Total Synthesis of WS9326A, a Potent Tachykinin Antagonist from *Streptomyces violaceoniger*[1)]

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Total synthesis of the cyclic peptide lactone WS9326A, a potent tachykinin antagonist isolated from *Streptomyces violaceoniger* strain 9326, has been achieved *via* Cbz–Thr(Boc–*allo*-Thr–Asn–Ser(Bzl))–(*E*)⊿MeTyr–Leu–D-Phe–OTce, which was cyclized (Phe and *allo*-Thr) using an active ester method with *N*-hydroxysuccinimide. Finally the unique *N*-acyl group, the 2-(1(*Z*)-pentenyl)cinnamoyl moiety, was introduced onto the amino group in the Thr unit. The key step of the synthesis involves the preparation of the *E*-isomer of the dehydro-*N*-methyltyrosine (⊿MeTyr) unit. The debenzoxylation reaction of the *threo*- and *erythro*-isomers of β-benzoxy-*N*-methyltyrosine derivatives gave exclusively the *Z*-isomer of Cbz–Thr–⊿MeTyr(MOM)–OMe, which was then converted to the desired *E*-isomer by photochemical isomerization of Cbz–Thr(TBDMS)–(*Z*)⊿MeTyr(MOM)–Leu–D-Phe–OTce at a later step.

Key words tachykinin antagonist; photochemical isomerization; cyclic peptide lactone

The cyclic peptide lactone WS9326A (**1**) (Fig. 1), isolated from *Streptomyces violaceoniger* strain 9326, shows strong antagonistic activity to substance P and neurokinin A receptors.[2)] Compound **1** consists of L-Thr, (*E*)-dehydro-*N*-methyltyrosine ((*E*)⊿MeTyr), L-Leu, D-Phe, L-*allo*-Thr, L-Asn, L-Ser, and the unique *N*-acyl group, 2-(1(*Z*)-pentenyl)cinnamic acid. Structural requirements for activity have been partially elucidated during structural assignment; namely, the dehydroamino acid and *N*-acyl group are essential for activity.[3)] A tetrahydro acyl group derivative of **1** is ten times more active than the natural compound (**1**) at neurokinin receptors (NK1, NK2).[2)] This derivative (code No. FK224) is currently in phase II clinical trials as a potential antiasthmatic agent. We describe here the first total synthesis of **1** *via* a cyclic peptide lactone (**21**) which has a free amino group in the Thr unit, allowing synthesis of various *N*-acyl derivatives, and we also report the synthesis of *N*-acyl derivatives using the intermediate **21**, as well as some structure–activity relationships.

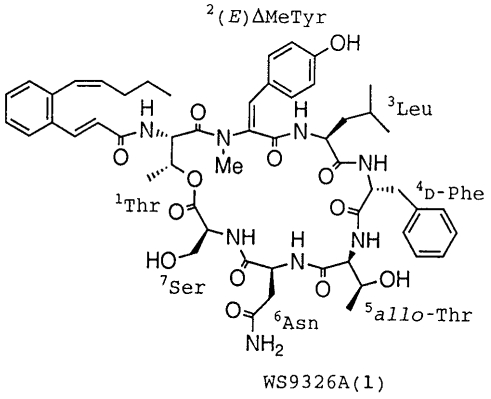

Fig. 1

Results and Discussion

Our retrosynthetic analysis is shown in Chart 1. The greatest challenge involved the preparation of the required *E*-isomer of the dehydro-*N*-methyltyrosine (⊿MeTyr) unit.[4)] Synthesis of the β-hydroxy-*N*-methyltyrosine derivative **9** was started from commercially available 4-hydroxy benzaldehyde. After protection of the hydroxy group with methoxymethyl (MOM),[5)] conjugation with Gly gave a *ca.* 1 : 1 mixture of *threo*- and *erythro*-isomers of the β-hydroxytyrosine unit. The diastereomeric mixture (**3**) was then *N*-methylated with dimethyl sulfate, followed by protection of the amino group with 2-nitrophenylsulfenyl (Nps) chloride[6)] and the carboxyl group as the methyl ester to give **6**. The *threo*- and *erythro*-isomers of **6** were separated by silica gel column chromatography using $CHCl_3$ as a solvent to give **6a** and **6b** (Chart 2).

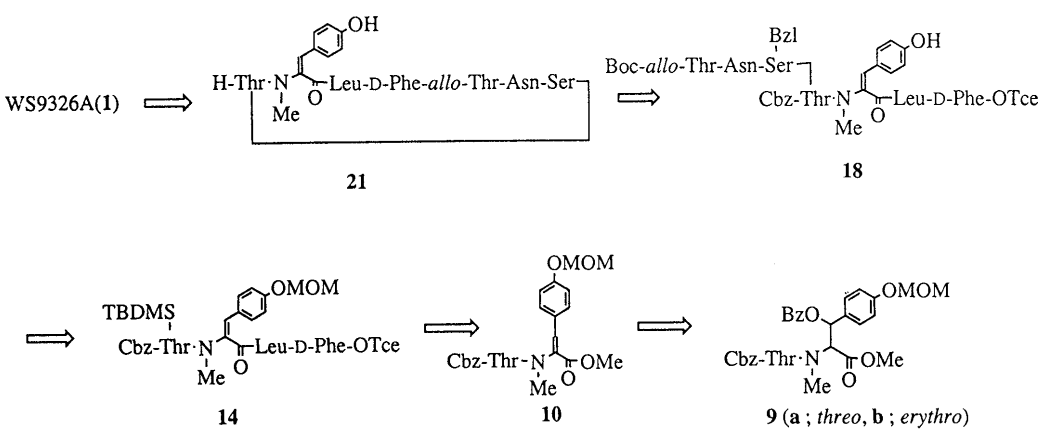

Chart 1. Retrosynthetic Route for WS9326A (**1**)

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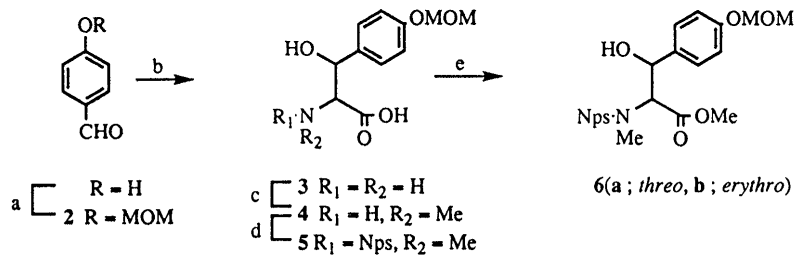
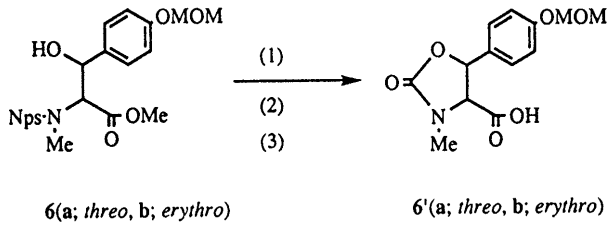


(a) CH_3OCH_2Cl, TEA, THF, rt, 1h; (b) Gly, KOH, EtOH, rt, 19h; (c) $(MeO)_2SO_2$, 1N NaOH, 90 °C, 20 min; (d) NpsCl, BSA, CH_2Cl_2, 0 °C, 2 h; (e) CH_2N_2 / ether.

Chart 2



(1) PhSH, TFA, CH_2Cl_2, rt, 10 min; (2) Cbz-Cl, BSA, CH_2Cl_2; (3) NaOH, 50 °C, 6 h.

Chart 3

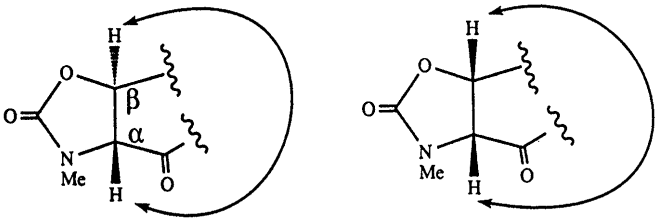
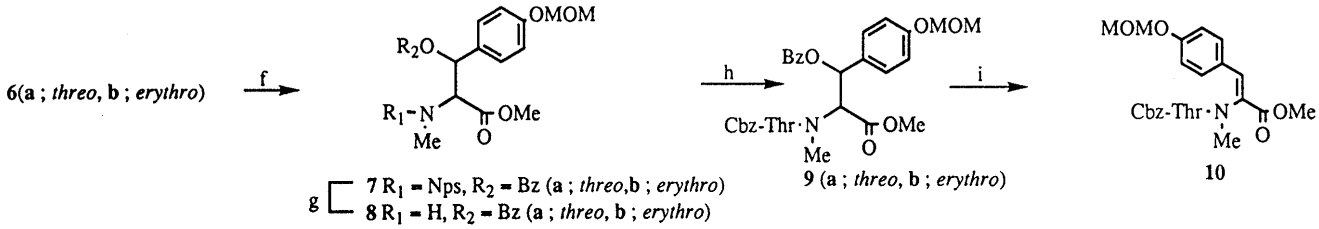


Fig. 2. Coupling Constants of Oxazolidone Derivative



(f) BzCl, DMAP, TEA, CH_2Cl_2, rt, 2d; (g) PhSH, TFA, CH_2Cl_2, 0 °C, 30 min; (h) Cbz-Thr-OH, EEDQ, CH_2Cl_2, rt, 20 min; (i) DBU, toluene, rt, 30 min.

Chart 4

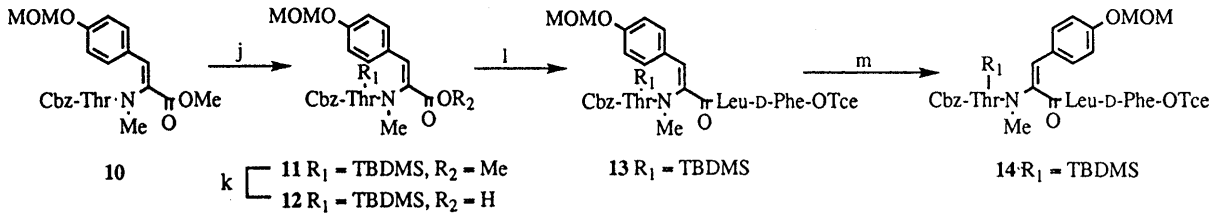
In order to verify the stereochemical integrity of **6a** and **6b**, the Nps group of both compounds was removed and the deprotected compounds were treated with benzyloxycarbonyl chloride, followed by cyclization to the corresponding 2-oxazolidone derivatives (**6'a**, **6'b**) (Chart 3). In the ^{1}H-NMR spectra of these two compounds, the vicinal coupling constants between the C_α-H and C_β-H protons verified **6'a** as the *threo* ($J_{\alpha-\beta}=5$ Hz) and **6'b** as the *erythro* ($J_{\alpha-\beta}=9$ Hz) isomer (Fig. 2).[7]

The hydroxy group of each of **6a** and **6b** was protected with benzoyl chloride to afford **7a** and **7b**. Removal of the Nps group of **7a** and **7b**, followed by coupling with Cbz–Thr–OH using 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ)[8] as a condensing reagent afforded *threo* (**9a**) and *erythro* (**9b**) isomers (Chart 4).

Both intermediates, **9a** (*threo*) and **9b** (*erythro*), gave exclusively the *Z*-isomer (**10**) as the elimination product upon treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The stereochemistry of the double bond in the dehydro peptide (**10**) was established by analysis of the nuclear Overhauser effect (NOE) difference spectra.[9] The *⊿*MeTyr unit of **10** showed NOE between the *N*-methyl protons at δ 3.14 and the aromatic protons at δ 7.46 (2',6'), indicating that *⊿*MeTyr had the *Z* configuration as shown.

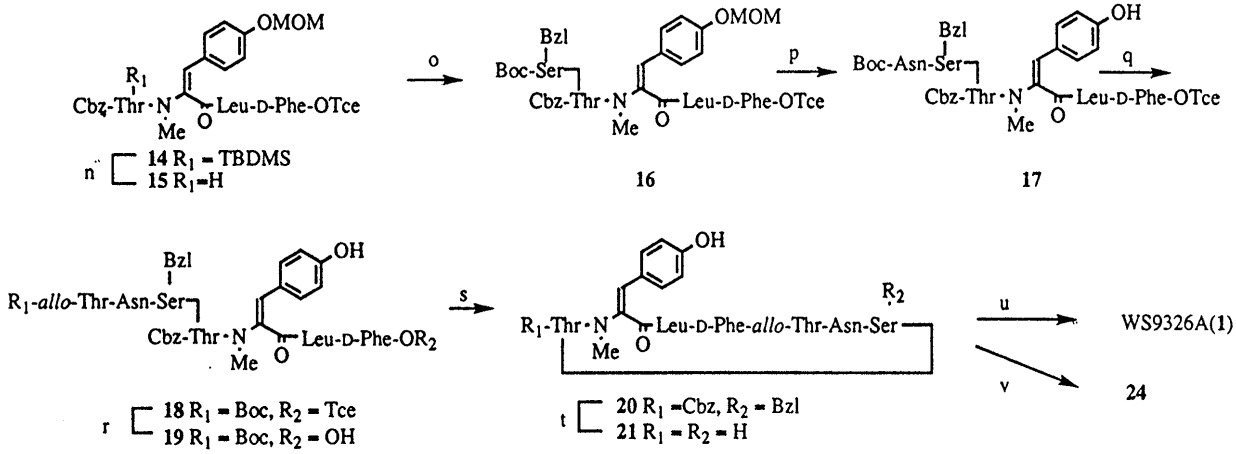
The desired *E*-isomer was obtained by photochemical isomerization of the tetrapeptide **13**.[10] Protection of the hydroxyl group of **10** with *tert*-butyldimethylsilyl chloride (TBDMSCl)[11] followed by removal of the methyl ester gave **12**. Synthesis of **13** was achieved by coupling **12** with H–Leu–D-Phe–OTce (**22**)[12] using EEDQ. Compound **13** in toluene–acetone (10 : 1) solution was irradiated with a high-pressure Hg lamp (100 W) for 1.5 h at 0 °C to afford a 2 : 1 mixture of the starting material (**13**) and the *E*-isomer **14** (Chart 5). It was known that the (*E*)*⊿*-amino acid ester is unstable to acid and base, and is easily converted to the (*Z*)*⊿*-amino acid.[13] The results might be related to the stability of the products, *i.e.*, a longer peptide chain at the C-terminal of *⊿*MeTyr (**13**) would impart a more stable conformation for the *E*-orientation (**14**) as compared with the methyl ester (**10**).

The stereochemistry of the double bond in compounds **13** and **14** was also established based on NOE difference



(j) TBDMSCl, imidazole, DMF, rt, 16 h; (k) 1N NaOH, 30 °C, 2 d; (l) H-Leu-D-Phe-OTce (**22**), EEDQ, CH_2Cl_2, rt, 15 h; (m) toluene : acetone = 10 : 1, hv (100 W), 0 °C, 1.5 h.

Chart 5



(n) 67% AcOH, 25 °C, 28 h; (o) Boc-Ser(Bzl)-OH, EDC·HCl, DMAP, CH_2Cl_2, rt, 12 h; (p) 4N HCl/dioxane, rt, 30 min, then Boc-Asn-OH, TEA, HOBT, EDC·HCl, CH_2Cl_2, rt, 1 h; (q) 4N HCl/dioxane, rt, 30 min, then Boc-*allo*-Thr-OH, TEA, HOBT, EDC·HCl, CH_2Cl_2, rt, 8 h; (r) 90% AcOH, Zn, rt, 9 h; (s)1) HONSu, EDC·HCl, CH_2Cl_2, rt, 15h; 2) TFA, rt, 30 min, then DMF pyridine, rt, 16 h; (t) HF-pyridine, rt, 1h; (u) 2-(1(*Z*)-pentenyl)cinnamoyl chloride (**23**), CH_2Cl_2, BSA, DMF, rt, 1 h.; (v) stearoyl chloride, pyridine, rt, 1h.

Chart 6

spectra as *Z* and *E*, respectively.[14] The results were consistent with the UV spectra of these compounds. The intensity of the UV absorption maximum of **13** (305 nm, ε 18500) was larger than that of **14** (284 nm, ε 8865), thereby confirming *Z* (**13**) and *E* (**14**) configurations in accordance with the result observed for *trans*-cinnamic acid.[15]

The *E*-isomer **14** was purified by silica gel column chromatography (*n*-hexane–EtOAc, 2 : 1) and allowed to react, after removal of the TBDMS protecting group, with Boc–Ser(Bzl)–OH using EDC hydrochloride (EDC·HCl)/*N*,*N*-dimethylamino pyridine (DMAP) to form the ester bond with the hydroxyl group in the Thr unit. Two successive peptide chain elongation reactions with Boc–Asn–OH and Boc–*allo*-Thr–OH using EDC/HOBT gave a linear protected peptide (**18**) which contained all the required amino acid units. Compound **18** was allowed to react, after removal of the trichloroethyl group on D-Phe, with HONSu using EDC·HCl to give an activated ester. The Boc group in the *allo*-Thr unit of the active ester was deprotected with TFA, and cyclization was achieved by a high dilution method[16] in pyridine to give cyclic peptide **20** in 40% overall yield (from the free acid). The Cbz and Bzl protecting groups of **20** were removed simultaneously with HF–pyridine to afford the free cyclic peptide lactone **21**.

The preparation of the *N*-acyl group has been reported previously,[3] and the route employed was followed in the present synthesis to afford 2-(1(*Z*)-pentenyl)cinnamoyl chloride (**23**). The acid chloride was coupled with **21** in the presence of *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and DMAP, and the product, which showed the same *Rf* value (0.59, $CHCl_3$–MeOH–H_2O, 65 : 25 : 4) as natural **1**, was purified by preparative TLC (Chart 6). Synthetic **1** was identical, in terms of ^{1}H-NMR, FAB mass spectra, IR and analytical RP-HPLC behavior (Fig. 3), with natural **1**.

The yield of the *N*-acyl group coupling reaction was very low (2.7%). In order to ascertain the reason, we synthesized a derivative using intermediate **21**. In the case of stearoyl as an *N*-acyl group, compound **24** was obtained in 13.8% yield. Thus, the former result was probably due to the structural complexity of the acyl group.

Synthetic **1** inhibits the binding of [^{3}H]substance P to a guinea pig lung membrane preparation with an IC_{50} value of 3.5×10^{-6} M, which is the same value as that of the natural product. On the other hand, compounds **21** and **24** showed values of $> 30 \times 10^{-6}$ M and $> 36 \times 10^{-6}$ M, respectively. These results indicate that the structure of the *N*-acyl group is important for the activity.

In conclusion, a stereoselective and convergent synthesis of WS9326A has been achieved. The synthetic route established in the present study should be useful for providing a variety of *N*-acyl derivatives to evaluate structure–activity relationships, in an effort to discover more potent derivatives.

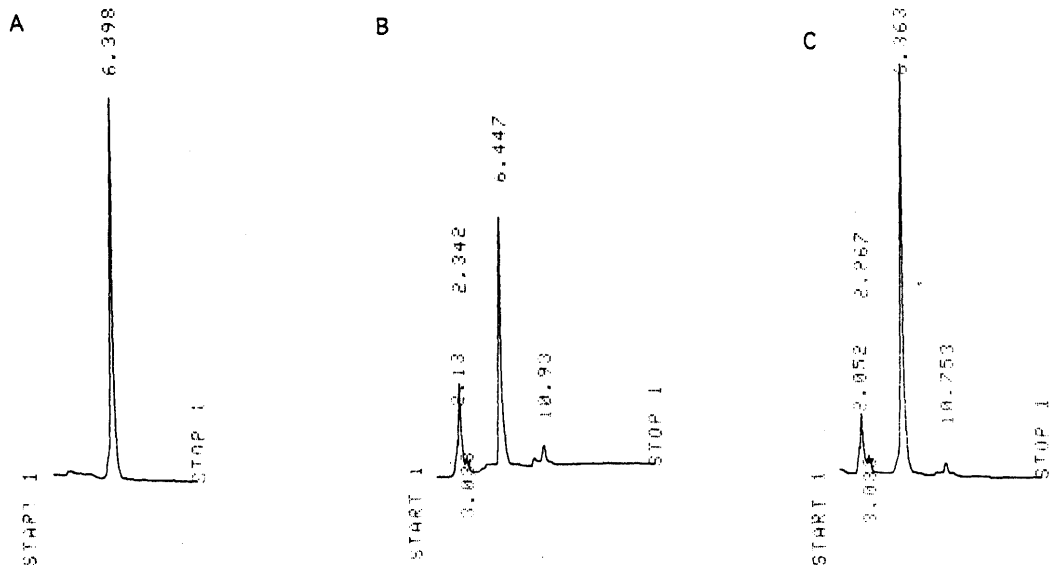


Fig. 3. HPLC Profiles of Natural and Synthetic WS9326A

RP-HPLC conditions: column YMC AM-303 (250 mm × 4.6 mm i.d.); eluate, MeOH : H_2O (80 : 20); flow rate, 1 ml/min; detection, UV 210 nm. A) Natural WS9326A, B) synthetic WS9326A, C) mixture of natural and synthetic WS9326A.

Experimental

Melting points (mp) were taken using a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO A-102 or a Perkin-Elmer 16PC FT-IR spectrophotometer. UV spectra were measured on a Hitachi 220A spectrophotometer. Optical rotations were determined with a JASCO DIP-140 polarimeter. ^{1}H- and ^{13}C-NMR spectra were measured on a Bruker AM200 or AM400WB NMR spectrometer. Mass spectra were measured on a VG ZAB-SE mass spectrometer. Analytical TLC was done on 2.0 × 6.5 cm precoated TLC plates (Silica gel 60F_{254}, layer thickness 0.25 mm) manufactured by E. Merck. Column chromatography was carried out with E. Merck Silica gel 60 (70—230 mesh ASTM). Acid hydrolysis of samples was conducted with 6 N HCl at 110 °C for 18 h in evacuated sealed tubes and amino acid analysis was performed on a JEOL JLC-500 amino acid analyzer system. N^{α}-Boc-amino acids and N^{α}-Cbz-amino acids were purchased from Peptide Institute, Inc., Osaka, Japan.

4-Methoxymethoxybenzaldehyde (2) Chloromethyl methyl ether (33 ml) was added to a solution of 4-hydroxybenzaldehyde (40.2 g) in tetrahydrofuran (THF) (400 ml) and Et_3N (80 ml) and the solution was stirred for 1 h at room temperature. The solvent was evaporated, and the residue was dissolved in ether. This solution was washed with 1 N NaOH, dried ($MgSO_4$), and evaporated. The residue was distilled under vacuum (84—85 °C/0.6 mmHg) to give **2** as a pale yellow oil (48.5 g, 88.7%). ^{1}H-NMR ($CDCl_3$) δ: 3.50 (3H, s), 5.26 (2H, s), 7.14 (2H, d, J=12 Hz), 7.83 (2H, d, J=12 Hz), 9.90 (1H, s).

β-Hydroxy-Tyr(MOM)-OH (3) Glycine (14.6 g, 0.2 mol) and **2** (48.5 g, 0.29 mol) were added to a suspension of KOH (26.8 g, 0.4 mol) in EtOH (500 ml) at room temperature, and the reaction mixture was stirred for 19 h. The solvent was evaporated, then the residue was dissolved in H_2O and acidified with HCl. The solution was washed with EtOAc and adjusted to pH 6 with $NaHCO_3$. A white solid precipitated and was collected by filtration to give **3** (9.2 g, 20%). The filtrate was put on a column of Diaion HP-20 (Mitsubishi Kasei Co.), which was washed with H_2O, and eluted with 90% MeOH. The eluate was evaporated, and the residual solid was rinsed with acetone to give additional **3** as a solid (12.0 g, 25.5%). mp 164—166 °C. *Rf* 0.57 (*n*-BuOH–AcOH–H_2O (5 : 2 : 3)). IR (KBr) cm^{-1}: 1610, 1510, 1400. ^{1}H-NMR (D_2O) a mixture of diastereomers (1 : 1) δ: 3.51 (6H, s), 3.90 (1H, d, J=4.6 Hz), 4.08 (1H, d, J=4.2 Hz), 5.27 (1H, d, J=4.6 Hz), 5.29 (4H, s), 5.35 (1H, d, J=4.2 Hz), 7.13 (2H, d, J=8.5 Hz), 7.16 (2H, d, J=8.5 Hz), 7.38 (2H, d, J=8.5 Hz), 7.44 (2H, d, J=8.5 Hz). FAB-MS m/z 242 $(M+H)^+$. *Anal.* Calcd for $C_{11}H_{15}NO_5 \cdot 1/2H_2O$: C, 52.80; H, 6.44; N, 5.60. Found: C, 52.88; H, 6.46; N, 5.60.

β-Hydroxy-MeTyr(MOM)-OH (4) A solution of **3** (21.0 g, 87 mmol) in 1 N NaOH (250 ml) was treated with $(MeO)_2SO_4$ (16.5 g, 130 mmol). The mixture was stirred for 20 min at 90 °C, then acidified with dilute HCl at 0 °C, washed with Et_2O, adjusted to pH 6.0 with 1 N NaOH and concentrated. The precipitate was collected by filtration to give **4** (5.2 g, 23%). Additional **4** was obtained from the filtrate in the same manner as described for **3** (6.0 g, 27%). mp 177—178 °C. *Rf* 0.54 (*n*-BuOH–AcOH–H_2O (5 : 2 : 3)). IR (KBr) cm^{-1}: 3100, 1600, 1375, 1350. ^{1}H-NMR (D_2O) mixture of diastereomers (1 : 1) δ: 2.68 (3H, s), 3.03 (3H, s), 3.50 (6H, s), 3.75 (1H, d, J=7.4 Hz), 3.88 (1H, d, J=10 Hz), 5.01 (1H, d, J=7.4 Hz), 5.02 (1H, d, J=10 Hz), 5.29 (4H, s), 7.12 (2H, d, J=8.5 Hz), 7.14 (2H, d, J=8.5 Hz), 7.42 (4H, d, J=8.5 Hz). FAB-MS m/z: 256 $(M+H)^+$. *Anal.* Calcd for $C_{12}H_{17}NO_5$: C, 56.46; H, 6.71; N, 5.49. Found: C, 56.24; H, 6.88; N, 5.51.

β-Hydroxy-N^{α}-Nps-MeTyr(MOM)-OH (5) A solution of NpsCl (11.2 g, 59 mmol) in CH_2Cl_2 (50 ml) was added to a solution of **4** (15.1 g, 59 mmol) and BSA (25 ml, 0.1 mol) in CH_2Cl_2 (150 ml), and the mixture was stirred for 2 h at 0 °C. BSA (10 ml, 40 mmol) and NpsCl (5.6 g, 30 mmol) were added, and the whole was stirred for 3 h at room temperature, then 1 N NaOH (200 ml) was added. The organic layer was washed with H_2O (300 ml) and the aqueous solutions were combined, acidified with dilute HCl, and extracted with EtOAc (300 ml). The organic solution was washed with H_2O (100 ml × 3), dried ($MgSO_4$), and evaporated to give **5** as a solid (20.5 g, 85%). mp 59—60 °C. *Rf* 0.34, 0.48 ($CHCl_3$–MeOH–AcOH (10 : 1 : 0.1)). IR (KBr) cm^{-1}: 3400, 1700, 1495, 1300. FAB-MS m/z: 409 $(M+H)^+$. HR-FAB-MS m/z: Calcd for $C_{18}H_{21}N_2O_7S$ $(M+H)^+$: 409.1069. Found: 409.1083.

β-Hydroxy-N^{α}-Nps-MeTyr(MOM)-OMe (6) A solution of **5** (20.0 g, 49 mmol) in EtOAc (100 ml) was treated with freshly prepared CH_2N_2 in Et_2O (80 ml). The mixture was stirred for 10 min, then evaporated. The residue was purified on a silica gel column (500 g, $CHCl_3$) to give **6** as an oil. (*threo* isomer (**6a**): 8.82 g (42.6%), *erythro* isomer (**6b**): 6.63 g (32.1%)).

threo Isomer (**6a**): Oil. *Rf* 0.40 (EtOAc–*n*-hexane (1 : 1)). IR (film) cm^{-1}: 3500, 2950, 1735, 1510. ^{1}H-NMR ($CDCl_3$) δ: 3.15 (3H, s), 3.50 (3H, s), 3.63 (3H, br s), 3.94 (1H, d, J=8 Hz), 5.18 (2H, s), 5.22 (1H, m), 7.02 (2H, d, J=8.5 Hz), 7.30 (2H, d, J=8.5 Hz), 7.15—7.70 (4H, m), 8.27 (1H, d, J=8 Hz). FAB-MS m/z: 423 $(M+H)^+$. HR-FAB-MS Calcd for $C_{19}H_{23}N_2O_7S$ 423.1226 $(M+H)^+$. Found 423.1233.

erythro Isomer (**6b**): Oil. *Rf* 0.31 (EtOAc–*n*-hexane (1 : 1)). IR (film) cm^{-1}: 3500, 2950, 1735, 1510. ^{1}H-NMR ($CDCl_3$) (three conformers (4 : 2 : 1) existed in the solvent) major conformer δ: 2.72 (3H, s), 3.50 (3H, s), 3.88 (3H, s), 3.90 (1H, m), 5.10 (1H, m), 5.22 (2H, s), 7.05—7.50 (8H, m), 8.20 (1H, m). FAB-MS m/z 423 $(M+H)^+$. HR-FAB-MS m/z: Calcd for $C_{19}H_{23}N_2O_7S$: $(M+H)^+$: 423.1226. Found: 423.1235.

β-Benzoxy-N^{α}-Nps-MeTyr(MOM)-OMe (*threo*) (7a) Et_3N (6.3 g, 63 mmol), DMAP (1.53 g, 12.5 mmol), and benzoyl chloride (8.8 g, 63 mmol) were added to a solution of **6a** (5.29 g, 12.5 mmol) in CH_2Cl_2 (50 ml). The solution was stirred for 2 d at room temperature, then

3-dimethylaminopropylamine (19 g, 0.19 mol) was added. The mixture was concentrated and the residue was dissolved in EtOAc (30 ml). This solution was washed with dilute HCl, $NaHCO_3$ and H_2O, then evaporated, and the residue was purified by silica gel column chromatography (200 g, *n*-hexane–EtOAc (5:2)) to give **7a** as a solid (5.40 g, 81.9%). mp 114—115 °C. *Rf* 0.26 (AcOEt–*n*-hexane (1:2)). IR ($CHCl_3$) cm^{-1}: 2950, 1740, 1515. FAB-MS *m/z*: 527 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{26}H_{27}N_2O_8S$ $(M+H)^+$: 527.1488. Found: 527.1520.

β-Benzoxy-N^{α}-Nps-MeTyr(MOM)-OMe (*erythro*) (7b) Et_3N (1.38 g, 12 mmol), DMAP (0.45 g, 3.6 mmol) and benzoyl chloride (1.92 g, 14 mmol) were added to a solution of **6b** (3.85 g, 9.1 mmol) in CH_2Cl_2 (30 ml). The mixture was stirred for 16 h at room temperature, then 3-dimethylaminopropylamine (3.3 g, 32 mmol) was added. The mixture was concentrated and the residue was dissolved in EtOAc (30 ml). This solution was washed with dilute HCl, $NaHCO_3$ and H_2O, then evaporated, and the residue was purified by silica gel column chromatography (150 g, *n*-hexane–EtOAc (5:2)) to give **7b** (4.49 g, 93.6%). *Rf* 0.23 (EtOAc–*n*-hexane (1:2)). IR (film) cm^{-1}: 2950, 1740, 1515. FAB-MS *m/z*: 527 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{26}H_{27}N_2O_8S$ $(M+H)^+$: 527.1488. Found: 527.1512.

β-Benzoxy-MeTyr(MOM)-OMe (*threo*) (8a) Thiophenol (4.8 ml, 46.7 mmol) and TFA (2.5 ml, 32.5 mmol) were added to a solution of **7a** (4.94 g, 9.4 mmol) in CH_2Cl_2 (50 ml) at 0 °C. The mixture was stirred for 30 min, then $NaHCO_3$ was added. The organic layer was washed with $NaHCO_3$ and brine, and evaporated. The residue was purified on a silica gel column (100 g, 5% MeOH/$CHCl_3$) to give **8a** (3.22 g, 91.9%). *Rf* 0.31 (EtOAc–*n*-hexane (1:1)). IR (film) cm^{-1}: 2950, 1730, 1510. ^{1}H-NMR (CD_3OD) δ: 2.35 (3H, br s), 3.42 (3H, s), 3.60 (3H, s), 3.75 (1H, d, J=5 Hz), 5.15 (2H, s), 6.16 (1H, d, J=5 Hz), 7.02, 7.37 (each 2H, d, J=9 Hz), 7.49 (2H, m), 7.62 (1H, m), 8.08 (2H, dd, J=8.5, 1 Hz). FAB-MS *m/z*: 374 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{20}H_{24}NO_6$ $(M+H)^+$: 374.1603. Found: 374.1615.

β-Benzoxy-MeTyr(MOM)-OMe (*erythro*) (8b) Thiophenol (7 ml, 68 mmol) and TFA (3 ml) were added to a solution of **7b** (4.48 g, 8.5 mmol) in CH_2Cl_2 (50 ml) at 0 °C. The mixture was stirred for 30 min, then $NaHCO_3$ was added. The organic layer was washed with $NaHCO_3$ and brine, and evaporated. The residue was purified on a silica gel column (100 g, 5% MeOH/$CHCl_3$) to give **8b** (2.29 g, 72.1%). *Rf* 0.25 (EtOAc–*n*-hexane (1:1)). IR (film) cm^{-1}: 2950, 1730, 1515. ^{1}H-NMR (CD_3OD) δ: 2.35 (3H, s), 3.42 (3H, s), 3.68 (3H, s), 3.70 (1H, d, J=7 Hz), 5.17 (2H, s), 6.10 (1H, d, J=7 Hz), 7.04, 7.37 (each 2H, d, J=9 Hz), 7.50 (2H, m), 7.63 (1H, m), 8.05 (2H, dd, J=8.5, 1 Hz). FAB-MS *m/z*: 374 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{20}H_{24}NO_6$ $(M+H)^+$: 374.1603. Found: 374.1619.

Cbz-Thr-β-benzoxy-MeTyr(MOM)-OMe (*threo*) (9a) EEDQ (2.9 g, 11.7 mmol) was added to a solution of Cbz-Thr-OH (3.7 g, 14.6 mmol) and **8a** (3.11 g, 8.3 mmol) in CH_2Cl_2 (50 ml) and the mixture was stirred for 20 h at room temperature, then evaporated. The residue was dissolved in EtOAc (50 ml). This solution was washed with dilute HCl, $NaHCO_3$ and H_2O, and evaporated. The residue was purified on a silica gel column (100 g, *n*-hexane–EtOAc (1:1)) to give **9a** (2.04 g, 40.2%). *Rf* 0.36 (3% MeOH/$CHCl_3$). IR (film) cm^{-1}: 3400, 2950, 1740 (shoulder), 1720. ^{1}H-NMR ($CDCl_3$) (three conformers (4:2:1) existed in the solvent) major conformer δ: 0.97 (3H, d, J=6 Hz), 3.28 (3H, s), 3.44 (3H, s), 3.50 (1H, m), 3.63 (3H, s), 4.50 (1H, d, J=10 Hz), 5.08 (2H, s), 5.13 (2H, s), 5.61 (1H, d, J=10 Hz), 5.91 (1H, d, J=7.5 Hz), 6.68 (1H, d, J=7.5 Hz), 7.02 (2H, d, J=8 Hz), 7.25—7.65 (10H, m), 8.01 (2H, m). FAB-MS *m/z*: 609 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{32}H_{37}N_2O_{10}$ $(M+H)^+$: 609.2448. Found: 609.2481.

Cbz-Thr-β-benzoxy-MeTyr(MOM)-OMe (*erythro*) (9b) A solution of Cbz-Thr-OH (0.95 g, 3.75 mmol) and **8b** (1.40 g, 3.75 mmol) in CH_2Cl_2 (20 ml) was treated with EEDQ (0.93 g, 3.75 mmol). The mixture was stirred for 14 h at room temperature, then evaporated. The residue was dissolved in EtOAc (30 ml). This solution was washed with dilute HCl, $NaHCO_3$ and H_2O, then evaporated. The residue was purified on a silica gel column (50 g, *n*-hexane–EtOAc (1:1)) to give **9b** (1.12 g, 49.1%). *Rf* 0.23 (3% MeOH/$CHCl_3$). IR (film) cm^{-1}: 2950, 1740, 1730 (shoulder). ^{1}H-NMR ($CDCl_3$) (two conformers (1:1) existed in the solvent) δ: 0.97 (3H, d, J=6 Hz), 1.04 (3H, d, J=6 Hz), 2.93 (3H, s), 2.99 (3H, s), 3.36 (1H, m), 3.40 (3H, s), 3.43 (3H, s), 3.73 (6H, s), 4.02 (1H, m), 4.33 (2H, m), 4.0—5.2 (8H, m), 5.60 (4H, m), 6.46 (2H, m), 7.0 (4H, m), 7.25—7.65 (20H, m), 8.02 (4H, m). FAB-MS *m/z*: 609 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{32}H_{37}N_2O_{10}$ $(M+H)^+$: 609.2448. Found: 609.2479.

Cbz-Thr-(*Z*)ΔMeTyr(MOM)-OMe (10) DBU (0.30 g, 2.0 mmol) was added to a solution of **9a** (1.20 g, 2.0 mmol) in toluene (20 ml). The mixture was stirred for 0.5 h at room temperature, then 7% HCl (10 ml) was added. The organic layer was washed with H_2O and $NaHCO_3$, dried ($MgSO_4$), and evaporated to give **10** (0.95 g, 99%) as a colorless oil. *Rf* 0.25 (3% MeOH/$CHCl_3$). $[\alpha]_D^{23}$ −7.7° (*c*=0.64, MeOH). IR (film) cm^{-1}: 3400, 2950, 1720. ^{1}H-NMR ($CDCl_3$) (three conformers (4:3:1) existed in the solvent) major conformer δ: 0.90 (3H, d, J=7 Hz), 3.15 (3H, s), 3.48 (3H, s), 3.70 (3H, s), 3.68 (1H, m), 4.23 (1H, dd, J=1, 10 Hz), 5.08 (2H, m), 5.22 (2H, m), 5.53 (1H, d, J=10 Hz), 6.98—7.50 (9H, m), 7.69 (1H, s). FAB-MS *m/z*: 487 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{25}H_{31}N_2O_8$ $(M+H)^+$: 487.2080. Found: 487.2094. The same compound **10** was obtained in a similar manner from **9b** (reaction time 2.5 h).

Cbz-Thr(TBDMS)-(*Z*)ΔMeTyr(MOM)-OMe (11) *tert*-Butyldimethylsilyl chloride (0.75 g, 5.0 mmol) and imidazole (0.34 g, 5.0 mmol) were added to a solution of **10** (1.0 g, 2.0 mmol) in DMF (10 ml). The mixture was stirred for 16 h at room temperature, then EtOAc (30 ml) and ice (50 g) were added. The organic layer was washed with dilute HCl, $NaHCO_3$ and H_2O, and evaporated. The residue was purified on a silica gel column (30 g, $CHCl_3$) to give **11** (1.21 g, 98%). *Rf* 0.27 (AcOEt–*n*-hexane (1:2)). $[\alpha]_D^{23}$ −55.9° (*c*=0.56, MeOH). IR (film) cm^{-1}: 2950, 1720. ^{1}H-NMR ($CDCl_3$) (two conformer (1:1) existed in the solvent) δ: −0.07 (3H, s), −0.04 (3H, s), 0.03 (3H, s), 0.05 (3H, s), 0.85 (18H, s), 0.91 (3H, d, J=6 Hz), 1.10 (3H, d, J=6 Hz), 3.12 (3H, s), 3.14 (3H, s), 3.44 (3H, s), 3.50 (3H, s), 3.72 (3H, s), 3.87 (1H, m), 3.91 (3H, s), 4.04 (1H, m), 4.20 (1H, dd, J=4,9 Hz), 4.31 (1H, dd, J=4,9 Hz), 4.65 (1H, d, J=12 Hz), 4.80 (1H, d, J=12 Hz), 5.10 (2H, m), 5.12 (2H, s), 5.18 (1H, d, J=9 Hz), 5.24 (2H, s), 5.44 (1H, d, J=9 Hz), 7.03 (2H, d, J=8 Hz), 7.10 (2H, d, J=8 Hz), 7.18—7.44 (10H, m), 7.44 (2H, d, J=8 Hz), 7.55 (2H, d, J=8 Hz), 7.68 (1H, s), 7.72 (1H, s). FAB-MS *m/z*: 601 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{31}H_{45}N_2O_8Si$ $(M+H)^+$: 601.2945. Found: 601.2959.

Cbz-Thr(TBDMS)-(*Z*)ΔMeTyr(MOM)-OH (12) A solution of **11** (0.95 g, 1.6 mmol) in MeOH was treated with 1 N NaOH (4.8 ml), and the mixture was stirred for 2 d at 30 °C then evaporated. The residue was dissolved in EtOAc (20 ml). This solution was washed with dilute HCl and H_2O, and evaporated to gave **12** as a colorless gum (0.81 g, 87.3%). *Rf* 0.34 (10% MeOH/$CHCl_3$). $[\alpha]_D^{23}$ −82.9° (*c*=1.06, MeOH). IR (film) cm^{-1}: 3300, 2950, 1720, 1700 (shoulder). ^{1}H-NMR ($CDCl_3$) (two conformers (1:1) existed in the solvent) δ: −0.05 (3H, s), −0.03 (3H, s), 0.03 (3H, s), 0.05 (3H, s), 0.85 (18H, s), 0.93 (3H, d, J=6 Hz), 1.12 (3H, d, J=6 Hz), 3.14 (3H, s), 3.16 (3H, s), 3.43 (3H, s), 3.50 (3H, s), 3.90 (1H, m), 4.08 (1H, m), 4.28 (1H, dd, J=4, 9 Hz), 4.37 (1H, dd, J=5, 9 Hz), 4.60 (1H, d, J=12 Hz), 4.80 (1H, d, J=12 Hz), 5.07 (2H, m), 5.13 (2H, s), 5.21 (1H, d, J=9 Hz), 5.25 (2H, s), 5.60 (1H, d, J=9 Hz), 7.05 (2H, d, J=8 Hz), 7.12 (2H, d, J=8 Hz), 7.18-7.43 (10H, m), 7.46 (2H, d, J=8 Hz), 7.58 (2H, d, J=8 Hz), 7.75 (1H, s), 7.83 (1H, s). FAB-MS *m/z*: 587 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{30}H_{43}N_2O_8Si$ $(M+H)^+$: 587.2788. Found: 587.2813.

Cbz-Thr(TBDMS)-(*Z*)ΔMeTyr(MOM)-Leu-D-Phe-OTce (13) Et_3N (1.25 g, 12.3 mmol) and EEDQ (3.04 g, 12.3 mmol) were added to a mixture of **12** (1.60 g, 2.73 mmol) and **22** (5.50 g, 12.3 mmol) in CH_2Cl_2 (50 ml) and the mixture was stirred for 15 h at room temperature. A white solid was filtered off, then **22** (2.23 g, 5 mmol), Et_3N (0.50 g, 5 mmol) and EEDQ (1.24 g, 5 mmol) were added to the filtrate. The mixture was stirred for 18 h and evaporated. The residue was dissolved in EtOAc (50 ml) and this solution was washed with dilute HCl, $NaHCO_3$ and H_2O, and evaporated. The residue was purified on a silica gel column (100 g, *n*-hexane–EtOAc (2:1)) to give **13** (0.87 g, 32.6%). *Rf* 0.30 (EtOAc–*n*-hexane (2:3)). $[\alpha]_D^{23}$ −31.5° (*c*=1.07, MeOH). IR (film) cm^{-1}: 2950, 1760, 1740 (shoulder), 1720, 1660. UV λ_{max} (MeOH) nm (ε): 305 (18500). ^{1}H-NMR ($CDCl_3$) δ: −0.22 (3H, s), −0.13 (3H, s), 0.70 (3H, d, J=6 Hz), 0.83 (9H, s), 0.92 (3H, d, J=6 Hz), 0.95 (3H, d, J=6 Hz), 1.60—1.85 (3H, m), 3.01 (1H, dd, J=7, 14 Hz), 3.18 (3H, s), 3.22 (1H, dd, J=5, 14 Hz), 3.32 (1H, q, J=6 Hz), 3.49 (3H, s), 3.81 (1H, d, J=7 Hz), 4.45 (1H, m), 4.47 (1H, d, J=12 Hz), 4.64 (1H, d, J=12 Hz), 4.92 (1H, m), 4.99 (1 Hz, d, J=12 Hz), 5.14 (1H, d, J=12 Hz), 5.21 (2H, s), 5.92 (1H, d, J=7 Hz), 7.10 (2H, d, J=8.5 Hz), 7.17 (2H, d, J=8 Hz), 7.20—7.4 (11H, m), 7.66 (1H, s), 7.70 (1H, d, J=7.5 Hz). FAB-MS *m/z*: 977 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{47}H_{64}Cl_3N_4O_{10}Si$ $(M+H)^+$: 977.3457. Found: 977.3463. Amino acid ratios in an acid hydrolysate: Thr 0.80, Leu 1.00, Phe 1.07, $MeNH_2$ 1.12.

Cbz-Thr(TBDMS)-(*E*)ΔMeTyr(MOM)-Leu-D-Phe-OTce (14) A solution of **13** (0.85 g, 0.87 mmol) in toluene (100 ml) and acetone (10 ml)

was irradiated with a UV lamp (100 W) for 1.5 h at 0 °C. The solvent was evaporated, and the residue was purified on a silica gel column (50 g, *n*-hexane–EtOAc (2 : 1)) to give **14** (0.18 g, 21.2%) and **13** (0.42 g, 49.5%). **14**: mp 40—42 °C. *Rf* 0.22 (*n*-hexane–EtOAc (2 : 1)). $[\alpha]_D^{21}$ −23.6° (*c* = 0.5, MeOH). IR (KBr) cm^{-1}: 3300, 1740 (shoulder), 1640. UV λ_{max} (MeOH) nm (ε): 284 (8865). ^{1}H-NMR ($CDCl_3$) (two conformers (2 : 1) existed in the solvent) major conformer δ: 0.10 (3H, s), 0.83 (3H, d, *J* = 6 Hz), 0.86 (3H, d, *J* = 6 Hz), 0.90 (9H, s), 1.13 (3H, d, *J* = 6 Hz), 1.40—1.76 (3H, m), 3.01 (1H, dd, *J* = 9, 14 Hz), 3.04 (3H, s), 3.27 (1H, dd, *J* = 5, 14 Hz), 3.46 (3H, s), 4.25 (1H, m), 4.44 (1H, m), 4.47 (1H, d, *J* = 8 Hz), 4.64 (1H, d, *J* = 12 Hz), 4.84 (1H, d, *J* = 12 Hz), 4.89 (1H, m), 5.01 (1H, d, *J* = 12 Hz), 5.14 (1H, d, *J* = 12 Hz), 5.17 (2H, s), 5.83 (1H, d, *J* = 8 Hz), 6.63 (1H, s), 6.97 (2H, d, *J* = 8.5 Hz), 7.00—7.37 (11H, m), 7.38 (2H, d, *J* = 8.5 Hz), 7.83 (1H, d, *J* = 7 Hz). FAB-MS *m/z*: 977 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{47}H_{64}Cl_3N_4O_{10}Si$ $(M+H)^+$: 977.3457. Found: 977.3472. Amino acid ratios in an acid hydrolysate: Thr 0.84, Leu 1.00, Phe 1.01, $MeNH_2$ 1.16.

Cbz–Thr–(*E*)⊿MeTyr(MOM)–Leu–D-Phe–OTce (15) Compound **14** (0.17 g, 0.17 mmol) was dissolved in 67% AcOH (10 ml), and the solution was stirred for 28 h at 25 °C, then concentrated. The residue was dissolved in EtOAc (20 ml). This solution was washed with $NaHCO_3$ and H_2O, and evaporated. The residue was rinsed with *n*-hexane to give **15** (0.15 g, 99.9%). mp 56—59 °C. *Rf* 0.18 (*n*-hexane–EtOAc (1 : 1)). $[\alpha]_D^{20}$ −44.4° (*c* = 0.5, MeOH). IR (KBr) cm^{-1}: 3250, 1740 (shoulder), 1635. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, d, *J* = 6 Hz), 0.85 (3H, d, *J* = 6 Hz), 1.15 (3H, d, *J* = 6 Hz), 1.35—1.70 (3H, m), 3.04 (1H, dd, *J* = 8, 14 Hz), 3.08 (3H, s), 3.27 (1H, dd, *J* = 5, 14 Hz), 3.46 (3H, s), 4.21 (1H, q, *J* = 6 Hz), 4.44 (1H, m), 4.61 (1H, d, *J* = 8 Hz), 4.65 (1H, d, *J* = 12 Hz), 4.84 (1H, d, *J* = 12 Hz), 4.92 (1H, m), 5.02 (1H, d, *J* = 13 Hz), 5.10 (1H, d, *J* = 13 Hz), 5.18 (2H, s), 5.98 (1H, d, *J* = 8 Hz), 6.56 (1H, s), 6.98 (2H, d, *J* = 8.5 Hz), 7.00 (1H, m), 7.13—7.40 (12H, m), 8.16 (1H, br s). FAB-MS *m/z*: 863 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{41}H_{50}Cl_3N_4O_{10}$ $(M+H)^+$: 863.2592. Found: 863.2622. Amino acid ratios in an acid hydrolysate: Thr 0.83, Leu 1.00, Phe 1.02, $MeNH_2$ 1.13.

Cbz–Thr(Boc–Ser(Bzl))–(*E*)⊿MeTyr(MOM)–Leu–D-Phe–OTce (16) Boc–Ser(Bzl)–OH (0.10 g, 0.34 mmol), EDC·HCl (65 mg, 0.34 mmol) and DMAP (4 mg, 0.03 mmol) were added to a solution of **15** (0.14 g, 0.16 mmol) in CH_2Cl_2 (5 ml) and the mixture was stirred for 12 h at room temperature. 3-Dimethylaminopropylamine (50 mg, 0.5 mmol) was added, and the whole was evaporated. The residue was dissolved in EtOAc (20 ml) and this solution was washed with diluted HCl and H_2O, then evaporated. The residue was purified by silica gel column chromatography (10 g, *n*-hexane–EtOAc (1 : 1)) to give **16** (0.16 g, 86.5%). mp 51—56 °C. *Rf* 0.38 (*n*-hexane–EtOAc (1 : 1)). $[\alpha]_D^{23}$ −61.7° (*c* = 0.5, MeOH). IR (KBr) cm^{-1}: 3300, 1730 (shoulder), 1700, 1640, 1495. FAB-MS *m/z*: 1140 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{56}H_{69}Cl_3N_5O_{14}$ $(M+H)^+$: 1140.3906. Found: 1140.3916. Amino acid ratios in an acid hydrolysate: Thr 0.86, Ser 0.92, Leu 1.00, Phe 1.09, $MeNH_2$ 1.25.

Cbz–Thr(Boc–Asn–Ser(Bzl))–(*E*)⊿MeTyr–Leu–D-Phe–OTce (17) Compound **16** (145 mg, 0.13 mmol) was dissolved in 4 N HCl in dioxane (3 ml) and anisole (0.1 ml). The mixture was stirred for 30 min at room temperature, then evaporated. The residue was dissolved in CH_2Cl_2 (3 ml). To this solution were added Boc–Asn–OH (35 mg, 0.15 mmol), Et_3N (13 mg, 0.13 mmol), HOBT (18 mg, 0.13 mmol) and EDC·HCl (29 mg, 0.15 mmol). The mixture was stirred for 1 h at room temperature, then 7% HCl (5 ml) was added. The organic layer was washed with H_2O, then evaporated, and the residue was purified by preparative TLC (6% MeOH/$CHCl_3$) to give **17** (110 mg, 71.5%). mp 94—97 °C. *Rf* 0.44 ($CHCl_3$–MeOH (10 : 1)). $[\alpha]_D^{23}$ −59.3° (*c* = 0.5, MeOH). IR (KBr) cm^{-1}: 3300, 1730 (shoulder), 1650, 1505. FAB-MS *m/z*: 1210 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{58}H_{71}Cl_3N_7O_{15}$ $(M+H)^+$: 1210.4073. Found: 1210.4092. Amino acid ratios in an acid hydrolysate: Asp 1.10, Thr 1.13, Ser 0.88, Leu 1.00, Phe 1.17, NH_3 1.47, $MeNH_2$ 1.30.

Cbz–Thr(Boc–*allo*-Thr–Asn–Ser(Bzl))–(*E*)⊿MeTyr–Leu–D-Phe–OTce (18) Compound **17** (105 mg, 87 μmol) was dissolved in 4 N HCl in dioxane (3 ml) and anisole (0.1 ml). The mixture was stirred for 30 min at room temperature, then evaporated, and the residue was dissolved in CH_2Cl_2 (3 ml). To this solution were added Boc–*allo*-Thr–OH (22 mg, 0.1 mmol), Et_3N (9 mg, 90 μmol), HOBT (12 mg, 88 μmol) and EDC·HCl (19 mg, 0.1 mmol). The whole was stirred for 8 h at room temperature, then 7% HCl (5 ml) was added. The organic layer was washed with H_2O, then evaporated, and the residue was purified by preparative TLC (6% MeOH/$CHCl_3$) to give **18** (62.1 mg, 54.6%). *Rf* 0.73 ($CHCl_3$–MeOH (5 : 1)). IR (KBr) cm^{-1}: 3300, 1740 (shoulder), 1650, 1500. FAB-MS *m/z*: 1311 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{62}H_{78}Cl_3N_8O_{17}$ $(M+H)^+$: 1311.4550. Found: 1311.4571. Amino acid ratios in an acid hydrolysate: Asp 1.01, Thr 1.81, Ser 0.76, Leu 1.00, Phe 1.07, NH_3 0.87, $MeNH_2$ 1.19.

Cbz–Thr(Boc–*allo*-Thr–Asn–Ser(Bzl))–(*E*)⊿MeTyr–Leu–D-Phe–OH (19) Zinc powder (30 mg) was added to a solution of **18** (58.5 mg, 45 μmol) in 90% AcOH (1 ml). The mixture was stirred for 9 h at room temperature, then further zinc powder (30 mg) was added to the mixture every hour until the starting material disappeared. The mixture was filtered and the filtrate was evaporated. The residue was dissolved in EtOAc (10 ml) and this solution was washed with H_2O, then evaporated. The residue was purified by preparative TLC (EtOAc–acetone–AcOH–H_2O (6 : 3 : 1 : 1)) to give **19** (43.5 mg, 82.6%). *Rf* 0.16 ($CHCl_3$–MeOH–AcOH (10 : 1 : 0.1)). $[\alpha]_D^{20}$ −53.3° (*c* = 0.82, MeOH). IR (KBr) cm^{-1}: 3330, 1735 (shoulder), 1650, 1505. FAB-MS *m/z*: 1181 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{60}H_{76}N_8O_{17}$ $(M+H)^+$: 1181.5406. Found: 1181.5435. Amino acid ratios in an acid hydrolysate: Asp 1.03, Thr 1.84, Ser 0.80, Leu 1.00, Phe 1.12, NH_3 0.95, $MeNH_2$ 1.29.

Cbz–Thr–(*E*)⊿MeTyr–Leu–D-Phe–*allo*-Thr–Asn–Ser(Bzl) υ-Lactone (20) HONSu (20.4 mg, 0.18 mmol) and EDC·HCl (8.2 mg, 43 μmol) were added to a solution of **19** (42 mg, 36 μmol) in CH_2Cl_2 (4 ml) and DMF (0.1 ml). The mixture was stirred for 15 h at room temperature, then EDC·HCl (4 mg, 20 μmol) was added every 1.5 h until the starting material disappeared. The solvent was evaporated and the residue was dissolved in EtOAc (10 ml). This solution was washed with diluted HCl and water, dried over $MgSO_4$, and evaporated. The residue was dissolved in TFA (1 ml) and anisole (0.1 ml). This mixture was stirred for 30 min at room temperature, then evaporated. The residue was dissolved in DMF (2 ml) and the solution was added to pyridine (40 ml). The reaction mixture was stirred for 16 h at room temperature, then evaporated, and the residue was purified by preparative TLC ($CHCl_3$–MeOH (10 : 1)) to give **20** (15.2 mg, 40.2%). *Rf* 0.77 ($CHCl_3$–MeOH–H_2O (65 : 25 : 4)). $[\alpha]_D^{23}$ +18.0° (*c* = 0.1, MeOH), IR (KBr) cm^{-1}: 3300, 1730 (shoulder), 1635, 1510. FAB-MS *m/z*: 1063 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{55}H_{67}N_8O_{14}$ $(M+H)^+$: 1063.4776. Found: 1063.4789. Amino acid ratios in an acid hydrolysate: Asp 1.00, Thr 1.72, Ser 0.79, Leu 1.00, Phe 1.08, NH_3 1.03, $MeNH_2$ 1.27.

H-Thr–(*E*)⊿MeTyr–Leu–D-Phe–*allo*-Thr–Asn–Ser υ-Lactone Acetate (21) Compound **20** (22 mg, 21 μmol) was dissolved in HF–pyridine (0.8 ml) and anisole (0.2 ml) in an N_2 gas-bag. The mixture was stirred for 1 h at room temperature, then several pieces of ice were added and the pH was adjusted to 8 with $NaHCO_3$. The mixture was applied to a column of Diaion HP-20 (10 ml), which was washed with H_2O, and eluted with MeOH. The product was purified by preparative TLC ($CHCl_3$–MeOH–H_2O (3 : 1 : 0.1)) to give **21** (13.0 mg, 74.9%). *Rf* 0.35 ($CHCl_3$–MeOH–H_2O (3 : 1 : 0.1)). IR (KBr) cm^{-1}: 3350, 1720 (shoulder), 1635, 1510. $[\alpha]_D^{23}$ −90.6° (*c* = 0.1, MeOH). FAB-MS *m/z*: 839 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{40}H_{55}N_8O_{12}$ $(M+H)^+$: 839.3939. Found: 839.3952. Amino acid ratios in an acid hydrolysate: Asp 1.07, Thr 1.89, Ser 0.84, Leu 1.00, Phe 1.10, NH_3 0.98, $MeNH_2$ 1.34.

***N*-2-(1(*Z*)-Pentenyl)-cinnamoyl-Thr–(*E*)⊿MeTyr–Leu–D-Phe–*allo*-Thr–Asn–Ser υ-Lactone (WS9326A, 1)** A solution of **21** (6.0 mg, 6.7 μmol) in CH_2Cl_2 (1.5 ml), BSA (30 ml, 127 mmol) and DMF (0.3 ml) was treated with 0.02 M 2-(1-pentenyl)cinnamoyl chloride (0.4 ml). The mixture was stirred for 1 h at room temperature, then DMAP (0.1 mg) was added. Further 2-(1-pentenyl)cinnamoyl chloride was added to the mixture every 30 min until the starting material disappeared. Then dilute HCl was added, and the organic layer was washed with H_2O, and evaporated. The residue was purified by preparative TLC ($CHCl_3$–MeOH–H_2O (65 : 25 : 4)) to give **1** (0.2 mg, 2.7%). *Rf* 0.59 ($CHCl_3$–MeOH–H_2O (65 : 25 : 4)). IR (KBr) cm^{-1}: 3300, 1730 (shoulder), 1610, 1510. FAB-MS *m/z*: 1037 $(M+H)^+$.

H-Leu–D-Phe–OTce Hydrochloride (22) EDC·HCl (7.8 g, 40 mmol) and DMAP (0.5 g, 4 mmol) were added to a solution of Boc–D-Phe–OH (9.85 g, 37.1 mmol) and trichloroethanol (5.55 g, 37.1 mmol) in CH_2Cl_2 (100 ml) and the mixture was stirred for 1.5 h at room temperature, then evaporated. The residue was dissolved in EtOAc (100 ml) and this solution was washed with dilute HCl, $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated. The residue was dissolved in toluene (100 ml) and 4 N HCl in EtOAc (100 ml) and the solution was stirred for 1 h at room temperature, then evaporated to give D-Phe–OTce hydrochloride (7.2 g, 58.3%).

A solution of Boc–Leu–OH·H_2O (5.35 g, 21.5 mmol) in CH_2Cl_2

(50 ml) was dried ($MgSO_4$) and filtered. D-Phe–OTce hydrochloride (7.15 g, 21.5 mmol), Et_3N (2.17 g, 21.5 mmol), HOBT (2.9 g), and EDC·HCl (4.5 g, 23.5 mmol) were added to the filtrate. The mixture was stirred for 1 h at room temperature, then evaporated. The residue was dissolved in EtOAc. This solution was washed with dilute HCl, $NaHCO_3$, and H_2O, dried ($MgSO_4$), and evaporated to give Boc–Leu–D-Phe–OTce (10.96 g).

A solution of Boc–Leu–D-Phe–OTce (7.2 g, 14.1 mmol) in toluene (100 ml) and 4 N HCl/EtOAc (100 ml) was stirred for 30 min at room temperature. Evaporation of the solvent gave **22** (6.2 g, 98.7%). mp 190—193 °C. IR (KBr) cm^{-1}: 3300, 3030, 1780, 1700. ^{1}H-NMR ($CDCl_3$) δ: 0.73 (3H, d, J=6 Hz), 0.82 (3H, d, J=6 Hz), 1.03 (1H, m), 1.45—1.80 (2H, m), 3.08 (1H, dd, J=11, 14 Hz), 3.36 (1H, dd, J=5, 14 Hz), 4.30 (1H, m), 4.63 (1H, d, J=12 Hz), 4.95 (1H, m), 4.98 (1H, d, J=12 Hz), 7.20—7.40 (5H, m), 8.16 (3H, br s), 8.63 (1H, d, J=8 Hz). FAB-MS m/z: 409 $(M+H)^+$. *Anal.* Calcd for $C_{17}H_{23}Cl_3N_2O_3 \cdot HCl$: C, 45.76; H, 5.42; N, 6.28. Found: C, 45.82; H, 5.37; N, 6.26.

2-(1(*Z*)-Pentenyl)cinnamoyl Chloride (23) $(COCl_2)_2$ (0.5 ml) and DMF (0.05 ml) were added to a solution of 2-(1(*Z*)-pentenyl)cinnamic acid (1.08 g) in CH_2Cl_2 (10 ml). The mixture was stirred for 1 h at room temperature under an N_2 atmosphere, then evaporated. The residue was dissolved in hexane and the solution was filtered. The filtrate was evaporated to give 2-(1(*Z*)-pentenyl)cinnamoyl chloride as a pale yellow oil (1.15 g). IR (neat) cm^{-1}: 1750, 1730, 1605, 1585. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.5 Hz), 1.45 (2H, m), 2.06 (2H, m), 5.95 (1H, dt, J=7, 11 Hz), 6.58 (1H, d, J=11 Hz), 6.66 (1H, d, J=16 Hz), 7.25-7.50 (3H, m), 7.69 (1H, m), 8.12 (1H, d, J=16 Hz).

2-Oxazolidone Derivative of 6 Thiophenol (0.2 ml) and TFA (0.5 ml) were added to a solution of **6a** (0.35 g, 0.83 mmol) in CH_2Cl_2. The mixture was stirred for 10 min at room temperature, then aqueous $NaHCO_3$ was added. The organic layer was evaporated to give β-hydroxy-MeTyr(MOM)–OMe (0.22 g).

β-Hydroxy-MeTyr(MOM)–OMe (0.13 g, 0.48 mmol) was dissolved in CH_2Cl_2 (5 ml), and Cbz–Cl (0.1 ml, 0.6 mmol) and BSA (0.2 ml, 0.81 mmol) were added. The mixture was stirred for 15 min at room temperature, then 3-dimethylaminopropylamine (0.1 ml) was added. The whole was evaporated, the residue was dissolved in EtOAc (10 ml), and the solution was washed with 7% HCl, $NaHCO_3$ and water, dried ($MgSO_4$), and evaporated to give Cbz-β-hydroxy-MeTyr(MOM)–OMe (0.19 g).

A solution of Cbz-β-hydroxy-MeTyr(MOM)–OMe (50 mg, 0.12 mmol) in MeOH was treated with 1 N NaOH (0.5 ml). The mixture was stirred for 6 h at 50 °C, and evaporated. The residue was dissolved in EtOAc, and this solution was washed with 7% HCl, dried ($MgSO_4$) and evaporated. The residue was purified by preparative TLC ($CHCl_3$–MeOH–AcOH (10 : 1 : 0.1)) to give 5-(4-methoxymethoxyphenyl)-3-methyl-2-oxazolidone-4-carboxylic acid (**6'a**) (21 mg, 60%). **6'a**: ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 3.02 (3H, s), 3.48 (3H, s), 4.16 (1H, d, J=5 Hz), 4.72 (2H, s), 5.46 (1H, d, J=5 Hz), 7.07 (2H, d, J=8 Hz), 7.32 (2H, d, J=8 Hz).

The same procedure was applied to **6b** to give **6'b**.

6'b: ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 2.83 (3H, s), 3.38 (3H, s), 4.30 (1H, d, J=9 Hz), 5.09 (2H, s), 5.58 (1H, d, J=9 Hz), 6.92 (2H, d, J=8 Hz), 7.23 (2H, d, J=8 Hz).

***N*-Stearoyl-Thr–(*E*)ΔMeTyr–Leu–D-Phe–*allo*-Thr–Asn–Ser υ-Lactone (24)** A solution of **21** (11.0 mg, 12 μmol) in pyridine (1 ml) was treated with 0.02 M stearoyl chloride in CH_2Cl_2 (0.6 ml). The mixture was stirred for 1 h at room temperature, then further stearoyl chloride was added to the mixture every 1 h until the starting material disappeared. MeOH (2 ml) was added to the mixture and the solvent was evaporated. The residue was dissolved in AcOEt (10 ml) and this solution was washed with dilute HCl and H_2O, and evaporated. The residue was purified by preparative TLC ($CHCl_3$–MeOH–H_2O (3 : 1 : 0.1)) to give **24** (2.0 mg, 13.8%). *Rf* 0.67 ($CHCl_3$–MeOH–H_2O (3 : 1 : 0.1)). IR (KBr) cm^{-1}: 3250, 1720 (shoulder), 1640, 1510. FAB-MS m/z: 1105 $(M+H)^+$.

References and Notes

1) All amino acids are the L enantiomer unless otherwise noted. Standard abbreviations for amino acids, protecting groups and peptides are used [*Eur. J. Biochem.*, **138**, 9—37 (1984)]. Other abbreviations include: Boc = *tert*-butyloxycarbonyl, Bzl = benzyl, DMF = *N*,*N*-dimethylformamide, EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, HOBt = 1-hydroxybenzotriazole, HONSu = *N*-hydroxysuccinimide, RP-HPLC = reverse-phase high-performance liquid chromatography, Tce = 2,2,2-trichloroethyl, TFA = trifluoroacetic acid, TEA = triethylamine.

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