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SYNTHESIS OF OPTICALLY ACTIVE (*R*)- AND (*S*)-β-ARGININE FROM PYROGLUTAMIC ACID

Yoko Yasuno, Akira Sawai, Ai Sekihara, and Tetsuro Shinada*

Graduate School of Science, Osaka City University, 3-3-138, Sugimoto, Sumiyoshi, Osaka 558-8585, Japan. E-mail: shinada@sci.osaka-cu.ac.jp

Dedicated to Professor Kaoru Fuji on cerebration on his 80th birthday

Abstract – The first synthesis of optically active β-arginine was achieved starting from commercially available pyroglutamic acid. The new synthetic protocol is characterized by the use of nitrile as a carboxylic acid surrogate which could be transformed to the corresponding 2-acyl-1,3-bis(1,1-dimethylethyl)imidodi-carbonic acid ester (active amide) via Pt-catalyzed hydration under the mild conditions. The active amide was converted to β-arginine and Boc-β-Arg-Val-OMe in good yield.

INTRODUCTION

Blastidicin S (**1**), LL-BM547β (**2**), and SF-2132 (**3**) are β-arginine containing natural products discovered by a screening program of antibiotic natural products (Figure 1). Blastidicin S (**1**) is a metabolite of *Streptomyces griseochromogenes*[1] and inhibits protein synthesis in both prokaryotic and eukaryotic cells

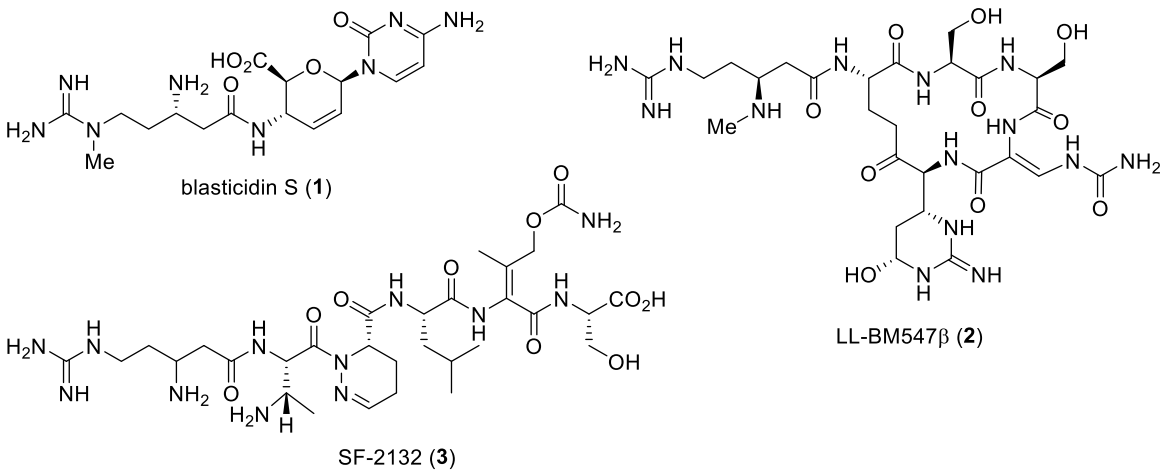


Figure 1. β-Arginine containing natural products

to lead strong growth inhibitory activity against a number of microorganisms such as *Piricularia oryzae*, *Escherichia coli*, and *Saccharomyces cerevisiae*.[2-4] LL-BM547β (**2**), isolated from *Nocardia* sp. Lederle culture BM547, is a structurally complex member of the viomycin family that reveals anti-tuberculosis activity.[5] SF-2132 (**3**), isolated form *Nocardiopsis* SF-2132 by Meiji Seika group, shows anti-bacterial activities against β-lactam antibiotic-resistant strains of *Pseudomonas* and *Escherichia*.[6] These natural products commonly share β-arginines **4**~**6** as one of the unusual amino acid components (Figure 2). The structure of blasticidic acid (**4**), a degradation product of **1**, was initially speculated by comparison of optical rotatory dispersion curves of **4** with those of structurally defined β-amino acids such as (*S*)-β-lysine.[7] The absolute structure of **4** was unambiguously determined by the single crystal X-ray diffraction analysis of **1**.[8] The absolute stereochemistry of *N*-methyl-β-arginine (**5**) of LL-BM547β (**2**) was supposed to be *S* by the chemical degradation of **2** to give **5** and comparison of the sign of optical rotation value of **5** ($[\alpha]_D$ +20, H_2O)[5] with that of structurally similar blasticidic acid (**4**) ($[\alpha]_D^{15}$ +25, (*c* 1, H_2O).[4] In contrast, the stereochemistry of β-arginine (**6**) of **3** has not been determined yet.

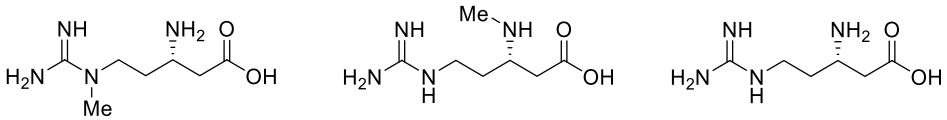
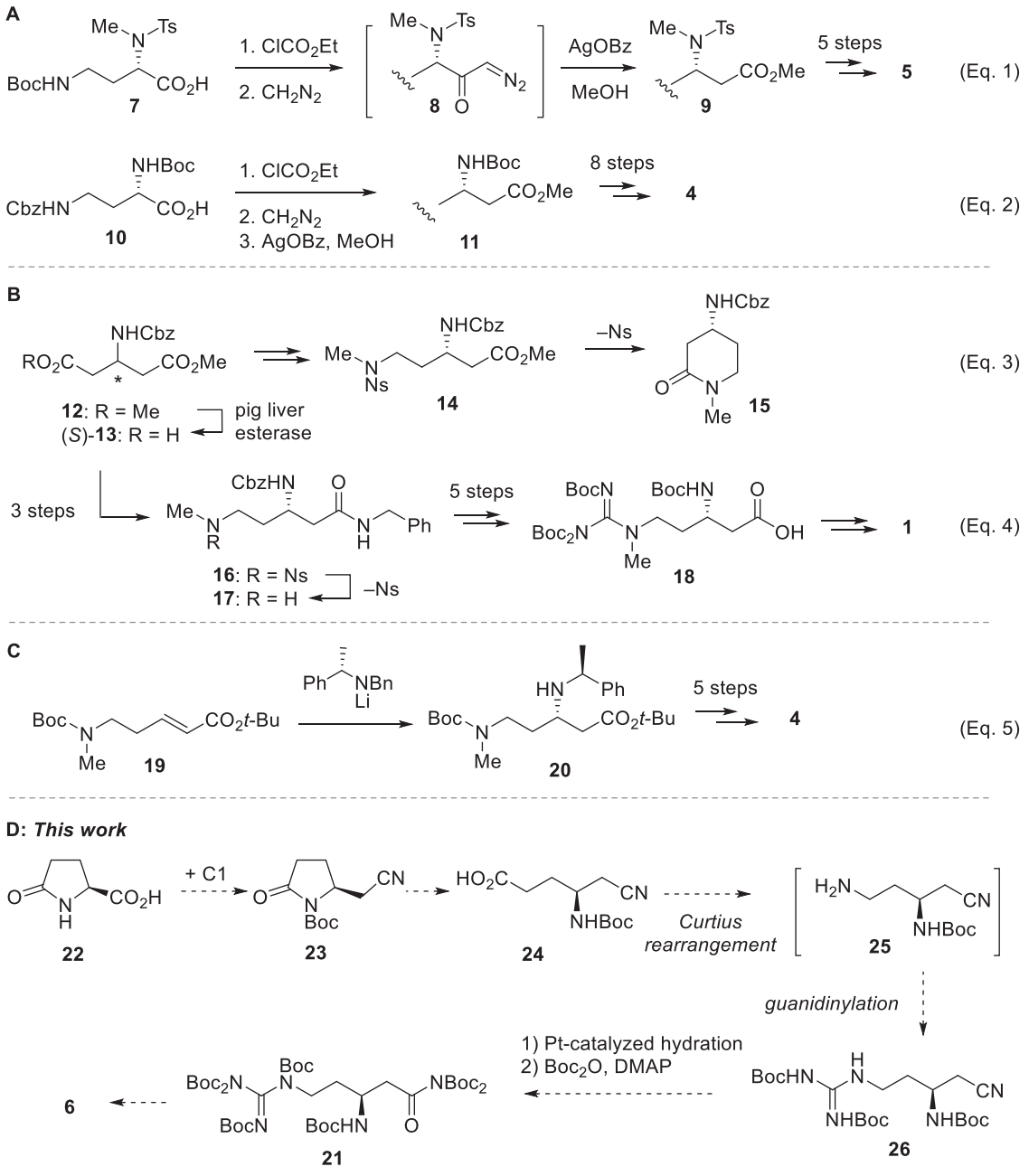


Figure 2. Structures of β-arginine units of **4**~**6**

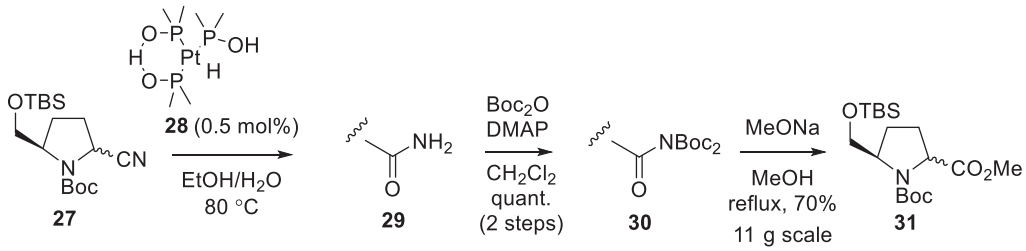
Authentic samples of (*R*/*S*)-β-arginine and its methyl derivatives **4**~**6** would aid the structure elucidation of β-arginine containing natural products. However, only a few synthetic examples of **4**~**6** have been reported (Scheme 1, **A**~**C**). In 1978, Shiba *et al.* reported the synthesis of **5** from **7** via Arndt-Eistert reaction of **8** (Eq. 1).[9] Three syntheses were performed for the synthesis of blasticidic acid (**4**) (**A** and **B**, Eqs. 2~4). Arndt-Eistert reaction was applied to the synthesis of **4** from (*S*)-**10** by Nomoto *et al.* (Eq. 2). Ichikawa *et al.* achieved the first total synthesis of blasticidin S (**1**) (Eq. 3).[10] In this study, optically active blasticidic acid derivative **18** was prepared by enzymatic desymmetrization of *meso*-diester **12** as a key step. The chiral half ester **13** was converted to *N*-methyl-(*S*)-β-arginine ester **14**. Initial attempts for the removal of the Ns group of **14** resulted in the formation of lactam **15**. The undesired lactam formation was successfully avoided by the protection of ester **14** as amide **16** (Eq. 4). Indeed, the deprotection of the 2-nitrobenzenesulfonyl (Ns) group of **16** gave amine **17** without cyclization. After guanidinylation, the resulting **18** was transformed to blasticidin S (**1**) for the first time. Sutherland *et al.* selectively prepared **4** by an aza-Michael reaction as a key step (Eq. 5).[11] Stereoselective addition of (*S*)-*N*-benzylphenethylamine to **19** gave **20** which was converted to **4** in 5 steps. In contrast to the

synthesis of **4** and **5**, structurally simple (*R*/*S*)-β-arginine **6** has not been synthesized yet.[12] Herein, we would like to report the first synthesis of optically active β-arginine **6** and its *t*-butoxycarbonyl (Boc)-protected derivative **21** (Scheme 1, **D**). The new synthesis features the use of commercially available pyroglutamic acid **22** as a chiral starting material. (*S*)-Pyroglutamic acid (**22**) would be transformed to nitrile **26** by a series of sequential transformations, C1 homologation, Curtius rearrangement, and guanidinylation reactions. Based on the Ichikawa's report, we chose nitrile as a stable carboxylic acid surrogate to avoid the problematic lactam formation. On the other hand, a mild reaction



Scheme 1. Synthesis of β-arginine derivatives. **A**: C1 homologation route, **B**: Desymmetrization route, **C**: Asymmetric Michael addition route, **D**: This work

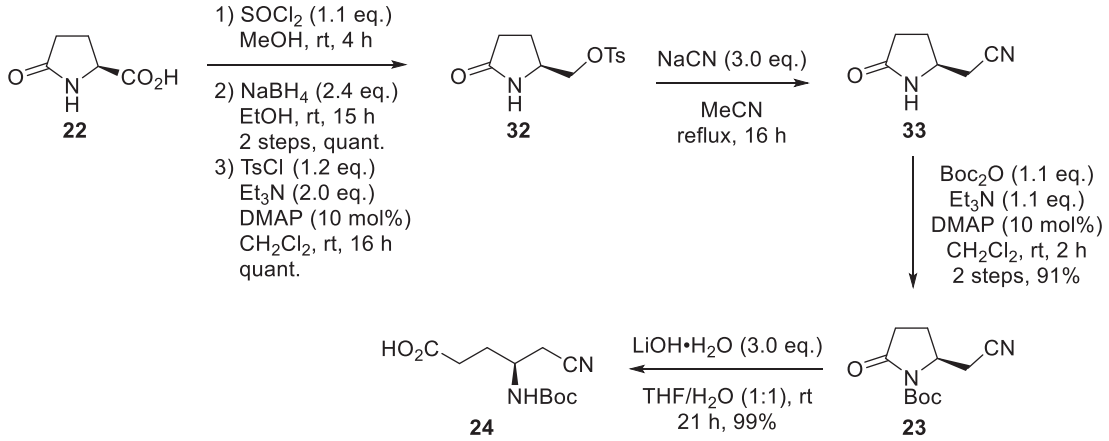
condition is required for the hydrolysis of nitrile **26** without loss of the Boc protection. We thought that the limitation would be overcome by application of our previous method for the conversion of nitrile **27** to ester **31** via di-Boc amide **30** (Scheme 2).[13]



Scheme 2. Mild transformation of nitrile **27** to ester **31**

RESULTS AND DISCUSSION

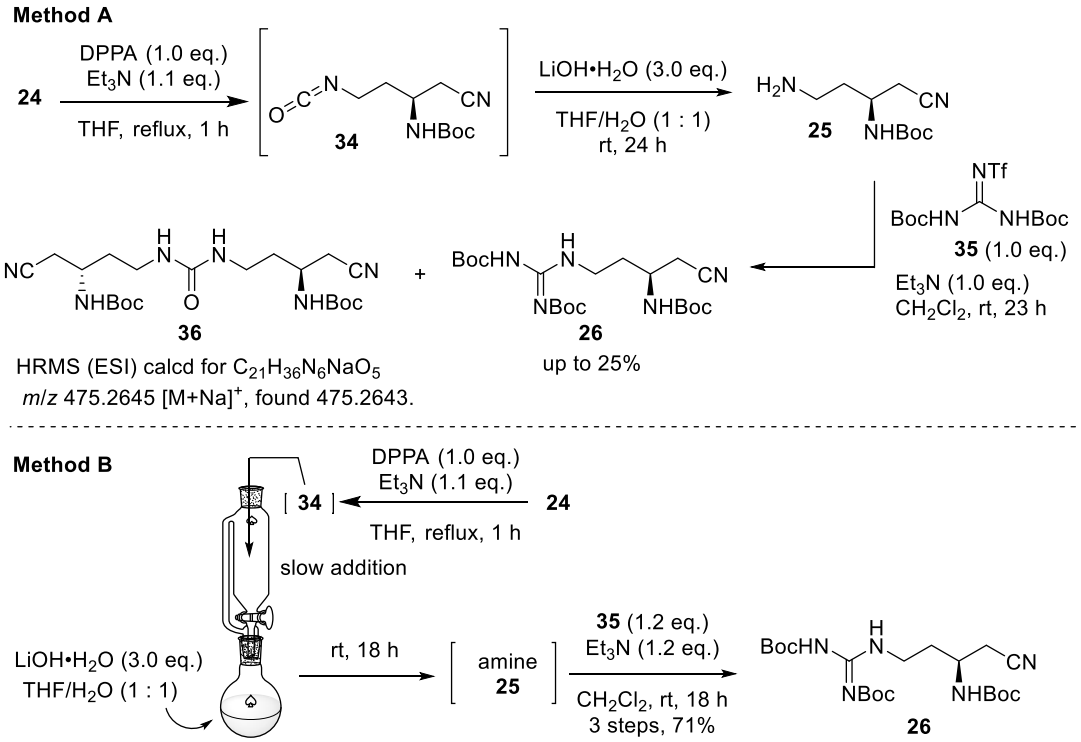
Synthesis of (*S*)-β-arginine (**6**) was commenced with one carbon homologation of (*S*)-pyroglutamic acid (**22**) (Scheme 3). According to the literature,[14] tosylate **32** was prepared from (*S*)-**22** in three steps. Treatment of **32** with NaCN in acetonitrile under reflux gave nitrile **33**. The ring opening reaction of **33** was achieved by the initial formation of *N*-Boc lactam **23** followed by treatment with aqueous LiOH to give carboxylic acid **24**.



Scheme 3. Synthesis of **24** from (*S*)-pyroglutamic acid (**22**)

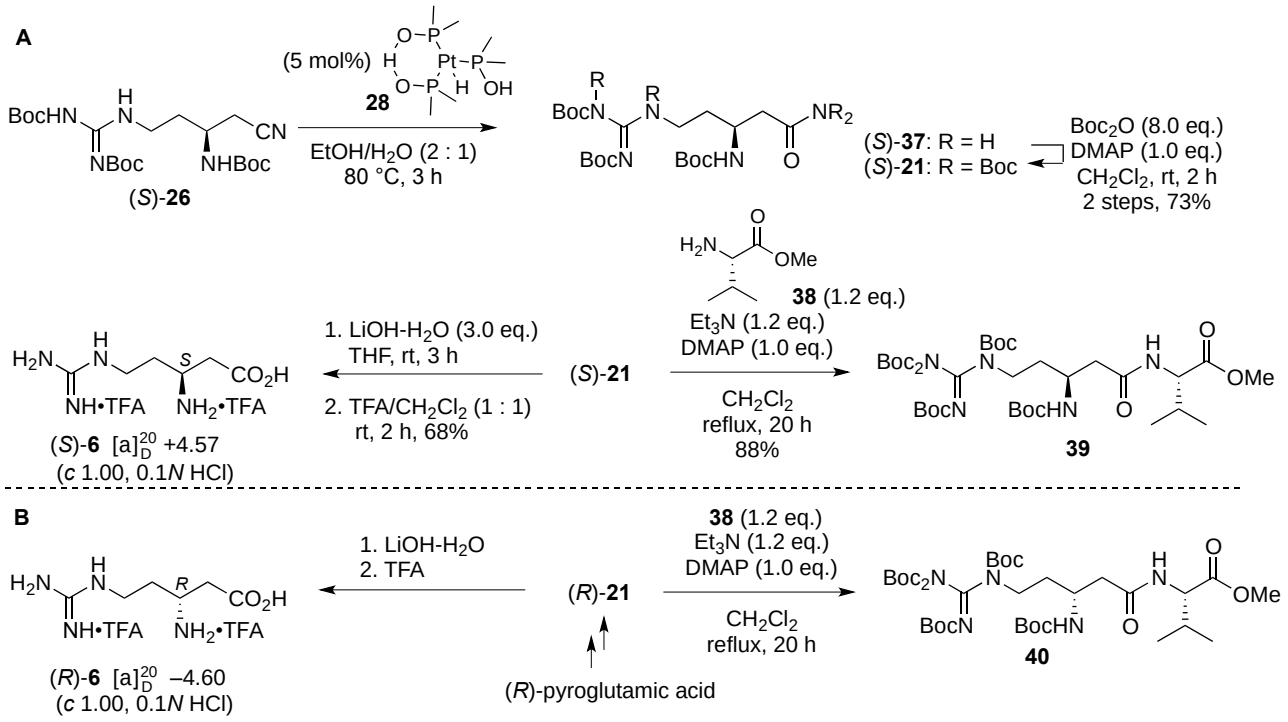
The initial attempts for the synthesis of **26** were failed to give rise to dimer **36** as a major product (Scheme 4, method **A**). Curtius rearrangement reaction of **24** was performed by diphenylphosphoryl azide (DPPA) and Et_3N under reflux to give isocyanate intermediate **34**. Then, aqueous LiOH solution was added to the reaction mixture including isocyanate intermediate **34** followed by treatment with Goodman reagent **35**[15] to give **26** in low yield (~25%) without reproducibility. These results indicated that the isocyanate intermediate **34** was trapped with amine **25** prior to the completion of the hydrolysis of

isocyanate **34**. This problem was solved by the reverse addition of isocyanate **34** to aqueous LiOH solution (method **B**). The resulting amine **25** was efficiently trapped with **35** to provide **26** in 71% yield. Only a negligible amount of urea **36** was detected under the conditions.



Scheme 4. One pot procedure for the synthesis of (*S*)-**26** from (*S*)-**24**

With nitrile **26** in hand, we next attempted the mild conversion of (*S*)-nitrile **26** to the active amide **21** and β-arginine (**6**) (Scheme 5, **A**). Addition of H_2O to nitrile **26** was achieved in the presence of 5 mol% of Pt catalyst **28** in EtOH at 80 °C.[13] The resulting amide **37** was activated as di-Boc amide **21** by treatment with an excess amount of Boc_2O in the presence of *N,N*-dimethyl-4-aminopyridine (DMAP). The active amide **21** was transformed to (*S*)-**6** by alkaline hydrolysis followed by treatment with trifluoroacetic acid (TFA) to furnish (*S*)-**6** ($[\alpha]_D^{20}$ +4.57, *c* 1.00, 0.1 *N* HCl). To test the synthetic potential of the active amide **21** for peptide coupling reaction, (*S*)-**21** was condensed with (*S*)-NH_2-Val-OMe **38**. The coupling reaction smoothly proceeded in the presence of Et_3N and DMAP in CH_2Cl_2 at reflux to provide **39** in 88% yield. In a similar manner, (*R*)-**6** ($[\alpha]_D^{20}$ –4.60, *c* 1.00, 0.1 *N* HCl) and dipeptide **40** were prepared from (*R*)-pyroglutamic acid (**22**), respectively (Scheme 5, **B**). ^{1}H-NMR data of **39** and **40** were depicted in Figure 3. Valine methyl resonances of **39** (0.86 and 0.82 ppm, each d, J = 6.6 Hz) and **40** (0.90 and 0.86 ppm, each d, J = 6.6 Hz) are distinctly distinguished each other (Supporting Information). These results confirm the optical purity of the synthetic β-arginine **6** as well as the facts that no epimerization took place in the new synthetic method.



Scheme 5. Synthesis of β-arginine and dipeptides.
A: synthetic route for (*S*)-**6** and **39**, **B**: synthetic route for (*R*)-**6** and **40**

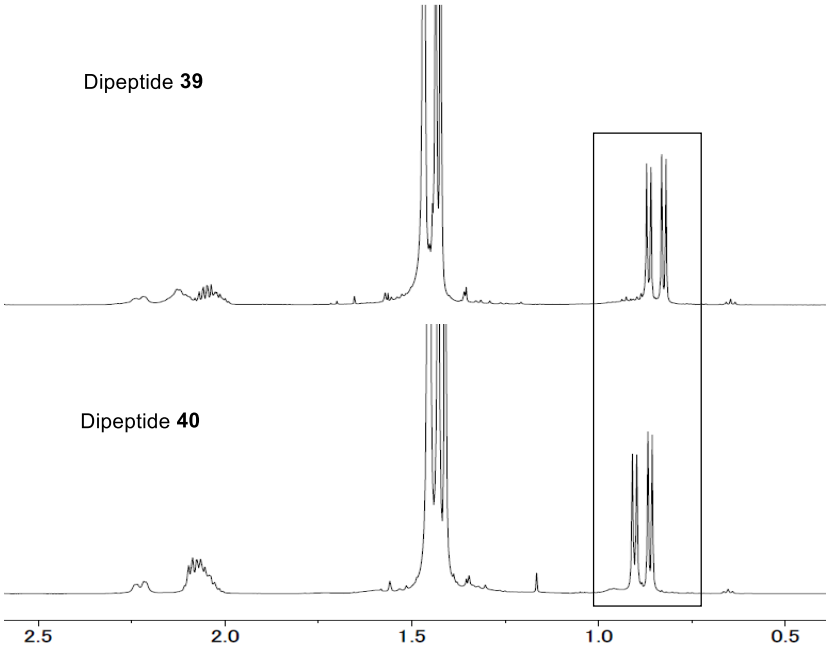


Figure 3. ^{1}H-NMR chart for 0.5~2.5 ppm of dipeptides **39** and **40** (600 MHz, C_6D_6)

CONCLUSION

In summary, we have developed an efficient synthetic method for the first synthesis of optically active β-arginine from easily available pyroglutamic acid as a chiral starting material. The synthetic

utility of the active amide **21** was demonstrated by the synthesis of β-arginine containing dipeptides. Total synthesis of β-arginine containing natural products is ongoing in our laboratory.

EXPERIMENTAL

General: All reagents and solvents were purchased from either Aldrich Chemical Company, Inc., Kanto Kagaku Co., Inc., Merck & Co., Inc., Nacalai Tesque Company, Ltd., Peptide Institute, Tokyo Kasei Kogyo Co., Ltd., or Wako Pure Chemical Industries, Ltd., and used without further purification unless otherwise indicated. Dichloromethane (CH_2Cl_2) was distilled from phosphorus pentoxide (P_2O_5). Acetonitrile (MeCN), ethanol (EtOH), and tetrahydrofuran (THF) of anhydrous grade were used. Optical rotations were taken on a JASCO P-1030 polarimeter with a sodium lamp (D line) using $CHCl_3$ or H_2O of a spectrochemical analysis grade. Melting points were determined with a Yanaco MP-21 melting point apparatus and were uncorrected. FTIR spectra were measured on a JASCO FT/IR-6200 infrared spectrophotometer. ^{1}H NMR spectra were recorded on Bruker AVANCE 300 (300 MHz), JEOL JNM-LA 400 (400 MHz), Bruker AVANCE 400 (400 MHz), or Bruker AVANCE 600 (600 MHz) spectrometer. Chemical shifts of ^{1}H NMR were reported in parts per million (ppm, δ) relative to $CHCl_3$ (δ = 7.26) in $CDCl_3$, C_6D_5H (δ = 7.15) in C_6D_6, or DSS (δ = 0.00) in D_2O. ^{13}C NMR spectra were recorded on Bruker AVANCE 300 (75 MHz), JEOL JNM-LA 400 (100 MHz), or Bruker AVANCE 400 (100 MHz) spectrometer. Chemical shifts of ^{13}C NMR were reported in ppm (δ) relative to $CHCl_3$ (δ = 77.0) in $CDCl_3$ or DSS (δ = 0.00) in D_2O. Low resolution mass spectra (LRMS) and high resolution mass spectra (HRMS) were obtained on JEOL JMS-T100LP for electrospray ionization (ESI) or direct analysis in real time (DART). All reactions were monitored by thin layer chromatography (TLC), which was performed with precoated plates (silica gel 60 F-254, 0.25 mm thickness, manufactured by Merck). TLC visualization was accompanied using UV lamp (254 nm) or a charring solution (ethanoic phosphomolybdic acid, aqueous potassium permanganate and butanolic ninhydrin). Daiso IR-60 1002W (40/63 μm) was used for flash column chromatography on silica gel. COSMOSIL® 140C_{18}-OPN was used for reversed-phase column chromatography.

(*S*)-*tert*-Butyl 2-(cyanomethyl)-5-oxopyrrolidine-1-carboxylate (23). To a solution of (*S*)-**32** (5.00 g, 20.2 mmol)[14] in MeCN (200 mL) was added NaCN (2.97 g, 60.7 mmol) at 0 °C under argon. The mixture was stirred for 16 h at reflux, cooled to room temperature, and filtrated through a thin Celite® pad. The filtrate was concentrated under reduced pressure to give a crude nitrile (*S*)-**32** which was subjected to the next reaction without purification. Boc_2O (5.1 mL, 22.2 mmol), Et_3N (3.1 mL, 22.2 mmol) and DMAP (247 mg, 2.02 mmol) were added to the crude nitrile in CH_2Cl_2 (200 mL) at 0 °C under argon. The mixture was stirred for 2 h at room temperature, quenched with sat. aq. NH_4Cl (200 mL), and extracted with EtOAc (150 mL × 3). The combined organic layers were washed with brine (200 mL), dried over

anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH = 100 : 1 to 50 : 1) to give (*S*)-**23** (4.10 g, 91% over 2 steps) as a white solid;

$[\alpha]_D^{24}$ –93.2 (*c* 0.59, $CHCl_3$); mp 88–89 °C (a colorless crystal, recrystallized from hexane/EtOAc); FTIR (neat) 2980, 2935, 1784, 1751, 1711, 1369, 1347, 1308, 1284, 1256, 1211, 1147, 1067, 1021 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.41–4.36 (m, 1 H), 2.88–2.71 (m, 3 H), 2.52 (ddd, *J* = 18.0, 9.9, 3.3 Hz, 1 H), 2.39–2.29 (m, 1 H), 2.07–1.98 (m, 1 H), 1.54 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 149.2, 116.5, 83.7, 53.6, 30.5, 27.7, 22.5, 22.2; HRMS (DART) calcd for $C_{11}H_{15}N_2O_3$ *m/z* 223.1083 $[M–H]^-$, found 223.1077. In a similar manner to the synthesis of (*S*)-**23**, (*R*)-**23** was prepared from (*R*)-**32** ($[\alpha]_D^{23}$ +94.7 (*c* 1.00, $CHCl_3$)).

(*S*)-4-((*tert*-Butoxycarbonyl)amino)-5-cyanopentanoic acid (24). To a solution of (*S*)-**23** (4.10 g, 18.3 mmol) in THF/H_2O (1 : 1, 180 mL) was added LiOH•H_2O (2.30 g, 54.8 mmol) at 0 °C. The mixture was stirred for 21 h at room temperature and acidified with AcOH to pH 5.0. After separation of the organic layer, aqueous layer was extracted with EtOAc (150 mL × 3). The combined organic layers were dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH = 100 : 1 to 30 : 1) to give (*S*)-**24** (4.10 g, 99%) as a white solid;

$[\alpha]_D^{24}$ –52.0 (*c* 0.40, $CHCl_3$); mp 111–112 °C (a colorless crystal, recrystallized from hexane/EtOAc); FTIR (neat) 333.6, 2979, 2934, 1717, 1705, 1700, 1696, 1684, 1558, 1540, 1521, 1507, 1457, 1419, 1385, 1368, 1252, 1220, 1165, 1057 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.80 (d, *J* = 8.7 Hz, 1 H), 3.91 (m, 1 H), 2.75 (dd, *J* = 16.8, 5.5 Hz, 1 H), 2.57 (dd, *J* = 16.8, 4.7 Hz, 1 H), 2.49 (t, *J* = 7.1 Hz, 2 H), 2.03–1.85 (m, 2 H), 1.45 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 155.2, 117.0, 80.6, 46.8, 30.3, 28.4, 28.2, 24.1; HRMS (DART) calcd for $C_{11}H_{19}N_2O_4$ *m/z* 243.1345 $[M+H]^+$, found 243.1342. In a similar manner to the synthesis of (*S*)-**24**, (*R*)-**24** was prepared from (*R*)-**23** ($[\alpha]_D^{25}$ +62.1 (*c* 1.00, $CHCl_3$)).

(*S*)-Nitrile 26. To a solution of (*S*)-**24** (3.18 g, 14.2 mmol) in THF (140 mL) were added DPPA (3.1 mL, 14.2 mmol) and Et_3N (2.2 mL, 15.6 mmol) at 0 °C under argon. The reaction mixture was stirred for 1 h at reflux to give crude isocyanate (*S*)-**34**, which was used in the next reaction without further purification. The mixture including (*S*)-**34** was added to a solution of LiOH•H_2O (1.79 g, 42.6 mmol) in H_2O (280 mL) over 1 h at 0 °C under argon. The reaction mixture was stirred for 18 h at room temperature and concentrated under reduced pressure to a crude amine (*S*)-**25**. To a solution of a crude amine in CH_2Cl_2 (140 mL) was added Goodman reagent **35**[15] (6.65g, 17.0 mmol) and Et_3N (2.4 mL, 17.0 mmol) at 0 °C under argon. The reaction mixture was stirred for 18 h at room temperature, quenched with 1*N* $NaHSO_4$ (300 mL), extracted with CH_2Cl_2 (200 mL × 3). The combined organic layers were washed with brine

(500 mL) and dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane/EtOAc = 30 : 1 to 5: 1) to give (*S*)-**26** (4.58 g, 71%) as a white solid;

$[\alpha]_D^{24}$ –39.7 (*c* 0.71, $CHCl_3$); mp 140–142 °C (decomp.) (a colorless crystal, recrystallized from hexane/EtOAc); FTIR (neat) 3328, 2979, 2934, 1717, 1636, 1616, 1559, 1506, 1417, 1388, 1365, 1327, 1289, 1251, 1228, 1153, 1130, 1052, 1025 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 11.4 (s, 1 H), 8.45 (brs, 1 H), 5.31 (brs, 1 H), 3.79 (dddd, *J* = 10.5, 7.9, 5.3, 5.3 Hz, 1 H), 3.57 (m, 1 H), 3.41 (ddd, *J* = 13.6, 6.8, 6.8 Hz, 1 H), 2.85–2.68 (m, 2 H), 2.03–1.80 (m, 2 H), 1.49 (s, 18 H), 1.44 (each s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 156.1, 155.1, 152.9, 117.2, 83.1, 79.7, 79.1, 45.6, 37.1, 32.8, 28.1, 28.0, 27.8, 23.3; HRMS (ESI) calcd for $C_{21}H_{37}N_5NaO_6$ *m/z* 478.2642 $[M+Na]^+$, found 478.2639. In a similar manner to the synthesis of (*S*)-**26**, (*R*)-**26** was prepared from (*R*)-**24** ($[\alpha]_D^{25}$ +24.2 (*c* 1.01, $CHCl_3$)).

Urea 35. ^1H NMR (300 MHz, $CDCl_3$) δ 5.24–4.38 (m, 4 H), 4.04–3.84 (m, 2 H), 3.60–3.40 (m, 2 H), 3.10–2.92 (m, 2 H), 2.72 (dd, *J* = 16.8, 5.2 Hz, 2 H), 2.61 (dd, *J* = 16.8, 5.0 Hz, 2 H), 1.91–1.65 (m, 4 H), 1.45 (s, 18 H); HRMS (ESI) calcd for $C_{21}H_{36}N_6NaO_5$ *m/z* 475.2645 $[M+Na]^+$, found 475.2643.

(*S*)-Boc-β-Arginine 21. To a solution of (*S*)-**26** (265 mg, 476 μmol) in EtOH/H_2O (2 : 1, 2 mL) was added Pt catalyst **28** (10.2 mg, 23.8 μmol), then the mixture was stirred at 80 °C. After 3 h, the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure to give a crude amide (*S*)-**37** which was subjected to the next reaction without further purification. To a solution of a crude amide (*S*)-**37** in CH_2Cl_2 (2 mL) were added Boc_2O (880 μL, 3.81 mmol) and DMAP (58.2 mg, 476 μmol) at 0 °C under argon. The mixture was stirred for 2 h at room temperature and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH = 100 : 1 to 50 : 1) to give (*S*)-**21** (303 mg, 73%) as a colorless amorphous solid;

$[\alpha]_D^{20}$ –6.26 (*c* 1.00, $CHCl_3$); FTIR (neat) 2980, 2935, 1810, 1772, 1733, 1717, 1647, 1558, 1539, 1507, 1457, 1395, 1369, 1244, 1220, 1155, 1121, 1104 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.13 (d, *J* = 8.9 Hz, 1 H), 3.97 (m, 1 H), 3.85–3.81 (m, 2 H), 3.14 (dd, *J* = 17.5, 4.6 Hz, 1 H), 2.99 (dd, *J* = 17.5, 6.1 Hz, 1 H), 1.95–1.89 (m, 2 H), 1.65–1.42 (m, 63 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 157.4, 155.2, 150.9, 149.1, 147.1, 143.5, 84.6, 83.2, 81.5, 78.8, 45.5, 44.4, 41.2, 31.5, 28.1, 28.0, 27.9, 27.7, 27.60, 27.55, 27.4; HRMS (ESI) calcd for $C_{41}H_{71}N_5NaO_{15}$ *m/z* 896.4844 $[M+Na]^+$, found 896.4833. According to the procedure for the synthesis of (*S*)-**21** from (*S*)-**26**, (*R*)-**21** was prepared from (*R*)-**26** ($[\alpha]_D^{22}$ + 6.24 (*c* 1.00, $CHCl_3$)).

(*S*)-β-Arginine (6). To a solution of (*S*)-**21** (70.8 mg, 81.0 μmol) in THF/H_2O (1 : 1, 2.0 mL) was added LiOH•H_2O (10.2 mg, 243 μmol) at 0 °C. The reaction mixture was stirred for 3 h at room temperature,

quenched with 1*N* HCl (3.0 mL), and extracted with EtOAc (5.0 mL × 3). The combined organic layers were washed with water (15 mL) and brine (15 mL), dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure to give a corresponding carboxylic acid (46.4 mg) as a colorless amorphous solid. TFA (1.0 mL) was added to a solution of the acid (38.4 mg, 56.9 μmol) in CH_2Cl_2 (1.0 mL) at 0 °C under argon. The reaction mixture was stirred at room temperature for 2 h and concentrated under reduced pressure. The residue was purified by reversed-phase column chromatography (1.0% aqueous TFA) and lyophilized to give (*S*)-**6** bisTFA salt (18.3 mg, 68%) as a colorless amorphous solid;

$[\alpha]_D^{20}$ +4.57 (*c* 1.00, 0.1*N* HCl); FTIR (neat) 3364, 3217, 1658, 1618, 1443, 1188, 1129 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 3.72 (m, 1 H), 3.34 (t, *J* = 7.1 Hz, 2 H), 2.87 (dd, *J* = 17.8, 4.7 Hz, 1 H), 2.74 (dd, *J* = 17.8, 7.7 Hz, 1 H), 2.10–1.96 (m, 2 H); ^{13}C NMR (75 MHz, D_2O) δ177.1, 166.0 (TFA, q, $^2J_{CF}$ = 38.9 Hz), 159.8, 119.4 (TFA, q, $^1J_{CF}$ = 282.9 Hz), 48.9, 40.2, 38.8, 33.9; HRMS (DART) calcd for $C_6H_{15}N_4O_2$ *m/z* 175.1195 $[M+H]^+$, found 175.1180. According to the procedure for the synthesis of (*S*)-**6** from (*S*)-**21**, (*R*)-**6** was prepared from (*R*)-**21** ($[\alpha]_D^{20}$ –4.60 (*c* 1.00, 0.1*N* HCl)).

Methyl ((*S*)-3-((*tert*-butoxycarbonyl)amino)-5-((*E*)-1,2,3,3-tetrakis(*tert*-butoxycarbonyl)guanidino)-pentanoyl)-L-valinate (39). To a solution of (*S*)-**21** (43.7 mg, 50.0 μmol) in CH_2Cl_2 (1.0 mL) were added (*S*)-valine methyl ester (**38**) (7.9 mg, 60.0 μmol), Et_3N (8.4 μL, 60.0 μmol), and DMAP (6.1 mg, 50.0 μmol) at 0 °C under argon. The mixture was stirred for 20 h at reflux, quenched with sat. aq. NH_4Cl (5.0 mL), extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine (30 mL), dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH = 100 : 1 to 50 : 1) to give **39** (30.1 mg, 88%) as a colorless amorphous solid;

$[\alpha]_D^{25}$ +2.1 (*c* 0.71, $CHCl_3$); FTIR (neat) 2977, 2934, 1803, 1734, 1718, 1653, 1647, 1559, 1539, 1507, 1457, 1394, 1368, 1279, 1240, 1220, 1141, 1103 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.34 (brs, 1 H), 5.27 (brs, 1 H), 4.48 (dd, *J* = 8.0, 5.1 Hz, 1 H), 3.92–3.73 (m, 3 H), 3.70 (s, 3 H), 2.51 (brs, 2 H), 2.20–2.08 (m, 1 H), 1.89 (q, *J* = 7.0 Hz, 2 H), 1.47–1.40 (m, 45 H), 0.90 (t, *J* = 8.0 Hz, 6 H); ^1H NMR (600 MHz, C_6D_6) δ 6.17 (d, *J* = 6.6 Hz, 1 H), 5.63 (d, *J* = 6.0 Hz, 1 H), 4.67 (dd, *J* = 8.1, 5.1 Hz, 1 H), 4.08–4.02 (m, 3 H), 3.29 (s, 3 H), 2.22 (m, 1 H), 2.12–1.99 (m, 4 H), 1.46 (s, 27 H), 1.43 (s, 9 H), 1.41 (s, 9 H), 0.86 (d, *J* = 6.6 Hz, 3 H), 0.82 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 170.5, 157.5, 155.6, 151.1, 147.3, 143.9, 83.6, 82.5, 81.9, 79.2, 57.1, 52.0, 46.7, 44.5, 41.1, 32.0, 31.3, 31.0, 28.3, 27.9, 27.9, 27.8, 18.9, 17.9; HRMS (ESI) calcd for $C_{37}H_{65}N_5NaO_{13}$ *m/z* 810.4477 $[M+Na]^+$, found 810.4487.

Methyl ((*R*)-3-((*tert*-butoxycarbonyl)amino)-5-((*E*)-1,2,3,3-tetrakis(*tert*-butoxycarbonyl)guanidino)-pentanoyl)-L-valinate (40). According to the procedure for the synthesis of **39** from (*S*)-**21**, **40** was

prepared by the coupling of (*R*)-**21** and (*S*)-valine methyl ester (**38**) in 86% yield; $[\alpha]_D^{25}$ –3.65 (*c* 1.00, $CHCl_3$); FTIR (neat) 2977, 2931, 1806, 1780, 1718, 1653, 1559, 1540, 1507, 1457, 1393, 1368, 1242, 1142, 1105 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.28 (brs, 1 H), 5.46 (brs, 1 H), 4.50 (dd, *J* = 8.6, 5.0 Hz, 1 H), 3.87–3.83 (m, 3 H), 3.72 (s, 3 H), 2.52 (brs, 2 H), 2.15 (m, 1 H), 1.92–1.89 (m, 2 H), 1.49–1.42 (m, 45 H), 0.94 (d, *J* = 6.9 Hz, 3 H), 0.90 (d, *J* = 6.9 Hz, 3 H); ^1H NMR (600 MHz, C_6D_6) δ 6.25 (d, *J* = 6.6 Hz, 1 H), 5.76 (d, *J* = 7.8 Hz, 1 H), 4.69 (dd, *J* = 8.4, 5.4 Hz, 1 H), 4.07–3.99 (m, 3 H), 3.29 (s, 3 H), 2.22 (m, 1 H), 2.10–2.01 (m, 4 H), 1.45 (s, 27 H), 1.43 (s, 9 H), 1.41 (s, 9 H), 0.90 (d, *J* = 6.6 Hz, 3 H), 0.86 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 170.8, 157.5, 155.6, 151.1, 147.3, 143.9, 83.7, 83.6, 83.5, 81.9, 79.2, 57.2, 52.1, 46.7, 44.6, 41.0, 32.0, 31.0, 28.4, 28.0, 27.9, 27.8, 19.0, 17.9; HRMS (ESI) calcd for $C_{37}H_{65}N_5NaO_{13}$ *m/z* 810.4477 $[M+Na]^+$, found 810.4483.

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