New Sugar-Mimic Alkaloids from the Pods of *Angylocalyx pynaertii*

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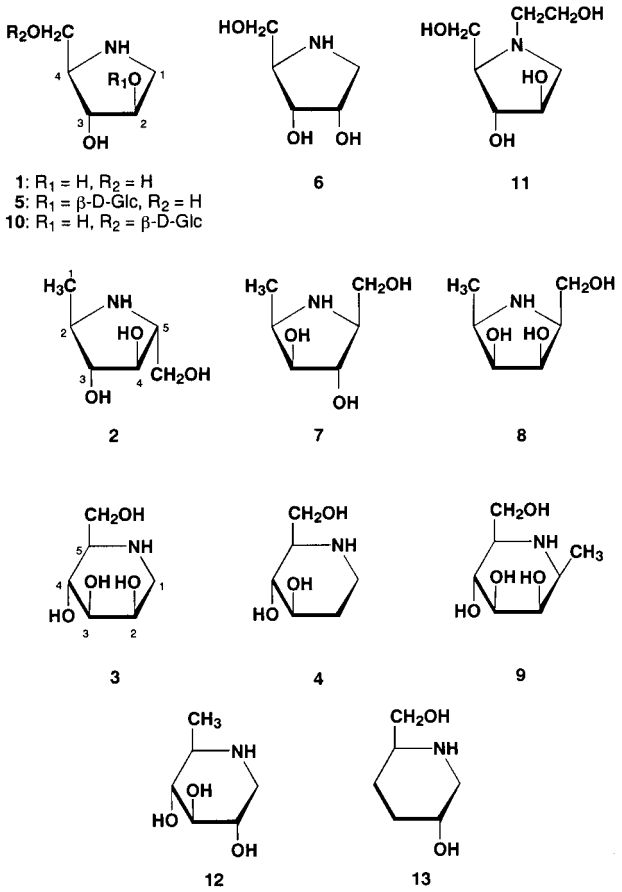
Chromatographic separation of the pod extract of *Angylocalyx pynaertii* resulted in the isolation of 13 sugar-mimic alkaloids (**1**−**13**). The structures of the new alkaloids were elucidated by spectroscopic methods as the 6-*O*-*β*-D-glucoside (**10**) and *N*-hydroxyethyl derivative (**11**) of 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) (**1**), 1,6-dideoxynojirimycin (**12**), and 1,3,4-trideoxynojirimycin (**13**). 2,5-Imino-1,2,5-trideoxy-L-glucitol (**7**), 2,5-dideoxy-2,5-imino-D-fucitol (**8**), and *β*-homofuconojirimycin (**9**), isolated from the pods as well as the bark, were very specific inhibitors of α-L-fucosidase with no significant inhibitory activity toward other glycosidases. In this work, 1,4-dideoxy-1,4-imino-D-ribitol (**6**) was found to be a better inhibitor of lysosomal *β*-mannosidase than 2,5-imino-1,2,5-trideoxy-D-mannitol (**2**). *N*-Hydroxyethyl-1-deoxynojirimycin (miglitol), which is commercially available for the treatment of diabetes, retained its inhibitory potential toward rat intestinal maltase and sucrase, whereas **11** and the synthetic *N*-hydroxyethyl derivative of 2,5-dideoxy-2,5-imino-D-mannitol markedly lowered or abolished their inhibition toward all enzymes tested.

The genus *Angylocalyx* (Leguminosae) consists of seven species of shrubs and trees growing in tropical African forests.[1] Six of them have been examined with regard to alkaloidal composition, and 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) (**1**) has been detected or isolated from all examined, including *Angylocalyx pynaertii* DeWild.[2] In 1993, Molyneux et al. isolated 2,5-imino-1,2,5-trideoxy-D-mannitol (6-deoxy-DMDP) (**2**), 1-deoxymannojirimycin (DMJ) (**3**), and fagomine (**4**), in addition to **1**, from the seeds of *A. pynaertii* and found that **2** is a weak competitive inhibitor of *β*-mannosidase.[3] In the course of a search for specific lysosomal glycosidase inhibitors from plants, we found that the extract of the bark of *A. pynaertii* shows a potent inhibitory activity toward rat α-L-fucosidase (IC_{50} = 3.1 *μ*g/mL) and reported that a thorough examination of the bark extract resulted in the isolation of 15 sugar-mimic alkaloids, including the known alkaloids **1**, **2**, **3**, 2-*O*-*β*-D-glucopyranosyl-DAB (**5**), *N*-methyl-DAB, 1,4-dideoxy-1,4-imino-D-ribitol (DRB) (**6**), 1,4-dideoxy-1,4-imino-D-xylitol, 2,5-imino-1,2,5-trideoxy-L-glucitol (**7**), 2,5-dideoxy-2,5-imino-D-fucitol (**8**), 2,5-imino-1,2,5-trideoxy-D-altritol, *N*-methyl-DMJ, 6-*O*-α-L-rhamnopyranosyl-DMJ (α-Rha-DMJ), *β*-L-homofuconojirimycin (*β*-HFJ) (**9**), 1-deoxyaltronojirimycin, and 1-deoxygulonojirimycin.[4] We previously reported that **7**, α-Rha-DMJ, and **9** are very potent competitive inhibitors of bovine epididymis α-L-fucosidase, with K_i values of 0.49, 0.06, and 0.0053 *μ*M, respectively.[4]

In this paper, we describe the isolation of 13 sugar-mimic alkaloids (**1**−**13**) from the pods of *A. pynaertii*, structural determination of new alkaloids **10**−**13**, and inhibitory activities of the alkaloids obtained in this work and chemically synthetic ones toward a variety of glycosidases.



Results and Discussion

We previously reported that the bark extract treated with Amberlite IR-120B (H^+ form) and Dowex1-X2 (OH^- form) showed a potent inhibitory activity toward rat α-L-fucosidase (IC_{50} = 3.1 *μ*g/mL).[4] The 50% aqueous EtOH extract of the pods was also similarly treated with ion-exchange resins and tested for inhibitory activity of rat

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epididymis glycosidases. The pod extract showed a more potent inhibitory activity ($IC_{50} = 0.23\ \mu g/mL$) than did the bark extract toward rat epididymis α-L-fucosidase.

The pods (2.2 kg) of *A. pynaertii* were extracted with 50% aqueous EtOH. Chromatographic separation of the extract afforded 13 alkaloids (**1**−**13**). Alkaloids **1**−**9**, other than **4**, were isolated from the pods as well. Alkaloid **4** was determined to be fagomine from the optical rotation and ^1H NMR and ^{13}C NMR spectral data. The structures of new alkaloids **10**−**13** isolated from the pods were determined from HRFABMS and various 1D and 2D NMR spectral data. We report here the structural determination of new alkaloids **10**−**13** and present full details on the structural determination of **7** and **8**, which were not reported in our previous paper.

Alkaloid **7** showed the molecular formula $C_6H_{13}NO_3$ by HRFABMS. The ^{13}C NMR spectral data revealed the presence of a single methyl (δ 15.4), a single methylene (δ 64.6), and four methine (δ 58.7, 68.5, 81.7, 82.3) carbon atoms, suggesting that **7** was an isomer of 6-deoxy-DMDP (**2**). Irradiation of H-5 enhanced the NOE intensity of H-2 and H-3, and a definite NOE between H-4 and C-6 (CH_2-OH) protons was also observed. These results suggest that H-2, H-3, H-4, and H-5 are in the α, α, *β*, and α orientations, respectively. Thus, alkaloid **7** was determined to be 2,5-imino-1,2,5-trideoxy-L-glucitol or its enantiomer. In the NMR spectra of **7**, minor signals were always observed, and no separations were observed using several chromatographic techniques. Since the intensity of the minor signals changed appreciably with different solvents and temperature in the NMR measurement, these minor signals were suggested to be due to those of the conformational isomer.

Alkaloid **8** showed the molecular formula $C_6H_{13}NO_3$ by HRFABMS. The ^{13}C NMR spectral data (δ 15.2, 58.1, 62.8, 63.4, 79.3, 80.9) suggest that **8** is also an isomer of 6-deoxy-DMDP. The definite NOEs between H-2 and H-3, between H-3 and H-4, and between H-4 and H-5 indicate that H-2, H-3, H-4, and H-5 are all in the α orientation (Figure 1a). Thus, alkaloid **8** was determined to be 2,5-dideoxy-2,5-imino-D-fucitol or its enantiomer.

Alkaloid **10** showed the molecular formula $C_{11}H_{21}NO_8$ by HRFABMS. The response to the naphthoresorcinol-sulfuric acid reagent and the characteristic anomeric proton (H-1′, δ 4.51, $J = 7.8$ Hz) and carbon (C-1′, δ 105.6) signals in the NMR suggested that **10** was a glycoside of an alkaloid. Six signals (δ 63.7, 72.6, 76.1, 78.6, 78.9, 105.6) in the ^{13}C NMR spectrum were completely in accord with those of the *β*-D-glucopyranosyl group in 6-*O*-*β*-D-glucopyranosyl-1-deoxynojirimycin[5] and 7-*O*-*β*-D-glucopyranosyl-α-homonojirimycin.[6] The strong NOEs from the anomeric proton to the C-5 hydroxymethyl protons (H-5a and H-5b) of the aglycone indicated that the *β*-glucosidic linkage site is C-5. The aglycone part was determined to be DAB from the decoupling and COSY experiments, the NOE effect between H-3 and H-5b (δ 4.13) or H-1*β* (δ 3.26), and ^{13}C NMR chemical shifts. Therefore, **10** was shown to be 5-*O*-*β*-D-glucopyranosyl-DAB. The 7.8 ppm downfield shift for C-5 and 1.3 ppm upfield shift for C-4 produced by the *β*-glucoside formation also supported this structure.

Alkaloid **11** showed the molecular formula $C_7H_{15}NO_4$ by HRFABMS. The ^{13}C NMR spectra showed the presence of three methine and four methylene carbon signals. The complete connectivity of the carbon and hydrogen atoms was defined from analysis of decoupling experiments and COSY and HMBC spectra. These experiments elucidated a $CH_2-CH-CH-CH-CH_2OH$ fragment, showing a linear sequence from the C-1 methylene group to the C-5 hydroxymethyl. The methine carbons at δ 78.3 and 81.7 were assigned to C-2 and C-3 bearing OH groups, and the methylene carbons at δ 59.1 and 62.2 to the *N*-hydroxyethyl group. The C-4 methine at δ 74.9 and C-1 methylene at δ 61.6 carbons must be bonded to the nitrogen of the pyrrolidine ring. Irradiation of H-4 enhanced the NOE signal intensity of H-2 and H-1α, and the NOE effect between H-3 and the C-5 (CH_2OH) protons were also observed (Figure 1b). These results suggest that H-2, H-3, and H-4 are in the α, *β*, and α orientations, respectively. Thus, alkaloid **11** was determined to be *N*-hydroxyethyl-DAB. Furthermore, the NMR spectral data and specific rotation of the *N*-hydroxyethyl derivative prepared by heating DAB (**1**) with 2-bromoethanol in the presence of anhydrous potassium carbonate in DMF were completely in accord with those of the natural product. The 2-deoxy derivative of **11**, *N*-(hydroxyethyl)-2-(hydroxymethyl)-3-hydroxypyrrolidine, has been isolated from the seeds of *Castanospermum australe*.[7]

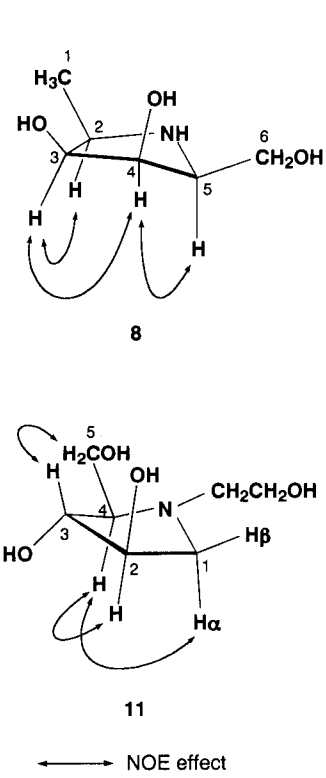

Figure 1. NOE interactions of 2,5-dideoxy-2,5-imino-D-fucitol (**8**) (a) and 1,4-dideoxy-1,4-imino-(hydroxyethyliminiumyl)-D-arabinitol (**11**) (b).

Alkaloid **12** showed the molecular formula $C_6H_{13}NO_3$ by HRFABMS. ^{13}C NMR spectral data revealed the presence of a single methyl (δ 19.8), a single methylene (δ 51.5), and four methine (δ 57.9, 73.9, 79.2, 80.9) carbon atoms. Decoupling experiments and COSY spectra elucidated a $CH_2-CH-CH-CH-CH-CH_3$ moiety, showing a linear sequence from the C-1 methylene group to the C-6 methyl. In the ^1H NMR spectrum, the large *J* values ($J_{2,3} = J_{3,4} = J_{4,5} = 9.3$ Hz) seen in the signals of H-3 and H-4 indicated an all *trans*-axial orientation of H-2, H-3, H-4, and H-5. An NOE effect between H-1ax and H-3 or H-5 indicated that the six-membered ring was in a $^4C_1$ chair conformation. Thus, alkaloid **12** was determined to be 1,6-dideoxynojirimycin. Although an *N*,*O*-tribenzyl derivative of **12** has been chemically synthesized as a structural unit of a pseudooligosaccharide type of inhibitor, its free base has not previously been characterized.[8]

Table 1. Concentration of Sugar-Mimic Furanoses and the Glucosides Giving 50% Inhibition of Various Glycosidases

	IC_{50} (μM)							
enzyme	**1**	**2**	**5**	**6**	**7**	**8**	**10**	**11**
α-glucosidase								
rice	120	–	–	1000	–	–	–	–
yeast	1.2	270	–	230	570	–	–	290
rat intestinal maltase	55	–	–	–	–	–	–	–
rat intestinal sucrase	16	–	–	95	–	–	–	250
rat intestinal isomaltase	5.8	–	–	17	–	–	–	84
α-mannosidase								
rat epididymis	150	–	–	–	–	–	–	–
β-mannosidase								
rat epididymis	290	93	–	18	–	–	–	–
α-galactosidase								
coffee bean	–[a]	–	–	–	–	–	–	700
β-galactosidase								
bovine liver	–	30	–	380	420	–	–	–
α-L-fucosidase								
bovine epididymis	–	150	–	–	1	35	–	–
amyloglucosidase								
Aspergillus niger	400	160	–	1000	510	–	–	620

[a] – indicates no inhibition (less than 50% inhibition at 1000 μM).

Table 2. Concentration of 1,4-Dideoxy-1,4-imino-D-arabinitol (DAB), 2,5-Dideoxy-2,5-imino-D-mannitol (DMDP), 1-Deoxynojirimycin (DNJ), and Their *N*-Hydroxyethyl (*N*-HE) Derivatives Giving 50% Inhibition of Various Glycosidases

	IC_{50} (μM)					
enzyme	DAB (**1**)	*N*-HE-DAB (**11**)	DMDP	*N*-HE-DMDP	DNJ	*N*-HE-DNJ
α-glucosidase						
rice	120	–[b]	300	1000	0.05	0.15
yeast maltase[a]	0.84	78	2.0	260	110	–
yeast isomaltase[a]	0.11	12	2.0	240	42	–
rat intestinal maltase	55	–	290	–	0.36	0.49
rat intestinal sucrase	16	250	–	–	0.21	0.19
rat intestinal isomaltase	5.8	84	91	–	0.30	1.10
α-mannosidase						
rat epididymis	150	–	–	–	320	–
β-mannosidase						
rat epididymis	290	–	14	–	–	–
β-galactosidase						
bovine liver	–	–	3.3	–	–	150
trehalase						
porcine kidney	2.5	–	200	–	41	700

[a] Activities of yeast maltase and isomaltase were determined using maltose and isomaltose, respectively, as substrate at pH 6.8. The released D-glucose was determined colorimetrically using Glucose B-test Wako. [b] – indicates no inhibition (less than 50% inhibition at 1000 μM).

Alkaloid **13** showed the molecular formula $C_6H_{13}NO_2$ by HRFABMS. ^{13}C NMR spectral data revealed the presence of four methylene (δ 29.0, 34.8, 54.1, 67.7) and two methine (δ 59.1, 70.0) carbon atoms. Decoupling experiments, COSY, and HMBC spectra revealed a $CH_2-CH-CH_2-CH_2-CH-CH_2OH$ moiety. The coupling pattern of H-1ax ($J_{1ax,2} = 10.2$, $J_{1ax,1eq} = 11.5$ Hz) indicated axial orientation of H-2. An NOE effect between H-1ax and H-3 or H-5 indicated that the six-membered ring was in a $^4C_1$ chair conformation. Thus, alkaloid **13** was determined to be 1,3,4-trideoxynojirimycin.

We previously reported that alkaloids **2**, **3**, **7**, **8**, and **9** are inhibitors of various α-L-fucosidases.[4] Among these alkaloids, **7** and **9** were very potent inhibitors of bovine epididymis α-L-fucosidase, with IC_{50} values of 1 and 0.01 μM, respectively.[4] The IC_{50} values of sugar-mimic furanoses (polyhydroxylated pyrrolidine alkaloids) toward various glycosidases are shown in Table 1. Although 6-deoxy-DMDP (**2**) has been reported to be a weak inhibitor of snail β-mannosidase,[3] DRB (**6**) was a better inhibitor of lysosomal β-mannosidase than 6-deoxy-DMDP. The isomers of 6-deoxy-DMDP, **7** and **8**, were very specific inhibitors of α-L-fucosidase with no significant inhibitory activity toward other glycosidases. DAB (**1**) is known to be a potent inhibitor of yeast α-glucosidase[9] and mammalian isomaltase.[10,11] Recently, DAB has been found to be a potent inhibitor of glycogen phosphorylase both in vitro and in vivo.[12,13] The *N*-methyl derivative of DAB was isolated from the bark, while *N*-hydroxyethyl-DAB (**11**) was found in the pods. The *N*-hydroxyethyl derivative of 1-deoxynojirimycin (DNJ), miglitol, is commercially available for the treatment of diabetes in several countries. We previously reported that the introduction of the *N*-alkyl residues to furanose-mimic alkaloids significantly lowered its inhibition toward glycosidases.[14] To investigate the change in potency and specificity of bioactivity by the introduction of a polar substituent to iminosugars, we prepared the *N*-hydroxyethyl derivatives of DMDP and DNJ. As shown in Table 2, the *N*-hydroxyethylation of DAB and DMDP markedly lowered or abolished its inhibition toward all enzymes tested. The derivatization of DNJ showed no change for rat intestinal maltase and sucrase but slightly lowered its potential toward rat isomaltase.

Although DNJ is a very potent inhibitor of various α-glucosidases,[11] the deoxygenation at C-6 to form **12** completely abolished its inhibition toward all α-glucosidases tested. This suggests a favorable interaction between the C-6 hydroxyl group and the active site of α-glucosidase. The 3,4-dideoxy derivative (**13**) of DNJ also showed no

significant inhibitory activity toward any glycosidases tested.

Experimental Section

General Experimental Procedures. The purity of samples was checked by HPTLC on silica gel $60F_{254}$ (E. Merck) using the solvent system PrOH−AcOH−H_2O (4:1:1), and a chlorine−*o*-tolidine reagent was used for detection. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL JNM-GX 400 spectrometer. Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP) in D_2O as an internal standard. The assignment of proton and carbon signals in NMR was determined from extensive homonuclear decoupling experiments, DEPT, ^{1}H−^{13}C COSY, and HMBC spectral data. FABMS were measured using glycerol as a matrix on a JEOL JMS-SX 102A spectrometer.

Plant Materials. The pods of *A. pynaertii* were collected on June 8, 1988, in the Republic of Cameroon.[3] The voucher specimen (No. 3738415) is deposited in the Missouri Botanical Garden Herbarium. The dried plant materials were pulverized with a ball mill to produce powder.

Extraction and Isolation. The pod powder (2.2 kg) of *A. pynaertii* was extracted three times with 6 L of 50% aqueous EtOH. The extract was applied to a column of Amberlite IR-120B (500 mL, H^+ form). The 0.5 M NH_4OH eluate was concentrated to give a brown syrup (46 g), which was chromatographed over an Amberlite CG-50 column (3.8 × 95 cm, NH_4^+ form) with H_2O as eluant (fraction size 15 mL). Fractions 28−63 and 64−90 were collected and concentrated to give 350 mg (pool I) and 580 mg (pool II), respectively. The 0.5 M NH_4OH eluate from the same column was concentrated to give a brown syrup of 3.45 g (pool III). Each pool was further chromatographed with Dowex 1-X2 (OH^- form) and/or CM-Sephadex C-25 (NH_4^+ form) columns to give alkaloids **11** (6 mg) from pool I, **3** (565 mg) and **12** (4 mg) from pool II, and **1** (1.03 g), **2** (51 mg), **4** (10 mg), **5** (22 mg), **6** (5 mg), **7** (729 mg), **8** (6 mg), **9** (36 mg), **10** (3 mg), and **13** (5 mg) from pool III.

2,5-Imino-1,2,5-trideoxy-D-glucitol (7): $[\alpha]_D$ +23.3° (*c* 0.64, H_2O); ^{1}H NMR (400 MHz, D_2O) the major conformer, δ 1.16 (3H, d, J = 6.8 Hz, CH_3), 3.01 (1H, ddd, J = 4.9, 4.9, 6.4 Hz, H-5), 3.32 (1H, dq, J = 4.4, 6.8 Hz, H-2), 3.68 (1H, dd, J = 6.4, 11.5 Hz, H-6a), 3.76 (1H, dd, J = 4.9, 11.5 Hz, H-6b), 3.88 (1H, dd, J = 2.2, 4.9 Hz, H-4), 3.96 (1H, dd, J = 2.2, 4.4 Hz, H-3); the minor conformer, δ 1.07 (3H, d, J = 6.6 Hz, CH_3), 3.54 (1H, ddd, J = 3.4, 5.9, 6.8 Hz, H-5), 3.65 (1H, dd, J = 5.9, 11.5 Hz, H-6a), 3.87 (1H, dd, J = 3.4, 11.5 Hz, H-6b), 3.94 (1H, dd, J = 6.8, 8.1 Hz, H-4), 4.03 (1H, dq, J = 6.6, 7.3 Hz, H-2), 4.05 (1H, dd, J = 7.3, 8.1 Hz, H-3); ^{13}C NMR (100 MHz, D_2O) the major conformer, δ 15.4 (C-1), 58.7 (C-2), 64.6 (C-6), 68.5 (C-5), 81.7 (C-3), 82.3 (C-4); the minor conformer, δ 17.9 (C-1), 57.3 (C-2), 65.9 (C-5), 66.6 (C-6), 77.6 (C-3), 78.3 (C-4); HRFABMS *m/z* 148.0972 [M + H]$^+$ ($C_6H_{14}NO_3$ requires 148.0974).

2,5-Dideoxy-2,5-imino-D-fucitol (8): $[\alpha]_D$ −11.1° (*c* 0.28, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 1.19 (3H, d, J = 6.8 Hz, CH_3), 3.53 (1H, dq, J = 3.7, 6.8 Hz, H-2), 3.60 (1H, ddd, J = 4.6, 6.4, 7.1 Hz, H-5), 3.69 (1H, dd, J = 7.1, 11.2 Hz, H-6a), 3.81 (1H, dd, J = 6.4, 11.2 Hz, H-6b), 4.02 (1H, dd, J = 2.0, 3.7 Hz, H-3), 4.27 (1H, dd, J = 2.0, 4.6 Hz, H-4); ^{13}C NMR (100 MHz, D_2O) δ 15.2 (C-1), 58.1 (C-2), 62.8 (C-6), 63.4 (C-5), 79.3 (C-4), 80.9 (C-3); HRFABMS *m/z* 148.0973 [M + H]$^+$ ($C_6H_{14}NO_3$ requires 148.0974).

1,4-Dideoxy-1,4-imino-(5-*O*-β-D-glucopyranosyl)-D-arabinitol (10): $[\alpha]_D$ +40.1° (*c* 0.16, H2O); ^{1}H NMR (400 MHz, D_2O) δ 2.97 (1H, br dd, J = 3.9, 12.5 Hz, H-1a), 3.26 (1H, dd, J = 5.6, 12.5 Hz, H-1b), 3.26-3.30 (1H, m, H-4), 3.33 (1H, dd, J = 7.8, 9.3 Hz, H-2′), 3.40 (1H, dd, J = 9.0, 9.8 Hz, H-4′), 3.49 (1H, ddd, J = 2.2, 5.9, 9.8 Hz, H-5′), 3.52 (1H, dd, J = 9.0, 9.3 Hz, H-3′), 3.74 (1H, dd, J = 5.9, 12.5 Hz, H-6′a), 3.82 (1H, dd, J = 7.6, 10.7 Hz, H-5a), 3.93 (1H, dd, J = 2.2, 12.5 Hz, H-6′b), 3.97 (1H, br, dd, J = 3.9, 4.9 Hz, H-3), 4.13 (1H, dd, J = 4.4, 10.7 Hz, H-5b), 4.21 (1H, ddd, J = 3.9, 3.9, 5.6 Hz, H-2), 4.51 (1H, d, J = 7.8 Hz, H-1′); ^{13}C NMR (100 MHz, D_2O) δ 53.4 (C-1), 63.7 (C-6′), 66.7 (C-4), 72.6 (C-5), 72.7 (C-4′), 76.2 (C-2′), 78.6 (C-3′), 78.9 (C-5′), 79.7 (C-2), 81.3 (C-3), 105.6 (C-1′); HRFABMS *m/z* 296. 1342 [M + H]$^+$ ($C_{11}H_{22}NO_8$ requires 296.1346).

1,4-Dideoxy-1,4-imino-(hydroxyethyliminiumyl)-D-arabinitol (11): $[\alpha]_D$ +54.7° (*c* 0.38, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 2.58 (1H, m, *N*-CH_2−), 2.61 (1H, m, H-4), 2.82 (1H, dd, J = 5.9, 11.2 Hz, H-1a), 3.02 (1H, m, *N*-CH_2−), 3.08 (1H, br dd, J = 2.2, 11.2 Hz, H-1b), 3.69−3.73 (4H, 2 × CH_2OH), 3.95 (1H, br dd, J = 2.9, 5.2 Hz, H-3), 4.13 (1H, ddd, J = 2.2, 2.9, 5.9 Hz, H-2); ^{13}C NMR (100 MHz, D_2O) δ 59.1 (*N*-CH_2−), 61.6 (C-1), 62.2 (*N*-CH_2-*C*H_2OH), 63.7 (C-5), 74.9 (C-4), 78.3 (C-2), 81.7 (C-3); HRFABMS *m/z* 178.1079 [M + H]$^+$ ($C_7H_{16}NO_4$ requires 178.1079).

***N*-Hydroxyethylation of Iminosugars.** 2-Bromoethanol (0.2 mL) and anhydrous K_2CO_3 (200 mg) were added to a solution of DAB (**1**) (100 mg) in DMF. The reaction mixture was heated overnight at 60 °C and diluted with MeOH. The diluted solution was applied to an Amberlyst 15 (20 mL) column, washed with MeOH, eluted with 1 M NH_4OH, and concentrated. The *N*-hydroxyethylated derivative was purified by Dowex 1-X2 (OH^- form) and Amberlite CG-50 (NH_4^+ form) chromatography with water as eluant to give **5** (95 mg) as a colorless oil. The *N*-hydroxyethylated derivatives of DMDP and DNJ were also synthesized in a similar manner.

2,5-Dideoxy-2,5-imino-(hydroxyethyliminiumyl)-D-mannitol (*N*-hydroxyethyl-DMDP): $[\alpha]_D$ −39.4° (*c* 0.89, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 51.0 (*N*-CH_2−), 62.1 (C-1,6), 62.3 (*N*-CH_2-*C*H_2OH), 70.8 (C-2,5), 81.1 (C-3,4); HRFABMS *m/z* 208.1183 [M + H]$^+$ ($C_8H_{18}NO_5$ requires 208.1185).

1,5-Dideoxy-1,5-imino-(hydroxyethyliminiumyl)-D-glucitol (*N*-hydroxyethyl-1-deoxynojirimycin, miglitol): $[\alpha]_D$ −11.0° (*c* 0.55, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 55.7 (*N*-CH_2−), 58.9 (C-1), 60.4 (C-6), 60.7 (*N*-CH_2-*C*H_2OH), 68.5 (C-5), 71.6 (C-2), 72.8 (C-4), 81.1 (C-3); HRFABMS *m/z* 208.1183 [M + H]$^+$ ($C_8H_{18}NO_5$ requires 208.1185).

1,6-Dideoxynojirimycin (12): $[\alpha]_D$ +37.8° (*c* 0.26, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 1.16 (3H, d, J = 6.4 Hz, CH_3), 2.46 (1H, dd, J = 10.7, 12.5 Hz, H-1ax), 2.52 (1H, dq, J = 6.4, 9.3 Hz, H-5), 3.01 (1H, dd, J = 9.3, 9.3 Hz, H-4), 3.07 (1H, dd, J = 5.1, 12.5 Hz, H-1eq), 3.28 (1H, dd, J = 9.3, 9.3 Hz, H-3), 3.51 (1H, ddd, J = 5.1, 9.3, 10.7 Hz, H-2); ^{13}C NMR (100 MHz, D_2O) δ 19.8 (C-6), 51.5 (C-1), 57.9 (C-5), 73.9 (C-2), 79.2 (C-4), 80.9 (C-3); HRFABMS *m/z* 148.0973 [M + H]$^+$ ($C_6H_{14}NO_3$ requires 148.0974).

1,3,4-Trideoxynojirimycin (13): $[\alpha]_D$ +36.6° (*c* 0.23, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 1.18 (1H, m, H-4ax), 1.35 (1H, m, H-3ax), 1.76 (1H, m, H-4eq), 2.07 (1H, m, H-3eq), 2.40 (1H, dd, J = 10.2, 11.5 Hz, H-1ax), 2.63 (1H, m, H-5), 3.16 (1H, ddd, J = 2.2, 4.6, 11.5 Hz, H-1eq), 3.43 (1H, dd, J = 7.3, 11.3 Hz, H-6a), 3.55 (1H, dd, J = 4.9, 11.3 Hz, H-6b), 3.65 (1H, m, H-2); ^{13}C NMR (100 MHz, D_2O) δ 29.0 (C-4), 34.8 (C-3), 54.1 (C-1), 59.1 (C-5), 67.7 (C-6), 70.0 (C-2); HRFABMS *m/z* 132.1024 [M + H]$^+$ ($C_6H_{14}NO_2$ requires 132.1025).

Glycosidase Inhibitory Activities. The enzymes α-glucosidases (from rice and yeast), α-galactosidase (from coffee beans), β-galactosidase (from bovine liver), amyloglucosidase (from *Aspergillus niger*), *p*-nitrophenyl glycosides, and disaccharides were purchased from Sigma Chemical Co. The rat epididymal fluid was purified from epididymis according to the method of Skudlarek et al.[15] Brush border membranes prepared from rat small intestine according to the method of Kessler et al.[15] were used as the enzyme source of rat intestinal maltase, sucrase, and isomaltase. The activity of rice α-glucosidase, rat intestinal glycosidases, and amyloglucosidase was determined using the appropriate disaccharides as substrates at the optimum pH of each enzyme. The released D-glucose was determined colorimetrically using the Glucose B-test Wako (Wako Pure Chemical Ind.). Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as a substrate at the optimum pH of each enzyme. The reaction was stopped by adding 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

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References and Notes

(1) Polhill, R. M. In *Advances in Legume Systematics*; Polhill, R. M., Raven, P. H., Eds.; Royal Botanic Gardens: Kew, 1981; pp 213−230.
(2) Nash, R. J.; Bell, E. A.; Williams, J. M. *Phytochemistry* **1985**, *24*, 1620−1622.
(3) Molyneux, R. J.; Pan, Y. T.; Tropea, J. E.; Elbein, A. D.; Lawyer, C. H.; Hughes, D. J.; Fleet, G. W. J. *J. Nat. Prod.* **1993**, *56*, 1356−1364.
(4) Asano, N.; Yasuda, K.; Kizu, H.; Kato, A.; Fan, J.-Q.; Nash, R. J.; Fleet, G. W. J.; Molyneux, R. J. *Eur. J. Biochem.* **2001**, *268*, 35−41.
(5) Asano, N.; Tomioka, E.; Kizu, H.; Matsui, K. *Carbohydr. Res.* **1994**, *253*, 235−245.
(6) Asano, N.; Nishida, M.; Kizu, H.; Matsui, K. *J. Nat. Prod.* **1997**, *60*, 98−101.
(7) Molyneux, R. J.; Pan, Y. T.; Tropea, J. E.; Bensom, M.; Kaushal, G. P.; Elbein, A. D. *Biochemistry* **1991**, *30*, 9981−9987.
(8) Liotta, L. J.; Bernotas, R. C.; Wilson, D. B.; Ganem, B. *J. Am. Chem. Soc.* **1989**, *111*, 783−785.
(9) Fleet, G. W. J.; Nicholas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash, R. J. *Tetrahedron Lett.* **1985**, *26*, 3127−3130.
(10) Scofield, A. M.; Fellows, L. E.; Nash, R. J.; Fleet, G. W. *Life Sci.* **1986**, *39*, 645−650.
(11) Asano, N.; Oseki, K.; Kizu, H.; Matsui, K. *J. Med. Chem.* **1994**, *37*, 3701−3706.
(12) Andersen, B.; Rassov, A.; Westergaard, N.; Lundgren, K. *Biochem. J.* **1999**, *342*, 545−550.
(13) Fosgerau, K.; Westergaard, N.; Quistorff, B.; Grunnet, N.; Kristiansen, M.; Lundgren, K. *Arch. Biochem. Biophysic.* **2000**, *380*, 274−284.
(14) Skudlarek, M. D.; Tulsiani, D. R. P.; Orgebin-Crist, M.-C. *Biochem. J.* **1992**, *286*, 907−914.
(15) Kessler, M.; Acuto, O.; Strelli, C.; Murer, H.; Semenza, G. *Biochim. Biophys. Acta* **1978**, *506*, 136−154.

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