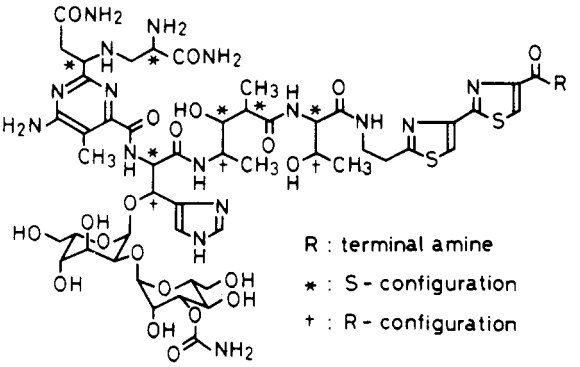
Synthesis of the Pyrimidine Moiety of Bleomycin

Sir:

Bleomycin (BLM) is an antitumor antibiotic first reported in 1966[1] and clinically used in the chemotherapy of squamous cell carcinomas and malignant lymphomas.[2] In addition to its

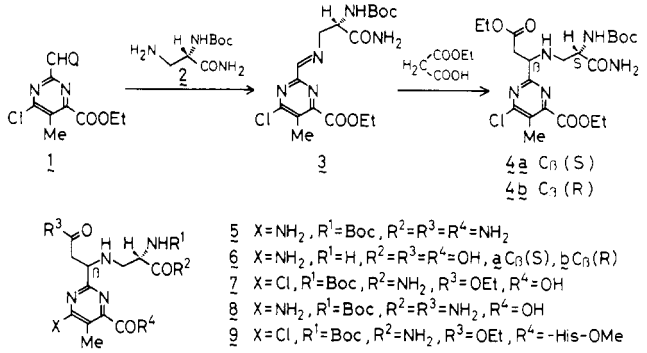


Bleomycin

medicinal importance, BLM is of great interest because of its unique mechanism of action[3] and unusual glycopeptide structure.[4] This communication describes the synthesis of the pyrimidine moiety of bleomycin, including a key pyrimidine intermediate (**4**), pyrimidobleonic acid[5,6] (**6a**), pyrimidoblamic acid (**8**), and a pyrimidoblamylhistidine equivalent (**9**), from the starting material ethyl 6-chloro-2-formyl-5-methylpyrimidine-4-carboxylate (**1**), which is readily accessible by the approach developed in our laboratories.[7]

The key features of the approach include (1) preparation of 2-formylpyrimidine derivative **1**, (2) formation of Schiff base **3**, (3) formation of key intermediate **4** by C–C bond formation at the C–N double bond, and (4) appropriate functionalization of the pyrimidine moiety to that of BLM.

The 2-formylpyrimidine **1**, efficiently prepared by the reaction of diethoxyacetamidine and ethyl α-ethoxalylpropionate followed by treatment with $POCl_3$[7] (68% overall yield), was treated with (S)-3-amino-2-[(*tert*-butoxycarbonyl)amino]propionamide[8,9] (**2**) in an equal molar ratio in CH_3CN in the presence of an activated molecular sieve at 25 °C for 22 h. The resulting Schiff base **3**[10] was caused to react with malonic acid monoethyl ester[11,12] at 25



5 X = NH_2, R^1 = Boc, R^2 = R^3 = R^4 = NH_2
6 X = NH_2, R^1 = H, R^2 = R^3 = R^4 = OH, a C_β(S), b C_β(R)
7 X = Cl, R^1 = Boc, R^2 = NH_2, R^3 = OEt, R^4 = OH
8 X = NH_2, R^1 = Boc, R^2 = R^3 = NH_2, R^4 = OH
9 X = Cl, R^1 = Boc, R^2 = NH_2, R^3 = OEt, R^4 = -His-OMe

°C for 24 h. The desired product **4** was obtained in 23% yield along with an elimination product[13] upon workup and chromatography on silica gel; R_f values for them were 0.46 and 0.86, respectively (silica gel plates, C_6H_6–AcOEt, 1:5). The product **4** showed a single spot on TLC, but it was separated cleanly by using high-performance TLC (HPTLC) and found to be a mixture of the two epimers **4a** and **4b** in about equal amounts (~10% yield each). R_f values for **4a** and **4b** were 0.58 and 0.44, respectively (Merck HPTLC plates, silica gel, Art. 5628, 10 × 10 cm, C_6H_6–EtOH, 9:1). Compounds **4a** [syrup; *m/e* 502 ($M^+ + 1$); $[\alpha]^{24}_D$ −3.5° (*c* 2, EtOH)] and **4b** [syrup; *m/e* 502 ($M^+ + 1$); $[\alpha]^{24}_D$ + 29.5° (*c* 2, EtOH)] were assigned to $S(C_\beta)S$ and $R(C_\beta)S$ configurations by correlating to degradation products from BLM as described below.

Thus, the epimeric mixture **4** was treated with saturated NH_3 in EtOH at 25 °C for a week and then at 40 °C for 3 days. The reaction mixture yielded an exhaustively aminated product **5** in 70% yield upon workup and chromatography on silica gel [syrup; silica gel plates, R_f 0.37 with BuOH–AcOH–H_2O, 3:1:2; *m/e* 425 ($M^+ + 1$) field desorption (FD)].[14] Compound **5** was hydrolyzed with 20% HCl at 50 °C for 5 days and purified with Dowex 1 (AcO^-). The eluate with 6% AcOH afforded a colorless powder (**6**) in about 50% overall yield from **4** upon workup (chromatography on microcrystalline cellulose, cellulose plates, R_f 0.18 with PrOH–Py–AcOH–H_2O, 15:10:3:12). The clean separation of the synthetic epimers **6** was successfully achieved by using the chelation compounds.[15] The epimeric mixture **6** was treated with $Cu(OAc)_2$ (1 equiv) in H_2O and subjected to high-pressure liquid chromatograph (high-pressure LC). Two sharp peaks were obtained in almost equal intensity, R_t 5.13 and 6.10 min, and the former peak was found identical with that of pyrimidobleonic acid, $[\alpha]^{26}_D$ −25.3° (*c* 0.75, 1 N HCl), derived from natural BLM,[5] and with a main peak derived from **4a** by similar procedures described above.[16,17] The latter peak was identical with that of epipyrimidobleonic acid, $[\alpha]^{26}_D$ −16° (*c* 0.75, 1 N HCl).[5,16] Therefore, **4a** has the same configuration as **6a** or the $S(C_\beta)S$ configuration, and **4b** is assigned to the $R(C_\beta)S$ configuration.

(1) Umezawa, H.; Maeda, K.; Takeuchi, T.; Okami, Y. *J. Antibiot.* **1966**, *19A*, 200.
(2) Umezawa, H. *Prog. Biochem. Pharmacol.* **1976**, *11*, 18.
(3) (a) Suzuki, H.; Nagai, K.; Yamaki, H.; Tanaka, N.; Umezawa, H. *J. Antibiot.* **1969**, *22*, 446. (b) Suzuki, H.; Nagai, K.; Akutsu, E.; Yamaki, H.; Tanaka, N.; Umezawa, H. *Ibid.* **1970**, *23*, 473. (c) Sugiura, Y.; Ishizu, K.; Miyoshi, K. *Ibid.* **1979**, *32*, 453. (d) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1976**, *73*, 814. (e) Takita, T.; Muraoka, Y.; Nakatani, T.; Fujii, A.; Iitaka, Y.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 1073.
(4) Takita, T.; Muraoka, Y.; Nakatani, T.; Fujii, A.; Umezawa, Y.; Naganawa, H.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 801.
(5) Umezawa, H. *Heterocycles* **1979**, *13*, 30. (*S*)-β-[(*S*)-2-amino-2-carboxyethyl]amino-β-(4-amino-6-carboxy-5-methylpyrimidin-2-yl)propionic acid was previously called pseudodipeptide[6] and now named pyrimidobleonic acid (**6a**), and the epimer *R* at the α position to the pyrimidine ring was named epipyrimidobleonic acid (**6b**). The epimeric acid **6b** was accompanied by isomerization during hydrolysis: Takita, T.; Muraoka, Y., unpublished results; also see ref 6.
(6) Takita, T. In "Bleomycin"; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979; pp 37–47.
(7) Part of the synthesis of the 2-formylpyrimidine **1** was reported at the U.S.–Japan Symposium held at the East–West Center, Honolulu, July 18–22, 1978; see: Umezawa, Y.; Morishima, H.; Yoshioka, T.; Otsuka, M.; Ohno, M., p 63 in ref 6. The detailed account will be published elsewhere.
(8) The amide **2** was prepared from *tert*-butoxycarbonyl-L-asparagine $[\alpha]^{20}_D$ −1.6° (*c* 1, MeOH)], commercially available by the following reactions: (a) Br_2-NaOH, (b) Z–Cl–NaOH, (c) CH_2N_2, (d) NH_3, (e) H_2–Pd/C.
(9) Hydrolysis of **2** with HCl afforded L-2,3-diaminopropionic acid, $[\alpha]^{20}_D$ +21.28° (*c* 1, NHCl),[6] confirming that essentially no racemization occurred during the procedures.
(10) The formation of the Schiff base **3** was clearly observed. ^1H NMR (Me_2SO-d_6) δ 8.36 (1 H, s, —CH=N—), 4.48 (2 H, q), ~3–4.07 (3 H, m), 2.48 (3 H, s), 1.40 (3 H, t), 1.32 (9 H, s); *m/e* 413 (M^+).
(11) Noordwijk, J. V.; Mellink, T. J.; Visser, B. J.; Wisse, J. H. *Recl. Trav. Chim. Pays-Bas* **1963**, *82*, 763.
(12) A Reformatsky reaction with an anil linkage was reported: Gilman, H.; Specter, M. *J. Am. Chem. Soc.* **1943**, *65*, 2255. However, application of this method to **3** did not afford any expected product.
(13) This compound was obtained in 48% yield, and the structure was determined to be ethyl 3-(4-chloro-6-ethoxycarbonyl-5-methylpyrimidin-2-yl)acrylate by IR, ^1H NMR, and mass spectral data.
(14) A triamide (R^1 = H in **5**) previously called triamide of pseudodipeptide was prepared from an *N*-acetyldehydroalanine and β-aminopropionic acid derivative obtained by degradation of BLM; see: Yoshioka, T.; Muraoka, Y.; Takita, T.; Maeda, K.; Umezawa, H. *J. Antibiot.* **1972**, *25*, 625.
(15) Muraoka, Y., p 92 in ref 6. High-pressure LC was performed on a Waters Associates A RADIAL-PAK A column (Radial Compression Separation System): solvent 0.1 M KH_2PO_4, flow rate 1.0 mL/min, 250, detector 254 nm.
(16) It was confirmed that the asymmetric carbon at the α position to the pyrimidine ring undergoes racemization to some extent during acid treatment; see also ref 5 and 6.
(17) The degradation products from BLM, **6a** and **6b**, were purified in the following way. The crude product obtained by acid-catalyzed hydrolysis of BLM[6] was treated with cupric acetate (1 equiv) in H_2O, and the resulting chelation compounds were subjected to column chromatography with a SP-Sephadex C-25 column. The first and second parts of the fractions afforded **6a** and **6b** in a 9:1 ratio,[5] respectively.

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The synthetic carbamoyl diester **4** has been found to be a suitable intermediate for further selective functionalization of the pyrimidine moiety of BLM. The compound **4** was selectively hydrolyzed to afford the carbamoyl acid ester **7** [syrup, *m/e* 474 ($M^+ + 1$) (FD), R_f 0.39 with BuOH–AcOH–H_2O, 4:1:1] in 93% yield by treatment with 0.1 N NaOH at 0 °C for 1 h. The hydrolyzed ethyl ester was clearly assigned to the one attached to the pyrimidine ring by UV shift (from 272 nm for **4** to 265 nm for **7**) and ^1H NMR data [only the lower signals for CO_2Et at δ 4.48 (q) and 1.42 (t) disappeared, and the signals for the side-chain ester at δ 4.15 (q) and 1.20 (t) remained in **7**]. Next, the acid ester **7** was subjected to amination with NH_3 at 40 °C for 6 days, and the resulting product was treated with dry EtOH, depositing a crystalline material, [mp 223–225 °C dec; $[\alpha]^{28}_D$ −32.8° (*c* 0.75, H_2O)] chromatographically homogeneous in fair yield.[18] Fortunately, it was found to be *tert*-butoxycarbonylpyrimidoblamic acid (**8**) with the desired *S*(C_β),*S* configuration by identification with the sample,[19] $[\alpha]^{28}_D$ −32.3° (*c* 0.75, H_2O), derived from pyrimidobleonic acid (**6a**) [mixed mp, IR, ^1H NMR, mass spectroscopy (FD), TLC, and high-pressure LC on chelation compound with Cu^{2+} and ORD]. These results are the first evidence for the partial structure of the pyrimidine moiety of BLM by direct comparison of the synthetic materials with the degradation products derived from BLM. Furthermore, **7** was treated with L-histidine methyl ester (2 equiv) in the presence of *N,N'*-carbonyldiimidazole (2 equiv) in DMF at 25 °C for 4 h. After workup and preparative chromatography (silica gel), pyrimidoblamylhistidine equivalent **9** was obtained in 40% yield [syrup, *m/e* 625 ($M^+ + 1$) (FD);[20] silica gel plates, R_f 0.32 with $CHCl_3$–EtOH, 4:1].

The research results described here provide a basis for further synthetic and transformational investigations relating to BLM and access to potentially useful analogues and open a route for a relay synthesis to BLM by using pyrimidobleonic acid and pyrimidoblamic acid available from natural BLM.

Acknowledgment. We thank Dr. H. Naganawa for obtaining spectroscopic data and Dr. Y. Muraoka for his advice of the chelation chemistry. This work was financially supported in part by grants-in-aid for Special Project Research from the Ministry of Education, Science and Culture of Japan.

(18) The deposited material was pure enough for further reactions but was obtained in only 10% yield, and the rest, including the epimer with *R,S* configuration and some of **8**, remained in the ethanol solution, but this step was found to be best for separation of the epimers.

(19) The acid **6a** derived from BLM was successively subjected to esterification (MeOH–HCl), selective hydrolysis of the methyl ester of the ring with $CuCO_3 \cdot Cu(OH)_2$, protection of the primary amine with Boc–S, and amination, affording **8**, namely *tert*-butoxycarbonylpyrimidoblamic acid.

(20) Compound **9** was negative for the ninhydrin test, showing absence of a primary amine, and the ^1H NMR spectra were well characterized and showed signals δ at δ 9.09 (d) for the amide (–CONH–) newly formed, 5.98 (d) for *tert*-butoxycarbonyl amide, and 6.12 (s) and 7.08 (s) for $CONH_2$.

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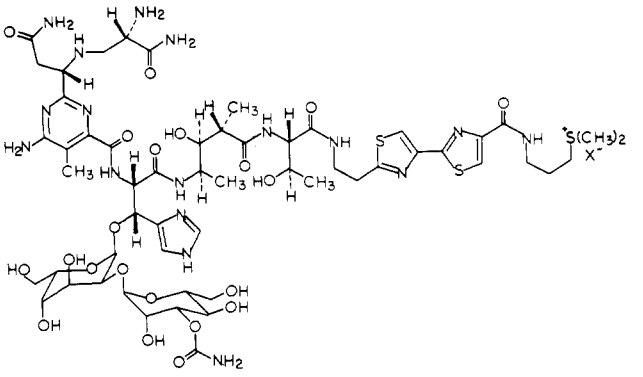
Received April 28, 1980

Synthesis of the Pyrimidine Moieties of Bleomycin and Epibleomycin

Sir:

The bleomycins are a family of glycopeptide-derived antibiotics with remarkable biochemical and biological properties.[1] At present, the bleomycins are of considerable interest because of their utility in the treatment of certain malignancies[2] and the identification of new bleomycins with properties that may enhance their effectiveness in the clinic.[3] A workable synthesis of bleomycin would be of obvious importance, in the sense that it would permit definition of the structural features requisite to the expression of anticancer activity. Previous studies have focused on the synthesis of tetrapeptide S[4] and its components,[5] and on the elaboration of L-*erythro*-β-hydroxyhistidine[6] and the carbohydrates[5d,7] present in the antibiotic. The preparation of the pyrimidine moiety of bleomycin has not been reported, although the chemistry of this portion of the molecule has been studied.[8] Described herein is the synthesis of the pyrimidine moieties of bleomycin (**1**) and epibleomycin.[9]



1

Ethyl 3-(6-carboethoxy-4-oxo-5-methylpyrimidin-2-yl)acrylate (**2**)[8b] was hydrogenated over 1% palladium-on-charcoal (2:1 EtOH–EtOAc, 12 h), affording pyrimidinylpropionate **3a** as a white solid (99%), mp 124–125 °C. Successive treatments of **3a** with $POCl_3$ (100 °C, 30 min) and NaN_3 (DMF, 25 °C, 12 h) gave azide **3b** as colorless needles in 83% overall yield from **3a**: mp 60–61 °C; IR (neat) 1725 (br), 1620 cm^{-1}; NMR [$CDCl_3$, $(CH_3)_4Si$] δ 1.26 (3 H, t, J = 7.0 Hz), 1.48 (3 H, t, J = 7.0 Hz), 2.93 (3 H, s), 3.13 (2 H, t, J = 6.0 Hz), 3.81 (2 H, t, J = 6.0 Hz), 4.16 (2 H, q, J = 7.0 Hz), 4.50 (2 H, q, J = 7.0 Hz); mass spectrum, *m/e* 279 (M^+). The absence of an azide stretching band in the infrared reflected the equilibrium between azide **3b** and tetrazole **3b'**.[11,12]

(1) (a) Umezawa, H. *Biomedicine* **1973**, *18*, 459. (b) Umezawa, H. In "Bleomycin: Current Status and New Developments"; Carter, S. K.; Crooke, S. T.; Umezawa, H., Eds.; Academic Press: New York, 1978; p 15 ff. (c) Hecht, S. M. In "Bleomycin: Chemical, Biochemical and Biological Aspects"; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979; p 1 ff.

(2) (a) Umezawa, H. *Prog. Biochem. Pharmacol.* **1976**, *11*, 18. (b) Ichikawa, T. *Ibid.* **1976**, *11*, 143. (c) Carter, S. K.; Blum, R. H. *Ibid.* **1976**, *11*, 158. (d) Bonadonna, G.; Tancini, G.; Bajetta, E. *Ibid.* **1976**, *11*, 172. (e) Depierre, A. *Ibid.* **1976**, *11*, 195. (f) Rygard, J.; Hansen, H. S. *Ibid.* **1976**, *11*, 205. (g) Rathert, P.; Lutzeyer, W. *Ibid.* **1976**, *11*, 223.

(3) (a) Tanaka, W. *J. Antibiot. (Tokyo)* **1977**, *30*, S-41. (b) Matsuda, A.; Yoshioka, O.; Ebihara, K.; Ekimoto, H.; Yamashita, T.; Umezawa, H. In "Bleomycin: Current Status and New Developments"; Carter, S. K.; Crooke, S. T.; Umezawa, H., Eds.; Academic Press: New York, 1978; p 299 ff.

(4) Levin, M. D.; Subrahamanian, K.; Katz, H.; Smith, M. B.; Burlett, D. J.; Hecht, S. M. *J. Am. Chem. Soc.* **1979**, *102*, 1452.

(5) (a) Cheng, K. Y. Z.; Cheng, C. C. *J. Heterocycl. Chem.* **1970**, *7*, 1439. (b) McGowan, D. A.; Jordis, U.; Minster, D. K.; Hecht, S. M. *J. Am. Chem. Soc.* **1977**, *99*, 8078. (c) Yoshioka, T.; Hara, T.; Takita, T.; Umezawa, H. *J. Antibiot. (Tokyo)* **1974**, *27*, 356. (d) Hecht, S. M.; Burlett, D. J.; Mushika, Y.; Kuroda, Y.; Levin, M. D., ref 1c, p 48 ff.

(6) (a) Takita, T.; Yoshioka, T.; Muraoka, Y.; Maeda, K.; Umezawa, H. *J. Antibiot. (Tokyo)* **1971**, *24*, 795. (b) Hecht, S. M.; Rupprecht, K. M.; Jacobs, P. M. *J. Am. Chem. Soc.* **1979**, *101*, 3982.

(7) (a) Omoto, S.; Takita, T.; Maeda, K.; Umezawa, S. *Carbohydr. Res.* **1973**, *30*, 239. (b) Minster, D. K.; Hecht, S. M. *J. Org. Chem.* **1978**, *43*, 3987.

(8) (a) Muraoka, Y.; Takita, T.; Maeda, K.; Umezawa, H. *J. Antibiot. (Tokyo)* **1970**, 252. (b) Yoshioka, T.; Muraoka, Y.; Takita, T.; Maeda, K.; Umezawa, H. *Ibid.* **1972**, *25*, 625. (c) Umezawa, Y.; Morishima, H.; Yoshioka, T.; Otsuka, M.; Ohno, M., ref 1c, p 63 ff.

(9) Kunishima, M.; Fujii, T.; Nakayama, Y.; Takita, T.; Umezawa, H. *J. Antibiot. (Tokyo)* **1976**, *29*, 853.

(10) Satisfactory spectral and analytical data were obtained for the new compounds reported.

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