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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 ethoxalpropionate 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 ethoxalpropionate; 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.

![Bleomycin A2 (1)](chart)

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 ethoxalypropionate 6\(^11\) in 30% yield. However, 7a gave bis(ethoxy-methyl)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).

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

| Table 1 |  
|--------|---|
| **5d** |  
| **11a**: R = C₂H₅ | **12a**: R = C₃H₇ |
| **11b**: R = n-C₃H₇ | **12b**: R = n-C₃H₇ |
| **11c**: R = n-C₃H₉ | **12c**: R = n-C₃H₉ |
| **11d**: R = n-C₃H₁₁ | **12d**: R = n-C₃H₁₁ |

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
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<tbody>
<tr>
<td>12a</td>
<td>3</td>
</tr>
<tr>
<td>12b</td>
<td>2</td>
</tr>
<tr>
<td>12c</td>
<td>2.5</td>
</tr>
<tr>
<td>12d</td>
<td>2</td>
</tr>
</tbody>
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on the pyrimidine nucleus is easily replaced by an amino group by treatment with ammonia at a later stage of the synthesis. 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.

Experimental

Melt Monk 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. IR spectra were recorded on a JASCO DS-402 G spectrometer.

Ethyl 2-Diethoxymethyl-6-hydroxy-5-methylpyrimidine-4-carboxylate (7d) — A solution of diethoxycetamidine 5d (334 mg, 2.3 mmol), ethyl ethoxylpropionate 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%). 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, brs). 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. Caled for C₁₇H₁₉O₂N₂: 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-Dinitrophenylhydrazine: mp 214–215.5 °C, MS m/e: 408.

Ethyl 5-Alkyl-2-diethoxymethyl-6-hydroxy-pyrimidine-4-carboxylate (12a–d) — A solution of diethoxycetamidine 5d (2.3 mmol), ethyl ethoxylalkanoate (11, R² = C₆H₄, n-C₃H₇, n-C₆H₁₃, n-C₆H₁₃, n-C₆H₁₃) (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. Caled for C₁₉H₁₃N₂O₆: 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. Caled for C₁₉H₁₃N₂O₆: 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, 1103 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 (d), 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. Caled for C₁₉H₁₃N₂O₆: 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. Caled for C₁₇H₁₉N₂O₆: C, 59.98; H, 8.29; N, 8.23. Found: C, 59.97; H, 8.27; N, 8.27.

References and Notes


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.


13) Other amidines (5, R¹=CO₂CH₃Ph or CH₂OCOPh) derived from cyanoformate¹⁴) or O-benzoylglycolonitrile¹⁵) afforded no pyrimidine derivative.


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.
