



# First synthesis of blastidic acid, a component amino acid in an antibiotic, blasticidin S

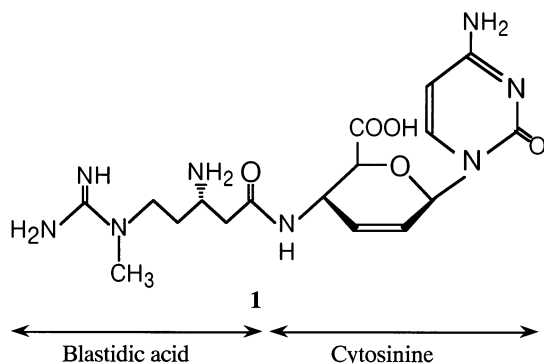
Shinya Nomoto\* and Akira Shimoyama

Department of Chemistry, University of Tsukuba, Tsukuba 305-8571, Japan

Received 29 November 2000; revised 20 December 2000; accepted 22 December 2000

**Abstract**—Blastidic acid, a component amino acid in an antibiotic, blasticidin S, was synthesized for the first time from  $\alpha,\gamma$ -diaminobutyric acid. The synthesis was achieved through elongation of the carbon chain of the starting amino acid, and *N*-methylation and amidation of an  $\omega$ -amino group. © 2001 Elsevier Science Ltd. All rights reserved.

Blasticidin S is an antibiotic produced by *Streptomyces griseochromogenes*, and has been used extensively as an excellent fungicide against *Piricularia oryzae*, a serious cause of rice blast disease in Japan.<sup>1</sup> The structure (1) was chemically determined by Otake et al.<sup>2</sup> and confirmed by X-ray analysis<sup>3</sup> to be composed of a guanidino  $\beta$ -amino acid moiety, blastidic acid, and a nucleoside moiety, cytosine. Although several groups have synthesized cytosine<sup>4,5</sup> and analogs of blasticidin S,<sup>6–8</sup> the synthesis of blastidic acid and total synthesis of this antibiotic have not hitherto been achieved. In this communication, we wish to report the first synthesis of L-blastidic acid from L- $\alpha,\gamma$ -diaminobutyric acid (A<sub>2</sub>bu).



In the synthetic path to the target, elongation of the carbon chain at the carboxyl end of A<sub>2</sub>bu, methylation of an  $\omega$ -amino group and amidation of a secondary

amino group must be performed in suitable order. The carbon chain elongation producing  $\beta$ -ornithine can be effected by the modified Arndt–Eistert reaction.<sup>9</sup> This conversion involves activation of a carboxyl group in an *N*-protected amino acid as a mixed anhydride, which is so reactive as to be liable to form a lactam with a guanidino or even a protected guanidino group. Therefore, the amidation should be carried out preferably after the Arndt–Eistert reaction. On the other hand, in preliminary studies on *N*-methylation reactions using *N* <sup>$\alpha$</sup> -*t*-butoxycarbonyl-A<sub>2</sub>bu, methyl iodide treatment of a tosylamino group in alkaline solution was revealed to be the most suitable method, introducing a methyl group in high yield without any side reaction. Since the methylation requires several hours under alkaline conditions, this procedure should not precede the chain elongation in order to avoid a possible racemization of A<sub>2</sub>bu. The Arndt–Eistert reaction could not be carried out in the presence of a tosylamino group, which is reactive enough toward a mixed anhydride. Therefore, we decided to perform the synthesis according to the following sequence of reactions: chain elongation, *N* <sup>$\omega$</sup> -tosylation, *N* <sup>$\omega$</sup> -methylation and amidation.

First, *N* <sup>$\alpha$</sup> -*t*-butoxycarbonyl-*N* <sup>$\gamma$</sup> -benzyloxycarbonyl-A<sub>2</sub>bu (**2**, Scheme 1) was prepared by *t*-butoxycarbonylation of *N* <sup>$\gamma$</sup> -benzyloxycarbonyl-A<sub>2</sub>bu derived from a copper complex of A<sub>2</sub>bu. The Arndt–Eistert reaction for **2** was conducted according to the conventional method<sup>9</sup> to give an oily  $\beta$ -ornithine derivative **3**, which was purified on a silica gel column to obtain the pure methyl ester **3**. The ester **3** was saponified to afford a crystalline carboxylic acid **4**, which was then hydrogenated with a palladium–charcoal catalyst to remove

**Keywords:** blasticidin S; blastidic acid; Arndt–Eistert reaction; amidation.

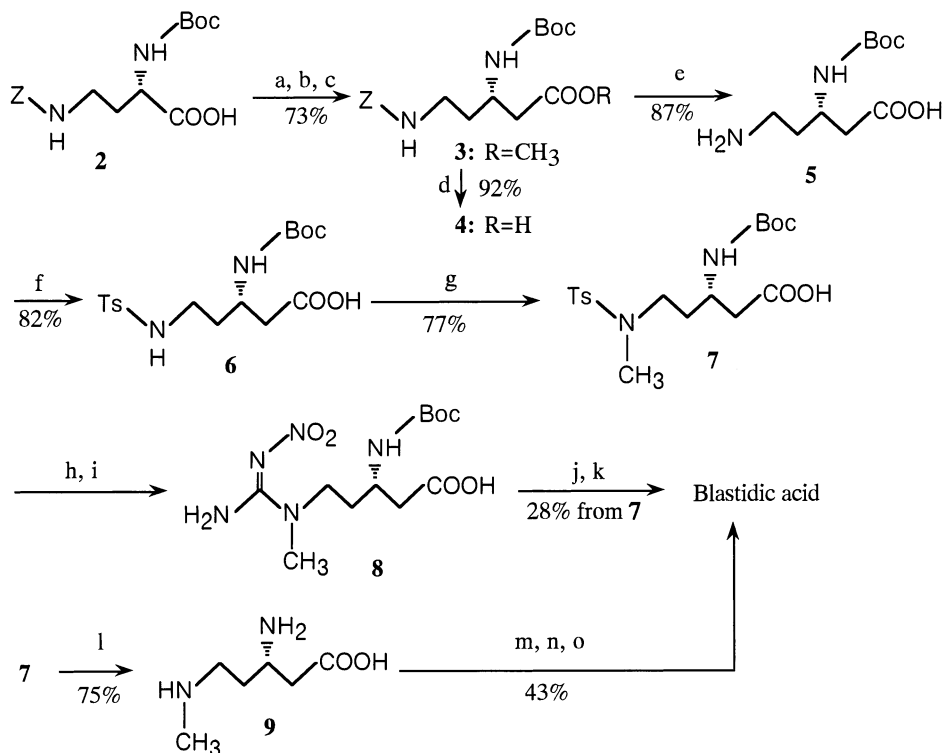
\* Corresponding author. Fax: +81 0 298 55 7610; e-mail: nomoto@staff.chem.tsukuba.ac.jp

the benzyloxycarbonyl group in **4**, resulting in formation of a crystal of **5**. After tosylation of the  $\delta$ -amino group in **5**, the resulting crystal of **6** was subjected to *N*-methylation with 10 equivalents of methyl iodide in a mixture (1:1) of 2 M NaOH aqueous solution and dioxane for 5 hours. The resulting *N*-methylated product was recrystallized from acetone–ether to give a pure crystal of **7**,  $^1\text{H}$  NMR (270 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  2.70 (s, 3H,  $N^\delta\text{-CH}_3$ ), 3.02 (m, 2H,  $\delta\text{-CH}_2$ ).

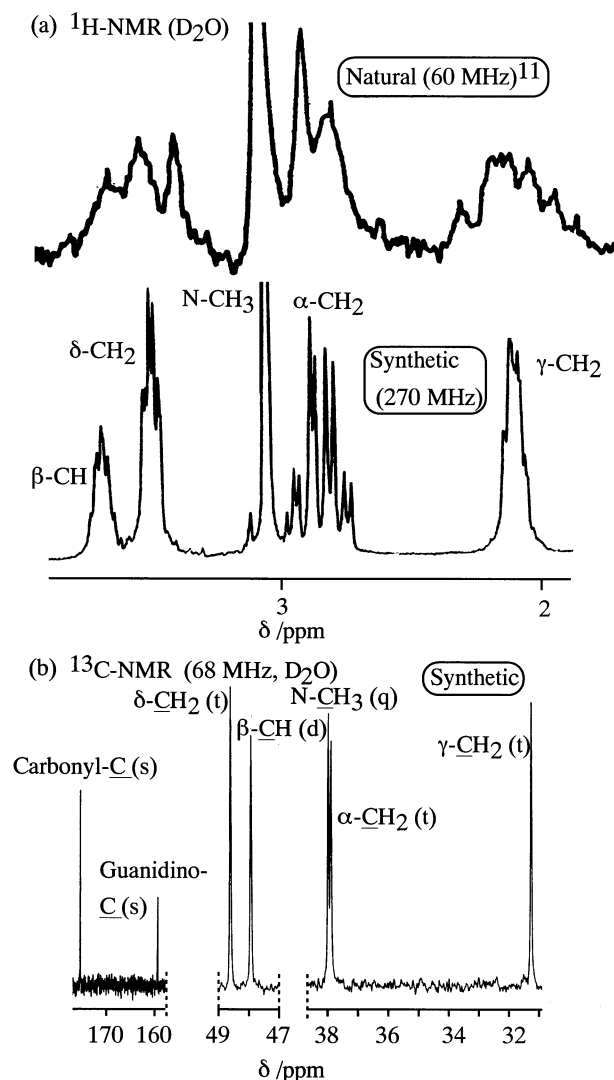
A tosyl group in **7** was selectively removed with sodium in liquid ammonia, and the deprotected product, without being isolated, was subjected to nitroamidination with *O*-methyl-*N*-nitroisourea in 2 M NaOH aqueous solution for 5 hours. The reaction mixture was extracted with ethyl acetate ten times to remove the unreacted isourea, adjusted to pH 3 and extracted with chloroform 30 times. It was difficult to extract the amidinated product completely from the water layer and also to separate the product from the unreacted isourea, but the product in water, without being isolated, could be deprotected as stated below. From the chloroform layer, the desired product **8** was obtained in 25% yield as a white solid,  $^1\text{H}$  NMR (270 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  3.04 (s, 3H,  $N^\delta\text{-CH}_3$ ), 3.50 (m, 2H,  $\delta\text{-CH}_2$ ). This compound is a protected form of blastidic acid, which can be used for peptide synthesis. *N*<sup>G</sup>-Nitro-*N*<sup>β</sup>-*t*-butoxycarbonylblastidic acid (**8**), thus obtained, was treated for 1 hour with trifluoroacetic acid to remove

the *t*-butoxycarbonyl group and then hydrogenated in the presence of palladium–charcoal in water for 20 hours to remove the nitro group. The deprotected product was applied to an Amberlite IRC120 column and eluted with 3 M HCl to afford the expected amino acid dihydrochloride, which was recrystallized from boiling ethanol containing a small amount of water to give needles<sup>10</sup> in 55% yield. In Fig. 1, the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the synthetic blastidic acid are shown and the  $^1\text{H}$  NMR spectrum agreed well with that of the natural blastidic acid.<sup>11</sup> From the above-stated water layer that resulted from the amidination, blastidic acid was also obtained in 14% yield after de-*t*-butoxycarbonylation with 6 M HCl and hydrogenation followed by chromatographic purification using an Amberlite column.

On the column chromatography, *N*<sup>δ</sup>-methyl- $\beta$ -ornithine (**9**) was recovered in 32% yield and was amidinated to give blastidic acid as follows. We found in this study that  $\beta,\omega$ -diamino acids form copper complexes, which can be utilized for selective modification of their  $\omega$ -amino groups. Thus, amino acid **9**, obtained also from **7** on deprotection with 25% HBr at 110°C for 2 hours, was converted to a copper complex in the usual manner and subjected to reaction with *O*-methylisourea for 24 hours at a room temperature. The reaction mixture was acidified with 6 M HCl, and  $\text{H}_2\text{S}$  was bubbled into the solution to remove copper ions. Chromatographic



**Scheme 1.** (a)  $\text{Cl-COOEt}$ , *N*-methylmorpholine,  $\text{AcOEt}$ ,  $-15^\circ\text{C}$ ; (b)  $\text{CH}_2\text{N}_2$ ,  $\text{AcOEt-Et}_2\text{O}$ ,  $-15^\circ\text{C}$ ; (c)  $\text{C}_6\text{H}_5\text{COOAg}$ ,  $\text{MeOH}$ ,  $0^\circ\text{C}$ ; (d) 2 M NaOH; (e)  $\text{H}_2/5\%$  Pd-C,  $\text{MeOH}$ ; (f)  $\text{Ts-Cl}$ , 1 M NaOH; (g)  $\text{MeI}$ , 2 M NaOH–dioxane; (h) Na, liq.  $\text{NH}_3$ ,  $-70^\circ\text{C}$ ; (i) *O*-methyl-*N*-nitroisourea, 2 M NaOH; (j)  $\text{CF}_3\text{COOH}$ ; (k)  $\text{H}_2/5\%$  Pd-C,  $\text{MeOH}$ ; (l) 25% HBr,  $110^\circ\text{C}$ ; (m)  $\text{CuCO}_3\cdot\text{Cu(OH)}_2$ ,  $\text{H}_2\text{O}$ ; (n) *O*-methylisourea, 1 M NaOH; (o)  $\text{H}_2\text{S}$ , 6 M HCl.



**Figure 1.** NMR spectra of natural and synthetic blastidic acid.

purification of the mixture on an Amberlite column afforded blastidic acid.

In conclusion, we have successfully constructed the blastidic acid moiety, which had not been synthesized ever since the structure was elucidated in 1965 although there was a need to establish a synthetic route to the total synthesis of blastidicin S and its analogs. The synthetic strategy designed here may provide a general method of obtaining structurally related amino acids such as pseudoblastidone and stendomycin, involved in cytomycin and stendomycin, respectively. We have also extended the scope of utility of diamino acid-copper complexes in selective modification of amino groups.

## References

1. Takeuchi, S.; Hirayama, K.; Ueda, K.; Sakai, H.; Yonehara, H. *J. Antibiot. Ser. A* **1958**, *11*, 1–5.
2. Otake, N.; Takeuchi, S.; Endo, T.; Yonehara, H. *Tetrahedron Lett.* **1965**, 1411–1419.
3. Omura, S.; Nawata, Y.; Saito, Y. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 1091.
4. Fox, J. J.; Watanabe, K. A. *Pure Appl. Chem.* **1971**, *28*, 475–487.
5. Kondo, T.; Nakai, H.; Goto, T. *Tetrahedron Lett.* **1972**, 1881–1884.
6. Leutzing, E. E.; Robins, R. K.; Townsend, L. B. *Tetrahedron Lett.* **1968**, 4475–4478.
7. Watanabe, K. A.; Wempen, I.; Fox, J. J. *Chem. Pharm. Bull.* **1970**, *18*, 2368–2371.
8. Watanabe, K. A.; Fox, J. J. *Chem. Pharm. Bull.* **1973**, *21*, 2213–2216.
9. Wakamiya, T.; Teshima, T.; Sakakibara, H.; Fukukawa, K.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1984–1989.
10. Synthetic blastidic acid: mp 203–205°C (dec.) (192–192.5°C (dec.)<sup>11</sup>); [ $\alpha$ ]<sub>D</sub><sup>18</sup> +21° (c 1, H<sub>2</sub>O) ([ $\alpha$ ]<sub>D</sub><sup>20</sup> +25° (c 1, H<sub>2</sub>O)<sup>11</sup>); retention time (min) on amino acid analysis performed by conventional methods 145 (cf. *N*<sup>δ</sup>-Me-β-Orn 120, β-Orn 116, Arg 111, Orn 91). Found: C, 31.68; H, 7.06; Cl, 26.27; N, 20.83%. Calcd for C<sub>7</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>2</sub>·1/4H<sub>2</sub>O: C, 31.65; H, 7.02; Cl, 26.69; N, 21.09%.
11. Otake, N.; Takeuchi, S.; Endo, T.; Yonehara, H. *Agric. Biol. Chem.* **1966**, *30*, 132–141.