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

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Dedicated to Professor Kaoru Fuji on celebration 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)imidodidicarbonyl 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-BM547 β (**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

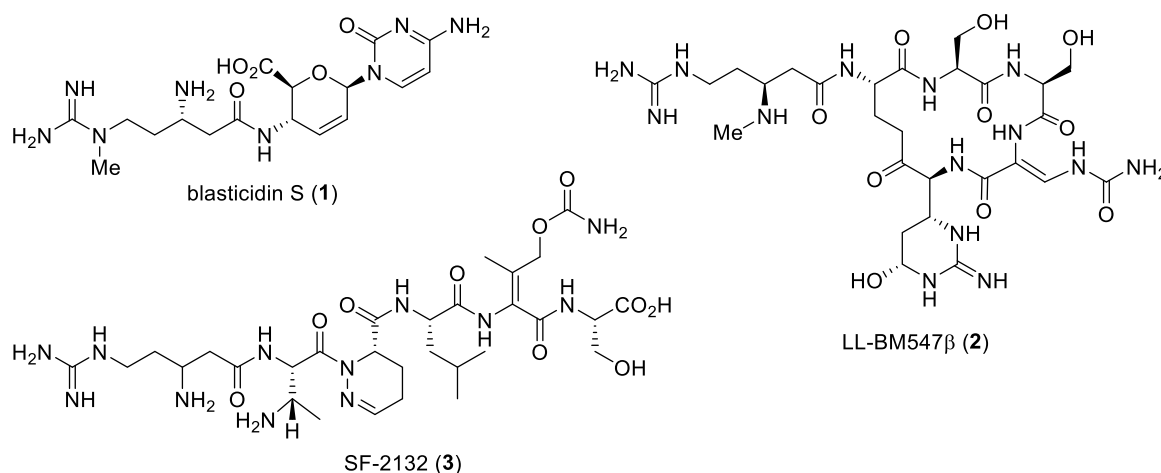


Figure 1. β -Arginine containing natural products

to lead strong growth inhibitory activity against a number of microorganisms such as *Piricularia oryzae*, *Escherichia coli*, and *Saccharomyces cerevisiae*.²⁻⁴ LL-BM547 β (**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 from *Nocardioopsis* 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-BM547 β (**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** ($[\alpha]_D^{+20}$, H₂O)⁵ with that of structurally similar blasticidic acid (**4**) ($[\alpha]_D^{+15}$, *c* 1, H₂O).⁴ In contrast, the stereochemistry of β -arginine (**6**) of **3** has not been determined yet.

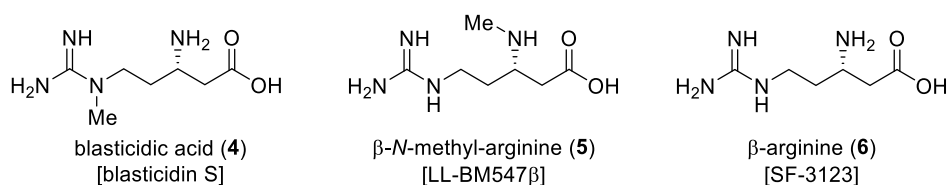
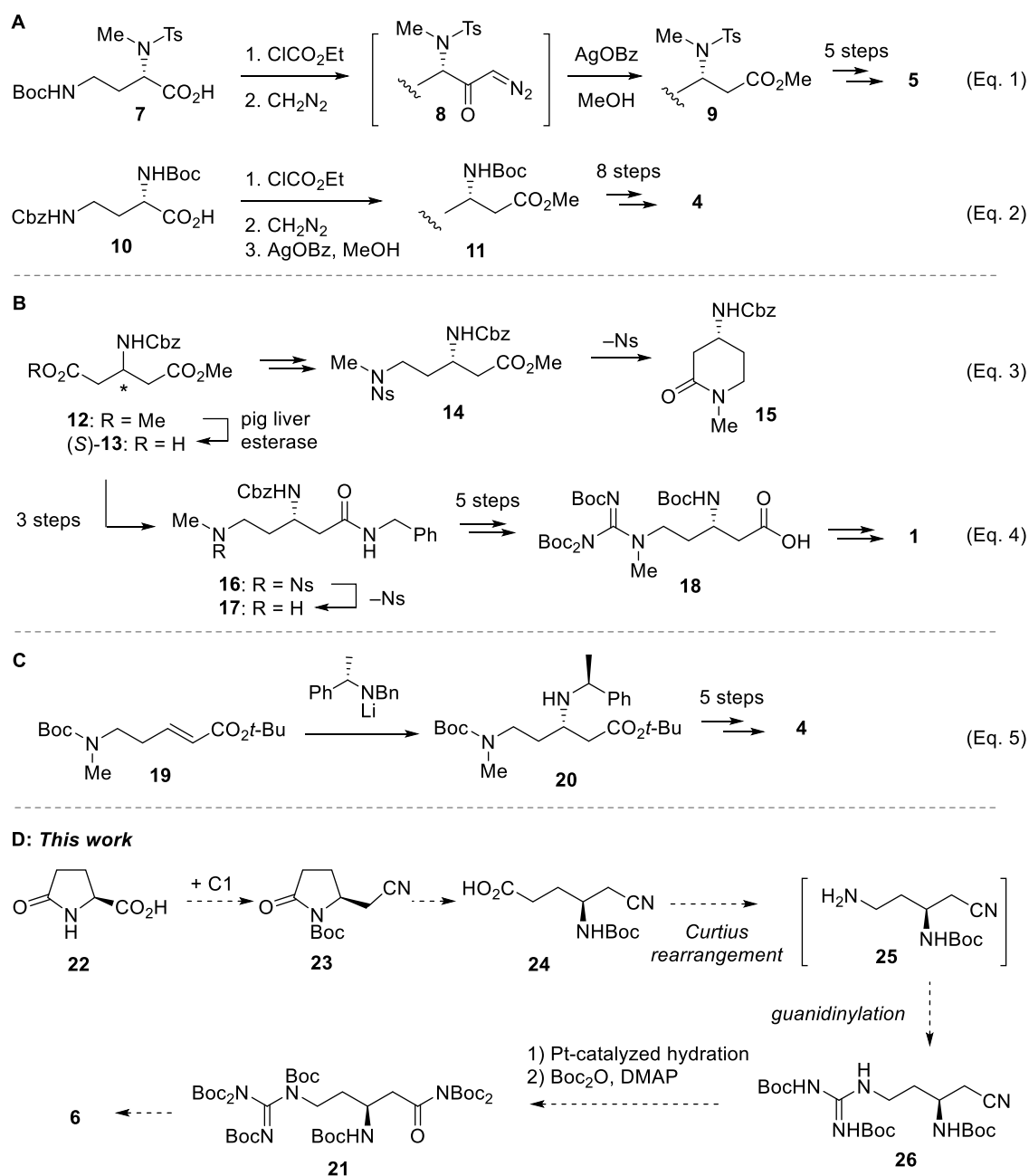


Figure 2. Structures of β -arginine units of **4**~**6**

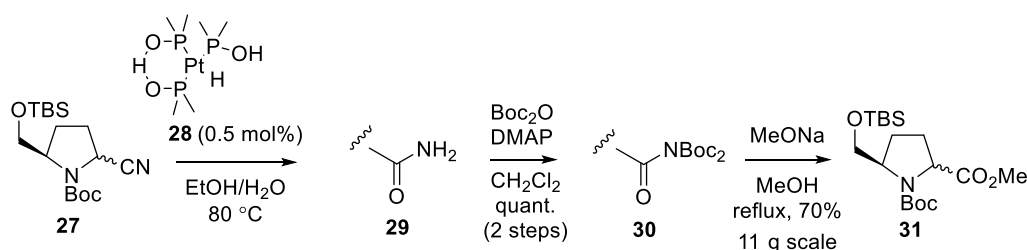
Authentic samples of (*R/S*)- β -arginine and its methyl derivatives **4**~**6** would aid the structure elucidation of β -arginine containing natural products. However, only a few synthetic examples of **4**~**6** have been reported (Scheme 1, **A**~**C**). In 1978, Shiba *et al.* reported the synthesis 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

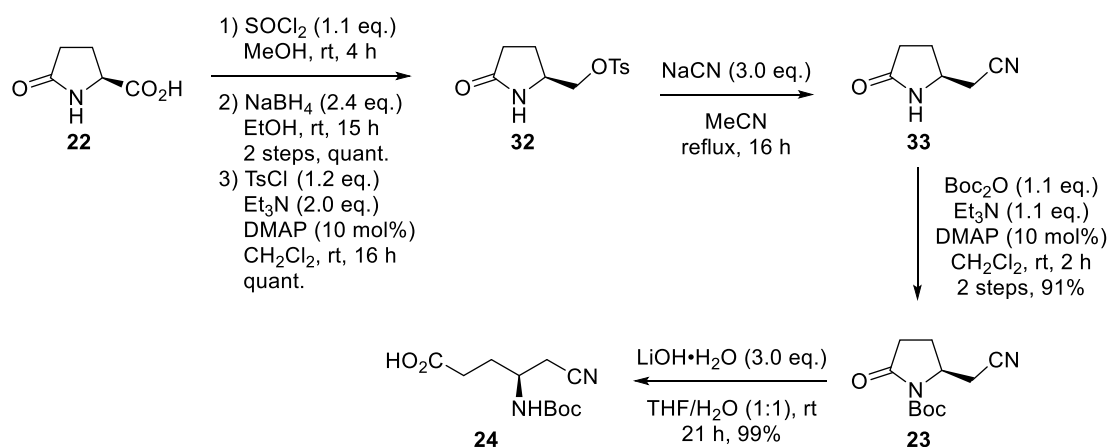
condition is required for the hydrolysis of nitrile **26** without loss of the Boc protection. We thought that the limitation would be overcome by application of our previous method for the conversion of nitrile **27** to ester **31** via di-Boc amide **30** (Scheme 2).¹³



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

RESULTS AND DISCUSSION

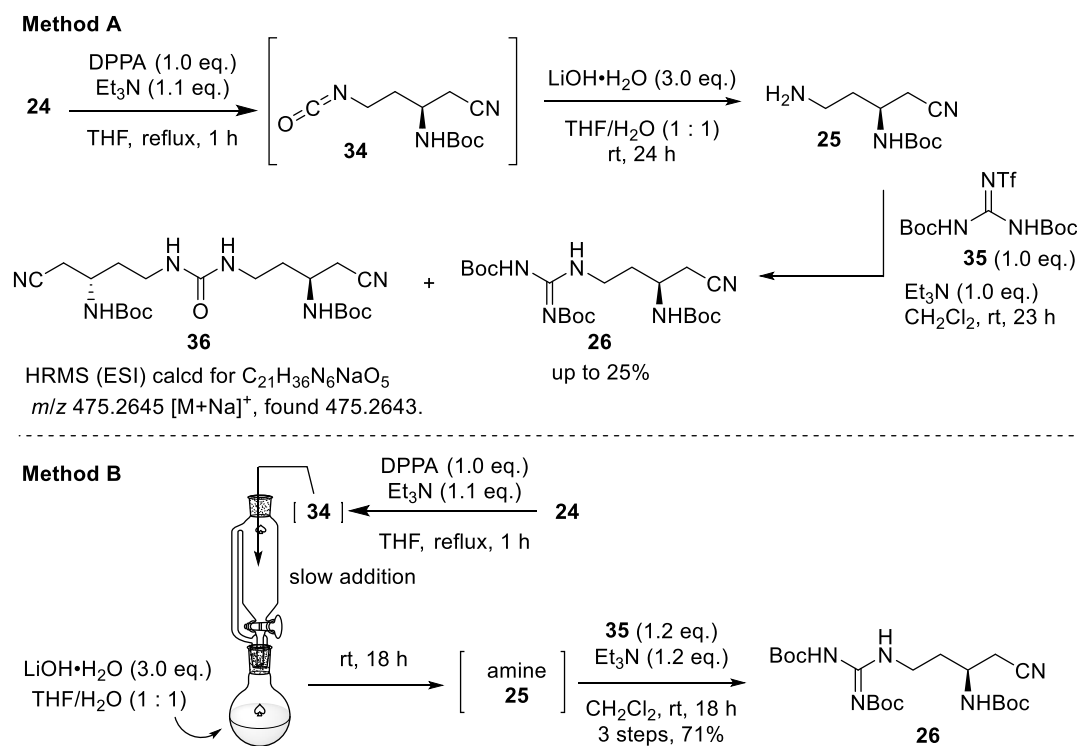
Synthesis of (*S*)- β -arginine (**6**) was commenced with one carbon homologation of (*S*)-pyroglutamic acid (**22**) (Scheme 3). According to the literature,¹⁴ tosylate **32** was prepared from (*S*)-**22** in three steps. Treatment of **32** with NaCN in acetonitrile under reflux gave nitrile **33**. 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**)

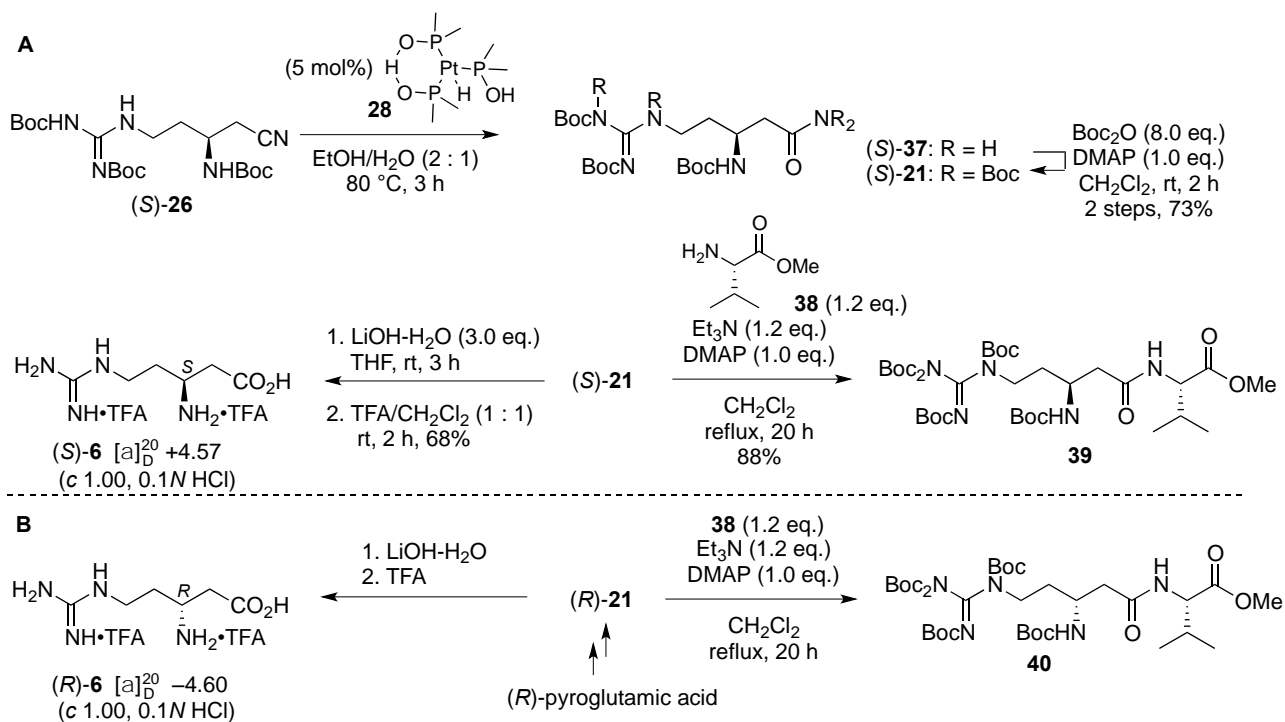
The initial attempts for the synthesis of **26** were failed to give rise to dimer **36** as a major product (Scheme 4, method A). Curtius rearrangement reaction of **24** was performed by diphenylphosphoryl azide (DPPA) and Et₃N under reflux to give isocyanate intermediate **34**. Then, aqueous LiOH 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**). 1H -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**

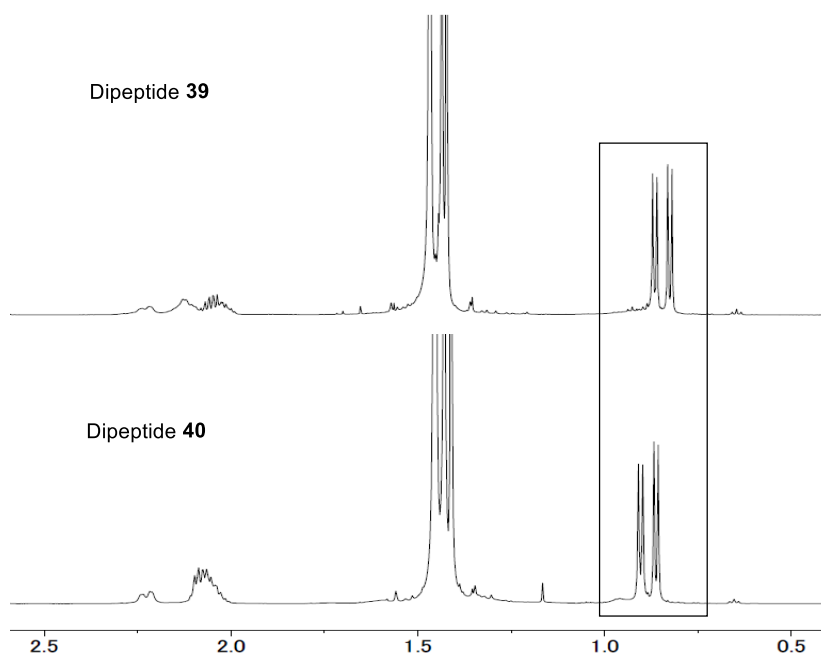


Figure 3. ¹H-NMR chart for 0.5~2.5 ppm of dipeptides **39** and **40** (600 MHz, C₆D₆)

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. ^1H 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 ^1H NMR were reported in parts per million (ppm, δ) relative to CHCl_3 ($\delta = 7.26$) in CDCl_3 , $\text{C}_6\text{D}_5\text{H}$ ($\delta = 7.15$) in C_6D_6 , or DSS ($\delta = 0.00$) 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 ($\delta = 77.0$) in CDCl_3 or DSS ($\delta = 0.00$) 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[®] 140C₁₈-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 \times 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 ($\text{CH}_2\text{Cl}_2/\text{MeOH} = 100 : 1$ to $50 : 1$) to give (*S*)-**23** (4.10 g, 91% over 2 steps) as a white solid;

$[\alpha]_{\text{D}}^{24} -93.2$ (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 $\text{C}_{11}\text{H}_{15}\text{N}_2\text{O}_3$ m/z 223.1083 $[\text{M}-\text{H}]^-$, found 223.1077. In a similar manner to the synthesis of (*S*)-**23**, (*R*)-**23** was prepared from (*R*)-**32** ($[\alpha]_{\text{D}}^{23} +94.7$ (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 $\text{LiOH}\cdot\text{H}_2\text{O}$ (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 \times 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 ($\text{CH}_2\text{Cl}_2/\text{MeOH} = 100 : 1$ to $30 : 1$) to give (*S*)-**24** (4.10 g, 99%) as a white solid;

$[\alpha]_{\text{D}}^{24} -52.0$ (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 $\text{C}_{11}\text{H}_{19}\text{N}_2\text{O}_4$ m/z 243.1345 $[\text{M}+\text{H}]^+$, found 243.1342. In a similar manner to the synthesis of (*S*)-**24**, (*R*)-**24** was prepared from (*R*)-**23** ($[\alpha]_{\text{D}}^{25} +62.1$ (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 $\text{LiOH}\cdot\text{H}_2\text{O}$ (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 1N NaHSO_4 (300 mL), extracted with CH_2Cl_2 (200 mL \times 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;

$[\alpha]_{\text{D}}^{24} -39.7$ (*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 $\text{C}_{21}\text{H}_{37}\text{N}_5\text{NaO}_6$ m/z 478.2642 $[\text{M}+\text{Na}]^+$, found 478.2639. In a similar manner to the synthesis of (*S*)-**26**, (*R*)-**26** was prepared from (*R*)-**24** ($[\alpha]_{\text{D}}^{25} +24.2$ (*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 $\text{C}_{21}\text{H}_{36}\text{N}_6\text{NaO}_5$ m/z 475.2645 $[\text{M}+\text{Na}]^+$, 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 ($\text{CH}_2\text{Cl}_2/\text{MeOH} = 100 : 1$ to 50 : 1) to give (*S*)-**21** (303 mg, 73%) as a colorless amorphous solid;

$[\alpha]_{\text{D}}^{20} -6.26$ (*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 $\text{C}_{41}\text{H}_{71}\text{N}_5\text{NaO}_{15}$ m/z 896.4844 $[\text{M}+\text{Na}]^+$, found 896.4833. According to the procedure for the synthesis of (*S*)-**21** from (*S*)-**26**, (*R*)-**21** was prepared from (*R*)-**26** ($[\alpha]_{\text{D}}^{22} + 6.24$ (*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 $\text{LiOH}\cdot\text{H}_2\text{O}$ (10.2 mg, 243 μmol) at 0 °C. The reaction mixture was stirred for 3 h at room temperature,

quenched with 1N 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 MgSO₄, 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 CH₂Cl₂ (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;

$[\alpha]_{\text{D}}^{20} +4.57$ (*c* 1.00, 0.1N HCl); FTIR (neat) 3364, 3217, 1658, 1618, 1443, 1188, 1129 cm⁻¹; ¹H NMR (300 MHz, D₂O) 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); ¹³C NMR (75 MHz, D₂O) δ 177.1, 166.0 (TFA, q, ²*J*_{CF} = 38.9 Hz), 159.8, 119.4 (TFA, q, ¹*J*_{CF} = 282.9 Hz), 48.9, 40.2, 38.8, 33.9; HRMS (DART) calcd for C₆H₁₅N₄O₂ *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** ($[\alpha]_{\text{D}}^{20} -4.60$ (*c* 1.00, 0.1N 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 CH₂Cl₂ (1.0 mL) were added (*S*)-valine methyl ester (**38**) (7.9 mg, 60.0 μmol), Et₃N (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. NH₄Cl (5.0 mL), extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine (30 mL), dried over anhydrous MgSO₄, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CHCl₃/MeOH = 100 : 1 to 50 : 1) to give **39** (30.1 mg, 88%) as a colorless amorphous solid;

$[\alpha]_{\text{D}}^{25} +2.1$ (*c* 0.71, CHCl₃); 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, CDCl₃) 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, C₆D₆) 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); ¹³C NMR (100 MHz, CDCl₃) 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 C₃₇H₆₅N₅NaO₁₃ *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 $\text{C}_{37}\text{H}_{65}\text{N}_5\text{NaO}_{13}$ m/z 810.4477 $[\text{M}+\text{Na}]^+$, found 810.4483.

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