Total Synthesis of Siastatin B and Its Enantiomer Using Carbohydrate as a Chiral Educt

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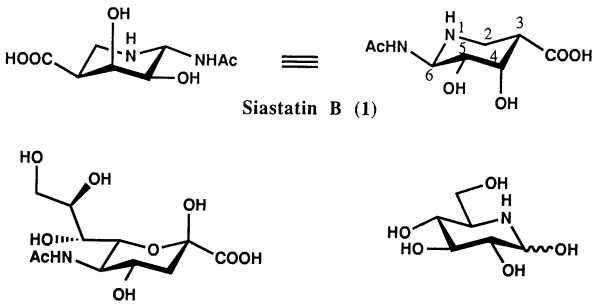
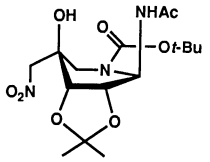
The enantioselective synthesis of siastatin B, an inhibitor of neuraminidase and its antipode from D-ribono-γ-lactone has stereospecifically been achieved. The total synthesis elucidated the absolute configuration of siastatin B as (3*S*,4*S*,5*R*,6*R*)-6-acetamido-4,5-dihydroxy-3-piperidinecarboxylic acid.

Cell-surface carbohydrates as glycoconjugates mediate cell-to-cell communication, cell-cell recognition, and the "social behavior" of cells. *N*-Acetylneuraminic acid as a terminal unit of cell-surface carbohydrates plays an essential role in various important biological functions such as immune response, oncogenesis, metastasis of tumors, sperm penetration, differentiation of neuronal cell, enhancement of neurite outgrowth, and as cellular receptors for hormons, toxins, bacteria, viruses, etc.[1] Neuraminidase and sialyltransferase, widely distributed among animal tissues, microorganisms, and viruses, are responsible for the control of these biological functions by the metabolism of cell-surface complex carbohydrates.[1b,1d,2]

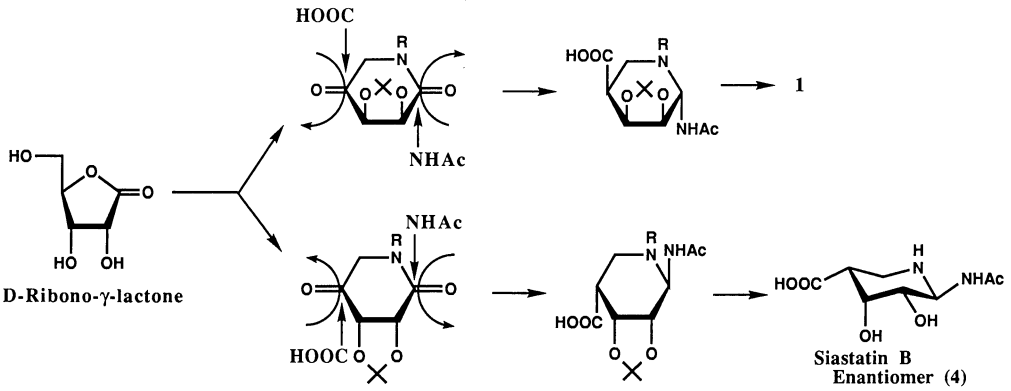
Siastatin B was isolated by Umezawa et al.[3] in 1974 from a culture filtrate of *Streptomyces verticillus* var. *quintum* MB695-A4. It inhibits neuraminidases isolated from various microorganisms and animal tissues as well as β-glucuronidase and *N*-acetyl-β-D-glucosaminidase. The relative configuration of siastatin B was determined as 6(*S*/*R*)-acetamido-4(*R*/*S*), 5-(*S*/*R*)-dihydroxypiperidine-3(*R*/*S*)-carboxylic acid by ^{1}H NMR and X-ray crystallographic studies.[3] We speculated from its biological activity that the absolute configuration of siastatin B should be that shown in **1** by analogy with *N*-acetylneuraminic acid (**2**). It is distinct from glycohydrolase inhibitors belonging to the sugar analogues having a piperidine ring such as nojirimycin (**3**)[4] and galactostatin,[5] and their congeners.[6] Here we wish to report the first total syntheses of siastatin B (**1**) and its antipode (**4**) with the full experimental details and discussions.[7]



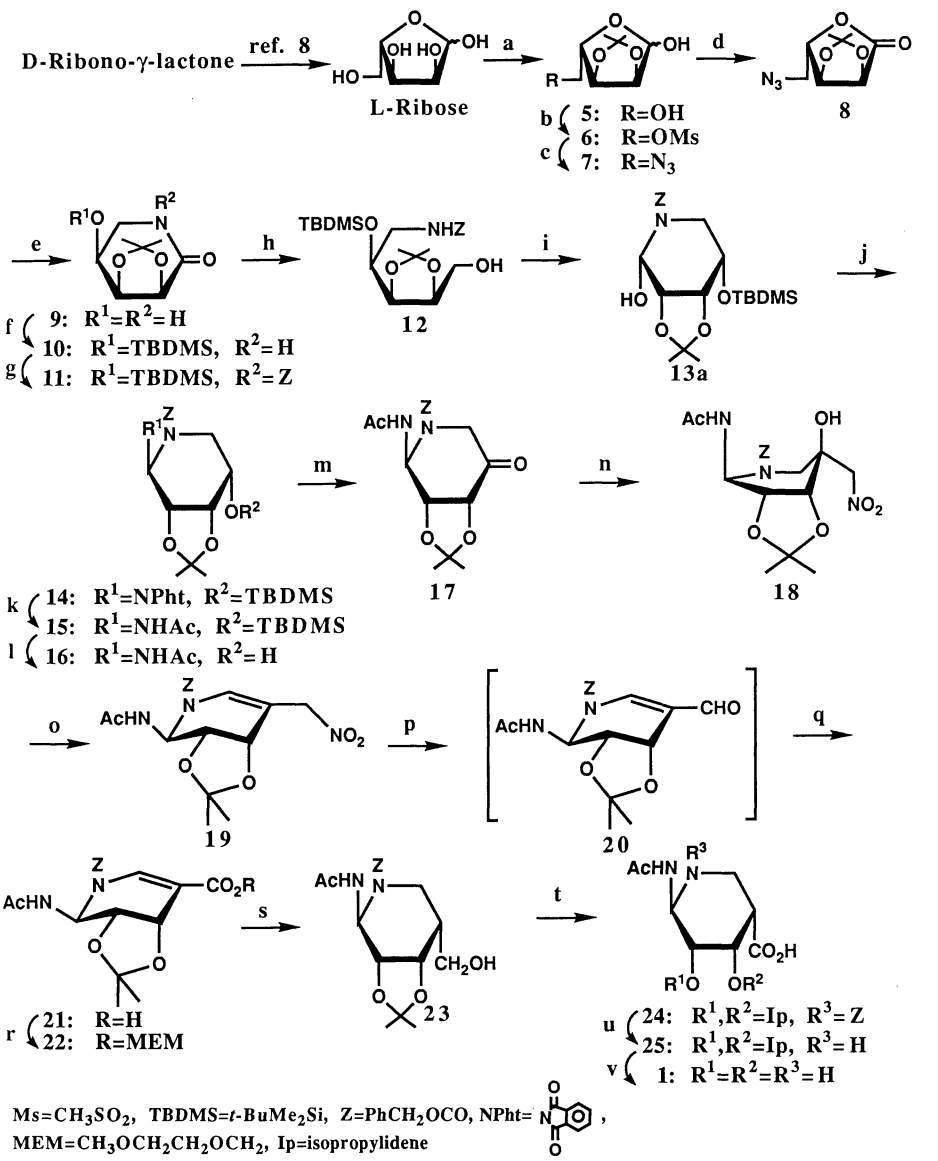


Results and Discussion

The strategy (Scheme 1) is based on an enantiodivergent method employing D-ribono-γ-lactone as a chiral source via stereospecific introduction of *N*-acetyl and carboxyl substituents into the key-intermediate-



Scheme 1.



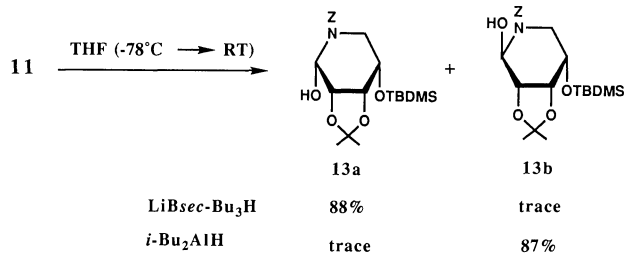
Ms=CH_3SO_2, TBDMS=*t*-$BuMe_2Si$, Z=$PhCH_2OCO$, NPht= , MEM=$CH_3OCH_2CH_2OCH_2$, Ip=isopropylidene

(a) *p*-TsOH, Me_2CO (b) CH_3SO_2Cl, C_6H_5N (c) NaN_3, DMSO (d) CrO_3/C_6H_5N, CH_2Cl_2 (e) H_2, Raney Ni, MeOH (f) *t*-$BuMe_2SiCl$, imidazole, DMF (g) $PhCH_2OCOCl$, NaH, DMF (h) $NaBH_4$, EtOH (i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2 (j) phthalimide, Ph_3P, DEAD, DMF (k) NH_2NH_2, MeOH; Ac_2O, C_6H_5N (l) n-Bu_4NF, THF (m) RuO_4, CH_2Cl_2, CCl_4 (n) CH_3NO_2, NaH, DME (o) *p*-TsOH, Ac_2O; K_2CO_3, C_6H_6, 100% (p) C_6H_5N, 38°C, 80% (q) $CH_3CH{=}C(CH_3)_2$/*t*-BuOH, $NaClO$-NaH_2PO_4, H_2O (r) MEMCl, (*i*-$Pr)_2NEt$, CH_2Cl_2 (s) $NaBH_4$, 1:10 CF_3CH_2OH/THF (t) PDC, DMF (u) H_2, 50% Pd/C, MeOH (v) 1M HCl, then Dowex 50W-X4 (H^+ form) eluted with NH_4OH

Scheme 2.

lactams. As shown in Scheme 2, the synthesis of lactam **9** began with L-ribose[8] prepared from D-ribono-γ-lactone which was transformed to 5-azido-5-deoxy-2,3-*O*-isopropylidene-L-ribonolactone (**8**) by protection of the 2,3-diol (*p*-$CH_3C_6H_4SO_3H$, CH_3COCH_3), azide formation (CH_3SO_2Cl, Py; NaN_3, DMSO), and oxidation (CrO_3/Py, CH_2Cl_2) in 89% yield. Hydrogenation of the azido group of **8** and ring expansion[9] with Raney Ni afforded the crystalline **9** in 88% yield. Hydroxyl and amide groups in **9** were protected with *t*-butyldimethylsilyl and benzyloxycarbonyl groups, respectively in 99% yield.

Stereospecific introduction of an axial hydroxyl group at C-2 was achieved by reduction of **11** with sodium borohydride ($NaBH_4$) to **12** in 96% yield, and Swern oxidation[10] to give aminal **13a** in 88% yield. A small coupling constant ($J<2$ Hz) between 2-H and 3-H in the ^1H NMR spectrum of **13a** is clearly indicative of an equatorial hydrogen. Strikingly a single stereoisomer controlled by an anomeric effect[11] results from this oxidation. One-step stereospecific transformation from lactam **11** into aminal **13a** was also best achieved by L-selectride reduction in THF in 88% yield. On the other hand, diisobutylaluminum hydride (DIBAH)



Scheme 3.

reduction in THF gave predominantly its epimer **13b** in 87% yield (Scheme 3). Stereoselectivity in L-selectride reduction was probably caused by hydride attack from a less sterically hindered side (upper side), whereas in DIBAH reduction it was controlled by a metal chelation formed between aluminum and oxygen atoms of isopropylidene group.

Displacement of the axial hydroxyl group of **13a** to the equatorial amino group proved troublesome until we discovered that Mitsunobu reaction[12] (PPh_3, diethyl azodicarboxylate, phthalimide) in *N,N*-dimethylformamide (DMF) gave the desired product **14**, quantitatively. A doublet (J=5 Hz) of a proton at C-2 in the ^{1}H NMR spectrum of **14** is clearly indicative of an axial hydrogen. Replacement of the amino substitution from phthaloyl to acetyl by treatment with hydrazine and acetic anhydride in pyridine, and removal of the *t*-butyldimethylsilyl group with tetrabutylammonium fluoride yielded **16**, quantitatively. Oxidation of the hydroxyl group was best achieved by treatment with ruthenium tetraoxide in dichloromethane to give ketone **17** in 99% yield.

After several attempts of nucleophilic addition to the ketone or Wittig reaction of **17**, condensation with nitromethane using sodium hydride in ethylene glycol dimethyl ether (DME) was found to proceed smoothly to give **18** as a single stereoisomer, quantitatively. The absolute stereochemistry at C-5 was best established as *S*-configuration by analogy with the stereochemistry of synthetic *N*-(*t*-butoxycarbonyl) antipode **26** determined by X-ray crystallographic analysis.[13] The structure of **18** was supported by the smooth base-catalyzed β-elimination of the acetoxyl group of the acetate of **18**.

Acetylation of **18** with a catalytic *p*-toluenesulfonic acid in acetic anhydride followed by base-catalyzed elimination of the acetoxyl group yielded exclusively endocyclic nitro olefin **19**, quantitatively. The structure of **19** was determined by its ^{1}H NMR spectrum which shows the methylene protons of the nitromethyl group at δ=4.78 and 5.16 (ABq, J=15 Hz), 4-H at δ=4.71 (d, J=6 Hz), and 6-H at δ=7.06 (s). Generally C-nitromethyl and acetoxy branched-glycopyranosides produce exocyclic nitromethylene derivative by the base-catalyzed elimination of the elements of acetic acid.[14] In our case, the preferential deprotonation of a proton at C-6 rather than a proton of nitromethyl group would lead to formation of endocyclic olefin **19**.

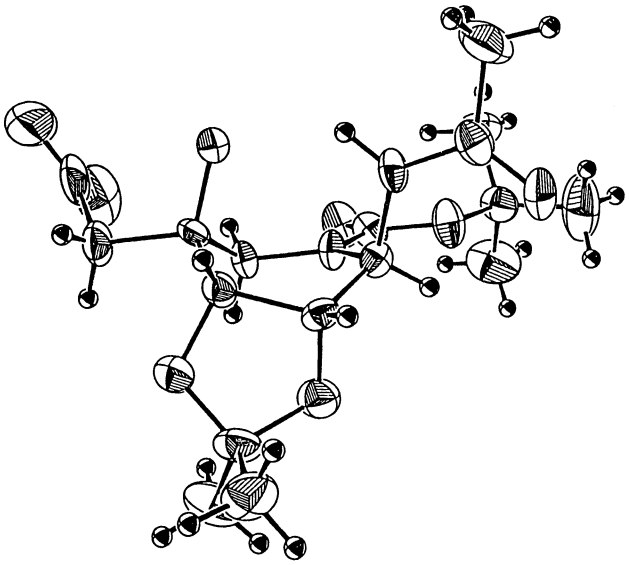


Fig. 1. X-Ray structure of **26**.[13]

Efforts to achieve the transformation of **19** into α,β-unsaturated aldehyde **20** by Nef and modified Nef reaction,[15] and by procedures employing either oxidizing[16] or reducing[17] agents were unpromising, and attention was then directed to the air oxidation under mild conditions. Endocyclic nitro olefin **19**, upon simply warming in pyridine, afforded α,β-unsaturated aldehyde **20**, which was converted to carboxylate **22** by a subsequent oxidation with sodium chlorite and protection with (2-methoxyethoxy)methyl (MEM) group in 55% yield. This process should be carried out by autoxidation[18] with air during a long reaction period (5 d) without positive introduction of air into the reaction medium.

Catalytic reduction[19] of the double bond in **22** accompanied by elimination of the hydroxyl group at C-4, and hydride reduction[20] of the double bond with or without combination of a transition metal also proceeded unfavorably and without chemoselectivity. In the reduction of free acid **21**, the same results as those mentioned above were also obtained. Therefore, **22** once was stereoselectively hydrogenated to α,β-saturated hydroxymethyl compound **23** with $NaBH_4$ in a mixture of 2,2,2-trifluoroethanol and tetrahydrofuran (1 : 10)[21] in 75% yield. Oxidation of **23** with pyridinium dichromate in *N,N*-dimethylformamide gave acid **24**, which was converted to crystalline **1** by removal of protecting groups. Its spectral properties (IR, ^{1}H NMR, ^{13}C NMR, mass spectrum) and specific rotation were identical with those of the natural siastatin B. Thus, the absolute configuration of siastatin B has been elucidated as the (3*S*,4*S*,5*R*,6*R*)-isomer **1**.

Enantiomer **4** was also synthesized by the same sequences used in the synthesis of **1** from 5-amino-5-deoxy-2,3-*O*-isopropylidene-D-ribonolactam[23] prepared from D-ribono-γ-lactone. Compound **4** was identical

in all respects with the synthetic and the natural **1** except for the sign of the specific rotation.

The synthetic **1** shows the same inhibitory effects as the natural siastatin B against neuraminidases prepared from *Clostridium perfringens, Streptomyces*, rat mammary gland, rat mammary liver, chorioallantoic membrane (IC_{50}=6, 20, 220, 340, and 100 μg mL^{-1}, respectively), β-glucuronidase (IC_{50}=8 μg mL^{-1}) and N-acetyl-β-D-glucosaminidase (IC_{50}=36 μg mL^{-1}).[3] In contrast, **4** shows almost no activities against neuraminidases mentioned above, whereas **4** demonstrates activity against β-glucuronidase (IC_{50}=50 μg mL^{-1}).

The authors wish to thank Mr. R. Sawa of our institute for the measurements of high-resolution mass spectra.

Experimental

General. Melting points were determined with a Yanagimoto apparatus and were uncorrected. IR spectra were determined on a Hitachi Model 260-10 spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. ^{1}H NMR spectra were recorded with JEOL GX-400 spectrometers. Chemical shifts are expressed in δ values (ppm) with tetramethylsilane as an internal standard. The mass spectra and the high-resolution mass spectra (HRMS) were taken by a Hitachi M-80H for secondary ionization and a JEOL SX102 for fast atom bomberdment.

2,3-*O*-Isopropylidene-L-ribofuranose (5). To a solution of L-ribose (4.1 g) in dry acetone (1.1 dm^3) was added *p*-toluenesulfonic acid monohydrate (13.8 g) at 10 °C, and the mixture was stirred at 10 °C for 1 h and then at room temperature for 3 h. After being quenched with $NaHCO_3$-saturated aqueous solution, evaporation of the solvent gave a viscous oil. The residue was taken up in acetone and the insoluble materials were separated. Evaporation of the solution afforded an oil, which was subjected to the column chromatography on silica gel. Elution with chloroform-methanol (10 : 1) gave a colorless oil of **6** (5 g, 96%): $[\alpha]_D^{21}$ +34.9° (*c* 0.88, CH_3OH); IR (CCl_4) 3350 (broad), 2980, 2940, 2870, 1460, 1385, 1375, 1320, 1270, 1240, 1210, 1140, 1105, 1070, 1040, 990, 920 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ=1.32 and 1.48 (3H each s, CH_3 of isopropylidene), 3.71 and 3.76 (2H ABq with a small coupling, J_{AB}=12, $J_{5,4}$=3 and $J_{5',4}$=2 Hz, H-5), 4.40 (1H broad s, $J_{1/2h}$=5 Hz, H-4), 4.58 (1H d, $J_{3,2}$=6 Hz, H-3), 4.85 (1H d, $J_{2,3}$=6 Hz, H-2) and 5.41 (1H s, H-1); MS (FAB, negative) *m/z* 379 ($2M^+-1$), 281, 235, 189 (M^+-1), 113, 71, 59. HR-FABMS (negative) Found: *m/z* 189.0761. Calcd for $C_8H_{13}O_5$: (M-H), 189.0763.

2,3-*O*-Isopropylidene-5-*O*-methylsulfonyl-L-ribofuranose (6). To a solution of **5** (4.86 g) in dry pyridine (172 ml) was added methanesulfonyl chloride (8.95 ml) at −30 °C, and the mixture was allowed to stand at the same temperature. After being quenched with 3% aqueous $NaHCO_3$ solution, the reaction mixture was diluted with a large amount of dichloromethane. The solution was washed with NaCl-saturated aqueous solution, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give a solid, which was subjected to the column chromatography on silica gel. Elution with toluene–acetone (1 : 1) gave colorless crystals of **6** (6.7 g, 98%): Mp 111—112 °C; $[\alpha]_D^{21}$ +9.2°(*c* 0.8, CH_3OH); IR ($CHCl_3$) 3500 (broad), 3030, 3000, 2960, 1390, 1380, 1370, 1350, 1280, 1240, 1185, 1170, 1105, 1080, 1050, 1000, 955 cm^{-1}. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of **6** shows a mixture of α- and β-isomer in a ratio of 3 : 1; a part of ^{1}H NMR of major isomer: δ=1.33 and 1.49 (each s, CH_3 of isopropylidene), 2.87 (d, $J_{OH,1}$=3 Hz, OH), 3.09 (s, CH_3SO_2), 4.65 (d, $J_{3,2}$=6 Hz, H-3), 4.73 (d, $J_{2,3}$=6 Hz, H-2) and 5.51 (d, $J_{1,OH}$=3 Hz, H-1); MS (FAB, positive) *m/z* 269 (M^++1), 251, 235, 115, 69, 43. HR-FABMS (positive) Found: *m/z* 269.0698. Calcd for $C_9H_{17}O_7S$: (M+H), 269.0695.

5-Azido-5-deoxy-2,3-*O*-isopropylidene-L-ribofuranose (7). To a solution of **6** (3.06 g) in dimethyl sulfoxide (22 ml) was added NaN_3 (5.42 g), and the mixture was stirred at 65 °C for 2 h. After addition of acetone (500 ml), the resulting precipitate was filtered off, and the residue was evaporated to give a solution. The solution was diluted with water (220 ml), and then the mixture was extracted with ether. The extract was washed with NaCl-saturated aqueous solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to the column chromatography on silica gel. Elution with toluene–acetone (10 : 1) gave a foamy glass of **7** (2.36 g, 96%): $[\alpha]_D^{26}$ +1.1° (*c* 0.89, $CHCl_3$); IR (CCl_4) 3610, 3450, 3000, 2950, 2100, 1383, 1380, 1270, 1240, 1210, 1160, 1105, 1080, 1040, 1000, 970 cm^{-1}; ^{1}H NMR spectrum ($CDCl_3$, 90 MHz) of **7** shows a mixture of α- and β-isomer in a ratio of 2 : 1; a part of ^{1}H NMR of major isomer: δ=1.30 and 1.47 (each s, CH_3 of isopropylidene), 3.17 (d, $J_{OH,1}$=4.5 Hz, OH), 4.63 (s, H-2 and H-3), and 5.48 (d, $J_{1,OH}$=4.5 Hz, H-1); MS (FAB, negative) *m/z* 214 (M^+-1), 199, 168, 138, 122, 46. HR-FABMS (negative) Found: *m/z* 214.0826. Calcd for $C_8H_{12}O_4N_3$: (M−H), 214.0828.

5-Azido-5-deoxy-2,3-*O*-isopropylidene-L-ribono-1,4-lactone (8). To dry pyridine (27.5 ml) was added CrO_3 (16.8 g) at 10 °C, and the mixture was diluted with dry dichloromethane. After the mixture was stirred at the same temperature for 15 min, a solution of **7** (3 g) in dichloromethane (98 ml) was added to the mixture. Then the resulting mixture was stirred vigorously at room temperature for 30 min. The supernatant was poured into $NaHCO_3$-saturated aqueous solution (300 ml). The solution was extracted with dichloromethane. The extract was washed with NaCl-saturated aqueous solution, dried over $MgSO_4$ and filtered. Evaporation of the filtrate gave an oil, which was subjected to the column chromatography on silica gel. Elution with toluene–acetone (5 : 1) yielded an oil (2.9 g, 96.9%): $[\alpha]_D^{25}$ −16.2° (*c* 0.77, $CHCl_3$); IR (CCl_4) 3010, 2960, 2120, 1810, 1465, 1450, 1395, 1385, 1360, 1275, 1220, 1175, 1160, 1105, 1085, 1005, 975, 970, 930, 910 cm^{-1}; ^{1}H NMR ($CDCl_3$, 90 MHz) δ=1.37 and 1.47 (3H each s, CH_3 of isopropylidene), 3.58 and 3.84 (2H ABq with a small coupling, J_{AB}=13.5 and $J_{5,4}$=3 Hz, H-5), 4.59 (1H d, $J_{3,2}$=6 Hz, H-3), 4.67 (1H d, $J_{4,5}$=3 Hz, H-4), and 4.84 (1H d, $J_{2,3}$=6 Hz, H-2); MS (FAB, positive) *m/z* 214 (M^++1), 167, 75, 57. HR-FABMS (positive) Found: *m/z* 214.0835. Calcd for $C_8H_{12}O_4N_3$: (M+H), 214.0827.

5-Amino-5-deoxy-2,3-*O*-isopropylidene-L-1,5-ribonolactam (9). A solution of **8** (2.9 g) in methanol (35 ml) was hydrogenated at room temperature in the presence of Raney Ni under atmosphere of hydrogen for 2 h. Filtration of the catalyst and evaporation of the filtrate gave a residue, which was subjected to the column chromatography on silica gel. Elution with chloroform–methanol (10 : 1) gave crystals of **9** (2.23 g, 87.7%): Mp 138—139 °C; $[\alpha]_D^{22}$ −16.4° (*c* 0.76, $CHCl_3$); IR (KBr) 3530, 3420, 3300, 2990, 2940, 2910, 1665, 1640, 1490,

1445, 1415, 1390, 1370, 1350, 1300, 1290, 1270, 1240, 1175, 1150, 1125, 1105, 1095, 1060, 1000, 990, 960, 950, 940 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ=1.38 and 1.49 (3H each s, CH_3 of isopropylidene), 3.00 (1H broad s, OH), 3.22 (1H dt, J=13 and 3 Hz, H-5), 3.46 (1H ddd, J=13, 7.8 and 3 Hz, H-5′), 4.06 (1H dt, J=7.8 and 3 Hz, H-4), 4.44 (1H d, J=7.6 Hz, H-2), 4.49 (1H dd, J=7.6 and 3 Hz, H-3), and 7.34 (1H broad s, NH); MS (FAB, positive) m/z 188 (M^++1), 172, 130, 114, 75. HR-FABMS (positive) Found: m/z 188.0934. Calcd for $C_8H_{14}O_4N$: (M+H), 188.0923.

5-Amino-4-*O*-(*t*-butyldimethylsilyl)-5-deoxy-2,3-*O*-isopropylidene-L-1,5-ribonolactam (10). To a solution of **9** (1.6 g) in DMF (31 ml) were added imidazole (3.1 g) and *t*-butyldimethylsilyl chloride (6.3 g), and the mixture was stirred at room temperature for 2 h. Addition of water and evaporation of the solvent gave an oil, which was dissolved in chloroform. The solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave a colorless crystalline residue, which was recrystallized from hexane to give colorless crystals of **10** (2.5 g, 98%): Mp 118—119 °C; $[\alpha]_D^{26}$ −17.9° (*c* 0.76, $CHCl_3$); IR (CCl_4) 3220, 3120, 3000, 2960, 2940, 2900, 2870, 1690, 1480, 1470, 1440, 1420, 1410, 1390, 1380, 1305, 1260, 1220, 1175, 1140, 1100, 1070, 1015, 995, 960, 940 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ=0.11 and 0.13 (3H each s, $(CH_3)_2$ of *t*-butyldimethylsilyl), 0.96 (9H s, $(CH_3)_3$ of *t*-butyldimethylsilyl), 1.43 and 1.63 (3H each s, CH_3 of isopropylidene), 3.16 (1H dt, J=12 and 4.6 Hz, H-5), 3.58 (1H ddd, J=12, 9.2 and 2 Hz, H-5′), 4.10 (1H ddd, J=9.2, 4.6, and 2 Hz, H-4), 4.35—4.45 (2H, H-3 and H-2), and 6.59 (1H broad s, NH); MS (FAB, positive) m/z 302 (M^++1), 286, 244, 186, 129, 73, 59. HR-FABMS (positive) Found: m/z 302.1776. Calcd for $C_{14}H_{28}O_4NSi$: (M+H), 302.1787.

5-*N*-(Benzyloxycarbonyl)-4-*O*-(*t*-butyldimethylsilyl)-5-deoxy-2,3-*O*-isopropylidene-L-1,5-ribonolactam (11). To a solution of **10** (8.18 g) in DMF (120 ml) was added NaH (1.96 g), and the mixture was stirred at room temperature. To the resulting mixture was added benzyloxycarbonyl chloride (16 g), and then the mixture was stirred at room temperature for 2 h. After filtration of the inorganic precipitate, the filtrate was neutrallized with acetic acid. Evaporation of the solvent gave an oil, which was dissolved in CH_2Cl_2. The solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate afforded an oil, which was subjected to the column chromatography on silica gel. Elution with a mixture of toluene–acetone (3 : 1) gave a colorless oil of **11** (11.6 g, 98%): $[\alpha]_D^{22}$ −32.1° (*c* 0.98, CCl_4); IR (CCl_4) 2960, 2940, 2900, 2860, 1785, 1730, 1500, 1475, 1470, 1390, 1385, 1370, 1300, 1250, 1210, 1190, 1160, 1110, 1085, 1060, 1025, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ=0.07 and 0.91 (3H each s, $(CH_3)_2$ of *t*-butyldimethylsilyl), 0.85 (9H s,$(CH_3)_3$ of *t*-butyldimethylsilyl), 1.38 and 1.52 (3H each s, CH_3 of isopropylidene), 3.47 (1H dd, J=16 and 5 Hz, H-5), 4.16 (1H dd, J=16 and 6.4 Hz, H-5′), 4.19 (1H overlapped with H-5′, H-4), 4.45 (1H dd, J=8.6 and 3 Hz, H-3), 4.55 (1H d, J=8.6 Hz, H-2), 5.28 and 5.32 (2H ABq, J=12.6 Hz, CH_2 of benzyl), and 7.25—7.50 (5H m, C_6H_5 of benzyl); MS (SIMS) m/z 436 (M^++1), 392, 378, 302, 276, 244, 226, 186, 143, 129. HR-FABMS (positive) Found: m/z 436.2154. Calcd for $C_{22}H_{34}O_6NSi$: (M+H), 436.2155.

(+)-(2*R*,3*R*,4*S*)-5-Benzyloxycarbonylamino-4-(*t*-butyldimethylsilyloxy)-2,3-(isopropylidenedioxy)-1-pentanol (12). To a solution of **11** (900 mg) in ethanol (20 ml) was added sodium borohydride (230 mg) at room temperature, and the mixture was stirred at the same temperature overnight. Addition of ethyl acetate and evaporation of the solvent gave a viscous oil, which was dissolved in CH_2Cl_2. The solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to the column chromatography on silica gel. Elution with a mixture of toluene–acetone (15 : 1) gave colorless crystals of **12** (872 mg, 96%): Mp 164 °C; $[\alpha]_D^{26}$ +21° (*c* 0.77, $CHCl_3$); IR ($CHCl_3$) 3460, 2980, 2960, 2930, 2900, 2870, 1720, 1515, 1475, 1465, 1460, 1380, 1370, 1250 (shoulder), 1145, 1115, 1100, 1080, 1035 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ=0.13 and 0.14 (3H each s, $(CH_3)_2$ of *t*-butyldimethyisilyl), 0.87 (9H s, $(CH_3)_3$ of *t*-butyldimethylsilyl), 1.44 and 1.60 (3H each s, isopropylidene), 2.60 (1H t, J=6.6 Hz, OH), 3.35 (1H dt, J=14 and 6.6 Hz, H-5), 3.47 (1H ddd, J=14, 7 and 6.6 Hz, H-5′), 3.62 (1H quintet, J=6.6 Hz, H-1), 3.72 (1H quintet, J=6.6 Hz, H-1′) 4.05—4.15 (2H, H-3 and H-4), 4.22 (1H q, J=6.6 Hz, H-2), 5.09 and 5.12 (2H ABq, J=12 Hz, CH_2 of benzyl), and 7.27—7.38 (5H m, C_6H_5 of benzyl); MS (SIMS) m/z 440 (M^++1), 382, 338, 306, 264, 248, 187, 131, 129. HR-FABMS (positive) Found: m/z 440.2468. Calcd for $C_{22}H_{38}O_6NSi$: (M+H), 440.2469.

(+)-(2*S*,3*S*,4*R*,5*S*)-*N*-(Benzyloxycarbonyl)-5-*O*-(*t*-butyldimethylsilyl)-3,4-*O*-isopropylidene-2,3,4,5-piperidinetetrol (13a). (a) From **12**. Dimethyl sulfoxide (0.6 ml) was added to the stirred solution of oxalyl dichloride (0.37 ml) in CH_2Cl_2 (5.8 ml) at −60 °C, and the mixture was stirred for 5 min. After addition of a solution of **12** (468 mg) in CH_2Cl_2 (1 ml) at −60 °C within 5 min, the mixture was stirred for 15 min. Addition of triethylamine (2.98 ml) and the mixture was stirred at the same temperature for 10 min, and then the mixture was allowed to warm to room temperature. After being quenched with water, the mixture was extracted with CH_2Cl_2. The extract was washed with NaCl-saturated aqueous solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to the column chromatography on silica gel. Elution with a mixture of toluene–acetone (20 : 1) gave a colorless solid of **13a** (408 mg, 88%). The solid was crystallized from a mixture of ether–hexane (1 : 1) to give colorless crystals of **13a**: Mp 106—107 °C; $[\alpha]_D^{22}$ +11° (*c* 0.83, $CHCl_3$); IR ($CHCl_3$) 3500, 3400, 3000, 2970, 2950, 2920, 2875, 1700, 1500, 1480, 1470, 1460, 1420, 1390, 1380, 1350, 1320, 1265, 1170, 1155, 1110, 1075, 1040, 1010, 960, 920 cm^{-1}; ^1H NMR ($CDCl_3$ 400 MHz, at 40 °C) δ=0.10 and 0.11 (3H each s, $(CH_3)_2$ of *t*-butyldimethylsilyl), 0.91 (9H s, $(CH_3)_3$ of *t*-butyldimethylsilyl), 1.32 and 1.39 (3H each s, isopropylidene), 3.38 (1H dd, J=11 and 6 Hz, H-6_{eq}), 3.44 (1H t, J=11 Hz, H-6_{ax}), 3.65 (1H broad s, OH), 4.37 (1H broad d with a small coupling constant, $J_{2,3}$=ca. 1.5 Hz, J=8 Hz, H-3), 4.41 (1H dd, J=8 and 2 Hz, H-4), 4.48 (1H ddd, J=11, 6, and 2 Hz, H-5), 5.14 and 5.20 (2H ABq, J=13 Hz, CH_2 of benzyl), 5.56 (1H broad s, $J_{1/2}$=ca. 1.5 Hz, H-2), and 7.25—7.38 (5H m, C_6H_5 of benzyl); MS (FAB, positive) m/z 420 (M^++1−H_2O), 376, 318, 286, 228, 129, 91; MS (FAB, negative) m/z 528 (M^+−1+Glycerin), 436 (M^+−1), 346, 246, 214, 183, 138, 110, 94, 71. HR-FABMS (positive) Found: m/z 460.2137. Calcd for $C_{22}H_{35}O_6NSiNa$: (M+Na), 460.2132.

(b) From **11**. To a solution of **11** (44 mg) in dry tetrahydrofuran (1 ml) was added L-selectride (1 mol dm^{-3} solution in THF, 0.12 ml) at −78 °C, and the mixture was allowed to warm to room temperature under stirring. Evaporation of the solvent gave a solid, which was dissolved in CH_2Cl_2. The solution was washed with NaCl-saturated aqueous solution,

dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave a solid. The solid was subjected to the preparative thin-layer chromatography on silica gel developed with a mixture of toluene–acetone (8 : 1) to give a colorless solid of **13a** (38.9 mg, 88%).

(+)-(2*R*,3*S*,4*R*,5*S*)-*N*-(Benzyloxycarbonyl)-5-*O*-(*t*-butyldimethylsilyl)-3,4-*O*-isopropylidene-2,3,4,5-piperidinetetrol (13b). To a solution of **11** (44 mg) in dry tetrahydrofuran (1 ml) was added diisobutylaluminum hydride (1 mol dm^{-3} solution in toluene, 0.12 ml) at −78 °C, and the mixture was allowed to warm to room temperature under stirring. Addition of NH_4Cl-saturated aqueous solution and evaporation of the solvent gave a solid, which was dissolved in CH_2Cl_2. The solution was washed with NaCl-saturated aqueous solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil. The oil was subjected to the preparative thin-layer chromatography on silica gel developed with a mixture of toluene–acetone (8 : 1) to give a colorless oil of **13b** (38.5 mg, 87%): $[\alpha]_D^{25}$ −11.7° (*c* 0.83, $CHCl_3$); IR ($CHCl_3$) 3400, 3020, 2970, 2950, 2880, 1710, 1505, 1470, 1425, 1390, 1360, 1345, 1330, 1310, 1295, 1265, 1195, 1170, 1100, 1060, 1020, 980, 960, 920 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz, at 40 °C) δ=0.13 (6H s, $(CH_3)_2$ of *t*-butyldimethylsilyl), 0.91 (9H s, $(CH_3)_3$ of *t*-butyldimethylsilyl), 1.37 and 1.52 (3H each s, isopropylidene), 3.38 (1H very broad s, H-6_{ax}), 3.66 (1H broad d, *J*=13 Hz, H-6_{eq}), 4.20 (1H dd, *J*=8 and 5 Hz, H-3), 4.32 (1H dd, *J*=8 and 4 Hz, H-4 overlapped with H-5), 5.17 (2H s, CH_2 of benzyl), 5.53 (1H d, *J*=5 Hz, H-2), and 7.25—7.5 (5H m, C_6H_5 of benzyl); MS (FAB, positive) *m/z* 420 (M^++1−H_2O), 376, 91, 73, 59; MS (FAB, negative) *m/z* 436 (M^+−1), 346, 306, 199, 138, 94, 46. HR-FABMS (positive) Found: *m/z* 460.2131. Calcd for $C_{22}H_{35}O_6NSiNa$: (M+Na), 460.2132.

(+)-(2*S*,3*R*,4*R*,5*S*)-*N*-(Benzyloxycarbonyl)-5-*O*-(*t*-butyldimethylsilyl)-3,4-*O*-isopropylidene-2-phthalimido-3,4.5-piperidinetriol (14). To the mixture of **13a** (1.2 g), triphenylphosphine (2.87 g) and phthalimide (1.61 g) in DMF (40 ml) was added dropwise diethyl azodicarboxylate under stirring, and the resulting mixture was stirred at room temperature overnight. Addition of water and evaporation of the solvent gave an oil, which was dissolved in ether. The solution was washed with water, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give a viscous solid, which was subjected to the column chromatography on silica gel. Elution with a mixture of toluene–acetone (10 : 1) gave a colorless solid of **14** (1.57 g, 100%). The solid was crystallized from a mixture of ether-hexane to give colorless crystals of **14**: Mp 129—130 °C; $[\alpha]_D^{26}$ +56.4° (*c* 0.72, CH_3OH); IR (KBr) 3000, 2950, 2930, 2900, 2860, 1775, 1720, 1700, 1500, 1475, 1460, 1420, 1395, 1385, 1355, 1335, 1320, 1255, 1245, 1215, 1200, 1165, 1135, 1115, 1100, 1080, 1040, 1020, 990, 970, 935, 920, 910 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ=0.13 and 0.86 (3H each s, $(CH_3)_2$ of *t*-butyldimethylsilyl), 0.89 (9H s, $(CH_3)_3$ of *t*-butyldimethylsilyl),1.33 and 1.50 (3H each s, isopropylidene), 3.35 (1H dd, *J*=13 and 2 Hz, H-6_{eq}), 4.00 (1H dd, *J*=13 and 7 Hz, H-6_{ax}), 4.32 (1H ddd, *J*=7, 4, and 2 Hz, H-5), 4.36 (1H dd, *J*=7 and 4 Hz, H-4), 4.69 (1H dd, *J*=7 and 5 Hz, H-3), 4.94 and 5.13 (2H ABq, *J*=12 Hz, CH_2 of benzyl), 5.92 (1H d, *J*=5 Hz, H-1), 7.16 (5H broad s, C_6H_5 of benzyl), and 7.65—7.8 (4H m, C_6H_4 of phthalimido); MS (FAB, positive) *m/z* 567 (M^++1), 420. HR-FABMS (positive) Found: *m/z* 567.2528. Calcd for $C_{30}H_{39}O_7N_2Si$: (M+H), 567.2526.

(−)-(2*S*,3*R*,4*R*,5*S*)-2-Acetamido-*N*-(benzyloxycarbonyl)-5-*O*-(t-butyldimethylsilyl)-3,4-*O*-isopropylidene-3,4,5-piperidinetriol (15). Compound **14** (460 mg) was dissolved in 1 mol dm^{-3} solution of hydrazine in methanol (20 ml), and the mixture was stirred at 30 °C for 1 d. Filtration of the resulting precipitate and evaporation of the filtrate gave a viscous solid. The residue was taken up in ether, and the solution was evaporated to give an oil. The oil was dissolved in pyridine (5 ml), and to the mixture was added acetic anhydride (0.7 ml), and then the mixture was allowed to stand at room temperature overnight. Addition of water and evaporation of the solvent gave an oil, which was dissolved in CH_2Cl_2. The solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to the column chromatography on silica gel. Elution with a mixture of toluene–acetone (10 : 1) gave a colorless oil of **15** (377 mg, 100%): $[\alpha]_D^{24}$ −46.1° (*c* 0.83, CCl_4); IR (CCl_4) 2960, 2940, 2900, 2870, 1700, 1520, 1500, 1475, 1470, 1455, 1420, 1390, 1380, 1315, 1260, 1215, 1190, 1175, 1160, 1125, 1070, 1010, 975, 950, 920 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ=0.09 and 0.10 (3H each s, $(CH_3)_2$ of *t*-butyldimethylsilyl), 0.89 (9H s, $(CH_3)_3$ of *t*-butyldimethylsilyl), 3.38 (1H t, *J*=11 Hz, H-6_{ax}), 3.50 (1H dd, *J*=11 and 5 Hz, H-6_{eq}), 4.04(1H ddd, *J*=11, 5, and 3.6 Hz, H-5), 4.38 (1H dd, *J*=7 and 3.6 Hz, H-4), 4.57 (1H dd, *J*=7 and 4 Hz, H-3), 5.15 (2H broad t, *J*=13 Hz, CH_2 of benzyl), 5.44 (1H broad s, H-2), and 7.25—7.4 (5H m, C_6H_5 of benzyl); MS (FAB, positive) *mlz* 479 (M^++1), 437, 420, 376, 345, 286, 228, 171, 129. HR-FABMS (positive) Found: *m/z* 479.2599. Calcd for $C_{24}H_{39}O_6N_2Si$: (M+H), 479.2578.

(−)-(2*S*,3*R*,4*S*,5*S*)-2-Acetamido-*N*-(benzyloxycarbonyl)-3,4-*O*-isopropylidene-3,4,5-piperidinetriol (16). To a solution of **15** (360 mg) in THF (18 ml) was added tetrabutylammonium fluoride (1 mol dm^{-3} solution in THF, 2.32 ml), and the mixture was allowed to stand at room temperature for 1 h. Evaporation of the solvent gave an oil, which was dissolved in CH_2Cl_2. The solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to the column chromatography on silica gel. Elution with a mixture of toluene–acetone (1 : 1) gave a colorless foam of **16** (283 mg, 100%): $[\alpha]_D^{26}$ −20.4° (*c* 0.74, $CHCl_3$): IR ($CHCl_3$) 3500, 3450, 3325, 3000, 2950, 1690, 1530, 1500, 1460, 1415, 1390, 1380, 1345, 1330, 1310, 1280, 1260, 1175, 1145, 1090, 1075, 1050, 1000, 975, 950, 900 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, at 40 °C) δ=1.36 and 1.47 (3H each s, isopropylidene), 1.91 (3H s, $COCH_3$), 2.36 (1H d, *J*=10 Hz, OH), 3.20 (1H t, *J*=11 Hz, H-6_{ax}), 3.66 (1H dd, *J*=11 and 5 Hz, H-6_{eq}), 3.95 (1H dt, *J*=11 and 5 Hz, H-5), 4.51 (1H dd, *J*=7 and 5 Hz, H-4), 4.70 (1H broad d with a small coupling (*J*=ca. 1.5 Hz), *J*=7 Hz, H-3), 5.12 and 5.20 (2H ABq, *J*=12 Hz, CH_2 of benzyl), 5.61 (1H broad s, H-2), and 7.25—7.40 (5H m, C_6H_5 of benzyl); MS (SIMS) *m/z* 365 (M^++1), 306, 262, 172, 117. HR-FABMS (positive) Found: *m/z* 365.1717. Calcd for $C_{18}H_{25}O_6N_2$: (M+H), 365.1713.

(−)-(4*R*,5*R*,6*S*)-6-Acetamido-*N*-(benzyloxycarbonyl)-4,5-(isopropylidenedioxy)-3-piperidinone (17). A solution of RuO_4 in CCl_4 prepared from RuO_2 (100 mg) and $NaIO_4$ (800 mg) in a mixture of H_2O (13 ml) and CCl_4 (14 ml) was added to a solution of **16** (240 mg) in CH_2Cl_2 (15 ml) until appearance of yellow color, and the mixture was stirred at room temperature for 1 h. After being quenched with 2-propanol, the mixture was diluted with CH_2Cl_2. The CH_2Cl_2 solution was washed with H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to the preparative thin-layer chromatography on silica gel developed with a

mixture of chloroform–methanol (10 : 1) to give a colorless oil of **17** (236 mg, 99%): $[\alpha]_D^{22}$ −56° (*c* 0.84, $CHCl_3$); IR ($CHCl_3$) 1740, 1700, 1680, 1520, 1500, 1460, 1420, 1390, 1380, 1320, 1270, 1200, 1165, 1110, 1080, 1040, 990, 950, 920 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, at 40 °C) δ=1.35 and 1.37 (3H each s, isopropylidene), 1.96 (3H s, $COCH_3$), 3.79 (1H broad d, *J*=16.5 Hz, H-6), 4.56 (1H d, *J*=6 Hz, H-4 or H-3 overlapped with H-6′), 4.72 (1H d, *J*=6 Hz, H-3 or H-4), 5.19 (2H broad s, CH_2 of benzyl), 5.53 (1H broad s, H-2), and 7.25—7.45 (5H m, C_6H_5 of benzyl); MS (FAB, positive) *m/z* 363 (M^++1), 319, 305, 292, 278, 260, 220, 170, 142, 113. HR-FABMS (positive) Found: *m/z* 363.1550. Calcd for $C_{18}H_{23}O_6N_2$: (M+H), 363.1556.

(+)-(2*S*,3*R*,4*R*,5*S*)-2-Acetamido-*N*-(benzyloxycarbonyl)-3,4-*O*-isopropylidene-5-(nitromethyl)-3,4,5-piperidinetriol (18). To a solution of **17** (1.16 g) in a mixture of dry 1,2-dimethoxyethane (8 ml) and nitromethane (4 ml) was added NaH (100 mg) at −20 °C, and the mixture was stirred at room temperature for 2 h. After being quenched with acetic acid, the mixture was diluted with $CHCl_3$. The solution was washed with $NaHCO_3$-saturated aqueous solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to the column chromatography on silica gel. Elution with a mixture of chloroform–methanol (20 : 1) gave a colorless foam of **18** (1.35 g, 99.6%): $[\alpha]_D^{22}$ +26° (*c* 0.9, $CHCl_3$): IR ($CHCl_3$) 3530, 3410, 3000, 2950, 1710, 1680, 1560, 1510, 1460, 1420, 1390, 1385, 1360, 1330, 1310, 1270, 1210, 1170, 1140, 1120, 1075, 980, 915 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, at 40 °C) δ=1.30 and 1.43 (3H each s, isopropylidene), 1.86 (3H s, $COCH_3$), 3.27 (1H d, *J*=13 Hz, H-6), 3.82 (1H d, *J*=13 Hz, H-6′), 4.23 (1H d with a small coupling, *J*=7 Hz, H-4), 4.45 and 4.66 (2H ABq, *J*=13 Hz, CH_2NO_2), 4.48 (1H broad d, *J*=7 Hz H-3), 5.10 and 5.24 (2H ABq, *J*=13 Hz, CH_2 of benzyl), 6.20 (1H broad s, H-2), 6.96 (1H broad d, *J*=9 Hz, –NHCO–), and 7.2—7.5 (5H m, C_6H_5 of benzyl); ^{13}C NMR ($CDCl_3$, 100 MHz) δ=169.00 (–C(O)–O–), 155.79 (–NCO–), 136.04, 128.47, 128.06, and 127.81 (phenyl), 109.65 ($(CH_3)\underline{C}$), 78.78 ($\underline{C}H_2NO_2$), 75.28 (C-4 or C-3), 73.01 (C-3 or C-4), 71.35 (C-5), 67.51 ($\underline{C}H_2$-Ph), 57.01 (C-2), 43.69 (C-6), 26.30 and 23.78 ($(\underline{C}H_3)_2$-C), and 23.20 ($NHCO\underline{C}H_3$); MS (SIMS) *m/z* 424 (M^++1), 365, 321, 274, 231, 174. HR-FABMS (positive) Found: *m/z* 424.1713. Calcd for $C_{19}H_{26}O_8N_3$: (M+H), 424.1720.

(+)-(2*S*,3*R*,4*S*)-2-Acetamido-*N*-(benzyloxycarbonyl)-5,6-didehydro-3,4-*O*-isopropylidene-5-(nitromethyl)-3,4-piperidinediol (19). To a solution of **18** (94 mg) in dry acetic anhydride (1.5 ml) was added *p*-toluenesulfonic acid (70 mg), and the mixture was stirred at room temperature overnight. Evaporation of the solvent gave an oil, which was dissolved in CH_2Cl_2. The solution was washed with $NaHCO_3$-saturated aqueous solution and NaCl-saturated aqueous solution, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give an oil. To a solution of this residue in dry benzene (3 ml) was added K_2CO_3 (39 mg), and the mixture was stirred at room temperature overnight. Evaporation of the solvent yielded an oil, which was taken in CH_2Cl_2. The solution was washed with NaCl-saturated aqueous solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate afforded an oil, which was subjected to the column chromatography on silica gel. Elution with a mixture of chloroform–methanol (20 : 1) gave a colorless foam of **19** (89 mg, 99%); $[\alpha]_D^{22}$ +69° (*c* 0.73, $CHCl_3$); IR ($CHCl_3$) 3570, 3460, 3300, 3000, 2950, 1735, 1685, 1560, 1505, 1460, 1430, 1410, 1385, 1380, 1370, 1330, 1305, 1290, 1275, 1240, 1200, 1170, 1120, 1085, 1040, 1010, 915 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ=1.31 and 1.37 (3H each s, isopropylidene), 1.95 (3H s, $COCH_3$), 4.47 (1H broad dd, *J*=6 and 2 Hz, H-3), 4.71 (1H d, *J*=6 Hz, H-4), 4.78 and 5.16 (2H ABq, *J*=15 Hz, CH_2NO_2), 5.21 and 5.32 (2H ABq, *J*=12 Hz, CH_2 of benzyl), 5.87 (1H broad d, *J*=8 Hz, NHCO), 6.35 (1H very broad s, H-2), 7.06 (1H s, H-6), and 7.3—7.45 (5H m, C_6H_5 of benzyl); MS (SIMS) *m/z* 406 (M^++1), 359, 315, 257, 245, 225. HR-FABMS (positive) Found: *m/z* 406.1623. Calcd for $C_{19}H_{24}O_7N_3$: (M+H), 406.1615.

(−)-(4*S*,5*R*,6*S*)-6-Acetamido-*N*-(benzyloxycarbonyl)-2,3-didehydro-4,5-(isopropylidenedioxy)-3-piperidinecarboxylic Acid (21) and Its Methoxyethoxymethyl Ester (22). Compound **19** (250 mg) was dissolved in pyridine (7 ml), and the solution was stirred at 38 °C for 1 week. Evaporation of the solvent gave an oil. This residue was roughly purified by the column chromatography on silica gel eluted with a mixture of chloroform–methanol (20 : 1) to give a crude product of **20** (189 mg). This crude product **20** (189 mg) was dissolved in a mixture of 2-methyl-2-propanol (2.9 ml) and 2-methyl-2-butene (0.475 ml), and to the mixture was added a solution of a mixture of $NaClO_2$ (456 mg) and NaH_2PO_4 (584 mg) in H_2O (2.9 ml). Then the mixture was vigorously stirred at room temperature for 1 d. After addition of CH_2Cl_2 (30 ml), the mixture was washed with NaCl-saturated aqueous solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate yielded a solid. A part of the solid was purified by the preparative thin-layer chromatography on silica gel developed with chloroform–methanol (10 : 1). The residue was served to the next step without purification. The residue was dissolved in dry CH_2Cl_2 (12 ml), and to the solution was added *N,N*-diisopropylethylamine (*i*-Pr_2NEt, 0.38 ml) and methoxyethoxymethyl chloride (MEMCl, 0.22 ml), and then the mixture was stirred at room temperature for 1 h. The mixture was washed with NaCl-saturated aqueous solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate afforded a solid, which was subjected to the column chromatography on silica gel. Elution with a mixture of chloroform–methanol (20 : 1) gave colorless crystals of **22** (162 mg, 55%).

21: Mp 188—190 °C (decomp); $[\alpha]_D^{21}$ −6.1° (*c* 0.18, CH_3OH); IR (KBr) 3425, 3050, 3000, 1730, 1665, 1570, 1470, 1440, 1400, 1350, 1320, 1290, 1250, 1180, 1150, 1120, 1090, 1030, 1000, 960, 920, 900 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ=1.36 and 1.42 (3H each s, isopropylidene), 1.90 (3H each s, $COCH_3$), 4.51 (1H dd, *J*=6 and 2.8 Hz, H-3), 5.04 (1H d, *J*=6 Hz, H-4), 5.25 and 5.41 (2H ABq, *J*=12 Hz, CH_2 of benzyl), 6.17 (1H d, *J*=2.8 Hz, H-2), 7.4—7.5 (5H m, C_6H_5 of benzyl), and 7.68 (1H d, *J*=ca. 1 Hz, H-6); MS (SIMS) *m/z* 413 (M^++Na), 391 (M^++1), 373, 333, 230, 140. HR-FABMS (negative) Found: *m/z* 389.1355. Calcd for $C_{19}H_{21}O_7N_2$: (M−H), 389.1349.

22: Mp 194—198 °C (decomp); $[\alpha]_D^{22}$ −4.1° (*c* 0.86, CH_3OH); IR ($CHCl_3$) 3000, 2950, 1740, 1710, 1650, 1500, 1460, 1405, 1390, 1380, 1330, 1290, 1280, 1220, 1170, 1100, 1090, 1060, 1030, 1010, 980, 960, 930 (shoulder) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ=1.32 and 1.39 (3H each s, isopropylidene), 1.92 (3H s, $COCH_3$), 3.35 (3H s, OCH_3, 3.53 and 3.79 (2H each t, *J*=5 Hz, $-OCH_2CH_2O-$), 4.46 (1H dd, *J*=6 and 3 Hz, H-3), 4.97 (1H d, *J*=6 Hz, H-4), 5.23 and 5.34 (2H ABq, *J*=12 Hz, CH_2 of benzyl), 5.35 and 5.47 (2H ABq, *J*=6 Hz, $-OCH_2O-$), 6.23 (1H d ,*J*=8 Hz, NHCO), 6.31 (1H broad d, *J*=8 Hz, H-2), 7.3—7.4 (5H m, C_6H_5 of benzyl), and 8.03 (1H s, H-6); ^{13}C NMR ($CDCl_3$ 100 MHz) δ=169.32 ($NH\underline{C}OCH_3$), 165.39 (C(O)OMEM), 152.52 ($NH\underline{C}(O)OCH_2$-Ph), 135.11 (C-6),

134.85, 128.65 and 128.36 (C_6H_5), 110.21 ($(CH_3)_2\underline{C}(O)_2$–), 108.88 (C-5), 89.14 (–OCH_2O–), 73.77 (C-3), 71.50 and 69.53 (–OCH_2CH_2O–), 69.19 ($\underline{C}H_2C_6H_5$), 67.14 (C-4), 58.98 (OCH_3), 58.48 (C-2), 27.88 and 26.44 ($(\underline{C}H_3)_2C(O)_2$–), and 22.96 ($NCO\underline{C}H_3$); MS (SIMS) *m/z* 501 ($M^+$+Na), 479 ($M^+$+1), 421, 373, 318, 269, 228, 181, 139, 122.

(+)-(2*S*,3*R*,4*S*,5*R*)-2-Acetamido-*N*-(benzyloxycarbonyl)-5-(hydroxymethyl)-3,4-*O*-isopropylidene-3,4-piperidinediol (23). To a solution of **22** (105 mg) in a mixture of tetrahydrofuran–2,2,2-trifluoroethanol (10:1, 10 ml) was added $NaBH_4$ (120 mg), and the mixture was stirred at room temperature for 3 h. After addition of H_2O, the mixture was extracted with CH_2Cl_2. The extract was washed with NaCl-saturated aqueous solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil. The oil was subjected to the preparative thin-layer chromatography on silica gel developed with a mixture of chloroform–methanol (15:1) to give a colorless foam of **23** (63 mg, 76%); $[\alpha]_D^{22}$ +7.0° (*c* 0.93, $CHCl_3$); IR (KBr) 3450, 3000, 2950, 2900, 1695, 1660 (shoulder), 1540, 1420, 1380, 1330, 1215, 1170, 1145, 1120, 1070, 1005, 990, 965 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ=1.34 and 1.43 (3H each s, isopropylidene), 1.94 (3H s, $NHCOCH_3$), 2.09 (1H broad m, H-5), 3.25 (1H t, *J*=13 Hz, H-6_{ax}), 3.59 (1H dd, *J*=13 and 5 Hz, H-6_{eq}), 3.76 and 3.78 (1H each s, $-CH_2OH$), 4.54 (1H dd, *J*=7 and 3 Hz, H-4), 4.64 (1H very broad s, H-3), 5.16 and 5.22 (2H ABq overlapped with H-2, *J*=12 Hz, CH_2 of benzyl and H-2), and 7.25—7.4 (5H m, C_6H_5 of benzyl); MS (SIMS) *m/z* 379 (M^++1), 320, 276, 218, 186, 170, 128. HR-FABMS (positive) Found: *m/z* 379.1841. Calcd for $C_{19}H_{27}O_6N_2$: (M+H), 379.1869.

***N*-(Benzyloxycarbonyl)-4,5-*O*-isopropylidenesiastatin B (24).** To a solution of **23** (45 mg) in DMF (0.45 ml) was added pyridinium dichromate (161 mg), and the mixture was stirred at room temperature overnight. After addition of H_2O, the mixture was extracted with ethyl acetate. The extract was washed with H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to the preparative thin-layer chromatography on silica gel to give a solid of **24** (35 mg, 75%). The solid was crystallized from a mixture of ethyl acetate and ether to yield colorless crystals of **24**: Mp 197—198 °C; $[\alpha]_D^{21}$ +24.7° (*c* 0.71, CH_3OH); IR (KBr) 3450, 3080, 3000, 2950, 1700 (shoulder), 1600, 1450, 1400, 1330, 1260, 1215, 1170, 1150, 1070, 1000, 960 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ=1.30 and 1.33 (3H each s, isopropylidene), 1.92 (3H s, $COCH_3$), 2.82 (1H ddd, *J*=13, 5.5, and 2.6 Hz, H-3), 3.59 (1H broad m, H-2_{ax}), 3.65 (1H dd, *J*=13 and 5.5 Hz, H-2_{eq}), 4.45 (1H broad d, *J*=8 Hz, H-5), 4.82 (1H dd, *J*=8 and 2.6 Hz, H-4), 5.13 and 5.20 (2H ABq, *J*=12 Hz, CH_2 of benzyl), 5.82 (1H broad s, H-6), and 7.2—7.45 (5H m, C_6H_5 of benzyl); MS (SIMS) *m/z* 415 (M^++Na), 334, 290, 244, 137. HR-FABMS (negative) Found: *m/z* 391.1495. Calcd for $C_{19}H_{23}O_7N_2$: (M–H), 391.1505.

4,5-*O*-Isopropylidenesiastatin B (25). A solution of **24** (26 mg) in methanol (5 ml) was hydrogenated at room temperature in the presence of 5% palladium on carbon under atmosphere of hydrogen for 1 h. Filtration of the catalyst and evaporation of the filtrate gave a solid of **25** (16.3 mg, 95%). The solid was crystallized from a mixture of ether and methanol to afford colorless crystals of **25**: Mp 159—160 °C (decomp); $[\alpha]_D^{19}$ +11.1° (*c* 0.67, CH_3OH); IR (KBr) 3440, 3000, 2940, 1660, 1590, 1450 (shoulder), 1410, 1390 (shoulder), 1320, 1245, 1230, 1180, 1160, 1115, 1085, 1070, 1020, 985, 950 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ=1.41 and 1.51 (3H each s, isopropylidene), 2.03 (3H s, $COCH_3$), 2.78 (1H dt, *J*=13 and 5 Hz, H-3), 2.92 (1H t, *J*=13 Hz, H-2_{ax}), 3.02 (1H dd, *J*=13 and 5 Hz, H-2_{eq}), 3.97 (1H dd, *J*=9 and 5 Hz, H-5), 4.45 (1H d, *J*=9 Hz, H-6), and 4.47 (1H t, *J*=5 Hz, H-4); MS (FAB, positive) *m/z* 259 (M^++1), 207, 115, 75, 57, 45; MS (FAB, negative) *m/z* 257 (M^+–1) 198, 151, 59. HR-FABMS (negative) Found: *m/z* 257.1138. Calcd for $C_{11}H_{17}O_5N_2$: (M–H), 257.1137.

Siastatin B (1). A solution of **25** (15 mg) in 1 M (1 M=1 mol dm^{-3}) hydrochloric acid (0.5 ml) was allowed to stand at room temperature for 1 h. Evaporation of the solvent gave crystals of HCl salt of **1**, which were subjected to the column chromatography on Dowex 50W-X4 (H^+). Elution with 0.5 M aqueous ammonia gave colorless solids of **1** (7.8 mg, 94%). The solid was crystallized from a mixture of methanol and water to yield colorless crystals of **1**: Mp 135—136 °C (decomp) (lit,[3] 137 °C); $[\alpha]_D^{20}$ +53° (*c* 0.25, H_2O) (lit,[3] +57.2°); IR (KBr) 3450, 3000, 2950, 1660, 1590, 1450, 1405, 1390 (shoulder), 1320, 1300 (shoulder), 1245, 1230, 1180, 1160, 1115, 1085, 1070, 1050 (shoulder), 1020, 985, 950 cm^{-1}; ^1H NMR (D_2O with a drop of 1 M HCl (pH ca. 1.0), 400 MHz) δ=2.11 (3H s, $COCH_3$), 3.14 (1H ddd, *J*=10, 8 and 3 Hz, H-3), 3.45—3.55 (2H m, H-2_{ax} and H-2_{eq}), 4.03 (1H dd, *J*=11 and 3 Hz, H-5), 4.58 (1H t, *J*=3 Hz, H-4), 5.07 (1H d, *J*=11 Hz, H-6); ^{13}C NMR (D_2O with a drop of 1 M HCl (pH ca. 1.0), 100 MHz) δ=176.28 (NHCO), 173.57 (CO_2H), 68.56 (C-4 and C-5), 60.74 (C-6), 43.58 (C-3), 39.35 (C-2), and 22.58 ($OC(O)CH_3$); MS (SIMS) *m/z* 219 (M^++1), 160, 75. HR-FABMS (negative) Found: *m/z* 217.0834. Calcd for $C_8H_{13}O_5N_2$: (M–H), 217.0825.

Enantiomers. The corresponding enantiomers were similarly obtained.

10 enantiomer: Yield 93%; mp 114—115 °C; $[\alpha]_D^{20}$ +21° (*c* 0.21, CH_3OH).

11 enantiomer: Yield 96%; $[\alpha]_D^{22}$ +33.6° (*c* 1.28, CCl_4).

12 enantiomer: Yield 92%; mp 161—162 °C; $[\alpha]_D^{23}$ –20.3° (*c* 0.92, $CHCl_3$).

13a enantiomer: yield 81%; mp 106—107 °C; $[\alpha]_D^{22}$ –10.9° (*c* 0.85, $CHCl_3$).

14 enantiomer: Yield 98%; mp 131—132 °C; $[\alpha]_D^{22}$ –52° (*c* 0.89, CH_3OH).

15 enantiomer: Yield 100%; $[\alpha]_D^{22}$ +48° (c 0.93, CCl_4).

16 enantiomer: Yield 95%; $[\alpha]_D^{22}$ +19.9° (*c* 0.82, $CHCl_3$).

17 enantiomer: Yield 98%; $[\alpha]_D^{21}$ +57.1° (*c* 0.81, $CHCl_3$).

18 enantiomer: Yield 100%; $[\alpha]_D^{25}$ +25.2° (*c* 0.9, $CHCl_3$).

19 enantiomer: Yield 95%; $[\alpha]_D^{22}$ –67.3° (*c* 0.88, $CHCl_3$).

21 enantiomer: Mp 187—189 °C; $[\alpha]_D^{22}$ +6.6° (*c* 0.23, CH_3OH).

22 enantiomer: Yield 60%; mp 194—198 °C (decomp); $[\alpha]_D^{22}$ +4.2° (*c* 0.82, CH_3OH).

23 enantiomer: Yield 81%; $[\alpha]_D^{22}$ –6.5° (*c* 0.75, $CHCl_3$).

24 enantiomer: Yield 70%; mp 197—199 °C; $[\alpha]_D^{21}$ –24.9° (*c* 0.76, CH_3OH).

25 enantiomer: Yield 90%; mp 158—159 °C (decomp); $[\alpha]_D^{21}$ –10.6° (*c* 0.83, CH_3OH).

4: Yield 95%; mp 138—140 °C (decomp); $[\alpha]_D^{21}$ –56° (*c* 0.15, H_2O).

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