Theoretical Studies on the Tautomeric Properties of Diamino-5-formamidopyrimidines

Piotr Cysewski

Ludwik Rydygier University of Medical Sciences in Bydgoszcz, Department of Clinical Biochemistry, Karlowicza 24, 85–092 Bydgoszcz, Poland

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The results of theoretical geometry prediction of formamidopyrimidine(fapy)-adenine and fapy-guanine tautomers are presented. Among 54 potential tautomeric structures of fapy-adenine the most stable structure corresponds to the diamino-keto isomer. The solvent effect has insignificant influence on the fapy-adenine tautomers succession. The fapy-guanine has 172 potential isomers. There are three most stable tautomers of this guanine derivative, which may exchange the order depending on the polarity of the environment. In vapour the most probable is the 4-enol-6-keto-diamino tautomer, while in water environment the 4,6-diketo-diamino isomer is dominant. A more polar solvent stabilises more polar fapy-guanine tautomers.

The geometric parameters and point-atomic charges corresponding to most probable tautomers are also supplied.

Introduction

Oxidative stress in cells is related to lack of equilibrium between prooxidant and antioxidant species (Halliwell, Aruoma, 1993) and (Cadet, Vigny, 1990). Such state occurs due to reactive oxygen agents (Demple, Levin, 1991) generated either by the cellular metabolism such as phagocytosis, mitochondrial respiration, xenobiotic detoxification, or by exogenous factors such as ionising radiation or chemical compounds performing red-ox reactions. The most important seems to be hydroxyl radicals, which can alter every macromolecule inside the living cell (Dizdaroglu, 1988) and (Halliwell, Aruoma, 1991). Due to its extreme reactivity, the $OH^{\cdot}$ radical generates various lesions in DNA such as base modifications and degradation products of deoxyribose chain and (Dizdaroglu, 1990). The broad spectrum of different derivatives one may found in the products of free radical caused degradation of DNA (Dizdaroglu, 1985). The oxidation of purine bases (von Sonntag, 1994) and (Steenken, 1992) may lead to both substitution and ring opening (Laval *et al.,* 1990), (Laval *et al.,* 1991) and (Olinski *et al.,* 1992). The first mechanism is related to oxidation mainly at C_2 and C_8 positions and produces such derivatives as 8-oxo-guanine, xanthine, 2-hydroxy-adenine and 8-oxo-adenine (Olinski *et al.,* 1992). The oxidation of C_8 atom results in ring opening and formation of 2,6-diamino-4-oxy-5-formamidopyrimidine and 4,6-diamino-5-formamidopyrimidine (O'Connor *et al.,* 1988) and (Neto *et al.,* 1992). These products are usually abbreviated as fapy-guanine (fapy-G) and fapy-adenine (fapy-A) (Lutgerink *et al.,* 1992) and (Muller *et al.,* 1995). Such DNA base lesions were identified in mammalian tissues such as cancerous female breast, lung, brain, and other human tissues (Malins, 1993). This evidence suggested that the base lesions are broadly present in the cancerous and microscopically normal tissues of a variety of eukaryotic organisms and are supposed to be one of the reasons of mutagenesis and carcinogenesis (Boiteux *et al.,* 1989) and (Shibutani *et al.,* 1991). Besides, it is generally assumed that the presence fapy-G and fapy-A strongly blocks DNA synthesis *in vitro* (Tudek *et al.,* 1992). These derivatives are cytotoxic and may have also promutagenic properties (Kamiya *et al.,* 1995) such as C → T transitions or G → T transversions (Palmer *et al.,* 1997). Additionally, oxidative guanine and adenine damage has been implicated in the pathology of different diseases such as Parkinson's disease (Alam *et al.,* 1997) and neuronal loss in Alzheimer's disease (Lyras *et al.,* 1997).

Reprint requests to Dr. Cysewski.
Fax: (04852) 3415933.
E-mail: piotrc@aci.amb.bydgoszcz.pl

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Knowledge of the biological significance of hydroxyl radical modified bases must be accomplished by theoretical studies concerning fundamental properties of such derivatives. This work deals with characteristics of energetic, structural and electrostatic properties of different tautomers of the fapy-guanine and fapy-adenine. Since intermolecular complex formation is strongly related to the tautomeric form this paper intend to find out the tautomers succession of all potential tautomers of fapy-A and fapy-G, which may occur in the DNA.

Until now only a few papers were published presenting high-level theory results of hydroxyl radical modified nucleic acid bases. Nonella *et al.* (1998) characterised protonated and co-ordinated purine derivatives on the semiempirical and Hartree-Fock minimal basis levels. They have studied xanthine, hypoxanthine, allopurinol and alloxanthine in H_1, H_3 H_9 tautomeric forms and protonated H_7 derivatives. Aida and Nishimura (1987) characterised the tautmomerism of 8-oxo-guanine. Miaskiewicz *et al.* (1993) studied the thymine glycol and 5,6-dihydro-thymine conformations. Recently, the tautomerism of hydroxyl radical adenine, guanine, cytosine and thymine derivatives was characterised (Cysewski P. *et al.*, 1995a; 1995b; 1996). Besides, the coding properties of 2-OH-adenine and 8-oxo-adenine were also the subject of calculations (Cysewski P., 1998).

Calculations

Purine derivatives can exist in different keto-enol and amino-imino tautomeric forms. The potential structures, which may be adopted by fapy-adenine and fapy-guanine are schematically presented in figures 1 and 2. Arrows indicate the proton migration or side group rotation. The N_9 centre was not taken into account since has no influence on the tautomeric equilibrium after purine connection to sugar moiety. In this paper the geometry optimisation was performed on standard routine of quantum chemistry. The Hartree-Fock (HF) and density factional theory (DFT) procedures was applied with the standard 6–31G** Gaussian basis functions (Frisch *et al.*, 1995). Such calculations are very time consuming and may not be applied to all 54 structures of fapy-A and 172 isomers of fapy-G. The selection of most probable

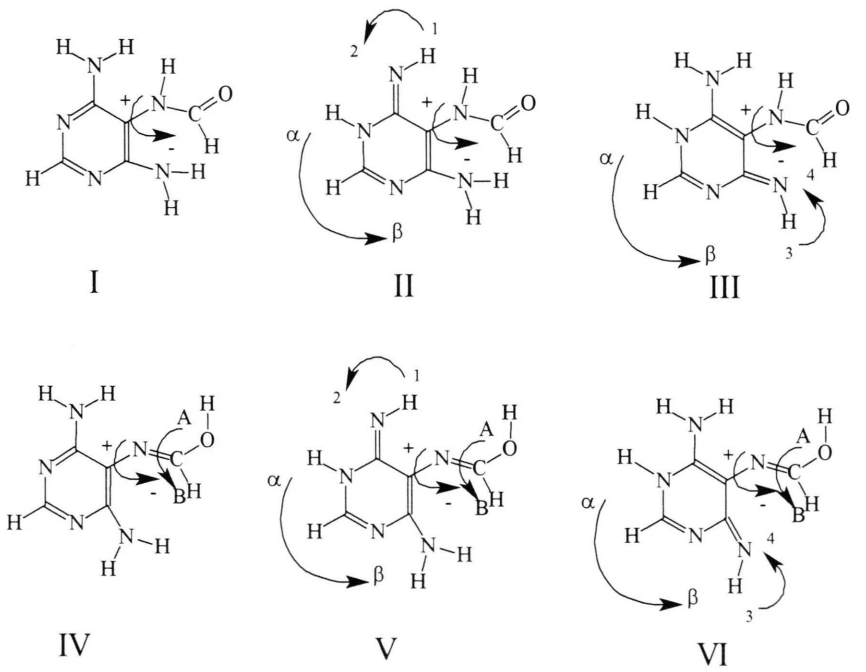


Fig. 1. Structures of all studied tautomers of fapy-adenine. All potential 54 isomers are grouped into six classes. Arrows indicate possible migration of hydrogen atom and rotation of side groups.

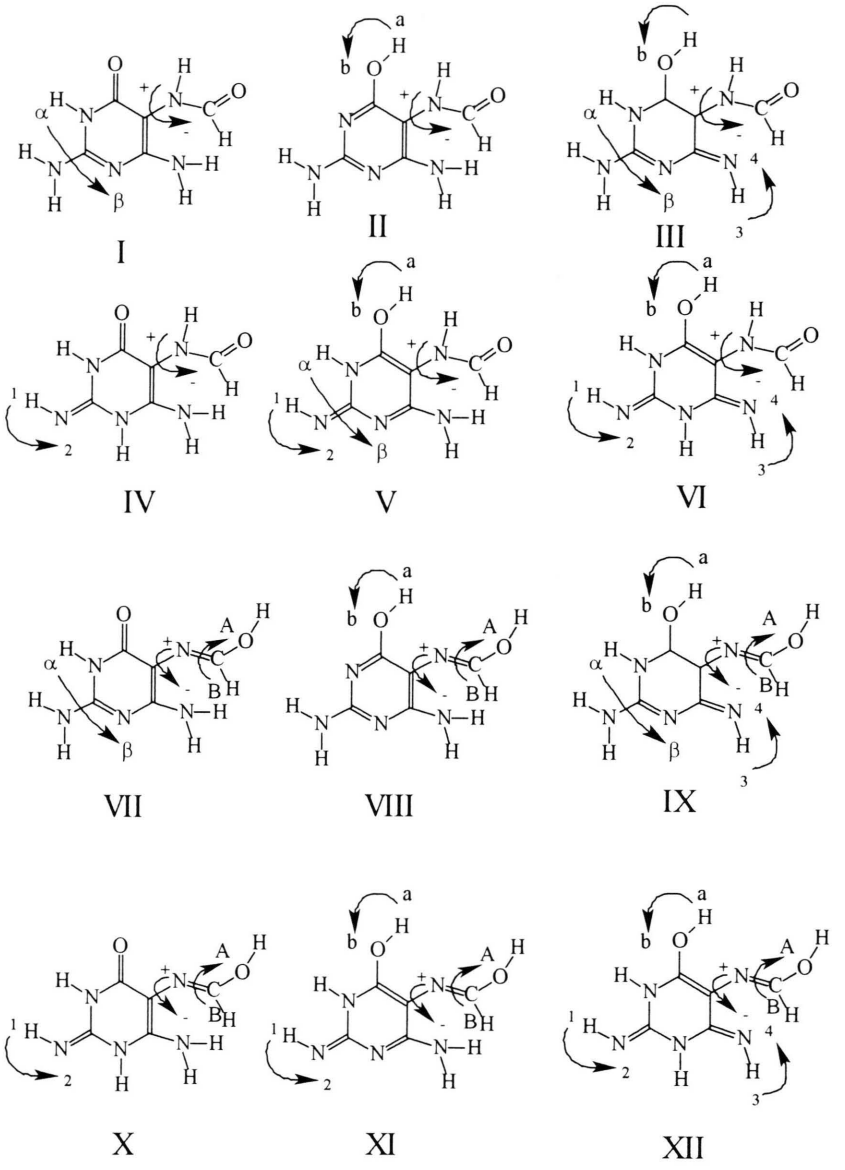


Fig. 2. Structures of all studied tautomers of fapy-guanine. The potential 172 were grouped into 12 sets.

structures was performed, based on the results of PM3 semiempririal calculations (Steward J. J. P., 1990). For these reasons all possible H_9 tautomers were constructed and optimised by means of PM3 approximation. This method was found (Cysewski P. *et al.*, 1995), as the most suitable semiempirical algorithm for characterising the hydroxyl radical modified DNA bases. Based on these results twelve most probable tautomers of fapy-A and fapy-G were selected for further analysis. After HF(6–31G**) geometry optimisation the total energy was corrected for electron correlation according to Molet-Pleser (MP2) algorithm (Steward J. J. P. *et al.*, 1994). Additionally, the density functional theory (DFT) methods were applied to molecular geometry optimisation. This methodology keeping the simplicity of one-particle approximation accounts for most static and dynamic elec-

trons correlation effects is characterised by great efficiency. Thus, the selected twelve tautomers of fapy-A and fapy-G were re-optimised on the basis of Becke-Perdev (BP86) (Perdev, J. P., 1996), Becke three parameters hybrid (B3LYP) (Becke A. D., 1993) and Vasko-Wilk-Nusair (VWM) (Vasko S. J. *et al.*, 1980) approximations implemented in Gaussian 94 (Frisch *et al.*, 1995).

Besides, importance of solvation effect on tautomer stability was estimated. For this reason self-consistent isodensity polarised continuum model (SCI-PCM) (Frisch *et al.*, 1995) was chosen to simulate water, acetone and cyclohexane solutions. In this model the solvent is not present explicitly and it is only the source of continuum field related to its dielectric constant. Solvents were declared by the following values of dielectric constant: 78.54 (water), 20.70 (acetone) and 2.02 (cyclohexane). Finally, electrostatic properties of most stable tautomeric form were obtained. Full geometry optimisation was performed for methyl-substituted fapy-adenine or fapy-guanine in their dominant tautomeric form. The methyl group was attached to mimic the N-glycosidic bond. Point atomic charges were estimated according to Merz-Singh-Kollman scheme (Frisch *et al.*, 1995). These data are necessary for the molecular dynamic simulations of DNA containing fapy-adenine or fapy-guanine.

All calculations were performed on the basis of the Gaussian-94 (Frisch *et al.*, 1995) program. No restrictions were imposed on molecule geometry during optimisation procedure.

Results and Discussion

Tautomerism of fapy-adenine

Fapy-adenine may be formed after adenine oxidation at C_8 centre and hydrolysis. As a result the C_8-N_9 bond breaks and five-member ring opening occurs. Due to free rotation of C_5-N_5-C_{5N}-O_5 branch the fapy-A structure is not flat. The presence of amino and keto groups allows for variety of tautomeric forms. Figure 1 presents 54 potential tautomers, which are grouped into six classes. First three sets correspond to structures with C_{5N}=O_5 keto group, while last three are characterised by the presence of enol O_5-H_5 group. The semiempirical calculations showed that the most probable isomers belong to I, II, IV and V groups. Thus, the presence of imido group formed by N_6 atom decreases significantly stability of the fapy-adenine. For this reason such tautomers were rejected from further analysis. The results of geometry optimisation ware presented in Table I and Figure 3. The solvent effect on the tautomers succession was presented in Table II. The comparison of the total energies allows the conclusion that the most stable tautomer of fapy-adenine has the amino-keto form. Results of both HF and DFT calculations lead to the same prediction. Although there are differences in the relative energies estimated by these two methods, the overall tautomers succession is similar. Furthermore, the presence of the solvent field does not affect the order of tautomers since in non-polar and highly polar environments the most stable is the amino-keto tautomer (**I**+).

Table I. The results of theoretical geometry optimisation of fapy-adenine tautomers. Presented data corresponds to total energies (E) expressed in atomic units. The symbols denote different methods described in the calculations section.

Fapy-A	$E^{HF}_{6-31G^{**}}$	$E^{MP2}_{6-31G^{**}}$	$E^{B3LYP}_{6-31G^{**}}$ [1]	$E^{BP}_{6-31G^{**}}$ [2]	$E^{VWM}_{6-31G^{**}}$ [3]
I+	−540.580519	−542.212006	−543.769547	−543.784452	−547.691818
II1a+	−540.553293	−542.187681	−543.746984	−543.763803	−547.671268
II2a+	−540.546358	−542.181343	−543.741717	−543.760394	−547.665374
II1b+	−540.526975	−542.163946	−543.721792	−543.738963	−547.647153
II2b+	−540.534667	−542.170702	−543.729210	−543.747790	−547.654572
IVB+	−540.546949	−542.184076	−543.745459	−543.762302	−547.667624
IVA+	−540.549979	−542.189707	−543.744738	−543.761250	−547.667236
Va1+	−540.534944	−542.179098	−543.743727	−543.765977	−547.666229
Va1−	−540.529732	−542.169803	−543.731806	−543.750793	−547.655508
Vb1+	−540.524156	−542.168494	−543.736286	−543.764243	−547.659030
Va2A+	−540.529517	−542.170282	−543.729978	−543.748874	−547.654249
Va2B+	−540.527951	−542.171804	−543.731449	−543.751844	−547.654287

[1] (Perdev, J. P., 1996), [2] (Becke A. D., 1993), [3] (Vasko S. J. *et al.*, 1980).

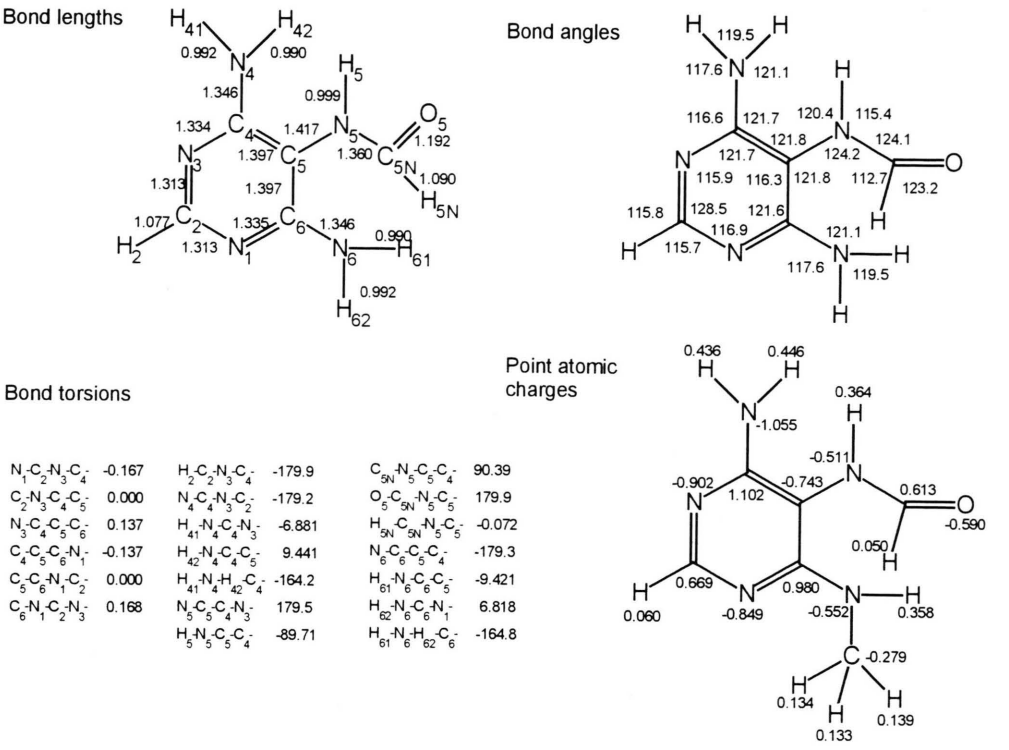


Fig. 3. The optimised geometry parameters estimated for most probable tautomer of fapy-adenine (I+). The bond lengths are expressed in angstroms, bond angles and dihedrals are presented in degrees. Point atomic charges were calculated according to Merz-Singh-Kollman scheme (Frisch *et al.*, 1995).

Table II. The impact of the solvent on tautomers succession of fapy-adenine and fapy-guanine. Presented values correspond to total energy (expressed in KJ/mol) of each tautomer related to the most stable tautomer in water. The reference point was set then on energy of + tautomer in water for fapy-adenine and tautomer **Ia+** in water for fapy-guanine, respectively.

	Fapy-Adenine					*Fapy-Guanine*			
	Water	Acetone	Cyclohexane	Vapour		Water	Acetone	Cyclohexane	Vapour
I+	0.0	2.9	34.4	49.7	Ia+	0.0	4.3	44.8	59.6
II1a+	63.2	67.0	102.9	121.2	Ib+	37.4	43.5	99.2	119.7
II2a+	72.3	77.0	118.9	139.4	IIa+	8.8	13.0	52.7	69.3
II1b+	100.2	106.5	164.1	190.3	IIb+	16.4	19.7	49.7	55.5
II2b+	96.2	101.7	151.4	170.1	VIIa	41.5	45.5	82.7	108.0
IVB+	96.1	98.9	124.9	137.8	VIIAa	42.0	45.8	81.5	94.1
IVA+	89.6	93.0	123.9	129.9	VIIb	78.5	84.9	141.1	155.5
Va1+	125.4	128.5	155.8	169.4	VIIBb	86.0	91.4	138.8	169.3
Va1-	137.0	140.2	168.8	183.1	VIIIAa+	85.5	89.2	123.2	128.1
Vb1+	142.6	146.4	180.3	197.7	VIIIBa+	68.2	71.1	98.2	105.7
Va2A+	145.6	148.5	175.9	183.6	VIIIbA+	91.4	94.5	123.1	126.7
Va2B+	153.7	156.3	180.6	187.7	VIIIbB+	72.5	74.9	97.0	112.1

The results presented in Table II show that the increases of the polarity does not change the difference between most stable tautomer **I**+ and the second one, **II1a**+. The optimised geometry parameters as well as point atomic charges corresponding to most probable tautomer are presented in figure 3. Since the part of fapy-adenine molecule responsible for the intermolecular complex formation has an analogous structure to non-modified adenine the formal coding abilities will be

similar. However, the charge dislocation may affect the actual pairing ability.

Tautomerism of fapy-guanine

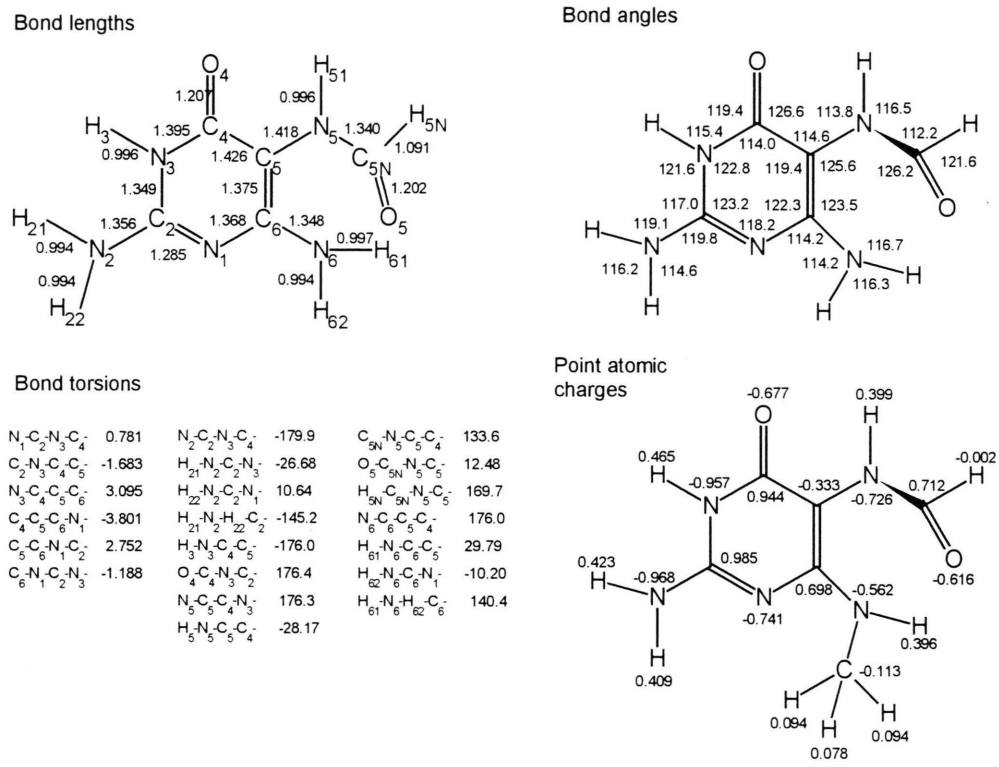
The fapy-guanine may be a result of oxidative hydroxyl radical attack on C_8 position and N_9-C_8 bond breaking. The presence of two amino and two keto groups is the source of many potential tautomeric structures. Additionally different orientations of O_5-C_{5N}-N_5-C_5 branch extend the number possible isomers. The O_5-C_{5N}-N_5-C_5 part may freely rotates around single C_{5N}-N_5 bond only if O_5=$C_{5\,N}$ carbonyl group is formed. When tautomeric form changes and H_{5N}-O_{C5} hydroxyl group appears the rotation is hindered due to formation of a double bond between C_{N5} and N_5 atoms. There are 172 potential tautomeric structures of fapy-guanine, which are schematically presented in figure 2. All structures were grouped into 12 classes. Each set consists of the same type of tautomer differing in conformations of side groups. The below and above plane orientations of O_{C5}-C_{N5}-N_5-C_5 part are denoted by plus and minus signs. All these structures were fully optimised by the semiempirical method. Taking into account values of the heat formation, twelve most probable structures were selected for further HF and DFT geometry optimisation. The results obtained are presented in Table III. It is concluded that the most stable tautomer corresponds to the diketo-diamino tautomer (**Ia**+). The second stable structure (**IIb**+), which is characterised by presence of an enol group connected to the C_4 carbon atom. Such conclusion may be drawn from MP2 and DFT results. The energy difference is less than 15kJ/mol and depends on the method of calculation. However, the HF(6–31G**) calculation predicted a reversed order of **Ia**+ and **IIb**+ tautomers. Due to such inconsistency additional calculations were performed on the dependence of the solvent field. The results of the solvent influence on tautomers succession are presented in Table II. From these data one can conclude that three most stable tautomers of fapy guanine may exchange the order depending on the polarity of the environment. In vapour state the tautomer **IIa**+ precedes the **Ia**+ and **IIb**+ ones. The increase of the environment polarity lead to the following succession of fapy-guanine tautomer: **Ia**+ > **IIb**+ > **IIa**+. However, in the presence of the water field the **Ia**+ tautomer precedes **IIa**+ and **IIb**+. This may be related to the polarity of each of tautomers. The dipole moment of isomer **Ia**+ is equal to 4.75D (debeys), while **IIa**+ and **IIb**+ tautomers are characterised by the following values of dipole moment: 3.36D and 2.45D, respectively. Thus, more polar solvent stabilises the more polar isomer. The geometrical parameters of two most stable fapy guanine tautomers are presented in figures 4 and 5. Additionally, point atomic charges estimated for N_6 methyl substituted analogues of **IIa**+ and **IIb**+ species are supplied.

It is noteworthy that the total energies of these three most stable tautomers of fapy guanine are close to each other. Thus, the actual fapy guanine

Table III. The results of theoretical geometry optimisation of fapy-guenine tautomers. Presented data corresponds to total energies (E) expressed in atomic units. The symbols denote different methods described in the calculations section.

Fapy-G	$E^{HF}_{6-31G^{**}}$	$E^{MP2}_{6-31G^{**}}$	$E^{B3LYP}_{6-31G^{**}}$ [(1)]	$E^{BP}_{6-31G^{**}}$ [(2)]	$E^{VWM}_{6-31G^{**}}$ [(3)]
Ia+	−615.462786	−617.271446	−619.017908	−619.040922	−623.357657
Ib+	−615.439915	−617.251356	−618.997477	−619.021258	−623.337538
IIa+	−615.459099	−617.261472	−619.003616	−619.023679	−623.341979
IIb+	−615.464379	−617.270926	−619.014546	−619.035511	−623.351719
VIIa	−615.444360	−617.250943	−618.997073	−619.018479	−623.337857
VIIAa	−615.449669	−617.260601	−618.982777	−619.032180	−623.346160
VIIb	−615.426278	−617.240246	−618.987105	−619.012532	−623.325983
VIIBb	−615.421048	−617.231115	−618.975094	−618.997378	−623.315261
VIIIAa+	−615.436727	−617.246098	−618.988735	−619.010576	−623.326512
VIIIBa+	−615.445261	−617.251535	−618.995444	−619.015660	−623.332175
VIIIbA+	−615.437263	−617.251452	−618.993770	−619.017896	−623.330086
VIIIbB+	−615.442795	−617.251601	−618.993070	−619.017329	−623.329886

[(1)] (Perdev, J. P., 1996), [(2)] (Becke A. D., 1993), [(3)] (Vasko S. J. *et al.*, 1980).



N_1-C_2-N_3-C_4	0.781	N_2-C_2-N_3-C_4	-179.9	C_{5N}-N_5-C_5-C_4	133.6
C_2-N_3-C_4-C_5	-1.683	H_{21}-N_2-C_2-N_3	-26.68	O_5-C_{5N}-N_5-C_5	12.48
N_3-C_4-C_5-C_6	3.095	H_{22}-N_2-C_2-N_1	10.64	H_{5N}-C_{5N}-N_5-C_5	169.7
C_4-C_5-C_6-N_1	-3.801	H_{21}-N_2-H_{22}-C_2	-145.2	N_6-C_6-C_5-C_4	176.0
C_5-C_6-N_1-C_2	2.752	H_3-N_3-C_4-C_5	-176.0	H_{61}-N_6-C_6-C_5	29.79
C_6-N_1-C_2-N_3	-1.188	O_4-C_4-N_3-C_2	176.4	H_{62}-N_6-C_6-N_1	-10.20
		N_5-C_5-C_4-N_3	176.3	H_{61}-N_6-H_{62}-C_6	140.4
		H_5-N_5-C_5-C_4	-28.17		

Fig. 4. The optimised geometry parameters estimated for most probable tautomer of fapy-guanine (Ia+). The bond lengths are expressed in angstroms, bond angles and dihedrals are presented in degrees. Point atomic charges were calculated according to Merz-Singh-Kollman scheme (Frisch *et al.*, 1995).

structure will be probably characterised by the mixture of keto and enol tautomers. This may have a significant impact on the coding abilities of fapy-guanine. The tautomer **Ia**+ has two acceptor centres located at H_{21} and H_3 hydrogen atoms and one donor centre located at the O_4 oxygen atom. Thus, the formal coding abilities may be denoted as (-++) and are similar to canonical guanine. In contrast IIb+ tautomer is characterised by two acceptor centres located at the H_{21} and H_4 hydrogen atoms and one donor centre located at the N_3 nitrogen atom. The formal coding properties may then be described as (+-+). Thus, the presence of this tautomer alters the pairing abilities of the fapy-guanine compared to canonical guanine.

Conclusions

The fapy-adenine and fapy-guanine may have an intricate structure due to five-member ring opening and formation of a O_{C5}-C_{N5}-N_5-C_5 branch. Results presented above allow the conclusion that the most probable fapy-adenine structure corresponds to di-amino form. Any isomerisation results in decrease of the molecule stability. Thus, fapy-adenine and canonical adenine have very similar ability of Watson-Crick-like hydrogen bond formation. However, the modification of the structure leads the significant charge dislocation which will influence the stability of intermolecular hydrogen bonded complexes. In the light of above results the second studied derivative, fapy-guanine, may be considered as a mixture of keto and enol tautomers. In the isolated state the enol form is more probable than keto one. However, the increase of the environment polarity led to the stabilisation of keto form over enol ones. Such tautomeric properties will have an impact on the pairing abilities of fapy-guanine. The keto form of fapy-G has the same coding potential as standard guanine. However, enol tautomers may form alternative dimers with standard nucleic acid bases.

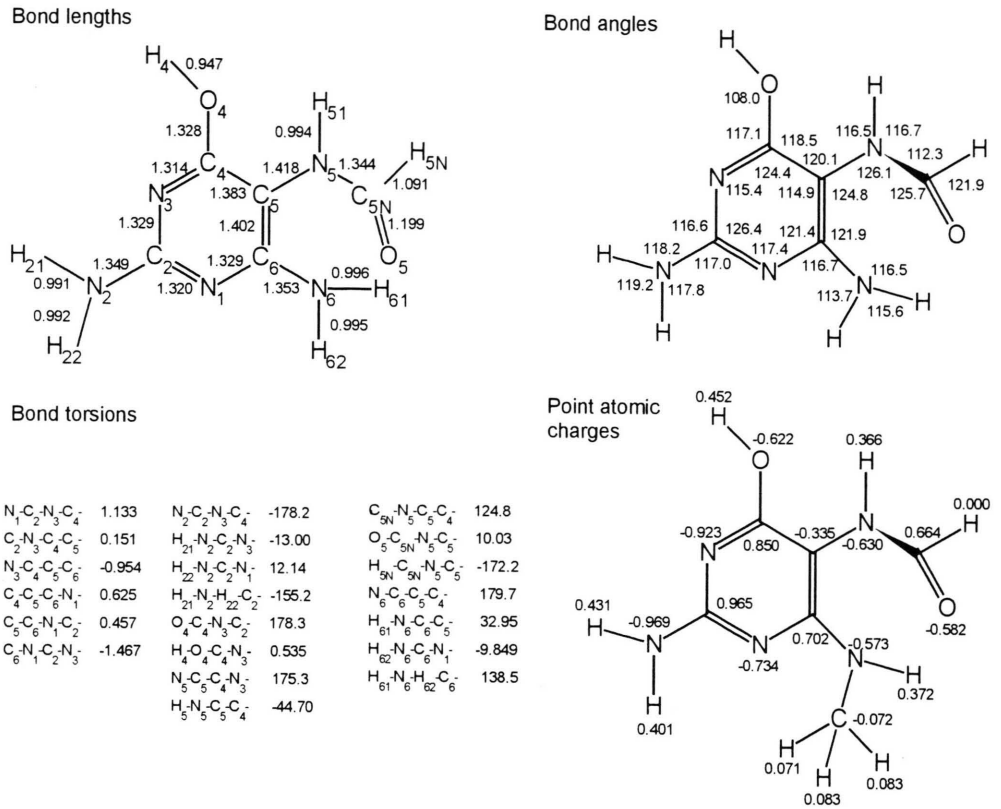


Fig. 5. The optimised geometry parameters estimated for second stabe probable tautomer of fapy-guanine (lib+). The bond lengths are expressed in angstroms, bond angles and dihedrals are presented in degrees. Point atomic charges were calculated according to Merz-Singh-Kollman scheme (Frisch *et al.*, 1995).

This may be the source of mispairing caused by oxidatively modified DNA fragments.

The presence of fapy-adenine or fapy-guanine in DNA will introduce steric hindrance due to O_{C5}-C_{N5}-N_5-C_5 part resulting from the ring opening. The value of the new dihedral angle is 90.4° and 133.6° for fapy-A and fapy-G, respectively. The almost perpendicular orientation of the O_{C5}-C_{N5}-N_5-C_5 branch is responsible for the "bulky" character of such derivatives. In DNA one may expect however, that the presence of neighbouring bases reduces value of the O_{C5}-C_{N5}-N_5-C_5 dihedral angle.

Acknowledgement

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