Research Article

Magnetic Resonance in Chemistry

Received: 7 January 2008 Revised: 19 February 2008 Accepted: 19 February 2008 Published online in Wiley Interscience: 28 March 2008

(www.interscience.com) DOI 10.1002/mrc.2226

NMR study of 5-substituted pyrazolo[3,4-*c*]pyridine derivatives

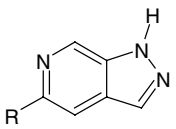
Orestis Tsikouris,[a,b] Tomáš Bartl,[b] Jaromír Toušek,[b] Nikolaos Lougiakis,[a] Tony Tite,[a] Panagiotis Marakos,[a] Nicole Pouli,[a] Emmanuel Mikros[a] and Radek Marek[b]*

Substituted pyrazolopyridines are potent inhibitors of phosphodiesterases and cyclin-dependent kinases. In this study, NMR was used to investigate the potential N1-H and N2-H tautomerism of 5-substituted pyrazolo[3,4-*c*]pyridine derivatives. Six compounds were fully characterized by using ^{1}H, ^{13}C, and ^{15}N chemical shifts and indirect $^{1}H–^{13}C$ and $^{1}H–^{15}N$ coupling constants. The ^{1}H NMR spectra were measured over a broad range of temperatures. All of the compounds were shown to exist predominantly in the N1-H tautomeric form. Complementary quantum-chemical calculations of the chemical shieldings and indirect spin-spin couplings support the structural conclusions drawn.

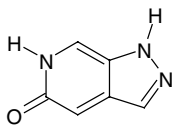
Keywords: NMR; ^{1}H; ^{13}C; ^{15}N; tautomerism; spin-spin coupling constant; purine; pyrazolo[3,4-*c*]pyridine; quantum-chemical calculation

Introduction

Substituted purines and purine analogs make up an important group of heterocyclic compounds. The tautomerism of isolated nucleic acid bases and several purine derivatives have been investigated extensively.[1] Tautomeric equilibria should be considered in any study of their binding modes with biological targets. A number of variously substituted pyrazolopyridines[2,3] have been shown to be potent inhibitors of phosphodiesterases,[4] matrix metalloproteinases,[5] glycogen synthase kinase-3,[6] and cyclin-dependent kinases.[7] Continuing our systematic study of pyrazolopyridines,[8] we describe here the NMR investigation of a number of 5-substituted pyrazolo[3,4-*c*]pyridine derivatives (compounds **1** – **6**).

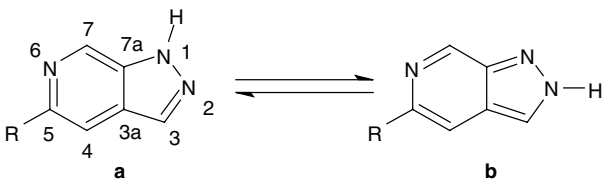


1: R = Cl
2: R = OCH_3
3: R = H
4: R = NH_2
5: R = $NHC(O)CH_3$



6

Generally, compounds **1**–**5** could exist in an N1-H or N2-H tautomeric form (Scheme 1). The relative populations of the individual tautomers are influenced by the temperature, the solvent, and the substitution pattern[9] – which modify the electron distribution within the molecule. We have thus prepared several compounds that bear at position 5, substituents with differing electronic and steric properties. The phenomenon of tautomerism was investigated systematically by using NMR spectroscopy, including low-temperature measurements.



a **b**

Scheme 1. N1-H and N2-H tautomeric forms in compounds **1**–**5**.

Experimental

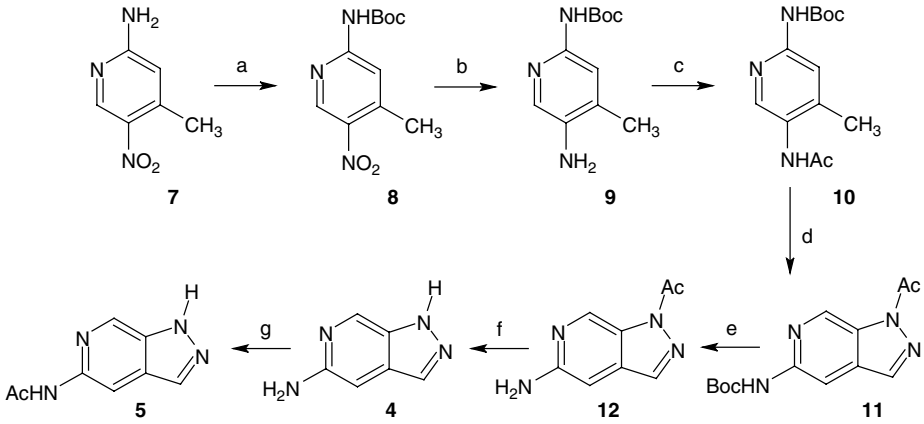
NMR spectroscopy

NMR spectra were recorded using a Bruker Avance DRX 500 spectrometer operating at frequencies of 500.13 MHz (^{1}H), and 125.77 MHz (^{13}C), a Bruker Avance 400 spectrometer operating at 400.13 MHz (^{1}H) and 100.61 MHz (^{13}C), and a Bruker Avance 300 spectrometer operating at frequencies of 300.13 MHz (^{1}H), 75.48 MHz (^{13}C), and 30.41 MHz (^{15}N). The NMR samples were prepared by dissolving approximately 10 mg of the 5-substituted pyrazolo[3,4-*c*]pyridine in 0.55 ml of methanol-d_4 or 0.50 ml of dimethyl sulfoxide (DMSO-d_6). The NMR spectra were measured at the various temperatures specified in the text and in the tables. The

* *Correspondence to: Radek Marek, National Center for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5/A4, CZ - 625 00 Brno, Czech Republic. E-mail: rmarek@chemi.muni.cz*

a *Department of Pharmacy, Division of Pharmaceutical Chemistry, University of Athens, Panepistimiopolis Zografou, Athens GR-15771, Greece*

b *National Center for Biomolecular Research, Masaryk University, Kamenice 5/A4, Brno CZ-62500, Czech Republic*



Scheme 2. (a) 1) NaH (60% in hexane), dry THF, rt, 1h, 2) Boc_2O, dry THF, rt, 2h; (b) Pd/C 10%, EtOH, 50 psi, 5h; (c) Ac_2O, CH_2Cl_2, rt, 12h; (d) AcOK, Ac_2O, isoamyl nitrite, C_6H_6, 90 °C, 14h; (e) CF_3CO_2H, CH_2Cl_2, rt, 16h; (f) NH_3 in MeOH, rt, 2h; (g) Ac_2O, CH_2Cl_2, rt, 12h.

Table 1. ^{1}H- and ^{15}N-NMR chemical shifts (δ in ppm) for compounds **1**–**6** in methanol-d_4 at 300 K

Compound	H-3	H-4	H-5	H-7	N-1	N-2	N-6
1	8.15	7.82	–	8.80	181.3	324.3	294.7
2[b]	8.04	7.08	–	8.62	177.4	320.3	271.3
3	8.20[a]	7.79	8.20[a]	8.99	181.2	318.6	294.0
4	7.88	6.85	–	8.51	176.8	317.7	268.4
5[c]	8.11	8.36	–	8.76	179.2	318.7	286.6
6	8.35	7.47	–	8.93	187.6	343.3	179.0

[a] Signal overlap.
[b] ^{1}H NMR: $\delta_{CH3} = 3.94$ ppm.
[c] ^{1}H NMR: $\delta_{CH3} = 2.37$ ppm; ^{15}N NMR, $\delta_{NHCO} = 140.2$ ppm.

^{1}H and ^{13}C NMR chemical shifts (δ in ppm) were referenced to the signal of methanol [$\delta = 3.31$ ppm for residual CHD_2OD (^{1}H); $\delta = 49.20$ ppm for CD_3OD (^{13}C)] or DMSO [$\delta = 2.49$ ppm for residual DMSO-d_5 (^{1}H); $\delta = 39.70$ ppm for DMSO-d_6 (^{13}C)]. The ^{15}N NMR chemical shifts were referenced to 1 M urea in DMSO-d_6 ($\delta = 77.0$ ppm)[10] and are reported relative to liquid ammonia.[11,12] No susceptibility correction was applied. The temperature was calibrated using the methanol sample.

A 5-mm QNP [^{13}C/^{19}F/^{31}P{^{1}H}] or a 5-mm multinuclear inverse BBI [^{1}H{BB}] probe with a self-shielded z-gradient coil was used to measure the ^{1}H, ^{13}C, and heteronuclear shift correlation spectra. ^{1}H NMR spectra: pulse 90°, relaxation delay 10 s, number of scans 4–16, resolution <0.005 ppm per point. ^{13}C NMR spectra: pulse 45°, relaxation 3 s, number of scans 512–8192, resolution <0.01 ppm per point. The ^{1}H–^{13}C GSQMBC[13] NMR experiment was adjusted for a long-range coupling of 7.5 Hz. ^{1}H–^{15}N GSQMBC and ^{1}H–^{15}N gs-HMBC[14,15] NMR experiments were adjusted for couplings ranging from 9 to 11 Hz (corresponding to 55.6–45.5 ms). Computer processing was performed with Bruker TopSpin software. Indirect spin–spin coupling constants (reported in hertz) are determined with an accuracy of ±0.5 Hz for $^1J_{H-C}$ and ±0.3 Hz for $^nJ_{H-C}$ and $^nJ_{H-N}$.

Quantum-chemical calculations

Calculations were performed at the DFT level, using the B3LYP functional as implemented in the Gaussian 03 program package.[16] Basis set 6-31G* was used to optimize the geometry and basis set 6-311G** to calculate the chemical shieldings [gauge-including atomic orbital (GIAO) approach][17] and indirect spin–spin coupling constants. Methods were selected based on our previous results.[18] The polarizable continuum model (PCM)[19] was employed to simulate solvent effects for calculating the NMR parameters. The ^{13}C and ^{15}N NMR chemical shifts were calculated by subtracting the shieldings of the individual atoms from the shielding of the standard [TMS for ^{13}C (186.40 ppm),[20] NH_3 for ^{15}N (245.07 ppm)[21]]. The ^{1}H–^{15}N coupling constants were recalculated from the ^{1}H–^{14}N couplings using a factor of −1.4027 ($\gamma_{N-15}/\gamma_{N-14}$). The optimized geometries of individual molecules can be obtained upon request from the author (JT).

Preparation of compounds

All starting chemicals were purchased from Aldrich Chemical Co. Melting points were determined on a Büchi apparatus and are uncorrected. Flash chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). Analytical thin layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck F-254 silica gel plates. Elemental analyses were performed on a Perkin-Elmer PE 240C Elemental Analyzer (Norwalk, CT, USA) and were within ±0.4% of the theoretical values.

Compounds **1, 2**, and **3** were prepared according to procedures in the literature.[22,23] The synthesis of **4** and **5** is depicted in Scheme 2: The compound **7** was first converted to the acetamide **10** by treatment with Boc anhydride, catalytic reduction, and acetylation.[24]

tert-Butyl-*N*-(1-acetylpyrazolo[3,4-*c*]pyridin-5-yl) carbamate (11)

Potassium acetate (425 mg, 4.33 mmol) and acetic anhydride (1.25 ml, 13.22 mmol) were added to a solution of **10** (850 mg, 3.21 mmol)[24] in dry benzene (20 ml) under argon. The reaction mixture was heated to boiling, isoamyl nitrite (1 ml, 7.44 mmol) was added, and the resulting mixture was refluxed at 80 °C for 14 h. The insoluble material was then filtered off, the solvent vacuum-evaporated, and the residue purified by column chromatography (silica gel), using a mixture of cyclohexane : ethyl acetate (8 : 2, v/v) as eluent, to give **11** (770 mg, 87% yield), mp 188 °C (EtOAc). ^{1}H-NMR (400 MHz, $CDCl_3$) chemical shifts in ppm: 1.62

Table 2. ^{13}C-NMR chemical shifts (δ in ppm) and $^{1}J_{H-C}$ coupling constants (Hz) for compounds **1**–**6** in methanol-d_4 at 300 K

Compound	C-3 ($^{1}J_{H3-C3}$)	C-3a	C-4 ($^{1}J_{H4-C4}$)	C-5	C-7 ($^{1}J_{H7-C7}$)	C-7a
1	134.39 (195.3)	131.42	115.17 (172.7)	141.30	135.34 (187.8)	137.86
2[b]	133.97 (192.2)	132.62	96.51 (166.9)	160.15	132.52 (184.5)	135.98
3	134.85 (192.2)	128.51	116.59 (167.0)	138.82	135.77 (184.5)	138.47
4	132.60[a] (191.2)	132.23	96.56 (164.1)	153.78	132.40[a] (182.2)	134.76
5[c]	134.82 (192.2)	130.40	105.27 (171.8)	144.54[a]	133.99 (185.2)	136.70
6	134.65 (197.4)	136.75	101.17 (173.0)	154.17	126.53 (192.7)	133.53

[a] Value obtained from the 2D ^{1}H–^{13}C chemical shift correlation spectrum.
[b] $\delta_{CH3} = 55.48$ ppm.
[c] $\delta_{CH3} = 23.96$ ppm, $\delta_{NHCO} = 171.86$ ppm.

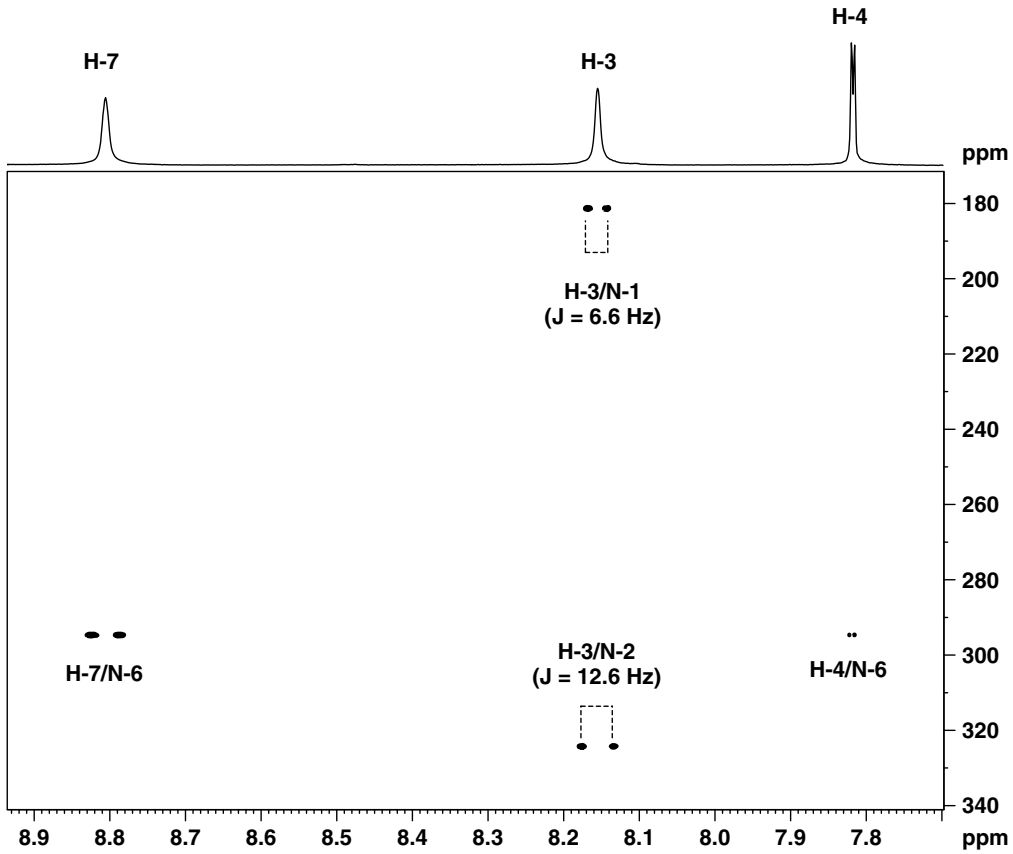


Figure 1. ^{1}H–^{15}N GSQMBC spectrum of **1** in methanol-d_4 at 300 K.

(s, 9H, $(CH_3)_3$), 2.78 (s, 3H, $COCH_3$), 8.11 (s, 1H, H-3), 8.36 (s, 1H, H-4), 9.51 (s, 1H, H-7), 9.78 (brs, 1H, D_2O exchang., NH). ^{13}C-NMR (50 MHz, $CDCl_3$) chemical shifts in ppm: 22.61(CH_3CO), 28.49 [$(CH_3)_3CO$], 81.35 [$(CH_3)_3CO$], 101.98 (C-4), 132.35 (C-3a), 133.94 (C-7a), 136.28 (C-7), 139.16 (C-3), 147.75 (C-5), 153.06 (OCONH), 170.29 ($COCH_3$). Partial elemental analysis calculated for $C_{13}H_{16}N_4O_3$: C, 56.51; H, 5.84; N, 20.28. Found: C, 56.39; H, 6.05; N, 20.16.

1-Acetylpyrazolo[3,4-*c*]pyridin-5-yl-amine (12)

Trifluoracetic acid (3.5 ml, 47.12 mmol) was added to a solution of **11** (470 mg, 1.7 mmol) in dry dichloromethane (20 ml) at 0 °C, and the resulting solution was stirred at laboratory temperature for 16 h. The pH was then adjusted to 8 with a saturated solution of $NaHCO_3$, the organic solvent was vacuum-evaporated, and the residue was extracted with ethyl acetate. The organic extracts were dehydrated with Na_2SO_4 and vacuum concentrated to dryness, and the residue was purified by column chromatography (silica gel), using a mixture of cyclohexane : ethyl acetate (6 : 4, v/v) as eluent, to give **12** (242 mg, 80% yield), mp 193–194 °C (EtOAc). ^{1}H-NMR (400 MHz, DMSO-d_6) chemical shifts in ppm: 2.64 (s, 3H, $COCH_3$), 6.00 (brs, 2H, D_2O exchang., NH_2), 6.73 (s, 1H, H-4), 8.31 (s, 1H, H-3), 8.99 (s, 1H, H-7). ^{13}C-NMR (50 MHz, DMSO-d_6) chemical shifts in ppm: 22.14 (CH_3), 95.04 (C-4), 128.79 (C-7a), 134.42 (C-3a), 135.03 (C-7), 139.32 (C-3), 156.45 (C-5), 169.51 ($COCH_3$). Partial elemental analysis calculated for $C_8H_8N_4O$: C, 54.54; H, 4.58; N, 31.80. Found: C, 54.78; H, 4.66; N, 31.59.

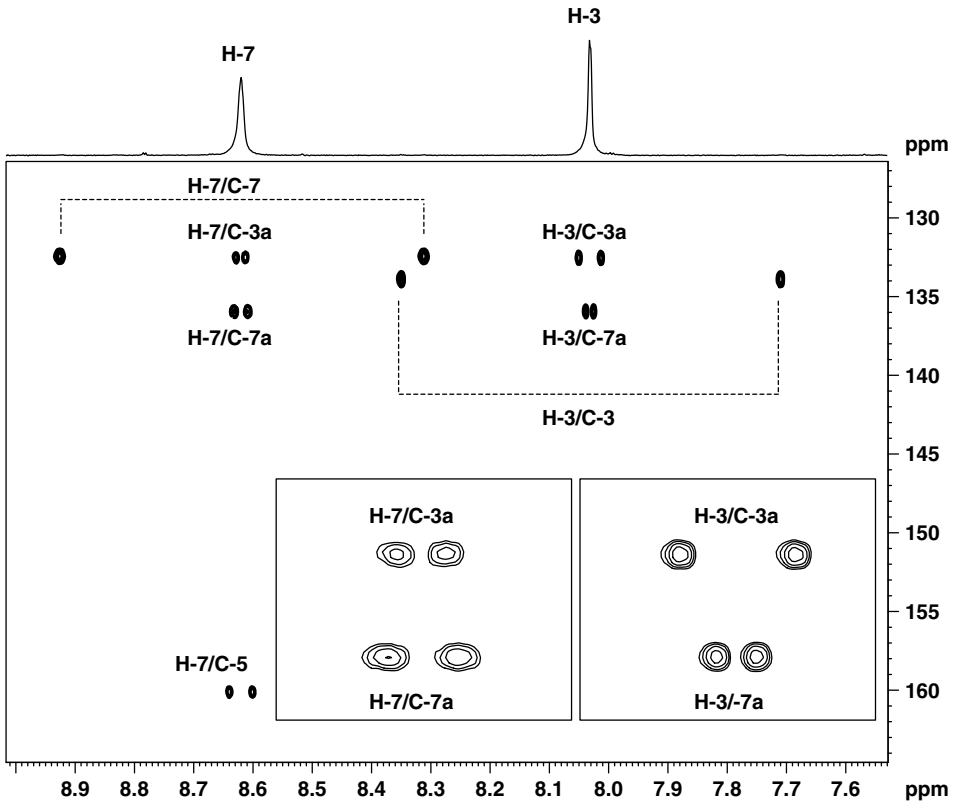


Figure 2. Portion of $^{1}H-^{13}C$ GSQMBC spectrum of **2** in methanol-d_4 at 300 K with additional details of correlation signals H3–C3a, H3–C7a and H7–C3a, H7–C7a.

Table 3. Selected $^{n}J_{H-C}$ coupling constants (Hz) for compounds **1**–**6** in methanol-d_4 at 300 K

Compound	$^{2}J_{H3-C3a}$	$^{3}J_{H3-C7a}$	$^{2}J_{H4-C3a}$	$^{3}J_{H4-C7a}$	$^{3}J_{H7-C3a}$	$^{2}J_{H7-C7a}$
1	11.4	4.0	2.0	5.9	4.2	7.1
2	11.4	3.4	2.0	6.0	4.8	6.6
3	12.4	5.4	4.6	6.5	4.1	8.5
4	11.3	3.8	3.0	6.1	4.3	6.4
5	11.2	4.3	– [a]	5.7	4.1	7.4
6	11.8	3.8	– [a]	7.0	4.8	2.4

[a] Not determined.

Table 4. Selected $^{n}J_{H-N}$ coupling constants (Hz) for compounds **1**–**6**, **13**, and **14** in methanol-d_4 at 300 K

Compound	$^{2}J_{H3-N2}$	$^{3}J_{H3-N1}$	$^{3}J_{H4-N6}$	$^{2}J_{H7-N6}$
1	12.6	6.6	2.0	10.4
2	12.4	6.5	3.1	9.8
3	12.7	7.3	– [a]	9.8
4	12.5	7.2	2.5	10.4
5	11.9	8.2	– [a]	10.5
6	12.5	7.0	3.0	2.7
13[c]	14.8	8.0	– [b]	–
14[c]	4.4	2.3	– [b]	–

[a] Not determined.
[b] Not published.
[c] Data from Ref. [8].

1*H*-Pyrazolo[3,4-*c*]pyridine (3)

The preparation of this compound has been reported previously.[22] Selected NMR data are listed below. ^{1}H-NMR (500 MHz, methanol-d_4) chemical shifts in ppm at 304 K: 7.80 (d, 1H, H-4), 8.20 (d, 1H, H-5), 8.21 (s, 1H, H-3), 8.99 (s, 1H, H-7); at 215 K: 7.89 (d, 1H, H-4), 8.23 (d, 1H, H-5), 8.29 (s, 1H, H-3), 9.04 (s, 1H, H-7); at 171 K: 7.93 (d, 1H, H-4), 8.25 (d, 1H, H-5), 8.34 (s, 1H, H-3), 9.08 (s, 1H, H-7). ^{13}C-NMR (125 MHz, methanol-d_4) chemical shifts in ppm at 171 K: 117.10 (C-4), 128.41 (C-7a), 134.84 (C-3), 135.77 (C-7), 138.06 (C-3a), 138.53 (C-5).

Pyrazolo[3,4-*c*]pyridin-5-yl-amine (4)

Compound **12** (120 mg, 0.68 mmol) was added to a saturated solution of ammonia in methanol (15 ml), and the resulting mixture was stirred at room temperature for 2 h. The solvent was removed in vacuo, and the residue was purified by column chromatography (silica gel), using a mixture of cyclohexane : ethyl acetate (2 : 8, v/v) as eluent, to give pure **4** (80 mg, 88% yield), mp 236–237 °C (dec) (EtOAc). ^{1}H-NMR (400 MHz, DMSO-d_6) chemical shifts in ppm: 5.34 (brs, 2H, D_2O exchange, NH_2), 6.62 (s, 1H, H-4), 7.84 (s, 1H, H-3), 8.50 (s, 1H, H-7), 12.98 (brs, 1H, D_2O exchange, NH). ^{13}C-NMR (50 MHz, DMSO-d_6) chemical shifts in ppm: 92.63 (C-4), 130.05 (C-3a), 131.15 (C-3), 131.96 (C-7), 132.66 (C-7a), 152.99 (C-5). ^{1}H-NMR (500 MHz, methanol-d_4) chemical shifts in ppm at 171 K: 6.90 (brs, 1H, H-4), 8.02 (brs, 1H, H-3), 8.56 (brs, 1H, H-7). Partial elemental analysis calculated for $C_6H_6N_4$: C, 53.72; H, 4.51; N, 41.77. Found: C, 53.52; H, 4.37; N, 41.63.

N-(Pyrazolo[3,4-*c*]pyridin-5-yl)acetamide (5)

Acetic anhydride (0.04 ml, 0.37 mmol) was added to a solution of **4** (50 mg, 0.37 mmol) in dry dichloromethane (5 ml), and the resulting solution was stirred at laboratory temperature for 12 h. The solvent was vacuum-evaporated, and the residue was purified by column chromatography (silica gel), using a mixture of cyclohexane : ethyl acetate (1 : 1, v/v) as eluent, to give **5** (40 mg, 60% yield). mp >250 °C (dec) (EtOAc). ^{1}H-NMR (400 MHz, DMSO-d_6) chemical shifts in ppm: 2.09 (s, 3H, $COCH_3$), 8.16 (s, 1H, H-3), 8.38 (s, 1H, H-4), 8.79 (s, 1H, H-7), 10.38 (brs, 1H, D_2O exchange, NHCO), 13.46 (brs, 1H, D_2O exchange, NH). ^{13}C-NMR (50 MHz, DMSO-d_6) chemical shifts in ppm: 23.82 (CH_3), 101.95 (C-4), 128.39 (C-3a), 132.53 (C-7), 133.18 (C-3), 134.60 (C-7a), 143.65 (C-5), 168.58 ($COCH_3$). ^{1}H-NMR (500 MHz, methanol-d_4) chemical shifts in ppm at 304 K: 2.19 (s, 3H, CH_3), 8.11 (s, 1H, H-3), 8.36 (s, 1H, H-4), 8.76 (s, 1H, H-5); at 204 K: 2.20 (s, 3H, CH_3), 8.19 (s, 1H, H-3), 8.50 (s, 1H, H-4), 8.81 (s, 1H, H-5); at 171 K: 2.21 (s, 3H, CH_3), 8.22 (s, 1H, H-3), 8.55 (s, 1H, H-4), 8.84 (s, 1H, H-5). ^{13}C-NMR (125 MHz, methanol-d_4) chemical shifts at 171 K: 23.87 (CH_3), 104.61 (C-4), 130.40 (C-7a), 134.17 (C-7), 134.67 (C-3), 136.24 (C-3a), 144.84 (C-5), 172.13 ($COCH_3$). Partial elemental analysis calculated for $C_8H_8N_4O$: C, 54.54; H, 4.58; N, 31.80. Found: C, 54.61; H, 4.49; N, 31.95.

1,6-Dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-5-one (6)

Sodium iodide (200 mg, 1.335 mmol) and trimethylsilylchloride (500 µl, 3.93 mmol) were added to a solution of **2** (70 mg, 0.469 mmol) in dry acetonitrile (20 ml) under an atmosphere of argon, and the resulting solution was refluxed for 17 h. The solvent was then evaporated to dryness, and the residue was purified by column chromatography (silica gel), using a mixture of dichloromethane : methanol (100 : 3, v/v) as eluent, to give compound **6** (35 mg, 55% yield). mp >250 °C (dec) (EtOH). ^{1}H-NMR (400 MHz, DMSO-d_6) chemical shifts in ppm: 7.28 (1H, s, H-4), 8.30 (1H, s, H-3), 8.96 (1H, s, H-7). ^{13}C-NMR (50 MHz, DMSO-d_6) chemical shifts in ppm: 99.00 (C-4), 127.58 (C-7), 132.41 (C-7a), 133.34 (C-3), 134.06 (C-3a), 153.59 (C-5). ^{1}H-NMR (500 MHz, methanol-d_4) chemical shifts in ppm at 303 K: 7.47 (d, 1H, H-4), 8.34 (d, 1H, H-3), 8.92 (t, 1H, H-7); at 171 K: 7.52 (bs, 1H, H-4), 8.38 (bs, 1H, H-3), 9.18 (bs, 1H, H-7). Partial elemental analysis calculated for $C_6H_5N_3O$: C, 53.33; H, 3.73; N, 31.10. Found: C, 53.02; H, 3.67; N, 30.84.

Results and Discussion

Low-temperature NMR spectroscopy

Low-temperature NMR spectroscopy was used to study the tautomeric equilibria of compounds **1**–**6**. It has been shown previously that separate signals for the individual tautomers of substituted purines[25] and purine analogs[8] can be detected at low temperatures. In order to examine this phenomenon, compounds **3**–**6** were studied at temperatures of 304–171 K. No significant changes in the ^{1}H NMR spectra were observed on decreasing the temperature. Thus, since only one set of ^{1}H and ^{13}C NMR signals was obtained for samples investigated at low temperature, these compounds probably exist in one predominant tautomeric form or undergo a rapid chemical exchange process. The ^{1}H and ^{13}C chemical shifts of compounds **3**–**6** at various temperatures can be found in the section Experimental.

Table 5. Selected experimental (in methanol-d_4) and calculated (with PCM_{MeOH}) J_{H-C} coupling constants (Hz) for compounds **1, 2**, and **6** (**a**: N1-H tautomer, **b**: N2-H tautomer)

Compound	$^1J_{H3-C3}$	$^1J_{H4-C4}$	$^1J_{H7-C7}$	$^2J_{H3-C3a}$	$^3J_{H3-C7a}$	$^2J_{H4-C3a}$	$^3J_{H4-C7a}$	$^3J_{H7-C3a}$	$^2J_{H7-C7a}$
1 (exp)	195.3	172.7	187.8	11.4	4.0	2.0	5.9	4.2	7.1
1a (calcd)	186.9	164.0	180.6	11.7	3.1	1.3	5.3	4.0	6.8
1b (calcd)	193.3	164.3	178.1	8.3	6.1	1.2	4.6	4.1	7.0
2 (expt)	192.2	166.9	184.5	11.4	3.4	2.0	6.0	4.8	6.6
2a (calcd)	184.6	158.4	176.3	11.6	3.2	1.4	5.6	4.2	6.4
2b (calcd)	191.2	158.4	174.5	8.3	6.2	1.3	4.9	4.2	6.7
6 (expt)	197.4	173.0	192.7	11.8	3.8	– [a]	7.0	4.8	2.4
6 (calcd)	186.6	157.5	179.4	11.9	3.1	1.2	7.6	5.7	0.8

[a] Not determined.

Table 6. Experimental (in methanol-d_4) and calculated (with PCM_{MeOH}) ^{15}N-NMR chemical shifts (δ in ppm) and selected indirect ^{1}H–^{15}N coupling constants (experimental data are absolute values) for compounds **1, 2**, and **6** (**a**, N1-H tautomer; **b**, N2-H tautomer)

Compound	N-1	N-2	$\Delta\delta$ (N2-N1)	N-6	$^2J_{H3-N2}$	$^3J_{H3-N1}$	$^3J_{H4-N6}$	$^2J_{H7-N6}$
1 (expt)	181.3	324.3	143.0	294.7	12.6	6.6	2.0	10.4
1a (calcd)	186.7	347.6	160.9	315.9	−13.0	−8.0	−0.6	−11.4
1b (calcd)	304.9	236.4	−68.5	317.2	−4.9	0.3	−0.7	−12.1
2 (expt)	177.4	320.3	142.9	271.3	12.4	6.5	3.1	9.8
2a (calcd)	182.0	345.6	163.6	283.2	−13.0	−8.0	−0.6	−10.8
2b (calcd)	300.6	234.5	−66.1	291.2	−5.2	0.4	−0.6	−11.4
6 (expt)	187.6	343.3	155.7	179.0	12.5	7.0	3.0	2.7
6 (calcd)	176.0	359.9	183.9	183.5	−13.2	−8.1	−2.2	−1.8

Table 7. Experimental (in methanol-d_4) and calculated (with PCM_{MeOH}) ^{13}C-NMR chemical shifts (δ in ppm) for compounds **1, 2**, and **6** (**a**, N1-H tautomer; **b**, N2-H tautomer) and theoretical populations of two tautomers (%) calculated using the Boltzmann equation

Compound	C-3	C-3a	C-4	C-5	C-7	C-7a	$\Delta\delta$ (C7a-C3)	% at 300 K
1 (expt)	134.39	131.42	115.17	141.30	135.34	137.86	3.5	–
1a (calcd)	141.73	138.71	124.31	155.54	140.28	144.43	2.7	98.9
1b (calcd)	132.17	133.99	124.18	155.82	152.39	153.04	20.9	1.1
;**2** (expt)	133.97	132.62	96.51	160.15	132.52	135.98	2.0	–
2a (calcd)	140.61	140.02	104.99	168.33	138.05	141.90	1.3	99.5
2b (calcd)	129.91	135.18	102.14	168.17	150.88	151.64	21.7	0.5
;**6** (expt)	134.65	136.75	101.17	154.17	126.53	133.53	−1.1	–
6 (calcd)	139.78	147.95	109.88	165.55	127.25	133.81	−6.0	– [a]

[a] Not calculated.

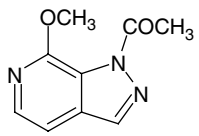
Ambient-temperature NMR spectroscopy

Since we did not observe any signal broadening or splitting at low temperatures, a full set of NMR data was recorded for compounds **1**–**6** at 300 K. The ^{1}H and ^{15}N NMR chemical shifts for **1**–**6** are summarized in Table 1, the ^{13}C NMR chemical shifts and single-bond coupling constants ($^1J_{H,C}$) in Table 2, and the heteronuclear long-range coupling constants in Tables 3 and 4.

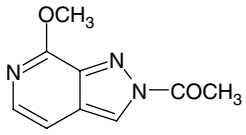
The NMR signals of three aromatic protons were assigned by using 2D shift correlation experiments. The interaction of H-3 with nitrogens N-1 and N-2 was observed in the ^{1}H–^{15}N GSQMBC spectrum (Fig. 1). This undoubtedly distinguishes H-3 from the other two hydrogens of the ring system. H-4 and H-7 are both coupled with N-6 but H-7 is expected to be more deshielded because of its position in the molecule. The assignment was confirmed unequivocally by using a NOESY experiment (mixing time 700 ms) for compound **2**. A NOE (nuclear Overhauser effect) cross-peak was obtained between the protons of the methyl group and H-4.

The signals of the six carbon atoms that form the heterocyclic skeleton were observed in the ^{13}C NMR spectrum. The carbons of the aromatic CH groups (C-3, C-4, and C-7) were easily assigned by observing their correlation peaks with the corresponding protons. The one-bond coupling constants measured for all compounds are summarized in Table 2.

A 2D ^{1}H–^{13}C GSQMBC experiment[13] was used to assign the signals of the quaternary carbons. A portion of the ^{1}H–^{13}C GSQMBC spectrum with additional details of the H3–C3a, H3–C7a and H7–C3a, H7–C7a cross-peaks is shown in Fig. 2. The ^{1}H–^{13}C coupling constants were extracted from the antiphase patterns of the cross-peaks[26] obtained in the GSQMBC spectra and are summarized in Table 3. In order to distinguish between C-3a and C-7a, the coupling constants $^2J_{H3-C3a}$, $^3J_{H3-C7a}$, $^2J_{H4-C3a}$, $^3J_{H4-C7a}$, $^3J_{H7-C3a}$, and $^2J_{H7-C7a}$ were analyzed. According to the DFT calculations, the values of the coupling constants $^2J_{H3-C3a}$ and $^2J_{H7-C7a}$ are larger for all compounds than those of $^3J_{H3-C7a}$ and $^3J_{H7-C3a}$ (see Table 5). The only exception is compound **6** which has a different electron distribution caused by the C=O group at position C-5. For this compound, the couplings of both protons (H-3 and H-7) are larger with C-3a than with C-7a. These observations allow us to assign C-3a and C-7a unequivocally. The values of other coupling constants are also in agreement with the theoretical data (Table 5). Finally, C-5 is assigned to the remaining resonance of the quaternary carbon found in the range of 141–160 ppm.



13



14

^{15}N NMR chemical shifts were obtained by using long-range ^{1}H–^{15}N chemical shift correlation experiments at the natural abundance of the ^{15}N isotope. The assignment of N-6 is straightforward because of its coupling with H-7 and H-4. The ^{1}H–^{15}N coupling constants were obtained from the antiphase (GSQMBC) and also the in-phase (gs-HMBC) splitting; the two values thus obtained for each coupling constant were then averaged to obtain the results presented in Table 4.

Several NMR parameters were analyzed and compared to determine the predominant tautomer in solution. Firstly, values of $^nJ_{H3-N}$ were compared with those reported recently for the N-1 and N-2 substituted analogs **13** and **14**.[8] Coupling constants $J_{H3-N2} = 11.9–12.7$ Hz and $J_{H3-N1} = 6.5–8.2$ Hz were obtained for compounds **1**–**6**. These values agree well with those obtained for regioisomer **13** ($J_{H3-N2} = 14.8$; $J_{H3-N1} = 8.0$ Hz). In contrast, $J_{H3-N2} = 4.4$ Hz and $J_{H3-N1} = 2.3$ Hz were measured for regioisomer **14**. Based on these tendencies observed for the indirect spin–spin coupling constants, we conclude that our compounds exist predominantly or exclusively in the N1-H form. The values of the ^{1}H–^{15}N coupling constants for the two tautomers **a** (N1-H) and **b** (N2-H) calculated by using the DFT method (see Table 6) correspond nicely with the experimental data and support our conclusion.

Further, the differences between the chemical shifts of N-1 and N-2 were employed to confirm unequivocally the structure drawn for the predominant tautomer (Table 6). The experimentally measured $\Delta\delta$ (N2-N1) values for compounds **1** and **2** are 143 ppm and 142 ppm, respectively. The values calculated for **1a** (160 ppm) and **2a** (163 ppm) using DFT agree well with the experimental data, whereas the values calculated for the N-2 tautomers (−68 ppm for **1b**, −66 ppm for **2b**) differ significantly from the experimental results. Finally, for all of the compounds the two nitrogen resonances were assigned to the N-1 and N-2 atoms on the basis of the chemical shifts. A hydrogen-bearing nitrogen atom is more shielded than its nonprotonated counterpart.[1] Therefore, the more shielded resonance was assigned to N-1.

The structural conclusions formulated above from the ^{15}N NMR data are also supported by the values of the ^{13}C chemical shifts. Protonation of a nitrogen atom increases the shielding of a neighboring carbon atom by about 10 ppm.[1] For compounds **1**–**6**, the tautomeric migration of a proton should affect mainly the C-7a and C-3 resonances. This effect is clearly evident from the DFT calculations (see Table 7). The experimentally measured differences in the chemical shifts $\Delta\delta$ (C7a-C3) are 3.5 ppm for **1** and 2.0 ppm for **2**. In accordance with the experimental results, the calculated $\Delta\delta$ values for the N-1 tautomers are 2.7 ppm (**1a**) and 1.3 ppm (**2a**). In contrast, the values $\Delta\delta$ (C7a-C3) of 20.9 ppm (**1b**) and 21.7 ppm (**2b**) were calculated for the N-2 tautomers.

Finally, the calculated energies support our conclusion that the N-1 form predominates (ΔE(**b** − **a**) is 2.7 kcal mol^{-1} for **1** and 3.2 kcal mol^{-1} for **2**).[9] According to the Boltzmann distribution (Table 7), at a temperature of 300 K, the difference in energy corresponds to an **a : b** ratio of about 99 : 1, with the population of the minor tautomer near or even below the limit of detection for conventional NMR spectroscopy. These results represent additional support for the conclusions made from the NMR data.

Conclusions

Using low-temperature NMR spectroscopy, it was found that in methanol all compounds either exist in one highly dominating tautomeric form or undergo a rapid chemical exchange process, even at temperatures as low as 171 K. The predominance of the N1-H form at laboratory temperature was determined by comparing the experimental $^2J_{NH}$, $^3J_{HN}$, $\Delta\delta_N$(N2-N1), and $\Delta\delta_C$(C7a-C3) values for our compounds with those published for the *N*-acyl analogs **13** and **14** and the values calculated using DFT. Theoretically calculated difference in energy favors the N1-H tautomer (**a** in Scheme 1), with about 99% of the population at 300 K. All of the compounds **1**–**6** were fully characterized by ^1H, ^{13}C, and ^{15}N chemical shifts and by ^1H–^{13}C and ^1H–^{15}N coupling constants.

Acknowledgements

This work was supported by the Ministry of Education of the Czech Republic (grants MSM0021622413 and LC06030 to TB, JT, and RM) and by the Ministry of Industry, Energy, and Technology of Greece (Research Grant, Program ENTER). We express our gratitude to the staff members of the supercomputing centers in Brno and Prague for CPU time.

References

[1] R. Marek, V. Sklenář, *Annu. Rep. NMR Spectrosc.* **2005**, *54*, 201.
[2] J. C. Milhavet, A. Gueiffier, L. Bernal, J. C. Teulade, *Heterocycles* **1999**, *51*, 1661.
[3] J. C. Milhavet, L. Bernal, A. Gueiffier, A. Contastin, J. P. Chapat, J. C. Teulade, A. Carpy, G. Grassy, *Arch. Pharm.* **1989**, *35*, 885.
[4] H. Ochiai, A. Ishida, T. Ohtani, K. Kusumi, K. Kishikawa, S. Yamamoto, H. Takeda, T. Obata, H. Nakai, M. Toda, *Bioorg. Med. Chem.* **2004**, *12*, 4089.
[5] H. Ito, J. Gardner-Thorpe, M. J. Zinner, S. W. Ashley, E. E. Whang, *Surgery* **2003**, *134*, 221.
[6] L. Meijer, M. Flajolet, P. Greengard, *Trends Pharmacol. Sci.* **2005**, *25*, 471.
[7] R. N. Misra, H.-Y. Xiao, D. B. Rawlins, W. Shan, K. A. Kellar, J. G. Mulheron, J. S. Sack, J. S. Tokarski, S. D. Kimball, K. R. Webster, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2405.
[8] V. N. Kourafalos, P. Marakos, E. Mikros, N. Pouli, J. Marek, R. Marek, *Tetrahedron* **2006**, *62*, 11987.
[9] I. Alkorta, J. Elguero, *J. Phys. Org. Chem.* **2005**, *18*, 719.
[10] D. S. Wishart, C. G. Bigam, J. Yao, F. Abildgaard, H. J. Dyson, E. Oldfield, J. L. Markley, B. D. Sykes, *J. Biomol. NMR* **1995**, *6*, 135.
[11] R. Marek, A. Lyčka, *Curr. Org. Chem.* **2002**, *6*, 35.
[12] R. Marek, A. Lyčka, E. Kolehmainen, E. Sievänen, J. Toušek, *Curr. Org. Chem.* **2007**, *11*, 1154.
[13] R. Marek, L. Králík, V. Sklenář, *Tetrahedron Lett.* **1997**, *38*, 665.
[14] W. Willker, D. Leibfritz, R. Kerssebaum, W. Bermel, *Magn. Reson. Chem.* **1993**, *31*, 287.
[15] R. Marek, J. Brus, J. Toušek, L. Kovács, D. Hocková, *Magn. Reson. Chem.* **2002**, *40*, 353.
[16] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, *Gaussian 03, Revision C.02*, Gaussian: Wallingford, **2004**.
[17] R. Ditchfield, *Mol. Phys.* **1974**, *27*, 789.
[18] J. Toušek, S. Van Miert, L. Pieters, G. Van Baelen, S. Hostyn, B. U. W. Maes, G. Lemière, R. Dommisse, R. Marek, *Magn. Reson. Chem.* **2008**, *46*, 42.
[19] J. Tomasi, B. Mennucci, R. Cammi, *Chem. Rev.* **2005**, *105*, 2999.
[20] A. K. Jameson, C. J. Jameson, *Chem. Phys. Lett.* **1987**, *134*, 461.
[21] C. J. Jameson, in *Encyclopedia of NMR*, vol. 2, (Eds: D. M. Grant, R. K. Harris), John Wiley: London, **1995**, pp 1273.
[22] H. E. Foster, J. Hurst, *J. Chem. Soc. Perkin Trans. I* **1973**, 2901.
[23] D. Chapman, J. Hurst, *J. Chem. Soc. Perkin Trans. I* **1980**, 2398.
[24] N. Lougiakis, P. Marakos, N. Pouli, J. Balzarini, *Chem. Pharm. Bull.* **2008**, *56*, in press.
[25] P. Sečkárová, R. Marek, K. Maliňáková, E. Kolehmainen, D. Hocková, M. Hocek, V. Sklenář, *Tetrahedron Lett.* **2004**, *45*, 6259.
[26] Y. Kim, J. H. Prestegard, *J. Magn. Reson.* **1989**, *84*, 9.

