Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 343–352

BIOORGANIC & MEDICINAL CHEMISTRY

gem-Diamine 1-*N*-Iminosugars of L-Fucose-type, the Extremely Potent L-Fucosidase Inhibitors

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Received 16 July 1999; accepted 23 October 1999

Abstract—An efficient route from D-ribono-γ-lactone to *gem*-diamine 1-*N*-iminosugars of L-fucose-type, a new family of glycosidase inhibitor, has been developed in a formation of a *gem*-diamine 1-*N*-iminopyranose ring by the Mitsunobu reaction of an aminal as a key step. The analogues were proved to be the extremely potent inhibitors against α-L-fucosidase (IC_{50} ~3 ng mL^{-1}, K_i ~5×10^{-9} M). The present study has shown that a cyclic methanediamine generated in media affects glycosidases as a real active-form of the *gem*-diamine 1-*N*-iminosugars of L-fucose-type.

Introduction

Current interest of glycosidase inhibitors has been directed toward new tools for unlabeling how glycoconjugates such as glycoproteins, glycolipids and proteoglycans regulate biological functions, and toward new drugs for the treatment of diseases associated with glycoconjugates biosynthesis and degradation, including cancer, metastasis of tumors, inflammatory disorders, viral and bacterial infections and so forth.[1,2] Various types of inhibitors have been designed based on the mechanism of the enzyme-assisted hydrolysis of glycosidic bonds and the structural reminiscent of natural inhibitors.[3,4] Among the effective glycosidase inhibitors are pyranoses and furanoses with the ring oxygen replaced by an imino group. In the course of our study on glycosidase inhibitors, we proposed a new family of glycosidase inhibitor, *gem*-diamine 1-*N*-iminosugars (**1**) in which an anomeric carbon atom is replaced by a nitrogen.[5,6] We considered that the protonated *gem*-diamine 1-*N*-iminosugars may mimic the putative glycosyl cation intermediate **2** formed during enzymatic glycosidic hydrolysis (Fig. 1).[7–11] They have shown highly potent and specific inhibition against glycosidases, and some of them have also shown potent suppression of experimental and spontaneous lung metastasis of tumor cells in mice.[5,6,12–17]

On the other hand, an α(1→3)-linked L-fucose residue in sialyl Lewis X tetrasaccharide (sLe^X, **3**) expressed on the surface of leukocyte and some kinds of tumor cells is essential for their adhesion to the endothelial basement membrane through cell-surface endothelial-leukocyte adhesion molecules (ELAMs) (Fig. 2).[18–21] It was also suggested that fucosidase in invasive human ovarian carcinoma cell mediates degradation of the sub-endothelial extracellular matrix.[22] Furthermore, *N*-methyl and 5-carboxymethyl-1-pentyl derivatives (**4** and **5**) of 1,5-dideoxy-1,5-imino-L-fucitol (**6**), α-L-fucosidase inhibitors have been shown to inhibit the cytopathic effect of human immunodeficiency virus (HIV) and yield of infectious virus (Fig. 3).[23,24] These findings have led us to an intensive search for small molecules as fucosidase inhibitors and as potential drug candidates for the treatment of reperfusion injury and other inflammatory disorders, HIV infection and tumor metastasis. We have recently communicated the synthesis of novel L-fucose-type 1-*N*-iminosugars, (2*S*,3*S*,4*R*,5*R*)-2-acetamido-5-methylpiperidine-3,4-diol (**7**) and (2*S*,3*S*,4*R*,5*R*)-5-methyl-2-trifluoroacetamidopiperidine-3,4-diol (**8**) (Fig. 4).[25] We now report full details of the syntheses together with the evaluation as L-fucosidase inhibitors and the relationships between structures and inhibitory activities for these candidates and their analogues, (2*S*,3*S*,4*R*,5*R*)-5-methyl-2-trichloroacetamidopiperidine-3,4-diol (**9**), (2*S*,3*S*,4*R*,5*R*)-5-methyl-2-phthalimidopiperidine-3,4-diol (**10**), (2*S*,3*S*,4*R*,5*S*)-5-methyl-2-trifluoroacetamidopiperidine-3,4-diol (**11**), (2*S*,3*S*,4*R*)-5-methylene-2-trifluoroacetamidopiperidine-3,4-diol (**12**), (2*R*,3*S*,4*R*,5*R*)-2-amino-5-methylpiperidine-3,4-diol (**13**) and (2*R*,3*S*,4*R*,5*R*)-2-amino-*N*-acetyl-5-methylpiperidine-3,4-diol (**14**) (Fig. 4).

Keywords: *gem*-diamine 1-*N*-iminosugar; L-fucosidase inhibitor; (2*S*,3*S*,4*R*,5*R*)-2-trifluoroacetamido-5-methylpiperidine-3,4-dial; cyclic methanediamine.

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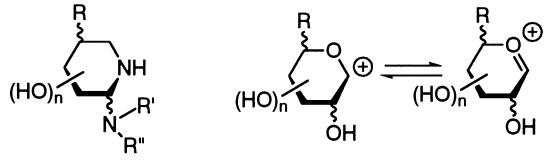
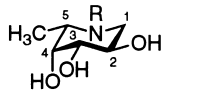
0968-0896/00/$ - see front matter
PII: S0968-0896(99)00289-8



1: R=CH_2OH/CO_2H, R',R"=substituent

2: R=CH_2OH/CO_2H

Figure 1. *gem*-Diamine 1-*N*-iminosugar (**1**) and glycopyranosyl cation (**2**).

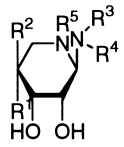


4: R=CH_3

5: R=$(CH_2)_5CO_2CH_3$

6: R=H

Figure 3. 1,5-Dideoxy-1,5-imino-L-fucitol (**6**) and analogues.



7: R^1=CH_3, R^2=R^3=R^5=H, R^4=$COCH_3$

8: R^1=CH_3, R^2=R^3=R^5=H, R^4=$COCF_3$

9: R^1=CH_3, R^2=R^3=R^5=H, R^4=$COCCl_3$

10: R^1=CH_3, R^2=R^5=H, R^3,R^4=Pht

11: R^1=R^3=R^5=H, R^2=CH_3, R^4=$COCF_3$

12: R^1,R^2=CH_2, R^3=R^5=H, R^4=$COCF_3$

13: R^1=CH_3, R^2=R^3=R^4=R^5=H

14: R^1=CH_3, R^2=R^3=R^4=H, R^5=$COCH_3$

Figure 4. *gem*-Diamine 1-*N*-iminosugars of L-fucose-type.

Results and Discussion

Synthesis

We have recently developed an efficient method of synthesis of multifunctionalized *gem*-diamine 1-*N*-imiosugars.[3] This methodology was employed conveniently for this synthesis. The synthesis of the pivotal intermediate, aminal **23** began with the known lactam **16**[26] which was converted into the diol **18** upon sodium borohydride reduction and removal of the protecting group in good yield. Selective protection of the hydroxymethyl group in **18** followed by the Dess–Martin oxidation[27] gave the ketone **20** in 95% yield. The Wittig reaction of **20** with methylenetriphenylphosphorane afforded the methylene **21** which was transformed into the monoalcohol **22** by removal of the protecting group in good yield. Stereoselective introduction of the hydroxyl group at C(2) was best achieved by the Swern oxidation[28] to give the key intermediate **23** as a sole product in 82% yield. The stereochemistry of **23** was established by its ^{1}H NMR spectrum. The ^{1}H NMR spectrum of **23** shows protons of C(2), C(3) and C(4) at δ 5.68 (d, $J<2$ Hz), 4.41 (dd, $J\sim2.0$ and 7.3 Hz) and 4.74 (d, $J=7.3$ Hz), respectively, indicative of an equatorial hydrogen at C(2). The same stereochemical outcome controlled by an anomeric effect[29] as those of the previous *gem*-diamine 1-*N*-iminosugar syntheses[5,6] was observed. This stereochemistry was later confirmed by X-ray crystallographic analysis of the hydrogenated compound **25**. Replacement of the aminal hydroxy group of **23** to the amino group was successfully carried out by the Mitsunobu reaction[30] (PPh_3, diethyl azodicarboxylate, phthalimide) in DMF to yield the iminophthalimide **24** in an excellent yield. Catalytic hydrogenation of **24** with palladium on carbon in methanol gave the desired product **25**, its epimer **26** and the rearrangement derivative **27** in 75, 5 and 18% yield, respectively. Hydrogenation of **27** under the same condition also gave efficiently **25** in good yield. However, the prolonged reaction period in reduction of **24** was rather inefficient. Compound **25** was crystallized from a mixture of toluene and *n*-hexane to yield a single crystal for X-ray diffraction analysis. The X-ray crystallographic analysis clearly indicated the desired absolute stereochemistry and a boat conformation (Fig. 5). On the other hand, the stereochemistry of **26** was estimated by comparison of the ^{1}H NMR spectra of **26** and its derivatives (**32** and **33**) with those of **25** and its derivatives (**28** and **30**), respectively. The large coupling constants ($J_{5,6ax}=11.5$–12.4 Hz) and the small coupling constants ($J_{4,5}=2.4$–3.4 Hz and $J_{2,3}=1.5$–2.0 Hz) of the ^{1}H NMR spectra of **25**, **28** and **30** indicate the same conformation as the B[3,7–11] boat conformation of **25** clarified by X-ray crystallographic analysis (Fig. 5), while the coupling constants ($J_{5,6ax}=12.7$–13.7 Hz, $J_{4,5}=6.0$–9.3 Hz and $J_{2,3}=1.5$–2.0 Hz) of the ^{1}H NMR spectra of **26**, **32** and **33** suggest their half-chair conformation. These results suggest that the methylene group of **24** easily rearranges under catalytic hydrogenation with palladium on carbon, and that hydrogenation may take place predominantly from the less sterically hindered side (a) of the double bond of **27** with

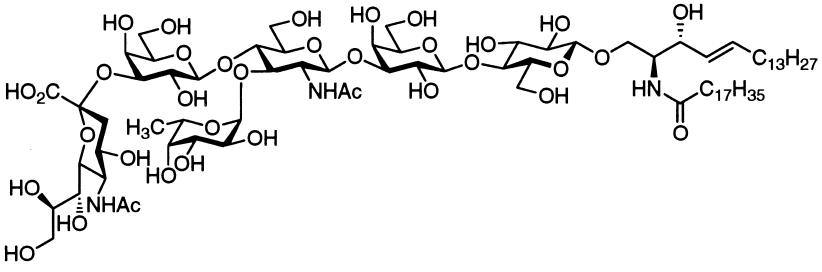


Figure 2. Sialyl Lewis X (**3**).

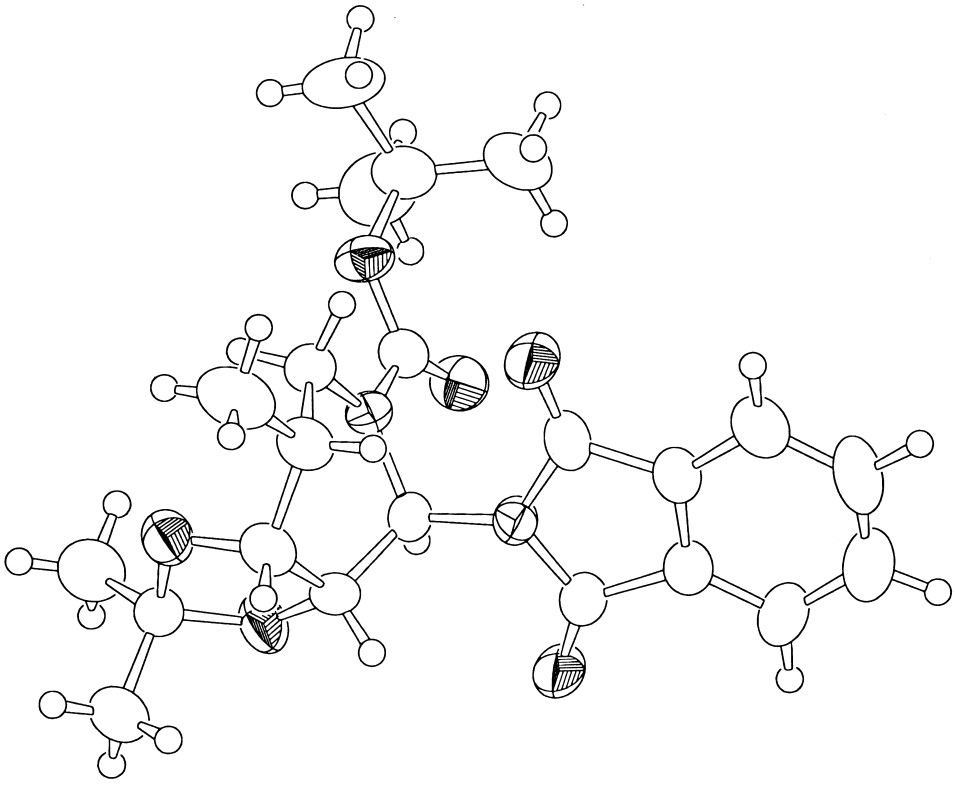


Figure 5. ORTEP drawing of compound **25**.

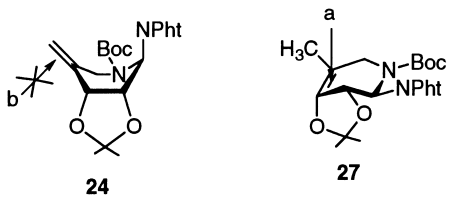
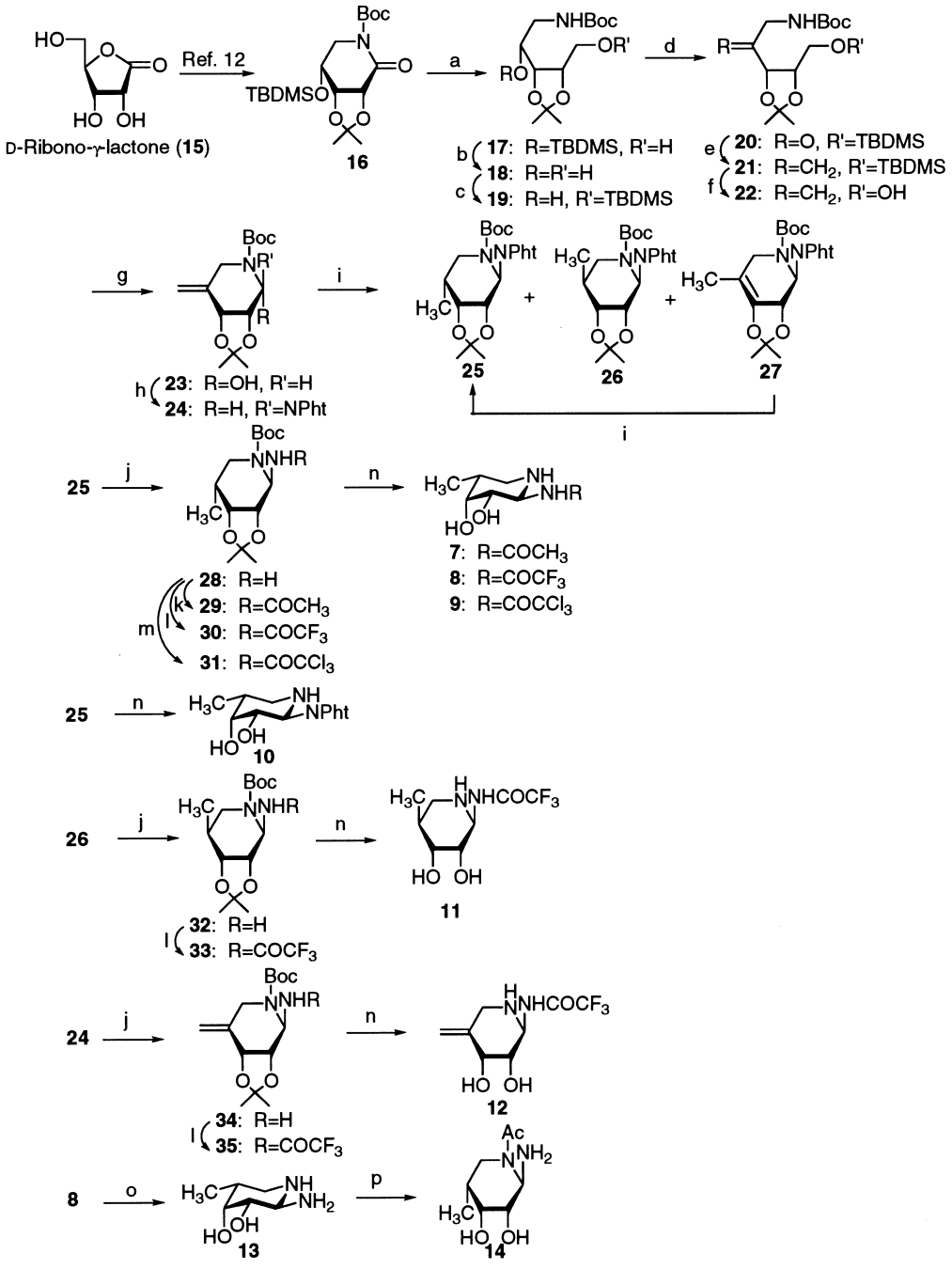


Figure 6. Possible mechanism of the sterochemical outcome in catalytic hydrogenation of **24**.

a boat conformation to lead **25** rather than from the less sterically hindered side (b) of the methylene group of **24** with a different boat conformation to lead **26** (Fig. 6). Hydrazinolysis of **25** afforded the amine **28** in 99% yield. Conventional acetylation, trifluoroacetylation and trichloroacetylation furnished the acetamide **29**, the trifluoroacetamide **30** and the trichloroacetamide **31** in excellent yields, respectively. Simultaneous removal of both the isopropylidene and *t*-butyloxycarbonyl groups in **29**, **30** and **31** with 4 M hydrogen chloride in dioxane resulted in the desired L-fucose-type 2-acetamido-, 2-trifluoroacetamido- and 2-trichloroacetamido-1-*N*-iminosugars **7**, **8** and **9** in excellent yields, respectively. And another L-fucose-type 2-phthalimido-1-*N*-iminosugar **10** was also similarly obtained from **25** in 94%. The large coupling constants (10.3–12.5 Hz) between H-2 and H-3 and between H-5 and H-6ax in ^{1}H NMR spectra of **7**, **8** and **9** are clearly indicative of their $^1C_4$ conformation. On the other hand, 6-deoxy-D-altrose-type 1-*N*-iminosugar **11**, the epimer of **8** and 5-methylene-D-*arabino*-hexopyranose-type 1-*N*-iminosugar **12**, the 5-methylene isomer of **8** were prepared by the similar sequences of reaction from **26** and **24** in good yields, respectively. The coupling constants ($J_{2,3}=5.9$, $J_{3,4}=2.7$, $J_{4,5}=7.8$, $J_{5,6}=4.4$ and $J_{5,6}=8.3$ Hz) of the ^{1}H NMR spectrum of **11** suggest its skew-boat conformation.

Biological activity

The inhibitory activities of synthesized 1-*N*-iminosugars (**7**–**12**) against glycosidases are summarized in Table 1. As expected, the L-fucose-type trifluoroacetamide (**8**) showed very strong, specific inhibition against α-L-fucosidase from bovine kidney, and the acetamide **7** also affected moderately the enzyme. Compound **8** was also proved to be a competitive inhibitor by Lineweaver–Burk plot and, the K_i value of **8** was elucidated as 5×10^{-9} M by Dixon plot. On the other hand, **7** and **8** showed no significant inhibition against all other D-glycosidases. Strikingly, the L-fucose-type trichloroacetamide (**9**) and phthalimide (**10**) also affected very potently α-L-fucosidase equivalent to the trifluoroacetamide (**8**). These results suggested that the common intermediate derived from these analogues **8**, **9** and **10** in the media might affect the enzyme as the real active-form. Then, we examined thoroughly the time-dependent alteration of the structures of the analogues **8**, **9** and **10** in ^{1}H NMR spectra. As expected, **8**, **9** and **10** were proved to be unstable in the conditions of the medium of citrate-phosphate buffer (pH 6.3, 37 °C) for L-fucosidase inhibition assay as well as acetate buffer (pH 5.0, 37°C) for other glycosidase inhibition assays. Their ^{1}H NMR spectra were suggestive of the existence of the common intermediate of the $^1C_4$-conformational methanediamine **13**. Compound **8**, **9** and **10** in methanol and water also showed similar ^{1}H NMR spectra after a



Scheme 1. (a) $NaBH_4$, EtOH, 0 °C to rt, (b) *n*-Bu_4NF, THF, rt, (c) *t*-$BuMe_2SiCl$, imidazole, DMF, rt, (d) Dess–Martin periodinane, CH_2Cl_2, (e) Ph_3PCH_3Br, $(Me_3Si)_2NLi$, THF, 0 °C to rt, (f) *n*-Bu_4NF, THF, rt, (g) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C to rt, (h) phthalimide, Ph_3P, DEAD, DMF, rt, (i) H_2/Pd-C, MeOH, rt, (j) $H_2NNH_2{\cdot}xH_2O$, MeOH, rt, (k) Ac_2O, DMAP, Py, rt, (l) $(CF_3CO)_2O$, Py, CH_2Cl_2, rt, (m) CCl_3COCl, Py, CH_2Cl_2, 0 °C, (n) 4 M HCl/dioxane, 0 °C to rt, (o) MeOH, 50 °C, (p) Ac_2O, Py, MeOH, rt.

few days, indicative of the presence of the same methanediamine **13**. Next we undertook the isolation of the common intermediate. After treatment of **8** in methanol at 50 °C for 13 h, evaporation of the solvent gave the pure methanediamine **13**. The ^1H NMR spectrum of **13** shows the similar coupling constants as those shown in the spectrum of **8**, indicating the $^1C_4$ conformation of the cyclic methanediamine. The ^{13}C NMR spectrum of **13** also shows peaks at δ 14.21 (5-CH_3), 33.52 (C-5), 44.26 (C-6), 72.25 (C-3 or C-4), 72.99 (C-4 or C-3) and 88.86 (C-2), supportive of the cyclic methanediamine. Furthermore, conventional *N*-acetylation (acetic anhydride, pyridine, CH_3OH) of **13** afforded the acetamide **14** different from **7** as a sole product. The structure of **14** was established by the ^1H NMR, IR and mass spectra, and the coupling constants of ^1H NMR spectrum ($J_{2,3}=J_{3,4}=3.7$, $J_{5,6eq}=4.4$ and $J_{5,6ax}=13.3$ Hz) also suggested the boat conformation of **14**. However, it is not clear at this stage why the ring imino-group was selectively acetylated to give **14**. Interestingly, **13** showed very

Table 1. Inhibitory activity of **7**–**14** against glycosidases

Enzyme	IC_{50} (M)							
	7	**8**	**9**	**10**	**11**	**12**	**13**	**14**
α-L-Fucosidase[a]	4.8×10^{-7} (0.11)[j]	1.1×10^{-8} (0.003)[j]	9.0×10^{-9} (0.003)[j]	1.3×10^{-8} (0.004)[j]	1.8×10^{-6} (0.50)[j]	7.0×10^{-7} (0.20)[j]	1.6×10^{-8} (0.003)[j]	$>2.7\times10^{-4}$ (>50)[j]
α-D-Glucosidase[b]	1.8×10^{-4}	4.7×10^{-5}	NT[k]	NT	NT	NT	5.4×10^{-5}	NT
β-D-Glucosidase[c]	1.0×10^{-5}	1.2×10^{-4}	NT	NT	NT	NT	1.4×10^{-4}	NT
α-D-Mannosidase[d]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT	$>5.5\times10^{-4}$	NT
β-D-Mannosidase[e]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT	$>5.5\times10^{-4}$	NT
α-D-Galactosidase[f]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT	$>5.5\times10^{-4}$	NT
β-D-Galactosidase[f]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT	$>5.5\times10^{-4}$	NT
β-D-Glucuronidase[g]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT	$>5.5\times10^{-4}$	NT
α-D-*N*-Acetylgalactosaminidase[h]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT	$>5.5\times10^{-4}$	NT
β-D-*N*-Acetylglucosaminidase[i]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT	$>5.5\times10^{-4}$	NT

[a]Bovine kidney.
[b]Baker's yeast.
[c]Almonds.
[d]Jack beans.
[e]Snail.
[f]*Escherichia coli*.
[g]Bovine liver.
[h]Chicken liver.
[i]Bovine epididymis.
[j]IC_{50} (μg/mL).
[k]Not tested.

strong inhibition against α-L-fucosidase equivalent to **8**, **9** and **10**, while **14** showed no inhibition against the enzyme. These results support the above speculation of which the common intermediate **13** affect the enzyme in the media, and seem also to support the hypothesis of which the protonated *gem*-diamine 1-*N*-iminosugars may mimic the presumed glycosyl cation (**2**) in the transition state of enzymatic reaction as shown in Figure 1. On the other hand, the acetamide **7** was stable in both methanol and water, also suggestive of the weak inhibition against L-fucosidase. Compounds **11** and **12** also inhibited weakly α-L-fucosidase. These results indicate that the 5-methyl group, its stereochemistry and the $^1C_4$-conformation play the important roles as the major factors for inhibition against L-fucosidase.

In summary, an efficient synthetic route involving the formation of a *gem*-diamine 1-*N*-iminopyranose ring by the Mitsunobu reaction of an aminal as a key step to L-fucose-type *gem*-diamine 1-*N*-iminosugars, from a readily available D-ribono-γ-lactone has been developed and has produced the extremely potent inhibitors of α-L-fucosidase. The present study indicates that the cyclic methanediamines may generally affect the enzymes as the real active-forms of the glycosidase inhibitors of the *gem*-diamine 1-*N*-iminosugars. That these *gem*-diamine 1-*N*-iminosugars are highly potent inhibitors of α-L-fucosidase further supports the hypothesis of our design of the new type inhibitor.

Experimental

General methods

Melting points were determined with a Yamato apparatus and were uncorrected. Optical rotations were measured with a Perkin–Elmer Model 241 polarimeter. ^1H NMR spectra were recorded with a Jeol GX-400 spectrometer. Chemical shifts are expressed in δ values (ppm) with tetramethylsilane (δ 0.00) for $CDCl_3$, with CD_2HOD (3.30) for CD_3OD, and with HDO (δ 4.65) for D_2O as an internal standard. The mass spectra were taken by Jeol SX102 for FAB and by Hitachi M-1200H for APCI (atmospheric pressure chemical ionization).

General procedures for enzyme inhibition assay

The enzymes, α-L-fucosidase (bovine kidney), β-glucuronidase (bovine liver), α-glucosidase (baker's yeast), β-glucosidase (almond), α-mannosidase (jack beans), β-mannosidase (snail), α-galactosidase (*Escherichia coli*), β-galactosidase (*E. coli*), α-*N*-acetylgalactosaminidase (chicken liver), and β-*N*-acetylglucosaminidase (bovine epididymis) were purchased from Sigma Chemical Co. α-L-Fucosidase was assayed using *p*-nitrophenyl α-L-fucopyranoside (1.5×10^{-3} M) as a substrate at pH 6.3 (0.025 M citrate-phosphate buffer). β-Glucuronidase was assayed using phenolphthalein mono-β-glucuronic acid (3.3×10^{-4} M) as a substrate at pH 5.0 (0.1 M acetate buffer). α- and β-glucosidases were assayed using *p*-nitrophenyl α-D-glucopyranoside (1.5×10^{-3} M) and β-D-glucopyranoside (2×10^{-3} M) as substrates at pH 6.3 (0.025 M citrate-phosphate buffer) and 5.0 (0.025 M acetate buffer), respectively. α- and β-Mannosidases were assayed using *p*-nitrophenyl α-D-mannopyranoside (2×10^{-3} M) and β-D-mannopyranoside (2×10^{-3} M) as substrates at pH 4.5 (0.05 M acetate buffer) and 4.0 (0.05 M acetate buffer), respectively. β-Galactosidase was assayed using *p*-nitrophenyl β-D-galactopyranoside (2×10^{-3} M) at pH 4.0 (0.025 M citrate-phosphate buffer). α-*N*-Acetylgalactosaminidase was assayed using *p*-nitrophenyl *N*-acetyl-α-D-galactosaminide (1×10^{-3} M) as a substrate at pH 4.0 (0.025 M citrate-phosphate buffer). β-*N*-Acetylglucosaminidase was assayed using

p-nitrophenyl *N*-acetyl-β-D-glucosaminide (1×10^{-3} M) at pH 4.0 (0.025 M citrate-phosphate buffer). The reaction mixture contained 0.5 mL of buffer, 0.1 mL of substrate solution and water or aqueous solution containing the test compound. The mixture was incubated at 37 °C for 3 min, and 0.01 mL of enzyme was added. After 0.5–1 h of reaction, 1.0 mL of 0.3 M glycine-sodium hydroxide buffer (pH 10.5) was added and the absorbance of the liberated nitrophenol or phenolyphthalein measured at 400 or 525 nm, respectively. The percentage inhibition was calculated by the formula (A−B)/A×100, where A is the nitrophenol liberated by the enzyme without an inhibitor and B is that with an inhibitor. The IC_{50} value is the concentration of inhibitor at 50% of enzyme activity.

1-(*t*-Butoxycarbonyl)amino-2,5-di-*O*-(*t*-butyldimethylsilyl)-1-deoxy-3,4-*O*-isopropylidene-D-ribitol (17). To a solution of **16**[26] (15.0 g, 37.4 mmol) in EtOH (500 mL) was added $NaBH_4$ (7.07 g, 186.8 mmol) at 0 °C, and the mixture was stirred at room temperature for 43 h. After quenching with H_2O, the mixture was further stirred for 30 min. Evaporation of the solvent gave a solid, which was dissolved in $CHCl_3$. The solution was washed with H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to column chromatography on silica gel. Elution with toluene:ethyl acetate (3:1) gave **17** as an oil (15 g, 99%): $[\alpha]_D^{27}$ −13.6° (*c* 0.91, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 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, H-5), 3.39 (1H, br t, *J* = 6.2 Hz, -OH), 3.59 and 3.72 (1H each, dt, *J* = 12 and 6.2 Hz, H-1), 4.08 (1H, t, *J* = 6.2 Hz, H-3), 4.12 (1H, br q, *J* = 6.2 Hz, H-4), 4.20 (1H, q, *J* = 6.2 Hz, H-2) and 5.84 (1H, br t, *J* = 6.4 Hz, -NHCO-); IR ($CHCl_3$) 1720 (C=O), 1520 (NH) cm^{-1}; FABMS *m*/*z* 406 $(M+H)^+$, 350, 306, 292, 248, 73, 57.41. Anal. $C_{19}H_{39}NO_5Si$ (C, H, N).

1-(*t*-Butoxycarbonyl)amino-1-deoxy-3,4-*O*-isopropylidene-D-ribitol (18). A solution of *n*-Bu_4NF in THF (1 M, 67.3 mL) was added to a solution of **17** (13.6 g, 33.6 mmol) in THF (200 mL), and the mixture was stirred at room temperature for 1 h. Evaporation of the solvent gave an oil, which was dissolved in $CHCl_3$. The solution was washed with water, and the aqueous phase was extracted three times with $CHCl_3$. The organic phases were combined, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to column chromatography on silica gel. Elution with $CHCl_3$:MeOH (19:1) gave **18** as an oil (9.2 g, 94%): $[\alpha]_D^{27}$ +37.7° (*c* 0.94, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.32 and 1.40 (3H each, s, CH_3 of isopropylidene), 1.43 (9H, s, COOC$(CH_3)_3$), 3.40 (1H, dd, *J* = 13.9 and 7.3 Hz, H-5), 3.44 (1H, dd, *J* = 13.9 and 3.4 Hz, H-5′), 3.62 (1H, dd, *J* = 11.2 and 6.3 Hz, H-1), 3.71 (1H, ddd, *J* = 9.3, 7.3 and 3.4 Hz, H-4), 3.81 (1H, dd, *J* = 11.2 and 5.9 Hz, H-1′), 3.96 (1H, dd, *J* = 9.3 and 6.4 Hz, H-3) and 4.25 (1H, br dd, *J* = 12.2 and 6.4 Hz, H-2); IR ($CHCl_3$) 1680 (C=O), 1510 (NH) cm^{-1}; FABMS *m*/*z* 292.4 $(M+H)^+$, 236.3, 178.2, 154.1, 136.1, 120.1, 107.1, 57.1. Anal. $C_{13}H_{25}NO_6$ (C, H, N).

1-(*t*-Butoxycarbonyl)amino-5-*O*-(*t*-butyldimethylsilyl)-1-deoxy-3,4-*O*-isopropylidene-D-ribitol (19). To a solution of **18** (1.0 g, 3.43 mmol) in D MF (10 mL) were added imidazole (491 mg, 7.21 mmol) and TBDMSCl (543 mg, 3.60 mmol), and the mixture was stirred at room temperature for 2 h. After quenching with H_2O, evaporation of the solvent gave an oil. The oil was dissolved in EtOAc, and 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 *n*-hexane:EtOAc (9:1) gave **19** as an oil (1.38 g, 99%): $[\alpha]_D^{27}$ +8.9° (*c* 0.99, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 0.11 (6H, s, $(CH_3)_2$ of *t*-butyldimethylsilyl), 0.91 (9H, s, $(CH_3)_3$ of *t*-butyldimethylsilyl), 1.31 and 1.40 (3H each, s, $(CH_3)_3$ of isopropylidene), 1.43 (9H, s, COOC$(CH_3)_3$), 3.03 (1H, dd, *J* = 13.9 and 7.1 Hz, H-5), 3.44 (1H, dd, *J* = 13.9 and 3.2 Hz, H-5′), 3.70–3.77 (2H, m, H-1 and H-4), 3.92–3.98 (2H, m, H-1 and H-3), 4.21 (1H, br dd, *J* = 11.5 and 5.6 Hz, H-2); IR ($CHCl_3$) 1710 (C=O), 1510 (NH) cm^{-1}; FABMS *m*/*z* 406.3 $(M+H)^+$, 350.2, 306.3, 292.2, 248.2, 142.1, 73.1, 57.1. Anal. $C_{19}H_{39}NO_6Si$ (C, H, N).

D-*erythro*-1-(*t*-Butoxycarbonyl)amino-5-*O*-(*t*-butyldimethylsilyl)-1-deoxy-3,4-*O*-isopropylidene-2-pentosulitol (20). To a solution of **19** (5.0 g, 12.3 mmol) in CH_2Cl_2 (100 mL) was added Dess–Martin periodinane (7.83 g, 18.5 mmol), and the mixture was stirred at room temperature for 4 h. After dilution with $CHCl_3$, the solution was washed with a saturated aqueous $NaHCO_3$ and H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to column chromatography on silica gel. Elution with *n*-hexane:EtOAc (9:1) gave **20** as an oil (4.78 g, 96%): $[\alpha]_D^{26}$ −35.8° (*c* 0.53, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.03 and 0.04 (3H each, s, $(CH_3)_2$ of *t*-butyldimethylsilyl), 0.86 (9H, s, $(CH_3)_3$ of *t*-butyldimethylsilyl), 1.35 and 1.56 (3H each, s, CH_3 of isopropylidene), 1.44 (9H, s, COOC$(CH_3)_3$), 3.68 (1H, dd, *J* = 11.7 and 2.4 Hz, H-1), 3.75 (1H, dd, *J* = 11.7 and 3.4 Hz, H-1′), 4.27 (2H, d, *J* = 4.4 Hz, H-5), 4.38–4.45 (1H, m, H-2), 4.56 (1H, d, *J* = 8.3 Hz, H-3) and 5.23 (1H, br s, NH); IR ($CHCl_3$) 1700 (C=O), 1500 (NH) cm^{-1}; FABMS *m*/*z* 404.3 $(M+H)^+$, 348.3, 304.3, 290.2, 246.2, 140.1, 73.1, 57.1. Anal. $C_{19}H_{37}NO_6Si$ (C, H, N).

1-(*t*-Butoxycarbonyl)amino-1,2-dideoxy-5-*O*-(*t*-butyldimethylsilyl)-3,4-*O*-isopropylidene-2-methylene-D-*erythro*-pentitol (21). To a solution of methylenetriphenylphosphorane, prepared from methyltriphenylphosphonium bromide (16.92 g, 47.4 mmol) and lithium bis(trimethylsilyl)amide (1.0 M, 45 mL) in THF (50 mL) from 0 °C to room temperature, was added a solution of **20** (4.78 g, 11.8 mmol) in THF (10 mL) at 0 °C, and the resulting mixture was stirred for 30 min. After quenching with acetic acid (2.94 mL, 47.4 mmol), the mixture was further stirred for 30 min. Evaporation of the solvent gave a solid, which was dissolved in $CHCl_3$. The solution was washed with H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to column chromatography on silica gel. Elution with *n*-hexane:EtOAc (9:1) gave **21** as an oil

(3.83 g, 81%): $[\alpha]_D^{28}$ −43.5° (*c* 0.87, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.04 and 0.06 (3H each, s, $(CH_3)_2$ of *t*-butyldimethylsilyl), 0.88 (9H, s, $(CH_3)_3$ of *t*-butyldimethylsilyl), 1.36 (3H, s, CH_3 of isopropylidene), 1.44 (12H, s, CH_3 of isopropylidene and $COOC(CH_3)_3$), 3.44 (1H, dd, *J* = 9.6 and 3.9 Hz, H-1), 3.55 (1H, br t, *J* = 9.6 Hz, H-1′), 3.69 (1H, dd, *J* = 15.1 and 3.9 Hz, H-5), 3.94 (1H, dd, *J* = 15.1 and 6.8 Hz, H-5′), 4.18–4.23 (1H, m, H-2), 4.66 (1H, d, *J* = 5.9 Hz, H-3), 5.15 and 5.33 (1H each, br s, methylene) and 5.39 (1H, br s, NH); IR ($CHCl_3$) 1710 (C=O), 1510 (NH) cm^{-1}; FABMS *m/z* 402.3 $(M+H)^+$, 346.3, 330.2, 288.2, 230.1, 226.3, 186.2, 154.1, 138.1, 73.1, 57.1. Anal. $C_{20}H_{39}NO_5Si$ (C, H, N).

1-(*t*-Butoxycarbonyl)amino-1,2-dideoxy-3,4-*O*-isopropylidene-2-methylene-D-*erythro*-pentitol (22). A solution of *n*-Bu_4NF in THF (1 M, 24.7 mL) was added to a solution of **21** (8.28 g, 20.6 mmol) in THF (100 mL), and the mixture was stirred at room temperature for 30 min. Evaporation of the solvent gave an oil, which was dissolved in $CHCl_3$. The solution was washed with H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to column chromatography on silica gel. Elution with $CHCl_3$:MeOH (24:1) gave **22** as an oil (5.98 g, 99%): $[\alpha]_D^{28}$ −49.3° (*c* 0.94, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.38 and 1.49 (3H each, s, CH_3 of isopropylidene), 1.45 (9H, s, $COOC(CH_3)_3$), 2.93 (1H, br t, *J* = 6.3 Hz, -OH), 3.49 (1H, br quintet, *J* = 5.9 Hz, H-1), 3.56–3.65 (1H, m, H-1′), 3.69 (1H, dd, *J* = 16.8 and 6.1 Hz, H-5′), 3.81 (1H, dd, *J* = 16.8 and 5.9 Hz, H-5), 4.31 (1H, br dd, *J* = 6.3 and 5.9 Hz, H-2), 4.68 (1H, d, *J* = 6.3 Hz, H-3), 4.93 (1H, br s, NH), 5.17 and 5.33 (1H each, br s, methylene); IR ($CHCl_3$) 1710 (C=O), 1520 (NH) cm^{-1}; FABMS *m/z* 288.3 $(M+H)^+$, 232.2, 174.2, 154.1, 112.1, 57.1. Anal. $C_{14}H_{25}NO_5$ (C, H, N).

(2*R*,3*R*,4*S*)-*N*-(*t*-Butoxycarbonyl)-3,4-*O*-isopropylidene-5-methylenepiperidine-2,3,4-triol (23). Dimethyl sulfoxide (1.78 mL, 25.1 mmol) was added to the stirred solution of oxalyl chloride (1.09 mL, 12.5 mmol) in CH_2Cl_2 (20 mL) at −78 °C, and the mixture was stirred for 20 min. After addition of a solution of **22** (900 mg, 3.13 mmol) in CH_2Cl_2 (24 mL) at −78 °C within 5 min, the mixture was stirred for 20 min. After addition of triethylamine (8.73 mL, 62.6 mmol), the mixture was stirred at the same temperature for 15 min, and then the mixture was allowed to warm to room temperature. After quenching with water, the mixture was extracted with CH_2Cl_2. The extract 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 toluene:acetone (19:1) gave **23** as a foam (734 mg, 82%): $[\alpha]_D^{29}$ −9.9° (*c* 0.45, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.37 and 1.44 (3H each, s, CH_3 of isopropylidene), 1.48 (9H, s, $COOC(CH_3)_3$), 3.17 (1H, br s,-OH), 3.82 and 4.19 (1H each, d, *J* = 14.2 Hz, H-6, 6′), 4.41 (1H, dd, *J* = 7.3 and 2.0 Hz, H-3), 4.74 (1H, d, *J* = 7.3 Hz, H-4), 5.25 and 5.33 (1H each, br s, methylene) and 5.68 (1H, br s, H-2); IR (KBr) 1690 (C=O) cm^{-1}; FABMS *m/z* 286 $(M+H)^+$, 271.1, 268.2, 230.1, 212.1, 168.1, 154.1, 110, 57.1. Anal. $C_{14}H_{23}NO_5$ (C, H, N).

(2*S*,3*S*,4*S*)-*N*-(*t*-Butoxycarbonyl)-3,4-*O*-isopropylidene-5-methylene-2-phthalimidopiperidine-3,4-diol (24). To the mixture of **23** (500 mg, 1.75 mmol), triphenylphosphine (1.38 g, 5.26 mmol) and phthalimide (773 mg, 5.26 mmol) in D MF (10 mL) was added dropwise diethyl azodicarboxylate (0.837 mL, 5.26 mmol) under stirring, and the resulting mixture was stirred at room temperature overnight. Addition of water and evaporation of the solvent gave an oil, which was dissolved in EtOAc. 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 column chromatography on silica gel. Elution with toluene:EtOAc (19:1) gave **24** as a foam (692 mg, 95%): $[\alpha]_D^{28}$ +57.6° (*c* 0.85, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.40 (3H, s, CH_3 of isopropylidene), 1.49 (12H, s, CH_3 of isopropylidene and $COOC(CH_3)_3$), 3.82 and 4.48 (1H each, d, *J* = 14.7 Hz, H-6, 6′), 4.75 (1H, d, *J* = 6.4 Hz, H-3), 5.00 (1H, d, *J* = 6.4 Hz, H-4), 5.19 and 5.32 (1H each, br s, methylene), 6.35 (1H, br s, H-2) and 7.72–7.84 (4H, m, phthalimido); IR ($CHCl_3$) 1775 (C=O), 1720 (C=O) cm^{-1}; FABMS *m/z* 415.3 $(M+H)^+$, 359.2, 315.2, 168.2, 148.1, 110.1, 57.1. Anal. $C_{22}H_{26}N_2O_6$ (C, H, N).

(2*S*,3*S*,4*R*,5*R*)-*N*-(*t*-Butoxycarbonyl)-3,4-*O*-isopropylidene-5-methyl-2-phthalimidopiperidine-3,4-diol (25) and (2*S*,3*S*,4*R*,5*S*)-*N*-(*t*-butoxycarbonyl)-3,4-*O*-isopropylidene-5-methyl-2-phthalimidopiperidine-3,4-diol (26) and (2*S*,3*S*)-*N*-(*t*-butoxycarbonyl)-4,5-didehydro-3,4-*O*-isopropylidene-5-methyl-2-phthalimidopiperidine-3,4-diol (27). A solution of **24** (3.69 g, 8.69 mmol) in MeOH (200 mL) was stirred with 10% palladium on carbon (1 g) under atmosphere of hydrogen at room temperature for 5 h. After removal of catalysts, evaporation of the solvent gave an oil, which was subjected to column chromatography on silica gel. Elution with toluene: AcOEt (9:1) gave **25** (2.73 g, 75%) and **26** (0.18 g, 5%) as a solid, and **27** (0.64 g, 18%) as a foam. Compounds **25** and **26** were crystallized from toluene-*n*-hexane to give their colorless crystals.

25: Mp 157–158 °C; $[\alpha]_D^{26}$ −32.9° (*c* 0.49, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.08 (3H, d, *J* = 6.8 Hz, 5-CH_3), 1.36 and 1.48 (3H each, s, CH_3 of isopropylidene), 1.38 (9H, s, $COOC(CH_3)_3$), 2.34–2.44 (1H, m, H-5), 3.19 (1H, br t, *J* = 11.5 Hz, H-6ax), 3.50 (1H, dd, *J* = 11.5 and 4.6 Hz, H-6eq), 4.33 (1H, dd, *J* = 7.3 and 3.4 Hz, H-4), 4.63 (1H, dd, *J* = 7.3 and 2.0 Hz, H-3), 6.05 (1H, d, *J* = 2.0 Hz, H-2) and 7.71–7.83 (4H, m, phthalimido); IR (KBr) 1770 (C=O), 1720 (C=O), 1700 (C=O), 1610 (C=O) cm^{-1}; FABMS *m/z* 417.3 $(M+H)^+$, 361.2, 317.2, 214.2, 170.2, 148.1, 112.1, 57.1: Anal. $C_{22}H_{28}N_2O_6$ (C, H, N).

26: Mp 137–138 °C; $[\alpha]_D^{26}$ +16.9° (*c* 0.47, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.12 (3H, d, *J* = 6.8 Hz, 5-CH_3); 1.33 and 1.51 (3H each, s, CH_3 of isopropylidene), 1.44 (9H, s, $COOC(CH_3)_3$), 1.78–1.88 (1H, m, H-5), 2.98 (1H, br t, *J* = 13.0 Hz, H-6ax), 3.90–4.02 (1H, m, H-6eq), 4.26 (1H, dd, *J* = 8.6 and 5.4 Hz, H-4), 4.30 (1H, dd, *J* = 5.4 and 1.5 Hz, H-3), 6.47 (1H, s, H-2) and 7.74–7.89 (4H, m, phthalimido); IR (KBr) 1770 (C=O), 1720 (C=O), 1700 (C=O), 1610 (C=O) cm^{-1}; FABMS *m/z* 417.3

$(M+H)^+$, 361.2, 317.2, 214.2, 170.2, 148.1, 112.1, 57.1. Anal. $C_{22}H_{28}N_2O_6$ (C, H, N).

27: $[\alpha]_D^{28}$ +55.5° (*c* 0.95, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.31 (9H, s, $COOC(CH_3)_3$), 1.43 and 1.58 (3H each, s, CH_3 of isopropylidene), 1.78 (3H, br s, 5-CH_3), 3.96 and 4.37 (1H each, d, *J* = 15.1 Hz, H-6, 6′), 4.95 (1h, br d, *J* = 6.8 Hz, H-3), 5.81 (1H, d, *J* = 6.8 Hz, H-2) and 7.73–7.88 (4H, m, phthalimido); IR ($CHCl_3$) 1780 (C=O), 1720 (C=O), 1700 (C=O) cm^{-1}; FABMS *m*/*z* 415.3 $(M+H)^+$, 359.2, 313.2, 255.2, 219.1, 168.2, 154.1, 148.1, 110.1, 57.1. Anal. $C_{22}H_{26}N_2O_6$ (C, H, N).

Synthesis of 25 from 27

Compound **25** was synthesized similarly from **27** as in the preparation of **25** from **24**; the yield was 75%.

(2*R*,3*S*,4*R*,5*R*)-2-Amino-*N*-(*t*-butoxycarbonyl)-3,4-*O*-isopropylidene-5-methylpiperidine-3,4-diol (28). To a solution of **25** (83 mg, 0.20 mmol) in MeOH (5 mL) was added hydrazine hydrate (0.5 mL), and the mixture was stirred at room temperature overnight. After dilution with $CHCl_3$, the resulting precipitates were filtered off. The filtrate 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 $CHCl_3$-MeOH (25:1) gave **28** as a foam (57 mg, 99%): $[\alpha]_D^{28}$ +14.3° (*c* 1.27, $CHCl_3$); ^1H NMR (CD_3OD) δ 1.00 (3H, d, *J* = 6.8 Hz, 5-CH_3), 1.31 and 1.35 (3H each, s, CH_3 of isopropylidene), 1.47 (9H, s, $COOC(CH_3)_3$), 2.28–2.38 (1H, m, H-5), 2.99 (1H, br t, *J* = 12.2 Hz, H-6ax), 3.22 (1H, br dd, *J* = 12.2 and 4.9 Hz, H-6eq), 4.30 (1H, br dd, *J* = 7.8 and 2.4 Hz, H-4), 4.33 (1H, dd, *J* = 7.8 and 1.5 Hz, H-3) and 4.90 (1H, d, *J* = 1.5 Hz, H-2); IR ($CHCl_3$) 1680 (C=O) cm^{-1}; FABMS *m*/*z* 287 $(M+H)^+$, 270.2, 214.2, 170.1, 112.1, 57.1. Anal. $C_{14}H_{26}N_2O_4$ (C, H, N).

(2*R*,3*S*,4*R*,5*R*)-2-Acetamido-*N*-(*t*-butoxycarbonyl)-3,4-*O*-isopropylidene-5-methylpiperidine-3,4-diol (29). To a solution of **28** (25 mg, 0.087 mmol) in CH_2Cl_2 (1 mL) were added pyridine (0.1 mL), acetic anhydride (0.1 mL) and 4-dimethylaminopyridine (2 mg), and the mixture was stirred at room temperature for 4 h. Evaporation of the solvent gave an oil, which was dissolved in ethyl acetate. 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 toluene:acetone (3:1) gave **29** as a foam (29 mg, 99%): $[\alpha]_D^{27}$ −34.3° (*c* 0.57, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.05 (3H, d, *J* = 6.8 Hz, 5-CH_3), 1.33 and 1.35 (3H each, s, CH_3 of isopropylidene), 1.46 (9H, s, $COOC(CH_3)_3$), 1.98 (3H, s, -$COCH_3$), 1.88–2.01 (1H, m, H-5), 3.01 (1H, br t, *J* = 12.3 Hz, H-6ax), 3.32 (1H, dd, *J* = 12.3 and 3.9 Hz, H-6eq), 4.26 (1H, dd, *J* = 7.3 and 2.0 Hz, H-4), 4.53 (1H, br d, *J* = 7.3 Hz, H-3) and 5.73 (2H, br s, H-2 and -NHCO-); IR ($CHCl_3$) 1680 (C=O) cm^{-1}; FABMS *m*/*z* 329.3 $(M+H)^+$, 273.2, 214.2, 170.2, 154.1, 112.1, 57.1. Anal. $C_{16}H_{28}N_2O_5$ (C, H, N).

(2*R*,3*S*,4*R*,5*R*)-*N*-(*t*-Butoxycarbonyl)-2-trifluoroacetamido-3,4-*O*-isopropylidene-5-methylpiperidine-3,4-diol (30). Compound **28** (120 mg, 0.419 mmol) was dissolved in CH_2Cl_2 (2 mL), and to the solution were added pyridine (0.1 mL), $(CF_3CO)_2O$ (0.1 mL) and 4-dimethylaminopyridine (10 mg) at 0 °C. The mixture was stirred at 0 °C for 30 min. Evaporation of the solvent gave an 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 solvent gave an oil, which was subjected to column chromatography on silica gel. Elution with toluene:EtOAc (10:1) gave **30** as a solid (159 mg, 99%): $[\alpha]_D^{25}$ −37.2° (*c* 0.46, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.08 (3H, d, *J* = 6.8 Hz, 5-CH_3), 1.35 (3H, s, CH_3 of isopropylidene), 1.46 (12H, s, CH_3 of isopropylidene and $COOC(CH_3)_3$), 1.82–1.95 (1H, m, H-5), 3.01 (1H, br t, *J* = 12.4 Hz, H-6ax), 3.39 (1H, dd, *J* = 12.4 and 3.9 Hz, H-6eq), 4.32 (1H, dd, *J* = 6.8 and 2.4 Hz, H-4), 4.52 (1H, dd, *J* = 6.8 and 2.0 Hz, H-3) and 5.77 (1H, br s, H-2); IR (KBr) 1720 (C=O), 1680 (C=O), 1530 (NH) cm^{-1}; FABMS *m*/*z* 383.2 $(M+H)^+$, 327.2, 214.2, 170.2, 154.1, 112.1, 57.1. Anal. $C_{16}H_{25}F_3N_2O_5$ (C, H, N).

(2*R*,3*S*,4*R*,5*R*)-*N*-(*t*-Butoxycarbonyl)-2-trichloroacetamido-3,4-*O*-isopropylidene-5-methylpiperidine-3,4-diol (31). Compound **28** (17 mg, 0.0594 mmol) was dissolved in CH_2Cl_2 (3 mL), and to the solution were added pyridine (19.2 μL, 0.238 mmol) and trichloroacetyl chloride (13.3 μL, 0.119 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min. After dilution with CH_2Cl_2 (15 mL), 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 toluene:EtOAc (10:1) gave **31** as a foam (25.6 mg, 99%): $[\alpha]_D^{26}$ −21.2° (*c* 0.5, MeOH); ^1H NMR ($CDCl_3$) δ 1.08 (3H, d, *J* = 6.8 Hz, 5-CH_3), 1.36 (3H, s, CH_3 of isopropylidene), 1.47 (12H, s, CH_3 of isopropylidene and $COOC(CH_3)_3$), 1.81–1.93 (1H, m, H-5), 3.01 (1H, br t, *J* = 12.1 Hz, H-6ax), 3.42 (1H, dd, *J* = 12.1 and 3.4 Hz, H-6eq), 4.34 (1H, dd, *J* = 6.4 and 2.0 Hz, H-4), 4.56 (1H, br d, *J* = 6.4 Hz, H-3), 5.75 (1H, br s, H-2) and 6.60 (1H, br s, -NHCO-); IR (KBr) 1720 (C=O), 1660 (C=O), 1520 (NH) cm^{-1}; FABMS *m*/*z* 433 $(M+2H)^+$, 431 $(M)^+$, 377, 375.16, 359.15, 270.31, 214.26, 170.24, 154.16, 57.10. Anal. $C_{16}H_{25}N_2O_5Cl_3$ (C, H, N).

(2*S*,3*S*,4*R*,5*R*)-2-Acetamido-5-methylpiperidine-3,4-diol (7). Compound **29** (20 mg, 0.0608 mmol) was dissolved in ether (2 mL), and to the solution was added 4 M HCl in 1,4-dioxane (0.4 mL) at 0 °C. The mixture was stirred at room temperature for 3 h. After addition of diethyl ether, the resulting precipitates were taken by centrifugation and washed with diethyl ether three times to give **7** as a colorless solid of its hydrochloride (8.8 mg, 65%): $[\alpha]_D^{27}$ −32.3° (*c* 0.41, MeOH); ^1H NMR (CD_3OD) δ 1.05 (3H, d, *J* = 6.8 Hz, 5-CH_3), 1.95–2.06 (1H, m, H-5), 2.05 (3H, s, $COCH_3$), 2.92 (1H, dd, *J* = 12.3 and 3.9 Hz, H-6eq), 3.05 (1H, br t, *J* = 12.3 Hz, H-6ax), 3.72 (1H, dd, *J* = 10.3 and 2.4 Hz, H-3), 3.84–3.87 (1H, br t, *J* = ~2.0 Hz, H-4) and 4.91 (1H, d, *J* = 10.3 Hz, H-2); IR (KBr) 1660 (C=O), 1550 (NH) cm^{-1}; FABMS *m*/*z* 189.2 $(M+H)^+$, 176.1, 154.1, 137.1, 120.1, 107, 89, 77. Anal. $C_8H_{16}N_2O_3 \cdot HCl$ (C, H, N). Calcd. Cl, 15.78; found Cl, 16.11.

(2*S*,3*S*,4*R*,5*R*)-2-Trifluoroacetamido-5-methylpiperidine-3,4-diol (8). Compound **8** as its hydrochloride was synthesized similarly from **30** as in the preparation of **7** from **29**; the yield was 97%: $[\alpha]_D^{28}$ −42.3° (*c* 0.46, MeOH); ^{1}H NMR (CD_3OD) δ 1.06 (3H, d, *J* = 6.8 Hz, 5-CH_3), 1.95–2.06 (1H, m, H-5), 2.97 (1H, dd, *J* = 12.5 and 4.4 Hz, H-6eq), 3.09 (1H, br t, *J* = 12.5 Hz, H-6ax), 3.84 (1H, dd, *J* = 10.3 and 2.4 Hz, H-3), 3.89 (1H, br t, *J* = 2.4 Hz, H-4) and 4.99 (1H, d, *J* = 10.3 Hz, H-2); IR (KBr) 1730 (C=O), 1555 (NH) cm^{-1}; FABMS *m*/*z* 243.2 $(M+H)^+$, 154.1, 137.1, 120.1, 107.1, 89, 77. Anal. $C_8H_{13}N_2O_3F_3$·HCl (C, H, N). Calcd. Cl, 12.72; found Cl, 13.04%.

(2*S*,3*S*,4*R*,5*R*)-2-Trichloroacetamido-5-methylpiperidine-3,4-diol (9). Compound **9** as its hydrochloride was synthesized similarly from **31** as in the preparation of **7** from **29**: the yield was 77%: $[\alpha]_D^{22}$ −37.8° (*c* 0.23, MeOH); ^{1}H NMR (CD_3OD) δ 1.07 (3H, d, *J* = 6.8 Hz, 5-CH_3), 1.96–2.02 (1H, m, H-5), 2.99 (1H, dd, *J* = 12.5 and 4.4 Hz, H-6eq), 3.07 (1H, br t, *J* = 12.5 Hz, H-6ax), 3.88–3.91 (1H, br t, *J* = ∼2.0 Hz, H-4), 3.94 (1H, dd, *J* = 10.3 and 2.4 Hz, H-3); IR (KBr) 1720 (C=O), 1530 (NH) cm^{-1}; FABMS *m*/*z* 293 $(M+2H)^+$, 291.08 $(M)^+$, 170.18, 154.09, 136.09, 130.13, 112.06, 107.04, 89.03, 77.04. Anal. $C_8H_{13}N_2O_3Cl_3$·HCl (C, H, N). Calcd. Cl, 43.04; found Cl, 43.47%.

(2*S*,3*S*,4*R*,5*R*)-5-Methyl-2-phthalimidopiperidine-3,4-diol (10). Compound **10** as its hydrochloride was synthesized similarly from **25** as in the preparation of **7** from **29**; the yield was 94%: $[\alpha]_D^{23}$ −19.6° (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD) δ 1.11 (3H, d, *J* = 6.8 Hz, 5-CH_3), 2.14–2.23 (1H, m, H-5), 3.10 (1H, dd, *J* = 12.2 and 4.4 Hz, H-6eq), 3.23 (1H, br t, *J* = 12.2 Hz, H-6ax), 3.99 (1H, br t, *J* = ∼2.0 Hz, H-4), 4.54 (1H, dd, *J* = 10.3 and 2.4 Hz, H-3), 5.44 (1H, d, *J* = 10.3 Hz, H-2) and 7.88–8.00 (4H, m, phthalimido); IR (KBr) 1780 (C=O), 1720 (C=O), 1690 (C=O) cm^{-1}; FABMS *m*/*z* 277.19 $(M+H)^+$, 265.17, 202.22, 170.17, 154.10, 136.09, 130.13, 107.04, 89.03, 77.04. Anal. $C_{14}H_{16}N_2O_4$·HCl (C, H, N). Calcd. Cl, 11.34; found Cl, 11.63%.

(2*S*,3*S*,4*R*,5*R*)-2-Amino-*N*-(*t*-butoxycarbonyl)-3,4-*O*-isopropylidene-5-methylpiperidine-3,4-diol (32). Compound **32** was synthesized similarly from **26** as in the preparation of **28** from **25**; the yield was 67.1%: $[\alpha]_D^{28}$ +58.9° (*c* 0.54, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.00 (3H, d, *J* = 6.4 Hz, 5-CH_3), 1.35 and 1.46 (3H each, s, CH_3 of isopropylidene), 1.48 (9H, s, $COOC(CH_3)_3$), 1.71–1.83 (1H, m, H-5), 2.67 (1H, br t, *J* = 12.7 Hz, H-6ax), 3.72 (1H, m, H-6eq), 3.78 (1H, dd, *J* = 9.3 and 4.9 Hz, H-4), 3.99 (1H, dd, *J* = 4.9 and 1.8 Hz, H-3) and 5.38 (1H, br s, H-2); IR ($CHCl_3$) 1680 (C=O) cm^{-1}; FABMS *m*/*z* 270.2 $(M\text{-}NH_2)^+$, 214.2, 170.1, 112.1, 57.1. Anal. $C_{14}H_{26}N_2O_4$ (C, H, N).

(2*S*,3*S*,4*R*,5*R*)-*N*-(*t*-Butoxycarbonyl)-2-trifluoroacetamido-3,4-*O*-isopropylidene-5-methylpiperidine-3,4-diol (33). Compound **33** was synthesized similarly from **32** as in the preparation of **30** from **28**; the yield was 77.9%: $[\alpha]_D^{27}$ +50.3° (*c* 0.54, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.05 (3H, d, *J* = 6.8 Hz, 5-CH_3), 1.36 and 1.49 (3H each, CH_3 of isopropylidene), 1.47 (9H, s, $COOC(CH_3)_3$), 1.82–1.93 (1H, m, H-5), 2.79 (1H, br s, H-6), 3.78 (1H, br d, *J* = 13.7 Hz, H-6′), 4.01 (1H, br t, *J* = 6.0 Hz, H-4), 4.24 (1H, br dd, *J* = ∼6.0 and ∼2.0 Hz, H-3), 5.80 (1H, br s, H-2); IR ($CHCl_3$) 1740 (C=O), 1700 (C=O), 1540 (NH) cm^{-1}; FABMS *m*/*z* 383.2 $(M+H)^+$, 327.2, 214.2, 170.2, 154.1, 112.1, 57.1. Anal. $C_{16}H_{25}N_2O_5F_3$ (C, H, N).

(2*S*,3*S*,4*R*,5*S*)-2-Trifluoroacetamido-5-methylpiperidine-3,4-diol (11). Compound **11** as its hydrochloride was synthesized similarly from **33** as in the preparation of **7** from **29**; the yield was 67.8%: $[\alpha]_D^{26}$ 22.4° (*c* 0.091, MeOH); ^{1}H NMR (CD_3OD) δ 1.11 (3H, d, *J* = 6.8 Hz, 5-CH_3), 2.18–2.30 (1H, m, H-5), 2.86 (1H, dd, *J* = 13.2 and 8.3 Hz, H-6ax), 3.30 (1H, dd, *J* = 13.2 and 4.4 Hz, H-6eq), 3.75 (1H, dd, *J* = 7.8 and 2.7 Hz, H-4), 3.98 (1H, dd, *J* = 5.6 and 2.7 Hz, H-3) and 5.23 (1H, d, *J* = 5.6 Hz, H-2); IR (KBr) 1730 (C=O), 1560 (NH) cm^{-1}; FABMS *m*/*z* 243.2 $(M+H)^+$, 154.1, 136.1, 130.2, 107.1, 89, 77. Anal. $C_8H_{13}N_2O_3F_3$·HCl (C, H, N). Calcd. Cl, 12.72; found Cl, 13.07%.

(2*R*,3*S*,4*S*)-2-Amino-*N*-(*t*-butoxycarbonyl)-3,4-*O*-isopropylidene-5-methylenepiperidine-3,4-diol (34). Compound **34** was synthesized similarly from **24** as in the preparation of **28** from **25**; the yield was 72%: $[\alpha]_D^{26}$ +4.0° (*c* 0.93, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.36 and 1.45 (3H each, s, CH_3 of isopropylidene), 1.48 (9H, s, $COOC(CH_3)_3$), 3.84 (1H, d, *J* = 14.2 Hz, H-6), 4.25 (1H, dt, *J* = 14.2 and 2.0 Hz, H-6′), 4.40 (1H, dd, *J* = 7.6 and 2.0 Hz, H-4), 4.70 (1H, d, *J* = 7.6 Hz, H-3), 5.07 (1H, br s, H-2) and 5.30 and 5.36 (1H each, br s, methylene); IR ($CHCl_3$) 1680 (C=O) cm^{-1}; FABMS *m*/*z* 268.1 $(M\text{-}NH_2)^+$, 212.1, 168.1, 154.1, 110, 57. Anal. $C_{14}H_{24}N_2O_4$ (C, H, N).

(2*R*,3*S*,4*S*)-*N*-(*t*-Butoxycarbonyl)-2-trifluoroacetamido-3,4-*O*-isopropylidene-5-methylenepiperidine-3,4-diol (35). Compound **35** was synthesized similarly from **34** as in the preparation of **30** from **28**; the yield was 89.2%: $[\alpha]_D^{26}$ −50.8° (*c* 0.87, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.37 (3H, s, CH_3 of isopropylidene), 1.48 (12H, s, CH_3 of isopropylidene and $COOC(CH_3)_3$), 3.87 (1H, d, *J* = 13.7 Hz, H-6), 4.25 (1H, dt, *J* = 13.7 and 2.0 Hz, H-6′), 4.44 (1H, br s, H-4), 4.74 (1H, d, *J* = 7.8 Hz, H-3), 5.41 and 5.45 (1H each, s, methylene), 6.09 (1H, dd, *J* = 7.8 and 1.5 Hz, H-2) and 6.26 (1H, br s, -NHCO-); IR ($CHCl_3$) 1730 (C=O), 1690 (C=O), 1520 (NH) cm^{-1}; FABMS *m*/*z* 381.1 $(M+H)^+$, 325.1, 279.1, 212.1, 168.1, 154.1, 110, 57.1. Anal. $C_{16}H_{23}N_2O_5F_3$ (C, H, N).

(2*S*,3*S*,4*S*)-2-Trifluoroacetamido-5-methylenepiperidine-3,4-diol (12). Compound **12** as its hydrochloride was synthesized similarly from **35** as in the preparation of **7** from **29**; the yield 50.6%: $[\alpha]_D^{26}$ −27.1° (*c* 0.09, MeOH); ^{1}H NMR (CD_3OD) δ 3.69 (1H, d, *J* = 13.2 Hz, H-6), 3.89 (1H, dd, *J* = 9.3 and 2.9 Hz, H-3), 4.00 (1H, d, *J* = 13.2 Hz, H-6′), 4.48 (1H, d, *J* = 2.9 Hz, H-4), 5.24 (1H, d, *J* = 9.3 Hz, H-2) and 5.35 and 5.43 (1H each, s, methylene); IR (KBr) 1725 (C=O), 1550 (NH) cm^{-1}; FABMS *m*/*z* 241.2 $(M+H)^+$, 168.2, 154.1, 136.1, 128.1, 110.1, 89, 77. Anal. $C_8H_{11}N_2O_3F_3$·HCl (C, H, N). Calcd. Cl, 12.82; found Cl, 13.17%.

(2*R*,3*S*,4*R*,5*R*)-2-Amino-5-methylpiperidine-3,4-diol (13). Compound **8** (30 mg) was dissolved in MeOH (3 mL), and the solution was stirred at 50 °C for 13 h. Evaporation of the solvent gave **13** as an oil of its hydrochloride (19.5 mg, 99%): $[\alpha]_D^{26}$ −24.9° (*c* 0.15, MeOH); ^{1}H NMR (CD_3OD) δ 1.04 (3H, d, J = 6.8 Hz, 5-CH_3), 1.95–2.02 (1H, m, H-5), 2.99–3.04 (2H, m, H-6, 6′), 3.55 (1H, dd, J = 8.8 and 2.5 Hz, H-3), 3.84 (1H, br t, J = 2.5 Hz, H-4) and 4.42 (1H, d, J = 8.8 Hz, H-2); ^{13}C NMR (CD_3OD, 100 MHz) δ 14.21 (CH_3), 33.52 (C-5), 44.26 (C-6), 72.25 (C-3 or C-4), 72.99 (C-4 or C-3) and 88.86 (C-2); IR (KBr) 3360, 2940, 1460, 1010 cm^{-1}; FABMS m/z 130.2 $(M\text{-}NH_2)^+$, 107.0, 89.0, 77.1.

(2*R*,3*S*,4*R*,5*R*)-*N*-Acetyl-2-amino-5-methylpiperidine-3,4-diol (14). To a solution of **13** (26.2 mg, 0.143 mmol) in MeOH (30 mL) were added acetic anhydride (0.2 mL) and pyridine (12.8 μL, 0.158 mmol), and the mixture was stirred at room temperature for 20 h. Evaporation of the solvent gave an oil, which was subjected to column chromatography on silica gel. Elution with $CHCl_3$–MeOH (50:1) gave **14** as an oil (14 mg, 51.9%): $[\alpha]_D^{24}$ +0.84° (*c* 0.45, MeOH); ^{1}H NMR (CD_3OD) δ 0.99 and 1.01 (total 3H, d each, J = 6.8 Hz, 5-CH_3), 1.62–1.72 and 1.73–1.83 (total 1H, m each, H-5), 2.17 and 2.18 (total 3H, s each, $COCH_3$), 2.64 and 3.12 (total 1H, br t each, J = 13.3 Hz, H-6ax), 3.37 and 4.09 (total 1H, dd each, J = 13.3 and 4.4 Hz, H-6eq), 3.45 and 3.59 (total 1H, br t each, J = 3.7 Hz, H-3), 3.73 (1H, br s, H-4) and 5.07 and 5.72 (total 1H, d each, J = 3.7 Hz, H-2); IR ($CHCl_3$) 1640 (C=O) cm^{-1}; APCI–MS m/z 189 $(M+H)^+$, 172 $(M\text{-}NH_2)^+$, 154, 130.

Acknowledgements

The authors are grateful to Dr. S. Kondo for his helpful discussion and encouragement. Thanks are due to Ms. H. Nakamura for X-ray crystallographic analysis. We also express appreciation to Ms. M. Kaneda for her technical assistance.

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