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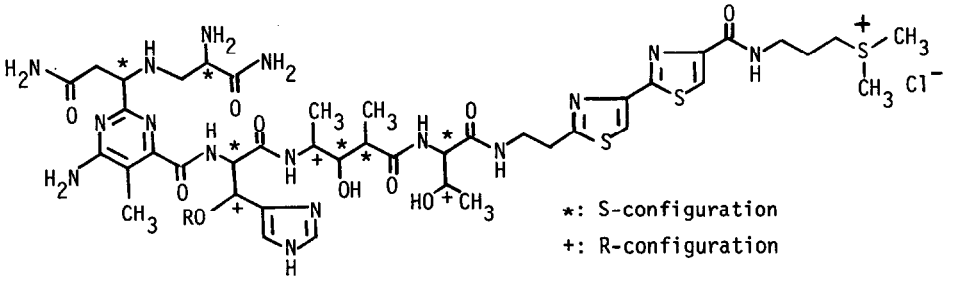
TOTAL SYNTHESIS OF DEGLYCO-BLEOMYCIN A2

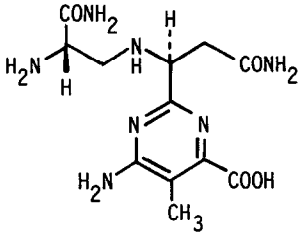
Tomohisa Takita*, Yoji Umezawa, Sei-ichi Saito,
Hajime Morishima, Hamao Umezawa
Institute of Microbial Chemistry
Kamiosaki, Shinagawa-ku, Tokyo 141, Japan
Yasuhiko Muraoka, Masanobu Suzuki
Pharmaceutical Division, Nippon Kayaku Co. Ltd.,
Shimo, Kita-ku, Tokyo 115, Japan
Masami Otsuka, Susumu Kobayashi and Masaji Ohno
Faculty of Pharmaceutical Sciences,
University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan

Summary: Deglyco-bleomycin A2, the aglycon of bleomycin A2, has been synthesized for the first time.

Bleomycin (BLM) is an antitumor antibiotic clinically used in the treatment of squamous cell carcinoma and malignant lymphoma.[1] In addition to its medicinal importance, BLM is of great interest because of its complicated structure[2,3] and unique mechanism of action.[3-6] BLM A2 (1-a) is the major component of the BLM clinically used at the present time. Deglyco-BLM A2 (1-b), the



(1-a) R: 2-O-(3-O-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl
(1-b) R: H



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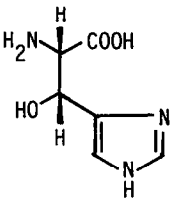

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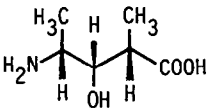

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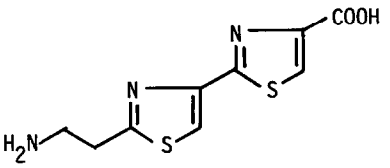

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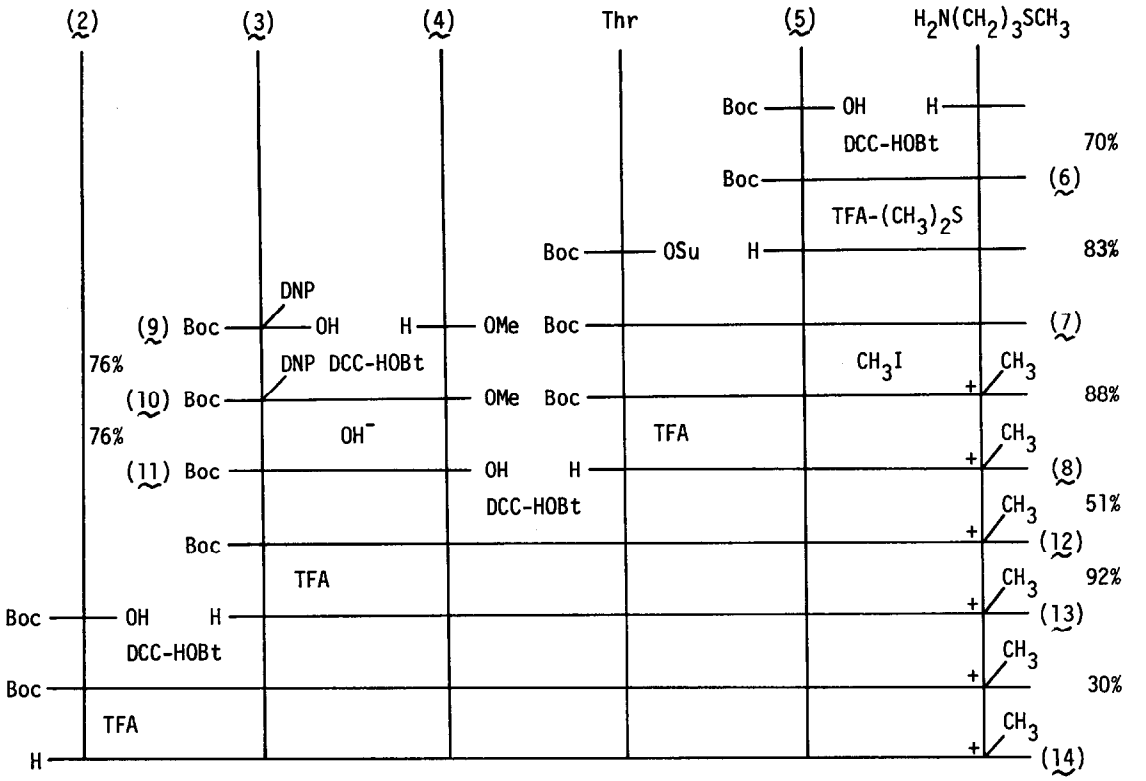
peptide part of BLM A2, consists of six components: pyrimidoblamic acid (2)[7], erythro-β-hydroxy-L-histidine (3), (2S,3S,4R)-4-amino-3-hydroxy-2-methylpentanoic acid (4), L-threonine (Thr), 2'-(2-aminoethyl)-2,4'-bithiazole-4-carboxylic acid (5), and (3-aminopropyl)-dimethylsulfonium chloride, the last being the terminal amine characteristic of BLM A2. These six components are connected to each other through peptide bonds in the above order to form deglyco-BLM A2. The novel amino acids, 2[7], 3[8] and 4[9] have already been synthesized by us, and 5 was first synthesized by Zee-Cheng and Cheng.[10] This communication describes the first total synthesis of deglyco-BLM A2, a process featuring the condensation of the following three fragments: pyrimidoblamic acid (2), polyfunctional dipeptide (3-4) and tripeptide S[11] (Thr-5-terminal amine). The process has the further advantage that racemization is minimized and it also provides a path to the efficient syntheses of BLM and its analogues.

Synthesis of tripeptide S. N-tert-Butoxycarbonyl-5[12] (Boc-5), mp 179-180°C, prepared by treatment of 5 with tert-butyl S-(4,6-dimethylpyrimidin-2-yl) thiocarbonate[13] (Boc-S) in almost quantitative yield, was coupled with 3-(methylthio)propylamine by dicyclohexylcarbodiimide in the presence of 1-hydroxybenztriazole (DCC-HOBt)[14] to give 6[12] [70%, colorless plates from AcOEt-Et_2O, mp 122°C]. The compound 6 was deprotected with trifluoroacetic acid (TFA) in the presence of dimethylsulfide to avoid S-alkylation and then treated with Boc-threonine N-hydroxy-succinimide ester[15] to afford 7[12] [83%, needles from AcOEt-iPr_2O, mp 106-107°C, $[\alpha]_D^{24}$=-20° (c=1, MeOH)]. The terminal amine residue was formed in situ by S-methylation of 7 with methyl iodide.[16] Tripeptide S (8) was obtained upon deprotection of the methylated product with TFA. The tripeptide S was transformed into the hydrochloride of the sulfonium chloride via the free base formed by treatment with Dowex 1 (OH^-) [88% yield from 7, $[\alpha]_D^{23}$=-15° (c=0.75, 0.1N HCl)]. The identity of the product was confirmed by TLC, IR and ^{1}H-NMR comparison with a natural sample[11], $[\alpha]_D^{23}$=-15° (c=0.75, 0.1N HCl).

Synthesis of dipeptide (3-4). Preliminary studies indicated that the protection of the imidazole function of 3 was necessary during the coupling with 4. Several protecting groups were examined and the 2,4-dinitrophenyl (DNP) group was selected for the synthesis. N^{α}-Boc-3, prepared by treatment of 3 with Boc-S in 86% yield, was reacted with 1-fluoro-2,4-dinitrobenzene to give N^{α}-Boc-N^{im}-DNP-3 (9) [77%, needles from MeOH, mp 107-113°C (dec.)]. The latter was coupled by DCC-HOBt in dimethylformamide (DMF) with the methyl ester of 4 (which was prepared by quantitative esterification of 4 with MeOH-HCl) to afford 10 in 76% yield. Treatment of 10 with 0.1N NaOH-MeOH (1:1) at room temperature gave 11 along with a small amount of Boc-3 and γ-lactam of 4.



After silica gel chromatography with AcOEt-EtOH-H_2O (10:5:1), 11 was isolated in 76% yield, $[\alpha]_D^{25}$=+11.5° (c=1, H_2O). The optical purity of 11 was strongly supported by a single spot in silica gel TLC [for example: Rf=0.59 BuOH-AcOH-H_2O (3:1:2)] and ^{1}H-NMR spectrometry [δ7.65 (1H, d, J=<1 Hz), 7.04 (1H, d, <1), 4.95 (1H, d, 7), 4.38 (1H, d, 7), 4.00 (1H, m, 7, 5), 3.63 (1H, q, 5, 7), 2.30 (1H, m, 7), 1.42 (9H, s), 1.15 (3H, d, 7), 1.08 (3H, d, 7) in MeOH-d_4].

Fragment condensation. The two fragments, 8 and 11, were prepared as described above. The first unambiguous synthesis of the remaining fragment, N^{α}-Boc-2, has been recently reported by us.[7] Although there are two free basic functions, a secondary amine and a 4-aminopyrimidine in N^{α}-Boc-2, the protection of these groups appeared to be unnecessary due to their poor reactivity.[7,17] Fragment 11 was coupled with tripeptide S (8) by DCC-HOBt in DMF. The product (12) was purified by Sephadex LH-20 chromatography developed with methanol (51% yield). The structure and optical purity of 12 were confirmed by TLC, ^{1}H-NMR and FDMS (M^+, m/z 840). Deprotection of 12 with TFA afforded pentapeptide (13) [92%, $[\alpha]_D^{26}$=+18° (c=0.5, 0.1N HCl)]. The coupling of 13 and N^{α}-Boc-2 was also successfully achieved by DCC-HOBt in DMF. After removal of the coupling reagents, the product was, without further purification, subjected to deprotection with TFA. To achieve a clear-cut separation, deglyco-BLM A2 (14) thus obtained was converted to a Cu(II)-complex and then purified by CM-Sephadex C-25 chromatography developed with linear gradient of NaCl[18]. The blue eluate containing deglyco-BLM A2 Cu(II)-complex was treated with excess EDTA under acidic

condition and charged on a column of Amberlite XAD-2. The column was washed with 5% EDTA·2Na followed by water to remove the chelated copper and NaCl.[19] The purified colorless deglyco-BLM A2 free of metal was obtained by elution with 0.002N HCl-MeOH (1:4) (30% yield from 13).

Deglyco-BLM has been found in a trace amount in the culture broth of BLM fermentation[20], and was also recently isolated from a mild acid hydrolysate of BLM.[21] The synthetic and natural samples of deglyco-BLM A2 were identical in all respects as measured by TLC, high pressure LC, ^{1}H- and ^{13}C-NMR, and optical rotation, $[\alpha]_D^{24}$ (c=0.5, 0.1N HCl), -15° (synthetic), -15° (natural). In particular, the optical purity was ascertained by the fine structure of the high magnetic field ^{1}H-NMR spectrum (250 M Hz). Thus, the total synthesis of deglyco-BLM A2 has been successfully achieved for the first time.

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