CHEMISTRY LETTERS, pp. 83-86, 1988.

Enantioselective Synthesis of erythro-β-Hydroxy-L-histidine, the Pivotal Amino Acid of Bleomycin-Fe(II)-O_2 Complex[1a)]

Takashi OWA, Masami OTSUKA, and Masaji OHNO*
Faculty of Pharmaceutical Sciences, The University of Tokyo,
Hongo, Bunkyo-ku, Tokyo 113

erythro-β-Hydroxy-L-histidine, a novel amino acid constituent of bleomycin, has been synthesized enantioselectively via the reaction of (N-pyruvylideneglycyl-D-phenylalaninato)copper(II) with imidazole-4-carbaldehyde.

Bleomycins (BLMs) are a group of antitumor antibiotics clinically used for the treatment of Hodgkin's lymphoma, tumors of testis, and carcinomas of skin, head, and neck.[2)] The potent activity of BLM is attributed to the oxygen activation[3)] and the DNA cleavage[4)] by the formation of a unique iron-chelate of the β-aminoalanine-pyrimidine-β-hydroxyhistidine moiety of the unusual glycopeptide (Fig. 1).[5)] Previously we have reported synthetic models of BLM in which erythro-β-hydroxy-L-histidine (**1**) was shown to be a key amino acid as an irreplaceable moiety of the metal binding site.[6)] The L-erythro stereochemistry of **1** appears to be particularly important in defining the spatial relationships between the metal binding site, the DNA binding site, and the disaccharide moiety. For further development of new man-designed BLMs, we required a facile access to **1** which is the subject of the present paper.

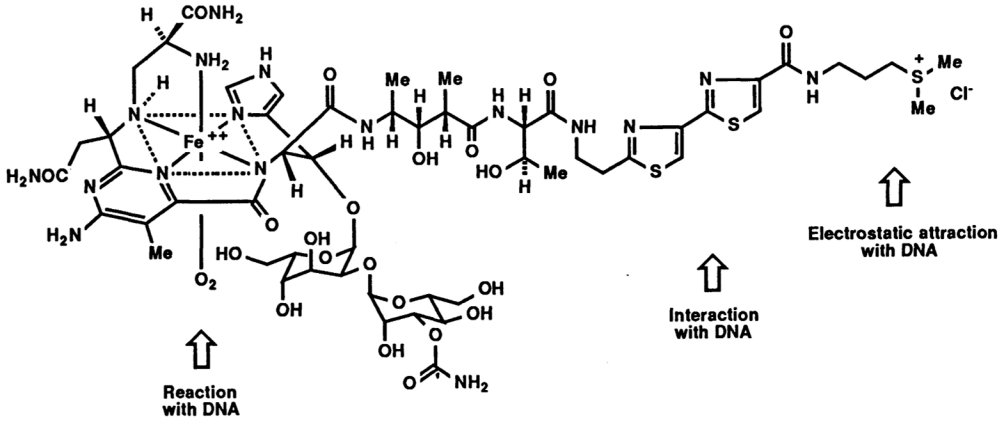
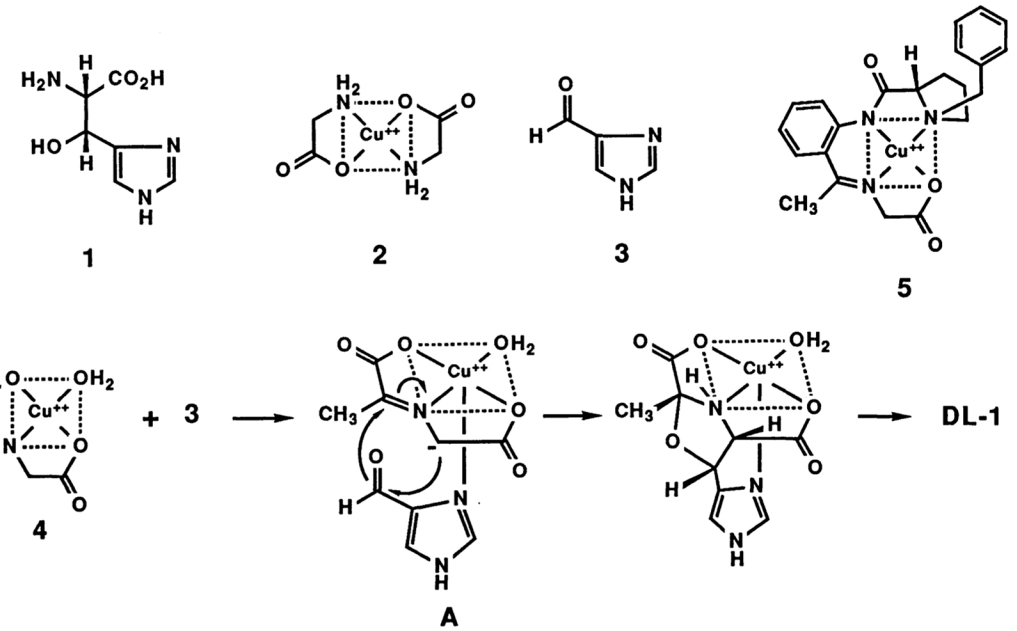


Fig. 1.

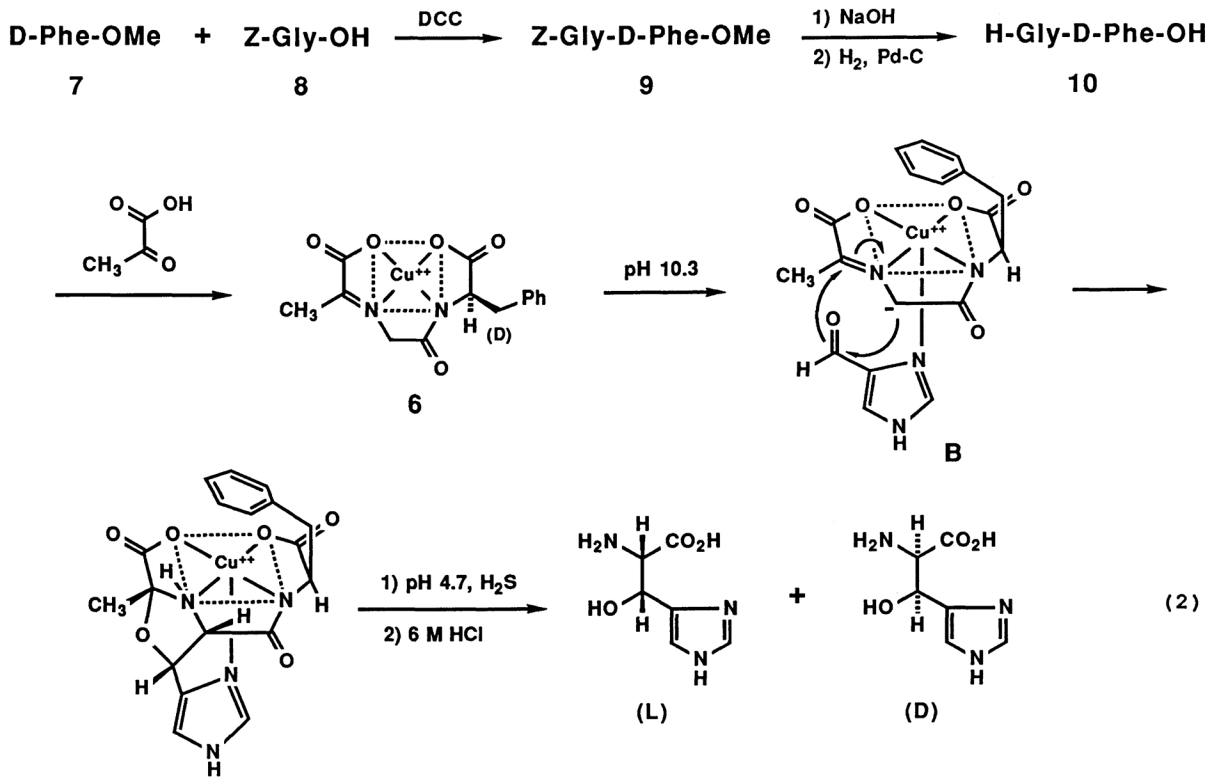
The first synthesis of erythro-β-hydroxy-L-histidine was reported by Takita et al. based on the Akabori reaction of bis(glycinato)copper(II) (**2**) and imidazole-4-carbaldehyde (**3**) to afford a mixture of four racemic threo and erythro isomers which had to be separated from each other.[7)] Yoshioka et al. improved this process by employing the Ishido's method[8)] using N-pyruvylidene-



glycinatocopper(II) (**4**), furnishing a racemic mixture of erythro-β-hydroxyhistidine, from which the desired L-erythro isomer was separated by a co-crystallization with D-tartaric acid.[9] The erythro selectivity of this process was accounted for by co-ordination of the imidazole to the metal (**A**, Eq. 1). It was considered that the L-erythro isomer **1** could be selectively available by shielding the front side of the co-ordination plane of **A** to avoid the undesired co-ordination of the imidazole. Although a complex containing N-benzylproline as a chiral auxiliary **5** has been reported for the enantioselective synthesis of threonine,[10] β-hydroxyhistidine could not be obtained by the reaction of **5** with aldehyde **3**. After extensive experimentations, we found that base-catalyzed reaction of (N-pyruvylideneglycyl-D-phenylalaninato)copper(II) **6** with aldehyde **3** afforded **1** stereoselectively.

Thus, D-phenylalanine methyl ester **7**[11] was coupled with N-benzyloxycarbonylglycine **8**[12] (DCC, THF-$CHCl_3$, -10 °C, overnight) to give the protected dipeptide **9** in 89% yield.[13] The protective groups in **9** were removed by ester hydrolysis (1 M NaOH, MeOH) followed by the hydrogenation (H_2, Pd-C, MeOH-AcOH-H_2O) to give glycyl-D-phenylalanine **10** in 89% yield; mp 262-265 °C (lit. 263-265 °C)[14a]; $[\alpha]_D^{23}$ -40.4° (c 2.5, water) (lit. $[\alpha]_D$ -41.0° (c 2.5, water)).[14a, 14b]

The copper complex **6** was prepared as follows. Pyruvic acid (1.15 ml, 16.0 mmol) was added to a suspension of the dipeptide **10** (3.55 g, 16.0 mmol) in water-EtOH (5-8 ml). The mixture was stirred at 40 °C for 30 min to give a yellow homogeneous solution of Schiff base, then $Cu(OAc)_2 \cdot H_2O$ (3.19 g, 16.0 mmol) was added. The resulting dark blue solution was stirred at 40 °C for 2 h and then at room temperature overnight. The precipitate of copper complex **6** was collected, washed successively with water, EtOH, and Et_2O, and then dried in vacuo. To a suspension of the copper complex **6** in water (16 ml) was added aldehyde **3**[15] (1.54 g, 16.0 mmol). After being stirred at room temperature for 2 h the pH of the solution was adjusted to 10.3-10.7 by adding anhydrous Na_2CO_3 and



the solution was stirred for further 5-7 h. Then, the solution was acidified to pH 4.3 with 1 M HCl and treated with H_2S gas. The precipitate of CuS formed was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in 6 M HCl (40 ml). The resulting solution was heated at 100-110 °C for 12-18 h to facilitate hydrolysis of the peptide bond, and was concentrated to dryness in vacuo. The residue containing glycine, D-phenylalanine, erythro-β-hydroxyhistidine, and unreacted **3** was separated by microcrystalline cellulose column chromatograohy (eluted with MeOH : H_2O : Pyridine = 8 : 2 : 0.4). Pauly- and ninhydrin-positive fractions were collected, treated with activated charcoal, and crystallized from EtOH to give erythro-β-hydroxyhistidine (1.35 g, 49.2%). The erythro-β-hydroxyhistidine was proved to be a mixture of L-form and D-form in a ratio of 3 : 1 by HPLC (DAICEL, CHIRALPAK WH; column 4.6 mm x 250 mm; solvent 0.5 mM $CuSO_4$; flow rate 1.0 ml / minute; temperature 25 °C), showing two peaks for L-erythro isomer **1** (retention time, 46 minutes) and D-erythro isomer **11** (retention time, 17 min). Although the enantioselectivity is not enough (50% ee), co-crystallization of this material with D-tartaric acid was carried out much smoothly to afford optically pure erythro-β-hydroxy-L-histidine; dp 203-204 °C (lit. 205 °C);[7] $[\alpha]_D^{25}$ +39.5° (c 1.0, water))(lit. $[\alpha]_D^{25}$ +40.0° (c 1.0, water))[7] The enantioselectivity of the reaction can be explained by considering that the desired conformationally restricted form **B** seems indeed preferred, but not exclusive. However, the present approach allowed us to synthesize 1-10 g of **1** enantioselectively for the first time and further study is now under progress to improve the enantioselectivity.

This study was finantially supported in part by Grants-in-Aid (No. 62114006)

for Special Project Research from the Ministry of Education, Science, and Culture, Japan, and Uehara Memorial Foundation.

References

1) a) Synthetic Studies on an Antitumor Antibiotic, Bleomycin. XXI; b) Part XVIII: M. Ohno and M. Otsuka, "Stereochemistry of Organic and Bioorganic Transformations," ed by W. Bartmann and K. B. Sharpless, VCH Verlagsgesellschaft, Weinheim (1987), pp. 147-167; c) Part XIX: M. Ohno, M. Otsuka, A. Kittaka, Y. Sugano, Y. Sugiura, T. Suzuki, J. Kuwahara, K. Umezawa, and H. Umezawa, Int. J. Exp. Clin. Chemotherapy, in press; d) Part XX: M. Otsuka, A. Kittaka, Y. Sugano, T. Owa, M. Ohno, Y. Sugiura, and H. Umezawa, Proceedings of Japan Symposium on Peptide Chemistry, in press.

2) H. Umezawa, Lloydia (Cinci), 1977, 67.

3) Y. Sugiura, J. Am. Chem. Soc., 102, 5208 (1980) and references cited therein.

4) H. Kasai, H. Naganawa, T. Takita, and H. Umezawa, J. Antibiot., 31, 1316 (1978); M. Chien, A. P. Grollman, and S. B. Horwitz, Biochemistry, 18, 96 (1977).

5) Y. Sugiura, T. Takita, and H. Umezawa, "Metal Ions in Biological Systems," ed by H. Sigel, Dekker, New York (1985), Vol. 19, pp. 81-108 and references cited therein.

6) A. Kittaka, Y. Sugano, M. Otsuka, M. Ohno, Y. Sugiura, and H. Umezawa, Tetrahedron Lett., 27, 3631 (1986); Y. Sugano, A. Kittaka, M. Otsuka, M. Ohno, Y. Sugiura, and H. Umezawa, ibid., 27, 3635 (1986); M. Otsuka, A. Kittaka, M. Ohno, T. Suzuki, J. Kuwahara, Y. Sugiura, and H. Umezawa, ibid., 27, 3639 (1986).

7) T. Takita, T. Yoshioka, Y. Muraoka, K. Maeda, and H. Umezawa, J. Antibiot., 24, 795 (1971).

8) T. Ichikawa, S. Maeda, T. Okamoto, Y. Araki, and Y. Ishido, Bull. Chem. Soc. Jpn., 44, 2779 (1971).

9) T. Yoshioka, Dissertation, The University of Tokyo, 1977; S. Saito, Y. Umezawa, T. Yoshioka, T. Takita, H. Umezawa, and Y. Muraoka, J. Antibiot., 36, 92 (1983); Multistep synthesis of optically active **1** starting with D-glucosamine has been reported; S. M. Hecht, K. M. Rupprecht, and P. M. Jacobs, J. Am. Chem. Soc., 101, 3982 (1979).

10) Y. N. Belokon', I. E. Zel'tzer, M. G. Ryzhov, M. B. Saporovskaya, V. I. Bakhmutov, and V. M. Belikov, J. Chem. Soc., Chem. Commun., 1982, 180.

11) M. Brenner and W. Huber, Helv. Chim. Acta, 36, 1109 (1953).

12) M. Bergmann and L. Zervas, Chem. Ber., 65, 1192 (1932).

13) All new compounds gave satisfactory spectral and analytical data.

14) a) R. Hirschmann, R. G. Strachan, H. Schwam, E. F. Schoenewaldt, H. Joshua, B. Barkemeyer, D. F. Veber, W. J. Paleveda, Jr., T. A. Jacob, T. E. Beesley, and R. G. Denkewalter, J. Org. Chem., 32, 3415 (1967); b) R. Katakai and M. Oya, J. Org. Chem., 37, 327 (1972).

15) J. R. Totter and W. J. Darby, Org. Synth., 24, 64 (1944); F. L. Pyman, J. Chem. Soc., 1916, 186.

(Received October 22, 1987)