Stereoselective Synthesis of β-Methoxytyrosine Derivatives for Identification of the Absolute Configuration of Callipeltin E

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Received 1 August 2007; revised 28 August 2007

Abstract: Asymmetric syntheses of all diastereoisomers of β-methoxytyrosine, an unusual amino acid contained in callipeltin A, were accomplished starting from a cinnamyl ester derivative. The stereochemistry of β-methoxytyrosine in callipeltin E was estimated to be 2*R*,3*R* by ^{1}H and ^{13}C NMR analyses of four diastereoisomeric tripeptides, each containing a β-methoxytyrosine isomer. These results obtained from the synthetic peptide derivatives were identical to D'Auria's results obtained by a degradative procedure.

Key words: callipeltin, cyclodepsipeptide, β-methoxytyrosine, marine sponge, unusual amino acid, anti-HIV activity

Callipeltin A (**1**) (Figure 1) is a novel cyclodepsipeptide isolated from the shallow water sponge *Callipelta*, collected in New Caledonia.[1] Callipeltin A (**1**) is the first natural peptide found to act against HIV. Additionally, it displays antifungal activity and potent cytotoxicity against a broad range of human carcinoma cell lines. It was also found that callipeltin A (**1**) was a selective and powerful inhibitor of the Na^+/Ca^+ exchanger and a positive ionotropic agent in guinea pig left atria.[2] These interesting biological activities as well as its unique structure have aroused interest.

Callipeltin A (**1**) is composed of a 22-membered macrocycle containing three unusual amino acids: β-methoxytyrosine (β-MeOTyr) (**2**), (2*R*,3*R*,4*S*)-4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (AGDHE), and (3*S*,4*R*)-3,4-dimethyl-L-glutamine (diMeGln). The configuration of β-MeOTyr (**2**) in callipeltin A (**1**) could not be determined due to its lability to acid. The total synthesis of callipeltin A (**1**) has not yet been achieved although several groups reported the synthesis of unusual amino acids contained in callipeltin A (**1**).[3] Since the stereostructure of β-MeOTyr (**2**) in callipeltin A (**1**) had not been identified in initial studies, preparations of all four stereoisomers of β-MeOTyr derivatives have been reported independently by three research groups: those of Hamada,[4] Joullié[5] and D'Auria.[6] The strategies used by the Hamada and Joullié research groups were based on the stereoselective addition of arylmetal reagents to serine aldehyde equivalents followed by methylation. For the preparation of the four stereoisomers of β-MeOTyr (**2**), a rather long sequence starting from L- and D-serine, respectively, was necessary. In contrast, D'Auria's group employed photo-assisted bromination of D- and L-tyrosine derivatives for the synthesis of intermediates as diastereomixtures. The stereochemistry of β-MeOTyr (**2**) in callipeltin A (**1**) was estimated to be 2*R*,3*R* by a comparison of oxidative degradation products obtained from callipeltin A (**1**) and four separately synthesized β-MeOTyr derivatives.[6] Based on this assignment, Lipton et al. recently reported the solid phase syntheses of callipeltin E (**3**)[7] (Figure 1) and a cyclized peptide, callipeltin B. The stereostructure of β-MeOTyr (**2**) in these natural products was confirmed to be identical with that in callipeltin A (**1**).[8]

In the course of studying callipeltin A (**1**), we have estimated the absolute configuration of β-MeOTyr (**2**) in callipeltin A (**1**) by a synthetic procedure instead of a degradative procedure. We herein report the simple asymmetric synthesis of all four diastereoisomers of protected β-MeOTyr **6** starting from a single cinnamyl ester derivative **5**. Sharpless asymmetric dihydroxylation[9] or aminohydroxylation[10] was used for each stereoselective synthesis. Identification of the absolute configuration of β-MeOTyr (**2**) was successfully achieved by a comparison of ^{1}H and ^{13}C NMR data of all four diasteroisomeric tripeptides, H-Gln-β-MeOTyr-*N*-Me-Ala-OMe (**4**), with natural products (Scheme 1).

Four diastereoisomers of protected β-MeOTyr **6** were prepared according to the route shown in Scheme 2. The synthesis begins with the conversion of 4-hydroxybenzaldehyde into 4-methoxyethoxymethoxycinnamyl ethyl ester (**5**) via protection with MEMCl and Horner–Emmons olefination in 95% overall yield. Os(VIII)/$(DHQD)_2AQN$-catalyzed asymmetric aminohydroxylation gave the desired *syn*-β-hydroxy-α-Boc-tyrosine derivatives as a major product with the use of *tert*-butyl carbamate[11] as the nitrogen source.[12] The reaction provided a mixture of two regioisomers with 7:1 regioselectivity in 85% chemical yield. The enantiomeric excess of the major product was determined to be 89% ee. The mixture of regioisomers was then treated with MeI and NaOH in DMSO–THF to give the methyl ether (2*S*,3*R*)-**6a** in 77% yield. After O-methylation, (2*S*,3*R*)-**6a** was separated from the undesired regioisomer by silica gel column chromatography. Application of this route to $(DHQ)_2AQN$, in-

SYNTHESIS 2007, No. 23, pp 3666–3672
Advanced online publication: 29.10.2007
DOI: 10.1055/s-2007-990848; Art ID: F14207SS

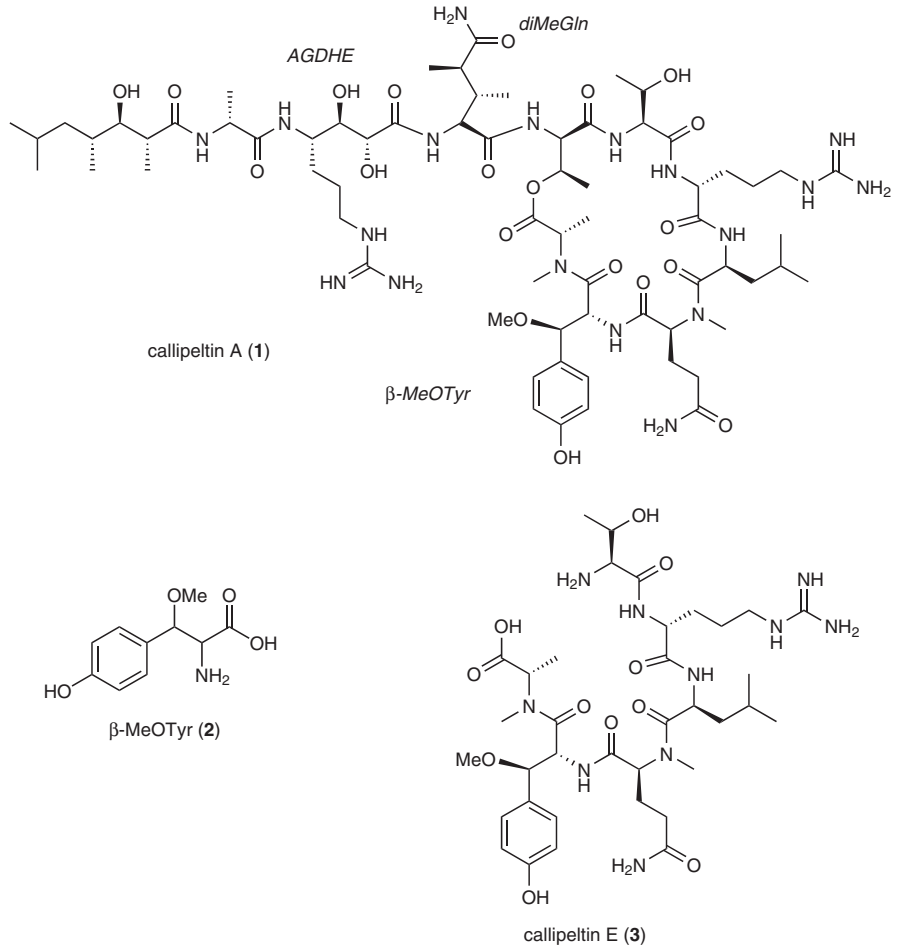
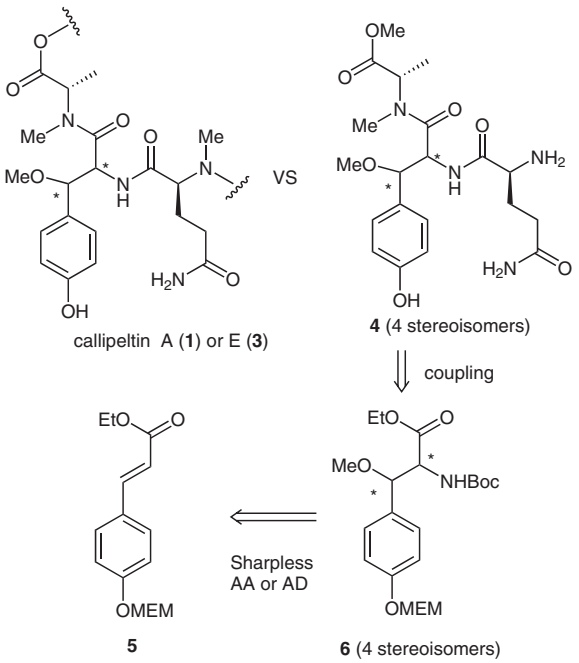


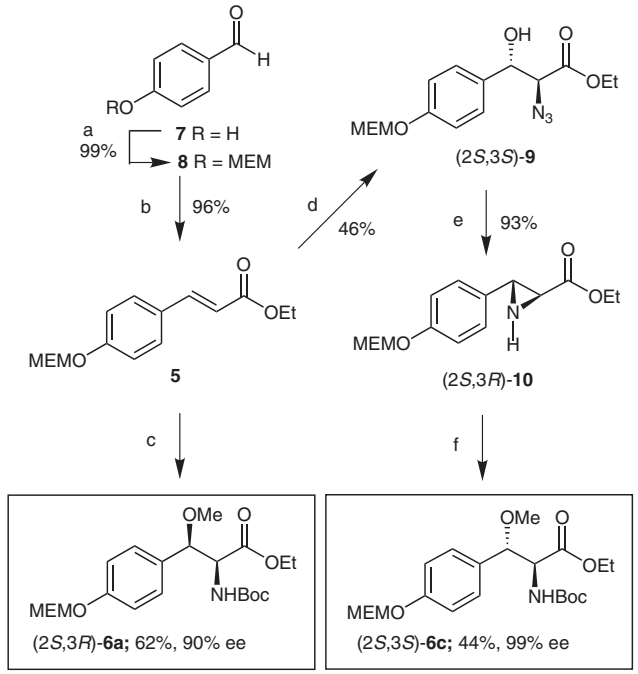
Figure 1 Callipeltin A (**1**), β-MeOTyr (**2**), and callipeltin E (**3**)



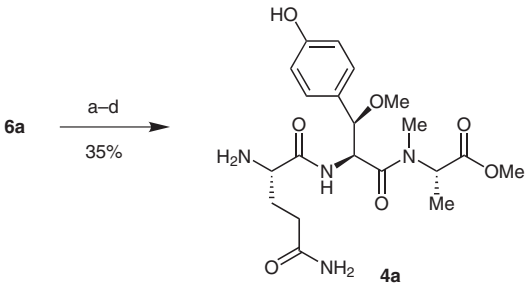
Scheme 1 Plan for synthesizing four diastereoisomeric tripeptides

stead of $(DHQD)_2AQN$, afforded the desired (2*R*,3*S*)-**6b** with similar regio- (8:1) and stereoselection (90% ee) and chemical yield.

Next, **5** was subjected to Sharpless asymmetric dihydroxylation in the presence of $(DHQ)_2PHAL$ (AD-mix α) and methanesulfonamide. The reaction proceeded smoothly to give (2*R*,3*S*)-diol in high yield with excellent optical purity (99% ee). The α-hydroxy group of diol was converted in azide (2*S*,3*S*)-**9** via 4-nitrobenzenesulfonylate[13] as a leaving group in 82% yield. Direct conversion of the hydroxy group of **9** into the methoxy group, however, was difficult to achieve since the retro-aldol reaction was dominant and the substrate decomposed to give the corresponding benzaldehyde and glycine derivatives. Thus, **9** was converted into aziridine **10** by treatment with Ph_3P in refluxing MeCN. Treatment of **10** with methanol in CH_2Cl_2 in the presence of $BF_3{\cdot}Et_2O$ and following Boc-protection of the amino group resulted in *N*-Boc-protected β-methoxytyrosine (2*S*,3*S*)-**6c** in 67% yield. This ring-opening process occurred through a stereospecific S_N2 manner at the C-3 position.[14] By switching the Sharpless chiral catalytic ligand to $(DHQD)_2PHAL$ (AD-mix β), the *anti* isomer (2*R*,3*R*)-**6d** was prepared with similar stereoselectivity (99% ee) and chemical yield.



Scheme 2 *Reagents and conditions*: (a) MEMCl, *i*-Pr_2NEt, CH_2Cl_2, 99%; (b) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, 96%; (c) i. $(DHQD)_2AQN$, $K_2OsO_2(OH)_2$, NaOH, *t*-BuOCl, $BocNH_2$, PrOH–H_2O (2:1), ii. MeI, NaOH, DMSO–THF (2:1); (d) i. $(DHQ)_2PHAL$, $K_2OsO_2(OH)_2$, K_2CO_3, $FeK_3(CN)_6$, $MeSO_2NH_2$, *t*-BuOH–H_2O (1:1), ii. 4-nitrobenzenesulfonyl chloride, Et_3N, CH_2Cl_2, iii. NaN_3, DMF, 60 °C; (e) Ph_3P, MeCN, reflux; (f) i. $BF_3{\cdot}OEt_2$, MeOH, CH_2Cl_2, –78 °C, ii. $(Boc)_2O$, Et_3N, DMAP, THF



Scheme 3 *Reagents and conditions*: (a) LiOH, *t*-BuOH–H_2O (1:1); (b) *N*-Me-Ala-OMe, HATU, HOAt, *i*-Pr_2NEt, CH_2Cl_2; (c) TFA–CH_2Cl_2 (1:1); (d) Fmoc-Gln(Trt)-OH, HATU, HOAt, Et_3N, CH_2Cl_2, then TFA–CH_2Cl_2 (1:1), then 20% piperidine–MeCN.

Each β-MeOTyr derivative **6a–d** thus obtained was then used for the preparation of the tripeptide esters **4a–d**. After hydrolysis of **6a** with LiOH, the product was condensed with *N*-Me-Ala-OMe using *O*-(7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)[15]/1-hydroxy-7-azabenzotriazole (HOAt)[16]/*i*-Pr_2NEt to give Boc-(2*S*,3*R*)-β-MeOTyr(MEM)-*N*-Me-Ala-OMe. After removal of the Boc group under acidic conditions (TFA–CH_2Cl_2 = 1:1, 30 min), the resulting deprotected dipeptide was condensed with Fmoc-Gln(Trt)-OH using HATU/HOAt/Et_3N to give the tripeptide. TFA-mediated deprotection of the trityl and MEM groups and subsequently Fmoc deprotection under standard conditions (20% piperidine in MeCN) proceeded

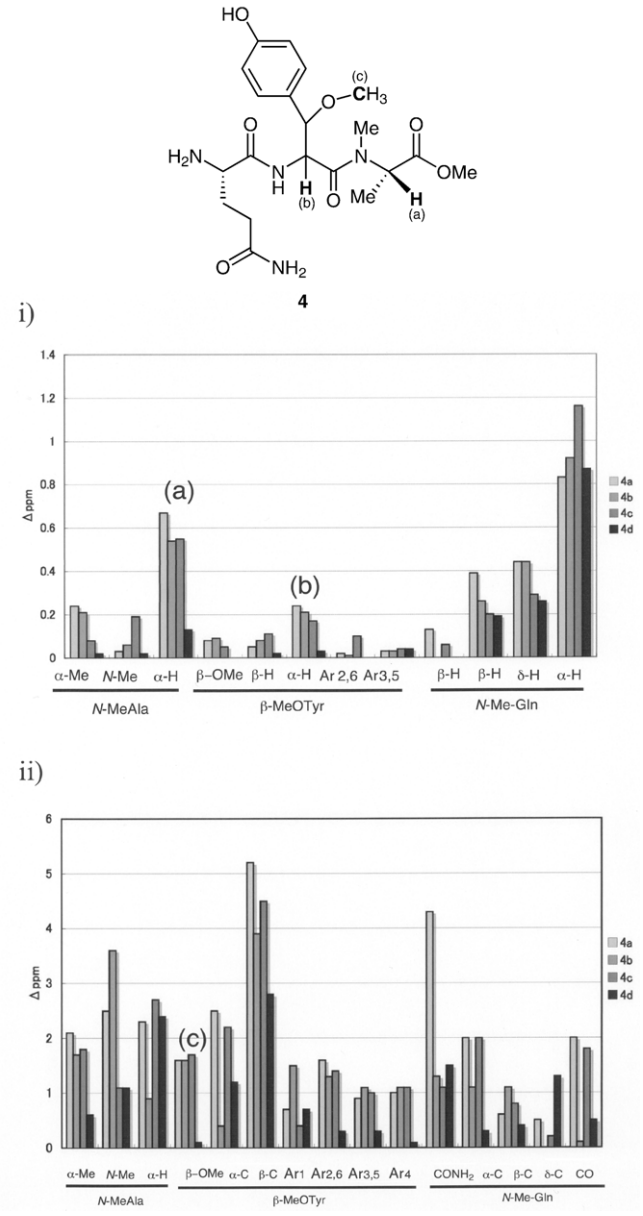


Figure 2 Comparison of chemical shifts of synthetic tripeptides **4a–d** and natural callipeltin E (**3**). i) ^1H NMR, ii) ^{13}C NMR.

cleanly to provide the desired tripeptide H-Gln-(2*S*,3*R*)-β-MeOTyr-*N*-Me-Ala-OMe (**4a**) after purification by preparative HPLC in moderate yield (Scheme 3). The remaining three diastereomeric tripeptides **4b**, **4c**, and **4d** were similarly synthesized starting from **6b**, **6c**, and **6d**, respectively.

^1H and ^{13}C NMR spectral data of the four synthetic tripeptides **4a–d** were then compared with those of callipeltin A (**1**) and E (**3**), a natural linear hexapeptide containing the Gln-β-MeOTyr-*N*-Me-Ala sequence. The chemical shifts of compound **4d** agreed very closely with those of the natural products, especially with linear callipeltin E (**3**). In particular, the α-CH chemical shifts of *N*-Me-Ala and β-MeOTyr of **4d** in the ^1H NMR spectra exhibited only 0.13 and 0.03 ppm differences from those reported for callipeltin E (**3**), whereas those for **4a–c** were 0.54–0.67 and

0.17–0.24 ppm [Figure 2 i); a,b]. In ^{13}C NMR spectroscopy, the methoxy chemical shift of β-MeOTyr of **4d** exhibited a 0.1 ppm difference, whereas the shifts of **4a–c** exhibited larger differences, 1.6–1.7 ppm [Figure 2 ii); c]. These observations permit the assignment of the absolute configuration at β-MeOTyr (**2**) in callipeltins as 2*R*,3*R*. This result matched D'Auria's conclusion that was based on the degradative method.[6]

In conclusion, we have succeeded in the simple synthesis of all four stereoisomers of the protected β-MeOTyr (**6**)[18] through Sharpless asymmetric aminohydroxylation or dihydroxylation. We synthesized tripeptide derivatives **4a–d** using each stereoisomer and identified the absolute configuration of β-MeOTyr (**2**) in callipeltins to be 2*R*,3*R* by ^1H and ^{13}C NMR analyses. Extension of these studies to the total synthesis of callipeltin A (**1**) will be reported in due course.

Amino acids and coupling reagents were purchased from Novabiochem. All manipulations were conducted under an inert atmosphere (N_2). All solvents were of reagent grade. THF was distilled from sodium and benzophenone ketyl. CH_2Cl_2 was distilled from CaH_2. All commercial reagents were of the highest purity available. Analytical TLC was performed on silica gel (60 F-254, Plates 0.25 mm). Column chromatography was carried out on Wakogel 60 (particle size, 0.063–0.200 mm). Analytical and preparative HPLC was performed on a Shimadzu SPD-10A YP instrument (OD 220 or 257 nm) equipped with the Nacalai tesque COSMOSIL 5C18-AR-II (4.6 × 150 mm or 10 × 250 mm) or Daicel CHIRALCEL OD-H (4.6 × 150 mm). ^1H and ^{13}C NMR spectrograms were recorded on a JEOL JNM-EX-400 or Bruker AM-300. Chemical shifts are expressed in ppm relative to TMS (0 ppm) or $CHCl_3$ (7.28 ppm for ^1H and 77.1 ppm for ^{13}C) or MeOH (3.30 ppm for ^1H and 49.0 ppm for ^{13}C). IR spectra were obtained on a PerkinElmer Model 1600 Series FT-IR spectrometer. Optical rotations were recorded on a JASCO DIP-370 polarimeter at the sodium D line. LRMS and HRMS were obtained on either a JEOL JMS-HX-211A (EI or FAB) or Bruker Autoflex-II (MALDI-TOF).

4-Methoxyethoxymethoxybenzaldehyde (8)

To a solution of 4-hydroxybenzaldehyde (**7**; 25.0 g, 204 mmol) in CH_2Cl_2 (500 mL) were added *i*-Pr_2NEt (107 mL, 614 mmol) and MEMCl (35.0 mL, 308 mmol) at 0 °C. The mixture was warmed to r.t., stirred for 28 h, and partitioned between H_2O (300 mL) and CH_2Cl_2 (200 mL). The organic layer was washed with brine (300 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (4:1 hexane–EtOAc) to give **8** (44.5 g, 99%) as a colorless oil.

IR (film): 2892, 1687, 1595, 1574, 1508, 1456, 1308, 1231, 1159, 1103, 980, 831 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 3.38 (s, 3 H), 3.55 (d, *J* = 2.4 Hz, 2 H), 3.84 (d, *J* = 2.7 Hz, 2 H), 5.36 (s, 2 H), 7.17 (dd, *J* = 6.0, 2.4 Hz, 2 H), 7.84 (m, 2 H), 9.91 (s, 1 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 58.9, 68.0, 71.4, 93.0, 116.2, 130.6, 131.7, 162.0, 190.6.

HRMS-EI: *m/z* $[M]^+$ calcd for $C_{11}H_{14}O_4$: 210.089; found: 210.0893.

Ethyl 3-(4′-Methoxyethoxymethoxyphenyl)prop-2-enoate (5)

To a solution of triethyl phosphonoacetate (28.0 mL, 143 mmol) and NaH (5.70 g, 143 mmol) in THF (320 mL) was added dropwise the aldehyde **8** (20.0 g, 95.0 mmol) at –10 °C. After stirring for 2.5 h, H_2O (150 mL) and EtOAc (200 mL) were added to the mixture. The organic layer was washed with brine (200 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (6:1 hexane–EtOAc) to give **5** (25.6 g, 96%) as a yellow oil.

IR (film): 3428, 2980, 1702, 1631, 1605, 1509, 1447, 1361, 1234, 1173, 1107, 990, 827 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.33 (t, *J* = 7.1 Hz, 3 H), 3.37 (s, 3 H), 3.55 (m, 2 H), 3.82 (m, 2 H), 4.25 (q, *J* = 7.1 Hz, 2 H), 5.30 (s, 2 H), 6.32 (d, *J* = 16.1 Hz, 1 H), 7.05 (d, *J* = 8.5 Hz, 2 H), 7.47 (d, *J* = 8.5 Hz, 2 H), 7.64 (d, *J* = 16.1 Hz, 1 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.2, 58.8, 60.1, 67.7, 71.4, 93.0, 116.2, 128.0, 129.3, 143.8, 158.7, 166.8.

HRMS-EI: *m/z* $[M]^+$ calcd for $C_{15}H_{20}O_5$: 280.1313; found: 280.1313.

Ethyl (2*S*,3*R*)-2-*tert*-Butoxycarbonylamino-3-(4-methoxyethoxymethoxyphenyl)-3-methoxypropanoate [(2*S*,3*R*)-6a]

To a vigorously stirred solution of NaOH (1.66 g, 39.9 mmol), *n*-PrOH (52.0 mL), and *tert*-butyl carbamate (2.41 g, 40.0 mmol) in H_2O (108 mL) was added dropwise *tert*-butyl hypochlorite (4.40 mL, 40.0 mmol). After 5 min, $(DHQD)_2AQN$ (669 mg, 0.70 mmol), olefin ester **5** (3.66 g, 13.0 mmol), and $K_2[OsO_2(OH)_4]$ (191 mg, 0.50 mmol) were added to the solution. After 19 h, aq $NaHSO_3$ (50 mL) and EtOAc (100 mL) were added. The organic layer was washed with brine (50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (2:1 hexane–EtOAc) to give hydroxy ester (4.79 g, 89%) as a mixture of regioisomers. To a stirred solution of the mixture of hydroxy ester (2.66 g, 6.40 mmol) in DMSO–THF (2:1, 21.0 mL) were added powder NaOH (768 mg, 19.2 mmol) and MeI (2.00 mL, 32.1 mmol), and then the mixture was stirred for 16 h at r.t. Aq 1 N HCl (20 mL) and EtOAc (50 mL) were added to the mixture. The organic layer was washed with brine (30 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (1:1 hexane–EtOAc) to give (2*S*,3*R*)-**6a** (1.70 g, 62%) as a colorless oil; $[\alpha]_D^{25}$ –33.0 (*c* 0.3, $CHCl_3$).

IR (film): 3442, 3385, 2973, 2366, 1720, 1605, 1508, 1359, 1302, 1165, 1096, 1010, 833 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.27 (t, *J* = 7.1 Hz, 3 H), 1.31 (s, 9 H), 3.23 (s, 3 H), 3.38 (s, 3 H), 3.56 (m, 2 H), 3.82 (m, 2 H), 4.17–4.28 (m, 2 H), 4.39 (dd, *J* = 9.3, 2.9 Hz, 1 H), 4.70 (d, *J* = 2.9 Hz, 1 H), 5.27 (s, 2 H), 7.03 (d, *J* = 8.3 Hz, 2 H), 7.23 (d, *J* = 8.5 Hz, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.3, 28.4, 57.0, 59.1, 61.5, 67.7, 71.7, 79.7, 82.5, 93.5, 116.1, 128.2, 130.5, 155.4, 157.1, 170.5.

HRMS-FAB: *m/z* $[M + H]^+$ calcd for $C_{21}H_{34}NO_8$: 428.2284; found: 428.2285.

The enantiomeric excess was determined to be 89% ee by HPLC using a DAICEL CHIRALCEL OD-H column (0.46 cm Φ × 25 cm); elution with 0.5% *i*-PrOH in hexane, t_R = 10.06 min for 2*R*,3*S*-isomer, t_R = 11.28 min for 2*S*,3*R*-isomer.

(2*R*,3*S*)-6b

$[\alpha]_D^{23}$ +34.6 (*c* 0.5, $CHCl_3$); 90% ee.

Ethyl (2*S*,3*S*)-2-Azido-3-hydroxy-3-(4′-methoxyethoxymethoxyphenyl)-2-propanoate [(2*S*,3*S*)-9]

Step 1; Intermediate (2R,3S)-Diol: To a solution of **5** (20.0 g, 71.0 mmol) in *t*-BuOH–H_2O (1:1, 714 mL) were added AD-mix α (125 g, 89.0 mmol) and $MeSO_2NH_2$ (10.1 g, 107 mmol) at 0 °C. After stirring at 4 °C for 24 h, aq $NaHSO_3$ (300 mL) and EtOAc (600 mL) were added. The organic layer was washed with brine (300 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (2:1 hexane–

EtOAc) to give (2*R*,3*S*)-diol (20.0 g, 89%); yellow oil; $[\alpha]_D^{23}$ +4.9 (*c* 1.1, $CHCl_3$).

IR (film): 3428, 2970, 2929, 1732, 1610, 1539, 1447, 1392, 1366, 1219, 1102, 1005, 837 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.27 (t, *J* = 7.1 Hz, 3 H), 2.65 (d, *J* = 6.8 Hz, 1 H), 3.10 (d, *J* = 5.9 Hz, 1 H), 3.39 (s, 3 H), 3.55 (m, 2 H), 3.81 (m, 2 H), 4.25 (q, *J* = 7.1 Hz, 2 H), 4.30 (m, 1 H), 4.94 (m, 1 H), 5.27 (s, 2 H), 7.05 (d, *J* = 8.8 Hz, 2 H), 7.33 (d, *J* = 8.5 Hz, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.1, 58.9, 62.0, 67.5, 71.5, 74.2, 75.0, 93.3, 115.9, 127.6, 133.4, 156.8, 172.5.

HRMS-FAB: *m*/*z* [M + H]$^+$ calcd for $C_{15}H_{22}O_7$: 314.1366; found: 314.1366.

Intermediate (2*S*,3*R*)-Diol

$[\alpha]_D^{22}$ –5.0 (*c* 1.1, $CHCl_3$).

Step 2; Intermediate α-Nosyl Product: To a solution of (2*R*,3*S*)-diol (1.30 g, 4.20 mmol) in DMF (14 mL) were added Et_3N (1.20 mL, 8.30 mmol) and 4-nitrobenzensulfonyl chloride (1.40 g, 6.30 mmol). The solution was stirred at –10 °C for 56 h, and then H_2O (20 mL) and Et_2O (100 mL) were added. The organic layer was washed with brine (50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (4:1 hexane–EtOAc) to give the α-nosyl product (2.60 g, 98%); colorless oil; $[\alpha]_D^{23}$ +45.0 (*c* 1.5, $CHCl_3$).

IR (film): 3420, 3106, 2923, 1742, 1737, 1611, 1532, 1375, 1344, 1310, 1225, 1187, 1094, 1009, 850, 839, 747 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.19 (t, *J* = 7.1 Hz, 3 H), 2.78 (s, 1 H), 3.35 (s, 3 H), 3.54 (m, 2 H), 3.81 (m, 2 H), 4.16 (q, *J* = 7.1 Hz, 2 H), 4.97 (d, *J* = 4.2 Hz, 1 H), 5.15 (s, 1 H), 5.23 (s, 2 H), 6.89 (d, *J* = 8.5 Hz, 2 H), 7.14 (d, *J* = 8.5 Hz, 2 H), 7.85 (d, *J* = 8.8 Hz, 2 H), 8.23 (d, *J* = 8.8 Hz, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.0, 59.0, 62.5, 67.8, 71.6, 73.1, 82.5, 93.4, 116.1, 124.1, 127.4, 129.1, 130.7, 141.4, 150.5, 157.4, 166.4.

HRMS-FAB: *m*/*z* [M + H]$^+$ calcd for $C_{15}H_{22}O_7$: 314.1366; found: 314.1369.

Enantiomer of α-Nosyl Product

$[\alpha]_D^{22}$ –43.2 (*c* 0.6, $CHCl_3$).

Azide (2S,3S)-9: To a stirred solution of the respective α-nosyl product (919 mg, 1.84 mmol) in DMF (6 mL) was added NaN_3 (599 mg, 9.21 mmol) at r.t. The mixture was heated at 55 °C for 45 h and then cooled down to r.t. H_2O (10 mL) and EtOAc (30 mL) were added and the organic layer was washed with brine (20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (2:1 hexane–EtOAc) to give (2*S*,3*S*)-**9** (382 mg, 54%); yellow oil; $[\alpha]_D^{22}$ +3.1 (*c* 1.0, $CHCl_3$).

IR (film): 3307, 2931, 2358, 2108, 1737, 1612, 1514, 1377, 1261, 1174, 1102, 1026, 838 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.28 (t, *J* = 7.1 Hz, 3 H), 3.39 (s, 3 H), 3.56 (m, 2 H), 3.73 (m, 2 H), 4.09 (d, *J* = 7.1 Hz, 1 H), 4.25 (m, 2 H), 4.97 (d, *J* = 7.1 Hz, 1 H), 5.24 (s, 2 H), 6.99 (d, *J* = 8.8 Hz, 2 H), 7.26 (d, *J* = 8.5 Hz, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.2, 59.1, 62.2, 66.9, 67.7, 71.6, 73.7, 93.4, 116.3, 127.9, 132.3, 157.5, 168.9.

HRMS-FAB: *m*/*z* [M + Na]$^+$ calcd for $C_{15}H_{21}N_3O_6$ + Na: 362.1328; found: 362.1332.

The enantiomeric excess was determined to be 99% ee by the Mosher method.[17]

(2*R*,3*R*)-9

$[\alpha]_D^{22}$ –3.1 (*c* 1.0, $CHCl_3$); 99% ee.

Ethyl (2*S*,3*R*)-2-*trans*-Aziridine-3-(4′-methoxyethoxymethoxyphenyl)-2-propanoate [(2*S*,3*R*)-10]

To a stirred solution of (2*S*,3*S*)-**9** (87.5 mg, 0.230 mmol) in MeCN (1.5 mL) was added Ph_3P (120 mg, 0.459 mmol). The mixture was heated at 40 °C for 22 h and then concentrated in vacuo. H_2O (5 mL) and EtOAc (20 mL) were added to the residue. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (4:1 hexane–EtOAc) to give (2*S*,3*R*)-**10** (72 mg, 93%); yellow oil; $[\alpha]_D^{23}$ –142.6 (*c* 1.0, $CHCl_3$).

IR (film): 3272, 2966, 1719, 1608, 1508, 1437, 1402, 1337, 1214, 1167, 1102, 1003, 814 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.31 (t, *J* = 7.1 Hz, 3 H), 2.54 (m, 1 H), 3.21 (d, *J* = 4.6 Hz, 1 H), 3.37 (s, 3 H), 3.55 (m, 2 H), 3.81 (m, 2 H), 4.21–4.29 (m, 2 H), 5.25 (s, 2 H), 6.99 (d, *J* = 8.8 Hz, 2 H), 7.19 (d, *J* = 8.5 Hz, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.4, 39.5, 40.1, 59.1, 61.9, 67.7, 71.7, 93.5, 116.3, 127.3, 131.2, 156.9, 171.7.

HRMS-FAB: *m*/*z* [M + H]$^+$ calcd for $C_{15}H_{22}NO_5$: 296.1498; found: 296.1499.

(2*R*,3*S*)-10

$[\alpha]_D^{25}$ +156.4 (*c* 1.2, $CHCl_3$).

Ethyl (2*S*,3*S*)-2-*tert*-Butoxycarbonylamino-3-(4′-methoxyethoxymethoxyphenyl)-3- methoxypropanoate [(2*S*,3*S*)-6c]

To a solution of (2*S*,3*R*)-**10** (526 mg, 1.68 mmol) in MeOH–CH_2Cl_2 (1:2, 1.8 mL) was added $BF_3{\cdot}OEt_2$ (0.212 mL, 1.68 mmol) at –78 °C. After stirring at –78 °C for 26 h, the mixture was added to sat. aq NH_4Cl (2 mL). To the mixture was added EtOAc (10 mL) and the organic layer was washed with brine (5 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to give the crude amino alcohol. To a solution of the crude amino alcohol in THF (2.5 mL) were added Et_3N (0.620 ml, 4.47 mmol) and $(Boc)_2O$ (650 mg, 2.98 mmol). After stirring for 20 h at r.t., H_2O (5 mL) and EtOAc (10 mL) were added. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (2:1 hexane–EtOAc) to give (2*S*,3*S*)-**6c** (498 mg, 67%); colorless oil; $[\alpha]_D^{27}$ –3.5 (*c* 1.0, $CHCl_3$).

IR (film): 3363, 2979, 2933, 1747, 1716, 1610, 1510, 1367, 1165, 1101, 1020, 1005, 867 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.26 (m, 3 H), 1.34–1.40 (s, 9 H), 3.31 (s, 3 H), 3.38 (s, 3 H), 3.56 (m, 2 H), 3.82 (m, 2 H), 4.23 (m, 2 H), 4.50 (m, 1 H), 4.56 (m, 1 H), 5.14 (d, *J* = 8.8 Hz, 1 H), 5.27 (s, 2 H), 7.04 (d, *J* = 6.6 Hz, 2 H), 7.22 (t, *J* = 8.5 Hz, 2 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.2, 28.2, 57.2, 58.4, 61.4, 67.6, 71.6, 79.6, 82.5, 93.5, 116.1, 128.1, 130.5, 155.4, 157.2, 170.3.

HRMS-FAB: *m*/*z* [M + Na]$^+$ Calcd for $C_{21}H_{33}NO_8$ + Na: 450.2104; found: 450.2108.

(2*R*,3*R*)-6d

$[\alpha]_D^{27}$ +2.4 (*c* 1.1, $CHCl_3$).

H-Gln-βMeOTyr-*N*-Me-Ala-OMe [(2*S*,3*R*)-4a]; Typical Procedure

To a solution of (2*S*,3*R*)-**6a** (200 mg, 0.470 mmol) in *t*-BuOH–H_2O (1:1, 3 mL) was added LiOH (39 mg, 0.940 mmol). The mixture was stirred for 2 h at r.t. and the solvent was removed in vacuo. The residue was extracted with EtOAc (10 mL) and the organic layer was washed with H_2O (5 mL), dried ($MgSO_4$), and evaporated in vacuo. To a solution of the residue in DMF (2 mL) were added N-

Me-Ala-OMe (108 mg, 0.705 mmol), *i*-Pr_2NEt (0.131 mL, 0.940 mmol), HATU (268 mg, 0.705 mmol), and HOAt (96 mg, 0.705 mmol) and the mixture was stirred for 30 min at r.t. The solvent was removed in vacuo. EtOAc (5 mL) and H_2O (2 mL) were added to the residue, and the organic layer was washed with brine (5 mL), dried ($MgSO_4$), and evaporated in vacuo. To a solution of the crude product in CH_2Cl_2 (1 mL) was added TFA (1 mL). The mixture was stirred for 30 min at r.t. and the solvent was removed in vacuo. To a solution of the residue in DMF (2 mL) were added Fmoc-*N*-Me-Gln(Trt)-OH (431 mg, 0.705 mmol), *i*-Pr_2NEt (0.131 mL, 0.940 mmol), HATU (268 mg, 0.705 mmol), and HOAt (96 mg, 0.705 mmol). The mixture was stirred for 30 min at r.t. and the product was similarly isolated as above. The resulting product was dissolved in CH_2Cl_2 (1 mL) and TFA (1 mL) was added. The mixture was stirred for 30 min at r.t. and the solvent was removed in vacuo. To a solution of the crude tripeptide in MeCN (2 mL) was added piperidine (0.50 mL). The mixture was stirred for 30 min at r.t. and the solvent was removed in vacuo. The residue was purified by preparative HPLC (MeCN–H_2O, 90:10) to give H-Gln-βMeOTyr-*N*-Me-Ala-OMe [(2*S*,3*R*)-**4a**; 408 mg, 35%]; white powder; $[\alpha]_D^{25}$ –70.8 (*c* 0.24, MeOH).

IR (film): 3264, 2929, 1739, 1683, 1616, 1515, 1205, 1172, 111, 838, 800 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.15 (d, *J* = 7.1 Hz, 3 H), 2.11 (m, 2 H), 2.43 (m, 2 H), 2.86 (s, 3 H), 3.21 (s, 3 H), 3.59 (s, 3 H), 4.04 (t, *J* = 6.6 Hz, 1 H), 4.37 (d, *J* = 8.8 Hz, 1 H), 4.46 (q, *J* = 7.1 Hz, 1 H), 4.95 (d, *J* = 8.8 Hz, 1 H), 6.82 (d, *J* = 8.5 Hz, 2 H), 7.21 (d, *J* = 8.5 Hz, 2 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.6, 26.9, 30.1, 33.1, 51.2, 52.1, 55.4, 55.5, 82.3, 115.2, 126.9, 128.8, 157.9, 168.2, 169.8, 171.4, 175.8.

HRMS-MALDITOF: *m*/*z* $[M + H]^+$ calcd for $C_{20}H_{31}N_4O_7$: 439.219; found: 439.245.

The following compounds **4b–d** were prepared in a manner similar to **4a**.

(2*R*,3*S*)-4b

From **6b**; yield: 17%; $[\alpha]_D^{25}$ –93.3 (*c* 0.15, MeOH).

IR (film): 3205, 2947, 1736, 1674, 1635, 1516, 1203, 1136, 1103, 839, 800, 760, 721 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.19 (d, *J* = 7.3 Hz, 3 H), 2.06 (m, 2 H), 2.32 (m, 2 H), 2.87 (s, 3 H), 3.21 (s, 3 H), 3.59 (s, 3 H), 4.27 (m, 1 H), 4.40 (d, *J* = 9.0 Hz, 1 H), 4.61 (q, *J* = 7.2 Hz, 1 H), 4.96 (d, *J* = 9.0 Hz, 1 H), 6.80 (d, *J* = 8.7 Hz, 2 H), 7.19 (d, *J* = 8.7 Hz, 2 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.0, 25.2, 29.0, 32.6, 51.3, 55.0, 55.4, 56.4, 82.6, 115.0, 128.7 130.7, 157.8, 171.2, 173.0, 173.3, 178.8.

HRMS-MALDITOF: *m*/*z* $[M + H]^+$ calcd for $C_{20}H_{31}N_4O_7$: 439.219; found: 439.174.

(2*S*,3*S*)-4c

From **6c**; yield: 18%; $[\alpha]_D^{25}$ +4.5 (*c* 0.1, MeOH).

IR (film): 3320, 2964, 2930, 1739, 1670, 1641, 1613, 1514, 1203, 845, 800 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.33 (d, *J* = 7.1 Hz, 3 H), 1.92 (m, 2 H), 2.28 (m, 2 H), 3.05 (s, 3 H), 3.10 (s, 3 H), 3.62 (s, 3 H), 3.71 (m, 1 H), 4.22 (d, *J* = 9.0 Hz, 1 H), 4.60 (q, *J* = 7.1 Hz, 1 H), 5.04 (d, *J* = 9.0 Hz, 1 H), 6.70 (d, *J* = 8.5 Hz, 2 H), 7.15 (d, *J* = 8.5 Hz, 2 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.9, 27.1, 31.5, 32.4, 51.2, 52.4, 55.3, 55.5, 83.0, 115.1, 128.6, 128.8, 158.0, 168.2, 169.4, 171.6, 179.9.

HRMS-MALDITOF: *m*/z $[M + H]^+$ calcd for $C_{20}H_{31}N_4O_7$: 439.219; found: 493.256.

(2*R*,3*R*)-4d

From **6d**; yield: 28%; $[\alpha]_D^{25}$ +3.8 (*c* 0.2, MeOH).

IR (film): 3315, 2964, 2929, 1739, 1670, 1641, 1612, 1511, 1203, 1137, 845, 800 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.43 (d, *J* = 7.3 Hz, 3 H), 1.72 (m, 2 H), 1.80 (m, 2 H), 2.88 (br s, 3 H), 3.15 (s, 3 H), 3.72 (s, 3 H), 4.00 (m, 1 H), 4.35 (d, *J* = 9.5 Hz, 1 H), 5.02 (q, *J* = 7.6 Hz, 1 H), 5.24 (dd, *J* = 9.5, 2.9 Hz, 1 H), 6.80 (d, *J* = 8.5 Hz, 2 H), 7.23 (d, *J* = 8.5 Hz, 2 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.1, 26.7, 31.5, 33.9, 52.6, 55.8, 56.9, 57.8, 83.7, 116.4, 116.5, 129.9, 130.1, 169.7, 171.4, 172.9, 181.6.

HRMS-MALDITOF: *m*/*z* $[M + H]^+$ calcd for $C_{20}H_{31}N_4O_7$: 439.219; found: 439.174.

Acknowledgment

We thank Dr. Hitoshi Hori (University of Tokushima) for useful discussions. We also thank Dr. Nobutaka Fujii (Kyoto University) and Dr. Takumichi Sugihara (Niigata University of Pharmacy and Applied Life Sciences) for the measurements of mass spectra. This work was supported in part by Chisso, Co. Ltd., and a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (Grant 16790084 to H.K.).

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