

## THE EFFECT OF CHLORINATION OF NUCLEOTIDE BASES ON THE CONFORMATIONAL PROPERTIES OF THYMIDINE MONOPHOSPHATE

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*Recent studies on Escherichia coli bacteria cultivation, in which DNA thymine was replaced with 5-chlorouracil have refreshed the problem of understanding the changes to physical properties of DNA monomers resultant from chemical modifications. These studies have shown that the replacement did not affect the normal activities and division of the bacteria, but has significantly reduced its life span. In this paper a comparative analysis was carried out by the methods of computational experiment of a set of 687 possible conformers of natural monomeric DNA unit (2'-deoxyribonucleotide thymidine monophosphate) and 660 conformers of 5-chloro-2'-deoxyuridine monophosphate – a similar molecules in which the natural nitrogenous base thymine is substituted with 5-chlorouracil. Structures of stable conformers of the modified deoxyribonucleotide have been obtained and physical factors, which determine their variation from the conformers of the unmodified molecule have been analyzed. A comparative analysis of the elastic properties of conformers of investigated molecules and non-covalent interactions present in them was conducted. The results can be used for planning experiments on synthesis of artificial DNA suitable for incorporation into living organisms.*

*Key words: DNA, conformational analysis, nucleotide base, deoxyribonucleotides, 5-chlorouracil, Escherichia coli.*

In a recent paper [1] it was reported that an international group of scientists working in xenobiology, a new brunch of life sciences, has succeeded in creating *Escherichia coli* bacteria, in which DNA thymine was substituted with 5-chlorouracil of synthetic origin – a substance which is toxic to other organisms. Having successfully replaced the natural nucleotide base, the scientists began to test the vital functions of the modified organisms. They observed that such substitution did not affect the normal vital functioning of the bacteria and its ability to divide normally, but it was found that these investigated organisms were not capable to transfer the modified fragments of DNA to the bacteria with natural DNA (containing thymine), in other words they cannot change the normal organisms' genomes. Moreover, these mutated bacteria could not live even for one day without "toxic" environment, because 5-chlorouracil which is vital for them is not found *in vivo*. It was also revealed that even in environments where 5-chlorouracil is available in sufficient quantities, the lifetime of the new bacteria is several times lower than for similar bacteria with natural DNA.

A reason for the reduced viability of bacteria with modified DNA can be modification of the AT pair donor-acceptor code in the DNA structure caused by the replacement of the methyl group of thymine with a chlorine atom. However, there is another possible mechanism for the effect of chlorination: changes in the conformational properties of nucleotides.

Thus, the objective of our study is to find out what kind of changes in conformational and elastic properties of the monomers of this bacteria have been caused by the substitution of thymine with 5-chlorouracil. In addition to the potential usefulness to explain the peculiarities of life of the bacteria with modified DNA, solution of this problem has independent significance, because it allows tracing the relationship of the chemical structure and physical properties of biomolecules.

### Materials and Methods

According to the main task of the work a detailed quantum-mechanical conformational analysis of molecules of 5'-thymidylic acid (5TA)

and 5-chloro-2'-deoxyuridine monophosphate (5CDUMP), deoxyribonucleotides of DNA with natural bases (thymine) and the base substituted with 5-chlorouracil, was carried out.

In all the molecules under study the phosphate group was electroneutral: the negative charge of the oxygen atoms was neutralized by a proton. Works [2-9] show that this model of the phosphate group works well in the quantum-mechanical studies of the conformational properties of DNA units.

It is known [10] that the conformation of the DNA strand units can be described by seven torsion angles, six of which determine the conformation of the sugar-phosphate skeleton and the seventh angle  $\chi$  characterizes the orientation of the nitrogenous base relative to the sugar. Fig. 1 shows the numbering system of atoms and designations of torsion angles for nucleotides.

The structures of possible conformers of the molecule 5TA were taken from [2], in which the quantum-mechanical conformational analysis of this molecule has been reported.

To get a full set of 5CDUMP conformers, the methyl group in each of the 660 unique structures of 5TA molecule was replaced by a chlorine atom. Next, the geometry of 660 nucleotide conformers with substituted base obtained in this way were optimized in package Gaussian 03 [11] by DFT B3LYP/6-31G(d, p) method with a tightened convergence criterion and high accuracy integration grid (*opt = tight* and *int = ultrafine* options).

Having 660 conformations of these two nucleotides – with a natural base (thymine) and with the bases substituted with 5-chlorouracil – we conducted a comparison of the conformational parameters, namely the torsion angles  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\epsilon$ ,  $\chi$ ,  $\zeta_1$ ,  $\zeta_2$ , P. We calculated the difference between torsion angles of the initial conformers with the natural bases and that of conformers with the modified base, each time selecting a pair of conformers whose conformation was most similar to each other.

Summarizing the absolute values of changes of each angle  $\alpha'$ ,  $\beta$ ,  $\gamma$ ,  $\epsilon$ ,  $\chi$ ,  $\zeta_1$ ,  $\zeta_2$ , P, it was found that the total change of angles  $\delta_{gen.}$  lies between  $1^\circ$  and  $353^\circ$ , and it was observed that "small" value  $\delta_{gen.}$  occurs much more frequently than "large" ones. Thus, it was necessary to find out what kind of magnitude of these changes should be considered as significant.

In order to establish such a "threshold" value for the sum of differences of the angles  $\delta_{gen.}$  (below we assume that the conformer had changed insignificantly after the base replacement if its  $\delta_{gen.}$  is less than this "threshold" value), statistical distribution of the number of the conformers on  $\delta_{gen.}$  was built (Fig. 2). For this purpose,  $\delta_{gen.}$  values were used for the horizontal axis while the values of the vertical axis show  $\frac{\Delta N}{\Delta \delta_{gen.}}$  – a frequency with which the conformers with such sum of angles differences occur, that is the values on the vertical axis show the number of conformers in which the angle difference lies within  $\delta_{gen.}$  and  $\delta_{gen.} + \Delta \delta_{gen.}$  (here the step  $\Delta \delta_{gen.}$  was

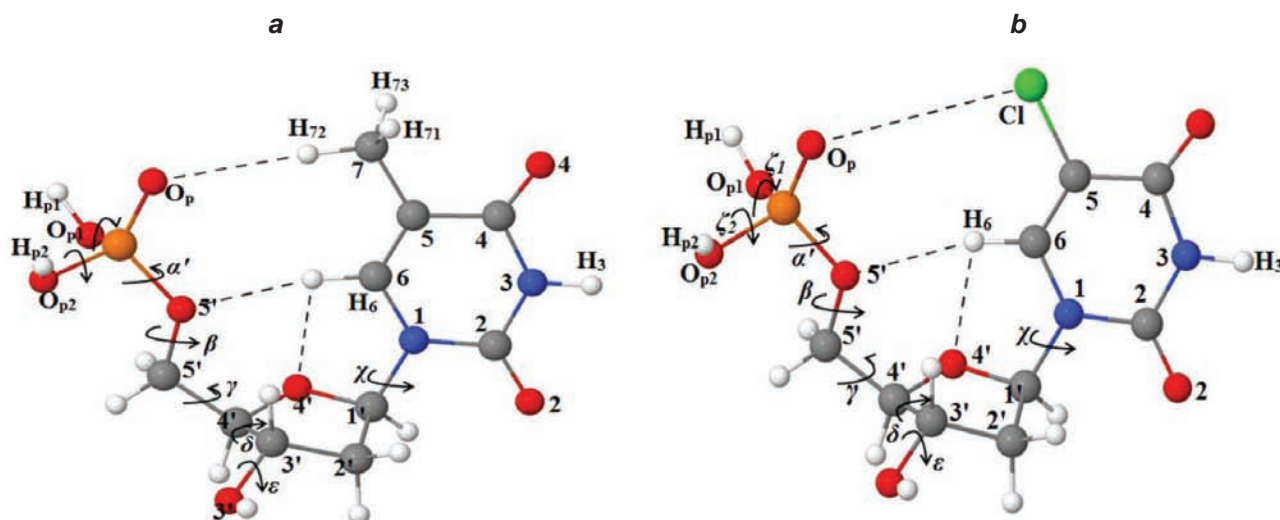


Fig. 1. The designation of the torsion angles and atoms of the molecule of 5'-thymidylic acid with natural base thymine (a) and with the nucleotide base substituted with 5-chlorouracil (b)

taken equal to  $5^\circ$ , which is close to the accuracy of the modeling methods used). We can say that the obtained dependence  $\frac{\Delta N}{\Delta \delta_{\text{gen.}}}$  of  $\delta_{\text{gen.}}$  has a sense similar to the distribution function density in the probability theory.

It was found that the resulting distribution  $\frac{\Delta N}{\Delta \delta_{\text{gen.}}}$  has a pronounced maximum in the area of the "small"  $\delta_{\text{gen.}}$ . The width of this peak was taken to be equal to the coordinate of the first minimum following the highest peak on the curve. It is this width equal to  $30^\circ$  which was taken as the threshold, and all the conformers with  $\delta_{\text{gen.}}$  below this threshold were regarded as having changed just slightly after the bases modification.

Then with the help of a special program developed by us to compare the conformations of the molecules, it was found that only 193 (of 660 in total) conformations of the 5TA molecule cannot be considered as having a "similar" (i.e., with the sum of differences of angles less than  $30^\circ$ ) conformers of 5CDUMP. At the same time, the number of 5CDUMP conformers which have no "close" conformers among the conformers of 5TA equals 166. It was found that this discrepancy is due to the fact that in addition to 660 conformers of 5TA molecule with natural bases there are others that have not been previously identified in [2]. For those of  $193 - 166 = 27$

structures of the 5CDUMP which were "missing", a chlorine atom was replaced by a methyl group and the obtained structures were optimized. It was found that during the geometry optimization their conformation changed insignificantly (the sum of the conformational parameters change did not exceed  $30^\circ$ ), so that it was concluded that additional 27 conformers of 5TA exist and, moreover, each of them has a "close" conformer of 5CDUMP. These 27 conformers of 5TA are those that have changed slightly upon the chlorination, and hence their detailed study is not needed.

Thus, we have identified 166 conformers with significant changes of conformational parameters caused by the replacement of thymine for the 5-chlorouracil. Further attention is focused on the examination of these 166 conformers only.

For all investigated conformers the spatial distribution of the electron charge density was determined at the same level of the theory, which geometry optimization was performed, and has been used for the non-covalent interactions analysis using R. Bader theory of atoms in molecules (QTAIM) [12]. The analysis was performed using the software package AimAll (version 8.11.29) [13].

In addition, for all conformers vibrational spectra were calculated and were used to determine the relaxed force constants, which characterize elastic properties of molecules [5, 14-16].

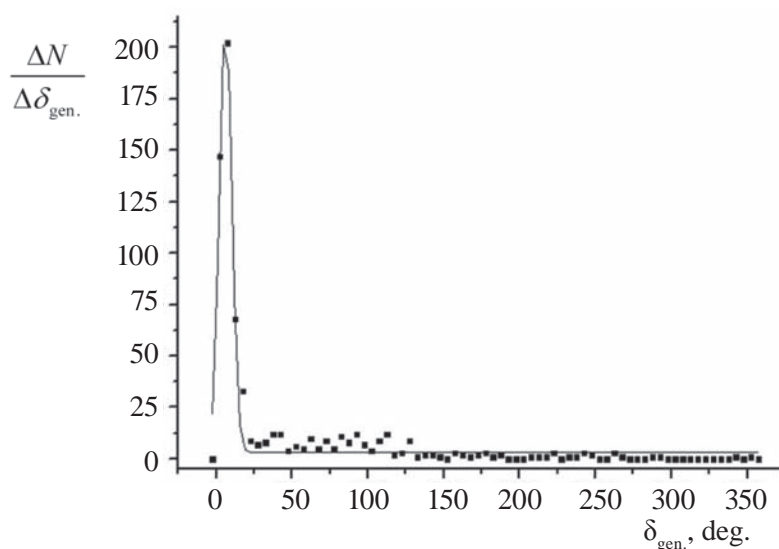


Fig. 2. The distribution of all conformers by the sum of differences of torsion angles caused by the replacement of the natural bases (thymine) to 5-chlorouracil in 5'-thymidylic acid. To construct the distribution the step  $\Delta \delta_{\text{gen.}} = 5^\circ$  was used

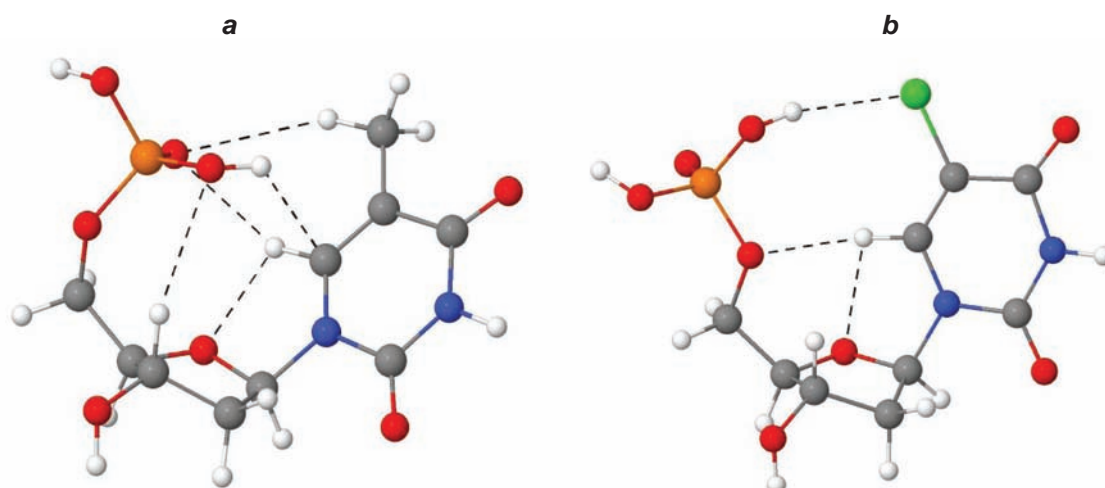


Fig. 3. The structure of the pairs of a conformers **136** of molecules of 5'-thymidylic acid (a) and 5-chloro-2'-deoxyuridine monophosphate (b) where the change of the torsion angles as a result of the modification is the largest among all

### Results and Discussion

Fig. 3 shows conformer 136, in which the change of the torsion angles caused by the modification is the largest among all:  $\Delta\alpha' = 70,7^\circ$ ,  $\Delta\beta = 82,6^\circ$ ,  $\Delta\gamma = 18,1^\circ$ ,  $\Delta\varepsilon = 1,8^\circ$ ,  $\Delta\chi = 28,3^\circ$ ,  $\Delta\zeta_1 = 85,6^\circ$ ,  $\Delta\zeta_2 = 54,5^\circ$ ,  $\Delta P = 43,7^\circ$ . Such changes of the torsion angles are significant and require a more detailed study of the underlying reason.

To understand the causes of changes in conformation of 5TA due to the nitrogenous base chlorination the above mentioned 166 conformers in which the most significant changes were observed, were divided into two groups. The first group, which contained 121 structures, collected the conformers

where a non-covalent bond (typically a hydrogen bond) involving a phosphate group appeared or disappeared as a result of base chlorination. The second group hosted the rest of 45 conformers in which the phosphate group is not involved in the formation of non-covalent bonds regardless of the nucleotide bases type. Typically, the restructuring of non-covalent bonds, in which the atoms of the phosphate group were involved, led to a change in its orientation, and the appropriate changes of torsion angles gave a decisive contribution (see below) to the total change of torsion angles caused by the nucleotide bases chlorination. However, 98 conformers have also been found (they have been identified as a

Table 1. The conformer numbers and their torsion angles (deg.) for the selected conformers of thymidine monophosphate

No.	$\alpha'$	$\beta$	$\gamma$	$\varepsilon$	$\chi$	$\zeta_1$	$\zeta_2$	P
6	-116	76	51	65	-111	-66	134	123
42	169	165	47	174	-137	-180	108	159
136	21	85	35	70	-177	-68	133	38

Table 2. The conformer numbers and their torsion angles (deg.) for the selected conformers of 5-chloro-2'-deoxyuridine monophosphate

No.	$\alpha'$	$\beta$	$\gamma$	$\varepsilon$	$\chi$	$\zeta_1$	$\zeta_2$	P
6	-106	70	50	65	-111	-71	138	119
42	173	158	49	176	-152	-103	178	169
136	-50	168	53	68	-166	-153	79	9



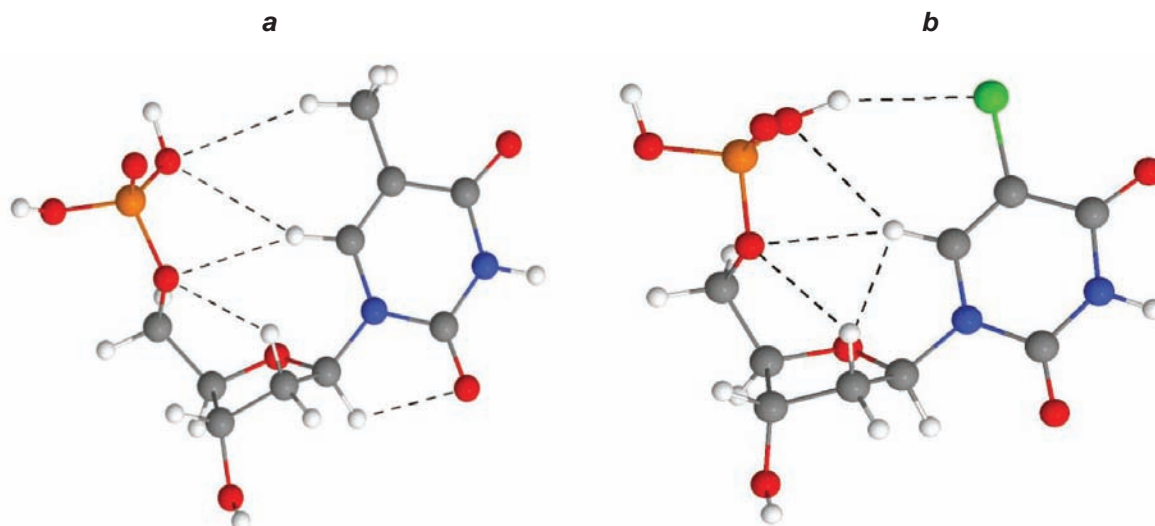


Fig. 4. The structure of the pairs of conformers **42** of 5'-thymidylic acid (a) and 5-chloro-2'-deoxyuridine monophosphate (b) molecules where the cause of conformational parameters changes is turning of the phosphate group due to the formation and / or rupture of  $C_7-H_{72} \cdots O_p$  and  $O_{p1}-H_{p1} \cdots Cl$  bonds

separate group, which partly crossed with the first or the second group), in which neither the twist of phosphate group, nor the turn of nucleotide bases around the  $C_1-N_1$  glycoside bond or change of the sugar conformation were not the cause which significantly influenced the change of the conformational parameters of the molecule.

The example of the conformers of the first group, which cause changes of conformational parameters, turning the phosphate group due to a rupture of non-covalent bond  $C_7-H_{72} \cdots O_p$  in the molecule with natural nucleotide bases (thymine) and formation of the  $O_{p1}-H_{p1} \cdots Cl$  bond in a modified molecule are shown in Fig. 4.

After analyzing the differences of angles  $\alpha'$ ,  $\beta$ ,  $\gamma$ ,  $\varepsilon$ ,  $\chi$ ,  $\zeta_1$ ,  $\zeta_2$ ,  $P$ , caused by the base chlorination in all conformers of the first group, it was found that a significant contribution to the sum of differences of all angles was made by the  $\alpha'$ ,  $\beta$ ,  $\zeta_1$ ,  $\zeta_2$  angles which, in turn, are responsible for the phosphate group rotation. The quantitative characterization of the contribution of the phosphate group rotation to the overall change of the molecule conformation was done by a process analogous to the process used for data analysis in Fig. 2. Namely, the distribution of the conformers by the ratio of  $\delta_{\alpha'\beta\zeta_1\zeta_2}/\delta_{gen.}$  of the total change  $\delta_{\alpha'\beta\zeta_1\zeta_2}$  of angles  $\alpha'$ ,  $\beta$ ,  $\zeta_1$ ,  $\zeta_2$  to the sum of differences of all angles  $\delta_{gen.}$  was built (Fig. 5). The resulting distribution has a distinct maximum, which covers a range of  $\delta_{\alpha'\beta\zeta_1\zeta_2}/\delta_{gen.}$  from 75 to 94%, and within which the

angles  $\alpha'$ ,  $\beta$ ,  $\zeta_1$ ,  $\zeta_2$  give the main contribution to  $\delta_{gen.}$ . The area under the main peak gives the number of conformers (equal to 57), in which the phosphate group rotation makes a major contribution to  $\delta_{gen.}$ . Thus, in this range of the  $\delta_{\alpha'\beta\zeta_1\zeta_2}/\delta_{gen.}$  ratio there are 57 conformers for which, therefore, the main cause of changes of the conformational parameters can be considered to be the phosphate group rotation. As the conformers of the first group were analyzed, we conclude that the mentioned rotation of the phosphate group is caused by the formation or rupture of hydrogen bonds involving its atoms.

Now let us consider the second group of 45 conformers that have no bonds involving a phosphate group. For these conformers we also built the distribution of their number by the ratio  $\delta_{\chi P}/\delta_{gen.}$  of the total change  $\delta_{\chi P}$  of  $\chi$  and  $P$  angles to the sum of differences of all angles  $\delta_{gen.}$ . But it was found that the resulting distribution has a distinct maximum at low ratios of  $\delta_{\chi P}/\delta_{gen.}$  indicating that in the majority of the conformers the angles  $\chi$  and  $P$  have changed slightly due to the base chlorination. However, there are also those conformers where  $\delta_{\chi P}/\delta_{gen.}$  is more than 75% (the threshold of 75% was taken by analogy with the previous case). The area under the curve in the region from 75 to 96% (maximum of  $\delta_{\chi P}/\delta_{gen.}$ ) gave 11 conformers for which angles  $\chi$  and  $P$  make the main contribution to  $\delta_{gen.}$ .

Thus, we can conclude that the main reason for the change of conformational parameters of the

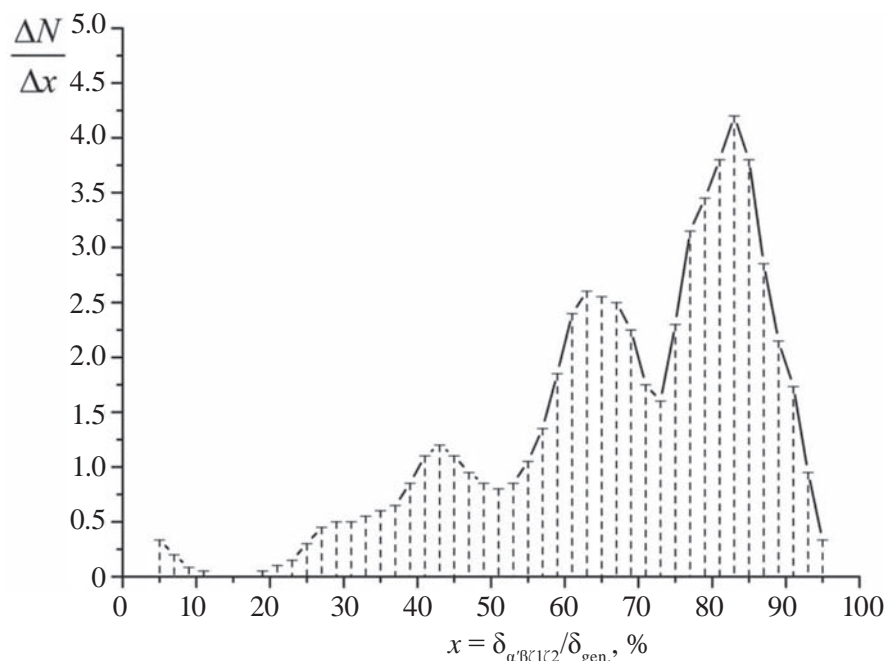


Fig. 5. The distribution of the conformers of the first group by a total contribution  $x = \delta_{\alpha\beta\zeta_1\zeta_2} / \delta_{gen.}$  of change of the angles  $\alpha'$ ,  $\beta$ ,  $\zeta_1$ ,  $\zeta_2$  to the total changes of all the conformational parameters, expressed as a percentage. To construct the distribution the step  $\Delta x = 2\%$  was used

found conformers is the rotation of the base around the  $C_1' - N_1$  bond (angle  $\chi$ ) and the change of the sugar conformation (angle  $P$ ).

Thus, the analysis of the obtained distributions made it possible to distinguish the conformers from the first and second groups, in which the change of the angles caused by chlorination comes from the phosphate group rotation or the nucleotide bases rotation and the concomitant changes in the conformation of the sugar ring. However, except for the mentioned conformers in the first group, as well as in the second, other conformers exist, which can not be said to have the change of the conformation "localized" in a particular fragment of the molecule. Such conformers were collected to the third group, and their total number was equal to 98.

Analyzing the conformers of the third group, which includes 64 conformers from the first group and 34 conformers from the second group, we have identified 17 in which none of the non-covalent bonds has changed as a result of chlorination of the molecule (see an example in Fig. 6).

These structures are of great interest for further research: for both checking the exhaustiveness of non-covalent interactions detection by QTAIM method, and finding the physical factors different

from non-covalent bonds but responsible for determination the conformation of biomolecules.

**DNA-like conformers.** Let us focus on the DNA-like conformers of 5TA and the impact of the nucleotide bases chlorination on their conformation.

Among all the 5TA conformers 14 DNA-like ones were found, and 9 DNA-like conformers were found among the conformers of 5CDUMP. The values of torsion angles of these conformers are shown in Tables 3 and 4.

It was found that not all DNA-like conformers of 5TA have remained DNA-like after the nucleotide base chlorination. For example, the angles in the conformers number **21**, **27**, **38**, **42**, **71** (see Table 3 and 4) of 5TA molecule were changed after modification so that they were no longer close to the angles of DNA-like conformers.

Consequently, the number of DNA-like conformers of 5CDUMP is less due to the fact that two BI-DNA-like conformers (**27** and **42**) and three AI-DNA-like conformers (**21**, **38**, **71**) of 5TA after modification were no longer close to DNA-like ones. This fact may indicate the need of greater (as compared to 5TA) consumption of free energy for changing the conformation of the 5CDUMP nucleotides for their "embedding" into the DNA during its

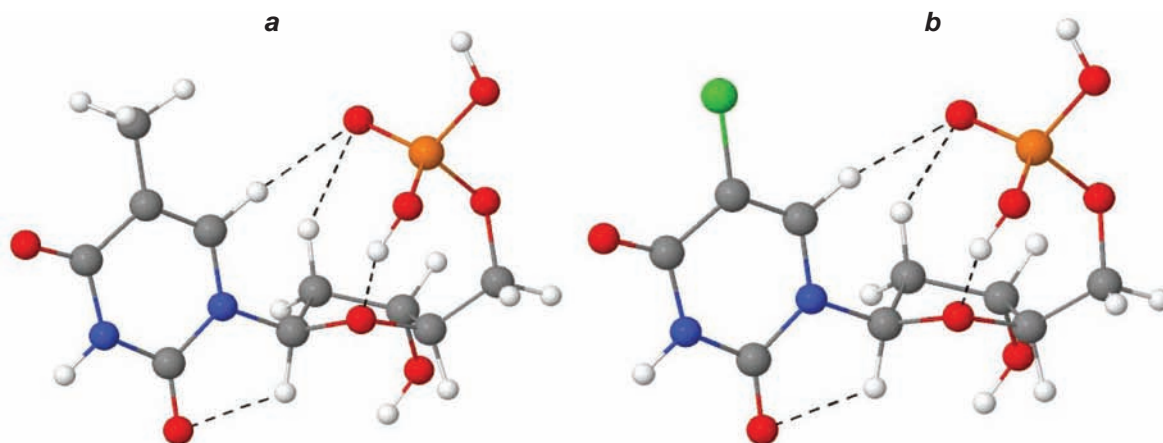


Fig. 6. The structure of the conformer **6**, which has no change in non-covalent bonds due to the substitution of the thymine base (a) with 5-chlorouracil (b). The differences of the torsion angles are:  $\Delta\alpha' = 10.0^\circ$ ,  $\Delta\beta = 6.5^\circ$ ,  $\Delta\gamma = 1.1^\circ$ ,  $\Delta\varepsilon = 0.1^\circ$ ,  $\Delta\chi = 0.4^\circ$ ,  $\Delta\zeta_1 = 5.3^\circ$ ,  $\Delta\zeta_2 = 4.4^\circ$ ,  $\Delta P = 4.3^\circ$  (total change of angles is  $32.1^\circ$ )

Table 3. The torsion angles (deg.) of DNA-like conformers of 5'-thymidylic acid

No. conformer	The form of DNA	$\alpha'$	$\beta$	$\gamma$	$\varepsilon$	$\chi$	$\zeta_1$	$\zeta_2$	P
<b>10</b>	BI	69	-173	55	176	-127	-123	154	158
<b>15</b>	BI	45	170	49	174	-136	-105	161	159
<b>17</b>	BI	69	166	58	173	-169	51	150	184
<b>22</b>	BI	-162	169	56	175	-122	-167	104	158
<b>27</b>	BI	42	164	49	173	-175	-127	-54	188
<b>42</b>	BI	169	165	47	174	-137	-180	108	159
<b>58</b>	BI	66	-172	58	177	-123	-138	-62	157
<b>21</b>	AI	44	168	51	-170	-165	-102	162	14
<b>30</b>	AI	-172	169	56	-168	-152	-103	175	21
<b>38</b>	AI	62	-169	58	-167	-160	-113	158	18
<b>41</b>	AI	-176	177	56	-172	-156	-171	103	18
<b>70</b>	AI	60	165	52	-166	-177	41	152	357
<b>71</b>	AI	58	-176	58	-166	-153	-133	-60	22
<b>135</b>	AII	-55	-164	-180	-173	-174	-158	104	358

biosynthesis, because the probability of 5CDUMP to adopt the DNA-like conformation is smaller than in the case of 5TA.

The total difference of the torsion angles  $\alpha'$ ,  $\beta$ ,  $\gamma$ ,  $\varepsilon$ ,  $\chi$ ,  $\zeta_1$ ,  $\zeta_2$ , P for nine DNA-like conformations acquired quite different values in comparison with the threshold of  $30^\circ$ : the smallest difference in torsion angles, which is  $34^\circ$ , was observed in conformer **41**, and the largest one of  $222^\circ$  in conformer **22**. All nine

pairs of nucleotide conformers with the natural and modified bases are shown in Figs. 7-15.

The values of torsion angles in the 5TA and 5CDUMP molecules were averaged over all DNA-like conformers and their mean-square deviation (using the formulae taken from [2] which take the periodicity of angular variables into account) were calculated. The obtained values were compared with the averaged experimental values [17-23] for crystal-

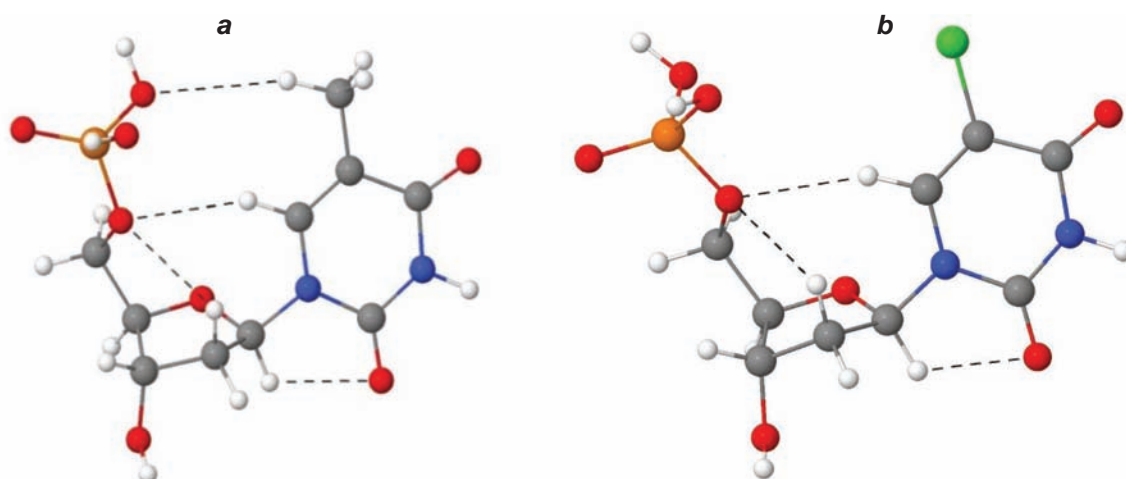


Fig. 7. The B-DNA-like conformer **10** with the thymine base (a) and with the base substituted by 5-chlorouracil (b)

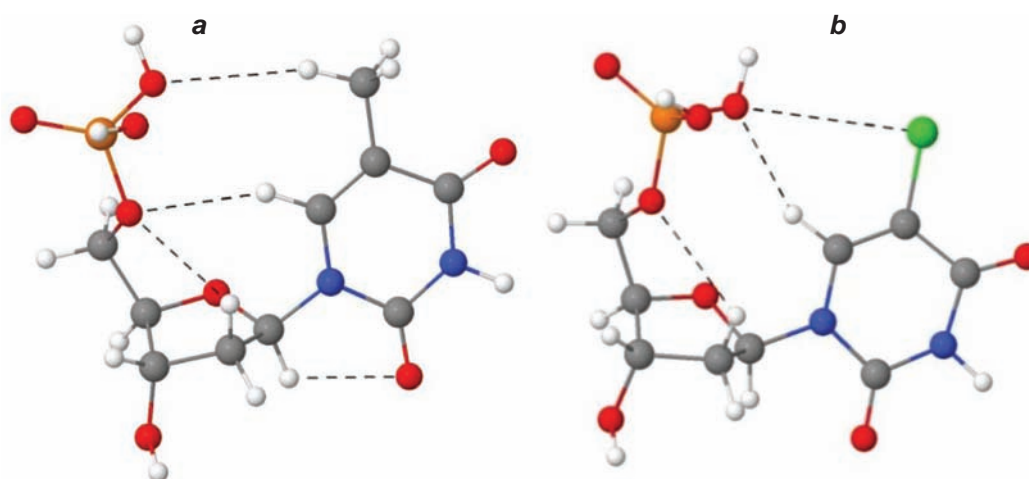


Fig. 8. The B-DNA-like conformer **15** with the thymine base (a) and with the base substituted by 5-chlorouracil (b)

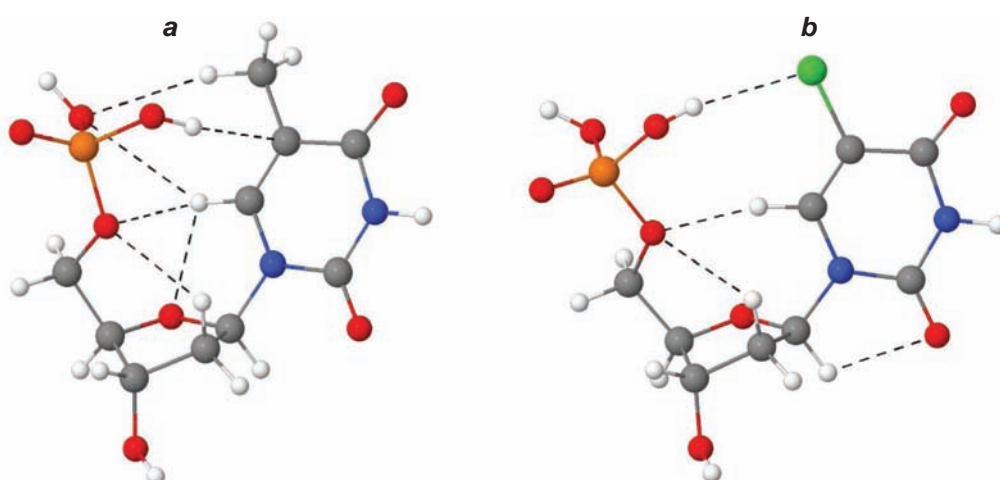


Fig. 9. The B-DNA-like conformer **17** with the thymine base (a) and with the base substituted by 5-chlorouracil (b)



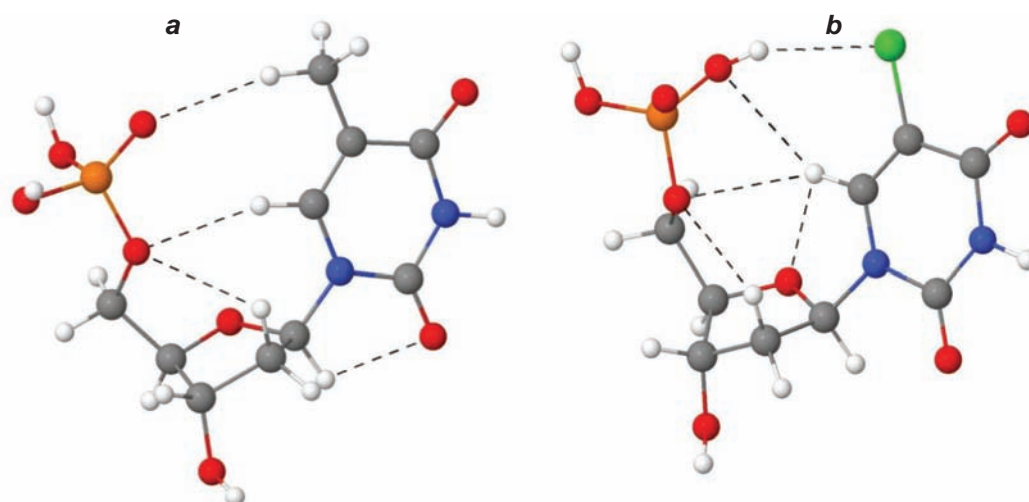


Fig. 10. The B-DNA-like conformer **22** with the thymine base (a) and with the base substituted by 5-chlorouracil (b)

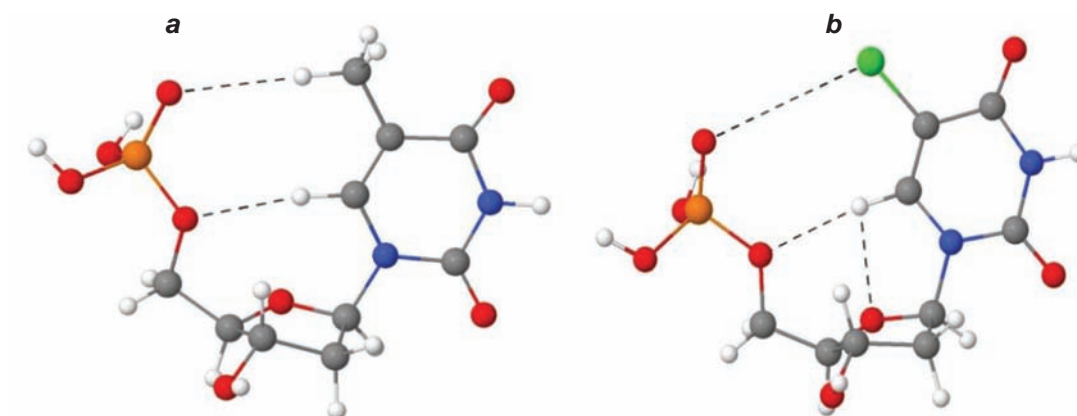


Fig. 11. The A-DNA-like conformer **30** with the thymine base (a) and with the base substituted by 5-chlorouracil (b)

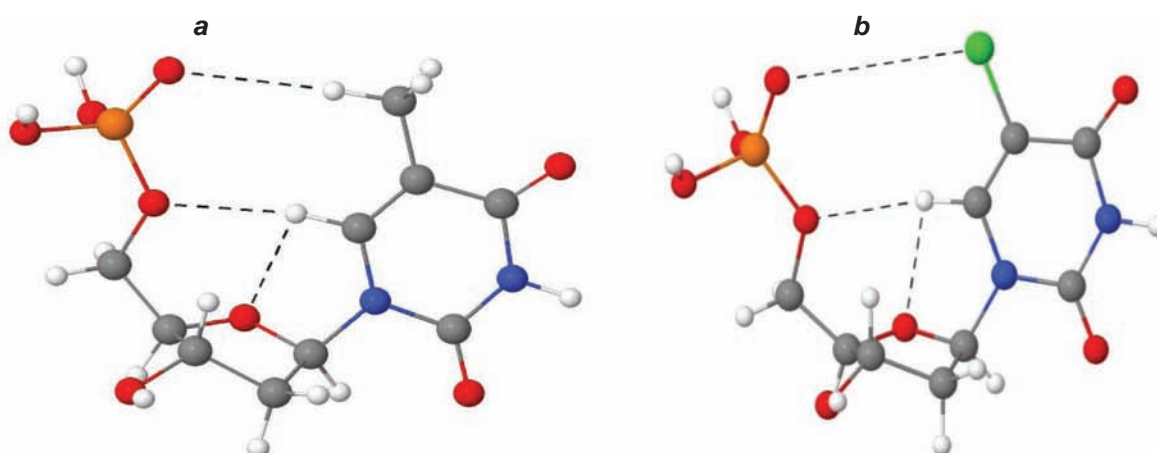


Fig. 12. The A-DNA-like conformer **41** with the thymine base (a) and with the base substituted by 5-chlorouracil (b)

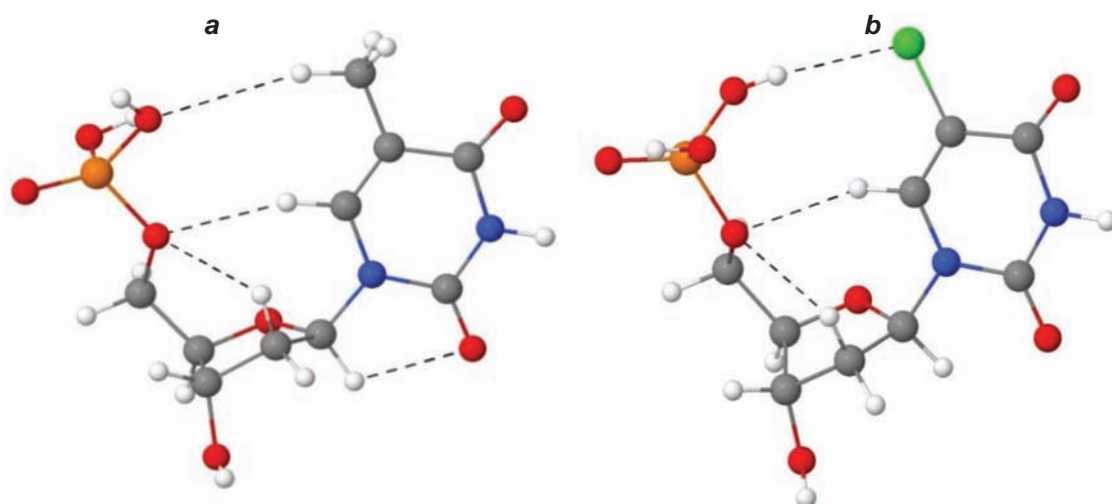


Fig. 13. The B-DNA-like conformer 58 with the thymine base (a) and with the base substituted by 5-chlorouracil (b)

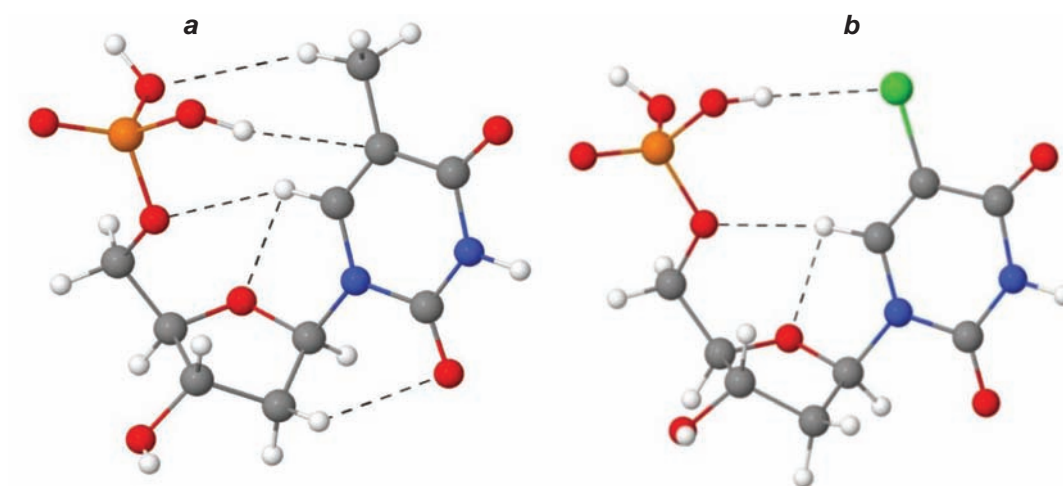


Fig. 14. The A-DNA-like conformer 70 with the thymine base (a) and with the base substituted by 5-chlorouracil (b)

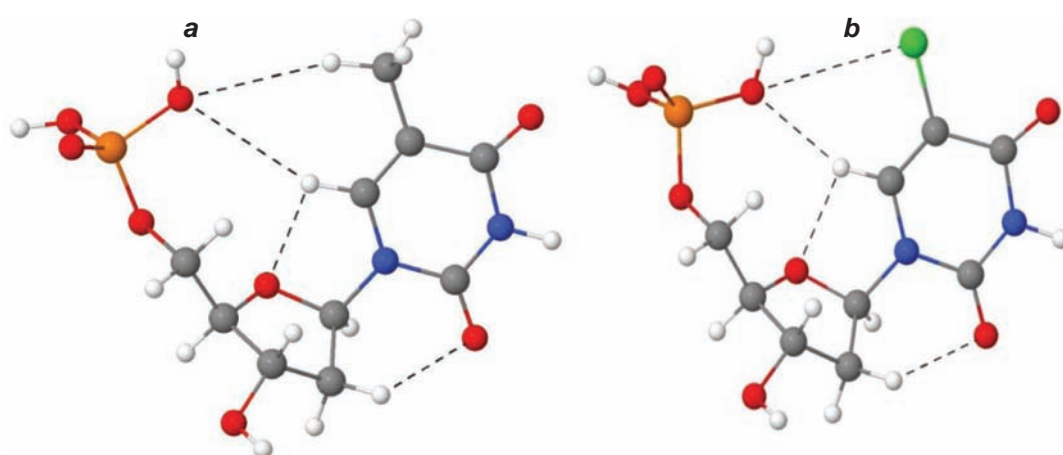


Fig. 15. The AII-DNA-like conformer 135 with the thymine base (a) and with the base substituted by 5-chlorouracil (b)

Table 4. The torsion angles (deg.) of DNA-like conformers of 5-chloro-2'-deoxyuridine monophosphate

No. conformer	The form of DNA	$\alpha'$	$\beta$	$\gamma$	$\varepsilon$	$\chi$	$\zeta_1$	$\zeta_2$	P
10	BI	58	-165	54	177	-132	-116	160	159
15	BI	45	152	47	176	-147	-106	170	153
17	BI	63	-170	57	178	-130	65	150	167
22	BI	174	158	49	176	-152	-103	178	169
58	BI	45	172	52	177	-143	-122	-76	170
30	AI	175	176	53	-165	-160	-102	173	19
41	AI	174	-178	53	-170	-161	-166	107	18
70	AI	51	-171	55	-161	-166	53	153	8
135	AII	-60	-140	-179	-172	-175	-153	110	360

line DNA (see Table 5). The comparison showed that the average conformational parameters (except for the angle  $\zeta$ ) of the obtained conformers are consistent with the experimental values in the case of 5TA conformers equally well as in the case of 5CDUMP.

In our study the angle  $\zeta$ , which in the DNA chain characterize the orientation of the  $O_3'$  atom of the previous nucleotide relative to the phosphate group of the current nucleotide, was defined as the torsion angle  $O_5'PO_pH$ , and therefore defined the orientation of the hydrogen atom in a phosphate group. The latter, of course, cannot serve as a good model of neighboring nucleotides, so it should not be expected for this angle to be consistent with the experimental value of  $\zeta$  in the crystalline DNA.

Thus, it was found that the averaged value of conformational parameters of obtained conformer of 5TA and 5CDUMP is close to the experimental values for crystalline DNA. At the same time, the fact that 5CDUMP has fewer DNA-like conformers as compared to 5TA indicates that the chlorination of the nucleotide bases affects the "depth" (and possibly the "form") of the minima at the nucleotide potential energy hypersurface rather than their location in space of the conformation parameters. Therefore, the question of how chlorination changes the force constant of the identified DNA-like conformers of both molecules is worth investigating.

Analyzing the data in the Table 6 and 7, we can conclude that due to the replacement of thy-

Table 5. The averaged angles (deg.) of AI- and BI-DNA-like conformers of 5'-thymidylic acid and 5-chloro-2'-deoxyuridine monophosphate in comparison with the averaged experimental values [17-23] for the crystalline DNA

Angels, deg.	$\alpha$	$\beta$	$\gamma$	$\varepsilon$	$\chi$	$\zeta$	P
<i>AI form of DNA</i>							
Experiment	-69 ± 14	175 ± 15	57 ± 10	-155 ± 14	-161 ± 11	-73 ± 9	18 ± 18
5TK	-66 ± 17	176 ± 9	55 ± 3	-168 ± 2	-160 ± 9	-168 ± 57	15 ± 8
5CDUMP	-72 ± 10	-178 ± 5	54 ± 1	-165 ± 4	-162 ± 3	-145 ± 56	15 ± 5
<i>BI form of DNA</i>							
Experiment	-62 ± 14	168 ± 25	51 ± 16	-173 ± 20	-108 ± 23	-98 ± 23	162 ± 18
5TK	-63 ± 15	173 ± 19	53 ± 4	175 ± 1	-141 ± 20	-170 ± 58	166 ± 13
5CDUMP	-71 ± 9	173 ± 17	52 ± 4	177 ± 1	-141 ± 9	-161 ± 54	164 ± 7

Note: Root-mean-square deviation are given after the sign «±»

Table 6. The relaxed force constant ( $\text{kcal}\cdot\text{mol}^{-1}\cdot\text{rad}^{-2}$ ) conformational parameters of the DNA-like conformers of thymidine monophosphate

No. conformer	The form of DNA	$\alpha'$	$\beta$	$\gamma$	$\varepsilon$	$\chi$	$\zeta_1$	$\zeta_2$	P
10	BI	5.8	3.4	26.5	6.4	8.9	1.8	3.6	10.3
15	BI	6.0	6.0	26.3	6.4	6.2	3.0	4.5	9.5
17	BI	13.8	8.8	34.9	6.4	18.5	7.5	5.9	7.5
22	BI	3.6	3.3	29.5	6.5	10.5	1.1	4.7	8.8
27	BI	5.5	8.8	27.9	6.4	15.3	0.4	4.8	3.4
42	BI	9.8	7.1	27.1	6.5	6.5	3.1	5.7	9.6
58	BI	7.5	2.1	28.6	6.4	9.6	3.1	3.7	9.9
21	AI	2.3	3.6	17.3	4.6	12.9	2.9	4.0	9.5
38	AI	6.2	3.2	23.4	4.0	12.1	2.3	4.5	11.2
41	AI	8.6	5.0	24.0	4.9	9.2	1.5	5.3	12.4
30	AI	9.1	3.4	24.6	4.5	5.6	5.7	2.2	10.0
70	AI	9.6	6.8	30.7	4.6	21.2	6.4	5.9	7.1
71	AI	7.7	2.0	23.7	4.0	5.3	2.1	3.4	9.3
135	AII	6.1	4.4	21.7	5.3	24.3	4.8	2.1	5.0

Table 7. The relaxed force constant ( $\text{kcal}\cdot\text{mol}^{-1}\cdot\text{rad}^{-2}$ ) conformational parameters of the DNA-like conformers of 5-chloro-2'-deoxyuridine monophosphate

No. conformer	The form of DNA	$\alpha'$	$\beta$	$\gamma$	$\varepsilon$	$\chi$	$\zeta_1$	$\zeta_2$	P
10	BI	3.8	1.4	21.7	6.2	6.0	2.0	3.6	9.1
15	BI	2.8	7.7	22.3	6.4	2.4	3.0	3.6	7.7
17	BI	13.8	34.9	8.8	6.4	18.5	7.5	5.9	7.5
22	BI	9.7	13.6	20.6	6.2	1.4	8.7	2.0	4.2
58	BI	6.2	8.0	26.7	6.2	1.1	1.0	3.1	7.6
30	AI	2.8	2.6	8.3	4.1	6.9	5.6	1.4	7.8
41	AI	7.1	4.8	20.8	4.7	12.5	1.5	4.9	9.1
70	AI	10.0	3.2	26.4	3.6	14.5	4.9	5.5	12.9
135	AII	6.3	14.6	27.8	5.2	24.6	6.3	1.7	6.2

mine with 5-chlorouracil the value of the force constant has changed insignificantly. In particular, the total difference of the force constant is less than  $28 \text{ kcal}\cdot\text{mol}^{-1}\cdot\text{rad}^{-2}$ . The following features have been identified: in all B-DNA-like conformers (**10**, **15**, **58** in Table 7) the force constant of the  $\chi$  angle decreases 9 times as compared to the natural nucleotide and the force constant of the angle  $\alpha'$  is somewhat reduced.

Meanwhile, for the A-DNA-like conformers this is not observed since the chlorination significantly changed only the constant of the conformer **30**, again reducing the constant for angle  $\alpha'$  and, even more surprisingly – also for such a "hard" angle as  $\gamma$ .

After analyzing the change of hydrogen bonds in DNA-like conformers of molecules 5TA and 5CDUMP, it has been observed that the  $\text{C}_6\text{H}\cdots\text{O}_5$  bond remains in the conformers number **30**, **41**, **70**,



**58, 10**, but it is lost in the conformer number **15** upon replacement of the natural base with its synthetic analogue. The  $C_6H \cdots O_4$  bond remains in conformers number **41, 70** and **135**.

After modification of the base the bond  $C_2H \cdots O_2$  remains in the conformers **10, 15** and **135**, but it has disappeared in the conformer number **70**, while the bond  $C_2H \cdots O_5$  remains in the conformer number **58**. In the conformers **30, 41** the bond  $C_7H \cdots O_p$  has disappeared, and bond  $C_7H \cdots O_{p1}$  has disappeared in the conformers number **10, 58, 135**, as well as the  $C_7H \cdots O_{p2}$  bond has disappeared in the conformers **15, 70**.

The bond  $C_1H \cdots O_2$  in conformer **10** has not been changed, but in conformers **58** and **15** it has disappeared after the chlorination of the molecule. The bond  $C_6H \cdots O_{p1}$  in conformer **135** has not been changed, but the bond  $C_6H \cdots O_{p2}$  has appeared in the conformer **15**.

As a result of replacing the natural base with 5-chlorouracil in conformers number **30, 41** the van der Waals contact  $Cl \cdots O_p$  has appeared, the bond  $Cl \cdots O_{p1}$  has appeared in the conformer **135**, and bond  $Cl \cdots O_{p2}$  has appeared in the conformer **15**. The bond  $O_{p2}H \cdots Cl$  has appeared in conformer **58**, while the bond  $O_{p1}H \cdots Cl$  has appeared in the conformer **70**, but the bond  $O_{p1}H \cdots C_5$  has disappeared in this conformer upon chlorination.

In conclusion, we conducted a comparative analysis of a set of 687 possible conformers of natural monomeric DNA unit (2'-deoxyribonucleotide thymidine monophosphate) and 660 conformers of 5-chloro-2'-deoxyuridine monophosphate – a similar molecule in which the natural nitrogenous base thymine is substituted with the 5-chlorouracil.

It was found that the modification of the nitrogenous base led to the significant changes in the conformation (the sum of difference of torsion angles higher than  $30^\circ$ ) in 166 (25% of the total) conformers of the studied 2'-deoxyribonucleotides.

As many as 121 conformers have been identified in which the hydrogen bond(s) involving the phosphate group atoms appeared or disappeared after chlorination, and 57 conformers – in which the change of the conformation has been caused mainly by the change in the conformation of the phosphate group (angles  $\alpha, \beta, \zeta_1, \zeta_2$ ). However, for 11 conformers the chlorination of the nitrogenous base led to its rotation around the glycoside bond (accompanied by the change in  $\chi$  angle) and changes the sugar conformation (the P angle).

Among conformers of the 2'-deoxyribonucleotide thymidine monophosphate the 4 A- and 5 B-DNA-like conformers were found, which are slightly less than in thymidine monophosphate (7 and 7 respectively). It was found that 2'-deoxyribonucleotide with the natural base has more conformers which are suitable for incorporation into the DNA without significant geometry distortion.

In all DNA-like conformers the non-covalent bonds (hydrogen or van der Waals) between chlorine atom (5-chloro-2'-deoxyuridine monophosphate) or methyl group (thymidine monophosphate) and the phosphate group atoms were found. In the natural 2'-deoxyribonucleotide the methyl group acted as a proton donor and in the modified 2'-deoxyribonucleotide the chlorine atom was a proton acceptor. In the latter case the formation of non-covalent bonds is energetically more favorable and it leads to the rotation of the phosphate group and changes in conformational parameters  $\alpha, \beta, \zeta_1, \zeta_2$ .

In 57 (35%) of 166 conformers having their structure sensitive to the substitution of the thymine with 5-chlorouracil, the main contribution to the total change of the conformational parameters was made by  $\alpha, \beta, \zeta_1, \zeta_2$  parameters. We can conclude that the main reason for the change of these 57 conformers lies in the formation of non-covalent bonds involving chlorine atom, which lead to the rotation of the phosphate group and changing the  $\alpha, \beta, \zeta_1, \zeta_2$  angles.

We have found the examples of conformers (98 in total), in which the mechanism of the effect of bases chlorination on conformational changes is not limited to the formation or destruction of the intramolecular non-covalent bonds, and hence can be the subject of further research.

## ВПЛИВ ХЛОРУВАННЯ НУКЛЕОТИДНОЇ ОСНОВИ НА КОНФОРМАЦІЙНІ ВЛАСТИВОСТІ ТИМІДИНМОНОФОСФАТУ

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Нещодавні дослідження з виведення бактерії *Escherichia coli*, в ДНК якої тимін був замінений 5-хлорурацилом, що майже не вплинуло на нормальну життєдіяльність та на поділ

бактерій, але призвело до значного скорочення часу її життя, актуалізували проблему розуміння змін у фізичних властивостях мономерів ДНК у разі їх хімічної модифікації. У роботі проведено порівняльний аналіз наборів із 687 можливих конформерів природної мономерної ланки ДНК (2'-дезоксирибонуклеотиду тимидинмонофосфату) та 660 конформерів 5-хлор-2'-дезоксирибонуклеотиду – аналогічної молекули, в якій природну азотисту основу тимін заміщено на 5-хлорурацил. Одержано структури стійких конформерів модифікованого дезоксирибонуклеотиду та проаналізовано фізичні чинники, що обумовлюють їх відмінність від конформерів немодифікованої молекули. Зроблено порівняльний аналіз пружних властивостей конформерів досліджених молекул та нековалентних взаємодій в них. Результати роботи можуть бути використані під час планування експериментів із синтезу штучних ДНК, придатних для їх включення в живі організми.

**Ключові слова:** ДНК, конформаційний аналіз, нуклеотидна основа, дезоксирибонуклеотид, 5-хлорурацил, *Escherichia coli*.

### ВЛИЯНИЕ ХЛОРИВОВАНИЯ НУКЛЕОТИДНОГО ОСНОВАНИЯ НА КОНФОРМАЦИОННЫЕ СВОЙСТВА ТИМИДИНМОНОФОСФАТА

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Недавние исследования по выведению бактерии *Escherichia coli*, в ДНК которой тимин был замещен 5-хлорурацилом, что почти не повлияло на нормальную жизнедеятельность и на деление бактерий, но привело к значительному сокращению времени ее жизни, актуализировали проблему понимания изменений в физических свойствах мономеров ДНК при их химической модификации. В работе проведен сравнительный анализ наборов из 687 возможных конформеров природного мономерного звена ДНК (2'-дезоксирибонуклеотида тимидинмонофосфата) и 660 конформеров 5-хлор-2'-дезоксирибонуклеотида – аналогичной молекулы, в которой природное азо-

тистое основание тимин замещено на 5-хлорурацил. Получены структуры устойчивых конформеров модифицированного дезоксирибонуклеотида и проанализированы физические факторы, обуславливающие их отличие от конформеров немодифицированной молекулы. Сделан сравнительный анализ упругих свойств конформеров исследованных молекул и нековалентных взаимодействий в них. Результаты работы могут быть использованы при планировании экспериментов по синтезу искусственных ДНК, пригодных для их включения в живые организмы.

**Ключевые слова:** ДНК, конформационный анализ, нуклеотидное основание, дезоксирибонуклеотид, 5-хлорурацил, *Escherichia coli*.

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Received 05.11.2014