Photoisomerizations of N^4-Hydroxycytosines

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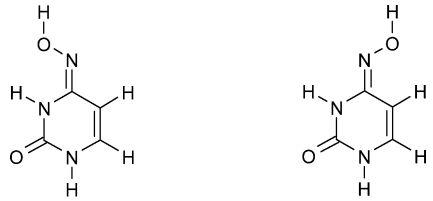
Received: December 9, 2005; In Final Form: February 28, 2006

A series of N^4-hydroxycytosines, unsubstituted or substituted with methyl groups at N(3) or C(5) atoms of the heterocyclic ring, was studied using the matrix-isolation method. Depending on the absence or presence of the methyl substituent at N(3) or C(5) atoms (or at both of them) the syn or anti form of the compounds (or a mixture of both forms) was trapped from the gas phase into a low-temperature matrix. Upon UV ($\lambda >$ 295 nm) irradiation of the matrixes the syn → anti as well as the anti → syn photoisomerization reactions were observed. The syn and anti isomers of N^4-hydroxycytosines were identified by comparing their experimental IR spectra with the theoretical spectra calculated at the DFT(B3LYP)/6-31G(d,p) level. For the majority of the studied compounds, the UV induced reactions led to a photostationary state. The position of the final photostationary state was found to be a sensitive function of weak interactions of a studied N^4-hydroxycytosine with the matrix environment: solid argon or solid nitrogen. However, not all of the studied photoisomerizations led to a classical photostationary state. For some of the investigated N^4-hydroxycytosines, the position of the photostationary state was shifted very strongly in favor of the photoproduct, whereas for some others the position was shifted so strongly in favor of the starting isomer that no photoisomerization was observed. These experimental findings were elucidated by theoretical investigations of the potential energy surfaces of the ground (S_0) and first excited (S_1) electronic states of N^4-hydroxycytosine. The crucial result of these calculations (carried out at the CASSCF level) was the localization of a conical intersection between S_0 and S_1 at a structure with perpendicular orientation of the hydroxylimino group with respect to the heterocyclic ring.

Introduction

N^4-Hydroxycytosines and N^4-methoxycytosines are products of reactions of cytosines with known mutagenic agents hydroxylamine and methoxylamine, respectively. Although the promutagenic activity of these derivatives of cytosine is well documented,[1] the molecular mechanism leading to a C → U(T) transition[2] is not fully understood. The dual functionality of N^4-hydroxy (and N^4-methoxy) cytosines (their ability to base pair like cytosine or uracil) is believed to be related to the possibility of syn or anti orientation of the hydroxylimino (or methoxylimino) group with respect to the N(3) atom of the pyrimidine ring.[3,4] This was documented by studies on base pairing with potentially complementary free bases in nonpolar solutions[5] and in self-complementary oligonucleotide duplexes,[6−9] as well as by selective 10^4-fold inhibition of thymidylate synthase activity by the syn conformer of N^4-hydroxycytidine monophosphate.[10] Studies of the structure of artificial oligonucleotide duplexes revealed that N^4-hydroxy (and N^4-methoxy) cytosines, in the imino tautomeric form with anti orientation of the hydroxylimino (or methoxylimino) group, form canonical Watson−Crick base pairs with adenine.[7−9,11] Nuclear magnetic resonance (^{1}H NMR) studies showed that the syn rotamer has a destabilizing effect on self-complementary oligonucleotide duplexes containing N^4-methoxycytosine−adenine base pair.[7] Cooling of such a duplex to low temperatures forces the N^4-methoxycytosine moiety to adopt the anti form, but the syn rotameric form is adopted again, when the sample is warmed to the temperature of the melting transition.

CHART 1: Syn and Anti Isomers of N^4-Hydroxycytosine



It was also demonstrated that two types of base pairs between guanine and N^4-hydroxy (or N^4-methoxy) cytosine are formed in artificial oligonucleotide duplexes.[6] One of the pairs has a "wobble" geometry, with N^4-hydroxy (or N^4-methoxy) cytosine in the syn-imino form, and the other is of canonical Watson−Crick structure, with the modified cytosine in the amino tautomeric form.

N^4-Hydroxycytosines in the gas phase, in solutions and in the solid state adopt predominantly (or exclusively) the imino-oxo tautomeric form.[3,4,12−14] From the chemical point of view such compounds are oximes with a C═N double bond directly attached to the six-membered pyrimidine ring. The hydroxyl group in the imino tautomers of these compounds can be oriented syn or anti with respect to the reference N(3) atom (Chart 1).

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10.1021/jp057186e CCC: $33.50
Published on Web 03/25/2006

Methylated derivatives of N^4-hydroxycytosine served in a number of studies as models of syn and anti isomers. It might be anticipated that the methyl substituent at N(3) would lead to preference for the anti form, whereas for the compounds methylated at C(5) the syn isomer would be favored. Experimental studies on methylated N^4-hydroxycytosines in the solid state[15] and in solution[3,4] confirmed these expectations; i.e., only the anti form of 3-methyl-N^4-hydroxycytosine and only the syn form of 5-methyl-N^4-hydroxycytosine were experimentally detected. 1,3,5-Trimethyl-N^4-hydroxycytosine represents an interesting case of a somewhat "overcrowded" molecule, with steric constraints to both syn and anti conformations. For this compound, a syn−anti equilibrium was observed in solution, with a preference of ca. 75% for the anti rotamer,[16] whereas the anti form was the only one observed in the solid state.[17]

In the present work the photochemical syn−anti isomerizations were investigated for monomers of N^4-hydroxycytosines. The matrix-isolation technique was applied to the studies of a series of N^4-hydroxycytosines, including the derivatives methylated at N(3) and/or C(5) positions. Moreover, the mechanism of the observed phototransformations has been investigated using the modern quantum chemical methods of theoretical photochemistry.

Experimental Section

Methylated N^4-hydroxycytosines (1,3-dimethyl-N^4-hydroxycytosine, 1,3,5-trimethyl-N^4-hydroxycytosine, 1,5-dimethyl-N^4-hydroxycytosine and 1,5-dimethyl-N^4-methoxycytosine) studied in the present work were synthesized according to the procedures described in refs 18 and 19. Solid samples of the compounds were heated in a miniature glass oven placed in the vacuum chamber of a continuous-flow helium cryostat. The vapors of the studied compound were deposited, together with a large excess of matrix gas, on a CsI window cooled to 10 K. Matrix gases of spectral purity, argon and nitrogen, were supplied by Linde AG and Technische Gase, Leipzig. The IR spectra were recorded using a Perkin-Elmer 580B spectrometer. Matrixes were irradiated with the light from a high-pressure mercury lamp HBO 200 fitted with a water filter and a cutoff filter transmitting the light with $\lambda > 295$ nm. The typical irradiation time was 2 h.

Computations

The ground-state equilibrium geometries of N^4-hydroxycytosine isomers have been determined with the second-order Møller−Plesset (MP2) method using the GAUSSIAN 98 program.[20] For the construction of the syn−anti rotamerization path in the ground state, the coordinate-driven minimum-energy-path (MEP) approach was adopted; i.e., for a given value of the N(3)−C(4)═N−O dihedral angle all remaining intramolecular coordinates were optimized.

Excitation energies and response properties have been calculated with the CC2 method,[21] which is a simplified and cost-effective variant of the coupled-cluster method with single and double excitations. CC2 can be considered to be the equivalent of MP2 for excited electronic states. The equilibrium geometries and the reaction path in the lowest excited singlet state of the system have been determined at the CC2 level, making use of the recently implemented analytic CC2 gradients.[22] These calculations were carried out with the TURBOMOLE program suite making use of the resolution-of-the-identity (RI) approximation for the evaluation of the electron-repulsion integrals.[23,24]

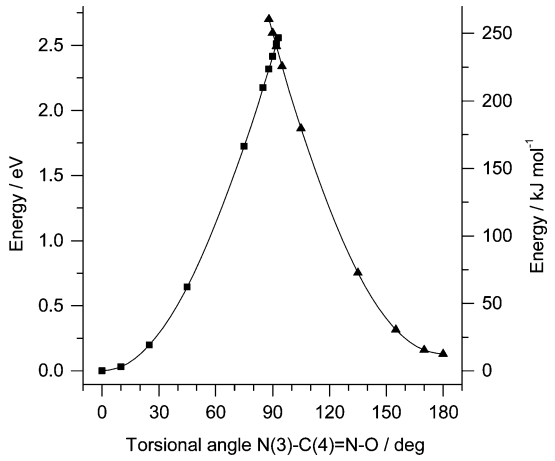


Figure 1. Potential energy profile for rotamerization in the ground state of N^4-hydroxycytosine determined at the MP2/cc-pVDZ level using the minimum energy path approach.

TABLE 1: CC2/cc-pVDZ Energy of Vertical Absorption (ΔE), Dipole Moment (μ), and Oscillator Strength (f) Calculated at the Ground-State Equilibrium of the Two Rotameric Forms of N^4−Hydroxycytosine

state	ΔE, eV	ΔE, kJ mol^{-1}	μ, D	f
		Syn Form		
S_0	0.0	0.0	2.24	
$^1\pi\pi$	5.00	482	2.73	0.21
$^1\pi\pi$	6.16	594	3.24	0.006
$^1n\pi$	6.23	601	2.21	0.002
		Anti Form		
S_0	(0.14)	(13)	2.48	
$^1\pi\pi$	4.69	453	3.72	0.19
$^1\pi\pi$	5.82	562	2.54	0.015
$^1n\pi$	6.03	582	5.59	0.003

The search for a conical intersection (CI) between the ground state and the lowest excited singlet state was performed with the complete active space self-consistent field (CASSCF) method. The active space for the CASSCF calculations was chosen to correlate six electrons distributed in six orbitals. The active space was constructed from the three HOMOs and the three LUMOs obtained from a RHF calculation performed at the guessed CI geometry. The conformation with perpendicular orientation of the hydroxylimino group was chosen as the starting structure for the search of the lowest-energy CI seam between the S_1 and S_0 states. The geometry of the CI point was fully optimized at the CASSCF level.

Having optimized the local syn and anti minima of the ground state and the conical intersection, a linearly interpolated internal-coordinate (LI-IC) reaction path was constructed. The LI-IC path is defined as the straight line in the multidimensional internal-coordinate space that connects a given initial local minimum with a given conical intersection. Single-point energy CASSCF calculations have been performed along each LI-IC path to obtain the potential-energy profiles of the S_1 and S_0 states. In these CASSCF calculations, the active space was defined in the same way as in the search of the conical intersection (six electrons were correlated in six molecular orbitals). Dunning's correlation-consistent basis set of double-ζ quality with polarization functions on all atoms (cc-pVDZ)[25] was utilized in the MP2, CC2 and CASSCF calculations.

For the sake of simulations of infrared spectra, the DFT-(B3LYP)/6-31G(d,p) calculations were performed for syn and anti isomers of N^4-hydroxycytosines studied in this work.[26−28] First the equilibrium geometries of the isomers in question were fully optimized and harmonic frequencies were then calculated

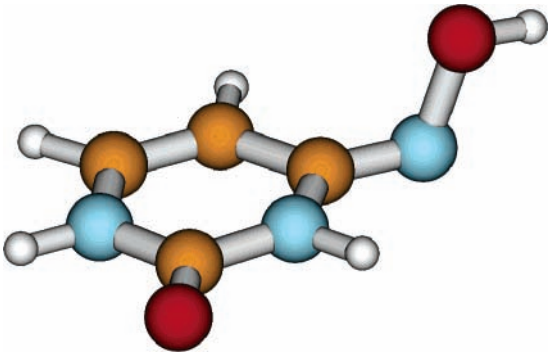


Figure 2. The optimized (CASSCF) structure of N^4-hydroxycytosine at the point of conical intersection between the S_0 and S_1 states.

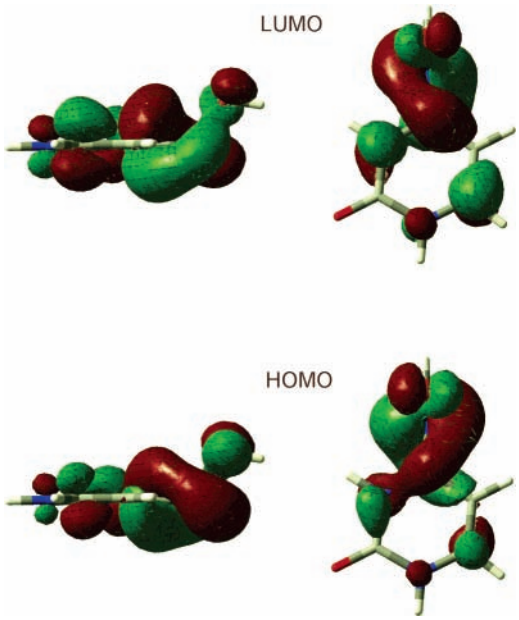


Figure 3. HOMO (lower panels) and LUMO (upper panels) natural orbitals of N^4-hydroxycytosine at the geometry of the conical intersection, presented as two projections with the heterocyclic ring perpendicular and parallel to the plane of observation.

at the same DFT(B3LYP)/6-31G(d,p) level. To correct for vibrational anharmonicity, basis set truncation and the neglected part of electron correlation, the calculated DFT wavenumbers were scaled down by a single factor of 0.98. The GAUSSIAN 98 program[20] was used in these calculations.

Results and Discussion

Potential Energy Surfaces in the Ground and Excited Electronic States. The geometries of the syn and anti rotamers of N^4-hydroxycytosine (Chart 1) have been optimized at the MP2/cc-pVDZ level of theory. At the optimized geometries both syn and anti isomers were found to be planar and moderately polar with dipole moments of about 2 D. The energy of the syn form was predicted, at the MP2/cc-pVDZ level, to be lower by 12.5 kJ mol^{-1}, with respect to the energy of the anti rotamer.

To determine the barrier for syn−anti rotamerization in N^4-hydroxycytosine on the ground-state potential energy (PE) surface, a minimum-energy path was determined at the MP2/cc-pVDZ level. The resulting PE profile is shown in Figure 1. The barrier for syn−anti isomerization was estimated to be as high as 242 kJ mol^{-1}, with respect to the energy of the syn form. This value is significantly higher than the previous prediction of the energy of the transition state between the two forms. In previous calculations,[14] carried out at the MP2/6-31G-

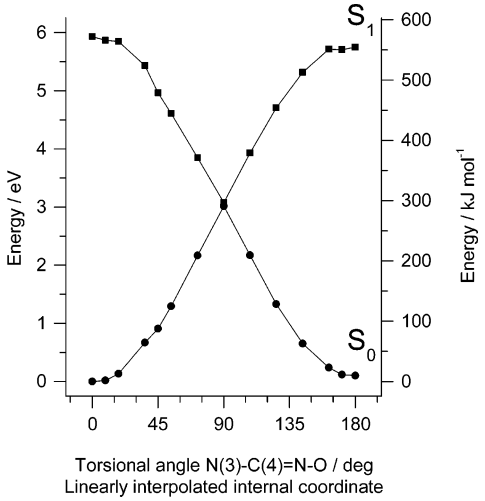
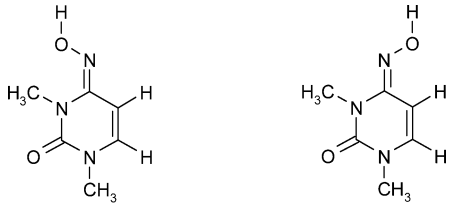


Figure 4. Potential energy profiles calculated for the ground S_0 and first excited singlet S_1 states of N^4-hydroxycytosine using the CASSCF-(6,6)/cc-pVDZ method. The points on PE surfaces of S_0 and S_1 were calculated along the linearly interpolated internal coordinate connecting the syn (or anti) minima with the point of conical intersection.

CHART 2: Syn and Anti Isomers of 1,3-Dimethyl-N^4-hydroxycytosine



(d,p) level (but without geometry optimization at the correlated level), the value of 185 kJ mol^{-1} was obtained as an estimate of the barrier height. A very high barrier for the syn → anti (or vice versa) conversion would undoubtedly preclude flips around the C=N bond on the ground electronic state PE surface, at least for isolated molecules of N^4-hydroxycytosine.

The energies of the vertical $S_0 \rightarrow S_1$ transitions and the corresponding oscillator strengths were calculated, with the aid of the CC2/cc-pVDZ method, at the ground-state equilibrium geometry of the two rotameric forms (Table 1). These calculations predict that for syn and anti forms the absorption to the lowest singlet state (which has the $\pi\pi^*$ orbital nature) is strongly allowed ($f \approx 0.2$). According to the CC2/cc-pVDZ calculations, the $S_0 \rightarrow S_1$ vertical transition in the anti rotamer should be shifted to the red by about 0.3 eV (29 kJ mol^{-1}), in comparison to the analogous transition in the syn form.

The geometries of the syn and anti forms of N^4-hydroxycytosine in the first excited singlet S_1 state were also optimized, at the CC2/cc-pVDZ level, under the constraint of C_s symmetry. Under this restriction the energy of the anti form in S_1 was predicted to be lower by 0.35 eV (34 kJ mol^{-1}) than the energy of the syn form in S_1. It turns out, however, that both rotamers are not stable in the S_1 state at the planar conformation, and unconstrained CC2 optimization of their geometry resulted in spontaneous twisting of the hydroxylimino group. Eventually, at structures with the orientation of the hydroxylimino group close to perpendicular, the calculations failed due to the lack of convergence of the CC2 iteration scheme.

Inspection of the CC2 results at the last converged points indicate that the S_0 and S_1 states become essentially degenerate near the perpendicular conformation of the hydroxylimino group.

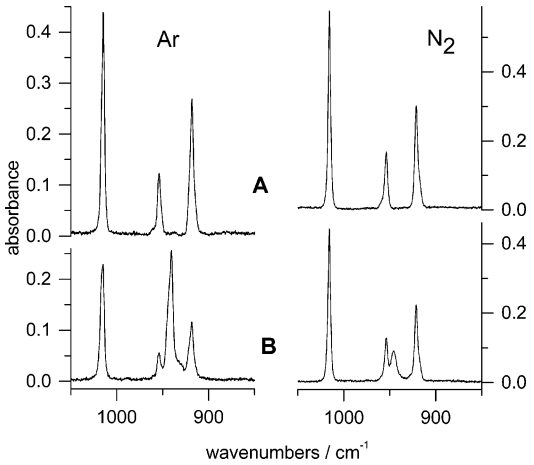
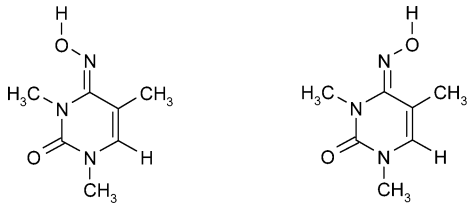


Figure 5. Portion of the infrared spectra of 1,3-dimethyl-N^4-hydroxycytosine isolated in Ar and N_2 matrixes. The spectra were recorded directly after deposition of the matrixes (A) and after prolonged UV (λ > 295 nm) irradiation (B).

This strongly suggests that there should be a conical intersection (CI) of the states near this conformation of the hydroxylimino group. Unfortunately, this effect cannot be well characterized at the CC2 level of theory due to natural limitations of the approximation, which cannot properly handle the degeneracy of the two states. On the other hand, the optimization of the CI geometry (starting from the CC2-guessed geometry) was possible at the CASSCF level. This optimization was carried out and structure of the CI point (optimized at the CASSCF level) is presented in Figure 2. The relevant HOMO and LUMO natural orbitals, resulting from the CASSCF calculation at the optimized CI geometry, are shown in Figure 3. Inspection of the orbitals shows that they are essentially orthogonal to each other and their single occupancy shows a biradicalic character of electron distribution in the molecule at the CI.

CHART 3: Syn and Anti Isomers of 1,3,5-Trimethyl-N^4-hydroxycytosine



syn **anti**

A barrierless access to the CI from the Franck−Condon area of the PE surface of the S_1 state has already been demonstrated by an unconstrained geometry optimization of the S_1 state at the CC2 level. This conjecture is also confirmed by the CASSCF calculation of the S_0 and S_1 potential-energy profiles along the respective LI-IC (linearly interpolated internal-coordinate) paths that connect the ground-state minima of the two rotameric forms with the CI between the S_0 and S_1 states. The plot of this profile, presented in Figure 4, shows that after excitation to S_1, the molecule can barrierlessly relax from the Franck−Condon area to the point of the conical intersection between S_1 and S_0.

The strong nonadiabatic interactions present at the CI provide a source of very efficient and fast internal conversion to the ground state. Because the CI between the PE surfaces of the S_1 and S_0 states occurs near the perpendicular conformation of the hydroxylimino group, the $S_1 \rightarrow S_0$ radiationless decay provides the driving force for the syn−anti photoisomerization of N^4-hydroxycytosines.

The representation of the ground and excited state PE surfaces (shown in Figure 4) clearly demonstrates that the system excited to S_1 can relax either to the initial isomer or to the structure with the hydroxylimino group rotated by 180°. In the latter case, the syn → anti (or anti → syn) photoisomerization occurs. An

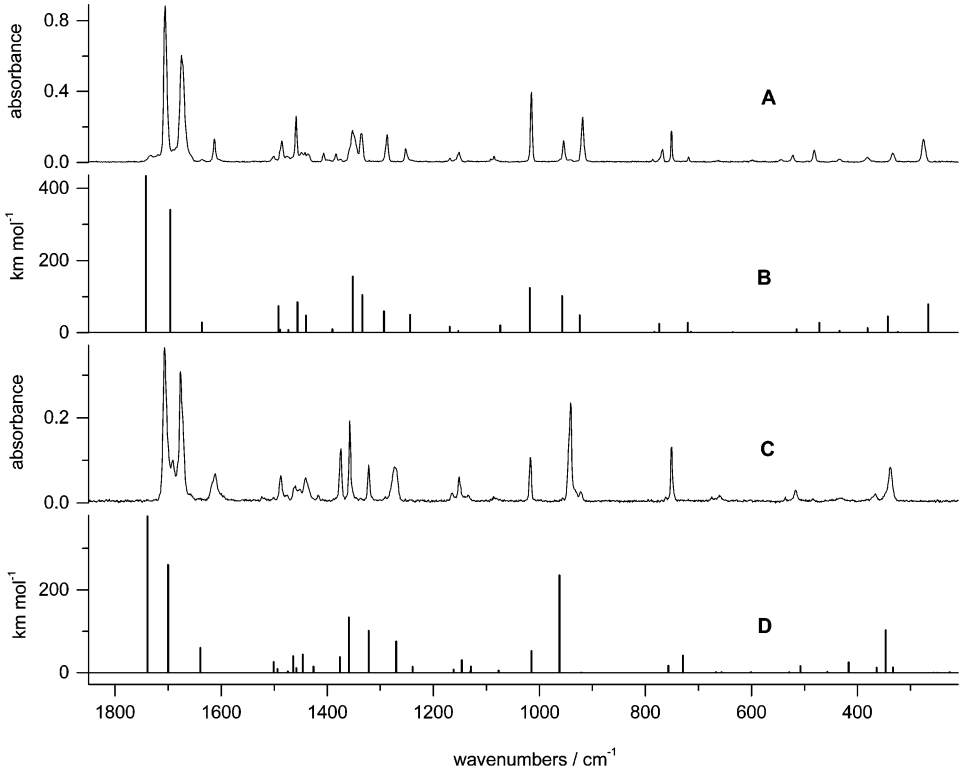


Figure 6. Infrared spectra of monomeric 1,3-dimethyl-N^4-hydroxycytosine: (A) experimental spectrum recorded after deposition of an Ar matrix; (B) theoretical spectrum of the anti rotamer of the imino-oxo tautomer of the compound; (C) experimental spectrum of the photoproduct generated upon UV (λ > 295 nm) irradiation; (D) theoretical spectrum of the syn rotamer of the imino-oxo tautomer of the compound. Theoretical spectra were calculated at the DFT(B3LYP)/6-31G(d,p) level. The theoretical frequencies were scaled by 0.98.

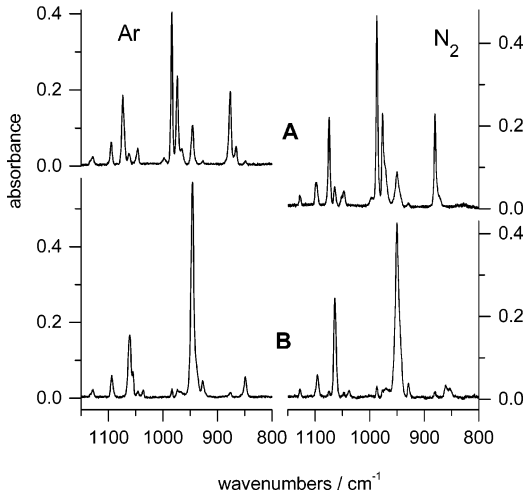
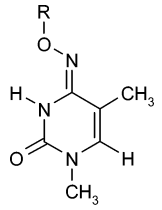


Figure 7. Portion of the infrared spectra of 1,3,5-trimethyl-N^4-hydroxycytosine isolated in Ar and N_2 matrixes. The spectra were recorded directly after deposition of the matrixes (A) and after prolonged UV (λ > 295 nm) irradiation (B).

apparent feature of the plot presented in Figure 4 is its approximate symmetry with respect to the torsion around the C═N bond. In the hypothetical case of exact symmetry, the system excited to S_1 would relax with equal probability to each of the two isomers. If the UV absorption coefficients of both forms were also equal, the final stage of the photoisomerization reaction would correspond to an equimolar mixture of the two isomers. For asymmetric systems such as N^4-hydroxycytosines, the deviations from symmetry of the S_0 and S_1 potential energy surfaces are expected to govern the direction of the observed photoreaction. However, if these deviations are small, then the syn → anti and anti → syn phototransformations should occur simultaneously and lead to a final, photostationary state.

CHART 4: Syn Isomers of 1,5-Dimethyl-N^4-hydroxycytosine (R = H) and 1,5-Dimethyl-N^4−Methoxycytosine (R = CH_3)



syn

***N^4*-Hydroxycytosine and 1-Methyl-*N^4*-hydroxycytosine.** Syn−anti photoisomerization reactions were recently investigated experimentally for the monomers of N^4-hydroxycytosine and 1-methyl-N^4-hydroxycytosine isolated in argon or nitrogen low-temperature matrixes.[29,30] Both compounds appeared directly after deposition of the matrixes exclusively in the imino-oxo tautomeric form with the syn orientation of the hydroxyl-imino group. These forms are stabilized (with respect to the anti isomers) by an attractive interaction between the lone electron pairs of the oxygen atom in the hydroxyl group and the hydrogen atom attached to the N(3) atom of the ring.

Upon UV irradiation the syn forms of N^4-hydroxycytosine and of 1-methyl-N^4-hydroxycytosine converted into the corresponding anti isomers. In both cases, the final stage of the photoreaction corresponded to a photostationary state. The percentage of the compound, converted from the syn isomer into the anti form until the photostationary state was achieved, was significantly changed when the argon matrix environment was replaced by solid nitrogen. For both compounds isolated in nitrogen matrixes, a clear shift of the position of the photostationary state in favor of a higher population of photoproduced anti isomers was observed.

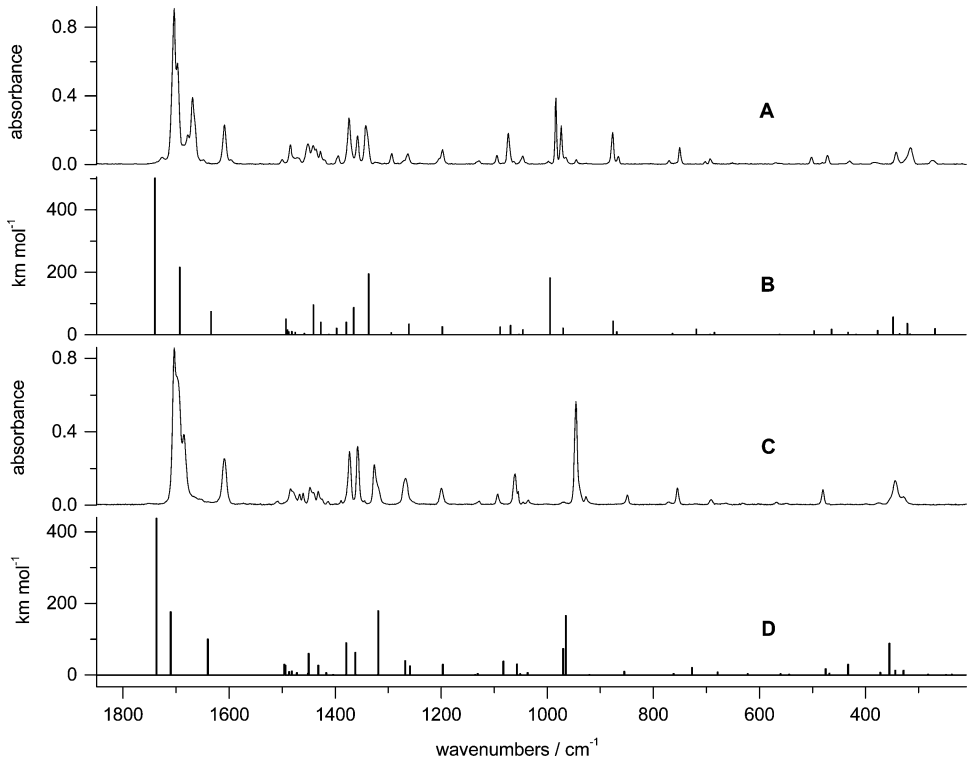


Figure 8. Infrared spectra of monomeric 1,3,5-trimethyl-N^4-hydroxycytosine: (A) experimental spectrum of the substrate of the UV-induced photoreaction; (B) theoretical spectrum of the anti rotamer of the imino-oxo tautomer of the compound; (C) experimental spectrum of the photoproduct generated upon UV (λ > 295 nm) irradiation; (D) theoretical spectrum of the syn rotamer of the imino-oxo tautomer of the compound. Experimental spectra were recorded for the compound isolated in an Ar matrix. Theoretical spectra were calculated at the DFT(B3LYP)/6-31G(d,p) level. The theoretical frequencies were scaled by 0.98.

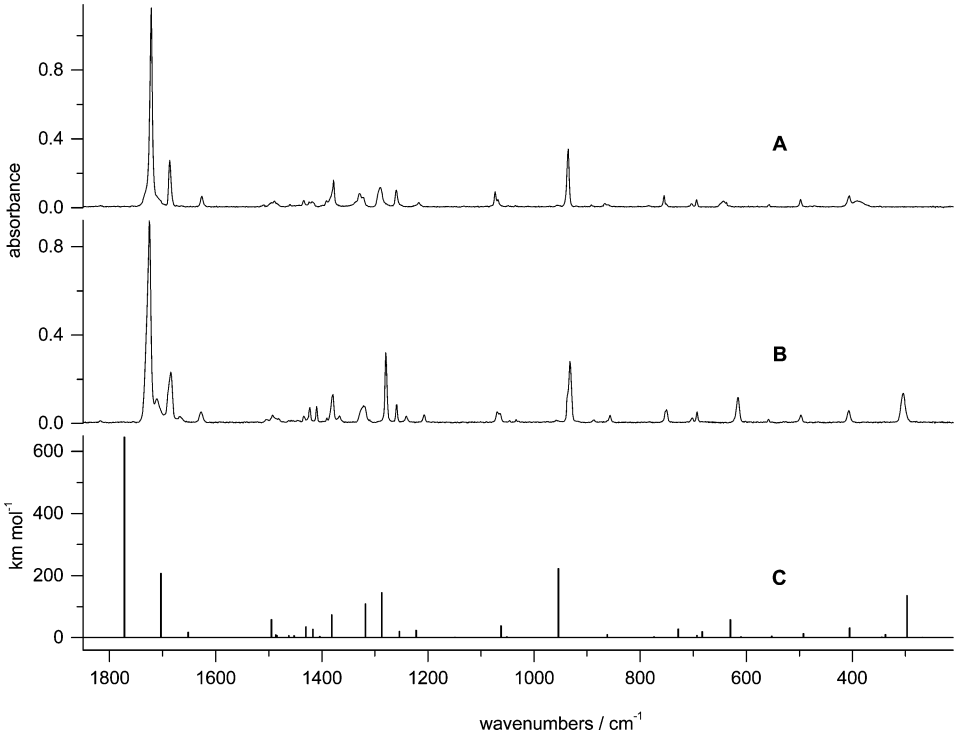


Figure 9. Infrared spectra of monomeric 1,5-dimethyl-N^4-hydroxycytosine: (A) experimental spectrum of the compound isolated in a nitrogen matrix; (B) isolated in an argon matrix; (C) theoretical spectrum of the syn rotamer of the imino-oxo tautomer of the compound. Theoretical spectra were calculated at the DFT(B3LYP)/6-31G(d,p) level. The theoretical frequencies were scaled by 0.98.

For N^4-hydroxycytosine,[30] the percentage of the anti form photoproduced for the compound isolated in an argon matrix was 28%, whereas for the nitrogen matrix the corresponding value was 54%. Similarly, in the case of 1-methyl-N^4-hydroxycytosine[29] the percentage of syn to anti conversion was 60% (in an argon matrix) and 80% (in a nitrogen matrix). These results demonstrate how sensitive the position of the photostationary state is to even the slightest changes in the shapes of the potential energy surfaces of the ground and excited electronic states, induced by weak interactions with matrix environment.

1,3-Dimethyl-N^4-hydroxycytosine. Methylation at the N(3) nitrogen atom (Chart 2) has a substantial effect on the structural and photochemical properties of a N^4-hydroxycytosine. The stabilizing interaction between the lone electron pairs of the hydroxyl group and the hydrogen atom of the N(3)H group does not exist in 1,3-dimethyl-N^4-hydroxycytosine. In addition, the N(3)-methyl derivative has a built in steric preference for the anti rotamer.

The experiments, carried out in the present work for 1,3-dimethyl-N^4-hydroxycytosine, have demonstrated that the anti rotamer is exclusively adopted by monomers of this compound isolated in argon or nitrogen low-temperature matrixes before any irradiation. In some respects, the pattern of the syn−anti photoisomerization reaction observed for matrix-isolated 1,3-dimethyl-N^4-hydroxycytosine must be different from that observed for N^4-hydroxycytosine and 1-methyl-N^4-hydroxycytosine. The main difference concerns the very fact that, in the case of 1,3-dimethyl-N^4-hydroxycytosine, the initial substrate of the photoreaction is the anti form, whereas in the case of N^4-hydroxycytosine and 1-methyl-N^4-hydroxycytosine the initial form was the syn rotamer.

Upon UV (λ > 295 nm) irradiation of matrix-isolated 1,3-dimethyl-N^4-hydroxycytosine the photoisomerization reaction transforming the anti isomer into the syn form was observed (see Figure 5). The bands of the initial IR absorption spectrum became weaker, whereas a set of new absorptions attributed to the syn isomer emerged. Even after prolonged irradiation, the photoreaction did not lead to complete transformation of the substrate into the photoproduct. At the final, photostationary stage of the anti → syn photoisomerization, the percentage of this conversion was equal to 64% (Ar matrix) or 28% (N_2 matrix). The matrix effect on the anti → syn photoisomerization in 1,3-dimethyl-N^4-hydroxycytosine might seem contradictory to the influence of the matrix environment on the final stage of the syn → anti photoisomerization in N^4-hydroxycytosine and 1-methyl-N^4-hydroxycytosine. For the two latter compounds, replacement of argon by a solid nitrogen environment resulted in conversion of a larger amount of the syn substrate into the anti photoproduct, whereas for 1,3-dimethyl-N^4-hydroxycytosine much less photoproduct was generated in the N_2 matrix. On the other hand, for all of the investigated N^4-hydroxycytosines (substituted and unsubstituted), the position of a photostationary state was systematically shifted in favor of a higher relative population of the anti isomer, when the argon matrix environment was replaced by nitrogen. The mechanism of transformation of very weak intermolecular interactions into significant shifts of photostationary states must be of such a delicate nature that detailed rationalization of the experimental findings described above seems to be beyond the current capabilities of theoretical photochemistry.

The initial and photoproduced isomers of 1,3-dimethyl-N^4-hydroxycytosine were identified by comparison of their experimental IR spectra with the spectra calculated at the DFT(B3LYP)/6-31G(d,p) level of theory. This comparison is presented in Figure 6. The IR spectra of the syn and anti isomers are quite similar to each other. There are no characteristic bands that would be present in the spectrum of one of the forms and absent in the spectrum of the other isomer. This reflects the nature of the isomerization that concerns only reorientation of a small fragment in a comparatively large molecule (consisting of 20 atoms). None of the chemical bonds changes its character on anti−syn transformation. Therefore, the infrared bands due

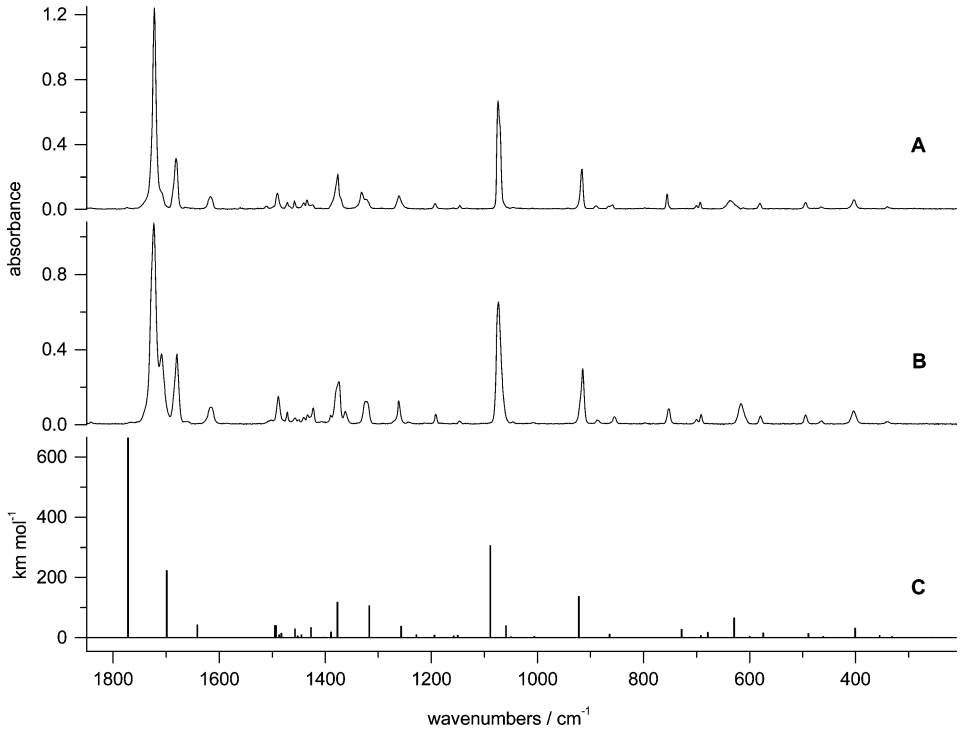


Figure 10. Infrared spectra of monomeric 1,5-dimethyl-N^4-methoxycytosine: (A) experimental spectrum of the compound isolated in a nitrogen matrix; (B) isolated in an argon matrix; (C) theoretical spectrum of the syn rotamer of the imino-oxo tautomer of the compound. Theoretical spectra were calculated at the DFT(B3LYP)/6-31G(d,p) level. The theoretical frequencies were scaled by 0.98.

to stretching vibrations are positioned at nearly the same frequencies as their counterparts in the spectrum of the other isomer. Such bands are not particularly useful for identification purposes. This concerns also the bands in the high-frequency range (above 3000 cm^{-1}), where the absorptions due to stretching vibrations of the OH groups (ν_{OH}) are expected. The ν_{OH} band at 3630 cm^{-1} (Ar) in the spectrum of the photoproduced syn isomer was found to differ only slightly in frequency from the analogous band observed at 3639 cm^{-1} (Ar) in the spectrum of the anti form (the substrate of the photoreaction). For the purpose of identification of the syn and anti isomers, the analysis of lower-frequency regions (1100−200 cm^{-1}) of the IR spectra is much more useful. The patterns of low-frequency IR bands due to in-plane and out-of-plane bending vibrations in the spectra of the two isomers are clearly different from each other. These patterns are well reproduced by the results of theoretical calculations (see Figure 6). Hence, a reliable identification of the syn and anti forms becomes feasible.

1,3,5-Trimethyl-N^4-hydroxycytosine. Introduction of two methyl groups at the N(3) and C(5) positions of N^4-hydroxycytosine, that is, in the direct vicinity of the isomerizing hydroxylimino group, modifies structural and photochemical properties of the resulting derivative (Chart 3), with respect to both 1-methyl-N^4-hydroxycytosine and 1,3-dimethyl-N^4-hydroxycytosine. For 1,3,5-trimethyl-N^4-hydroxycytosine in solutions, both anti and syn rotamers of the compound were previously[16] observed with relative populations of 75% and 25%, respectively.

The anti isomer of this compound was found to dominate also in the present matrix-isolation experiments. However, this domination was not so pronounced as in the case of the 1,3-dimethyl-N^4-hydroxycytosine. After deposition of the argon or nitrogen matrixes containing monomers of 1,3,5-trimethyl-N^4-hydroxycytosine, ca. 85% of the compound adopted the anti form, whereas ca. 15% was found in the syn structure. The infrared bands belonging to the spectra of each of the isomers could be separated thanks to the change of the relative population of the two forms that occurred upon UV ($\lambda > 295$ nm) irradiation. As can be seen in Figure 7, the UV-induced photoisomerization reaction led to conversion of nearly all of the initial anti isomer of 1,3,5-trimethyl-N^4-hydroxycytosine into the syn form. The final photostationary stage of the phototransformation (if in this case one can speak about a photostationary state at all) was so strongly shifted in favor of the syn form that replacement of argon matrix environment by solid nitrogen had no effect on the final result of the photoreaction.

The two forms involved in the photoisomerization reaction, the substrate and the product, observed for matrix-isolated 1,3,5-trimethyl-N^4-hydroxycytosine were assigned to the anti and syn isomers of the compound on the basis of comparison of the experimental IR spectra with the spectra calculated at the DFT(B3LYP)/6-31G(d,p) level of theory (see Figure 8). Analogously, as in the case of 1,3-dimethyl-N^4-hydroxycytosine, the ν_{OH} bands in the spectra of the syn and anti isomers were found at very similar frequencies: 3631 and 3633 cm^{-1} (Ar), respectively. The analysis of the low-frequency fragments of these spectra proved once again to be particularly useful for identification purposes.

1,5-Dimethyl-N^4-hydroxycytosine and 1,5-Dimethyl-N^4-methoxycytosine. Matrix isolation studies of 1,5-dimethyl-N^4-hydroxycytosine and 1,5-dimethyl-N^4-methoxycytosine (Chart 4) complete the gallery of possible behavior of N^4-hydroxycytosine derivatives upon UV excitation. Energy-lowering interaction between the N(3)H hydrogen atom and the lone electron pair of the hydroxylimino group contributes to stabilization of the syn isomers of these compounds. For 1,5-dimethyl-N^4-hydroxycytosine and 1,5-dimethyl-N^4-methoxycytosine, the relative stability of the syn form, with respect to the anti isomer, is also sterically conferred by repulsive interaction of the methyl group at C(5) and the hydroxylimino (or methoxylimino) group in the anti orientation.

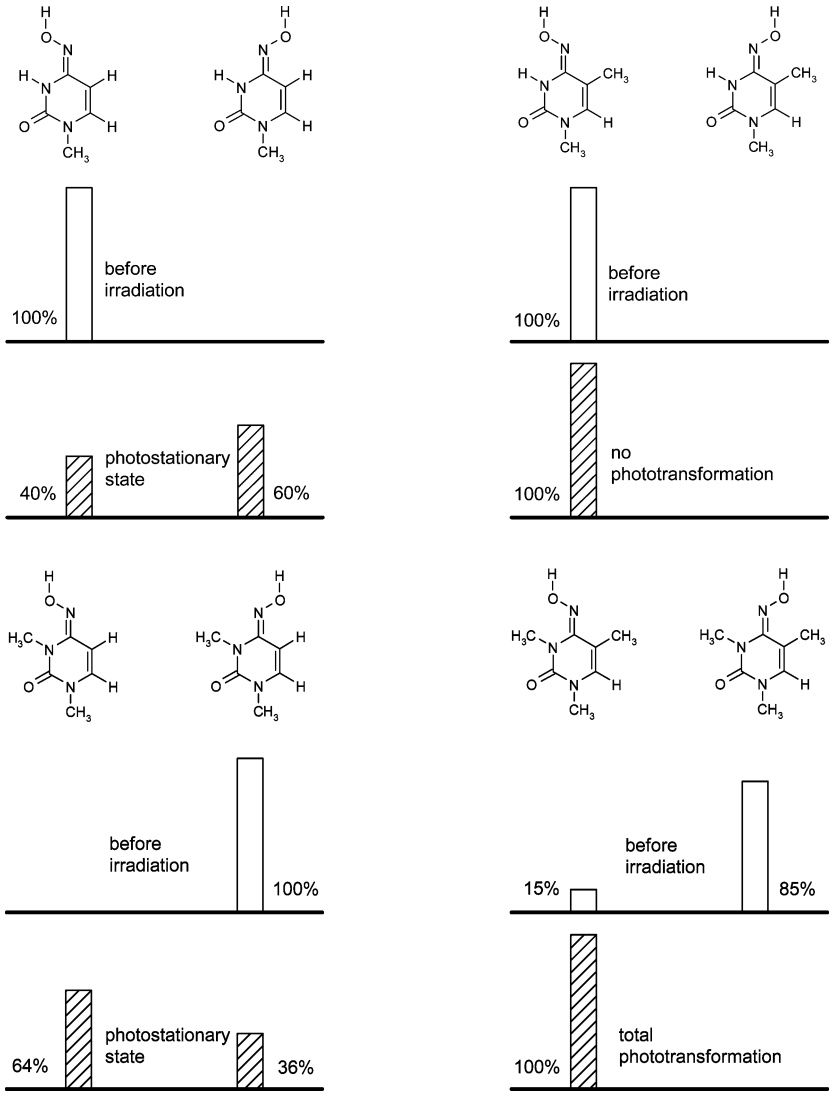


Figure 11. Illustration of the variety of photochemical behavior of N^4-hydroxycytosines. Empty bars show the ratio of syn and anti isomers before UV irradiation; dashed bars show the ratio of these isomers after prolonged UV (λ > 295 nm) irradiation. For 1-methyl-N^4-hydroxycytosine and 1,3-dimethyl-N^4-hydroxycytosine, the ratio of syn and anti isomers at a photostationary state concerns compounds isolated in Ar matrixes.

In the matrix-isolation experiments, carried out in the present work, both compounds were found to adopt exclusively the syn isomeric form in solid argon or nitrogen. The infrared spectra of 1,5-dimethyl-N^4-hydroxycytosine and 1,5-dimethyl-N^4-methoxycytosine are compared with the results of theoretical simulations in Figures 9 and 10, respectively. For 1,5-dimethyl-N^4-hydroxycytosine, the band due to the ν_{OH} vibration was found at 3650/3642 cm^{-1} (Ar). Neither this frequency nor that of the band due to the $\nu_{N(3)H}$ vibration (3446 cm^{-1}; Ar) indicates the presence of the intramolecular hydrogen bond between the proton of the N(3)H group and the oxygen atom of the hydroxylimino fragment. This is especially interesting because the syn isomer of N^4-hydroxycytosine is often considered[31] to be a molecule with an intramolecular hydrogen bond between N(3)H and the oxygen atom. For compounds with no intramolecular hydrogen bonding, such as 2(1*H*)-pyridinone[32] and 4(3*H*)-pyrimidinone,[33] the IR bands due to ν_{NH} vibrations were observed at 3438 and 3428 cm^{-1} (Ar). With respect to these values, no shift toward lower frequencies was observed for the $\nu_{N(3)H}$ band of 1,5-dimethyl-N^4-hydroxycytosine, as well as for the corresponding band found at 3445 cm^{-1} (Ar) in the spectrum of 1,5-dimethyl-N^4-methoxycytosine. This comparison indicates that the intramolecular hydrogen-bond-like interaction in the syn forms of 1,5-dimethyl-N^4-hydroxycytosine and 1,5-dimethyl-N^4-methoxycytosine must be very weak, despite the short distance detected in an X-ray study.[15] The reason for this is, most probably, the hybridization on the oxygen atom, which brings the majority of the electron density out of the plane of the molecule. Hence, the electron density at the position of the N(3)H proton must be low.

No changes in the IR spectra were observed for 1,5-dimethyl-N^4-hydroxycytosine and 1,5-dimethyl-N^4-methoxycytosine after the matrixes were irradiated with UV (λ > 295 nm) light. Apparently, in these cases, the syn form initially trapped in a matrix coincides with the form that would be photoproduced, if the reactant had the opposite (anti) orientation of the hydroxylimino group.

Concluding Discussion

The theoretical results obtained in the current work at the CC2 level (dynamically correlated, but not variational) as well as at the CASSCF level (variational, but largely neglecting the dynamical electron correlation effect) lead to the conclusion that there exists a low-lying conical intersection between the PE surfaces of the S_1 and S_0 states near the perpendicular orientation of the hydroxylimino group with respect to the heterocyclic ring of N^4-hydroxycytosine. On taking into account the barrierless

access of the conical intersection from the Franck−Condon regions of both isomers, an easy syn → anti (or anti → syn) conversion in N^4-hydroxycytosines should be expected upon excitation to S_1. This finding is crucial for description of the photophysical behavior of the system.

Photochemical isomerization reactions in both directions syn → anti and anti → syn have been experimentally observed for a number of N^4-hydroxycytosines (unsubstituted or substituted with methyl groups). The variety of photochemical behavior of N^4-hydroxycytosines is summarized in Figure 11. The final stage of syn → anti or anti → syn photoprocesses is a photostationary state. In some cases this state is very much shifted in favor of high population of a photoproduct (and an almost total conversion is observed), for some other species the photostationary state is shifted strongly in the direction of the substrate (and no phototransformation is observed). However, for the majority of the investigated derivatives of N^4-hydroxycytosine, the position of the photostationary state is located between these two extremes (and UV irradiation leads to partial conversion of one of the isomers into the other). It has also been demonstrated that the position of the photostationary state is usually a sensitive function of the weak interactions between the photoreactive molecule and the matrix environment.

Syn → anti and anti → syn phototransformations, similar to those described above, were also observed for matrix-isolated N^4-methoxycytosines[34] and N^2-hydroxyisocytosines.[35]

References and Notes

(1) Marfey, P.; Robinson, E.; *Mutat. Res.* **1981**, *86*, 155.
(2) Pavlov, Y. I.; Suslov, V. V.; Shcherbakova, P. V.; Kunkel, T. A.; Ono, A.; Matsuda, A.; Schaaper, R. M. *Mutat. Res.* **1996**, *357*, 1.
(3) Kierdaszuk, B.; Shugar, D. *Biophys. Chem.* **1983**, *17*, 285.
(4) Kierdaszuk, B.; Stolarski, R.; Shugar, D. *Eur. J. Biochem.* **1983**, *130*, 559.
(5) Shugar, D.; Kierdaszuk, B. *J. Biosciences* **1985**, *8*, 657.
(6) Nedderman, A. N. R.; Stone, M. J.; Williams, D. H.; Kong Thoo Lin, P.; Brown, D. M. *J. Mol. Biol.* **1993**, *230*, 1068.
(7) Stone, M. J.; Nedderman, A. N. R.; Williams, D. H.; Kong Thoo Lin, P.; Brown, D. M. *J. Mol. Biol.* **1991**, *222*, 711.
(8) Fazakerley, G. V.; Gdaniec, Z.; Sowers, L. C. *J. Mol. Biol.* **1993**, *230*, 6.
(9) Van Meervelt, L.; Moore, H. M.; Kong Thoo Lin, P.; Brown, D. M.; Kennard, O. *Nucleosides Nucleotides* **1990**, *9*, 467.
(10) Rode, W.; Zielinski, Z.; Dzik, J. M.; Kulikowski, T.; Bretner, M.; Kierdaszuk, B.; Ciesla, J.; Shugar, D. *Biochemistry* **1990**, *29*, 10835.
(11) Gdaniec, Z.; Ban, B.; Sowers, L. C.; Fazakerley, G. V. *Eur. J. Biochem.* **1996**, *242*, 271.
(12) Van Meervelt, L. *Acta Crystallogr. C* **1991**, *47*, 2635.
(13) Ramaekers, R.; Dehaen, W.; Adamowicz, L.; Maes, G. *J. Phys. Chem. A* **2003**, *107*, 1710.
(14) Les, A.; Adamowicz, L.; Rode, W. *Biochim. Biophys. Acta* **1993**, *1173*, 39.
(15) Shugar, D.; Huber, C. P.; Birnbaum, G. I. *Biochim. Biophys. Acta* **1976**, *447*, 274.
(16) Niedzwiecka-Kornas, A.; Kierdaszuk, B.; Stolarski, R.; Shugar, D. *Biophys. Chem.* **1998**, *71*, 87.
(17) Birnbaum, K. B.; Kierdaszuk, B.; Shugar, D. *Nucleosides Nucleotides* **1996**, *15*, 1805.
(18) Brown, D. M.; Hewlins, M. J. E.; Schell, P. *J. Chem. Soc. C* **1968**, 1925.
(19) Janion, C.; Shugar, D. *Biochem. Biophys. Res. Commun.* **1965**, *18*, 617.
(20) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople; J. A. *Gaussian 98*, revision A.7.; Gaussian, Inc.: Pittsburgh, PA, 1998.
(21) Christiansen, O.; Koch, H.; Jørgensen, P. *Chem. Phys. Lett.* **1995**, *243*, 409.
(22) Hättig, C. *J. Chem. Phys.* **2003**, *118*, 7751.
(23) Ahlrichs, R.; Bär, M.; Häser, M.; Horn, H.; Kölmel, C. *Chem. Phys. Lett.* **1989**, *162*, 165.
(24) Weigend, F.; Häser, M.; Patzelt, H.; Ahlrichs, R. *Chem. Phys. Lett.* **1998**, *294*, 143.
(25) Woon, D. E.; Dunning, T. H., Jr. *J. Chem. Phys.* **1993**, *98*, 1358.
(26) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098.
(27) Lee, C. T.; Yang, W. T.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
(28) Vosko, S. H.; Wilk, L.; Nusair, M. *Can. J. Phys.* **1980**, *58*, 1200.
(29) Stepanenko, T.; Lapinski, L.; Sobolewski, A. L.; Nowak, M. J.; Kierdaszuk, B. *J. Phys. Chem. A* **2000**, *104*, 9459.
(30) Lapinski, L.; Nowak, M. J.; Adamowicz, L. *Photochem. Photobiol.* **2001**, *74*, 253.
(31) Birnbaum, G. I.; Kulikowski, T.; Shugar, D. *Can. J. Biochem.* **1979**, *57*, 308.
(32) Nowak, M. J.; Lapinski, L.; Fulara, J.; Les, A.; Adamowicz, L. *J. Phys. Chem.* **1992**, *96*, 1562.
(33) Lapinski, L.; Fulara, J.; Nowak, M. J. *Spectrochim. Acta, Part A* **1990**, *46*, 61.
(34) Lapinski, L.; Ramaekers, R.; Kierdaszuk, B.; Maes, G.; Nowak, M. J. *J. Photochem. Photobiol. A* **2004**, *163*, 489.
(35) Lapinski, L.; Nowak, M. J.; Kwiatkowski, J. S.; Leszczynski, J. *Photochem. Photobiol.* **2003**, *77*, 243.