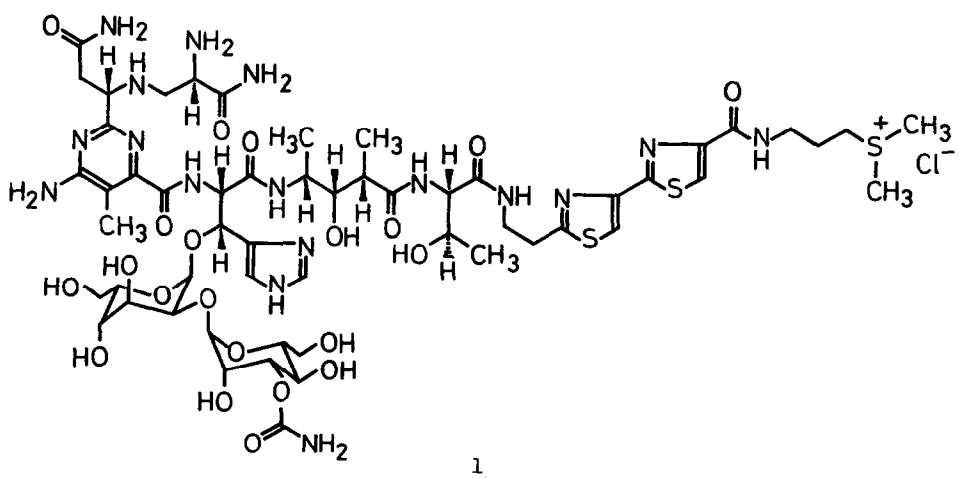
Tetrahedron Letters,Vol,23,No.5,pp 521-524,1982 0040-4039/82/050521-04$03.00/0
Printed in Great Britain

TOTAL SYNTHESIS OF BLEOMYCIN A2[1)]

Tomohisa Takita, Yoji Umezawa, Sei-ichi Saito, Hajime Morishima,
Hiroshi Naganawa, Hamao Umezawa*
Institute of Microbial Chemistry
Kamiosaki, Shinagawa-ku, Tokyo 141, Japan
Tsutomu Tsuchiya, Toshiaki Miyake, Shunji Kageyama, Sumio Umezawa
Institute of Bioorganic Chemistry
Ida, Nakahara-ku, Kawasaki 211, Japan
Yasuhiko Muraoka, Masanobu Suzuki
Pharmaceutical Division, Nippon Kayaku Co. Ltd.,
Shimo, Kita-ku, Tokyo 115, Japan
Masami Otsuka, Masatoshi Narita, Susumu Kobayashi and Masaji Ohno
Faculty of Pharmaceutical Sciences, University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan

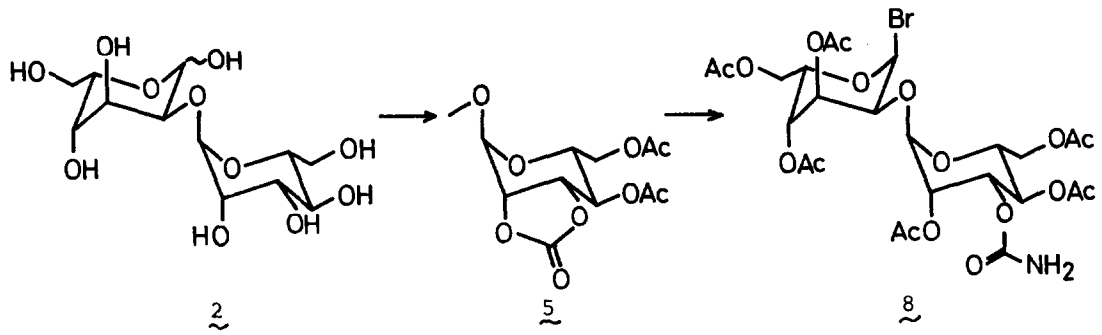
Summary: Bleomycin A2 has been synthesized for the first time.

Bleomycin (BLM) is an antitumor antibiotic clinically used in the treatment of squamous cell carcinoma, malignant lymphoma and testis tumors. BLM A2 (1)[2)], the major component of natural BLMs, consists of a linear hexapeptide named deglyco-BLM A2 and an O-carbamoyl disaccharide. We have already reported the syntheses of deglyco-BLM A2[3,4)] and 2-O-(α-D-mannopyranosyl)-L-gulose[5)], the disaccharide of BLM. Therefore, the total synthesis of BLM A2 is formally established by introduction of carbamoyl group to the disaccharide and glycosylation of deglyco-BLM A2 with the O-carbamoyl disaccharide. In this communication, we report the first total synthesis of BLM.



1

Introduction of carbamoyl group to the 3-hydroxyl group on the mannose moiety of the disaccharide of BLM



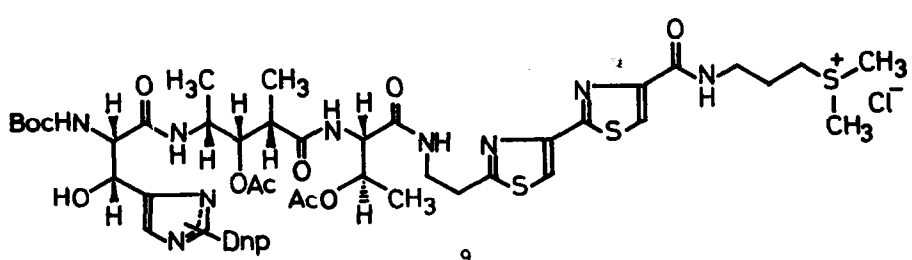
2 5 8

3,4,6-Tri-O-acetyl-2-O-(2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl bromide (8) was prepared from 2-O-(α-D-mannopyranosyl)-L-gulopyranose[5] (2) by the following pathway. Treatment of 2 with t-$BuMe_2SiCl$/imidazole (in DMF, room temp., 2 days) gave 6,6'-di- (3, 51%) and 1,6,6'-tri-O-silyl derivatives (3', 20%). Treatment of 3 and 3' with N,N'-carbonyldiimidazole (in THF, room temp., overnight) gave the corresponding 2',3'-cyclic carbonates (4, 58%; 4' 86%). Removal of the silyl protecting groups (with Bu_4NF in THF) from 4 and 4' followed by acetylation gave 1,3,4,6,4',6'-hexa-O-acetyl-2'-O:3'-O-carbonyl derivative (5, 65% from 4 and 59% from 4'). Dissolving of 5 in liquid ammonia (-75°C, then gradually to room temperature to evaporate ammonia) followed by acetylation gave a mixture of per-O-acetylated 2'-O-carbamoyl (6, 28%) and 3'-O-carbamoyl (7, 15%) β derivatives. The latter was led to 8 with HBr/CH_2Cl_2, 0°C, overnight, (^{1}H-NMR in $CDCl_3$: δ2.08, 2.09, 2.12, 2.15 (6H), 2.23 for acetyls; δ5.20 s, H-1'; δ6.43 slightly br. d, J 4.0 Hz, H-1), and used for the glycosylation in the total synthesis of BLM.

Glycosylation and total synthesis of BLM A2

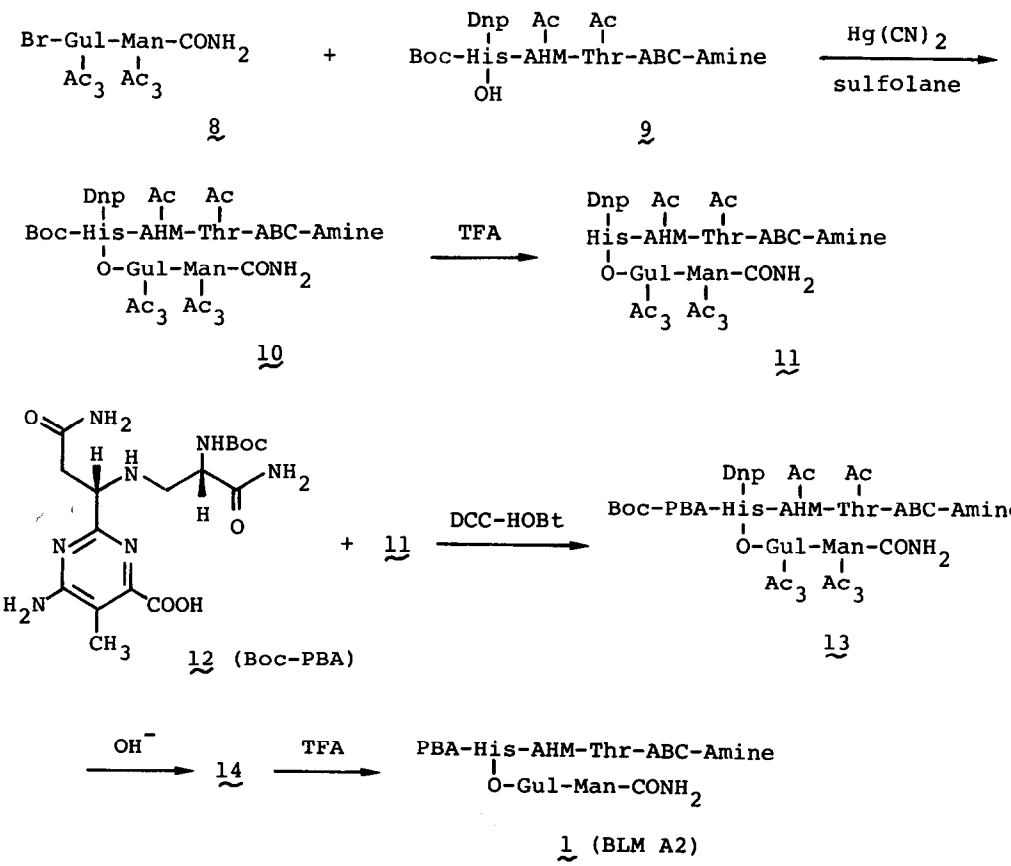
Several attempts to glycosylate a protected deglyco-BLM A2[4] with 8 failed due to undesired reactions. Therefore, to minimize the undesirable reactions, a protected pentapeptide 9, which was synthesized in the other paper[4], was chosen as the reactant for the glycosylation.

Protected pentapeptide 9 was allowed to react with 8 in anhydrous sulfolane in the presence of $Hg(CN)_2$ and molecular sieve 4A at 40°C overnight.



9

The reaction mixture was passed through a column of Sephadex LH-20 developed with methanol and an eluate containing a mixture of the reaction products including 10 was collected. The mixture was used without further separation in the following reactions because of the instability[6]. After treatment with TFA to remove the Boc-protecting group, the resulting mixture[6] containing 11 was allowed to react with Boc-protected pyrimidoblamic acid[7] (12) by DCC-HOBt in DMF overnight at room temperature. The resulting product[6] containing 13 was treated with 0.1M NaOH-MeOH (1:1) at room temperature overnight to deprotect the Dnp and acetyl groups. At this stage, the reaction products were analyzed by silica gel TLC developed with 10% $AcONH_4$-MeOH (1:1). The main product gave the Rf-value of 0.49, which was different from that of desired Boc-protected BLM A2 (14)[8] (Rf=0.47), although the spot of 14 was detected on the TLC. The mixture containing 14 was treated with TFA at 0°C for 30 min. to deprotect the Boc-group. The products were transformed to the Cu-complexes and analyzed by TLC under the same condition described above. The main product gave the Rf-value of 0.08 and BLM A2 was found at Rf 0.51 as a minor product. The spot of BLM A2 showed antimicrobial activity by bioautography.[9] Thus, the formation of BLM A2 was secured at this point. The



mixture containing BLM A2 Cu-complex was separated by a column chromatography of CM-Sephadex C-25, pretreated with 0.05 M sodium acetate buffer of pH 4.5, developed with a linear gradient of NaCl. A bioactive fraction containing BLM A2 and a fraction containing the main reaction product[10] were collected.

The BLM fraction was rechromatographed on CM-Sephadex C-25, pretreated with 0.05 M sodium phosphate buffer of pH 7.1, developed with a linear gradient of NaCl. The bioactive fraction was desalted and decoppered on an Amberlite XT-2 column by adsorption, washing (with EDTA) and desorption [with 0.002M HCl-MeOH (1:4)], and colorless metal-free BLM A2 was obtained. The synthetic and natural samples of BLM A2 were identical in all respects as measured by TLC, HPLC and ^{1}H-NMR. In particular, the purity including stereochemistry was ascertained by the fine structure of the high magnetic field ^{1}H-NMR spectrum (250 MHz). Thus, the total synthesis of BLM has been achieved for the first time.

References and Notes

1. Presented at the Seventh American Peptide Symposium, Madison, Wisconsin, U.S.A., June, 1981.
2. T. Takita, Y. Muraoka, T. Nakatani, A. Fujii, Y. Umezawa, H. Naganawa and H. Umezawa, J. Antibiot., 31, 801 (1978).
3. T. Takita, Y. Umezawa, S. Saito, H. Morishima, H. Umezawa, Y. Muraoka, Y. Suzuki, M. Otsuka, S. Kobayashi and M. Ohno, Tetrahedron Lett., 22, 671 (1981).
4. S. Saito, Y. Umezawa, H. Morishima, T. Takita, H. Umezawa, M. Narita, M. Otsuka, S. Kobayashi and M. Ohno, Tetrahedron Lett., following paper in this issue.
5. T. Tsuchiya, T. Miyake, S. Kageyama, S. Umezawa, H. Umezawa and T. Takita, Tetrahedron Lett., 22, 1413 (1981).
6. The compounds having Dnp-function are only stable under acidic and dark conditions.
7. Y. Umezawa, H. Morishima, S. Saito, T. Takita, H. Umezawa, S. Kobayashi, M. Otsuka, M. Narita and M. Ohno, J. Am. Chem. Soc., 102, 6630 (1980).
8. Authentic sample of Boc-protected BLM A2 (14) is obtained by butoxycarbonylation of natural BLM A2 with tert-butyl S-(4,6-dimethylpyrimidin-2-yl)thiocarbonate.
9) Among the reaction products, only the spot of BLM A2 showed the bioactivity.
10) The main reaction product was obtained by desalting and decoppering. The structure was elucidated to be an anomeric mixture of the N(imidazole)-glycoside of deglyco-BLM A2 by ^{1}H-NMR study. The chemical shifts (in D_2O) of the anomeric (α, δ6.24; β, 5.84) and imidazole (α, 7.36, 8.80; β, 7.18, 8.08) protons were distinctly different from those of BLM A2 (5.28, 7.52, 8.50) at pH 5.13∿5.18.

(Received in Japan 17 October 1981)