TOTALLY SYNTHETIC ANALOGUES OF SIASTATIN B

I. OPTICALLY ACTIVE 2-ACETAMIDOPIPERIDINE DERIVATIVES†

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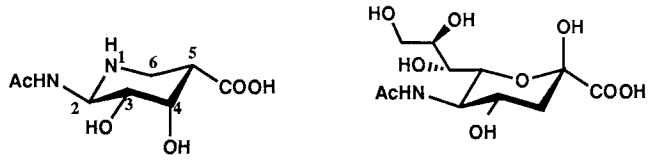
Totally synthetic analogues of siastatin B, optically active 2-acetamido-3,4,5-trihydroxypiperidines having the nitromethyl, aminomethyl and carboxyl branched groups at C-5 have been obtained from D-ribono-1,4-lactone by a stereospecific convergent method. Some analogues showed inhibitory activity against some glycosidases.

Poly- and multifunctional piperidines structurally related to carbohydrates have been isolated from microorganism cultures and plants. All resemble carbohydrate analogues in which the ring oxygen is replaced by nitrogen. Many of them are potent and specific inhibitors for glycosidases from various organisms, and they have many potential applications not only as molecular tools to investigate important biological processes but also as chemicals in medical and agricultural researches.[1] Siastatin B (**1**), the first natural inhibitor of neuraminidase, was isolated from a *Streptomyces* culture by UMEZAWA *et al.*[2] in 1974. It inhibits neuraminidases isolated from microorganisms and animal tissues as well as β-glucuronidase and *N*-acetyl-β-D-glucosaminidase and resembles sialic acid (*N*-acetylneuraminic acid, **2**) (Fig. 1). After achievement of the total synthesis[3,4] of **1**, we were interested in applying its strategy to the syntheses of analogues of **1**. Here, we wish to report the syntheses of 5-branched analogues of **1**, (+) and (−)-2-acetamido-3,4,5-trihydroxypiperidine having the nitromethyl (**3** and **4**), aminomethyl (**5** and **6**) and carboxyl (**7** and **8**) groups at C-5.

Synthesis

To investigate the influence of an additional hydroxyl group at the C-5 position of **1** by analogy with an anomeric center (C-2 position) of **2** and the effect of conversion of functional groups at C-5 on the inhibition of glycosidases and other biological activities, we prepared analogues of **1** modified at C-5. The related strategy of the total synthesis[3,4] of **1** and its antipode was effectively applied to these syntheses.

Fig. 1. Structures of siastatin B (**1**) and *N*-acetylneuraminic acid (**2**).



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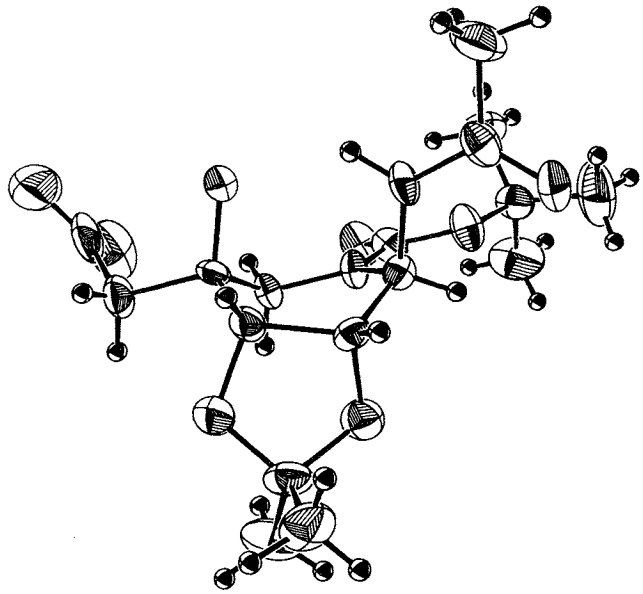
† A part of this paper was presented at the 2nd International Symposium on the Chemical Synthesis of Antbiotics and the Related Microbial Products, Abstracts, I-25, p. 68, Oiso, Japan, 1990.

Fig. 2. X-Ray molecular structure of antipode of **17**.

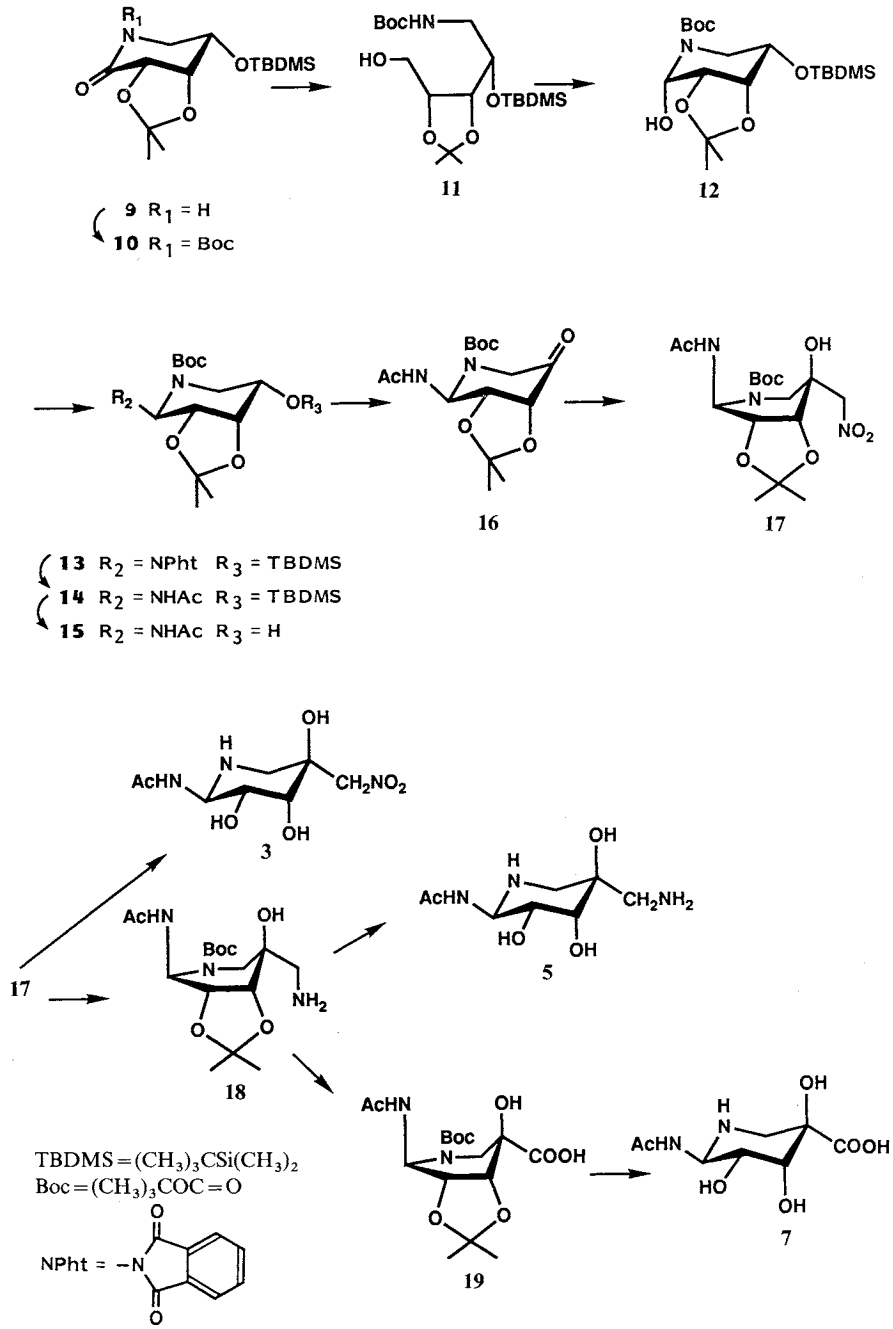


The crucial compound **17** for this synthesis was obtained from 5-amino-4-*O*-(*tert*-butyldimethylsilyl)-5-deoxy-2,3-*O*-isopropylidene-L-ribonolactam (**9**),[3,4] the intermediate for the synthesis of **1** in a straightforward manner. The acid labile *tert*-butoxycarbonyl (Boc) group[5] was employed as the protecting group of imino group of piperidine ring differentiating it from the benzyloxycarbonyl group in the total synthesis. Thus, the protection of the amide group of the lactam **9** with Boc group ($(t\text{-BuOCO})_2O$, NaH, DMF) gave *N*-protected lactam **10** in 96% yield.

Reduction of **10** with sodium borohydride to **11**, and subsequent Swern oxidation[6] afforded the aminal **12** stereospecifically by an anomeric effect[7] in 82% yield. Displacement of the axial hydroxyl group to the equatorial amino group was best achieved by Mitsunobu reaction[8] (PPh_3, diethyl azodicarboxylate, phthalimide) in *N*,*N*-dimethylformamide to give the iminophthalamide **13** in 87% yield. Replacement of the amino substituent in **13** from phthalyl to acetyl (NH_2NH_2, MeOH, then Ac_2O, pyridine) (compound **14**), removal of *tert*-butyldimethylsilyl group with tetrabutylammonium fluoride (compound **15**) and oxidation of the resulting hydroxyl group with ruthenium tetroxide furnished the acetamide ketone **16** in a good yield. Condensation of **16** with nitromethane[9] using sodium hydride in ethylene glycol dimethyl ether proceeded stereospecifically to give the adduct **17** in 69% yield. The stereochemistry at C-5 was proved to be of the *S*-configuration by X-ray crystallographic analysis of the corresponding antipode† (Fig. 2). A small coupling constant ($J = < 2$ Hz) between 2-H and 3-H in the ^1H NMR spectrum supported the half-chair conformation in agreement with the result of X-ray crystallographic analysis. The same stereochemical outcome as that of total synthesis discussed in the previous paper was observed.[4] (+)-(2*R*,3*R*,4*R*,5*S*)-2-Acetamido-3,4,5-trihydroxy-5-(nitromethyl)piperidine (**3**) was directly derived from **17** by removal of the protecting groups with 4 M HCl in dioxane in an excellent yield. Catalytic reduction of **17** with Raney Ni gave the aminomethyl compound **18**, which was converted upon acid treatment

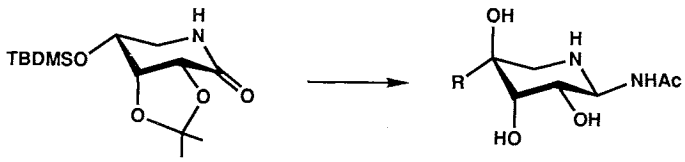
† The X-ray crystallographic analysis was carried out by Mr. YOSHIO KODAMA, Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd.

Scheme 1.



into (+)-(2*R*,3*R*,4*R*,5*R*)-2-acetamido-5-(aminomethyl)-3,4,5-trihydroxypiperidine (**5**) in a good yield. Ninhydrin oxidation[10] of the aminomethyl group in **18** to the aldehyde group and subsequent oxidation with sodium chlorite[11] afforded the carboxylic acid **19** in 23% yield. Removal of the protecting groups in **19** with acid resulted in (+)-(2*R*,3*R*,4*R*,5*R*)-2-acetamido-3,4,5-trihydroxypiperidine-5-carboxylic acid (5-hydroxysiastatin B, **7**) in 65% yield (Scheme 1). Small coupling constants ($J = \sim 2$ Hz) between 2-H

Scheme 2.



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4 R=CH_2NO_2
6 R=CH_2NH_2
8 R=COOH

Table 1. Inhibition (%) of siastatin B (**1**) and its analogues at 100 μg/ml against glycosidases.

Compound	α-Glucosidase (yeast)	β-Glucosidase (almond)	α-Mannosidase (soybean)	β-Glucuronidase (bovine liver)	α-Amylase (porcine pancreas)	β-Amylase (sweet potato)
1	3	24	2	85 (15.5)	0	6
3	89 (2.5)	34	0	3	0	34
4	76 (2.0)	56 (70.0)	0	8	0	15
5	23	32	8	38	0	7
6	0	3	6	24	0	3
7	7	8	9	77 (28.5)	0	0
8	7	6	6	2	0	9

( ): IC_{50}, μg/ml.

and 3-H in the ^1H NMR spectra of **18** and **19** are indicative of their half-chair or C_5^2-conformations caused by the fused isopropylidene and the bulky *N*-Boc groups.

Compounds **4**, **6** and **8** (Scheme 2), the corresponding antipodes of **3**, **5** and **7** were also synthesized from 5-amino-4-*O*-(*tert*-butyldimethylsilyl)-5-deoxy-2,3-*O*-isopropylidene-D-ribonolactam (**20**)[4] by the same sequences mentioned above.

Biological Activities

As shown in Table 1, compounds **3** and **4** both having the 5-nitromethyl group showed inhibitory activity against yeast α-glucosidase, and **4** also showed a weak effect on the inhibition of almond β-glucosidase. 5-Hydroxysiastatin B (**7**) as well as siastatin B (**1**) inhibited β-glucuronidase isolated from bovine liver, but **8** did not inhibit. All analogues affected neither other glycosidases (α-mannosidase from soybean, α-amylase from porcine pancreas, β-amylase from sweet potato) nor sialidases isolated from microorganisms (*Streptococcus* sp., *Arthrobacter ureafaciens* and *Clostridium perfringens*) and A/Aichi/2/68 (H3N2) strain of influenza virus. Siastatin B (**1**) itself had no inhibitory activity against these glycosidases and sialidases isolated from *A. ureafaciens* and A/Aichi/2/68 (H3N2) strain of influenza virus, whereas **1** demonstrated activity against sialidases isolated from *C. perfringens* and *Streptococcus* sp. (IC_{50} 50 and 6.29 μg/ml, respectively). Further evaluation of biological activities of these analogues are in progress.

Experimental

General Methods

Melting points were determined with a Yanagimoto apparatus and were uncorrected. IR spectra were determined on a Hitachi Model 260-10 spectrophotometers. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. ^1H NMR spectra were recorded with Jeol GX-400 and JNM-EX270

spectrometers. Chemical shifts are expressed in δ values (ppm) with tetramethylsilane as an internal standard. Mass spectra were taken by a Hitachi M-80H for secondary ionization and a Jeol SX102 in the FAB mode.

5-(*tert*-Butoxycarbonylamino)-4-*O*-(*tert*-butyldimethylsilyl)-5-deoxy-2,3-*O*-isopropylidene-L-ribonolactam (**10**)

To a solution of 5-amino-4-*O*-(*tert*-butyldimethylsilyl)-5-deoxy-2,3-*O*-isopropylidene-L-ribonolactam (**9**, 7.2 g) in DMF (144 ml) was added NaH (60% in oil, 8.1 g), and the mixture was stirred at room temperature for 2 hours. To the resulting mixture was added di-*tert*-butyl dicarbonate (20.9 g), and then the mixture was stirred at room temperature for 2 hours. After neutralization with acetic acid, evaporation of the solvent gave an oil, which was dissolved in CH_2Cl_2. The solution was washed with $NaHCO_3$-saturated aqueous solution, 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 CH_2Cl_2 gave a colorless solid. The solid was crystallized from hexane to give colorless crystals of **10** (9.2 g, 96%): MP 127~128°C; $[\alpha]_D^{25}$ −36.2° (*c* 0.69, $CHCl_3$); IR (KBr) cm^{-1} 3000 (sh), 2950, 2870, 1730, 1480, 1470, 1390, 1380, 1375, 1320, 1280, 1265, 1220, 1210, 1150, 1120, 1100, 1090, 1010, 990, 980, 920, 910; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.10 (6H, s, $-Si(CH_3)_2$), 0.87 (9H, s, $-SiC(CH_3)_3$), 1.38 and 1.52 (each 3H, s, isopropylidene), 1.51 (9H, s, $COOC(CH_3)_3$), 3.39 (1H, dd, J=13 and 1.4 Hz, 5-H_{eq}), 4.10 (1H, dd, J=13 and 6 Hz, 5-H_{ax}), 4.14 (1H, ddd, J=6, 3 and 1.4 Hz, 4-H), 4.43 (1H, dd, J=8.5 and 3 Hz, 3-H) and 4.51 (1H, d, J=8.5 Hz, 2-H); FAB-MS *m/z* 424 $(M+Na)^+$, 402 $(M+H)^+$, 386, 346, 302, 244, 73, 57.

(2*R*,3*R*,4*S*)-5-(*tert*-Butoxycarbonylamino)-4-*O*-(*tert*-butyldimethylsilyl)-2,3-*O*-isopropylidene-pentane-1,2,3,4-tetraol (**11**)

To a solution of **10** (11.26 g) in EtOH (280 ml) was added sodium borohydride (11.6 g) at room temperature, and the mixture was stirred overnight. Addition of EtOAc 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. The oil was purified by Kugelrohr distillation to afford a colorless oil (**11**) (10.9 g, 96%); $[\alpha]_D^{25}$ +13.1° (*c* 1.16, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3470, 3000, 2970, 2950, 2910, 2880, 1720, 1520, 1485, 1470, 1400, 1390, 1380, 1260, 1180, 1130, 1110, 1090, 1050, 950; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.14 (6H, s, $-Si(CH_3)_2$), 0.34 (3H, s, CH_3 of isopropylidene), 0.91 (9H, s, $-SiC(CH_3)_3$), 1.43 (12H, s, $COOC(CH_3)_3$ and CH_3 of isopropylidene), 3.32 (2H, br t, J=6.2 Hz, 5-H), 3.39 (1H, br t, J=6.2 Hz, OH), 3.59, 3.72 (each 1H, dt, J=12 and 6.2 Hz, 1-H), 4.08 (1H, t, J=6.2 Hz, 3-H), 4.12 (1H, br q, J=6.2 Hz, 4-H), 4.20 (1H, q, J=6.2 Hz, 2-H) and 5.84 (1H, very br t, J=6 Hz, NH); FAB-MS *m/z* 406 $(M+H)^+$, 350, 306, 292, 248, 73, 57, 41.

(2*S*,3*S*,4*R*,5*S*)-*N*-(*tert*-Butoxycarbonyl)-5-(*tert*-butyldimethylsilyloxy)-2,3,4-trihydroxy-3,4-*O*-isopropylidenepiperidine (**12**)

A solution of DMSO (6.6 ml) in CH_2Cl_2 (6.6 ml) was added to the stirred solution of oxalyl chloride (3.9 ml) in CH_2Cl_2 (45 ml) at −78°C, and the mixture was stirred for 2 minutes. After addition of a solution of **11** (6.0 g) in CH_2Cl_2 (30 ml) at −78°C within 5 minutes, the mixture was stirred for 15 minutes. Triethylamine (30.9 ml) was added and the mixture was stirred at the same temperature for 15 minutes, 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 (10:1) gave a colorless solid. The solid was crystallized from hexane and gave colorless crystals of **12** (4.9 g, 82%): MP 86~87°C; $[\alpha]_D^{25}$ +15.4° (*c* 0.68, $CHCl_3$); IR (KBr) cm^{-1} 3475, 3025 (sh), 3000, 2980, 2920, 2880, 1710, 1480, 1410, 1400, 1380, 1350, 1330, 1280, 1260, 1220, 1190, 1170, 1150, 1130, 1100, 1080, 1060, 1040, 1020, 960, 930; ^1H NMR ($CDCl_3$, 400 MHz, 40°C) δ 0.12 and 0.13 (each 3H, s, $-Si(CH_3)_2$), 0.92 (9H, s, $-SiC(CH_3)_3$), 1.34 and 1.44 (each 3H, s, isopropylidene), 1.48 (9H, s, $COOC(CH_3)_3$), 3.30 (1H, br dd, J=11 and 5.6 Hz, 6-H_{eq}), 3.35 (1H, t, J=11 Hz, 6-H_{ax}), 4.36 (1H, dd, J=7 and 1.6 Hz, 3-H), 4.40 (1H, dd, J=7 and 2.5 Hz, 4-H), 4.43 (1H, ddd, J=11, 5.6 and 2.5 Hz, 5-H) and 5.50 (1H, br s, 2-H); FAB-MS *m/z* 426 $(M+Na)^+$, 386, 286, 228, 188, 73, 57, 41.

(2*R*,3*R*,4*R*,5*S*)-*N*-(*tert*-Butoxycarbonyl)-5-(*tert*-butyldimethylsilyloxy)-3,4-dihydroxy-3,4-*O*-isopropylidene-2-phthalimidopiperidine (**13**)

To the mixture of **12** (870 mg), triphenylphosphine (2.26 g) and phthalimide (1.26 g) in DMF (35 ml) was added dropwise diethyl azodicarboxylate with stirring, and the resulting mixure was stirred at room temperature for 3 days. Addition of water and evaporation of the solvent gave an oil, which was dissolved in ether. The solution was washed with NaCl-saturated aqueous solution, 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 (30:1) gave a colorless solid. The solid was crystallized from a mixture of ether-hexane to give colorless crystals of **13** (999 mg, 87%): MP 136~137°C; $[\alpha]_D^{23}$ +54.5° (*c* 1.1, $CHCl_3$); IR (KBr) cm^{-1} 3425, 2975, 2950, 2925, 2880, 2850, 1770, 1720, 1700, 1470, 1460, 1455, 1405, 1390, 1380, 1370, 1350, 1330, 1250, 1210, 1200, 1150, 1125, 1105, 1085, 1065, 1000, 980, 960, 910; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.11 and 0.15 (each 3H, s, $-Si(CH_3)_2$), 0.92 (9H, s, $-SiC(CH_3)_3$), 1.32 (9H, s, $COOC(CH_3)_3$), 1.34 and 1.51 (each 3H, s, isopropylidene), 3.31 (1H, dd, J=13 and 3 Hz, 6-H_{eq}), 3.90 (1H, dd, J=13 and 7 Hz, 6-H_{ax}), 4.31 (1H, ddd, J=7, 4.2 and 3 Hz, 5-H), 4.35 (1H, dd, J=7 and 4.2 Hz, 4-H), 4.61 (1H, dd, J=7 and 4.2 Hz, 3-H), 5.99 (1H, d, J=4.2 Hz, 2-H), 7.72 and 7.84 (each 2H, m, phthalimido); FAB-MS *m/z* 533 $(M+H)^+$, 477, 433, 286, 228, 75, 57.

(2*S*,3*R*,4*R*,5*S*)-2-Acetamido-*N*-(*tert*-butoxycarbonyl)-5-(*tert*-butyldimethylsilyloxy)-3,4-dihydroxy-3,4-*O*-isopropylidenepiperidine (**14**)

To a solution of **13** (2.0 g) in MeOH (100 ml) was added anhydrous hydrazine (4.1 ml), and the mixture was stirred at 30°C overnight. Filtration of the resulting precipitate and evaporation of the filtrate gave a viscous solid. The residue was taken up in ether, and the ethereal solution was evaporated to give an oil. The oil was dissolved in pyridine (16 ml), and to the solution was added acetic anhydride (3.2 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 $CHCl_3$. 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 (6:1) gave a colorless oil of **14** (1.6 g, 94%): $[\alpha]_D^{26}$ +44.8° (*c* 1.0, CH_3OH); IR (KBr) cm^{-1} 3425, 3280, 2970, 2950, 2925, 2890, 2850, 1705, 1645, 1530, 1470, 1460, 1390, 1380, 1370, 1330, 1320, 1250, 1210, 1170, 1120, 1060, 990, 940, 910; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.12 and 0.13 (each 3H, s, $-Si(CH_3)_2$), 0.91 (9H, s, $-SiC(CH_3)_3$), 1.34 and 1.48 (each 3H, s, isopropylidene), 1.46 (9H, s, $COOC(CH_3)_3$), 1.99 (3H, s, $COCH_3$), 3.28 (1H, t, J=12 Hz, 6-H_{ax}), 3.44 (1H, dd, J=12 and 5 Hz, 6-H_{eq}), 4.02 (1H, ddd, J=12.5 and 3.4 Hz, 5-H), 4.38 (1H, dd, J=7 and 3.4 Hz, 4-H), 4.52 (1H, dd, J=7 and 4 Hz, 3-H) and 5.41 (1H, very br s, 2-H); FAB-MS *m/z* 445 $(M+H)^+$, 389, 345, 286, 228, 57.

(2*S*,3*R*,4*S*,5*S*)-2-Acetamido-*N*-(*tert*-butoxycarbonyl)-3,4,5-trihydroxy-3,4-*O*-isopropylidenepiperidine (**15**)

To a solution of **14** (1.57 g) in THF (79 ml) was added tetrabutylammonium fluoride (1 M solution in THF, 9.7 ml), and the mixture was allowed to stand at room temperature for 1.5 hours. Evaporation of the solvent gave an oil, 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, which was subjected to the column chromatography on silica gel. Elution with a mixture of toluene-acetone (1:1) gave a colorless oil of **15** (1.2 g, 99%): $[\alpha]_D^{25}$ +46.5° (*c* 1.0, CH_3OH); IR (KBr) cm^{-1} 3425, 3300 (sh), 2970, 2925, 1700, 1680, 1650, 1530, 1470, 1450, 1390, 1370, 1340, 1320, 1250, 1210, 1170, 1145, 1090, 1060, 990, 940; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.37, 1.49 (each 3H, s, isopropylidene), 1.46 (9H, s, $COOC(CH_3)_3$), 1.98 (3H, s, $COCH_3$), 2.43 (1H, br d, J=9.6 Hz, 5-OH), 3.08 (1H, t, J=11.6 Hz, 6-H_{ax}), 3.60 (1H, dd, J=11.6 and 4 Hz, 6-H_{eq}), 3.92 (1H, br m, 5-H), 4.50 (1H, dd, J=6.9 and 4 Hz, 4-H), 4.67 (1H, dd, J=6.9 and 2 Hz, 3-H), 5.61 (1H, br s, 2-H); FAB-MS *m/z* 331 $(M+H)^+$, 275, 216, 172, 114, 57.

(2*S*,3*R*,4*R*,5*S*)-2-Acetamido-*N*-(*tert*-butoxycarbonyl)-3,4,5-trihydroxy-3,4-*O*-isopropylidene-5-(nitromethyl)piperidine (**17**)

A solution of RuO_4 prepared from RuO_2 (484 mg) and $NaIO_4$ (3.9 g) in a mixture of H_2O (72 ml) and CCl_4 (72 ml) was added to a solution of **15** (1.15 g) in CH_2Cl_2 (40 ml) until appearance of a yellow

color, and the mixture was stirred at room temperature for 20 minutes. After being quenched with 2-propanol, the mixture was diluted with CH_2Cl_2. The CH_2Cl_2 solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil of **16** (900 mg). To a solution of **16** (900 mg) in a mixture of dry 1,2-dimethyoxyethane (7 ml) and nitromethane (3.5 ml) was added NaH (75 mg) at −20°C, and the mixture was stirred at room temperature for 1 hour. After being quenched with acetic acid, the mixture was diluted with $CHCl_3$. The solution was washed with $NaHCO_3$-saturated aqueous solution and water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave a solid. The solid was crystallized from a mixture of EtOAc and ether to give colorless crystals of **17** (929 mg, 69%): MP 181~183°C (dec); $[\alpha]_D^{26}$ +68.1° (*c* 1.0, CH_3OH); IR (KBr) cm^{-1} 3400, 3225, 2980, 2930, 1710, 1670, 1660, 1560, 1530, 1475, 1440, 1420, 1400, 1390, 1380, 1360, 1320, 1300, 1280, 1260, 1220, 1170, 1140, 1130, 1100, 1070, 1050, 1005, 980, 970, 950, 940; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.31, 1.53 (each 3H, s, isopropylidene), 1.45 (9H, s, $COOC(CH_3)_3$), 1.96 (3H, s, $COCH_3$), 3.17, 3.80 (each 1H, d, J=13.2 Hz, 6-H), 4.19 (1H, dd, J=6.9 and 2.3 Hz, 4-H), 4.47 and 4.70 (2H, ABq, J=13.5 Hz, CH_2NO_2), 4.51 (1H, d, J=6.9 Hz, 3-H), 6.12 (1H, br d, J=8.6 Hz with a small coupling, 2-H), 6.83 (1H, d, J=8.6 Hz, –NHCO–); FAB-MS *m/z* 390 $(M+H)^+$, 334, 275, 231, 173, 84, 57.

(2*R*,3*R*,4*R*,5*S*)-2-Acetamido-3,4,5-trihydroxy-5-(nitromethyl)piperidine (**3**)

Compound **17** (20 mg) was dissolved in 4 M hydrogen chloride in dioxane (0.4 ml), and the mixture was allowed to stand at room temperature for 2.5 hours. Another portion of 4 M hydrogen chloride in dioxane (0.5 ml) was added to the mixture, and then the reaction mixture was stirred at room temperature for 5 hours. Evaporation of the solvent gave a solid. The solid was throughly washed with ether to give a colorless amorphous solid of **3** as the hydrochloride (14.6 mg, 99.5%): $[\alpha]_D^{28}$ +32.9° (*c* 0.6, H_2O); IR (KBr) cm^{-1} 3530, 3360, 3220, 3040, 2920, 1680, 1570, 1535, 1450, 1435, 1420, 1395, 1375, 1340, 1310, 1290, 1260, 1230, 1220, 1180 (sh), 1160, 1140, 1120, 1080, 1060, 995, 980, 920, 910; ^1H NMR (D_2O, 400 MHz) δ 2.12 (3H, s, $COCH_3$), 3.37 (1H, dd, J=12.8 and 1.2 Hz, 6-H_{eq}), 3.58 (1H, d, J=12.8 Hz, 6-H_{ax}), 4.15 (1H, dd, J=2.8 and 1.2 Hz, 4-H), 4.26 (1H, dd, J=11 and 2.8 Hz, 3-H), 4.82 and 4.94 (2H, ABq, J=12.4 Hz, CH_2NO_2) and 5.12 (1H, d, J=11 Hz, 2-H); FAB-MS *m/z* 250 $(M+H)^+$, 231, 215, 75, 57, 45.

(2*S*,3*R*,4*R*,5*R*)-2-Acetamido-5-(aminomethyl)-*N*-(*tert*-butoxycarbonyl)-3,4,5-trihydroxy-3,4-*O*-isopropylidenepiperidine (**18**)

A solution of **17** (400 mg) in MeOH (8 ml) was hydrogenated with Raney Ni under 3.5 kg/cm^2 pressure of hydrogen gas at room temperature for 2 hours. Filtration of the catalyst and evaporation of the filtrate gave a colorless oil of **18** (369 mg, 100%): $[\alpha]_D^{28}$ +52.5° (*c* 1.0, CH_3OH); IR (KBr) cm^{-1} 3420, 3010, 2960, 1680, 1520, 1470, 1410, 1400, 1390, 1320 (sh), 1265 (sh), 1220, 1175, 1145, 1080, 1030, 1000, 970; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.29 and 1.40 (each 3H, s, isopropylidene), 1.45 (9H, s, $COOC(CH_3)_3$), 1.95 (3H, s, $COCH_3$), 2.55 (1H, br d, J=13 Hz, 6-H_{eq}), 3.00 (1H, br d, J=13 Hz, 6-H_{ax}), 3.12, 3.45 (2H, ABq, J=13 Hz, CH_2N), 4.00 (1H, dd, J=7 and 1.8 Hz, 4-H), 4.40 (1H, d with a small coupling, J=7 Hz, 3-H), 4.75 (1H, br s, OH), 6.27 (1H, br d with a small coupling, J=9 Hz, 2-H) and 7.38 (1H, br d, J=9 Hz, –NHCO–); FAB-MS *m/z* 360 $(M+H)^+$, 301, 245, 201, 57.

(2*R*,3*R*,4*R*,5*R*)-2-Acetamido-5-(aminomethyl)-3,4,5-trihydroxypiperidine (**5**)

A solution of **18** (42.3 mg) in a mixture of MeOH (0.43 ml) and 2 M hydrochloric acid (0.43 ml) was allowed to stand at room temperature overnight. After evaporation of the solvent, the residue was dissolved in 3 M hydrochloric acid, and the mixture was allowed to stand at room temperature for 2 hours. Evaporation of the solvent gave a foam, which was subjected to a column chromatography on Dowex 50W-X4 (H^+). Elution with 0.5 M NH_4OH gave a colorless amorphous solid of **5** (21.7 mg, 84%): $[\alpha]_D^{25}$ +4.61° (*c* 0.67, H_2O); IR (KBr) cm^{-1} 3400, 1650 (broad), 1560 (broad), 1460 (broad), 1380, 1320 (broad), 1100 (broad), 1060 (broad), 970, 920; ^1H NMR (D_2O, 400 MHz) δ 2.12 (3H, s, $COCH_3$), 3.25 (1H, dd, J=13.6 and 1.8 Hz, 6-H_{eq}), 3.27, 3.32 (2H, ABq, J=14 Hz, CH_2N), 3.50 (1H, d, J=13.6 Hz, 6-H_{ax}), 4.06 (1H, dd, J=3 and 1.8 Hz, 4-H), 4.29 (1H, dd, J=11 and 3 Hz, 3-H) and 5.11 (1H, d, J=11 Hz, 2-H); FAB-MS *m/z* 220 $(M+H)^+$, 161, 75, 57.

(2*S*,3*R*,4*R*,5*R*)-2-Acetamido-*N*-(*tert*-butoxycarbonyl)-3,4,5-trihydroxy-3,4-*O*-isopropylidenepiperidine-5-carboxylic Acid (**19**)

To a solution of **18** (264 mg) in a mixture of MeOH (13 ml) and water (13 ml) were added ninhydrin (327 mg) and $NaHCO_3$ (154 mg), and the mixture was stirred at room temperature overnight. To the mixture were added another portions of ninhydrin (131 mg) and $NaHCO_3$ (62 mg), and then the mixture was further stirred at room temperature for 5.5 hours. Evaporation of the solvent gave a solid, which was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with NaCl-saturated aqueous solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave a solid, which was dissolved in 2-methyl-2-propanol (3 ml). To the solution were added 2-methyl-2-butene (0.6 ml) and a solution of a mixture of $NaClO_2$ (664 mg) and NaH_2PO_4 (916 mg) in water (3 ml), and then the mixture was stirred at room temperature overnight. After separation of the mixture into 2-methyl-2-propanol and water layers, water layer was extracted with $CHCl_3$. 2-Methyl-2-propanol layer and extracts were combined and evaporated. The resulting oil was subjected to the preparative thin-layer chromatography on silica gel developed with a mixture of $CHCl_3$-MeOH (3:1) to give a colorless amorphous solid of **19** (64 mg, 23%): $[\alpha]_D^{26}$ +24.6° (*c* 1.0, MeOH); IR (KBr) cm^{-1} 3425, 3020, 2970, 1700 (sh), 1640 (sh), 1530, 1480, 1400, 1360, 1340 (sh), 1270, 1230, 1180, 1130, 1080, 1000, 980, 930; ^1H NMR ($CDCl_3$, 400 MHz, 40°C) δ 1.25 and 1.38 (each 3H, s, isopropylidene), 1.42 (9H, s, $COOC(CH_3)_3$), 1.88 (3H, s, $COCH_3$), 3.47 (1H, br d, J=10.5 Hz, 6-H_{eq} or 6-H_{ax}), 3.72 (1H, br d, J=10.5 Hz, 6-H_{ax} or 6-H_{eq}), 4.19 (1H, br s, 4-H), 4.30 (1H, br s, 3-H), 6.10 (1H, d with a small coupling, J=9 Hz, 2-H) and 7.33 (1H, br s, –NHCO–); SI-MS *m/z* 419 ($(M+2Na)^+-1$), 260, 238, 201, 198, 137, 115, 56.

(2*R*,3*R*,4*R*,5*R*)-2-Acetamido-3,4,5-trihydroxypiperidine-5-carboxylic Acid (**7**)

To a solution of **19** (45 mg) in MeOH (0.68 ml) was added 2 M hydrochloric acid (0.68 ml), and the mixture was allowed to stand at room temperature for 2.5 days. Evaporation of the solvent gave a solid. The solid was subjected to preparative thin-layer chromatography on silica gel developed with a mixture of $CHCl_3$-MeOH-conc NH_4OH (20:10:3) to give a colorless amorphous solid of **7** (18.3 mg, 65%): $[\alpha]_D^{24}$ +20.4° (*c* 0.74, H_2O); ^1H NMR (D_2O, 400 MHz) δ 2.05 (3H, s, $COCH_3$), 2.73 (1H, dd, J=14 and 1.2 Hz, 6-H_{eq}), 3.25 (1H, d, J=14 Hz, 6-H_{ax}), 3.84 (1H, dd, J=9.8 and 3.6 Hz, 3-H), 3.98 (1H, dd, J=3.6 and 1.2 Hz, 4-H) and 4.67 (1H, d, J=9.8 Hz, 2-H); FAB-MS *m/z* 257 $(M+Na)^+$, 235 $(M+H)^+$, 207, 115, 75, 57.

Enantiomers

The corresponding enantiomers were similarly prepared.

10 enantiomer: MP 122~125°C; $[\alpha]_D^{22}$ +32.4° (*c* 1.0, $CHCl_3$). **11** enantiomer: $[\alpha]_D^{22}$ −14.1° (*c* 1.0, $CHCl_3$). **12** enantiomer: MP 89~90°C; $[\alpha]_D^{22}$ −15.9° (*c* 1.0, $CHCl_3$). **13** enantiomer: MP 138~140°C; $[\alpha]_D^{24}$ −56.1° (*c* 1.0, MeOH). **14** enantiomer: $[\alpha]_D^{24}$ −43.9° (*c* 1.0, MeOH). **15** enantiomer: $[\alpha]_D^{27}$ −49.0° (*c* 1.0, MeOH). **17** enantiomer: MP 189~192.5°C (dec); $[\alpha]_D^{27}$ −56.9° (*c* 1.0, MeOH). **18** enantiomer: $[\alpha]_D^{24}$ −50.4° (*c* 1.0, MeOH). **19** enantiomer: $[\alpha]_D^{26}$ −25.9° (*c* 0.69, MeOH). **4**: $[\alpha]_D^{29}$ −30.5° (*c* 0.48, H_2O). **6**: $[\alpha]_D^{24}$ −5.1° (*c* 0.75, H_2O). **8**: $[\alpha]_D^{25}$ −18.7° (*c* 0.86, H_2O).

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