Communications to the editor

AN IMPROVED TOTAL SYNTHESIS OF BLEOMYCIN*

Sir:

In 1981, we were successful in the total synthesis of bleomycin (BLM) for the first time[1,2]. We have continued studies to increase the yield, and we now report an improved total synthesis of BLM.

The new strategy is: 1) the hydroxyl group of *erythro*-β-hydroxy-L-histidine (**1**) is first glycosidated with disaccharide (**2**), 2) tetrapeptide S[3] (**3**) is connected to the carboxyl group of the *O*-glycosidated **1**, and finally, 3) pyrimidoblamic acid (PBA)[4] (**4**) is linked to the amino group of the glycosidated pentapeptide.

Compound **1** was readily prepared as follows. DL-Mixture of **1** was stereoselectively synthesized by condensation of 4-formylimidazole[5] and *N*-pyruvylideneglycinatocopper (II)[6] at pH 10.3 (70 % yield) by a modification of ISHIDO's method for the preparation of α-amino-β-hydroxy acids[7,8]. This reaction was highly stereospecific; the *threo*-isomer could not be detected in the reaction product[8]**. The high stereospecificity of this reaction can be reasonably explained by coordination of the imidazole to the metal (Fig. 1). Compound **1** was isolated by resolution by diastereoisomeric co-crystallization with D(−)-tartaric acid from the DL-mixture quite efficiently.

For the selective *O*-glycosidation of **1**, three functional groups except the hydroxyl group of **1** were protected as follows. Methyl ester of **1** (**5**) was prepared by treatment of **1** with MeOH-HCl (94 %, mp 168~169°C). It was treated with *tert*-butyl *S*-(4,6-dimethylpyrimidin-2-yl)thiocarbonate (Boc-S)[10] to give **6** [45%, mp 132°C, $[\alpha]_D^{22}$ +13.1° (*c* 1, MeOH)]. The N_{im}-tosyl derivative of **6** (**7**) was prepared by treatment of **6** with tosyl chloride-Na_2CO_3 in dioxane-water [83%, mp 45~47°C, $[\alpha]_D^{26}$ +7.95° (*c* 0.73, MeOH)].

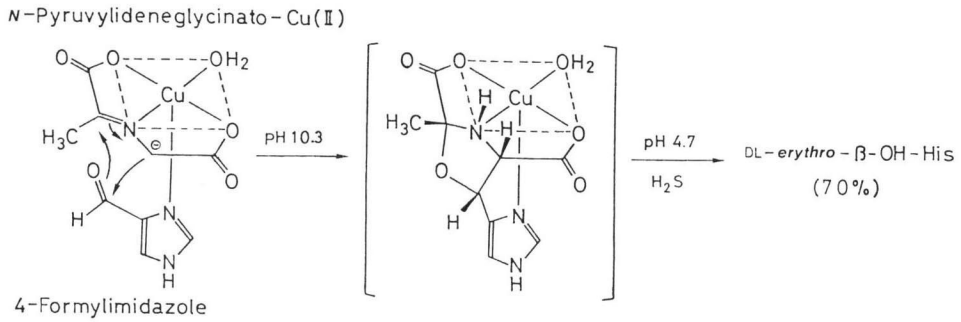
Compound **7** was reacted with the α-bromide of peracetyl **2** (**8**)[2] in CH_2Cl_2 using $AgClO_4$-$AgCO_3$ as a catalyst in the presence of molecular sieve 3A at room temperature for 3 hours. The main reaction product (**9**), which showed the Rf value 0.77 on a silica gel TLC developed with benzene-acetone (1:1), was isolated by silica gel chromatography developed with $CHCl_3$-MeOH (40:1). The secondary ion mass spectrum (SIMS) of **9** showed the MH^+ ion at *m/z* 1,059, which was in accord with the expected molecular weight. The ^1H NMR spectrum of **9** in $CDCl_3$ at 250 MHz showed that the signal of the C_1 proton of the gulose moiety appeared as a doublet (J = 3.5 Hz) at δ 5.12. This chemical shift and the small spin-spin coupling constant indicates that **9** is the expected α-*O*-glycoside. The yield of **9** was 21 % after purification.

All protective groups except the Boc-group of **9** were removed by mild alkaline treatment [0.1 N NaOH - MeOH (1:1) at 0°C for 15 hours]. The product (**10**) was, without purification, treated with dinitrofluorobenzene (DNFB) to give the N_{im}-DNP derivative (**11**). It was purified by Sephadex LH-20 (MeOH) and Amberlite XT-2 [0.002 N HCl - MeOH (1:4)] chromatography (71 %). The structure was confirmed by SIMS [*m/z* 805 (MH^+)] and ^1H NMR spectrometry. Compound **11** was coupled with tetrapeptide S (**3**)[3] by the DCC-HOBt method in DMF. The product was purified by Sephadex LH-20 and Amberite XT-2 chromatography to give **12** [51%, SIMS *m/z* 1,373 (M^+)]. It is important to note that the M^+, but not MH^+, appeared in the SIMS after the introduction of sulfonium cation. The Boc-group of **12** was deprotected with trifluoroacetic acid (TFA) to give **13** (91 %), and then, Boc-PBA[4] (**14**) was coupled with **13** by DCC-HOBt method in DMF. After purification by Sephadex LH-20 chromatography, **15** was obtained in 83 % yield. The SIMS of **15** neither gave the M^+ nor information on the molecular weight, though the M^+ of BLM A2 was easily observed at *m/z* 1,415 by our mass spectrometer (Hitachi M-80H). However, the ^1H NMR spectrum showed all expected signals and no others. The protective groups of **15** were successively removed by 0.1 N NaOH (DNP)

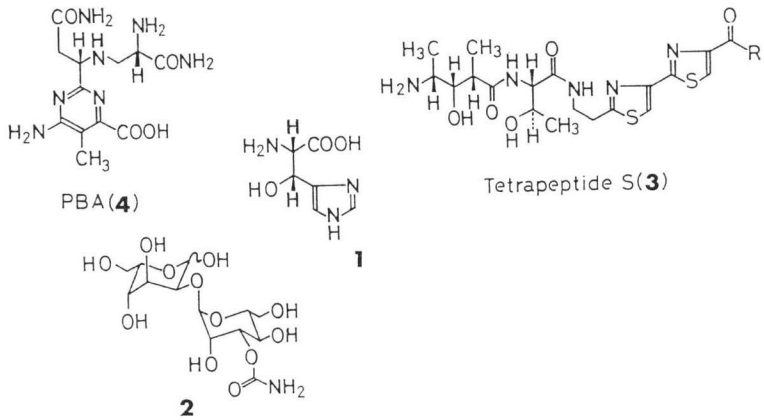
* Presented at the fourth International Conference on Organic Synthesis; Tokyo, Japan, August 27, 1982.

** Generally, *threo*-isomers are predominatly yielded by ISHIDO method[7,9].

Fig. 1. Synthesis of *erythro*-β-hydroxyhistidine (β-OH-His).



Drawing 1.



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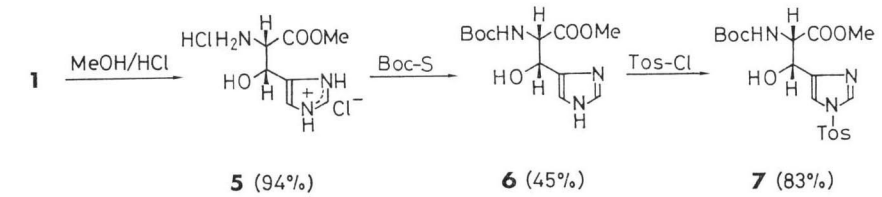

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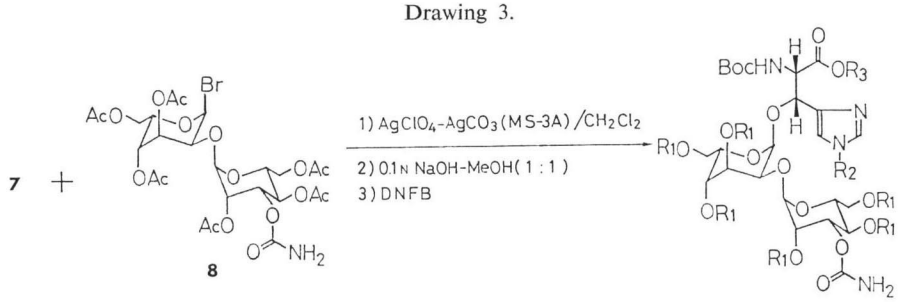
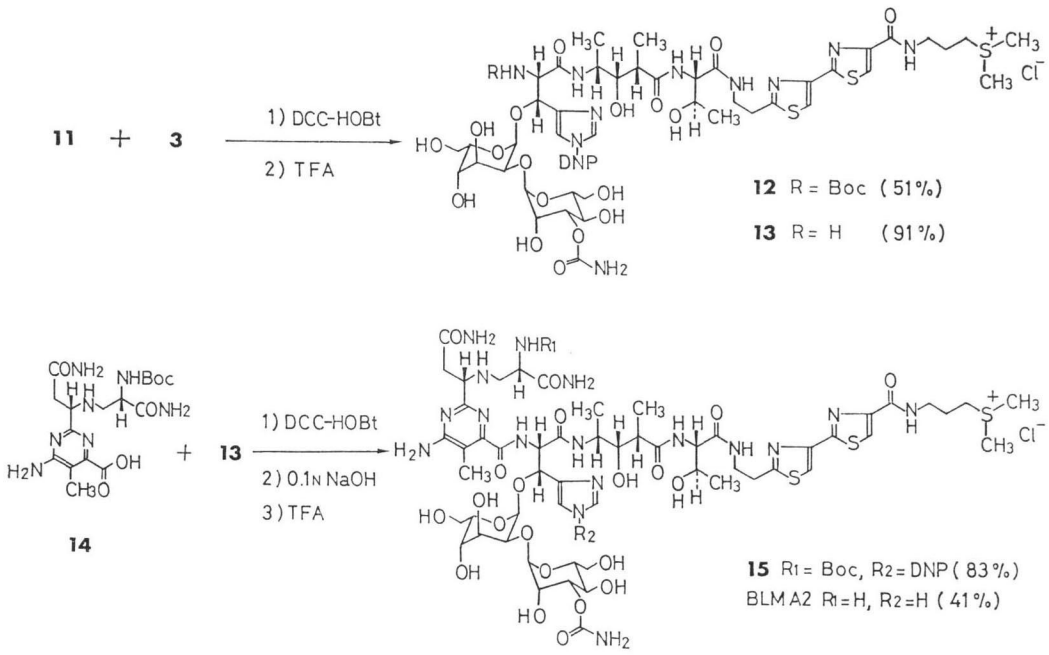


9 R_1=Ac, R_2=Tos, R_3=Me (21%)
10 R_1=H, R_2=H, R_3=H
11 R_1=H, R_2=DNP, R_3=H (71%)

Drawing 4.



and TFA (Boc), and the product was purified by the method described previously[2] to give pure BLM A2 (41%).

In this improved total synthesis of BLM, it must be emphasized that the desired α-*O*-glycoside is the main product in the glycosidation reaction. This success opens the way to prepare new synthetic BLMs, which may be more effective than natural BLMs. We are currently developing new useful BLMs by synthesis.

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(Received October 30, 1982)

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