Synthesis of Blastidic Acid and Cytosinine, Two Components of Blasticidin S

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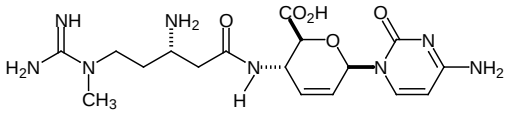
Received 28 August 2001

Abstract: Blastidic acid and cytosinine, two components of antibiotics blasticidin S, have been synthesized.

Key words: antibiotics, amines, cyanate, nucleosides, rearrangements

Blasticidin S (**1**) was isolated from *Streptomyces griseochromogenes* and at one time used as a fungicide for the prevention of rice blast in Japan.[1] The structural studies by Otake et al. revealed that blasticidin S (**1**) is a member of the peptidyl-nucleoside family of antibiotics as shown in Figure 1.[2]



Blasticidin S (**1**)

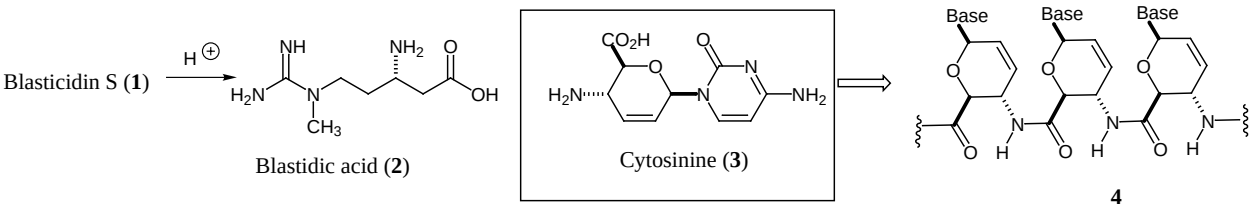
Figure 1

Careful acid hydrolysis of blasticidin S (**1**) yielded blastidic acid (**2**) and cytosinine (**3**), as shown in Scheme 1. Structurally, cytosinine (**3**) is characterized as a peculiar nucleoside possessing β-amino acid functionality, and this structural feature inspired us to propose a new type of DNA-analogue **4** based on a peptide backbone (peptide-nucleoside, PNA).[3] With this in mind, we here focused our attention on the synthesis of blasticidin S (**1**).

Although the first synthesis of cytosinine (**3**) was reported by Kondo and Goto,[4] there has been no report on the total synthesis of blasticidin S. A recent report on the synthesis of blastidic acid (**2**) by Nomoto prompted us to present our synthetic effort in this field.[5]

The synthesis of blastidic acid began with (3*S*)-3[(benzyloxycarbonyl])amino]-glutarate **6,** which was prepared by enantioselective hydrolysis of *meso*-diester **5** with pig liver esterase as reported by Ohno (Scheme 2).[6] Hence, our initial effort focused on the functional group interconversion of the carboxylic acid of **6** into the *N*-methyl guanidine moiety. Diborane reduction of the carboxylic acid **6** yielded the alcohol **7** in 74% yield, which was further converted into the benzyl amide **8** (54% yield) by reaction with benzylamine in refluxing benzene.[7] Transformation of the hydroxyl group of **8** into the *N*-methyl amine moiety was accomplished according to the protocol of Fukuyama.[8] Accordingly, *N*-methyl 2-nitrobenzenesulfonamide was alkylated with **8** under Mitsunobu conditions (DEAD, Bu_3P, CH_2Cl_2) to furnish **9** in 95% yield.[9] Deprotection of the *o*-nitrobenzenesulfonamide group of **9** was carried out by treatment with thiophenol and cesium carbonate in acetonitrile, giving the *N*-methylamine **10,** which further reacted with *N*,*N*-di-(*tert*-butoxycarbonyl)thiourea in the presence of mercuric chloride.[10] The bis-Boc-protected guanidine **11** was isolated in 75% overall yield from **9**.

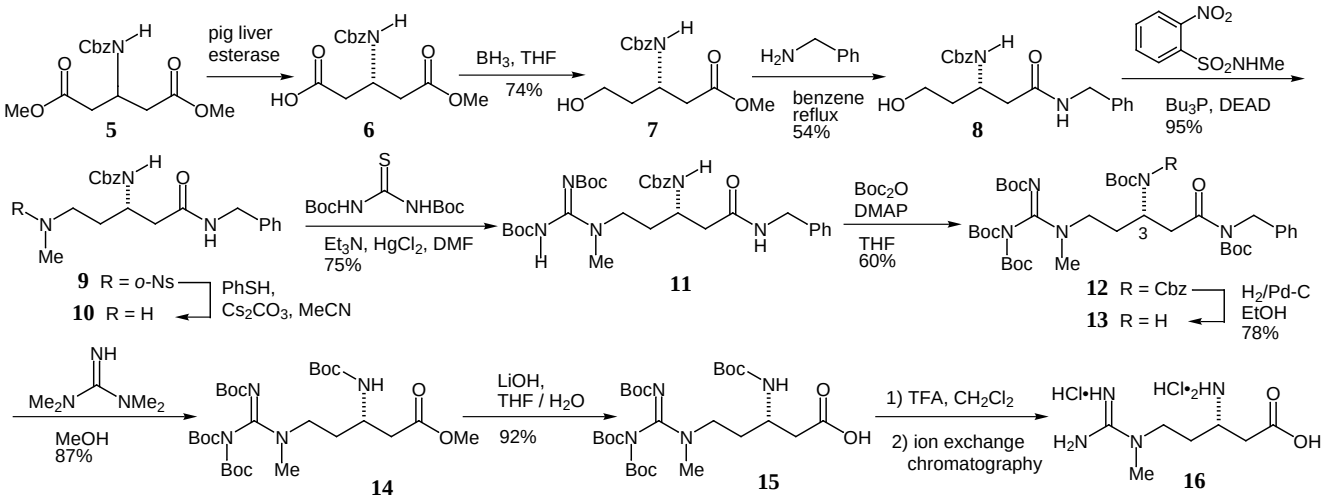
The final stage of the blastidic acid synthesis is the two-step process of amide activation and hydrolysis of the benzyl amide **11**.[11] Accordingly, benzylamide **11** was subjected to exhaustive acylation with di-*tert*-butyl dicarbonate in THF using DMAP as catalyst to afford the *N*-Boc imide **12** in 60% yield. During this acylation, the *tert*-butoxycarbonyl groups were also introduced onto the nitrogen atoms of both the benzyloxycarbonyl group and the Boc-protected *N*-methyl guanidine group. Although saponification of imide **12** was complicated by base-catalyzed β-elimination of the imide group at C-3, this reaction could be avoided by lowering the leaving group ability. Thus, imide **12** was transformed into the Boc-carbamate **13** by catalytic hydrogenolysis of the Cbz group of **12**. Methanolysis of the resulting compound **13** with tetramethyl guanidine in methanol furnished the methyl es-



Scheme 1

Synlett 2001, No. 11, 26 10 2001. Article Identifier:
1437-2096,E;2001,0,11,1763,1766,ftx,en;Y17301ST.pdf.

ISSN 0936-5214

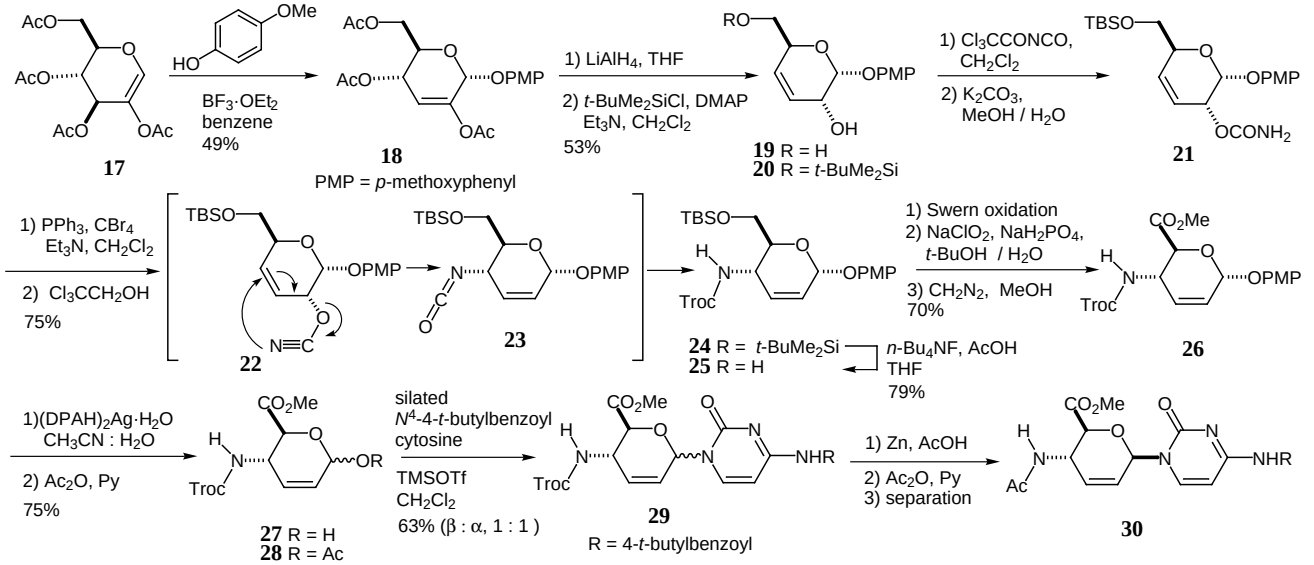


Scheme 2

ter **14** in 68% overall yield from **12**.[12] Finally, the methyl ester **14** was hydrolyzed with lithium hydroxide in aqueous THF to afford the Boc-protected blastidic acid **15,** which is ideal for the synthesis of blasticidin S.[13] Confirmation of the structure was performed by transforming **15** into the blastidic acid dihydrochloride **16** by treatment with TFA and purification using ion exchange chromatography. The ^{1}H and ^{13}C NMR spectra of our synthetic **16** were found to be identical with those of a sample prepared from natural blasticidin S.[14,15]

Cytosinine (**3**) has the structure of 2,3-dideoxy-4-amino-D-hex-2-enopyranoside (Scheme 1), and synthesis of such an unsaturated amino sugar moiety posed synthetic problems in previous studies, because they relied upon introducing the nitrogen substituent by replacement reaction with sodium azide.[4,16] To solve this problem, we have developed a new approach for the synthesis of cytosinine by using [3,3]-sigmatropic rearrangement of an allyl cyanate as shown in Scheme 3.[17]

Ferrier-type glycosylation of 2-acetoxy-tri-*O*-acetyl-D-glucal (**17**) with *p*-methoxyphenol catalyzed by boron trifluoride diethyl etherate gave **18** in 49% yield after recrystallization.[18] Treatment of **18** with lithium aluminum hydride gave the diol **19**,[19] which was selectively protected with *tert*-butyldimethylsilyl chloride and triethylamine in the presence of a catalytic amount of DMAP to afford the silyl ether **20** in 53% overall yield from **18**.[20] With the hex-3-enopyranoside **20** in hand, we have undertaken the allyl cyanate-to-isocyanate rearrangement of **20**. Thus, treatment of **20** with trichloroacetyl isocyanate followed by hydrolysis with potassium carbonate in aqueous methanol gave carbamate **21**. Dehydration of **21** with triphenylphosphine, carbon tetrabromide and triethylamine at –40 °C gave the allyl cyanate **22**, which underwent [3,3]-sigmatropic rearrangement at 0 °C for 60 min to afford the



Scheme 3

allyl isocyanate **23**. Since isolation of **23** using an aqueous work-up caused a decrease of yield due to the high reactivity of the isocyanate function, allyl isocyante **23** was transformed in situ into the trichloroethoxy carbamate **24** by reaction with 2,2,2-trichloroethanol.[21] The resulting carbamate **24** was isolated in 75% overall yield from **20** after chromatographic purification.

The next stage of the synthesis is the transformation of **24** into the corresponding 2,3-dideoxy-hex-2-enopyranouronate and cytosine glycosidation. Accordingly, the *tert*-butyldimethylsilyl group of **24** was removed with tetrabutylammonium fluoride in a mixture of acetic acid and tetrahydofuran to provide **25** in 79% yield. Two-step oxidation of **25** involving Swern oxidation followed by sodium chlorite oxidation and esterification of the resulting carboxylic acid with diazomethane in methanol furnished **26** in 70% overall yield for the three steps. Oxidative hydrolysis of *p*-methoxyphenyl glycoside **26** with silver (II) bis-(hydrogen dipicolinate) gave the unstable lactol **27**,[18] which was immediately subjected to acetic anhydride and pyridine to provide the acetyl glycoside **28** in 75% yield. Condensation of **28** with silated N^4-(4-*tert*-butylbenzoyl)cytosine[22] in the presence of TMSOTf afforded a 1:1 mixture of the fully protected cytosinine derivative **29** and its α-isomer in 63% yield.[23] Finally, **29** was transformed into **30** [24] which was identical with the product derived from natural blasticidin S.[25]

In summary, the synthesis of Boc-protected blastidic acid **15** was achieved in 9 steps starting from chiral carboxylic acid **6**. Allyl cyanate-to-isocyanate rearrangement has been successfully employed for the construction of an unsaturated amino sugar moiety of cytosinine, and our synthesis of the fully protected cytosinine derivative **29** required 13 steps starting from 2-acetoxy-tri-*O*-acetyl-D-glucal **17**. Further studies toward the total synthesis of blasticidin S are now underway in our laboratory.

Preparation of *p*-Methoxyphenyl 2,3,4,6-Tetradeoxy-4-trichloroethoxycarbonylamino-α-D-*erythro*-hex-2-enopyranoside 26 from 20

To a solution of **20** (28.2 g, 77.0 mmol) in CH_2Cl_2 (300 mL) cooled to 0 °C was added trichloroacetyl isocyanate (10.5 mL, 92.4 mmol). After stirring at 0 °C for 1 h, the reaction mixture was concentrated, and the resulting residue was dissolved in methanol (100 mL). Water (100 mL) and potassium carbonate (16.1 g, 231mmol) were added at 0 °C, and the cooling bath was removed. After stirring at room temperature for 2.5 h, the reaction mixture was concentrated under reduced pressure to remove methanol. The resulting aqueous phase was extracted with CH_2Cl_2 and the combined organic layer was dried (Na_2SO_4) and concentrated to afford the carbamate **21** (25.6 g), which was used for the next reaction without further purification.

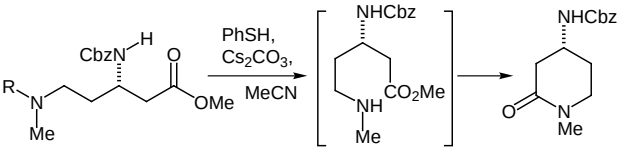
To a solution of the carbamate **21** (8.27 g, 20.2 mol), triphenylphosphine (13.3 g, 50.5 mmol) and triethylamine (7.04 mL, 50.5 mmol) in CH_2Cl_2 (170 mL) cooled to –40 °C under nitrogen atmosphere was added carbon tetrabromide (18.8 g, 56.6 mmol) in CH_2Cl_2 (30 mL). The reaction mixture was gradually warmed to 0 °C over 30 min and then stirred at 0 °C for 1 h. 2,2,2-Trichloroethanol (11.6 mL, 0.12 mol) was added, and stirring was continued at 0 °C for 2 h and then at r.t. overnight. The reaction mixture was washed with 1 N HCl and aq sat. $NaHCO_3$ solution, and dried (Na_2SO_4). Concentration under reduced pressure gave a crude residue, which was purified by silica gel chromatography to furnish **24** (10.1 g, 75% for the two steps).

Acknowledgement

We would like to thank Prof. T. Kondo, Nagoya University, for his generous gift of blastidic acid and cytosinine. This research was financially supported by a Grant-In-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture and by JSPS-RFTF.

References and Notes

(1) Takeuchi, K.; Hirayama, K.; Ueda, H.; Sakai, H.; Yonehara, H. *J. Antibiot., Ser. A* **1958**, *11*, 1.
(2) (a) Otake, N.; Takeuchi, S.; Endo, T.; Yonehara, H. *Tetrahedron Lett.* **1965**, 1405. (b) Otake, N.; Takeuchi, S.; Endo, T.; Yonehara, H. *Agric. Biol. Chem.* **1966**, *30*, 126. (c) Otake, N.; Takeuchi, S.; Endo, T.; Yonehara, H. *Agric. Biol. Chem.* **1966**, *30*, 132.
(3) (a) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497. (b) Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 1895.
(4) (a) Kondo, T.; Nakai, H.; Goto, T. *Tetrahedron Lett.* **1972**, 1881. (b) Kondo, T.; Nakai, H.; Goto, T. *Tetrahedron* **1973**, *29*, 1801.
(5) Nomoto, N.; Shimoyama, A. *Tetrahedron Lett.* **2001**, *42*, 1753.
(6) (a) Ohno, M.; Kobayashi, S.; Iimori, T.; Wang, V. F.; Izawa, T. *J. Am. Chem. Soc.* **1981**, *103*, 2405. (b) Ohno, M.; Otsuka, M. *Organic Reactions*, Vol. 37; John Wiley & Sons: New York, **1989**, 1.
(7) Since *N*-methylamine **32**, which was produced upon removal of the sulfonyl group of **31**, rapidly cyclized to form lactam **33**, benzyl amide was chosen as the carboxylic acid protecting group (Figure 2).



31 R = *o*-Ns **32** **33**

Figure 2

(8) (a) Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373. (b) Mitsunobu reactions of *N*-methyl *p*-toluenesulfonamide in THF afforded sulfonamide in moderate yield due to the formation of DEAD *N*-alkylation compound. In the case of *N*-methyl 2-nitrobenzenesulfonamide, such a by-product has never been observed. See: Henry, J. R.; Marcin, L. R.; McIntosh, M. C.; Scola, P. M.; Harris, G. D.; Weinreb, S. M. *Tetrahedron Lett.* **1989**, *30*, 5709.
(9) When we employed triphenylphosphine in this Mitsunobu reaction, we had difficulties in purification of product **9,** which shows a similar chromatographic behavior than triphenylphosphine oxide.
(10) Kim, K. S.; Qian, L. *Tetrahedron Lett.* **1993**, *34*, 7677.

(11) Flynn, D. L.; Zelle, R. E.; Grieco, D. A. *J. Org. Chem.* **1983**, *48*, 2424.
(12) Grehn, L.; Gunnarsson, K.; Ragnarsson, U. *Acta Chem. Scand., Ser. B* **1987**, *41*, 18.
(13) The synthesis of **15** was first reported in 1999 at the Chubu-Kansai Branch Joint Meeting and Symposium of the Japan Society for Bioscience, Biotechnology, and Agrochemistry at Gifu University. See the abstract on page 39.
(14) The specific rotation of our blastidic acid dihydrochloride (**16**) is found to be $[\alpha]^{20}_D$ = +13.3 (*c* 0.52, H_2O), while the values reported were $[\alpha]^{15}_D$ = +25.0 (*c* 1.0, H_2O; see reference 2), $[\alpha]^{18}_D$ = +21.0 (*c* 1.0, H_2O; see reference 5), and $[\alpha]^{26}_D$ = +9.1 (*c* 0.52, H_2O; see reference 15). Due to the discrepancy between these values, we felt it necessary to determine the optical purity of our synthetic **16**. Accordingly, compound **15** was transformed into (*R*)-(+)-α-methylbenzylamide **34**, and then the optical purity was confirmed to be >97%. Similar transformation of **15** using racemic α-methylbenzylamide gave a 1:1 mixture of the diastereoisomers to confirm the validity of this analysis (Figure 3).

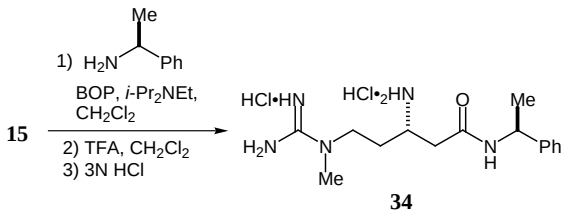


Figure 3

(15) Cooper, R.; Conover, M.; Patel, M. *J. Antibiot.* **1988**, 123.
(16) Watanabe, K. A.; Wempen, I.; Fox, J. J. *Chem. Pharm. Bull.* **1970**, *18*, 2368.
(17) Ichikawa, Y.; Kobayashi, C.; Isobe, M. *J. Chem. Soc., Perkin Trans. 1* **1996**, 377.
(18) We were extremely frustrated with a serious problem during transformation of the ethyl glycoside **35** into the corresponding acetyl glycoside, because acid-catalyzed hydrolysis of **35** resulted in rapid formation of pyrrole **36** (Figure 4). Taking this preliminary experiment into consideration, we employed *p*-methoxyphenyl glycosides, which can be hydrolyzed under neutral conditions. See: Noshita, T.; Sugiyama, T.; Kitazumi, Y.; Oritani, T. *Tetrahedron Lett.* **1994**, *35*, 8259.

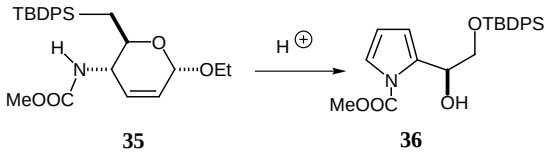


Figure 4

(19) (a) Isobe, M.; Ichikawa, Y.; Goto, T. *Tetrahedron Lett.* **1985**, *26*, 5199. (b) Ichikawa, Y.; Isobe, M.; Bai, D.-L.; Goto, T. *Tetrahedron* **1987**, *43*, 4737.
(20) Chaudhary, S. K.; Hernandez, O. *Tetrahedron Lett.* **1979**, *14*, 99.
(21) Ichikawa, Y.; Osada, M.; Ohtani, I.; Isobe, M. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1449.
(22) Will, D.; Breipohl, G.; Langner, D.; Knolle, J.; Uhlmann, E. *Tetrahedron* **1995**, *51*, 12069.
(23) (a) Schmidt, R. R.; Wagner, W. *Tetrahedron Lett.* **1983**, *24*, 4661. (b) Ueda, T.; Watanabe, S.-I. *Chem. Pharm. Bull.* **1985**, *33*, 3689. (c) Vorbruggen, H.; Ruh-Pohlenz, C. *Organic Reactions*, Vol. 55; John Wiley & Sons: New York, **2000**, 1.
(24) Due to the difficulties associated with separating a mixture of α- and β-isomers, we could not estimate the yields of this transformation.
(25) An authentic sample of **30** was prepared from cytosinine (**3**) by the following reaction sequence involving i) Ac_2O, Py; ii) CH_2N_2, MeOH; iii) MeOH, heated at reflux temperature; and then iv) 4-*tert*-butylbenzoyl chloride, Py.