Improved Synthesis of 3-Methylguanine

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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) has been used as a key intermediate for the syntheses of wyd and wybutine, the fluorescent minor bases from eukaryotic tRNAs. Of the two different syntheses of 4, we considered that the method of Townsend and Robins 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·H$_2$SO$_4$) with HCONH$_2$. Frihart et al. reported a modified procedure. We reexamined the reaction sequence for the synthesis of 4 (Chart 1) and wish to report results which clarify several discrepancies in the literature.

![Chart 1](image-url)

The starting material employed in the synthesis was 2,6-diamino-1-methyl-4-pyrimidone (1), which was first prepared by Roth et al. The correct structure was assigned later. The original authors purified 1 as the sulfate salt, 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. We prepared 1 according to Frihart et al. and obtained an analytical sample, mp 276—277°C (dec.), after repeated recrystallizations from H$_2$O. This sample gave a positive Beilstein test and the elemental analyses were consistent with the hemihydrate of the hemihydrochloride (1·1/2HCl·1/2H$_2$O). This was transformed into the sulfate (1·1/2H$_2$SO$_4$·H$_2$O), mp 265—266°C (dec.), after Roth et al. When treated with Amberlite IRA-402 (HCO$_3^-$), 1·1/2HCl 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$_2$O than the hemihydrochloride (1·1/2HCl).

Although Roth et al. reported 2,6-diamino-1-methyl-5-nitroso-4-pyrimidone (2) as the monohydrate, we obtained 2 as a hemihydrate in 92% yield by a procedure similar to that of Frihart et al. 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. Compound 2 was converted into 3·H$_2$SO$_4$ in 71% yield, in accord with the results in the literature, except that our sample was the monohydrate.

Townsend and Robins obtained the free base (4) by treatment of 3·H$_2$SO$_4$ with boiling HCONH$_2$ followed by recrystallization from H$_2$O. The NMR spectrum of 4 in (CD)$_3$SO

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was reported by Frihart et al. \(\delta 4.50 \ (3H, \ s), 8.08 \ (2H, \ br), 8.63 \ (1H, \ s)\).\(^{30}\) 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$_2$SO$_4$ after repeated recrystallizations of the crude product from H$_2$O. The free base (4) \(\delta 3.54 \ (3H, \ s, \ Me), 6.95 \ (2H, \ br, \ NH$_2$), 7.85 \ (1H, \ s, C$_4$-H)\) was obtainable by neutralization of an aqueous solution (pH 4) of the crude product. The sulfate salt (4-1/2H$_2$SO$_4$) was prepared from 4 according to Elion.\(^{14}\) Even the protons of 4-1/2H$_2$SO$_4$ were not found to resonate at such low field as reported by Frihart et al.\(^{30}\) (see “Experimental”).

Finally, we found that a crude sample of 4 prepared according to Townsend and Robins\(^{20}\) was contaminated by a more polar substance(s). Although this could be removed by treatment with charcoal,\(^{30}\) 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. Ultraviolet (UV) spectra were measured with a Hitachi 320 spectrophotometer using solutions in 95% acq. EtOH, 0.1 n acq. HCl (pH 1), 0.005 n phosphate buffer (pH 7), and 0.1 n acq. NaOH (pH 13). NMR spectra were recorded on a JEOL JNM-FX 100 NMR spectrometer in (CD$_3$)$_2$SO at 24.5°C using Me$_4$Si 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 Hemihydrachloride (1-1/2HCl) —— This was prepared from CH$_3$NH$_2$HCl (174 g, 2.56 mol), cyanoacamide (106 g, 1.28 mol), ethyl cyanacetate (261 g, 2.31 mol), and CH$_2$ONa (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% acq. HCl. The resulting precipitate was collected by centrifugation and recrystallized from boiling H$_2$O (1 l) to produce 1-1/2HCl-1/2H$_2$O (93.04 g, 24%, yield based on ethyl cyanacetate), mp 265—266°C (dec.). Further recrystallizations from H$_2$O gave colorless needles, mp 276—277°C (dec.), which were dried over P$_2$O$_5$ at 2 mmHg and 110°C for 3 h to give an analytical sample of the same mp. Anal. Calcd for C$_7$H$_5$N$_4$O$_2$-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$_7$H$_5$N$_4$O$_2$-1/2HCl-1/2H$_2$O: 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 $\lambda_{max}$ (pH 1) 267 nm (e 17800); $\lambda_{max}$ (pH 7) 266 (14600); $\lambda_{max}$ (pH 13) 266 (14200). NMR $\delta$: 3.29 (s, Me), 4.93 (s, C$_4$-H).

2,6-Diamino-1-methyl-4-pyrimidone Sulfate (1-1/2H$_2$SO$_4$) —— This was prepared from 1-1/2HCl-1/2H$_2$O (400 mg) according to Roth et al.\(^4\) (248 mg, 50% yield), mp 245—246°C (dec.). Recrystallizations from dilute aq. H$_2$SO$_4$ (pH 2) gave colorless plates, which were dried over P$_2$O$_5$ 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$_7$H$_5$N$_4$O$_3$-1/2H$_2$SO$_4$-H$_2$O: C, 28.98; H, 5.35; N, 27.04. Found: C, 28.93; H, 5.36; N, 27.10. UV $\lambda_{max}$ (pH 1) 267 nm (e 18000); $\lambda_{max}$ (pH 7) 266 (14500); $\lambda_{max}$ (pH 13) 266 (14100). NMR $\delta$: 3.03 (s, Me).

2,6-Diamino-1-methyl-4-pyrimidone (1) —— Amberlite IRA-402 (HCO$_2$-) (2 ml) was added to a warm solution of 1-1/2HCl-1/2H$_2$O (632 mg) in H$_2$O (80 ml). The mixture was poured into a column which was packed with another 2 ml of the same resin. The eluate and H$_2$O 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$_2$O several times, dried over P$_2$O$_5$ 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$_7$H$_5$N$_4$O$_3$-H$_2$O: C, 37.97; H, 6.37; N, 35.43. Found: C, 37.86; H, 6.42; N, 35.38. UV $\lambda_{max}$ (pH 1) 266 nm (e 14200); $\lambda_{max}$ (pH 7) 267 (18000); $\lambda_{max}$ (pH 7) 266 (14500); $\lambda_{max}$ (pH 13) 266 (14200). NMR $\delta$: 3.23 (s, Me), 4.74 (s, C$_4$-H).

2,6-Diamino-1-methyl-5-nitroso-4-pyrimidine (2) —— This was obtained as the hemihydrate (lit.\(^4\) monohydrate) (17.11 g, 92% yield) from 1-1/2HCl-1/2H$_2$O (17.48 g, 0.104 mol) according to Frihart et al.\(^{30}\) except that the reaction mixture was brought to pH 5 before any precipitate appeared. The product was suspended in H$_2$O (1.9 l) at 40°C and 10% acq. 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$_2$O, and dried to give a red solid (15.70 g). A portion of this sample was purified in a similar manner five times, dried over P$_2$O$_5$ 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$_7$H$_5$N$_4$O$_3$-1/2H$_2$O: C, 33.71; H, 4.53; N, 39.31. Found: C, 33.84; H, 4.38; N, 39.56. UV $\lambda_{max}$ (pH 1) 324 nm (unstable); $\lambda_{max}$ (pH 7) 324 (e 16000); $\lambda_{max}$ (pH 13) 303 (14000). NMR $\delta$: 2.87, 2.89, and 2.92 (a total of 3H, s each, Me), 7.26, 6.88, 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 (3H₂SO₄)—This was prepared from 2·1/2H₂O (22.74 g, 0.128 mol) according to Frihart et al. as the monohydrate (lit. anhydrous) (24.62 g, 71% yield), mp 240—
247°C (dec.). Recrystallizations from H₂O and drying over P₂O₅ at 2 mmHg and 100°C for 4 h gave colorless needles, mp 249—250°C (dec.) (lit. mp >300°C). Compound 3-H₂SO₄ was found to be unstable in alkaline solution.

Even at pH 7 it decomposes gradually. Anal. Calcd for C₃H₅N₂O·H₂SO₄·H₂O: C, 22.14; H, 4.88; N, 25.82. Found: C, 22.10; H, 4.81; N, 25.60. UV ƛₘₐₓ (pH 1) 264 nm (ε 14700); ƛₘₐₓ (pH 7) 286 (15000); ƛₘₐₓ (pH 13) 287 (unstable). NMR δ: 3.38 (s, Me₆).

3-Methylguanine (4)—ii) A mixture of 3-H₂SO₄·H₂O (37.48 g, 0.138 mol) and HCONH₂ (190 ml) was kept at 150°C for 4 h. The resulting precipitate was filtered off after being cooled, then washed successively with H₂O (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 H₂O (4.8 l) and the solution was brought to pH 8 with conc. NH₃, 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 H₂O (360 ml) and treated with charcoal. The resulting precipitate was collected by filtration, washed with H₂O, and dried to give P₂O₅ at 2 mmHg and 110°C for 7 h to give 4 (12.99 g, 57% yield), mp >300°C. Recrystallizations from H₂O and drying over P₂O₅ at 2 mmHg and 110°C for 3 h gave an analytical sample as colorless needles, mp >300°C. Anal. Calcd for C₃H₅N₂O·H₂O: C, 43.63; H, 4.27; N, 42.41. Found: C, 43.60; H, 4.11; N, 42.23. UV ƛₘₐₓ (pH 8) 238 nm (ε 8600), 266 (10700); ƛₘₐₓ (pH 1) 244 (shoulder) (7500), 263 (10200); ƛₘₐₓ (pH 7) 234 (8000), 268 (11000); ƛₘₐₓ (pH 13) 272 (13100). NMR: see the text.

The sulfate (1·1/2H₂SO₄) was prepared by recrystallization of 4 from 2N aq. H₂SO₄, mp >300°C. NMR δ: 3.60 (3H, s, Me₆), 8.08 (1H, s, C₆H₅—H).

ii) Compound 3-H₂SO₄·H₂O (3.00 g, 0.011 mol) was treated according to the literature. The crude product (1.85 g) was dissolved in boiling H₂O 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 H₂O, dried over P₂O₅ at 2 mmHg and 110°C for 3 h, and exposed to air until constant weight was reached. Anal. Calcd for C₃H₅N₂O·1/H₂SO₄·1/3H₂O: C, 37.78; H, 4.26; N, 36.71. Found: C, 37.48; H, 4.53; N, 36.82. UV ƛₘₐₓ (pH 8) 238 nm (ε 8700), 266 (10500); ƛₘₐₓ (pH 1) 244 (shoulder) (7500), 263 (10200); ƛₘₐₓ (pH 7) 234 (8100), 268 (10000); ƛₘₐₓ (pH 13) 272 (12800). NMR δ: 3.55 (3H, s, Me₆), 7.91 (1H, s, C₆H₅—H).

References