Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 16 (2005) 903–908

An efficient access to both enantiomers of pipecolic acid

Louis A. Watanabe,[a] Saori Haranaka,[b] Binoy Jose,[c] Minoru Yoshida,[c,d] Tamaki Kato,[b] Mitsuaki Moriguchi,[e] Kenji Soda[f] and Norikazu Nishino[b,c,*]

[a]*Faculty of Engineering, Kyushu Institute of Technology, Kitakyushu 804-8550, Japan*
[b]*Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu 808-0196, Japan*
[c]*CREST Research Project, Japan Science and Technology Agency, Saitama 332-001, Japan*
[d]*RIKEN, Saitama 351-0198, Japan*
[e]*Faculty of Engineering, Oita University, Oita 870-1192, Japan*
[f]*Faculty of Engineering, Kansai University, Suita, Osaka-fu 564-8680, Japan*

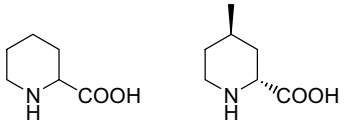
Received 14 December 2004; accepted 5 January 2005

Abstract—An efficient and convenient synthesis of both enantiomers of pipecolic acid has been developed using the intramolecular cyclization of 2-amino-6-bromohexanoic acid under mild conditions.

1. Introduction

Pipecolic acid **1**, a widespread natural non-proteinogenic amino acid, is an attractive synthetic target because it is a key constituent of many synthetic and natural bioactive molecules and useful building blocks for the preparation of peptides and peptide mimetics.[1] For example, biologically important natural products such as immunosuppressant FK506,[2] anesthetic bupivacaine,[3] anticancer agent VX710,[4] oxytoxin antagonist L-365209,[5] antifungal antibiotic demethoxyrapamycin,[6] antitumour antibiotic sandramycin,[7] phytotoxic metabolite Cyl-2[8] and antiprotozoal agent as well as histone deacetylase inhibitor apicidin,[9] trapoxins[10] etc. contain a pipecolic acid moiety. (2*R*,4*R*)-Methylpipecolic acid **2** is a key component for the preparation of a highly selective thrombin inhibitor.[11] The replacement of proteinogenic amino acids with cyclic imino acids has been carried out in structure–activity relationship studies and in search of new peptidomimetics that have improved pharmacological profiles.[12]

All these factors have contributed to the growing interest in finding a convenient and efficient synthetic route to pipecolic acid and related imino acids in high enantiomeric excess. Over the course of a project directed to the development of synthetic histone deacetylase inhibitors,[13] based on natural products such as Cyl-2, we found that a convenient route to enantiomerically pure pipecolic acids will be helpful. The great importance of **1** has fostered the development of many synthetic approaches involving enzymatic reactions,[14] alkylation of chiral glycine enolates,[15] derivatization of natural amino acids,[16] enantioselective reactions,[17] and resolution.[18] However, most of these methods have some limitations, such as tedious procedures, low yields, unavailability of starting materials etc. Therefore new and convenient methods for the preparation of optically active pipecolic acid are still required. We recently reported the synthesis of α-amino-ω-bromoalkanoic acid for side-chain modifications.[19] During the course of that work we explored a convenient route to L- and D-pipecolic acids.



1 **2**

2. Results and discussion

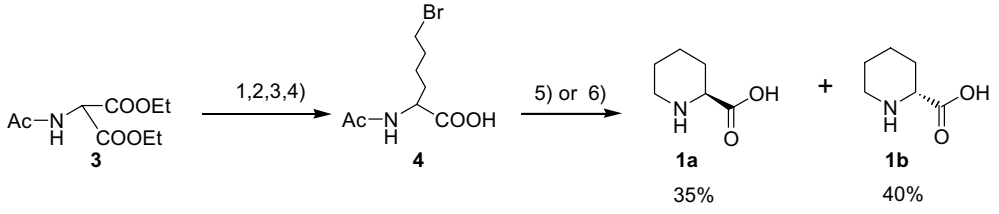
The synthesis of pipecolic acid started with commercially available diethyl acetamidomalonate **3** and

* Corresponding author. Tel./fax: +81 93 695 6061; e-mail: nishino@life.kyutech.ac.jp

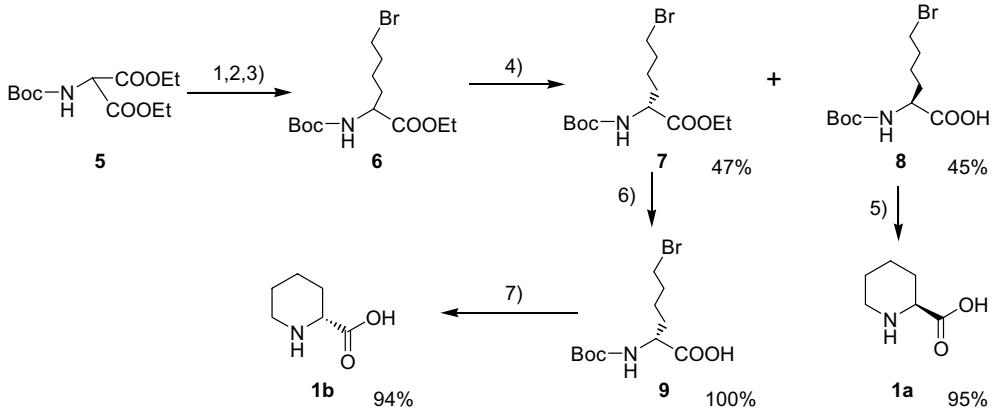
0957-4166/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2005.01.017

dibromobutane. An excess of dibromobutane was reacted with **3** in the presence of sodium ethoxide in absolute ethanol. One equivalent of 1 M NaOH was added at 0–5 °C to the reaction mixture and we obtained the corresponding monoacid monoester as a white solid after work-up. It was then decarboxylated by refluxing in toluene. After evaporation of toluene, the resulting acetyl-DL-2-amino-6-bromohexanoic acid ethyl ester as an oil was dissolved in ethanol and hydrolyzed using NaOH at 0–5 °C to yield acetyl-DL-2-amino-6-bromohexanoic acid **4** as a white powder in a quantitative yield after work-up. *Aspergillus genus* aminoacylase (TCI) was applied to resolve the racemic mixture. During the resolution we observed spontaneous intramolecular cyclization of L-2-amino-6-bromohexanoic acid to L-pipecolic acid **1a**. The reaction mixture was concentrated and then acidified with 1 M HCl to pH 3. The unreacted acetyl-D-2-amino-6-bromohexanoic acid (Ac-D-Ab6) was then extracted with ethyl acetate. The aqueous layer was applied to a column of ion-exchange resin (DOWEX 50W-X4) and L-pipecolic acid was eluted with 1 M ammonia. After solvent evaporation, L-pipecolic acid was obtained as a white solid and was recrystallized from acetone. Recovered Ac-D-Ab6 was treated with *Alcaligenes xylosoxydans* D-aminoacylase[20] to yield D-pipecolic acid **1b** (Scheme 1). In another run, D-pipecolic acid was obtained by the resolution of **4** using the D-aminoacylase. Yamaguchi and Ueki reported the cyclization of *N*-protected 2-amino-5-bromopentanoic acid ester to the corresponding proline derivatives using sodium hydride in THF.[21] They derived glutamic acid to *N*-protected 2-amino-5-bromopentanoic acid ester. However, to the best of our knowledge, there is no cyclization procedure reported for the synthesis of pipecolic acid from 2-amino bromohexanoic acid.

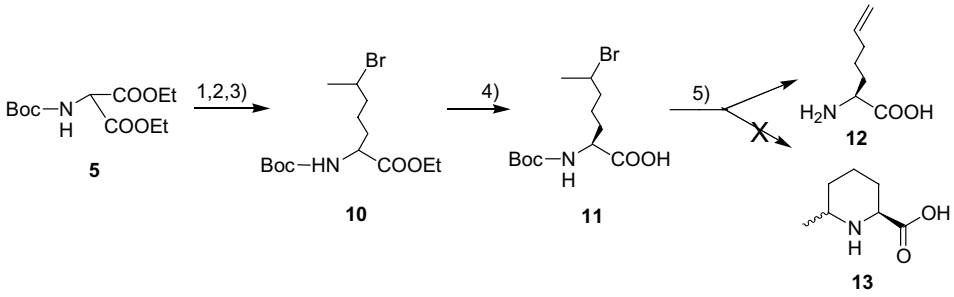
Even though the synthetic scheme for the preparation of pipecolic acid is simple, the extraction process is tedious and time consuming. For this reason, we have carried out the reaction with a different route, starting from diethyl Boc-aminomalonate **5**, as shown in Scheme 2. The initial steps are the same as described earlier. After refluxing in toluene, the resulting Boc-DL-amino-6-bromohexanoic acid ethyl ester (Boc-DL-Ab6-*O*Et) **6** was resolved to Boc-L-amino-6-bromohexanoic acid **8** using subtilisin Carlsberg from *Bacillus licheniformis* (Sigma) in a mixture of DMF and water (1/3, v/v) at pH 8. The pH was maintained at 8 by the addition of 1 M ammonia and the reaction was completed in 3 h. Then the unreacted Boc-D-Ab6-*O*Et **7** was removed by ether extraction. Compound **8** was extracted with ethyl acetate at pH 3 using citric acid. The Boc group of **8** was removed using 2 M HCl in dioxane to give corresponding amino acid hydrochloride. It was then cyclized to L-pipecolic acid in methanol by the addition of triethylamine. **7**, which was recovered after resolution, was then hydrolyzed using dilute NaOH. It was then processed as mentioned above to yield D-pipecolic acid. Thus, both enantiomers of pipecolic acid were prepared with high enantiomeric purity in high overall yield (27%) in five steps.



Scheme 1. Reagents and conditions: (1) (i) EtOH, sodium ethoxide, reflux, 30 min; (ii) 1,4-dibromobutane, reflux, 5 h, 73%; (2) NaOH aq, EtOH, 0 °C, 3 h, 85%; (3) toluene, reflux, 3 h, 97%; (4) NaOH aq, EtOH, 0 °C, 3 h, 81%; (5) L-aminoacylase, $CoCl_2 \cdot 6H_2O$, H_2O, pH 7, 38 °C, 24 h; (6) D-aminoacylase, H_2O, pH 7, 38 °C, 3 days.



Scheme 2. Reagents and conditions: (1) (i) EtOH, sodium ethoxide, reflux, 30 min; (ii) 1,4-dibromobutane, reflux, 5 h, 92%; (2) NaOH aq, EtOH, 0 °C, 3 h, 80%; (3) toluene, reflux, 3 h, 85%; (4) subtilisin, H_2O/DMF = 3/1, pH 8, 38 °C, 3 h; (5) (i) 2 M HCl/dioxane, 0 °C, 2 h; (ii) Et_3N, DMF, pH 8, 0 °C, 5 h; (6) NaOH aq, EtOH, 0 °C, 3 h; (7) (i) 2 M HCl/dioxane, 0 °C, 2 h; (ii) Et_3N, DMF, pH 8, 0 °C, 5 h.



Scheme 3. Reagents and conditions: (1) (i) EtOH, sodium ethoxide, reflux, 30 min; (ii) 1,4-dibromopentane, reflux, 3 h; (2) NaOH aq, EtOH, 0 °C, 3 h, 65%; (3) toluene, reflux, 3 h, 89%; (4) subtilisin, H_2O/DMF 3:1, pH 8, 38 °C, 3 h, 40%; (5) (i) 2 M HCl/dioxane, 0 °C, 2 h (ii) Et_3N, MeOH, pH 8, 0 °C, 1 h, 44%.

To examine the effect of a substituent at the 6-position on cyclization reaction, we proposed to attach a methyl group at the 6-position. We reasoned that the presence of a bulky methyl group at the 6-position could hinder the cyclization reaction or it could allow the formation of one enantiomer of pipecolic acid. We synthesized Boc-L-2-amino-6-bromoheptanoic acid **11** from **5** and 1,4-dibromopentane as shown in Scheme 3. After resolution using subtilisin, the unreacted D-compound was removed by extraction with ether. The L-compound was extracted using ethyl acetate under acidic conditions. After removal of the solvent, the Boc group was deprotected using 2 M HCl in dioxane. Dioxane was evaporated to give a white solid, which was then dissolved in DMF and two equivalent of triethylamine added. Formation of a new product was observed, but the reaction did not proceed further after 6 h. Another batch reaction was carried out in methanol using two equivalents of triethylamine. Similar results were obtained in 6 h and even under refluxing conditions no further changes were observed. The product was protected with a Boc group and benzyl group and purified by column chromatography. ^1H, ^{13}C and COSY NMR spectra showed that the product formed was 2-amino-6-heptenoic acid **12**. Thus, it is clear that the presence of a bulky methyl group at the 6-position of 2-amino-6-bromohexanoic acid hinders the formation of methyl pipecolic acid **13**.

3. Conclusion

In summary, we have developed a convenient method for the synthesis of L- and D-pipecolic acid based on the spontaneous cyclization of L- and D-2-amino-6-bromoalkanoic acid. The pipecolic acids can be obtained in very high enantiomeric purity in high yields. This approach is useful for the synthesis of substituted pipecolic acids, which give diversity in designing bioactive peptides.

4. Experimental

All reagents were used as purchased from commercial suppliers without further purification. Flash chromatography was performed using silica gel 60 (230–400 mesh) eluting with solvents as indicated. All compounds were routinely checked by thin layer chromatography (TLC) or high performance liquid chromatography (HPLC). TLC was performed on aluminum-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 F_{254}) with spots visualized by UV light or by heating the plate. Analytical HPLC were performed on a Hitachi instrument using: column I; chromolith performance RP-18e column (4.6 × 50 mm, Merck), the mobile phases used were A: H_2O with 10% CH_3CN and 0.1% TFA, B: CH_3CN with 0.1% TFA using a solvent gradient of A to B over 15 min with a flow rate of 2 mL/min, detection at 220 nm; column II; MCI GEL CRS10W (4.6 × 100 mm, Mitsubishi Chemical Corporation), the mobile phases used were 1.0 mM $CuSO_4$ with a flow rate of 0.2 mL/min, detection at 254 nm; column III; CHIRALCEL OD (4.6 × 250 mm, Daicel Chemical Industries, Ltd.), the mobile phases used were *n*-hexane with 5% 2-propanol and 0.1% TFA with a flow rate of 0.5 mL/min, detection at 220 nm. Melting points were determined with a Yamato melting point apparatus model MP-21. Electrospray ionization mass spectrometry (ESI-MS) were carried on an Applied Biosystems API 150 EX. FAB-mass spectra and high resolution mass spectra (HRMS) were measured on a JEOL JMS-SX 102A instrument. Optical rotations were measured on Jasco P-1020. NMR spectra were recorded on a Brucker 500 MHz spectrometer. Unless otherwise stated, all NMR spectra were measured in $CDCl_3$ solutions with reference to TMS. All ^1H shifts are given in parts per millions (s = singlet; d = doublet; t = triplet; m = multiplet). Assignments of proton resonances were confirmed, when possible, by selective homonuclear decoupling experiments or by correlated spectroscopy.

4.1. Synthesis of acetyl-DL-2-amino-6-bromohexanoic acid 4

Metallic sodium (2.19 g, 95 mmol) was added to absolute ethanol (100 mL) with stirring. After complete dissolution, diethyl acetamidomalonate **3** (21.08 g, 100 mmol) was added and refluxed for 30 min and then cooled down. 1,4-Dibromobutane (59 mL, 500 mmol) was added to the solution and refluxed for 5 h. The reaction was monitored by TLC. After completion of the reaction, aqueous NaOH (2 M, 50 mL) was added to the reaction mixture at 0 °C in different portions with

stirring. After selective hydrolysis, ethanol evaporated and the unreacted dibromoalkane was removed by extraction with diethyl ether under basic condition. Then the aqueous solution was extracted with ethyl acetate at pH = 3–4, by adding citric acid and washed with brine, dried and evaporated to obtain a white solid of monoester monoacid. It was then refluxed in toluene (100 mL) for 5 h and toluene then evaporated. The resulting oil (17.0 g, 60.9 mmol) was then dissolved in ethanol (50 mL) and treated with aqueous NaOH (2 M, 25 mL) at intervals of 20 min in portions at 0 °C. The stirring was continued for a further 3 h. After the evaporation of ethanol, the unreacted dibromoalkane was removed by extraction with diethyl ether under basic conditions. Then the aqueous solution was extracted with ethyl acetate, at pH = 3–4, by adding citric acid and washed with brine. Evaporation of ethyl acetate yield **4** as a white solid (12.5 g, 49%). Analytical RP HPLC: R_t: 3.22 min, >99% (column I); mp: 121–123 °C; ^{1}H NMR (500 MHz, CD_3OD) δ = 4.37 (m, 1H), 3.45 (t, 2H, $J_1 = J_2 = 6.5$ Hz), 1.99 (s, 3H), 1.91–1.67 (m, 4H), 1.54 (m, 2H); ^{13}C NMR (CD_3OD) δ = 175.4, 173.4, 53.5, 33.9, 33.4, 31.8, 25.5, 22.4; ESI-MS (*m*/*z*): 252 $(M+H)^+$, 274 $(M+Na)^+$.

4.2. Synthesis of L-pipecolic acid 1a

Compound **4** (10.3 g, 41.0 mmol) was dissolved in water (200 mL) and $CoCl_2{\cdot}6H_2O$ (48 mg, 0.2 mmol) was added. The pH of the solution was adjusted to 7 by the addition of 1 M NaOH. Then *Aspergillus genus* L-aminoacylase was added (2.0 g) and incubated at 38 °C. The pH of the solution was kept at 7 by the addition of 0.1 M NaOH. After 24 h, the mixture was concentrated and acidified with 1 N HCl. The unreacted Ac-D-Ab6 was extracted with ethyl acetate and evaporation of ethyl acetate yielded Ac-D-Ab6 as a white solid (4.6 g, 18.2 mmol). The aqueous solution was then applied to a column of Dowex 50W-X4 and elution with 1 M ammonia yielded L-pipecolic acid **1a** (1.7 g, 35%). TLC: R_f, 0.30 (1-butanol/acetic acid/pyridine/H_2O); >99% ee (determined by column II, R_t: 22.73 min); mp: 270–273 °C; $[\alpha]_D^{25} = -26.3$ (*c* 1, water); ^{1}H NMR (500 MHz, D_2O) δ = 3.58 (m, 1H), 3.41 (m, 1H), 3.01 (m, 1H), 2.22 (m, 1H), 1.91–1.54 (m, 5H); ^{13}C NMR (D_2O) δ = 175.9, 60.3, 44.9, 27.8, 23.1, 22.8; HRMS (FAB, *m*/*z*): $(M+H)^+$ found: 130.0842 (calcd for $C_6H_{12}O_2N$: 130.0868).

4.3. Synthesis of D-pipecolic acid 1b

Ac-D-Ab6 (3.5 g, 14 mmol) was dissolved in water (200 mL) and the pH of the solution adjusted to 8 by the addition of 1 M NaOH. Then *Alcaligenes xylosoxydans* D-aminoacylase was added (45 mg/45 mL H_2O) and incubated at 38 °C. The pH of the solution was kept at 7 by the addition of 0.1 M NaOH. The reaction was continued for three days and then the mixture concentrated. The concentrated aqueous solution was then applied to a column of Dowex 50W-X4 and elution with 1 M ammonia to yield D-pipecolic acid **1b** (1.4 g, 40%). TLC: R_f, 0.30 (1-butanol/acetic acid/pyridine/H_2O); >99% ee (determined by column II, R_t: 14.96 min); mp: 271–274 °C; $[\alpha]_D^{25} = +26.3$ (*c* = 1, water); ^{1}H NMR (500 MHz, D_2O) δ = 3.60 (m, 1H), 3.41 (m, 1H), 3.00 (m, 1H), 2.22 (m, 1H), 1.91–1.57 (m, 5H); ^{13}C NMR (D_2O) δ = 175.0, 59.4, 44.0, 26.9, 22.2, 21.9; HRMS (FAB, *m*/*z*): $(M+H)^+$ found: 130.0877 (calcd for $C_6H_{12}O_2N$: 130.0868).

4.4. Synthesis of Boc-D-2-amino-6-bromohexanoic acid ethyl ester 7 and Boc-L-2-amino-6-bromohexanoic acid 8

Metallic sodium (2.19 g, 95 mmol) was added to absolute ethanol (100 mL) with stirring. After complete dissolution, diethyl Boc-aminomalonate **5** (21.08 g, 100 mmol) was added and refluxed for 30 min and then cooled down. 1,4-Dibromobutane (59 mL, 500 mmol) was added to the solution and refluxed for 5 h. The reaction was monitored by TLC. After completion of the reaction, aqueous NaOH (2 M, 50 mL) was added to the reaction mixture at 0 °C in aliquots (10 mL) with stirring. After selective hydrolysis, ethanol was evaporated and the unreacted dibromoalkane removed by extraction with diethyl ether under basic condition. Then the aqueous solution was extracted with ethyl acetate at pH = 3–4, by adding citric acid and washed with brine, dried and evaporated to obtain monoester monoacid as a white solid. It was then refluxed in toluene (150 mL) for 5 h and toluene then evaporated. The resulting oil **6** (19.3 g, 59 mmol) was dissolved in a mixture of water (150 mL) and DMF (50 mL) and the pH of the solution adjusted to 8 by the addition of 1 M NH_3. Then subtilisin Carlsberg from *Bacillus licheniformis* was added (60 mg) and incubated at 38 °C. The pH of the solution was maintained at 8 by the addition of 1 M NH_3. The reaction was continued for 3 h and then the mixture concentrated. The unreacted Boc-D-Ab6-*O*Et **7** was removed by extraction with diethyl ether under basic conditions. The extraction containing unreacted **7** was washed with brine, dried and evaporated to yield a colourless oil **7** (9.8 g, 29%). The aqueous solution was extracted with ethyl acetate at pH = 3–4 by the addition of citric acid and then washed with brine, dried and evaporated to yield colourless oil **8** (8.0 g, 28%). Analytical RP HPLC: R_t: 6.75 min, 99% (column I); >99% ee (determined by column III, R_t: 14.78 min); $[\alpha]_D^{25} = -2.8$ (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 4.32 (m, 1H), 3.41 (t, 2H, $J_1 = J_2 = 7.0$ Hz), 1.93–1.67 (m, 4H), 1.56 (m, 2H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$) δ = 176.6, 155.6, 80.3, 53.1, 33.2, 32.1, 31.6, 28.3, 23.9; ESI-MS (*m*/*z*): 310 $(M+H)^+$, 332 $(M+Na)^+$.

4.5. Synthesis of L-pipecolic acid 1a

The Boc group of **8** (1.24 g, 4.0 mmol) was deprotected using 2 M HCl in dioxane (20 mL) to give the corresponding amino acid hydrochloride. After the evaporation of dioxane, it was dissolved in DMF (10 mL) and triethylamine (1.1 mL, 8.0 mmol) then added. The reaction was continued for 5 h and the pH of the solution was kept at 8 by the addition of triethylamine. The solution was filtered and then neutralized with acetic acid. Then the solution was concentrated and dissolved in water. The aqueous solution was then applied to a column of Dowex 50W-X4 with 1 M

ammonia elution. The solution was concentrated and crystallized from acetone to obtain L-pipecolic acid as a white solid **1a** (494 mg, 95%) TLC: R_f, 0.30 (1-butanol/acetic acid/pyridine/H_2O); 98% ee (determined by column II, R_t: 22.73 min); mp: 270–273 °C; $[\alpha]_D^{25} = -26.3$ (*c* 1, water).

4.6. Synthesis of Boc-D-2-amino-6-bromohexanoic acid 9

Boc-D-Ab6-*O*Et **7** (4.63 g, 14 mmol) was dissolved in ethanol (20 mL) and treated with aqueous NaOH (2 M, 7 mL) in intervals of 20 min in portions at 0 °C and then the stirring continued for 3 h. After evaporation of ethanol, the solution was extracted with diethyl ether under basic conditions. Then the aqueous solution was extracted with ethyl acetate under acidic condition and washed with brine. Ethyl acetate evaporation gave Boc-D-2-amino-6-bromo-hexanoic acid **9** as a colourless oil (4.33 g, 100%). Analytical RP HPLC: R_t: 6.75 min, 99% (column I); >99% ee (determined by column III, R_t: 15.39 min); $[\alpha]_D^{25} = +2.8$ (*c* 0.1, MeOH), ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 4.32$ (m, 1H), 3.41 (t, 2H, $J_1 = J_2 = 7.0$ Hz), 1.92–1.69 (m, 4H), 1.55 (m, 2H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$) $\delta = 177.1$, 155.6, 80.4, 53.1, 33.2, 32.1, 31.6, 28.3, 23.9; ESI-MS (*m*/*z*): 310 $(M+H)^+$, 332 $(M+Na)^+$.

4.7. Synthesis of D-pipecolic acid 1b

The Boc group of **9** (4.33 g, 14 mmol) was removed using 2 M HCl in dioxane (70 mL) to give the corresponding amino acid hydrochloride. After evaporation of dioxane, the solid product was dissolved in DMF (15 mL) and triethylamine (3.9 mL, 28 mmol) was added. The reaction was continued for 5 h and the pH of the solution kept at 8 by the addition of triethylamine. After filtration, the solution was neutralized with acetic acid. Then the solution was concentrated and dissolved in water. The aqueous solution was then applied to a column of Dowex 50W-X4 with 1 M ammonia elution. The solution was concentrated and crystallized from acetone to obtain D-pipecolic acid as a white solid **1b** (1.71 g, 94%) TLC: R_f, 0.30 (1-butanol/acetic acid/pyridine/H_2O); >99% ee (determined by column II, R_t: 14.96 min); mp: 271–274 °C; $[\alpha]_D^{25} = +26.3$ (*c* 1, water).

4.8. Synthesis of Boc-L-2-amino-6-bromoheptanoic acid 11

Metallic sodium (4.49 g, 195 mmol) was added to absolute ethanol (200 mL) with stirring. After complete dissolution, diethyl(Boc-amino)malonate **5** (55.1 g, 200 mmol) was added and refluxed for 30 min and then cooled down. 1,4-Dibromopentane (100 g, 435 mmol) was added to the solution and refluxed for 5 h. The reaction was monitored by TLC. After completion of the reaction, aqueous NaOH (2 M, 100 mL) was added to the reaction mixture at 0 °C in different portions with stirring. After selective hydrolysis, ethanol was evaporated and the unreacted dibromoalkane removed by extraction with diethyl ether under basic conditions. Then the aqueous solution was extracted with ethyl acetate at pH = 3–4, by the addition of citric acid and washed with brine, dried and evaporated to obtain the monoester monoacid as a white solid. It was then refluxed in toluene (150 mL) for 5 h and toluene then evaporated to give oil **10** (40.0 g, 57%). The resulting oil **10** (14.7 g, 42 mmol) was dissolved in mixture of water (110 mL) and DMF (40 mL). The pH of the solution was adjusted to 8 by the addition of 1 M NH_3. Then subtilisin Carlsberg from *Bacillus licheniformis* was added (50 mg) and incubated at 38 °C. The pH of the solution was maintained at 8 by the addition of 1 M NH_3. The reaction was continued for 3 h and then the mixture concentrated. The unreacted D-2-Boc-amido-6-bromo-heptanoic acid ethyl ester was removed by extraction with diethyl ether under basic condition. Then the aqueous solution was extracted with ethyl acetate at pH = 3–4, by the addition of citric acid and then washed with brine, dried and evaporated to obtain **11** as a colourless oil (5.6 g, 40%) Analytical RP HPLC: R_t: 7.35 min, 98% (column I); $[\alpha]_D^{25} = -2.0$ (*c* 0.5, MeOH), ^{1}H NMR (500 MHz, CD_3OD) $\delta = 4.16$ (m, 1H), 4.06 (m, 1H), 1.83–1.80 (m, 2H), 1.71–1.59 (m, 4H), 1.50–1.40 (m, 12H); ^{13}C NMR (CD_3OD) $\delta = 176.2$, 158.1, 80.5, 54.8, 52.2, 41.8, 32.3, 28.8, 26.9, 25.3; ESI-MS (*m*/*z*): 324 $(M+H)^+$, 346 $(M+Na)^+$.

4.9. Attempted synthesis of methyl pipecolic acid 13

The Boc group of **11** (3.7 g, 11.5 mmol) was removed using 2 M HCl in dioxane (58 mL) to give the corresponding amino acid hydrochloride. After evaporation of dioxane, it was dissolved in mixture of water (10 mL) and methanol (10 mL) and then triethylamine (3.2 mL, 23 mmol) added. The reaction was continued for 5 h and the pH of the solution maintained at 8 by the addition of triethylamine. After the evaporation of water and methanol, it was redissolved in a mixture of water (10 mL) and dioxane (10 mL) and Boc_2O (3.2 mL, 13.8 mmol) and triethylamine (2.4 mL, 17.3 mmol) were added at under 0 °C and stirred for 6 h. After the evaporation of dioxane, unreacted Boc_2O was removed by extraction with diethyl ether under basic conditions. Then the aqueous solution was extracted with ethyl acetate under acidic conditions and washed with brine. Evaporation of ethyl acetate give a colourless oil and the oil dissolved in DMF (10 mL), then benzyl bromide (2.1 mL, 17.3 mmol) and triethylamine (1.9 mL, 13.8 mmol) were added and stirred at 0 °C for 6 h. The reaction mixture was concentrated and the residue purified by silica gel chromatography using a mixture of hexane/ethyl acetate (8:1) to yield a colourless oil (2.7 g, 8.0 mmol). Analysis showed that the compound was Boc-L-2-amino-heptenoic acid benzyl ester **12**. Analytical RP HPLC: R_t: 10.34 min, 97% (column I); $[\alpha]_D^{25} = -27.7$ (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 7.31$–7.38 (m, 5H), 5.40 (m, 1H), 5.32 (m, 1H), 5.11–5.21 (m, 2H), 5.02 (d, 1H, $J = 8.5$ Hz), 4.33 (d, 1H, $J = 5.0$ Hz), 2.01 (m, 2H), 1.70–185 (m, 2H), 1.67 (m, 2H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$) $\delta = 172.7$, 155.3, 135.4, 129.3, 128.5, 128.4, 128.3, 128.1, 126.3, 114.9, 79.7, 66.9, 53.2, 33.1, 32.4, 28.3, 24.4; HRMS (FAB, *m*/*z*): $(M+H)^+$ found: 334.1997 (calcd for $C_{19}H_{28}O_4N$: 334.2018).

References

1. (a) Flynn, G. A.; Giroux, E. L.; Dage, R. C. *J. Am. Chem. Soc.* **1987**, *109*, 7914–7915; (b) Hamilton, S. G.; Wu, Y.-Q.; Limburg, D. C.; Wilkinson, D. E.; Vall, M. J.; Li, J. H.; Thomas, C.; Huang, W.; Sauer, H.; Ross, D. T.; Soni, R.; Chen, Y.; Guo, H.; Howaorth, P.; Valentine, H.; Liang, S.; Spicer, D.; Fuller, M.; Steiner, J. *J. Med. Chem.* **2002**, *45*, 3549–3557.
2. Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M. *J. Am. Chem. Soc.* **1987**, *109*, 5031–5033.
3. Adger, B.; Dyer, U.; Hutton, G.; Woods, M. *Tetrahedron Lett.* **1996**, *37*, 6399–6402.
4. Germann, U. A.; Shlyakhter, D.; Mason, V. S.; Zelle, R. E.; Duffy, J. P.; Galullo, V.; Armistead, D. M.; Saunders, J. O.; Boger, J.; Harding, M. W. *Anticancer Drugs* **1997**, *8*, 125–140.
5. Pettibone, D. J.; Clineschmidt, B. V.; Anderson, P. S.; Freidinger, R. M.; Lundell, G. F.; Koupal, L. R.; Schwartz, C. D.; Williamson, J. M.; Goetz, M. A.; Hensens, O. D.; Liesch, J. M.; Springer, J. P. *Endocrinology* **1989**, *125*, 217–222.
6. Vezina, C.; Kudelski, A.; Sehgal, S. N. *J. Antibiot.* **1975**, *28*, 721–726.
7. Boger, D. L.; Chen, J. H.; Saionz, K. W. *J. Am. Chem. Soc.* **1996**, *118*, 1629–1644.
8. Hirota, A.; Suzuki, A.; Aizawa, K.; Tamura, S. *Arg. Biol. Chem.* **1973**, *37*, 955–956.
9. (a) Darkin-Rattray, S. J.; Gurnett, A. M.; Myers, R. W.; Dulski, P. M.; Crumley, T. M.; Allocco, J. J.; Cannova, C.; Meinke, P. T.; Colletti, S. L.; Bednarek, M. A.; Singh, S. B.; Goetz, M. A.; Dombrowski, A. W.; Polishook, J. D.; Schmatz, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13143–13147; (b) Singh, S. B.; Zink, D. L.; Liesch, J. M.; Mosley, R. T.; Dombrowski, A. W.; Bills, G. F.; Darkin-Rattray, S. J.; Schmatz, D. M.; Goetz, M. A. *J. Org. Chem.* **2002**, *67*, 815–825.
10. Itagaki, H.; Nagashima, K.; Sugita, K.; Yoshida, H.; Kawamura, Y.; Yasuda, Y.; Matsumoto, K.; Ishii, K.; Uotani, N.; Nakai, H.; Terui, A.; Yoshimatsu, S. *J. Antibiot.* **1990**, *43*, 1524–1532.
11. Kikumoto, R.; Tamayo, Y.; Tezuka, T.; Tonomura, S.; Hara, H.; Ninomiya, K.; Hijikata, A.; Okamoto, S. *Biochemistry* **1984**, *23*, 85–90.
12. (a) Maison, W.; Kennedy, R.; Kemp, D. S. *Tetrahedron Lett.* **2001**, *42*, 4975–4977; (b) Smith, A. B., III; Benowitz, A. B.; Sprengeler, P. A.; Barbosa, J.; Gusman, M. C.; Hirchmann, R.; Schweiger, E. J.; Bolin, D. R.; Nagy, Z.; Campbell, R. M.; Cox, D. C.; Olson, G. L. *J. Am. Chem. Soc.* **1999**, *121*, 9286–9298.
13. (a) Furumai, R.; Komatsu, Y.; Nishino, N.; Khochbin, S.; Yoshida, M.; Horinouchi, S. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 87–92; (b) Komatsu, Y.; Tomizaki, K.; Tsukamoto, M.; Kato, T.; Nishino, N.; Sato, S.; Yamori, T.; Tsuruo, T.; Furumai, R.; Yoshida, M.; Horinouchi, S.; Hayashi, H. *Cancer Res.* **2001**, *61*, 4459–4466; (c) Nishino, N.; Jose, B.; Okamura, S.; Ebisusaki, S.; Kato, T.; Sumida, Y.; Yoshida, M. *Org. Lett.* **2003**, *5*, 5079–5082.
14. (a) Agematsu, H.; Fujii, T. *Bio Industry* **2002**, *19*, 40–47; (b) Nazabadioko, S.; Peréz, R. J.; Brieva, R.; Gotor, V. *Tetrahedron: Asymmetry* **1998**, *9*, 1597–1604; (c) Ng-Youn-Chen, M. C.; Serreqi, A. N.; Huang, Q. L.; Kazlauskas, R. J. *J. Org. Chem.* **1994**, *59*, 2075–2081; (d) Sánchez-Sancho, F.; Herradón, B. *Tetrahedron: Asymmetry* **1998**, *9*, 1951–1965.
15. (a) Myers, A. G.; Gleason, J. L.; Yoon, T.; Kung, D. W. *J. Am. Chem. Soc.* **1997**, *119*, 656–673; (b) Agami, C.; Kadouri-Puchot, C.; Kizirian, J. C. *Synth. Commun.* **2000**, *30*, 2565–2572; (c) Berrien, J. F.; Royer, J.; Husson, H. P. *J. Org. Chem.* **1994**, *59*, 3769–3774.
16. (a) Fujii, T.; Miyoshi, M. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 1341–1342; (b) Kisfaludy, L.; Korenczki, F. *Synthesis* **1982**, *9*, 163; (c) Ohtani, B.; Tsuru, S.; Nishimoto, S.; Kagiya, T. *J. Org. Chem.* **1990**, *55*, 5551–5553.
17. (a) Foti, C. J.; Comins, D. L. *J. Org. Chem.* **1995**, *60*, 2656–2657; (b) Fernandez-Garcia, C.; McKervey, M. A. *Tetrahedron: Asymmetry* **1995**, *6*, 2905–2906; (c) Ginesta, X.; Pericas, M. A.; Riera, A. *Tetrahedron Lett.* **2002**, *43*, 779–782.
18. (a) Portoghese, P. S.; Pazdernik, T. L.; Kuhn, W. L.; Hite, G.; Shafi'ee, A. *J. Med. Chem.* **1968**, *11*, 12–15; (b) Hochless, D. C. R.; Mayadunne, R. C.; Wild, S. B. *Tetrahedron: Asymmetry* **1995**, *6*, 3031–3037; (c) Eichhorn, E.; Roduit, J.; Shaw, N.; Heinzmann, K.; Kiener, A. *Tetrahedron: Asymmetry* **1997**, *8*, 2533–2536.
19. Watanabe, L. A.; Jose, B.; Kato, T.; Nishino, N.; Yoshida, M. *Tetrahedron Lett.* **2004**, *45*, 491–494.
20. (a) Wakayama, M.; Hayashi, S.; Yatsuda, Y.; Katsuno, Y.; Sakai, K.; Moriguchi, M. *Protein Expr. Purif.* **1996**, *7*, 395–399; (b) Wakayama, M.; Moriguchi, M. *J. Mol. Catal. B: Enzyme* **2001**, *12*, 15–25.
21. Yamaguchi, J.; Ueki, M. *Chem. Lett.* **1996**, *25*, 621–622.