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## Quantum-chemical investigation of the photoproduct of the reaction of two 1-methylthymine molecules: the pyrimidine(6-4)pyrimidone adduct

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**Abstract:** One of the products of the photochemical reaction induced by UV irradiation of the two 1-methylthymine molecules is pyrimidine(6-4)pyrimidone. Due to the low yield of this product, it is difficult to examine its geometry and vibrational spectrum. In this study, quantum chemical methods were used to characterize the structure of pyrimidine(6-4)pyrimidone. Its three conformers were optimized and their structures compared. Their vibrational frequencies in the harmonic approximation are discussed briefly. It was shown that the most significant changes in the infrared spectra arise from the formation of hydrogen bonds. All calculations were performed both in vacuum and in D<sub>2</sub>O.

**Keywords:** photochemistry, quantum chemistry, density functional theory.

### INTRODUCTION

The main chromophores for the absorption of UVA and UVB radiation in DNA and RNA are the nucleic acid bases. Most of the excited-state population of the nucleic acid bases in a low-pressure gas phase relaxes radiationlessly to the electronic ground state on an ultrafast timescale.<sup>1</sup> This fast relaxation prevents the creation of UV damage. Nevertheless, in the pyrimidine bases uracil and thymine, a long-lived dark state is formed to a minor extent.<sup>2–5</sup> Recent theoretical work<sup>6–8</sup> and supersonic jet experiments<sup>4</sup> have shown that this dark state is the T<sub>1</sub> state. In apolar solvents, a significant triplet quantum yield was observed, while in polar protic solvents, the triplet quantum yield was smaller.<sup>9</sup>

There is evidence that the triplet states of nucleic acids are involved in the formation of nucleic acids photo-lesions, which are mutagenic and carcinogenic for skin cells.<sup>10–13</sup> The most frequent photoreaction of nucleic acids is an intra-

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strand pyrimidine dimerization. This reaction covalently links two pyrimidine bases and yields mostly the cyclobutane pyrimidine dimer (CPD) and a small amount of the pyrimidine(6-4)pyrimidone adduct ((6-4)PP), Fig. 1. The former product is formed in a [2+2] cycloaddition reaction between adjacent bases, while the latter arises from a Paterno–Büchi cycloaddition, followed by thermal ring opening of the oxetane intermediate. The adduct exposed to UVB light can be further photochemically transformed to its Dewar valence photo-isomer, the so-called Dewar PP. In mammalian cells, UV-induced bipyrimidine photoproducts are removed *via* nucleotide excision repair (NER), by either transcription-coupled repair (TCR) or global genomic repair (GGR).<sup>14</sup> The repair kinetics and efficiencies of UV-induced damage vary considerably from one type of photolesion to another and also between different species.<sup>14</sup> Both PP products are less investigated than CPD because of the low reaction yield and difficult detection. Hence, more work is required to understand the mechanism of their formation and repair.

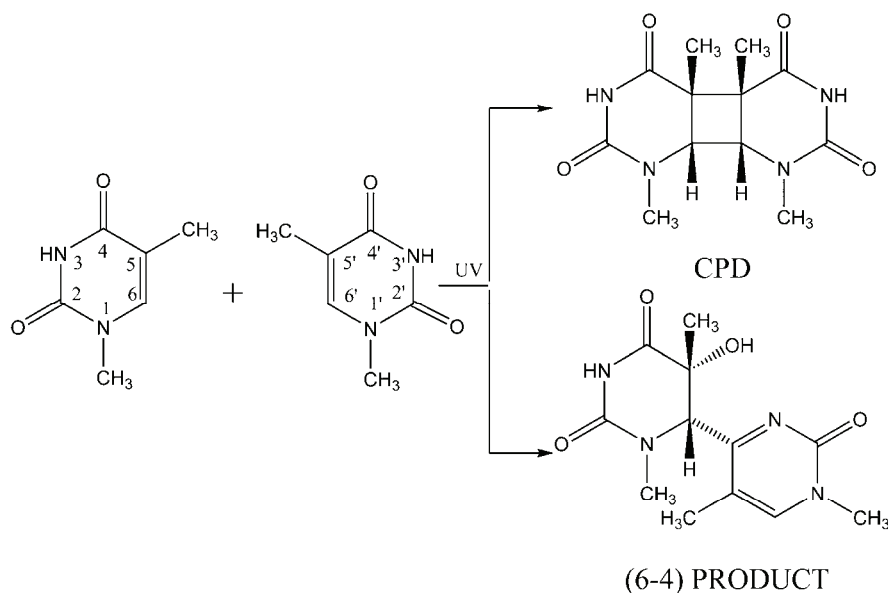


Fig. 1. Chemical structures and atom labels of the 1-methylthymine photoproducts: cyclobutane pyrimidine dimer (CPD) and pyrimidine(6-4)pyrimidone adduct.

In the present study, the pyrimidine(6-4)pyrimidone adduct, created by the photochemical reaction of two 1-methylthymine molecules, was analyzed by means of a quantum-chemical method. Thymine is a pyrimidine base that has the highest yield of dimerization photoproducts. The methylated compound was chosen for analysis because methylation in position one plays the role of sugar in

DNA. The ground state structure of (6-4)PP was characterized in vacuum and also in D<sub>2</sub>O. The solvent D<sub>2</sub>O was chosen because experiments concerning the detection of vibrational motion are usually performed in this solvent. Three conformers were found, and their structure and stability were characterized. In addition, their vibrational spectra were calculated and assigned.

#### COMPUTATIONAL DETAILS

All calculations were performed with the GAUSSIAN program package.<sup>15</sup> The density functional theory (DFT) with B3-LYP functional<sup>16</sup> was employed for electronic structure calculations. All calculations were realized in C<sub>1</sub> point-group symmetry. The optimization was performed with *tight* convergence criteria due to the presence of methyl groups and low frequency modes that involve methyl rotation. The Dunning's<sup>17</sup> correlation-consistent basis set cc-pVTZ (C, N, O, 10s5p2d1f /4s3p2d1f; H, 5s2p1d /3s2p1d) was used. In order to take into account the electrostatic interactions in the polar solvent (D<sub>2</sub>O), the Onsager Model,<sup>18,19</sup> in which the solute is imbedded in a spherical cavity of a continuum medium characterized by its dielectric constant, was employed. The dielectric constant of D<sub>2</sub>O was taken to be  $\epsilon = 78.06$ . All vibrational frequencies were calculated within the harmonic approximation.

#### RESULTS AND DISCUSSION

##### *The molecular geometries*

The ground state structure of (6-4)PP was optimized in vacuum and in D<sub>2</sub>O. Three conformers were found, which were labeled K1, K2 and K3 according to their stability. Their electronic energies with and without zero-point correction and their dipole moments are presented in Table I.

TABLE I. Electronic energy (relative to the most stable conformer in D<sub>2</sub>O), sum of the electronic and zero-point vibrational energy and dipole moment of the conformers in vacuum and in D<sub>2</sub>O

| Parameter  | K1    | K2    | K3    |
|--|-------|-------|-------|
| $E_{\text{el}} / \text{cm}^{-1}$                 | 1323  | 3235  | 3266  |
| $E_{\text{el+ZPV}} / \text{cm}^{-1}$             | 64309 | 66244 | 66227 |
| $\mu / \text{D}$                                 | 7.49  | 3.58  | 10.41 |
| $E_{\text{el,D}_2\text{O}} / \text{cm}^{-1}$     | 0     | 2872  | 537   |
| $E_{\text{el+ZPV,D}_2\text{O}} / \text{cm}^{-1}$ | 62985 | 65859 | 63544 |
| $\mu_{\text{D}_2\text{O}} / \text{D}$            | 9.71  | 4.77  | 14.02 |

The K1 conformer is the most stable one. Its optimized geometry with the most significant bond lengths is presented in Fig. 2. This structure was also found by Ai *et al.* using the CASSCF(10,8)/6-31G(d) method.<sup>11</sup> Its pyrimidone ring is planar. On the other hand, the pyrimidine ring is non-planar. The dihedral angles C<sub>2</sub>-N<sub>3</sub>-C<sub>4</sub>-C<sub>5</sub> and C<sub>2</sub>-N<sub>1</sub>-C<sub>6</sub>-C<sub>5</sub> in the pyrimidine ring are 15 and 42 degrees, respectively. The two rings are almost orthogonal (the dihedral angle C<sub>5</sub>-C<sub>6</sub>-C<sub>4</sub>'-N<sub>3</sub>' is 86 degrees). The C<sub>2</sub>=O and C<sub>4</sub>=O bond lengths are 1.21 and 1.22 Å, respectively. Ai *et al.*<sup>11</sup> found slightly shorter bonds, namely 1.19 and 1.20 Å.

There is a general tendency that the CASSCF bonds obtained by Ai *et al.* are shorter than are those found in this study. This is because the CASSCF method does not include the dynamic correlation required for the correct description of the electronic energy in the vicinity of the potential minimum. The C<sub>6</sub>'–N<sub>1</sub>' bond is 1.35 Å while the C<sub>4</sub>'–N<sub>3</sub>' bond is 1.30 Å long. This means that the former bond is weaker and is not strictly of a double bond character as is presented in the valence-bond structure in Fig. 1. The C<sub>6</sub>'–C<sub>4</sub>' bond is 1.54 Å long and it is a single bond that allows ring rotation. The O–H bond is orientated toward the oxygen that is connected to the C<sub>4</sub> atom. This orientation stabilizes the energy of the molecule by the formation of an intramolecular hydrogen bond. The hydrogen bond length (defined by the distance between the bridging hydrogen and the acceptor oxygen) amounts to 2.05 Å. There are 102 normal modes, which results in large zero-point energy, 62985 cm<sup>-1</sup>.

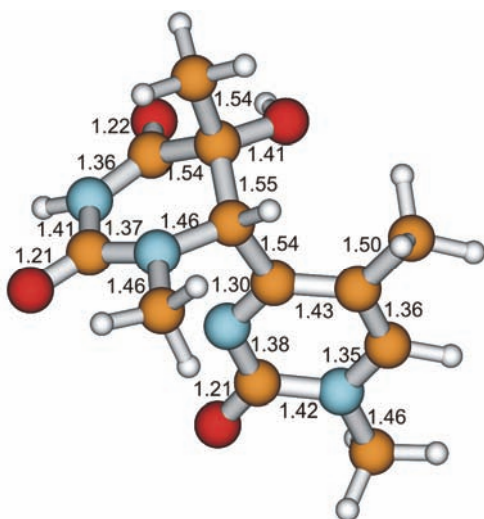


Fig. 2. The optimized geometry of the K1 conformer. All bond lengths are in Å.

The optimized geometry of the K2 conformer is displayed in Fig. 3. Its electronic energy is 1912 cm<sup>-1</sup> higher than that of the K1 conformer. Rotation around the C<sub>6</sub>–C<sub>4</sub>' bond can transform it into the K1 conformer. The dihedral angle C<sub>5</sub>–C<sub>6</sub>–C<sub>4</sub>'–N<sub>3</sub>' between the pyrimidine and pyrimidone rings is 111 degrees. The differences in the bond lengths in the K1 and K2 conformers are not larger than 0.01 Å. The pyrimidine bonds of the two conformers are more distinctive. This is due to the steric effects induced by the interaction of the methyl group from the pyrimidone ring and the pyrimidine ring in the K2 conformer. The intramolecular hydrogen bond is 2.11 Å. The zero-point energy of the K2 conformer is 24 cm<sup>-1</sup> larger than the zero-point energy of the K1 structure.

The K3 conformer is the least stable. Its electronic energy is  $31 \text{ cm}^{-1}$  higher than that of K2 and  $1943 \text{ cm}^{-1}$  higher than that of the K1 conformer. The optimized geometry of the K3 conformer with the most significant bond lengths is presented in Fig. 4. The angle between the pyrimidine and pyrimidone rings is  $57$  degrees. The dihedral angles  $\text{C}_2\text{-N}_3\text{-C}_4\text{-C}_5$  and  $\text{C}_2\text{-N}_1\text{-C}_6\text{-C}_5$  in the pyrimidine ring are  $3$  and  $44$  degrees, respectively. The K3 conformer has the lowest zero-point energy of all three conformers, it is  $25 \text{ cm}^{-1}$  lower than the zero-point energy of K1. The hydrogen bond in the K3 conformer is formed between the two rings and is of  $\text{O-H}\cdots\text{N}$  type in the contrast to hydrogen bonds in K1 and K2 conformers that are formed within the same ring, and both the donor and the acceptor are oxygen atoms. The hydrogen bond length in the K3 conformer amounts to  $1.9 \text{ \AA}$ . It lowers the dihedral angle between the two rings (it is close to  $90$  degrees in the most stable conformation), which increases the tension in the system, making it less stable.

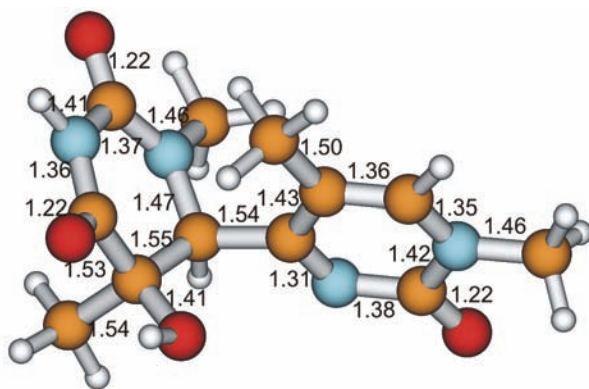


Fig. 3. The optimized geometry of the K2 conformer. All bond lengths are in  $\text{\AA}$ .

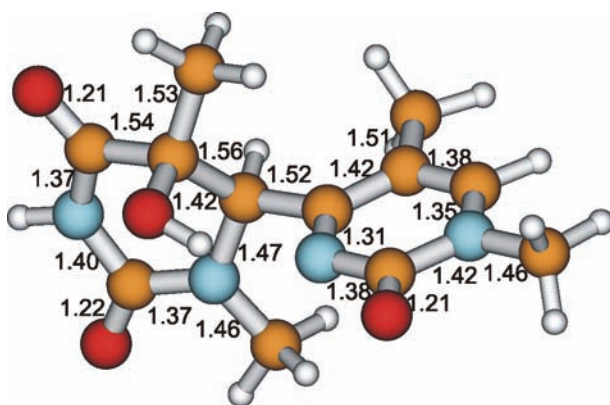


Fig. 4. The optimized geometry of the K3 conformer. All bond lengths are in  $\text{\AA}$ .

It is interesting to examine whether solvation in D<sub>2</sub>O modifies the relative stability of the three conformers. The K3 conformer has the largest dipole moment in vacuum (10.41 D) while the K2 conformer has the smallest (3.58 D). The dipole moment in the K1 conformer is 7.49 D. Therefore, it is expected that solvation would considerably modify the electronic energy of the K3 and K1 conformers. The increase of stability due to solvation is 1323, 363 and 2730 cm<sup>-1</sup> for K1, K2 and K3 conformers, respectively. The energy of the K3 conformer decreased the most so that it became the second most stable conformer, 537 cm<sup>-1</sup> higher in energy than the K1 conformer and 2335 lower in energy than the K2 conformer. This is expected due to this conformer having the largest value of the dipole moment. The dipole moments of the conformers in D<sub>2</sub>O are 9.71, 4.77 and 14.02 D, respectively. Although the solvation modifies the electronic energies, the bond lengths remain almost unchanged. The largest bond change was 0.01 Å.

#### *The vibrational properties*

The vibrational spectra were calculated in the harmonic approximation, Fig. 5. As mentioned before, there are 102 vibrational degrees of freedom. Attention will be focused on the O–H, N–H and C=O stretching vibrations and changes in the corresponding peak position and intensity upon conformational change will be analyzed. The frequencies and their intensities in vacuum and in D<sub>2</sub>O are listed in Table II.

First, the high frequency region above 3000 cm<sup>-1</sup> will be analyzed. This is the region of the X–H stretching modes (X = O, N, C). There are 14 C–H bonds, resulting in 14 C–H stretching modes. Due to the small difference in electronegativity of hydrogen and carbon atoms, the dipole moment does not drastically change upon such vibrational motions, which results in the small intensity of the corresponding peaks. On the other hand, the peak intensities that correspond to O–H and N–H stretching motion are generally large. Therefore, special attention will be paid to these two modes. As mentioned before, hydrogen bonds are formed in all three cases: in conformers K1 and K2, the proton acceptor is the oxygen atom of the pyrimidone ring, whereas in the K3 conformer, the proton acceptor is the nitrogen atom of the pyrimidine ring. Although the electronegativity of the oxygen atom is greater than that of the nitrogen atom, O–H···N bonds are usually stronger than O–H···O hydrogen bonds.<sup>20</sup> This is also the case with the (6-4)PP conformers; thus the harmonic  $\nu_{\text{OH}}$  frequencies equal 3659 and 3700 cm<sup>-1</sup> in K1 and K2, respectively, and 3540 cm<sup>-1</sup> in K3. The stronger hydrogen bond in K3 weakens the covalent O–H bond, which results in a lower O–H stretching frequency. In a polar solvent such as D<sub>2</sub>O, it is expected that hydrogen bonds between the solvent and the solute are formed (in this particular case, the oxygen atom of the hydroxyl group is supposed to play the role of an acceptor, and the solvent molecule of a donor). However, in order to account for the for-

mation of these bonds, it would be necessary to treat the solvent explicitly, which would require substantial memory and CPU time. These interactions are not included in the Onsager Model used in this work; hence, the intermolecular hydrogen bonds are not included. For this reason, the  $\nu_{\text{OH}}$  frequency in K1 undergoes a blue shift upon solvation. On the other hand, this band is red shifted in  $\text{D}_2\text{O}$  compared to vacuum for K2 and K3. Inclusion of the polar environment significantly increases the peak intensities in all cases. This change is especially pronounced for the K3 conformer, where the hydrogen bond is the strongest.

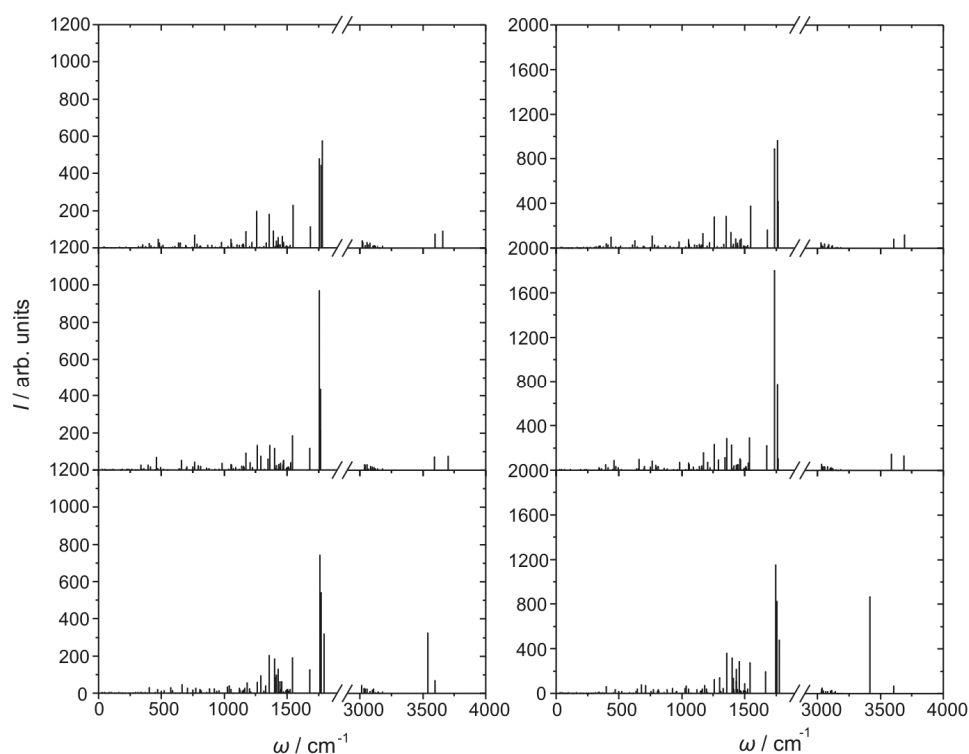


Fig. 5. Vibrational spectra of the conformers in vacuum (left) and  $\text{D}_2\text{O}$  (right). Upper row K1 conformer, middle row K2 conformer and lower row K3 conformer.

There is a break in frequency axis from 1850 to 2950  $\text{cm}^{-1}$ .

The situation with the  $\nu_{\text{NH}}$  mode is less complicated: neither the nitrogen nor the hydrogen atoms participate in hydrogen bonding. Therefore, the N–H stretching frequencies do not differ much between the three conformers, and neither after placing the molecules in  $\text{D}_2\text{O}$ . An exception is the significant intensity increase upon solvation of K2.

Considering the three C=O stretching modes, the values of the three frequencies do not drastically differ, neither in vacuum nor in  $\text{D}_2\text{O}$ . An exception is

$\nu_{C4=O}$ , *i.e.*, the role of an acceptor in hydrogen bonding weakens the  $C_4=O$  bond in K1 and K2 compared to that in K3; hence, in the last case, the frequency has the highest value. Inclusion of the solvent generally increases the peak intensity. An exception is the peak that corresponds to  $C_2=O$  stretching motion in K1 and K2, where the presence of the polar environment decreases the peak intensity.

TABLE II. Vibrational frequencies and infrared intensities of the O–H, N–H and C=O stretching vibrations of the three conformers in vacuum/D<sub>2</sub>O

| Parameter                             | K1        | K2         | K3         |
|---------------------------------------|-----------|------------|------------|
| $\nu_{O-H} / \text{cm}^{-1}$          | 3659/3691 | 3700/3686  | 3540/3416  |
| $I_{\nu_{O-H}} / \text{arb. units}$   | (91/117)  | (73/129)   | (324/868)  |
| $\nu_{N-H} / \text{cm}^{-1}$          | 3595/3607 | 3591/3586  | 3595/3606  |
| $I_{\nu_{N-H}} / \text{arb. units}$   | (73/82)   | (72/142)   | (68/72)    |
| $\nu_{C2=O} / \text{cm}^{-1}$         | 1781/1767 | 1767/1767  | 1769/1757  |
| $I_{\nu_{C2=O}} / \text{arb. units}$  | (575/418) | (433/103)  | (537/821)  |
| $\nu_{C4=O} / \text{cm}^{-1}$         | 1771/1761 | 1765/1761  | 1792/1776  |
| $I_{\nu_{C4=O}} / \text{arb. units}$  | (442/963) | (210/776)  | (319/476)  |
| $\nu_{C2'=O} / \text{cm}^{-1}$        | 1755/1736 | 1754/1736  | 1762/1746  |
| $I_{\nu_{C2'=O}} / \text{arb. units}$ | (479/890) | (969/1796) | (741/1147) |

#### CONCLUSIONS

In this contribution, the geometries and vibrational properties of pyrimidine(6-4)pyrimidone, a less stable product of a photoreaction of 1-methylthymine, were investigated. The bond lengths do not differ significantly between the three conformers and the factor that governs the stability of the conformers is the mutual orientation of the pyrimidine and pyrimidone rings. Due to the presence of polar groups, the order of stability of the three conformers changed when the polar environment was introduced (the polar environment mimics the solvent molecules, D<sub>2</sub>O).

The corresponding infrared spectra (peak position and intensities) are briefly discussed. The most noticeable changes concern the O–H stretching motion, since this hydroxyl group is involved in intramolecular hydrogen bonding. The vibrational frequencies were computed within the harmonic approximation. A detailed analysis would require inclusion of mode couplings, which would cause not only red shift of the O–H stretching band comparing to the harmonic value, but also band broadening and the appearance of additional peaks.

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## ИЗВОД

## КВАНТНО ХЕМИЈСКО ИСПИТИВАЊЕ ФОТОПРОИЗВОДА РЕАКЦИЈЕ ДВА МОЛЕКУЛА 1-МЕТИЛТИМИНА: ПИРИМИДИН(6-4)ПИРИМИДОН АДУКТА

МИРОСЛАВ М. РИСТИЋ, МИЛЕНА ПЕТКОВИЋ и МИХАЈЛО ЕТИНСКИ

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Један од производа фотохемијске реакције инициране UV зрачењем два молекула 1-метилтимина је пиримидин(6-4)пиримидон. Због малог приноса овог производа, тешко је испитати његову геометрију и вибрациони спектар. У овом раду коришћене су квантно хемијске методе да би се карактеризовала структура пиримидин(6-4)пиримидона. Његова три конформера су оптимизована и њихове структуре су поређене. Разматрана су вибрациона својства израчуната у хармонијској апроксимацији. Показано је да се најзначајније промене у инфрацрвеним спектрима јављају као последица образовања водоничних веза. Сви прорачуни су извршени и у вакууму и у D<sub>2</sub>O.

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