Homonojirimycin Isomers and N-Alkylated Homonojirimycins: Structural and Conformational Basis of Inhibition of Glycosidases

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A series of natural epimers of α-homonojirimycin and its N-alkylated derivatives have been prepared to investigate the contribution of the different chiral centers and conformation of the specificity and potency of inhibition of glycosidases. These epimers and N-alkylated derivatives are α-homonojirimycin (**1**), *β*-homonojirimycin (**2**), α-homomannojirimycin (**3**), *β*-homomannojirimycin (**4**), α-3,4-di-*epi*-homonojirimycin (**5**), *β*-4,5-di-*epi*-homonojirimycin (**6**), *N*-methyl-α-homonojirimycin (**7**), and *N*-butyl-α-homonojirimycin (**8**). Compound **1** was a potent inhibitor of a range of α-glucosidases with IC_{50} values of 1 to 0.01 μM. Compounds **2**, **3**, and **4** were surprisingly inactive as inhibitors of *β*-glucosidase and α- and *β*-mannosidases but were moderately good as inhibitors of rice and some mammalian α-glucosidases. Compound **4** was active in the micromolar range toward all α-glucosidases tested. Furthermore, compound **4**, which superimposes well on *β*-L-fucose, was a 10-fold more effective inhibitor of α-L-fucosidase than 1-deoxymannojirimycin (**12**) and **3**, with a K_i value of 0.45 μM. Only compounds **5** and **6** showed inhibitory activity toward α- and *β*-galactosidases (**6** with an IC_{50} value of 6.4 μM against α-galactosidase). The high-resolution structure of **1** has been determined by X-ray diffraction and showed a chair conformation with the C1 OH (corresponding to the C6 OH in 1-deoxynojirimycin) predominantly equatorial to the piperidine ring in the crystal structure. This preferred (C1 OH equatorial) conformation was also corroborated by ^{1}H NMR coupling constants. The coupling constants for **7** suggest the axial orientation of the C1 OH, while in **8** the C1 OH axial conformation was not observed. The C1 OH axial conformation appears to be responsible for more potent inhibition toward processing α-glucosidase I than α-glucosidase II. It has been assumed that the anti-HIV activity of alkaloidal glycosidase inhibitors results from the inhibition of processing α-glucosidase I, but **1**, **7**, and **8** were inactive against HIV-1 replication at 500 μg/mL as measured by inhibition of virus-induced cytopathogenicity in MT-4 cells. In contrast, the EC_{50} value for *N*-butyl-1-deoxynojirimycin (**11**), which also inhibits processing α-glucosidase I, was 37 μg/mL. Compound **7** has been shown to be a better inhibitor of α-glucosidase I than **1** and **8** both in vitro and in the cell culture system. These data imply that inhibition of HIV by glycosidase inhibitors can be due to factors other than simply inhibition of processing α-glucosidase I.

Introduction

Recently we found that a 50% aqueous EtOH extract of *Aglaonema treubii* Engl. (Araceae) after preliminary purification by ion-exchange resins shows potent inhibitory activity against rice α-glucosidase, with an IC_{50} value of 0.07 μg/mL. A thorough purification of the extract resulted in the isolation of 2,5-dideoxy-2,5-imino-D-mannitol (DMDP), α-homonojirimycin (**1**, Figure 1), *β*-homonojirimycin (**2**), α-homomannojirimycin (**3**), *β*-homomannojirimycin (**4**), α-3,4-di-*epi*-homonojirimycin (**5**), 7-*O*-*β*-D-glucopyranosyl-α-homonojirimycin (MDL 25,637), and 5-*O*-α-D-galactopyranosyl-α-homonojirimycin.[1] Furthermore, we recently have isolated *β*-4,5-di-*epi*-homonojirimycin (**6**) from the extract as well. α-Homonojirimycin (**1**) was first isolated from the neotropical liana *Omphalea diandra* and shown to be an inhibitor of several α-glucosidases.[2] Before the natural product (**1**) was isolated, its *β*-glucoside (MDL 25,637) had been designed as a potential drug for the treatment of diabetes mellitus.[3] This *β*-glucoside has also been shown to preferentially inhibit an α-glucosidase II of glycoprotein precessing[4] and to be a potent inhibitor of porcine kidney trehalase.[5,6] Very recently, α-homonojirimycin (**1**) and its *N*-methyl derivative (**7**) have been tested as inhibitors of the purified glycoprotein processing enzymes, α-glucosidases I and II, from mung bean seedlings.[7] Compound **7** was a reasonably good inhibi-

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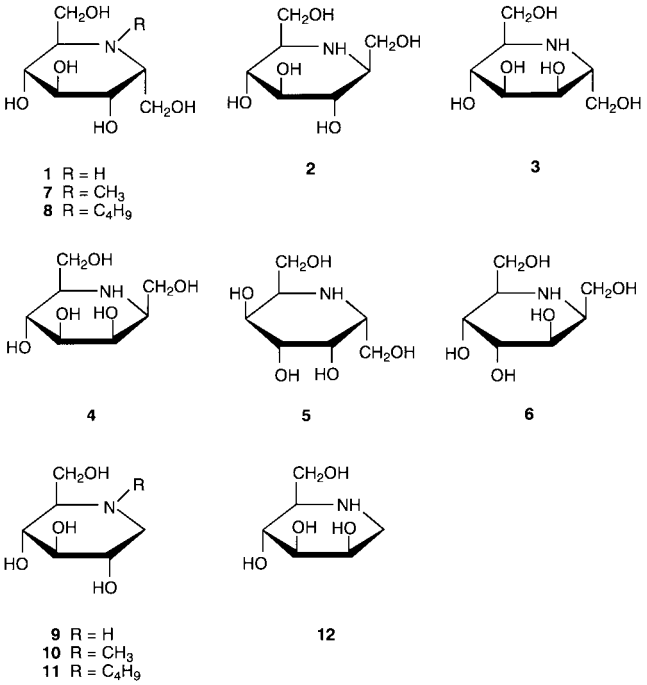

Figure 1.

tor of α-glucosidase I ($K_i = 1$ μM) and was about 3 times as effective against this enzyme as **1**. On the other hand, **1** inhibited α-glucosidase II with a K_i value of 1 μM, whereas **7** was a 30-fold less effective inhibitor than **1**.

1-Deoxynojirimycin (**9**) is a better inhibitor of α-glucosidase II than α-glucosidase I, as concentrations of 20 μM are required to give 50% inhibition of α-glucosidase I from *Saccharomyces cerevisiae* whereas concentrations of 2 μM are needed for α-glucosidase II.[8] It has been shown that the C6 OH group in **9** is predominantly equatorial to the piperidine ring, while that in the *N*-alkyl derivatives of **9** is predominantly axial.[9−11] This preferred C6 OH axial conformation appears to be an important structural feature of the *N*-alkyl derivatives of **9** exhibiting α-glucosidase I inhibition and antiviral activity.[12−14] However, the potencies of inhibition for purified calf liver α-glucosidase I in *N*-methyl-1-deoxynojirimycin (**10**) and *N*-butyl-1-deoxynojirimycin (**11**) are almost same, the K_i values being 0.07 and 0.09 μM, respectively,[15] whereas the anti-HIV-1 activity of **11** is obviously more potent than that of **10**.[10−12] We previously described that an unknown mechanism in addition to the potent inhibitory activity of **11** toward processing α-glucosidase I appeared to be involved in the inhibition of HIV-1.[11] 1-Deoxynojirimycin (**9**) has been found to also inhibit the formation of lipid-linked oligosaccharides, as the major lipid-linked oligosaccharide found in IEC-6 cells in the presence of **9** was $Man_9(GlcNAc)_2$-PP-dolichol, whereas in control cells it was $Glc_3Man_9(GlcNAc)_2$-PP-dolichol.[16] The *N*-methyl derivative of **9** does not affect the formation of lipid-linked saccharide intermediates. Recent studies have shown that **11** is a potent inhibitor of the glucosyltransferase-catalyzed biosynthesis of glucosylceramide (GlcCer), the key step in the biosynthetic pathway of GlcCer-based glycosphingolipids.[17,18] However, this activity is not shared by **9**. Thus, these glycosidase inhibitors suffer from a lack of specificity when given to animals and may cause many diverse effects. More specific inhibitors would have significant advantages as chemotherapeutic agents.

Table 1. Concentration of Homonojirimycin Isomers (μM) Giving 50% Inhibition of Glycosidases

	IC_{50} (μM)					
enzyme[a]	**1**	**2**	**3**	**4**	**5**	**6**
α-glucosidase						
rice	0.04	8.4	110	3.2	NI	0.7
rat intestinal maltase	0.34	15	46	4.6	NI	1.6
rat intestinal sucrase	0.17	7.2	27	3.0	410	0.8
rat liver lysosomal trehalase	0.26	8.2	NI	5.0	NI	2.7
porcine kidney	34	NI[b]	460	360	NI	140
α-galactosidase						
coffee bean	NI	NI	NI	NI	80	6.4
β-galactosidase						
bovine liver	NI	NI	NI	NI	160	86
rat intestinal lactase	NI	NI	NI	NI	290	130
α-L-fucosidase						
bovine epididymis	NI	NI	30	2.6	NI	38
rat epididymis	NI	NI	16	4.4	NI	20

[a] Rice α-glucosidase, maltase, sucrase, and trehalase were colorimetrically assayed by the D-glucose oxidase-peroxidase method using the appropriate disaccharide as substrate. Other enzyme activities were colorimetrically determined using *p*-nitrophenyl glycoside as substrate. [b] NI = less than 50% inhibition at 1000 μM.

Six homonojirimycin isomers isolated from *A. treubii* provided us with an opportunity to determine if, and how, the specificity of inhibition is altered with changes in chirality and the insertion of an hydroxymethyl group at the anomeric position of 1,5-dideoxy-1,5-iminohexopyranoses. Furthermore, in this paper we describe the comparison of changes in conformation and bioactivities (α-glucosidase inhibitory activity and anti-HIV-1 activity) by N-alkylation of α-homonojirimycin (**1**) and 1-deoxynojirimycin (**9**).

Results and Discussion

Inhibition of Glycosidases. The IC_{50} values of homonojirimycin isomers against various glycosidase are shown in Table 1.

1-Deoxynojirimycin (**9**) is a potent inhibitor of all types of mammalian α-glucosidases but is also a weak or moderate inhibitor of α-mannosidase, β-glucosidase, and β-galactosidase.[19] α-Homonojirimycin (**1**) inhibited α-glucosidases and trehalase to a similar extent as **9**, failing to have any activity toward other glycosidases tested. Thus, the insertion of a hydroxymethyl group at the anomeric position of **9** contributed to a greater selectivity. β-Homonojirimycin (**2**), with the β-glucose stereochemistry, has been synthesized chemically[20,21] and chemoenzymatically[22] and shown to be a very weak inhibitor of almond β-glucosidase.[22] However, in our assay, **2** showed less than 50% inhibition of almond, *Caldocellum saccharolyticum*, rat intestinal and rat liver lysosomal β-glucosidases even at 1000 μM, but it was a very specific inhibitor for α-glucosidase, with an IC_{50} value of ~10 μM. α-Homomannojirimycin (**3**) and β-homomannojirimycin (**4**) have been chemically synthesized and assayed for human liver glycosidases.[23] Compound **3** is a very weak inhibitor of human liver α-mannosidases, and the specificity and potency of **3** and 1-deoxymannojirimycin (**12**) are almost same, although **3** is more selective in not showing any significant inhibition of other human glycosidases. Compound **12**

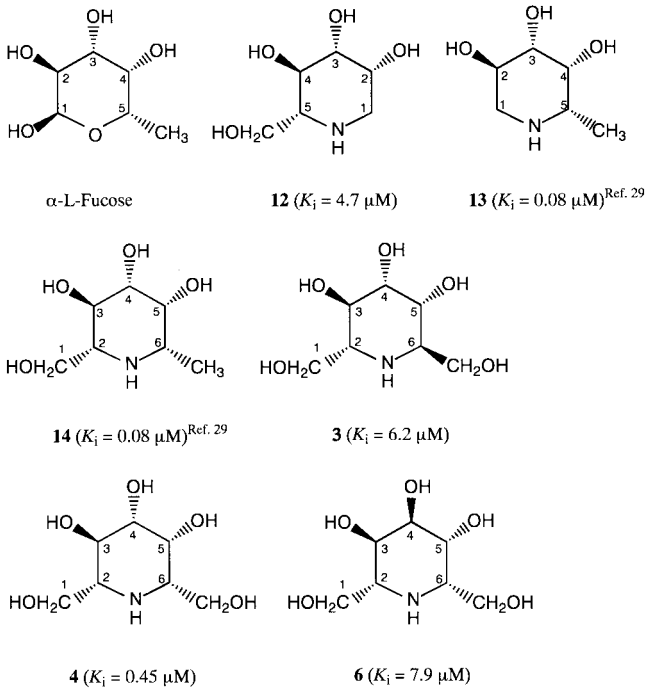

Figure 2.

is known as an inhibitor of glycoprotein processing α-mannosidase I[24,25] but is, in general, a much better inhibitor of α-L-fucosidase than of α-mannosidase.[26] Compound **4** unlike **3** is a potent inhibitor of human α-L-fucosidase (K_i = 4.5 μM).[23] We measured the inhibitory activities of **3** and **4** toward various glycosidases and found that **3** and **4** fail to inhibit α-mannosidases from jack bean, rat liver endoplasmic reticulum (ER), and rat liver lysosome, and β-mannosidases from snail and rat epididymis, respectively, although all the ring substituents are in the correct configuration (Table 1). However, **3** was a good inhibitor of rat digestive α-glucosidases and mammalian epididymis α-L-fucosidase, while **4** is a potent inhibitor of rice and rat α-glucosidases and mammalian epididymis α-L-fucosidase, with IC_{50} values of micromolar concentrations.

Fleet et al. synthesized 1-deoxyfuconojirimycin (**13**) from methyl D-glucopyranoside and showed that it is a very potent competitive inhibitor of bovine epididymis α-L-fucosidase (K_i = 4.8 nM).[27] They also showed that the introduction to **13** of an anomeric hydroxymethyl group to give β-L-homofuconojirimycin (**14**) does not diminish the inhibition of α-L-fucosidase.[28] This suggests that the presence of a substituent group on the anomeric position is not necessary for inhibition of α-L-fucosidase. From comparison of the extent of inhibitory activity with variation in structure or stereochemistry, Winchester et al.[29,30] have drawn certain broad conclusions that the common structural feature to all inhibitors of human α-L-fucosidase is the correct absolute stereochemistry of the three hydroxyl groups on the piperidine ring corresponding to C-2, C-3, and C-4 of L-fucose. α-Homomannojirimycin (**3**) has this minimum structural feature for inhibition of α-L-fucosidase but is not an inhibitor of the human liver enzyme.[23] However, in our present work, **3** was a good competitive inhibitor of bovine epididymis α-L-fucosidase, with a K_i value of 6.2 μM, as seen in Figure 2. This shows that there are marked differences in the inhibition by **3** of α-L-fucosidases from different species. Compound **4**, with its good superimposition onto β-L-homofuconojirimycin (**14**), is a more potent competitive inhibitor of α-L-fucosidase (K_i = 0.45 μM) than **3** but is less effective than **14**. This suggests that the hydrophobicity and the correct configuration of the C-6 substituent are important for the potent inhibition of α-L-fucosidase and that there might be a hydrophobic region in or around the active site. Inversion of the configuration at C-4 of **4** to give β-4,5-di-*epi*-homonojirimycin (**6**) decreased the potency of the competitive inhibition of α-L-fucosidase (K_i = 7.9 μM).

It is of great interest that β-4,5-di-*epi*-homonojirimycin (**6**) showed potent inhibitory activity toward all α-glucosidases tested and coffee bean α-galactosidase (Table 1). The epimerization at C-4 of **4** definitely enhances its inhibition toward α-glucosidases and α-galactosidase. In addition, it is a much better inhibitor of α-galactosidase (IC_{50} = 6.4 μM) than of β-galactosidase (IC_{50} = 86 μM).

Table 2. Coupling Constants for α-Homonojirimycin (**1**) and Its *N*-Methyl (**7**) and *N*-Butyl (**8**) Derivatives in D_2O

	coupling constants (Hz)							
compd	$J_{1a,2}$	$J_{1b,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{6,7a}$	$J_{6,7b}$
1	7.1	3.2	10.0	9.0	10.0	6.1	9.1	5.3
7	3.4	2.9	10.0	9.0	10.0	5.8	4.4	4.4
8	5.4	3.2	10.3	9.0	10.3	5.9	5.6	5.6

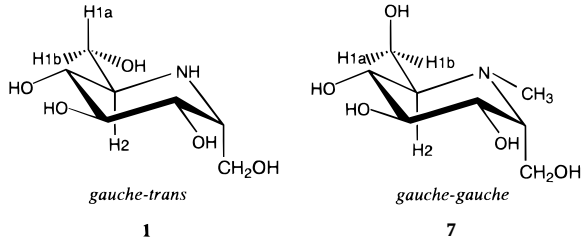

Figure 3.

Conformational Studies of α-Homonojirimycin and Its *N*-Alkyl Derivatives. The coupling constants measured from the 400 MHz NMR spectra in D_2O of α-homonojirimycin (**1**) and the *N*-methyl (**7**) and *N*-butyl (**8**) derivatives are shown in Table 2. Three large vicinal coupling constants, $^3J_{2,3}$, $^3J_{3,4}$, and $^3J_{4,5}$, suggest that these three compounds exist in aqueous medium in the chair conformation, and the coupling constant $^3J_{5,6}$ (6.1 Hz) of **1** shows that the anomeric (C6) hydroxymethyl group is in the axial orientation. A comparison of the magnitudes of these four coupling constants reveals that there is no significant difference in the ring conformation of these three compounds. The averaged $^3J_{1a,2}$ and $^3J_{1b,2}$ coupling constants relate to various rotamer populations about the C1−C2 bond. The coupling constants $^3J_{1b,2}$ (3.2 Hz) and $^3J_{1a,2}$ (7.1 Hz) for **1** suggest a large amount of the gauche−trans rotamer (Figure 3). On the other hand, the coupling constants $^3J_{1b,2}$ (2.9 Hz) and $^3J_{1a,2}$ (3.4 Hz) for **7** suggest a large population of the gauche−gauche rotamer. This preferred gauche−gauche conformation in **7** was not observed in **8**, as seen in the coupling constants $^3J_{1b,2}$ (3.2 Hz) and $^3J_{1a,2}$ (5.4 Hz), whereas the H6a and H6b of the *N*-butyl-1-deoxynojirimycin (**11**) (corresponding to the H1a and H1b of **8**) are in the gauche−gauche conformation.[11]

We have further determined the conformation of **1** in

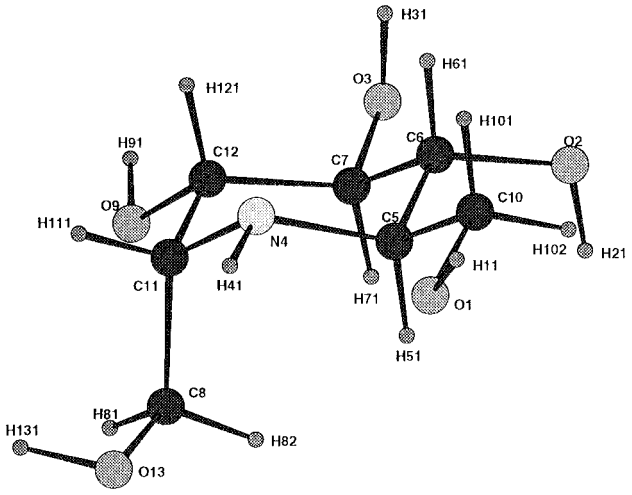

Figure 4.

Table 3. Concentration of α-Homonojirimycin (**1**) and Its *N*-Methyl (**7**) and *N*-Butyl (**8**) Derivatives (μM) Giving 50% Inhibition of α-Glucosidases[a]

	IC_{50} (μM)		
α-glucosidase	**1**	**7**	**8**
rice	0.04	0.06	4.2
rat intestinal sucrase	0.17	0.02	3.0
rat intestinal maltase	0.34	0.17	4.8
rat intestinal isomaltase	0.70	2.8	100
rat liver lysosmal	0.26	1.0	2.2
rat liver ER[b] glucosidase II	8.4	50	100

[a] Rice α-glucosidase, sucrase, maltase, and isomaltase activities were colorimetrically assayed by the D-glucose oxidase-peroxidase method using the corresponding disaccharide. [b] ER = endoplasmic reticulum.

solid state by X-ray crystallographic analysis. As seen in Figure 4, the six-membered ring is in a chair conformation with the anomeric (C6) hydroxymethyl group in an axial orientation and the other substituents in equatorial positions. Furthermore, the C1 OH group (corresponding to the C6 OH group of **9**) is predominantly equatorial to the piperidine ring in the crystal structure and the NH O6 intramolecular interaction stabilizes this preferred conformation.

Glycosidase Inhibitory Activity of *N*-Alkyl Derivatives of α-Homonojirimycin (1). The IC_{50} values of the *N*-alkyl derivatives of α-homonojimycin (**1**) against various α-glucosidases are shown in Table 3. Compound **1** is a potent inhibitor of rice, rat intestinal and liver lysosomal α-glucosidases and rat liver endoplasmic reticulum (ER) α-glucosidase II. *N*-Methylation of **1** enhanced inhibitory potential toward rat intestinal maltase and sucrase but reduced its inhibition of isomaltase, lysosomal, and ER enzymes. On the other hand, *N*-butylation of **1** significantly reduced its inhibition of all α-glucosidases tested. Recently it has been reported that the *N*-methyl derivative of **1** is a reasonably good inhibitor of mung bean α-glucosidase I, giving 50% inhibition at about 0.25 μg/mL (1.2 μM), whereas the parent compound **1** is only about 3-fold less effective against α-glucosidase I and the *N*-butyl derivative (**8**) is much less effective.[7]

It has been proposed that the C6 OH axial conformation of the *N*-alkyl derivatives of **9** best fit the active site of ER α-glucosidase I[9] or glucoamylase from *Aspergillus awamori*[31] and is responsible for their strong inhibitory activity. In fact compound **7** with this preferred conformation is a more potent inhibitor of α-glucosidase I than **1**, and unlike **11** compound **8**, which does not bear the C1 OH (corresponding to the C6 OH of **11**) group in a position perpendicular to the piperidine ring, is much less effective against α-glucosidase I.[7]

Inhibition of HIV Replication. The activity of α-homonojirimycin (**1**), its *N*-methyl (**7**) and *N*-butyl (**8**) derivatives against HIV-1 replication was investigated, based on the inhibition of virus-induced cytopathicity in MT-4 cells. Surprisingly, these compounds were all inactive against HIV-1, even at concentrations of 500 μg/mL, whereas the EC_{50} value for *N*-butyl-1-deoxynojirimycin (**11**) was 37 μg/mL.

Some sugar analogues capable of inhibiting N-linked oligosaccharide processing, such as 1-deoxynojirimycin (**9**) and castanospermine, and their derivatives, have been found to inhibit HIV replication and cellular cytopathicity.[13,32−34] Compound **11** is one of the most potent of these inhibitors. However, the exact mechanism of action of **11** as an inhibitor of HIV replication still remains to be determined. It is presumed, although not proven, that the anti-HIV activity of sugar analogues results from the inhibition of ER α-glucosidase I since there is a good correlation between potency of anti-HIV activity and that of α-glucosidase I inhibitory activity.[34−36] Karlsson et al.[14] have provided the first definitive evidence that α-glucosidase I inhibition occurs at the antiviral concentrations of **11** and proposed that the inhibition of this enzyme is a candidate mechanism for the antiviral activity of **11**. Fischer et al.[37] have reported that a major mechanism of action of **11** as an inhibitor of HIV replication is the impairment of viral entry at the level of post-CD4 binding, due to an effect on viral envelope components. The potency of inhibition for α-glucosidase I in *N*-methyl-1-deoxynojirimycin (**10**) and **11** are almost same, but the anti-HIV-1 activity of **11** is obviously more potent than that of **10**.[11−13] Therefore, the unknown mechanism of **11** in addition to inhibition of N-linked oligosaccharide processing appears to be involved in the inhibition of HIV-1. Compound **1**, and especially its *N*-methyl derivative (**7**), is more potent toward α-glucosidase I than either **9** or castanospermine.[7] However, **1** and **7** show no significant inhibition of HIV-1 replication even at 500 μg/mL. Inhibition of HIV by this class of compounds is not necessarilly correlated with inhibition of α-glucosidase I. These data imply that inhibition of HIV-1 is due to something other than inhibition of α-glucosidase I. It would be very important to determine what other factors are involved in the inhibition of this virus since that information may lead to novel sites of inhibition as well as to a new type of chemotherapeutic agent for AIDS or other viral diseases.

Experimental Section

General Methods. Optical rotation 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 as indicated using sodium 3-(trimethylsilyl)propionate (TSP) in D_2O and Me_4Si in pyridine-d_5−D_2O (3:1) as internal standards. MS were measured on a JEOL JMS-SX 102A spectrometer. Alkaloids were chromatographed on HPTLC Si gel $60F_{254}$ (E. Merck) using the solvent

system *n*-PrOH−AcOH−H_2O (4:1:1), and a chlorine-*o*-tolidine spray reagent was used for detection.

Preparation of Homonojirimycin Isomers and *N*-Alkyl Derivatives of α-Homonojirimycin. Homonojirimycin isomers were isolated from whole plants of *A. treubii* by the methods described in our previous paper.[1] The *N*-methyl (**7**) and *N*-butyl (**8**) derivatives of α-homonojirimycin were prepared and purified according to published procedures.[11]

α-Homonojirimycin (1): $[\alpha]_D$ +77.2 (*c* 0.57, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 2.87 (1H, ddd, J = 3.2, 7.1, 10.0 Hz, H-2), 3.21 (1H, dd, J = 9.0, 10.0 Hz, H-3), 3.29 (1H, ddd, J = 5.3, 6.1, 9.1 Hz, H-6), 3.50 (1H, dd, J = 9.0, 10.0 Hz, H-4), 3.57 (1H, dd, J = 7.1, 11.5 Hz, H-1a), 3.75 (1H, dd, J = 6.1, 10.0 Hz, H-5), 3.79−3.86 (2H, m, H-7a, H-7b), 3.90 (1H, dd, J = 3.2, 11.5 Hz, H-1b); ^{13}C NMR (100 MHz, D_2O) δ 56.9 (C-2), 59.1 (C-7), 59.7 (C-6), 64.8 (C-1), 74.4 (C-5), 74.9 (C-3), 77.1 (C-4); HRFABMS *m/z* 194.1026 $[M + H]^+$ ($C_7H_{16}O_5N$ requires 194.1028).

***β*-Homonojirimycin (2):** $[\alpha]_D$ −1.7 (*c* 0.35, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 2.66 (2H, ddd, J = 2.9, 6.6, 9.9 Hz, H-2, H-6), 3.25 (2H, dd, J = 9.2, 9.9 Hz, H-3, H-5), 3.39 (1H, t, J = 9.2 Hz, H-4), 3.64 (2H, dd, J = 6.6, 11.5 Hz, H-1a, H-7a), 3.89 (2H, dd, J = 2.9, 11.5 Hz, H-1b, H-7b); ^{13}C NMR (100 MHz, D_2O) δ 62.6 (C-2, C-6), 64.3 (C-1, C-7), 74.3 (C-3, C-5), 81.0 (C-4); HRFABMS *m/z* 194.1022 $[M + H]^+$ ($C_7H_{16}O_5N$ requires 194.1028).

α-Homomannojirimycin (3): $[\alpha]_D$ +4.3 (*c* 0.62, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 2.75 (1H, m, H-2), 3.15 (1H, ddd, J = 2.9, 6.8, 8.1 Hz, H-6), 3.65 (1H, t, J = 9.3 Hz, H-3), 3.68 (1H, dd, J = 2.9, 9.3 Hz, H-4), 3.69 (1H, dd, J = 6.8, 11.5 Hz, H-7a), 3.748 (1H, dd, J = 8.1, 11.5 Hz, H-7b), 3.751 (1H, dd, J = 5.8, 11.5 Hz, H-1a), 3.80 (1H, dd, J = 3.4, 11.5 Hz, H-1b), 4.02 (1H, t, J = 2.9 Hz, H-5); ^{13}C NMR (100 MHz, D_2O) δ 58.6 (C-2), 61.4 (C-6), 62.2 (C-7), 63.9 (C-1), 71.4 (C-3), 71.6 (C-5), 74.7 (C-4); HRFABMS *m/z* 194.1026 $[M + H]^+$ ($C_7H_{16}O_5N$ requires 194.1028).

***β*-Homomannojirimycin (4):** $[\alpha]_D$ +12.0 (*c* 0.27, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 2.59 (1H, ddd, J = 3.2, 5.4, 10.3 Hz, H-2), 2.87 (1H, dt, J = 1.7, 6.6 Hz, H-6), 3.56 (1H, dd, J = 2.5, 10.3 Hz, H-4), 3.59 (1H, t, J = 10.3 Hz, H-3), 3.62−3.71 (2H, H-7a, 7b), 3.74 (1H, dd, J = 5.4, 11.7 Hz, H-1a), 3.84 (1H, dd, J = 3.2, 11.7 Hz, H-1b), 4.01 (1H, dd, J = 1.7, 2.5 Hz, H-5); ^{13}C NMR (100 MHz, D_2O) δ 60.8 (C-6), 63.0 (C-2), 63.8 (C-1), 64.2 (C-7), 71.3 (C-3), 71.8 (C-5), 77.8 (C-4); HRFABMS *m/z* 194.1032 $[M + H]^+$ ($C_7H_{16}O_5N$ requires 194.1028).

α-3,4-Di-*epi*-homonojirimycin (5): $[\alpha]_D$ +39.1 (*c* 0.51, H_2O); ^{1}H NMR [400 MHz, pyridine-d_5−D_2O (3:1)] δ 3.65 (1H, ddd, J = 4.0, 4.8, 8.1 Hz, H-6), 3.87 (1H, ddd, J = 4.8, 6.2, 7.7 Hz, H-2), 4.21 (1H, dd, J = 7.7, 10.6 Hz, H-1a), 4.26 (1H, dd, J = 3.3, 6.2 Hz, H-3), 4.30 (1H, dd, J = 4.8, 10.6 Hz, H-1b), 4.39 (1H, t, J = 3.3 Hz, H-4), 4.42 (1H, dd, J = 4.8, 11.0 Hz, H-7a), 4.48 (1H, dd, J = 3.3, 4.0 Hz, H-5), 4.61 (1H, dd, J = 8.1, 11.0 Hz, H-7b); ^{13}C NMR (100 MHz, D_2O) δ 57.2 (C-2), 58.1 (C-6), 62.7 (C-7), 63.5 (C-1), 72.0 (C-3), 72.1 (C-4), 72.2 (C-5); HRFABMS *m/z* 194.1024 $[M + H]^+$ ($C_7H_{16}O_5N$ requires 194.1028).

***β*-4,5-Di-*epi*-homonojirimycin (6):** $[\alpha]_D$ +41.9 (*c* 0.43, H_2O); ^{1}H NMR(400 MHz, D_2O) δ 2.90 (1H, ddd, J = 3.2, 5.4, 10.5 Hz, H-2), 3.09 (1H, dt, J = 1.7, 6.6 Hz, H-6), 3.64 (1H, dd, J = 6.6, 11.2 Hz, H-7a), 3.68 (1H, dd, J = 6.6, 11.2 Hz, H-7b), 3.72 (1H, dd, J = 5.4, 11.7 Hz, H-1a), 3.78 (1H, dd, J = 3.2, 10.5 Hz, H-3), 3.82 (1H, dd, J = 3.2, 11.7 Hz, H-1b), 3.93 (1H, dd, J = 1.7, 3.9 Hz, H-5), 4.01 (1H, dd, J = 3.2, 3.9 Hz, H-4); ^{13}C NMR (100 MHz, D_2O) δ 56.8 (C-6), 58.2 (C-2), 64.3 (C-1, C-7), 68.9 (C-3), 72.3 (C-5), 73.8 (C-4); HRFABMS *m/z* 194.1029 $[M + H]^+$ ($C_7H_{16}O_5N$ requires 194.1028).

***N*-Methyl-α-homonojirimycin (7):** $[\alpha]_D$ +39.3 (*c* 0.56, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 2.53 (3H, s, NCH_3), 2.59 (1H, dt, J = 3.2, 10.0 Hz, H-2), 3.09 (1H, dt, J = 4.4, 5.8 Hz, H-6), 3.41 (1H, dd, J = 9.0, 10.0 Hz, H-3), 3.50 (1H, dd, J = 9.0, 10.0 Hz, H-4), 3.76 (1H, dd, J = 5.8, 10.0 Hz, H-5), 3.86 (1H, dd, J = 3.4, 12.7 Hz, H-1a), 3.89 (1H, dd, J = 2.9, 12.7 Hz, H-1b), 3.90 (1H, dd, J = 4.2, 12.2 Hz, H-7a), 3.94 (1H, dd, J = 5.4, 12.2 Hz, H-7b); ^{13}C NMR (100 MHz, D_2O) δ 41.1 (NCH_3), 58.1 (C-7), 60.6 (C-1), 64.0 (C-2), 69.4 (C-6), 72.8 (C-3), 73.1 (C-5), 77.4 (C-4); HRFABMS *m/z* 208.1179 $[M + H]^+$ ($C_8H_{18}O_5N$ requires 208.1185).

***N*-Butyl-α-homonojirimycin (8):** $[\alpha]_D$ +29.4 (*c* 1.08, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 0.91 (3H, s, NCH_3), 1.32 (2H, m, $NCH_2CH_2\mathbf{CH_2}CH_3$), 1.44 (1H, m, $NCH_2\mathbf{CH}_2CH_2CH_3$), 1.55 (1H, m, $NCH_2\mathbf{CH}_2CH_2CH_3$), 2.79−2.81 (3H, $N\mathbf{CH_2}$, H-2), 3.30 (1H, m, H-6), 3.41 (1H, dd, J = 9.0, 10.3 Hz, H-3), 3.51 (1H, dd, J = 9.0, 10.3 Hz, H-4), 3.80 (1H, dd, J = 5.9, 10.3 Hz, H-5), 3.84 (1H, dd, J = 5.4, 12.4 Hz, H-1a), 3.85 (2H, H-7a, H-7b), 3.94 (1H, dd, J = 3.2, 12.4 Hz, H-1b); ^{13}C NMR (100 MHz, D_2O) δ 16.1, 22.9, 32.7, 50.7 (*N*-butyl), 58.4 (C-7), 61.4 (C-1), 63.2 (C-2), 63.6 (C-6), 72.7 (C-5), 73.1 (C-3), 77.8 (C-4); HRFABMS *m/z* 250.1653 $[M + H]^+$ ($C_{11}H_{24}O_5N$ requires 250.1654).

Crystal Structure Determination of α-Homonojirimycin (1). Crystal Data. $C_7H_{15}NO_5$, M = 193.20 g mol^{-1}, orthorhombic, a = 6.807(1) Å, b = 8.706(1) Å, c = 14.656(2) Å, V = 868.5 Å^3 (by the least-squares refinement of the setting angles for 25 automatically centered reflections), space group $P2_12_12_1$, Z = 4, D_X = 1.48 g cm^{-3}, m = 10.27 cm^{-1}, transparent plate, crystal dimensions 0.05 × 0.35 × 0.7 mm^3.

Data Collection and Processing. Enraf-Nonius MACH3 diffractometer, ω−2θ scan mode with the ω scan width = 0.75 + 0.17 tan θ, ω scan speed 2.9−20.1° min^{-1}, graphite-monochromated Cu Kα radiation (λ = 1.5418 Å), measurement temperature 293 K, 2853 reflections were measured ($0 < \theta < 74°$, $-8 \le h \le 8$, $-10 \le k \le 10$, $-18 \le l \le 18$), 1658 unique reflections, 1295 reflections with $I > 3\sigma(I)$, crystal decay of 5.0%. Corrections for Lorentz and polarization effects were performed. Psi curve absorption corrections were in the range 1.0 to 1.27.

Structure Analyses and Refinement. Direct methods and full-matrix least-squares refinement were used. All non-hydrogen atoms were refined in anisotropic approximation. All carbon-bonded hydrogen atoms were placed in calculated positions and included in the final refinement with fixed positional and thermal parameters. The oxygen-bonded H atom was found in a difference map and not included in any cycle of refinement. A total of 120 parameters were refined from 1295 observations resulting in an observations/refined parameters ratio of 13.8. A Chebyshev weighting scheme[38] was applied. The Flack parameter[39]refined to 0.2(3). Refinement on F converged at R = 0.034 and R_w = 0.042. A final difference Fourier synthesis showed minimum and maximum residual electron densities of −0.27 and 0.26 e Å^{-3}. All crystallographic calculations were carried out using the CRYSTALS program package[40] on PC/AT-486. Neutral atom scattering factors were taken from the *International Tables for Crystallography*.[41]

Glycosidase Inhibitory Activities. The enzymes α-glucosidase (from rice), β-glucosidases (from almonds and *Caldocellum saccharolyticum*), α-mannosidase (from jack beans), β-mannosidase (from snail), α-galactosidase (green coffee beans), β-galactosidase (bovine liver), α-L-fucosidase (bovine epididymis), *p*-nitrophenyl glycosides, and disaccharides were purchased from Sigma Chemical Co. Brush border membranes, prepared from the intestine of male Wister rats by the method of Kessler et al.,[42] were used as the source of rat intestinal glycosidases. The partially purified lysosomal fraction prepared by the procedures of Tsuji et al.[43] was used as a source of lysosomal β-glucosidase and α-galactosidase.

The activities of rice α-glucosidase, rat intestinal glycosidases, and trehalase were determined using the appropriate disaccharides as substrates at the optimum pH of each enzyme. The released D-glucose was determined colorimetrically using 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.

Kinetics of Inhibition of α-L-Fucosidase. Kinetic parameters were determined by the double-reciprocal-plot method

of Lineweaver−Burk at increasing concentrations of *p*-nitrophenyl α-L-fucoside.

Antiviral Assays. MT-4 cells[44] and HTLV-III_B were used in the anti-HIV-1 assays. MT-4 cells (1×10^5/mL) were infected with HIV-1 at a multiplicity of infection (MOI) of 0.1. The cells were cultured in the presence of various concentrations of the test compounds. After a 4-day incubation at 37 °C, the number of viable cells were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[45] The cytotoxicities of the compounds were evaluated in parallel with their antiviral activity. This evaluation was based on the growth and viability of mock-infected cells, as monitored by the MTT method.

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