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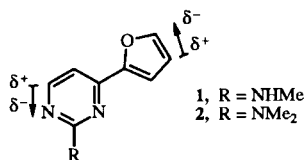
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Received September 8, 1995

Conformational transformations of the title compound **1** were studied using high-resolution  $^1\text{H}$ -nmr techniques, semi-empirical PM3 calculations and molecular dynamics. The unfused furan-pyrimidine ring system of **1** predominantly exists in an *s-trans* conformation in solution and the considerable  $\text{sp}^2$  character of the C2-amino bond results in the hindered rotation which is observed on the nmr time scale.

*J. Heterocyclic Chem.*, **33**, 1207 (1996).

Alkylamino-substituted heteroarylpyrimidines are ligands of serotonin neuroreceptors [1]. Some of them are potent antagonists at 5-HT<sub>2A</sub> receptors *in vivo* and have emerged as model compounds for defining steric relations within a topographic model of the 5-HT<sub>2A</sub> binding sites [1,2]. Large differences in biological responses of closely related derivatives have been noted. In order to obtain an insight into these puzzling effects at the molecular level of interaction, we have initiated conformational studies of substituted heteroarylpyrimidines [3]. The results obtained with 2-amino derivatives of 4-(2'-furyl)pyrimidine **1** and **2** by  $^1\text{H}$  nmr and molecular modeling methods are presented in this paper.



Selected spectra of 4-(2'-furyl)-2-(methylamino)pyrimidine (**1**) accumulated in acetone- $\text{d}_6$  from  $+40^\circ$  to  $-80^\circ$  are given in Figure 1. As the temperature decreases, all resonances with the exception of that for H5' progressively split into pairs of resonances with equal intensity of the two groups of signals within each pair. It can be suggested that compound **1** exists in acetone- $\text{d}_4$  at low temperature as an equimolar mixture of two conformers with the same orientation of the furylpyrimidine system and differing in the orientation of the methylamino substituent conjugated with the pyrimidine ring. The energy barrier of 10 kcal/mol was estimated for rotation of the methylamino group in **1** by using Gutowsky-Holm [4] and Eyring [5] approaches. This value compares well with the rotational barriers of 10.4 kcal/mol obtained for 4-(dimethylamino)pyrimidine and other dimethylamino-substituted pyrimidines and pyridines by  $^1\text{H}$  nmr temperature studies [6].

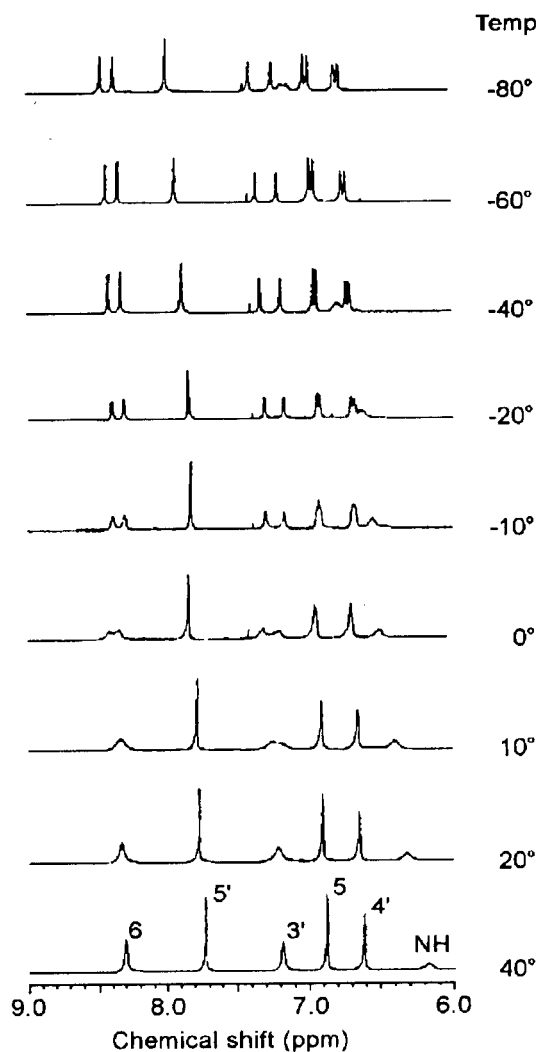


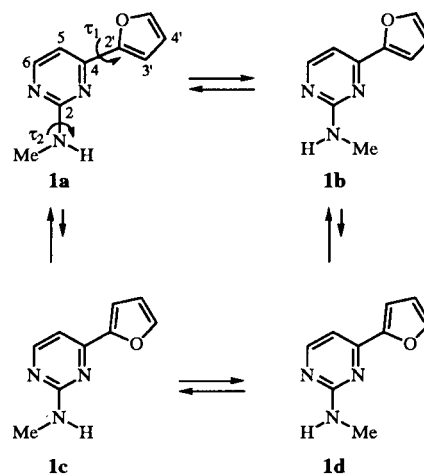
Figure 1. The  $^1\text{H}$  nmr spectra of **1** accumulated in acetone- $\text{d}_6$  solutions at different temperatures. The methyl signals undergoes splitting at  $-10^\circ$  (not shown). The spectra were taken at 400 MHz, the concentration of **1** was 20 mM, and tetramethylsilane was used as an internal reference. See the experimental section for coupling constants between protons (not seen in this figure, due to extensive reduction of the original spectra).

The aromatic protons in the vicinity of the methylamino group of the two conformers are in different chemical environments, thus causing the splitting of the aromatic resonances at low temperature. An interesting observation from Figure 1 is that the largest chemical shift difference is for H3', while the chemical shift for H5' is identical for these two conformers. This result strongly suggests that the 4-(2'-furyl)pyrimidine system of **1** adopts an *s-trans* orientation. An additional support for the *s-trans* conformation came from analysis of the phase sensitive NOESY spectrum of **1** accumulated at  $-70^\circ$  in acetone- $d_6$ . While all intra-ring nOe cross peaks for adjacent protons were observed, the inter-ring nOe cross peak between H5 of the pyrimidine and H3' of the furan was not seen.

For the dimethylamino analog **2** the splitting of the signal corresponding to methyl groups was observed at  $5^\circ$  in acetone- $d_6$ . The aromatic resonances were not split even at  $-95^\circ$  because the two conformers due to hindered rotation around the C2-N(CH<sub>3</sub>)<sub>2</sub> bond are identical. Again, the phase sensitive NOESY spectrum of **2** accumulated at  $-70^\circ$  in acetone- $d_6$  was fully consistent with the exclusive *s-trans* conformation of the 4-(2'-furyl)pyrimidine system.

Phase sensitive NOESY spectra of **1** were also accumulated at  $+45^\circ$  in acetone- $d_6$  and two additional solvents of higher dielectric constants, namely dimethyl sulfoxide- $d_6$  and deuterium oxide. It was of interest to find out whether or not the more polar *s-cis* conformation of the heterobiaryl system is present in a detectable amount under these conditions. The results are given in Table 1. As can be seen, a low-intensity cross peak corresponding to the interaction between H5 and H3' in the *s-cis* conformation was observed at  $+45^\circ$  in all three solvents. The relative intensities of the cross peaks *s-cis/s-trans* increase with increasing a dielectric constant of the solvent under the same temperature conditions. Due to many variables that affect intensity of nOe cross peaks, the integration results in Table 1 cannot be taken as a quantitative measure of the relative abundance of the *s-cis* and *s-trans* conformers. Nevertheless, the observed parallel relationship of an increased concentration of the minor *s-cis* conformer with increasing the dielectric

Scheme 1. Conformational transformation of **1** as obtained from <sup>1</sup>H nmr studies and supported by molecular modeling experiments. See Table 1 for additional information.



constant of solvent can be safely taken as an evidence that an intramolecular dipole-dipole interaction of the pyrimidine and furan rings is a primary factor which determines the preferred *s-trans* orientation. Due to conjugation, the two rings are co-planar in the first approximation. The results discussed above are summarized in Scheme 1.

In order to verify the results of the conformational analysis of **1** in solution, the molecular modeling studies were performed using the PM3 and molecular dynamics approaches. The results of the PM3 calculations for compound **1** are shown in Figure 2. It was found that the PM3 method gives co-planar conformations of the unfused furan-pyrimidine ring system ( $\tau_1 = 0^\circ$  or  $180^\circ$ ). The *s-trans* conformations ( $\tau_1 = 180^\circ$ ) are favored by 0.3-0.6 kcal/mol with respect to the *s-cis* conformations ( $\tau_1 = 0^\circ$ ) in the whole range of rotation of the 2-methylamino group ( $0^\circ < \tau_2 < 360^\circ$ ). Indeed, the two global energy minima, which differ only by 0.03 kcal/mol, refer to the *s-trans* conformation ( $\tau_1 = 180^\circ$ ) of the heterobiaryl ring system, and to the favorable rotamers of the 2-methylamino substituents at  $\tau_2 = 160^\circ$  and  $340^\circ$  (cf. conformers **1a** and **1b**, respectively, Scheme 1). The calculated rotation barrier around the C4-C2' bond at fixed  $\tau_2 = 160^\circ$  is 2.2 kcal/mol. The rotation barrier of the 2-methylamino group at fixed  $\tau_1 = 180^\circ$  is even higher and equals 5.7 kcal/mol (Figure 2b). The calculations predict correctly the lower rotational barrier around the C4-C2' bond and there is a good qualitative agreement between the experimental and calculated energy barrier for rotation of the methylamino group.

The results obtained in molecular dynamics experiments are collected in Table 1. In order to evaluate the *s-cis/s-trans* population ratio from the molecular dynamics simulation of **1**, the following criteria were defined:  $-60^\circ < \tau_1 < 60^\circ$  and  $120^\circ < \tau_1 < 240^\circ$  for *s-cis* and *s-trans* conformations of the

Table 1

Semiquantitative Populations of *S-cis* and *S-trans* Conformers of **1** as Obtained from Volume Integration of the NOESY Spectra and the Populations Calculated by Molecular Dynamics

Solvent	$\epsilon$	Temp (K)	$\Delta G^\circ$ (kcal/mol) [a]	<i>s-cis/s-trans</i>	
				NOESY	Molecular dynamics
acetone- $d_6$	21	203		0/100	18/82
		318	< -1.9	< 5/95	35/65
dimethyl sulfoxide- $d_6$	47	318	-0.7	23/77	44/56
deuterium oxide	78	318	-0.6	28/72	46/54

[a]  $\Delta G^\circ$  calculated from NOESY data using eq (2).

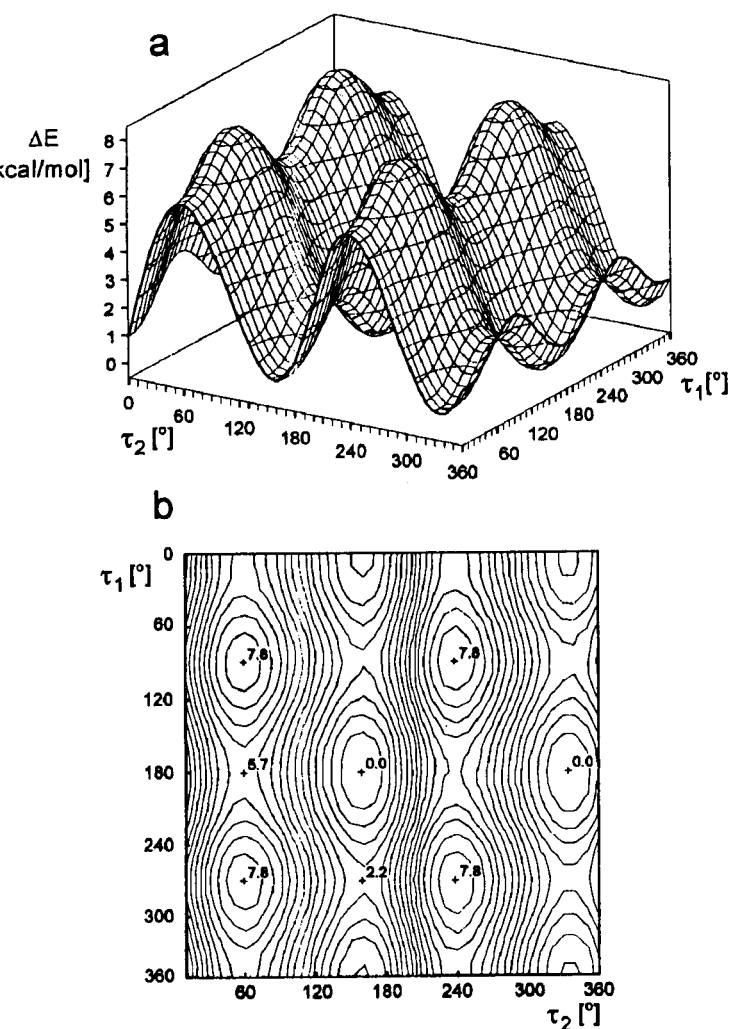


Figure 2. Surface (a) and contour map (b) of relative energies of conformations of **1** upon rotation of the inter-ring C4-C2' bond ( $\tau_1$ : N3-C4 C2'-O) and exocyclic C2-NHCH<sub>3</sub> bond ( $\tau_2$ : N3-C2-N-CH<sub>3</sub>), calculated by the PM3 method. The contour interval is 0.5 kcal/mol.

heterobiaryl fragment, respectively. It was found that the *s-trans* conformations of **1** dominate over *s-cis* conformations within the investigated range of temperatures and dielectric constants. Furthermore, the data presented in Table 1 clearly indicate that the effect of temperature and dielectric constant on the *s-cis/s-trans* population ratio, obtained from both molecular dynamics simulations and NOESY experiments, are similar. As observed in the NOESY experiments, the fraction of the more polar *s-cis* conformation increases with increasing a dielectric constant of the solvent. Solvents with a high dielectric constant partly compensate for the unfavorable dipole-dipole interactions of the *s-cis* conformation of **1**, allowing this conformation to be more populated. The same tendency is observed in molecular dynamics simulations, though the latter method may slightly overestimate the percentage of the *s-cis* conformations.

## EXPERIMENTAL

### 4-(2'-Furyl)-2-(methylamino)pyrimidine **1**.

A mixture of 2-chloro-4-(2'-furyl)pyrimidine [**7**] (0.40 g, 2.2 mmoles), tetrahydrofuran (10 ml) and methylamine (40% solution in water, 10 ml, 0.12 mole) in a teflon-lined Parr bomb was stirred at 25° for 14 hours. The resultant crystalline material was filtered, dissolved in water (25 ml), and the solution was adjusted to pH 10 with sodium hydroxide. Extraction with diethyl ether (3 x 50 ml) was followed by concentration and then silica gel chromatography with hexanes/dichloromethane as an eluent to give 0.35 g (91%) of **1**, mp 69-70° (from hexanes); <sup>1</sup>H nmr (deuteriochloroform, 35°):  $\delta$  3.04 (d, J = 5.0 Hz, 3H, NHCH<sub>3</sub>), 5.14 (broad s, 1H, exchangeable with deuterium oxide, NHCH<sub>3</sub>), 6.54 (dd, J = 3.6 and 1.6 Hz, 1H, H4'), 6.80 (d, J = 5.2 Hz, 1H, H5), 7.15 (dd, J = 3.6 and 0.8 Hz, 1H, H3'), 7.56 (dd, J = 1.6 and 0.8 Hz, 1H, H5'), 8.32 (d, J = 5.2 Hz, 1H, H6).

Anal. Calcd. for C<sub>9</sub>H<sub>9</sub>N<sub>3</sub>O: C, 61.70; H, 5.18; N, 23.99. Found: C, 61.59; H, 5.14; N, 24.07.

### 4-(2'-Furyl)-2-(dimethylamino)pyrimidine **2**.

A similar treatment of 2-chloro-4-(2'-furyl)pyrimidine [**7**] with dimethylamine followed by a similar workup gave compound **2** in an 84% yield, mp 48-49° (from hexanes); <sup>1</sup>H nmr (deuteriochloroform, 35°):  $\delta$  3.24 [s, 6H, N(CH<sub>3</sub>)<sub>2</sub>], 6.53 (dd, J = 3.6 and 1.6 Hz, 1H, H4'), 6.85 (d, J = 5.2 Hz, 1H, H5), 7.19 (dd, J = 3.6 and 0.8 Hz, 1H, H3'), 7.56 (dd, J = 1.6 and 0.8 Hz, 1H, H5'), 8.39 (d, J = 5.2 Hz, 1H, H6).

Anal. Calcd. for C<sub>10</sub>H<sub>11</sub>N<sub>3</sub>O: C, 63.47; H, 5.86; N, 22.21. Found: C, 63.39; H, 6.02; N, 21.92.

### <sup>1</sup>H NMR.

All spectra were taken on a Varian VXR-400 (400 MHz) spectrometer in 20 micromolar solutions. The 90° pulse width (pw 90) determination, relaxation time ( $T_1$ ) determination and accumulation of NOESY spectra were done without sample spinning to reduce deviations in the field homogeneity. The pw 90 and  $T_1$  determinations were conducted with **1** in acetone-d<sub>6</sub> at -70°. The pw 90 was found to be 17 microseconds and the average  $T_1$  of the aromatic protons in **1** was 2.6 ± 0.7 seconds. These values and optimized spectral widths were used in setting up subsequent NOESY accumulations for **1** and **2** in the phase sensitive mode. The relative abundance of the *s-cis* conformation was estimated by integration of the nOe cross peak corresponding to the interaction between H5 of the pyrimidine and H3' of the furan and normalized against the H5-H6 cross peak of the pyrimidine according to equation (1).

$$\eta_{H5-H6} \times (r_{H5-H6} / r_{H5-H3'})^6 = \eta_{(H5-H3')} 100\% \quad (1)$$

In this equation,  $\eta_{H5-H6}$  is the volume of the nOe cross peak between H5 and H6,  $r_{H5-H6}$  is the distance between H5 and H6,  $r_{H5-H3'}$  is the distance between H5 and H3', and  $\eta_{(H5-H3')} 100\%$  is the estimated nOe cross peak volume for the interaction between H5 and H3' if the molecule of **1** were 100% in the *s-cis* conformation [8]. The inter-proton distances were taken from the PM3 optimized geometry. The free energy difference for the *s-cis* and *s-trans* conformations was calculated from equation (2),

$$\Delta G^\circ = -RT \ln K_{eq} \quad (2)$$

where R is the gas constant, T is the coalescence temperature in K and  $K_{eq}$  is the equilibrium constant for the conformational interconversion.

### Computations.

Computations were run on an ESV 10/33 workstation using an integrated SYBYL 6.03 package (Tripos Associates, Inc.). The computations were conducted with the program MOPAC 5.0, as a constituent of the Advanced Computation Module of SYBYL, and by using the PM3 Hamiltonian. Low energy conformations were achieved under full geometry optimization with a gradient norm less than  $0.1 \text{ kcal}\cdot\text{mol}^{-1}\cdot\text{\AA}^{-1}$ . To investigate the rotational energy barriers, 1296 conformations were generated by a stepwise rotation of  $10^\circ$  around the interring C4-C2' ( $\tau_1$ ) and C2-NHCH<sub>3</sub> ( $\tau_2$ ) bonds. Each of these conformations was optimized using a PM3 method over all internal coordinates except for the two that define the relative orientation of both the 2-furyl and methylamino substituents (gradient norm  $<0.1 \text{ kcal}\cdot\text{mol}^{-1}\cdot\text{\AA}^{-1}$ ). Molecular dynamics experiments were performed under constant temperature and volume conditions with the following setup: Tripos force field was applied as the minimization procedure, charges were calculated as described [9], dielectric constants were within the range of  $21 < \epsilon < 78$ , simulations time of 400 picoseconds with a step of 1 femtosecond, and temperatures of 203 and 318 K.

### Acknowledgements.

This study was supported by grants of the Research Corporation, USA, the National Institutes of Health, USA (AI 27196), and the Polish State Committee for Scientific Research (6-P206-008-07, 1994-1996). Mr. A. J. Bojarski is a fellowship

holder of the Foundation for the Polish Science (1995). The ESV 10/33 workstation including SYBYL 6.03, was obtained with a partial support from the Foundation for the Polish Science equipment grant (J. L. M.) to the Institute of Pharmacology.

### REFERENCES AND NOTES

- [1] J. L. Mokrosz, L. Strekowski, B. Duszyńska, D. B. Harden, M. J. Mokrosz, and A. J. Bojarski, *Pharmazie*, **49**, 801 (1994).
- [2] M. J. Mokrosz, L. Strekowski, W. X. Kozak, B. Duszyńska, A. J. Bojarski, A. Kłodzińska, A. Czarny, M. T. Cegła, A. Dereń-Wesołek, E. Chojnacka-Wójcik, and J. L. Mokrosz, *Arch Pharm.-Pharm. Med. Chem.*, **328**, 659 (1995).
- [3] L. Strekowski, F. A. Taniou, S. Chandrasekaran, R. A. Watson, and W. D. Wilson, *Tetrahedron Letters*, **27**, 6045 (1986).
- [4] H. S. Gutowsky and H. C. Holm, *J. Chem. Phys.*, **25**, 1228 (1956).
- [5] S. Glasstone, K. J. Klaidier, and H. Eyring, *Theory and Rate Processes*, McGraw-Hill, New York, 1941.
- [6] M. Oki, *Applications of Dynamic NMR Spectroscopy to organic Compounds*, VCH Publishers, Deerfield Beech, 1985.
- [7] L. Strekowski, D. B. Harden, W. B. Grubb, S. E. Patterson, A. Czarny, M. J. Mokrosz, M. T. Cegła, and R. L. Wydra, *J. Heterocyclic Chem.*, **27**, 1393 (1990).
- [8] A. E. Derome, *Modern NMR Techniques for Chemistry Research*, Pergamon Press, New York, 1987.
- [9] SYBYL - Theory Manual, Tripos Associates Inc., St. Louis, USA, 1992, p 2066.