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Synthetic studies of the cyclic depsipeptides bearing the 3-amino-6-hydroxy-2-piperidone (Ahp) unit. Total synthesis of the proposed structure of micropeptin T-20

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Abstract—The first total synthesis of a cyclic depsipeptide possessing the 3-amino-6-hydroxy-2-piperidone (Ahp) unit was successfully achieved in a convergent manner by the oxidative construction of the Ahp unit at the later stage of the synthesis. This synthetic work provides data indicating that the structure of the target Ahp-depsipeptide, micropeptin T-20, should be re-examined.

1. Introduction

The 3-amino-6-hydroxy-2-piperidone (Ahp) unit has been recently characterized as a constituent in more than 60 cyclic depsipeptides derived from cyanobacteria.[1] These cyclic depsipeptides commonly consist of the 19-membered ring, and generally exhibit an interesting and significant inhibiting action against peptidic proteases. The unique unprecedented Ahp moiety will be biosynthetically derived from glutamate, and probably play an important role to exhibit biological activity because it will participate in converting the cyclic depsipeptide into a bioactive conformation due to the conformationally restricted structure and hydrogen bonding with the free hydroxyl group.

Micropeptin T-20 was isolated from the cyanobacterium *Microcyctis aeruginosa* collected from the freshwater Kang Krachan Dam in Thailand, and its structure was determined to be **1** (Fig. 1) by Kaya and co-workers.[1b] This cyclic depsipeptide strongly inhibits chymotrypsin with an IC_{50} of 2.5×10^{-9} M, but weakly inhibits tyrosinase with an IC_{50} of 5×10^{-3} M.

As a continuation of our interests in the synthetic studies of biologically active aquatic natural products,[2] we have investigated methods used for the synthesis of the Ahp peptides. Herein, we wish to report the total synthesis of micropeptin T-20 having the proposed structure **1**,[3] which revealed that natural micropeptin T-20 was not identical

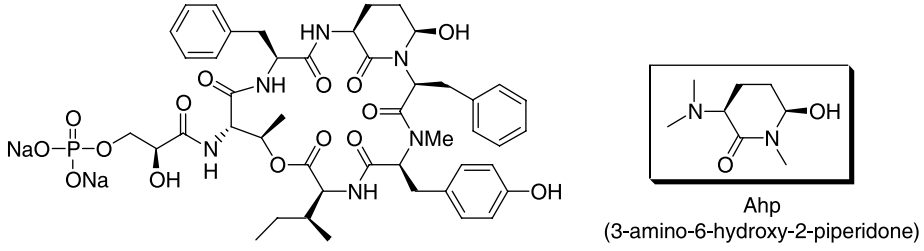

Figure 1. The proposed structure of micropeptin T-20 and the structure of the Ahp unit.

Keywords: Total synthesis; Cyclic depsipeptide; Macrolactamization; Hemiaminal; Cyanobacteria.
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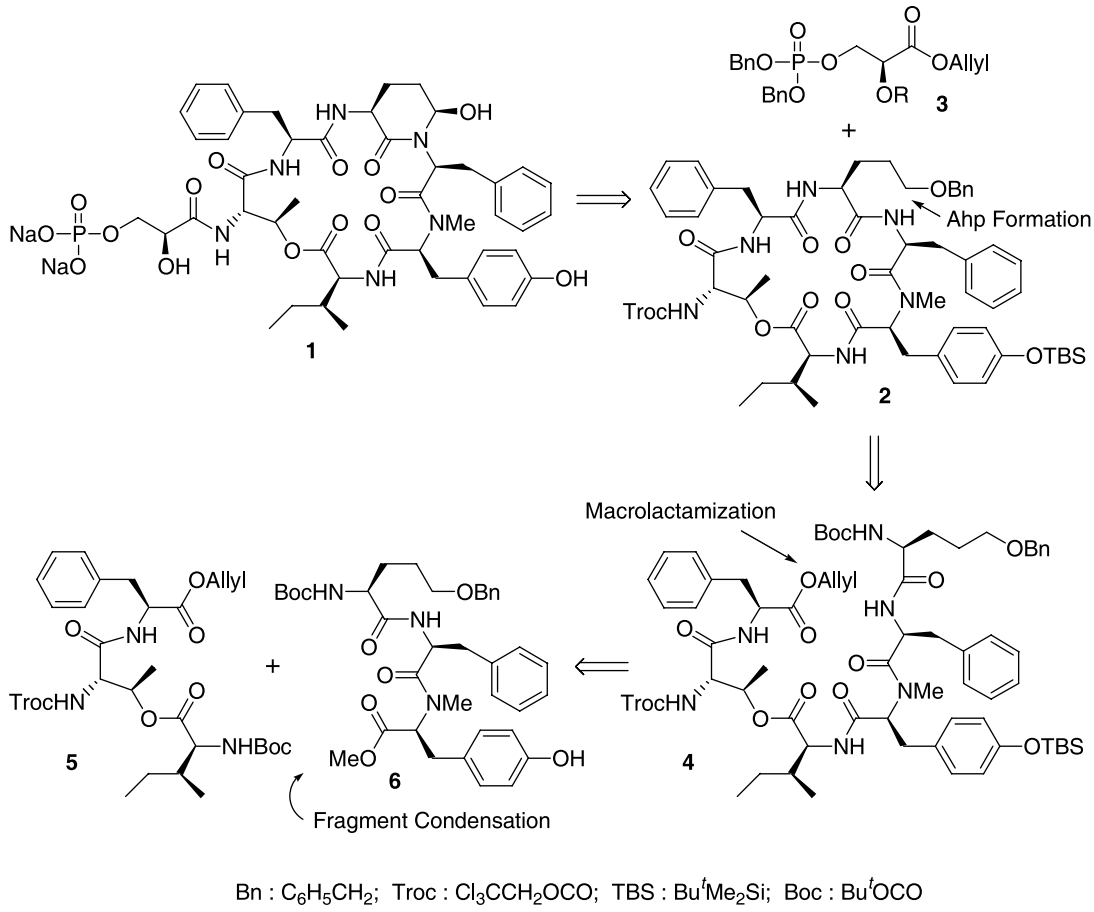


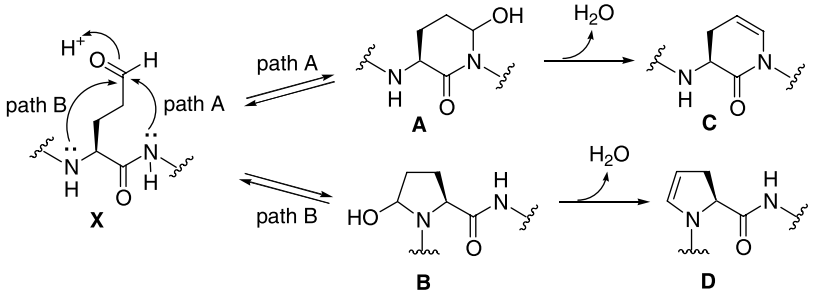
Scheme 1.

with the synthetic **1**. Thus the structure of micropeptin T-20 should be re-examined.

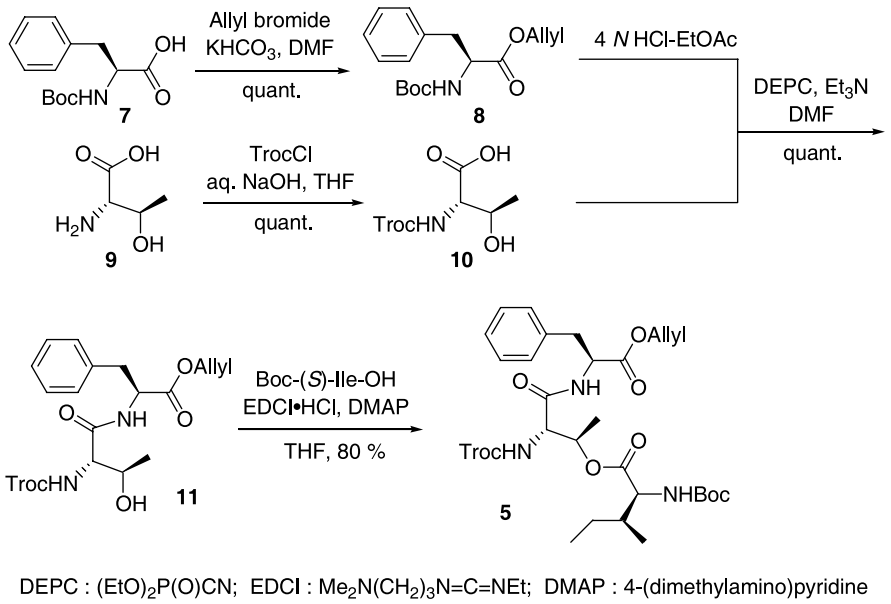
2. Synthetic strategy

Our synthetic plan for constructing micropeptin T-20 (**1**) is depicted in Scheme 1: (1) Preparation of the depsipeptide **5** and the tripeptide **6**, (2) fragment condensation of the depsipeptide **5** with the tripeptide **6**, (3) macrolactamization, (4) attachment of the side chain **3**, (5) oxidative cyclization to form the Ahp unit, and (6) deprotection. One of the most important problems to overcome in this synthesis lies in the formation of the hemiaminal-Ahp ring. The obvious starting material to construct the Ahp unit will be the aldehyde **X** prepared from glutamate, and aldehyde **X** will cyclize in two ways to give the 6-hydroxy-2-piperidone ring **A** (path A in Scheme 2) or 5-hydroxypyrrolidine ring **B** (path B). Control of the stereochemistry of the newly formed hydroxyl group will be another problem to solve. Furthermore, the hemiaminal ring is generally labile under both strong acidic and basic conditions, and it will easily undergo dehydration to produce the dehydro derivatives **C** or **D**, respectively.[4] Thus the construction of the Ahp unit should be done during the later stage of the synthesis.

As shown in the structure of micropeptin T-20 (**1**), it is a very polar compound because it has three hydroxyl groups in addition to the sodium phosphate function. Selective protection and deprotection of these polar groups will be another point to overcome. One of the most important problems to construct macrocyclic depsipeptides will be

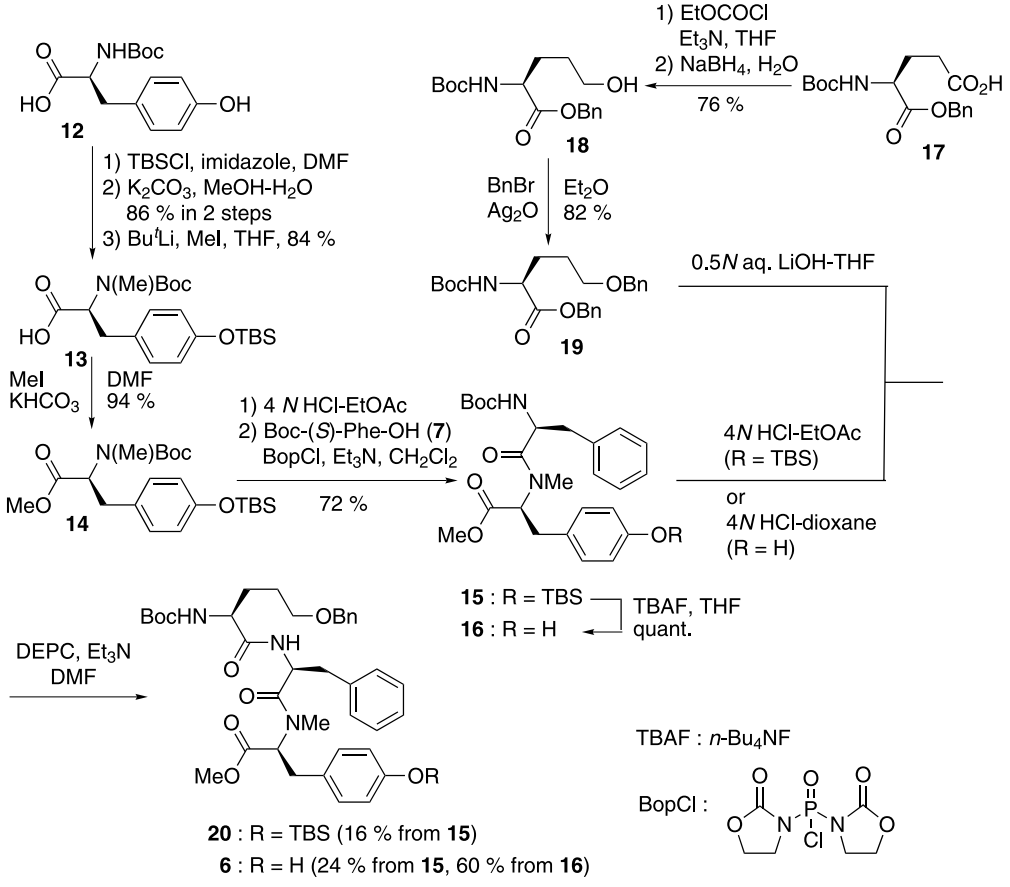


Scheme 2.



Scheme 3.

where the macrocyclization should be done and which will be better: macrolactamization or macrolactonization. Because the nucleophilicity of the amino group is generally higher than that of the hydroxyl group, macrolactamization will be preferred. An *N*-methylamino acid part in a linear peptide will form a bent structure, and it should be situated in the middle part of a cyclization precursor. This led us to synthesize the depsipeptide **5** and the tripeptide **6**. Coupling of these peptide fragments and then macrolactamization of the linear peptide **4** will give the 19-membered cyclic



Scheme 4.

depsipeptide **2**. After addition of the side chain fragment **3**, construction of the Ahp moiety followed by deprotection will finally afford micropeptin T-20 (**1**).

3. Results and discussions

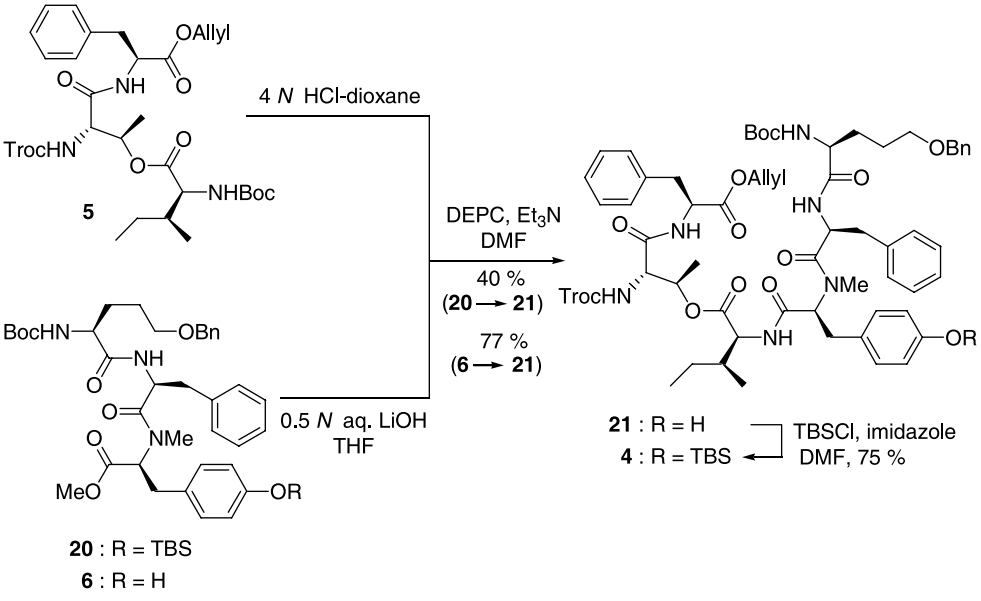
The synthesis of the depsipeptide **5** started from Boc-(*S*)-phenylalanine (**7**) by conversion to the corresponding allyl ester **8**, as shown in Scheme 3. After acidic deprotection of the *tert*-butoxycarbonyl (Boc) group of **8**, the resulting de-Boc derivative was coupled with trichloroethoxycarbonyl (Troc)-(*S*)-threonine (**10**) using diethyl phosphorocyanidate (DEPC, $(EtO)_2P(O)CN$) in the presence of triethylamine[5] to quantitatively give the dipeptide **11**. Esterification of **11** with Boc-(*S*)-isoleucine with *N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDCI·HCl) afforded the depsipeptide **5**.

The required *N*-methyl-(*S*)-tyrosine derivative (**13**) was prepared from Boc-(*S*)-tyrosine (**12**) by conversion to its *O*-*tert*-butyldimethylsilyl (TBS) derivative, which afforded the *N*-methyl derivative **13** according to our synthesis of the corresponding (*R*)-derivative,[6] as shown in Scheme 4. Esterification of the acid **13** gave the methyl ester **14**, whose Boc group was removed under acidic conditions. Coupling of the de-Boc derivative with Boc-(*S*)-phenylalanine (**7**) using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BopCl)[7] gave the dipeptide **15**.

The pentahomoserine derivative **19**, which will become a constituent of the Ahp unit, was prepared from Boc-(*S*)-glutamic acid benzyl ester (**17**) by its conversion to the mixed anhydride, reduction to the alcohol **18**,[8] and then benzylation. After the acidic deprotection of the Boc group of **15** and saponification of **19**, respectively, coupling by the DEPC method afforded a mixture of the tripeptides **20** and **6** in 16 and 24% yields, respectively. In contrast, the dipeptide **16**, obtained by removal of the TBS group from **15** with TBAF (Bu_4NF), underwent acidic deprotection and smoothly coupled with the carboxylic acid from **19** to give the tripeptide **6** in 60% yield,[9] as shown in Scheme 4. Preparation of the hexapeptide, the cyclization precursor, was first tried by coupling of the depsipeptide **5** with the O-TBS derivative **20**. After deprotection of each compound as shown in Scheme 5, coupling by the DEPC method afforded the hexapeptide **21** without the TBS function in 40% yield. It should be noted that removal of the TBS group again occurred in this case.[9] On the other hand, the coupling of **5** with the phenolic derivative **6** smoothly proceeded to give the hexapeptide **21** in 77% yield, which was converted to the O-TBS derivative **4**.

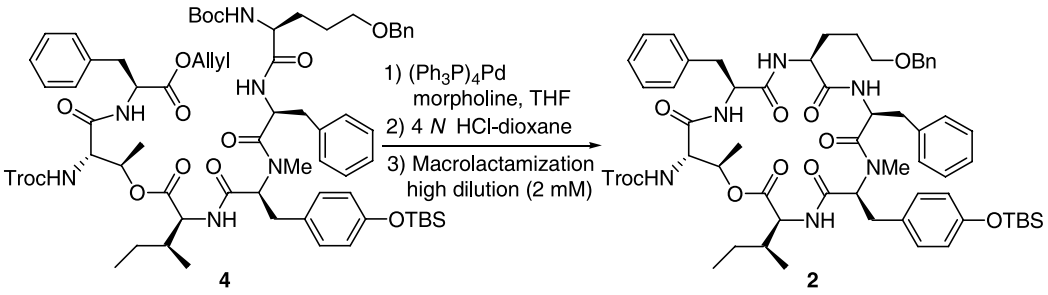
We are now at the stage of macrolactamization. After removal of the allyl group from the ester part of **4**, the Boc group was removed under acidic conditions. The resulting deprotected peptide underwent cyclization under high dilution conditions, as summarized in Table 1. So far, pentafluorophenyl diphenylphosphinate (FDPP, $(C_6H_5)_2P(O)OC_6F_5$)[10] in the presence of diisopropylethylamine in dichloromethane furnished best result, giving the macrolactam **2** in 84% yield. Unexpectedly, the yield of **2** using the corresponding pentafluorophenyl ester was low (23%).

Since there is no precedent on the construction of the Ahp ring in peptides, some model experiments were carried out, as shown in Scheme 6. First, the tripeptide **20** underwent a catalytic debenzylation to give the corresponding alcohol, which was subjected to oxidation with the Dess–Martin periodinane.[11] Neither the aldehyde **22** nor Ahp derivative was detected in the products. Next, the macrocyclic depsipeptide **2** underwent the catalytic removal of the benzyl function and then the product was analogously oxidized as described above. Again the aldehyde **23** was very labile and immediately transformed into a complex mixture. It was thought that the stability of the aldehyde might depend on the presence of the side chain which is attached at the *N*-terminal of threonine in the micropeptins and affects the conformation. Thus the construction of the

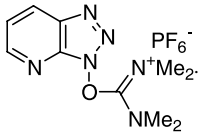


Scheme 5.

Table 1. Macrolactamization of the linear precursor **4**



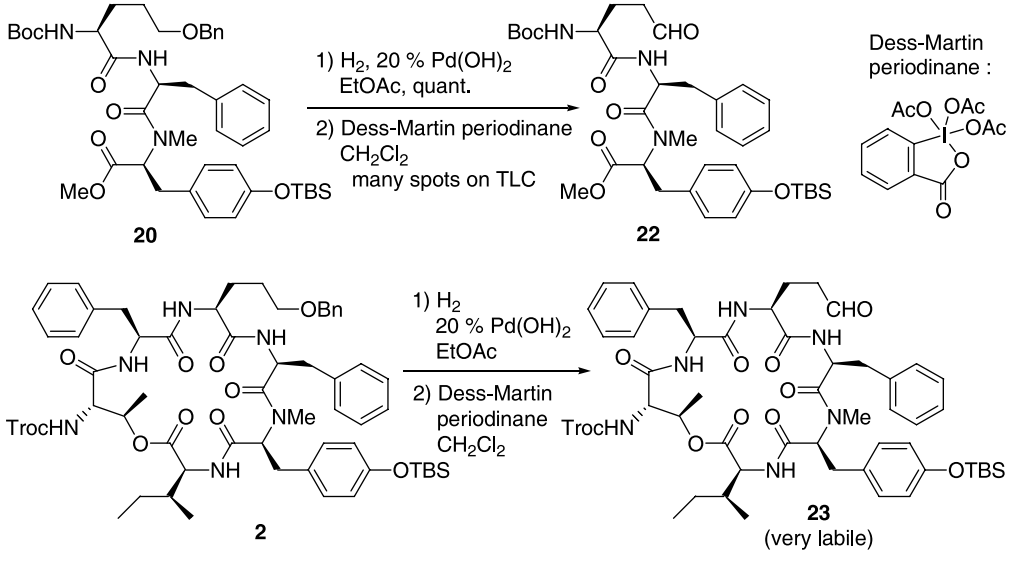
Entry	Coupling conditions	Time (h)	Yield (%)
1	DPPA, $NaHCO_3$, DMF, 4 °C	72	52
2	DEPC, $NaHCO_3$, DMF, 4 °C	96	44
3	HATU, Pr^i_2NEt, DMF, rt	14	38
4	HATU, Pr^i_2NEt, DMF, 4 °C	96	59
5	FDPP, Pr^i_2NEt, DMF, rt	14	57
6	FDPP, Pr^i_2NEt, CH_2Cl_2, rt	17	84

DPPA, $(C_6H_5O)_2P(O)N_3$; FDPP, $(C_6H_5)_2P(O)OC_6F_5$; HATU, 

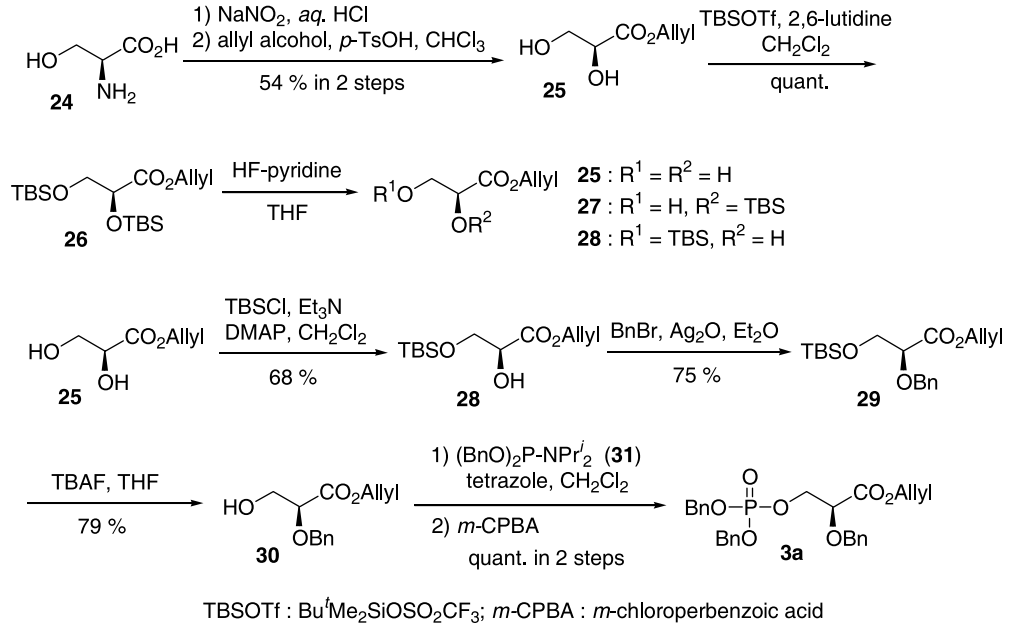
Ahp unit was postponed after the introduction of the side chain.

To prepare the side chain, (*S*)-glyceric acid allyl ester (**25**) obtained from (*S*)-serine (**24**)[12] was converted to the bis-TBS ester **26**, as shown in Scheme 7. The attempted selective deprotection of the bis-TBS ester **26** with HF-pyridine[13] afforded a mixture of the primary alcohol **27**, secondary alcohol **28**, and diol **25**. Thus the diol **25** was first converted to the monosilylated compound **28**,[14] which was transformed to the benzyl derivative **29**. Removal of the TBS group afforded the primary alcohol **30**. Phosphorylation of **30** failed using *o*-xylene *N*,*N*-diethylphosphoramidite[15] or the Mitsunobu reaction with dibenzyl phosphate.[16] The Mitsunobu reaction of the diol **25** with dibenzyl phosphate proceeded to give the mono phosphate in low yield. Finally, the primary alcohol **30** was treated with dibenzyl *N*,*N*-diisopropyl phosphoramidite (**31**) to give the phosphite, which was oxidized with *m*-chloroperbenzoic acid (*m*-CPBA)[17] to produce the desired side chain fragment **3a** in quantitative yield.

Since both the hydroxyl and phosphate residues of the side chain **3a** were protected by the benzyl group, the benzyl group of the homoserine part of the cyclic depsipeptide **2** should be removed before the introduction of the side chain. Although the hydrogenolytic removal of the benzyl group from **2** proceeded using 20% $Pd(OH)_2$, attempted removal of the Troc group using zinc in acetic acid failed. In contrast, removal of the Troc group from **2** rapidly proceeded to give the corresponding amino derivative using zinc in acetic acid, but hydrogenolysis did not give the

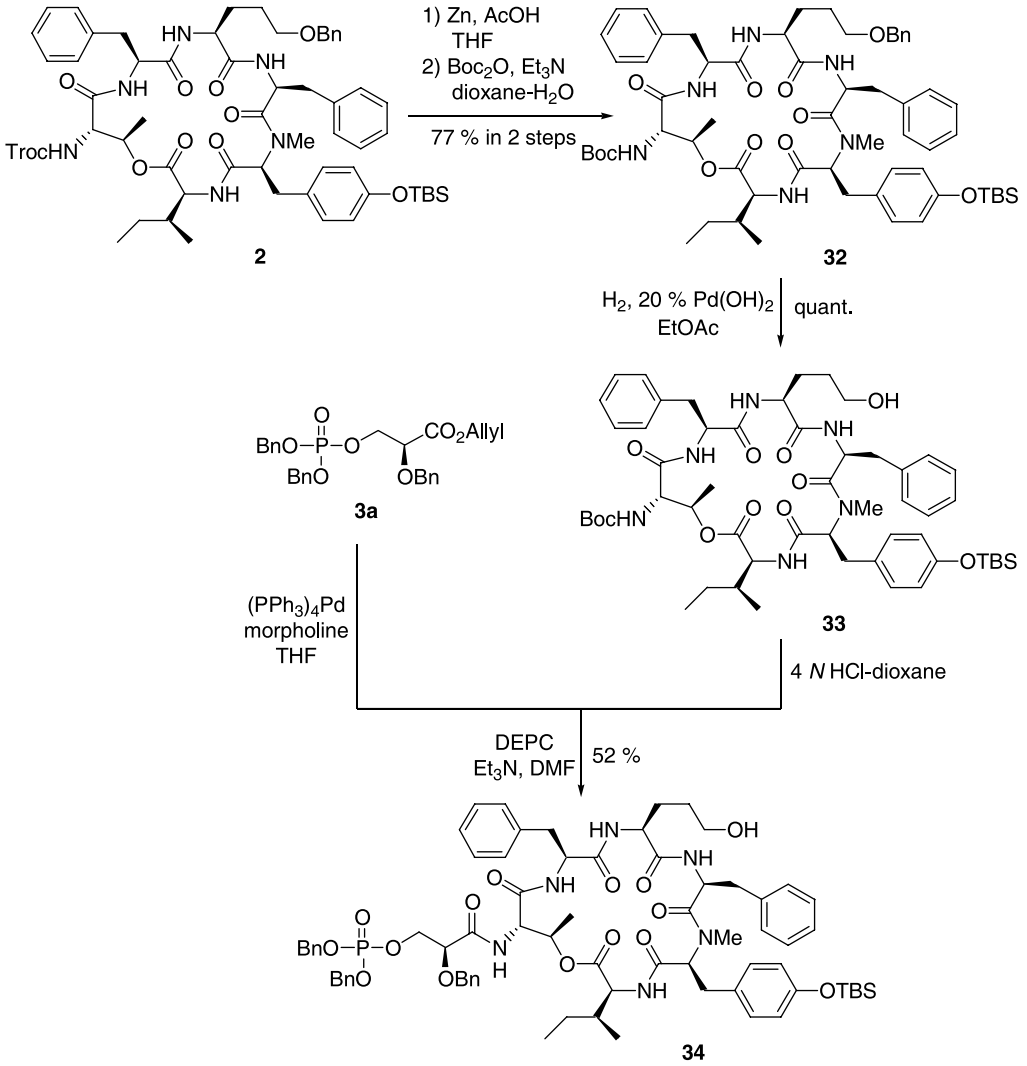


Scheme 6.

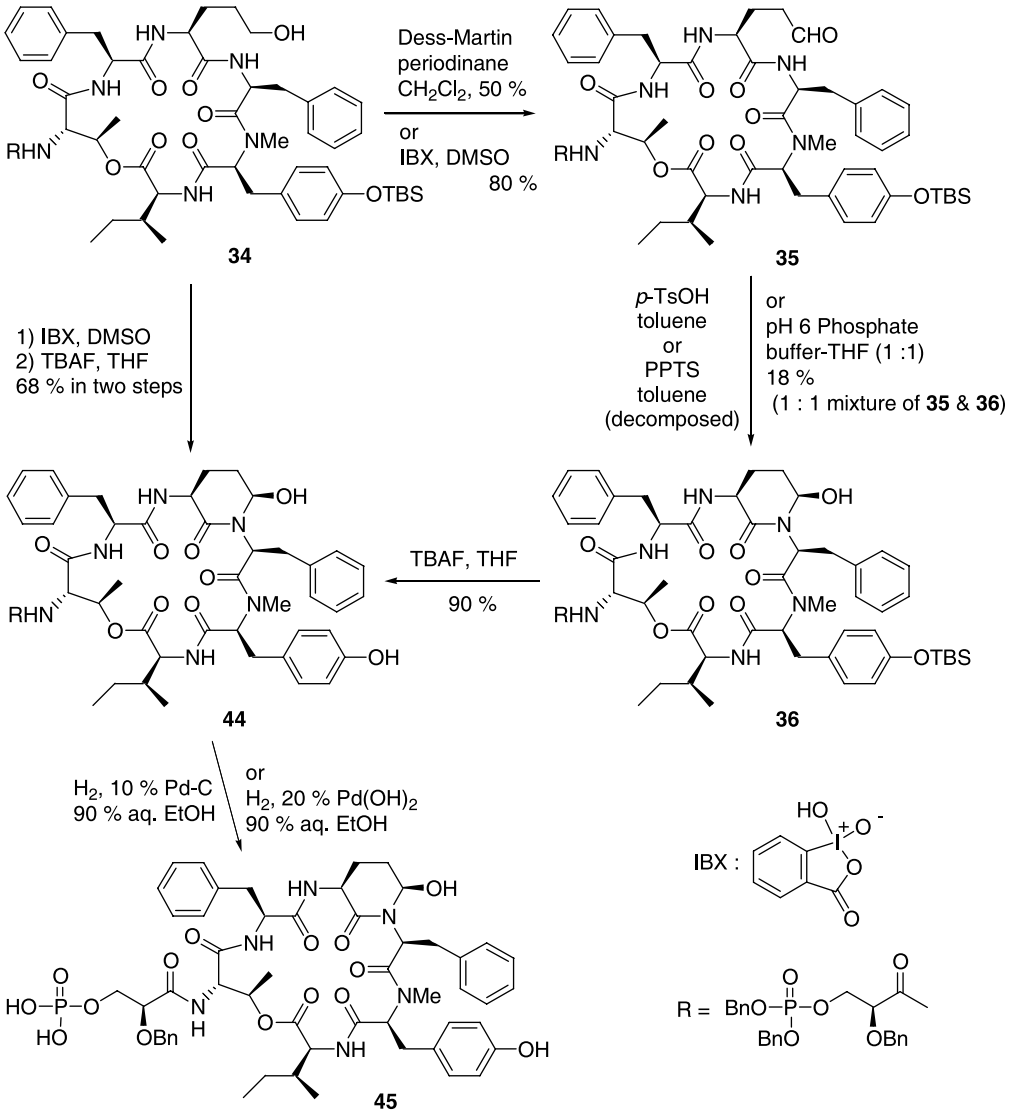


TBSOTf : $Bu^tMe_2SiOSO_2CF_3$; *m*-CPBA : *m*-chloroperbenzoic acid

Scheme 7.



Scheme 8.



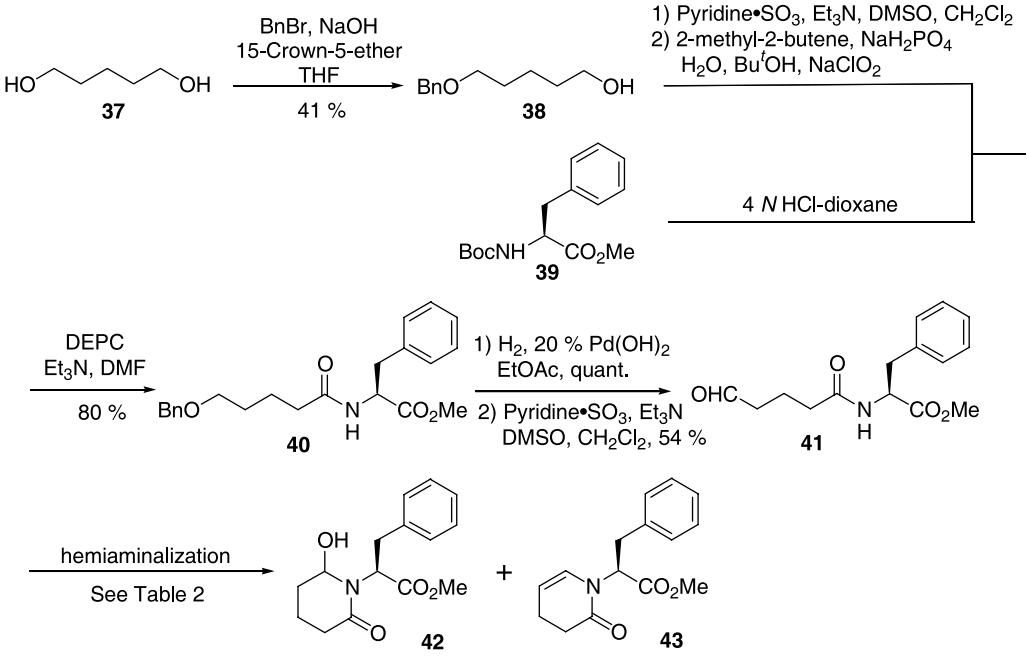
Scheme 9.

required *N*,*O*-deprotected compound under a variety of conditions. This might be due to the high polarity of the de-Troc product.

To overcome this difficulty, replacement of the Troc group with the less polar Boc group was undertaken. Thus, after removal of the Troc group of **2** with zinc in acetic acid as described above, the crude product was converted to the Boc derivative **32**, as shown in Scheme 8. The Boc derivative **32** smoothly underwent catalytic hydrogenolysis to give the Boc alcohol **33**, from which the Boc group was removed under acidic conditions. After removal of the allyl group from **3a**, the side chain fragment was coupled with the amine from **33** to give the Ahp precursor **34**.

The Ahp precursor **34** was oxidized with Dess–Martin periodinane to give the aldehyde **35** in 50% yield, as shown in Scheme 9. Alternatively, the use of 1-hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide (IBX), a milder oxidant,[18] afforded **35** in 80% yield, while oxidation with pyridinium dichromate resulted in the formation of a complex mixture. The aldehyde **35** was relatively stable compared to **23**. No equilibrium between the aldehyde **35** and the hemiaminal **36** was detected, and attempted cyclization of **35** using *p*-toluenesulfonic acid or pyridinium *p*-toluenesulfonate (PPTS) caused the gradual decomposition of the starting aldehyde **35**.

We now returned to the model experiments on the cyclization of the aldehyde to the hemiaminal. Thus, 1,5-pentanediol (**37**) was transformed into the monobenzyl ether **38**,[19] which was converted to the carboxylic acid and coupled with phenylalanine methyl ester from **39**, as shown in Scheme 10. The resulting amide **40** was converted to the aldehyde **41** through catalytic debenzylation followed by the Parikh–Doering oxidation. Cyclization of the aldehyde **41** to **42** was carried out under a variety of conditions, as summarized in Table 2. Many Lewis acids were not effective and resulted in no reaction or decomposition of the starting aldehyde **41** (entries 1–6 in Table 2). Alumina was also not useful, and cyclization followed by dehydration occurred using silica gel at higher temperature to give the dehydro derivative **43** (entries 7–9). Both *p*-toluenesulfonic acid and PPTS were ineffective (entries



Scheme 10.

Table 2. Hemiaminalization of the model compound **41**

Entry	Reagents	Solvents	Temperature (time)	Results
1	$ZnCl_2$	CH_2Cl_2	−78 °C (0.5 h) to rt (24 h)	No reaction
2	$MgBr_2$	Et_2O	0 °C (1 h) to rt (48 h)	Trace
3	$SnCl_4$	CH_2Cl_2	0 °C (1 h) to rt (1 h)	Complex mixture
4	$Sc(OTf)_3$	CH_2Cl_2	0 °C (2.5 h)	Trace
5	$TiCl_4$	CH_2Cl_2	0 °C (1 h)	Trace
6	$BF_3 \cdot Et_2O$	CH_2Cl_2	−78 °C (4 h) to rt (2 h)	Trace
7	Al_2O_3	CH_2Cl_2	0 °C (1 h) to rt (2 h)	No reaction
8	Al_2O_3	Toluene	80 °C (18 h)	Unknown product
9	SiO_2	Toluene	rt (1 h) to 80 °C (12 h)	Dehydro product **43** (62%)
10	*p*-TsOH	CH_2Cl_2	0 °C (1 h)	Trace (dehydro product **43**: major)
11	PPTS	CH_2Cl_2	0 °C (1 h) to rt (2.5 h)	Trace
12	0.1 M pH 6 phosphate buffer	THF	0 °C (0.5 h) to rt (20 h)	6-Hydroxy-2-piperidone **42** (66%)

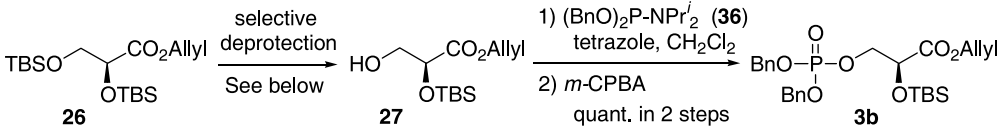
10–11). We thought at this stage that the addition of water to the solvent system might prevent the dehydration. In fact, treatment of the aldehyde **41** in tetrahydrofuran containing 0.1 M phosphate buffer (pH 6) afforded the desired piperidone **42** as a single isomer in 66% yield (entry 12). In the synthetic route to micropeptin T-20, removal of both the TBS and benzyl groups will be necessary after the construction of the Ahp unit. Thus, the stability of the model compound **42** toward hydrogenolysis and TBAF was examined. The hemiaminal **42** was stable to hydrogenolysis but treatment with TBAF caused dehydration to give the dehydro derivative **43** in only 30 min, which suggested the requirement of the careful treatment with TBAF.

The results of the above experiments indicated that both aldehyde and hemiaminal structures were labile to acidic conditions and the strong oxidative conditions were not necessary. After oxidation of the alcohol **34** with IBX, treatment of the aldehyde **35** with a pH 6 phosphate buffer afforded a 1:1 mixture of the starting aldehyde **35** and the Ahp derivative **36** in low yield, as shown in Scheme 9. Since the mixture was unseparable and labile, desilylation of the mixture was carried out with TBAF to give the Ahp derivative **44** as a single isomer, and the ^1H NMR spectrum of its Ahp part was completely identical with that of natural micropeptin T-20 (**1**), proving its stereochemistry as depicted. The conformational effect of the cyclic depsipeptide will govern the stereochemistry. The above experiments revealed that treatment of the aldehyde **35** with TBAF would give rise to the Ahp ring formation accompanied by desilylation. In fact, after oxidation of **34** with IBX, treatment with TBAF afforded the Ahp derivative **44** as a single isomer in good yield.

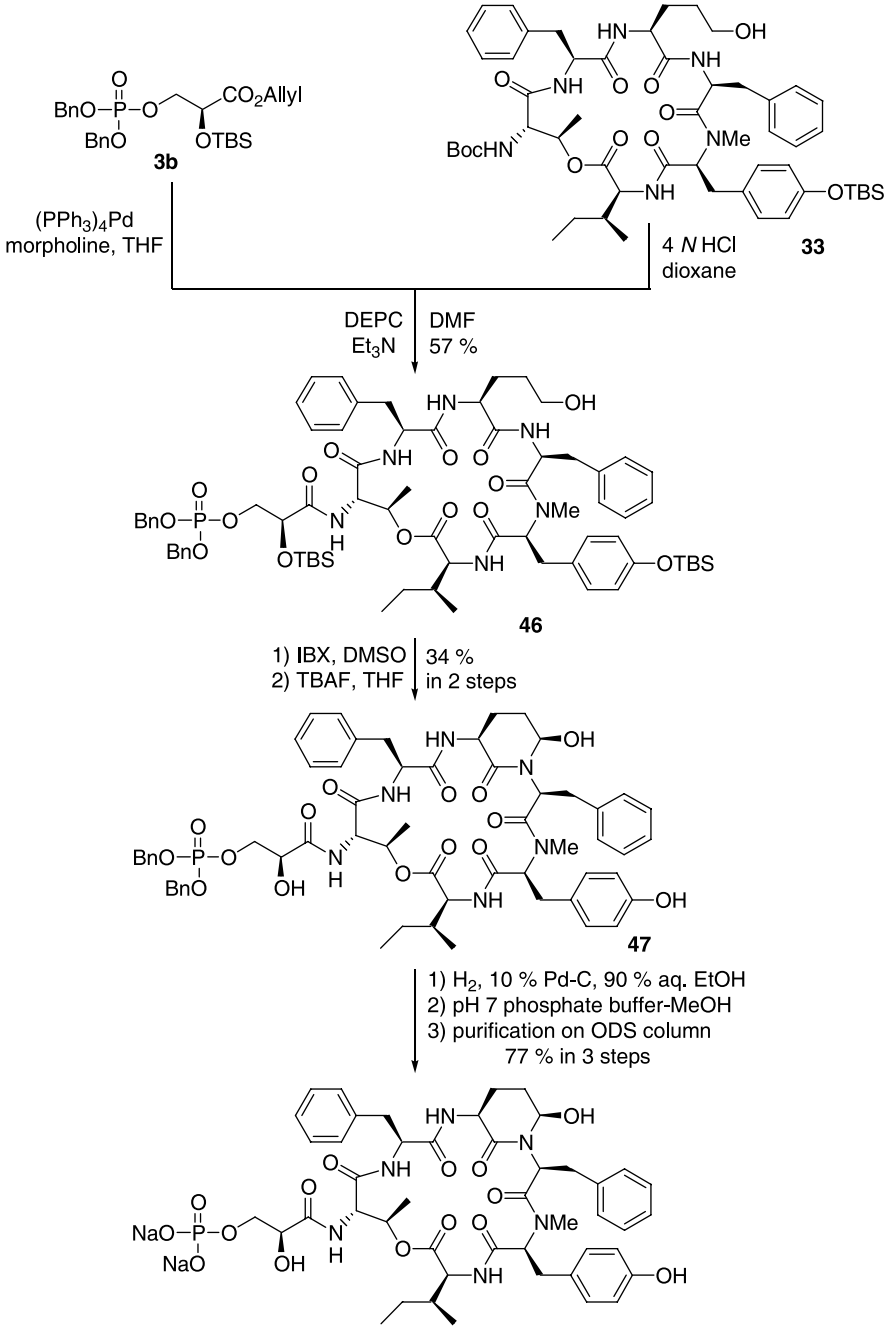
The stability of the Ahp structure will be due to cooperation of the resonance effect between the nitrogen and carbonyl group in the Ahp ring in addition to the anomeric and substituent effects. Since the dehydration in the Ahp nucleus will occur when the C5-axial hydrogen and the hydroxyl group are situated antiperiplanar, the freedom of the 6-membered chair conformation will affect the stability. This will be the reason for the different reactivities between the model compound **42** and the real substrate **44**.

Debenzylation at the side chain of **44** was attempted under a hydrogen atmosphere by using 10% Pd–C or 20% $Pd(OH)_2$

Table 3. Selective deprotection of **26**



Entry	Conditions	Temperature (time)	Results	Reference
1	3.5 M HF-pyridine, THF	rt (5 h)	Complex mixture	13
2	AcOH-H_2O-THF (13:7:3)	rt (9 h) −40 °C (4.5 h) to rt (11 h)	28%	21a
3	CF_3CO_2H-H_2O-THF (9:1:40)	0 °C (0.5 h) to rt (6 h)	54%	21a
4	Oxone, MeOH-H_2O (1:1) C	rt (6 h)	Trace	21b
5	$CeCl_3 \cdot 7H_2O$, NaI, MeCN	rt (15 h) to reflux (3 h)	62%	21c



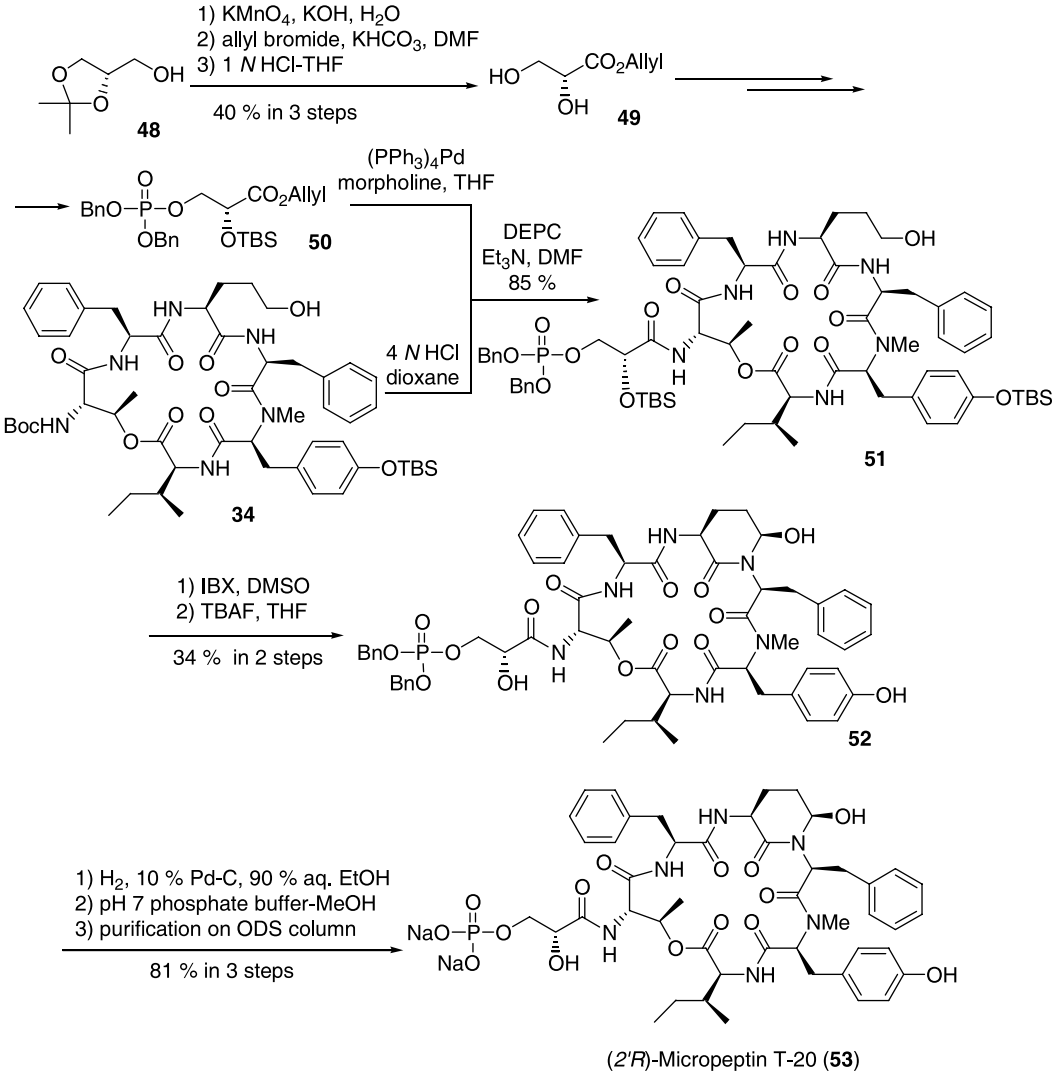
Scheme 11.

in 90% aqueous ethanol.[17,20] The product was revealed to be the monobenzylated derivative **45** (Scheme 9). Although both of the benzyl functions at the phosphate part were removed, the benzyl group at the ether function could not be removed at all. The addition of acetic acid with a longer reaction time resulted in cleavage of the side chain.

We now needed to renew the protective group at the glyceric phosphate side chain, therefore, the protection with the TBS group was reinvestigated, the results of which are shown in Table 3. So far, a combination of cerium chloride and sodium iodide[21c] toward **26** gave the best result that furnished the primary alcohol **27** in 62% yield. The alcohol **27** was analogously converted to the phosphate **3b** as the conversion of **30** to **3a** (See Scheme 7).

After introduction of the side chain **3b** to the cyclic depsipeptide **33**, oxidation of the alcohol **46** with IBX followed by treatment with TBAF afforded the Ahp peptide **47** as a single isomer. Catalytic removal of the benzyl function was easily accomplished over 10% Pd–C in 90% aqueous ethanol, as shown in Scheme 11. From the NMR spectral data, our synthetic micropeptin T-20 is apparently not the same compound reported by Kaya and co-workers.[1b] Comparison of the NMR spectra leads us to conclude that our synthetic compound has the structure **1** and it will be configurationally isomeric to natural micropeptin T-20. A complete similarity was observed in the spectral data of their cyclic depsipeptide part. However, a difference was seen at the methine proton at C2 of the glyceric acid part and methylene protons at C3; the ^{1}H signal of the synthetic material was 0.2 ppm higher than that of the natural one and the difference of 3 ppm was observed in their ^{13}C NMR spectra. This obvious spectral difference at the side chain was also observed after treatment with a pH 7 phosphate buffer followed by purification on an ODS column. Furthermore, pH and concentration proved not to influence the chemical shift of the NMR spectra. This structural discrepancy between the synthetic and natural micropeptin T-20 led us to synthesize another micropeptin T-20 having the (*R*)-glyceric acid phosphate residue.

The alcohol **48** was submitted to oxidation, allylation, and then acidic treatment to give the (*R*)-glyceric acid allyl ester (**49**), which was converted to the (*R*)-glyceric acid phosphate **50**, as shown in Scheme 12. After removal of



Scheme 12.

the allyl group, coupling with the amine from the cyclic depsipeptide **34** afforded **51** with the (*R*)-side chain. IBX oxidation and treatment with TBAF afforded **52**, which was converted to micropeptin T-20 (**53**) having the (*R*)-side chain as described above. However, this compound **53** was again not identical with natural micropeptin T-20.

Based on the preceding analysis and synthesis, we conclude that our synthesis proceeded as intended to correctly provide molecules possessing structures **1** and **53**. Although the method for the synthesis of the Ahp-containing cyclic depsipeptide was established,[22] this work strongly indicates that the structure of micropeptin T-20 should be revised. Unfortunately, however, since no natural micropeptin T-20 is presently available, structural re-assignment will require re-isolation.

4. Experimental

4.1. General information

Infrared spectra were recorded on a SHIMADZU FT IR-8100 spectrometer. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter with a sodium lump (λ=589 nm, D line) and are recorded as follows: $[\alpha]_D^T$ (C g/100 ml, solvent). ^{1}H NMR spectra were recorded on a JEOL EX-270 or α-500 spectrometer in deuterio solvent using tetramethylsilane (TMS) or $CHCl_3$ (δ 7.26 ppm) or CH_3OH (δ 3.30 ppm) as an internal standard. Data are described as follows: chemical shift, integration, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, br=broad, m=multiplet), coupling constants (Hz), and assignment. ^{13}C NMR spectra were recorded on a JEOL EX-270 (67.8 MHz) spectrometer with complete proton decoupling. Chemical shifts are described in ppm with the solvent as the internal standard ($CDCl_3$: δ 77.0 ppm, CD_3OD: δ 49.0 ppm). Analytical thin layer chromatography was performed on Merck Art. 5715, Kiselgel 60 F_{245}/0.25 mm thickness plates. Visualization was accomplished with UV light, phosphomolybdic acid, or ninhydrin solution followed by heating. Mass spectra were obtained on a JEOL JMS-SX 102A (EI) and JMS-AX 505HA (FAB) spectrometer. Column chromatography was performed with silica gel BW-820 MH or BW-200 MH (Fuji Silysia Co.). Solvents for extraction and chromatography were reagent grade. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl. Diethyl ether (Et_2O) was distilled from lithium aluminum hydride ($LiAlH_4$). Dichloromethane (CH_2Cl_2) was distilled from calcium hydride. All other commercially available reagents were used as received.

4.1.1. Boc-(*S*)-Phe-OAllyl (8). To a solution of Boc-(*S*)-Phe-OH (**7**) (3.0 g, 11.3 mmol) in DMF (30 ml) was added $KHCO_3$ (2.3 g, 23.0 mmol) and allyl bromide (1.4 ml, 16.2 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 14 h. The reaction was quenched with 1 M aqueous $KHSO_4$ and the mixture was extracted with EtOAc. The extracts were successively washed with H_2O, saturated aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and concentrated in vacuo to give **8** (3.6 g, quant.) as a colorless oil: $[\alpha]_D^{23}=+27.9$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3375, 2978, 1743, 1716, 1498, 1367, 1167; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.41 (9H, s, *tBu*), 3.02–3.14 (2H, m, Phe-3-*H*), 4.58–4.61 (3H, m, Phe-2-*H*, CO_2CH_2), 4.98 (1H, br, N*H*), 5.22–5.33 (2H, m, CO_2CH_2-$CHCH_2$), 5.78–5.93 (1H, m, $CO_2CH_2CHCH_2$), 7.12–7.23 (5H, m, C_6H_5); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 28.1, 38.2, 54.3, 65.7, 79.7, 118.7, 126.8, 128.0, 129.0, 131.4, 135.9, 154.9, 171.4; Anal. calcd for $C_{17}H_{23}NO_4$: C, 66.86, H, 7.59, N, 4.59. Found: C, 66.72, H, 7.63, N, 4.65.

4.1.2. Troc-(*S*)-Thr-OH (10). To a solution of (*S*)-Thr-OH (**9**) (15.0 g, 0.12 mol) in THF (120 ml) and 2.5 N aqueous NaOH (120 ml) was added 2,2,2-trichloroethoxycarbonyl chloride (10 ml×3, total 0.19 mol) three times every 1 h at 0 °C. The mixture was stirred at 0 °C for 6 h, and then concentrated. The residue was acidified with 1 M aqueous $KHSO_4$ and extracted with EtOAc (×3). The extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:1 to 1:3 then EtOAc only) to give **10** (23.8 g, 65%) as a colorless amorphous solid: $[\alpha]_D^{23}=+0.67$ (*c* 1.0, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3424, 1717, 1525, 1408, 1113; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.23–1.35 (3H, m, Thr-4-*H*), 4.37–4.42 (1H, m, Thr-3-*H*), 4.48–4.51 (1H, m, Thr-2-*H*), 4.69 (1H, d, *J*=12.2 Hz, OCH_2CCl_3), 4.76 (1H, d, *J*=11.8 Hz, OCH_2CCl_3), 6.22 (1H, br, N*H*); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 19.2, 59.1, 65.7, 68.0, 74.6, 95.1, 155.4, 173.9; Anal. calcd for $C_7H_{10}C_3NO_5\cdot 1/4EtOAc\cdot 1/2H_2O$: C, 29.34, H, 3.91, N, 4.39. Found: C, 29.13, H, 3.73, N, 4.70.

4.1.3. Troc-(*S*)-Thr-(*S*)-Phe-OAllyl (11). Boc-(*S*)-Phe-OAllyl (**8**) (1.00 g, 3.29 mmol) was treated with 4 N HCl–EtOAc (5 ml) at 0 °C and the mixture was stirred for 1.5 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless solid.

To a solution of the above crude amine salt and Troc-(*S*)-Thr-OH (**10**) (880 mg, 2.99 mmol) in DMF (6 ml) was added DEPC (0.72 ml, 4.74 mmol) and Et_3N (1.04 ml, 7.47 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 19 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous $KHSO_4$, H_2O, saturated aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=2:1 to 1:1) to give **11** (1.37 g, quant.) as a colorless amorphous solid: $[\alpha]_D^{24}=-8.8$ (*c* 1.0, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3324, 1740, 1662, 1538; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.17 (3H, d, *J*=6.2 Hz, Thr-4-*H*), 2.79 (1H, br, exchangeable with D_2O, O*H*), 3.05 (1H, dd, *J*=7.0, 13.8 Hz, Phe-3-*H*), 3.20 (1H, dd, *J*=5.9, 14.0 Hz, Phe-3-*H*), 4.08–4.15 (1H, m, Thr-3-*H*), 4.25–4.35 (1H, m, Thr-2-*H*), 4.60–4.80 (4H, m, CO_2CH_2CH, OCH_2CCl_3), 4.84–4.92 (1H, m, Phe-2-*H*), 5.25–5.36 (2H, m, $CO_2CH_2CHCH_2$), 5.81–5.95 (1H, br, $CO_2CH_2CHCH_2$), 7.10–7.33 (5H, m, C_6H_5); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 17.9, 37.6, 53.3, 58.7, 66.2, 66.8, 74.7, 95.2, 119.2, 127.2, 128.5, 129.1, 131.2, 135.5, 154.9, 169.9, 170.0; Anal. calcd for $C_{19}H_{24}Cl_3N_2O_6$: C, 47.37, H, 4.81, N, 5.81. Found: C, 47.54, H, 4.84, N, 5.51.

4.1.4. Troc-(*S*)-Thr[Boc-(*S*)-Ile]-(*S*)-Phe-OAllyl (5). To a solution of dipeptide **11** (50 mg, 0.10 mmol) and

Boc-(*S*)-Ile-OH (35 mg, 0.15 mmol) in THF (0.5 ml) was added DMAP (1.2 mg, 0.01 mmol) and EDCI·HCl (28 mg, 0.15 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 12 h. After dilution with EtOAc, the whole mixture was washed with 1 M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-200 MH, hexane–EtOAc=4:1) to give **5** (63 mg, 80%) as a colorless amorphous powder: $[\alpha]_D^{24}=+18.7$ (*c* 1.0, CHC_3); IR ν_{max} ($CHCl_3$) cm^{-1} 3437, 1747, 1716, 1697, 1684, 1506; ^1H NMR (270 MHz, $CDCl_3$) δ 0.88–0.93 (6H, m, Ile-5,6-*H*), 1.18–1.28 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.49 (9H, s, *tBu*), 1.67–1.80 (1H, m, Ile-3-*H*), 3.06–3.23 (2H, m, Phe-3-*H*), 4.02–4.10 (1H, m, Thr-2-*H*), 4.32–4.37 (1H, m, Ile-2-*H*), 4.59 (2H, d, $J=5.6$ Hz, CO_2CH_2CH), 4.73 (2H, s, OCH_2Cl_3), 4.84–4.92 (1H, m, Phe-2-*H*), 4.95–5.05 (1H, br, N*H*), 5.20–5.38 (3H, m, Thr-3-*H*, $CO_2CH_2CHCH_2$), 5.78–5.89 (1H, m, $CO_2CH_2CHCH_2$), 5.90–6.00 (1H, br, N*H*), 7.15–7.30 (6H, m, C_6H_5, N*H*); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 11.2, 14.9, 15.2, 25.2, 28.3, 36.7, 37.7, 53.7, 56.7, 58.4, 65.9, 69.7, 74.6, 80.2, 95.2, 118.9, 127.0, 128.5, 129.1, 131.3, 135.9, 153.9, 156.0, 167.3, 170.7, 171.2; Anal. calcd for $C_{30}H_{42}Cl_3N_3O_9$: C, 51.84, H, 6.09, N, 6.05. Found: C, 51.55, H, 6.07, N, 5.77.

4.1.5. Boc-(*S*)-Tyr(TBS)-OH. To a solution of Boc-(*S*)-Tyr-OH (**12**) (3.0 g, 10.6 mmol) in DMF (20 ml) was added TBSCl (4.8 g, 31.9 mmol) and imidazole (4.3 g, 63.6 mmol) at 0 °C. The solution was stirred at 0 °C for 1 h, then at room temperature for 12 h. After dilution with EtOAc, the whole mixture was washed with 1 M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. K_2CO_3 (2.1 g, 15 mmol) in H_2O (30 ml) was added to a solution of the above crude disilylate in THF (60 ml) and MeOH (30 ml). The mixture was stirred at room temperature for 48 h. After dilution with Et_2O, the whole mixture was successively washed with 1 M aqueous $KHSO_4$, H_2O, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=3:1) to give Boc-(*S*)-Tyr(TBS)-OH (3.6 g, 86%) as a colorless amorphous solid: $[\alpha]_D^{20}=+30.2$ (*c* 1.1, $CHCl_3$) ($[\alpha]_D^{25}=-30.4$ (*c* 1.5, $CHCl_3$) for (*R*)-isomer[6]); IR ν_{max}^{neat} cm^{-1} 3367, 1716, 1512, 1257, 1167; ^1H NMR (270 MHz, $CDCl_3$) δ 0.18 (6H, s, Si*Me*$_2$), 0.97 (9H, s, Si-*tBu*), 1.41 (9H, s, O-*tBu*), 3.01–3.14 (2H, m, Tyr-3-*H*), 4.50–4.60 (1H, br, Tyr-2-*H*), 4.85–4.95 (1H, br, N*H*), 6.77 (2H, d, $J=8.6$ Hz, Tyr-6,8-*H*), 7.04 (2H, d, $J=8.2$ Hz, Tyr-5,9-*H*); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −4.5, 18.1, 25.5, 28.2, 37.0, 54.2, 80.0, 120.0, 128.6, 130.3, 154.6, 155.3, 175.4; Anal. calcd for $C_{20}H_{33}NO_5Si$: C, 60.73; H, 8.41; N, 3.54. Found: C, 60.58; H 8.31; N; 3.52.

4.1.6. Boc-(*S*)-*N*-Me-Tyr(TBS)-OH (13). To a solution of Boc-(*S*)-Tyr(TBS)-OH (700 mg, 1.77 mmol) in THF (5.5 ml) was carefully added 1.54 N *t*-BuLi in pentane (2.5 ml, 3.89 mmol) at −78 °C. The solution was stirred at −78 °C for 30 min, then MeI (3.3 ml, 53.2 mmol) was added at 0 °C. After 1 h, the reaction mixture was allowed to warm to room temperature and stirred for 18 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and the mixture was extracted with EtOAc. The extracts were washed with 1 M aqueous $KHSO_4$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=5:1 to 3:1) to give **13** (607 mg, 84%) as a pale yellow amorphous solid: $[\alpha]_D^{23}=-55.1$ (*c* 1.0, $CHCl_3$) ($[\alpha]_D^{25}=+57.7$ (*c* 1.1, $CHCl_3$) for (*R*)-isomer[6]); IR ν_{max}^{neat} cm^{-1} 3200, 1705, 1512, 1257; ^1H NMR (270 MHz, $CDCl_3$) δ 0.17 (6H, s, Si*Me*$_2$), 0.97 (9H, s, Si-*tBu*), 1.36, 1.41 (9H, s×2, O-*tBu*), 2.65, 2.73 (3H, s×2, N*Me*), 2.98–3.25 (2H, m, Tyr-3-*H*), 4.41–4.72 (1H, m, Tyr-2-*H*), 6.76 (2H, d, $J=8.6$ Hz, Tyr-6,8-*H*), 7.05 (2H, d, $J=7.6$ Hz, Tyr-5,9-*H*); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −4.5, 18.0, 25.5, 28.0, 28.1, 32.4, 32.6, 33.8, 34.3, 60.0, 60.6, 80.3, 80.6, 120.0, 129.8, 154.2, 155.1, 156.1, 175.6; Anal. calcd for $C_{21}H_{35}NO_5Si$: C, 61.58; H, 8.61; N, 3.42. Found: C, 61.67; H 8.68; N; 3.21.

4.1.7. Boc-(*S*)-*N*-Me-Tyr(TBS)-OMe (14). To a solution of Boc-(*S*)-*N*-Me-Tyr(TBS)-OH (**13**) (1.22 g, 2.98 mmol) in DMF (10 ml) was added $KHCO_3$ (0.60 g, 5.96 mmol) and MeI (0.26 ml, 4.17 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 12 h. The reaction was quenched with 1 M aqueous $KHSO_4$ and the mixture was extracted with EtOAc. The extracts were successively washed with H_2O, saturated aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=10:1 to 5:1) to give **14** (1.19 g, 94%) as a colorless oil: $[\alpha]_D^{23}=-46.5$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 1747, 1699, 1512, 1257, 1170; ^1H NMR (270 MHz, $CDCl_3$) δ 0.16 (6H, s, Si*Me*$_2$), 0.96 (9H, s, Si-*tBu*), 1.36, 1.38 (9H, m, O-*tBu*), 2.67 (3H, s, N*Me*), 2.89–3.30 (2H, m, Tyr-3-*H*), 3.73 (3H, s, CO_2Me), 4.40–4.44, 4.83–4.87 (1H, m, Tyr-2-*H*), 6.75 (2H, d, $J=7.9$ Hz, Tyr-6,8-*H*), 6.95–7.06 (2H, m, Tyr-5,9-*H*); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −4.5, 18.0, 25.5, 28.1, 31.8, 32.7, 34.1, 34.6, 51.9, 59.5, 61.8, 79.7, 80.1, 120.0, 129.8, 154.3, 154.8, 171.5, 171.8; Anal. calcd for $C_{22}H_{37}NO_5Si$: C, 62.38; H, 8.80; N, 3.31. Found: C, 62.02; H, 8.80; N, 3.33.

4.1.8. Boc-(*S*)-Phe-(*S*)-*N*-Me-Tyr(TBS)-OMe (15). Boc-(*S*)-*N*-Me-Tyr(TBS)-OMe (**14**) (300 mg, 0.73 mmol) was treated with 4 N HCl–EtOAc (2.5 ml) at 0 °C and the mixture was stirred for 1 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless solid.

To a solution of the above crude amine salt and Boc-(*S*)-Phe-OH (**7**)(230 mg, 0.87 mmol) in CH_2Cl_2 (3 ml) was added Et_3N (0.25 ml, 1.82 mmol) and BopCl (220 mg, 0.87 mmol) at 0 °C. The reaction mixture was stirred at 4 °C for 15 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-200 MH, hexane–EtOAc=5:1) to give **15** (300 mg, 72%) as a colorless amorphous solid: $[\alpha]_D^{24}=-47.4$ (*c* 1.0, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3324, 1743, 1713, 1645, 1510, 1255, 1170; ^1H NMR (270 MHz, $CDCl_3$) δ 0.15 (6H, s, Si*Me*$_2$), 0.95 (9H, s, Si-*tBu*), 1.38 (9H, s, O-*tBu*), 2.73 (3H, s, N*Me*), 2.80–2.90 (2H, m, Phe-3-*H*), 2.90–3.05 (1H, m, Tyr-3-*H*), 3.15–3.25 (1H,

m, Tyr-3-*H*), 3.68 (3H, s, CO_2Me), 4.68–4.78 (1H, m, Tyr-2-*H*), 5.08–5.15 (2H, m, Phe-2-*H*), 6.68–6.80 (2H, m, Tyr-6,8-*H*), 6.93–6.99 (2H, m, Tyr-5,9-*H*) 7.15–7.30 (5H, m, C_6H_5); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −3.8, 18.7, 26.2, 28.9, 33.4, 34.4, 39.8, 52.2, 52.7, 59.6, 80.1, 120.7, 127.8, 128.9, 130.0, 130.2, 130.4, 137.0, 155.0, 155.5, 171.4, 172.6; Anal. calcd for $C_{31}H_{46}N_2O_6Si\cdot 1/2H_2O$: C, 64.22; H, 8.17; N, 4.83. Found: C, 64.57; H, 7.99; N, 5.13.

4.1.9. Boc-(*S*)-Phe-(*S*)-*N*-Me-Tyr-OMe (16). To a solution of dipeptide **15** (1.0 g, 1.75 mmol) in THF (1 ml) was added 1 N TBAF in THF (5.3 ml, 5.3 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h. After dilution with EtOAc, the whole mixture was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=2:1) to give **16** (797 mg, quant.) as a colorless amorphous powder: $[\alpha]_D^{24}=-19.6$ (*c* 1.1, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3324, 1741, 1698, 1646, 1516, 1250, 1169; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.37 (9H, s, *tBu*), 1.70–1.90 (1H, br, O*H*), 2.19–2.26 (1H, m, Tyr-3-*H*), 2.75 (3H, s, N*Me*), 2.79–2.88 (2H, m, Tyr-3-*H*, Phe-3-*H*), 2.91–3.04 (1H, m, Phe-3-*H*), 3.67 (3H, s, CO_2Me), 4.73–4.81 (1H, m, Tyr-2-*H*), 5.13–5.24 (2H, m, Phe-2-*H*, N*H*), 6.66–6.72 (2H, m, Tyr-6,8-*H*), 6.82–7.00 (2H, m, Tyr-5,9-*H*), 7.17–7.28 (5H, m, C_6H_5); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 15.1, 28.2, 31.5, 32.5, 33.7, 39.0, 51.5, 52.2, 59.0, 65.8, 79.9, 115.4, 126.7, 128.3, 129.4, 129.8, 136.1, 155.2, 170.8, 172.5.

4.1.10. Benzyl (*S*)-2-*tert*-butoxycarbonylamino-5-hydroxypentanoate (18). To a solution of Boc-(*S*)-Glu-OBn (**17**) (4.0 g, 11.8 mmol) in THF (60 ml) was added Et_3N (5.0 ml, 35.5 mmol) and ethyl chloroformate (3.4 ml, 35.5 mmol) at −10 °C under an argon atmosphere. The mixture was stirred at −10 °C for 1 h, and $NaBH_4$ (1.78 g, 47.2 mmol) in H_2O (60 ml) was added dropwise at −10 °C. The mixture was stirred at −10 °C for 1 h, then at room temperature for 2 h. The reaction was quenched with 1 M aqueous $KHSO_4$, and the mixture was extracted with EtOAc (×3). The extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:1) to give **18** (2.86 g, 76%) as a colorless oil: $[\alpha]_D^{24}=-3.9$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3367, 1738, 1713, 1517, 1165; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.43 (9H, s, *tBu*), 1.32–1.93 (5H, m, Phs-3,4-*H*, O*H*), 3.63 (2H, t, *J*=6.1 Hz, Phs-5-*H*), 4.32–4.45 (1H, m, Phs-2-*H*), 5.11–5.23 (3H, m, C$H_2C_6H_5$, N*H*), 7.35 (5H, s, C_6H_5); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 28.1, 29.1, 53.1, 61.7, 65.7, 66.9, 79.8, 128.3, 135.2, 155.4, 172.5; Anal. calcd for $C_{17}H_{25}NO_5$: C, 63.14; H, 7.79; N, 4.33. Found: C, 62.96; H, 7.63; N, 4.49.

4.1.11. Benzyl (*S*)-2-*tert*-butoxycarbonylamino-5-benzyloxypentanoate (19). To a solution of the pentahomoserine derivative **18** (1.4 g, 4.31 mmol) in Et_2O (14 ml) was added Ag_2O (4.0 g, 17.2 mmol) and BnBr (3.6 ml, 30.1 mmol) at room temperature under an argon atmosphere. The mixture was stirred for 14 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-200 MH, hexane–EtOAc=3:1) to give **19** (1.46 g, 82%) as a colorless oil: $[\alpha]_D^{22}=-4.2$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3367, 1743, 1714, 1498, 1161; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.42 (9H, s, *tBu*), 1.58–2.05 (4H, m, Phs-3,4-*H*), 3.45 (2H, t, *J*=6.0 Hz, Phs-5-*H*), 4.30–4.40 (1H, m, Phs-2-*H*), 4.46 (2H, s, $CH_2OCH_2C_6H_5$), 5.10–5.22 (3H, m, $CO_2CH_2C_6H_5$, N*H*), 7.29–7.34 (10H, m, $C_6H_5\times 2$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 25.4, 28.2, 29.3, 53.2, 66.8, 69.3, 72.7, 79.6, 127.4, 128.0, 128.2, 128.4, 135.3, 138.2, 155.3, 172.5; Anal. calcd for $C_{24}H_{31}NO_5$: C, 69.71; H, 7.56; N, 3.39. Found: C, 69.63; H, 7.68; N, 3.19.

4.1.12. Boc-(*S*)-Phs(Bn)-(*S*)-Phe-(*S*)-*N*-Me-Tyr-OMe (6) (Phs=pentahomoserine). Dipeptide **16** (700 mg, 1.22 mmol) was treated with 4 N HCl–dioxane (5 ml) at 0 °C and the mixture was stirred for 30 min. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless solid.

To a solution of the pentahomoserine derivative **19** (560 mg, 1.35 mmol) in THF (5 ml) was added 0.5 N aqueous LiOH (5 ml) at 0 °C. The mixture was stirred at room temperature for 12 h, then Et_2O was added. The separated aqueous layer was acidified with 1 M aqueous $KHSO_4$, salted out, and extracted with EtOAc (×2). The extracts were dried over Na_2SO_4, and concentrated in vacuo to give a crude carboxylic acid as a colorless oil which was used for the next step without further purification.

To a solution of the above crude amine salt and carboxylic acid in DMF (5 ml) was added DEPC (0.22 ml, 1.42 mmol) and Et_3N (0.42 ml, 3.05 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 18 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous $KHSO_4$, H_2O, saturated aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:1) to give **6** (477 mg, 60%) as a colorless amorphous powder: $[\alpha]_D^{24}=-2.6$ (*c* 1.0, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3303, 1741, 1693, 1518, 1228, 1170; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.49 (9H, m, *tBu*), 1.50–1.85 (5H, m, Phe-3,4-*H*, O*H*), 2.57 (3H, s, N*Me*), 2.68–2.95 (3H, m, Tyr-3-*H*, Phe-3-*H*), 3.20–3.27 (1H, m, Phe-3-*H*), 3.46–3.60 (2H, m, Phs-5-*H*), 3.70 (3H, s, CO_2Me), 3.90–4.05 (1H, m, Phs-2-*H*), 4.57 (2H, s, O$CH_2C_6H_5$), 4.80–4.89 (1H, m, Tyr-2-*H*), 5.00–5.10 (1H, m, Phe-2-*H*), 5.50–5.65 (1H, br, N*H*), 6.35–6.45 (1H, br, N*H*), 6.57 (2H, d, *J*=8.2 Hz, Tyr-6,8-*H*), 6.88 (2H, d, *J*=8.2 Hz, Tyr-5,9-*H*), 7.12–7.36 (10H, m, $C_6H_5\times 2$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 15.1, 25.4, 28.2, 30.0, 31.7, 33.6, 38.9, 50.0, 52.1, 54.0, 57.5, 65.7, 70.0, 73.1, 80.1, 115.4, 126.8, 127.1, 127.7, 127.9, 128.0, 128.2, 128.4, 129.4, 129.7, 130.2, 135.7, 137.4, 155.2, 170.7, 170.9, 171.4; Anal. calcd for $C_{37}H_{47}N_3O_8\cdot 1/2EtOAc\cdot 1/2H_2O$: C, 65.53; H, 7.33; N, 5.88. Found: C, 65.80; H, 7.07; N, 6.13.

4.1.13. Troc-(*S*)-Thr[Boc-(*S*)-Phs(Bn)-(*S*)-Phe-(*S*)-*N*-Me-Tyr-(*S*)-Ile]-(*S*)-Phe-OAllyl (21). Depsipeptide **6** (482 mg, 0.69 mmol) was treated with 4 N HCl–dioxane (3 ml) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 2 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless amorphous solid.

To a solution of tripeptide **6** (409 mg, 0.63 mmol) in THF (3 ml) was added 0.5 N aqueous LiOH (3 ml) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 1 h. After acidification with 1 M aqueous $KHSO_4$, the mixture was extracted with EtOAc (×3). The extracts were dried over Na_2SO_4, concentrated in vacuo to give a crude carboxylic acid as a colorless oil which was used for the next step without further purification.

To a solution of the above crude amine salt and carboxylic acid in DMF (4 ml) was added DEPC (0.11 ml, 0.76 mmol) and Et_3N (0.22 ml, 1.58 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 14 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous $KHSO_4$, H_2O, saturated aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:1) to give **21** (600 mg, 77%) as a colorless amorphous powder: $[\alpha]_D^{20}=-13.4$ (*c* 1.0, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3303, 1741, 1645, 1516, 1243; ^1H NMR (500 MHz, $CDCl_3$) δ 0.81–0.96 (6H, m, Ile-5,6-*H*), 1.11–1.27 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.38, 1.44 (9H, s×2, *tBu*), 1.58–1.90 (5H, m, Phs-3,4-*H*, O*H*), 1.95–2.06 (1H, br, Ile-3-*H*), 2.34–2.56 (1H, m, Tyr-3-*H*), 2.55–2.85 (5H, m, Phe(2)-3-*H*, N*Me*), 2.91–3.01 (1H, m, Tyr-3-*H*), 3.04–3.15 (2H, m, Phe(1)-3-*H*), 3.46–3.55 (2H, m, Phs-5-*H*), 3.96–4.05 (1H, br, Phs-2-*H*), 4.09–4.22 (1H, br, Thr-2-*H*), 4.37–4.51 (1H, br, Ile-2-*H*), 4.54–4.62 (6H, m, OCH_2Cl_3, $OCH_2C_6H_5$, CO_2CH_2CH), 4.65–4.89 (3H, m, Phe(1)-2-*H*, Phe(2)-2-*H*, Tyr-2-*H*), 5.20–5.28 (3H, m, Thr-3-*H*, $CO_2CH_2CHCH_2$), 5.79–5.84 (1H, m, $CO_2CH_2CHCH_2$), 6.64–6.73 (2H, m, Tyr-6,8-*H*), 6.87–6.91 (2H, m, Tyr-5,9-*H*), 7.11–7.35 (15H, m, C_6H_5×3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 10.8, 14.1, 15.1, 20.9, 25.0, 28.2, 33.0, 35.8, 36.4, 37.5, 37.8, 50.1, 53.2, 53.8, 56.5, 57.3, 57.7, 65.7, 65.8, 69.9, 70.5, 74.5, 79.2, 95.3, 115.4, 118.5, 126.7, 127.5, 127.7, 127.8, 128.1, 128.2, 128.3, 128.6, 128.9, 129.0, 130.0, 131.2, 135.6, 135.8, 137.5, 154.0, 155.0, 169.5, 170.5, 170.9, 171.8, 173.1; Anal. calcd for $C_{61}H_{77}Cl_3N_6O_{14}\cdot EtOAc\cdot H_2O$: C, 58.66; H, 6.59; N, 6.32. Found: C, 58.56; H, 6.38; N, 6.25.

4.1.14. Troc-(*S*)-Thr[Boc-(*S*)-Phs(Bn)-(*S*)-Phe-(*S*)-*N*-Me-Tyr(TBS)-(*S*)-Ile]-(*S*)-Phe-OAllyl (4). To a solution of hexapeptide **21** (960 mg, 0.78 mmol) in DMF (3 ml) was added imidazole (462 mg, 6.27 mmol) and TBSCl (473 mg, 3.13 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 12 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous $KHSO_4$, H_2O, saturated aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=2:1) to give **4** (788 mg, 75%) as a colorless amorphous powder: $[\alpha]_D^{20}=-2.6$ (*c* 0.9, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3302, 1741, 1682, 1645, 1510, 1255, 910; ^1H NMR (270 MHz, $CDCl_3$) δ 0.00, 0.10 (6H, s×2, Si*Me*$_2$), 0.83–0.97 (6H, m, Ile-5,6-*H*), 0.86, 0.93 (9H, s×2, Si-*tBu*), 1.14–1.28 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.36, 1.39 (9H, s×2, O-*tBu*), 1.60–2.05 (5H, br, Ile-3-*H*, Phs-3,4-*H*), 2.20–2.65 (2H, m, Phe(2)-3-*H*), 2.70–2.90 (4H, m, Tyr-3-*H*, N*Me*), 3.00–3.30 (3H, m, Tyr-3-*H*, Phe(1)-3-*H*), 3.38–3.70 (2H, m, Phs-5-*H*), 4.08–4.40 (3H, m, Phs-2-*H*, Thr-2-*H*, Ile-2-*H*), 4.40–4.70 (6H, m, OCH_2Cl_3, $OCH_2C_6H_5$, CO_2CH_2CH), 4.70–5.00 (3H, m, Phe(1)-2-*H*, Phe(2)-2-*H*, Tyr-2-*H*), 5.19–5.30 (3H, m, Thr-3-*H*, $CO_2CH_2CHCH_2$), 5.70–5.90 (1H, m, $CO_2CH_2CHCH_2$), 6.65–6.73 (2H, m, Tyr-6,8-*H*), 6.93–7.04 (2H, m, Tyr-5,9-*H*), 7.15–7.43 (15H, m, C_6H_5×3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −4.72, −4.62, 10.9, 15.2, 18.0, 25.5, 25.7, 28.3, 33.2, 36.6, 37.7, 49.8, 53.9, 58.1, 62.1, 65.8, 69.6, 70.5, 73.1, 74.6, 79.5, 95.3, 118.6, 120.1, 126.8, 126.9, 127.6, 127.7, 128.3, 128.5, 128.7, 129.0, 129.1, 129.6, 130.1, 131.3, 135.7, 136.1, 137.6, 153.8, 154.3, 155.0, 168.4, 169.9, 170.5, 171.6, 172.9; Anal. calcd for $C_{67}H_{91}Cl_3N_6O_{14}Si\cdot EtOAc$: C, 59.76; H, 6.99; N, 5.89. Found: C, 59.86; H, 6.98; N, 5.62.

4.1.15. Cyclo[Troc-(*S*)-Thr-(*S*)-Phe-(*S*)-Phs(Bn)-(*S*)-Phe-(*S*)-*N*-Me-Tyr(TBS)-(*S*)-Ile] (2) (entry 6 in Table 1). To a solution of hexapeptide **4** (2.07 g, 1.54 mmol) in THF (10 ml) was added $(PPh_3)_4Pd$ (0.18 g, 0.15 mmol) and morpholine (0.20 ml, 2.25 mmol) at room temperature. The mixture was stirred for 2 h. After dilution with EtOAc, the whole mixture was washed with 1 M aqueous $KHSO_4$, and brine, dried over Na_2SO_4, and concentrated in vacuo to give a crude carboxylic acid as a yellow amorphous powder which was used for the next step without further purification.

The above carboxylic acid was treated with 4 N HCl–dioxane (20 ml) at 0 °C for 1.5 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a yellow amorphous powder.

To a solution of the above deprotected hexapeptide in CH_2Cl_2 (700 ml) was added DIEA (1.30 ml, 7.50 mmol), and FDPP (1.15 g, 3.00 mmol) in CH_2Cl_2 (20 ml) at room temperature. The mixture was stirred at room temperature for 17 h, then concentrated. The residue was diluted with EtOAc, washed with 1 M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:1) to give cyclic peptide **2** (1.52 g, 84%) as a pale yellow amorphous powder: $[\alpha]_D^{24}=-27.1$ (*c* 0.9, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3303, 1739, 1645, 1512, 1259; ^1H NMR (270 MHz, $CDCl_3$) δ 0.01, 0.04 (6H, s, Si*Me*$_2$), 0.69–0.95 (6H, m, Ile-5,6-*H*), 0.88, 0.95 (9H, s×2, *tBu*), 1.10–1.50 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.50–1.90 (5H, br, Ile-3-*H*, Phs-3,4-*H*), 2.08–2.55 (2H, m, Phe(2)-3-*H*), 2.67, 2.80 (3H, s×2, N*Me*), 2.90–3.20 (2H, m, Tyr-3-*H*), 3.30–3.70 (4H, m, Phe(1)-3-*H*, Phs-5-*H*), 4.12–4.40 (3H, m, Phs-2-*H*, Thr-2-*H*, Ile-2-*H*), 4.40–4.60 (4H, m, OCH_2Cl_3, $OCH_2C_6H_5$), 4.60–4.80 (2H, m, Tyr-2-*H*, Phe(1)-2-*H*), 4.95–5.05 (1H, m, Phe(2)-2-*H*), 5.25–5.35 (1H, m, Thr-3-*H*), 6.65–6.72 (2H, m, Tyr-6,8-*H*), 6.88–6.93 (2H, m, Tyr-5,9-*H*), 7.01–7.34 (15H, m, C_6H_5×3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −4.7, −4.5, 10.7, 10.9, 14.9, 15.2, 17.9, 25.5, 25.6, 29.9, 30.7, 33.3, 36.5, 50.6, 51.3, 57.0, 57.5, 70.0, 70.4, 72.2, 74.7, 95.3, 120.1, 127.0, 127.2, 127.9, 128.5, 128.6, 128.9, 129.1, 129.7, 129.8, 130.0, 130.2, 135.9, 136.0, 137.6, 154.3, 154.4, 154.8, 168.7, 169.2, 170.2, 171.8, 172.1; Anal. calcd for $C_{59}H_{77}N_6O_{11}Si\cdot H_2O$: C, 59.11; H, 6.64; N, 7.01. Found: C, 58.97; H 6.62; N; 7.01.

(Entry 1 in Table 1) To a solution of the deprotected peptide from **4** (70 mg, 0.054 mmol) in DMF (25 ml) was added DPPA (23 μl, 0.11 mml) in DMF (2.5 ml) and $NaHCO_3$ (45 mg, 0.54 mmol) at 0 °C. The mixture was stirred at 4 °C for 72 h, then concentrated. The residue was purified as described above to give **2** (33 mg, 52%).

(Entry 2 in Table 1) To a solution of the deprotected peptide from **4** (77 mg, 0.056 mmol) in DMF (25 ml) was added DEPC (17 μl, 0.12 mmol) in DMF (3 ml) and $NaHCO_3$ (47 mg, 0.56 mmol) at 0 °C. The mixture was stirred at 4 °C for 96 h, then concentrated. The residue was purified as described above to give **2** (29 mg, 44%).

(Entry 3 in Table 1) To a solution of the deprotected peptide from **4** (76 mg, 0.058 mmol) in DMF (30 ml) was added HATU (67 mg, 0.18 mmol) and DIEA (50 μl, 0.29 mmol) at 0 °C. The mixture was stirred at 0 °C for 3 h, and at room temperature for 10 h, then concentrated. The residue was purified as described above to give **2** (26 mg, 38%).

(Entry 4 in Table 1) To a solution of the deprotected peptide from **4** (53 mg, 0.04 mmol) in DMF (20 ml) was added HATU (31 mg, 0.08 mmol) and DIEA (28 μl, 0.16 mmol) at 0 °C. The mixture was stirred at 4 °C for 96 h, then concentrated. The residue was purified as described above to give **2** (28 mg, 59%).

(Entry 5 in Table 1) To a solution of the deprotected peptide from **4** (208 mg, 0.16 mmol) in DMF (80 ml) was added FDPP (74 mg, 0.19 mmol) in DMF (5 ml) and DIEA (0.14 ml, 0.80 mmol) at room temperature. The mixture was stirred at room temperature for 14 h, then concentrated. The residue was purified as described above to give **2** (107 mg, 57%).

4.1.16. Allyl (*S*)-2,3-dihydroxypropanoate (25). To a solution of (*S*)-Ser-OH (**24**) (4.20 g, 40 mmol) in 0.4 M hydrochloric acid (200 ml) was added $NaNO_2$ (5.53 g, 80 mmol) in H_2O (100 ml) slowly at −10 °C. After addition, the mixture was allowed to warm to room temperature and stirred for 24 h, then concentrated. The residue was filtered to remove inorganic salt. Acetone–$CHCl_3$ (1:1) was added to the filtrate and the mixture was concentrated in vacuo. This work-up was repeated three times to remove H_2O completely. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo to give crude (*S*)-glyceric acid (9.92 g) as a yellow oil which was used for the next step without further purification.

To a solution of the above crude carboxylic acid (3.0 g) in allyl alcohol–$CHCl_3$ (1:2, 40 ml) was added *p*-TsOH (114 mg, 0.6 mmol) at room temperature. The mixture was refluxed for 3 h, then concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:2) to give **25** (957 mg, 54% in 2 steps) as a colorless oil: $[\alpha]_D^{25}=-21.1$ (*c* 1.2, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3389, 1740, 1207, 1120; ^1H NMR (270 MHz, $CDCl_3$) δ 2.40–2.70 (1H, br, exchangeable with D_2O, O*H*), 3.30–3.50 (1H, br, exchangeable with D_2O, O*H*), 3.80–3.95 (2H, br, CH_2OH), 4.30 (1H, br, C*H*OH), 4.70–4.73 (2H, m, CO_2CH_2), 5.26–5.40 (2H, m, $CO_2CH_2CHCH_2$), 5.86–6.00 (1H, m, $CO_2CH_2CHCH_2$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 64.0, 66.1, 71.8, 118.8, 131.3, 172.6; Anal. calcd for $C_6H_{10}O_4$: C, 49.31; H, 6.90. Found: C, 49.05; H 6.92.

4.1.17. Allyl (*S*)-2-hydroxy-3-(*tert*-butyldimethylsiloxy)-propanoate (28). To a solution of the allyl propanoate **25** (1.9 g, 13 mmol) in CH_2Cl_2 (40 ml) was added Et_3N (1.47 ml, 15.6 mmol), DMAP (63 mg, 0.52 mmol), and TBSCl (2.15 g, 14.3 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h, then at room temperature for 11 h. After dilution with Et_2O, the whole mixture was washed with 1 M aqueous $KHSO_4$, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=10:1) to give **28** (2.29 g, 68%) as a colorless oil: $[\alpha]_D^{25}=-7.6$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3496, 2930, 1747, 1254, 1128; ^1H NMR (270 MHz, $CDCl_3$) δ 0.03, 0.05 (6H, s×2, SiMe_2), 0.86 (9H, s, *tBu*), 3.04 (1H, d, J=7.9 Hz, O*H*), 3.83–3.98 (2H, m, CH_2OSi), 4.20–4.26 (1H, m, C*H*OH), 4.65–4.69 (2H, m, CO_2CH_2), 5.23–5.38 (2H, m, $CO_2CH_2CHCH_2$), 5.85–5.99 (1H, m, $CO_2CH_2CHCH_2$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.5, 25.6, 65.0, 65.9, 71.9, 118.7, 131.4, 172.3; Anal. calcd for $C_{12}H_{24}O_4Si$: C, 55.35; H, 9.29. Found: C, 55.17; H, 9.24.

4.1.18. Allyl (*S*)-2-benzyloxy-3-(*tert*-butyldimethyl-siloxy)propanoate (29). To a solution of the 2-hydroxy-propanoate **28** (1.22 g, 4.70 mmol) in Et_2O (20 ml) was added Ag_2O (3.27 g, 14.1 mmol) and BnBr (2.8 ml, 23.5 mmol) at room temperature under argon atmosphere. The mixture was stirred for 4 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-200 MH, hexane–Et_2O=20:1) to give **29** (1.24 g, 75%) as a colorless oil: $[\alpha]_D^{25}=-37.9$ (*c* 1.1, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 2955, 1751, 1450, 1257, 1132, 837; ^1H NMR (270 MHz, $CDCl_3$) δ 0.04 (6H, s, SiMe_2), 0.87 (9H, s, *tBu*), 3.91 (2H, t, J=5.2 Hz, CH_2OSi), 4.08 (1H, dd, J=4.7, 5.8 Hz, COC*H*), 4.53 (1H, d, J=11.8 Hz, C$H_2C_6H_5$), 4.62–4.66 (2H, m, CO_2CH_2), 4.76 (1H, d, J=11.9 Hz, C$H_2C_6H_5$), 5.17–5.38 (2H, m, $CO_2CH_2CHCH_2$), 5.85–5.99 (1H, m, $CO_2CH_2CHCH_2$), 7.27–7.38 (5H, m, C_6H_5); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.4, 18.1, 25.7, 64.1, 65.3, 72.4, 79.4, 118.5, 127.7, 127.9, 128.3, 131.7, 137.4, 170.5; Anal. calcd for $C_{19}H_{30}O_4Si\cdot 1/20$ hexane$\cdot 1/20$ Et_2O: C, 65.32; H, 8.77. Found: C, 65.68; H, 8.38.

4.1.19. Allyl (*S*)-2-benzyloxy-3-hydroxypropanoate (30). To a solution of the protected allyl propanoate **29** (460 mg, 1.31 mmol) in THF (2 ml) was added 1 N TBAF in THF (2.6 ml, 2.6 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h. After dilution with EtOAc, the whole mixture was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=3:1) to give **30** (251 mg, 79%) as a colorless oil: $[\alpha]_D^{25}=-85.1$ (*c* 1.1, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3453, 1747, 1454, 1190, 1122; ^1H NMR (270 MHz, $CDCl_3$) δ 2.10–2.25 (1H, br, O*H*), 3.90–3.95 (2H, m, CH_2OH), 4.12 (1H, dd, J=3.6, 5.1 Hz, COC*H*), 4.52 (1H, d, J=11.2 Hz, C$H_2C_6H_5$), 4.69 (2H, d, J=5.9 Hz, CO_2CH_2), 4.84 (1H, d, J=11.6 Hz, C$H_2C_6H_5$), 5.24–5.39 (2H, m, $CO_2CH_2CHCH_2$), 5.86–6.00

(1H, m, $CO_2CH_2CHCH_2$), 7.25–7.39 (5H, m, C_6H_5); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 63.3, 65.5, 72.6, 78.6, 118.6, 128.0, 128.1, 128.4, 131.5, 136.9, 170.1; Anal. calcd for $C_{13}H_{16}O_4 \cdot 1/5$ EtOAc: C, 65.28; H, 6.99. Found: C, 65.49; H, 6.80.

4.1.20. 2-Propenyl (*S*)-3-[(dibenzyloxyphosphinyl)oxy]-2-benzyloxypropanoate (3a). To a solution of the 3-hydroxypropanate **30** (20 mg, 0.08 mmol) in CH_2Cl_2 (1 ml) was added 1*H*-tetrazole (17 mg, 0.24 mmol) and *N*,*N*-diisopropyl dibenzyl phosphoramidite (**31**) (5.7 μl, 0.17 mmol) at room temperature. The mixture was stirred at room temperature for 1 h, then cooled to −30 °C. *m*-CPBA (70%, 40 mg, 0.16 mmol) was added and the mixture was stirred at −30 °C for 40 min. The reaction was quenched with saturated aqueous $NaHCO_3$ and the mixture was extracted with Et_2O. The extracts were successively washed with 1 M aqueous $KHSO_4$, H_2O, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=10:1 to 5:1 then 3:1) to give **3a** (40 mg, quant.) as a colorless oil: $[\alpha]_D^{22}=-28.4$ (*c* 1.1, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 1755, 1456, 1280, 1136, 1021; ^1H NMR (270 MHz, $CDCl_3$) δ 4.15–4.18 (1H, m, COC*H*), 4.21–4.36 (2H, m, $CHCH_2OP$), 4.53 (1H, d, $J=11.7$ Hz, $CHOCH_2C_6H_5$), 4.61–4.64 (2H, m, CO_2CH_2), 4.76 (1H, d, $J=11.6$ Hz, $CHOCH_2C_6H_5$), 4.99, 5.02 (4H, s×2, $P(OCH_2C_6H_5)\times2$), 5.21–5.35 (2H, m, $CO_2CH_2CHCH_2$), 5.81–5.93 (1H, m, $CO_2CH_2CHCH_2$), 7.26–7.36 (15H, m, $C_6H_5\times3$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 56.9, 67.2, 69.3, 72.7, 76.8, 119.0, 127.9, 128.0, 128.1, 128.4, 128.5, 131.4, 135.7, 136.9, 168.9; Anal. calcd for $C_{27}H_{29}O_7P$: C, 65.32; H, 5.89. Found: C, 65.05; H, 5.94.

4.1.21. Cyclo[Boc-(*S*)-Thr-(*S*)-Phe-(*S*)-Phs(Bn)-(*S*)-Phe-(*S*)-*N*-Me-Tyr(TBS)-(*S*)-Ile] (32). To a solution of cyclic peptide **2** (610 mg, 0.52 mmol) in THF (3 ml) was added Zn (900 mg) and AcOH (0.5 ml) at room temperature. The mixture was stirred for 4.5 h, and filtered through a pad of celite. The filtrate was concentrated in vacuo to give a crude amine salt as a colorless solid.

To a solution of the above crude amine salt in THF (3 ml) was added Boc_2O (340 mg, 1.55 mmol) and Et_3N (0.22 ml, 1.55 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 5 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous $KHSO_4$, H_2O, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:1) to give **32** (438 mg, 77%) as a colorless solid: $[\alpha]_D^{23}=-42.0$ (*c* 0.5, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3303, 1732, 1682, 1654, 1645, 1510, 1253, 914; ^1H NMR (270 MHz, $CDCl_3$) δ 0.01, 0.03, 0.07 (6H, s×3, SiMe_2), 0.65–0.90 (6H, m, Ile-5,6-*H*), 0.88, 0.95 (9H, s×2, Si-*tBu*), 1.15–1.30 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.41 (9H, s, O-*tBu*), 1.55–1.90 (5H, br, Ile-3-*H*, Phs-3,4-*H*), 2.35–2.75 (2H, m, Phe(2)-3-*H*), 2.66, 2.81 (3H, s×2, N*Me*), 2.86–3.18 (2H, m, Tyr-3-*H*), 3.35–3.50 (2H, m, Phe(1)-3-*H*), 3.35–3.70 (2H, m, Phs-5-*H*), 4.03–4.12 (1H, br, Phs-2-*H*), 4.30–4.60 (4H, br, Thr-2-*H*, Ile-2-*H*, Phe(1)-2-*H*, Tyr-2-*H*), 4.46, 4.48 (2H, s×2, $CH_2C_6H_5$), 4.85–5.05 (2H, br, Phe(2)-2-*H*, Thr-3-*H*), 6.64–6.72 (2H, m, Tyr-6,8-*H*), 6.85–6.96 (2H, m, Tyr-5,9-*H*), 7.01–7.40 (15H, m, $C_6H_5\times3$); ^{13}C NMR (67.8 MHz, CD_3OD) δ −4.5, −4.4, 11.3, 12.0, 15.1, 15.8, 17.6, 18.8, 26.1, 28.7, 30.4, 37.8, 51.2, 53.0, 56.7, 58.3, 72.8, 73.7, 80.7, 121.2, 127.5, 127.7, 128.5, 128.6, 128.7, 129.3, 129.4, 129.5, 129.9, 130.0, 130.3, 131.6, 137.5, 138.6, 139.6, 155.6, 157.3, 170.4, 171.6, 172.0, 173.2, 174.1, 174.3; Anal. calcd for $C_{61}H_{84}N_6O_{11}Si \cdot H_2O$: C, 65.22; H, 7.72; N, 7.48. Found: C, 65.61; H 7.64; N; 7.49.

4.1.22. Cyclo[Boc-(*S*)-Thr-(*S*)-Phe-(*S*)-Phs-(*S*)-Phe-(*S*)-*N*-Me-Tyr(TBS)-(*S*)-Ile] (33). To a solution of Boc protected cyclic peptide **32** (330 mg, 0.3 mmol) in EtOAc (2 ml) was added 20% $Pd(OH)_2$ (100 mg) under an argon atmosphere. The black slurry was stirred under 1 atom of H_2 at room temperature for 3 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo to give **33** (312 mg, quant.) as a colorless amorphous powder which was used for the next step without further purification. IR ν_{max} ($CHCl_3$) cm^{-1} 3303, 1730, 1682, 1651, 1645, 1634, 1512, 1454, 1257, 1170; ^1H NMR (270 MHz, $CDCl_3$) δ −0.02, 0.04 (6H, s×2, SiMe_2), 0.65–0.95 (6H, m, Ile-5,6-*H*), 0.87, 0.94 (9H, s×2, Si-*tBu*), 1.18–1.30 (5H, br, Ile-3-*H*, Phs-3,4-*H*), 1.44 (9H, s, O-*tBu*), 1.70–2.00 (5H, br, Ile-3-*H*, Phs-3,4-*H*), 2.46–2.57 (2H, m, Phe(2)-3-*H*), 2.80, 2.85 (3H, s×2, N*Me*), 2.95–3.20 (2H, m, Tyr-3-*H*), 3.36–3.40 (2H, m, Phe(1)-3-*H*), 3.45–3.48 (2H, br, Phs-5-*H*), 4.07–4.23 (1H, m, Phs-2-*H*), 4.25–4.65 (4H, m, Thr-2-*H*, Ile-2-*H*, Phe(1)-2-*H*, Tyr-2-*H*), 5.22–5.45 (2H, m, Phe(2)-2-*H*, Thr-3-*H*), 6.66–6.72 (2H, m, Tyr-6,8-*H*), 6.90–6.98 (2H, m, Tyr-5,9-*H*), 7.00–7.35 (10H, m, $C_6H_5\times2$).

4.1.23. (*S*)-2′-Benzyloxy-1′-cyclo[(*S*)-Thr-(*S*)-Phe-(*S*)-Phs-(*S*)-Phe-(*S*)-*N*-Me-Tyr(TBS)-(*S*)-Ile]-propanoate 3′-dibenzyl phosphate (34). Cyclic peptide **33** (30 mg, 30 μmol) was treated with 4 N HCl–dioxane (1 ml) at 0 °C and the mixture was stirred at 0 °C for 2 h, then at room temperature for 3 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless solid.

To a solution of phosphate **3a** (22 mg, 45 μmol) in THF (0.3 ml) was added $(PPh_3)_4Pd$ (5 mg, 4,5 μmol) and morpholine (6 μl, 68 μmol) at room temperature. The mixture was stirred for 3.5 h. After dilution with EtOAc, the whole mixture was washed with 1 M aqueous $KHSO_4$, and brine, dried over Na_2SO_4, and concentrated in vacuo to give a crude carboxylic acid as a yellow oil which was used for the next step without further purification.

To a solution of the above crude amine salt and carboxylic acid in DMF (0.5 ml) was added DEPC (7 μl, 45 μmol) and Et_3N (10 μl, 75 μmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 12 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous $KHSO_4$, H_2O, saturated aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by thin layer chromatography (hexane–EtOAc=1:4) to give **34** (21 mg, 52%) as a colorless solid: $[\alpha]_D^{24}=-11.1$ (*c* 1.0, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3303, 1738, 1651, 1518, 1263, 1020; ^1H NMR (270 MHz, $CDCl_3$) δ −0.05, −0.01 (6H, s×2, SiMe_2), 0.68–0.98 (6H, m, Ile-5,6-*H*), 0.87, 0.91 (9H, s×2, Si-*tBu*), 1.00–1.20 (3H, m, Thr-4-*H*), 1.20–1.55

(2H, m, Ile-4-*H*), 1.60–2.05 (5H, br, Ile-3-*H*, Phs-3,4-*H*), 2.50–2.90 (4H, m, Tyr-3-*H*, Phe(2)-*H*), 2.86 (3H, s, N*Me*), 3.00–3.35 (4H, m, Phs-5-*H*, Phe(1)-3-*H*), 3.50–3.70 (1H, br, O*H*), 3.90–4.20 (2H, br, Phs-2-*H*, Ga-2-*H*), 4.20–4.80 (6H, m, Thr-2-*H*, Ile-2-*H*, Phe(1)-2-*H*, Tyr-2-*H*, Ga-3-*H*), 4.52, 4.59 (2H, s×2, CHOCH_2C$_6$H$_5$), 4.80-5.15 (6H, m, Phe(2)-2-*H*, Thr-3-*H*, P(OCH_2C$_6$H$_5$)×2), 6.67–7.05 (4H, m, Tyr-5,6,8,9-*H*), 7.05–7.40 (20H, m, C$_6$*H*$_5$×4) 7.40–7.70 (5H, m, C$_6$*H*$_5$); ^{13}C NMR (67.8 MHz, CD_3OD) δ −4.4, −4.3, 12.2, 14.5, 15.0, 18.0, 18.9, 20.9, 26.1, 28.5, 29.7, 30.1, 30.5, 34.4, 37.0, 38.2, 51.4, 53.1, 56.2, 58.6, 61.5, 62.1, 63.7, 68.3. 70.7, 70.8, 70.9, 72.8, 74.0, 79.5, 79.7, 121.2, 127.5, 127.7, 129.0, 129.2, 129.3, 129.4, 129.5, 129.9, 130.0, 130.3, 131.0, 131.2, 131.6, 132.2, 136.8, 136.9, 137.6, 137.9, 138.4, 138.7, 155.6, 170.3, 170.6, 170.7, 171.5, 173.4, 174.1, 174.5; HRFABMS (*m*-nitrobenzyl alcohol) calcd for $C_{73}H_{93}N_6O_{15}PSi$ $[M+H]^+$: 1353.6284. Found: 1353.6309.

4.1.24. (*S*)-2′-Benzyloxy-1′-cyclo[(*S*)-Thr-(*S*)-Phe-(*S*)-Ahp-(*S*)-Phe-(*S*)-*N*-Me-Tyr(TBS)-(*S*)-Ile]-propanoate 3′-dibenzyl phosphate (44). To a solution of cyclic peptide **34** (35 mg, 26 μmol) in DMSO (0.7 ml) was added IBX (30 mg, 0.10 mmol) at room temperature. The mixture was stirred for 3 h. After dilution with EtOAc, the whole mixture was washed with H_2O (×2) and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, EtOAc only) to give aldehyde **35** (28 mg, 80%) as a colorless oil.

To a solution of the above aldehyde in THF (1 ml) was added 1 N TBAF in THF (20 μl) at 0 °C. The mixture was stirred at 0 °C for 20 min. After dilution with EtOAc, the whole mixture was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo to give **44** (25 mg, 85%) as a colorless oil: $[\alpha]_D^{27} = -130.1$ (*c* 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3389, 1732, 1675, 1670, 1667, 1651, 1634, 1517, 1456, 1261, 1020; ^{1}H NMR (270 MHz, $CDCl_3$) 0.74 (3H, t, *J*=6.6 Hz, Ile-5-*H*), 0.75 (3H, d, *J*=7.3 Hz, Ile-6-*H*), 0.88–0.95 (1H, m, Ile-4-*H*), 1.07 (3H, d, *J*=6.4 Hz, Thr-4-*H*), 1.40–1.55 (1H, m, Ile-4-*H*), 1.60–1.80 (2H, Ile-3-*H*, Ahp-5-*H*), 1.83–2.00 (2H, m, Ahp-4,5-*H*), 2.13–2.35 (1H, m, Tyr-3-*H*), 2.55–2.63 (1H, m, Ahp-4-*H*), 2.86 (3H, s, N*Me*), 2.88–3.00 (1H, m, Tyr-3-*H*), 3.05–3.45 (4H, m, Phe(1)-3-*H*, Phe(2)-3-*H*), 3.80 (1H, br, exchangeable with D_2O, O*H*), 3.85–3.95 (1H, m, Ahp-3-*H*), 4.00 (1H, br, Ga-2-*H*), 4.20–4.35 (2H, m, Ga-3-*H*), 4.38 (1H, br, exchangeable with D_2O, O*H*), 4.45 (1H, d, *J*=9.9 Hz, Thr-2-*H*), 4.56 (2H, s, CHOCH_2C$_6$H$_5$), 4.56–4.60 (1H, br, Ile-2-*H*), 4.78–4.90 (1H, br, Phe(1)-2-*H*), 4.90–4.95 (1H, br, Tyr-2-*H*), 5.00, 5.03 (4H, s×2, P(OCH_2C$_6$H$_5$)×2), 5.34 (1H, s, Ahp-6-*H*), 5.20–5.40 (2H, m, Phe(2)-2-*H*, Thr-3-*H*), 6.76 (2H, d, *J*=8.4 Hz, Tyr-6,8-*H*), 7.05 (2H, d, *J*=8.3 Hz, Tyr-5,9-*H*), 7.10–7.34 (25H, m, C$_6$*H*$_5$×5); ^{13}C NMR (67.8 MHz, CD_3OD) δ 11.5, 14.5, 16.5, 18.3, 18.9, 20.9, 26.2, 30.7, 31.6, 34.3, 36.4, 37.4, 38.8, 50.7, 52.5, 56.0, 56.2, 57.4, 61.5, 63.1, 70.7, 70.8, 70.9, 71.0, 74.0, 75.7, 116.6, 127.3, 127.5, 128.8, 129.0, 129.1, 129.2, 129.3, 129.5, 129.6, 130.0, 130.5, 131.7, 136.9, 137.0, 137.5, 138.0, 139.0, 157.5, 170.3, 170.8, 171.1, 172.8, 173.0, 174.5; HRFABMS (*m*-nitrobenzyl alcohol) calcd for $C_{67}H_{77}N_6O_{15}P$ $[M+H]^+$: 1237.5263. Found: 1237.5337.

4.1.25. 5-Benzyloxy-1-pentanol (38). To a solution of 1,5-pentanediol (**37**) (5.2 g, 50 mmol) in THF (100 ml) was added 15-crown-5-ether (0.3 ml, 3 mmol), finely grinded NaOH (12 g, 0.3 mol), and BnBr (6.0 ml, 50 mmol) at 10 °C. The mixture was stirred at 10 °C for 4 h. After dilution with EtOAc, the whole mixture was washed with brine (×3), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=2:1 to 1:1) to give **38** (4.0 g, 41%) as a colorless oil:^{1}H NMR (270 MHz, $CDCl_3$) δ 1.41–1.67 (7H, m, CH_2×3, O*H*), 3.48 (2H, t, *J*=6.3 Hz, CH_2OCH_2C$_6$H$_5$), 3.62 (2H, d, *J*=6.3 Hz, CH_2OH), 4.50 (2H, s, CH_2C$_6$H$_5$), 7.25–7.39 (5H, m, C$_6$*H*$_5$).

4.1.26. *N*-(5-Benzyloxypentanoyl)-(*S*)-phenylalanine methyl ester (41). To a solution of pentanol **38** (2.5 g, 13 mmol) in CH_2Cl_2 (15 mmol) was added Et_3N (9.1 ml, 65 mmol), and pyridine·SO_3 (10.3 g, 65 mmol) in DMSO (15 ml) slowly at 0 °C. The mixture was stirred at 0 °C for 20 min. The reaction was quenched with saturated aqueous $NaHCO_3$ and the mixture was extracted with Et_2O. The extracts were washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo to give a crude aldehyde as a colorless oil which was used for the next step without further purification.

To a solution of the above crude aldehyde in H_2O (7 ml) and *t*-BuOH (28 ml) was added 2-methyl-2-butene (6.9 ml, 65 mmol), NaH_2PO_4 (2.34 g, 19.5 mmol), and $NaClO_2$ (1.76 g, 19.5 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h. The reaction was quenched with 1 M $KHSO_4$ and the mixture was extracted with EtOAc. The extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give a crude carboxylic acid as a colorless oil which was used for the next step without further purification.

Boc-(*S*)-Phe-OMe (**39**) (4.2 g, 15 mmol) was treated with 4 N HCl–dioxane at 0 °C and the mixture was stirred for 2 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless amorphous powder.

To a solution of the above crude carboxylic acid and amine salt in DMF (40 ml) was added DEPC (2.4 ml, 15.6 mmol) and Et_3N (4.2 ml, 32.5 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 2 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M $KHSO_4$, H_2O, saturated aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=2:1 to 1:1) to give **40** (3.73 g, 80%) as a colorless oil: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.58–1.73 (4H, m, CH_2×2), 2.19 (2H, t, *J*=7.2 Hz, COCH_2), 3.00–3.17 (2H, m, Phe-3-*H*), 3.46 (2H, t, *J*=6.1 Hz, CH_2CH_2O), 3.71 (3H, s, CO_2*Me*), 4.47 (2H, s, OCH_2C$_6$H$_5$), 4.84–4.92 (1H, m, Phe-2-*H*), 5.90–5.95 (1H, br, N*H*), 7.06–7.33 (10H, m, C$_6$*H*$_5$×2).

4.1.27. *N*-(5-Formylbutanoyl)-(*S*)-phenylalanine methyl ester (41). To a solution of **40** (2.5 g, 6.8 mmol) in EtOAc (20 ml) was added 20% $Pd(OH)_2$ (500 mg) under an argon atmosphere. The black slurry was stirred under 1 atom of H_2

at room temperature for 3 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo to give the debenzylated alcohol (2.01 g, quant.) as a colorless oil which was used for the next step without further purification: IR ν_{max}^{neat} cm^{-1} 3306, 1745, 1649, 1534, 1219; ^1H NMR (270 MHz, $CDCl_3$) δ 1.51–1.58 (2H, m, C*H*$_2$), 1.64–1.75 (2H, m, C*H*$_2$), 2.23 (2H, t, J=7.2 Hz, COC*H*$_2$), 2.10–2.30 (1H, br, O*H*), 3.03–3.19 (2H, m, Phe-3-*H*), 3.60 (2H, t, J=6.1 Hz, C*H*$_2$OH), 3.73 (3H, s, CO_2*Me*), 4.86–4.93 (1H, m, Phe-2-*H*), 6.00–6.10 (1H, br, N*H*), 7.08–7.31 (5H, m, C_6*H*$_5$).

To a solution of the above alcohol (260 mg, 0.93 mmol) in CH_2Cl_2 (1.5 ml) was added Et_3N (0.65 ml, 4.65 mmol), and pyridine·SO_3 (740 mg, 4.65 mmol) in DMSO (1.5 ml) slowly at 0 °C. The mixture was stirred at 0 °C for 1 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and the mixture was extracted with Et_2O. The extracts were washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:2) to give **41** (140 mg, 54%) as a colorless oil: IR ν_{max}^{neat} cm^{-1} 3302, 1743, 1724, 1651, 1539, 1217; ^1H NMR (270 MHz, $CDCl_3$) δ 1.88–1.96 (2H, m, $CH_2CH_2CH_2$), 2.22 (2H, t, J=7.2 Hz, COC*H*$_2$), 2.45 (2H, t, J=7.1 Hz, C*H*$_2$CHO), 3.10–3.20 (2H, m, Phe-3-*H*), 3.76 (3H, s, CO_2*Me*), 4.85–4.92 (1H, m, Phe-2-*H*), 5.85–5.92 (1H, br, N*H*), 7.07–7.32 (5H, m, C_6*H*$_5$), 9.72 (1H, s, C*H*O).

4.1.28. Methyl (2*S*)-2-(6-hydroxy-2-piperidone-1-yl)-3-phenylpropanoate (42). To a solution of aldehyde **41** (15 mg, 54 μmol) in THF (0.3 ml) was added 0.1 M pH 6 phosphate buffer (0.3 ml) at 0 °C. The mixture was stirred at 0 °C for 30 min, then at room temperature for 20 h. After dilution with EtOAc, the whole mixture was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:1 to 1:3) to give **42** (10 mg, 66%) as a colorless oil: $[\alpha]_D^{22}$=−78.4 (*c* 0.82, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3389, 1738, 1629, 1300, 1234; ^1H NMR (270 MHz, $CDCl_3$) δ 1.45–1.73 (2H, m, piperidone-4-*H*), 1.86–2.10 (2H, m, piperidone-5-*H*), 2.20–2.50 (2H, m, piperidone-3-*H*), 3.10–3.25 (1H, br, exchangeable with D_2O, O*H*), 3.30–3.50 (2H, m, Phe-3-*H*), 3.81 (3H, s, CO_2*Me*), 3.98 (1H, dd, J=4.7, 9.1 Hz, piperidine-6-*H*), 4.07–4.15 (1H, m, Phe-2-*H*), 7.11–7.31 (5H, m, C_6*H*$_5$); ^{13}C NMR (67.8 MHz, CD_3OD) δ 16.4, 31.3, 33.1, 35.6, 52.6, 62.4, 83.0, 127.8, 129.6, 130.5, 139.5, 172.5, 172.8; Anal. calcd for $C_{15}H_{19}NO_4$: C, 64.97, H,6.91, N, 5.05. Found: C, 65.24, H, 6.88, N, 5.02.

4.1.29. Methyl (2*S*)-2-(3,4-dihydro-2-pyridone-1-yl)-3-phenylpropanoate (43). A colorless oil: $[\alpha]_D^{24}$=−55.4 (*c* 0.17, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1741, 1670, 1378, 1215; ^1H NMR (270 MHz, $CDCl_3$) δ 2.00–2.50 (4H, m, pyridone-3,4-*H*), 2.99–3.08 (1H, m, Phe-3-*H*), 3.35–3.43 (1H, m, Phe-3-*H*), 3.73 (3H, s, CO_2*Me*), 5.13–5.17 (1H, m, Phe-2-*H*), 5.27–5.34 (1H, m, pyridone-5-*H*), 6.04 (1H, d, J=7.8 Hz, pyridone-6-*H*), 7.15–7.28 (5H, m, C_6*H*$_5$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 19.8, 31.3, 35.8, 52.5, 56.6, 77.2, 106.8, 126.7, 127.0, 128.3, 128.9, 136.4, 159.9, 169.2, 170.7. HRMS calcd for $C_{15}H_{17}O_3N$: 259.1208. Found: 259.1210.

4.1.30. Allyl (*S*)-2,3-bis(*tert*-butyldimethylsiloxy)propanoate (26). To a solution of allyl propanoate (**25**) (1.55 g, 10.6 mmol) in CH_2Cl_2 (20 ml) was added 2,6-lutidine (5.0 ml, 42.4 mmol) and TBSOTf (7.3 g, 31.8 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h. After dilution with Et_2O, the whole mixture was successively washed with 1 M aqueous $KHSO_4$, H_2O, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–Et_2O=15:1) to give **26** (3.78 g, quant.) as a colorless oil: $[\alpha]_D^{23}$=−16.6 (*c* 1.1, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 2955, 2930, 2858, 1759, 1472, 1257, 1148, 1128; ^1H NMR (270 MHz, $CDCl_3$) δ 0.05 (6H, s, Si*Me*$_2$), 0.08, 0.09 (6H, s×2, Si*Me*$_2$), 0.88 (9H, s, *tBu*), 0.90 (9H, s, *tBu*), 3.73–3.87 (2H, m, *CH*$_2$OSi), 4.23 (1H, t, J=5.3 Hz, *CH*OSi), 4.62 (2H, d, J=5.6 Hz, CO_2CH_2), 5.22–5.37 (2H, m, $CO_2CH_2CHCH_2$), 5.85–5.97 (1H, m, CO_2CH_2-*CH*CH_2); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.3, −4.7, 18.4, 25.8, 65.0, 65.7, 72.7, 118.7, 131.5, 172.3; Anal. calcd for $C_{12}H_{24}O_4Si\cdot 1/2H_2O$: C, 56.35; H, 10.25. Found: C, 56.72; H, 10.07.

4.1.31. Allyl (*S*)-2-(*tert*-butyldimethylsiloxy)-3-hydroxypropanoate (27). To a solution of bis(TBS)propanoate **26** (100 mg, 0.26 mmol) in CH_3CN (2.5 ml) was added $CeCl_3\cdot 7H_2O$ (150 mg, 0.39 mmol) and NaI (40 mg, 0.26 mmol) at room temperature. The mixture was stirred at room temperature for 15 h, and refluxed for 3 h. The reaction was quenched with 1 N aqueous HCl, then concentrated. After dilution with EtOAc, the whole mixture was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=5:1) to give **27** (43 mg, 62%) as a colorless oil: IR ν_{max}^{neat} cm^{-1} 3496, 2958, 1759, 1615, 1255, 1140; ^1H NMR (270 MHz, $CDCl_3$) δ 0.10, 0.14 (6H, s×2, Si*Me*$_2$), 0.92 (9H, s, *tBu*), 2.15–2.24 (1H, br, O*H*), 3.80–3.86 (2H, br, *CH*$_2$OH), 4.33 (1H, t, J=4.6 Hz, *CH*OSi), 4.65 (2H, d, J=5.6 Hz, CO_2CH_2), 5.24–5.38 (2H, m, $CO_2CH_2CHCH_2$), 5.85–5.99 (1H, m, $CO_2CH_2CHCH_2$).

4.1.32. Allyl (*S*)-2-(*tert*-butyldimethylsiloxy)propanoate 3-dibenzyl phosphate (3b). To a solution of 3-hydroxypropanate **27** (150 mg, 0.57 mmol) in CH_2Cl_2 (2.0 ml) was added 1*H*-tetrazole (120 mg, 1.71 mmol) and *N*,*N*-diisopropyl dibenzyl phosphoramidite (0.23 ml, 0.69 mmol) at room temperature. The mixture was stirred at room temperature for 1.5 h, then cooled −30 °C. *m*-CPBA (70%, 280 mg, 1.14 mmol) was added and the mixture was stirred at −30 °C for 1 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and the mixture was extracted with Et_2O. The extracts were washed with 1 M aqueous $KHSO_4$, H_2O, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=5:1 to 3:1) to give **3b** (303 mg, quant.) as a colorless oil: $[\alpha]_D^{21}$=−12.8 (*c* 1.2, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 2955, 2930, 1759, 1456, 1283, 1260, 1153, 1018; ^1H NMR (270 MHz, $CDCl_3$) δ 0.07, 0.09 (6H, s×2, Si*Me*$_2$), 0.89 (9H, s, *tBu*), 4.11–4.18, 4.24–4.32 (2H, m, *CH*$_2$OP), 4.37–4.40 (1H, m, *CH*OSi), 4.60 (2H, d, J=5.6 Hz, CO_2CH_2), 5.01, 5.03 (2H, s×2, *CH*$_2C_6H_5$), 5.04, 5.06 (2H, s×2, *CH*$_2C_6H_5$), 5.21–5.34 (2H, m, $CO_2CH_2CHCH_2$), 5.83–5.95

(1H, m, $CO_2CH_2CHCH_2$), 7.33 (10H, s, $C_6H_5\times2$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.2, −4.9, 18.4, 25.7, 65.9, 68.9, 68.9, 69.3, 69.4, 71.6, 71.7, 118.8, 127.8, 128.3, 128.4, 131.4, 135.5, 135.6, 169.9; Anal. calcd for $C_{26}H_{37}O_7PSi$: C, 59.98; H, 7.16. Found: C, 59.89; H, 7.26.

4.1.33. (*S*)-2′-(*tert*-Butyldimethylsiloxy)-1′-cyclo[(*S*)-Thr-(*S*)-Phe-(*S*)-Phs-(*S*)-Phe-(*S*)-*N*-Me-Tyr(TBS)-(*S*)-Ile]-propanoate 3′-dibenzyl phosphate (46). Cyclic peptide **33** (270 mg, 0.27 mmol) was treated with 4 N HCl–dioxane (2 ml) at 0 °C and the mixture was stirred at 0 °C for 2.5 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless solid.

To a solution of phosphate **3b** (208 mg, 0.40 mmol) in THF (1.5 ml) was added $(PPh_3)_4Pd$ (46 mg, 0.04 mmol) and morpholine (52 µl, 0.60 mmol) at room temperature. The mixture was stirred for 1.5 h. After dilution with EtOAc, the whole mixture was washed with 1 M aqueous $KHSO_4$ and brine, dried over Na_2SO_4, and concentrated in vacuo to give a crude carboxylic acid as a yellow oil which was used for the next step without further purification.

To a solution of the above crude amine salt and carboxylic acid in DMF (2 ml) was added DEPC (62 µl, 0.4 mmol) and Et_3N (93 µl, 0.66 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 17 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc = 1:5 to $CHCl_3$–MeOH = 20:1) to give **46** (211 mg, 57%) as a colorless solid: $[\alpha]_D^{22} = -20.3$ (*c* 1.0, $CHCl_3$); IR $\nu_{max}(CHCl_3)$ cm^{-1} 3389, 3238, 2950, 2932, 1738, 1682, 1660, 1645, 1539, 1255, 1020; ^1H NMR (270 MHz, $CDCl_3$) δ 0.01, 0.08 (12H, s×2, $SiMe_2\times2$), 0.64–0.93 (6H, m, Ile-5,6-*H*), 0.82, 0.84 (18H, s×2, *tBu*×2), 1.03–1.44 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.60–2.00 (5H, m, Ile-3-*H*, Phs-3,4-*H*), 2.50–2.95 (4H, m, Tyr-3-*H*, Phe(2)-3-*H*), 2.78, 2.84 (3H, s×2, N*Me*), 3.05–3.45 (2H, m, Phe(1)-3-*H*), 3.50–3.80 (2H, m, Phs-5-*H*), 4.00–4.80 (7H, m, Phs-2-*H*, Ga-2-*H*, Thr-2-*H*, Ile-2-*H*, Tyr-2-*H*, Ga-3-*H*), 4.83–5.15 (6H, m, Phe(1)-*H*, Phe(2)-*H*, $P(OCH_2C_6H_5)\times2$), 5.20–5.30 (1H, br, Thr-3-*H*), 6.68–6.73 (2H, m, Tyr-6,8-*H*), 6.83–7.36 (22H, m, Tyr-5,9-*H*, $C_6H_5\times4$); ^{13}C NMR (67.8 MHz, CD_3OD) δ −5.1, −4.6, −4.5, −4.3, 12.1, 15.1, 17.9, 18.9, 26.1, 27.4, 29.7, 30.6, 34.4, 38.3, 51.3, 53.1, 56.1, 56.9, 58.6, 62.1, 63.7, 70.8, 70.9, 72.0, 72.8, 121.2, 127.6, 127.7, 127.8, 129.0, 129.2, 129.3, 129.4, 129.5, 129.6, 129.8, 130.1, 130.3, 131.3, 131.6, 136.9, 137.0, 137.7, 138.6, 155.7, 170.4, 170.5, 171.4, 171.5, 171.7, 173.4, 173.6, 174.6; HRFABMS (*m*-nitrobenzyl alcohol) calcd for $C_{72}H_{101}N_6O_{15}PSi_2$ $[M+H]^+$: 1377.6679. Found: 1377.6696.

4.1.34. (*S*)-2′-Hydroxy-1′-cyclo[(*S*)-Thr-(*S*)-Phe-(*S*)-Ahp-(*S*)-Phe-(*S*)-*N*-Me-Tyr-(*S*)-Ile]-propanoate 3′-dibenzyl phosphate (47). To a solution of cyclic peptide **46** (25 mg, 18 µmol) in DMSO (0.4 ml) was added IBX (21 mg, 75 µmol) at room temperature. The mixture was stirred for 3 h. After dilution with EtOAc, the whole mixture was washed with H_2O (×2) and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by thin layer chromatography ($CHCl_3$–MeOH = 10:1) to give aldehyde (9 mg, 36%) as a colorless solid.

To a solution of the above aldehyde in THF (0.4 ml) was added 1 N TBAF in THF (20 µl, 20 µmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then concentrated. The residue was purified by thin layer chromatography ($CHCl_3$–MeOH = 15:1) to give Ahp product **47** (5.5 mg, 95%) as a colorless solid: $[\alpha]_D^{24} = -29.6$ (*c* 0.21, $CHCl_3$); IR $\nu_{max}(CHCl_3)$ cm^{-1} 3389, 2930, 1732, 1681, 1651, 1635, 1539, 1456, 1294, 1140, 1022; ^1H NMR (270 MHz, CD_3OD) δ 0.72–0.95 (6H, m, Ile-5,6-*H*), 1.18–1.28 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.57–1.90 (4H, m, Ile-3-*H*, Ahp-4,5-*H*), 1.95–2.08 (1H, m, Tyr-3-*H*), 2.45–2.70 (2H, m, Tyr-3-*H*, Ahp-4-*H*), 2.81 (3H, s, N*Me*), 2.85–3.05 (2H, m, Phe(2)-3-*H*), 3.30–3.56 (2H, m, Phe(1)-3-*H*), 3.80–3.87 (1H, m, Ahp-3-*H*), 4.20–4.25 (3H, br, Ga-2,3-*H*), 4.47 (1H, d, *J* = 5.7 Hz, Ile-2-*H*), 4.53 (1H, br, Thr-2-*H*), 4.61–4.70 (1H, m, Phe(1)-*H*), 4.95–5.15 (6H, m, Tyr-2-*H*, Phe(2)-*H*, $P(OCH_2C_6H_5)\times2$), 5.15 (1H, br, Ahp-6-*H*), 5.44–5.53 (1H, br, Thr-3-*H*), 6.81 (2H, d, *J* = 8.2 Hz, Tyr-6,8-*H*), 6.88 (2H, d, *J* = 6.3 Hz, Tyr-5,9-*H*), 7.06–7.18 (10H, m, $C_6H_5\times2$), 7.35 (10H, s, $P(OCH_2C_6H_5)\times2$); ^{13}C NMR (67.8 MHz, CD_3OD) δ 11.4, 16.5, 18.4, 22.4, 26.2, 30.7, 31.7, 34.2, 36.4, 37.3, 38.7, 50.1, 50.7, 50.8, 52.6, 56.1, 57.5, 63.1, 70.7, 70.8, 70.9, 71.0, 72.0, 73.6, 75.7, 116.7, 127.4, 127.5, 128.9, 129.1, 129.3, 129.6, 129.8, 130.3, 130.5, 131.7, 136.9, 137.1, 137.5, 138.9, 157.7, 170.5, 170.7, 171.3, 172.7, 173.0, 174.6; HRFABMS (*m*-nitrobenzyl alcohol) calcd for $C_{60}H_{71}N_6O_{15}P$ $[M+Na]^+$: 1169.4613. found 1169.4620.

4.1.35. Micropeptin T-20 (1). To a solution of Ahp product **47** (10 mg, 4.3 µmol) in 90% aqueous EtOH (1 ml) was added 10% Pd–C (10 mg) under an argon atmosphere. The black slurry was stirred under 1 atom of H_2 at room temperature for 1 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo to give a deprotected product (7.8 mg, 89%) as a pale yellow solid.

The above deprotected product was treated with pH 7 phosphate buffer–MeOH (2:3, 1 ml) and the mixture was stirred at room temperature for 1 h. After concentration, the residue was purified by ODS silica gel column chromatography (BW-300 MH, H_2O then MeOH) to give **1** (6.8 mg, 77%) as a pale yellow solid: $[\alpha]_D^{23} = -24.6$ (*c* 0.15, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 0.87 (3H, t, *J* = 7.4 Hz, Ile-5-H), 0.91 (3H, d, *J* = 6.7 Hz, Ile-6-*H*), 1.07–1.15 (1H, m, Ile-4-*H*), 1.27 (3H, d, *J* = 6.4 Hz, Thr-4-*H*), 1.28–1.35 (1H, m, Ile-4-*H*), 1.60–1.66, 1.68–1.75 (2H, m, Ahp-5-*H*), 1.77–1.89 (2H, m, Ahp-4-*H*, Ile-3-*H*), 1.98–2.04 (1H, m, Tyr-3-*H*), 2.56–2.66 (1H, m, Ahp-4-*H*), 2.66–2.80 (2H, m, Phe(1)-3-*H*, Phe(2)-3-*H*), 2.85 (3H, s, N*Me*), 2.92–2.98 (1H, m, Tyr-3-*H*), 3.36–3.45 (2H, m, Phe(1)-3-*H*, Phe(2)-3-*H*), 3.83 (1H, ddd, *J* = 6.7, 9.4, 12.2 Hz, Ahp-3-*H*), 4.00–4.07 (1H, m, Ga-3-*H*), 4.13–4.18 (2H, m, Ga-2,3-*H*), 4.53 (1H, br, Thr-2-*H*), 4.65–4.71 (2H, m, Ile-2-*H*, Phe(1)-2-*H*), 4.90–4.96 (1H, m, Tyr-2-*H*), 5.03–5.06 (1H, m, Phe(2)-2-*H*), 5.16 (1H, br, Ahp-6-*H*), 5.46–5.52 (1H, m, Thr-3-*H*), 6.82 (2H, d, *J* = 8.5 Hz, Tyr-6,8-*H*), 6.89 (2H, d, *J* = 7.1 Hz, Tyr-5,9-*H*), 7.08–7.21 (10H, m, $C_6H_5\times2$); ^{13}C NMR (500 MHz, CD_3OD) δ 11.6, 16.7, 18.2, 22.4, 26.2,

30.6, 31.6, 34.3, 36.4, 37.4, 39.1, 50.8, 52.5, 55.8, 56.1, 57.4, 63.2, 68.3, 73.5, 73.7, 75.8, 116.7, 127.5, 127.7, 129.1, 129.4, 129.5, 130.1, 130.7, 131.8, 137.7, 139.0, 157.7, 190.9, 171.2, 171.5, 172.9, 173.3, 174.3, 174.6; HRFABMS (*m*-nitrobenzyl alcohol) calcd for $C_{46}H_{57}N_6Na_2O_{15}P$ $[M+H]^+$: 1011.3492. Found: 1011.3533.

4.1.36. Allyl (*R*)-2,3-dihydroxypropanoate (49). To a solution of alcohol **48** (1.0 g, 7.56 mmol) in H_2O (30 ml) was added KOH (1.0 g, 15.1 mmol) in H_2O (20 ml) and $KMnO_4$ (1.8 g, 11.3 mmol) in H_2O (30 ml) at 0 °C. The mixture was stirred at 0 °C for 2 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo. The residue was acidified to pH 4 with 1 M aqueous $KHSO_4$, salted out, and extracted with EtOAc (×3). The extracts were dried over Na_2SO_4, concentrated in vacuo to give a crude carboxylic acid as a colorless oil which was used for the next step without further purification.

To a solution of the above crude carboxylic acid in DMF (20 ml) was added $KHCO_3$ (1.5 g, 15.0 mmol) and allyl bromide (1.03 ml, 11.4 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 10 h. After dilution with Et_2O, the whole mixture was successively washed with 1 M aqueous $KHSO_4$, H_2O, saturated aqueous $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, and concentrated in vacuo to give a crude allyl ester as a colorless oil which was used for the next step without further purification.

The above crude ester was treated with 1 N aqueous HCl–THF (1:1, 20 ml) at room temperature. The mixture was stirred for 5.5 h, then concentrated. After dilution with EtOAc, the whole mixture washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:1) to give **49** (440 mg, 40% in 3 steps) as a colorless oil: $[\alpha]_D^{22}=+21.6$ (*c* 1.1, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3389, 2945, 1748, 1615, 1205, 1120, 1067; ^1H NMR (270 MHz, $CDCl_3$) δ 2.60–2.74 (1H, br, O*H*), 3.47–3.57 (1H, br, O*H*), 3.85–4.00 (2H, m, *CH*$_2$OH), 4.27–4.37 (1H, m, C*H*OH), 4.72 (2H, d, *J*=5.6 Hz, CO_2CH_2), 5.26–5.39 (2H, m, $CO_2CH_2CHCH_2$), 5.85–6.00 (1H, m, $CO_2CH_2CHCH_2$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 64.0, 65.5, 71.6, 119.1, 131.1, 172.5; Anal. calcd for $C_6H_{10}O_4\cdot 1/6H_2O$: C, 48.32; H, 6.98. Found: C, 48.43; H, 7.03.

4.1.37. Allyl (*R*)-2,3-bis(*tert*-butyldimethylsiloxy)propanoate. Obtained from **49** according to the preparation of **26**. A colorless oil: $[\alpha]_D^{23}+16.4$ (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 2955, 2930, 2887, 1759, 1472, 1257, 1148, 1128; ^1H NMR (270 MHz, $CDCl_3$) δ 0.05 (6H, s, Si*Me*$_2$), 0.08, 0.09 (6H, s×2, Si*Me*$_2$), 0.88 (9H, s, *tBu*), 0.90 (9H, s, *tBu*), 3.73–3.87 (2H, m, *CH*$_2$OSi), 4.29 (1H, t, *J*=5.3 Hz, *CH*OSi), 4.62 (2H, d, *J*=5.6 Hz, CO_2CH_2), 5.22–5.37 (2H, m, $CO_2CH_2CHCH_2$), 5.86–5.97 (1H, m, $CO_2CH_2CHCH_2$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.3, −4.9, 18.4, 25.8, 25.9, 65.4, 66.0, 74.0, 118.4, 131.8, 172.5. Anal. calcd for $C_{12}H_{24}O_4Si$: C, 57.70; H, 10.22. Found: C, 57.46; H, 10.36.

4.1.38. Allyl (*R*)-(*tert*-butyldimethylsiloxy)-3-hydroxypropanoate. Obtained from the above bis(TBS)propanoate according to the preparation of **27**. A colorless oil: IR ν_{max}^{neat} cm^{-1} 3474, 2932, 1755, 1615, 1257, 1142; ^1H NMR (270 MHz, $CDCl_3$) δ 0.10, 0.14 (6H, s×2, Si*Me*$_2$), 0.92 (9H, s, *tBu*), 2.15–2.24 (1H, br, O*H*), 3.80–3.86 (2H, br, *CH*$_2$OH), 4.32 (1H, t, *J*=4.6 Hz, *CH*OSi), 4.65 (2H, d, *J*=5.4 Hz, CO_2CH_2), 5.24–5.38 (2H, m, $CO_2CH_2CHCH_2$), 5.85–5.97 (1H, m, $CO_2CH_2CHCH_2$).

4.1.39. Allyl (*R*)-2-(*tert*-butyldimethylsiloxy)propanoate 3-dibenzyl phosphate (50). Obtained from the above mono(TBS)propanoate according to the preparation of **3b**. A colorless oil: $[\alpha]_D^{22}=+12.6$ (*c* 0.6, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 2955, 2930, 1759, 1456, 1283, 1260, 1153, 1018; ^1H NMR (270 MHz, $CDCl_3$) δ 0.07, 0.09 (6H, s×2, Si*Me*$_2$), 0.89 (9H, s, *tBu*), 4.11–4.18 (1H, m, *CH*$_2$OP), 4.24–4.32 (1H, m, *CH*$_2$OP), 4.37–4.40 (1H, m, *CH*OSi), 4.60 (2H, d, *J*=5.7 Hz, CO_2CH_2), 5.01, 5.03 (2H, s×2, *CH*$_2$C$_6$H$_5$), 5.04, 5.06 (2H, s×2, *CH*$_2$C$_6$H$_5$), 5.21–5.34 (2H, m, $CO_2CH_2CHCH_2$), 5.83–5.95 (1H, m, $CO_2CH_2CHCH_2$), 7.33 (10H, s, C$_6$*H*$_5$×2); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.2, −4.9, 18.4, 25.7, 65.9, 68.9, 69.0, 69.3, 69.4, 71.6, 71.7, 118.8, 127.8, 128.3, 128.4, 131.4, 135.5, 135.6, 169.9. Anal. calcd for $C_{26}H_{37}O_7PSi\cdot 1/2Et_2O$: C, 60.30; H, 7.59. Found: C, 60.42; H, 7.45.

4.1.40. (*R*)-2′-(*tert*-Butyldimethylsiloxy)-1′-cyclo[(*S*)-Thr-(*S*)-Phe-(*S*)-Phs-(*S*)-Phe-(*S*)-*N*-Me-Tyr(TBS)-(*S*)-Ile]-propanoate 3′-dibenzyl phosphate (51). Cyclic peptide **34** (100 mg, 0.10 mmol) was treated with 4 N HCl–dioxane (2 ml) at 0 °C and the mixture was stirred at 0 °C for 1 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless solid.

To a solution of phosphate **50** (80 mg, 0.15 mmol) in THF (1 ml) was added $(PPh_3)_4Pd$ (18 mg, 15 μmol) and morpholine (20 μl, 0.22 mmol) at room temperature. The mixture was stirred for 1 h. After dilution with EtOAc, the whole mixture was washed with 1 M aqueous $KHSO_4$ and brine, dried over Na_2SO_4, and concentrated in vacuo to give a crude carboxylic acid as a yellow oil which was used for the next step without further purification.

To a solution of the above crude amine salt and carboxylic acid in DMF (1 ml) was added DEPC (24 μl, 0.15 mmol) and Et_3N (35 μl, 0.25 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 13 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc=1:5) to give **51** (118 mg, 85%) as a colorless solid: $[\alpha]_D^{22}=-2.9$ (*c* 0.3, $CHCl_3$); IR ν_{max}($CHCl_3$) cm^{-1} 3389, 3282, 2957, 2990, 1735, 1682, 1649, 1510, 1259, 1021; ^1H NMR (270 MHz, $CDCl_3$) δ −0.06, −0.02 (6H, s×2, Si*Me*$_2$), 0.10, 0.12 (6H, s×2, Si*Me*$_2$), 0.68–0.93 (6H, m, Ile-5,6-*H*), 0.83, 0.93 (18H, s×2, *tBu*×2), 1.00–1.44 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.70–1.90 (5H, m, Ile-3-*H*, Phs-3,4-*H*), 2.55–2.67 (2H, m, Tyr-3-*H*), 2.82, 2.86 (3H, s×2, N*Me*), 2.75–2.96 (2H, m, Phe(2)-3-*H*), 3.03–3.28 (2H, m, Phe(1)-3-*H*), 3.58–3.68 (2H, m, Phs-5-*H*), 3.95–4.16 (1H, m, Phs-2-*H*), 4.18–4.33 (1H, m, Ga-2-*H*), 4.35–4.55 (4H, m, Ga-3-*H*, Ile-2-*H*, Thr-2-*H*), 4.55–4.65 (1H, m, Phe(1)-2-*H*), 4.93–5.10 (6H, m, Tyr-2-*H*, Phe(2)-2-*H*, P(O*CH*$_2$C$_6$H$_5$)×2), 5.20–5.30 (1H,

br, Thr-3-*H*), 6.66 (2H, d, $J=8.3$ Hz, Tyr-6,8-*H*), 6.94 (2H, d, $J=8.4$ Hz, Tyr-5,9-*H*), 7.15–7.31 (20H, m, $C_6H_5\times4$); ^{13}C NMR (67.8 MHz, CD_3OD) δ −5.0, −4.4, −4.3, 12.4, 14.9, 17.9, 18.3, 18.9, 19.0, 26.1, 26.4, 27.7, 29.8, 30.4, 34.4, 38.2, 38.5, 51.3, 52.9, 56.2, 56.6, 58.5, 62.1, 63.5, 70.8, 70.9, 73.2, 73.9, 121.2, 127.6, 129.1, 129.2, 129.3, 129.4, 129.6, 129.7, 129.9, 130.1, 131.1, 131.6, 133.0, 133.7, 136.9, 137.0, 137.7, 138.4, 155.6, 170.4, 170.5, 171.4, 172.0, 173.6, 174.3, 174.6; FABMS (*m*-nitrobenzyl alcohol) *m/z* 1380 $[M+H]^+$.

4.1.41. (*R*)-2′-Hydroxy-1′-cyclo[(*S*)-Thr-(*S*)-Phe-(*S*)-Phs-(*S*)-Phe-(*S*)-*N*-Me-Tyr(TBS)-(*S*)-Ile]-propanoate 3′-dibenzyl phosphate (52). To a solution of cyclic peptide **51** (20 mg, 14 μmol) in DMSO (0.4 ml) was added IBX (16 mg, 58 μmol) at room temperature. The mixture was stirred for 4 h. After dilution with EtOAc, the whole mixture was washed with H_2O (×2) and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by thin layer chromatography ($CHCl_3$–MeOH=10:1) to give aldehyde (9 mg, 45%) as a colorless solid.

To a solution of the above aldehyde in THF (0.3 ml) was added 1 N TBAF in THF (23 μl, 23 μmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then concentrated. The residue was purified by thin layer chromatography ($CHCl_3$–MeOH=10:1) to give Ahp product **52** (5.0 mg, 75%) as a colorless solid: ^{1}H NMR (270 MHz, CD_3OD) δ 0.85–0.94 (6H, m, Ile-5,6-*H*), 1.03–1.20 (2H, m, Ile-4-*H*), 1.25 (3H, d, $J=6.6$ Hz, Thr-4-*H*), 1.58–1.90 (4H, m, Ile-3-*H*, Ahp-4,5-*H*), 1.92–2.02 (1H, br, Tyr-3-*H*), 2.55–2.80 (3H, m, Ahp-4-*H*, Phe(1)-3-*H*, Phe(2)-2-*H*), 2.84 (3H, s, N*Me*), 2.85–3.02 (1H, m, Tyr-3-*H*), 3.20–3.40 (2H, m, Phe(1)-3-*H*, Phe(2)-3-*H*), 3.79–3.86 (1H, m, Ahp-3-*H*), 4.03–4.13, 4.18–4.27 (2H, m, Ga-3-*H*), 4.28–4.45 (1H, br, Ga-2-*H*), 4.52 (1H, br, Thr-2-*H*), 4.60 (1H, d, $J=6.0$ Hz, Ile-2-*H*), 4.65–4.70 (1H, m, Phe(1)-2-*H*), 4.95–5.13 (6H, m, Tyr-2-*H*, Phe(2)-2-*H*, $P(OCH_2C_6H_5)\times2$), 5.16 (1H, br, Ahp-6-*H*), 5.46–5.53 (1H, br, Thr-3-*H*), 6.80 (2H, d, $J=8.4$ Hz, Tyr-6,8-*H*), 6.88 (2H, d, $J=6.4$ Hz, Tyr-5,9-*H*), 6.98–7.25 (10H, m, $C_6H_5\times2$), 7.37 (10H, s, $P(OCH_2C_6H_5)\times2$).

4.1.42. (2′*R*)-Micropeptin T-20 (53). To a solution of Ahp product **52** (5.0 mg, 4.3 μmol) in 90% aqueous EtOH (0.4 ml) was added 10% Pd–C (4 mg) under an argon atmosphere. The black slurry was stirred under 1 atom of H_2 at room temperature for 1 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo to give a deprotected product (4.5 mg, quant.) as a colorless solid.

The above deprotected product was treated with pH 7 phosphate buffer–MeOH (2:3, 0.5 ml) and the mixture was stirred at room temperature for 0.5 h. After concentration, the residue was purified by ODS silica gel column chromatography (BW-300 MH, H_2O then MeOH) to give **53** (3.5 mg, 81%) as a colorless solid: $[\alpha]_D^{24}=-11.9$ (*c* 0.1, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 0.87 (3H, t, $J=7.5$ Hz, Ile-5-*H*), 0.91 (3H, d, $J=6.7$ Hz, Ile-6-*H*), 1.07–1.15 (1H, m, Ile-4-*H*), 1.24 (3H, d, $J=6.4$ Hz, Thr-4-*H*), 1.30–1.37 (1H, m, Ile-4-*H*), 1.60–1.90 (5H, m, Ahp-4,5-*H*, Ile-3-*H*), 1.98–2.05 (1H, m, Tyr-3-*H*), 2.56–2.62 (1H, m, Ahp-4-*H*), 2.68–2.79 (2H, m, Phe(1)-3-*H*, Phe(2)-3-*H*), 2.85 (3H, s, N*Me*), 2.92–2.98 (1H, m, Tyr-3-*H*), 3.35-3.44 (2H, m, Phe(1)-3-*H*, Phe(2)-3-*H*), 3.80–3.87 (2H, m, Ahp-3-*H*, Ga-3-*H*), 4.08–4.16 (1H, m, Ga-3-*H*), 4.30–4.33 (1H, m, Ga-2-*H*), 4.52 (1H, br, Thr-2-*H*), 4.65 (1H, d, $J=5.5$ Hz, Ile-2-*H*), 4.70 (1H, dd, $J=4.2$, 10.5 Hz, Phe(1)-2-*H*), 4.93–4.96 (1H, m, Tyr-2-*H*), 5.02–5.07 (1H, m, Phe(2)-2-*H*), 5.16 (1H, br, Ahp-6-*H*), 5.46–5.50 (1H, m, Thr-3-*H*), 6.84 (2H, d, $J=9.4$ Hz, Tyr-6,8-*H*), 6.89 (2H, d, $J=7.0$ Hz, Tyr-5,9-*H*), 7.09–7.20 (10H, m, $C_6H_5\times2$); ^{13}C NMR (500 MHz, CD_3OD) δ 11.6, 16.6, 18.1, 22.3, 26.3, 30.6, 31.7, 34.2, 36.4, 37.3, 39.0, 50.8, 52.6, 55.8, 56.1, 57.4, 63.2, 68.3, 73.8, 73.9, 75.8, 116.7, 127.6, 127.7, 129.1, 129.4, 129.6, 130.0, 130.6, 131.8, 137.7, 138.9, 157.7, 170.9, 171.2, 171.6, 172.9, 173.3, 174.2, 174.6; FABMS (*m*-nitrobenzyl alcohol) *m/z* 1011 $[M+H]^+$.

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