TOTALLY SYNTHETIC ANALOGUES OF SIASTATIN B

II. OPTICALLY ACTIVE PIPERIDINE DERIVATIVES HAVING TRIFLUOROACETAMIDE AND HYDROXYACETAMIDE GROUPS AT C-2†

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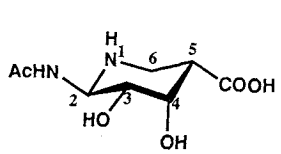
Siastatin B analogues, optically active 2-(trifluoroacetamide)-3,4,5-trihydroxypiperidines having nitromethyl, aminomethyl and carboxyl branched groups at C-5, and (+)-(2*R*,3*R*,4*R*,5*R*)-5-(aminomethyl)-3,4,5-trihydroxy-2-(hydroxyacetamido)piperidine have been obtained total synthetically from D-ribono-1,4-lactone. Some analogues have inhibitory activity against some glycosidases, and (+)-(2*R*,3*R*,4*R*,5*R*)-2-(trifluoroacetamido)-3,4,5-trihydroxypiperidine-5-carboxylic acid showed a marked inhibitory activity against β-glucuronidase.

Siastatin B (**1**), a neuraminidase inhibitor isolated from a *Streptomyces* culture,[1] was totally synthesized from L-ribose and the absolute configuration was related with that of sialic acid (*N*-acetylneuraminic acid, **2**) by us (Fig. 1).[2,3] In our studies on siastatin B analogues, some optically active compounds were synthesized, and (2*R*,3*R*,4*R*,5*S*)-2-acetamido-3,4,5-trihydroxy-5-(nitromethyl)piperidine and its enantiomer inhibiting α-glucosidase and (2*R*,3*R*,4*R*,5*R*)-2-acetamido-3,4,5-trihydroxypiperidine-5-carboxylic acid inhibiting β-glucuronidase were obtained.[4] In this paper, we describe the total syntheses of analogues of **1**, namely, (2*R*,3*R*,4*R*,5*S*)-2-(trifluoroacetamido)-3,4,5-trihydroxypiperidines and their enantiomers having nitromethyl (**3** and **4**), aminomethyl (**5** and **6**) and carboxyl (**7** and **8**) groups at C-5, and (2*R*,3*R*,4*R*,5*R*)-5-(aminomethyl)-3,4,5-trihydroxy-2-(hydroxyacetamido)piperidine (**9**).

Synthesis

In the course of our study[4] of the relationship between structure and biological activity, we were interested in the conversion of the functional group at the C-2 position, affecting the function of the imino group of the piperidine ring. The imino group probably interacts with the glycopyranosyl binding site to inhibit the enzymatic process. Thus, compounds (**3**~**8**) were effectively prepared from (+)-(2*R*,3*R*,4*R*,5*S*)-*N*-(*tert*-butoxycarbonyl)-5-*O*-(*tert*-butyldimethylsilyl)-3,4,5-trihydroxy-3,4-*O*-isopropylidene-2-phthal-

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



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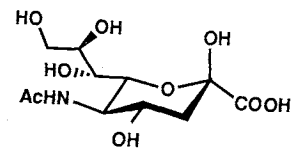

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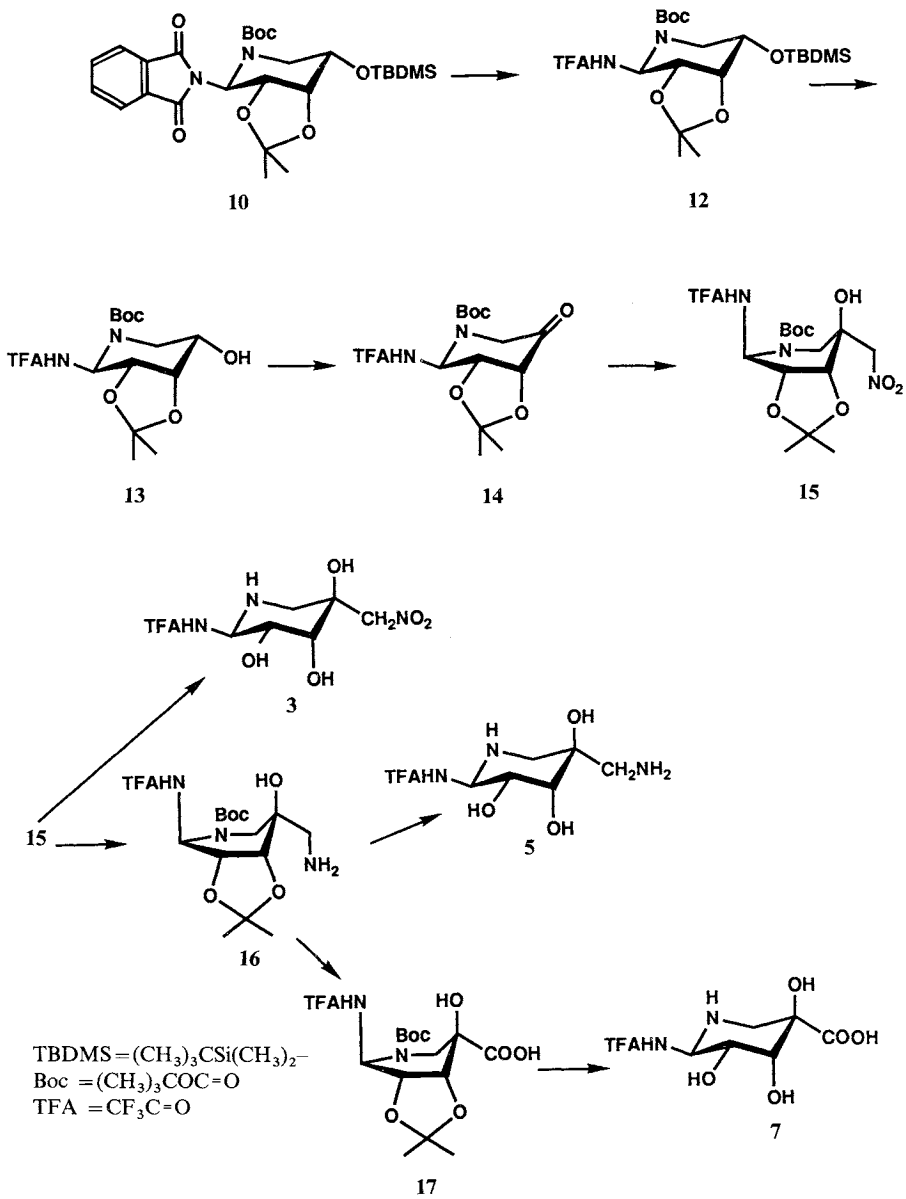
† A part of this paper was presented at the 2nd International Symposium on the Chemical Synthesis of Antibiotics and the Related Microbial Products, Abstracts, I-25, p. 68, Oiso, Japan, 1990.

imidopiperidine (**10**) and its antipode (**11**) by similar reaction sequences to that used for the synthesis of **1**.[2] Treatment of **10** with hydrazine and subsequent treatment with ethyl trifluoroacetate and diisopropylethylamine in *N*,*N*-dimethylformamide (DMF) gave the trifluoroacetamide **12** in 92% yield. Removal of the *tert*-butyldimethylsilyl group with tetrabutylammonium fluoride and oxidation of the resulting hydroxyl group of **13** with ruthenium tetroxide produced ketone **14** in a good yield. Condensation of **14** with nitromethane[5] using sodium hydride in 1,2-dimethoxyethane gave adduct **15** stereospecifically in 74% yield. The *S*-configuration at the C-5 position was assigned by analogy with the stereochemistry of (−)-(2*R*,3*S*,4*S*,5*R*)-2-acetamido-*N*-(*tert*-butoxycarbonyl)-3,4,5-trihydroxy-3,4-*O*-isopropylidene-5-

Scheme 1.

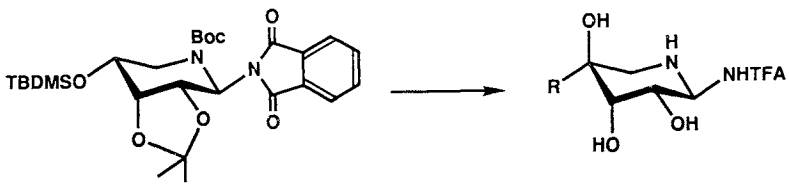


(nitromethyl)piperidine which had been determined by the X-ray crystallographic analysis shown in the preceding paper.[4] A small coupling constant ($J = < 2$ Hz) between 2-H and 3-H in the ^{1}H NMR spectrum is indicative of the half-chair or C_5^2-conformer of **15**. Removal of protecting groups in **15** with 4 M HCl in dioxane afforded (+)-(2*R*,3*R*,4*R*,5*S*)-2-(trifluoroacetamido)-3,4,5-trihydroxy-5-(nitromethyl)piperidine (**3**) in 90% yield. Catalytic reduction of **15** with Raney Ni (compound **16**) followed by removal of protecting groups with acid furnished (+)-(2*R*,3*R*,4*R*,5*R*)-5-(aminomethyl)-2-(trifluoroacetamido)-3,4,5-trihydroxypiperidine (**5**) in a good yield. Ninhydrin oxidation[6] of the aminomethyl group of **16** to the aldehyde group and subsequent oxidation with sodium chlorite[7] gave the carboxylic acid **17**, which was converted upon acid treatment into (+)-(2*R*,3*R*,4*R*,5*R*)-2-(trifluoroacetamido)-3,4,5-trihydroxypiperidine-5-carboxylic acid (**7**) in a good yield (Scheme 1).

The corresponding antipodes (**4**, **6** and **8**) were also obtained from **11** by the same sequence mentioned above (Scheme 2).

On the other hand, **9** was straightforwardly obtained from intermediate **16** (Scheme 3). Protection of the aminomethyl group in **16** with *tert*-butoxycarbonyl group (compound **18**) followed by removal of the *N*-trifluoroacetyl group gave **19** in 80% yield. Coupling of **19** with glycolic acid by use of 1,3-dicyclohexyl-

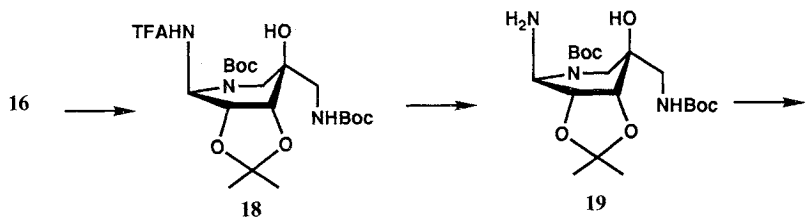
Scheme 2.



11

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

Scheme 3.



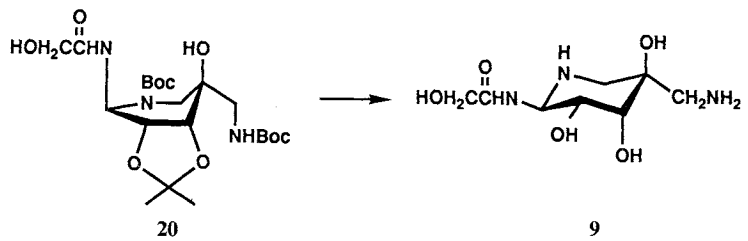


Table 1. Inhibition (%) of siastatin B (**1**) and its analogues at 100 μg/ml against glycosidases.

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	88 (2.2)	58 (60.0)	0	14	0	77 (16.8)
5	90 (1.9)	39	5	13	0	73 (10.0)
6	90 (1.6)	38	1	43	0	70 (16.5)
7	87 (7.7)	22	7	100 (0.02)	0	19
8	81 (5.3)	10	5	74 (37.0)	0	15
9	90 (4.4)	9	0	0	0	45

( ): IC_{50}, μg/ml.

carbodiimide (DCC) afforded *N*-glycolyl compound **20**, which was transformed into (+)-(2*R*,3*R*,4*R*,5*R*)-5-(aminomethyl)-3,4,5-trihydroxy-2-(hydroxyacetamido)piperidine (**9**) in a good yield upon treatment with acid (Scheme 3).

The ^{1}H NMR spectra of compounds **15**, **16**, **17**, **18**, **19** and **20** show small coupling constants ($J = \sim 2$ Hz) between 2-H and 3-H, while those of **3**, **5**, **7** and **9** show large coupling constants ($J = \sim 10$ Hz). These facts indicate that the fused isopropylidene and the bulky *N-tert*-butoxycarbonyl groups of compounds having branched substituents at the C-5 position are responsible for a conformational flip from C_2^5-type to C_5^2-type or half-chair type. Similar results were observed in the previous syntheses.[3,4]

Biological Activities

As shown in Table 1, all analogues tested showed inhibitory activity against α-glucosidase from yeast, and some of them also inhibited β-amylase from sweet potato. Remarkably, **7** affected strongly β-glucuronidase from bovine liver. No analogues showed inhibitory activity against other glycosidases (α-mannosidase from soybean, α-amylase from porcine pancreas) as well as sialidases isolated from microorganisms (*Streptococcus* sp., *Arthrobacter ureafaciens* and *Clostridium perfringens*) and A/Aichi/2/68 (H3N2) strain of influenza virus. Further evaluation of the biological activities of these analogues are under investigation.

Experimental

General Methods

Melting points were determined with a Yanagimoto apparatus and are 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 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.

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

To a solution of **10** (2.6 g) in MeOH (130 ml) was added anhydrous hydrazine (5 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 (2.1 g). The oil was dissolved in DMF (7.1 ml). To the solution were added diisopropylethylamine (7.1 ml) and ethyl trifluoroacetate (3.54 ml), and then 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 NaCl-saturated aqueous solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to column chromatography on silica gel. Elution with mixture of toluene-acetone (30:1) gave **12** as a colorless oil (2.23 g, 91.6%): $[\alpha]_D^{28}$ +39.7° (*c* 0.65, MeOH); IR (KBr) cm^{-1} 3000, 2980, 2950, 2930, 2890, 2850, 1730, 1700 (sh), 1520 (sh), 1470, 1460, 1390, 1385, 1370, 1340 (sh), 1310, 1250, 1200 (sh), 1165, 1120 (sh), 1070, 990, 950, 930, 920 (sh); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.91 (6H, s, $-Si(CH_3)_2$), 1.35, 1.50 (each 3H, s, isopropylidene), 0.47 (9H, s, $-Si(CH_3)_3$), 1.62 (9H, s, $COOC(CH_3)_3$), 3.30 (1H, t, J=11.5 Hz, 6-H_{ax}), 3.45 (1H, dd, J=11.5 and 5 Hz, 6-H_{eq}), 3.99 (1H, ddd, J=11.5, 5 and 3.6 Hz, 5-H), 4.41 (1H, dd, J=7 and 3.6 Hz, 4-H), 4.52, (1H, dd, J=7 and 4 Hz, 3H, 5.45 (1H, br s, 2-H) and 8.00 (1H, s, $-NHCOCF_3$); FAB-MS *m/z* 499 $(M+H)^+$, 443, 386, 286, 170 and 57.

(2*S*,3*R*,4*S*,5*S*)-*N*-(*tert*-Butoxycarbonyl)-2-(trifluoroacetamido)-3,4,5-trihydroxy-3,4-*O*-isopropylidenepiperidine (**13**)

To a solution of **12** (2.2 g) in THF (110 ml) was added tetrabutylammonium fluoride (1 M solution in THF, 55 ml), and the mixture was allowed to stand at room temperature overnight. 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 column chromatography on silica gel. Elution with a mixture of toluene-acetone (3:1) gave **13** as a cololress oil (1.69 g, 100%): $[\alpha]_D^{23}$ +48.2° (*c* 0.27, MeOH); IR (KBr) cm^{-1} 3425, 3025, 2990, 2950, 1740, 1705 (sh), 1540, 1520, 1480, 1460, 1400, 1380, 1360, 1330 (sh), 1310, 1260, 1200 (sh), 1170, 1100 (sh), 980, 950, 900; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.40, 1.52 (each 3H, s, isopropylidene), 1.47 (9H, s, $COOC(CH)_3)_3$), 2.35 (1H, d, J=9.9 Hz, 5-OH), 3.08 (1H, t, J=11.9 Hz, 6-H_{ax}), 3.66 (1H, dd, J=11.9 and 4.6 Hz, 6-H_{eq}), 3.87 (1H, m, 5-H), 4.55 (1H, dd, J=7 and 4 Hz, 4-H), 4.65 (1H, dd, J=7 and 2.3 Hz, 3-H), 5.63 (1H, br s, 2-H); FAB-MS *m/z* 385 $(M+H)^+$, 329, 313, 216, 172 and 57.

(2*S*,3*R*,4*R*,5*S*)-*N*-(*tert*-Butoxycarbonyl)-2-(trifluoroacetamido)-3,4,5-trihydroxy-3,4-*O*-isopropylidene-5-(nitromethyl)piperidine (**15**)

A solution of RuO_4 in CCl_4 prepared from RuO_2 (1.3 g) and $NaIO_4$ (10.2 g) in a mixture of H_2O (190 ml) and CCl_4 (190 ml) was added to a solution of **13** (1.4 g) in CH_2Cl_2 (45 ml) until the appearance of a yellow color, and the mixture was stirred at room temperature for 15 minutes. After being quenched with 2-propanol (4 ml), the mixture was diluted with CH_2Cl_2. The solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave solid **14** (1.3 g). To a solution of **14** (1.3 g) in a mixture of 1,2-dimethoxyethane (13 ml) and nitromethane (6 ml) was added NaH (90 mg) at −20°C, and the mixture was stirred at room temperature for 1.5 hours. After being quenched with acetic acid, the mixture was diluted with $CHCl_3$. The solution was washed with $NaHCO_3$-saturated aqueous solution and then water and dried over $MgSO_4$ and filtered. The filtrate was evaporated to give a solid. The solid was crystallized from a mixture of EtOAc and ether to give **15** as colorless crystals (1.2 g, 74%): MP 184~186°C (dec); $[\alpha]_D^{26}$ +60.6° (*c* 1.0, MeOH); IR (KBr) cm^{-1} 3330, 3225, 2975, 2930, 1740, 1670, 1555, 1530, 1460, 1415, 1380, 1360, 1340, 1320, 1300, 1265, 1215, 1165, 1140, 1110, 1070, 1060, 980, 970, 930, 910; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.33, 1.49 (each 3H, s, isopropylidene), 1.45 (9H, s, $COOC(CH_3)_3$), 3.17, 3.84 (each 1H, br d, J=12.9 Hz, 6-H), 4.21 (1H, dd, J=6.9 and 2 Hz, 4-H), 4.48, 4.70 (2H, ABq, J=13.9 Hz, CH_2NO_2), 4.49 (1H, dd, J=6.9 and 2.3 Hz, 3-H), 4.58 (1H, br s, 5-OH), 6.16 (1H, br d, J=8.6 Hz with a small coupling, 2-H), 7.85 (1H, br d, J=8.6 Hz, −NHCO−); FAB-MS *m/z* 444 $(M+H)^+$, 388, 231 and 57.

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

Compound **15** (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 (0.4 ml) was added to the mixture and then the reaction mixture was further stirred at room temperature overnight. Evaporation of the solvent gave a solid. The solid was throughly washed with ether to give a colorless amorphous solid of **9** as its hydrochloride (13.8 mg, 90%); $[\alpha]_D^{32}$ +22.7° (*c* 0.45, H_2O); IR (KBr) cm^{-1} 3400, 1730, 1640 (sh), 1570, 1470 (sh), 1440, 1400, 1230, 1190, 1110, 1090, 1020, 940, 920; ^1H NMR (D_2O, 400 MHz, 35°C) δ 3.33 (1H, br d with small couplings, J=13.6 Hz, 6-H), 3.54 (1H, dd, J=13.6 and 1.6 Hz, 6-H), 4.16 (1H, br s with small couplings, 4-H), 4.37 (1H, ddd, J=10.4, 3.2 and 1.6 Hz,

3-H), 4.81 and 4.96 (2H, ABq, $J=12.8$ Hz, $-CH_2NO_2$) and 5.14 (1H, dd, $J=10.4$ and 1.2 Hz, 2-H); FAB-MS m/z 304 $(M+H)^+$, 231, 191, 75 and 57.

(2*S*,3*R*,4*R*,5*R*)-5-(Aminomethyl)-*N*-(*tert*-butoxycarbonyl)-2-(trifluoroacetamido)-3,4,5-trihydroxy-3,4-*O*-isopropylidenepiperidine (**16**)

A solution of **15** (815 mg) in MeOH (16 ml) was hydrogenated at room temperature in the presence of Raney Ni under 3.5 kg/cm^2 pressure of hydrogen gas for 1 hour. Filtration of the catalyst and evaporation of the filtrate gave **16** as a cololress oil (747 mg, 98%): $[\alpha]_D^{28}$ +65.8° (*c* 1.0, MeOH); IR (KBr) cm^{-1} 3360, 3010, 2970, 1750, 1710, 1550, 1470, 1410, 1400, 1390 (sh), 1370, 1340, 1320, 1290, 1280, 1180, 1070, 990, 940; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.32, 1.43 (each 3H, s, isopropylidene), 1.45 (9H, s, $COOC(CH_3)_3$), 2.56, 3.00 (2H, ABq, $J=13$ Hz, $-CH_2NO_2$), 3.13 (1H, d, $J=13$ Hz, 6-H), 3.48 (1H, dd, $J=13$ and 1.8 Hz, 6-H), 4.03 (1H, dd, $J=7$ and 1.8 Hz, 4-H), 4.44 (1H, dd, $J=7$ and 2.2 Hz, 3-H), 4.97 (1H, br s, OH), 6.15 (1H, d with a small coupling, $J=10$ Hz, 2-H) and 8.58 (1H, br d, $J=10$ Hz, –NHCO–); FAB-MS m/z 414 $(M+H)^+$, 358, 301, 245, 201 and 57.

(2*R*,3*R*,4*R*,5*R*)-5-(Aminomethyl)-2-(trifluoroacetamido)-3,4,5-trihydroxypiperidine (**5**)

The procedure used for the preparation of **5** was similar to that used for the preparation of **3** from **15**; the yield of amorphous solid **5** hydrochloride was 98%: $[\alpha]_D^{24}$ +17.5° (*c* 0.55, H_2O); IR (KBr) cm^{-1} 3420, 3250 (sh), 3050, 2910, 1740, 1620, 1570, 1500, 1470, 1460, 1230, 1180, 1080, 1010, 950, 920; ^{1}H NMR (D_2O, 400 MHz) δ 3.25 (1H, dd, $J=14$ and 1.6 Hz, 6-H_{eq}), 3.26, 3.33 (2H, ABq, $J=14.4$ Hz, 5-CH_2), 3.51 (1H, d, $J=14$ Hz, 6-H_{ax}), 4.07 (1H, dd, $J=2.8$ and 1.6 Hz, 4-H), 4.37 (1H, dd, $J=10.8$ and 2.8 Hz, 3-H) and 5.18 (1H, d, $J=10.8$ Hz, 2-H); SI-MS m/z 274 $(M+H)^+$, 215, 201, 161, 75, 57 and 45.

(2*S*,3*R*,4*R*,5*R*)-*N*-(*tert*-Butoxycarbonyl)-2-(trifluoroacetamido)-3,4,5-trihydroxy-3,4-*O*-isopropylidenepiperidine-5-carboxylic Acid (**17**)

To a solution of **16** (747 mg) in a mixture of MeOH (37 ml) and water (37 ml) were added ninhydrin (965 mg) and $NaHCO_3$ (437 mg), and the mixture was stirred at room temperature overnight. 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 (8 ml). To the solution were added 2-methyl-2-butene (1.6 ml) and a solution of a mixture of $NaClO_2$ (1.54 g) and NaH_2PO_4 (2.14 g) in water (8 ml), and then the mixture was stirred at room temperature overnight. After separation of the mixture into organic and water layers, the water layer was extracted with $CHCl_3$. The organic layer and extract were combined and evaporated. The resulting oil was subjected to preparative thin-layer chromatography on silica gel, developing with a mixture of $CHCl_3$ - MeOH (3 : 1), to give **17** as a colorless amorphous solid (332 mg, 43%): $[\alpha]_D^{27}$ +36.6° (*c* 1.0, MeOH); IR (KBr) cm^{-1} 3410, 3010, 2970, 1750, 1700 (sh), 1640, (sh), 1540, 1470, 1410, 1400, 1360, 1310, 1270, 1230, 1180, 1070, 990, 930; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.26 (6H, br s, isopropylidene), 1.42 (9H, br s, $COOC(CH_3)_3$), 3.4~3.5, 3.5~3.7 (each 1H, br s, 6-H), 4.23 (1H, br s, 4-H), 4.38 (1H, br s, 3-H), 6.08 (1H, d with a small coupling, $J=8.5$ Hz, 2-H) and 8.3~8.6 (1H, br s, –NHCO–); FAB-MS m/z 451 $(M+Na)^+$, 395, 260, 238, 158, 115 and 57.

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

The procedures used for the preparation of **7** were similar to those used for the preparation of **3** from **15**; the yield amorphous solid **7** hydrochloride was 96%: $[\alpha]_D^{24}$ +19.7° (*c* 0.66, H_2O); IR (KBr) cm^{-1} 3430, 3350, 2950, 2870 (sh), 1750, 1730, 1575, 1450, 1410, 1280, 1240, 1190, 1185 (sh), 1110, 1105, 1100 (sh), 1000, 965, 940, 930; ^{1}H NMR (D_2O, 400MHz) δ 3.38 (1H, d with small couplings, $J=14$ Hz, 6-H_{eq}), 3.80 (1H, d, $J=14$ Hz, 6-H_{ax}), 4.24 (1H, br s with small couplings, 4-H), 4.36 (1H, dd, $J=10$ and 3 Hz, 3-H) and 5.21 (1H, d, $J=10$ Hz, 2-H); SI-MS m/z 289 $(M+H)^+$, 244, 177, 132, 115, 75, 57 and 45.

(2*S*,3*R*,4*R*,5*R*)-*N*-(*tert*-Butoxycarbonyl)-5-(*tert*-butoxycarbonylaminomethyl)-2-(trifluoroacetamido)-3,4,5-trihydroxy-3,4-*O*-isopropylidenepiperidine (**18**)

To a solution of **16** (83 mg) in MeOH (1.7 ml) were added diisopropylethylamine (0.12 ml) and di-*tert*-butyldicarbonate (0.138 ml), and the mixture was stirred at room temperature for 6 hours.

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 column chromatography on silica gel. Elution with a mixture of toluene-acetone (10:1) gave **18** as a colorless oil (94 mg, 91%): $[\alpha]_D^{24}$ +37° (*c* 1.0, MeOH); IR ($CHCl_3$) cm^{-1} 3490, 3380, 3050, 3020, 2970, 1745, 1710, 1540 (sh), 1525, 1410, 1390, 1320, 1280 (sh), 1270, 1180, 1070, 990, 930; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.32, 1.42 (each 3H, s, isopropylidene), 1.45 (9H, s, $COOC(CH_3)_3$), 1.48 (9H, s, $COOC(CH_3)_3$), 3.03 (1H, d, J=13 Hz, 6-H), 3.24, 3.37 (each 1H, dd, J=15 and 6 Hz, 5-CH_2), 3.64 (1H, dd, J=13 and 1.6 Hz, 6-H), 4.11 (1H, dd, J=7 and 1.6 Hz, 4-H), 4.42 (1H, dd, J=7 and 2 Hz, 3-H), 5.02 (1H, br t, J=6 Hz, NHCOO), 6.07 (1H, s, OH), 6.12 (1H, dd, J=9 and 2 Hz, 2-H) and 8.46 (1H, br d, J=9 Hz, $NHCOCF_3$); SI-MS m/z 514 $(M+H)^+$, 458, 402, 302, 245, 201, 75 and 57.

(2*S*,3*R*,4*R*,5*R*)-*N*-(*tert*-Butoxycarbonyl)-5-(*tert*-butoxycarbonylaminomethyl)-3,4,5-trihydroxy-2-(hydroxyacetamido)-3,4-*O*-isopropylidenepiperidine (**20**)

To a solution of **18** (94 mg) in MeOH (3 ml) was added a 7% aqueous solution of K_2CO_3 (5.4 ml), 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 water, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give **19** as an oil (67 mg). The oil was dissolved in DMF (1.2 ml), and to the solution were successively added glycolic acid (44 mg), 1-hydroxybenzotriazole hydrate (66 mg) and DCC (104 mg). Then the mixture was stirred at room temperature overnight. After filtration of the resulting precipitate, evaporation of the filtrate gave an oily residue. The residue was subjected to preparative thin-layer chromatography on silica gel, developing with a mixture of $CHCl_3$-MeOH (6:1), to give **20** as a colorless oil (59 mg, 68%): $[\alpha]_D^{24}$ +28° (*c* 0.4, MeOH); IR ($CHCl_3$) cm^{-1} 3460, 3370, 2990, 2950, 1670, 1515, 1460, 1395, 1390, 1370, 1300, 1270, 1260, 1165, 1095, 1070, 960; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.30, 1.40 (each 3H, s, isopropylidene), 1.44 (9H, s, $COOC(CH_3)_3$), 1.47 (9H, s, $COOC(CH_3)_3$), 3.05 (1H, d, J=13 Hz, 6-H), 3.22 and 3.38 (each 1H, dd, J=15 and 6 Hz, 5-CH_2), 3.60 (1H, br d with a small coupling, J=13 Hz, 6-H), 4.04 (2H, br s, $COCH_2$), 4.09 (1H, dd, J=7.5 and 2.2 Hz, 4-H), 4.42 (1H, br d with a small coupling, J=7.5 Hz, 3-H), 5.04 (1H, br t, J=6 Hz, NHCOO), 5.73 (1H, s, OH), 6.21 (1H, br d with a small coupling, J=9 Hz, 2-H) and 7.86 (1H, br d, J=9 Hz, NHCO); SI-MS m/z 476 $(M+H)^+$, 402, 302, 246, 201 and 57.

(2*R*,3*R*,4*R*,5*R*)-5-(Aminomethyl)-3,4,5-trihydroxy-2-(hydroxyacetamido)piperidine (**9**)

The procedures used for the preparation of **9** were similar to those used for the preparation of **3** from **15**; the yield of amorphous solid **9** hydrochloride was 84.6%: $[\alpha]_D^{28}$ +21.4° (*c* 0.51, H_2O); IR (KBr) cm^{-1} 3400, 3050 (sh), 2910 (sh), 1680, 1630, 1540, 1465, 1230, 1180, 1130, 1080, 990, 970, 920; ^1H NMR (D_2O, 400 MHz) δ 3.26, 3.33 (2H, ABq, J=14 Hz, 5-CH_2), 3.27 (1H, dd, J=13.2 and 1.6 Hz, 6-H_{eq}), 3.52 (1H, d, J=13.2 Hz, 6-H_{ax}), 4.07 (1H, dd, J=2.8 and 1.6 Hz, 4-H), 4.23 (2H, s, $COCH_2$), 4.37 (1H, dd, J=11.2 and 2.8 Hz, 3-H) and 5.19 (1H, d, J=11.2 Hz, 2-H); SI-MS m/z 236 $(M+H)^+$, 201, 161, 75 and 57.

Enantiomers

The corresponding enantiomers were similarly obtained.

12 enantiomer: $[\alpha]_D^{23}$ −42° (*c* 1.0, MeOH). **13** enantiomer: $[\alpha]_D^{24}$ −47° (*c* 1.0, MeOH). **15** enantiomer: MP 192~195°C (dec); $[\alpha]_D^{27}$ −67.9° (*c* 1.0, MeOH). **16** enantiomer: $[\alpha]_D^{23}$ −59.4° (*c* 1.0, MeOH). **17** enantiomer: $[\alpha]_D^{26}$ −36.5° (*c* 0.88, MeOH). **6**: $[\alpha]_D^{26}$ −17.5° (*c* 0.59, H_2O). **8**: $[\alpha]_D^{25}$ −19.6° (*c* 0.7, H_2O).

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