[Chem. Pharm. Bull. 33(2) 515—519 (1985)]

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

Masami Otsuka,[a] Susumu Kobayashi,[a] Masaji Ohno,*[,a]
Yoji Umezawa,[b] Hajime Morishima,[b]
and Hamao Umezawa[b]

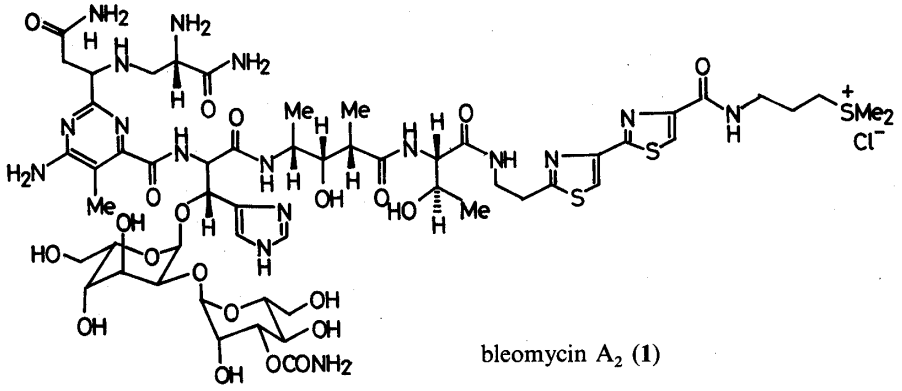
Faculty of Pharmaceutical Sciences, University of Tokyo,[a] Hongo, Bunkyo-ku, Tokyo 113, Japan and Institute of Microbial Chemistry,[b] Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received June 1, 1984)

An efficient synthesis of the pyrimidine nucleus of bleomycin was achieved. The reaction of diethoxyacetamidine 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 $POCl_3$.

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

Since the discovery[2] 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.



bleomycin A_2 (**1**)

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-forymylpyrimidine **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.

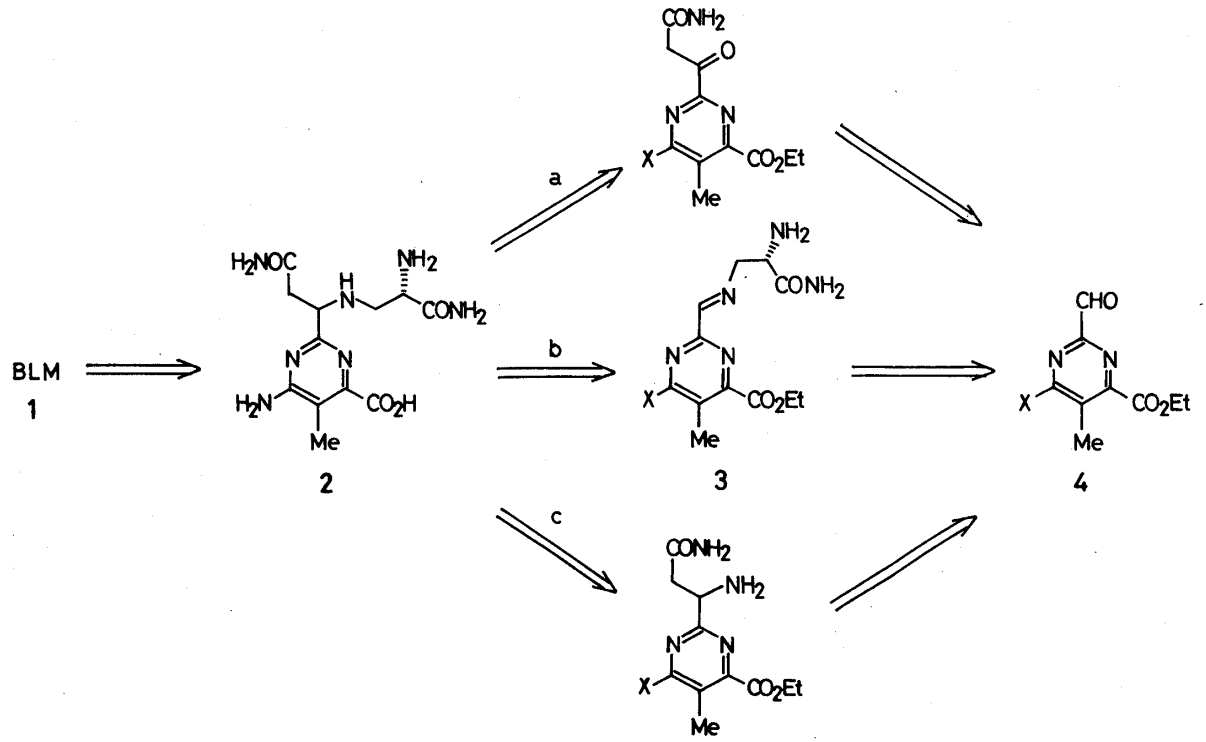


Chart 2

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 dichloroacetamidine **5a**[10] and ethyl ethoxalypropionate **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 $POCl_3$ (**10b**, 98%), followed by ozonolysis (64%) to afford **4** (X=Cl).

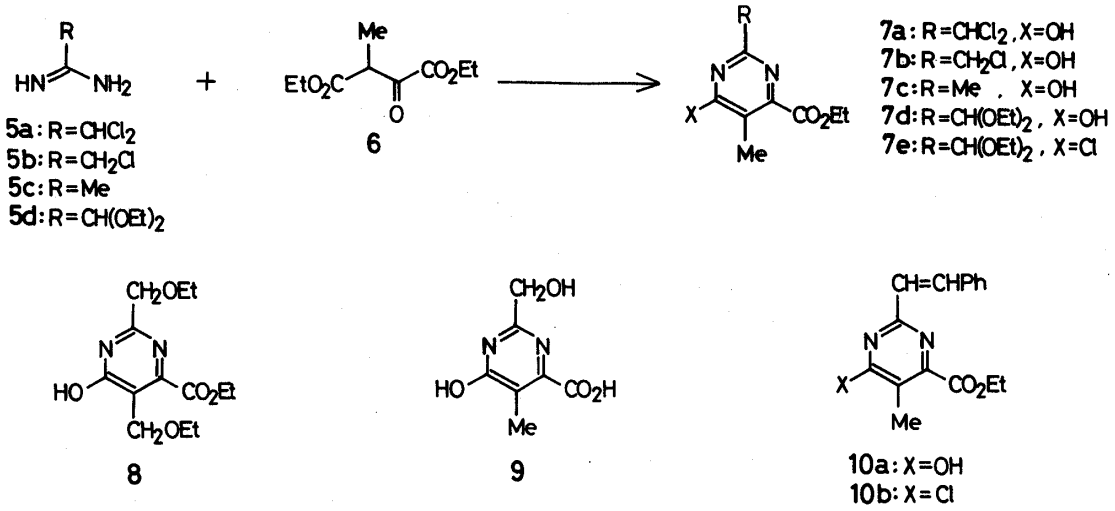
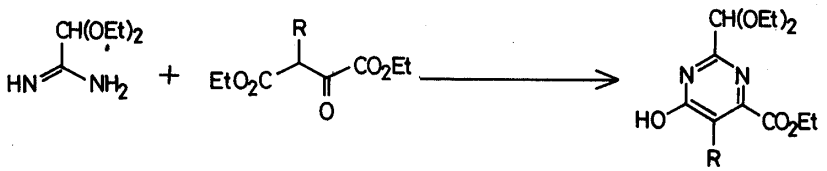


Chart 3

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 $POCl_3$ 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

TABLE I.



5d

11a: R=C_2H_5
11b: R=n-C_3H_7
11c: R=n-C_4H_9
11d: R=n-C_5H_{11}

12a: R=C_2H_5
12b: R=n-C_3H_7
12c: R=n-C_4H_9
12d: R=n-C_5H_{11}

	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 ammonia[18] 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, ^{1}H-NMR and ^{13}C-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 diethoxyacetamidine **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 C_6H_6–AcOEt (4 : 1) gave **7d** (496 mg, 76%). mp 109—110 °C (CH_2Cl_2–*n*-hexane). IR (KBr): 3420, 1725, 1660, 1075 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 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). ^{13}C-NMR ($CDCl_3$) 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 $C_{13}H_{20}O_5N_2$: 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 $POCl_3$ (20 ml) was heated at reflux for 1 h. After evaporation of the $POCl_3$ under reduced pressure, the residue was poured into ice-water and extracted with AcOEt. The extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed on silica gel and eluted with C_6H_6–AcOEt (5 : 1) to give **4** (X = Cl) (3.67 g, 85%). IR: 1725 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 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 diethoxyacetamidine **5d** (2.3 mmol), ethyl α-ethoxalylalkanoate (**11**, $R^2 = C_2H_5$, *n*-C_3H_7, *n*-C_4H_9, *n*-C_5H_{11}) (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 C_6H_6–AcOEt (4 : 1) to give **12a—d**.

12a: mp 61.5—63.0 °C (CH_2Cl_2–*n*-hexane). IR (KBr): 3440, 1730, 1663, 1097 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (12H, m), 2.63 (2H, q), 3.68 (4H, m), 4.39 (2H, q), 5.25 (1H, s). ^{13}C-NMR ($CDCl_3$) 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 $C_{14}H_{22}N_2O_5$: 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 (CH_2Cl_2–*n*-hexane). IR (KBr): 3430, 1730, 1665, 1103 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (14H, m), 2.59 (2H, m), 3.69 (4H, m), 4.38 (2H, q), 5.27 (1H, s). ^{13}C-NMR ($CDCl_3$) 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 $C_{15}H_{24}N_2O_5$: 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 (CH_2Cl_2–*n*-hexane). IR (KBr): 3430, 1730, 1665, 1105 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (16H, m), 2.64 (2H, m), 3.71 (4H, m), 4.41 (2H, q), 5.30 (1H, s). ^{13}C-NMR ($CDCl_3$) 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 $C_{16}H_{26}N_2O_5$: 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 (CH_2Cl_2–*n*-hexane). IR (KBr): 3420, 1730, 1660, 1105 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (18H, m), 2.61 (2H, m), 3.70 (4H, m), 4.40 (2H, q), 5.29 (1H, s). ^{13}C-NMR ($CDCl_3$) 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 $C_{17}H_{28}N_2O_5$: 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 cyanoformate[14] or *O*-benzoylglycolonitrile[15] 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).