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Synthesis and Activity of 1-*N*-Iminosugar Inhibitors, Siastatin B Analogues for α-*N*-Acetylgalactosaminidase and β-*N*-Acetylglucosaminidase

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Abstract—*N*-Acetylgalactosamine-based 1-*N*-iminosugars, new types of glycosidase inhibitor have been synthesized by modeling on siastatin B, isolated from a *Streptomyces* culture. The analogues of siastatin B were proved to be potent inhibitors for α-*N*-acetylgalactosaminidase and/or β-*N*-acetylglucosaminidase.

Introduction

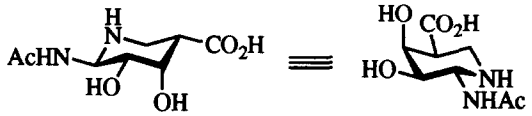
Many glycosidase inhibitors of azasugars have the potential to produce a number of beneficial pharmaceutical effects such as antihyperglycemic, antimetastatic, antifungal, antiviral activities, etc.[1]

A maltifunctional azasugar, siastatin B (**1**) was isolated as an inhibitor of β-glucuronidase as well as *N*-acetylneuraminidase from a *Streptomyces* culture.[2] Over the past several years, we have been interested in the design and synthesis of glycosidase inhibitor modeled on **1**.[3] This is in part due to its structure and shape highly reminiscent of a new type of 1-*N*-iminosugar inhibitor[4] of glycosidase. Compound **1** as a 1-*N*-iminosugar resembles structurally glucuronic acid (**2**) (Fig. 1). This led to the synthesis of highly potent β-glucuronidase inhibitors, (3*S*,4*S*,5*R*,6*R*)-6-(trifluoroacetamido)-4,5-dihydroxypiperidine-3-carboxylic acid (**3**)[3j] and (3*R*,4*R*,5*R*,6*R*)-6-(trifluoroacetamido)-3,4,5-trihydroxypiperidine-3-carboxylic acid (**4**) (Fig. 1).[3d]

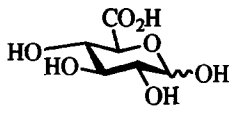
Compounds **3** and **4** were also found to show the inhibition of highly metastatic B16 variant (B16 BL6) and Lewis lung carcinoma (3LL) cell invasion through reconstituted basement membranes and the potent suppression of pulmonary metastasis of B16 BL6 cells in mice.[3j,3k] The other 1-*N*-iminosugar analogues (**5**, **6** and **7**)[3d] were also proved to be the good inhibitors for α-glucosidase (Fig. 2). On the other hand, N-linked oligosaccharides have a β-glycosidic bond between a *N*-acetylglucosamine and the amide nitrogen atom of an asparagine residue, and O-linked oligosaccharides involved an α-glycosidic bond between a *N*-acetylgalactosamine and the hydroxyl group of a serine or threonine residue. The activity of *N*-acetylglucosaminyltransferase V is associated with the metastatic potential of tumour cells.[5] A number of azasugar inhibitors of glycosidase have also been proved to affect glycosyltransferases.[4b,6] Under these circumstances our attention was focused on the synthesis of 1-*N*-iminosugar inhibitors (**8**–**13**) against α-*N*-acetylgalactosaminidase and β-*N*-acetylglucosaminidase described in this paper (Fig. 2).

Results and Discussion

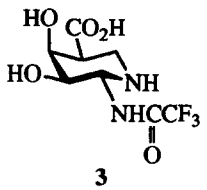
We have designed the inhibitors which feature hydroxyl and acetamide groups, a protonated heteroatom, and a ring conformation to mimic *N*-acetylgalactosamine or the transient intermediate present in the glycosidic bond-cleaving reaction. The hydroxymethyl group was also replaced by azidomethyl, aminomethyl, methylthiomethyl and methylsulfinylmethyl groups to examine the electrostatic effect of substituents at C-5 on affinity for glycosidases. Furthermore, in order to examine



Siastatin B (1)



Glucuronic acid (2)



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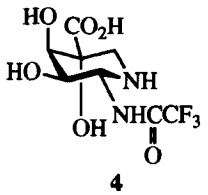

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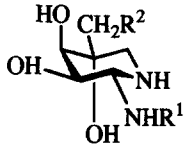
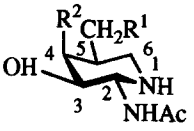
Figure 1.



5: R^1=$COCH_3$, R^2=NO_2
6: R^1=$COCF_3$, R^2=NO_2
7: R^1=$COCF_3$, R^2=NH_2



8: R^1=R^2=OH
9: R^1=N_3, R^2=OH
10: R^1=NH_2, R^2=OH
11: R^1=SCH_3, R^2=OH
12: R^1=$S(O)CH_3$, R^2=OH
13: R^1=OH, R^2=H

Figure 2.

whether *N*-acetylgalactosaminidase recognizes accurately the hydroxyl group at C-4, the hydroxyl group was removed. The hydroxymethyl analogue **8** was obtained by three routes of the total synthesis and the chemical modification of **1** (Scheme 1).

The intermediate **14**[3a,3b] in the total synthesis of **1** from L-ribose was efficiently transformed into **8** by catalytic hydrogenolysis and acid hydrolysis. Siastatin B methyl ester (**15**)[3h] was also converted into **8** by reduction with $NaBH_4$ in an excellent yield. The another synthesis utilized (2*S*,3*R*,4*S*,5*R*)-2-acetamido-*N*-(*tert*-butoxycarbonyl)-5-hydroxymethyl-3,4-*O*-isopropylidene-3,4-piperidinediol (**16**)[3i] easily derived from **1**. Compound **16** was straightforwardly converted to **8** by acid treatment. The azidomethyl analogue **9** was also obtained from **16** by sulfonation, subsequent displacement of sulfonate function with azide group, and acid hydrolysis. Catalytic hydrogenation of **18** and acid hydrolysis afforded the aminomethyl analogue **10**. On the other hand, the sulfonate **17** was transformed into methylsulfinylmethyl analogue **12** via methylthiomethyl analogue **11** through **20** by treatment with sodium thiomethoxide, acid hydrolysis, and oxidation. The 4-deoxy analogue **13**[3h] was also obtained from methyl ester of 4-deoxysiastatin B (**21**) easily derived from **1** by $NaBH_4$ reduction.

The inhibitory activities of synthesized analogues for some glycosidases are summarized in Table 1. As expected, all analogues except for **13** affected α-*N*-acetylgalactosaminidase from chicken liver. However, compounds **10**, **11** and **12** are a weaker inhibitors than **8** and **9**, indicating that a stronger electrostatic group at C-5 sometimes perturbs the binding to the enzyme and results in a weaker complex. The same situation was also observed for β-*N*-acetylglucosaminidase from bovine epididymis. Interestingly, compound **8** is a potent inhibitor not only of α-*N*-acetylgalactosaminidase but also of β-*N*-acetylglucosaminidase, whereas **13** is not effective.

In summary, the groups at the topographically equivalent position to those of 2-NHAc and 4-OH in *N*-acetylgalactosamine are the main determinant of specificity and potency of its inhibitor, while the binding group equivalent to the 4-OH in *N*-acetylglucosamine is not so important for specificity of its inhibitor. The equivalent of the 5-CH_2OH appears to assist in binding of inhibitors into the active site, but it is not essential for activity. That these 1-*N*-iminosugars are potent inhibitors of β-*N*-acetylglucosaminidase and α-*N*-acetylgalactosaminidase further supports the hypothesis of our design of the new type inhibitor.

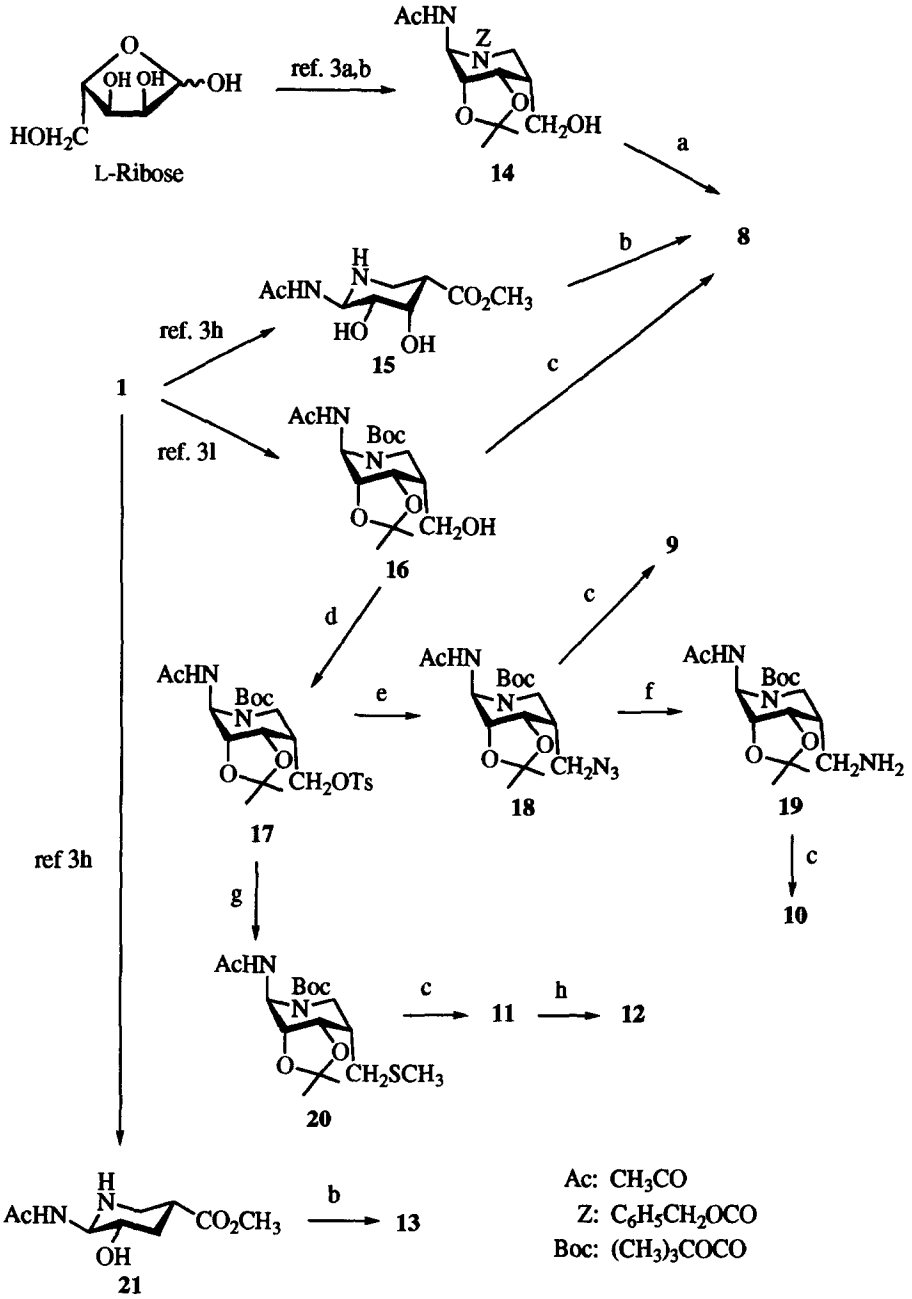
Experimental

IR spectra were determined on a Hitachi Model 260–10 spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. ^1H 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 Type I from baker's yeast,[7] β-glucosidase from almonds,[8] α-mannosidase from Jack beans,[9] β-mannosidase from Snail,[9] α-galactosidase from *Escherichia coli*,[10] β-galactosidase Grade IX from *E. coli*,[10] β-glucuronidase Type B-1 from bovine liver,[11] α-*N*-acetylgalactosaminidase from chicken liver,[12] and β-*N*-acetylglucosaminidase A from bovine epididymis[13], by methods similar to those described in references. All enzymes were purchased from Sigma Chemical Company.

(2*R*,3*R*,4*S*,5*R*)-2-Acetamido-5-hydroxymethylpiperidine-3,4-diol (8). Method A: A solution of **14** (52 mg) in methanol (10 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. The solid was dissolved in 4 M HCl in dioxane (1.5 mL), and the solution was stirred at room temperature for 1 h. Evaporation of the solvent gave crystal-HCl salt of **8**. The crystals were subjected to the preparative thin-layer chromatography on silica gel developed with chloroform:methanol:concd aqueous ammonia (20:10:3) to give a solid. The solid was further subjected to a column chromatography on LH20. Elution with methanol gave **8** (24 mg, 78%) as an amorphous solid; $[\alpha]_D^{25}$ +58.8° (*c* 0.37; CH_3OH); IR (KBr) ν_{max} 3400 (OH), 3270 (OH), 1675 (C=O), 1560 (C=O) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 2.04 (1H, m, 5-H), 2.06 (3H, s, $NCOCH_3$), 3.07–3.14 (2H, m, 6-H), 3.57 (1H, dd, J=10.8 and 7.3 Hz, Ha of —$C\underline{H}_2OH$) and 3.69 (1H, dd, J=10.8 and 6.3 Hz, Hb of —$C\underline{H}_2OH$), 3.72 (1H, dd, J=10.3 and 2.4 Hz, 3-H), 4.09 (1H, t, J=2.4 Hz, 4-H), 4.95 (1H, d, J=10.3 Hz, 2-H); FABMS *m/z* 205 (M+H, 100%), 146 (88), 75 (54), 57 (33), 45 (23); FAB-HRMS calcd for $C_8H_{17}N_2O_4$ (M+H): 205.1188. Found: 205.1193.



Scheme 1. (a) H_2, 5% Pd/C, MeOH; 4 M HCl/dioxane; (b) $NaBH_4$, EtOH; (c) 4 M HCl/dioxane; (d) $C_6H_5SO_2Cl$, Py; (e) NaN_3, DMF; (f) H_2, 5% Pd/C, MeOH–AcOEt; (g) CH_3SNa, DMF; (h) 30% H_2O_2/H_2O.

Table 1. IC_{50} (μg mL^{-1}) of siastatin B (**1**) and its analogues against glycosidases

Enzyme	1	8	9	10	11	12	13
α-Glucosidase[a]	>100	>100	>100	>100	>100	>100	>100
β-Glucosidase[b]	>100	>100	>100	>100	>100	>100	8.6
α-Mannosidase[c]	>100	>100	>100	>100	>100	>100	>100
β-Mannosidase[d]	>100	>100	>100	>100	>100	>100	>100
α-Galactosidase[e]	>100	>100	>100	>100	>100	>100	>100
β-Galactosidase[e]	>100	>100	>100	>100	>100	>100	>100
β-Glucuronidase[f]	15.5	>100	>100	>100	>100	>100	>100
α-*N*-Acetylgalactosaminidase[g]	>100	0.27	0.6	38	20	56	>100
β-*N*-Acetylglucosaminidase[h]	>100	0.42	45	>100	0.28	>100	>100

(a) Baker's yeast; (b) Almonds; (c) Jack beans; (d) Snail; (e) *Escherichia coli*; (f) Bovine liver; (g) Chicken liver; (h) Bovine epididymis.

Method B: To a solution of **15** (30 mg) in ethanol (1 mL) was added $NaBH_4$ (14 mg), and the mixture was stirred at room temperature for 6 h. After addition of ethyl acetate (1 mL), evaporation of the solvent gave a viscous solid. The solid was purified by similar methods described above to give an amorphous solid of **8** (90%).

Method C: Compound **16** (69 mg) was dissolved in 4 M HCl in dioxane (3 mL), and the solution was stirred at room temperature for 3 h. Evaporation of the solvent gave crystal-HCl salt of **8**. The solid was purified by the same methods described above to give **8** (63%) as an amorphous solid.

(2*S*,3*R*,4*S*,5*R*)-2-Acetamido-*N*-(*tert*-butoxycarbonyl)-3,4-*O*-isopropylidene-5-(*p*-toluene-sulfoxymethyl) piperidine-3,4-diol (17). To a solution of **16** (207 mg) in dry pyridine (4 mL) was added tosyl chloride (172 mg), and the mixture was stirred at room temperature overnight. After addition of water (50 μl) and dilution with chloroform, the solution was washed with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, and filtered. Evaporation of the solvent gave an oil, which was subjected to column chromatography on silica gel. Elution with toluene:acetone (5:1) gave **17** (252 mg, 84%) as a foamy solid: $[\alpha]_D^{25}$ +4.6° (*c* 0.45; $CHCl_3$); IR ($CHCl_3$) ν_{max} 1680 (br, C=O), 1500 (br, C=O), 1370 (SO_2), 1180 (SO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.25 and 1.35 (3H, each s, isopropylidene), 1.45 (9H, s, $C(O)OC(CH_3)_3$), 1.97 (3H, s, $NCOCH_3$), 2.33 (1H, br m, 5-H), 2.46 (3H, s, $C\underline{H}_3$—C6H5), 2.93 (1H, t, *J*=12.2 Hz, 6-Hax), 3.42 (1H, dd, *J*=12.2 Hz and 3.4 Hz, 6-Heq), 3.92 (1H, dd, *J*=10.0 and 8.1 Hz, Ha of —CH_2O—), 4.15 (1H, dd, *J*=10.0 and 6.1 Hz, Hb of —CH_2O—), 4.36 (1H, dd, *J*=7.3 and 2.0 Hz, 3-H), 4.58 (1H, br d with a small coupling, *J*=5.9 Hz, 4-H), 5.68 (2H, br m, 2-H and —NHCO—), 7.37 and 7.80 (each 2H, d, *J*=8.1 Hz, —C6H5—); FABMS *m/z* 499 (M+H, 7%), 443 (13), 340 (100), 110 (9), 57 (35); Anal. $C_{23}H_{34}N_2O_8S$ (C, H, N).

(2*S*,3*R*,4*S*,5*R*)-2-Acetamido-5-azidomethyl-*N*-(*tert*-butoxycarbonyl)-3,4-*O*-isopropylidenepiperidine-3,4-diol (18). To a solution of **17** (150 mg) in DMF (3 mL) was added NaN_3 (98 mg), and the mixture was stirred at 100 °C overnight. After dilution with chloroform, the solution was washed with water. The aqueous phase was extracted with chloroform. The organic phases were combined, dried over $MgSO_4$, and filtered. Evaporation of the solvent gave an oil. The oil was subjected to preparative thin-layer chromatography on silica gel developed with ethyl acetate to give **18** (105 mg, 95%) as a colorless oil; $[\alpha]_D^{25}$ +13.3° (*c* 0.81; $CHCl_3$); IR ($CHCl_3$) ν_{max} 2140 (N_3), 1690 (C=O), 1490 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$); d 1.33 and 1.44 (3H, each s, isopropylidene), 1.47 (9H, s, $C(O)OC(CH_3)_3$), 1.99 (3H, s, $NCOCH_3$), 2.99 (1H, t, *J*=12.2 Hz, 6-Hax), 3.28 (1H, dd, *J*=12.2 and 7.3 Hz, Ha of —$C\underline{H}_2N_3$), 3.48–3.57 (2H, m, 6-Heq and Hb of —$C\underline{H}_2N_3$), 4.20 (1H, dd, *J*=7.3 and 2.0 Hz, 3-H), 4.61 (1H, br d with a small coupling, *J*=4.9 Hz, 4-H), 5.68 (2H, br m, 2-H and —NHCO—); FABMS *m/z* 370 (M+H, 47%), 314 (86), 183 (79), 57 (100); Anal. $C_{16}H_{27}N_5O_5$ (C, H, N).

(2*R*,3*R*,4*S*,5*R*)-2-Acetamido-5-azidomethylpiperidine-3,4-diol (9). Compound **9** was obtained as an amorphous solid from **18** by a similar procedure to that used for the preparation of **8** from **16** (54%); $[\alpha]_D^{26}$ +25.7° (*c* 0.25; CH_3OH); IR (KBr) ν_{max} 3500 (OH), 3330 (OH), 3270 (OH), 2130 (N_3), 1630 (C=O), 1550 (C=O) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) d 1.87 (1H, m, 5-H), 1.98 (3H, s, $NCOCH_3$), 2.72 (1H, dd, *J*=12.0 and 4.6 Hz, 6-Heq), 2.78 (1H, t, *J*=12.0 Hz, 6-Hax), 3.28 (1H, dd, *J*=12.2 and 7.3 Hz, Ha of —CH_2N_3), 3.37 (1H, dd, *J*=9.3 and 2.9 Hz, 3-H), 3.44 (1H, dd, *J*=12.2 and 7.8 Hz, Hb of —CH_2N_3), 4.00 (1H, t, *J*=2.9 Hz, 4-H), 4.64 (1H, d, *J*=9.3 Hz, 2-H); FABMS *m/z* 230 (M+H, 98%), 171 (56), 75 (100), 57 (64), 45 (53); FAB-HRMS calcd for $C_8H_{16}N_5O_3$ (M+H): 230.1253. Found: 230.1272.

(2*S*,3*R*,4*S*,5*R*)-2-Acetamido-5-aminomethyl-*N*-(*tert*-butoxycarbonyl)-3,4-*O*-isopropylidenepiperidine-3,4-diol (19). A solution of **18** (104 mg) in a mixture of methanol (1 mL) and ethyl acetate (1 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 solvent gave a solid. The solid was subjected to preparative thin-layer chromatography on silica gel developed with chloroform:methanol:concd aqueous ammonia (90:10:1) to give **19** (105 mg, 95%) as an amorphous solid; $[\alpha]_D^{25}$ +34.3° (*c* 0.92; $CHCl_3$); IR ($CHCl_3$) ν_{max} 1665 (C=O), 1500 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) d 1.33 and 1.43 (3H, each s, isopropylidene), 1.46 (9H, s, $C(O)OC(CH_3)_3$), 1.93 (1H, m, 5-H), 1.97 (3H, s, $NCOCH_3$), 2.75 (1H, dd, *J*=12.7 and 6.4 Hz, Ha of —CH_2NH_2), 2.88 (1H, dd, *J*=12.7 and 7.8 Hz, Hb of —CH_2NH_2), 3.02 (1H, t, *J*=12.2 Hz, 6-Hax), 3.46 (1H, dd, *J*=12.2 and 3.9 Hz, 6-Heq), 4.46 (1H, dd, *J*=7.3 and 2.0 Hz, 3-H), 4.56 (1H, br d, *J*=5.9 Hz, 4-H), 5.76 (2H, br m, 2-H and —NHCO—); FABMS *m/z* 344 (M+H, 100%), 229 (47), 185 (78), 57 (94); Anal. $C_{16}H_{29}N_3O_5$ (C, H, N).

(2*R*,3*R*,4*S*,5*R*)-2-Acetamido-5-aminomethylpiperidine-3,4-diol (10). Compound **10** was obtained as an amorphous solid from **19** by a similar procedure to that used for the preparation of **8** from **16** (77%); $[\alpha]_D^{24}$ +21.5° (*c* 0.41; CH_3OH); IR (KBr) ν_{max} 3450 (br, OH), 3060 (br, OH), 1670 (br, C=O), 1550 (br, C=O) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) d 1.68 (1H, m, 5-H), 1.98 (3H, s, $NCOCH_3$), 2.61 (1H, dd, *J*=12.7 and 6.4 Hz, Ha of —CH_2NH_2), 2.65–2.80 (3H, m, 6-H and Hb of —$C\underline{H}_2NH_2$), 3.35 (1H, dd, *J*=9.8 and 2.4 Hz, 3-H), 4.04 (1H, t, *J*=2.4 Hz, 4-H), 4.61 (1H, d, *J*=9.8 Hz, 2-H); ^1H NMR of hydrochloride (400 MHz, CD_3OD) δ 2.05 (3H, s, $NCOCH_3$), 2.32 (1H, m, 5-H), 3.05 (1H, dd, *J*=13.2 and 6.3 Hz, Ha of —$C\underline{H}_2NH_2$),

3.10–3.17 (3H, m, 6-H and Hb of —C<u>H</u>$_2$NH$_2$), 3.77 (1H, dd, J=10.3 and 2.4 Hz, 3-H), 4.12 (1H, t, J=2.4 Hz, 4-H), 4.93 (1H, d, J=10.3 Hz, 2-H); FAB-MS *m/z* 204 (M+H, 100%), 145 (77), 115 (39), 75 (36), 57 (27); FAB-HRMS calcd for $C_8H_{18}N_3O_3$ (M+H): 204.1348. Found: 204.1358.

(2*S*,3*R*,4*S*,5*S*)-2-Acetamido-*N*-(*tert*-butoxycarbonyl)-3,4-*O*-isopropylidene-5-methylthiomethylpiperidine-3,4-diol (20). To a solution of **17** (150 mg) in DMF (3 mL) was added $NaSCH_3$ (105 mg), and the mixture was stirred at 110 °C overnight. After dilution with chloroform, the solution was washed with water. The aqueous phase was extracted with chloroform. The organic phases were combined, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil. The oil was subjected to preparative thin-layer chromatography on silica gel developed with ethyl acetate to give **20** (96 mg, 86%) as a colorless oil; $[\alpha]_D^{23}$ +31.7° (*c* 0.95; $CHCl_3$); IR ($CHCl_3$) ν_{max} 1685 (C=O), 1490 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.33 and 1.44 (3H, each s, isopropylidene), 1.46 (9H, s, C(O)OC(CH_3)$_3$), 1.99 (3H, s, NCOCH_3), 2.00 (1H, br m, 5-H), 2.16 (3H, s, —SCH_3), 2.49 (1H, dd, J=13.2 and 6.8 Hz, Ha of —CH_2S—), 2.70 (1H, dd, J=13.2 and 7.3 Hz, Hb of —CH_2S—), 3.01 (1H, t, J=12.2 Hz, 6-Hax), 3.56 (1H, dd, J=12.2 and 3.9 Hz, 6-Heq), 4.48 (1H, dd, J=7.3 and 2.4 Hz, 3-H), 4.57 (1H, dd, J=6.8 and 2.4 Hz, 4-H), 5.70 (2H, br m, 2-H and —NHCO—); FABMS *m/z* 375 (M+H, 37%), 319 (53), 216 (100), 57 (33); anal. $C_{17}H_{30}N_2O_5S$ (C, H, N).

(2*R*,3*R*,4*S*,5*S*)-2-Acetamido-5-methylthiomethylpiperidine-3,4-diol (11). Compound **11** was obtained as an amorphous solid from **20** by a similar procedure to that used for the preparation of **8** from **16** (61%); $[\alpha]_D^{24}$ +19.8° (*c* 0.09; CH_3OH); IR (KBr) ν_{max} 3500 (OH), 3350 (OH), 3300 (OH), 1640 (C=O), 1560 (C=O) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 1.83 (1H, m, 5-H), 1.98 (3H, s, NCOCH_3), 2.08 (3H, s, —SCH_3), 2.45 (1H, dd, J=13.2 and 7.3 Hz, Ha of —CH_2S—), 2.69 (1H, dd, J=13.2 and 7.8 Hz, Hb of —CH_2S—), 2.73–2.83 (2H, m, 6-H), 3.37 (1H, dd, J=9.8 and 2.4 Hz, 3-H), 4.06 (1H, t, J=2.4 Hz, 4-H), 4.64 (1H, d, J=9.8 Hz, 2-H); FABMS *m/z* 235 (M+H, 63%) 176 (100), 107 (54), 89 (46), 57 (42); FAB-HRMS calcd for $C_9H_{19}N_2O_3S$ (M+H): 235.1116. Found: 235.1108.

Diasteromeric mixture of (2*R*,3*R*,4*S*,5*S*)-2-acetamido-5-methylsulfinylmethylpiperidine-3,4-diol (12). To a solution of **11** (19 mg) in water (0.4 mL) was added a 30% hydrogen peroxide solution in water (0.1 mL), and the solution was stirred at room temperature for 4 h. Evaporation of the solvent gave an oil. The oil was purified by similar methods described in the preparation of **8** to give a diastereomeric mixture (18 mg, 87%) of **12** as an amorphous solid; IR (KBr) ν_{max} 3460 (br, OH), 3320 (br, OH), 3270 (br, OH), 1640 (br, C=O), 1560 (br, C=O), 1030 (br, S=O) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 1.99 (3H, s, NCOCH_3), 2.20–2.30 (1H, m, 5-H), 2.67 (3H, s, —$SOCH_3$), 2.70–3.00 (4H, m, 6-H and —CH_2SO—), 3.46 and 3.48 (total 1H, each t, J=2.4 and 2.7 Hz, 3-H), 3.98 (1/2H, t, J=2.4 Hz, a part of 4-H), 4.12 (1/2H, br s, a part of 4-H), 4.69 and 4.72 (total 1H, each d, J=2.0 Hz, 2-H): The ratio of the diastereomers (about 1:1) was estimated by ^1H NMR spectrum; FABMS *m/z* 251 (M+H, 41%), 219 (27), 192 (71), 176 (82), 107 (82), 69 (100), 57 (88); FAB-HRMS calcd for $C_9H_{19}N_2O_4S$ (M+H): 251.1066. Found: 251.1058.

(2*R*,3*S*,5*R*)-2-Acetamido-5-hydroxymethylpiperidine-3-ol (13). Compound **13** was obtained as an amorphous solid from **21** by a similar procedure to that used for the preparation of **8** from **15** (96%); $[\alpha]_D^{24}$ +42.3° (*c* 0.47; CH_3OH); IR (KBr) ν_{max} 3460 (OH), 3250 (OH), 1670 (C=O), 1565 (C=O) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 1.13 (1H, q, J=12.2 Hz, H-4ax), 1.70–1.82 (1H, m, 5-H), 1.99 (3H, s, NCOCH_3), 2.08 (1H, br d with small couplings, J=12.2 Hz, 4-Heq), 2.35 (1H, t, J=11.5 Hz, 6-Hax), 3.04 (1H, ddd, J=11.5, 4.0 and 2.0 Hz, 6-Heq), 3.34–3.46 (3H, m, —C<u>H</u>$_2$OH and 3-H), 4.15 (1H, d, J=9.3 Hz, 2-H); ^1H NMR of hydrochloride (400 MHz, D_2O) δ 1.49 (1H, q, J=12.2 Hz, 4-Hax), 2.12 (3H, s, NCOCH_3), 2.10–2.20 (1H, m, 5-H), 2.27 (1H, br d with small couplings, J=12.2 Hz, 4-Heq), 2.96 (1H, t, J=12.6 Hz, 6-Hax), 3.45 (1H, ddd, J=12.6, 4.0 and 2.0 Hz, 6-Heq), 3.57 (1H, dd, J=12.0 and 6.8 Hz, Ha of —C<u>H</u>$_2$OH), 3.64 (1H, dd, J=12.0 and 5.0 Hz, Hb of —C<u>H</u>$_2$OH), 3.88 (1H, ddd, J=12.2, 10.0 and 4.8 Hz, 3-H), 4.79 (1H, d, J=10.0 Hz, 2-H); FABMS *m/z* 189 (M+H, 100%), 130 (95), 75 (68), 57 (41), 45 (34); FAB-HRMS calcd for $C_8H_{17}N_2O_3$ (M+H): 189.1239. Found: 189.1242.

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References and Notes

1. (a) Look, G. C.; Fotsch, C. H.; Wong, C.-H. *Acc. Chem. Res.* **1993**, *26*, 182; (b) Nishimura, Y. *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1992; Vol. 10, pp 495–583; (c) Winchester, B.; Fleet, G. W. *Glycobiology* **1992**, *2*, 199.

2. Umezawa, H.; Aoyagi, T.; Komiyama, T.; Morishima, H.; Hamada, M.; Takeuchi, T. *J. Antibiotics* **1974**, *27*, 963.

3. (a) Nishimura, Y.; Wang, W.; Kondo, S.; Aoyagi, T.; Umezawa, H. *J. Am. Chem. Soc.* **1988**, *110*, 7249; (b) Nishimura, Y.; Wang, W.; Kudo, T.; Kondo, S. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 978; (c) Kudo, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *J. Antibiotics* **1992**, *45*, 954; (d) Nishimura,

Y.; Kudo, T.; Kondo, S.; Takeuchi, T. *J. Antibiotics* **1992**, *45*, 963; (e) Nishimura, Y.; Kudo, T.; Umezawa, Y.; Kondo, S.; Takeuchi, T. *Nat. Prod. Lett.* **1992**, *1*, 39; (f) Nishimura, Y.; Kudo, T.; Umezawa, Y.; Kondo, S.; Takeuchi, T. *Nat. Prod. Lett.* **1992**, *1*, 33; (g) Kudo, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *J. Antibiotics* **1992**, *45*, 1662; (h) Kudo, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *J. Antibiotics* **1993**, *46*, 300; (i) Nishimura, Y.; Umezawa, Y.; Kondo, S.; Takeuchi, T.; Mori, K.; Kijima-Suda, I.; Tomita, K.; Sugawara, K.; Nakamura, K. *J. Antibiotics* **1993**, *46*, 1883; (j) Nishimura, Y.; Kudo, T.; Kondo, S.; Takeuchi, T.; Tsuruoka, T.; Fukuyasu, H.; Shibahara, S. *J. Antibiotics* **1994**, *47*, 101; (k) Nishimura, Y.; Satoh, S.; Kondo, S.; Takeuchi, T.; Azetaka, M.; Fukuyasu, H.; Iizuka, Y.; Shibahara, S. *J. Antibiotics* **1994**, *47*, 840; (l) Satoh, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *Carbohyd. Res.*, in press; (m) Nishimura, Y. *Studies in Natural Products Chemistry*; Atta-ur-Rahamn, Ed.; Elsevier: Amsterdam, 1995; Vol. 16, pp. 75–123.

4. Very recently, 1-*N*-iminosugar inhibitors of glucosidase have been synthesized: (a) Jespersen, T. M.; Dong, W.; Sierks, M. R.; Skrydstrup, T.; Lundt, I.; Bols, M. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1778; (b) Ichikawa, M.; Igarashi, Y.; Ichikawa, Y. *Tetrahedron Lett.* **1995**, *36*, 1767.

5. (a) Dennis, J. W.; Laferte, S.; Waghorne, C.; Breitman, M. L.; Kerbel, R. S. *Science* **1987**, *236*, 582; (b) Dennis, J. W. *Cancer Surveys* **1988**, *7*, 573.

6. Kim, S. C.; Singh, A. N.; Rausher, F. *J. Biol. Chem.* **1988**, *263*, 10151.

7. Halvorson, H. O.; Ellias, L. *Biochim. Biophys. Acta* **1958**, *30*, 28.

8. Kobayashi, A. *Agr. Biol. Chem.* **1962**, *26*, 203.

9. Li, Y.-T. *J. Biol. Chem.* **1967**, *242*, 5474.

10. Craven, G. R.; Steers, Jr., E.; Anfinsen, C. B. *J. Biol. Chem.* **1965**, *240*, 2468.

11. Stahl, P. P. D.; Fishman, W. H. *Methods of Enzymatic Analysis*; Voigt, K. D., Ed.; Academic, New York, 1974; Vol. 4, pp 246–256.

12. Uda, Y.; Li, S.-C.; Li, Y.-T. *J. Biol. Chem.* **1977**, *252*, 5194.

13. Aoyagi, T.; Suda, H.; Uotani, K.; Kojima, F.; Aoyama, T.; Horiguchi, K.; Hamada, M.; Takeuchi, T. *J. Antibiotics* **1992**, *45*, 1404.

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