Phototautomeric Reaction, Tautomerism, and Infrared Spectra of 6-Thiopurine. Experimental Matrix Isolation and Quantum-Mechanical (Conventional ab Initio and Density-Functional Theory) Studies

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The infrared spectra of 6-thiopurine (6-mercaptopurine) isolated in Ar and N_2 low-temperature matrices show that the molecule exists as a mixture of thione N(1)H,N(7)H and thiol N(9)H tautomers. The photoreaction converting the thione N(1)H,N(7)H form of the compound into the thiol N(7)H form is observed for the matrix-isolated species. The assignments of the substrate to the thione N(1)H,N(7)H form and the photoproduct to the thiol N(7)H form are confirmed by good agreement between the experimental infrared spectra and the spectra theoretically predicted at the DFT(B3LYP)/6-31G(d,p) level. The thiol N(9)H tautomer is photochemically stable under the conditions of UV irradiation applied in the experiment ($\lambda > 320$ nm). The experimentally estimated ratio of tautomers in the matrix (non-UV-irradiated) [thiol N(9)H]/[thione N(1)H,N(7)H] = 0.24 correlates well with the theoretical estimate of the relative energies of the tautomeric forms of the compound predicted at conventional ab initio calculations [total energies including electronic energies calculated at the Hartree−Fock (HF)/6-31G(d,p) level corrected for electron-correlation contributions at the MP4(SDQ)/6-31G(d,p) level and zero-point energies at the HF/6-31G(d,p) level].

Introduction

An understanding of the physicochemical properties and tautomeric behavior of the purine and pyrimidine nucleic acid bases and their model systems is of fundamental importance in molecular biology and in relation to general aspects of chemical binding and physical chemistry.[1] Bases containing a sulfur instead of an oxygen atom have also been the subject of considerable interest because of their importance in biology (e.g., the sulfur bases and their derivatives are components of natural tRNAs; they show an important pharmacological activity; etc.).[2] The biological and pharmacological activities of 6-thiopurine (6-mercaptopurine) and its derivatives have been reviewed recently by Aarbakke et al.[3] Experiments with *Escherichia coli* cells treated with 6-thiopurine and exposed to near-UV radiation show that the photodynamic action of 6-thiopurine with UV can cause some kinds of DNA lesions that evoke lethality and mutation.[4] This compound was widely used as an effective inhibitor of cell growth and as a cell-killing agent. Hence, 6-thiopurine was clinically applied as a cytostatic antitumor drug,[5] and it is a well-known medicine, efficient in treatment of acute lymphoblastic or promyelocytic leukemia[6] and in inflammatory bowel disease.[7]

As a continuation of our efforts in predicting the tautomeric properties of the thioderivatives of the bases and the model systems,[8,9] we present here both experimental and quantum-mechanical studies of the structure of 6-thiopurine (6-mercaptopurine, thiohypoxanthine).

The molecule can formally exist in several tautomeric forms with the thiol forms in two conformations (cis or trans depending on the orientation of the SH group to the N(7) atom; see Figure 1). In this paper we present a quantum-mechanical estimate of the relative stabilities of several isolated tautomeric species of 6-thiopurine based on conventional ab initio methods [a comparison of the relative total energies of the tautomeric forms, i.e., the relative electronic energies calculated at the HF/6-31G(d,p) level and corrected by the relative electron-correlation energies predicted at the MP4(SDQ)/6-31G(d,p) level and the zero-point energies predicted at the HF/6-31G(d,p) level]. These results are compared with similar estimates based on DFT(B3LYP)/6-31G(d,p) calculations. However, the main focus of the present study is on the experimental data for which we present the infrared spectra of 6-thiopurine isolated in low-temperature Ar and N_2 matrixes, the UV-induced phototautomeric reaction which converts the thione N(1)H,N(7)H tautomer (**1**; initially, the most abundant) into the thiol N(7)H form (**2*t***), and the analysis of the infrared spectra of the observed species.

Experimental Section

6-Thiopurine was purchased from Fluka Chemie AG (Switzerland) and was purified by vacuum sublimation prior to the experiment. The sample was heated in a microoven placed in the vacuum chamber of the cryostat. The vapors of 6-thiopurine were deposited, together with large excess of argon, on a CsI (or sapphire for UV spectroscopy) window cooled to 10 K. Spectral-grade argon and nitrogen matrix gases were supplied by Linde AG (Germany) and VEB Technische Gase Werke Berlin (Germany), respectively. The infrared spectra were

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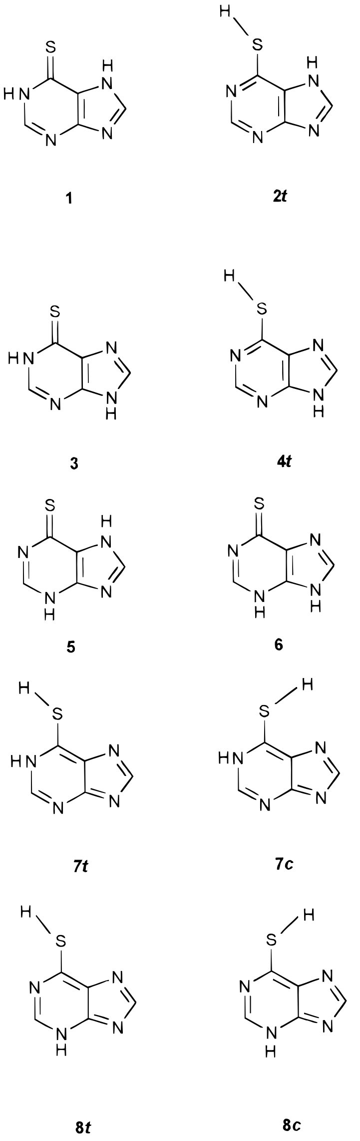


Figure 1.

recorded with a Perkin-Elmer 580B spectrometer. Integral intensities of the infrared (IR) absorption bands were measured by numerical integration.

The UV absorption spectra were taken using a 480-nm focal length monochromator (Digikroem 480), a deuterium lamp, and a Philips XP2020 photomultiplier. The signal was registered in the single-photon counting mode.

Matrices were irradiated with the light from a high-pressure HBO 200 mercury lamp fitted with a water filter and a cutoff filter (WG 320) transmitting light with $\lambda > 320$ nm. The time of irradiation was 45-min.

Computational Details. The geometries of the tautomeric forms of 6-thiopurine (Figure 1) were optimized using two quantum-mechanical approaches: (a) a conventional ab initio method at the Hartree−Fock (HF) level[10] and (b) the density-functional theory[11] with the combined Becke's three-parameter exchange functional and the gradient-corrected functional of Lee et al.[12] [DFT(B3LYP) method]. In all calculations, the planar structures of the tautomeric forms were assumed. At the HF-optimized geometries, additional calculations were performed to estimate the electron-correlation contributions to the total electronic energies of the tautomers in question. The calculations were carried out at the second, third, and fourth order of the Møller-Plesset perturbation theory[13] [MP2, MP3, and MP4-(SDQ)], applying the frozen-core (fc) approximation.[10] The lower level of perturbation theory, i.e., the MP2 method, was also used to calculate the dipole moments of the tautomers at the HF-optimized geometries. In all quantum-mechanical calculations, the standard 6-31G(d,p) basis set was used.[10] The harmonic vibrational wavenumbers and absolute intensities were calculated by means of the HF and DFT(B3LYP) methods for the corresponding optimized geometries.

All quantum-mechanical calculations were carried out using the GAUSSIAN 92/DFT program.[14] Note that the DFT(B3LYP) optimizations were carried out using the Opt = Tight and Int = FineGrid options whereas the IR spectra were predicted at the DFT level with the Int = FineGrid option.

Transformations of the force constant matrix in Cartesian coordinates allowed ordinary normal-coordinate calculations to be performed as described by Schachtschneider.[15] The internal coordinates were defined in the same way as those previously used for purine[16] except for the internal coordinates describing the stretching and the in-plane and out-of-plane bending vibrations of the NH and C═S groups attached to the pyrimidine ring which were chosen as for the 2-pyridinethiol/2(1*H*)-pyridinethione system.[9a] Potential-energy distribution (PED) analysis was performed according to the standard procedure.[17]

To correct for vibrational anharmonicity, basis set truncation, and the neglected part of electron correlation, the calculated DFT(B3LYP) wavenumbers were scaled down by a single factor of 0.98.

Results and Discussion

Tautomeric Stabilities. 6-Thiopurine may adopt several tautomeric forms (Figure 1). Table 1 collects the relative electronic energies computed at the HF/6-31G(d,p) level corrected by both the inclusion of the electron correlation contribution estimated at different levels of perturbation theory [MP2, MP3, and MP4(SDQ)] and the calculation of the zero-point vibrational energies at the HF/6-31G(d,p) level. For comparison, an estimate of the relative total energies of the tautomers using the DFT(B3LYP) approach is also presented in Table 1. In both cases, the temperature-dependent contributions to the free energies, which usually are computed with the rigid-rotor harmonic-oscillator ideal-gas approximation,[18] were neglected (compare, however, recent calculations for the tautomers of the DNA bases[19] and for the model systems[9m]).

The thione N(1)H,N(7)H form (**1**) is predicted to be the most stable by both approaches. The total energies of the cis and trans rotamers of the thiol N(9)H tautomer (**4*c*, 4*t***) predicted by the conventional ab initio approach are higher by 5.4 or 6.9 kJ/mol, respectively, than the energy of the most stable form, **1**. The thione N(1)H,N(9)H form (**3**) is predicted by this approach to be 9.9 kJ/mol higher in energy than the thione N(1)H,N(7)H tautomer. The energies of other tautomeric forms of 6-thiopurine are much higher. This prediction correlates very well with the experimental estimate of relative stabilities of the 6-thiopurine tautomers made in this paper (see Experimental Results).

An inspection of data collected in Table 1 shows that ΔZPE contributions calculated at both HF and DFT(B3LYP) levels are similar to each other. However, the DFT(B3LYP) approach yields different orders of stability for tautomers **3**, **4*t***, and **4*c*** relative to tautomer **1** compared to the prediction by a conventional ab initio approach (see Table 1). Note that the Δ*E*(tot) values predicted for these tautomers by the DFT-

TABLE 1: Relative Energies of the 6-Thiopurine Tautomers[a]

method	tautomers										
	2*t*	**2*c***	**3**	**4*t***	**4*c***	**5**	**6**	**7*t***	**7*c***	**8*t***	**8*c***
ab initio											
Δ*E*(el)											
HF	40.68	57.40	13.93	18.99	16.94	43.38	111.69	114.45	96.47	75.11	68.32
MP2	36.34	53.47	12.54	18.40	16.48	39.03	105.91	107.27	88.88	69.05	62.17
MP3	35.83	52.10	10.71	15.34	13.48	39.63	102.15	95.64	85.93	59.77	53.67
MP4(SDQ)	38.22	54.39	10.30	17.97	16.19	38.48	100.03	103.89	87.40	67.40	61.28
ΔZPE	−12.62	−13.84	−0.38	−11.08	−10.81	−1.33	−4.72	−13.00	−11.14	−10.46	−9.97
Δ*E*(tot)	25.60	40.55	9.92	6.89	5.38	37.15	95.31	90.89	76.26	56.94	51.31
DFT(B3LYP)											
Δ*E*(el)	47.28	58.50	13.06	28.97	26.84	38.45	104.33	110.25	92.31	72.71	66.06
ΔZPE	−11.57	−12.31	−0.38	−10.15	−9.81	−1.25	−4.86	−12.19	−10.47	−9.85	−9.31
Δ*E*(tot)	35.71	46.19	12.68	18.82	17.03	37.20	99.47	98.06	81.84	62.86	56.75

[a] All energies in kJ/mol. For the numbering of the tautomers see Figure 1. Energy of thione N(1)H,N(7)H tautomer (**1**) taken as reference. Trans (*t*) and cis (*c*) mean the conformation of the SH group relative to the N(7) atom, respectively. The zero-point energies, ΔZPE, calculated at the HF and DFT(B3LYP) levels are unscaled values. The ab initio Δ*E*(tot) value is a sum of the electronic energy calculated with the MP4(SDQ) contribution and the zero-point ΔZPE value calculated at the HF level.

TABLE 2: Rotational Constants and Dipole Moments of the 6-Thiopurine Tautomers[a,b]

	1, thione N(1)H,N(7)H		**3**, thione N(1)H,N(9)H		**4*t***, thiol (trans) N(9)H		**4*c***, thiol (cis) N(9)H	
quantity	HF	DFT	HF	DFT	HF	DFT	HF	DFT
A	1990.07	1952.08	2003.41	1963.80	2008.29	1965.47	2006.72	1960.09
B	1176.75	1154.42	1162.66	1140.68	1166.52	1146.33	1167.59	1150.99
C	739.49	725.42	735.70	721.56	737.91	724.04	738.12	725.16
μ	0.43 (0.52)	0.24	7.16 (6.01)	6.25	3.39 (3.31)	3.18	4.78 (4.65)	4.56

[a] Rotational constants *A*, *B*, and *C* in megahertz; dipole moment μ in debye units. [b] Dipole moments calculated with MP2(full) densities at the HF-optimized geometries are in the parentheses.

(B3LYP) method are significantly greater than those calculated by the conventional ab initio method. It is not unexpected because calculations of the relative energies of different tautomers of a molecule with sulfur at the MP4(SDTQ) or, at least, at the MP4(SDQ) level provide more reliable results than calculations using the DFT(B3LYP) method.[8,9j,l,m] This is why the MP4(SDQ) calculations are used here for comparison with an experimental estimate of the relative stabilities of the tautomers of 6-thiopurine.

Molecular Parameters. The optimized geometries of 6-thiopurine are not presented here, but in Table 2, we have collected the calculated rotational constants and dipole moments of the thione N(1)H,N(7)H form (**1**; the most favorable from an energetic point of view) and three other forms (**3**, **4t**, and **4c**) which are less energetically stable than **1**. Unfortunately, neither the rotational constants nor the dipole moment was measured for 6-thiopurine. However, the DFT(B3LYP) method provides theoretical data for these quantities congruent with the experimental data, when available (the reader is referred to our recent review[8] on this matter). For instance, the rotational constants predicted by the DFT(B3LYP) method for three tautomers of cytosine[9l] agree very well with the experimental data, and they are helpful in predicting which tautomers exist in the vapor phase. We expect that the data presented in Table 2 may be useful in future experimental work on 6-thiopurine.

The predicted dipole moments of tautomers **3**, **4*t***, and **4*c*** are significantly higher than the dipole moment of **1**. When going from a noninteracting environment (vapor phase and a rare-gas matrix) to an interacting environment (crystalline and a polar solution), the interaction between the tautomers and the environment may cause a change in the stabilities of the tautomers in question.

Experimental Results. The experimental spectrum of 6-thiopurine isolated in an Ar matrix is presented in Figure 2A. After irradiation of the matrix with light ($\lambda > 320$ nm) from the high-pressure mercury lamp, the spectrum changed considerably. The spectrum collected after the irradiation is shown in Figure 2B. Three sets of bands can be distinguished in these spectra. The first is the set of initially intense bands which disappear after the UV irradiation, the second is the set of bands of the photoproduct which appear after UV irradiation, and the third is the set of low-intensity bands which do not change upon irradiation of the matrix.

In the high-frequency region of the spectrum recorded before irradiation, three bands are observed. Two intense bands placed at 3460 and 3415 cm^{-1} vanish upon UV irradiation. The first band may be assigned to the stretching vibration of the NH bond in the imidazole ring. In the spectra of adenine and purine,[16] analogous bands were found at 3493 and 3498 cm^{-1}, respectively. The somewhat lower frequency observed in the present case may result from the interaction with the free electron pairs of the sulfur atom. The frequency of the second band is close to that previously observed for the stretching vibration of the NH group located in the pyrimidine ring in the direct vicinity of the C═S fragment. For example, in 4(3*H*)-pyrimidinethione,[9d] the analogous band was found at 3394 cm^{-1}. This suggests that the spectrum of the form which dominates in the matrix before irradiation and vanishes upon UV excitation belongs to one of the thione tautomers of 6-thiopurine. On the basis of the results of the theoretical estimates of relative energies of 6-thiopurine tautomers (Table 1), we consider the thione N(1)H,N(7)H form the most probable candidate for the carrier of the spectrum which vanishes upon UV irradiation. A comparison of the experimental spectrum recorded prior to UV irradiation of the matrix with the spectrum theoretically calculated for the thione N(1)H,N(7)H tautomer strongly supports the assignment of the most abundant form of 6-thiopurine to this tautomer. This comparison is shown in Figure 3.

The wavenumbers and relative intensities of the bands observed in the spectrum of the initially dominating form are

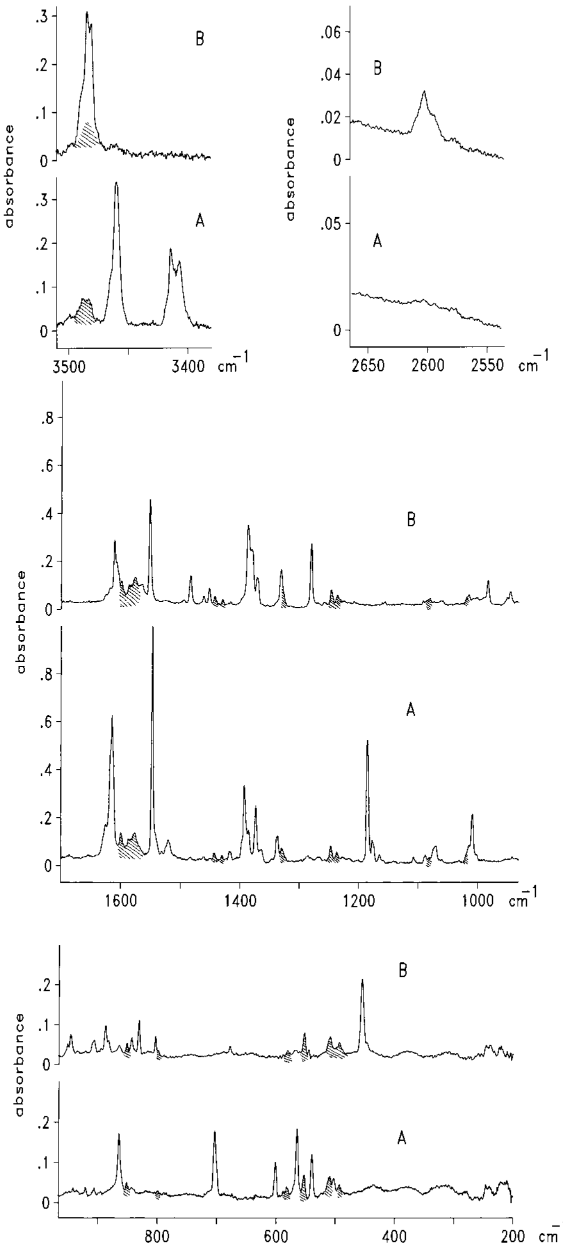


Figure 2. IR spectra of 6-thiopurine isolated in an Ar matrix: (A) after deposition of the matrix (10 K) and (B) after UV irradiation ($\lambda >$ 320 nm). The bands which did not change upon UV irradiation are marked as shaded areas.

collected in Table 3, together with the spectrum predicted at the DFT(B3LYP)/6-31G(d,p) level for the thione N(1)H,N(7)H tautomer. As could be expected for this thione form, two bands due to γN(1)H (at 703 cm^{-1}) and γN(7)H (at 539 cm^{-1}) vibrations are observed in the lower frequency part of the spectra. These bands can be easily identified because of their considerable shift toward higher frequencies in the N_2 matrix. The theoretical positions of these two bands (706 and 536 cm^{-1}) are in nearly perfect agreement with the measured positions (703 and 539 cm^{-1}, respectively). The third band due to coupled γC=S and γN(7)H vibrations was observed at 601 cm^{-1} (predicted band at 594 cm^{-1}). As can be seen (Table 3), the theoretical spectral pattern in the frequency range below 1000 cm^{-1} matches very well the experimentally observed spectra. In this region the correspondence between the experiment and theoretical data predicted with the harmonic approximation should be better than those at higher frequencies because of the lack of combinational tones.[8] The agreement observed in the present case confirms our assignment of the observed spectrum to the thione N(1)H,N(7)H tautomer of 6-thiopurine. At frequencies of 1200−1100 cm^{-1}, a strong band due to the normal mode with the contribution from the ν C=S vibration is usually observed in the IR spectra of other heterocyclic thione com-

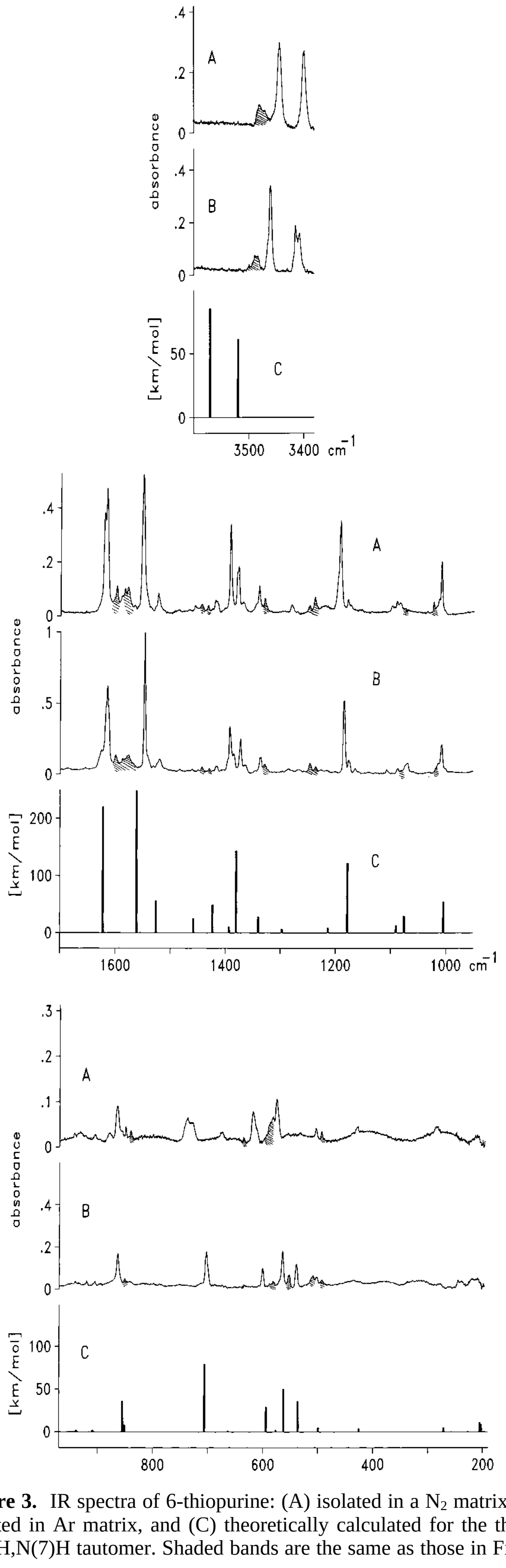


Figure 3. IR spectra of 6-thiopurine: (A) isolated in a N_2 matrix, (B) isolated in Ar matrix, and (C) theoretically calculated for the thione N(1)H,N(7)H tautomer. Shaded bands are the same as those in Figure 2.

TABLE 3: Experimental and Calculated IR Spectra of the Thione N(1)H,N(7)H Tautomer of 6-Thiopurine[a]

experimental Ar matrix		N_2 matrix		calculated DFT/6-31G(d,p)		
$\tilde{\nu}$, cm^{-1}	*I* rel	$\tilde{\nu}$, cm^{-1}	*I* rel	$\tilde{\nu}$,† cm^{-1}	A^{th}, km/mol	PED (%)
3465 sh						
3460	109	3443	158	3570	85	νN(7)H (100)
3418 sh						
3415	95	3399	131	3519	61	νN(1)H (100)
3407				3200	1.4	νC(8)H (99)
				3135	7	νC(2)H (100)
1625 sh						
1617 sh		1620				
1615	251	1616	185	1622	221	νC(5)C(6) (23), νC(2)N(3) (29)
1547	168	1551	189	1561	249	νC(2)N(3) (29), βN(1)H (28)
1520	22	1523	23	1526	56	νN(3)C(4) (19), νC(4)C(5) (13), νC(8)N(9) (11)
		1456	3	1458	25	νC(8)N(9) (29), βC(8)H (16), νC(4)C(5) (12), βr5 (12), νN(3)C(4) (11)
1417	8	1418	13	1423	49	βN(1)H (25), βN(7)H (19), νN(7)C(4) (16), νC(5)N(7) (11)
				1393	11	βC(2)H (35), νC(4)C(5) (12), νC(5)N(7) (11)
1393	105	1392	85			
1385				1380	144	νC(4)C(5) (16), νC(4)N(9) (14), νN(7)C(8) (13), βr4 (10), βN(7)H (10)
		1379	53			
		1377				
1373	68					
1364		1368	15	1340	28	βC(2)H (33), νC(4)N(9) (24)
1337	18	1340	32	1297	7	νC(8)N(9) (26), βC(8)H (15), νC(4)N(9) (10)
1285	9	1281	12			
1267	5					
		1219	11	1213	9	βC(8)H (38), βR1 (21)
1185	111	1192	100	1178	123	νN(1)C(6) (45), βN(1)H (19), νCS (15)
1176	22	1178	7			
1165	8	1173 sh				
1107	6	1094	23	1090	13	νN(1)C(2) (32), νN(7)C(8) (24), βN(7)H (19)
1088	7	1089				
1071	27	1084		1075	30	νN(7)C(8) (29), νN(1)C(2) (22), βN(7)H (14)
1014 sh		1013 sh				
1008	67	1009	51	1004	55	νC(4)N(9) (19), νCS (17), βr5 (17)
1000						
940	1	944	2	938	2	βr4 (69), νC(4)C(5) (15)
920	4	932	4			
905	4			909	2	γC(2)H (106)
		880	6			
864	50	865	39	855	36	βR1 (32), βr4 (11), νCS (10), βR2 (10), βR3 (10)
842	6	850	2	851	8	γC(8)H (102)
				773	0.1	τR1 (49), τr4 (38)
703	52	738	46	706	79	γN(1)H (102), γCS (10)
		730				
				670	0.2	νN(3)C(4) (27), βr5 (26), βR3 (13)
		678	?	663	1	τr5 (74), γCS (12), γN(7)H (10)
601	18	619	33	594	29	γCS (37), γN(7)H (24), τr4 (14), τR1 (10)
				576	2	βR2 (26), νC(5)C(6) (24), βr5 (15), βR3 (12)
564	42	582	29	562	50	τR1 (23), τr4 (20), τR2 (16), γN(7)H (15)
539	25	576		536	36	γN(7)H (52), τr5 (23), τR2 (13), γCS (12)
502	7	504	5	499	5	βR3 (36), βCS (18), βR1 (10)
		429	3	425	4	βR2 (48), νCS (28)
		285	6	271	5	τR3 (54), τRr (24), τr4 (21)
				205	11	βCS (67), βR3 (11)
				204	9	τR1(53), τR2 (30), τR3 (13)
				134	13	τR2 (4), τR1 (28), γCS (21)

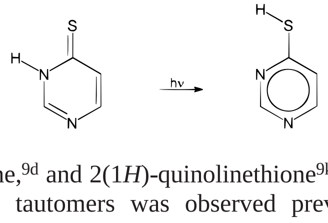
[a] The experimental wavenumbers ($\tilde{\nu}$) and the experimental integral intensities (*I*) were normalized so that the sum of the integrated absorbances of all normal modes observed experimentally was equalized to the sum of the absolute intensities (A^{th}) obtained in the calculations. Calculated harmonic wavenumbers scaled by a factor of 0.98 are represented by $\nu^{\dagger}$; potential energy distribution (PED) values lower than 10% are omitted. Abbreviations: rel, relative; ν, stretching; β, bending; τ, wagging; and γ, bending out-of-plane.

pounds.[8] In the spectrum considered here, the corresponding band was observed at 1185 cm^{-1} (Ar).

In the spectrum of the photoproduct, a weak band at 2603 cm^{-1} was recorded. Only one band (at 3485 cm^{-1}) has been found in the frequency region above 3300 cm^{-1}. The first band may be easily assigned to the stretching SH vibration and the second to the NH stretching in the imidazole ring. Hence, the form appearing after UV irradiation is most probably a thiol form of 6-thiopurine.

The photoreaction transforming the thione forms of 4(3*H*)-pyrimidinethione,[9d] (Scheme 1) 2(1*H*)-pyridinethione,[9a] 3(2*H*)-pyridazinethione,[9d] and 2(1*H*)-quinolinethione[9k] into the corresponding thiol tautomers was observed previously in this laboratory. It seems most reasonable to assume that the same type of reaction also occurs for 6-thiopurine. So, the photo-

SCHEME 1



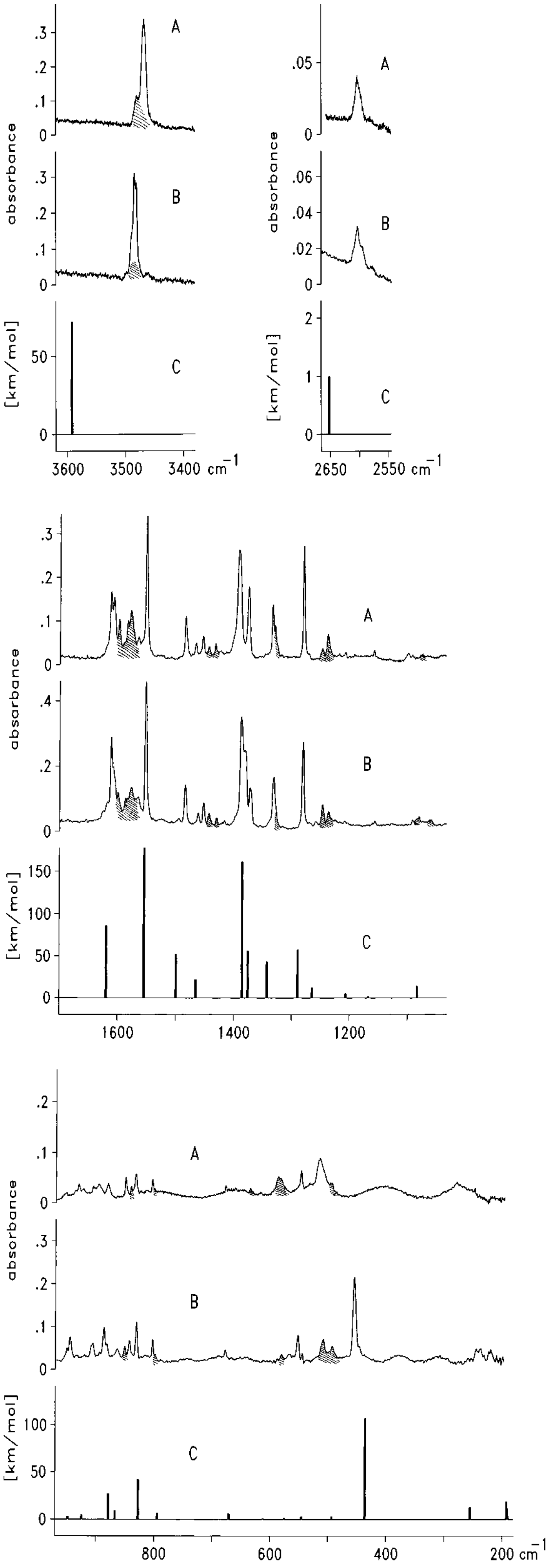


Figure 4. IR spectra of matrix-isolated 6-thiopurine after 45 min of UV irradiation ($\lambda > 320$ nm): (A) N_2 matrix, (B) Ar matrix and (C) theoretically calculated for the thiol N(7)H tautomer. Shaded bands are the same as those in Figure 2.

product resulting upon irradiation of the thione N(1)H,N(7)H tautomer of 6-thiopurine should be the thiol N(7)H form (**2*t***) of the compound. The experimental spectrum of the photoproduct is compared in Figure 4 with the spectrum theoretically predicted for the thiol N(7)H tautomer of 6-thiopurine. The similarity of these spectra is remarkable. It should be stressed that the theoretical spectrum calculated for the thiol N(9)H form agrees much less with the spectrum of the photoproduct. The same is the case for the theoretical spectrum of the thione N(1)H,N(9)H tautomer and the initial experimental spectrum of 6-thiopurine. Hence, the substrate and the product involved in the observed photoreaction may be assigned, without ambiguity, to the thione and thiol N(7)H forms, respectively, and the reaction scheme may be proposed as presented by Scheme 2.

The wavenumbers and IR intensities of the bands observed in the spectrum of the photoproduct are compared in Table 4 with the IR spectrum predicted at the DFT(B3LYP)/6-31G(d,p) level for the thiol N(7)H tautomer (**2*t***) of the molecules in question. In the low-frequency part of the experimental spectrum, a strong band was observed at 456 cm^{-1}. Because of the nitrogen matrix effect, this band can be easily assigned to the γNH vibration. In the theoretical spectrum of the thiol N(7)H tautomer, a single band owing to the γN(7)H vibration is predicted whereas for the thiol N(9)H tautomer the nearly equally intense bands (at 588 and 508 cm^{-1}) with dominating contributions from γN(9)H vibration were expected (Tables 5 and 6 in Supporting Information). The observation of only one intense band in this region supports our conclusion that the observed thiol tautomer is the N(7)H and not the N(9)H form.

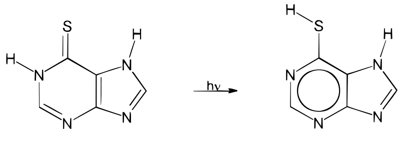
Along with the spectra of the thione N(1)H,N(7)H tautomer (before irradiation) and the thiol N(7)H tautomer (after irradiation), the spectrum of a third form was observed as a set of bands which do not change their intensities upon irradiation of the matrix. These bands (with the relative intensities given in parentheses) were observed at 3486 (27), {1600, 1587, 1557} (113), 1443 (7), 1430 (4), 1327 (9), {1247, 1236} (21), 1080 (3), 1018 (5), 851 (4), 798 (2), 580 (4), 552 (17), 509 (12), 493 (8) cm^{-1} (Ar).[20] The calculations of the relative energies of 6-thiopurine tautomers predict that the second of the most stable forms of the compound is the two rotamers of the thiol N(9)H tautomer. The energy difference of 5−7 kJ/mol between this tautomer and the thione N(1)H,N(7)H suggests that, in the vapors of 6-thiopurine which were subsequently trapped in the low-temperature matrix, the thiol N(9)H form should be populated in detectable abundance. The calculations were performed at a quite high level of theory [including electron-correlation contributions at the MP4(SDQ) level] which proves to provide reliable results as far as tautomeric equilibria in the gas phase are concerned. Moreover, the fact that the considered form does not react photochemically upon irradiation with UV light ($\lambda > 320$ nm) supports the supposition that it is not a thione form. From an energetic point of view (Table 1), the thione N(1)H,N(9)H form, which is higher in energy by 9.9 kJ/mol with respect to the thione N(1)H,N(7)H tautomer, should be also taken into account. This form should, however, react photochemically like the 4(3*H*)-pyrimidinethione or the thione N(1)H,N(7)H tautomer of 6-thiopurine. The other tautomeric forms are predicted to be so high in energy that one can safely rule out the possibility of their population at the temperature of vaporization (ca. 360 K) of the compound. The next argument in favor of the thiol N(9)H tautomer is the position of the experimentally observed band at 3486 cm^{-1} in the spectrum of the form which does not react photochemically. The band which grows at 3485 cm^{-1} in the spectrum of the thiol N(7)H tautomer overlaps with it. The predicted frequencies of the bands due to the stretching vibrations of the NH bonds in the imidazole ring are also nearly the same for the two forms: 3592 cm^{-1} for the thiol N(7)H tautomer and 3590 and 3592 cm^{-1} for the cis and trans rotamers of the thiol N(9)H form, respectively. Although

TABLE 4: Experimental and Calculated IR Spectra of the Thiol N(7)H Tautomer of 6-Thiopurine[a]

experimental Ar matrix		N2 matrix		calculated DFT/6-31G(d,p)		
$\tilde{\nu}$, cm^{-1}	I rel	$\tilde{\nu}$, cm^{-1}	I rel	$\tilde{\nu}$,† cm^{-1}	A^{th}, km/mol	PED (%)
3489 sh						
3485	139	3468	200	3592	72	νN(7)H (100)
3481						
				3191	1	νC(8)H (99)
				3126	25	νC(2)H (100)
2603	18	2601	23	2651	1	νSH (100)
1619 sh		1612				
1611	81	1607	98	1619	86	νC(5)C(6) (34), νC(5)N(7) (15)
1564	7					
1552	102	1551	121	1554	179	νN(3)C(4) (31), νC(4)C(5) (24), νN(1)C(2) (11)
1495	3					
1483	33	1484	32	1499	52	νC(8)N(9) (53), βC(8)H (18), νC(5)C(6) (10)
1462	8	1466	8			
				1465	22	βC(2)H (39), νC(2)N(3) (17), νC(6)N(1) (16)
1452	13	1454	13			
1388 1381	227	1392	177	1385	162	βN(7)H (35), νN(7)C(8) (25), βC(6)N(1) (16)
1372	46	1375	65	1375	56	νC(5)N(7) (22), νC(2)N(3) (18), νC(4)C(5) (17), βC(8)H (11)
1332	46	1334	30	1342	43	νC(4)N(9) (35), βC(2)H (17), νC(4)C(5) (10)
		1280	70	1289	57	νC(6)N(1) (25), βC(8)H (14), βC(2)H (14), νN(3)C(4) (10)
1281	76	1271 sh				
1259	3			1264	12	νC(2)N(3) (25),νC(8)N(9) (16),νC(6)N(1) (13), νN(1)C(2) (12), βC(8)H (12)
		1218	1			
1208	2	1208	3	1206	5	βR1 (32), νN(1)C(2) (19), βC(8)H (14)
1157	5	1158	3	1167	2	νN(1)C(2) (29), νC(5)N(7) (20), βC(8)H (11)
		1100	10			
1092	2	1093		1083	14	νN(7)C(8) (61), βN(7)H (28)
1015	4					
		991				
				974	46	νC(4)N(9) (20), β r5 (14), νCS (13), β SH (11), β R1 (10)
983	28	984	23			
950 sh						
945	23	953	3	948	3	γC(2)H (108)
907	11	936 932 924	10	924	5	βr4 (68), νC(4)C(5) (15)
887	26	896	13	878	27	βSH (57), βR1 (16)
881 sh						
864	8	880	8	867	9	γC(8)H (100)
843	8	850	10			
831	8	833	13	827	43	βSH (18), βR2 (16), βR1 (11), νCS (10), βr4 (10)
803	7	804	6	794	7	τR1 (58), τr4 (31)
678	5	678	2	671	6	βr5 (33), νN(3)C(4) (21), βR3 (20)
				658	0.1	τr5 (59), τRr (10)
				612	1	τr4 (29), τr5 (29), τR3 (24), τR1 (13)
567	3			575	2	βR2 (34), νC(5)C(6) (21), βR3 (11), βr5 (11)
545	5	548	10	545	4	γCS (36), τR2 (32), τRr (11)
				493	3	βR3 (36), βCS (16)
456	77	515	51	436	107	γN(7)H (98)
447 sh						
				396	0.2	νCS (53), βR2 (30)
				288	0.4	τR3 (37), τRr (23), τr4 (21), γCS (10)
		280	2	255	13	τSH (51), τR2 (18), τRr (14), τR3 (10)
				192	19	τSH (41), τRr (39)
				190	4	βCS (73)
				129	11	τR2 (42), γCS (28), τR1 (13), τR3 (13)

[a] See footnote to Table 3. In addition, the experimental intensities of the thiol N(7)H tautomer were multiplied by a factor of 1.29 (Ar) and 1.36 (N_2) times greater than those for the thione N(1)H,N(7)H form presented in Table 3.

SCHEME 2



the theoretical predictions of the infrared spectra may be somewhat inaccurate in predicting the absolute values of the wavenumbers because of implied approximations, they should, because of cancellation of errors, quite accurately predict the relative positions of the two bands because of very similar vibrations. Not only the band at highest frequency but also the observed bands in the lower frequency region of the spectrum of the nonreactive form agree well with the theoretically predicted spectrum of the thiol N(9)H form. The extracted spectrum of the nonreactive form is compared in Figure 5 with the theoretical spectra calculated for the cis and trans rotamers of the thiol N(9)H tautomer. As is shown in this figure, almost all intense bands predicted theoretically have their counterparts in the experimental spectrum. The theoretically predicted

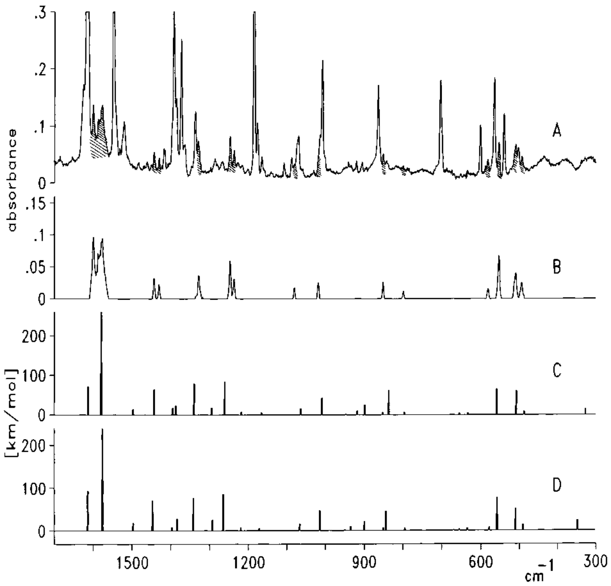


Figure 5. Comparison of the IR spectrum of the form of 6-thiopurine which does not react photochemically (shaded bands in trace A and the same bands extracted from the spectra of the compound before and after UV irradiation in trace B) with the spectra theoretically calculated for the trans (trace C) and cis (trace D) rotamers of the thiol N(9)H tautomer.

frequencies and IR intensities of the band spectra of the thiol N(9)H tautomer are listed in Tables 5 and 6 (Supporting Information) for the trans and cis rotamers, respectively. The theoretical spectra of the two rotamers are very similar to each other so that we cannot decide whether only one or both rotamers are present in the matrix. Nevertheless, the agreement between the theoretical and experimental spectra supports the assignment of the nonreactive form to the thiol N(9)H tautomer of 6-thiopurine. The theoretically predicted IR spectra of other tautomers were also compared with the experimental spectrum, but no agreement was found in any case.

An estimate of the relative population of the photochemically stable thiol N(9)H form with respect to the abundant thione N(1)H,N(7)H form was based on the experimentally measured integral intensities of the IR band due to the stretching vibrations of the imidazole NH groups in the spectra of the two species. To obtain values proportional to the concentrations, the experimental intensities were scaled by the theoretically predicted absolute intensities of the corresponding bands:

$$\frac{[\text{thiol N(9)H}]}{[\text{thione N(1)H,N(9)H}]} = \frac{I(\nu_{\text{N(9)H}_{\text{thiol N(9)H}}})\,A(\nu_{\text{N(7)H}_{\text{thione N(1)H,N(9)H}}})}{A(\nu_{\text{N(9)H}_{\text{thiol N(9)H}}})\,I(\nu_{\text{N(7)H}_{\text{thione N(1)H,N(9)H}}})}$$

where I is the integral intensity of an experimental band and A is the theoretically predicted absolute intensity.

It is difficult to estimate the difference between the theoretical and real values of $A(\nu\text{NH})$,[8] but in our estimation of the tautomeric ratio, these errors in absolute band intensities are of little significance because of their mutual cancellation.

Another measure of the relative population of both forms in the matrix is the ratio of the sum of the integrated intensities of assigned experimental bands divided by the theoretically predicted sums of absolute intensities of the respective bands:

$$\frac{[\text{thiol N(9)H}]}{[\text{thione N(1)H,N(9)H}]} = \frac{\sum I^{\text{thiol N(9)H}} \sum A^{\text{thione N(1)H,N(9)H}}}{\sum A^{\text{thiol N(9)H}} \sum I^{\text{thione N(1)H,N(9)H}}}$$

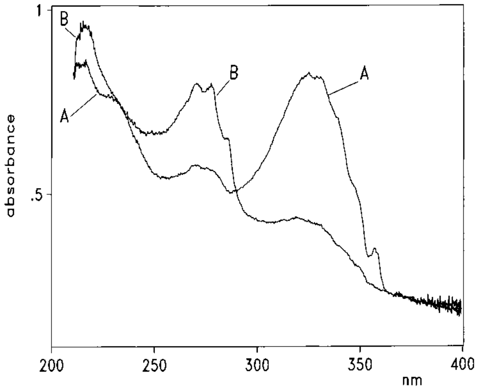


Figure 6. UV absorption spectra of 6-thiopurine isolated in an Ar matrix: (A) before UV irradiation and (B) after UV irradiation ($\lambda >$ 320 nm).

The first method results in a value of 0.23 for the thiol N(9)H/thione N(1)H,N(7)H ratio. Through the use of the second method with absolute intensities theoretically calculated for the cis or trans rotamers of the thiol N(9)H form, the value of 0.25 is again obtained. Hence, the ratio of populations of the tautomers in question may be evaluated as

$$\frac{[\text{thiol N(9)H}]}{[\text{thione N(1)H,N(9)H}]} = 0.24 \pm 0.03$$

This experimental value may be compared with the population ratio calculated from the theoretical predictions of relative internal energies of the tautomers. From the values in Table 1, the tautomeric ratio may be predicted at 360 K, that is, at the temperature of evaporation of the compound during the deposition of the matrixes. The ratio of the cis rotamer of the thiol N(9)H tautomer to the thione N(1)H,N(7)H is estimated to be equal to 0.166 whereas in the case of the trans rotamer the corresponding ratio is 0.095. Hence, the theoretically predicted cumulative thiol N(9)H/thione N(1)H,N(7)H ratio equals 0.26. The value is close to the experimental estimation.

In the above calculations the temperature of sublimation of the studied compound is applied because the ratio of tautomers in a low-temperature matrix corresponds to the frozen-gas-phase equilibrium. It is shown that the relative concentrations of tautomers observed in matrixes are virtually the same as those in the gas phase from which the matrix is formed.[21]

The electronic absorption and emission spectra of 6-thiopurine in aqueous solution and in their solid states were previously reported.[22,23] In the present work, the UV absorption spectra of matrix-isolated 6-thiopurine have been recorded (Figure 6). After irradiation of the matrix with UV light ($\lambda > 320$ nm) from a high-pressure mercury lamp, the long-wavelength part of the spectrum decreased and the short-wavelength region increased. This picture is similar to that previously observed for the thione–thiol phototautomeric reaction in matrix-isolated dithiouracil.[9p] Those observations provide the further support for the thione–thiol reaction scheme proposed for the present case.

Conclusions

6-Thiopurine in low-temperature inert gas matrixes is found to exist as a mixture of the dominating thione N(1)H,N(7)H tautomer and the minor thiol N(9)H form. The observed ratio is reproduced well by conventional ab initio calculations [electronic energies calculated at the HF/6-31G(d,p) level corrected for electron correlation at the MP4(SDQ)/6-31G(d,p) level and the zero-point energies at the HF/6-31G(d,p) level].

DFT(B3LYP)/6-31G(d,p) calculations fail to predict accurately the relative stabilities of the 6-thiopurine tautomers, and in general, this method is not recommended for the prediction of the thione−thiol tautomeric stabilities of mercapto compounds.

A photochemical reaction converting the thione N(1)H,N(7)H tautomer into the thiol N(7)H form is observed. The effect of this photoreaction is used for spectroscopic differentiation between the IR bands of the tautomeric forms existing in the matrix.

Good agreement between the experimental IR spectra of the detected tautomeric forms of 6-thiopurine and the spectra theoretically predicted at the DFT(B3LYP)/6-31G(d,p) level allows for positive identification of the considered species. The comparison between the experimental and theoretical spectra resulted with the assignment of the observed IR bands.

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Supporting Information Available: Tables of calculated [DFT(B3LYP)/6031G(d,p)] harmonic wavenumbers, absolute intensities, and assignments of modes of the bands for thiol N(9)H tautomer of 6-thiopurine (trans and cis forms corresponding to location of the SH group to N(7)H (Tables 5 and 6) (4 pages). Ordering information is given on any current masthead page.

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(20) The relative intensities are given in brackets, braces denote split bands for which cummulative intensity is given. Intensities are given in the same scale as those of the bands from the spectrum of the thione N(1)H,N(7)H form; see Table 3.

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