Sparsomycin Analogs. I. Synthesis of 5-Carboxy-6-methyluracil\(^1\)

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Methods for the synthesis of 5-carboxy-6-methyluracil (9), which is expected to be useful as an intermediate for the preparation of sparsomycin analogs, were investigated. A new route that leads to 9 from cyanoacetylurea \((8)\) via 2-cyano-3-oxobutanoylurea \((6a)\), 5-cyano-6-methyluracil \((7a)\), and 5-carbamoyl-6-methyluracil \((8)\), was developed. The total yield from 5 to 9 was 20.7%.

Keywords—5-carboxy-6-methyluracil; 5-cyano-6-methyluracil; 5-carbamoyl-6-methyluracil; 2-cyano-3-oxobutanoylurea; sparsomycin analogs

Sparsomycin \((1)\) was isolated in 1962 from the culture filtrate of *Streptomyces sparsogenes* var. *sparsogenes* sp. n. by Argoudelis et al.\(^2\) This compound was subjected to several preliminary biological tests, and exhibited moderate to high activity against several *in vivo* tumor systems, such as the Walker carcinosarcoma 256 and the sarcoma 180 solid tumor, in addition to moderate *in vitro* activity against various bacteria, fungi, and viruses.\(^3\)

The structure of this antibiotic was proposed by Wiley and MacKellar in 1970\(^4\) and recently a total synthesis of its enantiomer was independently reported by Ottenheijm et al.\(^5\) and Helquist et al.\(^6\) (Fig. 1).

The unique structural and biological properties of sparsomycin prompted us to investigate the structure-activity relationship of sparsomycin analogs. This report describes the synthesis of 5-carboxy-6-methyluracil, which is expected to be an intermediate for syntheses of sparsomycin analogs lacking the ethylene moiety.

5-Carboxy-6-methyluracil \((9)\) has been already prepared by the route shown in Chart 1 (path a) in Wiley and MacKellar's structural study\(^7\) of sparsomycin. In this synthetic route, 6-methyluracil \((2)\) was treated with formaldehyde to afford 5-hydroxymethyl-6-methyluracil \((3)\), then 3 was oxidized with CrO\(_3\) to afford 5-formyl-6-methyluracil \((4)\), and finally 4 was oxidized with KMnO\(_4\) in an alkaline medium to afford 9, in 43%, 20%, and 16% yields, respectively. Consequently the total yield from 2 to 9 was 1.4%. 4 has also been prepared by the oxidation of 3 with \(\text{K}_2\text{S}_2\text{O}_8\) containing AgNO\(_3\) in 63% yield.\(^7\)

When this figure is employed,
the total yield via path a is 4.3%. On the other hand, 4 has been directly prepared from 2 in 14% yield by the Reimer–Tiemann reaction (Chart 1, path b), and, if this figure is employed, the total yield from 2 to 9 (path b) would be 2.2%.

These poor yields are inappropriate for a synthetic route to sparsomycin analogs. Therefore we developed another route, shown in Chart 2, making use of 5-cyano-6-methyluracil (7a) as an intermediate.

Cyanocetylurea (5), prepared from cyanacetic acid and urea by the method in the literature, was treated with acetic anhydride in the presence of molten ZnCl₂ to give 2-cyano-3-oxobutanoic acid (6a) in 73% yield. The ureid 6a was treated with 10% NaOH to give a cyclized product in 89% yield.

In this cyclization reaction, two kinds of route were anticipated, i.e., route a and route b. If the reaction involves the initial nucleophilic attack of the amide nitrogen on the carbon atom of the cyano group, 5-acetyl-6-aminouracil (7a) should be formed. However, the infrared (IR) spectrum of our cyclization product showed a nitrile absorption band at 2230 cm⁻¹ and its mass spectrum showed the molecular ion peak of 5-cyano-6-methyluracil (7a) at m/e 151. On the basis of these data, the structure of this product was assigned as 7a. The elemental analysis data also supported this assignment.

An attempted hydrolysis of 7a with 1 N NaOH at 100° for 10 hr resulted in a quantitative recovery of unchanged 7a. 7a was hydrolyzed by heating with 90% H₂SO₄ to afford 5-carbamoyl-6-methyluracil (8) in 68% yield. Attempted alkaline hydrolysis of 8 was also unsuccessful. On the other hand, when 8 was heated with HCl–AcOH, 6-methyluracil (2) was formed with evolution of carbon dioxide.

Thus, the amide 8 was treated with n-butyl nitrite (n-BuONO) and HCl in acetic acid, and 5-carboxy-6-methyluracil (9) was successfully obtained in 47% yield. IR and NMR spectral data of 9 were identical with those of an authentic sample prepared from 2 according to the reported procedure.¹⁴b

Thus, we have developed a new route for the synthesis of 5-carboxy-6-methyluracil: the total yield from 5 to 9 was 20.7%.
Since 5-cyano-6-methyluracil (7a) and potent antitumor agents such as 5-fluorouracil (5-FU) and futrafal are structurally closely related, both having an electron-withdrawing group at position 5 of the uracil nucleus, we tested 7a for cytotoxic activity on HeLa cells by a colony formation method. However, 7a showed no activity at a concentration of 1, 10, or 100 μg/ml of the assay medium, while 5-FU as a control sample showed inhibition at a concentration of 1 μg/ml.

Synthesis of sparsomycin analogs lacking the ethylene moiety is being conducted in this laboratory.

**Experimental**

All melting points are uncorrected. IR spectra were taken on a JASCO IRA-2 spectrometer, mass spectra on a JEOL JMS-D100 mass spectrometer, UV spectra on a Hitachi 323 recording spectrophotometer, and NMR spectra on a JEOL JNM-MH-100 spectrometer with TMS as an internal standard. The abbreviations used are as follows: s, singlet; d, doublet; b, broad. For thinlayer chromatography, silica gel GF\textsubscript{254} (Merck) was used with the solvent system MeCOEt–Me\textsubscript{2}CO–H\textsubscript{2}O (7: 2: 1).

2-Cyano-3-oxobutanoyleurea (6a)—A mixture of 5.0 g (0.039 mol) of 5 (prepared from cyanacetic acid and urea in the presence of Ac\textsubscript{2}O in 82% yield by the reported method\textsuperscript{9}) 20 ml of Ac\textsubscript{2}O and 0.5 g of molten ZnCl\textsubscript{2} was heated gently over a small flame for several minutes until a solution was obtained. The solution was immediately cooled in an ice bath. The solidified product was filtered off, washed with Et\textsubscript{2}O–EtOH, and dried in vacuo. The crude product was recrystallized from EtOH to yield 4.94 g (73%) of 6a, colorless needles, mp 161° (dec.). Anal. Calcld for C\textsubscript{5}H\textsubscript{7}NO\textsubscript{3}: C, 42.61; H, 4.17; N, 24.84. Found: C, 42.79; H, 4.23; N, 24.88. IR ν\textsubscript{max} cm\textsuperscript{-1}: 2300 (CN), 1730, 1675. UV \(λ_{\text{max}}\) nm (e): 275 (80).

2-Cyano-3-oxopentanoyleurea (6b)—Acetylation of 5 (2.542 g, 0.02 mol) with (EtCO)\textsubscript{2}O (13 ml) and molten ZnCl\textsubscript{2} (0.25 g) afforded 1.5 g (41.0%) of 6b, mp 141–144° (dec.).

5-Cyano-6-methyluracil (7a)—4a (5.0 g, 0.03 mol) was mixed with 10% NaOH (20 ml, 0.05 mol). The mixture was shaken to give a solution, which solidified after a few minutes. The solidified mixture was heated at 60° for 2 min on a water bath, cooled to room temperature and acidified with 50% AcOH. The resulting precipitate was filtered off, washed with cold water, and dried in vacuo. The crude product was recrystallized from water to yield 4.03 g (89%) of 7a. Colorless prisms, mp above 300°. Anal. Calcld for C\textsubscript{5}H\textsubscript{7}NO\textsubscript{3}: C, 47.69; H, 3.33; N, 27.81. Found: C, 47.80; H, 3.08; N, 27.98. IR ν\textsubscript{max} cm\textsuperscript{-1}: 2230 (CN), 1720, 1658. MS m/e: 151 (M\textsuperscript{+}). UV \(λ_{\text{max}}\) nm (e): 278 (7900). Rf: 0.80.

5-Cyano-6-ethyluracil (7b)—Treatment of 6b (1.465 g, 8 mmol) as described for 7a followed by recrystallization from water gave 7b (139 mg, 11%) as colorless needles, mp 281° (dec.). Anal. Calcld for C\textsubscript{6}H\textsubscript{7}NO\textsubscript{3}: C, 50.00; H, 4.40; N, 24.99. Found: C, 50.04; H, 4.22; N, 24.81. IR ν\textsubscript{max} cm\textsuperscript{-1}: 2230 (CN), 1730, 1665. MS m/e: 165 (M\textsuperscript{+}). UV \(λ_{\text{max}}\) nm (e): 273.5 (11670).

5-Carbamoyl-6-methyluracil (8)—4a (1.51 g, 0.01 mol) was heated with 1.1 ml of H\textsubscript{2}O (0.661 mol) and 13.9 ml of conc. H\textsubscript{2}SO\textsubscript{4} at 110° for 16.5 hr. After cooling, the reaction mixture was poured over ice in a beaker. The precipitate was filtered off with suction, washed with cold water and recrystallized from water to yield 1.08 g (62%) of 8, colorless prisms, mp above 300°. Anal. Calcld for C\textsubscript{6}H\textsubscript{7}NO\textsubscript{4}: C, 41.15; H, 4.41; N, 23.99. Found: C, 41.42; H, 4.01; N, 23.92. IR ν\textsubscript{max} cm\textsuperscript{-1}: 1720, 1685. MS m/e: 180 (M\textsuperscript{+}).

NMR (DMSO-d\textsubscript{4}): 2.41 (s, 3H, CH\textsubscript{3}), 7.10 (b, 1H), 8.21 (b, 1H), 11.16 (b, 1H), 11.22 (b, 1H). UV \(λ_{\text{max}}\) nm (e): 268 (9065). Rf: 0.60.

5-Carbonyl-6-methyluracil (9)—Dry HCl gas was bubbled slowly through a solution of 0.169 g (0.001 mol) of the amide (8) in glacial acetic acid for 15 min. The solution was stirred and 0.210 g (0.002 mol) of α-BuONO was added to it. The reaction mixture was heated at 100° for 3 hr with stirring, then cooled. The solvent was removed in vacuo, and the product was recrystallized from water to give 0.080 g (47%) of 9. Colorless needles, mp 242° (dec.). Anal. Calcld for C\textsubscript{6}H\textsubscript{7}NO\textsubscript{4}: C, 49.02; H, 3.82; N, 15.91. Found: C, 41.27; H, 3.51; N, 16.34. IR ν\textsubscript{max} cm\textsuperscript{-1}: 1720, 1610. MS m/e: 170 (M\textsuperscript{+}). NMR (DMF-d\textsubscript{4}): 2.70 (s, 3H, CH\textsubscript{3}), 12.10 (b, 2H, NH), 13.78 (b, 1H, COOH). UV \(λ_{\text{max}}\) nm (e): 271 (8900). Rf: 0.30.

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**References and Notes**

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