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A Facile Synthesis of D-Glucose-type *gem*-Diamine 1-*N*-Iminosugars: A New Family of Glucosidase Inhibitors

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Abstract—*gem*-Diamine 1-*N* iminosugars of D-glucose-type, a new type of glycosidase inhibitors, have been synthesized from siastatin B, isolated from *Streptomyces* culture. 2-Trifluoroacetamido-1-*N*-iminosugar, (2*S*,3*R*,4*R*,5*R*)-2-trifluoroacetamido-5-hydroxymethylpiperidine-3,4-diol was proved to be a potent inhibitor for α-D- and β-D-glucosidases (IC_{50} 1.9×10^{-7} and 4.2×10^{-7} M, respectively). 2-Acetamido-1-*N*-iminosugar, (2*S*,3*R*,4*R*,5*R*)-2-acetamido-5-hydroxymethylpiperidine-3,4-diol also affected these enzymes (IC_{50} 2.9×10^{-6} and 5.4×10^{-6} M, respectively).

Introduction

The intense interest during the last decade in the chemistry, biochemistry and pharmacology of glycosidase inhibitors has led to many types of natural and synthetic inhibitors aiding in both unraveling of the mechanism of action of glycosidases and development of potential pharmaceuticals such as antiviral, antitumor, antimetastatic, antibacterial, antidiabetic agents, etc.[1–3] Various types of inhibitors have also been designed based on structures that resemble the glycosyl cations in a transition state of hydrolysis by glycosidases.[3,4] In the course of our study on glycosidase inhibitors, we proposed a new type of glycosidase inhibitors, *gem*-diamine 1-*N*-iminosugars (**1**) modeled on natural siastatin B (**3**) in which an anomeric carbon is replaced by a nitrogen atom (Fig. 1 and Scheme 1).[5] The *gem*-diamine 1-*N*-iminosugar (**1**) is likely to mimic a chair-like glycopyranosyl cation **4** of the presumed transient intermediates present in the glycosidic bond-cleaving reaction (Fig. 2).[6] The *gem*-diamine 1-*N*-iminosugars, particularly 2-trifluoroacetamido-1-*N*-iminosugars (**2**) have been proved to be very potent and specific glycosidase inhibitors.[7–9] Some *gem*-diamine 1-*N*-iminosugars of uronic acid-type have also shown the inhibition of invasion of metastatic tumor cells through reconstituted basement membrane and the potent suppression of experimental and spontaneous pulmonary metastasis in mice.[10] On the other hand, our need for specific glucosidase inhibitors arose in connection with projects on antitumor metastasis, anti-AIDS and anti-diabetes studies. The observation that some glycosidase inhibitors show antitumor metastasis[11] and anti-HIV activities,[12] and are also clinically useful for treatment of diabetes[13] prompted us to extend our study on *gem*-diamine 1-*N*-iminosugars to the synthesis of D-glucose-type 1-*N*-iminosugar inhibitors (**6**, **7**) against glucosidases (Fig. 3).

Results and Discussion

The configurations of siastatin B (**3**)[14] readily available from *Streptomyces* culture correspond to those of D-galactose as 1-*N*-iminosugar, and are also the correct configurations of D-glucose except for a configuration at C-4 position. Therefore, **3** was chosen as a starting material of the facile synthesis of **6** and **7** by epimerization at C-4 position. The synthesis of **6** and **7** was begun with the known derivatives **8**[15] and **9**,[16] respectively, obtained from **3**. Protection of hydroxyl groups with *tert*-butyldimethylsilyl (TBDMS) group in **9** gave the desired 4-hydroxy derivative **10** and the 3-hydroxy derivative **11** in 58 and 31% yield, respectively. An attempt of the direct epimerization of the hydroxyl group at C-4 by the Mitsunobu reaction[17] using several acids was unsuccessful. Attention was then directed to two-step epimerization, oxidation of the hydroxyl group and reduction of the resulting ketone. Oxidation of **10** with Dess–Martin periodinane[18] afforded the ketone **12** in 97% yield. Stereoselective reduction of **12** was best achieved with $LiBH_4$ in CH_3CN to yield the desired *gluco*-isomer **13** and the starting **10** in 89 and 8%, respectively. Simultaneous removal of both TBDMS and *tert*-butyloxycarbonyl groups of **13** with 4 M hydrogen chloride in dioxane resulted in the desired D-glucose-type 2-trifluoroacetamido-1-*N*-iminosugar **6** in 91% yield. The D-glucose-type 2-acetamido-1-*N*-imino-

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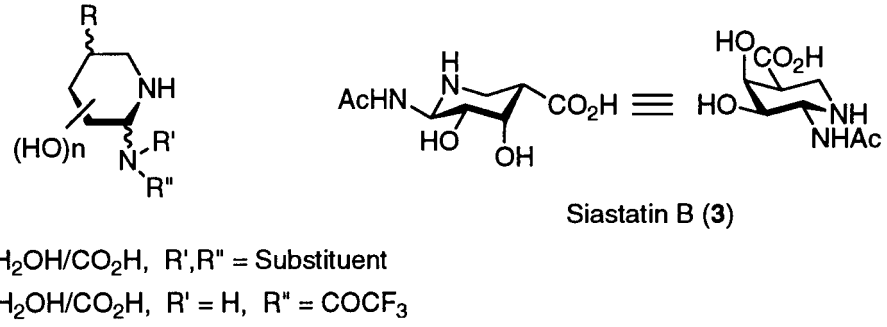
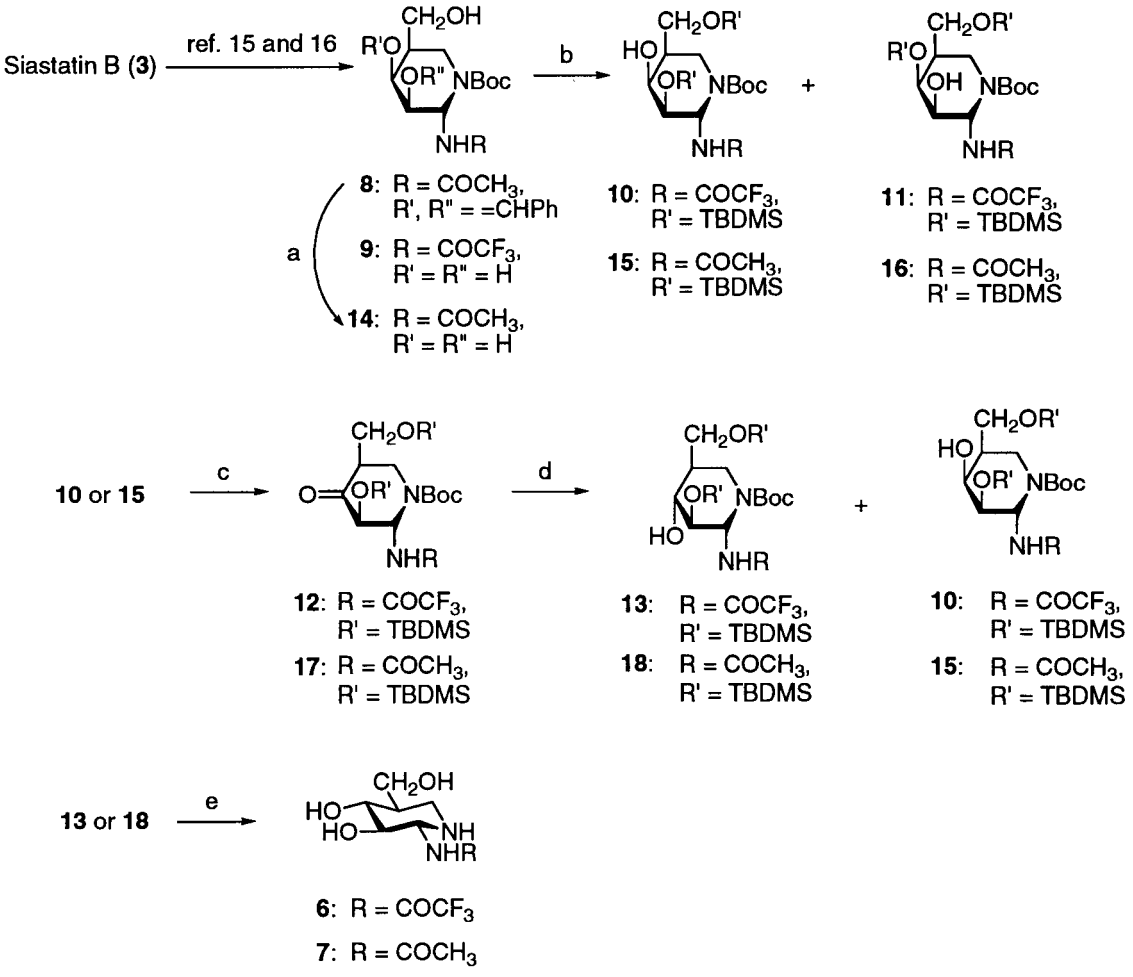


Figure 1.



Scheme 1. (a) H_2, 10% Pd/C, MeOH, 94% (b) TBDMSCI, imidazole, DMF, rt, (**9**→**10**, 58%; **14**→**15**, 50%) (c) Dess-Martin periodinane, CH_2Cl_2, rt, (**10**→**12**, 97%; **15**→**17**, 98%) (d) $LiBH_4$, CH_3CN, −50 °C, (**12**→**13**, 88%; **17**→**18**, 74%) (e) 4M HCl/dioxane, rt, (**13**→**6**, 91%; **18**→**7**, 80%).

sugar **7** was similarly synthesized starting from **14** obtained by hydrogenolysis of **8**[15] in a good yield.

The inhibitory activities of D-glucose-type *gem*-diamine 1-*N*-iminosugars (**6** and **7**) for α-glucosidase (baker's yeast), β-glucosidase (almonds), α- and β-galactosidases (*Aspergillus niger*), α-mannosidase (jack beans), β-mannosidase (snail), α-*N*-acetylgalactosaminidase (chicken liver), β-*N*-acetylglucosaminidase (bovine epididymis) and β-glucuronidase (bovine liver) are summarized in Table 1. As expected, **6** and **7** affected α-D- and β-D-glucosidases. These results seem to indicate that **6** and **7** may mimic a transient intermediate **4** and a glucopyranoside of grand-state in hydrolysis of α-D- and β-D-glucosidases, respectively. While 2-acetamido-1-*N*-iminosugar **7** bearing structural resemblance to *N*-acetylglucosamine also showed inhibition against β-D-*N*-acetylglucosaminidase, 2-trifluoroacetamido-1-*N*-iminosugar **6** showed no inhibition against this enzyme. Both **6** and **7** had also no inhibitory activities against α-*N*-acetylgalactosaminidase. On the other hand, D-galactose-type *gem*-diamine 1-*N*-iminosugars (**19** and

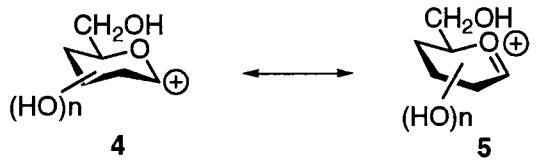


Figure 2. The presumed reaction intermediates in a transition state of D-glycosidase hydrolysis.

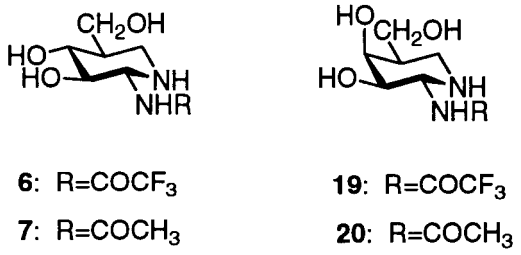


Figure 3. Structures of D-glucose and D-galactose-type *gem*-diamine 1-*N*-iminosugars (**6**, **7**, **19**, and **20**).

20) previously synthesized from **3** are already known to inhibit α- and β-D-galactosidases, β-D-glucosidase, and α-D-*N*-acetylgalactosaminidase at IC_{50} $1.3\times10^{-5}\sim1.6\times10^{-7}$ M.[16] It is also known that **20** affected β-D-*N*-acetylglucosaminidase at IC_{50} 2.1×10^{-6} M, but **19** showed no inhibitory activity against this enzyme at IC_{50} 3.2×10^{-4} M.[16] These results suggest that the axial 4-OH group and the equatorial 2-NHAc group are the main determinant of specificity and potency of the inhibitors against D-*galacto*-type hydrolases and *N*-acetylglucosaminidase, respectively. However, D-*gluco*-type hydrolases and *N*-acetylgalactosaminidase may roughly recognize the configuration of 4-OH group and the kinds of 2-amide group of the inhibitors, respectively. Further evaluation of biological activities (anti-HIV, antimetastatic, antidiabetes, etc.) of these compounds are now in progress.

In summary, a facile synthesis of novel *gem*-diamine 1-*N*-iminosugars of D-glucose-type have been achieved from natural siastatin B. The synthesis presented here should offer a useful approach to *gem*-diamine 1-*N*-iminosugars, promising to be potent glycosidase inhibitors. That these *gem*-diamine 1-*N*-iminosugars are potent inhibitors of D-glucosidases further supports the hypothesis of our design of the new type inhibitor.

Experimental

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 a JEOL JNM EX400 spectrometer. Chemical shifts are expressed in δ values (ppm) with tetramethylsilane as an internal standard. Mass spectra were taken by a JEOL JMS-SX102 in the FAB mode.

Enzyme inhibition assay

Inhibitory activities were assayed using α-glucosidase from baker's yeast,[19] β-glucosidase from almonds,[20] α-mannosidase from jack beans,[21] β-mannosidase from snail,[21] α-galactosidase from *Aspergillus niger*,[22] β-galactosidase from *Aspergillus niger*,[22] β-glucuronidase from bovine liver,[23] α-*N*-acetylgalactosaminidase from chicken liver,[24] and β-*N*-acetylglucosaminidase from bovine epididymis,[25] by methods similar to those described in the references. All enzymes were purchased from Sigma Chemical Company.

(2*S*,3*R*,4*S*,5*R*)-*N*-(*tert*-Butoxycarbonyl)-3-*O*-(*tert*-butyldimethylsilyl)-5-(*tert*-butyldimethylsilyloxy)methyl-2-trifluoroacetamidopiperidine-3,4-diol (10) and (2*S*,3*R*,4*S*,5*R*)-*N*-(*tert*-butoxycarbonyl)-4-*O*-(*tert*-butyldimethylsilyl)-5-(*tert*-butyldimethylsilyloxy)methyl-2-trifluoroacetamidopiperidine-3,4-diol (11). To a solution of **9**[16] (1.3 g, 3.63 mmol) in DMF (25 mL) were added imidazole (1.73 g, 12.7 mmol) and *tert*-butyldimethylsilyl chloride (1.91 g, 12.7 mmol), and the mixture was stirred at room temperature for 16 h. Evaporation of the solvent gave a viscous oil, which was dissolved in ethyl acetate. The solution was washed with saturated aqueous NaCl solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to a column chromatography on silica gel. Elution with *n*-hexane:ethyl acetate (9:1) gave a solid of **10** (1.23 g, 58%) and an oil of **11** (0.66 g, 30%). The former was crystallized from a mixture of toluene and hexane to give colorless needles.

10: $[\alpha]_D^{28}+42.5°$ (*c* 0.96, MeOH); mp 89 °C; IR ($CHCl_3$) ν_{max} 1680 (br, C=O), 1530 (br, C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.06, 0.11 and 0.12 (6H, 3H and 3H, s each, $(CH_3)_2$ of *tert*-butyldimethylsilyl), 0.89 and 0.90 (9H each, s, $(CH_3)_3$ of *tert*-butyldimethylsilyl), 1.47 (9H, s,

Table 1. Inhibitory activities (IC_{50} M) of siastatin B (**3**), **6**, and **7** against glycosidases

Enzyme	3	6	7
α-Glucosidase (baker's yeast)	$>4.6\times10^{-4}$	1.9×10^{-7}	2.9×10^{-6}
β-Glucosidase (almonds)	$>4.6\times10^{-4}$	4.2×10^{-7}	5.4×10^{-6}
α-Galactosidase (*Aspergillus niger*)	$>4.6\times10^{-4}$	$>3.2\times10^{-4}$	$>3.9\times10^{-4}$
β-Galactosidase (*Asoergillus niger*)	$>4.6\times10^{-4}$	1.9×10^{-4}	$>3.9\times10^{-4}$
α-Mannosidase (jack beans)	$>4.6\times10^{-4}$	2.2×10^{-5}	2.5×10^{-4}
β-Mannosidase (snail)	$>4.6\times10^{-4}$	3.2×10^{-6}	3.8×10^{-5}
α-*N*-Ac-Galactosaminidase (chicken liver)	$>4.6\times10^{-4}$	$>3.2\times10^{-4}$	$>3.9\times10^{-4}$
β-*N*-Ac-Glucosaminidase (bovine epididymis)	$>4.6\times10^{-4}$	$>3.2\times10^{-4}$	1.2×10^{-5}
β-Glucuronidase (bovine liver)	7.1×10^{-5}	$>3.2\times10^{-4}$	$>3.9\times10^{-4}$

$COOC(CH_3)_3$), 1.83∼1.93 (1H, m, H-5), 2.69 (1H, br s, -OH), 3.20 (1H, br t, J = 13.7 Hz, H-6ax), 3.63 (1H, dd, J = 10.8 and 7.8 Hz, -CH_2OTBDMS), 3.69 (1H, dd, J = 13.7 and 3.9 Hz, H-6eq), 3.75∼3.82 (2H, m, CH_2 OTBDMS and H-3), 3.98 (1H, br d, J = 2.4 Hz, H-4) and 5.47 (1H, br t, J = 8.8 Hz, H-2); FABMS m/z 587.4 $(M+H)^+$, 531.4, 487.4, 473.3, 374.4, 316.3, 242.3, 186.2, 73.1, 57.1; Anal. $C_{25}H_{49}N_2O_6Si_2F_3$ (C, H, N).

11: $[\alpha]_D^{28}$ +37.6° (*c* 1.0, MeOH); IR ($CHCl_3$) ν_{max} 1680 (br, C=O), 1530 (br, C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.05, 0.06, 0.11 and 0.15 (3H each, s, $(CH_3)_2$ of *tert*-butyldimethylsilyl), 0.90 and 0.95 (9H each, s, $(CH_3)_3$ of *tert*-butyldimethylsilyl), 1.46 (9H, s, $COOC(CH_3)_3$), 1.77∼1.85 (1H, m, H-5), 2.37 (1H, d, J = 7.8 Hz, -OH), 3.22∼3.32 (1H, m, H-6eq), 3.56 (1H, br t, J = 10.7 Hz, H-6ax), 3.58 (2H, dd, J = 5.9 and 2.9 Hz, -CH_2OH), 3.66 (1H, dt, J = 8.2 and 2.9 Hz, H-3), 4.17 (1H, br t, J = 2.9 Hz, H-4) and 5.55 (1H, br t, J = 8.2 Hz, H-2); FABMS m/z 587.4 $(M+H)^+$, 531.4, 487.4, 374.3, 316.3, 286.2, 242.3, 171.2, 73.1, 57.1; Anal. $C_{25}H_{49}N_2O_6Si_2F_3$ (C, H, N).

(2*S*,3*R*,5*R*)-*N*-(*tert*-Butoxycarbonyl)-3-*O*-(*tert*-butyldimethylsilyl)-5-(*tert*-butyldimethylsilyloxy)methyl-4-oxo-2-trifluoroacetamidopiperidine-3-ol (12). To a solution of **10** (190 mg, 0.324 mmol) in CH_2Cl_2 (10 mL) was added Dess–Martin periodinane (275 mg, 0.648 mmol), and the mixture was stirred at room temperature for 1 h. After dilution with $CHCl_3$, the solution was washed with saturated aqueous $NaHCO_3$ and water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to a column chromatography on silica gel. Elution with *n*-hexane:ethyl acetate (7:1) gave **12** (183 mg, 97%) as a solid. Crystallization from a mixture of toluene:*n*-hexane gave colorless needles: $[\alpha]_D^{27}$ +23.3° (*c* 0.7, MeOH); mp 166 °C; IR ($CHCl_3$) ν_{max} 1730 (C=O), 1700 (br, C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.03, 0.06 and 0.12 (3H, 6H and 3H, s each, $(CH_3)_2$ of *tert*-butyldimethylsilyl), 0.89 (18H, s, $(CH_3)_3$ of *tert*-butyldimethylsilyl), 1.47 (9H, s, $COOC(CH_3)_3$), 2.55∼2.63 (1H, m, H-5), 3.63 (1H, dd, J = 10.3 and 9.3 Hz, H-6ax), 3.99 (2H, m, -CH_2OTBDMS and H-6eq), 4.11 (1H, dd, CH_2OTBDMS), 4.74 (1H, d, 7.5 Hz, H-3), 5.17 (1H, br t, J = 7.5 Hz, H-2) and 7.30 (1H, br s, -NHCO-); FABMS m/z 585.5 $(M+H)^+$, 529.4, 471.4, 414.4, 372.4, 358.3, 314.3, 284.3, 226.2, 154.2, 73.1, 57.1; Anal. $C_{25}H_{47}N_2O_6Si_2F_3$ (C, H, N).

(2*S*,3*R*,4*R*,5*R*)-*N*-(*tert*-Butoxycarbonyl)-3-*O*-(*tert*-butyldimethylsilyl)-5-(*tert*-butyldimethylsilyloxy)methyl-2-trifluoroacetamidopiperidine-3,4-diol (13). To a solution of **12** (110 mg, 0.19 mmol) in CH_3CN (3 mL) was added $LiBH_4$ (8.2 mg, 0.38 mmol) at −50 °C, and the mixture was stirred at −50 °C for 1 h. After the reaction was quenched with saturated aqueous NH_4Cl solution, the mixture was diluted with $CHCl_3$. The solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the solvent gave an oil, which was subjected to a column chromatography on silica gel. Elution with *n*-hexane:ethyl acetate gave **13** (96.9 mg, 88%) as an oil: $[\alpha]_D^{25}$ +20° (*c* 1.0, MeOH); IR ($CHCl_3$) ν_{max} 1690 (br, C=O), 1540 (br, C=O); ^1H NMR ($CDCl_3$) δ 0.04, 0.06, 0.10 and 0.14 (3H each, s, $(CH_3)_3$ of *tert*-butyldimethylsilyl), 0.896 and 0.899 (9H each, s, $(CH_3)_3$ of *tert*-butyldimethylsilyl), 1.47 (9H, s, $COOC(CH_3)_3$), 1.83∼1.93 (1H, m, H-5), 2.25 (1H, d, J = 2.9 Hz, -OH), 3.31 (1H, brd, J = 11.7 Hz, H-6), 3.59 (1H, dd, J = 10.0 and 5.1 Hz, CH_2OTBDMS), 3.70∼3.81 (4H, m, CH_2OTBDMS, H-3, H-4 and H-6), 5.88 (1H, brs, H-2) and 8.11 (1H, d, J = 7.3 Hz, -NHCO-); FABMS m/z 587.5 $(M+H)^+$, 531.5, 487.4, 473.3, 374.4, 316.3, 242.3, 155.2, 73.1, 57.1; Anal. $C_{25}H_{49}N_2O_6Si_2F_3$ (C, H, N).

(2*S*,3*R*,4*R*,5*R*)-5-Hydroxymethyl-2-trifluoroacetamidopiperidine-3,4-diol hydrochloride (6). Compound **13** (40 mg, 0.068 mmol) was dissolved in HCl in 1,4-dioxane (4 M, 1.0 mL), and the mixture was kept at room temperature overnight. After addition of Et_2O, the resulting precipitates were taken by centrifugation and washed with Et_2O three times to give **6** (18.2 mg, 91%) as a colorless solid: $[\alpha]_D^{26}$ +35° (*c* 0.5, MeOH); IR (KBr) ν_{max} 1740 (br, C=O), 1570 (br, C=O) cm^{-1}; ^1H NMR (CD_3OD) δ 1.88∼2.00 (1H, m, H-5), 3.11 (1H, br t, J = 13.2 Hz, H-6ax), 3.42 (1H, dd, J = 13.2 and 4.4 Hz, H-6eq), 3.51 (1H, dd, J = 10.3 and 9.3 Hz, H-4), 3.67 (1H, dd, J = 11.2 and 6.4 Hz, -CH_2OH), 3.73 (1H, dd, J = 10.3 and 9.3 Hz, H-3), 3.84 (1H, dd, J = 11.2 and 3.9 Hz, -CH_2OH), 4.84 (1H, d, J = 9.3 Hz, H-2); FABMS m/z 259.1 $(M+H)^+$, 202.2, 154.1, 146.1, 136.1, 128.1, 107, 77.1, 57.1; Anal. $C_8H_{13}N_2O_4F_3 \cdot HCl \cdot H_2O$ (C, H, N); calcd: Cl, 11.34; found: Cl, 11.07.

(2*S*,3*R*,4*S*,5*R*)-2-Acetamido-*N*-(*tert*-butoxycarbonyl)-5-hydroxymethylpiperidine-3,4-diol (14). A solution of **8**[15] (1.2 g, 3.06 mmol) in methanol (120 mL) was hydrogenated at room temperature in the presence of 10% palladium on carbon under atmosphere of hydrogen for 6 h. The catalyst was filtered and evaporation of the filtrate gave a solid, which was subjected to a column chromatography on silica gel. Elution with $CHCl_3$:CH_3OH (3:2) gave **14** (877 mg, 94%) as a solid. It was crystallized from a mixture of ethanol and ether to give colorless needle crystals: $[\alpha]_D^{25}$ +50.5° (*c* 0.49, MeOH); Mp 201° (decomp); IR ($CHCl_3$) ν_{max} 1670 (br, C=O), 1650 (br, C=O), 1540 (br, C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (9H, s, $COOC(CH_3)_3$), 1.91∼1.98 (1H, m, H-5), 1.96 (3H, s, -NHCOCH_3), 3.10 (1H, dd, J = 14.2 and 3.4 Hz, H-6), 3.71 (1H, dd, J = 5.4 and 2.4 Hz, H-3), 3.73 (1H, dd, J = 11.2 and 8.1 Hz, -CH_2OH), 3.80 (1H, dd, J = 11.2 and 3.9 Hz, -CH_2OH), 3.99 (1H, dd, J = 5.4 and 3.2 Hz, H-4), 4.14 (1H, d with a small coupling, J = 14.2 Hz, H-6), 5.99 (1H, d, J = 2.4 Hz, H-2); FABMS m/z 305.3 $(M+H)^+$, 289.2, 249.2, 154.1, 146.2, 136.1, 107.1, 57.1; Anal. $C_{12}H_{24}N_2O_6$ (C, H, N).

(2*S*,3*R*,4*S*,5*R*)-2-Acetamido-*N*-(*tert*-butoxycarbonyl)-3-*O*-(*tert*-butyldimethylsilyl)-5-(*tert*-butyldimethylsilyloxy)-methylpiperidine-3,4-diol (15) and (2*S*,3*R*,4*S*,5*R*)-2-acetamido-*N*-(*tert*-butoxycarbonyl)-4-*O*-(*tert*-butyldimethylsilyl)-5-(*tert*-butyldimethylsilyloxy)methylpiperidine-3,4-diol (16). Compounds **15** and **16** were synthesized similarly as in the preparation of **10** and **11** from **9**.

15: yield, 50%; $[\alpha]_D^{23}$ +37.3° (*c* 1.0, MeOH); IR ($CHCl_3$) ν_{max} 1680 (br, C=O), 1500 (br, C=O) cm^{-1}; ^1H NMR

($CDCl_3$) δ 0.05, 0.10 and 0.14 (6H, 3H and 3H, s each, $(CH_3)_2$ of *tert*-butyldimethylsilyl), 0.89 and 0.94 (9H each, 2s, $(CH_3)_3$ of *tert*-butyldimethylsilyl), 1.46 (9H, s, $COOC(CH_3)_3$), 1.73∼1.82 (1H, m, H-5), 2.04 (1H, s, -NHCOC$\underline{H}_3$), 2.76 (1H, d, J = 6.8 Hz, -OH), 3.28∼3.37 (1H, m, H-6ax), 3.52∼3.63 (4H, m, -CH_2OTBDMS, H-3 and H-6eq), 4.14 (1H, br t, J = 2.9 Hz, H-4), 5.58 (1H, br t, J = 8.1 Hz, H-2), 7.40 (1H, br s, -NHCO-); FABMS m/z 533.3 $(M+H)^+$, 474.3, 374.3, 316.2, 242.2, 171.2, 73.1, 57.1; Anal. $C_{25}H_{52}N_2O_6Si_2$ (C, H, N).

16: yield, 49%; $[\alpha]_D^{27}$ +43.8° (*c* 0.91, MeOH); IR ($CHCl_3$) ν_{max} 1680 (br, C=O), 1500 (br, C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.05, 0.06, 0.12 and 0.17 (3H each, s, $(CH_3)_2$ of *tert*-butyldimethylsilyl), 0.89 and 0.90 (9H each, s, $(CH_3)_3$ of *tert*-butyldimethylsilyl), 1.46 (9H, s, $COOC(CH_3)_3$), 1.88∼1.97 (1H, m, H-5), 1.99 (3H, s, -NHCOC$\underline{H}_3$), 2.84 (1H, d, J = 3 Hz, -OH), 3.39∼3.53 (2H, m, H-6), 3.66 (1H, dd, J = 10.3 and 7.8 Hz, -C$\underline{H}_2$OTBDMS), 3.81 (1H, dd, J = 7.5 and 2.9 Hz, H-3), 3.85 (1H, dd, J = 10.3 and 6.8 Hz, -C$\underline{H}_2$OTBDMS), 3.89∼3.94 (1H, m, H-4), 5.63 (1H, br t, J = 8.0 Hz, H-2), 6.78 (1H, br s, -NHCO-); FABMS m/z 533.3 $(M+H)^+$, 474.3, 374.3, 316.2, 242.2, 186.2, 73.1, 57.1; Anal. $C_{25}H_{52}N_2O_6Si_2$ (C, H, N).

(2*S*,3*R*,5*R*)-2-Acetamido-*N*-(*tert*-butoxycarbonyl)-3-*O*-(*tert*-butyldimethylsilyl)-5-(*tert*-butyldimethylsilyloxy)-methyl-4-oxopiperidine-3-ol (17). Compound **17** was synthesized similarly as in the preparation of **12** from **10**; the yield was 98%: $[\alpha]_D^{28}$ +45° (*c* 0.84, MeOH); IR ($CHCl_3$) ν_{max} 1740 (br, C=O), 1680 (br, C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.06, 0.07 and 0.10 (6H, 3H and 3H, s, $(CH_3)_2$ of *tert*-butyldimethylsilyl), 0.88 and 0.89 (9H each, s, $(CH_3)_3$ of *tert*-butyldimethylsilyl), 1.47 (9H, s, $COOC(CH_3)_3$), 1.98 (3H, s, NHCOC$\underline{H}_3$), 2.53∼2.62 (1H, m, H-5), 3.62 (1H, br t, J = 9.8 Hz, H-6ax), 3.97 (2H, m, H-6eq and C$\underline{H}_2$OTBDMS), 4.08 (1H, dd, J = 13.7 and 4.9 Hz, -CH_2OTBDMS), 4.73 (1H, br s with a small coupling, H-3), 5.14 (1H, br s, H-2), 6.31 (1H, br s, -NHCO-); FABMS m/z 531.4 $(M+H)^+$, 472.3, 416.3, 372.3, 358.2, 314.3, 186.2, 73.1, 57.1; Anal. $C_{25}H_{50}N_2O_6Si_2$ (C, H, N).

(2*S*,3*R*,4*R*,5*R*)-2-Acetamido-*N*-(*tert*-butoxycarbonyl)-3-*O*-(*tert*-butyldimethylsilyl)-5-(*tert*-butyldimethylsilyloxy)-methylpiperidine-3,4-diol (18). Procedures used were similar to those used for the preparation of **13** from **12**; the yield was 74%: $[\alpha]_D^{26}$ +24.2° (*c* 0.73, MeOH); IR ($CHCl_3$) ν_{max} 1680 (br, C=O), 1510 (br, C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.05, 0.06, 0.10 and 0.14 (3H each, s each, $(CH_3)_2$ of *tert*-butyldimethylsilyl), 0.89 (18H, s, $(CH_3)_3$ of *tert*-butyldimethylsilyl), 1.47 (9H, s, $COOC(CH_3)_3$), 1.78∼1.87 (1H, m, H-5), 1.97 (3H, s, -NHCOC$\underline{H}_3$), 2.22 (1H, d, J = 2.4 Hz, -OH), 3.41 (1H, br d, J = 13.2 Hz, H-6), 3.58∼3.69 (1H, m, H-6), 3.61 (1H, dd, J = 10.3 and 5.4 Hz, -C$\underline{H}_2$OTBDMS), 3.69 (1H, br t, J = 4.2 Hz, H-3), 3.75 (2H, m, -C$\underline{H}_2$OTBDMS and H-4), 5.74 (1H, br s, H-2), 7.07 (1H, d, J = 8.3 Hz, NHCO-); FABMS m/z 533.5 $(M+H)^+$, 477.4, 374.3, 316.3, 186.2, 73.1, 57.1; Anal. $C_{25}H_{52}N_2O_6Si_2$ (C, H, N).

(2*S*,3*R*,4*R*,5*R*)-2-Acetamido-5-hydroxymethylpiperidine-3,4-diol (7). Procedures used were similar to those used for the preparation of **6** from **13**; the yield was 80%: $[\alpha]_D^{26}$ +45.4° (*c* 0.5, MeOH); IR (KBr) ν_{max} 1680 (br, C=O), 1550 (br, C=O) cm^{-1}; ^1H NMR (CD_3OD) δ 1.83∼1.97 (1H, m, H-5), 2.06 (1H, s, -NHCOC$\underline{H}_3$), 3.04 (1H, br t, J = 12.9 Hz, H-6ax), 3.36 (1H, dd, J = 12.9 and 4.4 Hz, H-6eq), 3.47 (1H, dd, J = 10.8 and 8.8 Hz, H-4), 3.60 (1H, dd, J = 10.3 and 8.8 Hz, H-3), 3.65 (1H, dd, J = 11.2 and 6.8 Hz, -C$\underline{H}_2$OH), 3.83 (1H, dd, J = 11.2 and 3.4 Hz, -C$\underline{H}_2$OH), 4.71 (1H, d, J = 10.3 Hz, H-2); FABMS m/z 205.2 $(M+H)^+$, 154.1, 146.1, 136.1, 107, 89, 77; Anal. $C_8H_{16}N_2O_4{\cdot}HCl{\cdot}H_2O$ (C, H, N); calcd: Cl, 13.70; found: Cl, 13.38.

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