these three species amount to 99.99 % *syn*-A, 6.1x10⁻³ % *anti*-A and 2.1x10⁻³ % *syn*-B1. The calculated energy barrier of the rotation *syn*-A → *anti*-A increases to 8.60 kcal mol⁻¹, i.e. it is 57% higher in comparison to the gas phase. The results are practically the same when we take into account the improved CPCM/SCS-MP2/6-31+G(d,p) energetics compared to CPCM/MP2/6-31+G(d,p) ones.

The water-assisted proton transfer process in inosine was investigated using *ab initio* MP2 and SCS-MP2 quantum chemical approaches. Solute-solvent clusters containing inosine molecule and five water molecules embedded in “bulk” solvent treated as polarizable continuum (CPCM/MP2/6-31+G(d,p) level of theory) were explored. The energy barrier of the water-assisted proton transfer reaction in inosine is found to be 12.9 kcal mol⁻¹ and the rate constant ($k = 6.68 \times 10^1 \text{ s}^{-1}$) is sufficiently large to generate the 6-enol tautomer. The analysis of the reaction profiles (Fig. 12) shows that the proton transfer processes occur through the asynchronous concerted mechanism.

Spectroscopic methods for study of tautomerism of DNA and RNA structural components in aqueous solutions have been of limited use. Vibrational IR and Raman spectroscopies are sensitive to identifying tautomeric forms of nucleobases and nucleosides in aqueous solutions. Infrared spectroscopy of water solutions is limited, however, by solvent absorption to the region from 1800 to 1500 cm⁻¹. More complete vibrational spectra of aqueous inosine may be obtained by Raman spectroscopy [76] because liquid water gives rise to only very weak Raman scattering over the region 2000-200 cm⁻¹.

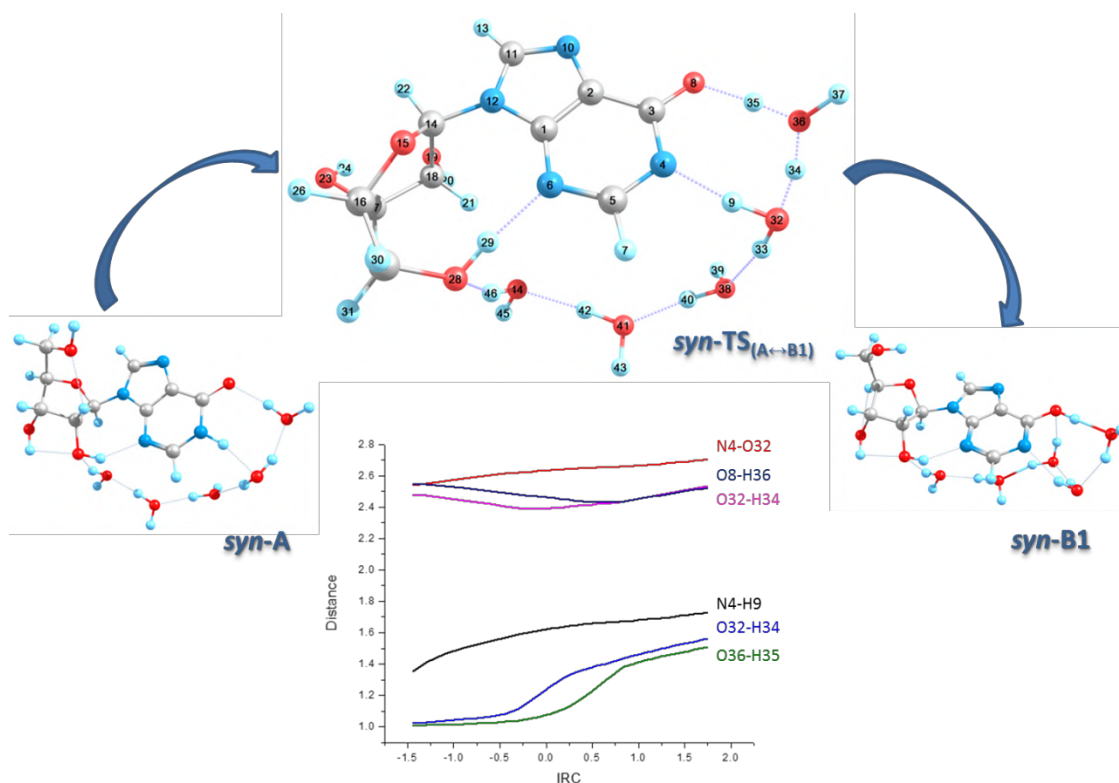


Figure 12. Structures of the five-hydrated tautomeric forms *syn-A* and *syn-B1* of inosine and respective transition state calculated at CPCM/MP2/6-31+G(d,p) level, and distances between two selected atoms (in Å) along the IRC (in $\text{amu}^{1/2} \text{ bohr}$).

We have calculated IR frequencies, intensities and Raman activities of inosine conformers (*syn*- and *anti*-) for tautomers A and B in gas phase and water. We considered the effect of aqueous hydration on IR and Raman spectra of inosine using approach in which the cluster including water molecules and inosine was embedded in polar medium (water) and the CPCM/MP2/6-31+G(d,p) calculations were performed. The cluster including five water molecules forming a chain between C=O group from hypoxanthine moiety and OH-group from ribose ring are attached to the tautomers A and B of the inosine *syn*- and *anti*-conformers. According to our results there is a good agreement between theoretical and experimental data.

Summing up, no band $\nu_{\text{O-H}}$ was found in the experimental IR and Raman spectra of inosine in water for enol form B. According to theoretical spectra of the *syn*- and *anti*-conformers of tautomer B in water solution this line was predicted to be shifted (because of hydrogen bonding formation with water molecules) around 2920 cm^{-1} . The C-H stretching frequency of the ribose ring was calculated to be around 2930 cm^{-1} and it overlaps with $\nu_{\text{O-H}}$ one but the intensity of these lines is different. Therefore, experimental IR and Raman spectra of inosine in water have no evidence of enol tautomer presence but according to our theoretical predictions as regards to kinetics of tautomeric conversion in water of inosine the enol form B should be presented in solution. We have shown [33, 39, 50, 77] that the calculated barriers in range $12\text{--}17 \text{ kcal mol}^{-1}$ for the water-assisted proton transfer reactions indicate that the tautomeric conversions are kinetically feasible processes. The detection of the rare tautomeric forms by spectroscopic methods is not possible and a probable exception might be fluorescence spectroscopy [77].

III. Keto-Enol Equilibria of Salicylideneanilines [78]

The cases discussed so far are examples of intermolecular tautomeric conversions in which solvent molecules are involved not only as a medium but also as a catalyst. In this section, a case of intramolecular proton transfer will be considered, in which the solvent will only be counted as a continuous medium.

Schiff bases are a great topic of basic research, that to date have an important place in organic chemistry and they have a great versatility in different fields of study. They have different biologic applications as antitumor agents, in the strengthening of immune response for cancer, in leukemia, in HIV, as anticonvulsant, antibacterials, antifungal, antiinflammatory, as prodrugs and as study models in the intramolecular hydrogen bond from cofactor pyridoxal-5-phosphate. They are also of interest because of their solvatochromic, thermochromic and photochromic properties with applications in optical recording technology, molecular electronics and photonics [79].

Schiff bases, derivatives of aromatic o-hydroxyaldehydes, are a class of compounds which have received attention owing to their interesting linear and nonlinear optical properties, biological activity, and technological applications. In salicylideneanilines and related Schiff bases, generally called anils, an intramolecular proton-transfer reaction between the enol-imine (E) and keto-amine (enaminone) (K) forms can occur both in solution and in the crystalline state (Fig. 13a). This reaction can be triggered either by light or by heat and can even be encountered in biological media. The associated photo- and thermochromisms make salicylideneaniline-like compounds intelligent materials, which can be used as molecular switches and memories.

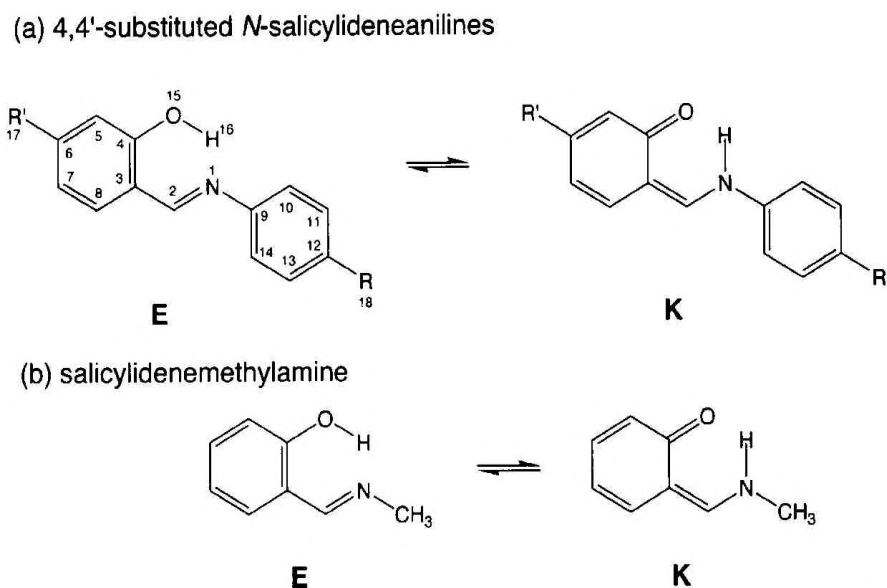


Figure 13. Enol (E) and keto (K) tautomeric forms of Schiff bases with intramolecular hydrogen bond: (a) *N*-salicylideneaniline ($R = H$, $R' = H$), **1**; *N*-salicylidene-4-bromoaniline ($R = Br$, $R' = H$), **2**; 4'-amino-*N*-salicylidene-4-bromoaniline ($R = Br$, $R' = NH_2$), **3**; 4'-amino-*N*-salicylideneaniline ($R = H$, $R' = NH_2$), **4**; 4'-amino-*N*-salicylidene-4-nitroaniline ($R = NO_2$, $R' = NH_2$), **5**; 4'-cyano-*N*-salicylideneaniline ($R = H$, $R' = CN$), **6**; *N*-salicylidene-4-formyl-aniline ($R = CHO$, $R' = H$), **7**; 4'-hydroxy-*N*-salicylideneaniline ($R = H$, $R' = OH$), **8**; *N*-salicylidene-4-aminoaniline ($R = NH_2$, $R' = H$), **9** (b) salicylidenemethylamine, **10**.

In Schiff bases with intramolecular H-bonds, such as derivatives of aromatic *o*-hydroxyaldehydes condensed with primary amines (Figure 13), the E form is usually the most stable one. Besides the modifications of the thermodynamically aspects (energies of reaction and energies of activation), varying the substituents has an impact on the other molecular properties: absorption and emission spectra, vibrational signatures, as well as linear and nonlinear optical (NLO) properties. We have investigated by several groups the NLO properties of substituted salicylideneanilines and in particular their variations upon switching between the K and E forms. The keto-enol tautomerization equilibrium, and, more particularly, the keto-amine/enol-imine equilibrium, has been investigated for a series of substituted salicylideneanilines in view of designing compounds with large contrast of first hyperpolarizabilities.

The E/K equilibrium for compounds 1-9 as well as the model salicylidenemethylamine compound 10 was studied in details at different computational levels: HF, B3LYP, MP2 и MP4//MP2 with 6-31G** and 6-31+G** basis sets. At all levels of approximation, the E form is the most stable. Considering compound 10, using the MP4//MP2 value as reference, the B3LYP approach underestimates the ΔG_{298} value by more than 50%, whereas the HF and MP2 approaches underestimate it by 1 kcal mol⁻¹ or less. Including solvent effects via the PCM scheme reduces ΔG_{298} substantially with respect to the gas-phase values. According to our results the E form is more stable than K one by 8.11 kcal mol⁻¹ and 7.15 kcal mol⁻¹ at MP2/6-31G(d,p) and MP2/6-31+G(d,p) levels, respectively. After single-point calculations at MP4/6-31G(d,p)//MP2/6-31G(d,p) and MP4/6-31+G(d,p)//MP2/6-31+G(d,p) theoretical levels the energy differences increase slightly - 8.19 kcal mol⁻¹ and 9.35 kcal mol⁻¹, respectively. When the solvent (water) taking into account as continuum approximation with long-range solvent polarization interactions the energy differences between E and K forms decrease dramatically - 3.09 kcal mol⁻¹ and 2.17 kcal mol⁻¹ at PCM/MP4/6-31G(d,p)//MP2/6-31G(d,p) and PCM/MP4/6-31+G(d,p)//MP2/6-31+G(d,p) levels, respectively.

Similar conclusions can be drawn for the activation Gibbs energy [$\Delta G^{\ddagger}_{298}$ (forward)]. The intramolecular proton transfer barrier is reduced considerably in water solution at the same theoretical levels. The most substantial decreasing is observed at MP4 level – more than two times. The exception is HF method that overestimates it by about 50%. This overestimation is not surprising, owing to the fact that accounting for electron correlation is necessary for a reasonable prediction of the activation energies, and that the contribution of the correlation energy is usually larger for transition structures than for equilibrium structures. Besides using the MP4//MP2 level of approximation (and the HF scheme), the activation Gibbs energy for the reverse reaction is always negative when the solvent effects are not taken into account. The activation energy is small for the reverse reaction, demonstrating the weak stability of the K form, which can easily convert to the E form. Together with a substantial (relative) stabilization of the K form, including solvent effects reduces the activation barrier and makes them positive for both the forward and reverse paths.

The main thermodynamic and kinetic data for the tautomeric equilibrium of compounds 1-9 are similar to salicylidenemethylamine.

The keto-enol tautomerization equilibrium, and, more particularly, the keto-amine/enol-imine equilibrium, has been investigated for a series of substituted salicylideneanilines in view of designing compounds with large contrast of first hyperpolarizabilities. The different compounds present a sufficiently large contrast of β between the E and K forms to allow its detection for at least one type of second-order NLO measurements (EFISH or HRS). The largest β values are mainly associated with species bearing a donor in the para position of the salicylidene ring and an acceptor on the other ring whereas the largest α values are generally found for the E form.

Conclusions:

The role of water as a solvent in intermolecular tautomeric conversions of nucleic acid structural units is essential. It is similar in the intramolecular proton transfer in the molecules of various salicylidenanilines. In the first case, the energy barriers of the proton transfer reactions are drastically reduced when the short-range interactions of the solute with the water molecules are taken into account. This is also observed when a combined approach is employed to investigate the effect of the solvent - clusters formed of solute molecule and water molecules situated in a solvent considered as a polarizable continuous medium are modeled. This approach most fully describes all interactions with the solvent, both short-range and long-range. When the influence of the solvent (water) is taken into account, as the continuous dielectric medium during the intramolecular proton transfer in the molecules of different salicylidenylamines, the energy differences between the enol and keto forms as well as the proton transfer barriers decrease.

The analysis of the reaction profiles of all intermolecular tautomeric conversions shows that the proton transfer occurs in nucleobase and nucleoside molecules proceeds in an asynchronous concerted mechanism, in one step, and without intermediates.

Perspectives

My scientific studies will proceed in several directions:

1. Clarifying of the reaction mechanisms in organic systems
2. Elucidating of the molecule structures using quantum-chemical methods
3. Tautomerism in organic compounds
4. Modeling of processes of prebiotic compounds formation

The following research interests are related to projects that I coordinate:

1. "Development of chalcones with strong antiparasitic properties", DNTS/India 01/5, 24.06.2013, BNSF

The determination of correlations between structure and activity in chalcones with strong antiparasitic properties against *P. falciparum* (causing malaria) by quantum chemical and statistic calculations for the SAR analysis will performed in the third phase of the project.

2. "Chemical characterization and evaluation of antiviral and antibacterial activity of extracts from *Graptopetalum paraguayense* E. Walther (*Crassulaceae*)", DN 19/16, 20.12.2017, BNSF

In the recent years a new scientific area is revealed and will continue - *Quantum-chemical and docking methods in phytochemistry*. The efforts will directed to detection of the active compounds from the plant *Graptopetalum paraguayense* E. Walther and examination of theirs binding expedient to viral enzymes active sites.

In addition I am a participant in of Centre of excellence "National center of mechatronics and clean technologies" BG05M2OP001-1.001-0008-C03, 2018-2023, WP1, section 1.2.1. *Modeling and development of materials for nonlinear optics and optoelectronics, optical sensors*.

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