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SYNTHETIC STUDIES ON CALLIPELTINS: STEREOSELECTIVE SYNTHESES OF (3*S*,4*R*)-3,4-DIMETHYL-L-PYROGLUTAMIC ACID AND FMOC-D-ALLOTHREONINE FROM SERINE DERIVATIVES

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Abstract – The non-proteinogenic amino acids contained in callipeltins, (3*S*, 4*R*)-3, 4-dimethyl-L-pyroglutamic acid and D-allothreonine, were synthesized from D- or L-serine. The stereoselective synthesis of two methyl groups of (3*S*, 4*R*)-3, 4-dimethyl-L-pyroglutamic acid was accomplished by diastereoselective hydrogenation and alkylation. Kinetic epimerization of the C-4 methyl substituent followed by Boc-deprotection with 10% TFA gave the desired (3*S*, 4*R*)-3, 4-dimethyl-L-pyroglutamic acid as a single isomer.

INTRODUCTION

Callipeltin A (**1**)[1] and B (**2**)[2] are cyclic depsipeptides isolated from a shallow water sponge of the genus *Callipelta* by Zampella *et al*. Callipeltins have antiviral and antifungal properties, and callipeltin A (**1**) is the first natural peptide found to act against HIV. Callipeltin A (**1**) was also found to be a selective and powerful inhibitor of cardiac interest as a regulator of myocardial contractility.[3] Similar biological activities were reported for cyclic depsipeptides isolated from other marine sponges.[4] Because of the unique structure containing unusual amino acid residues and biological properties of these natural products, intensive efforts have been directed toward their synthesis[5] including the solid phase synthesis of callipeltin B (**2**).[6] Although the total synthesis of callipeltin A (**1**) has not yet been achieved, several groups[7] have generated the unusual amino acids contained in callipeltins. We also reported the synthesis and identification of the absolute configuration of β-methoxytyrosine in callipeltin E to be 2*R*, 3*R*.[8]

As part of our efforts toward the total synthesis and elucidation of the structure-activity relationship of callipeltin B (**2**), we report herein the synthesis of two unusual amino acids contained in callipeltin B (**2**), (3*S*,4*R*)-3,4-dimethyl-L-pyroglutamic acid (**3**) and Fmoc-D-allothreonine (**4**), from a serine derivative. Two other groups, Lipton's group[9] and Hamada's group,[10] have achieved the synthesis of **3** using an intermediate derived from L-pyroglutamic acid, independently. The key step, the diastereoselective induction of methyl groups, was achieved by Micheal-type addition and subsequent enolate alkylation. In the present study, serine derivatives, a versatile synthon used in the synthesis of a variety of unusual amino acids, was employed as the starting compound for the synthesis of both **3** and **4**. For the total synthesis of callipeltin A (**1**), 3,4-dimethyl-L-glutamine has been briefly transformed from **3**.

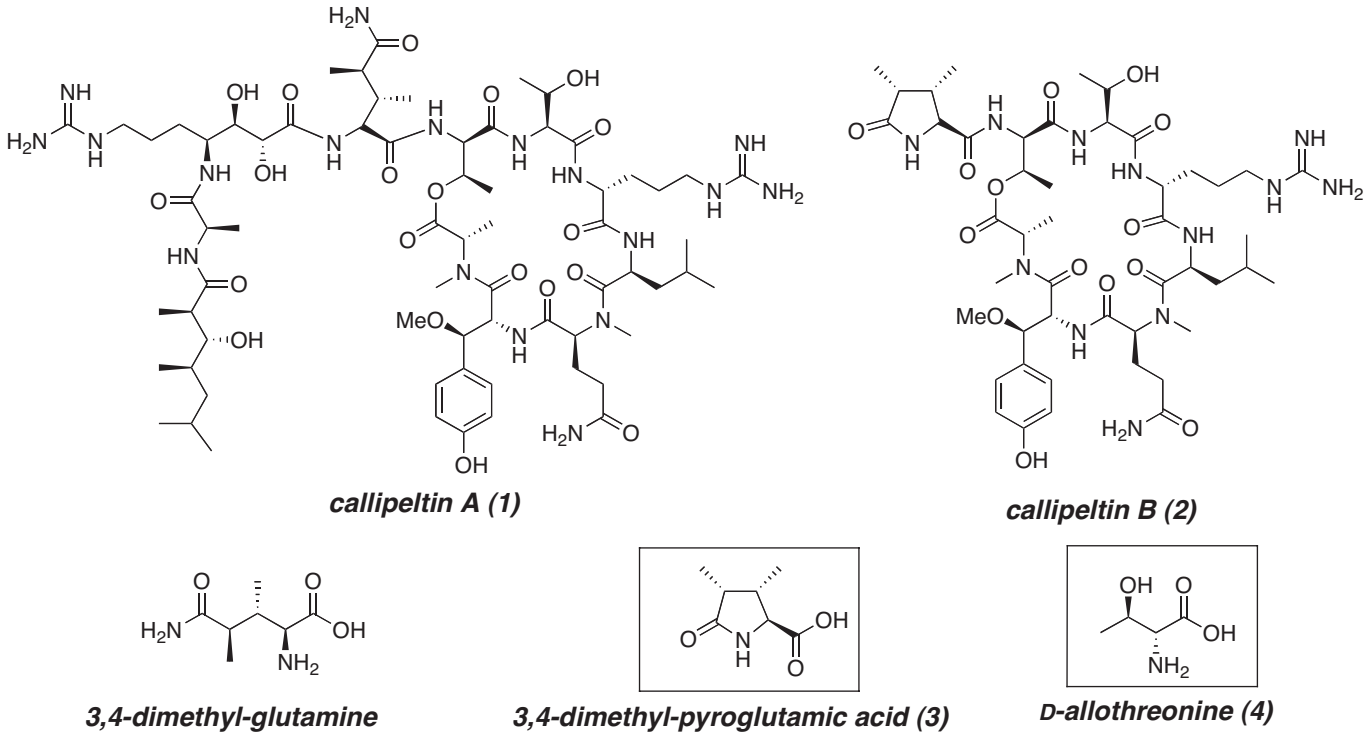


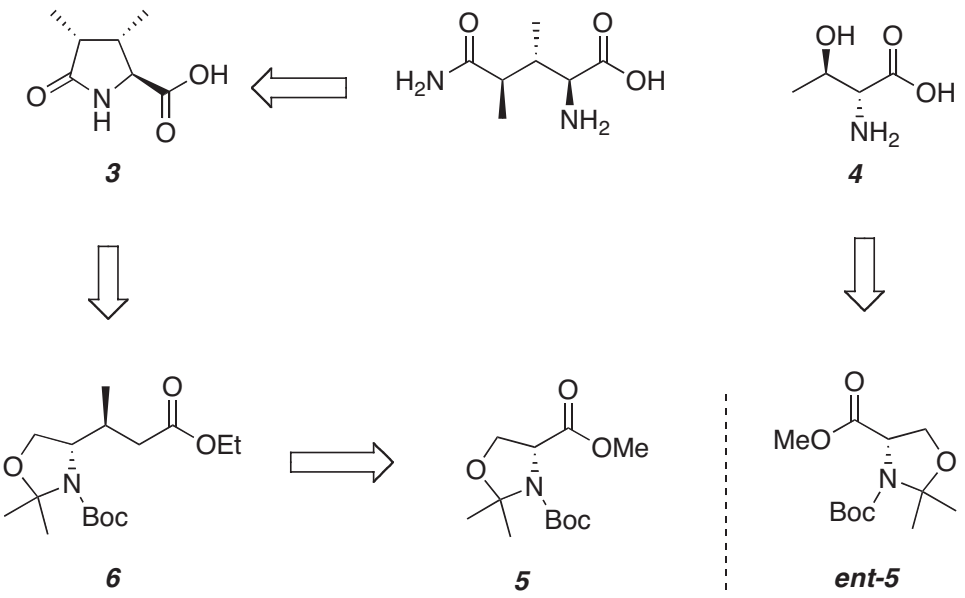
Figure 1

RESULTS AND DISCUSSION

Our method of generating **3** and **4** is outlined in Scheme 1. As a common starting compound, protected serine derivatives (**5**) prepared from D- or L-serine were selected. For the diastereoselective introduction of methyl groups, Hanessian's 1, 2-asymmetric induction reaction[11] was employed to give *anti*-product **6**.

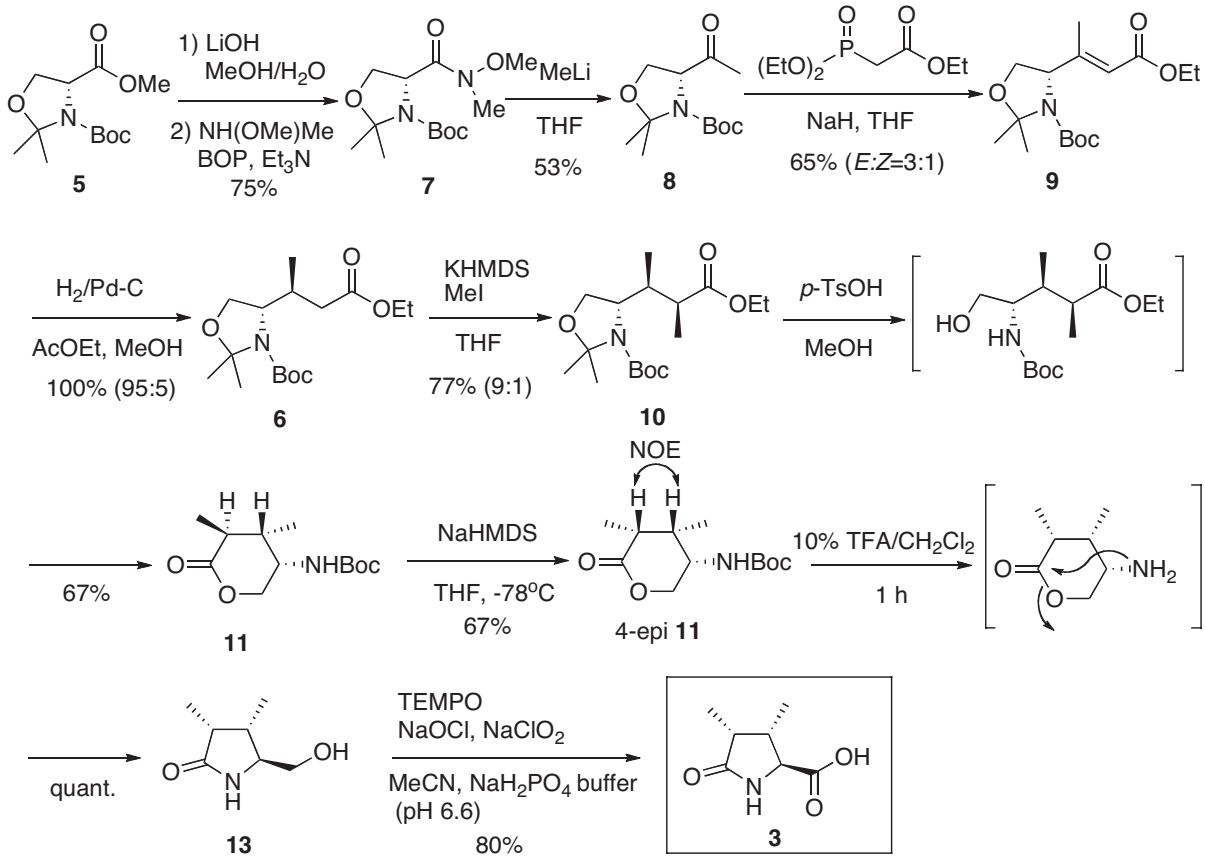
Scheme 2 shows the synthetic route for **3**. The protected serine derivative (**5**) derived from D-serine was first transformed through hydrolysis of methyl ester and subsequent Weinreb amidation into an amide (**7**) in 75% yield. Subsequent alkylation with methyllithium in THF afforded ketone (**8**) in 53% yield, which was then treated with triethyl phosphonoacetate to give the α,β–unsaturated ester (**9**) in 65% yield as *E*/*Z*

mixture (3:1). Diastereoselective hydrogenation of **9** as an *E*/*Z* mixture in the presence of 5% Pd/C gave the saturated ester (**6**) in a quantitative yield with stereoselection of 95:5. The desired *anti*-product was easily separated from the minor *syn*-product. The result indicates that the stereo-controlled reaction in the acyclic system exploiting internal 1, 2-asymmetric induction proceeded effectively.[11] Treatment of the potassium enolate of **6** with methyl iodide gave the *anti-syn* product (**10**) as a single product isolated in 77% yield, which suggests that diasteroselection in the alkylation of α-alkyl substituted enolate esters[12] is applicable to the *syn*-alkylation to yield α, β-dimethyl groups in **10**. Deprotection of acetonide (**10**) with a catalytic amount of *p*-TsOH gave hydroxyl amine, which cyclized to afford the corresponding δ-lactone (**11**) as a single diastereoisomer in 67% yield. The stereostructure of the two methyl groups of **11** was further confirmed by conversion to known hydroxyl γ-lactam (4-*epi*-**13**)[10] by Boc-deprotection and subsequent DBU treatment. The *trans*-product (**11**) was converted to the desired *cis*-lactone (4-*epi*-**11**) by kinetic epimerization of a α-methyl group of δ-lactone (**11**). Treatment with sodium hexamethyldisilazide in THF at -78 °C and stereoselective protonation gave a single diastereoisomer (4-*epi*-**11**) in 67% yield, as expected: NOE experiments showed the stereoinversion of the α-position.[11] The 4-*epi*-**11** was then efficiently converted to **13** without epimerization by treatment with 10% TFA/CH_2Cl_2 at 0°C over 10 h. Treatment of 4-*epi*-**11** with neat TFA gave undesired hydroxyl γ-lactam (4-*epi*-**13**) having *trans*-methyl groups on the quantitative acid-catalyzed epimerization of the α-methyl group. The same isomerization was observed by Lipton *et al*. in the synthesis of Fmoc-protected dimethylglutamine derivative by the indirect conversion of Boc to Fmoc group.[9a] Finally, the hydroxyl γ-lactam (**13**) was converted to 3, 4-dimethyl-pyroglutamic acid (**3**) by TEMPO-mediated oxidation[13] in 80% yield. The spectroscopic data for **3** are well consistent with the reported data.[9]



Scheme 1. Retrosynthetic analysis of **3** and **4**

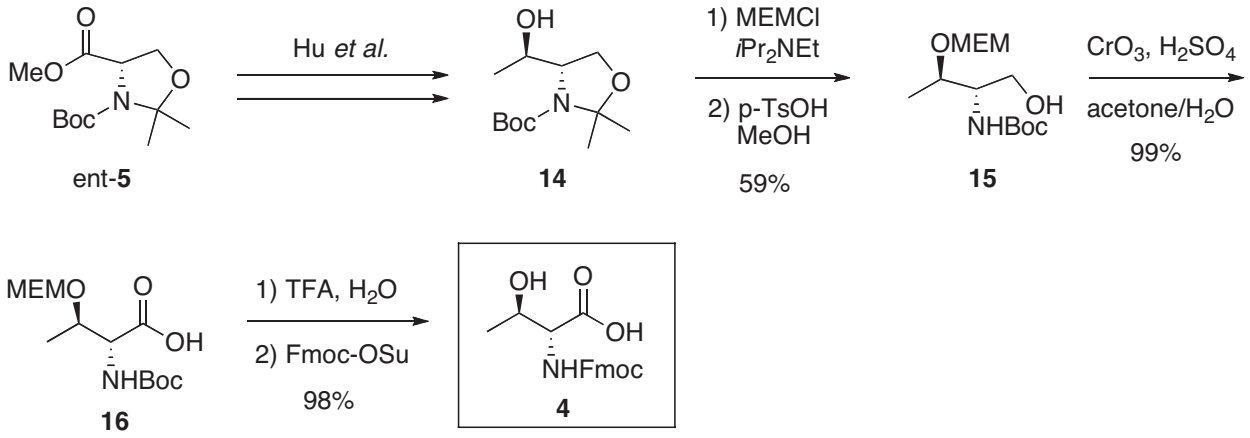
Transformation of the alcohol group in 4-*epi*-**13** to the desired 3, 4-dimethyl glutamine has already been completed by Hamada's group.[10,14]



Scheme 2. Synthetic route for **3**

Then, D-allothreonine was prepared from the protected L-serine derivative (*ent*-**5**) by the route shown in Scheme 3. First, the alcohol derivative (**14**) was prepared from L-serine in 24% overall yield (6 steps) according to a published procedure.[15] Protection of a hydroxyl group of **14** with MEMCl/*i*Pr$_2$NEt followed by deprotection of acetonide with a catalytic amount of *p*-TsOH afforded the corresponding alcohol (**15**) in 59% yield. Oxidation of the alcohol with Jones reagent gave D-allothreonine derivative (**16**) quantitatively. Deprotection of the Boc group followed by Fmoc protection afforded the desired Fmoc-D-allothreonine (**4**). The overall yield of **4** was 57% in 5 steps from **14**.

In conclusion, two unusual amino acid derivatives, (3*S*, 4*R*)-3, 4-dimethyl-L-pyroglutamic acid (**3**) and Fmoc-D-allothreonine (**4**), were synthesized stereoselectively starting from a serine derivative. In the synthesis of **3**, the stepwise diastereoselective introduction of methyl groups proceeded to give the desired compound as a single isomer. In addition, deprotection of the Boc group by 10% TFA/CH_2Cl_2 at 0 °C effectively suppressed the acid-catalyzed epimerization of the α-methyl group. The synthesis of other components of callipeltins and total synthesis on a solid support are now actively underway.



Scheme 3. Synthesis of *N*-Fmoc-D-alloThreonine (**4**)

EXPERIMENTAL

General. All manipulations were conducted under an inert atmosphere (N_2). All solvents were of reagent grade. THF was distilled from sodium and benzophenone ketyl. CH_2Cl_2 was distilled from CaH_2. All commercial reagents were of the highest purity available. Analytical TLC was performed on silica gel (60F-254, plates 0.25 mm). Column chromatography was carried out on Wakogel 60 (particle size, 0.063-0.200 mm). ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker AM-300. Chemical shifts are expressed in ppm relative to TMS (0 ppm) or $CHCl_3$ (7.28 ppm for ^{1}H and 77.0 ppm for ^{13}C). IR spectra were obtained on a HORIBA FREEXACT-II FT-710 spectrometer. Optical rotations were recorded on a HORIBA SEPA-300 polarimeter at the sodium D line. Low-resolution mass spectra (LRMS) and high-resolution mass spectra (HRMS) were obtained on either a JEOL JMS-HX-211A or a JMS-HX-110A (EI or FAB), or a JMS-T100LC (ESI).

(*R*)- *N*,*O*-Isopropylidenyl-2-(*N*-*tert*-butyloxycarbonylamino)-3-oxo-propanamide-*N*,*O*-dimethyl-hydroxyamine (7). To a solution of **5** (10.0 g, 38.6 mmol) in MeOH (100 mL) was added $LiOH{\cdot}H_2O$ (455 mg, 77.2 mmol) and the mixture was stirred for 4 h at room temperature. H_2O and Et_2O were then added, and the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. To a solution of crude product in CH_2Cl_2 (100 mL) were added Et_3N (7.81 mL, 77.2 mmol), BOP (20.0 g, 45.1 mmol), and NH(OMe)Me·HCl (4.52 g, 46.3 mmol) and the mixture was stirred for 3 h at room temperature. 1N HCl (pH=4) and Et_2O were added and the organic layer was washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified with silica gel column chromatography (hexane/AcOEt=2:1) to the give amide **7** (8.29 g, 28.8 mmol, 75%) as a colorless oil. $[\alpha]_D^{23}$ +31.5° (*c* 1.0, $CHCl_3$). IR (film) ν max cm^{-1}: 2978, 1707, 1685, 1390, 1101, 999, 849, 769. ^{1}H NMR ($CDCl_3$) δ: 1.42 (4.5H, s), 1.50 (4.5H, s), 1.53 (1.5H, s), 1.58 (1.5H, s), 1.69 (1.5H, s), 1.71 (1.5H, s), 3.22 (3H, s), 3.71

(1.5H, s), 3.75 (1.5H, s), 3.93 (0.5H, dd, *J*=9.0, 3.6 Hz), 3.98 (0.5H, dd, *J*=9.3, 3.0 Hz), 4.19 (0.5H, dd, *J*=9.0, 7.2 Hz), 4.20 (0.5H, dd, *J*=9.0, 7.5 Hz), 4.73 (0.5H, dd, *J*=7.5, 3.6 Hz), 4.81 (0.5H, dd, *J*=7.2, 3.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 24.6, 24.7, 25.5, 25.7, 28.35, 28.42, 32.6, 36.7, 57.7, 57.9, 61.2, 65.9, 66.1, 80.0, 80.5, 94.4, 95.0, 151.3, 152.2, 170.6, 171.4. HRFABMS $(M+H)^+$ calcd for $C_{13}H_{25}N_2O_5$: 289.1763, found: 289.1747.

(*R*)-*N*,*O*-Isopropylidenyl-4-(*N*-*tert*-butyloxycarbonylamino)-3-oxo-2-butanone (8). To a solution of **7** (4.15 g, 14.4 mmol) in THF (100 mL) was added MeLi (1.0 M in THF solution; 25.0 mL, 25.0 mmol) at -78 °C and the mixture was stirred for 1 h. H_2O and Et_2O were then added and the organic layer was washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified with silica gel column chromatography (hexane/AcOEt=2:1) to give the ketone **8** (1.80 g, 7.69 mmol, 53%) as a colorless oil. $[\alpha]_D^{23}$ +48.0° (*c* 1.0, $CHCl_3$). IR (film) ν max cm^{-1}: 2979, 1732, 1705, 1392, 1250, 1176, 1092, 850, 769. ^{1}H NMR ($CDCl_3$) δ: 1.21 (3H, s), 1.30 (9H, s), 1.46 (3H, s), 1.98 (3H, s), 3.73 (1H, brs), 3.93 (1H, brs), 4.16 (1H, brs). ^{13}C NMR ($CDCl_3$) δ: 23.3, 24.3, 25.0, 25.5, 25.9, 27.8, 64.7, 65.0, 79.9, 80.0, 93.9, 94.6, 150.9, 151.9, 205.7. HRFABMS $(M+H)^+$ calcd for $C_{12}H_{22}NO_4$: 244.1549, found: 244.1570.

(*S*) Ethyl *N*,*O*-isopropylidenyl-4-(*N*-*tert*-butyloxycarbonylamino)-3-methyl-5-oxo-2-pentenoate (9). To a solution of triethyl phosphonoacetate (2.49 mL, 11.1 mmol) in THF (20 mL) was added NaH (444 mg, 11.1 mmol) at 0 °C. The mixture was stirred for 1 h and then the ketone **8** (1.80 g, 7.41 mmol) was added dropwise. After 24 h of stirring, sat. aq. NH_4Cl and AcOEt were added, and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified with silica gel column chromatography (hexane/AcOEt=4:1) to give the ethyl ester **9** (1.50 g, 4.79 mmol, 65%) as a colorless oil. $[\alpha]_D^{23}$ +10.4° (*c* 1.0, $CHCl_3$). IR (film) ν max cm^{-1}: 2979, 1700, 1376, 1243, 1213, 1161, 1092, 1058, 852. ^{1}H NMR ($CDCl_3$) δ: 1.27 (3H, m), 1.39 (3H, brs), 1.52 (9H, brs), 1.68 (3H, brs), 2.14 (3H, s), 3.75 (1H, dd, *J*=9.3, 3.3 Hz), 4.13 (1H, dd, *J*=9.3, 7.2 Hz), 4.15 (2H, m), 4.32 (1H, dd, *J*=7.2, 3.3 Hz), 5.77 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 14.0, 14.7, 23.1, 25.5, 28.0, 29.4, 63.8, 67.1, 68.9, 79.8, 94.6, 115.8, 151.6, 156.6. HRFABMS $(M+H)^+$ calcd for $C_{16}H_{28}NO_5$: 314.1967, found: 314.1951.

(3*S*,4*S*) Ethyl *N*,*O*-isopropylidenyl-4-(*N*-*tert*-butyloxycarbonylamino)-3-methyl-5-oxo-pentanoate (6). To a solution of ethyl ester **9** (1.50 g, 4.81 mmol) in MeOH/AcOEt (1:1; 20 mL) was added Pd-C (50 mg) at room temperature. The mixture was stirred for 12 h in a hydrogen atmosphere. It was then filtered on a Celite pad and concentrated *in vacuo*. The crude product was purified with silica gel column chromatography (hexane/AcOEt=4:1) to give the ester **6** (1.52 g, 4.81 mmol, 100%) as a colorless oil.

$[\alpha]_D^{23}$ +6.3° (*c* 1.0, $CHCl_3$). IR (film) ν max cm^{-1}: 2979, 1736, 1697, 1458, 1375, 1257, 1176, 1084, 854. ^1H NMR ($CDCl_3$) δ: 0.87 (3H, d, *J*=5.4 Hz), 1.18 (3H, td, *J*=6.9, 1.8 Hz), 1.40 (9H, m), 1.41 (3H, brs), 1.53 (3H, brs), 2.05 (1H, m), 2.43 (2H, d, *J*=13.2 Hz), 3.73 (2H, m), 3.83 (1H, m), 4.06 (2H, qd, *J*=6.9, 1.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.1, 16.4, 26.1, 28.2, 32.5, 32.8, 36.4, 60.0, 61.0, 64.1, 79.8, 94.0, 152.4, 172.9. HRFABMS $(M+H)^+$ calcd for $C_{16}H_{30}NO_5$: 316.2124, found: 316.2126.

(2*S*,3*S*,4*S*) Ethyl *N*,*O*-isopropylidenyl-4-(*N*-*tert*-butyloxycarbonylamino)-2,3-dimethyl-5-oxo-pentanoate (10). To a solution of the ester **6** (1.52 g, 4.82 mmol) in THF (20 mL) was added NaHMDS (1.0 M in THF solution; 7.82 mL, 7.82 mmol) at -78 °C and the mixture was stirred for 30 min. Methyl iodide (0.675 ml, 10.8 mmol) was added, the solution was stirred for 2 h, sat. aq. NH_4Cl and AcOEt were added, and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified with silica gel column chromatography (hexane/AcOEt=4:1) to give the ethyl ester **10** (1.22 g, 3.70 mmol, 77%) as a colorless oil. $[\alpha]_D^{29}$ +3.0° (*c* 1.0, $CHCl_3$). IR (film) ν max cm^{-1}: 2979, 1734, 1697, 1456, 1383, 1255, 1176, 1086, 860. ^1H NMR ($CDCl_3$) δ: 0.93 (3H, d, *J*=7.2 Hz), 1.13 (3H, d, *J*=7.2 Hz), 1.26 (3H, t, *J*=6.7 Hz), 1.50 (9H, s), 1.52 (3H, s), 1.60 (3H, s), 2.34 (1H, brs), 2.52 (1H, m), 3.87 (3H, m), 4.15 (2H, q, *J*=6.9 Hz). ^{13}C NMR ($CDCl_3$) δ: 10.4, 11.5, 14.2, 14.3, 16.1, 22.9, 24.5, 26.7, 27.2, 28.3, 28.4, 32.6, 38.0, 40.5, 40.9, 59.9, 60.1, 60.2, 64.2, 66.6, 79.9, 93.8, 94.2, 152.4, 153.3, 175.7. HRFABMS $(M+H)^+$ calcd for $C_{17}H_{32}NO_5$: 330.2280, found: 330.2289.

(3*S*,4*S*,5*S*)-*N*-(*tert*-Butyloxycarbonyl)-5-amino-3,4-dimethyl-pyran-2-one (11). To a solution of the ethyl ester **10** (300 mg, 0.911 mmol) in MeOH (5 mL) was added *p*-TsOH (10 mg) at room temperature. After the solution had been stirred for 4 h, H_2O and AcOEt were added and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified with silica gel column chromatography (hexane/AcOEt=4:1) to give the lactone **11** (149 mg, 0.612 mmol, 67%) as a colorless oil. $[\alpha]_D^{27}$ -13.2° (*c* 1.1, $CHCl_3$). IR (film) ν max cm^{-1}: 3356, 2978, 1740, 1712, 1522, 1367, 1248, 1171. ^1H NMR ($CDCl_3$) δ: 1.22 (3H, d, *J*=6.9 Hz), 1.27 (3H, d, *J*=6.9 Hz), 1.45 (9H, s), 1.55 (1H, m), 2.21 (1H, dq, *J*=11.1, 6.9 Hz), 3.68 (1H, brs), 4.15 (1H, dd, *J*=12.0, 4.2 Hz), 4.33 (1H, dd, *J*=12.0, 3.6 Hz), 4.94 (1H, m). ^{13}C NMR ($CDCl_3$) δ: 13.9, 18.2, 28.1, 36.7, 40.0, 51.4, 51.8, 68.8, 79.6, 155.1, 174.5. HRFABMS $(M+H)^+$ calcd for $C_{12}H_{22}NO_4$: 244.1549, found: 244.1530.

(3*S*,4*S*,5*S*)-5-Hydroxymethyl-3,4-dimethyl-pyrrolidin-2-one (4-*epi*-13). To a solution of the lactone **11** (20 mg, 0.0821 mmol) in CH_2Cl_2 (1.0 mL) was added TFA (0.10 mL) at room temperature. After 1 h of stirring, the solvent was removed *in vacuo*. To a solution of crude product in THF (2 mL) was added DBU (5 αL, 0.0328 mmol) and then the mixture was stirred for 3 h at room temperature. To the mixture

were added H_2O and AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified with silica gel column chromatography ($CHCl_3$/MeOH=19:1) to give the lactam 4-*epi*-**13** (12 mg, 0.0821 mmol, 100%) as a colorless solid. Mp=81-84 °C. $[\alpha]_D^{23}$ -2.0° (*c* 1.0, $CHCl_3$). IR (film) ν max cm^{-1}: 3384, 2992, 2981, 1685, 1460, 1063. ^1H NMR ($CDCl_3$) δ: 1.12 (6H, brs), 2.16 (2H, brs), 3.58 (2H, m), 3.72 (1H, m), 4.71 (1H, brs), 7.51 (1H, brs). ^{13}C NMR ($CDCl_3$) δ: 13.1, 13.9, 39.8, 42.6, 57.3, 62.0, 181.6. HRFABMS $(M+H)^+$ calcd for $C_7H_{14}NO_2$: 144.1025, found: 144.1031.

(3*R*,4*S*,5*S*)-*N*-(*tert*-Butyloxycarbonyl)-5-amino-3,4-dimethyl-pyran-2-one (4-*epi*-11). To a solution of **11** (462 mg, 1.90 mmol) in THF (10 mL) was added NaHMDS (1.0 M in THF solution; 5.70 mL, 5.70 mmol) at -78 °C and the mixture stirred for 30 min. Sat. aq. NH_4Cl and AcOEt were then added and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified with silica gel column chromatography (hexane/AcOEt=2:1) to give the lactone 4-*epi*-**11** (311 mg, 1.28 mmol, 67%) as a colorless oil. $[\alpha]_D^{28}$ +13.8° (*c* 1.1, $CHCl_3$). IR (film) ν max cm^{-1}: 3238, 2976, 1743, 1701, 1281, 1255, 1163, 1101, 855, 792. ^1H NMR ($CDCl_3$) δ: 1.12 (3H, d, *J*=6.9 Hz), 1.17 (3H, d, *J*=6.9 Hz), 1.48 (9H, s), 2.08 (1H, ddq, *J*=10.2, 8.1, 6.9 Hz), 2.23 (1H, dq, *J*=10.2, 6.9 Hz), 3.72 (1H, ddt, *J*=8.1, 3.6, 1.2 Hz), 3.93 (1H, dd, *J*=10.8, 8.1 Hz), 4.22 (1H, dd, *J*=10.8, 3.6 Hz), 5.92 (1H, brs). ^{13}C NMR ($CDCl_3$) δ: 13.4, 13.8, 27.7, 39.9, 41.7, 53.8, 66.7, 82.8, 153.3, 179.6. HRFABMS $(M+H)^+$ calcd for $C_{12}H_{22}NO_4$: 244.1549, found: 244.1544.

(3*R*,4*S*,5*S*)-5-Hydroxymethyl-3,4-dimethyl-pyrrolidin-2-one (13). To a solution of the lactone 4-*epi*-**11** (311 mg, 1.28 mmol) in CH_2Cl_2 (10 mL) was added TFA (1 mL) at room temperature. After 1 h of stirring, the solvent was removed *in vacuo*. The product was purified with silica gel column chromatography ($CHCl_3$/MeOH=19:1) to give the lactam **13** (105 mg, 0.733 mmol, 57%) as a colorless solid. Mp=76-79 °C. $[\alpha]_D^{23}$ +81° (*c* 0.2, $CHCl_3$). IR (film) ν max cm^{-1}: 3325, 2962, 1682, 1458, 1267, 1065. ^1H NMR ($CDCl_3$) δ: 1.02 (3H, d, *J*=7.3Hz), 1.09 (3H, d, *J*=7.6 Hz), 2.25 (1H, m), 2.54 (1H, m), 3.33 (1H, m), 3.50 (1H, dd, *J*=11.2, 7.3 Hz), 3.74 (1H, dd, *J*=11.2, 2.9 Hz), 4.32 (1H, brs), 6.05 (1H, brs). ^{13}C NMR ($CDCl_3$) δ: 10.7, 13.8, 34.6, 39.8, 62.1, 64.6, 181.4. HREIMS $(M)^+$ calcd for $C_7H_{13}NO_2$: 143.0943, found: 143.0944.

(2*S*,3*S*,4*R*)-3,4-Dimethylpyroglutamic acid (3). To a solution of **13** (105 mg, 0.733 mmol) in MeCN-pH 6.6 NaH_2PO_4 buffer (1:1; 10 mL) were added a 10~15% NaOCl solution (12 mL), $NaClO_2$ (150 mg, 1.65 mmol) and TEMPO (15 mg, 0.1 mmol). The mixture was stirred for 48 h at room temperature. To the mixture were added 1N HCl and AcOEt. The organic layer was washed with brine, dried over $MgSO_4$,

and evaporated *in vacuo*. The product was purified with silica gel column chromatography ($CHCl_3$/MeOH=9:1) to give the dimethyl pyroglutamic acid **3** (92 mg, 0.587 mmol, 80%) as a yellow solid. $[\alpha]_D^{23}$ +41° (*c* 0.1, $CHCl_3$). IR (film) ν max cm^{-1}: 3262, 2979, 1719, 1654, 1243. ^1H NMR (D_2O) δ: 0.89 (3H, d, *J*=6.6 Hz), 0.97 (3H, d, *J*=6.2 Hz), 2.53 (2H, m), 3.80 (1H, d, *J*=3.9 Hz). ^{13}C NMR (D_2O) δ: 9.4, 13.7, 38.0, 39.2, 61.4, 176.2, 183.8. HRFABMS $(M+H)^+$ calcd for $C_7H_{12}NO_3$: 158.0817, found: 158.0815.

(2*S*,3*R*)-2-*N*-(*tert*-Butyloxycarbonyl)-amino-3-methoxyethoxymethoxybutan-1-ol (15). To a solution of **14** (3.04 g, 12.4 mmol) in CH_2Cl_2 (50 mL) were added *i*Pr_2NEt (3.75 g, 37.0 mmol) and MEMCl (2.31 g, 28.7 mmol) at 0 °C and the mixture stirred for 10 h at room temperature. 1N HCl and CH_2Cl_2 were then added and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified with silica gel column chromatography (hexane/AcOEt=4:1) to give the MEM ether (3.98 g, 11.9 mmol, 96%) as a colorless oil. ^1H NMR ($CDCl_3$) δ: 1.17 (3H, d, *J*=6.6 Hz), 1.49 (9H, s), 1.52 (3H, s), 1.58 (3H, s), 3.41 (3H, s), 3.56 (2H, t, *J*=4.2 Hz), 3.70 (2H, m), 3.76 (1H, m), 3.93 (1H, m), 4.08 (1H, dd, *J*=8.4, 1.8 Hz), 4.11 (1H, m), 4.77 (1H, d, *J*=6.9 Hz), 4.83 (1H, d, *J*=6.9 Hz). To a solution of the MEM ether (3.23 g, 9.67 mmol) in MeOH (50 mL) was added *p*-TsOH (10 mg) at room temperature and the mixture stirred for 10 h. Sat. aq. $NaHCO_3$ and AcOEt were then added and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo*. The product was purified with silica gel column chromatography (hexane/AcOEt=2:1) to give the alcohol **15** (1.78 g, 6.07 mmol, 63%) as a colorless oil. $[\alpha]_D^{25}$ -1.3° (*c* 0.6, $CHCl_3$). IR (film) ν max cm^{-1}: 3448, 3363, 2978, 2931, 1712, 1511, 1365, 1172, 1041, 856. ^1H NMR ($CDCl_3$) δ: 1.19 (3H, d, *J*=6.3 Hz), 1.45 (9H, s), 3.07 (1H, brs), 3.40 (3H, s), 3.54-3.63 (6H, m), 3.79 (1H, m), 4.11 (1H, q, *J*=5.7 Hz), 4.64 (1H, d, *J*=7.2 Hz), 4.77 (1H, d, *J*=7.2 Hz), 4.91 (1H, brs). ^{13}C NMR ($CDCl_3$) δ: 16.4, 28.3, 56.0, 59.0, 62.6, 67.0, 70.6, 71.6, 79.3, 93.0, 156.3. HRESIMS $(M+Na)^+$ calcd for $C_{13}H_{27}NO_6Na$: 316.1736, found: 316.1748.

(2*R*,3*R*)-2-*N*-(*tert*-Butyloxycarbonyl)-3-methoxyethoxymethyl-D-allothreonine (16). To a solution of **15** (1.36 g, 4.64 mmol) in acetone (20 mL) was added Jones reagent (3 mL; 2.67 M solution) at 0 °C and the mixture was stirred for 30 min. 2-Propanol (1 mL) was then added and the mixture was filtered on a Celite pad and concentated *in vacuo*. The crude product was treated with 1N HCl and extracted with AcOEt. The organic layer was dried over $MgSO_4$, and concentrated *in vacuo*. The product was purified with silica gel column chromatography ($CHCl_3$/MeOH=9:1) to give **16** (1.30 g, 4.63 mmol, 91%) as a colorless oil. $[\alpha]_D^{23}$ +1.4° (*c* 1.3, $CHCl_3$). IR (film) ν max cm^{-1}: 3459, 3330, 2978, 2934, 1716, 1506, 1367, 1167, 1039, 858. ^1H NMR ($CDCl_3$) δ: 1.21 (3H, d, *J*=6.3 Hz), 1.42 (9H, s), 3.28 (3H, s), 3.51-3.70 (5H, m), 4.31 (2H, m), 4.61-4.74 (2H, m), 5.35 (1H, m). ^{13}C NMR ($CDCl_3$) δ: 16.8, 28.2, 58.0, 58.8, 67.0,

71.6, 72.9, 79.9, 93.6, 156.1, 173.9. ESIMS $(M)^+$ calcd for $C_{13}H_{25}NO_7$: 307.16, found: 307.12.

(2*R*,3*R*)-2-*N*-(*tert*-9-Fluorenylmethyloxycarbonyl)-D-allothreonine (4). A solution of **16** (67 mg, 2.19 mmol) in 90% TFA/H_2O (2 mL) was stirred for 10 h at room temperature and the solvent was removed *in vacuo*. To a solution of the crude product in H_2O (2 mL) were added 1N NaOH (0.4 mL) and Fmoc-OSu (147 mg, 0.436 mmol) in dioxane (1 mL) and then the mixture was stirred for 15 min at room temperature. 1N HCl was added and the resulting precipitate was filtered to give **4** (73 mg, 0.214 mmol, 98%) as a white powder. All spectra for the synthetic **4** were well consistent with those reported. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, m), 2.65 (1H, brs), 4.16 (1H, brs), 4.35 (3H, brs), 5.39 (1H, brs), 6.05 (1H, brs), 7.30 (2H, m), 7.37 (2H, t, *J*=7.2 Hz), 7.58 (2H, brs), 7.73 (2H, d, *J*=7.2 Hz). ESIMS $(M+Na)^+$ calcd for $C_{19}H_{19}NO_5Na_7$: 364.12, found: 364.05.

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