[Chem. Pharm. Bull. 30(9)3392—3394(1982)]

Improved Synthesis of 3-Methylguanine

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(Received March 16, 1982)

An improved procedure for the synthesis of 3-methylguanine (4) from 2,6-diamino-1-methyl-4-pyrimidone (1) is presented. Elemental analyses and ultraviolet (UV) and nuclear magnetic resonance (NMR) spectral data are presented for the compounds involved in the reaction sequence.

Keywords——3-methylguanine; 4-pyrimidones; Traube synthesis; nitrosation; reduction; ring closure; UV; NMR

3-Methylguanine (4)[1] has been used as a key intermediate for the syntheses of wye[2] and wybutine,[3] the fluorescent minor bases from eukaryotic *t*RNAs^{Phe}. Of the two different syntheses of 4,[1] we considered that the method of Townsend and Robins[1b] was better for a large-scale preparation for economic reasons. They prepared 4 by cyclization of 2,5,6-triamino-1-methyl-4-pyrimidone sulfate ($3 \cdot H_2SO_4$)[4] with $HCONH_2$. Frihart *et al.* reported a modified procedure.[3b] We reexamined the reaction sequence for the synthesis of 4 (Chart 1) and wish to report results which clarify several discrepancies in the literature.[1b,3b,4]

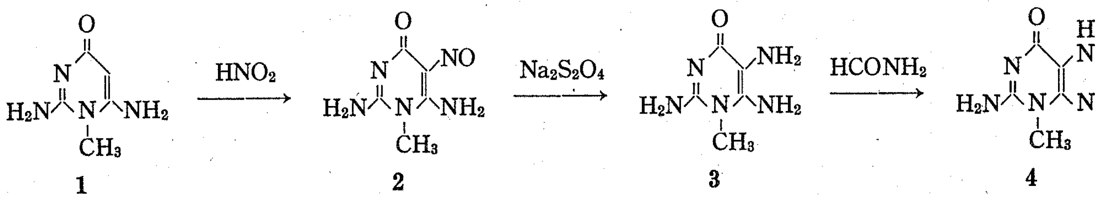

Chart 1

The starting material employed in the synthesis was 2,6-diamino-1-methyl-4-pyrimidone (1), which was first prepared by Roth *et al.*[4] The correct structure was assigned later.[5] The original authors purified 1 as the sulfate salt,[4] whereas Frihart *et al.* stated that the free base (1) had a melting point of 284°C, but did not carry out full characterization of this compound.[3b] We prepared 1 according to Frihart *et al.*[3b] and obtained an analytical sample, mp 276—277°C (dec.), after repeated recrystallizations from H_2O. This sample gave a positive Beilstein test and the elemental analyses were consistent with the hemihydrate of the hemihydrochloride ($1 \cdot 1/2HCl \cdot 1/2H_2O$). This was transformed into the sulfate ($1 \cdot 1/2H_2SO_4 \cdot H_2O$), mp 265—266°C (dec.), after Roth *et al.*[4] When treated with Amberlite IRA-402 (HCO_3^-), $1 \cdot 1/2HCl$ gave the free base (1) as the monohydrate, mp 230—232°C (dec.). The free base (1) was found to be much more soluble in H_2O than the hemihydrochloride ($1 \cdot 1/2HCl$).

Although Roth *et al.* reported 2,6-diamino-1-methyl-5-nitroso-4-pyrimidone (2) as the monohydrate,[4] we obtained 2 as a hemihydrate in 92% yield by a procedure similar to that of Frihart *et al.*[3b] The nuclear magnetic resonance (NMR) spectrum of 2 suggests that 2 is a mixture of tautomers or rotational isomers due to restricted rotation about the pyrimidone–NO bond.[6] Compound 2 was converted into $3 \cdot H_2SO_4$ in 71% yield, in accord with the results in the literature,[3b,4] except that our sample was the monohydrate.

Townsend and Robins obtained the free base (4) by treatment of $3 \cdot H_2SO_4$ with boiling $HCONH_2$ followed by recrystallization from H_2O.[1b] The NMR spectrum of 4 in $(CD_3)_2SO$

was reported by Frihart *et al.* [δ 4.50 (3H, s), 8.08 (2H, br), 8.63 (1H, s)].[3b] The chemical shift (4.50 ppm) seems farther downfield than would be expected for the 3-methyl protons of the free base (**4**). We obtained **4**·$1/5H_2SO_4$ after repeated recrystallizations of the crude product from H_2O. The free base (**4**) [δ 3.54 (3H, s, Me), 6.95 (2H, br, NH_2), 7.85 (1H, s, $C_{(8)}$–H)] was obtainable by neutralization of an aqueous solution (pH 4) of the crude product. The sulfate salt (**4**·$1/2H_2SO_4$) was prepared from **4** according to Elion.[1a] Even the protons of **4**·$1/2H_2SO_4$ were not found to resonate at such low field as reported by Frihart *et al.*[3b] (see "Experimental").

Finally, we found that a crude sample of **4** prepared according to Townsend and Robins[1b] was contaminated by a more polar substance(s). Although this could be removed by treatment with charcoal,[3b] we achieved a better result by lowering the reaction temperature to 150°C.

Thus, we have elaborated the procedure for the synthesis of **4** to make it more convenient and reproducible.

Experimental

Melting points are corrected. Ultravioled (UV) spectra were measured with a Hitachi 320 spectrophotometer using solutions in 95% aq. EtOH, 0.1 N aq. HCl (pH 1), 0.005 M phosphate buffer (pH 7), and 0.1 N aq. NaOH (pH 13). NMR spectra were recorded on a JEOL JNM-FX 100 NMR spectrometer in $(CD_3)_2SO$ at 24.5°C using Me_4Si as an internal standard. The abbreviations br and s denote broad and singlet, respectively. We are indebted to Mr. Y. Itatani and his associates at Kanazawa University for microanalyses and NMR spectroscopy.

2,6-Diamino-1-methyl-4-pyrimidone Hemihydrochloride (1·1/2HCl)——This was prepared from $CH_3NH_2\cdot HCl$ (174 g, 2.58 mol), cyanoguanidine (108 g, 1.28 mol), ethyl cyanoacetate (261 g, 2.31 mol), and CH_3ONa (265 g, 4.90 mol) according to the procedure of Roth *et al.*[4] except for the following work-up. After cooling, the reaction mixture was brought to pH 6 with 10% aq. HCl. The resulting precipitate was collected by centrifugation and recrystallized from boiling H_2O (1 l) to produce **1**·$1/2HCl\cdot 1/2H_2O$ (93.04 g, 24% yield based on ethyl cyanoacetate), mp 265—266°C (dec.). Further recrystallizations from H_2O gave colorless needles, mp 276—277°C (dec.), which were dried over P_2O_5 at 2 mmHg and 110°C for 3 h to give an analytical sample of the same mp. *Anal.* Calcd for $C_5H_8N_4O\cdot 1/2HCl$: C, 37.92; H, 5.41; N, 35.38. Found: C, 37.67; H, 5.66; N, 35.34. This sample regained moisture on exposure to air. *Anal.* Calcd for $C_5H_8N_4O\cdot 1/2HCl\cdot 1/2H_2O$: C, 35.88; H, 5.72; Cl, 10.59; N, 33.47. Found: C, 35.92; H, 5.71; Cl, 10.31; N, 33.39. UV $\lambda_{max}^{H_2O}$ (pH 1) 267 nm (ε 17800); $\lambda_{max}^{H_2O}$ (pH 7) 266 (14600); $\lambda_{max}^{H_2O}$ (pH 13) 266 (14200). NMR δ: 3.29 (s, Me), 4.93 (s, $C_{(5)}$–H).

2,6-Diamino-1-methyl-4-pyrimidone Sulfate (1·1/2H₂SO₄)——This was prepared from **1**·$1/2HCl\cdot 1/2H_2O$ (400 mg) according to Roth *et al.*[4] (248 mg, 50% yield), mp 245—246°C (dec.). Recrystallizations from dilute aq. H_2SO_4 (pH 2) gave colorless plates, which were dried over P_2O_5 at 2 mmHg and 100°C for 4 h then exposed to air until constant weight was reached, giving an analytical sample, mp 265—266°C (dec.). *Anal.* Calcd for $C_5H_8N_4O\cdot 1/2H_2SO_4\cdot H_2O$: C, 28.98; H, 5.35; N, 27.04. Found: C, 28.93; H, 5.35; N, 27.10. UV $\lambda_{max}^{H_2O}$ (pH 1) 267 nm (ε 18000); $\lambda_{max}^{H_2O}$ (pH 7) 266 (14500); $\lambda_{max}^{H_2O}$ (pH 13) 266 (14100).

2,6-Diamino-1-methyl-4-pyrimidone (1)——Amberlite IRA-402 (HCO_3^-) (2 ml) was added to a warm solution of **1**·$1/2HCl\cdot 1/2H_2O$ (632 mg) in H_2O (80 ml). The mixture was poured into a column which was packed with another 2 ml of the same resin. The eluate and H_2O washing (45 ml) of the column were combined and evaporated to dryness *in vacuo* to afford a colorless solid (597 mg, 100% yield), mp 229—230°C (dec.). This was recrystallized from H_2O several times, dried over P_2O_5 at 2 mmHg and 80°C for 7 h, then exposed to air until constant weight was reached, giving colorless needles, mp 230—232°C (dec.). *Anal.* Calcd for $C_5H_8N_4O\cdot H_2O$: C, 37.97; H, 6.37; N, 35.43. Found: C, 37.86; H, 6.42; N, 35.38. UV $\lambda_{max}^{95\%\ EtOH}$ 266 nm (ε 14200); $\lambda_{max}^{H_2O}$ (pH 1) 267 (18000); $\lambda_{max}^{H_2O}$ (pH 7) 266 (14500); $\lambda_{max}^{H_2O}$ (pH 13) 266 (14200). NMR δ: 3.23 (s, Me), 4.74 (s, $C_{(5)}$–H).

2,6-Diamino-1-methyl-5-nitroso-4-pyrimidone (2)——This was obtained as the hemihydrate (lit.[4] monohydrate) (17.11 g, 92% yield) from **1**·$1/2HCl\cdot 1/2H_2O$ (17.48 g, 0.104 mol) according to Frihart *et al.*[3b] except that the reaction mixture was brought to pH 5 before any precipitate appeared. The product was suspended in H_2O (1.9 l) at 40°C and 10% aq. NaOH was added to make a clear solution. The solution was then brought to pH 5 with AcOH. The resulting precipitate was filtered off, washed with H_2O, and dried to give a red solid (15.70 g). A portion of this sample was purified in a similar manner five more times, dried over P_2O_5 at 2 mmHg and 110°C for 4 h, then exposed to air until constant weight was reached to afford an analytical sample, mp>300°C. *Anal.* Calcd for $C_5H_7N_5O_2\cdot 1/2H_2O$: C, 33.71; H, 4.53; N, 39.31. Found: C, 33.84; H, 4.38; N, 39.56. UV $\lambda_{max}^{H_2O}$ (pH 1) 324 nm (unstable); $\lambda_{max}^{H_2O}$ (pH 7) 324 (ε 16000); $\lambda_{max}^{H_2O}$ (pH 13) 303 (14900). NMR δ: 2.87, 2.89, and 2.92 (a total of 3H, s each, Me), 7.26, 8.58, 10.92, and 11.26

(1H, each, br, NH's or NH's and OH('s)).

2,5,6-Triamino-1-methyl-4-pyrimidone Sulfate ($\mathbf{3\cdot H_2SO_4}$)——This was prepared from $2\cdot 1/2H_2O$ (22.74 g, 0.128 mol) according to Frihart *et al.*[3b] as the monohydrate (lit.[4] anhydrous) (24.62 g, 71% yield), mp 240—247°C (dec.). Recrystallizations from H_2O and drying over P_2O_5 at 2 mmHg and 100°C for 4 h gave colorless needles, mp 249—250°C (dec.) (lit.[3b] mp >300°C). Compound $3\cdot H_2SO_4$ was found to be unstable in alkaline solution. Even at pH 7 it decomposes gradually. *Anal.* Calcd for $C_5H_9N_5O\cdot H_2SO_4\cdot H_2O$: C, 22.14; H, 4.83; N, 25.82. Found: C, 22.10; H, 4.81; N, 25.60. UV $\lambda_{max}^{H_2O}$ (pH 1) 264 nm (ε 14700); $\lambda_{max}^{H_2O}$ (pH 7) 286 (11500); $\lambda_{max}^{H_2O}$ (pH 13) 287 (unstable). NMR δ: 3.38 (s, Me).

3-Methylguanine (4)——i) A mixture of $3\cdot H_2SO_4\cdot H_2O$ (37.48 g, 0.138 mol) and $HCONH_2$ (190 ml) was kept at 150°C for 4 h. The resulting precipitate was filtered off after being cooled, then washed successively with H_2O (50 ml) and EtOH (10 ml), and dried to give a pale yellow solid (29.77 g), mp >300°C. This was dissolved in boiling H_2O (4.8 l) and the solution was brought to pH 8 with conc. aq. NH_3 then treated with charcoal. After removal of the charcoal by filtration, the solution was concentrated *in vacuo* to *ca.* 350 ml. The mixture was heated to dissolve the precipitate, then treated again with charcoal. The resulting precipitate (15.44 g) was chromatographically homogeneous, but it gave a different infrared spectrum from that of an analytically pure sample. This product was again dissolved in hot H_2O (360 ml) and treated with charcoal. The resulting precipitate was collected by filtration, washed with H_2O, and dried over P_2O_5 at 2 mmHg and 110°C for 7 h to give **4** (12.99 g, 57% yield), mp >300°C. Recrystallizations from H_2O and drying over P_2O_5 at 2 mmHg and 110°C for 3 h gave an analytical sample as colorless needles, mp >300°C. *Anal.* Calcd for $C_6H_7N_5O$: C, 43.63; H, 4.27; N, 42.41. Found: C, 43.60; H, 4.11; N, 42.23. UV $\lambda_{max}^{95\%\ EtOH}$ 238 nm (ε 8600), 266 (10700); $\lambda_{max}^{H_2O}$ (pH 1) 244 (shoulder) (7500), 263 (10200); $\lambda_{max}^{H_2O}$ (pH 7) 234 (8000), 268 (11100); $\lambda_{max}^{H_2O}$ (pH 13) 272 (13100). NMR: see the text.

The sulfate ($1\cdot 1/2H_2SO_4$) was prepared by recrystallization of **4** from 2 N aq. H_2SO_4,[1a] mp >300°C. NMR δ: 3.60 (3H, s, Me), 8.08 (1H, s, $C_{(8)}$–H).

ii) Compound $3\cdot H_2SO_4\cdot H_2O$ (3.00 g, 0.011 mol) was treated according to the literature.[1b] The crude product (1.85 g) was dissolved in boiling H_2O and the solution was treated with charcoal. It was concentrated to *ca.* 400 ml to deposit a chromatographically pure solid (1.09 g), mp >300°C. For analysis, this was recrystallized from H_2O, dried over P_2O_5 at 2 mmHg and 110°C for 3 h, and exposed to air until constant weight was reached. *Anal.* Calcd for $C_6H_7N_5O\cdot 1/5H_2SO_4\cdot 1/3H_2O$: C, 37.78; H, 4.26; N, 36.71. Found: C, 37.48; H, 4.53; N, 36.62. UV $\lambda_{max}^{95\%\ EtOH}$ 238 nm (ε 8700), 266 (10500); $\lambda_{max}^{H_2O}$ (pH 1) 244 (shoulder) (7500), 263 (10200); $\lambda_{max}^{H_2O}$ (pH 7) 234 (8100), 268 (10900); $\lambda_{max}^{H_2O}$ (pH 13) 272 (12800). NMR δ: 3.55 (3H, s, Me), 7.91 (1H, s, $C_{(8)}$–H).

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