# Conformational Flexibility of Pyrimidine Ring in Nucleic Acid Bases

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# Chapter 21: Conformational Flexibility of Pyrimidine Ring in Nucleic Acid Bases

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**Abstract**

Nucleic acid bases (NAB) had been considered for many years to be planar and conformationally rigid. However, recently two possible sources of nucleobases nonplanarity had been found. Ab initio quantum chemical calculations using large basis sets augmented by inclusion of electron correlation and also recent experimental studies revealed that amino groups in isolated cytosine, guanine, and adenine adopt a nonplanar trigonal-pyramidal configuration. Since the values of amino group inversion barriers do not exceed approximately one kcal/mol this group possesses rather flexible geometry. A different source of nonplanarity of nucleobases originates from the high deformability of the pyrimidine ring. Transition of such a ring in uracil, thymine, cytosine, and guanine molecules from a planar equilibrium conformation to a sofa configuration characterized by a relevant torsion angle of $\pm 20^{o}$ entails an increase of energy by less than 1.5 kcal/mol. Therefore, at room temperature certain fraction of isolated DNA bases should possess nonplanar structure of the heterocyclic ring. This review summarizes recent theoretical studies on flexibility of the NABs.

**Keywords**

Nonplanarity, Conformational flexibility, DNA bases, 2'-deoxyribonucleotides

## 21.1 Introduction

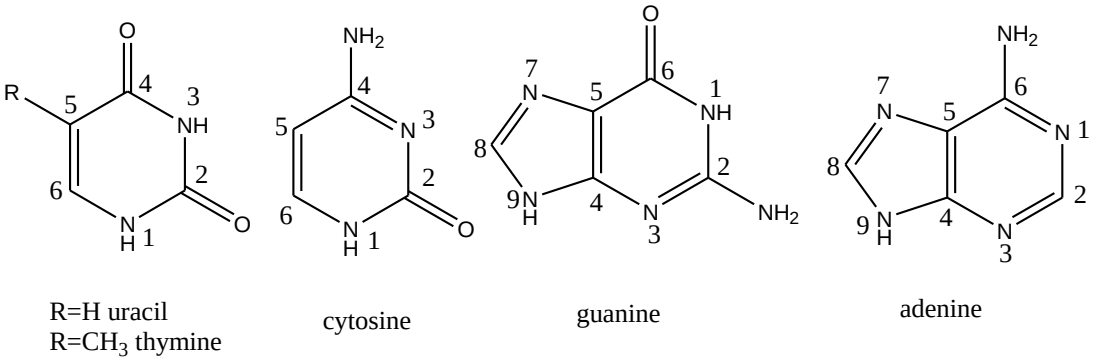
It is well recognized that DNA macromolecules are very flexible and can easily change their conformation depending on solvation, concentration, nature of counterions, and temperature [1]. Conformational flexibility of these biomolecules also plays an important role during interactions with proteins and drugs creating the most favorable conditions for molecular recognition. Therefore, molecular mechanism of DNA flexibility is a focus of many studies.

Usually conformational flexibility of these macromolecules is associated with easy conformational changes of backbone including rotation around the P-O bonds, change of ribose conformation, etc [2, 3]. Stacked nucleic acid bases are considered as quite rigid fragments of DNA playing the role of a rod for structure and dynamics of these biopolymers. In general, this is true because it was demonstrated that nucleotides are very flexible molecules with numerous minima on the potential energy surface [4-6]. However,

in the case of nucleobases commonly considered mechanism for deformation of stacked and hydrogen bonded base pairs involves for example flipping of bases accompanied by breaking of hydrogen bonds or stacking interactions [7-10].

On the other hand, during the last decade it has been demonstrated that the nucleic acid bases (see Scheme 1 for structures and atomic numbering schemes) also should be considered as quite flexible moieties. In particular, this concerns the configuration and orientation of amino group in cytosine, guanine and adenine, and conformational flexibility of pyrimidine ring in all bases. Such findings compel changes of the viewpoint on possible relaxation of DNA geometry during interactions with many drugs, metal ions and proteins. In addition, this phenomenon also influences molecules which are able to intercalate between two adjacent pairs of bases. In such a case, high conformational flexibility of nucleobases can create the most suitable conditions for penetration of intercalators and stabilization of their complexes with DNA.

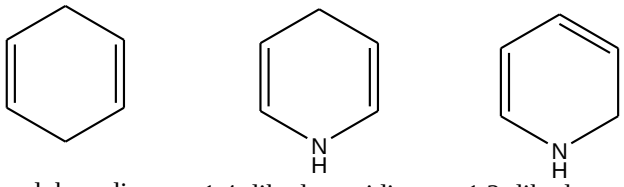
There is some imbalance in studying and describing those two phenomena. In spite that both of them were first predicted computationally [11, 12], only nonplanarity of amino-group had been confirmed experimentally [13] and attracted considerable attention in the literature (see, for example [14, 15]. In case of conformational flexibility of DNA bases only indirect experimental evidences have been revealed [16-18]. Therefore, in this review we discuss in detail the current status of investigations on the ability of pyrimidine ring of DNA bases to fluctuate between planar and nonplanar states.




**Scheme 1**. Structure and atomic numbering of nucleic acid bases.

## 21.2 Ab initio studies of conformational flexibility in nucleic acid bases

The assumption about conformational flexibility of pyrimidine rings of nucleic acid bases proceeds from results of investigation of conformational properties of various dihydroaromatic rings [19]. Conformational flexibility of six-membered dihydrocycles containing saturated carbon atoms within ring is well known for many years. For example, it is well recognized that 1,4-cyclohexadiene (Scheme 2) molecule may easily change planar equilibrium conformation to the boat one characterized by endocyclic torsion angles assuming values up to $30^{o}$ [20]. Such transformation of ring is accompanied by rather small changes of energy – less than 2 kcal/mol [21]. The same feature was revealed for 1,4-dihydropyridine (Scheme 2) and 1,4-dihydropyrimidine rings [22-24], despite of presence of different substituents at the saturated carbon atoms [25]. High conformational flexibility of such rings was explained by a balance of two opposite factors influencing conformation of dihydrocycle [20]: i) bending strain which is maximal at the planar geometry of ring and ii) 1,2-allylic strain which is minimal at the planar conformation. Therefore transition of ring from planar to boat conformation results in simultaneous decrease of bending strain and increase of 1,2-allylic strain leading to very small changes of total energy of molecule for a large range of values of endocyclic torsion angles.




1,4-cyclohexadiene 1,4-dihydropyridine 1,2-dihydropyridine

**Scheme 2**. Structure of 1,4-cyclohexadiene, 1,4-dihydroipyridine and 1,2-dihydroipyridine.

However, non-planar geometry of dihydroaromatic rings was found also in compounds which do not contain saturated carbon atoms within ring (see for example reference [26]). More detailed analysis of potential energy surface of various 1,4- and 1,2-dihydroaromatic rings revealed that all these molecules possess high conformational flexibility [27-30]. A transition between planar equilibrium and non-planar conformation (characterized by the values of endocyclic torsion angles up to 20$^{o}$) results in molecular energy increase by less than 1.5 kcal/mol. It was concluded that conformational flexibility is a general property of all types of dihydroaromatic rings [19].

In the case of absence of the saturated carbon atoms the bending and 1,2-allylic strains cannot be considered as the reasons for a flat potential energy surface around minimum. Therefore, it was suggested [27, 28] that the deviation of characteristics of cyclic conjugated systems from their aromatic features is responsible for conformational flexibility of such dihydroaromatic rings. In addition, it was demonstrated that the decrease of polarity of exocyclic double bonds in para-benzoquinone accompanied by decrease of concentration of π-electrons within ring results in reduction of conformational flexibility of dihydrocycle due to enhancement of degree of aromaticity. The opposite trend was found for derivatives of 1,4-, 1,2-dihydropyridine containing formally non-aromatic 7-π-electron cyclic conjugated system. From general viewpoint the pyrimidine rings in cytosine and guanine represent dihydroaromatic rings. Therefore taking into account results of investigation of conformational flexibility of different types of dihydrocycles it is possible to expect that these rings should also possess high conformational flexibility. The same conclusion should be derived for tetrahydroaromatic rings in uracil and thymine where cyclic conjugated system formally contains 8-π electrons and should be considered as antiaromatic. It was demonstrated that antiaromatic dihydrocycles are considerably more flexible than other dihydroaromatic rings [31].

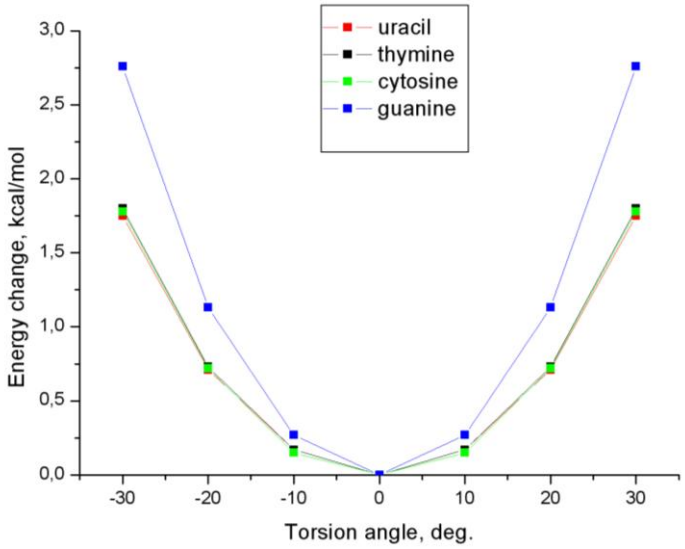
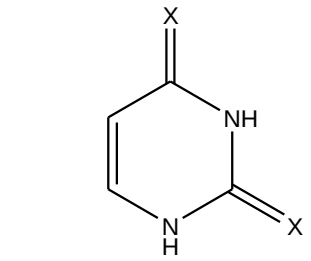


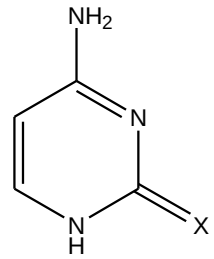

**Fig. 21.1** Dependence of energy change on value of the N1-C2-N3-C4 torsion angle in uracil and thymine, the C6-N1-C2-N3 torsion angle in cytosine and C2-N1-C6-C5 torsion angle in guanine. The data are from the MP2/6-31G(d,p) level of calculations [32].

The results of quantum-chemical studies using AM1 [12], HF [32] and MP2 [33] methods demonstrated that pyrimidine rings in uracil, thymine, cytosine and guanine possess high conformational flexibility. A change of relevant endocyclic torsion angle within $20^o$ results in energy increase by less than 1.5 kcal/mol (Fig.1). The most flexible rings are in uracil, thymine and cytosine and the most rigid one in guanine. This agrees well with general trends of conformational flexibility of six-membered rings without the saturated carbon atoms.

The main reason of high conformational flexibility of pyrimidine rings in nucleobases is deviation of a character of cyclic conjugated system from genuine aromatic features. Replacement of exocyclic C=O bond by less polar C=NH and $C=CH_2$ fragments leads to increased concentration of π-electrons within conjugated system of pyrimidine ring. This results in enhancement of anti-aromatic (for uracil and thymine) or non-aromatic (for cytosine and guanine) character of cyclic conjugated system within heterocycle [32], accompanying by considerable increase of conformational flexibility of ring (Fig. 21.2).




uracil X=O,
methyleneuracil $X=CH_2$




cytosine X=O,
methylenecytosine $X=CH_2$

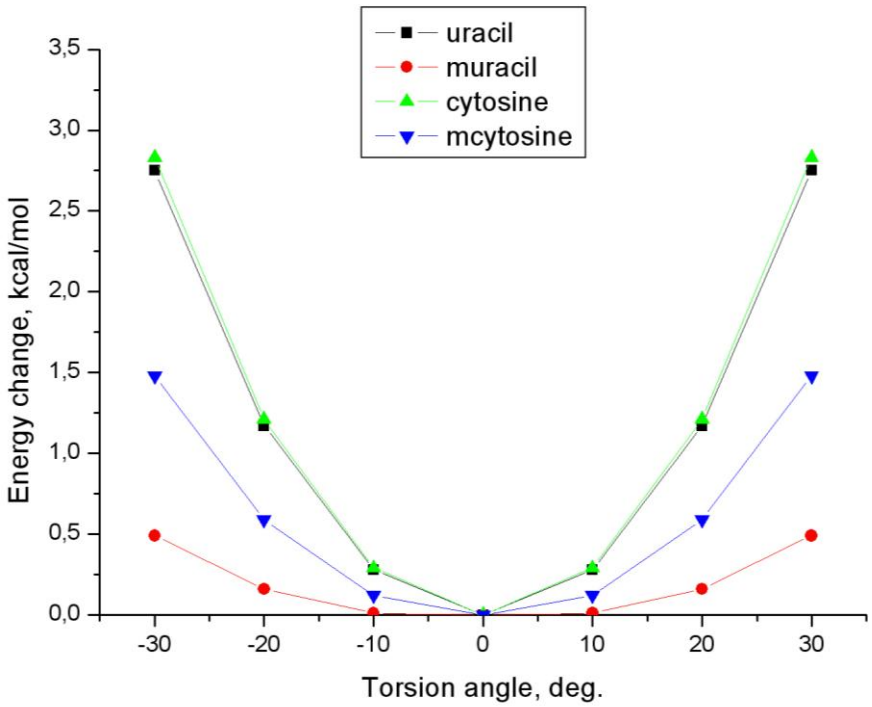



**Fig. 21.2** Dependence of energy change on the value of the N1-C2-N3-C4 torsion angle in uracil and the C6-N1-C2-N3 torsion angle in cytosine and their methylene analogues predicted at the HF/6-31G(d,p) level of calculations [33] .

High deformability of any molecular fragment usually results in existence of low-lying modes of normal vibrations [34]. A comparison of the easiest deformation modes resulted from scan of endocyclic torsion angle with a profile of ring out-of-plane normal vibrations demonstrates their remarkable agreement [33]. For example, in the case of uracil it is possible to suggest two directions of ring out-of-plane deformations associated with out-of-plane motions of the N1 and N3 atoms. In terms of endocyclic torsion angles these deformations may be described as change of the C6-N1-C2-N3 and C2-N3-C4-C5 torsion angles. Quantum-chemical calculations at different levels of theory [12, 32, 26] indicate that energy change revealed during scan for latter torsion angle is smaller (Table 21.1). This corresponds to lower vibrational frequency for out-of-plane vibration of the N3 atom [26].

**Table 21.1**. Frequencies of ring out-of-plane vibrations and change of energy during scans of the potential energy surface for nucleic acid bases calculated by MP2/6-31G(d,p) method [33].

| Molecule | Frequency, $cm^{-1}$ | Assignment | Torsion angle for scan | Value of torsion angle | | |
|---|---|---|---|---|---|---|
| | | | | 10 | 20 | 30 |
| Uracil | 131 | N(3) out-of-plane | N(1)-C(2)-N(3)-C(4) | 0.17 | 0.71 | 1.75 |
| | 158 | N(1) out-of-plane | C(6)-N(1)-C(2)-N(3) | 0.22 | 0.94 | 2.31 |
| Thymine | 136 | N(3) out-of-plane | N(1)-C(2)-N(3)-C(4) | 0.17 | 0.73 | 1.80 |
| | 106 | N(1) out-of-plane | C(6)-N(1)-C(2)-N(3) | 0.22 | 0.93 | 2.31 |
| Cytosine | 127 | N(1) out-of-plane | C(6)-N(1)-C(2)-N(3) | 0.15 | 0.72 | 1.78 |
| | 202 | NH2 wag. | C(2)-N(3)-C(4)-C(5) | 0.41 | 1.79 | 4.26 |
| Guanine | 129 | N(1) out-of-plane | C(2)-N(1)-C(6)-C(5) | 0.27 | 1.13 | 2.76 |
| | 151 | C=O wag. | C(4)-C(5)-C(6)-N(1) | 0.39 | 1.63 | 4.04 |

Thus, high conformational flexibility of pyrimidine ring in nucleic acid bases may be confirmed by experimental investigation of low-lying normal vibrations of these molecules. A comparison of calculated values of vibrational frequencies of such vibrations with experimental data for uracil [35, 36], thymine [37, 38], and guanine [29] demonstrates good agreement between theory and experiment. This may be considered as experimental confirmation of high conformational flexibility of pyrimidine ring in nucleic acid bases.

Another experimental verification of theoretical data may be provided by analysis of range of variation of endocyclic torsion angles in crystal structures containing uracil and cytosine fragments. It was demonstrated [32] that range of variation of the N1-C2-N3-C4 torsion angle in uracil and the C6-N1-C2-N3 torsion angle in cytosine amounts to $\pm 12\text{-}13^{o}$. For example, it was found that value of the C6-N1-C2-N3 in the crystal of 5-bromo-2'-deoxycytidine is $-7.1^{o}$, $-12.4^{o}$, and $-13.9^{o}$, respectively for three molecules in asymmetrical part of unit cell [39].

More detailed analysis of low-lying ring out-of-plane vibrational modes in internal coordinates revealed several interesting features concerning conformational flexibility of heterocycles in nucleic acid bases. It was demonstrated that endocyclic torsion angle characterized by the smallest energy change during the potential energy surface scan has the maximal amplitude of changes related to the corresponding normal vibrations [33]. This allows suggesting simple method for determination of suitable torsion angle for scan in any cyclic system.

An analysis of low-lying normal vibrations in nucleic acid bases revealed existence of two vibrational modes including ring out-of-plane deformations. There are the N1 and N3 out-of-plane motions in uracil and thymine, wagging vibrations of amino group in cytosine and carbonyl oxygen in guanine including also considerable out-of-plane pyrimidine ring deformation. An investigation of potential energy surface for these modes of deformation demonstrates that both these modes are very soft. The values of ring deformation energy during scan along relevant endocyclic torsion angle (up to $\pm 20^{o}$) do not exceed 2 kcal/mol. It

should be noted that deformation of pyrimidine ring during wagging vibrations of amino and carbonyl groups in cytosine and guanine results in considerable twist of endocyclic double bonds. Nevertheless, the value of ring deformation energy remains rather small.

Taking into account a good agreement between values of calculated and experimental ring out-of-plane vibrational frequencies and shape, character, and also energy of ring out-of-plane deformations, it is possible to suggest that the values of vibrational frequencies may be considered as universal and experimentally verified indicators of conformational flexibility of pyrimidine rings. However, a comparison of vibrational frequencies and ring deformation energies for out-of-plane motions of the N1 and N3 atoms in uracil and thymine indicates that the vibrational characteristics should be used for these purposes with cautions. In the case of uracil out-of-plane vibration of the N3 atom has lower values of both the frequency of normal vibration and ring deformation energy. It is difficult to assume that replacement of the hydrogen atom by methyl group in thymine should significantly influence intramolecular interactions within ring and, therefore, ring flexibility. In agreement with this expectation ring deformation energy for out-of-plane motion of the N3 atom in thymine is smaller as compared to the N1 atom. However, the values of frequency of the corresponding vibrations have an opposite order. The frequency of out-of-plane-vibration related to the N1 atom becomes lower then the one belonging to N3 atom. Detailed analysis of character of this vibrational mode indicates that this vibration involves also out-of-plane motions of methyl group. Therefore, kinetic effects, namely involvement of exocyclic substituent in molecular motions results in decrease of vibrational frequency without considerable changes of the potential energy surface [33].

An analysis of character and frequencies of normal vibrations and scan of relaxed potential energy surface represents two complementary approaches to investigation of conformational flexibility of pyrimidine rings in nucleic acid bases. A combined application of these approaches allows estimating population of conformations with non-planar rings for each molecule.

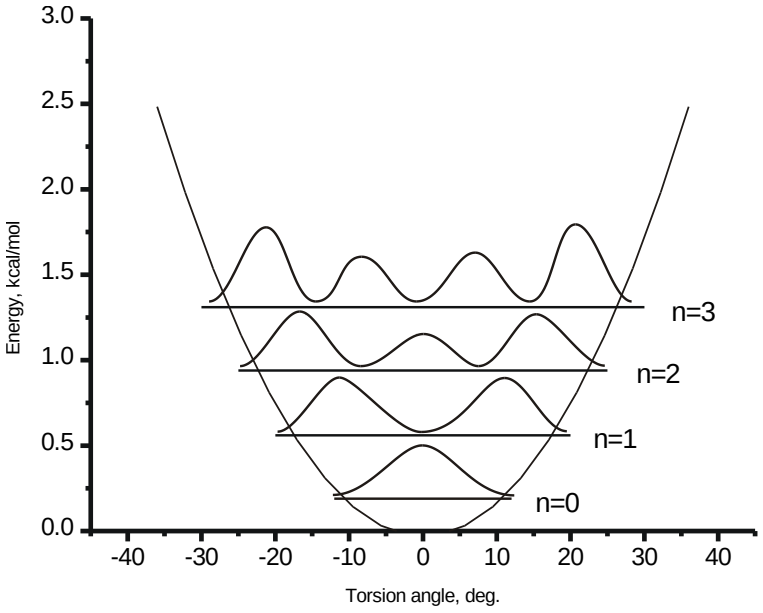



**Fig. 21.3** Probability density function for different vibrational levels of normal vibrations. Dependence of energy change on the value of torsion angle corresponding to the out-of-plane deformation of pyrimidine ring in uracil. MP2/6-31G(d,p) level calculations.

It is well-known that the position of maximum of probability density functions of low-lying levels of quantum mechanical harmonic oscillator strongly depends on a position of energy level [40]. For zero level this maximum falls roughly in the center of minimum of the potential function for vibration (Fig.3). However, for higher levels of harmonic

oscilations maxima of probability are located near potential wells. Therefore, molecules which populate these levels will possess geometry that differs considerably from the geometry of minimum of the potential energy.

In the case of discussed molecules it means that for the nucleobase molecules populating zero vibrational level for every vibration the planar or nearly planar geometry (Fig. 21.3) is the most probable. Molecules with considerably non-planar conformation of pyrimidine ring will populate higher vibrational levels. Therefore, we can estimate a fraction of molecules with essentially non-planar geometry based on population of vibrational levels characterized by number 1 and higher. Similar approach is used for analysis of structure and vibrational spectra of molecules with large amplitude motions [34].

**Table 21.2** Energy and population of harmonic vibrational levels for the lowest vibrational modes of nucleobases at 298 K. The values of harmonic vibrational frequencies calculated at the MP2/6-31G(d,p) level of theory [33] .

| Molecule | Frequency, $cm^{-1}$ | Level | Energy, kcal/mol | Population, % |
|---|---|---|---|---|
| Uracil | 131 | 0 | 0.19 | 47 |
| | | 1 | 0.56 | 25 |
| | | 2 | 0.94 | 13 |
| | | 3 and higher | ≥1.31 | 15 |
| | 158 | 0 | 0.23 | 53 |
| | | 1 | 0.68 | 25 |
| | | 2 | 1.13 | 12 |
| | | 3 and higher | ≥1.59 | 10 |
| Thymine | 136 | 0 | 0.20 | 48 |
| | | 1 | 0.58 | 25 |
| | | 2 | 0.97 | 13 |
| | | 3 and higher | ≥1.36 | 14 |
| | 145 | 0 | 0.21 | 50 |
| | | 1 | 0.62 | 25 |
| | | 2 | 1.04 | 12 |
| | | 3 and higher | ≥1.46 | 13 |
| Cytosine | 127 | 0 | 0.18 | 46 |
| | | 1 | 0.55 | 25 |
| | | 2 | 0.91 | 13 |
| | | 3 and higher | ≥1.27 | 16 |
| | 202 | 0 | 0.29 | 62 |
| | | 1 | 0.87 | 23 |
| | | 2 | 1.45 | 9 |
| | | 3 and higher | ≥2.03 | 6 |
| Guanine | 129 | 0 | 0.18 | 46 |
| | | 1 | 0.55 | 25 |
| | | 2 | 0.92 | 13 |
| | | 3 and higher | ≥1.29 | 16 |
| | 151 | 0 | 0.22 | 52 |
| | | 1 | 0.65 | 25 |
| | | 2 | 1.08 | 12 |
| | | 3 and higher | ≥1.52 | 11 |

The population of harmonic vibrational levels for every out-of-plane ring vibration may be evaluated using Boltzmann distribution function. These values for nucleic acid bases are listed in Table 21.2. As can be seen the population of the zero level for the lowest vibrations in molecules under study does not exceed 53%. Therefore, one can conclude that the remaining molecules have a non-planar geometry of pyrimidine ring. The degree of ring deformation may be approximately estimated based on ring deformation energy (Table 21.1)

and energy of populated levels (Table 21.2). For example, energy of the first vibrational level of the lowest vibration of uracil amounts to 0.56 kcal/mol. This corresponds to the ring deformation with the value of relevant torsion angle *ca.* ±17°. Therefore, one can conclude that 25% of uracil molecules in every moment of time possess a non-planar geometry of pyrimidine ring with the value of torsion angle about 17°. The degree of ring non-planarity increases with an increase of energy of the vibrational level. In uracil, for the third vibrational level (population 7%) the magnitude of relevant torsion angle is about 26°.

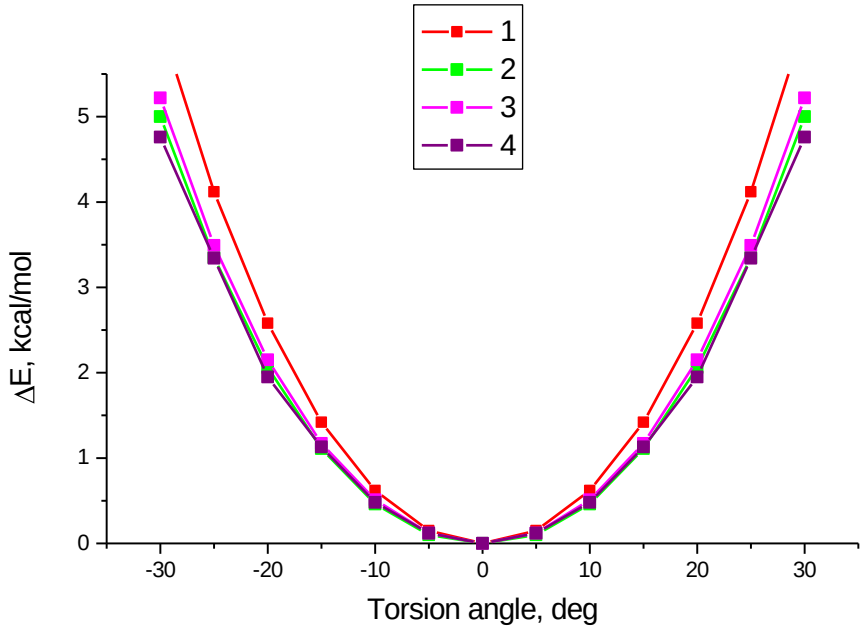
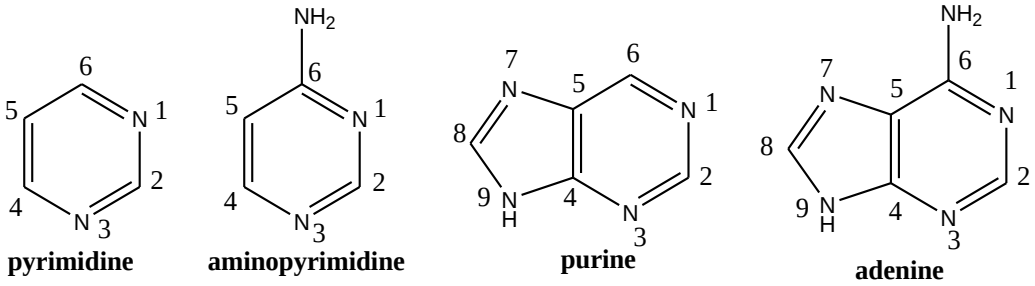



**Fig. 21.4** Dependence of energy change on value of the C4-C5-C6-N1 torsion angle in adenine (4) and aminopyrimidine (2), the N3-C4-C5-C6 torsion angle in pyrimidine (1) and purine (3). MP2/6-31G(d,p) level calculations.

Unlike in the case of uracil, thymine, cytosine, and guanine, the pyrimidine ring in adenine possesses clearly aromatic character. Therefore, it was expected that this ring should be conformationally rigid. However, an analysis of normal vibration of adenine demonstrated an existence of vibrational mode with frequency of only 166 $cm^{-1}$ [41] corresponding to the wagging motion of amino group involving considerable pyrimidine ring out-of-plane deformation. A scan of relaxed potential energy surface for such deformation of six-membered ring in adenine indicates that this heterocycle possesses notable degree of conformational flexibility despite of its aromatic character (Fig. 21.4). A change of value of endocyclic torsion angle (up to ±20°) results in energy increase less than 2 kcal/mol [41].




**Scheme 3**

An analysis of conformational flexibility of pyrimidine ring in related molecules (purine, aminopyrimidine and unsubstituted pyrimidine (Scheme 3)) indicates that the flat character of the potential energy surface around minimum is a general property of pyrimidine ring. A presence of amino group and fused imidazole ring only promotes increase of conformational flexibility of heterocycle.

**Table 21.3** Energy and population of harmonic vibrational levels for the lowest vibrational modes of adenine and related molecules at 298 K. The values of harmonic vibrational frequencies calculated at the MP2/cc-pvdz level of theory [41].

| | | Pyrimidine | Aminopyrimidine | Purine | Adenine |
|---|---|---|---|---|---|
| Freq., $cm^{-1}$ | | 354 | 201 | 232 | 166 |
| N=0 | E, kcal/mol | 0.51 | 0.29 | 0.33 | 0.24 |
| | Popul., % | 82 | 62 | 67 | 55 |
| N=1 | E, kcal/mol | 1.52 | 0.86 | 1.00 | 0.71 |
| | Popul., % | 15 | 24 | 22 | 25 |
| N=2 | E, kcal/mol | 2.54 | 1.44 | 1.66 | 1.19 |
| | Popul., % | 3 | 9 | 8 | 11 |
| N=3 | E, kcal/mol | 3.65 | 2.02 | 2.33 | 1.67 |
| | Popul., % | 0 | 4 | 3 | 5 |

Estimation of population of the ground and excited vibrational levels for ring out-of-plane vibrations demonstrates that even in the case of unsubstituted pyrimidine ring 18% of molecules possess considerably non-planar geometry of ring at every moment of time. In the case of adenine the population of non-planar conformations approaches almost 50% (Table 21.3).

Further investigation of conformational flexibility of aromatic rings indicates that this property is general for all molecules in this class including benzene [16, 42, 43]. The degree of conformational flexibility depends on presence of heteroatoms, fused rings, or substituents. Moreover, it was found that the transition of the ring into non-planar conformation with values of endocyclic torsion angles up to $\pm 30^{o}$ does not influence characteristics of electron density distribution [35] and aromaticity of benzene ring [44].

The most complete picture of conformational flexibility of pyrimidine rings in nucleic acid bases has been provided by molecular dynamics study of isolated molecules using ab initio Carr-Parinello method [45]. According to these studies the population of planar conformation of heterocycle does not exceed 20% for thymine, cytosine, and guanine and amounts to about 30% for adenine (Table 21.4). These values are considerably smaller as compared to estimations based on vibrational frequencies mentioned above. Such difference is quite natural because in the case of vibrational analysis only one the lowest ring out-of-plane normal mode is considered. However, there are also smaller contributions of the other ring out-of-plane vibrations not included in this analysis. Therefore, such estimation should be considered as an upper limit for assessment of population of planar conformation of ring.

An analysis of conformational flexibility of imidazole rings in guanine and adenine indicates that these heterocycles are much more rigid (Table 21.4). However, even in this case more than 30% of molecules have also considerably non-planar geometry of ring.

It should be noted that an investigations of conformational flexibility of pyrimdine rings in nucleic acid bases had considered only isolated molecules. Therefore, a question how this property is affected by intermolecular interactions in solutions or DNA macromolecules should be independently addressed. An investigation of conformational flexibility of rings in Watson-Crick adenine-thymine and guanine-cytosine pairs of bases demonstrated [46] that the formation of hydrogen bonds between bases virtually does not influence energy of pyrimidine rings deformation. This establishes the possibility for effective relaxation of geometry of hydrogen bonded nucleobases with respect to different steric clashes. In particular, it was found that pyrimidine rings in stacked dimers of uracil,

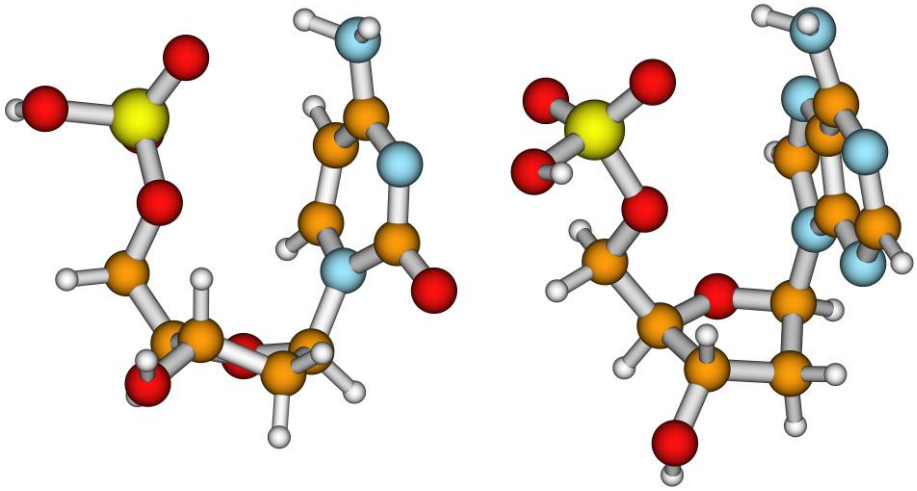
cytosine and also adenine-uracil, and uracil-cytosine pairs possess considerable non-planarity [47, 48]. However, a comparison of energy of interactions between planar and non-planar stacked bases indicates rather small difference in their values (about 1 kcal/mol).

**Table 21.4** Population of planar conformation of rings (with endocyclic torsion angles less than $10^{o}$ ) in nucleic acid bases according to Carr-Parinello molecular dynamics simulations [45].

| *Molecule* | *Ring* | *Population, %* |
|---|---|---|
| Thymine | Pyrimidine | 11.9 |
| Cytosine | Pyrimidine | 17.1 |
| Guanine | Pyrimidine | 17.8 |
| Guanine | Imidazol | 66.0 |
| Adenine | Pyrimidine | 30.3 |
| Adenine | Imidazole | 64.2 |

Therefore it is possible to conclude that high conformational flexibility of pyrimidine rings does not influence significantly the energy of stacking interaction between bases in DNA macromolecules.

An investigation of structure of polyhydrated complexes of adenine [48] and guanine [49] demonstrates that asymmetric shape of hydrogen environment may lead to significant non-planarity of the pyrimidine ring. High conformational flexibility allows adjustment of heterocycle conformations in order to provide conditions for maximal attraction between base and water. Therefore, it is possible that their conformational flexibility also remains in water solution despite of presence of numerous hydrogen bonds. This agrees well with small changes of value of ring out-of-plane vibration frequency in complex of cytosine with 13 water molecules [4, 50].



CMP AMP

**Fig. 21.5** Structure of orthogonal conformers of 2'-deoxyriboadenosine monophosphate (AMP) and 2'-deoxycytidine monophosphate (CMP) with non-planar pyrimidine ring according to calculations at the B3LYP/aug-cc-pvdz level [4].

A confirmation of the role of conformational flexibility of pyrimidine rings as efficient way for geometry relaxation was found during an investigation of conformational characteristics of canonical 2'-deoxyribonucleotides. It was demonstrated that formation of intramolecular hydrogen bonds between amino and phosphates groups of 2-deoxyriboadenosine monophosphate and 2'-deoxycytidine monophosphate results in

significant out-of-plane deformation of pyrimidine ring [4, 5, 51]. The values of endocyclic torsion angles are up to $23^{o}$ for cytosine and $13^{o}$ for guanine. This allows creating the most suitable conditions for formation of hydrogen bonds (Fig. 21.5). In addition to discussed theoretical studies, as it was already mentioned, there are also indirect experimental evidences of conformational flexibility DNA bases and related molecular species [16, 17, 52].

## 21.3 Conclusions

Contrary to earlier believes the recent computational studies have revealed that DNA bases are fairly flexible. There are two sources of conformational flexibility of nucleobases: nonplanarity of amino groups and high deformability of the pyrimidine ring. The conformational flexibility of pyrimidine rings which is a topic of this review represents very important way for relaxation of molecular geometry as a response to intermolecular interactions or steric clashes. This is especially important for understanding the mechanisms of molecular recognition, for example, during intercalation process. In such a case nucleic acid bases are able easily change their conformation in order to maximize interactions with intercalating agent without considerable changes of electronic properties or intermolecular interactions with neighboring bases.

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