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Synthetic Studies on Antifungal Cyclic Peptides, Echinocandins. Stereoselective Total Synthesis of Echinocandin D via a Novel Peptide Coupling

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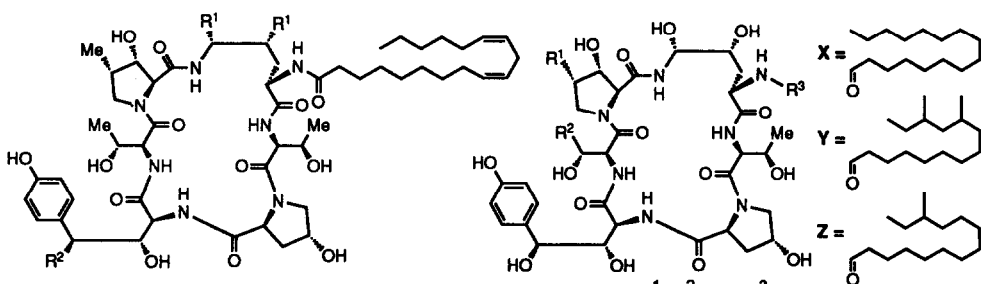
Abstract: *Synthetic studies on the novel fungicidal oligopeptides, echinocandins (**1b** and **1c**), are described. The constituent amino acids **5-8** were synthesized in a stereocontrolled manner from the chiral starting materials, **5a**, **6a** and **7a**, respectively. The coupling of these amino acids was characterized by the use of unprotected amino acid as the C-terminal and 2-pyridyl thiol ester as the N-terminal, and the coupling was performed in the presence of 1-(trimethylsilyl)imidazole (TMSIm) or a catalytic amount of tert-amine to give C-terminal free dipeptides, **14** and **16a**, respectively, which were converted to the pentapeptide **17a**, a common intermediate for the synthesis of **1b** and **1c**. The synthesis of **1c** was achieved by the cyclization of the hexapeptide **24b**.*

Echinocandins (**1a-1c**) are novel oligopeptide antibiotics isolated from a strain of *Aspergillus ruglosus* or *Aspergillus nidulans*.[1,2] These structures were shown to be a unique 21-membered cyclic hexapeptide by chemical degradation studies[1,2] in combination with X-ray crystallographic analysis of one of their derivatives.[3,4] Echinocandins attract growing interest due to their potent fungicidal activities against *Candida albicans*, a clinically important pathogen, by inhibiting the synthesis of 1,3-β-glucan, an integral component of the fungal cell wall.[5] A series of related natural products, aculeacins (**2**),[6] pneumocandins (**3a** and **3b**),[7-9] and mulundocandins (**4**)[10] have also been reported.

Figure 1.



Echinocandin
B (**1a**), R^1=R^2=OH
C (**1b**), R^1=OH, R^2=H
D (**1c**), R^1=R^2=H

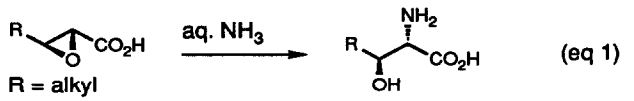
Aculeacine Aγ (**2**) R^1=R^2=Me, R^3=X
Pneumocandin A_0 (L-671,329) (**3a**)
R^1=Me, R^2=CH_2CONH_2, R^3=Y
Pneumocandin B_0 (L-688,786) (**3b**)
R^1=H, R^2=CH_2CONH_2, R^3=Y
Mulundocandin (**4**) R^1=Me, R^2=H, R^3=Z

These fungal metabolites vary in several of the amino acid residues, the lipophilic side chain and the extent of hydroxylation, yet all retain potent antifungal properties. Recent studies have demonstrated that these oligopeptides are effective in the treatment of *Pneumocysis carinii* infections, which are fatal pneumonitis among HIV patients and other immunocompromised host.[11]

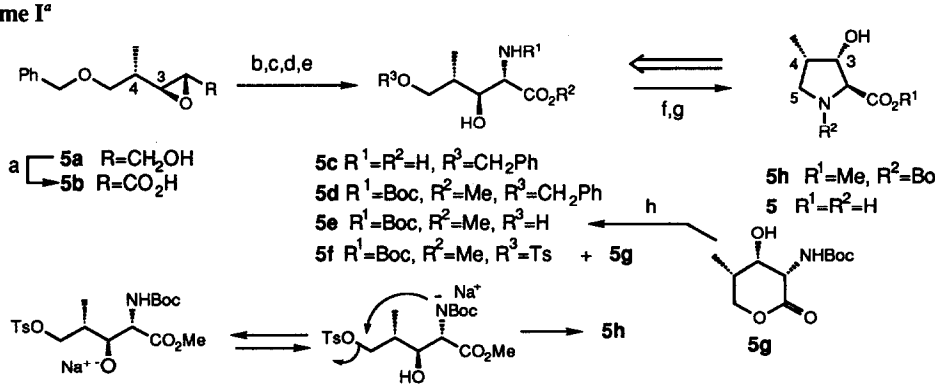
A feature common to the constituent amino acids of these peptides is the 1,2- or 1,3-amino hydroxyl system, which is often found in biologically active natural products.[12] Recent works from this labolatory have described stereoselective approaches for the syntheses of both *syn* and *anti* β/γ-hydroxy α-amino acids.[13] In order to extend the scope of application of these methods as well as to develop efficient coupling methods for the hydroxylated amino acids, we chose echinocandins as the synthetic target. Since echinocandins possessing a benzylic hydroxyl group are unstable toward acid and bases,[1,2] echinocandin C (**1b**) and D (**1c**) were chosen as the target. Herein we wish to detail the stereoselective synthesis of the constituent amino acids, and incorporation of these amino acids into an efficient synthesis of echinocandin D (**1c**).[14,15] The synthesis involves, in part, new peptide coupling reactions using unprotected amino acids. Approaches to the synthesis of **1b** are also disclosed.

Synthesis of (3*S*,4*S*)-3-hydroxy-4-methy-L-proline (Hmp) (5)

To date, Hmp (**5**) has been found only in the echinocandin-type peptide antibiotics.[16] Biosynthesis of **5** in pneumocandins (L-671329) (**3a**) is shown to be derived from L-leucine.[17] We planned the synthesis of **5** by disconnection of its N-C5 providing a leucine-type intermediate (e.g., **5c**) in which the adjacent 3*S*,4*S* chiral centers corresponded to those of the known epoxy alcohol **5a**.[18] Thus, the synthesis of **5** began with **5a**, which upon oxidation with pyridinium dichromate (PDC) in *N*,*N*-dimethylformamide (DMF) afforded in 79% yield the α,β-epoxy carboxylic acid **5b**. It has been reported that addition of amines to an α,β-epoxy carboxylic acid results in regioselective ring cleavage with inversion to give β-hydroxy α-amino acid (eq 1).[19]



Scheme I[a]



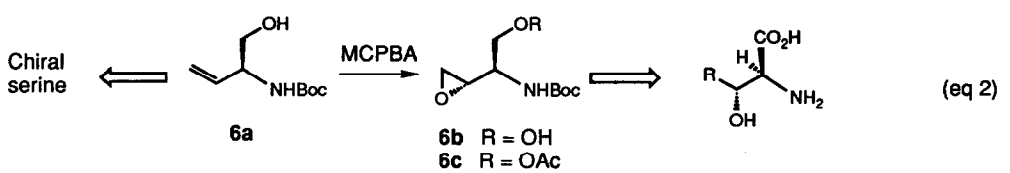
[a] (a) Pyridinium dichromate (PDC), DMF, room temperature, 45 h, 79%; (b) 28% aqueous NH_3, room temperature, 5 days, 72%; (c) (1) Boc-ON, Et_3N, dioxane/H_2O, room temperature, 4 h; (2) CH_2N_2, 93%: (d) H_2/10% Pd-C, MeOH, room temperature, 20 h; (e) TsCl, pyridine, room temperature, 8 h, 98% from **5d**; (f) 1.0 equiv of NaH, THF, 0 °C, 1.5 h, 62%; (g) (1) 0.5 N NaOH, CH_2Cl_2, 0 °C, 14 h; (2) CF_3COOH, CH_2Cl_2, room temperature, 30 min; (3) Dowex 50w x 4 (H^+ form; elution with 1 *N* NH_3, 86%; (h) (1) 0.5 N NaOH, 0 °C, 16 h; (2) CH_2N_2, 96%.

Thus, this was treated with 28% aqueous ammonia to give, as expected, the desired β-hydroxy α-amino acid **5c** (72%) as the exclusive regioisomer. In order to perform further chemical transformations, the amino acid **5c** was converted to the protected form **5d** with (1) 2-*tert*-butoxycarbonyloxyimino-2-phenylacetonitrile (Boc-ON)/triethylamine (Et_3N) and (2) CH_2N_2. Removal of the benzyl group of **5d** was carried out using 4 ~ 5 atm of H_2/10% Pd-C in MeOH to give a mixture of the desired diol **5e** and the lactone **5g**. The yields of the mixture were excellent but the ratio varied from **5e**/**5g** = 3:1 ~ 3:2 probably due to a slight difference in the reaction conditions. The mixture was separated after treatment with 1.0 equiv of *p*-toluenesulfonyl chloride (TsCl). The mixture gave the desired monotosylate **5f**, and the recovered lactone **5g** which was converted into **5f** by the following sequence of reactions; (1) hydrolysis with 0.5 *N* NaOH, (2) esterification of the resulting carboxylic acid with CH_2N_2, and (3) tosylation with TsCl/pyridine. The combined yields of **5f** from **5d** were always 85-90%. Initial attempts to obtain the pyrrolidine **5h** from the tosylate **5f** were not satisfactory because of poor yields; e.g., lithium diisopropylamide (15%), lithium bis-(trimethylsilyl)amide (30%), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) > (10%), and *N,N*-diisopropylethylamine (trace). Finally, the use of 1.0 equiv of NaH gave **5h** in 62% yield. The use of excess amounts of NaH (2 equiv) resulted in a decrease in yields (>5%). These results suggested that the reaction proceeded via an initial alkoxide formation and subsequent inter- or external proton migration from an amide group to form an anion at the amide terminal, which underwent S_N2 type substitution at the C5 tosyl group to give **5h**. Excess base might produce dianion both at the hydroxyl and the amide termini, which would accelerate side reactions such as retro-aldol and/or polymerization to give an unidentifiable mixture of products.

The protecting groups of **5h** were removed in two steps, (1) 0.5 *N* NaOH and (2) trifluoroacetic acid (TFA). Treatment of the resulting TFA salt with Dowex 50Wx4 (H^+ form, eluted with 1 *N* NH_3) gave **5** in 86% yield. The synthetic material showed completely identical physical constants and spectroscopic data with those reported.[1]

Synthesis of (3*R*)-hydroxy-L-homotyrosine (Hht) (6)

Recently, we reported the synthesis of chiral 2-amino-3-butenol derivative **6a** from L- or D-methionine,[20] which is useful not only as a masked form of chiral serine but also as a chiral synthon for the 1,2-amino hydroxyl system (eq 2).[13,21,22] In particular, the syn 1,2-amino hydroxyl system was constructed in a regio- and stereoselective manner by the epoxidation of **6a** with 2-chloroperbenzoic acid (MCPBA) followed by the nucleophilic opening of the resulting epoxide **6c**.[23]



6a

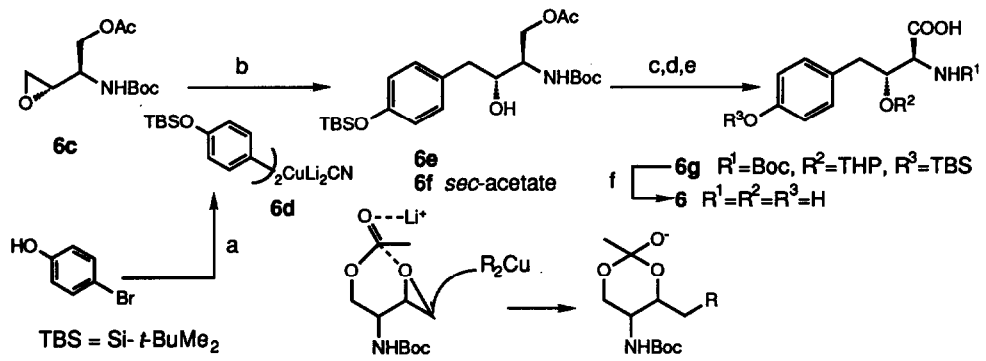
6b R = OH
6c R = OAc

(eq 2)

The epoxide **6c** possesses the requisite 2*R*,3*S* chiral centers corresponding to those of Hht (**6**). The coupling of **6c** with diaryl cuprate **6d**, prepared from 4-bromophenol, underwent regiospecific opening of the epoxide to give a mixture of the desired acetate **6e** (65%) and the *sec*-acetate **6f** (15%). Partial migration of the primary acetoxyl group to the secondary position might be attributed to the putative formation of an orthoester type intermediate (Scheme II).[23] It is noted that nucleophilic opening of the epoxide using a cuprate

reagent required its hydroxyl group to be protected with an electron-deficient group such as acetyl or benzoyl ester group. An electron-donating group such as *tert*-butyldimethylsily (TBS) or benzyl (Bzl) group was not suitable and resulted in reduced yields of products (<10%).[23] The primary acetate **6e** was converted into the *C*-terminal free Hht (**6g**) having appropriate protecting groups for the peptide coupling (*vide infra*). This transformation was performed in 65% overall yield by the following sequence of reactions; (1) protection of the *sec*-hydroxyl group with tetrahydropyranyl (THP) group, (2) removal of the acetoxyl group, and (3) oxidation with PDC/DMF. Further treatments of **6g** with TFA followed by Dowex 50Wx4 (elution with 1 *N* NH_3) afforded Hht (**6**) in 78% yield, which has not, yet, been isolated from echinocandins by the chemical degradation studies.[1,2,24]

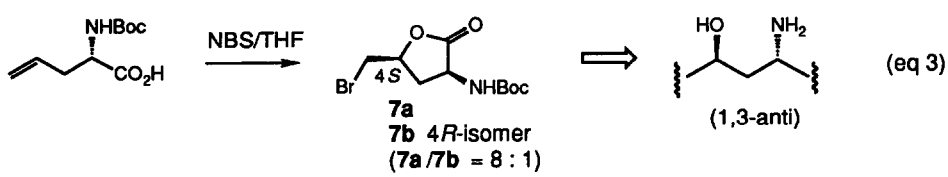
Scheme II[a]



[a](a) (1) *tert*-Butyldimethylsilyl chloride (TBSCl), imidazole, DMF, 0 °C, 1 h, room temperature, 30 min, 90%; (2) nBuLi, CuCN, THF, -78 °C, 2 h; (b) **6d**, THF, -78 °C, 30 min, 0 °C, 3 h, **6e**, 65% and **6f**, 15%; (c) dihydropyran, CSA, CH_2Cl_2, 0 °C, 1.5 h; (d) 0.1 equiv of K_2CO_3, MeOH, 0 °C~room temperature, 3.5 h; (e) PDC, DMF, room temperature, 24 h, 65% from **6e**; (f) (1) CF_3COOH, CH_2Cl_2, room temperature, 2 h; (2) Dowex 50w x 4 (H+ form; elution with 1 *N* NH_3), 78%.

Syntheses of γ-hydroxy α-amino acids, 4-hydroxyproline (7) and 4,5-dihydroxyornithine (8)

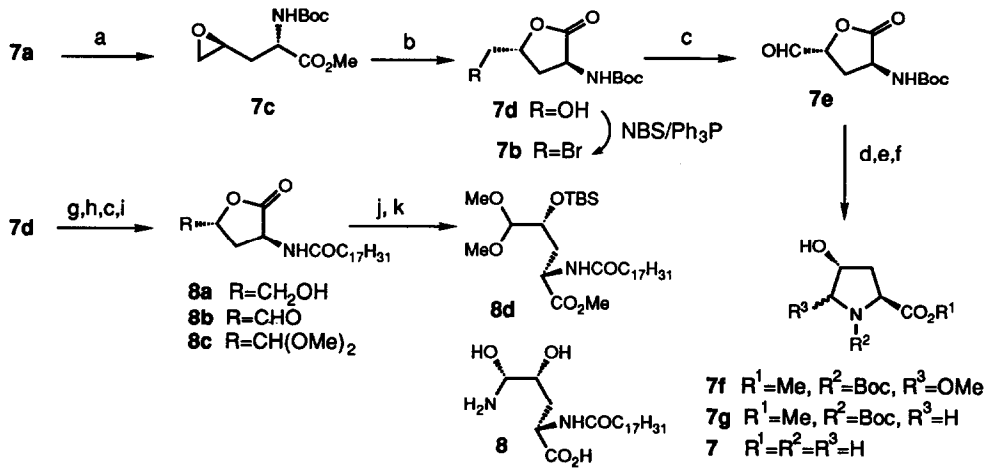
A feature common to the structures of 4-hydroxyproline (**7**) and 4,5-dihydroxyornithine (**8**) is the 1,3-amino hydroxyl system.[12,13] The pioneering work by Witkop et al.,[25] and our recent studies [13,26] concerning halolactonization of (2*S*)-2-amino-4-pentenoic acid (allylglycine) derivatives have demonstrated an efficient protocol for γ-lactonization (eq 3). This process was stereoselective to give *cis*-γ-butyrolactone **7a** as the major product which possessed 2*S*-amino and 4*S*-hydroxyl groups (1,3-anti stereochemistry).[26] Thus, we planned to synthesize both **7** and **8** from *cis*-γ-butyrolactone **7a**.



Due to the undesired C4 stereochemistry of **7a**, examined first was its inversion to the 4*R*-isomer. Methanolysis of **7a** yielded an epoxy ester **7c**, which upon treatment with 0.5 *N* NaOH followed by a catalytic amount of *dl*-camphorsulfonic acid (CSA), gave the desired *trans*-γ-butyrolactone **7d** (75% yield from **7b**).

This compound having the 4*R* configuration was ascertained by converting its hydroxyl group to the corresponding bromide (*N*-bromosuccinimide/triphenylphosphine), which was identical in all respects with the authentic **7b**, a minor isomer of the bromolactonization products of **7a**. Swern oxidation[27] of the alcohol **7d** gave the aldehyde **7e**. Ring opening of **7e** by methanolysis (*p*-TsOH/MeOH) was accompanied by a ring closure from the amino terminal to the aldehyde moiety to afford the desired pyrrolidine **7f** (78%). This was subjected to acid hydrolysis (60% acetic acid) followed by reductive amination[28] with sodium cyanoborohydride to give **7g** (41% from **7f**). Hydrolysis of the ester group with 0.5 *N* NaOH followed by the removal of the Boc group with TFA afforded **7**.[29]

Scheme III[a]



[a](a) K_2CO_3, MeOH, 0 °C, 30 min, 100%; (b) (1) 0.5 *N* NaOH, 0 °C, 14 h; (2) CSA, CH_2Cl_2, room temperature, 20 h, 75%; (c) $(COCl)_2$, CH_2Cl_2/DMSO, -78 °C, 15 min, -45 °C, 1 h, Et_3N, 0 °C, 15 min, 74%; (d) p-TsOH, MeOH, room temperature, 14 h; (e) (1) 60% AcOH, room temperature, 3 days; (2) $NaBH_3CN$, EtOH/60% AcOH, room temperature, 1 h; (f) (1) 0.5 *N* NaOH, 0 °C, 19 h; (2) TFA, CH_2Cl_2, room temperature, 15 min; (3) Dowex 50w x 4 (H^+ form; elution with 1 *N* NH_3), 41% from **7f**; (g) TFA, CH_2Cl_2, room temperature, 1 h; (h) $C_{17}H_{31}COSPy$, K_2CO_3, DMF, room temperature, 1 h; (i) 2,2-dimethoxypropane, CSA, CH_2Cl_2, room temperature, 14 h, 41% from **7d**; (j) (1) 0.5 *N* NaOH, 0 °C, 14 h; (2) CH_2N_2; (k) $Me_2(t\text{-Bu})SiOSO_2CF_3$, 2,6-lutidine, CH_2Cl_2, room temperature, 10 min, 87% from **8c**.

N^{α}-Linoleyl-γ,δ-dihydroxyornitine residue **8** was prepared as its protected form **8d** with both γ-and δ-hydroxyl groups protected to avoid γ-butyrolactonization and pyrrolidine formation. Thus, *trans*-lactone **7d** was converted to the dimethyl acetal **8c** (41% from **7d**) by the following sequence of reactions: (1) removal of the Boc group with TFA, (2) coupling of the linoleyl moiety using its thiopyridyl ester (*vide infra*), (3) Swern oxidation, and (4) acetal formation of the resulting aldehyde with 2,2-dimethoxypropane/CSA. Hydrolysis of **8c** with 0.5 *N* NaOH followed by a careful work-up (pH 3.5) gave carboxylic acid which was immediately esterified with CH_2N_2 to give an ester. The hydroxyl group of the ester was protected with the TBS group using Corey's method (*tert*-butyldimethylsilyl trifluoromethanesulfonate/2,6-lutidine)[30] to give **8d** (87% yield from **8c**).

Efficient coupling methods for the synthesis of C-terminal free dipeptides

A number of methods for peptide coupling have been reported. Almost all cases except the Shotten

Baumann method [31] employed amino esters for an amine component which always afforded dipeptides with an ester protected *C*-terminal. Therefore, it required removal of the ester group prior to carring out further coupling reactions. To our knowledge, only one example has been reported of the synthesis of *C*-terminal free peptide which employed *N*-trimethylsilyl-α-amino acid trimethylsilyl ester as the amine component. The *N,O*-bis-trimethylsilylated amino acid was reacted with active ester and the trimethylsilyl groups of the resulting dipeptide were removed under the work-up conditions.[32] For the total synthesis of echinocandins, mild conditions seemed to be essential in order to avoid side reactions such as racemization and β-elimination of the hydroxyl group of the amino acids. Thus, an efficient method for the coupling of functionalized amino acids was examined first. In order to carry out the entire process (preparation and coupling) under neutral conditions, the 2-pyridyl thiol ester was chosen as the acid component, although such methods have not often been used.[33] On the other hand, unprotected amino acids were chosen as the amine component which would provide the following advantages: (i) protection from racemization by zwitterion formation, (ii) shortening the synthetic sequences, and (iii) synthesis of carboxylic acid free peptide.

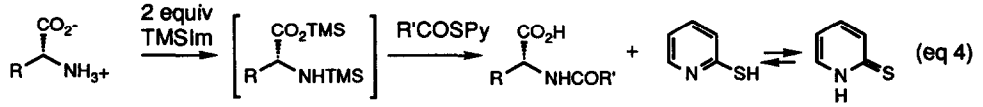
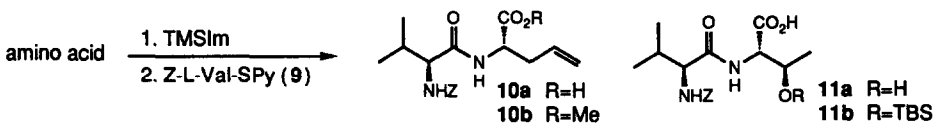


Table 1. Peptide coupling of unprotected amino acids with Z-L-Val-SPy (**9**) effected by TMSIm.[a]



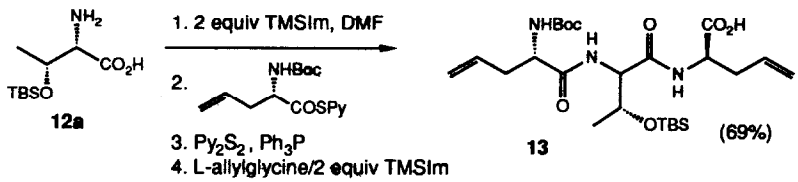
Amino acid	TMSIm	solvent	product	yield[b] (%)
L-allylglycine	1 equiv	DMF	**10a**	22
L-allylglycine	2 equiv	DMF	**10a**	84[c]
L-allylglycine	3 equiv	DMF	**10a**	trace
L-allylglycine	2 equiv	THF	**10a**	68
L-allylglycine	2 equiv	dioxane	**10a**	77
L-methionine	2 equiv	DMF	Z-L-Val-L-Met-OH	89
L-phenylalanine	2 equiv	DMF	Z-L-Val-L-Phe-OH	86[c]
L-threonine	3 equiv	DMF	**11a**	56[c]
O-TBS-L-threonine	2 equiv	DMF	**11b**	70[c]
L-proline	1 equiv	DMF	Z-L-Val-L-Pro-OH	trace

[a]All reactions were carried out at room temperature for 2-16 h. [b]Isolated yield. [c]Isolated as its methyl ester.

Unprotected amino acids exist in zwitterion form and are quite insoluble in organic solvents. We thought that *in situ* protection of the carboxylate moiety followed by its removal under the work-up would provide an answer to this problem (eq 4). As a model study, unprotected L-allylglycine was treated with 2 equiv of 1-(trimethylsilyl)imidazole (TMSIm) in DMF at room temperature. The suspension after 30 min gave a clear solution. To this solution was added a solution of *N*-benzyloxycarbonyl-L-valine 2-pyridyl thiol ester (Z-L-Val-Spy) (**9**)[34] in DMF. After work-up, Z-L-Valyl-L-allylglycine (**10a**) was obtained in 84% yield (Table 1).

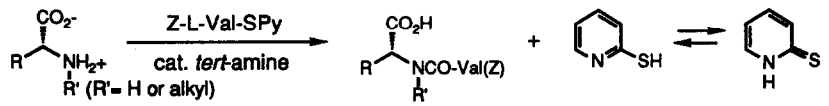
Thus, the entire process could be carried out in an one pot. In order to examine the extent of racemization, the peptide **10a** was esterified with CH_2N_2 to the corresponding methyl ester **10b**. On the other hand, a diastereomeric mixture of Z-L-valyl-D- and L-allylglycine methyl esters were prepared in the same manner as above. Comparison of this mixture with **10b** using ^1H NMR (360 MHz) and HPLC revealed that less than 1% (if any) of racemization in **10b** was encountered (see, experimental section). The use of 1.0 equiv of TMSIm resulted in a decrease in yield (22%). Only trace amounts of **10a** were obtained by the use of 3.0 equiv of TMSIm probably due to the formation of *N,N*-bistrimethylsilylamino trimethylsilyl ester. These results suggested that an initially formed *N*-trimethylsilylamino acid trimethylsilyl ester was an active form (eq 4) which subsequently coupled with the active ester **9** to give **10a**. As shown in Table I, DMF was found to be superior to other solvents. This method is applicable for other unprotected α-amino acids which gave the corresponding dipeptides in excellent yields. In the case of hydroxylated amino acids such as L-threonine, the reaction required 3 equiv of TMSIm. In this case, the *sec*-hydroxyl group might be also trimethylsilylated. In the case of *O*-TBS-L-threonine, 2 equiv of TMSIm was satisfactory and the yield was slightly improved to give **11b**. As an additional example, the tripeptide **13** was synthesized by this method in 4 steps in 69% overall yield (Scheme IV).[35] However, a limitation of this method was observed in the case of imino acids such as L-proline. The reaction gave only a trace amount of Z-L-Val-L-Pro-OH. This may be due to a much stronger zwitterion than that of amino acids. Therefore, the requisite TMS ester would not be formed *in situ*.

Scheme IV



It was found that *tert*-amines (triethylamine or *N,N*-diisopropylethylamine) catalyzed such peptide coupling (Table 2). Upon treatment of L-proline with **9**, Z-L-Val-L-Pro-OH was produced in excellent yield. This process did not require TMSIm. The formation of external ion pair (carboxyl group with *tert*-amine) might generate some nucleophilic character at the α-amino group which reacts with the activated ester. Exchange of the *tert*-amine salt between the product and substrate might occur; the resulting pyridine thiol or 2-thiopyridone would not form an ion pair with *tert*-amine due to its neutral nature. Therefore, the reaction proceeded in a catalytic manner. However, in the case of amino acids using *tert*-amine, the peptide coupling was very slow (~one week), probably because of poor nucleophilicity of primary amines. Thus, we developed the complementary methods for coupling of unprotected amino and imino acids.

Table 2. *tert*-Amine catalyzed peptide coupling of unprotected amino acids with Z-L-Val-SPy (**9**).[a]



amino acid	*tert*-amine	reaction time	product	yield[b] (%)
L-proline	0.2 equiv Et_3N	16 h	Z-L-Val-L-Pro-OH	89
L-proline	0.2 equiv iPr_2NEt	72 h	Z-L-Val-L-Pro-OH	85
L-proline	0.1 equiv DBU	66 h	Z-L-Val-L-Pro-OH	73
L-proline	no base	72 days	Z-L-Val-L-Pro-OH	37
L-azetidine-2-carboxylic acid (L-Aze-OH)	0.1 equiv iPr_2NEt	14 h	Z-L-Val-L-Aze-OH	86
L-allylglycine	0.1 equiv iPr_2NEt	6 days	**10a**	71
L-methionine	0.1 equiv iPr_2NEt	5 days	Z-L-Val-L-Met-OH	55

[a]All reactions were carried out at room temperature.[b]Isolated yields as its methyl ester.

Synthesis of the pentapeptide 17

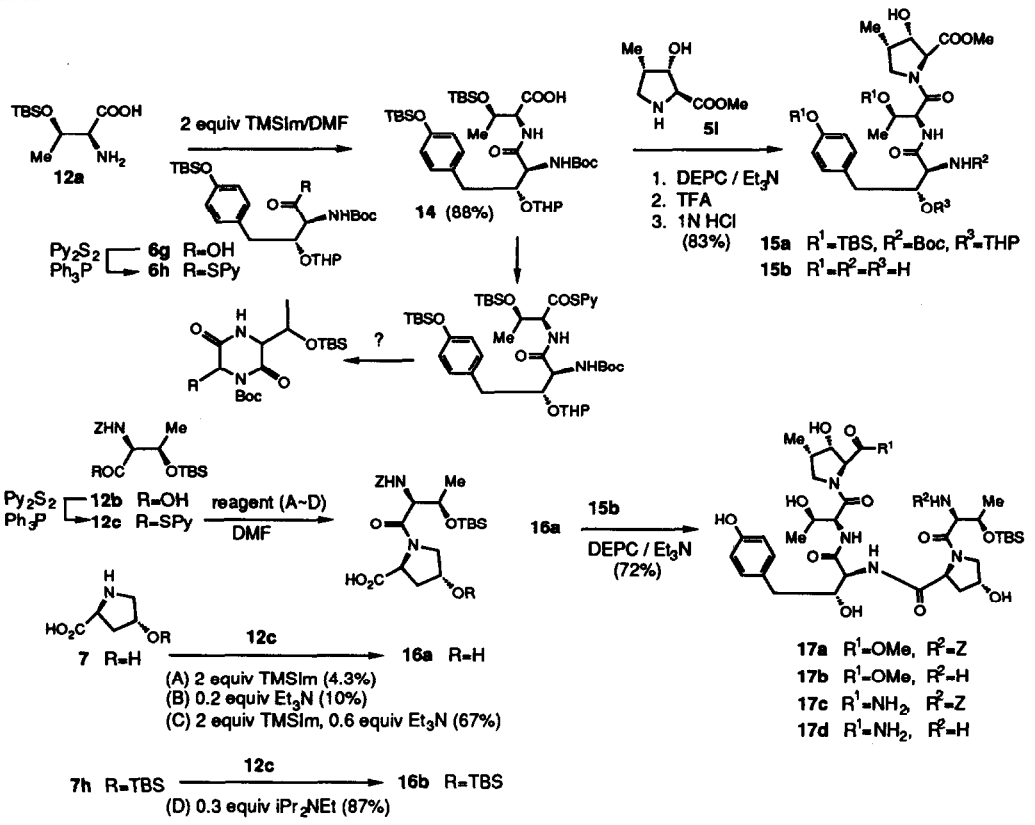
The structures of echinocandins led us to an imagination that there would be an internal hydrogen bonding between the two threonine moieties due to the presence of the two proline analogues which constrained their conformations to be relatively rigid ones. That the hemiaminal bond[36] connecting the fragment amino acid 1 (FA-1) and FA-6 could exist in a stable form might be due to the above mentioned reasons. Therefore, both *N*- and *C*-termini of the acyclic hexapeptide disconnected at the hemiaminal bond would be spatially proximal as shown in Scheme VI. Thus, the crucial cyclization steps for both echinocandin C (**1b**) and D (**1c**) were examined by the coupling of the acyclic hexapeptides **18b** and **22b**, and **24b**, respectively.

As mentioned in the previous section, peptide coupling by means of *O*-protected-L-threonine **12a** gave better yields than that of L-threonine (Table I). Thus, we examined the coupling of **6h** with **12a** by means of the TMSIm method (2 equiv of TMSIm/DMF) to give the desired dipeptide **14** in 88% yield. On the other hand, the thiol ester of the dipeptide **14** could not be obtained in satisfactory yield (~40%) due probably to an attack of the amino group to the resulting internal thiol group to produce a diketopiperazine derivative.[37] Therefore, the coupling of **14** with imino acid **5i**, prepared from **5h** with TFA, was performed using the Shioiri reagent, diethylphosphoryl cyanide (DEPC), which has been proven as one of the most effective coupling reagent to date.[38] This reaction proceeded smoothly yielding the tripeptide **15a** (83%), which upon deprotection with aqueous HCl afforded the tripeptide **15b** (FA-1~3), quantitatively, as a hydrochloride. Thus obtained **15b** was a diastereomerically homogeneous compound as ascertained by its ^1H NMR (360 MHz).

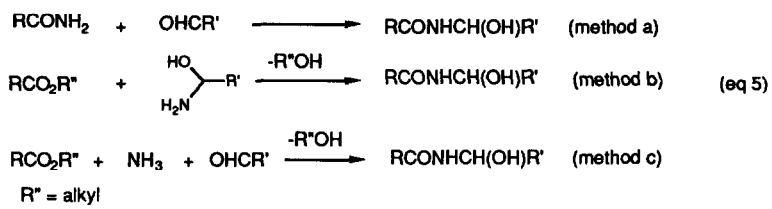
Coupling of the unprotected imine **7** with 2-pyridyl thiol ester **12c** using a catalytic amount of triethylamine (0.2 equiv) afforded Z-L-Thr-4-hydroxy-L-Pro-OH (**16a**) in only 10% yield. The yield was improved to 67% by the use of 0.9 equiv of triethylamine and 3.0 equiv of TMSIm (Scheme V). The use of

the *O*-TBS protected compound **7h** in the presence of 0.1 equiv of diisopropylethylamine gave **16b** in better yield (87%). The solubility of imino acid might be crucial and the TBS ether was quite soluble in the solvent. Thus obtained dipeptide **16a** (FA-4 and 5) was condensed with the tripeptide **15b** using DEPC to give in 72% yield the pentapeptide **17a**, a synthetic intermediate common to both **1b** and **1c**.

Scheme V



Approaches to the synthesis of echinocandin C (1b)

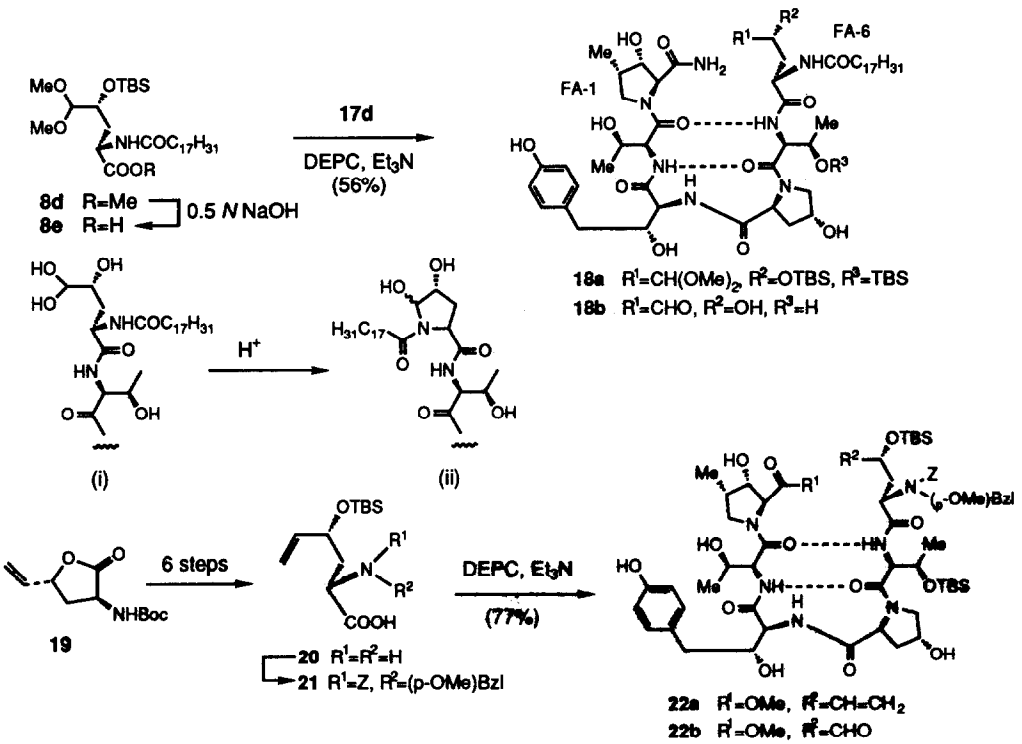


(eq 5)

There are only a few methods considerable for the preparation of the requisite hemiaminal bond of **1b** as shown in eq 5 (methods a~c). Some examples are reported for the hemiaminal bond formation by the method a which requires the coupling of $CONH_2$ at FA-1with the aldehyde at FA-6.[39] The methods b and c involve an amino acetal which would be labile as well as synthetically difficult. Therefore, our first attempts were the

use of method a. Thus, the pentapeptide **17a** was converted to the acid amide **17c** with NH_3/MeOH in 38% yield (62% of **17a** was recovered and recycled). After removal of the Z group of **17c** (H_2/10% Pd-C), this was then coupled with the carboxylic acid **8e** in the presence of DEPC to give a protected form of the desired hexapeptide **18a** (56% yield from **17c**). We expected that, upon removal of all protecting groups under the acidic conditions, the desired cyclization leading to **1b** would occur. However, in spite of our numerous attempts using a variety of acidic conditions (0.1 *N* HCl, 60% acetic acid, CSA, etc.), such product was not detected by TLC, HPLC and ^{1}H NMR. Under these conditions, all the protecting groups of **18a** were removed at once to give a protecting group-free hexapeptide **18b**, where the proton signal corresponding aldehyde was not observed by its ^{1}H NMR spectrum. The MS (SIMS) data of the product showed the parent ion peak at 1044 [$(M + H)^+$], which corresponded to a pyrrolidine (**ii**) produced from an aldehyde (**18b**) or its hydrate form (**i**). The proximal linoleyl amide group might add to the resulting aldehyde producing a stable pyrrolidine.[11b] Using this, further attempts for cyclization (TsOH, MS 4A, heat) were not successful, giving only a mixture of unidentifiable products.

Scheme VI



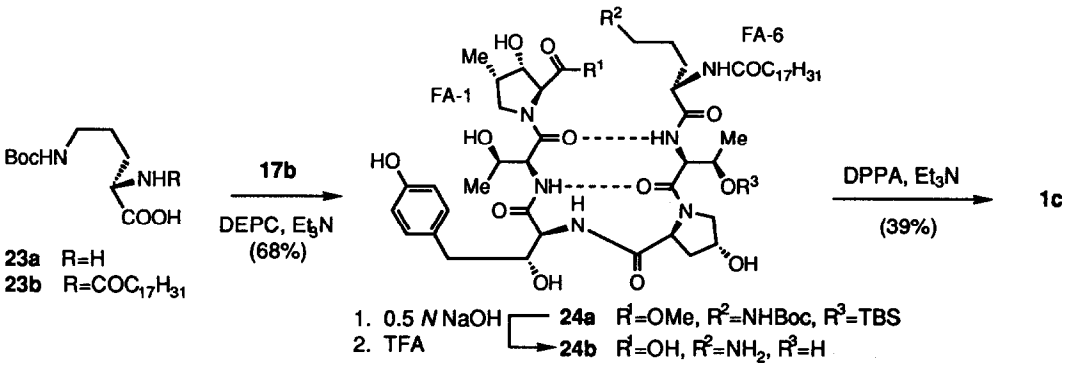
The next approach was method c which is a modified and tandem manner of method b; we expected that *in situ* ammonia would initially react with the aldehyde to form an aminal, which would simultaneously attack the ester group to give desired hemiaminal. In order to carry out this approach, the structure of ornithine moiety **8d** was modified to **21** in which the aldehyde was masked with the methylene group, and the

linoleylamide group was changed to the *N*-Z,*N*-(p-OMe)Bzl group. The synthesis of **21** was carried out from the *cis*-γ-butyrolactone **19** [26] by the following sequence of reactions: (1) Hydrolysis of **19** with 0.5 *N* NaOH, (2) esterification of the resulting carboxylic acid with diazomethane gave (2*S*,4*R*)-2-(*tert*-butoxycarbonyl)amino-4-hydroxy-5-hexenoic acid methyl ester, (3) protection of the C4 hydroxyl group with the TBS group (TBSCl, imidazole, DMF, 91%; 3 steps), (4) removal of the Boc group using *tert*-butyldimethylsilyl trifluoromethanesulfonate in the presence of 2,6-lutidine,[40] (5) hydrolysis of the ester group with 0.5 *N* NaOH gave (2*S*,4*R*)-2-amino-4-(*tert*-butyldimethylsilyl)oxy-5-hexenoic acid, (6) reductive amination of the resulting free amino acid with *p*-methoxybenzaldehyde using $NaBH_3CN$ (58%),[28,41] and (7) protection of the resulting *sec*-amino group with the Z group (benzyloxycarbonyl chloride, 0.5 *N* NaOH) gave **21** (39%). Condensation of **21** with the pentapeptide **17b** was effected by DEPC to give **22a** in 77% yield. Ozonolysis of **22a** gave the aldehyde **22b**. Upon treatment of **22b** under aqueous ammonia or methanolic ammonia, desired cyclized product could not be detected. Due to the above mentioned difficulties for the hemiaminal bond formation, we turned our attention to the synthesis of echinocandin D (**1c**).

Synthesis of echinocandin D (1c)

The *N*-linoleylornithine **23b** was prepared from *N*$^{\alpha}$-*tert*-butoxycarbonyl-L-ornithine **23a** in one step using the TMSIm method ($C_{17}H_{31}COSPy$, 2 equiv of TMSIm). This was coupled with amine **17b** using DEPC to give the desired hexapeptide **24a** in 68% yield. The isolated **24a** was diastereomerically homogeneous that was ascertained by its ^1H NMR analysis. Removal of the protecting groups were performed in two steps, (i) 0.5 *N* NaOH and (ii) TFA, to give **24b**. The cyclization was accomplished by means of diphenylphosphoryl azide (DPPA)[42] to give echinocandin D (**1c**) in 39% from **24a**. Synthetic **1c** was identical in all respects with those reported.[2] In addition, synthetic **1c** was converted into its tetrahydro derivative (H_2/Pd-C, 100%) and was found to be identical in all respects with a sample derived from the natural **1c**.

Scheme VII



Experimental

Melting points are uncorrected. ^1H NMR NMR spectra were recorded on one of the following instruments: JEOL FX 100, JEOL JNM-EX 400 and Nicolet NT-360. Chemical shifts are reported in ppm (δ) relative to $CHCl_3$ (δ = 7.26) in $CDCl_3$, CH_3OH (δ = 3.30) in CD_3OD or TSP (δ = 0.00) in D_2O. IR spectra

were measured either on a Hitachi 270-30 or on a Perkin Elmer FT-IR 1640 spectrophotometer. Mass spectra (MS) and high resolution mass spectra (HRMS) were obtained on a Hitachi M-80B spectrometer for secondary ionization mass spectrometry (SIMS) and electron-impact ionization (EI) or on a JEOL JMX-HX 110 for fast atom bombardment ionization (FAB). Optical rotations were taken on a Perkin Elmer 241 polarimeter. All reactions were carried out under N_2, monitored by thin layer chromatography (TLC), which was performed with precoated TLC plates (Merck). The organic solutions obtained by extractive workup were dried over anhydrous magnesium sulfate and concentrated under reduced pressure by a rotary evaporator, unless otherwise stated. Silica gel (Merck 60, 70-230 mesh) was used for column chromatography. HPLC was performed with a Develosil ODS-5 (*Nomura Chemical, Nagoya*). Yields are of chromatographically and spectroscopically (^{1}H NMR) pure materials, unless otherwise stated.

(2*R*,3*S*,4*S*)-5-Benzyloxy-4-methyl-2,3-epoxypentanoic acid (5b). To a mechanically stirred solution of (2*S*,3*S*,4*S*)-2,3-epoxy-4-methyl-5-benzyloxypentanol (**5a**) (7.7 g, 35 mmol) in DMF (150 mL) was added pyridinium dichromate (56.4 g, 150 mmol) at room temperature. After the reaction was stirred for 45 h, ethy ether (700 mL) and magnesium sulfate (100 g) was added successively to give an oily residue. The residue was dissolved in 0.5 *N* NaOH (150 mL), and extracted with ethyl ether. The aqueous layer was acidified with 1 *N* HCl to pH 3, and was extracted with ethyl ether, dried, and concentrated in vacuo to give **5b** (6.5 g, 79%) as an oil: IR ($CHCl_3$) 3300~2800, 1740 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 7.63 (1 H, br s), 7.32 (5 H, s), 4.52 (2 H, s), 3.48 (2 H, d, *J* = 6 Hz), 3.34 (1 H, d, *J* = 2 Hz), 3.23 (1 H, dd, *J* = 2, 6 Hz), 1.89 (1 H, m), 1.03 (3 H, d, *J* = 6 Hz); $[\alpha]^{25}_D$ +2.25° (*c* 1.6, $CHCl_3$); MS (EI) *m/z* 236 $(M)^+$, 161; HRMS (EI) *m/z* calcd for $C_{13}H_{16}O_4$ M^+ 236.1046, found 236.1021.

(2*S*,3*S*,4*S*)-2-Amino-3-hydroxy-4-methyl-5-benzyloxypentanoic acid (5c). A solution of **5b** (11.0 g, 47 mmol) in 28% aqueous ammonium hydroxide solution (250 mL) was stirred at room temperature for 5 days. The solvent was removed in vacuo to give **5c** as crude crystals, which, upon recrystallization from H_2O, gave **5c** (8.5 g, 72%) as coloreless crystals: mp 188 °C (decomp); IR (KBr) 3400, 3170, 2860, 1640, 1580, 1530 cm^{-1}; ^{1}H NMR (100 MHz, 1 *N* DCl/D_2O) δ 6.80 (5 H, m), 3.95 (2 H, s), 3.71 (1 H, d, *J* = 3 Hz), 3.31 (1 H, dd, *J* = 3, 9 Hz), 3.08 (1 H, dd, *J* = 5, 10 Hz), 2.91 (1 H, dd, *J* = 5.5, 10 Hz), 1.8~1.3 (1 H, m), 0.42 (3 H, d, *J* = 7 Hz); $[\alpha]^{25}_D$ +17.7° (*c* 1.0, 1 *N* HCl); MS (EI) *m/z* 254 $(M+H)^+$, 179; Anal. Calcd for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53. Found: C, 61.38; H, 7.56; N, 5.48.

(2*S*,3*S*,4*S*)-2-(*tert*-Butoxycarbonyl)amino-3-hydroxy-4-methyl-5-benzyloxypentanoic acid methyl ester (5d). To a solution of **5c** (2.31 g, 9.1 mmol) and triethylamine (1.50 g, 14.8 mmol) in dioxane and water (1/1, 30 mL) was added 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (2.95 g, 12.0 mmol). The mixture was stirred at room temperature for 4 h. Dioxane was evaporated in vacuo. The residue was extracted with ethyl ether. The aqueous layer was acidified to pH 3 with 1 *N* HCl, and extracted with ethyl ether 2 times. To the combined organic layer was added a solution of diazomethane in ethyl ether until esterification was completed. The solvent was evaporated in vacuo to give **5d** (3.10 g, 93%) as an oil: *Rf* 0.52 (ethyl acetate/benzene, 1/3); IR (film) 3440, 2980, 1740, 1710 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 7.26 (5 H, m), 5.54 (1 H, d, *J* = 8 Hz), 4.46 (2 H, s), 4.39 (1 H, dd, *J* = 3, 8 Hz), 3.71 (3 H, s), 3,8~3.3 (3 H, m), 2.3~1.8 (1 H, m), 1.39 (9 H, s), 0.97 (3 H, d, *J* = 7 Hz); $[\alpha]^{25}_D$ +47.0° (*c* 1.0, $CHCl_3$); MS (EI) *m/z* 368 $(M+H)^+$, 179. Anal. Calcd for $C_{19}H_{29}NO_6$: C, 62.10; H, 7.96; N, 3.81. Found: C, 62.10; H, 7.99; N, 3.82.

(2*S*,3*S*,4*S*)-2-(*tert*-Butoxycarbonyl)amino-3-hydroxy-4-methyl-5-(*p*-toluenesulfonyl)oxypentanoic acid methyl ester (5f). A solution of **5d** (1.65 g, 4.5 mmol) in methanol (50 mL) was stirred over 10% palladium on carbon (320 mg) under H_2 (4 atm) for 20 h at room temperature. The catalyst was filtered off, and the filtrate was concentrated in vacuo to give an oily mixture of **5e** and **5g** which was dissolved in pyridine (20 mL) along with *p*-toluenesulfonyl chloride (0.86 g, 4.5 mmol). After being stirred at room temperature for 8

h, the mixture was poured into ice-water, and extracted with ethyl acetate 3 times. The combined organic layer was washed with water, dried, and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (ethyl acetate/benzene, 1/9, then 1/6) to give **5g** (*Rf* 0.35 (ethyl acetate/benzene, 1/3); 500 mg, 45.3%) and **5f** (*Rf* 0.43 (ethyl acetate/benzene, 1/3); 1.06 g, 54.7%) . The lactone **5g** (500 mg, 2.0 mmol) was dissolved in THF (4.5 mL) and 0.5 *N* NaOH (4.5 mL) at 0 °C. After being stirred at 0 °C for 16 h, the solvent was evaporated in vacuo. The residue was extracted with ethyl ether. The aqueous layer was acidified to pH 3.5 with 1 *N* HCl, and was extracted with ethyl acetate 3 times. To the combined organic layer was added a solution of diazomethane in ethyl ether until the esterification was completed. The mixture was dried, and concentrated in vacuo. The residue, diol **5e**, was treated with *p*-toluenesulfonyl chloride (389 mg, 2.0 mmol) in pyridine (10 mL) and worked up in the same manner as described above. Purification by column chromatography on SiO_2 (ethyl acetate/benzene, 1/9, then 1/6) afforded **5f** (0.84 g, 96%). Total 1.90 g (98% from **5d**) of **5f** was obtained as an oil. **5f**: IR (film) 3400, 2980, 1750, 1710 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 7.72 (2 H, d, *J* = 8 Hz), 7.28 (2 H, d, *J* = 8 Hz), 5.50 (1 H, d, *J* = 7 Hz), 4.36 (1 H, dd, *J* = 2, 7 Hz), 4.16 (1 H, dd, *J* = 5, 10 Hz), 4.00 (1 H, dd, *J* = 4, 10 Hz), 3.70 (3 H, s), 3.8~3.5 (1 H, m), 3.5~2.9 (1 H, m), 2.39 (3 H, s), 2.2~1.8 (1 H, m), 1.39 (9 H, s), 0.96 (3 H, d, *J* = 7 Hz); $[\alpha]^{25}_D$ -5.7° (*c* 3.4, $CHCl_3$); MS (EI) *m/z* 272 $(M\text{-Boc-COOMe})^+$, 243, 173. Anal. Calcd for $C_{19}H_{29}NO_8S$: C, 52.89; H, 6.78; N, 3.25. Found C, 52.73; H, 6.84; N, 3.36. **5g**: IR ($CHCl_3$) 3440, 2980, 1746, 1714 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 5.40 (1H, m), 4.3~4.1 (2 H, m), 4.20 (2 H, s), 2.64 (1 H, s), 2.7~2.1 (1 H, m), 1.46 (9 H, s), 1.05 (3 H, d, *J* = 7 Hz); $[\alpha]^{25}_D$ +47.2° (*c* 1.02, $CHCl_3$); MS (EI) *m/z* 246 $(M+H)^+$, 189, 119. Anal. Calcd for $C_{11}H_{29}NO_5$: C, 53.86; H, 7.81; N, 5.71. Found: C, 53.80; H, 7.80; N, 5.65.

(3*S*,4*S*)-*N*-(*tert*-Butoxycarbonyl)-3-hydroxy-4-methyl-L-proline methyl ester (5h). To a stirred suspension of 60% NaH (80 mg, 2.0 mmol) in THF (6 mL) was added a solution of **5f** (880 mg, 2.0 mmol) in THF (5 mL), drop-by-drop, over 10 min. The suspension was stirred for 1.5 h at 0 °C. The reaction mixture was quenched with saturated aqueous ammonium chloride (5 mL). The mixture was extracted with ethyl ether 3 times. The combined organic layer was washed with water, dried, and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (ethyl acetate/benzene, 1/9, then 1/5) to give **5h** (328mg, 62%) as an oil: *Rf* 0.30 (ethyl acetate/benzene, 1/3); IR (film) 3420, 2970, 1750, 1710, 1690, 1680 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 4.4~4.1 (2 H, m), 3.72 (3 H, s), 3.9~3.5 (1 H, m), 3.14 (1 H, dt, *J* = 2, 10 Hz), 2.6~2.2 (1 H, m), 2.06 (1 H, d, *J* = 4 Hz), 1.45 and 1.39 (9 H, s), 1.06 (3 H, d, *J* = 7 Hz); $[\alpha]^{25}_D$ -24.2° (*c* 1.1, $CHCl_3$); MS (EI) *m/z* 259 M^+, 200. Anal. Calcd for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.58; H, 8.28; N, 5.11.

***(3S,4S)*-3-Hydroxy-4-methyl-L-proline (5).** A solution of **5h** (31 mg, 0.12 mmol) in THF (0.5 mL) and 0.5 *N* NaOH (0.26 mL) was stirred at 0 °C for 14 h. The mixture was acidified to pH 3 with 1 *N* HCl and extracted with ethyl acetate 2 times. The combined organic layer was washed with water, dried, and concentrated in vacuo. The residue was dissolved in trifluoroacetic acid (0.5 mL) and CH_2Cl_2 (0.5 mL). After being stirred at room temperature for 30 min, the solvent was evaporated in vacuo. The residue was passed through a column of Dowex 50w x 4 (100-200 mesh) ion exchange resin (H_2O, then 1 *N* aqueous NH_3) to give **5** (15 mg, 86%) as colorless crystals: *Rf* 0.30 ($CHCl_3$/MeOH/H_2O, 65/65/10); mp 260 °C (decomp); IR (KBr) 3300, 3060, 2960, 2850, 2630, 2450, 2280, 2050, 1610 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 4.41 (1 H, d, J = 4 Hz), 4.06 (1 H, br s), 3.58 (1 H, dd, *J* = 8, 11.5 Hz), 3.03 (1 H, t, *J* = 11.5 Hz), 2.24 (1 H, m), 1.04 (3 H, d, *J* = 6.5 Hz); $[\alpha]^{25}_D$ -27° (*c* 0.8, H_2O); MS (EI) *m/z* 146 $(M+H)^+$, 100.[1]

(2*R*,3*S*)-2-(*tert*-Butoxycarbonyl)amino-3,4-epoxybutanol (6b). To a solution of (2*R*)-(*tert*-butoxycarbonyl)amino-3-butenol (**6a**) (327 mg, 1.7 mmol) in CH_2Cl_2 (8 mL) was added 3-chloroperbenzoic acid [402 mg, 2.1 mmol (90% purity)]. After being stirred at room temperature for 17 h, the solution was poured into 5% aqueous potassium carbonate. The organic layer was separated, and the aqueous layer was

extracted with ethyl acetate 3 times. The combined organic layer was washed with brine, dried, and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (ethyl ether/n-hexane, 1/1, then ethyl ether) to give **6b** (213mg, 60%) as colorless crystals: *Rf* 0.31 (ethyl ether); mp 60~61.5 °C; IR ($CHCl_3$) 3420, 2970, 1690, 1490 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.85 (1 H, d, J = 8 Hz), 4.00~3.85 (1 H, m), 3.85~3.65 (2 H, m), 3.20~3.15 (1 H, m), 2.76 (1 H, dd, J = 4.5, 5 Hz), 2.70~2.55 (1 H, m), 2.62 (1 H, dd, J = 3, 5 Hz), 1.42 (9 H, s); $[\alpha]^{26}_D$ -17.8° (*c* 1.5, $CHCl_3$); MS (EI) *m/z* 204 $(M+H)^+$ 172. Anal. Calcd for $C_9H_{17}NO_4$: C, 53.19; H, 8.43; N, 6.89. Found: C, 52.92; H, 8.42; N, 6.70.

(2*R*,3*S*)-2-(*tert*-Butoxycarbonyl)amino-3,4-epoxybutyl acetate (6c). To a solution of **6b** (390 mg, 1.9 mmol) in THF (2 mL) was added triethylamine (196 mg, 1.9 mmol) and acetyl chloride (0.21 mL, 3.0 mmol) at 0 °C. After being stirred at 0 °C for 1 h, the mixture was poured into water and extracted with ethyl ether 2 times. The combined organic layer was washed with saturated aqueous sodium bicarbonate and brine, successively, dried, and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (benzene/ethyl acetate, 1/9, then ethyl acetate) to give **6c** (387mg, 82%) as colorless crystals: *Rf* 0.46 (ethyl acetate/benzene, 1/3); mp 39.5~41.0 °C; IR ($CHCl_3$) 3420, 2970, 1710, 1490 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 4.54 (1 H, br s), 4.3~3.9 (3 H, m), 3.02 (1 H, m), 2.65 (1 H, dd, J = 4.5, 5 Hz), 2.54 (1 H, dd, J = 2, 5 Hz), 2.00 (3 H, s), 1.35 (9 H, s); $[\alpha]^{25}_D$ -39.8° (*c* 1.7, $CHCl_3$); MS (EI) *m/z* 246 $(M+H)^+$, 190, 172. Anal. Calcd for $C_{11}H_{19}NO_5$: C, 53.86; H, 7.81; N, 5.71. Found: C, 53.56; H, 7.94; N, 5.66.

***O*-(*tert*-Butyldimethylsilyl)-4-bromophenol.** To a solution of 4-bromophenol (23.0 g, 0.13 mol) and imidazole (13.6 g, 0.20 mol) in DMF (70 mL) was added a solution of *tert*-butyldimethylsilyl chloride (22.7 g, 0.15 mol) in DMF (70 mL) at 0 °C, drop-by-drop, over 30 min. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 30 min. The mixture was poured into ice water (600 mL) and extracted with ethyl ether/n-hexane (1/1, 500 mL). The organic layer was washed with water 5 times, dried, and concentrated in vacuo. The residue was removed in vacuo to give the title compound (34.2 g, 90%) as a colorless oil: bp 137 °C (25 mmHg); IR (film) 2970, 2940, 2900, 2870, 1590, 1490 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 7.32 (2 H, dd, J = 2, 8 Hz), 6.72 (2 H, dd, J = 2, 8 Hz), 0.98 (9 H, s), 0.19 (6 H, s); MS (EI) *m/z* 288 and 286 M^+, 231, 229; HRMS (EI) *m/z* calcd for $C_{12}H_{19}BrOSi$ M^+ 286.0392, found 286.0391.

(2*R*,3*R*)-2-(*tert*-Butoxycarbonyl)amino-3-hydroxy-4-[4-(*tert*-butyldimethylsilyl)oxy]phenylbutyl acetate (6e). To a stirred solution of *O*-*tert*-butyldimethylsilyl-4-bromophenol (13.78 g, 48.0 mmol) in dry THF (30 mL) was added 1.6 M solution of *n*-butyl lithium in hexane (30 mL) at -78 °C. The solution was stirred at -78 °C for 1.5 h. This solution was transferred to a stirred cool (-78 °C) suspension of CuCN (2.22 g, 24.8 mmol) in dry THF (15 mL) via cannula. The mixture was stirred at -78 °C for 2 h. Thus obtained clear solution of **6d** was added to a cool (-78 °C) solution of **6c** (3.02 g, 12.3 mmol) in dry THF (30 mL) via cannula. The mixture was gradually warmed to 0 °C and stirred at 0 °C for 3 h. The mixture was then cooled to -78 °C, and saturated aqueous ammonium chloride (60 mL) was added. After being stirred at room temperature for 15 min, the mixture was passed through a celite pad, and the filtrate was extracted with ethyl ether 3 times. The combined organic layer was washed with water, dried, and concentrated in vacuo. The residue was subjected to a column chromatography on SiO_2 (ethyl ether/n-hexane, 2/3, then 3/1) to give a mixture of oily products, which were further purified by lobar column chromatography (Lichroprep Si-60 (Merck); ethyl acetate/benzene, 1/9) afforded **6e** (3.62 g, 65%) as an oil: *Rf* 0.47 (ethyl ether/n-hexane, 3/1); IR (film) 3460, 1728, 1612 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 7.06 (2 H, d, J = 8 Hz), 6.76 (2 H, d, J = 8 Hz), 4.97 (1 H, d, J = 9 Hz), 4.23 (1 H, dd, J = 7.5, 11 Hz), 4.07 (1 H, dd, J = 5.5, 11 Hz), 4.0~3.7 (2 H, m), 2.72 (2 H, br d, J = 6.5 Hz), 2.04 (3 H, s), 2.13 (1 H, br d, J = 3 Hz), 1.46 (9 H, s), 0.96 (9 H, s), 0.18 (6 H, s); $[\alpha]^{25}_D$ +14.2° (*c* 1.06, $CHCl_3$); MS (EI) *m/z* 454 $(M+H)^+$, 379, 319. Anal. Calcd for $C_{23}H_{39}NO_6Si$: C, 60.90; H, 8.66; N, 3.09. Found: C, 60.73; H, 8.74; N, 3.07.

(2*R*,3*R*)-2-(*tert*-Butoxycarbonyl)amino-3-(tetrahydropyranyl)oxy-4-[4-(*tert*-butyldimethylsilyl)oxy]-phenylbutyl acetate. To a solution of **6e** (3.88 g, 8.5 mmol) in CH_2Cl_2 (20 mL) was added 3,4-dihydro-2*H*-pyran (1.6 mL, 17.5 mmol) and *dl*-10-camphorsulfonic acid (20 mg, 0.086 mmol). The solution was stirred at 0 °C for 1.5 h. Sodium bicarbonate powder (20 mg, 0.24 mmol) was added to the reaction mixture. The mixture was concentrated in vacuo and purified by column chromatography on SiO_2 (ethyl ether/n-hexane, 1/9, then 1/3) to give the title compound (4.52 g, 98%) as an oil: *Rf* 0.43 (ethyl ether/n-hexane, 1/1); IR (film) 3470, 3360, 2950, 1750, 1720, 1610, 1510 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 7.05 (2 H, d, *J* = 8 Hz), 6.76 (2 H, d, *J* = 8 Hz), 5.0~4.8 (1 H, m), 4.64 (0.5 H, br s), 4.3~3.2, (6.5 H, m), 3.0~2.6 (2 H, m), 2.01 and 1.98 (3 H, s) 2.3~2.1 (0.5 H, m), 1.48 (9 H, s), 1.8~1.2 (5.5 H, m), 0.97 (9 H, s), 0.18 (6 H, s); MS (EI) *m/z* 453 $(M+H\text{-tetrahydropyranyl})^+$, 435, 319, 85. Anal. Cacld for $C_{28}H_{47}NO_7Si$: C, 62.54; H, 8.81; N, 2.60. Found: C, 62.41; H, 8.85; N, 2.50.

(2*R*,3*R*)-2-(*tert*-Butoxycarbonyl)amino-3-(tetrahydropyranyl)oxy-4-[4-(*tert*-butyldimethylsilyl)oxy]-phenylbutanol. To a solution of (2*R*,3*R*)-2-(*tert*-butoxycarbonyl)amino-3-(tetrahydropyranyl)oxy-4-[4-(*tert*-butyldimethylsilyl)oxy]phenylbutyl acetate (4.61 g, 8.6 mmol) in dry methanol (22 mL) was added potassium carbonate (60 mg, 0.43 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h. Additional potassium carbonate (20 mg, 0.14 mmol) was added to the mixture, and the mixture was stirred at 5 °C for 1 h, then at room temperature for 1.5 h. The reaction was quenched by saturated aqueous ammonium chloride. After removal of methanol in vacuo, the aqueous residue was extracted with ethyl acetate 2 times. The combined organic layer was washed with water, dried, and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (ether/n-hexane, 1/9, then 1/1) to give the title compound (3.47g, 82%) as an oil: *Rf* 0.60 (ethyl ether); IR ($CHCl_3$) 3456, 2960, 1710, 1610, 1506 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 7.06 (2 H, d, *J* = 8 Hz), 6.74 (2 H, d, *J* = 8 Hz), 5.00 (1 H, d, *J* = 8 Hz), 4.67 (0.5 H, m), 4.1~3.0 (6.5 H, m), 3.0~2.6 (2 H, m), 2.2~1.0 (6 H, m), 1.46 (9 H, s), 0.96 (9 H, s), 0.17 (6 H, s); MS (EI) *m/z* 496 $(M+H)^+$, 411, 364, 85. Anal. Cacld for $C_{26}H_{45}NO_6Si$: C, 62.99; H, 9.15; N, 2.83. Found: C, 62.79; H, 9.13; N, 2.74.

(2*S*,3*R*)-2-(*tert*-Butoxycarbonyl)amino-3-(tetrahydropyranyl)oxy-4-[4-(*tert*-butyldimethylsilyl)oxy]-phenylbutyric acid (6g). To a suspension of pyridinium dichromate (386 mg, 1.03 mmol) in DMF (0.8 mL) was added a solution of (2*R*,3*R*)-2-(*tert*-butoxycarbonyl)amino-3-(tetrahydropyranyl)oxy-4-[4-(*tert*-butyldimethylsilyl)oxy]phenylbutanol (102 mg, 0.21 mmol) in DMF (0.3 mL). The mixture was stirred at room temperature for 24 h. The mixture was diluted with ethyl ether, and magnesium sulfate was added. The mixture was filtered through a magnesium sulfate pad, and the filtrate was concentrated in vacuo to give **6g** (84 mg, 81%) as an oil: *Rf* 0.65 and 0.75 (MeOH/$CHCl_3$, 1/4); IR ($CHCl_3$) 3450, 2950, 1730, 1710, 1610, 1505 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 8.73 and 8.02 (1 H, br s), 7.06 (2 H, d, *J* = 8 Hz), 6.73 (2 H, d, *J* = 8 Hz), 5.54 and 5.30 (1 H, d, *J* = 8 Hz), 4.8~3.2 (5 H, m), 3.2~2.0 (2 H, m), 2.2~2.1 (0.5 H, m), 2.0~1.0 (5.5 H, m), 1.49 and 1.46 (9 H, s), 0.96, (9 H, s), 0.16 (6 H, s); MS (EI) *m/z* 465 $(M+H\text{-}COOH)^+$, 407. Elementary analytical data were measured by the use of its methyl ester, prepared by the esterification with diazomethane. Anal. for the methyl ester of **6g**: Calcd for $C_{27}H_{45}NO_7Si$: C, 61.45; H, 8.59; N, 2.65. Found: C, 61.91; H, 8.79; N, 2.64.

(3*R*)-3-Hydroxy-L-homotyrosine (6). A solution of **6g** (31 mg, 0.06 mmol) in CH_2Cl_2 (1 mL) and trifluoroacetic acid (1 mL) was stirred at room temperature for 2 h. The solvent was evaporated in vacuo. The residue was passed through a column of Dowex 50w x 4 (100-200 mesh) ion exchange resin (H_2O, then 1 *N* aqueous NH_3) to give **6** (10 mg, 78%) as colorless crystals: *Rf* 0.35 ($CHCl_3$/MeOH/H_2O, 65/65/10); mp 200~203 °C (decomp); IR (KBr) 3570, 3310, 1630, 1580, 1510 cm^{-1}; ^1H NMR (360 MHz, 1*N* DCl) δ 6.67 (2 H, d, *J* = 8.5 Hz), 6.33 (2 H, d, *J* = 8.5 Hz), 3.91 (1 H, ddd, *J* = 4, 5, 9 Hz), 3.58 (1 H, d, *J* = 4 Hz), 2.41 (1 H, dd, *J* = 5, 14 Hz), 2.28 (1 H, dd, *J* = 9, 14 Hz); $[\alpha]^{25}_D$ +54.1° (*c* 1.22, 1 *N* HCl); MS (EI) *m/z* 193 $(M\text{-}H_2O)^+$, 149, 107, 75. Anal. Calcd for $C_{10}H_{13}NO_4.H_2O$: C, 52.40; H, 6.60; N, 6.11. Found C, 52.86; H, 6.41; N, 6.14.

(2*S*,4*S*)-2-(*tert*-Butoxycarbonyl)amino-4-bromomethyl-γ-butyrolactone (7a). To a solution of *N*-*tert*-butoxycarbonyl-L-allylglycine (10.4 g, 48.3 mmol) in THF (70 mL) *N*-bromosuccinimide (8.9 g, 50.0 mmol) at 0 °C. The reaction was stirred at 0 °C for 20 min, quenched with saturated aqueous sodium bicarbonate (30 mL), and extracted with ethyl ether 2 times. The combined organic layer was washed with water, dried, and concentrated in vacuo to give crude crystals, which was recrystallized from ethyl ether/n-hexane (1/8) to give **7a** (10.3 g, 73%) as colorless crystals: *Rf* 0.33 (ethyl ether/n-hexane, 3/1); mp 119.5~120 °C; IR ($CHCl_3$) 3470, 3000, 1805, 1730, 1520 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 5.08 (1 H, d, J = 7 Hz), 4.61 (1 H, dq, J = 5, 10 Hz), 4.44 (1 H, ddd, J = 7, 9, 12 Hz), 3.61 (1 H, dd, J = 5, 12 Hz), 3.29 (1 H, dd, J = 5, 12 Hz), 2.92 (1 H, ddd, J = 5, 9, 13 Hz), 1.98 (1 H, ddd, J = 10, 12, 13 Hz), 1.42 (9 H, s); $[\alpha]^{23}_D$ +24.5° (*c* 1.0, $CHCl_3$); MS (EI) *m/z* 296 and 294 $(M+H)^+$, 240 and 238, 195 and 193. Anal. Calcd for $C_{10}H_{16}BrNO_4$: C, 40.82; H, 5.48; N, 4.76. Found: C, 40.79; H, 5.48; N, 4.76.

(2*S*,4*S*)-2-(*tert*-Butoxycarbonyl)amino-3,4-epoxypentanoic acid methyl ester (7c). A solution of **7a** (10.3 g, 35.0 mmol) and potassium carbonate (1.5 g, 10.9 mmol) in dry methanol (100 mL) was stirred at 0 °C for 30 min. The reaction was quenched with saturated aqueous ammonium chloride, and the methanol was removed in vacuo. The residue was extracted with ethyl ether 2 times. The combined organic layer was washed with water, dried, and concentrated in vacuo to give **7c** (8.63 g, 100%) as an oil: *Rf* 0.24 (ethyl ether/n-hexane, 3/1); IR (film) 3360, 2984, 1750, 1704, 1518 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 5.30 (1 H, d, J = 8 Hz), 4.42 (1 H, m), 2.98 (1 H, m), 2.74 (1 H, t, J = 5 Hz), 2.46 (1 H, dd, J = 2, 5 Hz), 2.3~1.8 (2 H, m), 1.41 (9 H, s); $[\alpha]^{26}_D$ -8.28° (*c* 1.74, $CHCl_3$); MS (EI) *m/z* 246 $(M+H)^+$, 190; HRMS (EI) *m/z* calcd for $C_{11}H_{20}NO_5$ $(M+H)^+$ 246.1342, found 246.1367.

(2*S*,4*R*)-2-(*tert*-Butoxycarbonyl)amino-4-hydroxymethyl-γ-butyrolactone (7d). A solution of **7c** (8.2 g, 33.4 mmol) in THF (70 mL) and 0.5 *N* NaOH (70 mL) was stirred at 0 °C for 14 h. After being concentrated in vacuo, the residue was extracted with ethyl ether. The aqueous layer was adjusted to pH 3.5 with 1 *N* HCl and extracted with ethyl ether. The organic layer was washed with water, dried, and concentrated in vacuo to give an oily residue, which was dissolved in CH_2Cl_2 (300 mL) along with *dl*-10-Camphorsulfonic acid (100 mg, 0.43 mmol). The mixture was stirred at room temperature for 20 h. The solution was washed with water, dried, and concentrated in vacuo to give crude crystals, which were washed with CH_2Cl_2 then recrystallized from water to give **7d** (5.76 g, 75%) as white crystals: *Rf* 0.57 (ethyl acetate); mp 208~209 °C; IR (nujol) 3464, 3352, 1760, 1738, 1698, 1520 cm^{-1}; ^1H NMR (100 MHz, d_6-DMSO) δ 7.29 (1 H, d, J = 8.5 Hz), 5.11 (1 H, t, J = 5 Hz), 4.54 (1 H, ddt, J = 3, 3.5, 7 Hz), 4.27 (1 H, dd, J = 8.5, 10 Hz), 3.58 (1 H, ddd, J = 3, 5, 11.5 Hz), 3.42 (1 H, ddd, J = 3.5, 3.5, 11.5 Hz), 2.34~2.08 (2 H, m), 1.36 (9 H, s); $[\alpha]^{25}_D$ -34.0° (*c* 0.3, MeOH); MS (EI) *m/z* 232 $(M+H)^+$, 175. Anal. Calcd for $C_{10}H_{17}NO_5$: C, 51.94; H, 7.41; N, 6.06. Found: C, 51.89; H, 7.40; N, 6.04.

(2*S*,4*R*)-2-(*tert*-Butoxycarbonyl)amino-4-formyl-γ-butyrolactone (7e). To a solution of oxalyl chloride (0.174 mL, 2.0mmol) in CH_2Cl_2 (5 mL) was added a solution of dimethyl sulfoxide (202 mg, 2.6 mmol) in CH_2Cl_2 (1 mL) at -78 °C. After being stirred at -78 °C for 15 min, a solution of **7d** (200 mg, 0.86 mmol) in CH_2Cl_2 (1 mL) and dimethyl sulfoxide (1 mL) was added. The reaction mixture was stirred at -78 °C for 15 min, then at -45 °C for 1 h. Then, triethylamine (0.85 mL, 6.1 mmol) was added at -45 °C, and the mixture was stirred at -45 °C for 20 min, and at 0 °C for 15 min. Water was added to the reaction mixture and the mixture was extracted with ethyl acetate 2 times. The combined organic layer was dried, and concentrated in vacuo. The residue was roughly purified by short pass column chromatography on SiO_2 (acetate/n-hexane, 1/1, then ethyl acetate) to give **7e** (147 mg, 74%) as an oil: *Rf* 0.50 (ethyl acetate). This was used for the next reaction without further purification.

(4*R*)-*N*-(*tert*-Butoxycarbonyl)-4-hydroxy-5-methoxyproline methyl ester (7f). A solution of **7e** (147 mg, 0.64 mmol) and *p*-toluenesulfonic acid monohydrate (16 mg, 0.084 mmol) in dry methanol (5 mL) was stirred at room temperature for 14 h. The reaction was quenched with sodium bicarbonate powder (8 mg, 0.095 mmol), and evaporated in vacuo. The residue was purified by column chromatography on SiO_2 (ethyl ether/n-hexane 1/1, then 4/1) to give a diastereomeric mixture of **7f** (137 mg, 78%) as an oil: *Rf* 0.50 (ethyl ether); IR (film) 3476, 2984, 1760, 1710 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 5.2~4.9 (1 H, m), 4.7~4.3 (1 H, m), 4.22 (1 H, br s), 3.75 and 3.74 (3 H, s), 3.43 (3 H, s), 2.32~1.76 (3 H, m), 1.44 (9 H, br s); MS (EI) *m/z* 276 $(M+H)^+$, 245, 175; HRMS (EI) *m/z* calcd for $C_{12}H_{21}NO_6$ M^+ 275.1369, found 275.1375.

(4*R*)-*N*-(*tert*-Butoxycarbonyl)-4-hydroxy-L-proline methyl ester (7g). A solution of **7f** (21 mg, 0.076 mmol) in 60% acetic acid (1 mL) was stirred at room temperature for 3 days. The solvent was evaporated in vacuo, and the residue was dissolved in ethanol (1 mL) and 60% acetic acid (0.5 mL). To this solution was added sodium cyanoborohydride (6.7 mg, 0.107 mmol), and the mixture was stirred at room temperature for 1 h. Water was added and the mixture was extracted with ethyl acetate 2 times. The combined organic layer was dried, and concentrated in vacuo. The residue was purified by preparative TLC (silica gel, ethyl ether) to give **7g** (7.7 mg, 41%) as an oil: *Rf* 0.32 (ethyl ether); IR (film) 3472, 1750, 1702 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 4.6~4.3 (1 H, m), 4.42 (1 H, dd, *J* = 8, 12 Hz), 3.74 (3 H, s), 3.8~3.4 (1 H, m), 3.59 (1 H, d, *J* = 4 Hz), 2.11 (1 H, dd, *J* = 5, 8 Hz), 2.5~1.8 (2 H, m), 1.42 (9 H, s); $[\alpha]^{25}_D$ -60.3° (*c* 0.7, $CHCl_3$); MS (EI) *m/z* 246 $(M+H)^+$, 190, 146; HRMS (EI) *m/z* calcd for $C_{11}H_{19}NO_5$ M^+ 245.1263, found 245.1289.

(4*R*)-4-Hydroxy-L-proline (7). A solution of **7g** (11.5 mg, 0.047 mmol) in THF (0.3 mL) and 0.5 *N* NaOH (0.14 mL) was stirred at 0 °C for 19 h. After removal of THF in vacuo, the residue was acidified to pH 3 with 1 *N* HCl and extracted with ethyl acetate. The organic layer was dried, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (0.5 mL) and trifluoroacetic acid (0.5 mL). After stirring at room temperature for 15 min, the solvent was evaporated in vacuo. The residue was passed through a column of Dowex 50w x 4 (100-200 mesh) ion exchange resin (H_2O, then 1 *N* aqueous NH_3) to give **7** (6.1 mg, 99%) as colorless crystals: *Rf* 0.63 ($CHCl_3$/MeOH/H_2O = 2:20:10); mp 260~265 °C (decomp); IR (nujol) 3292, 3144, 1594 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 4.69~4.64 (1 H, m), 4.33 (1 H, dd, *J* = 8, 10 Hz), 3.48 (1 H, dd, *J* = 4, 12.5 Hz), 3.34 (1 H, ddd, *J* = 1.5, 2, 12.5 Hz), 2.43 (1 H, ddt, *J* = 2, 8, 14 Hz), 2.15 (1 H, dddd, J = 0.5, 4, 10, 14 Hz); $[\alpha]^{25}_D$ -46.4° (*c* 1.0, *1* N HCl); MS (EI) *m/z* 132 $(M+H)^+$.[29]

(2*S*,4*R*)-2-(Linoleyl)amino-4-hydroxymethyl-γ-butyrolactone (8a). Preparation of linoleic acid 2-pyridyl thiolester: To a solution of linoleic acid (1.00 g, 3.6 mmol) in CH_2Cl_2 (15 mL) was added 2,2'-dipyridyl disulfide (0.84 g, 3.8 mmol) and triphenylphosphine (1.05 g, 4.0 mmol), successively. The mixture was stirred at room temperature for 1.5 h and the solvent was evaporated in vacuo. The residue was purified by column chromatography on SiO_2 (ethyl ether/n-hexane, 1/9, then 1/5) to give linoleic acid 2-pyridyl thiolester (1.23 g, 92%). Preparation of **8a**: A solution of **7d** (0.83 g, 3.6 mmol) in trifluoroacetic acid (4 mL) and CH_2Cl_2 (4 mL) was stirred at room temperature for 1 h. The solvent was evaporated in vacuo. Trace amounts of acid was removed azeotropically with benzene. The residue was dissolved in DMF (8 mL), and potassium carbonate (0.50 g, 3.6 mmol) was added. After being stirred for 5 min, linoleic acid 2-pyridyl thiolester (1.23 g, 3.3 mmol) in DMF (4 mL) was added to the mixture. The mixture was stirred at room temperature for 1 h, poured into water, and extracted with ethyl acetate 2 times. The combined organic layer was dried, and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (ethyl ether, then ethyl acetate) to give **8a** (1.15 g, 81%) as colorless crystals: *Rf* 0.50 (ethyl acetate); mp 80.5~81.5 °C; IR ($CHCl_3$) 3430, 2940, 1782, 1671 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 6.27 (1 H, d, *J* = 6.5 Hz), 5.5~5.1 (4 H, m), 4.8~4.5 (1 H, m), 4.58 (1 H, dd, *J* = 6.5, 10 Hz), 3.93 (1 H, ddd, *J* = 3, 6, 12.5 Hz), 3.66 (1 H, ddd, *J* = 3.5, 5.5, 12.5 Hz), 2.94 (1 H, dd, *J* = 5.5, 6 Hz), 2.9~2.7 (2 H, m), 2.62 (1 H, dd, *J* = 2.5, 10 Hz), 2.5~1.9 (7 H, m), 1.8~1.1 (16 H, m), 0.88 (3 H, t, *J* = 7 Hz); $[\alpha]^{25}_D$ -10.3°C (*c* 0.9, $CHCl_3$); MS (EI) *m/z* 393 M^+, 362; HRMS

(EI) *m/z* calcd for $C_{23}H_{39}NO_4$ M^+ 393.2879, found 393.2880.

(2*S*,4*R*)-2-*N*-(Linoleyl)amino-4-dimethoxymethyl-γ-butyrolactone (8c). To a solution of oxalyl chloride (0.1 mL, 1.2 mmol) in CH_2Cl_2 (3 mL) was added a solution of dimethylsulfoxide (118mg, 1.5 mmol) in CH_2Cl_2 (0.3 mL) at -78 °C. After being stirred at -78 °C for 10 min, a solution of **8a** (226 mg, 0.6 mmol) in CH_2Cl_2 (1.5 mL) was added to the mixture. The mixture was stirred at -78 °C for 15 min, and at -45 ~ -50 °C for 1 h. To this mixture, triethylamine (0.6 mL, 4.3 mmol) was added at -50 °C. Then the solution was warmed to 0 °C and stirred at the same temperature for 20 min. The reaction mixture was poured into water, and extracted with ethyl acetate 2 times. The combined organic layer was dried, and concentrated in vacuo. The residue was purified by short pass column chromatography onb SiO_2 (ethyl ether/n-hexane, 1/1, ethyl ether, then methanol/ethyl ether, 1/9) to give **8b** (167 mg, 0.43 mmol). The aldehyde **8b** was dissolved in CH_2Cl_2 (2 mL) and 2,2-dimethoxypropane (2 mL) along with *dl*-10-Camphorsulfonic acid (20 mg, 0.086 mmol). The reaction was stirred at room temperature for 14 h, quenched with sodium bicarbonate powder (20 mg, 0.24 mmol), and evaporated in vacuo. The residue was purified by column chromatography on SiO_2 (ethyl ether/n-hexane, 1/1, then ethyl ether) to give **8c** (127 mg, 51%) as an oil: *Rf* 0.31 (ethyl ether); IR ($CHCl_3$) 2940, 1787, 1676 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 5.87 (1 H, d, *J* = 6 Hz), 5.5~5.1 (4 H, m), 4.8~4.3 (3 H, m), 3.47 (3 H, s), 3.46 (3 H, s), 3.0~2.6 (3 H, m), 2.4~1.8 (7 H, m), 1.8~1.0 (16 H, m), 0.86 (3 H, t, *J* = 6 Hz); $[\alpha]^{25}_D$ -10.8° (*c* 2.8, $CHCl_3$); MS (EI) *m/z* 437 M^+. Anal. Calcd for $C_{25}H_{43}NO_5$: C, 68.61; H, 9.90; N, 3.20. Found: C, 68.68; H, 10.07; N, 3.05.

(2*S*,4*R*)-2-(Linoleyl)amino-4-(*tert*-butyldimethylsilyl)oxy-5-dimethoxypentanoic acid methyl ester (8d). A solution of **8c** (143 mg, 0.33 mmol) in THF (1 mL) and 0.5 *N* NaOH (0.7 mL) was stirred at 0 °C for 14 h. The solution was carefully acidified to pH 3.5 with 1 *N* HCl and extracted with ethyl ether 2 times. To this extract was added a solution of diazomethane in ethyl ether until esterification was completed. The mixture was dried, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (1.5 mL) along with 2,6-lutidine (160 mg, 1.49 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (236 mg, 0.89 mmol). The mixture was stirred at room temperature for 10 min, quenched with saturated aqueous ammonium chloride, and extracted with ethyl ether 2 times. The combined organic layer was washed with water, dried, and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (ethyl acetate/benzene, 1/13) to give **8d** (165 mg, 87%) as an oil: *Rf* 0.67 (ethyl acetate/benzene, 1/3); IR (film) 3290, 2920, 2850, 1750, 1650 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 6.37 (1 H, d, *J* = 7 Hz), 5.50~5.12 (4 H, m), 4.63 (1 H, dt, *J* = 5.5, 7 Hz), 4.16 (1 H, d, *J* = 5 Hz), 3.80 (1 H, dd, *J* = 5, 10 Hz), 3.70 (3 H, s), 3.40 (3 H, s), 3.38 (3 H, s), 2.76 (2 H, t, *J* = 6 Hz), 2.3~1.8 (8 H, m), 1.8~1.0 (16 H, m), 0.88 (9 H, s), 0.86 (3 H, t, *J* = 6 Hz), 0.08 (6 H, s); $[\alpha]^{25}_D$ -8.3° (*c* 0.7, $CHCl_3$); MS (EI) *m/z* 551 $(M\text{-}CH_3OH)^+$, 519, 494. Anal. Calcd for $C_{32}H_{61}NO_6Si$: C, 65.82; H, 10.53; N, 2.40. Found: C, 65.79; H, 10.74; N, 2.87.

General procedure for the peptide coupling using 1-(trimethylsilyl)imidazole. *N*-(Benzyloxycarbonyl)-L-valyl-L-allylglycine (10a). A suspension of L-allylglycine (230 mg, 2.0 mmol) and 1-(trimethylsilyl)imidazole (560 mg, 4.0 mmol) in dry DMF (5 mL) was stirred at room temperature for 30 min (The mixture turned to a clear solution). To this solution was added a solution of **9** (550 mg, 1.6 mmol) in dry DMF (3 mL). After being stirred for 2 h, 1 *N* HCl (5 mL) was added, and the mixture was stirred at room temperature for 30 min. Then the mixture was diluted with water, and extracted with ethyl acetate 3 times. The combined organic layer was washed with water, dried, and concentrated in vacuo. The crystalline residue was recrystallized from $CHCl_3$/n-hexane to give **10a** (467 mg, 84%) as white crystals: *Rf* 0.32 ($MeOH/CHCl_3$, 1/4). Spectroscopic data and physical constants were measured by the use of its methyl ester (prepared by esterification with diazomethane). Methyl ester of **10a**: *Rf* 0.49 (ethyl ether/n-hexane, 3/1); mp 116.5~117.0 °C; IR ($CHCl_3$) 3448, 1740, 1724, 1682, 1504 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.45~7.25 (5 H, m), 6.30 (1 H, d, *J* = 7.5 Hz), 5.72~5.58 (1 H, m), 5.33 (1 H, d, J = 8.5 Hz), 5.16~5.00 (4 H, m), 4.66 (1 H, dt, *J* = 6,

7.5 Hz), 4.02 (1 H, dd, J = 6.5, 8.5 Hz), 2.64~2.46 (2 H, m), 2.13 (1 H, octet, *J* = 6.5 Hz), 0.98 (3 H, d, *J* = 6.5 Hz), 0.93 (3 H, d, *J* = 6. 5 Hz); $[\alpha]^{25}_{D}$ +12.87° (*c* 1.01, $CHCl_3$); MS (EI) *m/z* 363 $(M+H)^+$, 254. In order to examine the extent of racemization of **10a**, the mixture of Z-L-Valyl-DL-allylglycine methyl ester was prepared in the same manner as above. In comparing their ^1H NMR (360 MHz), the following signals corresponding to the D-isomer were separated from those of the methyl ester of **10a**: δ 6.41 (1 H, d, *J* = 7.5 Hz), 5.31 (1 H, d, *J* = 8.5 Hz), 4.06 (1 H, dt, *J* = 6, 7.5 Hz), 0.97 (3 H, d, *J* = 6.5 Hz), 0.91 (3 H, d, *J* = 6. 5 Hz). The mixture of Z-L-Valyl-DL-allylglycine methyl ester showed separable peaks under the following HPLC conditions: column: Develosil ODS-5; column dimensions, 1.07 cm i.d. x 25 cm; flow rate, 1 mL/min; eluent, MeOH/H_2O, 58/42. Retention time of each compound: methyl ester of **10a**, 42 min; Z-L-Valyl-DL-allylglycine methyl ester, 42 min and 45 min. From these studies, less than 1% (if any) of racemization from the methyl ester of **10a** was found.

***N*-(Benzyloxycarbonyl)-L-valyl-L-methionine.** According to the general procedure, L-methionine (90 mg, 0.6 mmol) gave the title compound (168 mg, 89%) as white crystals: mp180-182 °C; IR (nujol) 3350, 3300, 1740, 1640 cm^{-1}; ^1H NMR (100 MHz, CD_3OD) δ 7.32 (5 H, s), 5.08 (2 H, s), 4.58 (1 H, dd, *J* = 4, 8 Hz), 3.96 (1 H, d, *J* = 8 Hz), 2.05 (3 H, s), 0.99 (3 H, d, *J* = 7 Hz), 0.96 (3 H, d, *J* = 7 Hz); $[\alpha]^{25}_{D}$ -22.57° (*c* 1.05, $CHCl_3$); MS (EI) *m/z* 276 $(M-(CH_3)_2S-CO_2)^+$, 203, 183.

***N*-(Benzyloxycarbonyl)-L-valyl-L-phenylalanine.** According to the general procedure, L-phenylalanine (99 mg, 0.6 mmol) gave the methyl ester of the title compound (171 mg, 86%): mp 142-143 °C; IR ($CHCl_3$) 3450, 3350, 1750, 1740, 1730, 1680 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 7.0-7.4 (5 H, m), 7.36 (5 H, s), 6.36 (1 H, d, *J* = 8 Hz), 5.34 (1 H, d, *J* = 9 Hz), 5.10 (2 H, s), 4.88 (1 H, dt, *J* = 6, 8, 8 Hz), 4.00 (1 H, dd, *J* = 6, 9 Hz), 3.72 (3 H, s), 3.10 (2 H, d, *J* = 6 Hz), 2.08 (1 H, quintet, *J* = 6 Hz), 0.93 (3 H, d, *J* = 6 Hz), 0.87 (3 H, d, *J* = 6 Hz); $[\alpha]^{25}_{D}$ +36.55° (*c* 1.1, $CHCl_3$); MS (EI) *m/z* 412 M^+, 304, 256.

***N*-(Benzyloxycarbonyl)-L-valyl-L-threonine (11a).** According to the general procedure, L-threonine (24 mg, 0.2 mmol) (in this case, 3 equiv of 1-(trimethylsilyl)imidazole (84 mg, 0.6 mmol) was used) gave the methyl ester of **11a** (40 mg, 56%) as white crystals: *Rf* 0.28 (ethyl ether); mp 141~142 °C; IR ($CHCl_3$) 3448, 1745, 1715, 1684, 1504 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 7.30 (5 H, m), 7.11 (1 H, d, *J* = 9 Hz), 5.71 (1 H, d, *J* = 9 Hz), 5.06 (2 H, s), 4.60 (1 H, dd, *J* = 2, 9 Hz), 4.5~4.1 (1 H, m), 4.09 (1 H, dd, *J* = 7, 9 Hz), 3.71 (3 H, s), 3.49 (1 H, d, *J* = 6 Hz), 2.3~1.9 (1 H, m), 1.14 (3 H, d, *J* = 6 Hz), 0.97 (3 H, d, *J* = 7 Hz), 0.93 (3 H, d, *J* = 7 Hz); $[\alpha]^{25}_{D}$ -8.3° (*c* 1.0, $CHCl_3$); MS (EI) *m/z* 366 M^+, 322.

***N*-(Benzyloxycarbonyl)-L-valyl-*O*-(*tert*-butyldimethylsilyl)-L-threonine (11b).** According to the general procedure, treatment of **12a** (38 mg, 0.16 mmol) with 1-(trimethylsilyl)imidazole (46 mg, 0.32 mmol) gave the methyl ester of **11b** (55 mg, 70%) as an oil: *Rf* 0.70 (ethyl acetate/benzene, 1/3); IR (film) 3300, 2964, 1720, 1670, 1516 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 7.33 (5 H, m), 6.38 (1 H, d, *J* = 8 Hz), 5.48 (1 H, d, *J* = 8 Hz), 5.11 (2 H, s), 4.6~4.3 (2 H, m), 4.14 (1 H, dd, *J* = 5, 8 Hz), 3.72 (3 H, s), 2.2~2.0 (1 H, m), 1.16 (3 H, d, *J* = 6 Hz), 1.03 (3 H, d, *J* = 7 Hz), 1.00 (3 H, d, *J* = 7 Hz), 0.85 (9 H, s), 0.06 (3 H, s), 0.00 (3 H, s); $[\alpha]^{25}_{D}$ +4.31° (*c* 1.16, $CHCl_3$); MS (EI) *m/z* 481 $(M+H)^+$ 423.

***N*-(*tert*-Butoxycarbonyl)-L-allylglycine 2-thiopyridyl ester.** To a stirred solution of Boc-L-allylglycine (9.3 g, 43.3 mmol) and triphenylphosphine (14.8 g, 56.3 mmol) in dry CH_2Cl_2 (100 mL) at room temperature was added 2,2'-dipyridyl disulfide (10.5 g, 47.6 mmol) in portions. The reaction mixture was stirred for 15 h and concentrated in vacuo to give an oily residue, which, upon column chromatography on SiO_2 (ethyl ether/hexane, 1/1), gave the title compound (12.2 g, 91%) as pale yellow prisms: mp 87-89 °C; IR (neat) 3230, 2984, 2950, 1712, 1576 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.56 (1 H, ddd, *J* = 5, 2, 1 Hz), 7.70 (1 H, ddd, *J* = 8, 7, 2 Hz), 7.53 (1H, ddd, *J* = 8, 2, 1 Hz), 7.22 (1H, ddd, *J* = 7, 5, 2 Hz), 5.72 (1 H, ddt, *J* = 17, 10,

7 Hz), 5.54 (1H, d, J = 8 Hz), 5.12 (2 H, m), 4.50 (1 H, dt, J = 7, 7 Hz), 2.54 (2 H, m), 1.43 (9 H, s).

***N*-(*tert*-Butoxycarbonyl)-L-allylglycyl-*O*-(*tert*-butyldimethylsilyl)-L-threonyl-L-allylglycine (13).** To a stirred suspension of **12b** (795 mg, 3.4 mmol) in DMF (7 mL) at room temperature was added trimethylsilyl imidazole (1.0 ml, 6.8 mmol). The reaction mixture was stirred for 2 h at room temperature. To this solution was added a solution of *N-tert*-butoxycarbonyl-L-allylglycine thiopyridyl ester (876 mg, 2.8 mmol) in CH_2Cl_2 (8 mL), dropwise, over 2 h. The mixture was stirred for 15 h, acidified to pH 3 with 3 *N* HCl, and extracted with EtOAc several times. The combined organic phase was washed with H_2O and brine, dried, and concentrated in vacuo to give *N*-(*tert*-butoxycarbony)-L-allylglycyl-*O*-(*tert*-butyldimethylsilyl)-L-threonine as a colorless oil. To this solution in CH_2Cl_2 (10 mL) was added triphenylphosphine (1.1 g, 4.2 mmol) and a solution of 2,2'-dipyridyl disulfide (740 mg, 3.4 mmol) in CH_2Cl_2 (3 mL), dropwise, over 2 h .The reaction mixture was stirred for 7 h, concentrated in vacuo to give an oily residue, which, upon purification by column chromatography on SiO_2 (ethyl ether/hexane, 3/1), gave the corresponding thiopyridyl ester (1.29 g, 87%) as a pale yellow oil. IR (neat) 3450, 3332, 2936, 2864, 1710, 1576, 1506 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.57 (1 H, ddd, J = 5, 2, 1 Hz), 7.66 (1 H, ddd, J = 8, 7, 2 Hz), 7.53 (1 H, ddd, J = 8, 2, 1 Hz), 7.26 (1 H, ddd, J = 7, 5, 2 Hz), 7.02 (1 H, d, J = 9 Hz), 5.80 (1 H, ddt, J = 17, 10, 7 Hz), 5.20 (1 H, br d, J = 17 Hz), 5.16 (1 H, br d, J = 10 Hz), 5.02 (1 H, d, J = 8 Hz), 4.60 (2 H, m), 4.32 (1 H, dt, J = 8, 7 Hz), 2.61 (2 H, m), 1.45 (9 H, s), 1.14 (3 H, d, J = 7 Hz), 0.89 (9 H, s), 0.03 (3 H, s), -0.01 (3 H, s). A stirred suspension of L-allylglycine (255 mg, 2.2 mmol) and trimethylsilyl imidazole (651 ml, 4.4 mmol) in DMF (5 mL) was stirred at room temperature for 1 h. To the reaction mixture was added a solution of the above pyridine thiol ester (966 mg, 1.8 mmol) in CH_2Cl_2 (8 mL) over 2 h. The reaction mixture was stirred for 15 h, acidified to pH 3 with 1 *N* HCl and extracted with EtOAc several times. The combined organic phase was washed with H_2O and brine, dried and concentrated in vacuo to give **13** as a colorless oil. This was purified as its methyl ester by treatment with diazomethane. The solvent was removed in vacuo to give an oily residue, which, upon purification by column chromatography on SiO_2 (ethyl ether/hexane, 1/1), gave the methyl ester of **13** (875 mg, 88%) as colorless powder: mp 135-136 °C; $[\alpha]^{28}_D$ -3.1° (*c* 0.89, MeOH); IR (neat) 3425, 3380, 2964, 2940, 2855, 1748, 1714, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30 (1 H, d, J = 9 Hz), 7.01 (1 H, d, J = 6 Hz), 5.73 (2 H, m), 5.05-5.25 (4 H, m), 5.00 (1 H, br s), 4.66 (1 H, q, J = 7 Hz), 4.41 (1 H, br s), 4.36 (1 H, dd, J = 6, 3 Hz), 4.19 (1 H, d, J = 6 Hz), 3.74 (3 H, s), 2.53 (4 H, m), 1.45 (9 H, s), 1.11 (3 H, d, J = 7 Hz), 0.93 (9 H, s), 0.16 (3 H, s), 0.14 (3 H, s,); MS (EI) *m/z* 542 $(M)^+$, 526, 497, 485. Anal. Calcd for $C_{26}H_{47}O_7N_3Si$: C, 57.64; H, 8.74; N, 7.76. Found: C, 57.66; H, 8.78; N, 7.70.

***N*-(Benzyloxycarbonyl)-*O*-(*tert*-butyldimethylsilyl)-L-threonine (12b).** To a solution of imidazole (2.42 g, 36 mmol) in dry DMF (3 mL) and *N*-(benzyloxycarbonyl)-L-threonine (3.00 g, 12 mmol) in dry DMF (7 mL) was added *tert*-butyldimethylsilyl chloride (5.38 g, 36 mmol) in dry DMF (10 mL) at 0 °C under N_2. The mixture was warmed to room temperature and stirred at the same temperature for 14 h. The reaction mixture was poured into ice-water (200 mL) and extracted with ethyl ether. The organic layer was dried, and concentrated in vacuo. The residue was dissolved in THF (24 mL) and 0.5 *N* KOH (24 mL), stirred at 0 °C for 4 h, and extracted with ethyl ether. The aqueous layer was acidified to pH 3 with 1 *N* HCl, and extracted with ethyl ether 3 times. The combined organic layer was washed with brine, dried, and concentrated in vacuo to give a crude solid which was washed with n-hexane to give **12b** (3.88g, 89%) as white crystals: *Rf* 0.51 (MeOH/$CHCl_3$, 1/9); mp 150.5~152.5 °C; IR (film) 3450, 2950, 1720 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 8.00~7.60 (1 H, br s), 7.35 (5 H, m), 5.70 (1 H, d, J = 8.5 Hz), 5.13 (2 H, s), 4.47 (1 H, dq, J = 2.5, 6.5 Hz), 4.32 (1 H, dd, J = 2.5, 8.5 Hz), 1.20 (3 H, d, J = 6.5 Hz), 0.86 (9 H, s), 0.08 (3 H, s), 0.06 (3 H, s); $[\alpha]^{23}_D$ +13.2° (*c* 1.0, $CHCl_3$); MS (EI) *m/z* 367 M^+, 310, 266, 159, 91. Anal. Calcd for $C_{18}H_{29}NO_5Si$: C, 58,83; H, 7.95; N, 3.81. Found: C, 59.01; H, 7.98; N, 3.81.

***O*-(*tert*-Butyldimethylsilyl)-L-threonine (12a).** A solution of **12b** (500 mg, 1.36 mmol) in ethanol (20 mL)

was stirred over 10% palladium on carbon (50 mg) under H_2 (1 atm) at room temperature for 17 h. The catalyst was filtered off, and the filtrate was concentrated in vacuo to give **12a** (318 mg, 100%) as a white powder: *Rf* 0.61 (MeOH/$CHCl_3$/H_2O, 65/65/10); mp 152~169 °C (decomp); IR (nujol) 3600~2300, 1720 cm^{-1}; ^1H NMR (100 MHz, CD_3OD) δ 4.54 (1 H, dq, *J* = 2, 6.5 Hz), 3.37 (1 H, d, *J* = 2 Hz), 1.28 (3 H, d, *J* = 6.5 Hz), 0.89 (9 H, s), 0.11 (3 H, s), 0.10 (3 H, s); $[\alpha]^{23}_D$ -28.7° (*c* 1.0, MeOH); MS (EI) *m/z* 234 $(M+H)^+$, 176. HRMS (EI) *m/z* calcd for $C_6H_{14}NO_3Si$ $(M\text{-butyl})^+$ 176.0740, found 176.0738.

General procedure for the peptide coupling using a catalytic amount of *tert*-amine. *N*-(Benzyloxycarbonyl)-L-valyl-L-proline. A suspension of L-proline (28 mg, 0.24 mmol), **9** (69 mg, 0.2 mmol), and *N,N*-diisopropylethylamine (7 μL, 0.04 mmol) in dry DMF (1.5 mL) was stirred at room temperature for 72 h. The reaction mixture was diluted with water, acidified to pH 2 with 1 *N* HCl, and extracted with ethyl acetate 3 times. The combined organic layer was dried, and concentrated in vacuo to give an oily residue, which was dissolved with ethyl ether and treated with diazomethane. The crude ester was purified by column chromatography on SiO_2 (ethyl ether/n-hexane, 1/1) to give the methyl ester of the title compound (62 mg, 85%) as an oil: IR (film) 3300, 1750, 1720, 1645 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 7.32 (5 H, s), 5.55 (1 H, d, *J* = 7 Hz), 5.04 (2 H, s), 4.50 (1 H, m), 4.32 (1 H, dd, *J* = 7, 9 Hz), 3.69 (3 H, s), 1.02 (3 H, d, *J* = 7 Hz), 0.95 (3 H, d, *J* = 7 Hz); $[\alpha]^{25}_D$ -93.0° (*c* 1.62, MeOH); MS (EI) *m/z* 363 $(M+H)^+$, 255.

***N*-(Benzyloxycarbonyl)-L-valyl-L-azetidine-2-carboxylic acid.** According to the general procedure, treatment of L-Aze-OH (53 mg, 0.52 mmol) with **9** (49 mg, 0. 43 mmole) in the presence of *N,N*-diisopropylethylamine (9 μL, 0.052 mmole) gave the methyl ester of the title compound (129 mg, 86%) as an oil: IR (film) 3300, 1750, 1740, 1720, 1660 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 7.32 (5 H, s), 5.55 (1 H, d, *J* = 9 Hz), 5.06 (2 H, s), 4.70 (1 H, dd, *J* = 7, 9 Hz), 4.28 (1 H, dd, *J* = 7, 8 Hz), 4.01 (1 H, dd, *J* = 7, 8 Hz), 3.73 (3 H, s), 1.8-2.8 (3 H, m), 1.02 (3 H, d, *J* = 6 Hz), 0.94 (3 H, d, *J* = 6 Hz); $[\alpha]^{25}_D$ -93.4° (*c* 1.1, $CHCl_3$); MS (EI) *m/z* 348 M^+, 206, 256.

(2*S*,3*R*)-2-(*tert*-Butoxycarbonyl)amino-3-(tetrahydropyranyl)oxy-4-[4-(*tert*-butyldimethylsilyl)oxy]-phenylbutyric acid 2-pyridyl thiolester (6h). To a solution of **6g** (1.03 g, 2.02 mmol) in CH_2Cl_2 (10 mL) was added triphenylphosphine (0.95 g, 3.62 mmol) and 2,2'-dipyridyl disulfide (0.71 g, 3.22 mmol), successively. The mixture was stirred at 40 °C for 8.5 h and at room temperature for 13 h. The mixture was concentrated in vacuo and subjected to a short pass column chromatography on SiO_2 (ethyl ether/n-hexane, 1/9, then 1/1) gave **6h** (0.85 g, 69%) as an oil. This compound was used for the next reaction without further purification. *Rf* 0.32 (ethyl ether/n-hexane, 1/1); IR (film) 2950, 1760, 1730, 1610, 1580, 1510 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 8.56 and 8.46 (each 1 H, d, *J* = 4 Hz), 7.8~7.4 (2 H, m), 7.3~7.1 (1 H, m), 7.06 (2 H, d, *J* = 8 Hz), 6.74 (2 H, d, *J* = 8 Hz), 5.75 and 5.51 (each 1 H, d, *J* = 10 Hz), 4.7~4.1 (3 H, m), 4.0~2.5 (4 H, m), 2.3~2.1 (0.5 H, m), 1.9~1.3 (5.5 H, m), 1.53 (9 H, s), 0.96 (9 H, s), 0.16 (6 H, s); MS (EI) *m/z* 464 (M-COSPy)$^+$.

(2*S*,3*R*)-2-(*tert*-Butoxycarbonyl)amino-3-(tetrahydropyranyl)oxy-4-[4-(*tert*-butyldimethylsilyl)oxy]-phenylbutyryl-*O*-(*tert*-butyldimethylsilyl)-L-threonine (14). A suspension of **12a** (52.7 mg, 0.23 mmol) and 1-(trimethylsilyl)imidazole (63.4mg, 0.45 mmol) in dry DMF (1 mL) was stirred at room temperature for 1 h. To this solution was added a solution of **6g** (90.3 mg, 0.15 mmol) in dry DMF (1.5 mL). The mixture was stirred at room temperature for 14 h. Water was added to the reaction mixture, and the resulting solution was acidified to pH 3 with 1 *N* HCl, and extracted with ethyl acetate 3 times. The combined organic layer was washed with brine, dried, and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (methanol/chloroform, 1/13) to give the dipeptide **14** (96.0 mg, 88%) as an amorphous powder: *Rf* 0.62 (MeOH/$CHCl_3$, 1/4); IR (film) 3360, 2940, 1722, 1680, 1514 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 7.07 and 7.03 (2 H, d, *J* = 8 Hz), 6.72 (2 H, d, *J* = 8 Hz), 6.4~6.0 (1 H, br s), 5.72 and 5.43 (1 H, d, *J* = 8 Hz), 4.9~4.0

(6 H, m), 3.6~3.1 (1 H, br s), 3.1~2.6 (2 H, m), 1.44 (9 H, s), 1.8~1.2 (6 H, m), 1.17 and 1.12 (3 H, d, J = 7 Hz), 0.96 and 0.88 and 0.86 (18 H, s), 0.16 and 0.15 and 0.10 and 0.07 (12 H, s); MS (SIMS) *m/z* 725 $(M+H)^+$. Anal. Calcd for $C_{36}H_{64}N_2O_9Si$: C, 59.63; H, 8.90; N, 3.86. Found: C, 59.40; H, 8.90; N, 3 .79.

(2*S*, 3*R*)-2-(*tert*-Butoxycarbonyl)amino-3-(tetrahydropyranyloxy-4-[4-(*tert*-butyldimethylsilyl)oxy]-phenylbutyryl-*O*-(*tert*-butyldimethylsilyl)-L-threonyl-3(*S*)-hydroxy-4(*S*)-methyl-L-proline methyl ester (15a). A solution of **5i** (23.6 mg, 91 mmol) in trifluoroacetic acid and CH_2Cl_2 (1/1, 2 mL) was stirred at room temperature for 30 min. The mixture was concentrated in vacuo, and extracted with ethyl ether. The aqueous layer was concentrated in vacuo. Trace amounts of water were removed azeotropically with benzene. The residue was dissolved in dry DMF (1 mL) along with **14** (49.6 mg, 68 mmol), diethylphosphoryl cyanide (15.1 mg, 93 mmol) and triethylamine (16.7 mg 165 mmol). The mixture was stirred at 0 °C for 15 h, diluted with benzene/ethyl acetate (2/1, 10 mL), and washed with 1 *N* HCl, water, saturated aqueous sodium bicarbonate, and brine successively. The organic phase was dried, and concentrated in vacuo to afford an oily residue, which, upon purification with column chromatography on SiO_2 (ethyl ether/n-hexane, 3/7, then 7/3), gave **15a** (49.4 mg, 83%) as an amorphous powder: *Rf* 0.55 (ethyl ether); IR (film) 3430, 2960, 1750, 1720, 1650 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.07 and 7.04 (2 H, d, J = 8 Hz), 6.74 and 6.73 (2 H, d, J = 8 Hz), 5.63 and 5.33 (1 H, d, J = 7.5 Hz), 4.75~3.80 (9 H, m), 3.72 and 3.71 (3 H, s), 3.60~3.20 (3 H, m), 2.92 (0.5 H, dd, J = 6, 14 Hz), 2.75~2.55 (1.5 H, m), 2.42~2.30 (2 H, m), 1.55~1.30 (6 H, m), 1.23 and 1.18 (3 H, d, J = 6 Hz), 1.09 and 1.07 (3 H, d, J = 6 Hz), 0.97 (9 H, s), 0.91 and 0.89 (9 H, s), 0.13 and 0.11 and 0.09 and 0.08 and 0.07 and 0.06 (12 H, s); MS (SIMS) *m/z* 866 $(M+H)^+$. Anal. Calcd for $C_{43}H_{75}N_3O_{11}Si_2$: C, 59.62; H, 8.73; N, 4.85. Found: C, 59.55; H, 8.70; N, 4.74.

3(*R*)-Hydroxy-L-homotyrosinyl-L-threonyl-3(*S*)-hydroxy-4(*S*)-methyl-L-proline methyl ester hydrochloride (15b). A solution of **15a** (29.7 mg, 34 mmol) and *dl*-10-camphorsulfonic acid (2.0 mg, 8.6 mmol) in methanol (0.5 mL) was stirred at room temperature for 20 h. The solvent was evaporated in vacuo. The residue was dissolved in trifluoroacetic acid/CH_2Cl_2 (1/1, 2 mL), and was stirred at room temperature for 20 min. The solvent was evaporated in vacuo. The residue was dissolved in THF/1*N* HCl (1/1, 1 mL), and was stirred at room temperature for 14 h. The reaction mixture was extracted with ethyl ether. The aqueous layer was concentrated in vacuo to give **15b** (17.0 mg, 100%) as a hygroscopic powder: *Rf* 0.34 ($CHCl_3$/MeOH/H_2O, 71/25/4); FT-IR (film) 3288, 1743, 1678, 1635, 1515 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 7.07 (1 H, d, J = 8 Hz), 6.70 (1 H, d, J = 8 Hz), 4.61 (1 H, d, J = 7 Hz), 4.38 (1 H, d, J = 1 Hz), 4.17 (1 H, d, J = 4 Hz), 4.06~3.88 (4 H, m), 3.70 (3 H, s), 3.55 (1 H, t, J = 10 Hz), 2.78 (1 H, dd, J = 10, 14 Hz), 2.61 (1 H, dd, J = 10, 14 Hz), 2.42~2.32 (1 H, m), 1.30 (3 H, d, J = 6 Hz), 1.06 (3 H, d, J = 6.5 Hz); $[\alpha]^{28}_D$ -11.82° (*c* 1.7, MeOH); MS (SIMS) *m/z* 454 $(M+H)^+$.

***N*-(Benzyloxycarbonyl)-*O*-(*tert*-butyldimethylsilyl)-L-threonine 2-pyridyl thiol ester (12c).** To a solution of **12b** (839 mg, 2.3 mmol) in dry CH_2Cl_2 (9 mL) was added 2,2'-dipyridyl disulfide (508 mg, 2.3 mmol) and triphenylphosphine (718 mg, 2.7 mmol), successively. The mixture was stirred at room temperature for 14 h, concentrated in vacuo, and subjected to a short-pass column chromatography on SiO_2 (ethyl ether/n-hexane, 1/9, then 2/3) to give **12c** (969 mg, 92%) as an oil: *Rf* 0.63 (ethyl ether/n-hexane, 3/1); IR (film) 3460, 2936, 1734 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 8.59 (1 H, d, J = 4 Hz), 7.81~7.18 (3 H, m), 7.38 (5 H, m), 5.68 (1 H, d, J = 9 Hz), 5.20 (2 H, s), 4.55 (1 H, dq, J = 2, 6 Hz), 4.40 (1 H, dd, J = 2, 9 Hz), 1.21 (3 H, d, J = 6 Hz), 0.86 (9 H, s), 0.04 (3 H, s), 0.00 (3 H, s); MS (EI) *m/z* 460 M^+ 402, 350, 322.

***N*-(Benzyloxycarbonyl)-*O*-(*tert*-butyldimethylsilyl)-L-threonyl-4(*R*)-hydroxy-L-proline (16a).** To a suspension of **7** (44 mg, 0.34 mmol) in dry DMF (1 mL) was added 1-(trimethylsilyl)imidazole (94 mg, 0.67 mmol) and triethylamine (20 mg, 0.20 mmol). The mixture was stirred at room temperature for 2.5 h. The thiol ester **12c** (103 mg, 0.22 mmol) was added to this solution, and the mixture was stirred at room temperature for 16 h. The solution was acidified to pH 3 with 1 *N* HCl and extracted with ethyl acetate 3

times. The combined organic layer was washed with brine, dried, and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (ethyl ether/n-hexane, 4/1, then methanol/chloroform, 1/4) to give **16a** (72 mg, 67.0%) as an amorphous powder: *Rf* 0.34 (MeOH/$CHCl_3$, 2/3); IR ($CHCl_3$) 3452, 2964, 1716, 1646 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 7.32 (5 H, m), 5.60 (1 H, d, *J* = 7 Hz), 5.07 (2 H, s), 4.73 (1 H, dd, *J* = 8, 9 Hz), 4.50 (1 H, m), 4.37 (1 H, dd, *J* = 6, 7 Hz), 4.2–3.7 (3 H, m), 3.67 (1 H, dd, *J* = 4, 11 Hz), 2.4~2.2 (2 H, m), 1.20 (3 H, d, *J* = 7 Hz), 0.85 (9 H, s), 0.07 (3 H, s), 0.06 (3 H, s); $[\alpha]^{20}_D$ -49.5° (*c* 3.07, MeOH); MS (SIMS) *m/z* 481 $(M+H)^+$. Elementary analytical data were measured by the use of its methyl ester (prepared by the esterification with diazomethane). Anal. for methyl ester of **16a**: Calcd for $C_{24}H_{38}N_2O_7Si$: C, 58.28; H, 7.74; N, 5.66. Found: C, 58.28; H, 7.75; N, 5.59.

***N*-(Benzyloxycarbonyl)-*O*-(*tert*-butyldimethylsilyl)-L-threonyl-4(*R*)-*O*-(*tert*-butyldimethylsilyl)-L-proline Methyl Ester (Methyl Ester of 16b).** To a solution of **7h** (84 mg, 0.34 mmol) and diisopropylethylamine (9.5 μL, 0.055 mmol) in DMF (1.2 mL) was added a solution of **12c** (82 mg, 0.18 mmol) in DMF (1.3 mL). The mixture was stirred at room temperature for 16 h. The solution was acidified to pH 3 with 1 *N* HCl and extracted with ethyl acetate 3 times. The combined organic layer was washed with brine, dried, and concentrated in vacuo. The residue dissolved in ethyl ether was esterified with diazomethane to give a crude ester which was purified by column chromatography on SiO_2 (ethyl ether/n-hexane, 1/9, then 3/7) to give the methyl ester of **16b** (95 mg, 87.0%) as an amorphous powder:; IR (film) 3450, 3304, 2960, 1750, 1725, 1644 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.34 (5 H, m), 5.47 (1 H, d, *J* = 8.8 Hz), 5.07 (2 H, s), 4.57 (1 H, dd, *J* = 7.8, 7.8 Hz), 4.48 (1 H, m), 4.42 (1 H, dd, *J* = 5.9, 8.8 Hz), 4.0 (1 H, quintet, *J* = 6.4 Hz), 3.87 (1 H, dd, *J* = 4.4, 10.4 Hz), 3.72 (3 H, s),, 3.70 (1 H, m), 2.19 (1 H, ddd, *J* = 4.0, 7.8, 12.7 Hz), 2.02 (1 H, ddd, *J* = 4.4, 7.8, 12.7 Hz), 1.20 (3 H, d, *J* = 6.4 Hz), 0.87 (9 H, s), 0.85 (9 H, s), 0.07 (3 H, s), 0.06 (6 H, s), 0.05 (3 H, s); $[\alpha]^{20}_D$ -33.3° (*c* 0.5, $CHCl_3$); MS (FAB) *m/z* 603 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{30}H_{52}N_2O_7Si_2$ M^+ 609.3391, found 609.3393.

***N*-(Benzyloxycarbonyl)-*O*-(*tert*-butyldimethylsilyl)-L-threonyl-4(*R*)-hydroxy-L-prolyl-3(*R*)-hydroxy-L-homotyrosinyl-L-threonyl-3(*S*)-hydroxy-4(*S*)-methyl-L-proline methyl ester (17a).** To a stirred solution of **15b** (153 mg, 0.31 mmol) and **16a** (165 mg, 0.34 mmol) in dry DMF (3 mL) was added diethylphosphoryl cyanide (56 mg, 0.34 mmol) and triethylamine (65 mg, 0.65 mmol), successively, at 0 °C. The mixture was stirred at 0 °C for 1 h, and at room temperature for 2 h. The reaction mixture was diluted with ethyl acetate (30 mL), and washed with 10% aqueous citric acid, water, saturated aqueous sodium bicarbonate, and brine successively. The organic phase was dried, and concentrated in vacuo to afford the crude pentapeptide, which, upon purification by column chromatography on SiO_2 (methanol/chloroform, 2/23), gave **17a** (205 mg, 72%) as an amorphous solid: *Rf* 0.45 (MeOH/$CHCl_3$, 3/17); IR ($CHCl_3$) 3400, 1734, 1638, 1516 cm^{-1}; ^1H NMR (360 MHz, d_6-DMSO) δ 9.09 (1 H, s), 8.20 (1 H, d, *J* = 9 Hz), 7.47 (1 H, d, *J* = 9.5 Hz), 7.37 (1 H, d, *J* = 8 Hz), 7.30 (5 H, m), 6.98 (2 H, d, *J* = 8.5 Hz), 6.58 (2 H, d, *J* = 8.5 Hz), 5.48 (1 H, d, *J* = 4.5 Hz), 5.17 (1 H, d, *J* = 3.5 Hz), 5.03 (1 H, d, *J* = 5 Hz), 5.00 (2 H, s). 4.75 (1 H, d, *J* = 5 Hz), 4.57 (1 H, t, *J* = 8 Hz), 4.42 (1 H, dd, *J* = 6, 8 Hz), 4.37 (1 H, m), 4.26 (1 H, t, *J* = 9.5 Hz), 4.20 (1 H, d, *J* = 1.5 Hz), 4.13 (1 H, dd, *J* = 2.5, 9 Hz), 4.40~3.98 (2 H,m), 3.91 (1 H, dq, *J* = 6, 9.5 Hz), 3.89 (1 H, dd, *J* = 8, 10 Hz), 3.84~3.74 (1 H, m), 3.76 (1 H, dd, *J* = 4.5, 11 Hz), 3.66 (1 H, d, *J* = 11 Hz), 3.62 (3 H, s), 3.4~3.2 (1 H, overlapping), 2.70 (1 H, dd, *J* = 8, 13 Hz), 2.53 (1 H, dd, *J* = 6, 13 Hz), 2.26~2.16 (1 H, m), 2.18~2.08 (1 H, m), 1.90 (1 H, ddd, *J* = 4.5, 8, 13 Hz), 1.18 (3 H, d, *J* = 6 Hz), 1.10 (3 H, d, *J* = 6 Hz), 0.93 (3 H, d, *J* = 7 Hz), 0.82 (9 H, s), 0.06 (3 H, s), 0.03 (3 H, s); $[\alpha]^{26}_D$ -83.9° (*c* 1.27, MeOH); MS (SIMS) *m/z* 916 $(M+H)^+$. Anal. Calcd for $C_{44}H_{65}N_5O_{14}Si$-H_2O: C, 56.57; H, 7.23; N, 7.50. Found: C, 56.28; H, 7.12; N, 7.24.

***N*-(Benzyloxycarbonyl)-*O*-(*tert*-butyldimethylsilyl)-L-threonyl-4(*R*)-hydroxy-L-prolyl-3(*R*)-hydroxy-L-homotyrosinyl-L-threonyl]-3(*S*)-hydroxy-4(*S*)-methyl-L-proline amide (17c).** A solution of **17a** (9.5 mg, 10 mmol) in dry methanol (1 mL) was placed in a two-neck flask on which dry ice condenser was attached.

Upon cooling the condenser at -78 °C by dry ice/ethanol, gaseous ammonia was passed through the flask until the total volume of the solution became ~2 mL. Then, dry ice was removed from the condenser, the reaction mixture was allowed to warm to room temperature, and stirred at room temperature for 5.5 days. The mixture was concentrated in vacuo and purified by preparative TLC (silica gel, methanol/chloroform, 2/9) to afford **17c** (3.5 mg, 38%) as an amorphous powder, and the strating **17a** (6.2 mg, 62%) was recovered. **17c**: *Rf* 0.29 ($MeOH/CHCl_3$, 1/4); IR (film) 3368, 2952, 1634, 1518 cm^{-1}; ^1H NMR (360 MHz, d_6-DMSO) δ 9.08 (1 H, s), 8.28 (1 H, d, *J* = 9 Hz), 7.48 (1 H, d, *J* = 9.5 Hz), 7.25 (1 H, d, *J* = 8.5 Hz), 7.14 (2 H, m), 6.99 (2 H, d, *J* = 8.5 Hz), 6.58 (2 H, d, *J* = 8.5 Hz), 5.21 (1 H, d, *J* = 5 Hz), 5.16 (1 H, d, *J* = 3.5 Hz), 5.10 (1 H, d, *J* = 5 Hz), 5.06 (1 H, d, *J* = 5.5 Hz), 4.64~4.56 (1 H, m), 4.59 (1 H, dd, *J* = 5.5, 8.5 Hz), 4.42~4.34 (1 H, m), 4.26 (1 H, dd, *J* = 8.5, 9 Hz), 4.12~3.96 (4 H, m), 3.96~3.88 (3 H, m), 3.76 (1 H, dd, *J* = 4, 10 Hz), 3.67 (1 H, d, *J* = 10 Hz), 3.18 (1 H, t, *J* = 10 Hz), 2.71 (1 H, dd, *J* = 8, 13 Hz), 2.56 (1 H, dd, *J* = 5.5, 13 Hz), 2.24~2.12 (2 H, m), 1.91 (1 H, ddd, *J* = 4.5, 8, 13 Hz), 1.20 (3 H, d, *J* = 4 Hz), 1.07 (3 H, d, *J* = 4.5 Hz), 0.93 (3 H, d, *J* = 5 Hz), 0.82 (9 H, s), 0.06 (3 H, s), 0.03 (3 H, s); $[\alpha]^{25}_D$ -75.7° (*c* 1.88, MeOH); MS (SIMS) *m/z* 901 $(M+H)^+$.

(2*S*,4*R*)-(Linoleyl)amino-4-(*tert*-butyldimethylsilyl)oxy-5-dimethoxypentanoyl-*O*-(*tert*-butyldimethylsilyl)-L-threonyl-4(*R*)-hydroxy-L-prolyl-3(*R*)-hydroxy-L-homotyrosinyl-L-threonyl-3(*S*)-hydroxy-4(*S*)-methyl-L-proline amide (18a). Preparation of **17d**: A solution of **17c** (42.0 mg, 47 mmol) in ethanol (0.5 mL) was stirred over 10% palladium on carbon (10 mg) under H_2 (1 atm) at room temperature for 14 h. The catalyst was filtered off, and the filtrate was concentrated in vacuo to give **17d** (35.8 mg, 100%) as an amorphous solid. Preparation of **8e**: A solution of **8d** (47.0 mg, 80 mmol) in THF (0.3 mL) and 0.5 *N* NaOH (0.165 mL) was stirred at 0 °C for 14 h. The mixture was carefully acidified to pH 3 with 0.5 *N* HCl, and extracted with ethyl acetate 2 times. The combined organic layer was washed with brine, dried, and concentrated in vacuo to give **8e** (45.9 mg, ~100%). Synthesis of **18a**: To a solution of **8e** (45.9 mg, 80 mmol) and **17d** (35.8 mg, 47 mmol) in dry DMF (1 mL) was added diethylphosphoryl cyanide (13.5 mg, 83 mmol) and triethylamine (8.4 mg, 83 mmol), successively, at 0 °C. The mixture was stirred at 0 °C for 4.5 h, then at room temperature for 20 h. The reaction mixture was diluted with ethyl acetate (10 mL), washed with 10% aqueous citric acid, water, saturated aqueous sodium bicarbonate, and brine successively. The organic phase was dried, and concentrated in vacuo to give crude hexapeptide, which, upon purification with column chromatography on SiO_2 ($MeOH/CHCl_3$, 3/17), gave **18a** (34.3 mg, 56%) as an amorphous powder: *Rf* 0.50 ($MeOH/CHCl_3$, 1/4); IR (film) 3352, 2934, 1646, 1520 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 7.08 (2 H, d, *J* = 8.5 Hz), 6.66 (2 H, d, *J* = 8.5 Hz), 5.40~5.26 (4 H, m), 4.74 (1 H, d, *J* = 5 Hz), 4.69 (1 H, d, *J* = 5 Hz), 4.67 (1 H, t, *J* = 8 Hz), 4.56 (1 H, t, *J* = 7 Hz), 4.52~4.46 (1 H, br s), 4.39 (1 H, d, *J* = 1 Hz), 4.34 (1 H, d, *J* = 2 Hz), 4.24~4.07 (5 H, m), 3.95 (1 H, dd, *J* = 8, 9.5 Hz), 3.91 (1 H, dd, *J* = 4, 11 Hz), 3.84 (1 H, dd, *J* = 6, 10.5 Hz), 3.78 (1 H, d, *J* = 10.5 Hz), 3.41 (3 H, s), 3.40 (3 H, s), 3.45~3.35 (1 H, overlapping), 2.83 (1 H, dd, *J* = 7.5, 13.5 Hz), 2.76 (2 H, t, *J* = 6 Hz), 2.74 (1 H, dd, *J* = 6.5, 13.5 Hz), 2.44~2.24 (2 H, m), 2.20 (2 H, t, *J* = 7.5 Hz), 2.24~2.00 (2 H, m), 2.05 (4 H, q, *J* = 6.5 Hz), 1.71 (1 H, ddd, *J* = 6.5, 7.5, 14.5 Hz), 1.64~1.54 (2 H, m), 1.40~1.26 (14 H, m), 1.24 (3 H, d, *J* = 6 Hz), 1.20 (3 H, d, *J* = 6 Hz), 1.04 (3 H, d, *J* = 7 Hz), 0.90 (3 H, t, *J* = 6 Hz), 0.91 (9 H, s), 0.89 (9 H, s), 0.13 (3 H, s), 0.12 (3 H, s), 0.10 (3 H, s), 0.09 (3 H, s); $[\alpha]^{26}_D$ -51.6° (*c* 1.11, MeOH); MS (SIMS) *m/z* 1318 $(M+H)^+$.

N^{α}-Linoleyl-N^{δ}-(*tert*-butoxycarbonyl)ornitine (23b). To a suspension of N^{δ}-*tert*-butoxycarbonylornitine (**23a**) (102 mg, 0.44 mmol) in dry DMF (1.5 mL) was added 1-(trimethylsilyl)imidazole (123 mg, 0.88 mmol). The mixture was stirred at room temperature for 2 h. To this solution was added linoleic acid 2-pyridyl thiolester (251 mg, 0.67 mmol) in dry DMF (1.5 mL). The mixture was stirred at room temperature for 14 h, then quenched with water, acidified to pH 3 with 0.5 *N* HCl, and extracted with ethyl acetate 3 times. The combined organic layer was washed with brine, dried, and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (ethyl ether/n-hexane, 4/1, methanol/chloroform, 1/4, then 3/7) gave **23b** (195 mg, 90%) as an oil: *Rf* 0.44 ($MeOH/CHCl_3$, 1/4); IR (film) 3328, 3212, 2932, 2860, 1722,

1700, 1680, 1534 cm^{-1}; ^1H NMR (100MHz, $CDCl_3$) δ 7.6~7.0 (1 H, m), 6.8~6.4 (1 H, m), 5.7~5.0 (4 H, m), 4.7~4.4 (1 H, m), 3.4~2.9 (2 H, m), 2.76 (2 H, t, *J* = 6 Hz), 2.4~1.1 (26 H, m), 1.44 (9 H, s), 0.88 (3 H, t, *J* = 6 Hz); $[\alpha]^{26}_D$ +16.7° (*c* 1.29, $CHCl_3$); MS (EI) *m/z* 495 $(M+H)^+$, 393, 376. HRMS (EI) *m/z* calcd for $C_{28}H_{50}N_2O_5$ $(M)^+$: 494.3717, found: 494.3701.

N^{α}-Linoleyl-N^{δ}-(*tert*-butoxycarbonyl)-L-ornitinyl-*O*-(*tert*-butyldimethylsilyl)-L-threonyl-4(*R*)-hydroxy-L-prolyl-3(*R*)-hydroxy-L-homotyrosinyl-L-threonyl]-3(*S*)-hydroxy-4(*S*)-methyl-L-proline methyl ester (24a). A solution of **17a** (171.7 mg, 0.19 mmol) in methanol (1.5 mL) was stirred over 10% palladium on carbon (32 mg) under H_2 (1 atm) at room temperature for 32 h. The catalyst was filtered off, and the filtrate was concentrated in vacuo to give **17b** (146.6mg, 100%) as an amorphous solid which was dissolved in dry DMF (2 mL) along with **23b** (102.0 mg, 0.21 mmol), diethylphosphoryl cyanide (33.6 mg, 0.21 mmol) and triethylamine (21.1 mg, 0.21 mmol). The mixture was stirred at 0 °C for 38 h, diluted with ethyl acetate (20 mL), and washed with 10% aqueous citric acid, water, saturated aqueous sodium bicarbonate, and brine, successively. The organic phase was dried, and concentrated in vacuo to afford crude hexapeptide, which was purified by column chromatography on SiO_2 (methanol/chloroform, 2/23) to give **24a** (159.6 mg, 68%) as an amorphous powder: *Rf* 0.47 (MeOH/$CHCl_3$, 2/3); IR ($CHCl_3$) 3328, 2936, 1646, 1516 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 7.08 (2 H, d, *J* = 8.5 Hz), 6.67 (2 H, d, *J* = 8.5 Hz), 5.40~5.26 (4 H, m), 4.70~4.60 (1 H, overlapping), 4.68 (1 H, d, *J* = 6.5 Hz), 4.63 (1 H, d, *J* = 6 Hz), 4.52~4.47 (1 H, m), 4.43 (1 H, dd, *J* = 5, 8.5 Hz), 4.38 (1 H, d, *J* = 1.5 Hz), 4.34 (1 H, d, *J* = 2.5 Hz), 4.21 (1 H, ddd, *J* = 2.5, 6.5, 7.5 Hz), 4.13 (1 H, dd, *J* = 1.5, 4.5 Hz), 4.09 (1 H, quintet, *J* = 6.5 Hz), 4.02 (1 H, quintet, *J* = 6 Hz), 3.92 (1 H, dd, *J* = 8, 10 Hz), 3.87 (1 H, dd, *J* = 7, 11 Hz), 3.83 (1 H, d, *J* = 11 Hz), 3.72 (3 H, s), 3.46 (1 H, t, *J* = 10 Hz), 3.02 (2 H, dt, *J* = 1.5, 6.5 Hz), 2.81 (1 H, dd, *J* = 7.5, 13.5 Hz), 2.76 (2 H, t, *J* = 6 Hz), 2.72 (1 H, dd, *J* = 6.5, 13.5 Hz), 2.41~2.26 (2 H, m), 2.24 (2 H, t, *J* = 7.5 Hz), 2.05 (4 H, q, *J* = 6.5 Hz), 2.14~2.01 (1 H, m), 1.82~1.71 (1 H, m), 1.64~1.54 (3 H, m), 1.42 (9 H, br s), 1.52~1.20 (16 H, m), 1.26 (3 H, d, *J* = 6 Hz), 1.24 (3 H, d, *J* = 6.5 Hz), 1.05 (3 H, d, *J* = 7 Hz), 0.90 (3 H, t, *J* = 6 Hz), 0.88 (9 H, s), 0.11 (6 H, s); $[\alpha]^{24}_D$ -25.0° (*c* 1.07, MeOH); MS (SIMS) *m/z* 1258 $(M+H)^+$.

Echinocandin D (1c). A solution of **24a** (159.6 mg, 0.13 mmol) in THF (0.5 mL) and 0.5 *N* NaOH (0.26 mL) was stirred at 0 °C for 19 h. In order to complete the reaction, additional 0.5 *N* NaOH (0.098 mL) was added to the mixture and was stirred at room temperature for 8 h. The reaction mixture was acidified to pH 3 with 1 *N* HCl, and extracted with ethyl acetate. The organic layer was dried, and concentrated in vacuo. The residue was treated with CH_2Cl_2/trifluoroacetic acid (1/1, 2 mL) at room temperature for 15 min. The solvent was evaporated in vacuo. The residue was dissolved in THF/1 *N* HCl (1/1, 2 mL), and the mixture was stirred at room temperature for 5 h. Then the solvent was evaporated in vacuo. Trace amounts of water were removed azeotropically with benzene. The residue was dissolved in dry DMF (25 mL) along with diphenylphosphoryl azide (52.4 mg, 0.19 mmol) and triethylamine (38.5 mg, 0.38 mmol). The mixture was stirred at 0 °C for 15 h, and concentrated in vacuo to give crude **1c**, which, upon purification by medium pressure column chromatography (Lichroprep RP-8 (Merck), MeOH/H_2O, 4/1), then preparative TLC (silica gel, $CHCl_3$/MeOH/H_2O, 71/25/4), gave **1c** (50 mg, 39%) as colorless crystals which were recrystallized from ethanol/water).[2] *Rf* 0.57 ($CHCl_3$/MeOH/H_2O, 71/25/4); mp 172~174 °C (decomp) ; IR (nujol) 3296, 1636 cm^{-1}; ^1H NMR (360 MHz, d_6-DMSO) δ 9.16 (1 H, br s), 8.03~7.56 (1 H, br s), 7.78 (1 H, d, *J* = 8.5 Hz), 7.64 (1 H, d, *J* = 7.5 Hz), 7.55 (1 H, d, *J* = 9 Hz), 7.47 (1 H, t, *J* = 5 Hz), 6.94 (2 H, d, *J* = 8 Hz), 6.65 (2 H, d, *J* = 8 Hz), 5.39~5.18 (9 H, overlapping), 4.75 (1 H, dd, *J* = 3.5, 9.5 Hz), 4.54 (1 H, br s), 4.47~4.39 (2 H, m), 4.30~4.13 (5 H, m), 4.01 (2 H, m), 3.88 (1 H, dd, *J* = 3, 11 Hz), 3.90~3.80 (1 H, overlapping), 3.72 (1 H, d, *J* = 11 Hz), 3.20 (1 H, t, *J* = 9.5 Hz), 3.14~2.92 (2 H, m), 2.74 (2 H, t, *J* = 6 Hz), 2.54 (1 H, dd, *J* = 6, 13 Hz), 2.45 (1 H, dd, *J* = 8, 13 Hz), 2.32~2.18 (2 H, m), 2.16~1.78 (4 H, overlapping), 2.02 (4 H, q, *J* = 6.5 Hz), 1.58~1.38 (3 H, m), 1.42~1.16 (16 H, m), 1.11 (3 H, d, *J* = 6 Hz), 1.04 (3 H, d, *J* = 6 Hz), 0.95 (3 H, d, *J* = 7 Hz), 0.86 (3 H, t, *J* = 7 Hz); $[\alpha]^{22}_D$ -46.1° (*c* 0.9, MeOH); MS (SIMS) *m/z* 1012 $(M+H)^+$.[2]

Tetrahydroechinocandin D. A solution of **1c** (12.1 mg, 12 mmol) in methanol (2 mL) was stirred over 10% palladium on carbon (2.4 mg) under H_2 (1 atm) at room temperature for 8 h. The catalyst was filtered off, and the filtrate was concentrated in vacuo to give the title compound (12.1 mg, 100%) as an amorphous solid:[2] *Rf* 0.57 ($CHCl_3$/MeOH/H_2O, 71/25/4); mp 180~185 °C (decomp); IR (nujol) 3330, 1628, 1518 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 7.02 (2 H, d, *J* = 8.5 Hz), 6.70 (2 H, d, *J* = 8.5 Hz), 5.0~4.7 (2 H, overlapping), 4.64 (1 H, dd, *J* = 7, 11.5 Hz), 4.57 (1 H, br s), 4.55~4.40 (1 H, m), 4.45~4.35 (3 H, m), 4.31 (1 H, d, *J* = 2 Hz), 4.30~4.20 (1 H, m), 4.19 (1 H, dd, *J* = 2, 4 Hz), 4.02 (1 H, dd, *J* = 3, 11 Hz), 3.9~3.8 (1 H, overlapping), 3.81 (1 H, d, *J* = 11 Hz), 3.53~3.40 (1 H, m), 3.38 (1 H, t, *J* = 9.5 Hz), 3.03~2.92 (1 H, m), 2.66 (1 H, dd, *J* = 6.5, 14 Hz), 2.56 (1 H, dd, *J* =8, 14 Hz), 2.50~2.40 (2 H, m), 2.24 (2 H, t, *J* = 7 Hz), 2.2~2.0 (1 H, overlapping), 2.08 (1 H, dt, *J* = 3, 13 Hz), 1.75~1.65 (2 H, m), 1.65~1.50 (3 H, m), 1.4~1.2 (28 H, m), 1.22 (3 H, d, *J* = 6.5 Hz), 1.18 (3 H, d, *J* = 6.5 Hz), 1.05 (3 H, d, *J* = 7 Hz), 0.90 (3 H, t, *J* = 7 Hz); ^1H NMR (360 MHz, d_6-DMSO) δ 9.18 (1 H, s), 8.00~7.80 (1 H, m), 7.79 (1 H, d, *J* = 8.5 Hz), 7.65 (1 H, d, *J* = 7.5 Hz), 7.55 (1 H, d, *J* = 9 Hz), 7.49 (1 H, t, *J* = 4.5 Hz), 6.95 (2 H, d, *J* = 8 Hz), 6.65 (2 H, d, *J* = 8 Hz), 5.27 (1 H, d, *J* = 5.5 Hz), 5.4~5.1 (4 H, overlapping), 4.75 (1 H, m), 4.54 (1 H, br s), 4.50~4.38 (2 H, m), 4.30~4.10 (5 H, m), 4.05~3.95 (2 H, m), 3.88 (1 H, dd, *J* = 3, 11 Hz), 3.9~3.8 (1 H, overlapping), 3.72 (1 H, d, *J* = 11 Hz), 3.21 (1 H, t, *J* = 9.5 Hz), 3.15~2.90 (2 H, m), 2.55 (1 H, dd, *J* = 9, 14 Hz), 2.45 (1 H, dd, *J* = 8, 14 Hz), 2.35~2.15 (2 H, m), 2.2~2.0 (2 H, m), 1.95 (1 H, dt, *J* = 3, 12 Hz), 1.95~1.75 (1 H, m), 1.6~1.3 (5 H, m), 1.4~1.1 (28 H, m), 1.10 (3 H, d, *J* = 6.5 Hz), 1.03 (3 H, d, *J* = 6.5 Hz), 0.95 (3 H, d, *J* = 7 Hz), 0.86 (3 H, t, *J* = 6.5 Hz); $[\alpha]^{22}_D$ -38.5° (*c* 0.61 MeOH); MS (SIMS) *m/z* 1016 $(M+H)^+$.

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