Nitrogen-in-the-Ring Pyranoses and Furanoses: Structural Basis of Inhibition of Mammalian Glycosidases

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Seven pyranoses and three furanoses with a nitrogen in the ring were prepared by chemical synthesis, microbial conversion, and isolation from plants to investigate the contribution of epimerization, deoxygenation, and conformation to the potency of inhibition and specificity of mammalian glycosidases. The seven pyranoses are **1**-deoxynojirimycin (**1**), the D-*manno* (**2**), D-*allo* (3), and D-*galacto* (**4**) isomers of **1**, fagomine (1,2-dideoxynojirimycin, **5**), and the D-*allo* (**6**) and D-*galacto* (**7**) isomers of **5**, while the three furanoses are 2,5-dideoxy-2,5-imino-D-mannitol (**8**), 1,4-dideoxy-1,4-imino-D-arabinitol (**9**), and 1,4-dideoxy-1,4-imino-D-ribitol (**10**). The 2-deoxygenation and/or 3-epimerization of **1** enhanced the potency for rat intestinal lactase and bovine liver cytosolic β-galactosidase. Especially compound **6** showed a potent inhibitory activity against both enzymes, and compound **8**, a mimic of β-D-fructofuranose, was a potent inhibitor of both β-galactosidases as well. Compound **4**, which has been known as a powerful α-galactosidase inhibitor, exhibited no significant inhibitory activity for most of mammalian β-galactosidases. In addition, compound **6** fairly retained a potency of **1** toward rat intestinal isomaltase. In this study, compound **8**, known as a processing α-glucosidase I inhibitor in cell culture, has been found to have no effect on processing α-glucosidase II, whereas **9** has been shown to be a good nonspecific inhibitor of intestinal isomaltase, processing α-glucosidase II, Golgi α-mannosidases I and II, and porcine kidney trehalase. It has been speculated that glycosidase inhibitors have structures which resemble those of the respective glycosyl cations. This broad inhibitory activity of **9** toward various glycosidases suggests that it superimposes well on the various glycosyl cations.

Introduction

Many alkaloidal sugar mimics with a nitrogen in the ring have been isolated from plants and microorganisms and inhibit various glycosidases in a reversible and competitive manner.[1-3] Glycosidases are involved in several important biological processes, such as digestion, the biosynthesis of glycoproteins, and the catabolism of glycoconjugates. Since glycosidase inhibitors have been proved to have the potential to produce antiviral, insect antifeedant, antidiabetic, and anticancer effects, as well as immune modulatory properties, they have attracted a lot of attention. 1-Deoxynojirimycin (**1**), which is a D-glucose analogue with an NH group substituting for the oxygen atom in the pyranose ring, has been shown to inhibit intestinal α-glucosidases and pancreatic α-amylase both *in vitro* and *in vivo*[4-6] and α-glucosidases I and II involved in N-linked oligosaccharide processing.[7,8] Compound **1** inhibits more strongly α-glucosidase II than α-glucosidase I, but N-alkylation of **1** resulted in analogues with increased potency and specificity for α-glucosidases I.[9-12] 1-Deoxymannojirimycin (**2**) is an inhibitor of rat liver Golgi α-mannosidase I but not an inhibitor of soluble (or endoplasmic reticulum, ER) α-mannosidase and Golgi α-mannosidase II.[8,13] The D-*galacto* isomer of **1** (1,5-dideoxy-1,5-imino-D-galactitol, **4**) has been chemically synthesized and shown to be a powerful competitive inhibitor of coffee bean α-galactosidase (K_i = 0.0016 μM).[14] This compound inhibited β-galactosidases of the microbial origin with K_i values that ranged from 0.16 to 12.5 μM. Fagomine (1,2-dideoxynojirimycin, **5**) has been shown to have some activity against mammalian gut α-glucosidase, but no other glycosidase inhibitory activity has been reported.[15-17] 2,5-Dideoxy-2,5-imino-D-mannitol (**8**) known as DMDP, an analogue of β-D-fructofuranse with a nitrogen in the ring, is a potent inhibitor of mouse intestinal α-glucosidases, β-glucosidase, and β-galactosidase.[17] In cell culture, compound **8** inhibited processing α-glucosidase I to accumulate $Glc_3Man_{7-9}(GlcNAc)_2$ types of N-linked oligosaccharides.[18] 1,4-Dideoxy-1,4-imino-D-arabinitol (**9**), which lacks one of the hydroxymethyl groups of **8**, is a potent inhibitor of yeast α-glucosidase.[19] 1,4-Dideoxy-1,4-imino-D-ribitol (DRB) has been enantiospecifically synthesized and proven to be a weaker competitive inhibitor than **9** of yeast α-glucosidase.[20-21]

Compounds **1** and **2** have been investigated in detail on the inhibition for processing glycosidases and other mammalian enzymes.[1,3] However, as to other pyranoses and furanoses with a nitrogen in the ring, the detailed and systematic study on the mammalian glycosidase inhibition has not been reported.

Having prepared nitrogen-in-the-ring pyranoses (**1**, **2**, **4**, **5**) and furanoses (**8**–**10**) described above and three more pyranose analogues (the D-*allo* isomer **3** of **1** and the D-*allo* isomer **6** and D-*galacto* isomer **7** of **5**) by isolation from plants, microbial conversion, and chemical synthesis, we have systematically investigated their potency and specificity toward the various forms of glycosidases which were purified or partially purified from mammalian tissues. In this paper, we attempt to find which functional groups are essential for inhibitory

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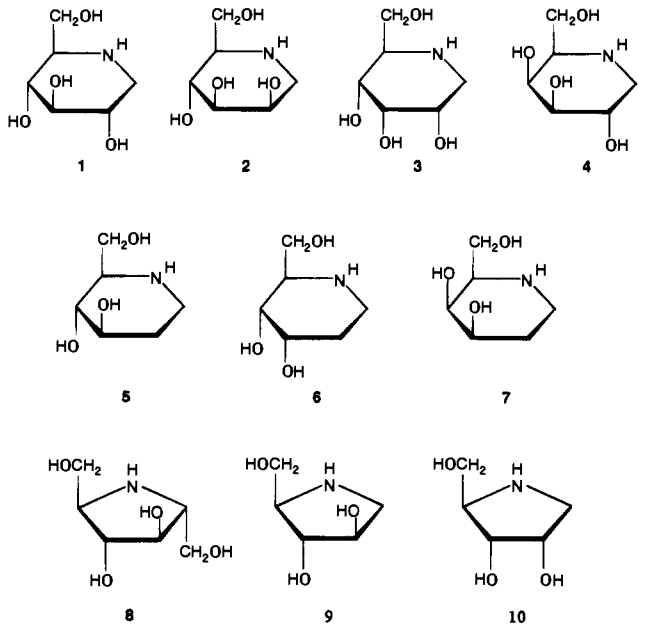
Chart 1



activity and specificity or what a common binding topography for the active glycosidase inhibitors is.

Results and Discussion

Preparation of Sugars with a Nitrogen in the Ring. 1-Deoxynojirimycin (1) was first found in mulberry tree, *Morus* sp.[22] We recently have isolated many nitrogen-in-the-ring sugars containing 1, **5**, **6**, **9**, and **10** from *Morus alba*.[23] 2,5-Dideoxy-2,5-imino-D-mannitol (**8**) was first isolated from the leaves of *Derris elliptica* (Leguminosae)[24] and later isolated from the seeds of *Lonchocarpus sericeus* (Leguminosae)[25] and the leaves of *Omphalea diandra* (Euphorbiaceae).[26] We isolated 1-deoxymannojirimycin (**2**) and **8** from the leaves of *Derris malaccensis* collected in the greenhouse of The Medicinal Plants Garden of Hokuriku University. The D-*allo* isomer of 1 (1,5-dideoxy-1,5-imino-D-allitol, **3**) was prepared by the microbial conversion with *Flavobacterium saccharophilum* from *N*-(benzyloxycarbonyl)-1-deoxynojirimycin (11). *F. saccharophilum* has glucoside 3-dehydrogenase [EC 1.1.99.13], which oxidizes the 3-OH group of D-glucose, D-galactose, 1,5-anhydro-D-glucitol, 1,5-anhydro-D-galactitol, the glucosides and galactosides, and the carba-oligosaccharides such as validamycins to give the corresponding 3-keto derivative.[27,28] We found that the incubation of a *F. saccharophilum* cell suspension containing 11 forms the *N*-benzyloxycarbonyl derivative of **3** by a redox reaction at C-3, as shown in Scheme 1. The preparation of the D-*galacto* isomers of 1 and **5** (**4** and **7**, respectively) was achieved by the chemical epimerization of the 4-OH group according to the method of Heiker and Scheller.[29]

Inhibition of Mammalian α-Glucosidases. The IC_{50} values of nitrogen-in-the-ring pyranoses and furanoses against various mammalian α-glucosidases are shown in Table 1.

1-Deoxynojirimycin (1) has been shown to be a potent inhibitor of all types of mammalian α-glucosidases.[4-8] The epimerization and/or deoxygenation of 1 markedly lowered or abolished its inhibition toward α-glucosidases. However, the D-*allo* isomers of 1 and fagomine (**5**) (**3** and **6**, respectively) fairly retained a potency toward intestinal isomaltase (IC_{50} values of 34 and 6.4 μM, respectively), while only the D-*manno* isomer **2** of 1 fairly retained a potency toward rat and bovine liver lysosomal α-glucosidases (IC_{50} values of 25 and 21 μM, respectively). Compound **9**, which loses one hydroxymethyl group from **8** and is a potent inhibitor of yeast α-glucosidase,[19] was an about 10-fold more effective inhibitor of isomaltase than of maltase. Compounds 1 and **9** were potent competitive inhibitors of ER α-glucosidase II involved in N-linked oligosaccharide processing, with K_i values of 1.3 and 9.7 μM, respectively (summarized in Table 5). Compound 1 has been reported to be a better inhibitor of α-glucosidase II than of α-glucosidase I, as concentrations of 20 μM are required to inhibit α-glucosidase I whereas concentrations of only 2 μM are needed for α-glucosidase II.[8] DMDP (**8**) inhibits α-glucosidase I in cell culture to accumulate glycoproteins with high-mannose structures that were mostly $Glc_3Man_{7-9}(GlcNAc)_2$ types,[18] but in the present work, it has no effect on α-glucosidase II at 1 mM. Thus, DMDP has proved to be a very specific inhibitor of α-glucosidase I.

Inhibition of Mammalian α-Mannosidases and α-L-Fucosidase. 1-Deoxymannojirimycin (**2**) inhibits *in vivo* conversion of high-mannose oligosaccharides to complex type oligosaccharides by blocking Golgi α-mannosidase I activity to form glycoproteins mostly consisting of high-mannose structures of $Man_9(GlcNAc)_2$ types.[13,30] As shown in Table 2, only compounds **2** and **9** were good inhibitors of Golgi α-mannosidase I with IC_{50} values of 25 and 53 μM, respectively, and inhibited Golgi α-mannosidase II in a competitive manner, with K_i values of 410 and 35 μM, respectively (Table 5).

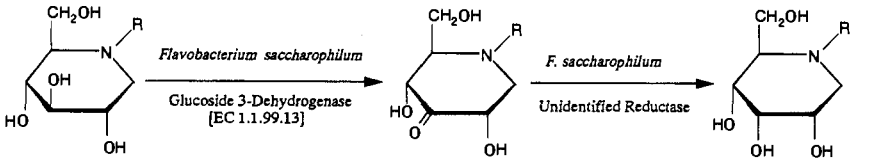
These compounds were further weak inhibitors of lysosomal and epididymal α-mannosidases but not inhibitors of soluble (or ER) α-mannosidases, which are presumed to be involved in the early steps of oligosaccharide processing.[31] In contrast, compound **8** was a weak inhibitor of soluble α-mannosidases, but it exhibited no appreciable inhibition for other α-mannosidases.

Compound **2** is also a good inhibitor of α-L-fucosidase in addition to α-mannosidase.[32] A weak inhibitory activity against α-L-fucosidase was observed in fagomine (**5**) which is the 2-deoxy derivative of 1.

Inhibition of Mammalian β-Galactosidases. As shown in Table 3, the deoxygenation at C-2 and/or epimerization at C-3 of 1 enhanced its inhibition toward rat intestinal lactase and bovine liver cytosolic β-galactosidase. The D-*allo* isomer **6** of **5** potently inhibited rat intestinal lactase and bovine liver cytosolic β-galactosidase in a competitive manner, with K_i values of 1.9 and 1.5 μM, respectively (Table 5). Compound **8** was also a potent inhibitor of these two enzymes, but both **6** and **8** exhibited no significant inhibition toward lysosomal and epididymal enzymes, which are optimally active in acidic conditions. 1,5-Dideoxy-1,5-imino-D-galactitol (**4**), which is an extremely powerful inhibitor of coffee bean α-galactosidase with a K_i value of 0.0016 μM,[14] showed a weak or no inhibitory activity toward mammalian β-galactosidases. Thus, 5-amino-5-deoxy-D-galactopyranose (D-*galacto*-nojirimycin) is a potent inhibitor of mammalian β-galactosidases,[14,33] whereas its 1-deoxy derivative (**4**) has been proven to be an extremely specific inhibitor of α-galactosidases in this study.

Inhibition of Mammalian Trehalases and β-Glu-

Scheme 1



1 R = H
11 R = -CO-O-$CH_2C_6H_5$

3 R = H
12 R = -CO-O-$CH_2C_6H_5$

Table 1. Concentration of Nitrogen-in-the-Ring Pyranoses and Furanoses (μM) Giving 50% Inhibition of Mammalian α-Glucosidases[a]

	α-glucosidase				
	rat intestine		rat liver		bovine
compd	maltase	isomal-tase	ER[b] glucosidase II	lysosomal	liver lysosomal
1	0.36	0.3	4.6	0.4	0.4
2	150	NI	NI	25	21
3	560	34	NI	NI	NI
4	NI[c]	940	NI	1000	NI
5	820	460	840	NI	520
6	500	6.4	NI	NI	NI
7	NI	NI	NI	NI	NI
8	NI	91	NI	92	170
9	55	5.8	20	100	89
10	NI	17	470	610	540

[a] Maltase and isomaltase were colorimetrically assayed by the D-glucose oxidase–peroxidase method using maltose and isomaltose, respectively, as substrate. Other enzyme activities were colorimetrically determined using *p*-nitrophenyl α-D-glucopyranoside as substrates. [b] ER = endoplasmic reticulum. [c] NI = less than 50% inhibition at 1000 μM.

Table 2. Concentration of Nitrogen-in-the-Ring Pyranoses and Furanoses (μM) Giving 50% Inhibition of Mammalian α-Mannosidases[a]

	α-mannosidase					
	rat liver					α-L-fucosidase
compd	Golgi I	Golgi II	lyso-somal	soluble	rat epididymis	bovine epididymis
1	NI[b]	NI	1000	NI	320	NI
2	25	500	570	NI	210	26
3	NI	NI	NI	NI	NI	NI
4	NI	NI	NI	NI	NI	NI
5	NI	1000	NI	NI	NI	140
6	NI	NI	NI	NI	NI	1000
7	NI	NI	NI	NI	NI	NI
8	NI	NI	NI	260	NI	NI
9	53	46	110	NI	84	NI
10	NI	800	1000	NI	NI	NI

[a] Golgi α-mannosidase I activity was assayed using HPLC and $Man_6(GlcNAc)_2$-PA as substrate, measuring the decrease of the substrate. Other α-mannosidase and α-L-fucosidase activities were colorimetrically determined using *p*-nitrophenyl α-D-mannopyranoside and *p*-nitrophenyl α-L-fucopyranoside, respectively, as substrate. [b] NI = less than 50% inhibition at 1000 μM.

cosidases. Compound **8** has been shown to be a good inhibitor of insect trehalase and almond β-glucosidase, with IC_{50} values of 55 and 78 μM, respectively,[32,34] but it was a much weaker inhibitor than **9** of mammalian trehalases. The epimerization at C-2 and/or deoxygenation at C-3 of **1** tend to enhance its inhibitory activity toward intestinal β-glucosidase (cellobiase), as seen in β-galactosidases. Although **8** was a good inhibitor of intestinal β-glucosidase, **9** exhibited no appreciable inhibition.

From the inhibitory activities toward α- and β-glucosidases, trehalases, and β-galactosidases, the relationship between **8** and **9** has been found to be very similar to that between nojirimycin and 1-deoxynojirimycin (**1**).[4,35]

Table 3. Concentration of Nitrogen-in-the-Ring Pyranoses and Furanoses (μM) Giving 50% Inhibition of Mammalian β-Galactosidases[a]

	β-galactosidase				
	rat intestine	rat liver	rat	bovine liver	
compd	lactase	lysosomal	epididymis	cytosolic	lysosomal
1	26	NI	NI	NI	NI
2	NI[b]	NI	NI	NI	NI
3	27	NI	NI	300	NI
4	30	390	500	NI	NI
5	15	NI	NI	38	NI
6	4	NI	NI	3.1	NI
7	NI	NI	NI	NI	NI
8	3.6	NI	NI	3.3	NI
9	260	NI	NI	1000	NI
10	17	NI	NI	580	NI

[a] Lactase activity was colorimetrically assayed by the D-glucose oxidase–peroxidase method using lactose as substrate. Other enzyme activities were colorimetrically determined using *p*-nitrophenyl β-D-galactopyranoside as substrate. [b] NI = less than 50% inhibition at 1000 μM.

Table 4 Concentration of Nitrogen-in-the-Ring Pyranoses and Furanoses (μM) Giving 50% Inhibition of Mammalian Trehalases and β-Glucosidases[a]

	trehalase		β-glucosidase	
compd	rat intestine	porcine kidney	rat intestine cellobiase	rat liver lysosomal
1	42	41	520	NI
2	NI[b]	NI	NI	NI
3	NI	NI	460	NI
4	NI	NI	NI	NI
5	NI	NI	NI	NI
6	NI	NI	100	NI
7	NI	NI	NI	NI
8	360	200	34	NI
9	25	4.8	NI	NI
10	NI	NI	230	NI

[a] Trehalase and cellobiase activities were assayed colorimetrically by the D-glucose oxidase–peroxidase method using trehalose and cellobiose, respectively, as substrate. Lysosomal β-glucosidase activity was determined colorimetrically using *p*-nitrophenyl β-D-glucopyranoside as substrate. [b] NI = less than 50% inhibition at 1000 μM.

Structure–Activity Relationship and Design of New Inhibitors. The way to the design of new glycosidase inhibitors would be to know the mechanism of action of glycosidases and the mode of binding of the inhibitors with the active site of glycosidases. Most work on these problems has focused on glucosidase inhibition. Reversible α-glucosidase inhibitors often have structures closely resembling glucose, that is, they have a common topographical property.

1-Deoxynojirimycin (**1**), a pyranose analogue with a nitrogen in the ring, superimposes well on castanospermine,[36,37] while 1,4-dideoxy-1,4-imino-D-arabinitol (**9**), a furanose analogue with a nitrogen in the ring,

Table 5. Measured K_i Values and Modes of Inhibition

inhibitor	enzyme	K_i (μM)[a]	inhibition[b]
1	ER α-glucosidase II	1.3	competitive
2	Golgi α-mannosidase II	410	competitive
6	rat intestinal lactase	1.9	competitive
	bovine liver β-galactosidase	1.5	competitive
9	ER α-glucosidase II	9.7	competitive
	Golgi α-mannosidase II	35	competitive

[a,b] Inhibition constant (K_i) and mode of inhibition were determined by the Lineweaver–Burk plot.

superimposes well on DMDP (**8**), australine,[15] and 7-*epi*-australine.[38] Compound 1 and castanospermine were potent inhibitors of all types of mammalian α-glucosidases. Australine and 7-*epi*-australine are ER α-glucosidase I inhibitors but not ER α-glucosidase II inhibitors.[39,40] In this study, **8** has been proven to be not an inhibitor of ER α-glucosidase II. Compound **9** was a good inhibitor of ER α-glucosidase II, but it is unlikely to be an ER α-glucosidase I inhibitor. It has been known that an anti-HIV activity correlates well with an inhibitory effect against ER α-glucosidase I.[40,41] Compound **9** has no significant anti-HIV activity.[42,43] whereas ER α-glucosidase I inhibitors (such as *N*-methyl-1-deoxynojirimycin, *N*-butyl-1-deoxynojirimycin, **8**, and 7-*epi*-australine) show an anti-HIV activity.[40,42,43]

On the other hand, any structural similarity of α-mannosidase inhibitors to α-D-mannose is not immediately obvious. Winkler and Holan[44] have reported a computer graphics-based structure–activity study of the activities of various α-mannosidase inhibitors on jack bean α-mannosidase, based on the results of molecular orbital calculations of the geometries and relative stabilities of the two half-chair forms of the mannopyranosyl cation. Their modeling studies attempted to find a common binding topography for the active mannosidase inhibitors that would also be inaccessible to the inactive mannose analogues. Although this model still presents some problems as pointed out by Elbein,[8] it is a vary attractive model. Compound **9** is a weak inhibitor of jack bean α-mannosidase with IC_{50} values of 320 (our data) and 100 μM in ref 19. Although **9** superimposes well on their binding model on the basis of the mannosyl cation geometry, it is cited as an inactive inhibitor because of its low activity toward jack bean α-mannosidase. However, in our present work, **9** was a good inhibitor of rat liver Golgi α-mannosidase I with an IC_{50} value of 53 μM, which is comparable to that of 1-deoxymannojirimycin (**2**) (IC_{50} = 25 μM). Furthermore **9** was also a good competitive inhibitor Golgi α-mannosidase II, with a K_i value of 35 μM, as shown in Table 5. Thus, compound **9** should be regarded as an active inhibitor with the superimposition on the mannosyl cation.

It is of much interest that 1,2,5-trideoxy-1,5-imino-D-*allo*-hexitol (**6**) showed a potent inhibitory activity toward intestinal lactase and bovine liver β-galactosidase. The deoxygenation at C-2 and/or epimerization at C-3 of 1 definitely enhance its inhibition toward β-galactosidase and β-glucosidase, as does the epimerization at C-2 (**10**) of **9**. Compound **10** has two ring hydroxyl groups lying in the same region of space as C-3 and C-4 of **6**, with the hydroxymethyl groups also being topographically equivalent. However, any structural similarity of **6** or **10** to β-D-galactose has not been obvious yet. Broad inhibitory activities of **9** toward various α-glucosidases, α-mannosidases, and trehalase suggest that it superimposes well on the various glycosyl cations.

There are marked differences in the inhibition by sugar analogues of the various forms of a glycosidase in different species and even within the same cell. The molecular modeling studies, having taken this differential inhibition into consideration, will provide useful information for the design of new classes of inhibitors and the modifications to existing glycosidase inhibitors which will enhance their activity.

Experimental Section

General Methods. Sugars with a nitrogen in the ring were chromatographed by HPTLC silica gel-60F_{254} (E. Merck) using solvent system *n*-PrOH–AcOH–H_2O (4:1:1), with detection by spraying with the chlorine-*o*-tolidine reagent. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Jeol JNM-GX 400 spectrometer as indicated in D_2O using sodium 3-(trimethylsilyl)propionate (TSP) as an internal standard. Mass spectrometry data were measured on a Jeol JMX-DX 300 JMA-DA 5000 spectrometer.

HPLC Analysis. Waters M-600E multisolvent delivery system equipped with a Hitachi Model F-1050 fluorescence spectrophotometer was used. HPLC anlaysis was performed using a μBondasphere 5 μm C18 column (0.46 × 15 cm) from Nihon Waters, Ltd. The elution buffer was 0.1 M acetic acid adjusted to pH 4.0 with triethylamine. The flow rate was 0.4 mL/min, and the column temperature was room temperature. For detection of PA–sugar chains, an excitation wavelength of 320 nm and an emission wavelength of 400 nm were used.

Materials. Porcine kidney trehalase (EC 3.2.1.28), bovine liver cytosolic β-galactosidase (EC 3.2.1.23), jack bean α-mannosidase (EC 3.2.1.24), and bovine epididymis α-L-fucosidase (EC 3.2.1.51) were purchased from Sigma Chemical Co. Various *p*-nitrophenyl glycosides and palatinose were purchased from Sigma Chemical Co. Other disaccharides were purchased from Wako Pure Chemical Industries. Pyridylamino (PA)–sugar chain "018" ($Man_6(GlcNAc)_2$-PA) was purchased from Takara Biochemicals.

Preparation and Isolation of Sugars with a Nitrogen in the Ring. 1-Deoxynojirimycin (1), fagomine (**5**), 3-*epi*-fagomine (**6**), 1,4-dideoxy-1,4-imino-D-arabinitol (**9**), and 1,4-dideoxy-1,4-imino-D-ribitol (**10**) were isolated from the roots of *M. alba*.[23] 2,5-Dideoxy-2,5-imino-D-mannitol (**8**) and 1-deoxymannojirimycin (**2**) were isolated from the leaves of *D. malaccensis* as follows. The leaves of *D. malaccensis* (150 g, dry weight) collected in the greenhouse of The Medicinal Plants Garden of Hokuriku University were extracted with hot water. The extract was applied to an Amberlite IR-120B column (200 mL, H^+ form) and eluted with 0.5 M NH_4OH. The concentrated eluate was applied to a Dowex 1-X2 column (1.8 × 95 cm, OH^- form) and eluted with water to give **8** (1.1 g) and **2** (24 mg), respectively. The ^{13}C NMR data and optical rotation of **8** were identical to those previously described.[24] The absolute structure of **2** was confirmed as 1,5-dideoxy-1,5-imino-D-mannitol (1-deoxymannojirimycin) by comparison with an authentic sample microbially converted from 1.[45]

1,5-Dideoxy-1,5-imino-D-glucitol (1): R_F 0.37; $[\alpha]_D$ +42.1° (*c* 1, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 51.5 (C-1), 63.3 (C-5), 64.2 (C-6), 73.6 (C-2), 74.4 (C-4), 81.3 (C-3); EIMS *m/z* 163 (M^+, 1), 132 (M − CH_2OH, 100). Anal. ($C_6H_{13}NO_4$) C, H, N.

1,5-Dideoxy-1,5-imino-D-mannitol (**2**): R_F 0.33; $[\alpha]_D$ −41.4° (*c* 0.74, H_2O) (lit.[46] $[\alpha]_D$ −39°); ^{13}C NMR (100 MHz, D_2O) δ 51.5 (C-1), 63.4 (C-5), 63.7 (C-6), 71.3 (C-4), 72.1 (C-2), 77.5 (C-3); EIMS *m/z* 163 (M^+, 1), 132 (M − CH_2OH, 100). Anal. ($C_6H_{13}NO_4$) C, H, N.

1,2,5-Trideoxy-1,5-imino-D-*arabino*-hexitol (**5**): R_F 0.36; $[\alpha]_D$ +19.5° (*c* 1, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 35.6 (C-2), 45.4 (C-1), 63.7 (C-5), 64.5 (C-6), 76.1 (C-3,4); EIMS *m/z* 147 (M^+, 2), 116 (M − CH_2OH, 100). Anal. ($C_6H_{13}NO_3$) C, H, N.

1,2,5-Trideoxy-1,5-imino-D-*allo*-hexitol (**6**): R_F 0.36; $[\alpha]_D$ +69.0° (*c* 0.5, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 33.8 (C-2),

41.2 (C-1), 58.6 (C-5), 64.9 (C-6), 70.7 (C-3), 72.4 (C-4); EIMS *m/z* 147 (M^+, 5), 116 (M − CH_2OH, 100). Anal. ($C_6H_{13}NO_3$) C, H, N.

2,5-Dideoxy-2,5-imino-D-mannitol (8): R_F 0.55; $[\alpha]_D$ +56.9° (*c* 0.54, H_2O); (lit.[24] $[\alpha]_D$ +56.4°); ^{13}C NMR (100 MHz, D_2O) δ 64.4 (C-2,5), 64.9 (C-1,6), 80.7 (C-3,4); EIMS *m/z* 163 (M^+, 2), 132 (M − CH_2OH, 100). Anal. ($C_6H_{13}NO_4$) C, H, N.

1,4-Dideoxy-1,4-imino-D-arabinitol (9): R_f 0.45; $[\alpha]_D$ +6.3° (*c* 1, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 53.4 (C-1), 64.8 (C-5), 68.0 (C-4), 80.2 (C-2), 81.8 (C-3); FABMS (glycerol matrix) *m/z* 134 (M + 1, 30).

1,4-Dideoxy-1,4-imino-D-arabinitol Hydrochloride (9·HCl). The free base (10 mg) was dissolved in H_2O (3 mL) and acidified to pH 4 with dilute aqueous HCl. The solution was then freeze-dried to give 11.7 mg (92% yield) of a colorless solid: $[\alpha]_D$ +34.9° (*c* 1, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 52.8 (C-1), 61.8 (C-5), 69.4 (C-4), 77.1 (C-2), 78.5 (C-3); EIMS *m/z* 133 (M^+, 2), 102 (M − CH_2OH, 100). Anal. ($C_5H_{11}NO_3$·HCl) C, H, N.

1,4-Dideoxy-1,4-imino-D-ribitol (10): R_F 0.36; $[\alpha]_D$ +42.0° (*c* 0.53, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 52.8 (C-1), 64.4 (C-5), 65.2 (C-4), 73.9 (C-2), 75.8 (C-3); FABMS (glycerol matrix) *m/z* 134 (M + 1, 25).

1,4-Dideoxy-1,4-imino-D-ribitol Hydrochloride (10·HCl). The free base (10 mg) was dissolved in H_2O (3 mL) and acidified to pH 4 with dilute aqueous HCl. The solution was then freeze-dried to give 11.0 mg (87% yield) of a colorless solid: $[\alpha]_D$ +52.7° (*c* 0.72, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 52.5 (C-1), 60.8 (C-5), 64.6 (C-4), 72.3 (C-2), 74.0 (C-3); EIMS *m/z* 133 (M^+, 3), 102 (M − CH_2OH, 100). Anal. ($C_5H_{11}NO_3$·HCl) C, H, N.

1,5-Dideoxy-1,5-imino-D-allitol (3). 1,5-Dideoxy-1,5-imino-D-allitol (**3**) was prepared from *N*-(benzyloxycarbonyl)-1-deoxyjirimycin (11) by microbial conversion with *F. saccharophilum*. The washed cells (22 g, wet weight) of *F. saccharophilum* were suspended in 200 mL of 50 mM phosphate buffer (pH 6.5). The cell suspensions containing 1 g of 11 were incubated at 30 °C for 2 days. The incubation mixture was centrifuged, and the supernatant was applied to an Amberlite XAD-4 column (20 mL), eluted with methanol, and concentrated. The concentrate was then applied to a Toyopearl HW-40S column (3 × 100 cm) and eluted with 20% methanol (v/v) to give the D-*allo* isomer of 11 (85 mg; **12**). A solution of **12** (80 mg) in 50% ethanol (v/v, 10 mL) and acetic acid (2 mL) was hydrogenated in the presence of 5% palladium on carbon (200 mg) for 8 h at room temperature. The reaction mixture was processed conventionally and purified by a Dowex 1-X2 column (20 mL, OH^- form) to give **3** (34 mg): R_F 0.46 (1, 0.44); $[\alpha]_D$ +25.7° (*c* 0.65, H_2O); ^1H NMR (400 MHz, D_2O) δ 2.78 (1H, dd, J = 8.1, 13.2 Hz, H-1_{ax}), 2.99 (1H, dd, J = 3.7, 13.2 Hz, H-1_{eq}), 3.19 (1H, ddd, J = 4.4, 6.2, 8.1 Hz, H-5), 3.59−3.65 (2H, H-2,3), 3.75 (1H, dd, J = 4.4, 8.1 Hz, H-4), 3.76−3.80 (2H, H-6a,6b); ^{13}C NMR (100 MHz, D_2O) δ 46.9 (C-1), 59.2 (C-5), 60.7 (C-6), 72.7 (C-2), 73.3 (C-4), 75.0 (C-3); EIMS *m/z* 163 (M^+, 1), 132 (M − CH_2OH, 100). Anal. ($C_6H_{13}NO_4$) C, H; N: calcd, 8.65; found, 8.58.

1,5-Dideoxy-1,5-imino-D-galactitol (4) Hydrochloride. 1,5-Dideoxy-1,5-imino-D-galactitol (**4**) hydrochloride was synthesized from 1 by the method of Heiker and Schueller:[29] R_F 0.38; $[\alpha]_D$ +49° (*c* 0.1, H_2O) (lit.[47] $[\alpha]_D$ +46.1°); ^{13}C NMR (100 MHz, D_2O) δ 49.1 (C-1), 62.1 (C-5), 63.0 (C-6), 67.7 (C-2), 70.4 (C-4), 76.0 (C-3); EIMS *m/z* 163 (M^+, 3), 132 (M − CH_2OH, 100). Anal. ($C_6H_{13}NO_4$·HCl) C, H, N.

1,2,5-Trideoxy-1,5-imino-D-*galacto*-hexitol (7). 1,2,5-Trideoxy-1,4-imino-D-*galacto*-hexitol (**7**) was prepared from fagomine (**5**) by the same method as that used to synthesize **4** from 1: R_F 0.31; $[\alpha]_D$ +19.0° (*c* 0.46, H_2O); ^1H NMR (400 MHz, D_2O) δ 1.16−1.73 (2H, H-2_{ax},2_{eq}), 2.62 (1H, ddd, J = 4.4, 11.0, 13.0 Hz, H-1_{ax}), 2.76 (1H, dt, J = 1.5, 6.6 Hz, H-5), 3.09 (1H, ddd, J = 2.6, 4.4, 13.0 Hz, H-1_{eq}), 3.63 (1H, dd, J = 6.6, 11.5 Hz, H-6a), 3.67 (1H, dd, J = 6.6, 11.5 Hz, H-6b), 3.75 (1H, ddd, J = 3.0, 6.5, 10.5 Hz, H-3), 3.92 (1H, dd, J = 1.5, 3.0 Hz, H-4); ^{13}C NMR (100 MHz, D_2O) δ 30.2 (C-2), 45.6 (C-1), 61.8 (C-5), 64.5 (C-6), 70.5 (C-4), 72.7 (C-3); EIMS *m/z* 147 (M^+, 4), 116 (M − CH_2OH, 100). Anal. ($C_6H_{13}NO_3$) C, H; N: calcd, 9.61; found, 9.52.

Preparation and Assay Method of Glycosidases in Rat Liver: ER α-glucosidase II was purified 197-fold from microsomes as previously described.[48] Enzyme activity was assayed at pH 6.8 by measuring the hydrolysis of *p*-nitrophenyl (PNP) α-D-glucopyranoside, the released *p*-nitrophenol being determined by its absorbance at 400 nm.

α-Mannosidases I and II were partialy purified 1.7- and 4.2-fold from Golgi membranes according to the methods of Tulsiani et al.[49,50] Soluble α-mannosidase was purified 1130-fold from the soluble fraction of liver homogenates according to the method of Bishoff and Kornfeld,[31] and lysosomal α-mannosidase was purified 705-fold from liver homogenates according to the method of Opheim and Touster.[51] Golgi α-mannosidase I activity was assayed at pH 5.5 using HPLC and $Man_6(GlcNAc)_2$-PA as substrate, measuring the decrease of the substrate. α-Mannosidase II (pH 5.5) and soluble (pH 6.5) and lysosomal (pH 4.5) α-mannosidase activities were determined colorimetrically using PNP α-D-mannopyranoside as substrate.

The partially purified lysosomal fraction prepared by the procedures of Tsuji et al.[52] was used as a source of lysosomal α- and β-glucosidases and β-galactosidase. These lysosomal enzyme activities were colorimetrically assayed at pH 4.0 using the corresponding PNP glycoside.

Preparation of Rat Epididymal Fluid. The epididymal fluid was purified from epididymis according to the method of Skudlarek et al.[53] The purified epididymal fluid was assayed at pH 5.2 for α-mannosidase and at pH 3.5 for β-galactosidase using the corresponding PNP glycoside.

Preparation of Bovine Liver Lysosome. The lysosome fraction partially purified by the same procedures as those used to prepare rat liver lysosome was assayed at pH 4.0 for bovine liver lysosomal β-galactosidase using PNP β-D-galactopyranoside.

Preparation of Brush Border Membranes from Rat Intestine. Brush border membranes prepared from rat small intestine according to the method of Kessler et al.[54] were assayed at pH 5.8 for rat digestive glycosidases using the appropriate disaccharide as substrate. The released D-glucose was determined colorimetrically using the commercially available glucose B-test Wako (Wako Pure Chemical Ind.).

Kinetics of Inhibition. The nature of the inhibition against enzymes and the K_i values were determined from the Lineweaver−Burk plot.

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