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Synthetic Studies on an Antitumor Antibiotic, Bleomycin. XIII.¹⁾ Synthesis of 2-Formylpyrimidine, a Key Intermediate for the Pyrimidine Moiety of Bleomycin

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An efficient synthesis of the pyrimidine nucleus of bleomycin was achieved. The reaction of diethoxyacetamide and ethyl ethoxalylpropionate gave ethyl 2-diethoxymethyl-6-hydroxy-5-methylpyrimidine-4-carboxylate in 76% yield; this product was then converted into ethyl 6-chloro-2-formyl-5-methylpyrimidine-4-carboxylate in 85% yield by treatment with POCl₃.

Keywords—bleomycin; diethoxyacetamide; pyrimidine; ethyl ethoxalylpropionate; formylpyrimidine

Since the discovery²⁾ of bleomycin (BLM) **1** by Umezawa in 1966, it has been found to show outstanding antitumor activity, having a therapeutic effect against squamous cell carcinomas and malignant lymphomas.³⁾ An early study showed that the antitumor activity of BLM is due to its ability to cause deoxyribonucleic acid (DNA)-strand scission,⁴⁾ which requires ferrous ions and oxygen.⁵⁾ The reductive activation of molecular oxygen by the BLM-Fe(II) complex was suggested, and oxygen-radical species were actually detected by a recent electron spin resonance (ESR) study.⁶⁾ Furthermore, in 1978, the structure of BLM was revised to **1**,⁷⁾ containing an open-chain carbamoylmethyl group instead of the previously proposed β -lactam ring.

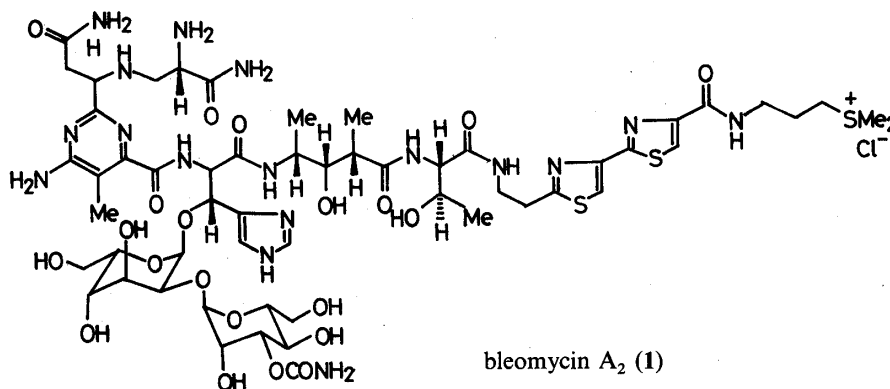


Chart 1

At the same time, a reasonable definite structure of the BLM-Fe(II) complex was proposed by Umezawa *et al.*⁸⁾ Five nitrogens in the pyrimidine moiety are thought to

participate in complexation with the ferrous ions to activate molecular oxygen at the sixth coordination site, causing DNA breakage.⁸⁾ We have initiated a synthetic approach to BLM leading to the total synthesis of BLM itself, in order (1) to understand the BLM action at the molecular level and to elucidate the unique chelation chemistry of BLM mentioned above, and (2) to obtain conclusive evidence for the structure **1**. Since BLM is a glycopeptide, total synthesis through fragment condensation may be the best practical choice.⁹⁾ The pyrimidine moiety was chosen to be synthesized first of all, because it was essential to prove the partial structure at an early stage of the total synthesis. Our synthetic strategy for the elaboration of the pyrimidine moiety is depicted in Chart 2. Three approaches were thought to be possible. They are the β -keto amide route (a), the Schiff base route (b), and the 1,4-addition route (c), and 2-formylpyrimidine **4** is a key synthon in all of them. The actual synthetic strategy we employed, after several fundamental investigations, was based on the Schiff base approach. Its key features were: (1) the preparation of the 2-formylpyrimidine derivative **4**, (2) the introduction of an acetate unit into the Schiff base **3**, and (3) the appropriate functionalization of the pyrimidine moiety. We describe here an efficient synthesis of 2-formylpyrimidine as a key synthon for the pyrimidine moiety of BLM.

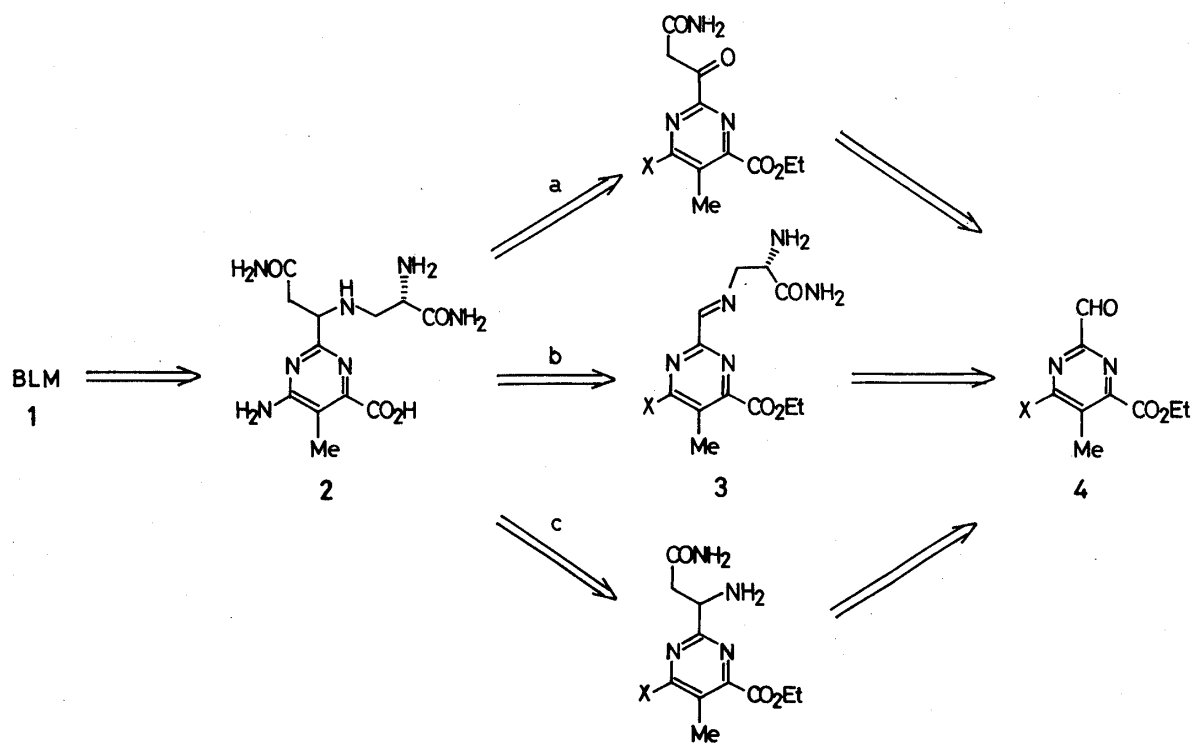


Chart 2

The pyrimidine moiety of BLM is unique, being different from those found in usual nucleosides. No efficient methods have yet been reported for the synthesis of such a polyfunctionalized pyrimidine nucleus bearing a variety of substituents on every carbon. At first, we planned to prepare the aldehyde **4** by transformation of a structurally related pyrimidine, **7a**, **7b**, or **7c**.⁹⁾ Dichloromethylpyrimidine **7a** was obtained from dichloroacetamide **5a**¹⁰⁾ and ethyl ethoxalpropionate **6**¹¹⁾ in 30% yield. However, **7a** gave bis(ethoxymethyl)pyrimidine **8** on treatment with sodium ethoxide. No expected product was obtained by hydrolysis under various conditions. Chloromethylpyrimidine **7b** might be convertible to **4**. Indeed, **7b** was hydrolyzed to **9**, which was then converted to **7d** in a good yield by esterification (EtOH-HCl), mild oxidation with MnO_2 , and acetalization, but the starting

material **7b** was obtained in only a 10% yield from chloroacetamidine **5b**.¹²⁾ 2,5-Dimethylpyrimidine **7c**, obtained from acetamidine **5c** and **6** in 20% yield, was also examined. The 2-methylpyrimidine derivative **7c** was selectively condensed with benzaldehyde in the presence of zinc chloride (**10a**, 45%), and the product **10a** was chlorinated with POCl₃ (**10b**, 98%), followed by ozonolysis (64%) to afford **4** (X=Cl).

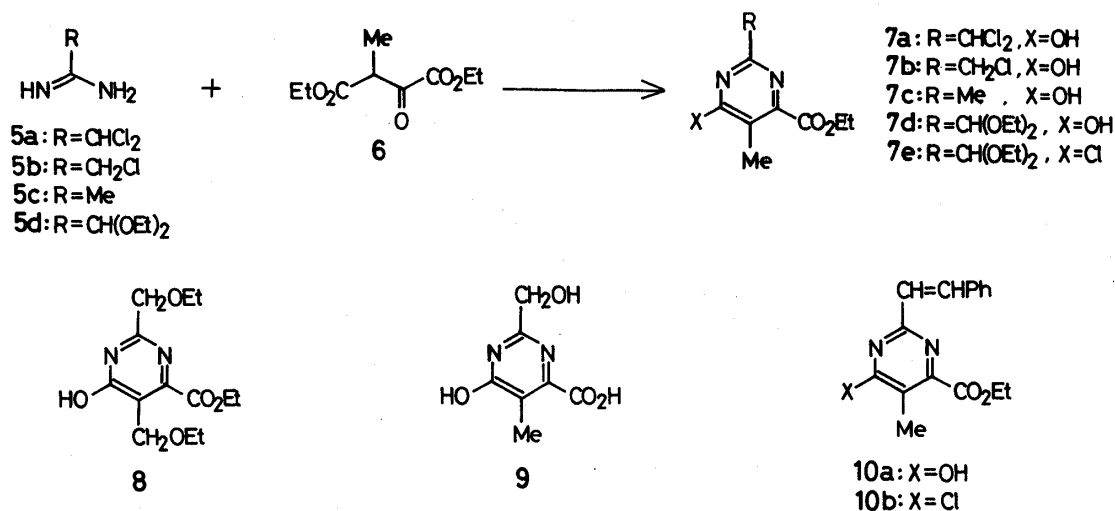
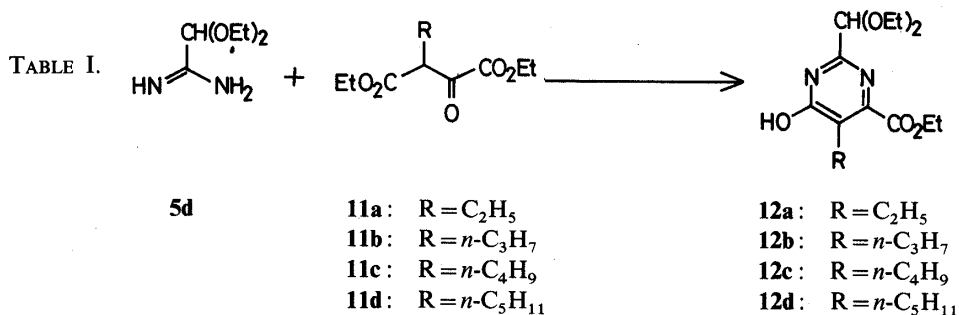


Chart 3

Although the aldehyde **4** and its equivalent **7d** could be obtained as described above, the overall yields, especially the yield of the pyrimidine-ring formation, were very poor, and so these methods would be impractical for a large-scale synthesis of **4**. After an extensive investigation of various substituted acetamidine derivatives,¹³⁾ a new method for a one-step synthesis of the acetal **7d** was successfully developed.

The reaction of diethoxyacetamidine **5d**¹⁶⁾ (1 eq) and **6** (1 eq) in the presence of triethylamine (2 eq) in EtOH (0.4 M solution)¹⁷⁾ under reflux for 2 h afforded **7d** directly in 76% yield. It was easily converted to **4** (X=Cl) by treatment with POCl₃ under reflux for 1 h (85% yield), showing that chlorination of the hydroxyl group took place together with the generation of the formyl group. Selective chlorination of **7d** is also possible by treatment with thionyl chloride in dimethylformamide (DMF), affording **7e** in 88% yield. The chloro group



	Time (h)	Yield (%)
12a	3	35
12b	2	32
12c	2.5	27
12d	2	28

on the pyrimidine nucleus is easily replaced by an amino group by treatment with ammonia¹⁸⁾ at a later stage of the synthesis.¹⁹⁾ The present method was found to be greatly superior to any conventional method, and can also be applied to the preparation of some analogues of **7d**, as shown in Table I, although the yields are not so good for higher alkyl groups.

The successful preparation of the key compound **4** in quantity allowed us to proceed to the next stage, *i.e.*, construction of the side chain of the pyrimidine moiety.¹⁹⁾

Experimental

Melting points were measured on a Yamato MP-21 apparatus and are uncorrected. Nuclear magnetic resonance, ¹H-NMR and ¹³C-NMR, spectra were obtained on a JEOL FX-100 spectrometer and chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS). Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Infrared (IR) spectra were recorded on a JASCO DS-402 G spectrometer.

Ethyl 2-Diethoxymethyl-6-hydroxy-5-methylpyrimidine-4-carboxylate (7d)—A solution of diethoxyacetamide **5d** (334 mg, 2.3 mmol), ethyl ethoxalylpropionate **6** (485 mg, 2.3 mmol) and triethylamine (465 mg, 4.6 mmol) in EtOH (11.5 ml) was refluxed under an argon atmosphere for 2 h. After removal of the solvent, the residue was chromatographed on silica gel. Fractions eluted with C₆H₆–AcOEt (4:1) gave **7d** (496 mg, 76%). mp 109–110 °C (CH₂Cl₂–*n*-hexane). IR (KBr): 3420, 1725, 1660, 1075 cm⁻¹. ¹H-NMR (CDCl₃) δ: 1.33 (9H, m), 2.24 (3H, s), 3.68 (4H, m), 4.40 (2H, q), 5.25 (1H, s), 10.20 (1H, br s). ¹³C-NMR (CDCl₃) δ: 165.34 (s), 163.34 (s), 154.62 (s), 149.84 (s), 125.77 (s), 98.53 (d), 63.06 (t), 61.94 (t), 15.01 (q), 14.13 (q), 11.65 (t). *Anal.* Calcd for C₁₃H₂₀O₅N₂: C, 54.92; H, 7.09; N, 9.85. Found: C, 55.00; H, 7.07; N, 9.55.

Ethyl 6-Chloro-2-formyl-5-methylpyrimidine-4-carboxylate (4) (X = Cl)—A solution of **7d** (5.18 g, 18 mmol) in distilled POCl₃ (20 ml) was heated at reflux for 1 h. After evaporation of the POCl₃ under reduced pressure, the residue was poured into ice-water and extracted with AcOEt. The extract was washed with water, dried over anhydrous Na₂SO₄ and concentrated. The residue was chromatographed on silica gel and eluted with C₆H₆–AcOEt (5:1) to give **4** (X = Cl) (3.67 g, 85%). IR: 1725 cm⁻¹. ¹H-NMR (CDCl₃) δ: 1.44 (3H, t), 2.59 (3H, s), 4.52 (2H, q), 10.05 (1H, s). MS *m/e*: 229 (M⁺ + 1), 228 (M⁺). 2,4-Dinitrophenylhydrazone: mp 214–215.5 °C, MS *m/e*: 408.

Ethyl 5-Alkyl-2-diethoxymethyl-6-hydroxypyrimidine-4-carboxylate (12a–d)—A solution of diethoxyacetamide **5d** (2.3 mmol), ethyl α-ethoxalylalkanoate (**11**, R² = C₂H₅, *n*-C₃H₇, *n*-C₄H₉, *n*-C₅H₁₁) (2.3 mmol) and triethylamine (4.6 mmol) in EtOH (11.5 ml) was refluxed for the period listed in Table I. After removal of the solvent, the residue was chromatographed on silica gel and eluted with C₆H₆–AcOEt (4:1) to give **12a–d**.

12a: mp 61.5–63.0 °C (CH₂Cl₂–*n*-hexane). IR (KBr): 3440, 1730, 1663, 1097 cm⁻¹. ¹H-NMR (CDCl₃) δ: 1.27 (12H, m), 2.63 (2H, q), 3.68 (4H, m), 4.39 (2H, q), 5.25 (1H, s). ¹³C-NMR (CDCl₃) δ: 165.39 (s), 162.07 (s), 154.67 (s), 150.14 (s), 130.69 (s), 98.29 (d), 63.20 (t), 62.03 (t), 19.74 (t), 15.01 (q), 14.13 (q), 13.01 (q). *Anal.* Calcd for C₁₄H₂₂N₂O₅: C, 56.36; H, 7.43; N, 9.39. Found: C, 56.60; H, 7.44; N, 9.27.

12b: mp 57.5–59.0 °C (CH₂Cl₂–*n*-hexane). IR (KBr): 3430, 1730, 1665, 1103 cm⁻¹. ¹H-NMR (CDCl₃) δ: 1.27 (14H, m), 2.59 (2H, m), 3.69 (4H, m), 4.38 (2H, q), 5.27 (1H, s). ¹³C-NMR (CDCl₃) δ: 165.43 (s), 162.46 (s), 154.71 (s), 150.43 (s), 129.33 (s), 98.34 (d), 63.11 (t), 61.94 (t), 28.07 (t), 21.30 (t), 15.01 (q), 14.18 (q). *Anal.* Calcd for C₁₅H₂₄N₂O₅: C, 57.67; H, 7.74; N, 8.97. Found: C, 57.37; H, 7.72; N, 9.01.

12c: mp 68.0–69.0 °C (CH₂Cl₂–*n*-hexane). IR (KBr): 3430, 1730, 1665, 1105 cm⁻¹. ¹H-NMR (CDCl₃) δ: 1.26 (16H, m), 2.64 (2H, m), 3.71 (4H, m), 4.41 (2H, q), 5.30 (1H, s). ¹³C-NMR (CDCl₃) δ: 165.43 (s), 163.00 (s), 154.86 (s), 150.29 (s), 129.43 (s), 98.53 (d), 63.01 (t), 61.89 (t), 30.78 (t), 25.92 (t), 22.95 (t), 15.01 (q), 14.13 (q), 13.84 (q). *Anal.* Calcd for C₁₆H₂₆N₂O₅: C, 58.88; H, 8.03; N, 8.58. Found: C, 58.76; H, 8.15; N, 8.49.

12d: mp 41.5–43.5 °C (CH₂Cl₂–*n*-hexane). IR (KBr): 3420, 1730, 1660, 1105 cm⁻¹. ¹H-NMR (CDCl₃) δ: 1.23 (18H, m), 2.61 (2H, m), 3.70 (4H, m), 4.40 (2H, q), 5.29 (1H, s). ¹³C-NMR (CDCl₃) δ: 165.49 (s), 162.71 (s), 154.77 (s), 150.29 (s), 129.52 (s), 98.45 (d), 63.06 (t), 61.94 (t), 32.06 (t), 28.36 (t), 26.17 (t), 22.51 (t), 15.01 (q), 14.13 (q), 13.99 (q). *Anal.* Calcd for C₁₇H₂₈N₂O₅: C, 59.98; H, 8.29; N, 8.23. Found: C, 59.97; H, 8.27; N, 8.27.

References and Notes

- 1) Part XII: M. Otsuka, A. Kittaka, T. Iimori, H. Yamashita, S. Kobayashi, and M. Ohno, *Chem. Pharm. Bull.*, **33**, 509 (1985).
- 2) H. Umezawa, K. Maeda, T. Takeuchi, and Y. Okami, *J. Antibiot.*, **19A**, 200 (1966). Although the first paper was written in 1966, bleomycin was actually discovered in 1963 at the Institute of Microbial Chemistry.
- 3) a) M. Ishizuka, H. Takayama, T. Takeuchi, and H. Umezawa, *J. Antibiot.*, **20A**, 15 (1967); b) M. Takeuchi and T. Yamamoto, *ibid.*, **21**, 631 (1968); c) T. Ichikawa, A. Matsuda, K. Miyamoto, M. Tsubosaki, T. Kaihara, K. Sakamoto, and H. Umezawa, *ibid.*, **20A**, 149 (1967); d) R. H. Blum, S. K. Carter, and K. Agre, *Cancer*, **31**, 903 (1973).

- 4) a) K. Nagai, H. Suzuki, N. Tanaka, and H. Umezawa, *J. Antibiot.*, **22**, 569 (1969); b) K. Nagai, H. Suzuki, N. Tanaka, and H. Umezawa, *ibid.*, **22**, 624 (1969); c) K. Nagai, H. Suzuki, N. Tanaka, and H. Umezawa, *Biochim. Biophys. Acta*, **179**, 165 (1969).
- 5) E. A. Sausville, J. Peisach, and S. B. Horwitz, *Biochem. Biophys. Res. Commun.*, **73**, 814 (1976).
- 6) Y. Sugiura and T. Kikuchi, *J. Antibiot.*, **31**, 1310 (1978).
- 7) T. Takita, Y. Muraoka, T. Nakatani, A. Fujii, Y. Umezawa, H. Naganawa, and H. Umezawa, *J. Antibiot.*, **31**, 801 (1978).
- 8) T. Takita, Y. Muraoka, T. Nakatani, A. Fujii, Y. Iitaka, and H. Umezawa, *J. Antibiot.*, **31**, 1073 (1978).
- 9) Y. Umezawa, H. Morishima, T. Yoshioka, M. Otsuka, and M. Ohno, "Bleomycin," ed. by S. M. Hecht, Springer-Verlag, New York, 1979, p. 63.
- 10) H. Schroeder and C. Grundmann, *J. Am. Chem. Soc.*, **78**, 2447 (1956).
- 11) R. F. B. Cox and S. M. McElvain, "Organic Syntheses," Coll. Vol. 2, ed. by A. H. Blatt, John Wiley and Sons, New York, 1943, p. 272.
- 12) F. C. Schaefer and G. A. Peters, *J. Org. Chem.*, **26**, 412 (1961).
- 13) Other amidines (**5**, $R^1 = CO_2CH_2Ph$ or CH_2OCOPh) derived from cyanoformate¹⁴⁾ or *O*-benzoylglycolonitrile¹⁵⁾ afforded no pyrimidine derivative.
- 14) Y. Nii, K. Okano, S. Kobayashi, and M. Ohno, *Tetrahedron Lett.*, **1979**, 2517.
- 15) J. F. Olin and T. B. Johnson, *Recl. Trav. Chim. Pays-Bas*, **50**, 72 (1931).
- 16) Diethoxyacetonitrile was prepared according to Erickson's method: J. G. Erickson, *J. Am. Chem. Soc.*, **73**, 1338 (1951). It was converted into diethoxyacetamidine **5d** according to the method of Schaefer and Peters: F. C. Schaefer and G. A. Peters, *J. Org. Chem.*, **26**, 412 (1961).
- 17) It was shown that the yield was markedly influenced by the concentration of the reactants; the present method is easily applicable on a 100 g scale.
- 18) G. W. Kenner and A. Todd, "Heterocyclic Compounds," Vol. 6, ed. by R. C. Elderfield, John Wiley and Sons, New York, N. Y., 1957.
- 19) Part XIV: M. Otsuka, M. Narita, M. Yoshida, S. Kobayashi, M. Ohno, Y. Umezawa, H. Morishima, S. Saito, T. Takita, and H. Umezawa, *Chem. Pharm. Bull.*, **33**, 520 (1985).