Fused Pyrimidines. 5. Methylated Pyrimido[4,5-*d*]pyrimidines [1]

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The Mannich reaction has been applied to pyrimidines possessing activating groups at positions 2, 4, and 6 and that have a methyl group attached at a ring nitrogen atom. The corresponding tetrahydropyrimido[4,5-*d*]pyrimidines were obtained. In one case there was a possibility of isomer formation. Chemical reactivity was not successful in determining the structure but through an nmr program called INAPT, a version of INEPT, we were able to assign the structure as that of **6**.

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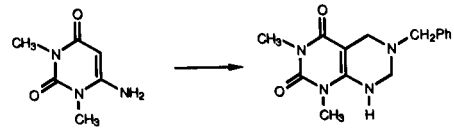
The pyrimido[4,5-*d*]pyrimidine ring system is isomeric with the pteridine ring and, therefore, is of interest as a model for biologically active compounds. The Mannich reaction serves as a convenient method for the formation of tetrahydro derivatives of this ring system, which may then serve as tetrahydrofolate analogs.

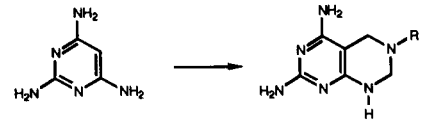
The first example of the preparation of a pyrimido[4,5-*d*]pyrimidine using a pyrimidine precursor in the Mannich reaction involved treatment of 6-amino-1,3-dimethyluracil, **1**, with aqueous formaldehyde and benzylamine [2]. The expected tetrahydropyrimido[4,5-*d*]pyrimidine, **2**, a compound with antidepressant properties, was readily obtained. We have also been interested in the use of the Mannich reaction with pyrimidines as a means of obtaining tetrahydropyrimido[4,5-*d*]pyrimidines [3,4]. Our previous efforts have been directed at obtaining 6-substituted-tetrahydropyrimido[4,5-*d*]pyrimidines, **4**, from formaldehyde, a variety of aralkylamines and 2,4,6-triaminopyrimidine, **3**. This pyrimidine was chosen because (a) the structure of the final product is unambiguous, that is, cyclization involving either the 4- or 6-amino groups gives the same product and (b) the resulting 2,4-diamino fused pyrimidines are related to biologically important molecules.

We decided to examine pyrimidine precursors that could afford isomeric products when the direction of cyclization could follow different pathways as well as extend the scope of this reaction. A simple example of this is a pyrimidine that is methylated on a ring nitrogen. In this report we describe the results of our initial efforts to study this cyclization, including the characterization of the product from the reaction of 2,4,6-triamino-1-methylpyrimidinium iodide, **5**, with methylamine and formaldehyde as well as the synthesis of two other ring *N*-methylatedpyrimido[4,5-*d*]pyrimidines.

Compound **5** was readily prepared from **3** by methylation using methyl iodide in refluxing methanol [5]. The location of the tautomeric imino group in **5** was not a factor since the compound was isolated and used as the hydroiodide salt. Treatment of **5** with aqueous formaldehyde and methylamine could lead either to the 1-methyl derivative, **6**, and/or to the 3-methyl derivative, **7**, depending on the direction of cyclization. However, only one product was isolated, in 78% yield, which had the expected composition of a Mannich product. There did not seem to be an *a priori* rationale for predicting the formation of one isomer over the other and so we sought chemical evidence for support of the correct structure. It is well documented that hydrolysis of amino groups on heterocyclic rings is a common route in the preparation of oxo heterocycles [6] and this hydrolysis reaction has been systematically studied [7]. We envisioned, therefore, that selective hydrolysis of the amino group at position 4 would give either structure **8** or **9**.



1 **2**

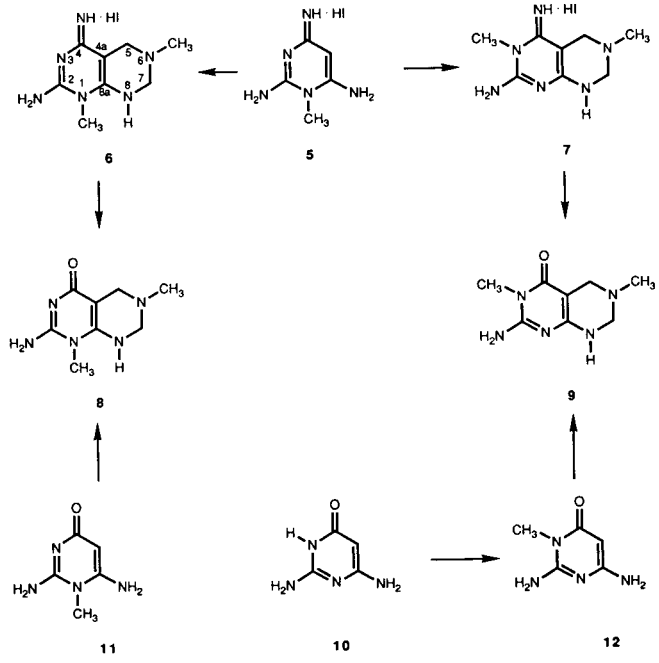


3 **4**

Before carrying out the hydrolysis reaction we prepared the two possible products, **8** and **9**, that would result from a selective hydrolysis. It is known that 2,4-diamino-6(1*H*)-oxopyrimidine, **10** can be methylated to give **12** [9]. Compound **11**, on the other hand, is derived from a primary synthesis involving methylguanidine and ethyl cyanoacetate [8]. These compounds were prepared according to the

literature procedures and each of these *N*-methylpyrimidines were subjected to the Mannich reaction conditions and resulted in the formation of **8** and **9**, respectively. These new fused pyrimidines were characterized by ^1H and ^{13}C nmr data and elemental analysis. With the two possible hydrolysis products in hand for comparison purposes we then subjected **6/7** to hydrolytic conditions that have been used to convert amino pyrimidines into oxo pyrimidines. Boiling 2 *N* sodium hydroxide has been used to convert 4-aminopyrimidines and 4-amino fused pyrimidines into the corresponding oxo derivatives without affecting the 2-amino group [7]. When compound **6/7** was treated with 2 *N* sodium hydroxide at reflux for periods of up to two days, the starting material was recovered unchanged. Not only did the amino/imino group resist hydrolysis, but the reduced pyrimidine ring portion of the molecule, which may be viewed as an aminal, was stable as well. In order to ascertain that feature of the molecule responsible for this behavior, the pyrimidine, **5**, was also subjected to reflux with 2 *N* sodium hydroxide for 4 hours and to heating with 50% aqueous hydrogen bromide at 100° for 2 hours. Acidic conditions are usually more harsh, with the 4-amino group undergoing hydrolysis first, followed by the 2-amino group [7]. In both instances the starting material was recovered, indicating that neither hydrolysis of the amino group nor rearrangement of the ring methyl group had occurred. Under alkaline conditions Dimroth rearrangements to give exocyclic alkylamino substituents frequently occur [10].

With the failure of this straightforward chemical resolution to the question of the structure of the product, **6** or **7**, we turned to spectroscopy in order to make this determination. A recently described nmr technique, called INAPT [11-13], was employed to distinguish between structures **6** and **7**. INAPT is a selective INEPT experiment that applies soft proton pulses to a specific proton resonance during the acquisition of a ^{13}C decoupled spectrum. This pulsing transfers proton magnetization to carbons no further than three bonds away. The results give long-range J-connectivities of protons and carbons separated by two and three bonds. The critical point in the analysis is the relationship of the original ring methyl group to one of the quaternary carbon atoms. This relationship was chosen because this technique is particularly effective for nonprotonated carbons. The protons of a methyl group at position 1 would be three bonds away from C-8a while the protons of a methyl group at position 3 would be five bonds distant (see the numbering system used on structure **6**). Therefore, we focused our attention on C-8a. Analysis of the ^1H nmr spectrum of **6/7** showed that the signals for the H-7 protons (at 4.00 ppm) were sufficiently isolated from those at position 5 (3.37 ppm) to allow their use in the



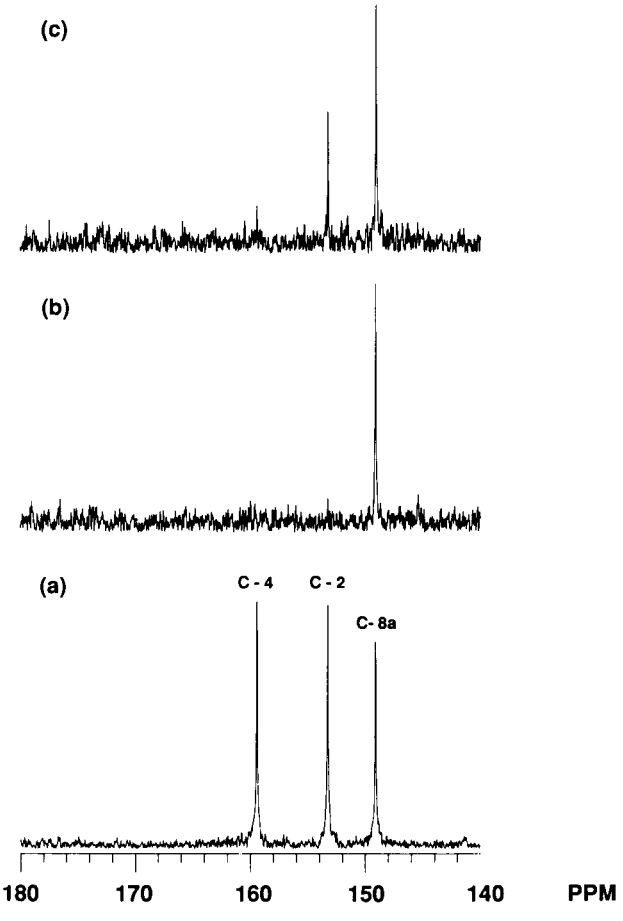


Figure 1. Spectra of **6** in DMSO-d_6. (a) Proton-decoupled ^{13}C nmr spectrum showing the main sp^2 carbon signals; (b) INAPT spectrum obtained by transfer from H-7 protons; (c) INAPT spectrum obtained by transfer from N-1-methyl protons.

INAPT experiment. The initial assignments of both proton and carbon signals is based on our earlier work [3,4] and on a recent detailed spectroscopic study of similar molecules [14].

The INAPT experiment carried out on **6**/**7** gave the results shown in Figure 1. Three clear resonances were observed for the critical sp^2 carbons in the decoupled ^{13}C spectrum (Figure 1a). Irradiation of the protons at H-7 left only one signal, which was assigned to the carbon at C-8a because of the limit of three bonds (Figure 1b). The carbons at C-2 and C-4 are five bonds away from the protons at position 7. Subsequent irradiation of the ring methyl group at 3.28 ppm, which could be located at either position 1 or position 3, resulted in two carbon signals, one attributed to C-8a and the other to C-2 (Figure 1c). Both of these carbons are three bonds from the methyl protons while C-4 is five bonds away. These results support the assignment of the structure of the product as that of compound **6**. The carbon signals for the 3-methyl compound, **7**, would not have included that of C-8a but rather the two signals for C-2 and C-4. The corresponding INAPT experiment was performed with **8**, the structure of which is unambiguous, and gave results consistent with those described above.

No clear explanation of the regiochemical cyclization to give exclusively (or nearly so) compound **6** can be offered at this time. This observation is currently under investigation.

EXPERIMENTAL

The ^1H and ^{13}C nmr spectra were recorded in DMSO-d_6 with TMS as the internal standard or in deuterium oxide with DSS as the internal standard on a QE-300 NMR spectrometer, at 300 MHz. For the INAPT experiments the following parameters were used: P1 = 16.10 μsec(180°), P2 = 8.05 μsec(90°), D3 = 25 msec, D5 = 1 sec, D6 = 11.90 msec. The purity of all new compounds was judged to be ≥95% by ^1H nmr and hplc. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. A DuPont TGA 2950 Thermogravimetric Analyzer was used to determine the amount of water in the analytical samples.

General Method for the Mannich Reaction.

A mixture of two equivalents of aqueous methylamine (40%) and two equivalents of formalin (37%) was stirred at room temperature for 15 minutes. After addition of methanol, one equivalent of the appropriate pyrimidine was added and the resulting mixture refluxed overnight.

2-Amino-1,4,5,6,7,8-hexahydro-4-imino-1,6-dimethylpyrimido[4,5-*d*]pyrimidine Hydroiodide (**6**).

After cooling the reaction mixture to room temperature the precipitate was filtered to give 78% white solid. Recrystallization from water gave pure **6**; ^1H nmr (DMSO-d_6): δ 2.31 (s, 3H, 6-N-CH_3), 3.28 (s, 3H, 1-N-CH_3), 3.37 (s, 2H, 5-CH_2), 4.00 (s, 2H, 7-CH_2), 6.99 (s, 2H, 2-NH_2), 7.44 (s, 2H, 4-NH_2), 7.80 (s, 1H, 8-N*H*); ^{13}C nmr (DMSO-d_6): δ 31.71 (6-N-*C*H_3), 40.27 (1-N-*C*H_3), 48.36 (5-*C*H_2), 62.92 (7-*C*H_2), 79.97 (4a-*C*), 149.07 (8a-*C*), 153.23 (2-*C*-NH_2), 159.38 (4-*C*-NH_2).

Anal. Calcd. for $C_8H_{15}IN_6 \cdot 0.5H_2O$: C, 29.02; H, 4.87; N, 25.38. Found: C, 29.21; H, 4.85; N, 25.34.

2-Amino-1,4,5,6,7,8-hexahydro-1,6-dimethyl-4(3*H*)-oxopyrimido-[4,5-*d*]pyrimidine (**8**).

After removing the solvent, the residue was washed with methanol/methylene chloride (2:8) to yield 34% of white solid. Recrystallization from ethanol/water gave pure **8**; ^1H nmr (deuterium oxide): δ 2.63 (s, 3H, 6-N-CH_3), 3.37 (s, 3H, 1-N-CH_3), 3.71 (s, 2H, 5-CH_2), 4.27 (s, 2H, 7-CH_2); ^{13}C nmr (deuterium oxide): δ 33.77 (6-N-*C*H_3), 41.76 (1-N-*C*H_3), 51.16 (5-*C*H_2), 65.12 (7-*C*H_2), 89.23 (4a-*C*), 151.72 (8a-*C*), 157.64 (4-*C*=O), 173.02 (2-*C*-NH_2).

Anal. Calcd. for $C_8H_{13}N_5O \cdot 1.25H_2O$: C, 44.12; H, 7.25; N, 32.17. Found: C, 44.40; H, 6.66; N, 32.24.

2-Amino-3,4,5,6,7,8-hexahydro-3,6-dimethyl-4(3*H*)-oxopyrimido-[4,5-*d*]pyrimidine (**9**).

The reaction mixture was filtered hot and the filtrate evaporated to give a solid residue. Absolute ethanol was added to the residue, heated to reflux, filtered hot, and the organic solution reduced in volume. After cooling in the refrigerator, the precipitate was filtered to give 56% of white solid. Recrystallization from absolute ethanol gave pure **9**; ^1H nmr (deuterium oxide): δ 2.32 (s, 3H, 6-N-CH_3), 3.30 (s, 3H, 3-N-CH_3), 3.39 (s, 2H, 5-CH_2), 4.01 (s, 2H, 7-CH_2); ^{13}C nmr (deuterium oxide): δ 30.67 (6-N-*C*H_3), 42.31 (3-N-*C*H_3), 51.27 (5-*C*H_2), 65.19 (7-*C*H_2), 85.27 (4a-*C*), 157.60 (8a-*C*), 159.80 (2-*C*-NH_2), 164.41 (4-*C*=O).

Anal. Calcd. for $C_8H_{13}N_5O$: C, 49.22; H, 6.71; N, 35.87. Found: C, 49.37; H, 6.91; N, 35.58.

Acknowledgements.

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REFERENCES AND NOTES

[1] Presented, in part, at the 13th International Congress of Heterocyclic Chemistry, Corvallis, OR, USA, August 1991.

[2] J. L. Bernier, A. Lefebvre, C. Lespagnol, J. Navarro, and A. Perio, *Eur. J. Med. Chem.-Chim. Ther.*, **12**, 341 (1977).

[3] T. J. Delia and S. M. Sami, *J. Heterocyclic Chem.*, **18**, 929 (1981).

[4] T. J. Delia, D. D. Kirt, and S. M. Sami, *J. Heterocyclic Chem.*, **20**, 145 (1983).

[5] D. J. Brown and N. W. Jacobson, *J. Chem. Soc.*, 3172 (1962).

[6] D. J. Brown, The Pyrimidines, Interscience, New York, NY, 1962, pp 229-231; see also Supplements I and II.

[7] R. B. Trattner, G. B. Elion, G. H. Hitchings, and D. M. Sharefkin, *J. Org. Chem.*, **29**, 2674 (1964).

[8] C. R. Frihart, A. M. Feinberg, and K. Nakanishi, *J. Org. Chem.*, **43**, 1644 (1978).

[9] H. Rokos and W. Pfleiderer, *Chem. Ber.*, **104**, 739 (1971).

[10] See reference [6], pp 373-374; see also Supplements I and II.
[11] A. Bax, *J. Magn. Reson.*, **57**, 314 (1984).
[12] A. Bax, J. A. Ferritti, N. Nashad, and D. M. Jerina, *J. Org. Chem.*, **50**, 3029 (1985).
[13] R. B. Taylor, D. G. Corley, M. S. Tempesta, Z. T. Fomum, J. F. Ayafor, J. I. Wandji, and P. N. Ifeadike, *J. Nat. Prod.*, **49**, 670 (1986).
[14] D. Gravier, J.-P. Dupin, F. Casadebaig, G. Hou, M. Petraud, J. Moulines, and B. Barbe, *J. Heterocyclic Chem.*, **28**, 391 (1991).