Organic Process Research & Development **1997**, *1,* 85–87

A Practical Synthesis of “Metabolite A_1” (AAMU) of Caffeine

D. Gala,*[,†] D. DiBenedetto,[†] F. Günter,[‡] M. Kugelman,[†] D. Maloney,[†] M. Cordero,[†] and I. Mergelsberg[‡]

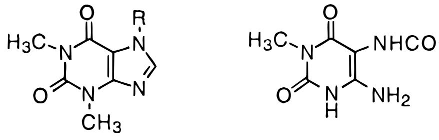
Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, New Jersey 07003, and Werthenstein Chemie, CH-6105, Schachen, Switzerland

Abstract:

A three-pot, preparative scale synthesis of 5-acetamido-6-amino-3-methyluracil, “metabolite A_1” (AAMU) (2) of caffeine, from readily available 6-aminouracil (3) in 72% overall yield is described.

Introduction

Xanthines such as caffeine and theophylline are of tremendous current interest due to their pharmacological properties. Their mode of action and metabolism have been of considerable research interest.[1] A convenient, short synthesis for the major metabolite of caffeine, previously identified as “metabolite A_1” (**2**),[2] and subsequently referred to as “AAMU” [1] (Figure 1), is of interest as it would make this compound readily available for more detailed studies. In the past, small amounts of this compound have been prepared by a multistep synthesis in moderate yields.[3] During the course of our work on pyrimidines, we have devised an efficient, convenient synthesis of this metabolite starting from commercially available uracil derivative **3** (Scheme 1). Note that an efficient *in situ* trapping of the diamine **6** and elimination of the cyclization of **2** to its xanthine analog were crucial in securing high yields for this synthesis. This paper describes our findings which have been used to prepare kilo size batches[4] of **2**. From commercially available radiolabeled compounds, this synthesis allows for a practical introduction of radiolabels at the methyl and/or each atom of the acetyl group of **2**, which is important for the metabolism research.



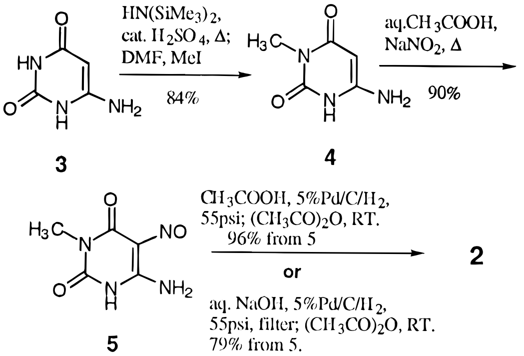
1 **2**

(a) R = CH_3 :Caffeine
(b) R = H :Theophylline

'Metabolite A_1'
(AAMU)

Figure 1.

Scheme 1



Results and Discussion

With an efficient chemistry (described later) for the conversion of nitrosopyrimidine **5**[5] to **2** at hand, attention was focused on a practical synthesis of this key intermediate **5**. Several publications[5] have described its synthesis; however, during the course of our work, a publication[6] describing a selective N-methylation of commercially available, inexpensive 6-aminouracil (**3**) to 3-*N*-methyl-6-aminouracil (**4**) appeared to be the most attractive route for the eventual preparation of large amounts of **5**. After some optimization, and minor modifications to render it practical, a synthesis suitable for a large scale preparation of compound **4** was instituted, and it is described in the Experimental Section. In short, on the basis of its efficiency, homogeneity,[7] and reproducibility, concd H_2SO_4 was a more suitable catalyst than $(NH_4)_2SO_4$ for a large scale silylation reaction. After the removal of most of the silylating reagent, DMF as a solvent (i) allowed for a complete removal of the residual silylating reagent and its byproducts and (ii) improved the solubility of the reactants, facilitating an efficient stirring, which ensured a complete methylation reaction with methyl iodide. In the course of this work an 84% yield for this conversion was maintained.[6]

Practical changes for a large scale preparation and isolation of **5** were also needed. Compound **5** is insoluble in most of the organic solvents, and during its synthesis under

---

[†] Schering-Plough Research Institute.

[‡] Werthenstein Chemie.

(1) (a) Carrillo, J. A.; Benitez, *J. Clin. Pharmacol. Ther.* **1994**, *55*, 293. (b) Rodopouloa, N.; Noemn, A. *Scand. J. Clin. Lab. Invest.* **1994**, *54*, 305. (c) Karlsson, J.-A.; Kjellin, G.; Persson, G. A. *J. Pharm. Pharmacol.* **1982**, *34*, 788. (d)Peet, N. P.; Lentz, N. L.; Meng, E. C.; Dudley, M. W.; Odgen, A. L.; Demeter, D. A.; Weintraub, J. R.; Bey, P. *J. Med. Chem.* **1990**, *33*, 3127. (e) Shimada, J.; Suzuki, F.; Nonaka, H.; Ishii, A. *J. Med. Chem.* **1992**, *35*, 924.

(2) (a) Branfman, A. R.; McComish, M. F.; Bruni, R. J.; Callahan, M. M.; Robertson, R.; Yesair, D. W. *Drug Metab. Dispos.* **1983**, *11*, 206. (b) Tang, B. K.; Grant, D. M.; Kalow, W. *Ibid.* **1983**, *11*, 218.

(3) (a) Bredereck, H.; Hennig, I., Pfleiderer, W. *Chem. Ber.* **1953**, *86*, 321, 333. (b) Fink, K.; Adams, W. S.; Pfleiderer, W. *J. Biol. Chem.* **1964**, *239*, 4250. (c) Pfleiderer, W. *Chem. Ber.* **1957**, *90*, 2272.

(4) In this paper, the laboratory procedures for the preparation of ≥100 g of the final product are described. Where applicable, the information that would facilitate the preparation of kilos of these intermediates/product is abbreviated in the footnotes (7, 10, 13, and 14).

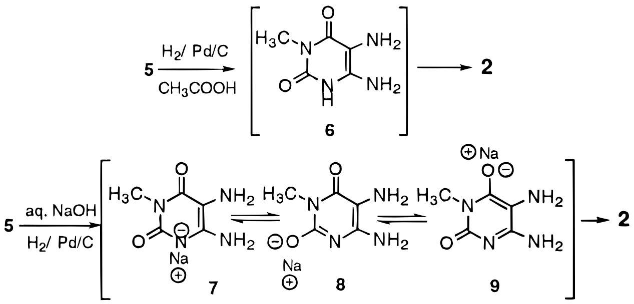
(5) (a) Wright, C. E. *J. Heterocycl. Chem.* **1976**, *13*, 539. (We thank Dr. B. Neustdat for this reference.) (b) Salas-Peregrin, J. M.; Moreno-Carretero, M. N.; Colacio-Rodriguez, E. *Can. J. Chem.* **1985**, *63*, 3573. (c) Zvilichovsky, G.; Feingers, J. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1507.

(6) Müller, C. E. *Tetrahedron Lett.* **1991**, *32*, 6539. The use of dimethyl sulfate in place of MeI led to a mixture of products resulting in a poor yield.The regioselectivity of this methylation reaction was confirmed by the selective INEPT ^{13}C-NMR method of Osterman et al.: Osterman, R. M.; McKittrick, B. A.; Chan, T,-M. *Tetrahedron Lett.* **1992**, *33*, 4867.

(7) Solid sulfates, e.g., $(NH_4)_2SO_4$, stay out of solution, rendering a complete silylation difficult on a large scale.

S1083-6160(96)00010-2 CCC: $14.00

Scheme 2



the standard conditions for nitrosation[5] large chunks (of **5** plus unreacted **4**) formed. This made monitoring this reaction and driving it to completion difficult. This was overcome by first refluxing the aqueous suspension of **4** to form a fine suspension, followed by a slow addition of aqueous $NaNO_2$ solution to the reaction mixture, which avoided the clumping.

The catalytic reduction[8] of **5** to 5,6-diamino-3-methyluracil (**6**) (Scheme 2) was first investigated in several solvents (e.g., DMF, DMA, THF, etc.). In all cases this reaction was incomplete after a prolonged period of time probably due to the sparing solubility of **5** in the above solvents.[9] On the other hand, a complete reduction of **5** was achieved in acetic acid (method A). Routine workup of this reaction (i.e., the removal of catalyst by filtration through a Celite pad) followed by acylation of **6** with Ac_2O in the filtrate led to a poor yield (22%) of **2**. It was conceivable that either diamine **6** and/or **2** (or its xanthine analog)[10] could remain with the catalyst on the Celite pad in precipitated form. Repeated extractions of the catalyst with hot acetic acid led to the recovery of **2**, thus raising the yields of this reaction to 95%.

The problems associated with either the adsorption or the lack of solubility of these compounds were circumvented by using an alternate basic reducing condition (method B) which utilized the fact that sodium salt **5** is soluble enough to allow reduction whereas **6** is fairly soluble in water. Thus, after the completion of the reduction, the catalyst was removed by filtration, leaving the sodium salt of diamine **6** (presumably in its salt forms **7**−**9,** Scheme 2) in the aqueous phase, which was acylated without isolation to obtain only the desired product. The lower yield in this case, compared to the acetic acid route described above (79% vs 95%), may be due to the adsorption of the intermediates (**6**−**9**) on the catalyst and/or due to the subsequent acylation at any of the five possible positions of pyrimidine (two amine nitrogens plus one amide nitrogen, and two oxygen atoms shown in structures **7**−**9**),[11] none of which precipitated with the product. For the incorporation of the radiolabel in the acetyl moiety, method B is more attractive than method A as it leads to less "radio-contaminated material", thus minimizing special handling required for the radiolabeled materials.

Conclusions

Efficient methods for the preparation of the major metabolite of caffeine, "metabolite A_1" (AAMU, or 5-acetamido-6-amino-3-methyluracil), from commercially available 6-aminouracil have been developed.

Experimental Section

NMRs were recorded on a Varian XL-300 with TMS as internal standard. Melting points were determined with Mel-Temp. The infrared spectra were recorded on a Mattson Galaxy 7000 FTIR. Chemical ionization mass spectra were obtained on an EXTREL 400-1 mass spectrometer.

6-Amino-3-methyluracil (4). To a stirred mixture of 6-aminouracil (**1**) (250 g, 1.97 mol) in hexamethyldisilazane (HMDS) (1.15 L, 5.45 mol) at rt was added concd H_2SO_4 (2.5 mL). The mixture was stirred at rt for an additional 5 min, and then heated to reflux (bath temperature 140 °C) for 6 h (complete consumption of **3** as judged by formation of a nearly clear solution). The reaction mixture was then subjected to a vacuum distillation (~48 °C, 100−75 mbar) to remove volatiles (~350 mL). The residue in the reaction flask was diluted with DMF (1.35 L), stirred for 10 min, and subjected to vacuum distillation as above to collect additional volatiles (100 mL) to remove most of the remaining HMDS (monitored with GC). The flask was then cooled to rt, treated with CH_3I (400 mL, 912 g, 6.4 mol), and stirred at room temperature for 48 h for complete reaction (HPLC, Brownlee Spheri-5 RP-18, 254 nm, 0.8 mL/min; mobile phase, 4.5 g of $(Me)_4NOH{\cdot}5H_2O$ in H_2O (1 L) adjusted to pH 6 with H_3PO_4).

Next, to the cooled (0 °C) reaction mixture was slowly added a saturated $NaHCO_3$ solution (2.5 L) while the reaction temperature[12] was maintained between 20 and 40 °C. The resultant off-white suspension was cooled to 0−5 °C,filtered, and washed with MeOH (2×300 mL) followed by water (2×500 mL).[13] The product was filtered and dried at 50 °C under a current of N_2 for 2 days to obtain an HPLC corrected (purity 96.7%) yield of 232.2 g (83.7%) of **4** (identical to the one obtained via the method of ref 6).

6-Amino-3-methyl-5-nitrosouracil (5). A stirred suspension of 6-amino-3-methyluracil (**4**) (480 g, 3.4 mol) in water (4.8 L) was refluxed for 3 h (a fine powder formed) and then cooled to rt. To this stirred suspension ws added glacial acetic acid (1.6 L, 30.6 mol). Next $NaNO_2$ (422 g, 6.12 mol) in water (3.5 L) was slowly added to this mixture over 1.5 h while the reaction temperature was maintained at 20 °C. The reaction mixture was stirred for an additional 0.5 h (consumption of **4** as judged by the above HPLC method) and filtered, and the solid was washed with water (4×2 L) and dried in a vacuum oven at 50 °C for 18 h to obtain 481 g (90% yield, corrected for 97.5% HPLC purity) of **5** as a purple solid (identical to the one obtained via the method of ref 5).

---

(8) Chemical reductions of nitrosopyrimidines lead to unstable 5,6-diamines: (a) Phillippossian, G.; Easlon, M. U.S. Patent 4,581,451; 1986. (b) *Chem. Abstr. 96* (17), **1982**, 142571*t*. (c) Muller, C. A. *Synthesis* **1993**, 125.

(9) Catalyst poisoning by diamine **6** remained a distinct possibility at this stage of our work.

(10) In the presence of acetic acid employed for the reduction, the reactive 5-amino group of diamine **6** can form sparingly soluble **2** or may intramolecularly dehydrate to its xanthine derivative (which too is extremely insoluble), either of which can remain with the catalyst on the silica pad during the filtration of the catalyst.

(11) The N vs O acylation/alkylation of amides is a well-precedented phenomenon, e.g.: Comins, D. L.; Jianhua, G. *Tetrahderon Lett.* **1994**, *35*, 2819.

(12) This reaction is quite exothermic in the beginning with evolution of CO_2 gas throughout.

(13) The excess of CH_3I in the filtrate was destroyed (monitor with GC) by stirring with 400 mL of 2-aminoethanol for 24 h.

5-Acetamido-6-amino-3-methyluracil (2). *Method A.*[14] A suspension of **5** (100 g , 0.59 mol) and 5% Pd/C (10 g) in acetic acid (1 L) was hydrogenated in a Parr shaker (55 psi, rt) overnight and then filtered thorough a Celite pad. The Celite pad was washed with acetic acid (125 mL) and saved for further extractions. The filtrate was treated with acetic anhydride (69.5 mL), stirred at rt for 3 h, then heated to 40 °C, and treated with additional acetic anhydride (60 mL) plus a catalytic amount of 4-(dimethylamino)pyridine. After stirring for 18 h, the resultant yellow reaction mixture was subjected to a vacuum distillation to obtain a light tan solid. This solid was stirred at rt in water (150 mL) for 1 h, filtered, and dried in a draft oven (50 °C, 18 h) to obtain 26.25 g (22.5%) of the product.

The Celite pad was washed with 250 mL of MeOH and then extracted with hot (100 °C) acetic acid (8 × 250 mL). Each extract was concentrated to a solid under vacuum. The first seven extracts each gave 12 g, whereas the last one led to 4 g of the product. The combined product was stirred at rt for 1 h in water (350 mL), filtered, and dried as above to obtain 85.4 g (73.2%) of additional light tan **2**. Thus a total of 111.6 g of **2** represents a 95.7% yield.

An analytical sample was prepared by suspending 0.63 g of the above sample in 40 mL of hot (100 °C) acetic acid, followed by treatment with 0.1 g of charcoal. After the filtration of charcoal, the amount of acetic acid was reduced under vacuum to 15 mL and the resultant light tan solution was cooled to 0 °C. Next, 25 mL of MeOH was added to this cold solution. This led to a white solid, which was filtered, washed with MeOH, water, and hexanes and then dried as above to obtain 0.3 g (47%) of the product, mp $>$300 °C. MS (EI): 198 (M^+). IR (Nujol): ν 1710, 1660, 1640 cm^{-1}. ^{1}H-NMR (d_6-DMSO): δ 1.95 (s, 3H, $COCH_3$), 3.1 (s, 3H, *N*-CH_3), 6.0 (s, 2H, NH_2), 8.4 (s, 1H, CONH), 10.45 (s, 1H, CONH). ^{13}C-NMR (d_6-DMSO): δ 22.6, 26.3, 86.9, 149.8, 150, 160.8, 170.5. Anal. Calcd for $C_7H_{10}N_4O_3 \cdot 1.25H_2O$: C, 38.1; H, 5.7; N, 25.4. Found: C, 38.5; H, 5.6; N, 25.3.

Method B. A mixture of **5** (1 g, 5.88 mmol) and 5% Pd/C (0.1 g) in 1 N aqueous NaOH (6.5 mL) was hydrogenated (55 psi, rt) overnight in a Parr shaker and then filtered through a Celite pad. The Celite pad was washed with a small amount of water. The combined filtrate plus washes was cooled to 0 °C with stirring. Next acetic anhydride (2 mL) was added, and the mixture was stirred at rt for 1.5 h. The resultant light yellow solid was filtered, washed with water followed by hexanes, and dried in a draft oven (50 °C, 18 h) to obtain 1.09 g (79% yield) of the product as a dihydrate.

Received for review October 29, 1995.⊗

OP960010D

---

(14) On a kilo scale this procedure was modified as follows to render it more convenient. To a suspension of **5** (2.9 kg, 17 mol) in acetic acid (25 L) was added 50% water wet 5% Pd/C (0.5 kg). This mixture was hydrogenated (55 psi, rt) overnight, and then acetic anhydride (5.41 kg, 53 mol) was added. This mixture was stirred at rt for 14 h, and then water (1.5 L) was added slowly over 1 h. The mixture of the product and Pd/C was filtered through a Celite pad. The Celite pad was extracted with hot (100 °C) acetic acid (4 × 30 L). The extracts were combined, concentrated to ~5 L under vacuum, and then filtered. The solid thus obtained was washed with a small amount of acetic acid and dried in a draft oven (50 °C) to obtain 2.69 kg (80%) of the product as a light tan solid. (Additional extraction could have led to more product.) Further work suggested that a Soxhlet extraction of the product can minimize the labor and the amount of acetic acid needed for this extraction. As a bonus, the product crystallizes out in the receiving flask, making its isolation in a purer form possible. This makes it a true three-pot synthesis.

⊗ Abstract published in *Advance ACS Abstracts,* December 1, 1996.