Synthetic approach to microsclerodermins: construction of three building blocks

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Dedicated to Professor Zhi-Tang Huang on the occasion of his 75th birthday

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Abstract

As an approach to the construction of microsclerodermins (**1**), the three building blocks **2**, **3**, and **4** were efficiently prepared from the carboxylic acids **6**, **16**, and **28**.

Keywords: Microsclerodermin, asymmetric alkylation, 2-hydroxy-3-pinanone, amino acid

Introduction

Microsclerodermins have been isolated from the lithistid marine sponge *Microscleroderma* sp. by Faulkner and co-workers.[1] They are a family of complex cyclic peptides that display potent anti-fungal and anti-proliferative activities. One of the representative peptides are microsclerodermins A and B whose structures have been determined to be **1a** and **1b**, respectively, as shown in Fig. 1. Our continuing interests in the synthesis of biologically active aquatic natural peptides[2] led to synthesize micro-sclerodermins. Toward this end, we have already accomplished the synthesis of four requisite building blocks:[3] the tryptophan derivative **2**,[3d] the

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hydroxypyrrolidinone equivalent **3**,[3d] 4-amino-3-hydroxybutanoic acid (GABOB) derivative **4**,[3d] and AMMTD **5**,[3a-c,e] shown in Fig. 1. We now report the details of the synthesis of **2**-**4**.[3d]

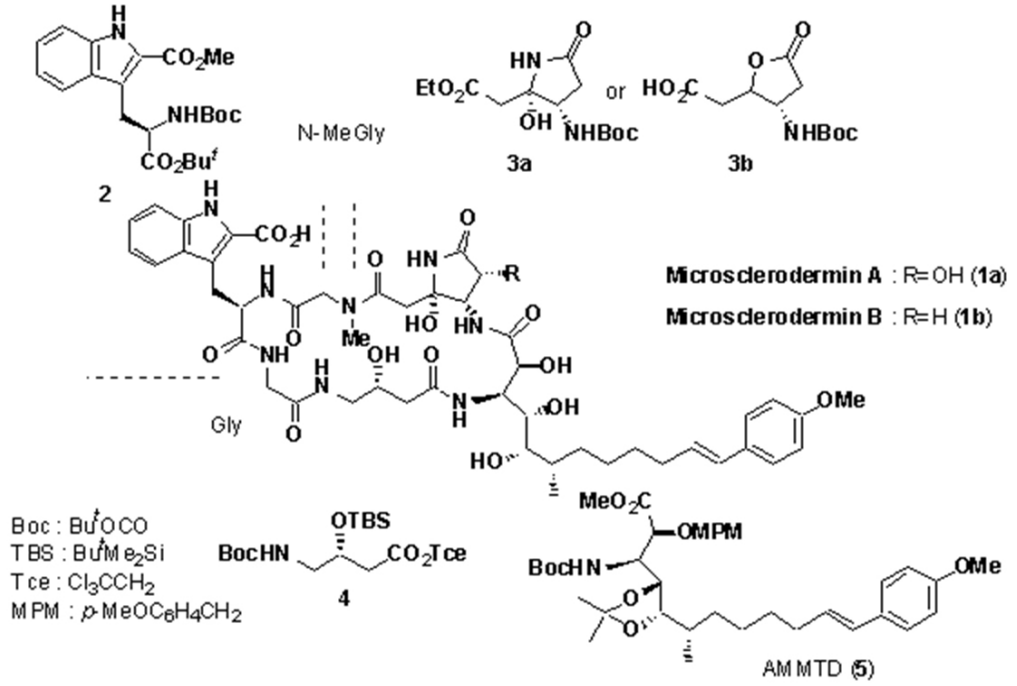
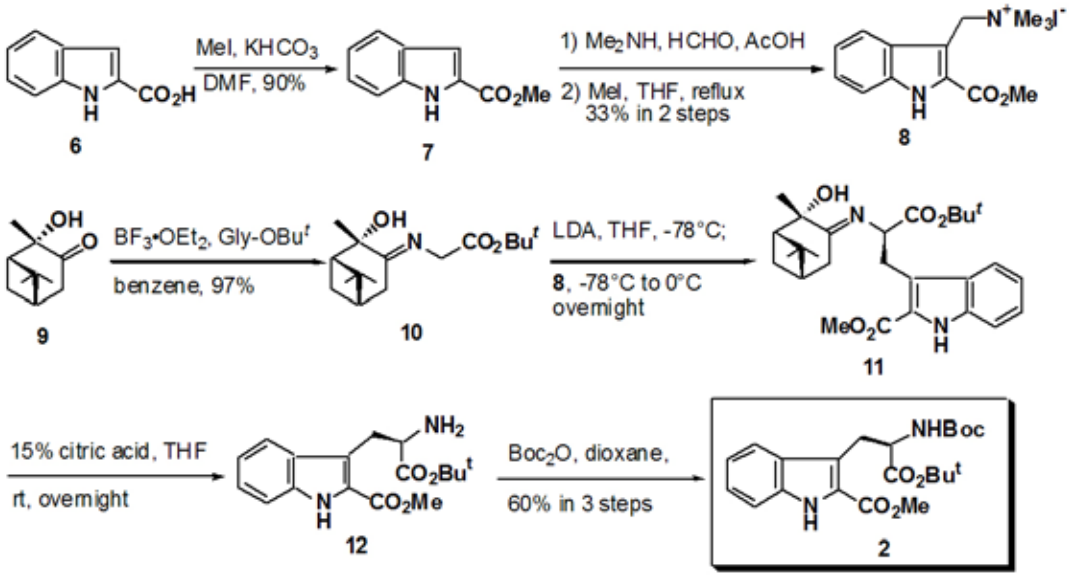


Figure 1

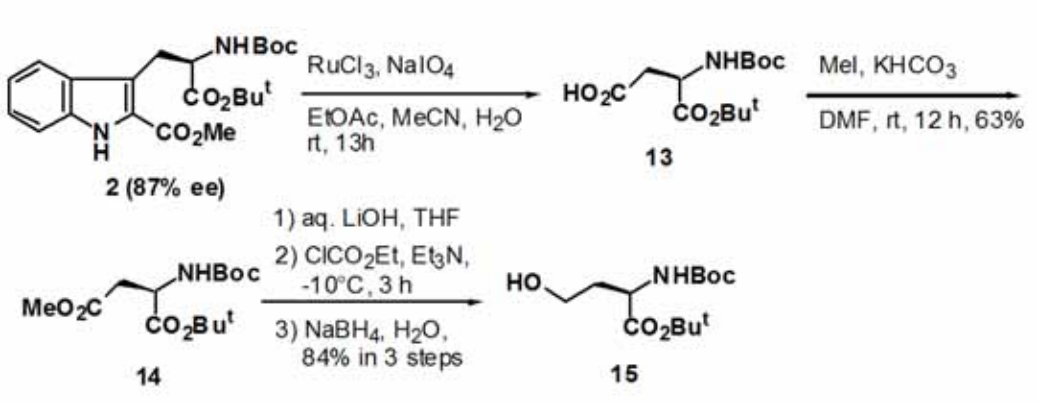
Results and Discussion

We first synthesized the tryptophan derivative **2**. We have already developed[4] the method for the asymmetric synthesis of α-amino acids by alkylation of the imine **10** derived from glycine ester and optically active 2-hydroxy-3-pinanone (HyPN, **9**). The alkylating agent **8** to carry out this method was prepared from 2-indolecarboxylic acid (**6**) by esterification with iodomethane, Mannich reaction, and then quaternization, as shown in Scheme 1. The corresponding bromo derivative was also prepared, but it was found to be labile and the chemical yield was variable. The imine **10** derived from (-)- HyPN (**9**)[4a,b] was lithiated with lithium diisopropylamide (LDA) and the resulting enolate was alkylated with **8** to give the alkylated imine **11**, which was directly treated under weak acidic conditions to remove the chiral auxiliary **9**. The resulting amine was converted to the corresponding *tert*-butoxycarbonyl (Boc) derivative **2** with Boc_2O. The enantiomeric excess of **2** was 87%, and it raised to 91% by recrystalization.



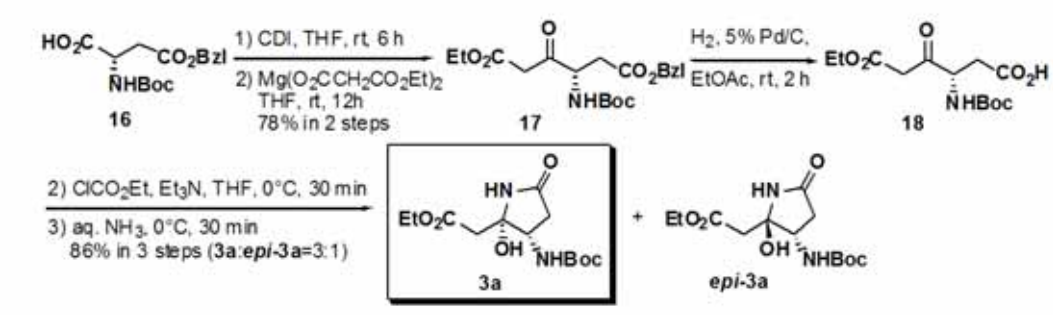
Scheme 1

The absolute configuration of the tryptophan derivative **2** should be (*R*),[4] but it was further unambiguously confirmed by transformation to the known homoserine derivative **15**, as shown in Scheme 2. Thus the tryptophan derivative **2** with 87% ee was oxidized with ruthenium tetroxide to give the aspartic acid derivative **13** which was purified as the methyl ester **14**. Although the compound **14** was known,[5] its specific rotation was not reported. Therefore, the compound **14** was further transformed to the homoserine derivative **15** in three steps, shown in Scheme 2. The homoserine derivative **15** thus obtained was completely identical with **15** derived from (*S*)-aspartic acid according to the known method,[6] but the sign of their specific rotations were opposite to each other: $[\alpha]_D^{25}$+32.3 (c 1.09,EtOH) for **15** from **2**, +37.1 (calcd. for **15** with 100% ee), reported[6] $[\alpha]_D^{25}$-37.5 (c 1, EtOH). These results clearly indicate that the absolute configuration of the tryptophan derivative **2** is (*R*), as expected.



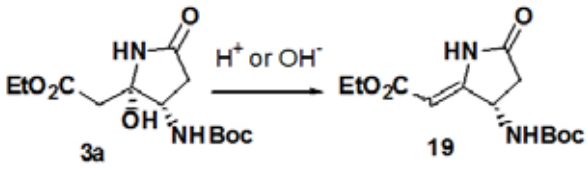
Scheme 2

The requisite pyrrolidinone derivative **3a** was prepared from *N*-Boc-(*S*)-aspartic acid 4-benzyl ester (**16**), as shown in Scheme 3. Homologation of **16** was achieved by treatment with 1,1'-carbonyldiimidazole (CDI) and then the magnesium salt of malonic acid half ester to give the β-keto ester **17**. After catalytic hydrogenolytic removal of the benzyl function, the resulting acid **18** was treated with ethyl chlorocarbonate-triethylamine and then aqueous ammonia. The products were revealed to be a diastereoisomeric mixture of the cyclic hemiaminals **3a** and ***epi*-3a** in a ratio of 3:1.[8]



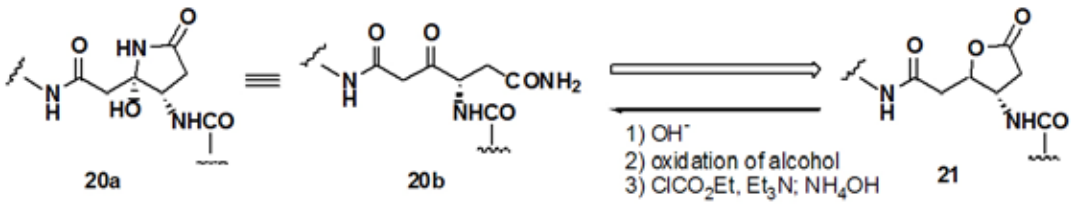
Scheme 3

The hemiaminal **3a** easily underwent the dehydration under both acidic and basic conditions to give theα,β-unsaturated ester **19**, shown in Scheme 4.



Scheme 4

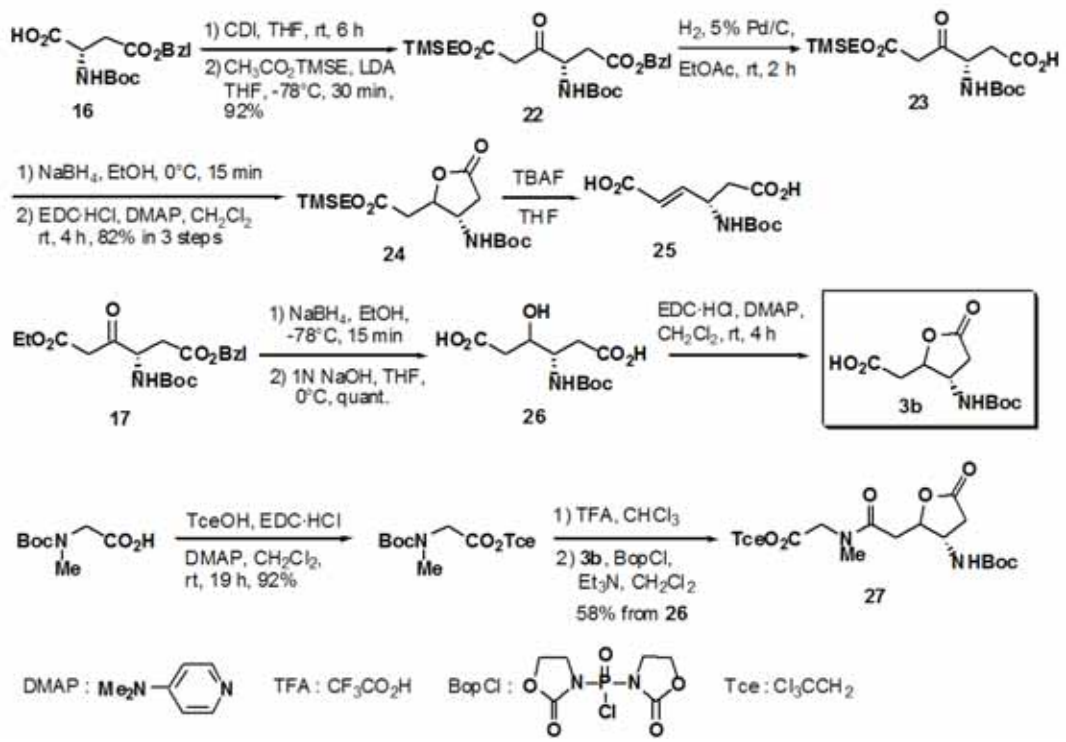
These results suggest that the synthesis of microsclerodermins could proceed via theγ-lactone **21**, which will give the keto-amide **20b**, equivalent to the hemiaminal **20a**, by alkaline hydrolysis, oxidation of the hydroxyl group, and amidation, as shown in Scheme 5.



Scheme 5

Thus, the γ-lactone trimethylsilylethyl (TMSE) ester **24** was prepared from **16** by transformation to the imidazolide, homologation with TMSE acetate,[9] catalytic removal of the benzyl function, reduction with sodium borohydride, and lactonization with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), as shown in Scheme 6. Attempted removal of the TMSE group with tetrabutylammonium fluoride (TBAF) did not give the γ-lactone **3b**, but resulted in the formation of the α,β-unsaturated dicarboxylic acid **25**.[10]

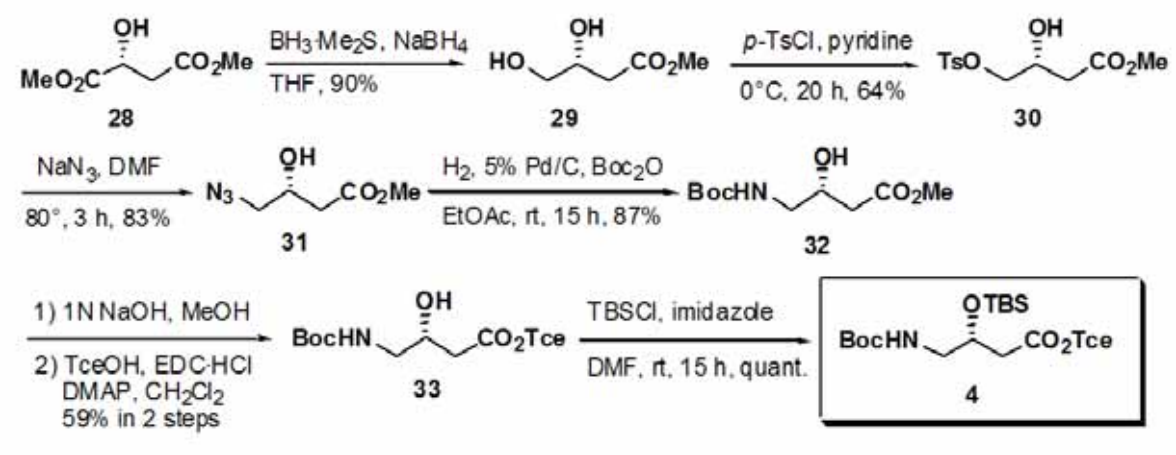
To prepare **3b** in more efficient way, the β-keto ester **17** was first transformed to the hydroxy dicarboxylic acid **26**, which underwent the lactonization with EDC to give **3b**, as shown in Scheme 6. The lactone **3b** was fully characterized by its transformation to the sarcosine derivative **27**. Thus, we could succeed in the efficient synthesis of **3b** in shorter steps.



Scheme 6

Finally, the synthesis of the required GABOB derivative **4** started from dimethyl (*R*)-malate, which was regioselectively reduced according to the known method,[11] as shown in Scheme 7. The resulting diol **29** was converted to the hydroxy azide **31** via the tosylate **30**. Catalytic hydrogenation of **31** in the presence of Boc_2O[12] afforded the Boc derivative **32**, whose methyl ester function was replaced with the trichloroethyl (Tce) one. The Tce ester **33** was finally converted to the required corresponding *tert*-butyldimethylsilyl (TBS) derivative **4**.

Thus we have succeeded in the efficient synthesis of the required building blocks **2**-**4** for the construction of microsclerodermins. The methods utilized here will have broad applicability to the synthesis of the other natural products.



Scheme 7

Experimental Section

General Procedures. Melting points were measured with a YAMATO MP-21 or YANAGIMOTO melting point apparatus (hot plate) and are uncorrected. Infrared spectra were recorded on a SHIMADZU FT IR-8100 spectrometer. Optical rotations were measured on a JASCO DIP-140 or DIP-1000 digital polarimeter with a sodium lump (λ=589 nm, D Line) and are recorded as follows:$[\alpha]_D^T$ (c g/100 ml, solvent).

^{1}H NMR spectra were recorded on a JEOL EX-270 (270 MHz) spectrometer. Chemical shifts are recorded in ppm from tetramethylsilane as the internal standard. Data are recorded as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz), and assignment. ^{13}C NMR spectra were recorded on a JEOL EX-270 (67.8 MHz) spectrometer with complete proton decoupling. Chemical shifts are recorded in ppm from tetramethylsilane with the solvent as the internal standard (deuterochloroform: δ 77.0 ppm). Mass spectra were obtained on a JEOL LMS-DX 300 spectrometer. Column chromatography was performed with silica gel BW-820MH or BW-200 (Fuji Davison Co.).

Solvents for extraction and chromatography were reagent grade. Tetrahydrofuran (THF) and benzene were distilled from sodium/benzophenone ketyl. Diethyl ether (Et_2O) was distilled from lithium aluminum hydride ($LiAlH_4$). Dichloromethane (CH_2Cl_2) was distilled from calcium hydride. Methanol (MeOH), ethanol (EtOH), and hexamethylphosphoric triamide (HMPA) were distilled from calcium hydride and stored over 4 molecular sieves. Diisopropylamine was distilled from

calcium hydride and stored over sodium hydroxide. *N*,*N*-Dimethylformamide (DMF) was dried over 4 molecular sieves. Triethylamine was dried over sodium hydroxide. All other commercially available reagents were used as received.

Methyl indole-2-carboxylate (7). To a stirred solution of indole-2-carboxylic acid (**6**) (16.16 g, 100 mmol) in DMF (200 ml) was added $KHCO_3$ (11.01 g, 110 mmol) and MeI (6.82 ml, 110 mmol) at 0°C. The mixture was stirred for 18 h at room temperature. After dilution with ether (600 ml), the mixture was washed with water (200 ml x 2) and 1M aqueous $KHSO_4$ (200 ml x 2), aqueous saturated $NaHCO_3$ (200 ml), saturated brine (200 ml), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude solid was purified by recrystallization (hexane/EtOAc) to give **7** (15.68 g, 90%) as colorless needles: mp 149-151°C (hexane/EtOAc); IR $\nu_{max}(CHCl_3)$ cm^{-1} 3021, 1703, 1215, 756; ^{1}H-NMR ($CDCl_3$) δ 3.95 (3H, s, CO_2Me) 7.13-7.44 (4H, m, Ar-H) 7.70 (1H, d, *J* = 8.0Hz, indole-8-H) 8.88 (1H, br, NH); ^{13}C-NMR ($CDCl_3$) δ 51.98 (CH_3) 108.76 (CH, Ar) 111.91 (CH, Ar) 120.73 (CH, Ar) 122.55 (CH, Ar) 125.33 (CH, Ar) 127.02 (4°, Ar) 127.40 (4°, Ar) 136.98 (4°, Ar) 162.64 (4°, C=O); Anal. calcd for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.45; H, 5.22; N, 7.74.

(2-(Methoxycarbonyl)indole-3-yl)methyl trimethyl ammonium iodide (8). To a stirred solution of **7** (14.015 g, 80 mmol) in AcOH (40 ml) was added aqueous Me_2NH (50%, 6.79 ml, 88 mmol) and aqueous HCHO (37%, 6.6 ml, 88 mmol) at room temperature. The mixture was stirred under reflux for 16 h. To the reaction mixture was added aqueous saturated $NaHCO_3$ at 0°C until the mixture become basic. The aqueous layer was extracted with ether (200 ml x 3), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by recrystallization (hexane/EtOAc) to give methyl 3-(*N*,*N*-dimethylaminomethyl)indole-2-carboxylate (6.202 g, 33%) as colorless prisms: mp 89-91°C; IR $\nu_{max}(CHCl_3)$ cm^{-1} 3346, 1705, 1456, 1254, 1209; ^{1}H-NMR ($CDCl_3$) δ 2.22 (6H, s, NMe_2) 3.85, 3.86 (s, 5H, CH_2N, CO_2Me) 7.05-7.29 (3H, m, Ar-H) 7.76 (1H, d, *J* = 8.3Hz, indole-8-H) 8.80 (1H, br, NH); ^{13}C-NMR ($CDCl_3$) δ 45.66 (CH_3, NMe_2) 51.77 (CH_3, CO_2Me) 52.79 (CH_2) 111.66 (CH, Ar) 120.46 (CH, Ar) 120.73 (4°, Ar) 121.63 (CH, Ar) 124.38 (4°, Ar) 125.55 (CH, Ar) 128.53 (4°, Ar) 135.74 (4°, Ar) 162.55 (4°, C=O).

To a stirred solution of give methyl 3-(*N*,*N*-dimethylaminomethyl)indole-2-carboxylate (5.087 g, 25 mmol) in THF (125 ml) was added MeI (1.55 ml, 25 mmol) at room temperature. The mixture was stirred under reflux for 20 h. After cooling, the precipitated solid was filtered and washed with benzene thoroughly to give **8** (9.82 g, quant.). The analytical sample was recrystallized with MeOH/benzene/hexane to give colorless needles: mp 218-220°C; IR $\nu_{max}(CHCl_3)$ cm^{-1} 3180, 1703, 1460, 1377, 1254; ^{1}H-NMR (CD_3OD) δ 3.19 (9H, s, NMe_3) 4.01

(3H, s, CO_2Me) 4.56 (2H, s, CH_2N) 7.28 (1H, t, *J* = 7.22Hz, Ar-H) 7.41 (1H, t, *J* = 6.9Hz, Ar-H) 7.57 (1H, d, *J* = 7.6Hz, Ar-H) 7.86 (1H, d, *J* = 7.9Hz, indole-8-H);; Anal. calcd for $C_{14}H_{19}IN_2O_2$: C, 44.93; H, 5.12; N, 7.49. Found: C, 44.49; H, 5.11; N, 3.33.

***tert*-Butyl(1*S*,2*S*,5*S*)-(2-hydroxy-2,6,6-trimethyl-bicyclo[3.1.1]hept-3-ylidene-amino)acetate (10).**[3b] To a stirred solution of Z-Gly-OBu*t* (10.98 mg, 41.4 mmol) in EtOAc (70 ml) was added 5% Pd/C (1g) under argon atmosphere at room temperature. The mixture was stirred for 1 h at room temperature under H_2 atmosphere. The mixture was filtered and concentrated *in vacuo*. The volatile amine was used for the next step without further purification.

In a 200 ml round bottled flask, equipped with a Dean Stark apparatus (molecular sieves type 4) and reflux condenser, the crude amine (41.4 mmol) was dissolved in benzene (60 ml), followed by addition of (-)-HyPN (9) (3.48 g, 20.7 mmol) and $BF_3{\cdot}Et_2O$ (0.13 ml, 1.1 mmol). The mixture was stirred under reflux for 15 h. The mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-820MH, 150 g, hexane:EtOAc = 2:1 to 1:1) to give **10** (5.56 g, 97%) as a colorless oil; IR ν_{max}^{neat} $_{cm}^{-1}$ 3432, 1743, 1655, 1369, 1151; ^{1}H-NMR ($CDCl_3$) δ 0.87 (3H, s, Me_2C) 1.33 (3H, s, Me_2C) 1.48 (9H, s, Me_3C) 1.52 (3H, s, $CH_3C(OH)$) 1.69-1.90 (2H decreased with D_2O to 1H, $CHCH_2CH$, OH) 2.00-2.10 (2H, m, $CHCH_2CH$) 2.30-2.40 (1H, m, $CHCH_2CH$) 2.47 (2H, s, $CH_2C{=}N$) 4.08 (2H, s, NCH_2CO_2).

Methyl 3-[(1*S*,2*S*,5*S*)-2-(2-hydroxy-2,6,6-trimethyl-bicyclo[3.1.1]hept-3-ylidene-amino-(2*S*)-*tert*-butoxycarbonyl-ethyl]-1*H*-indole-2-carboxylate (11). To a stirred solution of *i*-Pr_2NH (6.1 ml, 43.5 mmol) in THF (40 ml) was added 1.6 M solution of *n*-butyllithium in hexane (28 ml, 43.4 mmol) dropwise at -78°C. The mixture was stirred for 40 min at -78°C. To the LDA solution was added a solution of **10** (5.56 g, 19.8 mmol) in THF (20 ml plus 5 ml of THF rinse). The mixture was stirred for 30 min at -78°C. The ammonium salt 8 was added to the reaction mixture, followed by stirring at -78°C for 2 h and at 4°C for 11 h. The reaction was quenched with saturated aqueous NH_4Cl (50 ml). The aqueous layer was extracted with EtOAc (100 ml x 3), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-200, 150 g, hexane:EtOAc = 3:1) to give **11** as a light yellow oil, which was used for the next step without further purification; IR ν_{max}^{neat} $_{cm}^{-1}$ 3300, 1717, 1456, 1257; ^{1}H-NMR ($CDCl_3$) δ 1.12 (3H, s, Me_2C) 1.16 (3H, s, Me_2C) 1.28-1.91 (8H, m, $CH_3C(OH)$, $CHCH_2CH$, $CH_2C{=}N$) 1.45 (9H, s, CMe_3) 2.09 (2H, m, $CHCH_2CH$) 3.75 (2H, d, *J* = 4.3Hz, CH_2CHN) 4.02 (3H, s, CO_2Me) 4.19 (1H, t, *J* = 7.0Hz, CHN) 7.13-7.20 (3H, m, Ar-H) 7.81 (1H, d, *J* = 7.9Hz, indole-8-H) 8.73 (1H, brs, NH).

(*R*)-*tert*-Butyl 3-(2-(methoxycarbonyl)indol-3-yl)-2-*N*-(*tert*-butoxycarbonyl)-amino-propionate (2). To a stirred solution of the crude **11** (4.693 g, 9.9 mmol) in THF (40 ml) was added 15% aqueous citric acid (40 ml) at room temperature. The mixture was stirred for 1 day at room temperature. After evaporation of THF, EtOAc (30 ml) was added and the mixture was extracted with 15% aqueous citric acid (50 ml x 3). The aqueous layer was basified with $NaHCO_3$ in an ice bath, followed by salting out, and extracted with ether (100 ml x 3). The organic layer was washed with saturated brine (50 ml), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude amine **12** which was immediately used for the next step without further purification.

To a stirred solution of the crude amine (9.9 mmol) in dioxane (30 ml) was added Boc_2O (4.3 g, 19.8 mmol) at room temperature. The mixture was stirred for 11.5 h at room temperature. After evaporation of the volatile, the residue was purified by column chromatography (silica gel BW-200, 120 g, hexane:EtOAc = 6:1 to 4:1) to give **2** (2.605 g, 63% in 3 steps, 87%ee) as a colorless solid. The analytical sample was recrystallized with hexane/EtOAc to give **2** as colorless needles (91%ee); mp 129°C; $[\alpha]_D^{26}$ -7.45 (c 1.20, $CHCl_3$); IR $\nu_{max}(CHCl_3)$ cm^{-1} 3351, 2978, 1738, 1368, 1256, 1135; ^{1}H-NMR ($CDCl_3$) δ 1.31 (9H, s, CMe_3) 1.33 (9H, s, CMe_3) 3.46 (2H, d, *J* = 8.9Hz, CH_2CH) 3.97 (3H, s, CO_2Me) 4.50 (1H, dd, *J* = 6.6, 8.6Hz, $CHCH_2$) 5.31 (1H, brd, NH) 7.14-7.38 (3H, m, Ar-H) 7.74 (1H, d, *J* = 7.9Hz, indole-8-H) 8.78 (1H, brs, indole-NH); ^{13}C-NMR ($CDCl_3$) δ 27.78 (CH_3) 28.14 (CH_3, CH_2) 51.80 (CH_3) 55.25 (CH) 79.26 (4°, CMe_3) 81.47 (CMe_3) 111.77 (CH, Ar) 119.24 (4°, Ar CCO_2Me) 120.41 (CH, Ar) 120.81 (CH, Ar) 123.88 (4°, Ar) 125.69 (CH, Ar) 127.99 (4°, Ar) 135.86 (4°, Ar) 155.16 (4°, $NHCO_2$) 162.62 (4°, CO_2Me) 171.57 (4°, $CO_2{}^tBu$) ; Anal. calcd for $C_{22}H_{30}N_2O_6$: C, 63.14; H, 7.23; N, 6.69. Found: C, 62.94; H, 7.19; N, 6.68; HPLC analysis for **2**: column, Daicel Chiralcel OD; solvent, hexane/*i*-PrOH = 9:1; flow, 1.0 ml/min; retention time, 9.03, 9.70 min.

Boc-D-Asp(OH)-OBu^t (13). To a stirred solution of **2** (67.1 mg, 0.16 mmol, 87% ee) in EtOAc (0.4 ml), MeCN (0.4 ml), and H_2O (4 ml) was added $NaIO_4$ (856 mg, 4 mmol) and $RuCl_3{\cdot}nH_2O$ (2.0 mg, 0.0096 mmol) at room temperature. The mixture was stirred at room temperature for 12.5 h. Aqueous $KHSO_4$ (1M, 5 ml) was added to the reaction mixture and the aqueous layer was extracted with EtOAc (10 ml x 3). The organic layer was washed with saturated brine (10 ml), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give the crude carboxylic acid **13** which was used for the next step without further purification.

Boc-D-Asp(OMe)-OBu^t (14). To a stirred solution of the crude carboxylic acid **13** (0.16 mmol) in DMF (1 ml) was successively added $KHCO_3$ (24 mg, 0.24 mmol) and MeI (40 μl, 0.64 mmol) at 0°C. The mixture was stirred for 4 h at room temperature. After dilution with ether (30 ml), the

organic layer was washed with 1M aqueous $KHSO_4$ (10 ml), saturated brine (10 ml), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-200, 7 g, hexane:EtOAc = 10:1) to give **14** (30.4 mg, 63% in 2 steps) as a light yellow oil. $[\alpha]_D^{17}$ -16.60 (c 1.44, $CHCl_3$); IR $\nu_{max}(CHCl_3)$ cm^{-1} 3400, 1745, 1717, 1368, 1155; ^{1}H-NMR ($CDCl_3$) δ 1.44 (9H, s, CMe_3) 1.45 (9H, s, CMe_3) 2.76 (1H, dd, *J* = 5.0, 16.5Hz, CH_2CO_2Me) 2.95 (1H, dd, *J* = 4.6, 16.8Hz, CH_2CO_2Me) 3.69 (3H, s, CO_2Me) 4.43-4.46 (1H, m, CHNH) 5.44 (1H, d, *J* = 7.9Hz, NH). (lit.[5] *ent*-14: ^{1}H-NMR ($CDCl_3$) δ 1.45 (18H, s, CMe_3) 2.84 (2H, dd, CH_2CO_2Me) 3.70 (3H, s, CO_2Me) 4.45 (1H, m, CHNH) 5.42 (1H, brs, NH).).

Boc-D-HomoSer-OBu^t (15). To a stirred solution of **14** (29.3 mg, 0.097 mmol) in THF (0.5 ml) was added 0.5*N* aqueous LiOH (0.22 ml, 0.11 mmol) at 0°C. The mixture was stirred for 6 h at 0°C. After acidification with 1M aqueous $KHSO_4$, the aqueous layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give the crude carboxylic acid, which was used for the next step without further purification.

To a stirred solution of the carboxylic acid (0.97 mmol) in THF (0.5 ml) was added Et_3N (21 ml, 0.146 mmol) and $ClCO_2Et$ (14 ml, 0.146 mmol) successively at -10°C, followed by stirring for 30 min. Triethylammonium chloride was removed by filtration and washed with small amount of THF. A solution of sodium borohydride (8 mg, 0.21 mmol) in water (0.5 ml) was added dropwise to the filtrate at 0°C, followed by stirring at 0°C for 30 min. After being quenched with 1M aqueous $KHSO_4$ (3 ml), the mixture was extracted with EtOAc (10 ml x 3), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-200, 7 g, hexane:EtOAc = 3:1) to give **15** (21.0 mg, 82% in 3 steps) as a colorless oil. $[\alpha]_D^{14}$ 32.3° (c 1.09, $CHCl_3$) (lit.[6] *ent*-15 $[\alpha]_D^{25}$ +37.5° (c 1.09, $CHCl_3$)); IR $\nu_{max}(CHCl_3)$ cm^{-1} 3400, 1730, 1694, 1507, 1367, 1154; ^{1}H-NMR ($CDCl_3$) δ 1.45 (9H, s, CMe_3) 1.47 (9H, s, CMe_3) 1.50-1.53 (2H, m, $CHCH_2$) 2.10-2.17 (1H, m, $CHCH_2$) 3.00 (1H, br, disappeared with D_2O, OH) 3.59-3.70 (2H, m, CH_2OH) 4.31-4.38 (1H, m, CHN) 5.34 (1H, d, *J* = 7.6Hz, NH). The IR and ^{1}H-NMR spectra are identical with those of the reported ones.

α-Ethyl β-benzyl 3-oxo-4-*N*-(*tert*-butoxycarbonyl)aminoadipate (17). To a stirred solution of **16** (327 mg, 1.01 mmol) in THF (4 ml) was added carbonyldiimidazole (CDI) at 0°C. The mixture was stirred at room temperature for 6 h. In another flask, to a stirred suspension of magnesium salt of ethyl hydrogen malonate (0.735 mmol), prepared from ethyl hydrogen malonate (194 mg, 1.47 mmol) and magnesium ethoxide (84 mg, 0.735 mmol) by the method reported by S. Masamune,[7] in THF (4 ml) was added the solution of imidazolide *via* canula, and rinsed with THF (2 ml). The reaction mixture was stirred for 20 h at room temperature. After dilution with EtOAc (100 ml), the

mixture was washed with 1M aqueous $KHSO_4$ (30 ml), saturated brine (30 ml), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-200, 20 g, hexane:EtOAc = 5:1) to give **17** (291 mg, 78%) as a colorless solid: mp 46-48°C (hexane/ether); $[\alpha]_D^{18}$ -15.81 (c 1.35, $CHCl_3$); IR $\nu_{max}(CHCl_3)$ cm^{-1} 3400, 1715, 1505, 1456, 1163; ^{1}H-NMR ($CDCl_3$) δ 1.27 (3H, t, *J* = 7.3Hz, CH_3CH_2) 1.45 (9H, s,CMe_3) 2.81 (1H, dd, *J* = 4.3, 17.2Hz, CH_2CO_2Bzl) 3.05 (1H, dd, *J* = 5.0, 17.5Hz, CH_2CO_2Bzl) 3.61 (2H, d, *J* = 1.7Hz, EtO_2CCH_2) 4.18 (2H, q, *J* = 7.3Hz, CH_3CH_2) 4.56-4.61 (1H, m, CHN) 5.12 (2H, s, CH_2Ph) 5.64 (1H, brd, *J* = 8.9Hz, NH) 7.34-7.40 (5H, m, Ar-H); ^{13}C-NMR ($CDCl_3$) δ 13.96 (CH_3) 28.17 (CH_3) 35.25 (CH_2) 45.84 (CH_2) 55.94 (CH) 61.36 (CH_2) 66.82 (CH_2) 80.46 (4°, CMe_3) 128.14 (CH, Ar) 128.32 (CH, Ar) 128.51 (CH, Ar) 135.23 (4°, Ar) 155.18 (4°, $NHCO_2$) 166.84 (4°, ester C=O) 171.21 (4°, ester C=O) 201.29 (4°, ketone C=O); Anal. calcd for $C_{20}H_{27}NO_7$: C, 61.06; H, 6.92; N, 3.56. Found: C, 61.08; H, 6.87; N, 3.62.

α-Ethyl β-hydrogen (*S*)-3-oxo-4-*N*-(*tert*-butoxycarbonyl)aminoadipate (18). To a stirred solution of **17** (48.6 mg, 0.15 mmol) in EtOAc (0.5 ml) was added 5% Pd/C (20 mg) under argon atmosphere at room temperature. The mixture was stirred for 1.5 h at room temperature under hydrogen atmosphere, filtered through a pad of celite, and concentrated *in vacuo* to give the carboxylic acid **18** as a colorless oil, which was used for the next step without further purification; IR ν_{max}^{neat} cm^{-1} 3300-3400, 1715, 1693, 1514, 1395, 1254, 1165; ^{1}H-NMR ($CDCl_3$) δ 1.28 (3H, t, *J* = 6.9Hz, CH_3CH_2) 1.46 (9H, s, CMe_3) 2.68-2.75 (1H, m, CH_2CO_2H) 2.98 (1H, dd, *J* = 6.9Hz, CH_2CO_2H) 3.37 (2H, s, EtO_2CCH_2) 4.21 (2H, q, *J* = 6.9Hz, CH_3CH_2) 4.44 (1H, brm, CHN) 5.53 (1H, br, NH) 5-6 (1H, br, disappeared with D_2O, CO_2H).

(3*S*,4*S*)-3-*N*-(*tert*-butoxycarbonyl)amino-4-hydroxy-4-(ethoxycarbonyl)-methyl-pyrrolidin-2-one (3a) and (3*S*,4*R*)-3-*N*-(*tert*-butoxycarbonyl)amino-4-hydroxy-4-(ethoxycarbonyl)methylpyrrolidin-2-one (*epi*-3a). To a stirred solution of the crude carboxylic acid **18** (0.15 mmol) in THF (0.5 ml) was added Et_3N (23 μl, 0.165 mmol) and $ClCO_2Et$ (16 μl, 0.165 mmol) successively at 0°C. The mixture was stirred for 3 h. Aqueous NH_3 (28%, 0.1 ml) was added to the reaction mixture. The mixture was stirred for 30 min at 0°C. After addition of water (3 ml), the mixture was extracted with EtOAc (10 ml x 3), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-200, 7 g, hexane:EtOAc = 1:2) to give a diastereomeric mixture of **3a** and ***epi*-3a** (30 mg, 86%) as a colorless solid. The analytical sample was purified by further column chromatography (silica gel BW-200, CHCl3:MeOH = 50:1 to 10:1) to give the diastereomeric pure **3a** and ***epi*-3a**.

3a, less polar one: mp 128-132°C; $[\alpha]_D^{22}$ +2.75 (c 1.20, $CHCl_3$); IR $\nu_{max}(CHCl_3)$ cm^{-1} 3300, 1721, 1696, 1514, 1392, 1367, 1271, 1250, 1205, 1169, 756; ^{1}H-NMR ($CDCl_3$) δ 1.28 (3H, t, *J* = 7.3Hz, CH_3CH_2) 1.45 (9H, s, CMe_3) 2.37 (1H, dd, *J* = 9.9, 16.8Hz, $NHCOCH_2$) 2.60-2.70 (1H, m, $NHCOCH_2$) 2.66 (1H, d, *J* = 17.2Hz, EtO_2CCH_2) 2.99 (1H, d, *J* = 16.5Hz, EtO_2CCH_2) 4.16-4.24 (1H, m, CHNHBoc) 4.21 (2H, q, *J* = 7.3HZ, CH_3CH_2) 4.85 (1H, br, disappeared with D_2O, OH) 5.29 (1H, br, NHBoc) 7.21 (1H, br, decreased with D_2O, NHCO); ^{13}C-NMR ($CDCl_3$) δ 13.87 (CH_3) 28.16 (CH_3) 35.11 (CH_2) 42.46 (CH_2) 53.38 (CH) 61.15 (CH_2) 79.74 (4°, CMe_3) 85.48 (4°, C(OH)) 155.45 (4°, $NHCO_2$) 170.83 (4°, ester C=O) 175.41 (4°, amide C=O); Anal. calcd for $C_{13}H_{22}N_2O_6$: C, 51.65; H, 7.33; N, 9.27. Found: C, 51.29; H, 7.31; N, 9.18.

***epi*-3a**, more polar one: mp 131-133°C; $[\alpha]_D^{22}$ -8.07 (c 0.66, $CHCl_3$); IR $\nu_{max}(CHCl_3)$ cm^{-1} 3300, 1714, 1682, 1537, 1392, 1367, 1252, 1196, 1167, 753; ^{1}H-NMR ($CDCl_3$) δ 1.28 (3H, t, *J* = 7.3Hz, CH_3CH_2) 1.45 (9H, s, CMe_3) 2.15 (1H, dd, *J* = 3.3, 17.2Hz, $NHCOCH_2$) 2.75 (1H, d, *J* = 16.5Hz, EtO_2CCH_2) 2.92 (1H, d, *J* = 17.2Hz, EtO_2CCH_2) 2.99 (1H, d, *J* = 7.9, 16.5Hz, $NHCOCH_2$) 4.20 (2H, q, *J* = 7.3HZ, CH_3CH_2) 4.30 (1H, m, CHNHBoc) 4.48 (1H, br, disappeared with D_2O, OH) 5.01 (1H, br, NHBoc) 6.87 (1H, br, decreased with D_2O, NHCO); ^{13}C-NMR ($CDCl_3$) δ 13.94 (CH_3) 28.21 (CH) 36.42 (CH_2) 39.85 (CH_2) 56.30 (CH) 56.30 (CH) 61.27 (CH_2) 80.10 (4°, CMe_3) 88.94 (4°, C(OH)) 155.59 (4°, $NHCO_2$) 171.46 (4°, ester C=O) 175.68 (4°, amide C=O); Anal. calcd for $C_{13}H_{22}N_2O_6$: C, 51.65; H, 7.33; N, 9.27. Found: C, 51.24; H, 7.18; N, 9.15.

α-2-(Trimethylsilyl)ethyl β-benzyl 2-oxo-3-*N*-(*tert*-butoxycarbonyl)amino-adipate (22). To a stirred solution of **16** (3.57 g, 11.04 mmol) in THF (20 ml) was added CDI (2.86 g, 17.6 mmol) at 0°C. The mixture was stirred at room temperature for 6 h. In another flask, to a stirred solution of *i*-Pr_2NH (7.4 ml, 53 mmol) in THF (100 ml) cooled in an ice bath was added dropwise 1.6 M solution of n-butyllithium in hexane (33 ml, 52 mmol). The mixture was stirred for 30 min at 0°C. After cooling at -78°C, a solution of trimethylsilylethyl acetate (8.5 g, 53 mmol) in THF (50 ml) was added dropwise to the LDA solution. The mixture was stirred for 30 min at -78°C. The solution of the imidazolide was added to the solution of the lithium enolate *via* canula. The reaction mixture was stirred for 30 min at -78°C, and quenched with 1M aqueous $KHSO_4$ (100 ml). The aqueous layer was extracted with EtOAc (100 ml x 3). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-200, 200 g, hexane:EtOAc = 6:1 to 4:1) to give **22** (4.548 g, 92%) as a colorless oil: $[\alpha]_D^{23}$ -20.29 (c 1.02, $CHCl_3$); IR $\nu_{max}(CHCl_3)$ cm^{-1} 3400, 1745, 1715, 1514, 1456, 1252, 1165; ^{1}H-NMR ($CDCl_3$) δ 0.034 (9H, s, $SiMe_3$) 0.98-1.04 (2H, m, $TMSCH_2$) 1.45 (9H, s, CMe_3) 2.81

(1H, dd, *J* = 4.6, 17.2Hz, CH_2CO_2Bzl) 3.05 (1H, dd, *J* = 5.0, 17.5Hz, CH_2CO_2Bzl) 3.60 (2H, s, $TMSEO_2CCH_2$) 4.18-4.24 (2H, m, $TMSCH_2CH_2$) 4.57-4.60 (1H, m, CHN) 5.12 (2H, s, CH_2Ph) 5.63 (1H, d, *J* = 9.2Hz, NH) 7.34-7.39 (5H, m, Ar-H); ^{13}C-NMR ($CDCl_3$) δ -1.59 (CH3, $SiMe_3$) 17.19 (CH_2) 28.21 (CH3, CMe_3) 35.33 (CH_2) 46.03 (CH_2) 55.97 (CH) 63.81 (CH_2) 66.86 (CH_2) 80.52 (4°, CMe_3) 128.19 (CH, Ar) 128.37 (CH, Ar) 128.57 (CH, Ar) 135.25 (4°, Ar) 155.22 (4°, $NHCO_2$) 167.00 (4°, ester C=O) 171.26 (4°, ester C=O) 201.47 (4°, ketone C=O); Anal. calcd for $C_{23}H_{35}NO_7Si$: C, 59.33; H, 7.58; N, 3.01. Found: C, 59.44; H, 7.60; N, 2.89.

(3*S*,4*RS*)-3-*N*-(*tert*-Butoxycarbonyl)amino-4-[(trimethylsilyl)ethyl]methyl-butyrolactone (24). To a stirred solution of **22** (96 mg, 0.206 mmol) in EtOAc (1 ml) was added 5% Pd/C (40 mg) under argon atmosphere at room temperature. The mixture was stirred for 12 h under hydrogen atmosphere at room temperature, and filtered through a pad of celite, concentrated *in vacuo* to give the carboxylic acid **23** as a colorless oil, which was used for the next step without further purification.

To a stirred solution of the crude carboxylic acid **23** (0.206 mmol) in EtOH (1 ml) was added $NaBH_4$ (13 mg, 0.343 mmol) at 0°C. The mixture was stirred for 15 min at 0°C. The reaction mixture was quenched with 1M aqueous $KHSO_4$ (3 ml), extracted with $CHCl_3$ (10 ml x 3), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give the crude hydroxy acid as a colorless oil, which was used for the next step without further purification.

To a stirred solution of the crude hydroxy acid (0.206 mmol) in CH_2Cl_2 (2 ml) was added EDC·HCl (62 mg, 0.325 mmol) and DMAP (3 mg, 0.025 mmol) successively at 0°C. The mixture was stirred at 0°C for 4 h. After dilution with ether (30 ml), the mixture was washed with 1M aqueous $KHSO_4$ (10 ml), saturated aqueous $NaHCO_3$ (10 ml), saturated brine (10 ml), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-200, 7 g, hexane:EtOAc = 5:1 to 3:1) to give **24** (61 mg, 82% in 3 steps) as a colorless oil: $[\alpha]_D^{23}$ -0.01 (c 1.735, $CHCl_3$); IR ν_{max}($CHCl_3$) cm^{-1} 3400, 1790, 1738, 1715, 1537, 1368, 1287; ^{1}H-NMR ($CDCl_3$) δ 0.034 (9H, s, $SiMe_3$) 0.96-1.02 (2H, m, $TMSCH_2$) 1.43 (9H, s, CMe_3) 2.47 (1H, dd, *J* = 6.9, 18.2Hz, $NCHCH_2$) 2.71-2.87 (2H, m, $TMSEO_2CCH_2$) 3.01 (1H, dd, *J* = 8.6, 17.8Hz, $NCHCH_2$) 4.16-4.22 (3H, m, NCHCH, $TMSCH_2CH_2$) 4.60-4.66 (1H, m, CHN) 4.96 (1H, brd, *J* = 5.9Hz, NH); ^{13}C-NMR ($CDCl_3$) δ -1.97 (CH_3) -1.59 (CH_3) -1.22 (CH_3) 17.18 (CH_2) 28.23 (CH_3) 34.57 (CH_2) 35.11 (CH_2) 35.86 (CH_2) 38.25 (CH_2) 49.41 (CH) 51.50 (CH) 63.45 (CH_2) 79.24 (CH) 80.43 (4°, CMe_3) 81.45 (CH) 155.09 (4°, $NHCO_2$) 169.66 (4°, ester C=O) 169.88 (4°, lactone C=O) 173.90 (4°, lactone C=O); Anal. calcd for $C_{16}H_{29}NO_6Si$: C, 53.46; H, 8.13; N, 3.90. Found: C, 53.22; H, 7.94; N, 3.74.

(3*RS*,4*S*)-3-Hydroxy-4-(*N*-(*tert*-butoxycarbonyl)amino)adipic acid (26). To a stirred solution of **17** (128 mg, 0.325 mmol) in EtOH (1 ml) was added $NaBH_4$ (27 mg, 0.714 mmol) at -78°C. The mixture was stirred for 1 h at -78°C. Aqueous citric acid (15%, 3 ml) was added, and the mixture was allowed to warm to room temperature, and diluted with EtOAc (50 ml), washed with water (10 ml), saturated brine (10 ml), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give the crude alcohol as a pale yellow oil, which was used for the next step without further purification.

To a stirred solution of the crude alcohol (0.325 mmol) in THF (1 ml) was added dropwise 1*N* aqueous NaOH (0.9 ml, 0.9 mmol) at 0°C. The mixture was stirred for 5 h at 0°C, then transferred to a separating funnel with water (50 ml). The aqueous layer was washed with $CHCl_3$ (10 ml x 2), acidified with 1M aqueous $KHSO_4$ at 0°C and salted out. The mixture was extracted with EtOAc (10 ml x 5), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give the crude **27**, which was purified by recrystallization (EtOAc) to give **27** (92 mg, quant.) as a colorless solid: mp 148-151°C (EtOAc); $[\alpha]_D^{19}$ -0.09 (c 1.02, MeOH); IR $\nu_{max}(CHCl_3)$ cm^{-1} 3350, 3000-3500, 1692, 1460, 1377, 1165; ^{1}H-NMR (CD_3OD) δ 1.42 (9H, s, CMe_3) 2.38-2.44 (2H, m, CH_2CO_2H) 2.54-2.73 (2H, m, CH_2CO_2H) 3.85-3.93 (2H, m, CHOH, CHNH); Anal. calcd for $C_{11}H_{19}NO_7$: C, 47.65; H, 6.91; N, 5.05. Found: C, 47.41; H, 6.87; N, 5.08.

Boc-Sarcosine tricloroethylester (Boc-MeGly-OTce). To a stirred solution of Boc-sarcosine (3.78 g, 20 mmol) in CH_2Cl_2 (50 ml) was successively added TceOH (2.4 ml, 25 mmol), EDC·HCl (4.38 g, 23.0 mmol), and DMAP (244 mg, 2.0 mmol) at 0°C. The mixture was stirred at room temperature for 19 h. After dilution with CH_2Cl_2 (200 ml), the mixture was washed with 1M aqueous $KHSO_4$ (50 ml x 2), saturated aqueous $NaHCO_3$ (50 ml), saturated brine (50 ml), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-200, 200 g, hexane:EtOAc = 7:1 to 5:1) to give Boc-MeGly-OTce (5.892 g, 92%) as a colorless oil; IR ν_{max}neat cm^{-1} 1771, 1700, 1481, 1452, 1390, 1248, 1175; ^{1}H-NMR ($CDCl_3$) δ 1.43, 1.47 (9H, s, CMe_3) 2.96, 2.97 (3H, s, NMe) 4.07, 4.13 (2H, s, NCH_2) 4.78 (2H, s, CH_2CCl_3); ^{13}C-NMR ($CDCl_3$) δ 28.16, 28.21 (CH_3) 35.45, 35.54 (CH_3) 50.10, 50.65 (CH_2) 74.05, 74.12 (CH_2) 80.36, 80.43 (4°, CMe_3) 94.52 (4°, CCl_3) 155.12, 155.92 (4°, amide C=O) 168.40 (CO_2Tce); Anal. calcd for $C_{10}H_{16}Cl_3NO_4$: C, 37.46; H, 5.03; N, 4.37. Found: C, 37.41; H, 5.11; N, 4.41.

Boc-Lactone 27. To a stirred solution of **26** (345 mg, 1.25 mmol) in CH_2Cl_2 (13 ml) and DMSO (13 ml) were added EDC·HCl (358 mg, 1.88 mmol) and DMAP (16 mg, 0.13 mmol) at 0°C. The mixture was stirred for 17 h at room temperature. After dilution with ether (100 ml), the mixture was washed with 1M aqueous $KHSO_4$ (30 ml), saturated brine (30 ml), dried over $MgSO_4$, filtered,

and concentrated *in vacuo* to give the crude lactone carboxylic acid (134 mg). The aqueous layer was salted out and extracted with ether (10 ml x 10). The combined organic solution was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude **3b** (312 mg) as a colorless amorphous solid, which was used for the next step without further purification. (IR $\nu_{max}(CHCl_3)$ 1786 cm^{-1}(lactone C=O)).

To a stirred solution of Boc-MeGly-OTce (577 mg, 1.8 mmol) in $CHCl_3$ (4 ml) was added TFA (2 ml) at room temperature. The mixture was stirred for 1 h at room temperature., then concentrated *in vacuo* to give the light yellow residue. Toluene was added to the whole and the mixture was concentrated *in vacuo*. This work-up was repeated three times to remove the excess of TFA completely. The crude TFA salt was used for the next step without further purification.

To a stirred solution of the crude **3b** and the crude TFA salt of H-MeGly-OTce were added BopCl (510 mg, 2.0 mmol) and Et_3N (0.56 ml, 4.0mmol) at 0°C. The mixture was stirred for 2 days at 4°C. After dilution with EtOAc (100 ml), the mixture was washed with 1M aqueous $KHSO_4$ (30 ml), saturated aqueous $NaHCO_3$ (30 ml), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-200, 25 g, hexane : EtOAc = 1 : 1) to give **27** (302 mg, 58% from **26**) as a 5.3:1 diastereoisomeric mixture. A colorless oil: $[\alpha]_D^{19}$ -0.06 (c 0.80, $CHCl_3$); IR ν_{max}neat cm^{-1} 3335, 1771, 1705, 1651, 1522, 1367, 1279, 1169, 756; ^{1}H-NMR ($CDCl_3$) δ 1.44 (9H, s, CMe_3) 2.51 (1H, dd, *J* = 7.6, 18.1Hz, lactone CH_2) 2.83-3.15 (1H, dd, *J* = 7.6, 18.1Hz, N(Me)$COCH_2$, lactone CH_2) 3.13 (3H, s, NMe) 4.10-4.29 (3H, m, CHO, Sar CH_2) 4.69-4.84 (1H, m, CHNHBoc) 4.78 (2H, s, CH_2CCl_3) 5.28 (1H, br, NHBoc); Anal. calcd for $C_{16}H_{23}Cl_3N_2O_7 \cdot {}^1/_4$EtOAc: C, 41.52; H, 5.12; N, 5.70. Found: C, 41.50; H, 4.87; N, 5.61.

Methyl (*R*)-3,4-dihydroxybutanoate (29). In a 300 ml, three necked, round-bottled flask mounted with a reflux condenser and thermometer was placed a solution of (*R*)-dimethyl malate (**28**) (5 ml, 37.7 mmol) in THF (80 ml). To this was added 10.0-10.2 M borane-methylsulfide complex (3.9 ml, 39.0-39.8 mmol) dropwise at room temperature under stirring during 30 min. The solution was stirred at room temperature until evolution of hydrogen gas ceased (ca. 30 min). Then, the flask was cooled with a water-ice bath and stirring was continued for 30 min. To the solution was added $NaBH_4$ (72 mg, 1.90 mmol) in one portion (exothermic) under vigorous stirring at 0°C. When the exothermic reaction subsided, the water bath was removed and the reaction was stirred at room temperature for 30 min. MeOH (13 ml) and *p*-TsOH (360 mg, 1.9 mmol) were added to the reaction mixture, and the resulting slightly cloudy solution was stirred for 30 min at room temperature, followed by concentration to give a colorless gum. This was dissolved in benzene (40 ml) and

MeOH (40 ml), and the resulting solution was concentrated again: this operation was repeated. To the residue was added benzene (60 ml) and the solution was concentrated, which was repeated to eliminate MeOH and $B(OMe)_3$ as thoroughly as possible to give a clear, colorless gum. The residue was purified by column chromatography (silica gel BW-820MH, 150 g, hexane:EtOAc = 1:19 to EtOAc only) to give **29** (4.562 g, 90%) as a colorless oil.

Methyl (*R*)-3-hydroxy-4-*p*-toluenesulfonyloxybutanoate (30). To a stirred solution of **29** (130 mg, 0.969 mmol) in pyridine (2.5 ml) was added *p*-TsCl (194 mg, 1.02 mmol) at 0°C. The mixture was stirred at 4°C for 20 h. After dilution with EtOAc (50 ml), the mixture was washed with 1M aqueous $KHSO_4$ (20 ml x 2), saturated aqueous $NaHCO_3$ (20 ml) , saturated brine (20 ml), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-200, 20 g, hexane:EtOAc = 5:2 to 2:1) to give **30** (179 mg, 64%) as a white solid. The analytical sample was purified by recrystallization (hexane/ether): mp 81.5-82.5°C (hexane/ether); $[\alpha]_D^{26}$ +6.83 (c 1.1, $CHCl_3$); IR $\nu_{max}(CHCl_3)$ cm^{-1} 3580, 1732, 1599, 1439; ^{1}H-NMR ($CDCl_3$) δ 2.45 (3H, s, MeAr) 2.53-2.56 (2H, m, CH_2CO_2Me) 3.01 (1H, d, *J* = 5.0Hz, disappeared with D_2O, OH) 3.70 (3H, s, CO_2Me) 4.04 (2H, d, *J* = 5.0Hz, $TsOCH_2$) 4.22-4.28 (1H, m, CHOH) 7.36 (2H, d, *J* = 8.3Hz, SO_2Ar (m)) 7.80 (2H, d, *J* = 8.3Hz, SO_2Ar (o)); ^{13}C-NMR ($CDCl_3$) δ 21.56 (CH_3) 37.10 (CH_2) 51.93 (CH_3) 65.85 (CH) 71.96 (CH_2) 127.88 (CH) 129.88 (CH) 132.36 (4°, Ar) 145.08 (4°, Ar) 171.84 (4°, C=O); Anal. calcd for $C_{12}H_{16}O_6S$: C, 49.99; H, 5.59. Found: C, 49.88; H, 5.44.

Methyl (*R*)-3-hydroxy-4-azidobutanoate (31). To a stirred solution of **30** (50.5 mg, 0.175 mmol) in DMF (0.5 ml) was added NaN_3 (57 mg, 0.877 mmol) at room temperature. The mixture was stirred at 80 °C for 3 h. After dilution with EtOAc (50 ml), the mixture was washed with water (20 ml), 1M aqueous $KHSO_4$ (20 ml), saturated brine (20 ml), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-200, 7 g, hexane:EtOAc = 3:1) to give **31** (23.0 mg, 83%) as a colorless oil: $[\alpha]_D^{26}$ +19.63 (c 1.03, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3400, 2106, 1441, 1286, 1172; ^{1}H-NMR ($CDCl_3$) δ 2.54-2.57 (2H, m, CH_2CO_2Me) 3.15 (1H, s, disappeared with D_2O, OH) 3.36 (2H, dd, *J* = 4.3, 5.9Hz, CH_2N_3) 3.73 (3H, s, CO_2Me) 4.18-4.24 (1H, m, CHOH); ^{13}C-NMR ($CDCl_3$) δ 38.20 (CH_2) 51.91 (CH_3) 55.49 (CH_2) 67.20 (CH) 172.32 (4°, C=O); Anal. calcd for $C_5H_9N_3O_3$: C, 37.74; H, 5.70; N, 26.40. Found: C, 37.48; H, 5.68; N, 26.32.

Methyl (*R*)-3-hydroxy-4-*N*-(*tert*-butoxycarbonyl)aminobutanoate (32). To a stirred solution of **31** (1.76 g, 11.1 mmol) in EtOAc (20 ml) was added 5% Pd/C (500 mg) and Boc_2O (3.63 g, 16.6 mmol) under argon atmosphere. The black slurry was stirred under 1 atom of H_2 for 13 h at room

temperature. The mixture was filtered through a pad of celite (rinsed with EtOAc) and the filtrate was concentrated *in vacuo* and purified by column chromatography (silica gel BW-200, 70 g, hexane:EtOAc = 1:1) to give **32** (1.721 g, 87%) as a colorless oil: $[\alpha]_D^{26}$ +5.37° (c 1.06, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3400, 1738, 1715, 1520, 1368, 1271, 1252, 1169; ^{1}H-NMR ($CDCl_3$) δ 1.44 (9H, s, CMe_3) 2.48-2.51 (2H, m, CH_2CO_2Me) 3.30-3.38 (2H, m, NCH_2) 3.44 (1H, s, disappeared with D_2O, OH) 3.71 (3H, s, CO_2Me) 4.07-4.16 (1H, m, CHOH) 4.96 (1H, brs, NH); ^{13}C-NMR ($CDCl_3$) δ 28.26 (CH_3) 38.45 (CH_2) 45.46 (CH_2) 51.78 (CH_3) 67.62 (CH) 79.57 (4°, CMe_3) 156.54 (4°, NHC=O) 172.77 (4°, C=O); Anal. calcd for $C_{10}H_{19}NO_5$: C, 51.49; H, 8.21; N, 6.00. Found: C, 51.34; H, 8.43; N, 5.87.

2,2,2-Trichloroethyl (*R*)-3-hydroxy-4-*N*-(*tert*-butoxycarbonyl)aminobutanoate (33). To a stirred solution of **32** (1.721 g, 7.38 mmol) in MeOH (15 ml) was added 1*N* aqueous NaOH (8.9 ml, 8.9 mmol) at 0°C. The mixture was stirred at 0°C for 3.5 h, then acidified by addition of 1M aqueous $KHSO_4$, and salted out. The aqueous layer was extracted with $CHCl_3$ (30 ml x 4). The combined organic layer was washed with saturated brine (30 ml), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give the crude carboxylic acid as a colorless oil, which was used for the next step without further purification.

To a stirred solution of the crude carboxylic acid (7.38 mmol) and 2,2,2-trichloroethanol (1.1 ml, 11.5 ml) and DMAP (90 mg, 0.73 mmol) in CH_2Cl_2 (30 ml) was added EDC·HCl (1.7 g, 8.92 mmol) at 0°C. The mixture was stirred at room temperature overnight. After dilution with EtOAc (200 ml), the mixture was washed with 1M $KHSO_4$ (50 ml x 2), saturated aqueous $NaHCO_3$ (50 ml), saturated brine (50 ml), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-200, 70 g, hexane:EtOAc = 3:2) to give **33** (1.495 g, 59% in 2 steps) as a white solid: mp 70.5-71.5°C (hexane); $[\alpha]_D^{23}$ +2.08 (c 1.02, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3400, 1755, 1715, 1539, 1507, 1368, 1279, 1254, 1161; ^{1}H-NMR ($CDCl_3$) δ 1.45 (9H, s, CMe_3) 2.67 (2H, d, *J* = 6.3Hz, CH_2CO_2Tce) 3.13-3.23 (1H, dt, *J* = 6.3, 14.2Hz, NCH_2) 3.38-3.40 (2H decreased with D_2O to 1H, m, OH, NCH_2) 4.19 (1H, br, CHOH) 4.77 (2H, ABq, *J* = 12.2Hz, CH_2CCl_3) 4.97 (1H, brs, NH); ^{13}C-NMR ($CDCl_3$) δ 28.32 (CH_3) 38.69 (CH_2) 45.53 (CH_2) 67.63 (CH) 73.98 (CH_2) 79.87 (4°, CMe_3) 94.61 (4°, CCl_3) 156.78 (4°, NHC=O) 170.42 (4°, C=O); Anal. calcd for $C_{11}H_{18}Cl_3NO_5$: C, 37.68; H, 5.17; N, 3.99. Found: C, 37.72; H, 5.07; N, 3.79.

2,2,2-Trichloroethyl (*R*)-3-(*tert*-butyldimethyl)siloxy-4-*N*-(*tert*-butoxy-carbonyl)-aminobutanoate (4). To a stirred solution of **33** (1.245 g, 3.55 mmol) in DMF (10 ml) cooled in an ice bath was added imidazole (1.2 g, 17.6 mmol) and TBSCl (1.2 g, 7.96 mmol). The mixture was

stirred at room temperature for 15 h. After dilution with ether (200 ml), the mixture was washed with 1M aqueous $KHSO_4$ (30 ml x 2), saturated aqueous $NaHCO_3$ (30 ml) and saturated brine (30 ml), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel BW-200, 60 g, hexane:ether = 9:1 to 8:1) to give **4** (1.675 g, 100%) as a colorless oil: $[\alpha]_D^{23}$ +8.44 (c 0.98, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3400, 1759, 1713, 1504, 1368, 1254, 1161; ^{1}H-NMR ($CDCl_3$) δ 0.06 (3H, s, $SiMe_2$) 0.09 (3H, s, $SiMe_2$) 0.87 (9H, s, $SiCMe_3$) 1.44 (9H, s, CMe_3) 2.62 (2H, d, *J* = 5.9Hz, CH_2CO_2Tce) 3.24-3.29 (2H, m, NCH_2) 4.25-4.29 (1H, m, CHOTBS) 4.72 (2H, ABq, *J* = 12.2Hz, CH_2CCl_3) 4.71-4.78 (1H, brm, NH); ^{13}C-NMR ($CDCl_3$) δ -4.92 (CH_3) -4.74 (CH_3) 17.91 (4°, $SiCMe_3$) 25.69 (CH_3) 28.35 (CH_3) 39.71 (CH_2) 45.93 (CH_2) 68.08 (CH) 74.08 (CH_2) 79.44 (4°, $NHCO_2CMe_3$) 94.73 (4°, CCl_3) 155.86 (4°, NHC=O) 169.46 (4°, C=O); Anal. calcd for $C_{17}H_{32}Cl_3NO_5Si$: C, 43.92; H, 6.94; N, 3.01. Found: C, 43.72; H, 6.91; N, 2.94.

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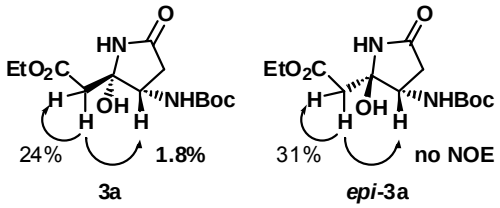
References

1. (a) Bewley, C. A.; Debitus, C.; Faulkner, D. J. *J. Am. Chem. Soc.* **1994**, *116*, 7631. (b) Faulkner, D. J.; Schmidt, E. W. *Tetrahedron* **1998**, *54*, 3043. (c) Qureshi, A.; Colin, P.; Faulkner, D. J. *Tetrahedron* **2000**, *56*, 3679.
2. For a recent review, see Shioiri, T.; Hamada, Y. *Synlett* **2001**, 184.
3. (a) Sasaki, S.; Hamada, Y.; Shioiri, T. *Tetrahedron Lett.* **1997**, *38*, 3013. (b) Sasaki, S.; Hamada, Y.; Shioiri, T. *Peptide Science- Present and Future*, ed. by Shimonishi, Y., Kluwer Academic Pub., Amsterdam, pp 536-537. (c) Sasaki, S.; Hamada, Y.; Shioiri, T. *Peptide Science 1998*, ed. by Kondoh, Protein Reseach Foundation, Osaka, pp 17-20. (d) Sasaki, S.; Hamada, Y.; Shioiri, T. *Synlett* **1999**, 453. (e) Sasaki, S.; Hamada, Y.; Shioiri, T. *Tetrahedron Lett*. **1999**, *40*, 3187.
4. (a) Yamada, S.; Oguri, T.; Shioiri, T. *J. Chem. Soc., Chem. Commun*. **1976**, 136. (b) Oguri, T.; Kawai, N.; Shioiri, T.; Yamada, S. *Chem. Pharm. Bull*. **1978**, *26*, 803. (c) Irako, N.; Hamada, Y.;

Shioiri, T. *Tetrahedron* **1995**, *51*, 12731. (d) Matsumoto, T.; Shioiri, T.; Osawa, E. *Tetrahedron* **1996**, *52*, 5961. (e) Matsumoto, T.; Shioiri, T.; Osawa, E. *Tetrahedron* **1996**, *52*, 5971. (f) Sugiyama, H.; Yokokawa, F.; Shioiri, T.; Katagiri, N.; Oda, O.; Ogawa, H. *Tetrahedron Lett.* **1999**, *40*, 2569. (g) Yokokawa, F.; Sugiyama, H.; Shioiri. T.; Katagiri, N.; Oda, O.; Ogawa, H. *Tetrahedron* **2001**, *57*, 4759-4766.

5. Ramalingam, K.; Woodward, R. W. *J. Org. Chem*. **1988**, *53*, 1900.
6. Ramsamy, K.; Olsen, R. K.; Emery, T. *Synthesis* **1982**, *42*. See also: Shioiri, T.; Irako, N.; Sakakibara, S.; Matsuura, F.; Hamada, Y. *Heterocycles* **1997**, *44*, 519.
7. Cf. Brooks, P. W.; Lu, L. D.-L.; Masamune, S. *Angew. Chem. Int. Ed. Engl.* **1979**, *18*, 72.
8. The configurations of **3a** and its epimer ***epi*-3a** were determined by NOE experiments of their NMR spectra.



9. (a) Soderquist, J. A.; Thompson, K. L. *J. Organomet. Chem.* **1978**, *159*, 237-249. (b) Hamada, Y.; Kondo, Y.; Shibata, M.; Shioiri, T. *J. Am. Chem. Soc*. **1989**, *111*, 669.
10. Treatment of **24** under alkaline conditions followed by relactonization with EDC afforded the required γ-lactone carboxylic acid **3b**.
11. Saito, S.; Ichikawa, T.; Kubota, A.; Koga, K.; Moriwake, T. *Tetrahedron* **1992**, *48*, 4067.
12. Deng, J.; Hamada, Y.; Shioiri, T. *Synthesis* **1998**, 627.