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Tetrahedron
Tetrahedron 62 (2006) 11987–11993

Synthesis and tautomerism study of 7-substituted pyrazolo[3,4-*c*]pyridines

Vassilios N. Kourafalos,[a] Panagiotis Marakos,[a] Emmanuel Mikros,[a,*] Nicole Pouli,[a,*] Jaromír Marek[b] and Radek Marek[c]

[a]*Department of Pharmacy, Division of Pharmaceutical Chemistry, University of Athens, Panepistimiopolis, 15771 Zografou, Greece*
[b]*Laboratory of Functional Genomics and Proteomics, Faculty of Science, Masaryk University, Kamenice 5/A2 Brno, CZ-62500, Czech Republic*
[c]*National Center for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5/A4 Brno, CZ-62500, Czech Republic*

Received 20 July 2006; revised 1 September 2006; accepted 21 September 2006
Available online 20 October 2006

Abstract—A number of 7-substituted pyrazolo[3,4-*c*]pyridine derivatives have been synthesized in order to investigate the N1–N2 tautomerism within this class of biologically interesting compounds. Tautomeric equilibrium has been studied using NMR ^{13}C, ^{15}N chemical shifts and heteronuclear ^{1}H–^{15}N and ^{1}H–^{13}C spin–spin couplings, in conjunction with X-ray crystallography. The N1 tautomer predominates in DMF solution in all the compounds tested.

1. Introduction

Biomolecules are often characterized by the presence of more than one basic atom and can exist in multiple tautomeric and protonated states, which should be considered in the study of their mode of interaction with their substrates, where hydrogen bonding between donor and acceptor motifs are usually involved. Within this context, the structure and the 7*H*–9*H* prototropic tautomeric equilibrium of isolated nucleic acid bases and several purine derivatives have been extensively investigated, both theoretically and experimentally.[1–5] Among a large number of purine-related heteroaromatic compounds that have also been studied, all five isomers of pyrazolopyridines have received much attention, as concerns their pharmacological activity. Some pyrazolopyridines are well-known non-sedative anxiolytic agents,[6] while some others can act as human enzyme inhibitors, for example, a number of differently substituted pyrazolopyridines have been recently shown to be potent inhibitors of phosphodiesterases,[7] matrix metalloproteinases,[8] glycogen synthase kinase-3,[9] and of cyclin-dependent kinases.[10]

We are involved in the synthesis and structure–activity relationship studies of some pyrazolo[3,4-*c*]pyridine nucleosides (Fig. 1, I and II, respectively), which can be viewed as singly modified (4-deaza) formycins.[11] Formycin A and formycin B (Fig. 1) are potent antibiotics with proven antiviral, immunodepressant, antitumor, and antimetabolic activities.[12]

The presence of the pyrazole ring in the formycins results in a N1–H/N2–H prototropic tautomerism and consequently at physiological pH they exist as mixture of tautomers. Concerning formycin A, it has been shown that in aqueous solution the N1–H tautomer is the predominant one (>94%), although protonation at N-4 was accompanied with the migration of the hydrogen from N-1 to N-2.[13] A shift in the tautomeric equilibrium for both formycins towards the

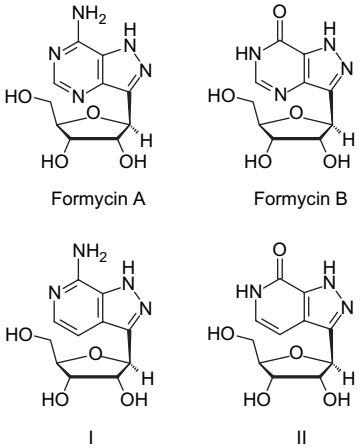


Figure 1. Structures of purine-like C-nucleosides.

* Corresponding authors. Tel.: +30 2107274813; fax: +30 2107274747; e-mail addresses: mikros@pharm.uoa.gr; pouli@pharm.uoa.gr

0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.09.081

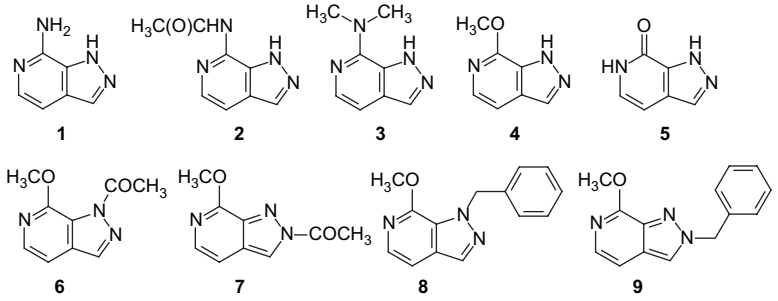


Figure 2. Structures of the examined pyrazolo[3,4-*c*]pyridines.

minor N2–H form was also observed upon complex formation with purine nucleoside phosphorylase (PNP), suggesting the preferential binding of a specific tautomer to this biologically important enzyme.[14]

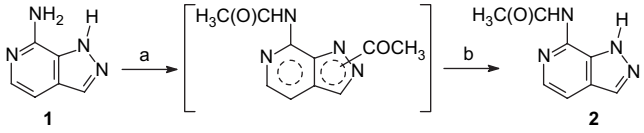
During initial studies, a peak broadening in the ^{1}H NMR spectra of compounds I and II was observed, which was attributed in the coexistence of tautomers, in accordance with the findings reported for the formycins.[15] In order to further investigate this phenomenon, we have selected a number of pyrazolo[3,4-*c*]pyridine derivatives (Fig. 2) and we have decided to study their tautomerism by low-temperature NMR spectroscopy.[16]

Relative populations of individual tautomers are generally influenced by temperature, solvent, and substitution pattern, which modifies the electronic distribution within the molecule. We have thus prepared compounds **1**–**5**, which bear 7-substituents with various electronic and steric properties. Moreover, since the ^{13}C and ^{15}N NMR chemical shifts were utilized to specify the protonation site of purines[16,17] we have considered the syntheses of N-1 and N-2 substituted analogues **6**–**9** as models for the determination of the spectral data. In addition to chemical shift analysis, heteronuclear coupling constants were revealed to be a useful tool for tautomeric equillibria studies, consequently all compounds lack a 3-substituent, in order to utilize the $J_{H3–Cx}$ and $J_{H3–Ny}$ couplings to accomplish the study.

2. Results and discussion

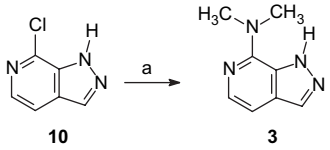
2.1. Synthesis

The syntheses of compounds **1**, **4**, and **5** (Fig. 2) has already been reported starting from 2-amino-3-nitro-4-picoline, which was suitably substituted and ring-closed in the presence of nitrosyl chloride or isoamylnitrite.[18] Compound **2** was prepared by acetylation of **1** and treatment of the resulting mixture of regio-isomers with methanolic ammonia (Scheme 1).



Scheme 1. Reagents and conditions: [a] acetic anhydride, rt, 12 h; [b] NH_3/CH_3OH, rt, 1 h, 92% (overall).

Compound **3** was prepared from 7-chloropyrazolo[3,4-*c*]-pyridine (**10**)[18b] upon treatment with an ethanolic solution of dimethylamine (Scheme 2).



Scheme 2. Reagents and conditions: [a] $(CH_3)_2NH/CH_3CH_2OH$, reflux, 12 h, 75%.

Compounds **6** and **7** were prepared by direct acetylation of **4**,[18a] whereas the benzyl substituted regio isomers **8** and **9**, resulted upon treatment of compound **4** with NaH, followed by addition of benzyl bromide to the generated anion. The substitution occurs readily at position 2, when the reaction is carried out at room temperature and this is probably due to the steric hindrance, exerted by the 7-substituent. On the other hand, analogous treatment of a DMF solution of **4** with acetic anhydride or benzyl bromide at reflux provided both 1- and 2-isomers, and from this mixture the 1-substituted analogue was isolated by column chromatography.

During the preparation of the 1-benzyl isomer **8**, a minor amount of a third product, apart from the 2-regio isomer **9**, was also isolated. This derivative was identified as the 2,6-dibenzylpyrazolo[3,4-*c*]pyridine-7-one (**11**, Fig. 3a), based on NMR analysis. Furthermore, compound **11** furnished crystals from methanol suitable for an X-ray diffraction

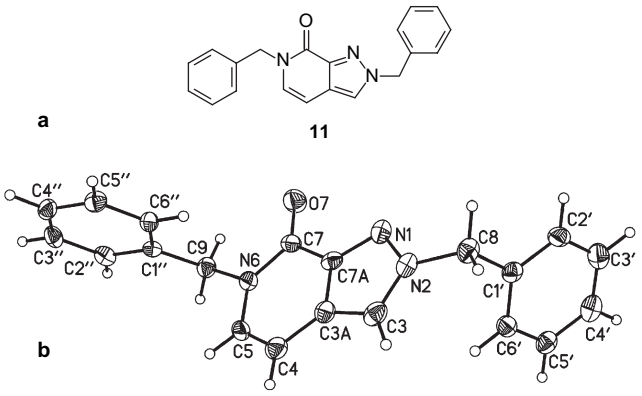
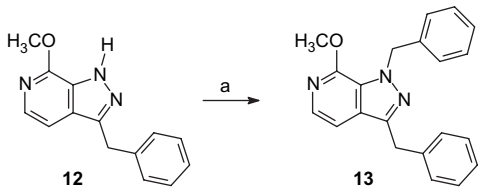


Figure 3. (a) Structure of compound **11** and (b) perspective view of the X-ray structure of compound **11**.

analysis, which provided the unambiguous assigment of its structure (Fig. 3b).

Taking into account the regioselective formation of the N-2 substituted isomers, we have also prepared the 3-benzyl analogue **13** (Scheme 3), in order to study the influence of a bulky 3-substituent in the N-alkylation. Thus, reaction of 3-benzyl-7-methoxypyrazolo[3,4-*c*]pyridine (**12**)[19] with benzyl bromide in the presence of sodium hydride, provided exclusively the 1-benzyl analogue **13**. This regio-isomer was identified by the use of the corresponding HMBC spectrum, where a cross-peak between the NCH_2Ph and C-7a is obvious and additionally, this methylene presented NOE with the 7-methoxy group.



Scheme 3. Reagents and conditions: [a] NaH, benzyl chloride, DMF, rt, 1 h, 68%.

2.2. NMR spectroscopy

Low-temperature NMR represents an important tool for examining tautomeric equilibrium.[16] 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.[20] Decreasing the temperature slows down the chemical exchange process. At low temperatures, separated signals of individual tautomers could be detected, however, this methodology has rarely been used in the field of purine chemistry since exchange studies of purine derivatives have been performed primarily in water.[21] Separation of the NMR signals of individual tautomers of purine derivatives at low temperatures has been recently described in DMF and DMSO/acetonitrile solutions.[16,22]

The ^{1}H NMR spectra of compounds **1**–**9** in DMF solution are characterized by four main signals: the AX system of H-4 and H-5 at 7.11–7.57 ppm and 7.50–7.93 ppm, respectively, a singlet at 8.20–8.65 ppm attributed to H-3 and a broad signal at ~14 ppm attributed to the proton suspected for tautomerism exchanging between N-1 and N-2. In all cases, except pyrazolopyridinone **5** and 7-dimethylamino derivative **3**, the H-3, H-4, and H-5 signals were sharp at room temperature.

The assignment of ^{13}C and ^{15}N spectra was performed using the corresponding 2D gHMBC and gHSQC[23] spectra and chemical shifts are summarized in Table 1.

Heteronuclear coupling constants were determined using the GSQMBC spectra[24] from the anti-phase doublets of the corresponding cross-peaks and the most important are summarized in Table 2.

The spectroscopic data concerning the pair of compounds **6**–**7** and **8**–**9** show that C-7a, C-3a, C-3, N-1, and N-2 chemical shifts and the J_{H-C} and J_{H-N} coupling constants can be a useful tool for the determination of the substitution site and/or the tautomeric form, as previously seen in the case of formycin and purine derivatives.[16,25] When N-1 is substituted, N-2 is deshielded (at ~326 ppm), as well as C-3 and C-3a (~135 ppm). When N-2 is substituted, N1 and C-7a are deshielded (at ~291 ppm and 138 ppm, respectively). Moreover, indirect spin–spin coupling constants of H-3 with N-1, N-2, and C-7a exhibit characteristic differences between N-1 and N-2 substitution and can be additionally used for tautomer discrimination.

Considering the above results the qualitative inspection of the spectroscopic data concerning compounds **1**–**5** and mainly N-1 and N-2 chemical shifts, suggests that in all cases the N1–H tautomer should be predominant in DMF solution.

In order to study the tautomeric equilibrium concerning compounds **1**–**5**, variable temperature studies have been performed, in a temperature range of 213–303 K. In the case of compound **5**, on lowering the temperature to 213 K, a second set of signals appeared in the ^{1}H NMR spectrum with relative intensities 100 and 7, which corresponds to the ratio of major and minor components 93.5:6.5.

2D ^{1}H–^{13}C and ^{1}H–^{15}N gHSQC, gHMBC, and GSQMBC spectra recorded at this temperature, allowed for the complete resonance assignment and determination of coupling constants. ^{1}H–^{15}N GSQMBC spectrum of compound **5** is shown in Figure 4. All characteristic NMR spectroscopic data are summarized in Table 3, along with the corresponding data for compounds **6** and **7**, in order to facilitate the comparison. All data suggest that the major component corresponds to the N1–H tautomer, while the minor component corresponds to N2–H.

No significant changes in the ^{1}H NMR patterns were observed on decreasing the temperature in all other derivatives **1**–**4**. One set of signals was detected, even if signals were

Table 1. ^{13}C and ^{15}N NMR chemical shifts (ppm) for compounds **1**–**9** and **11** in DMF-d_7 at 303 K

Compounds	C3	C3a	C4	C5	C7	C7a	N1	N2	N6
1	133.65	127.54	104.79	138.25	147.73	128.77	190.5	309.6	248.9
2	134.30	130.42	112.64	137.54	139.03	130.16	188.0	322.1	276.6
4	134.68	129.79	109.82	136.49	151.66	128.23	184.4	322.1	252.7
5	134.0	125.5	98.6	126.1	155.1	133.1	not obsd	not obsd	155.2
6	139.71	135.46	109.87	141.30	152.51	125.52	222.5[a]	325.7[a]	263.1[a]
7	123.46	126.73	109.78	137.94	157.93	140.44	291.5[a]	252.5[a]	252.3[a]
8	133.58	130.34	109.64	135.98	150.89	126.43	191.1	327.4	252.1
9	125.49	127.25	109.42	136.08	156.65	137.97	292.2	233.9	247.9
11	125.74	124.13	99.25	130.71	157.90	142.29	307.1	230.5	160.5

[a] Chemical shifts determined at 273 K.

Table 2. Characteristic J_{H-C} and J_{H-N} scalar coupling constants (Hz) of compounds **1–9** and **11** in DMF-d_7 at 303 K

	$^1J_{H3-C3}$	$^2J_{H3-C3a}$	$^3J_{H3-C7a}$	$^3J_{H3-N1}$	$^2J_{H3-N2}$
1	188.9	11.3	4.4	7.2	12.3
2	190.2	11.7	3.9	7.6	12.9
4	190.2	11.5	4.5	8.2	13.2
5	189.0	10.7	3.7	[a]	[a]
6	196.6	11.1	3.2	8.0	14.0
7	198.8	8.2	6.8	2.3	4.4
8	192.1	11.0	3.7	7.5	12.9
9	194.2	8.8	7.6	0.9	4.5
11	192.9	8.5	7.2	—	4.7

[a] Not observed.

Table 3. Characteristic ^{15}N and ^{13}C chemical shifts (δ in ppm) and indirect spin–spin coupling constants (J in Hz) of tautomers **5** *major* and **5** *minor* in DMF-d_7 at 213 K along with the corresponding spectroscopic data for derivatives **6** and **7**

	6	**7**	***5** major*	***5** minor*
	Chemical shifts			
N1	222.5	291.5	196.7	298.7
N2	325.7	252.5	319.1	221.7
C3	139.32	124.34	134.98	124.34
C3a	134.97	123.67	126.06	123.67
C7a	124.92	142.29	132.82	142.29
	Coupling constants			
J_{H3-N1}	8.0	2.3	6.9	[a]
J_{H3-N2}	14.0	4.4	13.2	6.4
J_{H3-C3a}	11.1	8.2	11.0	8.2
J_{H3-C7a}	3.2	6.8	5.5	6.4

[a] Not observed.

sharpened. In the case of compound **3** the two N-7 methyl group signals were clearly observed at 213 K, while a broad signal exists at room temperature (thus not considered in the tables), showing that an additional conformational equilibrium exists around the C7–N7 bond, due to the steric hindrance imposed by the bulk of the two methyl groups.

As mentioned above, from a qualitative point of view, the spectroscopic data for compounds **1–4** are in good agreement with the N1–H tautomer predominance. Simple AM1 semi-empirical calculations showed that the energy difference between N1–H and N2–H tautomers is ~3 kcal/mol in the case of the pyridinone **5**, while this difference is >5 kcal/mol for all other derivatives. These theoretical calculations are in agreement with the experimental observations described above, predicting that the population of N2–H tautomer should be undetectable by NMR. The calculated energy differences present an analogy to those performed on adenine and guanine[26] predicting that in both cases the N9–H was the most stable tautomer, followed by N7–H, however, concerning adenine, the N7–H was considerably less stable. In the case of derivative **4**, the experimental ^{15}N chemical shifts existing for the acetyl- and benzyl- derivatives **6–9** permit the theoretical calculation of the population ratio of the two tautomers at room temperature, according to equations similar to those described for the purine tautomerism.[5] The ratio was calculated to be 97/3 and, as the reliability of the approaches for determining the tautomeric ratios using averaged chemical shifts is approximately 10% and substitution at N-1 and N-2 is not the same as in compounds **6–9** and **4**, we conclude that the N2–H tautomer population is very low and undetectable by NMR at low temperature.

7-Methoxy-1*H*-pyrazolo[3,4-*c*]pyridine (**4**) furnished crystals from methanol suitable for a single-crystal X-ray analysis, which provided the unambiguous assignment of its structure (Fig. 5). The crystallographic analysis reveals that, in the solid state the proton is also localized at the nitrogen atom N1, confirming the NMR analysis.

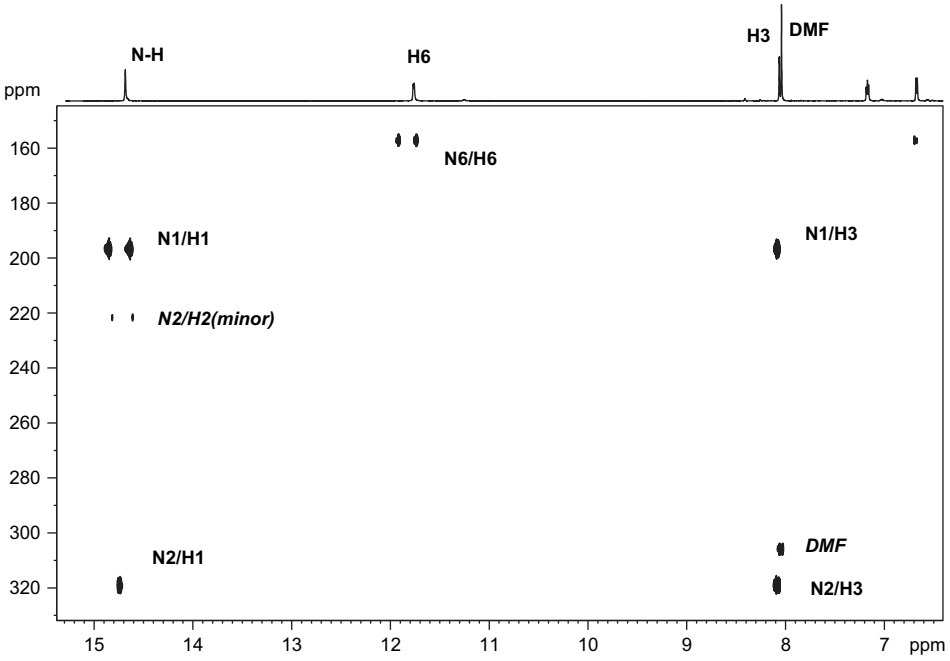


Figure 4. $^1H-^{15}N$ GSQMBC spectrum of compound **5** in DMF-d_7 at 213 K.

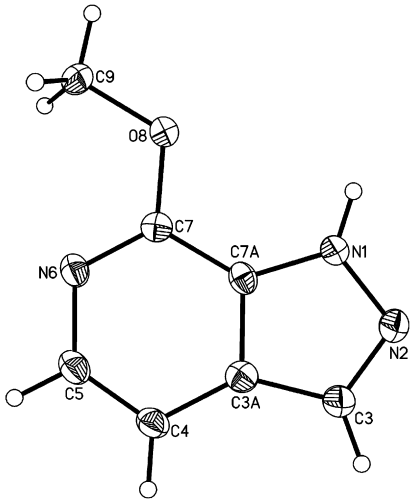


Figure 5. Perspective view of the X-ray structure of compound **4**.

3. Conclusions

In conclusion, we have shown that the N1–H tautomer of the 7-substituted pyrazolo[3,4-*c*]pyridines is predominant in solution. Only in the case of the pyrazolopyridinone **5** could the N2–H tautomer be clearly detected at appropriate NMR spectra, probably due to the rather small energy difference of the N1–H and N2–H pair of tautomers, which could be attributed to the relatively reduced aromatic character of the pyridine ring in both tautomers. On the other hand, the reduced aromaticity of the pyridine ring of the N2–H tautomer of compounds **1**–**4**, when compared to the corresponding N1–H tautomer, results in a high energy difference between them and this is in favor of the existence of the N1–H species. N-substitution of the 7-substituted pyrazolo[3,4-*c*]pyridine ring system occurs predominantly at the less hindered N-2. However, the presence of a 3-bulky substituent favors the regioselective formation of the N-1 isomer.

4. Experimental

4.1. Chemistry

All 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 silica gel F-254 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.

4.1.1. 7-Acetamido-1*H*-pyrazolo[3,4-*c*]pyridine (2). 7-Aminopyrazolo[3,4-*c*]pyridine[18b] (200 mg, 1.49 mmol) was stirred overnight with 5 ml of acetic anhydride, at room temperature. Acetic anhydride was removed under reduced pressure and the residue was dissolved in a saturated solution of ammonia in methanol. The solution was stirred at room temperature for 1 h, the solvent was vacuum evaporated and the residue was purified by column chromatography (silica gel) using a mixture of cyclohexane/ethylacetate 20/80 (v/v) as the eluent to give **2** (240 mg, 92%) as white crystals. Mp 263 °C (CH_2Cl_2). ^{1}H NMR (DMF-d_7, 300 MHz) δ 2.32 (s, 3H, CH_3), 7.57 (d, 1H, H-4, J_{4-5}=5.38 Hz), 7.93 (d, 1H, H-5, J_{5-4}=5.38 Hz), 8.22 (s, 1H, H-3). ^{13}C NMR (DMF-d_7, 50 MHz) δ 24.02 (CH_3), 112.64 (C-4), 130.16 (C-7α), 130.42 (C-3α), 134.30 (C-3), 137.54 (C-5), 139.03 (C-7), 171.10 (CO). Anal. Calcd for $C_8H_8N_4O$: C, 54.54; H, 4.58; N, 31.80. Found: C, 54.62; H, 4.43; N, 31.98.

4.1.2. 7-Dimethylamino-1*H*-pyrazolo[3,4-*c*]pyridine (3). 7-Chloro-1*H*-pyrazolo[3,4-*c*]pyridine[18b] (**10**, 150 mg, 0.98 mmol) was added to a saturated solution of dimethylamine in ethanol (15 ml) and the resulting solution was refluxed for 12 h. The solvent was evaporated and the residue was purified by column chromatography (silica gel) using a mixture of methylenechloride/methanol 90/10 (v/v) as the eluent, to give **3** (120 mg, 75%) as white crystals. Mp 260 °C (dec) (Et_2O/MeOH). ^{1}H NMR (DMF-d_7, 300 MHz) δ 3.65 [br s, 6H, $(CH_3)_2N$], 7.11 (br d, 1H, H-4), 7.50 (br, 1H, H-5), 8.46 (br s, 1H, H-3). Anal. Calcd for $C_8H_{10}N_4$: C, 59.24; H, 6.21; N, 34.54. Found: C, 59.51; H, 6.16; N, 34.32.

4.1.3. 1-Benzyl-7-methoxypyrazolo[3,4-*c*]pyridine (8). 7-Methoxy-1*H*-pyrazolo[3,4-*c*]pyridine[18a] (50 mg, 0.34 mmol) was dissolved in anhydrous DMF (7 ml) under argon. A suspension of NaH (60% in paraffin oil, 20 mg, 0.51 mmol), which had previously been washed with pentane, was added to the solution, under cooling and the mixture was stirred at room temperature for 30 min. Then, a solution of benzyl bromide (0.05 ml, 0.37 mmol) in anhydrous DMF (1 ml) was added and the reaction mixture was heated at reflux for 1 h. The solvent was vacuum evaporated and the product was purified by column chromatography (silica gel) using a mixture of cyclohexane/ethylacetate 80/20 (v/v) as the eluent, to give compounds **8**, **9**, and **11**.

4.1.4. Data for 1-benzyl-7-methoxypyrazolo[3,4-*c*]pyridine (8). Yield: 28%. Mp 147 °C (white crystals). ^{1}H NMR (DMF-d_7, 300 MHz) δ 4.13 (s, 3H, CH_3), 5.85 (s, 2H, CH_2), 7.26–7.36 (m, 5H, Ph), 7.37 (d, 1H, H-4, J_{4-5}=5.85 Hz), 7.75 (d, 1H, H-5, J_{5-4}=5.85 Hz), 8.20 (s, 1H, H-3). ^{13}C NMR (DMF-d_7, 50 MHz) δ 53.30 (CH_3), 54.73 (CH_2), 109.64 (C-4), 126.43 (C-7α), 127.79 [*C*H(Ph)], 128.04 [*C*H(Ph)], 128.82 [*C*H(Ph)], 130.34 (C-3α), 133.58 (C-3), 135.98 (C-5), 138.29 [*C*(Ph)], 150.89 (C-7). Anal. Calcd for $C_{14}H_{13}N_3O$: C, 70.28; H, 5.48; N, 17.56. Found: C, 70.35; H, 5.62; N, 17.38.

4.1.5. Data for 2-benzyl-7-methoxypyrazolo[3,4-*c*]pyridine (9). Yield: 32%. Oil. ^{1}H NMR (DMF-d_7, 300 MHz) δ 4.06 (s, 3H, CH_3), 5.80 (s, 2H, CH_2), 7.25 (d, 1H, H-4, J_{4-5}=6.06 Hz), 7.36–7.49 (m, 5H, Ph), 7.62 (d, 1H, H-5, J_{5-4}=6.06 Hz), 8.65 (s, 1H, H-3). ^{13}C NMR (DMF-d_7, 50 MHz) δ 53.61 (CH_3), 58.09 (CH_2), 109.42 (C-4), 125.49 (C-3), 127.25 (C-3α), 129.25 [*C*H(Ph)], 129.79 [*C*H(Ph)], 136.08 (C-5), 137.97 (C-7α), 138.02 [*C*(Ph)], 156.65 (C-7). Anal. Calcd for $C_{14}H_{13}N_3O$: C, 70.28; H, 5.48; N, 17.56. Found: C, 70.43; H, 5.38; N, 17.32.

4.1.6. Data for 2,6-dibenzylpyrazolo[3,4-*c*]pyridin-7-one (11). Yield: 15%. Mp 148 °C (white crystals). ^{1}H NMR

(DMF-d_7, 300 MHz) δ 5.23 (s, 2H, N^6–CH_2), 5.65 (s, 2H, N^2–CH_2), 6.53 (d, 1H, H-4, J_{4-5}=7.31 Hz), 7.23 (d, 1H, H-5, J_{5-4}=7.31 Hz), 7.25–7.44 (m, 10H, 2×Ph), 8.31 (s, 1H, H-3). ^{13}C NMR (DMF-d_7, 50 MHz) δ 50.74 (N^6–CH_2), 57.26 (N^2–CH_2), 99.25 (C-4), 124.13 (C-3α), 125.74 (C-3), 128.00 [*C*H(Ph)], 128.35 [*C*H(Ph)], 128.67 [*C*H(Ph)], 128.76 [*C*H(Ph)], 129.17 [*C*H(Ph)], 129.30 [*C*H(Ph)], 130.71 (C-5), 137.51 [*C*(Ph)], 139.12 [*C*(Ph)], 142.29 (C-7α), 157.90 (C-7). Anal. Calcd for $C_{20}H_{17}N_3O$: C, 76.17; H, 5.43; N, 13.32. Found: C, 76.38; H, 5.54; N, 13.07.

4.1.7. 1,3-Dibenzyl-7-methoxypyrazolo[3,4-*c*]pyridine (13). 3-Benzyl-7-methoxypyrazolo[3,4-*c*]pyridine[19] (**12**, 40 mg, 0.17 mmol) was dissolved in anhydrous DMF (7 ml) under argon. A suspension of NaH (60% in paraffin oil, 10 mg, 0.26 mmol) was added to the solution, under cooling and the mixture was stirred at room temperature for 30 min. A solution of benzyl bromide (0.03 ml, 0.19 mmol) in anhydrous DMF (1 ml) was then added and the reaction mixture was stirred at room temperature for 1 h. The solvent was vacuum evaporated and the product was purified by column chromatography (silica gel) using a mixture of cyclohexane/ethylacetate 85/15 (v/v) as the eluent, to give **13** (38 mg, 68%) as white crystals. Mp 89 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.08 (s, 3H, CH_3), 4.29 (s, 2H, C^3–CH_2), 5.78 (s, 2H, C^3–CH_2), 6.87 (d, 1H, H-4, J_{4-5}=5.85 Hz), 7.16–7.30 (m, 10H, 2×Ph), 7.60 (d, 1H, H-5, J_{5-4}=5.86 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 33.77 (C^3–*C*H$_3$), 53.50 (CH_3), 54.84 (N^1–*C*H$_2$), 108.89 (C-4), 126.52 [*C*H(Ph)], 127.55 [*C*H(Ph)], 127.69 (C-7α), 128.63 [*C*H(Ph)], 128.64 (C-3α), 128.80 [*C*H(Ph)], 135.27 (C-5), 138.05 [*C*(Ph)], 138.89 [*C*(Ph)], 144.60 (C-3), 150.93 (C-7). Anal. Calcd for $C_{21}H_{19}N_3O$: C, 76.57; H, 5.81; N, 12.76. Found: C, 76.29; H, 5.76; N, 12.88.

4.2. NMR spectroscopy

NMR spectra were recorded using a Bruker Avance DRX 500 spectrometer operating at frequencies of 500.13 MHz (^{1}H), 125.77 MHz (^{13}C), and 50.68 MHz (^{15}N), a Bruker Avance DRX 400 spectrometer operating at frequencies of 400.13 MHz (^{1}H), 100.61 MHz (^{13}C), and 40.54 MHz (^{15}N) 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). NMR spectra were measured at various temperatures specified in the text or in the tables. The ^{1}H and ^{13}C NMR chemical shifts (δ in ppm) were referenced to the signal of the solvent. The ^{15}N chemical shifts were referenced to liquid CH_3NO_2 (381.7 ppm)[27] and are reported relative to liquid NH_3. The 2D NMR experiments gHSQC, gHMBC, and GSQMBC were recorded as described previously[23,24] and were used for assigning the individual ^{1}H, ^{13}C, and ^{15}N resonances and for determining the ^{1}H–^{13}C and ^{1}H–^{15}N coupling constants.

4.3. X-ray diffraction analysis

The diffraction data were collected with a KM4CCD four-circle area-detector diffractometer (KUMA Diffraction, Poland) equipped with an Oxford Cryostream Cooler (Oxford Cryosystems, UK). Mo Kα radiation (monochromator Enhance, Oxford Diffraction, UK) was used in all measurements. We performed the ω-scan technique with different κ and φ offsets in order to cover the entire independent part of reflections in the 3–25° θ range. Cell parameters were refined from all the strong reflections. Data reduction was carried out using the program CrysAlis RED (Oxford Diffraction, UK). Direct methods in SHELXS-97,[28] were used to solve the structures, and the structures were refined by using SHELXL-97.[29] The tables were prepared for publication by using SHELXL and PARST,[30] and the figures were generated with ORTEP-III.[31] Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center (CCDC). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.

4.3.1. Crystal data for 4. CCDC Ref. No. 608944. Crystallized from methanol, $C_7H_7N_3O$, M_{rel}=149.16, T=120(2) K, Orthorhombic, λ=0.71073 Å, space group P_{bac}, a=11.3022(8) Å, b=7.4180(6) Å, c=16.2981(12) Å, V=1366.43(18) Å^3, Z=8, D_{calcd}=1.450 Mg/m^3, crystal size 0.40×0.30×0.20 mm, GOF=1.140, R=0.0402/0.0453 ($I>2\sigma(I)$/all data).

4.3.2. Crystal data for 11. CCDC Ref. No. 608945. Crystallized from methanol, $C_{20}H_{17}N_3O$, M_{rel}=315.37, T=120(2) K, Orthorhombic, λ=0.71073 Å, space group $P2_12_12_1$, a=5.8111(6) Å, b=10.0362(10) Å, c=26.568(3) Å, V=1549.5(3) Å^3, Z=4, D_{calcd}=1.352 Mg/m^3, crystal size 0.50×0.25×0.20 mm, GOF=0.948, R=0.0343/0.0480 ($I>2\sigma(I)$/all data).

Acknowledgements

The financial support of this work by a grant from the National Scholarship Foundation of Greece (IKY), from the Special Account for Research Grants Committee of the University of Athens (Program Kapodistrias) and by the Ministry of Education of the Czech Republic (MSM0021622413 to R.M., MSM0021622415 to J.M.) is gratefully acknowledged.

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