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Direct determination of tautomerism in purine derivatives by low-temperature NMR spectroscopy

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Abstract—An investigation of the tautomerism of the purine derivatives *N*,*N*-dimethyl-*N*′-(7(9)-*H*-purin-6-yl)-formamidine **1**, 6-chloropurine **3** and 6-methoxy purine **5** at low temperatures by NMR spectroscopy has been carried out. Knowledge of tautomeric equilibria is important for predicting N-alkylation positions, hydrogen-bonding patterns, and interactions with biological targets. In the NMR spectra of **1** and **5** at 213 K we observed two sets of signals, whereas at laboratory temperature there was only a single set of signals, reflecting the time-averaged contribution of both components. Based on characteristic values of ^{13}C and ^{15}N chemical shifts and of vicinal ^{1}H–^{13}C scalar coupling constants, the two components of **1** were determined to be the *N7*–H (71%) and *N9*–H (29%) tautomers and those of **5** as the *N7*–H (18%) and *N9*–H (82%) tautomers. The investigation of **3** revealed a substantial predominance of the *N9*–H tautomer without any separation of NMR signals at 213 K.
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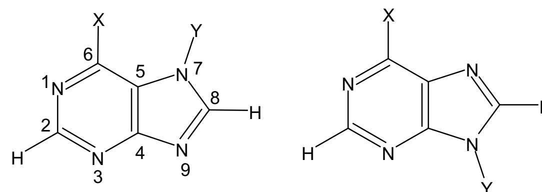
Purines play a significant role in living systems. They are not only one of the basic constituents of nucleic acids but they also interact with enzymes and other proteins as components of cofactors and signal molecules. Many commercially used antiviral and antitumor drugs are derivatives of naturally occurring purine bases (adenine and guanine). Modified purine derivatives, bearing diverse types of substituents on the carbon atoms of the purine moiety, display a broad spectrum of biological activities (antimycobacterial,[1] antibacterial,[2] antiviral,[3] cytostatic agents,[4] etc.). Biological and pharmacological effects are frequently linked to the ability of the purine to form supramolecular complexes with biological targets. Hydrogen bonds play a crucial role in such interactions. Various tautomers of purine bases usually coexist due to the presence of several nitrogen sites in the molecule. The preferred tautomers of purine derivatives are *N7*–H and *N9*–H species (Fig. 1),[5–7] probably due to their lower energy in comparison to *N1*–H and *N3*–H forms.[8] Relative populations of individual tautomers are generally influenced by the substitution (which modifies the electronic distribution within the molecule), environment (solvent), and temperature. The ability to form H-bonds as well as the regioselectivity of alkylation or glycosidation reactions, used in the synthesis of potential pharmaceuticals, are influenced by the tautomeric equilibria and by the electronic distribution around the purine skeleton.



1a: X = N=CHN(CH_3)$_2$, Y = H
2a: X = N=CHN(CH_3)$_2$, Y = CH_2CN
3a: X = Cl, Y = H
4a: X = Cl, Y = CH_2Ph
5a: X = OCH_3, Y = H
6a: X = OCH_3, Y = CH_2Ph

1b: X = N=CHN(CH_3)$_2$, Y = H
2b: X = N=CHN(CH_3)$_2$, Y = CH_2CN
3b: X = Cl, Y = H
4b: X = Cl, Y = CH_2Ph
5b: X = OCH_3, Y = H
6b: X = OCH_3, Y = CH_2Ph

Figure 1.

Keywords: Purine; NMR; Tautomerism.

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Tautomerism of several purine derivatives has been investigated by various methods—quantum chemical calculations,[9–11] IR matrix isolation/ab initio approach[12,13] and UV spectroscopy.[14] ^{13}C NMR chemical shifts were utilized to specify the protonation sites of purine ions,[15] in order to study differences between the ^{13}C chemical shifts of purine and *N*-alkylated purine derivatives,[16] and to quantitatively determine the tautomeric populations.[5] The pH dependence of ^{15}N NMR chemical shifts in several purine derivatives[6] and the tautomeric equilibria of adenine derivatives have been investigated spectroscopically.[8] In addition to NMR chemical shift analysis, purine derivatives and their tautomeric equilibria were also studied by the measurements of ^3J (^{13}C,^{1}H) coupling constants.[17,18] Low-temperature NMR represents an important tool for examining tautomeric equilibria. Fast chemical exchange among the individual components usually occurs at laboratory temperatures. The resulting NMR spectra correspond to a time-averaged contribution, which reflects Boltzmann populations of the individual tautomers. Decreasing the temperature slows down the chemical exchange process. At low temperatures, separated signals of individual tautomers could be detected. Many attempts have been made to determine the tautomeric equilibria of purine derivatives in various media. To the best of our knowledge, separation of the NMR signals of individual tautomers of purine derivatives at low temperatures, as described in this communication, has not been reported previously.

Determination of characteristic values of ^{15}N NMR chemical shifts[19] and vicinal $^3J_{H8,C4}$ and $^3J_{H8,C5}$ coupling constants[18] is an effective way to localize the position of H-atoms on the purine skeleton and to quantify the tautomeric populations in the presence of chemical exchange. Three different examples of low-temperature NMR investigations, including measurements of ^{13}C and ^{15}N NMR chemical shifts and vicinal $^3J_{H8,C4}$ and $^3J_{H8,C5}$ coupling constants, for 6-substituted purines (Fig. 1) N^6-(*N*,*N*-dimethylaminomethylidene)adenine **1**, 6-chloropurine **3**, and 6-methoxypurine **5** are discussed. These derivatives differ in the tautomeric populations and in the temperature dependence of their ^{1}H NMR spectra.

In the ^{1}H NMR spectrum of **1**—measured in DMF-d_7 at 303 K only one set of signals was detected. The signal of proton H2 was assigned by detecting the three-bond interaction with C4 and C6 (GHMBC,[20] optimized for J_{CH} = 7.5 Hz), whereas the signal of H8 was coupled by three-bond scalar interaction with C4 and C5. At lower temperatures, significant broadening of the proton signals (see Fig. 2) and a change in the H-8 chemical shift were detected. At 213 K we observed two different sets of sharp signals in the NMR spectrum with the relative intensities 10 and 4, which correspond to the ratio of major and minor components 71:29. For comparison purposes we also determined the ratio of the fast exchanging components at higher temperatures using the approach[17] based on the analysis of the $^3J_{H,C}$ values. Broadening of the signals at laboratory temperature prevented the measurement of multiple bond scalar coupling constants. Therefore, we selected a temperature of 333 K for this analysis. From the Boltzmann distribution of the tautomers determined by integrating the low-temperature ^{1}H NMR spectra, we calculated the energy difference and subsequently their populations at 333 K as 64% of the major and 36% of the minor component, respectively. The calculated percentage of *N7*–H tautomer is 60% by applying couplings for **2a** and **2b** and 56% using the values for **1a** and **1b** (neglecting the temperature shift changes, see Table 2). Here, the determined population of major tautomer (*N7*–H vide infra) is slightly higher than the population recently determined in a CD_3OD solution of the same compound based on the vicinal $^3J_{H,C}$ coupling constant analysis (~60% at 273 K).[18] As demonstrated for compound **1**, the reliability of the approaches for determining the tautomeric ratios using averaged chemical shifts and coupling constants is approximately ±10%. Based on the differences in chemical shifts of the two H8 protons observed at 213 K and considering that each proton shows two-bond correlations to two nitrogen atoms, one of which has a chemical shift typical for a pyrrole and the other for a pyridine-type nitrogen,[19] we deduced that the two sets of signals correspond to *N7*–H and *N9*–H tautomers. In order to determine the structures of the individual tautomers unambiguously, the characteristic values of ^{13}C and ^{15}N chemical shifts and ^{1}H–^{13}C coupling constants were measured and compared with the data of compounds alkylated at N7 or N9. The NMR chemical shifts of both tautomers **1a** and **1b** and their *N*-alkylated analogues **2a** and **2b** are listed in Table 1. Remarkable agreement between the experimental data of **1a** and **2a** with respect to **1b** and **2b** was obtained. For example, the N3 atom has a higher chemical shift in **1a** (255.4 ppm) and **2a** (256.4 ppm) than in **1b** (239.0 ppm) and **2b** (234.3 ppm). This change of N3 chemical shift is the typical marker of the pair of N7/N9 regioisomers.[21,22] The values obtained clearly indicate that the major tautomer of **1** detected at low temperature is the tautomer *N7*–H and the minor one is *N9*–H. An inspection of the vicinal $^3J_{H8,C4}$ and $^3J_{H8,C5}$ coupling constants (values were determined from the anti-phase doublets[23] of the corresponding cross-peaks in GSQMBC[24,25] spectra) also supports this conclusion. The value of the $^3J_{H8,C4}$ coupling constant is significantly higher than the $^3J_{H8,C5}$ coupling constant for *N7*-alkylated derivatives, whereas for *N9*-alkylated derivatives the opposite is true.[18] The tautomeric ratios obtained by various approaches are summarized in Table 2. The preference for compound **1** to exist as the *N7*–H tautomer could be explained by a stabilizing interaction between the lone pair of electrons on the nitrogen C6–N=C and the hydrogen atom on N7. In order to clarify this assumption, theoretical calculations are currently in progress.

Two sets of signals were also observed in the low temperature NMR spectrum of **5**. The values of the $^3J_{H8,C4}$ coupling constants of the major **5b** and minor **5a** tautomers are 5.9 and 12.7 Hz, respectively. To compare, the values for *N*-alkylated analogues are 5.0 Hz **6b** and 13.0 Hz **6a**. The clear analogy between **5a**–**6a** and **5b**–**6b** is also obvious by inspection of the values of

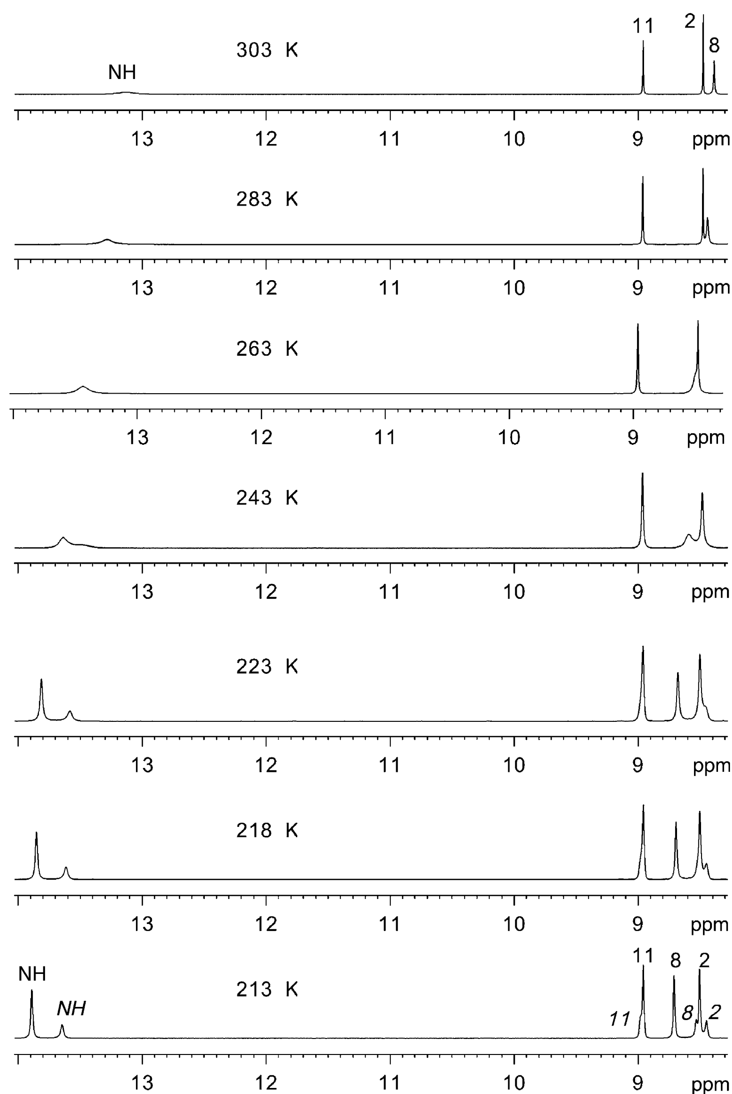

Figure 2. Temperature dependence of the ^{1}H NMR spectra of compound **1** in DMF-d_7.

Table 1. ^{13}C and ^{15}N NMR chemical shifts[a] (δ in ppm) and $^{3}J_{HC}$ coupling constants (Hz) of purine derivatives **1**–**6** in DMF-d_7 and DMSO-d_6 (10–50 mg /550 μL) at various temperatures

	C2	C4	C5	C6	C8	N1	N3	N7	N9	$^{3}J_{H8,C4}$	$^{3}J_{H8,C5}$	T (K)/solvent
1[b]	152.56	158.30	121.56	157.40	143.06	—[c]	—[c]	—[c]	—[c]	9.2	7.0	333/DMF-d_7
1a	152.53	161.48	118.11	155.32	145.26	246.4	255.4	149.2	247.0	12.5	4.9	213/DMF-d_7
1b	152.65	153.35	125.86	159.70	141.89	249.5	239.0	245.2	157.4	6.3	11.0	213/DMF-d_7
2a[d]	152.96	160.80	116.45	155.03	146.57	248.3	256.4	141.1	248.7	12.5	4.0	[e]/DMSO-d_6
2b[d]	152.57	151.25	125.00	159.50	142.11	252.2	234.3	246.6	146.2	4.5	11.5	[e]/DMSO-d_6
3	151.34	154.00	129.13	147.64	146.04	273.8	256.3	227.6	175.4	7.3	10.7	303/DMSO-d_6
3	152.25	153.67	131.19	149.31	146.87	274.1	254.7	242.7	161.9	6.7	11.4	[f]/DMF-d_7
4a	151.70	161.61	121.93	142.12	151.24	277.2	272.2	157.0	248.2	13.2	4.2	303/DMSO-d_6
4b	151.57	151.70	130.71	149.04	147.31	274.8	251.9	242.5	167.8	5.2	12.0	303/DMSO-d_6
5a	151.86	162.05	112.30	157.13	145.87	239.7	258.8	148.5	247.3	12.7	5.7	213/DMF-d_7
5b	152.23	153.56	120.91	160.75	142.89	239.2	243.3	241.0	159.7	5.9	11.4	213/DMF-d_7
6a	152.22	162.75	113.39	157.79	147.67	240.6	261.1	157.3	248.9	13.0	4.1	303/DMF-d_7
6b	152.43	153.13	121.80	161.49	144.29	239.9	240.5	241.5	165.1	5.0	11.7	303/DMF-d_7

NMR spectra were recorded at frequencies of 500.13 MHz (^{1}H), 125.77 MHz (^{13}C), and 50.68 MHz (^{15}N).

[a] N-15 chemical shifts measured indirectly, referenced to 1 M urea in DMSO-d_6 (77.0) and liquid CH_3NO_2 (381.7),[19] and reported relative to liquid NH_3.

[b] Chemical shifts from HMBC spectrum.

[c] Not obtained.

[d] Ref. 17.

[e] C-13 at 293 K, N-15 at 303 K.

[f] C-13 at 213 K, N-15 at 243 K.

Table 2. Populations of *N7*–H tautomers for compounds **1**, **3**, and **5** determined by various approaches

	A (%)	B (%)	C (%)	D (%)	E (%)	F (%)	G (%)	H (%)	I (%)	*T* (K)/solvent
1	71	—	—	—	—	—	—	—	—	213/DMF-d_7
1	—	64	55	57	59	60	61	55	—	333/DMF-d_7
3	—	—	—	—	22	21	21	8	16	303/DMSO-d_6
5	18	—	—	—	—	—	—	—	—	213/DMF-d_7
5	—	26	—	—	—	—	—	—	—	303/DMF-d_7

A: Experimental (integration of peaks in low-temperature ^{1}H NMR spectra). B: A recalculated for different temperature (Boltzmann distribution). C: Calculated from $^3J_{H8,C4}$ (using values of the individual tautomers —temperature dependence of the chemical shifts neglected). D: Calculated from $^3J_{H8,C5}$ (tautomers); E: Calculated from $^3J_{H8,C4}$ (using values of *N*-alkyl derivatives). F: Calculated from $^3J_{H8,C5}$ (*N*-alkyl derivatives). G: Calculated from ^{13}C NMR chemical shifts of C4 atoms. H: Calculated from ^{13}C NMR chemical shifts of C5 atoms. I: Calculated from ^{15}N NMR chemical shifts (*N*-benzyl derivatives).

the $^3J_{H8,C5}$ coupling constants and the ^{13}C and ^{15}N NMR chemical shifts (Table 1). At 213 K 82% of *N9*–H tautomer **5b** and 18% of the *N7*–H tautomer **5a** were determined by integration of the corresponding signals in the ^{1}H NMR spectrum. When recalculated for laboratory temperature (26% of *N7*–H), the ratio is in very good agreement with the results of a ^{13}C chemical shift study[5] where 32% of *N7*–H tautomer **5a** was determined.

In contrast to the previous studies, no significant changes in ^{1}H NMR patterns were observed on cooling the sample of 6-chloropurine **3**. One set of signals was detected both at laboratory temperature and at 213 K. From a qualitative point of view, the values of the $^3J_{H8,C4}$ and $^3J_{H8,C5}$ coupling constants for **3** are in a good agreement with the typical values for the *N9*-isomer. Therefore, *N9*–H is assumed to be the dominant tautomeric form under these conditions. Comparison of the ^{13}C and ^{15}N chemical shifts with the values measured for *N7*- and *N9*-alkylated derivatives confirmed this assumption (see Table 1). However, careful calculations using the values of the $^3J_{CH}$ coupling constants[17] indicated that the population of the *N7*–H tautomer (minor) is roughly 21% at 303 K (Table 2). The detected relative decrease in the interconversion barrier for **3** (compared to **1** and **5**) is currently under investigation.

The results discussed for three selected 6-substituted purines represent three different cases. In the first case **1**, two sets of signals were detected at low temperature. Based on the characteristic values of ^{13}C and ^{15}N chemical shifts, the $^3J_{H8,C4}$ and $^3J_{H8,C5}$ analysis, and on their comparison with the data for *N*-alkylated analogues, the signals were assigned unambiguously to the *N7*–H (major) and *N9*–H (minor) tautomeric forms. Similarly, two sets of signals were observed for **5** at low temperature, but in contrast to **1**, the analysis indicated that *N9*–H was the prevailing tautomer. In situations where only one set of signals was detected at low temperature, as in the case **3**, the characteristic values of the chemical shifts and coupling constants can be used to estimate the tautomer populations. However, this can be done only qualitatively using the values of vicinal *J*-coupling constants. If, in addition, chemical shifts and coupling constants for *N7*- and *N9*-alkylated analogues are known, the data can be evaluated quantitatively.[5,17]

The influence of various substituents on the tautomeric equilibrium is currently studied by a combination of experimental (NMR and X-ray diffraction) and theoretical methods. The scope and limitations of such a study for determining the tautomeric, protonation, and metal complexation processes in a number of purine derivatives are investigated.

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